EUROPEAN PHARMACOPOEIA

TENTH EDITION

Supplement 10.1

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Individual copies of texts published in this edition will not be supplied.

Subscribers to the current version (book or electronic) of the European Pharmacopoeia have access to an online archive version of all obsolete editions and supplements of the European Pharmacopoeia in PDF format.

A list of new reagents published during the course of this edition is available under 'Useful information' in Pharmeuropa Online.

NEW TEXTS

The following texts appear for the first time in the European Pharmacopoeia. They will be implemented on ***1 April 2020*** *at the latest.*

REVISED TEXTS

The following texts have been technically revised since their last publication. They will be implemented on ***1 April 2020*** *at the latest.*

CORRECTED TEXTS

The following texts have been corrected for Supplement 10.1 and specify 'corrected 10.1' above the title. These corrections are to be taken into account as soon as possible and not later than ***30 November 2019*** *(the end of the month following the month of publication of Supplement 10.1).*

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2.2.47. Capillary electrophoresis

5.25. Process analytical technology

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TEXTS WHOSE TITLE HAS CHANGED

The titles of the following texts have been changed in Supplement 10.1.

MONOGRAPHS

Herbal drugs and herbal drug preparations

Fraxinus chinensis bark (2452) *(previously Fraxinus rhynchophylla bark)*
Senna leaflet (0206) *(previously Senna leaf)*
Senna pods (0207) *(previously Senna pods, Alexandrian)*

Monographs

Mercaptopurine monohydrate (0096) *(previously Mercaptopurine)*

DELETED TEXTS

The following texts are deleted as of ***1 April 2020****.*

MONOGRAPHS

Herbal drugs and herbal drug preparations

Senna pods, Tinnevelly (0208)

Monographs

Insulin, bovine (1637)

2.2. Physical and physico-chemical methods

01/2010:20247 corrected 10.1

2.2.47. CAPILLARY ELECTROPHORESIS[(1)]

GENERAL PRINCIPLES

Capillary electrophoresis is a physical method of analysis based on the migration, inside a capillary, of charged analytes dissolved in an electrolyte solution, under the influence of a direct-current electric field.

The migration velocity of an analyte under an electric field of intensity E, is determined by the electrophoretic mobility of the analyte and the electro-osmotic mobility of the buffer inside the capillary. The electrophoretic mobility of a solute (μ_{ep}) depends on the characteristics of the solute (electric charge, molecular size and shape) and those of the buffer in which the migration takes place (type and ionic strength of the electrolyte, pH, viscosity and additives). The electrophoretic velocity (v_{ep}) of a solute, assuming a spherical shape, is given by the equation:

$$v_{ep} = \mu_{ep} \times E = \left(\frac{q}{6\pi\eta r}\right) \times \left(\frac{V}{L}\right)$$

q = effective charge of the solute,

η = viscosity of the electrolyte solution,

r = Stoke's radius of the solute,

V = applied voltage,

L = total length of the capillary.

When an electric field is applied through the capillary filled with buffer, a flow of solvent is generated inside the capillary, called electro-osmotic flow. The velocity of the electro-osmotic flow depends on the electro-osmotic mobility (μ_{eo}) which in turn depends on the charge density on the capillary internal wall and the buffer characteristics. The electro-osmotic velocity (v_{eo}) is given by the equation:

$$v_{eo} = \mu_{eo} \times E = \left(\frac{\varepsilon\zeta}{\eta}\right) \times \left(\frac{V}{L}\right)$$

ε = dielectric constant of the buffer,

ζ = zeta potential of the capillary surface.

The velocity of the solute (v) is given by:

$$v = v_{ep} + v_{eo}$$

The electrophoretic mobility of the analyte and the electro-osmotic mobility may act in the same direction or in opposite directions, depending on the charge of the solute. In normal capillary electrophoresis, anions will migrate in the opposite direction to the electro-osmotic flow and their velocities will be smaller than the electro-osmotic velocity. Cations will migrate in the same direction as the electro-osmotic flow and their velocities will be greater than the electro-osmotic velocity. Under conditions in which there is a fast electro-osmotic velocity with respect to the electrophoretic velocity of the solutes, both cations and anions can be separated in the same run.

The time (t) taken by the solute to migrate the distance (l) from the injection end of the capillary to the detection point (capillary effective length) is given by the expression:

$$t = \frac{l}{v_{ep} + v_{eo}} = \frac{l \times L}{\left(\mu_{ep} + \mu_{eo}\right) \times V}$$

In general, uncoated fused-silica capillaries above pH 3 have negative charge due to ionised silanol groups in the inner wall. Consequently, the electro-osmotic flow is from anode to cathode. The electro-osmotic flow must remain constant from run to run if good reproducibility is to be obtained in the migration velocity of the solutes. For some applications, it may be necessary to reduce or suppress the electro-osmotic flow by modifying the inner wall of the capillary or by changing the concentration, composition and/or pH of the buffer solution.

After the introduction of the sample into the capillary, each analyte ion of the sample migrates within the background electrolyte as an independent zone, according to its electrophoretic mobility. Zone dispersion, that is the spreading of each solute band, results from different phenomena. Under ideal conditions the sole contribution to the solute-zone broadening is molecular diffusion of the solute along the capillary (longitudinal diffusion). In this ideal case the efficiency of the zone, expressed as the number of theoretical plates (N), is given by:

$$N = \frac{\left(\mu_{ep} + \mu_{eo}\right) \times V \times l}{2 \times D \times L}$$

D = molecular diffusion coefficient of the solute in the buffer.

In practice, other phenomena such as heat dissipation, sample adsorption onto the capillary wall, mismatched conductivity between sample and buffer, length of the injection plug, detector cell size and unlevelled buffer reservoirs can also significantly contribute to band dispersion.

Separation between 2 bands (expressed as the resolution, R_s) can be obtained by modifying the electrophoretic mobility of the analytes, the electro-osmotic mobility induced in the capillary and by increasing the efficiency for the band of each analyte, according to the equation:

$$R_s = \frac{\sqrt{N}\left(\mu_{epb} - \mu_{epa}\right)}{4\left(\overline{\mu}_{ep} + \mu_{eo}\right)}$$

μ_{epa} and μ_{epb} = electrophoretic mobilities of the 2 analytes separated,

$\overline{\mu}_{ep}$ = mean electrophoretic mobility of the 2 analytes $\overline{\mu}_{ep} = \frac{1}{2}\left(\mu_{epb} + \mu_{epa}\right)$.

APPARATUS

An apparatus for capillary electrophoresis is composed of:

- a high-voltage, controllable direct-current power supply;
- 2 buffer reservoirs, held at the same level, containing the prescribed anodic and cathodic solutions;
- 2 electrode assemblies (the cathode and the anode), immersed in the buffer reservoirs and connected to the power supply;
- a separation capillary (usually made of fused-silica) which, when used with some specific types of detectors, has an optical viewing window aligned with the detector. The ends of the capillary are placed in the buffer reservoirs. The capillary is filled with the solution prescribed in the monograph;
- a suitable injection system;

(1) This chapter has undergone pharmacopoeial harmonisation. See chapter *5.8. Pharmacopoeial harmonisation.*

– a detector able to monitor the amount of substances of interest passing through a segment of the separation capillary at a given time; it is usually based on absorption spectrophotometry (UV and visible) or fluorimetry, but conductimetric, amperometric or mass spectrometric detection can be useful for specific applications; indirect detection is an alternative method used to detect non-UV-absorbing and non-fluorescent compounds;
– a thermostatic system able to maintain a constant temperature inside the capillary is recommended to obtain a good separation reproducibility;
– a recorder and a suitable integrator or a computer.

The definition of the injection process and its automation are critical for precise quantitative analysis. Modes of injection include gravity, pressure or vacuum injection and electrokinetic injection. The amount of each sample component introduced electrokinetically depends on its electrophoretic mobility, leading to possible discrimination using this injection mode.

Use the capillary, the buffer solutions, the preconditioning method, the sample solution and the migration conditions prescribed in the monograph of the considered substance. The employed electrolytic solution is filtered to remove particles and degassed to avoid bubble formation that could interfere with the detection system or interrupt the electrical contact in the capillary during the separation run. A rigorous rinsing procedure should be developed for each analytical method to achieve reproducible migration times of the solutes.

CAPILLARY ZONE ELECTROPHORESIS

PRINCIPLE

In capillary zone electrophoresis, analytes are separated in a capillary containing only buffer without any anticonvective medium. With this technique, separation takes place because the different components of the sample migrate as discrete bands with different velocities. The velocity of each band depends on the electrophoretic mobility of the solute and the electro-osmotic flow in the capillary (see General Principles). Coated capillaries can be used to increase the separation capacity of those substances adsorbing on fused-silica surfaces.

Using this mode of capillary electrophoresis, the analysis of both small (M_r < 2000) and large molecules (2000 < M_r < 100 000) can be accomplished. Due to the high efficiency achieved in capillary zone electrophoresis, separation of molecules having only minute differences in their charge-to-mass ratio can be effected. This separation mode also allows the separation of chiral compounds by addition of chiral selectors to the separation buffer.

OPTIMISATION

Optimisation of the separation is a complex process where several separation parameters can play a major role. The main factors to be considered in the development of separations are instrumental and electrolytic solution parameters.

Instrumental parameters

Voltage. A Joule heating plot is useful in optimising the applied voltage and capillary temperature. Separation time is inversely proportional to applied voltage. However, an increase in the voltage used can cause excessive heat production, giving rise to temperature and, as a result thereof, viscosity gradients in the buffer inside the capillary. This effect causes band broadening and decreases resolution.

Polarity. Electrode polarity can be normal (anode at the inlet and cathode at the outlet) and the electro-osmotic flow will move toward the cathode. If the electrode polarity is reversed, the electro-osmotic flow is away from the outlet and only charged analytes with electrophoretic mobilities greater than the electro-osmotic flow will pass to the outlet.

Temperature. The main effect of temperature is observed on buffer viscosity and electrical conductivity, and therefore on migration velocity. In some cases, an increase in capillary temperature can cause a conformational change in proteins, modifying their migration time and the efficiency of the separation.

Capillary. The dimensions of the capillary (length and internal diameter) contribute to analysis time, efficiency of separations and load capacity. Increasing both effective length and total length can decrease the electric fields (working at constant voltage) which increases migration time. For a given buffer and electric field, heat dissipation, and hence sample band-broadening, depend on the internal diameter of the capillary. The latter also affects the detection limit, depending on the sample volume injected and the detection system employed.

Since the adsorption of the sample components on the capillary wall limits efficiency, methods to avoid these interactions should be considered in the development of a separation method. In the specific case of proteins, several strategies have been devised to avoid adsorption on the capillary wall. Some of these strategies (use of extreme pH and adsorption of positively charged buffer additives) only require modification of the buffer composition to prevent protein adsorption. In other strategies, the internal wall of the capillary is coated with a polymer, covalently bonded to the silica, that prevents interaction between the proteins and the negatively charged silica surface. For this purpose, ready-to-use capillaries with coatings consisting of neutral-hydrophilic, cationic and anionic polymers are available.

Electrolytic solution parameters

Buffer type and concentration. Suitable buffers for capillary electrophoresis have an appropriate buffer capacity in the pH range of choice and low mobility to minimise current generation.

Matching buffer-ion mobility to solute mobility, whenever possible, is important for minimising band distortion. The type of sample solvent used is also important to achieve on-column sample focusing, which increases separation efficiency and improves detection.

An increase in buffer concentration (for a given pH) decreases electro-osmotic flow and solute velocity.

Buffer pH. The pH of the buffer can affect separation by modifying the charge of the analyte or additives, and by changing the electro-osmotic flow. In protein and peptide separation, changing the pH of the buffer from above to below the isoelectric point (pI) changes the net charge of the solute from negative to positive. An increase in the buffer pH generally increases the electro-osmotic flow.

Organic solvents. Organic modifiers (methanol, acetonitrile, etc.) may be added to the aqueous buffer to increase the solubility of the solute or other additives and/or to affect the degree of ionisation of the sample components. The addition of these organic modifiers to the buffer generally causes a decrease in the electro-osmotic flow.

Additives for chiral separations. For the separation of enantiomers, a chiral selector is added to the separation buffer. The most commonly used chiral selectors are cyclodextrins, but crown ethers, polysaccharides and proteins may also be used. Since chiral recognition is governed by the different interactions between the chiral selector and each of the enantiomers, the resolution achieved for the chiral compounds depends largely on the type of chiral selector used. In this regard, for the development of a given separation it may be useful to test cyclodextrins having a different cavity size (α-, β-, or γ-cyclodextrin) or modified cyclodextrins with neutral (methyl, ethyl, hydroxyalkyl, etc.) or ionisable (aminomethyl, carboxymethyl, sulfobutyl ether, etc.) groups. When using modified cyclodextrins, batch-to-batch variations in the degree of substitution of the cyclodextrins must be taken into account since it will influence the selectivity. Other factors controlling the resolution in chiral separations are

concentration of chiral selector, composition and pH of the buffer and temperature. The use of organic additives, such as methanol or urea can also modify the resolution achieved.

CAPILLARY GEL ELECTROPHORESIS

PRINCIPLE

In capillary gel electrophoresis, separation takes place inside a capillary filled with a gel that acts as a molecular sieve. Molecules with similar charge-to-mass ratios are separated according to molecular size since smaller molecules move more freely through the network of the gel and therefore migrate faster than larger molecules. Different biological macromolecules (for example, proteins and DNA fragments), which often have similar charge-to-mass ratios, can thus be separated according to their molecular mass by capillary gel electrophoresis.

CHARACTERISTICS OF GELS

2 types of gels are used in capillary electrophoresis: permanently coated gels and dynamically coated gels. Permanently coated gels, such as cross-linked polyacrylamide, are prepared inside the capillary by polymerisation of the monomers. They are usually bonded to the fused-silica wall and cannot be removed without destroying the capillary. If the gels are used for protein analysis under reducing conditions, the separation buffer usually contains sodium dodecyl sulfate and the samples are denatured by heating in a mixture of sodium dodecyl sulfate and 2-mercaptoethanol or dithiothreitol before injection. When non-reducing conditions are used (for example, analysis of an intact antibody), 2-mercaptoethanol and dithiothreitol are not used. Separation in cross-linked gels can be optimised by modifying the separation buffer (as indicated in the capillary zone electrophoresis section) and controlling the gel porosity during the gel preparation. For cross-linked polyacrylamide gels, the porosity can be modified by changing the concentration of acrylamide and/or the proportion of cross-linker. As a rule, a decrease in the porosity of the gel leads to a decrease in the mobility of the solutes. Due to the rigidity of these gels, only electrokinetic injection can be used.

Dynamically coated gels are hydrophilic polymers, such as linear polyacrylamide, cellulose derivatives, dextran, etc., which can be dissolved in aqueous separation buffers giving rise to a separation medium that also acts as a molecular sieve. These separation media are easier to prepare than cross-linked polymers. They can be prepared in a vial and filled by pressure in a wall-coated capillary (with no electro-osmotic flow). Replacing the gel before every injection generally improves the separation reproducibility. The porosity of the gels can be increased by using polymers of higher molecular mass (at a given polymer concentration) or by decreasing the polymer concentration (for a given polymer molecular mass). A reduction in the gel porosity leads to a decrease in the mobility of the solute for the same buffer. Since the dissolution of these polymers in the buffer gives low viscosity solutions, both hydrodynamic and electrokinetic injection techniques can be used.

CAPILLARY ISOELECTRIC FOCUSING

PRINCIPLE

In isoelectric focusing, the molecules migrate under the influence of the electric field, so long as they are charged, in a pH gradient generated by ampholytes having pI values in a wide range (poly-aminocarboxylic acids), dissolved in the separation buffer.

The three basic steps of isoelectric focusing are loading, focusing and mobilisation.

Loading step. Two methods may be employed:

- loading in one step: the sample is mixed with ampholytes and introduced into the capillary either by pressure or vacuum;
- sequential loading: a leading buffer, then the ampholytes, then the sample mixed with ampholytes, again ampholytes alone and finally the terminating buffer are introduced into the capillary. The volume of the sample must be small enough not to modify the pH gradient.

Focusing step. When the voltage is applied, ampholytes migrate toward the cathode or the anode, according to their net charge, thus creating a pH gradient from anode (lower pH) to cathode (higher pH). During this step the components to be separated migrate until they reach a pH corresponding to their isoelectric point (pI) and the current drops to very low values.

Mobilisation step. If mobilisation is required for detection, use one of the following methods.

- in the first method, mobilisation is accomplished during the focusing step under the effect of the electro-osmotic flow; the electro-osmotic flow must be small enough to allow the focusing of the components;
- in the second method, mobilisation is accomplished by applying positive pressure after the focusing step;
- in the third method, mobilisation is achieved after the focusing step by adding salts to the cathode reservoir or the anode reservoir (depending on the direction chosen for mobilisation) in order to alter the pH in the capillary when the voltage is applied. As the pH is changed, the proteins and ampholytes are mobilised in the direction of the reservoir which contains the added salts and pass the detector.

The separation achieved, expressed as ΔpI, depends on the pH gradient (dpH / dx), the number of ampholytes having different pI values, the molecular diffusion coefficient (D), the intensity of the electric field (E) and the variation of the electrophoretic mobility of the analyte with the pH ($-\mathrm{d}\mu$ / dpH):

$$\Delta\mathrm{pI} = 3 \times \sqrt{\frac{D(\mathrm{dpH} / \mathrm{d}x)}{E(-\mathrm{d}\mu / \mathrm{dpH})}}$$

OPTIMISATION

The main parameters to be considered in the development of separations are:

Voltage. Capillary isoelectric focusing utilises very high electric fields, 300 V/cm to 1000 V/cm in the focusing step.

Capillary. The electro-osmotic flow must be reduced or suppressed depending on the mobilisation strategy (see above). Coated capillaries tend to reduce the electro-osmotic flow.

Solutions. The anode buffer reservoir is filled with a solution with a pH lower than the pI of the most acidic ampholyte and the cathode reservoir is filled with a solution with a pH higher than the pI of the most basic ampholyte. Phosphoric acid for the anode and sodium hydroxide for the cathode are frequently used.

Addition of a polymer, such as methylcellulose, in the ampholyte solution tends to suppress convective forces (if any) and electro-osmotic flow by increasing the viscosity. Commercial ampholytes are available covering many pH ranges and may be mixed if necessary to obtain an expanded pH range. Broad pH ranges are used to estimate the isoelectric point whereas narrower ranges are employed to improve accuracy. Calibration can be done by correlating migration time with isoelectric point for a series of protein markers.

During the focusing step precipitation of proteins at their isoelectric point can be prevented, if necessary, using buffer additives such as glycerol, surfactants, urea or zwitterionic buffers. However, depending on the concentration, urea denatures proteins.

MICELLAR ELECTROKINETIC CHROMATOGRAPHY (MEKC)

PRINCIPLE

In micellar electrokinetic chromatography, separation takes place in an electrolyte solution which contains a surfactant at a concentration above the critical micellar concentration (*cmc*). The solute molecules are distributed between the aqueous buffer and the pseudo-stationary phase composed of micelles, according to the partition coefficient of the solute. The technique can therefore be considered as a hybrid of electrophoresis and chromatography. It is a technique that can be used for the separation of both neutral and charged solutes, maintaining the efficiency, speed and instrumental suitability of capillary electrophoresis. One of the most widely used surfactants in MEKC is the anionic surfactant sodium dodecyl sulfate, although other surfactants, for example cationic surfactants such as cetyltrimethylammonium salts, are also used.

The separation mechanism is as follows. At neutral and alkaline pH, a strong electro-osmotic flow is generated and moves the separation buffer ions in the direction of the cathode. If sodium dodecyl sulfate is employed as the surfactant, the electrophoretic migration of the anionic micelle is in the opposite direction, towards the anode. As a result, the overall micelle migration velocity is slowed down compared to the bulk flow of the electrolytic solution. In the case of neutral solutes, since the analyte can partition between the micelle and the aqueous buffer, and has no electrophoretic mobility, the analyte migration velocity will depend only on the partition coefficient between the micelle and the aqueous buffer. In the electropherogram, the peaks corresponding to each uncharged solute are always between that of the electro-osmotic flow marker and that of the micelle (the time elapsed between these two peaks is called the separation window). For electrically charged solutes, the migration velocity depends on both the partition coefficient of the solute between the micelle and the aqueous buffer, and on the electrophoretic mobility of the solute in the absence of micelle.

Since the mechanism in MEKC of neutral and weakly ionised solutes is essentially chromatographic, migration of the solute and resolution can be rationalised in terms of the retention factor of the solute (k'), also referred to as mass distribution ratio (D_m), which is the ratio of the number of moles of solute in the micelle to those in the mobile phase. For a neutral compound, k' is given by:

$$k' = \frac{t_R - t_0}{t_0 \times \left(1 - \frac{t_R}{t_{mc}}\right)} = K \times \frac{V_S}{V_M}$$

t_R = migration time of the solute,

t_0 = analysis time of an unretained solute (determined by injecting an electro-osmotic flow marker which does not enter the micelle, for instance methanol),

t_{mc} = micelle migration time (measured by injecting a micelle marker, such as Sudan III, which migrates while continuously associated in the micelle),

K = partition coefficient of the solute,

V_S = volume of the micellar phase,

V_M = volume of the mobile phase.

Likewise, the resolution between 2 closely-migrating solutes (R_s) is given by:

$$R_s = \frac{\sqrt{N}}{4} \times \frac{\alpha - 1}{\alpha} \times \frac{k'_b}{k'_b + 1} \times \frac{1 - \left(\frac{t_0}{t_{mc}}\right)}{1 + k'_a \times \left(\frac{t_0}{t_{mc}}\right)}$$

N = number of theoretical plates for one of the solutes,

α = selectivity,

k'_a and k'_b = retention factors for both solutes, respectively ($k'_b > k'_a$).

Similar, but not identical, equations give k' and R_s values for electrically charged solutes.

OPTIMISATION

The main parameters to be considered in the development of separations by MEKC are instrumental and electrolytic solution parameters.

Instrumental parameters

Voltage. Separation time is inversely proportional to applied voltage. However, an increase in voltage can cause excessive heat production that gives rise to temperature gradients and viscosity gradients of the buffer in the cross-section of the capillary. This effect can be significant with high conductivity buffers such as those containing micelles. Poor heat dissipation causes band broadening and decreases resolution.

Temperature. Variations in capillary temperature affect the partition coefficient of the solute between the buffer and the micelles, the critical micellar concentration and the viscosity of the buffer. These parameters contribute to the migration time of the solutes. The use of a good cooling system improves the reproducibility of the migration time for the solutes.

Capillary. As in capillary zone electrophoresis, the dimensions of the capillary (length and internal diameter) contribute to analysis time and efficiency of separations. Increasing both effective length and total length can decrease the electric fields (working at constant voltage), increase migration time and improve the separation efficiency. The internal diameter controls heat dissipation (for a given buffer and electric field) and consequently the sample band broadening.

Electrolytic solution parameters

Surfactant type and concentration. The type of surfactant, in the same way as the stationary phase in chromatography, affects the resolution since it modifies separation selectivity. Also, the log k' of a neutral compound increases linearly with the concentration of surfactant in the mobile phase. Since resolution in MEKC reaches a maximum when k' approaches the value of $\sqrt{t_{mc} / t_0}$, modifying the concentration of surfactant in the mobile phase changes the resolution obtained.

Buffer pH. Although pH does not modify the partition coefficient of non-ionised solutes, it can modify the electro-osmotic flow in uncoated capillaries. A decrease in the buffer pH decreases the electro-osmotic flow and therefore increases the resolution of the neutral solutes in MEKC, resulting in a longer analysis time.

Organic solvents. To improve MEKC separation of hydrophobic compounds, organic modifiers (methanol, propanol, acetonitrile, etc.) can be added to the electrolytic solution. The addition of these modifiers usually decreases migration time and the selectivity of the separation. Since the addition of organic modifiers affects the critical micellar concentration, a given surfactant concentration can be used only within a certain percentage of organic modifier before the micellisation is inhibited or adversely affected, resulting in the absence of micelles and, therefore, in the absence of partition. The dissociation of micelles in the presence of a high content of organic solvent does not always mean that the separation will no longer be possible; in some cases the hydrophobic interaction between the ionic surfactant monomer and the neutral solutes forms solvophobic complexes that can be separated electrophoretically.

Additives for chiral separations. For the separation of enantiomers using MEKC, a chiral selector is included in the micellar system, either covalently bound to the surfactant or added to the micellar separation electrolyte. Micelles that have a moiety with chiral discrimination properties include

salts of *N*-dodecanoyl-L-amino acids, bile salts, etc. Chiral resolution can also be achieved using chiral discriminators, such as cyclodextrins, added to the electrolytic solutions which contain micellised achiral surfactants.

Other additives. Several strategies can be carried out to modify selectivity, by adding chemicals to the buffer. The addition of several types of cyclodextrins to the buffer can also be used to reduce the interaction of hydrophobic solutes with the micelle, thus increasing the selectivity for this type of compound.

The addition of substances able to modify solute-micelle interactions by adsorption on the latter, is used to improve the selectivity of the separations in MEKC. These additives may be a second surfactant (ionic or non-ionic) which gives rise to mixed micelles or metallic cations which dissolve in the micelle and form co-ordination complexes with the solutes.

QUANTIFICATION

Peak areas must be divided by the corresponding migration time to give the corrected area in order to:

- compensate for the shift in migration time from run to run, thus reducing the variation of the response,
- compensate for the different responses of sample constituents with different migration times.

Where an internal standard is used, verify that no peak of the substance to be examined is masked by that of the internal standard.

CALCULATIONS

From the values obtained, calculate the content of the component or components being examined. When prescribed, the percentage content of one or more components of the sample to be examined is calculated by determining the corrected area(s) of the peak(s) as a percentage of the total of the corrected areas of all peaks, excluding those due to solvents or any added reagents (normalisation procedure). The use of an automatic integration system (integrator or data acquisition and processing system) is recommended.

SYSTEM SUITABILITY

In order to check the behaviour of the capillary electrophoresis system, system suitability parameters are used. The choice of these parameters depends on the mode of capillary electrophoresis used. They are: retention factor (k') (only for micellar electrokinetic chromatography), apparent number of theoretical plates (N), symmetry factor (A_s) and resolution (R_s). In previous sections, the theoretical expressions for N and R_s have been described, but more practical equations that allow these parameters to be calculated from the electropherograms are given below.

APPARENT NUMBER OF THEORETICAL PLATES

The apparent number of theoretical plates (N) may be calculated using the expression:

$$N = 5.54 \times \left(\frac{t_R}{w_h}\right)^2$$

t_R = migration time or distance along the baseline from the point of injection to the perpendicular dropped from the maximum of the peak corresponding to the component,

w_h = width of the peak at half-height.

RESOLUTION

The resolution (R_s) between peaks of similar height of 2 components may be calculated using the expression:

$$R_s = \frac{1.18 \times (t_{R2} - t_{R1})}{w_{h1} + w_{h2}}$$

$$t_{R2} > t_{R1}$$

t_{R1} and t_{R2} = migration times or distances along the baseline from the point of injection to the perpendiculars dropped from the maxima of two adjacent peaks,

w_{h1} and w_{h2} = peak widths at half-height.

When appropriate, the resolution may be calculated by measuring the height of the valley (H_v) between 2 partly resolved peaks in a standard preparation and the height of the smaller peak (H_p) and calculating the peak-to-valley ratio:

$$\frac{p}{v} = \frac{H_p}{H_v}$$

SYMMETRY FACTOR

The symmetry factor (A_s) of a peak may be calculated using the expression:

$$A_s = \frac{w_{0.05}}{2d}$$

$w_{0.05}$ = width of the peak at one-twentieth of the peak height,

d = distance between the perpendicular dropped from the peak maximum and the leading edge of the peak at one-twentieth of the peak height.

Tests for area repeatability (standard deviation of areas or of the area/migration-time ratio) and for migration time repeatability (standard deviation of migration time) are introduced as suitability parameters. Migration time repeatability provides a test for the suitability of the capillary washing procedures. An alternative practice to avoid the lack of repeatability of the migration time is to use migration time relative to an internal standard.

A test for the verification of the signal-to-noise ratio for a standard preparation (or the determination of the limit of quantification) may also be useful for the determination of related substances.

SIGNAL-TO-NOISE RATIO

The detection limit and quantification limit correspond to signal-to-noise ratios of 3 and 10 respectively. The signal-to-noise ratio (S/N) is calculated using the expression:

$$\frac{S}{N} = \frac{2H}{h}$$

H = height of the peak corresponding to the component concerned, in the electropherogram obtained with the prescribed reference solution, measured from the maximum of the peak to the extrapolated baseline of the signal observed over a distance equal to twenty times the width at half-height,

h = range of the background in an electropherogram obtained after injection of a blank, observed over a distance equal to twenty times the width at the half-height of the peak in the electropherogram obtained with the prescribed reference solution and, if possible, situated equally around the place where this peak would be found.

2.4. Limit tests

04/2020:20424

2.4.24. IDENTIFICATION AND CONTROL OF RESIDUAL SOLVENTS

The test procedures described in this general method may be used:

i. for the identification of the majority of Class 1 and Class 2 residual solvents in an active substance, excipient or medicinal product when the residual solvents are unknown;

ii. as a limit test for Class 1 and Class 2 solvents when present in an active substance, excipient or medicinal product;

iii. for the quantification of Class 2 solvents when the limits are greater than 1000 ppm (0.1 per cent) or for the quantification of Class 3 solvents when required.

Class 1, Class 2 and Class 3 residual solvents are listed in general chapter *5.4. Residual solvents*.

Three diluents are described for sample preparation and the conditions to be applied for head-space injection of the gaseous sample onto the chromatographic system. Two chromatographic systems are prescribed but System A is preferred whilst System B is employed normally for confirmation of identity. The choice of sample preparation procedure depends on the solubility of the substance to be examined and in certain cases the residual solvents to be controlled.

The following residual solvents are not readily detected by the head-space injection conditions described: formamide, 2-ethoxyethanol, 2-methoxyethanol, ethylene glycol, *N*-methylpyrrolidone and sulfolane. Other appropriate procedures should be employed for the control of these residual solvents.

When the test procedure is applied quantitatively to control residual solvents in a substance, then it must be validated.

PROCEDURE

Examine by gas chromatography with static head-space injection (*2.2.28*).

Sample preparation 1. This is intended for the control of residual solvents in water-soluble substances.

Sample solution (1). Dissolve 0.200 g of the substance to be examined in *water R* and dilute to 20.0 mL with the same solvent.

Sample preparation 2. This is intended for the control of residual solvents in water-insoluble substances.

Sample solution (2). Dissolve 0.200 g of the substance to be examined in *dimethylformamide R* (DMF) and dilute to 20.0 mL with the same solvent.

Sample preparation 3. This is intended for the control of *N,N*-dimethylacetamide and/or *N,N*-dimethylformamide, when it is known or suspected that one or both of these substances are present in the substance to be examined.

Sample solution (3). Dissolve 0.200 g of the substance to be examined in *1,3-dimethyl-2-imidazolidinone R* (DMI) and dilute to 20.0 mL with the same solvent.

In some cases none of the above sample preparation procedures are appropriate, in which case the diluent to be used for the preparation of the sample solution and the static head-space conditions to be employed must be demonstrated to be suitable.

Solvent solution (a). To 1.0 mL of *Class 1 residual solvent solution CRS*, add 9 mL of *dimethyl sulfoxide R* and dilute to 100.0 mL with *water R*. Dilute 1.0 mL of this solution to 100 mL with *water R*. Dilute 1.0 mL of this solution to 10.0 mL with *water R*.

The reference solutions correspond to the following limits:
- benzene: 2 ppm;
- carbon tetrachloride: 4 ppm;
- 1,2-dichloroethane: 5 ppm;
- 1,1-dichloroethene: 8 ppm;
- 1,1,1-trichloroethane: 10 ppm.

Solvent solution (b). Dissolve appropriate quantities of the Class 2 residual solvents in *dimethyl sulfoxide R* and dilute to 100.0 mL with the same solvent. Dilute in *water R* to give a concentration of 1/20 of the limits stated in Table 2 (see *5.4. Residual solvents*).

Solvent solution (c). Dissolve 1.00 g of the solvent or solvents present in the substance to be examined in *dimethyl sulfoxide R* or *water R*, if appropriate, and dilute to 100.0 mL with *water R*. Dilute to give a concentration of 1/20 of the limit(s) stated in Table 1 or 2 (see *5.4. Residual solvents*).

Blank solution. Prepare as described for solvent solution (c) but without the addition of solvent(s) (used to verify the absence of interfering peaks).

Test solution. Introduce 5.0 mL of the sample solution and 1.0 mL of the blank solution into an injection vial.

Reference solution (a) (Class 1). Introduce 1.0 mL of solvent solution (a) and 5.0 mL of the appropriate diluent into an injection vial.

Reference solution (a_1) (Class 1). Introduce 5.0 mL of the sample solution and 1.0 mL of solvent solution (a) into an injection vial.

Reference solution (b) (Class 2). Introduce 1.0 mL of solvent solution (b) and 5.0 mL of the appropriate diluent into an injection vial.

Reference solution (c). Introduce 5.0 mL of the sample solution and 1.0 mL of solvent solution (c) into an injection vial.

Reference solution (d). Introduce 1.0 mL of the blank solution and 5.0 mL of the appropriate diluent into an injection vial.

Close the vials with a tight rubber membrane stopper coated with polytetrafluoroethylene and secure with an aluminium crimp cap. Shake to obtain a homogeneous solution.

The following static head-space injection conditions may be used:

	Sample preparation procedure		
Operating parameters	**1**	**2**	**3**
Equilibration temperature (°C)	80	105	80
Equilibration time (min)	60	45	45
Transfer-line temperature (°C)	85	110	105
Carrier gas: *nitrogen for chromatography R* or *helium for chromatography R* at an appropriate pressure			
Pressurisation time (s)	30	30	30
Injection volume (mL)	1	1	1

The chromatographic procedure may be carried out using:

SYSTEM A

– a fused-silica capillary or wide-bore column 30 m long and 0.32 mm or 0.53 mm in internal diameter coated with *cyanopropyl(3)phenyl(3)methyl(94)polysiloxane R* (film thickness 1.8 µm or 3 µm);

– *nitrogen for chromatography R* or *helium for chromatography R* as the carrier gas, split ratio 1:5 with a linear velocity of about 35 cm/s;

– a flame-ionisation detector (a mass spectrometer may also be used or an electron-capture detector for the chlorinated residual solvents of Class 1);

maintaining the temperature of the column at 40 °C for 20 min, then raising the temperature at a rate of 10 °C per min to 240 °C and maintaining it at 240 °C for 20 min and maintaining the temperature of the injection port at 140 °C and that of the detector at 250 °C; or, where there is interference from the matrix, use:

SYSTEM B

– a fused-silica capillary or wide-bore column 30 m long and 0.32 mm or 0.53 mm in internal diameter coated with *macrogol 20 000 R* (film thickness 0.25 µm);

– *nitrogen for chromatography R* or *helium for chromatography R* as the carrier gas, split ratio 1:5 with a linear velocity of about 35 cm/s;

– a flame-ionisation detector (a mass spectrophotometer may also be used or an electron-capture detector for the chlorinated residual solvents of Class 1);

maintaining the temperature of the column at 50 °C for 20 min, then raising the temperature at a rate of 6 °C per min to 165 °C and maintaining it at 165 °C for 20 min and maintaining the temperature of the injection port at 140 °C and that of the detector at 250 °C.

Inject 1 mL of the gaseous phase of reference solution (a) onto the column described in System A and record the chromatogram under such conditions that the signal-to-noise ratio for 1,1,1-trichloroethane can be measured. The signal-to-noise ratio must be at least 5. A typical chromatogram is shown in Figure 2.4.24.-1.

Inject 1 mL of the gaseous phase of reference solution (a_1) onto the column described in System A. The peaks due to the Class 1 residual solvents are still detectable.

Inject 1 mL of the gaseous phase of reference solution (b) onto the column described in System A and record the chromatogram under such conditions that the resolution between acetonitrile and dichloromethane can be determined. The system is suitable if the chromatogram obtained resembles the chromatogram shown in Figure 2.4.24.-2 and the resolution between acetonitrile and dichloromethane is at least 1.0.

Inject 1 mL of the gaseous phase of the test solution onto the column described in System A. If in the chromatogram obtained, there is no peak which corresponds to one of the residual solvent peaks in the chromatograms obtained with reference solution (a) or (b), then the substance to be examined meets the requirements of the test. If any peak in the chromatogram obtained with the test solution corresponds to any of the residual solvent peaks obtained with reference solution (a) or (b) then System B is to be employed.

Inject 1 mL of the gaseous phase of reference solution (a) onto the column described in System B and record the chromatogram under such conditions that the signal-to-noise ratio for benzene can be measured. The signal-to-noise ratio must be at least 5. A typical chromatogram is shown in Figure 2.4.24.-3.

Inject 1 mL of the gaseous phase of reference solution (a_1) onto the column described in System B. The peaks due to the Class 1 residual solvents are still detectable.

Inject 1 mL of the gaseous phase of reference solution (b) onto the column described in System B and record the chromatogram under such conditions that the resolution between acetonitrile and 1,1,2-trichloroethene can be determined. The system is suitable if the chromatogram obtained resembles the chromatogram shown in Figure 2.4.24.-4 and the resolution between acetonitrile and 1,1,2-trichloroethene is at least 1.0.

Inject 1 mL of the gaseous phase of the test solution onto the column described in System B. If in the chromatogram obtained, there is no peak which corresponds to any of the residual solvent peaks in the chromatogram obtained with the reference solution (a) or (b), then the substance to be examined meets the requirements of the test. If any peak in the chromatogram obtained with the test solution corresponds to any of the residual solvent peaks obtained with reference solution (a) or (b) and confirms the correspondence obtained when using System A, then proceed as follows.

Inject 1 mL of the gaseous phase of reference solution (c) onto the column described for System A or System B. If necessary, adjust the sensitivity of the system so that the height of the peak corresponding to the identified residual solvent(s) is at least 50 per cent of the full scale of the recorder.

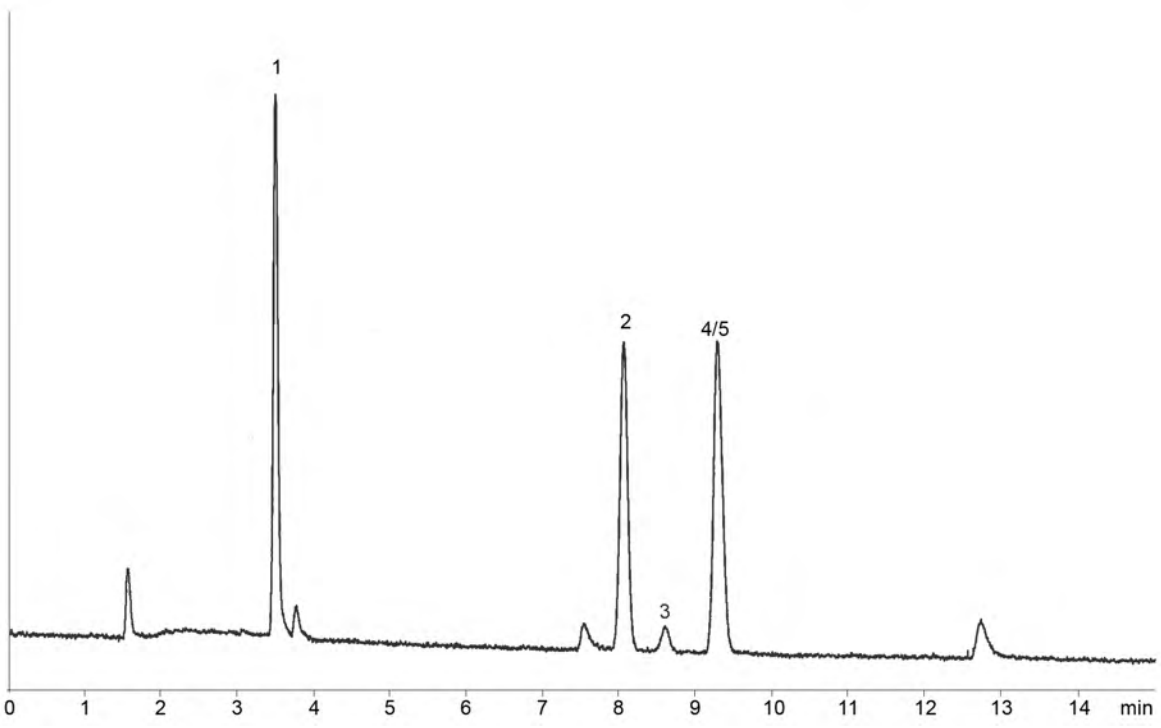
Inject 1 mL of the gaseous phase of reference solution (d) onto the column. No interfering peaks should be observed.

Inject 1 mL of the gaseous phase of the test solution and 1 mL of the gaseous phase of reference solution (c) on to the column. Repeat these injections twice more.

The mean area of the peak of the residual solvent(s) in the chromatograms obtained with the test solution is not greater than half the mean area of the peak of the corresponding residual solvent(s) in the chromatograms obtained with reference solution (c). The test is not valid unless the relative standard deviation of the differences in areas between the analyte peaks obtained from 3 replicate paired injections of reference solution (c) and the test solution, is at most 15 per cent.

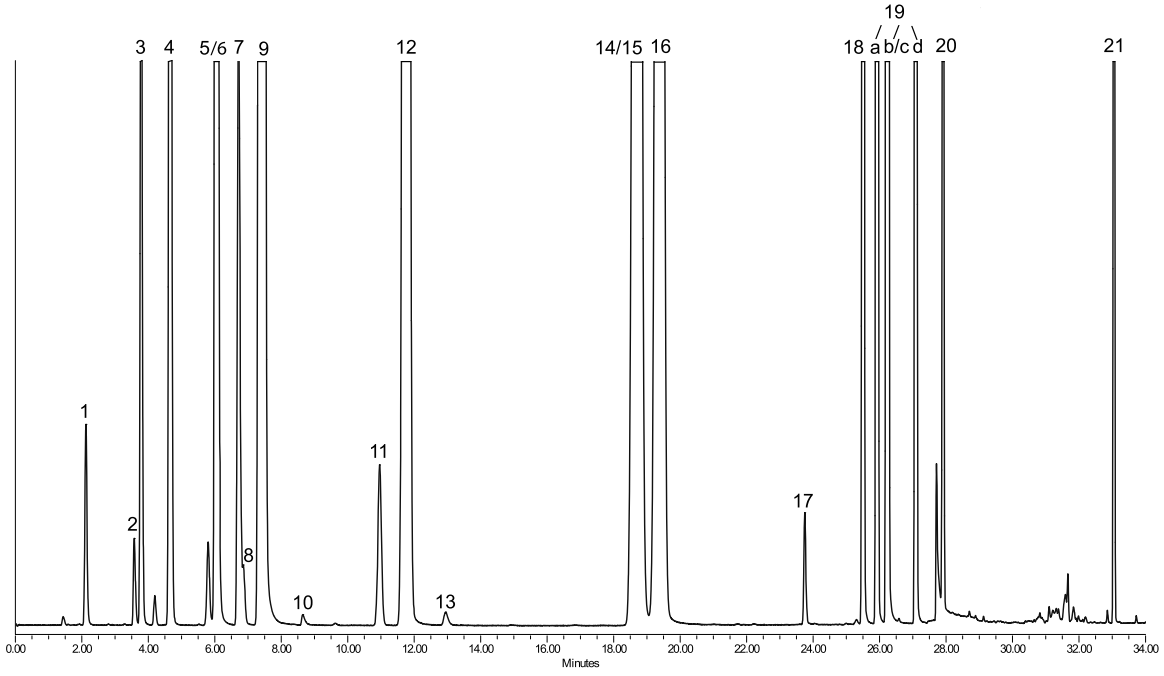
A flow diagram of the procedure is shown in Figure 2.4.24.-5.

When a residual solvent (Class 2 or Class 3) is present at a level of 0.1 per cent or greater then the content may be quantitatively determined by the method of standard additions.



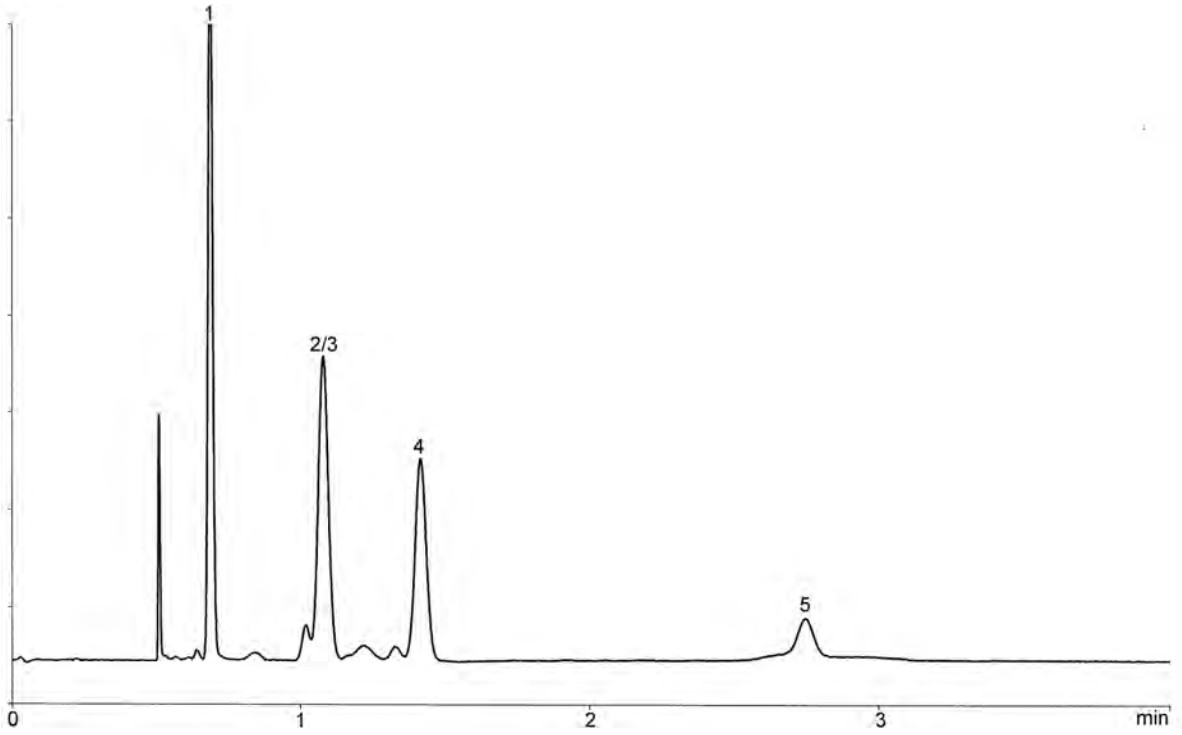
1. 1,1-dichloroethene 2. 1,1,1-trichloroethane 3. carbon tetrachloride 4. benzene 5. 1,2-dichloroethane

Figure 2.4.24.-1. – *Typical chromatogram of Class 1 solvents using the conditions described for System A and Procedure 1. Flame-ionisation detector*



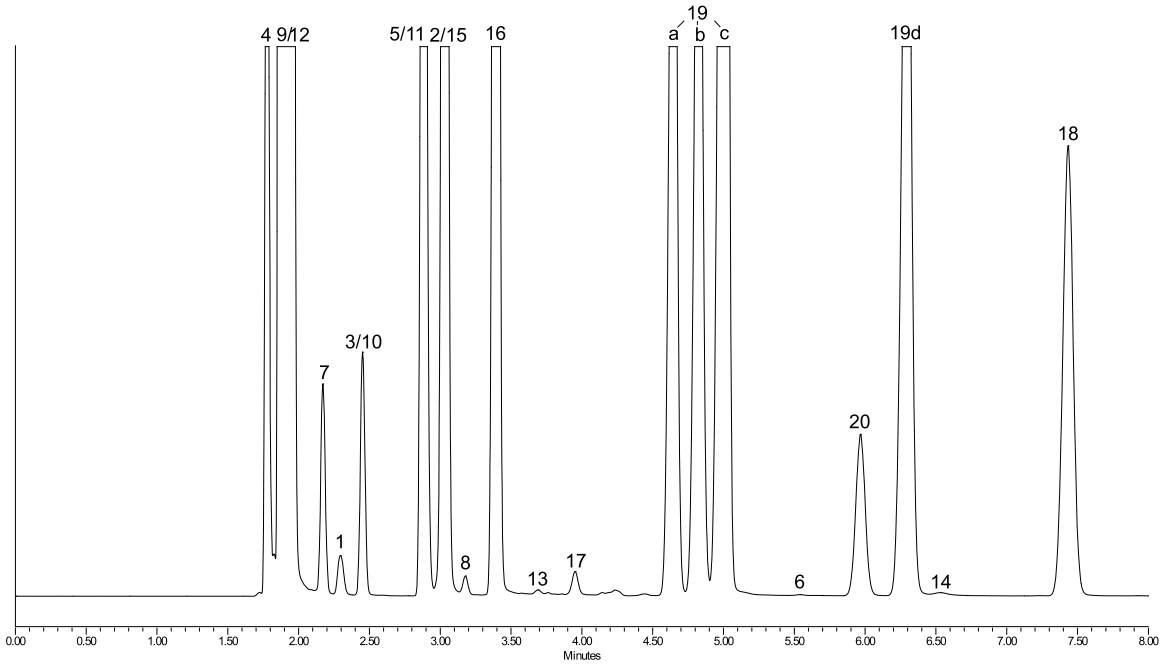
1. methanol
2. acetonitrile
3. dichloromethane
4. hexane
5. 1,2-dichloroethene
6. nitromethane
7. tetrahydrofuran
8. chloroform
9. cyclohexane
10. 1,2-dimethoxyethane
11. 1,1,2-trichloroethene
12. methylcyclohexane
13. 1,4-dioxane
14. pyridine
15. methylisobutylketone
16. toluene
17. methylbutylketone
18. chlorobenzene
19. xylene mix
a. ethylbenzene
b. *p*-xylene
c. *m*-xylene
d. *o*-xylene
20. cumene
21. tetralin

Figure 2.4.24.-2. – *Chromatogram of Class 2 solvents (solvent solution (b)) using the conditions described for System A and Procedure 1. Flame-ionisation detector*



1. 1,1-dichloroethene 2. 1,1,1-trichloroethane 3. carbon tetrachloride 4. benzene 5. 1,2-dichloroethane

Figure 2.4.24.-3. – *Chromatogram of Class 1 residual solvents using the conditions described for System B and Procedure 1. Flame-ionisation detector*



1. methanol	5. 1,2-dichloroethene	9. cyclohexane	13. 1,4-dioxane	17. methylbutylketone	20. cumene
2. acetonitrile	6. nitromethane	10. 1,2-dimethoxyethane	14. pyridine	18. chlorobenzene	21. tetralin (t_R = 27 min)
3. dichloromethane	7. tetrahydrofuran	11. 1,1,2-trichloroethene	15. methylisobutylketone	19. xylene mix a. ethylbenzene b. *p*-xylene c. *m*-xylene d. *o*-xylene	
4. hexane	8. chloroform	12. methylcyclohexane	16. toluene		

Figure 2.4.24.-4. – *Typical chromatogram of Class 2 residual solvents (solvent solution (b)) using the conditions described for System B and Procedure 1. Flame-ionisation detector*

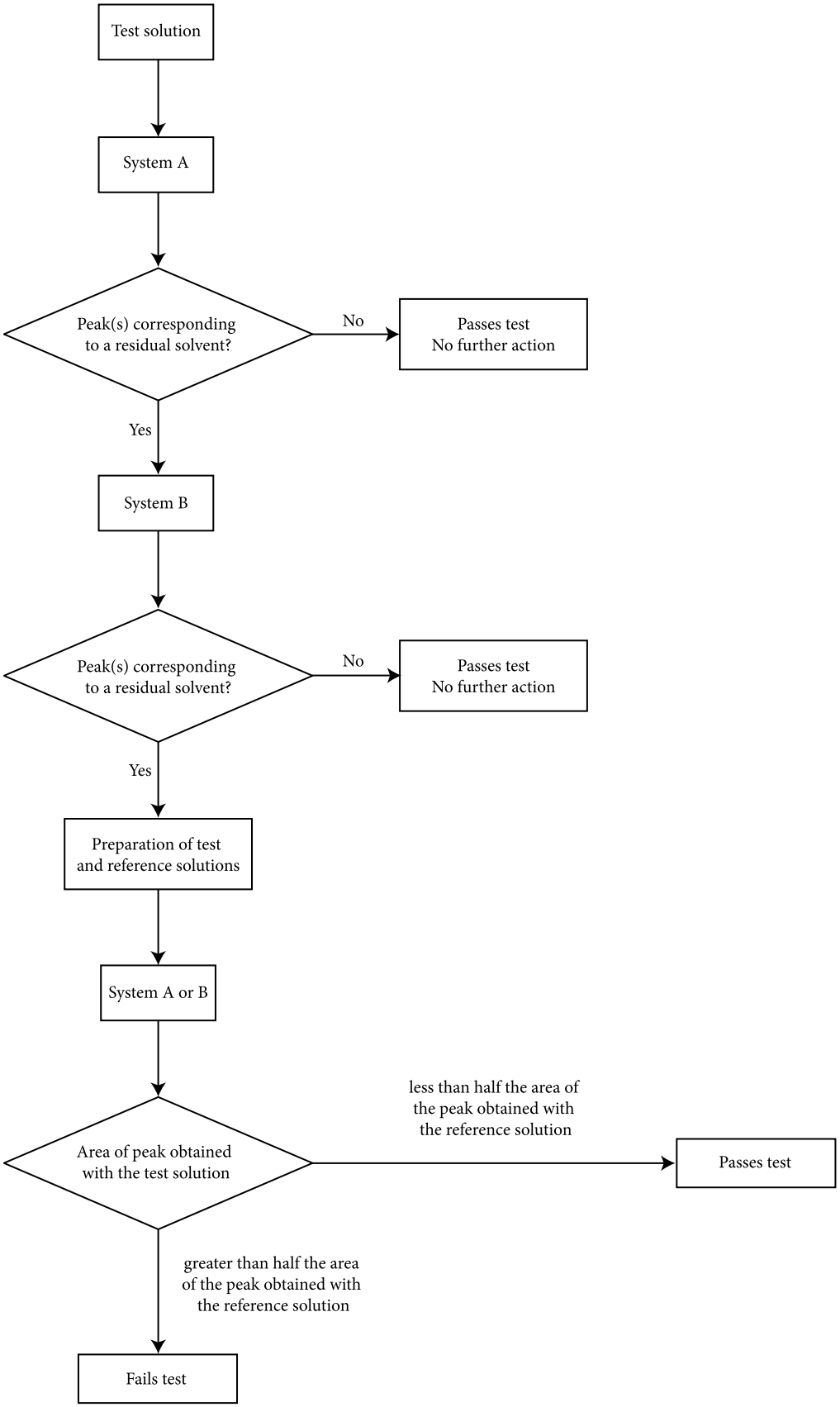


Figure 2.4.24.-5. – *Diagram relating to the identification of residual solvents and the application of limit tests*

4. Reagents

04/2020:40101

4.1.1. REAGENTS

Calcium chloride, anhydrous. $CaCl_2$. (M_r 111.0). *1014800.* [10043-52-4].

Content: minimum 98.0 per cent (dried substance).

White or almost white granules, deliquescent, very soluble in water, freely soluble in ethanol (96 per cent) and in methanol.

Loss on drying (*2.2.32*): maximum 5.0 per cent, determined by drying in an oven at 200 ± 10 °C.

Storage: in an airtight container, protected from moisture.

Convallatoxin. $C_{29}H_{42}O_{10}$. (M_r 550.6). *1207900.* [508-75-8]. 3β-[(6-Deoxy-α-L-mannopyranosyl)oxy]-5,14-dihydroxy-19-oxo-5β-card-20(22)-enolide. 5,14-Dihydroxy-19-oxo-3β-[(α-L-rhamnopyranosyl)oxy]-5β-card-20(22)-enolide.

White or slightly yellow, crystalline powder, slightly soluble in water, soluble in ethanol, and in acetone, slightly soluble in ethyl acetate.

mp: 235-242 °C.

Cymarin. $C_{30}H_{44}O_9$. (M_r 548.7). *1208000.* [508-77-0]. 3β-[(2,6-Dideoxy-3-*O*-methyl-β-D-*ribo*-hexopyranosyl)oxy]-5β,14-dihydroxy-19-oxocard-20(22)-enolide.

White or slightly yellow powder, slightly soluble in water, soluble in methanol.

mp: about 148 °C.

2,4-Dihydroxybenzaldehyde. $C_7H_6O_3$. (M_r 138.1). *1208100.* [95-01-2]. β-Resorcylaldehyde.

Dioxan solution. *1032002.*

Dissolve 1.00 g of *dioxan R* in *water R* and dilute to 100.0 mL with the same solvent. Dilute 5.0 mL of this solution to 100.0 mL with *water R* (0.5 mg/mL of dioxan).

Isorhamnetin-3-*O*-rutinoside. $C_{28}H_{32}O_{16}$. (M_r 625). *1208200.* [604-80-8]. 3-*O*-Methylquercetin-3-rutinoside. Narcissoside.

Methyl decanoate. $C_{11}H_{22}O_2$. (M_r 186.3). *1054000.* [110-42-9].

Content: minimum 99.0 per cent.

Clear, colourless or yellow liquid, soluble in light petroleum.

d_{20}^{20}: 0.871 to 0.876.

n_D^{20}: 1.425 to 1.426.

Molecular sieve. *1056600.* [70955-01-0].

Molecular sieve composed of sodium aluminosilicate. It is available as beads or powder with a pore size of 0.4 nm.

When reused, it is recommended that the molecular sieve be regenerated according to the manufacturer's instructions.

Raffinose. $C_{18}H_{32}O_{16}$. (M_r 504.4). *1208300.* [512-69-6]. β-D-Fructofuranosyl α-D-galactopyranosyl-(1→6)-α-D-glucopyranoside.

Rosuvastatin ethyl ester. $C_{24}H_{32}FN_3O_6S$. (M_r 509.6). *1208400.* [851443-04-4]. Ethyl (3*R*,5*S*,6*E*)-7-[4-(4-fluorophenyl)-2-(*N*-methylmethanesulfonamido)-6-(propan-2-yl)pyrimidin-5-yl]-3,5-dihydroxyhept-6-enoate.

Content: minimum 98 per cent.

White or pale yellow powder.

Sennoside A. $C_{42}H_{38}O_{20}$. (M_r 863). *1208500.* [81-27-6]. (9*R*,9′*R*)-5,5′-Bis(β-D-glucopyranosyloxy)-4,4′-dihydroxy-10,10′-dioxo-9,9′,10,10′-tetrahydro[9,9′-bianthracene]-2,2′-dicarboxylic acid.

Silica gel for chromatography, octadecylsilyl R1. *1110100.*

A very finely divided ultrapure silica gel, chemically modified at the surface by the bonding of octadecylsilyl groups.

Silica gel for chromatography, octadecylsilyl, with embedded polar groups, end-capped. *1177900.*

A very finely divided silica gel, chemically modified at the surface by the bonding of polar-embedded octadecylsilyl groups. To minimise any interaction with basic compounds, it is carefully end-capped to cover most of the remaining silanol groups.

Silica gel for chromatography, octylsilyl, solid core, end-capped. *1208600.*

Silica gel with spherical silica particles containing a non-porous solid silica core surrounded by a thin outer porous silica coating with octylsilyl groups. To minimise any interaction with basic compounds it is carefully end-capped to cover most of the remaining silanol groups.

Silica gel for chromatography, octylsilyl, with embedded polar groups, end-capped. *1152600.*

A very finely divided silica gel, chemically modified at the surface by the bonding of polar-embedded octylsilyl groups. To minimise any interaction with basic compounds, it is carefully end-capped to cover most of the remaining silanol groups.

Sodium laurilsulfate R1. *1208700.* [151-21-3].

Content: minimum 99.0 per cent.

04/2020:40201

4.2.1. PRIMARY STANDARDS FOR VOLUMETRIC SOLUTIONS

Potassium bromate. $KBrO_3$. (M_r 167.0). *2000300.* [7758-01-2].

Crystallise *potassium bromate R* from boiling *water R*. Collect the crystals and dry to constant mass in an oven at 180 ± 10 °C (*2.2.32*).

5.22. Names of herbal drugs used in traditional Chinese medicine

5.22. NAMES OF HERBAL DRUGS USED IN TRADITIONAL CHINESE MEDICINE

04/2020:52200 For transparency, this general chapter provides cross-references to the Chinese names in pinyin and in sinograms for herbal drugs used in traditional Chinese medicine for which a monograph is published in the Ph. Eur.

However, the titles in English, French and Latin are the only official names; the samples of herbal drugs must be labelled with at least one official title.

This general chapter is published for information.

Monograph number	Latin title	English title	Pinyin	Sinogram
2827	Abelmoschi corolla	Abelmoschi corolla	*huangshukuihua*	黄蜀葵花
2432	Acanthopanacis gracilistyli cortex	Acanthopanax bark	*wujiapi*	五加皮
2999	Achyranthis bidentatae radix	Achyranthes bidentata root	*niuxi*	牛膝
2472	Akebiae caulis	Akebia stem	*mutong*	木通
2554	Amomi fructus	Amomum fruit	*sharen*	砂仁
2555	Amomi fructus rotundus	Round amomum fruit	*doukou*	豆蔻
2712	Andrographidis herba	Andrographis herb	*chuanxinlian*	穿心莲
2661	Anemarrhenae asphodeloides rhizoma	Anemarrhena asphodeloides rhizome	*zhimu*	知母
2556	Angelicae dahuricae radix	Angelica dahurica root	*baizhi*	白芷
2557	Angelicae pubescentis radix	Angelica pubescens root	*duhuo*	独活
2558	Angelicae sinensis radix	Angelica sinensis root	*danggui*	当归
2435	Astragali mongholici radix	Astragalus mongholicus root	*huangqi*	黄芪
2559	Atractylodis lanceae rhizoma	Atractylodes lancea rhizome	*cangzhu*	苍术
2560	Atractylodis macrocephalae rhizoma	Atractylodes rhizome, largehead	*baizhu*	白术
1797	Aucklandiae radix	Aucklandia root	*muxiang*	木香
2561	Belamcandae chinensis rhizoma	Belamcanda chinensis rhizome	*shegan*	射干
2384	Bistortae rhizoma	Bistort rhizome	*quanshen*	拳参
2562	Bupleuri radix	Bupleurum root	*chaihu*	柴胡
2386	Carthami flos	Safflower flower	*honghua*	红花
2430	Citri reticulatae epicarpium et mesocarpium	Mandarin epicarp and mesocarp	*chenpi*	陈皮
2463	Clematidis armandii caulis	Clematis armandii stem	*chuanmutong*	川木通
2714	Codonopsidis radix	Codonopsis root	*dangshen*	党参
2454	Coicis semen	Coix seed	*yiyiren*	薏苡仁
2715	Coptidis rhizoma	Chinese goldthread rhizome	*huang lian*	黄连
2976	Corydalis rhizoma	Corydalis rhizome	*yan husuo*	延胡索
2890	Dioscoreae nipponicae rhizoma	Dioscorea nipponica rhizome	*chuanshanlong*	穿山龙
2473	Dioscoreae oppositifoliae rhizoma	Dioscorea oppositifolia rhizome	*shanyao*	山药
2563	Drynariae rhizoma	Drynaria rhizome	*gusuibu*	骨碎补
2564	Ecliptae herba	Eclipta herb	*mohanlian*	墨旱莲
2451	Ephedrae herba	Ephedra herb	*ma huang*	麻黄
2412	Eucommiae cortex	Eucommia bark	*duzhong*	杜仲
2718	Evodiae fructus	Evodia fruit	*wuzhuyu*	吴茱萸
2452	Fraxini chinensis cortex	Fraxinus chinensis bark	*qinpi*	秦皮
2565	Gardeniae fructus	Cape jasmine fruit	*zhizi*	栀子
2721	Gastrodiae rhizoma	Gastrodia rhizome	*tianma*	天麻
2722	Houttuyniae herba	Houttuynia herb	*yuxingcao*	鱼腥草
2566	Isatidis radix	Isatis root	*banlangen*	板蓝根
2634	Ligustici chuanxiong rhizoma	Szechwan lovage rhizome	*chuanxiong*	川芎
2431	Ligustici radix et rhizoma	Ligusticum root and rhizome	*gaoben*	藁本
2612	Lycii fructus	Barbary wolfberry fruit	*gouqizi*	枸杞子

Monograph number	Latin title	English title	Pinyin	Sinogram
2723	Lycopi herba	Lycopus lucidus herb	*zelan*	泽兰
2742	Magnoliae biondii flos immaturus	Magnolia biondii flower bud	*xinyi*	辛夷
2567	Magnoliae officinalis cortex	Magnolia officinalis bark	*houpo*	厚朴
2568	Magnoliae officinalis flos	Magnolia officinalis flower	*houpohua*	厚朴花
2474	Moutan cortex	Moutan bark	*mudanpi*	牡丹皮
2383	Notoginseng radix	Notoginseng root	*sanqi*	三七
3000	Ophiopogonis radix	Dwarf lilyturf tuber	*maidong*	麦冬
2424	Paeoniae radix alba	Peony root, white	*baishao*	白芍
2425	Paeoniae radix rubra	Peony root, red	*chishao*	赤芍
2727	Persicariae tinctoriae folium	Indigo plant leaf	*liaodaqingye*	蓼大青叶
2477	Piperis fructus	Pepper	*hujiao*	胡椒
2453	Piperis longi fructus	Long pepper	*bibo*	荜茇
2660	Platycodonis radix	Platycodon root	*jiegeng*	桔梗
2724	Polygoni cuspidati rhizoma et radix	Polygonum cuspidatum rhizome and root	*huzhang*	虎杖
2433	Polygoni multiflori radix	Fleeceflower root	*heshouwu*	何首乌
2726	Polygoni orientalis fructus	Polygonum orientale fruit	*shuihonghuazhi*	水红花子
2475	Poria	Poria	*fuling*	茯苓
2439	Prunellae spica	Common selfheal fruit-spike	*xiakucao*	夏枯草
2434	Puerariae lobatae radix	Kudzuvine root	*gegen (yege)*	葛根 (野葛)
2483	Puerariae thomsonii radix	Thomson kudzuvine root	*fenge*	粉葛
2569	Rehmanniae radix	Rehmannia root	*dihuang*	地黄
2663	Salviae miltiorrhizae radix et rhizoma	Salvia miltiorrhiza root and rhizome	*danshen*	丹参
2385	Sanguisorbae radix	Sanguisorba root	*diyu*	地榆
2428	Schisandrae chinensis fructus	Schisandra fruit	*wuweizi* (*bei wuweizi*)	五味子 (北五味子)
2438	Scutellariae baicalensis radix	Baical skullcap root	*huangqin*	黄芩
2450	Sinomenii caulis	Orientvine stem	*qingfengteng*	青风藤
2440	Sophorae flavescentis radix	Lightyellow sophora root	*kushen*	苦参
2639	Sophorae japonicae flos	Sophora flower	*huaihua*	槐花
2427	Sophorae japonicae flos immaturus	Sophora flower-bud	*huaimi*	槐米
2478	Stephaniae tetrandrae radix	Fourstamen stephania root	*fenfangji* (*hanfangji*)	粉防己 (汉防己)
2937	Typhae pollis	Typhae pollen	*puhuang*	蒲黄
2729	Uncariae rhynchophyllae ramulus cum uncis	Uncaria stem with hooks	*gou teng*	钩藤
2656	Zanthoxyli bungeani pericarpium	Zanthoxylum bungeanum pericarp	*huajiao*	花椒

5.25. Process analytical technology

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5.25. PROCESS ANALYTICAL TECHNOLOGY

This general chapter focuses on the interfacing of analytical techniques with the manufacturing process as a means of enhancing process control and improving process understanding. It should be noted that it is not within the scope of the chapter to give specific instructions for all possible process analytical technology sensors; instead, it offers a general approach to the integration of analytical techniques in the process environment together with aspects to be taken into consideration for the application of process analytical technology.

1. INTRODUCTION

Process analytical technology (PAT) can be defined as a system for designing, analysing and controlling manufacturing processes through timely measurements (i.e. during processing) of critical quality attributes (CQA) of raw and in-process materials and critical performance characteristics of processes in order to ensure the quality of the final product. It is important to note that the term 'analytical' in PAT is used in a broad sense to include chemical, physical and microbiological measurements conducted in an integrated manner and combined with data analysis. The goal of PAT is control of the manufacturing process and enhanced process understanding, guided by risk management. Interfacing manufacturing processes with analytical techniques is therefore essential in PAT, as it facilitates process development in accordance with quality by design (QbD) principles, enables real-time release testing (RTRT) and supports continuous manufacturing processes.

Time delays between obtaining a sample for testing, analysis of that sample and any consequent outcomes must be taken into consideration when applying PAT. When the analytical results are used on a continuous basis to monitor and control a process, it is important to minimise such delays. This can be achieved most effectively with sensor-based continuous measurement systems directly interfacing with the process stream during a specific unit operation. The sensors interfaced with the process continuously measure the process conditions and material characteristics within the process environment (*in situ*) and send the measured data (e.g. a spectrum) to an operating system where it is recorded and analysed, and where any necessary adjustments to processing conditions can be determined on a continuous basis. These *in situ* measurements can generate very large volumes of data representative of the process.

2. INTERFACING MODES

The interfacing of analytical techniques with the manufacturing process is central to the application of PAT. Results generated by analytical equipment interfaced with a process can be used for monitoring purposes and to ensure that the process is stable, for example via automated feedback or feedforward control loops.

The terms 'off-line', 'at-line', 'on-line' and 'in-line' describe interfacing modes (see Figure 5.25.-1). Measurements using on-line and in-line systems generally support rapid and automated process adjustments since they move the analytical techniques into the process stream. In contrast, off-line and at-line measurements involve transferring the samples away from the process stream to the analytical equipment.

Off-line measurements

These correspond to conventional analytical testing in which the sample is removed from the manufacturing process environment and tested in a laboratory, typically located away from the production environment. This transfer of samples away from the process stream can result in a significant time lag and generally does not permit immediate process adjustments. However, off-line measurements can nonetheless be useful for PAT purposes if relevant analytical data can be obtained within a time frame that is compatible with process dynamics.

At-line measurements

With at-line measurements, the sample is also removed (manually or automatically) from the process stream for testing, but the testing equipment is usually located within the production environment, i.e. in close physical proximity to the process stream, and testing can therefore take place with minimal delay. This results in a shorter time frame for obtaining analytical data, and also differentiates at-line measurements from off-line measurements. It is therefore possible to make process adjustments based on the results of at-line measurements.

On-line measurements

On-line measurements typically involve sensor-based measurements made under real-time conditions by diverting a portion of the material from the process stream directly into a measuring device and the results are available after a minimal time delay.

Depending on the nature of the test, e.g. whether or not it is detrimental to the product, the diverted portion may be returned to the process stream, otherwise it is discarded.

In-line measurements

In-line measurements are taken within the process stream by placing measuring devices, typically sensors, directly in contact with or into the process stream. No portions of the material are therefore removed from the process stream.

In-line measurements must also be non-detrimental to the product.

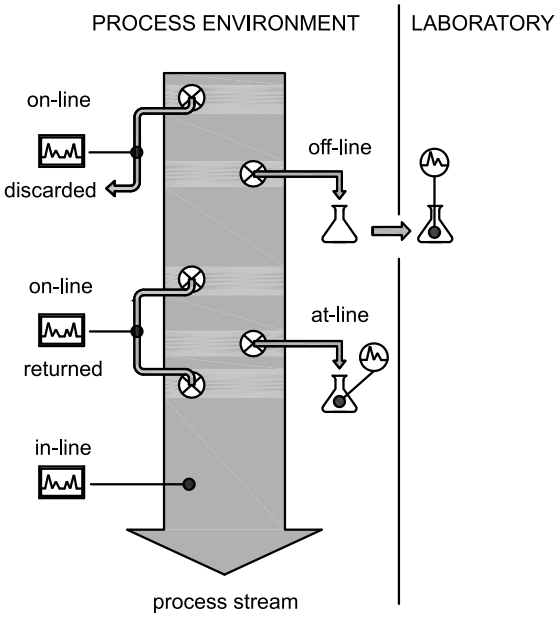

Figure 5.25.-1. – *Interfacing modes*

3. COMPARISON OF INTERFACING MODES

Both off-line and at-line measurements are based on analysis of discrete samples removed from a process stream or bulk material. These samples are considered representative of the material in the process stream at the time they are removed. Frequent at-line measurements also provide data supporting the application of PAT, as they are carried out within a short time frame and in the immediate vicinity of the process stream.

Neither in-line nor on-line measurements involve sampling in the conventional sense. The tested portion might not be separated from the process stream, and is usually smaller than a conventional sample.

Scale of scrutiny must be considered and will determine the frequency and duration of measurements. Furthermore, measurements can be influenced by physical attributes interfering with the acquisition properties of the measuring system. When measuring solids or suspensions, for example using spectroscopic methods, consideration must be given to the surface and bulk scattering properties of samples, and movement of the material. Influencing factors such as particle size, surface roughness and solid density can cause significant spectral differences, which must be taken into account when a method is designed and used in order to ensure that the process and material are described adequately.

In-line and on-line modes offer the advantage of fast data acquisition, allowing high measurement frequency, and thus enabling rapid continuous monitoring as well as immediate action and control of the process.

In some cases, PAT measurements can be used qualitatively to build models such as process trajectories or process signatures which can be used to characterise process variability and highlight unusual process behaviours. These models are not necessarily directly linked to a critical in-process quality attribute or performance characteristic. It is therefore recommended to demonstrate the causal relationship between the PAT measurements, the model and the relevant quality attributes or performance characteristics when using such models for process control purposes.

General validation principles also apply to in-line or on-line measurements although some validation procedures may be different to those for conventional quality control methods.

Analytical validation procedures for at-line and off-line measurements assume homogenous and authentic samples, which are crucial for the assessment of precision. However, this requirement is rarely fulfilled for on-line or in-line methods which are often used to measure processes in which the material rapidly changes and/or moves during the measurement, for example a fast-drying process or reaction monitoring. Comparison of results from on-line/in-line measurements with results from a reference test procedure would normally be needed.

4. STATISTICAL PROCESS CONTROL

Statistical process control (SPC) comprises a set of data analysis methods applied in order to monitor and control a process based on analysis of process variability (e.g. of CQAs). For PAT purposes, the process can be monitored using, for example, real-time data collected by process analysers.

Based on this data, SPC can be used to make process adjustments, when necessary, to maintain or attain a desired state and to ensure that the process remains under control. This includes, for example, trend analysis of quality attributes or process variables. SPC can also be used to measure process variability and process capability (i.e. the ability of a process to produce a product that complies with requirements), with a view to enhancing process understanding, thereby improving lifecycle management.

Rather than using univariate SPC methods to monitor several individual variables, it is often preferable to use multivariate statistical process control (MSPC) to analyse several variables simultaneously, taking into account potential correlations.

5. EUROPEAN PHARMACOPOEIA TEXTS SUPPORTING THE APPLICATION OF PAT

Typically, the analytical techniques described in the Ph. Eur. include qualification criteria designed for off-line analytical systems. These criteria are not always relevant or practical in a PAT setting, for instance when the equipment is specifically designed for on-line or in-line measurements. For this reason, certain general chapters (including *2.2.40. Near-infrared spectroscopy*, *2.2.48. Raman spectroscopy* and *2.9.47. Demonstration of uniformity of dosage units using large sample sizes*) have been revised or specifically elaborated to support and promote the use of the techniques in conjunction with PAT.

General Notices

Section 1.1, under Demonstration of compliance with the Pharmacopoeia, states that a substance can be demonstrated to be of pharmacopoeial quality on the basis of product design, together with the control strategy applied and data derived, for example, from validation studies of the manufacturing process.

The section also states that an enhanced approach to quality control could utilise PAT and/or real-time release testing strategies (including parametric release) as alternatives to end-product testing alone.

Absorption spectrophotometry, infrared

General chapter *2.2.24* has a wide variety of applications in the manufacturing process that enable PAT, such as reaction monitoring in chemical synthesis. Wavenumber shifts and acceptable tolerances for the reference material polystyrene are described for both transmission and ATR measurement modes.

Absorption spectrophotometry, ultraviolet and visible

General chapter *2.2.25* covers the use of PAT in the UV-Vis range using modern detectors such as photodiode arrays (PDA) or charge-coupled devices (CCD). Both transmission and diffuse reflection measurement modes are possible with off-line, at-line, on-line and in-line measurements. The table with reference wavelengths for the control of wavelength accuracy includes wavelengths in the UV range down to 180 nm.

X-ray fluorescence spectrometry

General chapter *2.2.37* includes references to modern equipment and the current applications of the XRF technique.

Substantial advances in miniaturisation and automation have led to the development of hand-held energy dispersive XRF (ED-XRF) spectrometers for rapid field measurements. XRF is potentially non-destructive, although it may cause sample instability. Hence, XRF lends itself to applications of PAT such as the analysis of unwanted trace catalysts in active pharmaceutical ingredients (API).

Near-infrared spectroscopy

General chapter *2.2.40* takes into account the increasing use of NIR spectroscopy for process monitoring and control, and the development of modern NIR spectrometers. NIR measurements can be performed off-line, but the technique also lends itself to at-line, on-line and in-line testing.

The 'Sample preparation/presentation' section includes moving materials or samples, and the use of fibre-optic probe systems in different measurement modes (i.e. transmission, diffuse reflection and transflection). The use of internal referencing for process analysis purposes is permitted when it is impossible to remove a probe for reference background data collection. A detailed table for the control of instrument performance depending on measurement mode and instrument used (benchtop, mobile or process instrument) is also provided. The chapter includes a section on qualitative analysis for identification and characterisation of a substance in addition to sections on limit analysis (e.g. for dryer end-point control) and trend analysis (for monitoring blend uniformity).

Raman spectroscopy

General chapter *2.2.48* includes Raman technologies that have potential uses for the application of PAT, including hand-held instruments.

Wavenumber shifts and acceptable tolerances for the reference materials cyclohexane, paracetamol and polystyrene (values derived from an inter-laboratory study) are described for both benchtop and hand-held Raman systems.

Demonstration of uniformity of dosage units using large sample sizes

General chapter *2.9.47* allows the determination of dosage unit uniformity in a PAT environment where the sample size is markedly greater than 30 units.

Compliance with the acceptance criteria of general chapter *2.9.47* is considered as evidence that the batch would also comply with general chapter *2.9.40. Uniformity of dosage units* if tested using a small sample size (e.g. 30-unit). Therefore, general chapter *2.9.47* does not constitute stand-alone acceptance criteria.

Alternative methods for control of microbiological quality

General chapter *5.1.6* describes alternative methods that might be used for the application of PAT in order to contribute to real-time or near-real-time microbiological quality control (e.g. test for microbiological examination of non-sterile products or for sterility based on laser-induced fluorescence) of in-process samples, active substances, medicinal products or excipients (particularly water). The chapter also provides guidance on how to validate these alternative methods.

Chemometric methods applied to analytical data

Chemometrics (general chapter *5.21*) has proven to be well suited for PAT. The investigation of large data tables and treatment of intricate signals, i.e. data collections with a hidden structure, of the type accumulated during measurements in PAT setups, requires alternative analytical tools to those used in a one-variable-at-a-time approach.

Chemical imaging

Chemical imaging (CI) (general chapter *5.24*) can be used to support PAT applications. CI measures spatial distribution and contributes to the understanding of the properties of materials such as finished products, pharmaceutical intermediates, excipients, APIs and starting materials.

The uses of CI include, for example, detection of defects at the sample surface such as cracks in tablet coatings and identification of foreign particles. It is a versatile tool used in process development and improvement, root cause analysis (e.g. for out-of-specification results), and may also be used to enhance process understanding.

Radiopharmaceutical preparations and starting materials for radiopharmaceutical preparations

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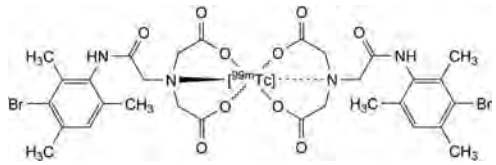
TECHNETIUM (^{99m}Tc) MEBROFENIN INJECTION

Technetii (^{99m}Tc) mebrofenini solutio iniectabilis



DEFINITION

Sterile solution of a complex of technetium-99m with mebrofenin. It may contain stabilisers and inert additives.

Content: 90 per cent to 110 per cent of the declared technetium-99m radioactivity at the date and time stated on the label.

PRODUCTION

It is prepared by dissolving [[2-(3-bromo-2,4,6-trimethylanilino)-2-oxoethyl]azanediyl]diacetic acid (mebrofenin) in *Sodium pertechnetate (^{99m}Tc) injection (fission) (0124)* or *Sodium pertechnetate (^{99m}Tc) injection (non-fission) (0283)* in the presence of a reducing agent such as a stannous salt.

CHARACTERS

Appearance: clear, colourless solution.

Half-life and nature of radiation of technetium-99m: see general chapter *5.7. Table of physical characteristics of radionuclides*.

IDENTIFICATION

A. Gamma-ray spectrometry.

Results: the most prominent gamma photon of technetium-99m has an energy of 0.141 MeV.

B. Examine the chromatogram obtained in the test for other radiochemical impurities (see Tests).

Results: the principal peak in the chromatogram obtained with the test solution is similar in retention time to the peak due to technetium-99m mebrofenin in the chromatogram obtained with the reference solution.

TESTS

pH (*2.2.3*): 4.0 to 7.5.

Sterility. It complies with the test for sterility prescribed in the monograph on *Radiopharmaceutical preparations (0125)*. The injection may be released for use before completion of the test.

Bacterial endotoxins (*2.6.14*): less than 175/*V* IU/mL, *V* being the maximum recommended dose in millilitres.

RADIOCHEMICAL PURITY

Technetium-99m mebrofenin: minimum 90 per cent of the total radioactivity.

Impurity A. Thin-layer chromatography (*2.2.27*).

Test solution. The preparation to be examined.

Reference solution (a). To 1 mL of a 1 g/L solution of *stannous chloride R* in *0.05 M hydrochloric acid* in a closed vial, add 2 mL of sodium pertechnetate (^{99m}Tc) injection (fission or non-fission). Use within 30 min after preparation.

Reference solution (b). Dissolve 40 mg of *mebrofenin CRS* in 2 mL of a 4 g/L solution of *sodium hydroxide R* and adjust to pH 6.5 with *1 M hydrochloric acid* or a 40 g/L solution of *sodium hydroxide R*. To this solution add 25 µL of a 20 mg/mL solution of *stannous chloride R* in *0.05 M hydrochloric acid* and 400 MBq of sodium pertechnetate (^{99m}Tc) injection (fission or non-fission) in a volume of 2 mL. Allow to stand for 30-60 min.

Plate: *TLC silica gel plate R*; use a glass-fibre plate.

Mobile phase: *water R*, *acetonitrile R* (40:60 *V/V*).

Application: about 5 µL.

Development: immediately, over 4/5 of the plate.

Drying: in air.

Detection: determine the distribution of radioactivity using a suitable detector.

Retardation factor: impurity A = 0.0 to 0.1.

System suitability: the retardation factor of the principal peak in the chromatogram obtained with reference solution (a) is not more than 0.1; the retardation factor of the principal peak in the chromatogram obtained with reference solution (b) is more than 0.7.

Limit:

– *impurity A*: maximum 5 per cent of the total radioactivity.

Other radiochemical impurities. Liquid chromatography (*2.2.29*).

Test solution. The preparation to be examined.

Reference solution. Use reference solution (b) of the test for impurity A.

Column:

– *size*: *l* = 0.25 m, Ø = 4.0 mm;
– *stationary phase*: *end-capped polar-embedded octadecylsilyl amorphous organosilica polymer R* (5 µm).

Mobile phase:

– *mobile phase A*: 3.85 g/L solution of *ammonium acetate R*;
– *mobile phase B*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 20	70	30
20 - 25	70 → 0	30 → 100
25 - 30	0	100

Flow rate: 1.0 mL/min.

Detection: radioactivity detector.

Injection: 20 µL.

Relative retention with reference to technetium-99m mebrofenin (retention time = about 20 min): impurity B = about 0.17.

Limits:

– *sum of the areas of the peaks eluted before the principal peak (corresponding to hydrophilic impurities, including impurity B)*: maximum 6 per cent of the total area of the peaks in the chromatogram obtained with the test solution.

RADIOACTIVITY

Determine the radioactivity using a calibrated instrument.

IMPURITIES

A. technetium-99m in colloidal form,

B. [^{99m}Tc]pertechnetate ion.

Sutures for human use

04/2020:90004

SUTURES FOR HUMAN USE: INTRODUCTION

Sutures for human use are medical devices as defined in Regulation (EU) 2017/745 and any subsequent amendments.

Monographs can be applied to show compliance with certain requirements therein.

Herbal drugs and herbal drug preparations

04/2020:1297

CALENDULA FLOWER

Calendulae flos

DEFINITION

Whole or cut, dried, and fully opened flowers that have been detached from the receptacle of the cultivated, double-flowered varieties of *Calendula officinalis* L.

Content: minimum 0.4 per cent of flavonoids, expressed as hyperoside ($C_{21}H_{20}O_{12}$; M_r 464.4) (dried drug).

IDENTIFICATION

A. The ligulate florets consist of a yellow or orange-yellow ligule, about 3-5 mm wide and about 7 mm in the middle part, with a 3-toothed apex and a hairy, partly sickle-shaped, yellowish-brown or orange-brown tube with a projecting style and a bifid stigma occasionally with a partly bent yellowish-brown or orange-brown ovary. The tubular florets, about 5 mm long, are present and consist of the yellow, orange-red or reddish-violet 5-lobed corolla and the yellowish-brown or orange-brown tube, hairy in its lower part, mostly with a partly bent yellowish-brown or orange-brown ovary.

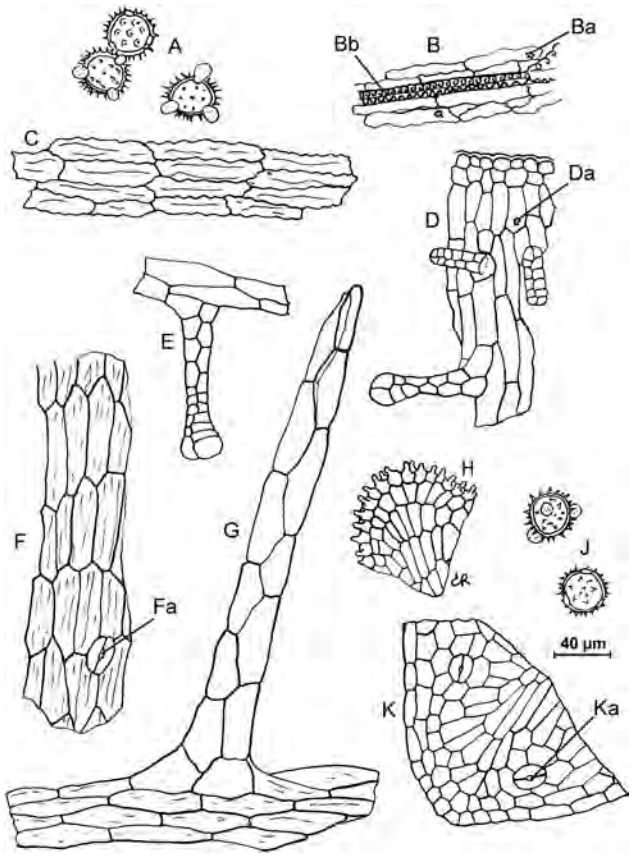


Figure 1297.-1. – *Illustration for identification test B of powdered herbal drug of calendula flower*

B. Microscopic examination (*2.8.23*). The powder is yellowish-brown. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters (Figure 1297.-1): fragments of epidermises of the corolla [C, F, K] containing light yellow oil droplets, some with fairly large anomocytic stomata (*2.8.3*) [Fa, Ka]; covering trichomes biseriate, multicellular and conical [G], usually fragmented, and glandular trichomes with a multicellular stalk [E], very abundant on the base of the corolla [D]; fragments of parenchyma of the corolla [B] containing prisms and very small cluster crystals of calcium oxalate [Ba, Da] and small vessels [Bb]; spherical pollen grains up to about 40 µm in diameter with a sharply spiny exine and 3 germinal pores [A, J]; occasional fragments of the stigmas with short, bulbous papillae [H].

C. High-performance thin-layer chromatography (*2.8.25*).

Test solution. To 0.5 g of the powdered herbal drug (355) (*2.9.12*) add 5.0 mL of *methanol R*. Sonicate for 15 min, filter or centrifuge and use the filtrate or supernatant.

Reference solution (a). Dissolve 3.0 mg of *chlorogenic acid R* and 4.0 mg of *isorhamnetin-3-O-rutinoside R* in *methanol R* and dilute to 10.0 mL with the same solvent.

Reference solution (b). Dilute 2.5 mL of reference solution (a) to 10.0 mL with *methanol R*.

Reference solution (c). Dissolve 2.5 mg of *hyperoside R* and 3 mg of *chlorogenic acid R* in *methanol R* and dilute to 10 mL with the same solvent.

Intensity marker: isorhamnetin-3-*O*-rutinoside.

Plate: *TLC silica gel F_{254} plate R* (2-10 µm).

Mobile phase: *anhydrous formic acid R*, *water R*, *ethyl acetate R* (10:10:80 *V/V/V*).

Application: 4 µL as bands of 8 mm.

Development: 70 mm from the lower edge of the plate.

Drying: in a current of air at room temperature for 5 min.

Detection: heat at 100-105 °C for 5 min; spray the warm plate with a 10 g/L solution of *diphenylboric acid aminoethyl ester R* in *methanol R*, then with a 50 g/L solution of *macrogol 400 R* in *methanol R* or, alternatively, dip the warm plate in a 5 g/L solution of *diphenylboric acid aminoethyl ester R* in *ethyl acetate R* and then in a 50 g/L solution of *macrogol 400 R* in *methylene chloride R*; allow to dry in air for about 1 min and examine in ultraviolet light at 366 nm.

System suitability: reference solution (c):

– the chromatogram shows in the middle third 2 distinct zones, which may be touching; the lower zone (chlorogenic acid) shows a light blue fluorescence and the upper zone (hyperoside) shows a yellow or orange fluorescence.

Results: see below the sequence of fluorescent zones present in the chromatograms obtained with reference solution (a) and the test solution. Furthermore, in the chromatogram obtained with the test solution, other faint to very faint blue, brown or orange fluorescent zones may be present.

Top of the plate	
	2 blue zones, faint to equivalent
———	———
	A greenish-yellow zone, faint
Chlorogenic acid: a light blue zone	A light blue zone, faint to equivalent
———	———
Isorhamnetin-3-*O*-rutinoside: a greenish-yellow zone	A greenish-yellow zone (isorhamnetin-3-*O*-rutinoside) A brown or orange zone, faint to equivalent
	A greenish-yellow zone
	A brownish-orange zone, very faint to faint
Reference solution (a)	**Test solution**

TESTS

Foreign matter (*2.8.2*): maximum 5 per cent of bracts and maximum 2 per cent of other foreign matter.

Loss on drying (*2.2.32*): maximum 12.0 per cent, determined on 1.000 g of the powdered herbal drug (500) (*2.9.12*) by drying in an oven at 105 °C for 2 h.

Total ash (*2.4.16*): maximum 10.0 per cent.

ASSAY

Stock solution. Into a 100 mL round-bottomed flask introduce 0.800 g of the powdered herbal drug (500) (*2.9.12*), 1 mL of a 5 g/L solution of *hexamethylenetetramine R*, 7 mL of *hydrochloric acid R1* and 20 mL of *acetone R*. Boil the mixture under a reflux condenser for 30 min. Filter the liquid through a plug of absorbent cotton into a 100 mL volumetric flask. Add the absorbent cotton to the residue in the round-bottomed flask and extract with 2 quantities, each of 20 mL, of *acetone R*, each time boiling under a reflux condenser for 10 min. Allow to cool to room temperature, filter the liquid through a plug of absorbent cotton, then filter the combined acetone solution through a filter-paper into the volumetric flask, and dilute to 100.0 mL with *acetone R* by rinsing the flask and filter. Introduce 20.0 mL of this solution into a separating funnel, add 20 mL of *water R* and extract the mixture with 1 quantity of 15 mL and then with 3 quantities, each of 10 mL, of *ethyl acetate R*. Combine the ethyl acetate extracts in a separating funnel, rinse with 2 quantities, each of 50 mL, of *water R*, filter the extract over 10 g of *anhydrous sodium sulfate R* into a 50 mL volumetric flask and dilute to 50.0 mL with *ethyl acetate R*.

Test solution. To 10.0 mL of the stock solution add 1 mL of *aluminium chloride reagent R* and dilute to 25.0 mL with a 5 per cent *V/V* solution of *glacial acetic acid R* in *methanol R*.

Compensation liquid. Dilute 10.0 mL of the stock solution to 25.0 mL with a 5 per cent *V/V* solution of *glacial acetic acid R* in *methanol R*.

Measure the absorbance (*2.2.25*) of the test solution after 30 min, by comparison with the compensation liquid at 425 nm.

Calculate the percentage content of flavonoids, expressed as hyperoside, using the following expression:

$$\frac{A \times 1.25}{m}$$

i.e. taking the specific absorbance of hyperoside to be 500.

A = absorbance at 425 nm;

m = mass of the herbal drug to be examined, in grams.

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DWARF LILYTURF TUBER

Ophiopogonis radix

DEFINITION

Dried root tuber of *Ophiopogon japonicus* (Thunb.) Ker Gawl., with the spindly roots removed.

Content: minimum 0.12 per cent of total saponins, expressed as ruscogenin ($C_{27}H_{42}O_4$; M_r 430.6) (dried drug).

IDENTIFICATION

A. It is fusiform with both ends slightly tapered, 1.5-3 cm long and 3-6 mm in diameter. The outer surface is whitish-yellow, fine and longitudinally wrinkled. The texture is hard, the fracture is yellowish-white and translucent, and the vascular system appears as a small ring in the centre.

B. Microscopic examination (*2.8.23*). The powder is yellowish-white. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters (Figure 3000.-1): numerous fragments of parenchyma consisting of suborbicular or elliptical cells [A]; different sized raphides of calcium oxalate, free [F] or included in parenchymatous cells [Aa], either fine, 12-65 µm long [Aa], or thicker, up to 130 µm long [F]; sclereids, usually in groups (surface view [E]), subsquare, rectangular or polygonal (20-57 µm in diameter) with distinct, dense channels; fragments of the endodermis with rectangular or elongated cells, with channelled and pitted walls (surface view [C]); fragments (transverse section [B]) showing the endodermis [Ba], sclereids with unevenly thickened walls [Bb] and parenchymatous cells [Bc]; thin xylem fibres [G], with oblique or square ends, slightly thickened walls, and oblique, cross-shaped or V-shaped pits; fragments of xylem [D] consisting of spiral or reticulate vessels [Da] and fibres [Db, Dc].

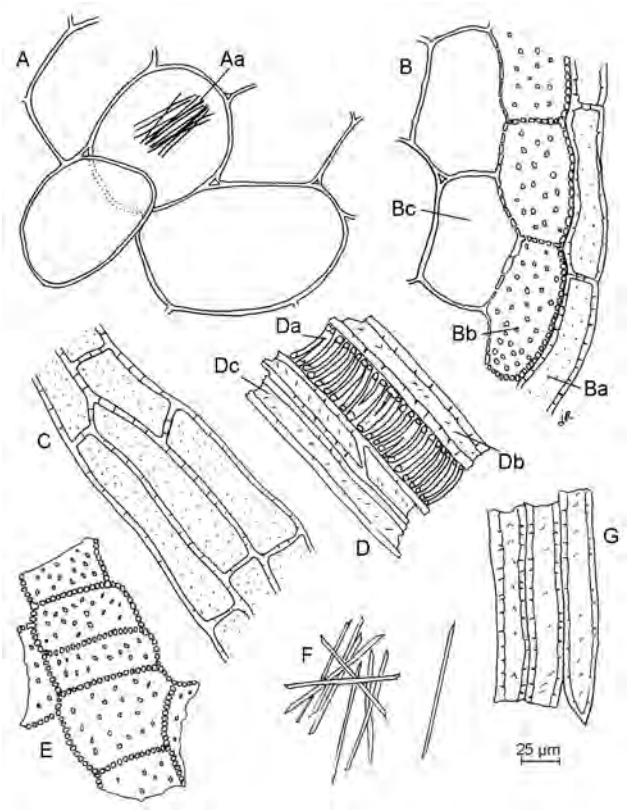


Figure 3000.-1. – *Illustration for identification test B of powdered herbal drug of dwarf lilyturf tuber*

C. High-performance thin-layer chromatography (*2.8.25*).

Test solution. To 2.0 g of the powdered herbal drug (355) (*2.9.12*) add 20 mL of *anhydrous ethanol R*. Sonicate for 30 min, filter and evaporate to dryness. Dissolve the residue in 0.5 mL of *anhydrous ethanol R*, centrifuge and use the supernatant.

Reference solution (a). Dissolve 1 mg of *β-sitosterol R* and 1 mg of *methylophiopogonanone A R* in 2.0 mL of *anhydrous ethanol R*.

Reference solution (b). Mix 1.0 mL of reference solution (a) and 3.0 mL of *anhydrous ethanol R*.

Reference solution (c). Dilute 3 µL of *isoeugenol R* and 6 µL of *methyleugenol R* in 20 mL of *toluene R*.

Intensity markers: β-sitosterol and methylophiopogonanone A.

Plate: *TLC silica gel F_{254} plate R* (2-10 µm).

Mobile phase: *ethyl acetate R*, *toluene R* (10:90 *V/V*).

Application: 5 µL as bands of 8 mm.

Development: 70 mm from the lower edge of the plate.

Drying: in a current of cold air for 5 min.

Detection: dip the plate in a 10 per cent *V/V* solution of *sulfuric acid R* in *anhydrous ethanol R*; heat the plate at 105 °C for 10 min and examine under white light.

System suitability: reference solution (c):

– the chromatogram shows 2 distinct zones in the middle third; the lower zone (isoeugenol) and the upper zone (methyleugenol) show a violet-red fluorescence.

Results: see below the sequence of zones present in the chromatograms obtained with reference solution (a) and the test solution. Furthermore, in the chromatogram obtained with the test solution, other faint fluorescent zones may be present.

Top of the plate	
	2 violet-red zones
_____	_____
Methylophiopogonanone A: a brownish-yellow zone	A brownish-yellow zone, faint to equivalent
_____	_____
	1 or 2 violet-red or reddish zones, faint to equivalent (possibly overlapping)
β-sitosterol: a violet-red zone	A violet-red zone
	A broad, intense violet-red zone
Reference solution (a)	**Test solution**

TESTS

Loss on drying (*2.2.32*): maximum 15.0 per cent, determined on 1.000 g of the powdered herbal drug (355) (*2.9.12*) by drying in an oven at 105 °C for 2 h.

Total ash (*2.4.16*): maximum 2.5 per cent.

Ash insoluble in hydrochloric acid (*2.8.1*): maximum 0.5 per cent.

ASSAY

Test solution. Introduce 1.20 g of the powdered herbal drug (355) (*2.9.12*) into a 50 mL round-bottomed flask, add 20.0 mL of *methanol R* and weigh. Boil under a reflux condenser for 2 h. Allow to cool to room temperature, weigh again and adjust to the original mass with *methanol R*. Mix thoroughly and filter through a membrane filter (nominal pore size 0.22 µm). Evaporate 12.5 mL of the filtrate to dryness under a current of *nitrogen R*. Dissolve the residue in 5 mL of *water R* and extract with 5 quantities, each of 5 mL, of *butanol R* saturated with *water R* using a separating funnel. Combine the butanol extracts in a separating funnel and wash with 2 quantities, each of 2.5 mL, of *dilute ammonia R1*. Evaporate to dryness under a current of *nitrogen R*. Dissolve the residue in a mixture of 20 volumes of *water R* and 80 volumes of *methanol R* and dilute to 25.0 mL with the same mixture of solvents. Evaporate 2.5 mL of the solution to dryness under a current of *nitrogen R*. Dissolve the residue in *perchloric acid R* and dilute to 10.0 mL with the same solvent. Transfer the solution to a test tube, stopper and heat at 80 °C in a water-bath for 15 min. Cool to room temperature.

Measure the absorbance (*2.2.25*) of the test solution at 397 nm using *perchloric acid R* as the compensation liquid.

Calculate the percentage content of total saponins, expressed as ruscogenin, using the following expression:

$$\frac{A \times 160}{m \times 196}$$

i.e. taking the specific absorbance of ruscogenin to be 196.

A = absorbance at 397 nm;

m = mass of the herbal drug to be examined used to prepare the test solution, in grams.

04/2020:2452

FRAXINUS CHINENSIS BARK

Fraxini chinensis cortex

DEFINITION

Whole or fragmented, dried branch or trunk bark of *Fraxinus chinensis* subsp. *rhynchophylla* (Hance) A.E.Murray (syn. *Fraxinus rhynchophylla* Hance), collected in spring or autumn.

Content: minimum 1.0 per cent for the sum of esculetin ($C_9H_6O_4$; M_r 178.1) and esculin ($C_{15}H_{16}O_9$; M_r 340.3) (dried drug).

IDENTIFICATION

A. The branch bark occurs as flexible, curved or channelled, rolled or folded pieces up to 60 cm long and 3 mm thick; the outer surface is whitish-grey to dark brownish-grey, sometimes in patches, and is smooth or slightly rough, dotted with whitish-grey, rounded lenticels; the inner surface is smooth, soft to the touch, yellowish-white or brown. The fracture is fibrous.

The trunk bark occurs as compact, rigid, slat-shaped pieces, up to 6 mm thick; the outer surface is brownish-grey, with fine longitudinal furrows and many reddish-brown lenticels, rounded or slightly split transversally; the inner surface is smooth, orange-brown. The fracture is fibrous.

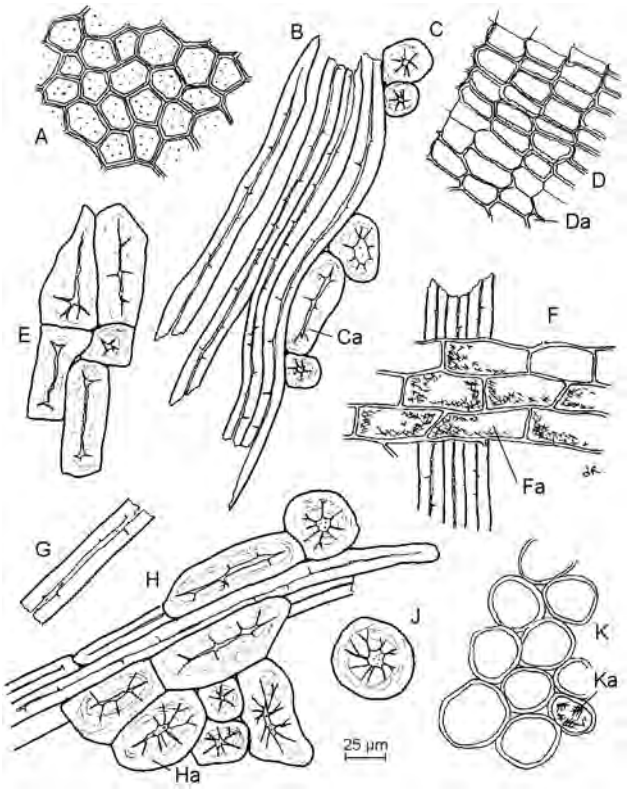


Figure 2452.-1. – *Illustration for identification B of powdered herbal drug of fraxinus chinensis bark*

B. Microscopic examination (*2.8.23*). The powder is brownish. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters (Figure 2452.-1): large sclereids up to 300 µm in diameter, single [J] or in groups [E], with a very narrow lumen and channelled walls with concentric striations; fragments of brownish cork consisting of polygonal cells with slightly thickened and pitted walls (surface view [A]) and of superimposed layers of cells with slightly thickened and channelled walls (transverse section [D]), sometimes associated with one or more layers of collenchyma [Da]; long fibres, usually fragmented, with very thick walls and a greatly reduced lumen, slightly channelled, isolated [B, G] or in groups [C, F, H], sometimes accompanied by sclereids [Ca, Ha] or medullary rays with rectangular cells containing tiny acicular crystals of calcium oxalate [Fa]; groups of parenchymatous cells [K], some containing tiny crystals of calcium oxalate [Ka].

C. To 0.1 g of the powdered herbal drug (355) (*2.9.12*) add 10 mL of *water R* previously heated to 60 °C. Allow to stand for 2 min and filter. Examined in ultraviolet light at 365 nm, the solution shows an intense blue fluorescence that fades considerably after the addition of 2 mL of *hydrochloric acid R*.

D. Thin-layer chromatography (*2.2.27*).

Test solution. To 0.25 g of the powdered herbal drug (355) (*2.9.12*) add 5 mL of *methanol R*. Heat in a water-bath at 60 °C for 1 min. Centrifuge and use the supernatant; filter, if necessary.

Reference solution. Dissolve 1 mg of *esculetin R* and 1 mg of *esculin R* in 2 mL of *methanol R*.

Plate: *TLC silica gel F_{254} plate R* (5-40 µm) [or *TLC silica gel F_{254} plate R* (2-10 µm)].

Mobile phase: *anhydrous formic acid R*, *water R*, *ethyl acetate R* (10:10:80 *V/V/V*).

Application: 10 µL as bands of 15 mm [or 8 mm].

Development: over a path of 10 cm [or 6 cm].

Drying: in air.

Detection: treat with a solution containing 10 g/L of *diphenylboric acid aminoethyl ester R* and 50 g/L of *macrogol 400 R* in *methanol R*; examine in ultraviolet light at 365 nm.

Results: see below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other fluorescent zones may be present in the chromatogram obtained with the test solution.

Top of the plate	
Esculetin: a greenish-yellow fluorescent zone	A greenish-yellow fluorescent zone (esculetin)
	A green fluorescent zone may be present
	A blue fluorescent zone may be present
Esculin: an intense blue fluorescent zone	An intense blue fluorescent zone (esculin)
	A whitish-blue fluorescent zone
Reference solution	**Test solution**

TESTS

Loss on drying (*2.2.32*): maximum 12.0 per cent, determined on 1.000 g of the powdered herbal drug (355) (*2.9.12*) by drying in an oven at 105 °C.

Total ash (*2.4.16*): maximum 5.0 per cent.

Ash insoluble in hydrochloric acid (*2.8.1*): maximum 2.0 per cent.

ASSAY

Liquid chromatography (*2.2.29*).

Test solution. To 0.500 g of the powdered herbal drug (355) (*2.9.12*) add 50.0 mL of *methanol R* and weigh. Heat on a water-bath under a reflux condenser for 1 h. Cool and weigh

again. Compensate for the loss of solvent with *methanol R* and mix. Filter through a membrane filter (nominal pore size 0.45 µm).

Reference solution (a). Dissolve 10.0 mg of *esculin CRS* in the mobile phase and dilute to 50.0 mL with the mobile phase.

Reference solution (b). Dissolve 10.0 mg of *esculetin CRS* in 10 mL of *acetonitrile R* and dilute to 50.0 mL with the mobile phase.

Reference solution (c). Mix 3.0 mL of reference solution (b) and 5.0 mL of reference solution (a) and dilute to 10.0 mL with the mobile phase.

Column:

– *size*: *l* = 0.15 m, Ø = 4.0 mm;

– *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase: *acetonitrile R*, 0.1 per cent *V/V* solution of *phosphoric acid R* (12:88 *V/V*).

Flow rate: 0.75 mL/min.

Detection: spectrophotometer at 334 nm.

Injection: 10 µL.

Run time: 1.5 times the retention time of esculetin.

Retention time: esculin = about 4.5 min; esculetin = about 8.5 min.

Identification of peaks: use the chromatogram obtained with reference solution (a) to identify the peak due to esculin and the chromatogram obtained with reference solution (b) to identify the peak due to esculetin.

System suitability: reference solution (c):

– *resolution*: minimum 5.0 between the peaks due to esculin and esculetin.

Calculate the percentage content of the sum of esculetin and esculin using the following expression:

$$\frac{A_1 \times m_2 \times p_1 \times 0.5}{A_2 \times m_1} + \frac{A_3 \times m_3 \times p_2 \times 0.3}{A_4 \times m_1}$$

A_1 = area of the peak due to esculin in the chromatogram obtained with the test solution;

A_2 = area of the peak due to esculin in the chromatogram obtained with reference solution (c);

A_3 = area of the peak due to esculetin in the chromatogram obtained with the test solution;

A_4 = area of the peak due to esculetin in the chromatogram obtained with reference solution (c);

m_1 = mass of the herbal drug to be examined used to prepare the test solution, in grams;

m_2 = mass of *esculin CRS* used to prepare reference solution (a), in grams;

m_3 = mass of *esculetin CRS* used to prepare reference solution (b), in grams;

p_1 = percentage content of esculin in *esculin CRS*;

p_2 = percentage content of esculetin in *esculetin CRS*.

04/2020:1220

HAWTHORN BERRIES

Crataegi fructus

DEFINITION

Dried false fruits of *Crataegus monogyna* Jacq. or *C. laevigata* (Poir.) DC. (syn. *C. oxyacantha* L.) or their hybrids or a mixture of these false fruits.

Content: minimum 0.06 per cent of procyanidins, expressed as cyanidin chloride ($C_{15}H_{11}ClO_6$; M_r 322.7) (dried drug).

IDENTIFICATION

A. The false fruit of *C. monogyna* is obovate or globular, generally 6-10 mm long and 4-8 mm wide, reddish-brown or dark red. The surface is pitted or, more rarely, reticulated. The upper end of the fruit is crowned by the remains of 5 reflexed sepals surrounding a small, sunken disc with a shallow, raised rim. The remains of the style occur in the centre of the disc with tufts of stiff, colourless hairs at the base. At the lower end of the fruit is a short length of pedicel or, more frequently, a small, pale, circular scar where the pedicel was attached. The receptacle is fleshy and encloses a yellowish-brown, ovoid fruit with a hard, thick wall containing a single, elongated, pale brown, smooth and shiny seed.

The false fruit of *C. laevigata* is up to 13 mm long. It contains 2-3 stony fruits, ventrally flattened, with short hairs at the top. The centre of the disc of the false fruit frequently bears the remains of the 2 styles.

B. Microscopic examination (*2.8.23*). The powder is greyish-red. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters (Figure 1220.-1): covering trichomes [F] from inside the disc that are long, unicellular, frequently bent, tapering to a point, with heavily thickened and lignified walls; fragments of the red outer layer of the receptacle (surface view [G]); fragments of the inner layers of the receptacle [A], some cells containing cluster crystals [Aa] or prisms [Ab] of calcium oxalate; occasional fragments [J, K] including groups of sclereids [Ka] and vascular bundles [Ja, Kb] associated with rows of cells containing prisms of calcium oxalate [Jb, Kc]; fragments of the pericarp [B] consisting of parenchyma including some cells containing cluster crystals of calcium oxalate [Ba] and groups of sclereids of various sizes with numerous pits [Bb]; thick-walled sclereids [E, H], channelled [E], some with conspicuously branched channels [H]; a few fragments of the testa [C] having an outer layer composed of hexagonal, mucilaginous cells [Ca] beneath which is a yellowish-brown pigment layer containing numerous prisms of calcium oxalate [Cb]; parenchyma of the endosperm and cotyledons consisting of cells containing aleurone grains and globules of fixed oil [D].

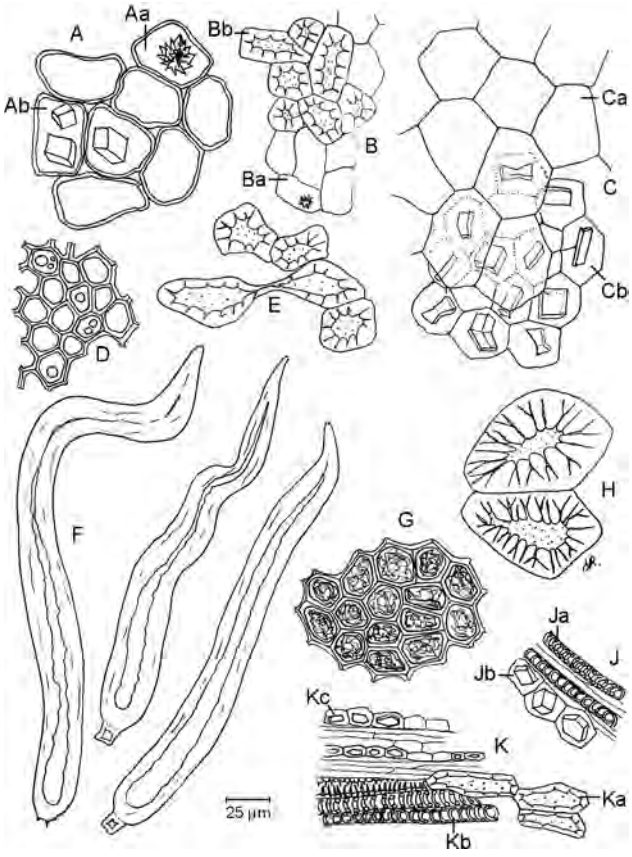


Figure 1220.-1. – *Illustration for identification test B of powdered herbal drug of hawthorn berries*

C. High-performance thin-layer chromatography (*2.8.25*).

Test solution. To 0.5 g of the powdered herbal drug (355) (*2.9.12*) add 5.0 mL of *methanol R*. Sonicate for 15 min, filter or centrifuge and use the filtrate or supernatant.

Reference solution (a). Dissolve 2.5 mg of *hyperoside R* and 3.5 mg of *rutoside trihydrate R* in *methanol R* and dilute to 10.0 mL with the same solvent.

Reference solution (b). Dilute 2.5 mL of reference solution (a) to 10.0 mL with *methanol R*.

Reference solution (c). Dissolve 2.5 mg of *hyperoside R* and 3 mg of *chlorogenic acid R* in *methanol R* and dilute to 10 mL with the same solvent.

Intensity marker: hyperoside.

Plate: *TLC silica gel F_{254} plate R* (2-10 µm).

Mobile phase: *anhydrous formic acid R*, *water R*, *ethyl acetate R* (10:10:80 *V/V/V*).

Application: 4 µL as bands of 8 mm.

Development: 70 mm from the lower edge of the plate.

Drying: in a current of air at room temperature for 5 min.

Detection: heat at 100-105 °C for 5 min; spray the warm plate with a 10 g/L solution of *diphenylboric acid aminoethyl ester R* in *methanol R*, then with a 50 g/L solution of *macrogol 400 R* in *methanol R* or, alternatively, dip the warm plate in a 5 g/L solution of *diphenylboric acid aminoethyl ester R* in *ethyl acetate R*, then in a 50 g/L solution of *macrogol 400 R* in *methylene chloride R*; allow to dry in air for about 1 min and examine in ultraviolet light at 366 nm.

System suitability: reference solution (c):

– the chromatogram shows in the middle third 2 distinct zones, which may be touching; the lower zone (chlorogenic acid) shows a light blue fluorescence and the upper zone (hyperoside) shows a yellow or orange fluorescence.

Results: see below the sequence of fluorescent zones present in the chromatograms obtained with reference solution (a) and the test solution. Furthermore, in the chromatogram obtained with the test solution, other faint blue, greenish and orange or yellow fluorescent zones may be present.

Top of the plate	
	1-2 blue zones, very faint to faint
_____	_____
	A yellow or orange zone, very faint to faint
Hyperoside: a yellow or orange zone	A yellow or orange zone, faint to equivalent (hyperoside)
	A light blue zone, faint (chlorogenic acid)
_____	_____
Rutoside: a yellow or orange zone	A yellow or orange zone, very faint (rutoside)
Reference solution (a)	**Test solution**

TESTS

Foreign matter (*2.8.2*): maximum 5 per cent of deteriorated false fruit and maximum 2 per cent of other foreign matter. It does not contain false fruits of other *Crataegus* species (*C. nigra* Waldst. et Kit., *C. pentagyna* Waldst. et Kit. ex Willd. and *C. azarolus* L.), which are characterised by the presence of more than 3 hard stones.

Loss on drying (*2.2.32*): maximum 12.0 per cent, determined on 1.000 g of the powdered herbal drug (355) (*2.9.12*) by drying in an oven at 105 °C for 2 h.

Total ash (*2.4.16*): maximum 5.0 per cent.

ASSAY

To 2.50 g of the powdered herbal drug (355) (*2.9.12*) add 30 mL of *ethanol (70 per cent V/V) R*. Heat under a reflux condenser for 30 min and filter. Wash the residue with 10.0 mL of *ethanol (70 per cent V/V) R*. Add to the filtrate 15.0 mL of *hydrochloric acid R1* and 10.0 mL of *water R*. Heat under a reflux condenser for 80 min. Allow to cool, filter and wash the residue with *ethanol (70 per cent V/V) R* until the filtrate is colourless. Dilute the filtrate to 250.0 mL with *ethanol (70 per cent V/V) R*. Evaporate 50.0 mL of this solution in a round-bottomed flask to about 3 mL and transfer to a separating funnel. Rinse the round-bottomed flask sequentially with 10 mL and 5 mL of *water R* and transfer to the separating funnel. Shake the combined solution with 3 quantities, each of 15 mL, of *butanol R*. Combine the organic layers and dilute to 100.0 mL with *butanol R*.

Measure the absorbance (*2.2.25*) of the solution at 555 nm.

Calculate the percentage content of procyanidins, expressed as cyanidin chloride, using the following expression:

$$\frac{A \times 500}{1200 \times m}$$

i.e. taking the specific absorbance of cyanidin chloride to be 1200.

A = absorbance at 555 nm;

m = mass of the substance to be examined, in grams.

04/2020:2950

RASPBERRY LEAF

Rubi idaei folium

DEFINITION

Whole or fragmented, dried leaves of *Rubus idaeus* L. harvested in spring or early summer.

Content: minimum 3.0 per cent of tannins, expressed as pyrogallol ($C_6H_6O_3$; M_r 126.1) (dried drug).

IDENTIFICATION

A. *Whole drug.* The leaf is imparipinnate, with 3, 5 or rarely 7 ovate to lanceolate leaflets with sharply serrate or doubly-serrate margins. The upper surface is dark green to brownish-green and weakly pubescent; the lower surface is silvery grey and densely tomentose with a prominent pinnate venation. The petiole is about 1-2 mm thick, green or occasionally reddish, slightly channelled on the upper surface, and sometimes shows small, straight prickles. The stipules are long and thin. The leaves of the short shoots are often simple, more or less 3-lobed, or they are trifoliate.

Fragmented drug. It is characterised by clumped fragments of leaves and petioles.

B. Microscopic examination (*2.8.23*). Reduce to a powder (710) (*2.9.12*). The powder is greyish-green and flocculent. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters (Figure 2950.-1): very numerous broken unicellular covering trichomes, either rigid with thickened walls [G], or twisted with slightly thickened walls [F]; fragments of the upper epidermis of the leaflet lamina (surface view [D]) with polygonal cells [Da] and rare, rigid, unicellular covering trichomes with thickened walls, measuring from 20 µm to more than 500 µm in length [Db], or their scars, often accompanied by palisade parenchyma [Dc] including some hypertrophied cells containing a cluster crystal of calcium oxalate [Dd]; fragments of the lower epidermis of the leaflet lamina [A] containing cells with thin, slightly lobed walls [Aa] and anomocytic stomata (*2.8.3*) (5-7 subsidiary cells) [Ab], with an abundant indumentum composed of unicellular, fine, twisted covering trichomes [Ac] up to 500 µm long; annular or spiral vessels from the petioles, rachis or principal veins [J], sometimes accompanied by cells of the pith with slightly thickened, pitted walls [Ja]; fragments of pericyclic fibres [C], frequently with a crystal sheath containing small cluster crystals of calcium oxalate [Ca]; fragments of the lamina (transverse section [H]) consisting of the upper epidermis [Ha], 1 or 2 layers of palisade parenchyma [Hb], including hypertrophied cells containing a cluster crystal of calcium oxalate [Hc], spongy parenchyma [Hd] and the lower epidermis with twisted unicellular covering trichomes [He]; free cluster crystals of calcium oxalate [E]; exceptionally, secretory trichomes with a biseriate, multicellular stalk and a globular, multicellular head, located on the upper epidermis of the leaflet veins [B].

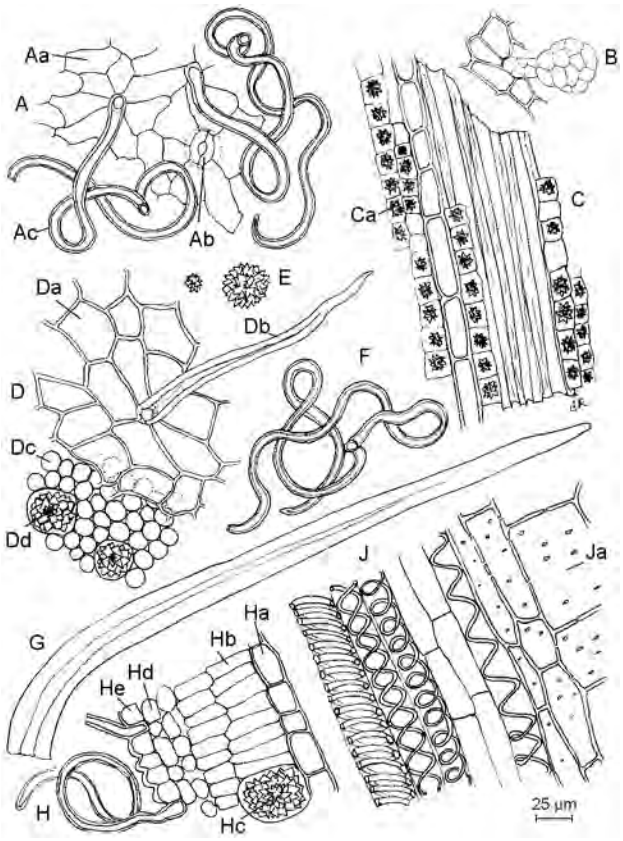


Figure 2950.-1. – *Illustration for identification test B of powdered herbal drug of raspberry leaf*

C. High-performance thin-layer chromatography (*2.8.25*).

Test solution. To 0.5 g of the powdered herbal drug (710) (*2.9.12*) add 10.0 mL of *methanol R*. Sonicate for 15 min, filter or centrifuge and use the filtrate or supernatant.

Reference solution (a). Dissolve 2.5 mg of *hyperoside R* and 3.5 mg of *rutoside trihydrate R* in *methanol R* and dilute to 10.0 mL with the same solvent.

Reference solution (b). Dilute 2.5 mL of reference solution (a) to 10.0 mL with *methanol R*.

Reference solution (c). Dissolve 3 mg of *hyperoside R* and 2.5 mg of *chlorogenic acid R* in *methanol R* and dilute to 10 mL with the same solvent.

Intensity marker: hyperoside.

Plate: *TLC silica gel F_{254} plate R* (2-10 µm).

Mobile phase: *anhydrous formic acid R*, *water R*, *ethyl acetate R* (10:10:80 *V/V/V*).

Application: 10 µL of the test solution and 4 µL of reference solutions (a), (b) and (c), as bands of 8 mm.

Development: 70 mm from the lower edge of the plate.

Drying: in a current of air at room temperature for 5 min.

Detection: heat at 100-105 °C for 5 min; spray the warm plate with a 10 g/L solution of *diphenylboric acid aminoethyl ester R* in *methanol R*, then with a 50 g/L solution of *macrogol 400 R* in *methanol R* or, alternatively, dip the warm plate in a 5 g/L solution of *diphenylboric acid aminoethyl ester R* in *ethyl acetate R*, then in a 50 g/L solution of *macrogol 400 R* in *methylene chloride R*; allow to dry in air for about 1 min and examine in ultraviolet light at 366 nm.

System suitability: reference solution (c):

– the chromatogram shows in the middle third 2 distinct zones, which may be touching; the lower zone (chlorogenic acid) shows a light blue fluorescence and the upper zone (hyperoside) shows a yellow or orange fluorescence.

Results: see below the sequence of fluorescent zones present in the chromatograms obtained with reference solution (a) and the test solution. Furthermore, in the chromatogram obtained with the test solution, other faint fluorescent zones, mainly light blue, greenish and yellow or orange, may be present, especially in the upper and lower thirds.

Top of the plate	
	A red zone
	A red zone, faint
———	———
	A light blue zone, faint to equivalent
	A greenish zone, faint (possibly overlapping with an orange zone)
Hyperoside: a yellow or orange zone	A yellow or orange zone, very faint to faint (possibly overlapping with a blue zone)
	A yellow or orange zone
———	———
Rutoside: a yellow or orange zone	
	A yellow or orange zone, faint to equivalent
Reference solution (a)	**Test solution**

TESTS

***Rubus fruticosus* L.** Microscopic examination (*2.8.23*). Examine the powdered herbal drug (710) (*2.9.12*) using *chloral hydrate solution R*. The presence of fasciculate-stellate covering trichomes indicates adulteration by *Rubus fruticosus* L.

Loss on drying (*2.2.32*): maximum 10.0 per cent, determined on 1.000 g of the powdered herbal drug (710) (*2.9.12*) by drying in an oven at 105 °C for 2 h.

Total ash (*2.4.16*): maximum 8.0 per cent.

ASSAY

Tannins (*2.8.14*). Use 1.000 g of the powdered herbal drug (710) (*2.9.12*).

04/2020:2569

REHMANNIA ROOT

Rehmanniae radix

DEFINITION

Dried root tuber of *Rehmannia glutinosa* (Gaertn.) DC. (syn. *Rehmannia glutinosa* (Gaertn.) Libosch. ex Fisch. & C.A. Mey.) with the crown and rootlets removed.

Content: minimum 0.2 per cent of catalpol ($C_{15}H_{22}O_{10}$; M_r 362.3) (dried drug).

IDENTIFICATION

A. The whole drug usually occurs as irregular or oblong masses, swollen in the centre, slightly tapering at both ends and bearing lenticular rootlet scars, 6-12 cm long and 3-6 cm thick. The fragmented drug occurs as slightly compressed or twisted slices. The outer surface is blackish-brown or blackish-grey, and is heavily shrunken with irregular transverse wavy lines. Fracture is difficult, with the cut surface being lustrous, blackish-brown or jet black and viscous.

B. Microscopic examination (*2.8.23*). The blackish-brown powder consists of rather sticky particles. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters (Figure 2569.-1): blackish-brown cork fragments consisting of polygonal cells (surface view [A]) or composed of superimposed layers (transverse section [E]); fragments of parenchyma [D], brown, consisting of polygonal or rectangular cells with thin, wavy or wrinkled walls, some containing orange-yellow oil droplets [Da]; vessels about 100-200 µm long and 40-60 µm in diameter, with reticulate or pitted walls [B, C], with the junction between vessels of similar diameter being clearly visible.

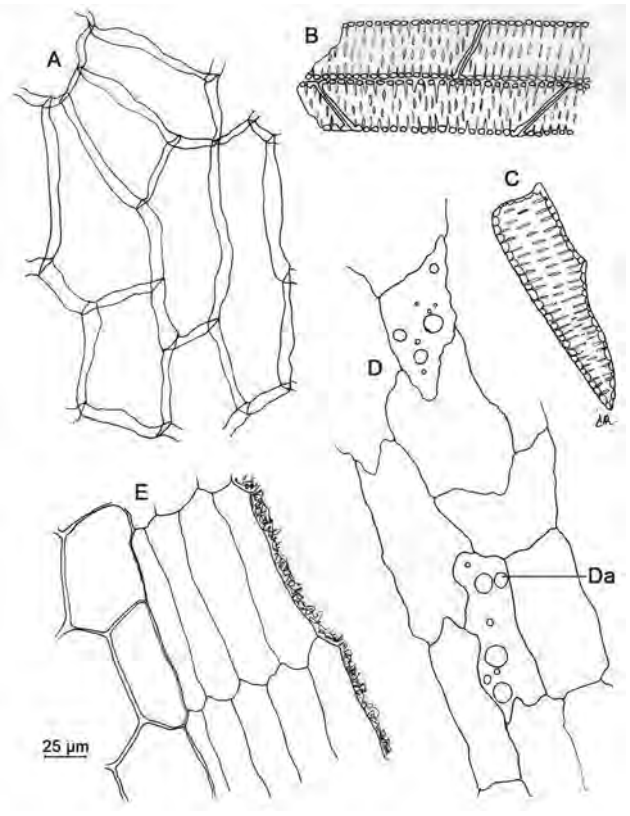


Figure 2569.-1. – *Illustration for identification test B of powdered herbal drug of rehmannia root*

C. High performance thin-layer chromatography (*2.8.25*).

Test solution. To 0.2 g of the powdered herbal drug (355) (*2.9.12*) add 5.0 mL of *methanol R*. Sonicate for 10 min, then filter or centrifuge the solution. Use the filtrate or supernatant.

Reference solution (a). Dissolve 10.0 mg of *sucrose R* and 10.0 mg of *raffinose R* in the minimum volume of *water R* and dilute to 2.0 mL with *methanol R*.

Reference solution (b). Mix 1.0 mL of reference solution (a) and 3.0 ml of *methanol R*.

Reference solution (c). Dissolve 10 mg of *fructose R* and 10 mg of *sucrose R* in the minimum amount of *water R* and dilute to 2 mL with *methanol R*.

Intensity marker: sucrose.

Plate: *TLC silica gel F_{254} plate R* (2-10 µm).

Mobile phase: *glacial acetic acid R*, *anhydrous formic acid R*, *water R*, *ethyl acetate R* (4:5:6:12 *V/V/V/V*).

Application: 2 µL as bands of 8 mm.

Development: 70 mm from the lower edge of the plate.

Drying: in a current of cold air for 5 min.

Detection: treat with a 10 per cent *V/V* solution of *sulfuric acid R* in *ethanol (96 per cent) R*, heat at 120 °C for 3 min and examine in ultraviolet light at 366 nm.

System suitability: reference solution (c):

– the chromatogram shows in the middle third 2 distinct zones, which may be touching; both the lower zone (sucrose) and the upper zone (fructose) show a brown colour.

Results: see below the sequence of zones present in the chromatograms obtained with reference solution (a) and the test solution. Furthermore, in the chromatogram obtained with the test solution, other faint blue fluorescent zones and faint brown zones may be present.

Top of the plate	
	A blue fluorescent zone, faint to intense
_____	_____
	A brown zone, faint
	A blue fluorescent zone, faint
Sucrose: a brown zone	A brown zone, faint to equivalent (sucrose)
_____	_____
Raffinose: a brown zone	A brown zone, faint to equivalent (raffinose)
	A brown zone, intense
	A brown zone, very faint
Reference solution (a)	**Test solution**

TESTS

Loss on drying (*2.2.32*): maximum 15.0 per cent, determined on 2.000 g of the powdered herbal drug (355) (*2.9.12*) by drying in an oven at 105 °C for 5 h.

Total ash (*2.4.16*): maximum 8.0 per cent.

Ash insoluble in hydrochloric acid (*2.8.1*): maximum 3.0 per cent.

ASSAY

Liquid chromatography (*2.2.29*).

Test solution. To 1.500 g of the powdered herbal drug (355) (*2.9.12*) add 50.0 mL of *water R*. Weigh and sonicate for 30 min at a temperature below 25 °C. Weigh again and compensate for the loss of solvent with *water R*. Shake well, then centrifuge for 10 min and filter the supernatant through a membrane filter (nominal pore size 0.45 µm).

Reference solution. Dissolve 5.0 mg of *catalpol CRS* in *water R* and dilute to 25.0 mL with the same solvent.

Column:

– *size*: *l* = 0.15 m, Ø = 4.6 mm;
– *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (3 µm);
– *temperature*: 25 °C.

Mobile phase:

– *mobile phase A*: *water for chromatography R*;
– *mobile phase B*: *acetonitrile R1*, *water for chromatography R* (5:95 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 3	90	10
3 - 20	90 → 70	10 → 30

Flow rate: 0.5 mL/min.

Detection: spectrophotometer at 210 nm.

Injection: 10 µL.

Retention time: catalpol = about 13 min.

System suitability: reference solution:

– *repeatability*: maximum relative standard deviation of 2.0 per cent determined on 6 injections.

Calculate the percentage content of catalpol using the following expression:

$$\frac{A_1 \times m_2 \times p \times 2}{A_2 \times m_1}$$

A_1 = area of the peak due to catalpol in the chromatogram obtained with the test solution;

A_2 = area of the peak due to catalpol in the chromatogram obtained with the reference solution;

m_1 = mass of the herbal drug to be examined used to prepare the test solution, in grams;

m_2 = mass of *catalpol CRS* used to prepare the reference solution, in grams;

p = percentage content of catalpol in *catalpol CRS*.

04/2020:0206

SENNA LEAFLET

Sennae foliolum

DEFINITION

Dried leaflets of *Senna alexandrina* Mill. (syn. *Cassia acutifolia* Delile and *Cassia angustifolia* Vahl).

Content: minimum 2.0 per cent of total hydroxyanthracene glycosides, expressed as sennoside B ($C_{42}H_{38}O_{20}$; M_r 863) (dried drug).

IDENTIFICATION

A. The green to brownish-green leaflets are thin, elongated, lanceolate, more or less asymmetrical at the base and mucronate at the apex, 15-50 mm long and 5-20 mm wide. Both surfaces of the lamina are covered with varying numbers of fine, short trichomes and may be marked with transverse or oblique lines. Pinnate venation is visible mainly on the abaxial surface and usually shows lateral veins anastomosing at the leaf margins.

B. Microscopic examination (*2.8.23*). The powder is light green or greenish-yellow. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters (Figure 0206.-1): fragments of epidermis [A, B, J, K] with polygonal cells [Aa, Ka], paracytic stomata (*2.8.3*) [Ab, Ac, Ba, Ja, Kb] and unicellular covering trichomes, conical in shape,

with warty walls (surface view [Ad], side view [G]), or their scars [Bb, Jb], frequently accompanied by palisade parenchyma [Ae, Jc]; isolated, fragmented covering trichomes [E]; fibres [F] accompanied by crystal sheaths containing prisms of calcium oxalate [Fa]; isolated prisms of calcium oxalate [D]; isolated cluster crystals of calcium oxalate [H]; fragments of median parenchyma from the lamina [C] with some cells containing cluster crystals of calcium oxalate [Ca], often accompanied by palisade parenchyma [Cb] and annular vessels [Cc].

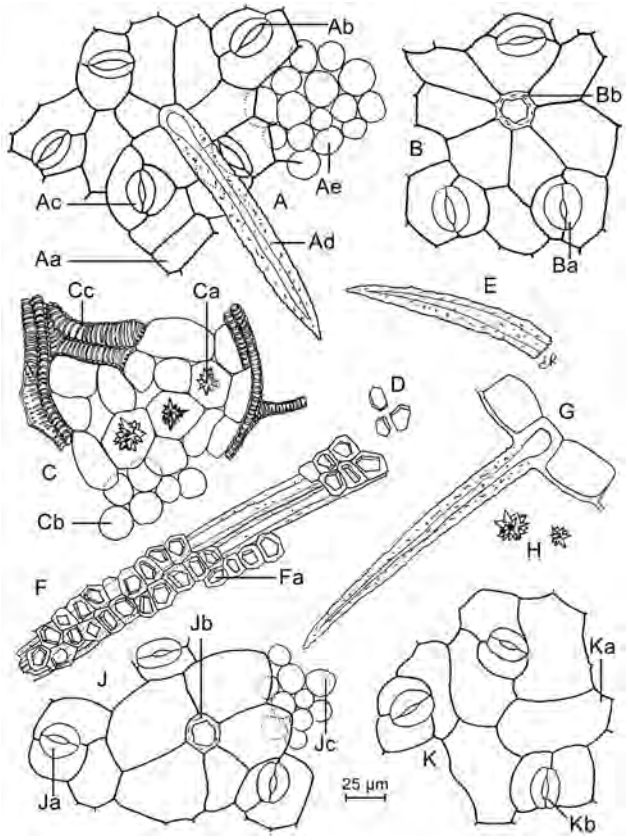


Figure 0206.-1. – *Illustration for identification test B of powdered herbal drug of senna leaflet*

C. High-performance thin-layer chromatography (*2.8.25*).

Solvent mixture: *ethanol (96 per cent) R*, *water R* (50:50 *V/V*).

Test solution. To 0.5 g of the powdered herbal drug (355) (*2.9.12*) add 5.0 mL of the solvent mixture. Sonicate for 10 min, then filter or centrifuge the solution. Use the filtrate or supernatant.

Reference solution (a). Dissolve 3 mg of *sennoside A R* and 3 mg of *sennoside B R* in a mixture of equal volumes of *ethanol (96 per cent) R* and a 1 g/L solution of *sodium hydrogen carbonate R* and dilute to 20.0 mL with the same mixture of solvents.

Reference solution (b). Dilute 2.5 mL of reference solution (a) to 10.0 mL with the solvent mixture.

Reference solution (c). Dissolve 10 mg of *senna extract HRS* in 1 mL of the solvent mixture (a slight residue may remain).

Intensity marker: sennoside A.

Plate: *TLC silica gel F_{254} plate R* (2-10 µm).

Mobile phase: *water R*, *ethyl acetate R*, *propanol R* (30:40:40 *V/V/V*).

Application: 1 µL of the test solution and 2 µL of reference solutions (a), (b) and (c), as bands of 8 mm.

Development: 70 mm from the lower edge of the plate.

Drying: in a current of air at room temperature for 5 min.

Detection: heat at 110 °C for 10 min; treat the warm plate with a 50 g/L solution of *potassium hydroxide R* in the solvent mixture and heat at 110 °C for 10 min; examine immediately in ultraviolet light at 366 nm.

System suitability: reference solution (c):

– the chromatogram shows 2 distinct zones, which may be touching, near the border between the lower and middle thirds; the lower zone (sennoside A) shows a light yellow fluorescence and the upper zone (sennoside D) shows a faint brownish-yellow fluorescence.

Results: see below the sequence of fluorescent zones present in the chromatograms obtained with reference solution (a) and the test solution. Furthermore, in the chromatogram obtained with the test solution, other blue and/or reddish fluorescent zones may be present, mainly above the zone due to sennoside A. For sennosides, R_F values vary slightly and the zones may be curved depending on the concentration.

Top of the plate	
	A light yellow zone, very faint to faint (sennoside C)
	A brownish-yellow zone, faint (sennoside D)
Sennoside A: a light yellow zone	A light yellow zone, equivalent (sennoside A)
Sennoside B: a brownish-yellow zone	A brownish-yellow zone, equivalent (sennoside B)
Reference solution (a)	**Test solution**

TESTS

Total anthraquinones (aloe emodin and rhein). Liquid chromatography (*2.2.29*). *Carry out the test protected from bright light.*

Solvent mixture: 1.0 g/L solution of *sodium hydrogen carbonate R*, *methanol R* (30:70 *V/V*).

Test solution. Introduce 1.00 g of the powdered herbal drug (355) (*2.9.12*) into a 250 mL screw-cap bottle and add 100.0 mL of the solvent mixture. Sonicate for 30 min and shake for 2 h. Filter through a membrane filter (nominal pore size 0.45 µm).

Reference solution (a). Dissolve 10 mg of *aloe emodin R* and 10.0 mg of *rhein CRS* in *tetrahydrofuran R* and dilute to 50.0 mL with the same solvent. Dilute 1.0 mL of the solution to 20.0 mL with the solvent mixture.

Reference solution (b). Dissolve 10 mg of *senna extract HRS* in 8 mL of the solvent mixture using sonication for 5 min and dilute to 10 mL with the solvent mixture (a slight residue may remain). Filter through a membrane filter (nominal pore size 0.45 µm).

Reference solution (c). Dissolve 5.0 mg of *sennoside B CRS* in 25 mL of *methanol R* using sonication and dilute to 50.0 mL with *water R*.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped propoxybenzene silica gel for chromatography R* (4 µm);
- *temperature*: 30 °C.

Mobile phase:

- *mobile phase A*: 1.275 per cent *V/V* solution of *anhydrous formic acid R*;
- *mobile phase B*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 3	87	13
3 - 40	87 → 37	13 → 63

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 270 nm.

Injection: 10 µL of the test solution and reference solutions (a) and (b).

Identification of peaks: use the chromatogram supplied with *senna extract HRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to isorhamnetin diglucoside and hydroxyanthracene glycosides (peaks 2-9); any shoulder appearing on the ascending part of the peak due to sennoside B (peak 3) is included in its area; peaks 4 and 5 may be co-eluted; peaks 7 and 8 may be co-eluted; use the chromatogram obtained with reference solution (a) to identify the peaks due to aloe emodin and rhein.

Relative retention with reference to sennoside B (peak 3, retention time = about 14.2 min): isorhamnetin diglucoside = about 0.93; hydroxyanthracene glycoside (peak 2) = about 0.98; hydroxyanthracene glycoside (peak 4) = about 1.01; hydroxyanthracene glycoside (peak 5) = about 1.02; hydroxyanthracene glycoside (peak 6) = about 1.07; hydroxyanthracene glycoside (peak 7) = about 1.09; hydroxyanthracene glycoside (peak 8) = about 1.11; hydroxyanthracene glycoside (peak 9) = about 1.13; aloe emodin = about 2.2; rhein = about 2.3.

System suitability: reference solution (b):

- *resolution*: minimum 3.0 between the peaks due to isorhamnetin diglucoside and hydroxyanthracene glycoside (peak 2).

Calculate the percentage content of total anthraquinones (aloe emodin and rhein) (*TA*), expressed as rhein, using the following expression:

$$\frac{A_1 \times m_2 \times p}{A_2 \times m_1 \times 10}$$

A_1 = sum of the areas of the peaks due to aloe emodin and rhein in the chromatogram obtained with the test solution;

A_2 = area of the peak due to rhein in the chromatogram obtained with reference solution (a);

m_1 = mass of the herbal drug to be examined used to prepare the test solution, in grams;

m_2 = mass of *rhein CRS* used to prepare reference solution (a), in grams;

p = percentage content of rhein in *rhein CRS*.

Calculate the percentage content of total anthraquinones with reference to the sum of total hydroxyanthracene glycosides (*THG*, see Assay) and total anthraquinones, using the following expression:

$$\frac{TA}{THG + TA} \times 100$$

Limit:

- *total anthraquinones (aloe emodin and rhein) expressed as rhein ($C_{15}H_8O_6$; M_r 284.2)*: maximum 7.0 per cent, calculated with reference to the sum of total hydroxyanthracene glycosides and total anthraquinones (dried drug).

Foreign matter (*2.8.2*): maximum 4 per cent.

Loss on drying (*2.2.32*): maximum 12.0 per cent, determined on 1.000 g of the powdered herbal drug (355) (*2.9.12*) by drying in an oven at 105 °C for 2 h.

Total ash (*2.4.16*): maximum 12.0 per cent.

Ash insoluble in hydrochloric acid (*2.8.1*): maximum 2.5 per cent.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for total anthraquinones (aloe emodin and rhein) with the following modification.

Injection: test solution and reference solution (c).

Calculate the percentage content of total hydroxyanthracene glycosides (peaks 2-9) (*THG*), expressed as sennoside B, using the following expression:

$$\frac{A_1 \times m_2 \times 2 \times p}{A_2 \times m_1}$$

A_1 = sum of the areas of the peaks due to hydroxyanthracene glycosides (peaks 2-9) in the chromatogram obtained with the test solution;

A_2 = area of the peak due to sennoside B in the chromatogram obtained with reference solution (c);

m_1 = mass of the herbal drug to be examined used to prepare the test solution, in grams;

m_2 = mass of *sennoside B CRS* used to prepare reference solution (c), in grams;

p = percentage content of sennoside B in *sennoside B CRS*.

STORAGE

Protected from moisture.

04/2020:0207

SENNA PODS

Sennae fructus

DEFINITION

Dried fruit of *Senna alexandrina* Mill. (syn. *Cassia acutifolia* Delile and *Cassia angustifolia* Vahl).

Content: minimum 2.0 per cent of total hydroxyanthracene glycosides, expressed as sennoside B ($C_{42}H_{38}O_{20}$; M_r 863) (dried drug).

IDENTIFICATION

A. Flattened, more or less reniform pods, yellowish-brown to greenish-brown with brown patches at the positions corresponding to the seeds, usually 35-60 mm long and

Herbal drugs

14-20 mm wide. At one end is a stylar point and at the other a short stalk. The pods contain 5-8 flattened, obovate seeds, green or pale brown, with a more or less prominent network of transverse, wavy ridges on the testa.

B. Microscopic examination (*2.8.23*). The powder is brown. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters (Figure 0207.-1): fragments of the epicarp (surface view [B, K]) with polygonal cells, anomocytic [Ba] or paracytic [Bb, Ka] stomata (*2.8.3*) and covering trichomes [Kb] or their scars [Bc]; isolated conical warty covering trichomes, usually bent [C]; fragments of the mesocarp (surface view [D]) with fibres [Da] in 2 crossed layers accompanied by a layer of cells containing calcium oxalate prisms [Db] and sometimes cells of the underlying endocarp [Dc]; fragments of the outer layers of the testa (transverse section [J]) covered by a thick cuticle [Ja] with palisade cells about 50 µm long, with thick walls and a reduced lumen [Jb], accompanied by the hypodermis with pillar-like cells [Jc]; palisade cells of the testa (surface view [N]) and fragments of the hypodermis of the seed forming rings (surface view [A]); fragments of cotyledons (surface view [F], transverse section [E]) consisting of small cells of the epidermis [Ea, Fa] and of the palisade tissue [Eb, Fb]; prisms and cluster crystals of calcium oxalate, free [De, Ga] or included in parenchyma [G]; fragments of vascular bundles [L] with spiral vessels [La] and fibres with moderately thickened and pitted walls [Lb]; sclereids [M, O] and fibres [H] accompanied by crystal sheaths of calcium oxalate [Ha] from fruit stalks.

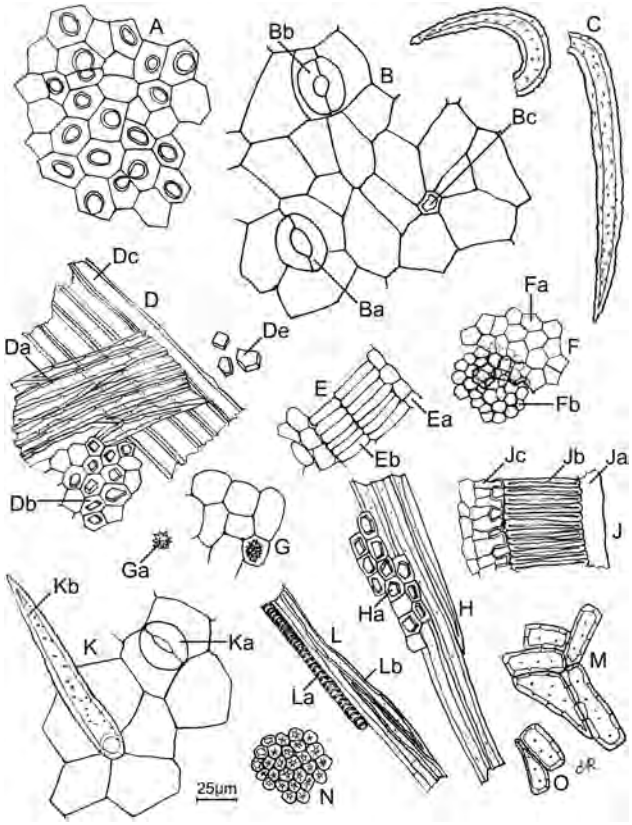


Figure 0207.-1. – *Illustration for identification test B of powdered herbal drug of senna pods*

C. High-performance thin-layer chromatography (*2.8.25*).

Solvent mixture: *ethanol (96 per cent) R*, *water R* (50:50 *V/V*).

Test solution. To 0.5 g of the powdered herbal drug (355) (*2.9.12*) add 5.0 mL of the solvent mixture. Sonicate for 10 min, then filter or centrifuge the solution. Use the filtrate or supernatant.

Reference solution (a). Dissolve 3 mg of *sennoside A R* and 3 mg of *sennoside B R* in a mixture of equal volumes of *ethanol (96 per cent) R* and a 1 g/L solution of *sodium hydrogen carbonate R* and dilute to 20.0 mL with the same mixture of solvents.

Reference solution (b). Dilute 2.5 mL of reference solution (a) to 10.0 mL with the solvent mixture.

Reference solution (c). Dissolve 10 mg of *senna extract HRS* in 1 mL of the solvent mixture (a slight residue may remain).

Intensity marker: sennoside A.

Plate: *TLC silica gel F_{254} plate R* (2-10 µm).

Mobile phase: *water R*, *ethyl acetate R*, *propanol R* (30:40:40 *V/V/V*).

Application: 1 µL of the test solution and 2 µL of reference solutions (a), (b) and (c), as bands of 8 mm.

Development: 70 mm from the lower edge of the plate.

Drying: in a current of air at room temperature for 5 min.

Detection: heat at 110 °C for 10 min; treat the warm plate with a 50 g/L solution of *potassium hydroxide R* in the solvent mixture and heat at 110 °C for 10 min; examine immediately in ultraviolet light at 366 nm.

System suitability: reference solution (c):

– the chromatogram shows 2 distinct zones, which may be touching, near the border between the lower and middle thirds; the lower zone (sennoside A) shows a light yellow fluorescence and the upper zone (sennoside D) shows a faint brownish-yellow fluorescence.

Results: see below the sequence of fluorescent zones present in the chromatograms obtained with reference solution (a) and the test solution. Furthermore, in the chromatogram obtained with the test solution, other blue and/or reddish fluorescent zones may be present, mainly above the zone due to sennoside A. For sennosides, R_F values vary slightly and the zones may be curved depending on the concentration.

Top of the plate	
	A light yellow zone, very faint to faint (sennoside C)
	A brownish-yellow zone, faint (sennoside D)
Sennoside A: a light yellow zone	A light yellow zone, equivalent to intense (sennoside A)
Sennoside B: a brownish-yellow zone	A brownish-yellow zone, intense (sennoside B)
Reference solution (a)	**Test solution**

Herbal drugs

TESTS

Total anthraquinones (aloe emodin and rhein). Liquid chromatography (*2.2.29*). *Carry out the test protected from bright light.*

Solvent mixture: 1.0 g/L solution of *sodium hydrogen carbonate R*, *methanol R* (30:70 *V/V*).

Test solution. Introduce 0.500 g of the powdered herbal drug (355) (*2.9.12*) into a 250 mL screw-cap bottle and add 100.0 mL of the solvent mixture. Sonicate for 30 min and shake for 2 h. Filter through a membrane filter (nominal pore size 0.45 µm).

Reference solution (a). Dissolve 10 mg of *aloe emodin R* and 10.0 mg of *rhein CRS* in *tetrahydrofuran R* and dilute to 50.0 mL with the same solvent. Dilute 1.0 mL of the solution to 20.0 mL with the solvent mixture.

Reference solution (b). Dissolve 10 mg of *senna extract HRS* in 8 mL of the solvent mixture using sonication for 5 min and dilute to 10 mL with the solvent mixture (a slight residue may remain). Filter through a membrane filter (nominal pore size 0.45 µm).

Reference solution (c). Dissolve 5.0 mg of *sennoside B CRS* in 25 mL of *methanol R* using sonication and dilute to 50.0 mL with *water R*.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped propoxybenzene silica gel for chromatography R* (4 µm);
- *temperature*: 30 °C.

Mobile phase:

- *mobile phase A*: 1.275 per cent *V/V* solution of *anhydrous formic acid R*;
- *mobile phase B*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 3	87	13
3 - 40	87 → 37	13 → 63

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 270 nm.

Injection: 10 µL of the test solution and reference solutions (a) and (b).

Identification of peaks: use the chromatogram supplied with *senna extract HRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to isorhamnetin diglucoside and hydroxyanthracene glycosides (peaks 2-9); any shoulder appearing on the ascending part of the peak due to sennoside B (peak 3) is included in its area; peaks 4 and 5 may be co-eluted; peaks 7 and 8 may be co-eluted; use the chromatogram obtained with reference solution (a) to identify the peaks due to aloe emodin and rhein.

Relative retention with reference to sennoside B (peak 3, retention time = about 14.2 min): isorhamnetin diglucoside = about 0.93; hydroxyanthracene glycoside (peak 2) = about 0.98; hydroxyanthracene glycoside (peak 4) = about 1.01; hydroxyanthracene glycoside (peak 5) = about 1.02; hydroxyanthracene glycoside (peak 6) = about 1.07; hydroxyanthracene glycoside (peak 7) = about 1.09; hydroxyanthracene glycoside (peak 8) = about 1.11; hydroxyanthracene glycoside (peak 9) = about 1.13; aloe emodin = about 2.2; rhein = about 2.3.

System suitability: reference solution (b):

- *resolution*: minimum 3.0 between the peaks due to isorhamnetin diglucoside and hydroxyanthracene glycoside (peak 2).

Calculate the percentage content of total anthraquinones (aloe emodin and rhein) (*TA*), expressed as rhein, using the following expression:

$$\frac{A_1 \times m_2 \times p}{A_2 \times m_1 \times 10}$$

A_1 = sum of the areas of the peaks due to aloe emodin and rhein in the chromatogram obtained with the test solution;

A_2 = area of the peak due to rhein in the chromatogram obtained with reference solution (a);

m_1 = mass of the herbal drug to be examined used to prepare the test solution, in grams;

m_2 = mass of *rhein CRS* used to prepare reference solution (a), in grams;

p = percentage content of rhein in *rhein CRS*.

Calculate the percentage content of total anthraquinones with reference to the sum of total hydroxyanthracene glycosides (*THG*, see Assay) and total anthraquinones, using the following expression:

$$\frac{TA}{THG + TA} \times 100$$

Limit:

- *total anthraquinones (aloe emodin and rhein) expressed as rhein ($C_{15}H_8O_6$; M_r 284.2)*: maximum 7.0 per cent, calculated with reference to the sum of total hydroxyanthracene glycosides and total anthraquinones (dried drug).

Foreign matter (*2.8.2*): maximum 1 per cent.

Loss on drying (*2.2.32*): maximum 12.0 per cent, determined on 1.000 g of the powdered herbal drug (355) (*2.9.12*) by drying in an oven at 105 °C for 2 h.

Total ash (*2.4.16*): maximum 9.0 per cent.

Ash insoluble in hydrochloric acid (*2.8.1*): maximum 2.0 per cent.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for total anthraquinones (aloe emodin and rhein) with the following modification.

Injection: test solution and reference solution (c).

Calculate the percentage content of total hydroxyanthracene glycosides (peaks 2-9) (*THG*), expressed as sennoside B, using the following expression:

$$\frac{A_1 \times m_2 \times 2 \times p}{A_2 \times m_1}$$

A_1 = sum of the areas of the peaks due to hydroxyanthracene glycosides (peaks 2-9) in the chromatogram obtained with the test solution;

A_2 = area of the peak due to sennoside B in the chromatogram obtained with reference solution (c);

m_1 = mass of the herbal drug to be examined used to prepare the test solution, in grams;

m_2 = mass of *sennoside B CRS* used to prepare reference solution (c), in grams;

p = percentage content of sennoside B in *sennoside B CRS*.

STORAGE

Protected from moisture.

Homoeopathic preparations

04/2020:2832

ADONIS VERNALIS FOR HOMOEOPATHIC PREPARATIONS[(1)]

Adonis vernalis ad praeparationes homoeopathicas

DEFINITION

Fresh aerial parts of *Adonis vernalis* L., collected during flowering.

IDENTIFICATION

The pithy, light green stem grows to a height of 10-40 cm, very occasionally up to 60 cm, and is up to 5 mm in diameter, erect, usually unbranched, round, often flattened towards the top, with longitudinal furrows. Younger shoots are slightly hairy but soon become completely glabrous.

The alternate and sessile leaves sheath the stem and are closer together in the upper part of the stem. They are glabrous or sparsely haired, 2- to 4-pinnate, and narrowly linear to filiform with entire margins, with downward-turned tips that are approximately 1 mm across. Brownish-black scale-like leaves are present at the base of the stem.

The single, erect or slightly overhanging, terminal flowers are normally 40-70 mm in size. The calyx has 5 greenish sepals with a covering of fine hairs on the outside. The sepals are broadly oval, half as long as the petals and pressed to the latter. The corolla consists of 10-20, normally 12, distinctly lustrous, deep yellow petals that are 20-40 mm in length, 6-10 mm wide with entire margins, elongated and tapering to a finely toothed point, and glabrous with clearly visible venation. The numerous stamens are an intense yellow. The numerous, rounded, apocarpous ovaries are covered all over with hairs; they are attached to a conical, convex receptacle and have a short, hooked style.

TESTS

Foreign matter (*2.8.2*): maximum 5 per cent.

Loss on drying (*2.2.32*): minimum 60.0 per cent, determined on 5.0 g of the comminuted herbal drug by drying in an oven at 105 °C for 2 h.

Mother tincture

The mother tincture complies with the requirements of the general monograph *Mother tinctures for homoeopathic preparations (2029)*.

DEFINITION

The mother tincture is prepared from the fresh aerial parts of *Adonis vernalis* L., collected during flowering.

Content: 0.01 per cent *m/m* to 0.05 per cent *m/m* of total cardenolic glycosides, expressed as cymarin ($C_{30}H_{44}O_9$; M_r 548.7).

PRODUCTION

The mother tincture is prepared from the fresh flowering aerial parts of *Adonis vernalis* L. according to the following methods as prescribed in the monograph *Methods of preparation of homoeopathic stocks and potentisation (2371)*:

- method 1.1.3;
- method 1.1.10, using ethanol (45 per cent *V/V*) and a maceration time of about 3 weeks.

CHARACTERS

Appearance: brown liquid.

IDENTIFICATION

Thin-layer chromatography (*2.2.27*).

Solvent mixture: *ethyl acetate R*, *methanol R* (50:50 *V/V*).

Test solution. To 10 mL of the mother tincture to be examined add 10 mL of *ethanol (50 per cent V/V) R* and 10 mL of *lead acetate solution R*. Boil for 2 min, then cool and centrifuge. Collect the supernatant and shake with 2 quantities, each of 15 mL of *ethyl acetate R*, centrifuging if an emulsion forms. Dry the combined organic phases over *anhydrous sodium sulfate R* and filter. Evaporate the filtrate to dryness and dissolve the residue in 0.5 mL of the solvent mixture.

Reference solution. Dissolve 10 mg of *convallatoxin R* and 10 mg of *cymarin R* in the solvent mixture and dilute to 10 mL with the solvent mixture.

Plate: *TLC silica gel plate R* (5-40 µm) [or *TLC silica gel plate R* (2-10 µm)].

Mobile phase: *water R*, *methanol R*, *ethyl acetate R* (8:11:81 *V/V/V*).

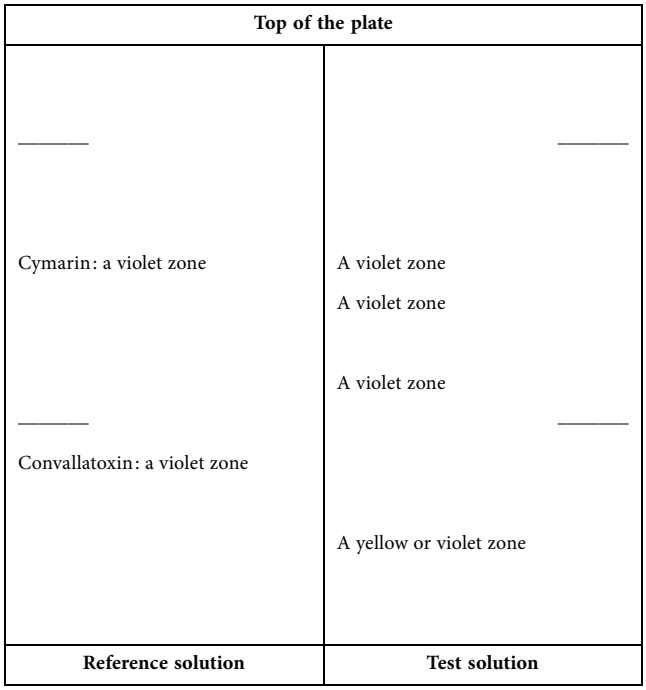
Application: 25 µL [or 20 µL] as bands of 20 mm [or 10 mm].

Development: over a path of 10 cm [or 6 cm].

Drying: in air until the solvents have evaporated.

Detection: treat with a mixture of equal volumes of *dilute sodium hydroxide solution R* and *dinitrobenzoic acid solution R*; examine in daylight.

Results: see below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other faint zones may be present in the chromatogram obtained with the test solution.



TESTS

Relative density (*2.2.5*): 0.930 to 0.956, where method 1.1.3 is used.

Ethanol (*2.9.10*): 40 per cent *V/V* to 50 per cent *V/V*, where method 1.1.10 is used.

Dry residue (*2.8.16*): minimum 3.6 per cent (where method 1.1.3 is used), minimum 1.0 per cent (where method 1.1.10 is used).

(1) FRENCH TITLE: Adonis vernalis pour préparations homéopathiques

ASSAY

Test solution. To 20.00 g of the mother tincture to be examined, add 12 mL of *lead subacetate solution R*. Shake and centrifuge. Collect the supernatant. Repeat the centrifugation twice using 5 mL of *water R* each time and collect the supernatant, then dilute to 50.0 mL with *water R*. To 10.0 mL of this solution add 10.0 mL of a 100 g/L solution of *anhydrous sodium sulfate R*. Filter. To 10.0 mL of the filtrate add 2.0 mL of *dinitrobenzoic acid solution R* and 1.0 mL of *1 M sodium hydroxide*.

Reference solution. Dissolve 10.0 mg of *cymarin R* in 20 mL of *ethanol (96 per cent) R* and dilute to 100.0 mL with *water R*. Dilute 5.0 mL of the solution to 20.0 mL with *water R*. To 10.0 mL of this solution, add 2.0 mL of *dinitrobenzoic acid solution R* and 1.0 mL of *1 M sodium hydroxide*.

Measure the absorbance (*2.2.25*) of the test solution and the reference solution at 540 nm several times during the first 12 min following the end of solution preparation until the maximum absorbance is reached, using a mixture of 10.0 mL of *water R*, 2.0 mL of *dinitrobenzoic acid solution R* and 1.0 mL of *1 M sodium hydroxide* as the compensation liquid.

Calculate the percentage content *m/m* of total cardenolic glycosides, expressed as cymarin, using the following expression:

$$\frac{A_1 \times m_2 \times p \times 0.25}{A_2 \times m_1}$$

A_1 = maximum absorbance of the test solution;

A_2 = maximum absorbance of the reference solution;

m_1 = mass of the mother tincture to be examined used to prepare the test solution, in grams;

m_2 = mass of *cymarin R* used to prepare the reference solution, in grams;

p = percentage content of cymarin in *cymarin R*.

04/2020:2676

MAGNESIUM FLUORATUM FOR HOMOEOPATHIC PREPARATIONS(2)

Magnesium fluoratum ad praeparationes homoeopathicas

MgF_2 M_r 62.3
[7783-40-6]

DEFINITION

Content: 98.5 per cent to 100.5 per cent of MgF_2.

CHARACTERS

Appearance: white or almost white powder or crystals.

Solubility: practically insoluble in water, very slightly soluble in dilute nitric acid and in concentrated sulfuric acid, practically insoluble in anhydrous acetic acid.

IDENTIFICATION

A. Mix 0.2 g with 2 g of *potassium hydrogen sulfate R* in a platinum crucible and melt at 800 °C. Cool, carefully take up the melt in 20 mL of *water R* and boil briefly. Cool and filter. Dilute 0.5 mL of the filtrate to 2 mL with *water R*. The solution gives the reaction of magnesium (*2.3.1*).

B. Mix 0.2 g with 1 g of *anhydrous sodium carbonate R* in a platinum crucible and melt at 850 °C. Cool, take up the melt in 10 mL of *dilute acetic acid R* and boil briefly. Cool, filter and dilute the filtrate to 20 mL with *water R*. To 0.4 mL of this solution add dropwise a mixture of 0.1 mL of *alizarin S solution R* and 0.1 mL of *zirconyl nitrate solution R*. The colour changes from red to yellow.

TESTS

Solution S. Boil 5.0 g with 100.0 mL of *distilled water R* under a reflux condenser for about 5 min. Allow to cool, then filter through an ashless filter paper (nominal pore size not greater than 2 µm).

Appearance of solution. Solution S is colourless (*2.2.2, Method II*).

Carbonates. Suspend 0.5 g in 5 mL of *carbon dioxide-free water R* and add 5 mL of *dilute acetic acid R*. Quickly stopper the test tube with a stopper fitted with a glass tube bent twice through 90° and which dips at the other end in *barium hydroxide solution R*. Warm the mixture. The barium hydroxide solution remains clear.

Chlorides (*2.4.4*): maximum 100 ppm.

Dilute 10 mL of solution S to 15 mL with *water R*.

Sulfates (*2.4.13*): maximum 200 ppm, determined on solution S.

Water-soluble matter: maximum 0.5 per cent.

Boil 2.00 g with 100 mL of *water R* for 5 min. Filter the hot solution, then cool the filtrate and dilute to 100 mL with *water R*. Evaporate 50 mL of this solution to dryness in an evaporating dish and dry at 100-105 °C. The residue weighs a maximum of 5 mg.

ASSAY

Thoroughly mix 0.100 g with 2 g of *potassium hydrogen sulfate R* in a platinum crucible and melt at 800 °C. Allow to cool, carefully take up the melt in 10 mL of *dilute hydrochloric acid R* and heat to dissolve. Dilute the solution to 100.0 mL with *water R*. Transfer 50.0 mL of this solution to a 500 mL conical flask and dilute to 300 mL with *water R*. Carry out the complexometric titration of magnesium (*2.5.11*).

1 mL of *0.1 M sodium edetate* is equivalent to 6.23 mg of MgF_2.

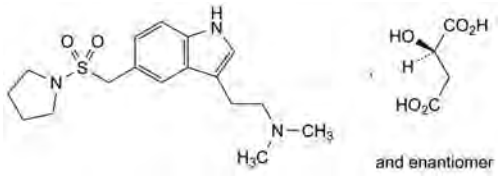
(2) FRENCH TITLE: Magnesia fluorata pour préparations homéopathiques

A

04/2020:2970

ALMOTRIPTAN MALATE

Almotriptani malas



$C_{21}H_{31}N_3O_7S$ M_r 469.6
[181183-52-8]

DEFINITION

N,*N*-Dimethyl-2-[5-[(pyrrolidine-1-sulfonyl)methyl]-1*H*-indol-3-yl]ethan-1-amine (*RS*)-2-hydroxybutanedioate.

Content: 98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance: white or slightly yellow, crystalline powder.

Solubility: soluble in water, slightly soluble in methanol, practically insoluble in anhydrous ethanol and in heptane.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *almotriptan malate CRS*.

B. Examine the chromatograms obtained in the assay.

Results: the principal peak in the chromatogram obtained with test solution (b) is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (b).

TESTS

Related substances. Liquid chromatography (*2.2.29*). *Carry out the test protected from light.*

Solvent mixture: *acetonitrile R*, *water R* (20:80 *V/V*).

Buffer solution. Dissolve 0.5 g of *sodium octanesulfonate monohydrate R* in about 900 mL of *water for chromatography R*. Add 5 mL of *phosphoric acid R*, adjust to pH 3.0 with *sodium hydroxide solution R* and dilute to 1000 mL with *water for chromatography R*.

Test solution (a). Dissolve 10.0 mg of the substance to be examined in the solvent mixture and dilute to 10.0 mL with the solvent mixture.

Test solution (b). Dissolve 25.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 mL with the solvent mixture. Dilute 5.0 mL of the solution to 50.0 mL with the solvent mixture.

Reference solution (a). Dilute 1.0 mL of test solution (a) to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b). Dissolve 25.0 mg of *almotriptan malate CRS* in the solvent mixture and dilute to 50.0 mL with the solvent mixture. Dilute 5.0 mL of the solution to 50.0 mL with the solvent mixture.

Reference solution (c). Dissolve 5 mg of *almotriptan for system suitability CRS* (containing impurity A) in the solvent mixture and dilute to 5 mL with the solvent mixture.

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm;
– *stationary phase*: *end-capped octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

– *mobile phase A*: *acetonitrile for chromatography R*, buffer solution (10:90 *V/V*);
– *mobile phase B*: buffer solution, *acetonitrile for chromatography R* (30:70 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	85	15
5 - 20	85 → 78	15 → 22
20 - 30	78 → 70	22 → 30
30 - 40	70	30

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 228 nm.

Injection: 5 µL of test solution (a) and reference solutions (a) and (c).

Identification of impurities: use the chromatogram supplied with *almotriptan for system suitability CRS* and the chromatogram obtained with reference solution (c) to identify the peak due to impurity A.

Relative retention with reference to almotriptan (retention time = about 27 min): malic acid = about 0.1; impurity A = about 0.96.

System suitability: reference solution (c):

– *resolution*: minimum 1.5 between the peaks due to impurity A and almotriptan.

Calculation of percentage contents:

– for each impurity, use the concentration of almotriptan malate in reference solution (a).

Limits:

– *impurity A*: maximum 0.5 per cent;
– *unspecified impurities*: for each impurity, maximum 0.10 per cent;
– *total*: maximum 0.7 per cent;
– *reporting threshold*: 0.05 per cent; disregard the peak due to malic acid.

Loss on drying (*2.2.32*): maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Mobile phase: *acetonitrile for chromatography R*, buffer solution (27:73 *V/V*).

Injection: 10 µL of test solution (b) and reference solution (b).

Run time: twice the retention time of almotriptan.

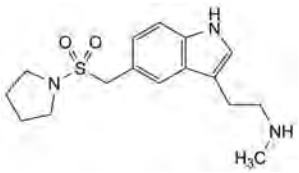
Retention time: almotriptan = about 9 min.

Calculate the percentage content of $C_{21}H_{31}N_3O_7S$ taking into account the assigned content of *almotriptan malate CRS*.

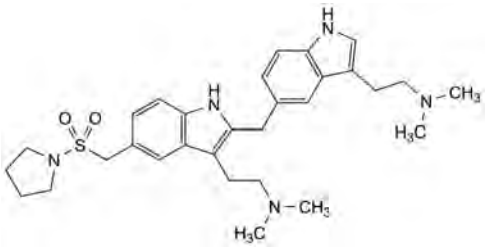
IMPURITIES

Specified impurities: *A*.

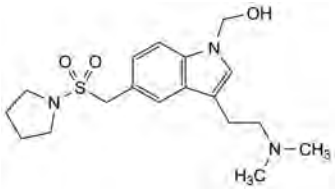
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *B*, *C*, *D*, *E*, *F*.



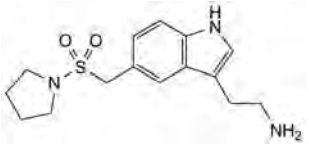
A. *N*-methyl-2-[5-[(pyrrolidine-1-sulfonyl)methyl]-1*H*-indol-3-yl]ethan-1-amine,



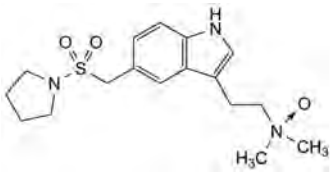
B. 2-[2-[[3-[2-(dimethylamino)ethyl]-1*H*-indol-5-yl]methyl]-5-[(pyrrolidine-1-sulfonyl)methyl]-1*H*-indol-3-yl]-*N*,*N*-dimethylethan-1-amine,



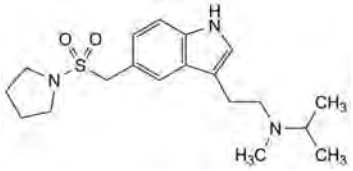
C. [3-[2-(dimethylamino)ethyl]-5-[(pyrrolidine-1-sulfonyl)methyl]-1*H*-indol-1-yl]methanol,



D. 2-[5-[(pyrrolidine-1-sulfonyl)methyl]-1*H*-indol-3-yl]ethan-1-amine,



E. *N*,*N*-dimethyl-2-[5-[(pyrrolidine-1-sulfonyl)methyl]-1*H*-indol-3-yl]ethan-1-amine *N*-oxide,

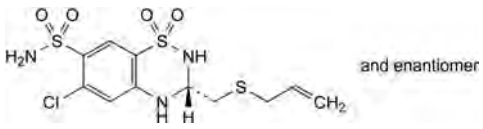


F. *N*-methyl-*N*-[2-[5-[(pyrrolidine-1-sulfonyl)methyl]-1*H*-indol-3-yl]ethyl]propan-2-amine.

07/2008:2185
corrected 10.1

ALTIZIDE

Altizidum



$C_{11}H_{14}ClN_3O_4S_3$ M_r 383.9
[5588-16-9]

DEFINITION

(3*RS*)-6-Chloro-3-[(prop-2-enylsulfanyl)methyl]-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide.

Content: 97.5 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance: white or almost white powder.

Solubility: practically insoluble in water, soluble in methanol, practically insoluble in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *altizide CRS*.

If the spectra obtained show differences, dissolve 50 mg of the substance to be examined and 50 mg of the reference substance separately in 2 mL of *acetone R* and evaporate the solvent. Precipitate by adding 1 mL of *methylene chloride R*. Evaporate to dryness and record new spectra using the residues.

TESTS

Impurity B. Thin-layer chromatography (*2.2.27*).

Test solution. Dissolve 0.200 g of the substance to be examined in *acetone R* and dilute to 2.0 mL with the same solvent.

Reference solution (a). Dissolve 10.0 mg of *altizide impurity B CRS* in *acetone R* and dilute to 25.0 mL with the same solvent.

Reference solution (b). To 1.0 mL of reference solution (a) add 1.0 mL of the test solution.

Reference solution (c). Dilute 5.0 mL of reference solution (a) to 10.0 mL with *acetone R*.

Plate: *TLC silica gel F_{254} plate R*.

Mobile phase: *acetone R*, *methylene chloride R* (25:75 *V/V*).

Application: 10 µL of the test solution and reference solutions (b) and (c).

Development: over 2/3 of the plate.

Drying: in air.

Detection: spray with a mixture of equal volumes of a 10 g/L solution of *potassium permanganate R* and a 50 g/L solution of *sodium carbonate R*, prepared immediately before use. Allow to stand for 30 min and examine in daylight.

System suitability: reference solution (b):

– the chromatogram shows 2 clearly separated spots.

Limit: any spot due to impurity B is not more intense than the principal spot in the chromatogram obtained with reference solution (c) (0.2 per cent).

Related substances. Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use, except reference solution (b).*

Test solution. Dissolve 50 mg of the substance to be examined in 5 mL of *acetonitrile R* and dilute to 25 mL with the mobile phase.

Reference solution (a). Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b). In order to produce impurity A *in situ*, dissolve 50 mg of the substance to be examined in 5 mL of *acetonitrile R* and dilute to 25 mL with *water R*. Allow to stand for 30 min.

Reference solution (c). Dissolve 4 mg of *furosemide CRS* in 2 mL of *acetonitrile R*, add 2 mL of the test solution and dilute to 100 mL with the mobile phase.

Column:

- *size*: *l* = 0.15 m, Ø = 3.9 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography with embedded polar groups R* (5 µm);
- *temperature*: 30 °C.

Mobile phase: *acetonitrile R*, *water for chromatography R* previously adjusted to pH 2.0 with *perchloric acid R* (25:75 *V/V*).

Flow rate: 0.7 mL/min.

Detection: spectrophotometer at 270 nm.

Injection: 5 µL.

Run time: twice the retention time of altizide.

Relative retention with reference to altizide (retention time = about 25 min): impurity A = about 0.15; furosemide = about 1.05.

System suitability: reference solution (c):

- *resolution*: minimum 1.0 between the peaks due to altizide and furosemide.

Limits:

- *impurity A*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water (*2.5.32*): maximum 0.5 per cent, determined on 50.0 mg.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances, with the following modifications.

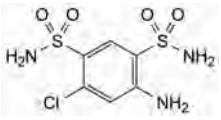
Test solution. Dissolve 25.0 mg of the substance to be examined in 2 mL of *acetonitrile R* and dilute to 25.0 mL with the mobile phase.

Reference solution. Dissolve 25.0 mg of *altizide CRS* in 2 mL of *acetonitrile R* and dilute to 25.0 mL with the mobile phase.

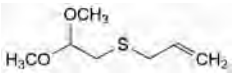
Calculate the percentage content of $C_{11}H_{14}ClN_3O_4S_3$ from the declared content of *altizide CRS*.

IMPURITIES

Specified impurities: A, B.



A. 4-amino-6-chlorobenzene-1,3-disulfonamide,

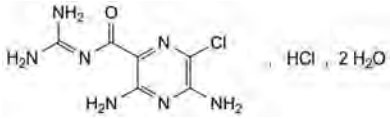


B. 3-[(2,2-dimethoxyethyl)sulfanyl]prop-1-ene.

04/2019:0651
corrected 10.1

AMILORIDE HYDROCHLORIDE DIHYDRATE

Amiloridi hydrochloridum dihydricum



$C_6H_9Cl_2N_7O,2H_2O$ M_r 302.1
[17440-83-4]

DEFINITION

3,5-Diamino-6-chloro-*N*-(diaminomethylidene)pyrazine-2-carboxamide hydrochloride dihydrate.

Content: 98.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance: pale yellow or greenish-yellow powder.

Solubility: slightly soluble in water and in anhydrous ethanol, practically insoluble in heptane.

IDENTIFICATION

First identification: A, C, D.

Second identification: B, C, D.

A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *amiloride hydrochloride CRS*.

B. Thin-layer chromatography (*2.2.27*).
Test solution. Dissolve 5 mg of the substance to be examined in *methanol R* and dilute to 10 mL with the same solvent.
Reference solution. Dissolve 5 mg of *amiloride hydrochloride CRS* in *methanol R* and dilute to 10 mL with the same solvent.
Plate: *TLC silica gel F_{254} plate R*.
Mobile phase: *concentrated ammonia R*, *propanol R* (30:70 *V/V*).
Application: 5 µL; the volume may be adapted according to the type of plate used.
Development: over 2/3 of the plate.
Drying: in air.
Detection: examine in ultraviolet light at 254 nm.
Results: the principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

C. Dissolve 25 mg of the substance to be examined in *water R* and dilute to 10 mL with the same solvent. 2 mL of the solution gives reaction (a) of chlorides (*2.3.1*); acidify with 0.5 mL of *dilute acetic acid R*, instead of *dilute nitric acid R*.

D. Water (see Tests).

TESTS

Free acid. Dissolve 1.0 g in a mixture of 50 mL of *methanol R* and 50 mL of *water R* and titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*). Not more than 0.3 mL of *0.1 M sodium hydroxide* is required to reach the end-point.

Related substances. Liquid chromatography (*2.2.29*).

Solution A. Dissolve 2.76 g of *sodium dihydrogen phosphate monohydrate R* in 850 mL of *water for chromatography R*, adjust to pH 3.0 with *phosphoric acid R* and dilute to 1.0 L with *water for chromatography R*.

Test solution. Dissolve 20 mg of the substance to be examined in 1 mL of *methanol R1* and dilute to 10.0 mL with solution A.

Reference solution (a). Dissolve 2 mg of *amiloride impurity A CRS* in 0.5 mL of *methanol R1*, add 0.5 mL of the test solution and dilute to 10.0 mL with solution A.

Reference solution (b). Dissolve 4 mg of *amiloride for peak identification CRS* (containing impurity C) in 0.5 mL of *methanol R1* and dilute to 2.0 mL with solution A.

Reference solution (c). Dilute 1.0 mL of the test solution to 100.0 mL with solution A. Dilute 1.0 mL of this solution to 10.0 mL with solution A.

Column:

- *size*: *l* = 0.125 m, Ø = 4.0 mm;
- *stationary phase*: *base-deactivated end-capped octylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 30 °C.

Mobile phase: dissolve 0.8 g of *sodium hexanesulfonate monohydrate R* in a mixture of 80 mL of *acetonitrile R1* and 920 mL of solution A.

Flow rate: 1.5 mL/min.

Detection: spectrophotometer at 210 nm.

Injection: 20 µL.

Run time: twice the retention time of amiloride.

Identification of impurities: use the chromatogram obtained with reference solution (a) to identify the peak due to impurity A; use the chromatogram supplied with *amiloride for peak identification CRS* and the chromatogram obtained with reference solution (b) to identify the peak due to impurity C.

Relative retention with reference to amiloride (retention time = about 10 min): impurity C = about 0.5; impurity A = about 0.8.

System suitability: reference solution (a):

- *resolution*: minimum 3.0 between the peaks due to impurity A and amiloride.

Calculation of percentage contents:

- for each impurity, use the concentration of amiloride hydrochloride dihydrate in reference solution (c).

Limits:

- *impurity C*: maximum 0.2 per cent;
- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- *total*: maximum 0.4 per cent;
- *reporting threshold*: 0.05 per cent.

Water (*2.5.12*): 11.0 per cent to 13.0 per cent, determined on 0.200 g.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in a mixture of 5.0 mL of *0.01 M hydrochloric acid* and 50 mL of *ethanol (96 per cent) R*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 mL of *0.1 M sodium hydroxide* is equivalent to 26.61 mg of $C_6H_9Cl_2N_7O$.

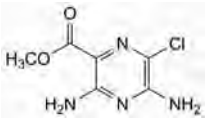
STORAGE

Protected from light.

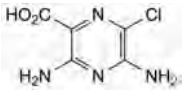
IMPURITIES

Specified impurities: C.

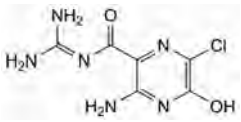
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *A, B.*



A. methyl 3,5-diamino-6-chloropyrazine-2-carboxylate,



B. 3,5-diamino-6-chloropyrazine-2-carboxylic acid,

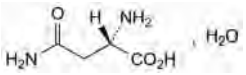


C. 3-amino-6-chloro-*N*-(diaminomethylidene)-5-hydroxypyrazine-2-carboxamide.

04/2020:2086

ASPARAGINE MONOHYDRATE

Asparaginum monohydricum



$C_4H_8N_2O_3,H_2O$ M_r 150.1

[5794-13-8]

DEFINITION

(2*S*)-2,4-Diamino-4-oxobutanoic acid monohydrate.

Content: 99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, crystalline powder or colourless crystals.

Solubility: slightly soluble in water, practically insoluble in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION

First identification: A, B, D.

Second identification: A, C, D.

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *asparagine monohydrate CRS*.

C. Thin-layer chromatography (*2.2.27*).

Test solution. Dissolve 10 mg of the substance to be examined in *water R* and dilute to 10 mL with the same solvent.

Reference solution. Dissolve 10 mg of *asparagine monohydrate CRS* in *water R* and dilute to 10 mL with the same solvent.

Plate: *TLC silica gel plate R.*

Mobile phase: *glacial acetic acid R*, *water R*, *butanol R* (25:25:50 *V/V/V*).

Application: 5 µL.

Development: over 2/3 of the plate.

Drying: at 110 °C for 15 min.

Detection: spray with *ninhydrin solution R* and heat at 105 °C for 10 min.

Results: the principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

D. Loss on drying (see Tests).

TESTS

Solution S. Dissolve with heating 2.0 g in *carbon dioxide-free water R* and dilute to 100 mL with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*): 4.0 to 6.0 for solution S.

Specific optical rotation (*2.2.7*): + 33.7 to + 36.0 (dried substance).

Dissolve 2.50 g in a 309.0 g/L solution of *hydrochloric acid R* and dilute to 25.0 mL with the same acid.

Related substances. Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution. Dissolve 0.100 g of the substance to be examined in *water R* and dilute to 10.0 mL with the same solvent.

Reference solution (a). Dilute 1.0 mL of the test solution to 100.0 mL with *water R.*

Reference solution (b). Dilute 1.0 mL of reference solution (a) to 10.0 mL with *water R.*

Reference solution (c). Dissolve 5.0 mg of *aspartic acid R* (impurity A) in *water R* and dilute to 10.0 mL with the same solvent. Dilute 1.0 mL of the solution to 10.0 mL with *water R.*

Reference solution (d). Dissolve 3.0 mg of *asparagine impurity C CRS* in 40 mL of the mobile phase using sonication and dilute to 50.0 mL with the mobile phase. Dilute 1.0 mL of the solution to 10.0 mL with *water R.*

Reference solution (e). Mix 5 mL of reference solution (c) with 2.5 mL of reference solution (a) and dilute to 10 mL with *water R.*

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 25 °C.

Mobile phase: dissolve 13.6 g of *potassium dihydrogen phosphate R* and 2.16 g of *sodium octanesulfonate R* in about 900 mL of *water for chromatography R.* Adjust to pH 2.2 with *phosphoric acid R* and dilute to 1000 mL with *water for chromatography R.* Add 5 mL of *acetonitrile R1.*

Flow rate: 0.7 mL/min.

Detection: spectrophotometer at 210 nm.

Injection: 20 µL.

Run time: twice the retention time of asparagine.

Identification of impurities: use the chromatogram obtained with reference solution (c) to identify the peak due to impurity A; use the chromatogram obtained with reference solution (d) to identify the peak due to impurity C.

Relative retention with reference to asparagine (retention time = about 6.6 min): impurity C = about 0.6; impurity A = about 1.2.

System suitability: reference solution (e):

- *resolution*: minimum 5.0 between the peaks due to asparagine and impurity A.

Calculation of percentage contents:

- for impurity A, use the concentration of impurity A in reference solution (c);
- for impurity C, use the concentration of impurity C in reference solution (d);
- for impurities other than A and C, use the concentration of asparagine monohydrate in reference solution (b).

Limits:

- *impurity A*: maximum 0.5 per cent;
- *impurity C*: maximum 0.1 per cent;
- *unspecified impurities*: for each impurity, maximum 0.05 per cent;
- *total*: maximum 0.8 per cent;
- *reporting threshold*: 0.03 per cent.

Chlorides (*2.4.4*): maximum 200 ppm.

Dilute 12.5 mL of solution S to 15 mL with *water R.*

Sulfates (*2.4.13*): maximum 200 ppm.

To 0.75 g add 2.5 mL of *dilute hydrochloric acid R* and dilute to 15 mL with *distilled water R.* Examine after 30 min.

Ammonium (*2.4.1, Method B*): maximum 0.1 per cent, determined on 10 mg.

Prepare the standard using 0.1 mL of *ammonium standard solution (100 ppm NH_4) R.*

Iron (*2.4.9*): maximum 10 ppm.

Dissolve 1.0 g in *dilute hydrochloric acid R* and dilute to 10 mL with the same acid. Shake 3 times with 10 mL of *methyl isobutyl ketone R1* for 3 min. Wash the combined organic phases with 10 mL of *water R* for 3 min. The aqueous phase complies with the limit test for iron.

Loss on drying (*2.2.32*): 10.5 per cent to 12.5 per cent, determined on 1.000 g by drying in an oven at 130 °C for 3 h.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

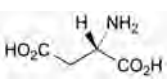
Dissolve 0.110 g in 5 mL of *anhydrous formic acid R.* Add 50 mL of *anhydrous acetic acid R.* Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 13.21 mg of $C_4H_8N_2O_3$.

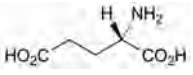
IMPURITIES

Specified impurities: A, C.

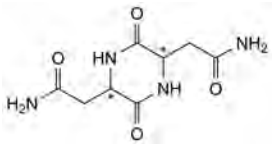
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034).* It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *B, D, E, F, G, H.*



A. (2*S*)-2-aminobutanedioic acid (aspartic acid),

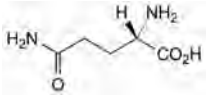


B. (2*S*)-2-aminopentanedioic acid (glutamic acid),

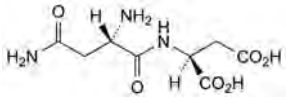


C. 2,2′-[(2Ξ,5Ξ)-3,6-dioxopiperazine-2,5-diyl]diacetamide,

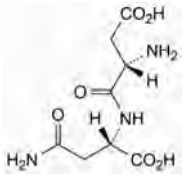
D. (2*E*)-but-2-enedioic acid (fumaric acid),



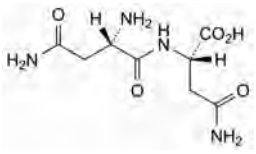
E. (2*S*)-2,5-diamino-5-oxopentanoic acid (glutamine),



F. (2*S*)-2-[[(2*S*)-2,4-diamino-4-oxobutanoyl]amino]butanedioic acid (asparaginylaspartic acid),



G. (2*S*)-4-amino-2-[[(2*S*)-2-amino-3-carboxypropanoyl]amino]-4-oxobutanoic acid (α-aspartylasparagine),

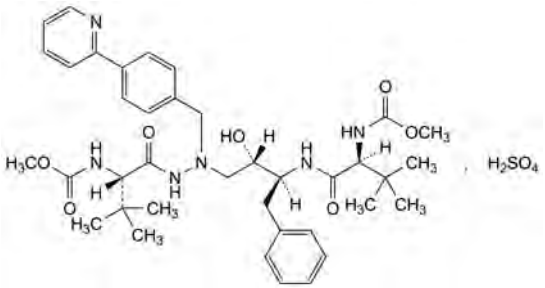


H. (2*S*)-4-amino-2-[[(2*S*)-2,4-diamino-4-oxobutanoyl]amino]-4-oxobutanoic acid (asparaginylasparagine).

04/2019:2898
corrected 10.1

ATAZANAVIR SULFATE

Atazanaviri sulfas



$C_{38}H_{54}N_6O_{11}S$ M_r 803
[229975-97-7]

DEFINITION

Methyl [(5*S*,10*S*,11*S*,14*S*)-11-benzyl-5-*tert*-butyl-10-hydroxy-15,15-dimethyl-3,6,13-trioxo-8-[[4-(pyridin-2-yl)phenyl]methyl]-2-oxa-4,7,8,12-tetraazahexadecan-14-yl]carbamate sulfate.

Content: 98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance: white or pale yellow, slightly hygroscopic, crystalline powder that may contain agglomerates.

Solubility: slightly soluble in water, freely soluble in ethanol (96 per cent), practically insoluble in heptane.

IDENTIFICATION

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *atazanavir sulfate CRS*.

C. It gives reaction (a) of sulfates (*2.3.1*).

TESTS

Specific optical rotation (*2.2.7*): − 44 to − 40 (anhydrous substance), measured at 25 °C.

Dissolve 0.100 g in 8 mL of *methanol R*, using sonication if necessary, and dilute to 10.0 mL with the same solvent.

Related substances. Liquid chromatography (*2.2.29*).

Solvent mixture. Mix equal volumes of *acetonitrile R1* and a freshly prepared 2.73 g/L solution of *potassium dihydrogen phosphate R* in *water for chromatography R* previously adjusted to pH 3.5 with *dilute phosphoric acid R*.

Test solution (a). Dissolve 20.0 mg of the substance to be examined in 40 mL of the solvent mixture, sonicate for 3 min and dilute to 50.0 mL with the solvent mixture.

Test solution (b). Dissolve 50.0 mg of the substance to be examined in 40 mL of the solvent mixture, sonicate for 3 min and dilute to 50.0 mL with the solvent mixture.

Reference solution (a). Dissolve 20.0 mg of *atazanavir sulfate CRS* in 40 mL of the solvent mixture, sonicate for 3 min and dilute to 50.0 mL with the solvent mixture.

Reference solution (b). Dilute 1.0 mL of test solution (a) to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (c). Dissolve 4 mg of *atazanavir for system suitability CRS* (containing impurity F) in 8 mL of the solvent mixture, sonicate for 3 min and dilute to 10 mL with the solvent mixture.

Reference solution (d). Dissolve 2.0 mg of *atazanavir impurity K CRS* in 9 mL of the solvent mixture, sonicate for 3 min and dilute to 10.0 mL with the solvent mixture. Dilute 5.0 mL of the solution to 100.0 mL with the solvent mixture. Dilute 3.0 mL of this solution to 20.0 mL with the solvent mixture.

Column:
- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (3.0 µm);
- *temperature*: 25 °C.

Mobile phase:
- *mobile phase A*: mix 25 volumes of *acetonitrile R1* and 75 volumes of a freshly prepared 2.73 g/L solution of *potassium dihydrogen phosphate R* previously adjusted to pH 3.5 with *dilute phosphoric acid R*;
- *mobile phase B*: mix 25 volumes of a freshly prepared 2.73 g/L solution of *potassium dihydrogen phosphate R* previously adjusted to pH 3.5 with *dilute phosphoric acid R*, and 75 volumes of *acetonitrile R1*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	100	0
5 - 45	100 → 0	0 → 100

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 215 nm.

Injection: 10 µL of test solution (a) and reference solutions (b) and (c).

Identification of impurities: use the chromatogram supplied with *atazanavir for system suitability CRS* and the chromatogram obtained with reference solution (c) to identify the peak due to impurity F.

Relative retention with reference to atazanavir (retention time = about 30 min): impurity F = about 0.99.

System suitability: reference solution (c):

– *resolution*: minimum 1.5 between the peaks due to impurity F and atazanavir.

Calculation of percentage contents:

– for each impurity, use the concentration of atazanavir sulfate in reference solution (b).

Limits:

– *unspecified impurities*: for each impurity, maximum 0.10 per cent;

– *total*: maximum 0.5 per cent;

– *reporting threshold*: 0.05 per cent; disregard any peak with a relative retention with reference to atazanavir of less than 0.2.

Impurity K. Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Mobile phase:

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	95	5
5 - 8	95 → 0	5 → 100
8 - 14	0	100

Injection: 20 µL of test solution (b) and reference solution (d).

Identification of impurities: use the chromatogram obtained with reference solution (d) to identify the peak due to impurity K.

Relative retention with reference to atazanavir (retention time = about 10 min): impurity K = about 0.4.

Calculation of percentage content:

– for impurity K, use the concentration of impurity K in reference solution (d).

Limit:

– *impurity K*: maximum 0.15 per cent.

Water (*2.5.32*): maximum 2.5 per cent, determined on 0.100 g by direct sample introduction.

Sulfated ash (*2.4.14*): maximum 0.2 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Mobile phase: solvent mixture.

Injection: test solution (a) and reference solutions (a) and (c).

Run time: 1.6 times the retention time of atazanavir.

Relative retention with reference to atazanavir (retention time = about 9.5 min): impurity F = about 0.94.

System suitability: reference solution (c):

– *resolution*: minimum 1.5 between the peaks due to impurity F and atazanavir.

Calculate the percentage content of $C_{38}H_{54}N_6O_{11}S$ using the chromatogram obtained with reference solution (a) and taking into account the assigned content of *atazanavir sulfate CRS*.

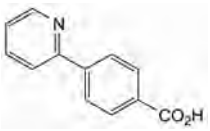
STORAGE

In an airtight container.

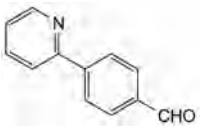
IMPURITIES

Specified impurities: K.

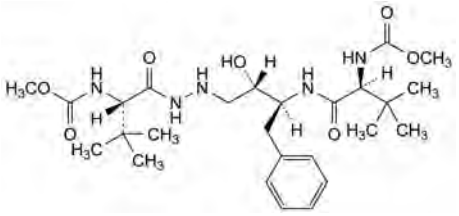
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *A, B, C, D, E, F, G, H, I, J.*



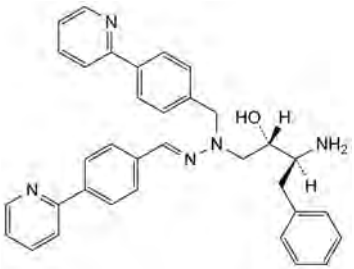
A. 4-(pyridin-2-yl)benzoic acid,



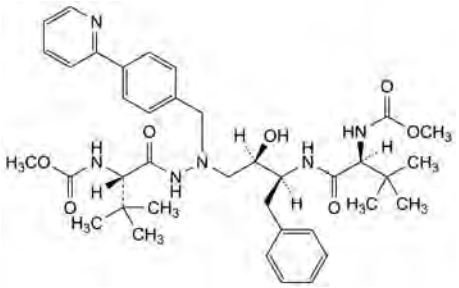
B. 4-(pyridin-2-yl)benzaldehyde,



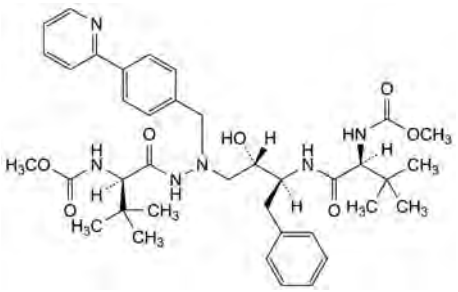
C. methyl [(5*S*,10*S*,11*S*,14*S*)-11-benzyl-5-*tert*-butyl-10-hydroxy-15,15-dimethyl-3,6,13-trioxo-2-oxa-4,7,8,12-tetraazahexadecan-14-yl]carbamate,



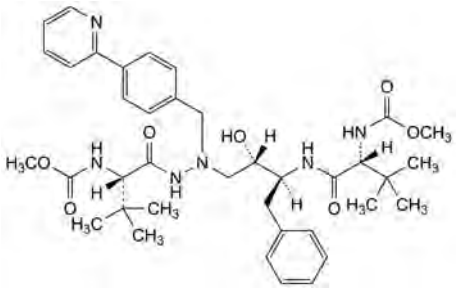
D. (2*S*,3*S*)-3-amino-4-phenyl-1-[(*E*)-1-[[4-(pyridin-2-yl)phenyl]methyl]-2-[[4-(pyridin-2-yl)phenyl]methylidene]hydrazin-1-yl]butan-2-ol,



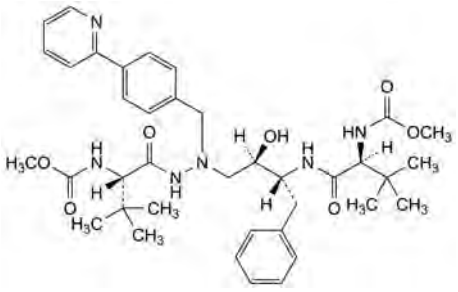
E. methyl [(5*S*,10*R*,11*S*,14*S*)-11-benzyl-5-*tert*-butyl-10-hydroxy-15,15-dimethyl-3,6,13-trioxo-8-[[4-(pyridin-2-yl)phenyl]methyl]-2-oxa-4,7,8,12-tetraazahexadecan-14-yl]carbamate,



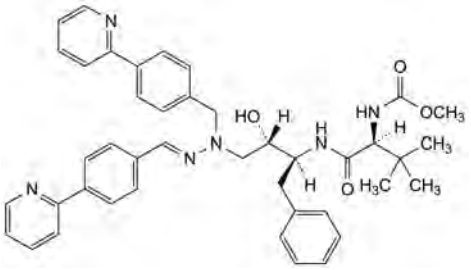
F. methyl [(5*R*,10*S*,11*S*,14*S*)-11-benzyl-5-*tert*-butyl-10-hydroxy-15,15-dimethyl-3,6,13-trioxo-8-[[4-(pyridin-2-yl)phenyl]methyl]-2-oxa-4,7,8,12-tetraazahexadecan-14-yl]carbamate,



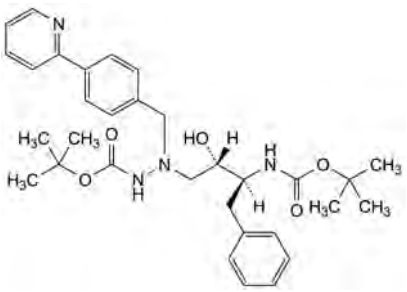
G. methyl [(5*S*,10*S*,11*S*,14*R*)-11-benzyl-5-*tert*-butyl-10-hydroxy-15,15-dimethyl-3,6,13-trioxo-8-[[4-(pyridin-2-yl)phenyl]methyl]-2-oxa-4,7,8,12-tetraazahexadecan-14-yl]carbamate,



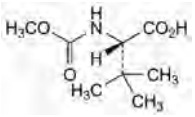
H. methyl [(5*S*,10*R*,11*R*,14*S*)-11-benzyl-5-*tert*-butyl-10-hydroxy-15,15-dimethyl-3,6,13-trioxo-8-[[4-(pyridin-2-yl)phenyl]methyl]-2-oxa-4,7,8,12-tetraazahexadecan-14-yl]carbamate,



I. methyl [(2*S*)-1-[[(2*S*,3*S*)-3-hydroxy-1-phenyl-4-[(*E*)-1-[[4-(pyridin-2-yl)phenyl]methyl]-2-[[4-(pyridin-2-yl)phenyl]methylidene]hydrazin-1-yl]butan-2-yl]amino]-3,3-dimethyl-1-oxobutan-2-yl]carbamate,



J. *tert*-butyl 2-[(2*S*,3*S*)-3-(*tert*-butoxyformamido)-2-hydroxy-4-phenylbutyl]-2-[[4-(pyridin-2-yl)phenyl]methyl]hydrazine-1-carboxylate,



K. (2*S*)-2-(methoxyformamido)-3,3-dimethylbutanoic acid.

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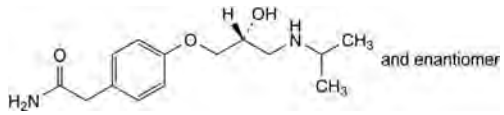
ATENOLOL

Atenololum



$C_{14}H_{22}N_2O_3$ M_r 266.3
[29122-68-7]

DEFINITION

2-[4-[(2*RS*)-2-Hydroxy-3-[(propan-2-yl)amino]propoxy]-phenyl]acetamide.

Content: 99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white powder.

Solubility: sparingly soluble in water, soluble in anhydrous ethanol, slightly soluble in methylene chloride.

IDENTIFICATION

First identification: B.

Second identification: A, C.

A. Melting point (*2.2.14*): 152 °C to 155 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *atenolol CRS.*

C. Thin-layer chromatography (*2.2.27*).
Test solution. Dissolve 10 mg of the substance to be examined in 1.0 mL of *methanol R.*
Reference solution. Dissolve 10 mg of *atenolol CRS* in 1.0 mL of *methanol R.*
Plate: *TLC silanised silica gel F_{254} plate R.*
Mobile phase: *concentrated ammonia R1*, *methanol R* (1:99 *V/V*).
Application: 10 µL.
Development: over 3/4 of the plate.
Drying: in air.
Detection: examine in ultraviolet light at 254 nm.

Monographs A-C

Results: the principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Solution S. Dissolve 0.10 g in *water R* and dilute to 10.0 mL with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and not more intensely coloured than intensity 6 of the range of reference solutions of the most appropriate colour (*2.2.2, Method II*).

Optical rotation (*2.2.7*): − 0.10° to + 0.10°, determined on solution S.

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 50 mg of the substance to be examined in 20 mL of the mobile phase and dilute to 25.0 mL with the mobile phase.

Reference solution (a). Dissolve 2 mg of *atenolol for system suitability CRS* (containing impurities B, F, G, I and J) in 1 mL of the mobile phase.

Reference solution (b). Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Column:

- *size*: *l* = 0.125 m, Ø = 4.0 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase: dissolve 1.0 g of *sodium octanesulfonate R* and 0.4 g of *tetrabutylammonium hydrogen sulfate R* in 1000 mL of a mixture of 20 volumes of *tetrahydrofuran R*, 180 volumes of *methanol R2* and 800 volumes of a 3.4 g/L solution of *potassium dihydrogen phosphate R*; adjust the apparent pH to 3.0 with *phosphoric acid R*.

Flow rate: 0.6 mL/min.

Detection: spectrophotometer at 226 nm.

Injection: 10 µL.

Run time: 5 times the retention time of atenolol.

Identification of impurities: use the chromatogram supplied with *atenolol for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities B, F, G, I and J.

Relative retention with reference to atenolol (retention time = about 8 min): impurity B = about 0.3; impurity J = about 0.7; impurity I = about 0.8; impurity F = about 2.0 (pair of peaks); impurity G = about 3.5.

System suitability: reference solution (a):

- *resolution*: minimum 1.4 between the peaks due to impurities J and I.

Limits:

- *impurity B*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
- *impurities F, G, I, J*: for each impurity, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.15 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
- *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Chlorides (*2.4.4*): maximum 0.1 per cent.

Dissolve 50 mg in a mixture of 1 mL of *dilute nitric acid R* and 15 mL of *water R*. The solution, without further addition of *dilute nitric acid R*, complies with the test.

Loss on drying (*2.2.32*): maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

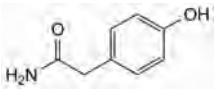
Dissolve 0.200 g in 80 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 26.63 mg of $C_{14}H_{22}N_2O_3$.

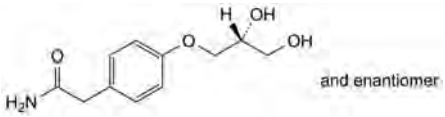
IMPURITIES

Specified impurities: B, F, G, I, J.

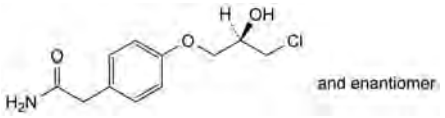
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *A, D, E, H.*



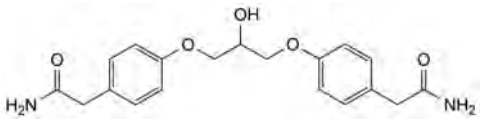
A. 2-(4-hydroxyphenyl)acetamide,



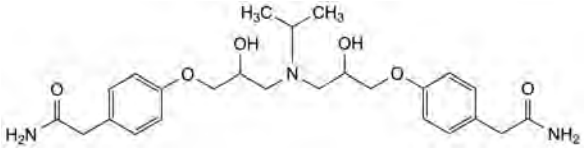
B. 2-[4-[(2*RS*)-2,3-dihydroxypropoxy]phenyl]acetamide,



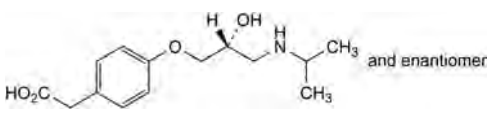
D. 2-[4-[(2*RS*)-3-chloro-2-hydroxypropoxy]phenyl]-acetamide,



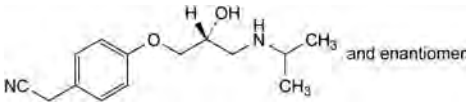
E. 2,2′-[(2-hydroxypropane-1,3-diyl)bis(oxy-4,1-phenylene)]diacetamide,



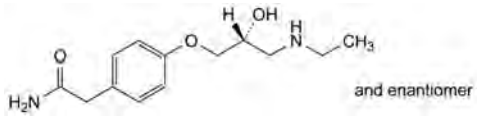
F. 2,2′-[[(propan-2-yl)azanediyl]bis[(2-hydroxypropane-3,1-diyl)oxy-4,1-phenylene]]diacetamide,



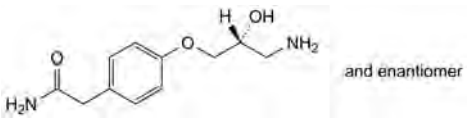
G. [4-[(2*RS*)-2-hydroxy-3-[(propan-2-yl)amino]-propoxy]phenyl]acetic acid,



H. [4-[(2*RS*)-2-hydroxy-3-[(propan-2-yl)amino]-propoxy]phenyl]acetonitrile,



I. 2-[4-[(2*RS*)-3-(ethylamino)-2-hydroxypropoxy]-phenyl]acetamide,



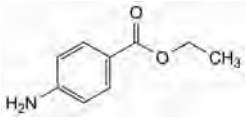
J. 2-[4-[(2*RS*)-3-amino-2-hydroxypropoxy]phenyl]-acetamide.

B

04/2020:0011

BENZOCAINE

Benzocainum



$C_9H_{11}NO_2$ M_r 165.2
[94-09-7]

DEFINITION

Ethyl 4-aminobenzoate.

Content: 99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, crystalline powder or colourless crystals.

Solubility: very slightly soluble in water, freely soluble in ethanol (96 per cent).

It shows polymorphism (*5.9*).

IDENTIFICATION

First identification: *A*.

Second identification: *B*.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *benzocaine CRS*.

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in *anhydrous ethanol R*, evaporate to dryness and record new spectra using the residues.

B. Melting point (*2.2.14*).

Determination A: determine the melting point of the substance to be examined.

Result A: 89 °C to 92 °C.

Determination B: mix equal parts of the substance to be examined and *benzocaine CRS* and determine the melting point of the mixture.

Result B: the absolute difference between the melting point of the mixture and the value obtained in determination A is not greater than 2 °C.

TESTS

Related substances. Liquid chromatography (*2.2.29*).

Solvent mixture: *acetonitrile R1*, *water for chromatography R* (50:50 *V/V*).

Test solution. Dissolve 25.0 mg of the substance to be examined in 5 mL of *acetonitrile R* and dilute to 50.0 mL with the solvent mixture.

Reference solution (a). Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b). Dissolve 5 mg of the substance to be examined and 5 mg of *4-nitrobenzoic acid R* (impurity E) in 10 mL of the solvent mixture. Dilute 1 mL of the solution to 50 mL with the solvent mixture.

Column:

- *size*: *l* = 0.10 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography compatible with 100 per cent aqueous mobile phases R* (3 µm);
- *temperature*: 35 °C.

Mobile phase:

- *mobile phase A*: dilute 1 mL of *perchloric acid R* to 100 mL with *water for chromatography R*; dilute 1 mL of this solution to 100 mL with *water for chromatography R*; mix 9 volumes of this solution and 1 volume of *acetonitrile R1*;
- *mobile phase B*: *acetonitrile R1*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	100	0
2 - 15	100 → 38.5	0 → 61.5

Flow rate: 1.5 mL/min.

Detection: spectrophotometer at 215 nm.

Injection: 10 µL.

Identification of impurities: use the chromatogram obtained with reference solution (b) to identify the peak due to impurity E.

Relative retention with reference to benzocaine (retention time = about 10 min): impurity E = about 0.9.

System suitability: reference solution (b):

- *resolution*: minimum 5.0 between the peaks due to impurity E and benzocaine.

Calculation of percentage contents:

- for each impurity, use the concentration of benzocaine in reference solution (a).

Limits:

- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- *total*: maximum 0.2 per cent;
- *reporting threshold*: 0.05 per cent.

Loss on drying (*2.2.32*): maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo*.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Carry out the determination of primary aromatic amino-nitrogen (*2.5.8*), using 0.400 g dissolved in a mixture of 25 mL of *hydrochloric acid R* and 50 mL of *water R*.

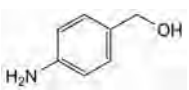
1 mL of *0.1 M sodium nitrite* is equivalent to 16.52 mg of $C_9H_{11}NO_2$.

STORAGE

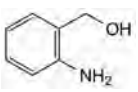
Protected from light.

IMPURITIES

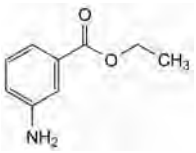
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *A, B, C, D, E, F, G, H*.



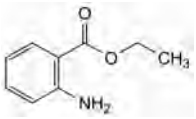
A. (4-aminophenyl)methanol,



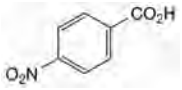
B. (2-aminophenyl)methanol,



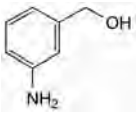
C. ethyl 3-aminobenzoate,



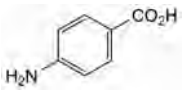
D. ethyl 2-aminobenzoate,



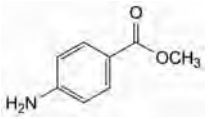
E. 4-nitrobenzoic acid,



F. (3-aminophenyl)methanol,



G. 4-aminobenzoic acid,



H. methyl 4-aminobenzoate.

C

04/2020:1497

CASTOR OIL, HYDROGENATED

Ricini oleum hydrogenatum

DEFINITION

Fatty oil obtained by hydrogenation of *Virgin castor oil (0051)* or *Refined castor oil (2367)* or a mixture of both. It consists mainly of the triglyceride of 12-hydroxystearic ((12*Ξ*)-12-hydroxyoctadecanoic) acid.

CHARACTERS

Appearance: fine, almost white or pale yellow powder or almost white or pale yellow masses or flakes.

Solubility: practically insoluble in water, slightly soluble in methylene chloride, very slightly soluble in anhydrous ethanol, practically insoluble in light petroleum.

IDENTIFICATION

A. Melting point (*2.2.14*): 83 °C to 88 °C.

B. Hydroxyl value (see Tests).

C. Composition of fatty acids (see Tests).

TESTS

Acid value (*2.5.1*): maximum 4.0, determined on 10.0 g dissolved in 75 mL of hot *ethanol (96 per cent) R*.

Hydroxyl value (*2.5.3, Method A*): 145 to 165, determined on a warm solution.

Iodine value (*2.5.4, Method A*): maximum 5.0.

Alkaline impurities. Dissolve 1.0 g with gentle heating in a mixture of 1.5 mL of *ethanol (96 per cent) R* and 3 mL of *toluene R*. Add 0.05 mL of a 0.4 g/L solution of *bromophenol blue R* in *ethanol (96 per cent) R*. Not more than 0.2 mL of *0.01 M hydrochloric acid* is required to change the colour of the indicator to yellow.

Composition of fatty acids. Gas chromatography (*2.4.22, Method A*) with the following modifications. Use the mixture of calibrating substances in Table 2.4.22.-3.

Test solution. Introduce 75 mg of the substance to be examined into a 10 mL centrifuge tube with a screw cap. Dissolve in 2 mL of *1,1-dimethylethyl methyl ether R1* by shaking and heat gently (50-60 °C). Add, when still warm, 1 mL of a 12 g/L solution of *sodium R* in *anhydrous methanol R*, prepared with the necessary precautions, and mix vigorously for at least 5 min. Add 5 mL of *distilled water R* and mix vigorously for about 30 s. Centrifuge for 15 min at 1500 *g*. Use the upper layer.

Reference solution. Dissolve 50 mg of *methyl 12-hydroxystearate CRS* and 50 mg of *methyl stearate CRS* in 10.0 mL of *1,1-dimethylethyl methyl ether R1*.

Column:

- *material*: fused silica;
- *size*: *l* = 30 m; Ø = 0.25 mm;
- *stationary phase*: *macrogol 20 000 R* (film thickness 0.25 µm).

Carrier gas: *helium for chromatography R*.

Flow rate: 0.9 mL/min.

Split ratio: 1:100.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 55	215
Injection port		250
Detector		250

Detection: flame ionisation.

Injection: 1 µL.

System suitability:

- *symmetry factor*: 0.7 to 1.5 for the peak due to methyl stearate in the chromatogram obtained with the test solution.

Calculate the fraction of each fatty acid using the following expression:

$$A_{x,s,c} / \sum A_{x,s,c} \times 100 \text{ per cent } m / m$$

$A_{x,s,c}$ = corrected peak area of the fatty acid in the test solution:

$$A_{x,s,c} = A_{x,s} \times R_c$$

$A_{x,s}$ = area of the peaks due to any specified or unspecified fatty acid methyl esters;

R_c = relative correction factor for the peak due to methyl 12-hydroxystearate:

$$R_c = \frac{m_{1,r} \times A_{2,r}}{A_{1,r} \times m_{2,r}}$$

R_c = 1 for peaks corresponding to each of the other specified fatty acids or any unspecified fatty acid;

$m_{1,r}$ = mass of methyl 12-hydroxystearate in the reference solution;

$m_{2,r}$ = mass of methyl stearate in the reference solution;

$A_{1,r}$ = area of the peak due to methyl 12-hydroxystearate in the chromatogram obtained with the reference solution;

$A_{2,r}$ = area of the peak due to methyl stearate in the chromatogram obtained with the reference solution.

Composition of the fatty-acid fraction of the oil:

- *palmitic acid*: maximum 2.0 per cent;
- *stearic acid*: 7.0 per cent to 14.0 per cent;
- *arachidic acid*: maximum 1.0 per cent;
- *12-oxostearic acid*: maximum 5.0 per cent;
- *12-hydroxystearic acid*: 78.0 per cent to 91.0 per cent;
- *any other fatty acid*: for each fatty acid, maximum 3.0 per cent.

STORAGE

In a well-filled container.

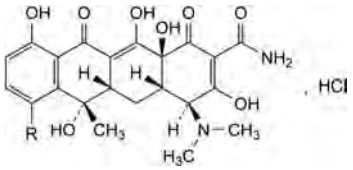
IMPURITIES

A. 12-oxooctadecanoic acid (12-oxostearic acid).

01/2017:0173
corrected 10.1

CHLORTETRACYCLINE HYDROCHLORIDE

Chlortetracyclini hydrochloridum



Compound	R	Molecular formula	M_r
Chlortetracycline hydrochloride	Cl	$C_{22}H_{24}Cl_2N_2O_8$	515.3
Tetracycline hydrochloride	H	$C_{22}H_{25}ClN_2O_8$	480.9

Chlortetracycline hydrochloride: [64-72-2]
Tetracycline hydrochloride: [64-75-5]

DEFINITION

Mixture of antibiotics, the main component being the hydrochloride of (4*S*,4a*S*,5a*S*,6*S*,12a*S*)-7-chloro-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (chlortetracycline hydrochloride), a substance produced by the growth of certain strains of *Streptomyces aureofaciens* or obtained by any other means.

Content:

– *chlortetracycline hydrochloride* ($C_{22}H_{24}Cl_2N_2O_8$): minimum 89.5 per cent (anhydrous substance);
– *tetracycline hydrochloride* ($C_{22}H_{25}ClN_2O_8$): maximum 6.0 per cent (anhydrous substance);
– *sum of the contents of chlortetracycline hydrochloride and tetracycline hydrochloride*: 94.5 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance: yellow powder.

Solubility: slightly soluble in water and in ethanol (96 per cent). It dissolves in solutions of alkali hydroxides and carbonates.

IDENTIFICATION

First identification: C, D.

Second identification: A, B, C.

A. Thin-layer chromatography (*2.2.27*).

Test solution. Dissolve 5 mg of the substance to be examined in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution (a). Dissolve 5 mg of *chlortetracycline hydrochloride CRS* in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution (b). Dissolve 5 mg of *chlortetracycline hydrochloride CRS*, 5 mg of *demeclocycline hydrochloride R* and 5 mg of *doxycycline R* in *methanol R* and dilute to 10 mL with the same solvent.

Plate: *TLC octadecylsilyl silica gel F_{254} plate R.*

Mobile phase: mix 20 volumes of *acetonitrile R*, 20 volumes of *methanol R* and 60 volumes of a 63 g/L solution of *oxalic acid R* previously adjusted to pH 2 with *concentrated ammonia R*.

Application: 1 µL.

Development: over 3/4 of the plate.

Drying: in air.

Detection: examine in ultraviolet light at 254 nm.

System suitability: the chromatogram obtained with reference solution (b) shows 3 clearly separated spots.

Results: the principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

B. To about 2 mg add 5 mL of *sulfuric acid R*. A deep blue colour develops and becomes bluish-green. Add the solution to 2.5 mL of *water R*. The colour becomes brownish.

C. It gives reaction (a) of chlorides (*2.3.1*).

D. Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection: test solution and reference solution (a).

Results: the principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

TESTS

pH (*2.2.3*): 2.3 to 3.3.

Dissolve 0.1 g in 10 mL of *carbon dioxide-free water R*, heating slightly.

Specific optical rotation (*2.2.7*): – 250 to – 235 (anhydrous substance).

Dissolve 0.125 g in *water R* and dilute to 50.0 mL with the same solvent.

Absorbance (*2.2.25*): maximum 0.40 at 460 nm.

Dissolve 0.125 g in *water R* and dilute to 25.0 mL with the same solvent.

Related substances. Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution. Dissolve 25.0 mg of the substance to be examined in mobile phase B and dilute to 25.0 mL with mobile phase B.

Reference solution (a). Dissolve 25.0 mg of *chlortetracycline hydrochloride CRS* in mobile phase B and dilute to 25.0 mL with mobile phase B.

Reference solution (b). Dilute 1.0 mL of the test solution to 100.0 mL with mobile phase B.

Reference solution (c). Dilute 1.0 mL of reference solution (b) to 10.0 mL with mobile phase B.

Reference solution (d). Dissolve 5 mg of *chlortetracycline for system suitability CRS* (containing impurities A, B, D, E, G, H, J, K and L) in mobile phase B and dilute to 5 mL with mobile phase B.

Reference solution (e). Dissolve 25.0 mg of *tetracycline hydrochloride CRS* in mobile phase B and dilute to 25.0 mL with mobile phase B. Dilute 5.0 mL of this solution to 100.0 mL with mobile phase B.

Column:

– *size*: *l* = 0.075 m, Ø = 4.6 mm;
– *stationary phase*: *end-capped octylsilyl silica gel for chromatography with embedded polar groups R* (3.5 µm);
– *temperature*: 45 °C.

Mobile phase:

– *mobile phase A*: to 725 mL of *water for chromatography R* add 50 mL of *perchloric acid solution R*, shake and add 225 mL of *dimethyl sulfoxide R*;
– *mobile phase B*: to 250 mL of *water for chromatography R* add 50 mL of *perchloric acid solution R*, shake and add 700 mL of *dimethyl sulfoxide R*;

Monographs A-C

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 46	100 → 0	0 → 100

Flow rate: 0.4 mL/min.

Detection: spectrophotometer at 280 nm.

Injection: 20 µL of the test solution and reference solutions (b), (c) and (d).

Identification of impurities: use the chromatogram supplied with *chlortetracycline for system suitability CRS* and the chromatogram obtained with reference solution (d) to identify the peaks due to impurities A, B, D, E, G, H, J, K and L.

Relative retention with reference to chlortetracycline (retention time = about 26 min): impurity D = about 0.5; tetracycline = about 0.6; impurity E = about 0.7; impurity B = about 0.8, impurity A = about 0.86; impurity G = about 0.9; impurity H = about 1.1; impurity J = about 1.4, impurity K = about 1.67; impurity L = about 1.71.

System suitability: reference solution (d):

- *resolution*: minimum 1.5 between the peaks due to tetracycline and impurity E; minimum 1.5 between the peaks due to impurities A and G; minimum 1.5 between the peaks due to impurities K and L; if necessary, adjust the concentration of dimethyl sulfoxide in mobile phase A.

Limits:

- *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity G = 1.4; impurity J = 0.3; impurity K = 0.4; impurity L = 0.4;
- *impurity A*: not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (4.0 per cent);
- *impurities B, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);
- *impurity J*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.3 per cent);
- *impurities D, G, H, L*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent);
- *impurity K*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.15 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.10 per cent);
- *sum of impurities other than A*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Water (*2.5.12*): maximum 2.0 per cent, determined on 0.300 g.

Sulfated ash (*2.4.14*): maximum 0.5 per cent, determined on 1.0 g.

Bacterial endotoxins (*2.6.14*): less than 1 IU/mg, if intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection: 10 µL of the test solution and reference solutions (a) and (e).

Calculate the percentage content of $C_{22}H_{24}Cl_2N_2O_8$ using the chromatogram obtained with reference solution (a) and taking into account the assigned content of *chlortetracycline hydrochloride CRS*. Calculate the percentage content of $C_{22}H_{25}ClN_2O_8$ using the chromatogram obtained with reference solution (e) and taking into account the assigned content of *tetracycline hydrochloride CRS*.

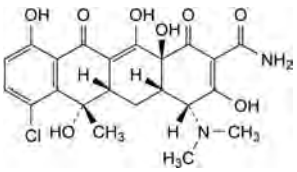
STORAGE

Protected from light. If the substance is sterile, store in a sterile, airtight, tamper-evident container.

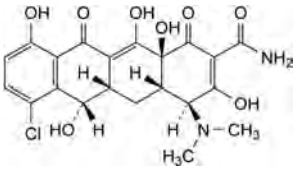
IMPURITIES

Specified impurities: A, B, D, E, G, H, J, K, L.

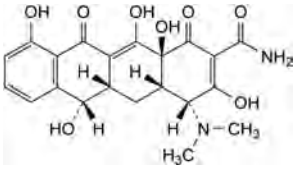
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *C, F, I.*



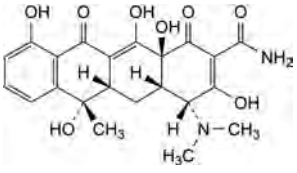
A. (4*R*,4a*S*,5a*S*,6*S*,12a*S*)-7-chloro-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (4-epichlortetracycline),



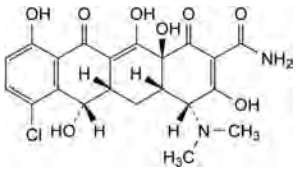
B. (4*S*,4a*S*,5a*S*,6*S*,12a*S*)-7-chloro-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (demeclocycline),



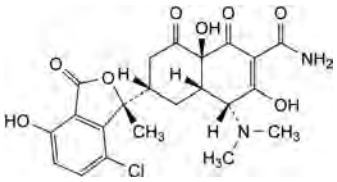
C. (4*R*,4a*S*,5a*S*,6*S*,12a*S*)-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (4-epidemethyltetracycline),



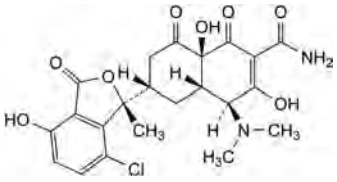
D. (4*R*,4a*S*,5a*S*,6*S*,12a*S*)-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (4-epitetracycline),



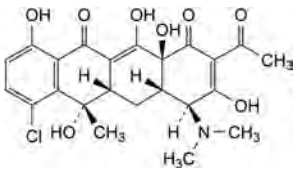
E. (4*R*,4a*S*,5a*S*,6*S*,12a*S*)-7-chloro-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (4-epidemethylchlortetracycline),



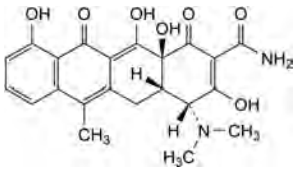
F. (4*R*,4a*S*,6*S*,8a*S*)-6-[(1*R*)-7-chloro-4-hydroxy-1-methyl-3-oxo-1,3-dihydro-2-benzofuran-1-yl]-4-(dimethylamino)-3,8a-dihydroxy-1,8-dioxo-1,4,4a,5,6,7,8,8a-octahydronaphthalene-2-carboxamide (4-epiisochlortetracycline),



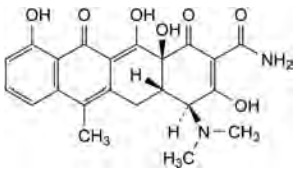
G. (4*S*,4a*S*,6*S*,8a*S*)-6-[(1*R*)-7-chloro-4-hydroxy-1-methyl-3-oxo-1,3-dihydro-2-benzofuran-1-yl]-4-(dimethylamino)-3,8a-dihydroxy-1,8-dioxo-1,4,4a,5,6,7,8,8a-octahydronaphthalene-2-carboxamide (isochlortetracycline),



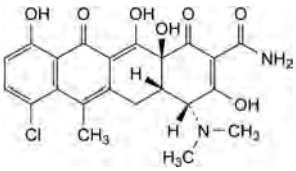
H. (4*S*,4a*S*,5a*S*,6*S*,12a*S*)-2-acetyl-7-chloro-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-4a,5a,6,12a-tetrahydrotetracene-1,11(4*H*,5*H*)-dione (2-acetyl-2-decarboxamidochlortetracycline),



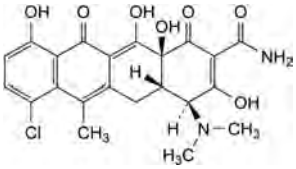
I. (4*R*,4a*S*,12a*S*)-4-(dimethylamino)-3,10,12,12a-tetrahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,11,12a-hexahydrotetracene-2-carboxamide (4-epianhydrotetracycline),



J. (4*S*,4a*S*,12a*S*)-4-(dimethylamino)-3,10,12,12a-tetrahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,11,12a-hexahydrotetracene-2-carboxamide (anhydrotetracycline),



K. (4*R*,4a*S*,12a*S*)-7-chloro-4-(dimethylamino)-3,10,12,12a-tetrahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,11,12a-hexahydrotetracene-2-carboxamide (4-epianhydrochlortetracycline),

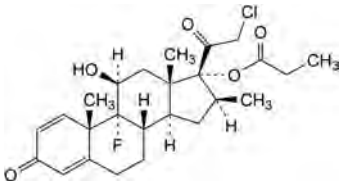


L. (4*S*,4a*S*,12a*S*)-7-chloro-4-(dimethylamino)-3,10,12,12a-tetrahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,11,12a-hexahydrotetracene-2-carboxamide (anhydrochlortetracycline).

04/2020:2127

CLOBETASOL PROPIONATE

Clobetasoli propionas



$C_{25}H_{32}ClFO_5$ M_r 467.0
[25122-46-7]

DEFINITION

21-Chloro-9-fluoro-11β-hydroxy-16β-methyl-3,20-dioxopregna-1,4-dien-17-yl propanoate.

Content: 97.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: practically insoluble in water, freely soluble in acetone, sparingly soluble in ethanol (96 per cent).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *clobetasol propionate CRS*.

TESTS

Specific optical rotation (*2.2.7*): + 112 to + 118 (dried substance).

Dissolve 0.250 g in *acetone R* and dilute to 25.0 mL with the same solvent.

Related substances. Liquid chromatography (*2.2.29*).

Test solution (a). Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 20.0 mL with the mobile phase.

Test solution (b). Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 mL with the mobile phase.

Reference solution (a). Dissolve 20.0 mg of *clobetasol propionate CRS* in the mobile phase and dilute to 100.0 mL with the mobile phase.

Reference solution (b). Dissolve the contents of a vial of *clobetasol impurity J CRS* in 2 mL of the mobile phase. To 0.5 mL of the solution add 0.5 mL of test solution (b) and dilute to 20 mL with the mobile phase.

Reference solution (c). Dissolve the contents of a vial of *clobetasol propionate for peak identification CRS* (containing impurities A, B, D and E) in 2 mL of the mobile phase.

Reference solution (d). Dilute 1.0 mL of test solution (a) to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Column:

- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 30 °C.

Mobile phase: mix 10 volumes of *methanol R1*, 42.5 volumes of a 7.85 g/L solution of *sodium dihydrogen phosphate monohydrate R* adjusted to pH 5.5 with a 100 g/L solution of *sodium hydroxide R* and 47.5 volumes of *acetonitrile for chromatography R*.

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 240 nm.

Injection: 10 µL of test solution (a) and reference solutions (b), (c) and (d).

Run time: 3 times the retention time of clobetasol propionate.

Identification of impurities: use the chromatogram obtained with reference solution (b) to identify the peak due to impurity J; use the chromatogram supplied with *clobetasol propionate for peak identification CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A, B, D and E.

Relative retention with reference to clobetasol propionate (retention time = about 11 min): impurity A = about 0.4; impurity B = about 0.6; impurity J = about 1.1; impurity D = about 1.2; impurity E = about 2.1.

System suitability: reference solution (b):

- *resolution*: minimum 2.0 between the peaks due to clobetasol propionate and impurity J.

Calculation of percentage contents:

- *correction factor*: multiply the peak area of impurity B by 0.6;
- for each impurity, use the concentration of clobetasol propionate in reference solution (d).

Limits:

- *impurities B, E*: for each impurity, maximum 0.3 per cent;
- *impurities A, D*: for each impurity, maximum 0.2 per cent;
- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- *total*: maximum 1.0 per cent;
- *reporting threshold*: 0.05 per cent.

Loss on drying (*2.2.32*): maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection: test solution (b) and reference solution (a).

Calculate the percentage content of $C_{25}H_{32}ClFO_5$ taking into account the assigned content of *clobetasol propionate CRS*.

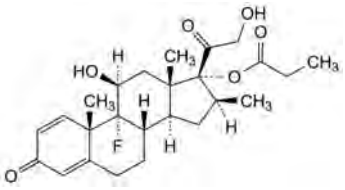
STORAGE

Protected from light.

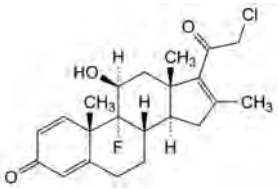
IMPURITIES

Specified impurities: A, B, D, E.

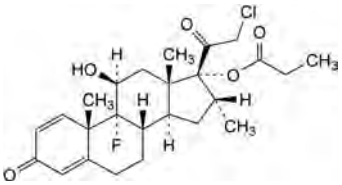
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *C, F, G, H, I, J, K.*



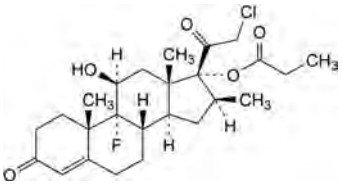
A. 9-fluoro-11β,21-dihydroxy-16β-methyl-3,20-dioxopregna-1,4-dien-17-yl propanoate (betamethasone 17-propionate),



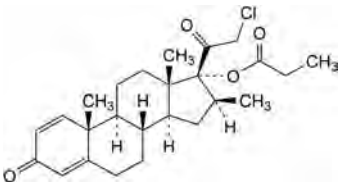
B. 21-chloro-9-fluoro-11β-hydroxy-16-methylpregna-1,4,16-triene-3,20-dione,



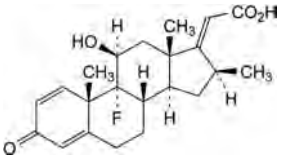
C. 21-chloro-9-fluoro-11β-hydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-17-yl propanoate,



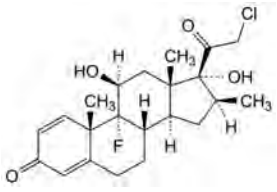
D. 21-chloro-9-fluoro-11β-hydroxy-16β-methyl-3,20-dioxopregn-4-en-17-yl propanoate (1,2-dihydroclobetasol 17-propionate),



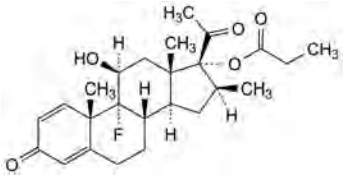
E. 21-chloro-16β-methyl-3,20-dioxopregna-1,4-dien-17-yl propanoate,



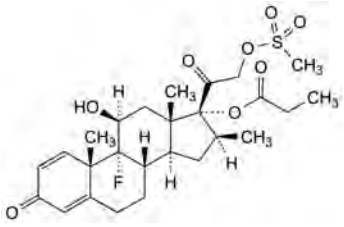
F. 9-fluoro-11β-hydroxy-16β-methyl-3-oxopregna-1,4,17(20)-trien-21-oic acid,



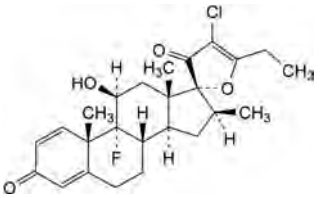
G. 21-chloro-9-fluoro-11β,17-dihydroxy-16β-methylpregna-1,4-diene-3,20-dione (clobetasol),



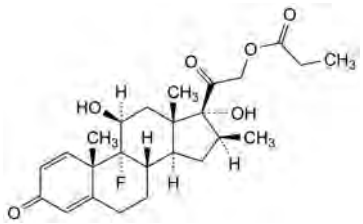
H. 9-fluoro-11β-hydroxy-16β-methyl-3,20-dioxopregna-1,4-dien-17-yl propanoate,



I. 9-fluoro-11β-hydroxy-16β-methyl-21-[(methanesulfonyl)-oxy]-3,20-dioxopregna-1,4-dien-17-yl propanoate,



J. (17*R*)-4′-chloro-5′-ethyl-9-fluoro-11β-hydroxy-16β-methylspiro[androsta-1,4-diene-17,2′-furan]-3,3′-dione (17α-spiro compound),



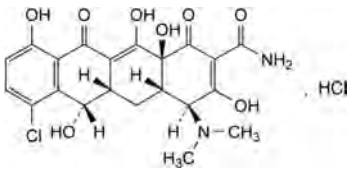
K. 9-fluoro-11β,17-dihydroxy-16β-methyl-3,20-dioxopregna-1,4-dien-21-yl propanoate (betamethasone 21-propionate).

D

01/2017:0176
corrected 10.1

DEMECLOCYCLINE HYDROCHLORIDE

Demeclocyclini hydrochloridum



$C_{21}H_{22}Cl_2N_2O_8$ M_r 501.3
[64-73-3]

DEFINITION

(4*S*,4a*S*,5a*S*,6*S*,12a*S*)-7-Chloro-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide hydrochloride.

Substance produced by certain strains of *Streptomyces aureofaciens*.

Content: 89.5 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance: yellow powder.

Solubility: soluble or sparingly soluble in water, slightly soluble in alcohol, very slightly soluble in acetone. It dissolves in solutions of alkali hydroxides and carbonates.

IDENTIFICATION

A. Thin-layer chromatography (*2.2.27*).

Test solution. Dissolve 5 mg of the substance to be examined in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution (a). Dissolve 5 mg of *demeclocycline hydrochloride CRS* in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution (b). Dissolve 5 mg of *demeclocycline hydrochloride CRS*, 5 mg of *chlortetracycline hydrochloride R* and 5 mg of *tetracycline hydrochloride R* in *methanol R* and dilute to 10 mL with the same solvent.

Plate: *TLC octadecylsilyl silica gel F_{254} plate R*.

Mobile phase: mix 20 volumes of *acetonitrile R*, 20 volumes of *methanol R* and 60 volumes of a 63 g/L solution of *oxalic acid R* previously adjusted to pH 2 with *concentrated ammonia R*.

Application: 1 µL.

Development: over 3/4 of the plate.

Drying: in air.

Detection: examine in ultraviolet light at 254 nm.

System suitability: the chromatogram obtained with reference solution (b) shows 3 clearly separated spots.

Results: the principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

B. To about 2 mg add 5 mL of *sulfuric acid R*. A violet colour develops. Add the solution to 2.5 mL of *water R*. The colour becomes yellow.

C. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

pH (*2.2.3*): 2.0 to 3.0.

Dissolve 0.1 g in *carbon dioxide-free water R* and dilute to 10 mL with the same solvent.

Related substances. Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Buffer 1: 22.2 g/L solution of *sodium edetate R* adjusted to pH 7.5 with a 40 g/L solution of *sodium hydroxide R*.

Buffer 2: 17.0 g/L solution of *tetrapropylammonium hydrogen sulfate R* adjusted to pH 7.5 with a 40 g/L solution of *sodium hydroxide R*.

Test solution. Dissolve 25.0 mg of the substance to be examined in a 1.0 g/L solution of *hydrochloric acid R* and dilute to 50.0 mL with the same solution.

Reference solution (a). Dissolve 25.0 mg of *demeclocycline hydrochloride CRS* in a 1.0 g/L solution of *hydrochloric acid R* and dilute to 50.0 mL with the same solution.

Reference solution (b). Dilute 1.0 mL of the test solution to 100.0 mL with a 1.0 g/L solution of *hydrochloric acid R*.

Reference solution (c). Dissolve 5 mg of *demeclocycline for system suitability CRS* (containing impurities A, B, C, E and G) in a 1.0 g/L solution of *hydrochloric acid R* and dilute to 10 mL with the same solution.

Column:

- *size*: *l* = 0.075 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octylsilyl silica gel for chromatography with embedded polar groups R* (3.5 µm);
- *temperature*: 40 °C.

Mobile phase:

- *mobile phase A*: *acetonitrile R*, *water for chromatography R*, buffer 1, buffer 2 (2:28:35:35 *V/V/V/V*);
- *mobile phase B*: *acetonitrile R*, buffer 1, buffer 2 (30:35:35 *V/V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	83	17
5 - 15	83 → 30	17 → 70
15 - 25	30	70

Flow rate: 1 mL/min.

Detection: spectrophotometer at 280 nm.

Injection: 10 µL of the test solution and reference solutions (b) and (c).

Identification of impurities: use the chromatogram supplied with *demeclocycline for system suitability CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A, B, C, E and G.

Relative retention with reference to demeclocycline (retention time = about 14 min): impurity C = about 0.3; impurity B = about 0.7; impurity A = about 0.8; impurity E = about 1.2; impurity G = about 1.6.

System suitability: reference solution (c):

- *resolution*: minimum 2.5 between the peaks due to impurities A and B.

Calculation of percentage contents:

- for each impurity, use the concentration of demeclocycline hydrochloride in reference solution (b).

Limits:

- *impurities A, B*: for each impurity, maximum 5.0 per cent;
- *impurities C, G*: for each impurity, maximum 0.3 per cent;
- *impurity E*: maximum 0.2 per cent;
- *any other impurity*: for each impurity, maximum 0.15 per cent;
- *total*: maximum 10.0 per cent;
- *reporting threshold*: 0.05 per cent.

Water (*2.5.12*): maximum 3.0 per cent, determined on 1.000 g.

Sulfated ash (*2.4.14*): maximum 0.5 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection: 10 µL of the test solution and reference solution (a).

Calculate the percentage content of $C_{21}H_{22}Cl_2N_2O_8$ using the chromatogram obtained with reference solution (a) and taking into account the assigned content of *demeclocycline hydrochloride CRS*.

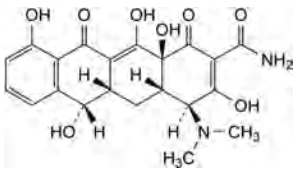
STORAGE

Protected from light.

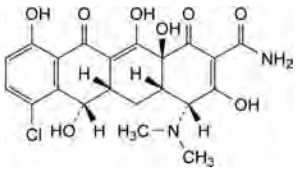
IMPURITIES

Specified impurities: A, B, C, E, G.

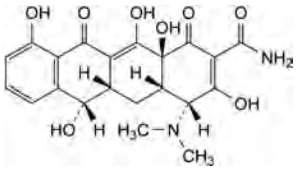
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *D, F.*



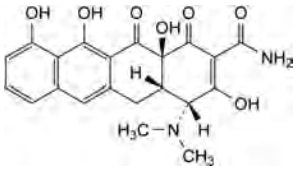
A. (4*S*,4a*S*,5a*S*,6*S*,12a*S*)-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (demethyltetracycline),



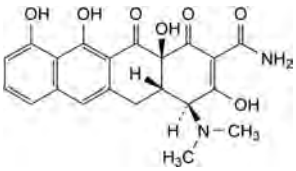
B. (4*R*,4a*S*,5a*S*,6*S*,12a*S*)-7-chloro-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (4-epidemeclocycline),



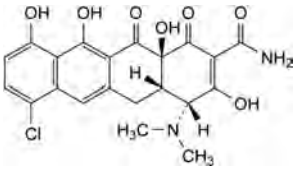
C. (4*R*,4a*S*,5a*S*,6*S*,12a*S*)-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (4-epidemethyltetracycline),



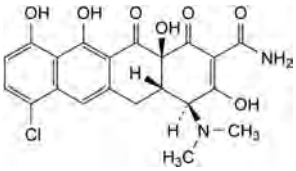
D. (4*R*,4a*S*,12a*S*)-4-(dimethylamino)-3,10,11,12a-tetrahydroxy-1,12-dioxo-1,4,4a,5,12,12a-hexahydrotetracene-2-carboxamide (4-epianhydrodemethyltetracycline),



E. (4*S*,4a*S*,12a*S*)-4-(dimethylamino)-3,10,11,12a-tetrahydroxy-1,12-dioxo-1,4,4a,5,12,12a-hexahydrotetracene-2-carboxamide (anhydrodemethyltetracycline),



F. (4*R*,4a*S*,12a*S*)-7-chloro-4-(dimethylamino)-3,10,11,12a-tetrahydroxy-1,12-dioxo-1,4,4a,5,12,12a-hexahydrotetracene-2-carboxamide (4-epianhydrodemeclocycline),

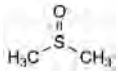


G. (4*S*,4a*S*,12a*S*)-7-chloro-4-(dimethylamino)-3,10,11,12a-tetrahydroxy-1,12-dioxo-1,4,4a,5,12,12a-hexahydrotetracene-2-carboxamide (anhydrodemeclocycline).

04/2020:0763

DIMETHYL SULFOXIDE

Dimethylis sulfoxidum



C_2H_6OS M_r 78.1
[67-68-5]

DEFINITION

(Methanesulfinyl)methane.

CHARACTERS

Appearance: colourless liquid or colourless crystals, hygroscopic.

Solubility: miscible with water and with ethanol (96 per cent).

IDENTIFICATION

First identification: C.

Second identification: A, B, D.

A. Relative density (see Tests).

B. Refractive index (see Tests).

C. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *dimethyl sulfoxide CRS*.

D. Dissolve 50 mg of *nickel chloride R* in 5 mL of the substance to be examined. The solution is greenish-yellow. Heat in a water-bath at 50 °C. The colour changes to green or bluish-green. Cool. The colour changes to greenish-yellow.

TESTS

Acidity. Dissolve 50.0 g in 100 mL of *carbon dioxide-free water R*. Add 0.1 mL of *phenolphthalein solution R1*. Not more than 5.0 mL of *0.01 M sodium hydroxide* is required to produce a pink colour.

Relative density (*2.2.5*): 1.100 to 1.104.

Refractive index (*2.2.6*): 1.478 to 1.480.

Freezing point (*2.2.18*): minimum 18.3 °C.

Absorbance (*2.2.25*). Purge with *nitrogen R* for 15 min. The absorbance, measured using *water R* as the compensation liquid, is not more than 0.30 at 275 nm and not more than 0.20 at both 285 nm and 295 nm. The substance to be examined shows no absorption maximum between 270 nm and 350 nm.

Related substances. Gas chromatography (*2.2.28*): use the normalisation procedure.

Test solution. The substance to be examined.

Reference solution (a). Dissolve 50 mg of the substance to be examined and 50 mg of *dimethyl sulfone R* (impurity A) in *methylene chloride R* and dilute to 100 mL with the same solvent.

Reference solution (b). Dilute 1.0 mL of the test solution to 10.0 mL with *methylene chloride R*. Dilute 0.5 mL of this solution to 100.0 mL with *methylene chloride R*.

Column:

- *material*: fused silica;
- *size*: *l* = 30 m, Ø = 0.25 mm;
- *stationary phase*: *methylpolysiloxane R* (5 µm).

Carrier gas: *helium for chromatography R*.

Flow rate: 1.5 mL/min.

Split ratio: 1:100.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 6	70
	6 - 24	70 → 250
Injection port		280
Detector		280

Detection: flame ionisation.

Injection: 0.4 µL.

Relative retention with reference to dimethyl sulfoxide (retention time = about 5.4 min): impurity A = about 1.2.

System suitability: reference solution (a):

- *resolution*: minimum 5.0 between the peaks due to dimethyl sulfoxide and impurity A.

Limits:

- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- *total*: maximum 0.15 per cent;
- *reporting threshold*: 0.05 per cent (reference solution (b)).

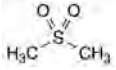
Water (*2.5.32*): maximum 0.2 per cent, determined on 1.00 g.

STORAGE

In an airtight, glass container, protected from light.

IMPURITIES

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): A.

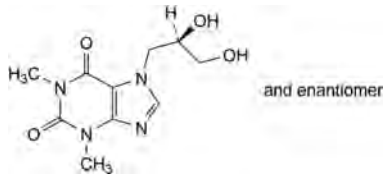


A. (methanesulfonyl)methane (dimethyl sulfone).

01/2017:0486
corrected 10.1

DIPROPHYLLINE

Diprophyllinum



$C_{10}H_{14}N_4O_4$ M_r 254.2
[479-18-5]

DEFINITION

7-[(2*RS*)-2,3-Dihydroxypropyl]-1,3-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione.

Content: 98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: freely soluble in water, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *diprophylline CRS*.

TESTS

Solution S. Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 50 mL with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity. To 10 mL of solution S add 0.25 mL of *bromothymol blue solution R1*. The solution is yellow or green. Not more than 0.4 mL of *0.01 M sodium hydroxide* is required to change the colour of the indicator to blue.

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 50 mg of the substance to be examined in *water R* and dilute to 50.0 mL with the same solvent.

Reference solution (a). Dilute 1.0 mL of the test solution to 100.0 mL with *water R*. Dilute 1.0 mL of this solution to 10.0 mL with *water R*.

Reference solution (b). Dissolve 5 mg of *etofylline CRS* (impurity C) in *water R* and dilute to 50 mL with the same solvent. Dilute 0.5 mL of the solution to 20 mL with the test solution.

Column:

- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography with embedded polar groups R* (3 µm);
- *temperature*: 30 °C.

Mobile phase: *methanol R*, *water for chromatography R* (10:90 *V/V*).

Flow rate: 0.7 mL/min.

Detection: spectrophotometer at 272 nm.

Injection: 10 µL.

Run time: 3 times the retention time of diprophylline.

Relative retention with reference to diprophylline (retention time = about 18 min): impurity C = about 1.1.

System suitability: reference solution (b):

– *peak-to-valley ratio*: minimum 5, where H_p = height above the baseline of the peak due to impurity C and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to diprophylline.

Limits:

– *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
– *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);
– *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Chlorides (*2.4.4*): maximum 400 ppm.

Dilute 2.5 mL of solution S to 15 mL with *water R*.

Loss on drying (*2.2.32*): maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

In order to avoid overheating in the reaction medium, mix thoroughly throughout and stop the titration immediately after the end-point has been reached.

Dissolve 0.200 g in 3.0 mL of *anhydrous formic acid R* and add 50.0 mL of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

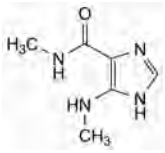
1 mL of *0.1 M perchloric acid* is equivalent to 25.42 mg of $C_{10}H_{14}N_4O_4$.

STORAGE

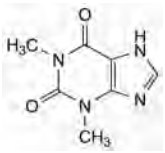
Protected from light.

IMPURITIES

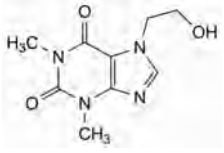
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *A, B, C, D.*



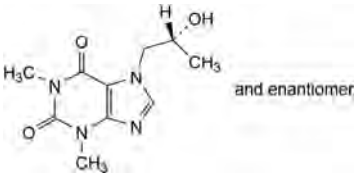
A. *N*-methyl-5-(methylamino)-1*H*-imidazole-4-carboxamide (theophyllidine),



B. 1,3-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione (theophylline),



C. 7-(2-hydroxyethyl)-1,3-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione (etofylline),

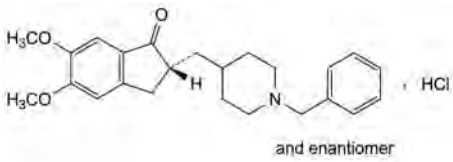


D. 7-[(2*RS*)-2-hydroxypropyl]-1,3-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione (proxyphylline).

04/2020:2582

DONEPEZIL HYDROCHLORIDE

Donepezili hydrochloridum



$C_{24}H_{30}ClNO_3$ M_r 416.0
[120011-70-3]

DEFINITION

(2*RS*)-2-[(1-Benzylpiperidin-4-yl)methyl]-5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-one hydrochloride.

Content: 98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: very slightly soluble in water, very soluble in methylene chloride, very slightly soluble in ethanol (96 per cent).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *donepezil hydrochloride CRS*.

B. Water (see Tests).

C. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Related substances. Liquid chromatography (*2.2.29*).

Solvent mixture: mobile phase B, mobile phase A (20:80 *V/V*).

Test solution (a). Dissolve 25.0 mg of the substance to be examined in the solvent mixture and dilute to 25.0 mL with the solvent mixture.

Test solution (b). Dilute 5.0 mL of test solution (a) to 10.0 mL with the solvent mixture.

Reference solution (a). Dilute 1.0 mL of test solution (a) to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b). Dissolve 15.0 mg of *donepezil hydrochloride CRS* in the solvent mixture and dilute to 30.0 mL with the solvent mixture.

Reference solution (c). Dissolve 5 mg of *donepezil for system suitability CRS* (containing impurities C, D and E) in the solvent mixture and dilute to 5 mL with the solvent mixture.

Column:

– *size*: *l* = 0.15 m, Ø = 4.6 mm;
– *stationary phase*: *end-capped phenylhexylsilyl silica gel for chromatography R* (3 µm).

Mobile phase:
- *mobile phase A*: add 15 mL of *triethylamine R* to about 900 mL of *water for chromatography R*, adjust to pH 6.8 with *phosphoric acid R* and dilute to 1000 mL with *water for chromatography R*;
- *mobile phase B*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	80	20
5 - 10	80 → 60	20 → 40
10 - 35	60	40

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 270 nm.

Injection: 100 µL of test solution (a) and reference solutions (a) and (c).

Identification of impurities: use the chromatogram supplied with *donepezil for system suitability CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities C + E, and impurity D.

Relative retention with reference to donepezil (retention time = about 15 min): impurities C and E = about 0.78; impurity D = about 0.83.

System suitability:
- *resolution*: minimum 2.0 between the peaks due to impurities C + E, and impurity D in the chromatogram obtained with reference solution (c);
- *signal-to-noise ratio*: minimum 30 for the principal peak in the chromatogram obtained with reference solution (a).

Calculation of percentage contents:
- for each impurity, use the concentration of donepezil hydrochloride in reference solution (a).

Limits:
- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- *total*: maximum 0.3 per cent;
- *reporting threshold*: 0.05 per cent.

Water (*2.5.12*): maximum 0.4 per cent, determined on 1.00 g.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

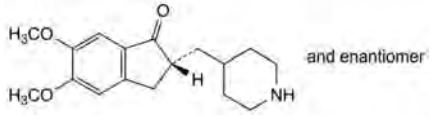
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection: 20 µL of test solution (b) and reference solution (b).

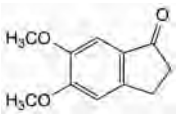
Calculate the percentage content of $C_{24}H_{30}ClNO_3$ taking into account the assigned content of *donepezil hydrochloride CRS*.

IMPURITIES

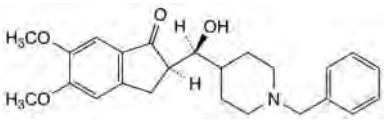
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *A, B, C, D, E, F, G.*



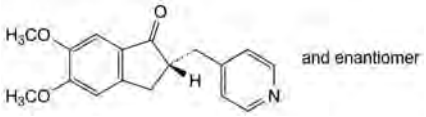
A. (2*RS*)-5,6-dimethoxy-2-[(piperidin-4-yl)methyl]-2,3-dihydro-1*H*-inden-1-one,



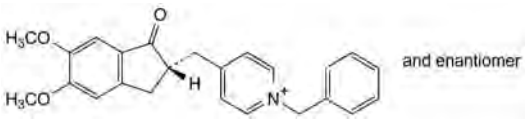
B. 5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-one,



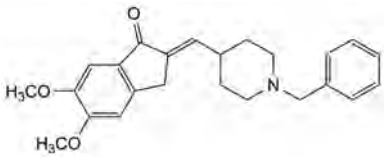
C. (2*R*)-2-[(*S*)-(1-benzylpiperidin-4-yl)(hydroxy)methyl]-5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-one,



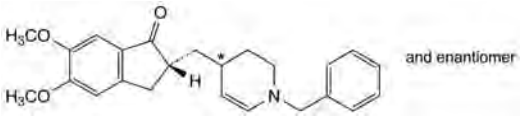
D. (2*RS*)-5,6-dimethoxy-2-[(pyridin-4-yl)methyl]-2,3-dihydro-1*H*-inden-1-one,



E. 1-benzyl-4-[[(2*RS*)-5,6-dimethoxy-1-oxo-2,3-dihydro-1*H*-inden-2-yl]methyl]pyridin-1-ium,



F. (2*E*)-2-[(1-benzylpiperidin-4-yl)methylidene]-5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-one,

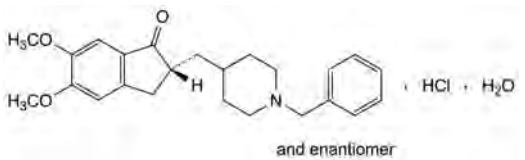


G. (2*RS*)-2-[(1-benzyl-1,2,3,4-tetrahydropyridin-4-yl)methyl]-5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-one.

04/2020:3067

DONEPEZIL HYDROCHLORIDE MONOHYDRATE

Donepezili hydrochloridum monohydricum



$C_{24}H_{30}ClNO_3,H_2O$ M_r 434.0
[884740-09-4]

DEFINITION

(2*RS*)-2-[(1-Benzylpiperidin-4-yl)methyl]-5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-one hydrochloride monohydrate.

Content: 98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: soluble in water, freely soluble in methylene chloride, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *donepezil hydrochloride monohydrate CRS*.

B. Water (see Tests).

C. It gives the reaction (a) of chlorides (*2.3.1*).

TESTS

Related substances. Liquid chromatography (*2.2.29*).

Solvent mixture: mobile phase B, mobile phase A (20:80 *V/V*).

Test solution (a). Dissolve 25.0 mg of the substance to be examined in the solvent mixture and dilute to 25.0 mL with the solvent mixture.

Test solution (b). Dilute 5.0 mL of test solution (a) to 10.0 mL with the solvent mixture.

Reference solution (a). Dilute 1.0 mL of test solution (a) to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b). Dissolve 15.0 mg of *donepezil hydrochloride CRS* in the solvent mixture and dilute to 30.0 mL with the solvent mixture.

Reference solution (c). Dissolve 5 mg of *donepezil for system suitability CRS* (containing impurities C, D and E) in the solvent mixture and dilute to 5 mL with the solvent mixture.

Column:
- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped phenylhexylsilyl silica gel for chromatography R* (3 µm).

Mobile phase:
- *mobile phase A*: add 15 mL of *triethylamine R* to about 900 mL of *water for chromatography R*, adjust to pH 6.8 with *phosphoric acid R* and dilute to 1000 mL with *water for chromatography R*;
- *mobile phase B*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	80	20
5 - 10	80 → 60	20 → 40
10 - 35	60	40

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 270 nm.

Injection: 100 µL of test solution (a) and reference solutions (a) and (c).

Identification of impurities: use the chromatogram supplied with *donepezil for system suitability CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities C + E, and impurity D.

Relative retention with reference to donepezil (retention time = about 15 min): impurities C and E = about 0.78; impurity D = about 0.83.

System suitability:
- *resolution*: minimum 2.0 between the peaks due to impurities C + E, and impurity D in the chromatogram obtained with reference solution (c);
- *signal-to-noise ratio*: minimum 30 for the principal peak in the chromatogram obtained with reference solution (a).

Calculation of percentage contents:
- for each impurity, use the concentration of donepezil hydrochloride monohydrate in reference solution (a).

Limits:
- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- *total*: maximum 0.3 per cent;
- *reporting threshold*: 0.05 per cent.

Water (*2.5.12*): 4.0 per cent to 6.0 per cent, determined on 0.200 g.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

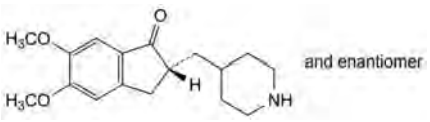
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection: 20 µL of test solution (b) and reference solution (b).

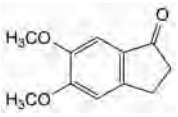
Calculate the percentage content of $C_{24}H_{30}ClNO_3$ taking into account the assigned content of *donepezil hydrochloride CRS*.

IMPURITIES

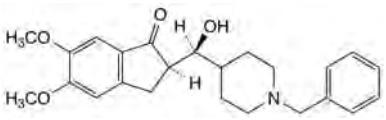
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *A, B, C, D, E, F, G.*



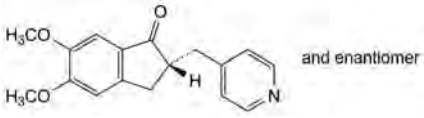
A. (2*RS*)-5,6-dimethoxy-2-[(piperidin-4-yl)methyl]-2,3-dihydro-1*H*-inden-1-one,



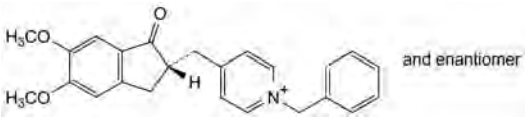
B. 5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-one,



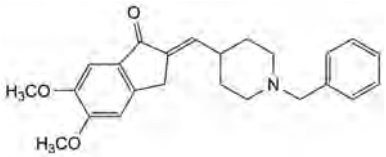
C. (2*R*)-2-[(*S*)-(1-benzylpiperidin-4-yl)(hydroxy)methyl]-5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-one,



D. (2*RS*)-5,6-dimethoxy-2-[(pyridin-4-yl)methyl]-2,3-dihydro-1*H*-inden-1-one,

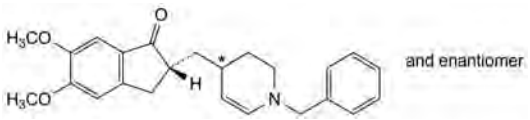


E. 1-benzyl-4-[[(2*RS*)-5,6-dimethoxy-1-oxo-2,3-dihydro-1*H*-inden-2-yl]methyl]pyridin-1-ium,



F. (2*E*)-2-[(1-benzylpiperidin-4-yl)methylidene]-5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-one,

Monographs D-K



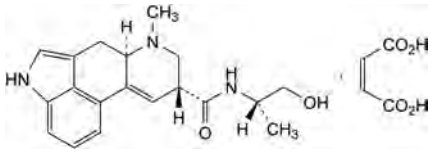
G. (2*RS*)-2-[(1-benzyl-1,2,3,4-tetrahydropyridin-4-yl)methyl]-5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-one.

E

04/2020:0223

ERGOMETRINE MALEATE

Ergometrini maleas



$C_{23}H_{27}N_3O_6$ M_r 441.5
[129-51-1]

DEFINITION

(8β)-*N*-[(2*S*)-1-Hydroxypropan-2-yl]-6-methyl-9,10-didehydroergoline-8-carboxamide (2*Z*)-but-2-enedioate.

Content: 98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white or slightly coloured, crystalline powder.

Solubility: sparingly soluble in water, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification: A.

Second identification: B.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *ergometrine maleate CRS*.

B. Thin-layer chromatography (*2.2.27*). *Prepare the solutions immediately before use.*

Solvent mixture: *concentrated ammonia R*, *ethanol (80 per cent V/V) R* (10:90 *V/V*).

Test solution. Dissolve 10 mg of the substance to be examined in the solvent mixture and dilute to 10 mL with the solvent mixture.

Reference solution. Dissolve 10 mg of *ergometrine maleate CRS* in the solvent mixture and dilute to 10 mL with the solvent mixture.

Plate: *TLC silica gel G plate R*.

Mobile phase: *water R*, *methanol R*, *methylene chloride R* (3:25:75 *V/V/V*).

Application: 5 µL.

Development: over 2/3 of the plate.

Drying: in a current of cold air.

Detection: spray with *dimethylaminobenzaldehyde solution R7* and dry in a current of warm air for about 2 min.

Results: the principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Solution S. Dissolve 0.100 g, without heating and protected from light, in 9 mL of *carbon dioxide-free water R* and dilute to 10.0 mL with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_5 or BY_5 (*2.2.2, Method II*).

pH (*2.2.3*): 3.6 to 4.4 for solution S.

Specific optical rotation (*2.2.7*): + 50 to + 56 (dried substance), determined on solution S.

Related substances. Liquid chromatography (*2.2.29*). *Carry out the test protected from light.*

Solvent mixture: *acetonitrile R*, *water R* (15:85 *V/V*).

Test solution. Dissolve 10.0 mg of the substance to be examined in 10 mL of the solvent mixture and dilute to 50.0 mL with the solvent mixture.

Reference solution (a). Dilute 1.0 mL of the test solution to 100.0 mL with *water R*. Dilute 1.0 mL of this solution to 10.0 mL with *water R*.

Reference solution (b). Dissolve the contents of a vial of *ergometrine impurity mixture CRS* (impurities D, E, F and I) in 1 mL of a mixture of 30 volumes of mobile phase B and 70 volumes of *water R*.

Column:

- *size*: *l* = 0.10 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography with extended pH range R* (3.5 µm);
- *temperature*: 30 °C.

Mobile phase:

- *mobile phase A*: 2 g/L solution of *ammonium carbamate R*;
- *mobile phase B*: *acetonitrile R*, *water for chromatography R* (50:50 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	85	15
5 - 10	85 → 65	15 → 35
10 - 15	65	35
15 - 20	65 → 20	35 → 80
20 - 25	20	80

Flow rate: 2.0 mL/min.

Detection: spectrophotometer at 310 nm.

Injection: 20 µL.

Identification of impurities: use the chromatogram supplied with *ergometrine impurity mixture CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities D, E, F and I.

Relative retention with reference to ergometrine (retention time = about 11.8 min): impurity D = about 1.3; impurity I = about 1.35; impurity E = about 1.43; impurity F = about 1.55.

System suitability: reference solution (b):

- *resolution*: minimum 2.0 between the peaks due to impurities D and I; minimum 1.5 between the peaks due to impurities I and E.

Calculation of percentage contents:

- for each impurity, use the concentration of ergometrine maleate in reference solution (a).

Limits:

- *impurity F*: maximum 0.15 per cent;
- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- *total*: maximum 0.3 per cent;
- *reporting threshold*: maximum 0.05 per cent.

Loss on drying (*2.2.32*): maximum 2.0 per cent, determined on 0.200 g by drying *in vacuo* at 80 °C for 2 h.

ASSAY

Dissolve 0.150 g in 40 mL of *anhydrous acetic acid R*. Titrate with *0.05 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.05 M perchloric acid* is equivalent to 22.07 mg of $C_{23}H_{27}N_3O_6$.

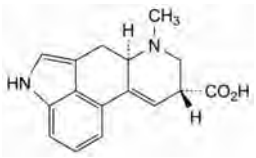
STORAGE

In an airtight, glass container, protected from light, at a temperature of 2 °C to 8 °C.

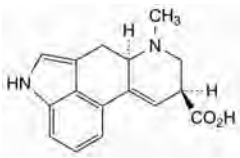
IMPURITIES

Specified impurities: F.

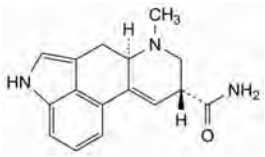
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *A, B, C, D, E, G, H, I.*



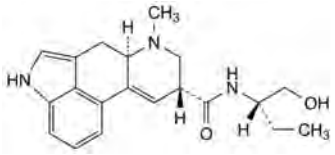
A. (8β)-6-methyl-9,10-didehydroergoline-8-carboxylic acid (lysergic acid),



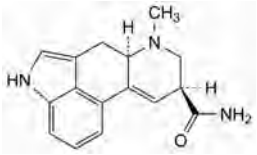
B. (8α)-6-methyl-9,10-didehydroergoline-8-carboxylic acid (isolysergic acid),



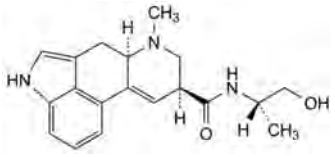
C. (8β)-6-methyl-9,10-didehydroergoline-8-carboxamide (ergine),



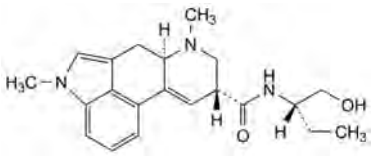
D. (8β)-*N*-[(2*S*)-1-hydroxybutan-2-yl]-6-methyl-9,10-didehydroergoline-8-carboxamide (methylergometrine),



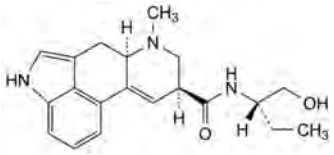
E. (8α)-6-methyl-9,10-didehydroergoline-8-carboxamide (erginine),



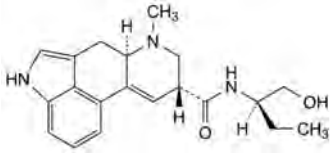
F. (8α)-*N*-[(2*S*)-1-hydroxypropan-2-yl]-6-methyl-9,10-didehydroergoline-8-carboxamide (ergometrinine),



G. (8β)-*N*-[(2*S*)-1-hydroxybutan-2-yl]-1,6-dimethyl-9,10-didehydroergoline-8-carboxamide (methysergide),



H. (8α)-*N*-[(2*S*)-1-hydroxybutan-2-yl]-6-methyl-9,10-didehydroergoline-8-carboxamide (methylergometrinine),

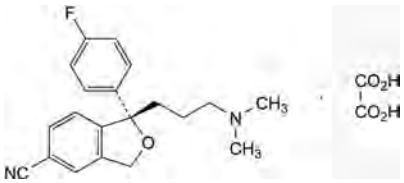


I. (8β)-*N*-[(2*R*)-1-hydroxybutan-2-yl]-6-methyl-9,10-didehydroergoline-8-carboxamide (2′-*epi*-methylergometrine).

07/2016:2733
corrected 10.1

ESCITALOPRAM OXALATE

Escitaloprami oxalas



$C_{22}H_{23}FN_2O_5$ M_r 414.4
[219861-08-2]

DEFINITION

(1*S*)-1-[3-(Dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydro-2-benzofuran-5-carbonitrile hydrogen oxalate.

Content: 99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: sparingly soluble in water, freely soluble in methanol, slightly soluble in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *escitalopram oxalate CRS*.

B. Enantiomeric purity (see Tests).

TESTS

Related substances. Liquid chromatography (*2.2.29*).

Buffer solution. Dissolve 3.4 g of *potassium dihydrogen phosphate R* in 900 mL of *water for chromatography R*, adjust to pH 3.0 with *phosphoric acid R* and dilute to 1000 mL with *water for chromatography R*.

Test solution. Dissolve 25 mg of the substance to be examined in mobile phase A and dilute to 50.0 mL with mobile phase A.

Reference solution (a). Dissolve 5 mg of *escitalopram for system suitability CRS* (containing impurity D) in mobile phase A and dilute to 10.0 mL with mobile phase A.

Reference solution (b). Dilute 1.0 mL of the test solution to 100.0 mL with mobile phase A. Dilute 1.0 mL of this solution to 10.0 mL with mobile phase A.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 45 °C.

Mobile phase:

- *mobile phase A*: *acetonitrile R*, buffer solution (10:90 *V/V*);
- *mobile phase B*: buffer solution, *acetonitrile R* (35:65 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)	Flow rate (mL/min)
0 - 2	95	5	1.0
2 - 37	95 → 65	5 → 35	1.0
37 - 47	65 → 0	35 → 100	1.0
47 - 62	0	100	2.0

Detection: spectrophotometer at 237 nm and at 254 nm.

Injection: 20 µL.

Identification of impurities: use the chromatogram supplied with *escitalopram for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peak due to impurity D.

Relative retention with reference to escitalopram (retention time = about 38 min): impurity D = about 0.98.

System suitability: reference solution (a) at 237 nm:

- *peak-to-valley ratio*: minimum 5.0, where H_p = height above the baseline of the peak due to impurity D and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to escitalopram.

Calculation of percentage contents:

- for each impurity, use the concentration of escitalopram oxalate in reference solution (b) at 237 nm.

Limits:

- *unspecified impurities at 237 nm and at 254 nm*: for each impurity, maximum 0.10 per cent;
- *total at 237 nm*: maximum 0.5 per cent;
- *reporting threshold at 237 nm*: 0.05 per cent.

Enantiomeric purity. Liquid chromatography (*2.2.29*): use the normalisation procedure.

Test solution. Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 mL with the mobile phase. Dilute 5.0 mL of the solution to 20.0 mL with the mobile phase.

Reference solution (a). Dissolve 5.0 mg of *citalopram hydrobromide CRS* (containing equal amounts of impurity K and escitalopram) in the mobile phase and dilute to 10.0 mL with the mobile phase. Dilute 5.0 mL of the solution to 20.0 mL with the mobile phase.

Reference solution (b). Dilute 1.0 mL of reference solution (a) to 50.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Column:

- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *protein derivative of silica gel for chiral separation R* (5 µm);
- *temperature*: 30 °C.

Mobile phase: *acetonitrile R*, *0.05 M phosphate buffer solution pH 7.0 R* (15:85 *V/V*).

Flow rate: 0.6 mL/min.

Detection: spectrophotometer at 240 nm.

Injection: 15 µL.

Run time: twice the retention time of escitalopram.

Identification of impurities: use the chromatogram obtained with reference solution (a) to identify the peak due to impurity K.

Relative retention with reference to escitalopram (retention time = about 11 min): impurity K = about 1.2.

System suitability: reference solution (a):

- *resolution*: minimum 1.3 between the peaks due to escitalopram and impurity K;
- *symmetry factor*: maximum 4.0 for the peak due to escitalopram; maximum 4.0 for the peak due to impurity K.

Limits:

- *impurity K*: maximum 2.0 per cent;
- *reporting threshold*: 0.10 per cent (reference solution (b)).

Water (*2.5.12*): maximum 1.0 per cent, determined on 0.250 g.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

ASSAY

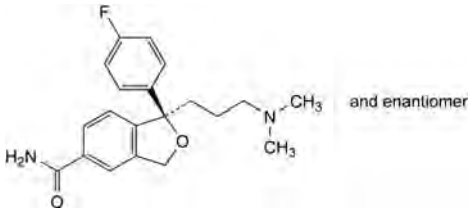
Dissolve 0.300 g in a mixture of 5.0 mL of *acetic anhydride R* and 75 mL of *glacial acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 41.44 mg of $C_{22}H_{23}FN_2O_5$.

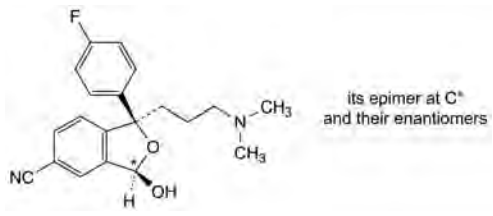
IMPURITIES

Specified impurities: K.

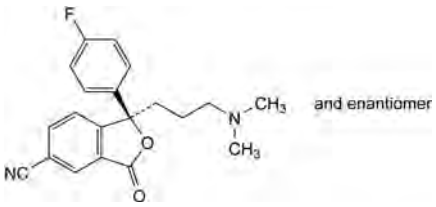
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *A, B, C, D, E, F, G, H, I, J, L, M.*



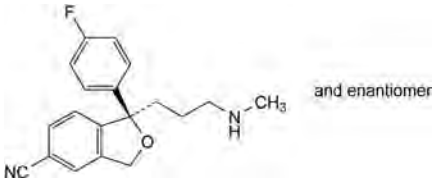
A. (1*RS*)-1-[3-(dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydro-2-benzofuran-5-carboxamide,



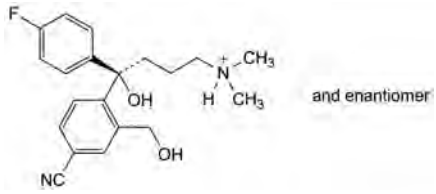
B. mixture of (1*RS*,3*RS*)-1-[3-(dimethylamino)propyl]-1-(4-fluorophenyl)-3-hydroxy-1,3-dihydro-2-benzofuran-5-carbonitrile and (1*RS*,3*SR*)-1-[3-(dimethylamino)propyl]-1-(4-fluorophenyl)-3-hydroxy-1,3-dihydro-2-benzofuran-5-carbonitrile,



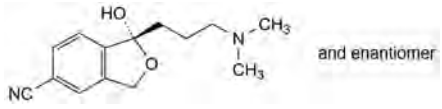
C. (1*RS*)-1-[3-(dimethylamino)propyl]-1-(4-fluorophenyl)-3-oxo-1,3-dihydro-2-benzofuran-5-carbonitrile,



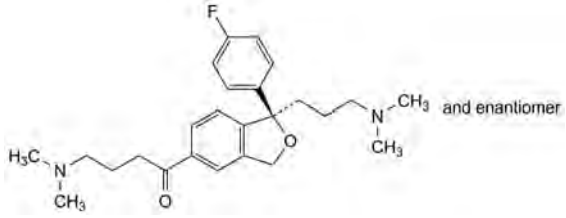
D. (1*RS*)-1-(4-fluorophenyl)-1-[3-(methylamino)propyl]-1,3-dihydro-2-benzofuran-5-carbonitrile,



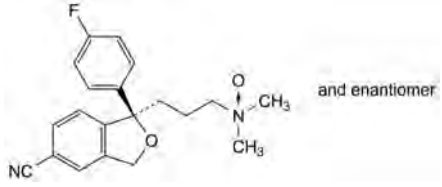
E. (4*RS*)-4-[4-cyano-2-(hydroxymethyl)phenyl]-4-(4-fluorophenyl)-4-hydroxy-*N*,*N*-dimethylbutan-1-aminium,



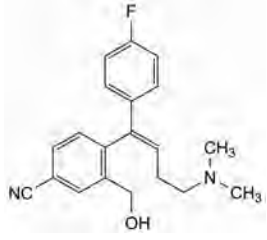
F. (1*RS*)-1-[3-(dimethylamino)propyl]-1-hydroxy-1,3-dihydro-2-benzofuran-5-carbonitrile,



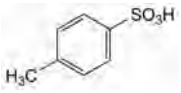
G. 4-(dimethylamino)-1-[(1*RS*)-1-[3-(dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydro-2-benzofuran-5-yl]-butan-1-one,



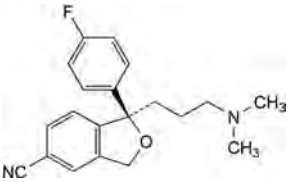
H. 3-[(1*RS*)-5-cyano-1-(4-fluorophenyl)-1,3-dihydro-2-benzofuran-1-yl]-*N*,*N*-dimethylpropan-1-amine *N*-oxide,



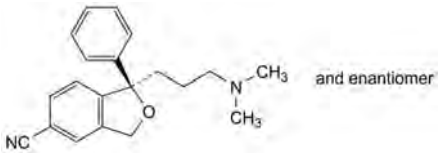
I. 4-[(1*Z*)-4-(dimethylamino)-1-(4-fluorophenyl)but-1-en-1-yl]-3-(hydroxymethyl)benzonitrile,



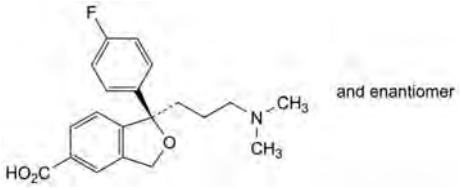
J. 4-methylbenzenesulfonic acid (*p*-toluenesulfonic acid),



K. (1*R*)-1-[3-(dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydro-2-benzofuran-5-carbonitrile,



L. (1*RS*)-1-[3-(dimethylamino)propyl]-1-phenyl-1,3-dihydro-2-benzofuran-5-carbonitrile,

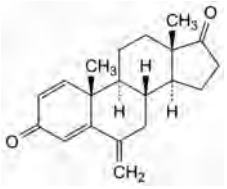


M. (1*RS*)-1-[3-(dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydro-2-benzofuran-5-carboxylic acid.

04/2020:2766

EXEMESTANE

Exemestanum



$C_{20}H_{24}O_2$ M_r 296.4
[107868-30-4]

DEFINITION

6-Methylideneandrosta-1,4-diene-3,17-dione.

Content: 98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance: white or slightly yellow powder.

Solubility: practically insoluble in water, freely soluble in methylene chloride, soluble in methanol.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *exemestane CRS*.

TESTS

Specific optical rotation (*2.2.7*): + 290 to + 298 (dried substance).

Dissolve 0.250 g in *methanol R* and dilute to 25.0 mL with the same solvent.

Related substances. Liquid chromatography (*2.2.29*). *Store the solutions protected from light.*

Solvent mixture: *water R*, *acetonitrile R* (25:75 *V/V*).

Test solution. Dissolve 25.0 mg of the substance to be examined in the solvent mixture and dilute to 25.0 mL with the solvent mixture.

Reference solution (a). Dissolve 5 mg of *exemestane for system suitability CRS* (containing impurity G) in the solvent mixture and dilute to 5 mL with the solvent mixture.

Reference solution (b). Dissolve 5 mg of *exemestane for peak identification CRS* (containing impurity I) in the solvent mixture and dilute to 5 mL with the solvent mixture.

Reference solution (c). Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (d). Dissolve 25.0 mg of *exemestane CRS* in the solvent mixture and dilute to 25.0 mL with the solvent mixture.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped polar-embedded octadecylsilyl amorphous organosilica polymer R* (3.5 µm);
- *temperature*: 40 °C.

Mobile phase:

- *mobile phase A*: *water for chromatography R*;
- *mobile phase B*: *acetonitrile for chromatography R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	75	25
5 - 35	75 → 55	25 → 45
35 - 45	55 → 5	45 → 95
45 - 50	5	95

Flow rate: 1.2 mL/min.

Detection: spectrophotometer at 247 nm.

Injection: 10 µL of the test solution and reference solutions (a), (b) and (c).

Identification of impurities: use the chromatogram supplied with *exemestane for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peak due to impurity G; use the chromatogram supplied with *exemestane for peak identification CRS* and the chromatogram obtained with reference solution (b) to identify the peak due to impurity I.

Relative retention with reference to exemestane (retention time = about 28 min): impurity G = about 1.03; impurity I = about 1.4.

System suitability: reference solution (a):

- *peak-to-valley ratio*: minimum 1.5, where H_p = height above the baseline of the peak due to impurity G and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to exemestane.

Calculation of percentage contents:

- for each impurity, use the concentration of exemestane in reference solution (c).

Limits:

- *impurity I*: maximum 0.15 per cent;
- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- *total*: maximum 0.5 per cent;
- *reporting threshold*: 0.05 per cent.

Loss on drying (*2.2.32*): maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection: test solution and reference solution (d).

Calculate the percentage content of $C_{20}H_{24}O_2$ taking into account the assigned content of *exemestane CRS*.

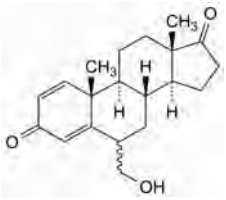
STORAGE

Protected from light.

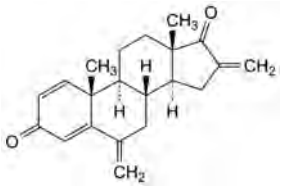
IMPURITIES

Specified impurities: *I.*

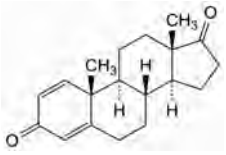
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *B, C, D, E, F, G, H, J.*



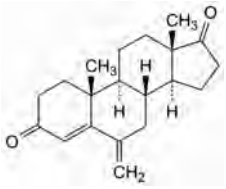
B. 6ξ-(hydroxymethyl)androsta-1,4-diene-3,17-dione,



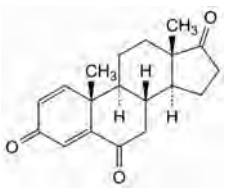
C. 6,16-dimethylideneandrosta-1,4-diene-3,17-dione,



D. androsta-1,4-diene-3,17-dione,

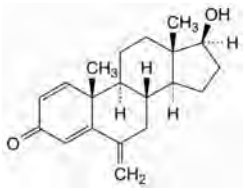


E. 6-methylideneandrost-4-ene-3,17-dione,

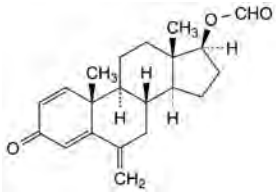


F. androsta-1,4-diene-3,6,17-trione,

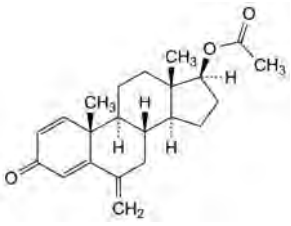
G. unknown structure,



H. 17β-hydroxy-6-methylideneandrosta-1,4-dien-3-one,



I. 6-methylidene-3-oxoandrosta-1,4-dien-17β-yl formate,



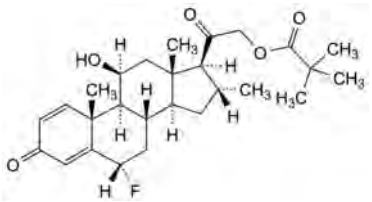
J. 6-methylidene-3-oxoandrosta-1,4-dien-17β-yl acetate.

F

04/2020:1212

FLUOCORTOLONE PIVALATE

Fluocortoloni pivalas



$C_{27}H_{37}FO_5$ M_r 460.6
[29205-06-9]

DEFINITION

6α-Fluoro-11β-hydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-21-yl 2,2-dimethylpropanoate.

Content: 96.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: practically insoluble in water, freely soluble in methylene chloride, sparingly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification: *A, B.*

Second identification: *C, D.*

A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *fluocortolone pivalate for ID and assay CRS.*

B. Examine the chromatograms obtained in the assay.
Results: the principal peak in the chromatogram obtained with test solution (b) is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (c).

C. Thin-layer chromatography (*2.2.27*).
Solvent mixture: *methanol R*, *methylene chloride R* (10:90 *V/V*).
Test solution. Dissolve 10 mg of the substance to be examined in the solvent mixture and dilute to 10 mL with the solvent mixture.
Reference solution (a). Dissolve 20 mg of *fluocortolone pivalate for ID and assay CRS* in the solvent mixture and dilute to 20 mL with the solvent mixture.
Reference solution (b). Dissolve 10 mg of *norethisterone CRS* in reference solution (a) and dilute to 10 mL with reference solution (a).
Plate: *TLC silica gel F_{254} plate R.*
Mobile phase: add a mixture of 1.2 volumes of *water R* and 8 volumes of *methanol R* to a mixture of 15 volumes of *ether R* and 77 volumes of *methylene chloride R.*
Application: 5 µL.
Development: over 3/4 of the plate.
Drying: in air.
Detection A: examine in ultraviolet light at 254 nm.
Results A: the principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).
Detection B: spray with *alcoholic solution of sulfuric acid R*, heat at 120 °C for 10 min or until the spots appear, then allow to cool; examine in daylight and in ultraviolet light at 365 nm.
Results B: the principal spot in the chromatogram obtained with the test solution is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm and size to the principal spot in the chromatogram obtained with reference solution (a).
System suitability: reference solution (b):
– the chromatogram shows 2 clearly separated spots.

D. Mix about 5 mg with 45 mg of *heavy magnesium oxide R* and ignite in a crucible until an almost white residue is obtained (usually less than 5 min). Allow to cool, add 1 mL of *water R*, 0.05 mL of *phenolphthalein solution R1* and about 1 mL of *dilute hydrochloric acid R* to render the solution colourless. Filter and add to the filtrate a freshly prepared mixture of 0.1 mL of *alizarin S solution R* and 0.1 mL of *zirconyl nitrate solution R*. Mix, allow to stand for 5 min and compare the colour of the solution with that of a blank prepared in the same manner. The test solution is yellow and the blank is red.

TESTS

Specific optical rotation (*2.2.7*): + 105 to + 110 (dried substance).

Dissolve 0.25 g in *methylene chloride R* and dilute to 25.0 mL with the same solvent.

Related substances. Liquid chromatography (*2.2.29*).

Test solution (a). Dissolve 25.0 mg of the substance to be examined in *acetonitrile R* and dilute to 25.0 mL with the same solvent.

Test solution (b). Dissolve 25.0 mg of the substance to be examined in a mixture of 50.0 mL of *acetonitrile R* and 50.0 mL of *water R*.

Reference solution (a). Dilute 1.0 mL of test solution (a) to 100.0 mL with *acetonitrile R*. Dilute 1.0 mL of this solution to 10.0 mL with *acetonitrile R*.

Reference solution (b). Dissolve 2 mg of *fluocortolone pivalate for system suitability CRS* (containing impurities C, D, E and F) in 2 mL of *acetonitrile R*.

Reference solution (c). Dissolve 25.0 mg of *fluocortolone pivalate for ID and assay CRS* in a mixture of 50.0 mL of *acetonitrile R* and 50.0 mL of *water R*.

Column:
– *size*: *l* = 0.25 m, Ø = 4.6 mm;
– *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase: *methanol R1*, *acetonitrile for chromatography R*, *water for chromatography R* (25:30:32 *V/V/V*).

Flow rate: 1.5 mL/min.

Detection: spectrophotometer at 243 nm.

Injection: 20 µL of test solution (a) and reference solutions (a) and (b).

Run time: twice the retention time of fluocortolone pivalate.

Identification of impurities: use the chromatogram supplied with *fluocortolone pivalate for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities C, D, E and F.

Relative retention with reference to fluocortolone pivalate (retention time = about 21 min): impurity C = about 0.9; impurity D = about 1.1; impurity E = about 1.4; impurity F = about 1.6.

System suitability: reference solution (b):
– *resolution*: minimum 1.5 between the peaks due to fluocortolone pivalate and impurity D.

Calculation of percentage contents:
– *correction factors*: multiply the peak areas of the following impurities by the corresponding correction factor: impurity E = 1.4; impurity F = 1.3;

– for each impurity, use the concentration of fluocortolone pivalate in reference solution (a).

Limits:

– *impurities C, D*: for each impurity, maximum 1.0 per cent;
– *impurity E*: maximum 0.2 per cent;
– *impurity F*: maximum 0.15 per cent;
– *unspecified impurities*: for each impurity, maximum 0.10 per cent;
– *total*: maximum 2.0 per cent;
– *reporting threshold*: 0.05 per cent.

Loss on drying (*2.2.32*): maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Mobile phase: *methanol R1*, *acetonitrile for chromatography R*, *water for chromatography R* (25:40:32 *V/V/V*).

Injection: 10 µL of test solution (b) and reference solutions (b) and (c).

Run time: 1.5 times the retention time of fluocortolone pivalate.

Relative retention with reference to fluocortolone pivalate (retention time = about 11 min): impurity D = about 1.1.

System suitability: reference solution (b):

– *resolution*: minimum 1.5 between the peaks due to fluocortolone pivalate and impurity D.

Calculate the percentage content of $C_{27}H_{37}FO_5$ taking into account the assigned content of *fluocortolone pivalate for ID and assay CRS*.

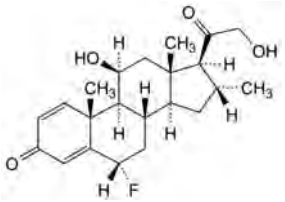
STORAGE

Protected from light.

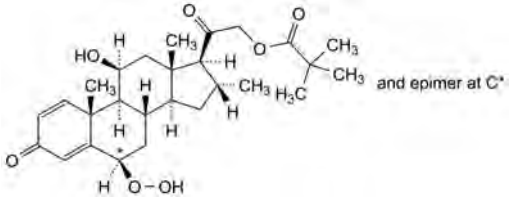
IMPURITIES

Specified impurities: C, D, E, F.

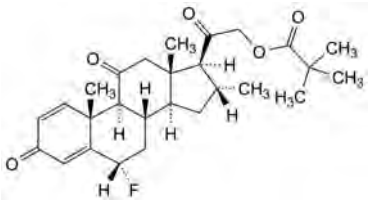
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *A, B.*



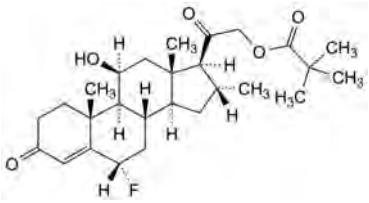
A. 6α-fluoro-11β,21-dihydroxy-16α-methylpregna-1,4-diene-3,20-dione (fluocortolone),



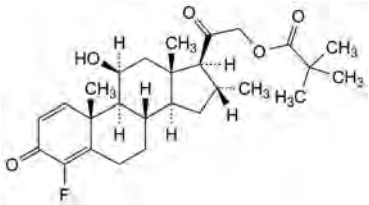
B. 6αβ-hydroperoxy-11β-hydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-21-yl 2,2-dimethylpropanoate,



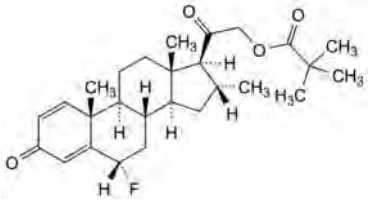
C. 6α-fluoro-16α-methyl-3,11,20-trioxopregna-1,4-dien-21-yl 2,2-dimethylpropanoate,



D. 6α-fluoro-11β-hydroxy-16α-methyl-3,20-dioxopregn-4-en-21-yl 2,2-dimethylpropanoate,



E. 4-fluoro-11β-hydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-21-yl 2,2-dimethylpropanoate,

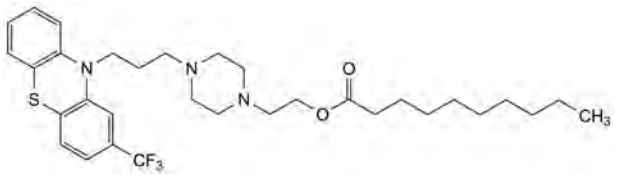


F. 6α-fluoro-16α-methyl-3,20-dioxopregna-1,4-dien-21-yl 2,2-dimethylpropanoate.

04/2020:1014

FLUPHENAZINE DECANOATE

Fluphenazini decanoas



$C_{32}H_{44}F_3N_3O_2S$ M_r 591.8
[5002-47-1]

DEFINITION

2-[4-[3-[2-(Trifluoromethyl)-10*H*-phenothiazin-10-yl]propyl]piperazin-1-yl]ethyl decanoate.
Content: 98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance: pale yellow, viscous liquid or yellow solid.
Solubility: practically insoluble in water, very soluble in anhydrous ethanol and in methylene chloride, freely soluble in methanol.

IDENTIFICATION

First identification: B, C.

Second identification: A, C.

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution. Dissolve 50.0 mg in *methanol R* and dilute to 100.0 mL with the same solvent. Dilute 1.0 mL of the solution to 50.0 mL with *methanol R*.

Spectral range: 230-350 nm.

Absorption maximum: 260 nm.

Shoulder: about 310 nm.

Specific absorbance at the absorption maximum: 570 to 630.

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation: apply 50 µL of a 25 g/L solution in *methylene chloride R* to a disc of *potassium bromide R*. Dry the discs at 60 °C for 1 h before use.

Comparison: *fluphenazine decanoate CRS*.

C. Thin-layer chromatography (*2.2.27*).

Test solution. Dissolve 10 mg of the substance to be examined in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution (a). Dissolve 10 mg of *fluphenazine decanoate CRS* in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution (b). Dissolve 5 mg of *fluphenazine enantate CRS* (impurity C) in reference solution (a) and dilute to 5 mL with the same solution.

Plate: *TLC octadecylsilyl silica gel F_{254} plate R.*

Mobile phase: *concentrated ammonia R1*, *water R*, *methanol R* (1:4:95 *V/V/V*).

Application: 2 µL.

Development: over 2/3 of the plate.

Detection: examine in ultraviolet light at 254 nm.

Retardation factors: fluphenazine decanoate = about 0.17; impurity C = about 0.26.

System suitability: reference solution (b):

– the chromatogram shows 2 clearly separated spots.

Results: the principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS

Related substances. Liquid chromatography (*2.2.29*). *Carry out the test protected from light and prepare the solutions immediately before use.*

Solvent mixture A: mobile phase A, mobile phase B (5:95 *V/V*).

Solvent mixture B: *water R*, *acetonitrile R* (5:95 *V/V*).

Test solution. Dissolve 10.0 mg of the substance to be examined in *acetonitrile R* and dilute to 50.0 mL with the same solvent.

Reference solution (a). Dissolve 5 mg of *fluphenazine enantate CRS* (impurity C) and 5 mg of *fluphenazine octanoate CRS* (impurity D) in *acetonitrile R* and dilute to 50 mL with the same solvent.

Reference solution (b). Dilute 5.0 mL of the test solution to 100.0 mL with solvent mixture A. Dilute 1.0 mL of this solution to 10.0 mL with solvent mixture A.

Reference solution (c). Dissolve 5.0 mg of *fluphenazine sulfoxide CRS* (impurity A) and 11.7 mg of *fluphenazine dihydrochloride CRS* (corresponding to about 10.0 mg of impurity B) in solvent mixture B and dilute to 100.0 mL with solvent mixture B. Dilute 1.0 mL of the solution to 50.0 mL with solvent mixture B.

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm;

– *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

– *mobile phase A*: 10 g/L solution of *ammonium carbonate R* adjusted to pH 7.5 with *dilute hydrochloric acid R*;

– *mobile phase B*: mobile phase A, *acetonitrile R*, *methanol R* (7.5:45:45 *V/V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 7	20	80
7 - 17	20 → 0	80 → 100
17 - 80	0	100

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 260 nm.

Injection: 10 µL.

Relative retention with reference to fluphenazine decanoate (retention time = about 34 min): impurity A = about 0.13; impurity B = about 0.33; impurity C = about 0.76; impurity D = about 0.82.

System suitability: reference solution (a):

– *resolution*: minimum 6.0 between the peaks due to impurities C and D.

Limits:

– *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.5 per cent);

– *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (1.0 per cent);

– *any other impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

– *total*: not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent);

– *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*): maximum 1.0 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C for 3 h.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

ASSAY

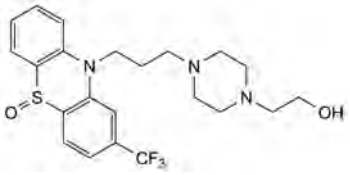
Dissolve 0.250 g in 30 mL of *glacial acetic acid R*. Using 0.05 mL of *crystal violet solution R* as indicator, titrate with *0.1 M perchloric acid* until the colour changes from violet to green.

1 mL of *0.1 M perchloric acid* is equivalent to 29.59 mg of $C_{32}H_{44}F_3N_3O_2S$.

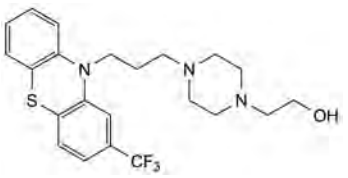
STORAGE

Protected from light.

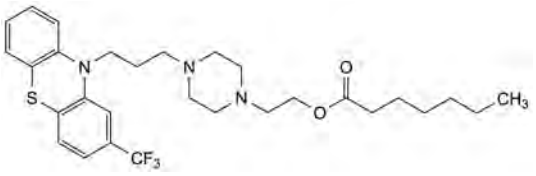
IMPURITIES



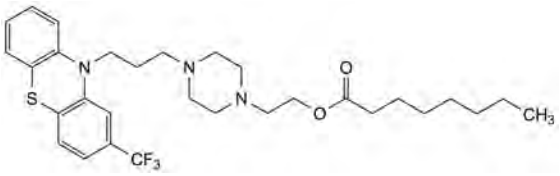
A. fluphenazine *S*-oxide (fluphenazine sulfoxide),



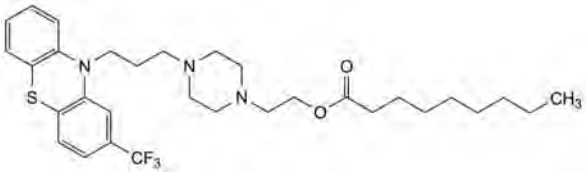
B. fluphenazine,



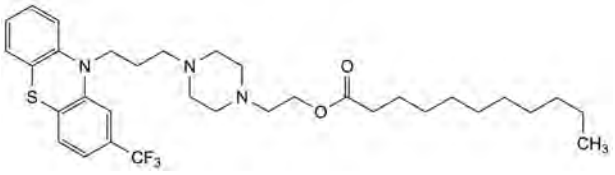
C. fluphenazine enantate,



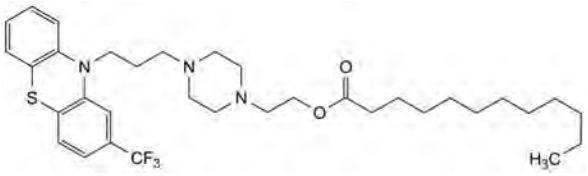
D. fluphenazine octanoate,



E. fluphenazine nonanoate,



F. fluphenazine undecanoate,

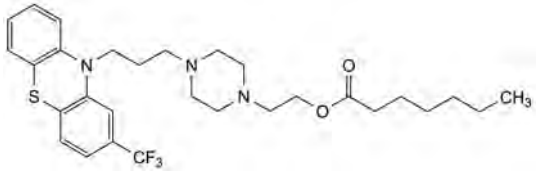


G. fluphenazine dodecanoate.

04/2020:1015

FLUPHENAZINE ENANTATE

Fluphenazini enantas



$C_{29}H_{38}F_3N_3O_2S$ M_r 549.7
[2746-81-8]

DEFINITION

2-[4-[3-[2-(Trifluoromethyl)-10*H*-phenothiazin-10-yl]propyl]piperazin-1-yl]ethyl heptanoate.

Content: 98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance: pale yellow, viscous liquid or yellow solid.

Solubility: practically insoluble in water, very soluble in anhydrous ethanol and in methylene chloride, freely soluble in methanol.

IDENTIFICATION

First identification: B, C.

Second identification: A, C.

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution. Dissolve 50.0 mg in *methanol R* and dilute to 100.0 mL with the same solvent. Dilute 1.0 mL of the solution to 50.0 mL with *methanol R*.

Spectral range: 230-350 nm.

Absorption maximum: 260 mm.

Shoulder: about 310 nm.

Specific absorbance at the absorption maximum: 610 to 670.

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation: apply 50 µL of a 25 g/L solution in *methylene chloride R* to a disc of *potassium bromide R*. Dry the discs at 60 °C for 1 h before use.

Comparison: *fluphenazine enantate CRS*.

C. Thin-layer chromatography (*2.2.27*).

Test solution. Dissolve 10 mg of the substance to be examined in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution (a). Dissolve 10 mg of *fluphenazine enantate CRS* in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution (b). Dissolve 5 mg of *fluphenazine decanoate CRS* (impurity C) in reference solution (a) and dilute to 5 mL with the same solution.

Plate: *TLC octadecylsilyl silica gel F_{254} plate R.*

Mobile phase: *concentrated ammonia R1*, *water R*, *methanol R* (1:4:95 *V/V/V*).

Application: 2 µL.

Development: over 2/3 of the plate.

Detection: examine in ultraviolet light at 254 nm.

Retardation factors: impurity C = about 0.17; fluphenazine enantate = about 0.26.

Sytem suitability: reference solution (b):

– the chromatogram shows 2 clearly separated spots.

Results: the principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS

Related substances. Liquid chromatography (*2.2.29*). *Carry out the test protected from light and prepare the solutions immediately before use.*

Solvent mixture: mobile phase A, mobile phase B (5:95 *V/V*).

Test solution. Dissolve 10.0 mg of the substance to be examined in *acetonitrile R* and dilute to 50.0 mL with the same solvent.

Reference solution (a). Dissolve 5 mg of *fluphenazine enantate CRS* and 5 mg of *fluphenazine octanoate CRS* (impurity D) in *acetonitrile R* and dilute to 50 mL with the same solvent.

Reference solution (b). Dilute 5.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (c). Dissolve 5.0 mg of *fluphenazine sulfoxide CRS* (impurity A) in *acetonitrile R* and dilute to 100.0 mL with the same solvent. Dilute 1.0 mL of the solution to 50.0 mL with *acetonitrile R*.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

- *mobile phase A*: 10 g/L solution of *ammonium carbonate R* adjusted to pH 7.5 with *dilute hydrochloric acid R*;
- *mobile phase B*: mobile phase A, *acetonitrile R*, *methanol R* (7.5:45:45 *V/V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 7	20	80
7 - 17	20 → 0	80 → 100
17 - 80	0	100

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 260 nm.

Injection: 10 µL.

Relative retention with reference to fluphenazine enantate (retention time = about 25 min): impurity A = about 0.2; impurity D = about 1.1.

System suitability: reference solution (a):

- *resolution*: minimum 6.0 between the peaks due to fluphenazine enantate and impurity D.

Limits:

- *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent);
- *any other impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *total*: not more than 3.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.6 per cent);
- *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*): maximum 1.0 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C for 3 h.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

ASSAY

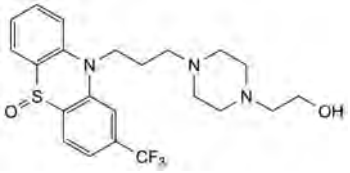
Dissolve 0.250 g in 30 mL of *glacial acetic acid R*. Using 0.05 mL of *crystal violet solution R* as indicator, titrate with *0.1 M perchloric acid* until the colour changes from violet to green.

1 mL of *0.1 M perchloric acid* is equivalent to 27.49 mg of $C_{29}H_{38}F_3N_3O_2S$.

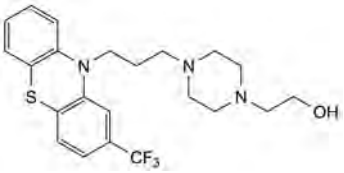
STORAGE

Protected from light.

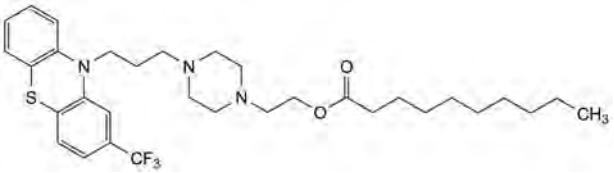
IMPURITIES



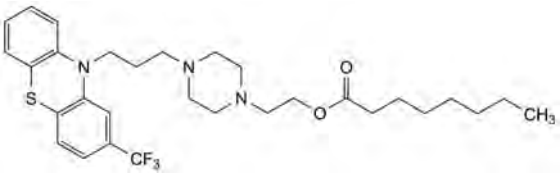
A. fluphenazine *S*-oxide (fluphenazine sulfoxide),



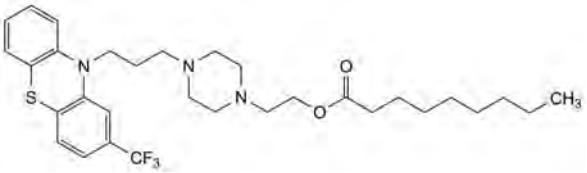
B. fluphenazine,



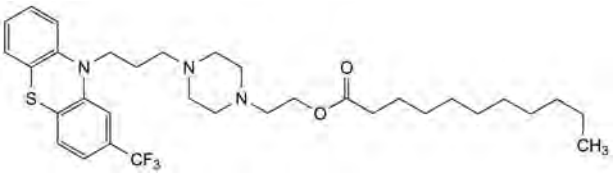
C. fluphenazine decanoate,



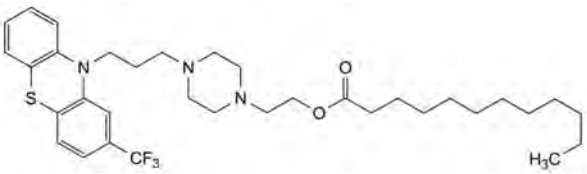
D. fluphenazine octanoate,



E. fluphenazine nonanoate,



F. fluphenazine undecanoate,



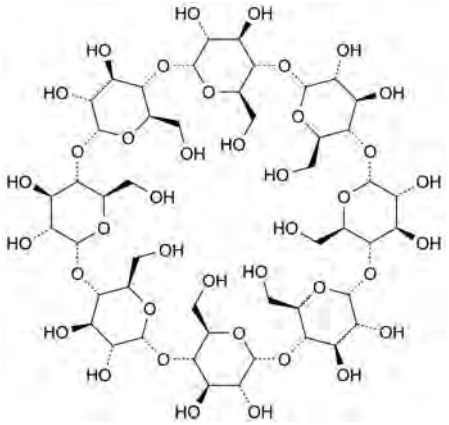
G. fluphenazine dodecanoate.

G

04/2018:2769
corrected 10.1

GAMMADEX

Gammadexum



$[C_6H_{10}O_5]_8$ M_r 1297
[17465-86-0]

DEFINITION

Cyclooctakis-(1→4)-(α-D-glucopyranosyl) (cyclomaltooctaose or γ-cyclodextrin).

Content: 97.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, amorphous or crystalline, hygroscopic powder.

Solubility: freely soluble in water, very slightly soluble in propylene glycol, practically insoluble in anhydrous ethanol and in methylene chloride. When dissolved in water, it forms a colloidal dispersion over time.

IDENTIFICATION

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *gammacyclodextrin CRS*.

TESTS

Solution S. Dissolve 1.000 g in *carbon dioxide-free water R* and dilute to 100.0 mL with the same solvent.

pH (*2.2.3*): 5.0 to 8.0.

Mix 1 mL of a 223.6 g/L solution of *potassium chloride R* and 30 mL of solution S.

Specific optical rotation (*2.2.7*): + 174 to + 180 (dried substance), determined on solution S after filtration through a membrane filter (nominal pore size 0.45 µm).

Reducing sugars: maximum 0.2 per cent.

Test solution. To 1 mL of solution S add 1 mL of *cupri-tartaric solution R4*. Heat on a water-bath for 10 min and allow to cool to room temperature. Add 10 mL of *ammonium molybdate reagent R1* and allow to stand for 15 min.

Reference solution. Prepare at the same time and in the same manner as for the test solution, using 1 mL of a 0.02 g/L solution of *glucose R*.

Measure the absorbances (*2.2.25*) of the 2 solutions at the absorption maximum at 740 nm using *water R* as the compensation liquid. The absorbance of the test solution is not greater than that of the reference solution.

Light-absorbing impurities. Examine solution S after filtration through a membrane filter (nominal pore size 0.45 µm). The absorbance (*2.2.25*) is not greater than 0.10 between 230 nm and 350 nm and not greater than 0.05 between 350 nm and 750 nm.

Related substances. Liquid chromatography (*2.2.29*).

Test solution (a). Dissolve 0.250 g of the substance to be examined in *water R* with heating, cool and dilute to 25.0 mL with the same solvent.

Test solution (b). Dissolve 25.0 mg of the substance to be examined in *water R* and dilute to 25.0 mL with the same solvent.

Reference solution (a). Dissolve 25.0 mg of *alfadex CRS* (impurity A), 25.0 mg of *betadex CRS* (impurity B) and 25.0 mg of *gammacyclodextrin CRS* (gammadex) in *water R* and dilute to 50.0 mL with the same solvent.

Reference solution (b). Dilute 5.0 mL of reference solution (a) to 50.0 mL with *water R*.

Reference solution (c). Dissolve 25.0 mg of *gammacyclodextrin CRS* in *water R* and dilute to 25.0 mL with the same solvent.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 30 °C.

Mobile phase: *methanol R*, *water for chromatography R* (5:95 *V/V*).

Flow rate: 1.5 mL/min.

Detection: differential refractometer maintained at a constant temperature (e.g. 35 °C).

Equilibration: with the mobile phase for about 3 h.

Injection: 50 µL of test solution (a) and reference solutions (a) and (b).

Run time: 4 times the retention time of gammadex.

Identification of impurities: use the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A and B.

Relative retention with reference to gammadex (retention time = about 7 min): impurity A = about 1.5; impurity B = about 3.2.

System suitability: reference solution (a):
- *resolution*: minimum 2.5 between the peaks due to gammadex and impurity A;
- *symmetry factor*: 0.8 to 1.8 for the peak due to gammadex.

Calculation of percentage contents:
- for impurities A and B, use the concentration of the corresponding impurity in reference solution (b);
- for impurities other than A and B, use the concentration of gammadex in reference solution (b).

Limits:
- *impurities A, B*: for each impurity, maximum 0.5 per cent;
- *sum of impurities other than A and B*: maximum 0.5 per cent;
- *reporting threshold*: 0.15 per cent.

Loss on drying (*2.2.32*): maximum 11.0 per cent, determined on 1.000 g by drying in an oven at 120 °C for 2 h.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Injection: test solution (b) and reference solution (c).

System suitability: reference solution (c):
- *symmetry factor*: 0.8 to 1.8 for the peak due to gammadex;

– *repeatability*: maximum relative standard deviation of 2.0 per cent determined on 5 injections.

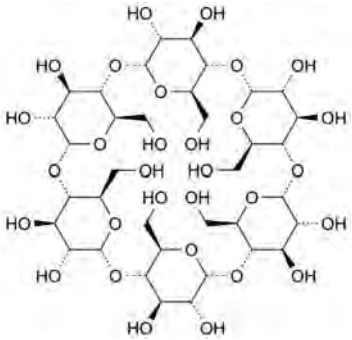
Calculate the percentage content of $[C_6H_{10}O_5]_8$ taking into account the assigned content of *gammacyclodextrin CRS*.

STORAGE

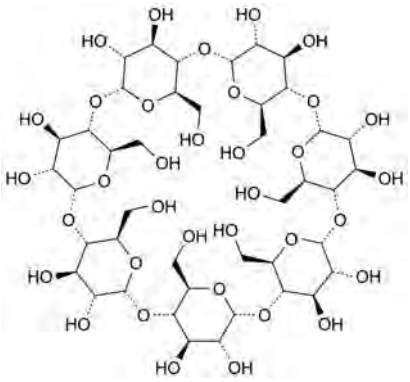
In an airtight container.

IMPURITIES

Specified impurities: A, B.



A. cyclohexakis-(1→4)-(α-D-glucopyranosyl) (alfadex or cyclomaltohexaose or α-cyclodextrin),



B. cycloheptakis-(1→4)-(α-D-glucopyranosyl) (betadex or cyclomaltoheptaose or β-cyclodextrin).

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INSULIN PREPARATIONS, INJECTABLE

Praeparationes insulini iniectabiles

Injectable insulin preparations comply with the requirements for Injections prescribed in the monograph on Parenteral preparations (0520).

DEFINITION

Injectable insulin preparations are sterile preparations of *Insulin, human (0838)*, *Insulin, porcine (1638)* or bovine insulin. They contain not less than 90.0 per cent and not more than the equivalent of 110.0 per cent of the amount of insulin stated on the label. They are either solutions or suspensions or they are prepared by combining solutions and suspensions.

PRODUCTION

The methods of preparation are designed to confer suitable properties with respect to the onset and duration of therapeutic action.

The following procedures are carried out in a suitable sequence, depending on the method of preparation:

- addition of suitable antimicrobial preservatives;
- addition of a suitable substance or substances to render the preparation isotonic with blood;
- addition of a suitable substance or substances to adjust the pH to the appropriate value;
- determination of the strength of the insulin-containing component or components followed, where necessary, by adjustment so that the final preparation contains the requisite number of International Units per millilitre;
- sterilisation by filtration of the insulin-containing component or components; once this procedure has been carried out all subsequent procedures are carried out aseptically using materials that have been sterilised by a suitable method.

In addition, where appropriate, suitable excipients are added and suitable procedures carried out to confer the appropriate physical form on the insulin-containing component or components. The final preparation is distributed aseptically into sterile containers which are closed so as to exclude microbial contamination.

TESTS

pH (*2.2.3*). The pH of the solution or suspension is 6.9 to 7.8, unless otherwise prescribed in the specific monograph.

Insulin in the supernatant. For injectable insulin preparations that are suspensions, not more than 2.5 per cent of the total insulin content, unless otherwise stated. Centrifuge 10 mL of the suspension at 1500 *g* for 10 min and carefully separate the supernatant and the residue. Determine the insulin content of the supernatant (*S*) by a suitable method, for example using the chromatographic conditions described under Assay. Calculate the percentage of insulin in solution using the following expression:

$$\frac{100S}{T}$$

where *T* is the total insulin content determined as described under the Assay.

Impurities with molecular masses greater than that of insulin. Examine by size-exclusion chromatography (*2.2.30*).

Test solution. Add 4 µL of *6 M hydrochloric acid R* per millilitre of the preparation to be examined, whether a suspension or a solution, to obtain a clear acid insulin solution. When sampling a suspension, agitate the material prior to sampling in order to obtain a homogeneous sample. If a suspension does not turn clear within 5 min of the initial addition of hydrochloric acid, add small aliquots of acid (less than 4 µL per millilitre) until a solution is obtained. Preparations with concentrations higher than 100 IU/mL need to be diluted with *0.01 M hydrochloric acid* to avoid overloading the column with insulin monomer.

Resolution solution. Use a solution of insulin (approximately 4 mg/mL), containing more than 0.4 per cent of high molecular mass proteins. An injectable insulin preparation, whether a solution or a suspension, that has been clarified with a sufficient amount of *6 M hydrochloric acid R*, containing the indicated percentage of high molecular mass proteins, or a solution prepared from insulin, dissolved in *0.01 M hydrochloric acid*, may be used. Insulin containing the indicated percentage of high molecular mass proteins may be prepared by allowing insulin powder to stand at room temperature for about ten days.

Maintain the solutions at 2 °C to 10 °C and use within 30 h (soluble insulin injection) or 7 days (other insulin preparations). If an automatic injector is used, maintain the temperature at 2 °C to 10 °C.

The chromatographic procedure may be carried out using:

- a column 0.3 m long and at least 7.5 mm in internal diameter packed with *hydrophilic silica gel for chromatography R* (5 µm to 10 µm), of a grade suitable for the separation of insulin monomer from dimers and polymers;
- as mobile phase at a flow rate of 0.5 mL/min a mixture consisting of 15 volumes of *glacial acetic acid R*, 20 volumes of *acetonitrile R* and 65 volumes of a 1.0 g/L solution of *arginine R*; filter and degas;
- as detector a spectrophotometer set at 276 nm.

Equilibration of the column. Before using a new column for chromatographic analysis, equilibrate by repeated injections of an insulin solution containing high molecular mass proteins. This can be done by at least three injections of the resolution solution. The column is equilibrated when repeatable results are obtained from two subsequent injections. If protamine-containing samples are to be analysed, the equilibration of the column is performed using a solution containing protamine.

Inject 100 µL of the resolution solution. When the chromatograms are recorded under the prescribed conditions, the retention times are: polymeric insulin complexes or covalent insulin-protamine complex: about 13 min to 17 min, covalent insulin dimer: about 17.5 min, insulin monomer: about 20 min, salts: about 22 min. If the sample solution contains preservatives, for example methyl paraben, *m*-cresol or phenol, these compounds elute later. The test is not valid unless the resolution, defined by the ratio of the height of the dimer peak to the height above the baseline of the valley separating the monomer and dimer peaks, is at least 2.0.

Inject 100 µL of the test solution. Record the chromatogram for approximately 35 min. In the chromatogram obtained, the sum of the areas of any peak with a retention time less than that of the insulin peak is not greater than 3.0 per cent (protamine containing preparations) or 2.0 per cent (non-protamine containing preparations) of the total area of the peaks. Disregard any peak with a retention time greater than that of the insulin peak.

Related proteins. Examine by liquid chromatography (*2.2.29*) as described under Assay, following the elution conditions as described in the table below:

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)	Comment
0 - 30	42	58	isocratic
30 - 44	42 → 11	58 → 89	linear gradient
44 - 50	11	89	isocratic

Maintain the solutions at 2 °C to 10 °C and use within 24 h. Perform a system suitability check (resolution, linearity) as described under Assay. If necessary, the relative proportions of the mobile phases may be adjusted to ensure complete elution of A21 desamido porcine insulin before commencement of the gradient. The profile of the gradient may also be adjusted to ensure complete elution of all insulin related impurities.

Inject 20 µL of the test solution and 20 µL of either reference solution (a), for insulin preparations containing 100 IU/mL, or reference solution (b), for insulin preparations containing 40 IU/mL. If necessary, adjust the injection volume to a volume between 10 µL and 20 µL in accordance with the results obtained in the test for linearity as described under Assay. Record the chromatograms for approximately 50 min. If necessary, make further adjustments to the mobile phase in order to ensure that the antimicrobial preservatives present in the test solution are well separated from the insulin and show a shorter retention time. A small reduction in the concentration of acetonitrile increases the retention time of the insulin peaks relatively more than those of the preservatives. In the chromatogram obtained with either reference solution (a), or reference solution (b), as appropriate, A21 desamido insulin appears as a small peak after the principal peak and has a retention time of about 1.3 relative to the principal peak, due to insulin. In the chromatogram obtained with the test solution, the area of the peak due to A21 desamido insulin is not greater than 5.0 per cent of the total area of the peaks; the sum of the areas of any other peaks, apart from those due to insulin and A21 desamido insulin is not greater than 6.0 per cent of the total area of the peaks. Disregard the peaks due to the preservatives and protamine (early eluting peaks).

Total zinc. Not more than the amount stated in the individual monograph, determined by atomic absorption spectrometry (*2.2.23, Method I*).

Use the following method, unless otherwise prescribed in the specific monograph.

Test solution. Shake the preparation gently and dilute a volume containing 200 IU of insulin to 25.0 mL with *0.01 M hydrochloric acid*. Dilute if necessary to a suitable concentration of zinc (for example 0.4 µg to 1.6 µg of Zn per millilitre) with *0.01 M hydrochloric acid*.

Reference solutions. Use solutions containing 0.40 µg, 0.80 µg, 1.00 µg, 1.20 µg and 1.60 µg of Zn per millilitre, freshly prepared by diluting *zinc standard solution (5 mg/mL Zn) R* with *0.01 M hydrochloric acid*.

Measure the absorbance at 213.9 nm using a zinc hollow-cathode lamp as source of radiation and an air-acetylene flame of suitable composition (for example 11 L of air and 2 L of acetylene per minute).

Zinc in solution. Where applicable, not more than the amount stated in the individual monograph, determined by atomic absorption spectrometry (*2.2.23, Method I*).

Test solution. Centrifuge the preparation to be examined and dilute 1 mL of the clear supernatant obtained to 25.0 mL with *water R*. Dilute if necessary to a suitable concentration of zinc (for example 0.4 µg to 1.6 µg of Zn per millilitre) with *water R*.

Reference solutions. Use solutions containing 0.40 µg, 0.80 µg, 1.00 µg, 1.20 µg and 1.60 µg of Zn per millilitre, freshly prepared by diluting *zinc standard solution (5 mg/mL Zn) R* with *0.01 M hydrochloric acid*.

Measure the absorbance at 213.9 nm using a zinc hollow-cathode lamp as source of radiation and an air-acetylene flame of suitable composition (for example 11 L of air and 2 L of acetylene per minute).

Bacterial endotoxins (*2.6.14*): less than 80 IU per 100 IU of insulin.

ASSAY

Examine by liquid chromatography (*2.2.29*).

Test solution. Add 4 µL of *6 M hydrochloric acid R* per millilitre of the preparation to be examined, whether a suspension or a solution, to obtain a clear solution. When sampling a suspension, shake the material prior to sampling in order to obtain a homogeneous sample. If a suspension does not turn clear within 5 min of the initial addition of acid, add small aliquots of acid (less than 4 µL per millilitre) until a solution is obtained. For a preparation containing more than 100 IU/mL, an additional dilution with *0.01 M hydrochloric acid* is necessary to avoid overloading the column.

Reference solution (a). For a preparation containing a single species of insulin, dissolve in *0.01 M hydrochloric acid*, as appropriate, the contents of a vial of *human insulin CRS*, *porcine insulin CRS* or *bovine insulin CRS* to obtain a concentration of 4.0 mg/mL. For a preparation containing both bovine and porcine insulins, mix 1.0 mL of a solution containing 4.0 mg of *bovine insulin CRS* per millilitre of *0.01 M hydrochloric acid* and 1.0 mL of a solution containing 4.0 mg of *porcine insulin CRS* per millilitre of *0.01 M hydrochloric acid*. *Reference solution (a) is used for the assay of insulin preparations containing 100 IU/mL.*

Reference solution (b). Dilute 4.0 mL of reference solution (a) to 10.0 mL with *0.01 M hydrochloric acid*. *Reference solution (b) is used for the assay of insulin preparations containing 40 IU/mL.*

Reference solution (c). Dissolve the contents of a vial of *human insulin CRS* in *0.01 M hydrochloric acid* to obtain a concentration of 4.0 mg/mL.

Reference solution (d). Dissolve the contents of a vial of *insulin porcine for system suitability CRS* in *0.01 M hydrochloric acid* to obtain a concentration of 4 mg/mL.

Reference solution (e). Dilute 1.0 mL of reference solution (a) to 10.0 mL with *0.01 M hydrochloric acid*.

Reference solution (f). Dilute 1.0 mL of reference solution (b) to 10.0 mL with *0.01 M hydrochloric acid*.

Resolution solution. Mix 1.0 mL of reference solution (c) and 1.0 mL of reference solution (d).

Maintain the solutions at 2 °C to 10 °C and use within 48 h. If an automatic injector is used, maintain the temperature at 2 °C to 10 °C.

The chromatographic procedure may be carried out using:

- a stainless steel column 0.25 m long and 4.6 mm in internal diameter packed with *octadecylsilyl silica gel for chromatography R* (5 µm);
- as mobile phase at a flow rate of 1 mL/min the following solutions prepared and maintained at a temperature not lower than 20 °C:

  Mobile phase A. Dissolve 28.4 g of *anhydrous sodium sulfate R* in *water for chromatography R* and dilute to 1000 mL with the same solvent; add 2.7 mL of *phosphoric acid R*; adjust the pH to 2.3, if necessary, with *ethanolamine R*; filter and degas;

  Mobile phase B. Mix 550 mL of mobile phase A with 450 mL of *acetonitrile R1*. Warm the solution to a temperature not lower than 20 °C in order to avoid precipitation (mixing of mobile phase A with acetonitrile is endothermic); filter and degas;
- as detector a spectrophotometer set at 214 nm;

maintaining the temperature of the column at 40 °C.

Elute with a mixture of 42 volumes of mobile phase A and 58 volumes of mobile phase B, adjusted if necessary.

Inject 20 µL of the resolution solution and 20 µL of reference solution (d). Record the chromatogram of the resolution solution until the peak corresponding to the principal peak in the chromatogram obtained with reference solution (d) is clearly visible. In the chromatogram obtained with the resolution solution, identify the peaks due to porcine insulin and human insulin. The test is not valid unless the resolution between the peaks due to human insulin and porcine insulin is at least 1.2. If necessary, adjust the concentration of acetonitrile in the mobile phase until this resolution is achieved.

Inject 20 µL of the test solution and 20 µL of either reference solutions (a) and (e), for insulin preparations containing 100 IU/mL, or 20 µL of reference solutions (b) and (f), for insulin preparations containing 40 IU/mL. If necessary, make further adjustments of the mobile phase in order to ensure that the antimicrobial preservatives present in the test solution are well separated from the insulin and show shorter retention times. A small reduction in the concentration of acetonitrile increases the retention time of the insulin peaks relatively more than those of the preservatives. If necessary, after having carried out the chromatography of a solution wash the column with a mixture of equal volumes of *acetonitrile R1* and *water for chromatography R* for a sufficient time to ensure elution of any interfering substances before injecting the next solution. The test is not valid unless the area of the principal peak in the chromatogram obtained with reference solution (a) or (b) is 10 ± 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (e) or (f). If this test fails, adjust the injection volume between 10 µL and 20 µL, in order to be in the linearity range of the detector.

Calculate the content of insulin plus A21 desamido insulin from the area of the peak due to the bovine, porcine or human insulin and that of any peak due to the A21 desamido insulin, using the declared content of insulin plus A21 desamido insulin in *bovine insulin CRS*, *porcine insulin CRS* or *human insulin CRS*, as appropriate. For preparations containing both bovine and porcine insulin use the sum of the areas of both the bovine and porcine insulin peaks and of the peaks due to the A21 desamido insulin(1) derivatives.

STORAGE

Unless otherwise prescribed, store in a sterile, airtight, tamper-evident container, protected from light, at a temperature of 2 °C to 8 °C. Insulin preparations are not to be frozen.

LABELLING

The label states:

- the potency in International Units per millilitre;
- the concentration in terms of the number of milligrams of insulin per millilitre (for preparations containing both bovine insulin and porcine insulin the concentration is stated as the combined amount of both insulins);
- where applicable, that the substance is produced by enzymatic modification of porcine insulin;
- where applicable, that the substance is produced by recombinant DNA technology;
- where applicable, the animal species of origin;
- that the preparation must not be frozen;
- where applicable, that the preparation must be resuspended before use.

(1) 100 IU are equivalent to 3.47 mg of human insulin, to 3.45 mg of porcine insulin and to 3.42 mg of bovine insulin.

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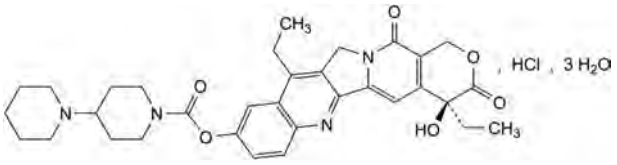
IRINOTECAN HYDROCHLORIDE TRIHYDRATE

Irinotecani hydrochloridum trihydricum



$C_{33}H_{39}ClN_4O_6,3H_2O$ M_r 677
[136572-09-3]

DEFINITION

(4*S*)-4,11-Diethyl-4-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1*H*-pyrano[3′,4′:6,7]indolizino[1,2-*b*]quinolin-9-yl 1,4′-bipiperidine-1′-carboxylate hydrochloride trihydrate.

Content: 98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance: pale yellow or yellow, crystalline powder.

Solubility: sparingly soluble in water, in ethanol (96 per cent) and in methanol.

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *irinotecan hydrochloride trihydrate CRS*.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness and record new spectra using the residues.

B. Water (see Tests).

C. It gives reaction (a) of chlorides (*2.3.1*).

Dissolve 0.10 g in *water R* and dilute to 50 mL with the same solvent.

TESTS

Appearance of solution. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution GY_2 (*2.2.2, Method II*).

Dissolve 0.200 g in *water R* with heating at 80 °C and dilute to 20 mL with the same solvent.

Enantiomeric purity. Liquid chromatography (*2.2.29*).

Solvent mixture: *diethylamine R*, *anhydrous ethanol R* (0.4:100 *V/V*).

Test solution. Dissolve 15.0 mg of the substance to be examined in the solvent mixture and dilute to 10.0 mL with the solvent mixture.

Reference solution (a). Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b). Dissolve the contents of a vial of *irinotecan for system suitability 1 CRS* (containing impurity L) in 1 mL of the solvent mixture.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *cellulose derivative of silica gel for chiral separation R* (10 µm).

Mobile phase: *diethylamine R*, *anhydrous ethanol R*, *hexane R* (0.2:50:50 *V/V/V*).

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 370 nm.

Injection: 20 µL.

Run time: 1.5 times the retention time of irinotecan.

Identification of impurities: use the chromatogram obtained with reference solution (b) to identify the peak due to impurity L.

Relative retention with reference to irinotecan (retention time = about 15 min): impurity L = about 0.7.

System suitability:

– *resolution*: minimum 1.5 between the peaks due to impurity L and irinotecan in the chromatogram obtained with reference solution (b).

Calculation of percentage content:

– for impurity L, use the concentration of irinotecan hydrochloride trihydrate in reference solution (a).

Limit:

– *impurity L*: maximum 0.15 per cent.

Related substances. Liquid chromatography (*2.2.29*). *Carry out the test protected from light.*

Solvent mixture: *acetonitrile R*, *methanol R*, mobile phase A (25:25:50 *V/V/V*).

Test solution. Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 mL with the solvent mixture.

Reference solution (a). Dissolve 50.0 mg of *irinotecan hydrochloride trihydrate CRS* in the solvent mixture and dilute to 50.0 mL with the solvent mixture.

Reference solution (b). Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (c). Dissolve the contents of a vial of *irinotecan for peak identification CRS* (containing impurities C and E) in 1 mL of the solvent mixture.

Reference solution (d). Dissolve 5 mg of *irinotecan for system suitability 2 CRS* (containing impurity M) in the solvent mixture and dilute to 10 mL with the solvent mixture.

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm;

– *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography with embedded polar groups R* (5 µm).

Mobile phase:

– *mobile phase A*: dissolve 2.72 g of *potassium dihydrogen phosphate R* in 950 mL of *water for chromatography R*, adjust to pH 3.5 with *dilute phosphoric acid R* and dilute to 1000 mL with *water for chromatography R*;

– *mobile phase B*: *methanol R2*, *acetonitrile R1* (40:60 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	80	20
2 - 42	80 → 30	20 → 70
42 - 47	30	70

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 220 nm.

Injection: 10 µL of the test solution and reference solutions (b), (c) and (d).

Identification of impurities: use the chromatogram supplied with *irinotecan for peak identification CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities C and E; use the chromatogram obtained with reference solution (d) to identify the peak due to impurity M.

Relative retention with reference to irinotecan (retention time = about 17 min): impurity M = about 0.9; impurity C = about 1.3; impurity E = about 1.5.

System suitability:

– *resolution*: minimum 2.0 between the peaks due to impurity M and irinotecan in the chromatogram obtained with reference solution (d).

Calculation of percentage contents:

– *correction factor*: multiply the peak area of impurity M by 1.3;

– for each impurity, use the concentration of irinotecan hydrochloride trihydrate in reference solution (b).

Limits:

– *impurities C, E, M*: for each impurity, maximum 0.15 per cent;

– *unspecified impurities*: for each impurity, maximum 0.10 per cent;

– *total*: maximum 0.5 per cent;

– *reporting threshold*: 0.05 per cent.

Water (*2.5.12*): 7.0 per cent to 9.0 per cent, determined on 0.100 g.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection: 10 µL of the test solution and reference solution (a).

Calculate the percentage content of $C_{33}H_{39}ClN_4O_6$ taking into account the assigned content of *irinotecan hydrochloride trihydrate CRS*.

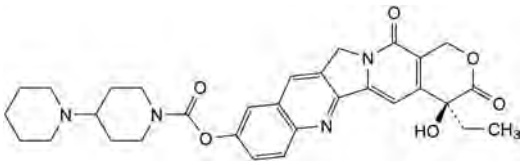
STORAGE

In an airtight container, protected from light.

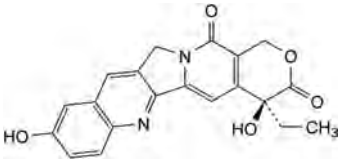
IMPURITIES

Specified impurities: C, E, L, M.

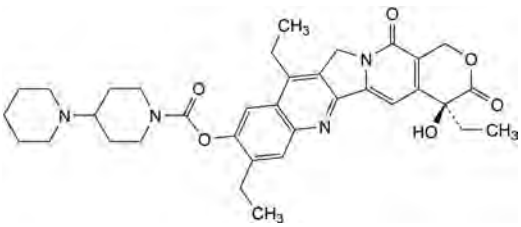
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *A, B, D, F, G, H, K.*



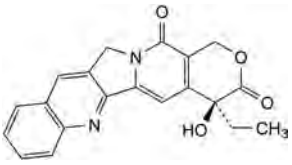
A. (4*S*)-4-ethyl-4-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1*H*-pyrano[3′,4′:6,7]indolizino[1,2-*b*]quinolin-9-yl 1,4′-bipiperidine-1′-carboxylate (11-desethyl irinotecan),



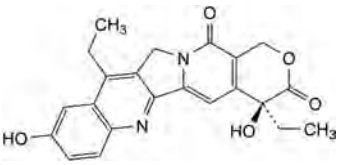
B. (4*S*)-4-ethyl-4,9-dihydroxy-1*H*-pyrano[3′,4′:6,7]-indolizino[1,2-*b*]quinoline-3,14(4*H*,12*H*)-dione (9-hydroxycamptothecin),



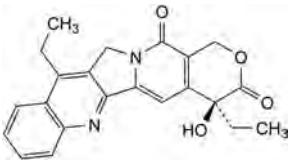
C. (4*S*)-4,8,11-triethyl-4-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1*H*-pyrano[3′,4′:6,7]indolizino[1,2-*b*]quinolin-9-yl 1,4′-bipiperidine-1′-carboxylate (8-ethyl irinotecan),



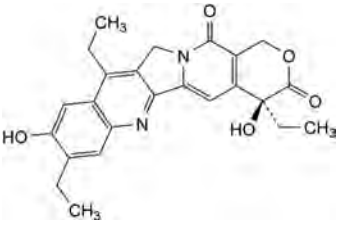
D. (4*S*)-4-ethyl-4-hydroxy-1*H*-pyrano[3′,4′:6,7]indolizino-[1,2-*b*]quinoline-3,14(4*H*,12*H*)-dione (camptothecin),



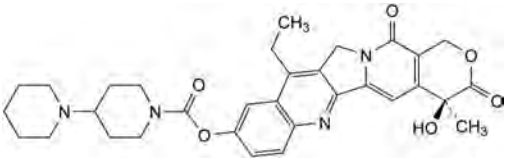
E. (4*S*)-4,11-diethyl-4,9-dihydroxy-1*H*-pyrano[3′,4′:6,7]-indolizino[1,2-*b*]quinoline-3,14(4*H*,12*H*)-dione (11-ethyl-9-hydroxycamptothecin),



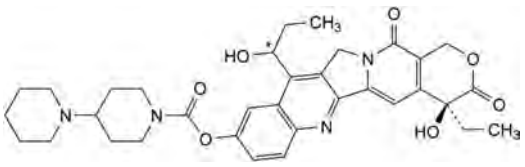
F. (4*S*)-4,11-diethyl-4-hydroxy-1*H*-pyrano[3′,4′:6,7]-indolizino[1,2-*b*]quinoline-3,14(4*H*,12*H*)-dione (11-ethylcamptothecin),



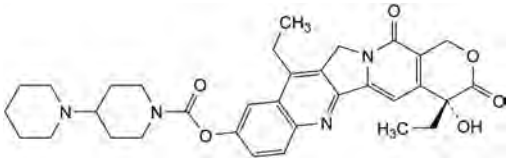
G. (4*S*)-4,8,11-triethyl-4,9-dihydroxy-1*H*-pyrano[3′,4′:6,7]-indolizino[1,2-*b*]quinoline-3,14(4*H*,12*H*)-dione (8,11-diethyl-9-hydroxycamptothecin),



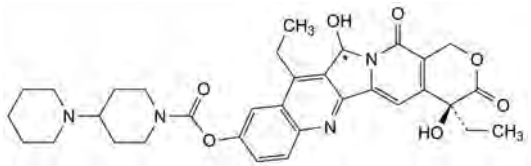
H. (4*S*)-11-ethyl-4-hydroxy-4-methyl-3,14-dioxo-3,4,12,14-tetrahydro-1*H*-pyrano[3′,4′:6,7]indolizino[1,2-*b*]quinolin-9-yl 1,4′-bipiperidine-1′-carboxylate (4-methyl irinotecan analogue),



K. (4*S*)-4-ethyl-4-hydroxy-11-(1-hydroxypropyl)-3,14-dioxo-3,4,12,14-tetrahydro-1*H*-pyrano[3′,4′:6,7]indolizino-[1,2-*b*]quinolin-9-yl 1,4′-bipiperidine-1′-carboxylate,



L. (4*R*)-4,11-diethyl-4-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1*H*-pyrano[3′,4′:6,7]indolizino[1,2-*b*]quinolin-9-yl 1,4′-bipiperidine-1′-carboxylate (irinotecan enantiomer).

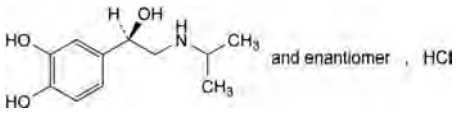


M. (4*S*)-4,11-diethyl-4,12-dihydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1*H*-pyrano[3′,4′:6,7]indolizino[1,2-*b*]quinolin-9-yl 1,4′-bipiperidine-1′-carboxylate (12-hydroxy irinotecan).

04/2020:1332

ISOPRENALINE HYDROCHLORIDE

Isoprenalini hydrochloridum



$C_{11}H_{18}ClNO_3$ M_r 247.7
[51-30-9]

DEFINITION

(1*RS*)-1-(3,4-Dihydroxyphenyl)-2-[(1-methylethyl)amino]-ethanol hydrochloride.

Content: 98.0 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: freely soluble in water, sparingly soluble in ethanol (96 per cent), practically insoluble in methylene chloride.

IDENTIFICATION

First identification: B, C, E.

Second identification: A, C, D, E.

A. Melting point (*2.2.14*): 165 °C to 170 °C, with decomposition.

B. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *isoprenaline hydrochloride CRS.*

C. Optical rotation (see Tests).

D. To 0.1 mL of solution S (see Tests) add 0.05 mL of *ferric chloride solution R1* and 0.9 mL of *water R*. A green colour is produced. Add dropwise *sodium hydrogen carbonate solution R*. The colour becomes blue and then red.

E. To 0.5 mL of solution S add 1.5 mL of *water R*. The solution gives reaction (a) of chlorides (*2.3.1*).

TESTS

Prepare the solutions immediately before use.

Solution S. Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 25.0 mL with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution B_7 or BY_7 (*2.2.2, Method II*).

pH (*2.2.3*): 4.3 to 5.5.

Mix 5 mL of solution S and 5 mL of *carbon dioxide-free water R*.

Optical rotation (*2.2.7*): – 0.10° to + 0.10°, determined on solution S.

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution (a). Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 5.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b). Dissolve 2.5 mg of *orciprenaline sulfate CRS* in the mobile phase and dilute to 100 mL with the mobile phase.

Reference solution (c). To 5 mL of reference solution (a) add 5 mL of reference solution (b).

Reference solution (d). Dissolve 6.0 mg of *isoprenaline impurity A CRS* in the mobile phase and dilute to 50.0 mL with the mobile phase. Dilute 10.0 mL of the solution to 50.0 mL with the mobile phase.

Column:

- *size*: *l* = 0.125 m, Ø = 4.0 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase: *methanol R*, 11.5 g/L solution of *phosphoric acid R* (5:95 *V/V*).

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 280 nm.

Injection: 20 µL.

Run time: 7 times the retention time of isoprenaline.

Identification of impurities: use the chromatogram obtained with reference solution (d) to identify the peak due to impurity A; use the chromatogram obtained with reference solution (b) to identify the peak due to orciprenaline.

Relative retention with reference to isoprenaline (retention time = about 4 min): orciprenaline = about 1.5; impurity A = about 1.8. If necessary, adjust the concentration of methanol in the mobile phase.

System suitability: reference solution (c):

- *resolution*: minimum 3.0 between the peaks due to isoprenaline and orciprenaline.

Limits:

- *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (d) (0.5 per cent);
- *unspecified impurities*: for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *total*: maximum 1.0 per cent;
- *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*): maximum 1.0 per cent, determined on 1.000 g by drying *in vacuo* at 15-25 °C for 4 h.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

In order to avoid overheating in the reaction medium, mix thoroughly throughout and stop the titration immediately after the end-point has been reached.

Dissolve 0.150 g in 10 mL of *anhydrous formic acid R* and add 50 mL of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

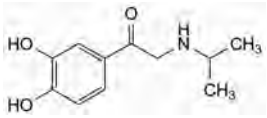
1 mL of *0.1 M perchloric acid* is equivalent to 24.77 mg of $C_{11}H_{18}ClNO_3$.

STORAGE

In an airtight container, protected from light.

IMPURITIES

Specified impurities: A.

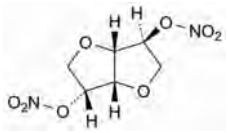


A. 1-(3,4-dihydroxyphenyl)-2-[(1-methylethyl)amino]-ethanone.

01/2008:1117
corrected 10.1

ISOSORBIDE DINITRATE, DILUTED

Isosorbidi dinitras dilutus



$C_6H_8N_2O_8$ M_r 236.1

DEFINITION

Dry mixture of isosorbide dinitrate and *Lactose monohydrate (0187)* or *Mannitol (0559)*.

Content: 95.0 per cent *m/m* to 105.0 per cent *m/m* of the content of 1,4:3,6-dianhydro-D-glucitol 2,5-dinitrate stated on the label.

CAUTION: undiluted isosorbide dinitrate may explode if subjected to percussion or excessive heat. Appropriate precautions must be taken and only very small quantities handled.

CHARACTERS

Appearance: undiluted isosorbide dinitrate is a fine, white or almost white, crystalline powder.

Solubility: undiluted isosorbide dinitrate is very slightly soluble in water, very soluble in acetone, sparingly soluble in ethanol (96 per cent).

The solubility of the diluted product depends on the diluent and its concentration.

IDENTIFICATION

First identification: A, C, D.

Second identification: B, C, D.

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation: discs prepared with the residue obtained in identification test D.

Comparison: *isosorbide dinitrate CRS*.

B. Thin-layer chromatography (*2.2.27*).

Test solution. Shake a quantity of the substance to be examined corresponding to 10 mg of isosorbide dinitrate with 10 mL of *ethanol (96 per cent) R* for 5 min and filter.

Reference solution. Shake a quantity of *isosorbide dinitrate CRS* corresponding to 10 mg of isosorbide dinitrate with 10 mL of *ethanol (96 per cent) R* for 5 min and filter.

Plate: *TLC silica gel G plate R.*

Mobile phase: *methanol R*, *methylene chloride R* (5:95 *V/V*).

Application: 10 µL.

Development: over a path of 15 cm.

Drying: in a current of air.

Detection: spray with freshly prepared *potassium iodide and starch solution R*; expose to ultraviolet light at 254 nm for 15 min and examine in daylight.

Results: the principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

C. Thin-layer chromatography (*2.2.27*).

Test solution. Shake a quantity of the substance to be examined corresponding to 0.10 g of lactose or mannitol with 10 mL of *water R*. Filter if necessary.

Reference solution (a). Dissolve 0.10 g of *lactose monohydrate R* in *water R* and dilute to 10 mL with the same solvent.

Reference solution (b). Dissolve 0.10 g of *mannitol R* in *water R* and dilute to 10 mL with the same solvent.

Reference solution (c). Mix equal volumes of reference solutions (a) and (b).

Plate: *TLC silica gel G plate R.*

Mobile phase: *water R*, *methanol R*, *anhydrous acetic acid R*, *ethylene chloride R* (10:15:25:50 *V/V/V/V*); measure the volumes accurately since a slight excess of water produces cloudiness.

Application: 1 µL; thoroughly dry the points of application.

Development A: over a path of 15 cm.

Drying A: in a current of warm air.

Development B: immediately, over a path of 15 cm, after renewing the mobile phase.

Drying B: in a current of warm air.

Detection: spray with *4-aminobenzoic acid solution R*, dry in a current of cold air until the acetone is removed, then heat at 100 °C for 15 min; allow to cool, spray with a 2 g/L solution of *sodium periodate R*, dry in a current of cold air, and heat at 100 °C for 15 min.

System suitability: reference solution (c):

– the chromatogram shows 2 clearly separated spots.

Results: the principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a) for lactose or to the principal spot in the chromatogram obtained with reference solution (b) for mannitol.

D. Shake a quantity of the substance to be examined corresponding to 25 mg of isosorbide dinitrate with 10 mL of *acetone R* for 5 min. Filter, evaporate to dryness at a temperature below 40 °C and dry the residue over *diphosphorus pentoxide R* at a pressure of 0.7 kPa for 16 h. The melting point (*2.2.14*) of the residue is 69 °C to 72 °C.

TESTS

Impurity A. Thin-layer chromatography (*2.2.27*).

Test solution. Shake a quantity of the substance to be examined corresponding to 0.10 g of isosorbide dinitrate with 5 mL of *ethanol (96 per cent) R* and filter.

Reference solution. Dissolve 10 mg of *potassium nitrate R* in 1 mL of *water R* and dilute to 100 mL with *ethanol (96 per cent) R*.

Plate: *TLC silica gel plate R.*

Mobile phase: *glacial acetic acid R*, *acetone R*, *toluene R* (15:30:60 *V/V/V*).

Application: 10 µL.

Development: over a path of 15 cm.

Drying: in a current of air until the acetic acid is completely removed.

Detection: spray copiously with freshly prepared *potassium iodide and starch solution R*; expose to ultraviolet light at 254 nm for 15 min and examine in daylight.

Limit:

– *impurity A*: any spot due to impurity A is not more intense than the principal spot in the chromatogram obtained with the reference solution (0.5 per cent, calculated as potassium nitrate).

Impurities B and C. Liquid chromatography (*2.2.29*).

Test solution (a). Sonicate a quantity of the substance to be examined corresponding to 25.0 mg of isosorbide dinitrate with 20 mL of the mobile phase for 15 min and dilute to 25.0 mL with the mobile phase. Filter the solution through a suitable membrane filter.

Test solution (b). Dilute 1.0 mL of test solution (a) to 10.0 mL with the mobile phase.

Reference solution (a). Sonicate a quantity of *isosorbide dinitrate CRS* corresponding to 25.0 mg of isosorbide dinitrate with 20 mL of the mobile phase for 15 min and dilute to 25.0 mL with the mobile phase. Filter the solution through a suitable membrane filter.

Reference solution (b). Dilute 1.0 mL of reference solution (a) to 10.0 mL with the mobile phase.

Reference solution (c). Dissolve 10.0 mg of *isosorbide-2-nitrate CRS* (impurity B) in the mobile phase and dilute to 10.0 mL with the mobile phase. Dilute 0.1 mL of this solution to 20.0 mL with the mobile phase.

Reference solution (d). Dissolve 10.0 mg of *isosorbide mononitrate CRS* (impurity C) in the mobile phase and dilute to 10.0 mL with the mobile phase. Dilute 0.1 mL of this solution to 20.0 mL with the mobile phase.

Reference solution (e). Dissolve 5 mg of *isosorbide-2-nitrate CRS* (impurity B) in the mobile phase and dilute to 10 mL with the mobile phase. To 1 mL of this solution add 0.5 mL of reference solution (a) and dilute to 10 mL with the mobile phase.

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm;

– *stationary phase*: *aminopropylsilyl silica gel for chromatography R* (10 µm).

Mobile phase: *anhydrous ethanol R*, *trimethylpentane R* (15:85 *V/V*).

Flow rate: 1 mL/min.

Detection: spectrophotometer at 210-215 nm.

Injection: 10 µL of test solution (a) and reference solutions (c), (d) and (e).

Retention time: isosorbide dinitrate = about 5 min; impurity B = about 8 min; impurity C = about 11 min.

System suitability: reference solution (e):

– *resolution*: minimum 6.0 between the peaks due to isosorbide dinitrate and impurity B.

Limits:

– *impurity B*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent);

– *impurity C*: not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (0.5 per cent).

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for impurities B and C with the following modifications.

Detection: spectrophotometer at 230 nm.

Injection: 20 µL of test solution (b) and reference solution (b).

If the areas of the peaks from 2 successive injections of reference solution (b) do not agree to within 1.0 per cent, then inject a further 4 times and calculate, for the 6 injections, the relative standard deviation.

System suitability: reference solution (b):

– *repeatability*: maximum relative standard deviation of 2.0 per cent after 6 injections.

Calculate the content of isosorbide dinitrate as a percentage of the declared content.

STORAGE

Protected from light.

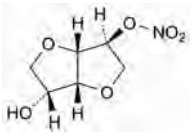
LABELLING

The label states the percentage content of isosorbide dinitrate.

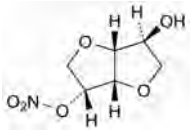
IMPURITIES

Specified impurities: A, B, C.

A. inorganic nitrates,



B. 1,4:3,6-dianhydro-D-glucitol 2-nitrate (isosorbide 2-nitrate),

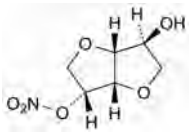


C. 1,4:3,6-dianhydro-D-glucitol 5-nitrate (isosorbide 5-nitrate, isosorbide mononitrate).

01/2008:1118
corrected 10.1

ISOSORBIDE MONONITRATE, DILUTED

Isosorbidi mononitras dilutus



$C_6H_9NO_6$ M_r 191.1

DEFINITION

Dry mixture of isosorbide mononitrate and *Lactose monohydrate (0187)* or *Mannitol (0559)*.

Content: 95.0 per cent *m/m* to 105.0 per cent *m/m* of the content of 1,4:3,6-dianhydro-D-glucitol 5-nitrate stated on the label.

CHARACTERS

Appearance: undiluted isosorbide mononitrate is a white or almost white, crystalline powder.

Solubility: undiluted isosorbide mononitrate is freely soluble in water, in acetone, in ethanol (96 per cent) and in methylene chloride.

The solubility of the diluted product depends on the diluent and its concentration.

IDENTIFICATION

First identification: A, C, D.

Second identification: B, C, D.

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation: discs prepared with the residue obtained in identification test D.

Comparison: *isosorbide mononitrate CRS*.

B. Thin-layer chromatography (*2.2.27*).

Test solution. Shake a quantity of the substance to be examined corresponding to 10 mg of isosorbide mononitrate with 10 mL of *ethanol (96 per cent) R* for 5 min and filter.

Reference solution. Dissolve 10 mg of *isosorbide mononitrate CRS* in *ethanol (96 per cent) R* and dilute to 10 mL with the same solvent.

Plate: *TLC silica gel G plate R*.

Mobile phase: *methanol R*, *methylene chloride R* (5:95 *V/V*).

Application: 10 µL.

Development: over a path of 15 cm.

Drying: in a current of air.

Detection: spray with freshly prepared *potassium iodide and starch solution R*. Expose to ultraviolet light at 254 nm for 15 min and examine in daylight.

Results: the principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

C. Thin-layer chromatography (*2.2.27*).

Test solution. Shake a quantity of the substance to be examined corresponding to 0.10 g of lactose or mannitol with 10 mL of *water R*; filter if necessary.

Reference solution (a). Dissolve 0.10 g of *lactose monohydrate R* in *water R* and dilute to 10 mL with the same solvent.

Reference solution (b). Dissolve 0.10 g of *mannitol R* in *water R* and dilute to 10 mL with the same solvent.

Reference solution (c). Mix equal volumes of reference solutions (a) and (b).

Plate: *TLC silica gel G plate R*.

Mobile phase: *water R*, *methanol R*, *anhydrous acetic acid R*, *ethylene chloride R* (10:15:25:50 *V/V/V/V*); measure the volumes accurately since a slight excess of water produces cloudiness.

Application: 1 µL; thoroughly dry the points of application.

Development A: over a path of 15 cm.

Drying A: in a current of warm air.

Development B: immediately, over a path of 15 cm, after renewing the mobile phase.

Drying B: in a current of warm air.

Detection: spray with *4-aminobenzoic acid solution R* and dry in a current of cold air until the acetone is removed; heat at 100 °C for 15 min and allow to cool; spray with a 2 g/L solution of *sodium periodate R* and dry in a current of cold air; heat at 100 °C for 15 min.

System suitability: reference solution (c):

– the chromatogram shows 2 clearly separated spots.

Results: the principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with

reference solution (a) for lactose or to the principal spot in the chromatogram obtained with reference solution (b) for mannitol.

D. Shake a quantity of the substance to be examined corresponding to 25 mg of isosorbide mononitrate with 10 mL of *acetone R* for 5 min. Filter, evaporate to dryness at a temperature below 40 °C and dry the residue over *diphosphorus pentoxide R* at a pressure of 0.7 kPa for 16 h. The melting point (*2.2.14*) of the residue is 89 °C to 91 °C.

TESTS

Impurity A. Thin-layer chromatography (*2.2.27*).

Test solution. Shake a quantity of the substance to be examined corresponding to 0.10 g of isosorbide mononitrate with 5 mL of *ethanol (96 per cent) R* and filter.

Reference solution. Dissolve 10 mg of *potassium nitrate R* in 1 mL of *water R* and dilute to 100 mL with *ethanol (96 per cent) R*.

Plate: *TLC silica gel plate R*.

Mobile phase: *glacial acetic acid R*, *acetone R*, *toluene R* (15:30:60 *V/V/V*).

Application: 10 µL.

Development: over a path of 15 cm.

Drying: in a current of air until the acetic acid is completely removed.

Detection: spray copiously with freshly prepared *potassium iodide and starch solution R*; expose to ultraviolet light at 254 nm for 15 min and examine in daylight.

Limit:

– *impurity A*: any spot due to impurity A is not more intense than the principal spot in the chromatogram obtained with the reference solution (0.5 per cent, calculated as potassium nitrate).

Impurities B and C. Liquid chromatography (*2.2.29*).

Test solution (a). Sonicate a quantity of the substance to be examined corresponding to 25.0 mg of isosorbide mononitrate with 20 mL of the mobile phase for 15 min and dilute to 25.0 mL with the mobile phase. Filter the solution through a suitable membrane filter.

Test solution (b). Dilute 1.0 mL of test solution (a) to 10.0 mL with the mobile phase.

Reference solution (a). Dissolve 25.0 mg of *isosorbide mononitrate CRS* in the mobile phase and dilute to 25.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b). Dissolve 10.0 mg of *isosorbide-2-nitrate CRS* (impurity C) in the mobile phase and dilute to 10.0 mL with the mobile phase. Dilute 0.1 mL of this solution to 20.0 mL with the mobile phase.

Reference solution (c). Sonicate a quantity of *isosorbide dinitrate CRS* (impurity B) corresponding to 10.0 mg of isosorbide dinitrate in 15 mL of the mobile phase for 15 min and dilute to 20.0 mL with the mobile phase. Filter the solution through a suitable membrane filter. Dilute 0.1 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (d). Dissolve 5 mg of *isosorbide mononitrate CRS* and 5 mg of *isosorbide-2-nitrate CRS* (impurity C) in the mobile phase and dilute to 10 mL with the mobile phase. Dilute 1 mL of this solution to 10 mL with the mobile phase.

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm;
– *stationary phase*: *aminopropylsilyl silica gel for chromatography R* (10 µm).

Mobile phase: *anhydrous ethanol R*, *trimethylpentane R* (15:85 *V/V*).

Flow rate: 1 mL/min.

Detection: spectrophotometer at 210-215 nm.

Injection: 10 µL of test solution (a) and reference solutions (b), (c) and (d).

Retention time: impurity B = about 5 min; impurity C = about 8 min; isosorbide 5-nitrate = about 11 min.

System suitability: reference solution (d):

– *resolution*: minimum 4.0 between the peaks due to impurity C and isosorbide 5-nitrate.

Limits:

– *impurity B*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent);
– *impurity C*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent).

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for impurities B and C with the following modifications.

Detection: spectrophotometer at 230 nm.

Injection: 20 µL of test solution (b) and reference solution (a).

If the areas of the peaks from 2 successive injections of reference solution (a) do not agree to within 1.0 per cent, then inject a further 4 times and calculate, for the 6 injections, the relative standard deviation.

System suitability: reference solution (a):

– *repeatability*: maximum relative standard deviation of 2.0 per cent after 6 injections.

Calculate the content of isosorbide mononitrate as a percentage of the declared content.

STORAGE

Protected from light.

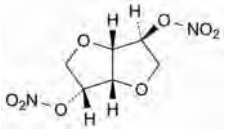
LABELLING

The label states the percentage content of isosorbide mononitrate.

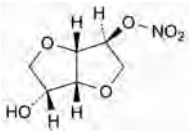
IMPURITIES

Specified impurities: A, B, C.

A. inorganic nitrates,



B. 1,4:3,6-dianhydro-D-glucitol 2,5-dinitrate (isosorbide dinitrate),



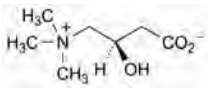
C. 1,4:3,6-dianhydro-D-glucitol 2-nitrate (isosorbide 2-nitrate).

L

01/2019:1339
corrected 10.1

LEVOCARNITINE

Levocarnitinum



$C_7H_{15}NO_3$ M_r 161.2
[541-15-1]

DEFINITION

(3*R*)-3-Hydroxy-4-(trimethylazaniumyl)butanoate.

Content: 98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance: white or almost white, crystalline powder or colourless crystals, hygroscopic.

Solubility: freely soluble in water, soluble in warm ethanol (96 per cent), practically insoluble in acetone.

IDENTIFICATION

First identification: A, B.

Second identification: A, C.

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation: discs, prepared using substance previously dried *in vacuo* at 50 °C for 5 h.

Comparison: *levocarnitine CRS*.

C. To 1 mL of solution S (see Tests) add 9 mL of *water R*, 10 mL of *dilute sulfuric acid R* and 30 mL of *ammonium reineckate solution R*. A pink precipitate is formed. Allow to stand for 30 min. Filter and wash with *water R*, with *ethanol (96 per cent) R* and then with *acetone R* and dry at 80 °C. The precipitate melts (*2.2.14*) at 147 °C to 150 °C.

TESTS

Solution S. Dissolve 5.00 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 50.0 mL with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*): 6.5 to 8.5.

Dilute 10 mL of solution S to 20 mL with *carbon dioxide-free water R*.

Specific optical rotation (*2.2.7*): − 32.0 to − 29.0 (anhydrous substance), determined on solution S at 25 °C.

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 0.100 g of the substance to be examined in the mobile phase and dilute to 20.0 mL with the mobile phase.

Reference solution (a). Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b). Dissolve 12.5 mg of *levocarnitine impurity A CRS* in *water R* and dilute to 50.0 mL with the same solvent. Dilute 2.0 mL of the solution to 20.0 mL with the mobile phase.

Reference solution (c). Dissolve 50 mg of the substance to be examined in 10 mL of reference solution (b).

Column:

– *size*: *l* = 0.30 m, Ø = 3.9 mm;

– *stationary phase*: *aminopropylsilyl silica gel for chromatography R* (10 µm);

– *temperature*: 30 °C.

Mobile phase: mix 35 volumes of a 6.81 g/L solution of *potassium dihydrogen phosphate R* previously adjusted to pH 4.7 with *dilute sodium hydroxide solution R*, and 65 volumes of *acetonitrile R1*.

Flow rate: 1 mL/min.

Detection: spectrophotometer at 205 nm.

Injection: 25 µL.

Run time: twice the retention time of levocarnitine.

Identification of impurities: use the chromatogram obtained with reference solution (b) to identify the peak due to impurity A.

Relative retention with reference to levocarnitine (retention time = about 10 min): impurity A = about 1.1.

System suitability: reference solution (c):

– *resolution*: minimum 1.5 between the peaks due to levocarnitine and impurity A.

Limits:

– *impurity A*: not more than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);

– *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

– *total (excluding impurity A)*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

– *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Chlorides (*2.4.4*): maximum 200 ppm.

Dilute 2.5 mL of solution S to 15 mL with *water R*.

Sulfates (*2.4.13*): maximum 300 ppm.

Dilute 5 mL of solution S to 15 mL with *distilled water R*.

Water (*2.5.12*): maximum 1.0 per cent, determined on 2.00 g.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.125 g in a mixture of 3 volumes of *anhydrous formic acid R* and 50 volumes of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 16.12 mg of $C_7H_{15}NO_3$.

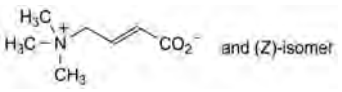
STORAGE

In an airtight container.

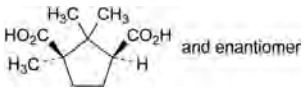
IMPURITIES

Specified impurities: A.

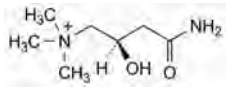
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *B, C, D.*



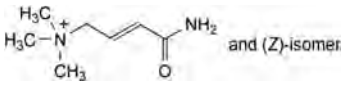
A. (2*EZ*)-4-(trimethylazaniumyl)but-2-enoate,



B. (1*RS*,3*SR*)-1,2,2-trimethylcyclopentane-1,3-dicarboxylic acid (camphoric acid),



C. (2*R*)-4-amino-2-hydroxy-*N,N,N*-trimethyl-4-oxobutan-1-aminium (carnitinamide),



D. (2*EZ*)-4-amino-*N,N,N*-trimethyl-4-oxobut-2-en-1-aminium.

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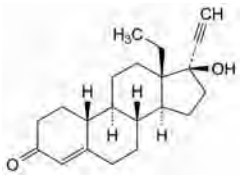
corrected 10.1

LEVONORGESTREL

Levonorgestrelum



$C_{21}H_{28}O_2$ M_r 312.5
[797-63-7]

DEFINITION

13-Ethyl-17-hydroxy-18,19-dinor-17α-pregn-4-en-20-yn-3-one.

Content: 98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: practically insoluble in water, sparingly soluble in methylene chloride, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *levonorgestrel CRS*.

TESTS

Specific optical rotation (*2.2.7*): − 35 to − 30.

Dissolve 0.200 g in *methylene chloride R* and dilute to 20.0 mL with the same solvent.

Related substances

A. Impurities A, B, H, K, M, O, S, U. Liquid chromatography (*2.2.29*).

Solvent mixture: *water R*, *acetonitrile R* (30:70 *V/V*).

Test solution. Dissolve 10.0 mg of the substance to be examined in 7 mL of *acetonitrile R* using sonication and dilute to 10.0 mL with *water R*.

Reference solution (a). Dissolve 5 mg of *levonorgestrel for system suitability 1 CRS* (containing impurities A, H, K, M, O and S) in 3.5 mL of *acetonitrile R* using sonication and dilute to 5 mL with *water R*.

Reference solution (b). Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (c). Dissolve 5.0 mg of *levonorgestrel impurity B CRS* in 35 mL of *acetonitrile R* and dilute to 50.0 mL with *water R*. Dilute 1.0 mL of the solution to 100.0 mL with the solvent mixture.

Reference solution (d). Dissolve 5.0 mg of *norethisterone CRS* (impurity U) in 35 mL of *acetonitrile R* and dilute to 50.0 mL with *water R*. Dilute 1.0 mL of the solution to 100.0 mL with the solvent mixture.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octylsilyl silica gel for chromatography with embedded polar groups R* (5 µm);
- *temperature*: 30 °C.

Mobile phase:
- *mobile phase A*: *acetonitrile R1*, *water for chromatography R* (40:60 *V/V*);
- *mobile phase B*: *acetonitrile R1*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 50	100 → 20	0 → 80

Flow rate: 0.7 mL/min.

Detection: spectrophotometer at 215 nm and, for impurity O, at 200 nm.

Injection: 50 µL.

Identification of impurities: use the chromatograms supplied with *levonorgestrel for system suitability 1 CRS* and the chromatograms obtained with reference solution (a) at 215 nm to identify the peaks due to impurities A, H, K, M and S, and at 200 nm to identify the peak due to impurity O; use the chromatogram obtained with reference solution (c) to identify the peak due to impurity B; use the chromatogram obtained with reference solution (d) to identify the peak due to impurity U.

Relative retention with reference to levonorgestrel (retention time = about 20 min): impurity H = about 0.5; impurity U = about 0.8; impurity K = about 0.85; impurity A = about 0.91; impurity M = about 0.95; impurity O = about 1.16; impurity B = about 1.26; impurity S = about 1.9.

System suitability:
- *signal-to-noise ratio*: minimum 60 for the principal peak in the chromatogram obtained with reference solution (b);
- *peak-to-valley ratio*: minimum 3.0, where H_p = height above the baseline of the peak due to impurity M and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity A in the chromatogram obtained with reference solution (a).

Calculation of percentage contents:
- *correction factors*: multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 0.4; impurity M = 3.1; impurity O = 2.6;
- for impurity B, use the concentration of impurity B in reference solution (c);
- for impurity U, use the concentration of impurity U in reference solution (d);
- for impurities other than B and U, use the concentration of levonorgestrel in reference solution (b).

Limits:

- *impurities A, B, K*: for each impurity, maximum 0.3 per cent;
- *impurity O at 200 nm*: maximum 0.3 per cent;
- *impurities M, S, U*: for each impurity, maximum 0.2 per cent;
- *impurity H*: maximum 0.15 per cent;
- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- *sum of impurities other than O*: maximum 1.0 per cent;
- *reporting threshold*: 0.05 per cent.

B. Impurities V and W. Liquid chromatography (*2.2.29*).

Solvent mixture: *water R*, *acetonitrile R* (30:70 *V/V*).

Test solution. Dissolve 10.0 mg of the substance to be examined in 7 mL of *acetonitrile R* using sonication and dilute to 10.0 mL with *water R*.

Reference solution (a). Dissolve 5 mg of *levonorgestrel for system suitability 2 CRS* (containing impurities V and W) in 3.5 mL of *acetonitrile R* using sonication and dilute to 5 mL with *water R*.

Reference solution (b). Dissolve 5.0 mg of *ethinylestradiol CRS* in 35 mL of *acetonitrile R* using sonication and dilute to 50.0 mL with *water R*. Dilute 3.0 mL of the solution to 100.0 mL with the solvent mixture.

Column:

- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography compatible with 100 per cent aqueous mobile phases R* (3 µm).

Mobile phase:

- *mobile phase A*: *acetonitrile R1*, *water for chromatography R* (40:60 *V/V*);
- *mobile phase B*: *water for chromatography R*, *acetonitrile R1* (10:90 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 1	92	8
1 - 3	92 → 82	8 → 18
3 - 6	82	18
6 - 16	82 → 60	18 → 40
16 - 21	60 → 0	40 → 100
21 - 32	0	100

Flow rate: 1 mL/min.

Detection: spectrophotometer at 200 nm.

Injection: 50 µL.

Identification of impurities: use the chromatogram supplied with *levonorgestrel for system suitability 2 CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities V and W.

Relative retention with reference to levonorgestrel (retention time = about 12 min): impurity W = about 0.9; impurity V = about 1.9.

System suitability: reference solution (a):

- *resolution*: minimum 2.8 between the peaks due to impurity W and levonorgestrel.

Calculation of percentage contents:

- for each impurity, use the concentration of ethinylestradiol in reference solution (b).

Limits:

- *impurity W*: maximum 0.3 per cent;
- *impurity V*: maximum 0.15 per cent.

Loss on drying (*2.2.32*): maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 45 mL of *tetrahydrofuran R*. Add 10 mL of a 100 g/L solution of *silver nitrate R*. After 1 min, titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*). Carry out a blank titration.

1 mL of *0.1 M sodium hydroxide* is equivalent to 31.25 mg of $C_{21}H_{28}O_2$.

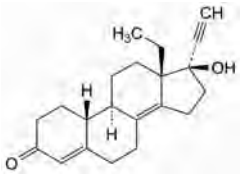
STORAGE

Protected from light.

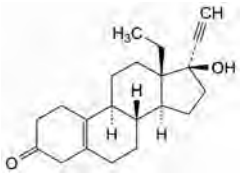
IMPURITIES

Specified impurities: A, B, H, K, M, O, S, U, V, W.

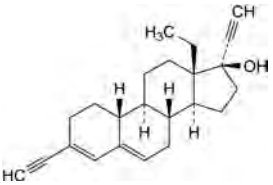
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *C, D, G, I, J, L, N, P, Q, R, T.*



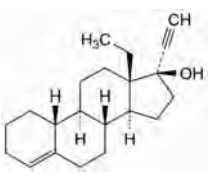
A. 13-ethyl-17-hydroxy-18,19-dinor-17α-pregna-4,8(14)-dien-20-yn-3-one,



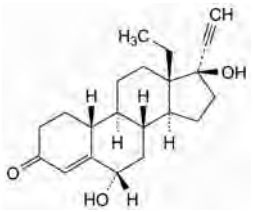
B. 13-ethyl-17-hydroxy-18,19-dinor-17α-pregn-5(10)-en-20-yn-3-one,



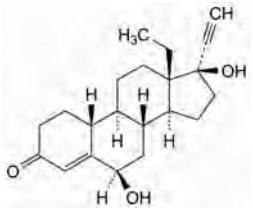
C. 13-ethyl-3-ethynyl-18,19-dinor-17α-pregna-3,5-dien-20-yn-17-ol,



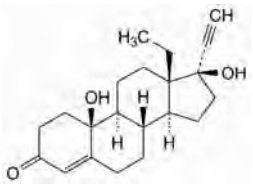
D. 13-ethyl-18,19-dinor-17α-pregn-4-en-20-yn-17-ol (3-deoxolevonorgestrel),



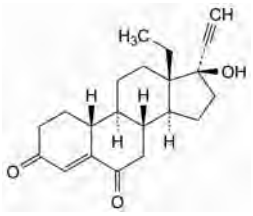
G. 13-ethyl-6α,17-dihydroxy-18,19-dinor-17α-pregn-4-en-20-yn-3-one (6α-hydroxylevonorgestrel),



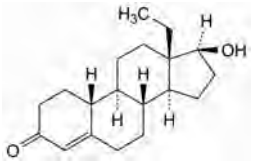
H. 13-ethyl-6β,17-dihydroxy-18,19-dinor-17α-pregn-4-en-20-yn-3-one (6β-hydroxylevonorgestrel),



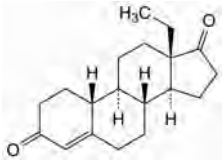
I. 13-ethyl-10,17-dihydroxy-18,19-dinor-17α-pregn-4-en-20-yn-3-one (10-hydroxylevonorgestrel),



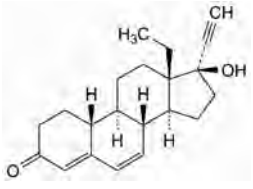
J. 13-ethyl-17-hydroxy-18,19-dinor-17α-pregn-4-en-20-yne-3,6-dione (6-oxolevonorgestrel),



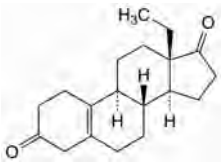
K. 13-ethyl-17β-hydroxygon-4-en-3-one (18-methylnandrolone),



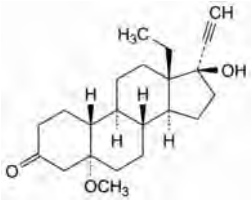
L. 13-ethylgon-4-ene-3,17-dione (levodione),



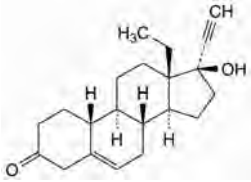
M. 13-ethyl-17-hydroxy-18,19-dinor-17α-pregna-4,6-dien-20-yn-3-one (Δ6-levonorgestrel),



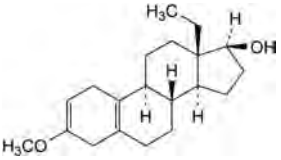
N. 13-ethylgon-5(10)-ene-3,17-dione (Δ5(10)-levodione),



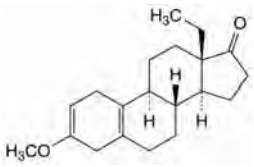
O. 13-ethyl-17-hydroxy-5α-methoxy-18,19-dinor-17α-pregn-20-yn-3-one (4,5-dihydro-5α-methoxylevonorgestrel),



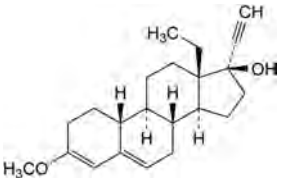
P. 13-ethyl-17-hydroxy-18,19-dinor-17α-pregn-5-en-20-yn-3-one (Δ5-levonorgestrel),



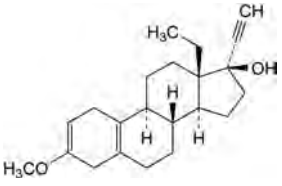
Q. 13-ethyl-3-methoxygona-2,5(10)-dien-17β-ol,



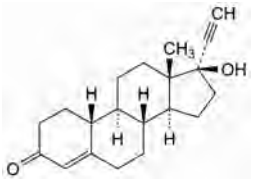
R. 13-ethyl-3-methoxygona-2,5(10)-dien-17-one,



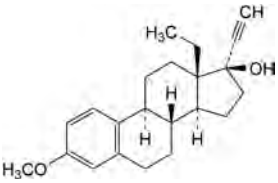
S. 13-ethyl-3-methoxy-18,19-dinor-17α-pregna-3,5-dien-20-yn-17-ol,



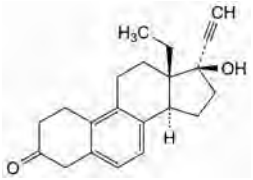
T. 13-ethyl-3-methoxy-18,19-dinor-17α-pregna-2,5(10)-dien-20-yn-17-ol,



U. 17-hydroxy-19-nor-17α-pregn-4-en-20-yn-3-one (norethisterone),



V. 13-ethyl-3-methoxy-18,19-dinor-17α-pregna-1,3,5(10)-trien-20-yn-17-ol,

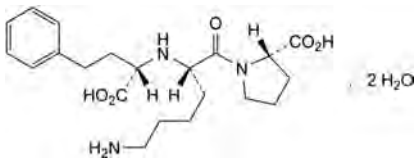


W. 13-ethyl-17-hydroxy-18,19-dinor-17α-pregna-5,7,9-trien-20-yn-3-one.

04/2020:1120

LISINOPRIL DIHYDRATE

Lisinoprilum dihydricum



$C_{21}H_{31}N_3O_5,2H_2O$ M_r 441.5
[83915-83-7]

DEFINITION

(2*S*)-1-[(2*S*)-6-Amino-2-[[(1*S*)-1-carboxy-3-phenylpropyl]-amino]hexanoyl]pyrrolidine-2-carboxylic acid dihydrate.

Content: 98.5 per cent to 101.5 per cent (anhydrous substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: soluble in water, practically insoluble in anhydrous ethanol and in heptane.

IDENTIFICATION

A. Specific optical rotation (*2.2.7*): − 47 to − 43 (anhydrous substance).

Dissolve 0.5 g in *zinc acetate solution R* and dilute to 50.0 mL with the same solvent.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *lisinopril dihydrate CRS*.

TESTS

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 50.0 mg of the substance to be examined in mobile phase A and dilute to 10.0 mL with mobile phase A.

Reference solution (a). Dissolve 5 mg of *lisinopril for system suitability A CRS* (containing impurities A and E) in mobile phase A and dilute to 1 mL with mobile phase A.

Reference solution (b). Dilute 1.0 mL of the test solution to 100.0 mL with mobile phase A. Dilute 1.0 mL of this solution to 10.0 mL with mobile phase A.

Reference solution (c). Dissolve the contents of a vial of *lisinopril impurity F CRS* in 1 mL of mobile phase A.

Reference solution (d). Dissolve 5 mg of *lisinopril for peak identification CRS* (containing impurity G) in mobile phase A and dilute to 1 mL with mobile phase A.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 50 °C.

Mobile phase:

- *mobile phase A*: mix 3 volumes of *acetonitrile R1* and 97 volumes of a 3.12 g/L solution of *sodium dihydrogen phosphate R* previously adjusted to pH 3.8 with *dilute phosphoric acid R*;
- *mobile phase B*: mix 20.5 volumes of *acetonitrile R1* and 79.5 volumes of a 3.12 g/L solution of *sodium dihydrogen phosphate R* previously adjusted to pH 3.5 with *dilute phosphoric acid R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	100	0
2 - 37	100 → 0	0 → 100
37 - 62	0	100

Flow rate: 1.6 mL/min.

Detection: spectrophotometer at 210 nm.

Injection: 50 µL.

Identification of impurities: use the chromatogram supplied with *lisinopril for system suitability A CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A and E; use the chromatogram obtained with reference solution (c) to identify the peak due to impurity F; use the chromatogram supplied with *lisinopril for peak identification CRS* and the chromatogram obtained with reference solution (d) to identify the peak due to impurity G.

Relative retention with reference to lisinopril (retention time = about 14 min): impurity A = about 0.7; impurity E = about 1.2; impurity F = about 1.9; impurity G = about 2.9.

System suitability:

- *resolution*: minimum 1.5 between the peaks due to lisinopril and impurity E in the chromatogram obtained with reference solution (a);
- *signal-to-noise ratio*: minimum 45 for the principal peak in the chromatogram obtained with reference solution (b).

Calculation of percentage contents:

- *correction factor*: multiply the peak area of impurity F by 2.1;
- for each impurity, use the concentration of lisinopril dihydrate in reference solution (b).

Limits:

- *impurities A, E, F*: for each impurity, maximum 0.2 per cent;
- *impurity G*: maximum 0.15 per cent;
- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- *total*: maximum 0.5 per cent;
- *reporting threshold*: 0.05 per cent; disregard any peak with a retention time less than 3 min.

Water (*2.5.12*): 8.0 per cent to 9.5 per cent, determined on 0.200 g.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

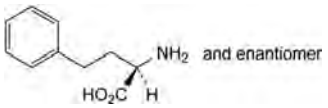
Dissolve 0.350 g in 50 mL of *water R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*) at the 1st point of inflexion.

1 mL of *0.1 M sodium hydroxide* is equivalent to 40.55 mg of $C_{21}H_{31}N_3O_5$.

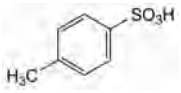
IMPURITIES

Specified impurities: A, E, F, G.

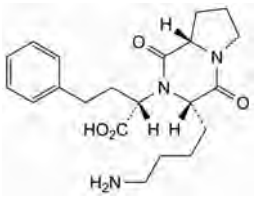
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *B, C, D, H, I, J.*



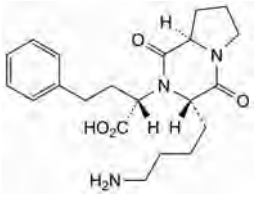
A. (2*RS*)-2-amino-4-phenylbutanoic acid,



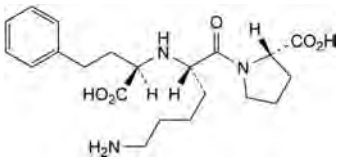
B. 4-methylbenzenesulfonic acid,



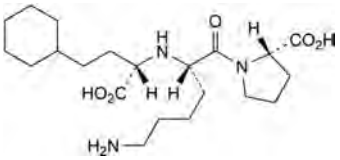
C. (2*S*)-2-[(3*S*,8a*S*)-3-(4-aminobutyl)-1,4-dioxohexahydropyrrolo[1,2-*a*]pyrazin-2(1*H*)-yl]-4-phenylbutanoic acid (*S*,*S*,*S*-diketopiperazine),



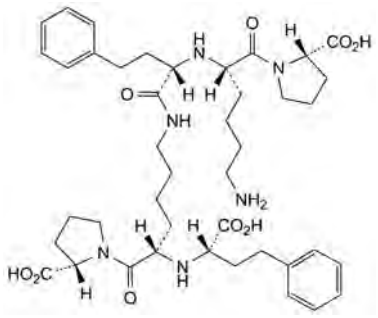
D. (2*S*)-2-[(3*S*,8a*R*)-3-(4-aminobutyl)-1,4-dioxohexahydropyrrolo[1,2-*a*]pyrazin-2(1*H*)-yl]-4-phenylbutanoic acid (*R*,*S*,*S*-diketopiperazine),



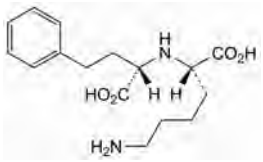
E. (2*S*)-1-[(2*S*)-6-amino-2-[[(1*R*)-1-carboxy-3-phenylpropyl]amino]hexanoyl]pyrrolidine-2-carboxylic acid (lisinopril *R*,*S*,*S*-isomer),



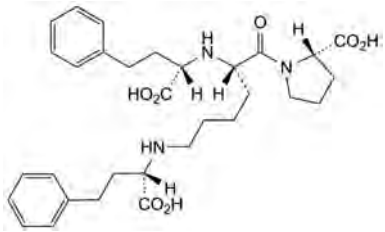
F. (2*S*)-1-[(2*S*)-6-amino-2-[[(1*S*)-1-carboxy-3-cyclohexylpropyl]amino]hexanoyl]pyrrolidine-2-carboxylic acid (cyclohexyl analogue),



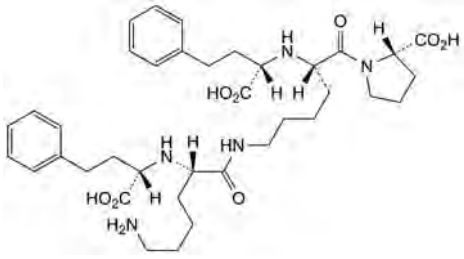
G. (2*S*)-1-[(2*S*)-6-amino-2-[[(2*S*)-1-[[(5*S*)-5-[[(1*S*)-1-carboxy-3-phenylpropyl]amino]-6-[(2*S*)-2-carboxypyrrolidin-1-yl]-6-oxohexyl]amino]-1-oxo-4-phenylbutan-2-yl]amino]hexanoyl]pyrrolidine-2-carboxylic acid (lisinopril dimer),



H. (2*S*)-6-amino-2-[[(1*S*)-1-carboxy-3-phenylpropyl]amino]-hexanoic acid,



I. (2*S*)-1-[(2*S*)-2,6-bis[[(1*S*)-1-carboxy-3-phenylpropyl]-amino]hexanoyl]pyrrolidine-2-carboxylic acid,



J. (2*S*)-1-[(2*S*)-6-[(2*S*)-6-amino-2-[[(1*S*)-1-carboxy-3-phenylpropyl]amino]hexanamido]-2-[[(1*S*)-1-carboxy-3-phenylpropyl]amino]hexanoyl]pyrrolidine-2-carboxylic acid.

M

04/2020:1342

MAIZE OIL, REFINED

Maydis oleum raffinatum

DEFINITION

Fatty oil obtained from the seeds of *Zea mays* L. by mechanical expression or by extraction. It is then refined.

PRODUCTION

The oil is prepared using materials and methods designed to ensure that the content of brassicasterol (*2.4.23*) in the sterol fraction of the oil is not greater than 0.3 per cent.

CHARACTERS

Appearance: clear, light yellow or yellow oil.

Solubility: practically insoluble in water and in ethanol (96 per cent), miscible with light petroleum (bp: 40-60 °C) and with methylene chloride.

Relative density: about 0.920.

Refractive index: about 1.474.

IDENTIFICATION

First identification: B.

Second identification: A.

A. Identification of fatty oils by thin-layer chromatography (*2.3.2*).

Results: the chromatogram obtained with the test solution is similar to the chromatogram obtained with the reference solution.

B. Composition of fatty acids (see Tests).

TESTS

Acid value (*2.5.1*): maximum 0.5, or maximum 0.3 if intended for use in the manufacture of parenteral preparations, determined on 10.0 g.

Peroxide value (*2.5.5, Method A*): maximum 10.0, or maximum 5.0 if intended for use in the manufacture of parenteral preparations.

Unsaponifiable matter (*2.5.7*): maximum 2.8 per cent, determined on 5.0 g.

Alkaline impurities (*2.4.19*). It complies with the test.

Composition of fatty acids (*2.4.22, Method A*). Use the mixture of calibrating substances in Table 2.4.22.-3.

Composition of the fatty-acid fraction of the oil:

- *sum of fatty acids of chain length less than* C_{16}: maximum 0.6 per cent;
- *palmitic acid*: 8.6 per cent to 16.5 per cent;
- *stearic acid*: maximum 3.3 per cent;
- *oleic acid and isomer*: 20.0 per cent to 42.2 per cent;
- *linoleic acid*: 39.4 per cent to 65.6 per cent;
- *linolenic acid*: 0.5 per cent to 1.5 per cent;
- *arachidic acid*: maximum 0.8 per cent;
- *eicosenoic acid*: maximum 0.5 per cent;
- *behenic acid*: maximum 0.5 per cent;
- *any other fatty acid*: for each fatty acid, maximum 0.5 per cent.

Water (*2.5.32*): maximum 0.1 per cent, determined on 1.00 g.

STORAGE

Protected from light, at a temperature not exceeding 25 °C.

LABELLING

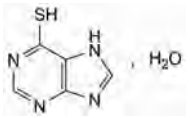
The label states:

- where applicable, that the substance is suitable for use in the manufacture of parenteral preparations;
- whether the oil is obtained by mechanical expression or by extraction.

04/2020:0096

MERCAPTOPURINE MONOHYDRATE

Mercaptopurinum monohydricum



$C_5H_4N_4S,H_2O$ M_r 170.2

[6112-76-1]

DEFINITION

7*H*-Purine-6-thiol monohydrate.

Content: 98.5 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance: yellow, crystalline powder.

Solubility: practically insoluble in water, slightly soluble in ethanol (96 per cent), practically insoluble in heptane. It dissolves in solutions of alkali hydroxides.

IDENTIFICATION

A. Dissolve 20 mg in 5 mL of *dimethyl sulfoxide R* and dilute to 100 mL with a 10.3 g/L solution of *hydrochloric acid R*. Dilute 5 mL of this solution to 200 mL with a 10.3 g/L solution of *hydrochloric acid R*. Examined between 230 nm and 350 nm (*2.2.25*), the solution shows only 1 absorption maximum, at 325 nm.

B. Dissolve about 20 mg in 20 mL of *ethanol (96 per cent) R* heated to 60 °C and add 1 mL of a saturated solution of *mercuric acetate R* in *ethanol (96 per cent) R*. A white precipitate is formed.

C. Dissolve about 20 mg in 20 mL of *ethanol (96 per cent) R* heated to 60 °C and add 1 mL of a 10 g/L solution of *lead acetate R* in *ethanol (96 per cent) R*. A yellow precipitate is formed.

TESTS

Related substances. Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution. Dissolve 12.0 mg of the substance to be examined in 2.5 mL of *methanol R* and dilute to 100.0 mL with a 0.1 per cent *V/V* solution of *anhydrous formic acid R*.

Reference solution (a). Dissolve 3.0 mg of *mercaptopurine impurity B CRS* and 4.5 mg of *mercaptopurine impurity A CRS* in mobile phase A and dilute to 250.0 mL with mobile phase A. Dilute 1.0 mL of the solution to 100.0 mL with mobile phase A.

Reference solution (b). Dilute 1.0 mL of the test solution to 100.0 mL with a 0.1 per cent *V/V* solution of *anhydrous formic acid R*.

Reference solution (c). Dissolve 3 mg of *mercaptopurine impurity D CRS* in 10 mL of *dimethyl sulfoxide R* and dilute to 250 mL with mobile phase A. Dilute 1 mL of the solution to 100 mL with mobile phase A.

Column:

- *size*: *l* = 0.10 m, Ø = 4.6 mm;

– *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (3 µm);
– *temperature*: 30 °C.

Mobile phase:
– *mobile phase A*: *methanol R*, 0.1 per cent *V/V* solution of *anhydrous formic acid R* (2:98 *V/V*);
– *mobile phase B*: *methanol R*, 0.1 per cent *V/V* solution of *anhydrous formic acid R* (50:50 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 8	100	0
8 - 20	100 → 0	0 → 100
20 - 25	0	100

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 260 nm.

Injection: 50 µL.

Autosampler: set at 4 °C.

Identification of impurities: use the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A and B; use the chromatogram obtained with reference solution (c) to identify the peak due to impurity D.

Relative retention with reference to mercaptopurine (retention time = about 6 min): impurity B = about 0.3; impurity A = about 0.5; impurity D = about 3.5.

System suitability: reference solution (a):
– *resolution*: minimum 5.0 between the peaks due to impurities B and A.

Calculation of percentage contents:
– *correction factor*: multiply the peak area of impurity D by 0.25;
– for impurities A and B, use the concentration of the corresponding impurity in reference solution (a);
– for impurities other than A and B, use the concentration of mercaptopurine monohydrate in reference solution (b).

Limits:
– *impurity A*: maximum 0.15 per cent;
– *impurities B, D*: for each impurity, maximum 0.10 per cent;
– *unspecified impurities*: for each impurity, maximum 0.10 per cent;
– *total*: maximum 0.3 per cent;
– *reporting threshold*: 0.05 per cent.

Water (*2.5.12*): 10.0 per cent to 12.0 per cent, determined on 0.250 g.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.100 g in 50 mL of *dimethylformamide R*. Titrate with *0.1 M tetrabutylammonium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M tetrabutylammonium hydroxide* is equivalent to 15.22 mg of $C_5H_4N_4S$.

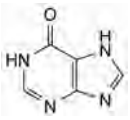
STORAGE

Protected from light.

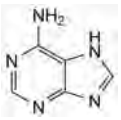
IMPURITIES

Specified impurities: A, B, D.

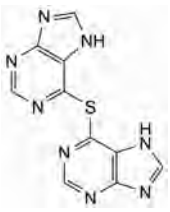
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *C.*



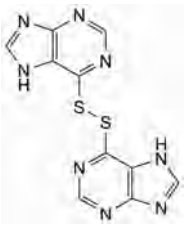
A. 1,7-dihydro-6*H*-purin-6-one (hypoxanthine),



B. 7*H*-purin-6-amine (adenine),



C. 6,6′-sulfanediyldi-7*H*-purine,

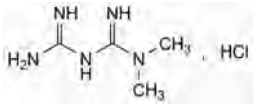


D. 6,6′-disulfanediyldi-7*H*-purine.

04/2020:0931

METFORMIN HYDROCHLORIDE

Metformini hydrochloridum



$C_4H_{12}ClN_5$ M_r 165.6
[1115-70-4]

DEFINITION

1,1-Dimethylbiguanide hydrochloride.

Content: 98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white crystals.

Solubility: freely soluble in water, slightly soluble in ethanol (96 per cent), practically insoluble in acetone and in methylene chloride.

IDENTIFICATION

First identification: B, E.

Second identification: A, C, D, E.

A. Melting point (*2.2.14*): 222 °C to 226 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *metformin hydrochloride CRS*.

C. Thin-layer chromatography (*2.2.27*).
Test solution. Dissolve 20 mg of the substance to be examined in *water R* and dilute to 5 mL with the same solvent.

Reference solution. Dissolve 20 mg of *metformin hydrochloride CRS* in *water R* and dilute to 5 mL with the same solvent.

Plate: *TLC silica gel G plate R*.

Mobile phase: *glacial acetic acid R*, *butanol R*, *water R* (10:40:50 *V/V/V*); use the upper layer.

Application: 5 µL.

Development: over 3/4 of the plate.

Drying: at 100-105 °C for 15 min.

Detection: spray with a mixture of equal volumes of a 100 g/L solution of *sodium nitroprusside R*, a 100 g/L solution of *potassium ferricyanide R* and a 100 g/L solution of *sodium hydroxide R*, prepared 20 min before use.

Results: the principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

D. Dissolve about 5 mg in *water R* and dilute to 100 mL with the same solvent. To 2 mL of the solution add 0.25 mL of *strong sodium hydroxide solution R* and 0.10 mL of *α-naphthol solution R*. Mix and allow to stand in iced water for 15 min. Add 0.5 mL of *sodium hypobromite solution R* and mix. A pink colour develops.

E. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S. Dissolve 2.0 g in *water R* and dilute to 20 mL with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*). Heat the solution to 50 °C and cool to room temperature.

Impurity F. Liquid chromatography (*2.2.29*).

Derivatisation solution. Prepare the solution immediately before use. Dilute 1 mL of *fluorodinitrobenzene R* in 100.0 mL of *acetonitrile R*.

Blank solution. To 5.0 mL of *acetonitrile R* add 100 µL of *triethylamine R1* and 1.0 mL of the derivatisation solution. Shake well and heat at 60 °C for 30 min. After cooling, dilute to 10.0 mL with *acetonitrile R*.

Test solution. Prepare the solution immediately before use. Suspend 10.0 mg of the substance to be examined in 5.0 mL of *acetonitrile R* and sonicate for 5 min. Add 100 µL of *triethylamine R1* and 1.0 mL of the derivatisation solution. Shake well and heat at 60 °C for 30 min. After cooling, dilute to 10.0 mL with *acetonitrile R*. Filter or centrifuge at 800 *g* for 5 min before use.

Reference solution. Dilute 1.0 mL of *metformin impurity F CRS* in 100.0 mL of *acetonitrile R*. Dilute 2.5 mL of the solution to 100.0 mL with *acetonitrile R*. To 1.0 mL of this solution add successively 5.0 mL of *acetonitrile R*, 100 µL of *triethylamine R1* and 1.0 mL of the derivatisation solution. Shake well and heat at 60 °C for 30 min. After cooling, dilute to 10.0 mL with *acetonitrile R*.

Column:

- *size*: *l* = 0.125 m, Ø = 3 mm;
- *stationary phase*: spherical *end-capped octadecylsilyl silica gel for chromatography R1* (5 µm);
- *temperature*: 30 °C.

Mobile phase:

- *mobile phase A*: *phosphoric acid R*, *water for chromatography R* (0.1:99.9 *V/V*);
- *mobile phase B*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	60 → 45	40 → 55
10 - 11	45 → 25	55 → 75
11 - 15	25	75

Flow rate: 0.7 mL/min.

Detection: spectrophotometer at 380 nm.

Injection: 5 µL.

Identification of impurities: use the chromatograms obtained with the blank solution and the reference solution to identify the peak due to the impurity F derivative.

Retention time: impurity F derivative = about 4 min.

System suitability: reference solution:

- *resolution*: minimum 3.0 between the peak due to the impurity F derivative and the nearby eluting peaks due to the derivatisation reagent.

Limit:

- *impurity F*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (0.05 per cent).

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution (a). Dissolve 20.0 mg of *metformin impurity A CRS* in *water R* and dilute to 100.0 mL with the same solvent. Dilute 1.0 mL of the solution to 200.0 mL with the mobile phase.

Reference solution (b). Dilute 1.0 mL of the test solution to 50.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 20.0 mL with the mobile phase.

Reference solution (c). Dissolve 10 mg of *melamine R* (impurity D) in about 90 mL of *water R*. Add 5 mL of the test solution and dilute to 100 mL with *water R*. Dilute 1 mL of this solution to 50 mL with the mobile phase.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *strong cation-exchange silica gel for chromatography R* (10 µm).

Mobile phase: 17 g/L solution of *ammonium dihydrogen phosphate R* adjusted to pH 3.0 with *phosphoric acid R*.

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 218 nm.

Injection: 20 µL.

Run time: twice the retention time of metformin.

Identification of impurities: use the chromatogram obtained with reference solution (a) to identify the peak due to impurity A; use the chromatogram obtained with reference solution (c) to identify the peak due to impurity D.

Relative retention with reference to metformin (retention time = about 14 min): impurity A = about 0.3; impurity D = about 0.4.

System suitability: reference solution (c):

- *resolution*: minimum 10 between the peaks due to impurity D and metformin.

Limits:

- *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.02 per cent);
- *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent);
- *total*: maximum 0.2 per cent;

– *disregard limit*: 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.03 per cent); do not disregard the peak due to impurity A.

Loss on drying (*2.2.32*): maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 5 h.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

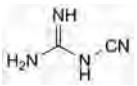
Dissolve 0.100 g in 4 mL of *anhydrous formic acid R*. Add 80 mL of *acetonitrile R*. Carry out the titration immediately. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 16.56 mg of $C_4H_{12}ClN_5$.

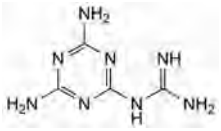
IMPURITIES

Specified impurities: A, F.

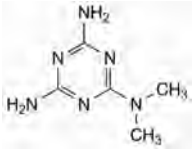
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *B, C, D, E.*



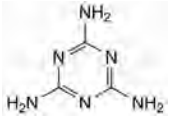
A. cyanoguanidine,



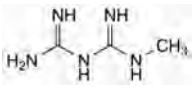
B. (4,6-diamino-1,3,5-triazin-2-yl)guanidine,



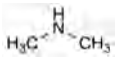
C. N^2,N^2-dimethyl-1,3,5-triazine-2,4,6-triamine (*N*,*N*-dimethylmelamine),



D. 1,3,5-triazine-2,4,6-triamine (melamine),



E. 1-methylbiguanide,

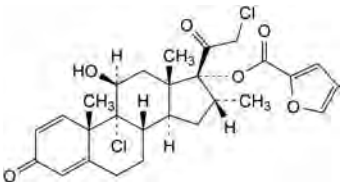


F. *N*-methylmethanamine (dimethylamine).

04/2020:1449

MOMETASONE FUROATE

Mometasoni furoas



$C_{27}H_{30}Cl_2O_6$ M_r 521.4

[83919-23-7]

DEFINITION

9,21-Dichloro-11β-hydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-17-yl furan-2-carboxylate.

Content: 98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white powder.

Solubility: practically insoluble in water, soluble in acetone and in methylene chloride, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification: A, D, E.

Second identification: B, C.

A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *mometasone furoate CRS*.

B. Thin-layer chromatography (*2.2.27*).
Test solution. Dissolve 10 mg of the substance to be examined in the mobile phase and dilute to 10.0 mL with the mobile phase.
Reference solution. Dissolve 10 mg of *mometasone furoate CRS* in the mobile phase and dilute to 10.0 mL with the mobile phase.
Plate: *TLC silica gel F_{254} plate R*.
Mobile phase: *methanol R*, *methylene chloride R* (10:90 *V/V*).
Application: 10 µL; the volume may be adapted based on the type of plate used.
Development: over 3/4 of the plate.
Drying: in air.
Detection: spray with a solution prepared as follows: dissolve 0.25 g of *2,4-dihydroxybenzaldehyde R* in *glacial acetic acid R*, dilute to 50 mL with the same solvent and add a mixture of 12.5 mL of *sulfuric acid R* and 37.5 mL of *glacial acetic acid R*; heat the plate at 90 °C for 35 min or until the spots appear and allow to cool; examine in daylight and in ultraviolet light at 365 nm.
Results: the principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

C. Mix 80 mg with 0.30 g of *anhydrous sodium carbonate R* and ignite in a crucible until an almost white residue is obtained. Allow to cool and dissolve the residue in 5 mL of *dilute nitric acid R*; filter. To 1 mL of the filtrate add 1 mL of *water R*. The solution gives reaction (a) of chlorides (*2.3.1*).

D. Examine the chromatograms obtained in the assay.
Results: the principal peak in the chromatogram obtained with test solution (b) is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (c).

Monographs L–P

E. Loss on drying (see Tests).

TESTS

Specific optical rotation (*2.2.7*): + 50 to + 55 (dried substance).

Dissolve 50.0 mg in *ethanol (96 per cent) R* and dilute to 10.0 mL with the same solvent.

Related substances. Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Solvent mixture. Mix 200 mL of *acetonitrile R* and 200 mL of *water R*, then add 0.4 mL of *acetic acid R*.

Test solution (a). Dissolve 25.0 mg of the substance to be examined in 15 mL of *acetonitrile R* and dilute to 50.0 mL with the solvent mixture.

Test solution (b). Dilute 5.0 mL of test solution (a) to 25.0 mL with the solvent mixture.

Reference solution (a). Dissolve 5 mg of *mometasone furoate for system suitability CRS* (containing impurities C and J) in 3 mL of *acetonitrile R* and dilute to 10 mL with the solvent mixture.

Reference solution (b). Dilute 1.0 mL of test solution (a) to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (c). Dissolve 25.0 mg of *mometasone furoate monohydrate CRS* in 15 mL of *acetonitrile R* and dilute to 50.0 mL with the solvent mixture. Dilute 5.0 mL of the solution to 25.0 mL with the solvent mixture.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase: *acetonitrile R, water for chromatography R* (50:50 *V/V*).

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 254 nm.

Injection: 20 µL of test solution (a) and reference solutions (a) and (b).

Run time: 3.5 times the retention time of mometasone furoate.

Identification of impurities: use the chromatogram supplied with *mometasone furoate for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities C and J.

Relative retention with reference to mometasone furoate (retention time = about 24 min): impurity C = about 0.9; impurity J = about 1.5.

System suitability: reference solution (a):

- *resolution*: minimum 2.5 between the peaks due to impurity C and mometasone furoate.

Limits:

- *impurity J*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.15 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
- *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*): maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

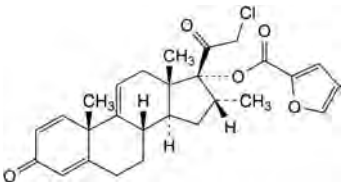
Injection: test solution (b) and reference solution (c).

Calculate the percentage content of $C_{27}H_{30}Cl_2O_6$ taking into account the assigned content of *mometasone furoate monohydrate CRS*.

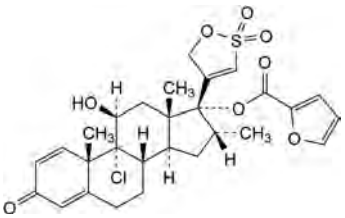
IMPURITIES

Specified impurities: *J*.

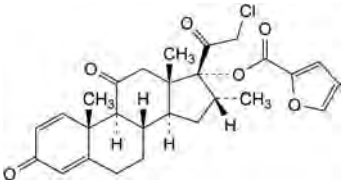
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *A, B, C, D, E, F, G, H, I, K, L, M, N, O, P, Q, R, S, T, U.*



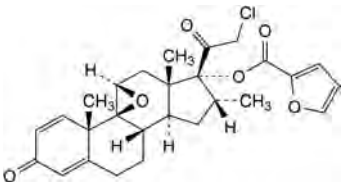
A. 21-chloro-16α-methyl-3,20-dioxopregna-1,4,9(11)-trien-17-yl furan-2-carboxylate,



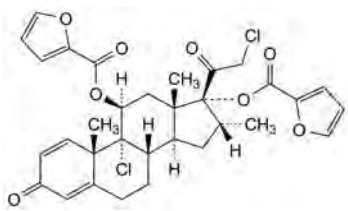
B. 9-chloro-17β-(2,2-dioxo-2,5-dihydro-1,2λ⁶-oxathiol-4-yl)-11β-hydroxy-16α-methyl-3-oxoandrosta-1,4-dien-17α-yl furan-2-carboxylate,



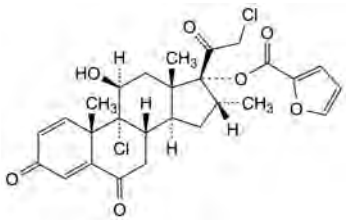
C. 21-chloro-16α-methyl-3,11,20-trioxopregna-1,4-dien-17-yl furan-2-carboxylate,



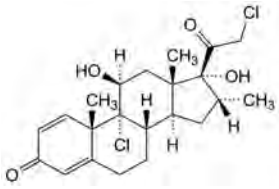
D. 21-chloro-9,11β-epoxy-16α-methyl-3,20-dioxo-9β-pregna-1,4-dien-17-yl furan-2-carboxylate,



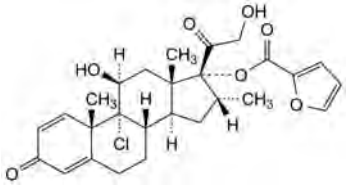
E. 9,21-dichloro-16α-methyl-3,20-dioxopregna-1,4-diene-11β,17-diyl di(furan-2-carboxylate),



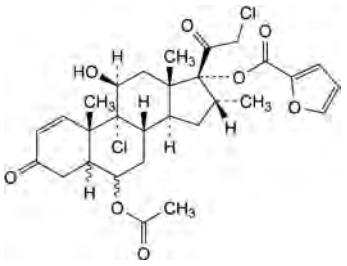
F. 9,21-dichloro-11β-hydroxy-16α-methyl-3,6,20-trioxopregna-1,4-dien-17-yl furan-2-carboxylate,



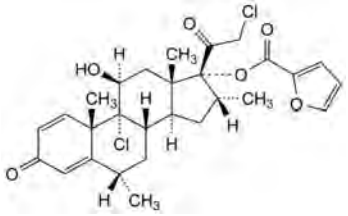
G. 9,21-dichloro-11β,17-dihydroxy-16α-methylpregna-1,4-diene-3,20-dione (mometasone),



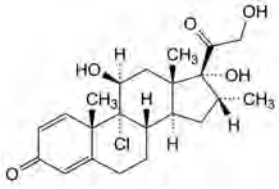
H. 9-chloro-11β,21-dihydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-17-yl furan-2-carboxylate,



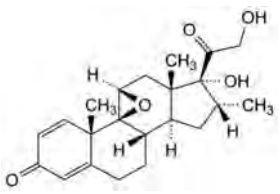
I. 6ξ-(acetyloxy)-9,21-dichloro-11β-hydroxy-16α-methyl-3,20-dioxo-5ξ-pregn-1-en-17-yl furan-2-carboxylate,



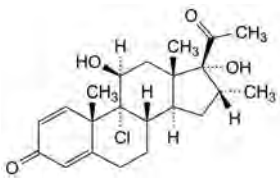
J. 9,21-dichloro-11β-hydroxy-6α,16α-dimethyl-3,20-dioxopregna-1,4-dien-17-yl furan-2-carboxylate,



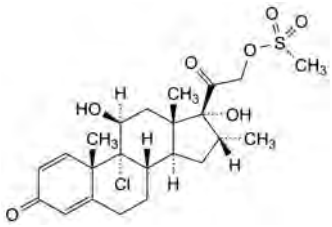
K. 9-chloro-11β,17,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione,



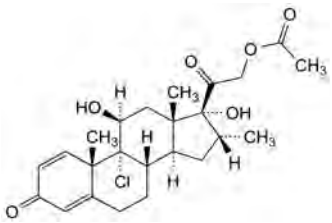
L. 9,11β-epoxy-17,21-dihydroxy-16α-methyl-9β-pregna-1,4-diene-3,20-dione,



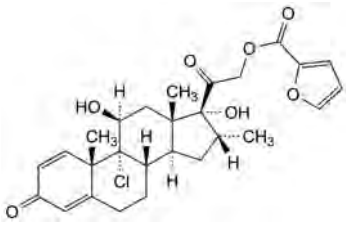
M. 9-chloro-11β,17-dihydroxy-16α-methylpregna-1,4-diene-3,20-dione,



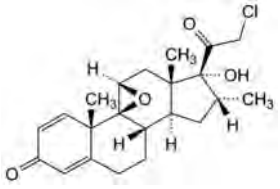
N. 9-chloro-11β,17-dihydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-21-yl methanesulfonate,



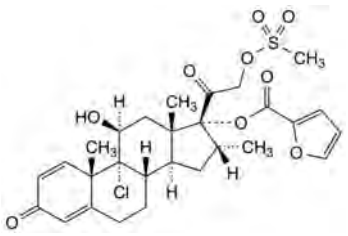
O. 9-chloro-11β,17-dihydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-21-yl acetate,



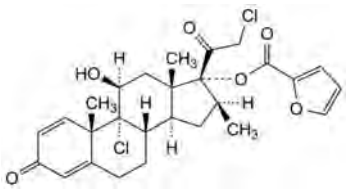
P. 9-chloro-11β,17-dihydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-21-yl furan-2-carboxylate,



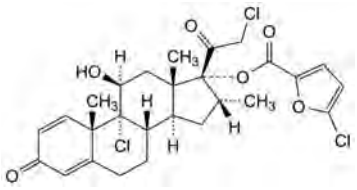
Q. 21-chloro-9,11β-epoxy-17-hydroxy-16α-methyl-9β-pregna-1,4-diene-3,20-dione,



R. 9-chloro-11β-hydroxy-21-[(methanesulfonyl)oxy]-16α-methyl-3,20-dioxopregna-1,4-dien-17-yl furan-2-carboxylate,



S. 9,21-dichloro-11β-hydroxy-16β-methyl-3,20-dioxopregna-1,4-dien-17-yl furan-2-carboxylate,



T. 9,21-dichloro-11β-hydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-17-yl 5-chlorofuran-2-carboxylate,

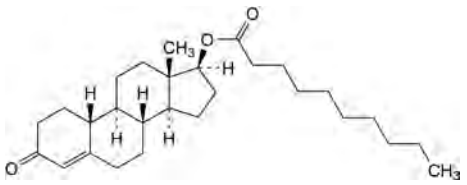
U. unknown structure.

N

01/2008:1992
corrected 10.1

NANDROLONE DECANOATE

Nandroloni decanoas



$C_{28}H_{44}O_3$ M_r 428.7
[360-70-3]

DEFINITION

3-Oxoestr-4-en-17β-yl decanoate.

Content: 97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: practically insoluble in water, very soluble in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION

A. Melting point (*2.2.14*): 34 °C to 38 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *nandrolone decanoate CRS*.

TESTS

Appearance of solution. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Dissolve 0.20 g in 10 mL of *methanol R*.

Specific optical rotation (*2.2.7*): + 35.0 to + 40.0 (dried substance).

Dissolve 0.200 g in *anhydrous ethanol R* and dilute to 20.0 mL with the same solvent.

Impurities A, B, C. Thin-layer chromatography (*2.2.27*).

Test solution. Dissolve 50 mg of the substance to be examined in *methylene chloride R* and dilute to 5.0 mL with the same solvent.

Reference solution (a). Dilute 1.0 mL of the test solution to 10.0 mL with *methylene chloride R*.

Reference solution (b). Dilute 1.0 mL of reference solution (a) to 10.0 mL with *methylene chloride R*.

Reference solution (c). Dilute 1.0 mL of reference solution (a) to 20.0 mL with *methylene chloride R*.

Reference solution (d). Dissolve 5 mg of *nandrolone decanoate for system suitability CRS* (containing impurities A, B, C) in 0.5 mL of *methylene chloride R*.

Plate: *TLC silica gel plate R*.

Mobile phase: *acetone R*, *heptane R* (30:70 *V/V*).

Application: 10 µL of the test solution and reference solutions (b), (c) and (d).

Development: over 2/3 of the plate.

Drying: in air.

Detection: treat with *alcoholic solution of sulfuric acid R* and heat at 130 °C until the spots appear. Examine in ultraviolet light at 366 nm.

Retardation factors: nandrolone decanoate = about 0.37; impurity A = about 0.45; impurity B = about 0.55; impurity C = about 0.58.

System suitability: reference solution (d):
- the chromatogram shows 4 clearly separated spots.

Limits:
- *impurity A*: any spot due to impurity A is not more intense than the principal spot in the chromatogram obtained with reference solution (b) (1.0 per cent);
- *impurities B, C*: any spot due to impurity B or C is not more intense than the principal spot in the chromatogram obtained with reference solution (c) (0.5 per cent).

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 25 mg of the substance to be examined in *methanol R* and dilute to 10.0 mL with the same solvent.

Reference solution (a). Dilute 1.0 mL of the test solution to 100.0 mL with *methanol R*. Dilute 1.0 mL of this solution to 10.0 mL with *methanol R*.

Reference solution (b). Dissolve 5 mg of *nandrolone decanoate for peak identification CRS* (containing impurities D, F, G, H, I, K, L) in *methanol R* and dilute to 2.0 mL with the same solvent.

Column:
- *size*: *l* = 0.15 m, Ø = 3.9 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
- *mobile phase A*: *water for chromatography R*;
- *mobile phase B*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	35	65
5 - 40	35 → 0	65 → 100
40 - 75	0	100
75 - 80	0 → 35	100 → 65

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 254 nm.

Injection: 20 µL.

Relative retention with reference to nandrolone decanoate (retention time = about 30 min): impurity D = about 0.05; impurity F = about 0.6; impurity K = about 0.7; impurity L = about 0.9; impurity G = about 0.97; impurity H = about 1.1; impurity I = about 1.2.

System suitability: reference solution (b):
- *peak-to-valley ratio*: minimum 1.5, where H_p = height above the baseline of the peak due to impurity G and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to nandrolone decanoate.

Limits:
- *correction factors*: for the calculation of contents, multiply the peak areas of the following impurities by the corresponding correction factor: impurity D = 0.5; impurity F = 0.6; impurity H = 1.1; impurity I = 1.3; impurity K = 0.8;
- *impurities D, F, G, H, I, K, L*: for each impurity, not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *total*: not more than 15 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*): maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at a pressure not exceeding 0.7 kPa for 4 h.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 10.0 mg in *anhydrous ethanol R* and dilute to 100.0 mL with the same solvent. Dilute 5.0 mL of this solution to 50.0 mL with *anhydrous ethanol R*. Measure the absorbance (*2.2.25*) at the absorption maximum at 240 nm. Calculate the content of $C_{28}H_{44}O_3$ taking the specific absorbance to be 407.

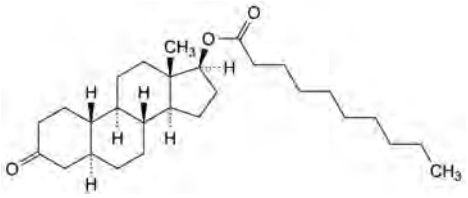
STORAGE

Under nitrogen, protected from light and at a temperature of 2 °C to 8 °C.

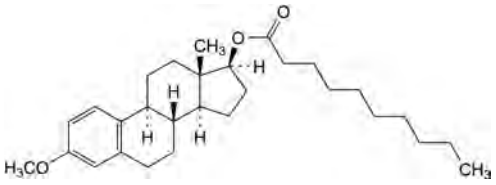
IMPURITIES

Specified impurities: A, B, C, D, F, G, H, I, K, L.

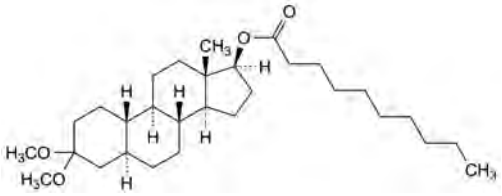
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *E, J.*



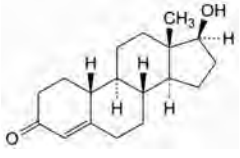
A. 3-oxo-5α-estran-17β-yl decanoate,



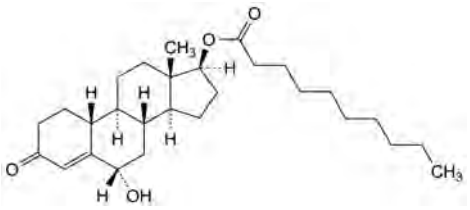
B. 3-methoxyestra-1,3,5(10)-trien-17β-yl decanoate,



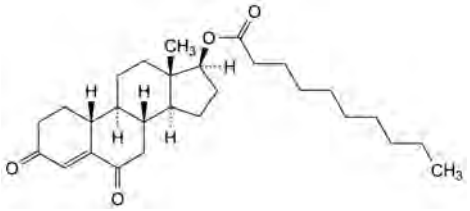
C. 3,3-dimethoxy-5α-estran-17β-yl decanoate,



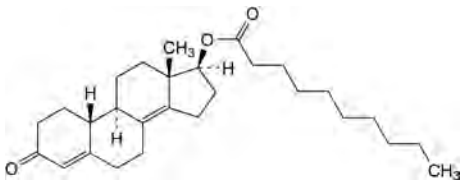
D. 17β-hydroxyestr-4-en-3-one,



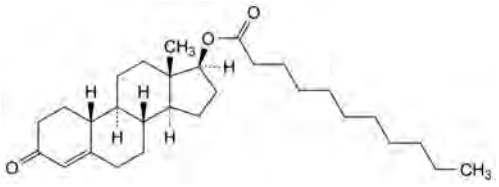
E. 6α-hydroxy-3-oxoestr-4-en-17β-yl decanoate,



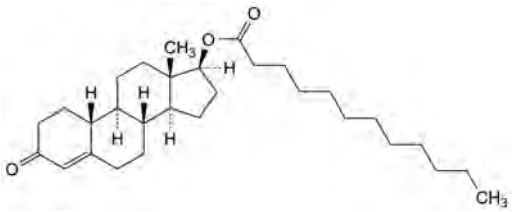
F. 3,6-dioxoestr-4-en-17β-yl decanoate,



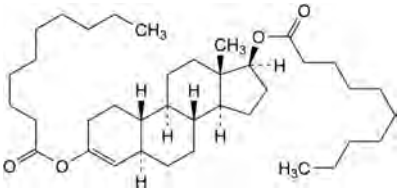
G. 3-oxoestra-4,8(14)-dien-17β-yl decanoate,



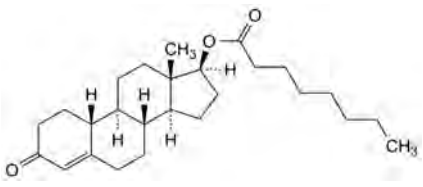
H. 3-oxoestr-4-en-17β-yl undecanoate,



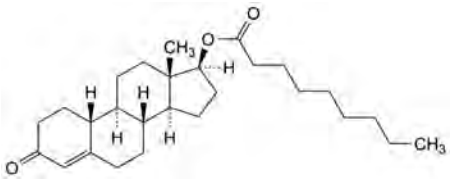
I. 3-oxoestr-4-en-17β-yl dodecanoate,



J. 5α-estr-3-ene-3,17β-diyl didecanoate,



K. 3-oxoestr-4-en-17β-yl octanoate,

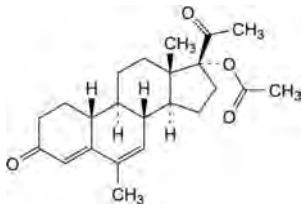


L. 3-oxoestr-4-en-17β-yl nonanoate.

04/2020:1551

NOMEGESTROL ACETATE

Nomegestroli acetas



$C_{23}H_{30}O_4$ M_r 370.5
[58652-20-3]

DEFINITION

6-Methyl-3,20-dioxo-19-norpregna-4,6-dien-17-yl acetate.

Content: 98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: practically insoluble in water, freely soluble in acetone, soluble in ethanol (96 per cent), practically insoluble in heptane.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *nomegestrol acetate for ID and assay CRS*.

TESTS

Appearance of solution. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_5 (*2.2.2, Method II*).

Dissolve 1.0 g in *methylene chloride R* and dilute to 10 mL with the same solvent.

Specific optical rotation (*2.2.7*): – 64.0 to – 60.0 (dried substance).

Dissolve 0.500 g in *anhydrous ethanol R* and dilute to 25.0 mL with the same solvent.

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 25.0 mg of the substance to be examined in *methanol R* and dilute to 50.0 mL with the same solvent.

Reference solution (a). Dissolve 25.0 mg of *nomegestrol acetate for ID and assay CRS* in *methanol R* and dilute to 50.0 mL with the same solvent.

Reference solution (b). Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (c). Dissolve 25.0 mg of *nomegestrol acetate impurity A CRS* in *methanol R* and dilute to 100.0 mL with the same solvent. Dilute 0.25 mL of the solution to 25.0 mL with reference solution (a).

Reference solution (d). Dissolve the contents of a vial of *nomegestrol acetate impurity B CRS* in 1 mL of *methanol R*.

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm;
– *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase: *acetonitrile for chromatography R*, *methanol R1*, *water for chromatography R* (24:38:38 *V/V/V*).

Flow rate: 1.3 mL/min.

Detection: spectrophotometer at 245 nm and at 290 nm.

Injection: 10 µL of the test solution and reference solutions (b), (c), (d).

Run time: 1.5 times the retention time of nomegestrol acetate.

Identification of impurities: use the chromatogram obtained with reference solution (c) at 245 nm to identify the peak due to impurity A; use the chromatogram obtained with reference solution (d) at 290 nm to identify the peak due to impurity B.

Relative retention with reference to nomegestrol acetate (retention time = about 17 min): impurity B = about 0.6; impurity A = about 1.1.

System suitability: reference solution (c) at 245 nm:

– *peak-to-valley ratio*: minimum 5, where H_p = height above the baseline of the peak due to impurity A and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to nomegestrol acetate.

Calculation of percentage contents:

– for each impurity at 245 nm, use the concentration of impurity A in reference solution (c);
– for each impurity at 290 nm, use the concentration of nomegestrol acetate in reference solution (b).

Limits:

– *impurity A at 245 nm*: maximum 0.2 per cent;
– *impurity B at 290 nm*: maximum 0.15 per cent;
– *unspecified impurities at 245 nm and at 290 nm*: for each impurity, maximum 0.10 per cent;
– *sum of impurities other than A at 245 nm and 290 nm*: maximum 0.3 per cent;
– *reporting threshold at 245 nm and at 290 nm*: 0.05 per cent.

Loss on drying (*2.2.32*): maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications:

Detection: spectrophotometer at 290 nm.

Injection: test solution and reference solution (a).

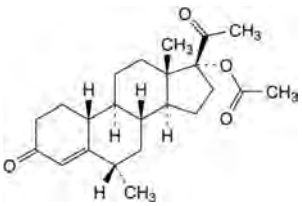
Calculate the percentage content of $C_{23}H_{30}O_4$ taking into account the assigned content of *nomegestrol acetate for ID and assay CRS*.

STORAGE

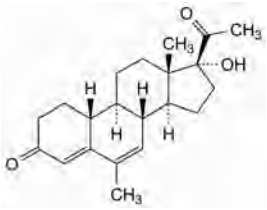
Protected from light.

IMPURITIES

Specified impurities: A, B.



A. 6α-methyl-3,20-dioxo-19-norpregn-4-en-17-yl acetate,



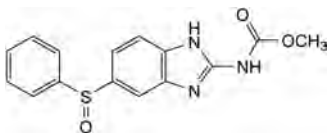
B. 17-hydroxy-6-methyl-19-norpregna-4,6-diene-3,20-dione (nomegestrol).

O

04/2020:1458

OXFENDAZOLE FOR VETERINARY USE

Oxfendazolum ad usum veterinarium



$C_{15}H_{13}N_3O_3S$ M_r 315.3
[53716-50-0]

DEFINITION

Methyl [5-(phenylsulfinyl)-1*H*-benzimidazol-2-yl]carbamate.

Content: 97.5 per cent to 100.5 per cent (dried substance).

CHARACTERS

Appearance: white or almost white powder.

Solubility: practically insoluble in water, slightly soluble in ethanol (96 per cent) and in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *oxfendazole CRS*.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *ethanol (96 per cent) R*, evaporate to dryness and record new spectra using the residues.

TESTS

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 mL with the mobile phase.

Reference solution (a). Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase.

Reference solution (b). In order to prepare impurity C *in situ*, add 0.25 mL of *strong hydrogen peroxide solution R* to 10 mL of the test solution, then dilute to 25 mL with the mobile phase.

Reference solution (c). Dissolve 5.0 mg of *fenbendazole CRS* (impurity A) in the mobile phase and dilute to 100.0 mL with the mobile phase. Dilute 1.0 mL of the solution to 20.0 mL with the mobile phase.

Reference solution (d). Dissolve 10.0 mg of *oxfendazole impurity B CRS* in the mobile phase and dilute to 100.0 mL with the mobile phase. Dilute 1.0 mL of the solution to 20.0 mL with the mobile phase.

Reference solution (e). Dissolve 5 mg of *oxfendazole with impurity D CRS* in the mobile phase and dilute to 20 mL with the mobile phase.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase: mix 36 volumes of *acetonitrile R* and 64 volumes of a 2 g/L solution of *sodium pentanesulfonate R* previously adjusted to pH 2.7 with a 2.8 per cent *V/V* solution of *sulfuric acid R*.

Flow rate: 1 mL/min.

Detection: spectrophotometer at 254 nm.

Injection: 20 µL.

Run time: 6 times the retention time of oxfendazole.

Identification of impurities: use the chromatogram obtained with reference solution (c) to identify the peak due to impurity A; use the chromatogram obtained with reference solution (d) to identify the peak due to impurity B; use the chromatogram obtained with reference solution (b) to identify the peak due to impurity C; use the chromatogram supplied with *oxfendazole with impurity D CRS* and the chromatogram obtained with reference solution (e) to identify the peak due to impurity D.

Relative retention with reference to oxfendazole (retention time = about 7 min): impurity C = about 0.4; impurity B = about 1.9; impurity D = about 2.7; impurity A = about 5.4.

System suitability: reference solution (b):

- *resolution*: minimum 4.0 between the peaks due to impurity C and oxfendazole.

Limits:

- *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (d) (2.0 per cent);
- *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (1.0 per cent);
- *impurities C, D*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);
- *unspecified impurities*: not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.20 per cent);
- *total*: maximum 3.0 per cent;
- *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent).

Loss on drying (*2.2.32*): maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 105 °C for 2 h.

Sulfated ash (*2.4.14*): maximum 0.2 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 3 mL of *anhydrous formic acid R*. Add 40 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

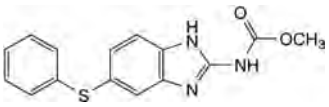
1 mL of *0.1 M perchloric acid* is equivalent to 31.53 mg of $C_{15}H_{13}N_3O_3S$.

STORAGE

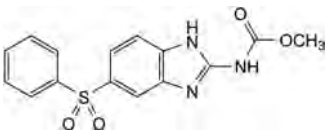
Protected from light.

IMPURITIES

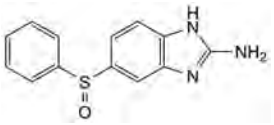
Specified impurities: A, B, C, D.



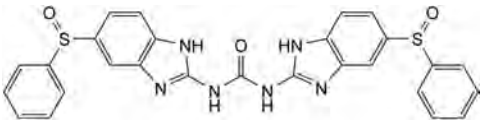
A. methyl [5-(phenylsulfanyl)-1*H*-benzimidazol-2-yl]carbamate (fenbendazole),



B. methyl [5-(phenylsulfonyl)-1*H*-benzimidazol-2-yl]carbamate,



C. 5-(phenylsulfinyl)-1*H*-benzimidazol-2-amine,

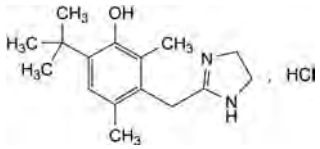


D. *N*,*N*′-bis[5-(phenylsulfinyl)-1*H*-benzimidazol-2-yl]urea.

01/2013:0943
corrected 10.1

OXYMETAZOLINE HYDROCHLORIDE

Oxymetazolini hydrochloridum



$C_{16}H_{25}ClN_2O$ M_r 296.8
[2315-02-8]

DEFINITION

3-[(4,5-Dihydro-1*H*-imidazol-2-yl)methyl]-6-(1,1-dimethylethyl)-2,4-dimethylphenol hydrochloride.

Content: 99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: freely soluble in water and in ethanol (96 per cent).

IDENTIFICATION

First identification: *A, D.*

Second identification: *B, C, D.*

A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *oxymetazoline hydrochloride CRS.*

B. Thin-layer chromatography (*2.2.27*).
Test solution. Dissolve 20 mg of the substance to be examined in a mixture of equal volumes of *ethyl acetate R* and *methanol R* and dilute to 5 mL with the same mixture of solvents.
Reference solution. Dissolve 20 mg of *oxymetazoline hydrochloride CRS* in a mixture of equal volumes of *ethyl acetate R* and *methanol R* and dilute to 5 mL with the same mixture of solvents.
Plate: *TLC silica gel G plate R.*
Mobile phase: *diethylamine R, cyclohexane R, anhydrous ethanol R* (6:15:79 *V/V/V*).
Application: 5 µL.
Development: over 2/3 of the plate.
Drying: in a current of warm air for 5 min, then allow to cool.
Detection: spray with a freshly prepared 5.0 g/L solution of *potassium ferricyanide R* in *ferric chloride solution R2*; examine in daylight.
Results: the principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

C. Dissolve about 2 mg in 1 mL of *water R*, then add 0.2 mL of a 50 g/L solution of *sodium nitroprusside R* and 0.2 mL of *dilute sodium hydroxide solution R*. Allow to stand for 10 min. Add 2 mL of *sodium hydrogen carbonate solution R*. A violet colour develops.

D. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Appearance of solution. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution BY_7 (*2.2.2, Method II*).

Dissolve 2.5 g in *water R* and dilute to 50 mL with the same solvent.

Acidity or alkalinity. Dissolve 0.25 g in *carbon dioxide-free water R* and dilute to 25 mL with the same solvent. Add 0.1 mL of *methyl red solution R* and 0.2 mL of *0.01 M hydrochloric acid*. The solution is red. Not more than 0.4 mL of *0.01 M sodium hydroxide* is required to change the colour of the indicator to yellow.

Related substances. Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution. Dissolve 50.0 mg of the substance to be examined in *water R* and dilute to 50.0 mL with the same solvent.

Reference solution (a). Dilute 5.0 mL of the test solution to 100.0 mL with *water R*. Dilute 2.0 mL of this solution to 100.0 mL with *water R*.

Reference solution (b). Dissolve 5.0 mg of *oxymetazoline impurity A CRS* and 5 mg of the substance to be examined in *water R* and dilute to 50.0 mL with the same solvent. Dilute 10.0 mL of the solution to 50.0 mL with *water R*.

Reference solution (c). Dilute 1.0 mL of reference solution (b) to 20.0 mL with *water R*.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography with embedded polar groups R* (5 µm).

Mobile phase:
- *mobile phase A*: 1.36 g/L solution of *potassium dihydrogen phosphate R* adjusted to pH 3.0 with *phosphoric acid R*;
- *mobile phase B*: *acetonitrile for chromatography R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	70	30
5 - 20	70 → 15	30 → 85
20 - 35	15	85

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 220 nm.

Injection: 10 µL.

Relative retention with reference to oxymetazoline (retention time = about 5.0 min): impurity A = about 0.9.

System suitability: reference solution (b):
- *resolution*: minimum 4.0 between the peaks due to impurity A and oxymetazoline.

Limits:
- *impurity A*: not more than 1.5 times the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.15 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

– *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water (*2.5.32*): maximum 0.3 per cent, determined on 1.00 g .

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

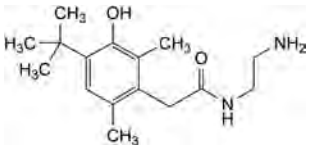
Dissolve 0.200 g in a mixture of 20 mL of *acetic anhydride R* and 20 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 29.68 mg of $C_{16}H_{25}ClN_2O$.

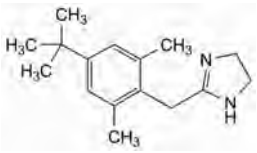
IMPURITIES

Specified impurities: *A*.

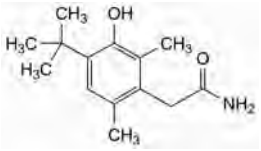
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *B, C, D, E*.



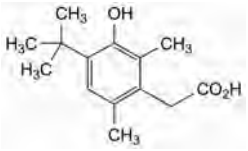
A. *N*-(2-aminoethyl)-2-[4-(1,1-dimethylethyl)-3-hydroxy-2,6-dimethylphenyl]acetamide,



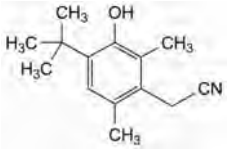
B. 2-[[4-(1,1-dimethylethyl)-2,6-dimethylphenyl]methyl]-4,5-dihydro-1*H*-imidazole (xylometazoline),



C. 2-[4-(1,1-dimethylethyl)-3-hydroxy-2,6-dimethylphenyl]-acetamide,



D. 2-[4-(1,1-dimethylethyl)-3-hydroxy-2,6-dimethylphenyl]-acetic acid,



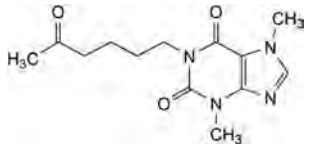
E. 2-[4-(1,1-dimethylethyl)-3-hydroxy-2,6-dimethylphenyl]-acetonitrile.

P

04/2020:0851

PENTOXIFYLLINE

Pentoxifyllinum



$C_{13}H_{18}N_4O_3$ M_r 278.3
[6493-05-6]

DEFINITION

3,7-Dimethyl-1-(5-oxohexyl)-3,7-dihydro-1*H*-purine-2,6-dione.

Content: 99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: soluble in water, freely soluble in methylene chloride, sparingly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification: A, B.

Second identification: A, C, D.

A. Melting point (*2.2.14*): 103 °C to 107 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *pentoxifylline CRS*.

C. Thin-layer chromatography (*2.2.27*).
Test solution. Dissolve 20 mg of the substance to be examined in *methanol R* and dilute to 10 mL with the same solvent.
Reference solution. Dissolve 20 mg of *pentoxifylline CRS* in *methanol R* and dilute to 10 mL with the same solvent.
Plate: *TLC silica gel F_{254} plate R*.
Mobile phase: *methanol R*, *ethyl acetate R* (15:85 *V/V*).
Application: 5 µL.
Development: over 2/3 of the plate.
Drying: in air.
Detection: examine in ultraviolet light at 254 nm.
Results: the principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

D. It gives the reaction of xanthines (*2.3.1*).

TESTS

Solution S. Dissolve 2.5 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 50 mL with the same solvent.

Appearance of solution. A 40 per cent *V/V* solution of solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_7 (*2.2.2, Method II*).

Acidity. To 8 mL of solution S add 12 mL of *carbon dioxide-free water R* and 0.05 mL of *bromothymol blue solution R1*. The solution is green or yellow. Not more than 0.2 mL of *0.01 M sodium hydroxide* is required to change the colour of the indicator to blue.

Related substances. Liquid chromatography (*2.2.29*).
Solution A: 5.44 g/L solution of *potassium dihydrogen phosphate R*.
Solvent mixture: *methanol R*, solution A (50:50 *V/V*).
Test solution. Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 25.0 mL with the solvent mixture.
Reference solution (a). Dilute 2.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 5.0 mL of this solution to 100.0 mL with the solvent mixture.
Reference solution (b). Dilute 10.0 mL of reference solution (a) to 50.0 mL with the solvent mixture.
Reference solution (c). Dissolve 2 mg of *caffeine R* (impurity F) and 2 mg of *theophylline R* (impurity C) in the solvent mixture, add 1 mL of the test solution and dilute to 10 mL with the solvent mixture.
Reference solution (d). Dissolve 5.0 mg of *caffeine R* (impurity F), 5.0 mg of *theobromine R* (impurity A) and 5.0 mg of *theophylline R* (impurity C) in the solvent mixture and dilute to 100.0 mL with the solvent mixture. Dilute 1.0 mL of the solution to 25.0 mL with the solvent mixture.
Column:
– *size*: *l* = 0.25 m, Ø = 4.0 mm;
– *stationary phase*: *base-deactivated end-capped octylsilyl silica gel for chromatography R* (5 µm);
– *temperature*: 30 °C.
Mobile phase:
– *mobile phase A*: *methanol R*, solution A (30:70 *V/V*);
– *mobile phase B*: solution A, *methanol R* (30:70 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 6	85	15
6 - 13	85 → 10	15 → 90
13 - 30	10	90
30 - 35	10 → 85	90 → 15
35 - 45	85	15

Flow rate: 1 mL/min.
Detection: spectrophotometer at 272 nm.
Injection: 10 µL.
Relative retention with reference to pentoxifylline (retention time = about 12 min): impurity A = about 0.3; impurity C = about 0.4; impurity F = about 0.5; impurity J = about 1.6.
System suitability: reference solution (c):
– *resolution*: minimum 4.0 between the peaks due to impurities C and F.
Limits:
– *impurities A, C, F*: for each impurity, not more than the area of the corresponding peak in the chromatogram obtained with reference solution (d) (0.1 per cent);
– *impurity J*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
– *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
– *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
– *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (b) (0.02 per cent).

Chlorides (*2.4.4*): maximum 100 ppm.
Place 20 mL of solution S in a separating funnel and shake with 2 quantities, each of 20 mL, of *2-methylpropanol R*. Dilute 10 mL of the aqueous layer to 15 mL with *water R*.

Sulfates (*2.4.13*): maximum 200 ppm, determined on 15 mL of solution S.

Monographs L-P

Loss on drying (*2.2.32*): maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 5 mL of *anhydrous acetic acid R*. Add 20 mL of *acetic anhydride R*. Titrate with *0.1 M perchloric acid* determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 27.83 mg of $C_{13}H_{18}N_4O_3$.

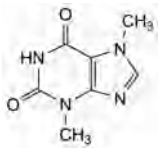
STORAGE

Protected from light.

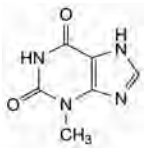
IMPURITIES

Specified impurities: *A, C, F, J.*

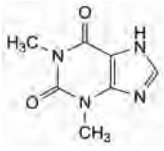
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *B, D, E, G, H, I, K.*



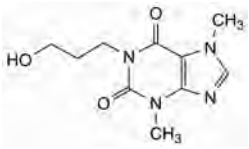
A. theobromine,



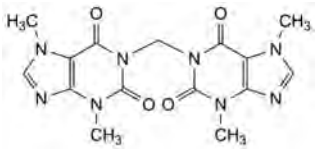
B. 3-methyl-3,7-dihydro-1*H*-purine-2,6-dione,



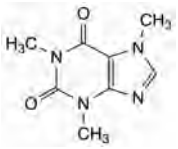
C. theophylline,



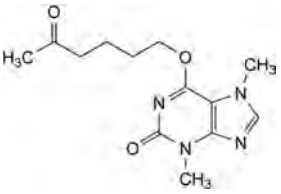
D. 1-(3-hydroxypropyl)-3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione,



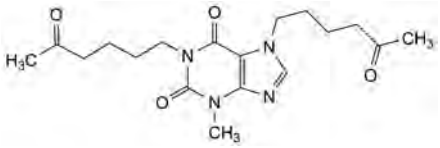
E. 1,1′-methylenebis(3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione),



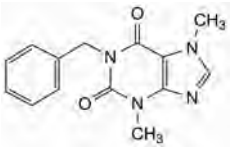
F. caffeine,



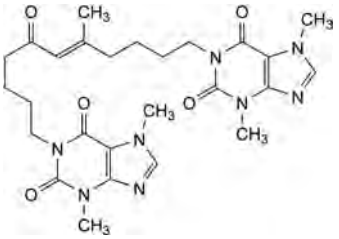
G. 3,7-dimethyl-6-(5-oxohexyloxy)-3,7-dihydro-2*H*-purin-2-one,



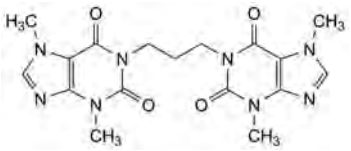
H. 3-methyl-1,7-bis(5-oxohexyl)-3,7-dihydro-1*H*-purine-2,6-dione,



I. 1-benzyl-3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione,



J. 1-[(5*E*)-11-(3,7-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purin-1-yl)-5-methyl-7-oxoundec-5-enyl]-3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione,

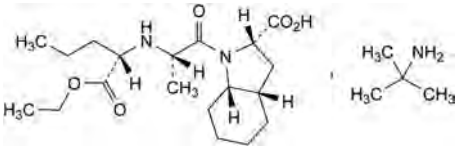


K. 1,1′-(propane-1,3-diyl)bis(3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione).

04/2020:2019

PERINDOPRIL *tert*-BUTYLAMINE

tert-Butylamini perindoprilum



$C_{23}H_{43}N_3O_5$ M_r 441.6

[107133-36-8]

DEFINITION

2-Methylpropan-2-amine (2*S*,3a*S*,7a*S*)-1-[(2*S*)-2-[[(1*S*)-1-(ethoxycarbonyl)butyl]amino]propanoyl]octahydro-1*H*-indole-2-carboxylate.

Content: 99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance: white or almost white, slightly hygroscopic, crystalline powder.

Solubility: freely soluble in water and in ethanol (96 per cent), soluble or sparingly soluble in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Specific optical rotation (*2.2.7*): – 69 to – 66 (anhydrous substance).

Dissolve 0.250 g in *ethanol (96 per cent) R* and dilute to 25.0 mL with the same solvent.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *perindopril tert-butylamine CRS*.

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in *methylene chloride R*, evaporate to dryness and record new spectra using the residues.

C. Examine the chromatograms obtained in the test for impurity A.

Results: in the chromatogram obtained with the test solution a spot is observed with the same R_F as the spot with the higher R_F in the chromatogram obtained with reference solution (c) (*tert*-butylamine).

TESTS

Impurity A. Thin-layer chromatography (*2.2.27*).

Test solution. Dissolve 0.20 g of the substance to be examined in *methanol R* and dilute to 10.0 mL with the same solvent.

Reference solution (a). Dissolve 5 mg of *perindopril impurity A CRS* in *methanol R* and dilute to 25.0 mL with the same solvent.

Reference solution (b). Dilute 5.0 mL of reference solution (a) to 20.0 mL with *methanol R*.

Reference solution (c). To 5 mL of reference solution (a) add 5 mL of a 20 g/L solution of *1,1-dimethylethylamine R* in *methanol R*.

Plate: *TLC silica gel plate R*.

Mobile phase: *glacial acetic acid R*, *toluene R*, *methanol R* (1:40:60 *V/V/V*).

Application: 10 µL of the test solution and reference solutions (b) and (c).

Development: over 2/3 of the plate.

Drying: in a current of warm air.

Detection: expose to iodine vapour for at least 20 h.

System suitability: reference solution (c):

– the chromatogram shows 2 clearly separated spots.

Limit:

– *impurity A*: any spot due to impurity A is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.25 per cent).

Stereochemical purity. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 20 mg of the substance to be examined in *ethanol (96 per cent) R* and dilute to 10.0 mL with the same solvent.

Reference solution (a). Dilute 1.0 mL of the test solution to 100.0 mL with *ethanol (96 per cent) R*. Dilute 1.0 mL of this solution to 10.0 mL with *ethanol (96 per cent) R*.

Reference solution (b). Dissolve 10 mg of *perindopril for stereochemical purity CRS* (containing impurity I) in *ethanol (96 per cent) R* and dilute to 5 mL with the same solvent.

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm;
– *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
– *temperature*: 50 °C for the column and the tubing preceding the column (the method has been developed with a temperature of 50 °C for at least 30 cm of the tubing preceding the column).

Mobile phase: mix, in the following order, 21.7 volumes of *acetonitrile R1*, 0.3 volumes of *pentanol R*, and 78 volumes of a 1.50 g/L solution of *sodium heptanesulfonate R* previously adjusted to pH 2.0 with a mixture of equal volumes of *perchloric acid R* and *water for chromatography R*.

Flow rate: 0.8 mL/min.

Detection: spectrophotometer at 215 nm.

Equilibration: minimum 4 h.

Injection: 10 µL.

Identification of impurities: use the chromatogram supplied with *perindopril for stereochemical purity CRS* and the chromatogram obtained with reference solution (b) to identify the peak due to impurity I.

Run time: 1.5 times the retention time of perindopril.

Relative retention with reference to perindopril (retention time = about 100 min): impurity I = about 0.9.

System suitability:

– the chromatogram obtained with reference solution (b) is similar to the chromatogram supplied with *perindopril for stereochemical purity CRS*;
– *signal-to-noise ratio*: minimum 3 for the principal peak in the chromatogram obtained with reference solution (a);
– *peak-to-valley ratio*: minimum 3, where H_p = height above the baseline of the peak due to impurity I and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to perindopril in the chromatogram obtained with reference solution (b).

Limits:

– *impurity I*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
– *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
– *disregard limit*: disregard any peak with a relative retention with reference to perindopril of less than 0.6 or more than 1.4.

Related substances. Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use or maintain them at a temperature below 10 °C.*

Test solution. Dissolve 60 mg of the substance to be examined in mobile phase A and dilute to 20.0 mL with mobile phase A.

Reference solution (a). Dissolve 3 mg of *perindopril for peak identification CRS* (containing impurities B, E, F, H and K) in 1 mL of mobile phase A.

Reference solution (b). Dilute 1.0 mL of the test solution to 200.0 mL with mobile phase A.

Reference solution (c). Dilute 1.0 mL of reference solution (b) to 10.0 mL with mobile phase A.

Column:

– *size*: *l* = 0.15 m, Ø = 4 mm;
– *stationary phase*: *end-capped octylsilyl silica gel for chromatography R* (5 µm);
– *temperature*: 60 °C for the column and the tubing preceding the column.

Mobile phase:

– *mobile phase A*: *water for chromatography R* adjusted to pH 2.5 with a mixture of equal volumes of *perchloric acid R*; and *water for chromatography R*;

– *mobile phase B*: 0.03 per cent *V/V* solution of *perchloric acid R* in *acetonitrile R1*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	95	5
5 - 60	95 → 40	5 → 60

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 215 nm.

Injection: 20 µL.

Identification of impurities: use the chromatogram supplied with *perindopril for peak identification CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities B, E, F, H and K.

Relative retention with reference to perindopril (retention time = about 25 min): impurity B = about 0.68; impurity K = about 0.72; impurity E = about 1.2; impurity F = about 1.6; impurity H = about 1.8 (impurity H may be eluted as 1 or 2 peaks).

System suitability: reference solution (a):

– *peak-to-valley ratio*: minimum 3, where H_p = height above the baseline of the peak due to impurity B and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity K.

Limits:

– *impurity E*: not more than 0.8 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.4 per cent);
– *impurity B*: not more than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);
– *impurities F, H*: for each impurity, not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
– *unspecified impurities*: for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
– *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);
– *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Water (*2.5.12*): maximum 1.0 per cent, determined on 0.50 g.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.160 g in 50 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 22.08 mg of $C_{23}H_{43}N_3O_5$.

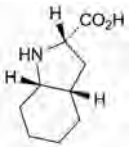
STORAGE

In an airtight container.

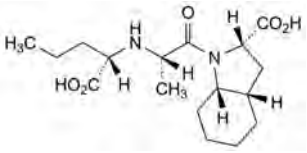
IMPURITIES

Specified impurities: A, B, E, F, H, I.

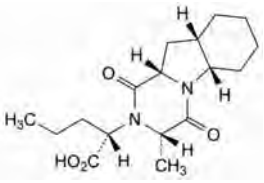
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *C, D, G, J, K, L, M, N, O, P, Q, R, S, T, U, V, W, X, Y, Z, AA, BB, CC.*



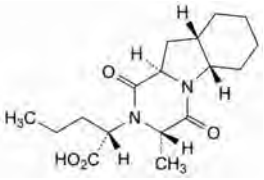
A. (2*S*,3a*S*,7a*S*)-octahydro-1*H*-indole-2-carboxylic acid,



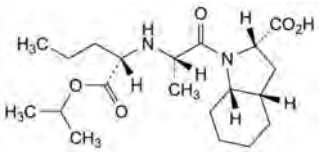
B. (2*S*,3a*S*,7a*S*)-1-[(2*S*)-2-[[(1*S*)-1-carboxybutyl]amino]-propanoyl]octahydro-1*H*-indole-2-carboxylic acid (perindoprilat),



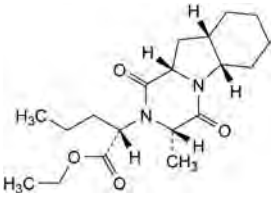
C. (2*S*)-2-[(3*S*,5a*S*,9a*S*,10a*S*)-3-methyl-1,4-dioxodecahydro-pyrazino[1,2-*a*]indol-2(1*H*)-yl]pentanoic acid,



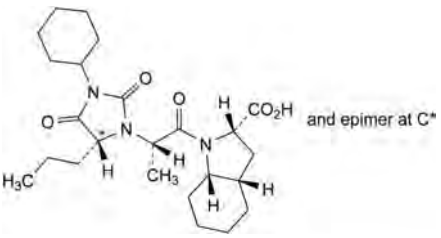
D. (2*S*)-2-[(3*S*,5a*S*,9a*S*,10a*R*)-3-methyl-1,4-dioxodecahydro-pyrazino[1,2-*a*]indol-2(1*H*)-yl]pentanoic acid,



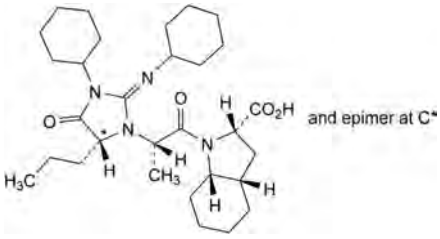
E. (2*S*,3a*S*,7a*S*)-1-[(2*S*)-2-[[(1*S*)-1-[(1-methylethoxy)-carbonyl]butyl]amino]propanoyl]octahydro-1*H*-indole-2-carboxylic acid,



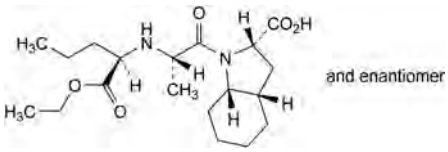
F. ethyl (2*S*)-2-[(3*S*,5a*S*,9a*S*,10a*S*)-3-methyl-1,4-dioxo-decahydropyrazino[1,2-*a*]indol-2(1*H*)-yl]pentanoate,



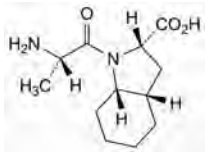
G. (2*S*,3a*S*,7a*S*)-1-[(2*S*)-2-[(5*RS*)-3-cyclohexyl-2,4-dioxo-5-propylimidazolidin-1-yl]propanoyl]octahydro-1*H*-indole-2-carboxylic acid,



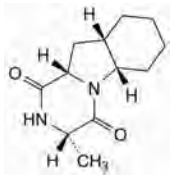
H. (2*S*,3a*S*,7a*S*)-1-[(2*S*)-2-[(5*RS*)-3-cyclohexyl-2-(cyclohexylimino)-4-oxo-5-propylimidazolidin-1-yl]propanoyl]octahydro-1*H*-indole-2-carboxylic acid,



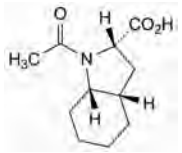
I. (2*RS*,3a*RS*,7a*RS*)-1-[(2*RS*)-2-[[(1*SR*)-1-(ethoxycarbonyl)-butyl]amino]propanoyl]octahydro-1*H*-indole-2-carboxylic acid ((±)-1″-*epi*-perindopril),



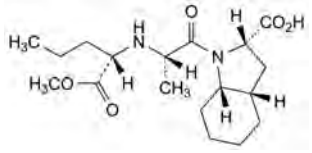
J. (2*S*,3a*S*,7a*S*)-1-[(2*S*)-2-aminopropanoyl]octahydro-1*H*-indole-2-carboxylic acid,



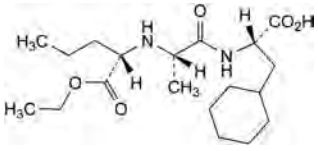
K. (3*S*,5a*S*,9a*S*,10a*S*)-3-methyldecahydropyrazino[1,2-*a*]indole-1,4-dione,



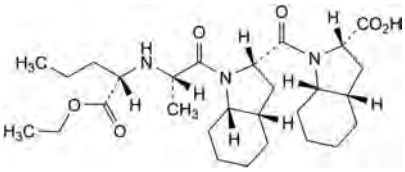
L. (2*S*,3a*S*,7a*S*)-1-acetyloctahydro-1*H*-indole-2-carboxylic acid,



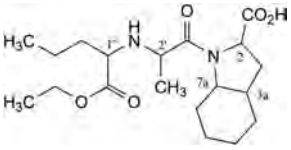
M. (2*S*,3a*S*,7a*S*)-1-[(2*S*)-2-[[(1*S*)-1-(methoxycarbonyl)-butyl]amino]propanoyl]octahydro-1*H*-indole-2-carboxylic acid,



N. (2*S*)-3-cyclohexyl-2-[[(2*S*)-2-[[(1*S*)-1-(ethoxycarbonyl)-butyl]amino]propanoyl]amino]propanoic acid,



O. (2*S*,3a*S*,7a*S*)-1-[[(2*S*,3a*S*,7a*S*)-1-[(2*S*)-2-[[(1*S*)-1-(ethoxycarbonyl)butyl]amino]propanoyl]octahydro-1*H*-indol-2-yl]carbonyl]octahydro-1*H*-indole-2-carboxylic acid,



1-[2-[[1-(ethoxycarbonyl)butyl]amino]propanoyl]octahydro-1*H*-indole-2-carboxylic acid,

P. (2*RS*,3a*RS*,7a*RS*)-, (2′*SR*)-, (1″*RS*)-: (±)-2′-*epi*-perindopril,

Q. (2*RS*,3a*RS*,7a*SR*)-, (2′*RS*)-, (1″*RS*)-: (±)-7a-*epi*-perindopril,

R. (2*RS*,3a*SR*,7a*RS*)-, (2′*RS*)-, (1″*RS*)-: (±)-3a-*epi*-perindopril,

S. (2*SR*,3a*RS*,7a*RS*)-, (2′*RS*)-, (1″*RS*)-: (±)-2-*epi*-perindopril,

T. (2*RS*,3a*RS*,7a*RS*)-, (2′*SR*)-, (1″*SR*)-: (±)-1″,2′-di-*epi*-perindopril,

U. (2*RS*,3a*RS*,7a*SR*)-, (2′*RS*)-, (1″*SR*)-: (±)-1″,7a-di-*epi*-perindopril,

V. (2*SR*,3a*SR*,7a*RS*)-, (2′*RS*)-, (1″*RS*)-: (±)-2,3a-di-*epi*-perindopril,

W. (2*SR*,3a*RS*,7a*RS*)-, (2′*RS*)-, (1″*SR*)-: (±)-1″,2-di-*epi*-perindopril,

X. (2*SR*,3a*RS*,7a*SR*)-, (2′*RS*)-, (1″*RS*)-: (±)-2,7a-di-*epi*-perindopril,

Y. (2*SR*,3a*RS*,7a*RS*)-, (2′*SR*)-, (1″*RS*)-: (±)-2,2′-di-*epi*-perindopril,

Z. (2*RS*,3a*SR*,7a*RS*)-, (2′*RS*)-, (1″*SR*)-: (±)-1″,3a-di-*epi*-perindopril,

AA. (2*RS*,3a*SR*,7a*SR*)-, (2′*RS*)-, (1″*RS*)-: (±)-3a,7a-di-*epi*-perindopril,

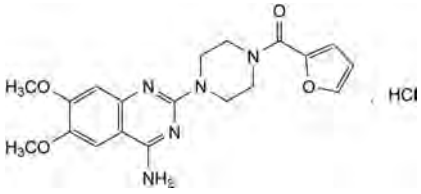
BB. (2*RS*,3a*SR*,7a*RS*)-, (2′*SR*)-, (1″*RS*)-: (±)-2′,3a-di-*epi*-perindopril,

CC. (2*RS*,3a*RS*,7a*SR*)-, (2′*SR*)-, (1″*RS*)-: (±)-2′,7a-di-*epi*-perindopril.

04/2020:0856

PRAZOSIN HYDROCHLORIDE

Prazosini hydrochloridum



$C_{19}H_{22}ClN_5O_4$ M_r 419.9

[19237-84-4]

DEFINITION

[4-(4-Amino-6,7-dimethoxyquinazolin-2-yl)piperazin-1-yl](furan-2-yl)methanone hydrochloride.

Content: 98.5 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance: white or almost white powder.

Solubility: very slightly soluble in water, slightly soluble in ethanol (96 per cent) and in methanol, practically insoluble in acetone.

IDENTIFICATION

First identification: A, C.

Second identification: B, C.

A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *prazosin hydrochloride CRS*.

B. Thin-layer chromatography (*2.2.27*).
Test solution. Dissolve 10 mg of the substance to be examined in a mixture of 1 volume of *diethylamine R*, 10 volumes of *methanol R* and 10 volumes of *methylene chloride R* and dilute to 10 mL with the same mixture of solvents.
Reference solution. Dissolve 10 mg of *prazosin hydrochloride CRS* in a mixture of 1 volume of *diethylamine R*, 10 volumes of *methanol R* and 10 volumes of *methylene chloride R* and dilute to 10 mL with the same mixture of solvents.
Plate: *TLC silica gel GF_{254} plate R*.
Mobile phase: *diethylamine R*, *ethyl acetate R* (5:95 *V/V*).
Application: 10 µL.
Development: over 2/3 of the plate.
Drying: in a current of warm air.
Detection: examine in ultraviolet light at 254 nm.
Results: the principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

C. Dissolve about 2 mg in 2 mL of *water R*. The solution gives reaction (a) of chlorides (*2.3.1*).

TESTS

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 mL with the mobile phase.

Reference solution (a). Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b). Dilute 1 mL of the test solution to 10 mL with the mobile phase. Use 1 mL of this solution to dissolve the contents of a vial of *prazosin impurity A CRS*.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase: mix 50 volumes of *methanol R* and 50 volumes of a solution containing 3.5 g/L of *sodium pentanesulfonate R* and 3.6 g/L of *tetramethylammonium hydroxide R* previously adjusted to pH 5.0 with *glacial acetic acid R*.

Flow rate: 1 mL/min.

Detection: spectrophotometer at 254 nm.

Injection: 20 µL.

Run time: 4 times the retention time of prazosin.

Identification of impurities: use the chromatogram obtained with reference solution (b) to identify the peak due to impurity A.

Relative retention with reference to prazosin (retention time = about 8 min): impurity A = about 0.8.

System suitability: reference solution (b):
- *resolution*: minimum 3.0 between the peaks due to impurity A and prazosin.

Calculation of percentage contents:
- for each impurity, use the concentration of prazosin hydrochloride in reference solution (a).

Limits:
- *impurity A*: maximum 0.2 per cent;
- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- *total*: maximum 0.5 per cent;
- *reporting threshold*: 0.05 per cent.

Iron: maximum 100 ppm.

Atomic absorption spectrometry (*2.2.23, Method I*).

Test solution. To 1.0 g add dropwise about 1.5 mL of *nitric acid R*. After fuming has subsided, evaporate on a water-bath and ignite by gradually raising the temperature from 150 °C to 1000 ± 50 °C, maintaining the final temperature for 1 h. Cool, dissolve the residue in 20 mL of *dilute hydrochloric acid R*, evaporate to about 5 mL and dilute to 25.0 mL with *dilute hydrochloric acid R*.

Reference solutions. Prepare the reference solutions using *iron standard solution (8 ppm Fe) R*, diluted as necessary with *water R*.

Source: iron hollow-cathode lamp.

Wavelength: 248 nm.

Flame: air-acetylene.

Water (*2.5.12*): maximum 0.5 per cent, determined on 1.00 g using a mixture of equal volumes of *methanol R* and *methylene chloride R* as solvent.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

In order to avoid overheating in the reaction medium, mix thoroughly throughout the titration and stop the titration immediately after the end-point has been reached.

Dissolve 0.350 g in a mixture of 20 mL of *anhydrous formic acid R* and 30 mL of *acetic anhydride R*. Titrate quickly with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 41.99 mg of $C_{19}H_{22}ClN_5O_4$.

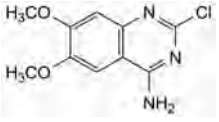
STORAGE

Protected from light.

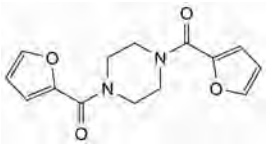
IMPURITIES

Specified impurities: A.

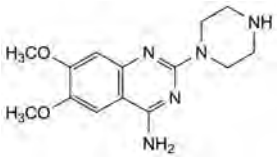
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *B, C, D, E.*



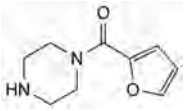
A. 2-chloro-6,7-dimethoxyquinazolin-4-amine,



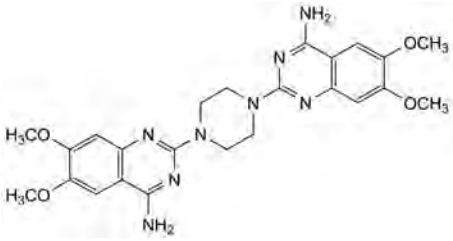
B. (piperazin-1,4-diyl)bis[(furan-2-yl)methanone],



C. 6,7-dimethoxy-2-(piperazin-1-yl)quinazolin-4-amine,



D. (furan-2-yl)(piperazin-1-yl)methanone,

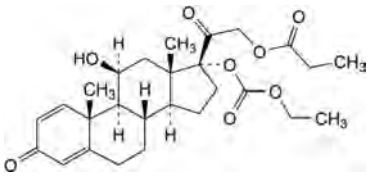


E. 2,2′-(piperazin-1,4-diyl)bis(6,7-dimethoxyquinazolin-4-amine).

04/2020:1467

PREDNICARBATE

Prednicarbatum



$C_{27}H_{36}O_8$ M_r 488.6
[73771-04-7]

DEFINITION

11β-Hydroxy-3,20-dioxopregna-1,4-diene-17,21-diyl 17-(ethyl carbonate) 21-propanoate.

Content: 97.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: practically insoluble in water, freely soluble in acetone and in ethanol (96 per cent), sparingly soluble in propylene glycol.

It shows polymorphism (*5.9*).

IDENTIFICATION

First identification: *A*.

Second identification: *B*.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *prednicarbate CRS*.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *ethanol (96 per cent) R*, evaporate to dryness on a water-bath and record new spectra using the residues.

B. Thin-layer chromatography (*2.2.27*).

Test solution. Dissolve 10 mg of the substance to be examined in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution. Dissolve 10 mg of *prednicarbate CRS* in the mobile phase and dilute to 10.0 mL with the mobile phase.

Plate: *TLC silica gel F_{254} plate R*.

Mobile phase: *methanol R*, *methylene chloride R* (10:90 *V/V*).

Application: 5 µL; the volume may be adapted based on the type of plate used.

Development: over 3/4 of the plate.

Drying: in air.

Detection: spray with a solution prepared as follows: dissolve 0.25 g of *2,4-dihydroxybenzaldehyde R* in *glacial acetic acid R*, dilute to 50 mL with the same solvent and add a mixture of 12.5 mL of *sulfuric acid R* and 37.5 mL of *glacial acetic acid R*; heat the plate at 90 °C for 35 min or until the spots appear and allow to cool; examine in daylight and in ultraviolet light at 365 nm.

Results: the principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Specific optical rotation (*2.2.7*): + 60 to + 66 (dried substance).

Dissolve 0.250 g in *ethanol (96 per cent) R* and dilute to 25.0 mL with the same solvent.

Related substances. Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution. Dissolve 30.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 mL with the mobile phase.

Reference solution (a). Dissolve 3 mg of *prednicarbate for system suitability A CRS* (containing impurities B, C, D, E and F) in the mobile phase and dilute to 5 mL with the mobile phase.

Reference solution (b). Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (c). Dissolve 30.0 mg of *prednicarbate CRS* in the mobile phase and dilute to 50.0 mL with the mobile phase.

Column:

- *size*: *l* = 0.125 m, Ø = 4 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase: *acetonitrile for chromatography R*, *water for chromatography R* (50:60 *V/V*).

Flow rate: 0.7 mL/min.

Detection: spectrophotometer at 243 nm.

Injection: 20 µL of the test solution and reference solutions (a) and (b).

Run time: twice the retention time of prednicarbate.

Identification of impurities: use the chromatogram supplied with *prednicarbate for system suitability A CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities B, C, D, E and F.

Relative retention with reference to prednicarbate (retention time = about 20 min): impurity B = about 0.25; impurity C = about 0.35; impurity D = about 0.39; impurity E = about 0.6; impurity F = about 1.2.

System suitability: reference solution (a):

– *resolution*: minimum 3.0 between the peaks due to prednicarbate and impurity F; minimum 1.5 between the peaks due to impurities C and D.

Calculation of percentage contents:

– for each impurity, use the concentration of prednicarbate in reference solution (b).

Limits:

– *impurity F*: maximum 0.8 per cent;
– *impurity C*: maximum 0.5 per cent;
– *impurity E*: maximum 0.3 per cent;
– *impurities B, D*: for each impurity, maximum 0.2 per cent;
– *unspecified impurities*: for each impurity, maximum 0.10 per cent;
– *total*: maximum 2.0 per cent;
– *reporting threshold*: 0.05 per cent.

Loss on drying (*2.2.32*): maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection: test solution and reference solution (c).

Calculate the percentage content of $C_{27}H_{36}O_8$ taking into account the assigned content of *prednicarbate CRS*.

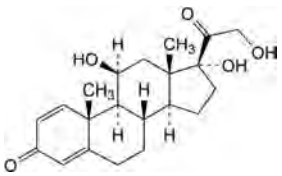
STORAGE

Protected from light.

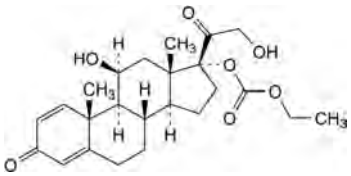
IMPURITIES

Specified impurities: B, C, D, E, F.

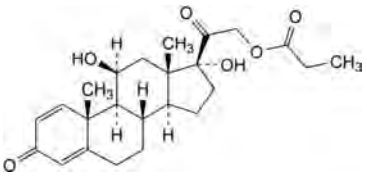
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use): A.



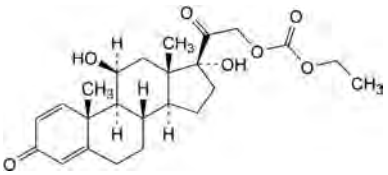
A. 11β,17,21-trihydroxypregna-1,4-diene-3,20-dione (prednisolone),



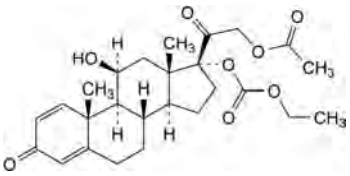
B. ethyl 11β,21-dihydroxy-3,20-dioxopregna-1,4-dien-17-yl carbonate (prednisolone 17-ethylcarbonate),



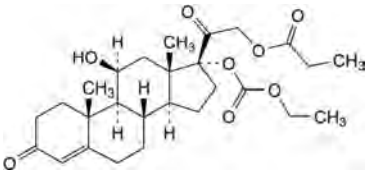
C. 11β,17-dihydroxy-3,20-dioxopregna-1,4-dien-21-yl propanoate (prednisolone 21-propanoate),



D. ethyl 11β,17-dihydroxy-3,20-dioxopregna-1,4-dien-21-yl carbonate (prednisolone 21-ethylcarbonate),



E. 11β-hydroxy-3,20-dioxopregna-1,4-dien-17,21-diyl 21-acetate 17-(ethyl carbonate) (prednisolone 21-acetate 17-ethylcarbonate),

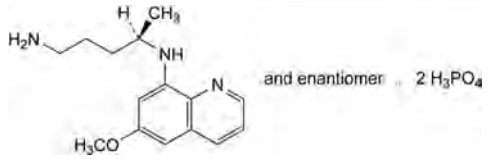


F. 11β-hydroxy-3,20-dioxopregn-4-ene-17,21-diyl 17-(ethyl carbonate) 21-propanoate (1,2-dihydroprednicarbate).

01/2019:0635
corrected 10.1

PRIMAQUINE DIPHOSPHATE

Primaquini diphosphas



$C_{15}H_{27}N_3O_9P_2$ M_r 455.3
[63-45-6]

DEFINITION

(4*RS*)-N^4-(6-Methoxyquinolin-8-yl)pentane-1,4-diamine bis(dihydrogen phosphate).

Content: 98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance: orange, crystalline powder.

Solubility: soluble in water, practically insoluble in ethanol (96 per cent).

mp: about 200 °C, with decomposition.

IDENTIFICATION

First identification: B, D.

Second identification: *A, C, D.*

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution (a). Dissolve 15 mg in a 1.03 g/L solution of *hydrochloric acid R* and dilute to 100.0 mL with the same solution.

Test solution (b). Dilute 5.0 mL of test solution (a) to 50.0 mL with a 1.03 g/L solution of *hydrochloric acid R*.

Spectral range: 310-450 nm for test solution (a); 215-310 nm for test solution (b).

Absorption maxima: at 332 nm and 415 nm for test solution (a); at 225 nm, 265 nm and 282 nm for test solution (b).

Specific absorbance at the absorption maxima:

- at 332 nm: 45 to 52 for test solution (a);
- at 415 nm: 27 to 35 for test solution (a);
- at 225 nm: 495 to 515 for test solution (b);
- at 265 nm: 335 to 350 for test solution (b);
- at 282 nm: 330 to 345 for test solution (b).

B. Infrared absorption spectrophotometry (*2.2.24*).

Examine the substances in discs prepared as follows: dissolve separately 0.1 g of the substance to be examined and 0.1 g of the reference substance in 5 mL of *water R*, add 2 mL of *dilute ammonia R2* and 5 mL of *methylene chloride R*, then shake. Dry the methylene chloride layer over 0.5 g of *anhydrous sodium sulfate R*. Prepare a blank disc using about 0.3 g of *potassium bromide R*. Apply dropwise to the disc 0.1 mL of the methylene chloride layer, allowing the methylene chloride to evaporate between applications. Dry the disc at 50 °C for 2 min.

Comparison: *primaquine diphosphate CRS*.

C. Thin-layer chromatography (*2.2.27*). *Carry out all operations as rapidly as possible, protected from light. Prepare the solutions immediately before use.*

Test solution. Dissolve 0.20 g of the substance to be examined in 5 mL of *water R* and dilute to 10 mL with *methanol R*. Dilute 1 mL of this solution to 10 mL with a mixture of equal volumes of *methanol R* and *water R*.

Reference solution. Dissolve 20 mg of *primaquine diphosphate CRS* in 5 mL of *water R* and dilute to 10 mL with *methanol R*.

Plate: *TLC silica gel GF_{254} plate R*.

Pretreatment: wash the plate with the mobile phase and allow to dry in air.

Mobile phase: *concentrated ammonia R*, *methanol R*, *methylene chloride R* (1:40:60 *V/V/V*).

Application: 5 µL.

Development: over 2/3 of the plate.

Drying: in air.

Detection: examine in ultraviolet light at 254 nm.

Results: the principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

D. Dissolve 50 mg in 5 mL of *water R*. Add 2 mL of *dilute sodium hydroxide solution R* and shake with 2 quantities, each of 5 mL, of *methylene chloride R*. The aqueous layer, acidified by addition of *nitric acid R*, gives reaction (b) of phosphates (*2.3.1*).

TESTS

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Use a freshly prepared solution. Dissolve 25.0 mg of the substance to be examined in mobile phase A, using sonication if necessary, and dilute to 50.0 mL with mobile phase A.

Reference solution (a). Dissolve 5 mg of *primaquine for system suitability CRS* (containing impurity A) in mobile phase A and dilute to 10 mL with mobile phase A.

Reference solution (b). Dilute 1.0 mL of the test solution to 100.0 mL with mobile phase A. Dilute 1.0 mL of this solution to 10.0 mL with mobile phase A.

Column:

- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octylsilyl silica gel for chromatography with embedded polar groups R* (5 µm);
- *temperature*: 25 °C.

Mobile phase:

- *mobile phase A*: *trifluoroacetic acid R*, *tetrahydrofuran R*, *acetonitrile R*, *water for chromatography R* (0.1/1/9/90 *V/V/V/V*);
- *mobile phase B*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	100	0
15 - 40	100 → 50	0 → 50

Flow rate: 1.2 mL/min.

Detection: spectrophotometer at 265 nm.

Injection: 25 µL.

Relative retention with reference to primaquine (retention time = about 13 min): impurity A = about 0.86.

System suitability: reference solution (a):

- *resolution*: minimum 2.0 between the peaks due to impurity A and primaquine.

Calculation of percentage contents:

- for each impurity, use the concentration of primaquine diphosphate in reference solution (b).

Limits:

- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- *total*: maximum 0.5 per cent;
- *reporting threshold*: 0.05 per cent.

Loss on drying (*2.2.32*): maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY

Dissolve 0.200 g in 40 mL of *anhydrous acetic acid R*, heating gently. Allow to cool and titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

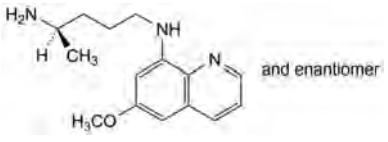
1 mL of *0.1 M perchloric acid* is equivalent to 22.77 mg of $C_{15}H_{27}N_3O_9P_2$.

STORAGE

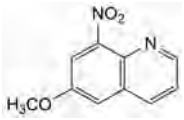
Protected from light.

IMPURITIES

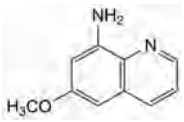
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *A, B, C, D.*



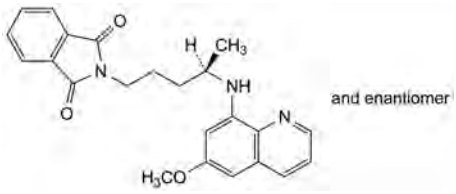
A. (4*RS*)-N^1-(6-methoxyquinolin-8-yl)pentane-1,4-diamine (quinocide),



B. 6-methoxy-8-nitroquinoline,



C. 6-methoxyquinolin-8-amine,

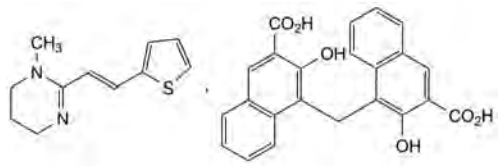


D. 2-[(4*RS*)-4-[(6-methoxyquinolin-8-yl)amino]pentyl]-1*H*-isoindole-1,3(2*H*)-dione.

04/2020:1680

PYRANTEL EMBONATE

Pyranteli embonas



$C_{34}H_{30}N_2O_6S$ M_r 594.7
[22204-24-6]

DEFINITION

1-Methyl-2-[(*E*)-2-(thiophen-2-yl)eth-1-en-1-yl]-1,4,5,6-tetrahydropyrimidine hydrogen 4,4′-methylenebis(3-hydroxynaphthalene-2-carboxylate).

Content: 98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance: pale yellow or yellow powder.

Solubility: practically insoluble in water, soluble in dimethyl sulfoxide, practically insoluble in methanol.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *pyrantel embonate CRS*.

TESTS

Related substances. Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use and protect from light.*

Solvent mixture. Mix 5 volumes of *glacial acetic acid R* and 5 volumes of *water for chromatography R*, then add 2 volumes of *diethylamine R* with cooling.

Test solution (a). Dissolve 0.800 g of the substance to be examined in 7 mL of the solvent mixture and dilute to 100.0 mL with *acetonitrile R*.

Test solution (b). Dilute 1.0 mL of test solution (a) to 10.0 mL with the mobile phase.

Reference solution (a). Dissolve 10 mg of *pyrantel impurity A CRS* in the solvent mixture, add 2.5 mL of test solution (b) and dilute to 50 mL with the solvent mixture. Dilute 2 mL of this solution to 100 mL with the solvent mixture.

Reference solution (b). Dilute 1.0 mL of test solution (b) to 200.0 mL with the mobile phase.

Reference solution (c). Dissolve 8.0 mg of *pyrantel impurity D CRS* in *acetonitrile R* and dilute to 10.0 mL with the same solvent. Dilute 1.0 mL of the solution to 100.0 mL with *acetonitrile R*.

Reference solution (d). Dissolve 8.0 mg of *pyrantel impurity C CRS* in *acetonitrile R* and dilute to 10.0 mL with the same solvent. Dilute 1.0 mL of the solution to 100.0 mL with *acetonitrile R*.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *silica gel for chromatography R* (5 µm);
- *temperature*: 30 °C.

Mobile phase: solvent mixture, *acetonitrile for chromatography R* (72:928 *V/V*).

Flow rate: 1 mL/min.

Detection: spectrophotometer at 288 nm and, for impurity D, at 238 nm.

Injection: 20 µL of test solution (b) and reference solutions (a), (b) and (d); for impurity D, 50 µL of test solution (a) and reference solution (c).

Run time: 4 times the retention time of pyrantel.

Identification of impurities: use the chromatogram obtained with reference solution (d) to identify the peak due to impurity C; use the chromatogram obtained with reference solution (c) to identify the peak due to impurity D.

Relative retention with reference to pyrantel (retention time = about 11 min): impurity C = about 0.3; embonic acid = about 0.5; impurity A = about 1.3; impurity D = about 2.2.

System suitability: reference solution (a):

- *resolution*: minimum 4.0 between the peaks due to pyrantel and impurity A.

Calculation of percentage contents:

- for impurity D, use the concentration of impurity D in reference solution (c);
- for impurity C, use the concentration of impurity C in reference solution (d);
- for impurities other than C and D, use the concentration of pyrantel embonate in reference solution (b).

Limits:

- *impurity D*: maximum 0.2 per cent;
- *impurity C*: maximum 0.10 per cent;
- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- *sum of impurities other than C and D (excluding embonic acid)*: maximum 0.3 per cent;
- *reporting threshold*: 0.05 per cent.

Chlorides (*2.4.4*): maximum 360 ppm.

To 0.46 g add 10 mL of *dilute nitric acid R* and 30 mL of *water R*. Heat on a water-bath for 5 min. Cool, dilute to 50 mL with *water R*, mix well and filter.

Sulfates (*2.4.13*): maximum 0.1 per cent.

To 0.50 g add 2.5 mL of *dilute nitric acid R* and dilute to 50 mL with *distilled water R*. Heat on a water-bath for 5 min, shake for 2 min, cool and filter.

Iron (*2.4.9*): maximum 75 ppm.

Ignite 0.66 g at 800 ± 50 °C for 2 h. Dissolve the residue in 2.5 mL of *dilute hydrochloric acid R* with gentle heating for 10 min. Cool and dilute to 50 mL with *water R*.

Loss on drying (*2.2.32*): maximum 1.0 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C for 3 h.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

To 0.450 g add 10 mL of *acetic anhydride R* and 50 mL of *glacial acetic acid R*, heat at 50 °C and stir for 10 min. Allow to cool (a clear solution is not obtained). Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*). Carry out a blank titration.

1 mL of *0.1 M perchloric acid* is equivalent to 59.47 mg of $C_{34}H_{30}N_2O_6S$.

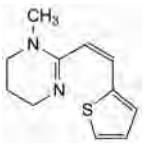
STORAGE

Protected from light.

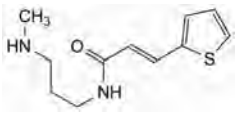
IMPURITIES

Specified impurities: C, D.

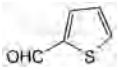
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *A, B.*



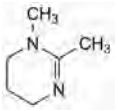
A. 1-methyl-2-[(*Z*)-2-(thiophen-2-yl)eth-1-en-1-yl]-1,4,5,6-tetrahydropyrimidine,



B. (*E*)-*N*-[3-(methylamino)propyl]-3-(thiophen-2-yl)prop-2-enamide,



C. thiophene-2-carbaldehyde,

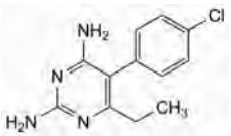


D. 1,2-dimethyl-1,4,5,6-tetrahydropyrimidine.

04/2020:0288

PYRIMETHAMINE

Pyrimethaminum



$C_{12}H_{13}ClN_4$ M_r 248.7

[58-14-0]

DEFINITION

5-(4-Chlorophenyl)-6-ethylpyrimidine-2,4-diamine.

Content: 99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, crystalline powder or colourless crystals.

Solubility: practically insoluble in water, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification: B.

Second identification: A, C.

A. Melting point (*2.2.14*): 239 °C to 243 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *pyrimethamine CRS.*

C. Thin-layer chromatography (*2.2.27*).

Solvent mixture: *methanol R*, *methylene chloride R* (10:90 *V/V*).

Test solution. Dissolve 0.1 g of the substance to be examined in the solvent mixture and dilute to 100 mL with the solvent mixture.

Reference solution. Dissolve 0.1 g of *pyrimethamine CRS* in the solvent mixture and dilute to 100 mL with the solvent mixture.

Plate: *TLC silica gel F_{254} plate R.*

Mobile phase: *methylene chloride R*, *propanol R*, *glacial acetic acid R*, *toluene R* (4:8:12:76 *V/V/V/V*).

Application: 20 µL.

Development: over 2/3 of the plate.

Drying: in air.

Detection: examine in ultraviolet light at 254 nm.

Results: the principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Solution S. Shake 1.0 g with 50 mL of *carbon dioxide-free water R* for 2 min and filter.

Appearance of solution. *Prepare the solution immediately before use.* Dissolve 0.25 g in a mixture of 1 volume of *methanol R* and 3 volumes of *methylene chloride R* and dilute to 10 mL with the same mixture of solvents. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).

Acidity or alkalinity. To 10 mL of solution S add 0.05 mL of *phenolphthalein solution R1*. The solution is colourless. Not more than 0.2 mL of *0.01 M sodium hydroxide* is required to change the colour of the indicator to pink. Add 0.4 mL of *0.01 M hydrochloric acid* and 0.05 mL of *methyl red solution R*. The solution is red or orange.

Related substances. Liquid chromatography (*2.2.29*).

Solvent mixture: mobile phase A, mobile phase B (50:50 *V/V*).

Test solution. Dissolve 10.0 mg of the substance to be examined in 4 mL of the solvent mixture using sonication and dilute to 10.0 mL with the solvent mixture.

Reference solution (a). Dissolve 10 mg of the substance to be examined and 10 mg of *pyrimethamine impurity B CRS* in 50 mL of the solvent mixture using sonication and dilute to 100 mL with the solvent mixture.

Reference solution (b). Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm;

– *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R1* (5 µm);
– *temperature*: 30 °C.

Mobile phase:
– *mobile phase A*: dissolve 2.72 g of *potassium dihydrogen phosphate R* in 900 mL of *water for chromatography R*, adjust to pH 8.0 with *ammonia R* and dilute to 1000 mL with *water for chromatography R*;
– *mobile phase B*: *acetonitrile R1*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 6	65	35
6 - 20	65 → 40	35 → 60
20 - 35	40 → 55	60 → 45

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 210 nm.

Injection: 10 µL.

Identification of impurities: use the chromatogram obtained with reference solution (a) to identify the peak due to impurity B.

Relative retention with reference to pyrimethamine (retention time = about 15 min): impurity B = about 0.8.

System suitability: reference solution (a):
– *resolution*: minimum 5.0 between the peaks due to impurity B and pyrimethamine.

Calculation of percentage contents:
– for each impurity, use the concentration of pyrimethamine in reference solution (b).

Limits:
– *unspecified impurities*: for each impurity, maximum 0.10 per cent;
– *total*: maximum 0.3 per cent;
– *reporting threshold*: 0.05 per cent.

Sulfates (*2.4.13*): maximum 80 ppm, determined on solution S. Prepare the standard using a mixture of 2.5 mL of *sulfate standard solution (10 ppm SO_4) R* and 12.5 mL of *distilled water R*.

Loss on drying (*2.2.32*): maximum 0.5 per cent, determined on 0.500 g by drying in an oven at 105 °C for 4 h.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 25 mL of *anhydrous acetic acid R* with gentle heating and allow to cool. Titrate with *0.1 M perchloric acid* determining the end-point potentiometrically (*2.2.20*).

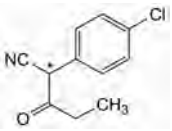
1 mL of *0.1 M perchloric acid* is equivalent to 24.87 mg of $C_{12}H_{13}ClN_4$.

STORAGE

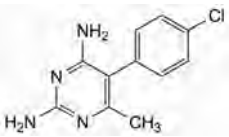
Protected from light.

IMPURITIES

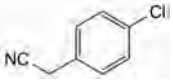
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *A, B, C, D.*



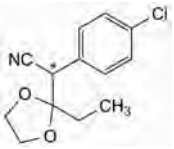
A. (2*Ξ*)-2-(4-chlorophenyl)-3-oxopentanenitrile,



B. 5-(4-chlorophenyl)-6-methylpyrimidine-2,4-diamine,



C. (4-chlorophenyl)acetonitrile,



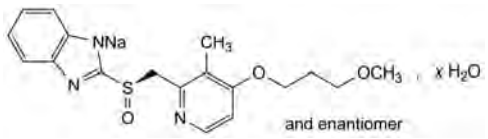
D. (*Ξ*)-(4-chlorophenyl)(2-ethyl-1,3-dioxolan-2-yl)acetonitrile.

R

04/2016:2331
corrected 10.1

RABEPRAZOLE SODIUM HYDRATE

Rabeprazolum natricum hydricum



$C_{18}H_{20}N_3NaO_3S,xH_2O$ M_r 381.4 (anhydrous substance)
[891191-56-3]

DEFINITION

Sodium 2-[(*RS*)-[[4-(3-methoxypropoxy)-3-methylpyridin-2-yl]methyl]sulfinyl]benzimidazol-1-ide hydrate.

Content: 97.5 per cent to 102.0 per cent (anhydrous substance).

It contains a variable quantity of water.

CHARACTERS

Appearance: white or slightly yellowish-white, hygroscopic, amorphous or crystalline powder.

Solubility: very soluble to freely soluble in water, freely soluble in anhydrous ethanol, practically insoluble in heptane.

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *rabeprazole sodium hydrate CRS*.
If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness and record new spectra using the residues.

B. Water (see Tests).

C. It gives reaction (a) of sodium (*2.3.1*).

TESTS

pH (*2.2.3*): 9.5 to 11.5.

Dissolve 0.100 g in *carbon dioxide-free water R* and dilute to 10 mL with the same solvent.

Related substances. Liquid chromatography (*2.2.29*). *Carry out the test protected from light.*

Solvent mixture: *methanol R*, *0.1 M phosphate buffer solution pH 11.3 R* (20:80 *V/V*).

Test solution (a). Dissolve 20.0 mg of the substance to be examined in the solvent mixture and dilute to 20.0 mL with the solvent mixture.

Test solution (b). Dilute 5.0 mL of test solution (a) to 50.0 mL with the solvent mixture.

Reference solution (a). Dilute 1.0 mL of test solution (a) to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b). Dissolve 5 mg of *rabeprazole for system suitability CRS* (containing impurities A and H) in the solvent mixture and dilute to 5 mL with the solvent mixture.

Reference solution (c). Dissolve 20.0 mg of *rabeprazole sodium hydrate CRS* in the solvent mixture and dilute to 20.0 mL with the solvent mixture. Dilute 5.0 mL of the solution to 50.0 mL with the solvent mixture.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 45 °C.

Mobile phase:
- *mobile phase A*: mix 5 volumes of *acetonitrile R* and 95 volumes of a 4.35 g/L solution of *dipotassium hydrogen phosphate R* previously adjusted to pH 7.0 with *phosphoric acid R*;
- *mobile phase B*: *methanol R*;
- *mobile phase C*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)	Mobile phase C (per cent *V/V*)
0 - 2	100	0	0
2 - 7	100 → 85	0	0 → 15
7 - 27	85 → 30	0 → 40	15 → 30
27 - 32	30 → 15	40 → 55	30

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 280 nm.

Autosampler: set at 6 °C.

Injection: 5 µL of test solution (a) and reference solutions (a) and (b).

Identification of impurities: use the chromatogram supplied with *rabeprazole for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A and H.

Relative retention with reference to rabeprazole (retention time = about 19 min): impurity A = about 0.9; impurity H = about 0.98.

System suitability: reference solution (b):
- *peak-to-valley ratio*: minimum 1.5, where H_p = height above the baseline of the peak due to impurity H and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to rabeprazole.

Calculation of percentage contents:
- for each impurity, use the concentration of rabeprazole sodium hydrate in reference solution (a).

Limits:
- *impurity A*: maximum 0.15 per cent;
- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- *total*: maximum 0.5 per cent;
- *reporting threshold*: 0.05 per cent.

Water (*2.5.12*): 1.5 per cent to 7.0 per cent, determined on 0.200 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection: test solution (b) and reference solution (c).

Calculate the percentage content of $C_{18}H_{20}N_3NaO_3S$ taking into account the assigned content of *rabeprazole sodium hydrate CRS*.

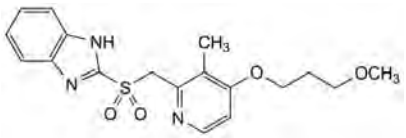
STORAGE

In an airtight container, protected from light.

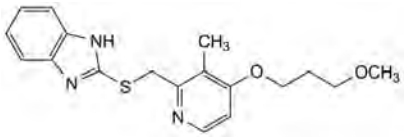
IMPURITIES

Specified impurities: *A*.

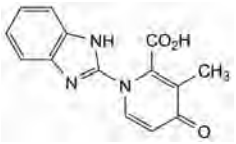
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *B, C, D, E, F, G, H, I, K*.



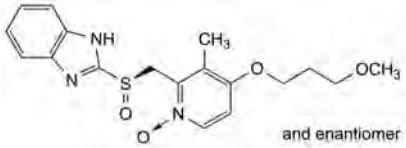
A. 2-[[[4-(3-methoxypropoxy)-3-methylpyridin-2-yl]-methyl]sulfonyl]-1*H*-benzimidazole,



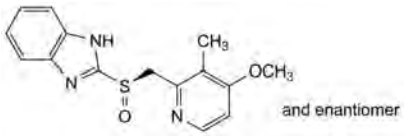
B. 2-[[[4-(3-methoxypropoxy)-3-methylpyridin-2-yl]-methyl]sulfanyl]-1*H*-benzimidazole,



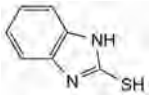
C. 1-(1*H*-benzimidazol-2-yl)-3-methyl-4-oxo-1,4-dihydropyridine-2-carboxylic acid,



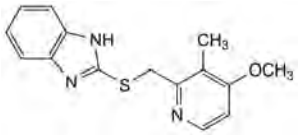
D. 2-[[(*RS*)-(1*H*-benzimidazol-2-yl)sulfinyl]methyl]-4-(3-methoxypropoxy)-3-methylpyridine 1-oxide,



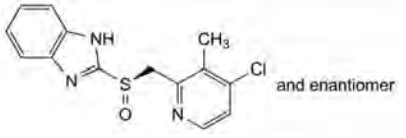
E. 2-[(*RS*)-[(4-methoxy-3-methylpyridin-2-yl)methyl]sulfinyl]-1*H*-benzimidazole,



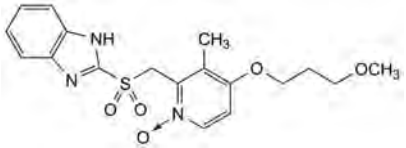
F. 1*H*-benzimidazole-2-thiol,



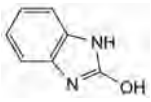
G. 2-[[(4-methoxy-3-methylpyridin-2-yl)methyl]sulfanyl]-1*H*-benzimidazole,



H. 2-[(*RS*)-[(4-chloro-3-methylpyridin-2-yl)methyl]sulfinyl]-1*H*-benzimidazole,



I. 2-[[(1*H*-benzimidazol-2-yl)sulfonyl]methyl]-4-(3-methoxypropoxy)-3-methylpyridine 1-oxide,

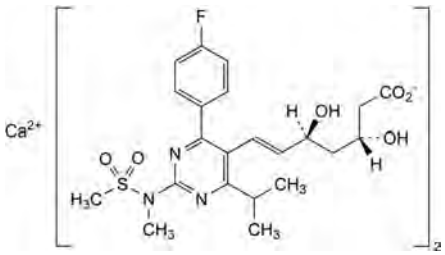


K. 1*H*-benzimidazol-2-ol.

04/2020:2631

ROSUVASTATIN CALCIUM

Rosuvastatinum calcicum



$C_{44}H_{54}CaF_2N_6O_{12}S_2$ M_r 1001
[147098-20-2]

DEFINITION

Calcium bis[(3*R*,5*S*,6*E*)-7-[4-(4-fluorophenyl)-2-(*N*-methylmethanesulfonamido)-6-(propan-2-yl)pyrimidin-5-yl]-3,5-dihydroxyhept-6-enoate].

Content: 97.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance: white or almost white, hygroscopic powder.

Solubility: slightly soluble in water, freely soluble in methylene chloride, practically insoluble in anhydrous ethanol.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *rosuvastatin calcium CRS*.

B. Enantiomeric purity (see Tests).

C. It gives reaction (b) of calcium (*2.3.1*).

TESTS

Enantiomeric purity. Liquid chromatography (*2.2.29*). *Carry out the test protected from light.*

Solvent mixture: *acetonitrile R*, *water R* (25:75 *V/V*).

Test solution. Dissolve 25.0 mg of the substance to be examined in 6 mL of *acetonitrile R* and dilute to 25.0 mL with *water R*.

Reference solution (a). Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b). Dissolve the contents of a vial of *rosuvastatin impurity G CRS* in 1 mL of the test solution.

Column:

– *size*: *l* = 0.15 m, Ø = 4.6 mm;
– *stationary phase*: *cellulose derivative of silica gel for chiral separation R* (5 µm);
– *temperature*: 35 °C.

Mobile phase: *acetonitrile for chromatography R*, 0.1 per cent *V/V* solution of *trifluoroacetic acid R* (25:75 *V/V*).

Flow rate: 0.5 mL/min.

Detection: spectrophotometer at 242 nm.

Injection: 10 µL.

Run time: 2.6 times the retention time of rosuvastatin.

Identification of impurities: use the chromatogram obtained with reference solution (b) to identify the peak due to impurity G.

Relative retention with reference to rosuvastatin (retention time = about 29 min): impurity G = about 0.9.

System suitability: reference solution (b):

– *resolution*: minimum 1.5 between the peaks due to impurity G and rosuvastatin.

Calculation of percentage content:

– for impurity G, use the concentration of rosuvastatin calcium in reference solution (a).

Limit:

– *impurity G*: maximum 0.15 per cent.

Impurity L. Liquid chromatography (*2.2.29*). *Carry out the test protected from light.*

Solvent mixture: *acetonitrile R*, *water R* (50:50 *V/V*).

Test solution. Dissolve 20.0 mg of the substance to be examined in 50 mL of *acetonitrile R* and dilute to 100.0 mL with *water R*.

Reference solution (a). Dissolve 5 mg of *rosuvastatin for impurity L identification CRS* in 10 mL of *acetonitrile R* and dilute to 20 mL with *water R*.

Reference solution (b). Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Column:

– *size*: *l* = 0.15 m, Ø = 4.6 mm;

– *stationary phase*: *end-capped solid core octylsilyl silica gel for chromatography R* (2.7 µm).

Mobile phase: to 650 mL of a 0.02 per cent *V/V* solution of *trifluoroacetic acid R*, add 350 mL of a mixture of 1 volume of *ethanol (96 per cent) R* and 2 volumes of *acetonitrile for chromatography R*.

Flow rate: 0.7 mL/min.

Detection: spectrophotometer at 243 nm.

Injection: 10 µL.

Run time: 3 times the retention time of rosuvastatin.

Identification of impurities: use the chromatogram supplied with *rosuvastatin for impurity L identification CRS* and the chromatogram obtained with reference solution (a) to identify the peak due to impurity L.

Relative retention with reference to rosuvastatin (retention time = about 22 min): impurity L = about 1.1.

System suitability: reference solution (a):

– *peak-to-valley ratio*: minimum 2.5, where H_p = height above the baseline of the peak due to impurity L and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to rosuvastatin.

Calculation of percentage content:

– *correction factor*: multiply the peak area of impurity L by 1.8;

– for impurity L, use the concentration of rosuvastatin calcium in reference solution (b).

Limit:

– *impurity L*: maximum 0.15 per cent.

Related substances. Liquid chromatography (*2.2.29*). *Carry out the test protected from light and prepare the solutions immediately before use.*

Solvent mixture: *acetonitrile R*, *water R* (25:75 *V/V*).

Test solution. Dissolve 35.0 mg of the substance to be examined in 12 mL of *acetonitrile R* and dilute to 50.0 mL with *water R*.

Reference solution (a). Dissolve 35.0 mg of *rosuvastatin calcium CRS* in 12 mL of *acetonitrile R* and dilute to 50.0 mL with *water R*.

Reference solution (b). Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (c). Dissolve 7 mg of *rosuvastatin for system suitability CRS* (containing impurities A, B and C) in 2.5 mL of *acetonitrile R* and dilute to 10 mL with *water R*.

Reference solution (d). Dissolve the contents of a vial of *rosuvastatin impurity mixture CRS* (impurities D and K) in 1 mL of the solvent mixture.

Reference solution (e). Dissolve 7 mg of *rosuvastatin for peak identification CRS* (containing impurity M) in 2.5 mL of *acetonitrile R* and dilute to 10 mL with *water R*.

Column:

– *size*: *l* = 0.15 m, Ø = 3.0 mm;

– *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (3 µm);

– *temperature*: 40 °C.

Mobile phase:

– *mobile phase A*: 1 per cent *V/V* solution of *trifluoroacetic acid R*, *acetonitrile for chromatography R*, *water for chromatography R* (1:29:70 *V/V/V*);

– *mobile phase B*: 1 per cent *V/V* solution of *trifluoroacetic acid R*, *water for chromatography R*, *acetonitrile for chromatography R* (1:24:75 *V/V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 30	100	0
30 - 50	100 → 60	0 → 40
50 - 60	60 → 0	40 → 100
60 - 70	0	100

Flow rate: 0.75 mL/min.

Detection: spectrophotometer at 242 nm.

Injection: 10 µL of the test solution and reference solutions (b), (c), (d) and (e).

Identification of impurities: use the chromatogram supplied with *rosuvastatin for system suitability CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A, B and C; use the chromatogram supplied with *rosuvastatin impurity mixture CRS* and the chromatogram obtained with reference solution (d) to identify the peaks due to impurities D and K; use the chromatogram supplied with *rosuvastatin for peak identification CRS* and the chromatogram obtained with reference solution (e) to identify the peak due to impurity M.

Relative retention with reference to rosuvastatin (retention time = about 25 min): impurity M = about 0.8; impurity A = about 0.9; impurity B = about 1.1; impurity C = about 1.5; impurity D = about 1.9; impurity K = about 2.0.

System suitability: reference solution (c):

– *resolution*: minimum 2.0 between the peaks due to rosuvastatin and impurity B.

Calculation of percentage contents:

– *correction factor*: multiply the peak area of impurity C by 1.4;

– for each impurity, use the concentration of rosuvastatin calcium in reference solution (b).

Limits:

– *impurity C*: maximum 0.8 per cent;

– *impurity B*: maximum 0.5 per cent;

– *impurity A*: maximum 0.2 per cent;

– *impurities D, K, M*: for each impurity, maximum 0.15 per cent;

– *unspecified impurities*: for each impurity, maximum 0.10 per cent;

– *total*: maximum 1.2 per cent;

– *reporting threshold*: 0.05 per cent.

Water (*2.5.12*): maximum 6.1 per cent, determined on 0.100 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection: test solution and reference solution (a).

Calculate the percentage content of $C_{44}H_{54}CaF_2N_6O_{12}S_2$ taking into account the assigned content of *rosuvastatin calcium CRS*.

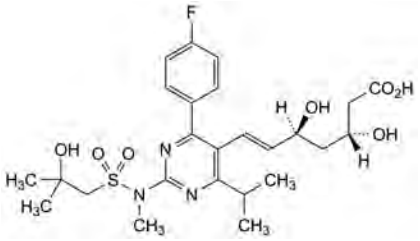
STORAGE

In an airtight container, protected from light, at a temperature of 2 °C to 8 °C.

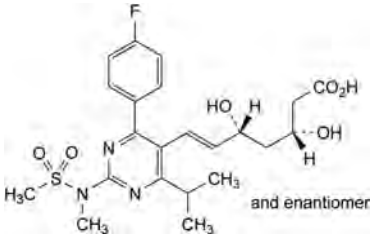
IMPURITIES

Specified impurities: A, B, C, D, G, K, L, M.

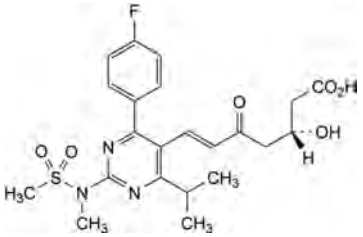
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *E, F, J.*



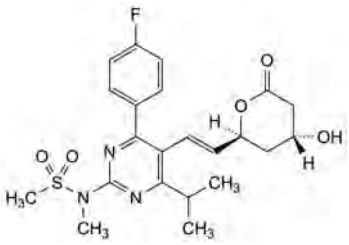
A. (3*R*,5*S*,6*E*)-7-[2-(2,*N*-dimethyl-2-hydroxypropane-1-sulfonamido)-4-(4-fluorophenyl)-6-(propan-2-yl)pyrimidin-5-yl]-3,5-dihydroxyhept-6-enoic acid,



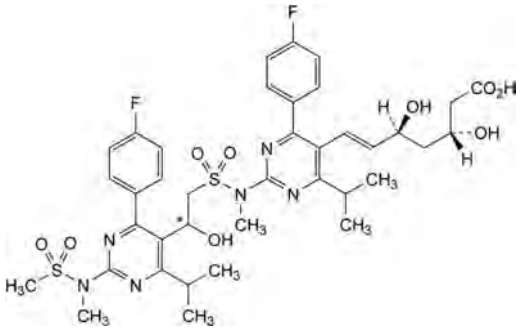
B. (3*RS*,5*RS*,6*E*)-7-[4-(4-fluorophenyl)-2-(*N*-methylmethanesulfonamido)-6-(propan-2-yl)pyrimidin-5-yl]-3,5-dihydroxyhept-6-enoic acid,



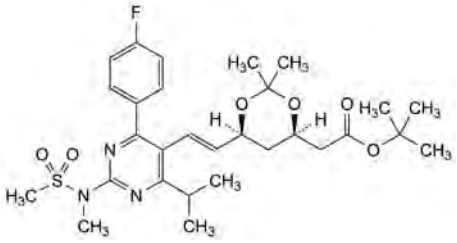
C. (3*R*,6*E*)-7-[4-(4-fluorophenyl)-2-(*N*-methylmethanesulfonamido)-6-(propan-2-yl)pyrimidin-5-yl]-3-hydroxy-5-oxohept-6-enoic acid,



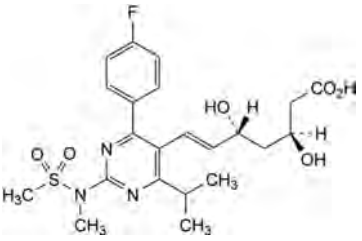
D. *N*-[4-(4-fluorophenyl)-5-[(1*E*)-2-[(2*S*,4*R*)-4-hydroxy-6-oxooxan-2-yl]ethen-1-yl]-6-(propan-2-yl)pyrimidin-2-yl]-*N*-methylmethanesulfonamide,



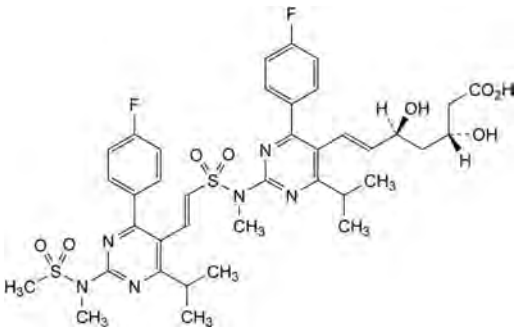
E. (3*R*,5*S*,6*E*)-7-[4-(4-fluorophenyl)-2-[(2*Ξ*)-2-[4-(4-fluorophenyl)-2-(*N*-methylmethanesulfonamido)-6-(propan-2-yl)pyrimidin-5-yl]-2-hydroxy-*N*-methylethane-1-sulfonamido]-6-(propan-2-yl)pyrimidin-5-yl]-3,5-dihydroxyhept-6-enoic acid,



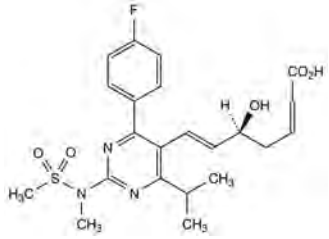
F. *tert*-butyl[(4*R*,6*S*)-6-[(1*E*)-2-[4-(4-fluorophenyl)-2-(*N*-methylmethanesulfonamido)-6-(propan-2-yl)pyrimidin-5-yl]ethen-1-yl]-2,2-dimethyl-1,3-dioxan-4-yl]acetate,



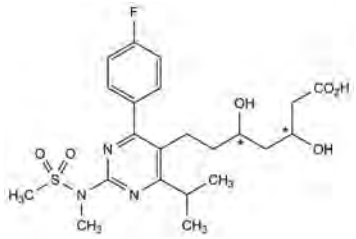
G. (3*S*,5*R*,6*E*)-7-[4-(4-fluorophenyl)-2-(*N*-methylmethanesulfonamido)-6-(propan-2-yl)pyrimidin-5-yl]-3,5-dihydroxyhept-6-enoic acid,



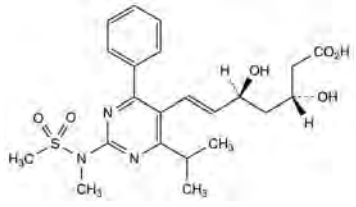
J. (3*R*,5*S*,6*E*)-7-[4-(4-fluorophenyl)-2-[(1*E*)-2-[4-(4-fluorophenyl)-2-(*N*-methylmethanesulfonamido)-6-(propan-2-yl)pyrimidin-5-yl]-*N*-methylethene-1-sulfonamido]-6-(propan-2-yl)pyrimidin-5-yl]-3,5-dihydroxyhept-6-enoic acid,



K. (2*E*,5*S*,6*E*)-7-[4-(4-fluorophenyl)-2-(*N*-methylmethanesulfonamido)-6-(propan-2-yl)pyrimidin-5-yl]-5-hydroxyhepta-2,6-dienoic acid,



L. (3*E*,5*E*)-7-[4-(4-fluorophenyl)-2-(*N*-methylmethanesulfonamido)-6-(propan-2-yl)pyrimidin-5-yl]-3,5-dihydroxyheptanoic acid,



M. (3*R*,5*S*,6*E*)-7-[2-(*N*-methylmethanesulfonamido)-4-phenyl-6-(propan-2-yl)pyrimidin-5-yl]-3,5-dihydroxyhept-6-enoic acid,

04/2020:3008

ROSUVASTATIN TABLETS

Rosuvastatini compressi

DEFINITION

Tablets containing *Rosuvastatin calcium (2631)*.

They comply with the monograph Tablets (0478) and the following additional requirements.

Content: 93.0 per cent to 105.0 per cent of the content of rosuvastatin ($C_{22}H_{28}FN_3O_6S$) stated on the label.

IDENTIFICATION

A. Record the UV spectrum of the principal peak in the chromatograms obtained with the solutions used in the assay, with a diode array detector in the range 210-400 nm.

Results: the UV spectrum of the principal peak in the chromatogram obtained with the test solution is similar to the UV spectrum of the principal peak in the chromatogram obtained with reference solution (a).

B. Examine the chromatograms obtained in the assay.

Results: the principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

TESTS

Related substances. Liquid chromatography (*2.2.29*). *Carry out the test protected from light and prepare the solutions immediately before use.*

Solvent mixture: *acetonitrile R*, *water R* (25:75 *V/V*).

Test solution. To an appropriate number of tablets (at least 6) add at least 50 mL of *water R* and shake vigorously until the tablets have disintegrated completely. Add a suitable volume of *acetonitrile R* and again shake vigorously. Add a suitable volume of *water R* to obtain a ratio of acetonitrile to water of 25:75 *V/V*. Filter through a poly(vinylidene difluoride) filter and dilute with the solvent mixture, if necessary, to obtain a concentration of rosuvastatin calcium of 1 mg/mL.

Reference solution (a). To 25.0 mg of *rosuvastatin calcium CRS* add 25 mL of *water R* and allow to stand for at least 10 min. Sonicate for about 30 min until dissolved, shaking every 10 min. Add 12.5 mL of *acetonitrile R*, mix well and dilute to 50.0 mL with *water R*. Dilute 1.0 mL of the solution to 50.0 mL with the solvent mixture.

Reference solution (b). Dissolve 7 mg of *rosuvastatin for system suitability CRS* (containing impurities A, B and C) in 2.5 mL of *acetonitrile R* and dilute to 10 mL with *water R*.

Reference solution (c). Dissolve the contents of a vial of *rosuvastatin impurity mixture CRS* (containing impurity D) in 1 mL of the solvent mixture.

Reference solution (d). Dissolve 2 mg of *rosuvastatin ethyl ester R* (impurity FP-A) in 20 mL of the solvent mixture. Dilute 1 mL of the solution to 100 mL with the solvent mixture.

Column:

- *size*: *l* = 0.15 m, Ø = 3.0 mm;
- *stationary phase*: *end-capped octadecylsilyl amorphous organosilica polymer for chromatography R* (3.5 µm);
- *temperature*: 40 °C.

Mobile phase:

- *mobile phase A*: 1 per cent *V/V* solution of *trifluoroacetic acid R*, *acetonitrile for chromatography R*, *water for chromatography R* (1:31:68 *V/V/V*);
- *mobile phase B*: 1 per cent *V/V* solution of *trifluoroacetic acid R*, *water for chromatography R*, *acetonitrile for chromatography R* (1:37:66 *V/V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 26	100	0
26 - 36	100 → 20	0 → 80
36 - 46	20	80

Flow rate: 0.7 mL/min.

Detection: spectrophotometer at 242 nm.

Injection: 10 µL.

Identification of impurities: use the chromatogram supplied with *rosuvastatin for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B and C; use the chromatogram

supplied with *rosuvastatin impurity mixture CRS* and the chromatogram obtained with reference solution (c) to identify the peak due to impurity D; use the chromatogram obtained with reference solution (d) to identify impurity FP-A.

Relative retention with reference to rosuvastatin (retention time = about 11 min): impurity A = about 0.9; impurity B = about 1.1; impurity C = about 1.7; impurity D = about 2.2; impurity FP-A = about 3.1.

System suitability: reference solution (b):

- *peak-to-valley ratio*: minimum 2.0, where H_p = height above the baseline of the peak due to impurity B and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to rosuvastatin.

Calculation of percentage contents:

- *correction factor*: multiply the peak area of impurity C by 1.4;
- for each impurity, use the concentration of rosuvastatin calcium in reference solution (a).

Limits:

- *impurities C, D*: for each impurity, maximum 1.5 per cent;
- *impurity FP-A*: maximum 0.5 per cent;
- *unspecified impurities*: for each impurity, maximum 0.2 per cent;
- *total*: maximum 2.5 per cent;
- *reporting threshold*: 0.1 per cent; disregard the peaks due to impurities A and B.

Dissolution (*2.9.3*, *Apparatus 2*). *Carry out the test protected from light.*

The tablets comply with the test and the acceptance criterion described below, unless otherwise justified and authorised.

Dissolution medium (0.05 M citrate buffer solution pH 6.6). Dissolve 147.0 g of *sodium citrate R* in 2 L of *water R*, add 3.3 g of *anhydrous citric acid R* and dilute to 10 L with the same solvent. If necessary, adjust to pH 6.6 ± 0.05 with *sodium citrate R* or *anhydrous citric acid R*. Use 900 mL of the medium.

Rotation speed: 50 r/min.

Time: 30 min.

Analysis. Liquid chromatography (*2.2.29*).

Test solution. The samples withdrawn from the dissolution vessel and filtered, using a poly(vinylidene difluoride) filter.

Reference solution. To 25.0 mg of *rosuvastatin calcium CRS* add 25 mL of *water R* and allow to stand for at least 10 min. Sonicate for about 30 min until dissolved, shaking every 10 min. Add 12.5 mL of *acetonitrile R*, mix well and dilute to 50.0 mL with *water R*. Dilute a suitable volume of the solution with the dissolution medium to obtain a concentration of rosuvastatin corresponding to the theoretical concentration of rosuvastatin in the test solution, based on the labelled content of the tablets.

Column:

- *size*: *l* = 0.05 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase: *phosphoric acid R*, *acetonitrile for chromatography R*, *water for chromatography R* (0.1:40:60 *V/V/V*).

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 242 nm.

Injection: 20 µL.

Run time: 5 min.

System suitability: reference solution:

- *repeatability*: maximum relative standard deviation of 1.0 per cent determined on 6 injections.

Calculate the amount of dissolved rosuvastatin ($C_{22}H_{28}FN_3O_6S$), expressed as a percentage of the content stated on the label, taking into account the assigned content of *rosuvastatin calcium CRS* and a conversion factor of 0.96.

Acceptance criterion:

- *Q* = 75 per cent after 30 min.

ASSAY

Liquid chromatography (*2.2.29*). *Carry out the test protected from light.*

Solvent mixture: *acetonitrile R*, *water R* (25:75 *V/V*).

Test solution. To an appropriate number of tablets (at least 6) add at least 50 mL of *water R* and shake vigorously until the tablets have disintegrated completely. Add a suitable volume of *acetonitrile R* and again shake vigorously. Add a suitable volume of *water R* to obtain a ratio of acetonitrile to water of 25:75 *V/V*. Filter through a poly(vinylidene difluoride) filter and dilute with the solvent mixture, if necessary, to obtain a concentration of rosuvastatin calcium of 0.1 mg/mL.

Reference solution (a). To 25.0 mg of *rosuvastatin calcium CRS* add 25 mL of *water R* and allow to stand for at least 10 min. Sonicate for about 30 min until dissolved, shaking every 10 min. Add 12.5 mL of *acetonitrile R*, mix well and dilute to 50.0 mL with *water R*. Dilute 1.0 mL of the solution to 5.0 mL with the solvent mixture.

Reference solution (b). Dissolve 7 mg of *rosuvastatin for system suitability CRS* (containing impurities A, B and C) in 2.5 mL of *acetonitrile R* and dilute to 10 mL with *water R*.

Column:

- *size*: *l* = 0.15 m, Ø = 3.0 mm;
- *stationary phase*: *end-capped octadecylsilyl amorphous organosilica polymer for chromatography R* (3.5 µm);
- *temperature*: 40 °C.

Mobile phase:

- *mobile phase A*: 1 per cent *V/V* solution of *trifluoroacetic acid R*, *acetonitrile for chromatography R*, *water for chromatography R* (1:31:68 *V/V/V*);
- *mobile phase B*: 1 per cent *V/V* solution of *trifluoroacetic acid R*, *acetonitrile for chromatography R* (1:100 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 14	100	0
14 - 15	100 → 10	0 → 90

Flow rate: 0.7 mL/min.

Detection: spectrophotometer at 242 nm.

Injection: 10 µL.

Identification of impurities: use the chromatogram supplied with *rosuvastatin for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peak to impurity B.

Relative retention with reference to rosuvastatin (retention time = about 11 min): impurity B = about 1.1.

System suitability:

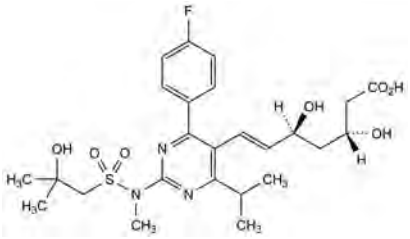
- *repeatability*: maximum relative standard deviation of 1.0 per cent determined on 6 injections of reference solution (a);
- *peak-to-valley ratio*: minimum 2.0, where H_p = height above the baseline of the peak due to impurity B and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to rosuvastatin in the chromatogram obtained with reference solution (b).

Calculate the percentage content of rosuvastatin ($C_{22}H_{28}FN_3O_6S$) taking into account the assigned content of *rosuvastatin calcium CRS* and a conversion factor of 0.96.

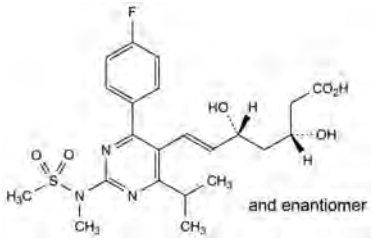
IMPURITIES

Specified impurities: C, D, FP-A.

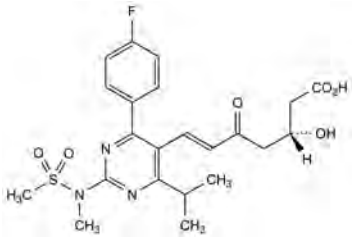
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph): *A, B, FP-B.*



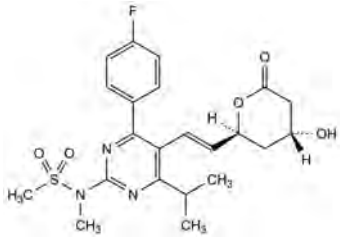
A. (3*R*,5*S*,6*E*)-7-[2-(2,*N*-dimethyl-2-hydroxypropane-1-sulfonamido)-4-(4-fluorophenyl)-6-(propan-2-yl)pyrimidin-5-yl]-3,5-dihydroxyhept-6-enoic acid,



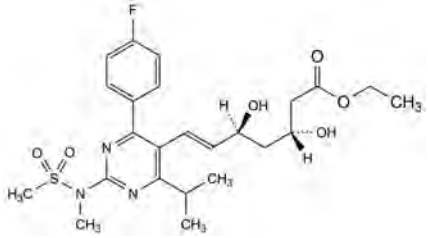
B. (3*RS*,5*RS*,6*E*)-7-[4-(4-fluorophenyl)-2-(*N*-methylmethanesulfonamido)-6-(propan-2-yl)pyrimidin-5-yl]-3,5-dihydroxyhept-6-enoic acid,



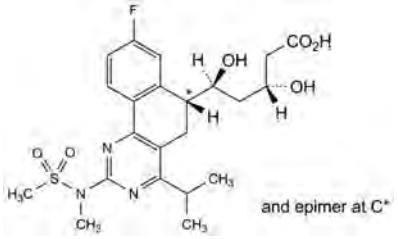
C. (3*R*,6*E*)-7-[4-(4-fluorophenyl)-2-(*N*-methylmethanesulfonamido)-6-(propan-2-yl)pyrimidin-5-yl]-3-hydroxy-5-oxohept-6-enoic acid,



D. *N*-[4-(4-fluorophenyl)-5-[(1*E*)-2-[(2*S*,4*R*)-4-hydroxy-6-oxooxan-2-yl]ethen-1-yl]-6-(propan-2-yl)pyrimidin-2-yl]-*N*-methylmethanesulfonamide,



FP-A. ethyl (3*R*,5*S*,6*E*)-7-[4-(4-fluorophenyl)-2-(*N*-methylmethanesulfonamido)-6-(propan-2-yl)pyrimidin-5-yl]-3,5-dihydroxyhept-6-enoate (rosuvastatin ethyl ester),



FP-B. (3*R*,5*S*)-5-[(6*RS*)-8-fluoro-2-(*N*-methylmethanesulfonamido)-4-(propan-2-yl)-5,6-dihydrobenzo[*h*]quinazolin-6-yl]-3,5-dihydroxypentanoic acid.

S

01/2019:0100
corrected 10.1

SODIUM SULFATE DECAHYDRATE

Natrii sulfas decahydricus

$Na_2SO_4,10H_2O$ M_r 322.2
[7727-73-3]

DEFINITION

Disodium sulfate decahydrate.

Content: 98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, crystalline powder or colourless, transparent crystals.

Solubility: freely soluble in water, practically insoluble in ethanol (96 per cent). It partly dissolves in its own water of crystallisation at about 33 °C.

IDENTIFICATION

A. It gives reaction (a) of sulfates (*2.3.1*).

B. It gives reaction (a) of sodium (*2.3.1*).

C. Loss on drying (see Tests).

TESTS

Solution S. Dissolve 5.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 mL with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity. To 10 mL of solution S add 0.1 mL of *bromothymol blue solution R1*. Not more than 0.5 mL of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Chlorides (*2.4.4*): maximum 200 ppm.

Dilute 5 mL of solution S to 15 mL with *water R*.

Calcium (*2.4.3*): maximum 200 ppm, if intended for use in the manufacture of parenteral preparations.

Dilute 10 mL of solution S to 15 mL with *distilled water R*.

Iron (*2.4.9*): maximum 40 ppm, if intended for use in the manufacture of parenteral preparations.

Dilute 5 mL of solution S to 10 mL with *water R*.

Magnesium: maximum 100 ppm, if intended for use in the manufacture of parenteral preparations.

To 10 mL of solution S add 1 mL of *glycerol (85 per cent) R*, 0.15 mL of *titan yellow solution R*, 0.25 mL of *ammonium oxalate solution R* and 5 mL of *dilute sodium hydroxide solution R* and shake. Any pink colour in the test solution is not more intense than that in a standard prepared at the same time in the same manner using a mixture of 5 mL of *magnesium standard solution (10 ppm Mg) R* and 5 mL of *water R*.

Loss on drying (*2.2.32*): 52.0 per cent to 57.0 per cent, determined on 1.000 g by drying at 30 °C for 1 h, then at 130 °C.

ASSAY

Pack 20 g of *strongly acidic ion-exchange resin R* into a glass column, at least 20 cm long and 20 mm in internal diameter, and cover it with *water R*. After 5 min, wash the resin with *water R* until the pH of the eluate is about 6 to 7 using a *pH indicator strip R*. Keep the resin covered with *water R*. Dissolve 1.100 g of the substance to be examined in 10 mL of *water R* in a beaker. Load the solution onto the column and pass through the resin at a flow rate of about 4 mL/min until the resin is just covered with the solution. Using about 200 mL of *water R*, rinse the beaker and pass the rinsings through the column at the same flow rate. Check that the pH of the last eluate is about 6 to 7 using a *pH indicator strip R*.

Titrate the eluate with *1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *1 M sodium hydroxide* is equivalent to 71.00 mg of Na_2SO_4.

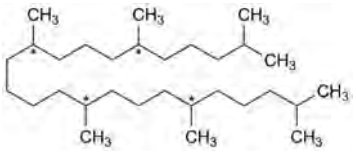
LABELLING

The label states, where applicable, that the substance is suitable for use in the manufacture of parenteral preparations.

04/2020:1630

SQUALANE

Squalanum



$C_{30}H_{62}$ M_r 422.8
[111-01-3]

DEFINITION

(6*Ξ*,10*Ξ*,15*Ξ*,19*Ξ*)-2,6,10,15,19,23-Hexamethyltetracosane (perhydrosqualene). It may be of vegetable (unsaponifiable matter of olive oil), animal (shark liver oil) or synthetic origin.

Content: 96.0 per cent to 103.0 per cent.

PRODUCTION

The origin of the squalane (vegetable, animal or synthetic) is stated by the manufacturer.

CHARACTERS

Appearance: clear, colourless, oily liquid.

Solubility: practically insoluble in water, miscible with most fats and oils, freely soluble in acetone and in cyclohexane, practically insoluble in ethanol (96 per cent).

Relative density: about 0.815.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *squalane CRS*.

B. Refractive index (see Tests).

TESTS

Appearance. The substance to be examined is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Refractive index (*2.2.6*): 1.450 to 1.454.

Acid value (*2.5.1*): maximum 0.2.

Iodine value (*2.5.4, Method A*): maximum 4.0.

Saponification value (*2.5.6*): maximum 3.0.

Total ash (*2.4.16*): maximum 0.5 per cent, determined on 1.000 g.

ASSAY

Gas chromatography (*2.2.28*).

Internal standard solution. To 1.0 mL of *dimethylacetamide R* add 100.0 mL of *heptane R*.

Test solution. Dissolve 0.100 g of the substance to be examined in the internal standard solution and dilute to 25.0 mL with the same solution.

Reference solution (a). Dissolve 0.100 g of *squalane CRS* in the internal standard solution and dilute to 25.0 mL with the same solution.

Reference solution (b). To 0.1 mL of *methyl erucate R* add 0.100 g of the substance to be examined, dissolve in the internal standard solution and dilute to 25 mL with the same solution.

Column:

- *material*: fused silica;
- *size*: *l* = 30 m, Ø = 0.32 mm;
- *stationary phase*: *methylpolysiloxane R* (film thickness 1 µm).

Carrier gas: *helium for chromatography R.*

Flow rate: 1.7 mL/min.

Split ratio: 1:12.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 39	60 → 290
	39 - 50	290
Injection port		275
Detector		300

Detection: flame ionisation.

Injection: 1 µL.

Relative retention with reference to squalane (retention time = about 41 min): internal standard = about 0.2; methyl erucate = about 0.9; cyclosqualane = 1.05.

System suitability: reference solution (b):

- *resolution*: minimum 5 between the peaks due to methyl erucate and squalane.

Calculate the percentage content of $C_{30}H_{62}$ using the chromatogram obtained with reference solution (a) and taking into account the assigned content of *squalane CRS*.

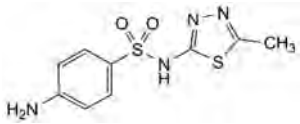
LABELLING

The label states the origin of squalane (vegetable, animal or synthetic).

04/2020:0637

SULFAMETHIZOLE

Sulfamethizolum



$C_9H_{10}N_4O_2S_2$ M_r 270.3

[144-82-1]

DEFINITION

4-Amino-*N*-(5-methyl-1,3,4-thiadiazol-2-yl)benzene-1-sulfonamide.

Content: 99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance: white or yellowish-white, crystalline powder or crystals.

Solubility: very slightly soluble in water, soluble in acetone, sparingly soluble in ethanol (96 per cent). It dissolves in dilute solutions of alkali hydroxides and in dilute mineral acids.

mp: about 210 °C.

IDENTIFICATION

First identification: A.

Second identification: B.

A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *sulfamethizole CRS*.

B. Thin-layer chromatography (*2.2.27*).
Test solution. Dissolve 30 mg of the substance to be examined in *acetone R* and dilute to 3 mL with the same solvent.
Reference solution. Dissolve 30 mg of *sulfamethizole CRS* in *acetone R* and dilute to 3 mL with the same solvent.
Plate: *TLC silica gel F_{254} plate R.*
Mobile phase: *methanol R*, *methylene chloride R* (15:85 *V/V*).
Application: 2 µL; the volume may be adapted based on the type of plate used.
Development: over 3/4 of the plate.
Drying: at 100-105 °C.
Detection A: examine in ultraviolet light at 254 nm.
Results A: the principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.
Detection B: spray with a 40 g/L solution of *copper acetate R* and examine in daylight.
Results B: the principal spot in the chromatogram obtained with the test solution is similar in colour to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Appearance of solution. The solution is not more intensely coloured than reference solution Y_5, BY_5 or GY_5 (*2.2.2, Method II*).

Dissolve 1.0 g in a mixture of 5 mL of *dilute sodium hydroxide solution R* and 5 mL of *water R*.

Acidity. To 1.25 g add 25 mL of *carbon dioxide-free water R* and heat at 70 °C for 5 min. Cool for about 15 min in iced water and filter. To 20 mL of the filtrate add 0.1 mL of *bromothymol blue solution R1.* Not more than 0.5 mL of *0.1 M sodium hydroxide* is required to change the colour of the indicator.

Related substances. Liquid chromatography (*2.2.29*).

Solvent mixture: *methanol R*, *water R* (15:85 *V/V*).

Test solution. Dissolve 20.0 mg of the substance to be examined in 1 mL of *methanol R*, sonicate if necessary and dilute to 20.0 mL with the solvent mixture.

Reference solution (a). Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b). Dissolve 2 mg of *sulfamethizole for system suitability CRS* (containing impurities B, C and D) in 0.1 mL of *methanol R*, sonicate if necessary and dilute to 2 mL with the solvent mixture.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography compatible with 100 per cent aqueous mobile phases R* (5 µm);
- *temperature*: 30 °C.

Mobile phase:

- *mobile phase A*: *acetic acid R, methanol R, water for chromatography R* (1:14:85 *V/V/V*);

– *mobile phase B*: *acetic acid R, methanol R* (1:99 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 20	100	0
20 - 40	100 → 30	0 → 70
40 - 45	30	70

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 254 nm.

Injection: 20 µL.

Identification of impurities: use the chromatogram supplied with *sulfamethizole for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities B, C and D.

Relative retention with reference to sulfamethizole (retention time = about 17 min): impurity B = about 0.23; impurity C = about 0.24; impurity D = about 1.9.

System suitability: reference solution (b):

– *resolution*: minimum 1.5 between the peaks due to impurities B and C.

Calculation of percentage contents:

– *correction factor*: multiply the peak area of impurity D by 1.4;
– for each impurity, use the concentration of sulfamethizole in reference solution (a).

Limits:

– *impurity D*: maximum 0.2 per cent;
– *unspecified impurities*: for each impurity, maximum 0.10 per cent;
– *total*: maximum 0.5 per cent;
– *reporting threshold*: 0.05 per cent.

Loss on drying (*2.2.32*): maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Carry out the determination of primary aromatic amino-nitrogen (*2.5.8*), using 0.2500 g and determining the end-point electrometrically.

1 mL of *0.1 M sodium nitrite* is equivalent to 27.03 mg of $C_9H_{10}N_4O_2S_2$.

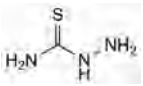
STORAGE

Protected from light.

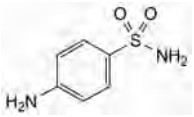
IMPURITIES

Specified impurities: *D*.

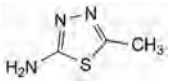
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *A, B, C*.



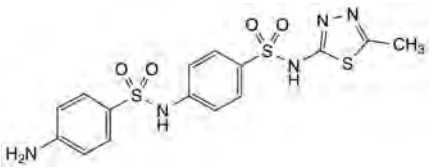
A. hydrazinecarbothioamide (thiosemicarbazide),



B. 4-aminobenzene-1-sulfonamide (sulfanilamide),



C. 5-methyl-1,3,4-thiadiazol-2-amine,



D. 4-(4-aminobenzene-1-sulfonamido)-*N*-(5-methyl-1,3,4-thiadiazol-2-yl)benzene-1-sulfonamide.

T

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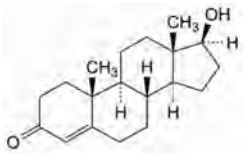
TESTOSTERONE

Testosteronum



$C_{19}H_{28}O_2$ M_r 288.4
[58-22-0]

DEFINITION

17β-Hydroxyandrost-4-en-3-one.

Content: 98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white crystalline powder, or colourless or yellowish-white crystals.

Solubility: practically insoluble in water, freely soluble in ethanol (96 per cent) and in methylene chloride, practically insoluble in fatty oils.

mp: about 155 °C.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *testosterone for ID and assay CRS*.

TESTS

Specific optical rotation (*2.2.7*): + 106 to + 114 (dried substance).

Dissolve 0.250 g in *anhydrous ethanol R* and dilute to 25.0 mL with the same solvent.

Related substances. Liquid chromatography (*2.2.29*).

Solvent mixture: *acetonitrile R*, *water R* (30:70 *V/V*).

Test solution. Dissolve 35.0 mg of the substance to be examined in 30 mL of *acetonitrile R* and dilute to 100.0 mL with *water R*.

Reference solution (a). Dissolve 9 mg of *testosterone for system suitability A CRS* (containing impurities C, G and K) in 8 mL of *acetonitrile R* and dilute to 25 mL with *water R*.

Reference solution (b). Dissolve 7.0 mg of *testosterone impurity I CRS* in 30 mL of *acetonitrile R* and dilute to 100.0 mL with *water R*. Dilute 1.0 mL of the solution to 100.0 mL with the solvent mixture.

Reference solution (c). Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (d). Dissolve 35.0 mg of *testosterone for ID and assay CRS* in 30 mL of *acetonitrile R* and dilute to 100.0 mL with *water R*.

Column:

- *size*: *l* = 0.10 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped solid core phenylhexylsilyl silica gel for chromatography R* (2.7 µm);
- *temperature*: 30 °C.

Mobile phase:

- *mobile phase A*: *water for chromatography R*;
- *mobile phase B*: *acetonitrile for chromatography R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2.7	65	35
2.7 - 7	65 → 63	35 → 37
7 - 10	63 → 10	37 → 90
10 - 12	10	90

Flow rate: 1.5 mL/min.

Detection: spectrophotometer at 242 nm, and for impurity I, at 290 nm.

Injection: 20 µL of the test solution and reference solutions (a), (b) and (c).

Identification of impurities: use the chromatogram supplied with *testosterone for system suitability A CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities C, G and K; use the chromatogram obtained with reference solution (b) to identify the peak due to impurity I.

Relative retention with reference to testosterone (retention time = about 4 min): impurity I = about 0.84; impurity K = about 0.86; impurity G = about 1.1; impurity C = about 1.3.

System suitability: reference solution (a):

- *peak-to-valley ratio*: minimum 2.0, where H_p = height above the baseline of the peak due to impurity G and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to testosterone.

Calculation of percentage contents:

- for impurity I, use the concentration of impurity I in reference solution (b);
- for impurities other than I, use the concentration of testosterone in reference solution (c).

Limits:

- *impurity C*: maximum 0.3 per cent;
- *impurity I at 290 nm*: maximum 0.2 per cent;
- *impurity K*: maximum 0.15 per cent;
- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- *total*: maximum 0.6 per cent;
- *reporting threshold*: 0.05 per cent.

Loss on drying (*2.2.32*): maximum 1.0 per cent, determined on 0.500 g by drying in an oven at 105 °C for 2 h.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection: 5 µL of the test solution and reference solution (d).

Calculate the percentage content of $C_{19}H_{28}O_2$ taking into account the assigned content of *testosterone for ID and assay CRS*.

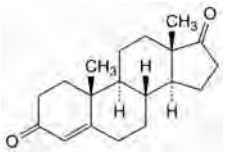
STORAGE

Protected from light.

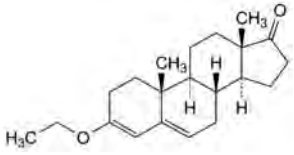
IMPURITIES

Specified impurities: C, I, K.

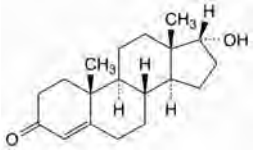
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *A, B, E, G, H, J, L, M*.



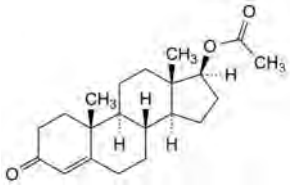
A. androst-4-ene-3,17-dione (androstenedione),



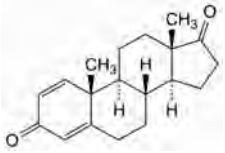
B. 3-ethoxyandrosta-3,5-dien-17-one (androstenedione ethylenolether),



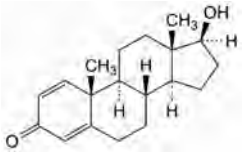
C. 17α-hydroxyandrost-4-en-3-one (17-*epi*-testosterone),



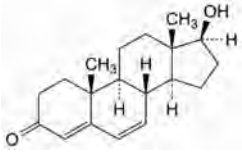
E. 3-oxoandrost-4-en-17β-yl acetate (testosterone acetate),



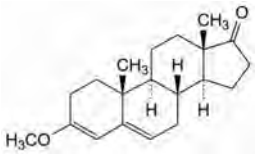
G. androsta-1,4-diene-3,17-dione (androstadienedione),



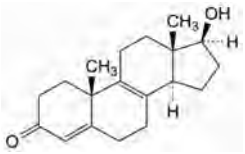
H. 17β-hydroxyandrosta-1,4-dien-3-one (boldenone),



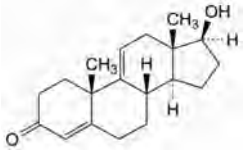
I. 17β-hydroxyandrosta-4,6-dien-3-one (Δ6-testosterone),



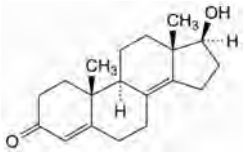
J. 3-methoxyandrosta-3,5-dien-17-one (androstenedione methylenolether),



K. 17β-hydroxyandrosta-4,8-dien-3-one,



L. 17β-hydroxyandrosta-4,9(11)-dien-3-one,

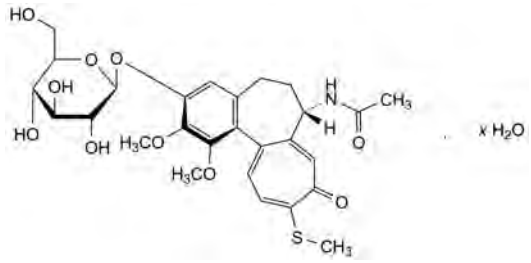


M. 17β-hydroxyandrosta-4,8(14)-dien-3-one.

04/2016:2814
corrected 10.1

THIOCOLCHICOSIDE HYDRATE

Thiocolchicosidum hydricum



$C_{27}H_{33}NO_{10}S,xH_2O$ M_r 563.6 (anhydrous substance)
[1622135-03-8]

DEFINITION

N-[(7*S*,12aR_a)-3-(β-D-Glucopyranosyloxy)-1,2-dimethoxy-10-(methylsulfanyl)-9-oxo-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl]acetamide.

Content: 96.0 per cent to 102.0 per cent (anhydrous substance).

It contains a variable quantity of water.

CHARACTERS

Appearance: yellow crystalline powder, slightly hygroscopic.

Solubility: sparingly soluble in water, slightly soluble in ethanol (96 per cent), practically insoluble in acetone.

mp: about 272 °C.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *thiocolchicoside hydrate CRS*.

TESTS

Appearance of solution. The solution is clear (*2.2.1*).

Dissolve 0.100 g in *methanol R* and dilute to 10 mL with the same solvent.

Specific optical rotation (*2.2.7*): – 600 to – 570 (anhydrous substance).

Dissolve 0.500 g in *water R* and dilute to 100.0 mL with the same solvent.

Related substances. Liquid chromatography (*2.2.29*). *Carry out the test protected from light.*

Test solution (a). Dissolve 20.0 mg of the substance to be examined in *methanol R* and dilute to 20.0 mL with the same solvent.

Test solution (b). Dilute 1.0 mL of test solution (a) to 10.0 mL with *methanol R*.

Reference solution (a). Dilute 1.0 mL of test solution (a) to 100.0 mL with *methanol R*. Dilute 2.0 mL of this solution to 10.0 mL with *methanol R*.

Reference solution (b). Dissolve 5 mg of *thiocolchicoside for system suitability CRS* (containing impurities D, E, G, H, K and L) in *methanol R* and dilute to 5 mL with the same solvent.

Reference solution (c). Dissolve 20.0 mg of *thiocolchicoside hydrate CRS* in *methanol R* and dilute to 20.0 mL with the same solvent. Dilute 1.0 mL of the solution to 10.0 mL with *methanol R*.

Column:

- *size*: *l* = 0.10 m, Ø = 2.1 mm;
- *stationary phase*: *end-capped ethylene-bridged phenylsilyl silica gel for chromatography (hybrid material) R* (1.7 µm);
- *temperature*: 25 °C.

Mobile phase:

- *mobile phase A*: dissolve 0.22 g of *ammonium formate R* in 350 mL of *water for chromatography R* and mix with 25 mL of *tetrahydrofuran R*;
- *mobile phase B*: dilute 45 µL of *anhydrous formic acid R* in 900 mL of *acetonitrile R* and mix with 100 mL of *tetrahydrofuran R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	99	1
2 - 6	99 → 92	1 → 8
6 - 7.5	92 → 70	8 → 30
7.5 - 8.8	70 → 50	30 → 50
8.8 - 9.2	50 → 2	50 → 98
9.2 - 10	2	98

Flow rate: 0.4 mL/min.

Detection: spectrophotometer at 370 nm.

Injection: 1.0 µL of test solution (a) and reference solutions (a) and (b).

Identification of impurities: use the chromatogram supplied with *thiocolchicoside for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities D, E, G, H, K and L.

Relative retention with reference to thiocolchicoside (retention time = about 6 min): impurity D = about 0.2; impurity H = about 0.4; impurity G = about 0.7; impurity K = about 0.8; impurity E = about 1.05; impurity L = about 1.11.

System suitability:

- *signal-to-noise ratio*: minimum 50 for the principal peak in the chromatogram obtained with reference solution (a);
- *peak-to-valley ratio*: minimum 1.5, where H_p = height above the baseline of the peak due to impurity E and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to thiocolchicoside in the chromatogram obtained with reference solution (b).

Calculation of percentage contents:

- *correction factors*: multiply the peak areas of the following impurities by the corresponding correction factor: impurity D = 1.7; impurity H = 2.8;
- for each impurity, use the concentration of thiocolchicoside hydrate in reference solution (a).

Limits:

- *impurity E*: maximum 1.0 per cent;
- *impurity H*: maximum 0.7 per cent;
- *impurity K*: maximum 0.3 per cent;
- *impurities D, G*: for each impurity, maximum 0.2 per cent;
- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- *total*: maximum 2.5 per cent;
- *reporting threshold*: 0.05 per cent; disregard the peak due to impurity L.

Water (*2.5.12*): maximum 2.5 per cent, determined on 0.500 g.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection: test solution (b) and reference solution (c).

Calculate the percentage content of $C_{27}H_{33}NO_{10}S$ taking into account the assigned content of *thiocolchicoside hydrate CRS*.

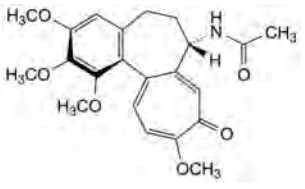
STORAGE

In an airtight container, protected from light.

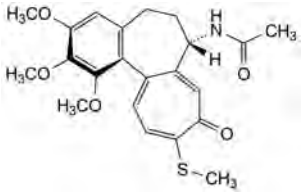
IMPURITIES

Specified impurities: D, E, G, H, K.

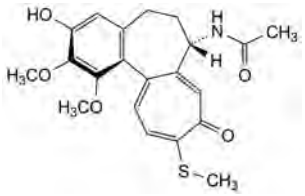
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *A, B, C, J, L.*



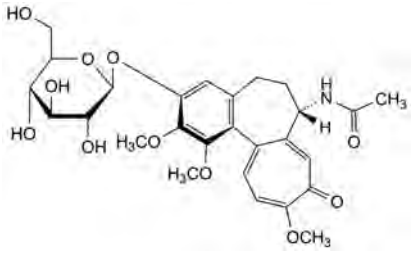
A. *N*-[(7*S*,12aR_a)-1,2,3,10-tetramethoxy-9-oxo-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl]acetamide (colchicine),



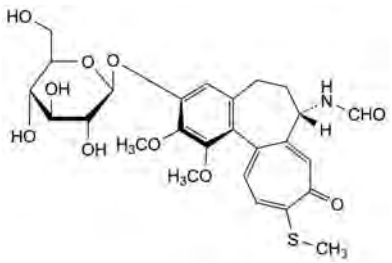
B. *N*-[(7*S*,12aR_a)-1,2,3-trimethoxy-10-(methylsulfanyl)-9-oxo-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl]acetamide (thiocolchicine),



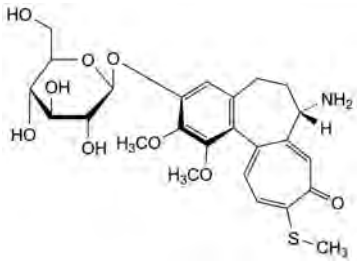
C. *N*-[(7*S*,12aR_a)-3-hydroxy-1,2-dimethoxy-10-(methylsulfanyl)-9-oxo-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl]acetamide (3-*O*-demethylthiocolchicine),



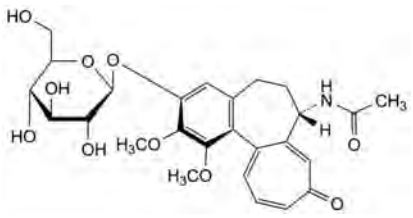
D. *N*-[(7*S*,12aR_a)-3-(β-D-glucopyranosyloxy)-1,2,10-trimethoxy-9-oxo-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl]acetamide (colchicoside),



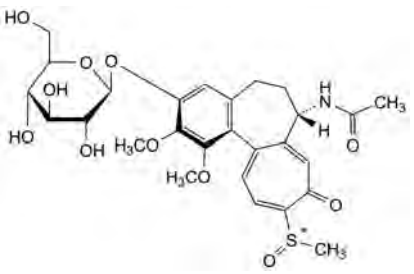
E. *N*-[(7*S*,12aR_a)-3-(β-D-glucopyranosyloxy)-1,2-dimethoxy-10-(methylsulfanyl)-9-oxo-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl]formamide (*N*-deacetyl-*N*-formylthiocolchicoside),



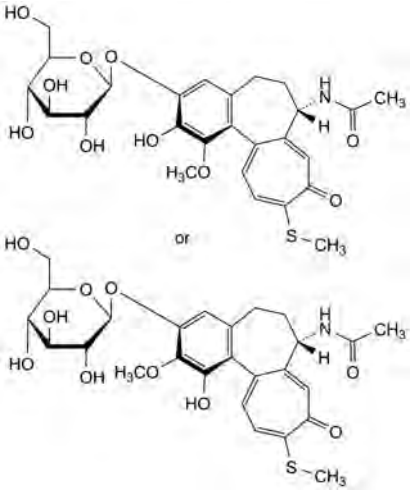
G. (7*S*,12aR_a)-7-amino-3-(β-D-glucopyranosyloxy)-1,2-dimethoxy-10-(methylsulfanyl)-6,7-dihydrobenzo[*a*]-heptalen-9(5*H*)-one (*N*-deacetylthiocolchicoside),



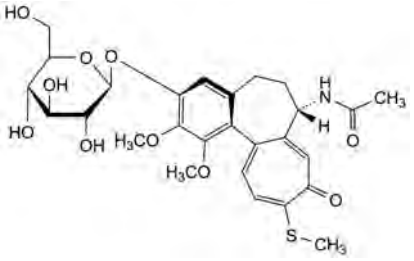
H. *N*-[(7*S*,12aR_a)-3-(β-D-glucopyranosyloxy)-1,2-dimethoxy-9-oxo-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl]acetamide (10-demethoxycolchicoside),



J. *N*-[(7*S*,12aR_a)-3-(β-D-glucopyranosyloxy)-1,2-dimethoxy-10-(methylsulfinyl)-9-oxo-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl]acetamide (thiocolchicoside *S*-oxide),



K. *N*-[(7*S*,12aR_a)-3-(β-D-glucopyranosyloxy)-2-hydroxy-1-methoxy-10-(methylsulfanyl)-9-oxo-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl]acetamide or *N*-[(7*S*,12aR_a)-3-(β-D-glucopyranosyloxy)-1-hydroxy-2-methoxy-10-(methylsulfanyl)-9-oxo-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl]acetamide,

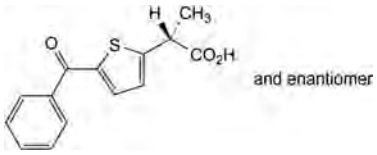


L. *N*-[(7*S*,12aS_a)-3-(β-D-glucopyranosyloxy)-1,2-dimethoxy-10-(methylsulfanyl)-9-oxo-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl]acetamide (12aS_a-thiocolchicoside).

04/2020:1157

TIAPROFENIC ACID

Acidum tiaprofenicum



$C_{14}H_{12}O_3S$ M_r 260.3
[33005-95-7]

DEFINITION

(2*RS*)-2-(5-Benzoylthiophen-2-yl)propanoic acid.

Content: 99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: practically insoluble in water, freely soluble in acetone, in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION

First identification: C.

Second identification: A, B, D.

A. Melting point (*2.2.14*): 95 °C to 99 °C.

B. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution. Dissolve 25.0 mg in *ethanolic hydrochloric acid R* and dilute to 50.0 mL with the same solvent. Dilute 1.0 mL of this solution to 50.0 mL with *ethanolic hydrochloric acid R*.

Spectral range: 220-350 nm.

Absorption maximum: 305 nm.

Shoulder: 262 nm.

Specific absorbance at the absorption maximum: 550 to 590.

C. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *tiaprofenic acid CRS*.

D. Thin-layer chromatography (*2.2.27*).

Test solution. Dissolve 10 mg of the substance to be examined in *methylene chloride R* and dilute to 10 mL with the same solvent.

Reference solution (a). Dissolve 10 mg of *tiaprofenic acid CRS* in *methylene chloride R* and dilute to 10 mL with the same solvent.

Reference solution (b). Dissolve 10 mg of *ketoprofen CRS* in *methylene chloride R* and dilute to 10 mL with the same solvent. Dilute 1 mL of this solution to 2 mL with reference solution (a).

Plate: *TLC silica gel F_{254} plate R.*

Mobile phase: *acetic acid R*, *methylene chloride R*, *acetone R* (1:20:80 *V/V/V*).

Application: 10 µL.

Development: over 3/4 of the plate.

Drying: in air.

Detection: examine in ultraviolet light at 254 nm.

Retardation factors: tiaprofenic acid = about 0.3; ketoprofen = about 0.4.

System suitability: reference solution (b):

– the chromatogram shows 2 clearly separated principal spots.

Results: the principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS

Appearance of solution. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Dissolve 2.0 g in *ethanol (96 per cent) R* and dilute to 20 mL with the same solvent.

Optical rotation (*2.2.7*): − 0.10° to + 0.10°.

Dissolve 0.50 g in *ethyl acetate R* and dilute to 10.0 mL with the same solvent.

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 20.0 mL with the mobile phase.

Reference solution (a). Dilute 1.0 mL of the test solution to 50.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b). Dilute 5.0 mL of reference solution (a) to 10.0 mL with the mobile phase.

Reference solution (c). Dissolve 10.0 mg of *tiaprofenic acid impurity C CRS* in the mobile phase and dilute to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 50.0 mL with the mobile phase.

Reference solution (d). Dilute 1 mL of reference solution (a) to 2 mL with reference solution (c).

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm;

– *stationary phase*: *silica gel for chromatography R* (5 µm).

Mobile phase: *water for chromatography R*, *glacial acetic acid R*, *hexane R*, *methylene chloride R* (0.25:20:500:500 *V/V/V/V*); add the water to the acetic acid, then hexane and methylene chloride; sonicate the mixture for 2 min. Do not degas with helium during analysis.

Flow rate: 1 mL/min.

Detection: spectrophotometer at 250 nm.

Injection: 20 µL.

Run time: twice the retention time of tiaprofenic acid.

Identification of impurities: use the chromatogram obtained with reference solution (c) to identify the peak due to impurity C.

Relative retention with reference to tiaprofenic acid (retention time = about 14 min): impurity C = about 0.86.

System suitability: reference solution (d):

– *resolution*: minimum 3.0 between the peaks due to impurity C and tiaprofenic acid.

Limits:

– *impurity C*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.2 per cent);

– *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);

– *sum of impurities other than C*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

– *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*): maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C for 3 h.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 25 mL of *ethanol (96 per cent) R*. Add 25 mL of *water R* and 0.5 mL of *phenolphthalein solution R*. Titrate with *0.1 M sodium hydroxide*.

1 mL of *0.1 M sodium hydroxide* is equivalent to 26.03 mg of $C_{14}H_{12}O_3S$.

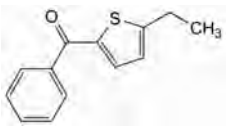
STORAGE

Protected from light.

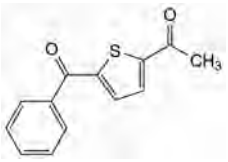
IMPURITIES

Specified impurities: C.

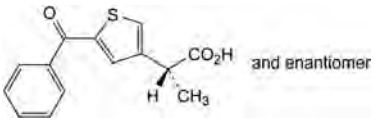
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *A, B, D, E, F.*



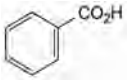
A. (5-ethylthiophen-2-yl)phenylmethanone,



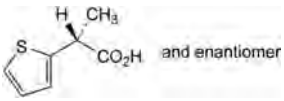
B. 1-(5-benzoylthiophen-2-yl)ethanone,



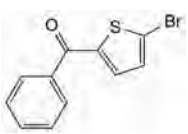
C. (2*RS*)-2-(5-benzoylthiophen-3-yl)propanoic acid,



D. benzoic acid,



E. (2*RS*)-2-(thiophen-2-yl)propanoic acid,

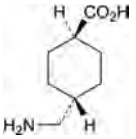


F. (5-bromothiophen-2-yl)phenylmethanone.

04/2020:0875

TRANEXAMIC ACID

Acidum tranexamicum



$C_8H_{15}NO_2$ M_r 157.2
[1197-18-8]

DEFINITION

(1*r*,4*r*)-4-(Aminomethyl)cyclohexane-1-carboxylic acid.

Content: 99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: freely soluble in water and in glacial acetic acid, practically insoluble in acetone and in ethanol (96 per cent).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *tranexamic acid CRS*.

TESTS

pH (*2.2.3*): 7.0 to 8.0.

Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 50 mL with the same solvent.

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 0.200 g of the substance to be examined in *water R* and dilute to 20.0 mL with the same solvent.

Reference solution (a). Dilute 1.0 mL of the test solution to 100.0 mL with *water R*. Dilute 1.0 mL of this solution to 20.0 mL with *water R*.

Reference solution (b). Dissolve 5.0 mg of *tranexamic acid impurity D CRS* in *water R* and dilute to 50.0 mL with the same solvent.

Reference solution (c). Dilute 5.0 mL of reference solution (b) to 100.0 mL with *water R*.

Reference solution (d). Dissolve 2.5 mg of *tranexamic acid impurity E CRS* in *water R* and dilute to 50.0 mL with the same solvent. Dilute 1.0 mL of the solution to 10.0 mL with *water R*.

Reference solution (e). Dissolve 2.5 mg of *tranexamic acid impurity C CRS*, 2.5 mg of *tranexamic acid impurity F CRS* and 7.5 mg of *tranexamic acid impurity B CRS* in 25 mL of *water R*. Mix 1 mL of the solution, 1 mL of reference solution (b) and 18 mL of the test solution.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase: dissolve 11.0 g of *anhydrous sodium dihydrogen phosphate R* in 500 mL of *water for chromatography R* and add 5 mL of *triethylamine R* and 1.4 g of *sodium laurilsulfate R1*; adjust to pH 2.0 with *phosphoric acid R* and dilute to 600 mL with *water for chromatography R*; add 400 mL of *methanol R2*.

Flow rate: 0.9 mL/min.

Detection: spectrophotometer at 210 nm.

Injection: 40 µL of the test solution and reference solutions (a), (c), (d) and (e).

Run time: 2.5 times the retention time of tranexamic acid.

Identification of impurities: use the chromatogram obtained with reference solution (c) to identify the peak due to impurity D; use the chromatogram obtained with reference solution (d) to identify the peak due to impurity E; use the chromatogram obtained with reference solution (e) to identify the peaks due to impurities B, C and F.

Relative retention with reference to tranexamic acid (retention time = about 10 min): impurity F = about 0.3; impurity C = about 1.1; impurity D = about 1.2; impurity E = about 1.3; impurity B = about 1.5.

System suitability: reference solution (e):

- *resolution*: minimum 2.0 between the peaks due to tranexamic acid and impurity C; minimum 1.5 between the peaks due to impurities C and D.

Calculation of percentage contents:

- *correction factors*: multiply the peak areas of the following impurities by the corresponding correction factor: impurity B = 1.3; impurity C = 0.4; impurity F = 0.6;
- for impurities C and D, use the concentration of impurity D in reference solution (c);
- for impurities E and F, use the concentration of impurity E in reference solution (d);
- for impurities other than C, D, E and F, use the concentration of tranexamic acid in reference solution (a).

Limits:

- *impurity B*: maximum 0.15 per cent;
- *impurities C, D, E, F*: for each impurity, maximum 0.05 per cent;
- *unspecified impurities*: for each impurity, maximum 0.05 per cent;
- *total*: maximum 0.2 per cent;
- *reporting threshold*: 0.03 per cent.

Halides expressed as chlorides (*2.4.4*): maximum 140 ppm.

Dissolve 1.2 g in *water R* and dilute to 50 mL with the same solvent.

Loss on drying (*2.2.32*): maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 2 h.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

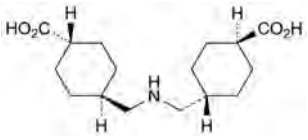
Dissolve 0.140 g in 20 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 15.72 mg of $C_8H_{15}NO_2$.

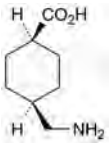
IMPURITIES

Specified impurities: B, C, D, E, F.

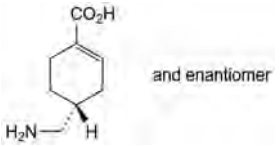
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *A.*



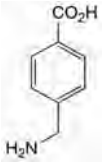
A. (1*r*,4*r*,1′*r*,4′*r*)-4,4′-[azanediylbis(methylene)]di(cyclohexane-1-carboxylic acid),



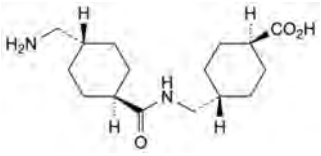
B. (1*s*,4*s*)-4-(aminomethyl)cyclohexane-1-carboxylic acid,



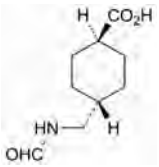
C. (4*RS*)-4-(aminomethyl)cyclohex-1-ene-1-carboxylic acid,



D. 4-(aminomethyl)benzoic acid,



E. (1*r*,4*r*)-4-[[(1*r*,4*r*)-4-(aminomethyl)cyclohexane-1-carboxamido]methyl]cyclohexane-1-carboxylic acid,



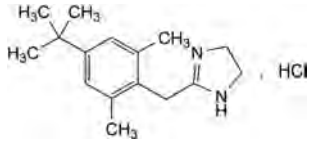
F. (1*r*,4*r*)-4-(formamidomethyl)cyclohexane-1-carboxylic acid.

X

01/2008:1162
corrected 10.1

XYLOMETAZOLINE HYDROCHLORIDE

Xylometazolini hydrochloridum



$C_{16}H_{25}ClN_2$ M_r 280.8
[1218-35-5]

DEFINITION

2-[4-(1,1-Dimethylethyl)-2,6-dimethylbenzyl]-4,5-dihydro-1*H*-imidazole hydrochloride.

Content: 99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: freely soluble in water, in ethanol (96 per cent) and in methanol.

IDENTIFICATION

First identification: A, E.

Second identification: B, C, D, E.

A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *xylometazoline hydrochloride CRS*.

B. Thin-layer chromatography (*2.2.27*).
Test solution. Dissolve 20 mg of the substance to be examined in *methanol R* and dilute to 5 mL with the same solvent.
Reference solution. Dissolve 20 mg of *xylometazoline hydrochloride CRS* in *methanol R* and dilute to 5 mL with the same solvent.
Plate: *TLC silica gel G plate R*.
Mobile phase: *concentrated ammonia R*, *methanol R* (5:100 *V/V*).
Application: 5 µL.
Development: over 2/3 of the plate.
Drying: in air.
Chlorine treatment: at the bottom of a chromatographic tank place a beaker containing a mixture of 1 volume of *hydrochloric acid R1*, 1 volume of *water R* and 2 volumes of a 15 g/L solution of *potassium permanganate R*. Close the tank and allow to stand for 15 min. Place the dried plate in the tank and reclose the tank. Leave the plate in contact with the chlorine vapour for 5 min. Withdraw the plate and place it in a current of cold air until the excess of chlorine is removed and an area of the coating below the points of application does not give a blue colour with a drop of *potassium iodide and starch solution R*.
Detection: spray with *potassium iodide and starch solution R*.
Results: the principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

C. Dissolve about 0.5 mg in 1 mL of *methanol R*. Add 0.5 mL of a freshly prepared 50 g/L solution of *sodium nitroprusside R* and 0.5 mL of a 20 g/L solution of *sodium hydroxide R*. Allow to stand for 10 min and add 1 mL of an 80 g/L solution of *sodium hydrogen carbonate R*. A violet colour develops.

D. Dissolve 0.2 g in 1 mL of *water R*, add 2.5 mL of *ethanol (96 per cent) R* and 2 mL of *1 M sodium hydroxide*. Mix thoroughly and examine in ultraviolet light at 365 nm. The solution shows no fluorescence or at most the same fluorescence as a blank solution prepared in the same manner. The identification is not valid unless a solution prepared in the same manner using *naphazoline hydrochloride CRS* instead of the substance to be examined shows a distinct bluish fluorescence.

E. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Appearance of solution. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Dissolve 2.5 g in *water R* and dilute to 50.0 mL with the same solvent.

Acidity or alkalinity. Dissolve 0.25 g in *carbon dioxide-free water R* and dilute to 25 mL with the same solvent. Add 0.1 mL of *methyl red solution R* and 0.1 mL of *0.01 M hydrochloric acid*. The solution is red. Not more than 0.2 mL of *0.01 M sodium hydroxide* is required to change the colour of the indicator to yellow.

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 50.0 mg of the substance to be examined in *water R* and dilute to 50.0 mL with the same solvent. Allow to stand for 1 h before injection.

Reference solution (a). Dilute 5.0 mL of the test solution to 100.0 mL with *water R*. Dilute 2.0 mL of this solution to 100.0 mL with *water R*.

Reference solution (b). Dissolve 5.0 mg of *xylometazoline impurity A CRS* and 5 mg of the substance to be examined in *water R* and dilute to 50.0 mL with the same solvent. Dilute 10.0 mL of this solution to 50.0 mL with *water R*.

Reference solution (c). Dilute 5.0 mL of reference solution (b) to 50.0 mL with *water R*.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography with embedded polar groups R* (5 µm).

Mobile phase:
- *mobile phase A*: 1.36 g/L solution of *potassium dihydrogen phosphate R* adjusted to pH 3.0 with *phosphoric acid R*;
- *mobile phase B*: *acetonitrile for chromatography R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	70	30
5 - 20	70 → 15	30 → 85
20 - 35	15	85

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 220 nm.

Injection: 10 µL.

Relative retention with reference to xylometazoline (retention time = about 7.2 min): impurity A = about 0.79.

System suitability: reference solution (b):
- *resolution*: minimum 2.5 between the peaks due to impurity A and xylometazoline.

Limits:
- *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.2 per cent);

– *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
– *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
– *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*): maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 25 mL of *anhydrous acetic acid R* and add 10 mL of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 28.08 mg of $C_{16}H_{25}ClN_2$.

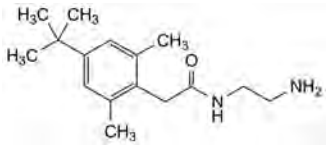
STORAGE

Protected from light.

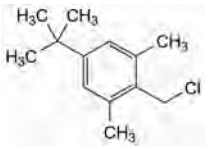
IMPURITIES

Specified impurities: A.

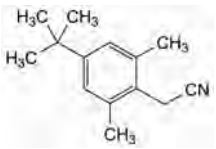
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *B, C, D, E, F.*



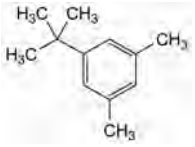
A. *N*-(2-aminoethyl)-2-[4-(1,1-dimethylethyl)-2,6-dimethylphenyl]acetamide,



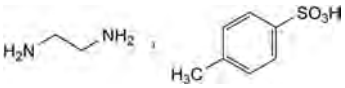
B. 2-(chloromethyl)-5-(1,1-dimethylethyl)-1,3-dimethylbenzene,



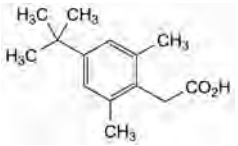
C. [4-(1,1-dimethylethyl)-2,6-dimethylphenyl]acetonitrile,



D. 1-(1,1-dimethylethyl)-3,5-dimethylbenzene,



E. ethane-1,2-diamine mono(4-methylbenzenesulfonate),



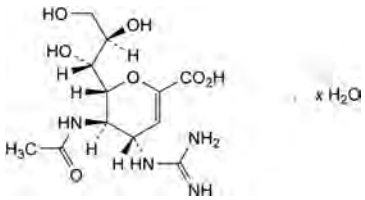
F. [4-(1,1-dimethylethyl)-2,6-dimethylphenyl]acetic acid.

Z

04/2020:2611

ZANAMIVIR HYDRATE

Zanamivirum hydricum



$C_{12}H_{20}N_4O_7,xH_2O$ M_r 332.3 (anhydrous substance)
[551942-41-7]

DEFINITION

(2*R*,3*R*,4*S*)-3-Acetamido-4-carbamimidamido-2-[(1*R*,2*R*)-1,2,3-trihydroxypropyl]-3,4-dihydro-2*H*-pyran-6-carboxylic acid hydrate.

Content: 97.0 per cent to 102.0 per cent (dried substance).

It contains a variable quantity of water.

CHARACTERS

Appearance: white or almost white, slightly hygroscopic powder.

Solubility: slightly soluble in water, practically insoluble in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *zanamivir hydrate CRS*.

TESTS

Specific optical rotation (*2.2.7*): + 36.0 to + 38.5 (dried substance).

Dissolve 0.250 g in 25.0 mL of *water R*; sonicate until dissolution is complete.

Related substances. Liquid chromatography (*2.2.29*).

Test solution (a). Dissolve 23.0 mg of the substance to be examined in 20 mL of *water R* and dilute to 50.0 mL with *acetonitrile R1*.

Test solution (b). Dilute 5.0 mL of test solution (a) to 50.0 mL with the mobile phase.

Reference solution (a). Dissolve 23.0 mg of *zanamivir for assay CRS* in 20 mL of *water R* and dilute to 50.0 mL with *acetonitrile R1*. Dilute 5.0 mL of the solution to 50.0 mL with the mobile phase.

Reference solution (b). Dissolve 5 mg of *zanamivir for system suitability CRS* (containing impurities A, B, C and E) in 6 mL of *water R* and dilute to 10 mL with *acetonitrile R1*.

Reference solution (c). Dilute 1.0 mL of test solution (a) to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (d). Dissolve 3.0 mg of *zanamivir impurity F CRS* in the mobile phase and dilute to 100.0 mL with the mobile phase.

Reference solution (e). Dilute 1.0 mL of reference solution (d) to 100.0 mL with the mobile phase. Dilute 3.0 mL of this solution to 20.0 mL with the mobile phase.

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm;
– *stationary phase*: *amino alkyl vinyl polymer for chromatography R* (5 µm);
– *temperature*: 30 °C.

Mobile phase: 0.7 g/L solution of *sulfuric acid R* previously adjusted to pH 5.5 with *dilute ammonia R3*, *acetonitrile R1* (40:60 *V/V*).

Flow rate: 1.5 mL/min.

Detection: spectrophotometer at 234 nm.

Preconditioning of the column: prior to first use, rinse with a 0.7 g/L solution of *ammonium sulfate R* at 1.5 mL/min at 30 °C for about 1 h; prior to each use, rinse with the mobile phase for at least 8 h.

Injection: 20 µL of test solution (a) and reference solutions (b), (c) and (e).

Run time: 3 times the retention time of zanamivir.

Identification of impurities: use the chromatogram supplied with *zanamivir for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B, C and E; use the chromatogram obtained with reference solution (e) to identify the peak due to impurity F.

Relative retention with reference to zanamivir (retention time = about 9 min): impurity F = about 0.3; impurity B = about 0.6; impurity C = about 0.75; impurity E = about 0.8; impurity A = about 2.6.

System suitability:

– *signal-to-noise ratio*: minimum 10 for the principal peak in the chromatogram obtained with reference solution (e);
– *peak-to-valley ratio*: minimum 2.5, where H_p = height above the baseline of the peak due to impurity E and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity C in the chromatogram obtained with reference solution (b).

Calculation of percentage contents:

– for impurity F, use the concentration of impurity F in reference solution (e);
– for impurities other than F, use the concentration of zanamivir hydrate in reference solution (c).

Limits:

– *impurity A*: maximum 0.5 per cent;
– *impurity B*: maximum 0.3 per cent;
– *impurity C*: maximum 0.2 per cent;
– *impurity F*: maximum 0.01 per cent;
– *unspecified impurities*: for each impurity, maximum 0.10 per cent;
– *total*: maximum 1.2 per cent;
– *reporting threshold*: 0.05 per cent, except for impurity F.

Loss on drying (*2.2.32*): 4.0 per cent to 9.0 per cent, determined on 1.000 g by drying *in vacuo* at 105 °C.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection: test solution (b) and reference solution (a).

Calculate the percentage content of $C_{12}H_{20}N_4O_7$ taking into account the assigned content of *zanamivir for assay CRS*.

STORAGE

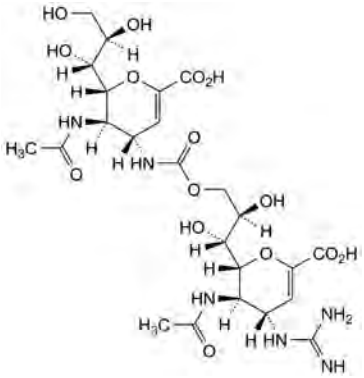
In an airtight container, protected from light.

IMPURITIES

Specified impurities: A, B, C, F.

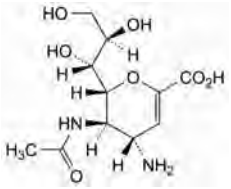
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general

acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *D, E, H.*

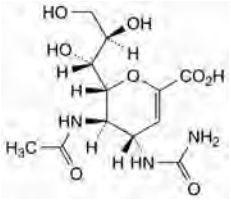


A. (2*R*,3*R*,4*S*)-3-acetamido-2-[(1*R*,2*R*)-3-[[[(2*R*,3*R*,4*S*)-3-acetamido-6-carboxy-2-[(1*R*,2*R*)-1,2,3-trihydroxypropyl]-3,4-dihydro-2*H*-pyran-4-yl]carbamoyl]oxy]-1,2-dihydroxypropyl]-4-carbamimidamido-3,4-dihydro-2*H*-pyran-6-carboxylic acid,

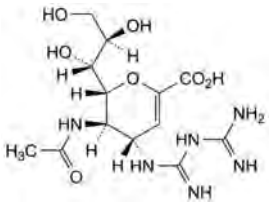
B. unknown structure,



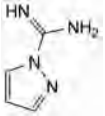
C. (2*R*,3*R*,4*S*)-3-acetamido-4-amino-2-[(1*R*,2*R*)-1,2,3-trihydroxypropyl]-3,4-dihydro-2*H*-pyran-6-carboxylic acid,



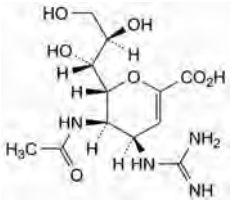
D. (2*R*,3*R*,4*S*)-3-acetamido-4-(carbamoylamino)-2-[(1*R*,2*R*)-1,2,3-trihydroxypropyl]-3,4-dihydro-2*H*-pyran-6-carboxylic acid,



E. (2*R*,3*R*,4*S*)-3-acetamido-4-(*N*′-carbamimidoyl-carbamimidamido)-2-[(1*R*,2*R*)-1,2,3-trihydroxypropyl]-3,4-dihydro-2*H*-pyran-6-carboxylic acid,



F. 1*H*-pyrazole-1-carboximidamide,

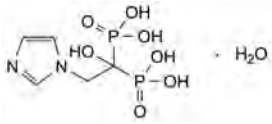


H. (2*R*,3*R*,4*R*)-3-acetamido-4-carbamimidamido-2-[(1*R*,2*R*)-1,2,3-trihydroxypropyl]-3,4-dihydro-2*H*-pyran-6-carboxylic acid.

04/2020:2743

ZOLEDRONIC ACID MONOHYDRATE

Acidum zoledronicum monohydricum



$C_5H_{10}N_2O_7P_2,H_2O$ M_r 290.1
[165800-06-6]

DEFINITION

[1-Hydroxy-2-(1*H*-imidazol-1-yl)ethane-1,1-diyl]bis-(phosphonic acid) monohydrate.

Content: 99.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: slightly soluble in water, practically insoluble in anhydrous ethanol and in heptane.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *zoledronic acid monohydrate CRS*.

B. Water (see Tests).

TESTS

Appearance of solution. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution B_7 or BY_7 (*2.2.2, Method II*).

Dissolve 0.5 g in a 4 g/L solution of *sodium hydroxide R* and dilute to 50.0 mL with the same solution.

pH (*2.2.3*): 1.8 to 2.8.

Dissolve 0.150 g in *carbon dioxide-free water R* and dilute to 50.0 mL with the same solvent. Sonicate for 10 min, if necessary.

Related substances. Liquid chromatography (*2.2.29*).

Solution A. Dissolve 10.8 g of *sodium octanesulfonate R* and 37 mg of *sodium edetate R* in *water for chromatography R*, add 10 mL of *perchloric acid R* and 2 mL of *phosphoric acid R* and dilute to 1000 mL with *water for chromatography R*.

Test solution. Dissolve 40.0 mg of the substance to be examined in the mobile phase, sonicate for 30 min, and dilute to 20.0 mL with the mobile phase.

Reference solution (a). Dissolve 2 mg of *zoledronic acid impurity A CRS*, 5 mg of *zoledronic acid impurity B CRS* and 2 mg of *sodium nitrate R* in the mobile phase and dilute to 50 mL with the mobile phase. To 1 mL of the solution add 7 mL of the mobile phase and dilute to 20 mL with the test solution.

Reference solution (b). Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped phenylhexylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 20 °C.

Mobile phase: *acetonitrile R1*, solution A (4:96 *V/V*).

Flow rate: 0.6 mL/min.

Detection: spectrophotometer at 215 nm.

Preconditioning: precondition the instrument and the column once before each series of injections as follows:
- *instrument*: rinse the instrument without a column with a 25 per cent *V/V* solution of *acetic acid R* at 5 mL/min for about 20 min. Then, rinse with *water for chromatography R* at 5 mL/min for about 2 h;
- *column*: rinse the column with the mobile phase at 0.6 mL/min for about 1 h. During the rinsing, inject the test solution 15 times, applying a run time of about 3 min for each injection.

Injection: 10 µL.

Run time: 5 times the retention time of zoledronic acid.

Identification of impurities: use the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A and B and the nitrate ion.

Relative retention with reference to zoledronic acid (retention time = about 6 min): nitrate = about 0.6; impurity B = about 0.7; impurity A = about 0.9.

System suitability: reference solution (a):
- *resolution*: minimum 1.5 between the peaks due to impurity A and zoledronic acid; minimum 1.5 between the peaks due to the nitrate ion and impurity B.

Calculation of percentage contents:
- *correction factor*: multiply the peak area of impurity B by 1.9;
- for each impurity, use the concentration of zoledronic acid monohydrate in reference solution (b).

Limits:
- *impurity B*: maximum 0.5 per cent;
- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- *total*: maximum 0.5 per cent;
- *reporting threshold*: 0.05 per cent, disregard the peak due to the nitrate ion.

Impurities E and F. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 50.0 mg of the substance to be examined in mobile phase B, sonicate if necessary, and dilute to 10.0 mL with mobile phase B.

Reference solution (a). Dissolve 95.0 mg of *anhydrous sodium dihydrogen phosphate R* and 79.0 mg of *phosphorous acid R* (impurity E) in *water R* and dilute to 100.0 mL with the same solvent.

Reference solution (b). Dissolve 34.0 mg of *sodium chloride R* in *water R* and dilute to 100.0 mL with the same solvent.

Reference solution (c). To 1.0 mL of reference solution (a) add 0.5 mL of reference solution (b) and dilute to 100.0 mL with mobile phase B.

Reference solution (d). Dilute 0.1 mL of reference solution (a) to 100 mL with mobile phase B.

Precolumn:
- *size*: *l* = 0.05 m, Ø = 4.0 mm;
- *stationary phase*: *anion-exchange resin R* (13 µm).

Column:
- *size*: *l* = 0.25 m, Ø = 4.0 mm;
- *stationary phase*: *anion-exchange resin R* (9 µm);
- *temperature*: 30 °C.

Mobile phase:
- *mobile phase A*: *carbon dioxide-free water R*;
- *mobile phase B*: 4.0 g/L solution of *sodium hydroxide R* in *carbon dioxide-free water R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 13	80 → 70	20 → 30
13 - 17	70 → 60	30 → 40
17 - 29	60	40

Flow rate: 1.0 mL/min.

Detection: conductivity detector; use a self-regenerating anion suppressor.

Injection: 20 µL.

Identification of impurities: use the chromatogram obtained with reference solution (a) to identify the peaks due to impurities E and F; use the chromatogram obtained with reference solution (b) to identify the peak due to the chloride ion.

Relative retention with reference to chloride (retention time = about 5 min): impurity E = about 1.2; impurity F = about 3.4.

System suitability:
- *resolution*: minimum 1.5 between the peaks due to the chloride ion and impurity E in the chromatogram obtained with reference solution (c);
- *signal-to-noise ratio*: minimum 10 for the peak due to impurity F in the chromatogram obtained with reference solution (d).

Calculation of percentage contents:
- for each impurity, use the concentration of the corresponding impurity in reference solution (c).

Limits:
- *impurities E, F*: for each impurity, maximum 0.15 per cent.

Water (*2.5.12*): 5.0 per cent to 7.5 per cent, determined on 0.100 g.

ASSAY

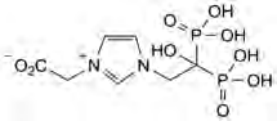
Dissolve 0.150 g in 50 mL of *carbon dioxide-free water R*. Sonicate for 10 min, if necessary. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*). Read the volume added at the 3rd inflexion point.

1 mL of *0.1 M sodium hydroxide* is equivalent to 9.07 mg of $C_5H_{10}N_2O_7P_2$.

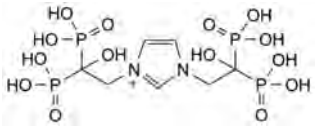
IMPURITIES

Specified impurities: B, E, F.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *A, C, D.*

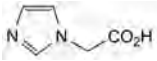


A. [1-(2-hydroxy-2,2-diphosphonoethyl)-1*H*-imidazol-3-ium-3-yl]acetate,

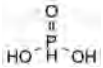


B. 1,3-bis(2-hydroxy-2,2-diphosphonoethyl)-1*H*-imidazol-3-ium,

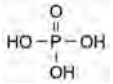
C. 1*H*-imidazole,



D. (1*H*-imidazol-1-yl)acetic acid,



E. phosphonic acid (phosphorous acid),

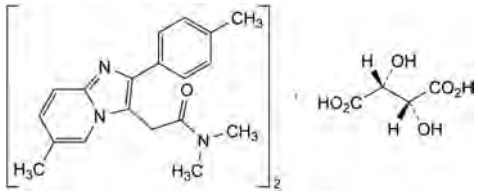


F. phosphoric acid.

04/2020:1280

ZOLPIDEM TARTRATE

Zolpidemi tartras



$C_{42}H_{48}N_6O_8$ M_r 765
[99294-93-6]

DEFINITION

Bis[*N*,*N*-dimethyl-2-[6-methyl-2-(4-methylphenyl)-imidazo[1,2-*a*]pyridin-3-yl]acetamide] (2*R*,3*R*)-2,3-dihydroxybutanedioate.

Content: 98.5 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance: white or almost white, hygroscopic, crystalline powder.

Solubility: slightly soluble in water, sparingly soluble in methanol, practically insoluble in methylene chloride.

IDENTIFICATION

First identification: A, C.

Second identification: B, C.

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation: dissolve 0.10 g in 10 mL of a 10.3 g/L solution of *hydrochloric acid R*. Add 10 mL of *water R*. Add dropwise with stirring 1 mL of *dilute ammonia R2*. Filter and collect the resulting precipitate. Wash the precipitate with *water R* and then dry at 105 °C for 2 h. Examine the precipitate as a disc.

Comparison: repeat the operations using 0.10 g of *zolpidem tartrate CRS*.

B. Thin-layer chromatography (*2.2.27*).

Test solution. Dissolve 50 mg of the substance to be examined in 5 mL of *methanol R*, add 0.1 mL of *diethylamine R* and dilute to 10 mL with *methanol R*.

Reference solution (a). Dissolve 50 mg of *zolpidem tartrate CRS* in 5 mL of *methanol R*, add 0.1 mL of *diethylamine R* and dilute to 10 mL with *methanol R*.

Reference solution (b). Dissolve 50 mg of *flunitrazepam CRS* in 5 mL of *methylene chloride R* and dilute to 10 mL with the same solvent. Mix 1 mL of this solution and 1 mL of reference solution (a).

Plate: *TLC silica gel F_{254} plate R*.

Mobile phase: *diethylamine R*, *cyclohexane R*, *ethyl acetate R* (10:45:45 *V/V/V*).

Application: 5 µL.

Development: over 2/3 of the plate.

Drying: in air.

Detection: examine in ultraviolet light at 254 nm.

Retardation factors: zolpidem = about 0.3; flunitrazepam = about 0.5.

System suitability: reference solution (b):

– the chromatogram shows 2 clearly separated spots.

Results: the principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dissolve about 0.1 g in 1 mL of *methanol R*, heating gently. 0.1 mL of this solution gives reaction (b) of tartrates (*2.3.1*).

TESTS

Appearance of solution. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 or BY_6 (*2.2.2, Method II*). *Prepare the solutions protected from light and carry out the test as rapidly as possible.*

Triturate 0.25 g with 0.125 g of *tartaric acid R*. Dissolve the mixture in 20 mL of *water R* and dilute to 25 mL with the same solvent.

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 mL with the mobile phase.

Reference solution (a). Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b). Dissolve 2.5 mg of *zolpidem for system suitability CRS* (containing impurities A and B) in the mobile phase and dilute to 5 mL with the mobile phase.

Column:

– *size*: *l* = 0.15 m, Ø = 3.9 mm;

– *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (4 µm).

Mobile phase: mix 18 volumes of *acetonitrile R*, 23 volumes of *methanol R* and 59 volumes of a 5.6 g/L solution of *phosphoric acid R* previously adjusted to pH 5.5 with *triethylamine R*.

Flow rate: 1.5 mL/min.

Detection: spectrophotometer at 254 nm.

Injection: 20 µL.

Run time: 4 times the retention time of zolpidem.

Identification of impurities: use the chromatogram supplied with *zolpidem for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A and B.

Relative retention with reference to zolpidem (retention time = about 7 min): tartaric acid = about 0.1; impurity A = about 0.8; impurity B = about 3.6.

System suitability: reference solution (b):

– *resolution*: minimum 2.0 between the peaks due to impurity A and zolpidem.

Calculation of percentage contents:

– for each impurity, use the concentration of zolpidem tartrate in reference solution (a).

Limits:

– *impurity B*: maximum 0.15 per cent;

– *unspecified impurities*: for each impurity, maximum 0.10 per cent;

– *total*: maximum 0.2 per cent;

– *reporting threshold*: 0.05 per cent; disregard the peak due to tartaric acid.

Water (*2.5.12*): maximum 3.0 per cent, determined on 0.500 g.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in a mixture of 20 mL of *anhydrous acetic acid R* and 20 mL of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*). Carry out a blank titration.

1 mL of *0.1 M perchloric acid* is equivalent to 38.24 mg of $C_{42}H_{48}N_6O_8$.

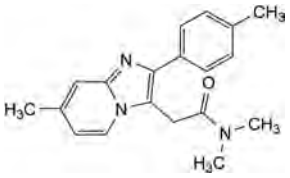
STORAGE

In an airtight container, protected from light.

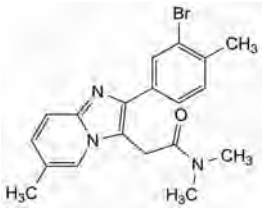
IMPURITIES

Specified impurities: B.

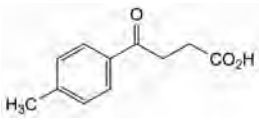
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *A, C, D, E, F.*



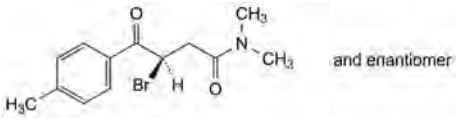
A. *N*,*N*-dimethyl-2-[7-methyl-2-(4-methylphenyl)-imidazo[1,2-*a*]pyridin-3-yl]acetamide,



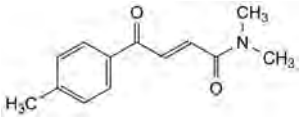
B. 2-[2-(3-bromo-4-methylphenyl)-6-methylimidazo[1,2-*a*]pyridin-3-yl]-*N*,*N*-dimethylacetamide,



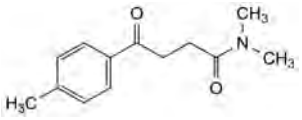
C. 4-(4-methylphenyl)-4-oxobutanoic acid,



D. (3*RS*)-3-bromo-*N*,*N*-dimethyl-4-(4-methylphenyl)-4-oxobutanamide,



E. (2*E*)-*N*,*N*-dimethyl-4-(4-methylphenyl)-4-oxobut-2-enamide,



F. *N*,*N*-dimethyl-4-(4-methylphenyl)-4-oxobutanamide.

INDEX

To aid users the index includes a reference to the supplement in which the latest version of a text can be found.

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means the monograph Altizide can be found on page 4374 of Supplement 10.1.

Note that where no reference to a supplement is made, the text can be found in the principal volume.

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