75 ml of *water R*. Allow to stand for 4 h, dilute to 200 ml with *water R* and filter through a suitable filter. To 100 ml of the filtrate add 0.5 ml of *sulphuric acid R*. Evaporate to dryness on a water-bath and ignite to constant mass at 600 ± 50 °C. The residue weighs a maximum of 5 mg.

Heavy metals (*2.4.8*): maximum 15 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (2 ppm Pb) R*.

ASSAY

Dissolve 0.200 g in 100 ml of *water R*. Carry out the complexometric titration of calcium (*2.5.11*).

1 ml of *0.1 M sodium edetate* is equivalent to 21.91 mg of $CaCl_2,6H_2O$.

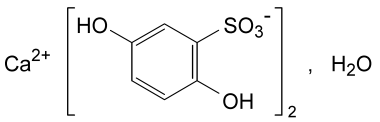
LABELLING

The label states, where applicable, that the substance is suitable for use in the manufacture of dialysis solutions.

01/2008:1183

CALCIUM DOBESILATE MONOHYDRATE

Calcii dobesilas monohydricus



$C_{12}H_{10}CaO_{10}S_2,H_2O$ M_r 436.4

DEFINITION

Calcium dobesilate monohydrate contains not less than 99.0 per cent and not more than the equivalent of 102.0 per cent of calcium di(2,5-dihydroxybenzenesulphonate), calculated with reference to the anhydrous substance.

CHARACTERS

A white or almost white, hygroscopic powder, very soluble in water, freely soluble in ethanol, very slightly soluble in 2-propanol, practically insoluble in methylene chloride.

IDENTIFICATION

A. Dissolve 0.100 g in *water R* and dilute to 200.0 ml with the same solvent. Dilute 5.0 ml of the solution to 100.0 ml with *water R*. Examined between 210 nm and 350 nm (*2.2.25*), the solution shows two absorption maxima, at 221 nm and 301 nm. The specific absorbance at the maximum at 301 nm is 174 to 181.

B. Mix 1 ml of *ferric chloride solution R2*, 1 ml of a freshly prepared 10 g/l solution of *potassium ferricyanide R* and 0.1 ml of *nitric acid R*. To this mixture add 5 ml of freshly prepared solution S (see Tests): a blue colour and a precipitate are immediately produced.

C. 2 ml of freshly prepared solution S (see Tests) gives reaction (b) of calcium (*2.3.1*).

TESTS

Solution S. Dissolve 10.0 g in *carbon dioxide-free water R* and dilute to 100 ml with the same solvent.

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

pH (*2.2.3*). The pH of solution S is 4.5 to 6.0.

Hydroquinone. Examine by thin-layer chromatography (*2.2.27*), using as the coating substance a suitable silica gel with a fluorescent indicator having an optimal intensity at 254 nm.

Test solution. Dissolve 2.0 g of the substance to be examined in *water R* and dilute to 10 ml with the same solvent.

Reference solution. Dissolve 10 mg of *hydroquinone R* in *water R* and dilute to 50 ml with the same solvent.

Apply to the plate 10 µl of each solution and dry the starting points in a current of cool air. Develop over a path of 15 cm using a mixture of 20 volumes of *methylene chloride R*, 30 volumes of *methyl acetate R* and 50 volumes of *ethyl acetate R*. Dry the plate in a current of hot air and examine in ultraviolet light at 254 nm. Any spot corresponding to hydroquinone in the chromatogram obtained with the test solution is not more intense than the principal spot in the chromatogram obtained with the reference solution (0.1 per cent).

Heavy metals (*2.4.8*). 1.0 g complies with limit test C for heavy metals (15 ppm). Prepare the standard using 1.5 ml of *lead standard solution (10 ppm Pb) R*.

Iron (*2.4.9*). 10 ml of solution S complies with the limit test for iron (10 ppm).

Water (*2.5.12*): 4.0 per cent to 6.0 per cent, determined on 0.500 g by the semi-micro determination of water.

ASSAY

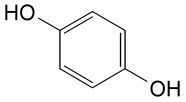
Dissolve 0.200 g in a mixture of 10 ml of *water R* and40 ml of *dilute sulphuric acid R*. Titrate with *0.1 M cerium sulphate*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M cerium sulphate* is equivalent to 10.45 mg of $C_{12}H_{10}CaO_{10}S_2$.

STORAGE

Store in an airtight container, protected from light.

IMPURITIES

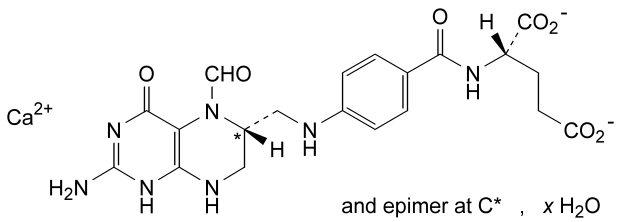


A. benzene-1,4-diol (hydroquinone).

01/2008:0978
corrected 6.0

CALCIUM FOLINATE

Calcii folinas



$C_{20}H_{21}CaN_7O_7,xH_2O$ M_r 511.5 (anhydrous substance)

DEFINITION

Calcium (2*S*)-2-[[4-[[[(6*RS*)-2-amino-5-formyl-4-oxo-1,4,5,6,7,8-hexahydropteridin-6-yl]methyl]amino]-benzoyl]amino]pentanedioate.

Content:
– *calcium folinate* ($C_{20}H_{21}CaN_7O_7$): 97.0 per cent to 102.0 per cent (anhydrous substance);
– *calcium* (Ca; A_r 40.08): 7.54 per cent to 8.14 per cent (anhydrous substance).

It contains a variable amount of water.

CHARACTERS

Appearance: white or light yellow, amorphous or crystalline powder.

Solubility: sparingly soluble in water, practically insoluble in acetone and in ethanol (96 per cent).

The amorphous form may produce supersaturated solutions in water.

IDENTIFICATION

First identification: A, B, D.

Second identification: A, C, D.

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation: discs.

Comparison: *calcium folinate CRS*.

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *water R* and add dropwise sufficient *acetone R* to produce a precipitate. Allow to stand for 15 min, collect the precipitate by centrifugation, wash the precipitate with 2 small quantities of *acetone R* and dry. Record new spectra using the residues.

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

Test solution. Dissolve 15 mg of the substance to be examined in a 3 per cent *V/V* solution of *ammonia R* and dilute to 5 ml with the same solvent.

Reference solution. Dissolve 15 mg of *calcium folinate CRS* in a 3 per cent *V/V* solution of *ammonia R* and dilute to 5 ml with the same solvent.

Plate: *cellulose for chromatography F_{254} R* as the coating substance.

Mobile phase: the lower layer of a mixture of 1 volume of *isoamyl alcohol R* and 10 volumes of a 50 g/l solution of *citric acid R* previously adjusted to pH 8 with *ammonia R*.

Application: 5 µl.

Development: over a path of 15 cm.

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 reaction (b) of calcium (*2.3.1*).

Carry out the tests and the assay as rapidly as possible, protected from actinic light.

TESTS

Solution S. Dissolve 1.25 g in *carbon dioxide-free water R*, heating at 40 °C if necessary, and dilute to 50.0 ml with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and its absorbance (*2.2.25*) at 420 nm is not greater than 0.60. Use *water R* as the compensation liquid.

pH (*2.2.3*): 6.8 to 8.0 for solution S.

Specific optical rotation (*2.2.7*): + 14.4 to + 18.0 (anhydrous substance), determined on solution S.

Acetone, ethanol and methanol. Head-space gas chromatography (*2.2.28*): use the standard additions method.

Test solution. Dissolve 0.25 g of the substance to be examined in *water R* and dilute to 10.0 ml with the same solvent.

Reference solution. Dilute 0.125 g of *acetone R*, 0.750 g of *anhydrous ethanol R* and 0.125 g of *methanol R* in *water R* and dilute to 1000.0 ml with *water R*.

Column:
– *material*: fused silica;
– *size*: *l* = 10 m, Ø = 0.32 mm;
– *stationary phase*: *styrene-divinylbenzene copolymer R*.

Carrier gas: *nitrogen for chromatography R*.

Flow rate: 4 ml/min.

Static head-space conditions that may be used:
– *equilibration temperature*: 80 °C;
– *equilibration time*: 20 min;
– *pressurisation time*: 30 s.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 6	125 → 185
	6 - 15	185
Injection port		250
Detector		250

Detection: flame ionisation.

Injection: at least 3 times.

Limits:
– *acetone*: maximum 0.5 per cent;
– *ethanol*: maximum 3.0 per cent;
– *methanol*: maximum 0.5 per cent.

Related substances. Liquid chromatography (*2.2.29*).

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

Reference solution (a). Dissolve 10.0 mg of *calcium folinate CRS* in *water R* and dilute to 10.0 ml with the same solvent.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with *water R*.

Reference solution (c). Dissolve 10.0 mg of *formylfolic acid CRS* (impurity D) in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with *water R*.

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

Reference solution (e). Dilute 5.0 ml of reference solution (c) to 10.0 ml with reference solution (b).

Column:
– *size*: *l* = 0.25 m, Ø = 4 mm;
– *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);
– *temperature*: 40 °C.

Mobile phase: mix 220 ml of *methanol R* and 780 ml of a solution containing 2.0 ml of *tetrabutylammonium hydroxide solution (400 g/l) R* and 2.2 g of *disodium hydrogen phosphate R*, previously adjusted to pH 7.8 with *phosphoric acid R*.

Flow rate: 1 ml/min.

Detection: spectrophotometer at 280 nm.

Injection: 10 µl of the test solution and reference solutions (b), (c), (d) and (e).

Run time: 2.5 times the retention time of folinate.

System suitability: reference solution (e):

– *resolution*: minimum 2.2 between the peaks due to folinate and impurity D.

Limits:

– *impurity D*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (1 per cent);

– *impurities A, B, C, E, F, G*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent);

– *sum of impurities other than D*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (2.5 per cent);

– *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (d) (0.1 per cent).

Chlorides (*2.4.4*): maximum 0.5 per cent.

Dissolve 67 mg in 10 ml of *water R* and add 3 ml of *acetic acid R*. Filter and wash the precipitate with 5 quantities, each of 5 ml, of *water R*. Collect the filtrate and washings and dilute to 100 ml with *water R*.

Heavy metals (*2.4.8*): maximum 50 ppm.

1.0 g complies with test F. Prepare the reference solution using 5 ml of *lead standard solution (10 ppm Pb) R*.

Platinum: maximum 20.0 ppm.

Atomic absorption spectrometry (*2.2.23, Method II*).

Test solution. Dissolve 1.00 g in *water R* and dilute to 100.0 ml with the same solvent.

Reference solutions. Prepare the reference solutions using *platinum standard solution (30 ppm Pt) R*, diluted as necessary with a mixture of 1 volume of *nitric acid R* and 99 volumes of *water R*.

Source: platinum hollow-cathode lamp.

Wavelength: 265.9 nm.

Water (*2.5.12*): maximum 17.0 per cent, determined on 0.200 g (ground to a very fine powder). Stir the substance to be examined in the titration solvent for about 6 min before titrating and use a suitable titrant that does not contain pyridine.

Bacterial endotoxins (*2.6.14*): less than 0.5 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Calcium. Dissolve 0.400 g in 150 ml of *water R* and dilute to 300 ml with the same solvent. Carry out the complexometric titration of calcium (*2.5.11*).

1 ml of *0.1 M sodium edetate* is equivalent to 4.008 mg of Ca.

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

Injection: test solution and reference solution (a).

System suitability:

– *repeatability*: maximum relative standard deviation of 2.0 per cent after 6 injections of reference solution (a).

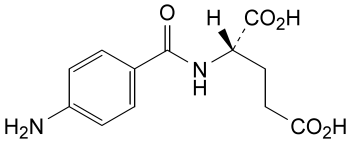
Calculate the percentage content of $C_{20}H_{21}CaN_7O_7$ from the declared content of *calcium folinate CRS*.

STORAGE

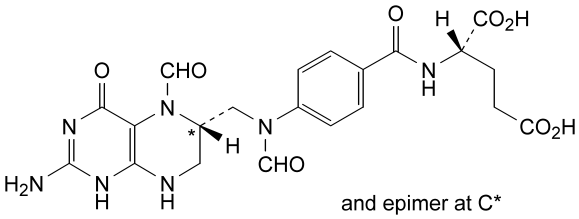
In an airtight container, protected from light. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

IMPURITIES

Specified impurities: *A, B, C, D, E, F, G*.

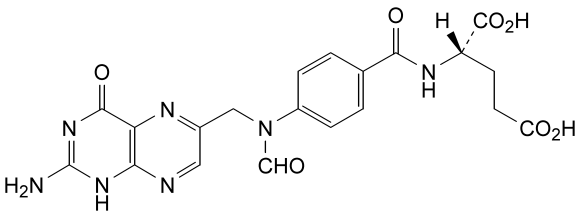


A. (2*S*)-2[(4-aminobenzoyl)amino]pentanedioic acid,

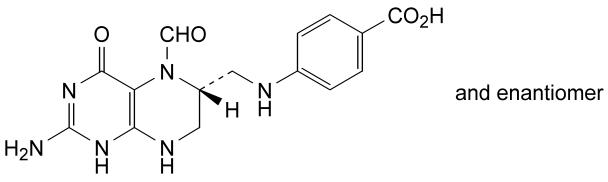


B. (2*S*)-2-[[4-[[[(6*RS*)-2-amino-5-formyl-4-oxo-1,4,5,6,7,8-hexahydropteridin-6-yl]methyl]formylamino]benzoyl]-amino]pentanedioic acid (5,10-diformyltetrahydrofolic acid),

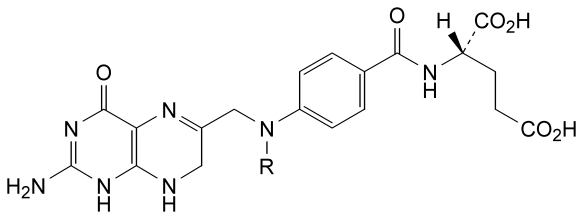
C. folic acid,



D. (2*S*)-2-[[4-[[(2-amino-4-oxo-1,4-dihydropteridin-6-yl)methyl]formylamino]benzoyl]amino]pentanedioic acid (10-formylfolic acid),



E. 4-[[[(6*RS*)-2-amino-5-formyl-4-oxo-1,4,5,6,7,8-hexahydropteridin-6-yl]methyl]amino]benzoic acid (5-formyltetrahydropteroic acid),



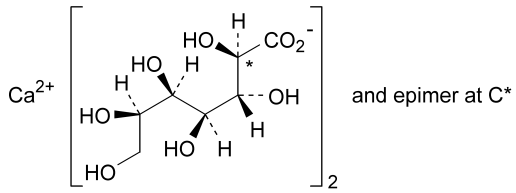
F. R = CHO: (2*S*)-2-[[4-[[(2-amino-4-oxo-1,4,7,8-tetrahydropteridin-6-yl)methyl]formylamino]benzoyl]amino]pentanedioic acid (10-formyldihydrofolic acid),

G. R = H: (2*S*)-2-[[4-[[(2-amino-4-oxo-1,4,7,8-tetrahydropteridin-6-yl)methyl]amino]benzoyl]amino]pentanedioic acid (dihydrofolic acid).

01/2008:1399
corrected 6.0

CALCIUM GLUCOHEPTONATE

Calcii glucoheptonas



$C_{14}H_{26}CaO_{16}$ M_r 490.4

DEFINITION

Mixture in variable proportions, of calcium di(D-*glycero*-D-*gulo*-heptonate) and calcium di(D-*glycero*-D-*ido*-heptonate).

Content: 98.0 per cent to 102.0 per cent of calcium 2,3,4,5,6,7-hexahydroxyheptanoate (dried substance).

CHARACTERS

Appearance: white or very slightly yellow, amorphous powder, hygroscopic.

Solubility: very soluble in water, practically insoluble in acetone and in ethanol (96 per cent).

IDENTIFICATION

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

Test solution. Dissolve 20 mg of the substance to be examined in 1 ml of *water R*.

Reference solution (a). Dissolve 20 mg of *calcium glucoheptonate CRS* in 1 ml of *water R*.

Reference solution (b). Dissolve 10 mg of *calcium gluconate CRS* in 0.5 ml of the test solution and dilute to 1 ml with *water R*.

Plate: *cellulose for chromatography R1* as the coating substance.

Mobile phase: a freshly prepared mixture of 20 volumes of *anhydrous formic acid R*, 20 volumes of *water R*, 30 volumes of *acetone R* and 30 volumes of *butanol R*.

Application: 10 µl as bands of 20 mm by 2 mm.

Development: in a tank previously allowed to saturate for 10 min, over a path of 12 cm.

Drying: in air.

Detection: spray with *0.02 M potassium permanganate*.

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).

B. 0.2 ml of (see Tests) gives reaction (b) of calcium (*2.3.1*).

TESTS

Solution S. Dissolve 10.0 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 not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

pH (*2.2.3*): 6.0 to 8.0 for solution S.

Reducing sugars: maximum 1 per cent, expressed as glucose.

Dissolve 1.0 g in 5 ml of *water R* with the aid of gentle heat. Cool and add 20 ml of *cupri-citric solution R* and a few glass beads. Heat so that boiling begins after 4 min and maintain boiling for 3 min. Cool rapidly and add 100 ml of a 2.4 per cent *V/V* solution of *glacial acetic acid R* and 20.0 ml of *0.025 M iodine*. With continuous shaking, add 25 ml of a mixture of 6 volumes of *hydrochloric acid R* and 94 volumes of *water R* until the precipitate dissolves, titrate the excess of iodine with *0.05 M sodium thiosulphate* using 1 ml of *starch solution R* added towards the end of the titration, as indicator. Not less than 12.6 ml of *0.05 M sodium thiosulphate* is required.

Cyanide. Dissolve 5.0 g in 50 ml of *water R* and add 2.0 g of *tartaric acid R*. Place this solution in a distillation apparatus (*2.2.11*). The plain bend adapter attached to the end of the condenser has a vertical part that is long enough to extend to 1 cm from the bottom of a 50 ml test-tube used as a receiver. Place 10 ml of *water R* and 2 ml of *0.1 M sodium hydroxide* into the receiver. Distil, collect 25 ml of distillate and dilute to 50 ml with *water R*. To 25 ml of this solution add 25 mg of *ferrous sulphate R* and boil for a short time. After cooling to about 70 °C add 10 ml of *hydrochloric acid R1*. After 30 min, filter the solution and wash the filter. A yellow spot appears on the filter; there is no blue or green spot.

Chlorides (*2.4.4*): maximum 100 ppm.

To 5 ml of solution S, add 10 ml of *water R*.

Sulphates (*2.4.13*): maximum 100 ppm, determined on solution S.

Iron (*2.4.9*): maximum 40 ppm.

Dilute 2.5 ml of to 10 ml with *water R*.

Heavy metals (*2.4.8*): maximum 10 ppm.

Dissolve 2.0 g in 10 ml of *buffer solution pH 3.5 R* and dilute to 20 ml with *water R*. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Loss on drying (*2.2.32*): maximum 5.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Bacterial endotoxins (*2.6.14*): less than 167 IU/g, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.