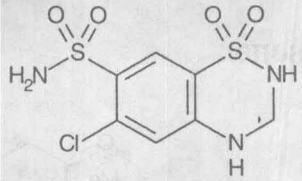
British Pharmacopoeia 2000

Volume 1

下册

Hydrochlorothiazide

corrected 1/00



$C_7H_8ClN_3O_4S_2$ 297.7 *58-93-5*

Hydrochlorothiazide complies with the requirements of the 3rd edition of the European Pharmacopoeia [0394]. These requirements are reproduced after the heading 'Definition' below.

Action and use Diuretic.

Preparations
Co-amilozide Oral Solution
Co-amilozide Tablets
Co-triamterzide Tablets
Hydrochlorothiazide Tablets

Ph Eur

DEFINITION

Hydrochlorothiazide contains not less than 98.0 per cent and not more than the equivalent of 102.0 per cent of 6-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulphonamide 1,1-dioxide, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, very slightly soluble in water, soluble in acetone, sparingly soluble in alcohol. It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION

First identification: *B.*
Second identification: *A, C, D.*

A. Dissolve 50.0 mg in 10 ml of *0.1M sodium hydroxide* and dilute to 100.0 ml with *water R*. Dilute 2.0 ml of this solution to 100.0 ml with *0.01M sodium hydroxide*. Examined between 250 nm and 350 nm (*2.2.25*), the solution shows absorption maxima at 273 nm and at 323 nm. The ratio of the absorbance measured at the maximum at 273 nm to that measured at 323 nm is 5.4 to 5.7.

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *hydrochlorothiazide CRS*.

C. 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 50 mg in *acetone R* and dilute to 10 ml with the same solvent.

Reference solution (a). Dissolve 50 mg of *hydrochlorothiazide CRS* in *acetone R* and dilute to 10 ml with the same solvent.

Reference solution (b). Dissolve 25 mg of *chlorothiazide R* in reference solution (a) and dilute to 5 ml with the same reference solution.

Apply separately to the plate 2 µl of each solution.
Develop over a path of 10 cm using *ethyl acetate R*. Dry the plate in a current of air and examine in ultraviolet light at 254 nm. 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). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

D. Gently heat about 1 mg with 2 ml of a freshly prepared 0.5 g/l solution of *chromotropic acid, sodium salt R* in a cooled mixture of 35 volumes of *water R* and 65 volumes of *sulphuric acid R*. A violet colour develops.

TESTS

Acidity or alkalinity Shake 0.5 g of the powdered substance to be examined with 25 ml of *water R* for 2 min and filter. To 10 ml of the filtrate, add 0.2 ml of *0.01M sodium hydroxide* and 0.15 ml of *methyl red solution R*. The solution is yellow. Not more than 0.4 ml of *0.01M hydrochloric acid* is required to change the colour of the indicator to red.

Related substances Examine by liquid chromatography (*2.2.29*).

Test solution. Dissolve 30.0 mg of the substance to be examined in 5 ml of a mixture of equal volumes of *acetonitrile R* and *methanol R*, using sonication if necessary, and dilute to 20.0 ml with *phosphate buffer solution pH 3.2 R1*.

Solvent solution. Dilute 50.0 ml of a mixture of equal volumes of *acetonitrile R* and *methanol R* to 200.0 ml with *phosphate buffer solution pH 3.2 R1*.

Reference solution (a). Dissolve 15.0 mg of *hydrochlorothiazide CRS* and 15.0 mg of *chlorothiazide CRS* in 25.0 ml of a mixture of equal volumes of *acetonitrile R* and *methanol R*, using sonication if necessary, and dilute to 100.0 ml with *phosphate buffer solution pH 3.2 R1*. Dilute 5.0 ml to 100.0 ml with the solvent solution.

Reference solution (b). Dilute 1.0 ml of the test solution to 50.0 ml with the solvent solution. Dilute 5.0 ml of this solution to 20.0 ml with the solvent solution.

The chromatographic procedure may be carried out using:
— a stainless steel column 0.1 m long and 4.6 mm in internal diameter packed with *octadecylsilyl silica gel for chromatography R* (3 µm),
— as mobile phase at a flow rate of 0.8 ml per minute a linear gradient programme using the following conditions:
Mobile phase A. To 940 ml of *phosphate buffer solution pH 3.2 R1* add 60.0 ml of *methanol R* and 10.0 ml of *tetrahydrofuran R* and mix,
Mobile phase B. To a mixture of 500 ml of *methanol R* and 500 ml of *phosphate buffer solution pH 3.2 R1* add 50.0 ml of *tetrahydrofuran R* and mix,
— as detector a spectrophotometer set at 224 nm.

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)	Comment
0–17	100→55	0→45	Linear gradient
17–30	55	45	Isocratic
30–35	55→100	45→0	Linear gradient
35–50	100	0	Isocratic
50=0	100	0	Return to initial eluent composition

Correspondence between Ph Eur general methods and Appendices of the British Pharmacopoeia is shown on page A8

Equilibrate the column for at least 20 min with *mobile phase A*. Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with 10 µl of reference solution (b) is at least 50 per cent of the full scale of the recorder.

Inject 10 µl of reference solution (a). When the chromatogram is recorded in the prescribed conditions, the retention times are: chlorothiazide about 7 min and hydrochlorothiazide about 8 min. The test is not valid unless the resolution between the peaks corresponding to chlorothiazide and hydrochlorothiazide is at least 2.5. If necessary, adjust slightly the composition of the mobile phase or the time programme of the linear gradient.

Inject separately 10 µl of the solvent solution as a blank, 10 µl of the test solution and 10 µl of reference solution (b). In the chromatogram obtained with the test solution: the area of any peak, apart from the principal peak, is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent); the sum of the areas of all peaks, apart from the principal peak, is not greater than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent). Disregard any peak due to the solvent solution and any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b).

Chlorides (*2.4.4*). Dissolve 1.0 g in 25 ml of *acetone R* and dilute to 30 ml with *water R*. 15 ml complies with the limit test for chlorides (100 ppm). Prepare the standard using 5 ml of *acetone R* containing 15 per cent *V/V* of *water R* and 10 ml of *chloride standard solution (5 ppm Cl) R*.

Loss on drying (*2.2.32*). Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 100°C to 105°C.

Sulphated ash (*2.4.14*). Not more than 0.1 per cent, determined on 1.0 g.

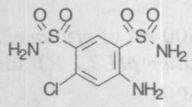
ASSAY

Dissolve 0.120 g in 50 ml of *dimethyl sulphoxide R*. Titrate with *0.1M tetrabutylammonium hydroxide*, determining the end-point potentiometrically (*2.2.20*) at the second point of inflexion.

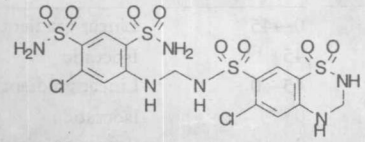
1 ml of *0.1M tetrabutylammonium hydroxide* is equivalent to 14.88 mg of $C_7H_8ClN_3O_4S_2$.

IMPURITIES

A. chlorothiazide,



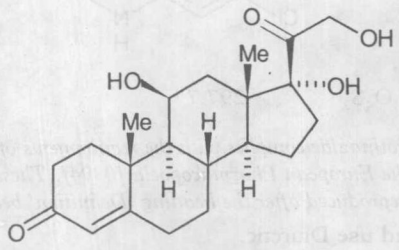
B. 4-amino-6-chlorobenzene-1,3-disulphonamide,



C. 6-chloro-*N*-[(6-chloro-7-sulphamoyl-2,3-dihydro-4*H*-1,2,4-benzothiadiazin-4-yl 1,1-dioxide)methyl]-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulphonamide 1,1-dioxide.

Ph Eur

Hydrocortisone



$C_{21}H_{30}O_5$ 362.5 *50-23-7*

Hydrocortisone complies with the requirements of the 3rd edition of the European Pharmacopoeia [0335]. These requirements are reproduced after the heading 'Definition' below.

Action and use Corticosteroid.

Preparations

Hydrocortisone Cream
Hydrocortisone and Clioquinol Cream.
Hydrocortisone and Neomycin Cream
Hydrocortisone Ointment
Hydrocortisone and Clioquinol Ointment

Ph Eur

DEFINITION

Hydrocortisone contains not less than 97.0 per cent and not more than the equivalent of 103.0 per cent of 11β,17,21-trihydroxypregn-4-ene-3,20-dione, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, practically insoluble in water, sparingly soluble in acetone and in alcohol, slightly soluble in methylene chloride.

It shows polymorphism.

IDENTIFICATION

First identification: A, B.
Second identification: C, D.

A. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *hydrocortisone 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 *acetone R*, evaporate to dryness on a water-bath and record new spectra using the residues.

B. 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 10 mg of the substance to be examined in a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R* and dilute to 10 ml with the same mixture of solvents.

Reference solution (a). Dissolve 20 mg of *hydrocortisone CRS* in a mixture of 1 volume of *methanol R* and 9 volumes of

Correspondence between Ph Eur general methods and Appendices of the British Pharmacopoeia is shown on page A8

methylene chloride R and dilute to 20 ml with the same mixture of solvents.
Reference solution (b). Dissolve 10 mg of *prednisolone CRS* in reference solution (a) and dilute to 10 ml with reference solution (a).

Apply separately to the plate 5 µl of each solution. Prepare the mobile phase by adding 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*. Develop over a path of 15 cm. Carry out a second development over a path of 15 cm using a mixture of 5 volumes of *butanol R* saturated with *water R*, 15 volumes of *toluene R* and 80 volumes of *ether R*. Allow the plate to dry in air. Examine in ultraviolet light at 254 nm. 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). Spray with *alcoholic solution of sulphuric acid R*. Heat at 120°C for 10 min or until the spots appear. Allow to cool. Examine the chromatograms in daylight and in ultraviolet light at 365 nm. 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). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

C. 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 (a). Dissolve 25 mg of the substance to be examined in *methanol R* and dilute to 5 ml with the same solvent. This solution is also used to prepare test solution (b). Dilute 2 ml of the solution to 10 ml with *methylene chloride R*.
Test solution (b). Transfer 0.4 ml of the solution obtained during preparation of test solution (a) to a glass tube 100 mm long and 20 mm in diameter and fitted with a ground-glass stopper or a polytetrafluoroethylene cap and evaporate the solvent with gentle heating under a stream of *nitrogen R*. Add 2 ml of a 15 per cent *V/V* solution of *glacial acetic acid R* and 50 mg of *sodium bismuthate R*. Stopper the tube and shake the suspension for 1 h in a mechanical shaker, protected from light. Add 2 ml of a 15 per cent *V/V* solution of *glacial acetic acid R* and filter into a 50 ml separating funnel, washing the filter with two quantities, each of 5 ml, of *water R*. Shake the clear filtrate with 10 ml of *methylene chloride R*. Wash the organic layer with 5 ml of *1M sodium hydroxide* and two quantities, each of 5 ml, of *water R*. Dry over *anhydrous sodium sulphate R*.
Reference solution (a). Dissolve 25 mg of *hydrocortisone CRS in methanol R* and dilute to 5 ml with the same solvent. This solution is also used to prepare reference solution (b). Dilute 2 ml of the solution to 10 ml with *methylene chloride R*.
Reference solution (b). Transfer 0.4 ml of the solution obtained during preparation of reference solution (a) to a glass tube 100 mm long and 20 mm in diameter and fitted with a ground-glass stopper or a polytetrafluoroethylene cap and evaporate the solvent with gentle heating under a stream of *nitrogen R*. Add 2 ml of a 15 per cent *V/V* solution of *glacial acetic acid R* and 50 mg of *sodium bismuthate R*. Stopper the tube and shake the suspension for 1 h in a mechanical shaker protected from light. Add 2 ml of a 15 per cent *V/V* solution of *glacial acetic acid R* and filter into a 50 ml separating funnel, washing the filter with two quantities, each of 5 ml, of *water R*. Shake the clear filtrate with 10 ml of *methylene chloride R*. Wash the organic layer with 5 ml of *1M sodium hydroxide* and two quantities, each of 5 ml, of *water R*. Dry over *anhydrous sodium sulphate R*.

Apply separately to the plate 5 µl of test solution (a), 5 µl of reference solution (a), 25 µl of test solution (b) and 25 µl of reference solution (b), applying the latter two in small quantities to obtain small spots. Prepare the mobile phase by adding 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*. Develop over a path of 15 cm. Carry out a second development over a path of 15 cm using a mixture of 5 volumes of *butanol R* saturated with *water R*, 15 volumes of *toluene R* and 80 volumes of *ether R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. The principal spot in each of the chromatograms obtained with the test solutions is similar in position and size to the principal spot in the chromatogram obtained with the corresponding reference solution. Spray with *alcoholic solution of sulphuric acid R* and heat at 120°C for 10 min or until the spots appear. Allow to cool. Examine the plate in daylight and in ultraviolet light at 365 nm. The principal spot in each of the chromatograms obtained with the test solutions 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 the corresponding reference solution. The principal spots in the chromatograms obtained with test solution (b) and reference solution (b) have an R_f value distinctly higher than that of the principal spots in the hromatograms obtained with test solution (a) and reference solution (a).

D. Add about 2 mg to 2 ml of *sulphuric acid R* and shake to dissolve. Within 5 min, an intense brownish-red colour develops with a green fluorescence which is particularly intense when examined in ultraviolet light at 365 nm. Add the solution to 10 ml of *water R* and mix. The colour fades and a clear solution remains. The fluorescence in ultraviolet light does not disappear.

TESTS

Specific optical rotation (*2.2.7*). Dissolve 0.250 g in *dioxan R* and dilute to 25.0 ml with the same solvent. The specific optical rotation is +150° to +156°, calculated with reference to the dried substance.

Related substances Examine by liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*
Test solution. Dissolve 25.0 mg of the substance to be examined in 2 ml of *tetrahydrofuran R* and dilute to 10.0 ml with *water R*.
Reference solution (a). Dissolve 2 mg of *hydrocortisone CRS* and 2 mg of *prednisolone CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase.
Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

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 *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm),
— as mobile phase at a flow rate of 1 ml/min a mixture prepared as follows: in a 1000 ml volumetric flask mix

Correspondence between Ph Eur general methods and Appendices of the British Pharmacopoeia is shown on page A8

220 ml of *tetrahydrofuran R* with 700 ml of *water R* and allow to equilibrate; dilute to 1000 ml with *water R* and mix again;

— as detector a spectrophotometer set at 254 nm, maintaining the temperature of the column at 45°C.

Equilibrate the column with the mobile phase at a flow rate of 1 ml/min for about 30 min.

Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with 20 µl of reference solution (b) is at least 50 per cent of the full scale of the recorder.

Inject 20 µl of reference solution (a). When the chromatograms are recorded in the prescribed conditions, the retention times are: prednisolone, about 14 min; hydrocortisone, about 15.5 min. The test is not valid unless the resolution between the peaks due to prednisolone and hydrocortisone is at least 2.2. If necessary, adjust the concentration of *tetrahydrofuran R* in the mobile phase.

Inject separately 20 µl of the solvent mixture of the test solution as a blank, 20 µl of the test solution and 20 µl of reference solution (b). Continue the chromatography of the test solution for four times the retention time of the principal peak. In the chromatogram obtained with the test solution: the area of any peak, apart from the principal peak, is not greater than half the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent): the sum of the areas of all the peaks, apart from the principal peak, is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent). Disregard any peak obtained with the blank run and any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b).

Loss on drying (*2.2.32*). Not more than 1.0 per cent, determined on 0.500 g by drying in an oven at 100°C to 105°C.

ASSAY

Dissolve 0.100 g in *alcohol R* and dilute to 100.0 ml with the same solvent. Dilute 2.0 ml of the solution to 100.0 ml with *alcohol R*. Measure the absorbance (*2.2.25*) at the maximum at 241.5 nm.

Calculate the content of $C_{21}H_{30}O_5$ taking the specific absorbance to be 440.

STORAGE

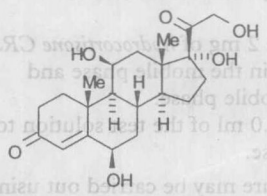
Store protected from light.

IMPURITIES

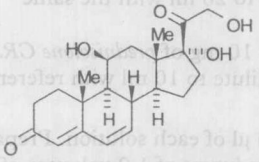
A. prednisolone,

B. cortisone,

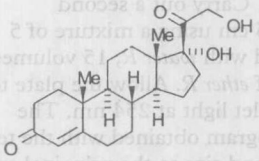
C. hydrocortisone acetate,



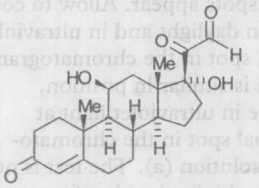
D. 6β,11β,17,21-tetrahydroxypregn-4-ene-3,20-dione (*6β-hydroxyhydrocortisone*),



E. 11β,17,21-trihydroxypregna-4,6-diene-3,20-dione (*Δ⁶-hydrocortisone*),



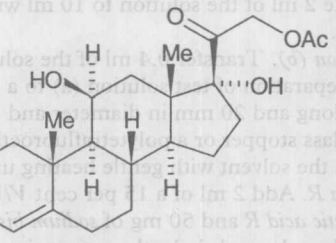
F. 17,21-dihydroxypregn-4-ene-3,20-dione (*Reichstein's substance*),



G. 11β,17-dihydroxy-3,20-dioxopregn-4-en-21-al.

Ph Eur

Hydrocortisone Acetate



$C_{23}H_{32}O_6$ 404.5 *50-03-3*

Hydrocortisone Acetate complies with the requirements of the 3rd edition of the European Pharmacopoeia [0334]. These requirements are reproduced after the heading 'Definition' below.

Action and use Corticosteroid.

Preparations

Hydrocortisone Acetate Cream
Hydrocortisone Acetate and Neomycin Ear Drops
Hydrocortisone Acetate and Neomycin Eye Drops
Hydrocortisone Acetate and Neomycin Eye Ointment
Hydrocortisone Acetate Injection
Hydrocortisone Acetate Ointment

Ph Eur

DEFINITION

Hydrocortisone acetate contains not less than 97.0 per cent and not more than the equivalent of 103.0 per cent of 11β,17,21-trihydroxypregn-4-ene-3,20-dione 21-acetate, calculated with reference to the dried substance.

Correspondence between Ph Eur general methods and Appendices of the British Pharmacopoeia is shown on page A8

CHARACTERS

A white or almost white, crystalline powder, practically insoluble in water, slightly soluble in ethanol and in methylene chloride.

It melts at about 220°C, with decomposition.

IDENTIFICATION

First identification: A, B.
Second identification: C, D, E.

A. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *hydrocortisone acetate CRS*.

B. 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 10 mg of the substance to be examined in a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R* and dilute to 10 ml with the same mixture of solvents.

Reference solution (a). Dissolve 20 mg of *hydrocortisone acetate CRS* in a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R* and dilute to 20 ml with the same mixture of solvents.

Reference solution (b). Dissolve 10 mg of *cortisone acetate R* in reference solution (a) and dilute to 10 ml with reference solution (a).

Apply separately to the plate 5 µl of each solution. Prepare the mobile phase by adding 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*. Develop over a path of 15 cm. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. 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). Spray with *alcoholic solution of sulphuric acid R*. Heat at 120°C for 10 min or until the spots appear. Allow to cool. Examine the plate in daylight and in ultraviolet light at 365 nm. 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). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

C. 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 (a). Dissolve 25 mg of the substance to be examined in *methanol R* and dilute to 5 ml with the same solvent. This solution is also used to prepare test solution (b). Dilute 2 ml of the solution to 10 ml with *methylene chloride R*.

Test solution (b). Transfer 2 ml of the solution obtained during preparation of test solution (a) to a 15 ml glass tube with a ground-glass stopper or a polytetrafluoroethylene cap. Add 10 ml of *saturated methanolic potassium hydrogen carbonate solution R* and immediately pass a stream of *nitrogen R* briskly through the solution for 5 min. Stopper the tube. Heat in a water-bath at 45°C protected from light for 2 h 30 min. Allow to cool.

Reference solution (a). Dissolve 25 mg of *hydrocortisone acetate CRS* in *methanol R* and dilute to 5 ml with the same solvent. This solution is also used to prepare reference solution (b). Dilute 2 ml of the solution to 10 ml with *methylene chloride R*.

Reference solution (b). Transfer 2 ml of the solution obtained during preparation of reference solution (a) to a 15 ml glass tube with a ground-glass stopper or a polytetrafluoroethylene cap. Add 10 ml of *saturated methanolic potassium hydrogen carbonate solution R* and immediately pass a stream of *nitrogen R* briskly through the solution for 5 min. Stopper the tube. Heat in a water-bath at 45°C protected from light for 2 h 30 min. Allow to cool.

Apply separately to the plate 5 µl of each solution. Prepare the mobile phase by adding 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*. Develop over a path of 15 cm. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. The principal spot in each of the chromatograms obtained with the test solutions is similar in position and size to the principal spot in the chromatogram obtained with the corresponding reference solution. Spray with *alcoholic solution of sulphuric acid R* and heat at 120°C for 10 min or until the spots appear. Allow to cool. Examine in daylight and in ultraviolet light at 365 nm. The principal spot in each of the chromatograms obtained with the test solutions 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 the corresponding reference solution. The principal spots in the chromatograms obtained with test solution (b) and reference solution (b) have an R_f value distinctly lower than that of the principal spots in the chromatograms obtained with test solution (a) and reference solution (a).

D. Add about 2 mg to 2 ml of *sulphuric acid R* and shake to dissolve. Within 5 min an intense brownish-red colour develops with a green fluorescence which is particularly intense when viewed in ultraviolet light at 365 nm. Add the solution to 10 ml of *water R* and mix. The colour fades and the fluorescence in ultraviolet light does not disappear.

E. About 10 mg gives the reaction of acetyl (*2.3.1*).

TESTS

Specific optical rotation (*2.2.7*). Dissolve 0.250 g in *dioxan R* and dilute to 25.0 ml with the same solvent. The specific optical rotation is +158° to +167°, calculated with reference to the dried substance.

Related substances Examine by liquid chromatography (*2.2.29*).

Test solution. Dissolve 25.0 mg of the substance to be examined in *methanol R* and dilute to 10.0 ml with the same solvent.

Reference solution (a). Dissolve 2 mg of *hydrocortisone acetate CRS* and 2 mg of *cortisone acetate R* in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

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 per minute a mixture prepared as follows: in a 1000 ml volumetric flask mix 400 ml of *acetonitrile R* with 550 ml of

Correspondence between Ph Eur general methods and Appendices of the British Pharmacopoeia is shown on page A8

water R and allow to equilibrate; adjust the volume to 1000 ml with *water R* and mix again,

— as detector a spectrophotometer set at 254 nm.

Equilibrate the column with the mobile phase at a flow rate of 1 ml per minute for about 30 min.

Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with 20 µl of reference solution (b) is not less than 50 per cent of the full scale of the recorder.

Inject 20 µl of reference solution (a). When the chromatograms are recorded in the prescribed conditions the retention times are: hydrocortisone acetate, about 10 min and cortisone acetate, about 12 min. The test is not valid unless the resolution between the peaks due to hydrocortisone acetate and cortisone acetate is at least 4.2; if necessary, adjust the concentration of acetonitrile in the mobile phase.

Inject separately 20 µl of the test solution and 20 µl of reference solution (b). Continue the chromatography for 2.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution: the area of any peak, apart from the principal peak, is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and not more than one such peak has an area greater than half the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent); the sum of the areas of all the peaks, apart from the principal peak, is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent). Disregard any peak due to the solvent and any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b).

Loss on drying (*2.2.32*). Not more than 0.5 per cent, determined on 0.500 g by drying in an oven at 100°C to 105°C.

ASSAY

Dissolve 0.100 g in *alcohol R* and dilute to 100.0 ml with the same solvent. Dilute 2.0 ml of the solution to 100.0 ml with *alcohol R*. Measure the absorbance (*2.2.25*) at the maximum of 241.5 nm.

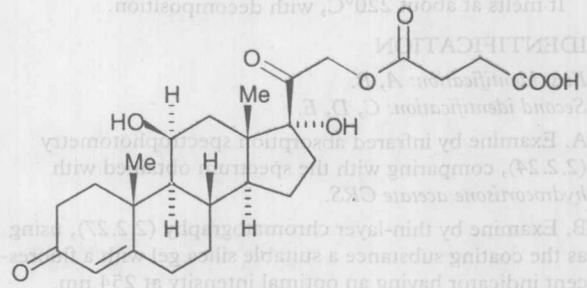
Calculate the content of $C_{23}H_{32}O_6$ taking the specific absorbance to be 395.

STORAGE

Store in a well-closed container, protected from light.

Ph Eur

Hydrocortisone Hydrogen Succinate



$C_{25}H_{34}O_8$ 462.5 *2203-97-6*

Hydrocortisone Hydrogen Succinate complies with the requirements of the 3rd edition of the European Pharmacopoeia [0768]. These requirements are reproduced after the heading 'Definition' below.

Action and use Corticosteroid.

Preparation
Hydrocortisone Sodium Succinate Injection

Ph Eur

DEFINITION

Hydrocortisone hydrogen succinate contains not less than 97.0 per cent and not more than the equivalent of 103.0 per cent of 11β,17α,21-trihydroxypregn-4-ene-3,20-dione 21-(hydrogen butanedioate), calculated with reference to the dried substance.

CHARACTERS

A white or almost white powder, hygroscopic, practically insoluble in water, freely soluble in acetone and in ethanol. It dissolves in dilute solutions of alkali carbonates and alkali hydroxides.

IDENTIFICATION

First identification: A, B.
Second identification: C, D.

A. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *hydrocortisone hydrogen succinate CRS*. Dry the substances before use at 100°C to 105°C for 3 h.

B. 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 10 mg of the substance to be examined in a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R* and dilute to 10 ml with the same mixture of solvents.

Reference solution (a). Dissolve 20 mg of *hydrocortisone hydrogen succinate CRS* in a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R* and dilute to 20 ml with the same mixture of solvents.

Reference solution (b). Dissolve 10 mg of *methylprednisolone hydrogen succinate CRS* in reference solution (a) and dilute to 10 ml with reference solution (a).

Apply separately to the plate 5 µl of each solution. Develop over a path of 15 cm using a mixture of 0.1 volumes of *anhydrous formic acid R*, 1 volume of *ethanol R*

Correspondence between Ph Eur general methods and Appendices of the British Pharmacopoeia is shown on page A8

and 15 volumes of *methylene chloride R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. 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). Spray the plate with *alcoholic solution of sulphuric acid R*. Heat at 120°C for 10 min or until the spots appear. Allow to cool. Examine in daylight and in ultraviolet light at 365 nm. 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). The test is not valid unless the chromatogram obtained with reference solution (b) shows two spots which may however not be completely separated.

C. 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 (a). Dissolve 25 mg of the substance to be examined in *methanol R* with gentle heating and dilute to 5 ml with the same solvent. (This solution is also used to prepare test solution (b)). Dilute 2 ml of the solution to 10 ml with *methylene chloride R*.

Test solution (b). Transfer 2 ml of the solution obtained during preparation of test solution (a) to a 15 ml glass tube with a ground-glass stopper or a polytetrafluoroethylene cap. Add 10 ml of a 0.8 g/l solution of *sodium hydroxide R* in *methanol R* and immediately pass a stream of *nitrogen R* briskly through the solution for 5 min. Stopper the tube. Heat in a water-bath at 45°C, protected from light, for 30 min. Allow to cool.

Reference solution (a). Dissolve 25 mg of *hydrocortisone hydrogen succinate CRS* in *methanol R* with gentle heating and dilute to 5 ml with the same solvent. (This solution is also used to prepare reference solution (b)). Dilute 2 ml of the solution to 10 ml with *methylene chloride R*.

Reference solution (b). Transfer 2 ml of the solution obtained during preparation of reference solution (a) to a 15 ml glass tube with a ground-glass stopper or a polytetrafluoroethylene cap. Add 10 ml of a 0.8 g/l solution of *sodium hydroxide R* in *methanol R* and immediately pass a stream of *nitrogen R* briskly through the solution for 5 min. Stopper the tube. Heat in a water-bath at 45°C, protected from light, for 30 min. Allow to cool.

Apply separately to the plate 5 µl of each solution. Prepare the mobile phase by adding 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*. Develop over a path of 15 cm. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. The principal spot in each of the chromatograms obtained with the test solutions is similar in position and size to the principal spot in the chromatogram obtained with the corresponding reference solution. Spray the plate with *alcoholic solution of sulphuric acid R*. Heat at 120°C for 10 min or until the spots appear. Allow to cool. Examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatograms obtained with the test solutions 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 the corresponding reference solution. The principal spot in each of the chromatograms obtained with test solution (b) and reference solution (b) has an R_f value distinctly higher than that of the principal spots in each of the chromatograms obtained with test solution (a) and reference solution (a).

D. Add about 2 mg to 2 ml of *sulphuric acid R* and shake to dissolve. Within 5 min, an intense brownish-red colour develops with a green fluorescence which is particularly intense when viewed in ultraviolet light at 365 nm. Add the solution to 10 ml of *water R* and mix. The colour fades and a clear solution remains. The fluorescence in ultraviolet light does not disappear.

TESTS

Appearance of solution Dissolve 0.10 g in 5 ml of *sodium hydrogen carbonate solution R*. The solution is clear (*2.2.1*).

Specific optical rotation (*2.2.7*). Dissolve 0.250 g in *ethanol R* and dilute to 25.0 ml with the same solvent. The specific optical rotation is +147° to +153°, calculated with reference to the dried substance.

Related substances Examine by liquid chromatography (*2.2.29*).

Test solution. Dissolve 25.0 mg of the substance to be examined in a mixture of equal volumes of *acetonitrile R* and *water R* and dilute to 10.0 ml with the same solvent.

Reference solution (a). Dissolve 2 mg of *hydrocortisone hydrogen succinate CRS* and 2 mg of *dexamethasone CRS* in 50 ml of *acetonitrile R* and dilute to 100.0 ml with *water R*.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with a mixture of equal volumes of *acetonitrile R* and *water R*.

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 per minute a mixture prepared as follows: in a 1000 ml volumetric flask mix 330 ml of *acetonitrile R* with 600 ml of *water R* and 1.0 ml of *phosphoric acid R* and allow to equilibrate; dilute to 1000 ml with *water R* and mix again,
— as detector a spectrophotometer set at 254 nm.

Equilibrate the column with the mobile phase at a flow rate of 1 ml per minute for about 30 min.

Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with 20 µl of reference solution (b) is not less than 50 per cent of the full scale of the recorder.

Inject 20 µl of reference solution (a). When the chromatograms are recorded in the conditions described above the retention times are: dexamethasone, about 12.5 min and hydrocortisone hydrogen succinate, about 15 min. The test is not valid unless the resolution between the peaks corresponding to dexamethasone and hydrocortisone hydrogen succinate is a least 5.0; if necessary, adjust the concentration of acetonitrile in the mobile phase.

Inject separately 20 µl of the test solution and 20 µl of reference solution (b). Continue the chromatography for twice the retention time of the principal peak. In the chromatogram obtained with the test solution: the area of any peak, apart from the principal peak, is not greater than half the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent); the sum of the areas of all the peaks, apart from the principal peak, is not greater than 0.75 times the area of the prin-

Correspondence between Ph Eur general methods and Appendices of the British Pharmacopoeia is shown on page A8

cipal peak in the chromatogram obtained with reference solution (b) (0.75 per cent). Disregard any peak due to the solvent and any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b).

Loss on drying (*2.2.32*). Not more than 4.0 per cent, determined on 1.000 g by drying in an oven at 100°C to 105°C.

Sulphated ash (*2.4.14*). Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.100 g in *alcohol R* and dilute to 100.0 ml with the same solvent. Dilute 2.0 ml to 100.0 ml with *alcohol R*. Measure the absorbance (*2.2.25*) at the maximum at 241.5 nm.

Calculate the content of $C_{25}H_{34}O_8$ taking the specific absorbance to be 353.

STORAGE

Store in an airtight container, protected from light.

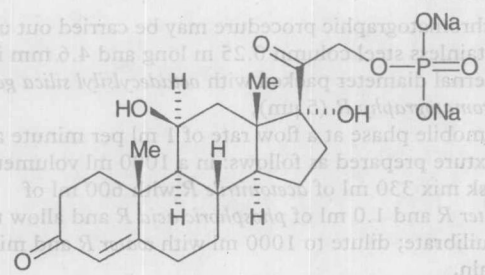
IMPURITIES

A. hydrocortisone,

B. hydrocortisone acetate.

Ph Eur

Hydrocortisone Sodium Phosphate



$C_{21}H_{29}Na_2O_8P$ 486.4 *6000-74-4*

Definition Hydrocortisone Sodium Phosphate is disodium 11β,17α-dihydroxy-3,20-dioxopregn-4-en-21-yl orthophosphate. It contains not less than 96.0% and not more than 103.0% of $C_{21}H_{29}Na_2O_8P$, calculated with reference to the anhydrous substance.

Characteristics A white or almost white powder; hygroscopic.

Freely soluble in *water*; practically insoluble in *absolute ethanol* and in *chloroform*.

Identification *Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.*

A. The *infrared absorption spectrum*, Apppendix II A, is concordant with the *reference spectrum* of hydrocortisone sodium phosphate *(RS 386)*.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a freshly prepared mixture of 60 volumes of *butan-1-ol*, 20 volumes of *acetic anhydride* and 20 volumes of *water* as the mobile phase. Apply separately to the plate 2 µl of each of the following solutions. Solution (1) contains 0.25% w/v of the substance being examined in *methanol*. Solution (2) contains 0.25% w/v of *hydrocortisone sodium phosphate BPCRS* in *methanol*. Solution (3) is a mixture of equal volumes of solutions (1) and (2). Solution (4) is a mixture of equal volumes of solution (1) and a 0.25% w/v solution of *betamethasone sodium phosphate BPCRS* in *methanol*. After removal of the plate, allow it to dry in air until the odour of solvent is no longer detectable, spray with *ethanolic sulphuric acid (20%)*, heat at 120° for 10 minutes and examine under *ultraviolet light (365 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The principal spot in the chromatogram obtained with solution (3) appears as a single, compact spot and the chromatogram obtained with solution (4) shows two principal spots with almost identical Rf values.

C. Dissolve 2 mg in 2 ml of *sulphuric acid*. A yellowish green fluorescence is produced immediately (distinction from betamethasone sodium phosphate, dexamethasone sodium phosphate and prednisolone sodium phosphate).

D. Heat gently 40 mg with 2 ml of *sulphuric acid* until white fumes are evolved, add *nitric acid* dropwise until oxidation is complete and cool. Add 2 ml of *water*, heat until white fumes are again evolved, cool, add 10 ml of *water* and neutralise to *litmus paper* with 5M *ammonia*. The resulting solution yields reaction A characteristic of *sodium salts* and reaction B characteristic of *phosphates*, Appendix VI.

Alkalinity pH of a 0.5% w/v solution, 7.5 to 9.0, Appendix V L.

Specific optical rotation In a 1% w/v solution, +121° to +129°, calculated with reference to the anhydrous substance, Appendix V F.

Inorganic phosphate Dissolve 25 mg in 10 ml of *water*, add 4 ml of 1M *sulphuric acid*, 1 ml of a 10% w/v solution of *ammonium molybdate* and 2 ml of *methylaminophenol–sulphite reagent* and allow to stand for 15 minutes. Add sufficient *water* to produce 25 ml and allow to stand for a further 15 minutes. The *absorbance* of a 4-cm layer of the resulting solution at 730 nm, Appendix II B, is not more than that of a 4-cm layer of a solution prepared by treating 10 ml of a 0.0036% w/v solution of *potassium dihydrogen orthophosphate* in the same manner, beginning at the words 'add 4 ml ...'.

Related substances Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 77 volumes of *dichloromethane*, 15 volumes of *ether*, 8 volumes of *methanol* and 1.2 volumes of *water* as the mobile phase. Apply separately to the plate 2 µl of each of three solutions in *methanol* containing (1) 1.0% w/v of the substance being examined, (2) 1.0% w/v of *hydrocortisone sodium phosphate BPCRS* and (3) 0.020% w/v of *hydrocortisone EPCRS*. After removal of the plate, allow it to dry in air for 5 minutes and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3).

Water Not more than 10.0%, Appendix IX C. Use 0.4 g.

Assay Dissolve 0.1 g in sufficient *water* to produce 200 ml. Dilute 5 ml to 100 ml with *water* and measure the *absorbance* of the resulting solution at the maximum at 248 nm, Appendix II B. Calculate the content of $C_{21}H_{29}Na_2O_8P$ taking 333 as the value of A(1%, 1 cm) at the maximum at 248 nm.

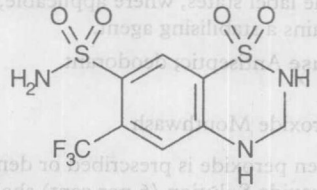
Correspondence between Ph Eur general methods and Appendices of the British Pharmacopoeia is shown on page A8

Storage Hydrocortisone Sodium Phosphate should be kept in a well-closed container and protected from light.

Action and use Corticosteroid.

Preparation
Hydrocortisone Sodium Phosphate Injection

Hydroflumethiazide



$C_8H_8F_3N_3O_4S_2$ 331.3 *135-09-1*

Definition Hydroflumethiazide is 3,4-dihydro-6-trifluoromethyl-2*H*-1,2,4-benzothiadiazine-7-sulphonamide 1,1-dioxide. It contains not less than 98.0% and not more than 102.0% of $C_8H_8F_3N_3O_4S_2$, calculated with reference to the dried substance.

Characteristics White or almost white, glistening crystals or crystalline powder; odourless or almost odourless.

Practically insoluble in *water*; soluble in *ethanol (96%)*; practically insoluble in *chloroform* and in *ether*.

Identification

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of hydroflumethiazide *(RS 181)*.

B. Dissolve 10 mg in 10 ml of 0.1M *sodium hydroxide*, add sufficient *water* to produce 100 ml and dilute 10 ml to 50 ml with 0.01M *sodium hydroxide*. The *light absorption* of the resulting solution, Appendix II B, in the range 230 to 350 nm exhibits two maxima, at 274 nm and 333 nm. The *absorbance* at the maxima is about 0.92 and about 0.19 respectively.

C. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and *ethyl acetate* as the mobile phase. Apply separately to the plate 5 µl of each of two solutions in *acetone* containing (1) 0.1% w/v of the substance being examined and (2) 0.1% w/v of *hydroflumethiazide BPCRS*. After removal of the plate, dry it in a current of air, examine under *ultraviolet light (254 nm)* and then reveal the spots by *Method I* and examine again. By each method of visualisation the principal spot in the chromatogram obtained with solution (1) corresponds in colour and intensity to that in the chromatogram obtained with solution (2).

Related substances Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and *ethyl acetate* as the mobile phase. Apply separately to the plate 10 µl of each of two solutions of the substance being examined in *acetone* containing (1) 1.0% w/v and (2) 0.010% w/v. After removal of the plate, dry it in a current of air and reveal the spots by *Method I*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

Loss on drying When dried to constant weight at 105°, loses not more than 0.5% of its weight. Use 1 g.

Sulphated ash Not more than 0.1%, Appendix IX A.

Assay Dissolve 0.3 g in 50 ml of *anhydrous pyridine* and carry out Method II for *non-aqueous titration*, Appendix VIII A, using 0.1M *tetrabutylammonium hydroxide VS* as titrant and determining the end point potentiometrically. Each ml of 0.1M *tetrabutylammonium hydroxide VS* is equivalent to 16.56 mg of $C_8H_8F_3N_3O_4S_2$.

Action and use Diuretic.

Preparation
Hydroflumethiazide Tablets

Hydrogen Peroxide Solution (30 per cent)

7722-84-1

Hydrogen Peroxide Solution (30 per cent) complies with the requirements of the 3rd edition of the European Pharmacopoeia [0396]. These requirements are reproduced after the heading 'Definition' below.

Action and use Antiseptic; deodorant.

When hydrogen peroxide is prescribed or demanded, Hydrogen Peroxide Solution (6 per cent) shall be dispensed or supplied.

Ph Eur

DEFINITION

Hydrogen peroxide solution (30 per cent) contains not less than 29.0 per cent *m/m* and not more than 31.0 per cent *m/m* of H_2O_2 (M_r 34.01). One volume of this solution corresponds to about 110 times its volume of oxygen. A suitable stabiliser may be added.

CHARACTERS

A colourless, clear liquid.

IDENTIFICATION

A. To 1 ml add 0.2 ml of *dilute sulphuric acid R* and 0.25 ml of *0.02M potassium permanganate* solution. The solution becomes colourless with evolution of gas.

B. To 0.05 ml add 2 ml of *dilute sulphuric acid R*, 2 ml of *ether R* and 0.05 ml of *potassium chromate solution R* and shake. The ether layer is blue.

C. It complies with the requirement for the content of H_2O_2.

TESTS

Acidity To 10 ml add 100 ml of *water R* and 0.25 ml of *methyl red solution R*. Not less than 0.05 ml and not more than 0.5 ml of *0.1M sodium hydroxide* is required to change the colour of the indicator.

Organic stabilisers Shake 20 ml with 10 ml of *chloroform R* and then with two quantities, each 5 ml, of *chloroform R*. Evaporate the combined chloroform layers under reduced pressure at a temperature not exceeding 25°C and dry the residue in a desiccator. The residue weighs not more than 10 mg (500 ppm).

Non-volatile residue Allow 10 ml to stand in a platinum dish until all effervescence has ceased, cooling if necessary.

Correspondence between Ph Eur general methods and Appendices of the British Pharmacopoeia is shown on page A8

Evaporate the solution to dryness on a water-bath and dry the residue at 100°C to 105°C. The residue weighs not more than 20 mg (2 g/l).

ASSAY

Dilute 1.00 g to 100.0 ml with *water R*. To 10.0 ml of this solution add 20 ml of *dilute sulphuric acid R*. Titrate with *0.02M potassium permanganate* until a pink colour is obtained.

1 ml of *0.02M potassium permanganate* is equivalent to 1.701 mg of H_2O_2 or 0.56 ml of oxygen.

STORAGE

Store protected from light; if the solution does not contain a stabiliser, store at a temperature below 15°C.

LABELLING

If the solution contains a stabiliser, the label states that the contents are stabilised. The competent authority may require that the name of the stabiliser be stated on the label.

CAUTION

It decomposes vigorously in contact with oxidisable organic matter and with certain metals and if allowed to become alkaline.

Ph Eur

Hydrogen Peroxide Solution (6 per cent)

Hydrogen Peroxide Solution

Definition Hydrogen Peroxide Solution (6 per cent) is an aqueous solution of hydrogen peroxide containing not less than 5.0% w/v and not more than 7.0% w/v of H_2O_2 (34.01), corresponding to about 20 times its volume of available oxygen. It may contain a suitable stabilising agent.

Characteristics A clear, colourless liquid. It decomposes in contact with oxidisable organic matter and with certain metals and if allowed to become alkaline.

Identification A. To 1 ml add 0.2 ml of 1M *sulphuric acid* and 0.25 ml of 0.02M *potassium permanganate*. The solution becomes colourless with evolution of gas.

B. Shake 0.05 ml with 2 ml of 1M *sulphuric acid*, 2 ml of *ether* and 0.05 ml of *potassium chromate solution*. The ether layer is blue.

C. Complies with the requirement for the content of H_2O_2.

Acidity Dilute 10 ml with 20 ml of *water* and add 0.25 ml of *methyl red solution*. Not less than 0.05 ml and not more than 1.0 ml of 0.1M *sodium hydroxide VS* is required to change the colour of the solution.

Organic stabilisers Shake 20 ml with successive quantities of 10, 5 and 5 ml of *chloroform*. Evaporate the combined chloroform extracts at a temperature not exceeding 25° at a pressure of 2 kPa and dry in a desiccator. Any residue weighs not more than 5 mg (250 ppm).

Non-volatile matter Place 10 ml in a platinum dish and allow to stand until effervescence has ceased, cooling if necessary. Evaporate the solution on a water bath. Any residue, when dried at 100° to 105°, weighs not more than 20 mg (0.2% w/v).

Assay Dilute 10 ml to 100 ml with *water*. To 10 ml of the resulting solution add 20 ml of 1M *sulphuric acid* and titrate with 0.02M *potassium permanganate VS*. Each ml of 0.02M *potassium permanganate VS* is equivalent to 1.701 mg of H_2O_2 or 0.56 ml of oxygen.

Storage Hydrogen Peroxide Solution (6 per cent) should be protected from light. If the solution does not contain a stabilising agent, it should be stored at a temperature not exceeding 15°. It should not be stored for long periods.

Labelling The label states, where applicable, that the solution contains a stabilising agent.

Action and use Antiseptic; deodorant.

Preparation
Hydrogen Peroxide Mouthwash

When hydrogen peroxide is prescribed or demanded, Hydrogen Peroxide Solution (6 per cent) shall be dispensed or supplied.

Hydrogen Peroxide Solution (3 per cent)

Dilute Hydrogen Peroxide Solution

Hydrogen Peroxide Solution (3 per cent) complies with the requirements of the 3rd edition of the European Pharmacopoeia [0395]. These requirements are reproduced after the heading 'Definition' below.

Action and use Antiseptic; deodorant.

When hydrogen peroxide is prescribed or demanded, Hydrogen Peroxide Solution (6 per cent) shall be dispensed or supplied.

Ph Eur

DEFINITION

Hydrogen peroxide solution (3 per cent) contains not less than 2.5 per cent *m/m* and not more than 3.5 per cent *m/m* of H_2O_2 (M_r 34.01). One volume of this solution corresponds to about ten times its volume of oxygen. A suitable stabiliser may be added.

CHARACTERS

A colourless, clear liquid.

IDENTIFICATION

A. To 2 ml add 0.2 ml of *dilute sulphuric acid R* and 0.25 ml of *0.02M potassium permanganate* solution. After a few seconds, the solution becomes colourless or slightly pink with evolution of gas.

B. To 0.5 ml add 1 ml of *dilute sulphuric acid R*, 2 ml of *ether R* and 0.1 ml of *potassium chromate solution R* and shake. The ether layer is blue.

C. It complies with the requirement for the content of H_2O_2.

TESTS

Acidity To 10 ml add 20 ml of *water R* and 0.25 ml of *methyl red solution R*. Not less than 0.05 ml and not more than 1.0 ml of *0.1M sodium hydroxide* is required to change the colour of the indicator.

Organic stabilisers Shake 20 ml with 10 ml of *chloroform R* and then with two quantities, each of 5 ml, of *chloroform R*. Evaporate the combined chloroform layers

Correspondence between Ph Eur general methods and Appendices of the British Pharmacopoeia is shown on page A8

under reduced pressure at a temperature not exceeding 25°C and dry the residue in a desiccator. The residue weighs not more than 5 mg (250 ppm).

Non-volatile residue Allow 10 ml to stand in a platinum dish until all effervescence has ceased. Evaporate the solution to dryness on a water-bath and dry the residue at 100°C to 105°C. The residue weighs not more than 20 mg (2 g/l).

ASSAY

Dilute 10.0 g to 100.0 ml with *water R*. To 10.0 ml of this solution add 20 ml of *dilute sulphuric acid R*. Titrate with *0.02M potassium permanganate* until a pink colour is obtained.

1 ml of *0.02M potassium permanganate* is equivalent to 1.701 mg of H_2O_2 or 0.56 ml of oxygen.

STORAGE

Store protected from light; if the solution does not contain a stabiliser, store at a temperature below 15°C.

LABELLING

If the solution contains a stabiliser, the label states that the contents are stabilised. The competent authority may require that the name of the stabiliser be stated on the label.

CAUTION

It decomposes in contact with oxidisable organic matter and with certain metals and if allowed to become alkaline.

Ph Eur

Hydrotalcite

$Al_2Mg_6(OH)_{16}CO_3,4H_2O$ 604.0 *12304-65-3*

Definition Hydrotalcite is a hydrated form of an aluminium magnesium basic carbonate corresponding to the formula $Al_2Mg_6(OH)_{16}CO_3,4H_2O$. It contains not less than 15.3% and not more than 18.7% of Al_2O_3 and not less than 36.0% and not more than 44.0% of MgO. The ratio of the content of Al_2O_3 to the content of MgO is not less than 0.40 and not more than 0.45.

Characteristics A white or almost white, free-flowing, granular powder.

Practically insoluble in *water*. It dissolves in dilute mineral acids with slight effervescence.

Identification

A. Dissolve 1.0 g in 20 ml of 2M *hydrochloric acid*. Effervescence occurs. Add 30 ml of *water*, boil, add 2M *ammonia* until just alkaline to *methyl red solution*, continue boiling for 2 minutes and filter, reserving the filtrate for test B. Wash the precipitate with 50 ml of a hot 2% w/v solution of *ammonium chloride* and dissolve in 15 ml of 2M *hydrochloric acid*. The resulting solution yields the reaction characteristic of *aluminium salts*, Appendix VI.

B. Dilute 1 ml of the filtrate obtained in test A to 10 ml with *water*. The resulting solution yields the reactions characteristic of *magnesium salts*, Appendix VI.

Alkalinity pH of a 4% w/v suspension in *carbon dioxide-free water*, 8.0 to 10.0, Appendix V L.

Neutralising capacity Mix 0.2 g with a small quantity of *water* to give a smooth paste and gradually add sufficient further quantities of *water* to produce 100 ml. Warm at 37°, add 100 ml of 0.1M *hydrochloric acid VS* previously heated to 37° and stir continuously for 1 hour using a paddle stirrer at a rate of about 200 revolutions per minute, maintaining the temperature at 37°, and titrate with 0.1M *sodium hydroxide VS* to pH 3.5. Subtract the volume of 0.1M *sodium hydroxide VS* from 100 ml to obtain the number of ml of 0.1M *hydrochloric acid VS* required for neutralisation. Not less than 260 ml of 0.1M *hydrochloric acid VS* is required to neutralise 1 g.

Arsenic Dissolve 0.33 g in 5 ml of 2M *hydrochloric acid*. The resulting solution complies with the *limit test for arsenic*, Appendix VII (3 ppm).

Heavy metals Dissolve 2.7 g in 20 ml of 5M *hydrochloric acid* and 10 ml of *water*, add 0.5 ml of *nitric acid* and boil for 30 seconds. Cool, add 2 g of *ammonium chloride* and 2 g of *ammonium thiocyanate* and extract with three 10-ml quantities of a mixture of equal volumes of *amyl alcohol* and *ether*. Add to the aqueous layer 0.1 ml of *phenolphthalein solution* and 13.5M *ammonia* until a pink colour is produced. Cool, add *glacial acetic acid* until the solution is decolorised and add a further 5 ml of *glacial acetic acid*. Filter, if necessary, and dilute the solution to 40 ml with *water*. 12 ml of the resulting solution complies with *limit test A for heavy metals*, Appendix VII. Use *lead standard solution (2 ppm Pb)* to prepare the standard (30 ppm).

Sodium Not more than 0.1% of Na when determined by Method II for *atomic emission spectrophotometry*, Appendix II D, measuring at 589 nm. To prepare the test solution dissolve 0.1 g in 4 ml of 5M *hydrochloric acid*, dilute to 200 ml with *water* and use *sodium standard solution (200 ppm Na)*, diluted if necessary with 0.1M *hydrochloric acid*, to prepare the standard solutions.

Chloride Dissolve 0.18 g in 10 ml of 2M *nitric acid*, boil, allow to cool and dilute to 100 ml with *water*. To 10 ml add 5 ml of *water*. The resulting solution complies with the *limit test for chlorides*, Appendix VII (0.3%).

Sulphate Dissolve 0.14 g in 15 ml of 1M *hydrochloric acid* and dilute to 100 ml with *water*. 15 ml of the resulting solution complies with the *limit test for sulphates*, Appendix VII (0.7%).

Loss on ignition When ignited at 800°, loses 40.0 to 50.0% of its weight. Use 1 g.

Assay

For Al_2O_3 Dissolve 0.3 g in 2 ml of 7M *hydrochloric acid*, add 250 ml of *water* and 50 ml of 0.05M *disodium edetate VS* and neutralise with 1M *sodium hydroxide* using *methyl red solution* as indicator. Heat the solution on a water bath for 30 minutes and allow to cool. Add 3 g of *hexamine* and titrate the excess of disodium edetate with 0.05M *lead nitrate VS* using *xylenol orange solution* as indicator. Each ml of 0.05M *disodium edetate VS* is equivalent to 2.549 mg of Al_2O_3.

For MgO Dissolve 0.125 g in the minimum volume of 7M *hydrochloric acid*, add 30 ml of *water*, 1 g of *ammonium chloride*, 10 ml of *triethanolamine*, 150 ml of *water* and 5 ml of *ammonia buffer pH 10.9* and titrate immediately with 0.05M *disodium edetate VS* using *mordant black 11 solution* as indicator. Each ml of 0.05M *disodium edetate VS* is equivalent to 2.015 mg of MgO.

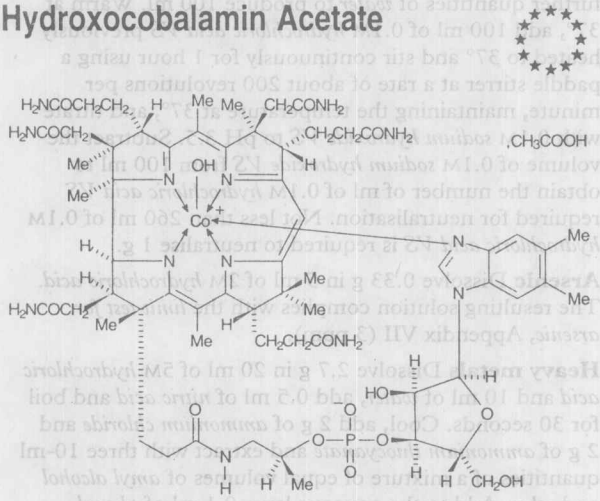
Action and use Antacid.

Preparation

Hydrotalcite Tablets

Correspondence between Ph Eur general methods and Appendices of the British Pharmacopoeia is shown on page A8

Hydroxocobalamin Acetate



$C_{62}H_{89}CoN_{13}O_{15}P,C_2H_4O_2$ 1406 *22465-48-1*

Hydroxocobalamin Acetate complies with the requirements of the 3rd edition of the European Pharmacopoeia [0913]. These requirements are reproduced after the heading 'Definition' below.

Action and use Vitamin B_{12} analogue used in treatment of vitamin B_{12} deficiency.

Preparation
Hydroxocobalamin Injection

Ph Eur

DEFINITION

Hydroxocobalamin acetate contains not less than 96.0 per cent and not more than the equivalent of 102.0 per cent of Coα-[α-(5,6-dimethylbenzimidazolyl)]-Coβ-hydroxocobamide acetate, calculated with reference to the dried substance.

CHARACTERS

A dark red, crystalline powder or dark red crystals, soluble in water, very hygroscopic. Some decomposition may occur on drying.

IDENTIFICATION

A. Dissolve 2.5 mg in a solution containing 0.8 per cent *V/V* of *glacial acetic acid R* and 10.9 g/l of *sodium acetate R* and dilute to 100 ml with the same solution. Examined between 260 nm and 610 nm (*2.2.25*), the solution shows three absorption maxima, at 274 nm, 351 nm and 525 nm. The ratio of the absorbance at the maximum at 274 nm to that at the maximum at 351 nm is 0.75 to 0.83. The ratio of the absorbance at the maximum at 525 nm to that at the maximum at 351 nm is 0.31 to 0.35.

B. Examine by thin-layer chromatography (*2.2.27*), using *silica gel G R* as the coating substance. *Carry out the test protected from light.*

Test solution. Dissolve 2 mg of the substance to be examined in 1 ml of a mixture of equal volumes of *alcohol R* and *water R*.

Reference solution. Dissolve 2 mg of *hydroxocobalamin CRS* in 1 ml of a mixture of equal volumes of *alcohol R* and *water R*.

Apply separately to the plate 10 µl of each solution. Develop in an unlined tank over a path of 12 cm using a mixture of 25 volumes of *dilute ammonia R1* and 75 volumes of *methanol R*. Allow the plate to dry in air. Examine in daylight. 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. It gives reaction (a) of acetates (*2.3.1*).

TESTS

Related substances Examine by liquid chromatography (*2.2.29*). *Use freshly prepared solutions and protect them from bright light.*

Test solution. Dissolve 10.0 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 5.0 ml of the test solution to 100.0 ml with the mobile phase.

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

Reference solution (c). Dissolve 25 mg of the substance to be examined in 10 ml of *water R*, warming if necessary. Allow to cool and add 1 ml of a 20 g/l solution of *chloramine R* and 0.5 ml of *0.05M hydrochloric acid*. Dilute to 25 ml with *water R*. Shake and allow to stand for 5 min. Inject immediately.

The chromatographic procedure may be carried out using:
— a stainless steel column 0.25 m long and 4 mm in internal diameter packed with *octylsilyl silica gel for chromatography R* (5 µm),
— as mobile phase at a flow rate of 1.5 ml per minute a mixture prepared as follows: mix 19.5 volumes of *methanol R* and 80.5 volumes of a solution containing 15 g/l of *citric acid R* and 8.1 g/l of *disodium hydrogen phosphate R*,
— as detector a spectrophotometer set at 351 nm,
— a loop injector,

Inject separately 20 µl of each solution and continue the chromatography for four times the retention time of the principal peak in the chromatogram obtained with reference solution (a). In the chromatogram obtained with the test solution, the sum of the areas of any peaks apart from the principal peak is not greater than the area of the principal peak in the chromatogram obtained with reference solution (a) (5 per cent). Disregard any peak whose area is less than that of the principal peak in the chromatogram obtained with reference solution (b). The test is not valid unless: the chromatogram obtained with reference solution (c) shows three principal peaks and the resolution between each pair of adjacent peaks is at least 3.0; the chromatogram obtained with reference solution (b) shows one principal peak with a signal-to-noise ratio of at least 5.

Loss on drying (*2.2.32*). 8.0 per cent to 12.0 per cent, determined on 0.400 g by drying at 100°C to 105°C at a pressure not exceeding 0.7 kPa.

ASSAY

Protect the solutions from light throughout the assay. Dissolve 25.0 mg in a solution containing 0.8 per cent *V/V* of *glacial acetic acid R* and 10.9 g/l of *sodium acetate R* and dilute to 1000.0 ml with the same solvent. Measure the absorbance of the resulting solution at the maximum at 351 nm (*2.2.25*).

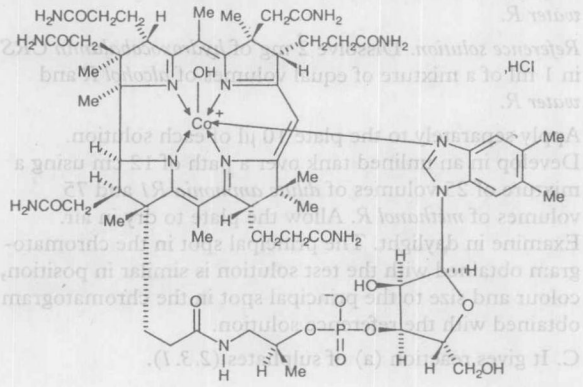
Calculate the content of $C_{64}H_{93}CoN_{13}O_{17}P$ taking the specific absorbance to be 187.

STORAGE

Store in an airtight container, protected from light, at a temperature of 2°C to 8°C.

Ph Eur

Hydroxocobalamin Chloride



$C_{62}H_{89}CoN_{13}O_{15}P,HCl$ 1383 *58288-50-9*

Hydroxocobalamin Chloride complies with the requirements of the 3rd edition of the European Pharmacopoeia [0914]. These requirements are reproduced after the heading 'Definition' below.

Action and use Vitamin B_{12} analogue used in treatment of vitamin B_{12} deficiency.

Preparation
Hydroxocobalamin Injection

Ph Eur

DEFINITION

Hydroxocobalamin chloride contains not less than 96.0 per cent and not more than the equivalent of 102.0 per cent of Coα-[α-(5,6-dimethylbenzimidazolyl)]-Coβ-hydroxocobamide chloride, calculated with reference to the dried substance.

CHARACTERS

A dark red, crystalline powder or dark red crystals, soluble in water, very hygroscopic. Some decomposition may occur on drying.

IDENTIFICATION

A. Dissolve 2.5 mg in a solution containing 0.8 per cent *V/V* of *glacial acetic acid R* and 10.9 g/l of *sodium acetate R* and dilute to 100 ml with the same solution. Examined between 260 nm and 610 nm (*2.2.25*), the solution shows three absorption maxima, at 274 nm, 351 nm and 525 nm. The ratio of the absorbance at the maximum at 274 nm to that at the maximum at 351 nm is 0.75 to 0.83. The ratio of the absorbance at the maximum at 525 nm to that at the maximum at 351 nm is 0.31 to 0.35.

B. Examine by thin-layer chromatography (*2.2.27*), using *silica gel G R* as the coating substance. *Carry out the test protected from light.*

Test solution. Dissolve 2 mg of the substance to be examined in 1 ml of a mixture of equal volumes of *alcohol R* and *water R*.

Reference solution. Dissolve 2 mg of *hydroxocobalamin CRS* in 1 ml of a mixture of equal volumes of *alcohol R* and *water R*.

Apply separately to the plate 10 µl of each solution. Develop in an unlined tank over a path of 12 cm using a mixture of 25 volumes of *dilute ammonia R1* and 75 volumes of *methanol R*. Allow the plate to dry in air. Examine in daylight. 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. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Related substances Examine by liquid chromatography (*2.2.29*). *Use freshly prepared solutions and protect them from bright light.*

Test solution. Dissolve 10.0 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 5.0 ml of the test solution to 100.0 ml with the mobile phase.

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

Reference solution (c). Dissolve 25 mg of the substance to be examined in 10 ml of *water R*, warming if necessary. Allow to cool and add 1 ml of a 20 g/l solution of *chloramine R* and 0.5 ml of *0.05M hydrochloric acid*. Dilute to 25 ml with *water R*. Shake and allow to stand for 5 min. Inject immediately.

The chromatographic procedure may be carried out using:
— a stainless steel column 0.25 m long and 4 mm in internal diameter packed with *octylsilyl silica gel for chromatography R* (5 µm),
— as mobile phase at a flow rate of 1.5 ml per minute a mixture prepared as follows: mix 19.5 volumes of *methanol R* and 80.5 volumes of a solution containing 15 g/l of *citric acid R* and 8.1 g/l of *disodium hydrogen phosphate R*,
— as detector a spectrophotometer set at 351 nm,
— a loop injector.

Inject separately 20 µl of each solution and continue the chromatography for four times the retention time of the principal peak in the chromatogram obtained with reference solution (a). In the chromatogram obtained with the test solution, the sum of the areas of any peaks apart from the principal peak is not greater than the area of the principal peak in the chromatogram obtained with reference solution (a) (5 per cent). Disregard any peak whose area is less than that of the principal peak in the chromatogram obtained with reference solution (b). The test is not valid unless: the chromatogram obtained with reference solution (c) shows three principal peaks and the resolution between each pair of adjacent peaks is at least 3.0; the chromatogram obtained with reference solution (b) shows one principal peak with a signal- to-noise ratio of at least 5.

Loss on drying (*2.2.32*). 8.0 per cent to 12.0 per cent, determined on 0.400 g by drying at 100°C to 105°C at a pressure not exceeding 0.7 kPa.

ASSAY

Protect the solutions from light throughout the assay. Dissolve 25.0 mg in a solution containing 0.8 per cent *V/V* of *glacial acetic acid R* and 10.9 g/l of *sodium acetate R* and dilute to 1000.0 ml with the same solvent. Measure the absorbance of the resulting solution at the maximum at 351 nm (*2.2.25*).

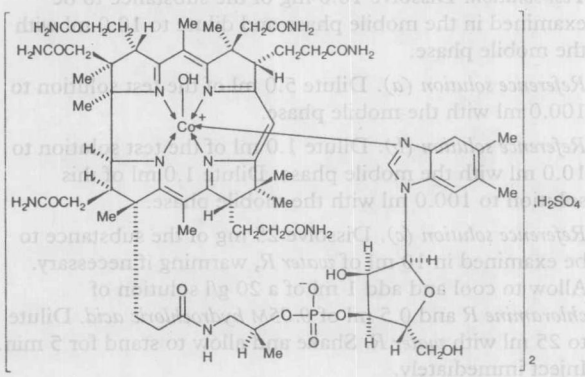
Calculate the content of $C_{62}H_{90}ClCoN_{13}O_{15}P$ taking the specific absorbance to be 190.

STORAGE

Store in an airtight container protected from light, at a temperature of 2°C to 8°C.

Ph Eur

Hydroxocobalamin Sulphate



$C_{124}H_{178}Co_2N_{26}O_{30}P_2,H_2SO_4$ 2791

Hydroxocobalamin Sulphate complies with the requirements of the 3rd edition of the European Pharmacopoeia [0915]. These requirements are reproduced after the heading 'Definition' below.

Action and use Vitamin B_{12} analogue used in treatment of vitamin B_{12} deficiency.

Preparation

Hydroxocobalamin Injection

Ph Eur

DEFINITION

Hydroxocobalamin sulphate contains not less than 96.0 per cent and not more than the equivalent of 102.0 per cent of di-(Coα-[α-(5,6-dimethylbenzimidazolyl)]-Coβ-hydroxocobamide) sulphate, calculated with reference to the dried substance.

CHARACTERS

A dark red, crystalline powder or dark red crystals, soluble in water, very hygroscopic. Some decomposition may occur on drying.

IDENTIFICATION

A. Dissolve 2.5 mg in a solution containing 0.8 per cent *V/V* of *glacial acetic acid R* and 10.9 g/l of *sodium acetate R* and dilute to 100 ml with the same solution. Examined between 260 nm and 610 nm (*2.2.25*), the solution shows three absorption maxima, at 274 nm, 351 nm and 525 nm. The ratio of the absorbance at the maximum at 274 nm to that at the maximum at 351 nm is 0.75 to 0.83. The ratio of the absorbance at the maximum at 525 nm to that at the maximum at 351 nm is 0.31 to 0.35.

B. Examine by thin-layer chromatography (*2.2.27*), using *silica gel G R* as the coating substance. *Carry out the test protected from light.*

Test solution. Dissolve 2 mg of the substance to be examined in 1 ml of a mixture of equal volumes of *alcohol R* and *water R*.

Reference solution. Dissolve 2 mg of *hydroxocobalamin CRS* in 1 ml of a mixture of equal volumes of *alcohol R* and *water R*.

Apply separately to the plate 10 µl of each solution. Develop in an unlined tank over a path of 12 cm using a mixture of 25 volumes of *dilute ammonia R1* and 75 volumes of *methanol R*. Allow the plate to dry in air. Examine in daylight. 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. It gives reaction (a) of sulphates (*2.3.1*).

TESTS

Related substances Examine by liquid chromatography (*2.2.29*). *Use freshly prepared solutions and protect them from bright light.*

Test solution. Dissolve 10.0 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 5.0 ml of the test solution to 100.0 ml with the mobile phase.

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

Reference solution (c). Dissolve 25 mg of the substance to be examined in 10 ml of *water R*, warming if necessary. Allow to cool and add 1 ml of a 20 g/l solution of *chloramine R* and 0.5 ml of *0.05M hydrochloric acid.* Dilute to 25 ml with *water R*. Shake and allow to stand for 5 min. Inject immediately.

The chromatographic procedure may be carried out using:
- a stainless steel column 0.25 m long and 4 mm in internal diameter packed with *octylsilyl silica gel for chromatography R* (5 µm),
- as mobile phase at a flow rate of 1.5 ml per minute a mixture prepared as follows: mix 19.5 volumes of *methanol R* and 80.5 volumes of a solution containing 15 g/l of *citric acid R* and 8.1 g/l of *disodium hydrogen phosphate R*,
- as detector a spectrophotometer set at 351 nm,
- a loop injector.

Inject separately 20 µl of each solution and continue the chromatography for four times the retention time of the principal peak in the chromatogram obtained with reference solution (a). In the chromatogram obtained with the test solution, the sum of the areas of any peaks apart from the principal peak is not greater than the area of the principal peak in the chromatogram obtained with refer-

ence solution (a) (5 per cent). Disregard any peak whose area is less than that of the principal peak in the chromatogram obtained with reference solution (b). The test is not valid unless: the chromatogram obtained with reference solution (c) shows three principal peaks and the resolution between each pair of adjacent peaks is at least 3.0; the chromatogram obtained with reference solution (b) shows one principal peak with a signal- to-noise ratio of at least 5.

Loss on drying (*2.2.32*). 8.0 per cent to 16.0 per cent, determined on 0.400 g by drying at 100°C to 105°C at a pressure not exceeding 0.7 kPa.

ASSAY

Protect the solutions from light throughout the assay. Dissolve 25.0 mg in a solution containing 0.8 per cent *V/V* of *glacial acetic acid R* and 10.9 g/l of *sodium acetate R* and dilute to 1000.0 ml with the same solvent. Measure the absorbance of the resulting solution at the maximum at 351 nm (*2.2.25*).

Calculate the content of $C_{124}H_{180}Co_2N_{26}O_{34}P_2S$ taking the specific absorbance to be 188.

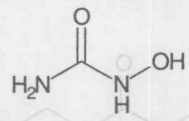
STORAGE

Store in an airtight container protected from light, at a temperature of 2°C to 8°C.

Ph Eur

Hydroxycarbamide
Hydroxyurea

For the purposes of product labelling in the United Kingdom, the pair of names given above shall be used together when the substance is stated as an active ingredient in a formulated preparation (see Supplementary Chapter II A, Changes in title).



$CH_4N_2O_2$ 76.05 *127-07-1*

Definition Hydroxycarbamide contains not less than 98.0% and not more than 100.5% of $CH_4N_2O_2$, calculated with reference to the dried substance.

Characteristics A white to off-white crystalline powder; odourless or almost odourless; hygroscopic.

Freely soluble in *water* and in hot *ethanol (96%)*.

Identification

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of hydroxycarbamide *(RS 182)*.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as that of the principal peak in the chromatogram obtained with solution (2).

Heavy metals Moisten 2.0 g in a silica crucible with *sulphuric acid*, ignite gently and carry out *limit test C for heavy metals*, Appendix VII, beginning at the words 'Allow to cool ...'. Use 3 ml of *lead standard solution (10 ppm Pb)* to prepare the standard (30 ppm).

Urea Carry out the method for *thin-layer chromatography*, Appendix III A, using a cellulose F precoated plate (Merck cellulose F plates are suitable), a mixture of 1 volume of *glacial acetic acid*, 1 volume of *water* and 4 volumes of *butan-1-ol* as the mobile phase and developing the chromatograms for 18 hours. Apply separately to the plate 50 µl of each of the following solutions in *water*, as several applications, containing (1) 2.0% w/v of the substance being examined and (2) 0.01% w/v of *urea*. After removal of the plate, dry it in a current of cold air, spray with a 1% w/v solution of *4-dimethylaminobenzaldehyde* in *ethanol (96%)* containing 2% v/v of *hydrochloric acid* and heat at 90° for 5 minutes. In the chromatogram obtained with solution (1), any spot corresponding to urea is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

Loss on drying When dried to constant weight at 60° at a pressure not exceeding 0.7 kPa, loses not more than 1.0% of its weight. Use 1 g.

Sulphated ash Not more than 0.2%, Appendix IX A.

Assay Carry out the method for *liquid chromatography*, Appendix III D, using three solutions in *water* containing (1) 0.10% w/v of the substance being examined, (2) 0.10% w/v of *hydroxycarbamide BPCRS* and (3) 0.10% w/v of *hydroxycarbamide BPCRS* and 0.4% w/v of *hydroxylamine hydrochloride*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *stationary phase C* (5 µm) (Spherisorb ODS 2 is suitable), (b) *water* as the mobile phase with a flow rate of 0.5 ml per minute and (c) a detection wavelength of 214 nm.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to hydroxycarbamide and hydroxylamine hydrochloride is at least 1.0.

Calculate the content of $CH_4N_2O_2$ using the declared content of $CH_4N_2O_2$ in *hydroxycarbamide BPCRS*.

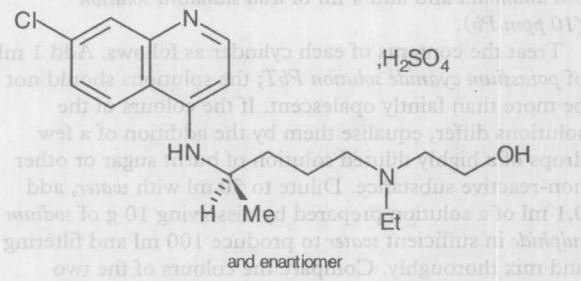
Storage Hydroxycarbamide should be kept in a well-closed container and protected from moisture.

Action and use Cytotoxic.

Preparation

Hydroxycarbamide Capsules/Hydroxyurea Capsules

Hydroxychloroquine Sulphate



and enantiomer

$C_{18}H_{26}ClN_3O,H_2SO_4$ 434.0 *747-36-4*

Definition Hydroxychloroquine Sulphate is (*RS*)-2-{*N*-[4-(7-chloro-4-quinolylamino)pentyl]-*N*-ethylamino}-ethanol sulphate. It contains not less than 98.0% and not

more than 100.5% of $C_{18}H_{26}ClN_3O,H_2SO_4$, calculated with reference to the dried substance.

Characteristics A white or almost white, crystalline powder; odourless or almost odourless.

Freely soluble in *water*; practically insoluble in *chloroform*, in *ethanol (96%)* and in *ether*.

Identification

A. Dissolve 0.1 g in 10 ml of *water*, add 2 ml of 2M *sodium hydroxide* and extract with two 20-ml quantities of *chloroform*. Wash the chloroform extracts with *water*, dry with *anhydrous sodium sulphate*, evaporate to dryness and dissolve the residue in 2 ml of *chloroform*. The *infrared absorption spectrum* of the resulting solution, Appendix II A, is concordant with the *reference spectrum* of hydroxychloroquine *(RS 183)*.

B. Yields the reactions characteristic of *sulphates*, Appendix VI.

Acidity pH of a 1% w/v solution, 3.5 to 5.5, Appendix V L.

Clarity and colour of solution A 10.0% w/v solution is not more than slightly turbid and not more than slightly yellow.

Lead Not more than 20 ppm when determined by the following method. Carefully heat 2.0 g for 10 minutes with 8 ml of *water* and 6 ml of *nitric acid* in a Kjeldahl flask. Cool, add 4 ml of *sulphuric acid* and heat until the mixture darkens. Continue heating, with the dropwise addition of *nitric acid*, until the liquid becomes colourless and white fumes of sulphur trioxide are produced. Add 3 ml of *water*, carefully evaporate until white fumes are again produced, cool and dilute to 18 ml with *water*. Add and dissolve 2 g of *citric acid*, make alkaline with 5M *ammonia* and add 1 ml of *potassium cyanide solution PbT*. Transfer to a separating funnel, add 10 ml of *dithizone solution*, shake vigorously and remove the lower layer. Repeat the extraction with two 5-ml quantities of *dithizone solution*. If, after the third extraction, the chloroform layer is bright red, continue the extraction with further 5-ml quantities of *dithizone solution* until the colour of the reagent no longer changes to bright red. Wash the combined chloroform solutions by shaking with 10 ml of *water* and then extract with two 10-ml quantities of 2M *hydrochloric acid*. Wash the combined acid solutions with 10 ml of *chloroform* and discard the chloroform. Transfer the solution to a *Nessler cylinder* and make alkaline with 5M *ammonia*. In a second *Nessler cylinder* mix 2 ml of 6M *acetic acid* with 20 ml of 2M *hydrochloric acid*, make alkaline with 5M *ammonia* and add 4 ml of *lead standard solution (10 ppm Pb)*.

Treat the contents of each cylinder as follows. Add 1 ml of *potassium cyanide solution PbT*; the solutions should not be more than faintly opalescent. If the colours of the solutions differ, equalise them by the addition of a few drops of a highly diluted solution of burnt sugar or other non-reactive substance. Dilute to 50 ml with *water*, add 0.1 ml of a solution prepared by dissolving 10 g of *sodium sulphide* in sufficient *water* to produce 100 ml and filtering and mix thoroughly. Compare the colours of the two solutions by a suitable method, such as by light reflected from a white tile through the Nessler cylinders. The colour of the solution in the first cylinder is not more intense than that of the solution in the second cylinder.

Chloride Dissolve 0.50 g in 50 ml of *water*. 15 ml of the resulting solution complies with the *limit test for chlorides*, Appendix VII (350 ppm).

Related substances Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 72 volumes of *methanol*, 25 volumes of *water* and 3 volumes of 13.5M *ammonia* as the mobile phase. Apply separately to the plate 2 µl of each of three solutions of the substance being examined in *water* containing (1) 5.0% w/v, (2) 0.050% w/v and (3) 0.025% w/v. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3).

Loss on drying When dried to constant weight at 105°, loses not more than 2.0% of its weight. Use 1 g.

Sulphated ash Not more than 0.2%, Appendix IX A.

Assay Dissolve 0.5 g in 10 ml of *water*, add 20 ml of 1M *sodium hydroxide* and extract with four 25-ml quantities of *chloroform*. Combine the chloroform extracts and evaporate to a volume of about 10 ml. Add 40 ml of *anhydrous acetic acid* and carry out Method I for *non-aqueous titration*, Appendix VIII A, using *oracet blue B solution* as indicator. Each ml of 0.1M *perchloric acid VS* is equivalent to 21.70 mg of $C_{18}H_{26}ClN_3O,H_2SO_4$.

Storage Hydroxychloroquine Sulphate should be protected from light.

Action and use Antimalarial.

Preparation

Hydroxychloroquine Tablets

Hydroxyethyl Salicylate

$C_9H_{10}O_4$ 182.2 *87-28-5*

Hydroxyethyl Salicylate complies with the requirements of the 3rd edition of the European Pharmacopoeia [1225]. These requirements are reproduced after the heading 'Definition' below.

Ph Eur

DEFINITION

Hydroxyethyl salicylate contains not less than 98.0 per cent and not more than the equivalent of 102.0 per cent of 2-hydroxyethyl 2-hydroxybenzoate.

CHARACTERS

An oily, colourless or almost colourless liquid, sparingly soluble in water, very soluble in acetone, in ether and in methylene chloride, freely soluble in alcohol.

Correspondence between Ph Eur general methods and Appendices of the British Pharmacopoeia is shown on page A8