TESTS

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

Dissolve 0.1 g in a 6 g/l solution of *potassium hydroxide R* and dilute to 20 ml with the same solution.

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 50 mg of the substance to be examined in the mobile phase and dilute to 25.0 ml with the mobile phase.

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

Reference solution (b). Dissolve 2 mg of *bumetanide impurity A CRS* and 2 mg of *bumetanide impurity B CRS* in the mobile phase and dilute to 10.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase.

Column:

– *size*: *l* = 0.15 m, Ø = 4.6 mm,
– *stationary phase*: *end-capped octylsilyl silica gel for chromatography R* (3.5 µm).

Mobile phase: mix 70 volumes of *methanol R*, 25 volumes of *water for chromatography R* and 5 volumes of a 27.2 g/l solution of *potassium dihydrogen phosphate R* previously adjusted to pH 7.0 with a 280 g/l solution of *potassium hydroxide R*; add *tetrahexylammonium bromide R* to this mixture to obtain a concentration of 2.17 g/l.

Flow rate: 1.0 ml/min.

Detection: spectrophotometer at 254 nm.

Injection: 10 µl.

Run time: 5 times the retention time of bumetanide.

Relative retention with reference to bumetanide (retention time = about 6 min): impurity B = about 0.4; impurity A = about 0.6; impurity D = about 2.5; impurity C = about 4.4.

System suitability: reference solution (b):

– *resolution*: minimum 2.0 between the peaks due to impurity A and impurity B.

Limits:

– *impurities A, B, C, D*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent),
– *other impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent),
– *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent),
– *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*): maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

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

ASSAY

Dissolve 0.300 g in 50 ml of *alcohol R*. Add 0.1 ml of *phenol red solution R*. Titrate with *0.1 M sodium hydroxide* until a violet-red colour is obtained. Carry out a blank titration.

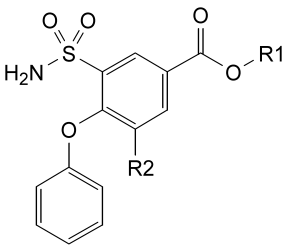
1 ml of *0.1 M sodium hydroxide* is equivalent to 36.44 mg of $C_{17}H_{20}N_2O_5S$.

STORAGE

Protected from light.

IMPURITIES

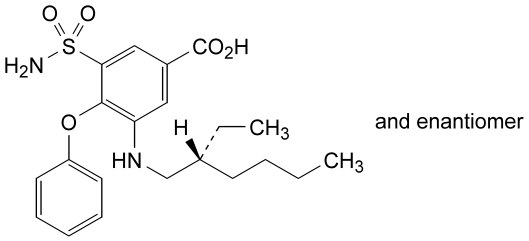
Specified impurities: A, B, C, D.



A. R1 = H, R2 = NO_2: 3-nitro-4-phenoxy-5-sulphamoylbenzoic acid,

B. R1 = H, R2 = NH_2: 3-amino-4-phenoxy-5-sulphamoylbenzoic acid,

C. R1 = C_4H_9, R2 = NH-C_4H_9: butyl 3-(butylamino)-4-phenoxy-5-sulphamoylbenzoate,

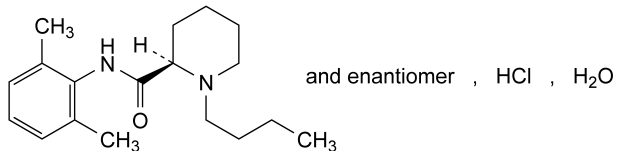


D. 3-[[(2*RS*)-2-ethylhexyl]amino]-4-phenoxy-5-sulphamoylbenzoic acid.

01/2008:0541
corrected 6.0

BUPIVACAINE HYDROCHLORIDE

Bupivacaini hydrochloridum



$C_{18}H_{29}ClN_2O,H_2O$ M_r 342.9
[14252-80-3]

DEFINITION

(2*RS*)-1-Butyl-*N*-(2,6-dimethylphenyl)piperidine-2-carboxamide hydrochloride monohydrate.

Content: 98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

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

Solubility: soluble in water, freely soluble in alcohol.

mp: about 254 °C, with decomposition.

IDENTIFICATION

First identification: A, D.

Second identification: B, C, D.

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation: discs of *potassium bromide R*.

Comparison: *bupivacaine hydrochloride CRS*.

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

Test solution. Dissolve 25 mg of the substance to be examined in *methanol R* and dilute to 5 ml with the same solvent.

Reference solution. Dissolve 25 mg of *bupivacaine hydrochloride CRS* in *methanol R* and dilute to 5 ml with the same solvent.

Plate: *TLC silica gel G plate R.*

Mobile phase: *concentrated ammonia R, methanol R* (0.1:100 *V/V*).

Application: 5 µl.

Development: over a path of 10 cm.

Drying: in air.

Detection: spray with *dilute potassium iodobismuthate solution R.*

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

C. Dissolve 0.1 g in 10 ml of *water R*, add 2 ml of *dilute sodium hydroxide solution R* and shake with 2 quantities, each of 15 ml, of *ether R*. Dry the combined ether layers over *anhydrous sodium sulphate R* and filter. Evaporate the ether, recrystallise the residue from *alcohol (90 per cent V/V) R* and dry under reduced pressure. The crystals melt (*2.2.14*) at 105 °C to 108 °C.

D. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S. Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

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

Acidity or alkalinity. To 10 ml of solution S add 0.2 ml of *0.01 M sodium hydroxide*; the pH (*2.2.3*) is not less than 4.7. Add 0.4 ml of *0.01 M hydrochloric acid*; the pH is not greater than 4.7.

Related substances. Gas chromatography (*2.2.28*).

Internal standard solution. Dissolve 25 mg of *methyl behenate R* in *methylene chloride R* and dilute to 500 ml with the same solvent.

Test solution. Dissolve 50.0 mg of the substance to be examined in 2.5 ml of *water R*, add 2.5 ml of *dilute sodium hydroxide solution R* and extract with 2 quantities, each of 5 ml, of the internal standard solution. Filter the lower layer.

Reference solution (a). Dissolve 10 mg of the substance to be examined, 10 mg of *bupivacaine impurity B CRS* and 10 mg of *bupivacaine impurity E CRS* in 2.5 ml of *water R*, add 2.5 ml of *dilute sodium hydroxide solution R* and extract with 2 quantities, each of 5 ml, of the internal standard solution. Filter the lower layer and dilute to 20 ml with the internal standard solution.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with the internal standard solution.

Reference solution (c). Dilute 5.0 ml of reference solution (b) to 10.0 ml with the internal standard solution.

Reference solution (d). Dilute 1.0 ml of reference solution (b) to 10.0 ml with the internal standard solution.

Column:

- *material*: fused silica,
- *size*: *l* = 30 m, Ø = 0.32 mm,
- *stationary phase*: *poly(dimethyl)(diphenyl)siloxane R* (film thickness 0.25 µm).

Carrier gas: *helium for chromatography R.*

Flow rate: 2.5 ml/min.

Split ratio: 1:12.

Temperature:

	Time (min)	Temperature (°C)
	0	180
Column	0 - 10	180 → 230
	10 - 15	230
Injection port		250
Detector		250

Detection: flame ionisation.

Injection: 1 µl.

Relative retention with reference to bupivacaine (retention time = about 10 min): impurity C = about 0.5; impurity A = about 0.6; impurity B = about 0.7; impurity D = about 0.8; impurity E = about 1.1; internal standard = about 1.4.

System suitability: reference solution (a):

- *resolution*: minimum 3.0 between the peaks due to bupivacaine and impurity E.

Limits:

- *impurity B*: calculate the ratio (*R*) of the area of the principal peak to the area of the peak due to the internal standard from the chromatogram obtained with reference solution (c); from the chromatogram obtained with the test solution, calculate the ratio of the area of the peak due to impurity B to the area of the peak due to the internal standard: this ratio is not greater than *R* (0.5 per cent),
- *any other impurity*: calculate the ratio (*R*) of the area of the principal peak to the area of the peak due to the internal standard from the chromatogram obtained with reference solution (d); from the chromatogram obtained with the test solution, calculate the ratio of the area of any peak, apart from the principal peak, the peak due to impurity B and the peak due to the internal standard, to the area of the peak due to the internal standard: this ratio is not greater than *R* (0.1 per cent),
- *total*: calculate the ratio (*R*) of the area of the principal peak to the area of the peak due to the internal standard from the chromatogram obtained with reference solution (b); from the chromatogram obtained with the test solution, calculate the ratio of the sum of the areas of any peaks, apart from the principal peak and the peak due to the internal standard, to the area of the peak due to the internal standard: this ratio is not greater than *R* (1.0 per cent),
- *disregard limit*: ratio less than 0.01 times *R* (0.01 per cent).

2,6-Dimethylaniline: maximum 100 ppm.

Dissolve 0.50 g in *methanol R* and dilute to 10 ml with the same solvent. To 2 ml of the solution add 1 ml of a freshly prepared 10 g/l solution of *dimethylaminobenzaldehyde R* in *methanol R* and 2 ml of *glacial acetic acid R* and allow to stand for 10 min. Any yellow colour in the solution is not more intense than that in a standard prepared at the same time and in the same manner using 2 ml of a 5 mg/l solution of *2,6-dimethylaniline R* in *methanol R.*

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

Dissolve 2.0 g in a mixture of 15 volumes of *water R* and 85 volumes of *methanol R* and dilute to 20 ml with the same mixture of solvents. 12 ml of the solution complies with limit test B. Prepare the standard using lead standard solution

(1 ppm Pb) obtained by diluting *lead standard solution (100 ppm Pb) R* with a mixture of 15 volumes of *water R* and 85 volumes of *methanol R*.

Loss on drying (*2.2.32*): 4.5 per cent to 6.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

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

ASSAY

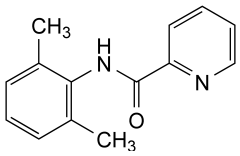
Dissolve 0.250 g in a mixture of 20 ml of *water R* and 25 ml of *alcohol R*. Add 5.0 ml of *0.01 M hydrochloric acid*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M ethanolic sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M ethanolic sodium hydroxide* is equivalent to 32.49 mg of $C_{18}H_{29}ClN_2O$.

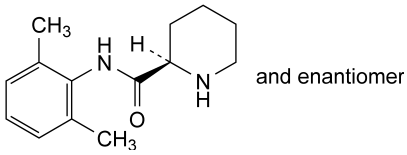
STORAGE

Protected from light.

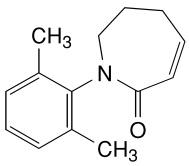
IMPURITIES



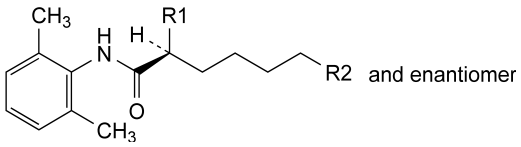
A. *N*-(2,6-dimethylphenyl)pyridine-2-carboxamide,



B. (2*RS*)-*N*-(2,6-dimethylphenyl)piperidine-2-carboxamide,

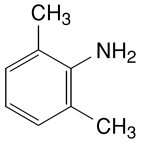


C. 1-(2,6-dimethylphenyl)-1,5,6,7-tetrahydro-2*H*-azepin-2-one,



D. R1 = R2 = Cl: (2*RS*)-2,6-dichloro-*N*-(2,6-dimethylphenyl)hexanamide,

E. R1 = H, R2 = NH-$(CH_2)_3$-CH_3: 6-(butylamino)-*N*-(2,6-dimethylphenyl)hexanamide,

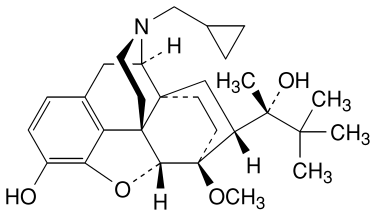


F. 2,6-dimethylaniline.

01/2008:1180
corrected 6.0

BUPRENORPHINE

Buprenorphinum



$C_{29}H_{41}NO_4$ M_r 467.6
[52485-79-7]

DEFINITION

Buprenorphine contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of (2*S*)-2-[17-(cyclopropylmethyl)-4,5α-epoxy-3-hydroxy-6-methoxy-6α,14-ethano-14α-morphinan-7α-yl]-3,3-dimethylbutan-2-ol, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, very slightly soluble in water, freely soluble in acetone, soluble in methanol, slightly soluble in cyclohexane. It dissolves in dilute solutions of acids.

It melts at about 217 °C.

IDENTIFICATION

Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the *Ph. Eur. reference spectrum of buprenorphine*.

TESTS

Solution S. Dissolve 0.250 g in *ethanol R* and dilute to 25.0 ml with the same solvent.

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

Specific optical rotation (*2.2.7*): – 103 to – 107, determined on and 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 the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a). Dissolve 5.0 mg of the substance to be examined in 2.0 ml of *methanol R*. Add 0.25 ml of *2 M hydrochloric acid R*.

Reference solution (b). Dilute 0.5 ml of the test solution to 200.0 ml with the mobile phase.

Reference solution (c). Dilute 0.65 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (d). Dilute 4.0 ml of reference solution (b) to 10.0 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),