A test for bovine viral diarrhoea virus antibodies is carried out; an acceptance criterion for the titre is established taking account of the risk assessment.

Composition. The content of a suitable selection of the following components is determined and shown to be within the expected range for the type of serum: cholesterol, α-, β- and γ-globulin, albumin, creatinine, bilirubin, glucose, serum aspartate transaminase (SAST, formerly SGOT - serum glutamic-oxaloacetic transaminase), serum alanine transaminase (SALT, formerly SGPT - glutamic-pyruvic transaminase), phosphorus, potassium, calcium, sodium and pH.

Tests carried out on the batch post-treatment

If bovine viral diarrhoea virus was detected before virus inactivation/removal, the following test for bovine viral diarrhoea virus is carried out after virus inactivation/removal.

Test for bovine viral diarrhoea virus. A validated test for bovine viral diarrhoea virus is carried out, for example by inoculation into susceptible cell cultures, followed by not fewer than 3 subcultures and detection by immunostaining. No evidence of the presence of bovine viral diarrhoea virus is found.

IDENTIFICATION

A. The electrophoretic pattern corresponds to that for serum and is consistent with the type (foetal or other) of bovine serum.

B. Bovine origin is confirmed by a suitable immunochemical method (*2.7.1*).

TESTS

Osmolality (*2.2.35*): 280 mosmol/kg to 365 mosmol/kg for foetal bovine serum and 240 mosmol/kg to 340 mosmol/kg for other types.

Total protein (*2.5.33*): 30 mg/mL to 45 mg/mL for foetal bovine serum and minimum 35 mg/mL for other types.

Haemoglobin: maximum 4 mg/mL, determined by a validated method, such as spectrophotometry.

Bacterial endotoxins (*2.6.14*): less than 10 IU/mL for donor bovine serum, less than 25 IU/mL for foetal bovine serum, less than 100 IU/mL for other types.

Sterility (*2.6.1*). It complies with the test. Use 10 mL for each medium.

Mycoplasmas (*2.6.7*). It complies with the test.

STORAGE

Frozen at – 10 °C or below.

LABELLING

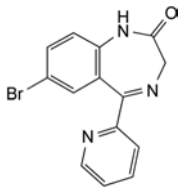
The label states:

– the type of serum;
– where applicable, that the serum has been inactivated and the inactivation method;
– where the serum has been inactivated by gamma irradiation, the target minimum dose of the irradiation procedure.

01/2008:0879

BROMAZEPAM

Bromazepamum



$C_{14}H_{10}BrN_3O$ M_r 316.2
[1812-30-2]

DEFINITION

7-Bromo-5-(pyridin-2-yl)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one.

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

CHARACTERS

Appearance: white or yellowish, crystalline powder.

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

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *bromazepam CRS*.

TESTS

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

Test solution. Dissolve 10.0 mg of the substance to be examined in 9 mL of a mixture of 1 volume of *acetonitrile R* and 8 volumes of *methanol R*. Dilute to 20.0 mL with an 11.33 g/L solution of *potassium dihydrogen phosphate R* previously adjusted to pH 7.0 with a 100 g/L solution of *potassium hydroxide R*.

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 5 mg of *bromazepam for system suitability CRS* (containing impurities A, B, C, D and E) in 5 mL of a mixture of 1 volume of *acetonitrile R* and 8 volumes of *methanol R*. Dilute to 10.0 mL with an 11.33 g/L solution of *potassium dihydrogen phosphate R* previously adjusted to pH 7.0 with a 100 g/L solution of *potassium hydroxide R*.

Column:

– *size*: *l* = 0.15 m, Ø = 4.6 mm;
– *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (3.5 μm);
– *temperature*: 50 °C.

Mobile phase: mix 5 volumes of *acetonitrile R*, 45 volumes of *methanol R* and 50 volumes of an 11.33 g/L solution of *potassium dihydrogen phosphate R* previously adjusted to pH 7.0 with a 100 g/L solution of *potassium hydroxide R*.

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 235 nm.

Injection: 20 μL.

Run time: 4 times the retention time of bromazepam.

Identification of impurities: use the chromatogram supplied with *bromazepam for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B, C, D and E.

Relative retention with reference to bromazepam (retention time = about 5 min): impurity D = about 1.4; impurity A = about 1.5; impurity C = about 1.6; impurity E = about 2.1; impurity B = about 2.2.

System suitability: reference solution (b):

– *resolution*: minimum 4.0 between the peaks due to bromazepam and impurity D and minimum 1.2 between the peaks due to impurities A and C.

Limits:

– *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 1.3; impurity B = 1.8; impurity E = 2.1;
– *impurities A, B, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
– *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

– *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 0.2 per cent, determined on 1.000 g by drying at 80 °C at a pressure not exceeding 2.7 kPa for 4 h.

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

ASSAY

Dissolve 0.250 g in 20 mL of *anhydrous acetic acid R*. Add 50 mL of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 31.62 mg of $C_{14}H_{10}BrN_3O$.

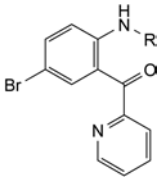
STORAGE

Protected from light.

IMPURITIES

Specified impurities: *A, B, E.*

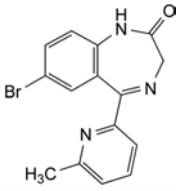
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical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



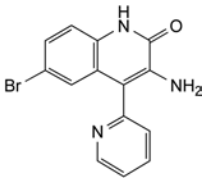
A. R = H: (2-amino-5-bromophenyl)(pyridin-2-yl)methanone,

B. R = CO-CH_2-Cl: *N*-[4-bromo-2-(pyridin-2-ylcarbonyl)phenyl]-2-chloroacetamide,

E. R = CO-CH_2-Br: 2-bromo-*N*-[4-bromo-2-(pyridin-2-ylcarbonyl)phenyl]acetamide,



C. 7-bromo-5-(6-methylpyridin-2-yl)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one,

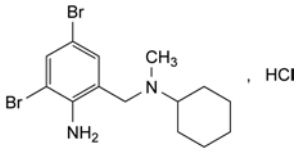


D. 3-amino-6-bromo-4-(pyridin-2-yl)quinolin-2(1*H*)-one.

01/2008:0706
corrected 6.0

BROMHEXINE HYDROCHLORIDE

Bromhexini hydrochloridum



$C_{14}H_{21}Br_2ClN_2$ M_r 412.6
[611-75-6]

DEFINITION

N-(2-Amino-3,5-dibromobenzyl)-*N*-methylcyclohexanamine hydrochloride.

Content: 98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: very slightly soluble in water, slightly soluble in alcohol and in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION

First identification: *A, E.*

Second identification: *B, C, D, E.*

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *bromhexine hydrochloride CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness and record new spectra using the residues.

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

Test solution. Dissolve 20 mg of the substance to be examined in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution. Dissolve 20 mg of *bromhexine hydrochloride CRS* in *methanol R* and dilute to 10 mL with the same solvent.

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

Mobile phase: *glacial acetic acid R, water R, butanol R* (17:17:66 *V/V/V*).

Application: 20 µL.

Development: over 3/4 of the plate.

Drying: in air.

Detection: examine in ultraviolet light at 254 nm.

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

C. Dissolve about 25 mg in a mixture of 1 mL of *dilute sulfuric acid R* and 50 mL of *water R*. Add 2 mL of *methylene chloride R* and 5 mL of *chloramine solution R* and shake. A brownish-yellow colour develops in the lower layer.

D. Dissolve about 1 mg in 3 mL of *0.1 M hydrochloric acid*. The solution gives the reaction of primary aromatic amines (*2.3.1*).

E. Dissolve about 20 mg in 1 mL of *methanol R* and add 1 mL of *water R*. The solution gives reaction (a) of chlorides (*2.3.1*).