Heavy metals (*2.4.8*): maximum 20 ppm.

2.0 g complies with test C. Prepare the reference solution using 4 mL of *lead standard solution (10 ppm Pb) R*.

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

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

ASSAY

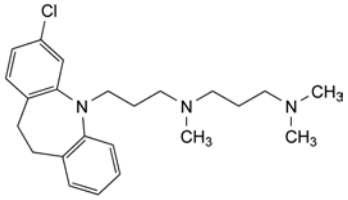
Dissolve 0.250 g in 50 mL of *alcohol R* and add 5.0 mL of *0.01 M hydrochloric acid*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 mL of *0.1 M sodium hydroxide* is equivalent to 35.13 mg of $C_{19}H_{24}Cl_2N_2$.

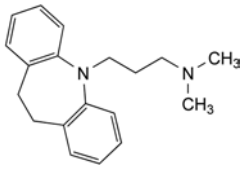
STORAGE

In an airtight container, protected from light.

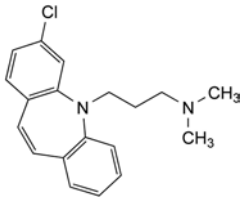
IMPURITIES



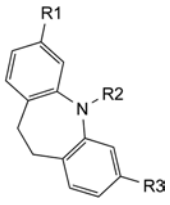
A. *N*-[3-(3-chloro-10,11-dihydro-5*H*-dibenzo[*b,f*]azepin-5-yl)propyl]-*N,N′,N′*-trimethylpropane-1,3-diamine,



B. 3-(10,11-dihydro-5*H*-dibenzo[*b,f*]azepin-5-yl)-*N,N*-dimethylpropan-1-amine (imipramine),



C. 3-(3-chloro-5*H*-dibenzo[*b,f*]azepin-5-yl)-*N,N*-dimethylpropan-1-amine,



D. R1 = R3 = Cl, R2 = CH_2-CH_2-CH_2-$N(CH_3)_2$: 3-(3,7-dichloro-10,11-dihydro-5*H*-dibenzo[*b,f*]azepin-5-yl)-*N,N*-dimethylpropan-1-amine,

E. R1 = R2 = R3 = H: 10,11-dihydro-5*H*-dibenzo[*b,f*]azepine (iminodibenzyl),

F. R1 = Cl, R2 = R3 = H: 3-chloro-10,11-dihydro-5*H*-dibenzo[*b,f*]azepine,

G. R1 = Cl, R2 = CH_2-CH=CH_2, R3 = H: 3-chloro-5-(prop-2-enyl)-10,11-dihydro-5*H*-dibenzo[*b,f*]azepine.

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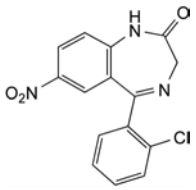
corrected 6.0

CLONAZEPAM

Clonazepamum



$C_{15}H_{10}ClN_3O_3$ M_r 315.7
[1622-61-3]

DEFINITION

5-(2-Chlorophenyl)-7-nitro-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one.

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

CHARACTERS

Appearance: slightly yellowish, crystalline powder.

Solubility: practically insoluble in water, slightly soluble in alcohol and in methanol.

mp: about 239 °C.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *Ph. Eur. reference spectrum of clonazepam*.

TESTS

Related substances. Liquid chromatography (*2.2.29*). *Carry out the test protected from light and prepare the solutions immediately before use.*

Solvent mixture: *tetrahydrofuran R, methanol R, water R* (10:42:48 *V/V/V*).

Test solution. Dissolve 0.100 g of the substance to be examined in *methanol R* and dilute to 20.0 mL with the same solvent. Dilute 1.0 mL to 10.0 mL with the solvent mixture.

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

Reference solution (b). Dissolve 5 mg of the substance to be examined and 5 mg of *flunitrazepam R* in the solvent mixture and dilute to 100.0 mL with the solvent mixture.

Reference solution (c). Dissolve 1.0 mg of *clonazepam impurity B CRS* in the solvent mixture and dilute to 20.0 mL with the solvent mixture. Dilute 1.0 mL of the solution to 100.0 mL with the solvent mixture.

Column:

– *size*: *l* = 0.15 m, Ø = 4.6 mm,
– *stationary phase*: *end-capped octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase: mix 10 volumes of *tetrahydrofuran R*, 42 volumes of *methanol R* and 48 volumes of a 6.6 g/L solution of *ammonium phosphate R* previously adjusted to pH 8.0 with a 40 g/L solution of *sodium hydroxide R* or *dilute phosphoric acid R*.

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 254 nm.

Injection: 10 µL.

Run time: 3 times the retention time of clonazepam.

Relative retention with reference to clonazepam (retention time = about 7 min): impurity B = about 2.1; impurity A = about 2.4.

System suitability: reference solution (b):

– *resolution*: minimum 1.8 between the peaks due to flunitrazepam and to clonazepam.

Limits:

– *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent),
– *impurity B*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent)
– *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent),
– *total*: not more than 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.

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

ASSAY

Dissolve 0.275 g in 50 mL of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

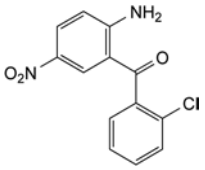
1 mL of *0.1 M perchloric acid* is equivalent to 31.57 mg of $C_{15}H_{10}ClN_3O_3$.

STORAGE

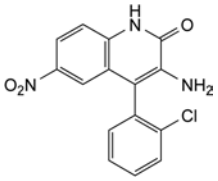
Protected from light.

IMPURITIES

Specified impurities: A, B.



A. (2-amino-5-nitrophenyl)(2-chlorophenyl)methanone,

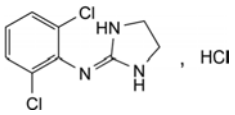


B. 3-amino-4-(2-chlorophenyl)-6-nitroquinolin-2(1*H*)-one.

01/2008:0477
corrected 6.3

CLONIDINE HYDROCHLORIDE

Clonidini hydrochloridum



$C_9H_{10}Cl_3N_3$ M_r 266.6
[4205-91-8]

DEFINITION

2,6-Dichloro-*N*-(imidazolidin-2-ylidene)aniline hydrochloride.

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

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: soluble in water and in anhydrous ethanol.

IDENTIFICATION

First identification: B, D.

Second identification: A, C, D.

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution. Dissolve 30.0 mg in *0.01 M hydrochloric acid* and dilute to 100.0 mL with the same acid.

Spectral range: 245-350 nm.

Absorption maxima: at 272 nm and 279 nm.

Point of inflexion: at 265 nm.

Specific absorbance at the absorption maxima:

– at 272 nm: about 18;
– at 279 nm: about 16.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *clonidine hydrochloride CRS.*

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

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

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

Plate: *TLC silica gel G plate R.*

Mobile phase: *glacial acetic acid R, butanol R, water R* (10:40:50 *V/V/V*); allow to separate, filter the upper layer and use the filtrate.

Application: 10 µL.

Development: over 2/3 of the plate.

Drying: in air.

Detection: spray with *potassium iodobismuthate solution R2.* Allow to dry in air for 1 h. Spray again with *potassium iodobismuthate solution R2* and then immediately spray with a 50 g/L solution of *sodium nitrite 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.

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

TESTS

Solution S. Dissolve 1.25 g in *carbon dioxide-free water R* and dilute to 25 mL with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_7 (*2.2.2, Method II*).

pH (*2.2.3*): 4.0 to 5.0 for solution S.

Related substances. Liquid chromatography (*2.2.29*).

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

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

Reference solution (b). Dissolve 5 mg of *clonidine impurity B CRS* in 2 mL of *acetonitrile R* and dilute to 5 mL with mobile phase A. To 1 mL of this solution, add 1 mL of the test solution and dilute to 10 mL with mobile phase A.

Column:

– *size*: *l* = 0.15 m, Ø = 3.0 mm;
– *stationary phase*: *propylsilyl silica gel for chromatography R* (5 µm);
– *temperature*: 40 °C.