TESTS

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

Dissolve 0.5 g in *water R* and dilute to 25 ml with the same solvent.

Acidity or alkalinity. Dissolve 0.20 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent. Add 0.2 ml of *methyl red solution R* and 0.2 ml of *0.01 M hydrochloric acid*. The solution is red. Add 0.4 ml of *0.01 M sodium hydroxide*. The solution is yellow.

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 10.0 mg of the substance to be examined in the mobile phase and dilute to 5.0 ml with the mobile phase.

Reference solution (a). Dissolve 8 mg of the substance to be examined and 4 mg of *betaxolol impurity A CRS* in 20.0 ml of the mobile phase.

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

Column:

– *size*: *l* = 0.25 m, Ø = 4 mm;
– *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase: mix 175 ml of *acetonitrile R* with 175 ml of *methanol R* and dilute the mixture to 1 litre with a 3.4 g/l solution of *potassium dihydrogen phosphate R*, previously adjusted to pH 3.0 with *phosphoric acid R*.

Flow rate: 1.5 ml/min.

Detection: spectrophotometer at 273 nm.

Injection: 20 µl.

Run time: 4 times the retention time of betaxolol.

System suitability: reference solution (a):

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

Limits:

– *impurities A, B, C, D, E*: for each impurity, not more than 0.3 times the area of the peak in the chromatogram obtained with reference solution (b) (0.3 per cent);
– *total*: not more than the area of the peak in the chromatogram obtained with reference solution (b) (1.0 per cent);
– *disregard limit*: 0.025 times the area of the peak in the chromatogram obtained with reference solution (b) (0.025 per cent).

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

Dissolve 2.0 g in 20 ml of *water R*. 12 ml of the solution complies with test A. Prepare the reference solution using 10 ml of *lead standard solution (1 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.

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

ASSAY

Dissolve 0.300 g in a mixture of 10.0 ml of *0.01 M hydrochloric acid* and 50 ml of *ethanol (96 per cent) R*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

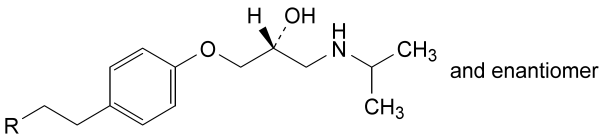
1 ml of *0.1 M sodium hydroxide* is equivalent to 34.39 mg of $C_{18}H_{30}ClNO_3$.

STORAGE

Protected from light.

IMPURITIES

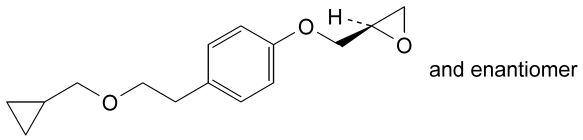
Specified impurities: *A, B, C, D, E.*



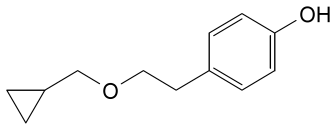
A. R = H: (2*RS*)-1-(4-ethylphenoxy)-3-[(1-methylethyl)-amino]propan-2-ol,

B. R = OH: (2*RS*)-1-[4-(2-hydroxyethyl)phenoxy]-3-[(1-methylethyl)amino]propan-2-ol,

E. R = O-CH_2-CH_2-CH_2-CH_3: (2*RS*)-1-[4-(2-butoxyethyl)-phenoxy]-3-[(1-methylethyl)amino]propan-2-ol,



C. 2-[[4-[2-(cyclopropylmethoxy)ethyl]phenoxy]methyl]-oxirane,

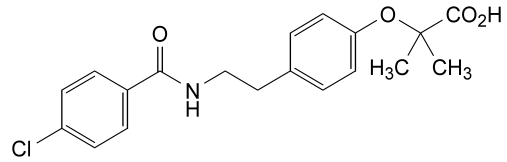


D. 4-[2-(cyclopropylmethoxy)ethyl]phenol.

01/2008:1394
corrected 6.0

BEZAFIBRATE

Bezafibratum



$C_{19}H_{20}ClNO_4$ M_r 361.8

[41859-67-0]

DEFINITION

2-[4-[2-[(4-Chlorobenzoyl)amino]ethyl]phenoxy]-2-methylpropanoic acid.

Content: 98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white crystalline powder.

Solubility: practically insoluble in water, freely soluble in dimethylformamide, sparingly soluble in acetone and in ethanol (96 per cent). It dissolves in dilute solutions of alkali hydroxides.

It shows polymorphism (*5.9*).

IDENTIFICATION

First identification: A, B.

Second identification: A, C.

A. Melting point (*2.2.14*): 181 °C to 185 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation: discs.

Comparison: *bezafibrate CRS*.

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in *methanol R* and evaporate to dryness. Dry the residues *in vacuo* at 80 °C for 1 h and record new spectra using the residues.

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

Test solution. Dissolve 10 mg of the substance to be examined in *methanol R* and dilute to 5 ml with the same solvent.

Reference solution. Dissolve 10 mg of *bezafibrate CRS* in *methanol R* and dilute to 5 ml with the same solvent.

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

Mobile phase: *glacial acetic acid R, methyl ethyl ketone R, xylene R* (2.7:30:60 *V/V/V*).

Application: 5 µl.

Development: over a path of 10 cm.

Drying: at 120 °C for at least 15 min.

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.

TESTS

Solution S. Dissolve 1.0 g in *dimethylformamide R* and dilute to 20 ml with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution BY_5 (*2.2.2, Method II*).

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a). Dilute 10.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (b). Dilute 5.0 ml of reference solution (a) to 50.0 ml with the mobile phase.

Reference solution (c). To 1 ml of the test solution, add 1 ml of *0.1 M hydrochloric acid* and evaporate to dryness on a hot plate. Dissolve the residue in 20 ml of the mobile phase.

Column:

– *size*: *l* = 0.125 m, Ø = 4 mm;
– *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase: mix 40 volumes of a 2.72 g/l solution of *potassium dihydrogen phosphate R* adjusted to pH 2.3 with *phosphoric acid R*, and 60 volumes of *methanol R*.

Flow rate: 1 ml/min.

Detection: spectrophotometer at 228 nm.

Injection: 20 µl.

Run time: the time necessary to detect the ester, which, depending on the route of synthesis, may be impurity C, D or E.

Retention time: impurity A = about 3 min; impurity B = about 3.5 min; bezafibrate = about 6.0 min; impurity C = about 9 min; impurity D = about 14 min; impurity E = about 37 min.

System suitability:

– *resolution*: minimum 5.0 between the 2 principal peaks in the chromatogram obtained with reference solution (c);
– *signal-to-noise ratio*: minimum 5 for the principal peak in the chromatogram obtained with reference solution (b).

Limits:

– *impurities A, B, C, D, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
– *total*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.75 per cent);
– *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Chlorides (*2.4.4*): maximum 300 ppm.

Dilute 10 ml of solution S to 50 ml with *water R*. Filter the resultant suspension through a wet filter previously washed with *water R* until free from chlorides. Prepare the standard using 9 ml of *chloride standard solution (5 ppm Cl) R* and 6 ml of *water R*.

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

2.0 g complies with test C. Prepare the reference solution using 2 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.

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

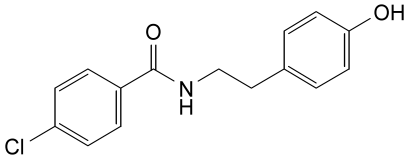
ASSAY

Dissolve 0.300 g in 50 ml of a mixture of 25 volumes of *water R* and 75 volumes of *ethanol (96 per cent) R*. Using 0.1 ml of *phenolphthalein solution R* as indicator, titrate with *0.1 M sodium hydroxide* until a pink colour is obtained. Carry out a blank titration.

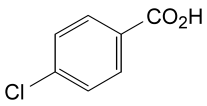
1 ml of *0.1 M sodium hydroxide* is equivalent to 36.18 mg of $C_{19}H_{20}ClNO_4$.

IMPURITIES

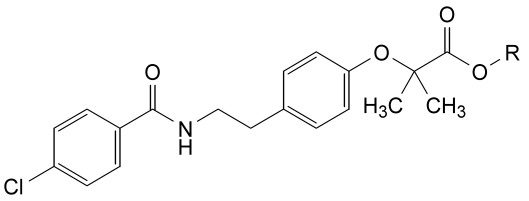
Specified impurities: *A, B, C, D, E*.



A. 4-chloro-*N*-[2-(4-hydroxyphenyl)ethyl]benzamide (chlorobenzoyltyramine),



B. 4-chlorobenzoic acid,



C. R = CH_3: methyl 2-[4-[2-[(4-chlorobenzoyl)amino]-ethyl]phenoxy]-2 methylpropanoate,

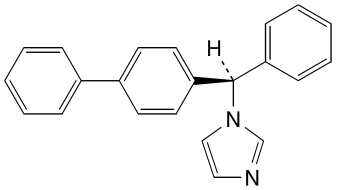
D. R = CH_2-CH_3: ethyl 2-[4-[2-[(4-chlorobenzoyl)amino]-ethyl]phenoxy]-2-methylpropanoate,

E. R = CH_2-CH_2-CH_2-CH_3: butyl 2-[4-[2-[(4-chlorobenzoyl)-amino]ethyl]phenoxy]-2-methylpropanoate.

01/2008:1395
corrected 6.0

BIFONAZOLE

Bifonazolum



and enantiomer

$C_{22}H_{18}N_2$ M_r 310.4
[60628-96-8]

DEFINITION

1-[(*RS*)-(Biphenyl-4-yl)phenylmethyl]-1*H*-imidazole.

Content: 98.0 per cent to 100.5 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: practically insoluble in water, sparingly soluble in anhydrous ethanol.

It shows polymorphism (*5.9*).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *bifonazole 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 *2-propanol R*, evaporate to dryness and record new spectra using the residues.

TESTS

Optical rotation (*2.2.7*): – 0.10° to + 0.10°.

Dissolve 0.20 g in 20.0 ml of *methanol R*.

Related substances. Liquid chromatography (*2.2.29*).

Buffer solution pH 3.2. Mix 2.0 ml of *phosphoric acid R* with *water R* and dilute to 1000.0 ml with the same solvent. Adjust to pH 3.2 (*2.2.3*) with *triethylamine R*.

Test solution. Dissolve 50.0 mg of the substance to be examined in 25 ml of *acetonitrile R* and dilute to 50.0 ml with buffer solution pH 3.2.

Reference solution (a). Dilute 0.25 ml of the test solution to 50.0 ml with buffer solution pH 3.2.

Reference solution (b). Dissolve 25.0 mg of *imidazole R* (impurity C) in *acetonitrile R* and dilute to 25.0 ml with the same solvent. Dilute 0.25 ml of this solution to 100.0 ml with buffer solution pH 3.2.

Reference solution (c). Dissolve 5.0 mg of *bifonazole impurity B CRS* in *acetonitrile R* and dilute to 5.0 ml with the same solvent.

Reference solution (d). Mix 0.25 ml of the test solution and 0.25 ml of reference solution (c) and dilute to 50.0 ml with buffer solution pH 3.2.

Column:

– *size*: *l* = 0.125 m, Ø = 4.6 mm;
– *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);
– *temperature*: 40 °C.

Mobile phase:

– *mobile phase A*: *acetonitrile R1*, buffer solution pH 3.2 (20:80 *V/V*);
– *mobile phase B*: buffer solution pH 3.2, *acetonitrile R1* (20:80 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 8	60	40
8 - 12	60 → 10	40 → 90
12 - 30	10	90

Flow rate: 1 ml/min.

Detection: spectrophotometer at 210 nm.

Injection: 50 µl of the test solution and reference solutions (a), (b) and (d).

Retention time: impurity B = about 4 min; bifonazole = about 4.5 min.

System suitability: reference solution (d):

– *resolution*: minimum 2.5 between the peaks due to impurity B and bifonazole.

Limits:

– *impurity B*: not more than 3 times the area of the corresponding peak in the chromatogram obtained with reference solution (a) (1.5 per cent);
– *impurity C*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.25 per cent);
– *impurities A, D*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
– *total*: not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (2 per cent);
– *disregard limit*: 0.1 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.

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

ASSAY

Dissolve 0.250 g in 80 ml of *anhydrous acetic acid 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 31.04 mg of $C_{22}H_{18}N_2$.