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.

E. Dissolve 20 mg in 2 ml of *sulphuric acid R*. The solution is colourless and shows blue fluorescence in ultraviolet light at 365 nm. Dissolve 0.1 g of *chloral hydrate R* in the solution. Within about 5 min, the colour changes to deep yellow and, after about 20 min, develops a brownish tinge.

TESTS

Related substances. 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. Prepare immediately before use.

Reference solution (a). Dissolve 5.0 mg of *glibenclamide impurity A CRS* and 5.0 mg of *glibenclamide impurity B CRS* in *methanol R* and dilute to 100.0 ml with the same solvent. Dilute 5.0 ml of the solution to 20.0 ml with *methanol R*.

Reference solution (b). Dilute 2.0 ml of the test solution to 100.0 ml with *methanol R*. Dilute 5.0 ml of this solution to 50.0 ml with *methanol R*.

Reference solution (c). Dissolve 5 mg of *gliclazide CRS* in *methanol R*, add 2 ml of the test solution and dilute to 100 ml with *methanol R*. Dilute 1 ml of this solution to 10 ml with *methanol R*.

Column:

– *size*: *l* = 0.10 m, Ø = 4.6 mm,
– *stationary phase*: spherical *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (3 µm),
– *temperature*: 35 °C.

Mobile phase:

– *mobile phase A*: mix 20 ml of a 101.8 g/l solution of freshly distilled *triethylamine R* adjusted to pH 3.0 using *phosphoric acid R*, and 50 ml of *acetonitrile R*; dilute to 1000 ml with *water R*,
– *mobile phase B*: mobile phase A, *water R*, *acetonitrile R* (20:65:915 *V/V/V*),

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	45	55
15 - 30	45 → 5	55 → 95
30 - 40	5	95
40 - 41	5 → 45	95 → 55
41 - 55	45	55

Flow rate: 0.8 ml/min.

Detection: spectrophotometer at 230 nm.

Injection: 10 µl.

Relative retention with reference to glibenclamide (retention time = about 5 min): impurity A = about 0.5; impurity B = about 0.6.

System suitability: reference solution (c):

– *resolution*: minimum 5.0 between the peaks due to glibenclamide and gliclazide.

Limits:

– *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.5 per cent),
– *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.5 per cent),
– *any other impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent), and not more than 2 such peaks have an area greater than half the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent),
– *total of other impurities*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent),
– *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

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

1.0 g complies with limit test D. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying (*2.2.32*): maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 100-105 °C.

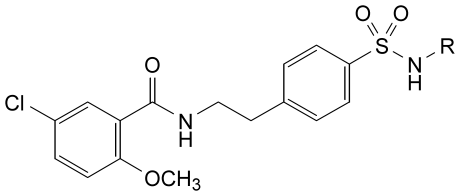
Sulphated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.400 g with heating in 100 ml of *alcohol R*. Titrate with *0.1 M sodium hydroxide*, using 1.0 ml of *phenolphthalein solution R* as indicator, until a pink colour is obtained.

1 ml of *0.1 M sodium hydroxide* is equivalent to 49.40 mg of $C_{23}H_{28}ClN_3O_5S$.

IMPURITIES



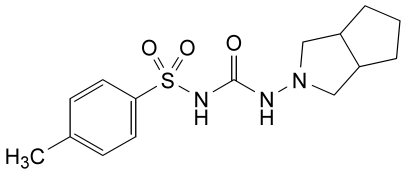
A. R = H: 5-chloro-2-methoxy-*N*-[2-(4-sulphamoylphenyl)ethyl]benzamide,

B. R = CO-OCH_3: methyl [[4-[2-[(5-chloro-2-methoxybenzoyl)amino]ethyl]phenyl]sulphonyl]carbamate.

01/2005:1524

GLICLAZIDE

Gliclazidum



$C_{15}H_{21}N_3O_3S$ M_r 323.4

DEFINITION

Gliclazide contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 1-(hexahydrocyclopenta[*c*]pyrrol-2(1*H*)-yl)-3-[(4-methylphenyl)sulphonyl]urea, calculated with reference to the dried substance.

CHARACTERS

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

IDENTIFICATION

Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *gliclazide CRS*. Examine the substances prepared as discs.

TESTS

Related substances. Examine by liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution. Dissolve 50.0 mg of the substance to be examined in 23 ml of *acetonitrile R* and dilute to 50.0 ml with *water R*.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with a mixture of 45 volumes of *acetonitrile R* and 55 volumes of *water R*. Dilute 10.0 ml of the solution to 100.0 ml with a mixture of 45 volumes of *acetonitrile R* and 55 volumes of *water R*.

Reference solution (b). Dissolve 5 mg of the substance to be examined and 15 mg of *gliclazide impurity F CRS* in 23 ml of *acetonitrile R* and dilute to 50 ml with *water R*. Dilute 1 ml of the solution to 20 ml with a mixture of 45 volumes of *acetonitrile R* and 55 volumes of *water R*.

Reference solution (c). Dissolve 10.0 mg of *gliclazide impurity F CRS* in 45 ml of *acetonitrile R* and dilute to 100.0 ml with *water R*. Dilute 1.0 ml of the solution to 100.0 ml with a mixture of 45 volumes of *acetonitrile R* and 55 volumes of *water R*.

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 0.9 ml/min a mixture of 0.1 volumes of *triethylamine R*, 0.1 volumes of *trifluoroacetic acid R*, 45 volumes of *acetonitrile R* and 55 volumes of *water R*,

– as detector a spectrophotometer set at 235 nm.

Inject 20 µl of reference solution (b). Adjust the sensitivity of the system so that the heights of the 2 principal peaks in the chromatogram obtained with reference solution (b) are at least 50 per cent of the full scale of the recorder. The test is not valid unless in the chromatogram obtained the resolution between the two principal peaks is at least 1.8.

Inject 20 µl of the test solution and 20 µl each of reference solutions (a) and (c). Continue the chromatography of the test solution for twice the retention time of gliclazide. In the chromatogram obtained with the test solution: the area of any peak corresponding to impurity F is not greater than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.1 per cent); the area of any peaks, apart from the principal peak and the peak due to impurity F, is not greater than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent); the sum of the areas of any such peaks is not greater than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent). Disregard any peak with an area less than 0.2 times that of the principal peak in the chromatogram obtained with reference solution (a).

Impurity B. Examine by liquid chromatography (*2.2.29*) as described in the test for related substances.

Test solution. Dissolve 0.400 g in 2.5 ml of *dimethyl sulphoxide R* and dilute to 10.0 ml with *water R*. Stir for 10 min, store at 4 °C for 30 min and filter.

Reference solution (a). Dissolve 20.0 mg of *gliclazide impurity B CRS* in *dimethyl sulphoxide R* and dilute to 100.0 ml with the same solvent. To 1.0 ml of the solution, add 12 ml of *dimethyl sulphoxide R* and dilute to 50.0 ml with *water R*.

Reference solution (b). To 1.0 ml of reference solution (a), add 12 ml of *dimethyl sulphoxide R* and dilute to 50.0 ml with *water R*.

Inject 50 µl of the test solution and 50 µl of reference solution (b). In the chromatogram obtained with the test solution, the area of any peak corresponding to impurity B is not greater than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (2 ppm).

Heavy metals (*2.4.8*). 1.5 g complies with limit test F for heavy metals (10 ppm). Prepare the standard using 1.5 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying (*2.2.32*). Not more than 0.25 per cent, determined on 1.000 g by drying in an oven at 100 °C to 105 °C for 2 h.

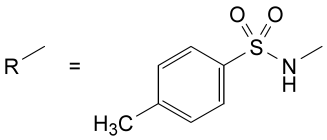
Sulphated ash (*2.4.14*). Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

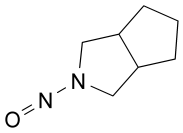
Dissolve 0.250 g in 50 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 32.34 mg of $C_{15}H_{21}N_3O_3S$.

IMPURITIES

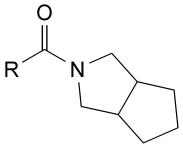


A. R-H: 4-methylbenzenesulphonamide,

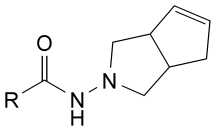


B. 2-nitroso-octahydrocyclopenta[*c*]pyrrole,

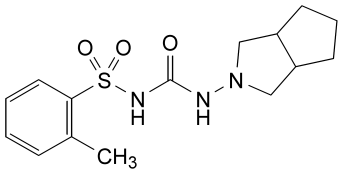
C. R-CO-O-C_2H_5: ethyl [(4-methylphenyl)sulphonyl]carbamate,



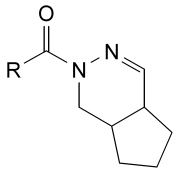
D. *N*-[(4-methylphenyl)sulphonyl]hexahydrocyclopenta[*c*]pyrrol-2(1*H*)-carboxamide,



E. 1-[(4-methylphenyl)sulphonyl]-3-(3,3a,4,6a-tetrahydrocyclopenta[*c*]pyrrol-2(1*H*)-yl)urea,



F. 1-(hexahydrocyclopenta[*c*]pyrrol-2(1*H*)-yl)-3-[(2-methylphenyl)sulphonyl]urea,

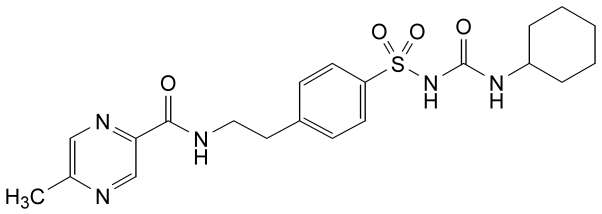


G. *N*-[(4-methylphenyl)sulphonyl]-1,4a,5,6,7,7a-hexahydro-2*H*-cyclopenta[*d*]pyridazine-2-carboxamide.

01/2005:0906

GLIPIZIDE

Glipizidum



$C_{21}H_{27}N_5O_4S$ M_r 445.5

DEFINITION

Glipizide contains not less than 98.0 per cent and not more than the equivalent of 102.0 per cent of 1-cyclohexyl-3-[[4-[2-[[(5-methylpyrazin-2-yl)carbonyl]amino]ethyl]phenyl]sulphonyl]urea, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, practically insoluble in water, soluble in methylene chloride, sparingly soluble in acetone, practically insoluble in alcohol. It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION

First identification: B.

Second identification: A, C, D.

A. Dissolve about 2 mg in *methanol R* and dilute to 100 ml with the same solvent. Examined between 220 nm and 350 nm (*2.2.25*), the solution shows two absorption maxima, at 226 nm and 274 nm. The ratio of the absorbance measured at the maximum of 226 nm to that measured at the maximum at 274 nm is 2.0 to 2.4.

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *glipizide CRS*. Examine the substances prepared as discs.

C. Examine the chromatograms obtained in the test for related substances in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Dissolve 50 mg in 5 ml of *dioxan R*. Add 1 ml of a 5 g/l solution of *fluorodinitrobenzene R* in *dioxan R* and boil for 2-3 min. A yellow colour is produced.

TESTS

Related substances. Examine by thin-layer chromatography (*2.2.27*), using *silica gel* GF_{254} *R* as the coating substance.

Test solution (a). Dissolve 0.20 g of the substance to be examined in a mixture of equal volumes of *methanol R* and of *methylene chloride R* and dilute to 10 ml with the same mixture of solvents.

Test solution (b). Dilute 1 ml of test solution (a) to 20 ml with a mixture of equal volumes of *methanol R* and *methylene chloride R*.

Reference solution (a). Dissolve 10 mg of *glipizide CRS* in a mixture of equal volumes of *methanol R* and *methylene chloride R* and dilute to 10 ml with the same mixture of solvents.

Reference solution (b). Dissolve 5 mg of *glipizide impurity A CRS* in a mixture of equal volumes of *methanol R* and *methylene chloride R* and dilute to 50 ml with the same mixture of solvents.

Reference solution (c). Dilute 0.5 ml of test solution (a) to 100 ml with a mixture of equal volumes of *methanol R* and *methylene chloride R*.

Reference solution (d). Dilute 4 ml of reference solution (c) to 10 ml with a mixture of equal volumes of *methanol R* and *methylene chloride R*.

Reference solution (e). Dilute 5 ml of test solution (a) to 10 ml with reference solution (b).

Apply to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 25 volumes of *anhydrous formic acid R*, 25 volumes of *ethyl acetate R* and 50 volumes of *methylene chloride R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. In the chromatogram obtained with test solution (a): any spot corresponding to glipizide impurity A is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent); any spot apart from the principal spot and the spot corresponding to glipizide impurity A is not more intense than the spot in the chromatogram obtained with reference solution (c) (0.5 per cent) and not more than two such spots are more intense than the spot in the chromatogram obtained with reference solution (d) (0.2 per cent). The test is not valid unless the chromatogram obtained with reference solution (e) shows two clearly separated spots.

Cyclohexylamine. Not more than 100 ppm, determined by gas chromatography (*2.2.28*), using *decane R* as internal standard.

Internal standard solution. Dissolve 25 mg of *decane R* in *hexane R* and dilute to 100 ml with the same solvent. Dilute 5 ml of this solution to 50 ml with *hexane R*.

Test solution (a). Dissolve 3.0 g of the substance to be examined in 50 ml of a 12 g/l solution of *sodium hydroxide R* and shake with two quantities, each of 5.0 ml, of *hexane R*. Use the combined upper layers.

Test solution (b). Dissolve 3.0 g of the substance to be examined in 50 ml of a 12 g/l solution of *sodium hydroxide R* and shake with two quantities, each of 5.0 ml, of the internal standard solution. Use the combined upper layers.

Reference solution. Dissolve 30.0 mg of *cyclohexylamine R* in a 17.5 g/l solution of *hydrochloric acid R* and dilute to 100.0 ml with the same acid. To 1.0 ml of this solution add 50 ml of a 12 g/l solution of *sodium hydroxide R* and shake with two quantities, each of 5.0 ml, of the internal standard solution. Use the combined upper layers.