System suitability: the chromatogram obtained with reference solution (b) shows 2 clearly separated spots or 2 clearly separated groups of spots.

Results: the principal spot or group of principal spots in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot or group of principal spots in the chromatogram obtained with reference solution (a) and to the spot or group of spots with the highest R_F value in the chromatogram obtained with reference solution (b).

C. Examine the chromatograms obtained in the test for composition.

Results: the 3 principal peaks in the chromatogram obtained with the test solution are similar in retention time to the 3 principal peaks in the chromatogram obtained with reference solution (a).

TESTS

Composition. Liquid chromatography (*2.2.29*): use the normalisation procedure.

Test solution. Dissolve 25 mg of the substance to be examined in 10 mL of *methanol R* and dilute to 25 mL with the mobile phase.

Reference solution (a). Dissolve 25 mg of *gramicidin CRS* in 10 mL of *methanol R* and dilute to 25 mL with the mobile phase.

Reference solution (b). Dilute 1.0 mL of reference solution (a) to 50.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm,
– *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm),
– *temperature*: 50 °C.

Mobile phase: *water R, methanol R* (29:71 *V/V*).

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 282 nm.

Injection: 20 µL.

Run time: 2.5 times the retention time of gramicidin A1.

Relative retention with reference to gramicidin A1 (retention time = about 22 min): gramicidin C1 = about 0.7; gramicidin C2 = about 0.8; gramicidin A2 = about 1.2; gramicidin B1 = about 1.9.

System suitability: reference solution (a):

– *resolution*: minimum 1.5 between the peaks due to gramicidin A1 and gramicidin A2,
– the chromatogram obtained is concordant with the chromatogram supplied with *gramicidin CRS*.

Composition:

– *sum of the contents of gramicidins A1, A2, B1, C1 and C2*: minimum 95.0 per cent,
– *ratio of the content of gramicidin A1 to the sum of the contents of gramicidins A1, A2, B1, C1 and C2*: minimum 60.0 per cent,
– *disregard limit*: the area of the peak due to gramicidin A1 in the chromatogram obtained with reference solution (b).

Related substances. Liquid chromatography (*2.2.29*) as described in the test for composition.

Limit:

– *any impurity*: maximum 2.0 per cent and not more than 1 peak is more than 1.0 per cent; disregard the peaks due to gramicidins A1, A2, B1, C1 and C2.

Loss on drying (*2.2.32*): maximum 3.0 per cent, determined on 1.000 g by drying over *diphosphorus pentoxide R* at 60 °C at a pressure not exceeding 0.1 kPa for 3 h.

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

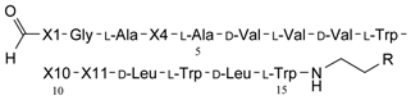
ASSAY

Carry out the microbiological assay of antibiotics (*2.7.2*), using the turbidimetric method. Use *gramicidin CRS* as the reference substance.

STORAGE

In an airtight container, protected from light.

IMPURITIES



Impurity	X1	X4	X10	X11	R
A	L-Val	Met	D-Leu	L-Trp	OH
B	L-Val	D-Leu	D-Leu	L-Trp	CH_2-OH
C	L-Ile	D-Leu	D-Leu	L-Phe	OH
D	L-Val	D-Leu	Met	L-Tyr	OH
E	L-Ile	D-Leu	D-Leu	L-Trp	CH_2-OH

A. [4-methionine]gramicidin A1,

B. gramicidin A1 3-hydroxypropyl,

C. gramicidin B2,

D. [10-methionine]gramicidin C1,

E. gramicidin A2 3-hydroxypropyl.

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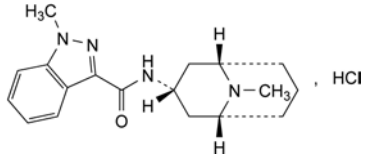
corrected 6.3

GRANISETRON HYDROCHLORIDE

Granisetroni hydrochloridum



$C_{18}H_{25}ClN_4O$ M_r 348.9
[107007-99-8]

DEFINITION

1-Methyl-*N*-[(1*R*,3*r*,5*S*)-9-methyl-9-azabicyclo[3.3.1]non-3-yl]-1*H*-indazole-3-carboxamide hydrochloride.

Content: 97.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white powder.

Solubility: freely soluble in water, sparingly soluble in methylene chloride, slightly soluble in methanol.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *granisetron hydrochloride CRS*.

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

TESTS

Solution S. Dissolve 0.2 g in *carbon dioxide-free water R* and dilute to 20 mL with the same solvent.

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

pH (*2.2.3*): 4.0 to 6.5 for solution S.

Impurity E. Thin-layer chromatography (*2.2.27*).

Solvent mixture: *water R, acetonitrile R* (20:80 *V/V*).

Test solution. Dissolve 0.25 g of the substance to be examined in the solvent mixture and dilute to 5 mL with the solvent mixture.

Reference solution. Dissolve 5.0 mg of *granisetron impurity E CRS* in the solvent mixture and dilute to 20.0 mL with the solvent mixture.

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

Mobile phase: *concentrated ammonia R, 2-propanol R, ethyl acetate R* (6.5:30:50 *V/V/V*).

Application: 2 µL.

Development: over half of the plate.

Drying: in air.

Detection: expose to iodine vapour for 30 min.

Limit:

– *impurity E*: any spot due to impurity E is not more intense than the principal spot in the chromatogram obtained with the reference solution (0.5 per cent).

Related substances. Liquid chromatography (*2.2.29*). *Carry out the test protected from light.*

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

Reference solution (a). Dilute 1.0 mL of the test solution to 50.0 mL with the mobile phase. Dilute 5.0 mL of this solution to 20.0 mL with the mobile phase.

Reference solution (b). Transfer 2 mL of the test solution to a colourless glass vial, stopper and expose the solution either to sunlight for 4 h or under a UV lamp for 16 h (partial degradation of granisetron to impurity C). A degradation of at least about 0.3 per cent of granisetron to impurity C must be obtained as shown by appearance of a corresponding peak in the chromatogram. If not, expose the solution once again to sunlight or under a UV lamp.

Reference solution (c). Dissolve 50.0 mg of *granisetron hydrochloride CRS* in the mobile phase and dilute to 50.0 mL with the mobile phase.

Reference solution (d). Dissolve the contents of a vial of *granisetron impurity A CRS* in 1 mL of the mobile phase.

Reference solution (e). Dissolve the contents of a vial of *granisetron impurity B CRS* in 1 mL of the mobile phase.

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm;
– *stationary phase*: spherical *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
– *temperature*: 40 °C.

Mobile phase: dilute 1.6 mL of *phosphoric acid R* to 800 mL with *water R*, add 200 mL of *acetonitrile R* and mix. Add 1.0 mL of *hexylamine R* and mix. Adjust to pH 7.5 ± 0.05 with freshly distilled *triethylamine R* (about 4 mL).

Flow rate: 1.5 mL/min.

Detection: spectrophotometer at 305 nm.

Injection: 10 µL of the test solution and reference solutions (a), (b), (d) and (e).

Run time: twice the retention time of granisetron.

Relative retention with reference to granisetron (retention time = about 7 min): impurity D = about 0.4; impurity B = about 0.5; impurity A = about 0.7; impurity C = about 0.8.

System suitability:

– *resolution*: minimum 3.5 between the peaks due to impurity C and granisetron in the chromatogram obtained with reference solution (b);
– *symmetry factor*: maximum 2.0 for the peak due to granisetron.

Limits:

– *correction factor*: for the calculation of content, multiply the peak area of impurity B by 1.7;
– *impurity B*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
– *impurity C*: not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
– *impurity A*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);
– *impurity D*: not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
– *any other impurity*: for each impurity, not more than 0.2 times 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) (1.0 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); disregard any peak due to the blank.

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

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

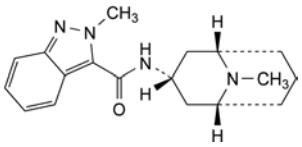
Injection: test solution and reference solution (c).

Calculate the percentage content of $C_{18}H_{25}ClN_4O$ using the declared content of *granisetron hydrochloride CRS*.

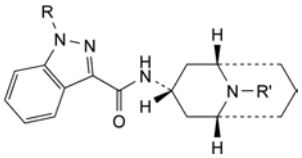
IMPURITIES

Specified impurities: *A, B, C, D, 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*): *F, G, H, I.*

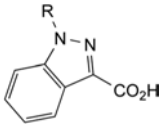


A. 2-methyl-*N*-[(1*R*,3*r*,5*S*)-9-methyl-9-azabicyclo[3.3.1]non-3-yl]-2*H*-indazole-3-carboxamide,



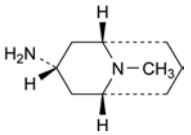
B. R = H, R′ = CH_3: *N*-[(1*R*,3*r*,5*S*)-9-methyl-9-azabicyclo[3.3.1]non-3-yl]-1*H*-indazole-3-carboxamide,

C. R = CH_3, R′ = H: *N*-[(1*R*,3*r*,5*S*)-9-azabicyclo[3.3.1]non-3-yl]-1-methyl-1*H*-indazole-3-carboxamide,

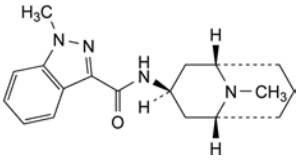


D. R = CH_3: 1-methyl-1*H*-indazole-3-carboxylic acid,

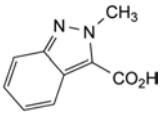
H. R = H: 1*H*-indazole-3-carboxylic acid,



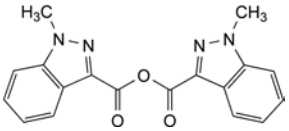
E. (1*R*,3*r*,5*S*)-9-methyl-9-azabicyclo[3.3.1]nonan-3-amine,



F. 1-methyl-*N*-[(1*R*,3*s*,5*S*)-9-methyl-9-azabicyclo[3.3.1]non-3-yl]-1*H*-indazole-3-carboxamide (*exo*-granisetron),



G. 2-methyl-2*H*-indazole-3-carboxylic acid,

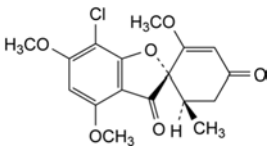


I. 1-methyl-1*H*-indazole-3-carboxylic anhydride.

01/2008:0182
corrected 6.0

GRISEOFULVIN

Griseofulvinum



$C_{17}H_{17}ClO_6$ M_r 352.8
[126-07-8]

DEFINITION

(1′*S*,3-6′*R*)-7-Chloro-2′,4,6-trimethoxy-6′-methylspiro[benzofuran-2(3*H*),1′-[2]cyclohexene]-3,4′-dione.

Substance produced by the growth of certain strains of *Penicillium griseofulvum* or obtained by any other means.

Content: 97.0 per cent to 102.0 per cent (dried substance).

PRODUCTION

The method of manufacture is validated to demonstrate that the product if tested would comply with the following test.

Abnormal toxicity. To each of 5 healthy mice, each weighing 17-22 g, administer orally a suspension of 0.1 g of the substance to be examined in 0.5-1 mL of *water R*. None of the mice dies within 48 h.

CHARACTERS

Appearance: white or yellowish-white, microfine powder, the particles of which generally have a maximum dimension of up to 5 µm, although larger particles that may exceed 30 µm may occasionally be present.

Solubility: practically insoluble in water, freely soluble in dimethylformamide and in tetrachloroethane, slightly soluble in anhydrous ethanol and in methanol.

mp: about 220 °C.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *griseofulvin CRS*.

B. Dissolve about 5 mg in 1 mL of *sulfuric acid R* and add about 5 mg of powdered *potassium dichromate R*. A dark red colour develops.

TESTS

Appearance of solution. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_4 (*2.2.2, Method II*).

Dissolve 0.75 g in *dimethylformamide R* and dilute to 10 mL with the same solvent.

Acidity. Suspend 0.25 g in 20 mL of *ethanol (96 per cent) R* and add 0.1 mL of *phenolphthalein solution R*. Not more than 1.0 mL of *0.02 M sodium hydroxide* is required to change the colour of the indicator.

Specific optical rotation (*2.2.7*): + 354 to + 364 (dried substance).

Dissolve 0.250 g in *dimethylformamide R* and dilute to 25.0 mL with the same solvent.

Related substances. Gas chromatography (*2.2.28*).

Internal standard solution. Dissolve 0.2 g of *diphenylanthracene R* in *acetone R* and dilute to 100.0 mL with the same solvent.

Test solution (a). Dissolve 0.10 g of the substance to be examined in *acetone R* and dilute to 10.0 mL with the same solvent.

Test solution (b). Dissolve 0.10 g of the substance to be examined in *acetone R*, add 1.0 mL of the internal standard solution and dilute to 10.0 mL with *acetone R*.

Reference solution. Dissolve 5.0 mg of *griseofulvin CRS* in *acetone R*, add 1.0 mL of the internal standard solution and dilute to 10.0 mL with *acetone R*.

Column:
- *material*: glass;
- *size*: *l* = 1 m, Ø = 4 mm;
- *stationary phase*: *diatomaceous earth for gas chromatography R* impregnated with 1 per cent *m/m* of *poly[(cyanopropyl)(methyl)][(phenyl)(methyl)]siloxane R*.

Carrier gas: *nitrogen for chromatography R*.

Flow rate: 50-60 mL/min.

Temperature:
- *column*: 250 °C;
- *injection port*: 270 °C;
- *detector*: 300 °C.

Detection: flame ionisation.

Run time: 3 times the retention time of griseofulvin.

Relative retention with reference to griseofulvin (retention time = about 11 min): dechloro-griseofulvin = about 0.6; dehydrogriseofulvin = about 1.4.

Calculate the ratio (*R*) of the area of the peak due to griseofulvin to the area of the peak due to the internal standard in the chromatogram obtained with the reference solution.

Limits:
- *dechloro-griseofulvin*: calculate the ratio of the area of the peak due to dechloro-griseofulvin to the area of the peak due to the internal standard in the chromatogram obtained with test solution (b): this ratio is not greater than 0.6 *R* (3.0 per cent);
- *dehydrogriseofulvin*: calculate the ratio of the area of the peak due to dehydrogriseofulvin to the area of the peak due to the internal standard in the chromatogram obtained with test solution (b): this ratio is not greater than 0.15 *R* (0.75 per cent).

Substances soluble in light petroleum: maximum 0.2 per cent.

Shake 1.0 g with 20 mL of *light petroleum R*. Boil under a reflux condenser for 10 min. Cool, filter and wash with 3 quantities,