Reference solution. Dilute 25 µl of *limonene R*, 100 µl of *citronellal R*, 25 µl of *citronellyl acetate R*, 25 µl of *citral R*, 25 µl of *geranyl acetate R*, 25 µl of *citronellol R* and 100 µl of *geraniol R* in 5 ml of *hexane R*.

Column:

– *material*: fused silica,

– *size*: *l* = 60 m, Ø = 0.25 mm,

– *stationary phase*: *macrogol 20 000 R* (0.2 µm).

Carrier gas: *helium for chromatography R*.

Flow rate: 1.0 ml/min.

Split ratio: 1:100.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 2	80
	2 - 26	80 → 150
	26 - 42	150 → 185
	42 - 49	185 → 250
Injection port		260
Detector		260

Detection: flame ionisation.

Injection: 1 µl of the reference solution, 0.2 µl of the test solution.

Elution order: the order indicated in the composition of the reference solution. Record the retention times of these substances.

System suitability: reference solution:

– *resolution*: minimum of 1.2 between the peaks due to geranyl acetate and citronellol.

Using the retention times determined from the chromatogram obtained with the reference solution, locate the components of the reference solution in the chromatogram obtained with the test solution.

Determine the percentage content of each of these components.

The percentages are within the following values:

– *limonene*: 1.0 per cent to 5.0 per cent,

– *citronellal*: 30.0 per cent to 45.0 per cent,

– *citronellyl acetate*: 2.0 per cent to 4.0 per cent,

– *neral*: less than 2.0 per cent,

– *geranial*: less than 2.0 per cent,

– *geranyl acetate*: 3.0 per cent to 8.0 per cent,

– *citronellol*: 9.0 per cent to 15.0 per cent,

– *geraniol*: 20.0 per cent to 25.0 per cent.

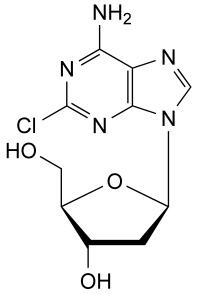
STORAGE

In a well-filled container, protected from light.

01/2008:2174

CLADRIBINE

Cladribinum



$C_{10}H_{12}ClN_5O_3$ M_r 285.7

DEFINITION

2-Chloro-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)-9*H*-purin-6-amine.

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

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: slightly soluble in water, soluble in dimethyl sulphoxide, slightly soluble in methanol, practically insoluble in acetonitrile.

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *cladribine CRS*.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined in the minimum volume of *methanol R* and evaporate to dryness. Dry the precipitate at 100 °C for 2 h and record a new spectrum using the residue.

TESTS

Solution S. Disperse 0.15 g in *carbon dioxide-free water R*, dilute to 50 ml with the same solvent and sonicate until dissolution is complete.

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

pH (*2.2.3*): 7.0 to 8.1 for solution S.

Specific optical rotation (*2.2.7*): – 21.0 to – 27.0 (anhydrous substance).

Dissolve 0.25 g in *dimethyl sulphoxide R* and dilute to 25.0 ml with the same solvent.

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

Test solution. Dissolve 40.0 mg of the substance to be examined in *dimethylformamide R* and dilute to 2.0 ml with the same solvent.

Reference solution (a). Dissolve 5.0 mg of *2-deoxy-D-ribose R* (impurity E) in *dimethylformamide R* and dilute to 25.0 ml with the same solvent. Dilute 3.0 ml of this solution to 10.0 ml with *dimethylformamide R*.

Reference solution (b). Dissolve 10.0 mg of *2-deoxy-D-ribose R* (impurity E) in *dimethylformamide R* and dilute to 5.0 ml with the same solvent. Mix 9 volumes of this solution with 1 volume of the test solution.

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

Mobile phase: *concentrated ammonia R, ethanol (96 per cent) R, ethyl acetate R* (20:40:40 *V/V/V*).

Application: 5 µl as bands of 10 mm; thoroughly dry the starting points in a current of warm air.

Development: over 2/3 of the plate.

Drying: in air, then heat at 45 °C for 10 min.

Detection: spray with a solution containing 0.5 g of *thymol R* in a mixture of 5 ml of *sulphuric acid R* and 95 ml of *ethanol (96 per cent) R*; heat at 110 °C for 20 min or until the spots appear.

System suitability: reference solution (b):

– the chromatogram shows 2 clearly separated spots.

Limit:

– *impurity E*: any spot due to impurity E is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.3 per cent).

Related substances. Liquid chromatography (*2.2.29*).

Solvent mixture: *acetonitrile R, water R* (10:90 *V/V*).

Test solution (a). Dissolve 25.0 mg of the substance to be examined in the solvent mixture and dilute to 5.0 ml with the solvent mixture.

Test solution (b). Dissolve 20.0 mg of the substance to be examined in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution (a). Dissolve 20.0 mg of *cladribine CRS* in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

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

Reference solution (c). Dilute 1.0 ml of reference solution (b) to 10.0 ml with the solvent mixture.

Reference solution (d). Dissolve 1.0 mg of *cladribine impurity C CRS* in reference solution (b) and dilute to 25.0 ml with the same solution.

Reference solution (e). Dilute 5.0 ml of reference solution (c) to 10.0 ml with the solvent mixture.

Reference solution (f). Dissolve 3 mg of *cladribine for peak identification CRS* (containing impurities A, B, C and D) in 2 ml of the solvent mixture.

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm;

– *stationary phase*: *base-deactivated octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

– *mobile phase A*: *water for chromatography R*;

– *mobile phase B*: *acetonitrile for chromatography R*;

– *mobile phase C*: 50 g/l solution of *phosphoric acid R* in *water for chromatography R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)	Mobile phase C (per cent *V/V*)
0 - 10	80 → 70	10 → 20	10
10 - 25	70 → 20	20 → 70	10
25 - 30	20	70	10
30 - 31	20 → 80	70 → 10	10
31 - 39	80	10	10

Flow rate: 0.8 ml/min.

Detection: spectrophotometer at 252 nm.

Injection: 20 µl of test solution (a) and reference solutions (c), (d), (e) and (f).

Identification of impurities: use the chromatogram supplied with *cladribine for peak identification CRS* to identify the peaks due to impurities A, B, C and D.

Relative retention with reference to cladribine (retention time = about 10 min): impurity A = about 0.33; impurity B = about 0.44; impurity C = about 0.73; impurity D = about 0.92.

System suitability: reference solution (d):

– *resolution*: minimum 4.5 between the peaks due to impurity C and cladribine.

Limits:

– *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity B = 1.7; impurity C = 0.8;

– *impurities A, C*: for each impurity, not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.3 per cent);

– *impurities B, D*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent);

– *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.10 per cent);

– *total*: not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent);

– *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (e) (0.05 per cent).

Water (*2.5.32*): maximum 0.5 per cent, determined on 0.100 g.

Bacterial endotoxins (*2.6.14*): less than 3 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

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

Injection: test solution (b) and reference solution (a).

Calculate the percentage content of $C_{10}H_{12}ClN_5O_3$ from the declared content of *cladribine CRS*.

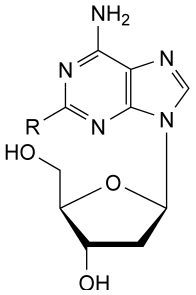
STORAGE

Protected from light, at a temperature of 2 °C to 8 °C. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

IMPURITIES

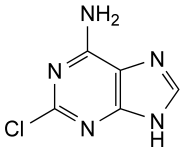
Specified impurities: A, B, C, D, E.

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.*

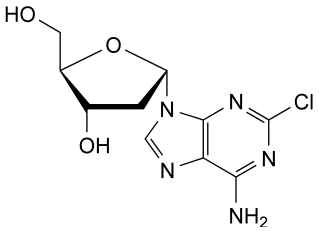


A. R = NH_2: 9-(2-deoxy-β-D-*erythro*-pentofuranosyl)-9*H*-purin-2,6-diamine,

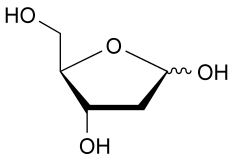
B. R = OCH_3: 9-(2-deoxy-β-D-*erythro*-pentofuranosyl)-2-methoxy-9*H*-purin-6-amine,



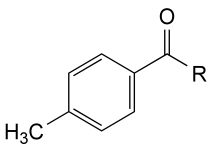
C. 2-chloro-7*H*-purin-6-amine (2-chloroadenine),



D. 2-chloro-9-(2-deoxy-α-D-*erythro*-pentofuranosyl)-9*H*-purin-6-amine,



E. 2-deoxy-D-*erythro*-pentofuranose (2-deoxy-D-ribose),



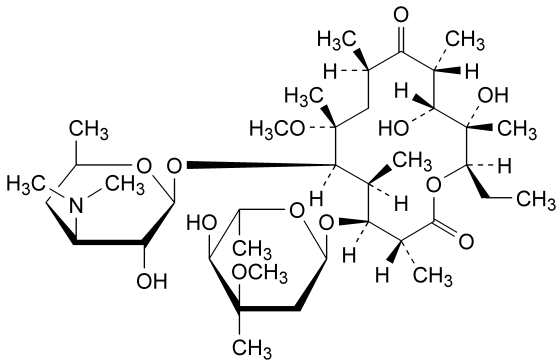
F. R = NH_2: 4-methylbenzamide,

G. R = OCH_3: methyl 4-methylbenzoate.

01/2008:1651
corrected 6.0

CLARITHROMYCIN

Clarithromycinum



$C_{38}H_{69}NO_{13}$ M_r 748
[81103-11-9]

DEFINITION

(3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*R*,12*R*,13*S*,14*R*)-4-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-14-ethyl-12,13-dihydroxy-7-methoxy-3,5,7,9,11,13-hexamethyl-6-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-*xylo*-hexopyranosyl]oxy]oxacyclotetradecane-2,10-dione (6-*O*-methylerythromycin A).

Semi-synthetic product derived from a fermentation product.

Content: 96.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: practically insoluble in water, soluble in acetone and in methylene chloride, slightly soluble in methanol.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *clarithromycin CRS*.

TESTS

Solution S. Dissolve 0.500 g in *methylene chloride R* and dilute to 50.0 ml with the same solvent.

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

Specific optical rotation (*2.2.7*): – 94 to – 102 (anhydrous substance), determined on solution S.

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 75.0 mg of the substance to be examined in 25 ml of *acetonitrile R1* and dilute to 50.0 ml with *water R*.

Reference solution (a). Dissolve 75.0 mg of *clarithromycin CRS* in 25 ml of *acetonitrile R1* and dilute to 50.0 ml with *water R*.

Reference solution (b). Dilute 5.0 ml of reference solution (a) to 100.0 ml with a mixture of equal volumes of *acetonitrile R1* and *water R*.