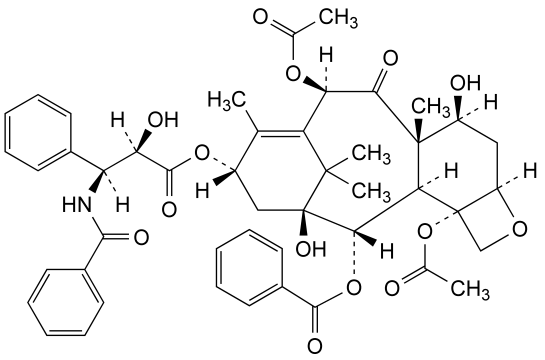
01/2009:1794

PACLITAXEL

Paclitaxelum



$C_{47}H_{51}NO_{14}$ M_r 854
[33069-62-4]

DEFINITION

5β,20-Epoxy-1,7β-dihydroxy-9-oxotax-11-ene-2α,4,10β,-13α-tetrayl 4,10-diacetate 2-benzoate 13-[(2*R*,3*S*)-3-(benzoylamino)-2-hydroxy-3-phenylpropanoate].

It is isolated from natural sources or produced by fermentation or by a semi-synthetic process.

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

CHARACTERS

Appearance: white or almost white, crystalline powder.

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

IDENTIFICATION

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *paclitaxel CRS*.

If the spectra obtained in the solid state show differences, dissolve 10 mg of the substance to be examined and the reference substance separately in 0.4 ml of *methylene chloride R*, evaporate to dryness and record new spectra using the residues.

TESTS

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

Dissolve 0.1 g in 10 ml of *methanol R*.

Specific optical rotation (*2.2.7*): – 49.0 to – 55.0 (anhydrous substance).

Dissolve 0.250 g in *methanol R* and dilute to 25.0 ml with the same solvent.

Related substances. Liquid chromatography (*2.2.29*).

A. Paclitaxel isolated from natural sources or produced by fermentation.

Test solution (a). Dissolve 20.0 mg of the substance to be examined in *acetonitrile R1* and dilute to 10.0 ml with the same solvent.

Test solution (b). Dilute 1.0 ml of test solution (a) to 20.0 ml with *acetonitrile R1*.

Reference solution (a). Dilute 1.0 ml of test solution (a) to 10.0 ml with *acetonitrile R1*. Dilute 1.0 ml of this solution to 100.0 ml with *acetonitrile R1*.

Reference solution (b). Dissolve 5.0 mg of *paclitaxel CRS* in *acetonitrile R1* and dilute to 5.0 ml with the same solvent. Dilute 2.0 ml of this solution to 20.0 ml with *acetonitrile R1*.

Reference solution (c). Dissolve 2.0 mg of *paclitaxel impurity C CRS* in *acetonitrile R1* and dilute to 20.0 ml with the same solvent.

Reference solution (d). Dilute 1.0 ml of reference solution (c) to 50.0 ml with *acetonitrile R1*.

Reference solution (e). To 1 ml of reference solution (b) add 1 ml of reference solution (c).

Reference solution (f). Dissolve 5 mg of *paclitaxel natural for peak identification CRS* (containing impurities A, B, C, D, E, F, H, O, P, Q and R) in *acetonitrile R1* and dilute to 5 ml with the same solvent.

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm;

– *stationary phase*: *diisopropylcyanopropylsilyl silica gel for chromatography R* (5 µm) with a specific surface area of 180 m^2/g and a pore size of 8 nm;

– *temperature*: 20 ± 1 °C.

Mobile phase:

– *mobile phase A*: *methanol R, water R* (200:800 *V/V*);

– *mobile phase B*: *methanol R, acetonitrile for chromatography R* (200:800 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 60	85 → 56	15 → 44
60 - 61	56 → 85	44 → 15
61 - 75	85	15

Flow rate: 1.0 ml/min.

Detection: spectrophotometer at 227 nm.

Injection: 10 µl of test solution (a) and reference solutions (a), (d), (e) and (f).

Identification of impurities: use the chromatogram supplied with *paclitaxel natural for peak identification CRS* and the chromatogram obtained with reference solution (f) to identify the peaks due to impurities A, B, C, D, E, F, H, O, P, Q and R.

Relative retention with reference to paclitaxel (retention time = about 50 min): impurities A and B = about 0.90; impurity R = about 0.93; impurity H = about 0.96; impurities Q and P = about 1.02; impurity C = about 1.05; impurity D = about 1.07; impurities O and E = about 1.15; impurity F = about 1.20.

System suitability: reference solution (e):

– *resolution*: minimum 3.5 between the peaks due to paclitaxel and impurity C.

Limits:

- *sum of impurities E and O*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *impurity R*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *sum of impurities A and B*: not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.4 per cent);
- *impurity C*: not more than 3 times the area of the corresponding peak in the chromatogram obtained with reference solution (d) (0.3 per cent);
- *impurity D*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- *sum of impurities P and Q*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- *impurity F*: not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (0.1 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *total*: not more than 15 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 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).

B. Paclitaxel produced by a semi-synthetic process.

Test solution. Dissolve 10.0 mg of the substance to be examined in *acetonitrile R1* and dilute to 10.0 ml with the same solvent.

Reference solution (a). Dilute 1.0 ml of the test solution to 10.0 ml with *acetonitrile R1*. Dilute 1.0 ml of this solution to 100.0 ml with *acetonitrile R1*.

Reference solution (b). Dissolve 5.0 mg of *paclitaxel CRS* in *acetonitrile R1* and dilute to 5.0 ml with the same solvent.

Reference solution (c). Dissolve 5 mg of *paclitaxel semi-synthetic for peak identification CRS* (containing impurities A, G, I and L) in *acetonitrile R1* and dilute to 5 ml with the same solvent.

Reference solution (d). Dissolve the contents of a vial of *paclitaxel semi-synthetic for system suitability CRS* (containing impurities E, H and N) in 1 ml of *acetonitrile R1*.

Column:

- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (3 µm) with a specific surface area of 300 m^2/g and a pore size of 12 nm;
- temperature: 35 °C.

Mobile phase:

- *mobile phase A*: *acetonitrile for chromatography R, water R* (400:600 *V/V*);
- *mobile phase B*: *acetonitrile for chromatography R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 20	100	0
20 - 60	100 → 10	0 → 90
60 - 62	10 → 100	90 → 0
62 - 70	100	0

Flow rate: 1.2 ml/min.

Detection: spectrophotometer at 227 nm.

Injection: 15 µl of the test solution and reference solutions (a), (c) and (d).

Identification of impurities: use the chromatogram supplied with *paclitaxel semi-synthetic for peak identification CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A, G, I and L; use the chromatogram supplied with *paclitaxel semi-synthetic for system suitability CRS* and the chromatogram obtained with reference solution (d) to identify the peaks due to impurities E, H and N.

Relative retention with reference to paclitaxel (retention time = about 23 min): impurity N = about 0.2; impurity G = about 0.5; impurity A = about 0.8; impurities M, J and H = about 0.9; impurity E = about 1.3; impurity I = about 1.4; impurity L = about 1.5; impurity K = about 2.2.

System suitability: reference solution (d):

- *resolution*: minimum 1.5 between the peaks due to impurity H and paclitaxel.

Limits:

- *correction factor*: for the calculation of content, multiply the peak area of impurity N by 1.29;
- *impurity A*: not more than 7 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.7 per cent);
- *impurity L*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *impurities E, I*: for each impurity, not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.4 per cent);
- *sum of impurities H, J and M*: not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.4 per cent);
- *impurities G, K, N*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (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 (a) (0.10 per cent);
- *total*: not more than 12 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.2 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).

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

Dissolve 1.0 g in *methanol R* and dilute to 20 ml with the same solvent. 12 ml of the solution complies with test B. Prepare the reference solution using 10 ml of lead standard solution (1 ppm Pb), obtained by diluting *lead standard*

solution (100 ppm Pb) R with *methanol R* and 2 ml of the test solution. To 12 ml of each solution, add 2 ml of *buffer solution pH 3.5 R*. Mix. Add 1.2 ml of *thioacetamide reagent R*. The substance will precipitate. Dilute to 40 ml with *methanol R*; the substance re-dissolves completely. Filter the solution through a membrane filter (pore size 0.45 µm). Compare the spots on the filters obtained with the different solutions. The substance to be examined complies with the test if any brownish-black colour in the spot obtained with the test solution is not more intense than that of the spot obtained with the reference solution.

Water (*2.5.32*): maximum 3.0 per cent, determined on 0.050 g.

Microbial contamination

TAMC: acceptance criterion 10^2 CFU/g (*2.6.12*).

Bacterial endotoxins (*2.6.14*): less than 0.4 IU/mg.

ASSAY

A. Paclitaxel isolated from natural sources or produced by fermentation.

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

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

   Calculate the percentage content of $C_{47}H_{51}NO_{14}$ from the declared content of *paclitaxel CRS*.

B. Paclitaxel produced by a semi-synthetic process.

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

   Injection: 10 µl of the test solution and reference solution (b).

   Calculate the percentage content of $C_{47}H_{51}NO_{14}$ from the declared content of *paclitaxel CRS*.

STORAGE

In an airtight container, protected from light.

LABELLING

The label states the origin of the substance:

– isolated from natural sources;

– produced by fermentation;

– produced by a semi-synthetic process.

IMPURITIES

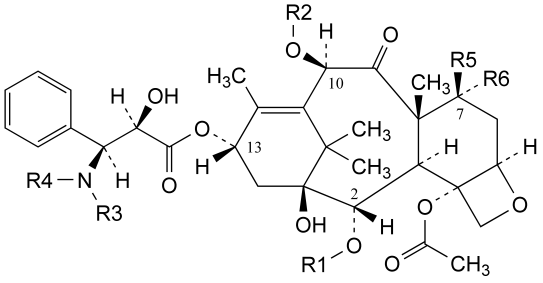
Test A

Specified impurities: A, B, C, D, E, F, O, P, Q, R.

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*): *H.*

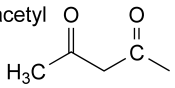
Test B

Specified impurities: A, E, G, H, I, J, K ,L, M, N.

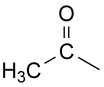


Abbreviations used

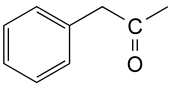
acetylacetyl Aa- =



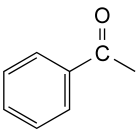
acetyl Ac- =



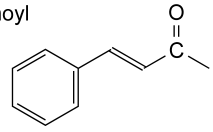
benzeneacetyl Ba- =



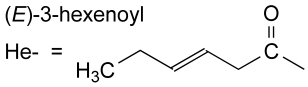
benzoyl Bz- =



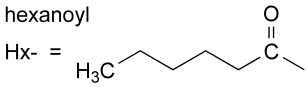
cinnamoyl Cn- =



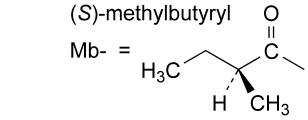
(*E*)-3-hexenoyl He- =



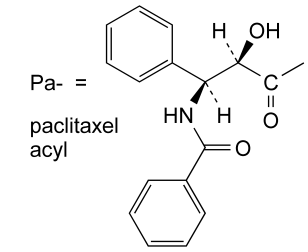
hexanoyl Hx- =



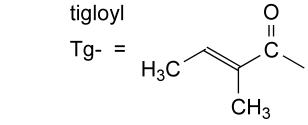
(*S*)-methylbutyryl Mb- =



Pa- = paclitaxel acyl



tigloyl Tg- =



A. R1 = Tg, R2 = Ac, R3 = Bz, R4 = R6 = H, R5 = OH: 2-*O*-debenzoyl-2-*O*-tigloylpaclitaxel,

B. R1 = Bz, R2 = Ac, R3 = Tg, R4 = R6 = H, R5 = OH: *N*-debenzoyl-*N*-tigloylpaclitaxel (cephalomannine),

C. R1 = Bz, R2 = Ac, R3 = Hx, R4 = R6 = H, R5 = OH: *N*-debenzoyl-*N*-hexanoylpaclitaxel (paclitaxel C),

D. R1 = Bz, R2 = Ac, R3 = Tg, R4 = R5 = H, R6 = OH: *N*-debenzoyl-*N*-tigloyl-7-*epi*-paclitaxel (7-*epi*-cephalomannine),

E. R1 = R3 = Bz, R2 = Ac, R4 = R5 = H, R6 = OH: 7-*epi*-paclitaxel,

F. R1 = Bz, R2 = Ac, R3 = Hx, R4 = CH_3, R5 = OH, R6 = H: *N*-debenzoyl-*N*-hexanoyl-*N*-methylpaclitaxel (*N*-methylpaclitaxel C),

G. R1 = R3 = Bz, R2 = R4 = R6 = H, R5 = OH: 10-*O*-deacetylpaclitaxel,

H. R1 = R3 = Bz, R2 = R4 = R5 = H, R6 = OH: 10-*O*-deacetyl-7-*epi*-paclitaxel,

I. R1 = R3 = Bz, R2 = Pa, R4 = R6 = H, R5 = OH: 10-*O*-[(2*R*,3*S*)-3-(benzoylamino)-2-hydroxy-3-phenylpropanoyl]-10-*O*-deacetylpaclitaxel,

J. R1 = R3 = Bz, R2 = Aa, R4 = R6 = H, R5 = OH: 10-*O*-deacetyl-10-*O*-(3-oxobutanoyl)paclitaxel,

K. R1 = R3 = Bz, R2 = Ac, R4 = R6 = H, R5 = O-Si(C_2H_5)$_3$: 7-*O*-(triethylsilanyl)paclitaxel,

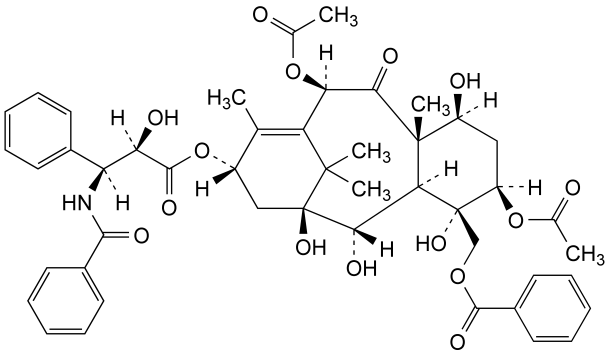
L. R1 = R3 = Bz, R2 = Ac, R4 = R6 = H, R5 = O-CO-CH_3: 7-*O*-acetylpaclitaxel,

O. R1 = Bz, R2 = Ac, R3 = Cn, R4 = R6 = H, R5 = OH: *N*-cinnamoyl-*N*-debenzoylpaclitaxel,

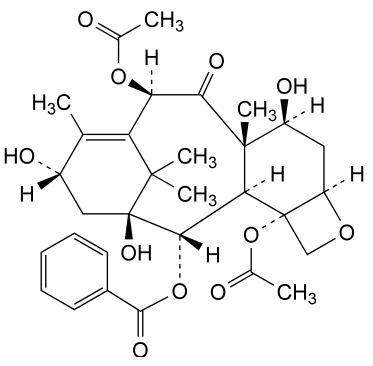
P. R1 = Bz, R2 = Ac, R3 = Ba, R4 = R6 = H, R5 = OH: *N*-debenzoyl-*N*-(phenylacetyl)paclitaxel,

Q. R1 = Bz, R2 = Ac, R3 = He, R4 = R6 = H, R5 = OH: *N*-debenzoyl-*N*-[(3*E*)-hex-3-enoyl]paclitaxel,

R. R1 = Bz, R2 = Ac, R3 = Mb, R4 = R6 = H, R5 = OH: *N*-debenzoyl-*N*-[(2*S*)-2-methylbutanoyl]paclitaxel,



M. 1,2α,4,7β-dihydroxy-9-oxotax-11-ene-5β,10β,13α,20-tetrayl 5,10-diacetate 20-benzoate 13-[(2*R*,3*S*)-3-(benzoylamino)-2-hydroxy-3-phenylpropanoate],



N. 13-*O*-de[(2*R*,3*S*)-3-(benzoylamino)-2-hydroxy-3-phenylpropanoyl]paclitaxel (baccatin III).

01/2009:0350

PANCREAS POWDER

Pancreatis pulvis

DEFINITION

Pancreas powder is prepared from the fresh or frozen pancreases of mammals. It contains various enzymes having proteolytic, lipolytic and amylolytic activities.

1 mg of pancreas powder contains not less than 1.0 Ph. Eur. U. of total proteolytic activity, 15 Ph. Eur. U. of lipolytic activity and 12 Ph. Eur. U. of amylolytic activity.

CHARACTERS

A slightly brown, amorphous powder, partly soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

A. Triturate 0.5 g with 10 ml of *water R* and adjust to pH 8 with *0.1 M sodium hydroxide*, using 0.1 ml of *cresol red solution R* as indicator. Divide the suspension into 2 equal parts (suspension (a) and suspension (b)). Boil suspension (a). To each suspension add 10 mg of *fibrin congo red R*, heat to 38-40 °C and maintain at this temperature for 1 h. Suspension (a) is colourless or slightly pink and suspension (b) is distinctly more red.

B. Triturate 0.25 g with 10 ml of *water R* and adjust to pH 8 with *0.1 M sodium hydroxide*, using 0.1 ml of *cresol red solution R* as indicator. Divide the suspension into 2 equal parts (suspension (a) and suspension (b)). Boil suspension (a). Dissolve 0.1 g of *soluble starch R* in 100 ml of boiling *water R*, boil for 2 min, cool and dilute to 150 ml with *water R*. To 75 ml of the starch solution add suspension (a) and to the remaining 75 ml add suspension (b). Heat each mixture to 38-40 °C and maintain at this temperature for 5 min.

To 1 ml of each mixture add 10 ml of *iodine solution R2*. The mixture obtained with suspension (a) has an intense blue-violet colour; the mixture obtained with suspension (b) has the colour of the iodine solution.

TESTS

Fat content. In an extraction apparatus, treat 1.0 g with *light petroleum R1* for 3 h. Evaporate the solvent and dry the residue at 100-105 °C for 2 h. The residue weighs not more than 50 mg (5.0 per cent).

Loss on drying (*2.2.32*). Not more than 5.0 per cent, determined on 0.50 g by drying at 60 °C at a pressure not exceeding 670 Pa for 4 h.

Microbial contamination

TAMC: acceptance criterion 10^4 CFU/g (*2.6.12*).

TYMC: acceptance criterion 10^2 CFU/g (*2.6.12*).

Absence of *Escherichia coli* (*2.6.13*).

Absence of *Salmonella* (*2.6.13*).

ASSAY

Total proteolytic activity. The total proteolytic activity of pancreas powder is determined by comparing the quantity of peptides non-precipitable by a 50 g/l solution of *trichloroacetic acid R* released per minute from a substrate of casein solution with the quantity of such peptides released by *pancreas powder (protease) BRP* from the same substrate in the same conditions.

Casein solution. Suspend a quantity of *casein BRP* equivalent to 1.25 g of dried substance in 5 ml of *water R*, add 10 ml of *0.1 M sodium hydroxide* and stir for 1 min.