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,

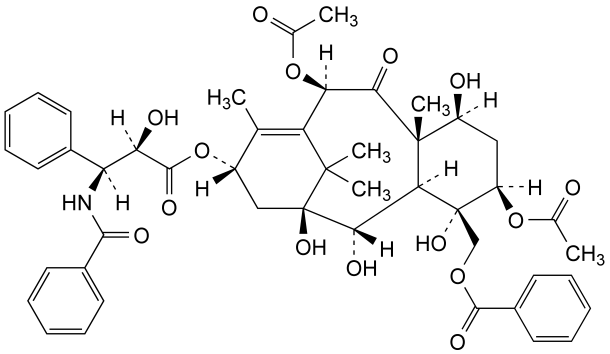
L. R1 = R3 = Bz, R2 = Ac, R4 = R6 = H, R5 = O-CO-CH_3: 7-*O*-acetylpaclitaxel,

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

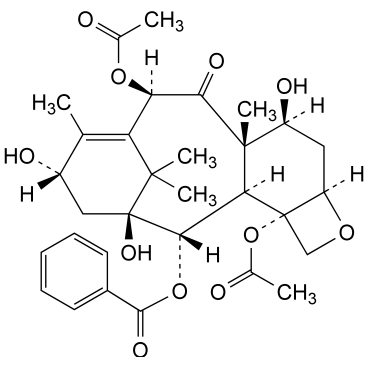
P. R1 = Bz, R2 = Ac, R3 = Ba, R4 = R6 = H, R5 = OH: *N*-debenzoyl-*N*-(phenylacetyl)paclitaxel,

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.

(Determine the water content of *casein BRP* prior to the test by heating at 60 °C *in vacuo* for 4 h.) Add 60 ml of *water R* and stir with a magnetic stirrer until the solution is practically clear. Adjust to pH 8.0 with *0.1 M sodium hydroxide* or *0.1 M hydrochloric acid*. Dilute to 100.0 ml with *water R*. Use the solution on the day of preparation.

Enterokinase solution. Dissolve 50 mg of *enterokinase BRP* in *0.02 M calcium chloride solution R* and dilute to 50.0 ml with the same solvent. Use the solution on the day of preparation.

For the test suspension and the reference suspension, prepare the suspension and carry out the dilution at 0-4 °C.

Test suspension. Triturate 0.100 g of the substance to be examined for 5 min adding gradually 25 ml of *0.02 M calcium chloride solution R*. Transfer completely to a volumetric flask and dilute to 100.0 ml with *0.02 M calcium chloride solution R*. To 10.0 ml of this suspension add 10.0 ml of the enterokinase solution and heat on a water-bath at 35 ± 0.5 °C for 15 min. Cool and dilute with *borate buffer solution pH 7.5 R* at 5 ± 3 °C to a final concentration of about 0.065 Ph. Eur. U. of total proteolytic activity per millilitre calculated on the basis of the stated activity.

Reference suspension. Prepare a suspension of *pancreas powder (protease) BRP* as described for the test suspension but without the addition of enterokinase so as to obtain a known final concentration of about 0.065 Ph. Eur. U. per millilitre calculated on the basis of the stated activity.

Designate tubes in duplicate T, T_b, S_1, S_{1b}, S_2, S_{2b}, S_3, S_{3b}; designate a tube B.

Add *borate buffer solution pH 7.5 R* to the tubes as follows:

B: 3.0 ml,

S_1 and S_{1b}: 2.0 ml,

S_2, S_{2b}, T and T_b: 1.0 ml.

Add the reference suspension to the tubes as follows:

S_1 and S_{1b}: 1.0 ml,

S_2 and S_{2b}: 2.0 ml,

S_3 and S_{3b}: 3.0 ml.

Add 2.0 ml of the test suspension to tubes T and T_b.

Add 5.0 ml of a 50 g/l solution of *trichloroacetic acid R* to tubes B, S_{1b}, S_{2b}, S_{3b} and T_b. Mix by shaking.

Place the tubes and the casein solution in a water-bath at 35 ± 0.5 °C. Place a glass rod in each tube. When temperature equilibrium is reached, add 2.0 ml of the casein solution to tubes B, S_{1b}, S_{2b}, S_{3b} and T_b. Mix. At time zero, add 2.0 ml of casein solution successively and at intervals of 30 s to tubes S_1, S_2, S_3 and T. Mix immediately after each addition. Exactly 30 min after addition of the casein solution, taking into account the regular interval adopted, add 5.0 ml of a 50 g/l solution of *trichloroacetic acid R* to tubes S_1, S_2, S_3 and T. Mix. Withdraw the tubes from the water-bath and allow to stand at room temperature for 20 min.

Filter the contents of each tube twice through the same suitable filter paper previously washed with a 50 g/l solution of *trichloroacetic acid R*, then with *water R* and dried.

A suitable filter paper complies with the following test: filter 5 ml of a 50 g/l solution of *trichloroacetic acid R* on a 7 cm disc of white filter paper; the absorbance (*2.2.25*) of the filtrate, measured at 275 nm using unfiltered trichloroacetic acid solution as the compensation liquid, is less than 0.04.

A schematic presentation of the above operations is shown in Table 0350.-1.

Table 0350.-1

	Tubes								
	S_1	S_{1b}	S_2	S_{2b}	S_3	S_{3b}	T	T_b	B
Buffer solution	2	2	1	1			1	1	3
Reference suspension	1	1	2	2	3	3			
Test suspension							2	2	
Trichloroacetic acid solution		5		5		5		5	5
Mix		+		+		+		+	+
Water-bath 35 °C	+	+	+	+	+	+	+	+	+
Casein solution		2		2		2		2	2
Mix		+		+		+		+	+
Casein solution	2		2		2		2		
Mix	+		+		+		+		
Water-bath 35 °C 30 min	+	+	+	+	+	+	+	+	+
Trichloroacetic acid solution	5		5		5		5		
Mix	+		+		+		+		
Room temperature 20 min	+	+	+	+	+	+	+	+	+
Filter	+	+	+	+	+	+	+	+	+

Measure the absorbance (*2.2.25*) of the filtrates at 275 nm using the filtrate obtained from tube B as the compensation liquid.

Correct the average absorbance values for the filtrates obtained from tubes S_1, S_2 and S_3 by subtracting the average values obtained for the filtrates from tubes S_{1b}, S_{2b} and S_{3b} respectively. Draw a calibration curve of the corrected values against volume of reference suspension used.

Determine the activity of the substance to be examined using the corrected absorbance for the test suspension ($T - T_b$) and the calibration curve and taking into account the dilution factors.

The test is not valid unless the corrected absorbance values are between 0.15 and 0.60.

Lipolytic activity. The lipolytic activity is determined by comparing the rate at which a suspension of pancreas powder hydrolyses a substrate of olive oil emulsion with the rate at which a suspension of *pancreas powder (amylase and lipase) BRP* hydrolyses the same substrate under the same conditions. *The test is carried out under nitrogen.*

Olive oil stock emulsion. In an 800 ml beaker 9 cm in diameter, place 40 ml of *olive oil R*, 330 ml of *acacia solution R* and 30 ml of *water R*. Place an electric mixer at the bottom of the beaker. Place the beaker in a vessel containing *ethanol (96 per cent) R* and a sufficient quantity of ice as a cooling mixture. Emulsify using the mixer at an average speed of 1000-2000 r/min. Cool to 5-10 °C. Increase the mixing speed to 8000 r/min. Mix for 30 min keeping the temperature below 25 °C by the continuous addition of crushed ice into the cooling mixture. (A mixture of calcium chloride and crushed ice is also suitable). Store the stock emulsion in a refrigerator and use within 14 days. The emulsion must not separate into 2 distinct layers. Check the diameter of the globules of the emulsion under a microscope. At least 90 per cent have a diameter below 3 µm and none has a diameter greater than 10 µm. Shake the emulsion thoroughly before preparing the emulsion substrate.

Olive oil emulsion. For 10 determinations, mix the following solutions in the order indicated: 100 ml of the stock emulsion, 80 ml of *tris(hydroxymethyl)aminomethane*

solution R1, 20 ml of a freshly prepared 80 g/l of *sodium taurocholate BRP* and 95 ml of *water R*. Use on the day of preparation.

Apparatus. Use a reaction vessel of about 50 ml capacity provided with:

– a device that will maintain a temperature of 37 ± 0.5 °C;

– a magnetic stirrer;

– a lid with holes for the insertion of electrodes, the tip of a burette, a tube for the admission of nitrogen and the introduction of reagents.

An automatic or manual titration apparatus may be used. In the latter case, the burette is graduated in 0.005 ml and the pH-meter is provided with a wide reading scale and glass-calomel or glass-silver-silver chloride electrodes. After each test the reaction vessel is evacuated by suction and washed several times with *water R*, the washings being removed each time by suction.

Test suspension. In a small mortar cooled to 0-4 °C, triturate carefully a quantity of the substance to be examined equivalent to about 2500 Ph. Eur. U. of lipolytic activity with 1 ml of cooled *maleate buffer solution pH 7.0 R* (lipase solvent) until a very fine suspension is obtained. Dilute the suspension with cold *maleate buffer solution pH 7.0 R*, transfer quantitatively to a volumetric flask and dilute to 100.0 ml with the cold buffer solution. Keep the flask containing the test suspension in iced water during the titration.

Reference suspension. To avoid absorption of water formed by condensation, allow the reference preparation to reach room temperature before opening the container. Prepare a suspension of *pancreas powder (amylase and lipase) BRP* as described for the test suspension using a quantity equivalent to about 2500 Ph. Eur. U.

Carry out the titrations immediately after preparation of the test suspension and the reference suspension. Place 29.5 ml of olive oil emulsion in the reaction vessel equilibrated at 37 ± 0.5 °C. Fit the vessel with the electrodes, a stirrer and the burette (the tip being immersed in the olive oil emulsion).

Put the lid in place and switch on the apparatus. Carefully add *0.1 M sodium hydroxide* with stirring to adjust to pH 9.2. Using a rapid-flow graduated pipette transfer about 0.5 ml of the previously homogenised reference suspension, start the chronometer and add continuously *0.1 M sodium hydroxide* to maintain the pH at 9.0. After exactly 1 min, note the volume of *0.1 M sodium hydroxide* used. Carry out the measurement a further 4 times. Discard the first reading and determine the average of the 4 others (S_1). Make 2 further determinations (S_2 and S_3). Calculate the average of the values S_1, S_2 and S_3. The average volume of *0.1 M sodium hydroxide* used should be about 0.12 ml per minute with limits of 0.08 ml to 0.16 ml.

Carry out 3 determinations in the same manner for the test suspension (T_1, T_2 and T_3). If the quantity of *0.1 M sodium hydroxide* used is outside the limits of 0.08 ml to 0.16 ml per minute, the assay is repeated with a quantity of test suspension that is more suitable but situated between 0.4 ml and 0.6 ml. Otherwise the quantity of the substance to be examined is adjusted to comply with the conditions of the test. Calculate the average of the values T_1, T_2 and T_3.

Calculate the activity in European Pharmacopoeia Units per milligram using the following expression:

$$\frac{n \times m_1}{n_1 \times m} \times A$$

n = average volume of *0.1 M sodium hydroxide* used per minute during the titration of the test suspension, in millilitres;

n_1 = average volume of *0.1 M sodium hydroxide* used per minute during the titration of the reference suspension, in millilitres;

m = mass of the substance to be examined, in milligrams;

m_1 = mass of the reference preparation, in milligrams;

A = activity of *pancreas powder (amylase and lipase) BRP*, in European Pharmacopoeia Units per milligram.

Amylolytic activity. The amylolytic activity is determined by comparing the rate at which a suspension of pancreas powder hydrolyses a substrate of starch solution with the rate at which a suspension of *pancreas powder (amylase and lipase) BRP* hydrolyses the same substrate under the same conditions.

Starch solution. To a quantity of *starch BRP* equivalent to 2.0 g of the dried substance add 10 ml of *water R* and mix. (Determine the water content of *starch BRP* prior to the test by heating at 120 °C for 4 h). Add this suspension, whilst stirring continuously, to 160 ml of boiling *water R*. Wash the container several times with successive quantities, each of 10 ml, of *water R* and add the washings to the hot starch solution. Heat to boiling, stirring continuously. Cool to room temperature and dilute to 200 ml with *water R*. Use the solution on the day of preparation.

For the test suspension and the reference suspension, prepare the suspension and carry out the dilution at 0-4 °C.

Test suspension. Triturate a quantity of the substance to be examined equivalent to about 1500 Ph. Eur. U. of amylolytic activity with 60 ml of *phosphate buffer solution pH 6.8 R1* for 15 min. Transfer quantitatively to a volumetric flask and dilute to 100.0 ml with *phosphate buffer solution pH 6.8 R1*.

Reference suspension. Prepare a suspension of *pancreas powder (amylase and lipase) BRP* as described for the test suspension, using a quantity equivalent to about 1500 Ph. Eur. U.

In a test tube 200 mm long and 22 mm in diameter, fitted with a ground-glass stopper, place 25.0 ml of starch solution, 10.0 ml of *phosphate buffer solution pH 6.8 R1* and 1.0 ml of an 11.7 g/l solution of *sodium chloride R*. Close the tube, shake and place in a water-bath at 25.0 ± 0.1 °C. When the temperature equilibrium has been reached, add 1.0 ml of the test suspension and start the chronometer. Mix and place the tube in the water-bath. After exactly 10 min, add 2 ml of *1 M hydrochloric acid*. Transfer the mixture quantitatively to a 300 ml conical flask fitted with a ground-glass stopper. Whilst shaking continuously, add 10.0 ml of *0.05 M iodine* immediately followed by 45 ml of *0.1 M sodium hydroxide*. Allow to stand in the dark at a temperature between 15 °C and 25 °C for 15 min. Add 4 ml of a mixture of 1 volume of *sulphuric acid R* and 4 volumes of *water R*. Titrate the excess of iodine with *0.1 M sodium thiosulphate* using a microburette. Carry out a blank titration adding the 2 ml of *1 M hydrochloric acid* before introducing the test suspension. Carry out the titration of the reference suspension in the same manner.

Calculate the amylolytic activity in European Pharmacopoeia Units per milligram using the following expression:

$$\frac{(n' - n)\, m_1}{(n'_1 - n_1)\, m} \times A$$

n = volume of *0.1 M sodium thiosulphate* used in the titration of the test suspension, in millilitres;

n_1 = volume of *0.1 M sodium thiosulphate* used in the titration of the reference suspension, in millilitres;

n' = volume of *0.1 M sodium thiosulphate* used in the blank titration of the test suspension, in millilitres;

n'_1 = volume of *0.1 M sodium thiosulphate* used in the blank titration of the reference suspension, in millilitres;

m = mass of the substance to be examined, in milligrams;

m_1 = mass of the reference preparation, in milligrams;

A = activity of *pancreas powder (amylase and lipase) BRP*, in European Pharmacopoeia Units per milligram.

STORAGE

Store in an airtight container.

01/2009:2403

PEA STARCH

Pisi amylum

DEFINITION

Pea starch is obtained from the seeds of *Pisum sativum* L.

CHARACTERS

Appearance: white or almost white, very fine powder.

Solubility: practically insoluble in cold water and in ethanol (96 per cent).

IDENTIFICATION

A. Examined under a microscope using equal volumes of *glycerol R* and *water R*, it presents a majority of large elliptical granules, 25-45 µm in size, sometimes irregular, or reniform. It also presents a minority of small rounded granules, 5-8 µm in size. Granules can present cracks or irregularities. Sometimes, granules show barely visible concentric striations. Exceptionally, granules show a slit along the main axis. Between orthogonally oriented polarising plates or prisms, the granules show a distinct black cross.

B. Suspend 1 g in 50 ml of *water R*, boil for 1 min and cool. A thin, cloudy mucilage is formed.

C. To 1 ml of the mucilage obtained in identification test B, add 0.05 ml of *iodine solution R1*. A dark blue colour is produced, which disappears on heating.

TESTS

pH (*2.2.3*): 5.0 to 8.0.

Shake 5.0 g with 25.0 ml of *carbon dioxide-free water R* for 60 s. Allow to stand for 15 min and shake again.

Foreign matter. Examined under a microscope using a mixture of equal volumes of *glycerol R* and *water R*, not more than traces of matter other than starch granules are present. No starch granules of any other origin are present.

Oxidising substances (*2.5.30*): maximum 20 ppm, calculated as H_2O_2.

Sulphur dioxide (*2.5.29*): maximum 50 ppm.

Iron (*2.4.9*): maximum 50 ppm.

Shake 1.0 g with 50 ml of *dilute hydrochloric acid R*. Filter. The filtrate complies with the test for iron.

Loss on drying (*2.2.32*): maximum 16.0 per cent, determined on 1.000 g by drying in an oven at 130 °C for 90 min.

Sulphated ash (*2.4.14*): maximum 0.6 per cent, determined on 1.0 g.

Microbial contamination

TAMC: acceptance criterion 10^3 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*).

01/2009:0682

PEPSIN POWDER

Pepsini pulvis

[9001-75-6]

DEFINITION

Pepsin powder is prepared from the gastric mucosa of pigs, cattle or sheep. It contains gastric proteinases, active in acid medium (pH 1 to 5). It has an activity not less than 0.5 Ph. Eur. U./mg, calculated with reference to the dried substance.

PRODUCTION

The animals from which pepsin powder is derived must fulfil the requirements for the health of animals suitable for human consumption.

CHARACTERS

A white or slightly yellow, crystalline or amorphous powder, hygroscopic, soluble in water, practically insoluble in ethanol (96 per cent). The solution in water may be slightly opalescent with a weak acidic reaction.

IDENTIFICATION

In a mortar, pound 30 mg of *fibrin blue R*. Suspend in 20 ml of *dilute hydrochloric acid R2*. Filter the suspension on a filter paper and wash with *dilute hydrochloric acid R2* until a colourless filtrate is obtained. Perforate the filter paper and wash the *fibrin blue R* through it into a conical flask using 20 ml of *dilute hydrochloric acid R2*. Shake before use. Dissolve a quantity of the substance to be examined, equivalent to not less than 20 Ph. Eur. U., in 2 ml of *dilute hydrochloric acid R2* and adjust to pH 1.6 ± 0.1. Add 1 ml of this solution to a test-tube containing 4 ml of the fibrin blue suspension, mix and place in a water-bath at 25 °C with gentle shaking. Prepare a blank solution at the same time and in the same manner using 1 ml of *water R*. After 15 min of incubation the blank solution is colourless and the test solution is blue.