Edition: BP 2025 (Ph. Eur. 11.6 update)

Pepsin

General Notices

(Pepsin Powder, Ph. Eur. monograph 0682)

9001-75-6

Action and use

Proteolytic enzyme.

Ph Eur

DEFINITION

Powder prepared from the gastric mucosa of pigs, cattle or sheep. It contains gastric proteinases, active in acid medium (pH 1 to 5).

Activity Not less than 0.5 Ph. Eur. U./mg (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

Appearance

White or slightly yellow, crystalline or amorphous powder, hygroscopic.

Solubility

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.

TESTS

Loss on drying (*2.2.32*)

Maximum 5.0 per cent, determined on 0.500 g by drying at 60 °C over *diphosphorus pentoxide R* 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

The activity of pepsin powder is determined by comparing the quantity of peptides, non-precipitable by *trichloroacetic acid solution R* and assayed using the *phosphomolybdotungstic reagent R*, which are released per minute from a substrate of *haemoglobin solution R*, with the quantity of such peptides released by *pepsin powder BRP* from the same substrate in the same conditions.

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

Avoid shaking and foaming during preparation of the test and reference solutions.

Test solution Immediately before use, prepare a solution of the substance to be examined expected to contain 0.5 Ph. Eur. U./mL in *dilute hydrochloric acid R2*; before dilution to volume, adjust to pH 1.6 ± 0.1, if necessary, using *1 M hydrochloric acid*.

Reference solution Less than 15 min before use, prepare a solution of *pepsin powder BRP* containing 0.5 Ph. Eur. U./mL in *dilute hydrochloric acid R2*; before dilution to volume, adjust to pH 1.6 ± 0.1, if necessary, using *1 M hydrochloric acid*.

Table 0682.-1

	Tubes								
	S_1	S_{1b}	S_2	S_{2b}	S_3	S_{3b}	T	T_b	B
Dilute hydrochloric acid R2 (mL)	0.5	0.5	0.25	0.25			0.25	0.25	1.0
Reference solution (mL)	0.5	0.5	0.75	0.75	1.0	1.0			
Test solution (mL)							0.75	0.75	
Trichloroacetic acid solution R (mL)		10.0		10.0		10.0		10.0	10.0
Mix		+		+		+		+	+
Water bath at 25 °C	+	+	+	+	+	+	+	+	+
Haemoglobin solution R (mL)		5.0		5.0		5.0		5.0	5.0
Mix		+		+		+		+	+
Haemoglobin solution R (mL)	5.0		5.0		5.0		5.0		
Mix	+		+		+		+		
Water bath at 25 °C, 10 min	+	+	+	+	+	+	+	+	+
Trichloroacetic acid solution R (mL)	10.0		10.0		10.0		10.0		

Tubes									
	S_1	S_{1b}	S_2	S_{2b}	S_3	S_{3b}	T	T_b	B
Mix	+		+		+		+		
Filter	+	+	+	+	+	+	+	+	+

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

Add *dilute hydrochloric acid R2* to the tubes as follows:

B: 1.0 mL

S_1 and S_{1b}: 0.5 mL

S_2, S_{2b} and T and T_b: 0.25 mL

Add the reference solution to the tubes as follows:

S_1 and S_{1b}: 0.5 mL

S_2 and S_{2b}: 0.75 mL

S_3 and S_{3b}: 1.0 mL

Add 0.75 mL of the test solution to tubes T and T_b.

Add 10.0 mL of *trichloroacetic acid solution R* to tubes S_{1b}, S_{2b}, S_{3b}, T_b and B. Mix by shaking.

Place the tubes and *haemoglobin solution R* in a water bath at 25 ± 0.1 °C. When temperature equilibrium is reached, add 5.0 mL of *haemoglobin solution R* to tubes B, S_{1b}, S_{2b}, S_{3b} and T_b. Mix.

At time zero add 5.0 mL of *haemoglobin solution R* successively and at intervals of 30 s to tubes S_1, S_2, S_3 and T.

Mix immediately after each addition.

Exactly 10 min after adding the *haemoglobin solution R*, stop the reaction by adding, at intervals of 30 s, 10.0 mL of *trichloroacetic acid solution R* to tubes S_1, S_2, S_3 and T (the use of a fast-flowing or blow-out pipette is recommended) and mix.

Filter the contents of each tube (samples and blanks) 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. Discard the first 5 mL of filtrate. Place 3.0 mL of each filtrate separately in a tube containing 20 mL of *water R*. Mix.

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

Add to each tube 1.0 mL of *sodium hydroxide solution R* and 1.0 mL of *phosphomolybdotungstic reagent R*, beginning with the blanks and then the samples of each set, in a known order.

A schematic presentation of the above operations is shown in Table 0682.-1.

After 15 min measure the absorbance (*2.2.25*) of solutions S_1, S_2, S_3, S_{1b}, S_{2b}, S_{3b} and T at 540 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}, S_{3b} respectively.

Draw a calibration curve of the corrected values against volume of reference solution used. Determine the activity of the substance to be examined using the corrected absorbance for the test solution (T - T_b) together with the calibration curve and taking into account the dilution factors.

STORAGE

Store in an airtight container, protected from light, at a temperature of 2 °C to 8 °C.

LABELLING

The label states the activity in European Pharmacopoeia Units per milligram.

Ph Eur