Trypsin

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Manufacturers, regulatory authorities, health authorities, researchers, and other stakeholders are invited to provide their feedback and comments on this draft proposal. Comments and samples received after the last date will not be considered by the IPC before finalizing the monograph.

Please send any comments you may have on this draft document to arnd-ipc@gov.in , with a copy to Dr. Gaurav Pratap Singh (email: gpsingh.ipc@gov.in) before the last date for comments.

Document History and Schedule for the Adoption Process

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Trypsin

Trypsin is a proteolytic enzyme obtained by the activation of trypsinogen extracted from the pancreas of mammals. It has an activity of not less than 0.5 microkatal per milligram, calculated with reference to the dried substance. In solution, it has maximum enzymic activity at pH 8; the activity is reversibly inhibited at pH 3, the pH at which it is most stable.

Category. Proteolytic enzyme.

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

Description. A white or almost white, crystalline or amorphous powder, hygroscopic if amorphous.

Identification

A. A 1.0 per cent w/v solution in *carbon dioxide-free water* (Solution A). Dilute 1 ml of solution A to 100 ml with *water*. In a depression in a white spot plate, mix 0.1 ml of the solution with 0.2 ml of *tosylarginine methyl ester hydrochloride solution*; a reddish violet colour develops within 3 minutes.

B. Dilute 0.5 ml of solution A to 5 ml with *water*. Add 0.1 ml of a 2 per cent w/v solution of *tosyl-lysyl-chloromethane hydrochloride*, adjusted to pH 7.0 and shake for 2 hours and dilute to 50 ml with *water*. In one of the depressions of a white spot plate, mix 0.1 ml of the solution with 0.2 ml of the *tosylarginine methyl ester hydrochloride solution*; no reddish violet colour develops within 3 minutes.

Tests

Appearance of solution. Solution A is not more opalescent than Opalescence standard OS3 (2.4.1).

pH (2.4.24). 3.0 to 6.0, determined in solution A.

Light absorption. Dissolve 30 mg in *0.001 M hydrochloric acid* and dilute to 100.0 ml with *0.001 M hydrochloric acid*. The resulting solution shows a specific absorbance of 13.5 to 16.5 at absorption maximum at 280 nm (2.4.7) and specific absorbance of 7.0 at absorption minimum at 250 nm (2.4.7).

Chymotrypsin.
Test solution. To 1.8 ml of *buffer solution pH 8.0*, and 7.4 ml of *water* and 0.5 ml of *0.2 M acetyltyrosine ethyl ester*. While shaking the solution, add 0.3 ml of solution A and start a timer. After exactly 5 minutes, measure the pH (2.4.24).

Reference solution. Prepare in the same manner as the test solution, replacing solution A by 0.3 ml of 0.05 per cent w/v solution of *chymotrypsin IPRS* and measure the pH exactly 5 minutes after adding the chymotrypsin.

The pH of the test solution is higher than that of the reference solution.

Loss on drying (2.4.19). Not more than 5.0 per cent, determined on 0.5 g by drying at 60° at a pressure not exceeding 0.7 kPa for 2 hours.

Microbial contamination (2.2.9). The total aerobic viable count is not more than 10^4 CFU per g and the total combined yeasts and molds count is not more than 10^2 CFU per g. 1 g is free from *Escherichia coli* and 10 g is free from *Salmonella*.

Assay. The activity of trypsin is determined by comparing the rate at which it hydrolyses *benzoylarginine ethyl ester hydrochloride* with the rate at which *trypsin IPRS* hydrolyses the same substrate under the same conditions.
Apparatus. Use a reaction vessel of about 30 ml capacity provided with:
– a device that will maintain a temperature of 25.0 ± 0.1°,

– a stirring device (for example, 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 device may be used. For the latter, the burette is graduated in 0.005 ml and the pH meter is provided with a wide range scale and glass-silver-silver chloride electrodes or other suitable electrodes.

NOTE – Store the test solutions and reference solutions at 0° to 5°.

Test solution. Dissolve a suitable quantity of the substance under examination in *0.001 M hydrochloric acid* and dilute to 25.0 ml with *0.001 M hydrochloric acid* to obtain a solution containing 700 nanokatals per millilitre.

Reference solution. Dissolve a suitable quantity of *trypsin IPRS* in *0.001 M hydrochloric acid* and dilute to 25.0 ml with *0.001 M hydrochloric acid* to obtain a solution containing 700 nanokatals per millilitre.

Store the solutions at 0° to 5°, Warm 1 ml of each solution to about 25° over 15 minutes and use 50 µl of each solution for each titration. Carry out the titration in an atmosphere of nitrogen. Transfer 10.0 ml of *0.0015 M borate buffer pH 8.0* to the reaction vessel and, while stirring, add 1.0 ml of a freshly prepared 0.686 per cent w/v solution of *benzoylarginine ethyl ester hydrochloride*. When the temperature is steady at 25.0 ± 0.1° (after about 5 minutes) adjust to pH 8.0, exactly with *0.1 M sodium hydroxide*. Add 50 µl of the test solution and start a timer. Maintain the pH at 8.0 by the addition of *0.1 M sodium hydroxide*, the tip of the microburette being immersed in the solution; note the volume added every 30 seconds. Follow the reaction for 8 minutes. Calculate the volume of *0.1 M sodium hydroxide* used per second. Carry out a titration in the same manner using the reference solution and calculate the volume of *0.1 M sodium hydroxide* used per second.

Calculate the activity in microkatals per milligram, using the following expression;

$$\frac{\mathrm{W1} \times \mathrm{V}}{W \times V1} \times A$$

Where, W1= weight of trypsin IPRS (in mg),
W= weight of the substance (in mg),
V= Volume of *0.1 M sodium hydroxide* used per second by the test solution,
V1= Volume of *0.1 M sodium hydroxide* used per second by the reference solution,
A= Activity of trypsin IPRS in microkatals per mg.

Storage. Store protected from light and moisture at a temperature of 2° to 8°.

Labelling. The label states the activity in microkatals per mg; for the amorphous substance, that it is hygroscopic.

Solubility. Sparingly Soluble in *water*.

4.2 GENERAL REAGENTS
Insert before **Tosylphenylanychloromethane**

Tosylarginine methyl ester hydrochloride solution; To 98.5 mg of *tosylarginine methyl ester hydrochloride*, add 5 ml of *tris(hydroxymethyl)aminomethane buffer pH 8.1* and shake to dissolve. Add 2.5 ml of *methyl red mixed solution* and dilute to 25.0 ml with *water*.

Tosyl-lysyl-chloromethane hydrochloride; *N*-Tosyl-L-lysyl-chloromethane hydrochloride; (3*S*)-7-Amino-1-chloro-3-(4-methylbenzenesulfonamido)heptan-2-one hydrochloride: $C_{14}H_{22}Cl_2N_2O_3S$ = 369.3.
General reagent grade of commerce.
mp, about 155˚. $[\alpha]_D^{20}$=-7 to -9 at a temperature of 20˚; $A^{1\%}_{1cm}$= 310 to 340, determined at 230 nm in *water*.