PRODUCTS THAT SHOW PARTICLES AFTER RECONSTITUTION. If a few particles remain when the preparation is reconstituted, it shall be demonstrated during validation studies that the potency is not significantly affected after passage of the preparation through the filter to be provided with the preparation.

CHARACTERS

Appearance: white or pale yellow, hygroscopic powder or friable solid.

Reconstitute the preparation to be examined as stated on the label immediately before carrying out the identification, tests (except those for solubility and water) and assay.

IDENTIFICATION

The assay for von Willebrand factor serves also to identify the preparation.

TESTS

Solubility. To a container of the preparation to be examined add the volume of the solvent stated on the label at the recommended temperature. The preparation dissolves completely with gentle swirling within 10 min, giving a clear or slightly opalescent, colourless or slightly yellow solution.

In addition, where the label states that the product may show a few particles after reconstitution, reconstitute the preparation as described on the label and pass it through the filter provided: the filtered solution is clear or slightly opalescent.

pH (*2.2.3*): 6.5 to 7.5.

Osmolality (*2.2.35*): minimum 240 mosmol/kg.

Total protein. If necessary, dilute an accurately measured volume of the preparation to be examined with a 9 g/l solution of *sodium chloride R*, to obtain a solution that may be expected to contain about 15 mg of protein in 2 ml. Place 2.0 ml of this solution in a round-bottomed centrifuge tube and add 2 ml of a 75 g/l solution of *sodium molybdate R* and 2 ml of a mixture of 1 volume of *nitrogen-free sulphuric acid R* and 30 volumes of *water R*. Shake, centrifuge for 5 min, decant the supernatant liquid and allow the inverted tube to drain on filter paper. Determine the nitrogen in the residue by the method of sulphuric acid digestion (*2.5.9*) and calculate the amount of protein by multiplying the result by 6.25. *For some products, especially those without a protein stabiliser, this method may not be applicable. Another validated method for protein determination must therefore be performed.*

Anti-A and anti-B haemagglutinins (*2.6.20*). Dilute the preparation to be examined with a 9 g/l solution of *sodium chloride R* to contain 6 IU of von Willebrand factor activity per millilitre. The 1 to 64 dilutions do not show agglutination.

Water. Determined by a suitable method, such as the semi-micro determination of water (*2.5.12*), loss on drying (*2.2.32*) or near infrared spectrophotometry (*2.2.40*), the water content is within the limits approved by the competent authority.

Sterility (*2.6.1*). It complies with the test for sterility.

Pyrogens (*2.6.8*). It complies with the test for pyrogens. Inject per kilogram of the rabbit's mass a volume of the preparation to be examined equivalent to not less than 100 IU of von Willebrand factor activity.

ASSAY

von Willebrand factor (*2.7.21*). The estimated potency is not less than 80 per cent and not more than 120 per cent of the stated potency. The confidence limits (P = 0.95) are not less than 80 per cent and not more than 120 per cent of the estimated potency.

Pending the availability of an International Standard for von Willebrand factor concentrate calibrated for use in the collagen-binding assay, only the ristocetin cofactor assay may be used.

Factor VIII (*2.7.4*). The assay is carried out where the factor VIII content is greater than 10 IU of factor VIII per 100 IU of von Willebrand factor activity. The estimated potency is not less than 60 per cent and not more than 140 per cent of the stated potency. The confidence limits (P = 0.95) are not less than 80 per cent and not more than 120 per cent of the estimated potency.

STORAGE

In an airtight container, protected from light.

LABELLING

The label states:

- the number of International Units of von Willebrand factor in the container;
- the number of International Units of factor VIII in the container, or that the content of factor VIII is less than or equal to 10 IU of factor VIII per 100 IU of von Willebrand factor activity;
- the amount of protein in the container;
- the name and quantity of any added substance;
- the name and volume of the liquid to be used for reconstitution;
- where applicable, that the preparation may show the presence of a few particles after reconstitution;
- that the transmission of infectious agents cannot be totally excluded when medicinal products prepared from human blood or plasma are administered.

01/2008:0912

HYALURONIDASE

Hyaluronidasum

[9001-54-1]

DEFINITION

Hyaluronidase is an enzyme extracted from mammalian testes (for example bovine testes) and capable of hydrolysing mucopolysaccharides of the hyaluronic acid type. It contains not less than 300 IU of hyaluronidase activity per milligram, calculated with reference to the dried substance. It may contain a suitable stabiliser.

PRODUCTION

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

CHARACTERS

A white or yellowish-white, amorphous powder, soluble in water, practically insoluble in acetone and in ethanol.

IDENTIFICATION

A solution containing the equivalent of 100 IU of hyaluronidase in 1 ml of a 9 g/l solution of *sodium chloride R* depolymerises an equal volume of a 10 g/l solution of *sodium hyaluronate BRP* in 1 min at 20 °C as shown by a pronounced decrease in viscosity. This action is destroyed by heating the hyaluronidase at 100 °C for 30 min.

TESTS

Appearance of solution. Dissolve 0.10 g of the substance to be examined in *water R* and dilute to 10 ml. The solution is clear (*2.2.1*).

pH (*2.2.3*). Dissolve 30 mg in 10 ml of *carbon dioxide-free water R*. The pH of the solution is 4.5 to 7.5.

Loss on drying (*2.2.32*). Not more than 5.0 per cent, determined on 0.500 g by drying at 60 °C at a pressure not exceeding 670 Pa for 2 h.

Bacterial endotoxins (*2.6.14*): less than 0.2 IU per IU of hyaluronidase.

ASSAY

The activity of hyaluronidase is determined by comparing the rate at which it hydrolyses *sodium hyaluronate BRP* with the rate obtained with the International Standard, or a reference preparation calibrated in International Units, using a slope-ratio assay.

Substrate solution. To 0.10 g of *sodium hyaluronate BRP* in a 25 ml conical flask add slowly 20.0 ml of *water R* at 4 °C. The rate of addition must be slow enough to allow the substrate particles to swell (about 5 min). Maintain at 4 °C and stir for at least 12 h. Store at 4 °C and use within 4 days.

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

Test solution. Dissolve a suitable amount of the substance to be examined in *hyaluronidase diluent R* so as to obtain a solution containing 0.6 ± 0.3 IU of hyaluronidase per millilitre.

Reference solution. Dissolve a suitable amount of *hyaluronidase BRP* in *hyaluronidase diluent R* so as to obtain a solution containing 0.6 IU of hyaluronidase per millilitre.

In a reaction vessel, mix 1.50 ml of *phosphate buffer solution pH 6.4 R* and 1.0 ml of the substrate solution and equilibrate at 37 ± 0.1 °C. At time t_1 = 0 (first chronometer) add 0.50 ml of the test solution containing E_t mg of the enzyme to be examined, mix, measure the viscosity of the solution using a suitable viscometer maintained at 37 ± 0.1 °C and record the outflow time t_2 using a second chronometer (graduated in 0.1 second intervals), several times during about 20 min (read on the first chronometer). The following viscometer has been found suitable: Ubbelohde microviscometer (DIN 51 562, Part 2), capillary type MII, viscometer constant about 0.1 mm^2/s^2.

Repeat the procedure using 0.50 ml of the reference solution containing E_r mg of *hyaluronidase BRP*.

Calculate the viscosity ratio from the expression:

$$\eta_r = \frac{k \times t_2}{0.6915}$$

k = the viscometer constant in mm^2/s^2 (indicated on the viscometer);

t_2 = the outflow time (in seconds) of the solution;

0.6915 = the kinematic viscosity in mm^2/s of the buffer solution at 37 °C.

Since the enzymatic reaction continues during the outflow time measurements, the real reaction time equals $t_1 + t_2/2$, half of the outflow time ($t_2/2$) for which a certain measurement is valid being added to the time t_1 at which the measurement is started. Plot $(\ln \eta_r)^{-1}$ as a function of the reaction time ($t_1 + t_2/2$) in seconds. A linear relationship is obtained. Calculate the slope for the substance to be examined (b_t) and the reference preparation (b_r).

Calculate the specific activity in International Units per milligram from the expression:

$$\frac{b_t}{b_r} \times \frac{E_r}{E_t} \times A$$

A = the specific activity of *hyaluronidase BRP* in International Units per milligram.

Carry out the complete procedure at least three times and calculate the average activity of the substance to be examined.

STORAGE

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

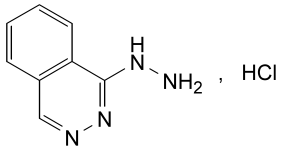
LABELLING

The label states the activity in International Units per milligram.

01/2008:0829

HYDRALAZINE HYDROCHLORIDE

Hydralazini hydrochloridum



$C_8H_9ClN_4$ M_r 196.6

[304-20-1]

DEFINITION

1-Hydrazinophthalazine hydrochloride.

Content: 98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: soluble in water, slightly soluble in ethanol (96 per cent), very slightly soluble in methylene chloride.

mp: about 275 °C, with decomposition.

IDENTIFICATION

First identification: B, E.

Second identification: A, C, D, E.

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution. Dissolve 50 mg in *water R* and dilute to 100 ml with the same solvent. Dilute 2 ml of this solution to 100 ml with *water R*.

Spectral range: 220-350 nm.

Absorption maxima: at 240 nm, 260 nm, 303 nm and 315 nm.

Absorbance ratio: A_{240}/A_{303} = 2.0 to 2.2.