Heparin (*2.7.5*): maximum 0.1 IU of heparin activity per International Unit of antithrombin III activity. It is necessary to validate the method for assay of heparin for each specific preparation to be examined to allow for interference by antithrombin III.

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 50 IU of antithrombin III, calculated from the activity stated on the label.

ASSAY

Assay of human antithrombin III (*2.7.17*).

The estimated potency is not less than 80 per cent and not more than 120 per cent of the potency stated on the label. The confidence limits (P = 0.95) are not less than 90 per cent and not more than 110 per cent of the estimated potency.

STORAGE

Protected from light, in an airtight container.

LABELLING

The label states:

- the content of antithrombin III expressed in International Units per container.
- the name and volume of solvent to be used to reconstitute the preparation,
- where applicable, the amount of albumin present as a stabiliser.

01/2008:1224
corrected 6.0

HUMAN COAGULATION FACTOR VII

Factor VII coagulationis humanus

DEFINITION

Human coagulation factor VII is a plasma protein fraction that contains the single-chain glycoprotein factor VII and may also contain small amounts of the activated form, the two-chain derivative factor VIIa. It may also contain coagulation factors II, IX and X and protein C and protein S. It is obtained from human plasma that complies with the monograph on *Human plasma for fractionation (0853)*.

The potency of the preparation, reconstituted as stated on the label, is not less than 15 IU of factor VII per millilitre.

PRODUCTION

The method of preparation is designed to minimise activation of any coagulation factor (to minimise potential thrombogenicity) and includes a step or steps that have been shown to remove or to inactivate known agents of infection; if substances are used for inactivation of viruses during production, the subsequent purification procedure must be validated to demonstrate that the concentration of these substances is reduced to a suitable level and that any residues are such as not to compromise the safety of the preparation for patients.

The specific activity is not less than 2 IU of factor VII per milligram of total protein, before the addition of any protein stabiliser.

The factor VII fraction is dissolved in a suitable liquid. Heparin, antithrombin and other auxiliary substances such as a stabiliser may be added. No antimicrobial preservative is added. The solution is passed through a bacteria-retentive filter, distributed aseptically into the final containers and immediately frozen. It is subsequently freeze-dried and the containers are closed under vacuum or under an inert gas.

CONSISTENCY OF THE METHOD OF PRODUCTION

The consistency of the method of production with respect to the activities of factors II, IX and X of the preparation, expressed in International Units relative to the activity of factor VII, shall be demonstrated.

The consistency of the method of production with respect to the activity of factor VIIa of the preparation shall be demonstrated. The activity of factor VIIa may be determined, for example, using a recombinant soluble tissue factor that does not activate factor VII but possesses a cofactor function specific for factor VIIa; after incubation of a mixture of the recombinant soluble tissue factor with phospholipids reagent and the dilution of the test sample in factor VII-deficient plasma, calcium chloride is added and the clotting time determined; the clotting time is inversely related to the factor VIIa activity of the test sample.

CHARACTERS

A hygroscopic powder or friable solid that may be white or almost white, pale yellow, green or blue.

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

It complies with the limits of the assay.

TESTS

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

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 reconstituted preparation with a 9 g/l solution of *sodium chloride R* to obtain a solution expected to contain about 15 mg of protein in 2 ml. To 2.0 ml of the solution in a round-bottomed centrifuge tube 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.

Activated coagulation factors (*2.6.22*). For each of the dilutions, the coagulation time is not less than 150 s.

Heparin. If heparin has been added during preparation, determine the amount present by the assay of heparin in coagulation factor concentrates (*2.7.12*). The preparation to be examined contains not more than the amount of heparin stated on the label and in any case not more than 0.5 IU of heparin per International Unit of factor VII.

Thrombin. If the preparation to be examined contains heparin, determine the amount present as described in the test for heparin and neutralise the heparin by addition of *protamine sulphate R* (10 µg of protamine sulphate neutralises 1 IU of heparin). In each of 2 test-tubes, mix equal volumes of the reconstituted preparation and a 3 g/l solution of *fibrinogen R*. Keep one of the tubes at 37 °C for 6 h and the other at room temperature for 24 h. In a third tube, mix a volume of the fibrinogen solution with an equal volume of a solution of *human thrombin R* (1 IU/ml) and place the tube in a water-bath at 37 °C. No coagulation occurs in the tubes containing the preparation to be examined. Coagulation occurs within 30 s in the tube containing thrombin.

Factor II. Carry out the assay of human coagulation factor II (*2.7.18*).

The estimated content is not more than 125 per cent of the stated content. The confidence limits (P = 0.95) are not less than 90 per cent and not more than 111 per cent of the estimated potency.

Factor IX. Carry out the assay of human coagulation factor IX (*2.7.11*).

The estimated content is not more than 125 per cent of the stated content. The confidence limits (P = 0.95) are not less than 80 per cent and not more than 125 per cent of the estimated potency.

Factor X. Carry out the assay of human coagulation factor X (*2.7.19*).

The estimated content is not more than 125 per cent of the stated content. The confidence limits (P = 0.95) are not less than 90 per cent and not more than 111 per cent of the estimated potency.

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 spectrometry (*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 equivalent to not less than 30 IU of factor VII.

ASSAY

Assay of human coagulation factor VII (*2.7.10*).

The estimated potency is not less than 80 per cent and not more than 125 per cent of the stated potency. The confidence limits (P = 0.95) are not less than 80 per cent and not more than 125 per cent of the estimated potency.

STORAGE

In an airtight container, protected from light.

LABELLING

The label states:

- the number of International Units of factor VII per container,
- the maximum content of International Units of factor II, factor IX and factor X per container,
- the amount of protein per container,
- the name and quantity of any added substances, including where applicable, heparin,
- the name and volume of the liquid to be used for reconstitution,
- that the transmission of infectious agents cannot be totally excluded when medicinal products prepared from human blood or plasma are administered.

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HUMAN COAGULATION FACTOR VIII

Factor VIII coagulationis humanus

DEFINITION

Human coagulation factor VIII is a preparation of a plasma protein fraction that contains the glycoprotein coagulation factor VIII together with varying amounts of von Willebrand factor, depending on the method of preparation. It is prepared from human plasma that complies with the monograph on *Human plasma for fractionation (0853)*.

The potency of the preparation, reconstituted as stated on the label, is not less than 20 IU of factor VIII:C per millilitre.

PRODUCTION

The method of preparation includes a step or steps that have been shown to remove or to inactivate known agents of infection; if substances are used for the inactivation of viruses, the subsequent purification procedure must be validated to demonstrate that the concentration of these substances is reduced to a suitable level and that any residues are such as not to compromise the safety of the preparation for patients.

The specific activity is not less than 1 IU of factor VIII:C per milligram of total protein before the addition of any protein stabiliser.

The factor VIII fraction is dissolved in a suitable liquid. Excipients such as a stabiliser may be added. No antimicrobial preservative is added. The solution is passed through a bacteria-retentive filter, distributed aseptically into the final containers and immediately frozen. It is subsequently freeze-dried and the containers are closed under vacuum or under an inert gas.

VALIDATION STUDIES

Products stated to have von Willebrand factor activity. For products intended for treatment of von Willebrand's disease it shall be demonstrated that the manufacturing process yields a product with a consistent composition with respect to von Willebrand factor. This composition may be characterised in a number of ways. For example, the number and the relative amount of the different multimers may be determined by sodium dodecyl sulphate (SDS) agarose gel electrophoresis (about 1 per cent agarose) with or without Western blot analysis, using a normal human plasma pool as reference; visualisation of the multimeric pattern may be performed using an immunoenzymatic technique and quantitative evaluation may be carried out by densitometric analysis or by other suitable methods.

Products that show flakes or particles after reconstitution for use. If a few small flakes or 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 provided.

CHARACTERS

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