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Injectable Insulin Preparations

General Notices

Insulin Preparations

(Ph. Eur. monograph 0854)

The provisions of this monograph apply to sterile preparations of Human Insulin and its analogues, Bovine Insulin and Porcine Insulin.

The provisions of this monograph apply to Injectable Insulin Preparations listed below.

For preparations of Human Insulin, the label states the approved code in lower case letters indicative of the method of production.

Preparations

Biphasic Isophane Insulin Injection

Insulin Injection

Insulin Aspart Injection

Insulin Lispro Injection

Insulin Zinc Suspension

Insulin Zinc Suspension (Amorphous)

Insulin Zinc Suspension (Crystalline)

Isophane Insulin Injection

Protamine Zinc Insulin Injection

Biphasic Insulin Aspart Injection

Ph Eur

Injectable insulin preparations comply with the requirements for Injections prescribed in the monograph Parenteral preparations (0520).

DEFINITION

Injectable insulin preparations are sterile preparations of *Insulin, human (0838)* or *Insulin, porcine (1638)*. They contain not less than 90.0 per cent and not more than the equivalent of 110.0 per cent of the amount of insulin stated on the label. They are either solutions or suspensions or they are prepared by combining solutions and suspensions.

PRODUCTION

The methods of preparation are designed to confer suitable properties with respect to the onset and duration of therapeutic action.

The following procedures are carried out in a suitable sequence, depending on the method of preparation:

— addition of suitable antimicrobial preservatives;

— addition of a suitable substance or substances to render the preparation isotonic with blood;

— addition of a suitable substance or substances to adjust the pH to the appropriate value;

— determination of the strength of the insulin-containing component or components followed, where necessary, by adjustment so that the final preparation contains the requisite number of International Units per millilitre;

— sterilisation by filtration of the insulin-containing component or components; once this procedure has been carried out all subsequent procedures are carried out aseptically using materials that have been sterilised by a suitable method.

In addition, where appropriate, suitable excipients are added and suitable procedures carried out to confer the appropriate physical form on the insulin-containing component or components. The final preparation is distributed aseptically into sterile containers which are closed so as to exclude microbial contamination.

TESTS

pH (*2.2.3*)

The pH of the solution or suspension is 6.9 to 7.8, unless otherwise prescribed in the specific monograph.

Insulin in the supernatant

For injectable insulin preparations that are suspensions, not more than 2.5 per cent of the total insulin content, unless otherwise stated. Centrifuge 10 mL of the suspension at 1500 *g* for 10 min and carefully separate the supernatant and the residue. Determine the insulin content of the supernatant (*S*) by a suitable method, for example using the chromatographic conditions described under Assay. Calculate the percentage of insulin in solution using the following expression:

$$\frac{S}{T}$$

where *T* is the total insulin content determined as described under Assay.

Impurities with molecular masses greater than that of insulin

Examine by size-exclusion chromatography (*2.2.30*).

Test solution Add 4 µL of *6 M hydrochloric acid R* per millilitre of the preparation to be examined, whether a suspension or a solution, to obtain a clear acid insulin solution. When sampling a suspension, agitate the material prior to sampling in order to obtain a homogeneous sample. If a suspension does not turn clear within 5 min of the initial addition of hydrochloric acid, add small aliquots of acid (less than 4 µL per millilitre) until a solution is obtained. Preparations with concentrations higher than 100 IU/mL need to be diluted with *0.01 M hydrochloric acid* to avoid overloading the column with insulin monomer.

Resolution solution Use a solution of insulin (approximately 4 mg/mL), containing more than 0.4 per cent of high molecular mass proteins. An injectable insulin preparation, whether a solution or a suspension, that has been clarified with a sufficient amount of *6 M hydrochloric acid R*, containing the indicated percentage of high molecular mass proteins, or a solution prepared from insulin, dissolved in *0.01 M hydrochloric acid*, may be used. Insulin containing the indicated percentage of high molecular mass proteins may be prepared by allowing insulin powder to stand at room temperature for about ten days.

Maintain the solutions at 2 °C to 10 °C and use within 30 h (soluble insulin injection) or 7 days (other insulin preparations). If an automatic injector is used, maintain the temperature at 2 °C to 10 °C.

The chromatographic procedure may be carried out using:

— a column 0.3 m long and at least 7.5 mm in internal diameter packed with *hydrophilic silica gel for chromatography R* (5 µm to 10 µm), of a grade suitable for the separation of insulin monomer from dimers and polymers;

— as mobile phase at a flow rate of 0.5 mL/min a mixture consisting of 15 volumes of *glacial acetic acid R*, 20 volumes of *acetonitrile R* and 65 volumes of a 1.0 g/L solution of *arginine R*; filter and degas;

— as detector a spectrophotometer set at 276 nm.

Equilibration of the column Before using a new column for chromatographic analysis, equilibrate by repeated injections of an insulin solution containing high molecular mass proteins. This can be done by at least 3 injections of the resolution

solution. The column is equilibrated when repeatable results are obtained from 2 subsequent injections. If protamine-containing samples are to be analysed, the equilibration of the column is performed using a solution containing protamine.

Inject 100 µL of the resolution solution. When the chromatograms are recorded under the prescribed conditions, the retention times are: polymeric insulin complexes or covalent insulin-protamine complex: about 13 min to 17 min, covalent insulin dimer: about 17.5 min, insulin monomer: about 20 min, salts: about 22 min. If the sample solution contains preservatives, for example methyl paraben, *m*-cresol or phenol, these compounds elute later. The test is not valid unless the resolution, defined by the ratio of the height of the dimer peak to the height above the baseline of the valley separating the monomer and dimer peaks, is at least 2.0.

Inject 100 µL of the test solution. Record the chromatogram for approximately 35 min. In the chromatogram obtained, the sum of the areas of any peak with a retention time less than that of the insulin peak is not greater than 3.0 per cent (protamine containing preparations) or 2.0 per cent (non-protamine containing preparations) of the total area of the peaks. Disregard any peak with a retention time greater than that of the insulin peak.

Related proteins

Examine by liquid chromatography (2.2.29) as described under Assay, following the elution conditions as described in the table below:

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)	Comment
0 - 30	42	58	isocratic
30 - 44	42 → 11	58 → 89	linear gradient
44 - 50	11	89	isocratic

Maintain the solutions at 2 °C to 10 °C and use within 24 h. Perform a system suitability check (resolution, linearity) as described under Assay. If necessary, the relative proportions of the mobile phases may be adjusted to ensure complete elution of A21 desamido porcine insulin before commencement of the gradient. The profile of the gradient may also be adjusted to ensure complete elution of all insulin related impurities.

Inject 20 µL of the test solution and 20 µL of either reference solution (a), for insulin preparations containing 100 IU/mL, or reference solution (b), for insulin preparations containing 40 IU/mL. If necessary, adjust the injection volume to a volume between 10 µL and 20 µL in accordance with the results obtained in the test for linearity as described under Assay. Record the chromatograms for about 50 min. If necessary, make further adjustments to the mobile phase in order to ensure that the antimicrobial preservatives present in the test solution are well separated from the insulin and show a shorter retention time. A small reduction in the concentration of acetonitrile increases the retention time of the insulin peaks relatively more than those of the preservatives. In the chromatogram obtained with either reference solution (a), or reference solution (b), as appropriate, A21 desamido insulin appears as a small peak after the principal peak and has a retention time of about 1.3 relative to the principal peak, due to insulin. In the chromatogram obtained with the test solution, the area of the peak due to A21 desamido insulin is not greater than 5.0 per cent of the total area of the peaks; the sum of the areas of any other peaks, apart from those due to insulin and A21 desamido insulin is not greater than 6.0 per cent of the total area of the peaks. Disregard the peaks due to the preservatives and protamine (early eluting peaks).

Total zinc

Not more than the amount stated in the individual monograph, determined by atomic absorption spectrometry (2.2.23, Method I).

Use the following method, unless otherwise prescribed in the specific monograph.

Test solution Shake the preparation gently and dilute a volume containing 200 IU of insulin to 25.0 mL with *0.01 M hydrochloric acid*. Dilute if necessary to a suitable concentration of zinc (for example, 0.4 µg to 1.6 µg of Zn per millilitre) with *0.01 M hydrochloric acid*.

Reference solutions Use solutions containing 0.40 µg, 0.80 µg, 1.00 µg, 1.20 µg and 1.60 µg of Zn per millilitre, freshly prepared by diluting *zinc standard solution (5 mg/mL Zn) R* with *0.01 M hydrochloric acid*.

Measure the absorbance at 213.9 nm using a zinc hollow-cathode lamp as source of radiation and an air-acetylene flame of suitable composition (for example 11 L of air and 2 L of acetylene per minute).

Zinc in solution

Where applicable, not more than the amount stated in the individual monograph, determined by atomic absorption spectrometry (2.2.23, Method I).

Test solution Centrifuge the preparation to be examined and dilute 1 mL of the clear supernatant obtained to 25.0 mL with *water R*. Dilute if necessary to a suitable concentration of zinc (for example 0.4 µg to 1.6 µg of Zn per millilitre) with *water R*.

Reference solutions Use solutions containing 0.40 µg, 0.80 µg, 1.00 µg, 1.20 µg and 1.60 µg of Zn per millilitre, freshly prepared by diluting *zinc standard solution (5 mg/mL Zn) R* with *0.01 M hydrochloric acid*.

Measure the absorbance at 213.9 nm using a zinc hollow-cathode lamp as source of radiation and an air-acetylene flame of suitable composition (for example 11 L of air and 2 L of acetylene per minute).

Bacterial endotoxins (*2.6.14*)

Less than 80 IU per 100 IU of insulin.

ASSAY

Examine by liquid chromatography (*2.2.29*).

Test solution Add 4 µL of *6 M hydrochloric acid R* per millilitre of the preparation to be examined, whether a suspension or a solution, to obtain a clear solution. When sampling a suspension, shake the material prior to sampling in order to obtain a homogeneous sample. If a suspension does not turn clear within 5 min of the initial addition of acid, add small aliquots of acid (less than 4 µL per millilitre) until a solution is obtained. For a preparation containing more than 100 IU/mL, an additional dilution with *0.01 M hydrochloric acid* is necessary to avoid overloading the column.

Reference solution (a) Dissolve, as appropriate, the contents of a vial of *human insulin CRS* or *porcine insulin CRS* in *0.01 M hydrochloric acid* to obtain a concentration of 4.0 mg/mL. *Reference solution (a) is used for the assay of insulin preparations containing 100 IU/mL.*

Reference solution (b) Dilute 4.0 mL of reference solution (a) to 10.0 mL with *0.01 M hydrochloric acid*. *Reference solution (b) is used for the assay of insulin preparations containing 40 IU/mL*.

Reference solution (c) Dissolve the contents of a vial of *human insulin CRS* in *0.01 M hydrochloric acid* to obtain a concentration of 4.0 mg/mL.

Reference solution (d) Dissolve the contents of a vial of *insulin porcine for system suitability CRS* in *0.01 M hydrochloric acid* to obtain a concentration of 4 mg/mL.

Reference solution (e) Dilute 1.0 mL of reference solution (a) to 10.0 mL with *0.01 M hydrochloric acid*.

Reference solution (f) Dilute 1.0 mL of reference solution (b) to 10.0 mL with *0.01 M hydrochloric acid*.

Resolution solution Mix 1.0 mL of reference solution (c) and 1.0 mL of reference solution (d).

Maintain the solutions at 2 °C to 10 °C and use within 48 h. If an automatic injector is used, maintain the temperature at 2 °C to 10 °C.

The chromatographic procedure may be carried out using:

— a stainless steel column 0.25 m long and 4.6 mm in internal diameter packed with *octadecylsilyl silica gel for chromatography R* (5 µm);

— as mobile phase at a flow rate of 1 mL/min the following solutions prepared and maintained at a temperature not lower than 20 °C:

Mobile phase A Dissolve 28.4 g of *anhydrous sodium sulfate R* in *water for chromatography R* and dilute to 1000 mL with the same solvent; add 2.7 mL of *phosphoric acid R*; adjust the pH to 2.3, if necessary, with *ethanolamine R*; filter and degas;

Mobile phase B Mix 550 mL of mobile phase A with 450 mL of *acetonitrile R1*. Warm the solution to a temperature not lower than 20 °C in order to avoid precipitation (mixing of mobile phase A with acetonitrile is endothermic); filter and degas;

— as detector a spectrophotometer set at 214 nm;

maintaining the temperature of the column at 40 °C.

Elute with a mixture of 42 volumes of mobile phase A and 58 volumes of mobile phase B, adjusted if necessary.

Inject 20 µL of the resolution solution and 20 µL of reference solution (d). Record the chromatogram of the resolution solution until the peak corresponding to the principal peak in the chromatogram obtained with reference solution (d) is

clearly visible. In the chromatogram obtained with the resolution solution, identify the peaks due to porcine insulin and human insulin. The test is not valid unless the resolution between the peaks due to human insulin and porcine insulin is at least 1.2. If necessary, adjust the concentration of acetonitrile in the mobile phase until this resolution is achieved.

Inject 20 µL of the test solution and 20 µL of either reference solutions (a) and (e), for insulin preparations containing 100 IU/mL, or 20 µL of reference solutions (b) and (f), for insulin preparations containing 40 IU/mL. If necessary, make further adjustments of the mobile phase in order to ensure that the antimicrobial preservatives present in the test solution are well separated from the insulin and show shorter retention times. A small reduction in the concentration of acetonitrile increases the retention time of the insulin peaks relatively more than those of the preservatives. If necessary, after having carried out the chromatography of a solution wash the column with a mixture of equal volumes of *acetonitrile R1* and *water for chromatography R* for a sufficient time to ensure elution of any interfering substances before injecting the next solution. The test is not valid unless the area of the principal peak in the chromatogram obtained with reference solution (a) or (b) is 10 ± 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (e) or (f). If this test fails, adjust the injection volume between 10 µL and 20 µL, in order to be in the linearity range of the detector.

Calculate the content of insulin plus A21 desamido insulin from the area of the peak due to the human or porcine insulin and that of any peak due to the A21 desamido insulin, using the declared content of insulin plus A21 desamido insulin in , *human insulin CRS* or *porcine insulin CRS* as appropriate. [1]

STORAGE

Unless otherwise prescribed, store in a sterile, airtight, tamper-evident container, protected from light, at a temperature of 2 °C to 8 °C. Insulin preparations are not to be frozen.

LABELLING

The label states:

— the potency in International Units per millilitre;

— the concentration in terms of the number of milligrams of insulin per millilitre;

— where applicable, that the substance is produced by enzymatic modification of porcine insulin;

— where applicable, that the substance is produced by recombinant DNA technology;

— where applicable, the animal species of origin;

— that the preparation must not be frozen;

— where applicable, that the preparation must be resuspended before use.

Ph Eur

[1] 100 IU are equivalent to 3.47 mg of human insulin and to 3.45 mg of porcine insulin.