Insulin preparations, injectable

**INSULIN PREPARATIONS, INJECTABLE**

Preparationes insulini injectabiles

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

**DEFINITION**

Injectable insulin preparations are sterile preparations of Insulin, human (0838), Insulin, bovine (1637) 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 substances 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 liquid and the residue. Determine the insulin content of the supernatant liquid (S) by a suitable method, for example using the chromatographic conditions described under Assay. Calculate the percentage of the insulin in solution from the expression:

\[
\frac{100S}{T}
\]

where \(T\) is the total insulin content determined as described under the 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;

**Injection:** 20 µl of the test solution.

Calculate the content of porcine insulin \(\text{C}_{256}\text{H}_{381}\text{N}_{65}\text{O}_{76}\text{S}_{6}\) plus A21 desamido porcine insulin from the area of the principal peak and the area of the peak corresponding to A21 desamido porcine insulin in the chromatograms obtained with the test solution and reference solution (b) and the declared content of porcine insulin plus A21 desamido porcine insulin in porcine insulin CRS.

**STORAGE**

In an airtight container, protected from light, at \(\pm 20 ^\circ C\) until released by the manufacturer. When thawed, insulin may be stored at \(\pm 3 ^\circ C\) and used for manufacturing preparations within a short period of time. To avoid absorption of humidity from the air during weighing, the insulin must be at room temperature.

See the information section on general monographs (cover pages)
-- 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 three injections of the resolution solution. The column is equilibrated when repeatable results are obtained from two 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:

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mobile phase A (per cent V/V)</th>
<th>Mobile phase B (per cent V/V)</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 30</td>
<td>42</td>
<td>58</td>
<td>isocratic</td>
</tr>
<tr>
<td>30 - 44</td>
<td>42 → 11</td>
<td>58 → 89</td>
<td>linear gradient</td>
</tr>
<tr>
<td>44 - 50</td>
<td>11</td>
<td>89</td>
<td>isocratic</td>
</tr>
</tbody>
</table>

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 approximately 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 acetic acid 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 litres of air and 2 litres 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 liquid obtained to 25.0 ml with water. 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 litres of air and 2 litres 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). For a preparation containing a single species of insulin, dissolve in 0.01 M hydrochloric acid, as appropriate, the contents of a vial of human insulin CRS, porcine insulin CRS or bovine insulin CRS to obtain a concentration of 4.0 mg/ml. For a preparation containing both bovine and porcine insulins, mix 1.0 ml of a solution containing 4.0 mg of bovine insulin CRS per millilitre of 0.01 M hydrochloric acid and 1.0 ml of a solution containing 4.0 mg of porcine insulin CRS per millilitre of 0.01 M hydrochloric acid. Reference solution (a) is used for the assay of insulin preparations containing 100 IU/ml.
**Insulin zinc injectable suspension**

**DEFINITION**

Insulin zinc injectable suspension is a sterile neutral suspension of bovine insulin and/or porcine insulin or of human insulin with a suitable zinc salt; the insulin is in a form which is practically insoluble in water.

**PRODUCTION**

Insulin zinc injectable suspension is prepared by carrying out the procedures described in the monograph on Insulin preparations, injectable (0854).

Insulin zinc injectable suspension is produced by mixing insulin zinc injectable suspension (crystalline) and insulin zinc injectable suspension (amorphous) in a ratio of 7 to 3.

---

(3) 100 IU are equivalent to 3.47 mg of human insulin, to 3.45 mg of porcine insulin and to 3.42 mg of bovine insulin.