**Sulphated ash (2.4.14):** maximum 2.5 per cent, determined on 0.200 g and calculated with reference to the dried substance.

**Bacterial endotoxins (2.6.14):** less than 10 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

**ASSAY**

Liquid chromatography (2.2.29).

*Test solution.* Dissolve 40.0 mg of the substance to be examined in 0.01 M hydrochloric acid and dilute to 10.0 ml with the same solvent.

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

*Reference solution (c).* 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 (a) and 1.0 ml of reference solution (b).

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

**Column:**
- size: l = 0.25 m, Ø = 4.6 mm,
- stationary phase: octadecylsilica gel for chromatography R (5 µm),
- temperature: 40 °C.

**Mobile phase:** mix 42 volumes of mobile phase A and 58 volumes of mobile phase B, adjusting the composition of the mixture if necessary.

Prepare and maintain the following solutions at a temperature of at least 20 °C:
- *mobile phase A:* dissolve 28.4 g of anhydrous sodium sulphate R in water R and dilute to 1000 ml with the same solvent; add 2.7 ml of phosphoric acid R; adjust to pH 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 R. Warm the solution to a temperature of at least 20 °C in order to avoid precipitation (mixing of mobile phase A with acetonitrile is endothermic); filter and degas.

**Flow rate:** 1 ml/min.

**Detection:** spectrophotometer at 214 nm.

**System suitability:**
- **resolution:** inject 20 µl of the resolution solution and 20 µl of reference solution (b). Record the chromatogram of the resolution solution until the peak corresponding to the principal peak in the chromatogram obtained with reference solution (b) 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 corresponding 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.
- **linearity:** inject 20 µl each of reference solutions (b) and (c). The test is not valid unless the area of the principal peak in the chromatogram obtained with reference solution (b) is 10 ± 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c). If this test fails, adjust the injection volume to between 10 µl and 20 µl in order that the responses are within the linearity range of the detector.

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

Calculate the content of porcine insulin C_{25s}H_{381}N_{65}O_{76}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 −20 °C until released by the manufacturer. When thawed, insulin may be stored at 5 ± 3 °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.

**LABELLING**

The label states where applicable, that the substance is free from bacterial endotoxins.

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**INSULIN PREPARATIONS, INJECTABLE**

**Präparationes 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 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, or a solution prepared from insulin, dissolved in 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, 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 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.

**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 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).
Insulin preparations, injectable

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 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 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 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 or porcine insulin CRS, or a defined quantity of 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.

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 porcine insulin CRS in 0.01 M hydrochloric acid to obtain a concentration of 4.0 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 octadecylsilyle 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 sulphate R in water 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 R. 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 R and water 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 bovine, porcine or human insulin and that of any peak due to the A21 desamido insulin, using the declared content of insulin plus A21 desamido insulin in bovine insulin CRS, porcine insulin CRS or human insulin CRS, as appropriate. For preparations containing both bovine and porcine insulin use the sum of the areas of both the bovine and porcine insulin peaks and of the peaks due to the A21 desamido insulin.(3)

STORAGE

Unless otherwise prescribed, store in a sterile, airtight, tamper-proof 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 (for preparations containing both bovine insulin and porcine insulin the concentration is stated as the combined amount of both insulins),
– 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.

01/2005:0837

INSULIN ZINC INJECTABLE SUSPENSION

Insulini zinci suspensio injectabilis

Insulin zinc injectable suspension complies with the monograph on Insulin preparations, injectable (0854) with the amendments prescribed below.

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.

CHARACTERS

A white suspension which on standing deposits a white sediment and leaves a colourless or almost colourless supernatant liquid; the sediment is readily resuspended by gently shaking. When examined under a microscope, the majority of the particles are seen to be rhombohedral crystals with a maximum dimension when measured from corner to corner through the crystal greater than 10 µm but rarely exceeding 40 µm; a considerable proportion of the particles are seen to have no uniform shape and a maximum dimension rarely exceeding 2 µm.

IDENTIFICATION

Examine the chromatograms obtained in the Assay. For preparations made from a single species of insulin (bovine, porcine or human), the position of the peak due to insulin in the chromatogram obtained with the test solution corresponds to that of the principal peak in the chromatogram obtained with the appropriate reference solution. For preparations made from a mixture of bovine and porcine insulin, the positions of the peaks due to the two insulins in the chromatogram obtained with the test solution correspond to those of the principal peaks in the chromatogram obtained with the appropriate reference solution.

TESTS

Insulin not extractable with buffered acetone solution:

63 per cent to 77 per cent of the total insulin content. Centrifuge a volume of the substance to be examined containing 200 IU of insulin and discard the supernatant liquid. Suspend the residue in 1.65 ml of water R, add 3.3 ml of buffered acetone solution R; stir for 3 min, again centrifuge, discard the supernatant liquid and repeat all the operations with the residue. Dissolve the residue using a suitable procedure, for example dissolve in 0.1 M hydrochloric acid to give a final volume of 2.0 ml. Determine the insulin content of the residue (R) and determine the total insulin content (T) of an equal volume of the suspension by a suitable method. Calculate the percentage of insulin not extractable with buffered acetone solution from the expression:

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

Total zinc: 0.12 mg to 0.25 mg per 100 IU of insulin, determined as described in the monograph on Insulin preparations, injectable (0854).

Zinc in solution: 20 per cent to 65 per cent of the total zinc is in the form of zinc in solution. Determine by the method described in the monograph on Insulin preparations, injectable (0854).

01/2005:0835

INSULIN ZINC INJECTABLE SUSPENSION (AMORPHOUS)

Insulini zinci amorphi suspensio injectabilis

Insulin zinc injectable suspension (amorphous) complies with the monograph on Insulin preparations, injectable (0854) with the amendments prescribed below.

DEFINITION

Insulin zinc injectable suspension (amorphous) is a sterile neutral suspension of bovine, porcine or human insulin complexed with a suitable zinc salt; the insulin is in a form which is practically insoluble in water.

(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.