Indometacin contains not less than 98.5 per cent and not more than the equivalent of 100.5 per cent of [1-(4-chlorobenzoyl)-5-methoxy-2-methylindol-3-yl]acetic acid, calculated with reference to the dried substance.

**CHARACTERS**

A white or yellow, crystalline powder, practically insoluble in water, sparingly soluble in alcohol.

**IDENTIFICATION**

*First identification: A, C.*

*Second identification: A, B, D, E.*

A. **Melting point (2.2.14):** 158 °C to 162 °C.

B. **Dissolve 25 mg in a mixture of 1 volume of 1 M hydrochloric acid and 9 volumes of methanol R and dilute to 100.0 ml with the same mixture of solvents. Dilute 10.0 ml of the solution to 100.0 ml with a mixture of 1 volume of 1 M hydrochloric acid and 9 volumes of methanol R. Examine between 300 nm and 350 nm (2.2.25), the solution shows an absorption maximum at 318 nm. The specific absorbance at the maximum is 170 to 190.

C. **Examine by infrared absorption spectrophotometry (2.2.24), comparing with the spectrum obtained with indometacin CRS.** Examine the substances in the solid state without recrystallisation.

D. **Dissolve 0.1 g in 10 ml of alcohol R, heating slightly if necessary.** To 0.1 ml of the solution add 2 ml of a freshly prepared mixture of 1 volume of a 250 g/l solution of hydroxylamine hydrochloride R and 3 volumes of dilute sodium hydroxide solution R. Add 2 ml of dilute hydrochloric acid R and 1 ml of ferric chloride solution R2 and mix. A violet-pink colour develops.

E. To 0.5 ml of the solution in alcohol prepared in identification test D, add 0.5 ml of dimethylaminobenzaldehyde solution R2. A precipitate is formed that dissolves on shaking. Heat on a water-bath. A bluish-green colour is produced. Continue to heat for 5 min and cool in iced water for 2 min. A precipitate is formed and the colour changes to light greyish-green. Add 3 ml of alcohol R. The solution is clear and violet-pink in colour.

**TESTS**

**Related substances.** Examine by thin-layer chromatography (2.2.27), using silica gel HF254 R as the coating substance. Prepare the slurry using a 46.8 g/l solution of sodium dihydrogen phosphate R.

**Test solution.** Dissolve 0.2 g of the substance to be examined in methanol R and dilute to 10 ml with the same solvent. Prepare immediately before use.

**Reference solution.** Dilute 1 ml of the test solution to 200 ml with methanol R.

Apply separately to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 30 volumes of light petroleum R and 70 volumes of ether R. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

**Heavy metals (2.4.8).** 2.0 g complies with limit test C for heavy metals (20 ppm). Prepare the standard using 4 ml of lead standard solution (10 ppm Pb) R.

**Loss on drying (2.2.32).** Not more than 0.5 per cent, determined on 1,000 g by drying in an oven at 105 °C.

**Sulphated ash (2.4.14).** Not more than 0.1 per cent, determined on 1.0 g.

**ASSAY**

Dissolve 0.300 g in 75 ml of acetone R, through which nitrogen R, free from carbon dioxide, has been passed for 15 min. Maintain a constant stream of nitrogen through the solution. Add 0.1 ml of phenolphthalein solution R. Titrate with 0.1 M sodium hydroxide. Carry out a blank titration. 1 ml of 0.1 M sodium hydroxide is equivalent to 35.78 mg of C19H16ClNO4.

**STORAGE**

Store protected from light.
**IMPURITIES**

A. 4-chlorobenzoic acid.

**INSULIN ASPART**

Insulinum aspartum

- H: Gly - Ile - Val - Glu - Gin - Cys - Thr - Ser - Ile - Cys - Ser -
  - Leu - Tyr - Gin - Leu - Glu - Asn - Tyr - Cys - Asn - OH
- H: Phe - Val - Asn - Gin - His - Leu - Cys - Gly - Ser - His - Leu -
  - Val - Glu - Ala - Leu - Tyr - Leu - Val - Cys - Gly - Arg -
- Gly - Phe - Phe - Tyr - Thr - Asp - Lys - Thr - OH

C_{236}H_{381}N_{65}O_{79}S_{6} M, 5826

**DEFINITION**

28^{b}I-L-Aspartate insulin (human).

Insulin aspart is a 2-chain peptide containing 51 amino acids. The A-chain is composed of 21 amino acids and the B-chain is composed of 30 amino acids. It is identical in primary structure to human insulin, except that it has aspartic acid instead of proline at position 28 of the B-chain. As in human insulin, insulin aspart contains 2 interchain disulphide bonds and 1 intrachain disulphide bond.

**Content:** 90.0 per cent to 104.0 per cent of insulin aspart C_{256}H_{381}N_{65}O_{79}S_{6} plus A21Asp insulin aspart, B3Asp insulin aspart, B3isoAsp insulin aspart and B28isoAsp insulin aspart (dried substance).

By convention, for the purpose of labelling insulin aspart preparations, 0.0350 mg of insulin aspart is equivalent to 1 unit.

**PRODUCTION**

Insulin aspart is produced by a method based on recombinant DNA (rDNA) technology under conditions designed to minimise the degree of microbial contamination.

**Prior to release the following tests are carried out on each batch of the final bulk product, unless exemption has been granted by the competent authority.**

**Host-cell-derived proteins.** The limit is approved by the competent authority.

**Single-chain precursor.** The limit is approved by the competent authority. Use a suitably sensitive method.

**CHARACTERS**

**Appearance:** white or almost white powder.

**Solubility:** practically insoluble in ethanol (96 per cent), in methanol and in aqueous solutions with a pH around 5.1. In aqueous solutions below pH 3.5 or above pH 6.5, the solubility is greater than or equal to 25 mg/ml.

**IDENTIFICATION**

A. Examine the chromatograms obtained in the assay.

**Results:** the principal peak in the chromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with reference solution (a).

B. Peptide mapping (2.2.55).

**SELECTIVE CLEAVAGE OF THE PEPTIDE BONDS**

**Test solution.** Prepare a 2.0 mg/ml solution of the substance to be examined in 0.01 M hydrochloric acid and transfer 25 μl of this solution to a clean tube. Add 100 μl of HEPES buffer solution pH 7.5 R and 20 μl of a 1 mg/ml solution of Staphylococcus aureus strain V8 protease R. Cap the tube and incubate at 25 °C for 6 h. Stop the reaction by adding 145 μl of sulphate buffer solution pH 2.0 R.

**Reference solution.** Prepare at the same time and in the same manner as for the test solution, but using insulin aspart CRS instead of the substance to be examined.

**CHROMATOGRAPHIC SEPARATION.** Liquid chromatography (2.2.29).

**Column:**
- size: l = 0.10 m, Ø = 4.6 mm,
- stationary phase: octadecysilyl silica gel for chromatography R (3 μm) with a pore size of 8 nm,
- temperature: 40 °C.

**Mobile phase:**
- mobile phase A: mix 100 ml of acetonitrile for chromatography R, 200 ml of sulphate buffer solution pH 2.0 R and 700 ml of water R; filter and degas;
- mobile phase B: mix 200 ml of sulphate buffer solution pH 2.0 R, 400 ml of acetonitrile for chromatography R and 400 ml of water R; filter and degas;

<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>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 60</td>
<td>90 → 30</td>
<td>10 → 70</td>
</tr>
<tr>
<td>60 - 65</td>
<td>30 → 0</td>
<td>70 → 100</td>
</tr>
<tr>
<td>65 - 70</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

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

**Detection:** spectrophotometer at 214 nm.

**Equilibration:** at initial conditions for at least 15 min. Carry out a blank run using the above-mentioned gradient.

**Injection:** 50 μl.

**System suitability:**
- the chromatograms obtained with the test solution and the reference solution are qualitatively similar to the chromatogram of insulin aspart digest supplied with insulin aspart CRS;
- in the chromatogram obtained with the reference solution, identify the peaks due to digest fragments I, II and III:
  - symmetry factor: maximum 1.5, for the peaks due to fragments II and III;
  - resolution minimum 8.0, between the peaks due to fragments II and III.

**Results:** the profile of the chromatogram obtained with the test solution corresponds to that of the chromatogram obtained with the reference solution.