METHYLTESTOSTERONE

Methyltestosteronum

C_{19}H_{28}O_{2}  M, 302.5

DEFINITION
Methyltestosterone contains not less than 97.0 per cent and not more than the equivalent of 103.0 per cent of 17β-hydroxy-17α-methyl-androst-4-en-3-one, calculated with reference to the dried substance.

CHARACTERS
A white or slightly yellowish-white, crystalline powder, practically insoluble in water, freely soluble in alcohol.

IDENTIFICATION
First identification: B.
Second identification: A, C.
A. Melting point (2.2.14): 162 °C to 168 °C.
B. Examine by infrared absorption spectrophotometry (2.2.24), comparing with the spectrum obtained with methyltestosterone CRS.
C. After examination of the chromatograms obtained in the test for related substances, spray the plate with a saturated solution of potassium dichromate R in a mixture of 30 volumes of water R and 70 volumes of sulphuric acid R and examine immediately in daylight. The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS
Specific optical rotation (2.2.7). Dissolve 0.250 g in alcohol R and dilute to 25.0 ml with the same solvent. The specific optical rotation is +79 to +85, calculated with reference to the dried substance.

Related substances. Examine by thin-layer chromatography (2.2.27), using as the coating substance a suitable silica gel containing a fluorescent indicator having an optimal intensity at 254 nm.

Test solution. Dissolve 0.2 g of the substance to be examined in a mixture of 1 volume of methanol R and 9 volumes of chloroform R and dilute to 10 ml with the same mixture of solvents.

Reference solution (a). Dissolve 20 mg of methyltestosterone CRS in 1 ml of a mixture of 1 volume of methanol R and 9 volumes of chloroform R.
Reference solution (b). Dilute 1 ml of the test solution to 100 ml with a mixture of 1 volume of methanol R and 9 volumes of chloroform R.
Reference solution (c). Dilute 5 ml of reference solution (b) to 10 ml with a mixture of 1 volume of methanol R and 9 volumes of chloroform R.

ASSAY
Dissolve 50.0 mg in alcohol R and dilute to 50.0 ml with the same solvent. Dilute 10.0 ml of the solution to 100.0 ml with alcohol R. Dilute 10.0 ml of this test solution to 100.0 ml with alcohol R. Measure the absorbance (2.2.25) at the maximum at 241 nm. Calculate the content of C_{19}H_{28}O_{2} taking the specific absorbance to be 540.

STORAGE
Store protected from light.

METHYLTIONINIUM CHLORIDE

Methylthioninii chloride

C_{19}H_{28}ClN_{3}S \cdot H_{2}O  M, 319.9 (anhydrous substance)

DEFINITION
3,7-Bis(dimethylamino)phenothiazin-5-yl chloride (methylene blue).
Content: 95.0 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance: dark blue, crystalline powder with a copper-coloured sheen, or green crystals with a bronze-coloured sheen.
Solubility: soluble in water, slightly soluble in ethanol (96 per cent).

IDENTIFICATION
A. Ultraviolet and visible absorption spectrophotometry (2.2.25).
Test solution. Dissolve 10 mg in dilute hydrochloric acid R and dilute to 100 ml with the same acid. Dilute 5 ml of the solution to 100 ml with dilute hydrochloric acid R.
Spectral range: 240-800 nm.
**Absorption maxima:** at 255-260 nm, 285-290 nm, 675-685 nm and 740-750 nm.

**B. Thin-layer chromatography (2.2.27).**

**Test solution.** Dissolve 10 mg of the substance to be examined in methanol \( R \) and dilute to 10 ml with the same solvent. Dilute 1 ml of the solution to 10 ml with methanol \( R \).

**Reference solution.** Dissolve 10 mg of methylthioninium chloride CRS in methanol \( R \) and dilute to 10 ml with the same solvent. Dilute 1 ml of the solution to 10 ml with methanol \( R \).

**Plate:** TLC silica gel plate \( R \).

**Mobile phase:** anhydrous formic acid \( R \), propanol \( R \) (20:80 \( V/V \)).

**Application:** 2 \( \mu l \).

**Development:** over a path of 8 cm.

**Drying:** in air, protected from light.

**Detection:** examine in daylight.

**Results:** the principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution. A secondary spot may appear above the principal spot in both chromatograms.

**C. Dissolve about 1 mg in 10 ml of water \( R \).** Add 1 ml of glacial acetic acid \( R \) and 0.1 g of zinc powder \( R \). Heat to boiling. The solution becomes colourless. Filter and shake the filtrate. It becomes blue on contact with air.

**D. Ignite 50 mg with 0.5 g of anhydrous sodium carbonate \( R \).** Cool and dissolve the residue in 10 ml of dilute nitric acid \( R \). Filter. The filtrate, without further addition of dilute nitric acid \( R \), gives reaction (a) of chlorides (2.3.1).

**TESTS**

**Methanol-insoluble substances:** maximum 10.0 mg (1.0 per cent).

To 1.0 g add 20 ml of methanol \( R \) and boil under a reflux condenser for 5 min. Filter through a tared sintered-glass filter (40) (2.1.2) and wash the filter with methanol \( R \) until a colourless filtrate is obtained. Dry the filter at 100 °C and weigh.

**Related substances.** Liquid chromatography (2.2.29).

**Test solution.** Dissolve 15.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

**Reference solution (a).** Dissolve 7.5 mg of methylthioninium impurity A CRS in the mobile phase and dilute to 50.0 ml with the mobile phase. To 1.0 ml of this solution add 1.0 ml of the test solution and dilute to 10.0 ml with the mobile phase.

**Reference solution (b).** Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

**Column:**
- **size:** \( l = 0.25 \text{ m}, \Theta = 4 \text{ mm} \),
- **stationary phase:** octadecysilyl silica gel for chromatography \( R \) (7 \( \mu m \)).

**Mobile phase:** mix 27 volumes of acetonitrile \( R \) and 73 volumes of a mixture of 3.4 ml of phosphoric acid \( R \) and 1000 ml of water \( R \).

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

**Detection:** spectrophotometer at 246 nm.

**Injection:** 20 \( \mu l \).

**Run time:** twice the retention time of methylthioninium.

**Relative retention** with reference to methylthioninium (retention time \( = \) about 11 min): impurity A = about 0.7.

**System suitability:** reference solution (a):
- **resolution:** minimum 1.5 between the peaks due to impurity A and methylthioninium. If necessary, adjust the concentration of acetonitrile in the mobile phase.

**Limits:**
- **impurity A:** not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (5.0 per cent),
- **any other impurity:** for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent),
- **sum of impurities other than A:** not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent),
- **disregard limit:** 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

**Metals.** Atomic emission spectrometry (2.2.22) in argon plasma, using as detector a conventional optical system or a mass spectrometer; in the case of a mass spectrometer, use indium as internal standard.

**Test solution.** In a 10 ml volumetric flask, dissolve with stirring 100 mg of the substance to be examined in 9 ml of water \( R \), add 100.0 \( \mu l \) of a 10 \( \mu g/ml \) solution of indium prepared from indium elementary standard solution for atomic spectrometry (1.000 g/l) \( R \) in nitric acid \( R \) which has been diluted fifty-fold with water \( R \). Dilute to 10 ml with water \( R \).

**Reference solutions.** Into a 100 ml volumetric flask, introduce 10.0 ml of a standard solution containing 1.00 \( \mu g/ml \) of each of the metals to be determined and prepared by dilution, with water \( R \), of each elementary standard solution for atomic spectrometry (1.000 g/l) \( R \) for the corresponding elements. Add 1.00 ml of a 10 \( \mu g/ml \) solution of indium prepared from indium elementary standard solution for atomic spectrometry (1.000 g/l) \( R \) in nitric acid \( R \) which has been diluted fifty-fold with water \( R \). Dilute to 100.0 ml with water \( R \).

**Blank solution.** Dilute one hundred-fold with water \( R \) the 10 \( \mu g/ml \) solution of indium used for the test and reference solutions.
Metixene hydrochloride

**EUROPEAN PHARMACOPOEIA 6.0**

**Optical detection**

<table>
<thead>
<tr>
<th>Element</th>
<th>Signal (nm)</th>
<th>Background 1 (nm)</th>
<th>Background 2 (nm)</th>
<th>Isotope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aluminium</td>
<td>396.15</td>
<td>396.05</td>
<td>396.25</td>
<td>27</td>
</tr>
<tr>
<td>Cadmium</td>
<td>214.44</td>
<td>214.37</td>
<td>214.51</td>
<td>114</td>
</tr>
<tr>
<td>Chromium</td>
<td>283.56</td>
<td>283.49</td>
<td>283.64</td>
<td>*</td>
</tr>
<tr>
<td>Copper</td>
<td>327.40</td>
<td>327.31</td>
<td>327.48</td>
<td>65</td>
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<tr>
<td>Tin</td>
<td>190.00**</td>
<td>189.90</td>
<td>190.10</td>
<td>118</td>
</tr>
<tr>
<td>Iron</td>
<td>238.20</td>
<td>238.27</td>
<td>238.14</td>
<td>*</td>
</tr>
<tr>
<td>Manganese</td>
<td>260.57</td>
<td>260.50</td>
<td>260.64</td>
<td>55</td>
</tr>
<tr>
<td>Mercury</td>
<td>253.70***</td>
<td>253.60</td>
<td>253.80</td>
<td>200</td>
</tr>
<tr>
<td>Molybdenum</td>
<td>202.03</td>
<td>202.02</td>
<td>202.04</td>
<td>95</td>
</tr>
<tr>
<td>Nickel</td>
<td>231.60</td>
<td>231.54</td>
<td>231.66</td>
<td>60</td>
</tr>
<tr>
<td>Lead</td>
<td>217.00**</td>
<td>216.90</td>
<td>217.10</td>
<td>208</td>
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<tr>
<td>Zinc</td>
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<td>213.80</td>
<td>213.91</td>
<td>66</td>
</tr>
<tr>
<td>Indium</td>
<td></td>
<td></td>
<td></td>
<td>115</td>
</tr>
</tbody>
</table>

*Element difficult, if not impossible, to be determined with a mass spectrometer as detector.
**Borderline sensitivity with conventional optical spectrometry.
***Mercury is often impossible to determine using conventional optical spectrometry; it may be quantified using a device for the determination of hydrides.

**Element Maximum content in ppm**

<table>
<thead>
<tr>
<th>Element</th>
<th>Maximum content in ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aluminium</td>
<td>1.00 × 10²</td>
</tr>
<tr>
<td>Cadmium</td>
<td>1.0</td>
</tr>
<tr>
<td>Chromium</td>
<td>1.00 × 10²</td>
</tr>
<tr>
<td>Copper</td>
<td>3.00 × 10²</td>
</tr>
<tr>
<td>Tin</td>
<td>10.0</td>
</tr>
<tr>
<td>Iron</td>
<td>2.00 × 10²</td>
</tr>
<tr>
<td>Manganese</td>
<td>10.0</td>
</tr>
<tr>
<td>Mercury</td>
<td>1.0</td>
</tr>
<tr>
<td>Molybdenum</td>
<td>10.0</td>
</tr>
<tr>
<td>Nickel</td>
<td>10.0</td>
</tr>
<tr>
<td>Lead</td>
<td>10.0</td>
</tr>
<tr>
<td>Zinc</td>
<td>1.00 × 10⁵</td>
</tr>
</tbody>
</table>

**Loss on drying (2.2.32):** 8.0 per cent to 22.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

**Sulphated ash (2.4.14):** maximum 0.25 per cent, determined on 1.0 g.

**ASSAY**

Dissolve 0.300 g in 30 ml of water R with heating. Cool, add 50.0 ml of potassium dichromate solution R1 and dilute to 100.0 ml with water R. Allow to stand for 10 min. Filter and discard the first 20 ml of filtrate. Introduce 50.0 ml of the filtrate into a flask with a ground-glass neck, add 50 ml of dilute sulphuric acid R and 8.0 ml of potassium iodide solution R. Allow to stand protected from light for 5 min, then add 80 ml of water R. Titrate with 0.1 M sodium thiosulphate using 2 ml of starch solution R, added towards the end of the titration, as indicator. Carry out a blank titration.

1 ml of 0.1 M sodium thiosulphate is equivalent to 10.66 mg of C₂₁H₂₁ClN₃S₂.

**STORAGE**

In an airtight container, protected from light.

**IMPURITIES**

*Specified impurities: A.*

A. 3-(dimethylamino)-7-(methylamino)phenothiazin-5-ylium.

01/2008:1347

**METIXENE HYDROCHLORIDE**

Metixeni hydrochloridum

C₂₀H₂₄ClNS·H₂O

M, 363.9
[7081-40-5]

**DEFINITION**

Metixene hydrochloride contains not less than 98.0 per cent and not more than the equivalent of 102.0 per cent of (RS)-1-methyl-3-[(9H-thioxanthen-9-yl)methyl]piperidine hydrochloride, calculated with reference to the dried substance.

**CHARACTERS**

A white or almost white, crystalline or fine crystalline powder, soluble in water, soluble in alcohol and in methylene chloride.

**IDENTIFICATION**

A. Examine by infrared absorption spectrophotometry (2.2.24), comparing with the spectrum obtained with metixene hydrochloride CRS.

B. It gives reaction (a) of chlorides (2.3.1).

**TESTS**

**Appearance of solution.** Dissolve 0.40 g in methanol R and dilute to 20.0 ml with the same solvent. The solution is clear (2.2.1) and not more intensely coloured than reference solution Y₆ (2.2.2, Method I).

**pH (2.2.3).** Dissolve 0.18 g in carbon dioxide-free water R heating if necessary at about 50 °C, cool and dilute to 10.0 ml with the same solvent. The pH of the solution, measured immediately, is 4.4 to 5.8.

**Related substances.** Examine by thin-layer chromatography (2.2.27), using a TLC silica gel plate R. Carry out the test rapidly and protected from light.

**Test solution.** Dissolve 50 mg of the substance to be examined in methylene chloride R and dilute to 5.0 ml with the same solvent.

**Reference solution (a).** Dissolve 5 mg of metixene hydrochloride CRS in methylene chloride R and dilute to 100.0 ml with the same solvent.

**Reference solution (b).** Dissolve 20 mg of thioxanthene CRS in 50 ml of methylene chloride R. Dilute 1.0 ml of the solution to 20.0 ml with methylene chloride R.