The following Draft proposal for Amendments are placed for stakeholders comments, if any. The comments may be sent to the Indian Pharmacopoeia Commission through email id lab.ipc@gov.in

General Notices
Page. 14,1080, 4242, 4156
Insert before Test Methods
Residual solvents. The requirements, guidance and information on residual solvents for pharmaceutical use are given in the chapter entitled Residual Solvents (5.4).

All IP articles are subject to relevant control of residual solvents, even when no test is specified in the individual monograph. If solvents are used during production, they must be of suitable quality. In addition, the toxicity and residual level of each solvent shall be taken into consideration, and the solvents limited according to the principles defined and the requirements specified in 5.4. Residual Solvent, using the general methods presented therein or other suitable methods.

2.5.5. Friability of Uncoated Tablets. Page 309
Insert at the end.
Effervescent tablets and chewable tablets may have different specifications as far as friability is concerned. In the case of hygroscopic tablets, a humidity controlled environment is required for testing.

A drum with dual scooping projections, or apparatus with more than one drum, for the running of multiple samples at one time, are also required.

Colchicine. Page 1690
Change to: Colchicine

\[
\text{C}_{22}\text{H}_{25}\text{NO}_6 \quad \text{Mol. Wt. 399.4}
\]

Colchicine is \(N\)-[(7S, 12aM)-1, 2, 3, 10-tetramethoxy-9-oxo-5, 6, 7, 9-tetrahydrobenzo[\(a\)]heptalen-7-yl] acetamide, an alkaloid which occurs in the corm and seeds of various species of *Colchicum*.

Colchicine contains not less than 97.0 per cent and not more than 102.0 per cent of \(\text{C}_{22}\text{H}_{25}\text{NO}_6\), calculated on the anhydrous basis.

*Note: Colchicine is extremely poisonous.*

**Category.** Gout suppressant

**Dose.** Initial dose, 1 mg; subsequent doses, 500 µg every two hours.

**Description.** A pale yellow, amorphous or crystalline powder.

**Identification**
Tests A, C and D may be omitted if test B is carried out. Test B may be omitted if tests A, C and D are carried out.

A. When examined in the range 230 nm to 400 nm (2.4.7), a 0.001 per cent w/v solution in ethanol (95 per cent) shows absorption maxima, at about 243 nm and 350 nm and the absorbance ratio of $A_{243}/A_{350}$ is 1.7 to 1.9.

B. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with colchicine RS or with the reference spectrum of colchicine. Ignore any peak at 1735 cm$^{-1}$.

C. To 0.5 ml of solution A (see tests), add 0.5 ml of dilute hydrochloric acid and 0.15 ml of ferric chloride solution. The solution is yellow and becomes dark green on boiling for 30 seconds. Cool, add 2 ml of methylene chloride and shake. The organic layer is greenish-yellow.

D. Dissolve 30 mg in 1.0 ml of ethanol (95 per cent) and add 0.15 ml of ferric chloride solution. A brownish red colour develops.

Tests

**Appearance of solution.** A 0.5 per cent w/v solution in carbon dioxide-free water (Solution A) is clear (2.4.1) and not more intensely coloured than reference solution GYS3 (2.4.1).

**Acidity or alkalinity.** To 10 ml of solution A, add 0.1 ml of bromothymol blue solution; either the solution does not change colour or it becomes green. Not more than 0.1 ml of 0.01 M sodium hydroxide is required to change the colour of the indicator to blue.

**Specific optical rotation** (2.4.22). -250° to -235°, determined in a 0.5 per cent w/v solution in ethanol (95 per cent) at 20°.

**Related substances.** Determine by liquid chromatography (2.4.14).

**Solvent mixture.** 50 volumes of methanol and 50 volumes of water.

**Test solution.** Dissolve 20 mg of the substance under examination in the solvent mixture and dilute to 20.0 ml with the solvent mixture.

**Reference solution (a).** Dissolve 5 mg of colchicine for peak identification RS (containing impurities A, E and G) in the solvent mixture and dilute to 5.0 ml with the solvent mixture.

**Reference solution (b).** A 0.001 per cent w/v solution of colchicine RS in the solvent mixture.

**Chromatographic system**
- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 450 volumes of a 0.68 per cent w/v solution of potassium dihydrogen orthophosphate and 530 volumes of methanol, cool to room temperature and dilute to 1000 ml with methanol, adjusted to the pH 5.5 with orthophosphoric acid,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20µl.

<table>
<thead>
<tr>
<th>Name</th>
<th>Relative Retention time</th>
</tr>
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<tr>
<td>Colchicine impurity E$^1$</td>
<td>0.6</td>
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<tr>
<td>Colchicine impurity B$^2$</td>
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<tr>
<td>Colchicine impurity A$^3$</td>
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<tr>
<td>Colchicine (Retention time: about 7 minutes)</td>
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</tr>
<tr>
<td>Colchicine impurity G$^4$</td>
<td>1.4</td>
</tr>
</tbody>
</table>

$^1$3-O-demethylcolchicine,
$^2$conformational isomer,
$^3$N-deacetyl-N-formylcolchicine,
Inject reference solution (a). Adjust the sensitivity of the system so that the peak-to-valley ratio is minimum 2.0, where \(H_p\) is height above the baseline of the peak due to impurity A and \(H_v\) is the height above the baseline of the lowest point of the curve separating this peak from the peak due to colchicines; the peak-to-valley ratio is minimum 2.0, where \(H_p\) is height above the baseline of the peak due to impurity B and \(H_v\) is the height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity A.

Inject the reference solution (b) and the test solution. Run the chromatogram 3 times the retention time of the principal peak. The area of any peak corresponding to colchicine impurity A is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3.0 per cent), the area of any peak corresponding to colchicine impurity G multiplied with correction factor 1.6, is not more than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent), the area of any peak corresponding to colchicine impurity E is not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and the sum of areas of all the secondary peaks is not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (4.0 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (0.05 per cent) and peak due to impurity B.

**Impurity F (Colchicine).** Not more than 0.2 per cent.

Dissolve 50 mg in 5 ml of water, add 0.1 ml of a 10.5 per cent w/v solution of ferric chloride. Any colour produced is not more than intense than that obtained by mixing 2.0 ml of ferric chloride colorimetric solution (FCS) with 1.0 ml of cobaltous chloride colorimetric solution (CCS) and 2.0 ml of cupric sulphate colorimetric solution (CSS) (2.4.1).

**Ethyl acetate.** Not more than 8.0 per cent w/w.

Determine by gas chromatography (2.4.13).

*Internal standard solution.* Dilute 0.5 ml of n-propyl alcohol to 100.0 ml with water.

*Test solution.* Dissolve 0.25 g of the substance under examination in 8 ml of water, add 1.0 ml of internal standard solution and dilute to 10.0 ml with water.

*Reference solution.* A 0.09 per cent w/w solution of ethyl acetate prepared by mixing 1.0 ml of ethyl acetate, 0.5 ml of diacetone alcohol, and 0.5 ml of n-propyl alcohol and diluted to 1000 ml with water.

**Chromatographic system**
- a fused silica column 30 m x 0.53 mm, packed with polyethylene glycol 20M (film thickness 1.0\textmu m),
- temperature:
  - column: 40\textdegree, hold for 20 minutes, 40\textdegree to 200\textdegree @ 20\textdegree per minute and hold for 10 minutes,
  - inlet port 180\textdegree and detector at 220\textdegree,
- split ratio: 15:1,
- flame ionization detector,
- flow rate: 5.72 ml per minute using nitrogen as the carrier gas,
- injection volume: 2 \mu l.

Inject the reference solution. The test is not valid unless the relative standard deviation of peak area ratio of ethyl acetate to the n-propyl alcohol and diacetone alcohol to n-propyl alcohol peak, from replicate injections is not more than 15.0 per cent.

Inject the reference solution and the test solution.

Calculate the percentage w/w of ethyl acetate by taking 0.901 g as the weight per ml at 20\textdegree (2.4.29).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.
**Water** (2.3.43). Not more than 2.0 per cent, determined on 0.5 g.

**Assay.** Determine by liquid chromatography (2.4.14).

**NOTE.** Protect the solutions from light and prepare the solutions immediately before use.

**Solvent mixture.** 50 volumes of methanol and 50 volumes of water.

**Test solution.** Dissolve 30 mg of Colchicine in about 50 ml of the solvent mixture and dilute to 100.0 ml with the solvent mixture. Dilute 1.0 ml of the solution to 50.0 ml and filter.

**Reference solution.** A 0.0006 per cent w/v solution of colchicine RS in the solvent mixture.

**Chromatographic system**
- a stainless steel column 25 cm x 4.6 mm packed with octylsilane bonded to porous silica (5µm),
- mobile phase: dilute 45 ml of 0.5 M monobasic potassium phosphate with water to 450 ml, add about 530 ml of methanol, cool to room temperature and dilute to 1000 ml with methanol, adjust to pH 5.5 with 0.5 M orthophosphoric acid,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 4500 theoretical plates and the relative standard deviation for replicate injections is not more than 2 per cent.

Inject the reference solution and the test solution.

Calculate the content of \( \text{C}_{22}\text{H}_{25}\text{NO}_6 \).

**Storage.** Store protected from light and moisture.

**Colchicine Tablets.** Page 1691

Change to: **Colchicine Tablets**

Colchicine Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of colchicine, \( \text{C}_{22}\text{H}_{25}\text{NO}_6 \).

**Usual strengths.** 250 µg; 500 µg.

**Identification**

A. Disperse a quantity of the powdered tablets containing 5 mg of Colchicine in 50 ml of methanol (50 per cent) and filter. Dilute 10 ml of the filtrate to 100 ml with methanol (50 per cent). When examined the solution in the range 220 nm to 400 nm (2.4.7), shows absorption maxima at about 246 nm and 352 nm.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

**Tests**

**Dissolution** (2.5.2).

Apparatus No. 1.

Medium. 500 ml of phosphate buffer pH 6.8,

Speed and time. 75 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14), using chromatographic system, as described under Related substances with 50 µl injection volume and spectrophotometer set at 243 nm.
NOTE - Carry out the test protected from light.

Test solution. Dilute the filtrate if necessary, with the dissolution medium to obtain a solution containing 0.0001 per cent w/v of colchicine.

Reference solution. A 0.0001 per cent w/v solution of colchicine RS in the dissolution medium.

Inject the reference solution and the test solution.

Calculate the content of C$_{22}$H$_{25}$NO$_6$, in the medium

D. Not less than 75 per cent of the stated amount of C$_{22}$H$_{25}$NO$_6$.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE - Carry out the test protected from light.

Test solution (a). Disperse a quantity of powdered tablets containing 5 mg of colchicine in 40 ml of methanol (50 per cent) with the aid of ultrasound and dilute to 50.0 ml with the same solvent, filter.

Test solution (b). Dilute 1 volume of test solution (a) to 100 volumes of methanol (50 per cent).

Test solution (c). Dilute 1 volume of test solution (b) to 10 volumes of methanol (50 per cent).

Reference solution. A 0.1 per cent w/v solution of colchicine for system suitability A RS in methanol (50 per cent).

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octylsilane bonded to porous silica (5 µm) (such as Lichrosorb RP8),
- mobile phase: A, water,
  B, methanol
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

<table>
<thead>
<tr>
<th>Time (in min.)</th>
<th>Mobile phase A (per cent w/v)</th>
<th>Mobile phase B (per cent w/v)</th>
</tr>
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<tr>
<td>38</td>
<td>52</td>
<td>48</td>
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</table>

The relative retention time with reference to colchicine (retention time- about 13 minutes) for impurity A is about 0.9.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to colchicine impurity A and colchicine peak is not less than 1.5.

Inject test solution (a), (b) and (c). In the chromatogram obtained with test solution (a), the area of any peak corresponding to colchicine impurity A is not more than 3.5 times the area of the principal peak in the chromatogram obtained with test solution (b) (3.5 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with test solution (b) (1.0 per cent) and the sum of areas of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with test solution (b) (5.0 per cent). Ignore any peak with an area less than the area of the principal peak in the chromatogram obtained with test solution (c) (0.1 per cent).
Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay with the following modifications.

Test solution. Disperse one tablet in 4 ml of the solvent mixture, with the aid of ultrasound and dilute to 5.0 ml with the solvent mixture, filter.

Inject reference solution (a) and the test solution.

Calculate the content of \( C_{22}H_{25}NO_6 \) in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

NOTE - Carry out the test protected from light.

Solvent mixture. 50 volumes of methanol and 50 volumes of water.

Test solution. Disperse 10 intact tablets in 40 ml of the solvent mixture, with the aid of ultrasound and dilute to 50.0 ml with the solvent mixture, filter.

Reference solution (a). Dissolve an accurately weighted quantity of colchicine RS in the solvent mixture and dilute with the solvent mixture to obtain a solution having a known concentration similar to the expected concentration of the test solution.

Reference solution (b). A 0.1 per cent w/v solution of colchicine for system suitability A RS in the solvent mixture.

Use chromatographic system as described under Related substances.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to colchicine impurity A and colchicine is not less than 1.5.

Inject reference solution (a) and the test solution.

Calculate the content of \( C_{22}H_{25}NO_6 \) in the tablets.

Storage. Store protected from light.

Dapsone. Page 1758

Para 1
Change to: Dapsone contains not less than 98.0 per cent and not more than 102.0 per cent of \( C_{12}H_{12}N_2O_2S \), calculated on the dried basis.

Assay
Change to: Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Reference solution. A 0.0025 per cent w/v solution of dapsone RS in the mobile phase.

Chromatographic system
- a stainless steel column 30 cm x 4.0 mm, packed with porous silica particles (10 µm),
- mobile phase: a mixture of 10 volumes of isopropyl alcohol, 10 volumes of acetonitrile, 10 volumes of ethyl acetate and 70 volumes of hexane,
- flow rate: 1 ml per minute,
Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2 per cent.

Inject the reference solution and the test solution. Run the chromatogram twice the retention time of the principal peak.

Calculate the content of \( \text{C}_{12}\text{H}_{12}\text{N}_{2}\text{O}_{2}\text{S} \).

**Disulfiram.** Page 1862

Change to: **Disulfiram**

![Disulfiram molecule]

\( \text{C}_{10}\text{H}_{20}\text{N}_{2}\text{S}_{4} \)  \hspace{1cm} \text{Mol.Wt. 296.5}

Disulfiram is thioperoxidicarbonic diamide\([\text{H}_{2}\text{N}(\text{C(S)})_{2}\text{S}_{2}]_{\text{tetraethyl-}}\); \text{Bis(diethylthiocarbamoyl)} disulfide.

Disulfiram contains not less than 98.0 per cent and not more than 102.0 per cent of \( \text{C}_{10}\text{H}_{20}\text{N}_{2}\text{S}_{4} \).

**Category.** Used in the treatment of alcoholism.

**Dose.** 500 mg as a single dose for 1 to 2 weeks; maintenance dose, 125 to 500 mg daily.

**Description.** A white to off-white, crystalline powder.

**Identification**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with disulfiram RS or with the reference spectrum of disulfiram.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

**Tests**

**Related substances.** Determine by liquid chromatography (2.4.14).

*NOTE.* Prepare the solutions immediately before use.

**Solvent mixture.** 50 volumes of mobile phase A and 50 volumes of mobile phase B.

**Test solution.** Disperse 0.2 g of Disulfiram in methanol and dilute to 100.0 ml with methanol. Dilute 10.0 ml of the solution to 20.0 ml with the solvent mixture.

**Reference solution (a).** A 0.01 per cent w/v solution of disulfiram RS in methanol. Dilute 10.0 ml of the solution to 100.0 ml with the solvent mixture.
Reference solution (b). A solution containing 0.005 per cent w/v of disulfiram RS and 0.001 per cent w/v of sulfiram RS in the solvent mixture.

Chromatographic system
- a stainless steel column 15 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- sample temperature: 4°,
- mobile phase: A. buffer solution prepared by dissolving 6.8 g of monobasic potassium phosphate in 1000 ml of water, adjusted to pH 7.0 with 45 per cent w/v solution of potassium hydroxide,
  B. methanol,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 250 nm,
- injection volume: 15 µl.

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<th>Name</th>
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<td>Diethyldithiocarbamic acid</td>
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<td>Tetraethylthiourea¹</td>
<td>0.69</td>
<td>0.91</td>
</tr>
<tr>
<td>Sulfiram²</td>
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<tr>
<td>Disulfiram</td>
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</tbody>
</table>

¹,1,1,3,3-Tetraethylthiourea.  
²Diethyldithiocarbamic thioanhydride.

Inject reference solution (b). The test is not valid unless the resolution between sulfiram and disulfiram is not less than 8.0.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to diethyldithiocarbamic acid, tetraethylthiourea and sulfiram, each of, is not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent), the area of any other secondary peak is not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent) and the sum of the areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

Selenium. Not more than 30 ppm, Determine by the Oxygen-Flask method (2.3.34).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Assay. Determine by liquid chromatography (2.4.14).

NOTE: Use freshly prepared solutions.
**Test solution.** Dissolve 50 mg of the substance under examination in 40 ml of *ethanol*, with the aid of ultrasound for 5 minutes and dilute to 50.0 ml with *ethanol*. Dilute 2.0 ml of the solution to 100.0 ml with the mobile phase.

**Reference solution.** A 0.1 per cent w/v solution of *disulfiram RS* in *ethanol*. Dilute 2.0 ml of the solution to 100.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 30 volumes of a buffer solution prepared by dissolving 6.8 g of *monobasic potassium phosphate* in 1000 ml of *water*, adjusted to pH 7.0 with 45 per cent w/v solution of *potassium hydroxide* and 70 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 250 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 1800 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of C\(_{10}\)H\(_{20}\)N\(_2\)S\(_4\).

**Storage.** Store protected from light and moisture.

**Disulfiram Tablets.** Page 1862

Change to: **Disulfiram Tablets**

Disulfiram Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of disulfiram C\(_{10}\)H\(_{20}\)N\(_2\)S\(_4\).

**Usual strengths.** 200 mg; 250 mg; 500 mg.

**Identification**

A. Extract a quantity of the powdered tablets containing 0.5 g of Disulfiram with 20 ml of *methanol*, filter, evaporate the filtrate to dryness and dry at 105º. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *disulfiram RS* or with the reference spectrum of disulfiram.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

**Tests**

**Disintegration** (2.5.1). Not more than 15 minutes, carry out the test without discs.

**Related substances.** Determine by liquid chromatography (2.4.14).

*NOTE*- Prepare the solutions immediately before use.

**Solvent mixture.** 50 volumes of mobile phase A and 50 volumes of mobile phase B.

**Test solution.** Disperse a quantity of the powdered tablets containing 0.2 g of Disulfiram in *methanol* and dilute to 100.0 ml with *methanol*. Dilute 10.0 ml of the solution to 20.0 ml with the solvent mixture.

**Reference solution (a).** A 0.01 per cent w/v solution of *disulfiram RS* in *methanol*. Dilute 10.0 ml of the solution to 100.0 ml with the solvent mixture.
Reference solution (b). A solution containing 0.005 per cent w/v of disulfiram RS and 0.001 per cent w/v of sulfiram RS in the solvent mixture.

Chromatographic system
- a stainless steel column 15 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- sample temperature: 4°,
- mobile phase: A. buffer solution prepared by dissolving 6.8 g of monobasic potassium phosphate in 1000 ml of water, adjusted to pH 7.0 with 45 per cent w/v solution of potassium hydroxide,
  B. methanol,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 250 nm,
- injection volume: 15 µl.

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²Diethyldithiocarbamic thioanhydride.

Inject reference solution (b). The test is not valid unless the resolution between sulfiram and disulfiram is not less than 8.0.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to diethyldithiocarbamic acid, tetraethylthiourea and sulfiram, each of, is not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent), the area of any other secondary peak is not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent) and the sum of the areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

NOTE- Prepare the solutions immediately before use.

Test solution. Weigh and powder 20 tablets. Weigh a quantity of the powder containing 0.1 g of Disulfiram in 70 ml of ethanol, with the aid of ultrasound with intermittent shaking for 30 minutes, dilute to 100.0 ml with ethanol and filter. Dilute 2.0 ml of the solution to 100.0 ml with the mobile phase.
Reference solution. A 0.1 per cent w/v solution of disulfiram RS in ethanol. Dilute 2.0 ml of the solution to 100.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 4.0 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 30 volumes of a buffer solution prepared by dissolving 6.8 g of monobasic potassium phosphate in 1000 ml of water, adjusted to pH 7.0 with 45 per cent w/v solution of potassium hydroxide and 70 volumes of methanol,
- flow rate: 1 ml per minute,
- spectrophotometer set at 250 nm,
- injection volume: 20 µl.

Run the chromatogram 1.5 times the retention time of disulfiram.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of C₁₀H₂₇N₂S₄ in the tablets.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Glycerin. Page 2183
Change to: Glycerin
Glycerol

Glycerin is propane-1,2,3-triol.

Glycerin contains not less than 98.0 per cent and not more than 101.0 per cent of C₃H₆O₃, calculated on the anhydrous basis.

Category. Lubricant; laxative; pharmaceutical aid (humectant).

Description. A clear, colourless or almost colourless, syrupy liquid; odourless; hygroscopic.

Identification

Test C may be omitted if tests A and B are carried out. Test A may be omitted if tests B and C are carried out.

A. Mix 5 ml of Glycerin with 1 ml of water. On the solution, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with glycerin RS or with the reference spectrum of glycerin.

B. Refractive index (2.4.27). 1.470 to 1.475, determined at 20°.

C. Relative density (2.4.29). 1.258 to 1.268, determined at 20°.

Tests
Appearance of solution. Dissolve 50.0 g of Glycerin in carbon dioxide-free water and dilute to 100.0 ml with the same solvent (solution A). Solution A is clear (2.4.1). Dilute 10 ml of solution A to 25 ml with water. The solution is colourless (2.4.1).

Acidity or alkalinity. To 50 ml of solution A, add 0.5 ml of phenolphthalein solution. The solution is colourless and not more than 0.2 ml of 0.1 M sodium hydroxide is required to produce a pink colour. Reserve this solution for the test for Esters.

Heavy metals (2.3.13). Dissolve 4.0 g of Glycerin in 2 ml of 0.1 M hydrochloric acid and dilute to 25 ml with water. The solution complies with the limit test for heavy metals, Method A (5 ppm).

Chlorides (2.3.12). 8.0 ml of solution A, complies with the limit test for chlorides (10 ppm).

Aldehydes. To 7.5 ml of solution A in a glass-stoppered flask, add 7.5 ml of water and 1.0 ml of decolourised pararosaniline solution, close the flask and allow to stand for 1 hour at 25°. The absorbance of the solution measured at 552 nm (2.4.7) is not more intense than that obtained in a standard solution prepared in the same manner by using 7.5 ml of formaldehyde standard solution (5 ppm CH₃O) and 7.5 ml of water. The test is not valid unless the standard solution is pink. (10 ppm).

Esters. Add 10 ml 0.1 M sodium hydroxide to the solution reserved in the test for Acidity or alkalinity. Boil under a reflux condenser for 5 minutes. Cool, add 0.5 ml of phenolphthalein solution and titrate with 0.1 M hydrochloric acid. Not less than 8.0 ml of 0.1 M hydrochloric acid is required to decolourise the solution.

Halogenated compounds. Not more than 35 ppm.

To 10 ml of solution A, add 1 ml of dilute sodium hydroxide solution, 5 ml of water and 50 mg of halogen-free nickel-aluminium alloy. Heat on a water-bath for 10 minutes, allow to cool and filter. Rinse the flask and the filter with water until 25 ml of filtrate is obtained. To 5 ml of the filtrate, add 4 ml of ethanol (95 per cent), 2.5 ml of nitric acid and 0.05 ml of silver nitrate solution and mix. Allow to stand for 2 minutes. Any opalescence in the solution is not more intense than that in a standard prepared at the same time by mixing 7.0 ml of chloride standard solution (5 ppm Cl), 4 ml of ethanol (95 per cent), 0.5 ml of water, 0.5 ml of nitric acid and 0.05 ml of silver nitrate solution.

Impurity A and related substances. Determine by gas chromatography (2.4.13).

Test solution. Dilute 10.0 ml of solution A to 100.0 ml with water.

Reference solution (a). A 5.0 per cent w/v solution of glycerin RS in water.

Reference solution (b). A 1.0 per cent w/v solution of diethylene glycol RS (Impurity A) in water.

Reference solution (c). Dilute 1.0 ml of reference solution (b) to 10.0 ml with reference solution (a). Dilute 1.0 ml of this solution to 20.0 ml with reference solution (a).

Reference solution (d). Mix 1.0 ml of the test solution and 5.0 ml of reference solution (b) and dilute to 100.0 ml with water. Dilute 1.0 ml of the solution to 10.0 ml with water.

Reference solution (e). Dilute 5.0 ml of reference solution (b) to 100.0 ml with water.

Chromatographic system
- a capillary column 30 m x 0.53 mm, packed with cyanopropylphenyl dimethylpolysiloxane (film thickness 3 µm), (Such as DB-624),
- temperature: column. 100° to 220° @ 7.5° per minutes, hold for 4 minutes, inlet port 220° and detector 250°,
- split ratio 1:10,
- flame ionization detector,
- linear velocity: 38 cm per second using nitrogen as the carrier gas,
injection volume: 0.5 µl.

Inject reference solution (d). The test is not valid unless the resolution between the peaks due to impurity A and glycerin is not less than 7.0.

Inject reference solution (c) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to impurity A is not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent); the area of any other secondary peak with retention time less than the retention of glycerin is not more than the area of the peak due to the impurity A in the chromatogram obtained with reference solution (c) (0.1 per cent); the sum of the areas of all the secondary peaks with retention time more than the retention time of glycerin is not more than 5 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (c) (0.5 per cent). Ignore any peak with an area less than 0.05 times of the area of the principal peak in the chromatogram obtained with reference solution (e) (0.05 per cent).

**Sugars.** Heat 10 ml of solution A with 1 ml of *dilute sulphuric acid* on a water-bath for 5 minutes. Add 3 ml of *dilute sodium hydroxide* (carbonate-free), mix and add drop wise 1 ml of freshly prepared *copper sulphate solution*; a clear blue solution is produced. Continue heating on the water-bath for 5 minutes; the solution remains blue and no precipitate is produced.

**Sulphated ash** (2.3.18). Not more than 0.01 per cent, determined on 5.0 g after heating to boiling and ignition.

**Water** (2.3.43). Not more than 2.0 per cent, determined on 1.0 g.

**Assay.** Mix 75 mg in 45 ml of *water*, add 25.0 ml of a mixture of 1 volume of 0.1 *M sulphuric acid* and 20 volumes of 0.1 *M sodium periodate*. Allow to stand protected from light for 15 minutes. Add 5 ml of a 50 per cent w/v solution of *ethylene glycol*, allow to stand protected from light for 20 minutes and titrate with 0.1 *M sodium hydroxide* using 0.5 ml of *phenolphthalein solution* as indicator. Carry out a blank titration.

1 ml of 0.1 *M sodium hydroxide* is equivalent to 0.00921 g of C₃H₈O₃.

**Storage.** Store protected from moisture.

**Hydroxychloroquine Sulphate.** Page 2234

Change to: **Hydroxychloroquine Sulphate**

C₁₈H₂₆CIN₃O,H₂SO₄  Mol Wt. 434.0

Hydroxychloroquine Sulphate is 2-[(RS)-4-[(7-chloroquinolin-4-yl)amino]pentyl]ethylamino]ethan-1-ol sulphate. Hydroxychloroquine Sulphate contains not less than 98.0 per cent and not more than 102.0 per cent of C₁₈H₂₆CIN₃O,H₂SO₄, calculated on the dried basis.

**Category.** Antiprotozoal.

**Description.** A white to off-white, crystalline powder.

**Identification**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum that obtained with *hydroxychloroquine sulphate RS* or with the reference spectrum of hydroxychloroquine sulphate.

B. It gives reaction (A) of sulphates (2.3.1).

**Tests**

**Related substances.** Determine by liquid chromatography (2.4.14).

*Test solution.* Dissolve 50 mg of substance under examination in mobile phase A and dilute to 50.0 ml with mobile phase A.

*Reference solution (a).* A 0.0001 per cent w/v solution of *hydroxychloroquine sulphate RS* in mobile phase A.
Reference solution (b). A solution containing 0.0001 per cent w/v each of desethyl hydroxychloroquine RS and hydroxychloroquine sulphate RS in mobile phase A.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 90 volumes of water, 10 volumes of acetonitrile and 0.2 volume of orthophosphoric acid,
  B. a mixture of 20 volumes of water, 80 volumes of acetonitrile and 0.1 volume of orthophosphoric acid,
- flow rate: 1 ml per minute,
- a gradient programme using the conditions given below,
- spectrophotometer set at 220 nm,
- injection volume: 10 µl.

<table>
<thead>
<tr>
<th>Time (in min.)</th>
<th>Mobile phase A (per cent v/v)</th>
<th>Mobile phase B (per cent v/v)</th>
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<tr>
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<td>0</td>
</tr>
<tr>
<td>2</td>
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<tr>
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<td>25.1</td>
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<td>50</td>
<td>100</td>
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<tr>
<th>Name</th>
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<th>Correction factor</th>
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<tr>
<td>Desethyl hydroxychloroquine¹</td>
<td>0.92</td>
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<td>Hydroxychloroquine sulphate</td>
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<tr>
<td>Hydroxychloroquine –O –sulphate²</td>
<td>1.4</td>
<td>1.34</td>
</tr>
<tr>
<td>4,7- dichloroquinoline</td>
<td>2.8</td>
<td>0.35</td>
</tr>
</tbody>
</table>

¹(RS)-2-[[4-[(7-chloro-4-quinolyl)amino] pentyl] amino] ethanol,
²(RS)-2-N-[(7-chloro-4-quinolyl amino) pentyl] –N- ethylaminoethanol-1-(hydrogen sulphate).

Inject reference solution (a) and (b). The test is not valid unless the resolution between the peaks due to desethyl hydroxychloroquine and hydroxychloroquine sulphate is not less than 1.0 in the chromatogram obtained with reference solution (b), the column efficiency in not less than 3000 theoretical plates, the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent in the chromatogram obtained with reference solution (a).

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to desethyl hydroxychloroquine is not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.4 per cent), the area of any peak corresponding to hydroxychloroquine-O-sulphate is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent), the area of any peak corresponding to 4,7-dichloroquinoline is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and the sum of the areas of all the secondary peaks is not more than 6 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.6 per cent). Ignore the peak due to sulphate and any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals (2.3.13). 1 g complies with the limit test for heavy metals, Method B (20 ppm).

Chlorides (2.3.12). Dissolve 0.7 g in 15.0 ml of water. The solution complies with the limit test for chlorides (350 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 2.0 per cent, determined on 1.0 g by drying in an oven at 105° for 2 hours.
Assay. Determine by liquid chromatography (2.4.14), as described under Related substances with the following modifications.

Test solution. Dissolve 50 mg of substance under examination in mobile phase A and dilute to 50.0 ml with mobile phase A. Dilute 1.0 ml of the solution to 10.0 ml with mobile phase A.

Reference solution. A 0.01 per cent w/v solution of hydroxychloroquine sulphate RS in mobile phase A.

Inject the reference solution and the test solution.

Calculate the content of C₁₈H₂₆ClN₃O.H₂SO₄.

Storage. Store protected from light.

Isopropyl Alcohol. Page 2326

Change to: Isopropyl Alcohol

Propan-2-ol

C₃H₈O  Mol. Wt. 60.1

Isopropyl Alcohol is propan-2-ol.

Category. Pharmaceutical aid (solvent).

Description. A clear and colourless liquid.

Identification

Tests A and D may be omitted if tests B and C are carried out. Test C may be omitted if tests A, B and D are carried out.

A. Weight per ml. (See test).

B. Refractive index. (See test).

C. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with isopropyl alcohol RS or with the reference spectrum of isopropyl alcohol.

D. To 1 ml, add 4 ml of water and mix. Carefully add 2 ml of 1 per cent w/v solution of dimethylaminobenzaldehyde in sulphuric acid, ensuring that the liquids do not mix; a bright reddish-violet ring forms immediately at the junction of the 2 liquids. After 2 to 5 minutes, the entire sulphuric acid layer turns violet.

Tests

Appearance of solution. Isopropyl Alcohol is clear (2.4.1) and colourless (2.4.1). A 5 per cent v/v solution of Isopropyl Alcohol in water remains clear (2.4.1) after 5 minutes.

Acidity or alkalinity. Gently boil 25 ml of Isopropyl Alcohol for 5 minutes, add 25 ml of carbon dioxide-free water and cool, protected from carbon-dioxide in the air. Add 0.1 ml of phenolphthalein solution. The solution is colourless. Not more than 0.6 ml of 0.01M sodium hydroxide is required to change the colour of the solution to pale pink.

Absorbance (2.4.7). When examined at the wavelength 230 nm, 250 nm, 270 nm, 290 nm and 310 nm, Isopropyl Alcohol shows an absorption maximum about 0.30, 0.10, 0.03, 0.02 and 0.01 respectively.

The absorbance is measured between 230 nm and 310 nm using water as the compensation liquid. The spectrum shows a steadily descending curve with no observable peaks or shoulders.
Refractive index (2.4.27). 1.376 to 1.379, determined at 20°.

Weight per ml (2.4.29). 0.785 g to 0.789 g, determined at 20°.

Peroxides. Place 8 ml of potassium iodide and starch solution in a 12-ml glass-stoppered cylinder of about 1.5 cm diameter. Fill completely with Isopropyl Alcohol, insert the stopper, shake vigorously and allow to stand in the dark for 30-minutes; no colouration is produced.

Benzene and related substances. Determine by gas chromatography (2.4.13).

Test solution (a). Isopropyl Alcohol.

Test solution (b). A 0.1 per cent v/v solution of 2-butanol in test solution (a).

Reference solution (a). A solution containing 0.1 per cent v/v each of 2-butanol and 1-propanol in test solution (a).

Reference solution (b). Dilute 0.1 ml of benzene to 100.0 ml with test solution (a). Dilute 0.2 ml of the solution to 100.0 ml with test solution (a).

Chromatographic system
- a fused silica column 30 m x 0.32 mm, packed with cyanopropylphenyl dimethylpolysiloxane (film thickness 1.8 µm), (Such as DB-624)
- temperature:
  - column. 40° hold for 12 minutes, 40° to 240° @ 10° per minute and hold at 240° for 10 minutes,
  - inlet port 280° and detector at 280°,
- split ratio: 1.5,
- flame ionization detector,
- linear velocity: 35 cm per second using nitrogen as the carrier gas,
- injection volume: 1 µl.

The retention time of benzene is about 10 minutes.

Inject reference solution (a). The test is not valid unless, the resolution between the peak due to 1-Propanol and 2-butanol is not less than 10.

Inject reference solution (b) and test solution (a). In the chromatogram obtained with test solution (a), the area of any peak corresponding to benzene is not more than 0.5 times the area of the benzene peak obtained with reference solution (b) (2 ppm). Record the chromatogram adjusting the sensitivity so that the height of the peak due to benzene represents least 10 per cent of full-scale deflection.

Inject reference solution (a) and test solution (b). In the chromatogram obtained with test solution (b), the area of all secondary peaks other than 2-butanol is not more than 3 times the area of the peak due to 2-butanol (0.3 per cent). Record the chromatogram adjusting the sensitivity so that the height of the 2 peaks following the principal peak in the chromatogram obtained with reference solution (a) represents at least 50 per cent of full-scale deflection.

Non-volatile substances. Not more than 20 ppm.

Evaporate 100 g on a water-bath after having verified that it complies with the test for peroxides and dry the residue at 105°. The residue weigh is not more than 2.0 mg.

Water (2.3.43). Not more than 0.5 per cent, determined on 5.0 g.

Storage. Store protected from light.

Solubility. Miscible with water, chloroform, ether and ethanol (95 per cent).
**Levocetirizine Hydrochloride.** Page 2413

**Related substances**

Change to: **Related substances.** Determine by liquid chromatography (2.4.14).

*NOTE-* Use the solutions within 16 hours.

**Test solution.** Dissolve 20 mg of the substance under examination in the mobile phase and dilute to 100.0 ml with the mobile phase, filter.

*Reference solution (a).* A solution containing 0.00002 per cent w/v each of levocetrizine dihydrochloride RS, levocetirizine amide RS and chlorobenzhydryl piperazine RS in the mobile phase.

*Reference solution (b).* A solution containing 0.02 per cent w/v of levocetirizine dihydrochloride RS and 0.00002 per cent w/v each of levocetirizine amide RS and chlorobenzhydryl piperazine RS in the mobile phase.

**Chromatographic system**

- a stainless steel column 25 cm x 4.6 mm packed with silica gel (5 µm),
- mobile phase: a mixture of 93 volumes of acetonitrile, 6.6 volumes of water and 0.4 volume of 1M sulphuric acid,
- flow rate: 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 20 µl.

<table>
<thead>
<tr>
<th>Name</th>
<th>Relative retention time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Levocetirizine</td>
<td>1.0</td>
</tr>
<tr>
<td>Chlorobenzhydryl piperazine1</td>
<td>1.3</td>
</tr>
<tr>
<td>Levocetirizine amide2</td>
<td>2.5</td>
</tr>
</tbody>
</table>

1(R)-1-{[(4-chlorophenyl)phenylmethyl]piperazine, 2(R)-2-{[(4-chlorophenyl)phenylmethyl]piperazin-1-yl}ethoxyacetamide.

Inject reference solution (a) and (b). The test is not valid unless the resolution between the peak due to chlorobenzhydryl piperazine and levocetirizine is not less than 3.0 and the tailing factor for levocetirizine is not more than 2.0 in the chromatogram obtained with reference solution (b) and the relative standard deviation for replicate injections for levocetirizine is not more than 5.0 per cent in the chromatogram obtained with reference solution (a).

Inject reference solution (a) and the test solution. Run the chromatogram 3 times the retention time of levocetirizine peak. The area of any peak corresponding to chlorobenzhydryl piperazine or levocetirizine amide is not more than twice the area of the peak due to chlorobenzhydryl piperazine or levocetirizine amide in the chromatogram obtained with reference solution (a) (0.2 per cent), the area of any other secondary peak is not more than the area of the peak due to levocetirizine in the chromatogram obtained with reference solution (a) (0.1 per cent) and the sum of areas of all the secondary peaks is not more than 5 times the area of the peak due to levocetirizine in the chromatogram obtained with reference solution (a) (0.5 per cent).

**Levocetirizine Tablets.** Page 2414

**Related substances**

Change to: **Related substances.** Determine by liquid chromatography (2.4.14).

**Test solution.** Disperse a quantity of powdered tablets containing 50 mg of Levocetirizine Dihydrochloride, in 20 ml of a mixture of 5.7 volumes of I M of sulphuric acid and 94.3 volumes of water, with the aid of ultrasound with intermittent shaking. Add 150 ml of acetonitrile, place the flask in an ultrasonic bath for 10 minutes, cool and dilute to 250.0 ml with acetonitrile. Centrifuge the solution and use the supernatant liquid.

*Reference solution (a).* A 0.0002 per cent w/v solution of levocetrizine dihydrochloride RS in the mobile phase.

*Reference solution (b).* A solution containing 0.02 per cent w/v of levocetirizine dihydrochloride RS and 0.00002 per cent w/v of chlorobenzhydryl piperazine RS in the mobile phase.

**Chromatographic system**

- a stainless steel column 25 cm x 4.6 mm packed with silica gel (5 µm),
– mobile phase: a mixture of 93 volumes of acetonitrile, 6.6 volumes of water and 0.4 volume of 1M sulphuric acid,
– flow rate: 1 ml per minute,
– spectrophotometer set at 230 nm,
– injection volume: 20 µl.

<table>
<thead>
<tr>
<th>Name</th>
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<tbody>
<tr>
<td>Levocetirizine</td>
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<tr>
<td>Chlorobenzhydryl piperazine(^{1,2})</td>
<td>1.4</td>
</tr>
<tr>
<td>Levocetirizin amide(^{1,3})</td>
<td>2.1</td>
</tr>
</tbody>
</table>

\(^{1}\) Process impurity controlled in drug substance and no need to report in drug product.
\(^{2}\)(R)-1-[(4-chlorophenyl)phenylmethyl]piperazine,
\(^{3}\)(R)-2-[(2-[(4-chlorophenyl)phenylmethyl]piperazin-1-yl]ethoxy)acetamide,

Inject reference solution (b). The test is not valid unless the resolution between the peak due to chlorobenzhydryl piperazine and levocetirizine is not less than 3.0, the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections for levocetirizine is not more than 1.0 per cent and for chlorobenzhydryl piperazine is not more than 5.0 per cent.

Inject reference solution (a) and the test solution. Run the chromatogram 2.3 times the retention time of levocetirizine peak. The area of any secondary peak is not more than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent) and the sum of areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent).

**Lincomycin Hydrochloride. Page 2441**

Change to: **Lincomycin Hydrochloride**

Lincomycin Hydrochloride Monohydrate

![Lincomycin Hydrochloride Monohydrate](image)

C\(_{18}\)H\(_{35}\)CIN\(_2\)O\(_8\)S, H\(_2\)O

Mol. Wt. 461.0

Lincomycin Hydrochloride consists mainly of methyl 6-amino-6,8-dideoxy-6-[[2S,4R]-1-methyl-4-propylproloidin-2-yl]carbonyl]amino]-1-thio-D-erythro-α-D-galacto-octopyranoside(lincomycin)hydrochloride monohydrate, a mixture of antibiotics produced by *Streptomyces lincolnensis* var. *lincolnensis* or obtained by any other means.

Lincomycin B Hydrochloride contains not more than 5.0 per cent and the sum of Lincomycin Hydrochloride, C\(_{18}\)H\(_{35}\)CIN\(_2\)O\(_8\)S and Lincomycin B Hydrochloride, C\(_{17}\)H\(_{33}\)CIN\(_2\)O\(_6\)S is not less than 96.0 per cent and not more than 102.0 per cent, calculated on the anhydrous basis.

**Category.** Antibacterial

**Dose.** Orally, the equivalent of 500 mg of lincomycin every 6 to 8 hours, 30 minutes before food; by intramuscular injection, the equivalent of 600 mg of lincomycin every 12 to 24 hours; by slow intravenous infusion, the equivalent of 600 mg of lincomycin every 8 to 12 hours.

**Description.** A white or almost white, crystalline powder.
Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with

*lincomycin hydrochloride RS* or with the reference spectrum of lincomycin hydrochloride.

B. A 1 per cent w/v solution gives reaction (A) of chlorides (2.3.1).

Tests

**Appearance of solution.** A 10.0 per cent w/v solution in *carbon dioxide-free water* (Solution A) is clear (2.4.1) and not more intensely coloured than reference solution YS6 (2.4.1).

**pH** (2.4.24). 3.5 to 5.5 determined in solution A.

**Specific optical rotation** (2.4.22). +135° to +150°, determined in 4.0 per cent w/v solution.

**Related substances.** Determine by liquid chromatography (2.4.14).

**Test solution.** Dissolve 25 mg of the substance under examination in the mobile phase and dilute to 10.0 ml with the mobile phase.

**Reference solution (a).** A 0.25 per cent w/v solution of lincomycin hydrochloride RS in the mobile phase.

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

**Reference solution (c).** Dissolve 5 mg of lincomycin hydrochloride for system suitability RS (containing impurities A, B and C) in 2 ml of the mobile phase.

**Chromatographic system**
- a stainless steel column 25 cm x 4.6 mm, packed with base-deactivated end capped octylsilane bonded to porous silica (5 µm),
- column temperature: 50°C,
- mobile phase: a mixture of 75 volumes of a buffer solution, prepared by dissolving 34 g of orthophosphoric acid in 900 ml of water, adjusted to pH 6.1 with concentrated ammonia, 17 volumes of acetonitrile and 8 volumes of methanol,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

<table>
<thead>
<tr>
<th>Name</th>
<th>Relative retention time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lincomycin impurity C&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.4</td>
</tr>
<tr>
<td>Lincomycin B</td>
<td>0.5</td>
</tr>
<tr>
<td>Lincomycin impurity A&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.7</td>
</tr>
<tr>
<td>Lincomycin (Retention time: about 10 minutes)</td>
<td>1.0</td>
</tr>
<tr>
<td>Lincomycin impurity B&lt;sup&gt;3&lt;/sup&gt;</td>
<td>1.2 and 1.3</td>
</tr>
</tbody>
</table>

<sup>1</sup> methyl 6,8-dideoxy-6-[[[2S,4R]-4-propylpyrrolidin-2-yl]carbonyl]amino]-1-thio-D-erythro-a-D-galacto-octopyranoside (N-desmethyl lincomycin),

<sup>2</sup> methyl 6,8-dideoxy-6-[[[2R,4R]-1-methyl-4-propylpyrrolidin-2-yl]carbonyl]amino]-1-thio-D-erythro-a-D-galacto-octopyranoside (a-amide epimer),

<sup>3</sup> methyl 6,8-dideoxy-6-[[[2S,4Z]-1-methyl-4-propylidenepyrrrolidin-2-yl]carbonyl]amino]-1-thio-D-erythro-a-D-galacto-octopyranoside (propylidene analogues).

Inject reference solution (c). The test is not valid unless the resolution between the peak due to lincomycin and 1st peak of impurity B is not less than 1.8.

Inject reference solution (b) and the test solution. Run the chromatogram 5.5 times the retention time of the principal peak for test solution. The area of any peak corresponding to lincomycin impurity A is not more than 0.5 times the area of principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent), the area of any peak corresponding to lincomycin impurity C is not more than 0.2 times the area of principal peak in the chromatogram.
obtained with reference solution (b) (0.2 per cent), the sum of areas of the peaks due to impurity B is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent), the area of any other secondary peak is not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.5 per cent.

Water (2.3.43). 3.1 per cent to 4.6 per cent, determined on 0.5 g.

Assay. Determine by liquid chromatography (2.4.14), as described under Related substances with the following modifications.

Inject reference solution (a) and the test solution.

Calculate the content of \( \text{C}_{18}\text{H}_{35}\text{ClN}_{2}\text{O}_{6}\text{S} \) (lincomycin) and \( \text{C}_{17}\text{H}_{33}\text{ClN}_{2}\text{O}_{6}\text{S} \) (lincomycin B).

Lincomycin Hydrochloride intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.5 Endotoxin Unit per mg.

Lincomycin Hydrochloride intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Labelling. The label states whether or not the material is intended for use in the manufacture of parenteral preparations.

Lincomycin Capsules. Page 2443

Change to: Lincomycin Capsules

Lincomycin Hydrochloride Capsules

Lincomycin Capsules contain Lincomycin Hydrochloride equivalent to not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of lincomycin, \( \text{C}_{16}\text{H}_{34}\text{N}_{2}\text{O}_{6}\text{S} \).

Usual strengths. 250 mg, 500 mg.

Identification

A. Extract a quantity of the capsules contents containing the equivalent of 0.2 g of lincomycin with a mixture of 4 volumes of chloroform and 1 volume of methanol; filter and evaporate the filtrate. Dissolve the oily residue in 1 ml of water, add acetone until precipitation begins and further add 20 ml of acetone. Filter the precipitate, wash with two 10 ml quantities of acetone, dissolve the residue in a little of the chloroform–methanol mixture (4:1), evaporate to dryness and dry at 60° at a pressure not exceeding 2 kPa for 4 hours. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with lincomycin hydrochloride RS or with the reference spectrum of lincomycin.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.
Tests

**Lincomycin B.** Determine by liquid chromatography (2.4.14), as described under Assay with the following modifications.

Inject the test solution. The area of any peak corresponding to lincomycin B is not more than 5 per cent of the area of the peak due to lincomycin, calculated by area normalisation method.

**Water** (2.3.43). Not more than 7.0 per cent, determined on 0.3 g of the contents of capsules.

**Other tests.** Comply with the tests stated under Capsules.

**Assay.** Determine by liquid chromatography (2.4.14).

**Test solution.** Mix the content of 20 capsules. Disperse a quantity of the mixed contents containing 50 mg of lincomycin in 50.0 ml of the mobile phase, shake mechanically for a minimum of 5 minutes and filter.

**Reference solution.** A 0.12 per cent w/v solution of lincomycin hydrochloride RS in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octysilane bonded to porous silica (5μm),
- column temperature: 45˚,
- mobile phase: a mixture of 78 volumes of a buffer solution prepared by dissolving 13.5 ml of orthophosphoric acid in 1000 ml of water, adjusted to pH 6.0 with ammonium hydroxide, 15 volumes of acetonitrile and 15 volumes of methanol,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 μl.

The relative retention time with reference to lincomycin for lincomycin B is about 0.5.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 4000 theoretical plates, the tailing factor is not more than 1.3 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of C18H34N2O6S in the capsules.

**Storage.** Store protected from moisture.

**Labelling.** The label states the strength in terms of the equivalent amount of lincomycin.

Methotrexate Tablets. Page 2557

**Dissolution**

Change to: Dissolution (2.5.2).

Apparatus No. 1,
Medium. 900 ml of 0.1 M hydrochloric acid,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Dilute a suitable volume of the filtrate with the same solvent and measure the absorbance of the resulting solution at the maximum at about 306 nm (2.4.7). Calculate the content of C20H22N8O5 in the medium from the absorbance obtained from a solution of known concentration of methotrexate RS.

D. Not less than 75 per cent of the stated amount of C20H22N8O5.
Niclosamide Tablets. Page 2720

5-Chlorosalicylic acid
Change to: 5-Chlorosalicylic acid. Not more than 60 ppm, determined by the following method. Boil a quantity of powdered tablets containing 0.5 g of niclosamide with 15 ml of water for 2 minutes, cool, filter through a membrane filter (pore size 0.45 µm), wash the filter and dilute the combined filtrate and washings to 20 ml with water (solution A). Dissolve 15 mg of 5-chlorosalicylic acid in 20 ml of methanol and add sufficient water to produce 100.0 ml. Dilute 1.0 ml of the solution to 100.0 ml with water (solution B). To 10.0 ml of each of solution A and B add separately 0.1 ml of ferric chloride solution; any violet colour produced in solution A is not more intense than that obtained in solution B.

Propylene Glycol. Page 3026
Change to: Propylene Glycol
1, 2-Propanediol; propane-1,2-diol

\[
\text{C}_3\text{H}_8\text{O}_2 \quad \text{Mol. Wt. 76.1}
\]

Propylene Glycol is propane-1,2-diol.

Propylene Glycol contains not less than 99.5 per cent of \(\text{C}_3\text{H}_8\text{O}_2\).

**Category.** Pharmaceutical aid (humectant; solvent).

**Description.** A clear, colourless, viscous liquid; practically odourless; hygroscopic.

**Identification**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with propylene glycol RS or with the reference spectrum of propylene glycol.

B. Diethylene glycol and Ethylene glycol. (see Tests)

C. In the test for Diethylene glycol and Ethylene glycol, the retention time of propylene glycol peak obtained with the test solution corresponds to the propylene glycol peak in the chromatogram obtained with the reference solution.

**Tests**

**Acidity.** Mix 1 ml of phenolphthalein solution with 50 ml of water and add 0.1 M sodium hydroxide until the solution is pink for 30 seconds. Add 10 ml of Propylene Glycol and titrate with 0.1M sodium hydroxide until the original pink colour returns and remains for 30 seconds, not more than 0.2 ml of 0.1 M sodium hydroxide consumed.

**Relative density** (2.4.29). 1.035 to 1.037.

**Diethylene glycol and Ethylene glycol.** Determine by gas chromatography (2.4.13).

**Test solution.** A solution containing 5.0 per cent w/v of Propylene Glycol and 0.01 per cent w/v of 2,2,2-trichloroethanol (internal standard) in methanol.
Reference solution. A solution containing 0.2 per cent w/v of propylene glycol RS, 0.005 per cent w/v of ethylene glycol RS, 0.005 per cent w/v of diethylene glycol RS and 0.01 per cent w/v of 2,2,2-trichloroethanol (internal standard) in methanol.

Chromatographic system
- a capillary column 30 m x 0.53 mm, packed with cyanopropylphenyl dimethylpolysiloxane (film thickness 3.0 µm) (Such as DB-624),
- temperature: column. 100° hold for 4 minutes, 100° to 120° @ 50° per minute and hold for 10 minutes, 120° to 220° @ 50° per minute and hold for 6 minutes, inlet port at 220° and detector at 250°,
- split ratio: 10:1,
- flame ionization detector,
- flow rate: 4.5 ml per minute, using nitrogen as the carrier gas,
- injection volume: 1µl.

<table>
<thead>
<tr>
<th>Name</th>
<th>Relative retention time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethylene glycol</td>
<td>0.8</td>
</tr>
<tr>
<td>Propylene glycol (Retention time about 4 minutes)</td>
<td>1.0</td>
</tr>
<tr>
<td>2,2,2-trichloroethanol</td>
<td>1.7</td>
</tr>
<tr>
<td>Diethylene glycol</td>
<td>2.4</td>
</tr>
</tbody>
</table>

Inject the reference solution. The test is not valid unless the resolution between the peaks due to ethylene glycol and propylene glycol is not less than 5.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the peak response ratio, each of, ethylene glycol and diethylene glycol relative to 2,2,2-trichloroethanol, is not more than the peak response ratio for ethylene glycol and diethylene glycol relative to 2,2,2-trichloroethanol in the chromatogram obtained with the reference solution (0.1 per cent).

Heavy metals (2.3.13). Dilute 5 ml of Propylene Glycol to 25 ml with water. The solution complies with the limit test for heavy metals, Method D (5 ppm), using 10 ml of lead standard solution (1 ppm Pb).

Chlorides (2.3.12). 15 ml of 23.8 per cent w/v solution of Propylene Glycol complies with the limit test for chlorides (70 ppm).

Sulphates (2.3.17). 10 ml of 25 per cent w/v solution of Propylene Glycol complies with the limit test for sulphates (60 ppm).

Sulphated ash (2.3.18). Not more than 0.007 per cent w/w, determined by heating 50 g of Propylene Glycol in 100 ml shallow dish until it burns, and ignite.

Water (2.3.43). Not more than 0.2 per cent, determined on 5.0 g.

Assay. Determine by gas chromatography (2.4.13).

Test solution. Propylene Glycol.

Chromatographic system
- a glass column 1 m x 4.0 mm, packed with support (40-60 mesh) high molecular weight tetrafluorethylene polymer coated with polyethylene glycol compound (avg. mol. wt. about 15,000), a high molecular weight compound of polyethylene glycol with a diepoxide linker,
- temperature: column. 120° to 200° @ 5° per minute,
inlet port 240° and detector 250°,
- a thermal-conductivity detector,
- using nitrogen as the carrier gas,

**NOTE** - Retention time of propylene glycol is about 5.7 minutes, and of three isomers of dipropylene glycol, when present, is about 8.2, 9.0, and 10.2 minutes, respectively.

Inject 10 µl of the test solution. Calculate the percentage of \(\text{C}_3\text{H}_8\text{O}_2\) by area normalization.

**Storage.** Store protected from moisture.

**Solubility.** Miscible with water, acetone, and chloroform; soluble in ether and is immiscible with fixed oils.

**Sulfasalazine Gastro-resistant Tablets.** Page 4516

**Dissolution**

Change to: **Dissolution (2.5.2).**

A. Apparatus No. 2,
Medium. 900 ml of 0.1 M hydrochloric acid,
Speed and time. 100 rpm and 120 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

**Test solution.** Dilute the filtrate, if necessary, with the dissolution medium.

**Reference solution.** A 0.0056 per cent w/v solution of sulfasalazine RS in 0.1M sodium hydroxide.

**Chromatographic system**
- a stainless steel column 25 cm × 4.6 mm, packed with octadecysilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 11 volumes of isopropanol, 7 volumes of acetonitrile, 22 volumes of water and 0.4 volume of glacial acetic acid,
- flow rate: 1 ml per minute
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of \(\text{C}_{13}\text{H}_{14}\text{N}_{4}\text{O}_{5}\text{S}\).

Complies with the acceptance criteria given under acid stage.

B. Apparatus No. 2,
Medium. 900 ml of phosphate buffer pH 7.5,
Speed and time. 100 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

**Test solution.** Dilute the filtrate, if necessary, with the dissolution medium.

**Reference solution.** A 0.0056 per cent w/v solution of sulfasalazine RS in 0.1M sodium hydroxide.
Use chromatographic system as described under test A.

Inject the reference solution. The test is not valid unless the relative standard deviation of replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

D. Not less than 85 per cent of the stated amount of C₁₈H₂₁N₃O₂S.

**Sumatriptan Injection.** Page 3285

Change to: **Sumatriptan Injection**

Sumatriptan Injection is a sterile isotonic solution of Sumatriptan Succinate in Water for Injections.

Sumatriptan Injection contains Sumatriptan Succinate equivalent to not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of sumatriptan, C₁₄H₂₁N₃O₂.

**Usual strength.** 6 mg sumatriptan per 0.5 ml.

**Description.** A clear, colourless to pale yellow solution.

**Identification**

To a volume of the injection containing equivalent to 29 mg of sumatriptan, add 1 ml of saturated sodium chloride solution and 1 ml of saturated sodium carbonate solution. Shake vigorously for 30 seconds, add two quantities of 2 ml of propan-2-ol, shake, allow to separate (this may take up to 24 hours) and discard the aqueous layer. Evaporate under a stream of nitrogen and dry at 100°C. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum obtained with sumatriptan RS treated in the same manner or with the reference spectrum of sumatriptan.

**Tests**

**pH** (2.4.24). 4.2 to 5.3.

**Impurities A and H.** Determine by liquid chromatography (2.4.14).

**Solvent mixture.** 75 volumes of 0.025 M sodium dihydrogen orthophosphate, adjusted to pH 6.5 and 25 volumes of acetonitrile.

**Test solution.** Dilute a volume of injection in the solvent mixture to obtain a solution containing 0.214 per cent w/v of sumatriptan.

**Reference solution (a).** Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture.

**Reference solution (b).** Dilute the contents of a vial of sumatriptan for system suitability RS (containing sumatriptan impurity A ([3-[2-(dimethylamino)ethyl]-2-[[3-[2-(dimethylamino)ethyl]-1H-indol-5-yl]methyl]-1H-indol-5-yl]-N-methylmethanesulphonamide) and sumatriptan impurity H ([3-[2-(dimethylamino)ethyl]-1H-indol-5-yl]methyl)-1H-indol-5-yl]-N-methylmethanesulphonamide) to 1.0 ml with the solvent mixture.

**Chromatographic system**

- a stainless steel column 25 cm × 4.6 mm, packed with silica (Such as Spherisorb silica S5W),
- mobile phase: a mixture of 10 volumes of 10 M ammonium acetate and 90 volumes of methanol,
- flow rate: 2 ml per minute,
- spectrophotometer set at 282 nm,
- injection volume: 20 μl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to sumatriptan and sumatriptan impurity A is not less than 1.5.

Inject reference solution (a) and the test solution. Run the chromatogram 5 times the retention time of the principal peak for test solution. The area of any peak corresponding to sumatriptan impurity A, multiplied with correction factor of 0.6, is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference
solution (a) (1.5 per cent) and the area of any peak corresponding to sumatriptan impurity H is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent).

**Related substances.** Determine by liquid chromatography (2.4.14).

**Solvent mixture.** 75 volumes of 0.025 M sodium dihydrogen orthophosphate, adjusted to pH 6.5 and 25 volumes of acetonitrile.

**Test solution.** Dilute a volume of injection in the solvent mixture to obtain a solution containing 0.214 per cent w/v of sumatriptan.

**Reference solution.** Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture.

**Chromatographic system**
- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Spherisorb ODS 1),
- mobile phase: a mixture of 75 volumes of a solution containing 0.97 g of dibutylamine, 0.735 g of orthophosphoric acid and 2.93 g of sodium dihydrogen orthophosphate in 750 ml of water, adjusted to pH 7.5 with 10 M sodium hydroxide, diluted to 1000 ml with water and 25 volumes of acetonitrile,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 282 nm,
- injection volume: 20 µl.

<table>
<thead>
<tr>
<th>Name</th>
<th>Relative retention time</th>
<th>Correction factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sumatriptan impurity D&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.2</td>
<td>-</td>
</tr>
<tr>
<td>Sumatriptan impurity 2&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Sumatriptan impurity 1&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0.4</td>
<td>0.2</td>
</tr>
<tr>
<td>Sumatriptan impurity E&lt;sup&gt;4&lt;/sup&gt;</td>
<td>0.5</td>
<td>-</td>
</tr>
<tr>
<td>Sumatriptan impurity B&lt;sup&gt;5&lt;/sup&gt;</td>
<td>0.6</td>
<td>-</td>
</tr>
<tr>
<td>Sumatriptan impurity F&lt;sup&gt;6&lt;/sup&gt;</td>
<td>0.7</td>
<td>-</td>
</tr>
<tr>
<td>Sumatriptan impurity C&lt;sup&gt;7&lt;/sup&gt;</td>
<td>0.8</td>
<td>-</td>
</tr>
<tr>
<td>Sumatriptan (Retention time is about 17 minutes)</td>
<td>1.0</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>1</sup>N,N-dimethyl-2-[5-[(methylsulfonyl)methyl]-1H-indol-3-yl]ethanamine N-oxide,
<sup>2</sup>-[3-[2-(dimethylamino)ethyl]-3-hydroxy-2-oxo-2,3-dihydro-1H-indol-5-yl]-N-methylmethanesulfonamide.
<sup>3</sup>3a-hydroxy-1,1-dimethyl-5-[[methyImino]sulfonyl][methyl]-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-b]indol-1-ium trifluoroacetate.
<sup>4</sup>-[3-[2-(aminoethy)l]-1H-indol-5-yl]-N-methylmethanesulfonamide
<sup>5</sup>R1 = R2 = H: N-methyl-3-[2-(dimethylamino)ethyl]-1H-indol-5-yl methanesulfonamide,
<sup>6</sup>R = H: N-methyl[2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indol-6-yl]methanesulfonamide
<sup>7</sup>R1 = CH2-OH, R2 = CH3: [3-[2-(dimethylamino)ethyl]-1-(hydroxymethyl)-1H-indol-5-yl]-N-methylmethanesulfonamide

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. Run the chromatogram 4 times the retention time of the principal peak for test solution. The area of any peak corresponding to sumatriptan impurity 1, multiplied with correction factor 0.2, is not more than 1.5 times the area of the principal peak in the chromatogram obtained with the reference solution (1.5 per cent), the area of any peak corresponding to sumatriptan impurity 2, multiplied with correction factor 0.3, is not more than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent) and the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent). The total impurity content in test for impurities A and H and the test for Related substances is not more than 4.0 per cent.

**Other tests.** Comply with the tests stated under Parenteral Preparations (Injections).

**Bacterial endotoxins** (2.2.3). Not more than 29.2 Endotoxin Units per mg of sumatriptan.

**Assay.** Determine by liquid chromatography (2.4.14).

**Solvent mixture.** 75 volumes of 0.025 M sodium dihydrogen orthophosphate, adjusted to pH 6.5 and 25 volumes of acetonitrile.
Test solution. Dilute a volume of injection in the solvent mixture to obtain a solution containing 0.021 per cent w/v of sumatriptan.

Reference solution. A 0.03 per cent w/v solution of sumatriptan succinate RS in the solvent mixture.

Use chromatographic system as described under Related substances.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of \( C_{14}H_{21}N_3O_2S \) in the injection. 1 mg of \( C_{14}H_{21}N_3O_2S \) is equivalent to 1.4 mg of \( C_{4}H_{6}O_4 \).

Storage. Store protected from light.

Temozolomide. Page 3325

Related substances
Change to: Related substances. Determine by liquid chromatography (2.4.14).

NOTE – Shake the solutions to dissolve. Do not sonicate.

Test solution. Dissolve 100 mg of the substance under examination in dimethylsulphoxide and dilute to 100.0 ml with dimethylsulphoxide. (NOTE – Use freshly opened bottle)

Reference solution (a). A 0.0001 per cent w/v solution of temozolomide RS in dimethylsulphoxide.

Reference solution (b). Mix 5 ml of 0.1 M hydrochloric acid and 5 ml of 0.1 per cent w/v solution of temozolomide RS in dimethylsulphoxide. Heat the mixture in a water-bath for 1 hour. [The preparation forms 2-azahypoxanthine, temozolomide acid, and aminoimidazolecarboxamide.]

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 96 volumes of 0.5 per cent v/v solution of glacial acetic acid in water and 4 volumes of methanol containing 0.094 per cent w/v of sodium 1-hexanesulphonate,
- flow rate: 1 ml per minute,
- spectrophotometer set at 270 nm,
- injection volume: 10 µl.

<table>
<thead>
<tr>
<th>Name</th>
<th>Relative retention time</th>
<th>Correction factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Azahypoxanthine</td>
<td>0.42</td>
<td>0.63</td>
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<tr>
<td>Temozolomide related compound A²</td>
<td>0.53</td>
<td>--</td>
</tr>
<tr>
<td>Temozolomide acid³</td>
<td>0.84</td>
<td>--</td>
</tr>
<tr>
<td>Temozolomide</td>
<td>1.0</td>
<td>--</td>
</tr>
<tr>
<td>Aminoimidazolecarboxamide⁴</td>
<td>1.37</td>
<td>0.4</td>
</tr>
<tr>
<td>Cyanotemozolomide⁵,⁶</td>
<td>2.3</td>
<td>--</td>
</tr>
</tbody>
</table>

1 4a,5-dihydro-4H-imidazo[4,5-d][1,2,3]triazin-4-one,
2 4-diaz-4H-imidazole-5-carboxamide,
3 3-methyl-4-oxo-3,4-dihydroimidazo[5,1-d][1,2,3,5]tetrazine-8-carboxylic acid,
4 5-aminoimidazole-4-carboxamide. Two peaks may be observed, use the sum of the peak areas for calculation,
5 3-methyl-4-oxo-3,4-dihydroimidazo[5,1-d][1,2,3,5]tetrazine-8-carbonitrile.
6 If possible from the manufacturing process.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to temozolomide acid and temozolomide is not less than 1.5.

Inject reference solution (a) and the test solution. Run the chromatogram 3.2 times the retention time of the principal peak. The area of any peak due to 2-azahypoxanthine is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent), the area of any peak due to temozolomide
impurity A is not more than 5 times the area of principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent), the area of any peak corresponding to temozolomide acid and aminomimidazolecarboxamide, each of, is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent), the area of any peak corresponding to cyanotemozol omide is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent) and the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and the sum of the areas of all the secondary peaks is not more than 8 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.8 per cent).

**Thiamine Hydrochloride.** Page 3354

**Related substances**

Change to: **Related substances.** Determine by liquid chromatography (2.4.14).

**Test solution.** Dissolve 0.35 g of the substance under examination in 15.0 ml of 5 per cent v/v solution of glacial acetic acid and dilute to 100.0 ml with water.

**Reference solution (a).** A 0.00035 per cent w/v solution of thiamine hydrochloride RS in water.

**Reference solution (b).** Dissolve the contents of a vial of thiamine for system suitability RS (containing thiamine impurities A, B and C) in 1.0 ml of a 0.75 per cent v/v solution of glacial acetic acid.

**Chromatographic system**
— a stainless steel column 25 cm x 4 mm, packed with endcapped octadecylsilane bonded to porous silica (5 μm),
— column temperature: 45°,
— mobile phase: A. a 0.38 per cent w/v solution of sodium hexanesulphonate, adjusted to pH 3.1 with orthophosphoric acid,
— B. methanol,
— a gradient programme using the conditions given below,
— flow rate: 1 ml per minute,
— spectrophotometer set at 248 nm,
— injection volume: 25 μl.

<table>
<thead>
<tr>
<th>Time (in min.)</th>
<th>Mobile phase A (per cent v/v)</th>
<th>Mobile phase B (per cent v/v)</th>
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<table>
<thead>
<tr>
<th>Name</th>
<th>Relative retention time</th>
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<tbody>
<tr>
<td>Thiamine impurity A&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.3</td>
</tr>
<tr>
<td>Thiamine impurity B&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.9</td>
</tr>
<tr>
<td>Thiamine (Retention time: about 30 minutes)</td>
<td>1.0</td>
</tr>
<tr>
<td>Thiamine impurity C&lt;sup&gt;3&lt;/sup&gt;</td>
<td>1.2</td>
</tr>
</tbody>
</table>

<sup>1</sup> 2-{3-[(4-amino-2-methylpyrimidin-5-yl)methyl]-4-methyl-1,3-thiazol-3-ium-5yl]ethyl sulfate (thiamine sulfate ester),
<sup>2</sup> 3-[(4-amino-2-methylpyrimidin-5-yl)methyl]-5-(2-hydroxyethyl)-4-methyl-1,3-thiazol-3-ium (desmethylothiamine),
<sup>2</sup> 3-[(4-amino-2-methylpyrimidin-5-yl)methyl]-5-(2-chloroethyl)-4-methyl-1,3-thiazol-3-ium (chlorothiamine),

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to thiamine impurity B and thiamine is not less than 3.0 and the resolution between the peaks due to thiamine and thiamine impurity C is not less than 2.0.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to thiamine impurity B is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent), the area of any peak corresponding to thiamine impurity A and
thiamine impurity C, each of, is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and the sum of the areas of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

**Thiamine Mononitrate. Page 3356**

**Related substances**

Change to: Related substances. Determine by liquid chromatography (2.4.14).

**Test solution.** Dissolve 0.35 g of the substance under examination in 15.0 ml of 5 per cent v/v solution of glacial acetic acid and dilute to 100.0 ml with water.

**Reference solution (a).** A 0.00035 per cent w/v solution of thiamine mononitrate RS in water.

**Reference solution (b).** Dissolve the contents of a vial of thiamine for system suitability RS (containing thiamine impurities A, B and C) in 1.0 ml of a0.75per cent v/v solution of glacial acetic acid.

**Chromatographic system**

— a stainless steel column 25 cm x 4 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm),
— column temperature: 45°,
— mobile phase: A. a 0.38 per cent w/v solution of sodium hexanesulphonate, adjusted to pH 3.1 with orthophosphoric acid,
  B. methanol,
— a gradient programme using the conditions given below,
— flow rate: 1 ml per minute,
— spectrophotometer set at 248 nm,
— injection volume: 25 µl.

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<tbody>
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<td>Thiamine impurity A&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.3</td>
</tr>
<tr>
<td>Thiamine impurity B&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.9</td>
</tr>
<tr>
<td>Thiamine (Retention time: about 30 minutes)</td>
<td>1.0</td>
</tr>
<tr>
<td>Thiamine impurity C&lt;sup&gt;3&lt;/sup&gt;</td>
<td>1.2</td>
</tr>
</tbody>
</table>

<sup>1</sup>2-[[4-amino-2-methylpyrimidin-5-yl]methyl]-4-methyl-1,3-thiazol-3-yl]ethyl sulfate (thiamine sulfate ester),
<sup>2</sup>3-[[4-amino-2-methylpyrimidin-5-yl]methyl]-5-(2-hydroxyethyl)-4-methyl-3-thiazol-3-im (desmethyinthiamine),
<sup>3</sup>3-[[4-amino-2-methylpyrimidin-5-yl]methyl]-5-(2-chloroethyl)-4-methyl-1,3-thiazol-3-im (chlorothiamine),

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to thiamine impurity B and thiamine is not less than 3.0 and the resolution between the peaks due to thiamine and thiamine impurity C is not less than 2.0.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to thiamine impurity B is not more than 6 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.6 per cent), the area of any peak corresponding to thiamine impurity C is not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.4 per cent), the area of any peak corresponding to thiamine impurity A is not more than 1.5 times the
area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and the sum of the areas of all the secondary peaks is not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

**Thyroxine Sodium.** Page 3370

**Identification**

Change to: A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *levothyroxine sodium RS* or with the reference spectrum of *levothyroxine sodium*.

B. To 20 mg, add 2 ml of 1 M sulphuric acid. Heat on a water-bath followed by heating carefully over a naked flame, increasing the temperature to about 600°. Continue ignition until most of the black particles have disappeared. Dissolve the residue in 2 ml of water; the solution gives reaction (A) of sodium salts (2.3.1).

**Liothyronine**

Change to: Related substances

Determine by liquid chromatography (2.4.14).

**Solvent mixture.** 30 volumes of mobile phase A and 60 volumes of ethanol (95 per cent).

**Test solution.** Dissolve 25 mg of the substance under examination in the solvent mixture and dilute to 50.0 ml with the solvent mixture. Dilute 10.0 ml of the solution to 25.0 ml with the solvent mixture.

**Reference solution (a).** A solution containing 0.0002 per cent w/v each of *levothyroxine sodium RS* and *liothyronine sodium RS* in the solvent mixture.

**Reference solution (b).** Dilute 1.0 ml of reference solution (a) to 10.0 ml with the solvent mixture.

**Chromatographic system**

- a stainless steel column 15 cm x 4.0 mm, packed with end-capped octadecylsilane bonded to porous silica (3 µm),
- mobile phase: A. 0.1 per cent w/v solution of orthophosphoric acid in water,
  - B. 0.1 per cent w/v solution of orthophosphoric acid in acetonitrile,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume: 25 µl.

<table>
<thead>
<tr>
<th>Time (in min.)</th>
<th>Mobile phase A (per cent v/v)</th>
<th>Mobile phase B (per cent v/v)</th>
</tr>
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<tbody>
<tr>
<td>0</td>
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<tr>
<td>55</td>
<td>70</td>
<td>30</td>
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</table>

**Name**

<table>
<thead>
<tr>
<th>Name</th>
<th>Relative retention time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liothyronine$^1$</td>
<td>0.5</td>
</tr>
<tr>
<td>levothyroxine (retention time about: 11 minutes)</td>
<td>1.0</td>
</tr>
<tr>
<td>levothyroxine impurity F$^2$</td>
<td>2.0</td>
</tr>
<tr>
<td>levothyroxine impurity G$^3$</td>
<td>2.4</td>
</tr>
</tbody>
</table>

$^1$(2S)-2-amino-3-[4-(4-hydroxy-3-iodophenoxy)-3,5-diiodophenyl]propanoic acid (liothyronine),

$^2$(2S)-2-amino-3-[4-(4-hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl]propanoic acid,

$^3$Unknown structure,
Inject reference solution (a). The test is not valid unless the resolution between the peaks due to levothyroxine and liothyronine is not less than 5.0.

Inject reference solution (a), (b) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to liothyronine is not more than the area of the corresponding peak of in the chromatogram obtained with reference solution (a) (1.0 per cent), the area of any peak corresponding to levothyroxine impurity F is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent), the area of any peak corresponding to levothyroxine impurity G is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent). The area of any other secondary peak is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent). The sum of the all the impurities is not more than 2.0 per cent. Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

**Loss of drying**
Change to: Water (2.3.43). 6.0 per cent to 12.0 per cent, determined on 0.1 g.

**Thyroxine Tablets.** Page 3371

**Identification**
Change to: In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Insert before **Uniformity of content**

**Dissolution** (2.5.2).

*NOTE: All containers that are in contact with solutions containing levothyroxine sodium are to be made of glass.*

Apparatus No. 1,
Medium. 500 ml of 0.2 per cent w/v solution of sodium lauryl sulphate in 0.01 M hydrochloric acid,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

**Test solution.** Dilute the filtrate, if necessary, with the dissolution medium.

**Reference solution.** A 0.01 per cent w/v solution of levothyroxine RS in methanol. Dilute suitably with the dissolution medium to obtain a solution of about the similar concentration as the test solution.

Chromatographic system

– a stainless steel column 25 cm x 4.6 mm, packed with nitrile groups chemically bonded to porous silica (5 µm),
– mobile phase: a mixture of 35 volumes of acetonitrile, 65 volumes of water and 0.05 volume of orthophosphoric acid,
– flow rate: 1.5 ml per minute,
– spectrophotometer set at 225 nm,
– injection volume: 100 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 4.0 per cent.

Inject the reference solution and the test solution.

D. Not less than 70 per cent of the stated amount of C₁₅H₁₀ I₄NNaO₄ in the medium.

**Liothyronine Sodium.** Determine by liquid chromatography (2.4.14).

*Solvent mixture.* 60 volumes of methanol, 40 volumes of water and 0.05 volume of orthophosphoric acid.
Solution A. Equal volumes of 0.02 M sodium hydroxide and methanol.

Test solution. Disperse a quantity of powdered tablets containing 100 µg of levothyroxine sodium in 10.0 ml of the mobile phase, add 2 glass beads and mix on a vortex mixer for 3 minutes. Centrifuge and use the supernatant.

Reference solution (a). A 0.04 per cent w/v solution of levothyroxine sodium RS in solution A.

Reference solution (b). A 0.04 per cent w/v solution of liothyronine sodium RS in solution A. Dilute 1.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution (c). Dilute 5.0 ml of reference solution (a) and 10.0 ml of reference solution (b) to 200.0 ml with the mobile phase.

Reference solution (d). Dilute 1.0 ml of reference solution (b) to 20.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with nitrile groups chemically bonded to porous silica (5 µm),
- mobile phase: 40 volumes of acetonitrile, 60 volumes of water and 0.05 volume of orthophosphoric acid,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume: 100 µl.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to levothyroxine and liothyronine is not less than 5.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent for levothyroxine peak.

Inject reference solution (d) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to liothyronine is not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (2.0 per cent).

Uniformity of content
Test solution. Line 3
Change from: 0.0002 per cent
  to: 0.000125 per cent

Reference solution. Line 1
Change from: 0.02 per cent
  to: 0.0125 per cent

Tiotropium Bromide Monohydrate. Page 3381
Change to: Tiotropium Bromide Monohydrate

![Tiotropium Bromide Monohydrate](image)

C_{19}H_{22}BrNO_{4}S_{3}, H_{2}O

Mol. Wt. 490.4

Tiotropium Bromide Monohydrate is (1R,2R,4S,5S,7S)-7-[[Hydroxy[di(thiophen-2-yl]acetyl]oxy]-9,9-dimethyl-3-oxa-9-azatricyclo[3.3.1.0^{2,4}]nonan-9-ium bromide monohydrate.
Tiotropium Bromide Monohydrate contains not less than 98.5 per cent and not more than 101.5 per cent of tiotropium bromide, C_{19}H_{22}NO_{4}S_{2}Br, calculated on the anhydrous basis.

**Category.** Bronchodilator.

**Dose.** 18 µg once daily.

**Description.** A white to off-white powder or crystals.

**Identification**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with tiotropium bromide monohydrate RS or with the reference spectrum of tiotropium bromide monohydrate.

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

**Tests**

**Appearance of solution.** A 1.0 per cent w/v solution is clear (2.4.1) and not more intensely coloured than reference solution YS6 (2.4.1).

**Impurities G and H.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

**NOTE-** Prepare the solutions immediately before use.

**Solvent mixture.** Dilute 1 volume of 3M hydrochloric acid to 100 volumes with methanol.

**Mobile phase.** A mixture of 10 volumes of water, 15 volumes of anhydrous formic acid, 35 volumes of acetonitrile and 50 volumes of methylene chloride.

**Test solution.** Dissolve 0.4 g of the substances under examination in the solvent mixture and dilute to 10.0 ml with the solvent mixture.

**Reference solution (a).** Dissolve the contents of a vial of tiotropium impurity mixture RS (containing impurities G and H) in 1.0 ml of the solvent mixture.

**Reference solution (b).** Mix 0.1 ml of the test solution with 0.1 ml of reference solution (a), and 20 µl of reference solution (b). Apply to the plate 10 µl of the test solution and reference solution (a), and 20 µl of reference solution (b). After development, dry the plate in air and expose to iodine vapors till spots appear (about 15 minutes), remove the plate and examine immediately. In the chromatogram obtained with the test solution, any secondary spot corresponding to each of, impurity G and impurity H is not more intense than the corresponding spot of impurity G and impurity H in the chromatogram obtained with reference solution (a) (0.1 per cent). The test is not valid unless the chromatogram obtained with reference solution (b) shows three clearly separated spots, of each, impurity G, impurity H and tiotropium with R\textsubscript{f} values of about 0.33, 0.38 and 0.64, respectively.

**Related substances.** Determine by liquid chromatography (2.4.14).

**NOTE - Carry out the test protected from light.**

**Test solution.** Dissolve 50 mg of the substance under examination in mobile phase B and dilute to 25.0 ml with mobile phase B.

**Reference solution (a).** Dilute 1.0 ml of the test solution to 100.0 ml with mobile phase B. Dilute 1.0 ml of the solution to 10.0 ml with mobile phase B.

**Reference solution (b).** Dissolve 4 mg of tiotropium for system suitability RS (containing impurity C) in 2.0 ml of mobile phase B.
Chromatographic system
- a stainless steel column 15 cm x 3.0 mm packed with propylsilane bonded to porous silica (3.5 µm),
- column temperature: 50°,
- mobile phase A: dissolve 1.0 g of sodium methanesulphonate and 5.0 g of potassium dihydrogen phosphate in 980 ml of water, adjusted to pH 3.0 with dilute phosphoric acid and dilute to 1000 ml with water;
- B: a mixture of 10 volumes of methanol, 40 volumes of acetonitrile and 50 volumes of mobile phase A;
- a gradient programme using the conditions given below,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 5µl.

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</table>

The relative retention time with reference to tiotropium (retention time: about 15 minutes) for impurity C is about 1.2.

Inject reference solution (b). The test is not valid unless the resolution between the peak due to tiotropium and impurity C is not less than 2.4.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

**Heavy metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). 2.5 per cent to 4.0 per cent, determined on 0.3 g.

**Assay.** Determine by liquid chromatography (2.4.14).

*Test solution.* Dissolve 20 mg of the substance under examination in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

*Reference solution.* A 0.002 per cent w/v solution of tiotropium bromide monohydrate RS in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 55 volumes of a buffer solution prepared by dissolving 3.85 g of ammonium acetate in 1000 ml of water, adjusted to pH 5.5 with dilute acetic acid and 45 volumes of methanol,
- flow rate: 1 ml per minute,
- spectrophotometer set at 235 nm,
- injection volume: 20 µl.
Inject the reference solution. The test is not valid unless the column efficiency in not less than 3000 theoretical plates, tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $\text{C}_{93}\text{H}_{122}\text{NO}_{4}\text{S}_{2}\text{Br}$.

**Storage.** Store protected from light and moisture.

**Solubility** (2.4.26.). Soluble in methanol, sparingly soluble in water, and practically insoluble in methylene chloride.