METHYLTHIONINII INJECTION
(METHYLTHIONIUM INJECTION)
DRAFT MONOGRAPH FOR INCLUSION IN
The International Pharmacopoeia
(July 2016)
DRAFT FOR COMMENTS

Should you have any comments on this draft, please send these to Dr Herbert Schmidt, Medicines Quality Assurance Programme, Technologies Standards and Norms, Department of Essential Medicines and Health Products, World Health Organization, 1211 Geneva 27, Switzerland; fax: (+41 22) 791 4730 or email: schmidt@who.int by 16 September 2016.

In order to speed up the process for receiving draft monographs and for sending comments, please let us have your email address (to bonnyw@who.int) and we will add it to our electronic mailing list. Please specify if you wish to receive monographs.

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Please send any request for permission to:
Dr Sabine Kopp, Group Lead, Medicines Quality Assurance Programme, Technologies Standards and Norms, Department of Essential Medicines and Health Products, World Health Organization, CH-1211 Geneva 27, Switzerland. Fax: (41-22) 791 4730; email: kopps@who.int.

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SCHEDULE FOR THE ADOPTION PROCESS OF DOCUMENT QAS/16.676:

Methylthioninium injection (Methylthioninii injectio)

<table>
<thead>
<tr>
<th>Event</th>
<th>Date</th>
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<tr>
<td>First draft received from a collaborating laboratory</td>
<td>April 2016</td>
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<tr>
<td>Discussion at informal consultation on quality control laboratory tools and specifications for medicines</td>
<td>9–11 May 2016</td>
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<tr>
<td>Draft monograph sent out for public consultation</td>
<td>July 2016</td>
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<tr>
<td>Presentation to WHO Expert Committee on Specifications for Pharmaceutical Preparations</td>
<td>October 2016</td>
</tr>
<tr>
<td>Further follow-up action as required</td>
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METHYLTHIONINIUM INJECTION
(METHYLTHIONINII INJECTIO)

[Note from the Secretariat. The monograph is proposed for inclusion in The International Pharmacopoeia.]

Description. A clear, dark blue solution.

Category. Antidote

Storage. Store at room temperature, protected from light.

Additional information. Strength in the current WHO Model List of Essential Medicines (EML): 10 mg/mL in 10 mL ampoule; other available strength: 5 mg/mL.

Requirements

Complies with the monograph for Parenteral preparations.

Definition. Methylthioninium injection is a sterile solution of Methylthioninium chloride in water for injection. It contains not less than 90.0% and not more than 110.0% of the amount of C_{16}H_{18}ClN_{3}S stated on the label.
Identity tests

• Any two of tests A, B and C may be applied.

A. Carry out the test as described under 1.14.4 High-performance-liquid chromatography using the conditions given under “Assay”, method A. The retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to the retention time of the peak due to methylthioninium in the chromatogram obtained with solution (2).

B. Carry out test as described under 1.14.1 Thin-layer chromatography using silica gel R6 as the coating substance and a mixture of 3 volumes of acetic acid R, 3 volumes of ethanol R and 4 volumes of water R as the mobile phase. Apply separately to the plate 1 µL of each of the following 2 solutions: For solution (A) dilute 1.0 mL of the injection to 20.0 mL with methanol R to obtain a solution with a concentration of 0.5 mg of the methylthioninium chloride per mL. For solution (B) dissolve 10.0 mg of methylthioninium chloride RS and dilute to 20.0 mL with a mixture of water R and methanol R (20:80 v/v). After removing the plate from the chromatographic chamber allow it to dry in air or in a current of cool air. Examine the chromatogram in daylight.

The principal spot obtained with solution (A) corresponds in position, appearance and intensity to that obtained with solution (B).
C. The absorption spectrum (1.6) of a 5 μg/mL solution in hydrochloric acid (~70 g/L) TS, when observed between 230 nm and 800 nm, exhibits 4 maxima at about 258 nm, 288 nm, 680 nm and 745 nm.

**pH value (1.13).** pH of the injection, 3.0–4.5

**Related substances**

Carry out test as described under 1.14.4 *High-performance liquid chromatography* using the chromatographic conditions as described under "Assay", method A.

Prepare the following solutions using as the diluent a mixture of 70 volumes of a 0.1% (v/v) solution of trifluoroacetic acid R (mobile phase A) and 30 volumes of acetonitrile R (mobile phase B).

For solution (1) dilute 1.0 mL of the injection to 20.0 mL to obtain a solution with a concentration of 0.5 mg of the methylthioninium chloride per mL. For solution (2) dilute 1.0 mL of solution (1) to 100.0 mL. For solution (3) dilute 5.0 mL of solution (2) to 50.0 mL. For solution (4) dissolve 2.5 mg methylthioninium chloride impurity A RS and dilute to 10.0 mL. Transfer 1.0 mL of this solution to a 10 mL volumetric flask and make up to volume with
solution (1). Alternatively, dry 100 mg of methylthioninium chloride R at 105 °C for 5 hours, dissolve 50 mg of the dried substance and dilute to 100.0 mL. Sonicate for 5 minutes.

Inject alternately 5 µL each of solutions (1), (2), (3) and (4).

Use the chromatograms obtained with solution (4) and solution (1) to identify the peak due to impurity A. Impurity A is eluted at the relative retention of about 0.8 with reference to methylthioninium (retention time about 11 minutes). The test is not valid unless the resolution between the peaks corresponding to methylthioninium and impurity A is at least 3.5.

In the chromatogram obtained with solution (1):

- the area of any peak corresponding to impurity A is not greater than 5 times the area of the principal peak obtained with solution (2) (5.0%);
- the area of any other impurity peak is not greater than two times the area of the principal peak obtained with solution (3) (0.20%);
- the sum of the areas of all impurity peaks, other than the peak corresponding to impurity A, is not greater than the area of the principal peak obtained with solution (2) (1.0%). Disregard any peak with an area less than 0.5 times the area of the principal peak obtained with solution (3) (0.05%).
Assay

- Either method A or B may be applied.

A. Carry out test as described under 1.14.4 *High-performance liquid chromatography* using a stainless steel column (10 cm x 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically-bonded phenylsilyl groups (3.5 µm).1

Use the following conditions for gradient elution:

- mobile phase A: 0.1% (v/v) solution of trifluoroacetic acid R;
- mobile phase B: acetonitrile R.

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Mobile phase A (% v/v)</th>
<th>Mobile phase B (% v/v)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–5</td>
<td>80</td>
<td>20</td>
<td>Isocratic</td>
</tr>
<tr>
<td>5–25</td>
<td>80 to 30</td>
<td>20 to 70</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>25–32</td>
<td>30</td>
<td>70</td>
<td>Isocratic</td>
</tr>
<tr>
<td>32–35</td>
<td>30 to 80</td>
<td>70 to 20</td>
<td>Return to initial composition</td>
</tr>
<tr>
<td>35–40</td>
<td>80</td>
<td>20</td>
<td>Re-equilibration</td>
</tr>
</tbody>
</table>

Operate with a flow of 1.0 mL/min. As a detector use an ultraviolet spectrophotometer set at a wavelength of 246 nm. Maintain the column temperature at 30 °C.

1 An X-Bridge Phenyl column and a Phenomenex Luna 3 µm Phenyl-Hexyl column were found suitable.
Prepare the following solutions using as diluent a mixture of 30 volumes acetonitrile R and 70 volumes of mobile phase A. For solution (1) dilute 5.0 mL of the injection to 50.0 mL. Dilute 5.0 mL of this solution to 50.0 mL to obtain a solution with a concentration of 0.1 mg of methylthioninium chloride per mL. For solution (2) dissolve 50.0 mg of methylthioninium chloride RS in 50.0 mL. Sonicate for 5 minutes. Dilute 5.0 mL of this solution to 50.0 mL.

Inject alternately 5 µL each of solutions (1) and (2). The test is not valid unless symmetry factor is not more than 2.0.

Measure the areas of the peak responses obtained in the chromatograms from solutions (1) and (2) and calculate the percentage content of methylthioninium chloride \( (C_{16}H_{18}ClN_3S) \) using the declared content of \( C_{16}H_{18}ClN_3S \) in methylthioninium chloride RS.

B. Prepare the following solutions using as diluent ethanol (~457 g/L) TS. Dilute 1.0 mL of the injection to 100.0 mL to obtain a solution with a concentration of 0.1 mg of methylthioninium chloride per mL. Dilute 2.0 mL of this solution to 100.0 mL. Measure the absorbance \( (I.6) \) of a 1 cm layer of the diluted solution at the maximum at about 664 nm and calculate the percentage content of methylthioninium chloride \( (C_{16}H_{18}ClN_3S) \).
using the absorptivity value of 2950 methylthioninium chloride. [Note from the Secretariat. The absorptivity value is so far based on a single determination. It is intended to perform further independent determinations to confirm the value.]

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains less than 2.5 IU of endotoxin per mg methylthioninium chloride.

Impurities

The impurities limited by the requirements of this monograph include those listed in the monograph for methylthioninium chloride.

Reagent to be established

Methylthioninium chloride R

Methylthioninium chloride of a suitable quality should be used.

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