METHYLTHIONINUM CHLORIDE
(METHYLTHIONINII CHLORIDUM)

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.
SCHEDULE FOR THE ADOPTION PROCESS OF DOCUMENT QAS/16.675:
METHYLTHIONINIMUM CHLORIDE (METHYLTHIONINII CHLORIDUM)

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<td>First draft received from a collaborating laboratory</td>
<td>April 2016</td>
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<td>Discussion at informal consultation on quality control</td>
<td>9–11 May 2016</td>
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<td>laboratory tools and specifications for medicines</td>
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METHYLTHIONINIUM CHLORIDE
(METHYLTHIONINII CHLORIDUM)

[Note from the Secretariat. It is proposed to revise the monograph on Methylthioninium chloride.]

Changes from the current monograph are indicated in the text by insert or delete.

Molecular formula. $\text{C}_{16}\text{H}_{18}\text{ClN}_{3}\text{S}$ (anhydrous); $\text{C}_{16}\text{H}_{18}\text{ClN}_{3}\text{S}\cdot\text{H}_{2}\text{O}$ (monohydrate);

$\text{C}_{16}\text{H}_{18}\text{ClN}_{3}\text{S}\cdot3\text{H}_{2}\text{O}$ (trihydrate); $\text{C}_{16}\text{H}_{18}\text{ClN}_{3}\text{S}\cdot5\text{H}_{2}\text{O}$ (pentahydrate).

Relative molecular mass. 319.9 (anhydrous); 337.9 (monohydrate); 373.9 (trihydrate); 409.9 (pentahydrate).

Graphic formula

n = 0 (anhydrous)

n = 3 (trihydrate)

n=0 (anhydrous)

n=1 (monohydrate)
n=3 (trihydrate)

n=5 (pentahydrate)

Chemical name. C.I. Basic Blue 9; 3,7-bis(dimethylamino)phenothiazin-5-iium chloride;


C.I. Basic Blue 9 monohydrate; 3,7-bis(dimethylamino)phenothiazin-5-iium chloride monohydrate; CAS Reg. No. 122965-43-9 (monohydrate).

C.I. Basic Blue 9 trihydrate; 3,7-bis(dimethylamino)phenothiazin-5-iium chloride trihydrate;

CAS Reg. No. 7220-79-3 (trihydrate).

C.I. Basic Blue 9 pentahydrate; 3,7-bis(dimethylamino)phenothiazin-5-iium chloride pentahydrate; CAS Reg. No. 32680-41-4 (pentahydrate).

Other name. Methylene blue

Description. Dark green crystals with a metallic lustre or a dark green, crystalline powder—odourless or almost odourless.

Solubility. Sparingly soluble in water R; slightly soluble in ethanol (~750 g/L) TS; practically insoluble in ether R.

Category. Antidote.
Storage. Methylthioninium chloride should be kept in a tightly closed container, protected
from light, at a temperature not exceeding 30 °C.

Additional information. Methylthioninium chloride is hygroscopic.

Requirements

Definition. Methylthioninium chloride contains not less than 97.0% and not more than
101.0% not less than 93.0% and not more than 102.0% (“Assay”, method A) or not less than
98.0% and not more than 102.0% (“Assay”, method B) of C₁₆H₁₈ClN₃S, calculated with
reference to the dried substance.

Identity tests

A. The absorption spectrum 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.

B. Dissolve 1 mg in 10 mL of water; a deep blue colour is produced. Add 2.0 mL of
hydrochloric acid (~70 g/l) TS and 0.25 g of zinc R powder; the colour of the solution
is discharged; filter and expose the filtrate to the air; the blue colour of the solution
reappears.
C. Mix 0.05 g with 0.5 g of anhydrous sodium carbonate R in a porcelain crucible.

Carefully heat the mixture to a red glow for 10 minutes. Cool, dissolve the residue in 10 mL of nitric acid (~130 g/l) TS and filter. The filtrate yields reaction A described under 2.1 General identification tests as characteristic of chlorides.

- Either tests A and F or any two of tests B, C, D or E together with test F may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from methylthioninium chloride RS or with the reference spectrum of methylthioninium chloride.

B. 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).

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.
D. Carry out the 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 2 \( \mu \text{L} \) of each of the following 2 solutions in methanol R containing (A) 0.1 mg of the test substance per mL and (B) 0.1 mg of methylthioninium chloride RS per mL. 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).

E. Dissolve 1 mg in 10 mL of water R; a deep blue color is produced. Add 2.0 mL of hydrochloric acid (~70 g/L) TS and 0.25 g of zinc R powder; the color of the solution is discharged. Filter and expose the filtrate to the air; the blue color of the solution reappears.

F. Mix 0.05 g of the substance to be investigated with 0.5 g of anhydrous sodium carbonate R in a porcelain crucible. Carefully heat the mixture to a red glow for 10 minutes. Cool, dissolve the residue in 10 mL of nitric acid (~130 g/L) TS and filter. The filtrate yields reaction A described under 2.1 General identification tests as characteristic of chlorides.
Copper or zinc. Prepare the following solutions. For solution (1) ignite 1.0 g in a porcelain crucible using as low a temperature as practicable, until all of the carbon is oxidized. Cool the residue, add 15 mL of nitric acid (~130 g/L) TS and boil for 5 minutes. For solution (2) separately prepare a reference solution by boiling a quantity of copper(II) sulfate R, equivalent to 200 μg of Cu, with 15 mL of nitric acid (~130 g/L) TS for 5 minutes. Filter separately the cooled test and reference solutions (1) and (2) and wash any residue with 10 mL of water. Combine the filtrate and washings of the test solution (1) and similarly combine the filtrate and washings of the reference solution (2); add to each an excess of ammonia (~100 g/L) TS and filter the solutions into 50 mL volumetric flasks. Wash the precipitates with small portions of water, adding the washings to the filtrates; dilute the contents of each flask with water to volume, mixing thoroughly. To 25 mL of each of the solutions add 10 mL of hydrogen sulfide TS; no turbidity is produced within 5 minutes (absence of zinc) and any dark colour produced in the test solution (1) is not more intense than that of the reference solution (2) (the copper content is not more than 0.20 mg/g).

Iron. Mix 4 g with 200 mL of water R in a long-necked, round-bottomed flask, add 15 mL of nitric acid (~1000 g/L) TS, heat carefully to boiling and continue boiling until the volume of liquid is reduced to about 20 mL. Allow to cool, add 10 mL of sulfuric acid (~1760 g/L) TS and mix. Heat to boiling and add small successive quantities of nitric acid (~1000 g/L) TS, cooling before each addition, until a colourless liquid is obtained. Heat until white fumes are evolved; if darkening occurs at this stage continue the treatment with nitric acid (~1000 g/L).
TS. Finally heat until white fumes are again evolved. Allow the colourless liquid to cool, add 25 mL of a saturated solution of ammonium oxalate R in water, and boil until the slight froth completely subsides. Cool, dilute to 50 mL with water; 5 mL of the diluted solution complies with the 2.2.4 Limit test for iron; not more than 0.10 mg/g.

**Sulfated ash.** Not more than 4.5 mg/g.

**Loss on drying.** Dry to constant weight at 105 °C for 5 hours; it loses not less than 80 mg/g and not more than 220 mg/g. (The dried substance may be used to produce solution (4) of the test “Related substances”).

**Foreign dyes.** Carry out the test as described under 1.14.1 Thin-layer chromatography, using as the coating substance a slurry prepared from silica gel R1 and a mixture of equal volumes of potassium dihydrogen phosphate (27.2 g/l) TS and disodium hydrogen phosphate (28.4 g/l) TS. As the mobile phase, use a mixture of 20 volumes of 1-propanol R, 4 volumes of anhydrous formic acid R, and 1 volume of water. Apply to the plate 2 μl of a solution prepared by dissolving 25 mg of the test substance in sufficient methanol R to produce 10 mL. After removing the plate from the chromatographic chamber, allow it to dry in an oven at 105°C. At an R_f value of about 0.5, 3-4 spots appear, placed very close to each other, the lowest spot being violet in colour and the others red, the intensity of the colour increasing in ascending order of the spots. No other spot is detected.
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) dissolve about 50 mg of the substance to be examined and dilute to 50.0 mL. Sonicate for 5 minutes. 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 the substance to be examined at 105°C for 5 h (the dried substance of the test “Loss on drying” may be used), dissolve 100 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), (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 the area of the principal peak
  obtained with solution (3) (0.10%);
- the sum of the areas of all impurity peaks, other than the peak corresponding to
  impurity A, is not greater than 5 times the area of the principal peak obtained with
  solution (3) (0.5%). Disregard any peak with an area less than 0.5 times the area of
  the principal peak obtained with solution (3) (0.05%).

Assay. Transfer about 0.3 g, accurately weighed, to a 100-mL volumetric flask, dissolve in 30-
ml of water by warming on a water-bath, and allow the solution to cool. While shaking, add
50.0 mL of potassium dichromate (0.0167 mol/l) VS, dilute to volume with water, and mix.
Repeat the shaking intermittently for 10 minutes, and filter; discard the first 20 mL of the
filtrate. Transfer 50.0 mL of the filtrate to a glass-stoppered flask, add 40 mL of sulfuric acid
(190 g/l) TS and 1 g of potassium iodide R, mix, and allow the closed flask to stand in the-
dark for 5 minutes. Add 100 mL of water and titrate with sodium thiosulfate (0.1 mol/l) VS, using starch TS as indicator, until a blue-green colour is obtained. Repeat the operation without the substance being examined and make any necessary corrections. Each mL of potassium dichromate (0.0167 mol/l) VS is equivalent to 10.66 mg of C\textsubscript{16}H\textsubscript{18}ClN\textsubscript{3}S.

Assay

• Either method A or B may be applied.

A. Carry out test as described under \textit{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). \textsuperscript{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>
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<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>
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</tbody>
</table>

\textsuperscript{1} An X-Bridge Phenyl column and a Phenomenex Luna 3 µm Phenyl-Hexyl column were found suitable.
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.

Prepare the following solutions using as diluent a mixture of 30 volumes of acetonitrile R and 70 volumes of mobile phase A. For solution (1) dissolve about 50 mg of the substance to be examined, accurately weighed, and dilute to 50.0 mL. Sonicate for 5 minutes. For solution (2) dissolve 50.0 mg of methylthioninium chloride RS and dilute to 50.0 mL. Sonicate for 5 min.

Inject alternately 5 µL each of solutions (1) and (2). The test is not valid unless the symmetry factor of methylthioninium is not more than 3.0.

Measure the areas of the peak responses obtained in the chromatograms from solutions (1) and (2) and calculate the percentage content of methylthioninium chloride \((\text{C}_{16}\text{H}_{18}\text{ClN}_3\text{S})\), using the declared content of \(\text{C}_{16}\text{H}_{18}\text{ClN}_3\text{S}\) in methylthioninium chloride RS.
B. Dissolve about 100 mg, accurately weighed, in sufficient ethanol (~457 g/L) TS to produce 250.0 mL. Dilute 5.0 mL of this solution to 100.0 mL with ethanol (~457 g/L) TS. Dilute 5.0 mL of this solution to 50.0 mL with ethanol (~457 g/L) TS. Measure the absorbance (1.6) 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_{3}S) 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.]

Additional requirements for Methylthioninium chloride for parenteral use

Complies with the monograph for Parenteral preparations.

Bacterial endotoxins. If intended for use in the manufacture of a parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins, carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 2.5 IU of endotoxin RS per mg.
**Impurities**

![Chemical structure](image)

**A. 3-(Dimethylamino)-7-(methylamino)phenothiazin-5-ium chloride (azure B)**

![Chemical structure](image)

**B. 3-Amino-7-(dimethylamino)phenothiazin-5-ium (azure A)**

![Chemical structure](image)

**C. 3-amino-7-(methylamino)phenothiazin-5-ium (azure C)**

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