Methylthioninium injection (Methylthioninii injectio)

2017-01

Description. A clear, dark blue solution.

Category. Antidote

Storage. Store at room temperature, protected from light. Do not refrigerate or freeze.

Additional information. Strength in the current WHO Model List of Essential Medicines: 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 $C_{16}H_{18}ClN_3S$ stated on the label.

Identity tests

-Either tests A and C or 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 Chromatography*, 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 per mL solution in hydrochloric acid (~70 g/L) TS, when observed between 230 nm and 800 nm, exhibits 4 maxima at about 255–260 nm, 285–290 nm, 670–680 nm and 740–750 nm.

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

Related substances

Carry out test as described under *1.14.1 Chromatography*, 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 10 mg of methylthioninium chloride RS (containing methylthioninium chloride and impurity A) and dilute to 10.0 mL.

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

Use the chromatograms obtained with solution (4) and the chromatogram supplied with methylthioninium chloride RS 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

Carry out the test as described under *1.14.1 Chromatography*, 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).

Use the following conditions for gradient elution:

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

mobile phase B: acetonitrile R.

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

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 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 20.0 mg of methylthioninium chloride RS in 200.0 mL.

Inject alternately 5 µL each of solutions (1) and (2). The test is not valid unless in the chromatogram obtained with solution (2) the symmetry factor of the methylthioninium peak 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.

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.