Edition: BP 2025 (Ph. Eur. 11.6 update)

Neostigmine Injection

General Notices

Action and use

Cholinesterase inhibitor.

DEFINITION

Neostigmine Injection is a sterile solution of Neostigmine Metilsulfate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of neostigmine metilsulfate, $C_{13}H_{22}N_2O_6S$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Dilute, if necessary, a volume of the injection containing 2.5 mg of Neostigmine Metilsulfate to 5 mL with *water*, shake with three 10-mL quantities of *ether* and discard the ether extracts. The *light absorption* of the aqueous solution, Appendix II B, in the range 230 to 350 nm exhibits two maxima, at 260 nm and 266 nm.
B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.
(1) Use the injection diluted, if necessary, with *water* to produce a solution containing 0.05% w/v of Neostigmine Metilsulfate.
(2) 0.05% w/v of *neostigmine metilsulfate EPCRS*.
(3) Equal volumes of solutions (1) and (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G*.
(b) Use the mobile phase as described below.
(c) Apply 10 µL of each solution.
(d) Develop the plate to 15 cm.
(e) After removal of the plate, dry in air and spray with *dilute potassium iodobismuthate solution*.

MOBILE PHASE

5 volumes of *water*, 10 volumes of *formic acid*, 35 volumes of *methanol* and 50 volumes of *chloroform*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

The principal spot in the chromatogram obtained with solution (3) appears as a single, compact spot.

C. To 1 mL add 0.5 mL of 5M *sodium hydroxide* and evaporate to dryness on a water bath. Heat rapidly on an oil bath to about 250° and maintain at this temperature for about 30 seconds. Cool, dissolve the residue in 1 mL of *water*, cool in ice

and add 1 mL of *diazobenzenesulfonic acid solution*. An orange-red colour is produced.

TESTS

Acidity

pH, 4.5 to 6.5, Appendix V L.

(3-Hydroxy)trimethylanilinium methyl sulfate

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Use the injection diluted, if necessary, with *water* to contain 0.05% w/v of Neostigmine Metilsulfate.
(2) Dilute 1 volume of solution (1) to 100 volumes with *water*.
(3) Add 0.05 mL of 5M *sodium hydroxide* to 1 mL of solution (1) and allow to stand for 5 minutes. Add 0.1 mL of 5M *hydrochloric acid* and use immediately.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Lichrospher 60 RP-select B is suitable).
(b) Use isocratic elution and the mobile phase described below.
(c) Use a flow rate of 1.1 mL per minute.
(d) Use an ambient column temperature.
(e) Use a detection wavelength of 215 nm.
(f) Inject 20 µL of each solution.

MOBILE PHASE

A 0.0015M solution of *sodium heptanesulfonate* in a mixture of 15 volumes of *acetonitrile* and 85 volumes of 0.05M *potassium dihydrogen orthophosphate* adjusted to pH 3.0 with *orthophosphoric acid*.

SYSTEM SUITABILITY

In the chromatogram obtained with solution (3) the principal peak has a retention time of about 6.8 minutes (neostigmine metilsulfate) and there is a peak with a relative retention time of about 0.5 ((3-hydroxy)trimethylanilinium methylsulfate).

LIMITS

In the chromatogram obtained with solution (1):

Identify any peak due to (3-hydroxy)trimethylanilinium methylsulfate using the chromatogram obtained with solution (3).

In the chromatogram obtained with solution (1):

the area of the peak due to (3-hydroxy)trimethylanilinium methylsulfate is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%).

ASSAY

Dilute a quantity containing 25 mg of Neostigmine Metilsulfate to 50 mL with *water*. Measure the *absorbance* of the resulting solution at the maximum at 260 nm, Appendix II B. Calculate the content of $C_{13}H_{22}N_2O_6S$ taking 14.35 as the value of A(1%, 1 cm) at the maximum at 260 nm.

STORAGE

Neostigmine Injection should be protected from light.