

20.7.05

**AOAC Official Method 971.41  
Neostigmine Methylsulfate  
in Drugs**

**Spectrophotometric Method  
First Action 1971  
Final Action 1975**

(Applicable only to injections.)

**A. Apparatus and Reagents**

(a) *Recording spectrophotometer.*—For measurement in range 230–350 nm.

(b) *Neostigmine methylsulfate standard solution.*—0.5 mg/mL. Accurately weigh 50 mg USP neostigmine methylsulfate Reference Standard of known purity, transfer to 100 mL volumetric flask, add 1 mL 0.5M H<sub>2</sub>SO<sub>4</sub>, **890.01** (see A.1.14), and dilute to volume with H<sub>2</sub>O.

**B. Preparation of Test Solution**

(a) *Interfering UV-absorbing preservatives absent.*—Transfer aliquot containing 5.0 mg neostigmine methylsulfate to 150 mL beaker and hydrolyze as in **D**.

(b) *Injection solutions containing phenol or parabens.*—Proceed as in **C**.

**C. Extraction of Interferences**

Transfer test solution aliquot (or dilution if necessary) containing 5.0 mg neostigmine methylsulfate to 125 mL separator, add 1 mL 0.05M H<sub>2</sub>SO<sub>4</sub>, and add H<sub>2</sub>O to total volume of 21 mL. Add 35 mL CHCl<sub>3</sub> and shake vigorously 2 min. Transfer CHCl<sub>3</sub> layer into second 125 mL separator containing wash solution of 10 mL H<sub>2</sub>O

and 1 mL 0.05M H<sub>2</sub>SO<sub>4</sub>; shake, let layers separate completely, and discard CHCl<sub>3</sub> phase. Repeat extraction with 5 additional 35 mL portions CHCl<sub>3</sub>. Rinse stem of each separator with CHCl<sub>3</sub> after last extraction and discard CHCl<sub>3</sub>. Combine aqueous layers in 150 mL beaker. Rinse each separator in succession with two 5 mL portions H<sub>2</sub>O, rinse stem of each separator with H<sub>2</sub>O, transfer rinsings to beaker, and proceed as in **D**.

**D. Hydrolysis**

Add 25 mL 10% NaOH solution (w/v) and H<sub>2</sub>O to ca 80 mL. Cover with watch glass and heat 30 min on vigorous steam bath. Cool, quantitatively transfer solution through loose glass wool plug, prewashed with 1% NaOH solution (w/v), to 250 mL volumetric flask, and dilute to volume with H<sub>2</sub>O. In separate beaker, similarly treat 10 mL aliquot standard neostigmine methylsulfate solution. Perform blank determination, omitting neostigmine methylsulfate. Proceed as in **E**.

**E. Determination**

Record spectra of test and standard solutions, relative to blank, in 1 cm cells, from 350 to 230 nm. Determine *A* of each solution by subtracting *A* at 350 nm from *A* at maximum, ca 239 nm.

$$\text{Neostigmine methylsulfate in test portion, mg/mL} = \left( \frac{A}{A} \right) 0.5 \quad (10/\text{mL test solution aliquot})$$

where *A* and *A* refer to test portion and standard, respectively.

Reference: *JAOAC* **54**, 21(1971).

CAS-51-60-5 (neostigmine methylsulfate)