AOAC Official Method 974.42
Neostigmine in Drugs

Chromatographic Method
First Action 1974
Final Action 1976

A. Apparatus

(**a**) *Spectrophotometer.*—Suitable for measurement in range 265–400 nm.

(**b**) *Chromatographic tube and tamping rod.*—*See* **967.31A** (*see* 19.1.02).

B. Reagents

(**a**) *Phosphate buffer.*—pH 5.8. Mix 1 volume 1M K_2HPO_4 (17.4 g/100 mL) with 4 volumes 1M KH_2PO_4 (13.6 g/100 mL). Adjust pH, using pH meter, to 5.80 ± 0.05 with either component.

(**b**) *Washed chloroform.*—Shake equal volumes $CHCl_3$ and H_2O in separator. Let layers separate 5 min and discard upper layer.

(**c**) *Washed ether.*—Shake equal volumes ether and H_2O in separator. Let layers separate 5 min and discard lower layer.

(**d**) *Bis(2-ethylhexyl) hydrogen phosphate* (*DEHP*) *solution.*—2.5%. Mix 2.5 mL DEHP with 97.5 mL H_2O-washed $CHCl_3$.

(**e**) *Diatomaceous earth.*—*See* **960.53B** (*see* 18.1.01).

(**f**) *Neostigmine standard solution.*—Dry USP neostigmine bromide Reference Standard 3 h in 105°C oven. Accurately weigh ca 5 mg dry standard, using microbalance, and transfer to 150 mL beaker. Add 2.0 mL pH 5.8 phosphate buffer, mix by swirling gently, and proceed as in **D**.

C. Preparation of Test Portion

(**a**) *Tablets.*—Accurately weigh portion powdered tablets containing 5 mg neostigmine bromide into 150 mL beaker, add 2.0 mL pH 5.8 phosphate buffer, mix by swirling gently, and proceed as in **D**.

(**b**) *Individual tablets.*—Transfer tablet to 50 mL centrifuge tube, powder if coated, and add 6.0 mL pH 5.8 phosphate buffer by pipet. Stopper, shake mechanically 30 min, and centrifuge 5 min at high speed. Pipet 2.0 mL clear supernate into 150 mL beaker and proceed as in **D**.

(**c**) *Ophthalmic solution.*—Dilute accurately measured volume of test solution to 2.5 mg neostigmine bromide/mL with pH 5.8 phosphate buffer solution. Pipet 2.0 mL diluted sample solution into 150 mL beaker and proceed as in **D**.

D. Determination

(*Note:* Use H_2O-washed solvents throughout.)

Treat standard and test portion solutions similarly. Add 3.0 g diatomaceous earth, mix with metal spatula until fluffy, and transfer quantitatively in 3 portions to chromatographic tube containing 1 g diatomaceous earth mixed with 0.5 mL pH 5.8 phosphate buffer. Pack uniformly. Dry-wash beaker with 0.2 g diatomaceous earth, transfer wash to tube, and pack uniformly. Wipe beaker and all apparatus used in column preparation with glass wool and add to column. Proceed without delay.

Wash column with 75 mL ether and then with 75 mL $CHCl_3$. Discard washings. Elute neostigmine bromide with 75 mL 2.5% DEHP solution into 500 mL separator containing 20 mL 0.05M H_2SO_4, **890.01** (*see* A.1.14). Complete elution with 25 mL $CHCl_3$. Add 175 mL isooctane to eluate and shake vigorously 2 min. Let stand ≥5 min to completely separate layers. Transfer lower aqueous layer to 250 mL beaker. Repeat extraction with two 20 mL portions 0.05M H_2SO_4, and combine aqueous layers in the 250 mL beaker.

Add 10 mL *10% NaOH solution (w/v)* to beaker, mix by swirling gently, cover with watch glass, and heat 45 min on vigorous steam bath. Cool, transfer quantitatively to 100 mL volumetric flask, dilute to volume with H_2O, and mix. Centrifuge portions of standard and test portion solutions. Record spectra of clear test portion and standard solutions between 400 and 255 nm against 1% NaOH (w/v) solution in 1 cm cells. Determine ΔA of each solution by subtracting A at 340 nm from A at maximum, ca 293.5 nm.

$$\text{Neostigmine bromide in final solution, mg} = (\Delta A/\Delta A') \times C \times 100$$

where C = mg neostigmine bromide standard/mL, and ΔA and $\Delta A'$ refer to test portion and standard, respectively.

Reference: *JAOAC* **57,** 725(1974).

CAS-114-80-7 (neostigmine bromide)