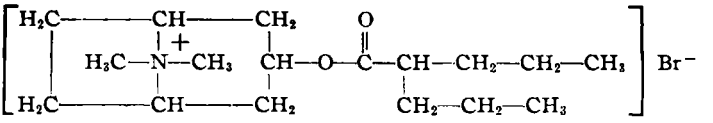
Qualitative and Quantitative Tests for Anisotropine Methylbromide

Provisional, unofficial monographs are developed by the Drug Standards Laboratory, in cooperation with the manufacturers of the drugs concerned, for publication in the *Journal of Pharmaceutical Sciences*. The ready availability of this information affords discriminating medical and pharmaceutical practitioners with an added basis for confidence in the quality of new drug products generally, and of those covered by the monographs particularly. Such monographs will appear on drugs representing new chemical entities for which suitable identity tests and assay procedures are not available in the published literature. The purity and assay limits reported for the drugs and their dosage forms are based on observations made on samples representative of commercial production and are considered to be reasonable within expected analytical and manufacturing variation.

8-METHYLTROPINIUM BROMIDE 2-propylpentanoate; $C_{17}H_{32}BrNO_2$; mol. wt. 362.36. The structural formula of anisotropine methylbromide may be represented as



Physical Properties.—Anisotropine methylbromide occurs as a white, glistening, odorless, bitter hygroscopic powder. It is freely soluble in chloroform and alcohol, soluble in water, slightly soluble in acetone, and practically insoluble in ether. The pH of a solution of anisotropine methylbromide in carbon dioxide-free water (1 in 100) is between 6 and 7.

Identity Tests.—A solution of anisotropine methylbromide (1 in 20) responds to the U.S.P. XVI tests for bromide.

Transfer about 50 mg. of anisotropine methylbromide to a 150-ml. beaker, dissolve in 50 ml. of water, and add 5 ml. of 20% sulfuric acid. Add slowly, with stirring, 10 ml. of a freshly prepared and filtered solution of ammonium reineckate (1 in 50); allow to stand 1 hour. Filter the pink precipitate through a sintered-glass filter of medium porosity, and wash it with three 2-ml. portions of ice cold water. Allow to air dry and recrystallize from 70% alcohol at 55°. Dry the crystals in a vacuum desiccator over phosphorus pentoxide for 16 hours: the reineckate melts with decomposition at 195–199°, U.S.P. XVI Class Ia.

A 1 in 400 solution of anisotropine methylbromide in water does not exhibit an ultraviolet absorption maximum in the wavelength range of from 220 to 360 mμ.

The infrared spectrum of a 0.5% dispersion of anisotropine methylbromide in potassium bromide in a disk of about 0.82-mm. thickness is shown in Fig. 1.

Purity Tests.—Dry about 1 Gm. of anisotropine methylbromide, accurately weighed, at 105° for 3 hours: it loses not more than 1.0% of its weight.

Char about 1 Gm. of anisotropine methylbromide, accurately weighed, cool the residue, add 1 ml. of sulfuric acid, heat cautiously until evolution of sulfur trioxide ceases, ignite, cool, and weigh: the residue does not exceed 0.1%.

Determine the heavy metals content by the U.S.P. XVI heavy metals test, method I: the heavy metals limit for anisotropine methylbromide is 20 p.p.m.

Transfer 500.0 mg. of anisotropine methylbromide, previously dried at 105° for 3 hours and accurately weighed, to a glass-stoppered 125-ml. conical flask. Add 50.0 ml. of 0.05 *N* sodium hydroxide, stopper, and allow to stand for exactly 1 hour. Prepare a blank in the same manner, omitting the anisotropine methylbromide. To each flask add 50.0 ml. of 0.05 *N* hydrochloric acid, 2 drops of phenolphthalein T.S., and titrate with 0.05 *N* sodium hydroxide. Subtract the volume of alkali consumed by the blank to obtain the volume required to neutralize the excess acid in the sample solution: not more than 2.5 ml. of 0.05 *N* sodium hydroxide is required.

Determine the nitrogen content by the U.S.P. XVI nitrogen determination, method II, using about 325 mg. of anisotropine methylbromide (previously dried at 105° for 3 hours and accurately weighed) and 0.1 *N* sulfuric acid for the titration. Each milliliter of 0.1*N* sulfuric acid is equivalent to 1.401 mg. of nitrogen (N). The amount of nitrogen found is not less than 3.79% and not more than 3.95% of the weight of the sample taken.

Assay

Bromine.—Transfer about 600 mg. of anisotropine methylbromide, previously dried at 105° for 3 hours and accurately weighed, to a 250-ml. conical flask; dissolve in 50 ml. of water. Add 50 ml. of methanol, 10 ml. of glacial acetic acid, and 2 or 3 drops of eosin Y T.S. Titrate with 0.1 *N* silver nitrate until the color of the precipitated silver bromide changes to pink. Each milliliter of 0.1 *N* silver nitrate is equivalent to 7.991 mg. of bromine (Br). The amount of bromine found is not less than 21.6% and not more than 22.5% of the weight of the sample taken.

Anisotropine Methylbromide.—Transfer about 700 mg. of anisotropine methylbromide, previously dried at 105° for 3 hours and accurately weighed, to a tall form 200-ml. beaker; dissolve in 30 ml. of glacial acetic acid. Add 10 ml. of mercuric acetate T.S. and 2 drops of methylrosaniline chloride T.S., and titrate with 0.1 *N* acetous perchloric acid to a

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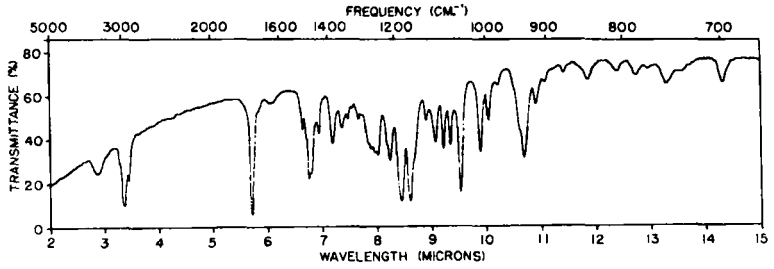

Fig. 1.—Infrared spectrum of anisotropine methylbromide in potassium bromide disk (0.5%); Perkin-Elmer model 21 spectrophotometer, sodium chloride prism.

blue end point. Perform a blank titration and make any necessary correction. Each milliliter of 0.1 *N* perchloric acid is equivalent to 36.24 mg. of $C_{17}H_{32}BrNO_2$. The amount of anisotropine methylbromide found is not less than 98.0% and not more than 102.0% of the weight of the sample taken.

DOSAGE FORMS OF ANISOTROPINE METHYLBROMIDE

Anisotropine Methylbromide Tablets

Identity Test.—Finely powder a number of anisotropine methylbromide tablets; transfer an amount equivalent to 50 mg. of anisotropine methylbromide to a 125-ml. conical flask. Add 50 ml. of water, and shake mechanically for 30 minutes. Filter through paper, discarding the first few milliliters of filtrate, and collect the remainder of the filtrate in a 125-ml. separator. Add 5 ml. of a solution prepared by mixing 1 volume of chloroform with 2 volumes of carbon tetrachloride; shake for 5 minutes, and allow the phases to separate. Discard the organic phase and bubble air through the aqueous phase for 5 minutes. Transfer the aqueous phase to a 150-ml. beaker and continue as directed under *Identity Tests* in the monograph for anisotropine methylbromide, beginning with ". . . add 5 ml. of 20% sulfuric acid . . ." The reineckate melts with decomposition at 195–199°, U.S.P. XVI Class Ia.

Assay.—*Standard Preparation.*—Transfer about 100 mg. of anisotropine methylbromide reference standard, previously dried at 105° for 3 hours and accurately weighed, to a 100-ml. volumetric flask; dilute to volume with water and mix.

Procedure.—Weigh and finely powder not less than 20 anisotropine methylbromide tablets. Transfer to a glass-stoppered 125-ml. flask an amount of powdered tablets, accurately weighed, equivalent to about 50 mg. of anisotropine methylbromide. Pipet in 50 ml. of water, stopper the flask, and shake mechanically for 30 minutes. Filter through dry paper, discarding the first 10 ml. of filtrate; transfer the remainder of the filtrate to a 125-ml. separator. Add 5 ml. of a solution prepared by mixing 1 volume of chloroform with 2 volumes of carbon tetrachloride, shake for 5 minutes, and allow the phases to separate. Discard the organic phase and bubble air through the aqueous phase for 5 minutes. Pipet 10 ml. of the aqueous phase into a graduated 15-ml. conical centrifuge tube; pipet 10 ml. of the *Standard Preparation* into a similar tube. Add 1.0 ml. of 20% sulfuric acid and 2.0 ml. of a freshly prepared and filtered solution of ammonium reineckate (1 in 50) to each tube. Stopper the tubes, mix by inversion, allow to stand for 1 hour, and centrifuge. Decant the supernatant liquid, and wash each precipitate with two 1-ml. portions of ice cold water, centrifuging and decanting each time. Dissolve the precipitates in acetone and dilute to 10.0 ml. with acetone Allow the solutions to stand for 1 hour, centrifuge if they show a haze, and determine the absorbances in 1-cm. cells with a suitable spectrophotometer at 525 mμ using acetone as the blank. Record the absorbance of the solution from the *Standard Preparation* as A_s and that from the tablets as A_u. Calculate the weight (in milligrams) of anisotropine methylbromide in the portion of the powdered tablets taken by the formula $0.5W \times A_u/A_s$, where W is the weight (in milligrams) of anisotropine methylbromide reference standard taken. The amount of anisotropine methylbromide found is not less than 93.0% and not more than 107.0% of the labeled amount.

Anisotropine Methylbromide Elixir

Identity Test.—Transfer a volume of anisotropine methylbromide elixir, equivalent to 50 mg. of anisotropine methylbromide, to a 150-ml. beaker. Dilute to 50 ml. with water and continue as directed under *Identity Tests* in the monograph for anisotropine methylbromide, beginning with ". . . add 5 ml. of 20% sulfuric acid. . ." but allowing the precipitate to stand 4 hours before filtering: the reineckate melts with decomposition at 195–199°, U.S.P. XVI Class Ia.

Assay.—*Standard Preparation.*—Prepare as directed in the *Assay* in the monograph for anisotropine methylbromide tablets.

Procedure.—Pipet a volume of anisotropine methylbromide elixir, equivalent to 10 mg. of anisotropine methylbromide, into a graduated 15-ml. conical centrifuge tube; dilute to 10.0 ml. with water. Pipet 10 ml. of the *Standard Preparation* into a similar tube and proceed as directed in the *Assay* in the monograph for anisotropine methylbromide tablets, beginning with "To each tube add 1.0 ml. of 20% sulfuric acid . . ." but allowing the precipitate to stand 4 hours before centrifuging. Calculate the weight (in milligrams) of anisotropine methylbromide in the volume of elixir taken by the formula $0.1W \times A_u/A_s$, where W is the weight (in milligrams) of the anisotropine methylbromide reference standard taken. The amount of anisotropine methylbromide found is not less than 93.0% and not more than 107.0% of the labeled amount.

DISCUSSION

U.S.P. and N.F. terminology for solubility, melting range, reagents, etc., have been used wherever feasible.

Anisotropine methylbromide[1] is a parasympatholytic agent which is used as a gastrointestinal

[1] Marketed as Valpin by Endo Laboratories, Inc., Richmond Hill, N. Y.

antispasmodic and visceral smooth muscle relaxant. It differs from atropine and most other ester antispasmodics in the substitution of an aliphatic for an aromatic side chain in the acid moiety of the ester. This substitution results in a compound which is highly resistant to hydrolysis. Under the conditions specified under *Purity Tests* in the monograph for anisotropine methylbromide, the volume of alkali consumed in the residual titration was zero so that the degree of saponification was nil. The melting range of the compound is not reproducible, but varies widely with the conditions of the test (initial temperature and rate of heating).

Identity Tests.—The inclusion of an ultraviolet absorption identity test is for the purpose of distinguishing anisotropine methylbromide from aromatic acid esters of tropine and its derivatives. The ultraviolet absorption spectra of atropine and homatropine derivatives show absorbance maxima in the vicinity of 251–252, 257–258, and 263–264 mμ due to the presence of tropate and mandelate moieties, respectively (1).

The identity test and the assay for anisotropine methylbromide in the tablets involve a similar procedure for the extraction and purification of the sample. If desired, the amounts called for in the assay may be increased proportionately so that the identity test and assay may be performed on the same treated tablet extract.

Quantitative Methods.—The assay for bromine (as bromide ion) determines the nonactive portion of the molecule but serves as a control on the purity of the bulk material. Analysis of anisotropine methylbromide by the indicator titration method gave an average value of 22.1 ± 0.0%[2] bromine (Br). Potentiometric titrations of anisotropine methylbromide (900 mg. in 50 ml. of water) by means of silver-calomel electrodes (the latter connected to the titration vessel with a saturated potassium nitrate–agar salt bridge), gave an average value of 22.1 ± 0.1%[2] bromine (Br). The calculated value for the bromine content is 22.05%.

The nonaqueous titration method for anisotropine methylbromide gave an average value of 100.0 ± 0.2%.[2] The indicator end point is very sharp and corresponds to a 200–225-mv. break in a potentiometric titration curve obtained under identical conditions.

The colorimetric reineckate method for the dosage forms is a variation of the assay in the U.S.P. XVI monograph for homatropine methylbromide tablets. Other variations of the method have appeared in the literature (2). The present method offers the convenience of carrying out precipitation, washing, and dilution in the same container. However, it suffers somewhat from loss of precision compared to the method in which the precipitate is transferred to a sintered-glass filter, washed, dissolved in acetone, and diluted to volume in a volumetric flask. The 15-ml. graduated centrifuge tubes were calibrated with water; only those found accurate at the 10.0-ml. mark were used. Analysis of commercial anisotropine methylbromide elixir by this method gave an average value of 95.3 ± 2.5%[2] of the labeled amount.

Prior to precipitating the reineckate the tablet assay requires the insertion of a wash step in order to remove interfering dye used to color the tablets. The red dye was found to be water-soluble in acid medium (absorbance maximum at about 550 mμ), but extractable from neutral medium with a chloroform–carbon tetrachloride mixture. A demonstration with fortified samples showed that the anisotropine methylbromide in the tablet samples survived the wash step completely. Analysis of commercial 10-mg. tablets gave an average value of 95.4 ± 2.4%[2] of the labeled amount.

[2] Maximum deviation from the mean value.

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