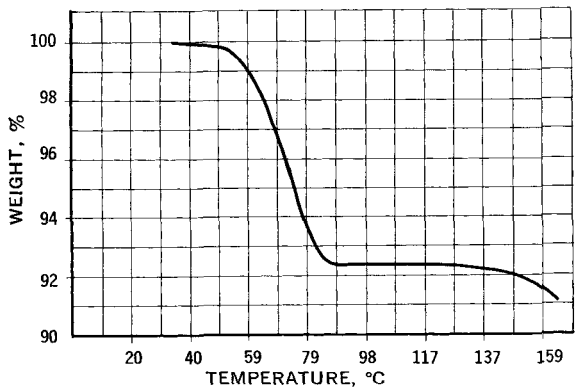

Figure 1—*Thermobalance plot of weight* versus *temperature for mephentermine sulfate.*

determination of the endpoint showed that both methods gave the same results.

Two recovery series were run, one prepared by dissolving 0.8942 g. of dried mephentermine sulfate in 40.0 ml. of water, the other by dissolving 0.5004 g. of dried mephentermine sulfate in enough water to make 25.00 ml. Seven replicate assays of 4-ml. aliquots of the first solution gave an average recovery of 99.2 ± 1.0% with a range of 98.4 to 101.0%.

Three replicates of 4-ml. aliquots of the second solution gave an average recovery of 100.3 ± 0.4% with a range of 100.0 to 100.7%.

DISCUSSION

The directions call for an indicator blank. If titration is carried out to the first color change, the blank is less than 1 drop (0.01 ml.); however, it is believed that some analysts will use the characteristic vivid green color. Titration to this point will give blanks of 0.02 to 0.05 ml.

The methods proposed are very much more rapid than those official in NF XII. The extraction process suggested for mephentermine sulfate injection is completed in less than 10 min. The results obtained, even though limited in number, appear to be within the expected analytical error.

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ACKNOWLEDGMENTS AND ADDRESSES

Received April 8, 1969, from the *School of Pharmacy, Temple University, Philadelphia, PA 19140*

Accepted for publication May 13, 1969.

Presented to the Pharmaceutical, Analysis and Control Section, APhA Academy of Pharmaceutical Sciences, Montreal meeting, May 1969.

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The authors thank Wyeth Laboratories, Radnor, Pa. for the sample of mephentermine sulfate and Smith Kline & French, Inc., Philadelphia, Pa. for the thermogravimetric determinations.

Fluorometric Determination of Atropine and Hyoscyamine in Tablets and Injections

LAURA A. ROBERTS

Abstract □ A fluorometric method has been developed for the quantitative determination of atropine and hyoscyamine in the presence of other belladonna constituents. Atropine and/or hyoscyamine is extracted with chloroform from basic solution, and an aliquot of the extract is added to a chloroform solution of eosine yellowish. This solution, appropriately diluted, is read on a fluorometer at an excitation wavelength of 475 mμ and an emission wavelength of 552 mμ. Scopolamine, atroscine, and other components do not produce a measurable fluorescence in this procedure and thus need not be separated from atropine or hyoscyamine.

Keyphrases □ Belladonna alkaloid dosage forms—analysis □ Atropine, hyoscyamine—determination □ Eosine yellowish—reagent □ Fluorometry—analysis

l-Hyoscyamine and atropine (*dl*-hyoscyamine) have become prominent anticholinergic and mydriatic agents (1). They are incorporated, alone or with other drugs (*e.g.*, scopolamine, barbiturates, and vitamins), in tablets, elixirs, injections, and capsules. Present official methods for the determination of atropine include a base-chloroform extraction followed by an acid-base titration (2), a base-chloroform extraction followed by a quantitative IR determinative step (3), and a perchloric acid titration in acetic acid (4). These procedures require relatively large amounts of atropine. Atropine, however, usually appears in submilligram amounts in most dosage forms.

A review of the literature revealed that much work has been done on the quantitative determination of tropane alkaloids in plant material and in commercial drug products. Most analytical approaches were characterized by colorimetric and titrimetric determinative steps. Nin'o (5) determined atropine in aluminum hydroxide with belladonna tablets using a column separation followed by reaction with *p*-dimethylaminobenzaldehyde. Zielinska-Sowicka *et al.* (6), Niezgodzki

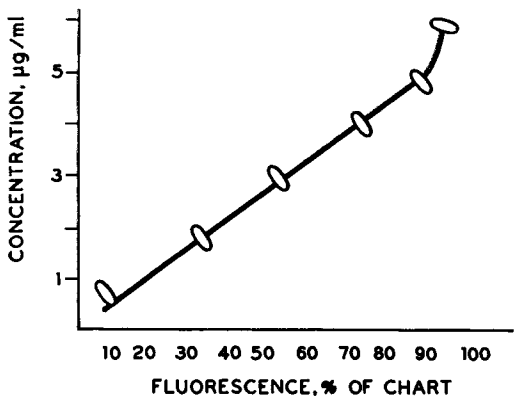

Figure 1—*Linearity of fluorometric measurement of atropine.*

and Manickowski (7), Saint-Firmin *et al.* (8), and Shipalov *et al.* (9) separated atropine group alkaloids by thin layer or paper chromatography and quantitated the alkaloids with Dragendorff reagent. Czyszewska *et al.* (10) performed an atropine content determination on prolonged action Bellergot tablets[1] containing ergotamine tartrate, atropine sulfate, and phenobarbital[2] (5-amino-2,3-dihydro-1,4-phthalazinedione, Winthrop Laboratories) by reaction with bromthymol blue following solvent extraction of the alkaloids. Koch *et al.* (11) utilized a nitration procedure, followed by reduction to the aromatic amine, diazotization, and coupling, to assay belladonna alkaloids in combination with phenobarbital. Toncheva (12), Ozimic (13), del Carmen Alvarez and Gomez-Serranillos (14), and Piasicka (15) employed a conventional acid-base titration as the determinative step after extraction of atropine from plant material. Timbekov and Kasymov (16), using potassium permanganate and potassium bromate as oxidizing agents, quantitatively determined hyoscyamine by amperometric titration.

Belladonna alkaloids give similar or identical reactions in all the above determinative steps. Thus, in an analysis, unless the alkaloids are separated by thin layer or paper chromatographic techniques, only total alkaloids can be determined. Considering the similar chemical structures of atropine-hyoscyamine and atroscine-scopolamine (alkaloids most commonly accompanying atropine and hyoscyamine in drugs), an analytical method that would quantitate atropine and hyoscyamine in the presence of these other alkaloids without any separation requirement would be the most desirable. A rapid fluorometric method for the determination of small amounts of atropine and hyoscyamine in the presence of other compounds has been reported by Ogawa *et al.* (17). This method was investigated by the author, modified, and adapted to the analysis of atropine and hyoscyamine alone and in combination with other pharmaceutical components.

The method is based on the fact that atropine and hyoscyamine form highly fluorescent salts with eosine yellowish, and these salts are soluble in chloroform. Scopolamine and atroscine in combination with eosine yellowish produce the same fluorescence spectra as atropine and hyoscyamine, but the intensity is 1/80 of that produced by atropine and hyoscyamine (17). Since these alkaloids also appear in submilligram amounts in drugs, they do not interfere with the atropine hyoscyamine assay procedure.

In the method developed, the alkaloids are extracted from a basic solution with chloroform, and an aliquot of the extract is added to a chloroform solution of eosine yellowish. This solution, appropriately diluted, is read on a fluorometer at an excitation wavelength of 475 mμ and an emission wavelength of 552 mμ. Excitation at 475 mμ was chosen because it produced a more intense emission at 552 mμ than the other possible, practical excitation wavelengths investigated, *i.e.*, 320 and 365 mμ. The blank emission is small at a 475 mμ excitation. Other constituents of belladonna besides the scopolamine and atroscine discussed were found not to interfere with the analysis (17).

The acid form of eosine yellowish fluoresces to a negligible extent. However, the salt form is highly fluorescent, and atropine and hyoscyamine are sufficiently strong bases to convert eosine yellowish to a salt in chloroform, resulting in the production of fluorescence. Tropine, a hydrolytic degradation product of atropine and hyoscyamine, also reacts with eosine yellowish to produce a measurable fluorescence. This method, along with the methods referenced above, cannot distinguish tropine from atropine or hyoscyamine. The basicities of scopolamine and atroscine are weakened by the presence of an electron withdrawing epoxide group in the molecule, and these compounds are unable to effectively ionize eosine yellowish to produce a measurable fluorescence. Scopine, a degradation product of scopolamine and atroscine, and scopoline, a rearranged amino-alcohol degradation product, behave in a similar manner.

Considering the reaction involved, the use of this method is precluded in the presence of strong organic bases not previously separated from atropine and hyoscyamine. Organic acids which might affect the reaction are separated from atropine and hyoscyamine in a basic-extraction step. Alcohol also interferes with the reaction; therefore, chloroform must be water-washed before use in this procedure, and pharmaceuticals to be analyzed must be alcohol-free. Benzyl alcohol, a preservative commonly found in atropine-containing drugs, does not produce measurable fluorescence when combined with eosine yellowish and thus does not interfere with the procedure.

The method is not applicable to methyl bromide or methyl nitrate derivatives of atropine and hyoscyamine.

Fluorescence was found to be proportional to concen-

Table I—Recovery of Atropine Sulfate and Hyoscyamine Hydrobromide from Simulated Pharmaceutical Preparations

Synthetic	Added	Found	% Recovery
No. 1	0.510 mg./ml. of atropine sulfate	0.513 mg./ml.	101
No. 2	1.25 mg. of hyoscyamine hydrobromide	1.23 mg.	98.4

[1] Bellergot.
[2] Luminal.

Table II—Analysis of Commercial Products

Dosage Form	Declared Composition	Amount	Determined	% of Label Claim Found
Injection	Atropine sulfate	15 mg./ml.	Atropine sulfate	98.3
	Benzyl alcohol	1%		
	Sulfuric acid	—		
Injection	Atropine sulfate	0.5 mg./ml.	Atropine sulfate	97.4
	Chlorobutanol	0.5%		
	Sulfuric acid	—		
Injection	Atropine sulfate	0.4 mg./ml.	Atropine sulfate	107
	Benzyl alcohol	1%		
		Per Tablet		
Tablets	Hyoscyamine sulfate	0.30 mg.	Hyoscyamine sulfate plus atropine sulfate	103
	Atropine sulfate	0.06 mg.		
	Scopolamine hydrobromide	0.02 mg.		
	Phenobarbital	45 mg.		
		Per Tablet		
Tablets	Hyoscyamine hydrobromide	0.225 mg.	Hyoscyamine plus atropine	96.9
	Atropine sulfate	0.019 mg.		
	Scopolamine	0.006 mg.		
	Vinbarbital	30 mg.		
		Per Tablet		
Tablets	Hyoscyamine	0.4507 mg.	Hyoscyamine plus atropine	98.4
	Atropine sulfate	0.0372 mg.		
	Scopolamine hydrobromide	0.0119 mg.		

tration in the range 1 to 5 mcg./ml. (Fig. 1). There was no change in fluorescence between 5 and 20 min. However, erroneous data result if the reaction is performed in sunlight or fluorescent light due to the hydrochloric acid produced by the decomposition of chloroform (16). Alcohol-free chloroform reacts with oxygen in the presence of light to form hydrochloric acid and phosgene.

EXPERIMENTAL

Apparatus—Spectrophotofluorometer—Capable of measuring fluorescence at an excitation wavelength of 475 mμ and an emission wavelength of 552 mμ, with a slit arrangement for routine work.[1] Parameters: use a xenon lamp, and set meter multiplier at 0.3, sensitivity to about 30, excitation wavelength at 475 mμ, and emission wavelength at 552 mμ.

Reagents—*Chloroform*—Water-washed, freshly prepared. Perform any operations with chloroform under subdued light.

Eosine Yellowish Solution—Dissolve 100 mg. of dye (FD&C Red No. 22, of disodium tetrabromofluorescein) in 100 ml. of water, acidify with 10% sulfuric acid, and extract with about 90 ml. of chloroform. Add the extract to a 100-ml. volumetric flask through a pledget of chloroform-moistened cotton. Dilute to volume with chloroform.

Reference Standard—Weigh 2.0 mg. of atropine or hyoscyamine into a 100-ml. volumetric flask and dilute to volume with chloroform. Alternatively, weigh 2.40 mg. of atropine sulfate or 2.25 mg. of hyoscyamine hydrochloride into a separatory funnel, make alkaline with 0.1 *N* sodium hydroxide, and extract with three 25-ml. portions of chloroform. Add each extract to a 100-ml. volumetric flask through a chloroform-moistened pledget of cotton. Dilute the solution to volume with chloroform.

PROCEDURE

Sample Treatment—(Perform these operations under subdued light.)

Solid Dosage Forms—Weigh an amount of finely powdered sample equivalent to about 2 mg. of atropine (or atropine plus hyoscyamine) into a separatory funnel. Add 5 ml. of 0.1 *N* NaOH and extract with four 20-ml. portions of chloroform. Add each extract to a 100-ml. volumetric flask through a chloroform-moistened pledget of cotton. Dilute the combined extracts to volume with chloroform and mix. Pipet 3.0 ml. of this solution into a 25-ml. volumetric flask, add 3.0 ml. of eosine yellowish solution, and dilute to volume with chloroform. Mix and let stand 10 min.

Concomitantly pipet 3.0 ml. of reference standard into a 25-ml. volumetric flask and proceed as above, beginning with "...add 3.0 ml. of eosine yellowish solution..."

Nonalcoholic Liquids—Pipet an amount of sample equivalent to 2.0 mg. of atropine (or atropine plus hyoscyamine) into a separator. Continue as for solid dosage forms, beginning with "add 5 ml. of 0.1 *N* NaOH..."

Determination—Adjust the fluorometer to 80% transmission with the fluorescence reference standard. Transfer about 4 ml. of the sample solution to a clean 10 × 10-mm. cell and read. Use 3.0 ml. of eosine yellowish solution plus 22.0 ml. of chloroform as a blank.

Calculate as follows: $100 \times C \times (F_u/F_s) = G$, where C is the concentration of the reference standard in milligrams per milliliter (before taking 3-ml. aliquot), F_u and F_s are, respectively, the fluorescence at 552 mμ of sample and standard, each corrected for the blank, and G is the amount in milligrams per milliliter of atropine hyoscyamine in the sample taken.

RESULTS AND DISCUSSION

Table I shows recoveries of atropine sulfate and hyoscyamine hydrobromide standards from simulated preparations consisting of atropine sulfate or hyoscyamine hydrobromide combined with drug components usually found with these belladonna alkaloids in commercial dosage forms. Synthetic No. 1 was a liquid containing 0.510 mg./ml. of atropine sulfate, 1% benzyl alcohol, and water. Synthetic No. 2 was a powder composed of 1.25 mg. of hyoscyamine hydrobromide, 1.03 mg. of scopolamine hydrobromide, 50.4 mg. of phenobarbital, and about 0.5 g. of lactose. Recoveries from both synthetics were good.

The above procedure has been applied to the determination of atropine and/or hyoscyamine in tablets and injections including those containing scopolamine, barbiturates, methyl and propyl parabens, benzyl alcohol, and chlorobutanol. The analyses of some of these commercial products are given in Table II. The analyses show good agreement with the label declarations.

The fluorometric procedure described here permits the determination of small quantities of hyoscyamine and atropine in the presence of scopolamine and atroscine. Conventional methods of analyses do not distinguish between these two groups of alkaloids. The fluorometric determinative step is sensitive, which is an advantage, considering the small amounts of hyoscyamine and atropine usually found in pharmaceuticals. Also, it is superior to an UV

[3] Aminco-Bowman Spectrophotofluorometer with a xenon energy source and slit arrangement No. 3, or equivalent instrument.

spectrophotometric determinative step because of the low absorptivity of atropine and hyoscyamine, *e.g.*, *a* of atropine sulfate = 6.31 in 0.1 *N* H_2SO_4 (18).

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ACKNOWLEDGMENTS AND ADDRESSES

Received April 7, 1969 from the *Food and Drug Administration, Department of Health, Education, and Welfare, San Francisco, CA 94102*

Accepted for publication June 5, 1969.

TECHNICAL ARTICLES

Automated Assay of Single Tablets of Digoxin

JAMES W. MYRICK

Abstract □ An automated analytical system has been used to determine the amount of digoxin in single tablets at a level of 0.25 mg. per tablet. The active ingredient in an alcoholic solution was oxidized with periodate; after removal of the excess periodate with arsenite, reaction with 2-thiobarbituric acid at 75° produced a visible color which was recorded from a colorimeter. Interference by dextrose, which may be present in the tablet, was eliminated by a simultaneous determination at a different wavelength. Relative standard deviation of the method was 0.7% for powdered tablet samples and 1.0% for an authentic tablet formulation.

Keyphrases □ Digoxin tablets—analysis □ Automated procedure—digoxin, single tablets □ Diagram–automated analysis, digoxin □ Colorimetric analysis—spectrophotometer

Digoxin, one of the active ingredients isolated from *Digitalis lanata*, is frequently used in the treatment of congestive heart conditions to increase the force of contraction and to increase cardiac tone. Patients placed under digitalis medication undergo two phases of administration—the initial course for digitalization and the second for maintenance. Both of these phases require individualized supervision to secure proper results. Since the duration of action of digoxin is one-third to one-seventh as long as digitoxin, the dosage of digoxin needed to control the patient must be accurately quantitated. Because of this need for accuracy, the USP (1) has established a content uniformity requirement in its monograph for digoxin tablets.

Various colorimetric assays for digoxin have been reported. Alkaline picrate was used as reagent by Baljet (2), xanthydrol by Pesez (3), 3,5-dinitrobenzoic acid by Tattje (4), acetone-phosphoric acid by Dequeker and Loobuyck (5), ferric chloride, acetic acid, and sulfuric acid by James *et al.* (6), 2,4-dinitrodiphenylsulfone by Tattje (7), thiobarbituric acid by Mesnard and Devaux (8), and *m*-dinitrobenzene by Houk *et al.* (9). An automated method based on acid-induced fluorescence was proposed by Khoury (10) but it was not reproducible in our laboratory because of instability of the fluorometer. Literature on applications of automated analyses to pharmaceutical formulations are now fairly extensive and several compilations exist (11). A survey of the colorimetric analytical methods indicated a lack of sensitivity or suitability for automation