Colorimetric Determination of Physostigmine in Pharmaceuticals

S. K. WAHBA KHALIL and B. D. PATTERSON

Abstract □ A colorimetric method for determination of physostigmine is suggested based on measurement of the yellow color produced when physostigmine dithiocarbamate reacts with copper. The color obeys Beer's law in the range of 65–400 mcg. The reaction was found to be specific for physostigmine in the presence of other alkaloids and nitrogen-containing substances, using a pH 8.6 buffer and sodium bicarbonate.

Keyphrases □ Physostigmine, analysis—colorimetric measurement of physostigmine dithiocarbamate–copper complex □ Colorimetry —analysis, physostigmine

A number of methods have been proposed for the quantitative determination of physostigmine in solution. The majority of the older methods have not proved completely satisfactory, either because of a lack of specificity for physostigmine or because of a lack of sensitivity for microquantities of the alkaloid.

Teare and Borst (1) recently developed a gas chromatographic method using the trimethylsilyl derivative of physostigmine. This method requires the use of equipment not always readily available, but it does represent a procedure for accurate determination of small quantities of physostigmine. In 1968, Fletcher and Davis (2) developed a method based on measuring the intensity of the yellow color of a physostigmine nitroso compound.

An earlier procedure, of Teare and Taylor (3), is an indirect titration method involving measurement of methylamine both before and after alkaline hydrolysis. Thus, physostigmine can be determined in the presence of its degradation products. However, the possibility exists that other ingredients in a preparation may yield methylamine on alkaline hydrolysis to interfere with the determination.

This paper describes a procedure based on the formation of a copper dithiocarbamate complex which is quantitative for microquantities of physostigmine. The method is a modification of a basic method for determination of primary and secondary amines (4, 5, 6). The modification permits measurement of physostigmine in the presence of its degradation products and other chemicals commonly included with physostigmine in pharmaceutical products. This procedure was applied to several commercial preparations containing physostigmine.

EXPERIMENTAL

Reagents—The following were used (all of analytical grade): physostigmine salicylate; saturated sodium bicarbonate solution; barbiturate buffer, pH 8.6, or an alkaline borate buffer, pH 8.6; copper sulfate 5% solution; benzene; and carbon disulfide.

Procedure—To 1 ml. of an aqueous solution of physostigmine salicylate, 0.05%, in a test tube, add 2 ml. of the buffer solution, 1 ml. saturated sodium bicarbonate solution, 1 ml. copper sulfate 5% solution, and 5 ml. carbon disulfide–benzene mixture (1:3).

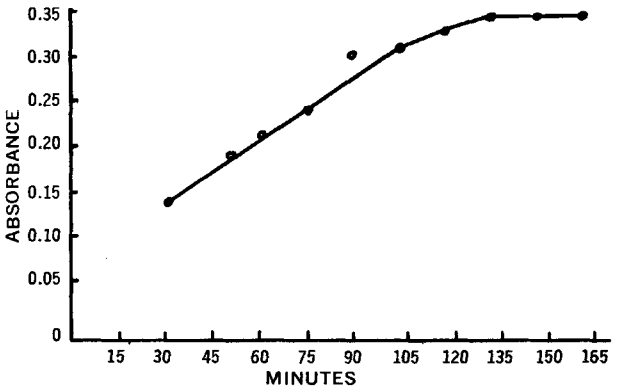

Figure 1—*Time for maximum color development.*

Shake the mixture periodically for 2.25 hr. Centrifuge the tube for 2 min. at 6000 r.p.m. to separate the two phases. Decant the organic layer immediately and measure the intensity of the yellow color using a Beckman DB spectrophotometer at 444 nm. against a blank, prepared under the same conditions using 1 ml. of water in place of the physostigmine solution.

To determine the amount of intact alkaloid present, the procedure must be run on two 1-ml. samples of physostigmine salicylate. In one sample the sodium bicarbonate solution is eliminated and 1 ml. of distilled water is added. In this case, the methylamine present due to decomposition of physostigmine reacts to give the copper dithiocarbamate complex while the intact physostigmine salicylate does not react. The difference in absorbance between the two sample tubes is a measure of intact physostigmine present in solution.

This procedure was also applied to solutions of other ingredients (atropine, pilocarpine, adrenaline, and benzethonium chloride) commonly included with physostigmine in commercial preparations at a variety of concentration levels. Finally, the procedure was applied to three commercial preparations to ascertain whether it is suitable as a possible assay procedure for physostigmine salicylate in commercial preparations. A comparison between the proposed method and Annino's method (7) was made.

RESULTS AND DISCUSSION

A standard curve was plotted using various concentrations of physostigmine salicylate solution. The color obeys Beer's law over a concentration range of 65–400 mcg. pure alkaloid and is stable indefinitely at room temperature in the organic layer after separation from the aqueous layer.

It was found, as shown in Fig. 1, that a period of 2.25 hr. was required to achieve maximum absorbance. However, the intensity of the color can be measured at any time after 15 min. if a standard is run concurrently using the same shaking conditions. Once the organic phase is separated from the aqueous phase, no further color development takes place and the color is stable for at least 24 hr.

This procedure is a modification of the copper dithiocarbamate method for detection of secondary amines. Although the previous

Table I—Recovery of Physostigmine Salicylate

Physostigmine, mcg./ml.	Physostigmine Found, mcg./ml.		Percent Recovery
100	99.7		99.7
200	201.8		100.9
300	301.2		100.4
400	398.6		99.6
		Average	100.2%

Table II—Comparison between Dithiocarbamate Method and Annino's Method (7).

Preparation	Dithiocarbamate Method: Labeled Amount, mg./ml.	Dithiocarbamate Method: Amount Found, mg./ml.	Dithiocarbamate Method: Percent Recovery	Annino's[a] Method, Percent Recovery
1. Eye drops containing physostigmine salicylate, methylcellulose, sodium bisulfite, sodium chloride, chlorobutanol, and citric acid	5	5.046	102.0	100.0
2. Injection containing physostigmine salicylate, sodium bisulfite, and benzyl alcohol	4	3.41	85.5	86.9
3. Injection containing physostigmine salicylate, l-hyoscyamine hydrobromide, benzyl alcohol, and sodium chloride	0.6	0.591	98.66	98.5

[a] Calculated as physostigmine.

reference (4) stated that tertiary aliphatic amines, aromatic amines, and secondary aliphatic–aromatic amines give a negative test, several alkaloids and other substances with primary, tertiary, and quaternary nitrogen groups were found that give a positive test, including atropine, pilocarpine, benzethonium chloride, urea, and some amino acids. Substitution of sodium bicarbonate for ammonium hydroxide in the procedure increased the specificity, eliminating positive reactions with all these chemicals except pilocarpine. The use of the pH 8.6 buffer further increased the specificity, with only physostigmine giving the color.

Different amounts of physostigmine were determined using this method. Recovery data are listed in Table I.

Three commercial preparations containing physostigmine were analyzed using this method. The results are listed in Table II.

REFERENCES

(1) F. W. Teare and S. I. Borst, *J. Pharm. Pharmacol.*, **21**, 277 (1969).

(2) G. Fletcher and D. J. G. Davis, *ibid.*, **20**, 108S(1968).

(3) F. W. Teare and D. W. Taylor, *ibid.*, **19**, 257(1967).

(4) F. Feigl, "Spot Tests in Organic Analysis," 6th ed., Elsevier, New York, N. Y., 1960, p. 274.

(5) G. R. Umbreit, *Anal. Chem.*, **33**, 1572(1961).

(6) B. M. Phillips, P. J. Kraus, and M. E. Stratmeyer, *J. Pharm. Sci.*, **54**, 803(1965).

(7) J. S. Annino, "Clinical Chemistry," 3rd ed., Little, Brown, Boston, Mass., 1964, p. 338.

ACKNOWLEDGMENTS AND ADDRESSES

Received October 12, 1970, from the *Department of Pharmacognosy, College of Pharmacy, North Dakota State University, Fargo, ND 58102*

Accepted for publication May 5, 1971.

The authors thank the following companies for supplying commercial preparations: Alcon Laboratories, Inc.; S. F. Durst & Co., Inc.; and Kremers-Urban Co.

Separation of Quingestanol Acetate from Sesame Oil Solution and Its Determination in Combinations with Ethinyl Estradiol or Quinestrol

MELVIN H. PENNER, DIMITRI C. TSILIFONIS, and LESTER CHAFETZ

Abstract □ Quingestanol acetate, norethindrone acetate 3-cyclopentyl enol ether, was not separable from its sesame oil dosage form vehicle as the intact compound using chromatography procedures that depend on differences in polarity. The steroid can be retained quantitatively on a Florisil column treated with a relatively high concentration of ammoniacal silver nitrate and then quantitatively eluted with ethanolic ammonium chloride. The progestin can be determined selectively in the presence of its products of solvolytic or oxidative degradation by UV spectrophotometry after treatment with sodium borohydride; the chromophore of the decomposition products is eliminated by this process while that of the intact compound is unaffected. Quinestrol or ethinyl estradiol, which may be coformulated with the progestin, does not interfere with its determination. Methods for the quantitative determination of the declared steroids in quingestanol acetate formulations and for the estimation of degradation products of the progestin are presented.

Keyphrases □ Quingestanol acetate capsules, combinations with ethinyl estradiol or quinestrol—separation, analysis □ Sesame oil formulations—separation of quingestanol acetate □ Column chromatography—separation, quingestanol acetate in capsules □ UV spectrophotometry—analysis, quingestanol acetate □ TLC—monitoring, quingestanol acetate separation

Quingestanol acetate (I), the cyclopentyl enol ether of norethindrone acetate (II), was shown to be a potent progestational and antiestrual agent (1, 2). As is typical of enol ethers, quingestanol acetate is sensitive to acid-catalyzed solvolysis. Both norethindrone acetate, the solvolysis product, and 6-hydroxynorethindrone acetate (III), formed by autoxidation, have been detected in aged samples of the drug and its dosage forms, but no other decomposition products have been observed. Optimum stability and activity of quingestanol acetate