ratio between the absorbance at 336 mμ in alkaline solution to that found in acid solution averaged 1.8.

SUMMARY

Pralidoxime chloride, commonly known as 2-PAM chloride, has been recommended for classification as standard type by the Medical Services, Department of Defense, for use in the therapy of nerve agent casualties. The item to be cataloged and issued consists of 5 Gm. of the oxime salt in a 20-ml. bottle with a rubber diaphragm closure; the addition of 13 ml. of sterile diluent gives a solution for parenteral use by medical personnel. The preparation of highly purified pralidoxime chloride, physical and chemical constants, the ultraviolet and infrared absorption spectra, tests for identification, and analytical procedures for the quantitative determination of the oxime in blood plasma, feces, and urine are given. A safety test, developed for the standard item to insure the absence of toxic impurities, and a procedure for determining the acute toxicity of pralidoxime chloride are also presented.

REFERENCES

(1) Kondritzer, A. A., *et al.*, *J. Pharm. Sci.*, **50**, 109(1961).
(2) Bloch, L. P., U. S. pat. 3,123,613 (March 3, 1964).
(3) Bliss, C. I., "The Statistics of Bioassay," Academic Press Inc., New York, N. Y., 1952.
(4) Finney, D. J., "Probit Analysis," 2nd ed., Cambridge University Press, London, England, 1952.
(5) O'Leary, J. F., *et al.*, *J. Pharmacol. Exptl. Therap.*, **132**, 50(1961).
(6) Lehman, R. A., and Nicholls, M. E., *Proc. Soc. Exptl. Biol. Med.*, **104**, 550(1960).
(7) Erdmann, V. W. D., and Englehard, H., *Arzneimittel-Forsch.*, **14**, (No. 1) 5(1964).
(8) Chemical Research and Development Laboratories, U. S. Army Edgewood Arsenal, Edgewood Arsenal, Md., unpublished data.
(9) Ellin, R. I., and Kondritzer, A. A., *Anal. Chem.*, **31**, 200(1959).

Analysis of Acetophenetidin in Dosage Forms by Nonaqueous Titration

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A simple nonaqueous titrimetric procedure is presented for the determination of acetophenetidin in powder, tablet, and capsule dosage forms. Titration is effected with perchloric acid in acetic acid–acetic anhydride using an acetic anhydride–chloroform–benzene mixture as the titration solvent. The end point is determined potentiometrically with a Fisher titrimeter equipped with a modified calomel–glass electrode system.

PHENACETIN tablets U.S.P. XVII (formerly acetophenetidin tablets U.S.P. XVI) are assayed (1) by a gravimetric procedure involving extraction with chloroform. The "British Pharmaceutical Codex" procedure (2) is similar to the U.S.P. method, while the "British Pharmacopoeia," 1953 (3), utilized ethanol as the extracting solvent. The method of the Association of Official Agricultural Chemists (4) involves iodination and gravimetric determination of the periodide or volumetric measurement of standard iodine solution consumed in the iodination. A variety of procedures have been proposed which are based on volumetric titration, colorimetry, and ultraviolet or infrared spectrophotometry. These have been applied to acetophenetidin and combinations of acetophenetidin with other drugs, including aspirin, caffeine, acetanilid, and aminopyrine. Connors (5) has reviewed the literature on this subject.

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Because of the weakly basic nature of acetophenetidin, application of nonaqueous titrimetry has been limited. Wollish *et al.* (6) titrated acetophenetidin after hydrolysis with hydrochloric acid to liberate the free amine, *p*-phenetidin, which was extracted with chloroform after making the solution alkaline. Titration was effected potentiometrically with perchloric acid in *p*-dioxane. A glass–calomel electrode system was employed.

The present study reports a simple nonaqueous titrimetric procedure by which acetophenetidin may be determined without preliminary treatment. The method is applied to dosage forms and combinations with other active constituents.

EXPERIMENTAL

Apparatus.—A Fisher titrimeter, model 35, was used for all titrations in this study. A conventional glass electrode and sleeve-type calomel electrode were equilibrated by immersing in acetic anhydride for 24 hr. before use. The calomel electrode was modified by replacing the aqueous bridge in the calomel cell with a 0.1 *M* solution of anhydrous lithium perchlorate in acetic anhydride as the supporting electrolyte as described by Wimer (7).

Reagents and Solutions.—Glacial acetic acid, acetic anhydride, chloroform, and benzene used in this investigation were A.C.S. reagent grade. Anhydrous lithium perchlorate and 72% double vacuum distilled perchloric acid were obtained from G. F. Smith Chemical Co., Columbus, Ohio. A solvent mixture consisting of 1 part acetic anhydride, 1 part chloroform, and 9 parts benzene (acetic anhydride–chloroform–benzene mixture) was prepared prior to use in titration. Acetophenetidin U.S.P. was dried at 60° for 2 hr. and used without further purification. A 0.1 *N* solution of perchloric acid in glacial acetic acid was prepared and standardized potentiometrically against primary standard potassium acid phthalate dissolved in glacial acetic acid. A 0.1 *N* solution of perchloric acid in an acetic acid–acetic anhydride mixture was prepared by diluting 8.5 ml. of 72% perchloric acid to 500 ml. with glacial acetic acid and diluting this to 1 L. with acetic anhydride. The solution was allowed to stand 24 hr. prior to standardization against potassium acid phthalate dissolved in glacial acetic acid

Method A.—Approximately 1 meq. of acetophenetidin, accurately weighed, was dissolved in 50 ml. of acetic anhydride with the aid of magnetic stirring. The solution was titrated potentiometrically with 0.1 *N* perchloric acid in glacial acetic acid. The end point was determined from the inflection of the curve obtained by plotting mv. *versus* ml. of titrant added. During the titration process, the titration beaker was covered with a rubber plate having holes for the passage of the electrodes and the buret tip. A series of ten samples was run by *Method A*. The data are reported in Table I. A typical titration curve is shown in Fig. 1.

Method B.—Approximately 1 meq. of acetophenetidin, accurately weighed, was dissolved in 50 ml. of acetic anhydride–chloroform–benzene solvent mixture. The solution was titrated potentiometrically with 0.1 *N* perchloric acid in

TABLE I.—DETERMINATION OF ACETOPHENETIDIN

Method A		*Method B*	
Amt. Weighed, Gm.	Recovery, %	Amt. Weighed, Gm.	Recovery, %
0.1784	102.00	0.1786	99.70
0.1797	101.40	0.1798	100.32
0.1803	100.90	0.1809	100.37
0.1778	100.22	0.1790	100.08
0.1800	99.01	0.1805	100.10
0.1779	100.18	0.1812	100.18
0.1817	101.08	0.1799	100.43
0.1810	99.44	0.1810	99.56
0.1743	101.01	0.1809	100.90
0.1800	99.01	0.1817	99.74
	Av. 100.43		Av. 100.14
	S.D. ±1.03		S.D. ±0.40

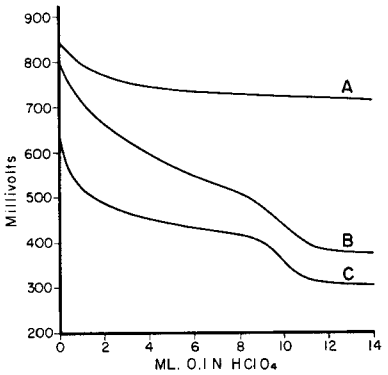

Fig. 1.—Typical titration curves for acetophenetidin. Key: A, 0.1 *N* $HClO_4$ in glacial acetic acid as titrant with glacial acetic acid as solvent; B, 0.1 *N* $HClO_4$ in glacial acetic acid as titrant with acetic anhydride as solvent; C, 0.1 *N* $HClO_4$ in acetic acid–acetic anhydride as titrant and acetic anhydride–chloroform–benzene mixture as solvent.

TABLE II.—ANALYSIS OF DOSAGE FORMS CONTAINING ACETOPHENETIDIN

Dosage Form	Active Ingredients Labeled Amt.[a]	Recovery, %
Tablet	Phenacetin, 5 gr. (324 mg.)	99.88 ± 1.08[b]
Tablet	Phenacetin, 2.5 gr. (162 mg.) Salol, 2.5 gr. (162 mg.)	103.51 ± 0.54
Tablet	Phenacetin, 2.5 gr. (162 mg.) Aspirin, 3.5 gr. (226 mg.) Phenobarbital, 1/4 gr. (16 mg.)	100.06 ± 0.12
Green tablet	Phenacetin, 2.5 gr. (162 mg.) Aspirin, 2.5 gr. (162 mg.) *d*-Amphetamine sulfate, 2.5 mg.	102.22 ± 1.06
Tablet	Phenacetin, 3 gr. (194 mg.) Aspirin, 2.5 gr. (162 mg.) Phenobarbital, 1/4 gr. (16 mg.) Hyoscyamine sulfate, 0.031 mg.	103.43 ± 1.43
Yellow tablet	Phenacetin, 2.5 gr. (162 mg.) Aspirin, 2.5 gr. (162 mg.) Amobarbital, 0.5 gr. (32 mg.) *d*-Amphetamine sulfate, 5 mg.	103.16 ± 2.19
Capsule	Phenacetin, 3 gr. (194 mg.) Aspirin, 2.5 gr. (162 mg.) Phenobarbital, 1/4 gr. (16 mg.) Hyoscyamine sulfate, 0.031 mg.	104.19 ± 1.28
Gray capsule	Phenacetin, 2.5 gr. (162 mg.) Aspirin, 3 gr. (194 mg.) Mephentermine sulfate, 7.5 mg. Promethazine HCl, 6.25 mg.	101.33 ± 0.60

[a] Per unit dosage form. [b] Standard deviation is based on at least 5 determinations.

acetic acid–acetic anhydride. The end point was determined as in *Method A*. Results for the analysis of a series of acetophenetidin samples are shown in Table I, and a typical titration curve is shown in Fig. 1.

Analysis of Dosage Forms.—*Method B* was applied to the determination of a number of dosage forms containing acetophenetidin in combination with other active ingredients. Twenty tablets were weighed and powdered. In the case of capsules, 20 capsules were emptied as completely as possible. An amount of the powder mass equivalent to about 1 meq. was accurately weighed and transferred to a 150-ml. beaker. The acetophenetidin in the sample was dissolved by stirring magnetically in 75 ml. of acetic anhydride–chloroform–benzene solvent mixture for at least 15 min. The sample was then titrated potentiometrically with 0.1 *N* perchloric acid in acetic acid–acetic anhydride solution. The acetophenetidin content was determined as described previously. The per cent recoveries are reported in Table II.

RESULTS AND DISCUSSION

The weakly basic nature of acetophenetidin presents difficulties in the selection of a suitable solvent system for a successful nonaqueous titration procedure. The method proposed by Wollish *et al.* (6) involves extensive pretreatment prior to nonaqueous titration. It was the objective of this study to develop a simple direct nonaqueous titration procedure applicable to acetophenetidin and its dosage forms.

Wimer (7) used acetic anhydride as a solvent for titrating a variety of amides and acylated amines. The titrant was perchloric acid in glacial acetic acid or dioxane and was shown to exhibit greater acidic behavior in acetic anhydride than in acetic acid. The active acid species appears to be the acetyl ion, CH_3CO^+, rather than the less acidic solvated proton. Wimer describes the titration as the reaction of acetyl perchlorate with an amide to form a salt, the success of the titration depending mainly on the extent of salt formation. End points were determined potentiometrically using a glass electrode and a modified sleeve-type calomel electrode system. This electrode system was employed in the present study.

Acetic anhydride as a nonaqueous titration medium, alone or in combination with other solvents, has proved useful for the determination of compounds which are too weakly basic in glacial acetic acid. Streuli (8) titrated such weak bases as acetanilid and caffeine in acetic anhydride using perchloric acid in acetic acid–acetic anhydride (1:1) as the titrant. Cowell and Selby (9) used a similar system for the determination of semicarbazones and phenylhydrazones. Fritz and Fulda (10) determined nicotinamide and xanthine derivatives in nitromethane–acetic anhydride (4:1) using perchloric acid in acetic acid as the titrant. Gremillion (11) titrated a number of weak organic bases with perchloric acid in acetic acid or acetic anhydride using acetic anhydride as the titration medium.

In preliminary studies a number of solvents in combination with acetic anhydride were tested as titration media for the analysis of dosage forms containing acetophenetidin. These included benzene, carbon tetrachloride, nitrobenzene, dioxane, chloroform, and acetonitrile. Of the systems tested, a mixture of acetic anhydride, chloroform, and benzene (1:1:9) was found to yield the most satisfactory end point in the titration of acetophenetidin. Typical titration curves are illustrated in Fig. 1. In acetic acid (curve A) no detectable inflection is apparent, although this solvent is an extremely useful one for the titration of stronger bases such as amines. With acetic anhydride as the solvent (curve B), a satisfactory end point was obtained. End point detection was further improved with acetic anhydride–chloroform–benzene solvent mixture as the titration solvent and perchloric acid in acetic acid–acetic anhydride as the titrant. This is illustrated by curve C. The inclusion of an excess of solvent having a low dielectric constant such as benzene and chloroform increases measurably the sensitivity of titrations in acetic anhydride.

A series of ten samples of acetophenetidin was analyzed in acetic anhydride (*Method A*) and in acetic anhydride–chloroform–benzene mixture (*Method B*). The data reported in Table I indicate better precision was realized with *Method B*.

Commercially available tablets and capsules of varying composition were analyzed for acetophenetidin content by *Method B* (Table II). The advantage of the proposed procedure is that preliminary treatment of the sample is not required. An aliquot of the powder mass is titrated directly after sufficient time is allowed for solution of the acetophenetidin. Commonly used excipients, coloring agents, insoluble fillers, diluents, and lubricants do not interfere. Weak bases comparable in strength to acetophenetidin will interfere, while acidic components and most salts will not. Bases which differ significantly in pKa from acetophenetidin should be differentially titratable. The last five dosage forms listed in Table II contain salts of organic bases in addition to acetophenetidin. Titration of synthetic mixtures indicated that these salts did not interfere with the determination of acetophenetidin.

REFERENCES

(1) "United States Pharmacopeia," 17th rev., Mack Publishing Co., Easton, Pa., 1965, p. 452.
(2) "British Pharmaceutical Codex," The Pharmaceutical Press, London, England, 1963, p. 1253.
(3) "British Pharmacopoeia," The Pharmaceutical Press, London, England, 1953, p. 412.
(4) "Official Methods of Analysis," 9th ed., Association of Official Agricultural Chemists, Washington, D. C., 1960, p. 481.
(5) Connors, K. A., "Pharmaceutical Analysis," Interscience Publishers, Inc., New York, N. Y., 1961, p. 550.
(6) Wollish, E. G., *et al.*, *Anal. Chem.*, **26**, 1753(1954).
(7) Wimer, D. C., *ibid.*, **30**, 77(1958).
(8) Streuli, C. A., *ibid.*, **30**, 997(1958).
(9) Cowell, D. B., and Selby, B. D., *Analyst*, **88**, 974 (1963).
(10) Fritz, J. S., and Fulda, M. O., *Anal. Chem.*, **25**, 1837(1953).
(11) Gremillion, A. F., *ibid.*, **27**, 133(1955).