Analysis of Acetaminophen and Barbiturate Combinations by Differentiating Nonaqueous Titration

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Abstract □ The difference in pKa values for acetaminophen and the barbituric acids permits differentiation of mixtures containing these drugs. Ion-exchange chromatography is used to separate acetaminophen and barbiturate from other active and inert constituents. Titrations are performed nonaqueously in dimethylformamide using tetrabutylammonium hydroxide as the titrant and a titrimeter equipped with a calomel and platinum electrode system.

Keyphrases □ Acetaminophen-barbiturate in combination—analysis by differentiating nonaqueous titration □ Barbiturate-acetaminophen in combination—analysis by differentiating nonaqueous titration □ Titrimetry, differentiating nonaqueous—analysis of acetaminophen-barbiturate in combination

Acetaminophen is a frequently used analgesic-antipyretic, alone as well as in combinations with other similar drugs in liquid and solid dosage forms. A number of studies have dealt with procedures for the analysis of the acetaminophen content in these preparations. These were referred to in an earlier paper (1), which described a nonaqueous titration procedure for dosage forms containing acetaminophen. Tablets were assayed by dissolving an aliquot of the powdered tablet mass in dimethylformamide and titrating potentiometrically with 0.1 *N* sodium methoxide. Additives and excipients in the formulation apparently did not interfere with the analysis. A drops dosage form was assayed similarly after evaporation of the solvent. An elixir dosage form was analyzed by extracting the acetaminophen from an aliquot by passage through a strong anion-exchange resin column. The weakly acidic acetaminophen was removed by the resin, while all nonionic components were washed from the column. Elution of the acetaminophen was effected with a solution of acetic acid in alcohol. After evaporation of the solvent, the residue was analyzed in the same manner as the tablets.

A previous report (2) described a differentiating titration procedure for acetaminophen and aspirin combinations. An aliquot of the powdered tablet mass was titrated potentiometrically in dimethylformamide with triethyl-*n*-butylammonium hydroxide. Preliminary separation of the components was not necessary.

More recently, a nonaqueous titration procedure for mixtures containing acetaminophen and salicylamide was described (3). Dimethylformamide was the titration solvent, and tetrabutylammonium hydroxide was the titrant. Titrations were performed potentiometrically, and the method was applied to synthetic mixtures and an assortment of commercially available complex solid dosage forms. Preliminary extraction of the active components was unnecessary.

The present report describes procedures for analyzing acetaminophen and barbiturate combinations. The difference in pKa values between acetaminophen, 9.92 (4), and barbiturates (phenobarbital), 7.42 (5), is sufficiently large to permit a satisfactory differentiating titration by the method described for the other combinations. The method is applied to several complex dosage forms.

EXPERIMENTAL

Apparatus—Titrations were performed potentiometrically with a titrimeter[1] equipped with a calomel and platinum electrode system. A 50-ml buret (1 cm i.d.) served as a chromatographic column. It contained a glass wool plug at the base to support the resin column.

Reagents and Chemicals—A strong anion-exchange resin[2] (200–400 mesh), acetaminophen, phenobarbital, and dimethylformamide were obtained from commercial sources and were the best quality available. All dosage forms were obtained commercially. All other chemicals and solvents employed in this study were reagent grade, and they were used without further purification.

Tetrabutylammonium hydroxide, 0.1 *N*, in benzene–methanol was prepared according to Cundiff and Markunas (6). The solution was standardized by dissolving 1 mEq of benzoic acid in 30 ml of dimethylformamide and titrating potentiometrically. The solution was restandardized at least weekly during the study.

Differentiating Titration of Synthetic Mixtures—Synthetic mixtures of acetaminophen and phenobarbital were prepared by weighing about equal amounts (50–200 mg) of each component into a 150-ml beaker and dissolving the mixture in 25 ml of dimethylformamide with the aid of magnetic stirring. The solution was titrated potentiometrically with 0.1 *N* tetrabutylammonium hydroxide.

Analysis of Acetaminophen–Barbiturate Dosage Forms—*Preparation of Column*—A strong anion-exchange resin column was prepared as described earlier (7).

Chromatography of Synthetic Mixtures—Synthetic mixtures containing approximately equal quantities (60–100 mg) of acetaminophen and phenobarbital were dissolved in 25 ml of 50% ethanol with the aid of magnetic stirring. The solution was transferred quantitatively to the resin column. The flow rate was adjusted to 1 ml/min. When the level of the solution for analysis reached the surface of the resin, three 20-ml portions of distilled water were added to the column. The washings were discarded and the resin column was then eluted with three 20-ml portions of

[1] Fisher, model 35, or Sargent-Welch recording titrator.
[2] Dowex 1-X8 (or equivalent).

Table I—Analysis of Acetaminophen and Phenobarbital, Alone and in Synthetic Mixtures

Run	Acetaminophen Recovery, %	Phenobarbital Recovery, %
1	101.1 ± 1.32[a]	—
2	—	101.1 ± 0.5
3	101.0 ± 0.7	102.4 ± 1.0
4[b]	97.1 ± 0.5	—
5[b]	—	101.7 ± 1.1
6[b]	97.8 ± 1.6	98.0 ± 0.5

[a] Standard deviation based on at least five determinations. [b] Runs 4, 5, and 6 were subjected to the ion-exchange chromatographic step prior to analysis.

50% acetic acid in ethanol. The eluate was collected in a 150-ml beaker and evaporated to dryness with the aid of a jet of clean air directed over the surface of the liquid. The residue was dissolved in 50 ml of dimethylformamide and titrated potentiometrically with 0.1 *N* tetrabutylammonium hydroxide.

Chromatography of Dosage Forms—Two tablet dosage forms and one capsule dosage form were analyzed by the described procedure. Twenty tablets were powdered or the contents of 20 capsules were removed as completely as possible, and the powder mass was weighed.

An aliquot of the tablet or powder mass containing about 150 mg of acetaminophen was transferred to a beaker containing 25 ml of 50% ethanol. The mixture was stirred magnetically for 10 min and was then transferred quantitatively to the resin column, using several 10-ml aliquots of 50% ethanol to aid in the transfer. The column was washed with three 20-ml portions of distilled water and was then eluted with 50% acetic acid in ethanol as described previously. The residue obtained from the evaporation of the eluate was titrated as described.

Since the ratio of acetaminophen to phenobarbital may range from 10:1 to as high as 30:1, it was necessary to analyze for the individual components in two aliquots: one containing the equivalent of about 150 mg of acetaminophen and the other of at least 80 mg of barbiturate.

RESULTS AND DISCUSSION

Earlier reports from this laboratory were concerned with the nonaqueous differentiating titration of mixtures of weak acids as synthetic combinations and as components in complex dosage forms. These included salicylic acid and benzoic acid (8), aspirin and barbiturates (9, 10), acetaminophen and aspirin (2), and acetaminophen and salicylamide (3). For each combination, the difference in pKa values was sufficiently large to permit satisfactory differentiating titrations in a nonaqueous solvent.

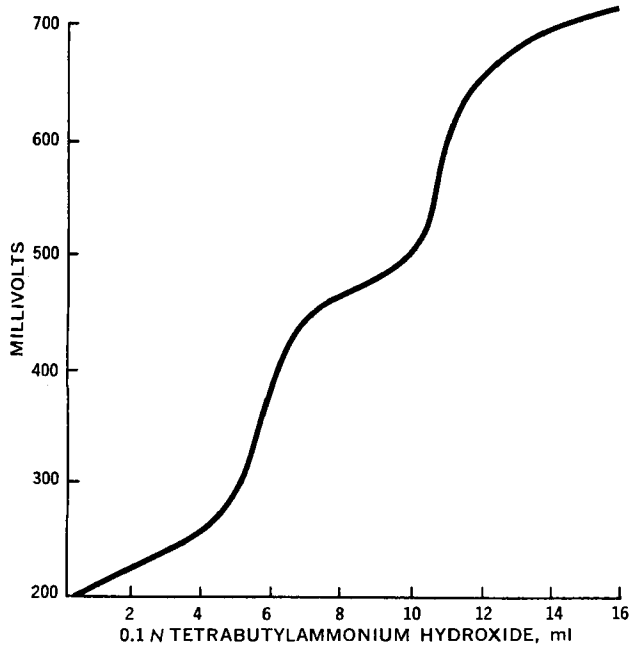

Figure 1—*A typical titration curve for a mixture containing acetaminophen and phenobarbital.*

Table II—Analysis of Dosage Forms Containing Acetaminophen and a Barbiturate by Differentiating Nonaqueous Titration

Dosage Form	Labeled Composition, mg/unit	Recovery, %
Tablet A	Phenobarbital, 16 Acetaminophen, 162 Phenacetin, 162 Atropine sulfate, 0.00065 Scopolamine hydrobromide, 0.0011 Hyoscyamine hydrobromide, 0.0325	102.5 ± 1.0 101.0 ± 0.2
Tablet B	Allobarbital, 15 Acetaminophen, 300	102.7 ± 0.9 103.2 ± 1.1
Capsule A	Sodium butabarbital, 10 Acetaminophen, 300 Mephenesin, 200	101.6 ± 0.8 103.6 ± 0.8

Acetaminophen and barbiturates are typical weak acids frequently used in analgesic-antipyretic preparations that very often contain other therapeutic agents. The difference in pKa values permits the differentiation by nonaqueous titration. Data are reported in Table I for the analysis of the individual components as well as for synthetic mixtures. A typical titration curve for the synthetic mixture is shown in Fig. 1. The first end-point corresponds to phenobarbital, and the second end-point is due to acetaminophen.

Since the presence of other components, active or inert, in complex dosage forms may interfere with the differentiating titration, an ion-exchange chromatographic procedure was developed for the separation of acetaminophen and the barbiturate from the other active and inert constituents. Solutions of acetaminophen and phenobarbital, individually and as mixtures, in 50% alcohol were subjected to ion-exchange chromatography. The strong anion-exchange resin removes all anions from solution, while all cations and soluble nonionic components appear in the eluate. The eluent, 50% acetic acid in alcohol, displaces the acetaminophen and phenobarbital from the column since the acetic acid is a stronger acid than either component. Other anions such as chloride and sulfate, which represent acids stronger than acetic acid, will not be displaced. The eluate, after removal of the solvent, is titrated nonaqueously as described in the *Experimental* section. Recovery data are shown in Table I. This procedure was then applied to one capsule and two tablet dosage forms.

For Tablet A (Table II), the bromide ion, sulfate ion, and any excipient anions are retained by the resin along with the acetaminophen and phenobarbital. The phenacetin, atropine, scopolamine, hyoscyamine, and any excipient cations and nonionic components appear in the eluate. Elution with acetic acid removes the acetaminophen and phenobarbital. In the case of Tablet B, allobarbital and acetaminophen are readily separated from the dosage form in the manner described. For Capsule A, the butabarbital anion and acetaminophen are retained by the column; the sodium cation appears in the eluate. Mephenesin, which is nonionic, also appears in the eluate. Because of the large ratio of acetaminophen to barbiturate in the dosage forms, it was necessary to use separate aliquots of the powder mass for analysis of each component. A sample size was taken that would require 3–10 ml of titrant for each analysis. For the barbiturate determination, titrations were carried out only to the first end-point; for acetaminophen, the difference between the first and second end-points was used for calculating recovery data.

REFERENCES

(1) J. Hunt, H. J. Rhodes, and M. I. Blake, *Can. J. Pharm. Sci.*, 6, 20(1971).

(2) M. I. Walash, S. P. Agarwal, and M. I. Blake, *ibid.*, 7, 115(1972).

(3) M. I. Blake and L. B. Shumaker, *J. Ass. Offic. Anal. Chem.*, **56**, 653(1973).
(4) G. B. Barlin and D. D. Perrin, *Quart. Rev.*, **20**, 75(1966).
(5) T. D. Edmonson and J. E. Goyan, *J. Amer. Pharm. Ass., Sci. Ed.*, **47**, 810(1958).
(6) R. H. Cundiff and P. C. Markunas, *Anal. Chem.*, **28**, 792(1956).
(7) M. I. Blake and D. A. Nona, *J. Pharm. Sci.*, **53**, 570(1964).
(8) M. I. Blake, *J. Amer. Pharm. Ass., Sci. Ed.*, **46**, 287(1957).
(9) S. L. Lin and M. I. Blake, *J. Pharm. Sci.*, **55**, 781(1966).
(10) *Ibid.*, **56**, 43(1967).

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Analysis of Acetaminophen, Phenylephrine Hydrochloride, Diphenhydramine Hydrochloride, and Ascorbic Acid in a Capsule Preparation

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Abstract □ A column chromatographic procedure was developed for separating acetaminophen, phenylephrine hydrochloride, and diphenhydramine hydrochloride in combination with ascorbic acid. While acetaminophen, which passes through an alginic acid column with the washings, is determined spectrophotometrically at 257 nm, phenylephrine hydrochloride and diphenhydramine hydrochloride are both eluted from the column with 0.01 *N* hydrochloric acid and determined simultaneously at two different wavelengths. Ascorbic acid is analyzed by a visual titration method, employing 2,6-dichloroindophenol as the titrant.

Keyphrases □ Acetaminophen—method for separation and analysis in combination with phenylephrine hydrochloride, diphenhydramine hydrochloride, and ascorbic acid □ Phenylephrine hydrochloride—method for separation and analysis in combination with acetaminophen, diphenhydramine hydrochloride, and ascorbic acid □ Diphenhydramine hydrochloride—method for separation and analysis in combination with acetaminophen, phenylephrine hydrochloride, and ascorbic acid □ Ascorbic acid—method for separation and analysis in combination with acetaminophen, phenylephrine hydrochloride, and diphenhydramine hydrochloride

Acetaminophen, phenylephrine hydrochloride, diphenhydramine hydrochloride, and ascorbic acid are used alone and in combination with other drugs in various pharmaceutical preparations. Several methods are available (1–7) for the analysis of these therapeutically active ingredients as pure substances and in combination. Smith (8) recently described a method for the separation and determination of sympathomimetic amines, antihistamines, and phenothiazines in various mixtures using polystyrene cation and quaternary ammonium anion resins packed into two assembled columns. Although this method might have served as the basis for the present study, a previously reported analytical procedure (9), which employed an alginic acid column with UV spectrophotometry and which was adequate for the separation and determination of acetaminophen, phenylephrine hydrochloride, codeine phosphate, and pyrilamine maleate in tablets or powder, was chosen. Therefore, the present report concerns the feasibility of utilizing alginic acid (10) for cation exchange in the analysis of pharmaceutical products. This investigation found that acetaminophen and ascorbic acid were not retained by the alginic acid but passed through the prepared column; phenylephrine hydrochloride and diphenhydramine hydrochloride were both eluted with 0.01 *N* hydrochloric acid and were simultaneously determined at 236 and 273 nm. Finally, ascorbic acid was analyzed by a visual titration method in an aliquot of the original solution using 2,6-dichloroindophenol.

Table I—Recovery of Phenylephrine Hydrochloride and Diphenhydramine Hydrochloride Mixed Standard in 50% Ethanol in 0.5% (v/v) Acetic Acid

	Phenylephrine Hydrochloride		Diphenhydramine Hydrochloride	
Mixture	Amount Taken, mg	Recovery, %	Amount Taken, mg	Recovery, %
1	7.5	98.3	15.0	98.3
2	7.5	101.8	15.0	99.5
3	15.0	99.4	30.0	101.4
4	15.0	99.1	30.0	98.2
5	7.5	98.7	15.0	98.3
6	15.0	100.5	30.0	101.2
7	7.5	101.3	15.0	100.7
8	7.5	101.4	15.0	98.6
9	15.0	99.6	30.0	98.3
10	7.5	99.4	15.0	98.8
Average, %	—	99.9	—	99.8
SD, %		±1.23		±1.29

EXPERIMENTAL

Apparatus—A recording spectrophotometer[1] with matched UV

[1] Beckman DB.