Determination of Aminophylline and Phenobarbital Combinations

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Procedures are presented for the analysis of theophylline (or aminophylline) and phenobarbital in various dosage forms by ion exchange chromatography and nonaqueous titration. Separation is effected on a column of Dowex 2-X8, a strong anion exchange resin. Theophylline and phenobarbital are retained by the column, while the ethylenediamine passes through with the water wash. Theophylline is eluted with 2 *M* HCl, and the phenobarbital is eluted with 50 per cent acetic acid in ethanol. Ethylenediamine is determined with standard hydrochloric acid; the theophylline and phenobarbital are determined nonaqueously with sodium methoxide in benzene–methanol.

A VARIETY OF techniques have been proposed for the analysis of theophylline (or aminophylline) and phenobarbital when used separately. A review of these methods is presented by Connors (1) for both theophylline and phenobarbital. The N.F. XI assay (2) for theophylline in theophylline sodium acetate and the U.S.P. XVI assay (3) for theophylline in aminophylline are both argentometric procedures. These methods are based on a procedure developed by Stevens and Wilson (4). The U.S.P. (5) recognizes a gravimetric method for the determination of phenobarbital in the elixir which involves chloroform extraction of the phenobarbital from the vehicle. Mattocks and Voshall (6) noted that this assay repeatedly yielded high results.

Although theophylline and phenobarbital combinations enjoy widespread usage, relatively few procedures have been proposed for the determination of this combination in a single dosage form. Bhattacharya and Banerjee (7) proposed a procedure which is essentially a combination of the official assays for the two individual compounds. In their method an excess of silver nitrate solution is added to an ammoniacal solution of the theophylline and phenobarbital; the solution is filtered. The barbiturate is extracted from the filtrate, dried, and weighed. The theophylline is determined by titrating the excess silver ion in the filtrate with standard thiocyanate, or the precipitate is dissolved in nitric acid and titrated with standard thiocyanate.

A volumetric procedure, which was conducted using ethanol or water as the solvent, was developed by Bartilucci and Discher (8). The titrant was aqueous sodium hydroxide, and titration was effected potentiometrically. A mixture of theophylline and phenobarbital or aminophylline and phenobarbital produced differential titration curves. The results indicated that phenobarbital and theophylline could be determined in this manner with relatively good accuracy, but that when aminophylline and phenobarbital mixture was titrated, the results were not entirely satisfactory.

Because of the accuracy attainable with spectrophotometry and the ability to determine individual components in a mixture when conditions are favorable, this technique lends itself to the determination of complex dosage forms such as theophylline and phenobarbital. It is usually necessary to carry out some preparative steps, such as solvent extraction, before the absorbance can be measured. Helgren (9) determined all three components of a dosage form containing theophylline, pentobarbital, and papaverine in this manner. The pentobarbital was extracted and determined separately, whereas the papaverine and theophylline were determined simultaneously. Yokoyama and Pernarowski (10) determined aminophylline and phenobarbital in the same solution. They employed the absorbance ratios in the analysis and were able to determine the concentration of the theophylline and phenobarbital with great accuracy. Comer and Bourne (11) proposed a method for the determination of a capsule containing aminophylline, amobarbital, and ephedrine. The aminophylline was determined by ultraviolet spectrophotometry after acidification and extraction with an organic solvent. The amobarbital was extracted from acid solution and ephedrine from alkaline solution. These two compounds were then determined by infrared spectrophotometry. An ultraviolet spectrophotometric procedure for aminophylline and phenobarbital mixtures was reported by Hyatt (12). The components were separated by extraction prior to determining the absorptivity.

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The present report describes procedures for determining theophylline (or aminophylline) and phenobarbital in various dosage forms by ion exchange chromatography and nonaqueous titration. When aminophylline is present, the ethylenediamine portion of the molecule is also determined. A modification of the general procedure is presented that eliminates interference by monobasic potassium phosphate which may be included in tablet dosage forms.

EXPERIMENTAL

Preparation of Ion Exchange Column.—A chromatographic column 60 cm. in length and 2.1-cm. i.d. was used. The column was fitted with a stopcock at its lower end. A plug of glass wool was inserted into the base of the column to support the resin. The strongly basic anion exchange resin, Dowex 2-X8, 50–100 mesh, was used in this investigation. It was prepared by suspending about 80 Gm. of the resin in distilled water and adding this as a slurry to the column. After the resin settled completely, the column was washed with 150 ml. of 2 *M* hydrochloric acid, 300 ml. of distilled water, 150 ml. of 5% sodium hydroxide solution, and finally with distilled water until the eluate was neutral. The acid wash was employed only when fresh resin was used. A solvent layer always was maintained above the resin.

General Assay Procedure.—A sample containing 100 mg. of aminophylline and 100 mg. of phenobarbital, accurately weighed, was dissolved in 50 ml. of 30% ethanol and transferred to the resin column, previously washed with 30% ethanol. When the sample solution just passed below the surface of the resin, 50 ml. of 30% ethanol was added to the column, followed by 250 ml. of distilled water. The flow rate of the eluate was maintained at 2 ml. per minute. The first 25 ml. of eluate was discarded. Approximately 150 ml. in the next fraction was collected in a conical flask. This fraction, containing the ethylenediamine, was evaporated by aeration to a volume of about 50 ml. The solution was then titrated with 0.1 *N* hydrochloric acid using methyl orange as the indicator. The column was next eluted with 2 *M* hydrochloric acid. About 110 ml. of eluate was collected at a flow rate of 2 ml. per minute. This fraction contained the theophylline as the hydrochloride salt. The solution was evaporated to dryness on a steam plate, and the residue was analyzed by nonaqueous titration by the procedure described below. The column was then washed with distilled water at full flow rate. About 30 ml. of eluate was collected; this solution was evaporated to dryness. The absence of a residue indicated that all theophylline had been removed from the column, and the phenobarbital was retained by it. The column was next eluted with 50% acetic acid in ethanol at a flow rate of about 2 ml. per minute. A volume of 150 ml. of eluate was collected and evaporated to dryness on a steam plate. The residue was analyzed for phenobarbital content by nonaqueous titration.

The theophylline or phenobarbital residue was dissolved in 50 ml. of dimethylformamide in a 100-ml. beaker. The solution, magnetically stirred, was titrated potentiometrically with 0.1 *N* sodium methoxide in benzene–methanol, prepared and standardized as described earlier (13). A Fisher titrimeter, equipped with a calomel and glass electrode system, was used for the potentiometric measurements.

Titration was also effected visually by adding 3 drops of azo violet indicator (saturated solution in benzene) to the titration beaker. The proper indicator color change was noted by using indicator solution in conjunction with a potentiometric titration. The color change corresponding to the graphic end point was from red to blue.

Modification in General Procedure.—For several of the dosage forms studied here, it was necessary to modify the general procedure. This usually involved simply a change in sample solvent. Theophylline and phenobarbital mixture was dissolved in 50% ethanol. The sample solution was added to the column, previously washed with 50% ethanol. The column was then washed with 50 ml. of 50% ethanol and 250 ml. of distilled water. From this point, the general procedure was followed. Since this dosage form contained theophylline, no ethylenediamine fraction was collected.

Aminophylline powder (100 mg.) was dissolved in 50 ml. of distilled water; the solution was transferred to the resin column. The column was washed with 250 ml. of distilled water. The general procedure was then followed. Commercially available aminophylline injection was analyzed by transferring 10 ml. (containing 250 mg. of aminophylline) with a pipet to the resin column. The column was washed with 250 ml. of distilled water, and the general procedure was applied for the elution and determination of ethylenediamine and theophylline.

Aminophylline and phenobarbital tablets were analyzed by weighing 20 tablets and reducing them to a fine powder in a mortar. A sample of the powder equivalent to approximately two tablets (200 mg. of theophylline and 60 mg. of phenobarbital) was weighed and placed in a 125-ml. conical flask. A volume of 75 ml. of 95% ethanol was added to the flask. The theophylline and phenobarbital were extracted on a mechanical shaker for 12–15 hours. The solution was then filtered, and the filtrate was transferred to the resin column. The column was then washed with 50 ml. of 95% ethanol and 250 ml. of distilled water. The theophylline and phenobarbital were eluted and determined by the general procedure.

DISCUSSION

The proposed procedure for determining aminophylline and phenobarbital is simple, accurate, and applicable to dosage forms. Theophylline and phenobarbital, weak acids, are adsorbed on the

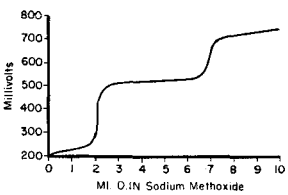

Fig. 1.—Titration curve of theophylline in the presence of residual hydrochloric acid.

TABLE I.—ANALYSIS OF THEOPHYLLINE AND PHENOBARBITAL COMBINATIONS

Sample	Theoretical or Labeled Amt., mg.	Compn.	Recovery, %	Official Requirement, %	Method
Theophylline	100		99.0 ± 1.3[c]		Proposed
Phenobarbital	100		102.6 ± 1.6		
Aminophylline	200	Theophylline	77.8 ± 1.0	78–83.5	Proposed
		Ethylenediamine	12.3 ± 0.4	12.8–14.1	
Aminophylline	250	Theophylline	78.0[d]	78–83.5	U.S.P.
		Ethylenediamine	13.8	12.8–14.1	
Aminophylline	100	Theophylline	80.5 ± 1.0	78–83.5	Proposed
Phenobarbital	100	Ethylenediamine	12.8 ± 0.5	12.8–14.1	
		Phenobarbital	97.3 ± 1.7		
Aminophylline injection	250[a]	Theophylline	90.3 ± 1.0	93–107[e]	Proposed
		Ethylenediamine	17.0 ± 0.6		
Aminophylline injection	250[a]	Theophylline	85.8[d]	93–107[e]	U.S.P.
		Ethylenediamine	15.2		
Aminophylline	100[b]	Theophylline	79.2 ± 1.8	78–83.5	Proposed
Phenobarbital tablets	30	Phenobarbital	99.8 ± 1.4		

[a] Per 10 ml. [b] Per tablet. [c] Standard deviation based on at least four determinations for samples determined by proposed method. [d] Results of official assay are averages of duplicate runs. [e] Based on labeled amount of aminophylline.

strongly basic anion exchange resin, Dowex 2-X8. Ethylenediamine, a base, is removed from the column with water and titrated in the eluate with standard acid. Hydrochloric acid (2 *M*) is used as the eluent for the theophylline which appears in the eluate as the hydrochloride. The phenobarbital, which is insoluble in 2 *M* HCl, remains adsorbed on the resin. Elution of the phenobarbital is effected with 50% acetic acid in ethanol. In preliminary studies the phenobarbital could be eluted with just ethanol, but large volumes were required to remove the phenobarbital completely. With acetic acid in ethanol, smaller volumes of eluent were required. It would appear that the hydrochloric acid eluent has displaced the phenobarbital from the exchange sites on the resin and that it is retained on the column by physical adsorption and, because of poor solubility in aqueous hydrochloric acid, is not removed by the eluent.

The eluates containing the theophylline and phenobarbital were evaporated to dryness. The residues were dissolved in dimethylformamide and titrated nonaqueously with sodium methoxide in benzene–methanol. The theophylline was present in the residue as the unstable hydrochloride. Prolonged heating on the steam plate was necessary to drive off all of the HCl. However, complete removal of the HCl was not necessary since potentiometrically it was possible to titrate differentially the theophylline and HCl. A typical titration curve is shown in Fig. 1. Visual titration is possible only when all the HCl has been driven away. Since this may create a source of error, potentiometric titration is recommended.

Commercially available tablets of aminophylline and phenobarbital were analyzed initially by extracting the active components with 30% alcohol. Consistently high results for theophylline were obtained. Upon request, the manufacturer identified the interfering component as monobasic potassium phosphate. Apparently the phosphate was eluted from the column as phosphoric acid along with the theophylline. The use of 95% ethanol as the extracting solvent dissolved only the theophylline and phenobarbital but not the monobasic potassium phosphate. An alternate procedure also proved effective. The eluate containing theophylline and phosphoric acid (from the Dowex 2-X8 column) was passed through the weakly basic anion exchange resin, Dowex 3-X8, which removed the phosphoric acid. The theophylline, a weak acid, appeared in the eluate which was evaporated to dryness. The residue was dissolved in dimethylformamide and titrated nonaqueously.

At present, there is no official assay for combinations of theophylline and phenobarbital or aminophylline and phenobarbital. The proposed method is applicable to both types of combinations as simple mixtures or in dosage forms. The results of the assay are reported in Table I. A comparison is made to the official assay for aminophylline powder and the injection. In general, favorable results were obtained when compared to the labeled amount and the official requirements. In addition to theophylline and phenobarbital combinations, the proposed procedure should be applicable to combinations containing other barbiturates, antihistamines, quinine, ephedrine, and other basic constituents or their salts. Modifications may be necessary when other acids are included in the formula.

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