UV Spectrophotometric Determination of Aminophylline, Amobarbital, and Ephedrine Hydrochloride in an Antiasthma Capsule Preparation

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Abstract □ A simple and rapid procedure for the determination of aminophylline, amobarbital, and ephedrine hydrochloride in a capsule preparation is described. Aminophylline and amobarbital are determined simultaneously using differential UV spectrophotometry; ephedrine hydrochloride is determined separately after elution from an alginic acid column with 0.1 *N* hydrochloric acid.

Keyphrases □ Aminophylline—UV spectrophotometric analysis, commercial capsule in combination with amobarbital and ephedrine hydrochloride □ Amobarbital—UV spectrophotometric analysis, commercial capsule in combination with aminophylline and ephedrine hydrochloride □ Ephedrine hydrochloride—UV spectrophotometric analysis, commercial capsule in combination with aminophylline and amobarbital □ UV spectrophotometry—analyses, aminophylline, amobarbital, and ephedrine hydrochloride in combination commercial capsule

The combination of aminophylline, ephedrine hydrochloride, and barbituric acid derivatives is frequently encountered in commercial and clinical preparations and in research situations. Procedures for the determination of these active ingredients alone or in combination in tablets, capsules, or syrups include gravimetric (1), titrimetric (2), spectrophotometric (3, 4), GLC (5), and IR (6) measurements.

The National Formulary (7) describes a method for the analysis of theophylline, phenobarbital, and ephedrine hydrochloride in a tablet formulation; the active components are separated using column partition chromatography and liquid–liquid extraction, followed by UV spectrophotometric determination of the isolated ingredients. However, this NF procedure is tedious and requires much care to obtain good results; the various steps are the largest sources of both systematic and random errors. Therefore, this work was planned to propose a uniform methodology that would reduce sample manipulation.

EXPERIMENTAL

Apparatus—A recording spectrophotometer[1] with matched 1- and 4-cm cells was used. The glass column was 50 × 2 cm and had a fine regulatory needle valve stopcock with a polytef insert[2].

Reagents—The following were used: alginic acid[3] cation-exchange resin, 40–100 mesh; 2 *N* hydrochloric acid in water; 0.1 *N* hydrochloric acid in water; 0.02 *N* sodium hydroxide in water; 50% ethanol in water; and 50% ethanol in 4% (v/v) acetic acid.

Standard Solutions—The following were used: ephedrine hydrochloride, 0.136 mg/ml in 0.1 *N* hydrochloric acid; aminophylline, 0.7 mg/ml in 50% ethanol in 0.4% (v/v) acetic acid; and amobarbital, 0.136 mg/ml in 50% ethanol in 0.4% (v/v) acetic acid. All standards were USP XVI, NF XIII, or BP 1973 quality[4].

Column Preparation—A slurry of about 4 g of alginic acid, previously soaked in water for 4 hr, was transferred to a chromatographic column and allowed to settle. The column was eluted with 2 *N* hydrochloric acid until the eluate gave an absorbance less than 0.005 between 230 and 330 nm. It was then washed with distilled water until the eluate was neutral to the litmus solution. Finally, a small pledget of glass wool was added to the column. After 20 ml of 50% ethanol in water was passed through the column, alginic acid was slightly tamped.

Sample Treatment—The average weight of not less than 20 capsules[5] was determined. An accurately weighed portion of powder, equivalent to about 67 mg of ephedrine hydrochloride, was transferred to a 50-ml volumetric flask, and 50% ethanol–acetic acid solution was added to the mark. A magnetic stirring bar was slipped into the flask, and the mixture was stirred for 10 min and then centrifuged for 2 min.

Determination—*Aminophylline and Amobarbital*—Pipet a 10-ml aliquot of the centrifuged sample onto the prepared alginic acid column. Place a 100-ml volumetric flask beneath the column and start collecting the eluate at a rate of 1 ml/min. Then add 50 ml of 50% ethanol (in water) in small portions, letting each drain through the column before successive additions. Remove the flask and dilute to volume with the same solvent. This solution contains aminophylline and amobarbital.

Transfer a 2-ml aliquot of the solution into each of two 50-ml volumetric flasks. Adjust the solution in one flask to pH 4 and the other to pH 10 by adding 0.02 *N* sodium hydroxide; then dilute to 50 ml with distilled water. Using the pH 4 solution as the reference and the pH 10 solution in the sample position, measure the absorbance at 285.5 and 240 nm (1-cm cells). Calculate the concentration of aminophylline and amobarbital in the tablets by determining the absorptivities at 285.5 and

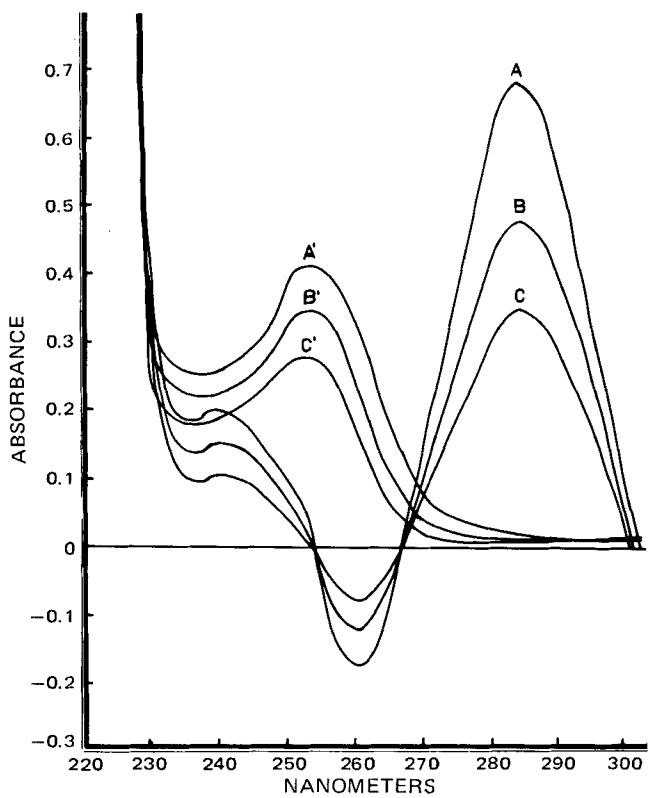

Figure 1—*Differential spectra of aminophylline (A, B, and C) and amobarbital (A′, B′, and C′).*

[1] Beckman DB.
[2] Brand, West Germany.
[3] British Drug Houses.
[4] E. Merck, Darmstadt, Germany.

[5] Capsules contained 130 mg of aminophylline, 25 mg of amobarbital, and 25 mg of ephedrine hydrochloride and are marketed as Emelasma.

Table I—Recovery of Aminophylline, Amobarbital, and Ephedrine Hydrochloride from Synthetic Mixtures in the Presence of an Excipient

Mixture	Aminophylline		Amobarbital		Ephedrine Hydrochloride	
	Added, mg	Recovery, %	Added, mg	Recovery, %	Added, mg	Recovery, %
1	351.0	97.9	67.5	101.3	68.3	98.7
2	349.5	98.6	68.0	99.2	67.5	100.0
3	350.3	98.0	67.9	98.8	67.3	99.0
4	349.4	99.3	66.8	97.9	67.0	98.4
5	349.9	99.1	67.5	98.2	67.4	98.8
6	350.7	97.5	69.3	99.6	66.5	98.7
7	348.8	99.7	67.3	98.1	67.4	99.6
8	348.7	98.2	66.9	98.8	68.0	98.3
9	347.2	98.6	66.9	97.9	66.9	98.7
10	350.2	98.6	67.4	98.5	67.1	98.5
Average, %		98.9		98.5		98.8
SD, %		±0.49		±0.44		±1.11

240 nm of both aminophylline and amobarbital standard solutions, taken individually, diluted as the sample, and adjusted to pH 4 (blank) and 10 (sample).

Ephedrine Hydrochloride—Wash the column with 20 ml of distilled water divided into two portions. Elute the column with 0.1 *N* hydrochloric acid at a rate of 2 ml/min, discarding the first 7 ml of the liquid. Collect the eluate in a volumetric flask to the 100-ml mark. Scan the sample and standard solutions between 330 and 230 nm, using 4-cm cells, against 0.1 *N* hydrochloric acid as the reference. Determine the absorptivity at the maximum (about 257 nm) for the ephedrine hydrochloride standard and calculate its concentration in the sample solution.

RESULTS AND DISCUSSION

A prepared column containing alginic acid retained ephedrine hydrochloride from an ethanolic solution and allowed aminophylline and amobarbital to pass through as washings. Subsequently, ephedrine hydrochloride was eluted with 0.1 *N* hydrochloric acid and determined spectrophotometrically at 257 nm. Aminophylline and amobarbital were volumetrically collected and quantitatively determined by UV differential spectrophotometry at 240 (maximum for amobarbital) and 285.5 (maximum for aminophylline) nm, using two aliquots of the same solution as the blank and sample at pH 4 and 10, respectively.

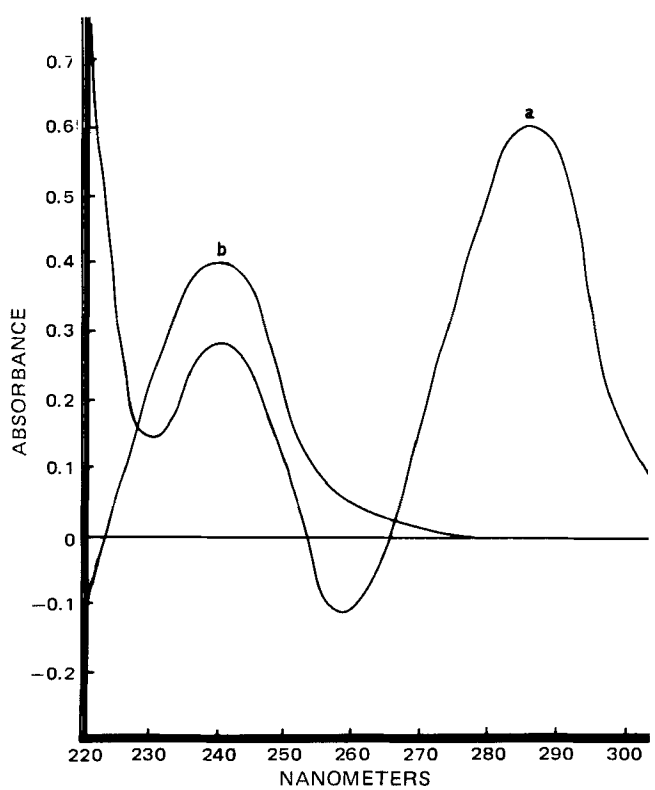

Figure 2—*Differential absorption spectra of aminophylline (a) and amobarbital (b) at pH 10.*

Synthetic mixtures of the three active ingredients in the presence of an excipient (light magnesium carbonate) were prepared and assayed by this method including the chromatographic step. Recovery results are given in Table I; analyses of commercial samples are reported in Table II.

The determination of aminophylline and amobarbital represented the main problem. An algebraic method for additive absorbances was not applicable, since the weight ratio of aminophylline to amobarbital was about 5:1. Therefore, the pH–chromophoric properties of the two compounds had to be considered. Both aminophylline and amobarbital are organic acids and have hydrogen atoms that can be ionized in alkaline media. When their absorption spectra are determined at different pH values that include molecular and ionic forms, isosbestic points are observed frequently. To find an eventual isosbestic point that might be employed as the baseline, differential absorption spectra of individually prepared solutions of aminophylline and amobarbital between pH 10 and 13 were examined.

A series of aminophylline solutions at pH 10, 11, 12, and 13 (using the same solutions at pH 4 as reference) showed two maxima at 240 and 285.5 nm and two isosbestic points at 253.5 and 263 nm. The differential curves determined at pH 11, 12, and 13 did not exhibit a significant change over those taken at pH 10, as would be expected from the pK value of aminophylline. When differential absorption spectra of amobarbital solutions were prepared following the same procedure, a gradual shift of the absorption maxima from 240 to about 254 nm was observed as the pH was increased from 10 to 13. Particular attention was focused on the absorption curves taken at pH 13, where the correlation between the isosbestic point at 253.5 nm for aminophylline and the maximum for amobarbital and the correspondence of the maximum for aminophylline at 285.5 nm with an insignificant absorbance for amobarbital seemed to be the ideal setup for the quantitative determination of the ingredients in the presence of one another.

Figure 1 illustrates the differential absorption spectra at pH 13 of three concentrations of aminophylline and three concentrations of amobarbital. Each solution contained the active ingredient in the weight ratio similar to that of the commercial preparation. However, while both compounds followed the Lambert–Beer law individually, their mixtures, over the same concentration range, did not. When these mixtures were scanned

Table II—Analyses of Commercial Product

Product	Aminophylline Found, % of Claim (130 mg)	Amobarbital Found, % of Claim (25 mg)	Ephedrine Hydrochloride Found, % of Claim (25 mg)
A	97.6	99.3	100.8
B	98.1	98.3	99.5
C	97.9	97.4	98.2
D	98.8	99.9	97.4
E	101.2	97.9	102.1
F	99.6	97.3	98.9
G	97.8	98.0	99.7

Table III—Recovery of Aminophylline and Amobarbital Mixed Standard in 50% Ethanol in 4% (v/v) Acetic Acid[a]

Mixture	Aminophylline		Amobarbital	
	Amount Taken, mg	Recovery, %	Amount Taken, mg	Recovery, %
A	350.0	100.7	67.0	97.4
B	350.0	98.9	67.0	98.6
C	360.0	99.3	69.0	99.1
D	340.0	97.5	65.0	99.4
E	300.0	97.9	57.5	101.3
F	350.0	98.6	67.0	100.6
G	360.0	99.1	69.0	99.8
H	350.0	97.7	67.0	97.8
I	350.0	98.6	67.0	98.9
J	340.0	98.9	65.0	99.4
Average, %	—	98.7	—	99.2
SD, %		±0.92		±1.67

[a] Synthetic mixes were prepared by dissolving an accurately weighed amount of both compounds in 50% ethanol in 4% acetic acid in a 50-ml volumetric flask. Each mixture was treated and diluted as the sample, excluding the chromatographic step.

between 320 and 220 nm, the absorption curves showed that the maximum at 285.5 nm remained unchanged and that the maximum at 254 nm was absent. The absorption at 240 nm increased significantly in intensity with respect to that of aminophylline determined individually at the same concentration.

The cause of this deviation at pH 13 may have been that the keto–enolic equilibrium of the doubly ionized form of amobarbital with the absorption maximum at 254 nm was probably shifted by a much larger concentration of the enolized aminophylline to exhibit an absorption maximum at 240 nm (singly ionized form). To eliminate such a possible keto–enolic equilibrium effect, a new approach was followed. The differential absorption curves at pH 10, previously plotted for the separated compounds, showed that aminophylline and amobarbital exhibited fairly strong maxima at about 240 nm and that there was a wide variation between the intensities of absorption of both components at 285.5 nm. Figure 2 shows the differential absorption spectra at pH 10 of aminophylline and amobarbital. These conditions seemed to be theoretically adequate for the quantitative determination of mixtures of aminophylline and amobarbital (8).

This hypothesis was verified experimentally when mixtures of the two substances, analyzed at pH 10, gave a positive response. The results in Table III show that the procedure is both accurate and precise. Compared to the NF procedure, the proposed method significantly shortens the time required for the analysis of aminophylline, amobarbital, and ephedrine hydrochloride, thus making possible the quantitative determination of more samples per day.

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Liquid Chromatography in Pharmaceutical Analysis VIII: Determination of Isoniazid and Acetyl Derivative in Plasma and Urine Samples

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Abstract □ Parameters are described for the qualitative and quantitative analysis of a mixture of isoniazid and its acetyl derivative. The compounds are chromatographed on an octadecylsilane column, using absolute methanol–distilled water (60:40) at pH 2.5 containing 0.01 *M* dioctyl sodium sulfosuccinate. The flow rate was 2.0 ml/min (2500 psig). The separation and quantification are applicable to plasma and urine samples. The determination in each biological fluid can be achieved in approximately 90 min with percentage accuracies for isoniazid of 5.25 and 7.45 and for the acetyl derivative of 4.47 and 1.56 in plasma and urine, respectively.

Keyphrases □ Isoniazid and acetyl derivative—high-pressure liquid chromatographic analyses, plasma and urine □ High-pressure liquid chromatography—analyses, isoniazid and acetyl derivative, plasma and urine □ Tuberculostatic antibacterials—isoniazid and acetyl derivative, high-pressure liquid chromatographic analyses, plasma and urine

Interest in high-pressure liquid chromatography (HPLC) for the determination of drugs and their metabolites in biological media led to the analysis of isoniazid (I) and its major metabolite, the acetyl derivative (II). Presently, I is very effective as an antituberculosis drug, and the determination of I and II is important in studies related to I disposition and metabolism. A review of analytical methodology concerning I and its metabolites is available (1).

This paper reports the HPLC separation of I and II and the application of the procedure to the quantification of these compounds in plasma and urine. The HPLC separation is effected utilizing ion-pair formation with dioctyl sodium sulfosuccinate on an octadecylsilane column. Generalities of ion-pair formation were reviewed extensively (2, 3). Some investigators (4–6) used ion-pair formation as a tool for effecting chromatographic analysis of selected organic ions. The mechanism for such a separation apparently depends upon the reversible formation of ion-pairs in the chromatographic system and the separa-