Quantitative GLC Analysis of Theophylline, Ephedrine Hydrochloride, and Phenobarbital Suspension

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Abstract □ A rapid GLC method was developed for the quantitative determination of theophylline, ephedrine hydrochloride, and phenobarbital in a suspension formulation. A simple extraction procedure was incorporated to separate ephedrine hydrochloride into one fraction (I) and theophylline and phenobarbital into a second fraction (II). An internal standard was added to each fraction, and the resulting solutions were chromatographed onto a single column. The internal standard and column temperature used were: α-naphthylamine and 150°, Fraction I; and hexobarbital and 200°, Fraction II. Quantitation was based on the sample–internal standard peak height ratio.

Keyphrases □ Theophylline, ephedrine hydrochloride, and phenobarbital suspensions—GLC analysis □ Ephedrine hydrochloride, theophylline, and phenobarbital suspensions—GLC analysis □ Phenobarbital, theophylline, and ephedrine hydrochloride suspensions—GLC analysis □ GLC—analysis, theophylline, ephedrine hydrochloride, and phenobarbital suspensions

Tablets and suspensions containing theophylline, ephedrine hydrochloride, and phenobarbital are frequently used for their actions of sedation and bronchodilation. The problem of developing a rapid and accurate assay for combinations of these components is well known (1). Although many methods are reported for the individual components, only a few reports concern the assay of mixtures of the three drugs.

A monograph for theophylline, ephedrine hydrochloride, and phenobarbital tablets is given in NF XIII (2). This represents the first recognition given by either the USP or NF to the mixture. The official assay, which also serves as the basis for identification, consists of separating the mixture into three fractions, each containing a single drug, followed by UV spectroscopy. Separation is accomplished by a laborious process involving two chromatographic columns and a liquid–liquid extraction. In addition to this time-consuming aspect, the official procedure is not directly adaptable to the mixture in suspension form.

Another assay for theophylline, ephedrine hydrochloride, and phenobarbital tablets was reported (1). This was a GLC method involving a single column and two separate sample preparations. A solution containing theophylline and phenobarbital was chromatographed directly after the solvent extraction of a tablet sample. Ephedrine was determined by treating a second tablet sample with sodium periodate to form benzaldehyde and chromatographing a solvent extract of this mixture. The oxidation step was included because ephedrine did not give a well-resolved peak. The assay was described as being rapid, specific, and precise. However, it applied only to tablets and could not be utilized directly for suspensions.

In recent years, GLC has become an increasingly important method for the analysis of theophylline, ephedrine hydrochloride, and phenobarbital, either individually or in combination with each other. This method is especially attractive because of its ability to provide both accuracy and convenience. The GLC methods used for these drugs in mixtures, such as dosage formulations and biological materials, frequently include solvent extraction (1, 3–9).

This report describes a rapid and accurate GLC method for the quantitative determination of theophylline, ephedrine hydrochloride, and phenobarbital contained in suspension. In the development of this procedure, the attainment of well-resolved peaks was sought through the use of a single column, a minimum number of sample preparations and injections, and a direct injection of the components. All of these conditions are successfully met in the described procedure.

EXPERIMENTAL

Apparatus—A gas chromatograph[1] equipped with a flame-ionization detector was used. The electrometer range was 10^2 with attenuation from 1 to 4 × 10^{-10} amp. The detector signal was fed to a 1-mv. recorder with a chart speed of 0.64 cm. (0.25 in.)/min. and a 1-sec.

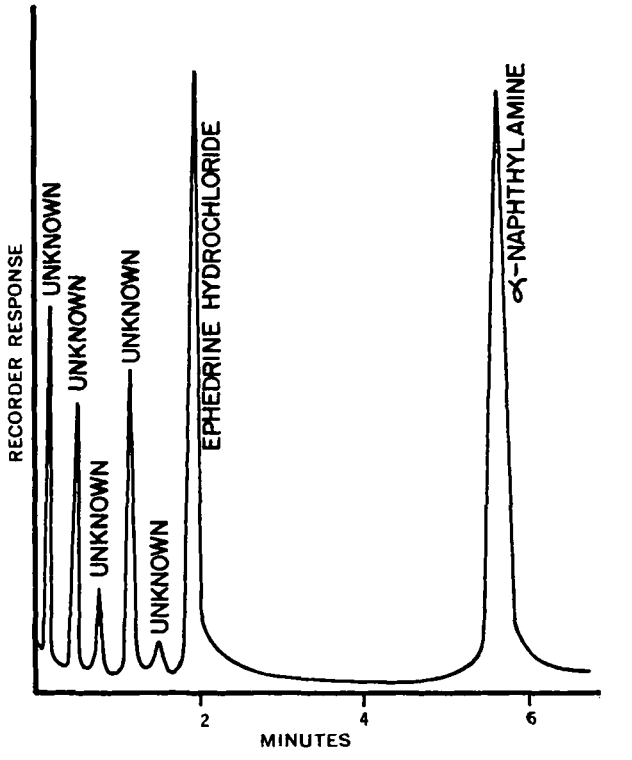

Figure 1—*Gas chromatogram of ephedrine hydrochloride in a commercial suspension. Conditions: 3% OV-17 on Gas Chrom Q; nitrogen flow rate, 75 ml./min.; column temperature, 150°; recorder chart speed, 0.64 cm./min.; and attenuation of electrometer, 2.0 × 10^{-10} amp.*

[1] Hewlett-Packard model 402.

Table I—Analysis of Theophylline, Ephedrine Hydrochloride, and Phenobarbital in a Synthetic Mixture[a]

	Theophylline		Ephedrine Hydrochloride		Phenobarbital	
	Found, mg./5.0 ml.	Recovery[b], %	Found, mg./5.0 ml.	Recovery[c], %	Found, mg./5.0 ml.	Recovery[d], %
Mean	65.2	100.3	11.9	99.2	3.9	97.5
Standard deviation	±0.09		±0.07		±0.04	
Coefficient of variation		±0.1		±0.6		±1.0

[a] Results obtained from six runs. [b] Each 5.0 ml. contained 65.0 mg. theophylline. [c] Each 5.0 ml. contained 12.0 mg. ephedrine hydrochloride. [d] Each 5.0 ml. contained 4.0 mg. phenobarbital.

Table II—Analysis of Theophylline, Ephedrine Hydrochloride, and Phenobarbital in a Commercial Suspension[a]

	Theophylline		Ephedrine Hydrochloride		Phenobarbital	
	Found, mg./5.0 ml.	Recovery[b], %	Found, mg./5.0 ml.	Recovery[c], %	Found, mg./5.0 ml.	Recovery[d], %
Mean	64.6	99.4	11.5	95.8	4.4	110.0
Standard deviation	±0.18		±0.09		±0.06	
Coefficient of variation		±0.3		±0.8		±1.4

[a] Results obtained from six runs. [b] Labeled amount of theophylline in 5 ml. was 65 mg. [c] Labeled amount of ephedrine hydrochloride in 5 ml. was 12 mg. [d] Labeled amount of phenobarbital in 5 ml. was 4 mg.

full-scale response. The gas flows were: nitrogen, 75 ml./min.; hydrogen, 35 ml./min.; and air, 280 ml./min.

GLC Column—A 1.8-m. (6-ft.) long, U-shaped, borosilicate glass tubing (4.0 mm. i.d.) was packed with 3% OV-17 on 100–120-mesh Gas Chrom Q[2]. The column was conditioned by heating at 100° for 1 hr., followed by increasing the temperature to 250° at a rate of 1°/min. and maintaining this temperature for 18 hr. with continuous carrier gas flow.

Operating Temperatures—The injection port, column, and detector temperatures for the assay of ephedrine hydrochloride were maintained at 200, 150, and 200°, respectively. For the assay of phenobarbital and theophylline, the injection port, column, and detector temperatures were maintained at 250, 200, and 250°, respectively.

Preparation of Ephedrine Hydrochloride Internal Standard Calibration Curve—*Internal Standard Solution*—A chloroform solution containing 12.5 mg. α-naphthylamine/ml. was used.

Standard Reference Solution—About 500 mg. ephedrine hydrochloride USP, accurately weighed, was dissolved in 10 ml. distilled water contained in a 125-ml. separator. The mixture was adjusted to pH 11 with 20% sodium hydroxide solution and extracted with four 25-ml. portions of chloroform. The extract was placed in a 100-ml. volumetric flask and diluted to volume with chloroform. The following quantities of this solution were pipeted into six 10-ml. volumetric flasks: 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 ml. To each flask were added 0.3 ml. of α-naphthylamine internal standard solution and sufficient chloroform to bring to volume. A 4-μl. sample of each solution was injected into the gas chromatograph. Four replicate samples of each concentration were injected, the peak heights were measured, and the average value for each concentration was determined. Upon plotting the ephedrine hydrochloride–α-naphthylamine peak height ratio to amount of ephedrine hydrochloride, a straight-line calibration curve was obtained over the 1.0–10.0-mcg. range at the corresponding range of peak height ratio values from 0.45 to 9.00.

Preparation of Phenobarbital Internal Standard Calibration Curve—*Internal Standard Solution*—Hexobarbital (0.2 mg./ml.) was dissolved in an equal volume of chloroform and methanol.

Standard Solution— Phenobarbital USP, accurately weighed, was dissolved in the hexobarbital internal standard solution to give a series of six solutions varying from about 0.2 to 3.9 mg./ml. The same GLC procedure for these solutions was followed as described for the ephedrine hydrochloride standard solution. Upon plotting the phenobarbital–hexobarbital peak height ratio to amount of phenobarbital, a straight-line calibration curve was obtained over the 0.7–15.4-mcg. range at the corresponding range of peak height ratio values from 0.40 to 8.60.

Preparation of Theophylline Internal Standard Calibration Curve—*Internal Standard Solution*—Hexobarbital internal standard solution, as described for the phenobarbital standard curve, was used.

Standard Solution—Theophylline NF, accurately weighed, was dissolved in hexobarbital internal standard solution to give a series of six solutions varying from about 0.8 to 21.0 mg./ml. The same GLC procedure was followed as described for the ephedrine hydrochloride standard solution. Upon plotting the theophylline–hexobarbital peak height ratio to amount of theophylline, a straight-line calibration curve was obtained over the 6.2–62.4-mcg. range at the corresponding range of peak height ratio values from 0.73 to 4.30.

Assay of a Synthetic Mixture—*Preparation*—A mixture containing the same quantities of theophylline, ephedrine hydrochloride, and phenobarbital as contained in a commercial suspension was formulated. Each 5 ml. contained 65.0 mg. theophylline, 12.0 mg. ephedrine hydrochloride, and 4.0 mg. phenobarbital in distilled water.

Ephedrine Hydrochloride Assay—To 5.0 ml. of the mixture was added 5 ml. distilled water, the pH was adjusted to 11 with 20% sodium hydroxide solution, and the solution was extracted two times with 12 ml. chloroform. The chloroform extract was placed into a 25-ml. volumetric flask containing 0.75 ml. of α-naphthylamine internal standard solution and diluted to volume with chloroform. Four microliters of the solution was injected into the gas chromatograph.

Phenobarbital and Theophylline Assay—To the aqueous phase remaining from the ephedrine hydrochloride assay, 4 g. of sodium chloride was added and the pH was adjusted to 4.8 with dilute hydrochloric acid. The mixture was extracted four times with 50 ml. chloroform. The chloroform extract was placed into a 200-ml. volumetric flask and diluted to volume with chloroform. With a pipet, 9.0 ml. of this solution was placed in a test tube and the solution was evaporated to dryness on a 65° water bath. One milliliter of the hexobarbital internal standard solution was pipeted into the tube to redissolve the residue. Four microliters of the solution was injected into the gas chromatograph. The results are given in Table I.

Assay of Commercial Suspension—The specific gravity of a commercial suspension[3] was determined. Approximately 6.4 g. of the suspension, accurately weighed, was mixed with 5 ml. of distilled water. This quantity, representing about 5 ml. of sample, was converted into volume using the specific gravity value. The resulting mixture was extracted and gas chromatographed by the same pro-

[2] Applied Science Laboratories, State College, Pa.

[3] Tedral suspension, Warner-Lambert Co.

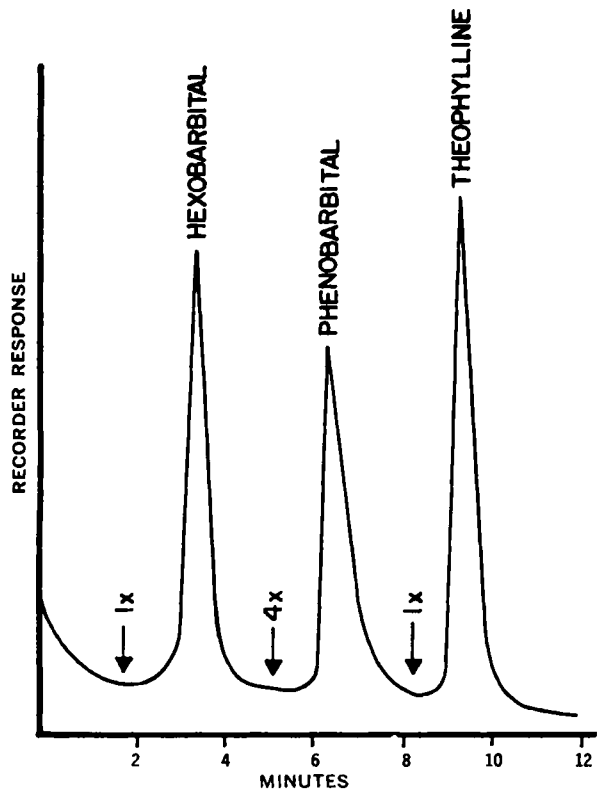

Figure 2—*Gas chromatogram of theophylline and phenobarbital in a commercial suspension. Conditions: 3% OV-17 on Gas Chrom Q; nitrogen flow, 75 ml./min.; column temperature, 200°; recorder chart speed, 0.64 cm./min.; and attenuation of electrometer, 1 × and 4 × 10^{-10} amp.*

cedure as used for the assay of a synthetic mixture. The results are given in Table II and Figs. 1 and 2.

RESULTS AND DISCUSSION

A GLC procedure for the quantitative determination of theophylline, ephedrine hydrochloride, and phenobarbital contained in a suspension formulation was presented. It was necessary to incorporate into the procedure a liquid–liquid extraction because of the varying concentration and polarity of the components. Through this process the sample was separated into two fractions, one containing ephedrine hydrochloride and the other containing theophylline and phenobarbital. Several additional substances, possible flavoring or coloring agents, were extracted by chloroform into the ephedrine extract. Complete elimination of these impurities was not attempted because they did not interfere with either the ephedrine or the internal standard peaks (Fig. 1).

The packing used in the GLC column was 3% OV-17 on Gas Chrom Q. A preliminary study showed that this packing gave the most desirable shape and resolution of peaks. In addition, it had the advantage of high thermal stability. Although peak tailing has been encountered with these drugs on various packing materials (6–8, 10), there was minimal peak tailing for all of the drugs in this investigation. The use of the silanized solid support of Gas Chrom Q probably minimized this problem.

A 4-μl. injection of each fraction was made and the resulting peak heights were measured. The peak height ratios of drug to internal standard were determined and compared to the appropriate calibration curve. Linear response was found in the calibration curves for the following ranges: ephedrine hydrochloride, 1.0–10.0 mcg.; phenobarbital, 0.7–15.4 mcg.; and theophylline, 6.2–62.4 mcg.

The procedure was used to assay a commercial suspension and a comparable synthetic mixture. Six replicate determinations were made of each preparation. Each extracted fraction was injected into the gas chromatograph four times and the average result was determined. The mean recoveries obtained (Table I) in the assay of the synthetic mixture for ephedrine hydrochloride, phenobarbital, and theophylline were 99.2 ± 0.6, 97.5 ± 1.0, and 100.3 ± 0.1%, respectively. Correspondingly, the mean recoveries for the commercial suspension (Table II) were: 95.8 ± 0.8, 110.0 ± 1.4, and 99.4 ± 0.3%, respectively. These latter values were calculated on the basis of the manufacturer's label claim. The standard deviation (Tables I and II) found for each drug in the two preparations indicated excellent precision for the procedure.

CONCLUSION

A rapid GLC method was developed for the determination of theophylline, ephedrine hydrochloride, and phenobarbital contained in a suspension formulation. A simple extraction procedure was incorporated to separate the components into two fractions. To each fraction an internal standard was added, and the resulting solutions were chromatographed onto a single column. Conditions of column packing, temperatures, internal standards, and carrier gas flow rate were selected to give well-resolved peaks. Quantitation was made on the basis of the peak height ratio of sample to internal standard. Accurate and precise values were obtained using this procedure.

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