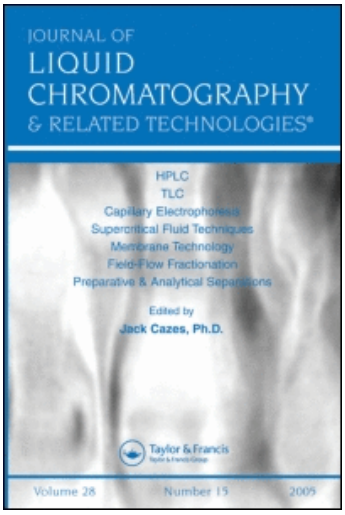

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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Shabbir U. Alvi[a]; Frank Castro[a]
[a] Quality Control Laboratory, Knoll Pharmaceutical Company Whippany, New Jersey

To cite this Article Alvi, Shabbir U. and Castro, Frank(1986) 'A Simultaneous Assay of Theophylline, Ephedrine Hydrochloride, and Phenobarbital in Suspensions and Tablets Formulations by High Performance Liquid Chromatography', Journal of Liquid Chromatography & Related Technologies, 9: 10, 2269 — 2279
To link to this Article: DOI: 10.1080/01483918608074147
URL: http://dx.doi.org/10.1080/01483918608074147

JOURNAL OF LIQUID CHROMATOGRAPHY, 9(10), 2269-2279 (1986)

A SIMULTANEOUS ASSAY OF THEOPHYLLINE, EPHEDRINE HYDROCHLORIDE AND PHENOBARBITAL IN SUSPENSIONS AND TABLETS FORMULATIONS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Shabbir U. Alvi* and Frank Castro

Quality Control Laboratory
Knoll Pharmaceutical Company
Whippany, New Jersey 07981

ABSTRACT

A simple reversed phase high-performance liquid chromatographic (HPLC) procedure is described for simultaneous determination of Theophylline, Ephedrine Hydrochloride and Phenobarbital in suspensions and tablets matrices. The separation was performed on a radial pak cartridge packed with octadecylsilane (C18) material using methanol and 0.01M monobasic potassium phosphate (33:67) as the mobile phase. Measurement was made by a uv-spectrophotometer at 215 nm and quantitation was based on the sample-internal standard peak area ratio. The respective precision and relative standard deviations from suspensions and tablets were: theophylline 95.27%, 0.06% and 101.35%, 0.16%, ephedrine hydrochloride 101.49%, 0.34% and 101.69%, 0.47% and phenobarbital 93.03%, 0.30% and 94.91%, 0.14%.

* Author to whom correspondence and reprint requests should be addressed.

 0148-3919/86/0910-2269$3.50/0

INTRODUCTION

Suspensions and tablets containing a mixture of theophylline, ephedrine hydrochloride are used for their action of bronchodilation and phenobarbital is used for mild sedation. An official analysis by HPLC is presented in the USP XXI (1) for the combination of theophylline, ephedrine and phenobarbital tablets. Shultz and Paveenbampen (2) reported a GLC method of suspension dosage form which requires derivative formation. The USP XXI method of theophylline, ephedrine and phenobarbital which was initially reported by Chen and Chaftez (3) requires an oxidation of ephedrine hydrochloride and the resulting benzaldehyde as an eluting peak is quantitated by HPLC and uv-detection. Tan etal (4) described and reported a relatively easy method for direct detection and quantitation of theophylline, ephedrine hydrochloride and phenobarbital in tablets using two different range of attenuation and changing the sensitivity during the analysis. However, none of these methods proposed an easy and convenient solution for routine analysis of suspensions and tablets dosage form.

This report describes a simultaneous, rapid HPLC procedure for the quantitative determination of three active components in suspensions and tablets formulation. This innovative reversed phase method also provides a solution to the problem of obtaining all the peaks on recorder scale and direct quantitation of ephedrine hydrochloride.

MATERIALS AND METHODS

Apparatus: The liquid chromatograph consisted of a pump [1], an automatic injector [2], a variable wavelength uv-spectrophotometer [3], and a plotter printer integrator [4]. A radial pak cartridge (8 mm x 10 cm) packed with octadecylsilane [5] (C18) 10 um particle material and a Z-Module [6] for the radial pak cartridge. A guard column [7] (2.5 cm x 3 mm) packed with reversed phase bondapak C18/corasil [8] material was attached before the analytical column.

Materials: Potassium phosphate monobasic [9], methanol [10], water [11] and chloroform. All the reagents and solvents were of HPLC grade except chloroform which was analytical reagent grade. Reference standard theophylline USP, ephedrine sulfate USP and phenobarbital USP [12] and reagent grade internal standard, guaifenesin USP.

Mobile Phase: 0.01M potassium phosphate monobasic adjusted to pH 5.7 and 5.5 by 1N NaOH for suspensions and tablets respectively. 67 volumes of 0.01M phosphate buffer and 33 volumes of methanol filtered through 0.45 micron membrane [13], mixed and degassed prior to use.

Chromatographic Conditions: Flow rate, about 2.0 mL/min. Column was equilibrated with mobile phase before making an injection. Detector range: 0.2 aufs measured at 215 nm at a chart speed 0.5 cm/min. Quantitation by peak area and internal standard.

Internal Standard: An initial concentration 2.0 mg/mL of internal standard (III) was prepared in methanol which was subsequently diluted in mobile phase to obtain a final 0.2 mg/mL concentration.

Standard Preparation: Approximately 59 mg of I [14], 24 mg each of II [15] and IV [16] were accurately weighed and transferred to a 100 mL volumetric flask.

For Suspensions: The weighed quantity of standards were dissolved in 30 mL of methanol and diluted to 100 mL in a volumetric flask with chloroform. A 20 mL aliquot was transferred into another 100 mL volumetric flask, diluted to mark with chloroform and mixed.

For Tablets: The weighed amount of standards were dissolved in 10 mL of methanol and diluted to 100 mL with chloroform in a volumetric flask. A 20 mL solution was pipetted into a 100 mL volumetric flask and diluted to mark with chloroform and mixed.

Sample Preparation: Suspensions: Sample was shaken on a mechanical shaker for about 45 minutes, sonicated and then 10 mL aliquot was pipetted into a 100 mL volumetric flask. Thirty milliliters (30 mL) methanol and 50 mL chloroform were added to this

flask and shaken for 45 minutes then diluted with chloroform, mixed and filtered through sodium sulfate anhydrous. A 20 mL portion was diluted with chloroform, mixed and filtered.

Tablets: No less than 10 tablets were weighed and average tablet weight was determined. The tablets were finely powdered and a portion of powder equivalent to one average tablet weight was weighed and quantitatively transferred into a 100 mL volumetric flask. Ten milliliters (10 mL) methanol and 50 mL chloroform were added and the mixture was shaken for 45 minutes on a mechanical shaker, then diluted to volume with chloroform, mixed and filtered. A 20 mL filtrate was further diluted to 100 mL with chloroform, mixed and filtered.

Procedure: Each standard preparation (10 mL) and sample preparation (10 mL) was pipetted into a glass stoppered flask and evaporated to dryness on a steam bath and under air current. The residue of standard and sample were reconstituted in 10 mL of internal standard and filtered into a HPLC vial.[17]

System Suitability: The column was equilibrated with mobile phase at a flow rate 2.0 mL/min. After a stable base line was obtained, 8 uL standard solution was injected into the liquid chromatograph. Four peaks obtained in the order of increasing retention times are theophylline, ephedrine, internal standard (Guaifenesin) and phenobarbital. The resolution factors Rs are calculated between the two neighboring peaks from the equation $2(t2-t1)/(w1+w2)$, where t2 and t1 are retention times of the two neighboring peaks, w1 and w2 are the widths at the base of the two respective peaks. The resolution Rs shall not be less than 1.5 between the two neighboring peaks and each peak should be completely resolved. The relative standard deviation (%RSD) of the six replicate injections of the standard solution shall not be more than 2.0% (calculated from the ratio of each peak area versus internal standard area).

Assay Method - Suspensions: Equal volumes (8 uL) of standard solution and sample solution were injected by means of automatic injector. Similarly for tablets (10 uL) equal volumes of standard

and sample preparation were injected into the liquid chromatograph, operated at room temperature.

Calculation: The amount of theophylline hydrous, ephedrine hydrochloride and phenobarbital were calculated in milligrams per 5 mL for suspensions and in milligrams per tablet for tablets from respective equation.

$$\frac{Ru}{Rs} \times \frac{Ws}{2} \times F \text{ and } \frac{Ru}{Rs} \times \frac{Ws}{Wu} \times A \times F$$

Where Ru and Rs are the respective response ratio of peak area of sample and standard. Ws and Wu are the weights in milligrams of standard and sample, respectively. F^{18} is the conversion factor for theophylline and ephedrine, and A is the average tablet weight in milligrams.

RESULTS AND DISCUSSIONS

The elution times of peaks corresponding to I, II, III and IV were in the increasing order of 4 minutes, 5.6 minutes, 8.4 minutes and 13 minutes, respectively. All the peaks were completely resolved and base line separation was achieved. Figure 1 shows a typical chromatogram of standard solution. The resolution factor (R) calculated according to the USP XXI (1) procedure between the two neighboring peaks were found to be 2.6, 4 and 5.5. The relative standard deviation of six replicate injections of standard were 0.11%, 0.42% and 0.19% for I, II and IV, respectively.

Linearity: Standard solutions ranging from 80% to 120% at 10% intervals were prepared then each solution (8 uL and 15 uL) were injected into the liquid chromatograph. The ratios of peak response versus internal standard were plotted against the respective concentration of active component. A straight line was obtained at each level (8 uL and 15 uL) for individual component, as evidenced by the correlation coefficient 0.9998, 0.9997 and 0.9998 corresponding to amount injected from 0.094 mg/ml to 0.141

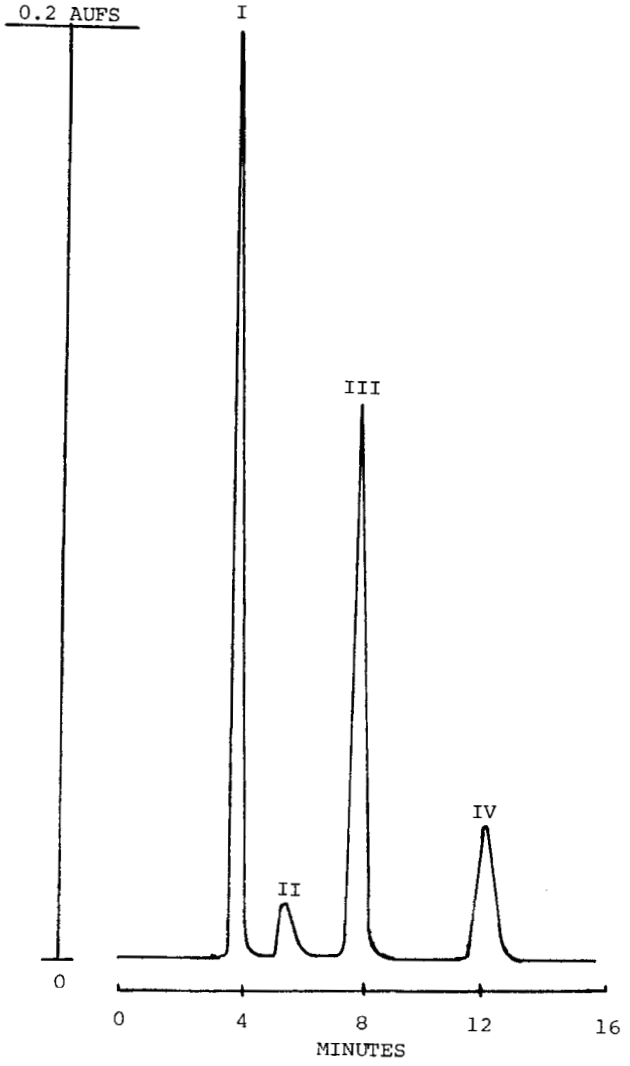

Figure 1 - Liquid chromatogram for standard preparation
Key: (I) Theophylline, (II) Ephedrine Sulfate
(III) Internal Standard (Guaifenesin) and
(IV) Phenobarbital

TABLE 1

System Precision Data

Injection No.	Theophylline	Ephedrine HCl	Phenobarbital
SUSPENSION: PERCENT RECOVERED			
1	95.25	101.76	92.60
2	95.30	101.98	93.22
3	95.30	101.12	92.92
4	95.27	101.23	93.26
5	95.34	101.25	92.30
6	95.17	101.59	92.87
Avg.	95.27	101.49	93.03
RSD, %	0.06	0.34	0.30
TABLET: PERCENT RECOVERED			
1	101.54	101.46	94.95
2	101.28	101.18	94.73
3	101.43	101.71	94.91
4	101.06	101.90	94.81
5	101.38	101.39	94.94
6	101.40	102.51	95.13
Avg.	101.35	101.69	94.91
RSD, %	0.16	0.47	0.14

mg/ml for I and 0.038 mg/mL to 0.058 mg/mL for II and IV, respectively.

System Precision: The method showed an excellent precision. The relative standard deviation of six replicate injections of Quadrinal suspensions [19] and Quadrinal tablets [19] were found to be, <1% for each active component as summarized in Table 1.

Recovery: The recovery of active components were determined by adding the known amount of I, II and IV in the placebo of quadrinal suspension and tablet, and assaying the mixture by the described procedure. An average recovery of 100% added amount for

suspension was 103.33%, 103.93% and 103.56%, and for tablet 101.4%, 100.56% and 97.79% corresponding to peaks I, II and IV, respectively. No interference from placebo was observed in chromatograms. The spiked placebo of quadrinal suspension and tablets showed early eluting potassium iodide and calcium salicylate peaks and a late eluting peak of methyl paraben in suspension only. No attempt was made to quantitate these peaks. Recovery which was also determined at 80% and 120% label claim, demonstrated that the method is linear. This data is presented in Table 2.

Limits of Detection: Standard solutions ranging from 0.03% to 0.001% of 100% were injected into the HPLC. Component I showed reproducibility up to a 0.001% level with % RSD 3.95, component II at 0.02% level showed % RSD 4.11 and component IV was detected at 0.01% level with % RSD 6.32.

TABLE 2

Recovery Data and Percent Label Claim

Component	PERCENT CLAIMED					
	80%	% RSD	100%	% RSD	120%	% RSD
	Suspension Percent Recovered (a)					
I	83.15	0.17	103.33	0.14	121.89	0.17
II	84.92	0.86	103.93	0.32	117.38	0.82
IV	86.77	0.25	103.56	0.06	124.75	0.42
	Tablet Percent Recovered (a)					
I	80.90	0.02	101.40	0.28	121.73	0.50
II	80.47	0.02	100.56	0.004	124.41	0.60
IV	77.46	0.14	97.79	0.15	114.41	0.52

(a) Average of two injections

The minimum amount detected at a sensitivity of 0.01 aufs and ratio peak to noise (S/N) greater than 12 were 1.2 ng, 9.6 ng and 4.8 ng corresponding to component I, II and IV, respectively.

Method Precision: The method was tested on four different lots including two lots of Conventional[20] tablets from different manufacturer and one lot each of Quadrinal[21] suspension and Quadrinal tablets. The samples were prepared by the described procedure. As shown in Table 3, the results obtained are in good

TABLE 3

Method Precision Data of Suspension & Tablet Dosage Form

Dosage Forms	Components	Percent Found	% RSD
Quadrinal Suspension[a,c]	I	100.74	0.05
	II	100.50	0.04
	IV	98.63	0.67
Quadrinal Tablets[a,c]	I	101.69	0.53
	II	101.27	0.79
	IV	96.27	0.92
Conventional Tablets[b,e]	I	98.81	0.21
	II	99.78	0.58
	IV	98.18	0.63
Conventional Tablets[d,e]	I	100.23	0.21
	II	100.27	0.95
	IV	101.57	0.92

a Knoll Pharm. Co.

b Tedral, Park Davis

c Average of Two Injection

d Primatine, Whitehall Labs

e Average of Six Injection

agreement with the label claim amount. This also shows that the method gives accurate results with satisfactory precision.

In essence this HPLC method provides an easy and accurate technique for simultaneous separation and quantitation of theophylline, ephedrine hydrochloride and phenobarbital in suspensions and tablets dosage form. The absorptivity of ephedrine is improved significantly by this procedure which eliminates an extra step of derivatization during the analysis (2), or oxidation of ephedrine in the sample preparation as required in the previous method (3), and therefore ephedrine can be measured directly from this method. The large difference in concentration of theophylline and phenobarbital presented no problem in keeping all the peaks on recorder scale because the wavelength is shifted away from the maxima of theophylline which necessitated the change of attenuation during analysis in the previous HPLC method (4). This method is being routinely used in this quality control laboratory.

FOOTNOTES

[1] M-6000A Waters Associates, Milford, MA 01757
[2] 710B WISP, Waters Associates
[3] M-481 LC Spectrophotometer, Waters Associates, Milford, MA 01757
[4] 730 Data Module, Waters Associates, Milford, MA 01757
[5] u-Bondapak C18, Waters Associates, Milford, MA 01757
[6] Z-Module, Waters Associates, Milford MA 01757
[7] Guard Column, Part #84550, Waters Associates, Milford MA 01757
[8] Bondapak C18/Corasil 37-50 microns, Waters Associates, Milford MA 01757
[9] Potassium Phosphate HPLC grade, Fisher Scientific
[10] Methanol HPLC grade, J.T. Baker Company
[11] Water HPLC grade from Milli-Q-System, Millipore Corp, Bedford,MA
[12] United States Pharmacopeial Convention, Rockville, MD
[13] Part #HVLP 047, Millipore Corp., Bedford, MA 01730
[14] Theophylline USP reference standard
[15] Ephedrine Sulfate USP reference standard
[16] Phenobarbital USP reference standard
[17] 4 mL vial assembly. Part #73018, Waters Associates, Milford, MA 01757

[18] The ratio of the molecular weights of theophylline hydrous versus theophylline anhydrous is 1.1 and the ratio of ephedrine hydrochloride versus one half ephedrine sulfate is 0.941. These ratio are used as a factor.

[19] Knoll Pharmaceutical Company

[20] USP tablets contain 130 mg, 24 mg and 8 mg of theophylline, ephedrine and phenobarbital, respectively.

[21] Quadrinal Suspension and Tablets, Knoll Pharm. Co., contain 32.5 mg and 65 mg of theophylline, 12 mg and 24 mg each of ephedrine and phenobarbital 160 mg and 320 mg of potassium iodide in suspension and tablet, respectively.

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