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High-Pressure Liquid Chromatographic Assay of Theophylline, Ephedrine Hydrochloride, and Phenobarbital Tablets

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Abstract □ An innovative high-pressure liquid chromatographic method is described in which theophylline, ephedrine hydrochloride (measured as benzaldehyde), and phenobarbital are determined simultaneously with butabarbital as the internal standard. Chromatographic conditions were selected to afford a pH sufficient for rapid oxidation of ephedrine and relatively high UV absorbance for the barbiturates and a detection wavelength near the maximum for benzaldehyde and the barbiturates and the minimum for theophylline. Chromatograms show peaks from iodate, theophylline, phenobarbital, butabarbital, and benzaldehyde, in order of increasing retention time, all within the dynamic range of a conventional recorder. Procedures are provided for the assay of conventional and sustained-action tablet formulations.

Keyphrases □ High-pressure liquid chromatography—simultaneous determination of theophylline, ephedrine hydrochloride, and phenobarbital, conventional and sustained-action tablet formulations □ Theophylline—simultaneous determination with ephedrine hydrochloride and phenobarbital by high-pressure liquid chromatography, conventional and sustained-action tablet formulations □ Ephedrine hydrochloride—simultaneous determination with theophylline and phenobarbital by high-pressure liquid chromatography, conventional and sustained-action tablet formulations □ Phenobarbital—simultaneous determination with theophylline and ephedrine hydrochloride by high-pressure liquid chromatography, conventional and sustained-action tablet formulations

The theophylline, ephedrine hydrochloride, and phenobarbital tablet is recognized in USP XX (1), which provides a laborious assay in which the drug components are separated by chromatography on two partition columns and solvent extraction and then determined by UV spectrometry. Elefant *et al.* (2) described a GLC assay for this formulation, and Schultz and Paveenbampen (3) reported one for a similar suspension dosage form. Both methods require derivative formation, and neither has proved satisfactory in speed and convenience.

High-pressure liquid chromatography (HPLC) affords easy separation of the three active components of the formulations; however, the enormous differences in their relative amounts and in their UV absorption maxima and absorptivities make their simultaneous determination by HPLC with UV detection a challenging analytical problem. This report describes an innovative solution to this problem, affording peaks within one dynamic range span of a recorder.

BACKGROUND

Conventional tablets, the USP formulation, declare 130 mg of hydrous theophylline, 24 mg of ephedrine hydrochloride, and 8 mg of phenobarbital; similar proportions are in sustained-action tablets. Hydrous theophylline shows a UV maximum at ~271 nm with an absorptivity (liters per gram centimeter) of ~48. Its UV spectrum is not affected greatly by pH. In acidic solution, ephedrine and phenobarbital have weak UV spectra due to their benzene ring structure, with maxima at ~256 nm and absorptivities of ~1.

Penner[1] developed a normal-phase HPLC method using detection at 254 nm and an attenuation change between elution of the phenobarbital and theophylline peaks to keep the latter on the recorder scale. Suraski and DiPede[2] developed this method further, using a separate injection of greater dilution to keep the theophylline peak within the recorder dynamic range. They suggested that use of a computing integrator could allow for one injection of all three drug components. In this method, ephedrine and phenobarbital are determined simultaneously and theophylline is determined separately, using the same chromatographic system. It is also possible to determine phenobarbital and theophylline together and ephedrine separately. The UV maximum of phenobarbital can be shifted to ~240 nm with an absorptivity of ~43 by raising the pH to 9–11, where the predominant UV chromophore is the monoanion of the ureide ring; however, a high pH is incompatible with HPLC column

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stability. The UV spectrum of ephedrine is not affected significantly by changes in pH.

One solution to the problems of simultaneous determination of all three drugs in conventional and sustained-action tablets is described here. A reversed-phase HPLC method was developed with butabarbital as the internal standard. The internal standard solution contains periodate, which oxidizes ephedrine to benzaldehyde without affecting theophylline or the barbiturates. UV detection is at 241 nm, at the maximum for the barbiturates and benzaldehyde and near the absorption minimum for theophylline, thus minimizing the concentration difference. All three drug components and the internal standard are on scale so the method can be employed using peak responses from a recorded chromatogram as well as from computerized data reduction systems.

EXPERIMENTAL

Apparatus—The HPLC instrument included a solvent delivery system[3], a microparticulate reversed-phase column of octadecylsilane bonded on silica[4], and a UV detector[5] set at 241 nm. An automatic sampler[6] and a computing integrator[7] were used.

Mobile Phase—Acetonitrile (240 ml) was mixed with pH 7.8, 0.01 *M* phosphate buffer (760 ml).

Internal Standard—Butabarbital sodium (25 mg) was dissolved in 1.7% (w/v) dibasic potassium phosphate solution (50 ml). Then 40 ml of this solution was mixed with 10 ml of 1% (w/v) sodium metaperiodate solution.

Standard Preparation—*Tablets*—About 120 mg of theophylline USP reference standard, 8 mg of phenobarbital USP reference standard, and 25 mg of ephedrine sulfate USP reference standard were weighed accurately and transferred to a 200-ml volumetric flask. The standards were dissolved in 10 ml of methanol and ~100 ml of chloroform and diluted to the mark with chloroform. Then 15.0 ml of this solution was pipetted into a 50-ml volumetric flask and diluted to volume with chloroform.

Sustained-Action Tablets—About 204 mg of theophylline USP reference standard, 25 mg of phenobarbital USP reference standard, and 53 mg of ephedrine sulfate USP reference standard were weighed accurately into a 200-ml volumetric flask. This mixture was dissolved in 10 ml of methanol and ~100 ml of chloroform and diluted to volume with chloroform. Then 20.0 ml of this solution was pipetted into a 100-ml volumetric flask and diluted to volume with chloroform.

Assay Preparation—*Tablets*—Not less than 20 tablets were weighed and finely powdered. A portion of the powdered tablets equivalent to about one average tablet weight was weighed accurately, transferred to a 200-ml volumetric flask, and shaken mechanically for 20 min with 10 ml of methanol and 100 ml of chloroform. Chloroform was added to the mark, the solution was mixed and filtered, and exactly 15.0 ml of the filtrate was diluted to 50 ml in a volumetric flask with chloroform.

Sustained-Action Tablets—Not less than 20 tablets were weighed and finely powdered. An amount of powder equivalent to about one average tablet weight was weighed accurately, transferred to a 200-ml volumetric flask, and shaken mechanically for 20 min with 10 ml of methanol and 100 ml of chloroform. Chloroform was added to the mark, and the solution was mixed and filtered. Then 20.0 ml of the filtrate was pipetted into a 100-ml volumetric flask and diluted to volume with chloroform.

Procedure—The assay preparation (10.0 ml) and standard preparation (10.0 ml) were pipetted into 25-ml glass-stoppered conical flasks and evaporated to dryness in a warm water bath with the aid of a stream of air. The residues were dissolved in 4.0 ml of the internal standard solution, and the flasks were stoppered and mixed. Then they were permitted to stand at room temperature for 30 min. Then 1.0 ml of 1% (v/v) aqueous propylene glycol solution was added to each flask, and the flasks were stoppered and mixed.

System Suitability—*Resolution*—A flow rate of 1.0 ml/min for the mobile phase was set, and the system was allowed to equilibrate until a stable baseline was obtained on the recorder. Then 10 μl of the standard preparation solution was injected into the chromatograph at 0.24-aufs detection sensitivity when the recorder was used or at a suitable attenuation when a computing integrator was used. (An attenuation setting of 16 was used for the instrument employed in these studies[7].)

In order of increasing elution time, the five peaks are iodate (from the reagent), theophylline, phenobarbital, butabarbital, and benzaldehyde (from ephedrine). They should be completely resolved, with resolution factors between each pair of neighboring peaks of not less than 1.5. The resolution factor, R, is calculated from $2(tr_y - tr_x)/(W_y + W_x)$, where tr_y and tr_x are the retention times for neighboring peaks x and y, the latter having the longer retention time, and W_x and W_y are the peak widths at the baseline for the respective peaks.

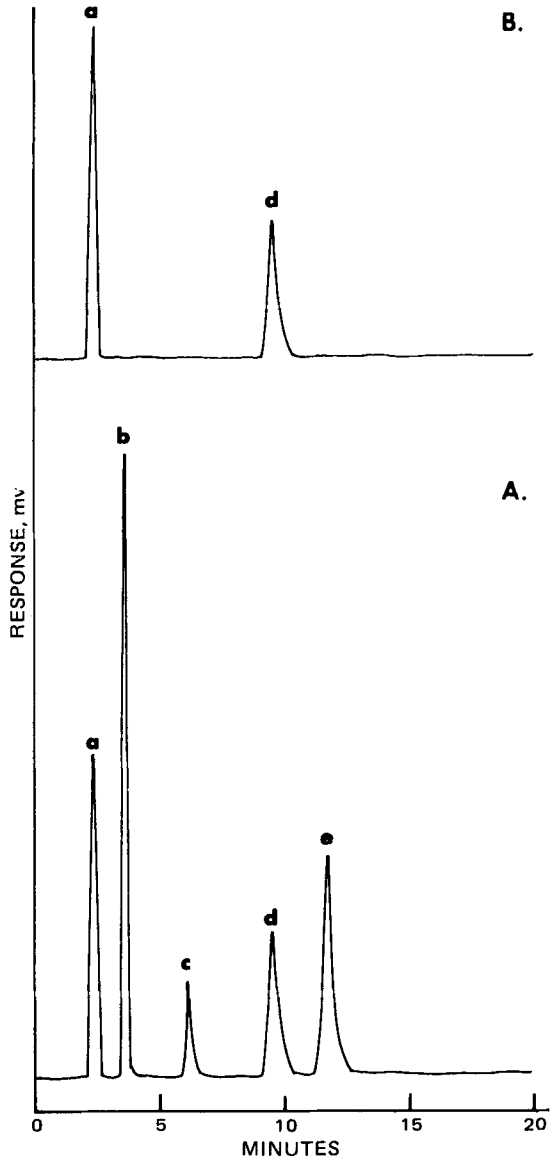

Figure 1—*Chromatograms of internal standard (B) and standard preparation (A). Key: a, iodate; b, theophylline; c, phenobarbital; d, butabarbital; and e, ephedrine as benzaldehyde.*

Linearity—Solutions at concentration levels of about 80, 90, 100, 110, and 120% of the appropriate standard preparation were prepared. Then each solution (10 μl) was injected into the liquid chromatograph. The concentrations of each active component were plotted against the respective ratios of its peak response to that of the internal standard. A straight line should be obtained in each instance.

Assay—Alternately 10 μl of the solutions from the standard preparation and the assay preparation was injected by means of a fixed-volume loop, and the peak responses were determined. The ratios of the peak responses were determined for theophylline, ephedrine hydrochloride as benzaldehyde, and phenobarbital to the internal standard in the chromatograms from the standard preparation and the assay preparation. The amount of each active ingredient, in milligrams per tablet, was calculated from $FC(R_U/R_S)(T/S)$, where C is the exact concentration, in milligrams per milliliter, of the assayed component in the standard preparation; R_U and R_S are the response ratios for each to the internal standard for the chromatograms from the assay preparation and the

[3] Waters Associates model 6000A.
[4] μBondapak ODS (30 cm × 4 mm), Waters Associates.
[5] Perkin-Elmer model LC-55.
[6] Perkin-Elmer model LC-420.
[7] Spectra-Physics model SP4100.

Table I—Dilution Factors [a]

Drug	Conventional Tablets	Sustained-Action Tablets
Theophylline	733.3	1000
Ephedrine hydrochloride	627.3	941
Phenobarbital	667	1000

[a] The theophylline factor includes 1.100, the ratio of the molecular weights of hydrous and anhydrous theophylline. The ephedrine hydrochloride factor includes 0.941, the ratio of the equivalent weights of the hydrochloride and sulfate salts.

standard preparation, respectively; T and S are the weights, in milligrams, of the average tablet and of the portion of the tablet taken for assay, respectively; and F represents the factors from dilutions (Table I).

RESULTS AND DISCUSSION

Figure 1 shows typical chromatograms for a conventional tablet formulation and a placebo mixture in which a reagent peak appears at ~2.3 min due to iodate, at ~3.5 min due to theophylline, at ~6.0 min due to phenobarbital, and at ~9.4 min due to butabarbital; benzaldehyde, from ephedrine, has a retention time of ~11.7 min. The chromatograms show no interference from the excipients and the oxidation products of propylene glycol, formaldehyde, and acetaldehyde.

Table II—Precision and Recovery Data

Trial	Percent Recovery: Theophylline	Ephedrine Hydrochloride	Phenobarbital
	Conventional Tablets		
1	98.4	99.2	97.8
2	99.4	97.6	100.8
3	98.1	98.0	96.6
4	98.7	100.1	99.6
5	100.3	99.2	100.0
6	98.4	97.5	96.3
Mean	98.9	98.6	98.5
RSD, %	0.83	1.07	1.91
	Sustained-Action Tablets		
1	98.6	98.3	98.1
2	101.4	100.7	100.3
3	99.3	100.0	99.7
4	100.7	100.0	99.7
5	100.0	101.4	100.3
6	100.0	99.3	99.7
Mean	100.0	100.0	99.6
RSD, %	0.99	1.08	0.81

Table III—Recovery Data at 80 and 120% Label Claim

Drug	Percent Found: 80	120
	Conventional Tablets	
Theophylline	82.2, 81.9	117.8, 119.5
Ephedrine hydrochloride	82.4, 82.6	120.3, 123.1
Phenobarbital	79.7, 79.0	121.4, 118.8
	Sustained-Action Tablets	
Theophylline	80.7, 80.7	117.9, 118.8
Ephedrine hydrochloride	79.4, 79.4	118.4, 118.6
Phenobarbital	79.1, 79.7	119.5, 120.3

The determination of ephedrine as benzaldehyde after periodate oxidation was shown to be stability indicating by Chafetz (4). Omission of periodate from the system had no effect on the retention times or response of theophylline, phenobarbital, or butabarbital. Ephedrine was moved to a retention time of ~20 min; however, its response was decreased by a factor of >200. Ephedrine can be visualized only by changing the detector to ~260 nm and using an increased concentration or lower attenuation.

The UV detection of phenobarbital and butabarbital at 241 nm is due to the monoanion form of the ureide ring, the unionized form having little absorbance in the near UV; hence, the incorporation of pH 7.8 buffer in the mobile phase. This mobile phase provides sufficient alkalinity to be in the pKa_1 range for the barbiturates and for a rapid reaction rate of periodate with ephedrine. (The apparent pH of the mobile phase is 8.3.)

Precision and recovery data for conventional and sustained-action theophylline, ephedrine hydrochloride, and phenobarbital tablets are shown in Tables II and III. Calculation of resolution factors between adjacent peaks provides values greater than 5. Peak response ratios *versus* concentrations for each component to the internal standard were rectilinear. Recovery was determined by adding known amounts of the drugs to the excipients, which included starch, lactose, stearic acid, and polyethylene glycol 6000 for conventional tablets and starch USP, lactose USP, stearic acid powder, polyethylene glycol 6000, guar gum, methylcellulose USP, sugar powder, ethylcellulose NF, magnesium stearate USP, carnauba wax, D&C Red No. 30 lake, and D&C Yellow No. 10 lake for sustained-action tablets.

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Catecholamine Analogs as Potential Antitumor Agents II

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Abstract □ Polyhydroxybenzylamine derivatives related to dopamine were synthesized and shown to have activity against murine P-388 lymphocytic leukemia. The 3,4,5-trihydroxy- and 2,3-dihydroxybenzylamine hydroiodides were active, as were several other catechol derivatives capable of *o*-quinone formation.

Keyphrases □ Catecholamine analogs—polyhydroxybenzylamine hydroiodides synthesized and evaluated for antitumor activity, mice □ Structure–activity relationships—catecholamine analogs synthesized and tested for antitumor activity, mice □ Antitumor activity—catecholamine analogs synthesized and evaluated for antitumor activity, mice

Phenalkylamines (1–4) and pyridine derivatives (5) possessing vicinal hydroxyl groups have antitumor activity against *in vivo* murine tumor models and related *in vitro* systems. A previous investigation (4) explored structure–

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