while the rest of the treated animals showed 48–77% inhibition of tumor growth as compared to the controls.

Figure 5 shows the effect of AB-163 against leukemia L1210 in BDF1 mice. In this experiment treatment was continued daily from day 1 to 11 post-inoculation. At optimal dose range, 25–30 mg./Kg., 20–30% of the animals survived for more than 45 days and are considered "cures." The remaining animals showed an average increase of survival time of 107–170%.

These results demonstrate that AB-163 is a much more effective compound against a spectrum of transplanted tumors than was AB-132(2).

EXPERIMENTAL

Into a 1-L. round-bottom flask equipped with a mechanical stirrer, pressure equalized addition funnel, thermometer, and a calcium chloride drying tube was introduced a solution of 15.6 Gm. (0.22 mole) of 2,2-dimethylaziridine, 22.2 (0.22 mole) of triethylamine in 350 ml. of toluene. The content of the flask was cooled to 0° by immersion in a mechanically refrigerated bath maintained at −3° to 0°, and a solution of 16.3 Gm. (0.1 mole) of ethyl dichlorophosphate in 100 ml. of toluene was slowly added. The reaction mixture was stirred for 24 hr. and then slowly warmed to room temperature and filtered. The precipitate contained nearly the theoretical amount of pure triethylamine hydrochloride. The filtrate was concentrated in a flash evaporator under reduced pressure, and the crude product was obtained as a viscous oil. The material was purified by high vacuum distillation and the analytically pure sample was obtained by collecting the fraction boiling at 70–71°/0.03–0.04 mm.; yield 17.3 Gm. (74%). This distillation had to be conducted with care because of the tendency of the material to decompose on overheating.

Anal.—Calcd. for $C_{10}H_{21}N_2O_2P$: C, 51.72; H, 9.05; N, 12.07. Found: C, 51.60; H, 9.18; N, 11.94.

Infrared absorption bands (Beckman IR-8): $\nu_{max.}^{CHCl_3}$ 2990, 1460 (broad), 1385, 1375, 1340, 1265, 1210 (broad), 1145, 1105, 1035, 960, 840 cm.$^{-1}$.

NMR absorption bands (in CCl_4, with tetramethylsilane as internal standard): 8.78 triplet (ester CH_3); 8.65 singlet (CH_3 ring-substituents); 8.05 doublet, P_H = 14 cps. (ring CH_2); 6.05, multiplet (ester CH_2).

Alkylation Studies—Comparative alkylating activities of I and AB-132 were determined at 80° by a previously described method (6).

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Determination of Ephedrine, Phenobarbital, and Theophylline in Tablets by Gas Chromatography

By MILTON ELEFANT, LESTER CHAFETZ, and JOSEPH M. TALMAGE

A gas chromatographic procedure is described for the rapid, specific, and precise determination of ephedrine, phenobarbital, and theophylline in tablets. Ephedrine is converted to benzaldehyde by periodate oxidation prior to chromatography, while the phenobarbital and theophylline are chromatographed directly after simple tablet disintegration in a co-solvent. Adaptation of the method to single tablet analysis is described.

THE PROBLEM of devising a convenient and accurate assay for combinations of ephedrine, a barbiturate, and theophylline or its complexes has become classical in the literature of pharmaceutical analysis. The literature has been reviewed by Connors (1) and, more recently, by Foreman and Blake (2). Most of the solutions described so far require lengthy and tedious liquid–liquid extractions, ion-exchange chromatography, and/or liquid–liquid partition chromatographic separations followed by quantitation of the separated drugs by gravimetry, titrimetry, or spectrophotometry. Since gas chromatography combines separation with quantitation, it appeared to be a singularly attractive means for assay of a tablet declaring ephedrine HCl, phenobarbital, and theophylline.

Gas chromatographic methods have been successfully applied to assay of barbiturates (3–7) and ephedrine (8–11); however, mixtures of ephedrine, phenobarbital, and theophylline present more difficulties in gas chromatography than the assay of the individual drugs because of their differences in functionality, polarity, and dose. The polar, strongly basic amine, ephedrine, tails badly on columns which give good separation and symmetrical peaks for the weakly acidic barbiturate and xanthine drugs. Ephedrine is quantitatively oxidized by periodate, and this observation has been made the basis of quantitative assays of the alkaloid by Wickström (12), who determined the acetaldehyde formed by colorimetry, and by Chafetz (13),

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Table I—Analysis of a Production Lot of Tablets

	Ephedrine HCl	Phenobarbital	Theophylline
Declared, mg./tablet	24.0	8.0	130.0
Found			
Chemical assay	23.7	7.9	130.0
GC assay trial *1*	23.4	7.9	132.0
2	23.6	7.8	135.0
3	23.8	7.8	135.0
4	23.8	7.8	129.0
5	23.6	7.7	133.8
GC av.	23.6	7.8	133.0
Relative S.D., %	±0.72	±0.9	± 1.9

who measured the benzaldehyde produced on oxidation by ultraviolet spectrophotometry.

Gas chromatography of the benzaldehyde produced by oxidation of ephedrine provided sharp peaks suitable for quantitation on a chromatographic column useful for phenobarbital and theophylline. Benzaldehyde conveniently chromatographed at 80° while the ureides are chromatographed at 250°. Moreover, it was difficult to find an oxidation medium for ephedrine which would serve also as a good solvent for eluting phenobarbital and theophylline from tablets. For these reasons, separate sample preparations and separate determinations were performed for ephedrine and the other two drugs. The use of separate chromatograms allowed use of closely resolved internal standards, with an advantage in precision and accuracy over programmed temperature technique. A procedure for assay of the three drugs in individual tablets is described.

EXPERIMENTAL

Instrumental Conditions—A Perkin-Elmer model 801 gas chromatograph equipped with dual flame ionization detectors and a Honeywell-Brown Electronik recorder was used. The column employed was a 6-ft. glass helix, 0.25 in. o.d., packed with 3% HI-EFF 8 BP on 100/120 mesh Gas Chrom Q (Applied Science Laboratories, Inc., State College, Pa.). Gas flow rates of 30 ml./min. for hydrogen and 40 ml./min. for helium were used.

Phenobarbital and Theophylline Assay—*Internal Standard*—Use an approximately 0.6 mg./ml. solution of 4,4′-methylene-bis-(*N*,*N*-dimethylaniline) (Eastman Organic) in equal volumes of chloroform and methanol.[1]

Mixed Standard—Dissolve accurately weighed amounts of about 13 mg. of phenobarbital and 200 mg. of anhydrous theophylline in 25.0 ml. of the internal standard solution.

Sample Preparation—Determine the average weight of not less than 10 tablets, and reduce the sample to a fine powder. Weigh accurately the equivalent of one average tablet weight of the composite sample, and transfer it to a 50-ml. glass-stoppered centrifuge tube. Add exactly 15.0 ml. of internal standard solution, stopper the mixture, and shake vigorously for 5 min. Centrifuge the mixture and use the clear supernatant solution for chromatography.

[1] The compound should be stored under nitrogen after opening to protect it from oxidation.

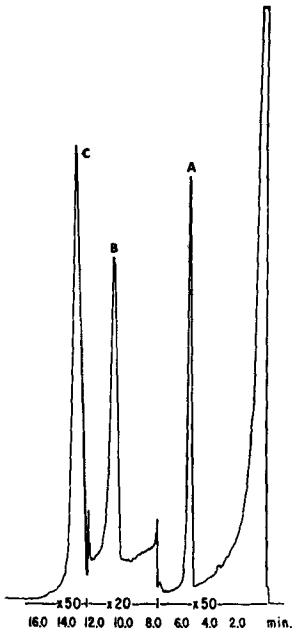

Fig. 1—Key: A, *internal standard;* B, *phenobarbital;* C, *theophylline.*

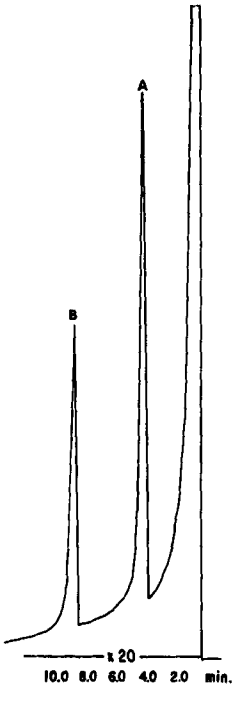

Fig. 2—Key: A, *benzaldehyde;* B, *internal standard.*

Procedure—Set the injector temperature at 325° and the column temperature at 250°. Inject in turn 2-μl. volumes of the sample and standard solutions at preselected attenuation settings.

Ephedrine Determination—*Internal Standard*—Use a solution of 0.06 ml. of benzyl alcohol in 100 ml. of ethyl acetate.

Standard Preparation—Dissolve approximately 65 mg. of ephedrine HCl, accurately weighed, in 25.0 ml. of saturated sodium bicarbonate solution.

TABLE II—ASSAY OF INDIVIDUAL TABLETS

Trial	Ephedrine HCl	Phenobarbital	Theophylline
1	23.6	7.7	127.0
2	23.2	7.6	128.0
3	24.1	7.7	127.0
4	23.9	7.6	127.0
5	23.8	7.7	127.0
6	23.8	7.6	127.0
7	23.6	7.8	130.0
8	23.6	7.8	126.0
9	23.4	7.7	126.0
10	23.8	7.8	126.0
Av.	23.7	7.7	127.0
Relative S.D.	1.1%	1.0%	0.9%

Pipet 10.0 ml. into a glass-stoppered centrifuge tube.

Sample Preparation—Accurately transfer the equivalent of one average tablet weight of composite sample into a glass-stoppered centrifuge tube. Add exactly 10.0 ml. of saturated sodium bicarbonate solution.

Procedure—Add 2.0 ml. of 10% sodium metaperiodate to the sample and standard preparations and shake 5 min. Pipet 10.0 ml. of internal standard solution into each tube, stopper, and shake for 3 min. Centrifuge the mixtures, and use the clear upper phases for chromatography.

Set the injector block temperature at 175° and the column temperature at 80°, and inject approximately 0.5 μl. of sample and standard solutions in turn. Program the column temperature at 6°/min. to a final temperature of 130° immediately after injection of each sample.

Assay of Individual Tablets—The procedures described above were employed with one modification; the single tablet was dispersed in the appropriate solvent by immersing the centrifuge tube in an ultrasonic cleaning bath (Maxomatic Ultrasonic, L. & R. Manufacturing Co., Kearny, N.J.).

Quantitation—Peak heights were employed according to the method described by Celeste and Turczan (14).

Sample—A commercially available tablet,[2] which has a label declaration of 24 mg. ephedrine HCl, 8.0 mg. phenobarbital, and 130 mg. of theophylline, was the only test preparation employed.

[2] Tedral is a registered trademark of Warner-Chilcott Laboratories, Morris Plains, N.J.

RESULTS

A production lot of tablets was analyzed by the gas chromatographic procedure, and the results were compared with values obtained by the conventional quality control methods. The data are presented in Table I. Figures 1 and 2 show typical chromatograms for phenobarbital and theophylline and for ephedrine, respectively.

The method was employed with equal success for the assay of individual tablets. The data obtained from assay of 10 individual tablets are presented in Table II. The average results and the relative standard deviations obtained do not differ appreciably from those obtained from powdered composite tablets.

SUMMARY

A gas chromatographic method has been developed for the determination of ephedrine, phenobarbital, and theophylline, and its application to the assay of composite and individual tablets has been described for a commercial tablet dosage form. Ephedrine is chromatographed after quantitative periodate oxidation to benzaldehyde at a lower temperature than the ureide drugs; thus, the method requires two sample preparations, two chromatograms, and one chromatography column. The procedure provided relative standard deviations of about 1% for each of the components of individual tablets, and it is rapid and selective.

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