Simultaneous High-Performance Liquid Chromatographic Determination of Theophylline, Ephedrine Hydrochloride, and Phenobarbital in Tablets

HENRY S. I. TAN [x], **PRAKONGSIRI C. BOONCONG, and SHARON L. FINE** *

Received June 13, 1980, from the *College of Pharmacy, University of Cincinnati–Medical Center, Cincinnati, OH 45267.* Accepted for publication December 8, 1980. *Present address: George Washington University Hospital, Washington, DC 20037.

Abstract □ A rapid reversed-phase high-performance liquid chromatographic (HPLC) method was developed for the simultaneous assay of theophylline, ephedrine hydrochloride, and phenobarbital in a tablet matrix. A methanolic extract of the powdered sample containing salicylamide as the internal standard was injected into the chromatograph. The HPLC system used methanol–0.007 *M* monobasic potassium phosphate (37:63, pH 2.3) as the mobile phase. Detection was at 254 nm, and quantitation was based on the drug–internal standard peak area ratio. This ratio was linear over a concentration range of 20.9–83.5 μg of theophylline, 4–15.9 μg of ephedrine hydrochloride, and 1.3–5.1 μg of phenobarbital. Overall recoveries (±*SD*) from three synthetic tablets were: theophylline, 99.8 ± 1.3%; ephedrine hydrochloride, 100.2 ± 0.6%; and phenobarbital, 99.4 ± 0.7%. The method was compared to the compendial method and also was applied to the assay of commercial tablets containing these three active ingredients. The proposed method is applicable to the assay of individual tablets.

Keyphrases □ High-performance liquid chromatography—simultaneous assay of theophylline, ephedrine hydrochloride, and phenobarbital □ Sedatives—simultaneous high-performance liquid chromatographic assay of theophylline, ephedrine hydrochloride, and phenobarbital □ Bronchodilators—simultaneous high-performance liquid chromatographic assay of theophylline, ephedrine hydrochloride, and phenobarbital

Tablets containing a mixture of theophylline, ephedrine hydrochloride, and phenobarbital are used as bronchodilators and sedatives. This dosage form was official in NF XIV and then was admitted to USP XX. No changes were made in the assay upon admission to USP XX, except that ephedrine sulfate instead of the hydrochloride now is used as the standard (1).

BACKGROUND

The compendial method is laborious and time consuming (~10 hr), involving the passage of the sample solution through two liquid chromatographic columns followed by partial or complete evaporation of the several liquid fractions obtained. The method also involves solvent extraction of one of the eluates, resulting in separation of the mixture into three fractions, each containing a single drug. The fractions are measured by UV spectroscopy.

Lach *et al.* (2) analyzed these tablets by UV spectroscopy, but a separate tablet sample was required for the assay of each drug. Measurement was made after each drug was isolated from the tablet matrix by a different solvent extraction.

GLC methods for the analysis of mixtures of these three drugs in tablets or suspensions were previously reported (3, 4). In these reports, ephedrine generally was assayed separately, and the phenobarbital and theophylline were chromatographed directly after solvent extraction. Ephedrine was converted to benzaldehyde prior to chromatography by treating a second sample with periodate (3). The suspensions were assayed by separating the sample into two fractions, one containing ephedrine and the other containing theophylline and phenobarbital, which then were gas chromatographed (4).

Quantitative determinations of single entities of theophylline or phenobarbital in various dosage forms and biological samples based on reversed-phase high-performance liquid chromatography (HPLC) were published previously and reviewed (5, 6). Some reversed-phase HPLC assays for ephedrine hydrochloride also were reported (7, 8).

This paper presents the simultaneous assay of theophylline, ephedrine hydrochloride, and phenobarbital in tablet mixtures by reversed-phase HPLC with salicylamide as the internal standard. The HPLC method is simple and rapid and is also more accurate and reproducible than the compendial method.

EXPERIMENTAL

Apparatus—The high-performance liquid chromatograph[1] was equipped with a constant-flow pump[2], a valve-type injector[3], a fixed-wavelength (254-nm) UV detector[4], and a strip-chart recorder[5].

Reagents and Materials—*l*-Ephedrine hydrochloride[6], theophylline[7], salicylamide[7], phenobarbital[8], 0.007 *M* KH_2PO_4 in water (adjusted to pH 2.3 with 85% aqueous phosphoric acid), and methanol[9] were used. Other chemicals used were analytical grade.

Chromatographic Conditions—A 25-cm × 4.5-mm i.d. 10-μm Partisil ODS II ITP column[10] was used at ambient temperature. The mobile phase was methanol–0.007 *M* KH_2PO_4 (pH 2.3, 37:63). This solution was filtered through a 5-μm membrane filter[11], degassed, and then pumped through the HPLC system at a rate of 1.2 ml/min. The detector was attenuated to 2.0 aufs for theophylline and salicylamide and to 0.05 aufs for ephedrine hydrochloride and phenobarbital.

Internal Standard Solution—The internal standard was salicylamide prepared as a 12-mg/ml solution in methanol.

Preparation and Assay of Standard Solution—Approximately 260 mg of theophylline, 50 mg of ephedrine hydrochloride, and 16 mg of phenobarbital were weighed accurately into a 100-ml volumetric flask. After ~25 ml of methanol was added, the mixture was placed in an ultrasonic bath for 5 min and the resulting solution was diluted to volume with methanol. Twenty milliliters of this solution was pipetted into a 25-ml volumetric flask. After addition of 1.0 ml of the internal standard solution, the solution was diluted to the mark with methanol and filtered through a 5-μm membrane filter[11]. By using a 25-μl syringe[12], three 20-μl portions of the filtrate were chromatographed under the described HPLC conditions and the average drug–salicylamide peak area ratio was determined.

Assay of Theophylline, Ephedrine Hydrochloride, and Phenobarbital Tablets—Twenty tablets were weighed and finely powdered. A portion of the powder equivalent to 8 mg of phenobarbital was weighed accurately and transferred to a 50-ml volumetric flask. Approximately 20 ml of methanol was added, and the mixture was placed in an ultrasonic bath for 5 min and then diluted to volume with methanol. Twenty milliliters of this mixture was pipetted into a 25-ml volumetric flask and treated as already described.

RESULTS AND DISCUSSION

A study of theophylline, ephedrine hydrochloride, and phenobarbital tablets from 13 manufacturers revealed that 45% of the tablet lots were not chemically equivalent, indicating a need for improvement in manufacturing and quality control (2). An assay for quality control use, par-

[1] Model ALC/GPC 244, Waters Associates, Milford, Mass.
[2] Model 6000A, Waters Associates, Milford, Mass.
[3] Model U6K, Waters Associates, Milford, Mass.
[4] Model 400, Waters Associates, Milford, Mass.
[5] Omniscribe Series A-5000, Houston Instruments, Austin, Tex.
[6] Sigma Chemical Co., St. Louis, Mo.
[7] Eastman Kodak Co., Rochester, N.Y.
[8] USP grade, Merck & Co., Rahway, N.J.
[9] HPLC grade, Fisher Scientific Co., Fair Lawn, N.J.
[10] Jones Chromatography Inc., Columbus, Ohio.
[11] Mitex type LS, Millipore Corp., Bedford, Mass.
[12] Series B-110, Precision Sampling Corp., Baton Rouge, La.

0022-3549/81/0700-0783$01.00/0
© 1981, American Pharmaceutical Association

Table I—Recovery Data from Synthetic Tablets

Tablet Component	Amount Weighed, mg/tablet	Amount Found[a], mg/tablet		Average Recovery, %	
		HPLC Method	USP XX Method	HPLC Method	USP XX Method
A Theophylline	129.3	129.4, 129.6	129.2, 130.2	100.2	100.3
Ephedrine hydrochloride	24.1	24.0, 24.1	26.2, 26.3	99.8	108.5
Phenobarbital	8.1	8.1, 8.1	8.6, 8.7	100.0	106.8
B Theophylline	130.1	131.0, 131.3	131.7, 132.7	100.8	101.6
Ephedrine hydrochloride	24.1	24.0, 24.2	25.4, 26.5	100.0	107.7
Phenobarbital	8.1	8.0, 8.1	8.9, 9.1	99.4	111.1
C Theophylline	121.3	119.1, 119.3	121.4, 122.4	98.3	100.5
Ephedrine hydrochloride	23.0	23.2, 23.2	24.6, 24.7	100.9	107.2
Phenobarbital	7.6	7.5, 7.5	8.2, 8.2	98.7	107.9

[a] Values of duplicate assays.

Table II—Recovery Data from Commercial Tablets [a]

Tablet Component	Amount Found[b], mg/tablet		Label Claim, %	
	HPLC Method	USP XX Method	HPLC Method	USP XX Method
A Theophylline	115.8, 116.3	118.4, 120.5	89.3	91.9
Ephedrine hydrochloride	24.2, 24.2	25.3, 25.8	100.8	106.5
Phenobarbital	7.8, 7.9	8.8, 8.9	98.2	110.7
B Theophylline	119.9, 121.5	118.5, 118.5	92.9	91.2
Ephedrine hydrochloride	24.1, 24.2	25.1, 25.4	100.6	105.2
Phenobarbital	8.3, 8.3	8.5, 8.5	103.8	106.3
C Theophylline	118.5, 119.2	—	91.5	—
Ephedrine hydrochloride	24.0, 24.1	—	100.2	—
Phenobarbital	8.3, 8.3	—	103.8	—

[a] Label claim per tablet: theophylline, 130 mg; ephedrine hydrochloride, 24 mg; and phenobarbital, 8 mg. [b] Values of duplicate assays.

ticularly for content uniformity and dissolution tests, should be simple, convenient, rapid, and accurate.

Theophylline, ephedrine hydrochloride, and phenobarbital vary greatly in acidity and polarity. In addition, the polar, strongly basic amine ephedrine has low molar absorptivity at its wavelengths of maximum absorption of 252, 257, and 263 nm. In the mobile phases that gave good symmetrical peaks for weakly acidic theophylline and phenobarbital and good separation between the three drugs at various pH values, ephedrine showed small, broad, and unsymmetrical peaks. However, the mobile phase of methanol–0.007 M KH_2PO_4 (pH 2.3, 37:63) produced a sharp and symmetrical ephedrine peak.

The proposed HPLC method simultaneously assays the three drugs; the tablet sample is dissolved in methanol, and this solution is injected into the chromatograph in the presence of salicylamide as the internal standard. The peaks of the four compounds were well resolved (Fig. 1), although it was necessary to change the attenuation of the detector during the run due to the relatively lower ephedrine hydrochloride and phenobarbital concentrations. Under the experimental conditions, the retention times were: ephedrine hydrochloride, ~3.3 min; theophylline, ~7.5 min; salicylamide, ~10.5 min; and phenobarbital, ~17 min. The average height equivalent to a theoretical plate ($\pm SD$) of the column ranged from 1.47 ± 0.05 mm for ephedrine hydrochloride, to 0.80 ± 0.03 mm for theophylline, to 0.34 ± 0.02 mm for phenobarbital ($n = 10$).

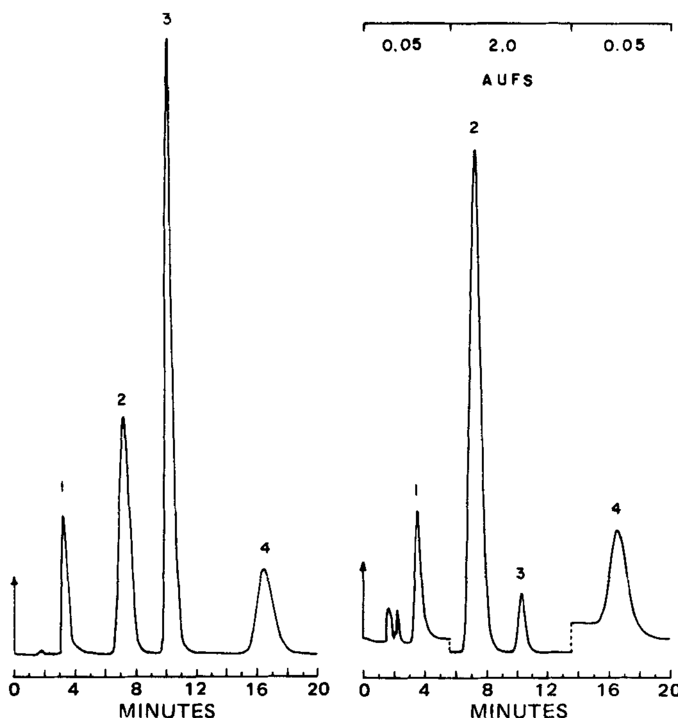

Figure 1—*Chromatogram of a mixture of ephedrine hydrochloride (1), theophylline (2), salicylamide (3), and phenobarbital (4) in methanol at 2.0 aufs.*

Figure 2—*Chromatogram of a commercial tablet. Key: 1, ephedrine hydrochloride; 2, theophylline; 3, salicylamide (internal standard); and 4, phenobarbital.*

Quantitation was based on the drug–internal standard peak area ratio. With these ratios, the linearity between the detector response at 254 nm and amount of drug injected was established for the three drugs. Linearity was obtained between 20.9 and 83.5 μg of theophylline, 4 and 15.9 μg of ephedrine hydrochloride, and 1.3 and 5.1 μg of phenobarbital. The working amount was 41.6 μg of theophylline, 7.7 μg of ephedrine hydrochloride, and 2.6 μg of phenobarbital. The regression equations ($\pm SD$, $n = 3$) for the drug–internal standard peak area ratio (A) and amount of drug injected (B, expressed in milligrams) were $A = (221.29 \pm 2.09)B + (0.90 \pm 0.11)$ for theophylline, $A = (5.24 \pm 0.13)B + (0.003 \pm 0.001)$ for ephedrine hydrochloride, and $A = (29.29 \pm 0.54)B - (0.000 \pm 0.002)$ for phenobarbital. The correlation coefficients ($\pm SD$, $n = 3$) were 0.9987 ± 0.0009, 0.9997 ± 0.0004, and 0.9997 ± 0.0001 for theophylline, ephedrine hydrochloride, and phenobarbital, respectively.

A tablet mixture containing the same amounts of theophylline, ephedrine hydrochloride, and phenobarbital as in the commercial tablet was prepared. When the tablet placebo[13] was subjected to the assay, a small peak appeared on the chromatogram (retention time of ~2 min), which could be attributed to one or more ingredients in the placebo. No peaks were observed in the region of the peaks of salicylamide and the three active ingredients. The recovery data from the synthetic tablets are presented in Table I. The overall recoveries ($\pm SD$) from three synthetic tablet mixtures were 99.8 ± 1.3% for theophylline, 100.2 ± 0.6% for ephedrine hydrochloride, and 99.4 ± 0.7% for phenobarbital.

For comparison, the synthetic tablets also were analyzed by the compendial method. Except for theophylline, the recoveries for ephedrine hydrochloride and phenobarbital were consistently higher (Table I). The compendial overall recoveries ($\pm SD$, $n = 3$) were 100.8 ± 0.7%, 107.8 ±

[13] The composition of the tablet placebo was: cornstarch, 52.66%; confectioners sugar, 38.89%; dextrose, 6.78%; sodium bisulfite, 0.15%; and magnesium stearate, 1.52%.

0.7%, and 108.6 ± 2.2% for theophylline, ephedrine hydrochloride, and phenobarbital, respectively.

The HPLC method was applied to the assay of commercial tablets (Table II). Again, the compendial results for ephedrine hydrochloride and phenobarbital were higher than those obtained by the proposed HPLC method. By coincidence, the theophylline content in the three commercial tablet samples investigated was low; the values were around the minimum limit of the compendial potency requirement range of 90.0–110.0% of the label claim. Figure 2 shows a typical chromatogram of the assay solution from commercial tablets.

The HPLC method is more accurate and precise and considerably less time consuming than the compendial method. The wide detector response range between theophylline and phenobarbital was due to the large difference in concentration and posed no problems. The change in the detector attenuation during the chromatographic run did not affect the results. The wide variation in polarity, which necessitated the prior separation of ephedrine in the GLC methods (4, 5), presented no problems under the proposed experimental conditions. The method also is applicable to the assay of individual tablets since the procedure is based on the quantities of drugs present in one tablet.

REFERENCES

(1) "The United States Pharmacopeia," 20th rev., United States Pharmacopeial Convention, Rockville, Md., 1980, p. 784.
(2) J. L. Lach, T.-F. Chin, and E. L. Parrott, *Am. J. Hosp. Pharm.*, **29,** 1043 (1972).
(3) M. Elefant, L. Chafetz, and J. M. Talmage, *J. Pharm. Sci.*, **56,** 1181 (1967).
(4) H. W. Schultz and C. Paveenbampen, *ibid.*, **62,** 1995 (1973).
(5) J. L. Cohen, in "Analytical Profiles of Drug Substances," vol. 4, K. Florey, Ed., Academic, New York, N.Y., 1975, p. 466.
(6) M. K. Chao, K. S. Albert, and S. A. Fusari, in *ibid.*, vol. 7, 1978, p. 359.
(7) F. Bailey and P. N. Brittain, *J. Chromatog.*, **83,** 431 (1973).
(8) V. D. Gupta and O. H. Shek, *Am. J. Hosp. Pharm.*, **33,** 1086 (1976).

ACKNOWLEDGMENTS

Presented at the Pharmaceutical Analysis and Control Section, APhA Academy of Pharmaceutical Sciences, St. Louis meeting, April 1981.

Immunological Studies of Poisonous Anacardiaceae: Production of Tolerance in Guinea Pigs Using 3-*n*-Pentadecylcatechol-"Modified" Autologous Blood Cells

EDNA S. WATSON ˣ, JAMES C. MURPHY, PHILIP W. WIRTH, MAHMOUD A. ElSOHLY, and PAUL SKIERKOWSKI

Received July 7, 1980, from the *Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, University, MS 38677.* Accepted for publication December 15, 1980.

Abstract ☐ The development of contact sensitivity to poison ivy urushiol in guinea pigs was prevented by intravenous injection of 3-*n*-pentadecylcatechol (I) coupled to autologous blood cells. Hartley, linebred, guinea pigs were treated with pentadecylcatechol-"modified" blood cells or sham-treated blood cells 2 weeks prior to attempted topical sensitization with I. Skin testing of all guinea pigs with 3-, 1-, and 0.3-μg doses of I applied in 5 μl of acetone to abdominal skin sites was begun 2 weeks after attempted sensitization and repeated at 2- or 4-week intervals thereafter for 6 months or until study termination. Profound tolerance to I was observed at all skin testing intervals in the group receiving haptenated red cells and did not weaken substantially with time. Contact sensitivity to I in control animals, however, waned with time; the study was terminated at 6 months because of the low sensitivity level of the control animals at that period. Complete or partial tolerance was induced in ~80% of the treated animals. The immune tolerance obtained by the single injection of pentadecylcatechol-associated red blood cells was of long duration and urushiol specific. This treatment also conferred tolerance to three unsaturated congeners of I. The allergenic potencies of the pentadecylcatechols declined with increasing saturation of the alkyl side chain. Binding studies using tritiated pentadecylcatechol showed that 81% of the activity incorporated into the red cell was membrane associated and that 19% was cell sap associated.

Keyphrases ☐ Urushiol—production of tolerance using 3-*n*-pentadecylcatechol with autologous blood cells, guinea pigs ☐ Poison ivy—production of tolerance to urushiol using 3-*n*-pentadecylcatechol with autologous blood cells, guinea pigs ☐ 3-*n*-Pentadecylcatechol—production of tolerance to urushiol, guinea pigs ☐ Immunology—production of tolerance to urushiol using 3-*n*-pentadecylcatechol, guinea pigs

Poison ivy (*Toxicodendron radicans*), poison oak (*T. diversilobum* and *T. quercifolium*), and poison sumac (*T. vernix*) are the main causes of contact dermatitis in the United States. One or more of these species is present in almost every state in the continental United States. These plants are so prevalent and insidious that ~80% of the U.S. population is allergic to them and 50% are clinically sensitive, *i.e.*, react to 2 μg of urushiol or less (1).

BACKGROUND

The dermatitogenic principles contained in the resin of these plants are a group of chemically related catechols, commonly referred to as urushiols, differing mainly in the length and degree of unsaturation of the 3-*n*-alk(en)yl side chain. Poison ivy urushiol was shown to be mainly (>95%) a mixture of 3-*n*-pentadec(en)ylcatechols with zero, one, two, or three double bonds in the C_{15} side chain (2–7). Poison oak urushiol, however, consists mainly (>98%) of the C_{17} homologs (2, 3, 6). A small percentage of the C_{15} congeners was found in poison oak urushiol, and a small percentage of the C_{17} homologs was found in poison ivy components (2, 3, 6). The analysis and identification of poison sumac urushiol are incomplete.

Contact of these catechols with the skin of susceptible individuals results in sensitization to all urushiols of the plant family Anacardiaceae (8–10). Once sensitivity is developed, it is difficult, if not impossible, to eliminate. Hyposensitization by administration of plant extracts is not regularly obtained. It requires large doses and months or years to be produced, and sensitivity is rapidly regained upon cessation of treatment (10, 11). The albino guinea pig is the animal of choice for studying sensitivity to these allergens (12). Tolerance to poison ivy was reported to be produced in guinea pigs by subcutaneous injection of pentadecylcatechol (I) in mineral oil or by oral administration of large doses of I prior to attempted sensitization (13). However, since urushiols and I itself are potent primary irritants and skin sensitizers, their use for producing tolerance in nonsensitive humans is heavily compromised.

Recently, a series of ring-substituted derivatives of I was studied for potential use in the production of immune tolerance to contact sensitivity in guinea pigs (14, 15). Some 6-substituted derivatives were reported to

0022-3549/81/0700-0785$01.00/0
© 1981, American Pharmaceutical Association