REFERENCES[5]

(1) J. L. Lach and T. F. Chin, *J. Pharm. Sci.*, **53**, 69 (1964).
(2) W. A. Pauli and J. L. Lach, *ibid.*, **54**, 1745 (1965).
(3) H. Schlenk, D. M. Sand, and J. A. Tillotson, *J. Am. Chem. Soc.*, **77**, 3587 (1955).
(4) C. A. Glass, *Can. J. Chem.*, **43**, 2652 (1965).
(5) D. French, *Adv. Carbohydr. Chem.*, **12**, 189 (1957).
(6) J. A. Thoma and L. Stewart, in "Starch Chemistry and Technology," vol. 1, R. L. Whistler and E. F. Paschall, Eds., Academic, New York, N.Y., 1965.
(7) F. Cramer and H. Hettler, *Naturwissenschaften*, **54**, 625 (1967), and references cited therein.
(8) R. Breslow and P. Campbell, *J. Am. Chem. Soc.*, **91**, 3085 (1969).
(9) J. Cohen and J. L. Lach, *J. Pharm. Sci.*, **52**, 132 (1963).
(10) J. L. Lach and J. Cohen, *ibid.*, **52**, 137 (1963).
(11) J. L. Lach and W. A. Pauli, *ibid.*, **55**, 32 (1966).
(12) A. C. O'Rourke and J. S. Kent (Syntex, Inc.), U.S. pat. 3,826,823 (1974).
(13) Ono Pharm. Co., West German pat. 2353-797 (1974).
(14) G. F. Thompson (Syntex Inc.), U.S. pat. 3,833,725 (1974).
(15) Ono Pharm. Co., Japanese pat. 8033-013 (1973).
(16) A. Hybl, R. E. Rundle, and D. E. Williams, *J. Am. Chem. Soc.*, **87**, 2779 (1965).
(17) P. V. Demarco and A. L. Thakkar, *Chem. Commun.*, **1970**, 2.
(18) A. L. Thakkar and P. V. Demarco, *J. Pharm. Sci.*, **60**, 652 (1971).
(19) T. Higuchi and K. A. Connors, in "Advances in Analytical Chemistry and Instrumentation," vol. 4, C. N. Reilley, Ed., Interscience, New York, N.Y., 1965, pp. 117–212.
(20) G. M. Barrow, "Physical Chemistry," McGraw-Hill, New York, N.Y., 1961, chap. 7.
(21) F. Cramer, *Angew. Chem.*, **73**, 49 (1967).
(22) G. Nemethy and H. A. Scheraga, *J. Chem. Phys.*, **36**, 3401 (1962).
(23) E. A. Lewis and L. D. Hansen, *J. Chem. Soc. Perkin Trans.*, **2**, 2081 (1973).
(24) W. A. Pauli and J. L. Lach, *J. Pharm. Sci.*, **54**, 1745 (1965).

[5] After the present study was completed, the authors became aware of the work of K. Uekama *et al.* on a very similar subject [*J. Pharm. Sci.*, **66**, 706 (1977)]. The present results are in general agreement in that the equilibrium constants for the molecular interaction are in the order of 10^3 mole^{-1} at 25°.

ACKNOWLEDGMENTS

Grateful appreciation is extended by S. G. Frank to The Upjohn Co. for a Summer Visiting Professorship in Pharmacy Research (1972), during which a portion of this project was completed.

Simultaneous Determination of Pseudoephedrine and Chlorpheniramine in Pharmaceutical Dosage Forms

AVRAHAM YACOBI ˣ, ZEE M. LOOK, and CHII-MING LAI

Received October 17, 1977, from the *Department of Biopharmaceutical Sciences, Research and Medical Affairs, Arnar-Stone Laboratories, Inc., McGaw Park, IL 60085.* Accepted for publication March 23, 1978.

Abstract □ A simple and sensitive high-pressure liquid chromatographic (HPLC) determination of pseudoephedrine and chlorpheniramine in a pharmaceutical dosage form is described. Quantities of 1.5 μg of pseudoephedrine and 0.1 μg of chlorpheniramine are sufficient to determine concentrations in an aqueous solution. Small volume samples, without any extraction procedures, can be treated for direct drug concentration measurement with a high-pressure liquid chromatograph. The stability-indicating property and the accuracy of this method are comparable to those of an established GLC method. The HPLC method can be applied directly and successfully for dissolution studies. The latter application eliminates the need for volume replacement or subsequent mathematical corrections.

Keyphrases □ Pseudoephedrine—high-pressure liquid chromatographic analysis, simultaneously with chlorpheniramine, in dosage forms □ Chlorpheniramine—high-pressure liquid chromatographic analysis, simultaneously with pseudoephedrine, in dosage forms □ High-pressure liquid chromatography—simultaneous analyses, pseudoephedrine and chlorpheniramine in dosage forms □ Adrenergics—pseudoephedrine, high-pressure liquid chromatographic analysis, simultaneously with chlorpheniramine, in dosage forms □ Antihistaminics—chlorpheniramine, high-pressure liquid chromatographic analysis, simultaneously with pseudoephedrine, in dosage forms

Some cough–cold–allergy dosage forms contain both pseudoephedrine hydrochloride, a nasal decongestant, and chlorpheniramine maleate, an antihistamine. These compounds are usually determined individually by GLC and/or UV spectrophotometry following TLC for drug separation. These methods require tedious extraction and lengthy reaction procedures. High-pressure liquid chromatographic (HPLC) systems also have been utilized for the determination of pseudoephedrine in cough–cold mixtures (1) and of chlorpheniramine in combination with other antihistamines (2) and antitussive preparations (3) and for quantitation of other antihistamines including chlorpheniramine in cough syrups (4).

This paper describes a suitable HPLC method for the simultaneous determination of pseudoephedrine and chlorpheniramine in pharmaceutical dosage forms.

EXPERIMENTAL

Instrumentation—A liquid chromatograph[1] was equipped with a UV detector operated at 254 nm and a nonpolar column[2] (30 cm long × 4 mm i.d.). The column was eluted with a mobile phase consisting of acetonitrile–methanol–sodium nitrate (35:40:25) and 1-heptanesulfonic acid[3] (0.001 *M* each), pH 5, at a flow rate of 2 ml/min. The output of the detector was recorded[4] at 10 mv.

A gas–liquid chromatograph[5] was equipped with a flame-ionization detector. A glass column (1.8 m long × 4 mm i.d.) was packed with 3% OV-17 on 100–120-mesh Chromosorb W-HP; helium was used as the carrier gas at a rate of 40 ml/min. The temperatures of the injection port, column, and detector were 225, 165, and 300°, respectively, for pseudoephedrine and 225, 235, and 300°, respectively, for chlorpheniramine.

[1] Model ALC/GPC 204, Waters Associates, Milford, Mass.
[2] μBondapak C_{18}, Waters Associates, Milford, Mass.
[3] Pic B-7 Waters Associates, Milford, Mass.
[4] Omniscribe recorder, Houston Instruments, Austin, Tex.
[5] Model 65, Beckman Instruments, Irvine, Calif.

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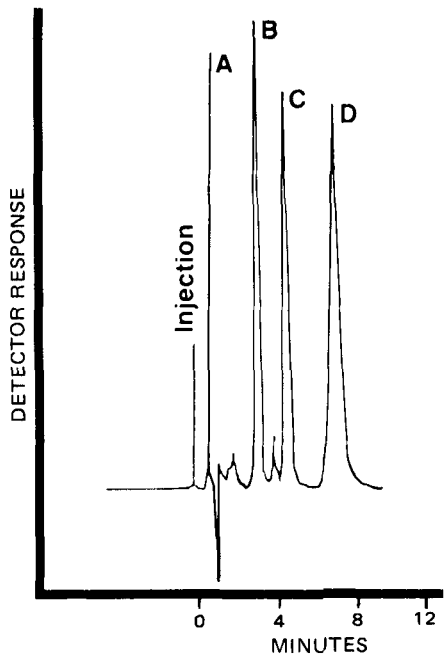

Figure 1—*Typical chromatogram obtained by HPLC analysis of the contents of sustained-release capsules. Key: A, maleic acid; B, pseudoephedrine; C, chlorpheniramine; and D, internal standard (chlorpromazine). The reaction times for A–D were 0.95, 3.1, 4.6, and 7.3, respectively.*

Pseudoephedrine also was determined spectrophotometrically with a UV spectrophotometer[6] at 256 nm.

Preparation—A stock solution containing pseudoephedrine hydrochloride[7] and chlorpheniramine maleate[8] (3.0 and 0.2 g, respectively, in 200 ml of purified water) was diluted to prepare a series of standard solutions for the determination of calibration curves for each drug. As the internal standard for the HPLC method, 5 mg of chlorpromazine hydrochloride[9] was dissolved in 100 ml of purified water. As internal standards for the GLC determination, 75 mg of mephentermine sulfate[10] (for pseudoephedrine) and 25 mg of pyrilamine maleate[9] (for chlorpheniramine) in 100 ml of purified water were used.

Sustained-release capsules[11] containing 120 mg of pseudoephedrine hydrochloride and 8 mg of chlorpheniramine maleate were sampled randomly, and their contents were ground to produce a fine powder. An accurate amount corresponding to one capsule weight was dissolved in purified water, and the solution was filtered through a filter paper[12]. The filtered solution then was diluted to prepare a series of working solutions with different concentrations. The final concentration of pseudoephedrine ranged from 0.3 to 3.6 mg/ml, and that of chlorpheniramine ranged from 20 to 240 μg/ml.

Capsules[13] containing 120 mg of pseudoephedrine hydrochloride also were processed in a similar manner to prepare a series of solutions, which were then determined by UV spectrophotometry.

For stability testing, solutions containing 600 μg of pseudoephedrine hydrochloride/ml and 40 μg of chlorpheniramine maleate/ml in water, 3 *N* NaOH, and buffers of various pH values (2.5, 4.5, 6.5, and 8) were prepared. The buffer solutions were heated at 90° for 1 hr. The water and alkaline solutions containing the drugs were heated for 24 hr at 100°. Then all solutions were allowed to cool overnight, adjusted for loss of volume, and subsequently assayed for pseudoephedrine and chlorpheniramine by the HLPC and GLC methods. The water solution, which was not exposed to heat, was used as the control.

Assay—For the HPLC method, 1 ml of each solution and 0.1 ml of the internal standard solution were mixed together and 5 μl was injected into the instrument. For the GLC methods[14], 2 ml of each solution and 2 ml of the internal standard solution were mixed together. Then about 2 g of sodium chloride, 2 drops of 10 *N* NaOH, and 2 ml of extraction solution (cyclohexane–benzene, 55:45) were added. The mixture was shaken and then centrifuged. Aliquots of 1–2 μl of the organic layer were injected into the instrument for determination of either pseudoephedrine or chlorpheniramine.

In both HPLC and GLC methods, the concentration of each drug was determined by means of peak height ratios of individual compounds and the respective internal standard. For UV spectrophotometry, solutions containing pseudoephedrine hydrochloride were determined directly. All samples were determined in duplicate.

Dissolution Study—For the dissolution test, an NF rotating basket assembly was used. The dissolution medium consisted of 900 ml of 0.01 *M* sodium phosphate buffer, pH 4.5. The contents of two capsules were put into the basket and immersed in the medium. The study was done in triplicate at 100 rpm and 37°. Serial 0.1-ml samples of the dissolution medium were taken immediately before and after immersion of the baskets and at appropriate sampling times. To each 0.1-ml sample, 0.05 ml of the internal standard (5 μg of chlorpromazine hydrochloride/ml) was added. The mixture was vortexed, and a 25-μl aliquot was injected for HPLC analysis.

Table I—Percent Error in Determination of Pseudoephedrine and Chlorpheniramine by the GLC and HPLC Methods

Drug	Method	Concentration Range[a], mg/ml	Percent Error[b] Range	Mean	*SD*
Pseudoephedrine hydrochloride	GLC	0.30–3.60	0.65–16.1	4.63	5.43
	HPLC		1.01–8.50	3.03	2.52
Chlorpheniramine maleate	GLC	0.02–0.24	0.48–4.73	1.73	1.45
	HPLC		0.92–3.84	1.92	1.20

[a] Total of seven concentrations. The assay for each concentration was done in duplicate. [b] These are absolute values and were determined by (absolute values of the difference between two determinations/concentration) × 100.

Table II—Precision and Reproducibility of the GLC and HPLC Methods for Determination of Pseudoephedrine and Chlorpheniramine in Sustained-Release Capsules

Sample	Relative Amount in Capsules, % Pseudoephedrine Hydrochloride GLC	HPLC	Chlorpheniramine Maleate GLC	HPLC
1	103.0	97.0	102.0	97.3
2	101.0	97.4	89.4	98.8
3	97.2	101.0	107.0	100.0
4	97.4	97.4	98.1	96.6
5	101.0	104.0	106.0	102.0
6	98.0	101.0	98.2	100.0
7	100.0	98.6	92.6	99.7
8	103.0	104.0	107.0	105.0
Mean	100.1	100.0	100.0	99.9
SD	2.35	2.88	6.68	2.65

RESULTS AND DISCUSSION

Figure 1 depicts a typical chromatogram obtained by the HPLC analysis of the contents of the sustained-release capsules. Chlorpheniramine maleate underwent hydrolysis in the system to chlorpheniramine and maleic acid, resulting in the earlier appearance of the latter on the chromatogram, which appears to be characteristic of maleic acid in this system. There was a clear separation of each component with no indication of any interference due to the hydrolysis of chlorpheniramine maleate or the presence of other molecules resulting from degradation of one of the compounds. The retention times of each species are listed in Fig. 1. These values show that the entire procedure for simultaneous analyses of pseudoephedrine and chlorpheniramine in the same sample can be completed within 10 min.

For comparison purposes, established stability-indicating GLC methods for the determination of pseudoephedrine and chlorpheniramine

[6] Model 25, Beckman Instruments, Irvine, Calif.
[7] Knoll, A. G., Whippany, N.J.
[8] E. M. Labs, Elmsford, N.Y.
[9] H. Reisman Corp., Orange, N.J.
[10] Arapahoe Chemicals, Inc., Boulder, Colo.
[11] Isoclor Timesule Capsules, Arnar-Stone Laboratories, Inc., McGaw Park, Ill.
[12] Whatman No. 1, W & R Balston, Ltd., London, England.
[13] Novafed Capsules, Dow Pharmaceuticals, Dow Chemical Co., Indianapolis, Ind.
[14] An unpublished in-house analytical procedure, Arnar-Stone Laboratories, McGaw Park, IL 60085.

Table III—Results of Stability Testing of Pseudoephedrine and Chlorpheniramine by the GLC and HPLC Methods

	Percent of Control[a]			
	Pseudoephedrine Hydrochloride		Chlorpheniramine Maleate	
Sample	GLC[b]	HPLC	GLC[b]	HPLC
pH 2.5	93.3 ± 4.32[c]	97.8 ± 2.35	99.3 ± 0.65	96.9 ± 1.17
pH 4.5	98.1 ± 1.26	97.4 ± 1.34	99.7 ± 3.39	100.3 ± 0.66
pH 6.5	97.5 ± 2.23	94.7 ± 1.63	97.3 ± 0.64	102.3 ± 1.26
pH 8.0	99.0 ± 1.29	95.6 ± 2.40	97.9 ± 0.40	100.4 ± 0.57
Water[d]	88.9 ± 1.54	78.2 ± 4.20	46.8 ± 1.68	47.4 ± 2.76
3 *N* NaOH[d]	80.8 ± 3.88	87.2 ± 2.34	29.2 ± 0.46	30.8 ± 0.55

[a] The control solution was prepared in water and reserved for assay together with the buffer solutions. The buffer solutions were heated for 1 hr at 90°. All solutions contained 600 μg of pseudoephedrine hydrochloride/ml and 40 μg of chlorpheniramine maleate/ml. [b] Pseudoephedrine and chlorpheniramine were determined individually by the GLC methods and simultaneously by the HPLC procedure. [c] Mean ± *SD*, $n = 4$. [d] These solutions were heated at 100° for 24 hr, $n = 6$.

were used. There were excellent correlations between: (*a*) the theoretical concentrations and those obtained by GLC and HPLC for pseudoephedrine and chlorpheniramine, respectively; (*b*) concentrations of pseudoephedrine determined by GLC and HPLC ($r = 0.976$, $p < 0.001$); and (*c*) concentrations of chlorpheniramine determined by GLC and HPLC ($r = 0.999$, $p < 0.001$). The lowest corresponding quantities injected into the HPLC system are 1.5 μg of pseudoephedrine hydrochloride and 0.1 μg of chlorpheniramine maleate.

Table I shows the values of the percent error involved with each procedure. For pseudoephedrine and chlorpheniramine, the mean values were 3.03 and 1.92% by HPLC and 4.63 and 1.73% by GLC, respectively. The variations in the measurement by HPLC were very low and comparable to those of the GLC procedures. Similar results were obtained when the HPLC method was tested against a UV spectrophotometric determination with respect to pseudoephedrine ($r = 0.989$, $p < 0.001$).

The precision and reproducibility of the HPLC method for the determination of potency of pseudoephedrine and chlorpheniramine in the sustained-release capsule, using a standard sample preparation and dilution procedure, were examined (Table II). The HPLC as well as the GLC method showed a high degree of accuracy, precision, and reproducibility. Table III compares the results of the stability testing of pseudoephedrine and chlorpheniramine. There was no statistically significant difference between the results obtained by the HPLC and the GLC methods, indicating that both methods are equally stability indicating.

Both compounds appeared to be stable at various pH values and moderate temperature conditions. However, when the aqueous and the alkaline samples were heated for about 24 hr, there was significant degradation of both molecules, particularly chlorpheniramine (Table III). The HPLC and GLC methods showed good separation of the parent compounds from their degradation products. The results were almost identical for both methods.

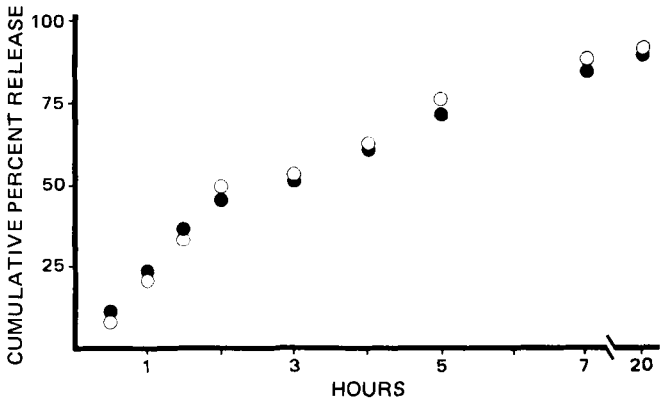

Figure 2—*Cumulative percentage released* versus *time profile of pseudoephedrine (○) and chlorpheniramine (●), observed from the dissolution test of a sustained-release capsule. Each data point represents the average of three values.*

Figure 2 shows the results for the dissolution test of the capsule content. About 85 and 92% of pseudoephedrine and chlorpheniramine, respectively, were released in 20 hr, comparable to results obtained in in-house dissolution studies utilizing the GLC method. The simplicity of this HPLC method for the determination of the two ingredients permits a rapid characterization of the dissolution profile of the drug with maximum accuracy. In addition, the size of samples (0.1 ml) taken from the dissolution medium is extremely small and eliminates the need for volume replacement or subsequent mathematical corrections.

The results indicate that the HPLC method can be considered for the determination of pseudoephedrine and chlorpheniramine in the evaluation of pharmaceutical dosage forms. This procedure does not involve any extraction or reaction steps. A sample can be easily mixed with an internal standard solution and analyzed *via* HPLC. The method is simple, rapid, accurate, stability indicating, and reproducible. Additionally, the sensitivity and reliability of the HPLC method over a wide range of concentrations will extend the use of this method to measurement of the urinary excretion of the active ingredients following ingestion of oral dosage forms containing one or both compounds. Such a study is presently in progress.

REFERENCES

(1) I. L. Honigberg, J. T. Stewart, and A. P. Smith, *J. Pharm. Sci.*, **63**, 766 (1974).

(2) A. G. Ghanekar and V. D. Gupta, *Am. J. Hosp. Pharm.*, **34**, 651 (1977).

(3) A. Menyharth, F. P. Mahn, and J. E. Heveran, *J. Pharm. Sci.*, **63**, 430 (1974).

(4) V. D. Gupta and A. G. Ghanekar, *ibid.*, **66**, 895 (1977).