REFERENCES

Ali, S.L. Ephedrine hydrochloride. In K. Florey (ed.), Analytical Profile of Drug Substances Vol. 15, pp. 223-281. Orlando : Academic Press, 1986.

British Pharmaceutical Codex London, U.K. : The Pharmaceutical Press, 1973.

British Pharmacopoeia London, U.K. : The United Kingdom for Her Majesty's Stationary Office, 1988.

Baker-10 SPE™. Application Guide Vol. II, Life Science Edition, Phillipsburg : The J.T. Baker Chemical Co., 1984.

Baum, R.G., Saetre, R. and Cantwell, F.F. Liquid Chromatography Columns of Microparticle Amberlite XAD-2 Anal. Chem. 52(1980) : 15-19.

Clarke, E.G.C., ed. Clarke's Isolations and Identification of Drugs (2 nd ed.), London : The Pharmaceutical Press, 1986.

Cox, N.; Meakin, B.J. and Davies, D.J.G. The Influence of some Antioxidations on the Photochemical decomposition kinetics of Promethazine hydrochloride. Journal of Pharmacy and Pharmacology 28 supplement (1976) : 45 P.

Critichfield, F.E. and Johnson, J.B. Reaction of Carbon disulfide with Primary and Secondary Aliphatic Amines as an Analytical Tool. Analytical Chemistry 28(1956) : 430-435.

Davidson, A.G. The Determination of Phenothiazine Drugs in Pharmaceutical Preparation by a Difference Spectrophotometric Method. Journal of Pharmacy and Pharmacology 28(1976) : 795-800.

Deavin, J.C. Recommended Methods for the Evaluation of Drugs Appendix : Recommended Procedure for the Determination of Ephedrine in Certain Pharmaceutical Preparation. Analyst 100(1975) : 136-141.

Fujimoto, J.M. and Wang, R.I.H. A Method of Identifying Narcotic Analgesics in Human Urine after Therapeutic Doses. Toxicology and Applied Pharmacology 16(1970) : 186-193.

Galente, R.N., Visalli, A.J. and Patel, D.M. Solid State Acetylation of Codeine Phosphate by Aspirin. Journal of Pharmaceutical Sciences 68(1979) : 1494-1498.

Gill, B.L. R. and Moffat, A.C. High Performance Liquid Chromatography Retention Data for 84 Basic Drugs of Forensic interest on a silica column using an aqueous methanol eluent. Journal of Chromatography 301(1984) : 165-172.

Glajch, J.L. and Kirkland, J.J. Optimization of Selectivity in Liquid Chromatography. Anal. Chem. 55(1983) : 319-336A.

Good, T.J. and Andrews, J.S. The Use of Bonded Phase Extraction Columns for Rapid Sample Preparation of Benzodiazepines and Metabolites from Serum for HPLC Analysis. J. Chromatogr. Sci. 19(1981) : 562-566.

Gupta, V.D. and De Lara, A.J.L. Colorimetric Determination of Chlorpheniramine maleate, Ephedrine hydrochloride and Guaiacolsulfonate potassium in a Cough Syrup. Journal of Pharmaceutical Sciences 64(1975) : 2001-2002.

Gupta, V.D. Simultaneous Quantitation of Acetaminophen, Aspirin, Caffeine, Codeine phosphate, Phenacetin and Salicylamide by High-Performance Liquid Chromatography. Journal of Pharmaceutical Sciences 69(1980) : 110-112.

Halstead, G.W. Determination of Amine Ingredients in Cough-Cold Liquids by Revered-Phase Ion-Pair High Performance Liquid Chromatography. Journal of Pharmaceutical Sciences 71(1982) : 1108-1112.

Leroy, P. and Nicolas, A. Determination of Secondary Amino Drugs as their Metal Dithiocarbamate Complexes by Reversed-Phase High-Performance Liquid Chromatography with Electrochemical Detection. Journal of Chromatography 317(1984) : 513-521.

Matsui, F. and French, W.N. Analysis of Binary Mixtures of Pharmaceutical Amines by the Acid Dye Technique. Journal of Pharmaceutical Sciences 60(1971) : 287-290.

Mathew et. al. Rapid Method for the Quantitative Measurement of Numerous Pharmaceutically Important Amines. Journal of the AOAC 55(1972) : 789-793.

McDowall, R.D. Sample Preparation for Biomedical Analysis. Journal of Chromatography 492(1989) : 21-32.

Mohammed, H.Y. and Cantwell, F.F. Liquid Chromatographic Analysis of Pharmaceutical Syrups Using Pre-column and Salt-Adsorption on Amberlite XAD-2, Anal. Chem. 50(1978) : 491-496.

Moore, C.M., Tebbett, I.R. and Schuerer, J. Extraction and Analysis of Codeine Morphine and Methadone from Medicinal Preparation. Microgram 22(1989) : 229-232.

Mutitadi, F.J. and Mahmoud, M.A.H. Codeine Phosphate in K. Florey (ed.), Analytical Profile of Drug Substances Vol. 10, pp. 93-138. New York : Academic Press, 1981.

Pound, N.J. and Sears, R.W. Analysis of Promethazine hydrochloride Formulation by High Speed Liquid Chromatography. Canadian Journal of Pharmaceutical Sciences 8(1973) : 84-88.

Shearer, C.M. and Miller, S.M. Promethazine hydrochloride. in K. Florey (ed.), Analytical Profile of Drug Substances Vol. 5, pp. 429-465. New York : Academic Press, 1976.

Sisco, W.R., Rittenhouse, C.T.; Everhart, L.A. and Mclaughlin, A.M. Simultaneous High Performance Liquid Chromatography Stability-Indicating Analysis of Acetaminophen, Codeine phosphate and Sodium benzoate in Elixir. Journal of Chromatography 354(1986) : 335-356.

Smith, R.V. and Stewart, J.T. A Textbook of Biopharmaceutic Analysis p. 108. Philadelphia : Lea & Febiger, 1981.

Sperling, A.R. Analysis of Promethazine hydrochloride in Syrups. Journal of Pharmaceutical Sciences 56(1967) : 98-100.

Stavchansky, S., Wallace, J.E. and Wu, P. Thermal and Photolytic Degradation Studies of Promethazine hydrochloride : A stability-Indicating. Journal of Pharmaceutical Sciences 72(1983) : 546-548.

Stevens, M.R. Gas Liquid chromatographic Analysis of Caffeine and Codeine phosphate in Pharmacetical Preparations. Journal of Pharmaceutical Sciences 64(1975) : 1686-1687.

Stewart, J.T., Reeves, T.S. and Honigberg, I.L. A Comparison of Solid Phase Extraction Techniques for Assays of Drugs in Aqueous and Human Plasma Samples. Anal. Lett. 17(1984) : 1811-1826.

The United State Pharmacopeia 22nd ed. Rochville, U.S.A.: The United States Pharmacopeial Convention, 1990.

Underberg, W.J.M. Oxidative Degradation of Pharmaceutically Important Phenothiazine. Journal of Pharmaceutical Sciences 67(1978) : 1128-1138.

Walker, T.S. Liquid Chromatographic Determination of Organic Nitrogenous Bases in Dosage Forms : A Progress Report. Journal of The AOAC 68(1985) : 539-542.

Wallace, J.E.; shinuk, E.L. Jr., Stavchansky, S. and Harris,S.C. Determination of Promethazine and other Phenothiazine Compounds by Liquid Chromatography with Electrochemical Detection. Analytical Chemistry 53(1981) : 960-962.

Weissman, N.; Lowe, M.L; Beattie, J.M. and Demetrious, J.A. A screening method for detection of Drugs of abuse in Human Urine. Clinical Chemistry. 17(1971) : 875-881.

Willson, I.D. A Rapid Method for the Isolation and Identification of Drug Metabolites from Human Urine Using Solid Phase Extraction and Proton NMR Spectroscopy. J. Pharm. Biomed. Anal. 4(5) (1986) : 663.

Yang, S.L., Wilken, L.O. and Clark, C.R. A High Performance Liquid Chromatographic Method for the Simultaneous Assay of Aspirin, Caffeine, Dihydrocodeine bitartrate and Promethazine hydrochloride in a capsule Formulation. Drug Development and Industrial Pharmacy 11(1985) : 799-814.

Appendix

Table 1 Variation of capacity factor (k′) with percent (%) methanol in mobile phase

Composition of mobile phase = Methanol : 20 mM Buffer at pH 7.0 (v/v)

Compound	k′ value* at various compositions of mobile phase			
	80:20	85:15	90:10	95:05
Promethazine hydrochloride	8.75	9.53	11.87	21.43
Codeine phosphate	6.38	7.63	9.93	21.05
Methylephedrine hydrochloride	4.03	5.15	7.53	15.05
Ethylephedrine hydrochloride	3.72	4.35	6.68	12.54
Ephedrine hydrochloride	2.42	2.97	4.33	8.28

$$* \; k' \text{ value} = \frac{t_R - t_O}{t_o}$$

where

t_R = retention time of solute

t_O = retention time of solvent or unretained solute

Table 2 Variation of capacity factor (k′) with pH of mobile phase

Composition of mobile phase = Methanol : 20 mM Buffer (90:10 , v/v)

Compound	k′ value at			
	pH 6.0	pH 6.5	pH 7.0	pH 7.5
Promethazine hydrochloride	12.81	13.58	12.54	9.92
Codeine phosphate	16.22	15.20	10.47	7.79
Methylephedrine hydrochloride	6.22	6.71	7.10	7.70
Ethylephedrine hydrochloride	5.19	5.52	5.82	6.32
Ephedrine hydrochloride	3.73	4.01	4.24	4.74

Table 3 Variation of capacity factor (k′) with concentration of ammonium acetate buffer (mM.)

Composition of mobile phase = Methanol : mM Buffer at pH 7.0 (90:10 , v/v)

Compound	k′ value at various buffer concentrations				
	10 mM	15 mM	20 mM	25 mM	30 mM
Promethazine hydrochloride	14.19	13.92	11.81	10.19	9.18
Codeine phosphate	13.03	12.53	9.82	8.73	8.05
Methylephedrine hydrochloride	9.08	8.73	7.67	6.32	4.48
Ethylephedrine hydrochloride	7.62	7.11	6.26	5.24	5.62
Ephedrine hydrochloride	5.23	5.29	4.23	3.53	3.01

Table 4 Approximate retention times of active ingredients and internal standard, and resolution factors of each peak pair in the proposed HPLC system

Compound	Retention time (min)	Resolution factor (R)
Ephedrine hydrochloride	3.92	
		1.26
Ethylephedrine hydrochloride	5.18	
		1.42
Codeine phosphate	6.88	
		1.46
Promethazine hydrochloride	8.92	

The resolution factor (R) is a measure of relative separation of two peaks. It is defined as follows :

$$R = \frac{2(t_2 - t_1)}{W_1 + W_2}$$

where

T_2 and T_1 = The retention times of the two peaks

W_1 and W_2 = The widths of the extrapolated straight portion of the peaks at baseline

When an R is greater than 1, the resolution between a pair of two adjacent peaks is complete.

Table 5 Efficiency of organic solvents used as eluting solvent in Solid Phase Extraction procedure for complete extraction of analytes.

organic solvent	Volume (ml)
Methanol	> 50
Methanol acidified with acid	> 50
Chloroform	35
Dichloromethane	15

Table 6 Linearities of Ephedrine hydrochloride, Codeine phosphate and Promethazine hydrochloride from synthetic mixtures in syrup USP using fixed amount of internal standard.

Ephedrine hydrochloride		Codeine phosphate		Promethazine hydrochloride	
Concentration (mcg/ml)	Peak area ratio[a] ($\times 10^{-2}$) $\pm$ SD	Concentration (mcg/ml)	Peak area ratio[a] ($\times 10^{-1}$) $\pm$ SD	Concentration (mcg/ml)	Peak area ratio ($\times 10^{-1}$) $\pm$ SD
40.37	11.48 $\pm$ 0.212	48.16	8.37 $\pm$ 0.530	20.06	20.15 $\pm$ 0.289
48.45	13.77 $\pm$ 0.021	57.79	10.57 $\pm$ 0.289	24.08	24.46 $\pm$ 4.202
56.51	15.98 $\pm$ 0.219	67.42	12.18 $\pm$ 0.141	28.09	28.12 $\pm$ 0.127
64.59	18.88 $\pm$ 0.516	77.06	13.78 $\pm$ 0.262	32.10	31.89 $\pm$ 0.289
72.66	20.49 $\pm$ 0.813	86.69	15.94 $\pm$ 0.099	36.12	36.40 $\pm$ 1.244
r^b = 0.9973		r^b = 0.9979		r^b = 0.9993	

[a] Mean $\pm$ standard deviation (n = 3)

[b] Correlation coefficient (n = 5)

Table 7 Intra-day precision

Trial	Percent labeled amount		
	Ephedrine hydrochloride	codeine phosphate	Promethazine hydrochloride
1	100.67	100.64	102.26
2	98.72	102.41	101.73
3	98.67	101.28	100.99
4	98.06	100.91	104.21
5	100.66	105.39	102.43
6	98.62	105.85	103.55
7	100.66	104.83	105.76
8	100.87	102.96	104.25
9	97.74	104.53	105.20
10	98.99	103.77	105.71
Mean	99.37	103.27	103.61
SD.[a]	1.214	1.916	1.698
% CV[b]	1.22	1.86	1.64

[a] Standard deviation

[b] % coefficient of variation

Table 8 Inter-day precision of 3 non-consecutive days

Compound	Mean concentration (% la ± SD)[a]	CV.[b] (%)
Ephedrine hydrochloride	99.93 ± 1.369	1.37
Codeine phosphate	101.93 ± 2.034	1.99
Promethazine hydrochloride	104.13 ± 2.129	2.04

[a] Percent labeled amount ± Standard deviation (n = 3)

[b] Coefficient of variation

Table 9 Recoveries of standards from syrup USP spiked with corresponding standards

Compound	Spiked concentration (mcg/ml)	Concentration found (mcg/ml)	Recovery* (%)
Ephedrine hydrochloride	40.37	40.16	99.49 ± 1.33
	56.52	56.33	99.67 ± 0.87
	72.66	72.50	99.78 ± 0.72
Codeine phosphate	48.16	48.32	100.34 ± 1.15
	67.42	68.69	101.89 ± 1.68
	86.69	89.22	102.92 ± 0.61
Promethazine hydrochloride	20.06	20.19	100.68 ± 0.86
	28.09	28.06	99.91 ± 0.58
	36.12	36.13	100.04 ± 1.14

* Mean ± standard deviation (n = 5)

Table 10 Percent labeled amounts of 5 commercial products according to the proposed method

Syrup No.	% labeled amount[a]		
	Ephedrine hydrochloride	Codeine phosphate	Promethazine hydrochloride
1	99.7 ± 0.85	101.1 ± 1.12	103.8 ± 1.65
2	103.2 ± 0.97	102.9 ± 1.05	102.6 ± 1.24
3	98.5 ± 1.02	103.1 ± 1.02	71.7 ± 1.59
4	98.3 ± 0.92	98.8 ± 1.02	90.7 ± 1.68
5	98.5 ± 0.98	98.6 ± 1.35	48.8 ± 1.70

[a] Mean ± standard deviation (n = 2)

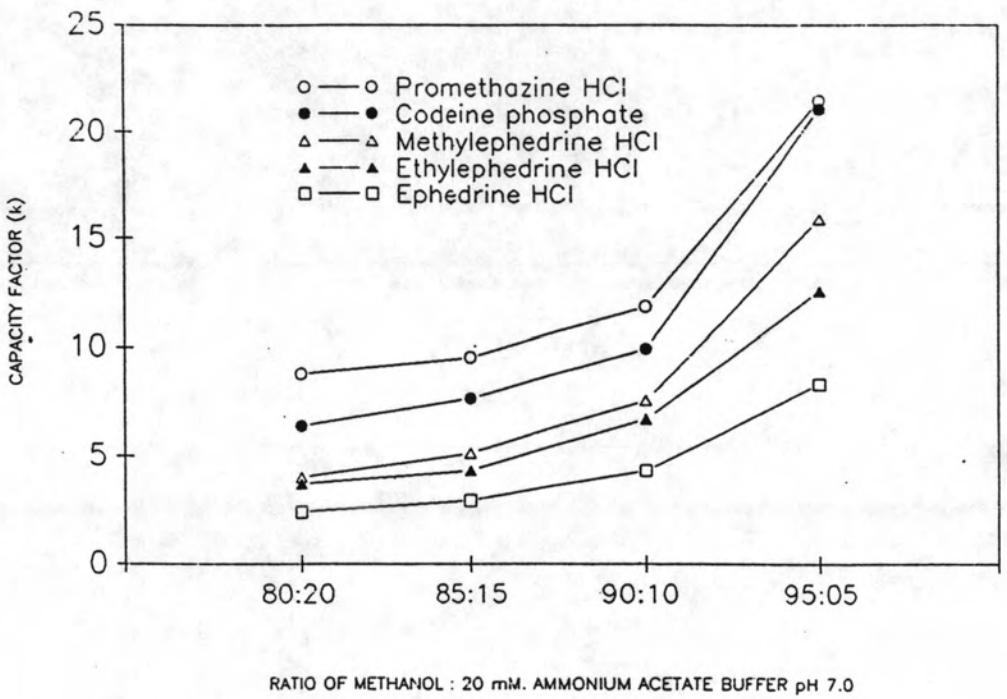

Figure 1 Capacity factor (k′) versus percent methanol in mobile phase

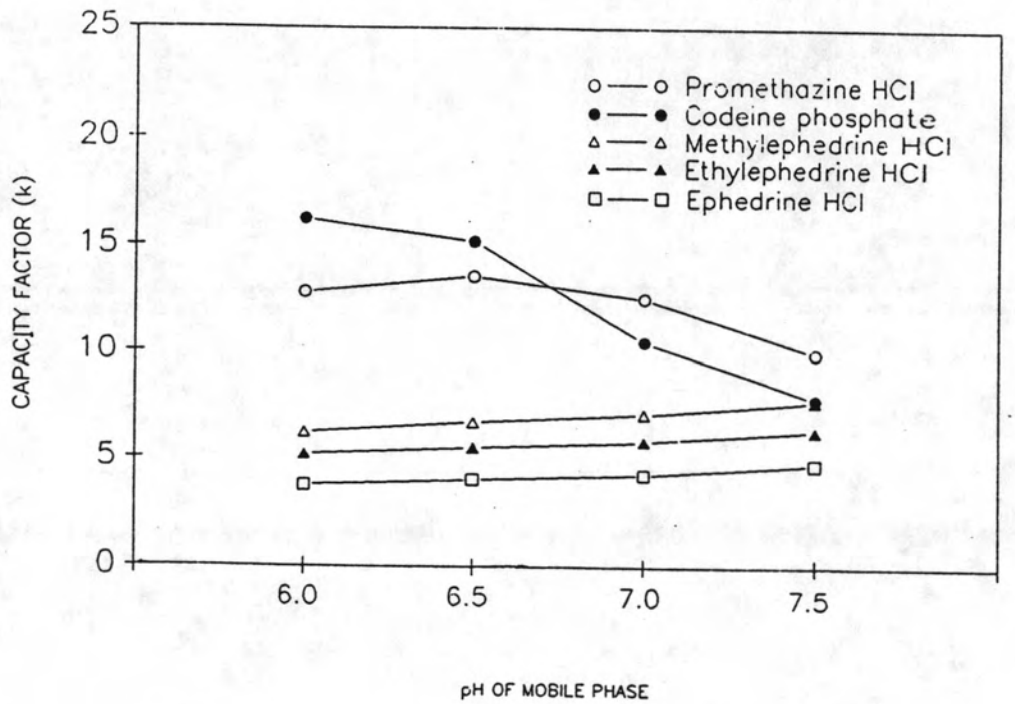

Figure 2 Capacity factor (k´) versus pH of mobile phase

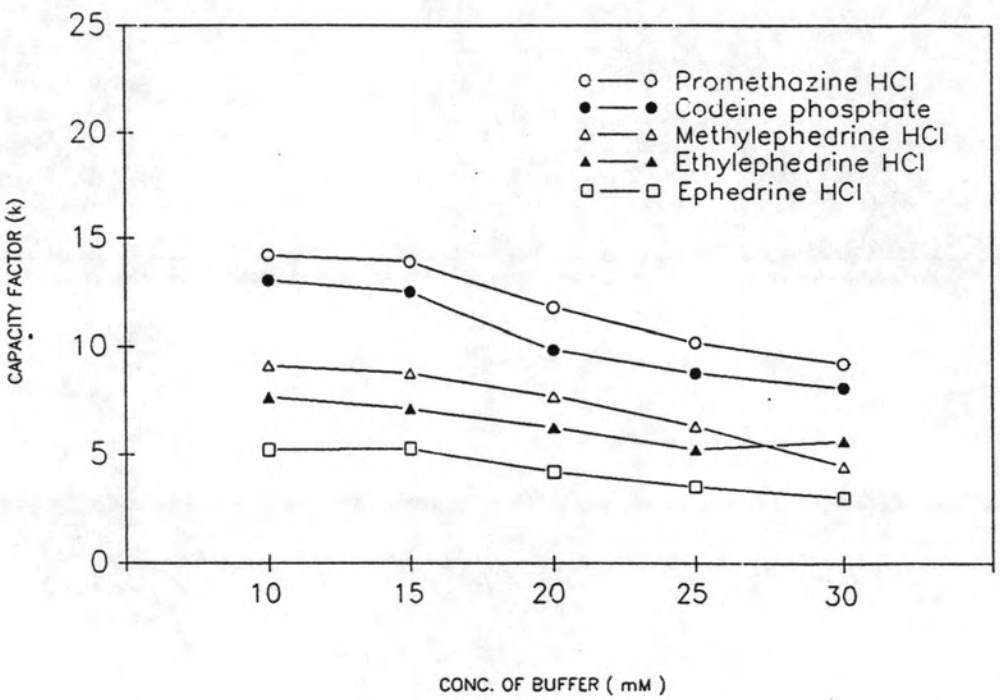

Figure 3 Capacity factor (k´) versus concentration of buffer in mobile phase

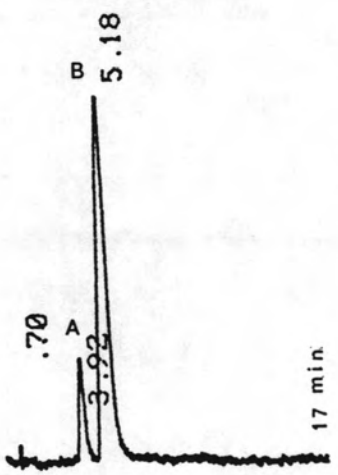

Figure 4 chromatogram of ephedrine hydrochloride and ethylephedrine hydrochloride

A = Ephedrine hydrochloride (R_t = 3.92 min)

B = Ethylephedrine hydrochloride,

(R_t = 5.18 min) an internal standard

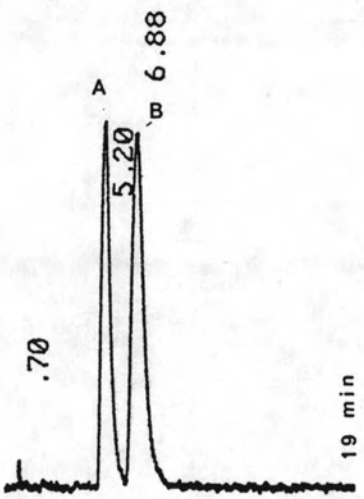

Figure 5 Chromatogram of codeine phosphate and ethylephedrine hydrochloride

A = Ethylephedrine hydrochloride (R_t = 5.20 min)

B = Codeine phosphate (R_t = 6.88 min)

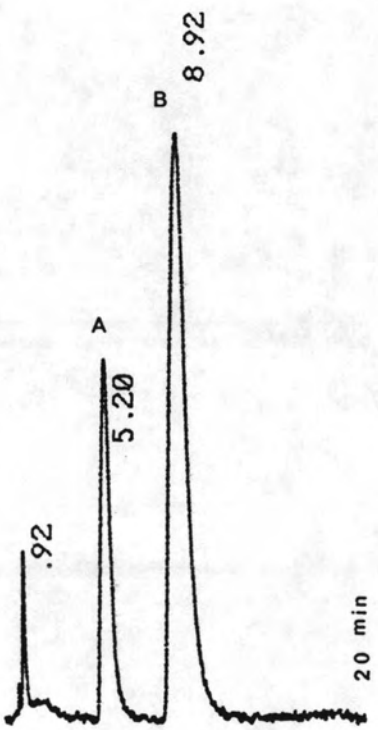

Figure 6 Chromatogram of promethazine hydrochloride and ethylephedrine hydrochloride

A = Ethylephedrine hydrochloride (R_t = 5.20 min)

B = Promethazine hydrochloride (R_t = 8.92 min)

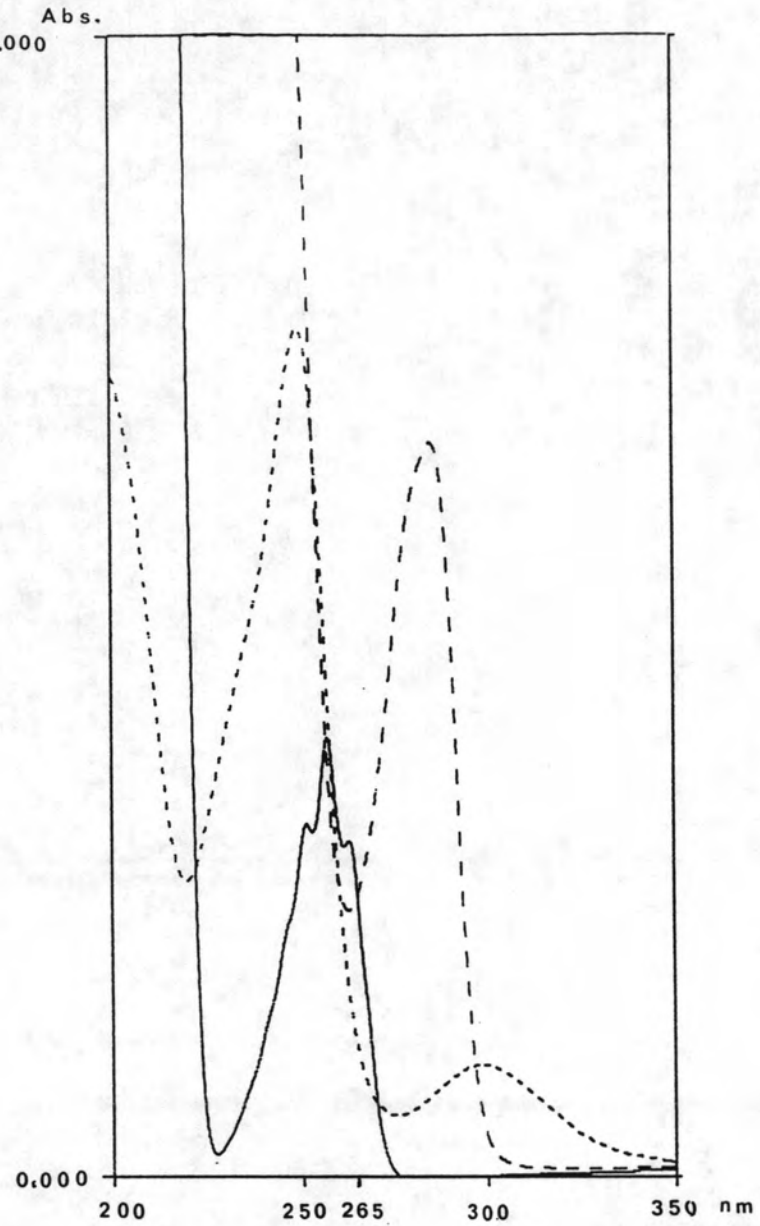

Figure 7 UV spectra of ephedrine hydrochloride, codeine phosphate and promethazine hydrochloride in the mobile phase

_____ ephedrine hydrochloride, 288 mcg/ml

- - - - codeine phosphate, 128 mcg/ml

..... promethazine hydrochloride, 8 mcg/ml

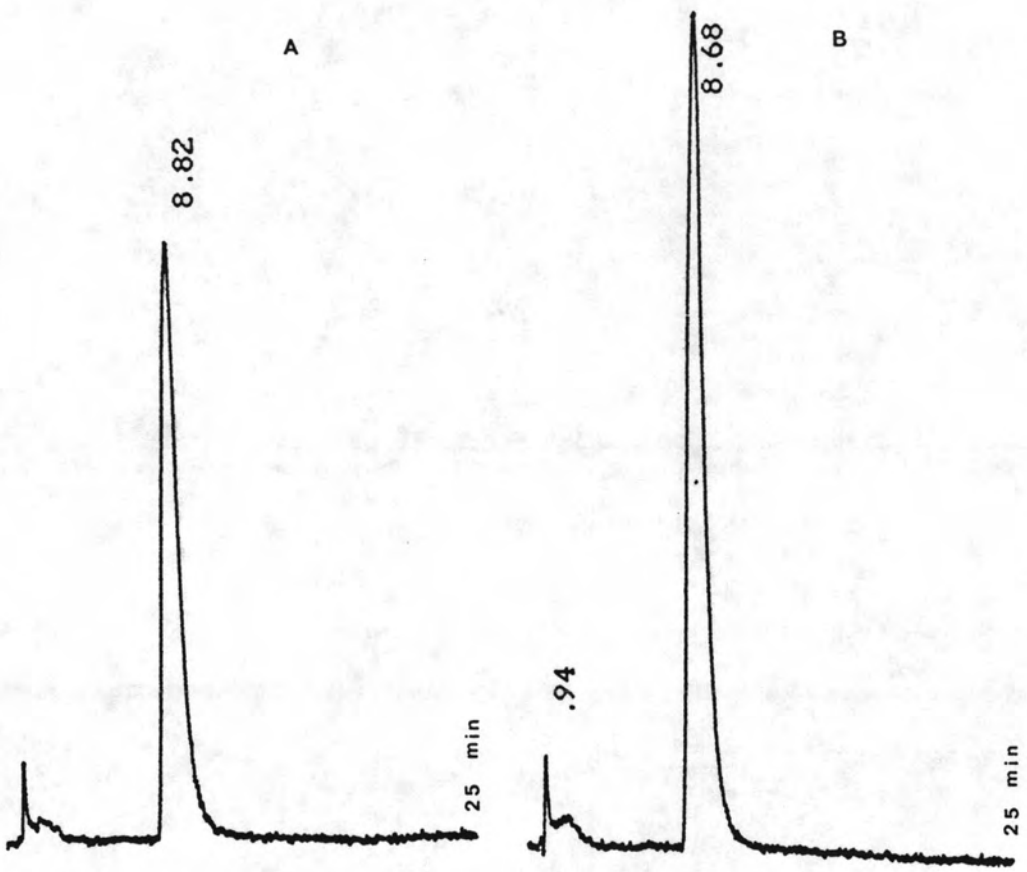

Figure 8 Chromatograms of freshly prepared promethazine hydrochloride, working standard and USP Reference standard

A = Working standard

B = USP reference standard

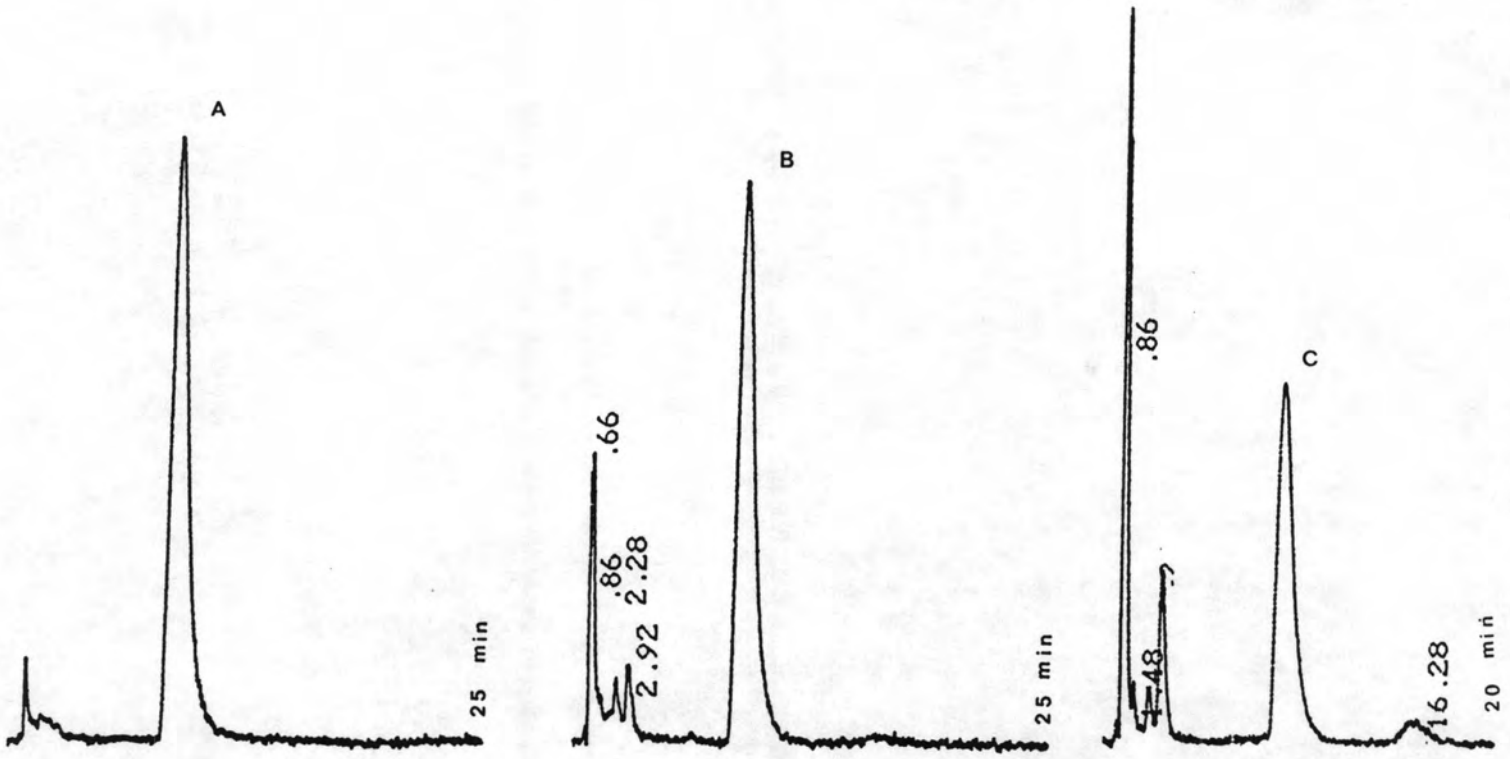

Figure 9 Chromatograms of standard promethazine hydrochloride (Rt 8.82)

A) freshly prepared

B) after 24 hours exposure to neon light (60 watt, 20 cm distance)

C) after 7 days exposure to neon light (60 watt, 20 cm distance)

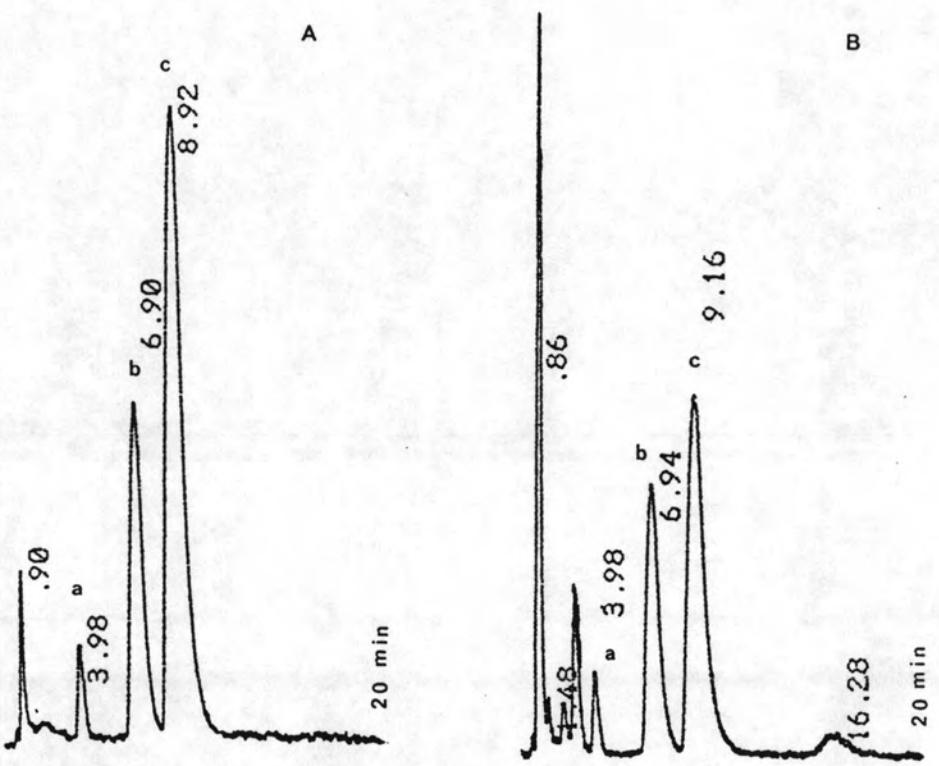

Figure 10 Chromatograms of a standard mixture of ephedrine hydrochloride, codeine phosphate and promethazine hydrochloride

(A) freshly prepared

(B) after 7 days exposure to neon light

a = ephedrine hydrochloride

b = codeine phosphate

c = promethazine hydrochloride

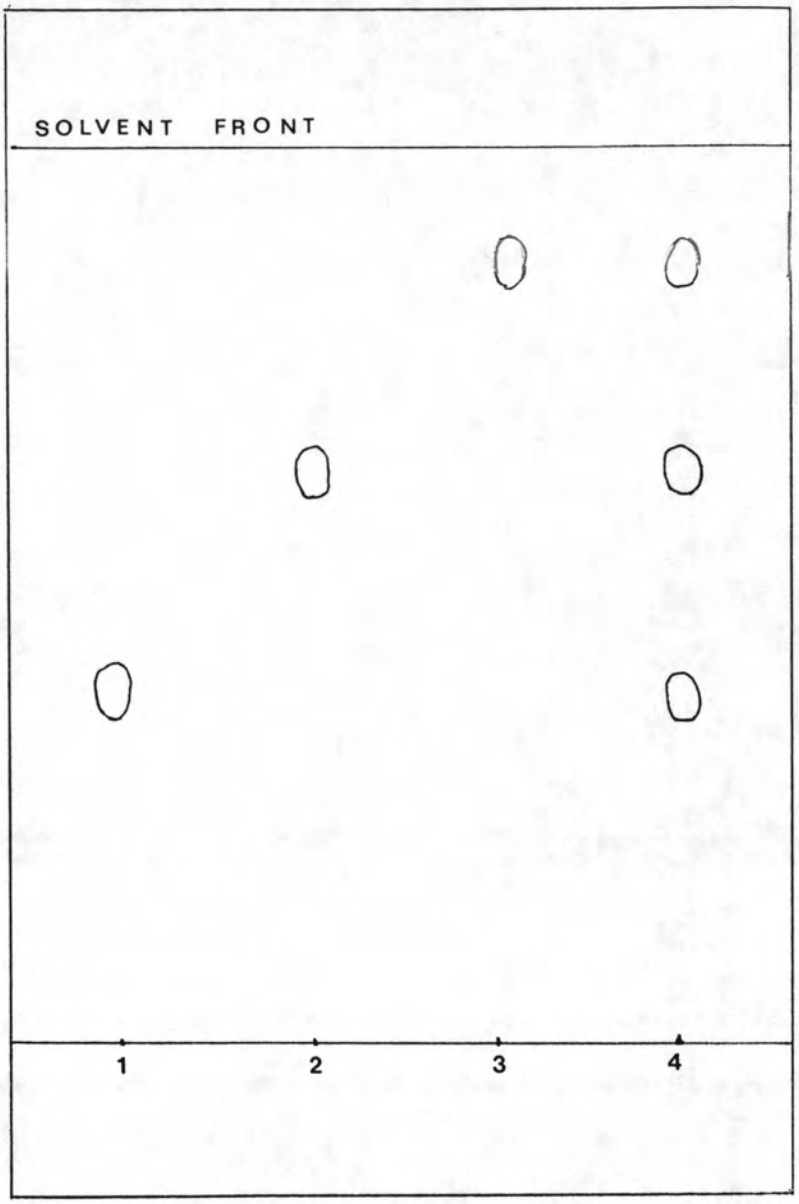

Figure 11 Thin-layer chromatogram[a] of standard ephedrine hydrochloride, codeine phosphate, promethazine hydrochloride and the eluate sample.

[a] mobile phase of Chloroform : Methanol = 9:1, detected under UV at 254 nm.

1 = ephedrine hydrochloride

2 = codeine phosphate

3 = promethazine hydrochloride

4 = the eluate sample processed according to the proposed SPE method

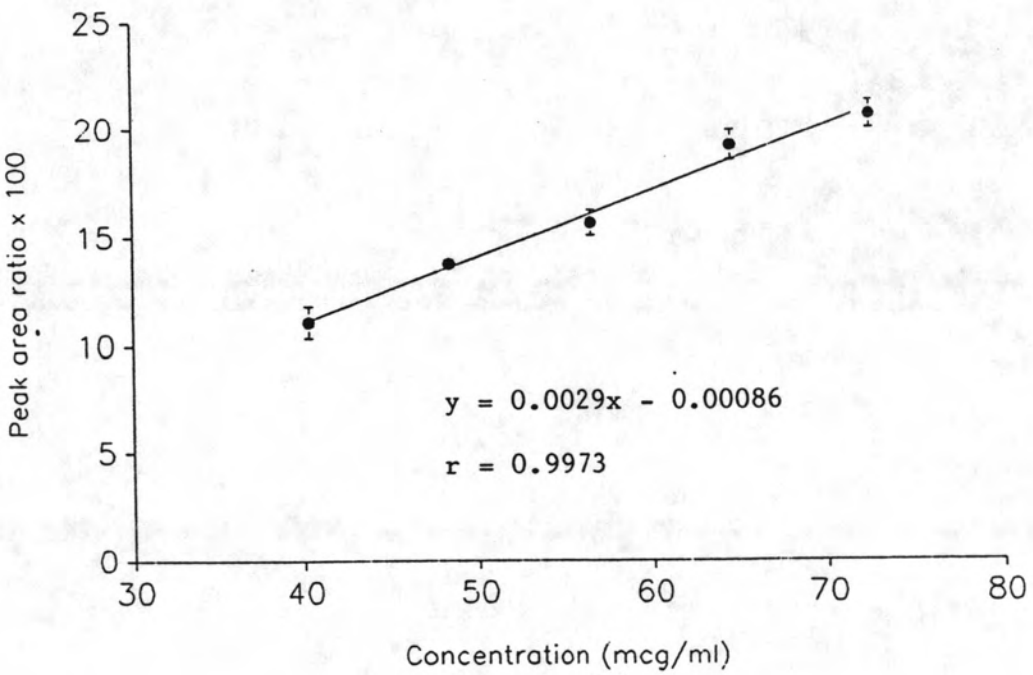

Figure 12 Peak area ratio versus concentration of ephedrine hydrochloride

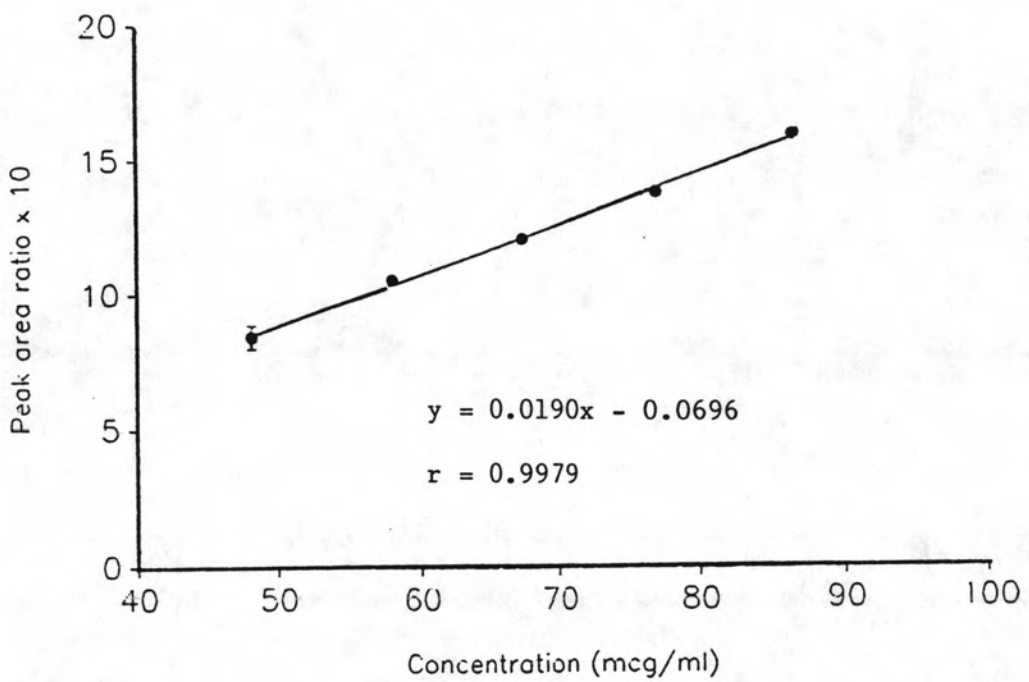

Figure 13 Peak area ratio versus concentration of codeine phosphate

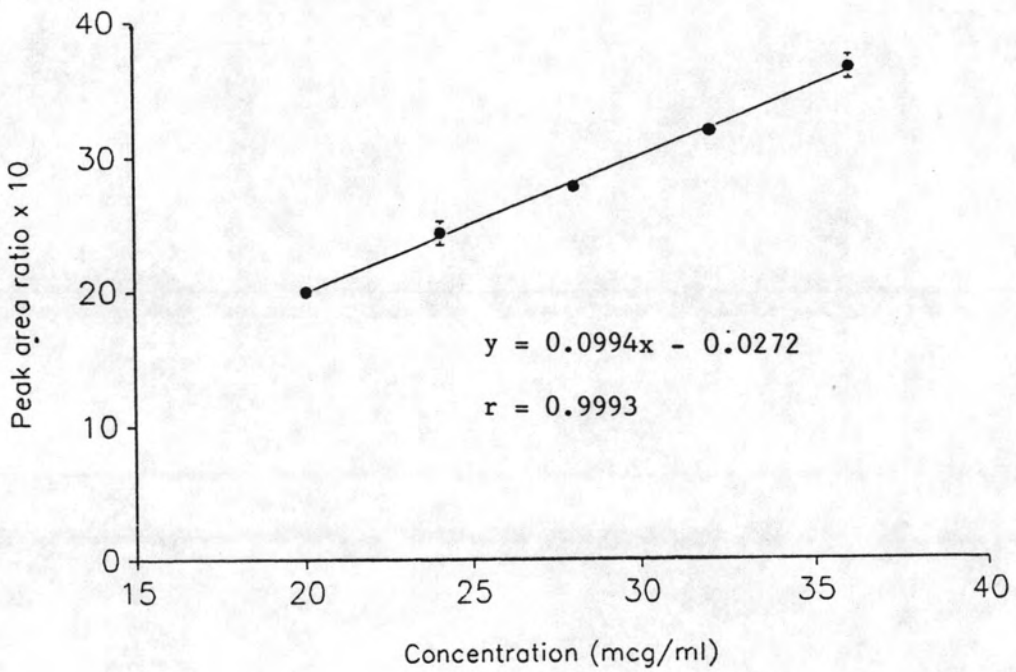

Figure 14 Peak area ratio versus concentration of promethazine hydrochloride

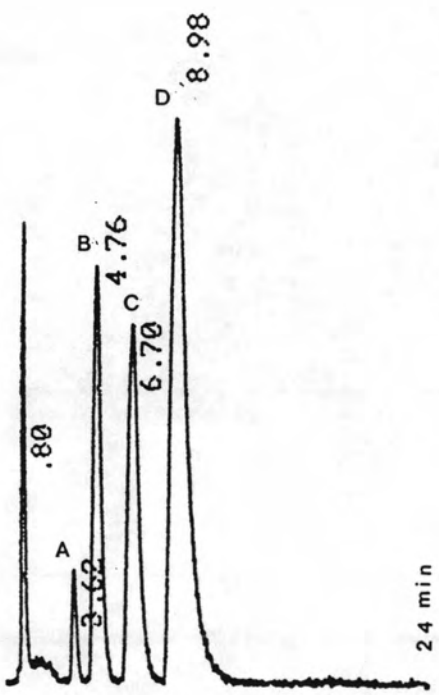

Figure 15 Chromatogram of the syrup USP spiked with ephedrine hydrochloride, codeine phosphate, promethazine hydrochloride and the internal standard

A = ephedrine hydrochloride

B = ethylephedrine hydrochloride, an internal standard

C = codeine phosphate

D = promethazine hydrochloride

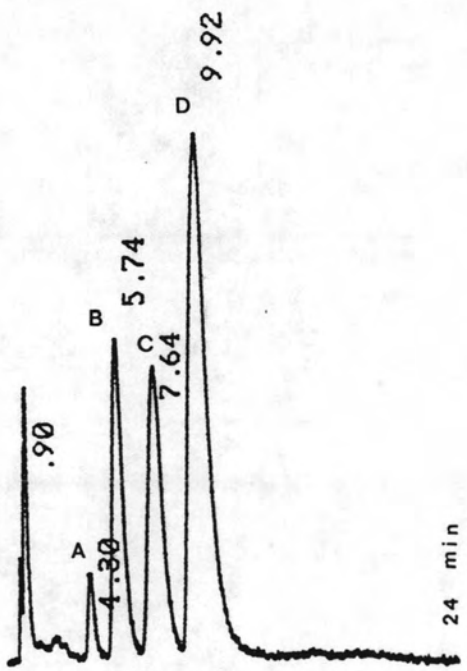

Figure 16 Chromatogram of syrup No. 1

A, B, C, D are the same as listed in Figure 15

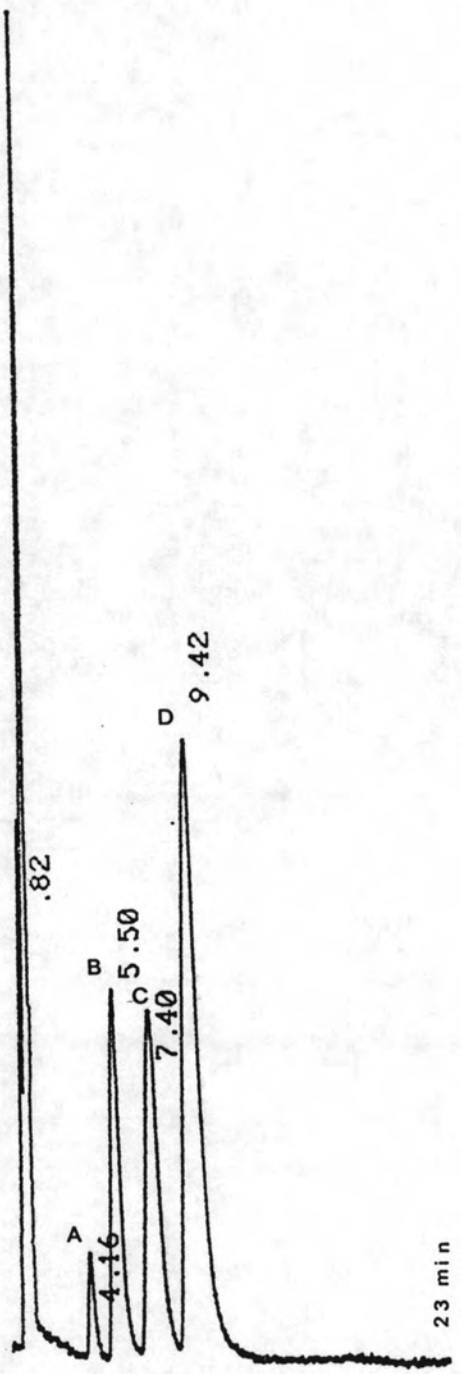

Figure 17 Chromatogram of syrup No. 2

A, B, C, D are the same as listed in Figure 15

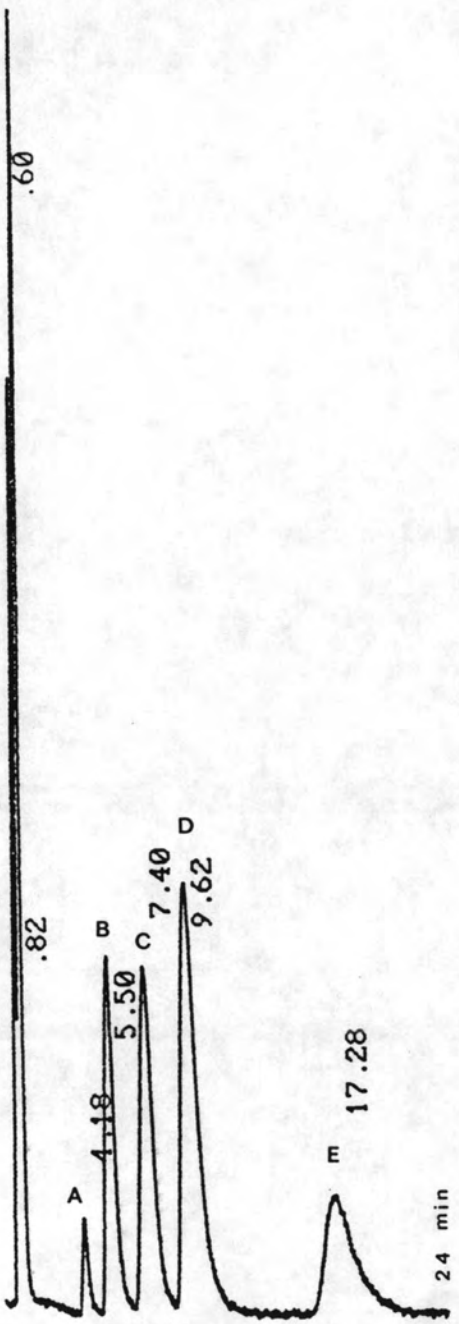

Figure 18 Chromatogram of syrup No. 3

A, B, C, D are the same as listed in Figure 15

E = unknown peak, suspected to be degradation products of promethazine hydrochloride

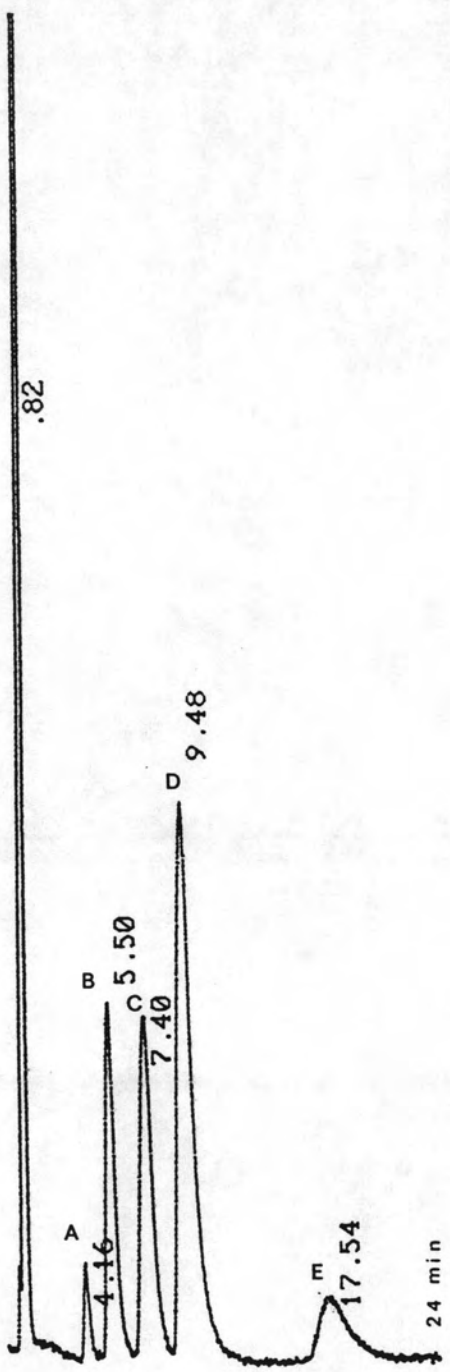

Figure 19 Chromatogram of syrup No. 4

A, B, C, D are the same as listed in Figure 15

E = is the same as described in Figure 18

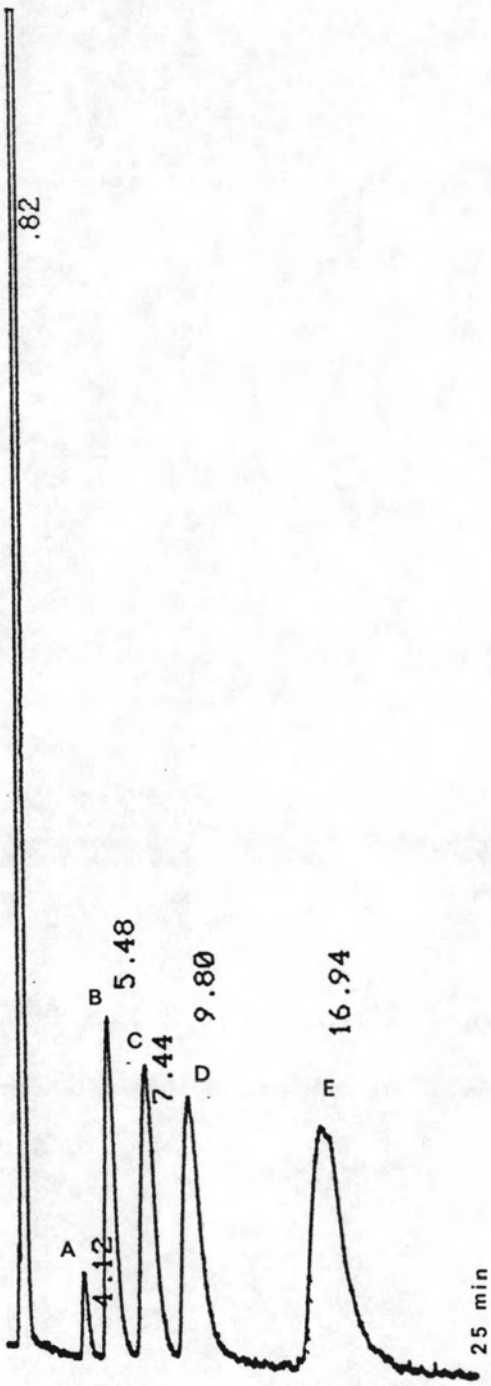

Figure 20 Chromatogram of syrup No. 5

A, B, C, D are the same as listed in Figure 15

E is the same as described in Figure 18

VITA

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