this study, to buffer the iodate–iodide reaction in the 3.4–4.0 pH range; in this range the reduction of iodate is almost instantaneous and no oxidation of the excess iodide by atmospheric oxygen occurs.

The ratio, thyroxine/$2I_2$, was determined on a series of six aliquots of a standard thyroxine solution ranging from 50 to 200 μg with the proposed oxidation and spectrophotometric procedures. The average (1.5131) of the determinations was 98.7% of the theoretical value (1.5304), and the standard deviation of the series was 0.0074.

Sixteen sodium levothyroxine tablet composites were extracted with butanol in separators, and aliquots of the extracts were analyzed by the low temperature ignition and the bromine oxidation procedures. The data obtained established that the two procedures yield concordant results; the greatest discrepancy between the two procedures (3.7%) was of the same order of magnitude as the greatest discrepancy (4.0%) within either individual procedure. On this basis, the bromine oxidation procedure was concluded to be as reliable as the ignition procedure and it is more convenient and simpler.

The scheme presented here for individual tablet analyses of sodium levothyroxine or sodium liothyronine tablets is an outgrowth of the more elaborate butanol shake-out procedure developed earlier in this investigation. By substituting the chloroform–2-propanol–acetic acid system for butanol and eliminating several washes, the procedure was shortened considerably and in no case did an emulsion form even after prolonged vigorous extractions in separators. However, because of attempts to keep the procedure as short as possible, difficulties were encountered during development of the individual tablet assay. The averages of the individual tablet analyses compared satisfactorily with those obtained by using the column assay of composites and weighed amounts of these composites equivalent to single tablets for all dosage levels of sodium levothyroxine tablets from Manufacturer A, the one brand of sodium liothyronine assayed, and, fortuitously, the two lots of sodium levothyroxine tablets from Manufacturer B that were initially assayed. The one dosage level of sodium levothyroxine tablets from Manufacturer D gave a substantial discrepancy between the averaged individual tablet analyses and the column assay of the composite sample. When additional dosage levels and lots from Manufacturer B were studied, more discrepancies were noted. Several attempts to remedy the situation were made without success until a sodium hydroxide treatment of an aliquot of the extract was included, as in the column assay. Since all of the samples analyzed produced some quantity of a gelatinous precipitate after sodium hydroxide treatment in the column assay, the discrepancies may have arisen as the effect of another excipient which is denatured by the sodium hydroxide treatment. In only two cases was there sufficient precipitate from the aliquot taken to warrant filtration in the individual tablet analyses.

This procedure, coupled with the extraction and spectrophotometric techniques, provides a very convenient method feasible for the determination of sodium levothyroxine or sodium liothyronine in individual tablets or composites in the range of 5–500 μg. The essential advantages of this procedure over the USP XVIII procedure (1) are realized in its sensitivity, precision, accuracy, and simplicity. Preliminary studies indicated that the proposed oxidation technique may be advantageous for the assay of certain other organic iodo compounds, and more definitive studies are in progress.

REFERENCES

(1) "The United States Pharmacopeia," 18th rev., Mack Publishing Co., Easton, Pa., 1970, pp. 642–646.

(2) J. E. Moody, J. R. Hohman, and G. B. Kaplan, *J. Pharm. Sci.*, **57**, 634(1968).

(3) J. J. Custer and S. Natelson, *Anal. Chem.*, **21**, 1005(1949).

(4) "The United States Pharmacopeia," 18th rev., Mack Publishing Co., Easton, Pa., 1970, p. 1036.

(5) *Ibid.*, p. 1024.

ACKNOWLEDGMENTS AND ADDRESSES

Received October 9, 1973, from the *Division of Drug Chemistry, Food and Drug Administration, Department of Health, Education, and Welfare, Washington, DC 20204*

Accepted for publication November 29, 1973.

* Present address: The United States Pharmacopeia, 12601 Twinbrook Parkway, Rockville, MD 20852

‡ Present address: Drugs Control Laboratory, Bombay 51, India.

ˣ To whom inquiries should be directed.

Liquid Chromatography in Pharmaceutical Analysis: Determination of Cough–Cold Mixtures

I. L. HONIGBERG ˣ, J. T. STEWART, and A. P. SMITH

Abstract □ Operating conditions are described for the qualitative and quantitative analysis of antihistaminic, antitussive, and analgesic compounds in cough–cold mixtures by high-pressure liquid chromatography. Twenty-one drugs were investigated. Determinations can be made in less than 30 min with an accuracy of 1–5%.

Keyphrases □ Antihistaminic, antitussive, and analgesic drugs—retention times, high-pressure liquid chromatography, application to analysis of cough–cold mixtures □ Analgesic, antihistaminic, and antitussive drugs—retention times, high-pressure liquid chromatography, application to analysis of cough–cold mixtures □ Antitussive, antihistaminic, and analgesic drugs—retention times, high-pressure liquid chromatography, application to analysis of cough–cold mixtures □ Cough–cold mixtures—analysis, high-pressure liquid chromatographic retention times of drug components □ High-pressure liquid chromatography—analysis, cough–cold mixtures, retention times of various antihistaminic, antitussive, and analgesic drugs

The recent introduction of commerically available, high-pressure liquid chromatography (HPLC) systems suggested a reinvestigation of the analysis of multicomponent pharmaceutical dosage forms. The cough–cold preparations which contain several therapeutic classes are exemplary of this type. The separation and quantitative analysis of these products present difficult problems to the pharmaceutical analyst. Mario and Meehan (1) reviewed the problems involved and used an all-glass GLC system because

Table I—Effect of Mobile Phase Composition on Retention Times[a]

Compound	Mobile Phase Composition[b] A Octadecyl	A Phenyl	B Octadecyl	B Phenyl	C Octadecyl	C Phenyl	D Octadecyl	D Phenyl	E Octadecyl	E Phenyl	F Octadecyl	F Phenyl
1. Pyrilamine maleate	91	88	105	94	97	88	114	106	113	91	99	106
	[c]	102	[c]	694	113	101	[c]	[c]	340	210	125	177
					602	425					215	
2. Aspirin	91	90	96	91	90	90	109	106	97	94	92	111
					118	109					128	
3. Pseudoephedrine hydrochloride	[c]	142	105	99	113	106	[c]	142	132	123	158	106
												134
4. Phenylpropanolamine hydrochloride	[c]	106	105	99	[c]	106	[c]	118	114	113	128	118
		196	273	215		277						
5. Isopropamide iodide	[c]	90	[c]	91	90	90	[c]	[c]	99	94	85	94
		106			113	106			189	177	120	156
											146	
6. Hydrocodone bitartrate	[c]	113	[c]	101	[c]	111	[c]	[c]	257	227	243	109
		466		338		300						224
7. Chlorpheniramine maleate	91	106	96	91	94	90	[c]	94	110	92	130	94
	[c]	[c]	[c]	[c]	113	104		[c]	708	225	514	248
						881						
8. Caramiphen edisylate	[c]	118	[c]	307	[c]	109	[c]	[c]	[c]	179	111	109
		520				168					239	142
9. Diphenhydramine hydrochloride	[c]	106	[c]	101	100	99	[c]	[c]	302	182	129	111
		118		606	421	101					198	161
		850			[c]	314						
10. Triprolidine hydrochloride	[c]	118	141	101	116	101	[c]	[c]	902	310	122	106
		[c]		[c]		104					648	277
						850						
11. Salicylamide	99		105				156	118	106			
12. Promethazine hydrochloride	170								1044			
13. Phenyltoloxamine citrate							[c]		109			
									535			
14. Bromodiphenhydramine hydrochloride									142	208	116	
									261		293	
									503			
15. Ephedrine sulfate									132			
16. Homatropine methylbromide									142			
17. Pheniramine maleate									112			
									272			
									436			
18. Brompheniramine maleate									109			
									768			
19. Acetaminophen									102			
20. Phenylephrine hydrochloride									107			
21. Codeine phosphate										180		

[a] Expressed as number of seconds elapsed between injection and attainment of the chromatographic peak maximum. [b] Solvent composition: A, 50 parts acetonitrile, 50 parts 0.1% ammonium carbonate, pH 8.50; B, 60 parts acetonitrile, 40 parts 0.1% ammonium carbonate, pH 8.60; C, 90 parts acetonitrile, 10 parts 0.1% ammonium carbonate, pH 8.90; D, 20 parts acetonitrile, 80 parts 1.0% ammonium acetate, pH 7.04; E, 60 parts acetonitrile, 40 parts 1.0% ammonium acetate, pH 7.40; F, 80 parts acetonitrile, 20 parts 1.0% ammonium acetate, pH 7.58. [c] Retention time greater than 1200 sec.

the free base form of the drugs interacted with the surface of metal columns. GLC was also used by Cometti *et al.* (2), but they encountered problems in finding suitable inert solid supports. Many of their columns were somewhat cumbersome to reproduce. Other investigators (3–5) reported problems with the use of GLC in the analysis of these preparations.

Onishi *et al.* (6) used countercurrent distribution in the determination of anticold preparations, but 50 transfers were needed to obtain quantitative separations. Other methods involved nonaqueous titrations (4), conductometric titrations (7), spectrophotometry (8), and column and thin-layer chromatography (4, 9). Difficulties in end-point perception and incomplete separations of drugs were disadvantages of these techniques.

Determination of drugs in cough–cold mixtures by HPLC overcomes or circumvents many shortcomings in the previously reported methods. In this paper, development of separations by HPLC of antihistaminic, antitussive, and analgesic drugs is reported. A quantitative study is described for a typical mixture. The preparation of samples is simple and rapid since the drugs can be analyzed in their salt form. Separation and analysis times require less than 30 min.

EXPERIMENTAL[1]

Reagents and Chemicals—Powdered samples (50 mg/10 ml) of pyrilamine maleate[2], phenylpropanolamine hydrochloride[3], chlor-

[1] A Waters associates liquid chromatograph (model ALC 202), equipped with an M-6000 pump, a UV monitor, a Infotronics integrator (model CRS-204) with digital printout, and Waters packed columns, 1.22 m long × 2.3 mm i.d., was used.

[2] Dorsey Laboratories, Lincoln, Neb.

[3] Aldrich Chemical Co., Milwaukee, Wis.

Table II—Calibration Data for Standard Drug Solutions

Compound	Concentration, mg/10 ml	*D/IS* Ratio[a]	Slope	Intercept	$r \pm s$
Pseudoephedrine hydrochloride	57.65	0.2542 ± 0.0017	0.00438	0.00401	0.9999 ± 0.002
	96.80	0.4306 ± 0.0046			
	201.90	0.8869 ± 0.0029			
Codeine phosphate	10.00	0.3284 ± 0.009			
	20.00	0.6417 ± 0.011	0.03269	−0.00400	0.9999 ± 0.0059
	40.00	1.3065 ± 0.003			
Triprolidine hydrochloride	2.50	0.4001 ± 0.0010	0.16695	−0.00985	0.9998 ± 0.008
	5.00	0.8360 ± 0.0035			
	10.00	1.6559 ± 0.0398			

[a] Data represent three replicate injections of standard solutions. *D/IS* is the ratio of the integrated area of the drug at some concentration divided by the integrated area of strychnine sulfate at a concentration of 5 mg/10 ml.

pheniramine maleate[4], diphenhydramine hydrochloride[5], phenindamine maleate[2], bromodiphenhydramine hydrochloride[5], phenyltoloxamine citrate[6], phenylephrine hydrochloride[7], brompheniramine maleate[8], ephedrine sulfate[9], aspirin[10], salicylamide[11], acetaminophen[11], hydrocodone bitartrate[12], isopropamide iodide[13], caramiphen edisylate[13], homatropine methylbromide[12], and promethazine hydrochloride[14] were used in the analytical procedure. In addition, pseudoephedrine hydrochloride[15], codeine phosphate[10], and triprolidine hydrochloride[15] were used in the analytical procedure and in the preparation of standard curves. All other chemicals and solvents utilized were the highest grade of commercially available materials.

Mobile Phases—The mobile phases, consisting of various percentages of acetonitrile and aqueous solutions of ammonium carbonate or ammonium acetate, were prepared fresh daily.

Internal Standard Solution—The stock internal standard solution (5 mg/ml) was prepared by dissolving strychnine sulfate in the appropriate mobile phase. Strychnine sulfate powder was recrystallized once from ethanol before use.

Standard Solutions for Calibration Curves—Into individual 10-ml volumetric flasks were added accurately weighed quantities of 57.65, 96.80, and 201.90 mg of pseudoephedrine hydrochloride. Stock solutions of codeine phosphate (250 mg/25 ml) and triprolidine hydrochloride (250 mg/100 ml) were prepared with the appropriate mobile phase [acetonitrile–aqueous 1% ammonium acetate (60:40)]. Volumes of 1, 2, and 4 ml of each stock solution were placed in 10-ml volumetric flasks. One milliliter of the internal standard stock solution in the same solvent was added to each flask. Additional mobile phase was then removed from the solvent reservoir and added to volume.

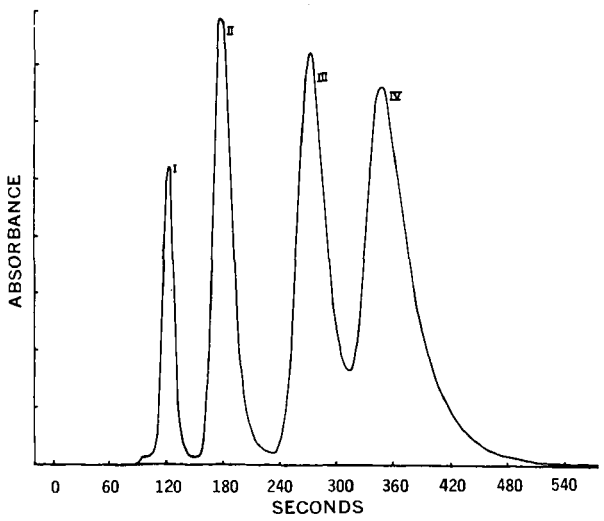

Figure 1—*Liquid chromatogram of a typical cough–cold preparation on diphenyldichlorosilane (phenyl) column with 60:40 acetonitrile–1% ammonium acetate. Key: I, pseudoephedrine hydrochloride; II, codeine phosphate; III, triprolidine hydrochloride; and IV, strychnine sulfate.*

Conditions for Chromatographic Separation and Quantification—The degassed mobile phase was pumped through columns containing monomolecular layers of octadecyltrichlorosilane (octadecyl) or diphenyldichlorosilane (phenyl) chemically bonded to a high efficiency pellicular packing, consisting of solid glass cores with a porous silica surface[16], at a flow rate of 1.4 ml/min (1000–1500 psig) at room temperature until a stable baseline was obtained. Replicate 10-μl injections of sample and standard solutions were made using a 25-μl syringe[17]. The chart recorder provided a record of drug elution from the column as peaks on a chromatogram. In all cases, the solute was measured by digital integration of the peak area[1].

RESULTS AND DISCUSSION

The purposes of this study were to determine the operating conditions for HPLC that would optimize the resolution of several therapeutic classes of heterogeneous compounds commonly found in cough–cold preparations in a reasonable time and to quantitate the analysis with a suitable level of precision.

The operating parameters studied were limited to solvent composition with respect to changing concentrations of acetonitrile and aqueous solutions of either 0.1% ammonium carbonate or 1% ammonium acetate, variations in the polarity of the reversed stationary phase, pH, and flow rate. The effect of the various parameters on the separation of the drugs studied is shown in Table I. Complete data are listed for Compounds 1–10, which were selected as representative structures of the 21 drugs studied.

Most compounds were separated best on the phenyl column using a 60:40 mixture (pH 7.4) of acetonitrile and aqueous 1% ammonium acetate. An increase in water concentration resulted in longer retention times on both columns with increased band spreading. Increases in acetonitrile concentration usually gave shorter retention times accompanied by multiple peaks on the chromatogram. Retention times were generally longer with the octadecyl column. In almost all cases, the compounds were eluted faster and the peaks were more symmetrical on the phenyl column when equivalent mobile phases were employed. A pH of 7.4 seemed essential for peak sharpness. Variations in pH had a marked effect on the number of species of the acidic or basic drugs that traveled through the column. This phenomenon is well documented in TLC. A flow rate of 1.4 ml/min, which resulted in pressures of 1000–1500 psig at room temperature, was the most satisfactory flow found for both octadecyl and phenyl columns since separations could be obtained with the various phases in less than 1200 sec.

[4] Schering Corp., Bloomfield, N.J.
[5] Parke-Davis and Co., Detroit, Mich.
[6] Bristol Laboratories, Syracuse, N.Y.
[7] Winthrop Laboratories, New York, N.Y.
[8] A. H. Robins and Co., Richmond, Va.
[9] Matheson, Coleman and Bell, East Rutherford, N.J.
[10] Merck Chemicals, Rahway, N.J.
[11] Eastman Organic Chemicals, Rochester, N.Y.
[12] Endo Laboratories, Garden City, N.Y.
[13] Smith Kline and French, Philadelphia, Pa.
[14] Wyeth Laboratories, Philadelphia, Pa.
[15] Burroughs Wellcome Co., Research Triangle Park, N.C.

[16] Corasil/C_{18} and Corasil/phenyl, 37–50 μm, Waters Associates, Milford, Mass.
[17] Model B-110, Precision Sampling Corp., Baton Rouge, La.

Table III—Analysis of Pseudoephedrine Hydrochloride, Codeine Phosphate, and Triprolidine Hydrochloride in Known Mixtures

Mixture	Added, mg	Found[a], mg	Accuracy, %
1. Pseudoephedrine hydrochloride	120.00	125.35 ± 4.47[b]	4.46
Codeine phosphate	20.00	20.01 ± 0.53	0.05
Triprolidine hydrochloride	5.00	5.14 ± 0.14	2.79
2. Pseudoephedrine hydrochloride	40.38	41.18 ± 1.89	1.98
Codeine phosphate	16.00	15.77 ± 0.17	1.46
Tripolidine hydrochloride	4.00	4.06 ± 0.09	1.50

[a] Based on three to five replicate determinations of known mixtures. [b] Confidence limits at $p = 0.05$.

A mixture of pseudoephedrine hydrochloride, codeine phosphate, and triprolidine hydrochloride was selected to demonstrate the utility of the separation and quantification method[18]. Figure 1 illustrates a chromatogram of the drugs being assayed. Various concentrations of standard solutions of each drug dissolved in the 60:40 acetonitrile–aqueous 1% ammonium acetate mixture were chromatographed using the phenyl column. Strychnine sulfate was added to each solution as the internal standard. The area under the curve for each peak on the chromatograms was integrated digitally. The ratio of each peak area to the area of the internal standard was calculated for each chromatogram. A linear regression line of these data at three concentrations of each drug gave the slope, intercept, and correlation coefficient for each calibration curve (Table II).

Known mixtures containing varied quantities of each drug were chromatographed, and the ratios of drug peak areas/internal standard peak areas *(D/IS)* were calculated. The overlap of the triprolidine and internal standard peaks in the mixture (Fig. 1) resulted in a smaller integrated area for the internal standard in the mixture *versus* its area in the calibration curves for codeine phosphate and pseudoephedrine. A suitable correction factor was applied to these two *D/IS* ratios in the mixture[19]. The *D/IS* ratios were then fed into a programmable calculator[20] along with slope and intercept data (see Table II) for each drug to obtain the concentration of the drugs in the mixture.

The data in Table III demonstrate the quantitative results obtained for two known mixtures. The utility of HPLC in the analysis of complex heterogeneous drug mixtures found in cough–cold dosage forms is clearly demonstrated. The accuracy range is from 1 to 5%.

[18] These three drugs are components of a commercially available dosage form, Actifed-C, marketed by Burroughs Wellcome Co. The expectorant, glyceryl guaiacolate, was not included as a part of this study.

REFERENCES

(1) E. Mario and L. Meehan, *J. Pharm. Sci.*, **59**, 538(1970).

(2) A. Cometti, G. Bagnasco, and N. Maggi, *ibid.*, **60**, 1074(1971).

(3) K. Goebbler, *Deut. Apoth.-Ztg.*, **111**, 1291(1971).

(4) G. Cavicchi, B. Cristofori, and M. Quaglio, *Boll. Chim. Farm.*, **108**, 682(1969); through *Chem. Abstr.*, **72**, 136507(1970).

(5) T. Reiss, *J. Ass. Offic. Anal. Chem.*, **53**, 609(1970).

(6) R. Onishi, K. Kawamura, and K. Yamamoto, *Yakugaku Zasshi*, **91**, 534(1971); through *Chem. Abstr.*, **75**, 52874(1971).

(7) K. Nikolic and Z. Cupic, *Acta Pharm. Jugoslav.*, **21**, 159(1971); through *Chem. Abstr.*, **76**, 103816(1972).

(8) F. Kagan and L. Kirichenki, *Farm. Zh. (Kiev)*, **26**, 32(1971); through *Chem. Abstr.*, **76**, 90102(1972).

(9) R. Hall and J. Levine, *J. Ass. Offic. Anal. Chem.*, **55**, 332(1972).

ACKNOWLEDGMENTS AND ADDRESSES

Received September 19, 1973, from the *Department of Medicinal Chemistry, School of Pharmacy, University of Georgia, Athens, GA 30602*

Accepted for publication December 20, 1973.

The authors express their appreciation to Mr. William Hester and Mr. Russell Plunkett for technical assistance.

[x] To whom inquiries should be directed.

[19] For example:

$$\frac{D_{\text{pseudoephedrine}}}{IS_T} \times \frac{IS_T}{IS_0} = \text{corrected } D/IS \text{ ratio for pseudoephedrine}$$

where $D_{\text{pseudoephedrine}}$ is the area of the pseudoephedrine in the mixture, IS_0 is the area of 10 μl of a 0.5-mg/ml solution of strychnine sulfate without triprolidine overlap, and IS_T is the area of 10 μl of a 0.5-mg/ml solution of strychnine sulfate in the presence of triprolidine.

[20] Olivetti-Underwood Programma 101.