Liquid Chromatography in Pharmaceutical Analysis IV: Determination of Antispasmodic Mixtures

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Abstract □ Parameters associated with the separation of antianxiety–antispasmodic agents were investigated using high-pressure liquid chromatography. Eight widely prescribed drugs were studied. The compounds were chromatographed on reversed-phase octadecyltrichlorosilane (C_{18}) or diphenyldichlorosilane (phenyl) columns, using mixtures of absolute methanol and distilled water buffered with ammonium dihydrogen phosphate, ammonium acid phosphate, or ammonium carbonate. A mixture of phenobarbital–propantheline bromide was selected to demonstrate the utility of the separation and quantification method. The mixture was chromatographed on a phenyl column, using absolute methanol–aqueous 1% ammonium dihydrogen phosphate (60:40) (pH 5.85) at a flow rate of 1.4 ml/min. Each determination can be achieved in approximately 15 min with an accuracy of 1–2%.

Keyphrases □ Antispasmodic mixtures—high-pressure liquid chromatographic analysis, method parameters □ Antianxiety–antispasmodic agents—high-pressure liquid chromatographic analysis, method parameters □ High-pressure liquid chromatography—analysis, antispasmodic mixtures

This paper reports an investigation of the parameters associated with the separation and quantification of antianxiety–antispasmodic agents as a continuation of the study into the use of high-pressure liquid chromatography (HPLC) in the analysis of multicomponent dosage forms. Previous reports (1–3) dealt with the separation, detection, and quantification of cough–cold and diuretic–antihypertensive mixtures by HPLC.

The compounds studied are included in listings of most prescribed drugs used singly or in combination for treatment of functional or organic disorders of the GI tract. The drugs included were scopolamine hydrobromide, hyoscyamine sulfate, isopropamide iodide, prochlorperazine dimaleate, clidinium bromide, chlordiazepoxide hydrochloride, propantheline bromide, and phenobarbital.

Scopolamine and hyoscyamine in dosage forms have been analyzed by many methods including aqueous and nonaqueous titrimetry (4, 5), UV and visible spectrophotometry (6, 7), and TLC and GLC (8–10). Isopropamide iodide was determined by colorimetry (11) and nonaqueous titrimetry (12). GC (13), polarographic (14), and fluorometric (15) methods were reported for prochlorperazine. Clidinium bromide and chlordiazepoxide were determined by nonaqueous titrimetry (16, 17). Other methods for chlordiazepoxide include fluorescence (18), GC (19), and colorimetry (20). Spectrophotometry (21) and nonaqueous titrimetry (22) are used in the analysis of propantheline bromide, while spectrophotometry (23), TLC (24), and GLC (25, 26) are available for the detection and quantification of phenobarbital.

The investigated HPLC operating parameters were limited to solvent composition, pH, and stationary phase polarity. The use of these data can be readily applied to the separation and quantification of selected multidrug dosage forms. The quantification of a typical antispasmodic mixture is described.

EXPERIMENTAL[1]

Reagents and Chemicals—Powdered samples of scopolamine hydrobromide[2], hyoscyamine sulfate[2], isopropamide iodide[3], prochlorperazine dimaleate[3], clidinium bromide[4], and chlordiazepoxide hydrochloride[4] were used. In addition, propantheline bromide[5] and phenobarbital[6] were used in the analytical procedure and in the preparation of standard curves. All other chemicals and solvents were the highest commercially available materials.

Table I—Calibration Data for Standard Drug Solutions

Compound	Final Concentration, mg[a]	D/IS Ratio[b]	Slope	Intercept	$r + s$
Phenobarbital	7.5	0.2397 ± 0.002[c]	0.03390	—0.03075	0.9985 ±0.017
	15.0	0.4534 ± 0.001			
	30.0	0.9944 ± 0.007			
Propantheline bromide	7.5	0.7079 ± 0.040	0.08094	0.13931	0.9985 ±0.041
	15.0	1.4110 ± 0.032			
	30.0	2.5484 ± 0.008			

[a] Total mg/10 ml solution. [b] Data represent three to five 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 *m*-terphenyl at a concentration of 1 mg/10 ml. [c] Confidence limits at $p = 0.05$.

Mobile Phases—The mobile phases consisted of various concentrations of absolute methanol mixed with aqueous solutions containing: *(a)* 1% ammonium dihydrogen phosphate, *(b)* 1% ammonium dihydrogen phosphate and 1% ammonium acid phosphate, or *(c)* 0.5% ammonium carbonate. The solutions were prepared fresh daily.

[1] A Waters Associates liquid chromatograph (model ALC 202), equipped with an M-6000 pump, a UV monitor (254 nm), and an Infotronics integrator (model CRS-204) with digital printout, and Waters packed columns (1.22 m long × 2.3 mm i.d.) was used.

[2] A. H. Robins, Richmond, Va.

[3] Smith Kline and French, Philadelphia, Pa.

[4] Hoffmann-La Roche, Inc., Nutley, N.J.

[5] G. D. Searle and Co., Chicago, Ill.

[6] American Pharmaceutical Co., New York, N.Y.

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

Mobile Phase Composition[b]	Chlordiazepoxide Hydrochloride		Scopolamine Hydrobromide		Propantheline Bromide		Hyoscyamine Sulfate		Isopropamide Iodide		Clidinium Bromide		Phenobarbital		Prochlorperazine Dimaleate	
	C_{18}	Phenyl	C_{18}	Phenyl	C_{18}	Phenyl	C_{18}	Phenyl	C_{18}	Phenyl	C_{18}	Phenyl	C_{18}	Phenyl	C_{18}	Phenyl
A	80[a] (24)[c] 100 (48)	80 (24)	83 (24)	84 (42)	111 (48) 194[e] (156)	120 (36)	86 (24)	86 (24)	93 (36)	90 (30)	106 (48)	94 (60)	79 (18)	72 (18)	—[d]	138[e] (66)
B	94 (30) 183 (78)	90 (24)	84 (30)	88 (18)	141 (84) 332[e] (318)	126 (42) 162[e] (72)	88 (30)	82 (24)	96 (48)	122 (42)	113 (60)	114[e] (30)	87 (18)	68[e] (18)	—[d]	256[e] (169)
C	156 (60) 540 (234)	113 (42)	92 (30)	83 (24) 114 (48)	264 (150) 998[e] (792)	153 (54) 208[e] (96)	90 (36)	88 (30) 112 (54)	150[e] (96)	150 (54)	156[e] (108)	89 (42) 136 (54)	92 (30)	68 (18)	—[d]	720[e] (594)
D	118 (42)	78 (24)	82 (30) 108 (48)	88 (30)	66 (30) 80 (36) 135 (72) 414 (396)	91 (30) 146 (60)	120 (60)	122 (36)	114 (90)	112 (30)	90 (30) 156 (108)	139 (54)	72 (24)	74 (24)	—[d]	302[e] (204)
E	104 (24) 216 (78)	79 (30)	100 (36) 196 (84)	87 (30)	84 (24) 123 (66) 207 (186) 318 (156) 954[e] (906)	187 (78)	138 (78)	120 (42)	146[e] (96)	118 (42)	210[e] (186)	134 (48)	84 (30)	74 (24)	—[d]	—[d]
F	144 (56) 524[e] (222)	92 (30)	124 (56)	90 (24)	66 (32) 104 (36) 224 (104) 436[e] (320)	244[e] (120)	172[e] (114)	112 (36)	222[e] (234)	110 (42)	372[e] (366)	135 (66)	86 (24)	72 (24)	—[d]	—[d]
G	82 (30) 99 (42)	78 (24)	86 (24)	78 (18)	74 (24) 156 (54) 276 (216) 916[e] (852)	268[e] (90)	180[e] (108)	180 (72)	130[e] (72)	146 (42)	103 (30) 241[e] (204)	188 (66)	74 (18)	72 (18)	—[d]	336[e] (276)
H	100 (30) 206 (78)	84 (30)	70 (24) 100 (30)	79 (24)	76 (30) 235 (102)	120 (24) 284 (178) 333[e] (198)	72 (30) 396[e] (270)	69 (24) 215[e] (90)	194[e] (162)	150[e] (66)	78 (30) 134 (48) 408[e] (432)	72 (18) 218[e] (54)	73 (18)	66 (18)	—[d]	—[d]

Table II—*Continued*

Mobile Phase Composition[b]	Chlordiazepoxide Hydrochloride		Scopolamine Hydrobromide		Propantheline Bromide		Hyoscyamine Sulfate		Isopropamide Iodide		Clidinium Bromide		Phenobarbital		Prochlorperazine Dimaleate	
	C_{18}	Phenyl	C_{18}	Phenyl	C_{18}	Phenyl	C_{18}	Phenyl	C_{18}	Phenyl	C_{18}	Phenyl	C_{18}	Phenyl	C_{18}	Phenyl
I	70	98	74	84	68	63	52	64	94	162[e]	62	222[e]	82	64	—[d]	—[d]
	(24)	(36)	(24)	(24)	(24)	(12)	(12)	(18)	(36)	(66)	(30)	(90)	(24)	(18)		
	154		144		100	309	86	90	414[e]		78					
	(66)		(54)		(48)	(108)	(90)	(24)	(558)		(30)					
	513				254	372[e]	552[e]	222[e]			250					
	(26)				(144)	(214)	(384)	(90)			(72)					
					1034						898[e]					
					(372)						(918)					

[a] Retention time expressed as seconds measured as elapsed time between injection and attainment of the chromatographic peak maximum. The eluted peaks were monitored using UV detection. [b] Solvent compositions and pH are given in Table III. [c] Base peak width expressed as seconds. [d] Retention time greater than 1800 sec. [e] Significant tailing was observed.

Table III—Solvent Compositions and pH

Mobile Phase	Components	Solvent Ratio	pH
A	Absolute methanol	60	5.85
	1% Ammonium dihydrogen phosphate	40	
B	Absolute methanol	50	5.50
	1% Ammonium dihydrogen phosphate	50	
C	Absolute methanol	40	5.50
	1% Ammonium dihydrogen phosphate	60	
D	Absolute methanol	60	8.20
	1% Ammonium dihydrogen phosphate	20	
	1% Ammonium acid phosphate	20	
E	Absolute methanol	50	7.90
	1% Ammonium dihydrogen phosphate	25	
	1% Ammonium acid phosphate	25	
F	Absolute methanol	40	7.60
	1% Ammonium dihydrogen phosphate	30	
	1% Ammonium acid phosphate	30	
G	Absolute methanol	60	8.65
	0.5% Ammonium carbonate	40	
H	Absolute methanol	50	8.70
	0.5% Ammonium carbonate	50	
I	Absolute methanol	40	8.80
	0.5% Ammonium carbonate	60	

Preparation of Drug Solutions—Separate solutions (150 mg/10 ml) of scopolamine hydrobromide, hyoscyamine sulfate, isopropamide iodide, and clidinium bromide were prepared by dissolving each powder in absolute methanol. Solutions of chlordiazepoxide hydrochloride (3 mg/10 ml), prochlorperazine dimaleate (30 mg/10 ml), and phenobarbital (30 mg/10 ml) were also prepared using methanol.

Internal Standard Solution—The stock internal standard solution (10 mg/10 ml) was prepared by dissolving *m*-terphenyl[7] in absolute methanol. *m*-Terphenyl, mp 85–87°, was purified before use by the method of France *et al.* (27).

Standard Solutions for Calibration Curves—Separate stock solutions (750 mg/100 ml) of phenobarbital and propantheline bromide were prepared in absolute methanol. Accurately pipetted volumes of 1.0, 2.0, and 4.0 ml of each stock solution were placed in 10-ml volumetric flasks. One milliliter of the internal standard stock solution was added to each flask, followed by the addition of absolute methanol to volume.

The three concentrations of each drug were subjected to a linear regression analysis, and the slope and intercept were calculated (Table I).

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[8], at a flow rate of 1.4 ml/min (2000 psig) at room temperature until a stable baseline was obtained. Replicate 15-μl injections of sample and standard solutions were made using a 25-μl syringe[9]. 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 purpose of this study was to determine the operating conditions for HPLC that would optimize the resolution of chemically unrelated therapeutic agents most commonly found in antispasmodic mixtures. Three operating parameters were studied: *(a)* variations in solvent composition containing absolute methanol with changing concentrations of distilled water; *(b)* variations in pH within the 5.5–8.8 range using ammonium acid phosphate, ammonium dihydrogen phosphate, or ammonium carbonate as inorganic buffers; and *(c)* variations in the nonpolar (reversed-phase) stationary phase in which the pellicular packing differed in polarity. The effects of these parameters on retention times and base

[7] Eastman Organic Chemicals, Rochester, N.Y.
[8] Corasil/C_{18} and Corasil/phenyl, 37–50 μm, Waters Associates, Milford, Mass.
[9] Model B-110, Precision Sampling Corp., Baton Rouge, La.

Table IV—Approximate Resolution (*Rs*) Values for Phenobarbital–Propantheline Bromide Mixture on Phenyl Column

Mobile Phase[a]	pH	*Rs* Value[b]
A	5.85	1.78
E	7.90	2.20
F	7.60	2.40
I	8.80	3.60

[a] Letters refer to solvent compositions in Table III. [b] See Footnote 10.

peak widths of the drugs are shown in Table II. In addition, Table II indicates those solvent systems that generate multiplets or tailing of single drug entities.

For the investigated drugs, the following generalizations can be inferred from the data in Table II. Retention time for most drugs on the octadecyl column increases at all pH ranges as the polarity of the mobile phase increases. This effect is also observed with the phenyl column in the acidic pH range. In basic pH ranges on the phenyl column, there is little change in retention time with a change in mobile phase polarity. In most cases, retention time for a drug is longer on the octadecyl column than on the phenyl column.

Changes in solvent polarity have a greater effect on the retention times of the drugs on either the octadecyl or phenyl column than do changes in pH of the mobile phase. However, clidinium bromide and prochlorperazine dimaleate show appreciable changes in retention time with changes in pH of the mobile phase. Drugs containing an ester group give multiple peaks in basic pH ranges, probably because of hydrolysis on the column.

Modifications in the polarity of stationary and mobile phases provided the following observations for the drugs examined. Chlordiazepoxide hydrochloride produces multiple peaks on the octadecyl column in almost all solvent systems while giving only single peaks on the phenyl column. Resolution of the frequently prescribed chlordiazepoxide–clidinium bromide combination can be observed in Solvent Systems B, C, D, E, F, and I (Tables II and III).

The concentration levels of scopolamine and hyoscyamine detected in this study were 100 times higher than the level commonly found in commercial dosage forms. In several cases, the more basic solvents (*i.e.*, G, H, and I) led to tailing and/or multiple peaks for hyoscyamine on the phenyl column. The tailing and/or multiple peaks are probably due to decomposition in alkaline media (28). Neither scopolamine nor hyoscyamine could be detected at therapeutic dose levels with the present instrumentation.

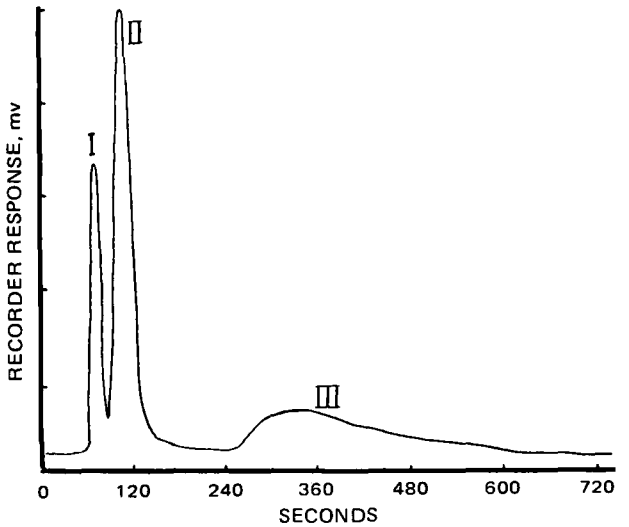

Figure 1—*Chromatogram of a phenobarbital–propantheline bromide mixture on diphenyldichlorosilane (phenyl) column with absolute methanol–1% ammonium dihydrogen phosphate (60:40). Key: I, phenobarbital; II, propantheline bromide; and III, m-terphenyl.*

Table V—Analysis of Phenobarbital–Propantheline Bromide in Known Mixture

Mixture	Milligrams Added	Milligrams Found[a]	Accuracy, %
Phenobarbital	15.0	15.262 ± 0.27[b]	1.75
Propantheline bromide	15.0	15.209 ± 0.20	1.39

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

A combination of long retention time and basic pH of the mobile phase resulted in multiple peaks for propantheline bromide on the octadecyl column.

A peak associated with iodide ion in isopropamide iodide appeared at the solvent front in all solvent systems and on both columns (not recorded in Table II).

Long retention times in basic solvents (*i.e.*, G, H, and I) led to multiple peaks for clidinium bromide, possibly due to hydrolysis.

Phenobarbital traveled close to the solvent front in all systems, and its retention time could not be distinguished from that of the solvent front.

Prochlorperazine dimaleate exhibited extremely long retention times and considerable tailing in all solvents at all pH's. This compound also produced a peak for maleate near the solvent front (not recorded in Table II).

Satisfactory quantification of a multicomponent dosage form is dependent on adequate resolution of the components. The value *Rs* (29), a theoretical parameter, is a reasonable measurement of the separation of two species on a column. It can be approximated for any two drugs included in this investigation from information contained in Table II[10]. A mixture of phenobarbital–propantheline bromide[11] was selected to exemplify this situation. Table IV shows calculated *Rs* values for the drug combination in various solvent systems and pH ranges on the phenyl column. Mobile phase A was chosen for the quantification since adequate resolution could be achieved in the shortest time.

Figure 1 illustrates a chromatogram of the assayed drugs. Various concentrations of standard solutions of each drug dissolved in the absolute methanol–aqueous 1% ammonium dihydrogen phosphate (60:40) mixture were chromatographed using the phenyl column. *m*-Terphenyl was added to each solution as the internal standard. The area under the curve for each peak on the chromatograms was determined with a digital integrator[1]. 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 V).

A known mixture containing a quantity of each drug was chromatographed, and the ratios of drug peak areas/internal standard peak areas (D/IS) were calculated for each drug. The constants (slope and intercept) for the linear regression equation shown in Table I were used to solve for drug concentration [D/IS = slope (concentration) + intercept]. The calculations were performed on a programmable calculator[12].

The data in Table V demonstrate the quantitative results obtained for the simulated dosage form. The utility of HPLC in the analysis of a phenobarbital–propantheline bromide mixture is clearly demonstrated with an accuracy of 1–2%.

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[10] It is possible to calculate the approximate resolution (*Rs*) of two components by the equation: $Rs = 2(t_2 - t_1)/(w_1 + w_2)$, where t_1 and t_2 are retention times and w_1 and w_2 are base peak widths of Compounds 1 and 2, respectively. In past experience, two components with an *Rs* value >1.5 showed satisfactory resolution for quantification by this analytical technique if the peak areas were approximately equal. A significant difference in peak areas for two components may require *Rs* values of 2 or greater.

[11] These two drugs are components of a commercially available dosage form, Pro-Banthine with Phenobarbital (G. D. Searle Co.).

[12] Olivetti-Underwood programma 101.

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ACKNOWLEDGMENTS AND ADDRESSES

Received October 30, 1974, from the *Department of Medicinal Chemistry, School of Pharmacy, University of Georgia, Athens, GA 30602*

Accepted for publication January 6, 1975.

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Analysis of Individual Thyroid Tablets

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Abstract □ A procedure was developed for the assay of individual thyroid tablets or composite samples equivalent to as little as 0.033 g (0.5 gr) of thyroid. The sample is ignited in a closed atmosphere of oxygen and, after a series of redox reactions, the iodine is determined spectrophotometrically as the triiodide ion. The results agree well with those obtained by the USP thyroid tablet assay.

Keyphrases □ Thyroid—analysis of individual tablets, spectrophotometric method measuring triiodide ion □ Iodine—analysis of individual thyroid tablets □ Spectrophotometry—analysis, individual thyroid tablets, measurement of triiodide ion

The USP thyroid tablet monograph assay (1) is not suitable for individual tablet analysis because the level of iodine in an individual tablet is usually too low to produce an acceptable titration volume. In an effort to provide an individual tablet analysis, the following method was developed. It also provides a satisfactory alternative to the compendial assay method (1).

The proposed method utilizes essentially the same chemical transformations that form the basis of the compendial monograph assay (Scheme I).

$$\text{thyroid} + Na_2CO_3 \longrightarrow I^- + \text{fusion products}$$

$$I^- + 3Br_2 + 3H_2O \xrightarrow{\text{boil}} IO_3^- + 6Br^- + 6H^+$$

$$IO_3^- + 5I^- + 6H^+ \longrightarrow 3I_2 + 3H_2O$$

Scheme I

In contrast to the compendial assay, which requires alkaline fusion of the equivalent of 1 g of thyroid and a final thiosulfate titration, the proposed procedure requires oxygen flask ignition of the equivalent of 30 mg of thyroid and a final spectrophotometric determination. The procedure is applicable to coated or uncoated tablets ranging from 0.033 to 0.4 g (0.5 to 6 gr) of thyroid. Potassium iodate is used as the standard, and the measurement is made on aliquots of the iodate solutions equivalent to 5–35 μg of iodine.

EXPERIMENTAL

Reagents—The standard potassium iodate solution is prepared by quantitatively diluting 0.05 *M* potassium iodate (2) stepwise to 1 in 2500 to obtain a solution equivalent to 2.538 μg I_2/ml.

The bromine–sodium acetate test solution is prepared as directed in Ref. 3.

Acetate buffer, pH 3.9, consists of 1.0 *M* sodium acetate in 5.5 *M* acetic acid.

Buffered potassium iodide is prepared just prior to use by mixing 1.5% (w/v) potassium iodide in pH 3.9 acetate buffer. The solution should remain colorless for several hours.

Assay Preparation—Oxygen ignition[1] was performed using 1-liter conical ignition flasks fitted with all-glass stopper sample holders.

Individual Tablets—Securely fasten one thyroid tablet in a suitable platinum basket in contact with a small strip of black Schöniger ignition paper. Suspend the basket from the sample

[1] Thomas-Ogg oxygen flask ignitor.