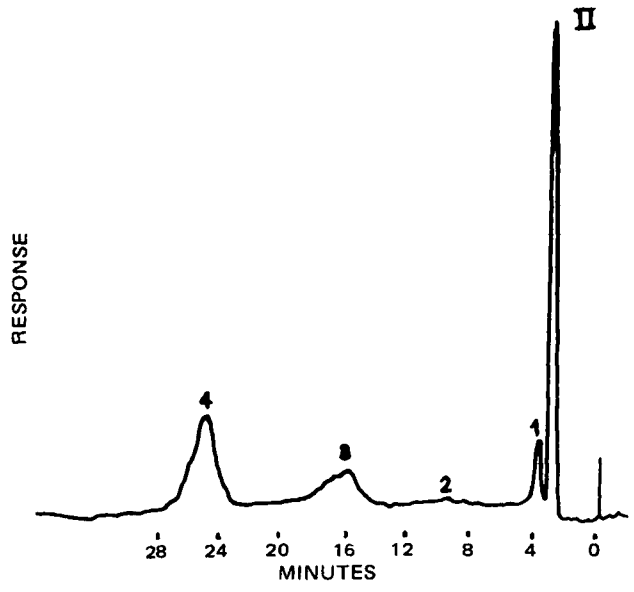

Figure 4—*Chromatogram of II and acid-degraded cephalexin derivative mixture.*

used in the assay of lysine and cephalexin. Cephalexin solutions stored under acidic conditions at 60° yielded four secondary peaks (retention times of 4.1, 11.0, 15.2, and 27.2 min), which could interfere with the chromatographic assay. Trinitrophenyl derivatives were prepared from mixtures containing 50% of acid-hydrolyzed cephalexin solutions and 50% of a pure cephalexin or pure lysine solution (Figs. 3 and 4), respectively. It is apparent that the peak of II was not affected by the acid decomposition products of cephalexin and that the peak of III was affected only minimally. The chromatogram of II after acid hydrolysis (1 hr) showed only two small additional peaks at 16.4 and 26.0 min.

The calibration factors used for the quantitative analysis of cephalexin and lysine are given in Table II. The low values of the intercept at the origin (a_0), when operating with the loop-injection technique but without the internal standard, allow use of this technique. Table II shows that similar calibration parameters were obtained by both techniques (with and without an internal standard), corresponding to the listed straight-line equations. The average response factors for II and III were 110.94 and 36.92 mm/mg with standard deviations of 0.76 and 1.05, respectively, for a set of 45 chromatograms. Sensitivity was three times higher for lysine than for cephalexin, but the sensitivity of the instrument allows lower detection limits of 0.1 and 0.3 μg/ml for lysine and cephalexin, respectively.

The high reproducibility and sensitivity of the HPLC method described here recommend it as a suitable procedure for the simultaneous analysis of cephalexin and lysine in mixtures of these two products for quality control and stability studies of pharmaceutical formulations containing cephalexin lysinate.

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Determination of Dextromethorphan Hydrobromide by High-Performance Liquid Chromatography Using Ion-Pair Formation

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Abstract □ The chromatographic retention behavior of dextromethorphan hydrobromide on an octadecylsilane column was investigated as a function of the pairing ion and the mobile phase composition. Dramatic increases in the capacity factor were observed with pairing ions containing more than eight carbons and with decreasing organic modifier (acetonitrile) concentration. Several pharmaceutically important amines exhibited similar behavior with respect to acetonitrile concentration. An analytical method was developed for dextromethorphan hydrobromide bulk drug and syrups and was applied to commercial preparations.

Keyphrases □ Dextromethorphan hydrobromide—high-performance liquid chromatographic analysis using ion-pair formation □ High-performance liquid chromatography—analysis, dextromethorphan hydrobromide, ion-pair formation □ Antitussives—dextromethorphan hydrobromide, high-performance liquid chromatographic analysis using ion-pair formation

Dextromethorphan hydrobromide [(+)-3-methoxy-17-methyl-9α,13α,14α-morphinan hydrobromide, I] is a centrally active antitussive which, unlike codeine, is devoid of analgesic properties and exhibits little or no depression of the central nervous system. Because of its lack of addiction potential, it is widely used in nonprescription

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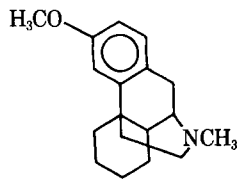

I

cough–cold preparations. The bulk drug and a syrup are described in USP XX (1). This drug is present in over 50 nonprescription antitussive preparations (2).

For USP XX, bulk I is analyzed by titration with acetous perchloric acid (1). The official method for the syrup employs extraction into hexane from a basic aqueous phase, back-extraction into dilute hydrochloric acid, and spectrophotometric quantitation. An automated system was developed which employs formation of an ion-pair with bromcresol green at pH 5.3, extraction of the ion-pair into chloroform, and measurement at 420 nm (3). This method has been used for the analysis of I in mixtures with guaifenesin and phenylpropanolamine in cough syrups. GLC after isolation by extraction into chloroform also has been employed (4). Reversed-phase high-pressure liquid chromatography (HPLC) has been used to measure I in syrups containing I and chlorpheniramine maleate (5). Ion-pair HPLC recently was employed to measure I in syrups along with pseudoephedrine hydrochloride and brompheniramine maleate (6). This report discussed a method that used a microparticulate octadecylsilane column with an acetonitrile–water mobile phase containing the sodium salt of octanesulfonic acid as the pairing ion.

A comprehensive study was made recently on the effects of the carbon chain length of the anionic ion-pairing reagents on the retention behavior of codeine, morphine, and

Table I—Comparison of Capacity Factors [a] for Several Amines Using Different Acetonitrile–Water Mixtures as the Mobile Phase

Amine	k'_A	k'_B	k'_B/k'_A
Dextromethorphan	4.60	97.9	21.3
Codeine	1.11	8.22	7.4
Methoxyphenamine	1.79	19.00	10.6
Tetracaine	3.16	45.60	14.4
Diazepam	2.02	14.22	7.0
Ephedrine	1.32	11.22	8.5
Cocaine	2.21	24.67	11.2
Naloxone	1.11	8.00	7.2
Morphine	0.89	5.56	6.2
Scopolamine	1.21	9.56	7.9
Lidocaine	1.84	18.33	10.0
Hydralazine	1.00	12.22	12.2
Meperidine	2.37	38.30	16.2
Methadone	5.63	>150	—
Methylphenidate	2.16	31.12	14.4
Quinine	4.26	>150	—

[a] k'_A is the capacity factor obtained in the 55:45 (v/v) acetonitrile–water mobile phase; k'_B is the capacity factor obtained in the 37:63 (v/v) acetonitrile–water mobile phase.

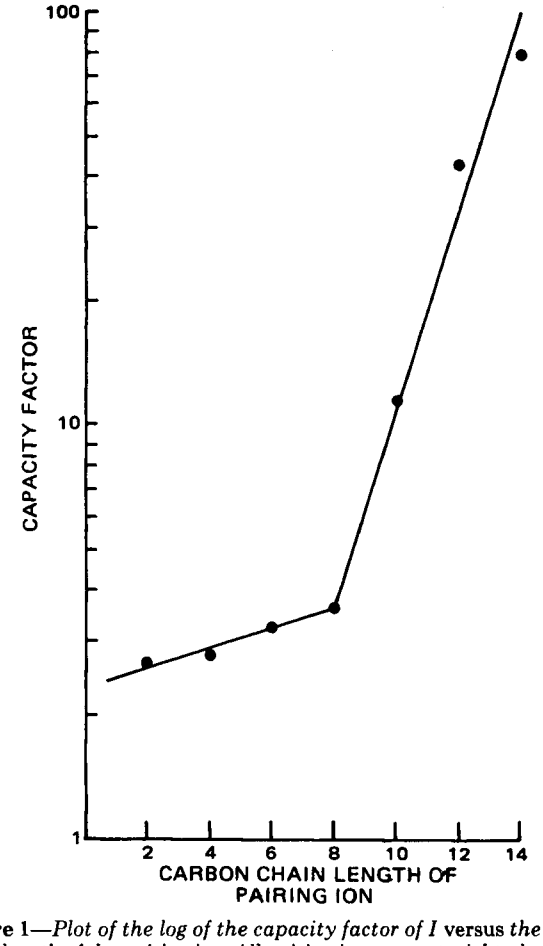

Figure 1—*Plot of the log of the capacity factor of I* versus *the carbon chain length of the pairing ion. All pairing ions were straight-chain alkyl sulfates or sulfonates. The mobile phase was the same as reported previously (7).*

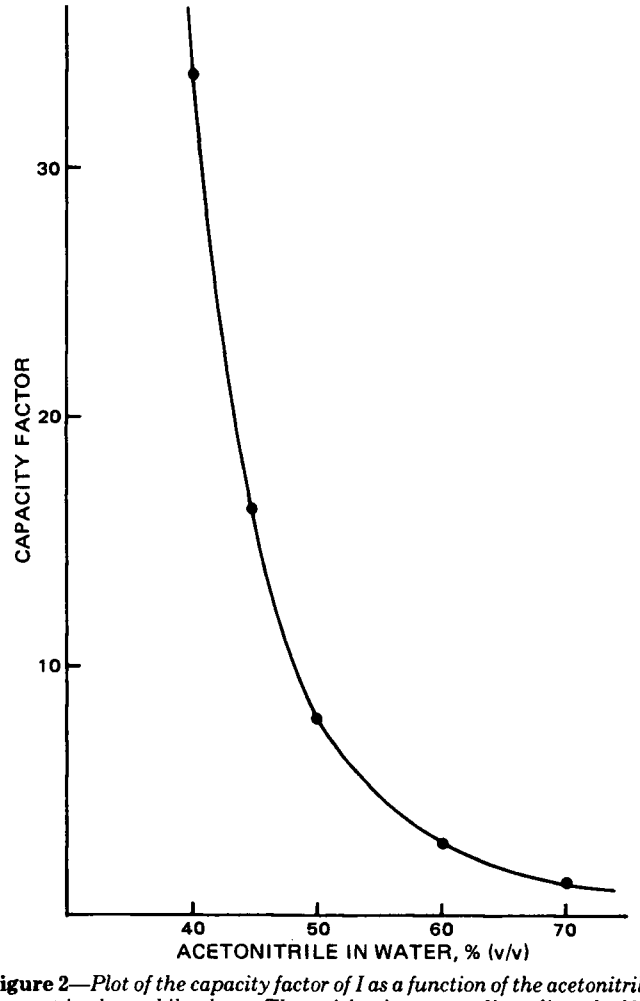

Figure 2—*Plot of the capacity factor of I as a function of the acetonitrile content in the mobile phase. The pairing ion was sodium dioctylsulfosuccinate.*

Table II—Analysis of Dextromethorphan Hydrobromide Syrup [a]

Recovery Data, mg/5 ml of placebo syrup		
Added, mg	Found, mg	Recovery, %
2.02	2.03	100.6
4.08	4.08	100.0
6.11	6.13	100.3
8.01	8.07	100.7
10.02	10.04	100.2
12.10	12.13	100.2
14.17	14.28	100.7
Average		100.4

Precision Data		
	Day 1, mg/5 ml	Day 2, mg/5 ml
	10.11	10.10
	10.14	10.06
	10.12	10.06
	10.13	10.08
	10.08	10.09
	10.10	10.14
Average	10.12	10.09
RSD, %	0.3	0.3

[a] The placebo syrup contained 100 mg of guaifenesin/5 ml and 4.75% alcohol.

ethylmorphine (7). In that study, dioctyl sodium sulfosuccinate was an effective ion-pairing reagent for the analysis of codeine in syrups. Utilizing the conditions developed for codeine as a starting point, a systematic study was undertaken to test the applicability of the method for dextromethorphan in syrups. This paper reports the results of this study and describes a reversed-phase ion-pair HPLC method for I in syrups and as the bulk drug. The chromatographic behavior of several pharmaceutically important amines also was examined using the conditions employed for the analysis of I.

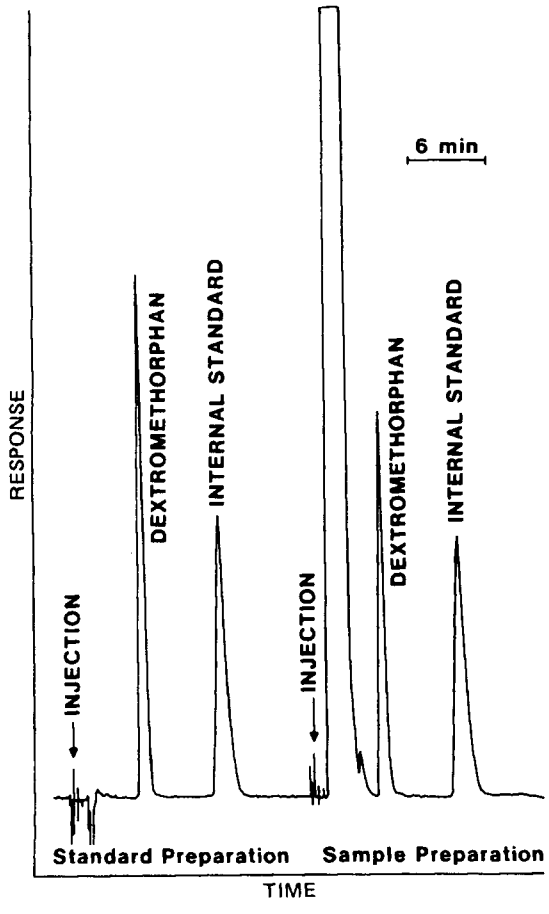

Figure 3—*Typical chromatographic tracings of a standard preparation and a sample preparation. The internal standard was testosterone propionate. The mobile phase was 55% (v/v) acetonitrile in water and contained 0.01 M ammonium nitrate and 0.005 M sodium dioctylsulfosuccinate. The pH of the mobile phase was adjusted to 3.3 with acetic acid. The flow rate was 2.0 ml/min.*

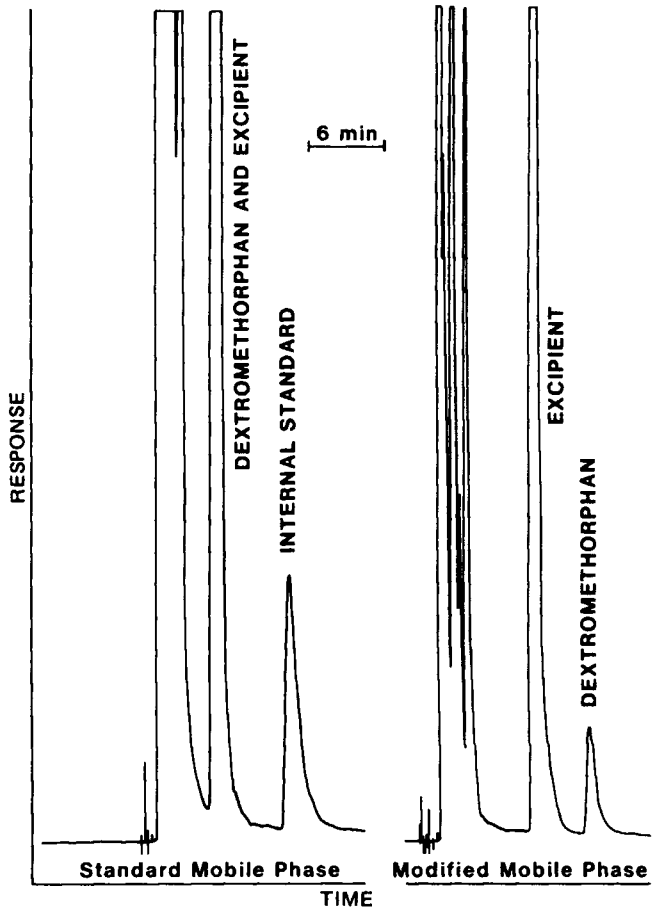

Figure 4—*Chromatographic tracings of a sample syrup preparation containing a large excipient interference. The standard mobile phase was the same as described in Fig. 3. The modified mobile phase was the same, except that the acetonitrile content was lowered from 55 to 45%. The flow rate was 2.0 ml/min.*

EXPERIMENTAL

Materials—Ethanesulfonic acid[1], sodium butanesulfonate[1], sodium hexanesulfonate[1], sodium octanesulfonate[1], sodium decylsulfate[1], sodium dodecylsulfate[1], sodium tetradecylsulfate[1], and sodium dioctylsulfosuccinate[2] were used as received. Dextromethorphan hydrobromide, codeine phosphate, methoxyphenamine hydrochloride, tetracaine hydrochloride, diazepam, ephedrine sulfate, cocaine hydrochloride, naloxone hydrochloride, morphine sulfate, scopolamine hydrobromide, lidocaine hydrochloride, hydralazine hydrochloride, meperidine hydrochloride, methadone hydrochloride, methylphenidate hydrochloride, and quinine sulfate were USP or NF quality.

The internal standard, testosterone propionate, was used as received[3]. Distilled-in-glass acetonitrile[4] and deionized water were used for all mobile phase preparations. All other chemicals were reagent grade. Samples of syrups containing dextromethorphan hydrobromide were obtained from a local wholesale drug distributor.

Apparatus—A modular high-performance liquid chromatograph

[1] Eastman Kodak Co., Rochester, NY 14650.
[2] Aldrich Chemical Co., Milwaukee, WI 53233.
[3] Steraloids, Wilton, N.H.
[4] Burdick & Jackson Laboratories, Muskegon, MI 49442.

Table III—Analyses of Dextromethorphan Hydrobromide Syrups

Syrup	Theory, mg/5 ml	Actual[a], mg/5 ml
A[b]	15.0	15.0 and 14.8
B[b]	15.0	15.0 and 14.9
C[c]	10.0	10.3 and 10.4
D[d]	15.0	15.1 and 15.4
E[e]	10.0	9.7 and 10.2
F[f]	3.5	3.3 and 3.1

[a] Two individual assays. [b] Contained 100 mg of guaifenesin/5 ml and 1.4% alcohol. [c] Contained 100 mg of guaifenesin and 12.5 mg of phenylpropanolamine hydrochloride/5 ml and 4.75% alcohol. [d] Contained 100 mg of guaifenesin/5 ml and 5% alcohol. [e] Contained 50 mg of guaifenesin and 12.5 mg of phenylpropanolamine hydrochloride/5 ml and 10% alcohol. [f] Contained 25 mg of guaifenesin/5 ml and 200 mg of sodium citrate/5 ml and 5% alcohol.

consisting of a reciprocating piston pump equipped with a pulse dampener[5], an automated loop injector[6], a UV detector[7] (254 nm), and a recorder[8] was used for all measurements. For quantitative measurements, data were collected and processed by a digital computer[9].

Mobile Phase—All mobile phases contained 0.005 *M* pairing ion and 0.01 *M* ammonium nitrate in acetonitrile–water. After mixing, the mobile phases were adjusted to pH 3.3 with acetic acid and filtered through a 5-μm filter. A mobile phase containing acetonitrile and water in a 55:45 ratio (v/v) was used for the final analysis of I.

Columns—A microparticulate octadecylsilane column[10] (10-μm particles, 30 cm × 4 mm) was obtained from commercial sources. When not in use, the column was flushed with acetonitrile–water (80:20 v/v) and stored in the same solvent. Flow rates of 2–3 ml/min gave adequate resolution with reasonable analysis times.

Quantitation of Dextromethorphan Hydrobromide in Syrup—Exactly 5.0 ml of the syrup was transferred quantitatively to a 125-ml erlenmeyer flask containing 40.0 ml of the internal standard solution (15 mg of testosterone propionate in 1 liter of the mobile phase) and mixed well. In some cases, 20.0 ml of the internal standard solution was used. When a clear solution did not form upon mixing, 5 ml of water was added to the mixture. Aliquots (25 μl) of the samples were chromatographed at a detector setting of 0.032 aufs. Concentrations were determined by comparison of peak height ratios from sample preparations to those from a standard preparation.

Bulk drug samples were prepared by adding accurately weighed samples (~10 mg) to 40.0 ml of the internal standard solution to produce the sample preparation.

RESULTS AND DISCUSSION

Dextromethorphan hydrobromide exhibited retention behavior similar to that of codeine under the conditions described previously (7). Figure 1 shows a plot of the log of the capacity factor for I as a function of the carbon chain length of the pairing ion. A dramatic increase in the capacity factor was observed with pairing ions containing more than eight carbons. When sodium dioctylsulfosuccinate (II) was used, a capacity factor of about 100 was obtained. This finding clearly represents a retention time far greater than that required for a reasonable retention time. Since I lacks the 4,5-ether linkage and the 6-hydroxyl group of codeine, the ion-pair resulting from I and II would be predicted to be considerably less polar than that resulting from codeine and II. Consequently, a reduction in the polarity of the mobile phase should reduce the capacity factor for I.

This idea was tested by measuring capacity factors of I as a function of the acetonitrile content in the mobile phase while keeping the pairing ion (II) and the pH constant. The results of this study are shown in Fig. 2. Below 60% (v/v) acetonitrile, the concentration of the organic modifier had a dramatic effect on the capacity factor. As a compromise between a short analysis time and resolution of I from syrup excipients, 55% acetonitrile was chosen for the analytical development. To test the generality of the observed relationship of the capacity factor and the acetonitrile concentration, several amines were studied using acetonitrile and water in ratios of 55:45 (v/v) and 37:63 (v/v) (II as the pairing ion). The results

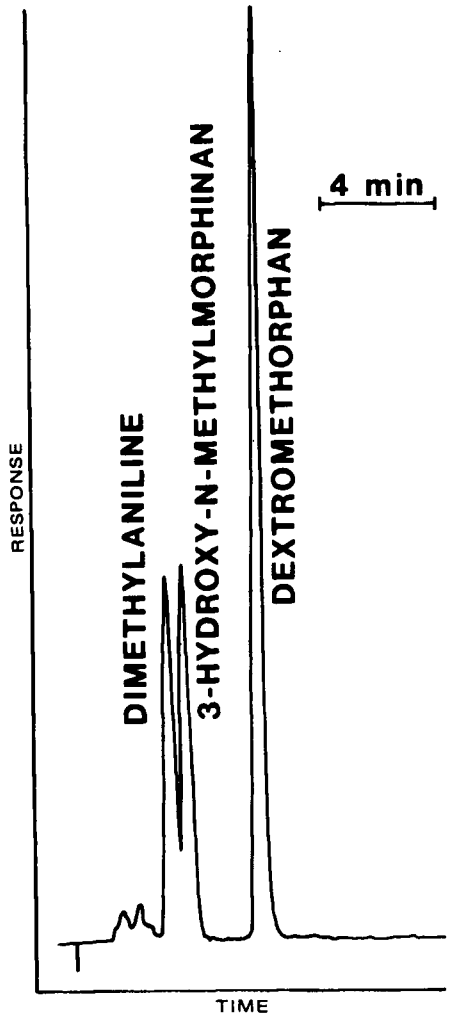

Figure 5—*Chromatographic tracing of a synthetic mixture of dimethylaniline, 3-hydroxy-N-methylmorphinan, and dextromethorphan. See Fig. 3 for the mobile phase composition and flow rate.*

are shown in Table I. With the exception of methadone and quinine, a seven- to 22-fold increase in the capacity factor was observed in going to the 37:63 acetonitrile–water mobile phase [this mobile phase has been used for the analysis of codeine (7)].

Typical chromatographic tracings for standard and sample prepara-

Table IV—Analysis of Bulk Dextromethorphan Hydrobromide

Recovery Data		
Added, mg	Found, mg	Recovery, %
8.08	8.09	100.2
9.10	9.15	100.6
9.85	9.71	98.6
10.77	10.73	99.7
11.86	11.85	99.2
Average		99.7
Precision Data		
Added, mg	Found, mg	Recovery, %
10.05	10.09	100.4
10.08	10.15	100.7
10.05	10.01	99.6
10.04	10.10	100.6
9.99	10.00	100.0
10.28	10.31	100.3
9.91	10.02	101.1
10.09	10.17	100.8
Average		100.4
RSD, %		0.5

[5] Model 110, Altex Corp., Berkeley, CA 94710.
[6] Upjohn LC Autosampler.
[7] UV III, Laboratory Data Control, Riviera Beach, Fla.
[8] Model XKR, Sargent Welch Co., Skokie, IL 60067.
[9] PDP 11, Digital Equipment Co., Maynard, MA 01754.
[10] μBondapak C_{18}, Waters Associates, Milford, MA 01757.

tions are shown in Fig. 3. Total analysis time was ~15 min. Spiked samples of a placebo syrup were analyzed by this method, giving quantitative recovery from 2.0 to 14.0 mg/5 ml (Table II). Replicate analyses of a single lot of syrup (n = 6) on 2 days gave precision (expressed as the relative standard deviation) of 0.3% (Table II).

Other commercially available syrups containing I were obtained and analyzed by this method. The results are summarized in Table III. Excellent agreement with the labeled contents was obtained. One syrup tested showed a rather large excipient peak that was not resolved from the I peak. To solve this problem, the percentage of acetonitrile in the mobile phase was reduced to 45% (v/v). Under these conditions, the excipient peak was resolved completely from the I peak (Fig. 4) but the internal standard peak did not elute in a reasonable time. Using peak height measurements, a value of 9.4 mg/5 ml (label claim was 10.0 mg/5 ml) was obtained for this syrup. This approach demonstrates that the acetonitrile content, as well as the carbon chain length of the pairing ion, can be manipulated to produce resolution of the I peak from interfering components in the mixture.

The method also was applied to the analysis of bulk drug samples. In this case, consideration must be given to the resolution of I and potential impurities arising from the synthetic process. Compound I is synthesized by the methylation of (+)-3-hydroxy-17-methyl-9α,13α,14α-morphinan (III) with phenyltrimethylammonium bromide. The by-product of the reaction is dimethylaniline. Compendial assays for I include tests for dimethylaniline and phenolic compounds (*e.g.*, III). Since dimethylaniline and III both are amines, trace amounts would be expected to be carried over in any acid–base extraction of I used for purification. A chromatographic tracing of a mixture of I, III, and dimethylaniline is shown in Fig. 5. The two potential impurities are well resolved from I. The results of the analyses of bulk I are shown in Table IV. Quantitative recovery and excellent precision are observed.

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High-Performance Liquid Chromatographic Assay for Benzocaine and *p*-Aminobenzoic Acid Including Preliminary Stability Data

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Abstract □ A high-performance liquid chromatographic assay was developed that separates and quantitates benzocaine and its primary degradation product, *p*-aminobenzoic acid. This method is rapid, sensitive, and specific. Preliminary stability data obtained with this method demonstrate its utility for this purpose.

Keyphrases □ Benzocaine—high-performance liquid chromatographic analysis with *p*-aminobenzoic acid, effects of pH, temperature, and phosphate ion on benzocaine stability □ *p*-Aminobenzoic acid—degradation product of benzocaine, high-performance liquid chromatographic analysis with benzocaine □ High-performance liquid chromatography—analysis, benzocaine and *p*-aminobenzoic acid, stability studies of benzocaine □ Degradation—benzocaine, effect of pH, temperature, and phosphate ion

In spite of the long history of benzocaine as a therapeutic agent, little information has appeared regarding its stability. It has been reported to be relatively unstable under certain conditions, and attempts have been made to decrease its degradation through the use of complexing agents (1) and surfactants (2). Benzocaine also may be unstable in the presence of pharmaceutical excipients such as citric acid, glucose, and cherry flavoring (3). This lack of information is due in part to the absence of a rapid, specific, stability-indicating assay for benzocaine in the presence of its degradation products.

Over the years, benzocaine has been assayed quantitatively by titration (4), diazotization (5), colorimetry (6), potentiometry (7), and crystal formation (8), all of which take advantage of the basic and aromatic character imparted by the aryl amino group. Some initial benzocaine stability work was undertaken by Higuchi and Lachman (1) using a partition spectroscopic method of analysis. GLC was used later for the analysis of benzocaine (9–11). However, recent data (12, 13) showed interference by *p*-hydroxybenzoates in the UV, GLC, and column chromatographic methods for benzocaine, thereby raising questions about the specificity of these commonly employed methods.

A recently developed high-performance liquid chromatographic (HPLC) assay was reported which measures intact benzocaine (14), but the report did not mention the separation or identification of the primary degradation product of benzocaine, *p*-aminobenzoic acid. This paper describes a rapid, precise, and sensitive method for the simultaneous analysis of benzocaine and *p*-aminobenzoic

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