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High-Performance Liquid Chromatographic Analysis of Codeine in Syrups Using Ion-Pair Formation

E. J. KUBIAK and J. W. MUNSON ˣ

Received July 25, 1979, from *Quality Control, The Upjohn Company, Kalamazoo, MI 49001.* Accepted for publication September 13, 1979.

Abstract □ The chromatographic behavior of morphine, codeine, and ethylmorphine was examined using reversed-phase high-performance liquid chromatography and ion-pair formation. Alkyl sulfonates and sulfates significantly increased the retention times for these compounds. The carbon chain length of the pairing ions was linearly related to the log of the capacity factors of these amine drugs. A mechanism for the increased retention based on ion-pair formation in the mobile phase is proposed. The use of dioctyl sodium sulfosuccinate as a pairing ion for codeine is described, and a method utilizing this pairing ion was developed for the quantitation of codeine in syrups. This method was applied successfully to various syrups containing codeine or codeine phosphate.

Keyphrases □ Codeine—analysis, high-performance liquid chromatography, ion-pair formation, effect of carbon chain length of pairing ions, syrups □ Ethylmorphine—analysis, high-performance liquid chromatography, ion-pair formation, effect of carbon chain length of pairing ions □ Morphine—analysis, high-performance liquid chromatography, ion-pair formation, effect of carbon chain length of pairing ions □ High-performance liquid chromatography, ion-pair formation—analysis of codeine, ethylmorphine, and morphine, effect of carbon chain length of pairing ion

The use of ion-pair formation to enhance retention time is becoming popular in reversed-phase high-performance liquid chromatography (HPLC). The counterions used to form ion-pairs generally are detergents such as alkyl sulfates and sulfonates or tetraalkylammonium salts. Anionic surfactants (sulfates and sulfonates) generally are used to form ion-pairs with cationic eluates, and cationic tetraalkylammonium salts are used with anionic eluates. By formation of the ion-pair, the apparent polarity of the eluate is decreased, leading to increased retention times in reversed-phase HPLC.

Since many drugs contain either acidic or basic functional groups, pH conditions can be chosen such that numerous drugs will form ion-pairs with these surfactants. The surfactant molecule often is referred to as the pairing ion, which gives rise to the term ion-pair HPLC. While ion-pair formation can be used in normal-phase HPLC, it is used more widely in reversed-phase HPLC. This technique also has been called soap chromatography (1, 2) because of the surfactants used.

BACKGROUND

The mechanism of retention enhancement has been attributed to two phenomena. The original concept closely follows classical ion-pair extraction techniques in which two oppositely charged molecules form an ion-pair in an aqueous solution and then partition as a complex into a nonpolar organic solvent. In the chromatographic analog of this concept, the analyte forms a complex with a pairing ion added to the mobile phase. The complex then partitions into the stationary phase (nonpolar), thus accounting for the increased analyte retention. An alternative mechanism suggests that the pairing ion first binds to the stationary phase to form an ion-exchanging surface due to the adsorbed charged pairing ions (3). Complex formation at the surface of the stationary phase thus is responsible for the increased analyte retention. Recently, application of solvophobic theory to a reversed-phase ion-pair chromatographic study led to the conclusion that the retention of the analyte involves ion-pair formation in the mobile phase and subsequent partitioning of the neutral complex to the stationary phase (4).

Codeine analysis by HPLC has been of interest. An early study (5) utilized columns of pellicular silica gel coated with polyethylene glycol 300 with an ethanol–heptane (1:10) mobile phase saturated with the stationary phase. In this system, codeine was well resolved from morphine, thebaine, and papaverine. However, quantitative codeine analysis at levels near 3 μg gave a relative error of ~9%. In a study of the liquid chromatographic behavior of alkaloids, the retention times of codeine and morphine on a microparticulate silica column were measured (6). Codeine eluted slightly before morphine with mobile phases of chloroform–methanol (three volume ratios) and ether–methanol (three volume ratios).

The quantification of morphine, codeine, and thebaine was accomplished by isocratic reversed-phase HPLC (7). With a microparticulate (10-μm) octadecylsilane column and a mobile phase of 0.1 *N* monobasic sodium phosphate in 25% acetonitrile–water, codeine in gum opium was measured with a relative standard deviation of 1.3%. Codeine also was separated from other opium alkaloids using a pellicular silica column and

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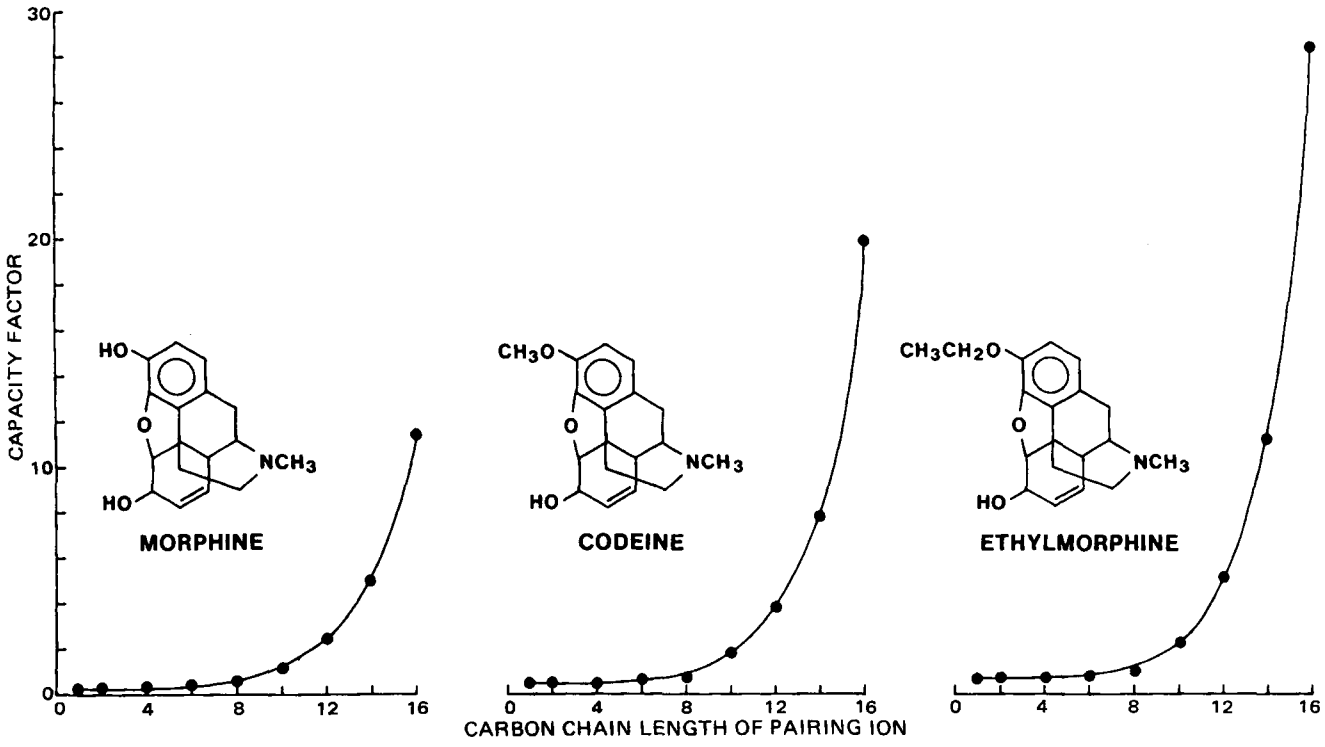


Figure 1—*Capacity factors of morphine, codeine, and ethylmorphine as a function of the carbon chain length of the alkylsulfonate and sulfate pairing ions using a microparticulate octadecylsilane column (10 μm).*

gradient elution with hexane and chloroform–methanol–diethylamine (400:300:1) (8). This methodology was applied to the analysis of morphine, cryptopine, thebaine, papaverine, codeine, and noscapine in opium (9).

The liquid chromatographic behavior of opium alkaloids using ion-pairing reagents in the mobile phase has been studied. With a microparticulate (10-μm) octadecylsilane column and a mobile phase of 0.005 *M* *n*-heptanesulfonic acid in methanol–water (40:60), several morphinan derivatives were separated (10). In this system, morphine eluted before codeine. Mixtures of morphine, codeine, thebaine, noscapine, and papaverine were separated with a similar chromatographic system (11). Codeine also was analyzed in liquid dosage forms with an octadecylsilane (10-μm) column and a water–methanol (87:13) mobile phase containing 0.05 *M* dibasic potassium phosphate (12). With this system, codeine was analyzed in the presence of guaifenesin, pheniramine maleate, phenylpropanolamine hydrochloride, and pyrilamine maleate.

This paper describes the effects of the carbon chain length of the pairing ion on the capacity factors for codeine, morphine, and ethylmorphine. Mechanisms are proposed to account for the retention of these compounds in reversed-phase ion-pair HPLC. The properties of dioctyl sodium sulfosuccinate as a pairing ion for codeine are described. An HPLC method for codeine syrups that requires minimum sample treatment is presented.

EXPERIMENTAL

Materials—Methanesulfonic acid[1], ethanesulfonic acid[1], sodium butanesulfonate[1], sodium hexanesulfonate[1], sodium hexanesulfate[1], sodium octanesulfonate[1], sodium decylsulfate[1], sodium dodecylsulfate[1], sodium tetradecylsulfonate[2], sodium tetradecylsulfate[1], sodium hexadecylsulfate[1], dioctyl sodium sulfosuccinate[3], codeine *N*-oxide[4], morphine hydrochloride[5], codeine phosphate[5], and ethylmorphine hydrochloride[5] were used as received.

Distilled-in-glass acetonitrile[6] and deionized water were used for all mobile phase preparations. The internal standard, diphenylamine, also was used as received. All other chemicals were reagent grade. Codeine syrups were obtained from a local drug wholesale distributor.

Apparatus—The modular high-performance liquid chromatograph consisted of a reciprocating piston pump equipped with a pulse dampener[7], an automated loop injector[8], a UV detector[9] (254 nm), and a recorder[10]. For quantitative measurements, data were collected and processed by a digital computer[11].

Mobile Phase—All mobile phases contained 0.005 *M* pairing ion and 0.01 *M* ammonium nitrate in acetonitrile–water (375:625). After mixing, the mobile phases were adjusted to pH 3.3 with acetic acid and filtered through a 5-μm filter. (Considerable foaming was observed with most mobile phases upon filtration.)

Columns—A microparticulate octadecylsilane column[12] (10 μm, 30 cm × 4 mm) and a microparticulate octasilane column[13] (10 μm, 25 cm × 4.6 mm) were obtained commercially. When not in use, these columns were flushed with acetonitrile–water (80:20) and stored in the same solvent. Flow rates of 2–3 ml/min gave adequate resolution with reasonable analysis time.

Quantitation of Codeine in Syrups—*Internal Standard Method*—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 (diphenylamine, 0.05 mg/ml in the mobile phase). After thorough mixing, 25-μl aliquots were chromatographed with a detector setting of 0.032 aufs. Concentrations were determined by comparison of the peak height ratios from sample preparations to those from a standard preparation (USP codeine phosphate reference standard).

External Standard Method—Exactly 5.0 ml of the syrup was added to 40.0 ml of the mobile phase and chromatographed in the same manner as the internal standard method. Concentrations were calculated by comparison of the peak heights of the codeine peaks.

RESULTS AND DISCUSSION

In preliminary studies, nitrate ions (as ammonium or sodium salts) significantly improved the peak shape and reduced tailing. This effect possibly was due to occupation of uncoated sites on the solid support

[1] Eastman Kodak Co., Rochester, NY 14650.
[2] Research Plus Laboratories, Denville, NJ 07834.
[3] Aldrich Chemical Co., Milwaukee, WI 53233.
[4] Courtesy of Dr. Arthur Jacobson, National Institute of Arthritis, Metabolism, and Digestive Disease.
[5] The Upjohn Co.
[6] Burdick & Jackson Laboratories, Muskegon, MI 49442.
[7] Model 110, Altex Corp., Berkeley, CA 94710.
[8] Upjohn LC Autosampler.
[9] UV III, Laboratory Data Control, Riviera Beach, Fla.
[10] Model XKR, Sargent Welch Co., Skokie, IL 60076.
[11] PDP 11, Digital Equipment Co., Maynard, MA 01754.
[12] μBondapak C_{18}, Waters Associates, Milford, MA 01757.
[13] RP-10A, Brownlee Laboratories, Santa Clara, CA 95050.

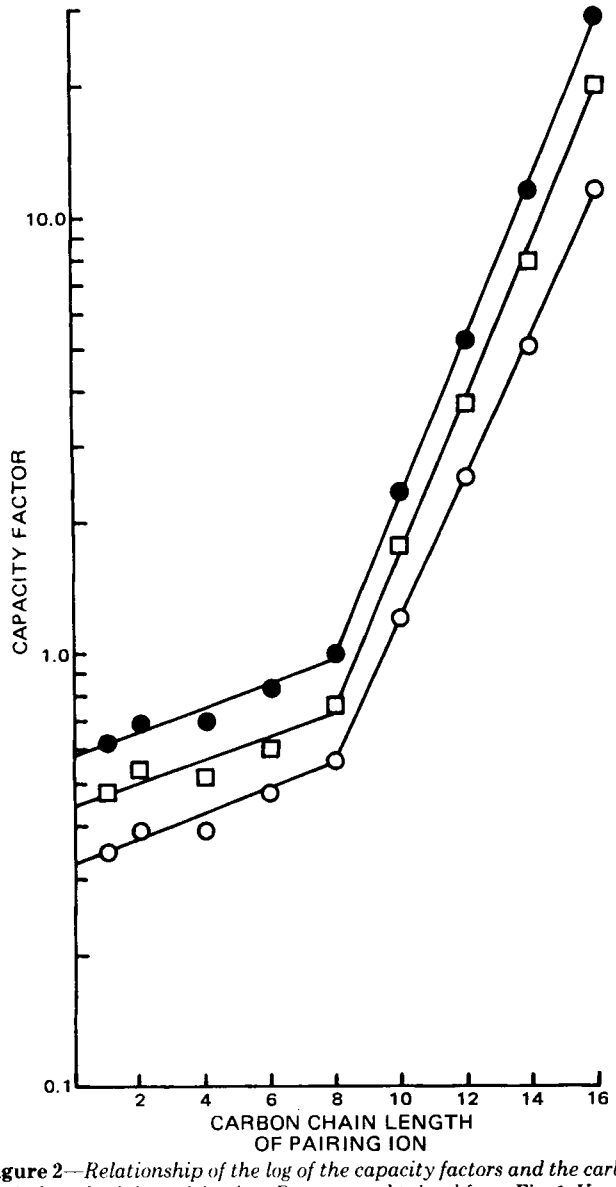


Figure 2—*Relationship of the log of the capacity factors and the carbon chain length of the pairing ion. Data were obtained from Fig. 1. Key:* ○, *morphine;* □, *codeine; and* ●, *ethylmorphine.*

material by the nitrate ions. Consequently, all mobile phase solutions contained 0.01 *M* ammonium nitrate. The mobile phase pH was adjusted to 3.3 to ensure that the amines would be protonated.

The effects of the carbon chain length of the pairing ion on the capacity factors of morphine, codeine, and ethylmorphine are shown in Fig. 1. Relatively minor effects were observed as the chain length was increased to eight carbons (octanesulfonate). Above this point, the capacity factors increased dramatically as the chain length was increased. The elution order for all systems was morphine, codeine, and ethylmorphine. This order is consistent with the polarity of the substitution on the aromatic ring (hydroxy, methoxy, and ethoxy, respectively). The shape of this curve suggests that the capacity factors are related logarithmically to the number of carbons in the pairing ions.

A plot of the log of the capacity factors of morphine, codeine, and ethylmorphine *versus* the carbon chain length of the pairing ion is shown in Fig. 2 (octadecylsilane column). A similar plot is shown in Fig. 3 for the octasilane column. The slopes of both plots are similar. The distinct break between the two linear portions of the curve suggests that two mechanisms may be operative in analyte retention.

Two distinct ion-pair mechanisms can be postulated to explain the behavior shown in Figs. 2 and 3. The first mechanism involves ion-pair formation in the mobile phase with subsequent partitioning into the stationary phase. This process is outlined in Scheme I, where D^+ is the protonated form of the amine drug, A^- is the anionic form of the pairing ion, and DA is the charge neutralized ion-pair:

$$D^+_M + A^-_M \rightleftharpoons DA_M$$

$$DA_M \rightleftharpoons DA_S$$

Scheme I

The subscripts *M* and *S* refer to the species in the mobile phase and the stationary phase, respectively.

The second mechanism, dynamic ion exchange, involves adsorption of the pairing ion onto the stationary phase surface followed by ion-pair formation at the interface (Scheme II):

$$A^-_M \rightleftharpoons A^-_S$$

$$A^-_S + D^+_M \rightleftharpoons DA_S$$

Scheme II

If the first mechanism is operative, one would expect the capacity factor to increase as the pairing-ion size increases. The increased chain length of the pairing ion should cause an increase in the hydrophobicity of the complex, thus favoring partitioning into the stationary phase. The portions of the curves in Figs. 2 and 3 that lie above a chain length of eight carbons show this behavior. This portion of the curve shows that each two additional methylene groups in the pairing ion increase the capacity by a factor of 2.2 (for the octadecylsilane column). This increase is in good agreement with the predicted value of 2.5 obtained from consideration of enhancement factors in a study of chromatographic retention of catecholamines paired with alkyl sulfates (4). In the same study, solvophobic theory was employed to deduce that ion-pair formation in the mobile phase was the primary mechanism for analyte retention.

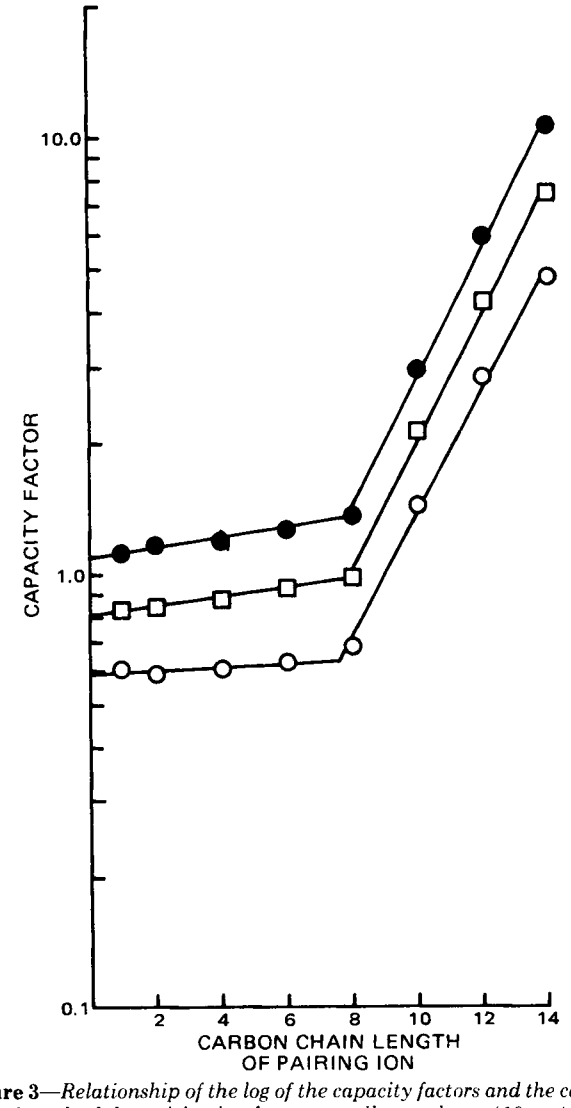


Figure 3—*Relationship of the log of the capacity factors and the carbon chain length of the pairing ion for an octasilane column (10 μm). Key:* ○, *morphine;* □, *codeine; and* ●, *ethylmorphine.*

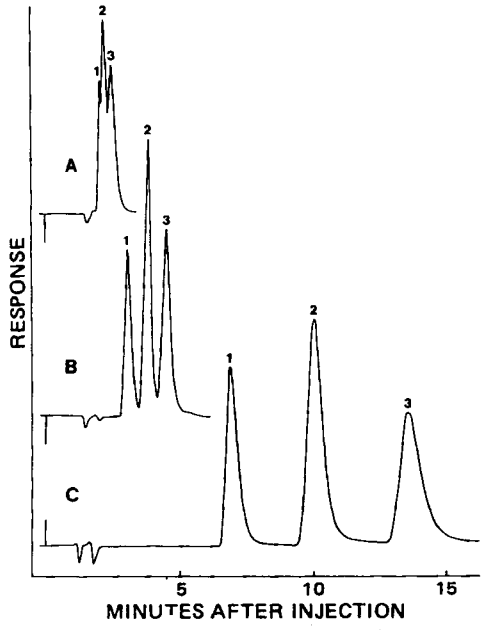


Figure 4—*Chromatograms of a mixture of morphine (1), codeine (2), and ethylmorphine (3) using various pairing ions. Key: A, sodium hexanesulfonate; B, sodium decylsulfate; and C, sodium tetradecylsulfate (octadecylsilane column).*

The retention mechanism when the ion-pairing reagent contains eight carbons or less is not straightforward. Since the shorter chain pairing ions were sulfonates and the longer chain pairing ions were sulfates, the possibility of sulfonates behaving differently from sulfates was considered. However, capacity factors measured with sodium hexanesulfonate and sodium hexanesulfate were the same (within experimental error); they also were the same for sodium tetradecylsulfonate and sodium tetradecylsulfate.

One is tempted to attribute this behavior to an ion-exchange mechanism, but the similarity of the curves in Figs. 2 and 3 weigh against it. If pairing-ion adsorption is significant, a difference in behavior when the chain length of the alkyl portion of the stationary phase is changed from eight to 18 would be expected. The plots presented in Figs. 2 and 3 are too similar to suggest that this mechanism is important. The behavior of the short chain pairing ions may be due to the ion-pair complex formed. Since there appears to be little increase in complex hydrophobicity with an increasing chain length up to eight, a mechanism is proposed to account for this behavior.

Examination of molecular models of codeine and octanesulfonate suggests that these molecules could form an ion-pair complex in which the hydrocarbon chain of the pairing ion aligns along the carbon backbone of the codeine molecule (ionic moieties of each molecule are juxtaposed), with essentially no portion of the carbon chain protruding beyond the codeine molecule. This type of complex formation would minimize the hydrophobic interactions of each molecule with the primarily aqueous solvent. For short carbon chains (eight or less), the ion-pair apparently is not appreciably less polar than the ion-pair formed from methanesulfonate and the protonated amine. In other words, the polarity is controlled primarily by the amine molecule. Consequently, little change in the capacity factor (Figs. 2 and 3) is observed with short pairing ions. On the other hand, pairing ions that contain more than eight carbon atoms would form ion-pairs in which the alkyl chain of the pairing ion would protrude beyond the codeine molecule. This protrusion results in greater complex hydrophobicity and hence greater retention. While these arguments can explain qualitatively the observed behavior, they do not rule out the possibility of dynamic ion exchange.

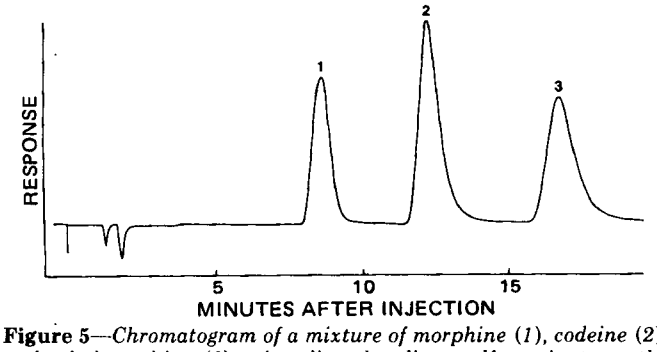


Figure 5—*Chromatogram of a mixture of morphine (1), codeine (2), and ethylmorphine (3) using dioctyl sodium sulfosuccinate as the pairing ion.*

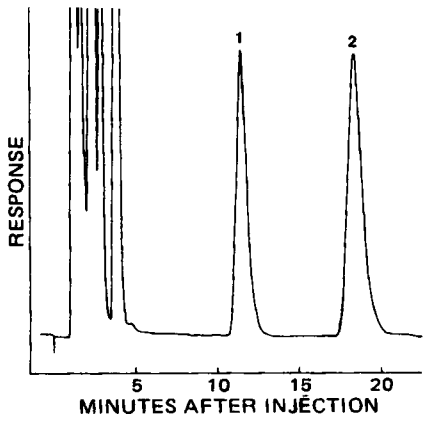
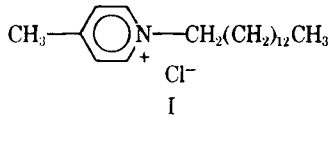


Figure 6—*Chromatogram of a codeine syrup using dioctyl sodium sulfosuccinate as the pairing ion. Peak 1 is codeine and peak 2 is diphenylamine, the internal standard (0.032 aufs).*

Data obtained in a study of the ion-pairing behavior of 4-methyl-1-tetradecylpyridinium chloride (I) lend support to the arguments presented here[14]. In this study, the capacity factor was measured as a function of the carbon chain length of the pairing ion (octadecylsilane column). Alkyl sulfates and sulfonates ranging from butanesulfonate to octadecylsulfate were used as pairing ions. No significant increase in capacity factor was observed until the alkyl chain length exceeded 12 carbons. These data are consistent with the concept of complex formation with association of the alkyl portions of both molecules of the ion-pair.

The effect of pairing ion chain length on compound resolution is shown in Fig. 4. Hexanesulfonate did not adequately resolve morphine, codeine, and ethylmorphine under these conditions, while decylsulfate and tetradecylsulfate gave resolutions adequate for peak height and peak area measurements, respectively. Furthermore, increasing the chain length increased the retention time to such an extent that nonionic species would have eluted well ahead of the amines. This latter aspect is important when direct injections of diluted syrups are contemplated.

In early studies, problems with pairing-ion solubilization were encountered with tetradecyl- and hexadecylsulfates. To circumvent this problem, dioctyl sodium sulfosuccinate (II) was investigated as a possible pairing ion for codeine. This molecule has the requisite solubility and a sufficiently long alkyl chain to produce adequate retention for codeine. Furthermore, this compound is readily available at low cost. The chromatogram for a morphine, codeine, and ethylmorphine mixture using II as the pairing ion is shown in Fig. 5. Under these conditions, II produced capacity factors similar to those produced by tetradecylsulfate. Also, II solubilization in the mobile phase was accomplished easily with stirring. Consequently, all assay development for codeine syrups was carried out using II as the pairing ion.



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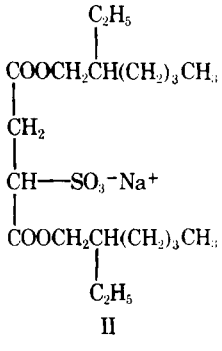

II

[14] Unpublished data.

Table I—Replicate Analyses [a] of Codeine Phosphate in a Single Syrup Lot

Day	Milligrams per 5 ml							
1	9.89,	9.96,	10.00,	10.00,	9.92,	9.97,	9.96,	9.89
2	10.12,	10.06,	10.08,	10.12,	10.07,	10.13		

[a] Internal standard method.

Table II—Analyses of Codeine Syrups (Milligrams per 5 ml)

Sample	Trial 1	Trial 2
A[a]	9.69[b]	9.66
B[c]	9.96[d]	9.99
C[e]	10.02	9.92
D[f]	10.21	10.05
E[g]	10.10	9.97

[a] Syrup contained 85 mg of terpin hydrate/5 ml and 10 mg of codeine/5 ml. [b] Expressed as free base. [c] Syrup contained (in 5 ml) 12.5 mg of phenylpropanolamine hydrochloride, 6.25 mg of pheniramine maleate, 6.25 mg of pyrilamine maleate, 100 mg of guaifenesin, and 10 mg of codeine phosphate. [d] Expressed as phosphate salt. [e] Syrup contained 100 mg of guaifenesin/5 ml and 10 mg of codeine phosphate/5 ml. [f] Syrup contained (in 5 ml) 5 mg of promethazine, 44 mg of potassium guaiacolsulfonate, and 10 mg of codeine phosphate. [g] Syrup contained (in 5 ml) 2 mg of brompheniramine maleate, 5 mg of phenylephrine hydrochloride, 5 mg of phenylpropanolamine hydrochloride, 100 mg of guaifenesin, and 10 mg of codeine phosphate.

Figure 6 shows a typical chromatogram of a 5.0-ml syrup sample (10.0 mg of codeine phosphate and 100 mg of guaifenesin/5 ml) diluted with 40.0 ml of the internal standard solution (diphenylamine, 0.05 mg/ml in the mobile phase). Excellent separation was obtained, with all syrup excipients (and guaifenesin) eluting at or near the solvent front. Codeine phosphate recovery from spiked placebo syrups averaged 100.5% with a relative standard deviation of 0.5% (n = 13) over 2.2–16.4 mg/5 ml (internal standard method). Replicate analyses of one lot of codeine syrup gave an average value of 9.95 mg/5 ml (theory is 10.0 mg/5 ml) with a relative standard deviation of 0.4% (n = 8). Analysis of the same lot on a different day gave an average of 10.10 mg/5 ml with a relative standard deviation of 0.3% (n = 6). The overall average of these data is 10.0 mg/5 ml with a relative standard deviation of 0.8% (n = 14) (Table I).

Five samples of syrups containing codeine or codeine phosphate were obtained through commercial sources and analyzed by the external standard method (Table II). Excellent agreement with the labeled codeine content was obtained. Even though some products contained other

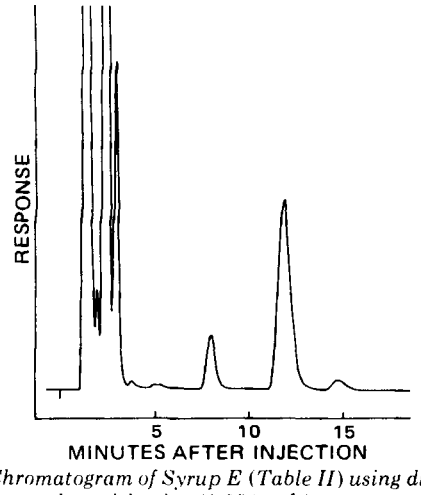

Figure 7—*Chromatogram of Syrup E (Table II) using dioctyl sodium sulfosuccinate as the pairing ion (0.064 aufs).*

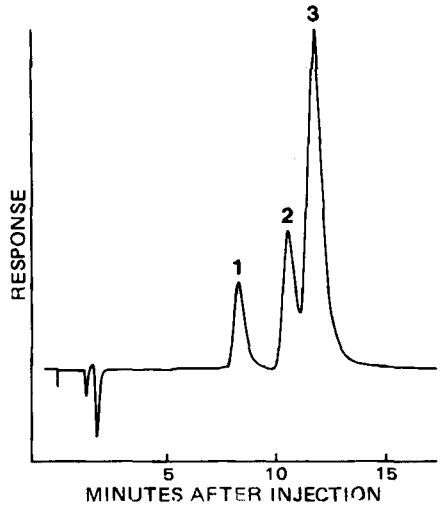

Figure 8—*Chromatogram of a mixture of morphine (1), codeine N-oxide (2), and codeine (3) using conditions used for the codeine assay.*

amine drugs, adequate resolution was obtained in all cases without modification of the mobile phase. The chromatographic tracing of the most complex mixture is shown in Fig. 7 (Product E).

The separation of codeine from its synthetic precursor, morphine, and a possible oxidation product, codeine N-oxide, is shown in Fig. 8. Even though the N-oxide was not completely resolved from codeine, it did not interfere when peak height measurements were used. No extraneous peaks were observed in any samples analyzed by this method, even though some samples were >1 year old.

The method described here is precise and accurate. Since the codeine elutes well after the solvent front, simple dilution of the syrups was the only sample preparation necessary. Total sample preparation and chromatographic analysis time is <20 min/sample. Dioctyl sodium sulfosuccinate has been shown to be a useful, inexpensive pairing ion for codeine analysis.

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ACKNOWLEDGMENTS

The authors thank Steve Mecker for assistance in obtaining morphine and ethylmorphine samples and Mark Fitch for preparation of placebo syrup formulations.