bioavailability determined from urine data may give a more accurate reflection of the true bioavailability. Thus, the feasibility of using urinary excretion data alone to determine bioavailability of furosemide is good, given the statistical agreement of absolute bioavailability determined by plasma and urine data. Also, the possibility of overestimation of bioavailability by plasma data because of enterohepatic recycling makes bioavailability determined from urine data appear more reliable.

The t_{max} and Cp_{max} determinations were not statistically significantly different for the tablet and solution. There was a trend toward later peak plasma concentrations following tablet administration, probably due to time required for tablet disintegration and dissolution. The seeming disagreement of Cp_{max} determinations presented in Table II and Fig. 1 is a function of mean data being graphically presented, while the means of individual subjects are presented in the table.

The bioavailabilities of the tablet and solution are essentially the same, though <70% of the dose was absorbed. This suggests the absorption may not be solely dependent on solubility, but may also be limited by absorption occurring only from a specific site in the GI tract. Site-limited absorption may explain intrasubject variability in absolute bioavailability.

In summary, the disposition of intravenous furosemide as determined by this study is in agreement with previous reports. The mean absolute bioavailability determined from cumulative urinary excretion data was 61 and 66% for the solution and tablet, respectively. The bioavailability determined with urine data may be more reliable than bioavailability determined with plasma data because of a possible enterohepatic recycling process. Site-limited absorption of furosemide is suggested.

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Determination of Amine Ingredients in Cough–Cold Liquids by Reversed-Phase Ion-Pair High-Performance Liquid Chromatography

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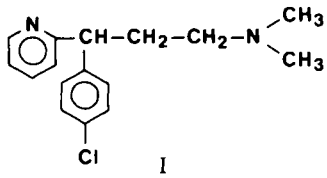
Abstract □ The chromatographic behavior of phenylephrine, codeine, pseudoephedrine, phenylpropanolamine, methoxyphenamine, pheniramine, pyrilamine, dextromethorphan, and chlorpheniramine was examined by reversed-phase, ion-pair high-performance liquid chromatography. An isocratic chromatographic system was devised for the analysis of cough–cold liquids containing these amine drugs by optimization of the mobile phase ionic strength, buffer pH, pairing ion concentration, and secondary ion concentration. Quantitative recovery and excellent precision were demonstrated for the simultaneous determination of phenylpropanolamine, dextromethorphan, and chlorpheniramine in a typical formulation. The method was successfully applied to various commercial cough–cold liquids for the analysis of a wide range of amine drugs.

Keyphrases □ Cough–cold liquids—determination of amine ingredients by reversed-phase, ion-pair high-performance liquid chromatography □ High-performance liquid chromatography—determination of amine ingredients in cough–cold liquids □ Amine drugs—determination in cough–cold liquids by reversed-phase ion-pair high-performance liquid chromatography

Cough–cold liquids are usually complex formulations containing several active ingredients and a broad spectrum of excipients such as dyes, flavors, sweeteners, and preservatives. Many of these products are designed to be multisymptom preparations typically containing a variety of basic amino compounds acting as antihistamines, decongestants, or cough suppressants. Some of the common amino agents utilized include phenylephrine, phenylpropanolamine, pseudoephedrine, pyrilamine, pheniramine, chlorpheniramine, codeine, and dextromethorphan. The analgesics, phenacetin and acetaminophen, are also commonly found in cough–cold liquids adding further complexity to the list of possible ingredients. Preservatives such as methyl- and propylparaben or sodium benzoate are normally present in a formulation. It was the purpose of this study to develop a simple high-performance liquid



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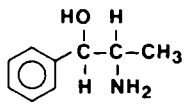

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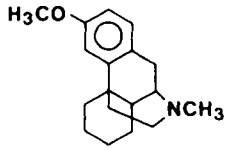

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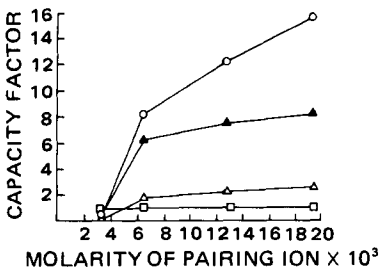

Figure 1—*Relationship between retention and pairing ion concentration of the mobile phase. Key: (△) phenylpropanolamine; (▲) dextromethorphan; (○) chlorpheniramine; (□) propylparaben. Mobile phase: 0.02* M *ammonium nitrate, 70% methanol, 1.5% glacial acetic acid. The pairing ion is sodium dioctyl sulfosuccinate.*

chromatographic (HPLC) procedure capable of determining the full range of amines commonly found in cough–cold liquids. Efforts were concentrated on the simultaneous determination of the antihistamine, chlorpheniramine (I); the decongestant, phenylpropanolamine (II); and the cough suppressant, dextromethorphan (III) in a typical cough–cold liquid. Applicability of the chromatographic separation to these and other amine drugs in a variety of different formulations was demonstrated by the analysis of cough–cold liquids from a variety of different sources.

BACKGROUND

Chromatographic determination of cough–cold amines has previously been performed using GLC (1–3) and ion-pair, reversed-phase HPLC (4–10). The GLC methods are generally less efficient due to the necessity of derivatization of some or all of the amines, and because the sample preparation usually involves an extraction step or evaporation of the sample and redissolution in a suitable solvent. Several of the available HPLC procedures were designed for the determination of one component and are not able to handle all of the amine ingredients found in combination products (4–6, 8). Another chromatographic system for the determination of pseudoephedrine, brompheniramine, and dextromethorphan elutes pseudoephedrine near the solvent front before major excipient peaks (7). This situation is undesirable, since excipient interferences are likely to arise when different formulations from a variety of sources are analyzed. The determination of codeine, phenylpropanolamine, pheniramine, and pyrilamine has also been described (10). This procedure fails to separate the two antihistamines, pheniramine and pyrilamine, and they must be quantitated separately by a spectrophotometric method.

Reversed-phase, ion-paired HPLC was chosen as the most suitable chromatographic system due to the desire for a sample preparation that would require only dilution of the cough–cold liquid prior to injection onto the column. The ideal chromatographic system would use a pairing ion that would not cause precipitation in the samples and yet provide enough retention to resolve the drugs of interest from the formulation excipients. All of the drugs would be adequately resolved and the analysis time should be sufficiently short (*i.e.*, ≤20 min). These goals were accomplished by the manipulation of the mobile phase ionic strength, mobile phase solvent strength, buffer pH, pairing ion concentration, and secondary ion concentration. The results of these studies and an HPLC method for a variety of cough–cold amines that requires a minimum of sample treatment are presented.

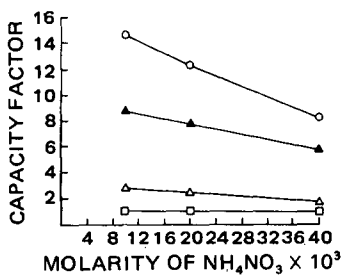

Figure 2—*Relationship between the capacity factor and the concentration of the secondary ion, ammonium nitrate. Key: (△) phenylpropanolamine; (▲) dextromethorphan; (○) chlorpheniramine; (□) propylparaben. Mobile phase: 70% methanol, 0.013* M *sodium dioctylsulfosuccinate, 1.5% glacial acetic acid.*

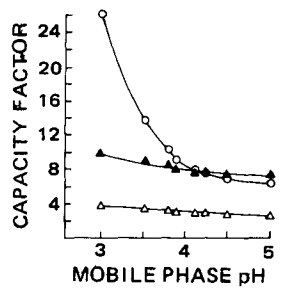

Figure 3—*Relationship between retention and pH of the mobile phase. Key: (△) phenylpropanolamine; (▲) dextromethorphan; (○) chlorpheniramine. Mobile phase: methanol–water–tetrahydrofuran–85% phosphoric acid (68:29:4:1); the sodium dioctyl sulfosuccinate concentration was 0.013* M. *Ammonium hydroxide was used for pH adjustment.*

EXPERIMENTAL

Materials—The various cough–cold ingredients, acetaminophen, guaifenesin, phenacetin, phenylephrine hydrochloride, codeine sulfate, ephedrine sulfate, phenylpropanolamine hydrochloride, methoxyphenamine hydrochloride, pheniramine maleate, benzphetamine hydrochloride, pyrilamine maleate, dextromethorphan hydrobromide, chlorpheniramine maleate, and α-aminopropiophenone were USP-NF quality. Sodium dioctyl sulfosuccinate[1], 3-hydroxy-*N*-methylmorphinan[2], *N*,*N*-dimethylaniline[1], acetophenone[1], methylparaben[2], and propylparaben[2] were used as received. Methanol and tetrahydrofuran were distilled in glass[3], and distilled water was used in all mobile phases. All other chemicals were reagent grade. The cough–cold liquids from various manufacturers were nonprescription products purchased at local drugstores.

Apparatus—A modular high-performance liquid chromatograph, consisting of a reciprocating piston pump equipped with a pulse dampener[4], an automated autosampler[5], a UV detector[6] (254 nm), and a recorder[7], was used for all measurements. For quantitative measurements, data were collected and processed by a digital computer[8].

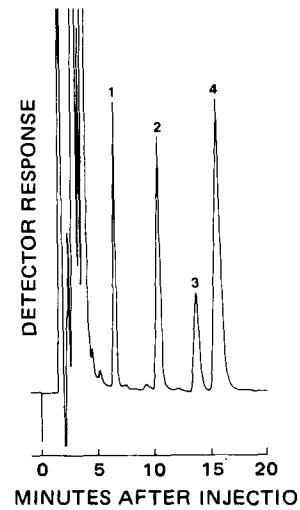

Figure 4—*Chromatogram of a typical cough–cold syrup containing phenylpropanolamine (Peak 1), benzphetamine (Peak 2, the internal standard), dextromethorphan (Peak 3), and chlorpheniramine (Peak 4) at 0.03 aufs.*

[1] Aldrich Chemical Co., Milwaukee, WI 53233.
[2] The Upjohn Co.
[3] Burdick & Jackson Laboratories, Muskegon, MI 49442.
[4] Model 110, Altex Corp., Berkeley, CA 94710.
[5] Model 8050, Varian Associates, Palo Alto, CA 94303.
[6] UVIII, Laboratory Data Control, Riviera Beach, Fla.
[7] Model XKR, Sargent Welch Co., Skokie, IL 60067.
[8] PDP 11, Digital Equipment Co., Maynard, MA 01754.

Table I—Replicate Analyses of Chlorpheniramine (I), Phenylpropanolamine (II), and Dextromethorphan (III) in a Single Cough–Cold Syrup Lot [a]

	I	II	III
Day 1	3.98	24.6	19.9
	4.00	25.1	20.1
	3.97	24.8	19.7
	3.90	24.9	19.6
	4.05	25.4	20.2
	3.96	24.6	19.7
	3.93	24.8	19.6
	3.97 ± 0.5	24.9 ± 0.2	19.8 ± 0.2
RSD	1.2%	1.1%	1.2%
Label	99.2	99.6	99.1
Day 2	3.98	25.0	19.9
	4.01	24.9	19.9
	4.03	25.1	20.1
	4.00	25.0	19.8
	3.96	25.1	19.9
	4.00	25.2	19.9
	4.01	25.1	20.0
	4.00 ± .02	25.1 ± 0.1	19.9 ± 0.1
RSD	0.5%	0.4%	0.4%
Label	100.0	100.2	99.6

[a] Internal standard method.

Mobile Phase—The mobile phase was prepared as follows. Methanol (680 ml) was added to 5.8 g of sodium dioctyl sulfosuccinate. Stirring was continued and 290 ml of distilled water, 40 ml of tetrahydrofuran, and 1 ml of 85% phosphoric acid were added. The pH was adjusted to 3.80 ± 0.05 with concentrated ammonium hydroxide solution. The solution was filtered through a 5-μm filter before use.

Separation between dextromethorphan and chlorpheniramine could be increased without affecting the other components by decreasing the pH of the mobile phase. If greater separation was necessary between phenylpropanolamine and the excipients, the methanol content of the mobile phase was decreased.

Columns—A microparticulate octadecylsilane column[9] (10-μm particles, 30 cm × 4 mm) was used throughout this study. The column was washed with a mobile phase consisting of methanol–water–tetrahydrofuran (68:29:4) when not in use to ensure long column life. The column temperature was ambient, column pressure ~2000 psi, injection volume ~50 μl, and the flow rate was 1.3 ml/min. These conditions gave satisfactory chromatography.

Internal Standard Solution—A solution with a concentration of ~7.0 mg/ml of benzphetamine hydrochloride was prepared.

Standard Preparation—About 8.3 mg of phenylpropanolamine hydrochloride, 6.7 mg of dextromethorphan hydrobromide, and 1.3 mg of chlorpheniramine maleate were accurately weighed, and 1.0 ml of the internal standard solution and ~35 ml of the mobile phase were added.

Sample Preparation—An appropriate amount of sample (accurately measured) was diluted with 1.0 ml of internal standard solution and ~30 ml of mobile phase. The resulting solution was mixed for 5 min. The amounts of commercial cough–cold liquid and mobile phase used were varied depending on the drug concentrations in the various products.

Procedure—The sample or standard (50 μl) was chromatographed using the conditions described. The detector setting was ~0.03 aufs. Concentrations were determined by comparison of peak height ratios from sample preparations to those from the standard preparation (USP reference standards were used).

RESULTS AND DISCUSSION

The primary requirements of the chromatographic system would be its ability to separate the amine drugs from the latest eluting excipient peak and yet maintain a chromatographic run time of <20 min. If adequate separation could be obtained, then sample preparation for the cough–cold liquids would merely require dilution of the sample with the mobile phase. Preliminary experiments indicated that the preservative, propylparaben, was the latest eluting excipient in the formulation chosen for initial development. Its retention behavior was compared with those of phenylpropanolamine, dextromethorphan, and chlorpheniramine in later studies to optimize the mobile phase.

The effects of the carbon chain length of the pairing ion on the capacity factors of phenylpropanolamine, dextromethorphan, and chlorpheniramine were initially investigated. The capacity factor of propylparaben was observed to remain nearly constant as the pairing ion was varied due to its presence in the unionized state at the pH of the initial mobile phase (pH 3.3). The chain length of alkylsulfonate or alkylsulfate pairing ions was varied from 6 to 20. It was observed that a pairing ion with a chain length of >12 carbons would be necessary to ensure adequate resolution of phenylpropanolamine from propylparaben. An upper limit on the size of the pairing ion was established by the fact that the C_{20} (eicosyl) pairing ion caused precipitates to form in the diluted syrup samples. Sodium dioctyl sulfosuccinate was ultimately chosen as the pairing ion based on its ability to produce adequate retention of phenylpropanolamine, dextromethorphan, and chlorpheniramine and its solubility, availability, and low cost.

By lowering the methanol content of the mobile phase, the resolution between propylparaben and phenylpropanolamine can be increased to obtain the desired degree of separation but only at the expense of extremely long retention times for chlorpheniramine. At this point it was clear that what was needed was greater selectivity between the amine drugs and propylparaben. Increasing the size of the pairing ion would increase the resolution between phenylpropanolamine and propylparaben, but this approach was unsuccessful due to the formation of precipitates when the samples were diluted with the mobile phase containing these larger pairing ions. Examination of a possible mechanism for retention in ion-paired, reversed-phase chromatography (Eqs. 1 and 2) suggested that a smaller, more soluble pairing ion could be used by increasing its concentration in the mobile phase to increase the k' of the amines while leaving the k' of propylparaben unaffected:

$$k' = \frac{V_s}{V_m}\frac{[R^+ I^-]_{org}}{[R^+]_{aq}} \quad \text{(Eq. 1)}$$

$$k' = \frac{V_s}{V_m} E\,[I^-]_{aq} \quad \text{(Eq. 2)}$$

where

$$E = \frac{[R^+ I^-]_{org}}{[R^+]_{aq}[I^-]_{aq}}$$

k' is the capacity factor, V_s is the volume of the stationary phase, V_m is

Table II—Analyses of Chlorpheniramine (I), Phenylpropanolamine (II), and Dextromethorphan (III) in Nonprescription Cough–Cold Liquids

Sample	Content, mg/ml I	II	III
A[a]	NP[b]	2.58 (103)[c]	C[d]
B	0.404 (101)	2.53 (101)	NP
C	0.681 (102)	C	0.676 (101)
D	0.133 (100)	NP	0.667 (100)
E	NP	1.22 (98)	0.998 (100)
F	0.396 (99)	NP	NP
G	0.401 (100)	NP	2.03 (102)
H	0.265 (99)	1.66 (100)	1.32 (99)

[a] Sample A was done by an external standard method. [b] NP refers to an amine not present in the formulation. [c] The numbers in parentheses refer to percent of label. [d] C refers to a chromatographic interference that prevented quantitation.

Table III—Approximate Retention of Various Cough–Cold Component Compounds [a]

Compound	Retention Time, min	k'
Acetaminophen	2.4	0.5
Guaifenesin	2.6	0.6
Phenacetin	2.9	0.7
Phenylephrine	5.1	2.1
Codeine	5.6	2.5
Ephedrine	7.0	3.2
Phenylpropanolamine	7.1	3.3
Methoxyphenamine	8.3	4.1
Pheniramine	11.0	5.9
Benzphetamine	11.2	6.0
Pyrilamine	14.4	8.0
Dextromethorphan	14.9	8.2
Chlorpheniramine	17.5	9.6

[a] Mobile phase: methanol–water–tetrahydrofuran–80% phosphoric acid (67:29:4:0.1) with 5.8 g of dioctyl sulfosuccinate adjusted to pH 3.8.

[9] μBondapak C_{18}, Waters Associates, Milford, MA 01757.

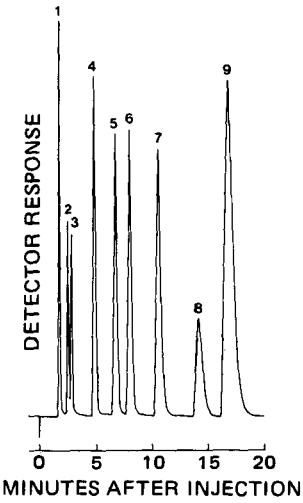

Figure 5—*Chromatogram demonstrating the retention behavior of a variety of cough–cold ingredients: maleic acid (1), guaifenesin (2), phenacetin (3), phenylephrine (4), phenylpropanolamine (5), methoxyphenamine (6), benzphetamine (7, internal standard), dextromethorphan (8), and chlorpheniramine (9). See Table III for mobile phase composition.*

the volume of the mobile phase, $[I^-]$ is the concentration of the pairing ion, $[R^+]$ is the concentration of the protonated amine, $[R^+ I^-]$ is the concentration of the ion pair, and E is the extraction constant (11).

The relationship between the capacity factors (k') and the pairing ion concentration for phenylpropanolamine, dextromethorphan, chlorpheniramine, and propylparaben is shown in Fig. 1. Equation 2 predicts k' to be proportional to the pairing ion concentration, and this type of behavior is observed in the early portions of the curves. The optimum pairing ion concentration was chosen as 0.013 M. As expected, the protonated form of propylparaben exhibited no change in capacity factor with increasing pairing ion concentration in the acetate buffer.

In the past, ammonium nitrate had been used in the mobile phase as a secondary ion to improve peak shape and reduce tailing (4, 5). It was initially included here based mainly on past history. Secondary ions compete with the pairing ion to form ion pairs and would be expected to decrease the retention of the amines by effectively decreasing the concentration of dioctyl sulfosuccinate ion pairs in the stationary phase. The effect of the nitrate ion concentration on the retention of phenylpropanolamine, dextromethorphan, chlorpheniramine, and propylparaben is shown in Fig. 2. The retention of the amines is observed to decrease with increasing nitrate ion concentration. As expected, the retention of propylparaben is unaffected by the concentration of the secondary ion. When ammonium nitrate was completely removed from the mobile phase, retention increased while tailing was changed very little. Tetrahydrofuran was added to the mobile phase and was found to be more effective in reducing tailing than nitrate ion.

Adequate resolution between propylparaben and phenylpropanolamine was obtained by increasing the retention with increased pairing ion concentration and removal of the secondary ion from the mobile phase, but the retention time of chlorpheniramine became excessive. The retention of chlorpheniramine was reduced relative to dextromethorphan, phenylpropanolamine, and propylparaben by changing from an acetate buffer to a carefully controlled phosphate buffer system of lower ionic strength. Figure 3 demonstrates the relationship between retention of the amines and mobile phase pH. In the pH region of 3–5, the capacity factors of phenylpropanolamine and dextromethorphan remain relatively constant, while chlorpheniramine's retention changes dramatically. The amine groups of phenylpropanolamine and dextromethorphan (pKa ~10) are fully protonated in this pH range and are behaving as monocations with respect to the ion-pairing system. Chlorpheniramine is capable of being protonated twice with reported pKa values of 4.0 (pyridinium group) and 9.2 (tertiary amine group) (12). In the pH 5.0 region, chlorpheniramine is exhibiting retention behavior more compatible with that of a monocation, while at pH 3.0 the increased retention is due to large concentrations of the dication form. For bivalent sample ions, the capacity factor is predicted to be proportional to the square of the pairing ion concentration (11), and the rapid increase in retention of chlorpheniramine at lower pH is consistent with this proposition. A mobile phase pH of 3.8 was chosen to shorten the chromatographic run time and still retain

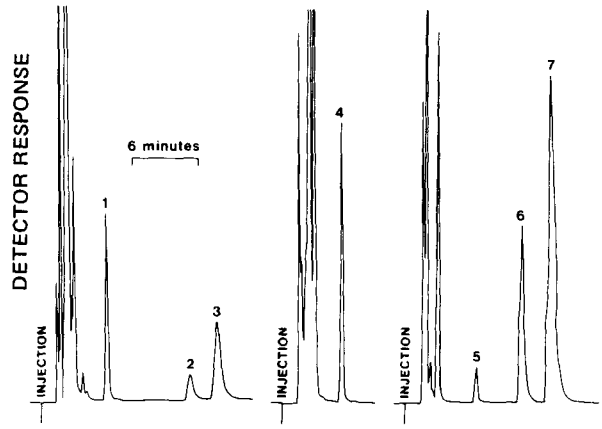

Figure 6—*Chromatograms of three different commercial cough–cold syrups demonstrating the separation of pseudoephedrine (1), dextromethorphan (2), chlorpheniramine (3), codeine (4), phenylpropanolamine (5), pheniramine (6), and pyrilamine (7) from the excipients found in these syrups.*

baseline separation of chlorpheniramine and dextromethorphan. Selectivity between dextromethorphan and chlorpheniramine is affected to such a great extent that the order of elution can be reversed by adjusting the pH of the mobile phase (Fig. 3).

Figure 4 shows a typical chromatogram of a 5.0-ml syrup sample (8.3 mg of phenylpropanolamine hydrochloride, 6.7 mg of dextromethorphan hydrobromide, and 1.3 mg of chlorpheniramine maleate/5 ml) spiked with 1.0 ml of internal standard solution and diluted to ~35 ml with mobile phase. Excellent separation of the three drugs and the internal standard were obtained with all syrup excipients eluting in the early portions of the chromatogram. The percent recovery of phenylpropanolamine, dextromethorphan, and chlorpheniramine from spiked syrup placebo averaged 100.0 ± 0.7 (n = 9), 99.9 ± 1.4 (n = 8), and 99.9 ± 1.1 (n = 8), respectively, over the ranges of 4.1–12.5, 3.2–9.9, and 0.8–1.9 mg/5 ml. Replicate analyses of one lot of cough–cold syrup gave average values of 24.9 ± 0.2 (phenylpropanolamine, theoretical: 25 mg/15 ml), 19.8 ± 0.2 (dextromethorphan, theoretical: 20 mg/15 ml), and 3.97 ± 0.05 (chlorpheniramine, theoretical: 4 mg/15 ml). Data for the first and second day analyses of this lot are given in Table I.

Eight samples of commercial cough–cold liquids containing phenylpropanolamine, dextromethorphan, or chloropheniramine, alone or in combination, were analyzed with no changes in the mobile phase or chromatographic parameters. Good agreement with the labeled content for phenylpropanolamine, dextromethorphan, and chlorpheniramine was obtained (Table II). In each case simple dilution of the sample with mobile phase was used, and no excipient inteference problems were noted with the exception of Sample C where an unknown excipient peak obscured phenylpropanolamine. However, dextromethorphan and chlorpheniramine were successfully quantitated in Sample C. In Sample A dextromethorphan was not adequately resolved from pyrilamine (also present in Sample A) to allow quantitation. Adjustment of the mobile phase pH would undoubtedly increase the separation of dextromethorphan and pyrilamine, since analogous behavior to that observed for chlorpheniramine and dextromethorphan in Fig. 3 would be expected in this case.

The retention behavior of other cough–cold ingredients was also investigated using this assay methodology to test the applicability of the chromatographic system to a wider range of product formulations. Table III lists the capacity factors (k') found for some common cough–cold drugs. Figure 5 contains a chromatogram of the separation of a synthetic mixture of many of these compounds. Commercial cough–cold liquids containing amine drugs other than or in addition to phenylpropanolamine, dextromethorphan, and chlorpheniramine were also investigated (Fig. 6). No excipient interferences were noted in commercial samples containing codeine, pseudoephedrine, methoxyphenamine, pheniramine, and pyrilamine. Analgesics such as acetaminophen eluted near the solvent front and were easily separated from the amine drugs of interest. While quantitation of these amine drugs was not attempted, the applicability of the present methodology to a wide range of amine drugs was confirmed.

The reversed-phase, ion-pairing HPLC procedure described is shown to be precise and accurate for the simultaneous determination of phenylpropanolamine, dextromethorphan, and chlorpheniramine in

cough–cold liquids. In addition, the results indicate that the methodology is applicable to a wide range of amine drugs commonly found in cough–cold liquids. The chromatographic system is capable of separating the amines of interest from the dyes, preservatives, and flavorings normally associated with a liquid cough–cold formulation. In all cases the sample preparation consisted of dissolution of the sample in the mobile phase and the total chromatographic run time was <20 min.

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Precise High-Performance Liquid Chromatographic Procedure for the Determination of Cefsulodin, a New Antipseudomonal Cephalosporin Antibiotic, in Plasma

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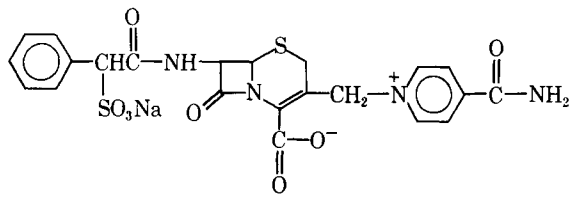
Abstract □ A simple and precise high-performance liquid chromatographic (HPLC) procedure was developed for the determination of cefsulodin, a new antipseudomonal cephalosporin antibiotic, in plasma. The analytical procedure involved ultrafiltration of samples that were buffered to prevent cefsulodin degradation, followed by injection into an HPLC system, utilizing a C_{18} reversed-phase analytical column, a mobile phase of acetonitrile–modified aqueous acetate buffer, and a UV spectrophotometric detector. Because of the simplicity of the procedure, the intraassay (~2%) and interassay (~3–4%) coefficients of variation were extremely low. Recoveries of drug were essentially quantitative in freshly buffered specimens and in those stored buffered and frozen for nearly 3 months. Calibration curves were rectilinear from the limit of quantification (~0.2 μg/ml) to 200 μg/ml, as demonstrated by regression correlation coefficients averaging >0.999 during routine analyses.

Keyphrases □ High-performance liquid chromatography—procedure for the determination of cefsulodin in plasma, new antipseudomonal cephalosporin antibiotic □ Cefsulodin—new cephalosporin antibiotic, high-performance liquid chromatographic determination □ Ultrafiltration—high-performance liquid chromatographic determination of cefsulodin in plasma, new antipseudomonal cephalosporin antibiotic

Cefsulodin sodium [3-(4-carbamoyl-1-pyridiniomethyl) - 7β - (D - α -sulfophenylacetamido) - ceph - 3 - em-4-carboxylate monosodium salt] (I), a semisynthetic cephalosporin antibiotic (Fig. 1)[1], has been shown to have excellent antipseudomonal activity (1, 2).



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Microbiological assays have traditionally been used for analysis of biological specimens containing antibiotics; however, such procedures are occasionally disadvantageous due to long analysis times, nonlinear calibration curves, inadequate specificity, and relatively poor precision. In addition, preliminary experiments suggested that problems might arise during microbiological assays for cefsulodin due to drug hydrolysis during incubation of inoculated analysis plates.

With these factors in mind, work was started to develop an alternate procedure which had adequate sensitivity, high precision, short analysis time, and which did not allow hydrolysis of the cefsulodin. High-performance liquid chromatography (HPLC) is ideally suited for the analysis of the relatively polar, nonvolatile cephalosporins. Since the therapeutic concentrations of these compounds are usually in the microgram per milliliter range, concentrating techniques are usually not required. However, the majority of the high molecular weight proteins and fibrin must be removed from plasma samples to prevent column filter and bed damage. Several HPLC procedures for cephalosporins, employing classical deproteination reagents such as tri-

Table I—Precision and Linearity of the HPLC Procedure for Cefsulodin

Concentration of Cefsulodin, μg/ml		Coefficient of Variation, %
Actual	Calculated[a]	
0.78	0.76 (0.74)[b]	4.5 (3.3)[b]
1.56	1.57 (1.57)	1.9 (1.7)
3.13	3.10 (3.13)	1.7 (0.4)
6.25	6.31 (6.44)	3.2 (2.1)
12.50	12.71 (12.70)	0.9 (0.6)
25.00	25.05 (25.17)	1.3 (0.4)
50.00	49.40 (50.41)	1.1 (0.4)
100.00	97.34 (98.87)	1.7 (0.3)

[a] Based on results of linear regression of means from quadruplicate determinations for each concentration using reciprocal variance weights (r = 0.9999). [b] Data in parentheses were obtained neglecting responses of the internal standard.

[1] Developed by Takeda Chemical Industries; also known as SCE-129. Currently under clinical investigation by Abbott Laboratories.

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