Bare Silica as a Reverse-Phase Stationary Phase: Liquid Chromatographic Separation of Antihistamines with Buffered Aqueous Organic Mobile Phases

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Abstract □ Unbonded silica gel is an effective support for reverse-phase liquid chromatographic separations of lipophilic amines. Simple buffered aqueous-organic mobile phases provide rapid isocratic separations of antihistamines. Phenylpropanolamine hydrochloride, chlorpheniramine maleate, and dextromethorphan hydrobromide are separated in 8 min on bare silica with a mobile phase of 75% methanol and 25% water which is 0.01 M in $(NH_4)_2HPO_4$. Quantitation is reproducible. A wide variety of additional compounds may be separated using the same mobile phase.

Keyphrases □ Bare silica—reverse-phase stationary phase, liquid chromatography □ Antihistamines—buffered aqueous organic liquid chromatographic mobile phases

The object of this work is to demonstrate that the use of silica in the reverse-phase mode is practical for the liquid chromatographic separation of lipophilic amines. The group of amines used as antihistamines, antitussives, and decongestants are frequently formulated together. When using C_{18}-bonded phase columns, it is necessary to use amine mobile-phase modifiers to ensure good retention and peak shape (1) in the ion-suppression mode. While C_{18}-bonded phases have been reported most often for the separation of these lipophilic amines, recent work (2) suggests that unbonded silica gel, with the maximum concentration of surface silanol groups, is a preferable stationary phase for these compounds.

Using unbonded silica as the stationary phase permits the analyst to separate a wide variety of amine compounds with a simple mobile phase of organic solvent and an aqueous phosphate buffer for pH control. The retention volumes are lower and the peaks are more symmetrical when silica, rather than a C_{18}-bonded support, is used as the stationary phase. However, because this use for unbonded silica is new, it is necessary to demonstrate long-term performance in routine operation.

Table I—Formulations

	Components	Concentration
Syrup		
A	Acetaminophen	625 mg/30 mL
	Phenylpropanolamine hydrochloride	25 mg/30 mL
	Chlorpheniramine maleate	2 mg/30 mL
	Dextromethorphan hydrobromide	20 mg/30 mL
B	Potassium guaiacolsulfonate	100 mg/5 mL
	Pseudoephedrine hydrochloride	30 mg/5 mL
	Chlorpheniramine maleate	2 mg/5 mL
	Dextromethorphan hydrobromide	10 mg/5 mL
E	Phenylpropanolamine hydrochloride	12.5 mg/5 mL
	Chlorpheniramine maleate	2 mg/5 mL
F	Guaifenesin	100 mg/5 mL
	Pseudoephedrine hydrochloride	30 mg/5 mL
	Dextromethorphan hydrobromide	10 mg/5 mL
Tablet		
C	Acetaminophen	325 mg
	Phenylpropanolamine hydrochloride	12.5 mg
	Dextromethorphan hydrobromide	10 mg
D	Pyrilamine maleate	25 mg
G	Theophylline	130 mg
	Ephedrine hydrochloride	24 mg
	Pyrilamine maleate	16.6 mg

This paper demonstrates that practical separations of commercial antihistamine formulations on unbonded silica in the reverse-phase mode result in reproducible analyses and good column life. In addition, the sample preparation is straightforward so that high throughput of samples is attained.

EXPERIMENTAL SECTION[1]

The salts[2], ammonium hydroxide[3], sodium hydroxide[3], hydrochloric acid[3], and phosphoric acid[3] used in this study were obtained commercially. Purified water[4], chromatographic-grade acetonitrile[5], and methanol[5] were used for mobile phase preparation. The mobile phases were filtered through 0.45-μm cellulose acetate filters[6] and deaerated under vacuum in an ultrasonic bath before use.

Seven different over-the-counter pharmaceutical formulations (tablets or syrups) were purchased in a local pharmacy and prepared as samples by dissolving 10 mL, or 1 tablet, in 100 mL of mobile phase and filtering through a 0.5-μm filter[7]. The composition of the various formulations is shown in Table I. A standard solution of formulation A was prepared from pharmaceutical grade materials obtained from a variety of sources. The concentrations of standards were designed to duplicate a 10:1 dilution of label concentration.

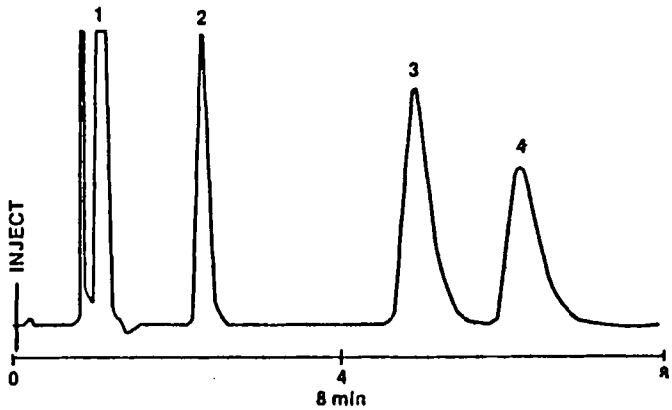

Figure 1—*Separation of a 1:10 solution of syrup A. Operating conditions were: solvent: 75% methanol, 25% H_2O, 0.01 M $(NH_4)_2HPO_4$, pH = 7.8; flow rate: 4 mL/min; 50-μL injection; detection at 254 nm; 0.05 AUFS. Key: (1) acetaminophen; (2) phenylpropanolamine; (3) chlorpheniramine; (4) dextromethorphan.*

[1] The chromatographic system was a Waters Associates, Inc., (Milford, Mass.) Model 244 ALC which included a Model 6000A Solvent Delivery System, Model U6K Injector, Model 480 Variable Wavelength Spectrophotometric Detector, and a Model R401 Refractometer. The column was Radial-PAK μPorasil Silica (8 mm × 10 cm) cartridges which were maintained under radial compression in the Z-Module Radial Compression Separation System (Waters). If excessive back pressure developed, the cartridge was reversed and the tubing to the detector was detached and sent to waste; the cartridge was flushed with mobile phase. When the pressure returned to the original value, the cartridge was returned to the original orientation and the module was reattached to the detector. The analog output of the absorbance (UV) was recorded with a Model 730 Data Module (printer, plotter, and integrator) (Waters) and the quantitation was done at 214 nm. Retention times were converted to retention volumes using the flow rate. Samples were injected automatically using the Model 710B Waters Intelligent Sample Processor (WISP). Automatic control of flow rate, injection, and data documentation was accomplished using the Model 720 System Controller.

[2] Fisher Scientific, Fair Lawn, N.J.

[3] Baker Chemical Co., Phillipsburg, N.J.

[4] Milli-Q System; Millipore Corp., Bedford, Mass.

[5] Waters Associates.

[6] HAWP 04700; Millipore Corp.

[7] Millex Filter; Millipore Corp.

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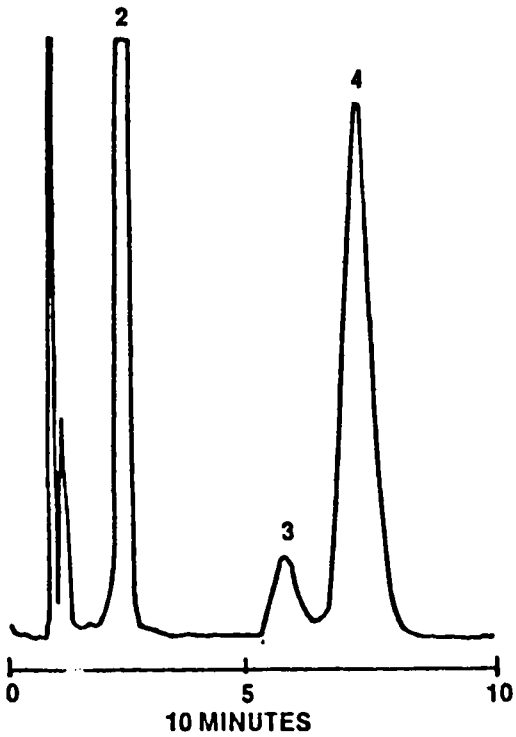

Figure 2—*Chromatogram of the standard solution. Conditions as in Fig. 1, except that detection was at 214 nm and 0.05 AUFS.*

RESULTS AND DISCUSSION

Development of the Separation—Formulation A, which had one nonionic component (acetaminophen) and three amines, was chosen as a demonstration case. The first separations were done with the acetonitrile–aqueous salt eluant described in a previous report (2). This separation resulted in inadequate resolution of chlorpheniramine (10.33 min) and dextromethorphan (11.10 min). Since methanol frequently exhibits different selectivity than acetonitrile (increased α) and weaker solvent strength (increased k'), it was evaluated at various aqueous salt concentrations. Using the organic content of 75% methanol and a salt concentration of 10 mM resulted in the optimized chromatogram shown in Fig. 1. The retention volumes were adequate and the resolution was more than baseline for chlorpheniramine and dextromethorphan. The relationship between the fraction of organic modifier (methanol) and the log retention volume was linear, as shown in a previous report (2).

Determination of Optimum Wavelength of Detection—For the separation shown in Fig. 1, detection was at 254 nm. The absorbance of acetaminophen (molar absorptivity = 13,200 at 248 nm) saturated the detector amplifier and gave a nonlinear response when a sufficient mass of the amines was injected for convenient detection; the amine components are present in much lower concentrations than acetaminophen. Inspection of the UV spectra of the various components indicated that acetaminophen has a minimum absorbance at 217 nm and that a significant improvement of signal strength occurs for phenylpropanolamine at 214 nm. Evaluation at 214 nm indicated that sensitive, reliable quantitation of the lipophilic amines was possible (Fig. 2). At 254 nm, sensitivity for phenylpropanolamine and chlorpheniramine was excellent, while sensitivity for dextromethorphan was not as good. At 214 nm, the relative sensitivity of chlorpheniramine is reduced compared with dextromethorphan and phenylpropanolamine. The quantification is better for all components at 214 nm. Even so, the absorbance of acetaminophen was still too high (from 26 to 350 times as much as the amines) for quantitation. When it was desirable to quantitate acetaminophen, it was necessary to decrease the sample size such that detection of dextromethorphan (254 nm) or chlorpheniramine (214 nm) was very difficult. A different method with greater retention is preferable for acetaminophen.

Table II—Reproducibility of Quantitation with Standard Solution [a]

Compound	Conc., mg/mL	*RSD*, %
Phenylpropanolamine hydrochloride	0.0847	±0.65
Chlorpheniramine maleate	0.00672	±4.53
Dextromethorphan hydrobromide	0.0669	±1.45

[a] n = 30; run completed overnight.

Table III—Linearity of Response with Sample Load

Compound	Slope	Intercept	Coefficient of Correlation
Phenylpropanolamine hydrochloride	0.01952	0.00223	0.9980
Chlorpheniramine maleate	0.02217	0.000038	0.9980
Dextromethorphan hydrobromide	0.01913	0.00239	0.9980

Table IV—Quantitative Analysis of Syrup A

Compound	Conc., mg/mL Found	Label Claim	% of Label Claim
Phenylpropanolamine hydrochloride	0.08008	0.08333	96.1
Chlorpheneramine maleate	0.00629	0.00667	94.3
Dextromethorphan hydrobromide	0.0632	0.06667	94.8

Determination of Reproducibility and Linearity—A standard solution was prepared from pharmaceutical grade materials which duplicated a 10:1 dilution of the label concentrations in Syrup A of phenylpropanolamine, chlorpheniramine, and dextromethorphan. Table II shows that the quantitation of the three bases in the standard solution is excellent: two components had <2% *SD* for a series of 30 injections, and the component present in lowest concentration had a <5% *SD*. The linearity of the system with sample load was evaluated. When plots of sample load in μg *versus* detector response in mg/mL were evaluated, the lines reported in Table III were obtained. The system was linear from 0.002 mg/mL to 0.022 mg/mL. The assay results of a 10:1 dilution of syrup A is shown in Table IV. The concentrations found were ~95% of label for all three components. This method was developed on a single column and several hundred injections were completed with comparable

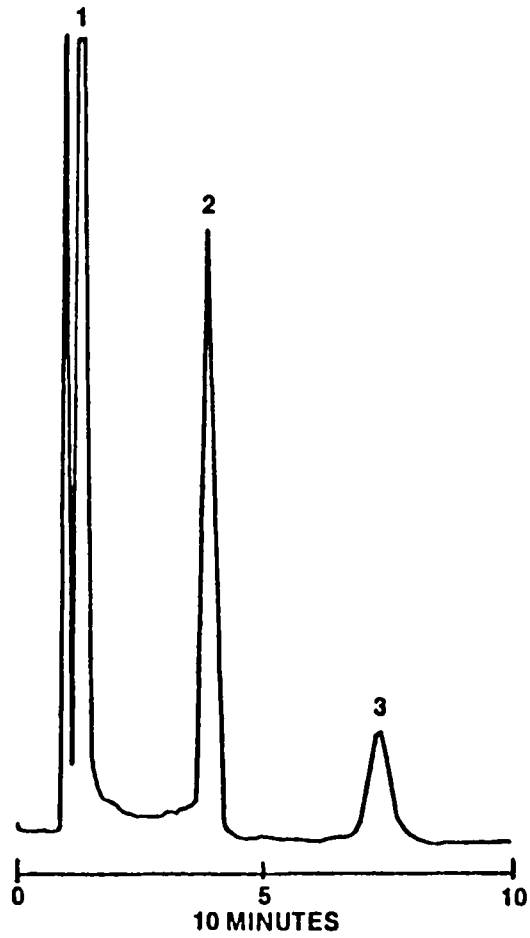

Figure 3—*Chromatogram of over-the-counter cough syrup F. Conditions as in Fig. 2. Key: (1) guaifenesin; (2) pseudoephedrine; (3) dextromethorphan.*

resolution: More than 800 injections were completed on a second column with no change in the separation.

Analysis of Additional Over-the-Counter Pharmaceutical Formulations—The six additional preparations, shown in Table I, were examined to determine whether the silica adsorbent was appropriate for other basic drugs. Chromatograms were obtained using the same mobile phase and detector wavelength as for quantitation of syrup A, and no attempt was made to optimize the separations. For example, Fig. 3 illustrates that other basic drugs are also eluted in this mobile phase system.

CONCLUSIONS

The separation of lipophilic bases on unbonded silica stationary phases in the reverse-phase mode is rapid and convenient. No amine modifiers or gradients are necessary for the separation; only simple buffers are required for pH control. Separations of several samples indicated that a variety of compounds may be separated with this system. Assay of a single sample indicated that linearity, reproducibility, and length of column life are excellent.

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Hypolipidemic Activity of Phthalimide Derivatives V: Reduced and Hydrolytic Products of Simple Cyclic Imides

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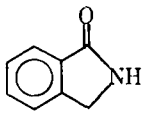
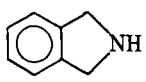
Abstract □ A series of cyclic imides and related compounds have previously been shown to possess hypolipidemic activity at the low dose level of 20 mg/kg/d. Hydrolytic and reduced products of the cyclic imides were synthesized and examined to discern if possible metabolic products were the active chemical species of these hypolipidemic agents. Phthalimide proved to be the most active cyclic imide tested. Unfortunately, the new products did not, in general, improve hypolipidemic activity in rodents. The exceptions were piperidine which demonstrated improved hypotriglyceridemic activity, and 3,4,5,6-dibenzohomopiperidin-2-one, which demonstrated improved hypocholesterolemic activity compared to phthalimide.

Keyphrases □ Cyclic imides—phthalimide derivatives, reduced and hydrolytic products, hypolipidemic activity □ Phthalimide derivatives—cyclic imides, hypolipidemic activity, reduced and hydrolytic products □ Hypolipidemic activity—phthalimide derivatives, cyclic imides, reduced and hydrolytic products

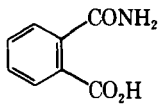
A series of cyclic imides including phthalimide (I), succinimide, 1,8-naphthalimide, and saccharin have been shown to be potent hypolipidemic agents in rodents (1). After conducting dose response studies on these compounds, the optimum dose appears to be 20 mg/kg/d ip when tested in mice. Thus, we have selected that dose for this structure-activity relationship study. The mode of action of these derivatives is different from standard therapeutic agents on the market in that they do not inhibit the regulating enzyme of cholesterol synthesis, HMG CoA reductase; rather, they regulate mitochondrial citrate exchange and the availability of acetyl CoA, the key intermediate required in the synthesis of fatty acids and cholesterol (1). These agents also decreased cholesterol absorption in the intestine and accelerated cholesterol excretion by the biliary route. The agents had no effect on appetite, organ weights, or body weight, and there was no evidence of organ toxicity or deleterious systemic effects (1). Since these are potentially hypolipidemic agents, we expanded the types of imide rings and examined a series of their reduced and hydrolytic products, which may be potential metabolic products of the parent imide yet retain pharmacological activity.



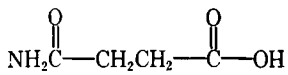
Phthalimidine (II)



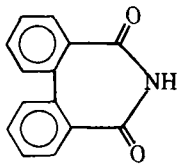
Isoindolin (III)



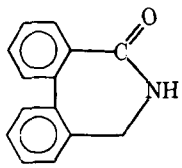
Phthalamic Acid (IV)



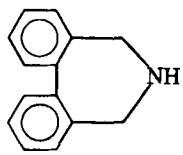
Succinamic Acid (IX)



Diphenimide (XVI)



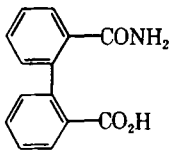
3,4,5,6-Dibenzohomo piperidin-2-one (XVII)



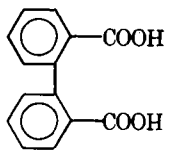
3,4,5,6-Dibenzohomo piperidine (XVIII)

$HO_2C(CH_2)_3CONH_2$

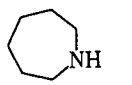
Glutaramic Acid (XIV)



Diphenamic Acid (XIX)



Diphenic Acid (XX)



Homopiperidine (XXIII)

$HO_2C(CH_2)_3CONH_2$

Adipamic Acid (XXIV)

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