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GENERAL REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR THE SEPARATION OF DRUGS USING TRIETHYLAMINE AS A COMPETING BASE

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SUMMARY

Triethylamine (TEA) was evaluated as a competing base for the retention control and peak shape improvement in the reversed-phase high-performance liquid chromatographic (RP-HPLC) analysis of selected acidic, basic, and neutral drugs. The effects of this amine on the capacity factor and theoretical plate number values of ephedrine, phenol, and sulfamerazine were examined on three unmodified commercial octadecylsilane chromatographic columns. Based on these results, a general RP-HPLC elution scheme using a μBondapak C_{18} 10-μm column, methanol–acetic acid–TEA–water mobile phases, and an ultraviolet detector was developed for more than 150 drugs of pharmaceutical interest. The proposed method was applied to the separation of groups of chemically or pharmacologically related drugs that included sympathomimetic amines, anthihistamines, phenothiazines, local anesthetics, Cinchona and tropane alkaloids, xanthines, sulfonamides, and steroids. In addition, paired-ion drugs such as physostigmine salicylate and combinations of ascorbic acid, benzoic acid, salicylic acid, pamoic acid, and 8-chlorotheophylline with various basic moieties were readily and effectively resolved into their ionic components using almost identical RP-HPLC conditions.

INTRODUCTION

This laboratory is currently working on the development of uniform and straightforward approaches to the analysis of drugs of pharmaceutical interest by reversed-phase high-performance liquid chromatography (RP-HPLC). Only a few such procedures have been described in the literature. For example, Lurie and Demchuk[1,2] described RP ion-pair HPLC conditions for the separation of a wide range of drugs of forensic importance that included ergot and opium alkaloids, phenylethylamine, local anesthetics, and barbiturates. Likewise, Hoogewijs and Massart[3], Detaevernier *et al.*[4], and De Smet *et al.*[5] reported standardized analytical strategies for analyzing basic drugs in pharmaceutical dosage forms by HPLC on bonded phases with polar and non-polar mobile phases. More recently, and at variance with the

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RP-HPLC modality, Jane *et al.*[6] have described HPLC conditions for the analysis of more than 450 basic drugs on unmodified silica columns with non-aqueous ionic eluents using photometric, fluorescence, and electrochemical oxidation detections.

In RP-HPLC, mobile phase additives represent the first form of *in situ* column modification for effecting selectivity changes as well as remediating peak asymmetry[7]. Many substances have been used to alter selectivity but alkylamines and alkylsulfonate ion-pairing reagents are the most common ones. Alkylamines act primarily by hydrogen bonding to non-derivatized silanol sites, thereby reducing adsorption and/or ion-exchange effects[8]. The addition of an alkylamine to a mobile phase can dramatically improve peak shapes with little loss of retention. In addition to their ability to reduce peak tailing, alkylamines are also useful as selectivity-enhancing agents.

A number of publications have dealt with the inclusion of amines in the eluent to control peak retention and to improve column efficiency. Eggers and Saint-Joly[9] studied the effects of amine modifiers on the RP chromatographic behavior of salbutamol. Hung *et al.*[10] investigated the effects of various organic amines on the ion-pair chromatographic analysis of tricyclic antidepressant drugs. Pennington and Schmidt[11] added tetraethylammonium ions to the mobile phase for the quantitative determination of mixtures of atropine, hyoscyamine, and scopolamine in pharmaceutical products. Cooke and Olsen[12] discussed the effect of a hydrophilic amine such as nonyl amine on the RP chromatographic behavior of a number of phenothiazines. The suitability of amines as silanol-masking agents and their effect on the retention characteristics of a variety of phenylethylamines[13], dibenzo-crown ethers and peptides[14], and tricyclic antidepressants[15], has also been considered.

This paper describes the use of triethylamine (TEA) as a mobile phase modifier for the RP-HPLC separation of acidic, basic, and neutral drugs; and examines the behavior of prototype drugs in terms of retentions and column efficiencies on three brands of unmodified octadecylsilane (ODS) columns. The positive influence of TEA on the resolving efficiency of methanol–acetic acid–TEA–water mobile phases was demonstrated by achieving the separation of mixtures of structurally related drugs and of several of their paired-ion combinations.

EXPERIMENTAL

Equipment and experimental conditions

The liquid chromatograph consisted of a Model 3500B solvent delivery system, a Model 770 variable-wavelength detector, a sampling valve fitted with a 10-μl sample loop (Spectra-Physics, Mountain View, CA, U.S.A.), and a Model 3380A recording integrator (Hewlett-Packard, Avondale, PA, U.S.A.). The chromatographic columns were a 10 μm μBondapak C_{18}, 300 × 3.9 mm I.D. (Waters Assoc., Milford, MA, U.S.A.), a 5 μm Zorbax ODS, 250 × 4.6 mm I.D. (DuPont, Wilmington, DE, U.S.A.), and a 5 μm Ultrasphere ODS, 250 × 4.6 mm I.D. (Beckman Instruments, Berkeley, CA, U.S.A.). All analyses were performed at ambient temperature with the mobile phase delivered at a flow-rate of 1.5 ml/min.

Chemicals

The test compounds used throughout the study were of reagent grade or better,

and were obtained from commercial sources. Mobile phases were prepared using HPLC-grade methanol (J. T. Baker, Phillipsburg, NJ, U.S.A.), reagent grade glacial acetic acid (Fisher Scientific, Fair Lawn, NJ, U.S.A.), reagent grade TEA (J. T. Baker), and water that had been double-distilled in glass.

Chromatographic solutions

Capacity factor (k') and theoretical plate number (N) values of model drugs were determined using solutions that contained 1 mg/ml of ephedrine, 0.1 mg/ml of phenol, and 0.05 mg/ml of sulfamerazine in methanol–acetic acid–water (22.5:1.5:76.0). To study chromatographic mobility behaviors as a function of the concentration of methanol in the mobile phase, test compounds were individually dissolved in methanol–water (1:1) to contain 0.5 mg/ml. Solutions of mixed sympathomimetic amines and tropane alkaloids were prepared in methanol–water (1:1) to contain 1 mg/ml of each component. Solutions of antihistamines, phenothiazines, local anesthetics, steroids, Cinchona alkaloids, sulfonamides, and xanthines were also prepared in methanol–water (1:1) to contain 0.25 mg/ml of each component. The prednisolone peak appearing during the separation of the steroids represents an impurity of prednisolone succinate. Solutions of the paired-ion drugs hydroxyzine pamoate, pyrantol pamoate, pyrvinium pamoate, and physostigmine salicylate were individually prepared in methanol–water (1:1) to contain 0.25 mg/ml of each sample. The paired-ion combinations of 8-chlorotheophylline and quinine were prepared by dissolving equal amounts of the corresponding moieties in methanol–water (1:1) to obtain solutions containing 0.25 mg/ml of each component.

RESULTS AND DISCUSSION

Normalization of the retention behavior of a solute can be achieved by incorporating TEA in the eluent to serve as a competing base for masking accessible surface silanol groups and for providing heterogeneity on the RP bonded surface. Kiel *et al.*[8] and Bij *et al.*[14] have previously shown that retention is practically independent of sample load if an amine is present in the eluent, and that short chain tertiary amine modifiers like TEA are highly effective in reducing or eliminating

TABLE I

EFFECTS OF TEA ON PEAK RETENTION AND COLUMN EFFICIENCY

k' and N values for ephedrine, phenol, and sulfamerazine on μBondapak C_{18} (A), Zorbax ODS (B), and Ultrasphere ODS (C) columns as a function of the concentration of TEA in the mobile phase. Mobile phases were mixtures of methanol–acetic acid–TEA–water [22.5:1.5:(0 or 1):(76 or 75)].

Drug	*k'*						*N*					
	0% TEA			*1% TEA*			*0% TEA*			*1% TEA*		
	A	*B*	*C*	*A*	*B*	*C*	*A*	*B*	*C*	*A*	*B*	*C*
Ephedrine	0.60	22.79	20.10	0.97	1.73	2.26	494	75	20	2784	2880	4900
Phenol	2.65	5.23	6.25	2.44	5.90	6.48	4018	10 076	14 604	3836	9423	13 395
Sulfamerazine	1.75	3.26	2.43	1.73	3.20	2.46	2832	4239	4746	2750	3823	5150

silanophilic interactions. Similar effects have been described for other amines[12,16,17].

The effects of TEA on k', a measure of retention, and on N, a measure of efficiency, were investigated on three model drugs by using mobile phases that only differed in the concentration of TEA present, and three brands of ODS RP columns. In this study k' is defined as $(t_R/t_0) - 1$, where t_R and t_0 are the retention times of the compound under investigation and a non-retained compound, respectively, and where t_0 was measured as the first distortion of the base line following the injection of water. Of the several methods of measuring column plate count, the peak width at half height method, *i.e.* $N = 5.54\ (t_R/W_{0.5})^2$, was found the most convenient.

As shown in Table I, although the concentration of amine in the eluent played little or no role in determining the k' and N values of the relatively neutral phenol and acidic sulfamerazine, irrespective of the column used, it however greatly influenced the values of the weak base ephedrine. Interestingly, in the case of ephedrine a sharp reduction in k' values occurred on the Zorbax ODS and Ultrasphere ODS columns, whereas the N values increased on all three columns used.

Plots of k' and N values against the concentration of TEA in the eluent revealed further relationships between the concentration of modifying amine and the chromatographic behavior. As illustrated with ephedrine (Fig. 1), these plots demonstrated that although on all three columns the same concentration of alkylamine, *i.e.*

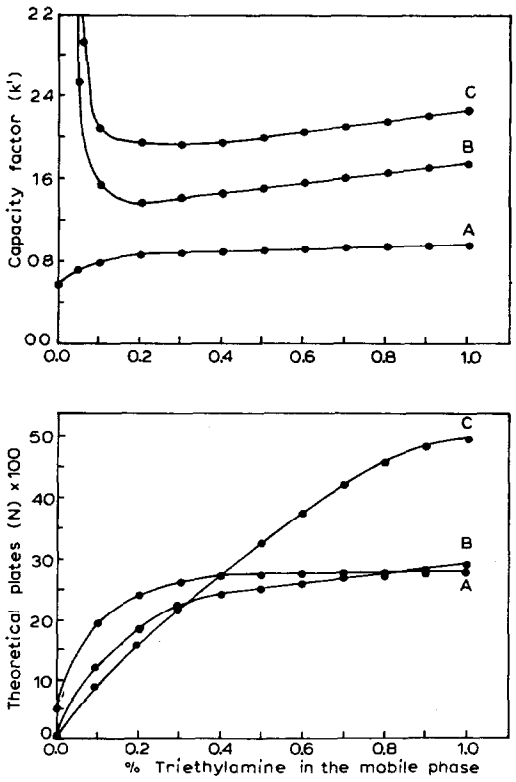


Fig. 1. Effect of the concentration of TEA in the mobile phase on k' and N values of ephedrine. See Table I for columns and mobile phases.

TABLE II

k' VALUES AS A FUNCTION OF THE CONCENTRATION OF METHANOL IN THE MOBILE PHASE

k' = capacity factor = $(t_R/t_0) - 1$, where t_0 = 2.5 min, were measured with a methanol–acetic acid–TEA–water (variable:1.5:0.5:variable, to yield 100 parts by volume) mobile phase.

Drug	*Parts of methanol per 100 parts of mobile phase*									
	0	*10*	*20*	*30*	*40*	*50*	*60*	*70*	*80*	*90*
Acetanilide	–	–	4.61	2.52	1.46	0.85	0.41	0.19	–	–
Acetophenazine	–	–	–	–	–	7.13	2.42	0.79	0.22	–
Acetyl sulfisoxazole	–	–	–	8.47	2.86	1.01	0.39	–	–	–
Aminopromazine	–	–	–	–	–	–	2.79	0.89	0.26	–
Amitriptyline	–	–	–	–	–	6.85	2.54	0.95	0.25	–
Amodiaquin	–	–	–	–	–	2.55	1.28	0.31	0.02	–
Amphetamine	–	2.33	1.63	0.85	–	0.32	–	–	–	–
Antazoline	–	–	–	–	3.20	1.50	0.48	0.08	–	–
Antipyrine	–	–	6.45	2.60	1.24	0.69	0.50	0.15	–	–
Atropine	–	12.12	4.26	1.09	0.75	0.36	0.18	–	–	–
Atropine methyl	–	9.18	3.19	0.89	0.63	0.31	0.16	–	–	–
Benzocaine	–	–	–	6.38	2.78	1.40	0.97	0.24	–	–
Benztropine	–	–	–	–	–	–	2.10	0.95	–	–
Bromodiphenhydramine	–	–	–	–	14.88	5.70	3.48	0.59	0.24	–
Brompheniramine	–	–	–	13.60	5.52	2.51	1.63	0.45	0.21	–
Bupivacaine	–	–	–	6.13	2.84	1.37	–	–	–	–
Butacaine	–	–	–	5.34	1.95	0.81	–	–	–	–
Butaperazine	–	–	–	–	–	33.04	9.04	2.57	0.80	0.21
Caffeine	–	–	4.11	1.48	0.74	0.46	0.31	0.10	–	–
Carbinoxamine	–	–	–	10.50	4.24	1.98	1.35	0.34	–	–
Chlorcyclizine	–	–	–	–	15.33	5.83	3.59	0.77	0.21	–
Chloroprocaine	–	–	–	0.92	0.44	0.22	–	–	–	–
Chloroquine	–	–	–	0.99	0.31	0.11	–	–	–	–
8-Chlorotheophylline	–	–	–	2.03	1.07	0.75	0.42	0.06	–	–
Chlorothiazide	–	–	–	0.31	0.23	0.09	–	–	–	–
Chlorpheniramine	–	–	–	10.05	4.51	2.11	1.35	0.34	0.12	–

(*Continued on p. 408*)

TABLE II (*continued*)

Drug	*Parts of methanol per 100 parts of mobile phase*									
	0	*10*	*20*	*30*	*40*	*50*	*60*	*70*	*80*	*90*
Chlorpromazine	–	–	–	–	–	11.28	3.86	1.20	0.41	0.05
Cinchonidine	–	–	–	–	2.93	1.23	–	–	–	–
Cinchonine	–	–	–	–	2.60	1.11	–	–	–	–
Clemizole	–	–	–	–	–	4.01	2.05	0.86	–	–
Cortisone acetate	–	–	–	–	–	–	2.19	0.84	0.18	–
Cyclizine	–	–	–	5.71	2.47	1.67	0.42	0.16	–	–
Cyclothiazide	–	–	–	–	2.51	0.75	0.24	–	–	–
Cycrimine	–	–	–	5.42	2.46	1.72	0.39	–	–	–
Desipramine	–	–	–	–	5.68	2.16	0.81	0.18	–	–
Dextromethorphan	–	–	–	4.61	2.14	1.55	0.40	0.18	–	–
Dibucaine	–	–	–	–	6.70	2.75	–	–	–	–
Dienestrol	–	–	–	–	16.44	4.70	1.43	0.32	–	–
Dietylstilbestrol	–	–	–	–	16.92	4.68	1.42	0.32	–	–
Dihydrocinchonidine	–	–	–	–	4.09	1.64	–	–	–	–
Dihydrocinchonine	–	–	–	–	3.64	1.48	–	–	–	–
Dihydroergocornine	–	–	–	–	4.26	1.36	0.41	–	–	–
Dihydroergocristine	–	–	–	–	8.22	2.36	0.67	–	–	–
Dihydroergocryptine	–	–	–	–	7.55	2.19	0.64	–	–	–
Dihydroquinidine	–	–	–	–	5.88	2.16	–	–	–	–
Dihydroquinine	–	–	–	–	7.91	2.64	–	–	–	–
Diphenhydramine	–	–	–	4.49	2.02	1.37	0.30	–	–	–
Diphenylpyraline	–	–	18.69	–	2.84	–	–	–	–	–
Doxylamine	–	–	4.24	2.03	1.04	0.71	0.16	–	–	–
Dyphylline	–	–	1.50	0.70	0.22	–	–	–	–	–
Ephedrine	2.72	1.70	1.04	0.55	–	–	–	–	–	–
Ergonovine	–	–	2.69	0.70	0.45	0.17	–	–	–	–
Ergotamine	–	–	–	–	–	4.82	1.55	0.45	–	–
Estradiol	–	–	–	–	–	–	4.67	1.59	0.41	–
Estradiol benzoate	–	–	–	–	–	–	–	–	3.53	0.68
Estradiol cypionate	–	–	–	–	–	–	–	–	7.45	1.32
Estradiol valerate	–	–	–	–	–	–	–	–	3.49	0.66
Estriol	–	–	–	–	–	–	0.99	0.35	0.02	–
Estrone	–	–	–	–	–	–	4.06	1.43	0.38	–

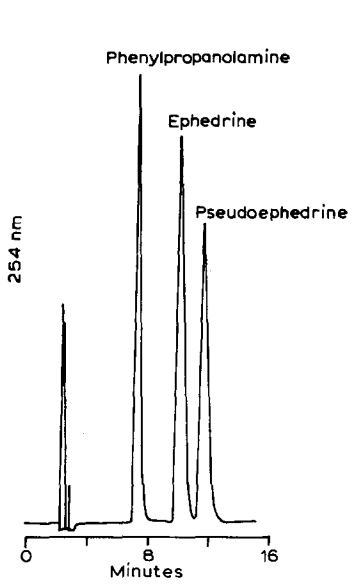
Ethinyl estradiol	–	–	–	–	–	–	4.12	1.45	0.32	–
Fluphenazine	–	–	–	–	–	–	6.99	1.93	0.53	0.03
Fluphenazine decanoate	–	–	–	–	–	–	–	–	3.49	1.12
Fluphenazine enanthate	–	–	–	–	–	–	–	–	2.49	0.50
Homatropine	–	4.66	1.69	0.50	0.34	0.20	0.09	–	–	–
Homatropine methyl	–	3.13	1.14	0.40	0.29	0.17	–	–	–	–
Hydrochlorothiazide	–	2.64	1.05	0.49	0.24	0.10	–	–	–	–
Hydroflumethazide	–	5.43	2.13	0.92	0.44	0.19	–	–	–	–
Hydrocortisone	–	–	–	–	–	–	1.27	0.56	0.11	–
Hydrocortisone acetate	–	–	–	–	–	–	2.25	0.91	0.21	–
Hydroxyamphetamine	1.25	0.90	0.50	–	–	0.09	–	–	–	–
Hydroxyzine	–	–	–	–	–	–	2.35	0.60	–	–
Hyoscyamine	–	12.82	4.76	1.09	0.75	0.36	0.18	–	–	–
Imipramine	–	–	–	–	–	5.25	2.14	0.84	0.21	–
Isoproterenol	0.71	0.34	0.18	–	–	–	–	–	–	–
Lidocaine	–	–	2.83	1.23	0.76	0.43	–	–	–	
Meclizine	–	–	–	–	–	–	–	–	1.94	0.48
Medroxyprogesterone acetate	–	–	–	–	–	–	–	3.12	0.77	–
Mephentermine	–	6.45	3.29	1.40	–	–	0.23	–	–	–
Mesoridazine	–	–	–	–	–	3.11	1.14	0.35	0.10	–
Mestranol	–	–	–	–	–	–	–	–	1.51	0.27
Methamphetamine	–	3.42	1.91	0.95	–	–	0.24	–	–	–
Methapyriline	–	–	–	5.24	2.14	1.08	0.73	0.18	–	–
Methotrimeprazine	–	–	–	–	–	–	2.41	0.58	0.21	–
Methoxyamphetamine	–	–	–	1.86	0.92	0.65	0.48	0.07	–	–
Methoxypromazine	–	–	–	–	–	–	2.17	0.54	0.14	–
Methyldopate	–	5.49	1.98	–	–	–	–	–	–	–
Methylparaben	–	–	–	–	–	1.44	0.74	0.27	–	–
Methyltestosterone	–	–	–	–	–	–	6.05	2.27	0.66	–
Naphazoline	–	–	–	2.52	1.23	0.66	0.47	0.09	–	–
Norethindrone	–	–	–	–	–	–	3.60	1.32	0.34	–
Norethindrone acetate	–	–	–	–	–	–	9.77	2.91	0.71	–
Nortriptyline	–	–	–	–	–	7.31	2.60	0.94	0.21	–
Oxyphencylcimine	–	–	–	–	11.63	4.78	2.79	0.67	0.21	–
Perchlorperazine	–	–	–	–	–	21.12	6.75	2.14	0.72	0.20
Perphenazine	–	–	–	–	–	–	5.12	1.59	0.50	0.06
Phenacetin	–	–	–	6.03	2.90	1.45	0.66	0.31	–	–

(*Continued on p. 410*)

TABLE II (*continued*)

Drug	*Parts of methanol per 100 parts of mobile phase*									
	0	*10*	*20*	*30*	*40*	*50*	*60*	*70*	*80*	*90*
Phenindamine	–	–	–	–	6.44	2.66	–	–	–	–
Pheniramine	–	–	–	3.40	1.62	0.84	0.65	0.17	–	–
Phenothiazine	–	–	–	–	–	–	5.51	1.56	0.47	–
Phenoxybenzamine	–	–	–	–	6.57	2.57	1.78	0.46	0.20	–
Phentermine	–	5.35	2.91	1.40	–	–	0.32	–	–	–
Phentolamine	–	–	–	6.87	2.41	1.50	1.05	0.13	–	–
Phenylpropanolamine	1.48	1.15	0.79	0.45	–	–	–	–	–	–
Phenylephrine	0.38	0.25	–	–	–	–	–	–	–	–
Phenyltoloxamine	–	–	–	–	7.63	3.03	2.00	0.41	0.14	–
Phthalylsulfathiazole	–	–	13.79	3.93	1.32	0.47	0.19	–	–	–
Physostigmine	–	–	–	1.60	0.57	–	–	–	–	–
Prednisolone	–	–	–	–	–	–	1.23	0.52	0.10	–
Prednisolone acetate	–	–	–	–	–	–	2.23	0.86	0.20	–
Prednisolone tebutate	–	–	–	–	–	–	14.73	4.00	0.86	–
Prednisone	–	–	–	–	–	–	0.85	0.36	0.04	–
Procaine	–	–	1.15	–	0.23	0.16	–	–	–	–
Prochlorperazine	–	–	–	–	21.12	6.75	3.57	2.14	0.72	–
Progesterone	–	–	–	–	–	–	10.36	3.39	0.91	–
Promazine	–	–	–	–	–	5.04	1.92	0.64	0.24	–
Promethazine	–	–	–	–	–	4.40	1.75	0.60	0.22	–
Protrophyline	–	–	–	–	–	5.76	2.03	0.73	0.13	–
Pseudoephedrine	3.52	1.96	1.07	0.55	–	–	–	–	–	–
Pyrantel	–	–	–	–	–	0.60	0.20	–	–	–
Pyrilamine	–	–	–	8.74	3.40	1.60	1.08	0.29	–	–
Pyrvinium	–	–	–	–	–	–	13.64	3.24	–	–
Quinidine	–	–	–	–	4.21	1.63	–	–	–	–
Quinine	–	–	–	–	5.49	1.89	–	–	–	–
Salicylamide	–	–	4.23	2.19	0.84	0.66	0.28	0.11	–	–
Salicylic acid	–	–	–	–	1.30	0.50	–	–	–	–
Scopolamine	–	3.98	1.63	0.55	0.40	0.21	0.11	–	–	–

Scopolamine aminoxide	–	3.32	1.40	0.48	0.34	0.21	0.10	–	–	–
Scopolamine methyl	–	5.46	2.12	0.65	0.47	0.26	0.12	–	–	–
Spironolactone	–	–	–	–	–	11.66	3.42	1.13	0.27	–
Succinylsulfathiazole	–	–	5.16	1.43	0.52	0.20	0.04	–	–	–
Sulfabenzamide	–	–	–	3.09	1.34	0.57	0.23	–	–	–
Sulfachlorpyridazine	–	–	8.45	2.01	0.93	0.43	0.20	–	–	–
Sulfadiazine	–	3.52	1.42	0.68	0.36	0.16	0.06	–	–	–
Sulfadimethoxine	–	–	–	6.90	2.56	1.00	0.42	0.09	–	–
Sulfamerazine	–	6.85	2.49	1.13	0.55	0.26	0.11	–	–	–
Sulfamethazine	–	–	4.06	1.72	0.80	0.38	0.17	–	–	–
Sulfamethizole	–	4.85	1.71	0.71	0.34	0.14	0.06	–	–	–
Sulfamethoxazole	–	–	5.46	2.21	0.97	0.41	0.20	–	–	–
Sulfamethoxypyridazine	–	–	4.35	1.79	0.80	0.36	0.16	–	–	–
Sulfanilamide	–	0.58	0.32	0.19	0.11	–	–	–	–	–
Sulfanilic acid	0.26	0.13	0.06	–	–	–	–	–	–	–
Sulfaphenazole	–	–	–	4.76	1.81	0.68	0.30	–	–	–
Sulfapyridine	–	5.49	1.98	0.89	0.45	0.21	0.11	–	–	–
Sulfathiazole	–	4.85	1.71	0.71	0.34	0.14	–	–	–	–
Sulfisomidine	–	4.07	1.61	0.65	0.34	0.17	0.07	–	–	–
Sulfisoxazole	–	–	6.57	2.54	1.04	0.43	0.17	–	–	–
Testosterone	–	–	–	–	–	–	4.48	1.75	0.52	–
Testosterone cypionate	–	–	–	–	–	–	–	–	8.07	1.48
Testosterone enanthate	–	–	–	–	–	–	–	–	7.12	1.33
Testosterone propionate	–	–	–	–	–	–	–	–	1.95	0.41
Tetracaine	–	–	–	12.16	4.55	2.06	1.33	0.33	–	–
Theobromine	–	–	1.23	–	0.29	0.19	–	–	–	–
Theophylline	–	–	2.08	0.87	0.48	0.31	–	–	–	–
Thioridazine	–	–	–	–	–	–	5.66	1.59	0.51	0.06
Thozylamine	–	–	–	5.31	2.22	1.10	0.75	0.19	–	–
Trichlormethiazole	–	–	7.47	2.89	1.26	0.56	0.21	–	–	–
Tiethylperazine	–	–	–	–	–	–	10.45	3.04	0.96	0.27
Triflupromazine	–	–	–	–	–	–	5.12	1.47	0.43	0.02
Trimeprazine	–	–	–	–	–	–	2.07	0.70	0.21	–
Tripelennamine	–	–	–	7.45	3.22	1.53	1.02	0.26	–	–
Triprolidine	–	–	–	15.90	5.74	2.40	1.52	0.35	–	–
Tropic acid	–	6.47	3.32	1.26	0.93	0.52	0.26	–	–	–



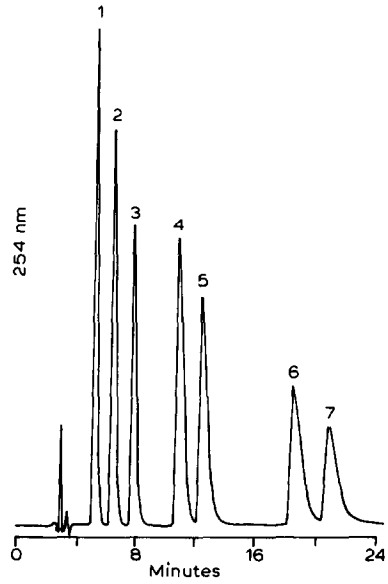
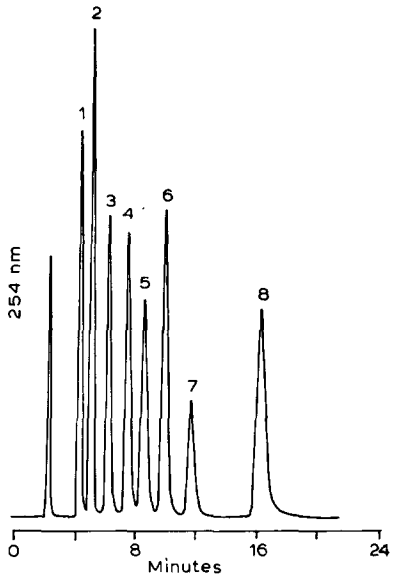


Fig. 2. Chromatographic separation of phenylpropanolamine and the diastereoisomers ephedrine and pseudoephedrine. Column, μBondapak C_{18}; mobile phase, methanol–acetic acid–TEA–water (5.0:1.5:0.5:93.0); detector sensitivity, 0.16 a.u.f.s.

Fig. 3. Chromatographic separation of sympathomimetic amines. Column, μBondapak C_{18}; mobile phase, methanol–acetic acid–TEA–water (15.0:1.5:0.5:83.0); detector sensitivity, 0.16 a.u.f.s. 1 = Hydroxyamphetamine; 2 = phenylpropanolamine; 3 = ephedrine; 4 = amphetamine; 5 = methamphetamine; 6 = phentermine; 7 = mephentermine.

0.2%, provided retention control, the same is not true of the peak shapes, as evidenced by the increase in N values with increasing amine in the eluent. Hence, control of N will require an amount of TEA that is dictated by the brand of column used. For example, whereas 0.5% of TEA was adequate for controlling the retention and peak shape of ephedrine on the μBondapak C_{18} and Zorbax ODS columns, a 1% concentration was needed on the Ultrasphere column. In any event, the same concentration (1.5%) of acetic acid in the mobile phase was sufficient to render the pH below 4.5, even at the maximum (1%) concentration of TEA added. Under these conditions weak bases will become ionized and weak acids will remain non-ionized.

Methanol–acetic acid–water mobile phases that contained 0.5% TEA, together with an arbitrarily selected RP-HPLC column (μBondapak C_{18}), were used to determine the chromatographic behavior of a large number of pharmaceutically important drugs, many of which are currently listed in the United States Pharmacopeia[18]. Table II gives the k' values for 166 compounds as a function of the concentration of methanol in the mobile phase. The data show that as the concentration of methanol increases the retention decreases, as would be expected in RP-HPLC[13]. From the compilation of k' values for a range of methanol concentrations in the mobile phase the most appropriate elution system may be selected for a given compound, whether this compound occurs singly or in combination with other listed compounds.



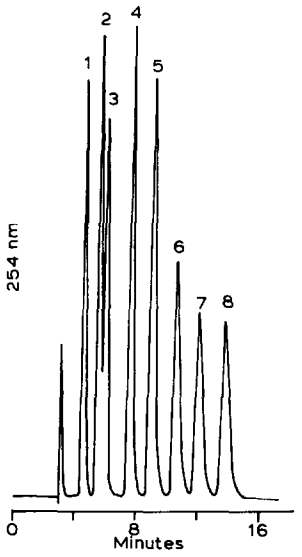


Fig. 4. Chromatographic separation of antihistamines. Column, μBondapak C_{18}; mobile phase, methanol–acetic acid–TEA–water (50.0:1.5:0.5:48.0); detector sensitivity, 0.16 a.u.f.s. 1 = Pheniramine; 2 = thozylamine; 3 = tripelennamine; 4 = chlorpheniramine; 5 = brompheniramine; 6 = phenindamine; 7 = phenyltoxamine; 8 = clemizole.

Fig. 5. Chromatographic separation of phenothiazines. Column, μBondapak C_{18}; mobile phase, methanol–acetic acid–TEA–water (70.0:1.5:0.5:28.0); detector sensitivity, 0.32 a.u.f.s. 1 = Mesoridazine; 2 = promethazine; 3 = acetophenazine; 4 = chlorpromazine; 5 = thioridazine; 6 = prochlorperazine; 7 = butaperazine; 8 = thiethylperazine.

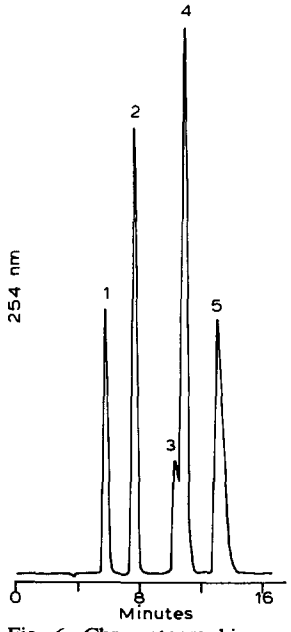


Fig. 6. Chromatographic separation of local anesthetics. Column, μBondapak C_{18}; mobile phase, methanol–acetic acid–TEA–water (50.0:1.5:0.5:48.0); detector sensitivity, 0.16 a.u.f.s. 1 = Lidocaine; 2 = butacaine; 3 = bupivacaine; 4 = benzocaine; 5 = tetracaine.

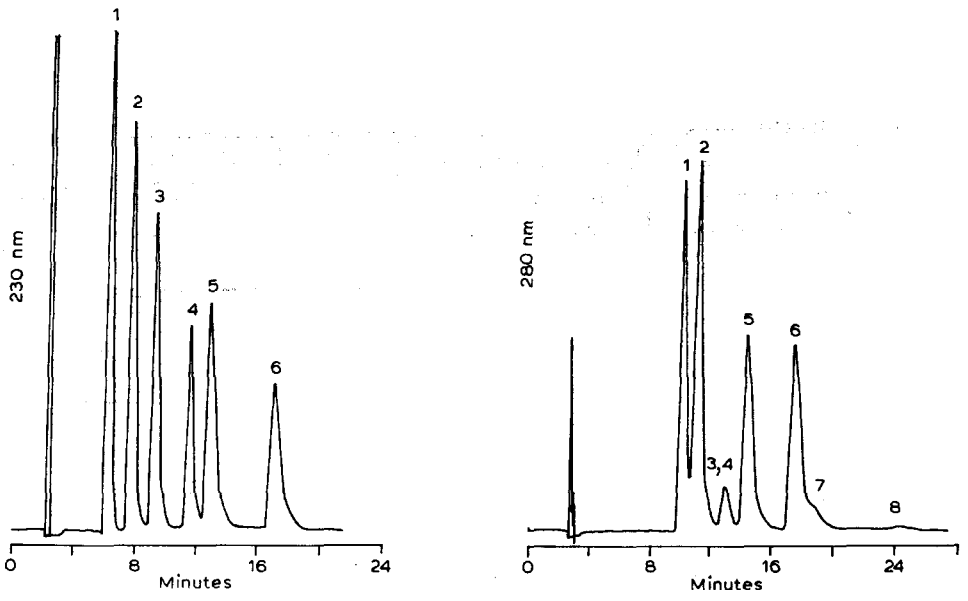


Fig. 7. Chromatographic separation of tropane alkaloids. Column, μBondapak C_{18}; mobile phase, methanol–acetic acid–TEA–water (15.0:1.5:0.5:83.0); detector sensitivity, 0.08 a.u.f.s. 1 = Homatropine; 2 = scopolamine; 3 = methscopolamine; 4 = tropic acid; 5 = atropine methyl; 6 = atropine.

Fig. 8. Chromatographic separation of Cinchona alkaloids. Column, μBondapak C_{18}; mobile phase, methanol–acetic acid–TEA–water (40.0:1.5:0.5:58.0); detector sensitivity, 0.64 a.u.f.s. 1 = Cinchonidine; 2 = cinchonine; 3 = dihydrocinchonine; 4 = dihydrocinchonidine; 5 = quinidine; 6 = quinine; 7 = dihydroquinidine; 8 = dihydroquinine.

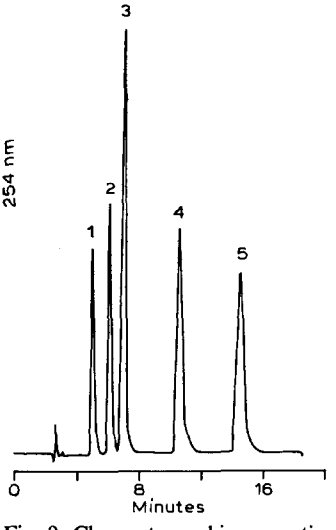


Fig. 9. Chromatographic separation of xanthines. Column, μBondapak C_{18}; mobile phase, methanol–acetic acid–TEA–water (25.0:1.5:0.5:73.0); detector sensitivity, 0.16 a.u.f.s. 1 = Theobromine; 2 = dyphylline; 3 = theophylline; 4 = caffeine; 5 = 8-chlorotheophylline.

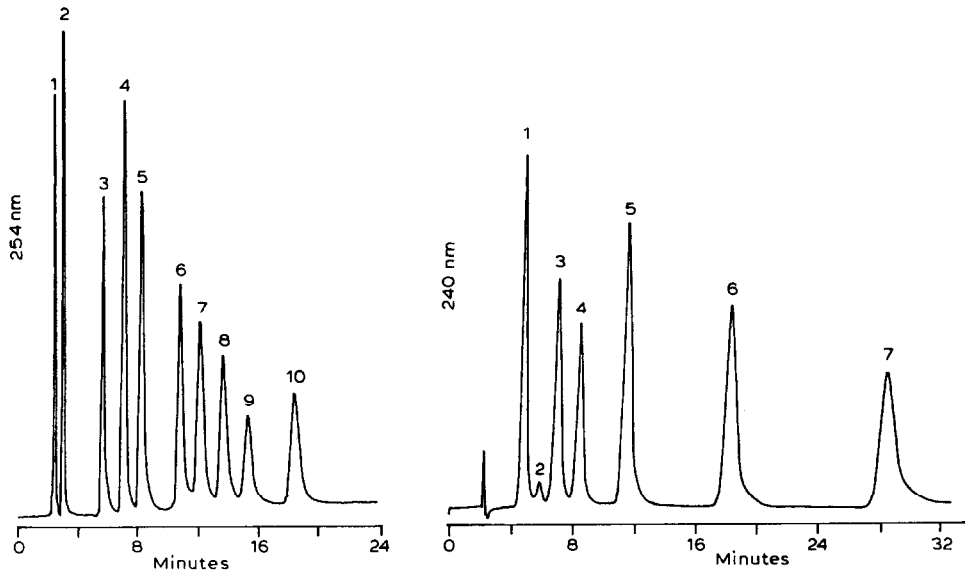


Fig. 10. Chromatographic separation of sulfonamides. Column, μBondapak C_{18}; mobile phase, methanol–acetic acid–TEA–water (20.0:1.5:0.5:78.0); detector sensitivity, 0.32 a.u.f.s. 1 = Sulfanilic acid; 2 = sulfanilamide; 3 = sulfadiazine; 4 = sulfapyridine; 5 = sulfamerazine; 6 = sulfamethizole; 7 = sulfamethazine; 8 = sulfamethoxazole; 9 = sulfisoxazole; 10 = sulfachlorpyridizine.

Fig. 11. Chromatographic separation of steroids. Column, μBondapak C_{18}; mobile phase, methanol–acetic acid–TEA–water (60.0:1.5:0.5:38.0); detector sensitivity, 0.32 a.u.f.s. 1 = Prednisone; 2 = prednisolone; 3 = prednisolone succinate; 4 = hydrocortisone acetate; 5 = norethindrone; 6 = methyltestosterone; 7 = progesterone.

From a qualitative point of view, a few structure–chromatographic behavior correlations become evident from the data in Table II. Among halogen-containing compounds, the halogenated derivatives consistently eluted after the corresponding parent compounds, as found for the pairs pheniramine and chlorpheniramine, cyclizine and chlorcyclizine, procaine and chlorprocaine, and theophylline and 8-chlortheophylline. Among phenolic compounds, hydroxylated ones eluted ahead of the parent compounds, as for hydroxyamphetamine and amphetamine. Compounds exhibiting multiple hydroxyl groups in the aromatic ring, like the catecholamines, were not retained by the ODS columns. An exception, however, was the catecholamine methyldopate, whose k' values are given in the same table.

The resolving efficiency of the proposed TEA-containing mobile phase systems for the RP-HPLC separation of groups of structurally or therapeutically related important drugs is illustrated in Figs. 2–11. Shown are chromatographic separations of sympathomimetic amines (Figs. 2 and 3), antihistamines (Fig. 4), phenothiazines (Fig. 5), local anesthetics (Fig. 6), tropane (Fig. 7) and Cinchona (Fig. 8) alkaloids, xanthines (Fig. 9), sulfonamides (Fig. 10), and steroids (Fig. 11). Whereas most of these separations entailed weakly basic drugs (Figs. 2–8), two included weakly acidic drugs (Figs. 9 and 10), and one included neutral steroids (Fig. 11). In general, excellent resolutions were obtained with a mobile phase containing 1.5% acetic acid, 0.5% TEA, and various ratios of a methanol–water mixture.

TABLE III

PAIRED-ION DRUGS ANALYZED BY RP-HPLC USING MOBILE PHASES CONTAINING TEA AS A MODIFIER

Drug	*Components*		*Main therapeutic use*
	Base	*Acid*	
Physostigmine salicylate	Physostigmine	Salicylic acid	Miotic, Belladonna alkaloids antidote
Hydroxyzine pamoate	Hydroxyzine	Pamoic acid	Tranquilizer, sedative
Pyrantel pamoate	Pyrantel	Pamoic acid	Anthelmintic
Pyrvinium pamoate	Pyrvinium	Pamoic acid	Anthelmintic
Dimenhydrinate	Diphenhydramine	8-Chlorotheophylline	Antihistaminic, antiemetic
Piprinhydrinate	Diphenylpyraline	8-Chlorotheophylline	Antihistaminic, antiemetic, sedative
Promethazine teoclate	Promethazine	8-Chlorotheophylline	Antihistaminic, antiemetic
Quinine ascorbate	Quinine	Ascorbic acid	Smoking deterrent
Quinine benzoate	Quinine	Benzoic acid	Antimalarial, analgesic
Quinine salicylate	Quinine	Salicylic acid	Antimalarial, analgesic

Based on the foregoing results, the same RP-HPLC system was applied to the resolution of a number of paired-ion drugs into their molecular components. Table III lists ten paired-ion drugs along with their chemical compositions and main therapeutic uses. Since without exceptions all the ionic components absorbed ultraviolet light above 230 nm, the proposed HPLC system will readily detect them in the

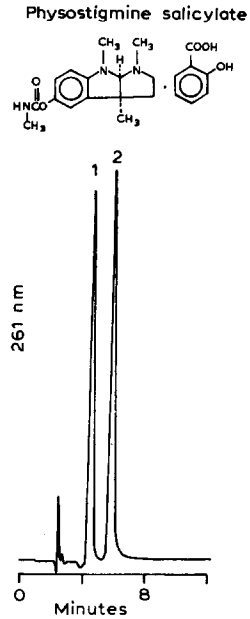


Fig. 12. Chromatographic separation of the components of physostigmine salicylate. Column, μBondapak C_{18}; mobile phase, methanol–acetic acid–TEA–water (35.0:1.5:0.5:63.0); detector sensitivity, 0.32 a.u.f.s. 1 = Physostigmine; 2 = salicylic acid.

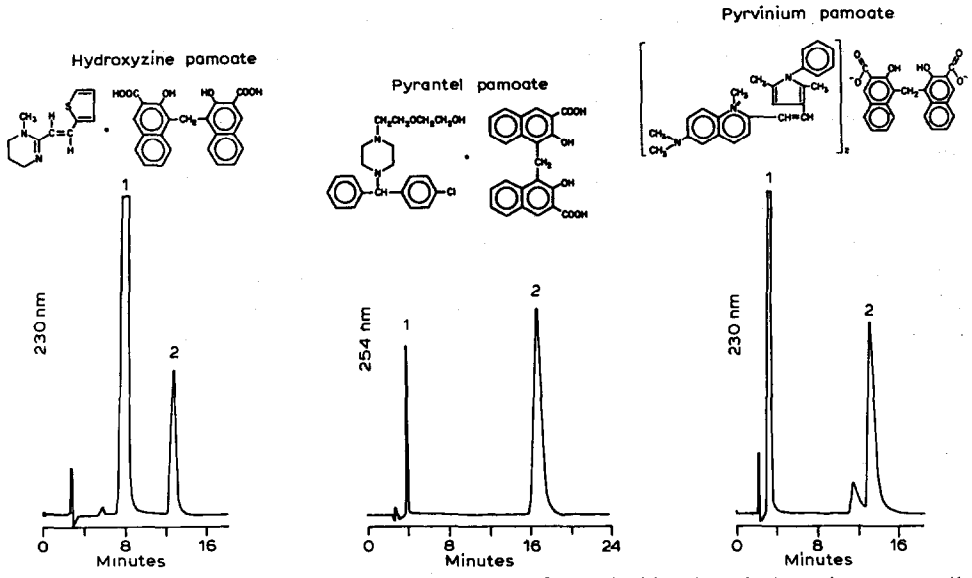


Fig. 13. Chromatographic separation of the components of the paired-ion drugs hydroxyzine pamoate (1 = pamoic acid; 2 = hydroxyzine), pyrantel pamoate (1 = pyrantel; 2 = pamoic acid), and pyrvinium pamoate (1 = pamoic acid; 2 = pyrvinium). Column, μBondapak C_{18}; mobile phases, methanol–acetic–TEA–water (58.0:1.5:0,.5:40.0) hydroxyzine pamoate; (50.0:1.5:0.5:48.0) pyrantel pamoate; and (67.0:1.5:0.5:31.0) pyrvinium pamoate.

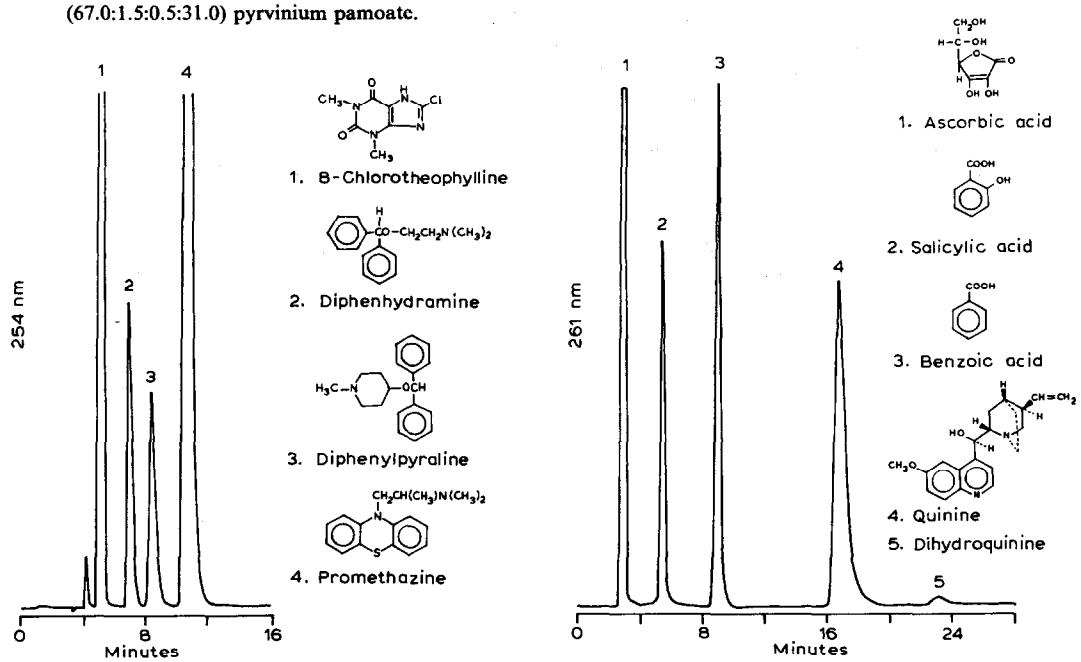


Fig. 14. Chromatographic separation of paired-ion combinations of 8-chlorotheophylline. Column, μBondapak C_{18}; mobile phase, methanol–acetic acid–TEA–water (60.0:1.5:0.5:38.0); detector sensitivity, 0.32 a.u.f.s.

Fig. 15 Chromatographic separation of paired-ion combinations of quinine. Column, μBondapak C_{18}; mobile phase, methanol–acetic acid–TEA–water (40.0:1.5:0.5:58.0); detector sensitivity, 0.64 a.u.f.s.

230–260 nm wavelength range. Chromatographic separations were achieved for the components of physostigmine salicylate (Fig. 12), pamoic acid combinations with hydroxyzine, pyrantel, and pyrvinium (Fig. 13), 8-chlorotheophylline pairs with diphenhydramine, diphenylpyraline, and promethazine (Fig. 14), and paired-ions of quinine with ascorbic, benzoic, and salicylic acids (Fig. 15).

In light of the results presented here, it is evident that the simultaneous addition of TEA and an organic acid such as acetic acid to a methanol–water mobile phase can provide both effective ion suppression of acidic drugs and ionization of basic ones, with the eventual improvement of column efficiency and peak shapes. A salient advantage to be gained from this type of mobile phase is the possibility of simultaneously analyzing a wide variety of weakly basic and acidic drugs and their paired-ion combinations using the same ODS column and ratio variations of the components of the quaternary mobile phase.

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