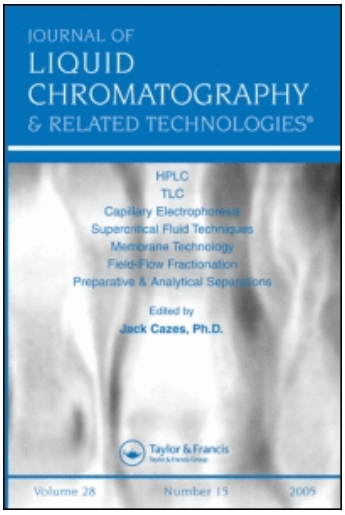


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Optimization of a Reverse Phase Ion-Pair Chromatographic Separation for Drugs of Forensic Interest Part II - Factors Effecting Selectivity

Ira S. Lurie[a]; Steven M. Demchuk[b]
[a] Drug Enforcement Administration Northeast Regional Laboratory, New York, New York [b] Drug Enforcement Administration Mid-Atlantic Regional Laboratory, Washington, D.C.

To cite this Article Lurie, Ira S. and Demchuk, Steven M.(1981) 'Optimization of a Reverse Phase Ion-Pair Chromatographic Separation for Drugs of Forensic Interest Part II - Factors Effecting Selectivity', Journal of Liquid Chromatography & Related Technologies, 4: 2, 357 — 374
To link to this Article: DOI: 10.1080/01483918108064823
URL: http://dx.doi.org/10.1080/01483918108064823

JOURNAL OF LIQUID CHROMATOGRAPHY, 4(2), 357-374 (1981)

OPTIMIZATION OF A REVERSE PHASE ION-PAIR CHROMATOGRAPHIC SEPARATION FOR DRUGS OF FORENSIC INTEREST PART II - FACTORS EFFECTING SELECTIVITY

Ira S. Lurie
Drug Enforcement Administration
Northeast Regional Laboratory
555 West 57th Street
New York, New York 10019
and
Steven M. Demchuk
Drug Enforcement Administration
Mid-Atlantic Regional Laboratory
460 New York Avenue, N.W.
Washington, D.C. 20537

ABSTRACT

Variables effecting selectivity for a reverse phase ion-pair chromatographic separation are examined for various drugs of forensic interest. Factors studied include type of stationary phase, ratio of water to methanol, size and concentration of counter-ion anc basicity of drug chromatographed. Most of the selectivity effects can be explained by Horvath's solvophobic theory.

INTRODUCTION

In a recent paper on reverse phase ion-pair chromatography for drugs of forensic interest, the resolution and time for analysis obtained were less than optimum[1]. Thus, it was desired to optimize

*Presented in part at the Eastern Analytical Symposium, October 31 thru November 2, 1979, New York City, New York.

the various separations for resolution and speed. For compounds with similar k' values, resolution can be expressed by the following relationship: $R = \frac{1}{4} (\alpha-1\alpha) (k'/k'+1) N^{\frac{1}{2}}$. This equation shows that small increases in alpha, the selectivity factor, affords an appreciable increase in resolution. Thus selectivity is an important parameter in optimizing a separation. This manuscript discusses the effect of column type, water-methanol ratio, counter ion size, counter ion concentration and basicity of a drug chromatographed on the selectivity factor for a reverse phase ion-pair chromatographic separation.

EXPERIMENTAL

The experimental conditions and procedures used are identical to those reported previously[2].

RESULTS AND DISCUSSION

Approximately 50 drugs of forensic interest including barbiturates, local anesthetics, phenethylamines, opium alkaloids, ergot alkaloids and other drugs of forensic interest were chromatographed using various stationary and mobile phases as previously indicated[2]. Tables 1-4 represent retention data for these drugs using the various mobile phases studied.

Effect of Stationary Phase on Selectivity

The role of the stationary phase on selectivity for three different columns was examined. They are Microbondapak C-18, Microbondapak Alkyl Phenyl and Microbondapak Cyanide. Wikby et. al.[3] has shown that for closely related solutes having different aliphatic character, selectivity would decrease in the order C18 > alkylphenyl > cyanide. This observa-

tion was based on the Horvath et. al.[4] model for retention in a reverse phase system from which can be derived that in a given mobile phase selectivity increases with the contact area between the non-polar portion of the solute and the stationary phase. Butabarbital and pentobarbital differ in their aliphatic character where an isobutyl group is replaced by an isopentyl group (Figure 1). In agreement with Wikby et. al.[3], the selectivity factor of butabarbital and pentobarbital decrease in the order C-18 < alkylphenyl < cyanide (Figure 2).

Phenobarbital and butabarbital do not follow this relationship (Figure 2), probably due to the presence of the phenyl ring in phenobarbital (Figure 1). Dipole and pi orbital interactions with the alkylphenyl and isopropyl cyano columns could enter into selectivity effects as well. On all three columns amobarbital and pentobarbital have a selectivity of approximately one which indicates equal retention as depicted in Figure 2. This is to be expected since both compounds have identical aliphatic character. Amobarbital and pentobarbital differ only in a pento group versus an isopento group (Figure 1). Pentobarbital and secobarbital have nearly identical selectivity on both the C-18 and alkylphenyl column (Figure 2). The selectivity of these barbiturates is slightly less on the cyano column which is illustrated in Figure 2. These compounds differ in that an ethyl group in pentobarbital is replaced by a propylene group (Figure 1). In this instance, interactions between the pi orbital in secobarbital and those in alkylphenyl and the cyano column could lead to higher selectivities than would be expected based on the Horvath et. al.[4]

TABLE 1

Capacity factors k'i and selectivity factors αji for the column Microbondapak-C18 and mobile phase methanol, water, 1% acetic acid and .005M alkylsulfonate.

Drug	40% Methanol MSA[1]		40% Methanol BSA[2]		40% Methanol HSA[3]	
	k'i	αji		αji		αji
Phenobarbital	1.85		1.87		1.97	
Butabarbital	2.83	1.53	2.81	1.50	2.98	1.51
Pentobarbital	5.76	2.03	5.61	2.00	6.11	2.05
Amobarbital	5.80	1.01	5.71	1.02	6.24	1.02
Secobarbital	8.02	1.38	7.99	1.40	8.56	1.37
Benzocaine	2.85		2.74		3.18	
Procaine	.237		.431		2.03	
Lidocaine	.819	3.46	1.27	2.95	5.68	2.80
Cocaine	1.77	2.17	2.66	2.09	9.29	1.64
Tetracaine	5.04	2.85	7.49	2.80	28.8	3.10
Acetominophen	.262		.260		.236	
Theophylline	.440	1.68	.441	1.70	.417	1.77
Caffeine	.744	1.69	.754	1.71	.726	1.74
Phenylpropanolamine	.263		.492		2.59	
Ephedrine	.328	1.25	.577	1.17	2.92	1.13
Amphetamine	.581	1.77	.935	1.62	4.13	1.41
Methamphetamine	.581	1.00	.935	1.00	4.30	1.04
Phentermine	.808	1.39	1.26	1.35	5.85	1.36
Methylphenidate	1.34	1.66	2.05	1.63	8.47	1.45
Antipyrene	1.42		1.41		1.52	
Morphine	0		.163		1.09	
Codeine	.225		.419	2.57	1.99	1.83
Acetylmorphine	.269	1.20	.500	1.19	2.31	1.16
Aminopyrene	.387	1.44	.834	1.67	2.04	
Strychine	.732	1.89	1.19	1.43	4.73	2.32
Acetylcodeine	1.05	1.43	1.58	1.33	6.19	1.31
Heroin	1.08	1.03	1.56	1.01+	6.11	1.01+
Thebaine	1.07	1.01+	1.61	1.03	6.24	1.02
Quinidine	1.02	1.05+	1.75	1.09	15.1	2.42
Quinine	1.38	1.35	2.30	1.31	18.6	1.23
Methapyrilene	2.12	1.54	3.05	1.33	14.0	1.33+
Narcotine	2.16	1.02	3.03	1.01+	11.3	1.24+
Papaverine	2.71	1.25	3.80	1.25	13.8	1.22
Mescaline	.307		.557		.270	
DMT	.501	1.63	.799	1.43	4.30	1.59
LSD	2.53		3.67		15.3	
Lampa	2.83	1.12	4.10	1.12	16.6	1.08
Iso-LSD	4.17	1.47	5.88	1.43	20.7	1.25
TCP	4.08	1.21	5.81	1.18	22.2	1.19
PCP	3.36		4.93		18.7	
Glutethimide	5.21		5.13		5.46	
Methaqualone	9.82		9.82		10.8	
Mecloqualone	12.1	1.23	12.3	1.25	13.0	1.20
Diazapam	21.6		20.9		23.0	
Phenmetrazine	.581		.820		3.64	
Phendimetrazine	.581	1.00	.874	1.07	3.87	1.06
MDA	.581		.935		4.13	
Diethylpropion	.819		1.24		4.73	

TABLE 1 (CONTINUED)

Capacity factors k'i and selectivity factors αji for the column Microbondapak-C18 and mobile phase methanol, water, 1% acetic acid and .005M alkylsulfonate.

Drug	30% Methanol MSA[1]		30% Methanol BSA[2]		30% Methanol HSA[3]	
	k'i	αji	k'i	αji	k'i	αji
Phenobarbital	3.43		3.39	1.47	3.93	
Butabarbital	5.10	1.49	4.99	2.19	5.90	
Pentobarbital	12.2	2.39	10.9	1.05	13.4	1.50
Amobarbital	12.3	1.01	11.5	1.40	13.8	2.27
Secobarbital	17.9	1.45	16.1		19.9	1.03
Benzocaine	5.73		5.76		6.84	1.44
Procaine	.845		1.21		4.89	
Lidocaine	1.90	2.25	2.56	2.11	13.5	2.76
Cocaine	6.09	3.20	6.91	2.70	31.0	2.30
Tetracaine	17.0	2.79	20.3	2.94	*	3.44
Acetominophen	.443		.424		.492	
Theophylline	.833	1.88	.860	2.03	.858	1.74
Caffeine	1.52	1.82	1.48	1.72	1.58	1.84
Phenylpropanolamine	.492		.796		4.50	
Ephedrine	.679	1.38	1.09	1.37	5.52	1.23
Amphetamine	1.01	1.49	1.47	1.35	8.28	1.50
Methamphetamine	1.25	1.24	1.72	1.17	9.44	1.14
Phentermine	1.65	1.32	2.29	1.33	13.3	1.41
Methylphenidate	3.65	2.21	4.68	2.04	26.1	1.96
Antipyrene	3.05		2.79		3.07	
Morphine	.225		.384		2.14	
Codeine	.753	3.35	1.04	2.71	4.87	2.28
Acetylmorphine	.906	1.20	1.25	1.20	6.17	1.26
Aminopyrene	1.25	1.38	1.64	1.31	4.97	1.24
Strychine	2.48	1.98	3.32	2.02	15.1	3.04
Acetylcodeine	3.40	1.37	4.45	1.34	19.9	1.32
Heroin	3.66	1.08	4.73	1.06	20.9	1.05
Thebaine	3.59	1.02+	4.70	1.01+	21.0	1.00
Quinidine	4.56	1.27	8.34	1.77	62.0	2.95
Quinine	6.41	1.41	11.5	1.38	85.1	1.38
Methapyrilene	7.38	1.15	10.6	1.08	44.9	1.90+
Narcotine	8.62	1.17	11.0	1.04	46.5	1.04
Papaverine	12.3	1.43	15.4	1.40	59.5	1.28
Mescaline	.850		1.19		6.34	
DMT	1.25	1.47	1.72	1.44	8.82	1.39
LSD	9.69		12.5		62.1	
Lampa	10.4	1.07	13.7	1.10	65.5	1.05
Iso-LSD	18.8	1.81	20.8	1.37	89.3	1.37
TCP	15.8	1.32	19.3	1.28	*	
PCP	12.0		15.1		65.6	
Glutethimide	11.3		10.8		13.4	
Methaqualone	28.1		24.8		30.2	
Mecloqualone	35.9	1.28	30.1	1.21	38.7	1.28
Diazapam	*		*		*	
Phenmetrazine	1.25		1.46		8.43	
Phendimetrazine	1.25	1.00	1.72	1.18	8.50	1.01
MDA	1.25		1.72		9.25	
Diethylpropion	2.32		2.92		12.8	

*retention greater than two hours
+successive capacity ratios are reversed

1-msa - methanesulfonate
2-bsa - butanesulfonate
3-hsa - heptanesulfonate

TABLE 2

Capacity factors k'i and selectivity factors αji, for the column Microbondapak Alkyl Phenyl and mobile phase methanol, water 1% acetic acid and .005M alkylsulfonate.

Drug	40% Methanol MSA[1]		40% Methanol BSA[2]		40% Methanol HSA[3]	
	k'i	αji	k'i	αji	k'i	αji
Phenobarbital	1.51		1.61		1.59	
Butabarbital	1.73	1.15	1.80	1.12	1.80	1.13
Pentobarbital	3.01	1.73	3.16	1.75	3.18	1.77
Amobarbital	2.96	1.02+	3.15	1.00	3.13	1.02+
Secobarbital	3.93	1.33	4.21	1.33	4.22	1.35
Benzocaine	2.16		2.37		2.27	
Procaine	.525		.628		1.63	
Lidocaine	.781	1.49	1.02	1.62	2.72	1.67
Cocaine	2.04	2.61	2.72	2.67	5.97	2.19
Tetracaine	3.68	1.80	5.09	1.87	11.4	1.91
Acetominophen	.294		.252		.282	
Theophylline	.620	2.11	.589	2.34	.575	2.04
Caffeine	1.14	1.69	1.13	1.71	1.02	1.74
Phenylpropanolamine	.310		.419		1.35	
Ephedrine	.395	1.27	.544	1.30	1.59	1.18
Amphetamine	.574	1.46	.737	1.35	2.03	1.27
Methamphetamine	.620	1.08	.848	1.15	2.27	1.12
Phentermine	.787	1.27	1.04	1.23	2.77	1.22
Methylphenidate	1.72	2.19	2.28	2.19	5.52	1.99
Antipyrene	1.46		1.51		1.46	
Morphine	.163		.242		.857	
Codeine	.456	2.80	.628	2.60	1.67	1.95
Aminopyrene	.500	1.10	.737	1.17	1.45	1.15+
Acetylmorphine	.554	1.11	.750	1.02	1.92	1.32
Strychine	1.67	3.01	2.28	3.04	5.31	2.77
Heroin	1.76	1.05	2.33	1.02	5.31	1.00
Acetylcodeine	1.80	1.02	2.38	1.02	5.45	1.02
Thebaine	1.85	1.03	2.52	1.06	5.61	1.03
Methapyrilene	2.43	1.31	3.71	1.47	7.49	1.34
Quinine	2.66	1.09	5.32	1.43	10.4	1.39
Quinidine	2.80	1.05	5.32	1.00	10.1	1.03+
Narcotine	4.08	1.46	5.42	1.02	11.4	1.13
Papaverine	4.25	1.04	5.55	1.02	10.6	1.08+
Mescaline	.388		.562		1.54	
DMT	.620	1.60	.848	1.51	2.27	1.47
LSD	3.52		4.80		10.5	
Lampa	3.93	1.12	5.26	1.12	11.5	1.10
Iso-LSD	4.72	1.20	6.15	1.16	12.1	1.05
TCP	4.09		5.32		11.8	
PCP	4.69	1.15	6.08	1.14	13.5	1.14
Glutethimide	4.25		4.45		4.34	
Methaqualone	9.67		9.92		9.22	
Mecloqualone	11.3	1.17	11.2	1.13	10.5	1.14
Diazapam	17.2		19.3		17.4	
Phenmetrazine	.620		.848		2.14	
Phendimetrazine	.620	1.00	.848	1.00	2.14	1.06
MDA	.688		.950		2.38	
Diethylpropion	.856		1.12		2.68	

TABLE 2 (CONTINUED)

Capacity factors k'i and selectivity factors αji, for the column Microbondapak Alkyl Phenyl and mobile phase methanol, water 1% acetic acid and .005M alkylsulfonate.

Drug	30% Methanol MSA[1]		30% Methanol BSA[2]		30% Methanol HSA[3]	
	k'i	αji	k'i	αji	k'i	αji
Phenobarbital	2.75		2.87		3.23	
Butabarbital	3.02	1.10	3.16	1.10	3.53	1.09
Pentobarbital	5.80	1.92	6.10	1.93	6.99	1.98
Amobarbital	5.80	1.00	6.04	1.01+	5.80	1.20+
Secobarbital	7.64	1.32	8.11	1.35	9.76	1.40
Benzocaine	4.28		4.52		5.02	
Procaine	1.00		1.38		4.19	
Lidocaine	1.26	1.26	1.72	1.25	6.00	1.43
Cocaine	4.82	5.02	6.32	3.66	19.2	3.2
Tetracaine	9.62	2.03	12.8	2.03	43.2	2.25
Acetaminophen	.392		.468		.478	
Theophylline	1.00	2.55	1.07	2.32	1.11	2.32
Caffeine	1.95	1.82	2.10	1.72	2.21	1.84
Phenylpropanolamine	.398		.603		2.25	
Ephedrine	.599	1.50	.841	1.39	2.91	1.32
Amphetamine	.794	1.33	1.10	1.31	3.81	1.31
Methamphetamine	1.00	1.26	1.38	1.25	4.69	1.23
Phentermine	1.22	1.22	1.68	1.22	5.67	1.21
Methylphenidate	3.51	2.88	4.65	2.77	15.6	2.75
Antipyrene	2.65		2.69		3.09	
Morphine	.305		.498		1.79	
Codeine	1.00	3.28	1.38	2.77	4.26	2.38
Aminopyrene	1.00	1.00	1.23	1.12+	3.26	1.31+
Acetylmorphine	1.23	1.23	1.63	1.32	5.21	1.60
Strychine	3.92	3.19	5.19	3.18	17.6	3.38
Heroin	4.58	1.17	5.96	1.15	18.8	1.07
Acetylcodeine	4.65	1.02	5.78	1.03+	19.0	1.01
Thebaine	4.96	1.07	6.43	1.11	19.8	1.04
Methapyrilene	5.31	1.07	7.89	1.23	27.1	1.37
Quinine	7.00	1.32	12.1	1.53	56.3	2.08
Quinidine	6.71	1.04+	11.9	1.02+	55.3	1.02+
Narcotine	13.1	1.95	16.7	1.40	53.5	1.03+
Papaverine	14.6	1.11	18.2	1.09	51.6	1.04+
Mescaline	.714		1.02		3.37	
DMT	1.19	1.67	1.66	1.63	5.25	1.56
LSD	10.2		15.5		45.8	
Lampa	11.2	1.10	17.2	1.11	49.7	1.09
Iso-LSD	15.5	1.38	21.4	1.24	54.9	1.10
TCP	9.91		14.6		47.8	
PCP	11.2	1.13	17.0	1.16	51.6	1.08
Glutethimide	8.92		9.02		10.0	
Methaqualone	23.8		25.2		26.2	
Mecloqualone	27.4	1.15	30.4	1.21	32.8	1.17
Diazapam	50.6		64.2		60.0	
Phenmetrazine	.962		1.38		4.47	
Phendimetrazine	1.00	1.04	1.38	1.00	4.69	1.05
MDA	1.14		1.56		5.25	
Diethylpropion	1.58		2.06		6.38	

*retention greater than two hours
+successive capacity ratios are reversed

1-msa - methanesulfonate
2-bsa - butanesulfonate
3-hsa - heptanesulfonate

TABLE 3

Capacity factors k'i and selectivity factors αji, for the column Microbondapak-CN and mobile phase methanol, water, 1% acetic acid and .005M alkylsulfonate.

Drug	40% Methanol MSA[1]		40% Methanol BSA[2]		40% Methanol HSA[3]	
	k'i	αji	k'i	αji	k'i	αji
Butabarbital	.513		.495		.618	
Phenobarbital	.704	1.37	.614	1.24	.804	1.30
Amobarbital	.744	1.10	.817	1.33	.981	1.22
Pentobarbital	.774	1.04	.817	1.00	.933	1.05+
Secobarbital	.944	1.22	.997	1.22	1.19	1.27
Benzocaine	.929		.915		1.03	
Procaine	.774		.817		.804	
Lidocaine	.913	1.18	.956	1.17	.957	1.19
Cocaine	1.64	1.80	1.76	1.84	1.54	1.61
Tetracaine	2.80	1.71	2.93	1.67	3.06	1.98
Theophylline	.134		.131		.122	
Caffeine	.177	1.32	.183	1.40	.179	1.47
Acetominophen	.199	1.12	.188	1.03	.203	1.13
Phenylpropanolamine	.493		.514		.522	
Ephedrine	.561	1.14	.584	1.14	.587	1.12
Amphetamine	.774	1.38	.817	1.39	.804	1.38
Methamphetamine	.774	1.00	.817	1.00	.804	1.00
Phentermine	.774	1.00	.817	1.00	.804	1.00
Methylphenidate	1.07	1.38	1.14	1.39	1.10	1.37
Antipyrene	.288		.280		.260	
Morphine	.410		.419		.412	
Aminopyrene	.416	1.01	.430	1.03	.412	1.00
Codeine	.561	1.35	.584	1.36	.562	1.36
Acetylmorphine	.774	1.38	.756	1.29	.758	1.35
Heroin	1.08	1.39	1.15	1.52	1.09	1.44
Acetylcodeine	1.11	1.03	1.17	1.02	1.13	1.03
Strychnine	1.29	1.16	1.32	1.13	1.33	1.18
Thebaine	1.44	1.12	1.42	1.08	1.36	1.02
Papaverine	1.50	1.04	1.58	1.11	1.41	1.04
Methapyrilene	1.88	1.25	1.85	1.17	1.87	1.33
Narcotine	1.89	1.00	1.98	1.07	1.95	1.04
Quinidine	1.95	1.03	2.13	1.08	1.83	1.07+
Quinine	2.07	1.06	2.24	1.05	1.91	1.04
Mescaline	.506		.560		.540	
DMT	1.22	2.41	1.23	2.20	1.20	2.22
LSD	2.26		2.46		2.29	
Lampa	2.49	1.10	2.70	1.10	2.48	1.08
Iso-LSD	2.69	1.08	2.92	1.08	2.55	1.03
TCP	1.52		1.63		1.53	
PCP	1.73	1.14	1.83	1.11	1.70	1.11
Glutethimide	1.01		.980		1.14	
Methaqualone	1.39		1.36		1.41	
Mecloqualone	1.76	1.27	1.74	1.28	1.86	1.32
Diazapam	2.35		2.34		2.56	
Phermetrazine	.774		.817		.804	
Phendimetrazine	.774	1.00	.817	1.00	.804	1.00
Diethylpropion	.774		.817		.804	
MDA	.851		.907		.949	

TABLE 3 (CONTINUED)

Capacity factors k'i and selectivity factors αji, for the column Microbondapak-CN and mobile phase methanol, water, 1% acetic acid and .005M alkylsulfonate.

Drug	30% Methanol MSA[1]		30% Methanol BSA[2]		30% Methanol HSA[3]	
	k'i	αji	k'i	αji	k'i	αji
Butabarbital	.859		.870		.833	
Phenobarbital	1.18	1.37	1.08	1.24	1.10	1.32
Amobarbital	1.45	1.23	1.33	1.23	1.36	1.24
Pentobarbital	1.43	1.01+	1.31	1.01+	1.31	1.04+
Secobarbital	1.82	1.27	1.65	1.26	1.69	1.29
Benzocaine	1.86		1.63		2.56	
Procaine	.859		.870		1.10	
Lidocaine	1.06	1.23	1.03	1.18	1.34	1.22
Cocaine	2.45	2.32	2.35	2.28	2.78	2.07
Tetracaine	5.36	2.19	4.91	2.09	6.34	2.28
Theophylline	.198		.174		.187	
Caffeine	.312	1.58	.283	1.58	.285	1.52
Acetominophen	.318	1.02	.286	1.01	.290	1.02
Phenylpropanolamine	.445		.487		.614	
Ephedrine	.537	1.21	.572	1.19	.738	1.20
Amphetamine	.810	1.50	.791	1.38	1.01	1.37
Methamphetamine	.859	1.06	.870	1.10	1.10	1.09
Phentermine	.859	1.00	.870	1.00	1.10	1.00
Methylphenidate	1.42	1.65	1.42	1.63	1.75	1.59
Antipyrene	.480		.451		.466	
Morphine	.407		.414		.534	
Aminopyrene	.407	1.00	.431	1.04	.542	1.01
Codeine	.636	1.56	.649	1.51	.809	1.49
Acetylmorphine	.859	1.35	.870	1.34	1.10	1.36
Heroin	1.64	1.91	1.54	1.77	1.87	1.70
Acetylcodeine	1.68	1.02	1.60	1.04	1.94	1.04
Strychnine	1.85	1.10	1.52	1.05+	2.11	1.09
Thebaine	2.28	1.23	2.15	1.41	2.58	1.22
Papaverine	2.84	1.25	2.65	1.23	3.15	1.22
Methapyrilene	2.77	1.02+	2.67	1.01	2.82	1.12+
Narcotine	3.35	1.21	3.12	1.17	3.70	1.31
Quinidine	2.85	1.18+	2.72	1.15+	3.08	1.20+
Quinine	3.18	1.12	3.04	1.12	3.44	1.12
Mescaline	.558		.565		.719	
DMT	1.50	2.69	1.50	2.65	1.82	2.53
LSD	4.27		3.93		4.69	
Lampa	4.80	1.12	4.38	1.11	5.15	1.10
Iso-LSD	5.41	1.13	4.90	1.13	5.62	1.09
TCP	2.25		2.18		2.74	
PCP	2.55	1.13	2.48	1.14	3.12	1.14
Glutethimide	2.07		1.85		1.86	
Methaqualone	3.21		2.80		2.86	
Mecloqualone	4.45	1.39	3.98	1.42	3.92	1.37
Diazapam	5.69		4.92		5.05	
Phermetrazine	.859		.870		1.10	
Phendimetrazine	.859	1.00	.870	1.00	1.10	1.00
Diethylpropion	.859		.870		1.10	
MDA	1.06		1.04		1.29	

*retention greater than two hours
+successive capacity factors are reversed

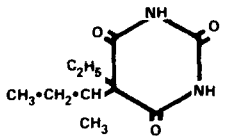
1-msa - methanesulfonate
2-bsa - butanesulfonate
3-hsa - heptanesulfonate

TABLE 4

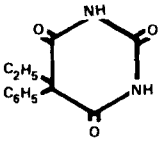
Capacity factors k'i and selectivity factors αji for mobile phase 20% methanol, 79% water, 1% acetic acid and 0.005M methanesulfonate acid using various columns.

Drug	Microbondapak C-18		Microbondapak Alkyl Phenyl		Microbondapak CN	
	k'i	αji	k'i	αji	k'i	αji
Phenobarbital	7.21	1.51	6.60		1.61	
Butabarbital	10.9	2.47	7.01	1.06	1.20	1.34+
Pentobarbital	26.9	1.05	15.2	2.17	2.04	1.70
Amobarbital	28.2	1.49	14.8	1.03+	2.11	1.03
Secobarbital	42.0		22.8	1.54	2.66	1.26
Benzocaine	12.7		10.1		2.56	
Procaine	2.17		2.65		1.09	
Lidocaine	4.10	1.90	2.73	1.03	1.29	1.18
Cocaine	17.1	4.17	14.7	5.39	3.43	2.67
Tetracaine	52.1	3.05	32.5	2.22	8.17	2.38
Acetominophen	.829		.863		.395	
Theophylline	1.76	2.12	2.02	2.34	.256	1.54+
Caffeine	3.58	2.03	4.34	2.03	.386	1.51
Phenylpropanolamine	.840		.743		.471	
Ephedrine	1.23	1.46	1.10	1.48	.587	1.25
Amphetamine	1.87	1.53	1.50	1.36	.860	1.46
Methamphetamine	2.47	1.32	2.02	1.36	.980	1.14
Phentermine	3.26	1.32	2.46	1.22	.980	1.00
Methylphenidate	9.36	2.92	8.95	3.64	1.82	1.86
Antipyrene	7.73		6.52		.726	
Morphine	.536		.849		.464	
Codeine	2.04	3.81	2.79	3.29	.778	1.68
Acetylmorphine	2.59	1.27	3.58	1.28	1.14	1.46
Aminopyrene	2.94	1.14	2.38	1.50+	.469	2.43+
Strychine	7.43	2.53	12.2	5.13	2.40	5.12
Acetylcodeine	12.3	1.66	16.4	1.34	2.40	1.00+
Heroin	13.9	1.13+	16.7	1.02	2.31	1.04+
Thebaine	13.0	1.07+	17.2	1.03	3.45	1.49
Quinidine	18.0	1.38	30.9	1.80	3.20	1.08+
Quinine	24.6	1.37	29.9	1.03+	3.64	1.14
Methapyrilene	21.7	1.13+	17.6	1.70+	3.57	1.02+
Narcotine	41.5	1.91	52.7	2.99	5.17	1.45
Papaverine	64.0	1.54	65.6	1.24	5.03	1.03+
Mescaline	2.47		1.77		.681	
DMT	2.77	1.12	2.67	1.51	1.85	2.72
LSD	37.1		37.2		7.10	
Lampa	40.0	1.08	42.6	1.15	8.01	1.13
Iso-LSD	74.1	1.85	58.0	1.36	9.33	1.13
TCP	41.5		31.1		3.27	
PCP	44.5	1.07	38.8	1.25	3.81	1.16
Glutethimide	29.6		24.2		3.15	
Methaqualone	*		*		5.51	
Mecloqualone	*		*	*	8.50	1.54
Diazapam	*		*		9.56	
Phermetrazine	2.02		1.92		.980	
Phendimetrazine	2.47	1.22	2.26	1.18	.980	1.00
MDA	2.38		2.30		1.18	
Diethylpropion	5.80		3.68		1.04	

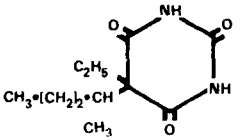
BUTABARBITAL



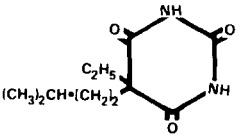
PHENOBARBITAL



PENTOBARBITAL



AMOBARBITAL



SECOBARBITAL

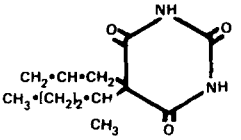


Figure 1 - Structures of barbiturates.

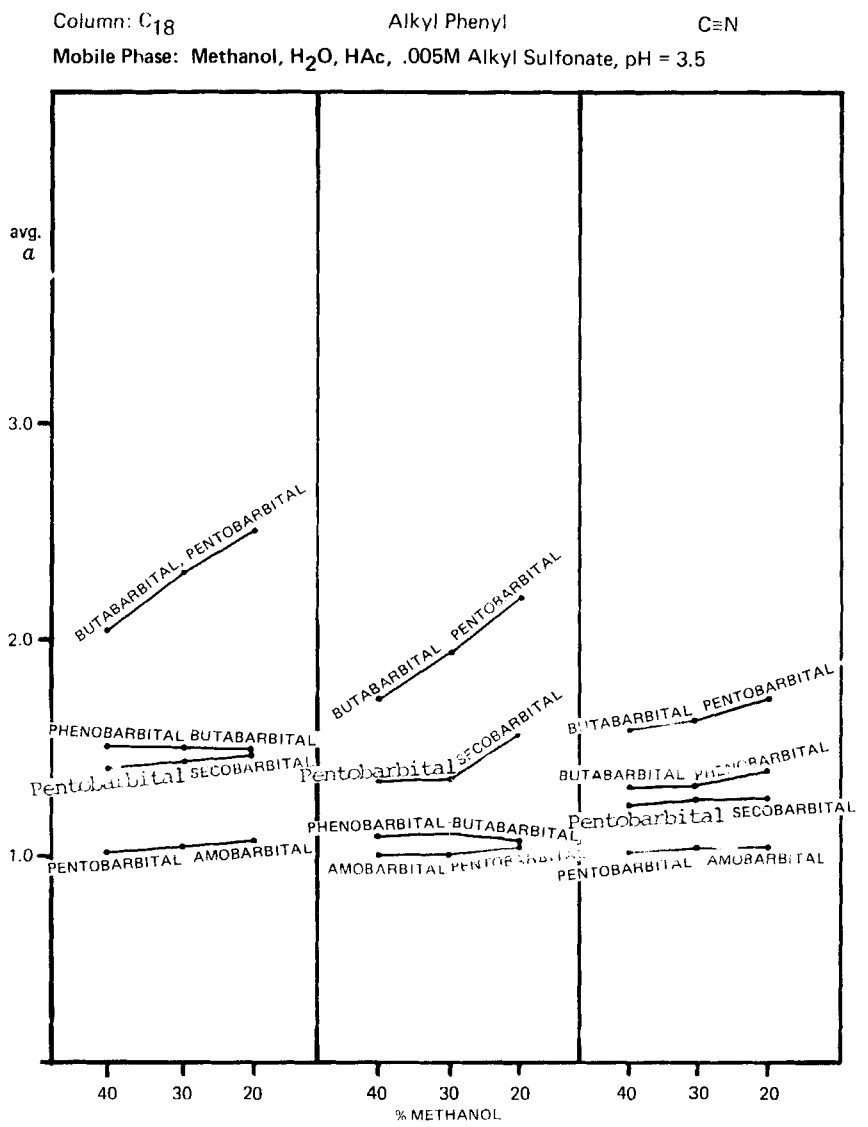


Figure 2 - Plot of average selectivity factors of barbiturates versus percent methanol for the Microbondapak C-18, Microbondapak Alkyl Phenyl and Microbondapak CN columns.

The decrease in selectivity on the cyanide column versus the alkylphenyl column could be explained by the small pi bonding character of the isopropyl cyano column.

As was shown earlier, based on the Horvath et. al.[4] model for reverse phase chromatography, for a given mobile phase selectivity increases with the contact area between non-polar substituents on the solute and the hydrocarbon ligands on stationary phase[3]. Horvath's theory for retention in reverse phase ion pair chromatography via an ion pair in mobile phase mechanism is also based on this model. Thus for solutes having different aliphatic character in a given mobile phase, selectivity would be expected to decrease in the order of C-18 < alkylphenyl < isopropyl cyano. For most bases studied the above relationship was not observed. No explanation could be offered for the above effect at this time.

Effect of Water-Methanol Ratio on Selectivity

It can be deduced from the Horvath et. al.[4] theory of retention in reverse phase chromatography that the selectivity of two solutes differing in aliphatic character on a given stationary phase will increase primarily with the surface tension of the eluent[3]. Therefore, increasing the ratio of water to methanol in the mobile phase should effect an increase in selectivity between two solutes differing in non-polar character. A similar effect is predicted based on the Karger et. al.[5] hydrophobic theory. Butabarbital and pentobarbital which differ in aliphatic character show an increase in selectivity with increased water in the mobile phase for all three

columns (Figure 2). The selectivity factor for amobarbital and pentobarbital which have identical aliphatic character is invariant to the water-methanol ratio (Figure 2). The phenobarbital:butabarbital:secobarbital pairs have selectivities that are also independent of the water-methanol ratio. The above effects are shown in Figure 2. No explanation is available for these effects. Tjaden et. al.[6] demonstrated that when using a water-methanol system on a silanized silica column, selectivity increases with the water-methanol ratio for barbiturates. The structures of these compounds differed only by non-polar substituents.

Horvath et. al.'s[5] theory for reverse phase ion pair chromatography would predict that the selectivity of two closely related solutes differing in aliphatic character would increase with the surface tension of the eluent. Earlier it was shown that the equilibrium constant for the binding of the neutral ion pair is proportional to the contact area between the ion pair and the stationary phase. This equilibrium constant would be proportional to the capacity factor. Wikby et. al.[3] showed that based on Horvath's theory of retention for reversed phase chromatography the following relationship: $d\log k'/d\,\Delta A = \alpha/940.7$ where gama is the surface tension and ΔA is the contact area of solute with the stationary phase. A similar relationship could be derived from the equilibrium constant for the adsorption of the ion pair on to the stationary phase: $\ln K = a - b + c\,\Delta A$, where the magnitude of this term depends primarily on the surface tension of the mobile phase and the contact area. For a given counter ion, an increase in selectivity

with increased water concentration was observed on all three columns for phenylpropanolamine and ephedrine, amphetamine and methamphetamine, (Figure 3) morphine and codeine, morphine and acetylmorphine, and codeine and acetylcodeine. The small increase in selectivity with the water-methanol ratio for heroin and acetylcodeine only occured on the C-18 column (Figure 3). In this instance the increased hydrocarbon character in heroin which has one more carbon atom than acetylcodeine is offset by the affinity of the polar carbonyl function in heroin for the polar mobile phase, see Figure 4. Positional isomers, methamphetamine and phentermine, LSD and LAMPA, and the stereoisomers quinine and quinidine, having identical aliphatic character exhibit no changes in selectivity with water-methanol ratio (Figure 3). LSD and Iso-LSD, diastereoisomers with identical aliphatic character, have selectivity values which increase with greater water-methanol ratios (Figure 3). The structures of these compounds are given in Figure 4.

Effect of Counter Ion Size on Selectivity

The retention of strong basic drugs on the C-18 and alkylphenyl column significantly increases with the size of the alkylsulfonate counter ion. The counter ion size has little effect on the k' values of weakly basic and acidic drugs. Therefore, for drug combinations consisting of strong and weak bases and acids, large changes in selectivity could result by changing the size of the counter ion. For certain base pairs on C-18 and alkylphenyl columns at a constant water-methanol ratio, the selectivity increases with decreasing size of the counter ion. Examples are codeine and acetylcodeine, phenyl-

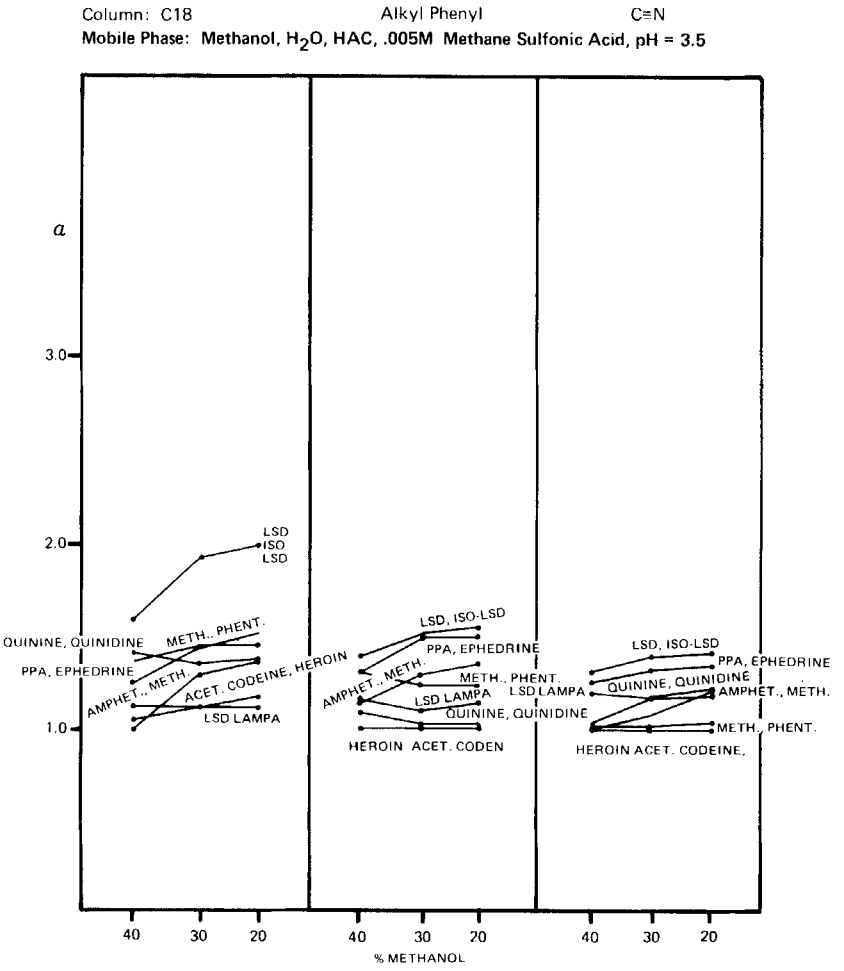
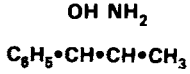
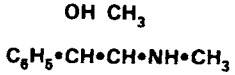


Figure 3 - Plot of selectivity factors with methanesulfonate counter ion versus percent methanol for the Microbondapak C-18, Microbondapak Alkyl Phenyl and Microbondapak CN columns.

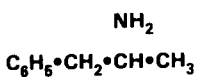
PHENYLPROPANOLAMINE



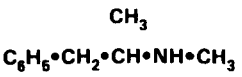
EPHEDRINE



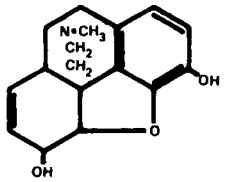
AMPHETAMINE



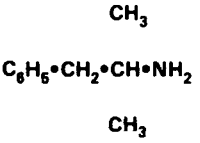
METHAMPHETAMINE



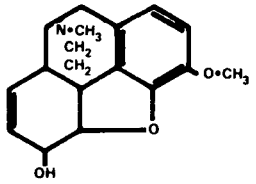
MORPHINE



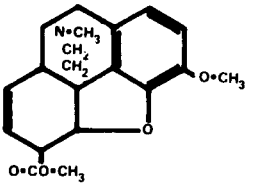
PHENTERMINE



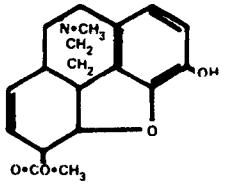
CODEINE



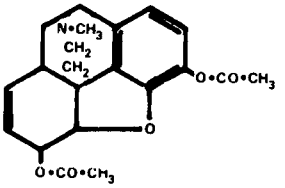
ACETYLCODEINE



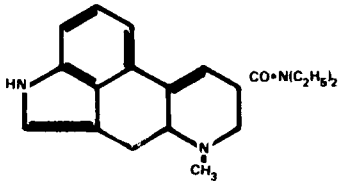
MONOACETYL MORPHINE



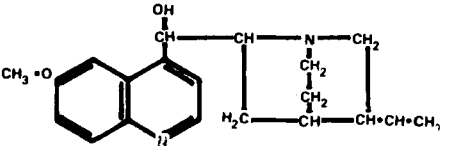
HEROIN



LSD, ISO-LSD



QUININE, QUINIDINE



LAMPA

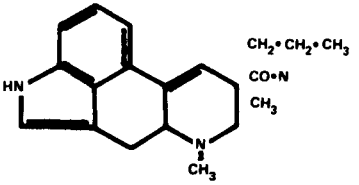


Figure 4 - Structures of basic drugs.

propanolamine and ephedrine, and lidocaine and cocaine. At the present time this effect can not be explained.

Effect of Counter Ion Concentration on Selectivity

For the ten basic solutes studied[2] only the selectivity of quinidine with other bases varied with counter ion concentration on both the C-18 and Alkyl Phenyl columns. The behavior of quinidine could be explained, as stated earlier[2], based on the two ionizable sites this compound has to interact with counter ion.

ACKNOWLEDGEMENTS

The authors are grateful to Mr. Jeffrey Weber for his review of the manuscript and to Dr. Mary Chaklos for her helpful discussions.

REFERENCES

1. Lurie, I., J. Ass. Off. Anal. Chem., 60, 1035-1040, 1977.
2. Lurie, I. S. and Demchuk, S. M., J. Liq. Chromatogr.
3. Wikby, A., Thalen, A. and Oresten, G., J. Chromatogr., 157, 65-74, 1978.
4. Horvath, C., Melander, W., and Molnar, I., J. Chromatogr., 125, 129-156, 1976.
5. Karger, B. L., Gant, J. R., Harthopf, A. and Weiner, P. H., J. Chromatogr., 128, 65-78, 1976.
6. Tjaden, J. C., Kraak, J. C., and Huber, J. F. K., J. Chromatogr. Biomed. Appl., 143, 183, 1977.