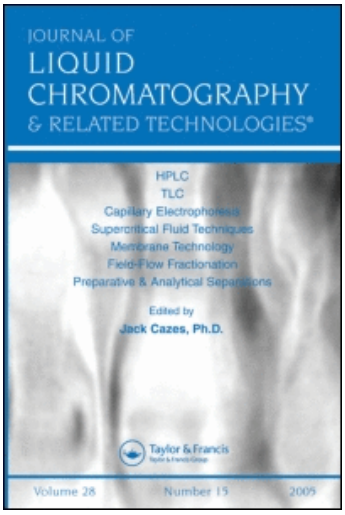

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A STUDY OF THE RETENTION BEHAVIOR OF SOME BASIC DRUG SUBSTANCES BY ION-PAIR HPLC

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ABSTRACT

Ion-pair HPLC of some basic drugs using alkane sulfonic acid as counter ion is described. Various ways to manipulate the retention of the compounds are discussed.

INTRODUCTION

The technique of high-performance liquid chromatography (HPLC) has become very useful for the analysis of pharmaceutical preparations and, especially, the use of reverse-phase mode has gained immense popularity. It is estimated that at the present time about 80 percent of all liquid chromatographic analyses are carried out by reverse-phase chromatography (1). Although reverse-phase HPLC has been proven to be very satisfactory for non-ionic or weakly acidic molecules, the analysis of ionic or strongly basic compounds has not been that successful. Long solute retention and unsatisfactory tailing have been the two major drawbacks.

One useful approach to analyze the ionic molecules is by ion-pair chromatography popularized by Schill et al in Sweden (2-3) and by Higuchi et al (4-5) in the U.S.A. Ion-pair HPLC has recently been reviewed by Tomlinson et al (6). Briefly, when an ionic species in aqueous medium comes in contact with an ionic species of opposite charge (counter ion), it forms an ion-pair (coulombic association). The ion-pair has a low polarity and transfers itself to organic phase.

The solute retention mechanism in reverse-phase ion-pair HPLC is governed by such factors as the nature of the column i.e., the type of organic moiety bonded to silica matrix, the hydroorganic composition of the mobile phase, the nature of the organic solvent and the nature of the counter ion. The contributions of the above parameters on the retention of some basic drug substances are discussed in this paper.

EXPERIMENTAL

A modular chromatographic unit consisting of a solvent delivery system, model #M-6000A from Waters Associates, a variable wavelength U.V. - VIS detector, model #SF-770 from Schoeffel Instruments, and a strip-chart recorder, model #56 from Perkin-Elmer was used. The columns were obtained from the following commercial sources: μ-Bondapak C_{18}, μ-Bondapak Phenyl, μ-Bondapak CN and μ-Bondagel from Waters Associates, Chromegabond C_8 and Chromegabond C_6H_{11} from E.S. Industries.

The physical characteristics of the silica matrices of both μ-Bondapak and Chromegabond columns are similar (particle size:

10 μ, irregular type; pore diameters: ≃ 100 Å; surface area: 300-350 meter^2/g). However, bonding and packing procedures of these columns are probably different. The obvious difference between these columns is in their organic contents. The Chromegabond columns have about twice the organic contents compared to μ-Bondapak columns. The μ-Bondagel column was made by bonding an ether group to silica particles (Si-$(RO)_xCH_3$). Silica gel used in μ-Bondagel is similar to μ-Bondapak except the fact that the pore diameters in μ-Bondagel are about 1000Å (7). All sulfonic acids (or their sodium salts) were purchased from Eastman Kodak and were used as received. All solvents were of chromatographic quality. All drugs were of U.S.P./N.F. quality. The drugs were dissolved in the appropriate mobile phase and were injected in quantities to obtain a 50-60% of the full scale response in the strip-chart recorder (10 mv).

The retention volume (V_R) was calculated by multiplying retention time with the flow rate. The capacity factor, k^1, was calculated as $(V_R-V_0)/V_0$ where V_R is the apparent retention volume of the solute and V_0 is the dead volume of the column. The tailing factor (T_L) was calculated by drawing a perpendicular from the peak maxiumum to the baseline and dividing the first half of the baseline (x) with the second half (y) and multiplying the ratio with 100 i.e., $T_L = (x/y) \times 100$.

THEORETICAL

Notable contributions on the theoretical priniciple of ion-pair chromatography have been made by Higuchi and Michaelis (4),

Schill et al (2), Horvath et al (8-9), Kraak and Huber (10) and Karger et al (11-12). A brief outline of the ion-pairing principle is given below.

If a cation, Q^+, in aqueous phase comes in contact with an ion of opposite charge (counter ion, hetaeron), X^-, and extracted in an organic phase, the equilibrium can be expressed as

$$Q^+_A + X^-_A \rightleftharpoons QX_O \qquad \text{(Equation 1)}$$

where subscripts A and O represent aqueous and organic phases, respectively.

The extraction coefficient, E_{QX}, is measured as:

$$E_{QX} = \frac{(QX)_O}{(Q^+)_A (X^-)_A} \qquad \text{(Equation 2)}$$

The distribution ratio of Q^+ is expressed as:

$$D_{Q^+} = \frac{(QX)_O}{(Q^+)_A} \qquad \text{(Equation 3)}$$

Substituting the value of $(QX)_O/Q^+_A$ from Equation 2, the Equation 3 can be rearranged as:

$$D_{Q^+} = E_{QX} (X^-)_A \qquad \text{(Equation 4)}$$

The capacity factor (k^1) of Q^+ is expressed as

$$k^1_{Q^+} = D_{Q^+} \cdot V_s \cdot V_m^{-1} \qquad \text{(Equation 5)}$$

where V_s and V_m are the volumes of stationary and mobile phases in the column, respectively.

Substituting the value of the distribution coefficient of Q^+ from Equation 4 to Equation 5, the capacity factor of Q^+ can

be expressed as:

$$k^1_{Q^+} = E_{QX} \cdot (X^-)_A \cdot V_s \cdot V_m^{-1} \qquad \text{(Equation 6)}$$

Equation 6 shows that $k^1_{Q^+}$ will depend on the nature and concentration of the counter ion, X^- and volume ratio of stationary and mobile phases. $k^1_{Q^+}$ is also largely dependent on extraction coefficient, E_{QX}. The value of E_{QX} will change when different stationary phases (different organic moieties in this case) are used or if the composition of the mobile phase is altered.

RESULTS AND DISCUSSION

1. The Type of Organic Bonded to Silica Matrix

The retention properties of seventeen compounds using six different types of bonded columns are shown in Table I. V_R, k^1 and T_L represent the apparent retention volume, capacity factor and tailing factor (peak symmetry), respectively. The data show that the retentions in general are higher in alkyl bonded columns. This can be explained using the general principle of ion-pair extraction. The affinity of the ion-pair is higher towards n-alkyl chain stationary phases and, thus, the extraction coefficient (E_{QX}) is higher resulting in an increase in retention. Horvath, et al (8-9) have recently presented solvophobic theory to explain the retention properties of ion-pair molecules. According to solvophobic theory the solute binds itself to the stationary phase surface and

TABLE I - RETENTION OF VARIOUS SOLUTES IN DIFFERENT COLUMNS

COMPOUND	-OCTADECYL			-OCTYL			-PHENYL			-CYCLOHEXYL			-CYANO			-ETHER		
	V_R	k^1	T_L	V_R	k^1	T_L	V_R	k^1	T_L	V_R	k^1	T_L	V_R	k^1	T_L	V_R	k^1	T_L
PHENIRAMINE	12.6	4.2	58	-	-	-	9.4	2.6	56	9.2	1.15	43	5.0	0.6	46	3.9	0.5	28
PHENYLEPHRINE	4.8	1.0	69	6.9	1.4	33	4.3	0.7	62	5.0	0.3	50	3.8	0.3	44	3.0	0.2	40
ANTAZOLINE	23.8	8.8	66	55.6	17.8	16	16.8	5.5	71	8.6	1.3	53	6.8	1.2	57	3.2	0.2	51
CODEINE	5.5	1.3	50	11.2	1.8	24	5.6	1.2	75	6.5	0.8	90	4.2	0.4	50	3.3	0.3	47
EPINEPHRINE	4.1	0.7	43	5.9	1.0	40	3.8	0.5	44	4.8	0.3	60	3.6	0.2	50	3.0	0.2	43
PAPAVERINE	12.6	4.2	72	24.0	7.1	11	13.8	4.3	80	9.4	1.5	77	4.7	0.5	60	3.3	0.3	57
QUINIDINE	19.9	5.0	72	32.2	10.3	15	14.7	4.7	67	9.0	1.4	41	5.3	0.8	43	3.6	0.4	33
N-ACETYL PROCAINAMIDE	7.8	1.4	53	8.6	1.9	26	5.2	1.0	67	7.0	0.8	67	4.1	0.3	56	3.2	0.2	38
PROCAINAMIDE	7.0	1.1	50	7.7	0.9	24	4.8	0.9	50	6.5	0.8	69	4.1	0.3	70	3.3	0.3	38
CAFFEINE	4.1	0.7	49	5.2	0.8	39	5.2	1.0	46	5.2	0.4	60	3.5	0.2	50	2.5	0.0	33
XYLOCAINE	9.1	2.7	70	17.4	4.9	16	6.8	1.6	43	6.4	0.7	64	4.5	0.5	40	3.1	0.2	25
SCOPOLAMINE	6.1	1.5	55	9.7	2.3	24	5.8	1.2	50	5.9	0.6	50	4.4	0.4	46	3.2	0.2	38
ATROPINE	7.2	2.0	64	12.1	3.1	17	6.6	1.6	33	6.4	0.7	50	4.4	0.5	29	3.2	0.2	36
NAPHAZOLINE	10.8	3.4	77	28.3	6.9	21	8.9	2.5	33	7.2	1.0	56	4.9	0.6	29	3.2	0.2	47
EPHEDRINE	6.6	1.6	67	11.6	2.9	20	5.5	1.1	43	5.4	0.5	67	4.1	0.3	36	3.0	0.2	25
CHLORPHENIRAMINE	23.1	8.5	59	-	-	-	14.0	4.4	52	10.6	1.9	43	6.1	1.0	48	3.7	0.5	21
PHENYLPROPA-NOLAMINE	6.3	1.6	67	11.0	2.8	19	5.2	1.0	42	5.1	0.4	46	4.2	0.4	36	2.8	0.1	25

SOLVENT: 50:49:1 (V/V); Water:Methanol:AcOH containing 0.005 M Heptane Sulphonic Acid Sodium Salt, pH ≈ 4.0
Flow = 1.5 ml/min.

the retention of the solute depends on the contact area between solute and the stationary phase. A larger contact area will increase the retention as seen in the case n-alkyl chain stationary phases in Table I.

One of the aims of this study was to explore if any correlation of retention exists between different type columns. Although it is realized that some difference exists between μ-Bondapak and Chromegbond series of columns, a comparison using regression analysis of $\log_e k^1$ of compounds tested on these columns give a correlation coefficient of 0.9724 which is significant. The higher retentions in -octyl column were mainly due to higher organic content of this column (V_s term of Equation 6); the contributions of other column parameters were not significant. The relationship of -octadecyl bonded column with other columns is given in Table II.

TABLE II

Relationship of Solute Retention of C_{18} Bonded Column to Other Columns

COLUMNS	r^2	SLOPE	INTERCEPT
$-C_{18}$ Vs $-C_8$	0.9724	1.2400	0.31
$-C_{18}$ Vs $-C_6H_5$	0.9530	0.8741	-0.18
$-C_{18}$ Vs C_6H_{11}	0.8693	0.6317	-0.76
$-C_{18}$ Vs -CN	0.7915	0.5439	-1.24

Results shown in Table II indicate that if the retention of a particular solute in a particular type of column is known, then the retention of that solute on other columns can be somewhat predictable.

The retention properties in -phenyl and -cyclohexyl columns are worth noting. In general, the retentions are much higher in -phenyl column compared to -cyclohexyl column. This probably could be attributed to the interaction of electrical charges between -phenyl group and the solute molecules. Both -cyano and -ether bonded columns did not seem to interact with the solute molecules. Thus, the k^1 of all the solutes were very low in these columns.

The retention data provided in Table I could be very useful in selecting the proper column for a desired dosage form analysis. For example, the separation of ephedrine and chlorpheniramine in a cough or cold formulation would take too long a time in an -octadecyl bonded column, whereas the same separation could be achieved in a -phenyl column in a much shorter time. Also, separation of pheniramine and pyrilamine could not be achieved in an -octadecyl bonded column, but the separation would be possible in a -phenyl column.

The following studies were carried out using an octadecyl siloxane bonded column (u-Bondapak C_{18}).

2. Hydroorganic Composition of the Mobile Phase

The retention volumes of all compounds increased with the increased amount of water in the mobile phase. A linear

relationship for each drug was obtained by plotting percent water in the mobile phase versus V_R^{-1} of the drugs. A change in water-methanol composition of the mobile phase essentially changes the extraction coefficient term, E_{QX} of Equation 6, in favor of the stationary phase resulting in an increase in the retention. This can also be explained on the basis of solvophobic theory. An increase of water will increase the surface tension of the mobile phase. The relationship between solute retention and the surface tension has been expressed by Horvath (8) as $\log_e k^1 = A + B\gamma$ where A and B are contributions due to other parameters and γ is the surface tension of the mobile phase. Thus, an increase of the surface tension of the mobile phase will result in an increase in retention of the solutes.

3. Nature of the Organic Modifier

A replacement of one organic modifier with another can lead to changes in both retention volume and selectivity of the ion-pair. Three solvents, viz., methanol, acetonitrile and tetrahydrofuran (THF) were studied for this purpose. The ratio of the organic modifier to water was adjusted in such a way that the overall polarities of the mobile phases were approximately the same. The effect of various organic modifiers on the retention of the drugs is shown in Table III.

Composition of the mobile phase: (a) Methanol: H_2O:AcOH, 49:50:1 (V/V); (b) Acetonitrile: H_2O:AcOH,42:57:1 (V/V); THF: H_2O:

TABLE III

Effect of Organic Modifiers on Retention

Drugs	Retention Volume of Drugs (ml)		
	Methanol	Acetonitrile	Tetrahydrofuran
	(a)	(b)	(c)
Phenylephrine	4.8	3.3	5.5
Procainamide	7.0	3.5	4.8
Caffeine	4.1	3.3	3.2
Naphazoline	10.8	5.6	10.2
Quinidine	19.9	5.2	13.5

AcOH, 29:75:1 (V/V). All solvents contained heptane sulfonic acid to yield 0.005 M solution.

4. The Nature of the Counter Ion

The effect on the retention of drugs on changing the alkyl chain length of the counter ion (sulfonic acid) in the mobile phase has been discussed in detail by Koziol et al (13). Briefly, the plot of the carbon number of the alkyl chain versus $\log_e$ retention volume showed a linear relationship for some drugs. For others, however, the plot was dual sloped. An increase in the carbon chain length of the counter ion changes the E_{QX} term of Equation 6 in favor of the stationary phase causing an increase in retention. Also, with the increasing

carbon chain length of the counter ion, the contact area of the ion-pair complex with the stationary phase will increase resulting in an overall increase in retention.

CONCLUSION

Ion-pair chromatography can be successfully applied in dosage form analyses. By appropriate adjustment of experimental parameters, many complex separations can be achieved with relative ease.

In general, the retention of the drugs can be adjusted in the following way:

(i) The alkyl bonded columns, e.g. C_{18}, C_8, etc. produce longer retention compared to phenyl or cyclohexyl bonded columns. The higher organic content of the column will produce longer retention.

(ii) An increase in the water ratio of the mobile phase will increase retention.

(iii) An increase in the carbon chain length of the counter ion will increase retention.

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REFERENCES

(1) Horvath, C. and Melander, W., Amer. Lab., 10, 17, 1977.

(2) Schill, G., Modin, R. and Presson, B-A., Acta. Pharm. Suecica, 2, 119, 1965.

(3) Schill, G., Acta Pharm. Suecica, 2, 13, 1965.

(4) Higuchi, T. and Michaelis, A., Anal. Chem., 40, 1925, 1968.

(5) Higuchi, T., Michaelis, A., and Rytting, J.H., Anal. Chem., 43, 287, 1971.

(6) Tomlinson, E., Jefferies, T.M., and Riley, C.M., Chromatogr. Rev., 159, 315, 1978.

(7) Waters Associates, Bulletin #D 13, July 1977.

(8) Horvath, C., Melander, W., and Molnar, I., J. Chromatogr., 125, 129, 1976.

(9) Horvath, C., Melander, W., Molnar, I. and Molnar, P., Anal. Chem., 49, 2295, 1977.

(10) Kraak, J.C. and Huber, J.F.K., J. Chromatogr., 102, 333, 1974.

(11) Persson, B-A, Karger, B.L., J. Chromatogr., 12, 521, 1974.

(12) Karger, B.L, Su, S.C., Marchese, S., and Persson, B-A., J. Chromatogr., 12, 678, 1974.

(13) Koziol, T., Jacob, J.T., and Achari, R.G., J. Pharm. Sci. to be published in Spetember 1979.