Journal of Chromatography, 475 (1989) 321–329
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 21 560

REVERSED-PHASE THIN-LAYER AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF AROMATIC ALKOXY AND HYDROXY ACIDS

MARGIT BIDLÓ-IGLÓY
Institute for Drug Research, P.O. Box 82, H-1325 Budapest (Hungary)
(First received November 29th, 1988; revised manuscript received April 4th, 1989)

SUMMARY

The R_M and log k' values of aromatic alkoxy and hydroxy acids showed a linear correlation with the methanol and acetonitrile concentration of the mobile phases in reversed-phase thin-layer and high-performance liquid chromatography. Values of R_M and log k' extrapolated to 0% organic solvent content, however, were different in mobile phases containing methanol and acetonitrile, respectively. Correlations of log k' values with the corresponding R_M values were linear in both solvent systems. The elution order was examined with respect to the structures of the acids. Linear correlations of the chromatographic parameters with the carbon atom number of the substituent alkyl group in the molecules was found in the case of homologous compounds. However, the chromatographic parameters of di- and trimethoxy acids could not be predicted on the basis of the corresponding parameters of the monomethoxy acids. Experimental data were used to optimize the separation of the acids.

INTRODUCTION

The detection, identification and determination of organic acids, including alkoxy acids, by chromatography is an important field of organic analysis. Several methods are given in the literature for the separation of such compounds by reversed-phase high-performance liquid chromatography (HPLC) and for studying their structure–retention correlations[1–9].

Several authors investigated the log k'–R_M correlation of different compounds in reversed-phase chromatography[10–13]. Recently, Pietrogrande *et al.*[14] compared the retention data of several compounds of pharmaceutical interest using reversed-phase HPLC and thin-layer chromatography (TLC). They pointed out the difference in retention when using methanol or acetonitrile as a mobile phase component. With the aid of R_M–log k' correlations, the chromatographic parameters measured by different chromatographic techniques can be compared. On the other hand, HPLC separation can be modelled by rapid, simple TLC tests.

In previous work[15] HPTLC of aromatic alkoxy acids on reversed phases and silica gel was studied. The object of the present paper is to compare reversed-phase

0021-9673/89/$03.50

TABLE I

EXPERIMENTALLY MEASURED R_M AND $\log k'$ VALUES AT DIFFERENT PERCENTAGES OF METHANOL IN MOBILE PHASES OF PHOSPHATE BUFFER 0.1 *M* (pH 2)

Acid	*90% methanol*		*80% methanol*		*70% methanol*		*60% methanol*		*50% methanol*		*40% methanol*	
	R_M	*log k'*	R_M	*log k'*	R_M	*log k'*	R_M	*log k'*	R_M	*log k'*	R_M	*log k'*
Benzoic	−0.659	−0.68	−0.432	−0.47	−0.176	−0.241	0.105	0.096	0.347	0.367	0.753	0.674
2-Methoxybenzoic	−0.689	−0.759	−0.477	−0.645	−0.347	−0.470	−0.052	−0.078	0.269	0.236	0.602	0.463
3-Methoxybenzoic	−0.550	−0.614	−0.347	−0.398	−0.213	−0.169	0.158	0.198	0.501	0.453	0.865	0.824
4-Methoxybenzoic	−0.630	−0.600	−0.410	−0.417	−0.231	−0.191	0.158	0.175	0.477	0.481	0.826	0.796
2,4-Dimethoxybenzoic	−0.954	−1.060	−0.689	−0.980	−0.575	−0.718	−0.327	−0.333	−0.017	−0.078	0.269	0.192
3,4-Dimethoxybenzoic	−0.689	−0.782	−0.550	−0.646	−0.288	−0.427	−0.070	0.039	0.288	0.208	0.630	0.536
3,4,5-Trimethoxybenzoic	−0.659	−0.759	−0.524	−0.598	−0.213	−0.345	0.017	0.031	0.327	0.317	0.689	0.673
4-Butoxy-3,5-dimethoxybenzoic	−0.158	−0.268	−0.087	−0.019	0.347	0.346	0.865	0.826	–	–	–	–
4-Decyloxy-3,5-dimethoxybenzoic	0.501	0.440	0.865	1.058	–	–	–	–	–	–	–	–
4-Oxybenzoic	−0.865	−1.019	−0.788	−0.914	−0.525	−0.718	−0.347	−0.330	−0.052	−0.116	0.194	0.168
3-Methoxy-4-oxybenzoic	−0.753	−1.019	−0.720	−0.914	−0.550	−0.699	−0.327	−0.305	−0.035	−0.085	0.288	0.213

TLC and HPLC retention data and to examine the correlation between the elution orders and chemical structures of the compounds studied. The knowledge obtained can be used for an improved separation of isomeric acids. Since some of these compounds are possible intermediates, decomposition products or metabolites of pharmaceutically active products, the improved HPLC separation system can be used in purity and stability control.

EXPERIMENTAL

The following acids were tested: benzoic, 2-methoxy-, 3-methoxy-, 4-methoxy-, 2,4-dimethoxy-, 3,4-dimethoxy-, 3,4,5-trimethoxy-, 4-hydroxy-, 4-hydroxy-3-methoxy-, 4-*n*-butoxy-3,5-dimethoxy- and 4-*n*-decyloxy-3,5-dimethoxybenzoic acid.

Benzoic acid (purum) was obtained from Reanal (Budapest, Hungary), the mono- and dialkoxy acids (purum) from Fluka (Buchs, Switzerland); trialkoxy acids were synthesized in this institute.

TLC tests were carried out on RP-18 F_{254s} (Merck, Darmstadt, F.R.G.) 20 cm × 10 cm plates. The plates were developed in a sandwich chamber. HPLC measurements were performed on an LKB (Bromma, Sweden) liquid chromatograph, with a 2150 Model HPLC pump and UV detection at 240 nm (LKB variable wavelength detector). A Nucleosil C_{18} 5-μm column (200 mm × 4 mm; Bio-Separation Techniques, Budapest, Hungary) was used. The mobile phases consisted of mixtures of 0.1 *M* phosphate buffer (pH 2) and acetonitrile, and 0.1 *M* phosphate buffer (pH 2) and methanol, respectively. The concentration of acetonitrile ranged from 20 to 45% (v/v), that of methanol from 40 to 90% for the methoxy acids. Acids containing *n*-butoxy and *n*-decyloxy groups were tested only in buffer–methanol mixtures containing 80 and 90% methanol. The flow-rate was 1 or 1.5 ml/min. Solvents used in the mobile phases were HPLC grade (Interchemia, Budapest, Hungary). Measurements were replicated three times.

RESULTS AND DISCUSSION

Values of $R_M = \log [(1 - R_F)/R_F]$ and $\log k' = \log [(t_R - t_0)/t_0$ for acids were determined by chromatography in different mobile phases. The t_0 values were measured by injecting aqueous potassium dichromate solution and measuring the retention time, as described in the literature[11]. Tables I and II present the experimentally measured R_M and $\log k'$ values at different methanol and acetonitrile concentrations in the mobile phase. The R_M values in 20% acetonitrile were not taken into consideration in the correlation calculations because of the low R_F values and elongated spots of most of the acids. From these data the correlation equations were obtained (Table III).

Considering the correlation equations, it is seen that, using methanol in the mobile phases, the slopes are approximately 1 and the intercepts are approximately zero in the R_M–$\log k'$ correlation. The change of the buffer concentration was not taken into consideration when examining the dependence of the retention of organic solvent concentration in the mobile phase. Preliminary experiments showed that a two-fold dilution of the buffer in this concentration range caused only slight changes in the retention of these acids; the change in organic solvent concentration was much more important.

TABLE II

EXPERIMENTALLY MEASURED R_M AND log k' VALUES AT DIFFERENT PERCENTAGES OF ACETONITRILE IN MOBILE PHASES CONSISTING OF PHOSPHATE BUFFER 0.1 *M* (pH 2)

Acid	*45% methanol*		*35% methanol*		*30% methanol*		*25% methanol*		*20% methanol*	
	R_M	*log k'*	R_M	*log k'*	R_M	*log k'*	R_M	*log k'*	R_M	*log k'*
Benzoic	−0.087	0.120	0.250	0.443	0.525	0.722	0.602	0.719	0.885	
2-Methoxybenzoic	−0.105	0.049	0.140	0.327	0.477	0.605	0.501	0.584	0.741	
3-Methoxybenzoic	0.0	0.163	0.327	0.521	0.630	0.813	0.630	0.838	1.057	
4-Methoxybenzoic	−0.035	0.129	0.327	0.488	0.630	0.780	0.630	0.806	1.025	
2,4-Dimethoxybenzoic	−0,288	−0.010	−0.052	0.270	0.308	0.551	0.389	0.515	0.664	
3,4-Dimethoxybenzoic	−0.250	0.046	0.070	0.263	0.389	0.564	0.477	0.562	0.767	
3,4,5-Trimethoxy-benzoic	−0.176	0.049	0.105	0.369	0.477	0.674	0.602	0.696	0.937	
4-Hydroxybenzoic	−0.368	−0.250	−0.194	0.007	0.087	0.32	0.122	0.248	0.377	
4-Hydroxy-3-methoxybenzoic	−0.347	−0.250	−0.158	0.022	0.105	0.336	0.140	0.270	0.319	

The R_M and log k' values extrapolated to zero organic solvent content, $R_{M,0}$ and log k'_0, respectively, are different in most cases, when approached by the methanol and acetonitile containing mobile phase series, respectively. This is in accord with the literature[12,14]. The $R_{M,0}$ and log k'_0 values of 2,4-dimethoxybenzoic acid, however, were practically equal when measured in methanol or acetonitrile containing mobile phases.

Values of $R_{M,0,CH_3OH}$, $R_{M,0,CH_3CN}$, log k'_{0,CH_3OH} and log k'_{0,CH_3CN} are presented in Table IV. From these data the following correlations were calculated:

$$R_{M,0,CH_3OH} = 1.115\ R_{M,0,CH_3CN} + 0.147\ (r = 0.913)$$

$$\log k'_{0,CH_3OH} = 0.968 \log k'_{0,CH_3CN} + 0.241\ (r = 0.938).$$

The linear correlation makes it possible to convert R_M values measured by TLC into HPLC log k' values.

Considering the individual retention data, it was seen that for HPLC separation of methoxy acids, mobile phases with lower acetonitrile concentration are suitable.

Fig. 1 and 2 show the HPLC separation of aromatic acids containing one or more methoxy groups. When interpreting the elution orders of these acids on the basis of $R_{M,0}$ and log k'_0 values, respectively, some problems arise. Considering the effect of the substituent groups on the retention, the polar hydroxy group, as expected, reduced the retention of 4-hydroxy- and 3-methoxy-4-hydroxybenzoic acid compared to that of benzoic or 3-methoxybenzoic acid. The effect of the methoxy group, however, is not unambiguous. When comparing the retention data with those of benzoic acid it is seen that the 2-methoxy group decreases but the 3- and 4-methoxy groups increase the retention. The retention of 2,4- and 3,4-dimethoxy-benzoic acids, however, is less than that of benzoic acid. 3,4,5-Trimethoxybenzoic acid shows nearly equal retention properties to those of benzoic acid, although it contains three substituent groups, which individually increase retention. None of the R_M or log k' values of di- and trimethoxy acids can be calculated on the basis of additivity of ΔR_M or Δ log k'

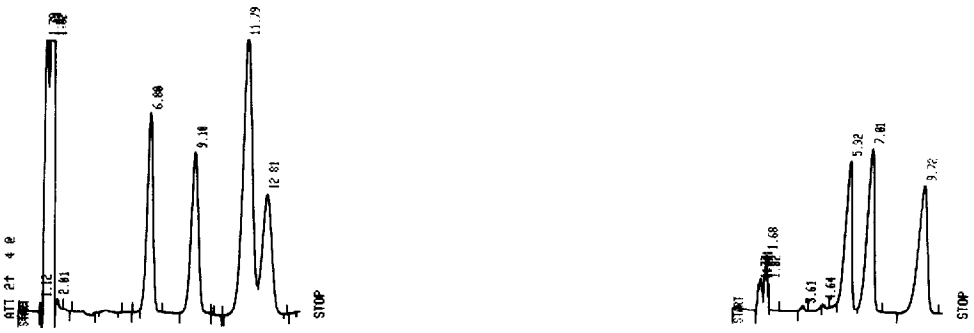


Fig. 1. HPLC separation of aromatic monomethxoy acids. Column: Nucleosil C_{18}, 5 μm, 200 × 4 mm. Mobile phase: acetonitrile–0.1 M phosphate buffer (pH 2) (20:80). Flow-rate: 1.5 ml/min. Detection: UV at 240 nm. Elution order: 2-methoxybenzoic, benzoic, 4-methoxybenzoic and 3-methoxybenzoic acid at 6.8, 9.1, 11.79 and 12.81 min respectively.

Fig. 2. HPLC separation of aromatic acids containing more than one methoxy group. Conditions as in Fig. 1. Elution order: 2,4-dimethoxy-, 3,4-dimethoxy- and 3,4,5-trimethoxybenzoic acid at 5.92, 7.01 and 9. 72 min respectively.

TABLE III

CORRELATION EQUATIONS FOR MOBILE PHASES CONTAINING METHANOL OR ACETONITRILE CALCULATED FROM EXPERIMENTALLY OBTAINED R_M VALUES

c_{CH_3OH} is the concentration of methanol; c_{CH_3CN} is the concentration of acetonitrile in the mobile phase, respectively.

Compound	*Correlation equations*
Mobile phases containing methanol	
Benzoic acid	$R_M = -0.027\ c_{CH_3OH} + 1.75$ ($r = 0.997$); $\log k' = -0.027\ c_{CH_3OH} + 1.74$ ($r = 0.995$); $\log k' = 1.009\ R_M - 0.026$ ($r = 0.998$)
2-Methoxybenzoic acid	$R_M = -0.025\ c_{CH_3OH} + 1.46$ ($r = 0.989$); $\log k' = -0.026\ c_{CH_3OH} + 1.49$ ($r = 0.987$); $\log k' = 1.013\ R_M - 0.092$ ($r = 0.993$)
3-Methoxybenzoic acid	$R_M = -0.028\ c_{CH_3OH} + 1.92$ ($r = 0.986$); $\log k' = -0.029\ c_{CH_3OH} + 1.93$ ($r = 0.996$); $\log k' = 1.027\ R_M - 0.006$ ($r = 0.996$)
4-Methoxybenzoic acid	$R_M = -0.030\ c_{CH_3OH} + 1.95$ ($r = 0.992$); $\log k' = -0.029\ c_{CH_3OH} + 1.90$ ($r = 0.994$); $\log k' = 0.964\ R_M - 0.045$ ($r = 0.971$)
2,4-Dimethoxy-benzoic acid	$R_M = -0.023\ c_{CH_3OH} + 1.11$ ($r = 0.993$); $\log k' = -0.027\ c_{CH_3OH} + 1.24$ ($r = 0.989$); $\log k' = 1.103\ R_M - 0.075$ ($r = 0.985$)
3,4-Dimethoxybenzoic acid	$R_M = -0.024\ c_{CH_3OH} + 1.49$ ($r = 0.985$); $\log k' = -0.027\ c_{CH_3OH} + 1.61$ ($r = 0.989$); $\log k' = 1.018\ R_M - 0.063$ ($r = 0.986$)
3,4,5-Trimethoxybenzoic acid	$R_M = -0.027\ c_{CH_3OH} + 1.71$ ($r = 0.993$); $\log k' = -0.029\ c_{CH_3OH} + 1.79$ ($r = 0.994$); $\log k' = 1.076\ R_M - 0.048$ ($r = 0.997$)
4-Hydroxybenzoic acid	$R_M = -0.021\ c_{CH_3OH} + 1.01$ ($r = 0.993$); $\log k' = -0.025\ c_{CH_3OH} + 1.13$ ($r = 0.987$); $\log k' = 1.137\ R_M - 0.032$ ($r = 0.990$)
4-Hydroxy-3-methoxybenzoic acid	$R_M = -0.021\ c_{CH_3OH} + 1.06$ ($r = 0.980$); $\log k' = -0.026\ c_{CH_3OH} + 1.22$ ($r = 0.988$); $\log k' = 1.165\ R_M - 0.064$ ($r = 0.986$)

Mobile phases containing acetonitrile

Benzoic acid	$R_M = -0.030\ c_{CH_3CN} + 1.36$ ($r = 0.984$); $\log k' = -0.029\ c_{CH_3CN} + 1.48$ ($r = 0.988$); $\log k' = 0.968\ R_M + 0.181$ ($r = 0.995$)
2-Methoxybenzoic acid	$R_M = -0.028\ c_{CH_3CN} + 1.21$ ($r = 0.975$); $\log k' = -0.031\ c_{CH_3CN} + 1.43$ ($r = 0.985$); $\log k' = 0.892\ R_M + 0.165$ ($r = 0.993$)
3-Methoxybenzoic acid	$R_M = -0.031\ c_{CH_3CN} + 1.46$ ($r = 0.984$); $\log k' = -0.032\ c_{CH_3CN} + 1.68$ ($r = 0.985$); $\log k' = 1.027\ R_M + 0.178$ ($r = 0.999$)
4-Methoxybenzoic acid	$R_M = -0.032\ c_{CH_3CN} + 1.49$ ($r = 0.982$); $\log k' = -0.032\ c_{CH_3CN} + 1.65$ ($r = 0.985$); $\log k' = 0.995\ R_M + 0.165$ ($r = 0.999$)
2,4-Dimethoxybenzoic acid	$R_M = -0.031\ c_{CH_3CN} + 1.14$ ($r = 0.977$); $\log k' = -0.027\ c_{CH_3CN} + 1.24$ ($r = 0.965$); $\log k' = 0.802\ R_M + 0.259$ ($r = 0.976$)
3,4,5-Trimethoxybenzoic acid	$R_M = -0.035\ c_{CH_3CN} + 1.46$ ($r = 0.966$); $\log k' = -0.035\ c_{CH_3CN} + 1.63$ ($r = 0.985$); $\log k' = 0.847\ R_M + 0.234$ ($r = 0.987$)
4-Hydroxybenzoic acid	$R_M = -0.023\ c_{CH_3CN} + 0.71$ ($r = 0.978$); $\log k' = -0.026\ c_{CH_3CN} + 0.93$ ($r = 0.943$); $\log k' = 1.079\ R_M + 0.176$ ($r = 0.987$)
4-Hydroxy-3-methoxybenzoic acid	$R_M = -0.023\ c_{CH_3CN} + 0.73$ ($r = 0.981$); $\log k' = -0.024\ c_{CH_3CN} + 0.88$ ($r = 0.917$); $\log k' = 1.137\ R_M + 0.168$ ($r = 0.983$)

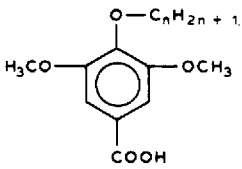
TABLE IV

VALUES OF $R_{M,0,CH_3OH}$, $R_{M,0,CH_3CN}$, log k'_{0,CH_3OH} AND log k'_{0,CH_3CN} FOR THE ACIDS

Acid	$R_{M,0,CH_3OH}$	$R_{M,0,CH_3CN}$	log k'_{0,CH_3OH}	log k'_{0,CH_3CN}
Benzoic	1.75	1.36	1.74	1.48
2-Methoxybenzoic	1.46	1.21	1.49	1.43
3-Methoxybenzoic	1.92	1.46	1.83	1.68
4-Methoxybenzoic	1.95	1.49	1.90	1.65
2,4-Dimethoxybenzoic	1.11	1.14	1.24	1.24
3,4-Dimethoxybenzoic	1.49	1.31	1.61	1.33
3,4,5-Trimethoxybenzoic	1.71	1.46	1.79	1.63
4-Hydroxybenzoic	1.01	0.71	1.13	0.93
4-Hydroxy-3-methoxybenzoic	1.06	0.73	1.22	0.88

increments of the substituents. This anomaly was not observed in the silica gel TLC of these compounds[15]. The elution order on silica gel can be predicted on the basis of additivity of ΔR_M increments. Probably, steric effects are more important in reversed-phase chromatographic retention, than in silica gel chromatography.

The acids containing butoxy and decyloxy groups were chromatographed only in mobile phases containing 80 and 90% methanol because of their strong mutual interaction with the reversed stationary phase. Together with 3,4,5-trimethoxybenzoic acid, they can be considered as members of the following homogous series:



$n=1$ 3,4,5-trimethoxybenzoic acid
$n=4$ 4-*n*-butoxy-3,5-dimethoxybenzoic acid
$n=10$ 4-*n*-decyloxy-3,5-dimethoxybenzoic acid

The R_M and log k' values were determined in buffer–methanol mixtures and 90% methanol and correlated with the carbon atom number, n_C. Linear correlations were observed:

90% methanol: $R_M = 0.126n_C - 0.736$ $(r=0.993)$
$\log k' = 0.131n_C - 0.851$ $(r=0.996)$
80% methanol: $R_M = 0.148n_C - 0.674$ $(r=0.999)$
$\log k' = 0.183n_C - 0.770$ $(r=0.999)$

REFERENCES

1 B. A. Charpentier and J. R. Cowles, *J. Chromatogr.*, 208 (1981) 132.
2 M. C. Spanjer and C. L. de Ligny, *J. Chromatogr.*, 253 (1982) 32.
3 E. Burtscher, H. Binder, R. Concin and O. Bobleter, *J. Chromatogr.*, 252 (1982) 167.
4 K. V. Casteele, H. Geiger and Ch. F. Van Sumere, *J. Chromatogr.*, 258 (1983) 111.

5 J. M. Andersen and W. B. Pedersen, *J. Chromatogr.*, 259 (1983) 131.
6 T. Hanai and J. Hubert, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 7 (1984) 524.
7 J. G. Buta, *J. Chromatogr.*, 295 (1984) 506.
8 C. A. Chang and Q. Wu, L. Tan, *J. Chromatogr.*, 361 (1986) 199.
9 J. Macek and E. Smolková-Keulemansová, *J. Chromatogr.*, 333 (1985) 309.
10 U. A. Th. Brinkman and G. de Vries, *J. Chromatogr.*, 192 (1980) 331.
11 M. Bieganowska and E. Soczewiński, *J. Chromatogr.*, 205 (1981) 451.
12 K. Glowniak and M. Bieganowska, *J. Chromatogr.*, 370 (1986) 281.
13 M. C. Guerra and A. M. Barbaro, *J. Chromatogr.*, 320 (1985) 281.
14 M. C. Pietrogrande, P. A. Borea and G. L. Biagi, *J. Chromatogr.*, 447 (1988) 404.
15 M. Bidló-Iglóy, in H. Kalász and L. S. Ettre (Editors), *Chromatography 85'*, Publishing House of the Hungarian Academic of Sciences, Budapest, 1986, p. 453.