

CHROMOSYMP. 422

## CHROMATOGRAPHY OF AROMATIC ACIDS ON ION EXCHANGERS

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### SUMMARY

The chromatographic behaviour of aromatic acids was determined on an octadecyl- (TSK LS 410), a propylamino- (Chromosorb LC-9), a diethylaminoethyl- (TSK IEX 540 DEAE) and a sulphonyl-bonded silica gel (TSK IEX 510 SP) in sodium phosphate buffer with acetonitrile. The dissociation constants and the maximum capacity ratios are related to the  $pK_a$  and  $\log P$  values. The maximum capacity ratios correlate well with their  $\log P$  values on the octadecyl-bonded packing. The  $pK_a$  values obtained on ion exchangers correlate well with those obtained on the octadecyl-bonded packing. The  $pK_a$  values decrease on anion exchangers in the order 510 SP > 410 > 540 DEAE > Chromosorb NH<sub>2</sub>.

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### INTRODUCTION

The retention of aromatic acids is optimized in reversed-phase liquid chromatography by using a combination of partition coefficients ( $\log P$ ) and dissociation constants ( $pK_a$ ) on octadecyl-bonded silica gels in pH-controlled acetonitrile-water mixtures. The  $\log P$  and  $pK_a$  values are related to the structures of the solutes in the system<sup>1</sup>. However, the chromatographic behaviour of aromatic acids on ion-exchange resins cannot be predicted directly from the structure of the acids<sup>2</sup>, as the resin matrix, which is a polystyrene-divinylbenzene copolymer, is very hydrophobic.

In this work, the chromatographic behaviour of aromatic acids was determined on some ion exchangers whose matrices are very polar. The observed dissociation constants and maximum capacity ratios are correlated with those obtained on an octadecyl-bonded silica gel to measure ion-ion interactions between the acids and ion-exchange groups on the packings.

### EXPERIMENTAL

The details of the instrument have been described previously<sup>1</sup>. The cation exchanger was a sulphonyl-bonded silica gel (TSK IEX 510 SP) from Toyo Soda (Tokyo, Japan). The anion exchangers were a diethylaminoethyl-bonded silica gel (TSK

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TABLE I  
 $pK_a$  VALUES AND MAXIMUM CAPACITY RATIOS ( $\log k'$ ) OF ACIDS

Compound	$\log P$	$pK_a^*$	510 SP		410 ODS		$NH_2$		540 DEAE	
			$pK_a$	$\log k'$	$pK_a$	$\log k'$	$pK_a$	$\log k'$	$pK_a$	$\log k'$
Mandelic acid	1.26	3.380	3.364	-0.260	3.385	0.232	2.871	0.150	3.033	0.616
3-Methoxymandelic acid	1.33	3.330	3.351	-0.269	3.348	-0.316	2.886	0.149	3.018	0.633
3,4,5-Trihydroxybenzoic acid	0.72	4.320	4.461	-0.081	4.322	-0.495	4.353	0.281	4.119	0.848
3,4-Dihydroxybenzoic acid	0.99	4.450	4.545	-0.064	4.471	-0.180	4.126	0.087	4.081	0.736
3,5-Dihydroxybenzoic acid	0.94	3.940	4.090	-0.066	4.066	-0.242	3.474	-0.056	3.790	0.801
2,4-Dihydroxybenzoic acid	1.30	3.250	3.169	0.006	3.158	0.081	2.742	0.107	2.987	1.024
4-Hydroxybenzoic acid	1.28	4.580	4.571	-0.056	4.572	0.262	3.851	-0.072	4.159	0.653
3-Hydroxybenzoic acid	1.37	4.070	4.117	-0.042	4.107	0.246	3.540	-0.005	3.819	0.724
4-Hydroxy-3-methoxybenzoic acid	1.31	4.470	4.486	-0.111	4.487	0.148	3.751	-0.022	4.049	0.662
Benzoic acid	1.94	4.200	4.178	-0.002	4.200	0.765	3.548	0.054	3.842	0.678
3-Methoxybenzoic acid	1.99	4.090	4.063	-0.009	4.084	0.883	3.508	0.072	3.760	0.712
2-Hydroxybenzoic acid	2.18	2.821	2.799	0.021	2.831	0.912	2.072	0.201	2.514	1.009
4-Chlorobenzoic acid	2.517	3.960	3.911	0.134	3.909	1.325	3.409	0.111	3.650	0.818
4-Bromobenzoic acid	2.726	3.980	3.880	0.206	3.878	1.429	3.430	0.130	3.610	0.872
4-Methoxybenzoic acid	1.86	4.480	4.477	-0.015	4.462	0.834	3.745	0.007	4.055	0.635
4-Methylbenzoic acid	2.297	4.340	4.374	0.070	4.363	1.128	3.639	0.020	3.964	0.687

4-Ethylbenzoic acid	2.827	4.350	4.397	0.137	4.364	1.515	3.636	0.038	3.961	0.710
3,4-Dihydroxyphenylacetic acid	0.98	4.387	4.403	0.216	4.371	-0.156	3.782	0.030	4.017	0.636
2,5-Dihydroxyphenylacetic acid	0.80	3.964	4.100	-0.260	3.979	-0.254	3.588	-0.052	3.850	0.699
4-Hydroxyphenylacetic acid	1.26	4.433	4.403	-0.219	4.397	0.112	3.746	-0.014	4.007	0.582
4-Hydroxy-3-methoxybenzoic acid	1.18	4.394	4.408	-0.263	4.411	0.177	3.730	0.028	3.972	0.583
Phenylacetic acid	1.94	4.300	4.315	-0.155	4.323	0.787	3.679	0.066	3.923	0.567
2-Hydroxyphenylacetic acid	1.47	4.225	4.184	-0.171	4.236	0.382	3.618	0.007	3.895	0.695
3-Methoxybenzoic acid	1.99	4.261	4.304	-0.176	4.294	0.842	3.674	0.069	3.902	0.583
4-Methoxybenzoic acid	1.95	4.398	4.380	-0.184	4.393	0.808	3.729	0.058	3.964	0.560
4-Chlorophenylacetic acid	2.473	4.216	4.182	0.018	4.171	1.269	3.561	0.095	3.821	0.715
3,4-Dihydroxycinnamic acid	1.15	4.545	4.594	0.006	4.537	0.066	4.021	0.086	4.206	0.907
4-Hydroxycinnamic acid	1.46	4.630	4.603	0.012	4.572	0.354	3.880	-0.014	4.163	0.785
Cinnamic acid	2.32	4.380	4.378	0.088	4.362	1.165	3.703	0.089	3.952	0.786
Hydroxycinnamic acid	2.25	4.579	4.650	-0.099	4.671	1.107	3.865	0.003	4.182	0.555
3-Methoxycinnamic acid	2.37	4.307	4.330	0.069	4.318	1.253	3.698	0.089	3.921	0.786
4-Hydroxy-3-methoxycinnamic acid	1.51	4.558	4.564	-0.046	4.544	0.444	3.874	0.011	4.068	0.720
3-( <i>p</i> -Methoxyphenyl)propionic acid	2.063	-	4.682	-0.142	4.682	1.084	3.874	-0.009	4.216	0.543
4-Phenylbutyric acid	2.522	4.719	4.786	-0.044	4.785	1.418	3.955	-0.010	4.260	0.551
2-Hydroxyhippuric acid	1.55	-	3.450	-0.152	3.513	0.434	3.063	0.137	3.243	0.758
Indoleacetic acid	1.92	-	4.626	0.097	4.597	0.785	3.871	0.089	4.109	0.795

\* From ref. 1.

IEX 540 DEAE) from Toyo Soda and a propylamino-bonded silica gel (Chromosorb LC-9) from Manville Products (Denver, CO, U.S.A.). The octadecyl-bonded silica gel was TSK LS 410 from Toyo Soda. These packings were laboratory packed in 15 or 25 cm  $\times$  4.0 or 4.6 mm I.D. stainless-steel tubes. The columns were thermostated in a water-bath at 40°C. The eluent was 0.02 *M* sodium phosphate buffer containing 20% (v/v) of acetonitrile.

## RESULTS AND DISCUSSION

The observed dissociation constants and the maximum capacity ratios are listed in Table I with their log *P* values.

The correlation coefficient (*r*) is 0.87 (*n* = 30) between the *pK<sub>a</sub>* values on the octadecyl- and propylamino-bonded silica gels. This value becomes 0.99 (*n* = 27) when those compounds which show an *ortho* effect are eliminated from the calculation; such compounds are 2,4-dihydroxybenzoic acid, 2-hydroxy benzoic acid and 2,5-dihydroxyphenylacetic acid. Our results show that the propylamino-bonded silica gel gives *pK<sub>a</sub>* values on average 0.606 smaller than the octadecyl-bonded silica gel and the DEAE ion exchanger gives *pK<sub>a</sub>* values on average 0.363 smaller than the octadecyl-bonded silica gel. The correlation coefficient between *pK<sub>a</sub>* values obtained and predicted from the reference values is 0.98 (*n* = 33). The *pK<sub>a</sub>* values obtained on the cation exchanger are 0.025 higher than those obtained on the octadecyl-bonded silica gel and the correlation coefficient between the observed and predicted values is 0.996 (*n* = 36).

One of the important factors in predicting retention times of the acids is their maximum capacity ratio obtained from the following equation:

$$k' = (k'_m + k'_i \cdot K_a/[H^+]) / (1 + K_a/[H^+])$$

The correlation between the log *P* values of acids and the maximum capacity ratios observed on both a polystyrene gel and an octadecyl-bonded silica gel is excellent<sup>3,4</sup>. In this experiment, the correlation coefficient between the log *P* values and the maximum capacity ratios obtained on the octadecyl-bonded silica gel is 0.991 (*n* = 36).

However, the maximum capacity ratios obtained on ion exchangers are not related to their log *P* values, owing to the different mechanism of the retention. The maximum capacity ratios are, therefore, related to dissociation constants, which indicate the acidity of the acids. The correlation coefficient is not satisfactory. The value on the DEAE ion exchanger is 0.53 (*n* = 28) and the value on the propylamino ion exchanger is 0.64 (*n* = 26). The acids that are complete outliers show either an *ortho* effect or have a 3,4-dihydroxy group.

The minimum capacity ratios are close to zero and the dissociation constant can be predicted. However, there are some problems in predicting the maximum capacity ratios on ion exchangers.

## CONCLUSION

The value of ion-ion interactions can be measured, even if the values are different for the type of ion-exchange group and the batch of products. The dissociation

constant of aromatic acids in ion-exchange system can therefore be used to predict the maximum capacity ratio for the calculation of the capacity ratios of the acids in ion-exchange liquid chromatography.

#### REFERENCES

- 1 T. Hanai and J. Hubert, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, in press.
- 2 T. Hanai and J. Hubert, *Chromatographia*, 17 (1983) 633.
- 3 T. Hanai, K. C. Tran and J. Hubert, *J. Chromatogr.*, 239 (1982) 385.
- 4 T. Hanai and J. Hubert, *J. Chromatogr.*, 239 (1982) 527.