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OPTIMISATION IN CHROMATOGRAPHY

THEORY AND APPLICATION TO THE SEPARATION OF AROMATIC ACIDS IN REVERSED-PHASE LIQUID CHROMATOGRAPHY

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SUMMARY

Plate theory is applied to the separation of eluted peaks in chromatography. It is shown that the peak elution volumes V_A and V_B for two solutes A and B can be used to define a separation factor S [$S = (V_B - V_A)/(V_A + V_B)$] which is simply related to the chromatographic resolution R_s; $S = 2R_s/N^{\frac{1}{2}}$ where N is the number of theoretical plates of the column.

It is shown that the use of the relative volatility factor α for optimisation of chromatographic performance can lead to inconsistencies when the column dead volume V_0 is not much smaller than V_A and V_B and that the use of S avoids these inconsistencies and is more straightforward particularly since it does not require measurement of V_0. Application of both approaches is made to the optimisation of the pH for separation of aromatic acids in water–methanol elution on octadecyl silica.

INTRODUCTION

The application of plate theory in chromatography and the development of the window diagram approach[1] to optimisation of performance has brought important rationalisation to the practice of chromatography. The optimisation has usually involved the use of the relative volatility parameter α. The application of plate theory to chromatographic separation is considered and an alternative approach is developed.

THEORETICAL

Chromatographic separation

This can be defined for two solutes A and B with actual elution volumes V_A and V_B by the chromatographic resolution, R_s,

$$R_s = (V_B - V_A)/(w_A + w_B)$$

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where w_A and w_B are the widths (2 σ) of the eluted peaks at the points of inflection. The ratio is commonly referred to[2,3] as the chromatographic resolution, R_s. As Snyder[2] has discussed the effective separation depends critically on the value of R_s, especially when the eluted peaks are of different size. He suggests than when $R_s > 1.25$ peak overlap is minimal, and also shows that peak width is effectively independent of peak height. For "exact" separation[4] (6 σ) $R_s = 1.5$.

Without making unnecessary assumptions such as $w_A = w_B$ (ref. 4), substitution for w_A and w_B from plate theory[5] gives

$$R_s = (V_B - V_A)/[2N^{\frac{1}{2}}(v_m + K_A v_s) + 2N^{\frac{1}{2}}(v_m + K_B v_s)]$$

where v_m and v_s are the volumes per plate of the mobile and stationary phase respectively and K_A and K_B are the respective partition coefficients. Since

$$V_A = N(v_m + Kv_s) \text{ and } V_B = N(v_m + K_B v_s)$$
$$R_s = N^{\frac{1}{2}}(V_B - V_A)/2(V_A + V_B)$$
$$2R_s/N^{\frac{1}{2}} = (V_B - V_A)/(V_A + V_B)$$

the right hand side can be conveniently defined as the separation parameter S. If this is evaluated for any pair of solutes the number of plates (N) required for a defined criterion of separation (R_s) can immediately be calculated. Thus for optimisation of chromatographic separation the maximum value of S should be found. S is very easily evaluated from the peak elution volumes or peak elution times (t) of solutes A and B (at constant flow-rates)

$$S = (V_B - V_A)/(V_A + V_B) = (t_B - t_A)/(t_A + t_B)$$

Comparison of S and relative volatility (α)

Optimisation of chromatographic separation has been developed by Purnell and co-workers[1,4,6–8] in terms of the relative volatility $\alpha = V_B'/V_A' \equiv (V_B - V_0)/(V_A - V_0)$. Evaluation of α requires the evaluation of V_0 which can be difficult in high-performance liquid chromatography (HPLC). Moreover when V_0 is not significantly less than V_A (or V_B), a condition possible in HPLC but unlikely in gas chromatography, the use of α can result in the wrong criterion for optimisation. For example, in a silica column with a polar eluent I we have had low elution volumes such as $V_B = 3.20$, $V_A = 3.10$, $V_0 = 3.00$ ml. With a less polar eluent II the values were 6.00, 5.00 and 3.00 ml, respectively. For eluent I $\alpha = 2.0$ but a better separation (A and B separated by 1.0 ml) is achieved for eluent II. If the column is reasonably assumed to have 10,000 plates for eluent I $2w_A = 0.124$ and $2w_B = 0.128$ ml and peaks are not separated even with $R_s = 1.0$ while for eluent II $2w_A = 0.20$ and $2w_B = 0.24$ ml and the peaks are well separated. On the other hand for eluent I $S = 0.016$ and for eluent II $S = 0.091$ correctly predicting the better separation with eluent II.

In practice providing V_0 is not very similar to V_A, S and α can give similar results. An illustrative application follows.

EXPERIMENTAL

The chromatographic system was composed of a microprocessor-controlled Altex Model 100A dual-piston reciprocating pump electronically interfaced with Hewlett-Packard Model HP 3380A reporting integrator which received the output from a Pye-Unicam LC–UV detector. A Rheodyne Model 7120 valve fitted with a 20-μl sample loop was used to inject the sample solutions. Narrow-bore stainless-steel tubing 1/16 in. O.D. was used to connect the pump, injection valve, column and detector.

The eluents were made up using glass-distilled water, AnalaR grade methanol (BDH, Poole, Great Britain), laboratory-grade phosphoric acid (BDH) and B.P.-grade sodium hydroxide (Steetley, Basingstoke, Great Britain). Benzoic (BA; BDH) 3,4-dihydroxybenzoic (3,4-DHBA; Koch-Light, Colnbrook, Great Britain), phenylacetic (PA; Fisons, Loughborough, Great Britain), 2-hydroxyphenylacetic (2-HPA; Aldrich, Gillingham, Great Britain), and 4-hydroxyphenylacetic (4-HPA; Sigma, St. Louis, MO, U.S.A.) acids were used as purchased. The salicylic acid (SA) was a gift from J. R. Turner, University College, Swansea, Great Britain.

Hypersil ODS 5-μm column packing was purchased from Shandon Southern Products (Runcorn, Great Britain).

A 250 × 4.6 mm I.D., 316 stainless-steel column was packed with Hypersil ODS slurried in methanol (4.0 g in 40 ml). The packing was performed at 6000 p.s.i. using a Haskel Model MCP71 air-driven pump with methanol as the pressurising solvent. After packing the column was conditioned with methanol (100 ml) and the number of theoretical plates was found to be 10,500 by injecting anthracene and using a methanol–water (80:20) mixture as eluent.

All eluents were deaerated ultrasonically prior to use. Methanol–aqueous phosphate buffer was used as the mobile phase, the pH of the aqueous phase being adjusted to the required value prior to mixing. The column was flushed with methanol for at least 20 min when changing eluent. The LC–UV detector was used at 254 nm. The flow-rate was measured using a fixed-volume flowmeter during the development of each chromatogram. The seven acids were chromatographed individually and as a seven component mixture for each eluent. The column was thermostatted at 24.5°C during elution.

RESULTS

The elution volumes of the six acids are shown in Table I as a function of the pH of the buffer added to methanol to give a volume composition of 70:30. Using a linear interpolating of elution volume between pH values the window diagrams shown in Figs. 1 and 2 were constructed.

In Fig. 1 values of S are plotted against pH and the maximum value of S is 0.0893 at pH 2.972.

Using the relationship developed earlier

$$2R_s/N^{\frac{1}{2}} = S$$

TABLE I

VARIATION IN RETENTION VOLUME (V) OF AROMATIC ACIDS WITH AQUEOUS PHASE PH–REVERSED-PHASE CHROMATOGRAPHY ON OCTADECYLSILICA USING METHANOL–AQUEOUS PHOSPHATE BUFFER (30:70)

Acid	*V (ml)*					
	pH					
	2.5	*3.0*	*4.0*	*5.0*	*6.0*	*7.0*
BA	24.96	23.78	17.06	7.19	4.64	4.36
SA	25.48	16.15	7.28	5.59	5.39	5.57
3,4-DHBA	5.24	5.13	4.70	3.44	2.81	2.76
PA	20.58	19.90	15.64	7.89	5.37	5.33
2-HPA	10.34	10.10	8.72	6.06	5.28	5.46
4-HPA	7.82	7.69	6.54	4.25	3.25	3.21

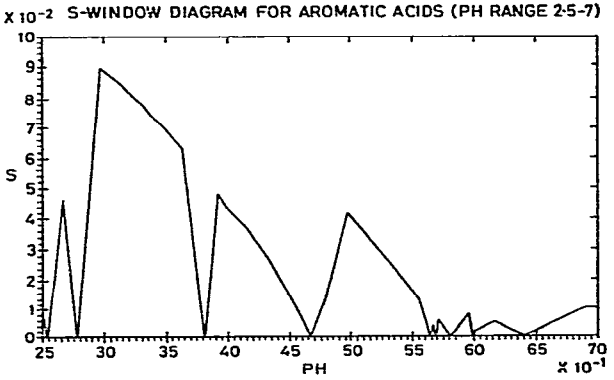

Fig. 1. Plot of separation parameter S against pH giving an S-window diagram for the separation of aromatic acids on octadecyl silica with methanol–aqueous buffer (30:70) eluent.

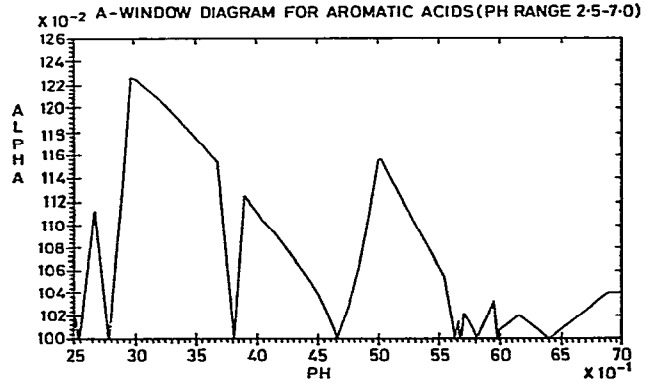

Fig. 2. Plot of the relative volatility factor (α) agains pH giving an α-window diagram for the separation of aromatic acids on octadecyl silica with methanol–aqueous buffer (30:70) eluent.

the maximum resolution (the best window) for the most difficultly separated pairs of solutes (SA, PA and BA, PA) is given by

$$R_s = SN^{\frac{1}{2}}/2 = 4.58$$

This shows that since "exact separation"[4] occurs at $R_s = 1.5$ the acids are completely resolved under these conditions.

Fig. 2 shows that on the basis of α plots the best window ($\alpha = 1.226$) occurs at pH 2.967. This is essentially the same as given by S, that since the elution volumes are reasonable in relation to V_0 (2.66 ml). The α plots however required the measurement of V_0 since $\alpha = (V_B - V_0)/(V_A - V_0)$. There is some uncertainty in V_0 measurements; in this case it corresponds to the elution volume of 3,4-DHPA in pure methanol. No such measurement is required in obtaining S values. The α value can now be used to calculate the required number of theoretical plates. The formula used[6] is

$$n_{req.} = 36\left(\frac{\alpha}{\alpha - 1}\right)^2\left(\frac{k' + 1}{k'}\right)^2$$

This equation is tacitly based on $R_s = 1.5$ and requires k', the capacity factor, to be calculated. k' has to be related either to one of the components, or the mean of the two. It is given by $(V_A - V_0)/V_0$ or $(V_B - V_0)/V_0$ or the mean of the two. When $V_A - V_0$ and $V_B - V_0$ are very small the choice is very important. This introduces a further approximation.

Thus it is to be concluded that the use of the separation parameter requires less measurements than that of α; in systems where V_0 is similar to the elution volumes of the solutes, it does not give erroneous windows and it gives a more direct measurement of the number of theoretical plates for a stipulated resolution (R_s) or directly gives the resolution since the number of theoretical plates for a column is usually known.

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