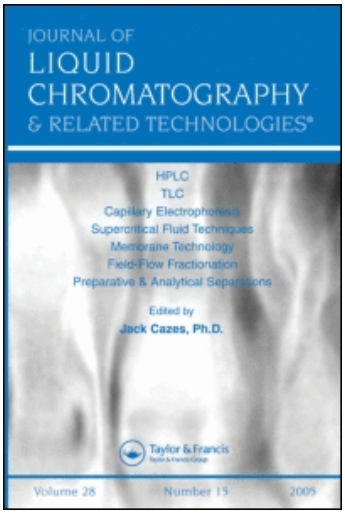

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CONVERTING GRADIENT RETENTION TIMES TO ISOCRATIC RETENTION TIMES IN REVERSED PHASE LIQUID CHROMATOGRAPHY

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ABSTRACT

Various methods of converting gradient retention times in reversed phase liquid chromatography to isocratic retention times are compared. The two main methods investigated were the analytical solution to $dV_m = dV'/k$, and the numerical solution to $dV_m = dV/(1+k)$. Other methods investigated involved using a quadratic expression for the dependence of log retention factor on mobile phase composition instead of the usual linear function and also inclusion of a correction for extra gradient delay due to void volume of the column. No method gave a good agreement between isocratic retention times estimated from gradient data and experimental isocratic retention times. The method which gave the best agreement was the numerical integration of $dV_m = dV/(1+k)$ using the usual linear relationship between log of retention factor and mobile phase composition. A modern spreadsheet was used for all calculations.

INTRODUCTION

The variation of the capacity or retention factor with solvent strength in reversed phase chromatography has usually been described by the log linear equation:

$$\ln k = \ln k_o - S \Phi \qquad (1)$$

where k is the retention factor, k_o a constant, S the solvent strength constant, and Φ the volume fraction of higher solvent strength mobile phase component. Schoenmakers et al.[1] have derived, using solubility parameters, a quadratic equation, equation (2), to describe the same variation. However they have argued that the linear equation is sufficient to describe the variation for practicable purposes.

$$\ln k = \ln k_o - S1 \Phi - S2 \Phi^2 \qquad (2)$$

A major use of equation (1) is to estimate isocratic retention times from gradient data. This is an advantage because one gradient run can cover the whole mobile phase composition range and thus elution is certain to occur in a reasonable time period for solutes showing normal chromatographic behaviour. The method of converting gradient data to isocratic data involves integration of a variable over time, or over volume, as the volume fraction mobile phase composition varies over time for the gradient experiment. Equation (3) can be derived in a few lines as shown by Said.[2]

$$dl = (u / (1 + k)) dt \qquad (3)$$

Here l is the distance moved by the solute along the column, u is the mobile phase velocity, k is the retention factor, and t is time. A more convenient form of equation (3) is to convert to mobile phase volume terms. This is easily done by substituting $dV_m = A\ dl$, $u = F/A$, and $dt = dV/F$ into equation (3). Here V_m is void volume, V is mobile phase volume, A is the average cross sectional area available to the mobile phase, L is the column length, and F is the flow rate. Equation (4) is obtained.

$$dV_m = dV / (k + 1) \qquad (4)$$

Equation (4) cannot be integrated analytically to estimate isocratic retention volumes from gradient retention volumes. Snyder,[3] Schoenmakers,[4] and Jandera[5] have all used equation (5) to estimate corrected retention volumes in gradient elution work.

$$dV_m = dV' / k \qquad (5)$$

The volume terms in equations (4) and (5) have different meanings. In equation (5), V' is the corrected mobile phase volume. The origin of equation (5) comes from arguments,[6] or the derivations by Drake,[7] and Freiling.[8,9] Equation (5) has the advantage of being able to be integrated analytically when linear gradients are used. The solution shown is from Herman et al.,[10] although all solutions give the same result. This solution is shown in equation (6).

$$t_r = (t_g / S \Delta\phi) \ln[S \Delta\phi t_o k_o \exp(- S \phi_i)/t_g + 1] + t_o + t_d \qquad (6)$$

Here t_g is the gradient time, $\Delta\phi$ is the change in mobile phase composition during t_g, t_o is the unretained solute time, ϕ_i is the initial mobile phase composition, t_d is the gradient delay to the start of the column, and S and k_o are the constants from equation (1). If t_o and t_d are known, then S and k_o can be found for each solute from two or more gradient runs. The retention times during further gradient and/or isocratic runs can now be predicted. These works have been well received by the chromatographic community as evidenced by the number of papers published on the subject. A convenient compendium of papers concerned with this approach, and other methods, has been published by Glajch and Snyder.[11]

Normally two gradient runs have been used to determine the constants k_o and S from equation (1). However DeGalan and coworkers[12,13] and more recently Snyder and Dolan,[14] have used one gradient run to start the optimisation procedure based on the discovery of a linear relationship between S and ln k_o, by Schoenmakers et al.[15] The constants for this relationship were found by a least squares fit to data from a large number of compounds.

Although equation (5) has the advantage of an analytical solution, it may not be expected to give accurate results in all cases. The error would be expected to be greatest for polar compounds where k is small. This is because the value of the right hand side of equation (5) goes to infinity as k goes to zero. Equation (4) gives the correct result as k goes to zero. Equation (4) requires numerical integration for a solution. However this is not as onerous as in previous years, due to the advent of the modern spreadsheet. A modern spreadsheet, such as EXCEL®, can be used to numerically integrate equation (4) very easily and without the need to write computer programs in a specialist language. In this paper a modern spreadsheet is used to investigate the effect of using equation (4) and equation (5) on isocratic retention times predicted from gradient retention times.

Quarry et al. have investigated both the effects of non ideal equipment[16] and the normal assumptions about processes occurring in the column on the calculation of isocratic retention times from gradient retention times. They allowed for a gradient delay to the start of the column but did not allow for a further gradient delay due to the void volume of the column. As the eluents

move through the column, a further gradient delay occurs which may be larger than their considered gradient delay depending on the void volume of the column. The effect of this further gradient delay is expected to be small as in the gradient experiment compounds spend a large portion of their time on the column at the beginning of the column. This paper also investigates the effect of this further gradient delay and the effect of using a quadratic function, such as equation (2), to express the dependence of log retention factor on mobile phase composition.

EXPERIMENTAL

Materials and Methods

All experiments were carried out with a GBC LC1150 pump (GBC Scientific Equipment Pty. Ltd., Dandenong, Victoria, Australia), a Rheodyne 7010 injector with a 20 μL sample loop, a LINEAR UVIS200 detector (Linear Instruments Corp., Nevada, USA), and a Lichrosphere 100, RP18, 5 μm, 125 mm column (E. Merck, Darmstadt, Germany). The detector was set at 260 nm.

The column was thermostated at 25°C. Data collection was done with a 12 bit A/D board and a 386 PC. The mobile phase was HPLC grade methanol (Fisons(AAG) Pty. Ltd. Homebush, NSW, Australia) and deionised water. Mobile phase compositions were made by the equipment and assumed to be correct. The flow rate was always 1.0 mL/min. The void volume was assumed to be the start of the first baseline variation observed after injection. This was 1.07 mL.

Linear gradient shapes, produced by the equipment, were checked with no column and mixtures of methanol and methanol plus 10% acetone. It was found that the equipment produced slightly curved gradients for gradient rates greater than about 4% per minute. Therefore, gradients for estimation of the constants S and k_o were confined to less than 3% per minute. The gradient delay to the start of the column was measured by extrapolating the gradient shape check plots to the baseline so that initial curvature was neglected. This volume was 1.13 mL. 20 μL of sample was always injected at time zero, i.e. the gradient delay time before the gradient reached the start of the column was always 1.13 divided by the flow rate in mL/min.

Helium sparging was used to degas the mobile phase components. Retention times used for all calculations were as reported by the software. The synthetic mixture was made from laboratory reagent grade materials, of various origin, dissolved in methanol. The five substances used were o-cresol, benzyl acetone, phenyl ethyl ether, ethyl benzene, and dimethyl phthalate. The choice of these substances was simply because of their shelf availability in the authors

laboratory and their ability to be detected by UV. The corresponding chromatogram peaks were not identified. Peak tracking was done was by visual inspection of areas. Some crossover occurred.

Calculation Methods

All calculations were done using the spreadsheet EXCEL, version 5, (Microsoft Corporation, Redmond, WA, USA). The constants S and ln k_0, from equation (1), were found for the five compounds from between five and seven isocratic runs from 0.3 volume fraction methanol to 0.9 volume fraction methanol in increments of 0.1 volume fraction. This was done by finding values of S and ln k_0 that minimised the sum of the squares of the differences between the estimated retention times and the experimental retention times, divided by the experimental retention time. The differences were divided by the experimental retention time so that results would not be biased towards large retention times. The addin SOLVER was used to find the values that minimised the sum automatically. The constants S1, S2, and ln k_0, from equation (2) were found from the isocratic data in the same way.

For any mixture with unknown elution characteristics, at least one gradient run, from low initial organic modifier to high final organic modifier, is usually required to make sure that all compounds, that can elute, have eluted. Equation (1), when substituted into either equations (4) or (5), contains two unknowns, ln k_0 and S, and requires at least two runs to determine these two constants. Using the information published by Schoenmakers et al.,[9] it is possible to estimate these constants when only one chromatogram is available. However, for this work, five gradient runs at each of four different gradient rates, were used so that the effects of experimental error would be minimised. Equation (6) and the EXCEL addin SOLVER® were used to find the best fit values of S and ln k_0, for each compound, from the five gradient runs. As for the isocratic data, this was done by finding values of S and ln k_0 that minimised the sum of the squares of the differences between the estimated gradient retention times and the experimental gradient retention times, divided by the experimental gradient retention time.

To find S and ln k_0 values from the gradient data using equation (4), columns of the value of the integral in equation (7), up to time t, were made against time at intervals of 0.5 secs. This was done for the five gradient runs with different starting compositions for each peak and at a constant gradient rate. Initially, guessed values of S and ln k_0 were used. The calculated retention time was found using the LOOKUP function for when the value of the integral equaled t_0. As for the isocratic data, the correct values of S and ln k_0 were assumed to be those that minimised the sum of the squares of the differences between the estimated retention times and the experimental retention times,

divided by the experimental retention time. These values were found by SOLVER. As SOLVER requires a continuous function to find a minimum, values of the integral were linearly interpolated when the LOOKUP function was used. To take into account the extra gradient delay due to the void volume of the column, the value of the integral to time t, was added to the normal gradient delay to be subtracted in equation (7). Equation (7) is obtained by combining equations (1) and (4) and including a linear gradient.

The values S1, S2, and ln k_0, from equation (2) could be found from the gradient data by the same procedures. The equation used is shown in equation (8).

$$t_0 = {}_0\int^{te} dt / (1+ \exp(\ln k_0 - S (\Phi i + (\Phi f - \Phi i) (t - td) / tg)))) \qquad (7)$$

$$t_0 = {}_0\int^{te} dt/(1+\exp(\ln k_0 - S1(\Phi i+(\Phi f-\Phi i)(t-td)/tg)) - S2(\Phi i+(\Phi f-\Phi i)(t-td)/tg))^2)) \qquad (8)$$

where t is the time, t_0 is the void volume time for the flow rate used, te is the elution time for a peak, Φi is the gradient start composition, Φf is the gradient finish composition, t_d is the gradient delay to column start, and tg is the gradient time from Φi to Φf. A copy of the spreadsheet used in these calculations is available from the author on request.

RESULTS AND DISCUSSION

The peaks are referred to in the discussion only as peak A through to peak E, in order of elution at high volume fraction of methanol. Peaks B and C showed crossover at low methanol volume fractions. Isocratic retention times at a flow rate of 1.0 mL/min are recorded in Table 1 for various methanol frac-

Table 1

Isocratic Retention Times in Seconds for the Eluents at a Flow Rate of 1.0mL/min

Vf (Methanol)	A	B	C	D	E
0.9	74	83	96	103	125
0.8	85	103	128	149	214
0.7	108	147	195	256	436
0.6	153	232	299	442	874
0.5	258	444	506	879	2134
0.4	551	995	866		
0.3	1453				

tions from 0.30 to 0.90. These data were fitted to equation 1 and it was found that isocratic retention times could be explained with a percentage deviation of 3.3%. Using equation 2, the data can be explained with a percentage deviation of 1.6%. Using the F test, the inclusion of the quadratic term was found to be significant at the 0.01 level. Dolan et al.[17] have stated that errors in predicting isocratic retention times from equation (1) and two isocratic runs are not serious provided that the runs are performed with mobile phase compositions differing by at least 10% in the higher solvent strength component. The results presented here tend to contradict this view. However it is always a matter of conjecture as to whether the improvement in prediction is worth the extra work involved to include the quadratic term.

Gradient retention times, at flow rates of 1.0 mL/min and gradient start and finish methanol fractions of 0.20 and 0.90 for various gradient times and various gradient rates are recorded in Table 2. The gradient data were used to estimate isocratic retention times that could be compared with the experimentally observed isocratic retention times reported in Table 1. To estimate these isocratic retention times, the constants S and ln k_o from equation (1), or the constants S1, S2, and ln k_o from equation (2) were found. Five different methods were used to estimate these two different sets of constants. These methods were the analytical solution to equation (6), the numerical solution to equation (7), the numerical solution to equation (7) with an added correction for the gradient delay due to the void volume of the column, the numerical solution to equation (8), and the numerical solution to equation (8) with an added correction for the gradient delay due to the void volume of the column. These are labelled methods 1 through 5, respectively. The ability of each method to estimate the experimental isocratic retention times was judged by the average percentage deviation of the estimated retention times from the experimental retention times. These figures are shown in Table 3. When the isocratic retention times are predicted from the constants found from gradient data via methods one to five, it can be seen that using the analytical method (1) of estimating S and ln k_o is considerably worse than any of the other methods. The numerical integration with two constants method (2) is best and with an average percentage deviation of 5.4%, seems reasonable compared to the 3.3% found from the isocratic fit. Incorporation of a correction for gradient delay due to void volume of the column results in the predicted isocratic times having slightly worse agreement. The reason for this is unknown. Both methods using three constants are also slightly worse than the uncorrected two constant method. This is in spite of the three constant model being a significantly better fit to the isocratic data than the two constant model. Also shown in Table 3 are the sum of the squares of the deviations of the estimated isocratic or gradient retention times, divided by the respective experimental times. These data show that the three constant models are also significantly better than the two constant models in explaining the gradient data. However this does not translate into predicting isocratic retention times better.

Table 2

Gradient Retention Times in Seconds for the Five Eluents at Gradient Rates of 0.778%, 1.167%, 1.55%, and 2.33% Methanol Per Minute, Respectively*

Initial Methanol Volume Fraction	Gradient Time in Minutes	A	B	C	D	E
0.1	115.7	2190	2619	1965	3063	3850
0.2	102.9	1490	1912	1520	2359	3144
0.3	90	879	1271	1094	1667	2343
0.4	77.14	489	738	718	1100	1720
0.5	64.29	251	398	456	682	1119
0.6	51.43	148	215	276	384	627
0.1	77.14	1628	1951	1613	2285	2827
0.2	68.52	1206	1507	1300	1843	2347
0.3	60	780	1029	955	1357	1824
0.4	51.43	414	628	636	945	1359
0.5	42.86	240	369	427	612	958
0.6	34.29	134	198	260	355	587
0.1	57.86	1326	1575	1421	1882	2292
0.2	51.43	1028	1262	1152	1539	1913
0.3	45	682	902	862	1176	1538
0.4	38.57	424	608	622	866	1200
0.5	32.14	234	353	410	589	868
0.6	25.71	145	216	266	353	551
0.1	38.6	1068	1238	1175	1442	1690
0.2	34.3	816	984	951	1190	1432
0.3	40	581	743	743	949	1186
0.4	25.71	380	516	548	713	934
0.5	21.43	227	328	380	498	700
0.6	17.14	144	207	259	335	483

* The final methanol composition was always 100%. The flow rate was 1.0 mL/min.

Table 4 shows the sum of the squares of the fractional deviations from the gradient retention times of the first two methods for each of the five different compounds in order of decreasing polarity. In each case it can be seen that the gradient data is explained better for the least polar compound. This was expected for method 1, the analytical solution to equation (5). However, it also occurs for the methods using the numerical solution to equation (4). Also

Table 3

Percentage Deviations of Estimated Isocratic Retention Times from Experiment and Sum of Squares of Deviations of Fractional Retention Times from Experiment Using Different Methods of Estimation*

	Isocratic 2 Constant	Isocratic 3 Constant	Gradient 1	Gradient 2	Gradient 3	Gradient 4	Gradient 5
Deviation /%	3.3	1.6	21	5.4	7.4	7.3	9.0
Sum of squares	0.047	0.010	0.158	0.040	0.040	0.018	0.019

* See text for Description of methods.

shown in Table 4 are the average percentage deviations when predicting the isocratic retention times for each compound. When method 1 (analytical solution) is used, a definite trend towards better prediction as the compound becomes less polar, is observed. When the numerical solution is used, the trend is not so prominent but still present. Most of the error in prediction of isocratic times by the analytical method occurs in the early eluting compounds.

Table 5 shows the constants from equations (1) and (2), ln k_o, S, S1, and S2, obtained from the isocratic data, and from the gradient data. Only data for compounds A, the most polar, and E, the least polar, are shown. Other com-

Table 4

Sum of Squares of Fractional Deviations from Gradient Retention Times and Average Percent Deviations of Isocratic Retention Times Predicted from Gradient Times for the Five Compounds

Sum of Squares	A	B	C	D	E
Method 1	0.086	0.015	0.046	0.007	0.004
Method 2	0.011	0.004	0.020	0.003	0.003
Average % Deviation					
Method 1	27.2	23.4	18.2	19.7	14.7
Method 2	7.0	7.8	2.7	4.7	4.5

Table 5

Comparison of Values of the Constants from Equations (1) and (2) Found from Isocratic and Gradient Data*

	A			E		
	S or S1	S2	ln k_0	S or S1	S2	ln k_0
Isocratic	8.84		5.63	7.48		6.29
Method 1	14.28		7.67	9.63		8.21
Method 2	9.37		5.85	8.72		7.77
Method 3	9.25		5.73	8.71		7.70
Isocratic	13.27	-4.50	6.64	8.69	0.01	7.80
Method 4	10.72	-1.54	6.12	6.09	2.33	7.04
Method 5	11.06	-1.98	6.13	4.96	3.30	6.65

* Various estimation methods used as described in the text.

pounds show intermediate behaviour. The agreement between the values estimated from the two different sets of data is not good. However, agreement in all cases improves as polarity decreases. The agreement is best when method 2, the numerical method without any correction, is used. Similar to prediction of isocratic retention times, correction for gradient delay due to void volume of the column and the introduction of an extra slope constant (S2) make agreement worse. This is again, in spite of the extra constant, producing a significantly improved fit to the gradient data.

These results indicate that there is still more to learn about translating gradient data into isocratic data. The fact that both isocratic and gradient data are better fitted by a model with three constants in the relationship between ln k and mobile phase composition and, yet, this model gives worse prediction of isocratic retention times than the two-constant model, indicates that some aspect of the processes involved is not understood. Also the fact that a logical correction to the void volume, although small, also causes slightly worse prediction of isocratic times from gradient times is another indication that all is not known.

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