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Computer simulation of isocratic retentions of alkylketones using gradient data

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ABSTRACT

The use of computer simulation software for high-performance liquid chromatographic (HPLC) method development is considered. In particular, gradient elution data entered into DryLab G/plus are used to predict isocratic retention times. The motivation was to establish whether data generated in a research-grade, gradient environment might be used to simulate accurately isocratic HPLC conditions applicable to a process monitoring operation. Good agreement between experimentally obtained and computer-predicted retention times for a homologous series of alkylketones was found for the conversion from gradient to isocratic elution conditions.

INTRODUCTION

Many chromatographers are still reluctant to use gradient elution high-performance liquid chromatography (HPLC), even though the theory of gradient elution is now well developed [1–3]. In general, gradient elution has been regarded as a research technique and has been excluded from process monitoring work, except for special applications. This situation may have developed for several reasons, including the following [4]: (1) laboratories involved with routine separations do not always have HPLC equipment that is suitable for gradient elution methods; (2) when compared with isocratic methods, gradient elutions methods are believed to be less precise; (3) because gradient elution is a more complex technique than isocratic elution, method development is more difficult; and (4) because gradient elution procedures are instrument specific, methods developed on one gradient system often do not perform in the same way when using another instrument [5].

Gradient elution does, however, have a number of advantages over the use of isocratic experiments for method development [4]. First, when gradient elution is used, fewer trial-and-error adjustments in solvent strength are required when changing from one solvent to another. Second, since early bands overlap in isocratic separations, it is difficult to establish how the resolution changes as the solvent strength is varied. As early overlapping bands are not often encountered in gradient elution, it is possible to increase the resolution during exploratory runs. Third, gradient elution experiments make it easier to locate compounds that are eluted either very early or

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very late. With isocratic separations, early-eluted compounds are often lost in the solvent front and late-eluted compounds disappear into the baseline or overlap with the next sample. Fourth, as gradient elution method development works for both gradient elution and isocratic methods, if it turns out that the final method needed to separate a sample is gradient elution, no time will have been wasted with isocratic runs that do not work.

This paper explores the possibility of using DryLab G/plus (LC Resources) for the purpose of developing isocratic methods. DryLab G/plus is one of a group of personal computer programs available for HPLC method development [4,6–11]. Although DryLab I/plus can predict isocratic retention from either gradient or isocratic input, DryLab G/plus has the distinct advantage that it can be used to develop both gradient and isocratic methods, whereas DryLab I/plus can only be used for isocratic method development.

DryLab G has been reported to predict correctly retention times for various gradient and isocratic separations [12]. It was noted that the prediction of retention times for certain isocratic conditions was susceptible to errors in the measured dwell volume. However, the predicted resolution was not as seriously affected as resolution is a function of the difference between retention times.

To use DryLab G/plus to predict isocratic retention, the program must be "tricked". This is done by entering data for two initial gradient runs into the program and then selecting Option 6.5–Multi-Segment Gradients. When asked for the number of segments in the gradient to be predicted, one segment is entered. The percentage of organic entered for the start and finish of the gradient is the same value as the percentage of organic of the isocratic run desired. A long gradient time, here 100 min, is used to ensure that all compounds in the sample are eluted.

EXPERIMENTAL

Equipment

An LC/9533 ternary-gradient liquid chromatograph (IBM Instruments, Danbury CT, USA) was used with a Model 7125 sample injector (Rheodyne, Cotati, CA, USA) with a 20-μl loop. A circulating water-bath (Model T9; P.M. Tamson, Zoetermeer, Netherlands) was used for temperature control (23.0 ± 0.2°C) of the column compartment. A variable-wavelength UV detector (Model 9523; IBM Instruments) was used. Chromatograms were processed with a Model 3390A reporting integrator (Hewlett-Packard, Palo Alto, CA, USA); this supplied values for retention times, peak area and peak area/peak height ratio, which were used to determine the band width and column plate number when necessary.

The column dead volume, $V_m = t_o F$ (where t_o is the elution time of an unretained peak and F is the flow-rate), was measured from the retention time of sodium nitrate (70% acetonitrile as mobile phase) as $V_m = 2.16$ ml [13]. The equipment dwell volume, $V_D = t_D F$ (where t_D is the dwell time of the gradient equipment), was determined by two methods. First, a blank gradient was run without the column (see Fig. 1 in ref. 14). From this method, a value of 4.5 ml was obtained at 2.0 ml/min and 4.1 ml at 1.0 ml/min. The second method used the DryLab G/plus program. Retention data for 20- and 60-min gradients were entered into the program and retention times for an isocratic run of 73% acetonitrile were predicted and compared with values obtained

experimentally, while changing the dwell volume. The best agreement between experimental and predicted retention times was found for a dwell volume of 3.9 ml. As both methods gave similar values, 4.5, 4.1 and 3.9 ml, an average was taken, giving a dwell volume value of 4.2 ml. An extra-column band broadening of $\sigma_{ec} = 0.04$ ml was used. This value was again determined by using the DryLab G/plus software. Predicted and experimental band width values for both a flat and a steep gradient (60 and 12 min) were compared while changing the extra-column value. The best agreement was found for band width values when $\sigma_{ec} = 0.04$ ml.

Materials

Columns were 25.0 × 0.46 cm I.D., packed with octyl-bonded silica (Zorbax, 880952706; Mac-Mod Analytical, Chadds Ford, PA, USA). The column packing was a spherical silica support, 5 μm in diameter, with 100 Å pores. Columns were evaluated for plate number at frequent intervals during the study; a test mixture of uracil, acetophenone, nitrobenzene, methyl benzoate and toluene was used with methanol–water (70:30, v/v) as the mobile phase. The initial plate number for toluene was *ca.* 14 000 for a flow-rate of 0.8 ml/min.

Apart from the column test mixture described above, the following sample of a homologous series of nine alkylketones was used: 2-pentanone, 2-hexanone, 2-heptanone, 2-octanone, 2-nonanone, 2-decanone, 2-undecanone, 2-dodecanone and 2-tridecanone. These compounds were selected to be well resolved in all gradient runs, so as to allow accurate measurements of retention time and band width for every peak [15].

For the gradient separations of the alkylketones, solvent A was 0.1% phosphoric acid in distilled, deionized water and solvent B was 0.1% phosphoric acid in acetonitrile. All gradients were linear and were run from 10 to 100% B. In the isocratic separations, the percentage of organic refers to the total percentage acetonitrile content of the solvent.

Chemicals

Individual alkylketones were obtained from Aldrich (Milwaukee, WI, USA) and Fluka (Ronkonkoma, NY, USA). Acetonitrile (HPLC quality) was obtained from Fisher Scientific (Fair Lawn, NJ, USA) and methanol (HPLC quality) from Burdick and Jackson (Muskegon, MI, USA). Phosphoric acid was obtained from J. T. Baker (Phillipsburg, NJ, USA). Water used to prepare the chromatographic mobile phase was first condensed from steam, then passed through an organic removal cartridge, two mixed ion-exchange resin cartridges, followed by a 0.20-μm filter (Nanopure four-module system with pump; Sybron/Barnstead, Boston, MA, USA). The water would qualify as Type I ASTM standard water having a specific resistivity greater than 18 MΩ cm.

Computer simulations

The personal computer used to run the DryLab software was an IBM-PC/XT compatible, containing an 8087 math coprocessor chip, 640K of RAM memory, a monochrome graphics printer card, with both a 30 Mbyte hard disk and a 5.25-in. floppy disk drive (DryLab LCS Liquid Chromatography Simulator; LC Resources, Lafayette, CA, USA). Associated with this computer assembly was a high-resolution

monochrome monitor (GM-1230; Casper, Santa Clara, CA, USA) and a dot-matrix printer (MX-100; Epson-America, Torrance, CA, USA).

Predictions of band width required estimates of parameters X and Y, where X is the ratio of the volume of mobile phase outside the pores to the total volume of mobile phase and Y is the ratio of solute diffusion coefficients inside and outside the pores. Based on the use of an octyl-bonded packing of 100 Å pore size, values of X = 0.75 and Y = 0.40 were used, as suggested in the DryLab G/plus Users' Manual [16,17].

Computer predictions of band width with DryLab G/plus require a value of the Knox parameter A [16,18]. This was determined using the DryLab G/plus software in much the same way that the extra-column band broadening value was obtained. Predicted and experimental band width values for both a flat and a steep gradient (60 and 12 min) were used. The best agreement was found between predicted and experimental values for A = 0.90.

RESULTS AND DISCUSSION

Fig. 1 shows a comparison of an experimental and a simulated (DryLab G/plus) chromatogram. Tables I–X contain the results of the comparisons of experimental and predicted retention times for the isocratic separations. To generate the data in Tables I–V, retention data for nine alkylketones separated by a 20-min and a 60-min gradient with a flow-rate of 1.5 ml/min was used as input to DryLab G/plus. The program was then used as described earlier to predict the retention time for a number of different isocratic separations of the same nine ketones. In Tables VI–X, a flow-rate of 2.0 ml/min was used for both the gradient input data and the predicted isocratic runs.

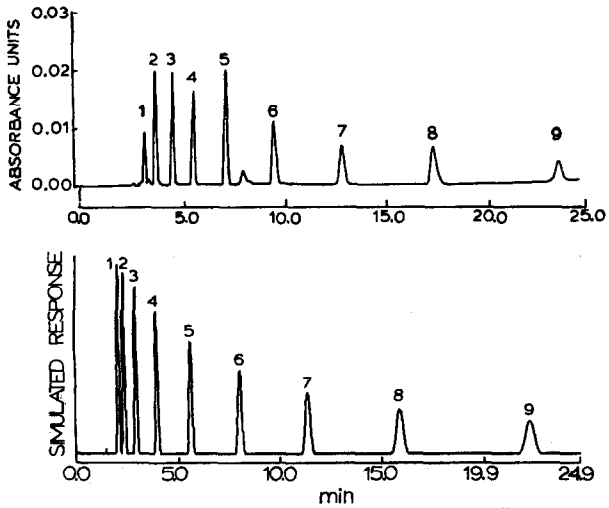

Fig. 1. Comparison of experimental and simulated (DryLab G/plus) chromatograms for an isocratic separation of nine alkylketones. Mobile phase: acetonitrile–aqueous 0.1% H_3PO_4 (70:30, v/v); flow-rate, 1.5 ml/min. Peaks: 1 = 2-pentanone; 2 = 2-hexanone; 3 = 2-heptanone; 4 = 2-octanone; 5 = 2-nonanone; 6 = 2-decanone; 7 = 2-undecanone; 8 = 2-dodecanone; 9 = 2-tridecanone.

TABLE I

EXPERIMENTAL AND PREDICTED RETENTION TIMES FOR THE ISOCRATIC SEPARATION OF ALKYLKETONES USING ACETONITRILE–AQUEOUS H_3PO_4 (70:30) AT A FLOW-RATE OF 1.5 ml/min

Solute	t_g (min)		Retention errors (±)	
	Exptl.	Calc.[a]	t_g[b]	Δt_g[c]
2-Pentanone	2.77	2.06	0.71	0.21
2-Hexanone	3.25	2.33	0.92	0.13
2-Heptanone	3.93	2.88	1.05	0.05
2-Octanone	4.91	3.91	1.00	0.30
2-Nonanone	6.28	5.58	0.70	0.42
2-Decanone	8.27	7.99	0.28	0.49
2-Undecanone	11.13	11.34	0.21	0.45
2-Dodecanone	15.19	15.85	0.66	0.38
2-Tridecanone	21.13	22.17	1.04	
Average			0.73	0.30

[a] DryLab G/plus predictions based on experimental data for 20- and 60-min gradients.
[b] Experimental minus predicted retention times (absolute values).
[c] Experimental minus predicted retention time differences, $\Delta t_g = t_2 - t_1$.

Differences in predicted and experimental retention times are expressed in two ways. The first compares experimental and predicted times directly: the absolute value of the experimental value minus the predicted value. The second uses the relationship between retention time differences, Δt_g. As R_s is proportional to the differ-

TABLE II

EXPERIMENTAL AND PREDICTED RETENTION TIMES FOR THE ISOCRATIC SEPARATION OF ALKYLKETONES USING ACETONITRILE–AQUEOUS H_3PO_4 (75:25) AT A FLOW-RATE OF 1.5 ml/min

Solute	t_g (min)		Retention errors (±)	
	Exptl.	Calc.[a]	t_g[b]	Δt_g[c]
2-Pentanone	2.60	1.91	0.69	0.22
2-Hexanone	2.98	2.07	0.91	0.11
2-Heptanone	3.45	2.43	1.02	0.07
2-Octanone	4.21	3.12	1.09	0.15
2-Nonanone	5.20	4.26	0.94	0.26
2-Decanone	6.60	5.92	0.68	0.38
2-Undecanone	8.50	8.20	0.30	0.29
2-Dodecanone	11.26	11.25	0.01	0.35
2-Tridecanone	15.09	15.43	0.34	
Average			0.66	0.20

[a–c] See Table I.

TABLE III

EXPERIMENTAL AND PREDICTED RETENTION TIMES FOR THE ISOCRATIC SEPARATION OF ALKYLKETONES USING ACETONITRILE–AQUEOUS H_3PO_4 (80:20) AT A FLOW-RATE OF 1.5 ml/min

Solute	t_g (min)		Retention errors (±)	
	Exptl.	Calc.[a]	t_g[b]	Δt_g[c]
2-Pentanone	2.48	1.80	0.68	0.18
2-Hexanone	2.76	1.90	0.86	0.16
2-Heptanone	3.15	2.13	1.02	0.07
2-Octanone	3.68	2.59	1.09	0.08
2-Nonanone	4.37	3.36	1.01	0.20
2-Decanone	5.31	4.50	0.81	0.27
2-Undecanone	6.60	6.06	0.54	0.30
2-Dodecanone	8.36	8.12	0.24	0.29
2-Tridecanone	10.84	10.89	0.05	
Average			0.70	0.19

[a-c] See Table I.

ence in retention times for two adjacent bands $(t_2 - t_1) = \Delta t_g$, errors in resolution can be related to the error in Δt_g. Because this takes into account the resolution between peaks, this method often gives a more accurate comparison of a predicted and experimental chromatogram than does the direct comparison of predicted and experimental retention times.

TABLE IV

EXPERIMENTAL AND PREDICTED RETENTION TIMES FOR THE ISOCRATIC SEPARATION OF ALKYLKETONES USING ACETONITRILE–AQUEOUS H_3PO_4 (85:15) AT A FLOW-RATE OF 1.5 ml/min

Solute	t_g (min)		Retention errors (±)	
	Exptl.	Calc.[a]	t_g[b]	Δt_g[c]
2-Pentanone	2.30	1.71	0.59	0.14
2-Hexanone	2.50	1.77	0.73	0.15
2-Heptanone	2.80	1.92	0.88	0.06
2-Octanone	3.17	2.23	0.94	0.06
2-Nonanone	3.63	2.75	0.88	0.10
2-Decanone	4.32	3.54	0.78	0.20
2-Undecanone	5.18	4.60	0.58	0.26
2-Dodecanone	6.32	6.00	0.32	0.26
2-Tridecanone	7.88	7.82	0.06	
Average			0.64	0.15

[a-c] See Table I.

TABLE V

EXPERIMENTAL AND PREDICTED RETENTION TIMES FOR THE ISOCRATIC SEPARATION OF ALKYLKETONES USING ACETONITRILE–AQUEOUS H_3PO_4 (90:10) AT A FLOW-RATE OF 1.5 ml/min

Solute	t_g (min)		Retention errors (±)	
	Exptl.	Calc.[a]	t_g[b]	Δt_g[c]
2-Pentanone	2.31	1.65	0.66	0.12
2-Hexanone	2.46	1.68	0.78	0.12
2-Heptanone	2.68	1.78	0.90	0.08
2-Octanone	2.96	1.98	0.98	0.00
2-Nonanone	3.32	2.34	0.98	0.08
2-Decanone	3.78	2.88	0.90	0.12
2-Undecanone	4.39	3.61	0.78	0.18
2-Dodecanone	5.15	4.55	0.60	0.20
2-Tridecanone	6.16	5.76	0.40	
Average			0.78	0.11

[a-c] See Table I.

The results of this study show that although the actual experimental and predicted retention times may not be exact (as indicated by the errors in predicted retention times in Table I–X), the corresponding errors in resolution are low. The errors in predicted retention times range from 0.64 to 1.05 min, with an average of 0.78 min.

TABLE VI

EXPERIMENTAL AND PREDICTED RETENTION TIMES FOR THE ISOCRATIC SEPARATION OF ALKYLKETONES USING ACETONITRILE–AQUEOUS H_3PO_4 (70:30) AT A FLOW-RATE OF 2.0 ml/min

Solute	t_g (min)		Retention errors (±)	
	Exptl.	Calc.[a]	t_g[b]	Δt_g[c]
2-Pentanone	1.98	1.52	0.46	0.22
2-Hexanone	2.32	1.64	0.68	0.17
2-Heptanone	2.81	1.96	0.85	0.11
2-Octanone	3.51	2.55	0.96	0.02
2-Nonanone	4.50	3.52	0.98	0.12
2-Decanone	5.92	5.06	0.86	0.24
2-Undecanone	7.95	7.33	0.62	0.29
2-Dodecanone	10.82	10.49	0.33	0.28
2-Tridecanone	15.03	14.98	0.05	
Average			0.64	0.18

[a-c] See Table I.

TABLE VII

EXPERIMENTAL AND PREDICTED RETENTION TIMES FOR THE ISOCRATIC SEPARATION OF ALKYLKETONES USING ACETONITRILE–AQUEOUS H_3PO_4 (75:25) AT A FLOW-RATE OF 2.0 ml/min

Solute	t_g (min)		Retention errors (±)	
	Exptl.	Calc.[a]	t_g[b]	Δt_g[c]
2-Pentanone	1.97	1.42	0.55	0.22
2-Hexanone	2.25	1.48	0.77	0.18
2-Heptanone	2.63	1.68	0.95	0.17
2-Octanone	3.18	2.06	1.12	0.11
2-Nonanone	3.93	2.70	1.23	0.04
2-Decanone	4.99	3.72	1.27	0.05
2-Undecanone	6.45	5.23	1.22	0.03
2-Dodecanone	8.49	7.30	1.19	0.01
2-Tridecanone	11.39	10.21	1.18	
Average			1.05	0.10

[a-c] See Table I.

The agreement of the corresponding error in resolution or Δt_g ranges from 0.07 to 0.30 min, with an average of 0.15 min, indicating good agreement between the experimental and predicted chromatograms.

TABLE VIII

EXPERIMENTAL AND PREDICTED RETENTION TIMES FOR THE ISOCRATIC SEPARATION OF ALKYLKETONES USING ACETONITRILE–AQUEOUS H_3PO_4 (80:20) AT A FLOW-RATE OF 2.0 ml/min

Solute	t_g (min)		Retention errors (±)	
	Exptl.	Calc.[a]	t_g[b]	Δt_g[c]
2-Pentanone	1.83	1.34	0.49	0.18
2-Hexanone	2.04	1.37	0.67	0.15
2-Heptanone	2.32	1.50	0.82	0.15
2-Octanone	2.71	1.74	0.97	0.11
2-Nonanone	3.23	2.15	1.08	0.04
2-Decanone	3.95	2.83	1.12	0.02
2-Undecanone	4.93	3.83	1.10	0.06
2-Dodecanone	6.24	5.20	1.04	0.03
2-Tridecanone	8.09	7.08	1.01	
Average			0.92	0.09

[a-c] See Table I.

TABLE IX

EXPERIMENTAL AND PREDICTED RETENTION TIMES FOR THE ISOCRATIC SEPARATION OF ALKYLKETONES USING ACETONITRILE–AQUEOUS H_3PO_4 (85:15) AT A FLOW-RATE OF 2.0 ml/min

Solute	t_g (min)		Retention errors (±)	
	Exptl.	Calc.[a]	t_g[b]	Δt_g[c]
2-Pentanone	1.75	1.28	0.47	0.15
2-Hexanone	1.91	1.29	0.62	0.13
2-Heptanone	2.12	1.37	0.75	0.12
2-Octanone	2.40	1.53	0.87	0.11
2-Nonanone	2.78	1.80	0.98	0.06
2-Decanone	3.28	2.24	1.04	0.00
2-Undecanone	3.95	2.91	1.04	0.02
2-Dodecanone	4.83	3.81	1.02	0.02
2-Tridecanone	6.03	5.03	1.00	
Average			0.86	0.08

[a-c] See Table I.

TABLE X

EXPERIMENTAL AND PREDICTED RETENTION TIMES FOR THE ISOCRATIC SEPARATION OF ALKYLKETONES USING ACETONITRILE–AQUEOUS H_3PO_4 (90:10) AT A FLOW-RATE OF 2.0 ml/min

Solute	t_g (min)		Retention errors (±)	
	Exptl.	Calc.[a]	t_g[b]	Δt_g[c]
2-Pentanone	1.71	1.23	0.48	0.11
2-Hexanone	1.83	1.24	0.59	0.11
2-Heptanone	1.98	1.28	0.70	0.09
2-Octanone	2.18	1.39	0.79	0.09
2-Nonanone	2.44	1.56	0.88	0.04
2-Decanone	2.78	1.86	0.92	0.00
2-Undecanone	3.22	2.30	0.92	0.03
2-Dodecanone	3.78	2.89	0.89	0.06
2-Tridecanone	4.51	3.68	0.83	
Average			0.78	0.07

[a-c] See Table I.

CONCLUSIONS

This study suggests that, although it was not intended for that purpose, the computer program DryLab G/plus can be used to predict isocratic retention from gradient input. Using DryLab G/plus for isocratic method development is advantageous over using DryLab I/plus, which also serves that purpose, because DryLab G/plus does not limit its user just to isocratic method development. If DryLab I/plus is used, only isocratic methods can be developed. If DryLab G/plus is used, the development of both isocratic and gradient elution method is possible.

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