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Adam P. Schellinger · Yun Mao · Peter W. Carr

Use of DRYLAB to compare octadecylsilane and carbon supports for reversed-phase chromatography of triazine herbicide test solutes

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Abstract One of the most important aspects of method development in reversed-phase high-performance liquid chromatography (RPLC) is the accurate prediction of the resolution as one or more experimental variables (usually the mobile-phase composition) are changed. By use of commercially available computer simulation programs, e.g. Drylab 2000, data from a few initial experimental runs are used to predict how the separation will vary with composition so that a computer program can then rapidly locate the optimum conditions. Assumption of a linear relationship between log k′ (retention factor) and ϕ (volume fraction of organic modifier in the element) minimizes the number of initial runs required for a separation and is usually adequate when the range of ϕ is not large. In this work the linearity of plots of log k′ against ϕ for octadecylsilane-coated silica (ODS) and carbon-coated zirconia (C-ZrO_2) columns were compared and the plots were used to determine whether prediction of the separation of triazine herbicides was adequate. Analysis of the slopes and intercepts of such plots are presented for both types of columns.

Keywords Drylab · Carbon-coated zirconia · Triazine herbicides

Introduction

A current goal of work in this laboratory is the development of a computer simulation program that will accurately predict separations using the new concept of thermally tuned tandem column (T^3C) chromatography [1, 2, 3, 4]. T^3C chromatography requires two columns with very different selectivity toward the sample constituents. Carbon-coated zirconia (C-ZrO_2) and octadecylsilane-coated silica (ODS) columns have radically different selectivity towards non-electrolytes. ODS-bonded phases have been used for many different types of samples in RPLC. Carbon-type phases are less familiar but have been used to separate a variety of compounds [5], including carbohydrates [6], enantiomeric amines [7, 8, 9, 10], ionizable compounds of medical interest [11], chiral and isomeric solutes [12, 13, 14, 15], numerous congeners [16, 17, 18], and monoamine oxidase inhibitory drugs [19]. Most work with carbon phases focuses on the separation of structurally similar compounds, which are difficult to resolve on conventional bonded phases.

Because carbon phases are relatively unfamiliar and the use of computer software to optimize separations using them has not yet been reported, we felt it appropriate to study the applicability of a well established approach to automated method development (Drylab) to this important sub-class of RPLC. Of particular importance is the extent to which carbon-type phases conform to linear solvent strength theory (LSST – see below). A recent article has described methods which can be used to improve the accuracy of computer simulation with Drylab on those occasions when when it suffers from errors from the HPLC equipment, random variations in the separation, solvent demixing (gradient elution), and failure of the modeling equations fitting retention time to mobile-phase composition and temperature [20]. In the light of this work, in this study the accuracy of predicted separations on carbon-clad zirconia and ODS columns has been compared as the mobile-phase composition was changed within a small range. Although not related to the major goals of this paper, the slopes and intercepts obtained by use of the LSST relationship were compared for both columns to see if the "S-log k'_w compensation" effect occurs for the triazine herbicide solutes [21].

Dedicated to Professor David M. Hercules on the occasion of his 70th birthday

A.P. Schellinger · Y. Mao · P.W. Carr (✉)
Department of Chemistry, University of Minnesota,
Smith and Kolthoff Halls,
207 Pleasant Street SE, Minneapolis, MN, 55455, USA
e-mail: carr@chem.umn.edu

Experimental

Instruments

All chromatography was performed with a Hewlett–Packard (HP; Wilmington, DE, USA) 1100 instrument equipped with a quaternary pump, a vacuum degasser, an autosampler, a thermostatted column compartment, a variable-wavelength UV detector, and ChemStation software. A prototype of an auxiliary heating apparatus was obtained from Systec (Minneapolis, MN, USA) for use as the hot zone for the C-ZrO_2 column. The Systec heating apparatus can be used to 200 °C and comprises a mobile-phase heat-exchanger, mobile-phase pre-heater, and a column jacket. Before reaching the detector the column effluent must be cooled to below 50 °C. Use of a 30-bar back-pressure regulator at the detector outlet prevented mobile-phase boiling.

Columns

A 5 cm×0.46 cm internal diameter (i.d.) carbon-clad zirconia column (ZirChrom Carb; C-PICA, 49 m^2 mL^{-1}; 1.6% carbon content; 3 μm particle size) was obtained from ZirChrom Separations (Anoka, MN, USA). The ODS column (5 cm×0.46 cm i.d.) was a Zorbax SB-C18 column from MacMod Analytical (Chadds Ford, PA, USA) packed by use of a method described elsewhere (80-Å pores; 125 m^2 mL^{-1}; 10% carbon loading; 5 μm particle size) [22]. The ODS and C-ZrO_2 columns are stable to 80 °C and 200 °C, respectively. The ODS column was operated at 30 °C and the C-ZrO_2 column at 60 °C unless stated otherwise.

Reagents

All reagents used were reagent-grade or better. Acetonitrile (ACN) was Burdick and Jackson HPLC-grade (Allied Signal, Muskegon, MI, USA). HPLC water was obtained from a Barnsted Nanopure deionizing system (Dubuque, IA, USA) with an "organic-free" cartridge followed by a 0.2-μm particle filter. Carbon dioxide was removed from the water by boiling. A 0.45 μm filter (Lida Manufacturing, Kenosha, WI, USA) was used to filter the water before use. The triazine herbicides were purchased from Supelco (Bellefonte, PA, USA). Acetone and uracil were purchased from Aldrich (Milwaukee, WI, USA).

Mobile-phase composition

The mobile-phase compositions used as the basis for predicting separations were 0.25 and 0.40 ACN–water (v/v). These compositions were chosen because the ϕ range (organic fraction in water) was not large and provided adequate separation on both columns. Unless otherwise stated, comparison of experimental data with predicted data was done at ϕ=0.30. Additional data at ϕ=0.20 and 0.60 (v/v) were obtained to broaden the range of mobile-phase composition, to enable better testing for curvature of plots in log k′ against ϕ and better comparison of the relative linearity of the ODS and C-ZrO_2 columns. Machine-mixed mobile phase was considered adequate for the purposes of this study. The reproducibility of separations performed by use of machine-mixed mobile phase was tested on a daily basis. Because the exact composition of the mobile phase used for the separation was unknown, due to errors in machine-mixing of the mobile phase, all separations required for estimation of linearity and prediction of separation were obtained on the same day.

Chromatographic Conditions

The flow rate was 1.00 mL min^{-1} unless stated otherwise. The sample (volume 1.0 μL) contained different concentrations (1–2 mg mL^{-1}) of analyte dissolved in pure acetonitrile. The dead volume for the ODS column was determined by triplicate injection of uracil in ACN. For the carbon column the dead volume was determined by triplicate injection of acetone in ACN. Because this measure of the dead volume varies with ϕ, average results for several mobile-phase compositions were used to provide a single dead volume that was used for all calculations. Uracil was injected without any column (using a zero volume connector), under similar chromatographic conditions, to determine the extra-column dead time (t_{ex}). The system dwell volume (V_d) was determined by use of the conventional method [23].

Prediction of isocratic retention data involves estimation of the retention factor (k′) for each component and plotting log k′ against ϕ. Solute retention factors were calculated by use of the equation:

$$k' = \frac{t_r - t_o}{t_o - t_{ex}} \quad (1)$$

where t_r is the retention time of the solute, t_o is the dead time of the column (average values of 0.490 min for ODS and 0.679 min for C-ZrO_2, respectively) and t_{ex} is the extra-column dead time of the instrumentation (found to be 0.073 min). Retention can be estimated over a narrow range of composition (two ϕ values) by use of linear solvent-strength theory (LSST):

$$\log k' = \log k'_w - S\phi \quad (2)$$

where k'_w and S, the y-intercept and slope, respectively, of a plot of log k′ against ϕ, are constant for each given solute and are specific to the stationary and mobile phases and column temperature used [23]. More specifically, k'_w is the value of k′ extrapolated to pure water as mobile phase (ϕ=0). A linear fit is assumed to provide a good approximation for interpolated and modestly extrapolated mobile phase compositions. Deviations from linearity are commonly observed when a wide range of mobile phase compositions is used.

When calculating resolution, the theoretical plate count (N) was estimated to be 5000 for a single 5-cm column based on observed plate-count values. Resolution was calculated as:

$$R_s = \frac{\sqrt{N}}{4} \cdot \frac{t_{r_2} - t_{r_1}}{t_{r_{average}}} = \frac{\sqrt{N}}{4} \cdot \frac{(\alpha - 1) \cdot k'}{\alpha \cdot (1 + k')} \quad (3)$$

where R_s is the resolution, $t_{r\ average}$ is the average retention time of adjacent peaks, N is the assumed plate count, α is the selectivity (or separation factor, k'_2/k'_1). The k′ values used were for the first peak of the pair (lowest retention time).

Drylab was used to predict separation conditions as the mobile-phase composition is changed, by use of the equations given above [24, 25, 26]. Drylab estimates enable input of a plate count (N) and band width (W) and uses these values to predict resolution. The retention factor is determined from the extra-column dead volume and column dead times based on the input data. For these reasons the retention times predicted with Drylab are used to obtain values for the retention factor (and selectivity) and resolution, by using Eqs. (1) and (3) to make direct comparisons between predicted and experimental data.

When experimental and predicted data for a separation were compared, a previous study [27] was most helpful and indicated that the error in the predicted retention factor (denoted ∂f), where f is the volume fraction of the organic modifier in the mobile phase, should be used. Error in the predicted retention factor is calculated as:

$$\partial f = \frac{-\left[\log\left(\frac{k''}{k'}\right)\right]}{S} \quad (4)$$

where k″ and k′ are, respectively, the predicted and experimental retention factors, and S the slope given in Eq. 2 is a constant for a given solute – typically 3.5 to 4.5 for small molecules in HPLC systems.

Results and discussion

Some environmental toxicants, e.g. the triazine herbicides, are slightly soluble in water. This has caused con-

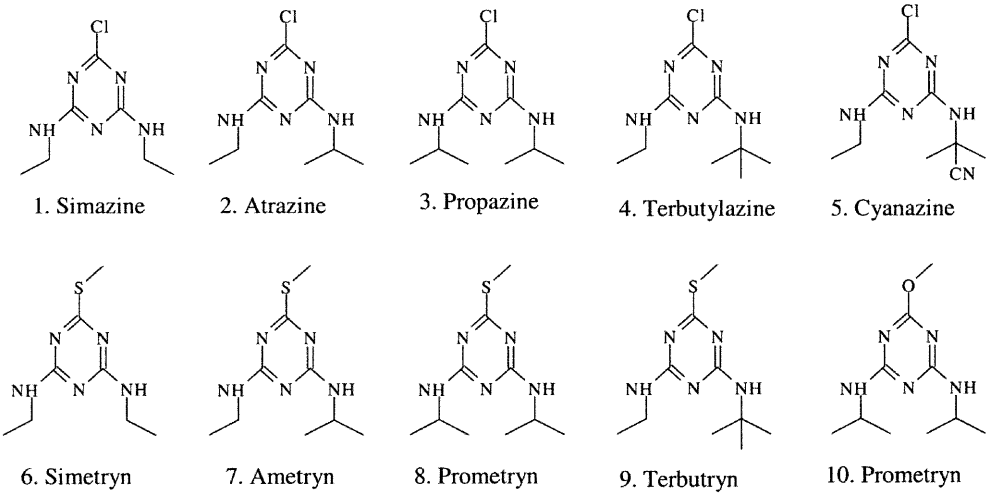


Fig. 1 Structures of the ten triazine herbicides

Table 1 Retention factors of the triazines on the ODS and C-ZrO_2 columns as a function of mobile-phase composition

	ODS[a]					C-ZrO_2[b]				
Mobile phase composition (ϕ)	0.2	0.25	0.3	0.4	0.6	0.2	0.25	0.3	0.4	0.6
Analytes										
Simazine	6.01	3.67	2.50	1.45	0.77	23.94	13.85	8.97	4.36	2.09
Cyanazine	7.66	4.28	2.72	1.45	0.77	12.01	6.86	4.40	2.24	1.08
Simetryn	13.00	7.03	4.29	2.10	0.98	70.64	38.97	23.79	10.97	3.89
Atrazine	13.79	7.59	4.67	2.25	0.98	16.76	9.59	6.16	3.13	1.31
Prometon	21.94	10.66	5.93	2.57	1.24	10.39	6.02	3.95	2.12	1.08
Ametryn	30.99	15.32	8.48	3.45	1.24	48.34	26.19	15.65	6.81	2.09
Propazine	32.56	16.38	9.13	3.68	1.36	13.02	7.22	4.60	2.39	1.18
Terbutylazine	48.22	22.40	11.67	4.30	1.62	22.22	11.82	7.11	3.24	1.40
Prometryn	74.74	34.15	17.34	5.94	1.62	32.05	17.10	10.10	4.36	1.55
Terbutryn	103.77	43.97	21.08	6.71	1.72	60.90	30.73	17.24	6.81	2.38

siderable environmental concern and engendered interest in the separation and analysis of these compounds. Conventional bonded phases have limited selectivity for their separation. Subtle differences in shape and polarity make these congeners interesting candidates for separation on carbon adsorbents. Carbon adsorbents have also been used to remove many environmentally hazardous chemicals in the petroleum and chemical industry [28].

Comparison of the linearity of plots of log k′ against ϕ for the ODS and C-ZrO_2 columns

To compare the linearity of the relationship between log k′ and ϕ for the ODS and C-ZrO_2 phases the retention of a set of ten triazines (Fig. 1) was measured for five mobile-phase compositions (ϕ=0.20, 0.25, 0.30, 0.40, and 0.60); the k′ values obtained are listed in Table 1. Least-squares regression was performed for each solute; the results are given in Table 2. For most analytes the linearity of the correlations were the same or marginally better on the C-ZrO_2 column than on the ODS column, suggesting that computer-aided optimization on a carbon-type phase should be just as accurate and facile as on an ODS phase for this type of analyte.

The F-statistic tests for differences between the average residual of the fit to Eq. (2) show the plots of log k′ against ϕ are equally linear on both phases (Table 2). Prometon is the only analyte for which deviation from linearity is clearly different and its adherence to Eq. (2) is slightly worse on ODS than on the carbon phase. We have no explanation for this observation. In view of the importance of dipolar and π–π interactions on the carbon phase [5, 29], we were mildly surprised to see the carbon phase behave so simply.

The curvature in the log k′ vs. ϕ plots is apparent in Fig. 2. The root mean square residuals for the individual analytes on the two phases are compared in Fig. 3, which shows that log k′ for all the components are more accurately, or similarly, predicted for the C-ZrO_2 column than for ODS. Predictions are extremely accurate within a short range of mobile phase strengths of approximately ϕ=0.25 but worsen outside this range. The average residual from a linear fit for both stationary phases at all compositions is at most 0.08, which is acceptable. Overall, from Table 2 and Fig. 3 it is apparent that the fit of log k′

Table 2 Comparison of log k'_w, S, and linearity for the ODS (30 °C) and C-ZrO_2 (60 °C) columns

Analyte	log k'_w[a]		–S[b]		sd[c] ×10^{-4}		r^{2d}		F_{exp}[e,f]	ϕ[g]	
	ODS	C-ZrO_2	ODS	C-ZrO_2	ODS	C-ZrO_2	ODS	C-ZrO_2	ODS/C-ZrO_2	ODS	C-ZrO_2
Simazine	1.74	2.26	3.46	3.69	2.47	1.03	0.998	0.999	5.71	0.215	0.341
Cyanazine	1.94	2.00	3.96	3.97	4.03	1.91	0.998	0.999	4.46	0.238	0.251
Simetryn	2.20	2.74	4.04	3.79	4.22	3.28	0.998	0.998	1.66	0.297	0.460
Atrazine	2.23	2.10	4.02	3.73	3.23	2.33	0.998	0.999	1.93	0.306	0.294
Prometon	2.53	1.89	4.58	3.78	6.92	2.30	0.997	0.999	9.04	0.333	0.235
Ametryn	2.71	2.65	4.65	4.12	4.88	2.44	0.998	0.999	4.02	0.367	0.401
Propazine	2.74	2.00	4.64	3.87	4.00	3.31	0.998	0.998	1.46	0.374	0.258
Terbutylazine	2.98	2.32	5.05	4.24	6.01	2.73	0.998	0.999	4.86	0.392	0.311
Prometryn	3.21	2.51	5.25	4.32	5.37	2.38	0.998	0.999	5.08	0.422	0.349
Terbutryn	3.42	2.84	5.61	4.59	7.24	3.20	0.998	0.999	5.11	0.430	0.401
Average	2.57	2.33	4.53	4.01							
Median	2.62	2.29	4.61	3.92							
Range	1.67	0.95	2.15	0.90							

[a]Average y-intercept from least-squares regression of log k′ against ϕ[h]
[b]Average slope from least-squares regression of log k′ against ϕ[h]
[c]Average residual from least-squares regression of log k′ against ϕ[h]
[d]Correlation coefficient from least-squares regression of log k′ against ϕ[i]
[e]Experimental F-ratio ($Sd^2_{ODS}/Sd^2_{C-ZrO_2}$)
[f]Critical F-ratio at a significance level of 0.05 and df=3 (numerator and denominator): 6.39
[g]Mobile-phase composition predicted from log k'_w and S values, setting log k′=1 in Eq. (2)
[h]Averages were obtained from least-squares regression of log k′ against ϕ, using both ϕ=0.25, 0.40 and ϕ=0.25, 0.30, and 0.40
[i]Values were obtained by using ϕ=0.25, 0.30, and 0.40, because the value obtained by using ϕ=0.25 and 0.40 will always be 1.000

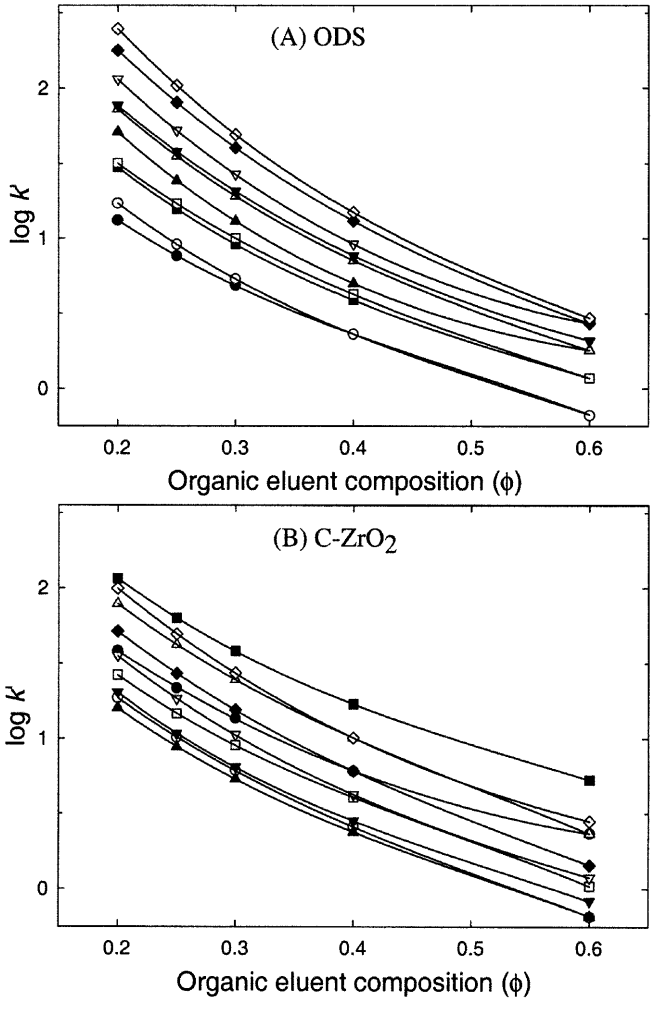


Fig. 2 Dependence of triazine log k′ values on ϕ for (**A**) the ODS column at 30 °C and (**B**) the C-ZrO_2 column at 60 °C. The lines were fitted by polynomial regression (ϕ=0.20, 0.25, 0.30, 0.40 and 0.60). Solutes: Simazine ●; Cyanazine ❍; Simetryn ●; Atrazine ❑; Prometon ▲; Ametryn △; Propzazine ▼; Terbutylazine ▽; Prometryn ♦; Terbutryn ◊

against ϕ for the C-ZrO_2 phase is somewhat more linear than for the ODS column.

Predictions of separation with two initial runs on both columns

Predicted and experimental retention times and critical pairs were compared, and then the selectivity, resolution, and retention factor were compared using predicted Drylab retention times and Eqs. (1) and (3) only (resolution is the most important comparison). The data were predicted for ϕ=0.30 using two initial runs for ϕ=0.25 and 0.40 with Drylab software. We must remark that Drylab was not used to optimize the separation with the two initial runs of ϕ=0.25 and 0.40 because we were concerned only with the accuracy of Drylab-predicted and experimental k′ values. The C_{18} bonded phase was used as the designated column with Drylab for both the ODS and C-ZrO_2 columns, because Drylab did not enable selection of the C-ZrO_2 column when this investigation was performed. Drylab's ability to fit the experimental data on the two different phases was examined for both retention time and the selectivity, by comparing the percent absolute error and the standard deviation for measured and predicted k′ values for each solute. The average over all solutes is reported in Table 3. The error and standard deviation for both

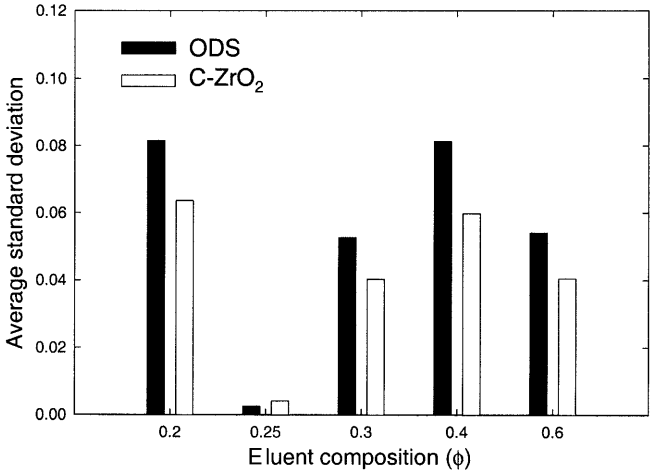


Fig. 3 Average residuals for the ten triazines from least squares regression of the data using Eq. (2) for the five mobile phase compositions (ODS 30 °C; C-ZrO_2 60 °C)

Table 3 Error and standard deviation in Drylab-predicted retention time (t_r) and selectivity[a] (α) for triazine separation at ϕ=0.30 from a linear fit of log k′ against ϕ (ϕ=0.25 and 0.40)

	ODS (30 °C)	ODS (80 °C)	C-ZrO_2 (60 °C)	C-ZrO_2 (130 °C)
Average absolute error in t_r (min)	0.076	0.057	0.059	0.035
Average standard deviation in t_r (min)	0.523	0.257	0.444	0.069
Average absolute error in α	0.013	0.012	0.011	0.009
Average standard deviation in α	0.012	0.012	0.009	0.007

Table 4 Drylab-predicted and experimental critical pairs for ϕ=0.30 using a linear fit of log k′ against ϕ (at ϕ=0.25 and 0.40)

Conditions	Predicted	Experimental
ODS (30 °C)	6.7	6.7
ODS (80 °C)	1.2	1.2
C-ZrO_2 (60 °C)	2.7	2.7
C-ZrO_2 (130 °C)	2.7	2.7

Table 5 Drylab-predicted[a] and experimental critical-pair resolution at ϕ=0.30 using a linear fit of log k′ against ϕ (ϕ=0.25 and 0.40)

Conditions	Predicted	Experimental
ODS (30 °C)	1.18	1.30
ODS (80 °C)	0.72	1.07
C-ZrO_2 (60 °C)	1.00	0.79
C-ZrO_2 (130 °C)	0.00	0.00

[a]Drylab-predicted critical-pair resolution was calculated by using the predicted retention times and Eq. (3) with N=5000

retention time and selectivity were lower for the C-ZrO_2 column than for the ODS column at both high and low temperatures (Table 3). The average absolute error and

Table 6 Comparison of error (∂f) in the predicted retention factor for ODS (30 °C) and C-ZrO_2 (60 °C) at ϕ=0.30[a]

Analyte	ODS	Carbon
Simazine	–0.006	–0.007
Cyanazine	–0.007	–0.006
Simetryn	–0.008	–0.008
Atrazine	–0.007	–0.006
Prometon	–0.010	–0.007
Ametryn	–0.008	–0.004
Propazine	–0.008	–0.006
Terbutylazine	–0.009	–0.006
Prometryn	–0.009	–0.007
Terbutryn	–0.010	–0.007

[a]Eq. (4) was used to predict the error (∂f) and the retention factors k′ and K" were calculated by use of Eq. (1)

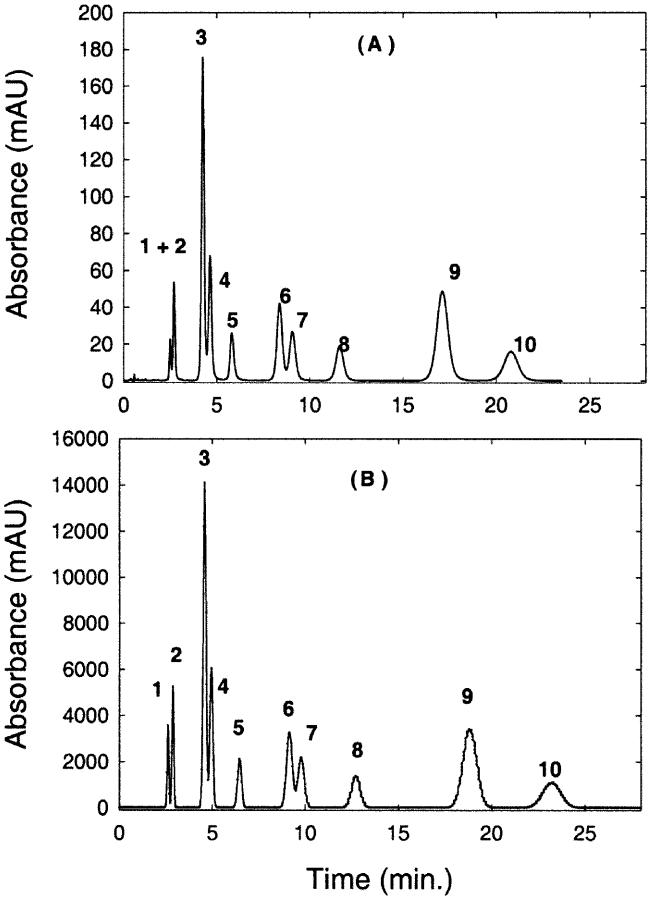


Fig. 4 Comparison of experimental (**A**) and Drylab-predicted (**B**) chromatograms for separation of the triazines on the ODS column. Conditions: temperature 30 °C, ϕ=0.30 (v/v ACN). The Drylab-predicted chromatogram was based on LSST and initial data acquired at ϕ=0.25 and 0.40

average standard deviation for predicted and experimental separation factor (selectivity) of the components on both columns were <0.013 and <0.012, respectively.

We show in Table 4 the predicted and actual critical pair for the separations. The same critical pair was observed for predicted and experimental separation at ϕ=0.30 on both

columns. With this accuracy it is assumed that predicted and experimental resolution of the critical-pair should be similar so the optimum mobile phase composition can be chosen for a separation. As is apparent from Table 5, this assumption is incorrect. Drylab over-predicts the resolution of the critical-pair for the C-ZrO_2 column (60 °C) to a lesser extent than it under-predicts critical-pair resolution for the ODS column (80 °C). As is apparent in Table 6, Drylab over-predicts the retention factor for the C-ZrO_2 column to a lesser extent than it over-predicts it on the ODS column. The actual and predicted chromatograms for the separation on each column were of similar shape, as is apparent from Figs. 4 and 5, although retention-time differences between the predicted and actual chromatograms are large. A possible reason for inaccurate prediction of the resolution, retention times, and retention factors for the columns is due to a non-linear fit of the plot of log k′ against ϕ plot. This suggests that an even smaller range of mobile phase composition around ϕ=0.30 should be used in this study. Another reason for errors in prediction on the C-ZrO_2 column could be the use of peak maximum retention time instead of center of mass retention time for the asymmetric experimental peaks (Fig. 5), i.e. predictions for the C-ZrO_2 column might have been more accurate if the peak asymmetry had been considered. Resolution is probably over-predicted by use of the theoretical plate count value of 5000, which is slightly higher than the values observed.

Differences between the selectivities of the ODS and C-ZrO_2 columns

An easy way to compare differences between the selectivity of two columns is to compare log k′ for each component separated at the same mobile phase composition on each column. As performed in a previous study [3], the two plots shown in Fig. 6 clearly show the dramatic differences between the selectivity of the ODS and C-ZrO_2 columns. Columns with similar selectivity would lead to similar log k′ values for each component at a given mobile phase composition. Differences between column selectivity are apparent in Fig. 6 from the existence of cross-

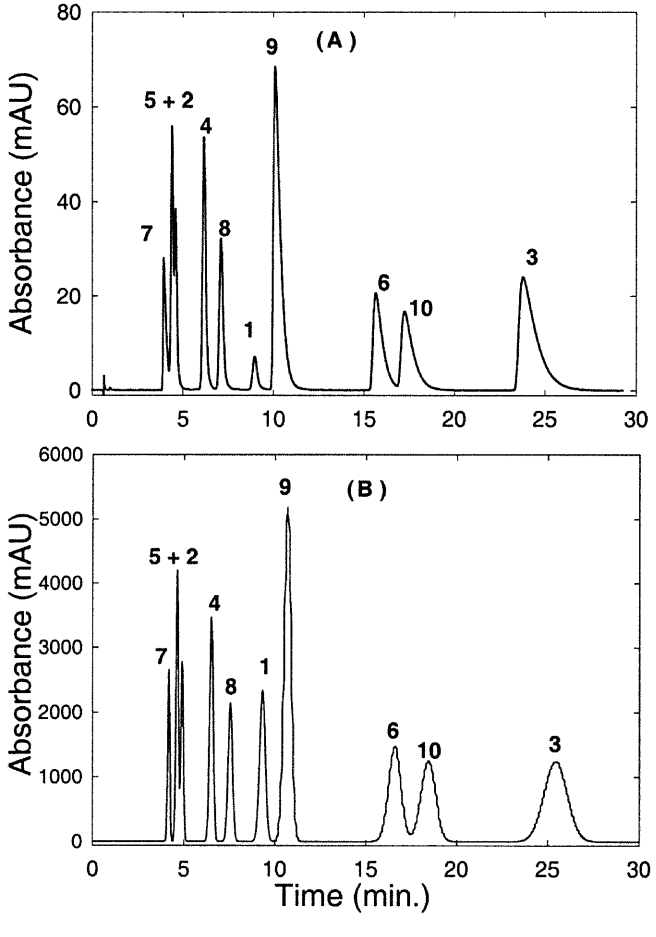


Fig. 5 Comparison of experimental (**A**) and Drylab-predicted (**B**) chromatograms for separation of the triazines on the C-ZrO_2 column. Conditions: temperature 60 °C, ϕ=0.30 (v/v ACN). The Drylab-predicted chromatogram was based on LSST and initial data acquired at ϕ=0.25 and 0.40

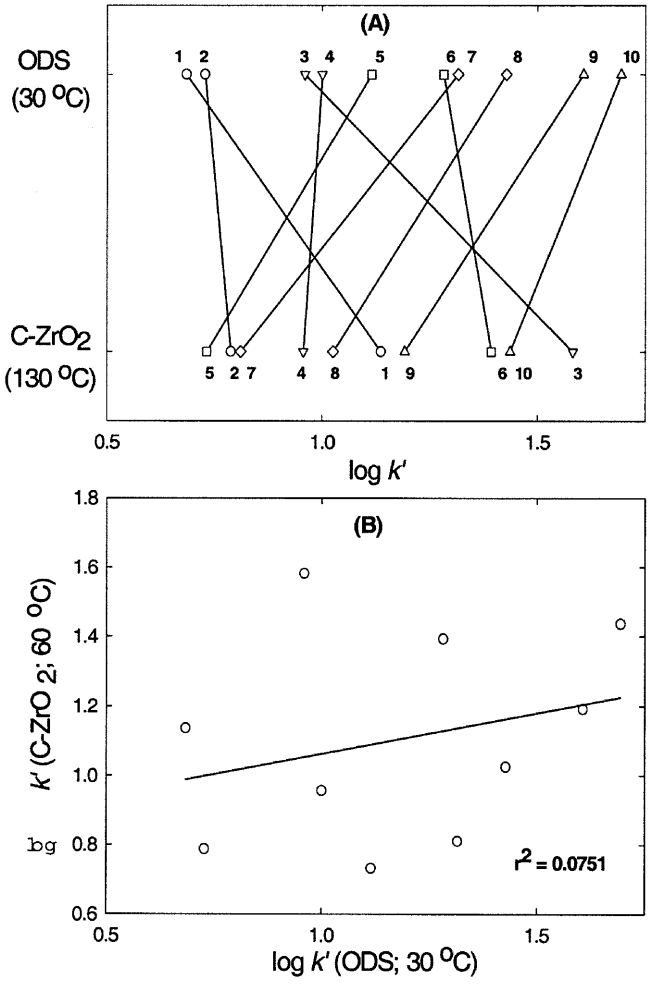


Fig. 6 **A.** Comparison of the order of elution (log k′) of the triazines on C-ZrO_2 and ODS. **B.** Plot of log k′ on C-ZrO_2 against that on ODS for the triazines at ϕ=0.30

ing lines (part A) and from the small correlation coefficient (part B) from least-squares regression of log k′ (C-ZrO_2) against log k′ (ODS).

Relationships between log k'_w and S

With the exception of simazine, cyanazine, and simetryn, both log k'_w (for all three components) and S (for simazine only) are larger on ODS than on C-ZrO_2 (Table 2). The range in log k'_w and S values is also smaller on the C-ZrO_2 column than on the ODS column (Table 2). By setting k′ equal to 1.00 in Eq. (2), comparison of ϕ on each column helps show differences between log k'_w and S values on each column (Table 2). It is interesting that ϕ (k′=1.0) values are fairly similar on both columns, in view of the fact that the ODS phase has a much larger surface area (several hundred $m^2\ g^{-1}$) than the C-ZrO_2 phase (30 $m^2\ g^{-1}$). It is clear that chemical interactions of the analyte with the carbon surface are stronger than those with the ODS surface.

Of particular interest, but not related to the main focus of this research, was the relationship between k'_w and S for triazines which vary only in the number of methyl groups on the herbicide (a pseudo-homologous series). A detailed explanation of the importance of these relationships on ODS and C-ZrO_2 has been presented in previous studies [3, 21]. As seen in Table 7, least squares regressions of log k'_w (ODS) against log k'_w (C-ZrO_2) and of S (ODS) against S (C-ZrO_2) along with log k'_w against S on one phase were performed for all the solutes and for the solutes that differ only by methyl groups with either a –Cl group (simazine, atrazine, and propazine) or a –SCH_3 group (simetryn, ametryn, and prometryn). There was no correlation between log k'_w on the two phases when the entire set of solutes was tested but there was a weak correlation between S on the two different phases. For all solutes regression of log k'_w against S on the ODS phase resulted in better correlation than the same regression on the C-ZrO_2 phase. This suggests a more complex retention mechanism on the C-ZrO_2 column.

The interesting aspect of Table 7 is the high correlation for each regression performed within a sub-class when only the number of methyl groups is varied. The correlation for the ODS column is superior to that for the C-ZrO_2 column for the regressions of log k'_w against S. The results of this study of the homologous series of triazine herbicides agree well with those from another study which investigated the pseudo-"S–log k'_w compensation" effect [21].

Table 7 Correlation of various least square regressions involving log k'_w and S

Linear regression	r^{2a}	r^{2b}	r^{2c}
log k'_w (C-ZrO_2) against log k'_w (ODS)	0.199	0.980	0.988
S (C-ZrO_2) against S (ODS)	0.779	0.911	0.984
log k'_w (ODS) against S (ODS)	0.979	1.000	1.000
log k'_w (C-ZrO_2) against S (C-ZrO_2)	0.356	0.809	0.945

[a]All the triazine herbicides were used in the regression
[b]Subsituents with a –Cl subsituent and an increasing number of methyl substituents (from lowest to highest – simazine, atrazine, and propazine) were used in the regression
[c]Subsituents with a –SCH_3 subsituent and an increasing number of methyl substituents (from lowest to highest– simetryn, ametryn and prometryn) were used in the regression

Conclusions

From this study, several major conclusions can be drawn. First, a more linear plot of log k′ against ϕ for the triazine congeners is obtained for the C-ZrO_2 column than for the ODS column, over a wide range (ϕ =0.20 to 0.60) of mobile phase strength. Second, on the basis of only two initial experimental runs, prediction of the separation on the C-ZrO_2 column is better than that on the ODS column. Predictions of retention time on both columns are adequate at both low and high temperatures (error and standard deviation in selectivity <0.05 and <0.02, respectively). Drylab can be used to provide an estimate of resolution and optimum conditions but does not seem to provide accurate chromatograms when only two experimental runs are performed over a 0.15 range in ϕ. The C-ZrO_2 column has been shown to be superior to the ODS column for prediction of the separation for triazine herbicides as mobile-phase composition is varied. Last, there is no relationship between log k'_w and S for either column, or between log k'_w on one column and S on the other column.

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