Computer-Assisted Optimization of Reversed-Phase HPLC Isocratic Separations of Neutral Compounds

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Rational selection of optimized experimental conditions for chromatographic separation of analytes is realized nowadays by means of specialized computer programs. Two such programs, DryLab (LC Resources, Walnut Creek, California, USA) and ChromSword (Merck KGaA, Darmstadt, Germany), were compared in this research. The aim of the study was to compare the optimization of chromatographic separations of neutral compounds performed isocratically in reversed-phase high performance liquid chromatography (HPLC) systems. A detailed description and discussion of the differences in predicted and experimental chromatographic retention parameters is reported. The conclusion reached is that the two programs provide identical predictions of retention data, when predictions are based on two initial experimental chromatographic measurements. An additional option of ChromSword, employing the quantitative structure-retention relationships (QSRR), appears to provide satisfactory predictions of the separation when molecular structural data along with retention data from a single initial experimental run are used as inputs. Predictions based on molecular structure alone are not very accurate and are not likely to provide useful separation information.

Introduction

Nowadays high performance liquid chromatography (HPLC) has an established place among other analytical techniques. This separation technique is commonly used in pharmaceutical and chemical laboratories (1, 2). HPLC method optimization is usually realized by trial-and-error on the basis of an individual's intuitive knowledge of the chromatographic process. HPLC method development is often time-consuming and involves an intensive exploitation of equipment and a large consumption of chemicals.

Over the years, a substantial progress in computer program development has assisted chromatographers in method development. Now, one can perform the optimization of separation conditions by means of specialist computer programs, thus reducing the trial-and-error factor (3–6).

Attempts to apply special software to facilitate HPLC method development have been reported since the late 1970s. Presently, a number of computer packages are commercially available that comprise modes for multifunctional optimizations of HPLC separations. Furthermore, each year new papers are published reflecting the progress in this area (7–9). The technical level of these programs is already high, but they still deserve continuous scientific investigations. Operational software programs allow one or more chromatographic process variables to be changed in time and, based on the response to a given change, to predict separation as a function of those variables. To that group of software programs belong: DryLab (LC Resources, Walnut Creek, California, USA), ICOS (Agilent Technologies, Palo Alto, California, USA), DIAMOND (Unicam, Cambridge, UK) and Turbo LC Plus (PerkinElmer, Norwalk, Connecticut, USA). There are also expert systems that claim to be able to predict the best initial separation conditions for a defined molecular structure. These include ChromSword (Merck KGaA, Darmstadt, Germany), EluEx (CompuDrug, Budapest, Hungary), ProDigest-LC

(Synthetic Peptides, Alberta, Canada) and ACD software (Advanced Chemistry Development, Toronto, Canada).

It was of interest to perform a comparative study of the most widely used software programs in each of the two general groups mentioned above. For that reason, the DryLab and ChromSword software draw special practical and theoretical attention because both apply the linear solvent strength model of retention. ChromSword is additionally supported by a quantitative structure-retention relationship (QSRR)–based procedure.

This study has been undertaken in order to analyse the possibilities of optimization of chromatographic separations performed under isocratic conditions. The essential problem was the description and evaluation of predictive errors and a further assessment of the accuracy of predictions generated by both programs.

DryLab

DryLab allows chromatographic separations to be modelled on a personal computer. DryLab modelling is based on input data from two or more scouting experimental runs. Separations are optimized in a computer by simulating new runs under different conditions (3). The mathematical basis for this software is the linear-solvent-strength (LSS) model that describes retention in reversed-phase HPLC by means of the following relationship (4):

$$\log k = \log k_w - Sf \qquad [1]$$

where k is the solute retention factor, k_w is the extrapolated value of k for water as mobile phase ($f = 0$), S is a constant for a given solute and a given HPLC system, and f is the volume fraction of the strong solvent in the mobile phase.

Linear model is applied when using retention data from two experimental runs as input data for predictions.

ChromSword

ChromSword also enables the user of HPLC to model chromatographic separations on a personal computer (5). This software is able to predict new separation conditions on the basis of the structural formulae of solutes (entered by drawing on a monitor screen) and/or on the basis of retention data from preliminary experimental runs. Isocratic predictions can be created based on the following:

- molecular structure of analytes alone (preliminary predictions for the next optimization steps – "first guess")
- retention data from experimental runs alone
- molecular structural data of analytes plus retention data from one, two or more experimental runs that are used to improve molecular descriptors of the solutes chromatographed under specific conditions.

Isocratic ChromSword predictions are based on two different retention models. The first one is based on Equation 1 (when one applies retention data from two runs as input data). The second one is based on a two-layer continuum model of the chromatographic system. The equation proposed for calculation of retention is as follows (5):

$$\ln k = a(V)^{2/3} + b(\Delta G) + c \qquad [2]$$

where k is the solute retention factor, V is molecular volume of a solute, ΔG is energy of interaction of a solute with water, and a, b and c are parameters that are determined by the characteristics of a reversed-phase column in the eluent being used (5).

Experimental

Apparatus: Chromatographic measurements were made on a liquid chromatographic system consisting of a pump (HPLC Pump Model 2200, Bischoff Analysentechnik und Geräte GmbH, Germany), a detector (HPLC-Spectrophotometer Lambda 1000, Bischoff Analysentechnik und Geräte GmbH, Germany) and an integrator (Chromato-Integrator D-2500, Merck Hitachi, Vienna, Austria).

The column was 15.0 × 0.46 cm i.d., packed with octadecyl-bonded silica (Kromasil KR100-5C18, Eka Nobel AB, Bohus, Sweden).

The injected sample volume was 20 µL. All chromatographic investigations were performed at 21 °C with a flow-rate of 1 mL/min.

Chemicals: Methanol (MeOH) and acetonitrile (ACN), both HPLC supra-gradient, were from Biosolve (Bio-Lab, Jerusalem, Israel). Water was prepared with the Milli-Q Water Purification System (Millipore Corp., Bedford, Massachusetts, USA).

Each mobile phase composition was prepared separately. Mobile phases used in initial experimental runs were as follows: 70/30 MeOH/H_2O (v/v) or 60/40 ACN/H_2O (v/v) (run 1), and 80/20 MeOH/H_2O (v/v) or 75/25 ACN/H_2O (v/v) (run 2). Predictions were simulated for: 75/25 MeOH/H_2O (v/v) or 70/30 ACN/H_2O (v/v).

The analytes were toluene (Aldrich Chemical Co., Milwaukee, Wisconsin, USA), naphthalene (Eindhoven University of Technology, The Netherlands), ethylbenzene (Merck-Schuchardt, Hohenbrunn, Germany), propylbenzene (Aldrich-Chemie, Stenheim, Germany), phenanthrene (Eindhoven University of Technology, The Netherlands), butylbenzene (Aldrich-Chemie, Stenheim, Germany), anthracene (Eindhoven University of Technology, The Netherlands) and amylbenzene (Aldrich Chemical Co., Milwaukee, Wisconsin, USA). Analysed mixtures of compounds were dissolved in the relevant mobile phase.

Computer simulations: Computer simulations for prediction of retention were performed with DryLab for Windows, Version 2.0 and with ChromSword for Windows, Version 1.1. Computer simulations performed with DryLab were based on two initial experimental runs. Retention data from these experiments were input data for predictions. The same procedure of predictions is also included and was undertaken in the instance of ChromSword. Simulations based exclusively on retention data from initial experimental runs are based on the linear model (Equation 1) in both programs. Hence, predicted retention data in DryLab and ChromSword in such investigation are the same. Additionally in ChromSword, predictions are made on the basis of molecular structure alone or using molecular structure in combination with retention data from one, two or more experimental runs.

Procedure to evaluate the differences in predicted and experimental retention data: A comparison of the predicted and the experimental retention data was performed according to reference 6. Errors in predicted values of retention time, $t_{R,}$ can be expressed in terms of an equivalent change (error), df, in the volume fraction of the organic modifier and f for the predicted separation. Therefore, the predicted value of t_R (k) for a mobile phase composition $f - df$ will correspond to the correct value of t_R (k) for a mobile-phase composition f. So, in the first part of the calculations the experimental and the predicted retention factors were compared on the basis of the following equation (6):

$$df = -[\log (k''/k)]/S \qquad [3]$$

where df is an error in predicted retention factor expressed as equivalent change in f, f is the volume fraction of the strong solvent in mobile phase, k" is the predicted value of solute retention factor, k

is the experimental value of solute retention factor, and S is the constant for a given solute and a given HPLC system (for small molecules this can be approximated to 4.2).

The second part of the calculations uses the relationship between df differences. Errors in Δt_R (k) for adjacent peaks i and j, as a result of errors in individual values of t_R (k), can be expressed as (6):

$$ddf = (df)_j - (df)_i \quad [4]$$

where ddf is an error in predicted values of resolution (R_S), $(df)_j$ and $(df)_i$ are the values of df for adjacent bands i and j. Additionally, statistical evaluation of the predictive errors was performed on a personal computer employing a statistical package (StatSoft, Tulsa, Oklahoma, USA). The procedure of variance analysis was executed and the T-Tukey test was used.

Results and Discussion

The results of the comparison of experimental and predicted retention data are presented in Tables 1 and 2.

Table 1 provides results for experiments performed with neutral compounds in methanol/water as the mobile phase. Average absolute df and ddf values given in Table 1 prove very accurate predictions based either on two experimental runs (option available in both DryLab and ChromSword) or on molecular parameters of solutes combined with experimental retention data from two chromatographic runs (option available in ChromSword).

Similarly, accurate predictions were obtained with the use of molecular structural data and retention data from a single initial experiment. These predictions are not as accurate as those achieved above, especially when one considers df values, but they do provide valuable separation information. Another situation was found in the instance of computer simulations based on molecular structure alone. Such predictions were not very accurate. The predicted separation was charged with a considerable error in comparison with experimentally found separation. Moreover, software was not able to distinguish between structural isomers (e.g., phenanthrene and anthracene) (Figure 1). In this particular instance, the prediction based on molecular structure allowed the whole analysis time to be estimated.

Similar results were obtained for neutral compounds using acetonitrile as the organic modifier of the mobile phase (Table 2). Although in this instance the prediction based on molecular structure plus data from a single initial experiment was charged with a larger error when compared with the simulation of

Table 1: *A Comparison of Experimental and Calculated Retention Data for the Isocratic Separation of Neutral Compounds Using 75/25/MeOH/H_2O.*

Solute	$k_{exptl.}$	$k_{calc.}$[1]	df	ddf	$k_{calc.}$[2]	df	ddf	$k_{calc.}$[3]	df	ddf	$k_{calc.}$[4]	df	ddf
Toluene	2.85	2.84	0.0004	0.0001	2.81	0.0015	0.0006	2.99	0.0048	0.0004	3.64	0.0253	0.0103
Naphthalene	4.09	4.07	0.0005	0.0005	4.01	0.0020	0.0006	4.27	0.0044	0.0008	4.73	0.0150	0.0377
Ethylbenzene	4.30	4.30	0.0000	0.0007	4.24	0.0015	0.0011	4.52	0.0051	0.0008	7.16	0.0527	0.0110
Propylbenzene	7.02	6.97	0.0007	0.0001	6.85	0.0025	0.0001	7.32	0.0043	0.0038	10.51	0.0417	0.0315
Phenanthrene	10.78	10.71	0.0007	0.0000	10.53	0.0024	0.0000	10.83	0.0005	0.0031	9.76	0.0103	0.0182
Butylbenzene	11.49	11.42	0.0006	0.0004	11.22	0.0025	0.0003	11.89	0.0035	0.0033	15.13	0.0285	0.0001
Anthracene	12.84	12.71	0.0011	0.0002	12.50	0.0028	0.0006	12.81	0.0003	0.0024	9.76	0.0284	0.0151
Amylbenzene	18.86	18.63	0.0013		18.26	0.0033		19.36	0.0027		21.44	0.0133	
Mean:			0.0007	0.0003		0.0023	0.0005		0.0032	0.0021		0.0269	0.0177

[1] Based on two initial experiments (DryLab and ChromSword).
[2] Based on molecular structure + two initial experiments (ChromSword).
[3] Based on molecular structure + single initial experiment (ChromSword).
[4] Based on molecular structure alone (ChromSword).

Table 2: *A Comparison of Experimental and Calculated Retention Data for the Isocratic Separation of Neutral Compounds Using 70/30/ACN/H_2O.*

Solute	$k_{exptl.}$	$k_{calc.}$[1]	df	ddf	$k_{calc.}$[2]	df	ddf	$k_{calc.}$[3]	df	ddf	$k_{calc.}$[4]	df	ddf
Toluene	2.41	2.45	0.0017	0.0003	2.38	0.0013	0.0003	2.32	0.0038	0.0001	3.15	0.0277	0.0100
Naphthalene	3.10	3.16	0.0020	0.0002	3.07	0.0010	0.0011	2.98	0.0039	0.0015	3.68	0.0177	0.0261
Ethylbenzene	3.43	3.49	0.0018	0.0002	3.36	0.0021	0.0011	3.25	0.0055	0.0026	5.24	0.0438	0.0150
Propylbenzene	5.23	5.33	0.0020	0.0003	5.07	0.0032	0.0009	4.84	0.0080	0.0013	6.91	0.0288	0.0273
Phenanthrene	6.20	6.30	0.0017	0.0002	5.96	0.0041	0.0007	5.66	0.0094	0.0016	6.11	0.0015	0.0131
Butylbenzene	7.04	7.14	0.0015	0.0004	6.72	0.0048	0.0001	6.33	0.0110	0.0001	6.11	0.0147	0.0018
Anthracene	7.93	8.07	0.0018	0.0001	7.58	0.0047	0.0012	7.14	0.0109	0.0029	8.98	0.0129	0.0080
Amylbenzene	12.08	12.31	0.0020		11.41	0.0059		10.58	0.0137		11.53	0.0048	
Mean:			0.0018	0.0002		0.0034	0.0008		0.0083	0.0015		0.0190	0.0145

[1] Based on two initial experiments (DryLab and ChromSword).
[2] Based on molecular structure + two initial experiments (ChromSword).
[3] Based on molecular structure + single initial experiment (ChromSword).
[4] Based on molecular structure alone (ChromSword).

separation in methanol/water system.

It is seen from Tables 1 and 2 that combining molecular parameters of neutral solutes together with experimental data from two runs does not appear to improve predictions in comparison with the program option treating retention data alone as input data. In fact, the predictions may be better when they are performed by means of molecular structural data accompanied with data from a single experiment. Moreover, predictions based on molecular structure plus data from a single initial experiment seem to be comparable to predictions using retention data from two initial experiments.

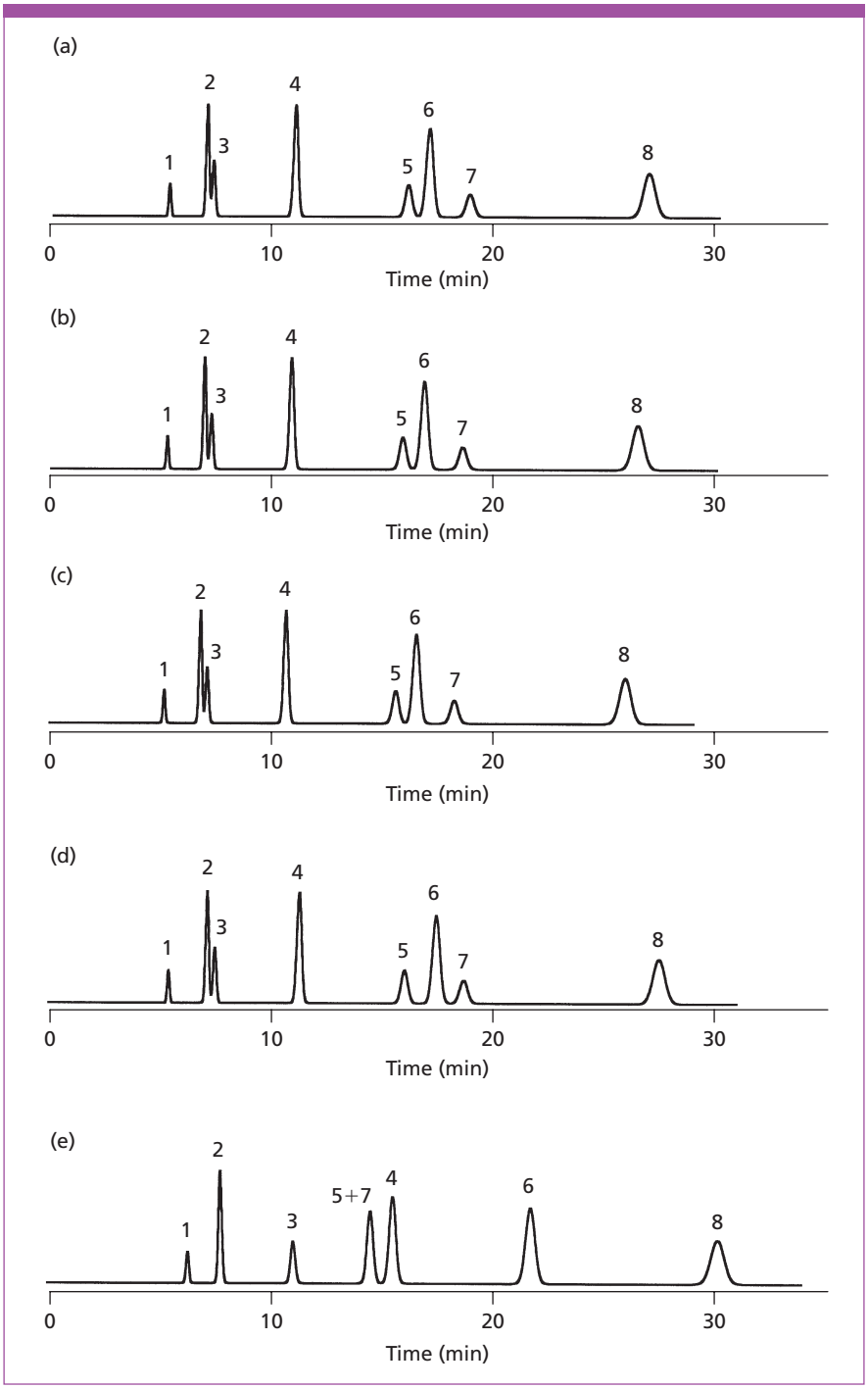

Figure 1: *A comparison of experimental versus predicted chromatograms for isocratic separation of neutral compounds using 75:25 MeOH:H_2O; (a) experimental separation, (b) predicted separation on the basis of two initial experiments (DryLab and ChromSword), (c) predicted separation on the basis of molecular structure + two initial experiments (ChromSword), (d) predicted separation on the basis of molecular structure + single initial experiment (ChromSword), (e) predicted separation on the basis of molecular structure alone (ChromSword).*
Peaks: 1 = toluene, 2 = naphthalene, 3 = ethylbenzene, 4 = propylbenzene, 5 = phenanthrene, 6 = butylbenzene, 7 = anthracene, 8 = amylbenzene.

Statistical evaluation of the results was also performed. Predictive errors (*df* values) for analytes were compared for four investigated options (i.e., two experiments; two experiments plus molecular structure; single experiment plus molecular structure; molecular structure alone). Variance analysis with the T-Tukey test revealed that there are no statistically significant differences between three tested options: two experiments' runs; two experiments plus molecular structure; and single experiment plus molecular structure. Predictions based on these three options can be treated as statistically equivalent (Table 3) at significance level $p = 0.05$.

Another situation involves predictions based on the option associated with the use of molecular structural data alone. It appeared that differences are much greater here in comparison with those obtained with the use of the three above mentioned options. It is seen in Figure 2, in which the average values of the predictive error, *df*, standard errors of estimate and standard deviations are presented.

Conclusions

Both computer programs, DryLab and ChromSword, are equally accurate in predicting isocratic retention data for neutral compounds analysed in methanol-containing mobile phase as well as in acetonitrile, when retention data from two initial experiments (both DryLab and ChromSword) or molecular structure plus data from two initial experiments (ChromSword) are used as input data.

Computer calculations based on molecular structure and single initial experiment data (ChromSword) are not as accurate as those indicated above, but are also able to deliver useful information on separations.

Predictions based on molecular structure alone are not very accurate. They do not give correct information, especially when one considers structural isomers (the same molecular formula results in the same retention time). In this option, predictions of retention data differ considerably from those obtained experimentally. However, predictions based on molecular structure of analytes alone should be treated as "first guess" only (5). Hence, in the instance of unsatisfactory separation, one can continue the process of optimization of chromatographic separations by including additional information by means of retention

Table 3: *Correlation Matrix for Four Tested Computer Options: (a) Two Initial Experiments, (b) Molecular Structure + Two Initial Experiments, (c) Molecular Structure + Single Initial Experiment and (d) Molecular Structure Alone.*

	a	b	c	d
a	1.00000	0.99997	0.95315	0.00001
b	0.99997	1.00000	0.96315	0.00001
c	0.95315	0.96315	1.00000	0.00001
d	0.00001	0.00001	0.00001	1.00000

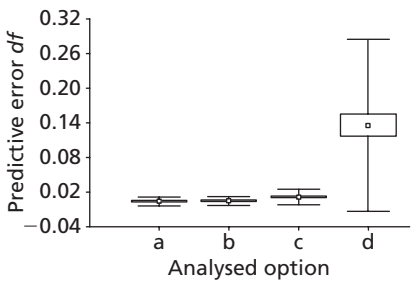

Figure 2: *A comparison of the average value of the predictive error, standard errors of estimate and standard deviations for four tested computer options: (a) two initial experiments, (b) molecular structure + two initial experiments, (c) molecular structure + single initial experiment and (d) molecular structure alone.*

data from one, two or more experiments.

Nonetheless, the idea of using information on structural properties of analytes in the process of optimization of separation conditions, which can be realized by means of ChromSword software, deserves further development. It appears especially promising when information from molecular structure is supported by retention data.

The limited success of the present version of the program can be attributed to the rather low reliability of the QSRR model advocated by Galushko and co-workers (7, 8) and expressed by Equation 2. In that QSRR model the bulkiness-related dispersive interactions of analytes with the components of the chromatographic systems are accounted for by the partial molar volume descriptor, V. That is most probably a fairly reliable parameter reflecting the non-specific structural inputs to analyte retention. Much less reliable appears to be the structural descriptor ΔG, which was intended to reflect differences in electrostatic interactions involving the analytes. Our recent observations (9, 10) suggest a QSRR model based on the following structural descriptors of analytes from molecular modelling: water-accessible molecular surface area, total dipole moment and a local molecular polarity parameter, (e.g., the highest electron excess on the most negatively charged atom). That new QSRR model in preliminary studies has been found to significantly improve the predictions of chromatographic separations.

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