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Training software for high-performance liquid chromatography

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Abstract

A computer simulation program of reversed-phase high-performance liquid chromatography was developed for training purposes. Experimental retention values of 75 organic compounds on a reversed-phase column at four different percentages of organic modifiers were reduced to a two-parameter retention model with the modifier content as variable. Modifiers used were acetonitrile, methanol and tetrahydrofuran. Isocratic and programmed solvent composition were included together with the usual experimental parameters available in modern HPLC equipment, such as UV diode array and refractive index detection. Instrument specifications were made variable within wide ranges. Detailed dispersion data were made available as tabulated output.

Keywords: Training software, LC; Computer simulation; Mobile phase composition

1. Introduction

1.1. Purpose of simulation

Simulation programs of separation methods, based on actual experimental data, can provide a valuable tool, e.g., method development and optimisation. In many such programs, entering new experimental results will eventually lead to further refinement of the model describing the behaviour of a certain combination of experimental conditions (equipment, type of column, solvent composition and sample). The ultimate purpose of the above-mentioned simulation is an exact model and perfect predictability. Obviously, many experiments will have to be carried out in order to achieve this. In addition, working with this kind of software tool requires thorough understanding of the behaviour of the system.

Whereas the programs in the above-mentioned category are invaluable in assisting experienced high-performance liquid chromatography (HPLC) practitioners, they are less suitable as a training tool for novices.

The present contribution therefore focuses on the use of simulation for training purposes: to provide the trainee with a powerful tool to acquire that understanding. For such programs, high speed and a friendly user-interface are more important than perfect agreement between simulated and experimental results. Most commonly used parameters should be incorporated in the simulation, so that potential users of the technique become acquainted with the effect of these parameters by either trial and error or (in a guided series of experiments) by systematic investigation.

In a review article on training software for another

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separation technique, namely electrophoresis, the above mentioned software categories were distinguished in more detail [1].

The use of computer simulation in training situations in analytical chemistry has a number of distinct advantages. Experiments can be performed in a safe, cheap and extremely fast way, with a high degree of reproducibility and flexibility. In this way, potential users of the technique can practise the use of suitable parameters for tuning sensitivity, selectivity and efficiency, and can become familiar with the practical ranges for different parameters.

1.2. Virtual laboratory software

The HPLC simulation program forms part of a virtual laboratory, developed at our institute, that also contains modules for gas chromatography (GC) [2], capillary zone electrophoresis (CZE) [3,4], chiral separations in capillary CZE [5,6] and micellar electrokinetic chromatography (MEKC) [7]. The programs share a common database. This shared database, and some other features, make it possible to switch from one simulator to another with the same sample composition, provided that migration/retention data are available for these components. As the application areas of some of the techniques do overlap, this is an interesting feature for comparison.

2. Experimental

2.1. Computer software and hardware

The computer program was written as a DOS application with the usual VGA resolution. The user interface was much the same as that of previously developed simulators [2–7] that have been used in our University and elsewhere for some years. The calculation times for a gradient run are within a fraction of a second when using a 486 or Pentium type processor, giving instant response to parameter changes. The program requires only about 300 kB of harddisk space. The total virtual laboratory, including the HPLC simulator fits on a 1.4 MB diskette. Details on the availability of the software can be obtained from the author by e-mail (j.c.reijenga@tue.nl).

2.2. Retention data

Retention data were obtained from Zorbax-C_8 packing material (Du-Pont), particle size 5 μm, pore size 100 Å. Organic modifiers were acetonitrile (ACN), methanol (MeOH) and tetrahydrofuran (THF), mixed with water to concentrations of 50, 40, 30 and 20% (v/v). The dataset of retention factors was a generous gift of Tan et al. [8,9]. The resulting retention factors k were linearised as log (k) vs. modifier content, according to the relation:

$$\log(k) = \log(k_0) - \beta\theta \quad (1)$$

where β is the fraction of organic modifier and θ a modifier specific parameter. Correlation coefficients were generally good. For acetonitrile the average value was 0.98 (with a standard deviation of 0.05), for methanol 0.996 (SD 0.01) and for tetrahydrofuran 0.98 (SD 0.06). Parameters log (k_0) and θ were stored in the database for each combination of sample component and modifier type. In case of missing values for some combinations, log (k_0) was set to 99 and θ to 0 so that the corresponding peak would not show up in the chromatogram because the resulting retention factor corresponds to an ''infinite'' retention time. For an overview of the retention model data see Tables 1 and 2.

2.3. Spectral data

UV spectra for many organic components are available, although not easily in electronic form. Spectral data were obtained from Sadtler Research Laboratories, Philadelphia, PA, USA. For inclusion of spectral data in the simulation database, it was decided to implement a severe data reduction. Earlier experiment showed that many UV spectra in the wavelength, λ, range 200–400 nm, as a first rough approximation, can be fitted to the following five-parameter model, consisting of an exponential and a Gaussian term:

$$\epsilon = a_1 \exp\left[(\lambda - 200)/a_2\right] + a_3 \exp\left[-(\lambda - a_4)^2/2a_5^2\right] \quad (2)$$

from which coefficients a_1 to a_5 were determined using a five-parameter curve fit. Starting values for the curve fit were estimated from the original

Table 1
Retention parameters on Zorbax-C_8 column according to Eq. (1)

	ACN		MeOH		THF	
	Log (k_0)	θ	Log (k_0)	θ	Log (k_0)	θ
Ethyl ether	1.403	3.159	2.708	4.939	0.291	1.310
Acetonitrile			−0.598	2.791	−1.220	0.532
2-Propanol	−0.766	1.755	0.748	3.541	−0.927	1.322
Methanol					−1.086	2.330
1-Butanol	1.123	3.979	2.438	4.895	0.948	3.686
Cyclohexanol	2.044	5.236	3.681	5.980	1.923	5.347
Acetone	−0.542	1.299	0.387	3.785	−1.312	0.949
2-Butanone	0.728	2.706	1.718	4.759	−0.398	1.250
Cyclopentanone	0.943	3.106	1.939	4.960	−0.207	1.658
2-Hexanone	3.299	5.934	4.671	7.417	1.951	4.175
n-Propylformate	2.229	4.320	2.735	5.113	1.215	2.662
n-Butylacetate	4.001	6.659	5.308	7.458	2.984	5.383
Ethylpropionate	2.731	5.022	3.884	6.309	1.360	2.544
Ethylbutyrate	3.853	6.309	5.155	7.320	2.861	5.006
n-Propionitrile	0.487	2.250	0.907	4.112	−0.299	1.428
n-Nitropropane	2.388	4.384	2.285	4.663	1.791	3.772
n-Valeronitrile	2.972	5.383	3.570	6.332	1.734	3.838
Butyraldehyde	1.368	3.120	2.614	5.471	0.948	3.224
222-Trifluoroetanol	0.726	2.858	0.746	3.168		
Methylenechloride	2.485	4.251	1.974	3.277	2.582	4.580
$CHCl_3$	3.770	5.862	3.693	5.153	4.593	7.909
Dibromomethane	3.199	5.254	2.911	4.428	3.604	6.304
N,*N*-Dimethyl formamide	−1.306	2.102	0.016	4.198		
N,*N*-Diethyl formamide	0.741	3.864	2.397	6.233		
Dimethyl sulfoxide	−2.779	1.679	−1.489	4.016		
N,*N*-Dimethyl acetamide	−1.170	2.042	0.749	5.029		
N,*N*-Diethyl acetamide	1.103	4.513	2.979	6.777		
Dioxane	−0.741	1.458			−1.404	0.675
Benzene	4.225	6.615	4.537	6.210	4.233	7.205
Toluene	5.481	8.305	5.755	8.059	5.406	9.123
Benzaldehyde	3.148	5.883	4.046	6.981	2.446	5.183
Acetophenone	3.490	6.546	4.758	7.928	2.514	5.397
Propiophenone	4.472	7.433	5.985	8.936	3.853	7.320
Benzonitrile	3.599	6.447	4.318	7.239	2.985	6.067
m-Tolunitrile	4.524	7.516	5.801	8.771	3.990	7.631
Nitrobenzene	3.993	6.638	4.487	6.749	4.113	7.790
m-Nitrotoluene	5.210	8.234	5.741	8.669	5.292	9.657
Anisole	4.259	6.880	5.036	7.322	4.470	8.561
Methyl benzoate	4.276	6.859	5.879	8.625	3.840	7.338
Ethyl benzoate	5.465	8.704	6.903	10.373	4.921	8.996
Phenol	2.366	5.208	3.065	5.883	3.090	6.403

spectra. The quality of the fit varied strongly from one component to another with correlation coefficients between 0.9 and 0.99. The five parameters thus obtained were assumed independent from the type and amount of organic modifier added to the water. For training purposes, the above mentioned approximation was considered sufficient. Spectra for non-UV absorbing components, not found in the Sadtler index, were estimated: zero values for a_3 and very small values for a_1 and a_2 resulting in ''universal'' response at 200 nm, the lowest wavelength value. For those components where no spectral information was available, a_2 was set to 10 nm, a_4 to 254 nm and a_5 to 20 nm as default values. In Fig. 1,

Table 2
Retention parameters on Zorbax-C_8 column according to Eq. (1)

	ACN		MeOH		THF	
	Log (k_0)	θ	Log (k_0)	θ	Log (k_0)	θ
m-Cresol	3.390	6.673	4.258	6.910	4.003	7.850
Benzylalcohol	1.940	5.100	3.145	6.056	1.637	4.393
2-Phenyl ethanol	2.769	6.341	4.050	6.811	2.368	5.685
3-Phenyl propanol	3.864	7.995	5.321	8.128	3.489	7.601
N-Benzyl formamide	1.959	5.842	3.368	7.060		
Fluorobenzene	4.510	7.097	4.871	6.604	4.722	8.200
Chlorobenzene	5.736	8.835	6.051	8.623	5.926	10.387
Ethylbenzene	5.931	9.210	6.823	9.924	5.558	9.809
n-Propylbenzene	7.121	10.822			6.606	11.582
o-Xylene	5.928	9.187	7.027	10.293	5.498	9.636
Mesitylene	6.960	10.511			6.373	11.110
Bromobenzene	5.334	8.566	6.314	9.855	6.241	10.880
Iodobenzene	5.875	9.302	6.940	10.661	5.681	10.488
n-Butylbenzene	7.228	11.145			5.973	10.569
tert.-Butylbenzene	6.675	10.454			5.630	9.993
Biphenyl	7.295	11.513			6.661	12.227
Naphthalene	6.048	9.728	7.186	11.006	5.587	10.431
Anthracene	6.894	11.029				
Benzophenone	6.621	10.497	7.377	11.858	5.896	10.709
Benzylcyanide	3.945	7.147	4.535	7.909	3.764	7.847
Benzylbromide	6.024	9.330	6.349	9.279	6.098	10.564
o-Nitrobenzyl bromide	5.833	9.312	5.765	9.256		
o-Nitrobenzyl chloride	5.466	8.768	5.667	8.444		
o-Nitrotoluene	5.072	8.147	5.576	8.658	5.143	9.475
p-Nitrotoluene	5.092	8.094	5.653	8.623	5.125	9.342
p-Cresol	3.448	6.758	4.193	6.823	3.977	7.783
o-Cresol	3.568	6.737	4.185	6.680	4.240	8.043
p-Ethylphenol	3.703	6.316	5.484	8.008	5.081	9.420
o-Chlorophenol	4.354	8.257	4.700	7.085	5.442	10.316
p-Chlorotoluene	6.115	9.544	7.456	11.329	5.869	10.626
p-Bromotoluene	6.395	9.924			6.130	11.133
p-Dichlorobenzene	6.221	9.774	7.226	10.730	6.156	11.041

the UV spectrum of *o*-cresol is given, together with the five-parameter fit. In this case, the following typical values of the parameters a_1–a_5 were obtained: 1.4, 11.4 nm, 0.24, 271 nm and 8.1 nm, respectively. Coefficient of correlation was 0.992 in this case.

3. Results and discussion

3.1. Simulated instrument hardware

In the simulator, column length (50–500 mm), internal diameter (0.1–25 mm), packing porosity (0.3–0.7) and particle diameter (1–250 μm) could be varied. The pump delivered a variable flow (0.02–2 ml/min) of a perfectly mixed water–modifier mixture of either isocratic or programmed composition. This program consisted of three plateau values, separated by a variable but linear gradient. The injection was by means of a sample loop of variable volume. The detector was operated in either the refractive index mode or the UV mode with the wavelength set between 200 and 400 nm. The volume (1–1000 μl) and pathlength of the detector cell (0.1–20 mm) and the time constant of the detector electronics (0.01–5 s) could be varied independently. These latter three parameters clearly range outside usual instrument specifications. For novice training, these should have a realistic value,

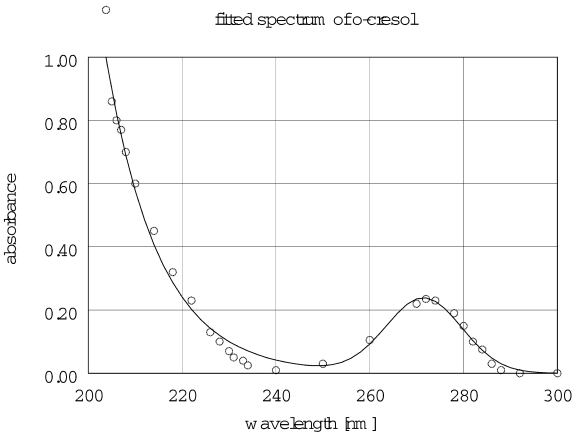

Fig. 1. Example of a five-parameter fit (curve) for the literature UV spectrum (markers) of *o*-cresol (0.142 g/l, 1 mm pathlength).

but more advanced users might obtain useful insight into equipment specifications by modifying them. As a special example for this: it is obvious that a long pathlength and a small detector cell volume are favourable, but there is an obvious trade-off, because the noise increases as the cross-sectional detector area decreases.

Although not of primary importance in selectivity tuning in HPLC, the column oven could be thermostated (0–50°C). The temperature affected the noise (depending on the detector mode), the eluent viscosity (−2%/°C) and all retention factors equally (−3%/°C).

The pressure drop over the column was calculated and displayed. This pressure drop depended on the solvent composition, temperature, flow and column parameters according to the usual relationships.

3.2. Contributions to separation efficiency

Extra-column contributions to total efficiency were limited to those originating from the injection volume, detector volume and detector time constant with the usual equations, and assuming mutual independence. The dispersion due to sample overload (other than the injection volume) was not taken into account, because of lack of data on the individual absorption isotherms. Anyhow, the peak shapes were symmetrical Gaussians in all cases. In-column effects Eddy diffusion, diffusional resistance in the mobile phase and kinetic resistance. All relevant equations can be found in chapter 3 in Ref. [10].

In the main menu of the program, the Option Dispersion command lists all individual plate height contributions, total plate height and plate number for each component in the sample mixture, so that the user has a complete overview of dispersion.

3.3. Modelling retention

In a separate window, isocratic retention input data can be shown as a plot of log (k) vs. modifier content for each of the sample components. The total analysis time to be calculated was made variable between 80 s and 80 min with the restriction that the time grid resolution (of the VGA hardware) was always 1/640 times the total analysis time. That means a time grid resolution of 1 s at 10.67 min.

For gradient elution, the same grid resolutions were chosen as mentioned above. At each time grid point, all local solvent compositions, corresponding retention factors, and resulting linear velocities were calculated, after which components were correspondingly displaced along the column until the detector was reached. The gradient composition was displayed on the screen along with the chromatogram. The gradient program consists of three variable isocratic levels (0–20 min), separated by two linear gradients of equal, variable slope (0–30%/min).

Retention was modelled to correspond to Eq. (1). Three column stationary phases were incorporated: C_2, C_8 and C_{18} modified silica, where the retention factors were assumed to be proportional to the stationary phase ligand carbon number, a very simple model which is in fact an over-estimation of the actual case. Naturally, we did not model possible effects of residual silanol groups, which one might expect in practice. An additional aspect is that of extrapolation: as the model is based on data in the modifier range 20–50%, one can expect systematic deviations from experimental practice at concentrations beyond that range. This resulted in especially annoying simulation results of a number of homologue series, where crossover of retention times at high modifier contents may occur. Some data moulding was found necessary in order to avoid such

improbable results. When extrapolating retention factors to zero modifier concentrations, users are warned not to use these values for calculating solid-phase sample clean-up recovery for obvious reasons.

Selectivity and efficiency changes are easily visualised: in Fig. 2A, a test mixture of seven components was separated using 40% acetonitrile, in Fig. 2B the same mixture using 40% methanol and in Fig. 2C using 40% tetrahydrofuran. Imagine the time gain when illustrating this in a practical course using real instruments.

3.4. UV diode array detection

The detector response to concentration was modelled according to Beer's law. The random noise level was taken proportional to the ratio of path length and volume of the cell, inversely proportional to the detector time constant, and inversely proportional to the cross-sectional area. The wavelength dependence of the detector noise was modelled according to the equation:

$$\text{Noise} = C \cdot (254/\lambda)^3 \quad (3)$$

in which C is a constant and λ is the wavelength in nm. Finally the noise was also made proportional to the operating temperature with a factor of 5% increase per degree. The diode array detector could be used in different ways. The detector could be preset for any wavelength in the range 200–400 nm. The chromatogram can be scanned in the time axis, using the cursor keys, during which the corresponding spectrum is displayed, in a separate window. In addition, a three-dimensional plot of intensity vs. time and wavelength can be plotted on the screen, also indicating, in red, the chromatogram at the preset wavelength. For each of the pure components in the sample, input spectra can be shown in a separate window.

3.5. Refractive index detection

The refractive index detector response was made proportional to the molar mass and the concentration of the sample component. The random noise level was proportional to the ratio of path length and volume of the cell and also inversely proportional to the detector time constant. Finally the noise was also made proportional to the square of the Kelvin operating temperature. Naturally, both the noise level itself and its temperature dependence were much higher than in UV detection. The alternate use of either refractive index and or diode-array UV de-

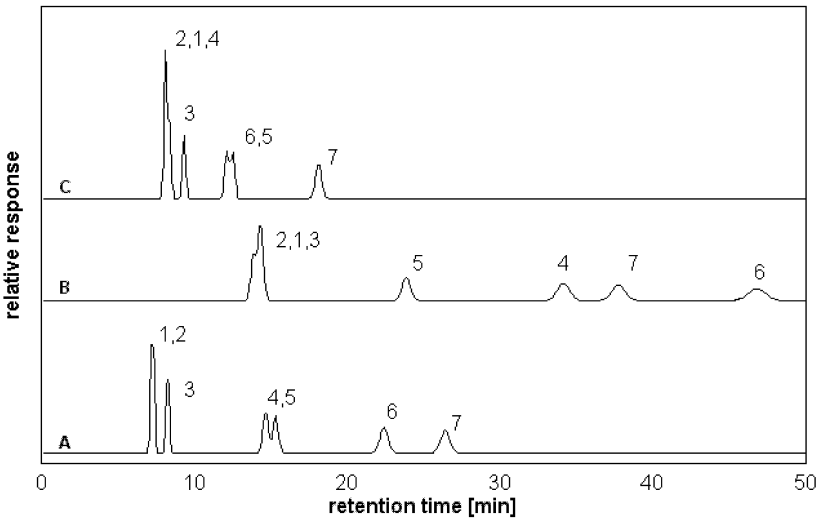

Fig. 2. HPLC separation of a mixture of *m*-cresol (1), *p*-cresol (2), *o*-cresol (3), methyl benzoate (4), benzene (5), ethylbenzoate (6) and toluene (7) using 40% modifier on a 130×4.7 mm C_{18} column, 5 μm particles, 0.7 ml/min. The modifier is acetonitrile (A), methanol (B) or tetrahydrofuran (C).

tection offers attractive possibilities to illustrate the aspects to be considered in trace analysis.

3.6. Using the programme in education

Experience with previously developed simulation software in analytical chemistry education indicates that the software can be applied on several levels and phases of the educational process. During a lecture, the software is used by the teacher to visualise the effect of any of the many parameters and variables available. The software is also used to generate example mixtures for textbooks, web-based illustrations or handouts, with the same purpose of visualisation. Because any mixture of sample components in the library can be made, and separately saved, in a practical course, different assignment can make use of the most suitable mixture and/or instrument settings. Such a course assignment usually consists of providing instruments settings and a sample mixture, and the task to systematically change one of the parameters within a given range, plotting or listing the results, and answering a number of questions, relating to the interpretation of the measured effect.

Once the student has also worked with real instruments and samples, more advanced simulation assignments are given, e.g., optimising separation or detection of a sample mixture, using combinations of several equipment settings.

4. Conclusions

The HPLC simulation software is part of an integrated package of simulators, also comprising in addition GC, MEKC and CZE. The fields of application of these analytical separation techniques show varying degree of overlap. This is especially the case for HPLC, GC and MEKC. In Fig. 3 a MEKC separation at 25°C in a typical MEKC buffer is shown of the same sample mixture as in Fig. 2. Also in Fig. 3, the isothermal GC determination of the same sample mixture is shown, using a non-polar column. Note the different selectivities and efficiencies. Needless to say that each technique should be further optimised in order to make a fair and realistic comparison between HPLC, MEKC and GC for the particular separation. In that respect, there are additional aspects besides selectivity and analysis time to be considered: detection limit is only one. For actual samples, with an unknown matrix composition (which is inherently impossible to simulate), the situation is more complicated.

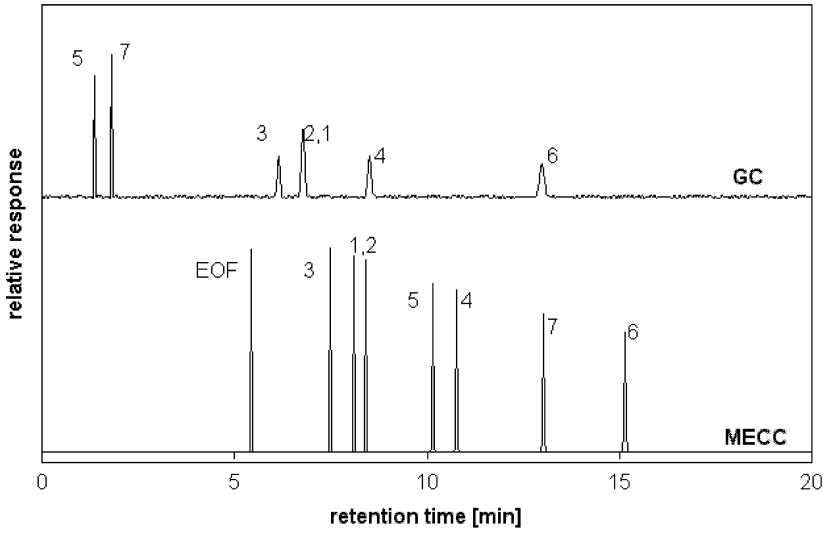

Fig. 3. The same mixture as in Fig. 2, analysed with MEKC at 25°C and with a non-polar column in capillary GC at 100°C. The detector response of both techniques is not necessarily the same.

In conclusion, we must strongly emphasise that it is not our intention to abolish HPLC equipment from the training laboratory. An integration of real and simulated experiments in our experience gives valuable training practice within a reasonable time, especially since the simulations can be done in between real experiments, while waiting for the experimental chromatogram to finish or column equilibration.

Acknowledgements

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