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Use of a threshold criterion in the computer-assisted optimization of chromatographic separations

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Abstract

A threshold criterion based on the calculation of the degree of overlap of adjacent peaks was used for the computer-based optimization of the binary mobile phase composition in HPLC. The procedure was applied to the chromatographic separation of selected phenylurea herbicides and some of their aniline degradation products. The optimization procedure was based on the modelling of retention, peak widths and peak shapes by the general exponential function, which allows non-symmetrical tailed peaks also to be described. The results from the optimization were presented as a contour map, which gives a good impression of the quality of separation over the whole range of two-dimensional space.

1. Introduction

Computer-based optimization methods in chromatography have been encouraged by a need to define method development strategies for automated chromatographic instruments. A number of optimization strategies have been developed specifically for chromatographic systems [1]. The primary purpose of optimization methods in chromatography is to obtain an adequate separation of the solutes of interest from all the other components in a reasonable analysis time. The procedures for the efficient location of the optimum in chromatographic optimization can be broadly divided into three categories: simultaneous grid-search methods, self-finding sequential methods such as the simplex method and interpretative methods [2]. Grid-search methods require large numbers of experiments, inevitable for finding the optimum [3] and the simplex procedure often locates a secondary, local maximum rather than the global optimum [4]. Regression designs start from the individual retention times and are based on the fact that a few well chosen chromatograms suffice to fit a simple model [5–10]. Many more criteria for optimization in chromatography have been discussed in the literature [3].

In this work, we used a threshold criterion introduced recently [11] in a computer-assisted optimization of the binary mobile phase composition in the HPLC of six phenylurea herbicides and some of their substituted aniline degradation products.

2. Experimental

2.1. Apparatus

Reversed-phase HPLC was performed using a Waters Model 501 pump with a Vydac 5-μm C_{18}

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column (250 × 4.6 mm I.D.) and a Waters Model 484 variable-wavelength UV detector. The flow-rate of the mobile phase was 1.5 ml/min in all experiments. The detection wavelength was 250 nm. Chromatograms were recorded and translated into ASCII code by a Waters Baseline 810 integrator.

2.2. Chemicals

Methanol was of spectrophotometric grade and was supplied by Janssen Chimia (Brussels, Belgium). The standards of phenylurea herbicides (monolinuron, linuron, isoproturon, chlorbromuron, metbromuron and chlortoluron) were supplied by the Research Institute of Chemical Technology (Bratislava, Slovak Republic). Substituted anilines (*o*-chloro-*m*-methylaniline, *p*-chloroaniline, *p*-bromaniline, isopropylaniline and 3,4-dichloroaniline) were supplied by the Department of Organic Chemistry of the Pharmaceutical Faculty of Comenius University (Bratislava, Slovak Republic).

2.3. Computer program

We used a laboratory-written computer program for the optimization, which consists of a deconvolution procedure and optimization procedure for finding the optimum. The program was written in PASCAL and runs on IBM-PC computers.

3. Optimization method

3.1. Criterion

The optimization criterion that was used in the optimization procedure is based on the quantification of the degree of overlap of K adjacent peaks. The criterion can be expressed by the following equation:

$$F = \sum_{1}^{N} I_k + \frac{c_{max} - c}{c_{max}}$$

where N is the number of components, I_k is a Boolean expression and equals one if the degree of overlap is under the threshold value p_k and zero in the opposite case, c_{max} is the maximum acceptable cost of analysis and c is the cost of an individual analysis. The degree of overlap of peak K is defined as the ratio of the overlapped area of the peak with m adjacent peaks and the total area of peak K. In the secondary part of the criterion analysis time, consumption of the mobile phase, etc., can be included. The criterion is discussed in detail elsewhere [12].

3.2. Optimization procedure

The optimization procedure requires the following information: the retention curves of all components; the peak parameters (obtained by a deconvolution procedure); and the dependences between capacity factors and the peak widths. All steps of the optimization procedure are described below.

4. Results and discussion

4.1. Modelling of retention

The retention was modelled with changing solvent composition. It is generally recognized that for most solutes the dependence between ln k' and φ (percentage of methanol in the mobile phase) is not linear when considering the full range of eluent compositions and therefore it may become necessary to express the functional dependence of ln k' on φ as a second-order polynomial, but over the limited range of methanol–water compositions ($1 < k' < 10$) the ln k'–φ dependence of most solutes is linear according to [13].

$$\ln k' = \ln k_0 - S\varphi \quad (1)$$

The correlation coefficients of the linear dependences (mostly higher than 0.99) and the slopes and intercepts of Eq. 1 for individual compounds are given in Table 1.

Table 1
Determination of slopes and intercepts of the dependences in Eqs. 1 and 2 and the choice of the shape parameters *a* and *b* included in the GEX function

Compound	Ln k_0	S	Correlation coefficient, r	A (min)	B (min)	Correlation coefficient, r	a	b
o-Chloro-*m*-methylaniline	2.065	5.09	0.99456	0.0945	0.1096	0.99956	0.90	5.00
p-Chloroaniline	3.837	7.20	0.99921	0.0995	0.1128	0.99325	1.55	5.00
p-Bromoaniline	4.395	7.86	0.99875	0.0997	0.1199	0.99762	1.80	5.10
Monolinuron	5.000	8.44	0.99936	0.0889	0.1109	0.99744	1.80	5.05
Isopropylaniline	4.762	7.70	0.99653	0.0890	0.1160	0.98532	1.95	5.05
Metbromuron	5.395	8.81	0.99921	0.0986	0.1199	0.99936	1.88	4.95
Isoproturon	5.337	8.63	0.99962	0.0950	0.1090	0.99712	2.03	4.90
Chlortoluron	5.884	9.28	0.99935	0.0897	0.1138	0.98965	1.76	5.00
3,4-Dichloroaniline	5.624	9.00	0.99982	0.0982	0.1173	0.99351	1.50	5.25
Linuron	6.572	9.89	0.99957	0.0986	0.1170	0.99663	1.87	5.00
Chlorbromuron	6.751	9.97	0.99952	0.0908	0.1184	0.98796	2.06	4.90

4.2. *Modelling of peak shapes*

Peak asymmetry can arise from a variety of instrumental and chromatographic sources. They include incomplete resolution of sample components, slow kinetic processes, chemical reactions and also the formation of column voids [14]. In reversed-phase chromatography the presence of tailing peaks of basic compounds can be explained by their interactions with acidic sites on reversed stationary phases by ion-exchange processes. Therefore, the peaks of substituted anilines are non-Gaussian but right tailed. Any peak in a chromatogram is characterized by several parameters such as retention time, peak height, peak width and shape parameters. We have described all peaks in a chromatogram by the generalized exponential function (GEX) [15]. This function is shown to be very general. For a given set of K peaks it may be represented by

$$h = \sum_{i=1}^{K} \left\{ h_{m,i} t_i^{(b_i-1)} \exp\left[\frac{b_i - 1}{a_i} \cdot (1 - t_i^{a_i}) \right] \right\}$$

where

$t_i = (t - t_{0,i})/(t_{R,i} - t_{0,i})$
$t_{0,i}$ = start of a peak i in minutes;
$t_{R,i}$ = retention time of a peak i;
$h_{m,i}$ = height of a peak i;
a_i, b_i = shape parameters of a peak i;
h = signal of a peak i at time t.

The retention time $t_{R,i}$ can be calculated from the ln k'–φ dependences relatively accurately (see Table 2). The start of a peak was calculated according to the experimentally measured dependence between $t_{R,i} - t_{0,i}$ and the capacity factor as

$$t_{R,i} - t_{0,i} = A + Bk' \quad (2)$$

Eq. 2 follows from basic chromatographic theory. Assuming that the start of a peak is a fixed number C of standard deviations away from its top, we obtain

$$t_{R,i} - t_{0,i} = C\sigma_i = C \cdot \frac{t_{R,i}}{\sqrt{N_i}}$$

$$t_{R,i} - t_{0,i} = C \cdot \frac{t_d}{\sqrt{N_i}} + C \cdot \frac{t_d}{\sqrt{N_i}} \cdot k'_i$$

This equation predicts the slope B and intercept A to be equal. Table 2 shows that this bears out reasonably in practice. As can be seen from Table 2, this dependence is linear for all the solutes tested. Peak heights were calculated from the individual peak areas, which were the inputs to the optimization procedure. The peak

Table 2
Comparison of experimentally measured and predicted values of capacity factors and degrees of overlap under the global optimum conditions

Compound	k'		DO_k	
	Predicted	Experimental	Predicted	Experimental
o-Chloro-*m*-methylaniline	0.69	0.67	0.00	0.00
p-Chloroaniline	1.46	1.48	0.28	0.40
p-Bromoaniline	1.87	1.88	0.31	0.42
Monolinuron	2.59	2.61	2.07	3.12
Metobromuron	3.20	3.18	60.40	67.96
Isoproturon	3.30	3.29	54.60	61.90
Chlortoluron	4.17	4.19	1.09	0.99
3,4-dichloroaniline	3.67	3.68	3.81	2.93
Isopropylaniline	2.90	2.89	5.92	5.99
Linuron	6.21	6.29	0.04	0.073
Chlorobromuron	7.16	7.21	0.03	0.057

asymmetry was calculated for all peaks with changing mobile phase composition and hence with changing capacity factors. We observed that the peak asymmetry varied only very slightly or not at all over the limited range of capacity factors. Therefore, only one deconvolution procedure was needed for obtaining the shape parameters a and b (see Table 1).

The algorithm of the deconvolution procedure is shown in Fig. 1 [16], where x is a vector of parameters of function $f(x)$, ε is the precision of the minimization procedure, k is a constant which prevents the method from accumulating errors due to the method of calculation of vector h, ω specifies the method of minimization (ω = 0, Fletcher–Reeves; $\omega = 1$, Polak–Rieber), i is a counter, g and h are the vectors and OPTIM(x, h) is a function which optimizes the value of λ for which the function $f(x+\lambda h)$ has a minimum. For a chromatogram consisting of m data points $[(h_i, t_i)$ and $i = 1, 2, \ldots, m]$ and K overlapping peaks, the function $f(x)$ from Fig. 1 may now be written as

$$f(x) = \sum_{i=1}^{m} (h_i - h_{\mathrm{exp},i})^2$$

where $h_i = \Sigma_{j=1}^{K} h_{j,i}$, $h_{\mathrm{exp},i}$ is the value of the recorded signal in a chromatogram at time t_i and h_i represents the sum of K GEX functions at the same time.

The deconvolution program was interfaced with a Baseline 810 workstation and loading of data was done automatically. A typical deconvolution using our program for four peaks takes *ca.* 2 min on a PC-AT 386 computer, which is an acceptable run time. With all the above-mentioned parameters, the optimization program can calculate the degree of overlap for an individual peak at a given composition of mobile phase and also the value of the F criterion.

Fig. 2 shows the result of optimization (x-axis = degree of overlay, y-axis = mobile phase composition, z-axis = F criterion). The figures in the middle of the zone indicate the number of sufficiently resolved peaks in this zone, meaning the number of peaks with a degree of overlap equal to or smaller than the given value of the threshold p_k. The choice of p_k depends on the required peak purities (*e.g.*, acceptable error of the method). To understand better the meaning of p_k, the dependence of the chromatographic resolution of the degree of overlap DO_A or DO_B of two equal Gaussian peaks is shown in Fig. 3. The p_k value can be selected analogously to the threshold values of other threshold criteria (*e.g.*, min $k_\omega \cap R_{s,\min} \geq x$). The slice of Fig. 2 for a given value of threshold 10% (which is responsible for a chromatographic resolution of *ca.* 0.85) is shown in Fig. 4. The optimum (nine peaks were separated with a degree of overlap $\leq$

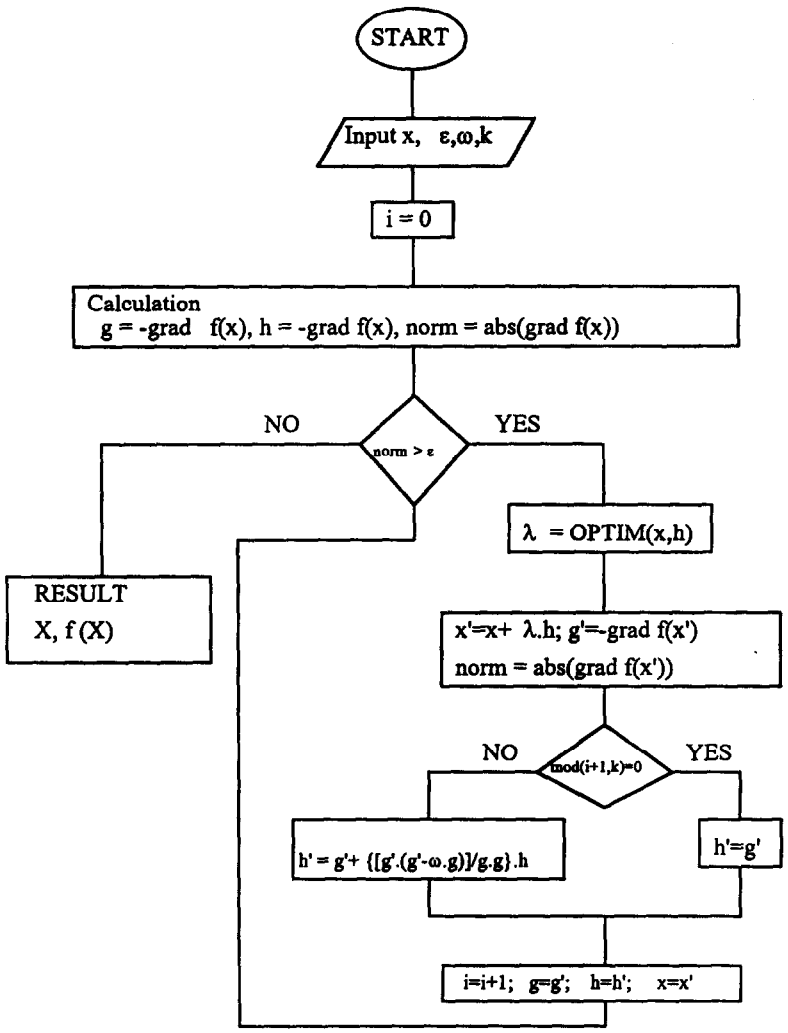

Fig. 1. Algorithm of the minimization procedure included in the deconvolution procedure [17].

10%) lies between 45 and 51% of methanol in the mobile phase. The global optimum for eleven components was found at $x = 6\%$, $y = 48\%$, $z = 9$. Two peaks (metbromuron and isoproturon) were strongly overlapped, but the degree of overlap for the other components was below 6% of the total area of the peak. Real and simulated chromatograms of ten components (metbromuron was excluded) measured with 48% of methanol in the mobile phase are shown in Figs. 5 and 6. In Table 3, the individual degrees of overlap for the set of measured and simulated chromatograms are compared.

5. Conclusions

The threshold criterion was used for the computer-assisted optimization of the binary mobile phase composition for the HPLC separation of selected phenylurea herbicides and some of their aniline degradation products. The F criterion counts the number of sufficiently resolved peaks realistically. It also reflects the height ratio of adjacent peaks and also the influence of other peaks that can interfere with the peak being investigated. The presentation of results as in Fig. 2 gives a good indication of the quality of

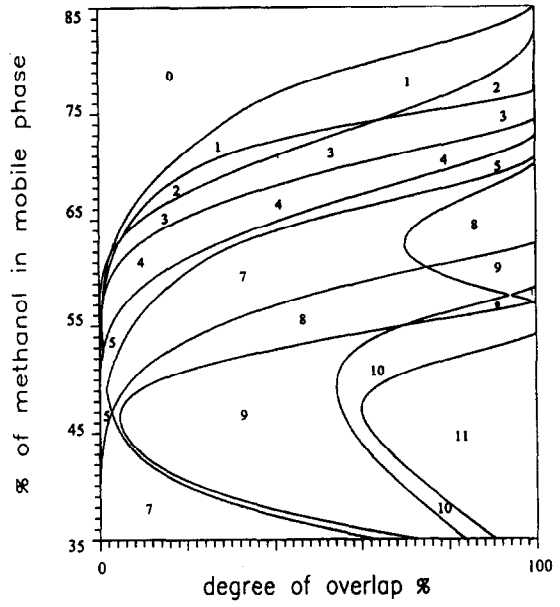

Fig. 2. Results of optimization procedure for eleven components. For details, see text.

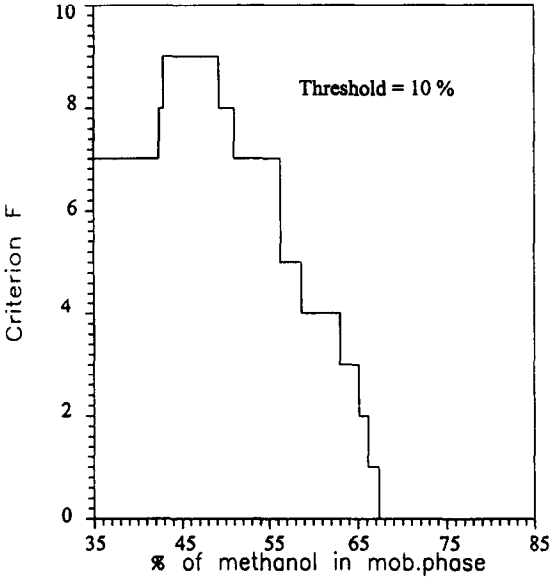

Fig. 4. Dependence of F criterion on the mobile phase composition if the threshold p_k is chosen to be 10% of the total area of the peak investigated.

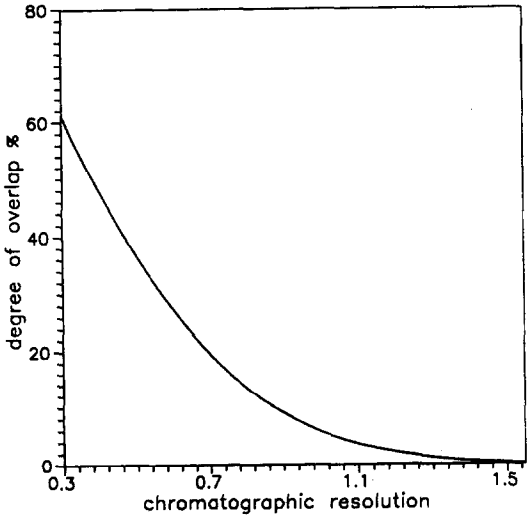

Fig. 3. Dependence of chromatographic resolution on the degree of overlap of two equal Gaussian peaks.

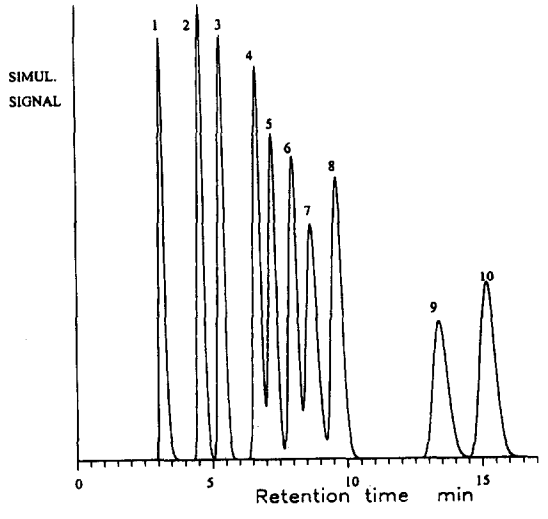

Fig. 5. Chromatogram simulated with the parameters corresponding to the global optimum (48% of methanol in the mobile phase). Peaks: 1 = *o*-chloro-*m*-methylaniline; 2 = *p*-chloroaniline; 3 = *p*-bromoaniline; 4 = monolinuron; 5 = isopropylaniline; 6 = isoproturon; 7 = 3,4-dichloroaniline; 8 = chlortoluron; 9 = linuron; 10 = chlorbromuron.

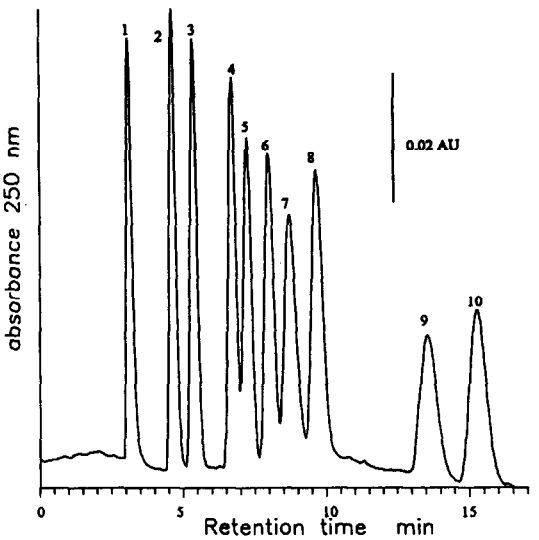

Fig. 6. Experimentally measured chromatogram under the global optimum conditions. Column, Vydac C_{18}, 5 μm (250 × 4.6 mm I.D.); flow-rate, 1.5 ml/min; UV detection at 250 nm. Peaks as in Fig. 5.

separation not only for the given value of the threshold but also over the whole range of p_k values. The results presented indicate relatively good agreement between the simulations and experiments.

6. References

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