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Optimization of chromatographic methods by a combination of optimization software and expert systems

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SUMMARY

Expert systems and other sophisticated computer programs can be of great benefit for the optimization of chromatographic separations. However, two factors have seriously hindered their proliferation. First, the area of method development for chromatography encompasses such a great variety and such a large amount of knowledge and expertise that it is not realistic to try and cover the entire area with a single program. Second, computer programs may be very complex to use, so that only experts can apply them. Steps towards the solution of both problems are described. Three different computer programs, two of which are expert systems, are used coherently for method optimization. Each system can assist the chromatographer in performing a certain well-defined task. The selectivity-optimization system (Diamond) is a package of conventional computer programs. Therefore, we refrain from calling it an expert system. One expert system is specifically applied to reduce the level of expertise required for applying this package. The most difficult decision that a Diamond user needs to make is the selection of the most appropriate optimization criterion. This decision can be made with the help of the expert system for CRIterion SElection (CRISE). The second expert system (System-Optimization System, SOS) is used to transform the chromatogram with optimum selectivity that results from Diamond into the optimum overall method by establishing the best column dimensions, flow-rate, instrumentation, injected amount, *etc.* An example is presented to demonstrate that the coherent use of several sophisticated computer programs can make method development in chromatography both better and easier.

INTRODUCTION

The major steps in developing a chromatographic method can be identified as

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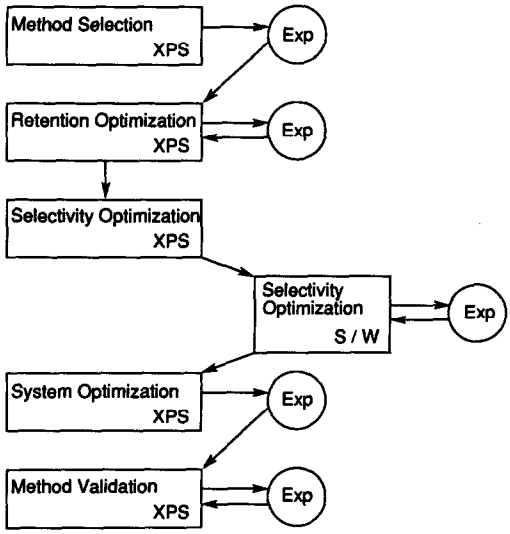

Fig. 1. Major steps in developing a chromatographic method. Boxes represent software (S/W) and circles represent chromatographic experiments (Exp). XPS denotes expert system.

follows[1,2] (Fig. 1): (i) method selection; (ii) retention optimization; (iii) selectivity optimization; (iv) system optimization; and (v) method validation.

Method selection is the process of selecting the appropriate chromatographic method [*e.g.*, gas chromatography (GC) or liquid chromatography (LC); normal-phase or reversed-phase LC; ion-exchange or ion-pairing LC, etc.] and the appropriate conditions (temperature, mobile-phase composition, pH, etc.) to elute the sample components as (reasonably) sharp, (reasonably) symmetrical peaks. Method selection is based on an understanding of the different chromatographic techniques and, most of all, on knowledge about the sample and its components. The goal of the method-selection step is to obtain chromatographic peaks for all the sample components of interest. It is important that none of these compounds remains on the column. Therefore, it is better at this stage when retention is too low than when it is too high. A typical chromatogram that may be obtained is the top one in Fig. 2.

In the *retention-optimization* step, the peaks obtained in the chromatogram after the method selection will be moved into the optimum range of capacity factors, usually by varying the composition of the mobile phase. It may be possible to predict the conditions for optimum retention based on a knowledge of the chromatographic process and the initial chromatogram. It may be necessary to improve this prediction after a second chromatogram has been obtained. The resulting chromatogram may typically be the second one in Fig. 2. In this chromatogram, all peaks are eluted in the optimum retention range, but not all of them are separated.

If the retention times of all peaks are in the optimum range, but the separation is not satisfactory, there are two possible solutions, selectivity optimization and system optimization.

Selectivity optimization[3] is the process that aims at improving the separation by altering the retention of the individual sample components relative to each other. To

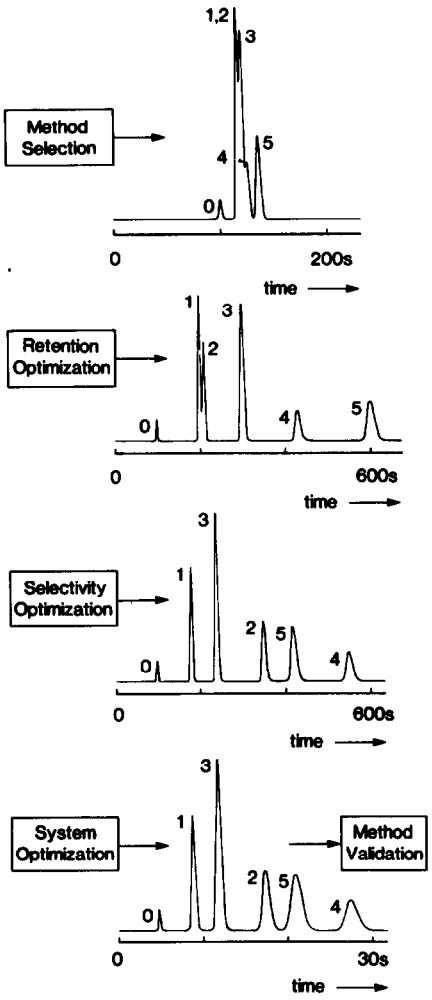

Fig. 2. Schematic illustration of a series of chromatograms that may be obtained during the development of a chromatographic method.

change the selectivity, either the stationary phase (GC or LC) or the mobile phase (LC) will usually be changed. Selectivity optimization is not usually a predictable process. Therefore, a number of sophisticated experimental optimization procedures have been applied to or developed for chromatography[3–5]. The desired result of the selectivity-optimization process is a chromatogram in which the peaks are more evenly distributed, as is illustrated in Fig. 2.

System optimization can be used either if the resolution is higher than required or if the separation is not good enough. In the first instance it may lead to a considerable reduction in the analysis time and in the second case it may be used to increase the resolution. Improving the sensitivity (signal-to-noise ratio) of the method may also be one of the goals of the system-optimization process. Parameters considered during this process may typically be the dimensions of the column, the flow-rate,

sample volume, etc. The effects of these parameters are to a large extent predictable, so that much of the system optimization can be based on calculations[6,7] or computer simulations[8]. An example of a result of a system-optimization step is shown in Fig. 2.

The final step in the method-development process is the *method validation*. It will always be necessary to demonstrate the applicability of the proposed method for the intended purpose. This very purpose will determine the extent to which a method will need to be validated. Generally, the more often a method is intended to be applied, the more different people will be using it and the greater the consequences of the results obtained by the method, the more extensive will be the testing[1]. Method validation will typically involve setting up systematic test programmes, performing series of experiments and evaluating the results. Experimental designs and statistics are important aspects of this process.

In recent years, there has been much interest in the use of computer programs to assist the chromatographer in developing chromatographic methods (see, *e.g.*, refs. 3, 4 and 9). An ideal situation may be one in which the user (chromatographer) can consult a single computer program, through which he or she can direct the method-development process, control the instrument, collect the data and evaluate the results. Within such a computer program a number of modules may exist to assist the user in the different steps of the method-development process. In other words, all the boxes in Fig. 1 may be called upon by the computer program. At present, such an ideal situation cannot yet be approached. In this paper, a step is made towards such an ideal situation by looking at the coherent application of several sophisticated software programs for optimizing liquid-chromatographic separations. The three programs that have been used in the present work are the following (Fig. 3): an expert system for the selection of the most suitable criterion for selectivity optimization[10]; a program for the optimization of selectivity by varying the composition of the mobile phase[11]; and an expert system for optimizing the column, operating conditions and instrumentation[12].

Expert system for criterion selection

The expert system for criterion selection represents one of the aspects of selectivity optimization, as is illustrated in Fig. 4. In order for an experimental selectivity-

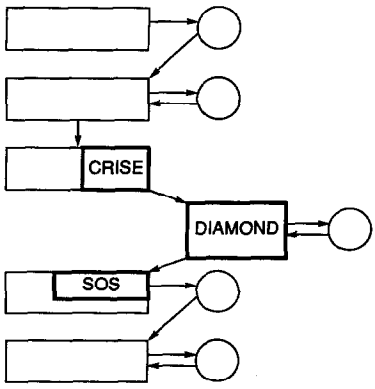

Fig. 3. Three computer programs studied in this work in relation to the scheme in Fig. 1.

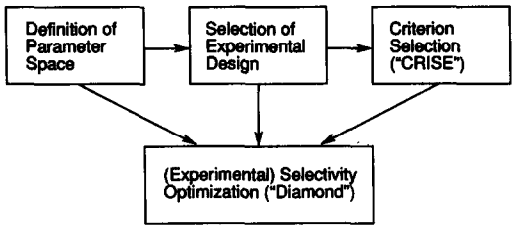

Fig. 4. Major aspects of selectivity optimization in chromatography.

optimization procedure (bottom) to be performed, decisions are needed on (i) the parameter space, *i.e.*, the parameters (variables) that will be considered together with their minimum and maximum values (limits); (ii) the experimental design, *i.e.*, the pattern according to which the necessary experiments will be performed; and (iii) the optimization criterion, *i.e.*, the parameter that will be used to judge the quality of the chromatogram. The goal of the selectivity-optimization process is to find the set of conditions (within the parameter space) that results in the best possible value for the optimization criterion. This set of conditions is referred to as the optimum.

There is not a single optimization criterion that is always the best one to use. Which criterion should be used will depend on the method to be developed, the optimization procedure to be used, the characteristics of the sample and the possibilities of the user. The expert system for CRIterion SElection (CRISE) has been described in detail elsewhere[10]. It assists the user in selecting the most appropriate optimization criterion.

Selectivity optimization

The selectivity-optimization procedure used has been described elsewhere[11,13]. The Diamond package features an interpretive optimization procedure, including (i) the definition of an (approximately) isoeluotropic triangle, with binary mixtures of, *e.g.*, methanol–water, acetonitrile–water and tetrahydrofuran–water at the corners, ternary mixtures along the sides and quaternary mixtures in the middle; (ii) the recording of ten three-dimensional (3-D) diode-array chromatograms equally distributed throughout the triangle; (iii) the labelling of the peaks in the 3-D chromatograms to determine the retention times of each individual solute at each composition; (iv) the modelling of the retention surfaces for all individual solutes; and (v) the calculation of the response surface (optimization criterion *vs.* composition) for the entire chromatogram.

System optimization

The expert system for system optimization has been described in detail[12,14]. The mobile and stationary phase are not altered in this process, but the column dimensions, flow-rate, etc., may be changed. Based on an initial chromatogram and a set of initial conditions, the program selects the best possible column from a column database created by the user and combines this with the best possible detector (cell) from the detector database and with the best possible time constant from a list of possible values. The optimum result is defined as (i) the resolution for all relevant pairs of peaks must exceed a minimum value specified by the user; (ii) the signal-to-

noise ratio for the smallest relevant peak must exceed a minimum value specified by the user; and (iii) the required analysis time should be as short as possible. Together with the optimum column, detector cell and time constant, the system recommends the optimum flow-rate and sample size and predicts the required analysis time, the "critical resolution" (*i.e.*, the lowest value observed for the resolution between a relevant pair of peaks) and the pressure drop over the column. It also provides an explanation of its reasoning in the form of a bar chart and some additional advice to the user[14].

EXPERIMENTAL

The expert system for criterion selection (CRISE) was implemented in the commercially available expert-system shell KES (Knowledge Engineering System; Software Architecture and Engineering, Arlington, VA, U.S.A.; release 2.4). The expert system runs on an Apollo workstation and on an IBM PC.

A prototype version of the Philips Scientific (Cambridge, UK) Diamond package for the selectivity optimization in high-performance liquid chromatography was run on a Philips 3202 personal computer (IBM/AT compatible). Data acquisition was performed using a Philips Scientific 4120 diode-array detector and the PU 6003 diode-array datastation running on the 3202 computer. Three-dimensional chromatograms were recorded at ten points in the isoeluotropic solvent triangle and peak labelling could be performed using the routines available within the Diamond package[11].

The version of the system-optimization expert system used was written in Pascal for a MicroVAX workstation (Digital Equipment, Maynard, MA, U.S.A.). An extensive description of this system has been given elsewhere[14]. An IBM-PC version of this system is now commercially available through Philips Scientific.

The practical example considered in this study concerns the separation of ten phenolic priority pollutants: phenol, 4-nitrophenol, 2,4-dinitrophenol, 2-chlorophenol, 2-nitrophenol, 2,4-dimethylphenol, 2-methyl-4,6-dinitrophenol, 4-chloro-3-methylphenol, 2,4-dichlorophenol and 2,4,6-trichlorophenol. The column used was a Dynamax axially compressed RP-18 column from Raynin (Emeryville, CA, U.S.A.). Further details on the experimental procedures can be found elsewhere[15].

RESULTS AND DISCUSSION

Expert system for criterion selection

The CRISE expert system is thought to be applicable to a wide variety of optimization procedures in which non-programmed (*e.g.*, isocratic) separations are being optimized. It is difficult to test the usefulness of the system with only one particular optimization strategy. Therefore, we have validated the expert system by applying it to a selection of ten literature reports, which were selected so as to represent as good a selection of different selectivity-optimization procedures as possible. This selection is summarized in Table I. Although most of the applications deal with LC, one of them (Val8) deals with selectivity optimization in GC and one (Val10) with supercritical-fluid chromatography (SFC).

From all ten reports, the information relevant for selecting the optimization

TABLE I

SUMMARY OF THE TEN APPROACHES TO SELECTIVITY OPTIMIZATION SELECTED FROM THE LITERATURE

No.	*Authors*	*Ref.*	*Method description*
Val1	Eppert *et al.*	16	Two-dimensional window diagrams
Val2	Cooper and Hurtubise	17	Window diagrams
Val3	Billiet *et al.*	18	Iterative optimization
Val4	Goldberg *et al.*	19	Sentinel[a]
Val5	Berridge and Morrisey	20	Simplex optimization
Val6	Conlon	21	Pesos
Val7	Haddad and Sekulic	22	Optimization with tailed peaks[a]
Val8	Hinshaw and Ettre	23	Selectivity tuning[a] (GC)
Val9	Naish *et al.*	11	Diamond
Val10	Schoenmakers	24	Interpretive optimization[a] (SFC)

[a] Tested with or without allowing system (column) optimization to take place after selectivity optimization.

criteria was selected and presented to both the human expert (P.J.S.) and the expert system. In some instances more than one possible answer was considered to certain questions asked by the expert system. This led for some of the test cases to more than one consultation. The resulting advice from the expert system is summarized in Table II.

The elementary criterion selected was either the resolution (R_s), the separation factor (S), the separation factor corrected for variations in the plate count between different solutes or between different experiments (S_N) or the peak-valley ratio (P). The expert system will recommend whether or not it is advisable to correct the elementary criterion for large variations in peak heights between different peaks or for peak asymmetry[25]. Also, it reveals whether or not the use of weighting factors (preferably 0 for irrelevant peaks and 1 for all relevant peaks) is recommended.

After selecting the most appropriate elementary criterion, the expert system will select the global optimization criterion, *i.e.*, the criterion that can be used to characterize the quality of the separation in the entire chromatogram. A fixed-threshold criterion implies that the analysis time is minimized, while the lowest value for the elementary criterion observed in the chromatogram does not fall below a specified value. For example, a minimum resolution of 1.5 may be specified. Two-criterion optimization[26] involves the simultaneous optimization of retention and resolution, then finding the proper trade-off between these two parameters at the end of the optimization process.

When the system-optimization system is available for further improving the results of the selectivity-optimization process, this can be taken into account during the selection of the criterion. It can be specified at this stage whether or not columns of different length and/or particle size will be considered in the system-optimization step. By taking the possibilities of the system-optimization into account at this stage, a better overall optimum may be found in the end[24].

The best possible distribution of all peaks over the chromatogram may be selected as the global optimization criterion, if the analysis time does not vary greatly

TABLE II

SUMMARY OF THE ADVICE PRESENTED BY THE EXPERT SYSTEM FOR CRITERION SELECTION (CRISE) FOR A TOTAL OF NINETEEN RUNS IN RELATION TO THE TEN DIFFERENT APPROACHES TO SELECTIVITY OPTIMIZATION LISTED IN TABLE I

WF indicates whether or not the use of weighting factors (0 and 1) is recommended. R_s denotes the resolution, S the separation factor, S_N the separation factor corrected for variations in the plate count and P the peak-valley ratio[10]. L is the column length and d_p the particle size.

No.	*Runs*	*Recommendations of CRISE*				
		Elementary criterion	*Global criterion*		*Corr.*[a]	*WF*
Val1	2	S_N		Fixed threshold	PHR	No
			or[b]:	two-criterion optimization	PHR	No
Val2	4	S_N		Fixed threshold	PHR	No
		or[c]: S	or[d]:	best distribution	PHR	No
Val3	2	R_s		Fixed threshold	PAS	Yes
			or[b]:	two-criterion optimization	PAS	Yes
Val4	2	S_N		Fixed threshold	None	No
			or[e]:	minimum analysis time (L variable)	None	No
Val5	1	P		Fixed threshold	N/A	No
Val6	1	P		Fixed threshold	N/A	No
Val7	2	R_s		Fixed threshold	PAS	No
			or[e]:	minimum analysis time (L variable)	PAS	No
Val8	1	S_N		Minimum analysis time (L variable)	PHR	No
Val9	2	R_s		Best distribution	PAS	Yes
			or[f]:	minimum analysis time (L and d_p variable)	PAS	Yes
Val10	2	S		Fixed threshold	None	No
			or[e]:	Best distribution in $\sim$ minimum time (L variable)	None	No

[a] Recommended corrections: PHR = peak-height ratio, PAS = peak asymmetry.
[b] When no *a priori* decision on a threshold value can be made.
[c] Depending on whether or not the plate count can be measured for one peak.
[d] Depending on whether minimum analysis time or best distribution of peaks is desired.
[e] Depending on whether system (column) optimization is allowed after selectivity optimization.
[f] Depending on whether the best distribution of peaks on a given column or the shortest analysis time on a column of optimum length and with optimum particle size is desired.

with variations of the conditions (within the parameter space). A good distribution of peaks may also be considered as a sort of secondary criterion, while emphasis is put on the shortest possible analysis time (see Val10 in Table II).

The main conclusions of the validation of the expert system for criterion selection were that (i) the expert system provided clear and unambiguous answers for each consultation and (ii) the expert system and the human expert provided the same

answers for all ten cases (nineteen consultations) considered during the validation. These conclusions imply that the expert system performs as intended. It does not imply that the expert system always provides the correct advice, because the correctness of the knowledge supplied to the expert system by the human expert has not been rigorously validated.

For a full explanation of optimization criteria, the reader is referred to refs. 3, 10 and 24.

Selectivity optimization

In this study we focused on the optimization part of the Diamond package. Establishing the elution order (and the retention times) of the ten individual solutes from the ten three-dimensional chromatograms will not be discussed in this paper. Based on the correct assignment of all the solutes, the retention surfaces for each solute can be calculated using piecewise-quadratic interpolation between the ten experimental data points[11]. Once the retention times of each solute can be calculated at each composition, a response surface can be calculated. Such a surface shows the variation of the overall optimization criterion with the mobile phase composition. An example is shown in Fig. 5. In this example, the minimum value for the separation factor [$S = (k_2 - k_1)/(2 + k_1 + k_2)$] is used as the optimization criterion. Response surfaces can be presented as quasi-three-dimensional plots (Fig. 5a) or as contour plots. In the latter instance, the total range of criterion values is divided in a number of equal sub-ranges, each of which is displayed in a certain shade of grey (as in Fig. 5b) or, preferably, in a different colour. In Fig. 5b the highest point on the surface is indicated by the cursor.

At the bottom of Fig. 5b a so-called stick chromatogram is displayed. This gives the position of all the peaks in the chromatogram on a ln $(1 + k)$ scale. On this scale all peaks are of equal width (if the plate count N is constant throughout the chromatogram). Hence the stick diagram provides an illustration of the expected separation at the position of the cursor, which can be anywhere in the triangle.

A number of different optimization criteria can be applied within the present prototype version of Diamond. A list of these is presented in Table III, together with a short description of each of the optimization criteria.

Within the Diamond system the optimization of the mobile-phase composition takes place in a so-called isoelutropic plane. This implies that the (binary) compositions at the vertices have been selected such that the analysis time (retention time of the last peak, t_ω) is roughly constant (typically within a factor of 2–3). This will then also apply for all other (ternary and quaternary) compositions that can be formed by blending the three binary mixtures in different ratios. In the present example t_ω varies from about 15 to about 44 min, *i.e.* by about a factor of three.

One of the possible recommendations of the CRISE system is to use a threshold criterion, which implies that the retention time is minimized in the range of compositions for which S_{min} exceeds a certain minimum ("threshold") value. In Diamond, a slightly different criterion is implemented, which yields similar results. This is the STMIN criterion in Table III, which equals S_{min}/t_ω. This criterion locates the optimum at an S_{min} value close to the maximum value. This can be understood as follows.

The minimum value for the separation factor observed in the chromatogram varies from 0 (when two solutes "cross over") to about 0.04 if all peaks are considered

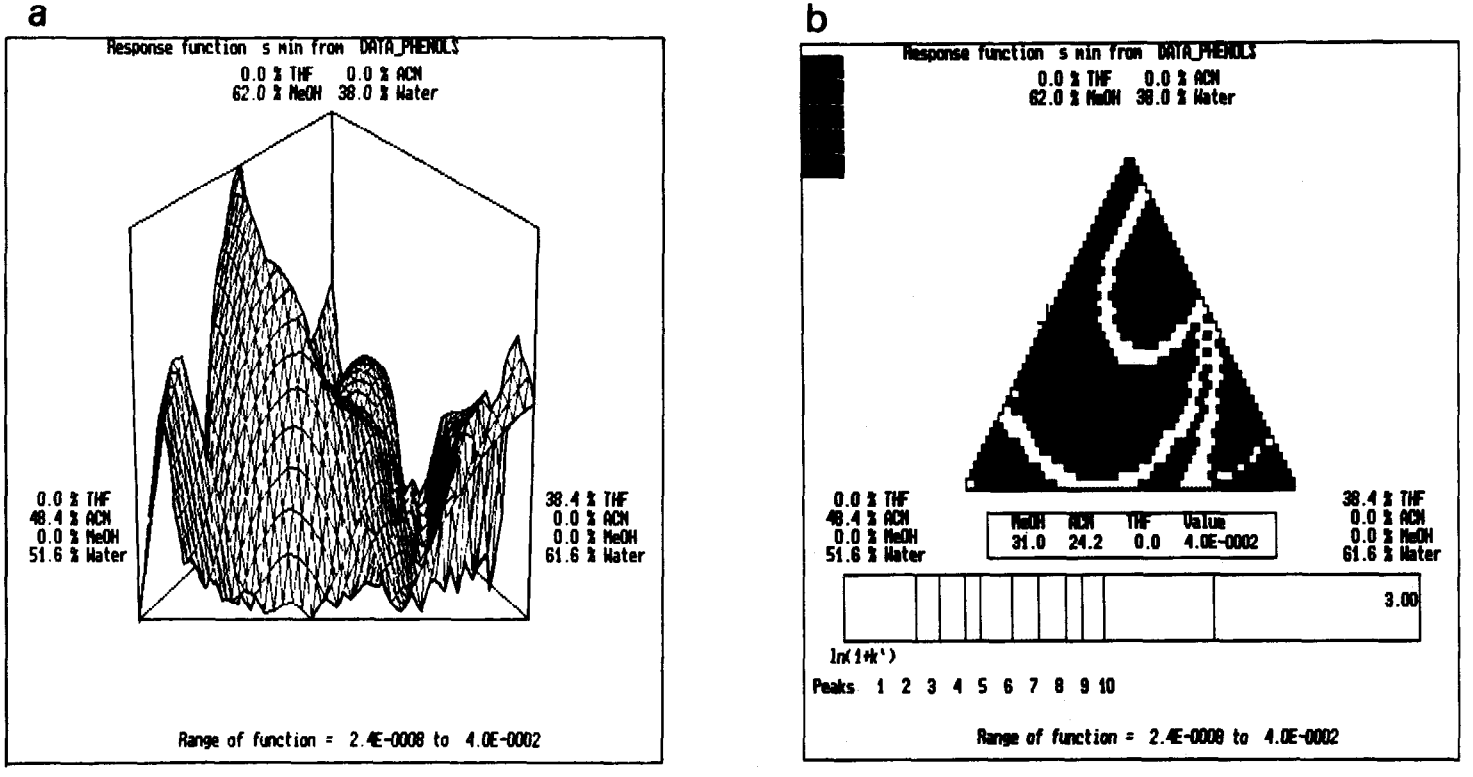

Fig. 5. Example of a response surface for the phenol sample. Criterion: S_{min}; relevant peaks: all. (a) Pseudo-three-dimensional plot; (b) contour plot. In (b) the range of S_{min} values from 0.00 to 0.04 has been divided into equal sub-ranges. The highest point on the contour is indicated by the cursor. The white areas are lowest. Below the triangle is a stick chromatogram on a ln (1 + k') scale (explained in text). ACN = Acetonitrile; MeOH = methanol; THF = tetrahydrofuran.

TABLE III

DESCRIPTION OF THE OPTIMIZATION CRITERIA USED IN THE SELECTIVITY-OPTIMIZATION PROGRAM DIAMOND

For a detailed explanation of all criteria, see refs. 3, 10 and 24.

Abbreviation	*Criterion*	*Description*
TNE	$[t_{ne}]_{r,d}$	Minimum required analysis time, allowing the column length to vary (with the flow-rate and particle size constant) during a subsequent system-optimization step
RSTAR	r^*	Best (most equal) distribution of all relevant peaks over the chromatogram
SMIN	S_{min}	Lowest value for the separation factor (proportional to resolution) between a relevant pair of peaks in the chromatogram
STMIN	S_{min}/t_ω	Corresponds to S_{min} divided by the required analysis time. This criterion approximates a "fixed-threshold" one, *i.e.*, to reach a required (resolution) target in the shortest possible time
RNT	$[r^*]_{nt}$	As TNE, but also paying some attention to the best possible distribution of peaks

to be relevant, and from 0 to about 0.07 if only components 7, 8 and 9 are relevant. Because t_ω varies by no more than a factor of three, the retention time can compensate for no more than a factor of three variation in S_{min}. Hence the criterion STMIN will locate the optimum at a composition where S_{min} is at least 35% of its highest value. At the predicted optimum composition, S_{min} will be higher than about 0.014 if all peaks are considered (> 0.025 for components 7, 8 and 9 only). The criterion STMIN thus behaves similarly to a fixed-threshold criterion, in which the optimum is located at the composition where S_{min} exceeds a certain minimum value and t_ω is as small as possible.

The other four criteria can potentially be selected by the expert system as the most appropriate optimization criterion for an interpretive method, such as that employed in Diamond. The expert system distinguishes between two different situations for applying the system-optimization system (SOS) after the selectivity-optimization step. A different criterion may be used depending on whether the column length or both the column length and the particle size will be allowed to vary within SOS. The criteria currently incorporated in Diamond correspond to the former situation.

Table IV shows some of the optima predicted by the Diamond system for the sample of ten phenolic solutes. If all ten solutes are considered to be relevant, four out of five criteria yield the same optimum composition, whereas the fifth criterion locates the optimum in the same area. For complex samples this is likely to be the case, because there will not be many regions in the triangle in which all peaks can be separated. The optimum predicted with the majority of the optimization criteria is illustrated by the chromatogram in Fig. 6a. All ten solutes are seen to be separated in about 20 min using the same column, flow-rate, etc., as were used to record the ten initial 3-D chromatograms.

If not all ten solutes are considered to be relevant and if changes in the elution order occur when the composition is varied, the selection of different criteria may well lead to the prediction of different optimum compositions. This is illustrated in Table

TABLE IV

SUMMARY OF OPTIMA FOUND FOR THE SEPARATION OF TEN PHENOLIC SOLUTES

Optimum No.	*Relevant peaks*	*Criteria*	*Composition (%)*		
			CH_3OH	*ACN*[a]	*THF*[b]
1	All	TNE, SMIN, STMIN, RNT	31.0	24.2	0.0
2	All	RSTAR	26.3	26.6	1.0
3	7,8,9	TNE, RNT	7.7	15.7	21.1
4	7,8,9	RSTAR	1.6	46.0	1.0
5	7,8,9	SMIN	17.0	1.2	1.0
6	7,8,9	STMIN	7.7	41.1	1.0

[a] Acetonitrile.
[b] Tetrahydrofuran.

IV for the example in which only components 7, 8 and 9 are considered to be relevant. In this instance the criteria TNE and RNT yield the same optimum composition, but in all other instances the predicted optima are located at significantly different positions in the triangle. The location of the different optima is illustrated in Fig. 7.

Fig. 8a illustrates that if only three peaks are considered to be relevant (optimum No. 3 in Table IV), the program (correctly) ignores the quality of the separation between irrelevant peaks. Notably, peaks 3, 4 and 5 are all poorly resolved at the optimum composition. However, the resolution of the relevant peaks 7, 8 and 9 and the resolution between these peaks and all the irrelevant peaks is very good.

System optimization

When the system-optimization system SOS is consulted for the predicted optimum chromatograms, it suggests that much can still be gained in terms of the required analysis time. The system was consulted for all six optima listed in Table IV. A

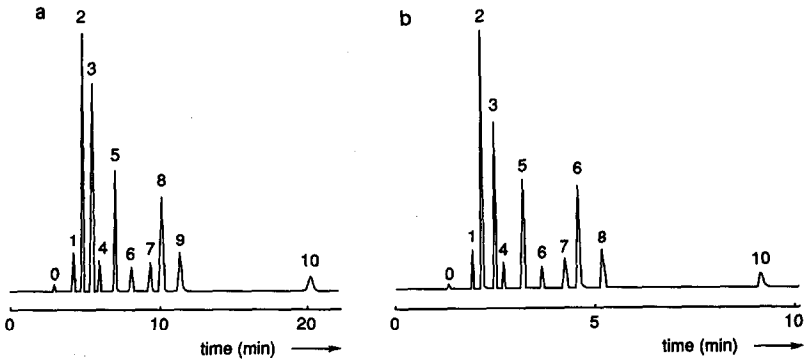

Fig. 6. Predicted optimum chromatograms for the separation of all ten phenolic solutes. For the mobile-phase composition and the criteria used see Table IV. (a) Optimum predicted by the Diamond system on the column used to record the ten 3-D chromatograms. (b) Optimum predicted by the SOS system using the optimum column (see Table V).

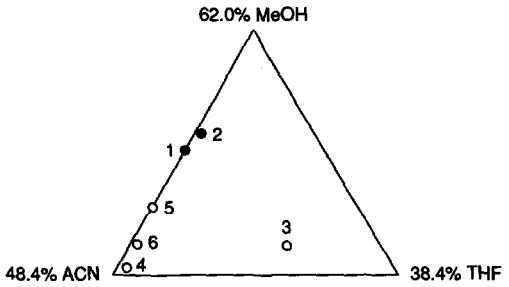

Fig. 7. Location of the different optima listed in Table IV in the isoeluotropic triangle. Closed circles correspond to situations in which all solutes are considered to be relevant. For the open circles only components 7, 8 and 9 were relevant.

minimum resolution of 2 and a required signal-to-noise ratio of 200 were specified for all situations. Three "standard" detector cells (8, 2.4 and 1.2 μl, as Nos. 1, 2 and 3, respectively) were included in the detector database and time constants of 50, 100, 200 and 500 ms were allowed. The maximum distortion factors for extra-column dispersion in the time and volume domains[12,14] were allowed to be the 0.5 and 0.2, respectively. The overall minimum and maximum values for the flow-rate were 0.02 and 10 ml/min, respectively. The overall pressure limits were 10 and 250 bar. A

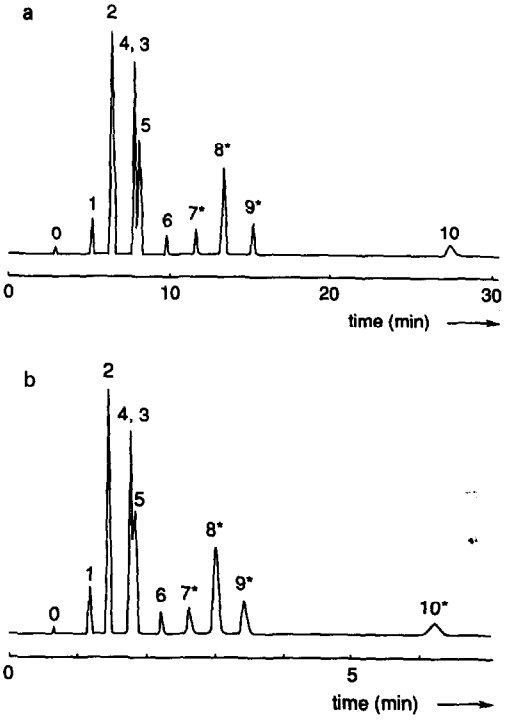

Fig. 8. As Fig. 6, but now only solutes 7, 8 and 9 (indicated by asterisks) are considered to be relevant.

TABLE V

SUMMARY OF THE CONSULTATION OF THE "SOS" SYSTEM FOR THE PREDICTED OPTIMA LISTED IN TABLE IV

Top: summary of the column database. Bottom: results for each of the optima. t_D is the required analysis time for the column and conditions used in the Diamond system (*i.e.*, before SOS); t_S is the required analysis time for the optimum predicted by the SOS system.

Column No.	*Length (cm)*	*I.D. (mm)*	*Particle size (μm)*
1	30	4.6	10
2	25	4.6	8
3	25	4.6	5
4	15	4.6	5
5	10	4.6	3
6	25	2	8
7	25	1	8

Optimum No. (Table IV)	t_D *(s)*	*Optimum column*				
		Column No. (above)	*Detector cell*	*Time constant*	*Flow-rate (ml/min)*	t_S *(s)*
1	1208	5	3	200	0.61	551
2	1229	3	2	500	0.89	1036
3	1645	4	2	500	1.92	373
4	990	5	3	200	0.76	362
5	2240	4	2	500	2.18	448
6	1060	5	3	200	1.02	228

maximum amount of 20 μl of sample (20 times the initial amount) was allowed. The column database is summarized in Table V (top). Column 1 was the column used to record the 3-D chromatograms for the Diamond program and the initial plate count was taken as 10 000.

The resulting optimum separations predicted by the SOS system are summarized in Table V (bottom). In all situations it can be seen that the predicted analysis time after system optimization is (much) lower than before. When all peaks need to be separated, the required number of plates is fairly large, but the analysis time can still be reduced by about a factor of two. When only peaks 7, 8 and 9 are relevant, the initial number of plates is higher than needed and the analysis time can be reduced by factors of 3–5 for the different optima. The initial column was never suggested to be the optimum choice by the SOS system. The system lists the best possible results that can be obtained on all (valid) columns. For example, if column 1 were to be selected, the analysis time at optimum conditions for a minimum resolution of 2 between relevant peaks would be 1652, 2073, 746, 1085, 833 and 809 for the six different optima. A comparison of these values with the numbers listed in the last column in Table V (bottom) illustrates the benefits of the SOS system.

The predicted optimum separations corresponding to the chromatograms in Figs. 6a and 8a (and to the optima Nos. 1 and 3 in Tables IV and V) are shown in Figs. 6b and 8b. The selectivity does not change on going from Fig. 6a to 6b or from

Fig. 8a to 8b, *i.e.*, the relative retention times are constant. However, the absolute retention times are reduced considerably.

CONCLUSIONS

We have tried to demonstrate that different computer programs can be used coherently for developing chromatographic methods. We have described some careful steps on the road towards an integrated system for method development in chromatography. In this work, different software programs were used as such, while no attempts were made to actually integrate the software into one program or even one computer. In fact, the three different programs used required three different computers. Integrating several expert systems into one system is one of the goals of the "Expert Systems for Chemical Analysis" project[2]. One of the laboratories involved in this work (Vrije Universiteit Brussel) is currently trying to develop a connection between different programs for performing the tasks of method development and method optimization, including the optimization of retention, selectivity and the chromatographic system. The overall system will appear to the user as a single computer program.

In this work we have demonstrated the value of using a combination of different computer programs. An expert system was used to select the most appropriate selectivity-optimization criterion. This system yielded the same advice as the human expert. A systematic procedure for optimizing the mobile-phase composition in reversed-phase LC was used in combination with several different optimization criteria. In some instances, the optimum composition can vary greatly once a different optimization criterion has been selected. The resulting optimum chromatogram can be subjected to an expert system for system optimization. This system predicts the best possible column, instrumentation and operating conditions based on the optimum chromatogram found during the selectivity optimization.

The use of these three systems together offers great advantages to the user. The selection of the most appropriate optimization criterion is difficult and only a few specialists are thought to master this area. Without the best optimization criterion, systematic selectivity-optimization procedures will not be used correctly and will not produce the best possible results. The expert system for criterion selection can thus make selectivity-optimization procedures easier to use and make them yield better results.

Likewise, the results of the selectivity-optimization process can be much improved by consulting the system-optimization system afterwards. This may result in much shorter analysis times and a much better sensitivity for the proposed method. The best results can be obtained if the possibilities of the system-optimization system are borne in mind during the selection of the optimization criterion, illustrating how the different systems interact together. The system-optimization system may also be consulted to decide on whether or not selectivity optimization is required. It can be rapidly consulted to see what kind of separation may be achieved without selectivity optimization. If this is thought to be adequate, the selectivity optimization can be forfeited.

There is much work to be done in demonstrating the applicability of the software programs discussed in this work, in verifying the correctness of the advice

offered by the expert system and in experimentally validating the methods proposed by systematic procedures such as ours. For example, the effect of the variability of retention and selectivity between different column materials will need to be considered. A good deal of work is in progress at the moment, but with the increasing availability of expert systems for chromatographers we feel that both research and applications in the area will blossom in the near future.

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