Journal of Chromatography, 592 (1992) 43–49
Elsevier Science Publishers B.V., Amsterdam

CHROMSYMP. 2450

Use of solvent selectivity optimization procedures for high-performance liquid chromatographic method development

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ABSTRACT

Solvent optimization procedures for high-performance liquid chromatography (HPLC) method development have been used in the past, but have never become routinely established owing to the complexity of the task involved. A unique solvent optimization software package has been developed by Philips Research that reduces the complexity of the problem. The optimization software utilizes four solvents and data from a UV diode-array system, and requires only eleven experiments in order to produce an optimized isocratic method. It has proved to be a powerful tool for solvent optimization in HPLC method development, and has proved its ability to resolve complex chromatographic problems, reducing method development time considerably. The system produces isocratic methods which, when compared with similar gradient methods, have analysis times reduced typically by a factor of 3–4. The fact that isocratic rather than gradient methods are produced leads to the production of more robust methods that can be easily transferred to other HPLC laboratories for routine testing or quality control purposes.

INTRODUCTION

High-performance liquid chromatography (HPLC) has developed into one of the most useful and widely used analytical techniques. The range and variety of compounds that can be analysed by HPLC, coupled with its relative ease of use, have led to applications in fields ranging from industrial chemistry to biological science.

Chromatographers often spend a considerable amount of effort on method development, which can be a time-consuming and costly procedure, owing to the number of interactive variables involved. The composition of the mobile phase is the most influencial parameter with respect to solute selectivity, and is therefore highly significant in maximizing resolution for complex samples. It was thought that a mobile phase (solvent) optimization procedure would be of valuable assistance in reducing method development time and providing quicker, more robust HPLC methods [1].

The optimization of the mobile phase for HPLC separations can be classified into three main groups [1]: (1) grid search methods, in which a large number of experiments (typically between 50 and 100) are carried out and the best is chosen; (2) sequential methods, where the results of previous experiments are used to select a subsequent set of conditions, an example of this approach being the simplex method [2], in which typically between 25 and 30 chromatograms are required, but often local (and not global) optima are found; and (3) interpretive methods, where a model of retention data with varying solvent compositions is set up. Computer modelling of this data provides the optimum solvent composition. Only 7–10 chromatograms are required, and global optima are achieved.

This paper describes the use of an interpretive optimization software package developed by Philips Research. The use of reversed-phase separations of positional isomers and the analysis of aromatic carboxylic acids and amines will be demon-

0021-9673/92/$05.00

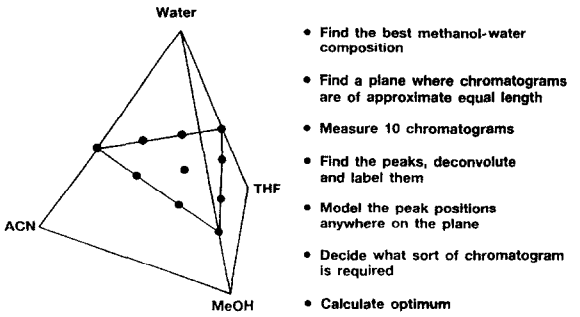

Fig. 1. Outline of the optimization procedure.

strated. The system was also used in a number of different modes of HPLC, including ion-pair, ion-suppression and normal-phase chromatography.

DESCRIPTION OF SOLVENT OPTIMIZATION SOFTWARE

The solvent optimization software package ("Diamond") has been specifically written by Philips Research for use on a diode-array detector. A brief outline of the procedure used by the solvent optimization system is given in Fig. 1.

The corners of the tetrahedron represent pure solvents, water, acetonitrile, methanol and tetrahydrofuran. For these solvents it is possible to define a plane which joins points of equal solvent strength. This is called the isoeluotropic plane, on which the total run time of the chromatograms will remain constant. The initial software calculates the location of the plane using data from a methanol–water gradient and solvent strength rules [3]. Once the plane has been located, the ten chromatograms (whose compositions are selected by the optimization software) are then run and the diode-arry data collected. After data collection, each chromatogram is processed; this involves (i) peak detection (using the second-derivative chromatogram) followed by deconvolution of any overlapping peaks using principal component analysis (PCA), (ii) reconstruction of chromatograms and spectra, using iterative target transformation factor analysis (ITTFA) [4] and (iii) spectral matching against a reference set (both peak spectra and peak concentrations can be used for peak tracking and subsequent labelling, with a least-squares fit being applied for spectral matching).

For each peak, a mathematical retention model is fitted to the ten points on the plane. This is done using a piece-wise quadratic model. The end result of this process is that a "retention surface" is generated, which maps the movement of a peak with changing solvent composition. From the retention surfaces for all of the components, response functions can be calculated (and a response surface generated), which measure the quality of the chromatograms anywhere on the plane.

The global optimum solvent composition can be selected from this response surface and chromatograms at these points can be predicted. A number of response functions can be calculated depending on the criteria used to define a good chromatogram. The response functions available are described in detail elsewhere [5]. The response function can be adapted for all of the peaks or for a group of peaks which may be of interest. Thus, an optimum could be selected specifically for analytes of interest. Previous work [6] has shown that comparisons of predicted with actual data can be made, with typical errors of 1–2% being obtained.

EXPERIMENTAL

Chromatographic apparatus

The HPLC apparatus consisted of a Philips PU 4100 quaternary pump and a Philips PU 4021 diode-array detector. The samples were injected using a Valco C6W injection valve fitted with a 20-μl loop.

Software packages used for this work were the Philips PU 6003 diode-array detector software and Philips PU 6100 solvent optimization software. These packages were run on a Philips PU 3203 computer.

The HPLC columns used were 250 mm × 4.6 mm I.D. stainless-steel columns packed with (i) 5-μm octadecylsilane (ODS) packing material (Phase Separations, Clwyd, UK), (ii) 5-μm base-deactivated silica (BDS) packing material (Shandon, Runcorn, UK) and (iii) 5-μm cyanopropyl-bonded-silica (S5-CN) packing material (Phase Separations).

Chemicals and reagents

The solvents used included acetonitrile (ACN) (Romil Chemicals, Loughborough, UK) of far-UV

grade, methanol (MeOH), (Hichrom, Reading, UK) of HPLC grade. Tetrahydrofuran (THF), hexane, dichloromethane, 2-propanol and pentanesulphonic acid (all of HPLC grade) were from Fisons, Loughborough, UK. Water was purified by means of a Millipore Milli-Q system. Sample and standard materials were supplied by Aldrich (Gillingham, Dorset, UK), and BP Chemicals (Hythe and Hull, UK).

Solvent optimization

The solvent optimization package has been used in a number of different application areas to solve particular problems. The performance of the system was tested with different chromatographic modes, varying from the use of reversed-phase HPLC to ion-pair, ion-suppression and normal-phase chromatography.

RESULTS AND DISCUSSION

Positional isomers

The separation of two positional isomers was optimized using reversed-phase HPLC, and the results were compared with an initial gradient method which was developed previously [7]. This proved to be a particularly testing example as the polarity and UV spectra of the isomers were very similar. The UV spectra differed by only 2 nm, but nevertheless were sufficiently different to allow accurate peak tracking of the two components.

The response function, S_{min}, was used for the optimization in order that the maximum resolution could be obtained between the two components. The response map or "solvent triangle" is produced, which is shown in contour form in Fig. 2. From this, the resolution of the two components can be observed at any given solvent composition. The optimum solvent composition is shown (cursor position, bottom right), with the chromatogram represented by a stick diagram showing the resolution obtained between the two components.

A comparison of the initial gradient method with the optimized isocratic method is demonstrated in Fig. 3. It can be seen that the resolution is significantly improved and the analysis time reduced by a factor of three using the optimized isocratic method.

Ion-pair HPLC

An initial gradient HPLC method was developed for the determination of aromatic amines. The gra-

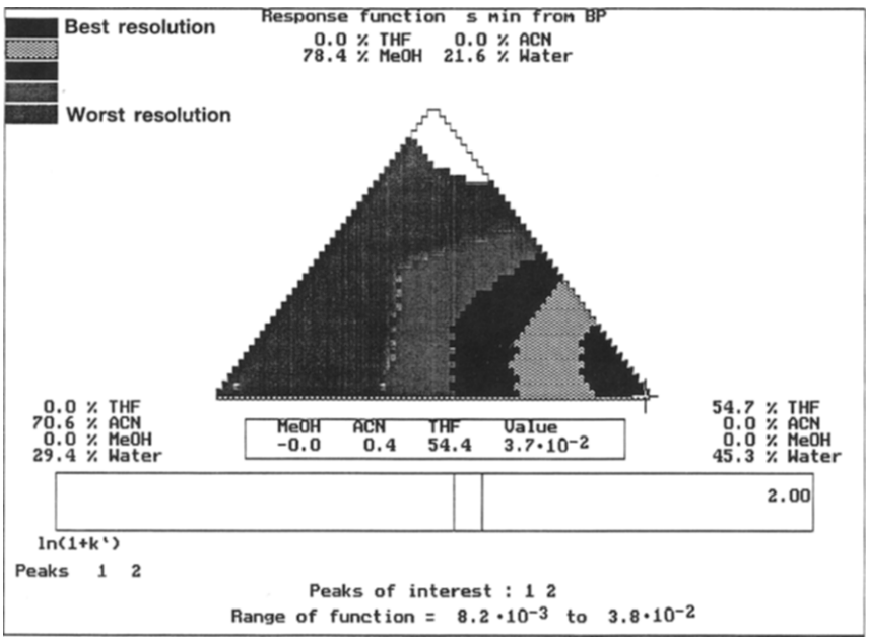

Fig. 2. Contour plot of the response function S_{min}.

dient method was subsequently optimized to produce a simpler isocratic method.

The response function R^* was chosen for the optimization in order to produce an even separation of all components. A comparison of the optimized method with the gradient method (Fig. 4) shows a reduction by a factor of three in the total analysis time. The original gradient method also suffers from solvent background peaks from the sulphonic acid ion-pairing agent, which were visible at the detection wavelength used. This was later overcome by using the purer sodium salt (Fig. 5A), which was not available for this analysis. The isocratic method however, did not suffer from solvent background effects.

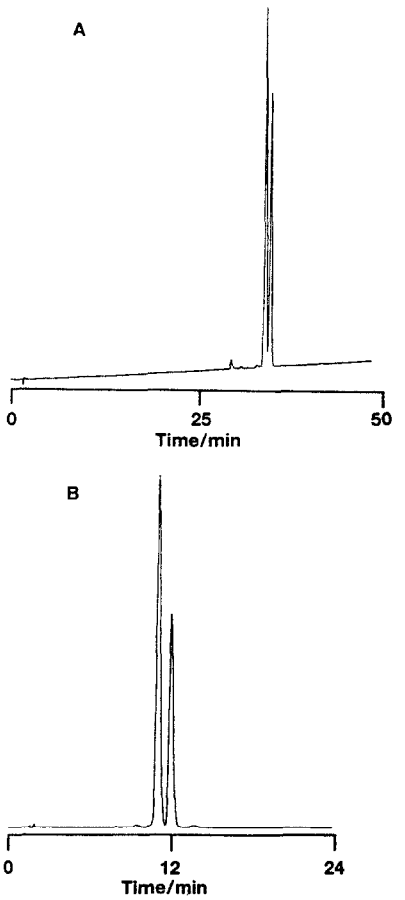

Fig. 3. Comparision of the initial gradient method with the optimized isocratic method. (A) Initial gradient method. Chromatographic conditions: mobile phase, (A) water–acetonitrile (80:20), (B) water–acetonitrile (20:80); gradient, 50–100% B in 50 min; column, 250 × 4.6 mm I.D. ODS; injector, Valco C6W, 10-μl loop; flow-rate, 1 ml/min; detector, UV, 280 nm. (B) Optimized isocratic method. Chromatographic conditions as in (A) except mobile phase, water–acetonitrile–methanol–THF (43:2:6:49).

Ion-pair suppresion HPLC

The technique used for the analysis of a mixture of aromatic amines and acids has been termed ion-

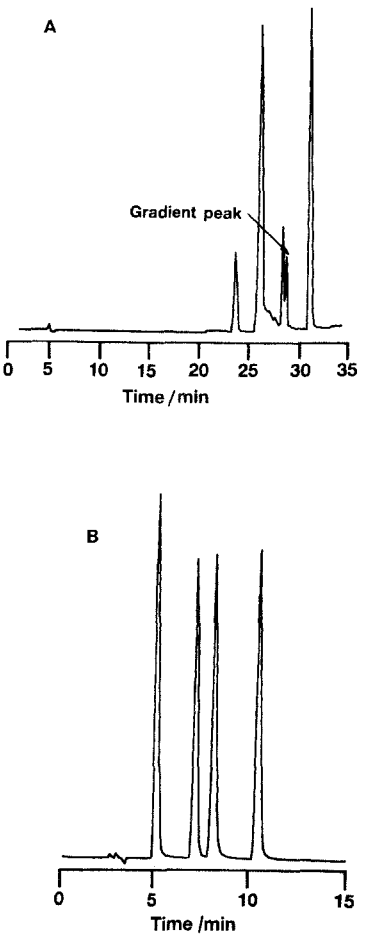

Fig. 4. Comparison of the initial gradient method with the optimized isocratic method. (A) Initial gradient method. Chromatographic conditions: mobile phase. (A) Aqueous 5 mM pentanesulphonic acid (pH 2). (B) methanol–water (60:40)–5 mM pentanesulphonic acid (pH 2); gradient: 0% B, 0–15 min, 0–20% B, 15–30 min, 20–100% B, 30–35 min, 100% B, 35–40 min; column, 250 × 4.6 mm I.D. BDS; flow-rate, 1 ml/min; injector, Valco C6W, 20-μl loop; detector, UV, 225 nm. (B) Optimized isocratic method. Chromatographic conditions as in (A) except mobile phase, water–acetonitrile (97:3)–5 mM pentanesulphonic acid (pH 2).

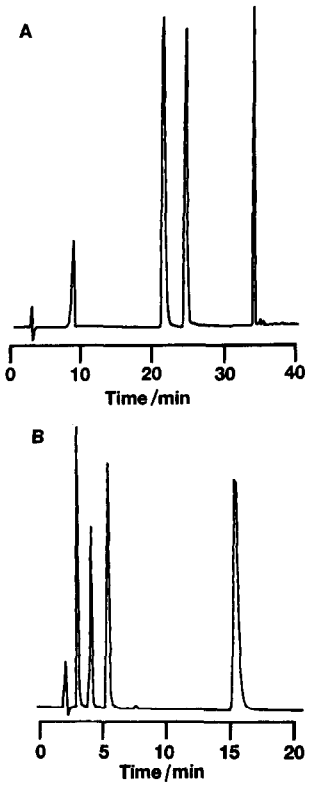

Fig. 5. Comparison of the initial gradient method with the optimized isocratic method. (A) Initial gradient method. Chromatographic conditions: mobile phase, (A) aqueous 5 m*M* sodium pentanesulphonate, (B) acetonitrile–water (70:30)–5 m*M* sodium pentanesulphonate; gradient: 5% B, 0–5 min, 5–15% B, 5–20 min, 15–100% B, 20–25 min; column, 250 × 4.6 mm I.D. BDS; flow-rate, 1.3 ml/min; injector, Valco C6W, 10-μl loop; detector, UV, 230 nm. (B) Optimized isocratic method. Chromatographic conditions as in (A) except mobile phase, water–THF (69:31)–5 m*M* sodium pentanesulphonate.

pair suppression. This uses sodium pentanesulphonate, which acts as an ion pair towards amines and a suppressor towards acidic functional groups.

The optimization procedure using the response function S_{min} predicted an optimum for the four-component separation in the "THF corner". A comparison of the initial gradient method with the optimized method (Fig. 5) again demonstrates the significantly reduced analysis time achieved.

Another important aspect of the optimization software is its value when used in conjunction with preparative chromatography, where only certain components may be of interest. Optimization for selected components can be performed, which may allow purer fractions to be collected.

Ion-suppression HPLC

The use of ion-suppression reversed-phase chromatography enabled a mixture of phthalate components to be analysed. When this method was optimized using the response function, S_{min} the optimum was again to be found in the "THF corner". This is shown in Fig. 6 as a response surface, which is an alternative view to the contour map display. Most of the methods developed within our laboratory are gradient elution methods with acetonitrile or methanol as the organic modifier. These solvents have a low UV absorbance, which is essential in gradient elution chromatography, particularly with low-wavelength UV detection. However, it has been observed that in the examples demonstrated here, THF is often the best solvent in terms of analyte selectivity, and as isocratic methods are produced the higher UV absorbance of THF is immaterial. This demonstrates the particular use of solvent selectivity optimization, whereby the best solvent for the component separation is selected.

Normal-phase HPLC

The optimization software was primarily developed to be used with reversed-phase chromatography. It can, however, be used to optimize separations for temperature, pH, buffer concentration or for normal-phase chromatography. Care is needed, however, when using these other modes, especially when applying this to normal-phase chromatography.

The reversed-phase solvents water, acetonitrile, methanol and THF were replaced with hexane, 2-propanol, dichloromethane and THF. The initial location of the isoeluotropic plane has to be done manually, as there are no solvent strength rules for normal-phase solvents programmed into the software. However, this can be done using a knowledge of the solvent strengths and, after location of the plane, the optimization procedure is the same as that outlined previously.

A method was developed originally to separate a five-component test mixture (2-nitrophenol, bromoacetophenone, dinitrobenzene, 4-butylphenol and 4-bromophenol) using a normal-phase gradient system. The solvent system was then optimized,

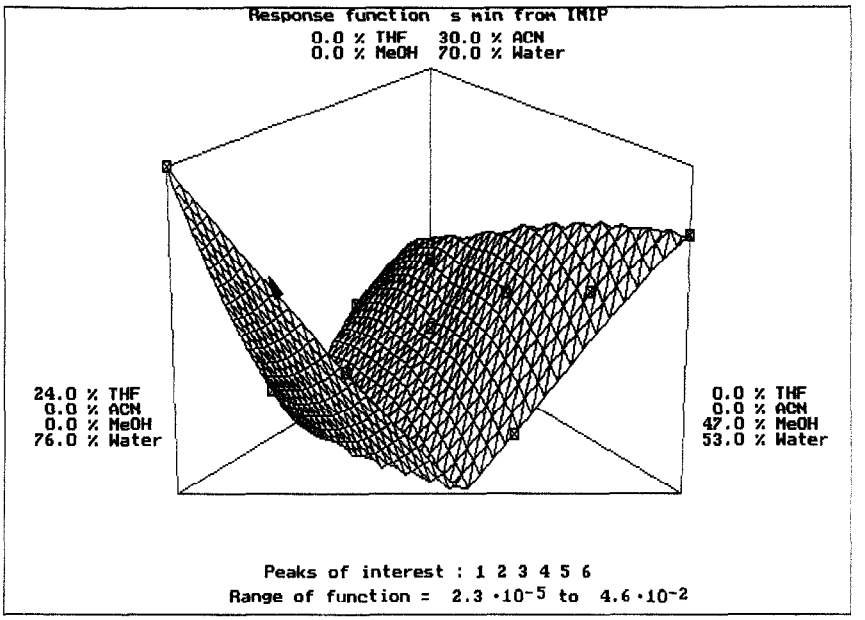

Fig. 6. Response surface of the S_{min} function.

producing an isocratic method with an analysis time of one third of the gradient method.

The effect of three different response functions (S_{min}, St_{min} and R^*) on the position of the optima were studied. A comparison of the chromatograms obtained at each optimum is shown as a stick diagram in Fig. 7. The S_{min} function produced an optimum to provide maximum resolution of the two least resolved peaks. The St_{min} function produced an optimum using the same criteria but biased for minimum retention time, thus providing reduced analysis time. If the most even spacing of peaks is required, the R^* function is used, and a more even distribution of peaks is observed.

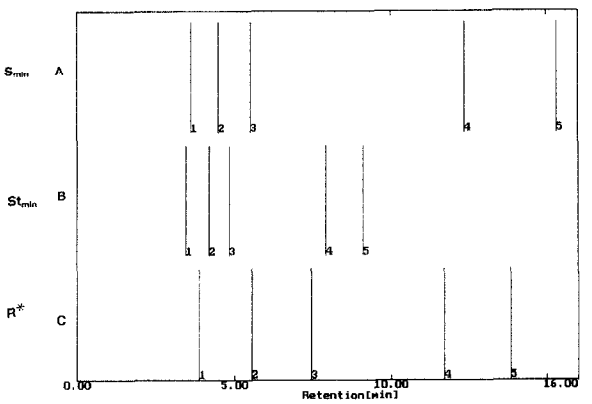

Fig. 7. Chromatograms obtained using the response functions (A) S_{min}, (B) St_{min} and (C) R^*. (A) Mobile phase: 2-propanol–dichloromethane–hexane (0.7:11.4:87.9); (B) mobile phase: 2-propanol–dichloromethane–THF–hexane (0.4:18.4:0.9:80.3); (C) mobile phase: 2-propanol–hexane (1.1:98.9). Components: 1 = 2-nitrophenol; 2 = bromoacetophenone; 3 = dinitrobenzene; 4 = 4-butylphenol; 5 = 4-bromophenol.

CONCLUSIONS

The solvent optimization software package described here provides a unique optimization facility which allows methods to be optimized for resolution and analysis times. It provides a user-friendly environment where parameters can be readily modified, unlike "black box" systems in which optima are produced without user consultation. Optima are produced according to criteria which are manually selected, and the user can inspect and modify data at each stage. The optimization software does have its limitations; it can only be used for analytes with a UV response and the software itself is expensive. However, approximately 80% of the work we carry out utilizes UV detectors and, in a busy laboratory,

the software could pay for itself within 1–2 years on savings in analysis time alone.

It has already proved an invaluable tool for HPLC method development and has proved a significant benefit within our laboratory, reducing analysis times typically by one third. It has the capability to optimize on selected components, which should prove useful in preparative chromatographic applications. It has proved to be a highly beneficial chromatographic tool for developing HPLC methods and in reducing method development time. The optimized methods are generally more robust than existing gradient methods and are therefore readily transferable to other HPLC laboratories for routine analysis or quality control purposes.

ACKNOWLEDGEMENTS

The author thanks Dr. R. Lynch and Dr. J. Hann, Philips Scientific (Cambridge, UK), for their help and advice throughout this work, and BP Research for granting permission to publish this paper.

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