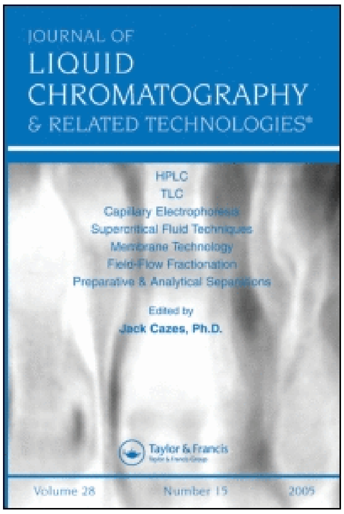

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AN EXPERT SYSTEM APPROACH TO ELUENT OPTIMISATION IN REVERSE-PHASE LIQUID CHROMATOGRAPHY WITH MULTICHANNEL DETECTION

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ABSTRACT

The optimisation of eluent composition in reversed-phase liquid chromatography is a complex process, which often requires the expenditure of a significant amount of effort on the part of the chromatographer. To assist in this task an expert system has been developed which uses a gradient elution experiment to determine the appropriate initial solvent strength, followed by response-surface modelling using an iterative regression method to determine the mobile phase composition for optimum resolution. Spectral information from a multichannel diode array detector is used to provide retention information for this model. Peak homogeneity is assessed by a number of independent modules, the output from which is interpreted by the expert system and used to validate the response surface model constructed by the optimisation procedure.

INTRODUCTION

The use of expert systems, which can be described as computer programs with the ability to process knowledge as well as numerical data, is becoming increasingly important within the field of analytical chemistry (1). Such systems have been developed to assist in the interpretation of experimental results derived, for instance, from a fluorimeter (2) or infra-red spectrophotometer (3), and to guide the analyst in the selection of the appropriate technique for a given analysis (4) or in the establishment of the appropriate instrument conditions once a method has been selected (5). The system described in this paper falls into the last of these categories, in that it has the capability to select the composition of the mobile phase for use in reversed-phase liquid chromatography so as to meet predefined analytical requirements.

To establish the mobile phase composition for a separation by HPLC, that gives the required resolution of the components of a given sample, within a predefined analysis time, requires the adjustment of a number of interacting variables. The parameters available to the analyst include the mode of separation; type of column to be used; and in reversed-phase chromatography, the identity and concentration of the organic modifier(s), the pH of the mobile phase, the identity of the buffer used, if any, and the identity and concentration of any additional components in the mobile phase. The adjustment of these parameters to achieve the desired separation can be a time consuming operation, requiring both an appropriate experimental methodology and information about the likely behaviour of the analyte.

Although several strategies are available for the automated determination of optimum conditions (6), most implementations rely upon a search of the parameter space to locate the optimum conditions, with the inherent need to perform a large number of trial separations. To reduce the number of experiments required during the optimisation process, the Authors have developed an expert system approach to optimisation (7,8). This expert system has the capacity to use the multivariate information extracted from the chromatograms produced during the initial stages of the optimisation procedure, to predict a suitable composition of the mobile phase to resolve each separately identifiable solute in an unknown mixture. This initial prediction is iteratively refined until the final optimum mobile phase composition is established. The available information content from each chromatogram is enhanced by the use of a photodiode array detector (9) to provide spectral information for all eluting peaks.

The photodiode array detector or PDA provides an extra dimension of information from a single chromatogram, as a result of its ability to collect ultra-violet or visible spectral information "on the fly" during the elution process. Thus in place of the conventional chromatogram, a three-dimensional spectrochromatogram is produced, as illustrated in Figure 1, in which the axes are time, wavelength and absorbance. The multi-variate nature of the data produced by a PDA allows the use of sophisticated chemometric tools, which may increase the useable information content of a spectrochromatogram by means of statistical manipulation (10).

OPTIMISATION STRATEGY

The strategy adopted by the expert system at the start of the optimisation process is to establish the initial solvent strength required for the separation by means of a water-methanol

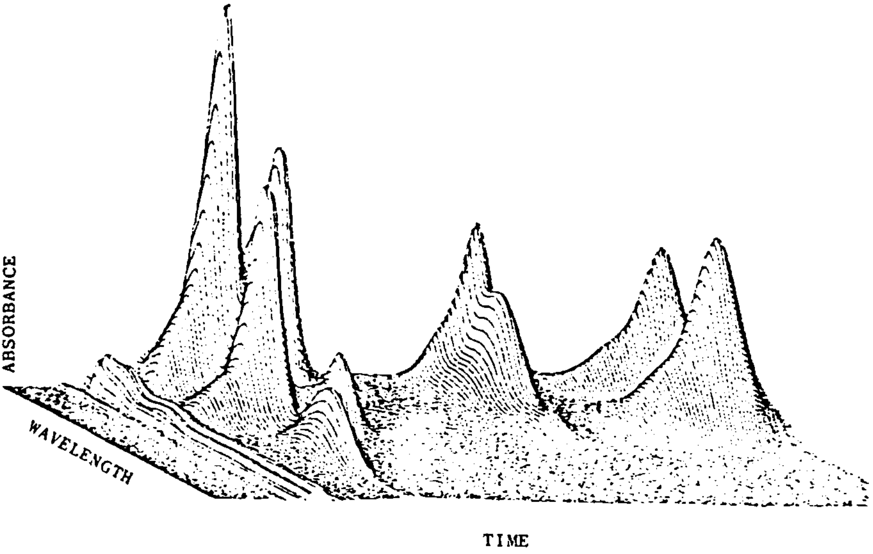

Figure 1: Spectrochromatogram of the test mixture.

gradient elution experiment. Then a model is constructed of the response surface over the solvent triangle bounded by isoeluotropic water-modifier mixtures of equivalent eluting power to that of the initial mixture (11). The modifiers added to the aqueous component at this stage are methanol, acetonitrile and tetrahydrofuran for reversed-phase separations. Other solvents could be introduced as may prove neccessary.

The construction of the response surface model is achieved by the iterative regression method (12). In this approach a logarithmic relationship is initially assumed to hold between the concentration of each component of the mobile phase and the capacity ratio (k') of each detected component. From this relationship a prediction is made of the resolution (R_s) between all pairs of identifiable solutes at all points within the solvent triangle. Thus the conditions can be predicted to give the best separation of the *worst resolved* pair of solutes. A separation is attempted using this predicted mobile phase composition, and the data produced are then incorporated into the model to give a more accurate description of the relationship between concentration and k'. This allows an improved prediction of the optimum mobile phase composition to be made. The iterative cycle of prediction, experimentation and refinement of the model response surface continues either until no further improvement in resolution is produced, or until a pre-determined resolution is achieved.

The iterative regression approach to mobile phase optimisation relies upon the correct and unambiguous identification of each solute in each of the chromatograms. Should this not be achievable, due perhaps to co-elution of two or more components or the close similarity of the uv-absorption spectra, the system then employs a modified Simplex procedure (13) to enable the optimisation to continue. This is possible since the Simplex procedure makes no assumptions based upon spectral data. To utilise the Simplex method the overall quality of each chromatogram must be described by a suitable function. This requires a numerical value, calculated according to an appropriate response function (14). The optimisation proceeds by comparing the value of the response function produced by three different mobile phase compositions. The mobile phase conditions giving the smallest value for the response function are discarded. A new composition for the mobile phase is then predicted by reflecting this discarded point about the line connecting the two remaining data points.

After each separation carried out under the control of the Simplex optimisation strategy, the spectro-chromatographic data are examined in an attempt to acquire sufficient spectral information for unambiguous assignation of identity to be made. As soon as this information becomes available, due to the improvement in resolution achieved, the control of the optimisation process is passed to the iterative regression algorithm for further refinement.

ASSESSMENT OF PEAK HOMOGENEITY

The iterative regression optimisation strategy requires that the retention time of each solute be established for each of the distinct sets of separation conditions employed. To assist in this, each eluting peak is characterised by a spectral profile, produced by a multi-channel diode array detector, which should enable it to be associated with a particular component in the mixture. The assignment of spectral profiles is straightforward for peaks containing only one component unless two or more components exhibit similar spectra, when other factors such as peak area may be employed to reduce any ambiguity. However the process is more complicated in cases of co-elution, when estimates of the true spectral profiles of the contributing components can only be produced after a suitable deconvolution of the matrix of (A, λ, t) data by means of a chemometric method. A number of these powerful statistical tools are available, including, for example, iterative target testing factor analysis (15). Deconvolution may require the expenditure of a significant amount of computing time, particularly when using a bench-top microcomputer. For this reason, the expert system is provided with a number of simpler tools to assess the homogeneity of each detected peak so that the use of deconvolution can be restricted to cases where the chromatographic peak is shown to be non-homogeneous.

The tests for assessing peak homogeneity available to the system have been described previously (6) and include: comparison of the normalised spectral profiles recorded on the upslope and the downslope of the peak; evaluation of the derivative total-absorbance chromatogram;

examination of the absorbance-ratio chromatogram; determination of the apparent retention time as a function of detector wavelength; and examination of the ratio of time-domain derivative chromatograms. These tests are described below.

Comparison of Normalised Spectral Profiles

Spectra are extracted from the data matrix at times before and after the peak where the amplitude of the total absorbance signal is approximately one third of that recorded at the maximum. These spectra are then normalised at the wavelength of maximum absorption in the first spectrum. The square root of the sum of squares (RSS) difference between the two profiles is calculated. This value reflects the variation in the spectral profile across the peak and is indicative of the degree of heterogeneity of the peak.

Evaluation of the Derivative Total Absorbance Chromatogram

The profile of a differentiated curve is often a more sensitive indicator of the existence of an additional, minor component than is the profile of the original zero-order curve (16). To make use of this effect an approximation to the derivative of the total absorbance chromatogram with respect to time is obtained by a simple difference mechanism: $\frac{dA}{dt}(i) = \frac{(A_{(i+1)} - A_{(i-1)})}{2}$. The number of zero crossings in the derivative profile is determined. For a pure peak one zero crossing is expected. To reduce the effects of noise in the derivative signal in determining the number of zero-crossings, a crossing is only accepted as valid if the value of the total absorbance chromatogram at that time is above a threshold of 0.1. The first derivative chromatogram is further differentiated and the number of zero crossings in the second derivative chromatogram determined. Two such crossings are expected for a pure peak. The derivative chromatograms produced by a pure peak are illustrated in Figure 2a. The test is designed to detect variations from this ideal produced by the presence of one or more additional components. Such variations are illustrated in Figure 2b.

Examination of the Absorbance Ratio Chromatogram

The ratio of absorbances measured at two wavelengths should be invariant with time for an homogeneous peak (17). This test locates the time at which maximum absorbance occurs (t_{max}) and identifies the wavelength of maximum absorbance (λ_{max}) and the wavelength which gives half this absorbance value at this time ($\lambda_{\frac{1}{2}max}$). The ratio of the signals at these two wavelengths is then calculated as a function of time across the peak for all points at which the signal is greater than an experimentally determined cut-off value. The exclusion of any signal of low value concurs two benefits on the final profile. Firstly any distortion due to the effects of noise on the ratiogram is

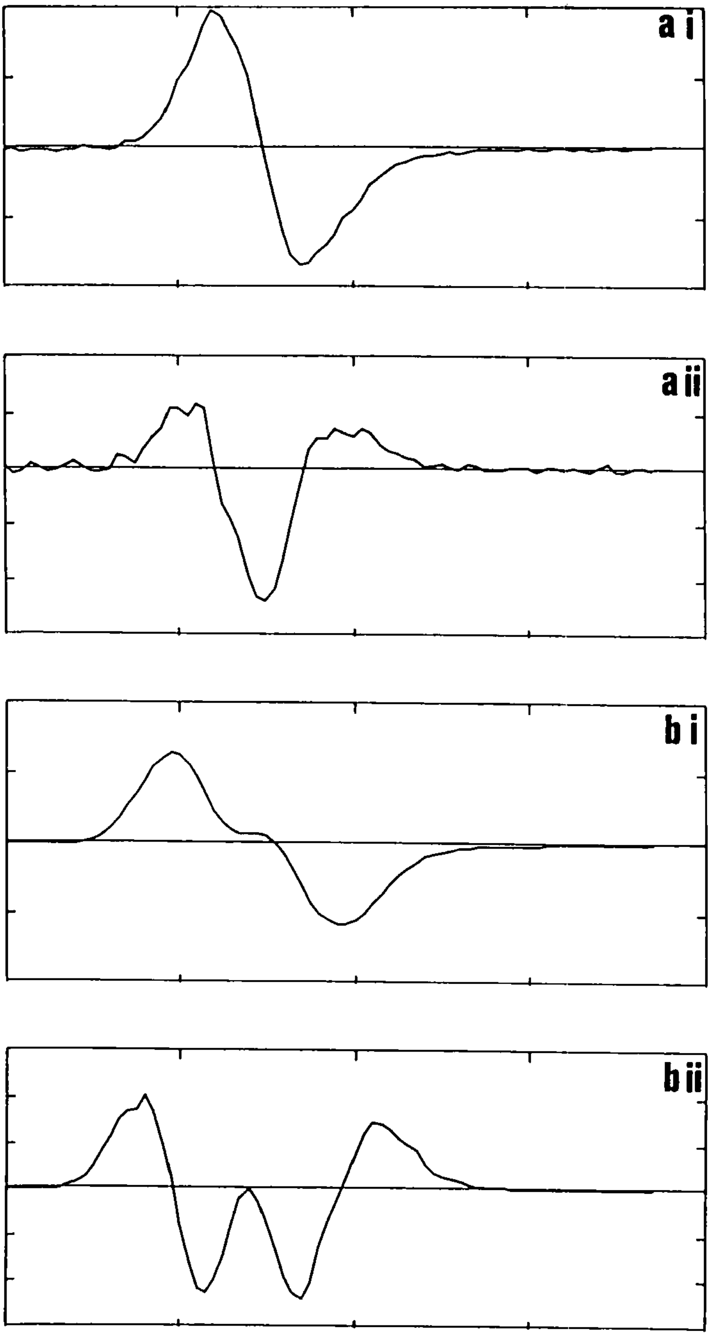

Figure 2: First (i) and second (ii) time-derivative chromatograms of a pure (a) and a composite (b) peak.

reduced, and secondly the chance of an inadvertant division by zero is eliminated. The RSS deviation from the value of the ratio at t_{max} is determined and reported. Ideally this should be zero, or for a real system not exceed the variation in signal due to detector noise. The presence of additional components distorts the ideal square-wave profile of the ratiogram (Figure 3a), as illustrated by Figure 3b.

Determination of the Apparent Retention Time as a Function of Wavelength

The retention time for a given component will be independent of the wavelength used to monitor the chromatogram. Where a peak is actually due to the co-elution of two or more components, a variation in apparent retention time with detector wavelength may be observed (18). To quantify this variation the value of t_{max} is determined for each detector channel and the difference between the largest and smallest values reported. To avoid reporting of noise signals for wavelengths at which the solute does not absorb, no retention time is recorded for wavelengths with a maximum signal of less than 0.1 AU.

Examination of the Ratio of Time-Domain Derivative Chromatograms

The idea of absorbance ratio evaluation may be extended into the derivative domain (19). This routine extracts chromatograms at λ_{max} and at $\lambda_{½max}$, normalises them at t_{max} and then calculates the time derivative for each chromatogram. The ratio of the chromatogram at $\lambda_{½max}$ to that at λ_{max} (which should be unity) is then calculated and the RSS deviation computed and reported, as for the absorbance ratio chromatogram.

Since the accuracy of the above tests is affected to different degrees by a number of factors (e.g. changes in the peak profile, the signal-to-noise ratio of the detector and the degree of similarity between the component spectra) the interpretation of the information provided by the tests requires consideration of these factors, when they can be determined. These factors are considered by rules contained within the expert system, which directs the interpretation of these test results and provides information on their reliability. The modular nature of the expert system facilitates the incorporation of additional homogeneity tests at any stage during the development of the system.

IMPLEMENTATION OF THE EXPERT SYSTEM

As implied above, the expert system is constructed from a number of modular routines, the internal operation of each module being independent of the rest of the system. The decision-making modules are written in microProlog (17), to which an extension has been provided (18) enabling it to call data capture and numerical processing modules written in other languages, such

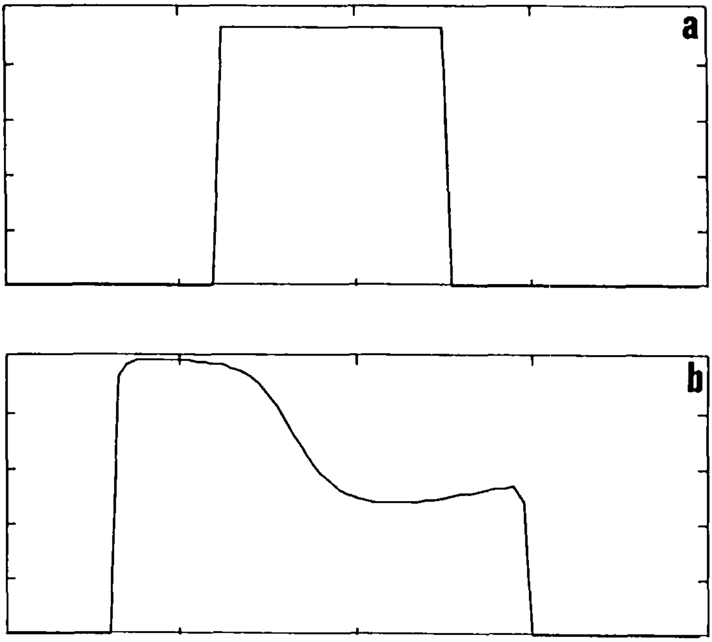

Figure 3: Absorbance ratiograms of a pure (a) and a composite (b) peak.

as Pascal, Fortran and Basic. The system is hosted on an IBM PS/2 microcomputer, with data from the photodiode array detector passed to the computer via an RS232C serial data link.

The present system requires the column to be preselected, and an operator to prepare the mobile phases, operate the pumps and perform the injections. It is our intention to automate these tasks under the direct control of the computer at a later stage in the development of the optimisation system.

MATERIALS AND EQUIPMENT

HPLC-grade methanol, acetonitrile and tetrahydrofuran (Rathburn Chemicals, Walkerburn, UK) were used as received. All eluents were filtered through Millipore® 0.45 µm filters in all glass apparatus and degassed under reduced pressure in an ultrasonic bath for 10 minutes.

Eluent delivery was achieved by the use of two PU4010 pumps under the control of a PU4850 chromatography data station (Pye Unicam Ltd., Cambridge, UK). The detector employed was a Pye-Unicam PU4021 diode array detector, connected to the microcomputer via an RS232C serial interface. The 100 x 5 mm column was packed with 5-µm Hypersil ODS.

EXPERIMENTAL

The operation of the expert system has been demonstrated for the optimisation of the separation of a synthetic mixture. The test mixture contained diethyl phthalate, 3-hydroxybenzaldehyde, n-propyl-p-hydroxybenzoate and phenol dissolved in methanol at a concentration of 20mg/ml.

The initial gradient elution experiment indicated that the required eluting power would be provided by a mixture of methanol and water (60:40 v/v). Equivalent combinations of acetonitrile - water (52:48 v/v) and tetrahydrofuran - water (42:58 v/v) were also prepared. The sample was injected onto the chromatograph using these three isoeluotropic mobile phases and each detected peak examined by the suite of purity checks described above. Retention times were established for each of the four components in each of the three isocratic chromatograms. These data were used to construct a mathematical model of the response surface, as described in the foregiong sections. A contour plot of the lines of equal separation for the worst resolved pair of solutes at each eluent combination is illustrated in Figure 4. In addition to the predicted global optimum composition, with a separation of over 40 arbitrary units, the system predicts the existence of two local maxima: one located at the apex representing 100% methanol - water, with a separation of 32 arbitrary units; the other is located at the apex for 100% THF - water and has a separation of under 5 arbitrary units.

A chromatogram was then carried out under the conditions predicted by this model and the retention data derived from this chromatogram was used to modify the model to produce a refined estimate of the required mobile phase composition. The process of continual refinement was continued until the new predicted mobile phase composition converges with the current actual composition.

DISCUSSION

The system described in this paper illustrates some of the potential benefits which may be derived from the incorporation of a degree of artificial intelligence into an optimisation strategy. To enhance the utility of the system, a number of additional facilities are intended. These include additional tests for peak homogeneity; improved rules to guide the interpretation of these tests; direct computer control of the chromatographic hardware; and rule-based modules to assist in the initial selection of column type and organic modifiers.

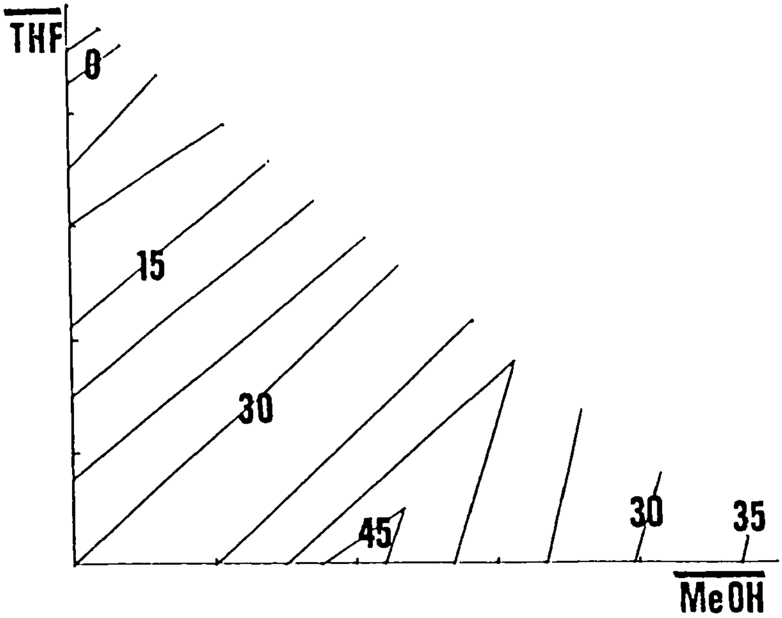

Figure 4: Contour plot of predicted resolution between the worst resolved pair of components.

The contours indicate the separation, in arbitrary units, of the least resolved pair of solutes at all points in the solvent triangle, as predicted by the first stage of the iterative regression optimisation routine. A value of zero indicates mobile phase compositions under which coelution of two components occurs. The best resolution is predicted to occur in a mobile phase containing 40% methanol-water with 0% tetrahydrofuran-water, and hence 60% acetonitrile-water.

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