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Expert systems in chromatography. Results of the ESCA project

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Abstract

The final results of the ESCA project (Expert Systems for Chemical Analysis) are presented This is one of the major projects in the field of expert systems for chromatography Expert systems have been developed that cover the important areas of method development in LC In the last part of the project attention was concentrated on two issues, the study of integration possibilities of the different stand-alone systems and the important aspect of validation and evaluation of the developed expert systems The integration studies and the results of the validation and evaluation are discussed

Keywords Expert systems, Liquid chromatography, ESCA project

The results of automation efforts by manufacturers of chromatographic instruments have led to an increased applicability of chromatographic instruments for routine analysis The bottleneck of analysis is situated mainly in the development of an optimum method and in the interpretation of the results These processes usually require a lot of expertise and experience to solve the problems that arise for each particular case Also, the quality control stage becomes an increasingly important aspect to be automated As a result of automation, the numbers of analyses and results have grown so much that automatic quality monitoring is necessary In view of the increasing demands of good laboratory and management practice (GLP and GMP), this aspect will become even more important The incorporation of expertise and experience in instruments is therefore the next step to be taken

Expert systems are software programs in which human expertise is implemented Therefore, they seem to be the right approach for further automation of instruments In other areas of chemistry they have already been demonstrated to be useful [1–3] In chromatography, a large amount of research has been carried out in a joint re-

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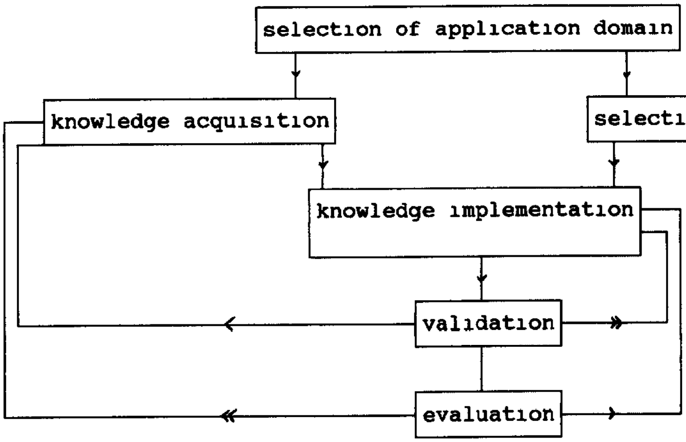

Fig 1 Different steps in the development process of expert systems

search project on the applicability of expert systems in chemical analysis (ESCA) [4] Partners from industry and universities have cooperated to study the possibilities of the expert system approach in LC In the first stage the development of LC methods for pharmaceutical compounds was selected as an application area Within this area expert systems were developed that are representative of the whole area of method development selection of initial method parameters, optimization of selectivity and instrumentation and finally validation of the method obtained As part of the project all aspects of the expert system building process have been investigated Aspects of integration and cooperation of expert systems have also been covered

The project started in May 1987 and officially finished in May 1990 During this period intermediate results and findings have been communicated by means of presentations and so far about 25 papers have been published in international journals and numerous lectures and posters have been presented In this paper an overview of the most important results is presented

EXPERT SYSTEM DEVELOPMENT PROCESS

The process of the development of expert systems consists of several stages and has many different aspects It requires close cooperation between workers who have the necessary application knowledge and expertise and those responsible for the implementation of the expert systems ("knowledge engineers") The different tasks are shown schematically in Fig 1 It shows clearly the sequence that has to be followed, the interaction between tasks and the loops that can occur

The aim of the project was to study and demonstrate the application of expert systems in chromatography Therefore, it was felt that a single application was too limited to demonstrate the objective For that reason, a number of (relatively) small domains were selected based on criteria of usefulness, difficulty and variety These domains were derived from LC method development as shown in Fig 2 This process resulted originally in four expert systems

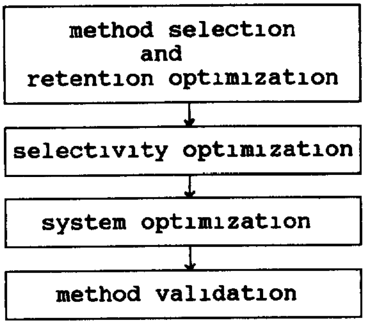

Fig 2 Stages in the chromatographic method development

Knowledge acquisition is the process of extracting knowledge as complete as possible from the chromatography expert This knowledge should then be implemented in a choosen tool by the knowledge engineer, which in this instance was either a chemometrician or a software specialist The latter requires more time to become familiar with the domain knowledge, but may realise better systems in terms of completeness, consistency and appearance Chemometricians, on the other hand, can acquire more chemical knowledge in a shorter period of time, but the quality of the resulting software can be inferior

To implement expert systems in a computer, a software tool is required This tool can be a standard computer language such as Pascal or C However, the implementation of expert systems with classical languages requires considerable software engineering experience and a large effort in terms of manpower In recent years dedicated tools for developing expert systems have become available These tools are often referred to as "expert system shells" The available tools range from simple to very sophisticated One of the purposes of ESCA was also to evaluate the suitability of these tools Therefore, it was necessary to select some suitable tools to implement the applications

This selection was based on the implementation of a small test knowledge base in different tools of varying size (large, mid-sized and small, see Table 1) The test knowledge base contained essential features of the final knowledge and was obtained from earlier work by De Smet et al [5]

TABLE 1

List of tools evaluated

Size	Name	Origin	Running on
Small	Delfi 2	Netherlands	PC
Medium	Goldworks [a]	USA	PC
	KES [a]	USA	PC
	Mylog	France	PC
	Nexpert object [a]	USA	PC
Large	S1	USA	Workstation
	KEE	USA	Workstation
	Knowledge Craft	USA	Workstation

[a] These were the tools selected

TABLE 2

Summary of development environment of the expert systems

Domain	Shell	Expertise centre [a]	Knowledge engineering centre [a]
Method selection and retention optimization	KES	VUB Organon	VUB
Optimization systems			
Selectivity optimization	KES	VUB Philips NL	VUB
System optimization	Nexpert object + Pascal	Philips NL	Philips Hamburg
Method validation systems			
Repeatability system	Goldworks	Unicam UK	KUN
Ruggedness system	Goldworks	Unicam UK	KUN

[a] VUB = Free University Brussels, Belgium, KUN = Catholic University Nijmegen, Netherlands, Philips NL = Research Lab , Eindhoven, Netherlands, Philips Hamburg = Research lab , Hamburg, Germany, Organon = Analytical R&D Labs , Oss, Netherlands, Unicam UK = Unicam, Cambridge, UK

In general, it was concluded that large tools such as KEE or Knowledge Craft were too complicated They also require large workstations or microcomputers to run on The small tools were clearly not adequate, e g , limited implementation possibilities, limited or no access to external databases and poor quality of the end user and knowledge engineering interface It was finally decided to use a selected group of mid-sized tools, which had the additional advantage of running on normal PCs [6] Table 2 summarizes the eventual tools from which the systems were built

Expert systems can only be expected to be useful in practice if they are reliable Conventional software products can be tested thoroughly through a number of standard procedures However, for testing expert systems no standard procedures exist With expert systems both the software and the knowledge base must be reliable and correct The fact that the knowledge base often contains a lot of heuristic knowledge poses specific problems to the testing phase [7,8] Considering the increasing demands of GLP, this part in the development process cannot be overestimated In view of this, considerable effort was devoted to the validation and evaluation of expert systems during the last part of the ESCA project

EXPERT SYSTEMS OF ESCA

The expert systems that were developed in the ESCA project can be divided into two categories stand-alone systems and integrated systems A complete overview is given in Table 3 In this section the stand-alone systems are considered

Method selection always starts with the choice of the chromatographic mode, be it GC or LC In LC a further refinement can be made by choosing, for example, the normal- or reversed-phase mode, and further a C_8 or a cyano phase In this way a decision tree can be built

DASH (Drug Analysis System in HPLC) is the system that assists in the selection of LC starting conditions for the purity check of pharmaceutical compounds Because of the complexity of the relationship between the structure (input) and suitable percentage of modifier (output), this system was developed only for heterocyclic basic compounds As most of these compounds are new chemical entities, there is no literature available on LC analyses for these compounds [9]

LABEL is an expert system that was developed by one of the partners (VUB) before ESCA started It selects a method for the LC of drugs in pharmaceutical formulations (label claim analysis) [5] It was included in the project because it covers the situation that one sample must be analysed for different compounds This is in contrast to DASH Compounds that are subjected to a purity check usually contain less then 5% of unknown impurities Optimization is then usually not required LABEL was added to be able to study the integration of method selection systems with optimization expert systems

LIT is a small expert system that helps to select all important parameters of a literature method and that checks whether a literature method can be treated by SLOPES

When the experimental results are not satisfactory, all three expert systems have an extension by which adaptations of the method are suggested in order to obtain an acceptable retention range of the compounds

The next step in method development is selectivity optimization This step typically involves the optimization of the mobile phase composition in order to obtain an optimum distribution of the peaks over the chromatogram

SLOPES (SeLectivity OPtimization Expert System) is an expert system which typically addresses one of the important aspects of selectivity optimization Initially attention was focused on the selection of an appropriate optimization criterion In the past, many optimization criteria have been put forward However, it should be recognized that a single criterion is not always the best one in all situations [10] SLOPES will help the chromatographer to select the most appropriate criterion, which will then be used to judge the quality of the chromatogram [11] This selection of a criterion depends, for example, on the selected experimental design and on the objective of the optimization (e g , best spreading of peaks

TABLE 3

Overview of the ESCA expert systems

Method development stage	Stand-alone expert systems [a]	Integrated expert systems (INT) [a]	
1 Initial method selection and retention optimization	DASH, LABEL, LIT	INT I DASH LABEL LIT +	
2 Selectivity optimization	SLOPES	SLOPES	
3 System optimization	SOS	INT II	INT III
4 Validation	REPS RES	SOS + REPS	SOS + RES

[a] Names are explained in the text

or minimum analysis time) Once the optimum selectivity has been obtained the mobile phase composition and stationary phase are kept constant for the next step, system optimization

SOS (System Optimization expert System [12]) can be used here to select a column with the shortest analysis time from a column set given by the user In addition, the user should also provide a set of available detector cells and a list of allowed time constants Finally, some limits should be given, such as the required minimum resolution between a relevant pair of peaks, maximum pressure drop and maximum flow-rate Within these constraints SOS recommends the column, instrument parameters and optimum flow-rate It predicts also the required analysis time and the critical resolution A result of a consultation and the experimental verification is shown in Fig 3

The final step in method development is the validation of the method This means that the quality of the results should be guaranteed to a certain extent The importance of validation is still increasing in view of increasing GLP demands The level of validation depends mainly on the intended use of the method A higher level of validation is required if, for example, the method is to be used in a large number of laboratories over a long period of time Methods to be used for regulatory analysis need the highest level of

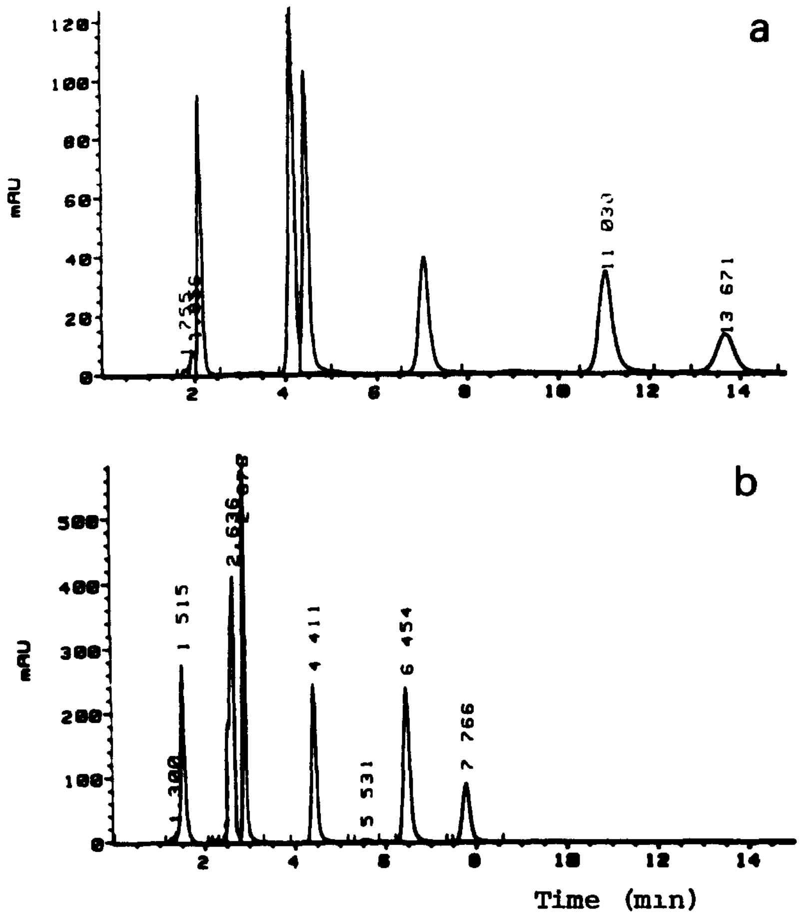

Fig 3 Optimization with the SOS expert system (a) Chromatogram before optimization, (b) chromatogram obtained with the conditions as advised by SOS, by which an analysis time and a resolution of 6 2 min and 1 6, respectively, were predicted

validation Validation of a method requires the testing of its specificity, precision, accuracy and limitations These so-called performance characteristics may be tested by validation procedures Many of these procedures make use of complex mathematics and rely on statistical designs, such as the Plackett–Burman design (e g , for precision testing) Expert systems can be of great help here in guiding the (inexperienced) user in the set-up of such advanced designs, in the calculation and in the interpretation of the results In the ESCA project, precision testing was chosen as the most challenging item in method validation to demonstrate the applicability of expert systems Precision testing involves, in fact, three separate parts repeatability, reproducibility and ruggedness In the repeatability test the analysis is repeated a number of times under identical conditions This is in contrast to the reproducibility test, where different conditions, e g , different instruments in different laboratories, are applied Finally, in a ruggedness test the effect of small changes in the operating conditions, i e , temperature, flow-rate and mobile phase composition, on precision is tested Repeatability and ruggedness testing were the subject of different expert systems

REPS (Repeatability testing System [13,14]) is an expert system in which Goldworks software is combined with the Lotus 1-2-3 spreadsheet package The expert system is used to select test procedures for repeatability Based on the usage requirements, an experimental design is set up The spreadsheet can run the algorithms and calculates the variances for peak areas and heights and retention times The expert system is able to interpret the results and to perform a diagnosis based on how the above parameters vary together An example of a rule can illustrate how a diagnosis proposal is reached For instance, if the variance of retention time and the variance in peak areas are large, and the variance of peak heights is small, then it is concluded that the problem of repeatability is imprecision of the flow-rate

RES (Ruggedness Expert System [15,16]) is a modular expert system in which the Goldworks software is combined with the procedural language C It is intended to assist in the proper set-up of a complete ruggedness test This involves heuristical (experience-based) knowledge to select the proper factors (and appropriate levels) to which the method should be rugged Statistical knowledge is necessary to choose a proper design based on the selected factors and the intended usage of the method The experimental results are interpreted If applicable system suitability criteria are provided, factors that cause problems are identified

INTEGRATION STUDIES [17,18]

The stand-alone expert systems described above all tackle a specific sub-problem of the method development process These systems are implemented in different shells and run on different hardware Ideally, the chromatographer should be able to consult the system that is needed in a specific situation as part of a complete method development expert system Also, from the viewpoint of the knowledge engineers it was seen as a challenging task to integrate stand-alone systems of different origins Because of the three knowledge engineer centres involved in the project, it was decided to study three partial integrations (see Figs 4–6)

There are two important aspects to this First, the analytical experts had to realize that to produce meaningful integrations it was necessary to fill knowledge gaps between the different stand-alone systems, so that additional knowledge acquisition was inevitable This resulted in considerable extensions of the existing systems and in the addition of new expert systems, such as LABEL and LIT Second, the knowledge engineers had the difficult task of linking sub-systems of different origins into an acceptable architecture

INT I [17] The structure of the architecture of INT I is given in Fig 4 In this scheme the supervisor is the essential part, having the strategic knowledge to route the end user to the different expert systems INT I is a typical example for which relatively much additional knowledge was necessary for the integration and integration was necessary in order to obtain a suitable system As

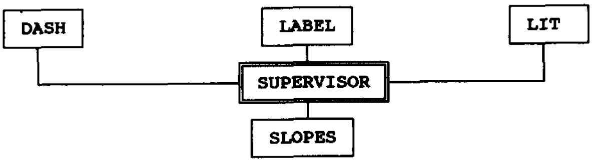

Fig 4 Structure of the integrated system 1 (INT I)

an extension, it is felt that the integration with the SOS system is necessary because using SOS strongly influences the selection of the optimization criterion

INT II [19] Figure 5 shows the structure of the second integrated system Two of the five subsystems (REPS and SOS) are the original stand-alone systems whereas the other modules were built to add flexibility to the system This architecture allows the user to consult the system in three different situations It can be used to assess the repeatability of a new method or to check the repeatability of a previously validated method Also, the possibility of using the system as a trouble-shooting tool turned out to be a valuable feature

INT III Another possibility to link stand-alone expert systems is shown in Fig 6 In the ruggedness expert system (RES) the system optimization system (SOS) is incorporated as an extra module The scheduler has knowledge on when to activate which module The different modules take care of the different tasks in the ruggedness test and the SOS module has been added to provide solutions for problems that have been detected by the diagnosis module SOS can be used in a number of situations Primarily, SOS can help to improve a method when the resolution has fallen below a critical level during the ruggedness testing SOS can then propose new conditions based on the requirement for higher resolution Both systems had to be adapted slightly and/or extended in order to make a sensible integration

These studies show that integration often results in complex structures, i e , they are less user friendly This endorsed in fact the original decision to build limited stand-alone systems On the other hand, it was shown that integration is useful in situations where the chromatographer often has to switch between systems Similar conclusions have also been reported elsewhere [20]

VALIDATION AND EVALUATION OF THE ESCA EXPERT SYSTEMS

Considerable attention was paid to the testing of the expert systems [21,22] It is important to note that systems were tested with special emphasis on their performance rather than on appearance aspects such as a nice user interface The latter should, however, be of sufficient quality to make an understandable system Two main stages have been distinguished, the validation and the evaluation stage

The validation process involved checking the software and testing the knowledge base by the responsible expert The procedure that was followed involved the selection of a number of test cases by the expert The expert solved the test cases manually, while the expert system was also

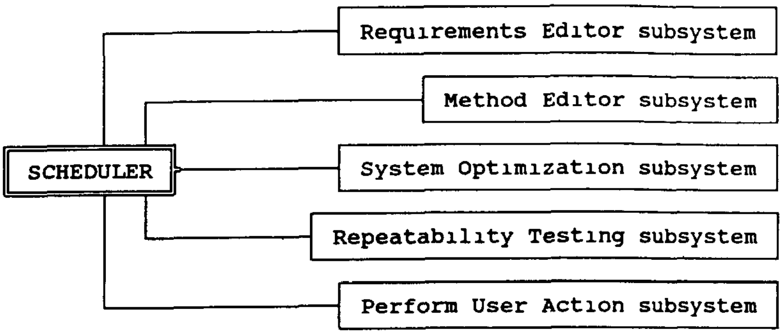

Fig 5 Structure of the integrated system 2 (INT II)

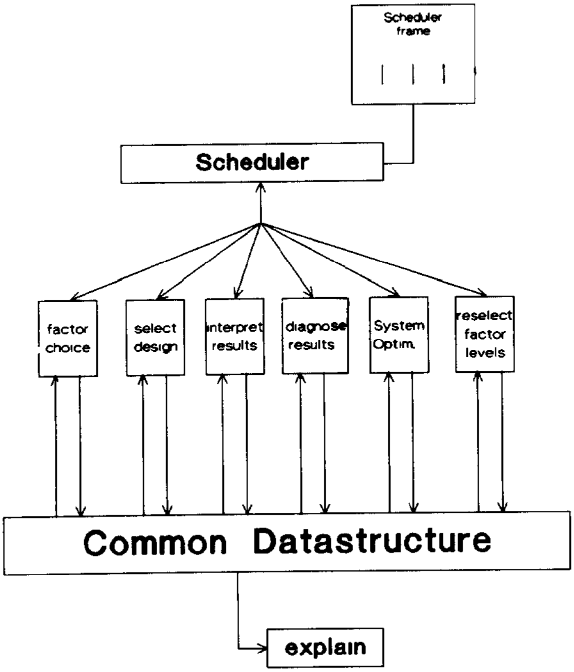

Fig 6 Structure of the integrated system 3 (INT III)

consulted The test cases were selected within the scope of the systems and to make use of as much of the knowledge base as possible Whenever differences between the expert systems solution and that of the expert were seen, the cause of the discrepancy was identified This led to the addition of missing knowledge or to the correction of existing knowledge To decide on the proper performance of the systems, a set of pass/fail criteria were defined by the knowledge engineer and the expert prior to testing The systems were improved until agreement was reached between the expert and the expert system After validation the systems were subjected to the second stage, the external evaluation

The evaluation phase consisted of testing the expert system in practical situations, to evaluate the system's performance in daily practice Generally, these tests were performed by external evaluators, i e , chromatographers not involved in building the system Unbiased problem cases were put to the expert system All inputs and outputs of the systems were registered and, whenever possible and appropriate, verified with experiments A list of performance criteria were identified by the knowledge engineers and the experts for each system These criteria took into account aspects of the man/machine interface, the consistency of the system and its limitations A list of criteria is given in Table 4

The evaluations were carried out by different persons, ranging from experts in method development to students with little or no experience Summarizing the evaluations of the three integrated systems the following conclusions can be drawn The user friendliness expressed among others in a good user interface, clear screen text and easy help functions was judged to be good in

TABLE 4

Example of evaluation criteria

Man–machine interface (user interface)	Choice of phrases
	Explanation
	Operation (mouse, keyboard, file input, etc)
	Usability/ease of use
Consistency testing	Accuracy (correct answer, quality of advice)
	Reproducibility (repeatability, same input same output)
	Robustness of software (does the system lock up or fall over)
	Ruggedness (small changes in input small changes in output, similar cases, similar answers)
System limits	Conflict (two rules with the same input give a different output)
	Missing rules (input leads to no realistic output)
	Are essential parts missing?
	Are there examples of strange answers in extreme cases, e g , incomplete input, nonsense output?
	Technical content do the systems do a useful job?

INT III and INT III Some of these aspects are closely related to the quality of the shell KES (INT I) belongs to the older generation of shells in which the above features can be improved

With respect to the knowledge, a great variety exists between the systems The knowledge collected in INT I is most complex and generally of heuristic nature Although this system was restricted to basic pharmaceutical compounds, there is still a lot of chemical and analytical knowledge to add The knowledge in the other systems is better defined and proved to be complete in a broad field of applications The evaluators found the item "factor choice in the ruggedness module very flexible On the other hand, they asked for more flexibility in the experimental design

Case study pH optimization

In the following example an interesting application of expert systems is shown in which algorithmic-based knowledge is combined with heuristic knowledge The complexity of some steps in the method development process will be demonstrated

INT I deals with method selection and selectivity optimization In this system three modules are present for the method selection and subsequent retention optimization After an experiment it has to be decided whether the selectivity has to be optimized The expert system adequately helps to select a method for the selectivity optimization, viz , sequential or simultaneous approach, and which parameters have preferably

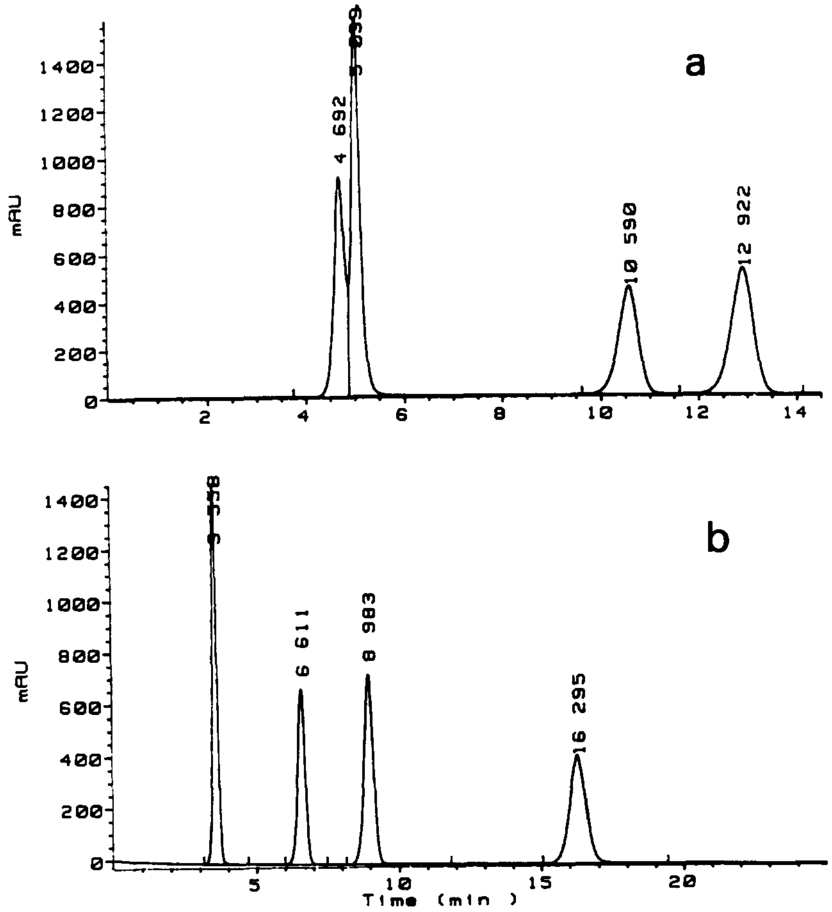

Fig 7 Optimization with SLOPES (a) Chromatogram in one of the experiments, (b) result obtained after optimization with SLOPES

to be optimized, viz, percentage of modifier, mixture design, temperature, pH The next step is to carry out the optimization It was chosen to implement only the software tools to carry out pH optimization in a simultaneous approach The pH optimization was selected because this is a relatively new area For other types of optimization one can use commercially available software tools

Before the experiments for the pH optimization can be carried out, the parameter space has to be defined, an experimental design has to be selected and a criterion has to be chosen for the calculation of the optimum In these three modules heuristic knowledge for the selection of parameter space for acids and for bases, chemometric knowledge for the selection of an appropriate design and algorithmic knowledge for the calculation of the optimum is used

In Fig 7, two chromatograms are shown, one before and one after pH optimization The optimization was done by means of SLOPES The main limitation of the system is to describe accurately the retention behaviour of each solute over the parameter space selected (retention surface) It is well known that the relationship between retention and pH is an S-shaped curve The calculation of the retention surface through the measuring points cannot be done by a quadratic function However, to keep the number of measurements small it was decided to fit a quadratic function through the data points and to study the pH variation over only a small pH range of 3 units By this means a reasonably accurate pH optimization could be achieved

Main results of the evaluation

Expert systems can provide very powerful assistance during method development because of the heuristic knowledge (expertise and experience of a specialist) that is implemented in these systems However, during the evaluation phase of all the expert systems it became clear that the attitude towards expert systems is strongly dependent on the expertise level of the evaluator The accessibility of the specialist's expertise was clearly appreciated by inexperienced users The introduction of the expert systems resulted for those users in a considerable amount of time saving in the development process Experienced users could appreciate the quality of advice given by the systems They are more interested, however, in comparing the expertise in the systems with their own experience When the strategy implemented in the system did not agree with their own expertise, it resulted in dissatisfaction with the expert system, because their own experience, probably better adapted to their specific situation, is not considered by the system This is especially the case when the knowledge domain of the expert system is strongly susceptible to individual opinions This gives rise to a second aspect, that at present expert systems are not flexible enough (Minor) changes that could result in a better performing expert system for a particular situation may be impossible to make by the user Only the knowledge engineer who is aware of the structure of the knowledge base is able to make such changes without unexpected consequences

A third conclusion of the evaluation is that the main attention should be devoted to the integration of expert systems with laboratory instruments When this is realized, expert systems can be used to obtain rapidly accurate advice while the user remains free to choose his or her own approach

Conclusions

The ESCA project can be considered as a pioneer project for the application of expert system technology in analytical chemistry Method development in LC was selected as the application expertise area The expertise that has been considered in the project covers the important areas of method development in LC

It was only possible to cover this large area because many recognized experts participated in the project It would be almost impossible to find a single expert to cover all the different aspects of method development

Different expert systems resulted from the project Most of these are still in the research phase, but a few have been further developed for commercialization (system optimization system, ruggedness system) From the results of validation and evaluation it can be concluded that expert systems are potentially very useful for method development in LC The benefits of the systems are that method development can be done more consistently and more efficiently and that better optimized and validated methods are produced Even when the systems were not yet complete this conclusion became clear

Expert systems still have to find their way into the chromatographic laboratory Users will have to accept computer programs that assist during tasks such as method development This requires, as stated above, expert systems that are flexible and easy to integrate It should be possible to add new knowledge or to adapt the system according to changes in the application environment Research on this subject remains necessary

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