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Feasibility study for the construction of an integrated expert system in high-performance liquid chromatography

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ABSTRACT

An integrated expert system consisting of several stand-alone expert systems was developed to assist the chromatographer in the determination of optimum high-performance liquid chromatographic conditions, *i.e.*, after a good "first guess", an elution within a reasonable analysis time and with adequate resolution. The implementation and linking of the systems were performed by means of the expert system building tool KES. The knowledge incorporated in this expert system is described.

INTRODUCTION

The selection of initial conditions ("first guess"), retention optimization and selectivity optimization are steps that need to be performed during high-performance liquid chromatographic (HPLC) method development. Expertise is required to perform these steps within a certain time constraint, and these constraints surely exist in the pharmaceutical world. In this field, the application of expert systems was investigated within ESPRIT (European Strategic Programme for Research and Development in Information Technology). The ESCA (Expert Systems in Chemical Analysis) project was supported by the EEC to study the feasibility of building complex expert systems for HPLC method development. Such expert systems would contain the experts' chemical knowledge, which could then be applied by (pharmaceutical) analysts less experienced in the field. Prior to the building of an expert system, the knowledge was acquired for each of the above steps. Once the knowledge acquisition was completed, stand-alone expert systems were developed for each of these steps. Then, in a later stage, these stand-alone expert systems were linked together. Of course, this latter process required additional knowledge to direct the user through the integrated system. Our aim in this paper is to describe the results of the feasibility study for the construction of an integrated system from stand-alone systems and, in particular, the chemical knowledge incorporated in this integrated system. The integration strategy from the software point of view has already been described

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elsewhere [1]. It is stressed that this is a feasibility study and that much more work would be needed to achieve a complete operational system.

OVERALL STRUCTURE OF THE INTEGRATED SYSTEM

The first task in the development of an HPLC method is the selection of initial chromatographic conditions. In the present integrated system, such first-guess conditions are selected by the expert systems LABEL, DASH and LIT, depending of the application field. After carrying out the first experiment, the retention time range of the solutes is evaluated. If there are solutes with capacity factors (k') outside the desired range, the retention optimization expert systems LABEL', DASH' and LIT' are consulted. At this level, a chromatogram is obtained in which all solutes elute within a reasonable time. However, two or more peaks may still overlap. The selectivity optimization expert system SLOPES is then consulted. The strategic knowledge necessary to route the end user to the different expert systems during the method development is incorporated in the SUPERVISOR expert system. A survey of the integrated system is presented in Fig. 1.

EXPERT SYSTEM BUILDING TOOL

The implementation of the different stand-alone expert systems was performed in KES (Knowledge Engineering System; release 2.5), written in C-language and based on the use of production rules. The tool reasons by backward and by forward chaining. External links to databases, spreadsheets and other processes are provided within KES. KES is embedded in C and this feature was used to link the different stand-alone expert systems without modification of the knowledge bases.

DESCRIPTION OF THE CHEMICAL KNOWLEDGE

LABEL–LABEL'

The expert system LABEL selects initial chromatographic conditions for the label claim analysis of pharmaceutical formulations on a cyanopropyl column used in different chromatographic modes. The knowledge incorporated in LABEL has already been described [2,3].

The main task of the expert system LABEL' is to situate the capacity factor in a suitable range. The retention optimization is performed by increasing or decreasing the percentage of organic modifier in the mobile phase, starting from the first-guess composition. As three different chromatographic modes are applied on the cyanopropyl column, three sets of rules for retention optimization are incorporated in LABEL'. An example of the rules for the "reversed-phase (RP) with buffer" chromatographic mode is given in the Appendix. The other sets of rules, namely for the "reversed-phase with water" and the normal-phase (NP) chromatographic mode, are similar. However, different rates of increase or decrease in the percentage of organic modifier are applied. In the RP chromatographic mode methanol is used as the modifier, while in the NP mode dichloromethane–hexane mixtures are used.

DASH–DASH'

The expert system DASH (Drug Analysis System

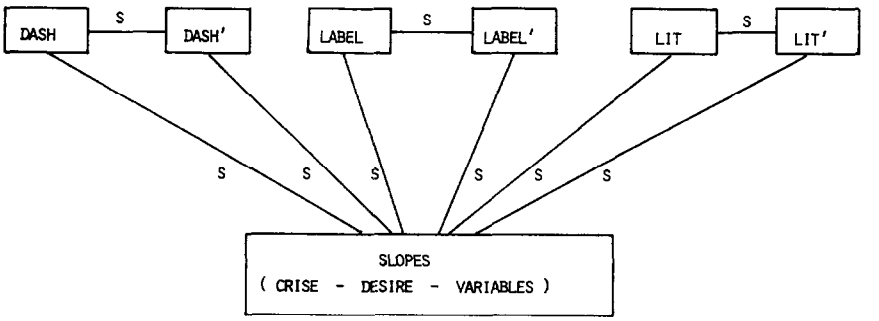

Fig. 1. Overall structure of the integrated system. S = Supervisor.

in HPLC) was originally developed for the purity control of basic compounds, namely CNS (central nervous system)-active and cardiovascular drugs. This expert system determines the initial chromatographic conditions to obtain a capacity factor between 3 and 10. The chemical knowledge incorporated in the expert system DASH has been described in the literature [4]. In more than 75% of all cases, correct predictions were obtained for the original family of substances. However, such a result cannot be expected for a wider range of pharmaceutical compounds. Therefore, the expert system DASH' was introduced, which performs retention optimization on the basis of the first guess result [5].

LIT–LIT'

The expert system LIT deals with HPLC methods selected from the literature by the end user for application in practice. This expert system functions in fact as a kind of filter to use only methods which can be treated further by the other expert system modules, more specifically the retention optimization expert system LIT' and the selectivity optimization expert system SLOPES. The system therefore checks a number of parameters. With regard to the knowledge in SLOPES, for example, all columns used in reversed-phase chromatography are admitted, whereas in normal-phase chromatography only the LiChrosorb cyanopropyl column can be used. This is not due to a lack of quality of other columns, but to the available knowledge in the team. In addition, several other restrictions were imposed (Table I).

TABLE I

PARAMETERS ADMITTED WITHOUT RESTRICTIONS (A) AND NON-ADMITTED (NA) BY THE EXPERT SYSTEM LIT

Parameter	A	NA
Mol.wt. < 1500	*	
Amino acids		*
Peptides		*
Proteins		*
Sugars		*
Inorganic cations/anions		*
Chiral separations		*
Ion-pair chromatography		*
Ion chromatography		*
Gel-permeation chromatography		*
Peak-shape additives	*	
HPLC instrumentation	*	
Detection	*	
Matrices	*	

The knowledge for the retention optimization is incorporated in the expert system LIT'. This step is carried out by comparing the experimental and the literature chromatograms. Three different cases for consulting LIT' have to be distinguished (Table II). In the first case the concentration organic modifier in the mobile phase will be decreased and in the second case it will be increased; in the third case the expert system will advise another brand of the particular type of column tested and the same strategy as mentioned above is followed. If the same elution pattern (case 3) persists, then the expert system will surrender. In all cases the end user has the possibility of stopping consulting the retention optimization expert system, once the experimental result is satisfactory.

SLOPES

The expert system for selectivity optimization consists of three different modules, namely VARIABLES, DESIRE and CRISE. In VARIABLES, the relevant optimization parameters and their boundaries are selected. Thereafter, the type of the experimental design and the location of the experiments are determined in DESIRE. Finally, the most suitable optimization criterion to describe the quality of a chromatogram is selected in CRISE. The stand-alone expert system CRISE has already been described [6]. Therefore, only the chemical knowledge incorporated in VARIABLES and DESIRE will be discussed.

TABLE II

DIFFERENT CASES FOR THE CONSULTATION OF THE EXPERT SYSTEM LIT

1. At least one relevant peak elutes with a significantly smaller retention time in comparison with the expected result
2. At least one relevant peak elutes with a significantly larger retention time in comparison with the expected result
3. At least one of the early-eluting peaks has a smaller retention time, and at least one of the late-eluting peaks is retained more strongly, in comparison with the expected result

VARIABLES

This expert system selects the optimization variables and the boundaries within which the optimization will be carried out. This depends of the chromatographic mode: in NPLC and RPLC with water the only possible variable is the solvent selectivity. In RPLC with buffer there are two possibilities: one can attempt to use the solvent selectivity or one can optimize the solvent strength and the pH simultaneously. The selection of the variables in RPLC with buffer is performed by determining the acid–base status of the components [2]. Once the acid–base characteristics of the compounds have been determined, the optimization variables and their boundaries are selected. This knowledge is outlined in Fig. 2.

The user is also asked whether solute/sample degradation can occur owing to one of the organic modifiers. In NPLC this leads to discarding one organic modifier, *i.e.*, carrying out the optimization with the base solvent (hexane) and the remaining modifier(s). In RPLC the organic modifier can be replaced. In RPLC with buffer, for example, methanol can be replaced with acetonitrile.

DESIRE

In the expert system DESIRE the most suitable experimental design is selected first. The possibilities considered in the integrated expert system are as follows.

(i) A simplex design, which belongs to the group of the sequential designs. In a sequential design only a few experiments are defined initially. The following experiments are based on the results of the first experiments [7–9].

(ii) A Doehlert design, which belongs to the group of factorial or simultaneous designs. In contrast to the sequential designs, all the experiments are defined before performing the experiments. In comparison with some of the common factorial designs, such as the central composite design, the Doehlert design is very economical. Only seven experiments are required for two optimization parameters. In this case the design takes the form of a centred

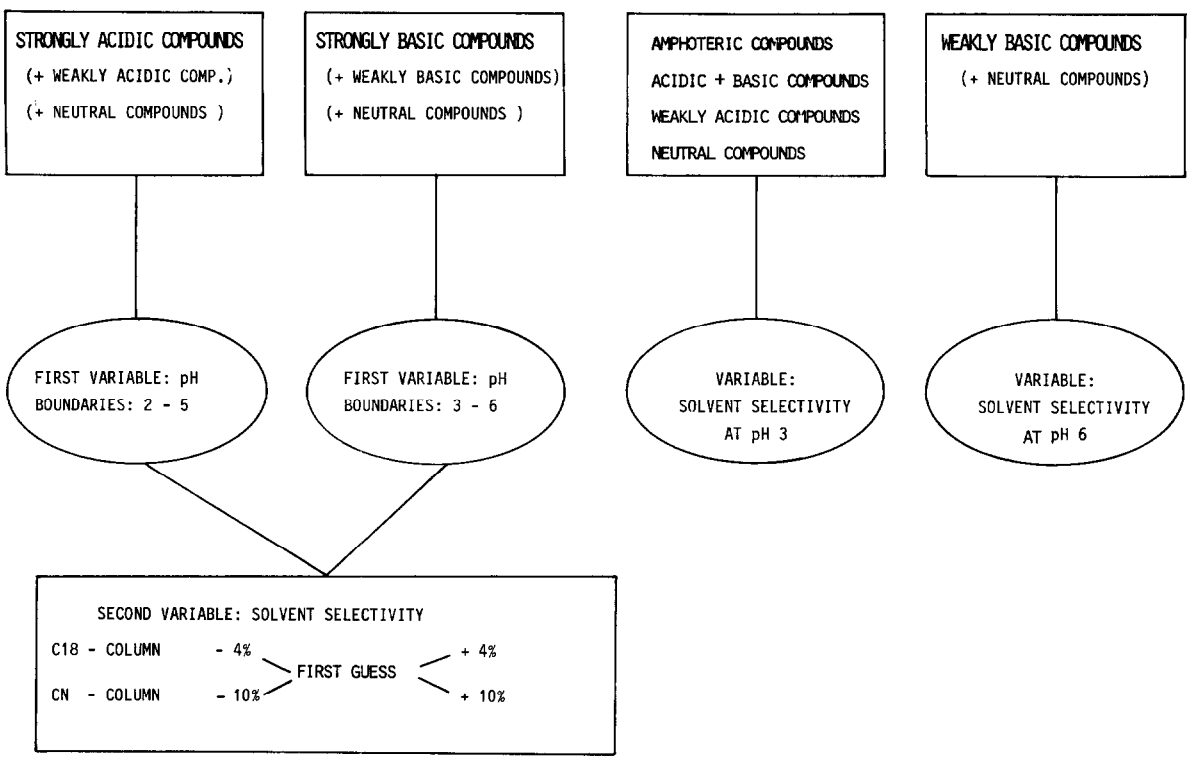

Fig. 2. Selection of the optimization variables and their boundaries.

hexagon. Such a design permits a uniform description of the parameter space [10].

(iii) A mixture design, which is a special factorial design due to a constraint on the variables, *i.e.*, the sum of the selected parameters is constant. This approach was originally developed by Glajch *et al.* [11].

The algorithm for each design was implemented as a separate computer program. Knowledge concerning the selection of the appropriate design was included in the expert system but, as this is only a study designed to investigate whether integration is feasible, only the Doehlert design was actually included in the integrated system. The Doehlert design was selected from the three above, as it is more novel and it is therefore more interesting to obtain information about its use. The necessary connections for the inclusion of the other algorithms are already provided in the integrated expert system.

Once a design has been selected, the number and the location of the experiments to be carried out are determined. Thereafter the correctness of the approach is investigated. Finally, the response function is predicted and the optimum located.

Selection of the design

Generally, a factorial approach is preferred to a simplex procedure. Only when more than two variables have to be optimized simultaneously is the simplex design selected. VARIABLES recommends a maximum of two optimization parameters. The option of a simplex design is provided for further extension in the future, because in a few cases (*e.g.*, ion-pair chromatography) for which the knowledge is not yet incorporated in this system, more than two variables may be selected. When there are one or two optimization parameters different situations arise (Fig. 3).

Because only the Doehlert design was actually implemented, only this one will be discussed in more detail.

Optimization strategy

The Doehlert design is constructed knowing the boundaries of both variables. The variable with the largest effect on the response, the pH, is tested on five levels and the other variable, the solvent strength, on three levels. The experiments are listed in Table III.

The Doehlert design is a simultaneous approach where all the experiments are defined before performing the first one. Within the expert system, the user may carry out the experiments in a sequential way. One may start with four experiments (Nos. 1, 2, 3 and 4 in Table III). After these four experiments, the user may then decide that one of them gives sufficiently good results and stop. Afterwards, if still necessary, the remaining experiments can be carried out. The knowledge for this part of the system is represented in Fig. 4.

General evaluation

The module of the expert system that deals with the evaluation of the experimental results consists of two parts. First, the system checks whether the capacity factors of the compounds are situated in an acceptable range for at least four experiments. If not, the boundaries for the optimization parameters recommended by VARIABLES are incorrect. New upper and lower limits must be selected in the area of optimum retention times. This knowledge is also outlined in Fig. 4. The second part of the evaluation concerns the effect of the variables on the selectivity coefficient α. If neither the pH nor the solvent strength has an effect on α, the parameters have not been selected correctly. The system then advises to optimize solvent selectivity and to use a mixture design. In all other situations the optimization procedure can be continued. Depending on the user's choice between the sequential and the simultaneous approach, different paths will be followed. A survey of this part of the system is presented in Fig. 5.

Location of the optimum

After selection of the optimization criterion with CRISE and entering all experimental data [retention time (t_r), dead time (t_0) and peak width at half height ($W_{1/2}$)] for each solute at each of the data points, the response function is modelled. The model used is a quadratic relationship between log k' (or log $W_{1/2}$) and the variables. The following model equation is used:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2$$

where Y represents log k' or log $W_{1/2}$, and X_1 and X_2 are the pH and the concentration organic modifier, respectively. The β-coefficients are calculated by

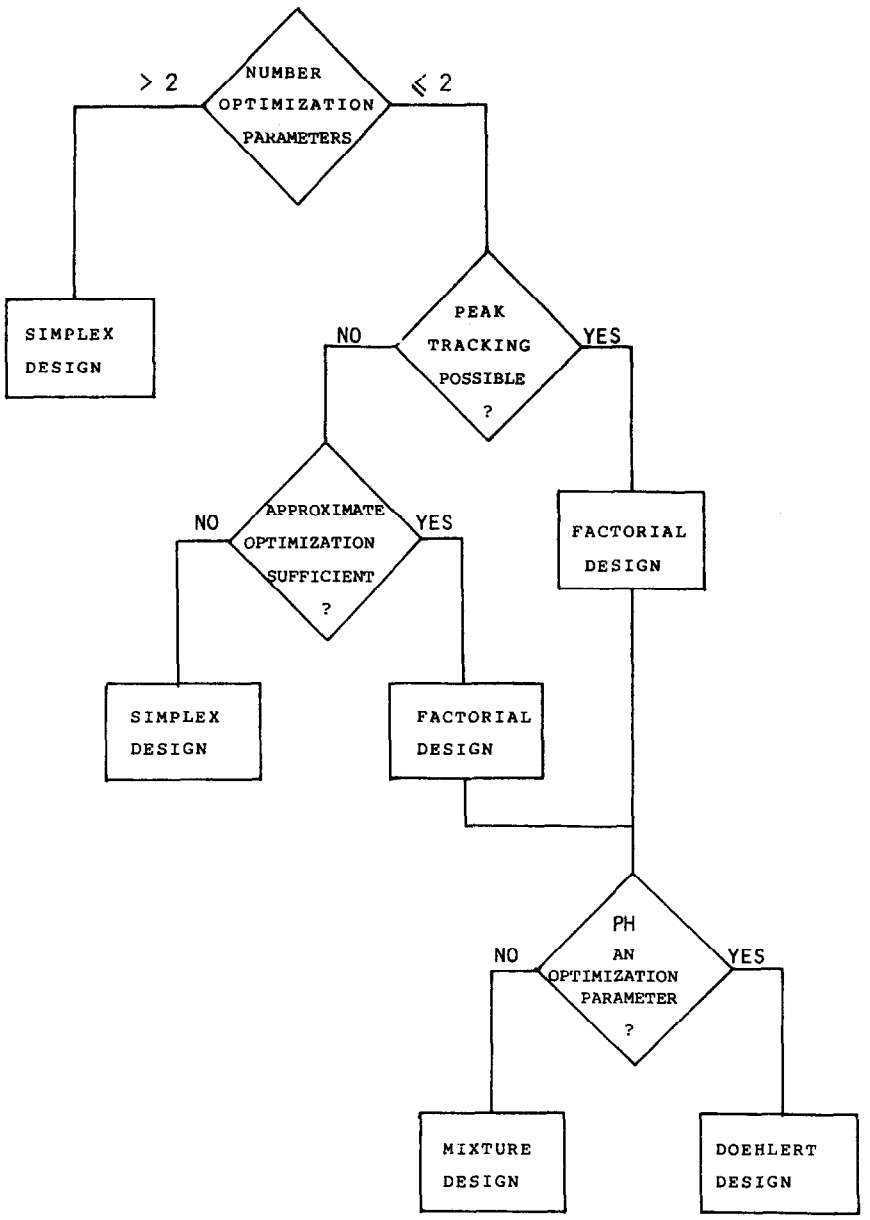

Fig. 3. Selection of the experimental design.

regression. Once these coefficients are known for all solutes, the response can be predicted and the optimum located. If the experimental result differs significantly from the predicted value, a recalculation of the optimum response is carried out, taking the results of the experiment at the initially predicted optimum into account. Once there is no significant difference between the predicted and the experimental results, the user has the possibility either of stopping or of predicting the response under other experimental conditions (Fig. 6). The latter may be useful, for example, if the optimum is situated on the low pH boundary and the user prefers a higher pH.

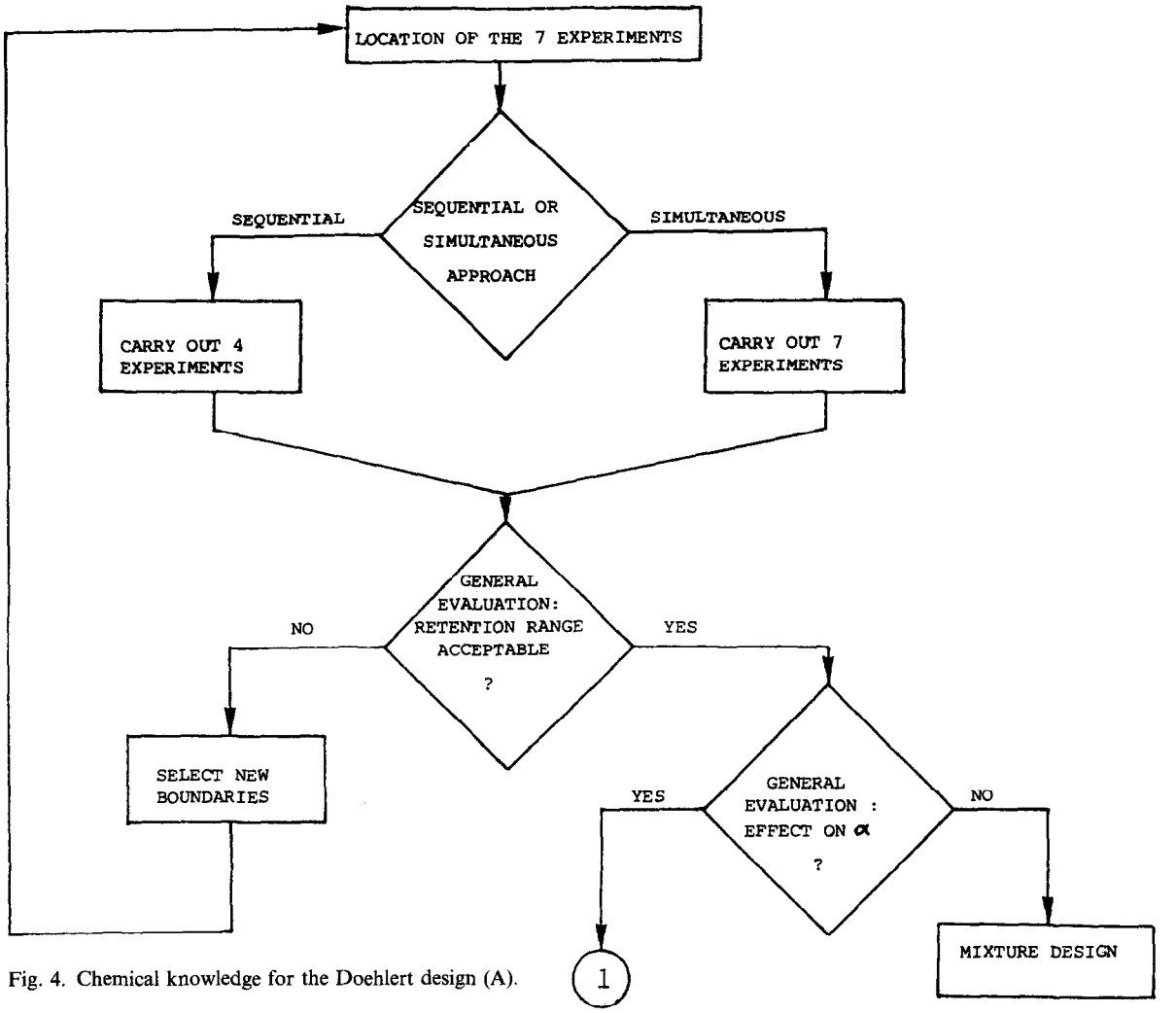

Fig. 4. Chemical knowledge for the Doehlert design (A).

TABLE III

LOCATION OF THE EXPERIMENTS IN THE DOEHLERT MATRIX DESIGN (IN NORMALIZED UNITS)

The upper and lower values for both parameters are +1 and −1, respectively.

No.	X_1 (=pH)	X_2 (=% organic modifier)
1	+0.5	−0.866
2	−0.5	−0.866
3	+0.5	+0.866
4	−0.5	+0.866
5	+1	0
6	0	0
7	−1	0

SUPERVISOR

The SUPERVISOR expert system contains the strategic knowledge necessary to route the end user to the different expert systems. The SUPERVISOR starts by soliciting a problem statement from the user and identifying the problem as belonging to LABEL, DASH or LIT. The link from LABEL, DASH or LIT to the SUPERVISOR is unidirectional, *i.e.*, once the problem statement is transmitted to one of the expert systems, a complete advice is given (Table IV) and afterwards the system will not be addressed again. Once the first-guess experiment has been carried out, the results are communicated to the SUPERVISOR for evaluation.

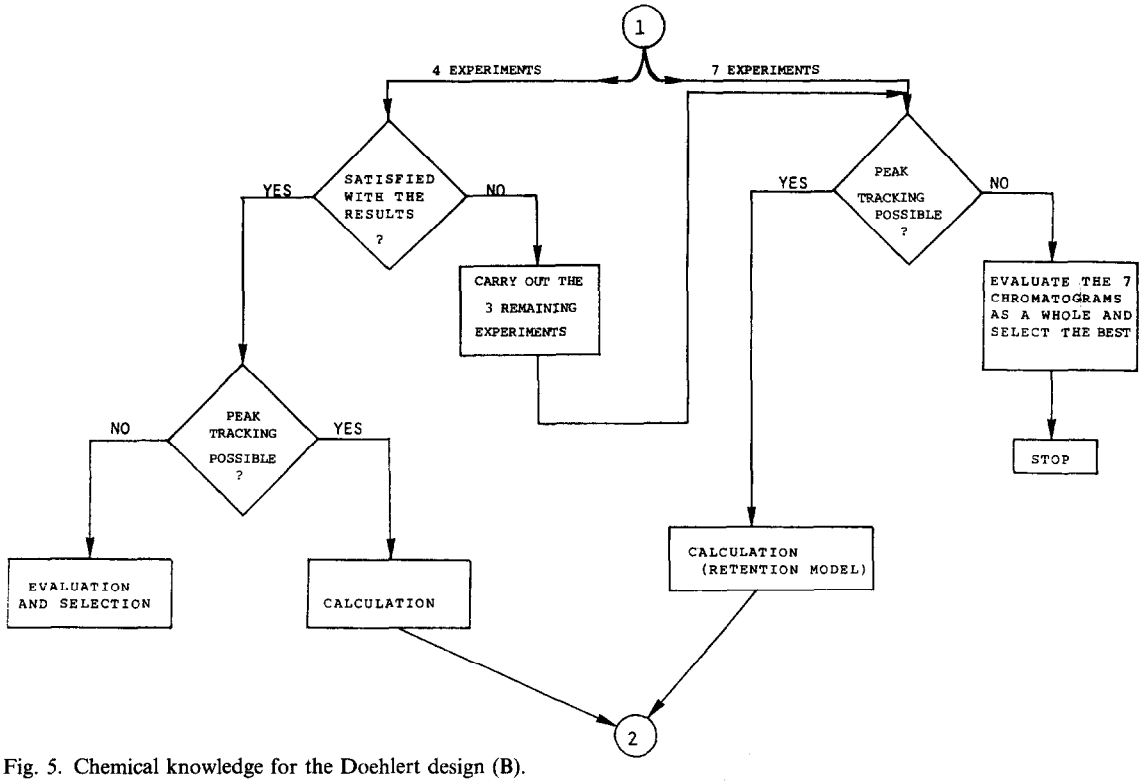

Fig. 5. Chemical knowledge for the Doehlert design (B).

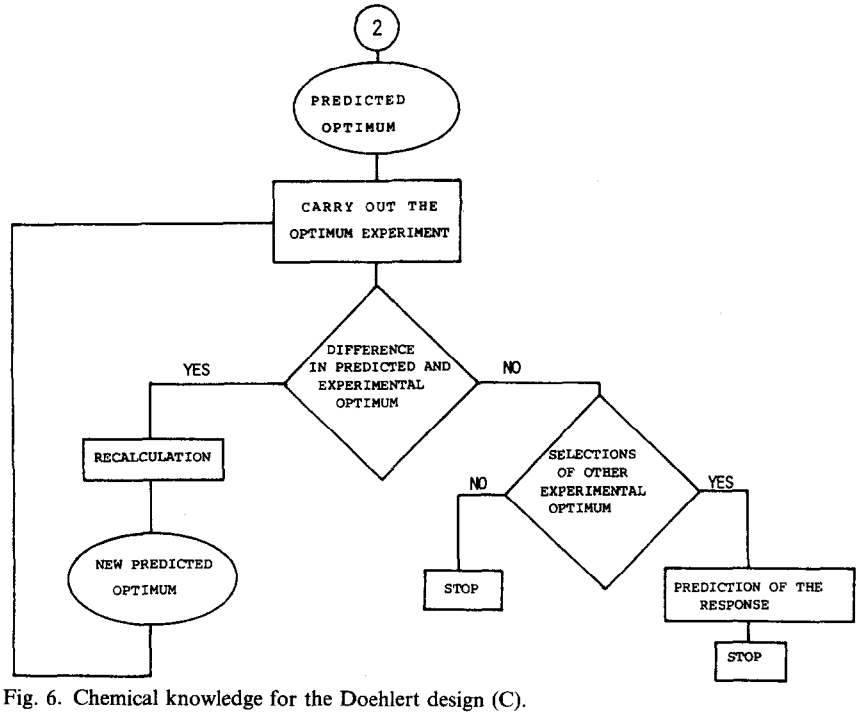

Fig. 6. Chemical knowledge for the Doehlert design (C).

For this purpose default values are applied (Table V). The conclusion of this evaluation is always submitted to the user, who has the possibility of overruling the decision of the SUPERVISOR. If the user agrees with the decision of the SUPERVISOR, then one is routed to the retention optimization expert systems.

The philosophy of the further interaction between LABEL′ and the SUPERVISOR is represented in Fig. 7. Second and third guesses are permitted, but not a fourth. The second and third guesses are obtained from LABEL′. If the third guess is unsuccessful, then the SUPERVISOR routes back to the advice obtained from LABEL and the second preference is tried out in the same way as the first approach. This process continues until either a satisfactory result is obtained or all possibilities have been exhausted. In the latter instance the retention optimization failed and the user has to find another way to solve the problem.

When gradient elution is recommended by LABEL (as a possible, but not a preferred approach) and the third guess is still unsuccessful, the user is advised to perform a gradient elution. The knowledge on gradient optimization is, however, not incorporated in the integrated system.

The rules to route to the retention optimization system DASH′ have already been described [4].

When LIT is used, the user has to compare the experimental result with the published result in the literature method. If the user is satisfied, no retention optimization with LIT′ is suggested by the SUPERVISOR. If the result is unsatisfactory, the SUPERVISOR routes to LIT′.

After successful application of LABEL′, DASH′ or LIT′, the retention times are situated in an acceptable range. However, the resolution may need to be enhanced for partially or completely overlapping peaks. For this purpose the selectivity optimization expert system can be consulted. To decide whether or not selectivity optimization is required,

TABLE IV

ADVICE FROM THE FIRST-GUESS EXPERT SYSTEMS LABEL, DASH AND LIT

LABEL	→ RP with water → RP with buffer → NP → Gradient elution → Three approaches in order of decreasing preference, for instance: first approach: RP with water second approach: RP with buffer third approach: NP
DASH	→ a single approach
LIT	→ one literature system at one time

TABLE V

DEFAULT VALUES APPLIED BY THE SUPERVISOR FOR THE CONSULTATION OF THE EXPERT SYSTEM LABEL′

$1 < k' < 3$ (for $n = 1$ or 2; n = number of substances)
$1 < k' < 5$ (for $n = 3$ or 4)
$1 < k' < 10$ (for $n > 4$)

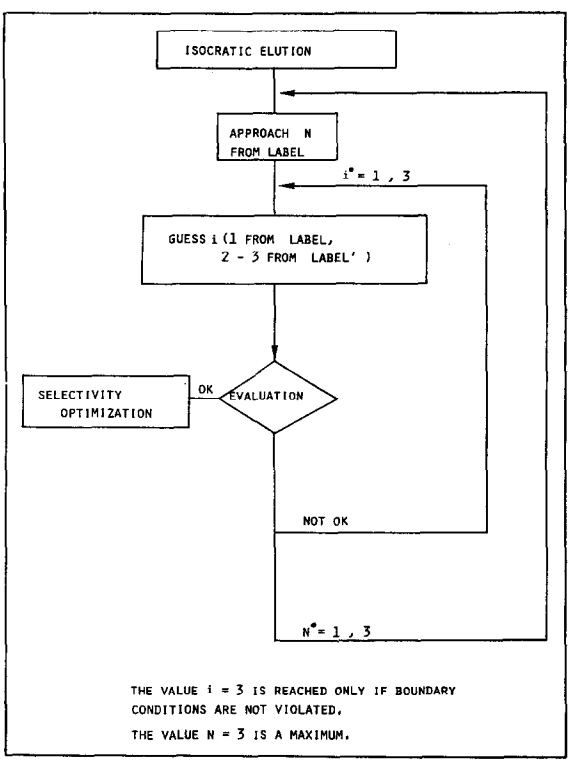

Fig. 7. Interaction between LABEL′ and the SUPERVISOR.

TABLE VI

RULES FOR THE CONSULTATION OF THE SELECTIVITY OPTIMIZATION EXPERT SYSTEM SLOPES

If $N_{found} \geq N_{exp}$, which means that all relevant peaks have been detected (case of LABEL, $N_{found} = N_{exp}$) or that an impurity has been found (case of DASH, $N_{found} > N_{exp}$), the selectivity optimization is not required
If $N_{found} < N_{exp}$, then the SUPERVISOR will route to the selectivity optimization expert system

TABLE VII

CHARACTERISTICS OF THE TEST COMPOUNDS

Compound	Acid/base status	Functional group
(1) Org 9731	Neutral/slightly basic	NH_2–C=N–OH
(2) Acid	Acidic	–COOH
(3) Amide	Neutral	NH_2–C=O
(4) Amidine	Basic	NH_2–C=NH

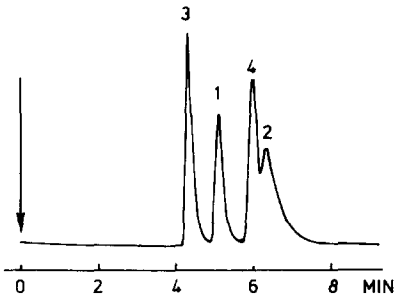

Fig. 8. Chromatogram of Org 9731 and related compounds after consultation of LABEL. Drugs as in Table VII. For experimental conditions, see Table VIII.

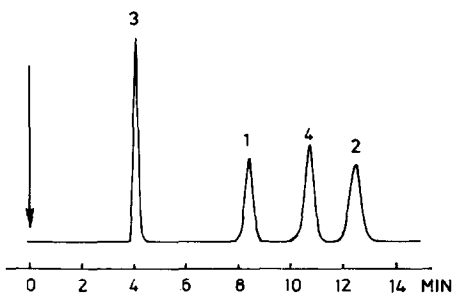

Fig. 9. Chromatogram of Org 9731 and related compounds after consultation of LIT. Drugs as in Table VII. For experimental conditions, see Table VIII.

the SUPERVISOR applies rules, which compare the number of peaks in the experimentally obtained chromatogram (N_{found}) with the expected number of peaks (N_{exp}). These rules are listed in Table VI.

EXAMPLE OF AN APPLICATION

A separation method was developed for Org 9731 and three related compounds. Some characteristics of the test compounds are shown in Table VII. In a first step the first-guess expert systems were consulted. As the fluorine atom in Org. 9731 was not present in the list of structural elements in DASH,

TABLE VIII

CHARACTERISTICS OF THE LABEL AND LIT METHODS

Parameter	LIT	LABEL
Column (dimensions, particle size)	μBondapak C_{18} (300 × 3.9 mm I.D., 10 μm)	LiChrosorb CN (250 × 4.0 mm I.D., 5 μm)
Basic solvent (%) (pH, μ)	Aqueous buffer (60%) (pH 3.5, μ = 0.1)	Phosphate buffer (80%) (pH 3.0, μ = 0.05)
Modifier (%)	Methanol (40%)	Methanol (20%)
Additive (%)	Amine (10%)	–
Flow-rate	1.5 ml/min	1.0 ml/min
Detector	Diode-array	UV–VIS
Temperature	30°C	Ambient

the consultation of this expert system was impossible. Concerning the expert system LIT, a method from the literature was available, which was in this case a method developed within Organon. The expert system LABEL was also consulted. The chromatograms obtained after carrying out the advice of LABEL and LIT (Table VIII) are shown in Figs. 8 and 9, respectively. In both instances acceptable retention times were obtained and further retention optimization was therefore unnecessary. At this level the best method was selected. As the separation selectivity and also the chromatographic performance, such as peak shapes and plate counts, were found to be better with the LIT method, this was selected for further selectivity optimization. The Org 9731 mixture consisted of an acidic, a basic and two neutral solutes. In such a situation the expert system advises an optimization at constant pH. However, in order to continue with the selectivity optimization the mixture was considered to consist of only basic and neutral solutes.

The Doehlert design was performed in a sequential way. As the mixture was not so complex, four experiments for the description of a first-order model were expected to be sufficient. The experiments are listed in Table IX (Nos. 1, 2, 3 and 4). The experimental results were evaluated. The variables and their boundaries were found to be correctly selected. Subsequently the optimization criterion was selected in CRISE. This expert system advised a threshold criterion (resolution) with an *a priori* value of 1.5. The optimum was predicted and then verified experimentally. As shown in Fig. 10, the predicted optimum was unsatisfactory. Resolution between

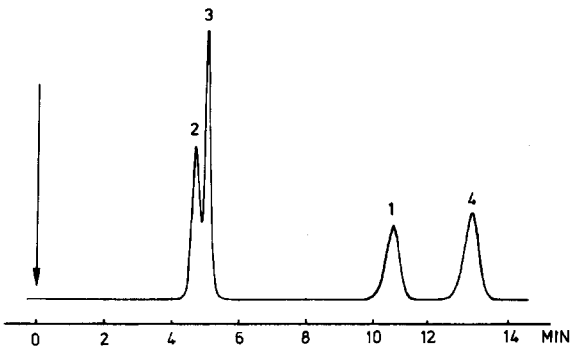

Fig. 10. Optimum for Org 9731 and related compounds after four experiments in the Doehlert design, using a threshold criterion as global optimization criterion. Mobile phase: aqueous buffer (pH 5.7)–methanol (61:39). Drugs as in Table VII. For other experimental conditions, see Table VIII.

compounds 2 and 3 was moderate, with a value of 0.87. The predicted retention times and peak widths were not in agreement with the experimental results. The result obtained with the input method (LIT) was clearly better. This illustrates that the first-order model was not accurate enough for the description of the response surface. For this reason the three remaining experiments were performed (Nos. 5, 6 and 7 in Table IX).

The same route as described for the four experiments was followed for further consultation. Depending on the criterion selected, different optima can be obtained. Two optimization criteria were

TABLE IX

LOCATION OF THE EXPERIMENTS IN THE DOEHLERT MATRIX DESIGN FOR THE ORG 9731 MIXTURE

No.	X_1 (=pH)	X_2 (=% organic modifier)
1	5.2	36
2	3.7	36
3	5.2	44
4	3.7	44
5	6.0	40
6	4.5	40
7	3.0	40

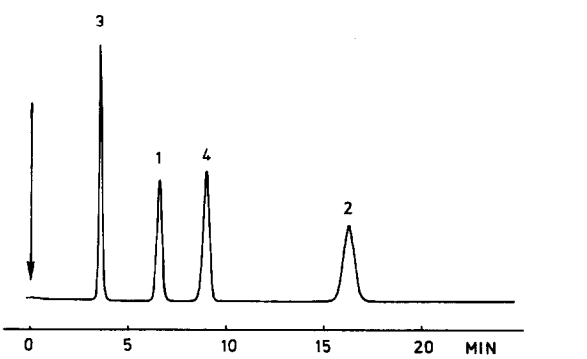

Fig. 11. Optimum for Org 9731 and related compounds after seven experiments in the Doehlert design, using required analysis time as global optimization criterion. Mobile phase: aqueous buffer (pH 3.0)–methanol (57:43). Drugs as in Table VII. For other experimental conditions, see Table VIII.

investigated, namely a threshold criterion (resolution) and also the required analysis time at constant pressure drop. In the first instance the separation is performed on the same column, whereas in the second the column length and/or particle size may be optimized in a later stage. The predicted retention time was 7.3 min (minimum resolution of 1.5) and 16.3 min (minimum resolution of 4.2), respectively. As shown in Fig. 11, the experimental results are in reasonable agreement with the predicted values. In the second instance the system was reconsulted to investigate whether the optimum was correctly selected. The difference between the "old" and the new optima was very small, so the supplementary experimental data point fitted very well in the calculated response surface.

CONCLUSIONS

This study was carried out to investigate the feasibility of building complex expert systems for method development in HPLC. It can be concluded that such a complex expert system can be developed by first dividing the problem into several smaller sub-problems. For instance, one can first investigate separately the first-guess stage, retention optimization and selectivity optimization. The knowledge acquisition and implementation for these sub-problems result in several smaller expert systems. In a later stage these systems can be linked.

The system developed here is not a complete system for HPLC method development in the sense that many application areas and many types of HPLC methods are not covered. However, the example demonstrates that the present system can be applied to many real problems.

We believe that using the approach described in this paper, one should be able to develop complete systems for specific areas, such as reversed-phase HPLC for basic drugs in biological media. It seems too ambitious to envisage building an expert system for the whole of HPLC. The main difficulty is that technology changes rapidly, so that the knowledge incorporated in expert systems should also change. It is feasible to adapt expert systems in a restricted area to these changes, but it seems nearly impossible (at least at an acceptable cost) to do so for a system covering the whole area.

ACKNOWLEDGEMENTS

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APPENDIX: EXAMPLE OF RULES FOR LABEL' (FOR THE RP + BUFFER MODE)

(I) $n = 1$

If $k' < 0.5$, then use -20% methanol in the mobile phase.

If $0.5 < k' < 1$, then use -10% methanol in the mobile phase.

If $k' > 5$, then use $+20\%$ methanol in the mobile phase.

If $3 < k' < 5$, then use $+10\%$ methanol in the mobile phase.

(II) $n = 2$

If k'_1, $k'_2 < 1$, then use -20% methanol in the mobile phase.

If $k'_1 < 0.5$ and $1 < k'_2 < 3$, then use -10% methanol in the mobile phase.

If $k'_1 < 0.5$ and $k'_2 > 3$, then the user decides if -10% methanol in the mobile phase is applied.

If $1 < k'_1 < 3$ and $k'_2 > 3$, then use $+10\%$ methanol in the mobile phase.

If k'_1, $k'_2 > 5$, then use $+20\%$ methanol in the mobile phase.

(III) $n = 3$ or 4

If k' of all compounds <1, then use -30% methanol in the mobile phase.

If k' of all compounds >10, then use $+30\%$ methanol in the mobile phase.

If k' of all compounds is between 5 and 10, then use $+20\%$ methanol in the mobile phase.

If the number of compounds with $k' < 1$ is larger than the number of compounds with $k' > 1$, then use -20% methanol in the mobile phase.

If the number of compounds with $k' < 1$ is smaller than or equal to the number of compounds with $k' > 1$, then use -10% methanol in the mobile phase.

If the number of compounds with $k' > 5$ is larger than the number of compounds with $k' < 5$, then use $+20\%$ methanol in the mobile phase.

If the number of compounds with $k' > 5$ is smaller than or equal to the number of compounds with $k' < 5$, then use $+10\%$ methanol in the mobile phase.

If there are compounds with $k' < 1$ and $k' > 5$, then the user decides if -10% methanol in the mobile phase is applied.

(IV) $n > 4$

If k' of all compounds <1, then use -40% methanol in the mobile phase.

If k' of all compounds >20, then use $+40\%$ methanol in the mobile phase.

If k' of all compounds is between 10 and 20, then use $+20\%$ methanol in the mobile phase.

If the number of compounds with $k' < 1$ is larger than the number of compounds with $k' > 1$, then use -20% methanol in the mobile phase.

If the number of compounds with $k' < 1$ is smaller than or equal to the number of compounds with $k' > 1$, then use -10% methanol in the mobile phase.

If the number of compounds with $k' > 10$ is larger than the number of compounds with $k' < 10$, then use $+20\%$ methanol in the mobile phase.

If the number of compounds with $k' > 10$ is smaller than or equal to the number of compounds with $k' < 10$, then use $+10\%$ methanol in the mobile phase.

If there are compounds with $k' < 1$ and $k' > 10$, then the user decides if -10% methanol in the mobile phase is applied.

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