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Validation of an expert system for the selection of initial high-performance liquid chromatographic conditions for the analysis of basic drugs

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SUMMARY

A prototype expert system was built which gives advice on the liquid chromatographic conditions for the analysis of basic compounds. In the validation process, the correct implementation of the knowledge and the advice of the expert system on real samples has been checked. For more than 50 compounds, the consultation of the expert system resulted in 75% correct proposals. Rules have been implemented to guide the operator in case the first proposal results in retention times that are not in the desired range.

INTRODUCTION

Several groups are working on the applicability of expert systems in (liquid) chromatography[1–7], which indicates a broad interest both from industry and universities in these types of computer systems. Within ESPRIT (European Strategic Programme for Research and Development in Information Technology), a programme supported by the EEC, a group of scientists is working on a joint project, "Application of Expert Systems in Chemical Analysis" (ESCA)[8]. The aim of this project is to demonstrate the applicability of expert systems in high-performance liquid chromatography (HPLC), particularly applied to pharmaceutical analysis. This project covers the whole field of method development. The scheme shown in Fig. 1 became the basis of our work within ESCA. Based on this scheme, four different stand-alone expert systems were developed[9–12].

The selection of the initial HPLC conditions, which is the first step in method development (Fig. 1), requires specific knowledge and expertise. For example, several studies[13] are directed at finding the relationship between chemical structure and chromatographic retention. Further, the chromatographic behaviour of basic pharmaceutical substances in HPLC is strongly influenced by the type of column packing, the

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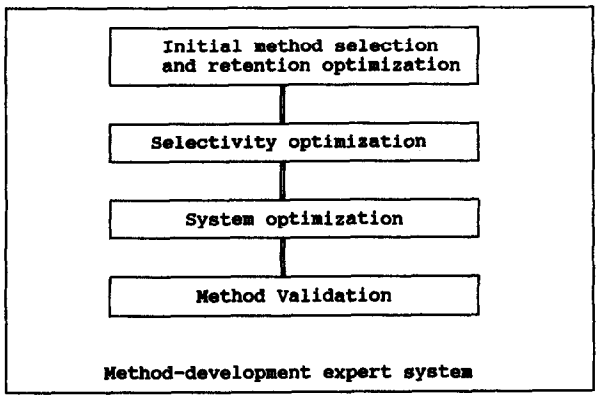

Fig. 1. Outline of an integral expert system for method development in HPLC. This paper concerns the initial method selection and retention optimization.

pH of the mobile phase and the concentration and type of buffer ions. This results in many choices to be made by the chromatographer.

In order to assist the chromatographer, an expert system has been developed for the selection of initial HPLC conditions[11]. On the basis of HPLC data for about 600 different Organon compounds and literature data, rules were defined and a knowledge base was built. The knowledge was implemented in KES (Knowledge Engineering System; Software A&E Architecture), a mid-sized expert system shell which runs on an IBM-PC.

In this paper we present details on the implementation of chemical knowledge and the validation of the expert system. Attention is also focused on the necessity to expand the chemical knowledge. This will be required when this expert system has to be combined with the expert systems on selectivity and system optimization to form a single, integrated expert system for method selection.

DESCRIPTION OF THE EXPERT SYSTEM

The present expert system, also called DASH (Drug Analysis System in HPLC) was originally developed as a first-guess system for method selection and retention optimization of basic drugs. The system is used in the purity control of drugs, synthesized within Organon, and predicts conditions for isocratic elution only.

In the first step of drug development, a large number of compounds are synthesized. Before these compounds are screened in pharmacological tests, they are subjected to HPLC analysis to check their purity. As most of these compounds are submitted for analysis only once, optimization of either the selectivity or the analysis time is not required. However, the "first-guess" HPLC conditions should preferably result in capacity factors (k') between 3 and 10 in order to obtain optimum resolution in an acceptable time.

In a previous paper[11], data-flow diagrams are shown outlining the reasoning process of this expert system. In the expert system rules are implemented on the selection of the column dimensions, flow-rate, pH and detection wavelengths. Another important part of the chemical knowledge is the calculation of the percentage of

methanol in the mobile phase in order to obtain a k' of about 5. To do so, the chemical structure of a compound has to be broken down into structural elements. Each element is linked to a percentage of methanol which can be positive or negative and which can be pH dependent. The proposed mobile phase composition is based on the calculation of the polarity of structural fragments that are not affected by the pH and of fragments for which the polarity depends strongly on pH, *viz.*, N-containing groups. The final percentage of methanol is calculated by summing all contributions which correspond with the structural elements. In order to prevent the expert system from suggesting unrealistic percentages of methanol, constraining rules are also implemented.

IMPLEMENTATION, PROBLEMS AND SOLUTIONS

DASH is implemented in the expert system shell KES (Knowledge Engineering System, Software A&E Architecture, release 2.4). The knowledge base operates on an IBM PS/2 computer. No major difficulties were met in the implementation of the knowledge of DASH.

However, some problems were observed in the use of the tool KES. Most of the attention was focused on building classes, each with the appropriate variables, resulting in a clear, frame-like structure. KES does not really allow splitting up the rule base into different parts. By using clearly arranged classes and by defining the class to which each rule applies, a splitting up of the rules has been achieved to a certain extent.

The major difficulty is to provide a good user interface. Some questions, automatically introduced by KES, may result in some confusion for the user. It is not possible to avoid this and the only way to make the output as clear as possible is to choose understandable variable names.

Another disadvantage is that an answer given by the user cannot easily be changed afterwards. When mistakes have been entered, the user has to stop the consultation and to start it all over again. He can only change/correct previous answers when he knows the attribute names of the variables. In situations in which this is really necessary, some actions can be defined so that control of the answers is performed and questions can be reanswered. It is not a feature provided by KES, but it must be built-in by the knowledge engineer.

TEST PROCEDURE

The advice of the expert system concerns the following items: column type, column size (conventional or microbore), mobile phase (type and composition), flow-rate and detector. Ideally, the system should respond in such a way that a capacity factor in the range 3–10 is obtained for the compound.

In order to consult the expert system, the following input is required: (i) character of the main component (*e.g.*, quaternary N compound, salt), UV activity of the counter ion; (ii) polarity of the (main) component; this information is obtained by the expert system through a list of structural elements, which together comprise the molecule; (iii) impurities present (if applicable); this information can be obtained either from the chemist or from an analysis with other techniques, *e.g.*, NMR; (iv) availability of detector types.

The following features are tested: (i) completeness, applicability and robustness of the system (capability of handling incomplete or poor-quality data); (ii) quality and consistency of the expert system advice; (iii) accuracy of the chemical knowledge for the analysis of specific basic compounds in the estimation of the percentage of methanol.

VALIDATION PROCESS

Approach

The validation procedure consisted of two processes: (i) validating the software, *i.e.*, the completeness and the robustness of the system, and (ii) validation of the chemical knowledge by following the advice of the expert system for a large number of CNS-active drugs. An expert system which is only suitable for specific Organon compounds is limited. Therefore, in a later stage, during the evaluation process, the suitability of the expert system for a broader range of compounds, *viz.*, compounds with pK_a values between 3 and 10 (measured for the protonated compound), will be tested.

The expert system was initially tested by the consultation of the system with twenty reference compounds. It was verified whether the system gave the same advice as the expert intended it to. In other words, the correct implementation of all the rules and the correct functioning of the inference engine is controlled. The expert system has been validated further by the analysis of more than 50 basic compounds. These compounds are mainly CNS-active drugs and varied in pK_a value from 5 to 9. Also, some compounds were tested for which the chemical knowledge in DASH was expected to be inadequate to give a correct first guess. The compounds were analysed on a Nova-Pak C_{18} or a μBondapak C_{18} column at both pH 7.4 and 4.0. The buffer consisted of a 0.05 *M* tetramethylammonium hydroxide buffer solution acidified with concentrated phosphoric acid to pH 7.4 or 4.0. In order to obtain more analytical information, the compounds were analysed preferably at three methanol percentages. In this way, plots of log k' *versus* percentage of methanol could be drawn for all compounds.

Pass/fail criteria

The system is considered to fail if: (1) no answer is obtained (software/hardware failure); (2) a clearly incorrect answer is obtained, e.g., percentage of methanol out of range; (3) the experimentally obtained capacity factor is outside the range $3 \leqslant k' \leqslant 10$.

In the first instance, the reason for the failure should be identified. If it is the software, it should be identified whether it is due to a bug or to incorrect or missing knowledge. In the second and third instances incorrect and/or missing knowledge should also be identified. If possible, the system should be modified and, eventually, rules should be altered and/or added.

RESULTS AND DISCUSSION

Validation of the implementation

The correct implementation of all the rules was tested in DASH (version 1.1) by

checking all the possibilities suggested by the expert system and by entering wrong and/or incomplete data. A few reasoning mistakes were found and, therefore, some rules were changed. A general problem remains that when a wrong answer is entered, the complete consultation has to be carried out again.

The consistency of the advice of the expert system was found to be good. In order to improve the clarity of the advice, extra text was added in two parts of the output. For example, when the user has only a refractive index detector available, a suggestion is added on the necessary sample concentration.

Calculation of the percentage of modifier

The rules for calculating the percentage of methanol were implemented correctly. This was checked by consulting the expert system for twenty nitrogen-containing compounds, the structures of which varied considerably. We found that when an LC expert, who is not familiar with organic chemical structures, consulted the system, more than 50% of the structures were incorrectly translated into the structural elements. Three suggestions were made which have already been implemented: (1) after entering the structural elements, the total number of N, C, O, S and Cl atoms is shown on the screen; the number of H atoms will not be shown, because this cannot be calculated; (2) if the user notices that a mistake has been made, *e.g.*, when the chemical formula is not correct (see 1), then it must be possible to reintroduce the structural elements; (3) a concise user manual is necessary, in which it is explained how a structure can be translated into the structural elements.

The number of structural elements is still the subject of discussion. On the one hand, it was found that some structural elements or groups were missing, *e.g.*, F, Br and C=S. Moreover, there is also the need for more structural elements to express differences in types of nitrogen-containing moieties (see below). On the other hand, the system should remain practical. This means that all structural elements must preferably be shown on one screen and the user should not become confused by too many choices. In conclusion, the number of structural elements must be restricted to (i) the possible atoms, (ii) regularly appearing small groups and (iii) small groups which strongly influence the polarity of a compound.

The best alternative would be to use a system by which the complete chemical structure can be entered. We are now studying the use of DARC, a computer program for the storage and retrieval of chemical structures developed by Télésystème (Paris, France). A small additional program was written for DARC, in which the available structural elements are defined. The link between DARC and DASH is now off-line, *i.e.*, the DARC system only generates a list of structural elements, and not yet on-line. Using a DARC–DASH coupling there is no obvious limitation to the available number of structural elements while the user-friendliness is clearly improved.

Accuracy of the chemical knowledge

The accuracy of the chemical knowledge for calculating the percentage of methanol was investigated by analysing more than 50 compounds synthesized at Organon (most of them both at pH 7.4 and 4.0). The compounds were all analysed at least at two different percentages of methanol, so that two or more data points were obtained with k' values larger than 3. The percentage of correct answers, the average difference in the percentage of methanol found experimentally for $k'=5$ and the percentage

suggested by the expert system, and the slope of log k' *versus* percentage of methanol are shown in Table I.

Most of the compounds studied were analogues of the compounds shown in Fig. 2A. For these types of compounds a good score of more than 75% was obtained in all instances. Generally, the results were slightly better at pH 7.4 than at pH 4.0. Also, compounds were analysed with structural moieties such as those shown in Fig. 2B. For these types of compounds additional nitrogen-containing structural elements have to be defined in order to improve the accuracy of the expert system advice, especially at pH 4.0.

More meaningful are the data on the differences between the experimental percentages of methanol and the results of DASH. The average difference can, if necessary, be reduced by changing the starting level ("zero level") of the methanol percentage. The standard deviation on the average difference illustrates the accuracy of the expert system. These data can also be translated into selectivity (α) values. Using the average slope of the log k' *versus* methanol percentage curve for a Nova-Pak C_{18} column at pH 7.4, an average k' of 5.2 is calculated. Further, it can be calculated that the criterion $3 \leqslant k' \leqslant 10$ corresponds to an allowable variation in the percentage of methanol of $+5$ and -7%.

Limitations to the accuracy of retention optimization

Although this is not really part of the validation process, it is important to describe the expected accuracy of the system. There are some factors which cause small changes in retention behaviour, such as (i) column-to-column reproducibility, (ii) changes in the pH of a solution when methanol is added and (iii) shifts in pK_a values of compounds with variation in the percentage of methanol. However, these factors can usually be kept under control in practice.

More serious problems are encountered with isomers, *e.g.*, *cis–trans* isomers.

TABLE I

VALIDATION OF THE CHEMICAL KNOWLEDGE FOR THE CALCULATION OF THE PERCENTAGE OF METHANOL (%M) IN THE MOBILE PHASE

Column	*No. of compounds*	*% good score* ($3 \leqslant k' \leqslant 10$)	*% M exp. − DASH ± S.D.*[c]	*Slope of log k' vs. %M ± R.S.D.*[c]
Nova-Pak C_{18}				
pH 7.4	24	87	0.5 ± 4.3	−0.044 ± 12%
pH 4.0	23	78	1.6 ± 4.8	−0.041 ± 18%
μBondapak C_{18}				
pH 7.4	17	88	0.3 ± 4.7	−0.036 ± 10%
pH 4.0	17	76	0.3 ± 8.6	−0.030 ± 11%
pH 4.0	15[a]	87	−2.1 ± 4.8	
Nova-Pak C_{18}				
pH 7.4	12[b]	75	−0.5 ± 5.1	−0.044 ± 16%
pH 4.0	5[b]	40	9.2 ± 8.5	

[a] Deleting two outliers from the previous result.

[b] Miscellaneous drugs with N-containing moieties as shown in Fig. 2B.

[c] S.D. = Standard deviation; R.S.D. = relative standard deviation; %M exp. − DASH = difference in %M found experimentally for $k' = 5$ and suggested by the expert system.

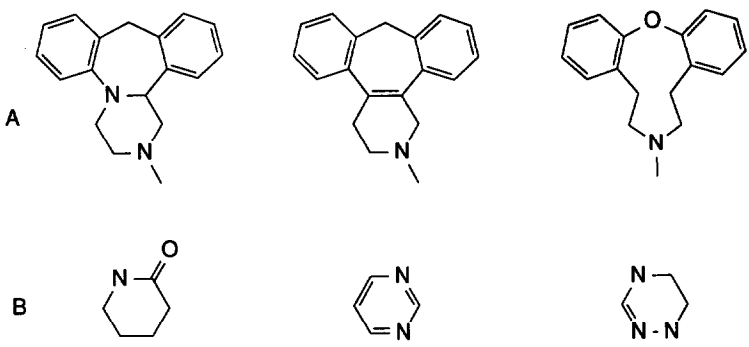

Fig. 2. Chemical structures of (A) three Organon compounds and (B) three structural elements.

The expert system does not take stereochemical effects into account, although differences in the required methanol percentages can easily amount to 5% for *cis–trans* isomers.

A second limitation is inherent in the method chosen for calculating the percentage of methanol. When, for example, a chlorine atom is added to an aromatic ring of a molecule, the influence of the chlorine on the overall polarity will depend on the polarity of that molecule. This effect is not considered by the expert system. For both polar and non-polar compounds, the advice is obtained to increase the percentage of methanol by 7%.

During the development of the expert system, several compounds which differed only in one atom were analysed. These "pairs" of compounds were analysed at pH 7.4, 6.0 and 4.0. The variation in pH results in a variation of the polarity and, therefore, of the retention behaviour. To compensate for this the percentage of methanol has to be changed. The results are give in Table II. It can be seen that the largest contribution of an atom that reduces the polarity, such as chlorine, is at a low pH. For an atom that increases the polarity, such as oxygen, the effect is largest at a high pH.

For calculating the percentages of methanol, DASH uses the average contributions listed in Table II. However, this will generally result in a predicted percentage of methanol that is too high for very non-polar compounds and too low for very polar compounds. If necessary, a rule can be formulated to correct for this effect.

TABLE II

CONTRIBUTION OF SOME STRUCTURAL ELEMENTS AT DIFFERENT pH VALUES EXPRESSED AS A PERCENTAGE OF METHANOL AT $k' = 5$

Moiety	*No. of "pairs"*	*pH 7.4*	*pH 6.0*	*pH 4.0*
Cl on aromatic group	7	5	5	9
O in ether; positioned between two aromatic groups	3	−8	−8	−3
S atom; positioned between two aromatic groups	2	1	1	4
CH_3	3	5	5	7

The third, and most important, limitation is the exact pK_a value of a compound. The pK_a value determines the degree of protonation at a certain pH. In turn, the degree of protonation strongly influences the polarity of a compound. A separate expert system would be necessary to calculate or estimate the pK_a value. Even when this would be possible, it remains questionable whether it is possible to translate the pK_a value into the percentage of methanol needed in the mobile phase. Therefore, in our opinion, it is more straightforward to define additional types of nitrogen-containing moieties in order to enhance the applicability of the expert system to other classes of drugs.

Generally, it can be concluded that most of the emphasis must be directed at avoiding serious mistakes in the calculation of the percentage of methanol and not at improving the accuracy further within the range of $3 \leqslant k' \leqslant 10$. For instance, great improvements can be obtained for the two outliers in Table I, for which the "error" of DASH in calculating the percentages of methanol was 11 and 22%.

Limitation of stationary phases

In most instances, the expert system suggests the use of either a Nova-Pak or a μBondapak C_{18} column. However, several other C_{18} phases are also available for the analysis of basic substances. From the literature[14] rules can be derived, *e.g.*, using data on the loading of C_{18} phases and the silanol activity of the stationary phases, by which the percentages of methanol can be roughly translated from one column to another.

Expected impurities

When impurities are expected, there is one variable available in DASH to influence the separation, *viz.*, the pH of the mobile phase. When the user has to separate *cis–trans* isomers, the expert system recommends the use of a pH of 7.4. When other impurities are expected, the reasoning is that when the impurity is slightly more polar the best separation can be obtained at a pH where the major compound is not protonated and, therefore, relatively non-polar. For basic compounds this can be achieved at a high pH. When the impurity is much more polar than the major compound, the resolution should not become too large and, therefore, a low pH is recommended. The opposite reasoning process is followed when the impurity is slightly or much less polar than the compound of interest.

A limiting factor is that the user has to decide on the differences in polarity. As an alternative, the expert system can calculate the differences in polarity. The expert system must then be able to judge, based on additional rules, which pH should be preferred. Especially for structurally related compounds, this option can be very powerful. When at both pH values the differences in polarity (methanol percentage) are too large, gradient elution can be suggested.

Retention optimization: DASH'

During the validation of DASH, it was found that in more than 75% of the consultations a correct advice was given by the expert system. However, we expect that the results will be less satisfactory when the range of compounds is enlarged during the evaluation. Therefore, an extension of the expert system is desirable, so that a bad first guess can easily be transformed into a good second guess. This exten-

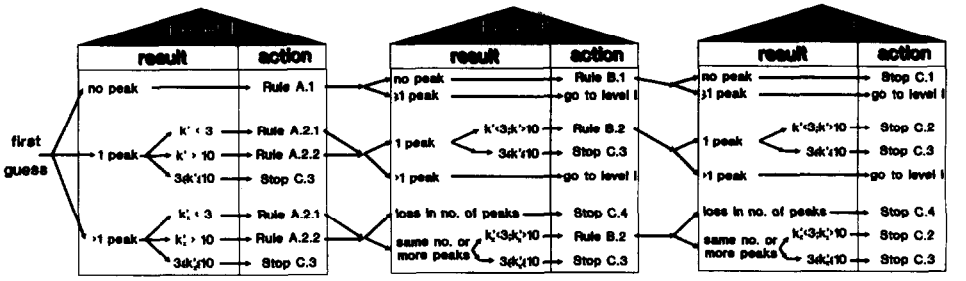

Fig. 3. The decision process in DASH' for a next guess. The rules for a next experiment (A and B) and to stop (C) are given in Table III. For further information, see text.

sion of DASH, specifically meant for retention optimization, is called DASH'.

A flow diagram of DASH' and the rules implemented in DASH' are shown in Fig. 3 and Table III, respectively. A consultation of DASH can yield up to a maximum of six guesses. This does not seem to be very effective, but the maximum number will only be reached in exceptional cases. In principle DASH' can also be consulted when a reversed-phase LC first-guess method taken from the literature failed.

TABLE III

RULES FOR THE CALCULATION OF A NEXT GUESS

A. After first guess:
1. No peak is recorded; increase % methanol by an absolute amount of 20%.
2. The compound of interest has:
   2.1. $k' < 3$; decrease % methanol with an absolute amount of $10 \times (5\text{-}k')\%$
   2.2. $k' > 10$;
   Nova-Pak C_{18}; pH 7.4 and pH 4.0: calculate % methanol for $k' = 5$ from the curve obtained by the first-guess data point (% methanol, k') and the fact that the slope of the % methanol–log k' curve is expected to be -0.044 (pH 7.4) and -0.041 (pH 4.0).
   μBondapak C_{18}; pH 7.4 and pH 4.0: calculate % methanol for $k' = 5$ from the curve obtained by the first-guess data point (% methanol, k') and the fact that the slope of the % methanol–log k' curve is expected to be -0.036 (pH 7.4) and -0.030 (pH 4.0).
   2.3. $3 \leqslant k' \leqslant 10$; retention optimization is finished.

B. After second, third, etc., guess:
1. Still no peak is recorded; increase sample amount by a factor of 10.
2. The compound of interest had $k' < 3$ or > 10:
   2.1. If during first guess no peak was recorded, then go to A.2.
   2.2. Use both data points and fit a linear curve through the % methanol–log k' points; calculate % methanol for $k' = 5$.

C. Rules to stop:
1. If after third guess still no peak is recorded, then the method is not suitable for this compound. When only a UV detector was used, the expert system will suggest the use of a refractive index detector, a sample concentration of > 5 mg/ml and the first-guess conditions.
2. If three successive guesses result in $k' < 3$ or > 10, then the method is not suitable for this compound. An alternative method can be suggested, *e.g.*, when $k' < 3$, use a PIC reagent.
3. When $3 \leqslant k' \leqslant 10$, the retention optimization is finished. If two or more peaks are observed, then calculate the resolution.

Knowledge on the average slope of the log k' *versus* percentage of methanol lines is important for obtaining an accurate second guess. The slopes listed in Table I are used in DASH' (rules A.2.2 in Table III). Another option is that the user can ask for a certain k', and DASH' will calculate the percentage of methanol. Because generally log k' *versus* percentage of methanol curves are only linear for k' values higher than 2 or 3, the calculation can only be expected to be accurate for k' values of 2 and higher.

CONCLUSIONS

From this validation, it can be concluded that the knowledge base is correctly implemented in the expert system. Improvements made in DASH, partly as a result of the validation, are (i) the presentation of a formula for the chemical composition combined with a possibility of correcting the answers and (ii) a system for further retention optimization when the first guess is not successful. This second extension called DASH, will also give the user the possibility to obtain capacity factors other than 5.

A problem was observed with the currently available structural elements. In order to keep the expert system practical, the number of structural elements is limited in the present set-up of the consultation. Investigations are in progress to combine DASH with a system in which the total chemical structure can be entered.

The accuracy of the advice based on the chemical knowledge for the method selection of the tested compounds is acceptable. Improvements can be obtained for compounds with other types of nitrogen-containing groups, the retention of which cannot be predicted accurately by the present system.

One of the problems with DASH, when it is to be combined with the expert systems for system optimization and selectivity optimization, is that DASH in principle only calculates the best LC conditions for one compound at a time. Probably some kind of repetitive consultation with a possibility of comparing the results will lead to a system which can also predict the initial LC conditions for a mixture of compounds.

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