Journal of Chromatography, 550 (1991) 87–100
Elsevier Science Publishers B.V., Amsterdam

CHROMSYMP. 2251

Prediction of initial high-performance liquid chromatographic conditions for selectivity optimization in pharmaceutical analysis by an expert system approach

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ABSTRACT

Initial mobile phase compositions can be selected from any physicochemical properties of solutes which can be correlated with their high-performance liquid chromatography (HPLC) retention. In this study, octanol–water partition coefficients (log P values) calculated from molecular increments are converted to HPLC retention data to achieve capacity factors (k') within a range 0.5–5. The eluent compositions are calculated to produce k' values of 0.5 (OP_{min}) and 5 (OP_{max}) for all solutes of interest. From the various eluent compositions obtained as OP_{min} values, the highest value (the strongest eluent composition, $OP_{min,ST}$) and as OP_{max} values, the lowest value (the weakest eluent composition, $OP_{max,WE}$), are chosen to calculate the optimum composition for the initial experiments (OP_{IN}). Using the suggested mobile phase composition, OP_{IN}, the k' values of each component are predicted and checked. The first guess experiment can be started with the suggested eluent composition. Pass–fail criteria have been established to evaluate the experimental data; the capacity factors of all components should be within the range 0.5–10, and the peak asymmetry factors should be between 0.8 and 1.8. For the direction of the second and third guess experiments, special rules followed by different actions are formulated. The applicability of the developed expert system is demonstrated through the separation of a model mixture containing solutes with widely different chemical structures.

INTRODUCTION

The selection of suitable experimental conditions for initial separations involves more than merely choosing an appropriate packing material to fill a tube of some prejudged dimension and a mobile phase to achieve suitable retentions. The approach suggested in this paper includes the following considerations.

Separation mode selection

The selection of phase system is a highly empirical task as a result of the unpredictable selectivity of one phase system for a new analytical problem. A single pack-

0021-9673/91/$03.50

ing material may be used in several different chromatographic modes to carry out different types of separations. The specified preknowledge regarding molecular weight, solubility and ionic properties of the solutes provide an useful source of information for the selection of the separation mode. A change in separation mode may be achieved simply by changing the composition of the mobile phase or by using various additives in the eluent. In the following discussion, only the principles relating to reversed-phase separations will be considered.

Phase system selection

Phase system selection includes decisions about two different things: stationary and mobile phase selections. All the experiments discussed here have been performed on chemically bonded octadecylsilica stationary phases with reversed-phase eluents.

The primary goal of the initialization experiments is to achieve an acceptable capacity factor (k') range and peak shape for all of the solutes of interest. Basically there are four different routes to achieve this goal.

Empirical route without any preknowledge of the sample. The experiments start using high organic concentrations in the eluent (for instance, 70–80% methanol in water at a pH of about 2.2 or 8.0 that can suppress possible ionization). The eluent composition is modified step by step on the basis of the capacity factor of the last eluting peak (a greater modification in the eluent strength is made if $k'_{last} < 1.0$; only a slight modification is made if $k'_{last} < 3$).

Empirical route considering the physicochemical properties of the solutes of interest. The mobile phase is selected according to the type of solute(s) (solubility, polarity in ion supression media and basicity). A recommended procedure has been reported by Gazdag *et al.* [1].

Application of gradient elution for the selection of initial mobile phase compositions. The application of gradient elution of initial method development has some advantages relative to the use of isocratic elution for several reasons: (1) for unknown samples, it is more likely that gradient elution will reveal the presence of compounds that might be lost due to their early elution with the solvent front or to their disappearance in the baseline as late eluting peaks under isocratic conditions; (2) the separation characteristics of sample components with a large k' range can be established more easily in a few gradient experiments (several isocratic runs would be required to obtain the same information); (3) the suitability of isocratic elution for the effective separation of a sample can be confirmed by gradient elution.

Several approaches have been described previously [2–5] to predict isocratic separations from gradient elution.

Computer-aided selection of initial mobile phase compositions. The initial mobile phase compositions can be selected on the basis of any physicochemical properties of the solutes which can be correlated with their HPLC retention. There are two different methods for this.

In the first approach (individual retention concept) suggested by Maris *et al.* [6], a calculation is carried out based on retention increments and obtaining a total retention factor for each individual solute to achieve a k' value of 3. The computer-aided selection of initial mobile phase composition is based on the calculation of the polarity of the structural fragments that are not affected by the pH (pH independent increments) and of fragments, the polarity of which strongly depends on the pH (pH

dependent increments). The mobile phase composition is given by summing all the contributions which correspond to the structural elements.

In the second approach (total retention concept), log P values (the logarithm of octanol–water partition coefficients) are calculated from molecular increments. Based on the algorithms published by Valkó [7,8] and a relatively large database, the log P data are transformed to HPLC retention data to achieve k' values within a range 0.5–5. The eluent compositions are calculated to produce a k' value of 0.5 (OP_{min}) and 5 (OP_{max}) for all solutes of interest. From the compositions obtained as OP_{min} values for all compounds in the mixture, the highest value is chosen and referred to as $OP_{min,ST}$ (the strongest eluent composition) and from the OP_{max} values the lowest value is chosen and referred to as $OP_{max,WE}$ (weakest eluent composition). From the two values the optimum composition for the initial experiments (OP_{IN}) is calculated according to the equation: $OP_{IN} = (OP_{max,WE} + OP_{min,ST})/2$.

BASIC PRINCIPLES

Fig. 1. shows the structural modules of the initialization expert system used in this study. From the initial conditions given in Fig. 1, only the mobile phase selection will be discussed here; the other conditions (column type and dimension, detection wavelength and flow-rate) were the same during the experiments. The structural elements for the prediction of the initial mobile phase composition are shown in Fig. 2.

As can be seen from Fig. 2, the input data correspond to the structural formulas of the compounds of interest. In the first step from the structural data the log P values

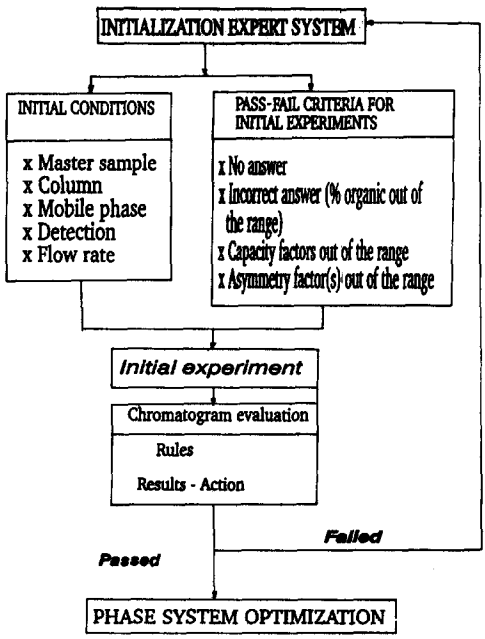


Fig. 1. Construction of the initialization expert system.

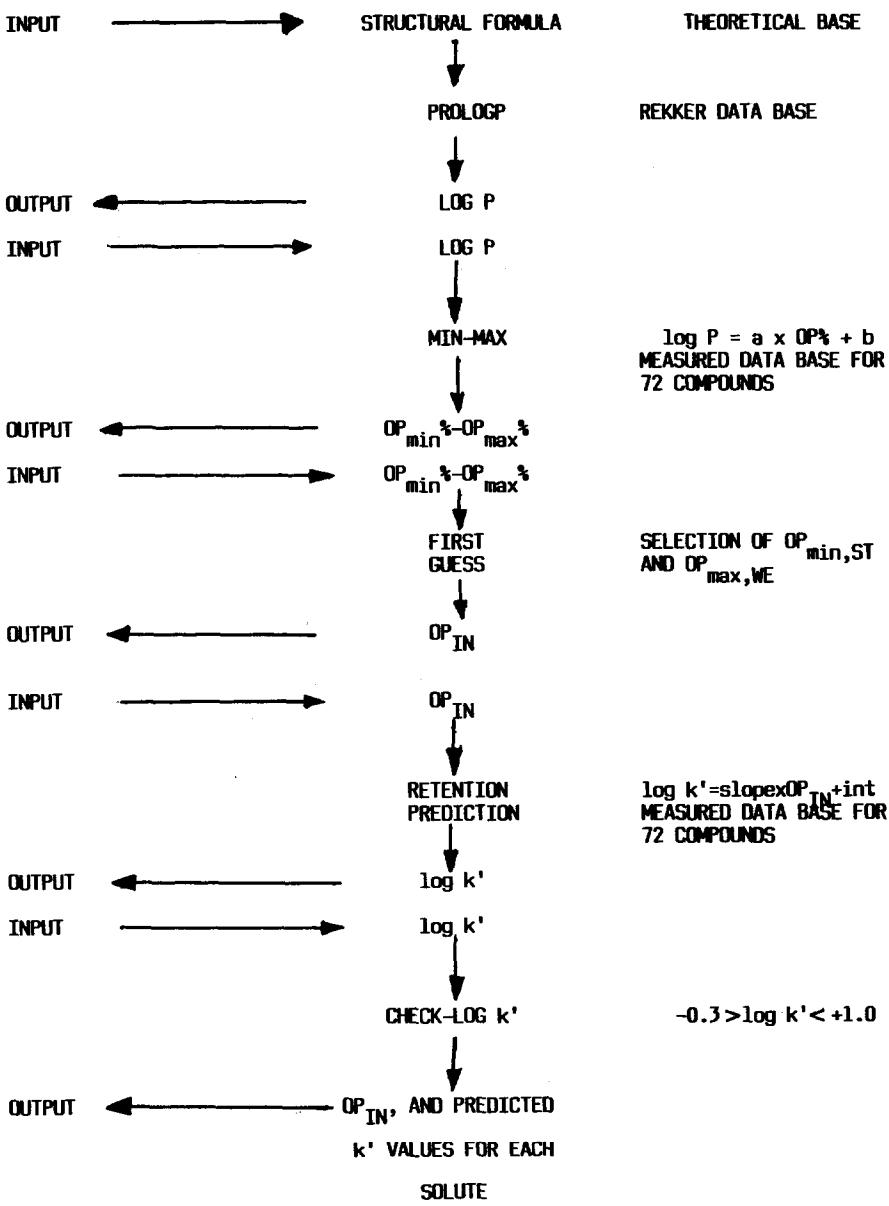


Fig. 2. Prediction of initial mobile phase composition for first guess experiment.

are calculated by the PrologP program based on the Rekker database (Compudrug, Budapest, Hungary). The calculated log P values are transformed in the next step into organic solvent concentrations in the eluent using our MinMax database, which calculates the organic solvent concentration for all components resulting in capacity factors of 0.5 (OP_{min} and 5 (OP_{max}) for each individual component. In the first guess block from the listed percentage organic solvent concentrations, the highest value of

OP_{min} (the strongest eluent composition giving $k' = 0.5$, $OP_{min,ST}$) and the lowest value of OP_{max} (weakest eluent composition giving $k' = 5$, $OP_{max,WE}$) are selected and their average is used as the first estimated eluent composition, OP_{IN}. As OP_{IN} is weaker than $OP_{min,ST}$ and stronger than $OP_{max,WE}$, the predicted values of k' for all components are estimated to be within a k' range 0.5–5. Using the suggested mobile phase composition, OP_{IN}, the k' values of each component are predicted and checked. If the predicted k' values are within the required range, the first guess experiment can be started with the suggested eluent composition.

PASS–FAIL CRITERIA FOR INITIAL EXPERIMENTS

In accordance with the main aim of the initialization experiments, criteria can be formulated for passing the experiments from the initial stage to the optimization stage. It is obvious that these criteria differ from those used for phase system optimization. In this study the following pass–fail criteria were established to evaluate the experimental data: (1) no answer (faulty operation occurs, or the PrologP program does not work); (2) capacity factors are out of range (k' of any single solute is < 0.5 or > 10); (3) peak asymmetry factor is out of the range (A_{sf} for any solute is < 0.8, or > 1.8); and (4) incorrect answer (percentage organic calculated for OP_{IN} is out of the range). If either the $OP_{min,ST}$ value or the $OP_{max,WE}$ value is out of range ($> 90\%$ or $< 1\%$ acetonitrile in the eluent are required to sufficiently elute one or more compounds in the mixture within a k' range 0.5–5), then as a first approximation the $OP_{min,ST}$ is taken as equal to 100% or $OP_{mas,WE}$ is taken as equal to zero in the OP_{IN} calculation. In this instance the predicted k' value of this particular compound will be automatically out of the range and its k' value can be adjusted to the correct value in the second or third guess experiments (see later example).

INITIAL PHASE SYSTEM: RULES

The initial mobile phase selection is based on between one and three experimental runs under predicted isocratic conditions. The first experiment is performed using a mobile phase containing acetonitrile and aqueous phosphate buffer at pH 4.5 in the suggested ratio (system I.1 in Table I). The eluent composition in the next guess experiment is dependent on the capacity factors of the first and/or the last peaks as well as on the peak asymmetry observed. The necessary steps are directed by several rules, including the change in acetonitrile concentration, using methanol in the eluent or a change in the eluent pH. The mobile phase compositions wich may be utilized in the second and third guess experiments are given in Table I.

As a general rule the following principles are used in our study: (1) if only one capacity factor criterion is out of range (k' of first eluted peak, $k'_F < 0.5$ or k' of last eluted peak, $k'_L > 10$) and the peak asymmetry factor (A_{sf}) is acceptable for each compound, the acetonitrile concentration is recalculated according to Table I (system I.1 in Table I); (2) if both capacity factor criteria are out of range, but the peak asymmetry factor is acceptable for each peak, then methanol is used in the eluent at an identical eluent strength and pH (system II.1 in Table I); (3) if only one asymmetry factor criterion is out of range, and the capacity factors are within range, the same eluent composition is used as in case (1), but the pH of the eluent is adjusted to pH 2.2

ABLE I

ITIAL PHASE SYSTEMS AND EXPERIMENTAL CONDITIONS

olumn: Hypersil ODS, 5 μm, 150 × 4 mm I.D. Flow-rate: 0.85 ml/min. Detection: 254 nm.

	Mobile phases[a]	
	(I) Acetonitrile (ACN)–water	(II) Methanol (MeOH)–water
	System I.1, pH 4.5	*System II.1, pH 4.5*
) $k'_F < 0.05$	ACN (%) = 0.84 × ACN_I (%)	MeOH (%) = 0.80 × $MeOH_I$ (%)
) $k'_L > 15$	ACN (%) = 1.2 × ACN_I (%)	MeOH (%) = 1.3 × $MeOH_I$ (%)
) $0.05 < k'_F < 0.5$	ACN (%) = ACN_I (%)–15 log $(0.5/k'_I)$	MeOH (%) = $MeOH_I$ (%)–25 log $(0.5k'_I)$
) $10 < k'_L < 15$	ACN (%) = ACN_I (%) + 15 log $(k'_I/10)$	MeOH (%) = $MeOH_I$ (%) + 25 log $(k'_I/10)$
	System I.2, pH 2.2	*System II.2, pH 2.2*
) $k'_F < 0.05$	ACN (%) = 0.84 × ACN_I (%)	MeOH (%) = 0.80 × $MeOH_I$ (%)
) $k'_L > 15$	ACN (%) = 1.2 × ACN_I (%)	MeOH (%) = 1.3 × $MeOH_I$ (%)
) $0.05 < k'_F < 0.5$	ACN (%) = ACN_I (%)–20 log $(0.5/k'_I)$	MeOH (%) = $MeOH_I$ (%)–30 log $(0.5k'_I)$
) $10 < k'_L < 15$	ACN (%) = ACN_I (%) + 20 log $(k'_I/10)$	MeOH (%) = $MeOH_I$ (%) + 30 log $(k'_I/10)$
	System I.3, pH 7.8	*System II.3, pH 7.8*
) $k'_F < 0.05$	ACN (%) = 0.84 × ACN_I (%)	MeOH (%) = 0.80 × $MeOH_I$ (%)
) $k'_L > 15$	ACN (%) = 1.2 × ACN_I (%)	MeOH (%) = 1.3 × $MeOH_I$ (%)
) $0.05 < k'_F < 0.5$	ACN (%) = ACN_I (%)–20 log $(0.5/k'_I)$	MeOH (%) = $MeOH_I$ (%)–30 log $(0.5k'_I)$
) $10 < k'_L < 15$	ACN (%) = ACN_I (%) + 20 log $(k'_I/10)$	MeOH (%) = $MeOH_I$ (%) + 30 log $(k'_I/10)$

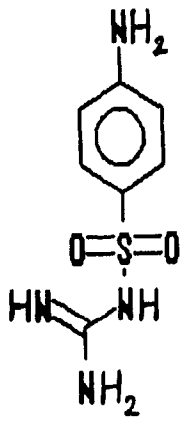
Subscript I refers to the organic solvent concentration in the previous experiment.

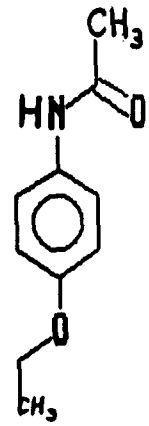
(A_{sf} is less than 0.8, system I.2 in Table I) or to pH 7.8 (A_{sf} is higher than 1.8, system I.3 in Table I); (4) if both asymmetry factor criteria are out of range, and the capacity factors are within range, methanolic eluent is used at an identical eluent strength and pH as in case (1) (system II.1 in Table I); (5) if only one capacity factor criterion and one peak asymmetry criterion are out of range, a recalculated acetonitrile concentration with different pH (system I.2 for peak leading, or system I.3 for peak tailing) is used; (6) if both capacity factor criteria and one peak asymmetry criterion are out of range, methanol is used in the second guess experiment at an identical eluent strength but with a different pH (system II.2 for peak leading, or system II.3 for peak tailing); (7) if one capacity factor criterion and both peak asymmetry factor criteria are out of range, a recalculated methanol concentration with the same pH (system II.1 in Table I) is used; (8) if both the capacity factor and peak asymmetry factor criteria are out of range, the expert system is not able to advise on further experimental conditions. This case requires special considerations (using normal phase chromatography, ion pair chromatography or other techniques based on secondary chemical equilibria).

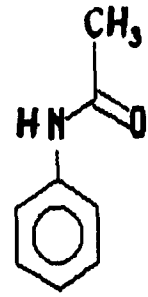
Table II summarizes the rules to be used for the direction of next guess experiments.

CHECKING THE EXPERT SYSTEM FOR A SELECTED MODEL MIXTURE

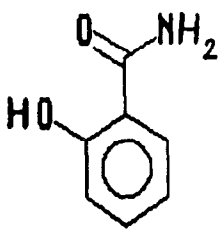
To control the operation of the initial expert system a model mixture (the components are shown in Fig. 3) was selected. The calculated data for log P, OP_{min}, OP_{max}, log k' and k' are also shown in Fig. 3.

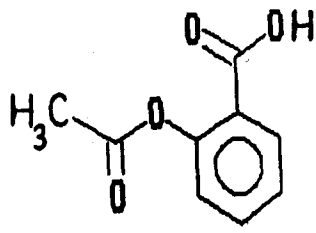


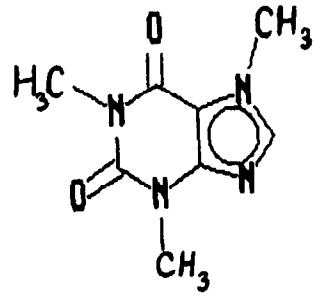




	Sulfaguanidine	Phenacetin	Acetanilide
log P	-2.384	1.128	0.529
$OP\%_{min}$	12.0%	55.7%	48.2%
$OP\%_{max}$	-16% (0)	12.4%	7.4%
log k	-0.751	0.369	0.265
k'	0.180	2.340	1.840







	Salicylamide	Acetylsal acid	Caffeine
log P	0.236	1.037	-0.912
$OP\%_{min}$	44.6%	54.5%	30.2%
$OP\%_{max}$	5%	11.6%	-4.4% (0)
log k	0.157	0.315	-0.285
k'	1.435	2.067	0.519

SUGGESTED ACETONITRILE CONCENTRATION: 28%

Fig. 3. Model compounds used for checking the expert system.

As can be seen from Fig. 3, the OP_{max} values of sulphaguanidine (−16%) and caffeine (−4.4%) are out of the percentage organic range, therefore $OP_{max,WE}$ was selected to be zero. $OP_{min,ST}$ was 55.7% for phenacetin, and the calculated OP_{IN} is

TABLE II

RULES FOR DIRECTION OF NEXT GUESS EXPERIMENTS

First guess				Second guess				Third guess			
Results			Action: rule	Results			Action: rule	Results			Action: rule
Capacity factor		Peak symmetry[a]		Capacity factor		Peak symmetry[a]		Capacity factor		Peak symmetry[a]	
First peak	Last peak			First peak	Last peak			First peak	Last peak		
>0.5	<10	0.8–1.8	STOP, A.1	>0.5	<10	0.8–1.8	STOP, B.1	>0.5	<10	0.8–1.8	STOP, C.1
<0.5	<10	0.8–1.8	A.21	<0.5	<10	0.8–1.8	B.210, B.212 B.213, B.214 STOP, B.211	<0.5	<10	0.8–1.8	C.21–C.24 C.31–C.34 C.41–C.43 C.51–C.53 C.61–C.63 C.71–C.73 C.81, C.82
>0.5	>10	0.8–1.8	A.22	>0.5	>10	0.8–1.8	B.311, B.312 B.313, B.314 STOP, B.310	>0.5	>10	0.8–1.8	C.91–C.94 C.101–C.104 C.111–C.113 C.121–C.123 C.131–C.133 C.141–C.143 C.151, C.152
<0.5	>10	0.8–1.8	A.23	<0.5	>10	0.8–1.8	B.410, B.412 B.413 STOP, B.411	<0.5	>10	0.8–1.8	C.161–C.164 C.171–C.174 C.181, C. 182 C.191, C.192

>0.5	<10	<0.8 <1.8	A.31	>0.5	<10	<0.8 <1.8	B.5	Other cases:	No advise
>0.5	<10	>0.8 >1.8	A.32	>0.5	<10	>0.8 >1.8	B.6		
>0.5	<10	<0.8 >1.8	A.33	>0.5	<10	<0.8 >1.8	B.7		
<0.5	<10	<0.8 <1.8	A.411	<0.5	<10	<0.8 <1.8	B.8		
>0.5	>10	<0.8 <1.8	A.412	>0.5	>10	<0.8 <1.8	B.9		
<0.5	<10	>0.8 >1.8	A.421	<0.5	<10	>0.8 >1.8	B.10		
>0.5	>10	>0.8 >1.8	A.422	>0.5	>10	>0.8 >1.8	B.11		
<0.5	<10	<0.8 >1.8	A.431	<0.5	<10	<0.8 >1.8	B.120		
							STOP, B.121		
							STOP, B.122		
>0.5	>10	<0.8 >1.8	A.432	>0.5	>10	<0.8 >1.8	B.130		
							STOP, B.131		
							STOP, B.132		
<0.5	>10	<0.8 >1.8	STOP, A.44	<0.5	>10	<0.8 >1.8	STOP, B.14		

[a] The data refer to the investigation of a model mixture; for one peak A_{sf} should be less than 0.8, and for another one higher than 1.8.

27.9% acetonitrile. Using this eluent composition, all the predicted capacity factors with the exception of k' for sulphaguanidine are within range.

Using system I.1 with an acetonitrile–water ratio of 27.9:72.1, the chromatogram shown in Fig. 4 is obtained; a comparison of the caculated and observed values of the capacity factors is given in Table III.

From the chromatogram in Fig. 4 and data in Table III, the following important information can be drawn: (1) the predicted and observed elution orders are in good agreement; (2) the predicted and observed k' values are also in good agreement with the exception of acetylsalicylic acid and phenacetin; (3) two criteria are out of range, the k' value of sulphaguanidine is less than 0.5 (for explanation, see earlier) and the peak asymmetry factor of acetylsalicylic acid is less than 0.8.

The necessary action is directed according to rule A.411 (Table IV), suggesting a recalculation of the eluent strength and the use of system I.2 (pH 2.2). The recalculated eluent concentration is 22% acetonitrile, and the chromatogram obtained is shown in Fig. 5 (k' data are also presented in Table III).

The data presented in Table III for a 22% acetonitrile concentration show that the predicted and observed elution orders are the same; the change of eluent pH has no influence on it. Similarly, a good correlation of the predicted and observed k' values can be seen, and the peak asymmetry factors of all components are within the required range. The necessary action is directed according to rule B.213 (Table IV), leading to a recalculated acetonitrile concentration of 18%. The chromatogram obtained using this eluent is shown in Fig. 6, and the data are given in Table III.

As can be seen from Fig. 6 and Table II, if 18% acetonitrile is used a k' value of only 0.4 can be obtained for sulphaguanidine, but the phenacetin retention achieved a critical value (close to the upper limit of the k' criterion), which requires further initial experiments to stop (Table IV). Therefore this eluent composition is suggested for phase system optimization. (It should be noted that peak separation criterion as

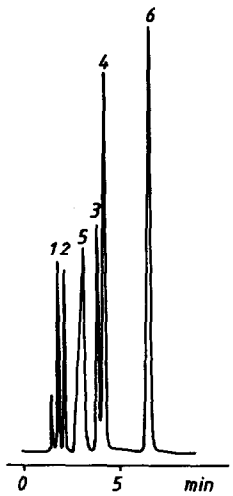


Fig. 4. Chromatogram obtained in first guess experiment. Eluent: acetonitrile–phosphate buffer (pH 4.5) (27.9:72.1). Other conditions as in Table I. Peaks: 1 = sulphaguadinine; 2 = caffeine; 3 = salycilamide; 4 = acetamide; 5 = acetylsalicylic acid; 6 = phenacetin.

TABLE III

RESULTS IN THE FIRST, SECOND AND THIRD GUESS EXPERIMENTS

Compounds	System	Capacity factors		Peak asymmetry
		Predicted	Found	
First guess	I.1. 27.9% acetonitrile			
Sulphaguanidine		0.180	0.201	1.05
Caffeine		0.519	0.400	0.95
Salicylamide		1.435	1.550	1.18
Acetanilide		1.840	1.775	1.03
Acetylsalicylic acid		2.067	1.257	0.67
Phenacetin		2.340	3.267	1.07
Second guess	I.2. 22.0% acetonitrile			
Sulphaguanidine		0.259	0.391	1.08
Caffeine		0.783	0.688	1.20
Salicylamide		2.043	2.503	1.48
Acetanilide		2.649	2.820	1.33
Acetylsalicylic acid		3.013	4.325	1.30
Phenacetin		3.197	5.879	1.43
Third guess	I.2. 18.0% acetonitrile			
Sulphaguanidine		0.324	0.400	1.05
Caffeine		1.033	1.023	1.21
Salicylamide		2.582	3.634	1.38
Acetanilide		3.428	4.049	1.18
Acetylsalicylic acid		3.864	7.105	1.32
Phenacetin		3.945	9.583	1.27

TABLE IV

RESULTS AND ACTIONS IN THE FIRST, SECOND AND THIRD GUESS EXPERIMENTS

Results	Rule	Criteria failed			Actions
		First guess	Second guess	Third guess	
First guess experiment	A.411	$k' < 0.5$ $A_{sf} < 0.8$			Use system I.2. with recalculated eluent strength for k'_F
Second guess experiment	B.213		$k' < 0.5$		Recalculate system I.2. for k'_F
Third guess experiment	C.81			$k' < 0.5$	Look for first eluting compound(s), if it is the same use B.213, if it is different use 1:1 mixture of A.411 and B.213 for optimization

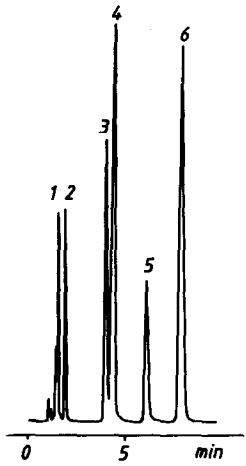


Fig. 5. Chromatogram obtained in second guess experiment. Eluent: acetonitrile–phosphate buffer (pH 2.2) (22:78). Other conditions as in Table I. For peak identification see Fig. 4.

resolution is not included in the pass–fail criteria of the initial expert system. Although practically baseline separation has been achieved for all components in the mixture during the initial experiments, in most instances two or more peaks may overlap in the initial system, therefore the separation of these overlapping compounds should be the target of further optimization steps.)

CONCLUSIONS

From the experimental data the following general conclusions can be drawn.

(1) The expert system based on the generation of solubility data (log *P*) from the structural formulas of compounds of interest and on their transformation to retention data on two different levels (upper boundary 10, lower boundary 0.5), resulting

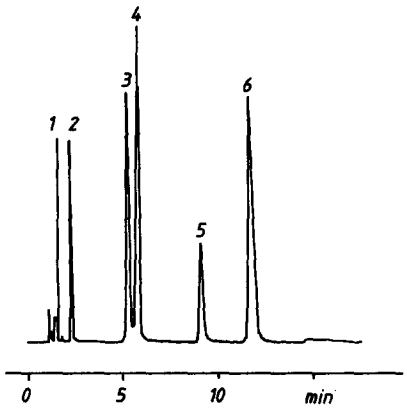


Fig. 6. Chromatogram obtained in third guess experiment. Eluent: acetonitrile–phosphate buffer (pH 2.2) (18:82). Other conditions as in Table I. For peak identification see Fig. 4.

in a suggested eluent composition for the initial experiments, can be successfully applied even if compounds differing widely in chemical structures can be separated.

(2) An acceptably good correlation to the predicted and observed elution orders has been found.

(3) The expert system can also be used in cases where one of the calculated eluent compositions for the upper or lower boundaries is out of range. In this concept zero is used for $OP_{max,WE}$ and 100 for $OP_{min,ST}$, but the experimental data also suggest using the calculated data. For a first guess experiment the expert system suggested a 16% acetonitrile concentration to achieve a k' of 5 sulphaguanidine ($OP_{max,WE}$) and 55.7% acetonitrile concentration to achieve a k' of 0.5 for phenacetine. Using these values for calculating OP_{IN}, a 19.9% acetonitrile concentration is proposed for the first guess experiment. Possibly the same k' value for sulphaguanidine should have been obtained (k' is about 0.4) in the first guess, as was obtained in the third guess.

(4) Although the main aim of the expert system concept used for the initial eluent selection in this study was to suggest a mobile phase composition providing symmetrical peaks which elute within a definite capacity factor range, a relatively good correlation between the predicted and observed capacity factors was seen. A general conclusion can be drawn from these experiments, namely, a much better correlation can be obtained for peaks with a k' value below 5 than for the peaks eluting with higher retentions. This observation possibly derives from the calculation of the first guess experiment using a k' value for the upper boundary of 5 instead of 10. However, the linearized equations between log P and OP% as well as between OP% and log k' are valid within a definite range of eluent composition; therefore according to our assumption, the calculation for the first guess experiment may give more reliable results within this narrower k' range than if it is extended to a larger k' range.

From the initial experiments some special information may be obtained, which me be a useful source of information for further optimization steps. The content of such information is also a function of the number and the necessary changes in the conditions of the initial experiments. The information obtained from the initial experiments are as follows: (1) elution order of the peaks; (2) number of peak-clusters to be resolved; (3) validity of the linear relationship of log k' *versus* the percentage organic curve; (4) dependence of elution order on the nature and concentration of organic solvents used for eluent preparation (methanol *versus* acetonitrile); (5) the dependence of solute retention on the pH of the eluent and (6) the estimation of difficulties during the optimization experiments.

ACKNOWLEDGEMENT

The authors are grateful to Dr. F. Darvas (Compudrug, Budapest, Hungary) for making the PrologP program available.

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