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EXPERT SYSTEM FOR THE SELECTION OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC METHODS IN PHARMACEUTICAL ANALYSIS

VALIDATION OF THE RULES FOR THE SELECTION OF THE MOBILE PHASE

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SUMMARY

The rules for the selection of the mobile phase and the validation performed on 44 pharmaceutical preparations, containing one to five active compounds, are described. These rules are incorporated into an expert system, called LABEL, for the selection of high-performance liquid chromatographic methods in pharmaceutical analysis. A single stationary phase type is used, namely a nitrile or cyanopropyl (CN) column, which can be used in both normal-phase (NP) and reversed-phase (RP) chromatography. Three mobile phase systems were evaluated on this column type: NP, RP with water and RP with buffer. LABEL selects one of these three systems on the basis of the rules incorporated for the mobile phase selection, checks if the addition of ion-suppressing agents to the eluting agent is necessary and finally gives the starting composition of the mobile phase in each of the three systems. For this selection the number of compounds in the sample, the acid–base properties and the hydrophobicity of the solutes are the more important factors. The validation of the rules on 44 pharmaceutical preparations resulted in an immediate success in 82% of all cases. In half of the remaining cases, the system proposed can be adapted with a minor change in conditions, so that it can also be used in practice.

INTRODUCTION

In the last few years, many efforts have been made to develop systematic optimization strategies for high-performance liquid chromatographic (HPLC) methods. Usually, these strategies deal with the optimization of mobile phase parameters such as solvent strength, solvent selectivity, pH and flow-rate. Excellent overviews were given by Berridge[1] and Schoenmakers[2]. Most HPLC separations are performed in the reversed-phase (RP) mode and the different optimization strategies developed have been mainly applied in this mode. However, for some separation problems, normal-phase (NP) chromatography is more suitable[3].

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In our laboratory, a general approach for the separation of pharmaceutical compounds was developed with the use of a single stationary phase type, namely a cyanopropyl or nitrile (CN) column, which can be applied in both the RP and NP modes owing to its intermediate polarity properties[4–6]. A separation strategy applied with success in many instances consists in carrying out a gradient elution from which a suitable solvent strength is determined for isocratic elution. Afterwards the solvent selectivity is changed to enhance the resolution between peaks while keeping the solvent strength constant. In the RP mode, one can use eluting agents with water as the basis solvent but it is also possible to replace it by a buffer solution. A systematic study of the parameters influencing the retention of basic, acidic and neutral drugs with mobile phases containing buffers was carried out on a CN column[7]. In this way, it is possible to chromatograph drugs on a CN column with three different mobile phase systems: NP, RP with water and RP with buffer. In the first two mobile phase systems, ion-suppressing agents can be added to the eluting agent to improve the peak shape. The nature of this factor depends on the acid–base properties of the pharmaceutical compounds being chromatographed.

The selection of a single column type offers the advantage that a more manageable number of possible combinations of stationary phase with mobile phases is obtained with which successful separations are possible in most instances. As one can now choose from three mobile phase systems, the problem to be solved is which mobile phase system to select for a certain separation problem. Until now, not much attention has been paid to this point in the literature and it is usually treated by most chromatographers by habit or by trial-and-error experiments. This selection can be incorporated into an expert system. Expert systems are software products that can offer intelligent advice on problems requiring some expertise. The existing optimization strategies can then be coupled to an expert system since the software is available. In our laboratory, the feasibility of the integration of experimental optimization methods into an expert system has been demonstrated[8]. In this way, one could obtain an expert system that gives advice about the different steps in the development of an HPLC method, starting with the initial selection of the chromatographic mode and finishing with the optimized separation of the compounds of interest.

In a previous paper, the knowledge base of the expert system LABEL was described[9]. LABEL is an expert system that selects suitable HPLC systems for the label claim analysis of pharmaceutical preparations. For a certain separation problem, the system gives advice about the detection mode (UV detection at fixed or variable wavelength and electrochemical detection in the oxidative mode), the chromatographic mode and the starting mobile phase composition to use in the selected mode so that a "first guess" system for an HPLC analysis is obtained. In this way, one has two strategies for selecting suitable starting mobile phase compositions for pharmaceutical analysis, *viz.*, the strategy incorporated in the expert system and the strategy with the gradient elution, and both have advantages and limitations.

In this paper, the rules incorporated in the expert system for the selection of the chromatographic mode and the starting mobile phase will be described and validated; those for detection will be considered elsewhere[10]. For all the samples examined in this study, UV detection was always used. The validation of the rules was performed on commercially available pharmaceutical formulations.

EXPERIMENTAL

Apparatus

The HPLC instrumentation included two Varian 5060 liquid chromatographs equipped with a Rheodyne loop injector (loop volumes used, 10, 50 and 100 μl). One chromatograph was equipped with a variable-wavelength UV detector (Varian UV-100 detector, a.u.f.s. 0.05) and the other with a Varian fixed-wavelength UV detector (254 nm, a.u.f.s. 0.08). The chromatograms were recorded with a Varian Vista CDS 401 chromatographic data system. Two HPLC instruments were used for practical reasons, one for the RP and the other for the NP mode; if we had used only one instrument, much time would have been spent in switching from the one chromatographic mode to the other by rinsing with solvents that are compatible with the two systems.

The columns used were 250 × 4 mm I.D. stainless-steel columns packed with LiChrosorb CN of particle size 5 μm and obtained from Merck (Darmstadt, F.R.G.). The flow-rate was 1 ml/min in the RP and 2 ml/min in the NP mode. All experiments were carried out at ambient temperature. For the determination of the dead time of the chromatographic system, methanol in the RP and *n*-hexane in the NP mode were injected.

Standards and reagents

All drugs were of pharmaceutical purity, methanol, dichloromethane and *n*-hexane were all of liquid chromatographic grade and glacial acetic acid and chloroform were of analytical-reagent grade, all obtained from Merck. Doubly distilled water which was further purified with a Water-I system (Gelman Sciences, Ann Arbor, MI, U.S.A.) was used for the mobile phase. Propylamine of analytical-reagent grade was purchased from Fluka (Buchs, Switzerland). In the RP with buffer mode, a phosphate buffer was used with a pH of 3 and an ionic strength of 0.05 or 0.02 for the mobile phase. The buffer solution used for the extraction of basic drugs was also a phosphate buffer of pH 3 with an ionic strength of 0.4, which contained sodium octylsulphate ($5 \cdot 10^{-2}$ *M*) as counter ion (Merck). For the preparation of the buffer solutions, phosphoric acid (H_3PO_4) (1 *M*) and sodium dihydrogenphosphate ($NaH_2PO_4 \cdot H_2O$) of analytical-reagent grade (Merck) were used. The buffer solution used for the extraction of acidic drugs was a phosphate buffer of pH 5.5 prepared from $NaH_2PO_4 \cdot H_2O$ and disodium hydrogenphosphate ($Na_2HPO_4 \cdot 2H_2O$) (Merck). Tri-*n*-octylamine (0.1 *M*), used as a counter ion in the ion-pair extraction of acidic drugs, was of analytical-reagent grade from Janssen Chimica (Beerse, Belgium).

Sample preparation

Table I lists the pharmaceutical preparations analysed. For the tablets and the coated tablets, a number of tablets were pulverized and homogeneously mixed and an aliquot of the resulting powder was suspended in a suitable solvent. After ultrasonification for 20 min and centrifugation, the clear supernatant was diluted and the diluted solution was injected into the HPLC system. In the RP mode, the solvent for dissolution was methanol and the supernatant was diluted with water. In the NP mode, dichloromethane was used to dissolve the active compounds and *n*-hexane for dilution of the supernatant. The supernatant was diluted until peaks were obtained that fell

TABLE I
LIST OF THE DIFFERENT PHARMACEUTICAL FORMULATIONS

No.	*Name*	*Active compounds*	*Amount or concentration*	*Dosage form*
1	Aacicortisol	Hydrocortisone sodium succinate	133.7 mg	Ampoules
2	Acid A Vit	Tretinoine	0.5 mg/ml	Lotio
3	Afebryl	Acetylsalicylic acid	300 mg	Effervescent tablets
		Ascorbic acid	300 mg	
		Paracetamol	200 mg	
4	Algotropyl	Promethazine hydrochloride	5 mg	Suppository
		Paracetamol	200 mg	
5	Androcur	Cyproterone acetate	50 mg	Tablets
6	Arovit	Retinol	50 000 IU	Coated tablets
7	Asept'acqua	Merbromin	20 mg/ml	Solution
8	Atropine Cusi	Atropine sulphate	5 mg/ml	Ophthalmic solution
9	Beneurol	Thiamine hydrochloride	300 mg	Coated tablets
10	Buscopan	Hyoscine butylbromide	10 mg	Tablets
11	Buscopan	Metamizol	250 mg	Ampoules
	Compositum	Hyoscine butylbromide	20 mg	
12	Cibalgine	Allobarbital	30 mg	Tablets
		Propyphenazone	220 mg	
13	Desclidium	Viquidil hydrochloride	100 mg	Capsules
14	Exidol	Glafenine	200 mg	Tablets
15	Flagyl	Metronidazole	500 mg	Suppository
16	Frisium	Clobazam	10 mg	Tablets
17	Haldol	Haloperidol	5 mg/ml	Ampoules
18	Hoofd- en zenuwpijn	Acetylsalicylic acid	350 mg	Powder
	poeders	Caffeine	50 mg	
		Phenacetin	200 mg	
19	Inderal	Propranolol hydrochloride	10 mg	Tablets
20	Insidon	Opipramol hydrochloride	50 mg	Coated tablets
21	Isoptine	Verapamil hydrochloride	40 mg	Coated tablets
22	Kevopril	Quinupramine	2.5 mg	Tablets
23	Largactil	Chlorpromazine	40 mg/ml	Solution
24	Masteron	Drostanolone propionate	100 mg/ml	Ampoules
25	Migrane Kranit	Caffeine	85 mg	Tablets
		Phenobarbital	30 mg	
		Paracetamol	200 mg	
		Propyphenazone	150 mg	
		Ethaverine hydrochloride	20 mg	
26	Minidiab	Glipizide	5 mg	Tablets
27	Monazone	Mofebutazon	250 mg	Coated tablets
28	Monotrean	Papaverine	40 mg	Coated tablets
		Quinine hydrochloride	100 mg	
29	Negram	Nalidixic acid	500 mg	Tablets
30	Nitrobaat	Nitroglyerin	1 mg	Tablets
31	Noscaphan	Dextromethorphan hydrobromide	3.5 mg per 5 ml	Syrup
		Guaifenesin	35 mg per 5 ml	
		Noscapine hydrochloride	3.5 mg per 5 ml	
32	Polistine-T-Caps	Carbinoxamine maleate	12 mg	Capsules
33	Priamide	Isopropamide iodide	5 mg	Coated tablets
34	Primperan	Metoclopramide hydrochloride	10 mg	Tablets
35	Prolopa	Levodopa	100 mg	Capsules
		Benserazide	25 mg	

TABLE I *(continued)*

No.	*Name*	*Active compounds*	*Amount or concentration*	*Dosage form*
36	Sedergine	Acetylsalicylic acid	330 mg	Effervescent tablets
		Ascorbic acid	200 mg	
37	Solubacter	Triclocarban	10 mg/g	Solution
38	Tagamet	Cimetidine	200 mg	Tablets
39	Torecan	Thiethylperazine dimaleate	10 mg	Coated tablets
40	Trinitrine Cafeinee	Nitroglycerin	0.5 mg	Coated tablets
		Caffeine	30 mg	
41	Trinitrine Papaverine	Nitroglycerin	0.3 mg	Coated tablets
		Papaverine hydrochoride	5 mg	
42	Uro-S3	Phenazopyridine hydrochloride	50 mg	Coated tablets
		Sulphadiazine	67 mg	
		Sulphamerazine	67 mg	
		Sulphathiazole	67 mg	
43	Vascoril	Cinepazet maleate	300 mg	Tablets
44	Vesalium	Haloperidol	0.3 mg	Coated tablets
		Isopropamide iodide	2 mg	

within scale at the detector attenuation stated. The following pharmaceutical preparations were treated in this way: Androcur, Arovit, Beneurol, Cibalgine, Exidol, Frisium, Inderal, Migraine Kranit, Monazone, Monotrean, Negram, Nitrobaat, Priamide, Primperan, Tagamet, Trinitrine Caffeinee, Trinitrine Papaverine, Uro-S_3 and Vesalium. For some tablets and coated tablets, the mobile phase was used to dilute the clear supernatant. This was the case with following pharmaceutical preparations: Buscopan, Isoptine, Kevopril, Minidiab and Vascoril.

For Insidon and Torecan, which are determined in the NP mode, an ion-pair extraction for basic drugs was carried out. A number of tablets were pulverized and homogeneously mixed. An aliquot of the resulting powder, equivalent with the mean weight of one tablet, was suspended in 50 ml of water for Insidon and in 50 ml of 0.2 *M* hydrochloric acid for Torecan. After ultrasonification and centrifugation, 5 ml of the clear supernatant were transferred into a centrifuge tube and 10 ml of phosphate buffer containing sodium octylsulphate ($5 \cdot 10^{-2}$ *M*) and 5 ml of chloroform were added. After gently shaking for 30 min in a shaking bath and centrifugation, the chloroform phase was diluted with the mobile phase.

For the determination of active compounds in capsules, the capsules were opened and the contents homogeneously mixed. Then the same sample preparation procedure was followed as for the tablets and the coated tablets. Desclidium was diluted with the mobile phase while Polistine-T-Caps and Prolopa were diluted with water.

Powders (Hoofdpijn en Zenuwpijn poeders) were treated in the same way as capsules, after homogeneously mixing.

The effervescent tablets Afebryl and Sedergine were both treated in the same way. One tablet was dissolved in 25 ml of water and shaken for 10 min. To 5 ml of this

solution, 5 ml of phosphate buffer (pH 5.5 and ionic strength 0.1) and 10 ml of chloroform containing tri-*n*-octylamine (0.1 *M*) were added and the mixture was shaken for 30 min. After centrifugation, the chloroform phase was diluted with the mobile phase and injected into the HPLC system

In the set of pharmaceutical formulations, two dosage forms as suppositories were included, namely Algotropyl and Flagyl. One Algotropyl suppository was placed in a centrifuge tube, 50 ml of methanol were added and the mixture was shaken for 30 min at 40°C. After centrifugation, the clear supernatant was diluted with methanol. For Flagyl, the same procedure was carried out but the suppository was dissolved in water and the supernatant diluted with water.

Ampoules, solutions and ophthalmic solutions were treated in an analogous manner for the sample pretreatment. Asept'acqua, Atropine Cusi, Buscopan Compositum and Largactil were diluted with water. Solubacter was diluted with methanol. A Masteron ampoule was diluted with dichloromethane. The pharmaceutical preparation Aacicortisol contains ampoules with the active compound, hydrocortisone sodium succinate, as powder and ampoules with the dissolution solvent for injection. An aliqout of the powder was dissolved in chloroform and further diluted with the mobile phase used in the NP mode. For Haldol, an ion-pair extraction for basic drugs was carried out. The contents of one ampoule were placed in a 50-ml volumetric flask and diluted to volume with 10^{-2} *M* hydrochloric acid. To 5 ml of this solution, 10 ml of phosphate buffer (pH 3 and ionic strength 0.4) containing $5 \cdot 10^{-2}$ *M* sodium octylsulphate and 5 ml of chloroform were added and further treated as for Insidon and Torecan.

Acid-A-Vit lotion was diluted with dichloromethane before injection. Noscaphan syrup was diluted with water before injection.

Detection

UV detection was used for all the samples and the wavelength was 254 nm except for Atropine Cusi, Nitrobaat, Noscaphan, Prolopa, Trinitrine Papaverine (220 nm) and Buscopan, Trinitrine Caffeinee (235 nm).

Software and hardware

The expert system, LABEL, is implemented in a software tool called KES (Knowledge Engineering System, Software Architecture & Engineering, Arlington, VA, U.S.A.; release 2.3). It runs on Apollo, Vax and IBM/AT computers.

RESULTS AND DISCUSSION

Description of the rules for mobile phase selection

The different decisions that have to be taken for the selection of the mobile phase on a CN column are represented in a decision tree (Fig. 1). The parameters determining the direction followed in the decision tree are primarily the number of compounds to be separated in the sample, the acid–base properties and the hydrophobicity of the compounds of interest. The hydrophobicity of a compound is expressed in this paper as the number of carbon atoms present in the molecule. We are aware that this parameter is not the only important factor for determining the hydrophobic character of a compound but, as will be shown later, it seems to be sufficient for the purposes of this system.

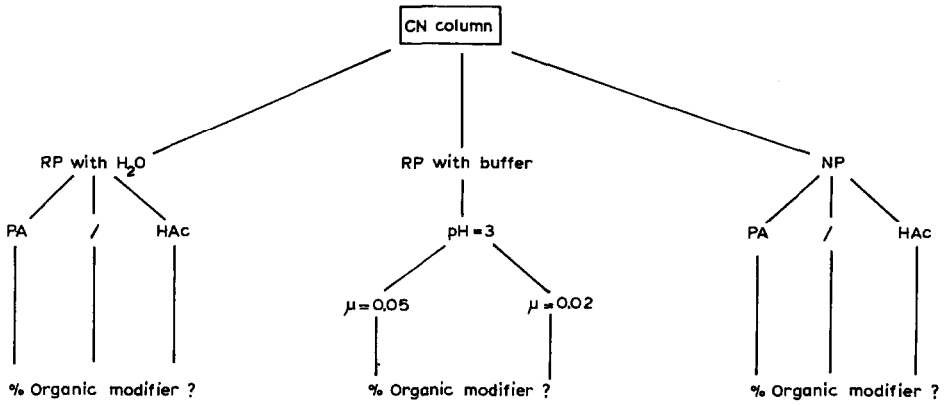

Fig 1. Decision tree for the selection of the mobile phase on a CN column. RP = reversed phase; NP = normal phase; /, no ion-suppressing agent; μ = ionic strength of the buffer.

The number of compounds in the sample and the hydrophobicity of the compounds determine if the RP or the NP mode should be used. For samples with one compound with a carbon number larger than 20 the NP mode is advised, otherwise the RP mode with water is used. For samples containing two or more compounds, the RP mode with water is advised, except when there are two or more compounds with a carbon number smaller than 10, then the NP mode is used.

The addition of ion-suppressing agents to the mobile phase depends on the

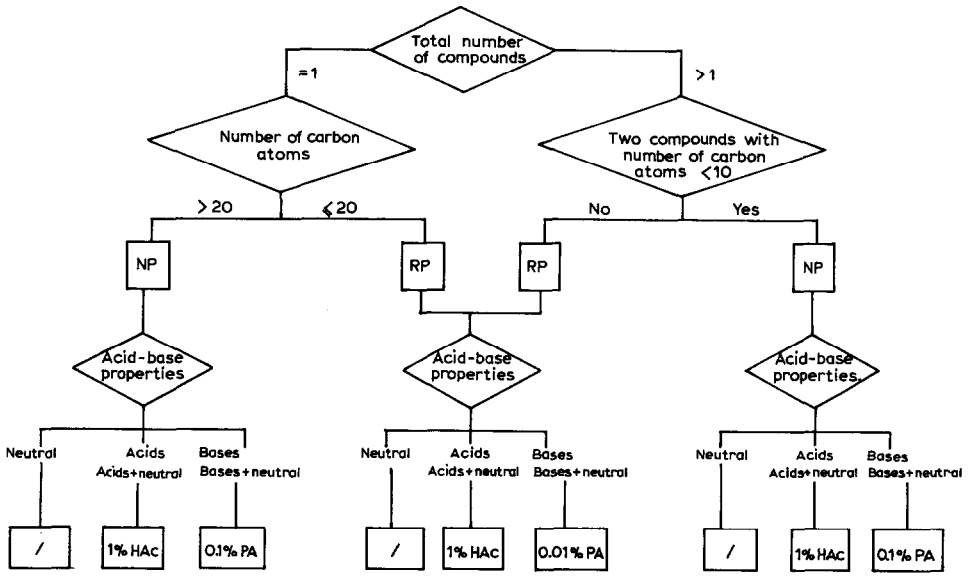

Fig. 2. Pathway followed by the expert system for the selection of the chromatographic mode and the kind of ion-suppressing agent to use in the mobile phase. RP = reversed phase with water; NP = normal phase; /, no ion-suppressing agent.

acid–base status of the compounds to be analysed. Propylamine (PA) is added to the eluting agent when basic drugs or a mixture of basic and neutral compounds are chromatographed (0.01% in the RP mode with water and 0.1% in the NP mode). Acetic acid (HAc) is added to the mobile phase for the chromatography of acidic drugs or mixtures of acidic and neutral compounds (1% HAc in the NP mode and the RP mode with water). The parameters that are important for the mobile phase selection and the relationship between them are outlined in Fig. 2.

All the rules formulated so far are abrogated when the following situations occur: when the use of electrochemical detection is necessary (which is never the case in this study), when basic and acidic drugs have to be determined in one mixture, when amphoteric compounds are present in the sample and when the sample contains fat-soluble vitamins. The chromatographic mode to use in the first three situations is RP with buffer. When the sample contains fat-soluble vitamins, the chromatographic mode advised is NP. More details are given in ref. 9.

For the determination of the acid–base status of a compound, the expert system can give some advice if the end user does not know it. The acid–base status of a compound can be determined by the expert system in three different ways. First, a list of different classes of drugs is available, wherein the compounds are classified as acidic, basic or neutral drugs. Second, the acid–base status of a compound is determined by the expert system by considering the anion or the cation accompanying the active compound[11]. Finally, it is also possible to know the acid–base status of a drug by considering the presence of functional groups in the molecule. There is a list available wherein different functional groups are classified as strongly basic, weakly basic, strongly acidic and weakly acidic[11]. Depending on the presence of these groups in the chemical structure, a compound can be classified as basic or acidic. More details of the determination of the acid–base status are given in ref. 9. The expert system LABEL also gives the starting mobile phase composition in the chromatographic mode selected. This mobile phase composition is not the optimal one for the sample being chromatographed. The main condition required of the starting mobile phase composition is that the solutes are chromatographed in a suitable capacity factor range. For the determination of the optimal mobile phase composition, the existing optimization strategies can be coupled to the expert system. These optimization strategies can then take the starting mobile phase composition as a starting point.

In all the chromatographic modes, the number of carbon atoms of a compound is also an important parameter for the selection of the starting mobile phase composition. The mean of all the carbon atoms in a sample is calculated and this value determines the volume percentage of organic modifier to use in the starting mobile phase composition. In the RP mode, methanol is used as organic modifier and in the NP mode, dichloromethane. An overview of the rules in the different chromatographic modes is presented in Table II. In the RP with buffer mode the volume percentage of organic modifier is restricted to 70% in order to avoid solubility problems with the buffer solution. From Table II, one observes that in the RP with water mode, two sets of rules determining the starting mobile phase composition are formulated: a distinction is made when basic drugs on the one hand and acidic or neutral drugs on the other have to be analysed. A larger volume percentage of organic modifier in the mobile phase is necessary for the chromatography of basic drugs in order to obtain suitable capacity factors for the solutes chromatographed because of the stronger interaction

of the basic compounds with the residual silanol groups. In the RP with buffer mode three classes of rules are distinguished depending on the kind of drugs to be analysed: the first is applied when basic, neutral or mixtures of both are chromatographed, the second for acidic and mixtures of acidic and neutral drugs and the third for mixtures of basic, acidic and neutral compounds. In this last class of rules, a subdivision is made depending on the number of acidic and basic drugs present in the sample. A larger volume percentage of organic modifier is needed when the number of basic drugs is larger than the number of acidic compounds[7]. The first two classes of rules in the RP with buffer mode are usually applied when electrochemical detection is necessary or when the samples contain amphoteric compounds, which are then considered as neutral compounds for the selection of the starting mobile phase composition (see also Table II).

For the buffer solution, one usually applies a pH of 3 and an ionic strength of 0.05 (ref. 7). An exception is made when electrochemical detection in the oxidative mode is used for solutes with a number of carbon atoms smaller than 10. In this instance an ionic strength of 0.02 is used for the buffer solution.

The expert system also contains rules to determine if a gradient elution is suitable for a certain separation problem. When the difference in the number of carbon atoms between two solutes in a mixture is larger than 15, the expert system advises, in addition to an isocratic mobile phase composition, also a gradient elution.

Validation of the rules for mobile phase selection

Initially, 50 pharmaceutical formulations were selected at random from the Belgian Drug repertory 1987. For the different formulations, LABEL was consulted to obtain an HPLC system. For six pharmaceutical formulations, no mobile phase was advised as these samples contain compounds that cannot be determined by UV or electrochemical detection in the oxidative mode. The validation was then performed on the 44 remaining pharmaceutical formulations. In this set, there are thirty formulations with one active compound, nine with two, three with three, one with four and one with five. In the pharmaceutical formulations containing acetylsalicylic acid a supplementary compound, namely salicylic acid, is taken into account to establish the mobile phase composition, as the latter is a known major degradation product of the former.

Two criteria have to be fulfilled in order to be able to conclude that the recommended chromatographic mode and the starting mobile phase composition are suitable for the pharmaceutical formulation being analysed. The first is that the compounds have to elute with a capacity factor (k') between 0.5 and 10 and the second is that the asymmetry factor (a_S) calculated at 10% of the peak height should not exceed 2. These limits take into account that the mobile phase composition given by the expert system is considered as a starting point, a first guess, and not as an optimal one. In the latter instance, the range for the criteria would have been more restricted.

Table III shows the k' and a_S values obtained in the recommended HPLC systems for all the drugs in the pharmaceutical formulations. For 36 pharmaceutical preparations, the two criteria are fulfilled. For Afebryl, Sedergine and Hoofdpijn- en Zenuwpijn poeders, all containing acetylsalicylic acid, different k' values, situated in the required range, are obtained although the same mobile phase composition is used. This is due to the fact that different CN columns were used. For Migraine Kranit

TABLE II

RULES FOR THE DETERMINATION OF THE STARTING MOBILE PHASE COMPOSITION IN THE DIFFERENT CHROMATOGRAPHIC MODES

$$C_i = \frac{C_1 + C_2 + \ldots + C_n}{n}$$

where C_i is the mean of the number of carbon atoms of all the compounds in the sample, $C_1, C_2, \ldots, C_n$ are the number of carbon atoms in compound $1,2,\ldots, n$ and n is the number of compounds in the sample.

*Mode**	C_i	*Mobile phase*
NP (with PA, HAc and /)	$C_i < 5 \rightarrow 70\%$	dichloromethane
	$5 \leqslant C_i < 10 \rightarrow 65\%$	dichloromethane
	$10 \leqslant C_i < 15 \rightarrow 60\%$	dichloromethane
	$15 \leqslant C_i < 20 \rightarrow 55\%$	dichloromethane
	$20 \leqslant C_i < 25 \rightarrow 50\%$	dichloromethane
	$25 \leqslant C_i < 30 \rightarrow 45\%$	dichloromethane
	$30 \leqslant C_i < 35 \rightarrow 40\%$	dichloromethane
	$35 \leqslant C_i < 40 \rightarrow 35\%$	dichloromethane
	$40 \leqslant C_i < 45 \rightarrow 30\%$	dichloromethane
	$C_i \geqslant 45 \rightarrow 25\%$	dichloromethane
RP with water (with HAc or /)	$C_i < 5 \rightarrow 5\%$	methanol
	$5 \leqslant C_i < 10 \rightarrow 10\%$	methanol
	$10 \leqslant C_i < 15 \rightarrow 25\%$	methanol
	$15 \leqslant C_i < 20 \rightarrow 40\%$	methanol
	$20 \leqslant C_i < 25 \rightarrow 55\%$	methanol
	$25 \leqslant C_i < 30 \rightarrow 70\%$	methanol
	$30 \leqslant C_i < 35 \rightarrow 85\%$	methanol
	$C_i \geqslant 35 \rightarrow 100\%$	methanol
RP with water (with PA)	$C_i < 5 \rightarrow 35\%$	methanol
	$5 \leqslant C_i < 10 \rightarrow 45\%$	methanol
	$10 \leqslant C_i < 15 \rightarrow 55\%$	methanol
	$15 \leqslant C_i < 20 \rightarrow 65\%$	methanol
	$20 \leqslant C_i < 25 \rightarrow 75\%$	methanol
	$25 \leqslant C_i < 30 \rightarrow 85\%$	methanol
	$30 \leqslant C_i < 35 \rightarrow 95\%$	methanol
	$C_i \geqslant 35 \rightarrow 100\%$	methanol
RP with buffer (the sample contains basic, neutral or basic + neutral drugs)**	$C_i < 5 \rightarrow 5\%$	methanol
	$5 \leqslant C_i < 10 \rightarrow 10\%$	methanol
	$10 \leqslant C_i < 15 \rightarrow 20\%$	methanol
	$15 \leqslant C_i < 20 \rightarrow 30\%$	methanol
	$20 \leqslant C_i < 25 \rightarrow 40\%$	methanol
	$25 \leqslant C_i < 30 \rightarrow 50\%$	methanol
	$30 \leqslant C_i < 35 \rightarrow 60\%$	methanol
	$C_i \geqslant 35 \rightarrow 70\%$	methanol
RP with buffer (the sample contains acidic or acidic + neutral drugs)**	$C_i < 5 \rightarrow 3\%$	methanol
	$5 \leqslant C_i < 10 \rightarrow 5\%$	methanol
	$10 \leqslant C_i < 15 \rightarrow 10\%$	methanol
	$15 \leqslant C_i < 20 \rightarrow 15\%$	methanol
	$20 \leqslant C_i < 25 \rightarrow 20\%$	methanol
	$25 \leqslant C_i < 30 \rightarrow 25\%$	methanol
	$30 \leqslant C_i < 35 \rightarrow 30\%$	methanol
	$35 \leqslant C_i < 40 \rightarrow 35\%$	methanol
	$C_i \geqslant 40 \rightarrow 40\%$	methanol

TABLE II *(continued)*

*Mode**	C_i	*Mobile phase*
RP with buffer [the sample contains basic + acidic or basic + acidic + neutral drugs and the number of acidic drugs < the number of (basic + neutral drugs)]	$C_i < 5 \rightarrow 5\%$	methanol
	$5 \leqslant C_i < 10 \rightarrow 10\%$	methanol
	$10 \leqslant C_i < 15 \rightarrow 20\%$	methanol
	$15 \leqslant C_i < 20 \rightarrow 30\%$	methanol
	$20 \leqslant C_i < 25 \rightarrow 40\%$	methanol
	$25 \leqslant C_i < 30 \rightarrow 50\%$	methanol
	$30 \leqslant C_i < 35 \rightarrow 60\%$	methanol
	$C_i \geqslant 35 \rightarrow 70\%$	methanol
RP with buffer [the sample contains basic + acidic or basic + acidic + neutral drugs and the number of acids ⩾ the number of (basic + neutral drugs)]	$C_i < 5 \rightarrow 5\%$	methanol
	$5 \leqslant C_i < 10 \rightarrow 10\%$	methanol
	$10 \leqslant C_i < 15 \rightarrow 15\%$	methanol
	$15 \leqslant C_i < 20 \rightarrow 20\%$	methanol
	$20 \leqslant C_i < 25 \rightarrow 25\%$	methanol
	$25 \leqslant C_i < 30 \rightarrow 30\%$	methanol
	$30 \leqslant C_i < 35 \rightarrow 35\%$	methanol
	$35 \leqslant C_i < 40 \rightarrow 40\%$	methanol
	$C_i \geqslant 40 \rightarrow 45\%$	methanol

* NP = normal phase; RP = reversed phase; PA = propylamine; HAc = acetic acid; / = no ion-suppressing agent.

** For the selection of the starting mobile phase composition, the amphoteric drugs have been considered as neutral compounds.

tablets, the expert system advises an isocratic mobile phase composition and a gradient elution as this formulation contains solutes for which the difference in the number of carbon atoms is larger than 15 [caffeine (C_8), paracetamol (C_8) and ethaverine hydrochloride (C_{24})]. Both chromatographic systems give suitable results for this particular case and one would prefer the isocratic one for analysis.

For eight formulations the criteria are not fulfilled: Atropine Cusi, Buscopan, Desclidium, Masteron, Noscaphan, Priamide, Prolopa and Vesalium. For these formulations, the separation strategy with the gradient elution, developed in our laboratory[4–6], has then been carried out as an alternative, to check if the selected chromatographic mode is not suitable or if the advised starting mobile phase composition for these samples is not appropriate. The separation strategy consists in carrying out a gradient elution from which an isocratic mobile phase composition with a suitable solvent strength is determined so that the drugs elute within a suitable k' range. To obtain the isocratic mobile phase composition from the gradient elution, the geometric mean of the volume percentages of organic modifier at which each drug elutes in the gradient elution is calculated and multiplied with an experimentally determined factor, 3/4 (refs. 4–6).

For Atropine Cusi, the mobile phase composition calculated from the gradient elution was methanol–water–PA (75:25:0.01) and resulted in $k' = 11.7$ and $a_S = 2.5$. Neither of the two strategies gives an acceptable solution. A mobile phase composed of methanol–PA (100:0.01) did result in a k' value of 6.3 but the a_S value was still 2.1. This suggests that the advised RP with water mode is not suitable for this compound. The same conclusion can be drawn for Buscopan because in the NP mode the active

TABLE III

k' VALUES AND a_S VALUES OF THE COMPOUNDS IN THE MOBILE PHASE SYSTEM RECOMMENDED BY THE EXPERT SYSTEM.

*No.**	*Active compounds*	*Recommended mobile phase system***	k'	a_S
1	Hydrocortisone sodium succinate	NP with HAc CH_2Cl_2–Hex–HAc (45:55:1)	2.0	1.2
2	Tretinoine	NP with HAc CH_2Cl_2–Hex–HAc (50:50:1)	1.0	1.1
3	Acetylsalicylic acid	NP with HAc	2.0	1.1
	Ascorbic acid	CH_2Cl_2–Hex–HAc	8.4	1.5
	Paracetamol	(65:35:1)	2.3	1.1
	(Salicylic acid)		2.5	1.1
4	Paracetamol	RP with buffer	0.6	1.0
	Promethazine hydrochloride	CH_3OH–B (15:85)	5.5	1.5
5	Cyproteronacetate	NP with / CH_2Cl_2–Hex (45:55)	4.7	1.4
6	Retinol	NP with / CH_2Cl_2–Hex (50:50)	1.7	1.0
7	Merbromin	RP with HAc CH_3OH–H_2O–HAc (55:45:1)	0.7	1.0
8	Atropine sulphate	RP with PA CH_3OH–H_2O–PA (65:35:0.01)	17.0	3.0
9	Thiamine hydrochloride	RP with PA CH_3OH–H_2O–PA (55:45:0.01)	0.7	1.0
10	Hyoscine butylbromide	NP with PA CH_2Cl_2–Hex–PA (50:50:0.1)	>20	/
11	Metamizol	RP with buffer	0.5	1.0
	Hyoscine butylbromide	CH_3OH–B (20:80)	1.3	1.1
12	Allobarbital	RP with HAc	0.6	1.2
	Propyphenazone	CH_3OH–H_2O–HAc (25:75:1)	1.2	1.3
13	Viquidil hydrochloride	RP with PA CH_3OH–H_2O–PA (75:25:0.01)	17.5	1.6
14	Glafenine	RP with PA CH_3OH–H_2O–PA (65:35:0.01)	0.6	1.1
15	Metronidazole	RP with / CH_3OH–H_2O (10:90)	0.8	1.1
16	Clobazam	RP with / CH_3OH–H_2O (40:60)	1.2	1.0

TABLE III *(continued)*

*No.**	*Active compounds*	*Recommended mobile phase system***	*k'*	a_S
17	Haloperidol	NP with PA CH_2Cl_2–Hex–PA (50:50:0.1)	1.8	1.0
18	Acetylsalicylic acid	NP with HAc	1.2	1.1
	Phenacetin	CH_2Cl_2–Hex–HAc	3.9	1.3
	Caffeine	(65:35:1)	4.6	1.4
	(Salicylic acid)		1.9	1.1
19	Propanolol hydrochloride	RP with PA CH_3OH–H_2O–PA (65:35:0.01)	7.6	1.8
20	Opipramol hydrochloride	NP with PA CH_2Cl_2–Hex–PA (50:50:0.1)	4.9	1.5
21	Verapamil hydrochloride	NP with PA CH_2Cl_2–Hex–PA (45:55:0.1)	0.9	1.0
22	Quinupramine	NP with PA CH_2Cl_2–Hex–PA (50:50:0.1)	9.7	1.2
23	Chlorpromazine	RP with PA CH_3OH–H_2O–PA (65:35:0.01)	5.9	1.1
24	Drostanolone propionate	NP with / CH_2Cl_2–Hex (50:50)	>20	/
25	Caffeine	RP with Buffer	0.8	1.0
	Phenobarbital	CH_3OH–B	1.1	1.0
	Paracetamol	(20:80)	0.6	1.0
	Propyphenazone		1.9	1.2
	Ethaverine hydrochoride		5.0	1.5
25	Caffeine	Gradient elution	1.3	1.0
	Phenobarbital	CH_3OH–B (0:100)	1.9	1.0
	Paracetamol	↓ 20 min	0.9	1.0
	Propyphenazone		4.1	1.1
	Ethaverine hydrochloride	CH_3OH–B (50:50)	6.6	1.1
26	Glipizide	NP with HAc CH_2Cl_2–Hex–HAc (50:50:1)	10.7	1.9
27	Mofebutazon	RP with HAc CH_3OH–H_2O–HAc (25:75:1)	1.1	1.1
28	Papaverine	RP with PA	0.6	1.0
	Quinine hydrochoride	CH_3OH–H_2O–PA (75:25:0.01)	2.1	1.0
29	Nalidixic acid	RP with HAc CH_3OH–H_2O–HAc (25:75:1)	1.3	1.9
30	Nitroglycerin	RP with HAc CH_3OH–H_2O–HAc (5:95:1)	2.3	1.3

(Continued on p. 38)

TABLE III *(continued)*

*No.**	*Active compounds*	*Recommended mobile phase system***	*k'*	a_s
31	Guaifenesin	RP with PA	0	/
	Dextromethorphan hydrobromide	CH_3OH–H_2O–PA (65:35:0.01)	0.3	1.0
	Noscapine hydrochloride		0.5	1.0
32	Carbinoxamine maleate	RP with PA CH_3OH–H_2O–PA (65:35:0.01)	5.9	1.3
33	Isopropamide iodide	NP with PA CH_2Cl_2–Hex–PA (50:50:0.1)	>20	/
34	Metoclopramide hydrochloride	RP with PA CH_3OH–H_2O–PA (55:45:0.01)	10.2	1.5
35	Levodopa	RP with buffer	0.3	1.0
	Benserazide	CH_3OH–B (10:90)	0.5	1.0
36	Acetylsalicylic acid	NP wih HAc	2.0	1.1
	Ascorbic acid	CH_2Cl_2–Hex–HAc	8.4	1.5
	(Salicylic acid)	(65:35:1)	2.5	1.1
37	Triclocarban	RP with / CH_3OH–H_2O (25:75)	10.0	1.6
38	Cimetidine	RP with PA CH_3OH–H_2O–PA (55:45:0.01)	0.7	1.0
39	Thiethylperazine dimaleate	NP with PA CH_2Cl_2–Hex–PA (50:50:0.1)	2.5	1.3
40	Nitroglycerin	NP with HAc	2.0	1.2
	Caffeine	CH_2Cl_2–Hex–HAc (65:35:1)	3.8	1.8
41	Nitroglycerin	RP with buffer	2.2	1.0
	Papaverine hydrochloride	CH_3OH–B (15:85)	2.7	1.4
42	Sulphadiazine	RP with buffer	1.0	1.0
	Sulphamerazine	CH_3OH–B	1.0	1.0
	Sulphathiazole	(15:85)	1.2	1.0
	Phenazopyridine hydrochloride		1.3	1.2
43	Cinepazet maleate	NP with PA CH_2Cl_2–Hex–PA (50:50:0.1)	2.2	1.2
44	Isopropamide iodide	RP with PA	0	/
	Haloperidol	CH_3OH–H_2O–PA (75:25:0.01)	1.0	1.0

* The numbers correspond to the pharmaceutical formulations in Table I.

** RP = reversed phase; NP = normal phase; B = buffer solution (see Experimental); HAc = acetic acid; Hex = *n*-hexane; PA = propylamine; / (in the recommended mobile phase system) = no ion-suppressing agent).

compound, hyoscine butylbromide, does not elute, even with the gradient elution. However, it is possible to chromatograph it in the RP with buffer mode (see Buscopan Compositum).

For Desclidium, the condition of the asymmetry factor is fulfilled but the mobile phase composition given by the expert system and that determined from the gradient elution resulted in too strong a retention. When 100% methanol (containing 0.01% of PA) is used a k' value of 8.5 is recorded. The same phenomenon is observed for Masteron. The expert system advises a mobile phase composition of methanol–water–PA (50:50:0.01), with which the drug is not eluted. The isocratic composition calculated from the gradient elution is methanol–water–PA (75:25:0.01), resulting in $k' = 17.5$ and $a_S = 1.8$. By increasing the proportion of methanol, the k' value obtained falls within the required range.

For two of the three drugs present in the Noscaphan syrup, the proposed mobile phase composition (methanol–water–PA; 65:35:0.01) gives too small a retention. With the separation strategy, a mobile phase composed of methanol–water–PA (30:70:0.01) is used and both criteria are fulfilled for two of the three drugs. The other drug, guaiphenesin, possesses ten carbon atoms, which is a limiting value for exhibiting retention on a CN column in the RP mode. To obtain a smaller k' value for isopropamide iodide in Priamide, 100% dichloromethane (containing 0.1% of PA) was used, but even then a k' value of 15 and an a_S value of 1.3 were recorded. These results suggest that the NP mode is not to be recommended, but this compound has also been chromatographed in the RP mode (see Vesalium) without success as it exhibits no retention. Isopropamide iodide is a quaternary ammonium derivative and ion-pair chromatography would perhaps offer a suitable solution[12]. Prolopa capsules contain levodopa, which does not exhibit sufficient retention on a CN column in the RP mode, even without an organic modifier in the mobile phase. Levodopa belongs to the group of the catecholamines, for which ion-par chromatography has been used in many studies.

A possible solution for the exceptions revealed by carrying out the validation step is to put them in a database that could be coupled to the expert system. This database could then be continuously updated when more pharmaceutical preparations are analysed.

Sample preparation

As the set of pharmaceutical preparations contain different dosage forms, different approaches for the sample preparation were applied. In the RP mode, usually methanol was applied for the dissolution of the active compounds and the dilution was performed with water. In the NP mode, dichloromethane was the solvent for dissolution and *n*-hexane for dilution. In some instances, problems of solubility occurred when water in the RP or *n*-hexane in the NP mode was used for the dilution. The dilution was then performed with the mobile phase rather than with the solvents for dissolution. When the solvent in which the compounds are dissolved possesses a stronger solvent strength than the mobile phase, the compounds are chromatographed with a bad peak shape[13]. This phenomenon is illustrated in Fig. 3, where cinepazet maleate, the active compound of Vascoril tablets was diluted with different solvents. The sample preparation was carried out in the first instance as described under Experimental for the determination in the NP mode, *viz.*, dissolution in

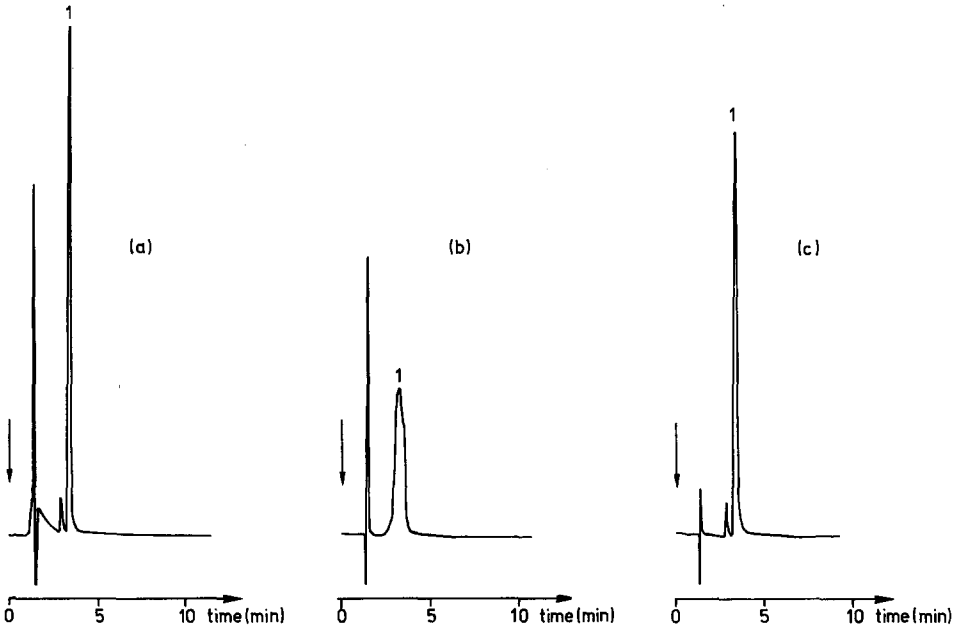

Fig. 3. Chromatograms obtained after injection of cinepazet maleate in different solution solvents: (a) *n*-hexane; (b) dichloromethane; (c) the mobile phase. The same concentration was injected. Mobile phase: dichloromethane–*n*-hexane–PA (50:50:0.1).

dichloromethane and dilution with *n*-hexane (Fig. 3a). A good peak shape was observed for this drug. However, after a certain time, problems arose because cinepazet maleate was only sparingly soluble in *n*-hexane. Therefore, the dilution was performed with dichloromethane and the chromatogram obtained (Fig. 3b) had a bad peak shape. Fig. 3c shows the chromatogram obtained when the dilution was performed with the mobile phase, which gave an acceptable result. This phenomenon was not observed for all compounds (see Experimental). For example, Acid A Vit lotio, containing tretinoine, is an ethanolic solution that has been diluted with dichloromethane and a good peak shape is obtained although the solvent strength of the injected solvent is stronger than that of the mobile phase. For Algotropyl suppositories, the supernatant was injected in a methanolic solution without problems of peak shape.

For some pharmaceutical preparations, an ion-pair extraction, which has been used in our laboratory for the determination of basic drugs in pharmaceutical preparations and in biological materials[14,15], was necessary to permit the determination of the active compounds in the chromatographic mode selected. This applied, for example, to Insidon and Torecan tablets, containing opipramol hydrochloride and thiethylperazine dimaleate, respectively. The expert system advises the NP mode, but these compounds are not soluble in dichloromethane. Dissolution in methanol and dilution with dichloromethane resulted in a broad solvent peak and a bad peak shape. This problem was solved by carrying out an ion-pair extraction with sodium octylsulphate as counter ion. The chloroform phase was then diluted with the mobile

phase. The same procedure also has to be applied to Haldol ampoules, as they contain an aqueous solution of haloperidol which was not compatible with the NP system selected. For Masteron ampoules, dilution with dichloromethane was possible as these ampoules contain an oil solution of drostanolone propionate.

The expert system advises the NP mode for both of the effervescent tablets, although the tablets must first be dissolved in water. In this way, it was necessary to carry out an extraction with a solvent that was miscible with the mobile phase. With a classical liquid–liquid extraction, it was not possible to extract the compounds of interest into the organic phase. Then, an ion-pair extraction with tri-*n*-octylamine, which has been applied in our laboratory for the extraction of colour additives[16], was used with success.

CONCLUSION

The rules incorporated in an expert system for the selection of suitable mobile phase systems for the chromatography of drugs in pharmaceutical preparations on a CN column were validated. It is possible to formulate these rules using a fairly rough parameter for the determination of the hydrophobic character of a compound, namely the number of carbon atoms in a molecule. The selection of one of the three chromatographic modes which can be used on a CN column was performed in addition to the determination of a starting mobile phase composition. In 82% of all instances, success was achieved in a manner that probably could not have been done better by a human chromatographic expert. In three of the eight remaining instances, simple adaptation of the volume ratio of the mobile phase solvents was sufficient. For the other five pharmaceutical formulations, it can be concluded that the selected chromatographic mode was not appropriate, which was also confirmed by the results obtained with the separation strategy involving gradient elution.

The use of an expert system for HPLC methods in a laboratory has the advantage that technicians or other workers with less chromatographic experience are able to start an analysis without the help or intervention of the chromatographic expert. The expert system can always be updated with new knowledge and rules. LABEL was developed for use with the CN column only. However, this column type is very interesting as it can be applied in different chromatographic modes. If one had chosen different stationary phase types to apply in the RP and NP modes, the number of rules would have been larger because, in that event, supplementary rules would be necessary to decide between the column types to use.

The rules formulated in this paper were developed for the chromatography of drugs on a certain type of CN column, namely a LiChrosorb CN column. Probably, if the rules were to be transferred to another type of CN column from a different manufacturer different results would be obtained. It is well known that even different columns from the same manufacturer, packed with the same type of stationary phase but emanating from different batches, can provide different results. It is then not possible, for a certain separation problem, to transfer unchanged the chromatographic system developed on one column to another. However, the results given in this paper were obtained on four different LiChrosorb CN columns so that the problem of column reproducibility has been taken into account in this validation. It can be stated that this problem does not influence the success of finding a suitable HPLC system

with the two criteria used and their range premised. It should be suitable for the rules developed for this kind of CN column to be transferred to the same type of columns emanating from a different manufacturer by carrying out only small modifications. This study is now under investigation.

In this paper, rules were also validated for the selection of the starting mobile phase composition for a certain separation problem. In previous papers, a separation strategy was described in which a suitable solvent strength for isocratic elution was determined from a gradient elution. This strategy was also applied in this paper to samples for which the expert system does not give a suitable solution and, in most instances, does not give an appropriate solution either. Comparing the two strategies, one can state that with the expert system it is possible to find a suitable mobile phase composition by carrying out one experiment. This is certainly not valid for the separation strategy, as one has first to perform a gradient elution and afterwards an isocratic elution, resulting in a minimum of two experiments. When some compounds coelute in the gradient elution, the position of each compound has to be determined as one has to know the volume percentage at which each compound elutes for the determination of the mobile phase composition in the isocratic elution. At the other hand, the separation strategy can be carried out on CN columns emanating from any manufacturer or even on another column type, whereas this is not true for the expert system. In this way, one would more quickly obtain a suitable HPLC system with the separation strategy.

Some attention has also been paid to the sample preparation of the pharmaceutical preparations. Despite the fact that no general approach has been formulated in LABEL until now, one can state that for some formulations, one can certainly incorporate in the expert system some more general rules about the dissolution and dilution of the samples in relation to the chromatographic mode used. This aspect will be incorporated in the expert system and further validated.

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REFERENCES

1 J. C. Berridge, *Techniques for the Automated Optimization of HPLC Separations*, Wiley-Interscience, New York, 1985.
2 P. J. Schoenmakers, *Optimization of Chromatograhic Selectivity*, Elsevier, Amsterdam, 1986.
3 S. R. Abboth, *J. Chromatogr. Sci.*, 18 (1980) 540.
4 M. De Smet, G. Hoogewijs, M. Puttemans and D. L. Massart, *Anal. Chem.*, 56 (1984) 2662.
5 M. De Smet and D. L. Massart, *J. Pharm. Biomed. Anal.*, 6 (1988) 277.
6 M. De Smet and D. L. Massart, *J. Liq. Chromatogr.*, in press.
7 M. De Smet and D. L. Massart, *J. Chromatogr.*, 410 (1987) 77.
8 Y. Z. Hu, A. Peeters, G. Musch and D. L. Massart, *Anal. Chim. Acta*, in press.
9 M. De Smet, G. Musch, A. Peeters, L. Buydens and D. L. Massart, *Anal. Chem.*, in press.
10 G. Musch and D. L. Massart, in preparation.
11 M. R. De Taevernier, Y. Michotte, L. Buydens, M. P. Derde, M. De Smet, L. Kaufman, G. Musch, J. Smeyers-Verbeke, A. Thielemans, L. Dryon and D. L. Massart, *J. Pharm. Biomed. Anal.*, 4 (1986) 297.
12 J. A. De Schutter and P. De Moerloose, *J. Chromatogr.*, 437 (1988) 83.
13 L. R. Snyder and J. J. Kirkland, *Introduction to Modern Liquid Chromatography*, Wiley-Interscience, New York, 1979, Ch. 19.
14 G. Hoogewijs and D. L. Massart, *J. Pharm. Biomed. Anal.*, 2 (1984) 449.
15 G. Hoogewijs and D. L. Massart, *J. Pharm. Biomed. Anal.*, 3 (1985) 165.
16 M. Puttemans, L. Dryon and D.L. Massart, *Anal. Chim. Acta*, 165 (1984) 245.