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Advances in expert systems for high-performance liquid chromatography

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ABSTRACT

The development of modules for the selection of the separation mode, stationary phase and mobile phase and for peak identification in expert systems for high-performance liquid chromatography is discussed. Both the rules and methods used in these modules and their theoretical basis are included. A program to select the separation mode and the stationary and mobile phases has been developed in which there are two modes of entry, the molecular structure of the sample and provision of the sample name. In the peak identification module, three methods for off-line peak identification, by transfer of retention values from one column to another column, by the relationship between retention values and molecular structure parameters and by the interaction index, and also a method for on-line peak identification by combination of the UV spectral parameters with the retention values, have been developed.

INTRODUCTION

In the last few years, much effort has been devoted to developing strategies for an expert system for chromatography (ESC)[1–4]. In a previous paper[1], a strategy for the development of an ESC was established that can be divided into three parts: a knowledge and chromatogram base, an inference engine and a user interface. The knowledge base consists of a set of facts and rules for the chromatographic subject. The chromatogram base contains a compilation of published chromatograms. The inference engine or rule interpreter is responsible for extracting the desired information from the knowledge base and explaining how the answer was obtained. The user interface allows interaction with the system through dialogues and a display of the reasoning results, chromatograms and explanation.

For a chromatographic analysis, the following modules should be included:

(1) selection of the sample pretreatment and detection method;
(2) selection of the separation modes and column system;
(3) optimization of operating conditions;
(4) peak identification and on-line quantification; and
(5) diagnosis of the hardware system.

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A computer program package to support the ESC task modules has been developed[1], and the knowledge, rule and method for modules 3 and 5 and the on-line quantitation section in module 4 have been reported. In this paper, we discuss the selection of the mode of separation, the mobile and stationary phases in module 2 and peak identification in module 4.

SELECTION OF THE SEPARATION MODE AND THE STATIONARY AND MOBILE PHASES

The column system is the central element for the chromatographic separation of a mixture. The selection or optimization of the column dimensions and packing size, which often affect column efficiency, can be obtained from some well known relationships[5,6]. However, in most instances, the column efficiency in high-performance liquid chromatography (HPLC) is almost constant (about 10 000 plates) for a given column, and the peak capacity of such a column is about 45 at capacity factor, $k' = 5.0$[7]. Therefore, there is enough space to arrange the compounds inside the column for the separation of most samples. The key problem is the selectivity, or how to distribute the compounds to suitable positions inside the column. Chemical effects on selectivity, such as the type and amount of the mobile and stationary phases, pH and temperature, are often the most important factors.

For the selection of a column system, the following two requirements have been put forward[8]:

(1) a relative separation factor (α) value > 1.16 is needed at $k' = 0.4$, and $\alpha > 1.06$ is feasible if $k' \approx 5$;

(2) the retention of all compounds in the sample should be adjusted to be in the range $0.4 < k' < 30$.

In our opinion, reversed-phase (RP) chromatography is to be recommended for the separation mode as often as possible because it is the simplest to use, and the reproducibility of the qualitative and quantitative data is the best of all the separation modes. In order to recommend a column system, two modes of user entry have been designed to interface the computer with the user: molecular structure of the compounds and provision of the sample name. Table I lists the functional groups used for the entry of the molecular structure, each of which has associated with it the molecular structure parameters such as the Van der Waals volume and the hydrogen bonding energy.

TABLE I

FUNCTIONAL GROUPS ENTERED BY THE USER IN THE ENTRY MODE OF THE MOLECULAR STRUCTURE IN THE EXPERT SYSTEM FOR HPLC

>C<	-OH	-NH2	-SH	-Cl
≡CH	-CHO	-NH-	>S=O	-Br
>CH2	>C=O	-NO2	-S-	-I
-CH3	-COO-	-ONH	>C=S	-F
>C=C<	-COOH	$-NH3^+$	-SOO-	-OPO2
-C≡C-	-O-	-N=O	-SO3H	-PO3H2
-C6H5	$-COO^-$	-ONO-	-S-S-	$-PO3H^-$
-C10H7		>N-	$-SO3^-$	-CN

Before entry of the functional groups of each compound in the sample, the following questions concerning the behaviour of the sample to be analysed must be answered:

(1) how many compounds does the sample contain?;
(2) is the sample to be separated by class?;
(3) does the sample contain homologues?;
(4) does the sample contain positional isomers?;
(5) does the sample contain configurational isomers?;
(6) does the sample contain racemic components?

Question 1 should be answered by the number of compounds of interest, question 2 defines the purpose of the analytical separation, question 3 aims to enter the molecular structure as efficiently as possible because the homologues differ only in the number of methylene groups and questions 4, 5 and 6 help to define the behaviour of the sample to determine the difficulty of separating its components.

In RP-HPLC, the capacity factor of a solute is determined by the hydrophobic (or dispersive) interactions and hydrophilic interactions (mainly the hydrogen bonding interaction) simultaneously. The retention value of a solute increases with increasing hydrophobic interaction, or the number of non-polar functional groups such as CH_3, CH_2 and C_6H_5, and decreases with increasing hydrophilic interaction or the number of polar functional groups such as OH and COOH. Therefore, there is a critical value of the ratio of non-polar to polar functional groups. Solutes contained in the sample with a ratio of non-polar to polar functional groups greater than the critical value should be separated by RP chromatography, otherwise another mode of separation is recommended. Such a critical value can be roughly estimated by entering the molecular structure parameters through the user interface. For organic ions, electrostatic and hydrophobic interactions also exist simultaneously, and there is a critical value of the ratio of non-polar to polar and ionic functional groups. Such a critical value can be estimated empirically. According to the purpose of the separation and the physico-chemical behaviour of the compounds in the sample, the mode of separation can be selected by the inference engine.

From the information on the molecular structures, it is possible to determine the most difficult substance pair to be resolved in the sample. The principles of molecular interactions needed to select suitable mobile and stationary phases are as follows:

(1) For samples containing only non-polar compounds with no π-electron interactions, the selectivity and retention are determined only by the dispersive interactions, and are simultaneously changed by varying the mobile phase composition and the type of organic modifier, which means that the selectivity and retention change together. Therefore, a cheap and less toxic organic solvent is recommended for the organic modifier. If the selectivity with a given column system is insufficient to provide the α value required, the situation can be improved by selecting a solvent with a weaker dispersive interaction as the organic modifier or a packing material with a stronger dispersive interaction as the stationary phase.

(2) For samples containing compounds with selectivity determined only by the dispersive interactions, but retention by the various types of molecular interactions, a stationary phase with a strong dispersive interaction and weak or no other types of interaction is preferred. An organic solvent with a weak dispersive interaction and strong other interactions is selected as the organic modifier.

(3) When the dipole moment interaction is the main factor determining the selectivity, but the retention is determined by various types of molecular interactions, for the same reason as in rule 2, a stationary phase with a strong dipole moment interaction and weak other types of molecular interactions and an organic modifier with a weak dipole moment interaction and strong other types of molecular interactions are to be preferred. Otherwise, if the stationary phase has no or a weak dipole moment interaction, then an organic modifier with a strong dipole moment interaction and moderate other types of molecular interactions is to be preferred.

(4) When hydrogen bonding is the main factor in determining the selectivity, but the retention is determined by the various types of molecular interactions, a stationary phase with a strong hydrogen bonding interaction and weak other types of molecular interactions and an organic modifier with a weak hydrogen bonding interaction and strong other types of molecular interaction are to be preferred. If the stationary phase used has no or a weak hydrogen bonding interaction, then an organic modifier with a strong hydrogen bonding interaction and moderate other types of molecular interaction is to be preferred.

(5) When the selectivity is determined by the π-electron interaction, but the retention value is determined by various types of molecular interactions, a stationary phase with a strong π-electron interaction and weak other types of the molecular interactions and an organic modifier with a weak π-electron interaction and strong other types of molecular interaction is to be preferred. If the stationary phase used has no or a weak π-electron interaction, then an organic modifier with a strong π-electron interaction and moderate other types of molecular interaction is to be preferred.

In RP chromatography, where water is used as the weak solvent and ODS packing material is used most widely, the key problem is how to select the strong solvent[9]. In practice, methanol, acetonitrile and tetrahydrofuran are generally used. We consider that methanol alone is sufficient to separate most samples. However, if the compounds in the sample differ only in the position of a double bond, then an acetonitrile–water mixture is recommended for the mobile phase. If the compounds have very strong hydrophobic interactions and cannot be eluted even with methanol as the mobile phase, then a ternary mobile phase containing methanol–water and a solvent with a strong hydrophobic interaction such as tetrahydrofuran or acetone, or even a non-aqueous mobile phase, is recommended.

The additive reagent can be selected from the information on the functional groups contained in the compounds. If the compounds in the sample have an amino group, then addition of a suitable amount of organic amine to mobile phase is recommended in order to eliminate the strong interaction between the free silanol groups and the amino groups. If the compounds in the sample have functional groups such as phenolic OH, COOH or H_2PO_4, then a suitable amount of phosphate buffer or ammonium acetate is recommended in order to adjust the acidity in the mobile phase.

In normal-phase (NP) liquid chromatography, the interaction between the solute and the stationary phase provides the main contribution to the selectivity and retention, and hexane is generally used as the weak solvent in the mobile phase. The strong solvent is used to adjust the retention of solutes in a suitable range. Therefore, the strong solvent can be recommended according to information on the functional groups present in the solutes. Strong solvents used in normal-phase chromatography and their interaction properties have been reported by Snyder[10]. If the compounds in

the sample have the functional groups OH, COOH or NH_2, then isopropanol is recommended. If the compounds in the sample have only COO, CN, NO_2 and C=O groups, ethyl acetate, acetone or acetonitrile is recommended as the strong solvent. If the compounds in the sample have only –O– and phenyl groups, diethyl ether is recommended as the strong solvent. If the compounds also have H_2PO_4, COOH or OH groups, then methanol–acetonitrile or even an aqueous solution is recommended. If the compounds have these functional groups simultaneously, the solvent can be selected using the above sequence. As an example, the results for the recommendation of the column system through the entry of molecular structure for ten bile acids and some saccharides are listed below:

Samples: ten bile acids, TUDCA, GUDCA, TCA, GCA, TCDCA, GCDCA, DCA, TDCA, TLCA and GLCA.
Entry mode: recommendation from structure.
Mode of separation: (reversed-phase) chromatography.
Column: silica-based bonded-phase C_{18}.
Mobile phase: methanol–water.
Additive agent: small amount of phosphoric acid or acetic acid.
Possibility factor: 0.9702.
Explanation: This kind of sample can be separated by RP-HPLC ($0.4 < k' < 30$). C_{18} is a common packing material for RP-HPLC. Methanol–water is sufficient to separate most samples. The acids can dissociate in RP-HPLC; the additive agent can prevent the dissociation.

Sample: saccharides of fucose, mannose and galactose.
Entry mode: recommendation from structure.
Mode of separation: (normal-phase) chromatography.
Column: silica-based bonded-phase NH_2.
Mobile phase: methanol–acetonitrile + suitable amount of water.
Additive agent: none.
Possibility factor: 0.8938.
Explanation: Sample is a strongly polar hydrophilic mixture, NP chromatography is suitable. Compounds have a strong acceptor ability for hydrogen bonding, NH_2 packing material should be selected.

CHROMATOGRAPHIC PEAK IDENTIFICATION

The other problem to be solved in an expert system of chromatography is peak identification. There are many methods[11–13] such as from retention values and mass and UV spectrometry that can be used in liquid chromatography. Here, we shall discuss the possibility of peak identification in liquid chromatography from retention values and from a combination of retention values with UV spectra.

Peak identification from retention values

It is generally observed in RP-HPLC that the retention values differ when column systems with the same mobile phase concentration but ODS packing material from different sources or even from the same source but from different batches are

used. This causes a difficulty in using retention data from the literature. In addition, the retention value is also affected by the nature of the mobile phase in a given column system. Therefore, how to transfer retention data from one ODS packing material to another with a given mobile phase and from one mobile phase to another with a given packing material is the first problem to be solved.

In another paper[14], the fundamental elution equation describing the effect of the mobile phase composition on k' is given as follows:

$$\ln k' = a + b \ln C_b + cC_b \quad (1)$$

where C_b is the volume fraction of the strong solvent and a, b and c are constants for a given chromatographic system. It has been observed that $b \ln C_b$ approaches a small constant value and is negligible in RP-HPLC, so that eqn. 1 can be simplified to

$$\ln k' = a + cC_b \quad (2)$$

The constant c is determined mainly by the properties of the mobile phase and approaches a constant for a certain solute with different ODS packing materials, and a is determined by the properties of the mobile and stationary phases. The constant c in

TABLE II

CALCULATED AND EXPERIMENTAL VALUES OF TRANSFERRED k' FOR 11 COMPOUNDS ON NUCLEOSIL C_{18} AT DIFFERENT CONCENTRATIONS OF METHANOL

Compound	k'	*Methanol concentration, C_b (%)*						
		95	*90*	*85*	*80*	*70*	*60*	*50*
Benzene	Exp.	0.45	0.59	0.79	1.08	1.99	3.94	7.60
	Calc.	0.42	0.58	0.80	1.10	2.08	3.93	7.43
Naphthalene	Exp.	0.72	1.02	1.51	2.29	5.29	14.16	
	Calc.	0.65	0.99	1.50	2.28	5.24	12.02	
Biphenyl	Exp.	0.85	1.29	2.03	3.40	9.24	27.90	
	Calc.	0.81	1.30	2.10	3.39	8.80	22.87	
Phenanthrene	Exp.	1.26	1.94	3.17	5.40	15.58		
	Calc.	1.15	1.92	3.20	5.33	14.82		
Anthracene	Exp.	1.32	2.08	3.47	5.91	17.29		
	Calc.	1.24	2.09	3.52	5.94	16.91		
Chrysene	Exp.	2.48	4.20	7.42	14.55	50.47		
	Calc.	2.27	4.19	7.71	14.21	48.23		
Anisole	Exp.	0.45	0.58	0.77	1.06	2.02	3.82	8.06
	Calc.	0.41	0.57	0.78	1.06	2.00	3.76	7.06
Benzyl alcohol	Exp.	0.21	0.25	0.30	0.38	0.62	1.00	1.76
	Calc.	0.20	0.26	0.33	0.42	0.67	1.10	1.79
Acetophenone	Exp.	0.33	0.39	0.48	0.63	1.09	1.93	3.76
	Calc.	0.31	0.41	0.54	0.71	1.24	2.15	3.73
p-Nitrotoluene	Exp.	0.45	0.61	0.81	1.18	2.36	5.06	11.00
	Calc.	0.40	0.57	0.81	1.16	2.34	4.72	9.54
Butyl benzoate	Exp.	0.73	1.08	1.69	2.69	7.26	21.23	
	Calc.	0.70	1.12	1.81	2.93	7.62	19.85	

the fundamental elution equation for different ODS packing materials with the same mobile phase makes it possible to transfer k' from one ODS packing material to another with different mobile phases investigated with only one isocratic experiment, from which the difference in constant a for the two kinds of ODS packing material can be determined. Table II shows data for the transfer of the capacity factor of eleven compounds from YQG-ODS to Nucleosil-ODS, where the constant c for both materials is considered to be the same and the constant a for Nucleosil-ODS can be determined by knowing constant c and $\ln k'$ after one preliminary isocratic experiment at 0.90 volume fraction of methanol on Nucleosil-ODS.

For peak identification from retention values in column systems with the same packing material and different mobile phases, a_I and c_I in the column system with mobile phase I can be correlated with a_{II} and c_{II} in the column system with mobile phase II as follows:

$$\begin{aligned} c_{II} &= c' + Bc_I \\ a_{II} &= a' + Aa_I \end{aligned} \tag{3}$$

and substituting eqn. 3 into eqn. 2, we have

$$\ln k' = C + Aa_I + Bc_I C_b \tag{4}$$

where $C = a' + Bc_b$. Eqn. 4 can be used to predict the capacity factor of the solute in the column system with mobile phase II from data for mobile phase I. Table III lists the capacity factors of some aromatic hydrocarbons transfered from a column system with a methanol–water mobile phase to one with acetonitrile–water. Serious errors in predicting retention values may be caused by differences in the π-electron interactions and hydrogen bonding energies for methanol and acetonitrile.

TABLE III

COMPARISON OF CAPACITY FACTORS MEASURED (k'_e) IN A COLUMN SYSTEM WITH ACETONITRILE–WATER AS THE MOBILE PHASE AND DEVELOSIL-ODS AS STATIONARY PHASE WITH THOSE CALCULATED (k'_c) FROM VALUES WITH METHANOL–WATER AS THE MOBILE PHASE

Solute	*Acetonitrile–water*									
	95:5		*90:10*		*80:20*		*70:30*		*60:40*	
	k'_e	k'_c	k'_e	k'_c	k'_e	k'_c	k'_e	k'_c	k'_e	k'_c
Benzene	0.43	0.47	0.62	0.61	1.03	1.06	1.72	1.84	2.84	3.19
Naphthalene	0.68	0.70	0.98	0.95	1.73	1.72	3.14	3.11	5.82	5.64
Biphenyl	0.76	0.89	1.15	1.22	2.20	2.30	4.28	4.33	8.61	8.15
Phenanthrene	1.06	1.08	2.19	2.53	3.02	3.11	5.90	5.95	12.05	11.38
Anthracene	1.38	1.14	2.08	1.58	4.11	3.02	6.55	5.78	13.55	11.05
Chrysene	1.79	1.74	2.78	2.44	5.86	4.85	9.21	9.60	19.28	19.03

Peak identification from the relationship between retention values and molecular structure parameters

Peak identification from the physico-chemical parameters of the solutes in a given column system has attracted much attention. In our expert system for chromatography, a possible means of identifying the peaks using the relationship between molecular structure parameters and retention value has been established. For samples containing only non-polar compounds, the retention value is determined only by the dispersive interaction between the solute and the mobile and stationary phases. The parameters a and c in eqn. 2 increase linearly with increasing Van der Waals volume of a solute, which can be calculated by obtaining information on the molecular structure via the user interface. For example, in a column system with acetonitrile–water as the mobile phase and YCM-phenyl as the stationary phase, the relationship between a, c and the Van der Waals volume can be expressed as

$$\begin{aligned} a &= 0.7444 + 0.0217V_w \\ c &= -1.6832 - 0.0195V_w \end{aligned} \quad (5)$$

Table IV lists the capacity factors of hydrocarbons measured experimentally and calculated from the Van der Waals volumes at different mobile phase compositions of acetonitrile–water. It can be seen from the data that the retention value data predicted from the Van der Waals volume for non-polar compounds are acceptable.

For samples containing a homologous series of compounds with the same hydrogen bonding, dipole moment and π-electron interactions, peak identification must take into account all of these interactions simultaneously. The contribution of the hydrogen bonding energy, dipole moment and π-electron interaction to the parameters a and c must be experimentally corrected for owing to the difficulty in correctly measuring the hydrogen bonding energy and the π-electron interaction. For example, for peak identification of homologous compounds such as alkylbenzenes or alcohols,

TABLE IV

COMPARISON OF EXPERIMENTAL CAPACITY FACTORS (k'_e) FOR *n*-ALKANES WITH THOSE CALCULATED (k'_c) FROM VAN DER WAALS VOLUMES AT DIFFERENT MOBILE PHASE COMPOSITIONS

Experimental data are from Hanai and Hubert[15].

Compound	*Acetonitrile–water*							
	80:20		*70:30*		*60:40*		*50:50*	
	k'_e	k'_c	k'_e	k'_c	k'_e	k'_c	k'_e	k'_c
Pentane	0.604	0.565	1.22	1.08	2.15	2.07	4.14	3.95
Hexane	0.679	0.653	1.41	1.31	2.66	2.62	5.52	5.24
Heptane	0.784	0.753	1.63	1.58	3.36	3.31	7.36	6.93
Octane	0.900	0.870	1.99	1.91	4.27	4.19	10.12	9.19
Decane	1.21	1.16	2.83	2.79	6.80	6.71	18.71	16.14
Dodecane	1.57	1.55	4.11	4.08	10.69	10.75	–	–

we can first input values of the parameters a and c for only one compound of each homologous series, *e.g.*, butylbenzene and decyl alcohol as follows:

decyl alcohol: $a = 2.0175$, $c = -2.9191$, $V_w = 113.78$;
butylbenzene: $a = 2.3356$, $c = -3.1164$, $V_w = 90.20$.

The contributions of the hydrogen bonding energy, dipole moment and π-electron interaction to the parameters a and c for these two homologues can be calculated from the linear relationship between parameters a and c, and the Van der Waals volume as shown in eqn. 5:

Alcohols:
$$\Delta a = 2.0175 - 0.7444 - (0.0217 \cdot 113.78) = -1.196$$
$$\Delta c = -2.9191 + 1.6832 + (0.0195 \cdot 113.78) = 0.9828$$

Alkylbenzenes:
$$\Delta a = 2.3556 - 0.7444 - (0.0217 \cdot 90.20) = -0.3468$$
$$\Delta c = -3.7164 + 1.6832 + (0.0195 \cdot 90.20) = 0.3257$$

The contributions of the various types of molecular interactions to the parameters a and c can now be expressed as follows:

Alcohols:
$$a = 0.7444 + 0.0217V_w - 1.1967 = -0.4516 + 0.0217V_w$$
$$c = -1.6832 - 0.0195V_w + 0.9828 = -0.7004 - 0.0195V_w \quad (6)$$

Alkylbenzenes:
$$a = 0.7444 + 0.0217V_w - 0.3468 = 0.3976 + 0.0217V_w$$
$$c = -1.6832 - 0.0195V_w + 0.3257 = -1.3575 - 0.0195V_w \quad (7)$$

The data in Table V are values of the parameters a and c calculated from eqns. 6 and 7 for compounds in these homologous series. Table VI lists experimentally measured capacity factors *versus* those calculated from a and c shown in Table V with different acetonitrile–water mobile phase compositions. Comparing the calculated and experimentally measured capacity factors, the agreement is very close, hence the method developed for peak identification of homologous compounds is acceptable.

Peak identification from the interaction index

For samples containing compounds with different polar functional groups, it is difficult to identify chromatographic peaks by predicting the retention value from the molecular structure parameters because it is difficult to describe correctly the contributions of the hydrogen bonding. π-electron interaction and the molecular configuration on the retention value. These contributions must be determined experimentally in order to identify a chromatographic peak. As mentioned above, the parameter c in the fundamental elution equation reflects the mutual interaction forces

TABLE V

PARAMETERS a AND c FOR HOMOLOGUES OF n-ALKYL ALCOHOLS AND ALKYLBENZENES CALCULATED BY CORRECTING FOR CONTRIBUTIONS OF HYDROGEN BONDING, DIPOLE MOMENT AND π-ELECTRON INTERACTION

Experimental data are from Hanai and Hubert[15].

Compound	*a*	*c*	*Compound*	*a*	*c*
Butyl alcohol	0.6855	−1.722	Toluene	1.699	−2.518
Pentyl alcohol	0.907	−1.922	Ethylbenzene	1.911	−2.717
Hexyl alcohol	1.129	−2.121	Propylbenzene	2.133	−2.917
Heptyl alcohol	1.351	−2.269	Butylbenzene	2.356	−3.116
Octyl alcohol	1.573	−2.520	Hexylbenzene	2.799	−3.515
Decyl alcohol	2.018	−2.919	Heptylbenzene	3.021	−3.715
Dodecyl alcohol	2.461	−3.318	Octylbenzene	3.243	−3.914
Tetradecyl alcohol	2.905	−3.717	Nonylbenzene	3.469	−4.114
Hexadecyl alcohol	3.350	−4.116	Decylbenzene	3.687	−4.313

between the solute and the mobile phase and is a constant for a given mobile phase. In our expert system for chromatography, the parameter c is defined as the "interaction index" and is used to identify the peak. The interaction index can be separated into

TABLE VI

COMPARISON OF EXPERIMENTALLY MEASURED CAPACITY FACTORS (k'_e) WITH THOSE CALCULATED FROM THE PARAMETERS a AND c (k'_c) SHOWN IN TABLE V AT DIFFERENT MOBILE PHASE COMPOSITIONS

Compound	*Acetonitrile–water*							
	80:20		*70:30*		*60:40*		*50:50*	
	k'_e	k'_c	k'_e	k'_c	k'_e	k'_c	k'_e	k'_c
Toluene	0.47	0.48	0.84	0.88	1.51	1.49	2.69	2.64
Ethylbenzene	0.55	0.56	1.02	0.95	1.91	1.88	3.57	3.52
Propylbenzene	0.63	0.64	1.23	1.26	2.41	2.36	4.73	4.75
Butylbenzene	0.73	0.74	1.49	1.50	3.06	2.95	6.27	6.42
Hexylbenzene	0.97	0.97	2.18	2.13	4.90	4.59	11.0	11.6
Heptylbenzene	1.12	1.11	2.63	2.54	6.19	5.80	14.6	15.6
Octylbenzene	1.29	1.27	3.19	3.04	7.85	7.29	19.3	21.1
Nonylbenzene	1.51	1.45	3.89	3.60	10.0	9.09	25.8	28.1
Decylbenzene	1.72	1.66	4.65	4.30	12.6	11.4	33.9	36.0
Butyl alcohol	0.20	0.18	0.30	0.30	0.45	0.40	0.67	0.54
Pentyl alcohol	0.23	0.22	0.37	0.36	0.57	0.54	0.88	0.76
Hexyl alcohol	0.27	0.26	0.44	0.44	0.72	0.69	1.17	1.07
Heptyl alcohol	0.34	0.31	0.58	0.55	0.98	0.88	1.65	1.47
Octyl alcohol	0.36	0.36	0.64	0.65	1.15	1.12	2.06	2.01
Decyl alcohol	0.48	0.49	0.94	0.95	1.82	1.78	3.61	3.70
Dodecyl alcohol	0.64	0.65	1.38	1.36	2.95	2.81	6.34	6.64
Tetradecyl alcohol	0.85	0.87	2.01	1.93	4.73	4.38	11.1	11.8
Hexadecyl alcohol	1.14	1.15	2.94	2.79	7.59	6.88	19.6	21.2

TABLE VII

VALUES OF V_w, μ_A^2 AND X_n FOR SOME SOLUTES AND COMPARISON OF THE CALCULATED INTERACTION INDICES WITH THOSE MEASURED IN A METHANOL–WATER MOBILE PHASE

Solute	V_w	μ_A^2	X_n	*c(exp.)*	*c(calc.)*	*Error*
Benzene	48.36	0.0	0.0	− 6.52	− 6.63	−0.11
Chlorobenzene	57.48	2.89	−0.76	− 8.01	− 7.85	0.16
Toluene	59.51	1.11	0.0	− 7.53	− 7.55	−0.02
p-Xylene	70.66	0.01	0.0	− 8.66	− 8.57	0.09
Phenol	52.83	4.93	−0.43	− 6.08	− 6.24	−0.16
Nitrobenzene	62.64	15.8	−2.38	− 6.84	− 7.07	−0.23
1,3,5-Trimethylbenzene	81.81	0.017	0.0	− 9.70	− 9.53	0.17
1,2,4,5-Tetramethylbenzene	92.96	0.01	0.0	−10.32	−10.50	−0.18
p-Chlorotoluene	68.99	3.69	−1.05	− 9.19	− 9.07	0.12
p-Nitrotoluene	73.79	17.6	−3.03	− 7.83	− 8.59	−0.76
p-Methylphenol	63.98	5.52	−0.55	− 7.09	− 7.23	−0.14
1,4-Dichlorobenzene	67.32	0.0	−0.25	− 9.30	− 8.71	0.59
1,4-Dihydroxybenzene	59.82	1.96	0.94	− 5.37	− 5.41	−0.04
p-Chloronitrobenzene	72.12	5.66	−0.44	− 7.65	− 7.72	−0.07
p-Chlorophenol	62.31	4.84	−0.83	− 7.69	− 7.78	−0.09
p-Chloroaniline	65.86	9.0	−1.01	− 7.05	− 7.12	−0.07
1,4-Dinitrobenzene	76.92	0.0	1.46	− 6.63	− 6.38	0.25
p-Nitroaniline	70.66	37.7	−5.22	− 6.40	− 5.90	0.50
Naphthalene	73.96	0.0	0.0	− 9.09	− 8.87	0.22
Phenanthrene	98.95	0.0	0.0	−11.15	−11.03	0.12
Anthracene	102.12	0.0	0.0	−11.42	−11.30	0.12
Pyrene	109.04	0.0	0.0	−11.75	−11.91	−0.16
Chrysene	134.64	0.0	0.0	−14.07	−14.14	−0.07

non-polar, polar and non-specific interaction indices as shown in the following equation[16]:

$$c = l_1 + l_2 V_w + l_3 \mu_A^2 + l_4 X_n \tag{8}$$

where V_w and μ_A are the Van der Waals volume and dipole moment of the solute, respectively, X_n is the non-specific interaction energy including hydrogen bonding, π-electron interaction, etc., which can be obtained by comparison of a compound having non-specific interactions with a compound without these forces, and l_1, l_2, l_3 and l_4 are constants related to the physico-chemical behaviour of the mobile phase. Table VII lists the Van der Waals volume, dipole moment, X_n and the interaction index c measured and calculated for various solutes. The interaction index database has been established, but not yet completed.

By this method, the peak order in the published chromatograms stored in the chromatogram base in our expert system for various classes of compounds has been systematically examined.

Peak identification from UV spectra and retention values

UV detection is most widely used in HPLC. The combination of the UV

TABLE VIII

UV SPECTRAL PARAMETER *Sp* OF FIVE INTERMEDIATES OF SYNTHETIC DYESTUFFS UNDER DIFFERENT SEPARATION CONDITIONS IN ION-PAIR REVERSED-PHASE LIQUID CHROMATOGRAPHY

Solute	*Sp at different concentrations of methanol (v/v) in a mobile phase of methanol–buffer containing 20 mmol $(C_2H_7)_4$ NBr 7 mmol KH_2PO_4 and with pH 5.50*			
	0.30	*0.25*	*0.20*	*0.15*
Phenylamine-3-sulphonic acid	225.8	225.7	225.4	225.1
Phenylamine-2-sulphonic acid	248.5	248.3	247.0	248.0
4-Methylphenylamine-2-sulphonic acid	232.0	230.7	231.2	230.9
Naphthylamine-5-sulphonic acid	242.1	241.9	242.0	240.6
2-Naphthylamine-5-sulphonic acid	226.9	226.1	226.9	225.6
	Sp at different concentrations of the ion-pair reagent $(C_2H_7)_4$ NBr (mmol) in a mobile phase of methanol–buffer (0.3:0.7) with 7 mmol KH_2PO_4 and with pH 5.50			
	25	*20*	*10*	
Phenylamine-3-sulphonic acid	225.9	225.4	226.4	
Phenylamine-2-sulphonic acid	247.8	247.0	247.4	
4-Methylphenylamine-2-sulphonic acid	231.8	231.2	231.5	
Naphthylamine-5-sulphonic acid	242.2	242.0	241.4	
2-Naphthylamine-5-sulphonic acid	226.3	226.9	225.1	
	Sp at different pH values in a mobile phase of methanol–buffer (0.3:0.7) with 20 mmol $(C_2H_7)_4$ NBr ion-pair reagent, 7 mmol KH_2PO_4 and pH adjusted with HCl			
	5.50	*3.75*	*3.10*	
Phenylamine-3-sulphonic acid	225.8	225.7	224.5	
Phenylamine-2-sulphonic acid	248.5	252.2	252.7	
4-Methylphenylamine-2-sulphonic acid	232.0	232.0	230.4	
Naphthylamine-5-sulphonic acid	242.1	237.7	234.7	
2-Naphthylamine-5-sulphonic acid	226.9	226.4	225.4	

TABLE IX

UV SPECTRAL PARAMETERS *Sp*, PREDICTED CAPACITY FACTORS AND THE COMPOUND NAME IDENTIFIED BY THE ESC

Peak No.	*Sp*	*k'*	*Compound name*
1	208.9	1.24	1,4-Dinitrobenzene
2	196.0	2.24	Anisole
3	201.4	2.70	4-Phenylphenol
4	203.0	5.89	Naphthalene
5	186.2	6.64	Triphenylmethanol
6	191.0	9.56	Biphenyl
7	204.1	14.0	Anthracene
8	195.6	19.1	Pyrene
9	215.1	27.7	Chrysene

spectrum and the retention value is one way to identify a chromatographic peak on-line. In our expert system for chromatography, a programmable multi-wavelength UV detector has been installed, making it possible to measure the UV spectrum of a solute on-line. An identification parameter P of the UV spectrum is defined as:

$$P = Sp \cdot area \tag{9}$$

$$Sp = \frac{\sum_{i=0}^{n} \varepsilon_{i(\lambda_i)} \lambda_i}{\int_{\lambda_0}^{\lambda_n} \varepsilon_{i(\lambda)} d\lambda}$$

where *area* is the area of the UV spectrum, λ and ε are the wavelength and corresponding absorption coefficient and the summation is over a number of different wavelengths. Light is detected over the range λ_0–λ_n. For a given solute, the P and *area* are affected by many factors such as mobile phase composition, pH and amount of solute. However, Sp is determined only by ε and λ, and for a fixed wavelength range Sp is a spectral parameter indicative of the absorption of light by the solute. In a certain region, Sp is not affected by the red or blue shift of the UV spectrum with changing physico-chemical behaviour of the solution, and therefore is important for peak identification in HPLC. The reliability of the use of Sp has been investigated in ion-pair reversed-phase liquid chromatography by changing the mobile phase composition, the concentration of the ion-pair reagent and the pH value for 28 intermediates in the synthesis of dyestuffs. Table VIII lists values of Sp obtained under various separation conditions for five compounds in ion-pair reversed-phase liquid chromatography.

Sp values for the 28 synthetic dyestuff intermediates and 20 aromatic compounds have been stored in the on-line peak identification module. Table IX lists experimentally measured values of Sp and k' for nine aromatic compounds.

CONCLUSION

We have discussed the development of a module for the selection of the separation mode, the stationary phase and the mobile phase and possible approaches to a peak identification module for an HPLC expert system. In order to widen its range of application, the rules and methods for these modules should be further tested, corrected and expanded.

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