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Note

Automated optimization of reversed-phase liquid chromatographic separations using a computer-assisted retention prediction

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During the past decade, high-performance liquid chromatography (HPLC), especially reversed-phase HPLC (RPLC), has developed rapidly and is now accepted as a reliable and versatile analytical tool for separation and quantitation of mixtures soluble in liquids. A wide variety of commercially available instruments completely satisfy the requirements of most routine analytical and semi-preparative separations. However, in practical separations, finding a good combination of mobile phase and stationary phase is usually the most difficult and time-consuming task which faces the chromatographers. This task has traditionally been carried out by trial and error, based on personal experience and intuition. Therefore, many attempts have been made systematically to optimize separation conditions[1–6].

One such method is to predict the retention behaviour as a function of mobile phase composition using theoretical models[7–9]. In order to predict the retention of any solute, a clear understanding of the retention mechanism is required. In recent years, much effort has been directed to investigating the mechanism of solute retention in RPLC, chromatographically and/or in combination with spectroscopic measurements[10–12]; great progress has been made. At present, "solvophobic theory" introduced by Horváth and co-workers[13–15] is generally acknowledged as one of the most consistent theories to describe solute distribution phenomena in RPLC. According to this entropically driven interaction model, it is anticipated that physicochemical parameters such as solute surface area, partition coefficient between two immiscible phases and aqueous solubility may be correlated with the retention in RPLC. Quantitative structure–retention relationships (QSRRs)[16,17] may be useful in predicting retention.

Based on the QSRR studies of aromatic compounds on an octadecyl silica (C_{18}) stationary phase[18–20], we have been investigating a computer-assisted system for retention prediction (RPS)[21–23]. In this system, the chromatogram of any aromatic solute under chosen conditions such as mobile phase composition, flow-rate, etc., can be predicted with the aid of a microcomputer. In addition, the RPS can be modified so as to yield the optimum separation condition for the solutes of interest.

The aim of this paper is to demonstrate the performance of the RPS in automated optimization of separation conditions in RPLC.

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EXPERIMENTAL

The liquid chromatographic system consisted of a microfeeder MF-2 (Azuma Electric, Co., Tokyo, Japan) as a pump and a Uvidec-100 II UV detector (Jasco, Tokyo, Japan) set at 210 nm. The column temperature was maintained at 20 ± 0.1°C by a DW-620 thermostat (Komatsu, Tokyo, Japan).

The reversed-phase column was a PTFE tubing (12 cm × 0.5 mm I.D.) packed with Jasco FineSIL C_{18} (5 μm). The mobile phase comprised HPLC grade acetonitrile (Kanto Chemicals, Tokyo, Japan) and purified water. All of the test substances were commercially available products from many sources.

The computer system was a 16-bit microcomputer NEC PC-9800 (Nippon Electric, Co., Tokyo, Japan), and the programs were written in BASIC.

RESULTS AND DISCUSSION

From QSRR studies on alkylbenzenes[18], polycyclic aromatic hydrocarbons (PAHs)[19] and substituted benzene derivatives[20], the retention descriptions shown in Table I had already been obtained. In the equations, X is the volume fraction of organic modifier in the mobile phase, log P is the partition coefficient in 1-octanol–water[24] and a measure of the hydrophobicity of a solute, F_1 is the correlation factor proposed by Schabron *et al.*[25], π is a hydrophobic substituent constant[26], σ is a Hammett's constant[26] and (HA-HD) represents the proton accepting ability of a solute molecule as suggested by Hansch and Leo[26]. If X and the parameters characteristic of a solute are known, the logarithm of the capacity factor, log k', can be determined for given chromatographic conditions. The RPS is constructed on the basis of this concept.

In this investigation, the C_{18} microcolumn was used as a typical example. For constructing the RPS, several standard materials (four or five substances from each group of compounds) were analyzed and their capacity factors determined at various mobile phase compositions. Then the retention descriptions shown in Table I for each data set were obtained as a function of X by multi-regression analyses. These descriptions were sufficient to predict the retention of any solute at given chromatographic conditions, which is the main function of the RPS[23]. However, the another function of the RPS which is the purpose of this work, *i.e.*, to yield the optimum separation conditions for any solutes of interest, requires knowledge of N, the theoretical plate number of the column used, because in order to control the separation

TABLE I

RETENTION DESCRIPTIONS FOR AROMATIC COMPOUNDS ON A C_{18} COLUMN AS A FUNCTION OF ORGANIC MODIFIER CONCENTRATION IN THE MOBILE PHASE

$f_{ai}(X)$, $f_{si}(X)$ and $f_{pi}(X)$ are functions of X where i = 1, 2 and 3.

Compound group	*Retention description*
Alkylbenzenes, PAHs	$\log k' = f_{a1}(X) \log P + f_{a2}(X) F_1 + f_{a3}(X)$
Substituted benzenes except phenols	$\log k' = f_{s1}(X)\pi + f_{s2}(X)$ (HA−HD) $+ f_{s3}(X)$
Phenols	$\log k' = f_{p1}(X)\pi + f_{p2}(X)\pi\sigma(1 - \pi) + f_{p3}(X)$

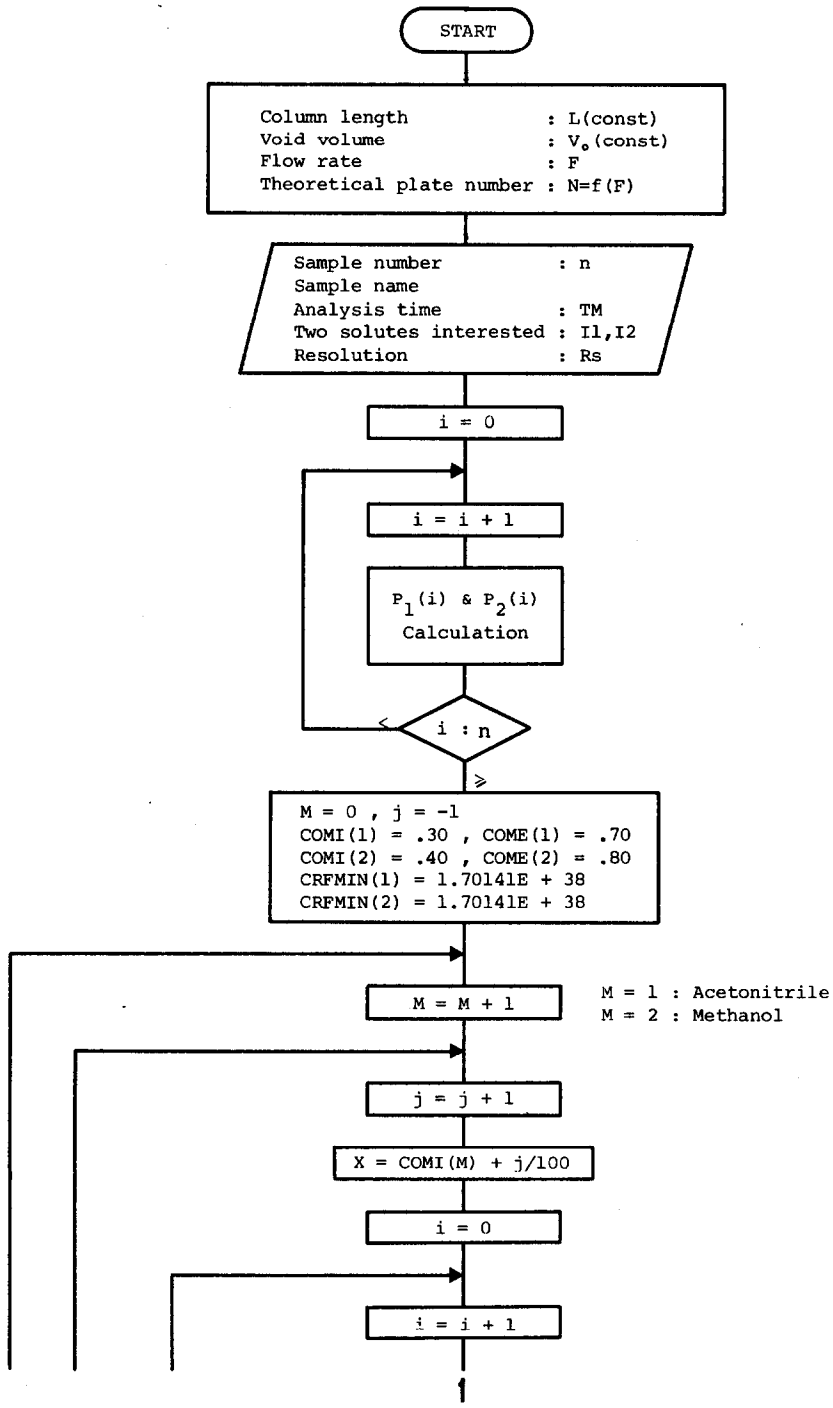
START
Column length : L(const)
Void volume : V_o(const)
Flow rate : F
Theoretical plate number : N=f(F)
Sample number : n
Sample name
Analysis time : TM
Two solutes interested : I1,I2
Resolution : Rs
i = 0
i = i + 1
P_1(i) & P_2(i) Calculation
i : n
<
≥
M = 0 , j = -1
COMI(1) = .30 , COME(1) = .70
COMI(2) = .40 , COME(2) = .80
CRFMIN(1) = 1.70141E + 38
CRFMIN(2) = 1.70141E + 38
M = M + 1
M = 1 : Acetonitrile
M = 2 : Methanol
j = j + 1
X = COMI(M) + j/100
i = 0
i = i + 1

Fig. 1.

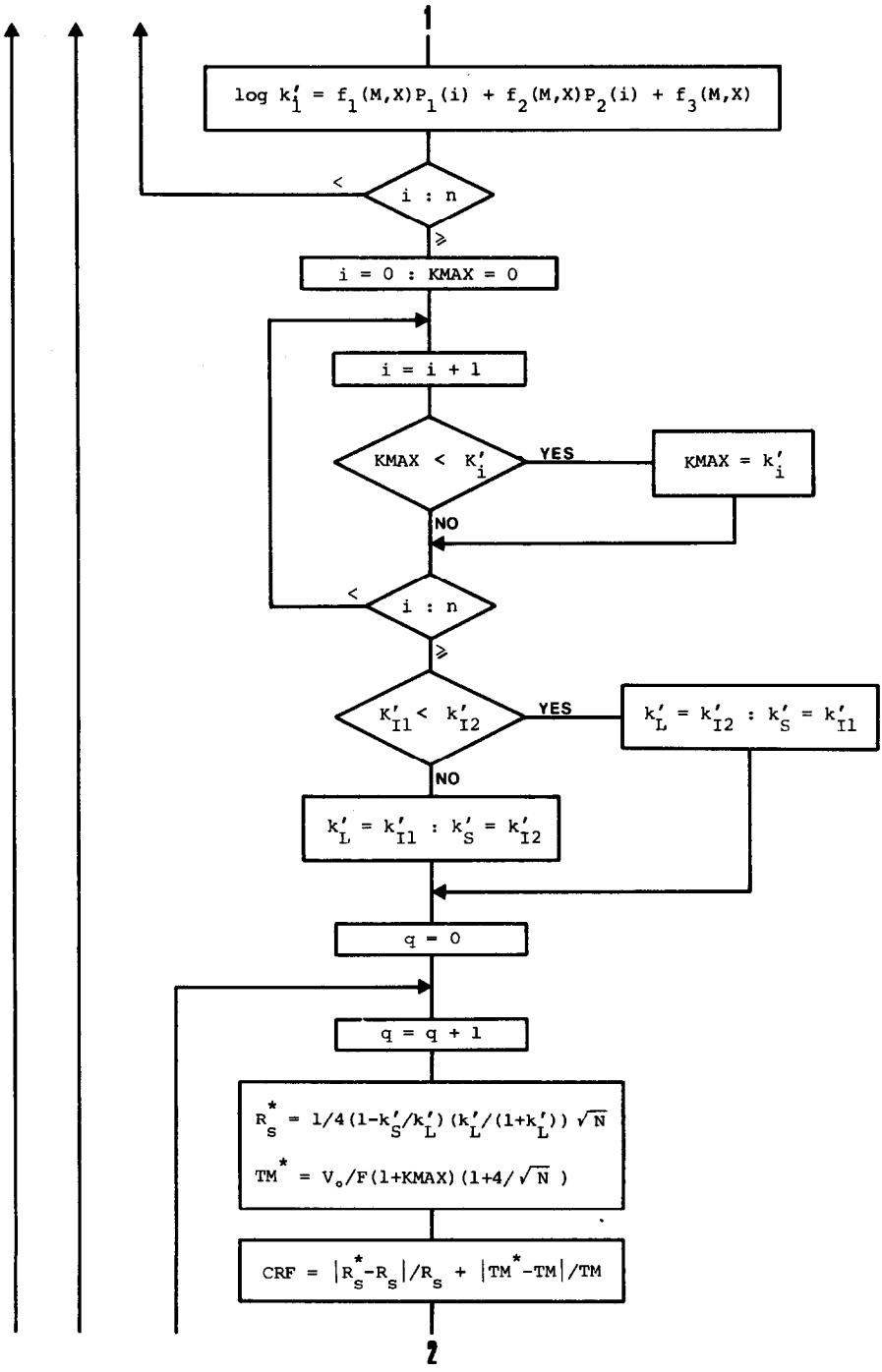

Fig. 1.

(Continued on p. 330)

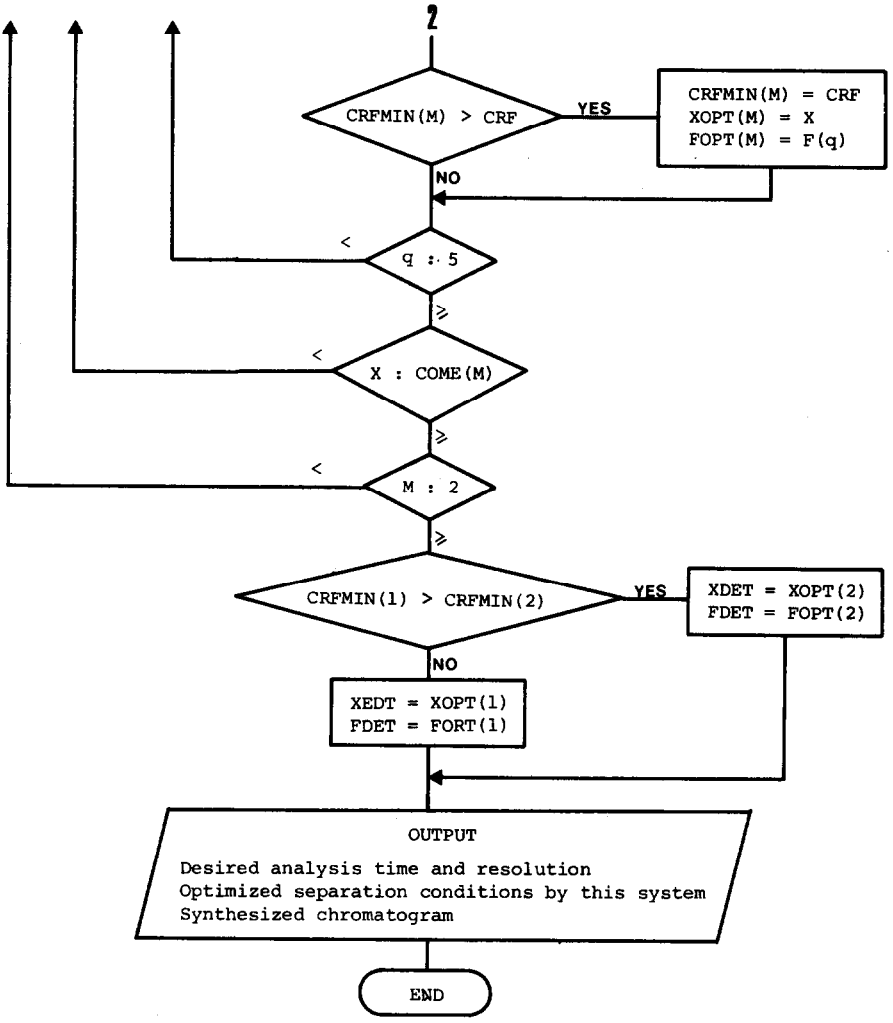

Fig. 1. Flow-chart for the optimization of separation conditions in RPLC.

or resolution, R_s, we must know how R_s varies with experimental parameters such as k' and N. R_s for two closely spaced bands can be expressed as[27]

$$R_s = (1/4)\,(1 - k'_S/k'_L)\,\sqrt{N}[k'_L/(1 + k'_L)] \tag{1}$$

where k'_S and k'_L are the capacity factors for bands S and L, respectively. Although N is generally a function of the linear velocity of the mobile phase, an approximate relationship for the column used was derived from some experiments

$$N = 3482/F^{0.4} \tag{2}$$

where F is the mobile phase flow-rate.

The flow-chart of this function of the RPS is shown in Fig. 1.

When the RPS is used to obtain the optimum separation conditions, the following data should be input interactively after accessing the function on the CRT of the computer; (i) the names or the chemical formulae of interesting solutes; (ii) the analysis time requested; (iii) the resolution for two interesting solutes.

Upon input of the compound names or chemical formulae, the computer calculates suitable descriptors for the compounds and then capacity factors for the solutes at various mobile phase compositions are predicted step-by-step with an interval of $X = 0.01$ for both aqueous acetonitrile and methanol mobile phases. In this instance the range of X is from 0.3 to 0.7 for the acetonitrile system and from 0.4 to 0.8 for the methanol system. After calculation of the capacity factors for the desired solutes, the resolution, R_s, for each step is estimated at five different flow-rates such as 1, 2, 4, 8 and 16 μl/min in order to find the conditions under which the most strongly retained solute is eluted in the requested analysis time and with the resolution closest to that desired. These calculations are performed by the use of the equation

$$\mathrm{CRF} = |\mathrm{TM} - \mathrm{TM}^*|/\mathrm{TM} + |R_S - R_S^*|/R_S \quad (3)$$

where CRF is the chromatographic response function, TM is the analysis time, R_S is the resolution and the superscript * means calculated, *i.e.*, predicted value. The conditions closest to those requested should be obtained when the value of CRF is minimal. At this stage, the user can make choose whether resolution or analysis time is to take priority. In the case of resolution, the first term of eqn. 3 should be given zero weighting, while the second term should be so weighed if analysis time is to be given priority. With these calculations, the RPS can find the optimum separation conditions for the desired solutes, and then yields the corresponding numerical values of the mobile phase composition, flow-rate, analysis time and resolution; finally it draws the idealized chromatogram under the optimum separation conditions.

To demonstrate the potential of the RPS, two experiments were performed. In Fig. 2 is shown the separation of a test mixture of aniline derivatives. The chemical formulae of three aniline derivatives such as aniline, N-methylaniline and N-ethylaniline were input and then an analysis time of 10 min and a resolution for N-methyl- and N-ethylanilines of 1.2 were requested. The RPS responded by predicting the retention data of the three compounds, under the conditions desired by user. It also predicted that an analysis time of 11 min and a resolution of 1.1 could be obtained with 65% acetonitrile as the mobile phase and at a flow-rate of 4 μl/min. The predicted chromatogram appeared on the CRT and was printed out on a printer connected with the computer. An analysis was then carried out experimentally at the optimum separation conditions predicted by the RPS. The actual analysis time for the separation was found to be 11.5 min, in excellent agreement with that predicted. In the second example a mixture was prepared containing six alkylbenzenes. The same procedure was performed and the RPS yielded the optimum separation conditions. Reference to Fig. 3 indicates an optimum at *ca.* 67% acetonitrile as mobile phase and a flow-rate of 4 μl/min. The chromatogram obtained experimentally at 65% acetonitrile and a flow-rate of 4 μl/min shows the retention time of the last eluted peak to be 31 min, compared with the predicted time of 29 min; this difference

```
***************************** COMPOUND NAME INPUT ****************************

1. alkylbenzenes(example:C6H5CH3) ? 0


2. polycyclic aromatic hydrocarbons(example:dibenz(a,c)anthracene) ? 0


3. substituted benzenes(example:C6H5NHCH3) ? 3
   No. 1  C6H5NH2
   No. 2  C6H5NHCH3
   No. 3  C6H5NHC2H5


4. phenols(example:o-methylphenol) ? 0



1. analysis time (min) ? 10            SAMPLE LIST
2. two solutes' numbers of inter-
   est ? 2,3                           No.  1  C6H5NH2
   resolution for these compounds      No.  2  C6H5NHCH3
   to be desired ? 1.2                 No.  3  C6H5NHC2H5


column   : FineSIL C18-5
column dim.: 0.5mm i.d. x 12cm
eluent   : CH3CN:H2O = 65 : 35
flow rate: 4 (ul/min)
void time: 5 (min)
theoretical plate number: 2000


************************ RETENTION DATA ************************
compound                    k'              retention time (min)
C6H5NH2                     0.65              8.26
C6H5NHCH3                   0.88              9.41
C6H5NHC2H5                  1.10             10.48
****************************************************************


****************** CONDITIONS DESIRED BY USER ******************
 analysis time : 10  (min)
 solutes of interest :C6H5NHCH3  &  C6H5NHC2H5
 resolution of these solutes to be desired : 1.2
****************************************************************


************** CONDITIONS ATTAINED IN THIS SYSTEM ***************
 analysis time : 11  (min)
 resolution to be attained : 1.1
****************************************************************
```

Fig. 2.

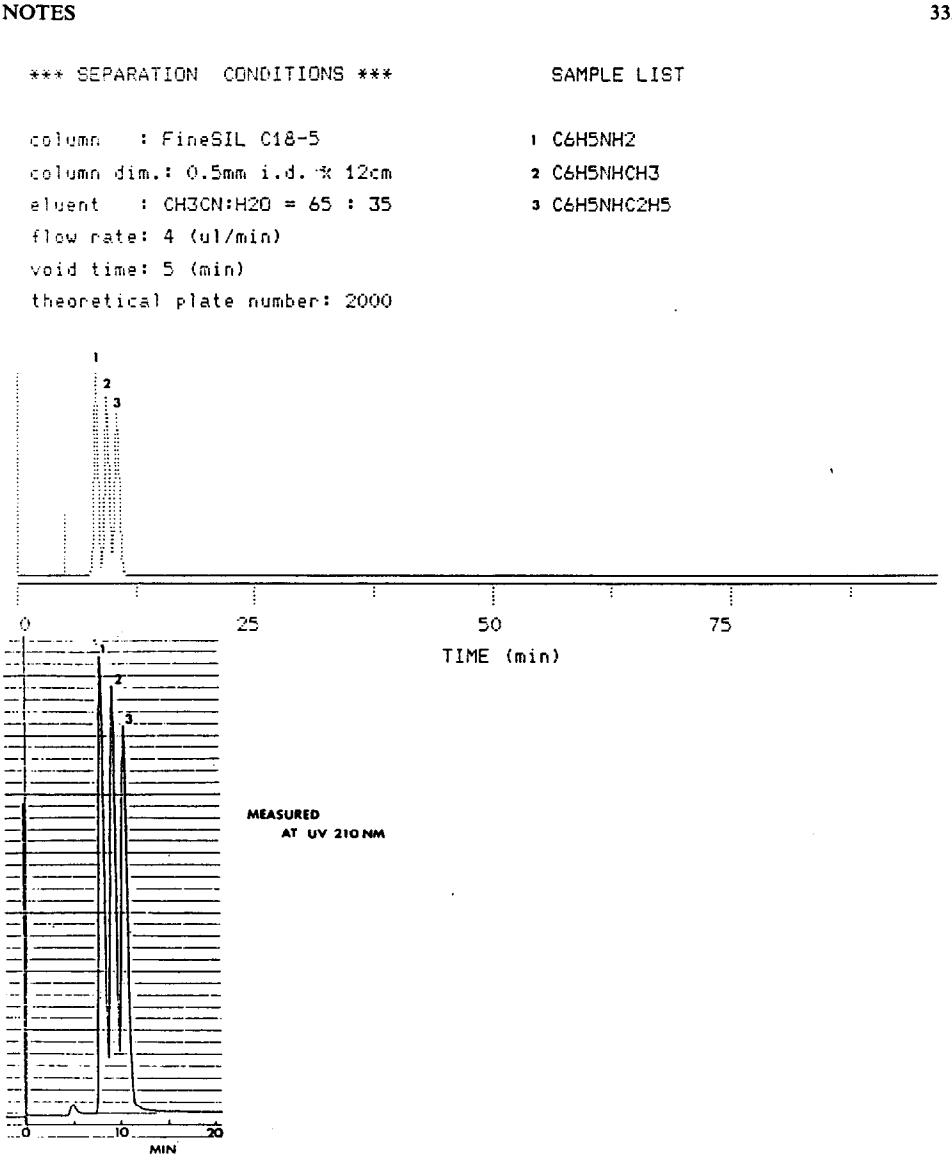

Fig. 2. Example of the optimization of the separation of aniline derivatives. Input information is underlined.

is reasonable because of the slightly lower concentration of acetonitrile in the mobile phase used experimentally.

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************************ RETENTION DATA ************************
compound                    k'              retention time (min)
C6H5CH3                     0.99             9.97
C6H5C2H5                    1.33            11.66
C6H5C3H7                    1.79            13.94
C6H5C4H9                    2.40            17.00
C6H5C5H11                   3.22            21.10
C6H5C6H13                   4.32            26.60
****************************************************************

****************** CONDITIONS DESIRED BY USER ******************
 analysis time : 30  (min)
 solutes of interest :C6H5CH3  &  C6H5C2H5
 resolution of these solutes to be desired : 1.3
****************************************************************

************** CONDITIONS ATTAINED IN THIS SYSTEM **************
 analysis time : 29  (min)
 resolution to be attained : 1.6
****************************************************************
```

```
*** SEPARATION  CONDITIONS ***          SAMPLE LIST

column    : FineSIL C18-5               1 C6H5CH3
column dim.: 0.5mm i.d. x 12cm          2 C6H5C2H5
eluent    : CH3CN:H2O = 67 : 33         3 C6H5C3H7
flow rate: 4 (ul/min)                   4 C6H5C4H9
void time: 5 (min)                      5 C6H5C5H11
theoretical plate number: 2000          6 C6H5C6H13
```

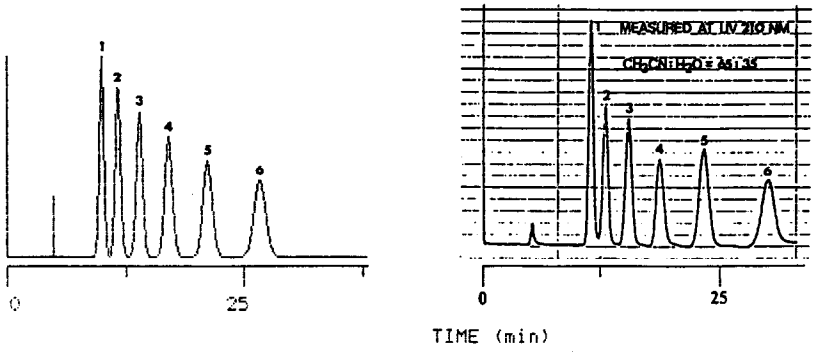

Fig. 3. Example of the optimization of the separation of six alkylbenzenes.

CONCLUSION

Two examples have been given of how the RPS can be used in automated optimization of chromatographic separations, and the optimization of reversed-phase separations has been demonstrated to be a practical reality.

In the current RPS, alkylbenzenes, PAHs and substituted benzene derivatives can be accessed to determine the optimum separation conditions with two mobile phase systems of aqueous acetonitrile and methanol. We now intend to widen the applicability of this system to other groups of compounds and more complicated mobile phase systems such as ternary solvents and/or gradient elution.

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