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MICROCOMPUTER-ASSISTED LIQUID CHROMATOGRAPHIC SEPARATION SYSTEM (MCASYST) FOR METHOD DEVELOPMENT AND DATA HANDLING

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SUMMARY

A computer-assisted liquid chromatographic separation system (MCASYST) has been developed for method development and related data handling, focusing especially on automated identification of separated components. The system has six main functions: retention prediction system, liquid chromatographic database system, automated identification system, automated optimization system for separation conditions, data loading program from a UV multi-channel detector and UV spectral database system. The performance and potential of the MCASYST system are discussed with respect to the analysis of phenylthiohydantoin-amino acids.

INTRODUCTION

Liquid chromatography (LC) has shown rapid development owing to recent progress in column and instrumental technologies and the introduction of high-efficiency columns and well designed devices including data handling systems. Unfortunately even with these advances, separation in the minimum time, under satisfactory conditions with maximum efficiency (*i.e.*, with optimized separation conditions), is not easily accomplished. In order to find the optimum separation conditions, the chromatographer first surveys information on the requirements of the particular separation. This is usually achieved by inspection of a large number of publications and data collections. The next step requires experimentation until the optimum separation conditions suitable for the particular purpose are found. This traditional procedure is usually performed by trial-and-error experiments which require experience and intuition from the investigator. Even after the proper separation conditions have been selected, the investigator still has to perform intricate qualitative and quantitative data analysis. When the analysis requires a rapid solution, as is usually the case, the aforementioned problem can often be insurmountable.

Current developments in the size and power of computers have changed the situation for analysts who have to solve complex problems. It is expected that the

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application of microcomputers will enhance the power of LC separations, especially in method development where a rapid response is required. Computer-assisted analysis can improve conventional procedures conveniently and economically. In computerized LC systems, finding the best separation conditions via manual literature searching would be replaced by retrieving information from a comprehensive LC database system which includes details of column materials, detection systems, retention information and reference information. Using an LC database system, the analyst can easily obtain the information required for his analysis if the required data have been stored in the database. The analyst then enters the optimization process in his instrumentation. In contrast to the conventional method, which is based largely on the experience and intuition of the analyst, there are several computerized methods for optimization. Some of them involve a statistical approach, such as simplex[1–3], DryLab methods as proposed by Snyder and co-workers[4–7] and a retention prediction approach based on quantitative structure–retention relationships (QSRR)[8,9]. The first two approaches require some actual experiments to optimize separations. The last can be performed without any practical experiments or chemicals after one has established the relationships between retention and selected descriptors, that is, the analyst needs to perform 'dry' experiments using computers.

For the identification of separated components, the retention prediction system[10–13] can be a useful approach. The system can also provide information on candidate compounds approximately identified in the mixture automatically[14,15]. In addition to these functions, one can predict chemical or physico-chemical properties of compounds from their retention information if necessary[8]. In this way, automation and systemization of LC analysis can be realized by the microcomputer-assisted separation system (MCASYST), which is based on the retention prediction concept developed by the authors in recent years[14–20]. The basic structure and components of this MCASYST system are illustrated schematically in Fig. 1.

The components are (1) REPRES, which can predict the retention time for particular compound groups such as polycyclic aromatic hydrocarbons[10], amino acids [11,12] or small peptides[13] (2) CIDBaS, an LC database which provides the basic information for LC separations[16], (3) ID.SYS, an automated identification system that can identify compounds automatically using the retention prediction con-

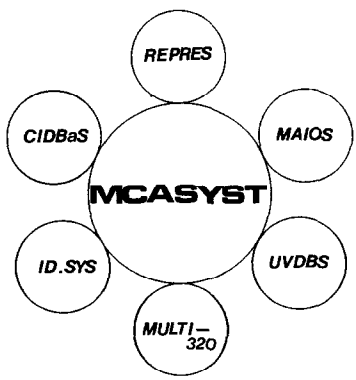

Fig. 1. MCASYST components and structure.

cept[14,15], (4) MAIOS, an automated optimization system[17,18] that optimizes separation conditions for particular compound groups such as amino acids, and offers the minimum analysis time to satisfy the maximum resolution for the compound groups desired, (5) MCASYST MULTI-320, a chromatographic data station for a multi-channel UV detection system which works as the data processor to interpret the information obtained by the multi-channel UV detector, *i.e.*, automated identification using retention and UV spectral information for the separated compounds, and (6) UVDBS, a UV spectral database system[19] that can store UV spectra taken from the literature or spectra measured in the laboratory. Employing a direct connection between this system and a computer that controls the UV spectrometer or using a digitizer via an RS232C connection, the data storage can be performed conveniently. The UV database acts as the basis of the spectral matching process to identify the components separated by LC.

In order to show the potential of the MCASYST system, the analysis of phenylthiohydantoin (PTH)-amino acids has been tested, because the demands of this analysis are very high in biotechnology and biochemistry.

EXPERIMENTAL

The LC system consisted of a Model 880-PU pump and Multi-320 multi-channel detector (Jasco, Tokyo, Japan) which allows the simultaneous detection of the UV spectral range from 195 to 350 nm at 5-nm intervals. The column used was Capcell-pak C_{18} polymer-coated silica (Shiseido, Yokohama, Japan), 250 mm × 4.6 mm I.D. at 30°C. The mobile phases were mixtures of acetonitrile, tetrahydrofuran and sodium acetate buffer and the flow-rate was 1 ml/min. Standard samples of commercially available PTH-amino acids were dissolved in acetonitrile at a concentration of 0.1 μg/ul.

The MCASYST system was constructed on an NEC PC 9801- VX2 16-bit personal computer with an MS-DOS operating system (Nippon Electric, Tokyo, Japan). The same computer was used to control the multi-channel detector.

RESULTS AND DISCUSSION

As the basics of retention prediction and automated identification by the computer-assisted system have been reported previously[10–23], the description here focuses on the utility of the MCASYST functions for the peak identification of a mixture of PTH-amino acids. However, to obtain a better understanding of the system and to pay specific attention to how to construct the retention prediction system for the Capcell-pak column, a short introduction to the retention prediction concept for PTH-amino acids will be presented.

If a highly correlated relationship between log k' (where k' = capacity factor) of PTH-amino acids and any physico-chemical properties of the compounds can be made, a database of those properties (*i.e.*, a table of descriptors) can be employed to predict the retention of these compounds using the following relationship:

$$\log k' = f(p) \tag{1}$$

where p is the descriptor, which for PTH-amino acids should be the R' values described previously[18]. As the R' values for PTH-amino acids have been determined by LC experiments with a particular stationary phase (Inertsil ODS; Gasukuro Kogyo, Iruma, Japan) in previous work[18,22], two possibilities exist for using R' values for the retention prediction of PTH-amino acids in this work: either to use R' values determined with the Inertsil ODS column or to redetermine R' values with the present column. With respect to the universal use of R' values the former is to be preferred. In fact, retention prediction with a Chemcosorb ODS column (Chemco, Osaka, Japan) has been made accurately by using R' values determined with the Inertsil column[22]. However, the difference in selectivity with different stationary phases, even though they are composed of the same octadecylsilica (ODS) bonded phases, may cause a lower accuracy in actual retention prediction results if one uses a different type of ODS phase. Both the Inertsil and the Chemcosorb are silica-based ODS, but the Capcell-pak is based on silica with a polymer coating on its surface. Generally, silanol effects should be more reduced with this material than with silica-based ODS phases. Therefore, in this work, we redetermined R' values with the Capcell-pak column, using the process to determine R' values that have been described elsewhere[11,18,22]. The re-determined R' values with the Capcell-pak are termed R^* in this paper in order

TABLE I

COMPARISON OF MEASURED RETENTION TIME WITH PREDICTED DATA FOR 20 PTH-AMINO ACIDS BY USING R^* VALUES

Column, Shiseido Capcell-pak C_{18}; mobile phase, acetonitrile–THF–buffer (40.0:1.2:58.8); column temperature, 30°C.

PTH-amino acid	R^*[a]	*Retention time (min)*		*Relative error (%)*
		Measured	*Predicted*	
L-Aspartic acid	37.33	2.93	2.93	0
L-Glutamic acid	38.13	2.87	2.89	−0.7
L-Arginine	33.66	3.05	3.17	−3.9
L-Histidine	32.15	3.22	3.29	−2.2
L-Asparagine	32.49	3.22	3.26	−1.2
L-Glutamine	30.04	3.27	3.47	−6.1
DL-Serine	29.41	3.39	3.53	−4.1
DL-Glycine	24.36	4.09	4.13	−1.0
DL-Alanine	19.01	4.96	5.05	−1.8
L-Tyrosine	16.79	5.30	5.55	−4.7
δ-Threonine	12.46	6.74	6.77	−0.4
DL-Valine	8.73	8.18	8.17	0.1
L-Proline	8.66	8.35	8.20	1.8
DL-Methionine	8.94	8.06	8.08	−0.2
DL-Norvaline	7.24	9.10	8.84	2.9
DL-Isoleucine	2.30	12.3	11.6	5.7
DL-Tryptophan	4.05	9.97	10.5	−5.3
L-Phenylalanine	2.34	11.1	11.6	−4.5
L-Leucine	1.00	12.8	12.6	1.6
L-Lysine	1.34	11.7	12.3	−5.1

[a] R^* values were determined with the Capcell-pak column.

to differentiate them from the R' values determined with the Inertsil ODS column. The R^* values are summarized in Table I.

The retention prediction equation with the Capcell-pak column was determined in several experiments using five standard PTH-amino acids as described previously[11-23]. The equation was regressioned with respect to the mobile phase composition, here the acetonitrile concentration X in the mobile phase. The equation obtained is

$$\log k' = (1.126 \cdot 10^{-3} X - 0.0757) \cdot R^* - 0.0490X + 2.680 \quad (2)$$

The performance of this equation was tested by comparison of the measured retention data for 20 PTH-amino acids and the values predicted by this equation for a mobile phase containing 40% acetonitrile. The results are given in Table I. The accuracy of the retention prediction is excellent, *i.e.*, within 6%. The results obtained using R' values determined with the Inertsil ODS column are given in Table II. It appears that the prediction accuracy using R^* is naturally higher than that using R' values. R^* values were therefore incorporated in the MCASYST system as the basic database of the retention descriptor for PTH-amino acids with the Capcell-pak column.

To identify the unknown peaks that appeared in the chromatogram of a PTH-

TABLE II

COMPARISON OF MEASURED RETENTION TIME WITH PREDICTED DATA FOR 20 PTH-AMINO ACIDS BY USING R' VALUES

Column, Shideido Capcell-pak C_{18}; mobile phase, acetonitrile–THF–buffer (40.0:1.2:58.8); column temperature, 30°C.

PTH-amino acid	R'^a	*Retention time (min)*		*Relative error (%)*
		Measured	*Predicted*	
L-Aspartic acid	37.35	2.93	2.86	2.4
L-Glutamic acid	34.13	2.87	3.06	−6.6
L-Arginine	30.61	3.05	3.34	−9.5
L-Histidine	29.34	3.22	3.46	−7.4
L-Asparagine	29.06	3.22	3.49	−8.4
L-Glutamine	27.40	3.27	3.67	−12.2
DL-Serine	26.94	3.39	3.72	−9.7
DL-Glycine	22.02	4.09	4.43	−8.3
DL-Alanine	18.21	4.96	5.19	−4.6
L-Tyrosine	14.84	5.30	6.07	−14.5
δ-Threonine	12.26	6.74	6.91	−2.5
DL-Valine	9.51	8.18	8.01	2.1
L-Proline	9.45	8.35	8.04	3.7
DL-Methionine	9.32	8.06	8.09	−0.4
DL-Norvaline	8.26	9.10	8.59	5.6
DL-Isoleucine	4.22	12.3	10.9	11.4
L-Tryptophan	3.65	9.97	11.3	−13.3
L-Phenylalanine	3.58	11.1	11.3	−1.8
L-Leucine	3.01	12.8	11.7	8.6
L-Lysine	1.00	11.7	13.3	−13.7

[a] R' values were determined with the Inertsil ODS column.

amino acids mixture, the several functions of the MCASYST should be employed. The first is CIDBaS for the information survey on the separation of PTH-amino acids, the second is to use the function of MAIOS for optimization of the separation conditions with the most suitable LC separation systems found by the CIDBaS system, the third is to utilize the MCASYST MULTI-320 function to facilitate the retention data and UV spectral data supplied from the multi-channel detector and the fourth is to access ID.SYS for automated identification and UVDBS for the UV spectral matching process. The basic algorithm is shown in Fig. 2.

In order to find LC systems suitable for the separation of PTH-amino acids, the first step in the analysis is the search of the LC database (CIDBaS). The search was performed by the retrieval key 'AMINACD' (abbreviation for amino acids). The CIDBaS now has 600 data with chromatograms and 1200 data without chromatograms. The search found 57 amino acid information items in the total stored data, from which information about PTH-amino acids was found for only three of the retrieved 57 data. The three retrieved data for the PTH-amino acids are shown in Fig. 3. The stationary phases used to obtain the published data were dimethylphenethyl, PTH-AA and octadecylsilica for data Nos. 77, 79 and 125, respectively. The most likely to be used in this investigation is the information of data No. 125, where a microcolumn packed with octadecylsilica was used under isocratic conditions with a mobile phase containing acetonitrile, tetrahydrofuran (THF) and sodium acetate buffer. As we had available an octadecyl column supplied by Shiseido and an LC pump for isocratic elution, it was decided that the approach for the analysis should use this column and a ternary mobile phase system of acetonitrile–THF–sodium acetate buffer.

The second step is the optimization of the LC system selected by the database search. The optimization criterion used in this system is the maximization of the sum of resolutions for each pair of solutes in the shortest analysis time. The function of MAIOS in the MCASYST system was utilized and the column information for Capcell-pak was retrieved from the column database previously stored in the MCASYST as demonstrated in Fig. 4. The MAIOS function was then applied with the desired condition of a 20-min analysis time for PTH-amino acids. The output from the operation of the MCASYST is shown in Fig. 5. The optimum condition for PTH-amino

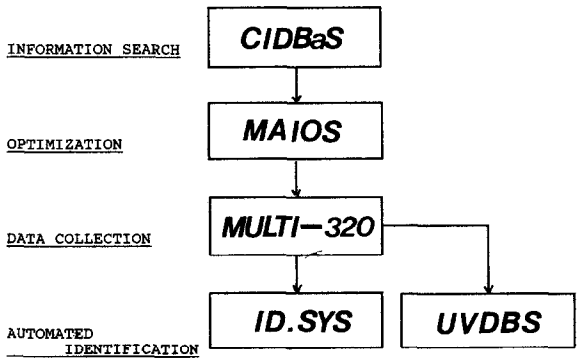

Fig. 2. Algorithm for use of MCASYST for PTH-amino acid separation.

A

```
********************************** No. 77 ********************************
compound            : PTH-amino acids
matrix              :
column              : dimethylphenethyl
separation mode     : REVERSED PHASE
column length       : 80 (mm)                column diameter: 6.2 (mm)
elution mode        : GRADIENT (CONTINUOUS)
mobile phase        : phosphoric acid + NaOH 4.0% to 16.5%, acetonitrile 5.5%
                       to 23.8%, tetrahydrofuran 90.5% to 59.7%.
flow-rate (ml/min): 2                        temperature (°C): 35
sample size         :
detector            : ULTRAVIOLET ···  0 (nm)
remarks             : RETENTION TIME= 25 (min)
authors             : J.L.Glajch  J.J.Kirkland
coden(year)         : JCHSBZ( 1986  )
journal/title       : J.Chromatogr.Sci.,25,4 / Mobile-Phase Optimization in G
                      radient-Elution HPLC for the Separation of the Phenylth
                      iohydantoin-Amino Acids.
comment             : optimization for PTH-amino acids analysis.  4 chromatog
                      rams
*UP ARROW:PREVIOUS SCREEN/RETURN:Next screen/*:Get this Cgram/HELP:Main menu*
```

```
#### CHROMATOGRAM ###

File Index NO.                77
Comment                        AMINACD

Theoretical Plate Number  50000 counts
Chart Speed                 6.9 mm/min
```

NO.	RETENTION	ABS	COMPOUND
1	3.200	0.363	Carboxymethyl cysteine
2	6.100	0.816	Asparagine
3	7.000	0.539	Histidine
4	7.400	0.600	Serine
5	7.800	0.713	Glutamine
6	8.050	0.566	Threonine
7	8.500	0.736	Glycine
8	9.600	0.461	Asparitc acid
9	10.980	0.432	Arginine
10	11.600	0.607	Alanine
11	12.450	0.484	Glutamic acid
12	15.100	0.432	Proline
13	16.250	0.482	Tyrosine
14	16.750	0.332	Valine
15	17.500	0.371	Methionine
16	19.500	0.263	Isoleucine
17	19.900	0.279	Phenylalanine
18	20.400	0.214	Leucine
19	21.200	0.266	Tryptophan
20	23.870	0.157	Lysine

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···· CHROMATOGRAM ·····
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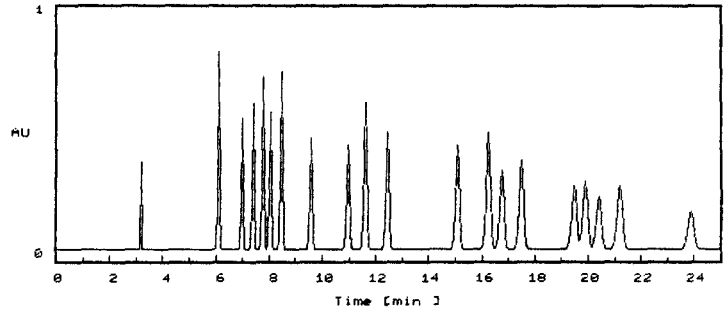

Fig. 3.

(Continued on p. 468

B

```
********************************** No. 79 ********************************
compound          : PTH-amino acids
matrix            :
column            : PTH-AA
separation mode   : REVERSED PHASE
column length     : 100 (mm)              column diameter: .54 (mm)
elution mode      : GRADIENT (CONTINUOUS)
mobile phase      : 0.01M NaOAc. CH3OH. CH3CN.
flow-rate (ml/min): .01                   temperature (°C):
sample size       : 0.004     (gram)
detector          : ULTRAVIOLET ···  270 (nm)
remarks           : RETENTION TIME= 60 (min)
authors           : F.Yang
coden(year)       : HCJCDB( 1986  )
journal/title     : J.High Resolu.Chromatogr / Chromatogr.Commun.,9,84 / Fu
                    sed-Silica Microparticulate Packed Column HPLC-Split Fl
                    ow Gradient Elution.
comment           : gradient conditions should be refered in the original.
                    4 chromatogrms
*UP ARROW:PREVIOUS SCREEN/RETURN:Next screen/*:Get this Cgram/HELP:Main menu*
```

CHROMATOGRAM

File Index NO. 79
Comment AMINACD

Theoretical Plate Number 45000 counts
Chart Speed 2.9 mm/min

NO.	RETENTION	ABS	COMPOUND
1	3.649	0.008	ASP
2	4.220	0.002	CYS
3	5.177	0.006	GLU
4	14.852	0.006	ASN
5	15.615	0.002	SER
6	17.482	0.004	THR
7	18.755	0.006	GLY.GLN
8	22.150	0.004	HIS
9	23.508	0.005	ALA
10	28.769	0.001	ARG
11	31.825	0.007	TYR
12	32.504	0.007	VAL
13	33.183	0.005	PRO
14	34.201	0.006	MET
15	37.850	0.005	ILE
16	39.802	0.004	LEU
17	41.414	0.004	PHE
18	44.809	0.004	TRP
19	51.598	0.003	LYS

···· CHROMATOGRAM ·····

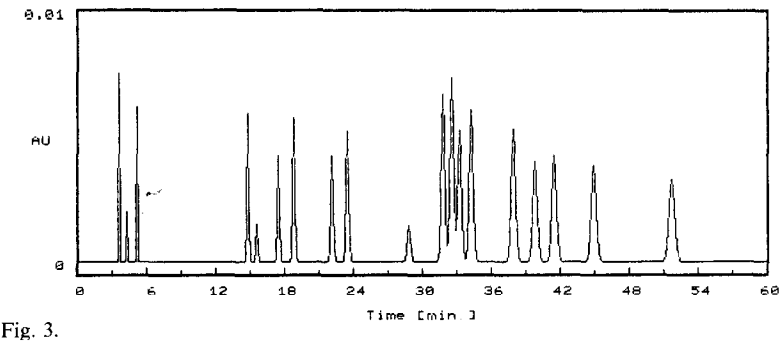

Fig. 3.

C

```
************************************ No. 125 ********************************
compound          : PTH amino acid
matrix            :
column            : ODS
separation mode   : REVERSED PHASE
column length     : 500 (mm)              column diameter: .32 (mm)
elution mode      : ISOCRATIC
mobile phase      : 30% CH3CN in THF / 0.01M sodium acetate (pH5.4) = 2 / 9
                    8.
flow-rate (ml/min): .002                  temperature (°C):
sample size       :
detector          : ULTRAVIOLET ···  254 (nm)
remarks           : RETENTION TIME= 450 (min)
authors           : M. Okamoto  K. Jinno  M. Yamagami
coden(year)       : JOCRAM( 1987  )
journal/title     : J.Chromatogr.,396,345 / Retention Behaviour of Phenylth
                    iohydantoin Amino Acid  in Micro High-Performance Liqui
                    d Chromatography with Octadecyl Bonded Glasses and Sili
                    cas.
comment           :
*UP ARROW:PREVIOUS SCREEN/RETURN:Next screen/*:Get this Cgram/HELP:Main menu*


#### CHROMATOGRAM ###

File Index NO.              125
Comment                      AMINACD

Theoretical Plate Number  17000 counts
Chart Speed                0.3 mm/min

 NO.      RETENTION      ABS      COMPOUND

  1         29.500      0.432    solvent
  2         34.243      0.591    L-aspartic acid
  3         39.286      0.303    L-glutamic acid
  4         48.810      0.338    L-arginine Hydrochloride
  5         51.191      0.932    His.Arg.Gln.Ser
  6         73.810      0.185    DL-glycine
  7        104.762      0.195    DL-a-alanine
  8        134.524      0.215    L-tyrosine
  9        169.048      0.052    delta-threonine
 10        219.048      0.172    DL-valine
 11        229.762      0.106    L-proline. DL-methionine
 12        251.190      0.123    DL-norvaline
 13        361.905      0.046
 14        376.190      0.106    DL-isoleucine
 15        384.524      0.243    DL-tryptophan
 16        390.476      0.192    L-phenylalanine
 17        427.381      0.066    L-leucine


···· CHROMATOGRAM ·····
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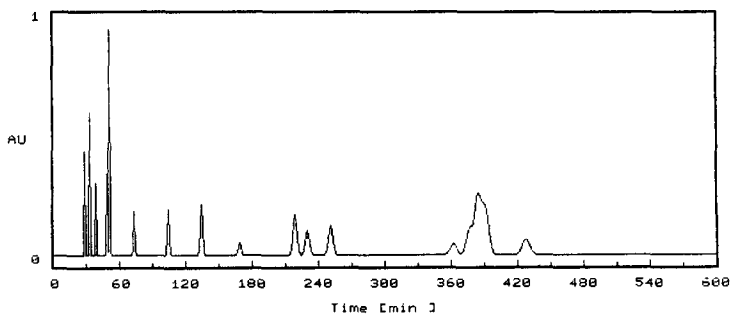


Fig. 3. Output of retrieval of CIDBaS LC database system concerning information on PTH-amino acids. (A) data No. 77; (B) data No. 79; (C) data No. 125.

```
$ DATA LIST $

RECORD NUMBER = 18

1. COLUMN       ( 35) : Shiseido Capcell-pak C18
2. MODE         (  5) : LC
3. MOBILE PHASE ( 24) : acetonitrile/buffer
4. TEMPERATURE  ( 10) : 30°C
5. FLOW RATE    ( 10) : 1 mL/min
6. PLATE NUMBER (  7) : 6000
7. DATA TABLE   ( 20) : PTH.TBL
8. COMPOUNDS    ( 20) : PTH-amino acids
6. COMMENT      (128) : M.Yamagami

EQUATION  : K(x,Q)=(1.126E-03*x-0.0757)*Q-0.0490*x+2.680

SURE (Y/N)  Y
```

Fig. 4. Output of small database for packing materials in the MCASYST. Information on the Capcell-pak C_{18} column.

acid analysis in 20 min with the Capcell-pak column is to use 35% acetonitrile in the mobile phase and the expected analysis time should be 20.2 min. Therefore, the actual analysis was performed under these conditions. The chromatogram actually obtained is shown in Fig. 6, where a mixture of several PTH-amino acids was used as a sample.

The data obtained by the multi-channel detector were transferred to the system using the MCASYST MULTI-320 function and the calculation of k' was performed in the system automatically[14]. Then ID.SYS was used to find the appropriate R^*

```
#### Retention Prediction System Version 4.0/MCASYST #### LC ###

 1. Separation Condition:
  1)Column Void Time (min)          : 2.13
  2)Number of Condition Parameters  : 1
    X(1): acetonitrile concentration value = 35.0
  3)Number of Descriptors
    D(1):R*
  4)Column                          : Shiseido Capcell-pak C18
  5)Plate Number                    : 6000
  6)Flow Rate                       : 1 mL/min
  7)Column Temperature              : 30 °C
 2. Retention Data
-------------------------------------------------------------
 NO.       COMPOUNDS          R*      Tr       k'
-------------------------------------------------------------
  1   PTH DL-methionine      8.94    11.5     4.38
  2   PTH L-proline          8.66    11.7     4.48
  3   PTH DL-norvaline       7.24    12.9     5.05
  4   PTH DL-tryptophan      4.05    16.1     6.57
  5   PTH L-phenylalanine    2.34    18.3     7.59
  6   PTH DL-isoleucine      2.30    18.3     7.61
  7   PTH L-lysine           1.34    19.7     8.25
  8   PTH L-leucine          1.00    20.2     8.48

#############################################################
```

Fig. 5. Output of MCASYST for the optimized conditions of PTH-amino acid analysis by MAIOS. The desired condition is an analysis time of 20 min.

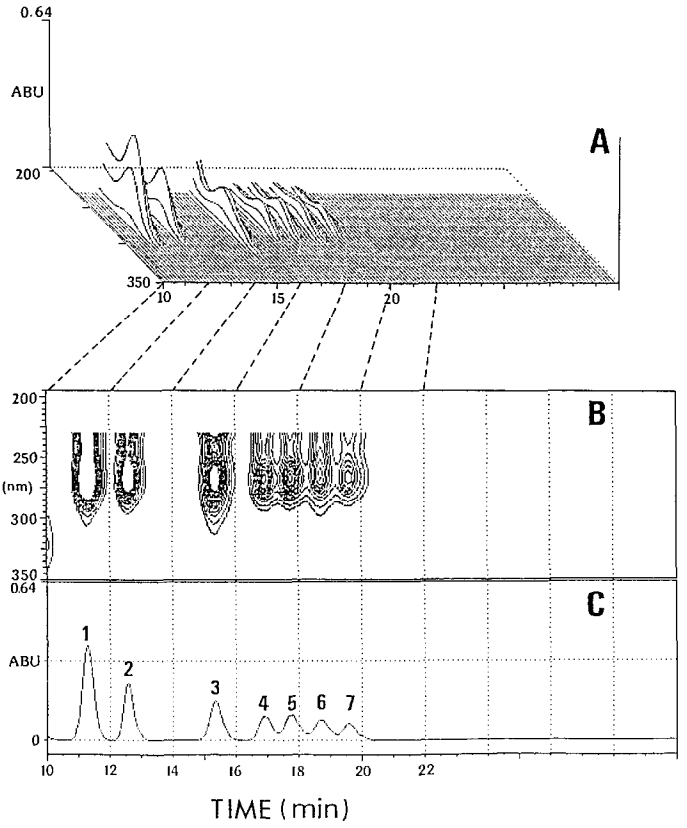
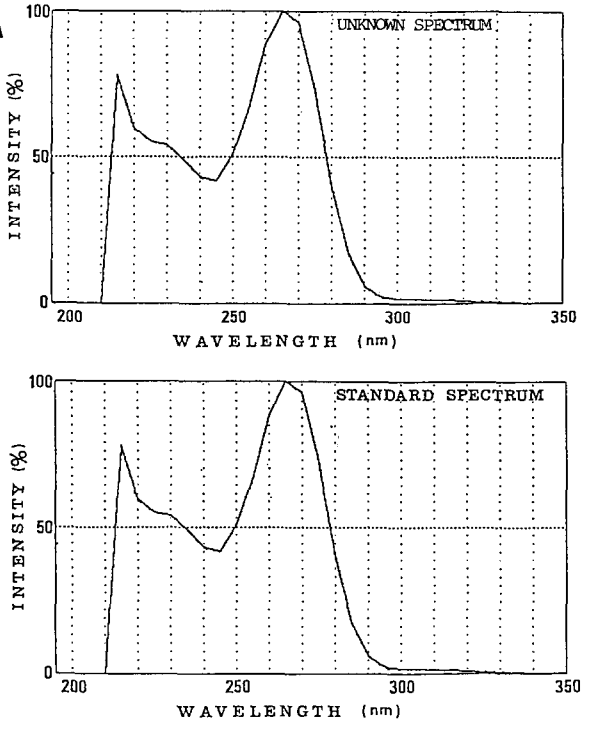


Fig. 6. Three-dimensional chromatogram of a mixture of PTH-amino acids. (A) Three-dimensional; (B) contour map; (C) typical UV chromatogram at 254 nm. Mobile phase; acetonitrile–THF–buffer (35.0:1.3:63.7).

values for the peak's k' using eqn. 2, and it found the candidates from the database of R^* for several PTH-amino acids. The candidates selected within the preset maximum permissible relative error will be listed together with the corresponding correlation coefficients, which indicate the measure of the probability of the predicted identification. As a parallel step, UV spectral matching is performed using the UVDBS function of the MCASYST for the respective peaks. The UV database can also search the standard UV spectra of PTH-amino acids stored in the database to obtain spectral matching[19].

Finally, the system decides the candidate PTH-amino acids contained in the sample solution by combining the retention prediction concept and the UV spectral matching process. Fig. 7 shows the output of the UV spectral search for peak No. 2 (retention time 12.6 min). It is indicated in the tabular output from the system shown in Fig. 7 that the component is PTH-norvaline with a high correlation coefficient of 1.00. The upper spectrum is the measured one and the lower spectrum is the standard in the UV spectral database. These two spectra are very similar, but the other candidates such as PTH-leucine, PTH-glutamic acid, PTH-serine and PTH-arginine, also

A



B

UV SPECTRAL SEARCH

NO.	COMPOUNDS	CORRELATION
1	PTH DL-norvaline	1.000
2	PTH L-leucine	0.999
3	PTH L-glutamic acid	0.999
4	PTH DL-serine	0.999
5	PTH L-arginine	0.999

Fig. 7. Output of the UV spectral search for peak No. 2 in the chromatogram in Fig. 6 at the retention time of 12.6 min. (A) UV spectrum; (B) system output.

have high correlation coefficients by the UV spectral matching process. Therefore, as it may be a false conclusion that the component at 12.6 min is PTH-norvaline, a retention prediction search process is required. The result of the retention search process for the peak is demonstrated in Fig. 8. The system indicates that this component is PTH-norvaline as the first candidate, and PTH-proline, PTH-valine and PTH-methionine are also selected as possible candidates. In comparison with the results of the UV spectral search, it seems that this component should be PTH-norvaline with a high probability, because the other compounds did not appear in either search results.

The identification of peak No. 1 in the chromatogram was performed successively. The peak seems to include at least two components because of its greater width than that of peak No. 2. Therefore, the peak deconvolution procedure which is also functioned in the MCASYST system, was applied to this peak. The result is demon-

RETENTION SEARCH

ACETONITRILE CONCENTRATION	: 35.0 vol %
RETENTION TIME OF UNKNOWN SOLUTE	: 12.6 min
COLUMN VOID TIME	: 2.13 min
CAPACITY FACTOR	: 4.90
THE MAXIMUM PERMISSIBLE ERROR	: 10.0 %

NO.	COMPOUNDS	R*value	CORRELATION
1	PTH DL-norvaline	7.24	0.837
2	PTH L-proline	8.66	0.418
3	PTH DL-valine	8.73	0.381
4	PTH DL-methionine	8.94	0.271

Fig. 8. Output of the retention search for peak No. 2 in the chromatogram in Fig. 6.

strated in Fig. 9. It appears that this peak contains two components which were eluted at 11.3 and 11.5 min, respectively. The identification of the first peak at 11.3 min was tried by the MCASYST system with ID.SYS and UVDBS. It was confirmed that the component eluted at 11.3 min is PTH-methionine or PTH-valine, and PTH-methionine is the first candidate because it received a higher probability than PTH-valine by both searching processes. In this procedure UV spectral matching was performed by using deconvoluted spectra.

The component at 11.5 min was identified as PTH-proline because the UV spectral search gave the top ranking for this solute and the retention search offered no components that were found in the spectral search except PTH-proline. The other peaks, No. 3 at 15.3 min, No. 5 at 17.8 min, No. 6 at 18.7 min and No. 7 at 19.6 min, were easily assigned by both the UV spectral search and the retention prediction search as PTH-tryptophan, PTH-isoleucine, PTH-lysine and PTH-leucine, respectively.

Peak No. 4 at 16.9 min was a problem, however. As shown in Fig. 10, the UV spectral search gave PTH-phenylalanine as the first candidate, but four other candidates also gave very high correlations. The retention prediction search also gave three candidates for this peak, such as PTH-tryptophan, PTH-phenylalanine and PTH-isoleucine. By both searching processes, PTH-phenylalanine and PTH-isoleucine remained as highly possible candidates with the same probability for the component at

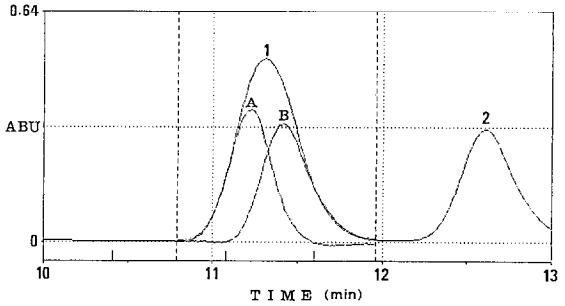


Fig. 9. Peak deconvolution procedure for peak No. 1 in the chromatogram in Fig. 6.

PEAK NO. 4

SPECTRAL SEARCH

NO.	COMPOUNDS	CORRELATION
1	PTH L-phenylalanine	1.000
2	PTH DL-methionine	0.999
3	PTH L-histidine	0.999
4	PTH DL-isoleucine	0.998
5	PTH DL-valine	0.997

RETENTION SEARCH (ERROR less than 10 %)

NO.	COMPOUNDS	R* value	CORRELATION
1	PTH DL-tryptophan	4.05	0.734
2	PTH L-phenylalanine	2.34	0.528
3	PTH DL-isoleucine	2.30	0.510
4	PTH L-lysine	1.34	0.096

SYSTEM OUTPUT

NO.	COMPOUNDS	CORRELATION	BAR GRAPH
1	PTH L-phenylalanine	0.727	*******
2	PTH DL-isoleucine	0.713	*******

Fig. 10. Output of the identification routine by MCASYST for peak No. 4 in the chromatogram in Fig. 6.

TABLE III

SUMMARY OF THE IDENTIFICATION ROUTINE BY MCASYST FOR A MIXTURE OF PTH-AMINO ACIDS

Peak No. in Fig. 6	*Retention time (min)*	*Actual component (PTH-)*	*Two major candidates identified by*		
			UV search (PTH-)	*RPS search (PTH-)*	*MCASYST output (PTH-)*
1	11.3	Methionine	Methionine Histidine	Methionine Valine	Methionine Valine
1	11.5	Proline	Proline Tyrosine	Methionine Valine	Proline –
2	12.6	Norvaline	Norvaline Leucine	Norvaline Proline	Norvaline –
3	15.3	Tryptophan	Tryptophan Tyrosine	Tryptophan –	Tryptophan –
4	16.9	Phenylalanine	Phenylalanine Methionine	Tryptophan Phenylalanine	Phenylalanine Isoleucine
5	17.8	Isoleucine	Isoleucine Valine	Phenylalanine Isoleucine	Isoleucine –
6	18.7	Lysine	Lysine Glysine	Isoleucine Phenylalanine[a]	Lysine –
7	19.6	Leucine	Norvaline Leucine	Lysine Leucine	Leucine –

[a] Lysine was found as the third candidate.

this retention time. However, as peak No. 5 at 17.8 min has been assigned as PTH-isoleucine as the first candidate, the peak at 16. 9 min must be identified as PTH-phenylalanine.

The results of the above searching processes are summarized in Table III and clearly indicate that the accuracy of this MCASYST system is excellent.

CONCLUSION

The results demonstrate the potential of the system in selecting PTH-amino acids that have similar and/or identical UV spectra and retention times from using the retention prediction concept for a number of compounds stored in the database, and the excellent accuracy of the identification capacility of the system. The MCASYST system works very well for practical applications, especially focusing on simple tasks. This computer-assisted approach can improve analytical techniques so that they become more convenient and faster with higher accuracy, including data interpretation and method development.

MCASYST is useful in assisting scientists to perform complicated analyses.

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