Journal of Chromatography, 605 (1992) 87–94
Elsevier Science Publishers B.V., Amsterdam

CHROM. 24 192

Development of directly coupled supercritical fluid chromatography with packed capillary column–mass spectrometry with atmospheric pressure chemical ionization

Kozo Matsumoto, Satoru Nagata, Hideo Hattori and Shin Tsuge

Department of Applied Chemistry, School of Engineering, Nagoya University, Furo-Cho, Chikusa-Ku, Nagoya 464 (Japan)

(First received November 6th, 1991; revised manuscript received March 18th, 1992)

ABSTRACT

Semi-micro packed column supercritical fluid chromatography was combined with atmospheric pressure chemical ionization mass spectrometry through a vacuum nebulization interface originally developed for high-performance liquid chromatography (HPLC)–mass spectrometry. High-purity carbon dioxide (99.999%) used as the mobile phase was delivered through a conventional HPLC pump to the semi-micro packed capillary column maintained at 100°C in an oven. The injected samples were introduced into the ion source with the aid of the nebulizing gas. To obtain the optimum results, the effects of various experimental parameters of the system were studied using Triton X-100 as a test sample. Under the optimum conditions, polyethylene glycol 400, polystyrene A-300, fat-soluble vitamins and polycyclic aromatic hydrocarbons were analysed.

INTRODUCTION

Compared with gas chromatography (GC), supercritical fluid chromatography (SFC) can be applied to less volatile compounds and compared with high-performance liquid chromatography (HPLC) it can be applied to the compounds with a shorter separation time and with a high resolving power. On the other hand, mass spectrometry (MS) possesses outstanding characteristics for the identification of isolated compounds with extremely high sensitivity. SFC–MS systems combining the characteristics of both techniques have been developed [1–6]. Many SFC–MS systems developed use capillary separation columns and chemical ionization (CI) or electron impact (EI) ionization in MS. With capillary separation columns, in general, hardly any drop in pressure is observed and the number of theoretical plates is larger than that of packed separation columns. In a capillary column, however, only a small sample size can be loaded and a splitter is usually installed after the injector. In additon, the analysis time with capillary columns is longer than that with packed columns.

In a previous paper, we reported an SFC–MS system with a capillary column and the CI mode with a vacuum nebulization interface [6]. In this system, a restrictor at the end of the capillary separation column was inserted into the ion source. Therefore, fluctuations of the flow-rate of the mobile phase caused changes in the ion source pressure and affected the observed mass chromatograms. In addition, mobile phase pump pressures above 300 atm could not be used to maintain a low vacuum in the ion source of the quadrupole mass spectrometer.

On the other hand, when atmospheric pressure ionization (API) is used with the SFC–MS system, API can ionize sample molecules at ambient pres-

Correspondence to: Dr. K. Matsumoto, Department of Applied Chemistry, School of Engineering, Nagoya University, Furo-Cho, Chikusa-Ku, Nagoya 464, Japan.

0021-9673/92/$05.00

sure without affecting the high vacuum of the mass spectrometer even if fluctuations of the flow-rate of the mobile phase occur and the mobile phase elutes into the ion source, as both the SFC and MS instruments can be operated almost independently of intervention by the API source. Huang *et al.* [7] reported a packed column SFC–APCI-MS system, using a common HPLC packed column (100 mm × 4.6 mm I.D.), a direct liquid introduction interface with a 20-μm pinhole and a triple quadrupole mass spectrometer [7]. They succeeded in the analysis of steroids extracted from equine urine.

In this study, a packed column SFC–API-MS system was developed, in which a semi-micro packed column was used as a separation column to attain splitless injection of samples for large sample loads and more rapid separation than with capillary columns. In this system, the vacuum nebulizing interface originally developed for HPLC–MS coupling was modified and incorporated into the nebulizer for transferring the SFC effluent to the API source. As API is a "soft" and highly efficient ionization method, it is suitable for the analysis of thermally unstable and oligomeric compounds which are amenable to SFC. The optimum conditions for the SFC–API-MS system were examined by using Triton X-100 as a test sample. The system was then applied to the analysis of various samples such as polyethylene glycol, polystyrene, vitamins and polycyclicaromatic hydrocarbons.

EXPERIMENTAL

The packed-column SFC–API-MS system used is shown schematically in Fig. 1. It is composed of three main parts: a supercritical fluid chromatograph, a nebulizing interface and a mass spectrometer. High-purity carbon dioxide was delivered to the packed separation column (5) through the pump of a Shimadzu Model LC-5A high-performance liquid chromatograph (2) which was operated in the pressure-programmed mode. The pump head was cooled to about 0°C with a micro-cooler (Netsudenshi). A fused-silica capillary tube (18 cm × 0.53 mm I.D.) packed with Kaseisorb LC ODS-300-5 3 (5 μm) (Tokyo Kasei) was used as the separation column, which was heated to 100°C in an oven (4). An integral-type restrictor with a 20-μm pinhole was connected to the end of the separation column through a stainless-steel transfer line (6) (1 m × 0.1 mm I.D.) heated at the same temperature of the oven. For modification of the mobile phase, methanol was added at a flow-rate of 10 μl/min with another HPLC pump (9). The mass spectrometer used was a Hitachi Model M-2000 double-focusing instrument equipped with an API system for HPCL–MS. The operating conditions were accelerating voltage 4 kV, ion-multiplier voltage 1.5 kV, scan rate 8 s from m/z = 0 to 1875 and corona discharge current 10 μA.

A vacuum nebulizing interface developed for

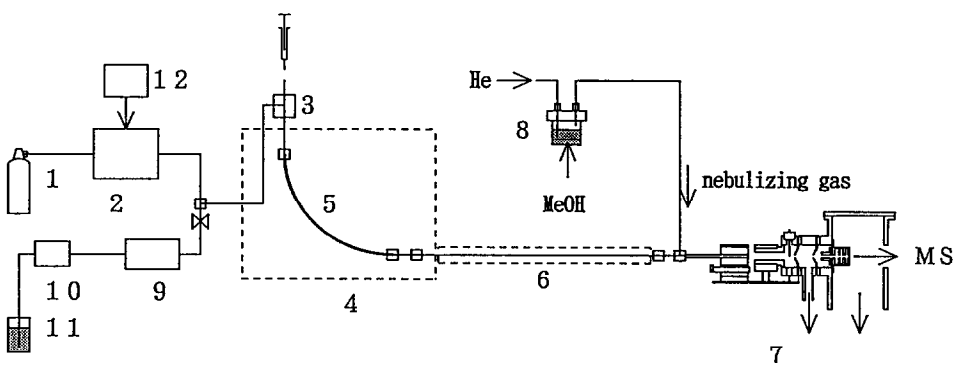


Fig. 1. Schematic diagram of the packed column SFC–API-MS system. 1 = CO_2 reservoir; 2 = HPLC pump modified for SFC; 3 = loop injector (0.5 μl); 4 = oven; 5 = semi-micro packed column; 6 = heated transfer line; 7 = API-MS interface; 8 = bubble saturator; 9 = HPLC pump for modifier; 10 = degasser; 11 = modifer reservoir; 12 = pressure programming controller.

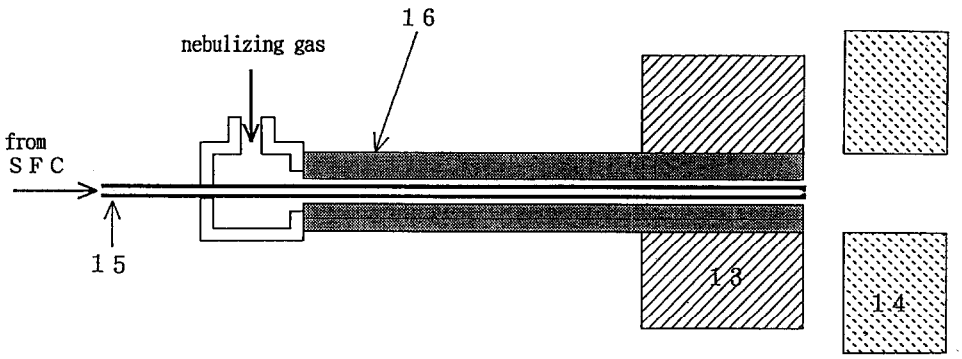


Fig. 2. Schematic diagram of the nebulizer nozzle. 13 = Nebulizer heater; 14 = desolvation chamber; 15 = restrictor (20 cm × 0.375 mm O.D. × 50 μm I.D.); 16 = stainless-steel sheath (13 cm × 1/16 in. O.D. × 0.41 mm I.D.).

HPLC–MS was applied in this SFC–MS system. Fig. 2 shows the modified interface for the SFC–MS system used in this work. The expansion of the supercritical fluid carbon dioxide at the outlet of the restrictor involves an endothermic effect under atmospheric pressure. As a result, the tip of the restrictor was cooled until it was covered with frost. Therefore, to attain stable and continuous nebulization, a nebulizing gas was supplied around the nozzle, the temperature of which was maintained between 200 and 400°C. The nebulizing gas (helium) saturated with methanol contained in a bubble saturator was introduced through the coaxial space between the restrictor and a stainless-steel sheath (13 cm × 1/16 in. O.D. × 0.41 mm I.D.). The desolvation chamber was also heated independently to 400°C to promote the desolvation of the sample molecules.

RESULTS AND DISCUSSION

In order to determine the optimum experimental conditions, various factors such as the temperatures of the nebulizer and the desolvation chamber, position of the nozzle tip, the species and the flow-rate of the nebulizing gas and the drift voltage for the API source were studied.

Effect of nebulization temperature

Fig. 3 shows reconstructed total ion current (RTIC) chromatograms of Triton X-100 obtained at various nebulization temperatures. Except for the nebulization temperature, the SFC–MS system was operated under the same experimental conditions: oven temperature 100°C, pressure programming mode from 150 to 350 kg/cm^2 at a rate of 10 kg/cm^2 · min, desolvation temperature 400°C, nebulizing gas flow-rate 200 ml/min and drift voltage 50 V. An aliquot (0.5 μl) of Triton X-100 solution disolved in methanol (3%) was injected through a loop injector. The three chromatograms A, B and C were obtained at nebulization temperatures of 200, 350 and 400°C, respectively. The chromatogram at 200°C (A) shows disturbed peaks and a lower peak intensity at higher molecular regions compared

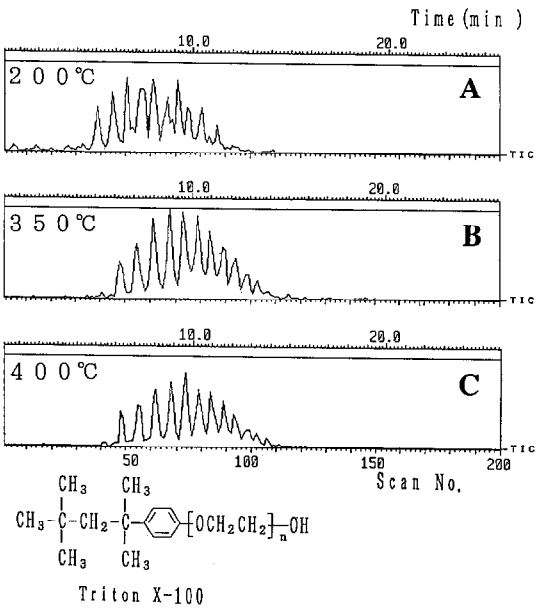


Fig. 3. Effect of nebulizer heater temperature on reconstructed total ion current (RTIC) chromatograms of Triton X-100. (A) 200°C; (B) 350°C; (C) 400°C.

with that obtained at 350°C (B). This effect is due to unstable nebulization caused by an insufficient heat supply to the nebulizer tip. On the other hand, the chromatogram at 400°C (C) gives lower intensity peaks than that at 350°C, but their shapes are very similar. This result suggests that the sample is partly decomposed by overheating. The nebulization temperature of 350°C was empirically decided to be the optimum for this sample.

Effect of the desolvation chamber temperature

When the desolvation temperature was kept at about 200°C, intense cluster ions of methanol and water and adduct ions of the sample molecules with methanol and water were observed in the mass spectrum, where the molecular and/or quasi-molecular ion peaks of the sample molecules were very difficult to identify. To reduce the cluster and adduct ions, the temperature of the desolvation chamber was kept at 400°C, which was the maximum temperature of this mass spectrometer.

Position of the nozzle tip

The position of the nozzle tip was varied under the optimum experimental conditions determined above. When the tip was located out of the nebulizing sheath, the observed peaks on the chromatograms became broad especially at longer retention times because of the insufficient heat supply. On the other hand, when the tip was withdrawn into the sheath, the peak heights of the chromatograms became lower because of the partial thermal decomposition of the sample molecules. Therefore, in this work, the position of the nozzle tip was adjusted to meet the edge of the nebulizing sheath.

Flow-rate of nebulizing gas

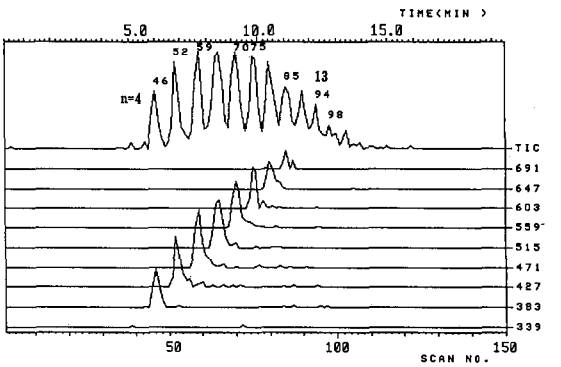
No significant influence of the flow-rate of the nebulizing gas below 400 ml/min was observed on the resulting mass chromatograms. However, without the nebulizing gas, the nebulization became unstable and the peak intensities on the chromatogram became weaker. On the other hand, at flow-rate above 400 ml/min, the observed peak intensities became smaller because of the shorter residence time of the sample molecules in the ion source. Therefore, in this work, the flow-rate of the nebulizing gas was fixed at about 200 ml/min. In addition, methanol saturation of the nebulizing gas (helium) was effective in to attaining "softer" ionization compared with pure helium.

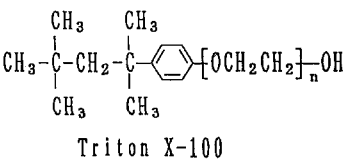
Drift voltage

For the HPLC–API-MS system, a drift voltage of 150 V is usually used to obtain stronger (protonated) molecular peaks. In this SFC–API-MS system, a lower voltage, *e.g.*, 50 V, proved to be suitable to attain "soft" ionization because of the presence of carbon dioxide used as the mobile phase (*ca.* 200 ml/min at NTP).

Applications

Fig. 4 shows the mass chromatograms of Triton X-100 observed under the optimum conditions: nebulization temperature 350°C, desolvation chamber temperature 400°C, nebulizing gas flow-rate 200 ml/min, drift voltage 50 V, column oven temperature 100°C and column head pressure 150–350 atm at a programming rate of 10 atm/min. The components were eluted within 15 min and gave clearly separated peaks on the chromatograms. As shown in Fig. 5, identification of the components (degree of polymerization, *DP*) was performed with the corresponding mass spectra on which protonated molecular peaks, MH^+, appeared as the main peaks with some adduct ions. The components from *DP* = 3 to 15 for this sample were identified. Takeuchi





Triton X-100

Fig. 4. RTIC and reconstructed ion current (RIC) chromatograms of Triton X-100.

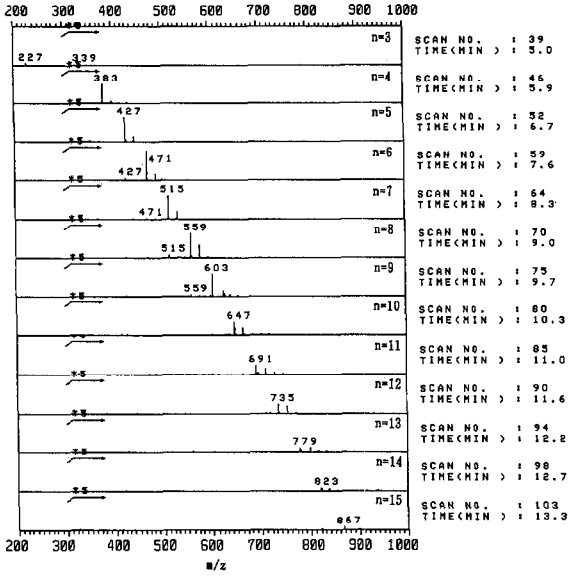
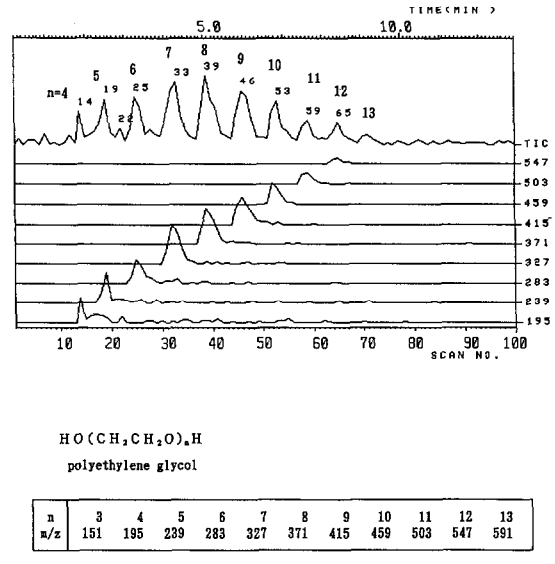


Fig. 5. Mass spectra of Triton X-100.



$HO(CH_2CH_2O)_nH$

polyethylene glycol

n	3	4	5	6	7	8	9	10	11	12	13
m/z	151	195	239	283	327	371	415	459	503	547	591

Fig. 6. RTIC and RIC chromatograms of polyethylene glycol 400.

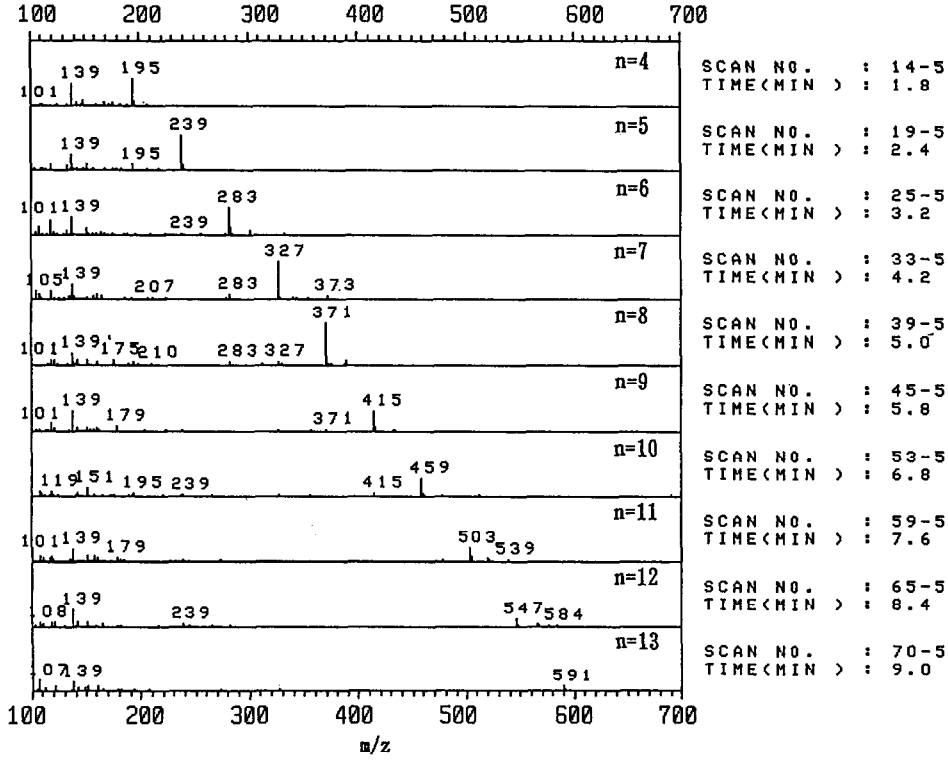


Fig. 7. Mass spectra of polyethylene glycol 400.

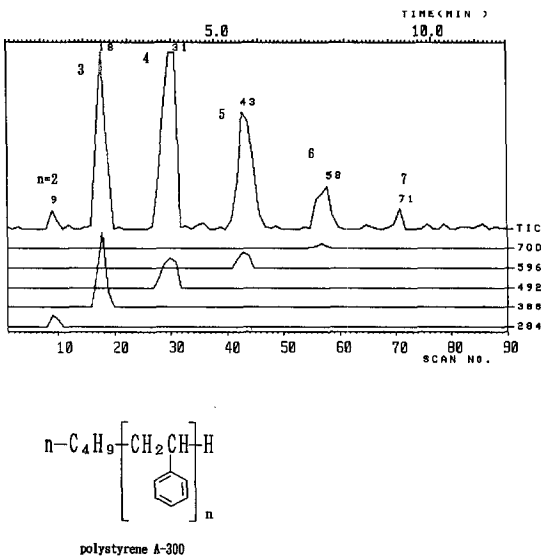


Fig. 8. RTIC and RIC chromatograms of polystyrene A-300.

et al. [8] reported the analysis of oligomers of DP = 3–14 by use of capillary HPLC–fast atom bombardment MS.

Fig. 6 shows mass chromatograms of polyethylene glycol 400 obtained under the same experimental conditions as in Fig. 4 except for the lower nebulization temperature of 300°C for the thermally labile Triton-X. The components were clearly resolved within 10 min. The identification of the peaks was also peformed with the corresponding mass spectra shown in Fig. 7, on which strong protonated molecular peaks were observed.

Fig. 8 shows the observed mass chromatograms of polystyrene A-300. The experimental conditions were the same as in Fig. 6, but the pressure was programmed from 200 to 350 atm at 10 atm/min. The components of the sample were eluted within 10 min, giving symmetrical and smooth peaks. As shown in Fig. 9, the identification of the components was performed from the mass spectra, on which aduct ions, $[MH + CH_3OH - CH_3]^+$, appeared in higher mass regions at m/z 284, 388, 492, 596, 700 and 804 for n = 2, 3, 4, 5, 6 and 7, respectively. In addition, fragmentation is observed, giving peaks of $[MH - C_6H_5]^+$ and $[MH - (C_6H_5 + C_6H_6)]^+$, *e.g.*, at m/z 293 and 215 for n = 3.

Fig. 10 shows the mass chromatograms of a mixture of fat-soluble vitamins (vitamin K_1, E and D_3 and vitamin A acetate) obtained under the same conditions as in Fig. 8. The samples were dissolved in methanol (0.25% each). All the components were eluted within 5 min. Although vitamin D_3, E and K_1 were not separated under these conditions on the RTIC chromatogram, they were discriminated on the RIC chromatograms. As shown in Fig. 11, they gave protonated molecular peaks, $[MH]^+$, on the mass spectra. Vitamin A acetate, however, gave a fragment peak of $[MH - AcOH]^+$ at m/z 269 without giving the protonated molecular peak on the mass spectrum.

Finally, polycyclic aromatic hydrocarbons

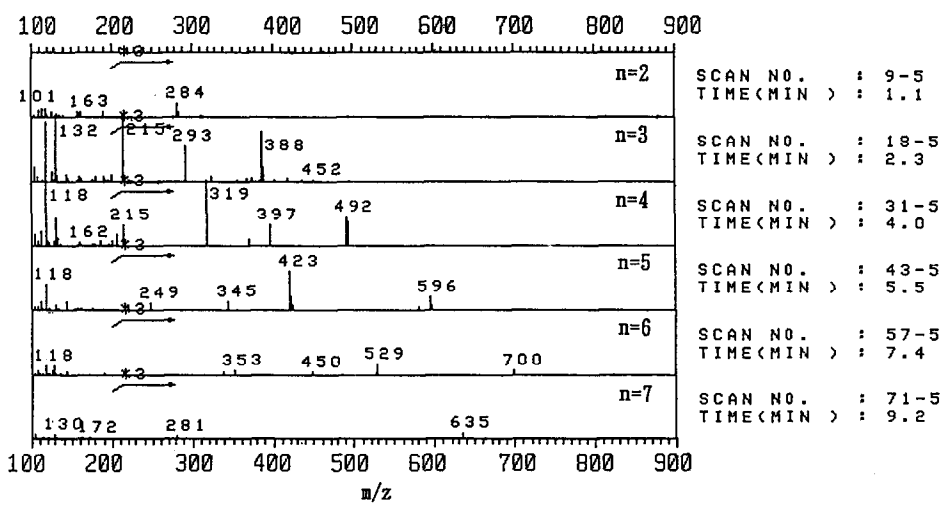


Fig. 9. Mass spectra of polystyrene A-300.

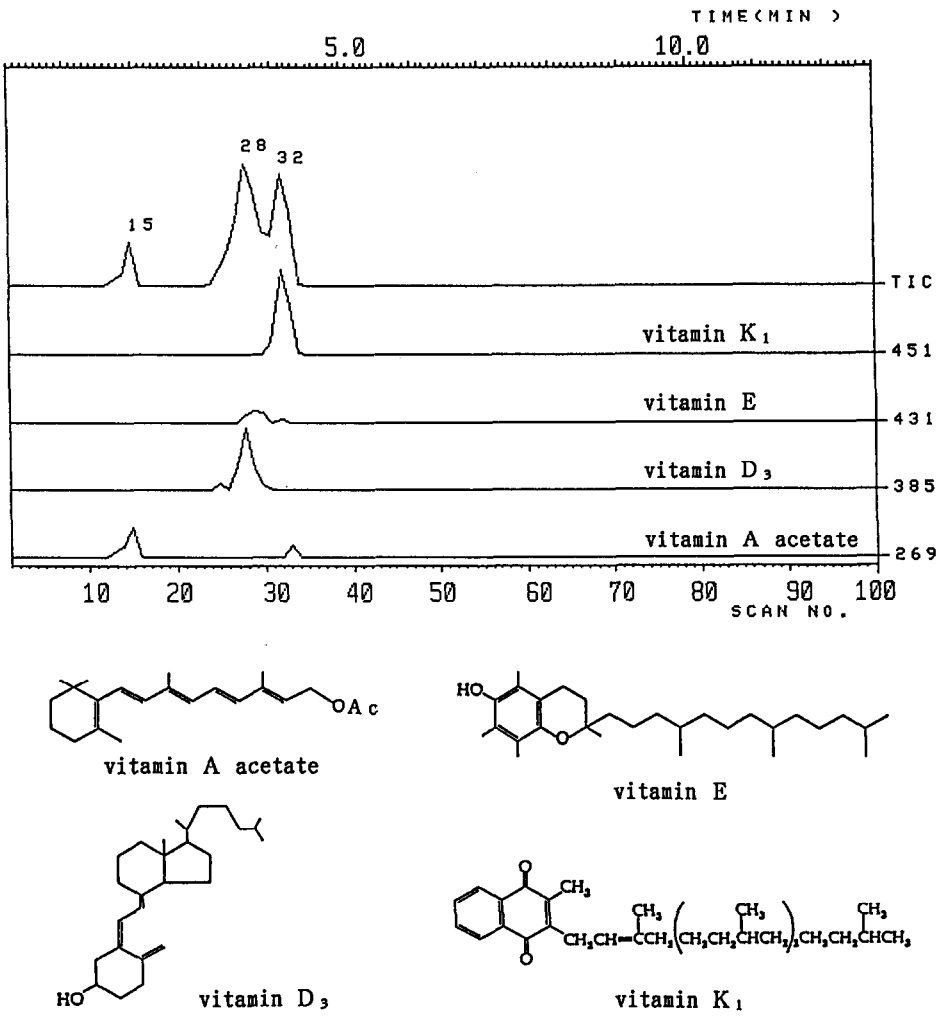


Fig. 10. RTIC and RIC chromatograms of the vitamin mixture.

(PAHs) are chosen as test samples which are not suitable for ordinary API detection because of their low polarity [9]. However, as shown by the following results, PAHs could be analysed using SFC–API-MS with the methanol-saturated helium as the nebulizing gas, which assisted the ionization of PAHs.

Fig. 12 shows the mass chromatograms of PAHs under the same conditions as in Fig. 6 except for the starting pressure of 100 atm. Samples (naphthalene, MW = 128; acenaphthylene, MW = 152; fluorene, MW = 166; anthracene, MW = 180; fluoranthene, MW = 202; pyrene, MW = 202; chrysene, MW = 228; and benzo[*e*]pyrene, MW = 252) dissolved in benzene (0.2% each) were analysed. These compounds gave $[M]^+$ and $[MH]^+$ ions of comparable intensity on their mass spectra without any appreciable fragment peaks. For naphthalene, its $[M]^+$ (*M/z* 128) was recorded on the RIC chromatogram, because the protonated molecular peak, $[MH]^+$, overlapped with the methanol tetramer peak, $[(CH_3OH)_4 + H]^+$, at *m/z* 129.

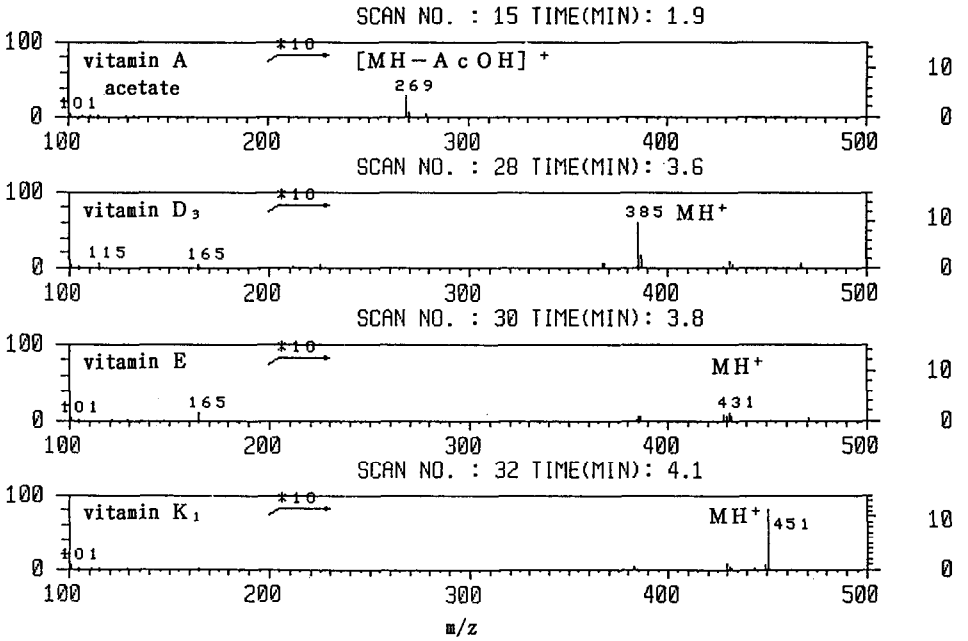


Fig. 11. Mass spectra of the vitamin mixture.

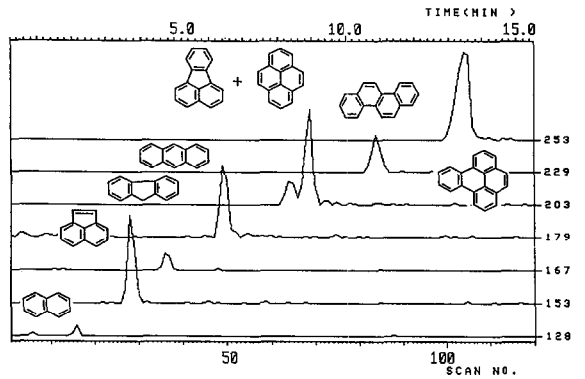


Fig. 12. RIC chromatogram of the PAH mixture. *m/z* 128 = Naphthalene; 153 = acenaphthylene; 167 = fluorene; 179 = anthracene; 203 = fluoranthene and pyrene; 229 = chrysene; 253 = benzo[*e*]pyrene.

CONCLUSIONS

A packed column SFC–APCI-MS system was developed with the use of a modified vacuum nebulization interface. The analysis of the thermally labile compounds such as Triton X-100, polyethylene glycol 400, polystyrene A-300 and fat-soluble vitamins was performed. Polycyclic aromatic hydrocarbons, for which conventional HPLC–APCI-MS did not give any appreciable sensitivity because of their low polarity, could be analysed with this system with the aid of a methanol-saturated nebulizing gas.

REFERENCES

1 S. M. Sheeley and V. N. Reinhold, *J. Chromatogr.*, 474 (1989) 83.
2 E. C. Huang, B. J. Jackson, K. E. Markides and M. L. Lee, *Anal. Chem.*, 60 (1988) 2715.
3 V. N. Reinhold, D. M. Sheeley, J. Kuei and G. Her, *Anal. Chem.*, 60 (1988) 2720.
4 H. T. Kalinoski and L. O. Hargiss, *J. Chromatogr.*, 474 (1989) 69.
5 J. D. Pinkston, D. J. Bowling and T. E. Delaney, *J. Chromatogr.*, 474 (1989) 97.
6 K. Matsumoto, N. Sato, S. Tsuge and M. Taguchi, *Mass Spectrosc.*, 39 (1991) 43.
7 E. Huang, J. Henion and T. R. Covey, *J. Chromatogr.*, 511 (1990) 257.
8 T. Takeuchi, S. Watanabe, N. Kondo, M. Goto and D. Ishii, *Chromatographia*, 25 (1988) 523.
9 *Hitachi Technical Data*, MS No. 43, Hitachi, Naka.