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New supercritical fluid chromatography interface probe for electrospray and atmospheric pressure chemical ionization mass spectrometry

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Abstract

A new supercritical fluid chromatography interface probe has been constructed for atmospheric pressure ionization mass spectrometry (API-MS). The use of API-MS effectively separates the ionization and mass analysis regions. Furthermore, a sheath-liquid flow of 20 μl/min in electrospray ionization provides an attractive way of optimising the separation and ionization conditions independently of each other. The new probe allows for easy change of ionization mode, between electrospray and atmospheric pressure chemical ionization, which provides more freedom of choice when an analytical problem has to be solved. © 1997 Elsevier Science B.V.

Keywords: Supercritical fluid chromatography interface probe; Interfaces, SFC–MS; Mass spectrometry

1. Introduction

Supercritical fluid chromatography (SFC) has been demonstrated for the separation of a wide range of moderately polar compounds, including polymers and thermally labile analytes. Open tubular columns can be deactivated enough to allow for the use of neat CO_2 as mobile phase, thus making it possible to use the universal flame ionization detector (FID). A relatively high resolution can also be obtained with open tubular columns at temperatures where thermally labile compounds still can be separated. Packed column SFC usually requires addition of modifiers and thus makes it possible to elute more polar solutes. Mass spectrometry (MS) was identified early as an important detector for the SFC techniques [1]. The research in interfacing SFC with MS has now been carried on for almost 20 years and resulted in several review articles [2–7].

The increasingly popular mild ionization techniques which are performed at/or near atmospheric pressure have several advantages in combination with SFC when compared to conventional low pressure ionization techniques. The gas-phase ionization in a corona discharge ion source i.e., atmospheric pressure chemical ionization (APCI) has been described for both open tubular and packed column SFC [8–13]. The interfaces used have mainly been commercially available LC–APCI-MS probes with minor modifications to allow for the use of a restrictor and restrictor tip heating. This heating should compensate for the adiabatic cooling of the expanding supercritical fluid and thereby avoid restrictor plugging and assure complete vaporisation of the mobile phase before ionization. More sophisticated interfaces have also been reported [11,12,14]

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where the whole transfer line was heated in order to avoid peak deformation and irreproducible retention times. SFC–MS via electrospray ionization (ESI) has also been investigated using both open tubular and packed columns [8,14–18]. Sadoun et al. [14] reported the coupling of SFC via ESI using packed columns with 1–30% of methanol–water modifier in CO_2. The increasing flow-rate during the mobile-phase composition gradient separation made it difficult to maintain a constant ion current. The authors suggested that this problem could be overcome by the use of a post-column addition of a suitable solvent. This problem was, however, not observed in a more recent report [17]. In a paper by Pinkstone and Baker [15] the combination of open tubular column SFC with a modified pneumatically assisted electrospray (ionspray) interface was reported. A liquid-sheath flow of methanol at 30 μl/min, containing ammonium acetate, was employed. The baseline was, however, unstable and it was suggested that the volatility of methanol caused an uneven spray. It was also shown that the addition of 20% water to the methanol improved the situation. The ion signal was further improved by increasing the flow-rate to 100 μl/min in combination with the use of a heated air gun (turbo-ionspray). The ionspray interface was later modified to allow for the use of packed columns [18] and besides the liquid-sheath flow, an extra liquid flow for post-column pressure regulation was added.

The criteria for optimal performance during ionization will always be different for ESI and APCI, respectively. In ESI it is important to control the factors that influence the optimum liquid-sheath flow-rate like, for example, liquid-sheath composition, mobile-phase flow, sheath-flow capillary and SFC restrictor dimensions as well as their relative position to each other. In APCI, on the other hand, the temperature in the ionization region will be the most important parameter since it has a direct effect on water cluster formation and hence sensitivity.

In this paper, an interface for coupling micro-column separation techniques to API-MS allowing easy alteration between ESI and APCI modes is described. The chromatographic performance of this dual interface is demonstrated for the use in open tubular SFC with carbon dioxide mobile phase.

2. Experimental

2.1. Sample and solution preparation

Test mixtures of polyethylene glycols (PEG 600), (Merck Darmstadt, Germany) and polypropylene glycol (PPG 425), (Aldrich, Milwaukee, WI, USA) were dissolved in methanol (Merck) and injected at a concentration range of 0.4–3.2 mg/ml. Stearic acid methyl ester, pentacosanoic acid methyl ester, trilaurin, cholesterol, cholesterol palmitate, 9-phenylantracene and 9,10-diphenylantracene were all obtained from Sigma (St. Louis, MO, USA). These analytes were dissolved in toluene (Merck) for chromatographic analysis at a concentration range of 0.9–1.6 μg/ml.

The sheath flow liquid was a solution of methanol–water (75:25) with the addition of 1 m*M* formic acid. Methanol of LC gradient grade and formic acid (analytical-reagent grade) were supplied by Merck and used without further purification. Water was obtained from a Milli-Q plus purification system (Millipore, Bedford, MA, USA). The sheath liquid was degassed using ultrasonication prior to use.

2.2. Interface design

The construction of the new SFC–MS interface was based on experience with an earlier in-house design previously reported [11]. The changes made to the heated transfer line are the following; (1) a stainless steel capillary, 0.75 mm I.D.×1 mm O.D., (G. Kinnvall, Sparreholm, Sweden) was used instead of the fused-silica capillary, 0.7 mm I.D., which became brittle after some time of use, (2) a tee connection (1/8 in.; 1/16 in.; 1/16 in., SGE; 1 in.=2.54 cm) was added to the oven end of the probe and an extra PTFE tube 1/8 in. was inserted into the 1/4 in. PTFE tube in order to have two separate layers of preheated compressed air which eliminate local temperature variations. This is schematically shown in Fig. 1a, (3) the heating wire which act as an "active insulator" has a diameter of 0.25 mm, resistance of 27 Ω/m and was wound at 1 mm/turn, (4) a high temperature ceramic fiber sleeving insulation (Dalfratex S-47, Cape Insulation Products,

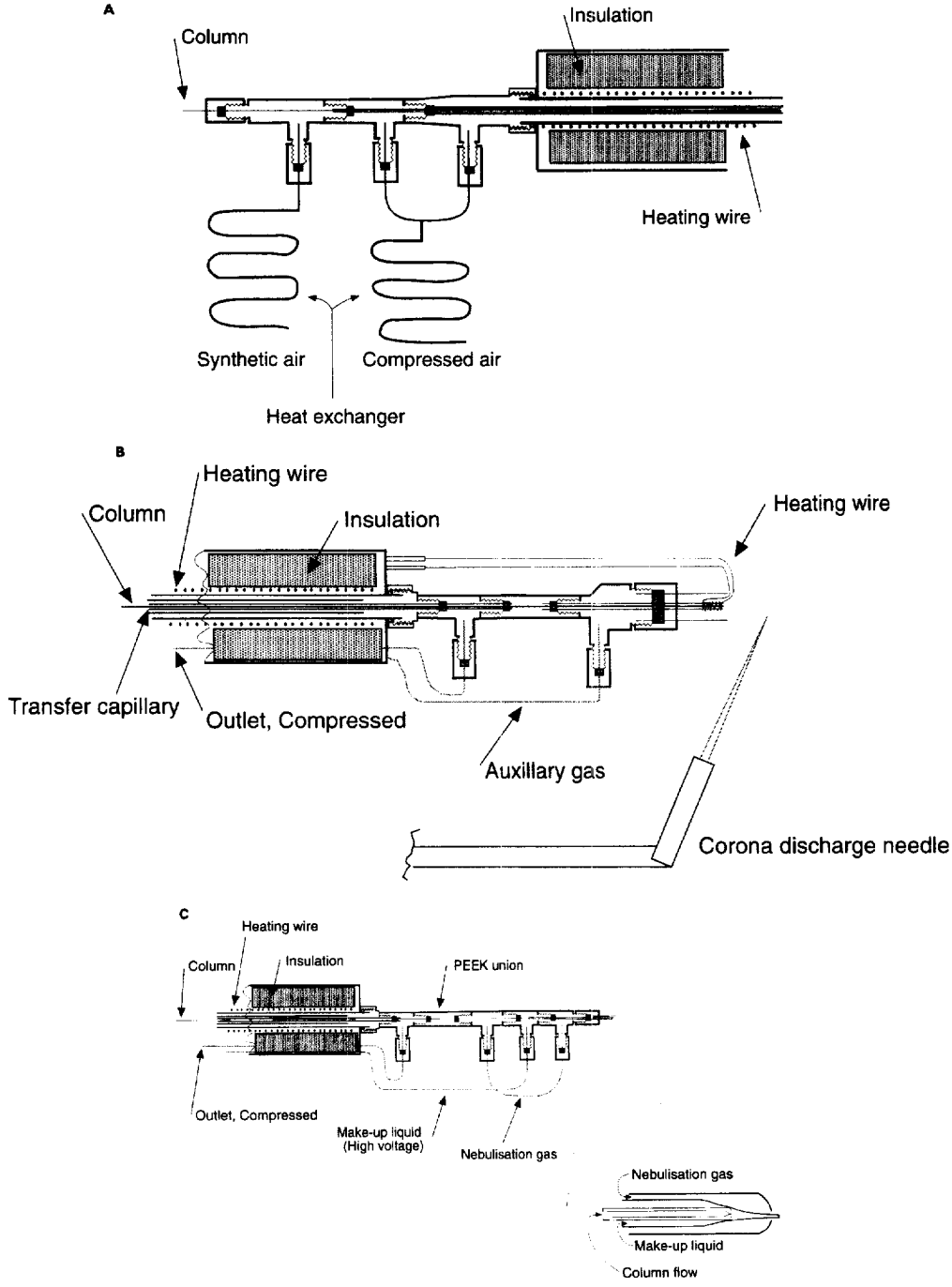

Fig. 1. Schematic drawings of the different interface probe parts of (a) assembly in oven, (b) assembly in ion source, i.e., APCI mode and (c) assembly in ion source i.e., ESI mode.

Washington, UK) with a 5 mm wall thickness was used as an outer insulation layer.

The front end of the interface probe was modified to make it possible to alter between the APCI or ESI mode. The original heated nebuliser probe supplied by Sciex (Concord, ON, Canada) was used as a shell and modified in the following way; a 1/4 in. stainless-steel nut (SGE, Austin, TX, USA) was welded to the front end of the empty probe. This stainless-steel nut provided a base for further connections and a leak tight seal between the insulated heated transfer line and the ion source.

For the SFC-APCI mode, further SGE connections were used as schematically shown in Fig. 1b. The nebuliser capillary was made from a 0.7 mm I.D. fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) where the front end I.D. was reduced to approximately 300 μm with the aid of a small acetylene–oxygen welding flame. The restrictor tip heating wire was placed around the nebuliser capillary to minimise the disturbance of the eluting gas flow profile. The nebuliser gas was synthetic air of FID quality (AGA, Stockholm, Sweden) with a flow-rate of 0.4 l/min. Furthermore, an extra quarts tube, 4 mm I.D.×6 mm O.D., was used for a co-axial auxiliary gas flow of 1 l/min, of synthetic air (AGA) to aid in minimising the chemical background. Furthermore, the protecting polyimide layer was burned off the tips of the fused-silica capillaries entering the interface since disturbances from the polyimide coating otherwise may occur as described by Thomas et al. [10].

For the SFC-ESI mode, the design is schematically shown in Fig. 1c. An extra stainless-steel tee (SGE) and a fused-silica capillary, 320 μm I.D.×430 μm O.D (Polymicro Technologies) were used to facilitate addition of a make-up sheath-liquid flow. The end of this sheath-liquid flow capillary was drawn out in a small welding flame and cut with a sapphire knife to produce a fine tip with an I.D. of 50 μm and O.D. of 150 μm. A nebuliser capillary was made from a 0.7 mm I.D. fused-silica capillary (Polymicr Technologies) where the front end I.D. was reduced to approximately 200 μm as described above. The sheath-liquid flow capillary was positioned inside the nebuliser capillary with the tip sticking out approximately 0.5 mm. A nebulisation gas (AGA) flow of 0.6 l/min was used. A stereo microscope (Carton Model SCZ, Carton Optical Industries, Tokyo, Japan) with 80× magnification was used to visually monitor the assembling of the spray devise. The high voltage was connected to the sheath-liquid tee connection (SGE) and a PEEK union (Upchurch Scientific, Oak Harbor, WA, USA) was used as a high voltage insulator between the heated transfer line and the front connections. An LC pump PU 980 (Jasco, Tokyo, Japan) operating at a constant flow of 10–20 μl/min to maintain the liquid sheath flow was used.

2.3. MS conditions

A PE-Sciex API III$^+$ triple quadrupole mass spectrometer (Concord, ON, Canada) equipped with a point to plane corona discharge ion source was used for this study. For the APCI mode, the discharge current was 2 μA and the potential was set to 650 V at the interface plate, 50 V at the orifice and 30 V at the first focusing RF-only quadrupole (Q0). For the ESI mode, a potential of 4 kV was applied to the sheath-liquid tee. The volumetric flow of the dry nitrogen counter current curtain gas (99.9999% purity, 6.0 AGA and heated to 50°C) was 1.2 l/min and 1.4 l/min over the sampling orifice for APCI and ESI, respectively. The ion source probe was directed 1–2 mm (APCI) and 5 mm (ESI) off-axis relative to the orifice to avoid injection of solvent vapour into the mass analyser. Mass scale calibration was performed using protonated water clusters obtained by a decrease of the dry nitrogen curtain gas flow. Data was acquired by selected ion monitoring (SIM) at unit mass resolution with a dwell time of 30 ms (unless stated otherwise) in order to more closely examine the chromatographic peak shape.

2.4. Chromatographic conditions

Open tubular column SFC was performed using a Lee Scientific series 600 chromatograph (Dionex, Sunnyvale, CA, USA) with SFC-grade carbon dioxide (L'Air Liquide, Paris, France) as mobile phase. The retention gap technique described by Chester and Innis [19,20] was employed for direct injections (30 s) into a 5 m, 50 μm I.D. capillary using a helium actuated injection valve, CI4W Valco Instruments (Houston, TX, USA), equipped with a 60 nl

sample rotor loop. Separations were performed with 30% biphenyl-substituted or 50% cyanopropyl-substituted methylpolysiloxane columns (Dionex) with 0.25 μm stationary phase film thickness, 10 m×50 μm I.D. using a density program at 100°C. The retention gap was connected to the analytical column using a zero dead volume union connection, (1/16 in.×1/16 in., SGE) using a single graphitised vespell ferrule as reported recently [21]. An integral restrictor [22] of 1–2 μm was made from the analytical column end as described earlier [11]. The FID temperature was 400°C.

3. Results and discussion

During recent years, there has been an increasing need to develop on-line microprobes to interface the chromatograph with the MS detector since miniaturization has resulted in faster and more efficient separations. At the same time, lower flow-rates to the atmospheric pressure ion source result in unsurpassed ionization efficiency and higher sensitivity. When highly efficient micro-analytical techniques are hyphenated to MS detection, accurate performance throughout the interface has to be retained. Second, the interface should not add chemical background to the analysis, and finally, it should be easy to use and change column, restrictor and ionization mode. These criteria have been guidelines for the interface design presented in this paper.

In APCI, chemical background in the low m/z range (below m/z 150) can be a problem and a leak-tight seal between the insulated heated transfer line and the ion source was essential. In micro-column separations with a low mobile-phase flow-rate and small cross section area of the nebulised effluent, neutral solutes influence the surrounding ion source gas to a larger extent when compared to conventional LC-ACI. This could result in increased chemical mass spectral background. The combination of a co-axial auxiliary gas flow and an ion source exhaust pump reduced this background. It was noted that the use of the exhaust pump also improved the peak shapes, possibly by reducing the re-circulation of sample vapour into the ionization region. In addition, the orifice potential was found to have a strong influence on the background cluster ions, but an increased orifice potential might also induce fragmentation of the analytes of interest.

For ESI, the positioning of the column restrictor tip relative to the sheath-liquid flow capillary was critical due to the fragile nature of the drawn out sheath-liquid flow capillary and the stereo microscope was of great use during the assembling process of the spray device. This positioning resulted in fixation of the column at two places, i.e., one ferrule in the oven and one ferrule close to the spray tip. This approach gave stable, reproducible results but had some limitations regarding ease of restrictor/column change.

The use of SFC–ESI-MS has been reported [15] to give asymmetric peaks as a result of cold trapping of solutes at the nebuliser tip. This asymmetry could be reduced by heating either the mobile phase or the restrictor at the expense of a decreased ion current. In Fig. 2A–C, it is evident that peak asymmetry was not a problem in the separation of PEG-600 and similar peak shapes were obtained using FID, APCI and ESI detection, respectively.

While the chromatographic performance was preserved through the interface and ionization, it was found that the elution profile of individual components in a polymeric mixture displayed different intensities in APCI and ESI modes as illustrated for PPG 425 in Fig. 3A and B. The APCI trace was similar to the FID profile. Upon closer examination of the $(M+H)^+$, $(M+NH_4)^+$ and $(M+Na)^+$ adduct ion signals, shown in Fig. 4A–C, it was easy to see the strong influence that solvent composition and the analyte affinity for different cations have on the elution profile when using ESI. In SFC–ESI-MS, the composition of the make-up liquid is expected to influence the ion production in a similar way as in LC–ESI-MS where different kind of ion signal suppression effects has been reported [23–31]. The understanding of the ESI mechanism when using supercritical fluids is, however, not yet complete and needs further study. For example, pneumatically assisted ESI (ionspray) [32] has been used to assist nebulisation at higher liquid flow-rates in order to increase the ion yield and it has recently been reported that a supercritical fluid also can assist nebulisation at higher flow-rates [16]. Both ESI and pneumatically assisted ESI at low liquid flow-rates, below 100 μl/min, are considered to be concen-

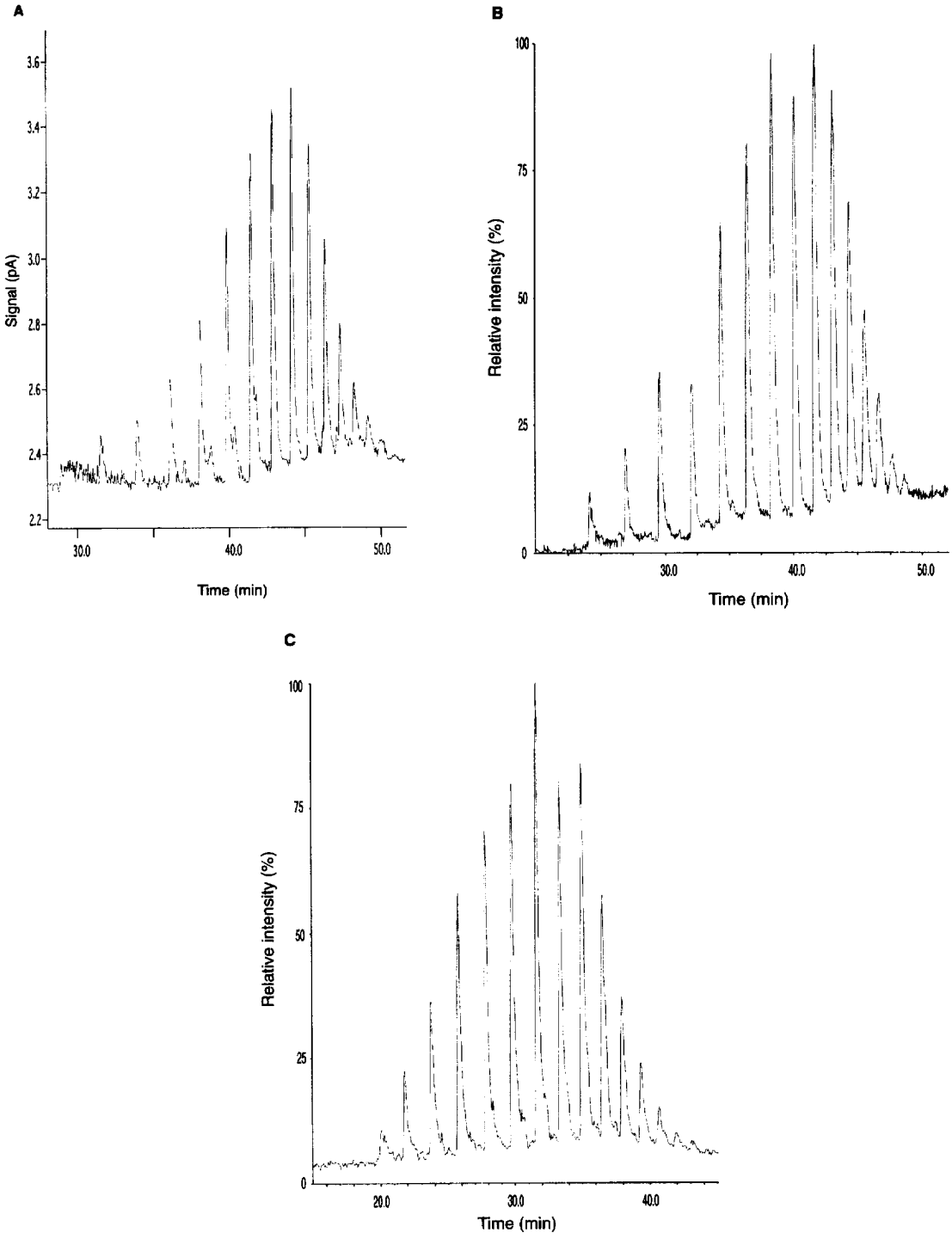

Fig. 2. Open tubular column SFC separation of PEG 600 using (A) FID (B) APCI and (C) ESI. Conditions: CO_2 at 100°C; cyanopropyl column; density program 0.12 g/ml (6 min), 0.0064 g/ml min to 0.1445 g/ml, 0.04 g/ml min to 0.43 g/ml and 0.01 g/ml min to 0.75 g/ml. For detection conditions see Section 2.3 Section 2.4.

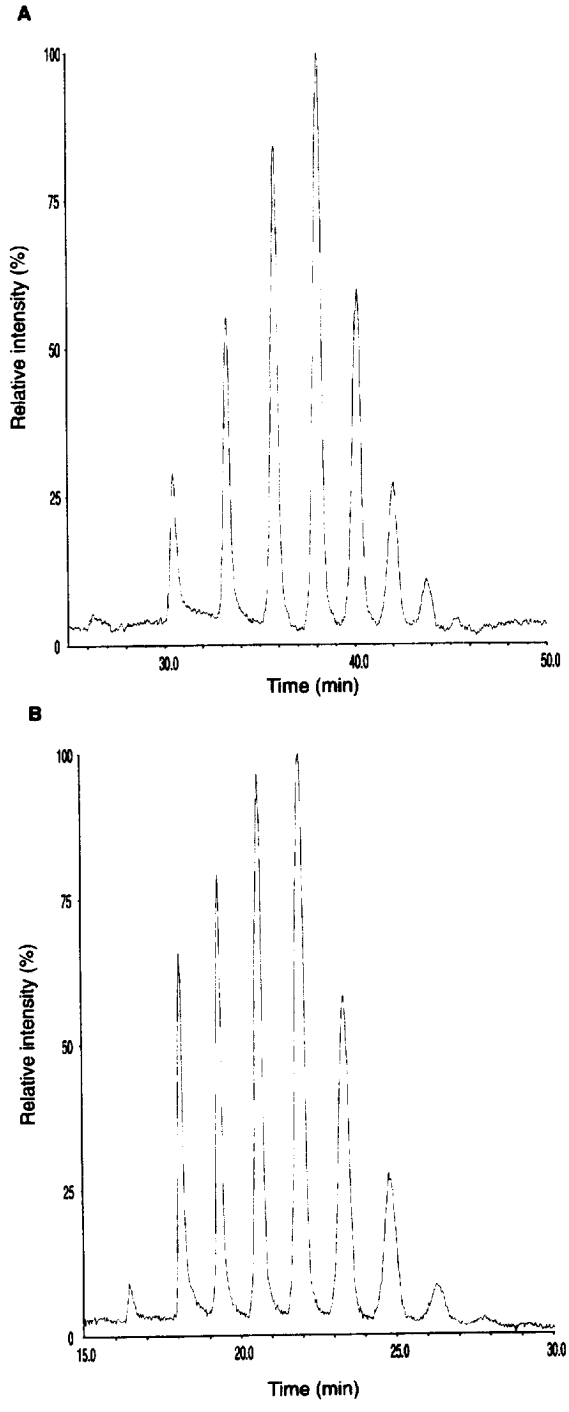

Fig. 3. Open tubular column SFC separation of PPG 425 using (A) APCI and (B) ESI. Conditions: CO_2 at 100°C; biphenyl column; density program 0.12 g/ml (6 min), 0.0064 g/ml min to 0.1445 g/ml, 0.04 g/ml min to 0.43 g/ml and 0.01 g/ml min to 0.75 g/ml. For detection conditions see Section 2.3.

tration-sensitive detection techniques [33,34] and therefor, for open tubular SFC–ESI-MS lower liquid-sheath flow-rate is expected to give improved sensitivity. In this study, a liquid-sheath flow-rate of 10 μl/min produced unstable baseline and ion signals (data not shown) which could be restored by increasing the flow-rate to 20 μl/min.

Control of ion source chemistry is important in SFC–APCI-MS and to obtain optimal signal and spectral quality, the spray has to be almost axial to the orifice. Care needs to be taken, however, to avoid problems with extensive clustering with water and other polar molecules that can occur in the free-jet expansion from atmospheric pressure to high vacuum. Optimisation of the interface in the APCI mode, was performed with continuous slow supercritical fluid extraction (SFE) as described previously [11] using an oven temperature of 40°C, a CO_2 pressure of 95 atm (1 atm=101 325 Pa) and stearic acid methyl ester loaded onto a small LC pre-column. It was found essential to set the SFC oven temperature close to the subsequent SFC separation temperature, otherwise, different linear flow of nebuliser gas velocities would be obtained allowing the nebulised effluent to penetrate the dry nitrogen curtain gas plume and cause protonated water clusters to appear in the spectra. Fig. 5 shows an example of the system performance in APCI mode. A test solution containing nonpolar to medium polar analytes present in the low pg levels was injected. It is believed that the detection limit of the APCI system described here might be decreased even further if the nebuliser design is modified to allow for a lower nebuliser gas flow and still retain a stable ion signal. A low nebulisation gas flow also allow for a more in-line axial position of the spray and thereby an enhanced ion sampling situation.

4. Conclusions

The possibility to easily change between APCI and ESI ionization modes in SFC–MS has been demonstrated. This tool is expected to be of high value for analysis of polymers and thermolabile compounds. With this thermostated interface, the total SFC mobile phase flow from the column is introduced to the ionization region. In APCI, a

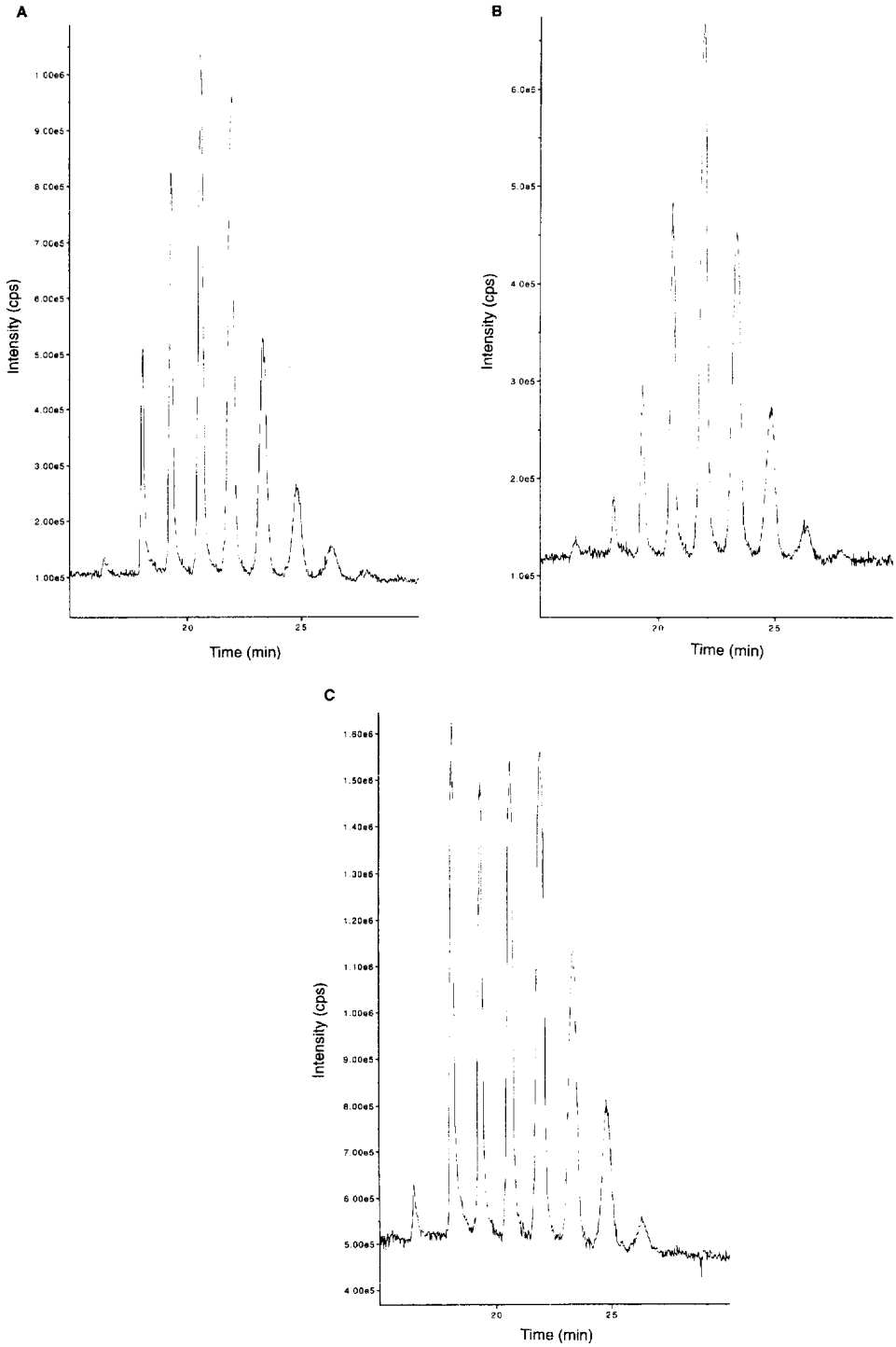

Fig. 4. Elution profiles for the different ESI adduct ions of PPG 425 for (A) $(M+H)^+$, (B) $(M+NH_4)^+$ and (C) $(M+Na)^+$. Conditions as in Fig. 3.

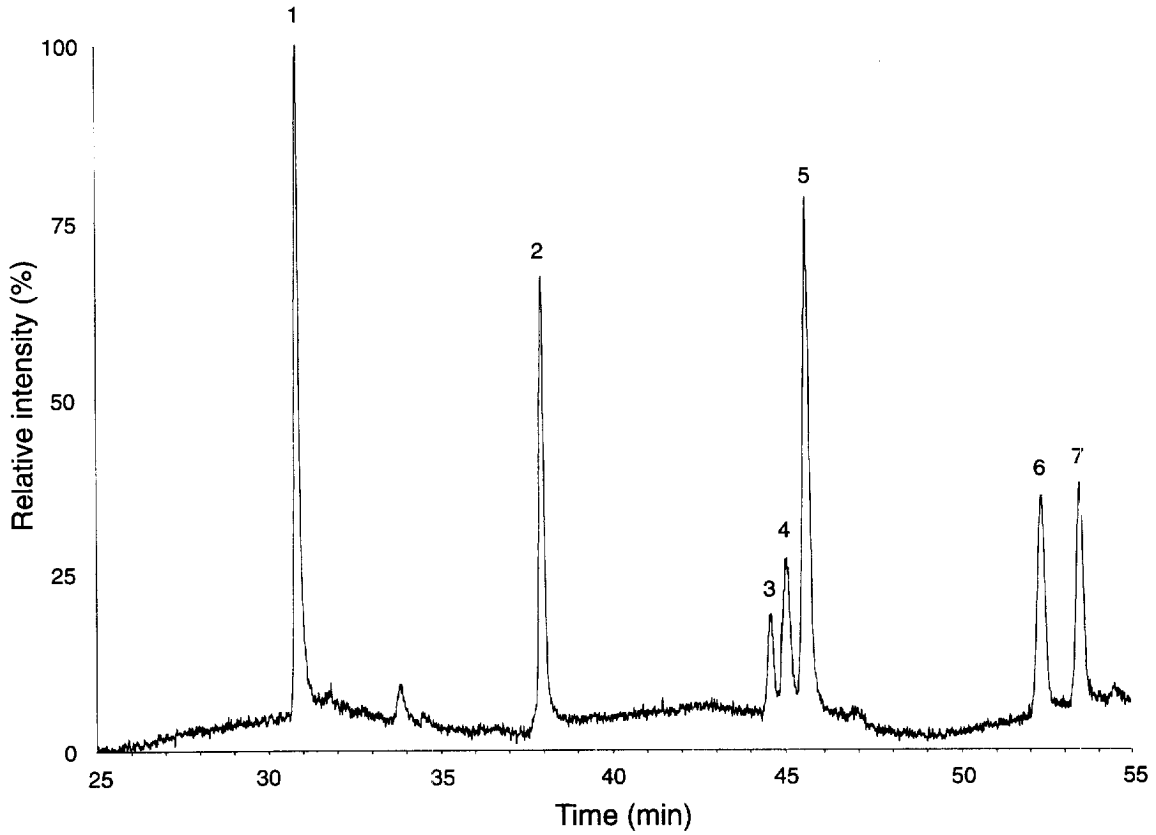

Fig. 5. Open tubular column SFC separation with APCI-MS total ion trace, SIM of $(M+H)^+$ with a dwell time of 50 ms. Conditions: CO_2 at 100°C; biphenyl column; density program 0.12g g/ml (6 min), 0.0064 g/ml min to 0.1445 g/ml, 0.04 g/ml min to 0.43 g/ml and 0.01 g/ml min to 0.75 g/ml. Peak identification: (1) stearic acid methyl ester (2) pentacosanoic acid methyl ester (3) trilaurin (4) 9-phenylantracene (5) cholesterol palmitate (6) 9,10-diphenylantracene (7) cholesterol. For detection conditions see Section 2.3.

heated region around the restrictor tip is used to compensate for the adiabatic cooling of the expanding mobile phase. In ESI, a liquid-sheath flow serves the same purpose. Quantitative aspects of the SFC interface probe are presently being studied. Furthermore, the probe could also be used for other microseparation techniques like high temperature open tubular LC–MS that has been reported recently [21].

Acknowledgements

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