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Capillary column supercritical fluid chromatography–atmospheric pressure ionisation mass spectrometry
Interface performance of atmospheric pressure chemical ionisation and electrospray ionisation

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Abstract

A supercritical fluid chromatography interface probe for atmospheric pressure ionisation mass spectrometry (API-MS) with the advantage of convenient switch between ionisation modes [atmospheric pressure chemical ionisation (APCI) and electrospray ionisation (ESI)] has recently been reported [P.J.R. Sjöberg, K.E. Markides, J. Chromatogr. A, 785 (1997) 101]. In order to obtain a stable ion signal and a low minimum detectable quantity, the design of the spray devise has to be optimised. For easy optimisation in the APCI mode, the corona needle was mounted directly on the interface probe. To compensate for the adiabatic cooling of the expanding mobile phase in the APCI mode, a heated region around the restrictor tip was used. In comparison, ESI required no additional heat, which might also prevent fragmentation for thermolabile compounds. As the mobile phase used was neat CO_2, a low flow of make-up liquid was utilised in the ESI mode for transfer of the analytes from the expanding CO_2 gas to the liquid phase before ionisation. The low make-up liquid flow in the ESI mode was sufficient for preventing the restrictor from becoming blocked. Factors that influence the ion signal intensity and stability have been studied. In APCI mode, corona needle position, nebuliser gas flow and gas additives were studied and in ESI mode, spray capillary assembly dimension and position, liquid flow-rate and composition were studied. The achievable detection limits were in the 50–0.1 pg (i.e., 290 fmol–140 amol) range. The detection limit in APCI mode was improved by a factor of about 20–25 compared to an earlier design [L.N. Tyrefors, R.X. Moulder, K.E. Markides, Anal. Chem. 65 (1993) 2835].

Keywords: Interfaces, SFC–MS; Instrumentation; Supercritical fluid chromatography–mass spectrometry; Atmospheric pressure ionisation; Detection, SFC; Anthracenes; Phenylamines; Fatty acids

1. Introduction

Supercritical fluid chromatography (SFC) has been demonstrated for the separation of a wide range of moderately polar compounds, including polymers, chiral and thermally labile analytes. Neat CO_2 as mobile phase can be used with well deactivated open tubular columns, thus making it possible to use the universal flame ionisation detection (FID). A relatively high resolution can also be obtained with these open tubular columns at temperatures where thermally labile compounds still can be separated.

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Packed column SFC, on the other hand, usually requires addition of modifiers with the added benefit of eluting more polar solutes. Mass spectrometry (MS) which can provide a selective and sensitive detection was early identified as an important detector for the different SFC techniques [3]. The research in interfacing SFC with MS has now been carried on for more than 20 years and resulted in several review articles [4–10].

The increasingly popular mild ionisation techniques which are performed at/or near atmospheric pressure, i.e., atmospheric pressure chemical ionisation (APCI) and electrospray ionisation (ESI), has several advantages in combination with SFC when compared to conventional low-pressure ionisation techniques [11]. A short summary of these developments were presented in a previous paper [1]. Due to the difference in general ionisation mechanism for ESI and APCI, the criteria for optimal performance during ionisation will also be different for the two ionisation techniques. In the pneumatically assisted ESI mode, which has been characterised as a concentration sensitive technique [12,13], it is important to control the factors that influence the optimum sheath liquid flow-rate like, for example, liquid sheath composition, mobile-phase flow, sheath liquid capillary and SFC restrictor dimensions as well as their relative position to each other. In the APCI mode, on the other hand, residence time versus reaction time for the ions in the ion source are important together with flow dynamics and thermochemistry of the gas phase [14–16] which will be influenced by the reactant ions present and the gas temperature.

In this paper, the performance of the previously reported interface [1] were studied with the aim of obtaining further knowledge on the limiting parameters for interfacing SFC with MS. The most influential instrumental and chemical parameters were investigated in order to optimise ion current signal and stability in ESI and APCI modes.

2. Experimental

2.1. Sample and solution preparation

Test mixtures of antracene, 9-phenylantracene, 9,10-diphenylantracene, diphenylamine, 2-nitrodiphenylamine, squalene, cholesterol, cholesteryl palmitate, α-tocopherol, α-tocopherol acetate, stearic acid methyl ester, pentacosanoic acid methyl ester, trilaurin, tripalmitin, tristearin and trioleinthe were dissolved in toluene from Merck (Darmstadt, Germany) – the compounds were all obtained from Sigma (St. Louis, MO, USA) except diphenylamine which was obtained from Merck. The sheath liquid flow was a solution of methanol–water (90:10, v/v) with the addition of 250 μ*M* sodium acetate. Methanol [liquid chromatography (LC) gradient grade), 2-propanol, ethyl acetate, chloroform, formic acid and sodium acetate were all of analytical-reagent grade quality and supplied by Merck. Sodium hydroxide (97–98%) was obtained from Eka Nobel (Bohus, Sweden). 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 laboratory design previously reported [2]. The present interface design has been reported elsewhere [1]. For the SFC–APCI mode, a minor but practically important modification was made compared to the previously reported design [1]. A special holder for the corona discharge needle was constructed in PTFE and mounted directly on the probe with a small screw as shown in Fig. 1. The nebuliser capillary was made from a 850 μm O.D.×700 μm I.D. fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) where the front end I.D. was reduced with the aid of a small acetylene–oxygen welding flame. In the first design [1] the I.D. was reduced to approximately 300 μm but later it has been further reduced to approximately 250 μm. The nebuliser gas was synthetic air of FID quality (AGA, Stockholm, Sweden) and with the 250 μm I.D. nebuliser capillary the gas flow was 0.2 l/min unless otherwise stated. The restrictor tip heating wire was placed around the nebuliser capillary to minimise the disturbance of the eluting gas flow profile. The potential applied to the restrictor tip heating wire was 6 V. The temperature of the nebulised gas stream into the

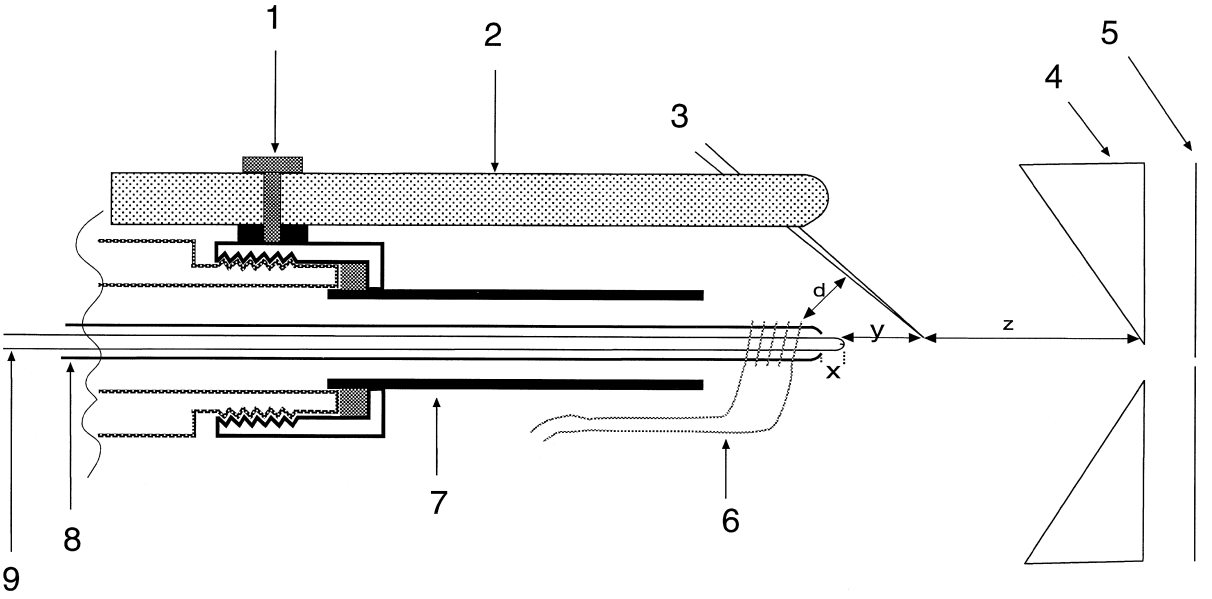

Fig. 1. Schematic drawing of the APCI interface probe tip. (1) Screw, (2) needle holder, (3) corona discharge needle, (4) curtain plate, (5) orifice plate, (6) restrictor tip heating wire, (7) auxiliary gas capillary, (8) nebuliser gas capillary, and (9) capillary column with integral restrictor.

ionisation region was measured in a separate experiment by thermocouple situated 1 mm in front of the nebuliser capillary. The co-axial auxiliary gas flow of synthetic air (AGA) was set to 1.0 l/min in all experiments to aid in minimising the chemical background.

For the SFC–ESI mode, the design was similar as previously reported [1], except that a fused-silica sheath liquid capillary of 400 μm O.D.×250 μm I.D. from Chrompack (Nacka, Sweden) was used. The end of this sheath liquid capillary was as in the original design [1] drawn out in a small welding flame and cut with a sapphire or cemented metal carbide knife to produce a fine tip with an I.D. of approximately 35 μm and an O.D. of approximately 80 μm. The column integral restrictor was first tapered before the tip I.D. was reduce to 1–2 μm. The fabrication of the tapered column restrictor was done by the aid of a fusion splicer (PFS500 Fiber Splicer, Power Technology, Little Rock, AK, USA). By positioning the column in the electric arc and simultaneously applying a slight dragging force to one end of the column a small tapered waist can be fabricated. The column is then cut at the waist and the tapered end are once again placed in the electric arc in order to reduce the tip I.D. to 1–2 μm. The spray assembly is schematically shown in Fig. 2.

The sheath liquid capillary was positioned inside the nebuliser capillary (850 μm O.D.×700 μm I.D., tip I.D. 250 μm, Polymicro Technologies) with the tip sticking out approximately 0.5 mm. A nebulisation gas (AGA) flow of 0.6 l/min was used. A stereo microscope (SMZ-1B, Nikon, 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 polyether ether ketone (PEEK) union (Upchurch Scientific, Oak Harbour, WA, USA) was used as a high-voltage insulator between the heated transfer line and the front connections. A Harvard Apparatus (South Natick, MA, USA) syringe infusion pump Model 22 was utilised with a 250 μl gas-tight syringe (Hamilton, Reno, NV, USA) for delivering the liquid sheath flow.

2.3. MS conditions

A PE-Sciex API III$^+$ triple quadrupole mass

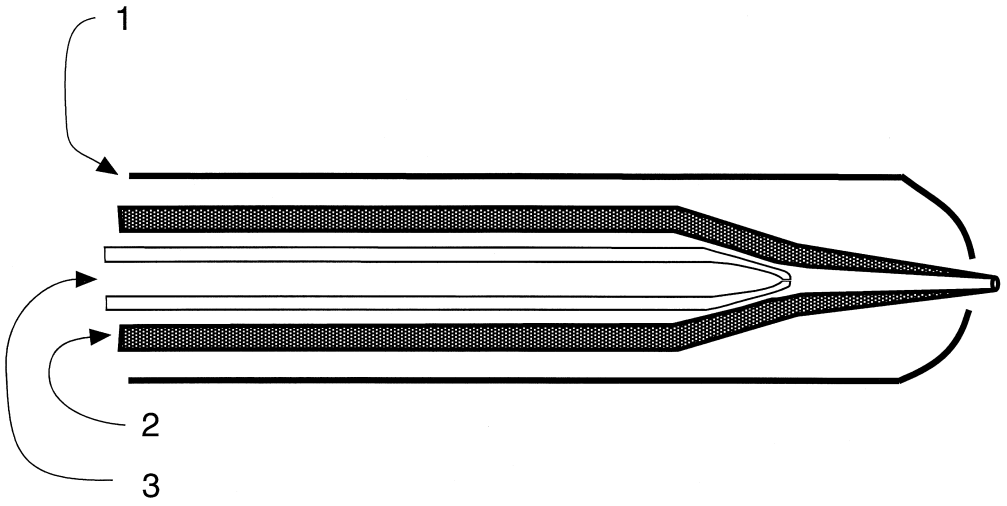

Fig. 2. Schematic drawing of the ESI interface probe tip. (1) Nebuliser gas capillary, (2) sheath liquid capillary, and (3) capillary column with tapered integral restrictor,

spectrometer (Concord, Canada) equipped with a point-to-plane corona discharge ion source was used for this study. For the APCI mode, the discharge current was 1 μA and the distance to the interface plate was 5 mm unless otherwise stated. The potential was set to 650 V at the interface plate, 60 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 60°C) was 1.2–1.4 l/min over the sampling orifice. 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 in APCI mode using protonated water clusters obtained by a decrease of the dry nitrogen curtain gas flow. Data was acquired either by selected ion monitoring (SIM) or full scan approximately 150–950 u both at unit mass resolution. A dwell time of 30 ms (unless stated otherwise) was used in SIM mode. Optimisation of the interface in the APCI mode, was performed with continuous slow supercritical fluid extraction (SFE) as described previously [2] using an oven temperature of 90°C, a CO_2 pressure of 120–140 atm and with pentacosanoic acid methyl ester (approx. 50 μg) loaded onto a small C_{18} LC pre-column (1 atm=101 325 Pa).

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 [17,18] was employed for direct injections (30 s) into a 5 m×50 μm I.D. capillary using a helium actuated injection valve, CI4W Valco (Houston, TX, USA), equipped with a 60-nl sample rotor loop. Separations were performed with a 30%-biphenyl-substituted column (Dionex) with 0.25 μm stationary phase film thickness, 10 m×50 μm I.D. using a density program at 100°C, (start density 0.1675 g/ml, density gradient 0.01 g ml^{-1} min^{-1} to 0.20 g/ml, then 0.05 g ml^{-1} min^{-1} to 0.50 g/ml and finally 0.04 g ml^{-1} min^{-1} to 0.76 g/ml). 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 vespel ferrule as reported recently [19] (1 in.=2.54 cm). An integral restrictor [20] of 1–2 μm was made from the analytical column end as described above.

3. Results and discussion

When highly efficient micro-analytical techniques are hyphenated to MS detection, accurate performance throughout the interface has to be retained. Although miniaturisation of separation techniques has resulted in enhanced speed and efficiency, it also requires lower flow-rates and puts higher demands on optimum connections in order to avoid dead volumes and band broadening. At the same time, lower flow-rates to the atmospheric pressure ion source result in improved ionisation and sampling efficiency which will give higher sensitivity. Since the first report of an SFC–MS interface probe for APCI and ESI [1] further efforts has been made to improve ion signal stability, sensitivity and also to achieve faster and easier optimisation.

3.1. APCI interface performance

All commercial interfaces for APCI are designed for use with a standard LC column having a mobile phase flow of about 1 ml/min. This high flow-rate produce a quite large nebulised aerosol and the position of the corona discharge needle will have a broad optimum.

As predicted by theory [14,16,21], highest sensitivity in APCI will be obtained if the density of the neutral analyte and reagent ions are high. In micro-column separations with a low mobile-phase flow-rate and small cross section area of the nebulised effluent it is important that the corona needle tip is positioned close to the nebulised effluent in order to obtain optimal sensitivity. In Fig. 1, the APCI probe tip is shown were a special needle holder made of PTFE is attached directly to the probe. The holder was constructed in such a way that it allows for easy position of the needle tip in front of the small cross section off the nebulised column effluent. The needle tip position relative to the nebuliser capillary was adjusted with the aid of a stereo micro scope. The optimisation procedure was greatly simplified when the probe could be freely moved around in the ion source and the optimum position relative to the sampling orifice could be found more easily. The optimal position of the needle tip relative the nebuliser capillary was first determined with the needle mounted in the original holder supplied with the instrument.

In this study it also became clear that a stable corona current is important if a stable ion current should be obtained. The API III$^+$ instrument has the possibility to monitor and store the discharge current while the instrument is collecting data. In Fig. 3, traces of ion current and discharge currents (2 μA) are shown when continuous slow SFE was used. Just before 1 min, the heating wire voltage (6 V) was turned on and, as can be seen in the figure, both the ion and discharge currents becomes unstable. When the distance (*d* in Fig. 1) between the heating wire and needle was increased by sliding the heating wire backwards, only a small spike appeared, in the otherwise stable ion current trace, when the heating

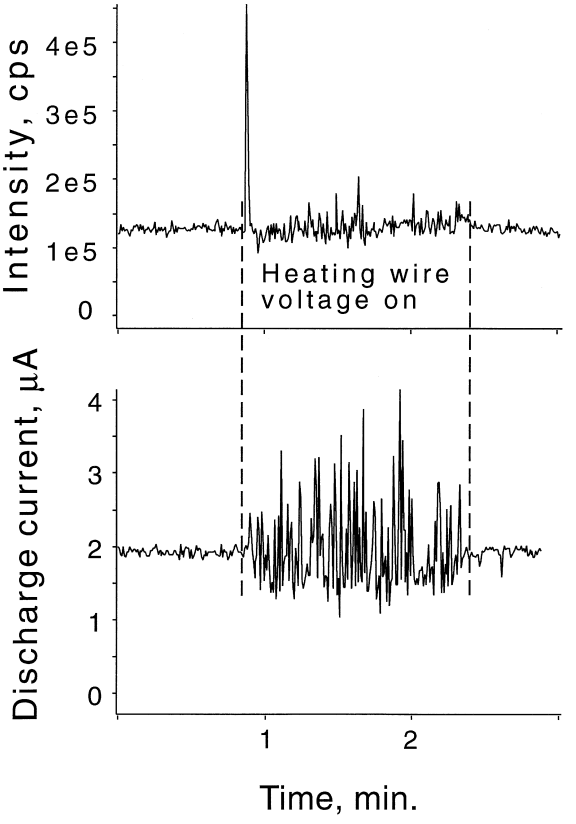

Fig. 3. Continuous slow SFE–APCI traces of ion current (m/z= 391, dioctylphtalate) and discharge currents (2 μA) for a distance *d* (Fig. 1) of 1 mm.

wire voltage was turned on. The distance *d* needs to be at least 1.5 mm to avoid this signal disturbance.

The distance between the corona needle and interface plate (distance *z* in Fig. 1) is an important parameter that will influence the residence time/reaction time for the ions in the ion source. APCI with a corona needle is a unipolar ion source, i.e., all ions present are either positive or negative. At high ion densities, unipolar ion sources become space charge dominated, i.e., coulombic repulsion between ions leads to decreased ion density which will induce ion sampling losses and reduced absolute sensitivity [16]. In order to maximise the ion density, the ions should be sampled as fast as possible, i.e., using a small needle/orifice distance. Reduction of the needle/orifice distance will, however, also cause a decreased ion residence time which will induce loss in sensitivity. In our study, both the signal and signal-to-noise ratio (S/N) was optimised at a corona needle interface plate distance, *z*, of 5 mm. When the distance was decreased from 15 mm to 5 mm, in 2–3 mm increments, the signal increased by a factor of about 2–17 and S/N increased by a factor of about 1–21 for the compounds listed in Table 1. When the distance was further decreased to 3 mm, the signal decreased by a factor of 5–30 and S/N decreased by a factor of 1–27. Optimum sensitivity for ambient air monitoring were reported [22] to be obtained at a needle/orifice distance of 5 mm using a Sciex TAGA 6000 MS–MS instrument. In a more recent paper, Lee et al. [23] investigated the effect of the needle/orifice distance and reported that optimum sensitivity were obtained at a distance of 3 mm. Several processes influence the ion transport in a high pressure ion source and that could probably explain the differences in the reported optimum needle/orifice distances. The main processes influencing the ion transport have been reported to be electrostatic transport, i.e., ion drift in the direction of the electrical field; diffusive transport from higher to lower concentrations and convective transport, where ions are transported with the gas stream [16]. The importance of gas flow dynamics in APCI has been considered and several different nebuliser capillaries with different tip I.D. were thus evaluated using continuous slow SFE. In the original design [1], a tip I.D. of 300 μm was used and the ion signal was optimised at a nebuliser gas flow of 0.4 l/min. In this design a capillary I.D. of 250 μm was used and the ion signal was optimised at a nebuliser gas flow of 0.2 l/min. Both set-ups give a linear gas flow-rate of about 160 m/s. The ion signals in APCI were also found to be influenced by the ion source exhaust pump. When the pump was turned on, the background signal decreased slightly and the analyte signal became unstable possibly caused by a more turbulent gas flow in the ion source. It has been reported that the use of the exhaust pump improved the peak shapes, possibly by reducing the re-circulation of sample vapour into the ionisation region [1,19]. However, when only small amount of analytes (low pg) were injected and separated, no such effect was apparent and the ion source exhaust pump was therefore left off.

When continuous slow SFE–APCI was used, the relative position of the restrictor tip to the nebuliser capillary and corona discharge needle was manipulated. A positioning of the restrictor tip about 0.2 mm from the needle tip resulted in an unstable signal (RSD=26%), but as the distance, *x* in Fig. 1, was increased slightly to about 0.7 mm a more stable signal (RSD=6%) was obtained. The optimum distance, *y* in Fig. 1, between the nebuliser capillary

Table 1
Detection limits in SIM mode, $S/N=3$

Compound	APCI (pg)	ESI (pg)
Antracene	50	nd
9-Phenylantracene	0.4	nd
9,10-Diphenylantracene	2	nd
Diphenylamine	0.3	0.2[c]
2-Nitrodiphenylamine	2	2
Squalene	0.1	1
Cholesterol	0.4[a]	na
Cholesteryl palmitate	0.8[b]	na
α-Tocopherol	40	na
α-Tocopherol acetate	0.1	0.2
Stearic acid methyl ester	1.1	1.0
Pentacosanoic acid methyl ester	0.3	0.2
Trilaurin	0.4	0.3
Tripalmitin	0.5	2
Tristearin	0.7	4
Triolein	0.3	3

nd=Not detected.
na=Not analysed.
[a] $[M+H\text{-}H_2O]^+$.
[b] $[M+H\text{-}C_{16}H_{32}O_2]^+$.
[c] $[M+H]^+$.

and corona discharge needle, 0.8 mm, was first determined with the needle mounted in the original holder.

The potential applied to the restrictor tip heating wire in combination with the gas flows will influence the gas temperature which is an important parameter in the APCI process [15]. The ion current for pentacosanoic acid methyl ester optimised at an applied voltage to the restrictor tip heating wire of 6 V which corresponds to a gas temperature of 120–130°C. If the applied potential was to low, the risk of restrictor plugging increased, on the other hand, if the applied potential was to high, the ion current signal became more unstable and the risk of thermal induce fragmentation increased.

Control of ion source chemistry is important in SFC–APCI-MS in order to obtain optimal quality of both signal and spectra. A stable generation of reagent ions could be achieved by addition of solvent vapour to the nebuliser gas. Several different solvents were tested including water, methanol, ethanol and 2-propanol. The response for different analytes were evaluated and, as expected, the gas phase thermochemistry was of importance. Difference in proton affinity or gas phase basicity (GB) between the analyte and the reagent ions as well as solvation energy differences are important parameters for analyte sensitivity. An in depth discussion can be found elsewhere [14,15,24]. In the recent study, for example, the sensitivity decreased by a factor of 10 for stearic acid methyl ester when water (GB=660 kJ/mol) was replaced with methanol (GB=724 kJ/mol) as reagent ion solvent. On the other hand, the sensitivity for diphenylamine and trilaurin did not change probably because the GB difference between the analyte and the reagent ions in this case was sufficiently large. This indicates that, to obtain high analyte sensitivity it can be advantageous to use capillary SFC with neat CO_2 whenever possible for analytes with low GB instead of packed column SFC or LC where methanol is a common component in the mobile phase.

In the APCI mode, due to improvements of our last reported design the detection limit for antracene was decreased by a factor of 20 from 1 ng [2] to 50 pg and trilaurin by a factor of 25 from 10 pg to about 0.4 pg. In a recent publication [30] utilisation of a commercial LC–APCI interface without any modifications resulted in a detection of the triacylglycerols (1,3-dipalmitoyl-2-oleoyl-*sn*-glycerol) of about 200 pg at a S/N of 5 using scan mode. In this study, triacylglycerols could be detected at a level of 0.3–0.7 pg in the APCI mode which corresponds to an improvement by a factor of at least 280. In the same report [30] the optimum distance between the restrictor and corona needle was quite large, i.e., 2.2–2.4 cm, compared to our design were the distance is only about 0.7 mm. In our design, the signal decreased by a factor of 7 when the corona needle restrictor tip distance was increased to 2 mm with the same nebuliser gas flow and the needle position relative to the sampling orifice. This clearly points out that it is important to optimise the interface hardware.

3.2. ESI interface performance

Optimisation of the interface in the ESI mode was first performed with pentacosanoic acid methyl ester dissolved in the make-up liquid followed by addition through continuous slow SFE. The analyte ion signals, $(M+H)^+$ and $(M+Na)^+$, optimised at a rather high electrospray voltage of 4 kV with a distance between the emitter and the counter electrode of 15 mm. Furthermore, detailed experiments were performed in order to study the ionisation mechanism and the results obtained indicates that a mixed ionisation mechanism between liquid phase ionisation, i.e., ESI, and gas phase ionisation, i.e., APCI is present. The detailed results of these experiments are presented elsewhere [25].

For the ESI mode, 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 a stereo microscope was of great help during the assembling process of the spray device. In the previous design [1] an integral restrictor was made directly from the square cut analytical column end. In this new design, see Fig. 2, the column end was first tapered as described above before the integral restrictor was made. The tapered restrictor in combination with a smaller I.D. (250 μm) sheath liquid capillary made it possible to reduce the make-up liquid flow-rate from 20 μl/min used in the original design [1] to about 4 μl/min without significant increase in baseline noise. When the flow-rate was further decreased the

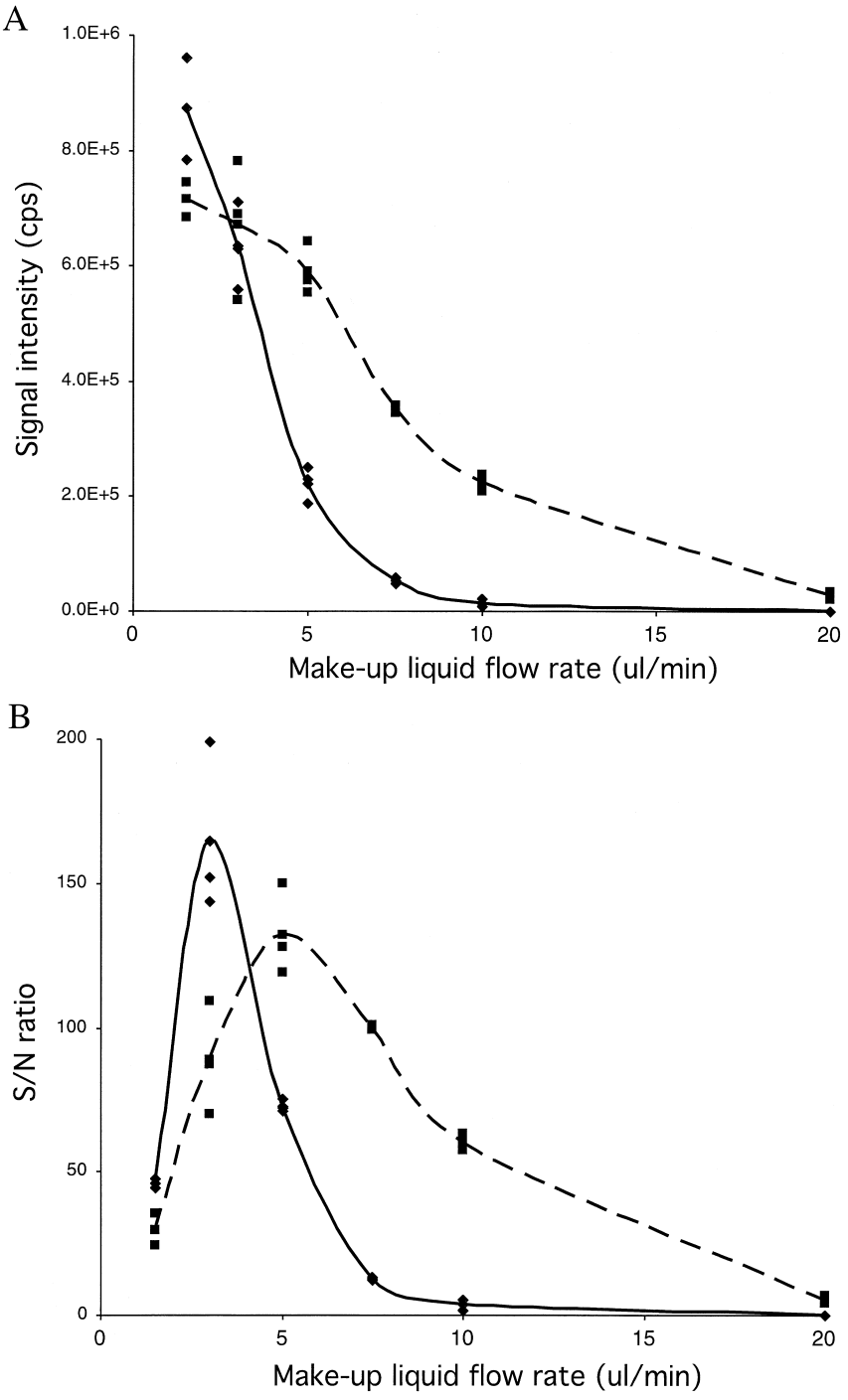

Fig. 4. (A) Signal level and (B) *S*/*N* ratio for pentacosanoic acid methyl ester at different make-up liquid flow-rates, showing the ions ♦ $(M+H)^+$ and ■ $(M+Na)^+$. The sheath liquid solvent was water–methanol (10:90, v/v) with 250 μ*M* of sodium acetate.

baseline noise increased with occasional spikes. The signal level and the *S*/*N* ratio for pentacosanoic acid methyl ester can be seen in Fig. 4. Pneumatically assisted ESI or ion spray has been characterised as a concentration sensitive ionisation technique in the flow range of 1–100 μl/min [12,13]. The $(M+Na)^+$ ion signal increased by a factor of about 20 as the make-up flow-rate was decreased from 20 μl/min to 5 μl/min. The decrease in dilution of the column effluent is, however, only a factor of 4, which could indicate that ion desolvation is more efficient at lower liquid flow-rates. When the *S*/*N* ratio was considered, see Fig. 4B, it could be concluded that the ratio maximise at 3 μl/min for the $(M+H)^+$ ion and at 5 μl/min for the $(M+Na)^+$ ion. The reason for the increase in the $(M+H)^+$ ion signal when the flow-rate was decreased could be an effect of an increased H^+ concentration produced from the electrochemical oxidation of water or increased influence of gas phase ionisation [25].

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 [26]. In this study, several different make-up liquid solvent combinations where tested, including: methanol–water (90:10); methanol–2-propanol–water (45:45:10); as well as methanol–ethyl acetate–water (80:10:10) and methanol–chloroform (30:70). The best performance was achieved with methanol–water (90:10). Most of the ion signals decreased when more hydrophobic solvents were used, like 2-propanol and chloroform, which partly could be explained by the theory that the analyte partitioning between the droplet surface and bulk solution [27] is shifted towards the latter. For ESI of triglycerides, methanol–chloroform (30:70) with 10 m*M* of sodium acetate has previously been used [28]. In this study, however, the sodiated triglyceride ion signals decreased by a factor of about 3 and the total electrospray current decreased by a factor of about 1.7 when methanol–water (90:10) was replaced by

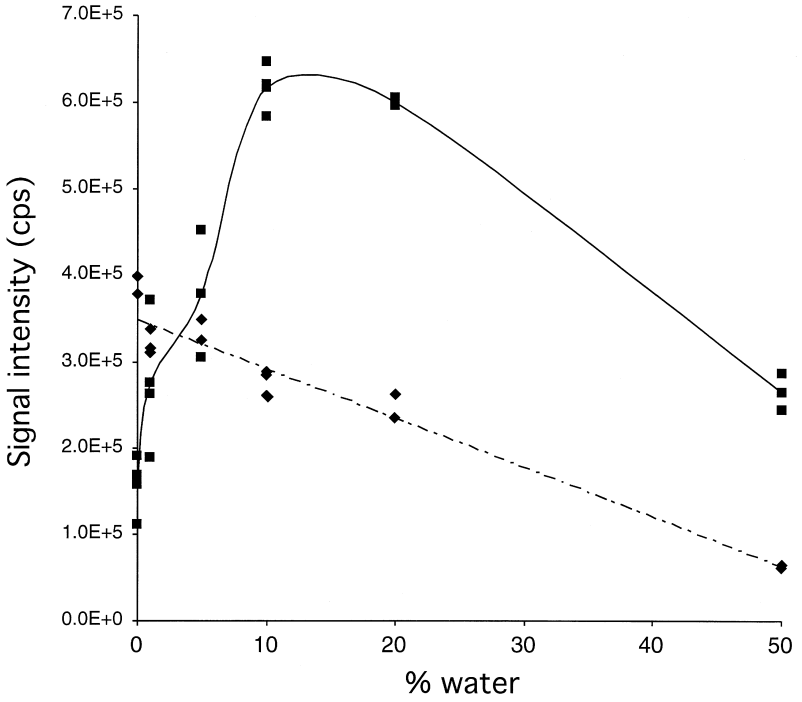

Fig. 5. Signal level as a function of water content of the sheath liquid solvent for stearic acid methyl ester [♦ $(M+Na)^+$] and diphenylamine [■ $(M+H)^+$]. The sheath liquid consisted of water–methanol with 250 μ*M* of sodium acetate.

methanol–chloroform (30:70). In both experiments the additive was sodium acetate at a concentration of 250 μ*M*.

Several alternative additives to a methanol–water (90:10) make-up liquid where tested, including formic acid, sodium hydroxide and sodium acetate. The additives were tested at a concentration of 50 μ*M*, while sodium acetate were additionally tested at 250 and 500 μ*M*. The best overall performance was achieved with 250 μ*M* of sodium acetate.

When methanol–water was used as make-up liquid with the addition of 250 μ*M* of sodium acetate, the general trend for the analytes tested was that the sodiated ion adduct signal increased when the water content was decreased which is shown in Fig. 5 for stearic acid methyl ester. The *S*/*N* ratio was optimised at a water content of 10–20%. For analytes that preferred to form protonated ion adducts, for example diphenylamine, the ion signal was also optimised at 10–20% of water as can be seen in Fig. 5. When the water content was decreased to 5% or lower, the ion signal became slightly more unstable and the *S*/*N* ratio decreased which agree with earlier observations by Pinkston and Baker [29].

3.3. Comparing APCI with ESI

When comparing the API methods it was found, as expected, that the fragmentation pattern differed. For example, the fragmentation of several triacylglycerols i.e., the elimination of different fatty acyl residues $(M\text{-}RCOO)^+$, was compared between APCI and ESI modes. No significant difference in the degree of fragmentation was observed when the ratio between $(M+H)^+$ and $(M\text{-}RCOO)^+$ was compared. Less fragmentation in ESI mode was, however, observed when the $(M+Na)^+/(M\text{-}RCOO)^+$ ratio in the ESI mode was compared with the $(M+H)^+/(M\text{-}$

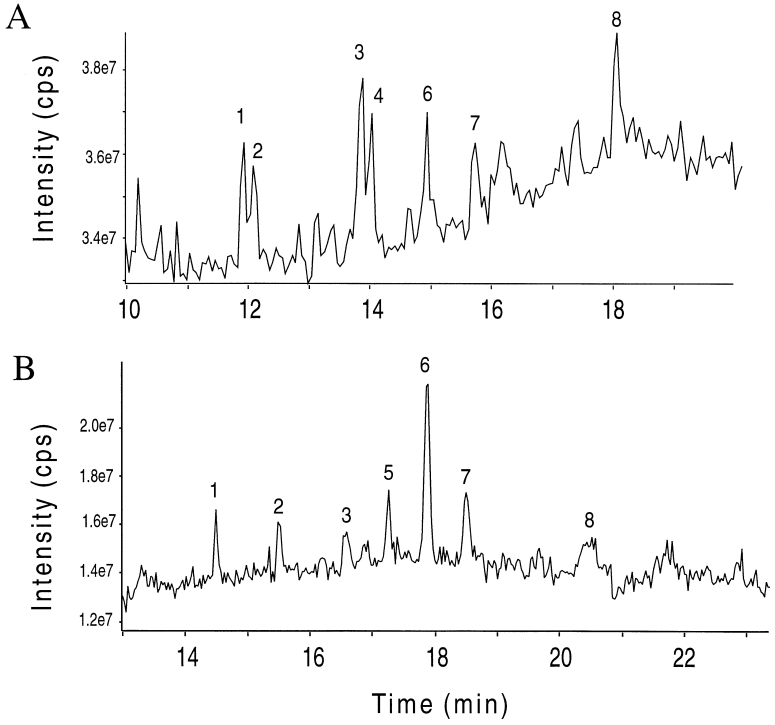

Fig. 6. SFC–MS performance at full scan in (A) APCI mode and (B) ESI mode. A test solution was injected at (APCI) 30–50 pg levels and (ESI) 150–250 pg levels. Scan ranges were in (APCI) 150–900 and in (ESI) 160–950. Step size was 0.5 u with a dwell time of 1 ms. In the APCI mode, water vapour was added to the nebuliser gas. In the ESI mode, the sheath liquid solvent was water–methanol (10:90, v/v) with 250 μ*M* of sodium acetate at a flow-rate of 4 μl/min. For SFC conditions, see text. Peaks: 1=stearic acid methyl ester, 2=diphenylamine, 3=pentacosanoic acid methyl ester, 4=squalene, 5=2-nitrodiphenylamine, 6=α-tochopherol, 7=trilaurine, 8=tristearin.

$RCOO)^+$ ratio in the APCI mode. No sodiated fragment ions were observed in the ESI and it is well known from the literature that sodiated ions are more difficult to fragment compared to protonated ions.

Fig. 6 shows another comparative example of the system performance in APCI and ESI mode. A test solution, containing the compounds listed in Table 1, was injected and several peaks were clearly visible at low pg levels even in the full scan mode. The detection limit for both ionisation modes were evaluated using three replicate injections in SIM detection mode and the results are shown in Table 1. In the ESI mode, the make-up flow-rate were set as low as possible (i.e., 4 μl/min) without significantly increase of the baseline noise. The difference in ionisation mechanism in APCI and ESI result, as expected, in non detectability of some neutral analytes in ESI but the two sources otherwise stands out as two comparable alternatives

4. Conclusions

This study has clearly shown the importance in optimising the hardware when microcolumn separation should be interfaced to MS. The sensitivity achievable for the test compounds in this study were, in ESI mode, in the low femtomole range and, in APCI mode, in the mid-attomole range for most of the compounds tested.

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