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Packed-column supercritical fluid chromatography coupled with electrospray ionization mass spectrometry

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ABSTRACT

A new approach to combined supercritical fluid chromatography–mass spectrometry (SFC–MS) was explored using a two-pump supercritical fluid chromatograph, and a packed column with the outlet directly interfaced to an electrospray (ES) ion source attached to a single quadrupole mass spectrometer. The experimental set-up is described and preliminary results are reported for mobile phase flow-rates in the range 0.15–1 ml/min, using CO_2 modified by the addition of 1–30% (v/v) of a polar organic solvent. The combined system has a greater potential for the analysis of polar molecules by MS than earlier SFC–MS instruments that utilized capillary column SFC directly coupled to the ion source of a chemical ionization mass spectrometer. A liquid-phase ionization process was utilized for solute ion formation, and it could be applied to the determination of high-mass and polar molecules separated by packed column SFC; however, the MS response is dependent on the mobile phase composition and the SFC–ES-MS instrument is still limited to the determination of low-mass samples owing to cold trapping on some critical surfaces.

INTRODUCTION

Several methods have been devised during the last decade to provide an effective coupling of supercritical fluid chromatography with mass spectrometry (SFC–MS). Nevertheless, the results have not convinced MS instrument manufacturers to promote any of these methods strongly, and SFC–MS users have to custom-build their machines. As a consequence, the number of instruments in routine use remains low. In a previous paper [1], the reasons for this particular situation were discussed, and it is now clear that SFC–MS coupling is not as easy to conceive as was thought in the early 1980s. In particular, the method referred to as direct coupling or direct fluid interfacing, consisting of directly connecting a capillary SFC column to the ion source of a chemical ionization mass spectrometer, has been plagued by inherent limitations, the most severe one being the poor MS response with high-mass molecules when the supercritical fluid pressure is varied over a wide interval, and with ionization produced by charge exchange with $CO_2^{+\bullet}$ primary ions.

In contrast, the situation of combined liquid chromatography–mass spectrometry (LC–MS) is brighter today. After many years of progression at a moderate rate [2], LC–MS has progressed rapidly in recent years because it has benefited from the explosive development of atmospheric pressure MS sources utilizing electrospray (ES) ionization [3] (no distinction is made here between electrospray and its pneumatically assisted variant called ionspray). Because LC–MS and

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SFC–MS have often followed similar routes, it is not surprising that the atmospheric pressure ion source (API) has been considered for potential use in a combined SFC–MS instrument, although the number of reports of this union is still low.

With one exception in which cationization with lithium primary ions was used for solute ionization [4], other reports on SFC–MS with an API source have described the production of sample ions from supercritical solutions by gas-phase ionization in a corona discharge, following total solution vaporization. Because of the gas-phase nature of the ionization process, the restrictor at the column end was generally set at temperatures well above 200°C. For example, gas-phase ionization by corona discharge was used together with packed column SFC, eluted with either neat CO_2 or CO_2 modified by the addition of a few percent of a polar organic solvent, for the determination of steroids in biological matrices [5], polyaromatic hydrocarbons in coal tar and sand oil extracts [6] and various synthetic mixtures including polyethylene glycol and polystyrene oligomers and vitamins [7].

We have assembled an instrument that is similar to those described by others [5–7], with the notable exception that the corona needle electrode was omitted. Supercritical solutions of polar basic solutes in CO_2 modified with organic solvents were introduced at mobile phase flow-rates compatible with the use of packed columns, *i.e.*, 150–1000 μl/min. We observed that by maintaining the restrictor at a low temperature, and by establishing an electrical potential of a few kilovolts between the restrictor end and the MS sampling orifice, both solvent and sample ions were produced. The conditions appeared to be identical with those existing in conventional electrospray ionization, a liquid-phase ionization process that is known to require the presence of charged liquid droplets. Such charged liquid droplets can be transiently obtained during decompression and cooling of the expanding subcritical jet at the restrictor outlet, thus allowing solvent-derived cluster ions and preformed sample ions to escape from the charged droplets into the gas phase, and be mass analysed. The light-scattering detector modified for SFC monitoring [8] is another case of an SFC detector that similarly utilizes liquid droplets transiently formed in the decompressed SFC fluid. In this paper we describe the experimental set-up, report preliminary results and discuss possibilities and limitations of electrospray ionization applied to SFC–MS coupling.

EXPERIMENTAL

Chemicals

HPLC-grade solvents were obtained from Merck. Atrazine was obtained from Cluzeau Info Labo (Sainte-Foy-la-Grande, France) and 1-chloro-2-amino pyridine (M_r = 128) and 1-chloro-2-(N-*tert.*-butylcarbamate)pyridine (M_r = 228) were provided by the Biochemistry Department of the CEA in Saclay (France). In this text, the mass 128 and 228 pyridine derivatives are referred to as Py-I and Py-II, respectively.

Supercritical fluid chromatography (Fig. 1)

High-purity (grade N48) liquid carbon dioxide supplied in cylinders with a dip tube (Air Liquide, Le-Plessis-Robinson, France) was introduced directly into a Gilson (Villiers-le-Bel, France) Model 305 single-piston reciprocating pump, fitted with a Model 10SC pump head, and modified for SFC operation. The polar solvent modifier was pumped by a Gilson Model 307 pump equipped with a Model 5SC pump head.

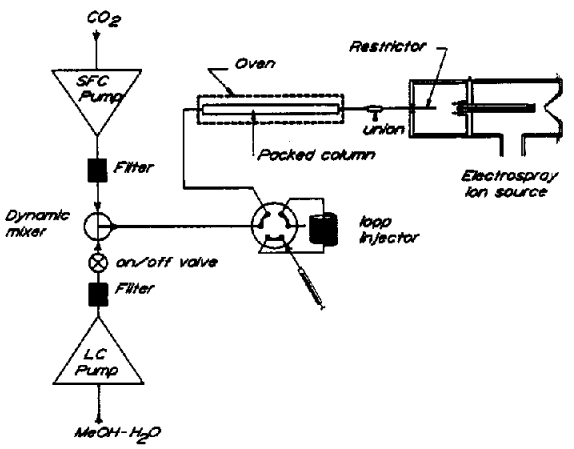

Fig. 1. Schematic diagram of the packed column SFC system utilizing gradient elution of a polar modifier in CO_2.

Both effluents were combined using a Gilson Model 811C dynamic mixer. The sample injector was either a Rheodyne Model 7520 fitted with a 5.0-μl sample loop or a Valco Model LC206 with a 1-μl internal loop, depending on the analytical SFC column diameter. Columns were either 150 mm × 2.1 mm I.D. 5-μm Zorbax Rx-C_{18} or 150 mm × 1 mm I.D. 5-μm Hypersil C_8. The column temperature was maintained constant in the range 40–100°C by an HPLC-type column heater and controller (Model Croco-Cil, Cluzeau Info Labo). Higher column temperatures, up to 200°C, were obtained by placing the column inside the oven of a gas chromatograph. The restrictor was attached at one end to the analytical column by a zero-dead-volume connector (Valco, part number CEF 1.5). The other end was positioned directly in the electrospray ion source, as described below. Valco and Rheodyne equipment and analytical columns were purchased from Touzart et Matignon (Vitry-sur-Seine, France).

SFC–MS interface (Fig. 2)

The end tip of the restrictor was located at *ca.* 5 cm and in direct line of sight with the ion sampling aperture. Linear restrictors were generally used and were made of 25–30 cm long fused-silica tubing (SGE, Villeneuve-St-George, France) of 60 and 25 μm I.D. when coupled to 2.1 and 1 mm I.D. columns, respectively. Proper electrospray ionization requires that the nebulizer be electrically conducting, to avoid disturbing charge build-up. Consequently, the polyimide coating of the fused-silica tube was covered with an electrically conducting layer of nickel-doped polyurethane paint (Aero 7465 antistatic paint; MAP, Pamiers, France) over a length of *ca.* 10 cm. In general, the restrictor end was kept at ground potential, and was only warmed by the surrounding nitrogen bath at 80°C in the atmospheric pressure ion source.

Another restrictor type was used occasionally and consisted of a stainless-steel tube (30 cm × 1 mm I.D.) crimped at the terminal end, such that a pressure drop of 100 bar was measured when the mobile phase flow-rate was 1 ml/min of CO_2. The restrictor end in the API source was heated at temperatures up to 300°C by a coil of resistance wire (Thermocoax, Suresnes, France).

Mass spectrometry (Fig. 2)

The mass spectrometer was a Nermag Model R1010C (Quad Service, Argenteuil, France) single-quadrupole instrument equipped with an Analytica (Branford, CT, USA) electrospray source and a Spectral-30, version 3.70, data system (P2A Système, Vincennes, France). With the exception of the above sample-introduction device, the Analytica electrospray source was used without modification, and typical operating voltages are listed in Fig. 2. A stream of dry nitrogen, at a flow-rate of 9 l/min and a temperature of 80°C, built a small positive pressure into the ES source that prevented the introduction of contaminants from the laboratory atmosphere, assisted solution vaporization and broke heavy ion clusters prior to MS analysis.

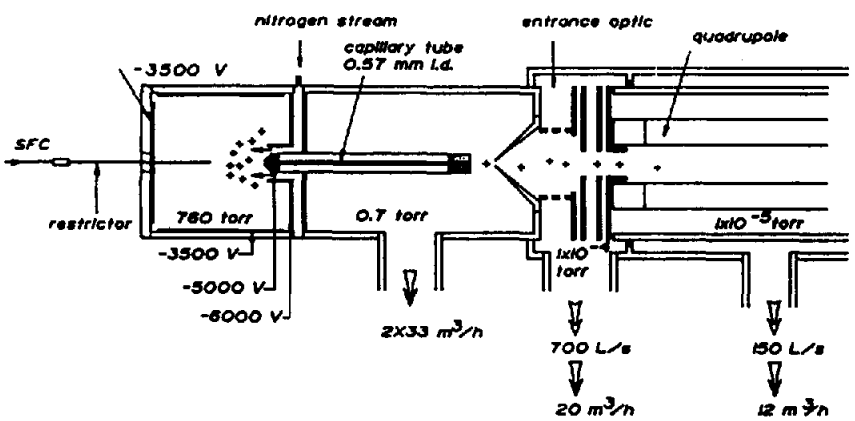

Fig. 2. Schematic diagram of the SFC–MS interface and electrospray ion source.

RESULTS AND DISCUSSION

Fluid temperature conditions

Both solvent cluster ions and solute-derived ions are observed using the described instrument when the supercritical fluid temperature is kept within a given range of temperature conditions, for a given flow-rate of the mobile phase. The temperature was varied either by changing the column oven temperature, up to 200°C, with the metallized fused-silica restrictor end only warmed by the nitrogen bath at 80°C into the ES source, or alternatively by keeping the column oven at 80°C and by varying the pinched metal restrictor temperature up to 300°C, using the resistor wire. Both conditions produce the same effects. Constant ion production is observed over a large temperature interval (typically 40–100°C) and is followed by a regularly decreasing ion signal at higher temperatures. As an example (Fig. 3), using a 25 μm I.D. fused-silica restrictor, the 1 mm I.D. SFC column and a fluid flow-rate of 250 μl/min, the plot of the MH^+ ion abundance for atrazine ($MH^+ = 216$) as a function of the column oven temperature declines regularly above 120°C and vanishes above 200°C. The regular ion current decrease for temperatures above 120°C also affects the ion currents (not shown) for solvent-derived clusters at low masses, and no total ion current is observed above 200°C. A similar trend was observed when the column oven temperature was kept constant at 80°C and the pinched metal restrictor tip was heated, causing the disappearance of any ion current at restrictor temperatures above 200°C. The workable temperature range at a nearly constant solute response is large enough to encompass common SFC requirements. Nevertheless, reducing the fluid flow-rate narrowed the operating temperature interval, thus showing that heat power to the vaporizing fluid is the relevant experimental factor.

This general SFC–ES-MS trend is similar to the decrease in solvent and solute ions in a thermospray experiment when heat power to the capillary nebulizer exceeds some upper temperature limits [9]. It is now established that optimum ion production in thermospray MS occurs when nearly complete vaporization of the liquid solution is achieved, with a mist of small droplets emerging from the capillary nebulizer. There is much evidence that electrospray ionization can occur in a conventional thermospray ion source, but the process is frequently obscured by gas-

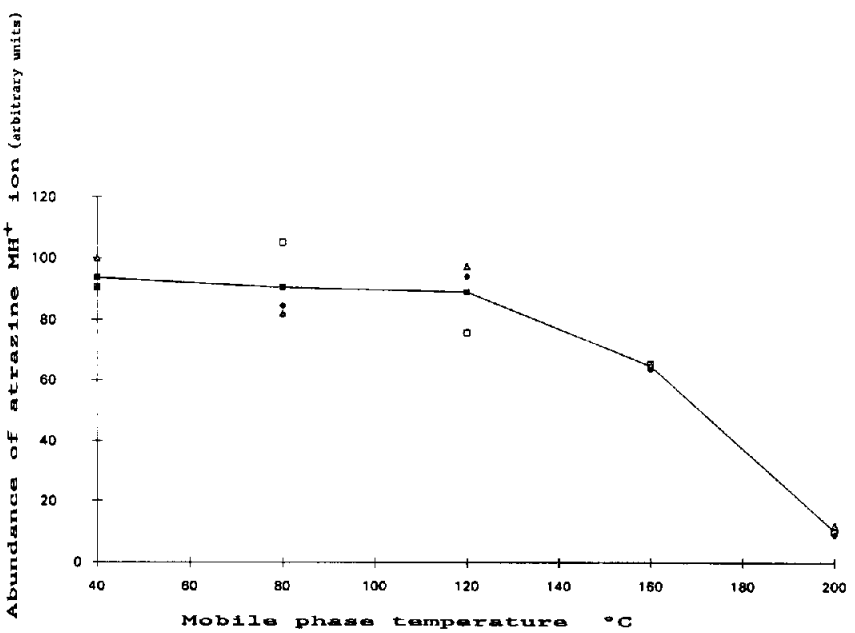

Fig. 3. Effect of the mobile phase temperature on the intensity of the atrazine MH^+ ion. Linear metallized fused-silica restrictor, 25 cm × 25 μm I.D.; 1 mm I.D. SFC column; mobile phase composition, 8% MeOH–H_2O (95:5, v/v) in 250 μl/min of CO_2. Data from three sets of experiments with the plot joining mean values.

phase CI reactions. Too high a heat power applied to the vaporizing fluid causes total evaporation inside the capillary, a condition that is known to be undesirable, and in particular, excessive heat is at the origin of thermally induced dissociations frequently observed in thermospray mass spectra of fragile solute molecules [10].

We believe that a same limitation exists with our system. Fluid decompression and cooling produce liquid droplets that are charged by the electrical field beyond the nebulizer end tip. This droplet formation makes possible electrospray ionization in our API source. When excessive heat power is applied prior to fluid decompression, a dry gaseous jet is formed at the nebulizer tip and no ion current is produced.

It should be noted that, under our experimental conditions, low-mass volatile samples can also be ionized by gas-phase CI reactions induced by methanol-derived clusters. During SFC–MS analysis of some low-mass compounds, *e.g.*, Py-I, a decreasing abundance of the solvent cluster ions was recorded when the solute eluted, thus producing an inverted chromatographic elution profile. Such a reactant ion behaviour generally proves the occurrence of gas-phase CI reactions.

SFC mobile phase composition

Solvent and sample ion generation strongly depends on the mobile phase composition. Pure CO_2 produced no ion for any investigated fluid temperature and flow-rate and API source voltage conditions. The similarity with the absence of ions when non-polar solvents are used in thermospray experiments is also notable [10]. On the other hand, solvent- and solute-derived ions were present when a minimum amount of a polar solvent was added to the mobile phase. Currents for solvent ions (Figs. 4 and 5) and protonated sample molecules (Fig. 6) were recorded when the percentage of a polar mixture of methanol, containing 5% of water, was varied at a constant CO_2 flow rate of 1 ml/min. The observed solvent-derived ions and clusters (Fig. 4) correspond to H_3O^+ (m/z 19), $(CH_3OH)_nH^+$ with $n = 1$, 2 (m/z 33, 65), and a mixed cluster $(H_2O)(CH_3OH)\,H^+$ (m/z 51). The presence of an ion at m/z 61 is more difficult to explain, unless one postulates a reaction involving CO_2 and MeOH that would lead to $CH_3COOH_2^+$; this would be the only visible ion arising from CO_2. Other frequently observed ions in SFC–MS studies using pure CO_2 and a CI source, *e.g.*, $CO_2^{+\bullet}$ and higher cluster ions [11], are totally absent here. The ion current trace of the atrazine MH^+ ion (not shown) exhibits a behaviour identical with the total solvent ion response (Fig. 5), with the same maximum when 2–3% of polar modifier is added to CO_2. The presence of 5% of

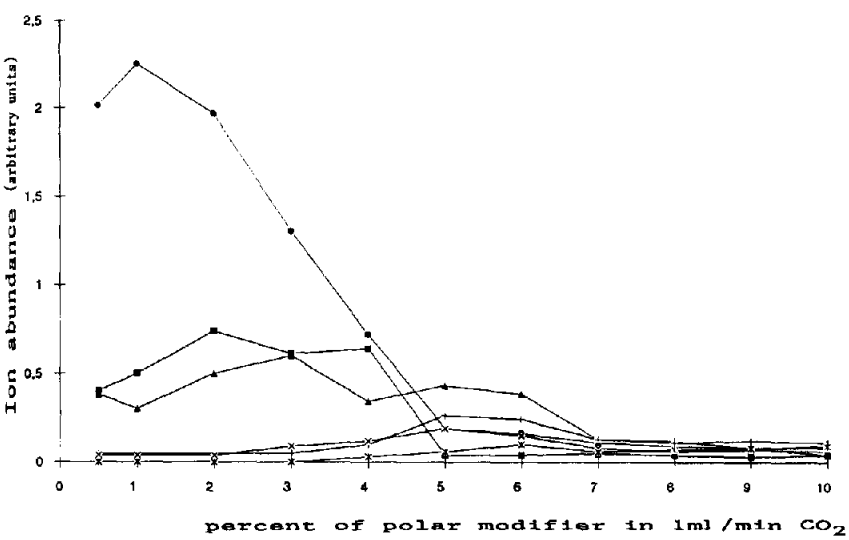

Fig. 4. Effect of the mobile phase composition on solvent-derived ion abundances. Oven temperature, 80°C; linear metallized fused-silica restrictor, 25 cm × 60 μm I.D.; 2.1 mm I.D. SFC column eluted with CO_2 at 1 ml/min and modified with MeOH–H_2O (95:5, v/v). m/z ratio of recorded ions: ▲ = 19; ● = 33; + = 51; × = 61; ■ = 65; * = 83.

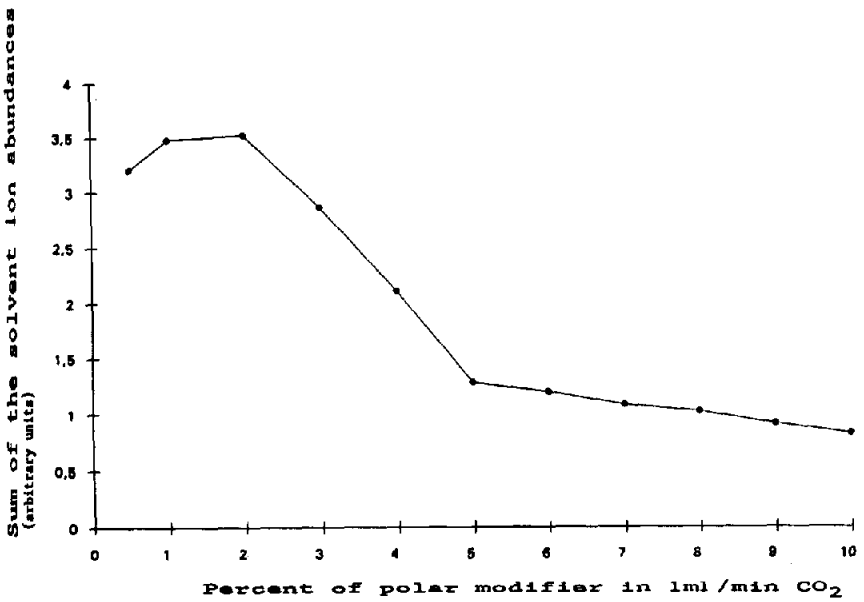

Fig. 5. Sum of solvent ion intensities as a function of the mobile phase composition. Experimental conditions as in Fig. 4.

water in the polar liquid additive was not found to be essential, because similar curves, with a weak signal at m/z 19 and no visible ion at m/z 51, but with intense methanol-derived ions and protonated solute ions, were obtained regularly in later experiments with pure methanol added to CO_2.

Whereas CO_2 does not appear to play any direct role in the ion formation, because of the lack of any abundant CO_2-derived primary ions (with the possible exception of the m/z 61 ion), the methanol- and solute-derived ion currents remain a function of the total mobile phase flow-rate. For example, the plot of the intensity

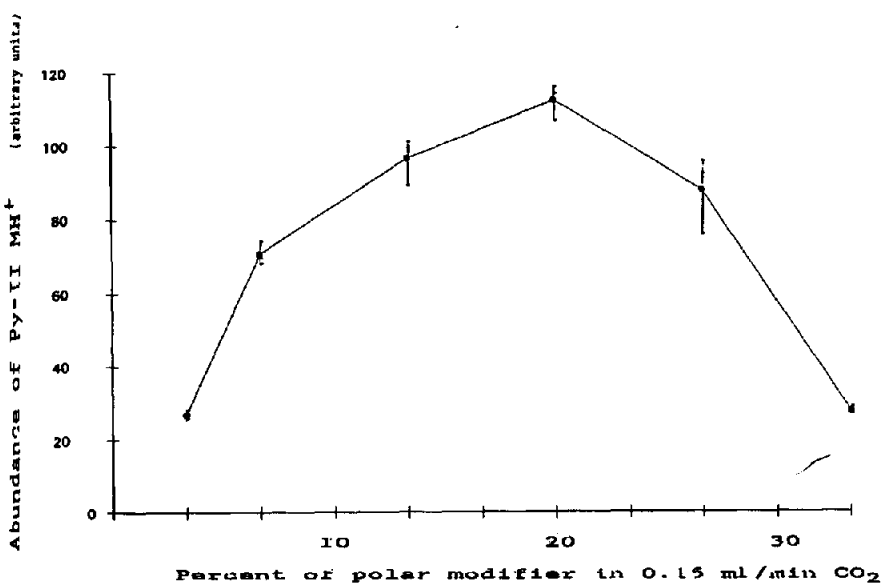

Fig. 6. MH^+ ion intensity for Py-II, at m/z 229, as a function of the mobile phase composition. Experimental conditions as in Fig. 3, except mobile phase temperature and flow-rate, 120°C and 150 μl/min, respectively. Added polar modifier: MeOH–H_2O (95:5, v/v).

of the MH^+ ion for Py-II at m/z 229 as a function of the mobile phase composition showed a maximum at a different solvent composition (Fig. 6) when the 1 mm I.D. column was eluted with 150 μl/min of mobile phase. The total ion current trace for solvent ions (not presented) shows a similar curve. For both curves, the ion formation optimum is shifted to 20% of polar modifier, compared with the 2–3% values observed when the mobile phase flow-rate is 1 ml/min. Despite the apparently different experimental conditions, it should be noted that 20% of a 150 μl/min flow and 2–3% of a 1 ml/min flow provide roughly the same 20–30 μl/min of polar modifier to the electrospray region. A strong effect of the CO_2 flow-rate on this sample ion was also observed (Fig. 7) when a 25 μl/min constant flow-rate of polar modifier and a variable flow-rate of CO_2 in the interval 50–350 μl/min flowed through the column, while monitoring the Py-II MH^+ ion during sample elution. We cannot fully explain such a strong influence for different CO_2 flow-rates.

A problem will arise when planning to achieve real SFC–ES-MS separations with a gradient of mobile phase composition, because constant ion formation appears not to be possible. Methods to overcome this limitation, probably by the use of post-column addition of a suitable solvent additive, will be needed. The second consequence is the preferable choice of the 1 mm I.D. column over the 2.1 mm I.D. column for SFC–MS because it allows a wider interval of possible solvent composition. On the other hand, the use of supercritical fluid mixtures with 1–10% of polar modifier at very low CO_2 flow-rates (less than 100 μl/min) was found to be experimentally difficult to achieve reproducibly. The use of very narrow packed SFC columns, of I.D. less than 1 mm, was precluded for this reason.

Nevertheless, there is at least one very important positive influence of the presence of CO_2, namely the increase in the tolerable input of mobile phase flow-rate into the API source. The basic Analytica ES source is limited to very small liquid flow-rate inputs, below 5 μl/min [12]. Much higher flow-rates were possible in our experiments, because the decompressing CO_2 acts as a nebulizer gas. We believe that CO_2 has the same beneficial influence on the tolerable fluid input as the added gas in an ionspray experiment [13].

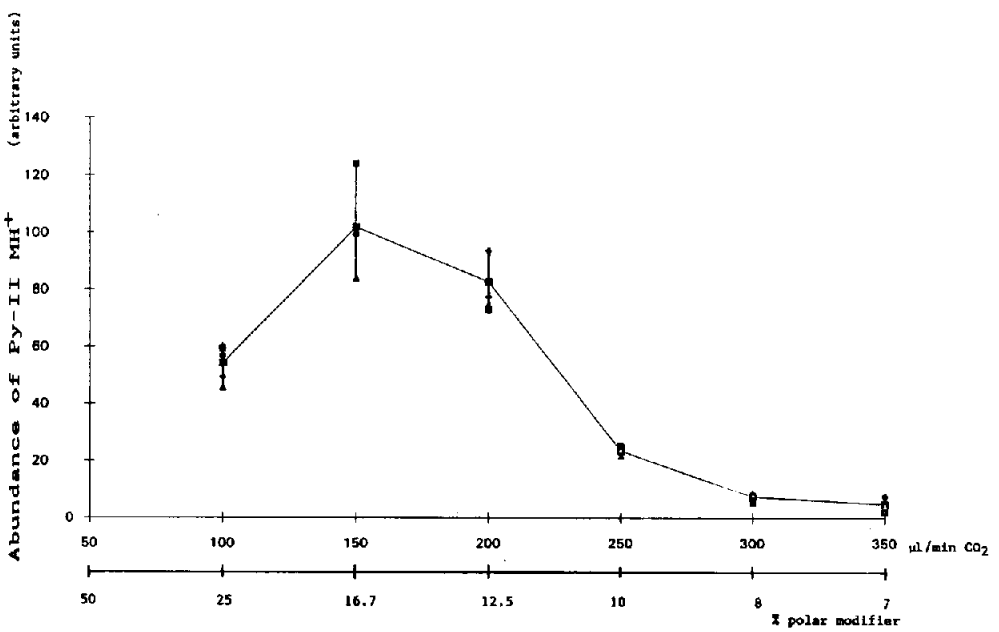

Fig. 7. MH^+ ion intensity for Py-II, at m/z 229, as a function of CO_2 flow-rate. Experimental conditions as in Fig. 3, except, oven temperature, 40°C; constant addition of 25 μl/min of MeO–H_2O (95:5, v/v) in a variable flow-rate of CO_2.

Limit of detection and response curve for model compounds

The optimum operating conditions derived from the data in Fig. 4 were selected for the response study of the atrazine MH^+ ion as a function of the injected sample amount, except that the column oven temperature was 80°C. Under these conditions, atrazine is poorly retained and elutes with k' close to 0. The response curve (Fig. 8) is similar to those often produced on electrospray ionization, with a lower detection limit in the low picogram range (Fig. 9), and a rapid signal saturation when nanogram amounts of sample are injected. Although this could be a source of difficulty when analysing real sample mixtures, the observed response curve is different from that in similar SFC–MS studies with gas-phase ionization using a corona discharge [5]. Corona discharge ionization generally exhibits a wider dynamic range of response, and the observed difference is further proof of the liquid-phase ionization mechanism believed to exist under our experimental conditions.

Application to a simple mixture and limitations of the system

The separation of a mixture of four herbicides, containing basic nitrogen functions, was reported previously [1], and was an example of the good sensitivity of the instrument to this class of compounds. Another SFC–MS example, corresponding to the separation of the two pyridine derivatives Py-I and Py-II (Fig. 10), illustrates the major difficulty to be overcome in future experiments. The total ion current and selected ion current profiles for solute MH^+ ions were recorded using the 1 mm I.D. column. The poor peak shape of the second peak is notable and was frequently observed for heavier compounds. Intact molecule-derived ions were obtained for many organic compounds, including mixtures of polyethylene oligomers with molecular masses above 1000, but the eluting peaks were generally useless owing to excessive tailing. We believe that peak asymmetry is due to cold trapping at the nebulizer tip. The peak asymmetry was reduced when the restrictor or the mobile phase was heated, but this also decreased the ion currents (Fig. 3), hence the operating temperature was often a compromise between conservation of chromatographic peak integrity and good MS sensitivity. We expect to improve the situation by the design of an optimized nebulizer, rather than the crude system used in this work. We have observed that most of the trapped sample is retained on the outer surface of the capillary nebulizer end, and a liquid sheet of methanol, or another suitable polar solvent, could continuously wash this surface. Post-SFC column addition of the polar additive would also provide ES ionization more independent of the chromatographic requirements of the mobile phase composition.

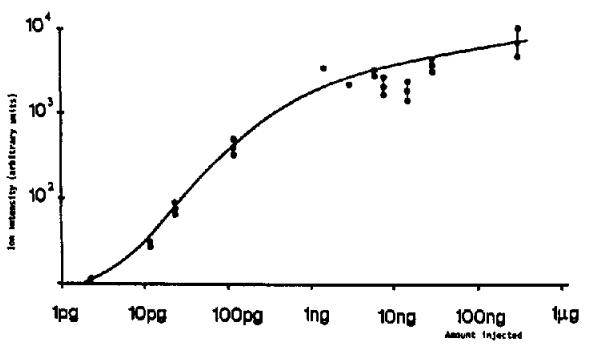

Fig. 8. Atrazine MH^+ ion intensity as a function of the amount injected. Instrument conditions as in Fig. 4, except for a constant solvent composition of 3% of polar additive in CO_2.

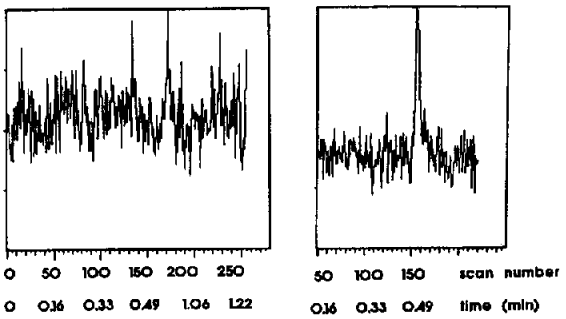

Fig. 9. Detection limit of atrazine MH^+ ion with signal-to-noise ratio = 2 (left) and 5 (right). Conditions as in Fig. 8. Sample concentration, (left) 2 and (right) 10 nmol/l; sample amount deposited on column, (left) 10.4 fmol or 2.2 pg and (right) 52 fmol or 11 pg.

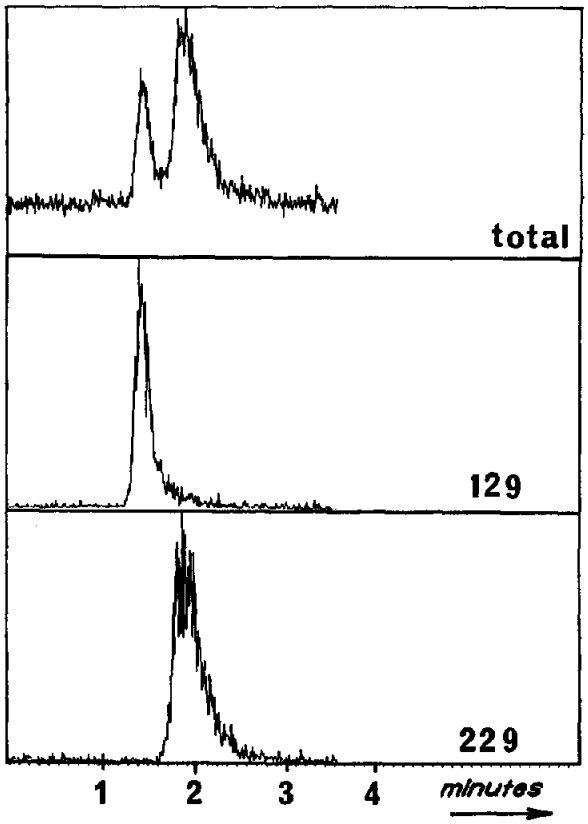

Fig. 10. SFC–MS analysis of a mixture of two pyridine derivatives. Top trace: total ion current recording for ions with $m/z > 100$. Middle and bottom traces: selected ion current profiles for solute MH^+ ions (m/z 129 and 229). Instrument conditions as in Fig. 3, except oven temperature, 50°C, and a constant fluid composition of 3% of polar modifier in CO_2.

CONCLUSIONS

The possibility of producing solute ions from a packed SFC column in the absence of a corona discharge has been demonstrated, using a standard electrospray ion source of the type normally designed for liquid solution sampling. A polar modifier must be present for the production of sample ions. Some of the polar additive could be added postcolumn to provide SFC–ES-MS conditions more independent of the chromatographic requirements, especially when using a gradient of mobile phase composition. At present the potential application of the liquid-phase ionization process involved in the ion formation mechanism to the determination of high-mass and polar samples is not fully exploited, because our required mild nebulizing conditions are still too disturbing to the conservation of chromatographic peak integrity.

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