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Journal of Chromatography A, 705 (1995) 205–217

JOURNAL OF CHROMATOGRAPHY A

Development of an "in-source" thermospray-type interface for on-line capillary liquid chromatography–mass spectrometry

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First received 28 December 1994; revised manuscript received 23 February 1995; accepted 3 March 1995

Abstract

The design and the construction of an "in-source" thermospray-type capillary interface for LC–MS experiments on a bench-top quadrupole mass spectrometer is described. The interface consists of a small diameter fused capillary tube which is introduced directly into the CI source through the solid probe inlet and is oriented in the beam axis. In order to produce average linear velocities comparable to those used in conventional thermospray and compatible with the optimum operating conditions of capillary LC–MS systems, a fused-silica transfer capillary of 10 μm is used. Heating of the interface is provided by the ion source and ions are generated using external mode ionization (filament-on). The effect on performance of several operating parameters, such as source temperature, mobile phase composition, sampling distance in the source and flow-rate have been investigated. The results obtained in experiments conducted on the effect of interface temperature and flow-rate demonstrate that the system is very stable under optimal operating conditions which are compatible with the optimum performance of capillary liquid chromatography systems. The interface developed can be used with a series of mobile phases (normal and reversed) and the ionization features in terms of sensitivity are in all cases essentially comparable. The system yields reproducible results and allows picogram range sensitivity to be achieved in flow injection mass spectrometric or liquid chromatographic experiments involving low-molecular-mass polar organic compounds.

1. Introduction

The development of techniques allowing the introduction of liquid samples into the ion source of a mass spectrometer has considerably increased the range of compounds that can be analyzed by mass spectrometry. Alternative ionization techniques for liquid introduction such as thermospray (TSP) [1–5], continuous-flow fast atom bombardment (CF-FAB) [6,7] and more recently the atmospheric pressure ionization (API) techniques such as atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI) [8–10] are most widely used. From the various LC–MS interfaces available, those that allow the introduction of the entire eluent into the ion source are the most appealing for the analysis of low-molecular-mass polar organic compounds. These types of interface use flow-rates that are compatible with the optimum range of operation of miniaturized liquid chromatographic systems and within the pumping capacity of most commercial mass spectrometers

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0021-9673/95/$09.50
SSDI 0021-9673(95)00315-0

that have CI capabilities [11]. The use of systems introducing liquids directly into the ion source can lead to three types of operating conditions that are interesting for mass spectrometric analysis. These systems can produce a stable liquid surface under vacuum (continuous-flow FAB), a stable stream of liquid droplets that can be further desolvated, as produced by pneumatic nebulizers with or without electrical assistance (electrospray), or alternatively a steady stream of gas and droplets as obtained with capillary thermal nebulizers (direct liquid introduction, thermospray).

Of the different liquid introduction systems, thermospray has proved to be a useful method. The technique has been widely accepted in the different areas of organic compound analysis such as food and agriculture [12–14], biomedical [15–17] and environmental [18–21] sciences, and is available as an accessory on many mass spectrometers. It is applicable to a wide range of analytes including volatile and nonvolatile, polar and labile molecules, analyzed under a variety of chromatographic conditions [22]. In particular, thermospray ionization can usually provide parent molecular ions from polar and moderate molecular mass compounds ($M_r < 1000$) that are not amenable to analysis using more conventional ionization techniques [23]. In addition, ionization is achieved with the use of filament-on or filament-off, and the abundance of the different fragment ions in the spectra is strongly dependent on the type and concentration of the analyte and the composition of the mobile phase. In this coupling method for liquid chromatography–mass spectrometry, which involves controlled partial vaporization of the LC effluent before the ion source of the mass spectrometer, it is vital that the heat input be properly controlled so that complete vaporization does not occur inside the capillary. As a result of heating, the liquid is nebulized and partially vaporized and the analytes are carried into the ion source as microdroplets or particles in a supersonic jet of vapor. Thermospray can be used successfully for flow-rates in the range of 0.5–2 ml/min. However, the standard thermospray interface cannot be used with lower flow-rates such as those used in capillary liquid chromatography (1–10 μl/min) because the applied heat and the flow-rate become unbalanced which leads to severe instability. Furthermore the use of a large internal diameter vaporizer does not allow the proper average linear velocities leading to thermospray conditions to be achieved.

Over the course of the last decade, liquid chromatography has been subject to a rapid evolution. The application of integrated electronics has lead to the automation and miniaturization of chromatography [24] and this trend has also been reflected in the miniaturization of liquid chromatography. A number of reports have appeared describing the use and advantages of miniaturized liquid chromatographic systems [25]. The low flow-rates used in such systems (1–10 μl/min) allow the introduction of the total liquid chromatographic eluent into the mass spectrometer, thus reducing the total quantity of sample injected necessary to achieve the same signal with respect to systems that require splitting of the eluent.

Previous work in our laboratory has lead to the development of a simple and inexpensive direct liquid introduction (DLI) system that uses a conventional GC–MS interface and that can be used to couple capillary liquid chromatography to mass spectrometry [26]. Experiments conducted with this system have shown that it can be stable under appropriate conditions, that it is reproducible and that it allows picogram range sensitivity to be routinely achieved for low-molecular-mass organic compounds. However, this interface presents limitations in the analysis of more polar compounds.

In order to allow the analysis of thermally labile or involatile compounds and to ensure the direct coupling of capillary liquid chromatography, the design and construction of an in-source capillary thermospray-type interface was undertaken. The interface consists of a small fused capillary tube that allows the direct introduction of liquid samples through a continuous flow loop injection system. Arpino and Beaugrand [27] have demonstrated that it is feasible to use capillary tubes with narrow inside diameters (10 μm) as flow restrictors to nebulize the liquid

solution in order to obtain thermospray conditions with flow-rates compatible with the use of micro-columns. In order to reproduce conditions comparable to those obtained in conventional thermospray, vacuum nebulization of the chromatographic eluents through a fused capillary tube should meet the following requirements: (i) an input liquid flow-rate small enough to allow the normal operation of the mass spectrometer using the available pumping equipment, (ii) an input liquid flow-rate greater than the rate of evaporation of the solvent at the nebulizer orifices, and finally (iii) an operating pressure within the limits fixed by the HPLC pump. The aim of this work was, therefore, to design and construct a thermospray-type interface that meets all these requirements and that would allow the analysis of polar substances.

The scope of applications of the interface has been determined and the influence on performance of operating parameters such as source temperature, flow-rate and vaporizing distance studied. The stability and reproducibility of the system have been investigated along with the effect of type and mobile phase composition on the ionization efficiency.

2. Experimental

2.1. Mass spectrometer

Analyses were performed on a VG TRIO-1 mass spectrometer (VG MassLab, Manchester, UK) equipped with differential pumping (analyzer 50 l/s, source 240 l/s). Data handling capability and control of the instrument were provided by the LAB BASE data system. The ion source pressure (as read on the vacuum gage) was maintained in the range of $2 \cdot 10^{-4}$ to $8 \cdot 10^{-4}$ Torr (1 Torr = 133.322 Pa) and unless otherwise specified the source temperature was maintained at 150°C.

2.2. LC–MS experiments

The capillary system used consisted of a Carlo-Erba (Milano, Italy) Phoenix-20 pump connected to a Valco Model C14W injector with a 60-nl sample loop. The capillary columns used were laboratory made (220 × 0.25 mm I.D.) and packed with 5-μm particles (Spherisorb ODS-2). Typical flow-rates used were between 2.5 and 5 μl/min. In the experiments the sample was introduced by a Valco C14W valve (Valco Instruments Co., Houston, TX, USA). This valve permits direct connection between capillary tubing or LC columns with a minimum dead volume. Detection at 254 nm for the LC–UV experiments was achieved with an ISCO μLC-10 variable-wavelength detector equipped with a 60-nl flow-cell.

2.3. Interface

The capillary thermospray-type interface developed for the mass spectrometer is shown in Fig. 1. It consists of a fused-silica capillary tubing which is introduced directly into the CI source through the solid probe inlet and is oriented in the beam axis. In order to produce average linear velocities comparable to those obtained in conventional thermospray, a fused-silica capillary of 18 mm × 10 μm I.D. is used. Heating of the interface is provided by the ion source and ionization is achieved using external-mode ionization (filament-on mode).

2.4. Chemicals

The compounds used in this study such as gramine, picolinic acid, geraniol, cineole, umbel-

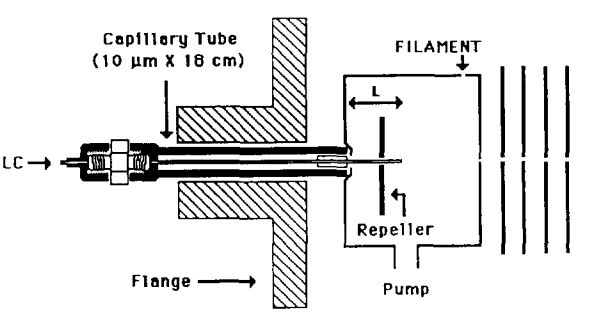


Fig. 1. Schematic of the thermospray interface. L is the sampling distance.

liferone, caffeine, theophylline, theobromine, piperidine, benzonitrile, chloroaniline, chloramphenicol, fluoranthene, anthracene, coumaric acid, ferrulic acid, caffeic acid, *trans*-cinnamic acid, phloroglucinol, salicyl alcohol, adenosine, toluene, ethylbenzene, butylbenzene and amylbenzene were purchased from Aldrich (Milwaukee, WI, USA). All compounds were used without further purification, and the mobile phases were prepared using HPLC-grade (Aldrich) acetonitrile, ethyl acetate, hexane and distilled, deionized water (Milli-Q, Millipore, Bedford, MA, USA).

2.5. Preparation of mobiles phases

The eluents were carefully prepared by mixing appropriate volumes of distilled, deionized water and organic modifiers. In all instances, the solvents were filtered (0.45-μm filter) and degassed in an ultrasonic bath for at least 30 min prior to their use.

3. Results and discussion

In general, most thermospray/plasmaspray ion sources consist of a cylindrical tube with the vaporizer probe at one end and the pumping exit at the other, the vaporizer probe being the heart of the thermospray system. In conventional commercial interfaces, and in most laboratory-built probes as well, vaporizer capillaries of 100–150 μm are usually used. These interfaces are used in conjunction with conventional liquid chromatographic columns and can accommodate flow-rates in the range 0.5–2 ml/min. However, these interfaces, because of their large internal diameter, cannot be used for lower flow-rates, such as those used in capillary liquid chromatography systems (1–10 μl/min). Hirter et al. [28] and Arpino and Beaugrand [29] reported the use of vaporizer capillaries of 10, 25 and 50 μm I.D. in order to introduce typical amounts of liquid between 40 and 80 μl/min. However, these flow-rates are still much higher than the average flow-rates used in capillary liquid chromatographic systems.

The capillary thermospray interface used in this work was designed to fit a VG TRIO-1 mass spectrometer and is shown schematically in Fig. 1. It has the same outside dimensions (7.9 mm O.D.) as the solid probe, and so can be introduced into the mass spectrometer through the solid probe inlet. Basically the interface consists of a stainless-steel tube of 12.5 cm $\times$ 7.9 mm O.D. which allows the introduction of the fused-silica capillary. A teflon disk of 1 cm $\times$ 7.9 mm fitted at the end (near the source) of the stainless steel tube serves as a holder for the capillary tube. The chromatographic column is fitted to the capillary tube with a Valco union. Through this capillary the total effluent of the capillary column is introduced directly into the ion source of the mass spectrometer without a desolvation chamber. In order to develop a thermospray-type interface that can be used for work with packed capillary columns (flow-rates 2–5 μl/min) and still produce average linear velocities comparable to those obtained in conventional thermospray ($\approx$900 mm/s), a fused-silica tubing of 18 mm $\times$ 10 μm I.D. was used as vaporizing capillary. This vaporizer capillary is connected to an injector and a pumping system. The characteristics of the in-source capillary thermospray-type interface system are (i) the absence of heating element for the vaporizer capillary, heating being provided by the ion source, and (ii) the absence of a desolvation chamber in contrast to the systems proposed by Hirter and Arpino which use an electrically heated vaporizer capillary and a desolvation chamber. The main appeal of the interface developed is its simplicity and the fact that it requires, except for the probe, no modification to the mass spectrometer such as the addition of a desolvation chamber or additional pumping device.

3.1. Operating parameters

Several parameters that are known to have an effect on the overall performance were studied in order to evaluate the interface and to determine its optimum operating conditions. The main factors affecting the performance of the interface are the temperature settings of the source, the

mobile phase composition and the flow-rate into the ion source of the mass spectrometer. The temperature of the interface governs the rate of evaporation of the mobile phase whereas the flow-rate affects both the evaporation rate and the pressure within the ion source and the composition of the plasma that affects the ionization efficiency. In this instance, another parameter that was found to have an influence on the performance of the system was the sampling distance (L in Fig. 1) which is defined as the length of capillary introduced into the ion source. The conditions (average linear velocities) used in the study were chosen so as to be similar to those involved in conventional thermospray. Optimization of the different parameters was done using the solvent ion intensities [30].

In order to reproduce the average linear velocity obtained with the conventional systems, flow-rates in the order 2.0–7.0 μl/min had to be used. However, a constraint on the flow-rate range that could be used was imposed by the pumping capacity of the mass spectrometer (flow-rates <6.0 μl/min) and the optimum range of operation of miniaturized liquid chromatography (flow-rates >2.0 μl/min). Thus, flow-rates used in this study ranged from 2.0 μl/min to 6.0 μl/min. Under those restrictions, the average linear velocities vary from 530 to 1500 mm/s. The optimization of operating parameters was initially done with a flow of 4.0 μl/min (linear velocity = 900 mm/s) which is similar to the operating conditions in conventional thermospray at a flow rate of 1 ml/min.

The effect of the interface temperature, that is provided by the ion source, on the stability of the plasma was studied. Temperature values have to be such that just enough heat is supplied to almost completely vaporize the liquid as it passes through the vaporizer and the total heat transferred must be equal to the heat required to convert the liquid to vapor at the exit temperature and pressure. If the temperature of the heater is too high then vaporization will tend to occur inside the capillary. On the other hand, if insufficient heat is supplied, superheated liquid will emerge and begin to vaporize in the ion source, thus, creating instability. Both these

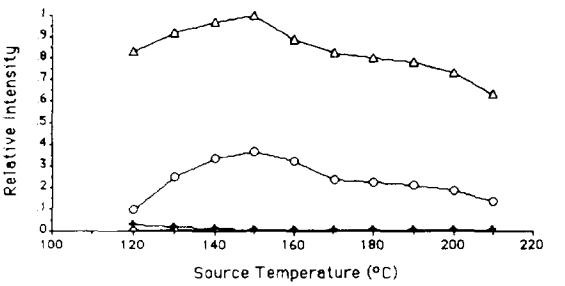


Fig. 2. Relative intensity of solvent ions (acetonitrile–water, 50:50, v/v) as a function of the source temperature. (△) 83, (○) 42, (+) 19, (◇) 18.

conditions will lead to unsatisfactory performance. As can be seen from Fig. 2, that shows the intensities of the solvent ions versus the source temperature, the results obtained when the temperature is varied at constant flow-rate (ca. 4.0 μl/min) of the mobile phase indicate that all ions reached a maximum intensity in the same temperature region of 140–160°C. This temperature region was found to provide extremely stable operating conditions. Partial to severe instability was encountered at both ends of this temperature range for reasons discussed previously. However, the optimum temperature for thermally labile compounds appeared to maximize at lower source temperature (135–145°C), higher temperature increasing the extent of fragmentation as can be predicted.

Another factor that can affect the stability and the operating conditions is the flow-rate. Its value has to be such that the input liquid flow-rate is greater than the rate of vaporization while the pressure, within the chemical ionization source, is adequate to maintain the ionization efficiency and sensitivity. The flow-rate in the system has an effect on the ion source pressure which affects the composition of the plasma and therefore the ionization characteristics of the system. If LC–MS experiments are to be conducted, additional constraints will be imposed on the flow-rate, since it will have to be such that the chromatographic conditions are also optimized in order to maintain chromatographic performance. Hence, the effect of the flow-rate was investigated at the optimum temperature of

the source (150°C). For flow-rate values below 2.0 μl/min, the system is quite unstable, resulting in important variation in the signal. As the flow-rate approaches values of 3.0 μl/min the overall operating conditions tend to stabilize. The range of flow-rates corresponding to stable operating conditions was found to be between 2.5 and 5.0 μl/min corresponding, depending on the solvent used, to indicated source pressures in the range of $2 \cdot 10^{-4}$ to $8 \cdot 10^{-4}$ Torr. This range of flow-rates is directly compatible with the use of 250 μm I.D. packed capillary columns.

As mentioned earlier, the flow-rate affects the ion source pressure and consequently the composition of the plasma. In order to assess the influence of the flow on ionization efficiency the composition of the plasma was monitored over the flow-rate or the pressure range that corresponds to the optimum performance of the system. Several mobile phase compositions were studied in these experiments for both normal and reversed modes, and typical results obtained with a binary mixture of acetonitrile–water (50:50) are shown in Fig. 3. The figure, which gives the ion profiles generated by an ACN–H_2O (50:50) mixture as a function of the flow-rate, indicates that the major ions corresponding to the protonated dimer and monomer of acetonitrile at m/z 83 and m/z 42 decrease in intensity as the flow-rate is increased well above the value of 3 μl/min. Thus, the prefered flow-rate for LC–MS experiments should be chosen in the range of 2–3.5 μl/min and this range approximately corresponds to the optimum linear velocity of the 250 μm capillary columns.

The composition of the mobile phase also has an effect on the quality of the mass spectra obtained since it will determine the reacting species and, thus, the features of the spectra in the filament-on mode. In order to investigate this effect and characterize the background signal (chemical noise) generated by the interface in the filament-assisted mode with varying mobile phase compositions, studies were performed with different solvent compositions. The systems studied were binary mixtures of acetonitrile–water (reversed-phase) and ethyl acetate–hexane (normal-phase) with different solvent ratios (25%, 50%, 75% 100%). Fig. 4A indicates that the major ion for the reversed-phase system (acetonitrile–water) corresponds to the protonated dimer of acetonitrile (m/z 83). Ions at m/z 18 and 19 that correspond to H_2O^+ and H_3O^+, respectively, have much weaker intensities than those generated by acetonitrile. These results are

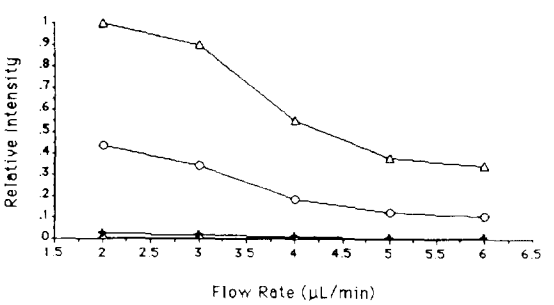


Fig. 3. Relative intensity of the solvent ions (acetonitrile–water, 50:50, v/v) as a function of the flow-rate. (△) 83, (○) 42, (+) 19, (◇) 18.

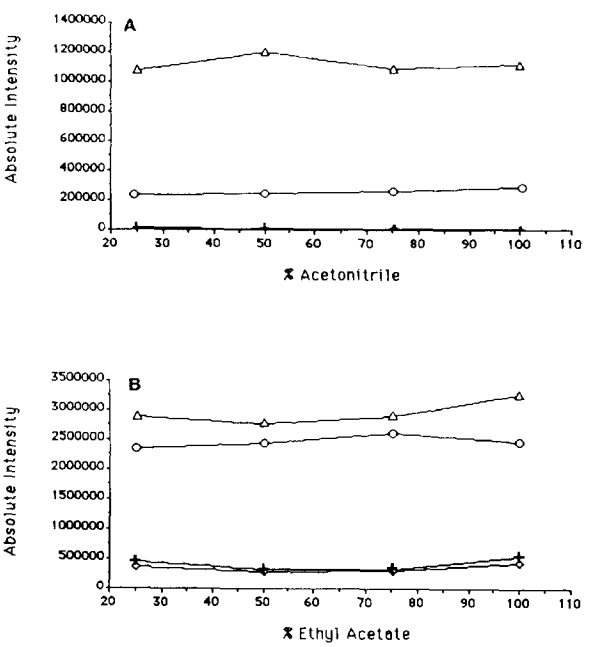


Fig. 4. Variation of the plasma obtained as a function of the mobile phase composition. (A) Acetonitrile–water: (△) 83, (○) 42, (+) 19, (◇) 18. (B) Hexane–ethyl acetate: (△) 177, (○) 89, (+) 85, (◇) 57.

explained by the higher proton affinity of acetonitrile as compared to that of water (approximately 70 kJ/mol higher). Experiments with filament-on thermospray have demonstrated that this mode of operation ionizes analytes by conventional chemical ionization reactions with solvent-derived ions [31] which are also observed in direct liquid introduction LC–MS. The plasma compositions obtained with the capillary in-source thermospray-type interface in the filament-assisted mode differ from those previously observed for ACN–H_2O in direct liquid introduction LC–MS [26] in that the major ion present is the protonated dimer of acetonitrile at m/z 83 instead of the protonated monomer at m/z 42 that is observed in DLI [26]. This reflects the differences in the vaporization conditions that exist between the two types of interfaces.

The results obtained in the filament-assisted mode with the normal-phase systems used (ethyl acetate–hexane) are similar to those obtain for acetonitrile–water mixtures. Fig. 4B indicates that the major ion corresponds to the protonated dimer of ethyl acetate (m/z 177). Ions at m/z 85 corresponding to hydride ion abstraction from hexane have much weaker intensities than those generated from ethyl acetate. From Fig. 4A,B, it can also be observed that the absolute intensities of the major ions of both systems are relatively stable for solvent ratios going from 25% to 100%. Thus, it can be expected that the ionization conditions in the source will be similar over that range of mobile phase compositions.

The efficiency of ionization of several types of compounds was investigated in the filament-assisted mode with various mobile phase compositions using mixture ratios within the determined range. In these experiments, several mobile phase compositions for the two different chromatographic modes, acetonitrile–water (reversed-phase) and ethyl acetate–hexane (normal-phase), were used. The effect of mobile phase composition on ionization was determined by injecting a constant quantity of model compounds and monitoring the relative intensities of the major ions as the composition of the binary mixtures varied. Caffeine (200 ng) was used for the reversed-phase system while chloroaniline (200 ng) and benzonitrile (200 ng) were used for the normal-phase mode. Fig. 5A (reversed-phase) and 5B (normal-phase) show the results obtained for both chromatographic systems. The relative intensities of ions for the model compounds seem to be stable over the range of compositions studied in both systems. These results appear interesting since they indicate that the system can maintain ionization performance in both modes over the composition range 25–100%. In the buffer ionization mode, the efficiency of ionization is greatly reduced which does not, as a general rule, make this mode appealing.

The sampling distance (L in Fig. 1) is a parameter, particular to this interface, that has an effect on the overall performance. This parameter was found to be extremely important since its value determines the heated length of the capillary interface and the vaporizing zone. Furthermore, the sampling distance can directly

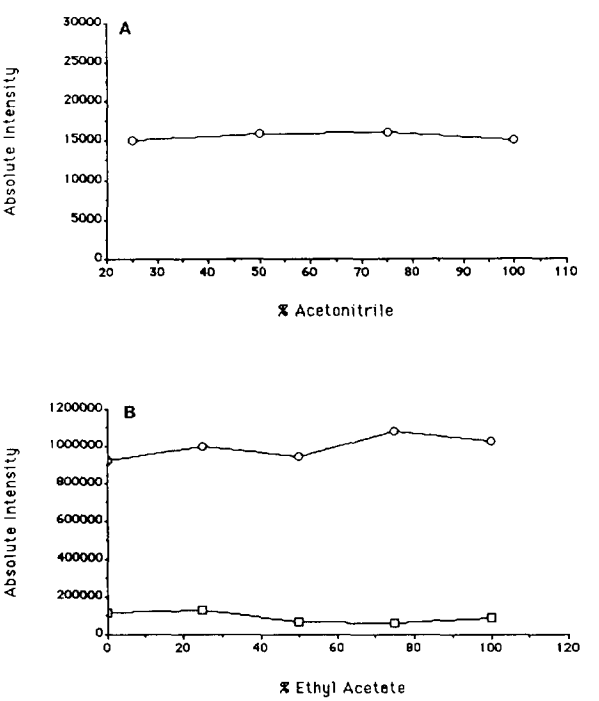


Fig. 5. Variation of the response of model compounds as a function of the mobile phase composition. (A) (○) Caffeine (acetonitrile–water); (B) (○) benzonitrile, (□) chloroaniline (hexane–ethyl acetate).

affect the sensitivity of the system by allowing the path travelled by the ions between the ion slit and the orifice of the interface to be varied. As can be seen from Fig. 6, which gives the relative intensity of solvent ions as a function of the sampling distance, the ion intensities increase with the distance up to a certain maximum, 12.5 mm, at which a drop in intensities occurs. This implies that the heated length of the capillary has to be equal to 12.5 mm in order to meet the proper vaporization conditions. This capillary length of 12.5 mm corresponds to a distance of 3 mm between the ion slit exit and the orifice of the interface. Partial to severe instability of the signal was encountered for shorter heated lengths.

3.2. Spectral and analytical features

The evaluation of the spectral and analytical features achieved with the in-source capillary interface was based on the quality of the mass spectra and on the sensitivity obtained for several types of organic compounds under typical operating conditions. Table 1 shows the structures of the different compounds that were used for the evaluation. In all cases, the protonated species were observed as the major ion and fragment ions were present only to a minor extent. Generally, for full scan spectra the injection of 1–50 ng was needed in order to produce an interpretable spectrum. Typical data obtained are presented in Fig. 7A,B which shows the mass spectra of adenosine (Fig. 7A) and chloramphenicol (Fig. 7B) obtained using acetonitrile as mobile phase. The mass spectra obtained for these compounds were found to be in all points comparable to those obtain under typical thermospray filament-on conditions [32].

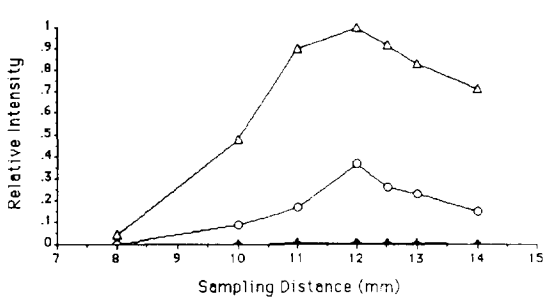
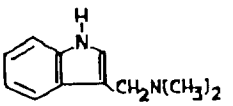
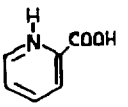
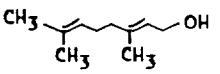
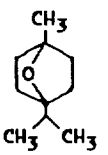
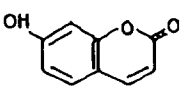
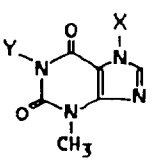
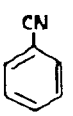
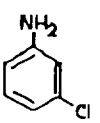
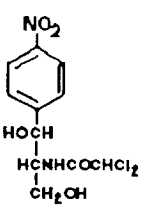


Fig. 6. Relative intensity of solvent ions (acetonitrile–water, 50:50, v/v) as a function of the sampling distance. (△) 83, (○) 42, (+) 19, (◇) 18.

A series of experiments were conducted to evaluate the sensitivity of the interface developed and to determine the detection limits that can be obtained in the scanning mode and in single-ion monitoring (SIM). The data of Table 2, which gives the detection limits measured in both modes (signal-to-noise ratio >3), indicate that limits of detection ranging from 2 to 23 ng were obtained for the different analytes in the scanning mode and ranging from 5 to 30 pg in the single-ion monitoring mode of the parent-molecular ion $[M+H]^+$. The sensitivity observed in both modes with the capillary interface developed is comparable to that obtained with conventional thermospray/plasmaspray (filament-on) interfaces and the detection limits in the order of low nanogrammes in the scanning mode and low pg in the single-ion monitoring mode are comparable. Another important analytical feature is the linearity of the source signal. The results shown in Fig. 8 indicate that the signal is found to be linear over at least 3 orders of magnitude for compounds such as benzonitrile and fluoranthene in the single-ion mode. The system proved to be linear from 2 to 1100 pg in the scanning mode and from 2 to 1000 ng in the single-ion monitoring mode for the two compounds respectively.

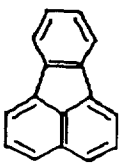
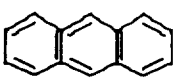
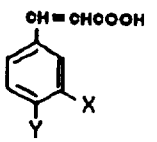
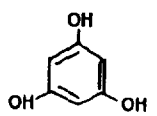
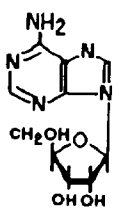
An important factor, when interfacing chromatographic methods to mass spectrometry, is the ability of the interface to preserve chromatographic performance. As mentioned previously, in the type of interface under discussion the flow-rate used has to be compatible with the optimum linear velocity of the mobile phase while allowing the pressure in the ion source to be in the range required for efficient ionization. The situation is similar for the chromatographic separation where the interface must not act as a dilution volume that can reduce the separation efficiency. The latter situation has been demonstrated to occur in LC–CF–FAB-MS systems where the droplet can be considered as a dilution

Table 1
Typical applications of the capillary thermospray interface

Compound	Structure	Ion intensity [a]
Gramine		175 (100)
Piconilic acid		124 (100)
Geraniol		155 (100), 137 (10)
Cineole		155 (100), 137 (35)
Umbelliferone		163 (100), 145 (20)
Caffeine (X = CH_3, Y = CH_3) Theophylline (X = H, Y = CH_3) Theobromine (X = CH_3, Y = H)		195 (100) 181 (100) 181 (100)
Benzonitrile		104 (100)
Chloroaniline		128 (100)
Chloramphenicol		323 (100), 305 (7)

(Table continued on p. 214)

(Table 1 contd.)

Fluoranthene		203 (100)
Anthracene		179 (100)
p-Coumaric acid (X = H, Y = OH) Ferulic acid (X = OCH_3, Y = OH) Caffeic acid (X = OH, Y = OH) *trans*-Cinnamic acid (X = H, Y = H)		165 (100), 147 (40) 195 (100), 177 (25) 181 (100), 163 (30) 149 (100), 131 (30)
Phloroglucinol		127 (100), 108 (20)
Adenosine		268 (100), 136 (18)

[a] Ion intensity (%) (base peak = 100), in parentheses.

Table 2
Typical limits of detection obtained with the capillary thermospray interface

Compound	Detection limit	
	Repetitive scanning[a] (ng)	Selected-ion monitoring (pg)
Chloramphenicol	10	12
Adenosine	23	30
Ranitidine	6	9
Caffeine	3	5
Butylbenzene	7	10
Chloroaniline	2	4
Fluoranthene	3	3
Benzonitrile	10	10
Phenylhexane	2	5

[a] Scan speed of 1 s over a mass range 10–550.

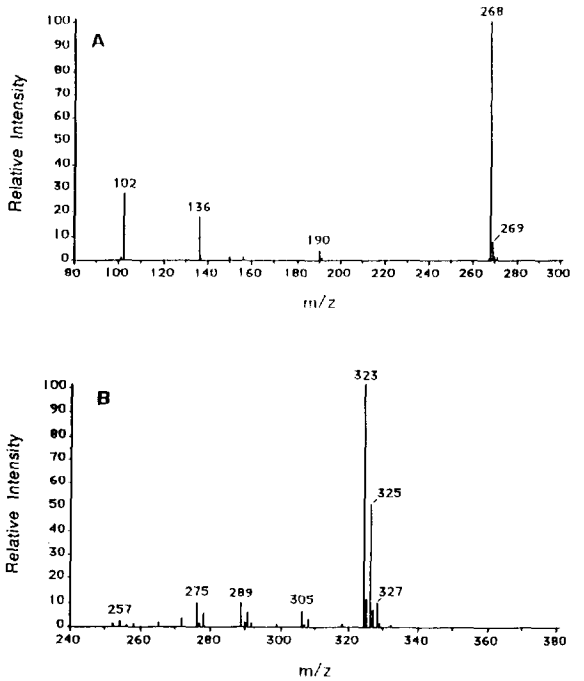


Fig. 7. Mass spectrum of (A) adenosine and (B) chloramphenicol obtained with the capillary in-source thermospray interface.

volume [33]. In order to investigate the possible band broadening that can occur in the interface, a series of experiments were conducted in which the chromatographic peak widths obtained in the μ-TSP interface were compared to those obtained in a conventional LC–UV capillary system. The results of these experiments are depicted in Fig. 9. The figure shows the chromatographic separation of a mixture of alkylbenzenes (toluene, ethylbenzene and propylbenzene) monitored with a UV detector (Fig. 9A) and that observed with the in-source capillary thermospray-type interface (Fig. 9B). Examination of

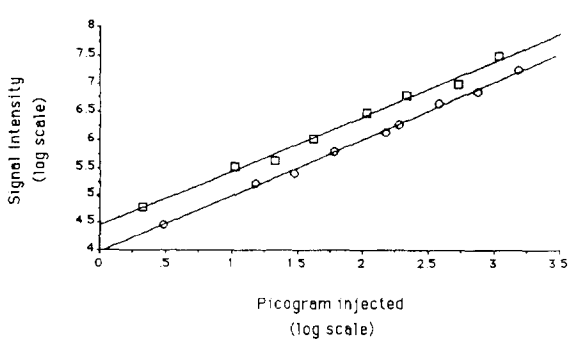


Fig. 8. Calibration curve for parent molecules ions of (○) fluoranthene and (□) benzonitrile.

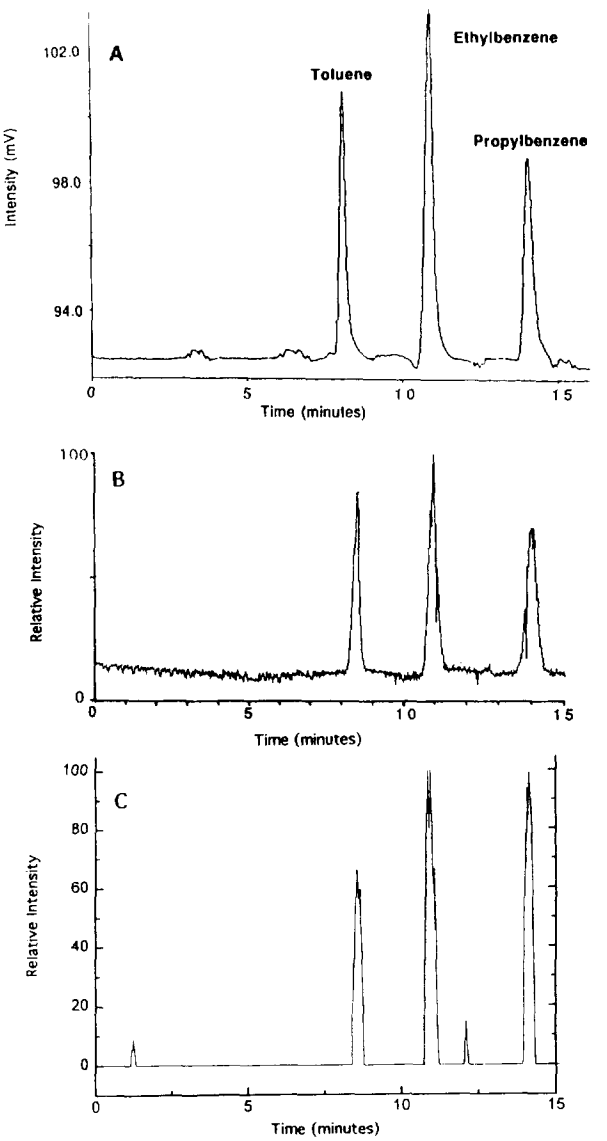


Fig. 9. Separation of a mixture of alkylbenzenes on a 20 cm × 0.25 mm I.D. fused—silica column packed with ODS-2 (5 μm); mobile phase acetonitrile–water (72:25), obtained with (A) UV detector, (B) capillary thermospray interface, (C) treated with the algorithm TICFilt.

the data reveals that the interface developed does not significantly alter the chromatographic profile and that it is not acting as a mixing chamber. Thus, this interface can be advantageously used for LC–MS analysis and also for real-time monitoring of reactions in solution or as a liquid introduction system for mass spectrometry or tandem mass spectrometry (MS–MS) analysis.

The in-source thermospray-type interface, albeit interesting in terms of its performance in the LC–MS analysis of polar molecules of low-molecular-mass, can present limitations when analyzing unknown substances. In the selected-ion monitoring mode the heavy background originating from the mobile phase can usually be avoided by the judicious choice of the m/z ratios that are monitored. However, when analyzing mixtures containing unknown compounds in the repetitive scanning mode, the background contributes a very significant signal that can totally mask signals due to analytes present in small concentrations. Thus, it is preferable to use background treatment algorithms that can eliminate most of the undesirable signal in the TIC as well as in the mass spectra. Fig. 9C shows the TIC that results from the treatment of the LC–MS data by TICFilt, a computer program that we have developed for that purpose [34]. The program allows the detection of small eluting peaks in the TIC generated by direct coupling LC–MS or DLI techniques and provides spectra from which interfering ions have been removed. Hence, the use of a background treatment algorithm minimizes the adverse effect of the important signal generated by the ionization of the mobile phase.

Another difficulty that can be encountered when using direct introduction systems such as the one described is partial or complete clogging of the capillary interface due to the presence of involatile materials. Partial or complete clogging of the vaporizer capillary has been experienced only when the system was used with more concentrated nonvolatile samples with injected quantities above 1 μg or occasionally due to the slow deposition over a long period of time of nonvolatile solvent impurities. The partial clogging can lead to the deviation of the spray resulting in a decrease of the sensitivity. However, this situation is easily detected and remedied.

4. Conclusions

The simple capillary thermospray-type interface that has been described in this work can be very useful to interface capillary liquid chromatography to mass spectrometry and also as a stand-alone system that can be use for flow injection analysis by mass spectrometry or tandem mass spectrometry. The performance of the capillary thermospray-type interface is satisfactory for the analysis of low-molecular-mass polar compounds that cannot always be analyzed by other techniques. It is a reliable system that is easy to operate and offers good stability when operated under optimum conditions. The interface developed has been used to analyze a wide range of compounds and good average sensitivity was obtained both in the repetitive scanning and selected-ion monitoring modes. Detections limits in the low nanogram and low picogram range are obtained in these respective modes of operation. Qualitatively, the mass spectra obtained for a series of compounds with different structures and polarity were found to be in all points comparable to those obtained under typical thermospray conditions. The analytical features of this type of interface can further be improved by the use of appropriate data treatment software that extend the applicability of the technique in the area of analysis of unknown materials.

Acknowledgment

The authors acknowledge the financial support of the Natural Sciences and Engineering Council of Canada (NSERC) and Hydro-Quebec, which permitted this study.

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