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DIRECT COUPLING OF A MICRO HIGH-PERFORMANCE LIQUID CHROMATOGRAPH AND A MASS SPECTROMETER

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SUMMARY

A simple and inexpensive interface for the introduction of the total effluent from a micro high-performance liquid chromatograph into a mass spectrometer is described. At flow-rates up to 15 μl/min this interface works reliably for extended periods. The samples are detected mass spectrometrically in the chemical ionization mode where the solvent (acetonitrile–water) acts as reagent gas. The performance of the interface is described and the analysis of a mixture of polycyclic aromatic hydrocarbons and phenols is reported.

INTRODUCTION

Considerable efforts have been made in recent years to develop an interface capable of coupling the instruments in combined high-performance liquid chromatography (HPLC)–mass spectrometry (MS)[1–16]. The current state of the art has been reviewed recently[17]. The following approaches have been explored for direct HPLC–MS coupling: a moving wire/belt system which allows the sample to be transported into the ion source while the LC solvent is evaporated in a system of vacuum locks[1,7]; a silicone-rubber membrane separator[5]; 1% splitting of the LC effluent into the MS, the LC solvent being used as a chemical ionization (CI) reagent gas[2,3,8,10,11]; flash vaporization using a laser beam[9]; the use of an atmospheric-pressure ionization (API) source[6]; a jet separator[13]; and a vacuum nebulizer[14]. If buffer solutions are used as LC solvents, a modified segmented flow extractor can be added to the interface[16]. With all of these interfaces the LC solvent either has to be evaporated or split prior to introduction into the mass spectrometer as a result of the limited pumping capabilities of commercial mass spectrometers.

With the advance of micro high-performance liquid chromatographs[18] in which the flow-rate of the LC solvent is in the range of microlitres instead of millilitres per minute, interfacing may become simpler. Such interfaces have been described recently[12–15]. Henion and Maylin[15] have shown that the total effluent from a micro liquid chromatograph can be introduced into the ion source of a commercial quadrupole mass spectrometer operated in the CI mode where the LC solvent acts as reagent gas. This approach appears to be particularly attractive as the interface is

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simple and inexpensive and no modifications of the mass spectrometer are required. We have therefore constructed an HPLC–MS interface based on the concept of Henion and Maylin, and its performance has been extensively tested.

EXPERIMENTAL

Apparatus

A JASCO Familic-100 N micro high-performance liquid chromatograph was used. This instrument is equipped with a 500-μl gas-tight micro syringe pump. The flow-rate can be varied from 1 to 29 μl/min (in 1 μl/min steps) and the volume of sample injected can be varied from 0.1 to 1.9 μl (in 0.1-μl steps). The pressure of the solvent is approximately 100 kg/cm^2. The instrument was equipped with a UV detector (UVI-DEC-100 II) with a quartz capillary micro cell (cell capacity 0.3 μl) and variable wavelength. Micro LC columns made of PTFE tubing (0.5 mm I.D., 14–27 cm long) filled with SS-10-ODS-A (JASCO) were used.

The mass spectrometer employed was a Varian-MAT 44S equipped with a 500 l/sec turbomolecular pump at the ion source (instead of the standard 270 l/sec pump). The instrument was operated in the CI mode (electron current 0.8 mA, electron energy 400 eV, source temperature 250–330°C, source pressure 450–500 μbar).

The interface

The interface, which is very simple, is shown in Fig. 1. It consists of a stainless-steel capillary (0.1 mm I.D., 0.2 mm O.D., 30 cm long). To improve the mechanical stability the capillary is enveloped by a second stainless-steel capillary used in conventional HPLC techniques (0.25 mm I.D.) and a tube made of steel or brass (5 mm O.D.). A gas-tight connection to the end of the micro LC column is made via an intermediate capillary, a PTFE tube and a Swagelok connection. No filter is used in the system, in order to avoid any additional dead volume. Several dimensions of the interface were varied systematically in order to obtain the minimal dead volume and optimal peak shape. A steel capillary was preferred to a glass capillary[15] as a flow restrictor owing to its higher mechanical stability and the fact that such a capillary can be either heated or cooled easily (see below) and coupled simply to the micro HPLC column. An inner diameter of the metal capillary of 0.1 mm was chosen as this was the smallest diameter available.

The interface is introduced into the ion source via a sliding rod similar to a standard gas chromatographic (GC) insertion probe using an O-ring as a seal and the

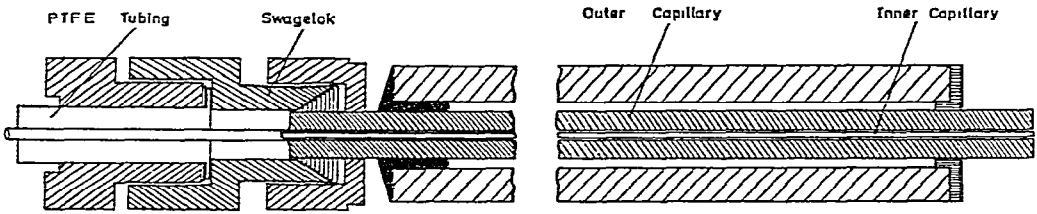

Fig. 1. HPLC–MS interface. For details and dimensions, see text.

GC vacuum lock system. The interface was positioned in such a way that the inner capillary was a distance of 1 cm from the ionization chamber. If the total LC effluent was introduced into the CI box, the pressure in this box was too high for CI conditions. By pulling the direct insertion probe (located opposite the interface capillary) back slightly an additional leak in the CI box was generated, which allowed the pressure in the CI box to be adjusted to its optimum value. Under these operating conditions the pressure in the source housing remained below 10 nbar.

Samples

Mixtures of acetonitrile and water were used as HPLC solvents, as discussed below. Acetonitrile (Baker, Phillipsburg, NJ, U.S.A.) was further purified by distillation and water was purified by double distillation. All other compounds were purchased from Fluka (Buchs, Switzerland) and used without further purification.

RESULTS AND DISCUSSION

Performance of the interface

The general performance of the interface was tested using naphthalene and diuron as samples and acetonitrile–water as solvent. Reproducible spectra could be obtained only when the ion source pressure could be kept constant over the entire measuring period. It was found that it is sometimes difficult to maintain a constant source pressure at high flow-rates (>15 ml/min) and with solvents with a high water content [*e.g.*, acetonitrite–water (30:70)], while at low or medium flow-rates ($\leqslant$15 μl/min) the source pressure indicated on the pirani gauge remained constant to within less then 1% throughout a complete measuring cycle (*ca.* 30 min). The strong pressure fluctuations frequently observed at high flow-rates must be due to uneven evaporation of the solvent at the end of the interface capillary.

The peak width as a function of time was tested with naphthalene as an example. If the end of the micro LC column was connected directly to the interface, the peak width of *m/z* 129 in the chromatogram of naphthalene with MS detection was only *ca.* 60% of that observed with the UV detector: injection of 1 μl of solvent (containing $2 \cdot 10^{-8}$ g of naphthalene) at a flow-rate of 15 μl/min led to a peak width at half-height of *ca.* 12 sec in the chromatogram with MS detection. It was observed qualitatively that the peak width increases with increasing sample concentration and when compounds of low volatility are studied, as a result of the longer residence time in the ionization chamber. Moreover, if the effluent passes first through the UV detector before it enters the mass spectrometer further peak broadening is observed. On comparing four identical interfaces that we had constructed, the peak width in the chromatogram of naphthalene with MS detection was found to be constant to within $\pm$10%.

The sensitivity of the system was tested in the single-ion monitoring mode. The sensitivity depends strongly on the compound studied. With naphthalene (*m/z* 129) a lower detection limit of $4 \cdot 10^{-9}$ g was found (signal-to-noise ratio 4:1), while with diuron (*m/z* 233) the detection limit at the same signal-to-noise ratio varied between $4 \cdot 10^{-11}$ and $4 \cdot 10^{-10}$ g (1-μl injection, flow-rate 15 μl/min). In the scan mode the sensitivity was lower by a factor of about 50 if a mass range of 100 a.m.u. was scanned in 8 sec. The sensitivity is apparently lower than that reported by Henion and Maylin[15],

although different compound classes were studied. However, the mass spectrometric sensitivity should be good enough for many applications and in favourable instances it is comparable to that of the UV detector.

Considerable efforts have been made to improve the interface further. For example, tungsten wires of 0.09 mm O.D. and different lengths were introduced into the central capillary to enhance the flow restriction. This led to an 80% reduction in the size of the capillary orifice. However, in various tests no improvement of either the peak shape or sensitivity could be found. As no solvent splitting is used, the amount of solvent entering the ion source is independent of the diameter of the interface orifice. Reduction of the orifice size leads only to a higher pressure build-up in front of the interface. A reduction of the orifice size also did not lead to a reduction of the pressure fluctuations sometimes observed at high flow-rates.

In addition, the position of the end of the central capillary was varied. Further introduction of the capillary into the CI box did not lead to any improvement of the performance of the interface.

In the present configuration of the interface, the end of the central capillary is 1 cm from the CI box. As the CI box is kept at a temperature of at least 250°C, the end of the capillary is heated. It might be expected that cooling of the capillary might improve the performance of the interface. Thus the interface reported by Melera[8], in which a 1% split of the HPLC effluent is introduced into the CI source of a mass spectrometer, uses a cooling system. To examine this effect, an interface was developed in which the central capillary was surrounded by a cooling jacket. However, with this interface it was impossible to maintain a constant source pressure. Heating of the end of the capillary is obviously a prerequisite for proper performance of the interface. We conclude that the interface described under Experimental shows the optimal performance.

This interface was tested over a period of 4 months. If high flow-rates (>15 μl/min) are avoided, the interface operates without any problems. Thus no plugging of the capillary was observed throughout the experiments, although no filter between the micro column and the interface was used. Although a rather aggressive solvent (containing at least 18% of water) was used, the cathode of the ion source lasted for at least 2 months. It may be possible that the lifetime of the cathode would be reduced if an extremely high water content (*e.g.*, 70%) were used. However, our experience so far does not allow reliable conclusions to be drawn.

Separation of polycyclic aromatic hydrocarbons and phenols

Using the above interface, the separation and mass spectrometric detection of two mixtures of polycyclic aromatic hydrocarbons and phenols were studied.

Polycyclic aromatic hydrocarbons (PAHs) are of considerable environmental significance. Although PAHs can be separated by gas chromatography[19], today more rapid separation by HPLC is sometimes preferred. As the separation of PAHs by reversed-phase HPLC is well established, these compounds are particularly suitable for testing on-line micro HPLC–MS coupling.

For this purpose a mixture of 11 commercially available PAHs was prepared. For the reversed-phase separation of PAHs, acetonitrile–water (82:18) has been proposed as a solvent[20]. However, using this solvent an adequate separation could not be achieved with the micro column used in this work. A reasonable separation was

possible with acetonitrile–water (40:60) and a flow-rate of 25 μl/min, as shown in Fig. 2a. With this solvent only chrysene and benzanthracene could not be separated. It is conceivable that an even better separation would be possible under other conditions. However, the degree of separation illustrated in Fig. 2a appeared to be adequate for testing the coupling.

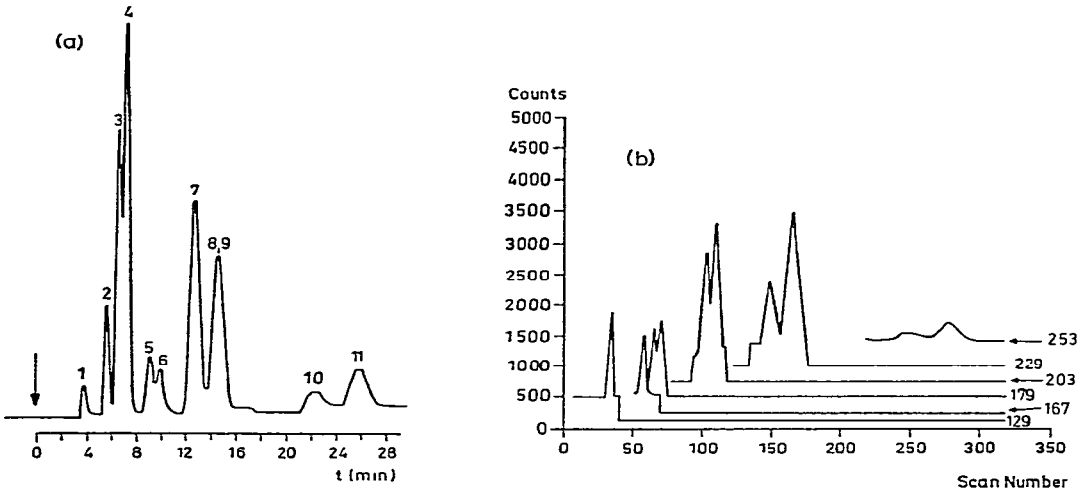

Fig. 2. Comparison of chromatograms of polycyclic aromatic hydrocarbons with (a) UV detection and (b) MS detection. Mobile phase; acetonitrile–water (40.60). Peaks: 1 = naphthalene (*m/z* 129); 2 = fluorene (*m/z* 167); 3 = phenanthrene (*m/z* 179); 4 = anthracene (*m/z* 179); 5 = fluoranthene (*m/z* 203); 6 = pyrene (*m/z* 203); 7 = triphenylene (*m/z* 229); 8, 9 = chrysene and benzanthracene (*m/z* 229); 10 = perylene (*m/z* 253); 11 = 3,4-benzpyrene (*m/z* 253).

The individual components of the mixture were studied first. Their spectra (after background subtraction) are characterized by a dominant $[M + H]^+$ ion, while fragments are almost completely absent. Pyrene is shown as an example in Fig. 3. Spectra are usually run at $m/z > 110$, as at lower masses ions formed by ion–molecule reactions of the solvent dominate the spectrum. The low-intensity signals at

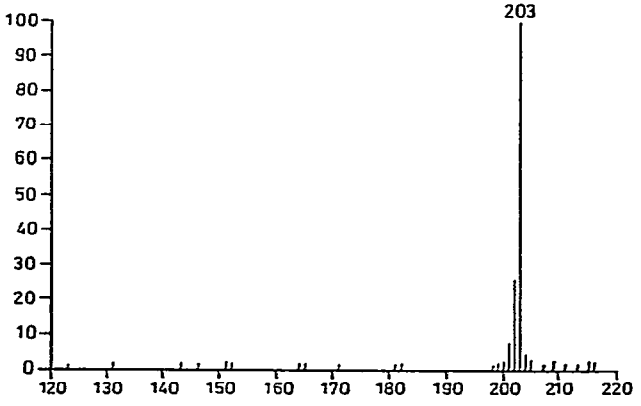

Fig. 3. Mass spectrum of pyrene.

lower masses are probably due to minor impurities in the solvent. A relatively abundant molecular ion demonstrates that the ionization conditions are intermediate between that of electron impact and chemical ionization. As the mass spectra essentially give information only on the molecular weight of the compound, the technique is particularily suitable for operation in the single-ion monitoring (SIM) mode. To this end, 1 μl of the mixture of PAHs (containing $4 \cdot 10^{-8}$ g of each component except perylene, the concentration of which was considerably lower owing to its poor solubility in the solvent used) were injected onto the column. As the mass spectrometer–computer system at present cannot be operated in the SIM mode, the mass spectrometer was scanned repetitively over selective mass ranges covering the molecular ions of all compounds, *i.e.*, *m/z* 120–135, 160–185, 195–210, 220–235 and 245–260, the successive mass intervals being selected according to the UV detector trace. Subsequently the $[M + H]^+$ ions of all components were plotted as a function of the scan number, which gave the chromatogram shown in Fig. 2b.

This chromatogram with MS detection parallels the UV detector trace closely, except that the relative intensity differs considerably, owing first to the different absorption coefficients of the PAHs at 254 nm and secondly to the different mass spectrometric sensitivities of the various compounds. Perylene is detectable only as a faint peak in the chromatogram with MS detection owing to its low concentration in the mixture. Unfortunately, peaks that are not completely resolved on the UV detector trace originate from isomeric compounds and are therefore also not completely resolved in the chromatogram with MS detection. Moreover, chrysene and benzanthracene have identical molecular weights and are therefore not separated in the chromatogram with MS detection. Much lower sample concentrations could be analysed if the spectrometer were operated in the SIM mode.

The second mixture used to test the HPLC–MS coupling contained five

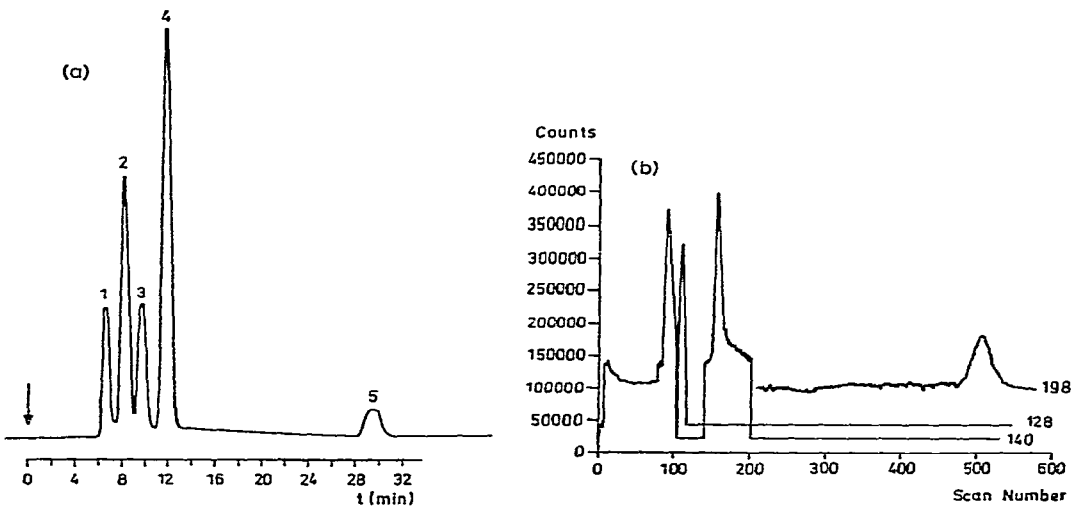

Fig. 4. Comparison of chromatograms of phenols with (a) UV detection and (b) MS detection. Mobile phase: acetonitrile–water (40:60). Peaks: 1 = phenol (*m/z* 94); 2 = 4-nitrophenol (*m/z* 140); 3 = 2-chlorophenol (*m/z* 128); 4 = 2-nitrophenol (*m/z* 140); 5 = 2,4,6-trichlorophenol (*m/z* 198).

phenols (Fig. 4a). Phenols, which are present in small concentrations in surface water and even drinking water, are of particular concern as they are toxic to fish and other aquatic life. Gas chromatography is the standard separation technique used for phenols. However, the use of HPLC, which allows a faster separation, has been proposed[21]. We chose this example as it demonstrates some limitations of the proposed coupling. It was reported that optimal separation of phenols on a reversed-phase column can be achieved in a minimum of time using gradient elution with water–acetonitrile–1% acetic acid as the mobile phase[21]. The currently available micro HPLC instruments do not allow gradient elution. Further, the presence of even small amounts of acetic acid had an adverse effect on the performance of the coupling: first, it was difficult to maintain a constant ion source pressure, and secondly, the lifetime of the filament seemed to be reduced by the presence of acetic acid. An adequate separation of the five phenols was achieved with a solvent of acetonitrile–water (40:60), as shown in Fig. 4a (little effort was made to find the optimal mobile phase for this system, so the HPLC separation can probably be further improved). Using these conditions the pure compounds were studied first. Phenol, with a molecular ion at *m/z* 94, could not be detected even in amounts as high as 10^{-6} g as a result of the abundant background signals observed at masses below *m/z* 110, as mentioned above. This result supports the previous statement that only compounds with molecular ions above *m/z* 110 can be identified reliably. For 2-chlorophenol and 2,4,6-trichlorophenol the molecular ions at *m/z* 128 and 198 were the most abundant ions, while the spectra of the nitrophenols were dominated by the protonated molecule at *m/z* 140. Using these ions, chromatograms with MS detection were obtained as a function of the scan number in the same fashion described for the PAHs. The result is shown in Fig. 4b.

CONCLUSIONS

At low to medium flow-rates (up to 15 μl/min) and low to medium water contents of the reversed-phase mobile phase (preferably below 50%), the proposed interface works reliably. Components of mixtures can be identified by MS with good sensitivity, although a further improvement in sensitivity is desirable and conceivable. If stable compounds are studied the spectra are dominated by protonated molecular ions with little if any fragmentation. Thus the MS information is reduced to the determination of the molecular weight, which in addition to the retention time is a valuable parameter for characterizing a given compound. As a result of the high abundance of the molecular or quasi-molecular ion, the MS detection of the components of a mixture in the single-ion monitoring (SIM) mode is of particular advantage. Thus the technique seems to be suitable for checking for the presence or absence of a compound in a mixture in routine analyses. Although not yet studied systematically, it is likely that only thermally stable compounds are amenable to this system as the end of the interface capillary is heated by the ion source, which is at 250°C. As the amount of solute injected on to the column is small (1 μl in our work, 0.1 μl in the work of Henion and Maylin[15]), the total solute must be concentrated to this small volume in order to obtain maximal sensitivity.

These studies have shown that operating conditions that are optimal for normal HPLC cannot be adopted directly in micro HPLC separation. Thus it is necessary to

obtain more experience with the micro HPLC technique and to improve it further. In particular, the availability of gradient elution and micro-capillaries[18] is desirable.

In spite of the limitations of the HPLC–MS coupling described here, its simple and inexpensive construction makes it attractive for many applications.

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