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MICRO POST-COLUMN EXTRACTION SYSTEM FOR INTERFACING REVERSED-PHASE MICRO LIQUID CHROMATOGRAPHY AND MASS SPECTROMETRY*

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SUMMARY

A system is described that interfaces micro reversed-phase liquid chromatography with direct liquid introduction liquid chromatography–mass spectrometry via a post-column liquid–liquid extraction system. This system allows the on-line transfer of analytes from an aqueous mobile phase to an organic extraction phase which is then admitted to the mass spectrometer. The operation of the system is demonstrated in two examples: the hydrophobic extraction of polycyclic aromatic hydrocarbons and the ion-suppressed extraction of the acidic organic pesticides 2,4-D, 2,4,5-T and Silvex and the non-chlorinated parent compound, anisic acid.

INTRODUCTION

The on-line combination of liquid chromatography (LC) and mass spectrometry (MS) has in recent years become an attractive approach to the analysis and characterization of non-volatile or thermally labile organic compounds. LC–MS interfacing techniques have developed rapidly and the field has been reviewed extensively[1]. Owing to the dissimilarity of the two techniques, their combination has required compromises to be made to each. The admittance of LC mobile phases to an MS system limits the choice of sample ionization techniques in the mass spectrometer owing to the relatively high source pressures, whereas the requirements of the MS vacuum system limit the character and flow-rate of the LC mobile phase that can be allowed into the mass spectrometer. In most instances, the result of these requirements is that only completely volatile mobile phases can be admitted to the mass spectrometer at flow-rates below 50 μl/min, and that only chemical ionization techniques can be used in the mass spectrometer.

Although the development of micro LC techniques has made practical (and

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even attractive) mobile phase flow-rates in the range 1–50 μl/min, the requirement for completely volatile mobile phases severe limitations on the chromatography, especially when reversed-phase separations are used.

It is common in reversed-phase LC, particularly in the analysis of biological samples, to use non-volatile additives and modifiers in the mobile phase. These non-volatile components can include inorganic buffers, ion-pairing reagents, reagents for chemical derivatization and even metal ions and salts.

Vouros *et al.*[2–4] have approached this problem via the use of an on-line segmented-flow liquid–liquid extraction system. In such a system, the analytes are selectively extracted from the LC mobile phase containing non-volatile components into an organic phase in which the non-volatile inorganic salts are insoluble. This work was applied using conventional LC (4.6 mm I.D. columns) and a moving-belt transport LC–MS interface that removes the bulk of the mobile phase by heat radiation and vacuum. However, with thermally labile analytes, the use of heating in such a system may lead to thermal degradation of the analytes[5].

In this paper, we describe the combination of two systems on which we have recently reported, an on-line micro post-column extraction system[6] and a helium jet nebulizing direct liquid introduction (DLI) LC–MS interface[7]. This system allows the use of non-volatile mobile phase components with micro LC and DLI LC–MS interfacing.

EXPERIMENTAL

A schematic diagram of the analytical system is shown in Fig. 1. Although each of the subsystems has been previously described[6–8], the combination as shown here is novel.

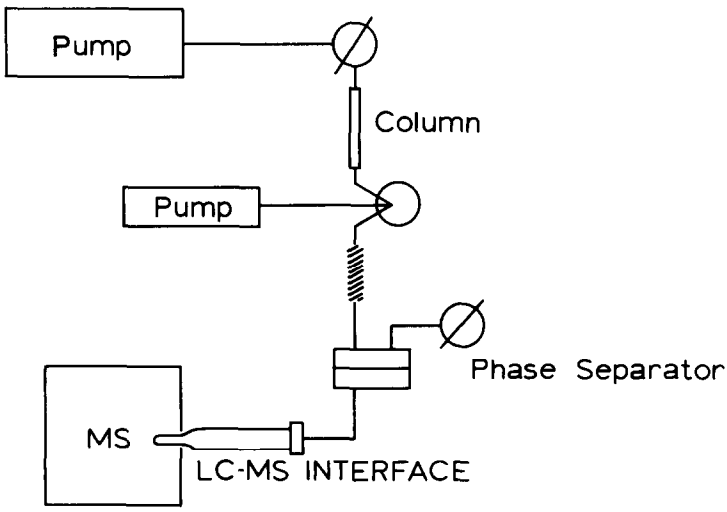

Fig. 1. Schematic diagram of the total apparatus used, consisting of miniaturized liquid chromatograph extraction system and mass spectrometer.

LC

The miniaturized LC mobile phase was delivered using a Gilson Villiers-le-Bel, France) Model 302 pump capable of isocratic flow-rates from 5 to 5000 μl/min. A Kontron (Zürich, Switzerland) Model 312 pulse damper was used to ensure essentially pulseless flow. Samples were introduced into the system using a home-made micro injector valve[8] with a 0.05 or 0.5 μl internal loop volume. Method development was performed using one of two modified detectors, *viz.*, a Varian (Walnut Creek, CA, U.S.A.) Fluorochrom filter fluorimeter or a Waters Millipore (Millford, MA, U.S.A.) Model 440 254-nm UV detector. Both detectors had been modified so that the flow cell volumes were approximately 0.6 μl and the variance contributions of the detectors were below 1.0 μl^2. All connections were made with Valco Instruments (Houston, TX, U.S.A.) 1/16 in. zero dead volume unions.

The microbore columns consisted of 10-cm lengths of 0.7 mm I.D., 1/16 in. O.D. glass-lined stainless-steel tubing (Scientific Glass Engineering, Melbourne, Australia). The column inlet and outlet fittings were Valco 1/16 in. zero dead volume fittings (with 0.25 mm I.D. bores) into which 0.5 μm stainless-steel screens had been placed. The columns were packed with 5 μm LiChrosorb RP-18 or RP-2 bonded phases (Merck, Darmstadt, F.R.G.) using a slurry packing method. These columns yield reduced plate heights of 2.5–3.5.

For the post-column extraction system, a home-made syringe pump that delivers flows in the range 10–1000 μl/min was used. This pump has a 50-ml volume and has been pressure tested to 400 bar without leakage. The aqueous organic effluent and organic extracting liquid were mixed in a home-made tee in which three 0.18 mm I.D. stainless-steel capillaries were joined at a 30° angle. The extraction capillary consisted of 30 cm of 0.3 mm I.D. PTFE tubing. The aqueous and organic segments were separated using a micro membrane phase separator with a total internal volume of 0.5 μl. The design of the membrane separator is shown in Fig. 2. The unit consists of two stainless-steel blocks, each of which has a shallow groove of 0.25 μl volume machined into it. Two stainless-steel pins allow the grooves to be precisely aligned.

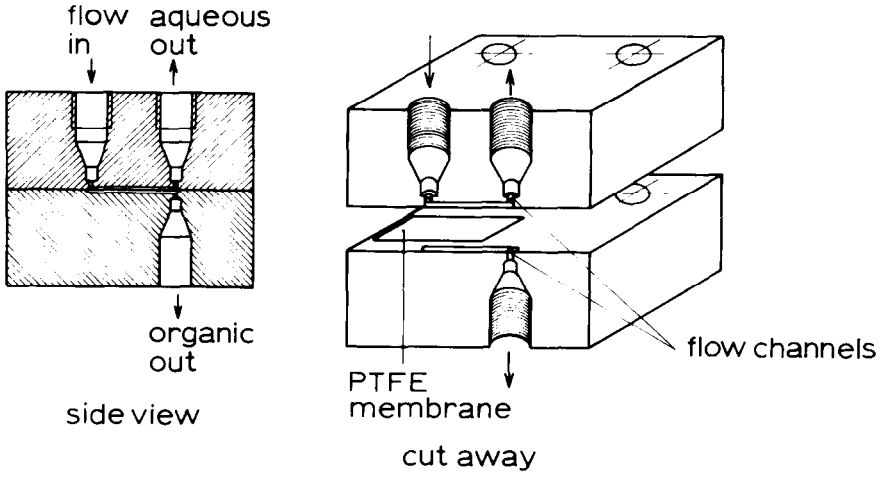

Fig. 2. Schematic diagram of the miniaturized phase separator.

The inlets and outlets are machined into the blocks to accommodate Valco 1/16 in. zero dead volume male fittings. The membrane material is a Fluorophore Type FH PTFE filter (Waters, Millipore). This hydrophobic material is permeable to organic non-polar solvents, but excludes aqueous solutions provided that it has not been previously wetted by water. Care must be taken to ensure that the membranes are never solely in contact with aqueous segments, as this destroys their ability to exclude water. The aqueous outlet of the phase separator is connected to a micro needle valve that allows the back-pressure (and the flow through the membrane) to be adjusted.

Mass spectrometer

The mass spectrometer was a Finnigan (Sunnyvale, CA, U.S.A.) Model 4021 quadrupole instrument with Varian (Palo Alto, CA, U.S.A.) Model M4 and H32 diffusion pumps for the vacuum system. No additional vacuum system (such as a cryogenic pump) was used. A Finnigan Model 2100 INCOS data system was used for data acquisition and processing.

DLI LC–MS interface

The DLI LC–MS interface is shown schematically in Fig. 3. The outer stainless-steel jacket is designed to fit the mass spectrometer's solid probe inlet. The LC effluent enters the MS source compartment through a 0.1 mm I.D. × 0.21 mm O.D. fused-silica capillary. The fused-silica capillary is coaxial with a 0.3 mm I.D. × 1/16 in. O.D. stainless-steel capillary, and extends approximately 0.5 mm past the end of the stainless-steel capillary into the MS source. Pressurized helium (1–2 bar) flows around the fused-silica capillary into the source.

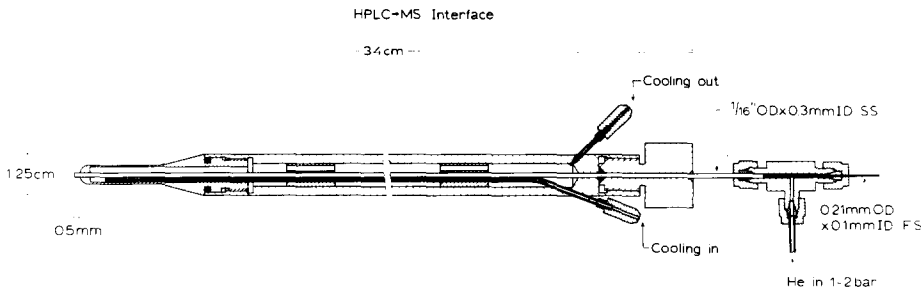

Fig. 3. Schematic diagram of the LC–MS interface.

Operating conditions

For post-column extraction LC–MS, the following conditions were used; LC mobile phase flow-rate, 25 μl/min; extracting liquid flow-rate, 50 μl/min; and MS source temperature, 250–300°C. Under these conditions, the organic flow into the MS source was adjusted using the micro needle valve to yield 0.8–0.9 Torr in the ionization chamber and consequently $0.8 \cdot 10^{-4}$–$0.9 \cdot 10^{-4}$ Torr in the analyser. The liquid flow into the MS was between 30 and 40 μl/min. The electron multiplier was operated at 1150 V with the dynode at −3 kV. Mass scans were made at 100 daltons sec^{-1}.

Reagents and chemicals

All solvents were of LC grade. *n*-Hexane, cyclohexane, dichloroethane, methanol and dioxane were obtained from J. T. Baker (Deventer, The Netherlands) and were used as received. Water was purified in-house using a Milli-Q system (Waters, Millipore). Potassium phosphate was obtained from J. T. Baker. Polycyclic aromatic hydrocarbons (naphthalene, fluorene and phenanthrene) were obtained from Fluka Buchs, Switzerland) and the acidic organic pesticides (anisic acid, 2,4-D, 2,4,5-T and Silvex) were obtained as gifts from Sandoz (Basle, Switzerland).

RESULTS

The application of the system is demonstrated below in two examples, the hydrophobic extraction of polynuclear aromatic hydrocarbons (PAHs) and the ion-suppressed extraction of organic acids.

The analysis of PAHs was used primarily as a probe to investigate the feasibility of the system. It was known from previous work[7] that the PAHs were compatible with the DLI LC–MS system and, owing to their hydrophobicity, they are well extracted into non-polar organic solvents. Fig. 4 shows the analysis of three PAHs using *n*-hexane and cyclohexane as extracting solvents. In this example, a standard mixture of naphthalene, fluorene and phenanthrene was separated on a 10 cm × 0.7 mm I.D. column packed with 5 μm RP-18 using methanol–water (70:30) as the mobile phase. As the mobile phase (and separated analytes) flows out of the analytical column, it is mixed with the organic extracting liquid, resulting in a segmented flow through the 0.3 mm I.D. PTFE capillary. In this system, the immiscible liquids form a train of alternating aqueous and organic segments each approximately 20 nl in volume. When the analytes elute from the column, an equilibrium is established between the aqueous and organic phases. The two liquid phases are then separated in the membrane phase separator, resulting in a flow of organic liquid (without any aqueous segments) that directly enters the DLI LC–MS interface and is nebulized into the MS source as a result of a combination of vaporization and nebulization by a helium gas jet. In the MS source, the analytes are ionized by a chemical ionization process that uses the organic extraction liquid (now a vapour) as a reaction gas. Fig. 4A and B show the reconstructed ion currents for three PAHs extracted by *n*-hexane and cyclohexane, respectively. The difference in the signal-to-noise ratios is largely dependent on the background signal of the solvents. This is discussed in more detail below.

The analysis of four organic acids is shown in Fig. 5. In this example, the reversed-phase separation is carried out in a buffered solution at a relatively low pH (3.0) to suppress the ionization of the acids and thereby increase their lipophilicity. In this separation, dioxane was used as a modifier. It was found, during the course of this work, that large proportions (>90%) of dioxane in water could be used with the membrane phase separator, whereas with methanol, acetonitrile and tetrahydrofuran, lower proportions had to be used to avoid penetration of the aqueous phase through the membrane. Following the chromatographic separation, the column effluent was mixed with 1,2-dichloroethane, which was then admitted to the mass spectrometer after phase separation. Owing to the electronegativity of the Cl atoms in the chlorinated acids (see Fig. 6 for structures), they produce relatively strong nega-

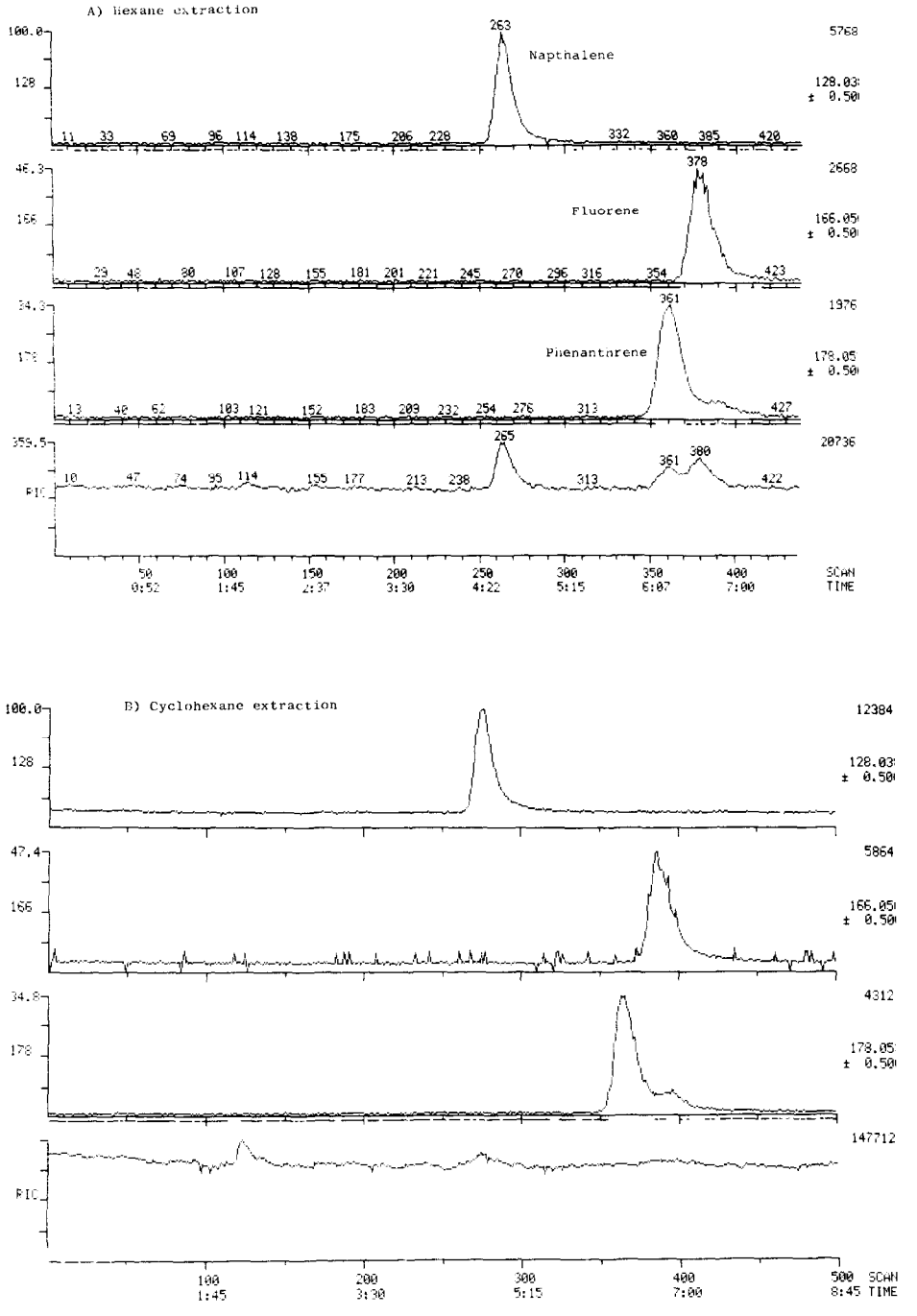

Fig. 4. PAH mass chromatograms for (A) *n*-hexane extraction and (B) cyclohexane extraction. Column, 10 × 0.7 mm I.D. GLT; stationary phase, 5 μm LiChrosorb RP-18; mobile phase, methanol–water (70:30); flow-rate, 25 μl; sample concentration, 1 mg/ml; sample volume, 59 nl.

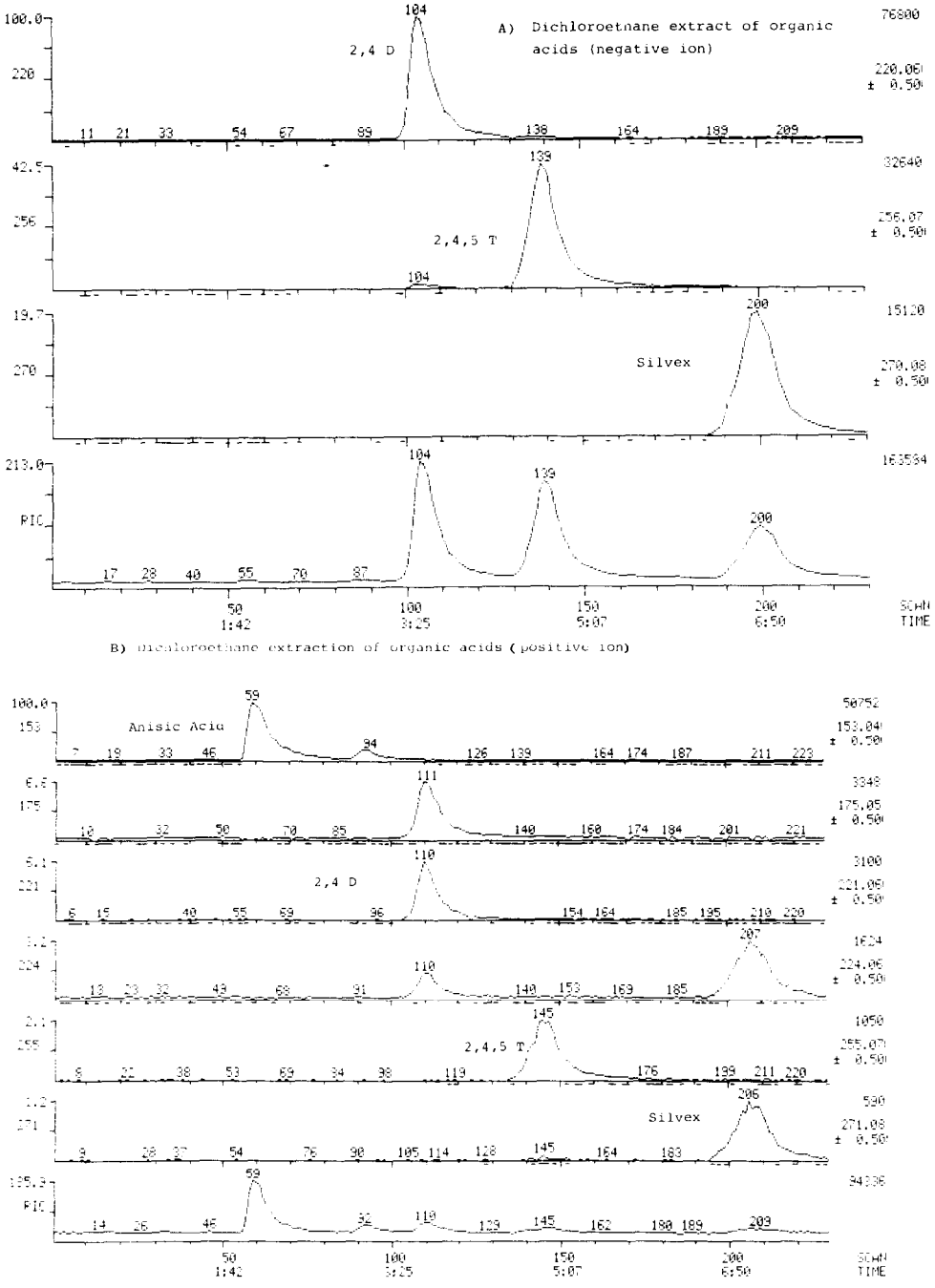

Fig. 5. Organic acid mass chromatograms for (A) negative ion and (B) positive ion. Column, 10 cm × 0.7 mm I.D. GLT; stationary phase, 5 μm LiChrosorb RP-2; mobile phase, dioxane–0.1 M NaH_2PO_4 (pH 3.0) (50:50); flow-rate, 25 μl/min; extraction solvent, dichloroethane; extraction flow-rate, 50 μl/min; sample concentration, 1 mg/ml for each component; sample volume, 50 nl.

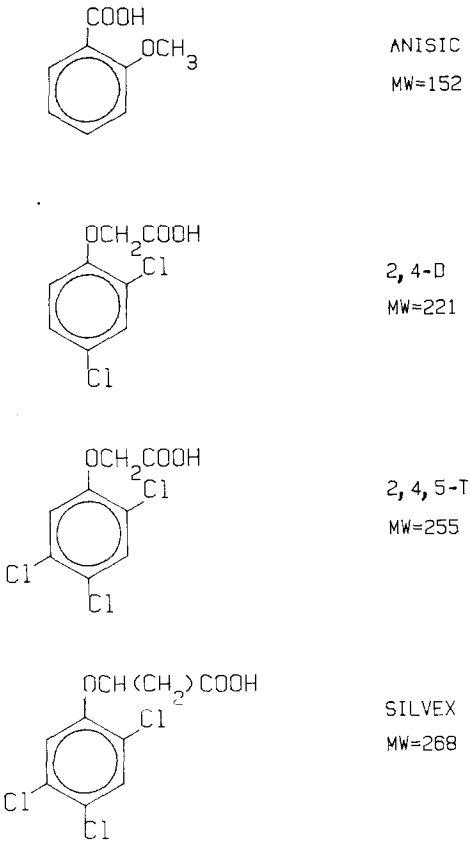

Fig. 6. Structures of organic acids investigated. MW = Molecular weight.

tive ion signals (Fig. 5A) compared with the positive ion signals (Fig. 5B). In addition to the increased signal, the negative chemical ionization produces a simpler spectrum (Fig. 7A) than the positive chemical ionization (Fig. 7B).

DISCUSSION

There are three main reasons for applying post-column extraction to LC–MS interfacing: increase in physical separation selectivity, compatibility of non-volatile mobile phases with MS and optimization of MS selectivity and sensitivity. For each of these, the character of the extraction solvent plays a primary role.

The first point is an advantage characteristic of all post-column extraction systems. Through the use of extraction procedures, it is possible to isolate an analyte from a complex matrix or to remove interfering matrix components and thereby decrease detection limits. The extraction solvent has a profound effect on the distribution coefficient of the analyte for a given aqueous phase. The mechanisms involved in both examples presented here are very straightforward, *i.e.*, the preferential solubility of hydrophobic compounds in a non-polar solvent relative to a polar solvent.

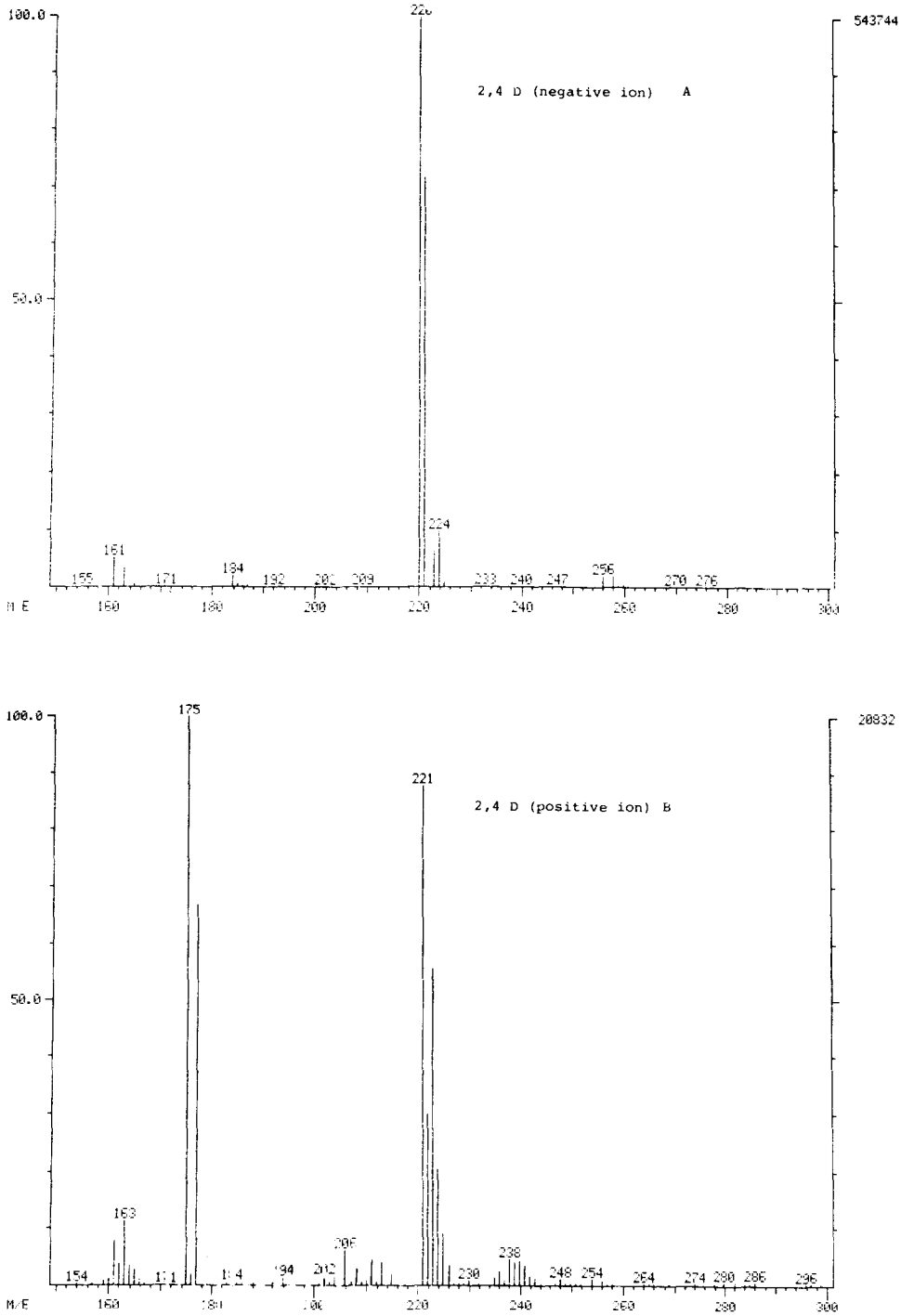

Fig. 7. Mass spectra for 2,4-D: (A) negative ion; (B) positive ion. Conditions as in Fig. 5.

This process is essentially analogous to the chromatographic mechanism used. It is possible in principle, though, to use an extraction mechanism that is dissimilar to the chromatographic system used and thus increase further the selectivity of the physical separation.

A second rationale for employing a post-column extraction system with LC–MS lies in the incompatibility of non-volatile buffers and additives often used in LC mobile phases with DLI LC–MS. According to Majors *et al.*[9], over 60% of reported separations are performed by reversed-phase LC, with ion-exchange LC accounting for another 10%. It is very common in these systems to adjust the selectivity of the separation through the addition of non-volatile components to the mobile phase, such as inorganic buffers, ion-pairing reagents, metal salts and cherlating or complexing agents. Although it is possible, in some instances, to substitute volatile additives in such systems, it is more desirable for the optimization of the chromatographic selectivity to be, as far as possible, independent of the MS requirements. In this work, the analysis of organic acids utilized a phosphate buffer at pH 3.0 to suppress the ionization of the acids and to demonstrate the feasibility of using non-volatile buffers in DLI LC–MS. Although similar results might have been obtained in this instance by using a volatile organic acid, such as acetic acid, to suppress ionization, this is not always the case, and the application clearly demonstrates the principle. Even in the absence of non-volatile mobile phase components, a high water content can cause nebulization problems with this (and other) DLI LC–MS interfaces. Whereas a high water content may be disadvantageous for LC–MS, it is often useful for extraction systems to optimize the distribution coefficient of the analytes.

Finally, the use of post-column extraction systems can be useful in LC–MS by providing a means of adjusting the sensitivity and selectivity of the MS analysis. Because, in DLI LC–MS, the liquid that is admitted to the MS source acts as a reaction gas, it is possible through the use of such a system to adjust the characteristics of the ionization process to some extent, independent of the chromatographic phase system. This can be demonstrated by pointing out that in the analysis of organic acids shown above, if a non-halogenated extraction liquid was used, such as *n*-hexane or benzene, no negative ion signals were recorded (although a reduced positive ion signal was present). It should be noted that the use of such a post-column extraction system is not restricted to simple extractions, but can include one- and two-phase reactions, ion-pair formation, metal complexation, etc., to produce enhanced MS characteristics. In short, the interface can be used as a chemical reactor to render analytes more suitable to mass detection.

On the other hand, the solvent entering the mass spectrometer will produce a spectrum itself. In some instances, this will yield a background signal that interferes with the analytical signal. This can be seen by comparing the *n*-hexane and cyclohexane spectra shown in Fig. 8. The presence of significant masses above 85 daltons for cyclohexane may cause difficulties in detecting analytes with masses between 100 and 200 daltons. This effect is demonstrated in Fig. 4. The single ion current signals are more intense for the PAHs when cyclohexane is used for the extraction rather than *n*-hexane, which is largely due to the higher extraction efficiency of cyclohexane. In the reconstructed ion current signals, however, which are the sum of the signals from 100 to 200 daltons, the background is so high when cyclohexane is used that it is not possible to recognize any chromatographic signals. In spite of the lower

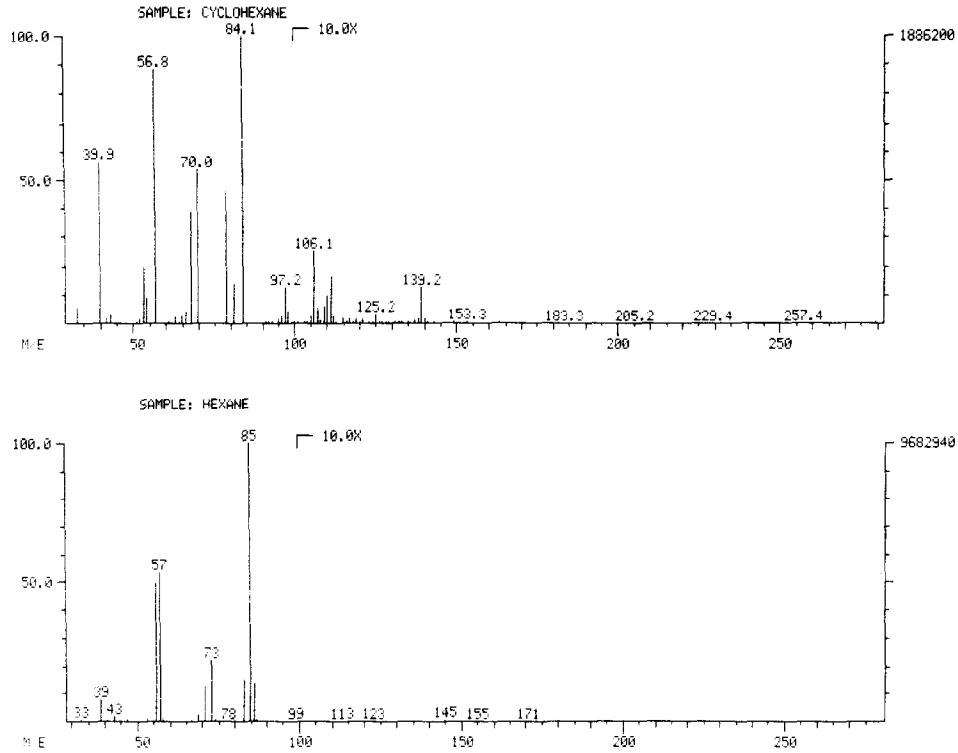

Fig. 8. *n*-Hexane and cyclohexane background mass spectra.

single ion current intensities in *n*-hexane, though, clearly recognizable peaks are present in the reconstructed ion current. Hence, unless it is known either when a peak is eluting or what the identity (or at least mass spectrum) of an analyte is, using cyclohexane as an extracting solvent it will be difficult to identify the pertinent analytical signal.

In previous work[7,8], the band broadening contribution of the extraction system and the LC–MS interface were evaluated. By comparing the dispersion in the LC–MS interface with that of the fluorescence detector used in the previous work it is possible to state that under typical conditions the combination of the extraction system and the LC–MS interface contributes approximately 1.5–2 sec^2 variance.

CONCLUSIONS

A system has been described that allows the interfacing of micro LC and mass spectrometry via an on-line post-column liquid–liquid extraction system.

It should be noted that the examples given are meant to demonstrate the feasibility of the total system and of the extraction system in this context. It is possible, in principle, to utilize such an extraction system with other micro LC systems or micro-flow injection analysis, or to use other LC–MS interfacing principles.

The work we have reported follows the work of Vouros and co-workers[2–4],

with two major differences. First, we have developed an extraction system based on a membrane phase separator that is compatible with miniaturized LC, as opposed to the earlier work using a gravimetric separator and conventional (4.6 mm I.D.) columns. Second, we have combined the extraction principle with direct liquid introduction LC–MS as compared with the transport system utilized by Vouros and co-workers. The use of the system presented here allows the high mass sensitivity analysis of low sample volumes and minimizes the thermal degradation of labile analytes.

In principle, all the work done by Vouros and co-workers[2–4] should also be applicable to this system. Further investigation along these lines, *i.e.*, with the use of ion-pair formation or two-phase reactions, are in progress.

ACKNOWLEDGEMENTS

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