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QUANTITATIVE TRACE ANALYSIS BY REVERSED-PHASE LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY

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SUMMARY

In order to overcome difficulties in spraying aqueous solvents into the vacuum of a mass spectrometer, an ultrasonic spraying device has been constructed. The vibration is achieved by means of magnetostriction in the nickel inlet tube itself. Applications to aliphatic acid determination in a shale oil process water and to determination of valproic acid, an anti-convulsant, in human serum (SRM 1599) are presented.

INTRODUCTION

The development of an on-line combination of liquid chromatography (LC) and mass spectrometry (MS) is being pursued in a number of laboratories, spurred on by the demonstrated great effectiveness of gas chromatography (GC)–MS. A number of LC–MS interfaces have resulted. Several reviews describe the operating principles, construction and performance of these devices[1–6]. Three methods, direct liquid injection (DLI), evaporation onto a moving belt, and DLI with sample pre-concentration, are now offered commercially.

Although DLI alone is intrinsically simple to implement, a large fraction of the sample usually must be discarded. (Exceptions are interfaces with micro-LC instruments, *e g.* that of Henion[7], and the more complex, rapid evaporation approach of Blakely *et al.*[8]) The moving belt has the advantage of concentrating the solute, thereby allowing a greater proportion of the sample to be introduced into the ion source of the mass spectrometer

A system which pre-concentrates the liquid stream and introduces the concentrate by DLI conceivably combines many of the advantages of ordinary DLI and the moving-belt techniques. We have described an interface which was designed and built with this aim in mind[9] The interface device concentrates a liquid stream by allowing it to flow down a resistance-heated stationary wire. Some of the residual liquid is drawn into the mass spectrometer through a metal capillary tube with a needle valve at the ion source end (see Fig. 1). The liquid sprays from the needle valve into the ion source of a conventional, differentially-pumped, quadrupole mass spectrometer

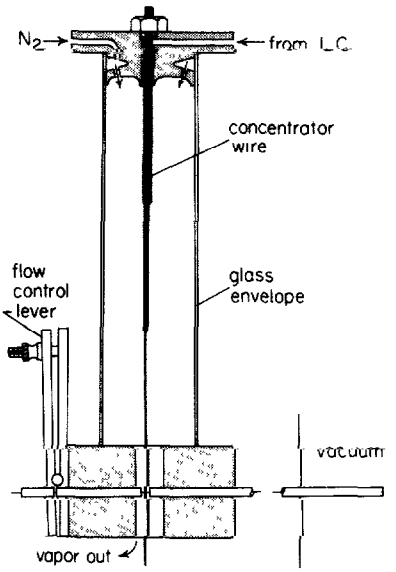


Fig 1 Concentrator wire and inlet probe for normal-phase LC–MS

This system operated satisfactorily with typical solvents used in normal-phase LC, but difficulties were encountered with the aqueous solvents used in reversed-phase LC. The liquid which issued from the tip did not spray well, accumulating in a drop at the orifice, and often freezing owing to evaporative cooling. The trouble apparently arises from the large surface tension and high heat of vaporization of water solutions Conductive and radiative heating were tried in attempts to get a dispersal of liquid from the tip, as was the introduction of an atomizing gas (see Fig. 2). After these approaches failed, ultrasonic vibration of the probe was attempted. The use of ultrasonics in LC–MS interfaces has been reported by other researchers[10–12] The vibration which we employ is a longitudinal one and is produced by using the nickel inlet tube itself as a magnetostrictive oscillator, details of which are given below

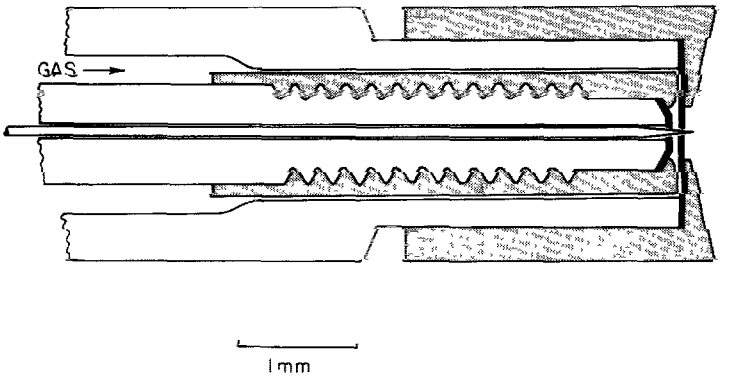


Fig 2 Cross section of probe tip with provision for concentric admission of atomizing gas

EXPERIMENTAL

Concentrator wire

The construction of the concentrator used is substantially similar to that previously described[9]. The wire is composed of three segments, a large diameter piece at the top with a high electrical resistance, an intermediate diameter piece in the center with intermediate electrical resistance, and a small diameter piece at the bottom with low electrical resistance. This provides for a high rate of evaporation where the flow-rate is high, and a low rate of evaporation at the bottom where the flow-rate is low. The feedback circuit for controlling the current input to the wire has been eliminated, and an adjustable constant-current supply is now being used.

DLI probe

A sectional drawing of the ultrasonic DLI is shown in Fig. 3. The inlet tube is 15 cm long, and the resonant frequencies are 16,000 Hz for vibration as 1/2 wavelength, and 50,000 Hz for vibration as 3/2 wavelength. A discussion of magnetostriction can be found in the textbook by Bozorth[13]. Dead volume in the inlet tube was minimized by re-drawing the nickel tubing. Annealed nickel tubing (1.5 mm O.D., 0.25 mm I.D.) was reduced to 1.25 mm O D., 0.15 mm I.D. in several steps, using simple steel dies with lanolin or soap as the lubricant. The tip is formed into a needle valve in order to control the rate of flow of liquid into the mass spectrometer vacuum. The stem of the valve is a 0.10-mm tungsten wire ground to a sharp point. The seat was made by swaging the nickel inlet tube down upon the pointed tungsten wire, then withdrawing the wire. This leaves a durable tapered seat that closely matches the wire (see Fig. 4).

Amplitude gain is provided by having the driving section *ca* 4 mm in diameter, while the driven portion is *ca.* 1.2 mm in diameter. The probe is supported on a diaphragm of phosphor bronze soldered at the node. This also serves as the vacuum seal.

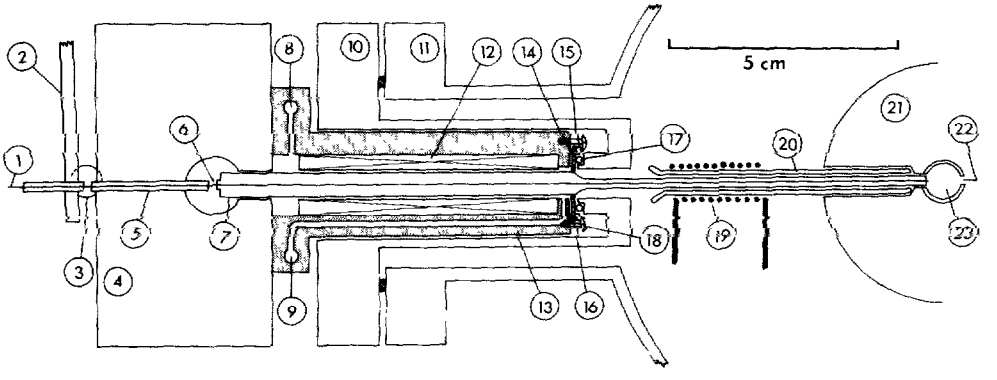
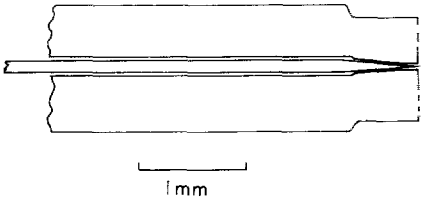


Fig 3 Cross section of the ultrasonic DLI probe assembly. 1 = Flow control wire; 2 = flow control lever, 3 = fulcrum balls; 4 = brass support block, 5 = guide tube, 6 = liquid entrance; 7 = inlet tube; 8 = cooling-water outlet; 9 = cooling-water inlet; 10 = re-entrant flange, 11 = instrument flange; 12 = driving solenoid; 13 = nylon support, 14 = gasket, 15 = support ring; 16 = phosphor-bronze diaphragm; 17 = O-ring, 18 = retaining screw, 19 = pick-up coil; 20 = glass support sleeve; 21 = ion-source block, 22 = viewing port; 23 = ion-source cavity.



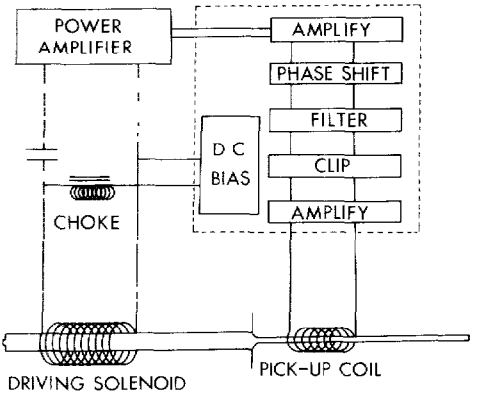


Fig 4 Cross section of needle valve with swaged seat.

Fig 5 Feedback circuit for ultrasonic DLI probe

The principles of operation of the driving/feedback electronics are shown in Fig. 5. The signal from the pick-up coil is amplified and clipped to constant amplitude. It is then filtered with a variable-bandwidth, variable-frequency filter, providing rejection of unwanted modes of vibration. The phase of the signal is adjusted and fed to a power amplifier, the output of which is variable up to 75 W. An adjustable direct current is supplied separately to the driving solenoid, with the power amplifier and direct current supply being isolated from each other by a capacitor and a choke. Water cooling is provided for the driving section and solenoid to carry away the heat which is produced in operation

Mass spectrometer

The mass spectrometer used is a variation of the Extranuclear Laboratories SpectrEL*, equipped for differential pumping and fitted with an electron-impact ion source. The source was usually operated in the temperature range 150–180°C. Liquid influx rates were in the range 5–20 μl/min A flange opposite the DLI inlet flange, previously occupied by a solids probe inlet, was fitted with a viewing window through which the tip of the probe could be examined with a telescope during operation

The source volume is pumped by a 450-l/sec turbomolecular pump and the quadrupole volume is pumped by a 280-l/sec diffusion pump This combination allows the vacuum system to be opened rapidly for repairs and modifications.

Liquid chromatography

The liquid chromatograph used was a conventional commercial instrument equipped with a loop injector and an octadecylsilane column. Injections of 20 μl were used for the examples presented. Solvents used were spectro- or HPLC-grade, although solvent quality was not found to be critical for the analyses discussed below

* Certain commercial equipment, instruments or materials are identified in this paper to specify adequately the experimental procedure Such identification does not imply recommendation or endorsement by the National Bureau of Standards, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose

All solvents used in these analyses contained 0.25% (v/v) of acetic acid for suppression of ionization. The flow-rate was 1 ml/min through the chromatographic column for all work This system was used for both the quantitative analysis of aliphatic acids in a shale oil process water and the analysis of an anti-convulsant, valproic acid, in serum.

The chromatography of the aliphatic acids was performed by isocratic or gradient runs of 35–80% methanol in water as shown in Table I Determinations of valproic acid in serum were performed on NBS Standard Reference Material 1599 by elution with 100% water for 5 min, followed by a linear 3-min 0–80% methanol gradient. While the water and gradient were being run, a stream of 80% methanol in water was run on the concentrator wire, and at the end of the gradient the analytical stream was switched to the concentrator wire. This procedure avoided contaminating the mass spectrometer with the salts and proteins present in the serum sample The LC arrangement for this analysis is shown in Fig. 6

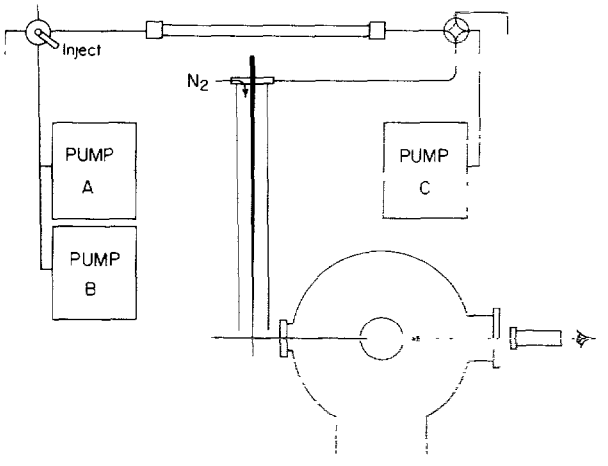


Fig 6 Connections of liquid chromatograph and mass spectrometer with provision for auxiliary stream, used in analysis of serum samples

RESULTS AND DISCUSSION

Concentrator performance

The same concentrator which was previously used for normal-phase solvents was used for the water–methanol solvents of the present study. About 95% evaporation of the solvent could be obtained with a 1-ml/min flow-rate for solvents containing 10–50% water, 90–50% methanol Because of concern that there would be insufficient methanol to prevent freezing at the tip of the probe, measurements were made of the methanol concentration in the residual liquid. Samples were collected and the methanol content inferred from refractive index measurements. With a starting solution of 80% methanol, the residual liquid contained 25% methanol at about 95% evaporation; with a starting solution of 60% methanol, the residual liquid contained about 10% methanol at about 95% evaporation. This methanol content proved to be high enough for the probe to function without plugging by freezing and without generating large ice crystals within the ion source of the mass spectrometer.

DLI probe performance

An early model of the ultrasonic probe had the needle valve seat formed from a 0.05-mm thickness film of poly(vinylidene fluoride) as shown in Fig. 7. This soft seat gave good flow control and a tight shut-off seal. It operated well with the conventional (non-ultrasonic) interface used with normal-phase solvents, but was subject to rapid wear when used with the oscillating probe. It was therefore replaced with the needle valve described in the experimental section, which had a hard seat formed integrally with the tip (Fig. 4).

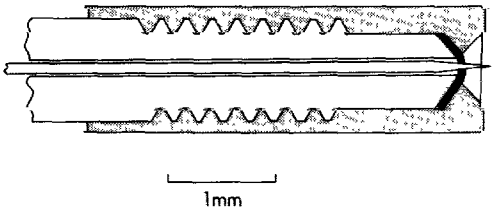


Fig. 7 Cross section of probe tip with soft needle valve seat made of poly(vinylidene fluoride) film

Trials of the hard seat ultrasonic probe in a vacuum test chamber showed that the tip vibrated with an amplitude of 20–60 μm. When liquid was admitted, the tip of the probe became covered with a liquid film from which a fog of droplets 20–25 μm in diameter emanated. In the mass spectrometer, pure water could be sprayed without freezing at the tip at a source block temperature of 200°C, and 20% methanol in water could be sprayed at 100°C. Trials with 5% (w/v) salt solutions showed no tendency for evaporative clogging, but viscous solutions, *e.g.*, above 15% (w/v) glycerol in water, could not be sprayed The diameter of the droplets was estimated by allowing them to strike a microscope slide The size of the wetted spots and an estimate of the wetting angle allowed calculation of the droplet volume.

A number of experiments were made in attempts to improve the performance of the DLI probe. It is believed that smaller droplets would improve the sensitivity of the mass spectrometer to larger, less volatile, molecules, and that a well-directed stream of droplets, rather than a diffuse fog, is desirable[14]. Both increasing the frequency and increasing the vibrational amplitude were tried in order to obtain smaller droplets. Raising the frequency to *ca.* 50,000 Hz, where the inlet tube resonates as 3/2 wavelength, gave smaller amplitude but about the same spraying characteristics as vibration at 16,000 Hz. Since the amount of useful input power is limited by the magnetic saturation of the driving section, attempts to increase vibrational amplitude were focused on changes in the geometry of the inlet tube and investigations into sources of damping. The amplitude proved to be less sensitive to the geometry of construction than had been expected. The taper at the node and the location of the supporting diaphragm did not seem to be particularly critical, for example. One model was constructed where the taper from the node to the spray tip was exponential, but no improvement in action was seen. Observable damping occurs when the flow control wire is adjusted so that it strikes against the seat of the needle valve, with a 20–30% diminution in vibrational amplitude.

An attempt to obtain a directed stream of small droplets was made by con-

structing a tip for the probe in which the diaphragm in the probe was allowed to vibrate against a flat-ended flow control wire as shown in Fig 8. It was hoped that the impact would generate a pumping action, where localized high pressure would cause a spurt of liquid with enough momentum to tear itself free of the diaphragm. The first model was constructed with a poly(vinylidene fluoride) film, through which a hole was made with pulses from a focused laser. A second was built with a stainless steel diaphragm from a commercial supplier. Both of these probes failed immediately from particles plugging the 10-μm orifices. This appears to be due to the considerable liquid surface which is exposed, and to the moving parts employed in the interface, making it difficult to exclude contamination from microscopic particles.

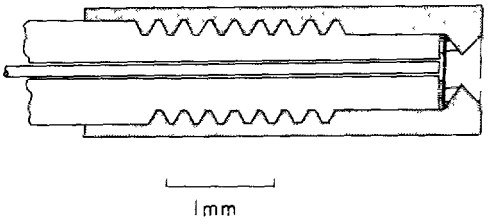


Fig. 8 Cross section of probe tip with 10-μm orifice and flat-ended flow-control wire

Quantitative determinations

The rapid analysis of industrial and energy-related effluents to determine concentrations of possibly toxic and/or hazardous organic constituents presents a formidable task Determination of individual trace organic compounds in complex matrices generally involve extraction of the compounds from the matrix, possibly column chromatographic clean-up and derivatization of the analytes, followed by quantitation using GC, LC, or GC–MS with internal or external standards. The results presented below show two examples of the use of LC–MS system described in this paper for the direct quantitative analysis of trace organic compounds by injection of a complex mixture onto the reversed-phase LC column without any prior extraction or derivatization

The first such analysis is the examination of the acidic components in a shale oil process water. Using the present LC–MS system the process water was injected directly onto the column and a chromatogram was obtained in 15 min, from which the peak heights of analytes of interest could be measured and compared to peak heights for the same compounds from an external standard solution. Four aliphatic acids, butanoic (C_4) through heptanoic (C_7), were determined Fig. 9 contains the chromatograms for the shale oil process water (top) and the external standard solution (bottom) obtained using the single ion records at m/z 131 $[(M + H)^+]$ to quantitate heptanoic acid. The results for all four acids examined are summarized in Table I. Included in the data presented are the results of the LC–MS analyses, the chromatographic conditions under which the compound was eluted and the preconcentration achieved on the interface concentrator wire. For comparison purposes, results obtained by the much more time-consuming approach of solvent extraction and GC–MS analysis are also presented In general, the results agree well, with the exception of butanoic acid. The GC MS measurements are only for the *n*-acids, whereas branched acids with the same molecular weight were not separated in the LC separation used. GC–MS

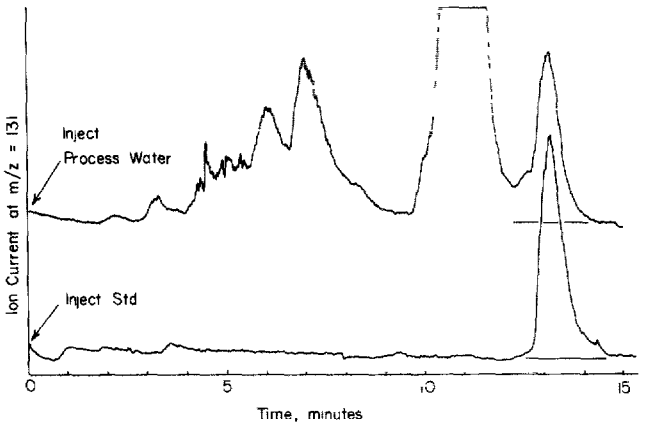


Fig 9 Single-ion record at m/z = 131 from determination of heptanoic acid in a shale oil process water Upper trace, process water, lower trace, standard solution of *n*-heptanoic acid.

examination of the C_4 acids indicated the presence of sufficient 2-methylpropanoic acid to account for the difference in LC–MS and GC–MS results.

In a second example, valproic acid (an anticonvulsant drug) was determined in NBS Standard Reference Material (SRM) 1599, Anticonvulsants in Human Serum. Two independent methods were used in the certification analyses of this SRM[15]. The first involved extraction from serum and GC analysis and the second, extraction, derivatization and LC analysis. Using the present LC–MS system in the reversed-phase mode, serum was directly injected onto the LC column and analysis, including comparison with an external standard, was ompleted within 20 min. Quantitation was achieved by comparison of peak areas of the valproic acid from serum to peak areas of standards injected shortly after the sample. A typical chromatogram, with single-ion monitoring at m/z 145 [$(M + H)^+$], is depicted in Fig. 10. Note that the valproic acid peak is broadened somewhat by the buffering effect of the serum. Table

TABLE I

DETERMINATION OF ALIPHATIC ACIDS IN A SHALE OIL PROCESS WATER

Acid	*Level by LC–MS* ($\mu g/ml \pm \sigma$)	*Level by GC–MS** ($\mu g/ml \pm \sigma$)	*Approx concentration factor obtained*	*Chromatographic conditions*
Butanoic	91 ± 10**	49 6 ± 7 6	1	35% Methanol, isocratic
Pentanoic	84 ± 10	71 9 ± 8 9	3	40–60% Methanol gradient in 5 min
Hexanoic	87 ± 13	87 8 ± 4 4	6	50–75% Methanol gradient in 10 min
Heptanoic	136***	144 5 ± 9 2	8	50–80% Methanol gradient in 10 min

* Specific for straight-chain acids.

** Includes 2-methylpropanoic acid

*** Average of only 2 determinations, 133 and 139 $\mu g/ml$.

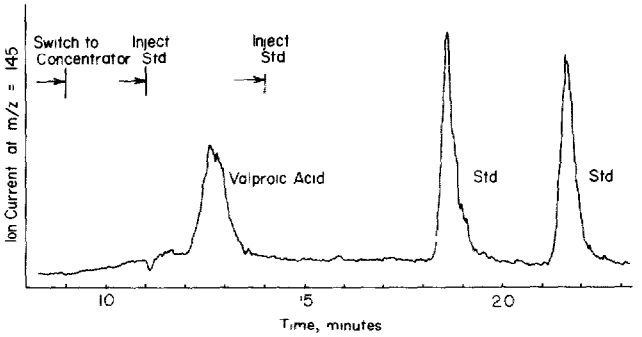


Fig 10. Single-ion record at $m/z = 145$ from determination of vaproic acid in serum, showing valproic acid peak followed by peaks from two subsequently injected standards.

TABLE II

DETERMINATION OF VALPROIC ACID IN HUMAN SERUM (SRM 1599)

Level	*LC–MS**	*Certified***
Low	13 5 ± 1.4	14 5 ± 0 3
Medium	65.4 ± 10	69 ± 1 0
High	139 ± 12	143 ± 1.0

* Mean ± standard deviation of a single measurement
** Mean ± standard error of the mean

II contains the results obtained by LC–MS analysis of the serum, as well as the certified concentrations for the SRM.

CONCLUSIONS

The LC–MS interface described in this paper has been successfully applied, in the reversed-phase LC mode, to the direct quantitative analysis of individual organic compounds in complex matrices. The system permits preconcentration of LC effluents and their introduction into the mass spectrometer when operated with solvent mixtures up to 65% water, and, under some conditions, 100% water.

The precision of analyses reported in this paper are better for the other, more time-consuming methods than for the LC–MS analyses, but the other methods involved a greater number of analyses, bracketing internal standards or standard addition experiments in many cases, and more sophisticated data handling hardware than were available for the LC–MS analyses. While rapid quantitative analyses of organic compounds in complex samples do not yet involve a routine transfer of methodology from a stand-alone LC to the LC–MS system, we belive that the examples described in this paper present adequate evidence for the utility and potential of this technique for achieving accurate and rapid trace organic analyses.

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