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DIRECT ANALYSIS OF LIQUID CHROMATOGRAPHIC EFFLUENTS

W. H. McFADDEN and H. L. SCHWARTZ
Finnigan Corporation, Sunnyvale, Calif. (U.S.A.)
and
S. EVANS
Finnigan Instruments, Ltd., Hemel Hempstead, Herts. (Great Britain)

SUMMARY

An experimental combined liquid chromatography–mass spectrometry (LC–MS) system has been constructed which transfers the LC effluent on a stainless-steel belt, 0.32 cm wide. When travelling at a speed of 2–4 cm/sec, the belt will transport the total effluent from an LC system with a solvent flow-rate of up to 0.85 ml/min. The solvent is evaporated in two consecutive vacuum lock chambers. The solute continues on the belt to a vaporization chamber where it is volatilized into the ion source of the mass spectrometer. Sample yields are in the range 25–40%. Minimal peak broadening of 1–3 sec occurs due to the time the sample passes through the vaporization chamber although tailing may occur for some polar compounds due to adsorption on the metal surfaces of the ion source. The detection limit for the current experimental system is less than 1 ng for carbaryl. Although yield precision is low, a linear calibration curve was obtained for methyl stearate in the range of 1–100 ng.

INTRODUCTION

The increasing use of liquid chromatography (LC) in organic separations has led to a demand for analysis by combined liquid chromatography–mass spectrometry (LC–MS). The techniques of combined gas chromatography–mass spectrometry (GC–MS)[1] have proven to be of tremendous value to the chemist and have provided many invaluable methods for qualitative and quantitative analysis of unknowns. Combination LC–MS would make possible the same types of analyses in LC and, in addition, would provide a sensitive (nanogram-level) universal detector that is not readily available for LC in current practice.

There are two operational characteristics of LC that make this process basically incompatible with MS. First, the quantity of carrier or mobile phase is much greater than can be handled by conventional MS vacuum systems. Secondly, the types of samples most frequently analysed by LC are not amenable to vaporization at the elevated temperatures generally required for MS.

A typical LC method uses a solvent flow-rate of 1 ml/min which, depending on

the molecular weight, corresponds to a gas volume in the range of 150 atm ml/min (for isooctane) to 1200 atm ml/min (for water). A modern GC–MS vacuum system will handle 1–2 atm/ml/min or up to 10–20 atm ml/min if configured for chemical ionization. Obviously, unless the LC–MS interface provides a large solute/solvent enrichment, sample utilization will be very low, possibly less than 1%. For a versatile system that does not have to compromise either the LC method or the MS operational mode, the LC–MS interface must provide sample/solvent enrichment greater than 10^3, and preferably as high as 10^5.

The necessity for vaporizing a sample during MS analysis will always remain an incompatibility in LC–MS. One of the most important applications of LC is with thermally sensitive samples that cannot be heated to the necessary GC temperatures. Yet, in order to perform a MS analysis, the sample must be vaporized and often, temperatures well above 250° will be necessary. Fortunately, the time for a rapid vaporization into the mass spectrometer ion chamber may be designed to be quite short, possibly less than a second. In contrast, the residence time for passage through a GC–MS system, from the point of injection to the MS ion-source, may be 100–1000 sec. Consequently, many samples that cannot stand the extreme time/temperature exposure in the GC–MS system can be flash vaporized into the ion chamber in an LC–MS mode. Ultimately of course, compounds will be encountered that cannot withstand even the short temperature cycle for a flash vaporization and LC–MS will not be practical. (An exception to this statement would occur with the proposed LC–MS interface employing a molecular beam enrichment process that does not involve metal/sample contact prior to ionization[2].)

EXPERIMENTAL METHODS

Four basic approaches are currently being studied for LC–MS interfacing, *viz.*

(1) A simple split of the LC effluent in which a maximum of 0.01 ml of liquid is vaporized into the ion chamber[3–5].

(2) Use of an atmospheric pressure ionization source (API) through which the entire effluent is vaporized[6].

(3) Enrichment of the effluent using a membrane separator[7].

(4) Enrichment of the effluent by evaporation of solvent in vacuum lock interface chambers during mechanical transport of the solution[8].

Use of a direct introduction method (1 above), involves certain disadvantages. If the mass spectrometer is operated in an electron impact (EI) mode[5], less than 0.1% of the sample can be utilized and such waste cannot generally be tolerated. Even when used in a chemical ionization (CI) mode[3], only 1–2% of the sample can be injected and the solvent carrier liquid must function as the CI reagent gas. This poses restrictions on both the MS ionization process and the LC solvent selection, but it has been demonstrated that these restrictions are not serious for most sample/solvent systems. In both cases (EI or CI), the sample must be vaporized but McLafferty has pointed out that vaporization from a solution does not require as high a temperature as vaporization of pure sample. The main disadvantage of this splitter technique is the low utilization of sample.

The combination of an atmospheric pressure ionization source with an LC–MS system is attractive in view of the high sensitivity this source can achieve for selected

gaseous samples[5]. Potentially, picogram samples might be analyzed but the current state-of-art has only shown analysis of LC fractions at the nanogram level. Even this is a notable achievement, considering that the solute is present at a mole fraction in the range of 10^{-9} and interference by solvent effects or impurities is inevitable. For some special chemical systems (very high proton affinity of solute) the API/LC–MS combination may prove to have a high sensitivity, but in any event, prior sample/solvent enrichment would substantially improve performance. The API source is also limited in that it generally provides information only about the quasi molecular ion.

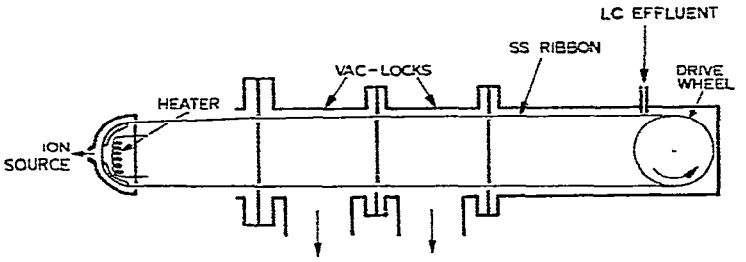
One attempt has been made to use a dimethyl silicone polymer membrane to effect enrichment of the LC effluent in a manner similar to the technique used in the GC–MS interface[7]. By restricting the LC operation solely to reversed-phase chromatography, it was suggested that the membrane would be preferentially permeated by the low polarity solutes (*e.g.*, anthracene, etc.) and would reject the more polar solvents. However, even with the paucity of published data on permeabilities in dimethyl silicone rubbers[9], it does not appear that a high leverage can be attained by this selective process and since the publication on the LC–MS membrane interace did not show any enrichment data or indicate that any attempt was made to determine this all important parameter, the general utility of this membrane interface is open to doubt. Further research is necessary.

The fourth approach to LC–MS interfacing involves transport of the sample into the mass spectrometer on a continuously moving wire[8]. In current practice, the wire is passed through a small trough containing the LC effluent and then into two vacuum lock interface chambers where the solvent is evaporated. The residual solute is then carried into the ion source region where it is thermally vaporized. Since virtually all solvent is removed, the mass spectrometer process and chromatographic solvent selection need not be compromised in favor of the LC–MS interface. On the other hand, the uptake of solution on the thin wire (0.013 cm diameter) is quite inefficient and less than 1% of the sample is carried into the mass spectrometer by this process.

The interface method described in this paper is an extension of Scott's moving wire system in which increased sample utilization is attained by transporting the effluent on a ribbon rather than on a wire. Consider, for example, a ribbon 0.32 cm wide traveling at a speed of 2.5 cm/sec. This ribbon will carry away a liquid film 0.2 mm thick from a solvent flow of 1 ml/min and if the solvent film can be evaporated without loss of solute, then the ribbon will transport virtually 100% of the solute into the mass spectrometer. Sample utilization will then depend only on the efficiency of the flash vaporization step. In practice, some sample is lost by spray processes and the flash vaporization cannot be fully efficient for all compounds. Nevertheless, yields in the range of 25–40% have been attained with an LC–MS ribbon interface system.

The ribbon transport LC–MS interface is illustrated in Figs. 1a and 1b. LC effluent is taken up on the stainless-steel ribbon (3.2 mm wide, 0.05 mm thick) and transported to the vac-locks. Partial evaporation which occurs prior to passage through vac-lock No. 1 can be aided by a combination of heat, gas flow, or vacuum as desired. Removal of solvent is completed in vac-locks No. 1 and No. 2 so that less than 10^{-7} g/sec of solvent enters the mass spectrometer. Vac-lock No. 1 is pumped with a 500 l/min forepump equipped with an oil mist eliminator and vac-lock No. 2 is pumped with a 300 l/min forepump. Depending on the tolerances set for the vac-lock interface pieces, these chambers are maintained in the pressure ranges 1–20 Torr

(a)



(b)

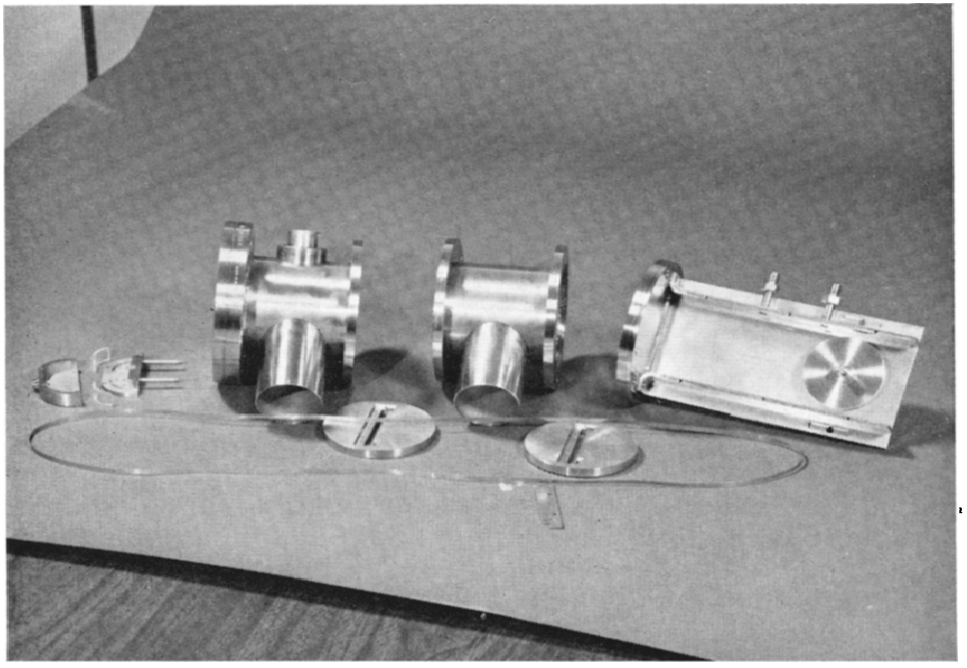


Fig. 1. (a) Schematic of LC–MS belt interface. (b) Photograph of unassembled LC–MS experimental interface.

(vac-lock No. 1) and 0.1–0.5 Torr (vac-lock No. 2) so that the mass spectrometer analyzer section can be pumped to a satisfactory level around 10^{-6} Torr.

Flash vaporization of the sample occurs by thermal and radiant heating in a small chamber butted directly to the solid-probe entrance of the mass spectrometer ion source. A Finnigan Model 3200 quadrupole was used throughout these experiments. Heat input is provided by a Nichrome heater contained in a quartz tube. The belt travel distance through the chamber is 6.5 cm, so that for most belt speeds (2–4 cm/sec), point residence time is 2–3 sec. By comparison with the temperature of vaporization for solid probe samples, it appears that the belt temperature rises to within 20–30° of the chamber temperature. (Direct belt temperature monitoring has not yet been performed.)

Detail of the vacuum lock assemblies is shown in Fig. 2. The slot for passage of the belt is formed by two "L"-shaped sapphire pieces which are attached to the

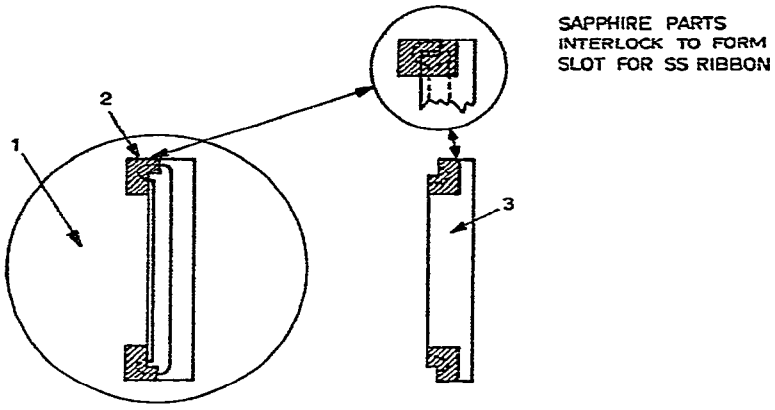


Fig. 2. Details of sapphire slits in vacuum lock interface parts.

stainless-steel flange or vacuum closure bar by epoxy cement. The belts used are either 0.05 or 0.075 mm thick and the slot tolerance is set as illustrated to be 0.075 mm greater than the belt thickness (*i.e.*, either 0.125 or 0.15 mm). The belt width is 0.317 cm and the slot width is 0.325 cm. These vacuum interlock dimensions permit attainment of reasonable vacuum levels, as previously discussed.

RESULTS

The LC–MS interface shown in Fig. 1 has been operated on an experimental basis for several months. An attempt was made to study the factors influencing the per cent yield and precision of sample utilization but during much of this time, progress has been hampered by frequent mechanical failure of the belt system. Because the belt must travel through six slits with dimensions of fairly close tolerance, any small imperfection (*e.g.*, belt width oversized, slight kink in belt, sapphire pieces not set properly, belt out of line) will impede the belt motion and eventually lead to a hangup or belt seizure. As a consequence, although the apparatus has operated successfully for many LC–MS functions on an intermittent basis, the mechanical failures must be eliminated before the system can be considered for reliable analytical service.

Efficiency of solvent removal through the two vacuum locks is very high, particularly for lower boiling solvents such as hexane. With approximately 10^{-2} g/sec (1 ml/min) of hexane flowing on to the belt, the hexane mass spectral background indicates that around 10^{-7} g/sec is entering the ion source. The enrichment of sample/solvent is therefore in the range of 10^5. Lower boiling solvents such as pentane or methylene dichloride give higher enrichment and should be selected whenever possible. Higher boiling solvents such as toluene, dioxane, or isooctane should be avoided since they require heat input for efficient solvent evaporation.

With the current apparatus, optimum performance could be attained with a solvent flow-rate of up to 0.85 ml/min. At a higher solvent flow-rate, sample is lost due to spray evaporation at the first vacuum lock.

The sample transfer efficiency of the belt interface system was determined by comparing the peak from repeated samples of methyl stearate (10^{-8} g) with the signal from comparable samples introduced by a direct probe. Yields were in the range of

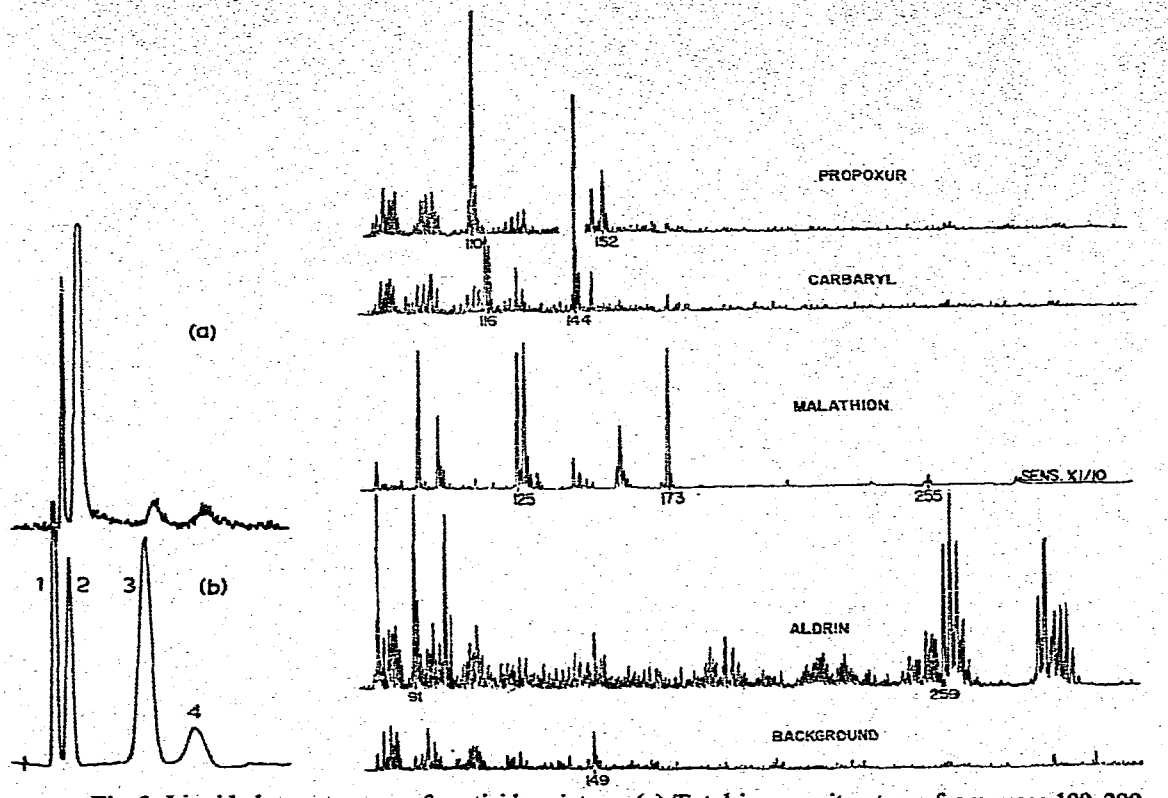


Fig. 3. Liquid chromatogram of pesticide mixture. (a) Total ion monitor trace from mass 100–290. (b) UV absorption curve, $\lambda = 254$ nm. Column, Duropack Carbowax 400 on Porasil; solvent, pentane; flow-rate, 0.5 ml/min. 1 = Aldrin; 2 = malathion; 3 = carbaryl; 4 = propoxur.

Fig. 4. Mass spectra of pesticides eluted from LC column and transported to mass spectrometer by LC–MS belt interface.

25–40% at optimum temperature conditions. If the temperature is too high or too low, the yield is reduced, but fortunately, there is a broad maximum for temperature optimization. For methyl stearate, the optimum range was found to be 135–175°. In general, the vaporization chamber should be around the temperature used for solid probe introduction of the samples under study.

The sample transfer efficiency stated above is considered to be a reasonable state-of-the-art achievement. Unfortunately, the precision of these yields is very poor and repeated sample yields will vary over the range 25–40%. Such erratic behavior results from an uneven build-up of the effluent film on the belt so that irregular losses occur due to spray evaporation. In future, an attempt will be made to increase the evaporation prior to the first vacuum lock, thus reducing the possibility of spray losses at this point.

To compare the LC–MS interface with a conventional UV detector, the exit line from a Jasco Uvidec 100 LC detector was brought into direct contact with the stainless-steel belt of the interface system. The chromatographic separation of a mixture of pesticides was monitored first by the UV detector and then by the mass spectrometer. Fig. 3 presents the chromatogram obtained with this tandem dual

detector system. Reasonable chromatographic fidelity is maintained during MS detection and very little cross contamination occurs of one compound with adjacent neighbors. The mass spectra obtained at the top of each peak are shown in Fig. 4. (Oscillographic traces rather than computer-generated bar graphs are presented here in order to show more realistically the actual quality of spectra produced in the LC–MS mode.)

Some chromatographic peak broadening must occur during the sample vaporization process. The amount of this distortion will depend on the residence time of a point as the belt passes through the vaporization chamber and on the nature of the vaporization process. The length of travel through the chamber is 6.5 cm so that for belt speeds of 2–4 cm/sec, the residence time will be in the range 1.5–3 sec. If vaporization is occurring during most of this period, the residence time should define the peak broadening. Fig. 5 compares the peak shapes obtained for carbaryl samples from the UV detector with those obtained with the LC–MS interface at a belt speed of 2.2 cm/sec. The width at half the peak height is broader for the mass spectral record by about 2 sec, consistent with that expected. Some tailing is observed, particularly for the larger (200-ng) sample, which is most likely due to adsorption effects on the metal surfaces in the ion chamber and vaporization chamber. Such tailing will be expected for many polar compounds of the type encountered in LC.

The belt recycle period is 38 sec at the speed of 2.2 cm/sec. In general, if the temperature of the vaporization chamber is in the optimum range, "ghost" peaks from carry-over on the second cycle are less than 5%. Usually they are not observed.

The sensitivity of the experimental LC–MS system was tested by monitoring

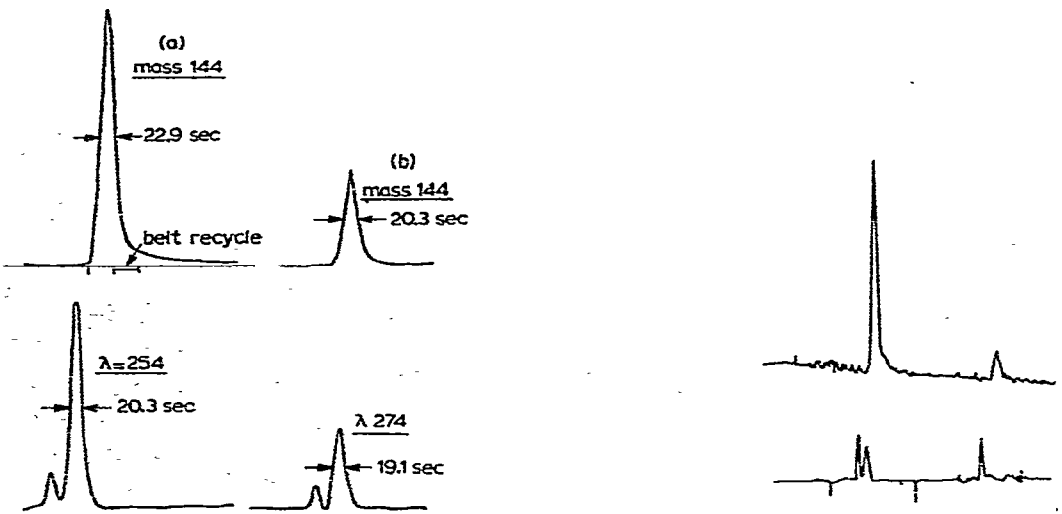


Fig. 5. Comparison of LC peak shape from UV detector (bottom traces) and single-ion monitor (top traces). (a) 200 ng carbaryl; (b) 20 ng carbaryl.

Fig. 6. Detection limit for carbaryl using LC–MS belt interface. First peak, 20 ng; second peak, 2 ng. Top trace, single-ion monitor, mass 144; bottom trace, UV detector, $\lambda = 254$ nm. First peak in UV trace is solvent front.

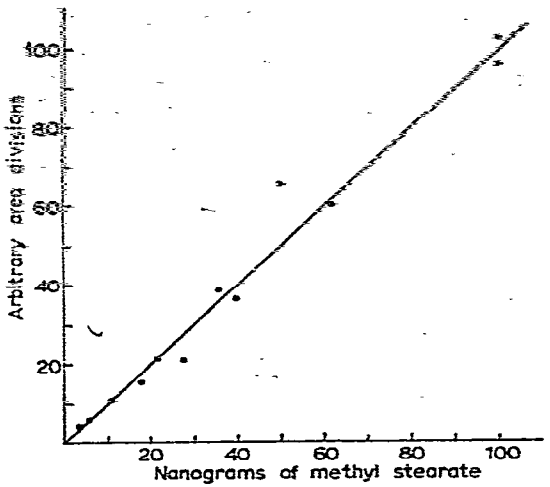


Fig. 7. Calibration curve for methyl stearate introduced through LC–MS interface. Belt speed 2.5 cm/sec; vaporization chamber at 160°; solvent flow-rate, 0.55 ml/min. One arbitrary area division represents 40 mV sec.

the mass 144 peak during injection of nanogram samples of carbaryl (Fig. 6). The 2-ng peak was significantly above the noise level, suggesting a detection limit of less than 1 ng.

Although the reproducibility of yield for sample transfer is not precise with the current system, a calibration curve was constructed from data taken over a limited sample size range using methyl stearate. The results, shown in Fig. 7, indicate a linear vaporization yield in the range of 1–100 ng.

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