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Note

Characterization of the direct-probe open-tubular liquid chromatography–mass spectrometry interface parameters

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We recently reported the design of an interface coupling open-tubular liquid chromatography (OTLC) (using columns of 2–10 μm I.D.) and mass spectrometry (MS)[1]. Coupling of OTLC and MS presents several advantages over other methods of LC–MS coupling. The low flow-rates utilized in OTLC (<0.1 μl/min) permit introduction of the entire effluent into the mass spectrometer. The resultant source pressures are low enough for the production of electron impact (EI) spectra.

The direct-probe OTLC–MS interface that we developed[1] has several advantages over previous OTLC–MS interface designs[2–4]. The direct liquid introduction (DLI)-OTLC–MS interface requires additional liquid flow for proper operation which prohibits operation of the mass spectrometer in the EI mode[2,3]. In the capillary vapor jet inlet interface, complete vaporization of the solvent takes place inside the capillary tubes[5,6]. This can lead to precipitation of less volatile analytes in the capillary tube[4]. Our direct-probe OTLC–MS interface (a capillary vapor-jet variation) uses a tapered column tip which decreases the evaporation surface and increases the effluent's linear velocity. This causes the vaporization to take place very near the orifice which eliminates the deposition of less volatile analytes in the capillary column[1].

A variety of parameters such as the orifice diameter, mobile phase velocity and temperature can influence the performance of the interface. The orifice diameter can be viewed as a non-variable interface parameter, while the mobile phase velocity and temperature can be varied during operation. We have recently reported several applications of OTLC–MS to the separation and analysis of pesticides[1], PNAs[1], pesticide metabolites[7] and herbicide metabolites[8]. Here we report the results of our study of the optimization of these parameters for the direct probe OTLC–MS interface and the results of our study of the range of compounds for which this interface is applicable. For this study we have used flow injection analysis in order to remove any effects of the chromatographic process on the behaviour of the interface.

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EXPERIMENTAL

OTLC

The OTLC system used in this work has been described previously[1]. A brief description follows. The mobile phase is kept under helium pressure in a 70-ml reservoir. Flow-rates are varied by varying the head pressure and linear velocities are determined from the column volume and measurement of the dead time of the column. The reservoir is connected to a stainless-steel tee with $\frac{1}{16}$-in. stainless-steel tubing through a 4-port injection valve and an in-line filter. This tee holds the OTLC column with a Vespel ferrule. Since no mechanical pumps are used, the flow-rate is pulse free. Injection are made by introducing a plug of sample solution onto the column[1,7]. The amount injected is controlled by the injection time; at a typical flow-rate of 1 nl/s, a 1-s injection results in a 1-nl injection volume. For this work only untreated fused-silica columns (1 m × 10 μm I.D.) (Polymicro Technologies, Phoenix, AZ, U.S.A.) containing no stationary phase are employed.

MS

Two Finnigan 3300 quadrupole mass spectrometers were used for this work: a chemical ionization mass spectrometer, previously modified for negative ion detection, and an electron ionization mass spectrometer. A Finnigan/Incos 2300 data system is interfaced to both mass spectrometers.

OTLC–MS interface

The interfacce probe has been described previously[1]. A description follows. The probe is constructed from an 8 in. long hollow stainless-steel shaft, on which a copper tip is silver soldered. The temperature of this tip is controlled and monitored by a cartridge heater and a thermocouple connected to an Omega 4001 temperature controller (Omega Engineering, Stamford, CT, U.S.A.). A 12-in. long piece of $\frac{1}{16}$-in. stainless-steel tubing goes through the entire probe and protrudes 2 mm beyond the copper probe tip. The OTLC column goes through this $\frac{1}{16}$-in. stainless-steel tubing and it protrudes beyond the stainless-steel tube an additional 2 mm.

Tapering

Tapering of the fused-silica tubing was achieved by quickly drawing the tubing out in a hot methane–air flame. This was done with 800 p.s.i. air flowing through the column. In case of coated columns the heat and air served to pyrolyze the stationary phase and to remove it from the column end to avoid plugging. The air flow also allowed one to determine whether or not the column was still open by immersing the column tip in water, and observing the formation of bubbles. Examination by electron microscopy (Model DS-130 electron microscope, International Scientific Instruments, Milpitas, CA, U.S.A.) of twelve tapered column ends with outer diameters varying from 150 to 20 μm and inner diameters varying from 10 to 1.5 μm showed that the ratio of the I.D. to the O.D. of the capillary did not change, even at the smallest diameters. It is therefore possible to calculate the taper orifice by measuring the outer diameter using a 100× optical microscope. A comparison of the twelve orifice diameters calculated from the O.D. and directly measured with an electron microscope resulted in a maximum of 5% difference between these two methods. To obtain accurate I.D. to

O.D. ratios, the O.D. and I.D. of the capillary need to be measured (with the optical 100 × microscope) prior to tapering, but with the polyimide coating removed. This is more reliable than using the manufacturer's data on I.D., O.D. and thickness of the polyimide coating.

Sample injection

To evaluate the performance of the interface, 10-s long injections were made, which ideally should result in the formation of broad flat-topped peaks. This allows study of the vaporization process over an extended period of time.

Reagents

The mobile phases used were water, methanol, acetonitrile (HPLC-grade, Fischer) and mixtures thereof. Cholesterol, isoleucine, adenine and adenosine were obtained from Sigma (St. Louis, MO, U.S.A.). Epinephrine, naphthalene, acridine, nitropyrene, pyrene, and perylene were obtained from Aldrich (Milwaukee, WI, U.S.A.). The pesticides were obtained from the U.S. Environmental Protection Agency (EPA Pesticides & Industrial Chemicals Repository, Research Triangle Park, NC, U.S.A.).

RESULTS AND DISCUSSION

The purpose of this study was to investigate the effect of the three main parameters (orifice diameter, mobile phase velocity and temperature) on the performance of the capillary interface. The acquired knowledge can be used to optimize operating conditions and expand the limits of application to compounds of lower volatility.

Selection of test compounds

To avoid effects due to other influences, such as chemical ionization (CI) reagent gas, the experiments were done under EI conditions. To further eliminate effects due to molecular ion fragmentation, we selected naphthalene as a relatively volatile standard compound (mol.wt. = 128, b.p. = 218°C) and perylene as a standard compound of lower volatility (mol. wt. = 252, b.p. = 400°C) since these compounds do not fragment appreciably. Preliminary tests have indicated that perylene could be detected with acceptable peak shape only under optimum interface conditions[1]. Toluene was used as the internal standard since, under all testing conditions, it was easily vaporized and detected, resulting in ideal peak shapes.

Goodness of fit

For a variety of compounds the long injections resulted in badly spiking and tailing peaks. We devised a quantitative means to evaluate such peak shapes by comparing the peak shape of an easily vaporized compound with that of a compound of lower volatility. The compounds were coinjected. The degree of overlap gives an indication of the effectiveness of vaporization of that compound. The goodness of fit (GOF)[9] is direct measure of this degree of overlap. For the interface evaluation experiments, the GOF between the square topped peak from the internal standard (toluene) and the peak of the test compound (perylene or naphthalene) was calculated.

As the interface performance degrades, the amount of overlap decreases, which results in a larger number for the goodness of fit. A perfect peak will overlap entirely with the internal standard and have a GOF value equal to 1. The GOF is calculated as follows:

$$\text{GOF} = \sum_{S} \left| \frac{I_C}{A_C} - \frac{I_S}{A_S} \right|$$

Where S is the scan number, I_C is the intensity for the compound at that scan, A_C is the total area of the square topped peak, I_S is the intensity of the internal standard, and A_S is the area of the internal standard. The values of the GOF for several different peak shapes are shown in Fig. 1.

Absolute area and relative area

The absolute peak areas are calcuated for all three compounds. These peak areas indicate a high transfer efficiency for the interface. The relative areas are calculated for perylene and naphthalene with respect to toluene in order to closely examine the results due to differences in vaporization. All values are the average of five replicates.

Effect of orifice diameter

In this experiment seven different taper orifice diameters were evaluated: 1.5, 2.0, 3.0, 4.1, 4.9, 6.0 and 10 μm (no taper). Taper diameters smaller than 1.5 μm required mobile phase head pressures in excess of that which can safely be used with this OTLC

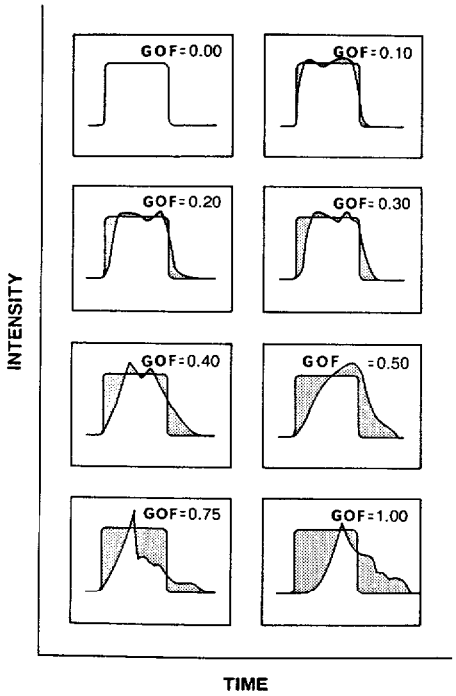

Fig. 1. Values for the GOF values calculated for increasingly degraded peak shapes.

system. The probe tip temperature for these experiments was 300°C, at which temperature a stable vapor-jet is formed resulting in constant source pressure, and the mobile phase linear velocity was held at 1 cm/s (a normal OTLC linear flow-rate for these diameters).

The effect of the orifice diameter on peak shape is shown in Fig. 2A. For the volatile compound, naphthalene, the effect is small, and little variation in the GOF as a function of diameter is observed. This is not the case for perylene for which peak shapes become unacceptable for taper diameters above 2 μm. At taper diameters of 4 μm and larger, perylene could not be detected.

The transfer efficiency as a function of orifice diameter and expressed by the absolute peak areas (Fig. 2B) is the highest at smaller diameters for both test compounds and the internal standard. It decreases and then becomes constant for naphthalene and toluene above 3 μm. For perylene no signal can be obtained at the larger diameters.

The difference in volatality between the two test compounds and the resulting effect of interface parameters is shown in Fig. 2C. The relative peak area for naphthalene does not change with orifice diameter, while the relative peak area for perylene drops rapidly to zero for orifice diameters of 3 μm or greater.

Effect of mobile phase velocity

The effects of mobile phase velocities from 0.25 to 3.4 cm/s have been evaluated at a probe tip temperature of 300°C and at a taper diameter of 1.5 μm (Fig. 3).

The effect of the mobile phase velocity on peak shape is shown in Fig. 3A. Even at low velocities the GOF indicates acceptable peak shapes, and the GOF remains relatively constant throughout the range of velocities evaluated.

Above a linear mobile phase velocity of 0.5 cm/s there is a significant increase in peak area as a function of mobile phase velocity (Fig. 3B), as would be expected due to the increase in the mass flux of the analyte. At lower velocities, however, there is very little increase in area as a function of eluent velocity. Apparently there is a critical minimum linear velocity required for the formation of a vapor jet which allows efficient introduction and detection of the analytes. The relative areas (Fig. 3C) show a decrease as a function of mobile phase velocity. This effect is more pronounced for perylene than for naphthalene.

Effect of temperature

Two processes can take place as a function of temperature: vaporization and thermal degradation. High probe tip temperatures are needed to vaporize compounds of lower volatility, but excessive temperatures can lead to thermal degradation of analytes. Ideally, the minimum probe tip temperature which satisfactory vaporizes analytes is the optimum probe tip temperature. This temperature, however, is compound dependent.

Naphthalene, for example, gives a peak shape that is chromatographically acceptable above 200°C, and the GOF remains constant at the good value of approximately 0.15 (Fig. 4A). Perylene, in constrast, requires temperatures above 275°C.

The effect of temperature on efficiency of vaporization (*i.e.* the absolute peak area) has also been examine (Fig. 4B). The absolute area for the volatile toluene and

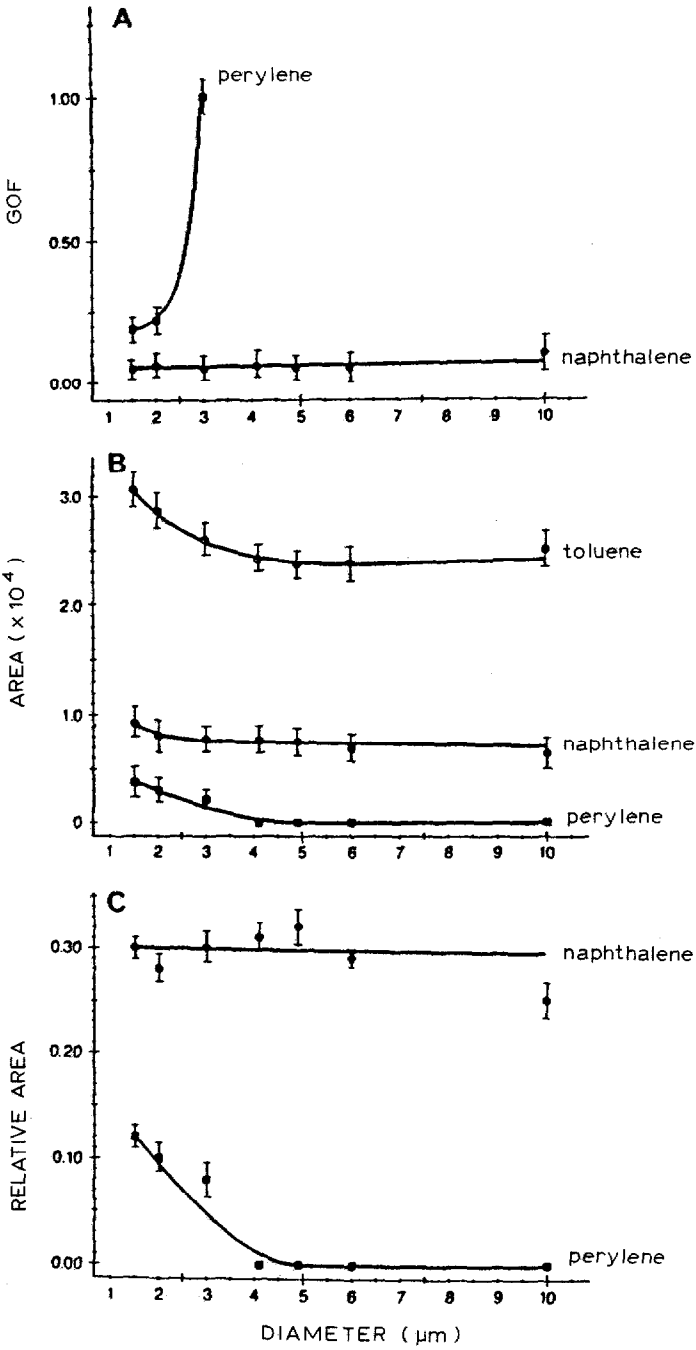

Fig. 2. Effect of the orifice diameter on interface performance.(A) Effect on GOF; (B) effect on absolute area; (C) effect on relative peak area.

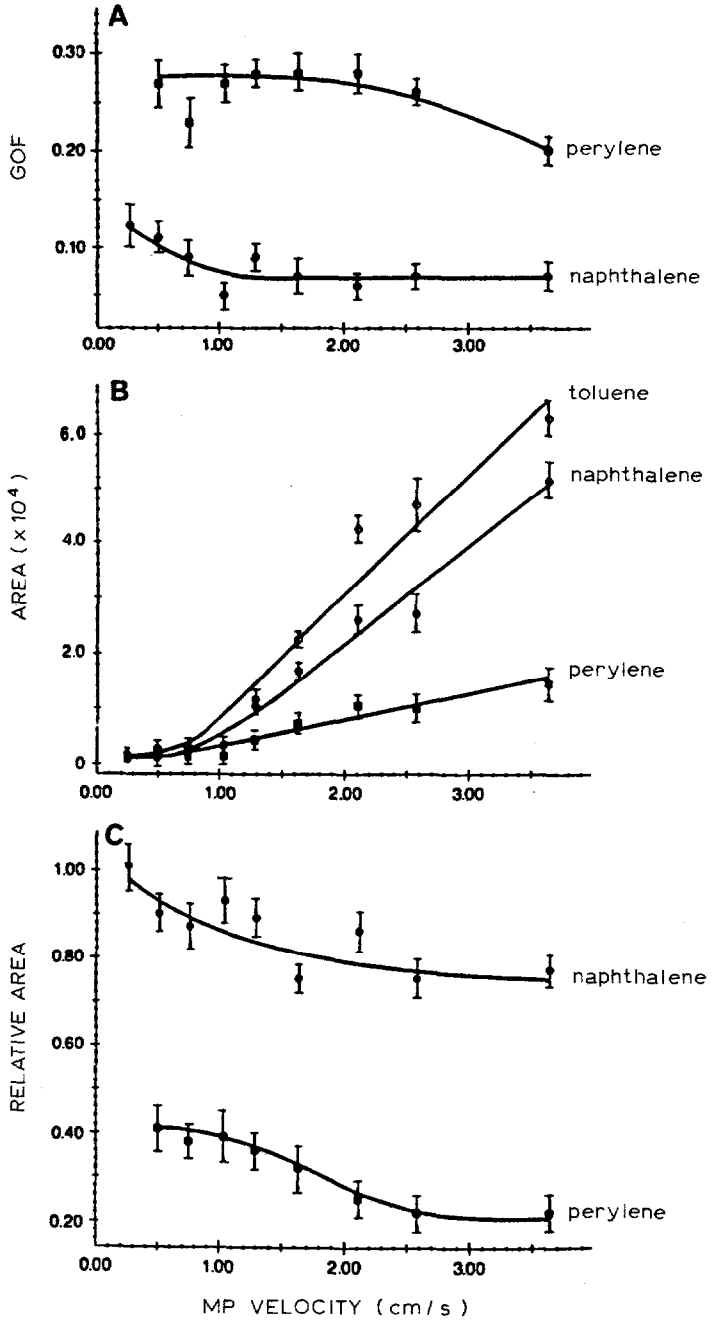

Fig. 3. Effect of mobile phase velocity on interface performance. (A) Effect on GOF; (B) effect on absolute area; (C) effect on relative peak area.

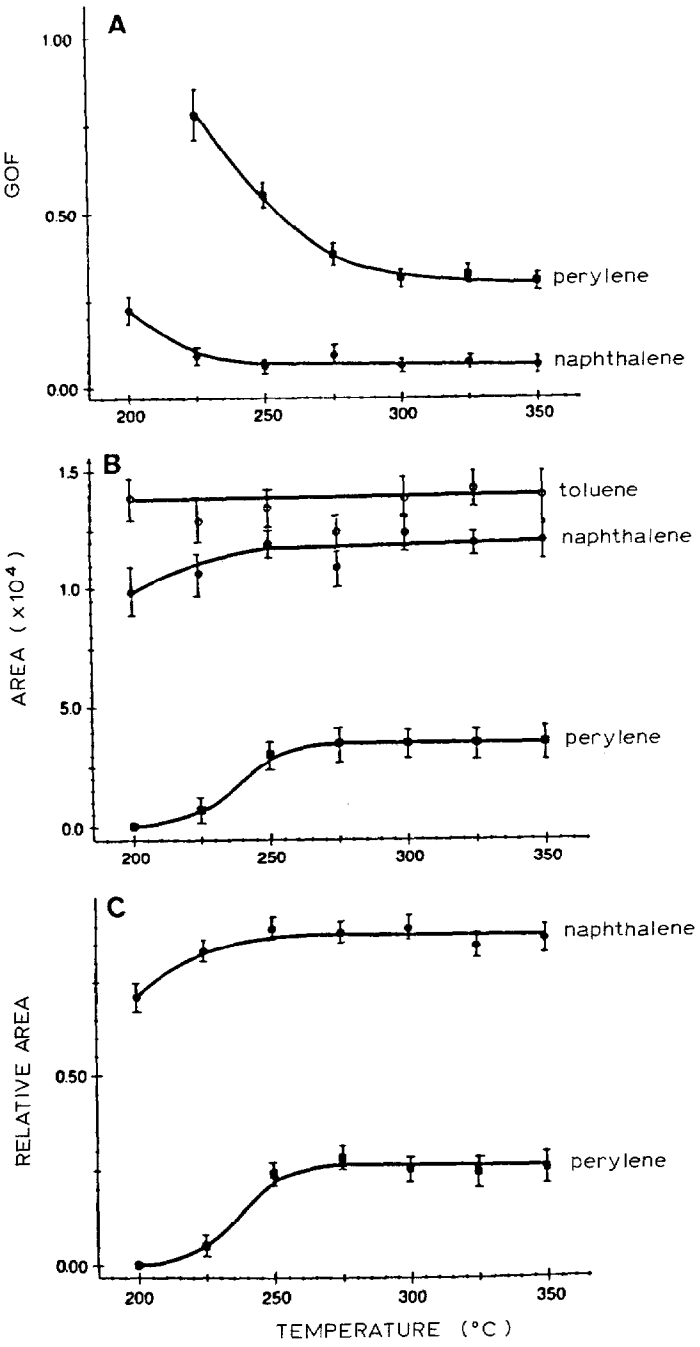

Fig. 4. Effect of probe tip temperature on interface performance. (A) Effect on GOF; (B) effect on absolute peak areas; (C) effect on relative peak areas.

naphthalene remain constant from 200 to 300°C. Perylene can not even be detected at 200°C, but the perylene peak area is constant from 250 to 350°C. The relative areas for both test compounds also remains constant above 250°C (Fig. 4C).

Thermal degradation

Cholesterol was used to examine the effect of probe tip temperature on thermal degradation. It is well known that cholesterol readily undergoes dehydration as a result of thermal degradation. A comparison was made between a mass spectrum obtained with a solid probe (Fig. 5A), under which conditions little thermal degradation takes place, a mass spectrum obtained from 100 ng by direct probe (Fig. 5B) (the lowest amount of analyte which gave a useful spectrum by direct probe on this system) and a mass spectrum obtained with the OTLC system from a 50-ng injection at a probe tip temperature of 325°C (Fig. 5C). More water loss (m/z 368) is observed under OTLC conditions. The remainder of the spectra compare well. The degree of thermal degradation was further studied by examining the relative intensities of $M^{+\cdot}$ (m/z = 386) and $[M-H_2O]^{+\cdot}$ (m/z = 368) as a function of probe tip temperature (Fig. 6). It is clear that the thermal degradation reached a maximum at 225°C and that the amount of degradation is limited. We can conclude that, although some thermal degradation of labile compounds can occur, it does not preclude their analysis.

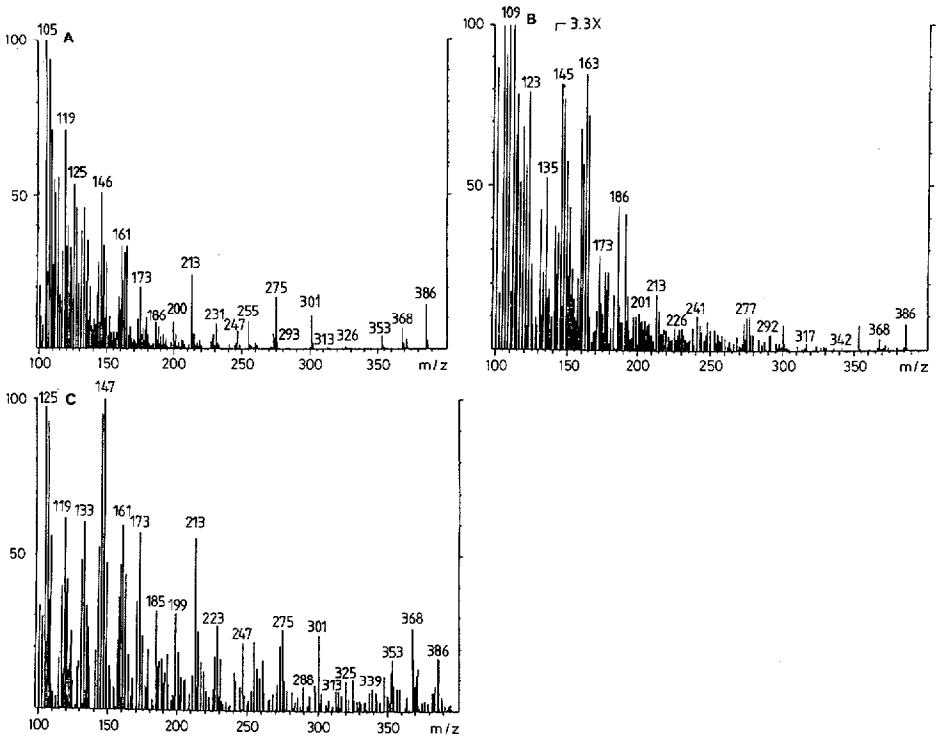

Fig. 5. (A) EI mass spectrum of cholesterol obtained on 100 μg with solid insertion probe. (B) EI mass spectrum of 100 ng of cholesterol obtained with solid insertion probe. (C) EI mass spectrum of cholesterol obtained on 50 ng by OTLC. Probe tip temperature was 325°C, solvent was methanol.

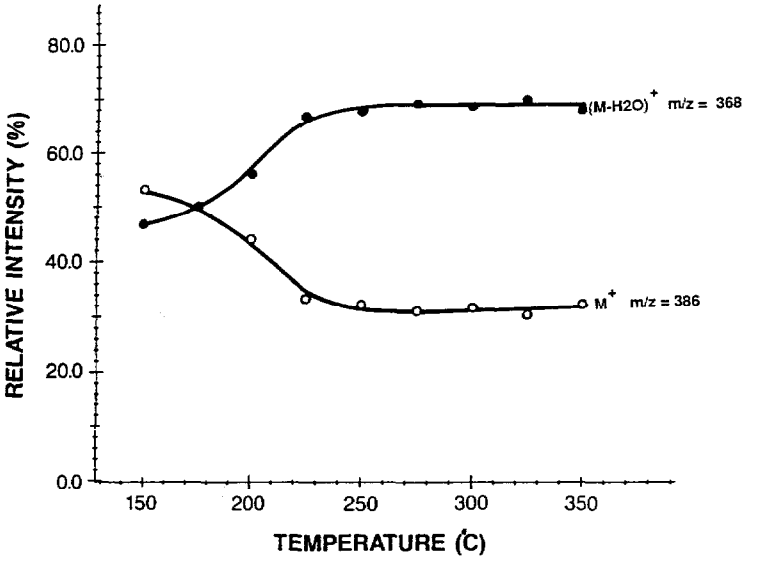

Fig. 6. Effect of probe tip temperature on loss of water in cholesterol. Relative intensity of parent ion (m/z = 386) (open circles) and $[M-18]^{+\cdot}$ (m/z = 368) (closed circles) as a function of probe tip temperature.

TABLE I

RANGE OF APPLICABILITY

Experimental conditions: probe tip temperature was 300°C; orifice diameter was 1.5 μm; mobile phase velocity was 1 cm/s. All data obtained with selected ion monitoring and EI ionization (except trifluralin, which was done under methane negative CI conditions).

Compounds	*Molecular weight*	*b.p. (°C)*	*MDQ**	*GOF*
Isoleucine	131	–	–**	–
Adenine	135	–	–	–
Adenosine	267	–	–	–
Epinephrine	183	–	1 ng	0.6
Benzene	78	80	10 pg	0.05
Naphthalene	128	218	10 pg	0.10
Anthracene	178	342	10 pg	0.10
Pyrene	202	404	10 pg	0.15
Perylene	252	400	20 pg	0.25
Nitropyrene	247	–	50 pg	0.10
Catechol	110	245	50 pg	0.15
Acridine	179	346	20 pg	0.10
Propazine	229	–	10 pg	0.15
Trifluralin	335	–	1 pg	0.15
Cholesterol	384	360	5 ng	0.4

* MDQ = minimum detectable quantity defined as a signal-to-noise ratio of 3:1 under selected ion monitoring.

** No mass spectral peaks observed.

A comparison of Fig. 5B and C indicates that the OTLC system provides approximately a ten-fold increase in sensitivity over the direct probe since the capillary injection can deliver a higher sample flow into the source than does the direct probe.

CONCLUSION

The parameters controlling the performance of this capillary interface for OTLC–MS have been systematically investigated. The importance of tapering and temperature had previously been demonstrated[1]. Without a tapered tip most compounds used with this interface could not be detected. For the OTLC system used in our laboratory, and orifice diameter of approximately 1.5 μm, mobile phase velocities above 0.25 cm/s, and temperatures around 300°C will give optimal operating conditions.

For the OTLC system we used, a 1.5-μm I.D. orifice diameter is a practical lower limit allowing safe helium head pressures, but the experiments show that smaller orifice diameters might give better results. With high pressure pumps these smaller diameters should not present a problem.

As is illustrated with the EI spectra of cholesterol, the thermal degradation introduced by the interface is limited. This is probably due to the short residence time of the compound at the hot column tip.

Since the interface requires vaporization of the analytes, its applicability is limited to non-polar to moderately polar compounds, with molecular weight below approximately 400 g/mol. This is demonstrated in Table I. The compounds examined were selected to cover a wide range of boiling points and polarities. While this limitation poses somewhat of a restriction on its utility, it should prove useful for the analysis of compounds such as pesticides[7].

A similar probe is being developed in our laboratory for use with a magnetic sector mass spectrometer. From results published by Alborn and Stenhagen[10] the high electrostatic fields may improve the performance of the interface ad increase its range of applicability.

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