

Determination of phthalates in water using fiber introduction mass spectrometry

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Fiber introduction mass spectrometry (FIMS)—a direct coupling of SPME and MS—using selective ion monitoring (SIM) was used to detect and quantify dimethylphthalate (DMP), diethylphthalate (DEP) and dipropylphthalate (DPP) in mineral water. In FIMS, a chromatographic silicone septum is the only barrier between ambient and the high-vacuum mass spectrometer, permitting direct introduction of the SPME fiber into the ionization region of the equipment. After their thermal desorption and ionization and dissociation, the extracted phthalates are detected and quantitated by MS. Three types of SPME fibers were screened for best analyte sorption/desorption behaviors: 100 μm polydimethylsiloxane (PDMS), 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB) and 65 μm Carbowax/divinylbenzene (CW/DVB). The PDMS/DVB and CW/DVB fibers were then evaluated for precision, and quantitative figures of merit were assessed for extractions using the PDMS/DVB fiber, which displayed the best overall performance. FIMS with the PDMS/DVB fiber allows simple extraction and MS detection and quantitation of DMP in water with good linearity and precision, and at concentrations as low as 3.6 $\mu\text{g L}^{-1}$. The LD and LQ of FIMS are below the maximum phthalate concentration allowed by the USEPA for drinking water (6 $\mu\text{g L}^{-1}$).

Introduction

Solid phase microextraction (SPME) has become a very popular technique for the extraction and pre-concentration of organic analytes. SPME uses a fused silica fiber coated by thin films of pure polymeric extracting phases (polydimethylsiloxane (PDMS), polyacrylate) or dispersions of solid adsorbents in polymers (PDMS/divinylbenzene (DVB), PDMS/Carboxen), both able to sorb analytes from different matrixes. SPME is normally preferred over other concurrent techniques such as liquid–liquid extraction because it uses no extracting solvents allowing fast and simple operation.¹ Although SPME was originally devised for coupling to gas chromatography (GC), couplings to other separation techniques such as HPLC² and CE³ are also becoming popular. Use of SPME directly combined to non-chromatographic techniques is also of great interest, especially for specific or highly selective detection devices. For example, Mester *et al.*⁴ employed SPME to extract inorganic mercury and methylmercury from fish tissue after alkylation, using inductively coupled plasma mass spectrometry (ICP-MS) for detection and quantitation, reaching detection limits as low as 0.19 ng (Hg) mL⁻¹. A special heated interface was designed to allow thermal desorption of SPME extracts and introduction in the ICP-MS instrument. Fraguero *et al.*⁵ employed headspace SPME and quartz furnace atomic absorption spectrometry to quantitate the anti-knocking agent methylcyclopentadienyl manganese tricarbonyl in water and gasoline, detecting down to 0.71 ng (Mn) mL⁻¹. Using a special SPME device, that is, a home-made sorbent-coated stainless steel probe for direct

insertion into a FTIR spectrometer, Yang and Tsai⁶ combined SPME and attenuated total reflection IR spectroscopy to quantify aromatic amines at $\mu\text{g L}^{-1}$ concentrations in water. In a similar fashion, Wittkamp *et al.*⁷ reported a special device used to extract analytes using the same principle of SPME, for detection and quantitation with conventional UV absorption spectroscopy.

Nearly direct coupling of SPME with MS for organic analysis was reported by Péres *et al.*⁸ A GC-MS system, in which the column was replaced by a short uncoated capillary, was used to transfer volatile analytes from cheese samples desorbed from a SPME fiber to a mass spectrometer without chromatographic separation. However, as demonstrated by the technique known as membrane introduction mass spectrometry, MIMS,⁹ and more specifically by trap & release MIMS,^{10–12} a silicone membrane can act both as the interface between the ambient and the mass spectrometer under high vacuum and as an efficient extraction device. Desorption of analytes previously extracted and pre-concentrated in the silicone membrane occurring inside the mass spectrometer results in much improved sensitivity, detectability, and analytical output. Therefore, we recently introduced a technique similar to T&R-MIMS in which the first “fully-direct” coupling of SPME with mass spectrometry was demonstrated, and termed it fiber introduction mass spectrometry (FIMS).¹³ In FIMS, a simple home-made holder introduces commercial SPME fibers directly inside the ionization region of a conventional quadrupole mass spectrometer, between two filaments delivering 70 eV electrons. The combination of high vacuum and heating by irradiation from the filaments causes desorption of the analytes from the fiber, which are immediately ionized producing characteristic molecular and

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fragment ions. Mass (m/z) monitoring of selective ionic fragments allows detection and quantitation of desired target analytes. FIMS was also recently adapted by Riter *et al.* to a portable ion trap MS instrument,¹⁴ in which a special heated interface placed away from the ion source was designed for thermal desorption from the SPME fiber. FIMS was first applied to real samples by van Hout *et al.*,¹⁵ who quantified concentrations down to 1 ng mL^{-1} of lidocaine in urine with good precision (RSD < 15%), after short (1 min) extraction with $30 \text{ }\mu\text{m}$ PDMS fibers and total analysis time of 3 min. FIMS has also been recently coupled to laser desorption ionization time-of-flight mass spectrometry.¹⁶

In this paper, we address the application of FIMS to the extraction and detection and quantitation of phthalic acid esters (phthalates) in water. Phthalates are semi-volatile organic compounds widely used as additives in plastics (plastifiers), and are therefore common contaminants in the environment and foods and beverages stored in plastic containers. The possible adverse health effects and environmental damage potential of phthalates is currently a matter of debate and interest.¹⁷ Some phthalates have been pointed out as potential carcinogenic¹⁸ and teratogenic¹⁹ agents for humans. However, it is the possible action of phthalates as endocrine disrupters in humans that has caused the most serious concerns as contaminants in water and food.^{20,21} Therefore, tolerance limits for organic phthalates in several matrixes have been set by the corresponding regulatory bodies; *e.g.*, for drinking water, according to USEPA regulations,²² the maximum allowed concentration of di(2-ethylhexyl) phthalate is $6 \text{ }\mu\text{g L}^{-1}$. The detection and quantitation of organic phthalates is almost universally performed either by GC or HPLC, following extraction and clean-up steps based on liquid-liquid extraction,²³ SPE²⁴ or SPME.²⁵ In this work we propose the use of FIMS as a much simpler alternative for the detection and quantitation of organic phthalates in water. The method here developed was applied as a proof-of-principle case to detect and quantify three main phthalates in plastic-bottled mineral water.

Experimental

Chemicals and materials

SPME fibers (Supelco, Bellefonte, PA) coated with $100 \text{ }\mu\text{m}$ PDMS (polydimethylsiloxane), $65 \text{ }\mu\text{m}$ PDMS/DVB (divinylbenzene) and $65 \text{ }\mu\text{m}$ CW (Carbowax)/DVB (Supelco Inc., Bellefonte, PA) were tested. Prior to first use the fibers were conditioned in a GC injector port according to the supplier instructions. All extractions were performed in 16 ml septum-sealed glass V-vials (Wheaton, Millville, NJ) under agitation with Teflon-coated magnetic stirring bars. Dimethylphthalate (DMP), diethylphthalate (DEP), di-*n*-propylphthalate (DPP) and methanol (Aldrich, Milwaukee, WI) with the highest available purity were employed. All test aqueous samples were prepared using deionized water obtained from a Milli-Q purifier (Millipore, Billerica, MA). Samples of mineral water bottled in 500 mL PET bottles were obtained in the local market.

Mass spectrometry

All MS measurements were performed using an Extrel (Pittsburgh, PA) mass spectrometer fitted with a high-transmission $\frac{3}{4}$ inch quadrupole, as described elsewhere;²⁶ the experimental set-up of the interfacing between MS and SPME here employed is shown in detail and discussed in ref. 13. Detection and quantitation of the analytes was performed by selective ion monitoring (SIM) of characteristic ionic fragments of m/z 163 for DMP, of m/z 177 for DEP and of m/z 209 for DPP.

Extraction procedure

Aqueous test solutions of the phthalates were prepared by serial dilution of 1 g L^{-1} methanolic stock solutions. Aliquots of samples (5 mL) placed in the glass vial were extracted for 30 min at room temperature ($23 \text{ }^\circ\text{C}$) under constant stirring of 1200 rpm and direct immersion of the fibers. The extracted analytes were immediately desorbed inside the mass spectrometer after the extraction. The desorption time was 40 s for all extractions; no carry-over between runs was observed under these conditions. To select the best fiber for the experiments, signal to noise ratios were measured after extractions from $50 \text{ }\mu\text{g L}^{-1}$ solutions of each analyte. The MS gain and electron multiplier high voltage were, respectively, 1×10^{11} and 1200 V. Repeatability was assessed after sextuplicate analysis of $50 \text{ }\mu\text{g L}^{-1}$ aqueous test solutions of the analytes carried out in the same day, using PDMS/DVB and CW/DVB fibers; for the reproducibility tests, three series of sextuplicate extractions of these solutions were carried out during non-consecutive days. Analytical curves for the concentration range between 10 and $100 \text{ }\mu\text{g L}^{-1}$ and using PDMS/DVB fiber were estimated to assess quantitative figures of merit of the method. Limits of detection (LD) and quantitation (LQ) were calculated from signal-to-noise ratios (S/N) estimated from data collected from extractions of $50 \text{ }\mu\text{g L}^{-1}$ aqueous solutions of the analytes and blank runs.²⁷ The optimized procedure was applied to detect and quantify the analytes in two samples of plastic-bottled mineral water. Recovery experiments were also performed after extractions of samples of both brands of mineral water spiked with $25 \text{ }\mu\text{g L}^{-1}$ of each analyte.²⁸

Results and discussion

Fig. 1 shows the FIMS signal profile obtained for SIM of the fragment ion of m/z 163 for extractions of a $50 \text{ }\mu\text{g L}^{-1}$ DMP solution with the three tested fibers, as well as blanks resulting from extraction of deionized water under the same conditions and background profiles for a clean fiber. The profiles for the other analytes are similar. The background for all fibers is negligible when compared to the blank and analytical signals, with no spurious signal from degradation products. Sorbent coatings of the three fibers tested here are therefore stable under the desorption conditions. Deterioration of the coating was observed for PDMS/DVB only after *ca.* 150 desorption cycles. For the three tested SPME fibers, at least two peaks can be discerned in the sample signal profiles. The signal for the fragment ion of m/z 163 which is used for the detection of the analyte (identified comparing blank and sample plots) have a

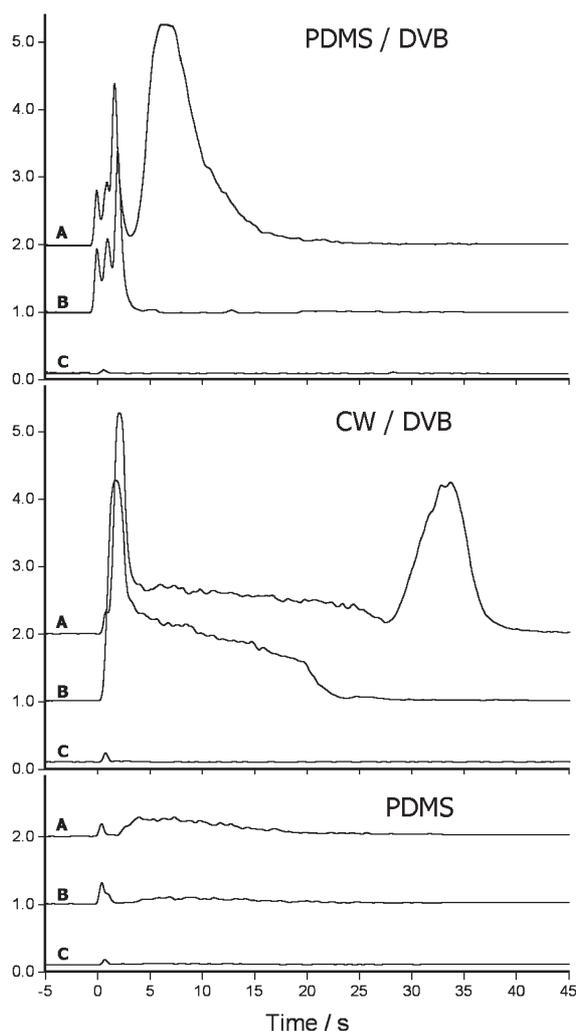


Fig. 1 (A) FIMS signal profile using SIM of the ion of m/z 163 for $50 \mu\text{g L}^{-1}$ DMP aqueous solution extraction, (B) deionized water extraction and (C) fiber background for PDMS/DVB, CW/DVB and PDMS fibers. Time and intensity scales are the same for the three plots. Point zero in time scale corresponds approximately to the moment of exposition of the fiber to the MS filaments.

maximum at $t \approx 7$ s for PDMS/DVB, $t \approx 33$ s for CW/DVB and appears as a flat and large peak at $t \approx 5$ – 20 s for PDMS. For all fibers, additional peaks with monitoring times shorter than that corresponding to the DMP signal appear both in the sample and blank plots: for PDMS a minute peak immediately after the exposure of the fiber to the MS chamber; for CW/DVB, a broad peak between $t \approx 0$ – 20 s, and a group of unresolved sharp peaks for $t \approx 0$ – 3 s for PDMS/DVB. The peaks appearing almost immediately after the insertion of the fibers inside the mass spectrometer were attributed to desorption of water, which was either sorbed or mechanically entrapped in the pores of the coatings (PDMS/DVB, CW/DVB), and also wetting the surface of the three fibers, including the non-porous and non-polar PDMS fiber. It can be presumed that, when the fiber is submitted to the heating and vacuum inside the MS ionization chamber, initially the water retained or sorbed by the coating is vaporized. The insertion of a PDMS/DVB or CW/DVB fiber saturated with

water causes the appearance of transient and intense signals for all m/z ions monitored, raising therefore the base line. Similar non-specific signals spread through all monitored m/z interval (chemical noise) can be observed in techniques such as CE-MS (capillary electrophoresis coupled to MS), where relatively large amounts of solvents or other extraneous substances can also be introduced in the ionization chamber.³⁰ This phenomenon has been associated with the formation, ionization and detection of solvent clusters (in this case, water clusters) originated from the introduction of large quantities of solvents inside the MS vacuum and ionization zone. Residual current caused by impact of a large number of neutral molecules in the MS electron multiplier can also account, at least partially, for the non-specific water peak. Apart from the generation of these spurious peaks in the SIM plots, the water vapor introduced in the system presumably causes a transient but significant rise on the local pressure, reducing instantaneously the efficiency of electron ionization and the capacity of the filaments to transfer heat to the fiber. Also, in view of the large enthalpy of vaporization of water (40.7 kJ mol^{-1})²⁹ this is a remarkable consuming process, which also cools the fiber. Therefore, effective heating of the SPME fiber by the MS ionization filaments seems to occur only after all water sorbed or entrapped is released from the fiber and its vapor is removed from the MS chamber by the vacuum pumps. Only when the bulk of the water vapor is removed is the SPME fiber effectively heated and heavier and less volatile analytes such as the phthalates are desorbed. This model is consistent with the different delays in the appearance of the DMP signal for PDMS/DVB and CW/DVB fibers. Being more polar, the CW/DVB fiber should retain larger amounts of water, and the desorption of the analytes will occur later when compared to PDMS/DVB. For the PDMS fiber, only a residual water signal is observed: the coating of this fiber is not porous and non-polar PDMS does not sorb significant amounts of water, and only a diminutive quantity of water will remain wetting its surface after its removal from the sample. It is therefore possible that the SPME fibers will act as limited fractionation devices, since large amounts of highly volatile analytes can be desorbed faster than heavier analytes—as it seems to happen with the water and phthalates. Further investigation are underway to study this aspect of SPME-MS desorption kinetics.

Considering the poor analyte SIM signal (Fig. 1), the PDMS fiber was excluded from the remaining experiments. Figs. 2 and 3 show signal profiles obtained using the CW/DVB and PDMS/DVB fibers, respectively, for extractions of aqueous test samples containing $50 \mu\text{g L}^{-1}$ of the 3 phthalates. The PDMS/DVB fiber displays far better extraction efficiency for each of the 3 phthalates when compared to the CW/DVB fiber. This result is consistent with the properties of the fiber coatings, which are both dispersions of a solid adsorbent (divinylbenzene, DVB) on polymeric phases, PDMS or CW. The PDMS/DVB coating, being less polar, is expected to have greater affinity towards aromatic esters such as the phthalates than the highly polar CW/DVB coating. Another remarkable aspect observed in Figs. 2 and 3 is that desorption times are nearly the same for each analyte, for both fibers. Therefore, the difference between their volatilities is not enough to cause

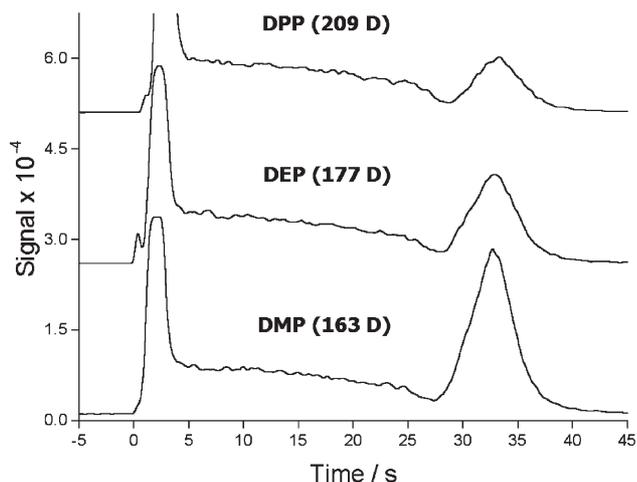


Fig. 2 FIMS signal profiles using SIM for $50 \mu\text{g L}^{-1}$ aqueous solution of DMP (m/z 163), DEP (m/z 177) and DPP (m/z 209) after extraction with the CW/DVB fiber.

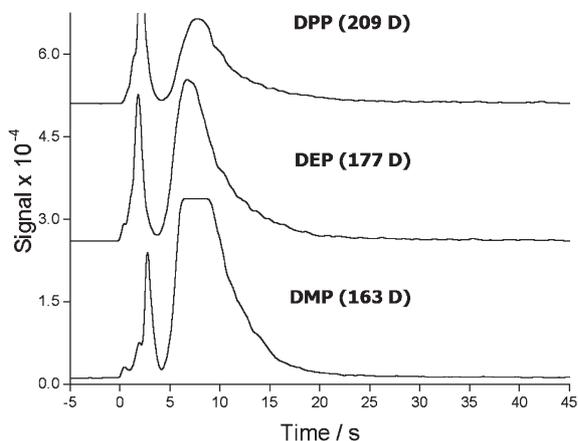


Fig. 3 FIMS signal profiles using SIM of the ion of m/z 163 for $50 \mu\text{g L}^{-1}$ aqueous solution of DMP, DEP (m/z 177) and DPP (m/z 209) after extraction with the PDMS/DVB fiber.

separation in desorption peaks such as it occurs with the phthalates and water, as discussed above.

Table 1 shows measurements performed to test repeatability and reproducibility using extractions with both the CW/DVB and PDMS/DVB fibers. For both fibers, precision is adequate with RSD ranging from 7.9% to 8.3% (PDMS/DVB) and 10.2% to 10.9% (CW/DVB). Moreover, the inter-day precision is also adequate with RSD less than 4.0% for PDMS/DVB and 9.3% for CW/DVB. Along with the reduced extraction

Table 1 Repeatabilities and reproductibilities (expressed as RSD%) after replicate extractions of $50 \mu\text{g L}^{-1}$ aqueous test solutions of the analytes using the PDMS/DVB and CW/DVB fibers

Analyte	Repeatability		Reproducibility	
	PDMS/DVB	CW/DVB	PDMS/DVB	CW/DVB
DMP	7.9	10.4	4.0	5.6
DEP	8.3	10.9	2.7	4.5
DPP	8.0	10.2	3.5	9.3

Table 2 Slope a and intercepts b and correlation coefficients r for analytical curves and limits of detection LD and quantitation LQ in $\mu\text{g L}^{-1}$ for PDMS/DVB extraction and FIMS detection and quantitation of phthalates in water

	DMP	DEP	DPP
$a \times 10^{-3}$	4.7 ± 0.4^a	4.4 ± 0.3	2.6 ± 0.2
$b \times 10^{-3}$	-45 ± 21	-47 ± 16	-32 ± 10
r	0.988	0.992	0.990
LD ^b	3.6	3.6	5.1
LQ ^b	12	12	17

^a Uncertainty of a and b are expressed as the corresponding estimates of standard deviations. ^b LD and LQ defined as the concentration of analyte producing a signal of 3.3 and 10 times the noise level, respectively.³²

efficiency compared to PDMS/DVB, the worse precision of the CW/DVB fiber analysis can be attributed to its larger retention of water, which causes more disturbance in the analyte signal due to variation of base line and a larger background peak. Therefore, the PDMS/DVB fiber displays the overall best performance and was selected for the remaining assays.

Table 2 shows therefore figures of merit for quantitation of the 3 phthalates after extraction with the PDMS/DVB fiber. The quality of fitting for all analytes can be regarded as acceptable, with correlation coefficients ranging from 0.988 to 0.992. A Student t -test performed using the intercepts of the analytical curves and their standard deviations indicated that they can be considered as statistically null. The sensitivities, expressed as the slopes of the analytical curves, increase in the order DMP > DEP > DPP. The limits of detection ranged from $3.6 \mu\text{g L}^{-1}$ and $5.1 \mu\text{g L}^{-1}$, which are smaller than the US EPA regulation limit of $6 \mu\text{g L}^{-1}$ for the similar contaminant (DEHP) in drinking water. Table 3 shows the data for the application of the FIMS method to mineral water samples bottled in PET bottles. The three analytes were monitored in samples #A and #B. DPP levels in both samples and DMP level in sample #B were below their quantitation limits. As for the recoveries, except for one case all figures are above 70%, which also can be considered as adequate. When using an adsorption-based fiber such as PDMS/DVB, it is possible that problems related to competition between analytes and matrix components for adsorptive sites on the fiber and displacement of adsorbed target species by the matrix components prejudice the linearity and accuracy of quantitative data obtained.³¹ Although this inter-species competition did not affect the results presented herein, such competition should be always checked when fibers such as PDMS/DVB are employed.

Table 3 Concentrations C ($\mu\text{g L}^{-1}$) of phthalates in PTE-bottled mineral water determined by FIMS after extraction using the PDMS/DVB fiber and recoveries R (%) from the same samples spiked with $25 \mu\text{g L}^{-1}$ of each analyte

Analyte	Sample #A		Sample #B	
	C	R	C	R
DMP	16	74	n.q. ^a	82
DEP	23	67	15	78
DPP	n.q.	85	n.q.	93

^a Species detected but below limit of quantitation.

Conclusions

The fully-direct coupling of SPME with mass spectrometry—the novel FIMS technique—proved to be a suitable alternative to quantitate phthalic acid esters in aqueous samples. The technique allows simultaneous quantitation of $\mu\text{g L}^{-1}$ levels of DMP, DEP and DPP with good accuracy and precision. Speed and simplicity are the main benefits of the FIMS technique which uses no chromatographic separation, and detection is performed in less than one minute. Also, selective detection is possible by monitoring characteristic 70 eV EI fragment ions. As the result of the special conditions inside the MS ionization chamber and the properties of the fiber coatings, time-resolution between analytes with high and low volatility can occur, which can be used to improve selectivity. This quasi-chromatographic feature of the FIMS technique may be beneficial for more complex samples, and is currently being investigated in our laboratory for the determination of phthalates and other analytes of environmental relevance.

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