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Detection of substituted benzenes in water at the pg/ml level using solid-phase microextraction and gas chromatography–ion trap mass spectrometry

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ABSTRACT

Solid-phase microextraction (SPME) is combined with gas chromatography–ion trap mass spectrometry (GC–IT-MS) for the analysis of benzene, toluene, ethyl benzene and xylene isomers (BTEX) in water. SPME is a recent technique for extracting organics from an aqueous matrix into a stationary phase immobilized on a fused-silica fiber. The analytes are thermally desorbed directly in the injector of a gas chromatograph.

The wide linear dynamic range (five orders of magnitude) and pg sensitivity of the ion trap mass spectrometer in its full scan mode is an ideal detector for identifying and quantifying the analytes extracted with an SPME device.

The combined method SPME–GC–IT-MS, using fibers coated with a 100-μm polydimethylsiloxane coating, showed a limit of quantitation (LOQ) of 50 pg/ml benzene in water. This corresponds to 5 pg of benzene absorbed onto the fiber. The limit of detection (LOD) was 15 pg/ml benzene. For *o*-xylene spiked at 50 pg/ml in water 50 pg were absorbed by the fiber indicating an LOQ and LOD 10 times better than for benzene. The detection limits obtained exceed the requirements of both the United States Environmental Protection Agency method 524.2 and the Ontario Municipal/Industrial Strategy for Abatement program, which range from 30 to 80 pg/ml and 500 to 1100 pg/ml, respectively.

The linearity of the method extended over five orders of magnitude. Relative standard deviation ranged from 2.7 to 5.2% for 15 ng/ml BTEX in water and from 5.5 to 7.5% for 50 pg/ml BTEX in water. SPME–GC–IT-MS was used to evaluate the contamination level in laboratory, potable and wastewater sources.

INTRODUCTION

Contamination of water by organic pollutants is a common environmental problem. Simple highly sensitive analytical techniques are required to detect and quantitate pollutants in water at trace levels. The first step in the analytical process involves extraction of the contaminants from the water matrix. This step is commonly achieved using liquid–liquid extraction, solid-phase extraction or purge-and-trap techniques. These methods either use large amounts of organic solvents, are labour intensive, difficult to automate and apply in the field or are expensive.

Solid-phase microextraction (SPME) is an excellent alternative to the aforementioned techniques. In SPME the analytes are extracted into a stationary phase which is attached to a length of fused-silica fiber [1]. The fiber is contained within a microsyringe for protection and ease of sampling. SPME completely eliminates solvents and consists of only a few simple steps. The fiber is withdrawn into the needle of the microsyringe and the needle of the syringe is used to pierce the sample vial. The fiber is then exposed to the aqueous sample where the ana-

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lytes partition between the stationary film on the fiber and the aqueous phase. The fiber is then withdrawn from the sample and inserted into the injector of a gas chromatograph where the analytes are thermally desorbed. In SPME an exhaustive extraction does not occur but an equilibrium is established as analytes partition between the stationary phase and the aqueous phase. At equilibrium a linear relationship exists between the number of moles of an analyte absorbed by the fiber and the analyte concentration in the aqueous phase [1]. For a large aqueous sample volume this relationship is described by:

$$n_s = KV_sC_{aq} \quad (1)$$

where n_s is the number of moles of the analyte in the stationary phase, K is the distribution constant of the analyte partitioning between the stationary and aqueous phase, V_s is the volume of the stationary phase and C_{aq} is the concentration of the analyte in the aqueous phase. High sensitivity can be ensured by using thick (large V_s) and selective (large K) stationary phases. The whole extraction and analyte transfer process usually takes only a few minutes [2] and can be easily automated [3].

The main objective of this paper is to demonstrate that in addition to its simplicity, SPME can be implemented in methods which meet both the United States Environmental Protection Agency (USEPA) and the Ontario Municipal/Industrial Strategy for Abatement (MISA) specifications for the analysis of environmental samples. These methods usually require gas chromatography (GC)–mass spectrometry (MS) separation and quantitation. Ion trap mass spectrometry (IT-MS) was used in this study. With the introduction of automatic gain control (AGC) [4] and axial modulation [5] quadrupole ion trap mass spectrometers have become an attractive alternative to conventional quadrupole mass filter spectrometers for environmental analysis. Ion trap mass spectrometers are capable of picogram detection limits in a full scan mode. Picogram detection limits can be achieved with a conventional quadrupole spectrometer when operating in the selected ion monitoring (SIM) mode, but in this case valuable spectral information is lost.

EXPERIMENTAL

An SPME device is shown in Fig. 1 and was prepared as follows. A 12-inch length of 30 gauge stainless steel tubing was obtained from Hamilton (Reno, NV, USA). Fused-silica capillary fibers coated with a 100 μm (± 5 μm) polydimethylsiloxane film were obtained from Polymicro Technologies (Tuscon, AZ, USA). A 1-cm portion of the coating was scraped from a short length (5 cm) of coated fiber. The stripped portion of fiber was then dipped into epoxy resin (Epo-Tek 353ND, Billerica, MA, USA) and inserted into the stainless steel tubing. The glue was cured for 1 min at approximately 150°C. The stainless steel was then inserted up through the needle of a Hamilton (7105) syringe (plunger wire removed) until it protruded from the top, cut to a length of 20.5 cm and glued in place with a dab of standard 5-min epoxy glue. Once the glue had cured the plunger button was replaced, the exposed fiber was trimmed to 1 cm and the syringe assembly was leak checked in a gas chromatograph injector using a Gow-Mac helium leak detector. Leaks may arise where the needle joins the syringe body and also at the top of the syringe barrel. These can be corrected by tightening the knurled nut which holds the needle in place.

A Varian Saturn benchtop gas chromatograph–ion trap mass spectrometer was used for the separation and analysis of benzene, toluene, ethyl benzene and xylene isomers (BTEX). The gas chromatograph was a Varian 3400 equipped with a Septum Programmable Injector and oven cryogenics. Separations were conducted using a Supelco 60 m x 0.25 mm I.D. VOCOL column (1.0 μm film) or a Supelco 30 m x 0.25 mm I.D. VOCOL column (1.5 μm film). The chromatographic conditions were as follows for the 60 m column:

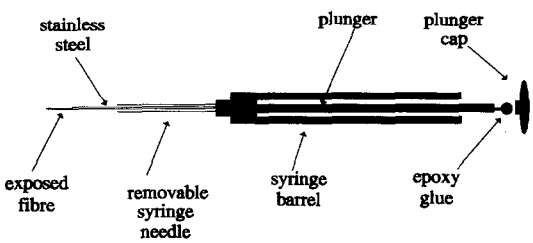

Fig. 1. A solid-phase microextraction device.

Injector:	25°C for 0.10 min, 250°C/min to 150°C, hold 3 min.
Column:	−5°C for 2 min, 20°C/min to 150°C, hold 3.25 min, ramp ballistically to 200°C, hold 3 min.
Transfer line:	220°C
Ion trap manifold:	220°C

For the 30-m column the oven was programmed as follows: 10°C for 1 min, 15°C/min to 90°C, 3°C/min to 105°C, ramp ballistically to 200°C, hold 2 min.

The mass spectrometer was tuned to perfluorotributylamine (PFTBA) in accordance with the MISA program. When tuned to PFTBA the ITS also met the tuning criteria for 4-bromofluorobenzene (BFB) as required by USEPA method 524.2 [6]. The electron multiplier and the AGC target were set automatically. The electron multiplier sets to give a multiplier gain of 10^5 and the target sets to achieve a valley to isotope ratio of 25% for mass 131 of perfluorotributylamine and its isotope 132. The filament emission current was set so that the ionization time was almost at the maximum value (25 ms) for the lowest concentration of BTEX analyzed. The segment breaks were left at their default values of 10–99/100–249/250–399/400–650 amu; however, the segment tune factors were set at 115/100/100/85 in order to pass both the PFTBA and BFB tune. The mass range scanned was 35–250 amu during BTEX analysis. The ions used for quantitation were as follows: benzene = 78, toluene = 91, 92, ethyl benzene = 91, 106 and xylene isomers = 106, 91.

Spiking standards were prepared in methanol (OmniSolve; BDH, Toronto, Canada) and spiked into Milli-Q (Millipore, USA) reagent water inside sealed 60-ml hypovials. The hypovials contained a stirbar and 0.5 ml of headspace to prevent the sample from wicking up the syringe needle. An absorption time of 30 min was used to ensure that equilibrium had been reached. Desorption was performed with the fiber just above the restriction in the glass insert of the SPI injector as this is the optimum location [3]. Each day a column blank was followed by a fiber blank and a water blank to determine the extent of any laboratory contamination.

The limit of quantitation (LOQ) and limit of detection (LOD) were determined initially from calibration curves and confirmed by analyzing five standards spiked with BTEX at the LOQ. The LOQ was defined as an analyte signal ten times the baseline noise. The LOD was defined as a signal three times the baseline noise.

The mass absorbed at equilibrium was obtained from a calibration curve constructed by injecting (1 μl) triplicate injections of BTEX standards prepared in methylene chloride (OmniSolve; BDH, Toronto, Canada).

Precision was determined by analyzing nine solutions containing 15 ng/ml BTEX on a single day with a single experimenter. This was repeated with five solutions containing 50 pg/ml BTEX. The relative standard deviation was calculated by dividing the standard deviation by the mean and multiplying by 100.

Carryover was investigated by analyzing a 1.5 ng/ml standard and then running consecutive fiber blanks to determine the fraction of the original mass desorbed remaining on the fiber.

Background contamination was examined in Milli-Q reagent water, deionized water, tap water and natural spring water (Aberfoyle, Canada).

RESULTS AND DISCUSSION

Benzene, toluene, ethyl benzene, and xylene isomers were used as target analytes in the investigation of SPME–GC–IT-MS since they are common groundwater pollutants. Their presence may arise from incomplete combustion of gasoline, leaking storage tanks or accidental spills into the environ-

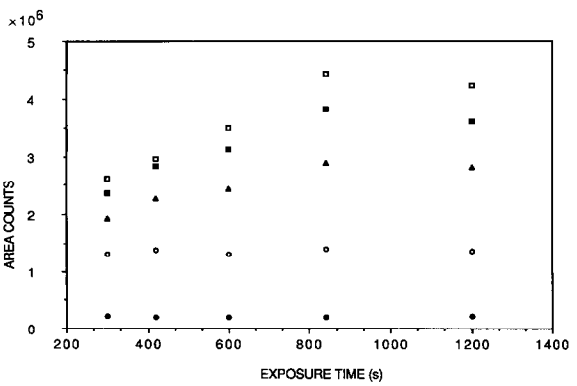

Fig. 2. Absorption profile for benzene, toluene, ethyl benzene and xylene isomers using 1 cm of a 100-μm polydimethylsiloxane-coated fiber. All compounds have reached equilibrium in 14 min using a conventional laboratory magnetic stirring plate. ● = Benzene; ○ = toluene; ■ = ethyl benzene; □ = *m*- and *p*-xylene; ▲ = *o*-xylene.

TABLE I

DISTRIBUTION CONSTANTS, LIMITS OF DETECTION, LIMITS OF QUANTITATION, PRECISION OF SPME, K_{ow} VALUES, AND METHOD DETECTION LIMITS (MDL) AS REQUIRED BY MISA AND USEPA REGULATIONS

Analyte	$\log_{10} K^a$	$\log_{10} K_{ow}$	LOD (pg/ml)	LOQ (pg/ml)	Precision 50 pg/ml (%)	Precision 15 ng/ml (%)	MDL	
							(MISA) (pg/ml)	USEPA (pg/ml)
Benzene	2.30	2.13[b]	15	50	7.3	5.3	500	30
Toluene	2.88	2.69[b]	5	15	6.7	3.2	500	80
Ethyl benzene	3.33	2.84[c]	2	7	7.2	3.6	600	60
m- and *p*-Xylene	3.31	3.20[d] *meta* 3.15 *para*	1	4	6.5	6.5	1100	90
o-Xylene	3.26	2.77	1.5	5	5.5	2.7	500	60

[a] Experimentally determined.
[b] Ref. 8.
[c] Ref. 9.
[d] Ref. 10.

ment. A quick, sensitive method is required to detect the presence of these contaminants in groundwater at the pg/ml level.

The first step in developing a method for SPME is to establish the time required for all target analytes to reach equilibrium. Previous work with a 56-μm film thickness showed that all BTEX components had reached equilibrium in under 6 min [3]. The distribution constants for BTEX analytes partitioning between the 56-μm coating and water were found to be similar to the analytes octanol–water partition coefficients (K_{ow}) [3]. In order to maximize the sensitivity of this technique the thickest available polydimethylsiloxane coated fiber (100 μm) that could be accommodated inside the syringe needle was used. Fig. 2 shows that all analytes have attained equilibrium in 14 min when using a 100-μm coating. The extraction process is limited by the mass transfer of analytes through a thin static aqueous layer at the fiber–solution interface [2]. The longer equilibration times for ethyl benzene and the xylene isomers relative to benzene and toluene are to be expected since these compounds have higher (K_{ow}) values and a greater mass must diffuse across the unstirred layer before equilibrium is reached. Using eqn. 1 the distribution constants for BTEX in water can be calculated for the 100-μm polydimethylsiloxane coating. As shown in Table I, using a 100 μm coating the calculated distribution constants are similar to the K_{ow} values.

In the following experiments a 30-min extraction time was chosen. This is twice the equilibration time but convenient since the time required to complete one chromatographic run and cool down for the next was about half an hour.

After establishing the equilibration time, the linear dynamic range and mass detection limits of the ion trap were investigated by injecting the target analytes dissolved in methylene chloride. A signal-to-noise ratio of 10:1 was obtained for 4 pg of benzene, which is at the LOQ and suggests a LOD of approximately 1 pg. Similar values were obtained

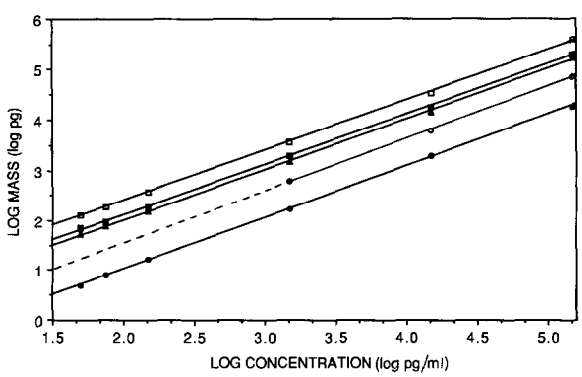

Fig. 3. The linearity of the 100-μm coated fiber and the mass spectrometer is shown over four orders of magnitude from 50 pg/ml to 150 ng/ml. Toluene is linear from 1.5 ng/ml upwards because of background contamination. Symbols as in Fig. 2.

for toluene, ethyl benzene and the xylene isomers. The mass spectrometer was found to give a linear response over five orders of magnitude, up to 100 ng BTEX using conventional syringe injections.

To determine the LODs and the dynamic range of the SPME–GC–IT-MS method aqueous standards were prepared ranging from 50 pg/ml to 1.5 μg/ml BTEX in water. Excellent linearity was obtained for 50 pg/ml to 150 ng/ml for all compounds except toluene (Fig. 3). Toluene was linear from 1.5 pg/ml to 150 pg/ml. This poor linear range was due to reagent water contamination and is discussed below. A signal-to-noise ratio of 10:1 was obtained for 50 ppt benzene in water indicating an LOD of 15 ppt and an LOQ of 50 ppt. The mass of benzene absorbed by the fiber at the LOQ was 5 pg. This agrees favourably with the results obtained using syringe injections. This low LOQ demonstrates that the coating on the fiber is sufficiently stable to withstand the thermal stress of desorption and does not produce background which might coelute with the target compounds. The mass absorbed at equilibrium for 50 pg/ml *o*-xylene was 50 pg, indicating an LOQ and LOD ten times lower than that obtained for benzene. A signal-to-noise ratio in excess of 100:1 confirms this. This greater sensitivity results from the higher K values (Table I) since for analytes with large distribution constants a greater amount of analyte diffuses into the coating before equilibrium is reached [2]. These extremely low detection limits were obtained without any sample preconcentration or special tuning of the mass spectrometer.

LODs and LOQs were determined for all compounds by examining the signal-to-noise ratios at 50 pg/ml and extrapolating from Fig. 3. The method detection limits for BTEX were all less than those required by the USEPA method 524.2 [6] and also by the MISA program [7] In fact, with the exception of benzene, even without extrapolation 50 pg/ml is below the detection limits required by the more stringent USEPA method. Based upon the signal-to-noise ratios obtained for 50 pg/ml BTEX the calculated LODs and LOQs should be easily attainable but, preparing standards at these low levels requires an extremely clean room for sample preparation. A summary of these results is shown in Table I.

BTEX analytes with the largest distribution constants have the lowest detection limits and therefore the maximum concentration that can be analyzed will also be lower. For example when sampling a 1.5 μg/ml BTEX sample the mass of ethyl benzene and xylene isomers absorbed exceeds the capacity of the ion trap. This results in peak splitting and extremely poor resolution between ethyl benzene and *m*- and *p*-xylenes. In this case a thinner stationary phase could be used. Benzene which has a low K value remains linear up to 1.5 μg/ml.

The relative standard deviation of this method for samples containing 15 ng/ml BTEX ranged from 2.7 for *o*–xylene to 5.2 for benzene (Table I). The precision is expected to be the worst for benzene since this is the most volatile compound present. At 50 pg/ml the precision ranged from 5.5 to 7.3% (Table I).

Carryover is an important issue to address since before any trace sampling can be undertaken a pristine fiber is required. The extent of carryover after sampling a 1.5 ng/ml standard is shown in Table II. The results indicate there is no detectable carryover for either benzene or toluene after sampling a 1.5 ng/ml BTEX standard. The amount of ethyl benzene, *m*- and *p*-xylenes, and *o*-xylene present after one injection was 0.17, 0.19 and 0.05%, respectively. Since a greater mass is absorbed at equilibrium

TABLE II

PERCENT OF BTEX REMAINING ON FIBER AFTER 1.5 ng/ml STANDARD

n/d = Not detected.

	Benzene	Toluene	Ethyl benzene	*m*- and *p*-Xylene	*o*-Xylene
1.5 ng/ml	100	100	100	100	100
1st blank	n/d	n/d	0.17	0.19	0.05
2nd blank	n/d	n/d	0.03	0.02	0.03

for those compounds with larger K_{ow} values and they tend to have higher boiling points it is not surprising that they show the greatest carryover [2]. All compounds have decreased to less than 0.03% of their original levels by the second fiber blank. Carryover can be reduced by increasing the desorption temperature, but this results in the slow degradation of the coating. In the experiments reported in this work a fiber blank was analyzed before and after the analysis of any standards or unknown samples to ensure proper quantitation.

Samples of a totally unknown composition or suspected to have an extremely high level of contamination should be screened using a thin film (15 μm) polydimethylsiloxane coating or a shorter fiber beforehand. A new fiber could also be used for each sample since the cost of a fiber is negligible. Alternatively, a less sensitive detector such as a flame

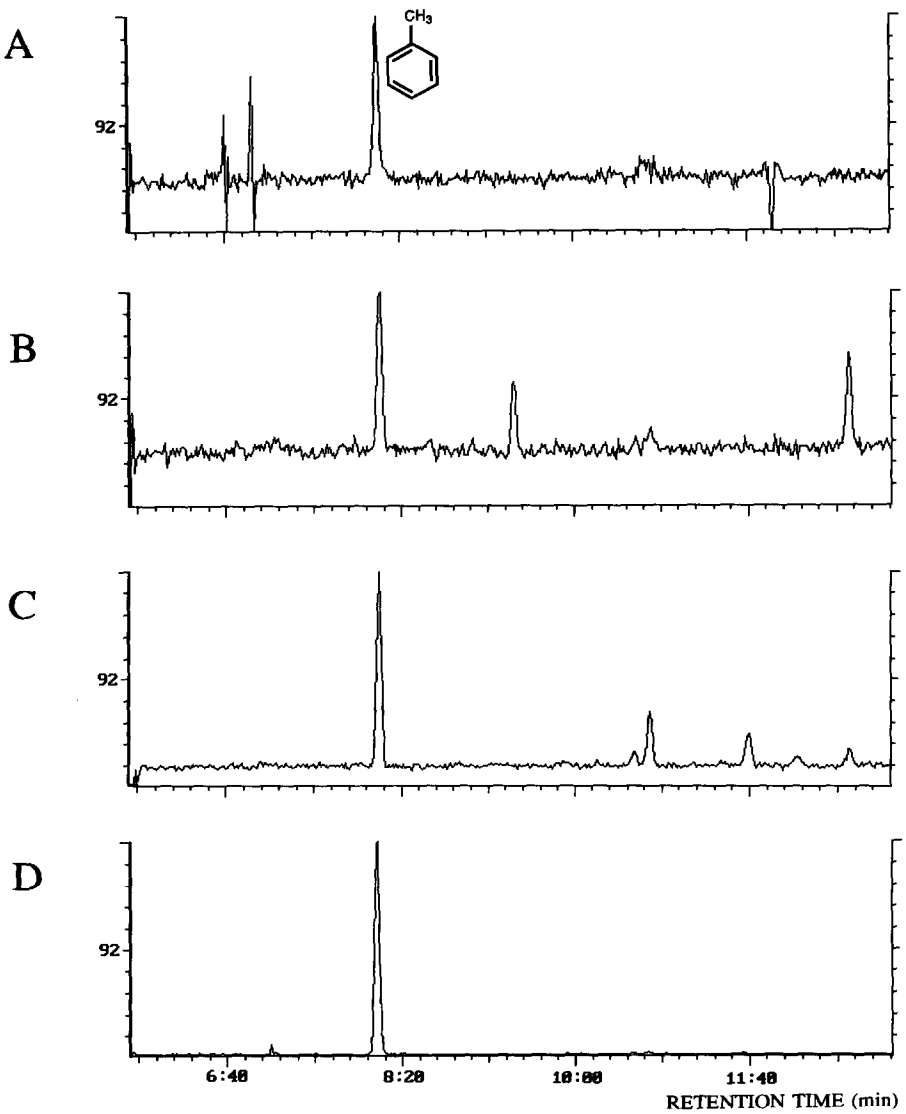

Fig. 4. Comparison of toluene background contamination in four different water sources. (A) Milli-Q reagent water, (B) regular tap water, (C) natural spring water and (D) deionized tap water.

ionization detector could be used if the positive identification a mass spectrometer provides is not required.

Milli-Q purified water was used in all experiments to prepare the standard solutions. When analyzing blanks toluene was found to be present in this water at about 15 pg/ml (see Fig. 4A) but was also detected at levels as high as 150 pg/ml, which explains the nonlinearity of the calibration curve in Fig. 3 for this analyte.

In the search for a clean water blank several sources were examined. The chemistry building tap water contained approximately 15 pg/ml toluene (Fig. 4B). Natural spring water purchased in a local grocery store contained toluene at 90 pg/ml (Fig. 4C). The biggest surprise was associated with the large amount of toluene in the deionized water of the chemistry building (Fig. 4D). Toluene contamination in the deionized water ranged from 0.5 ng/ml to greater than 10 ng/ml. It appears that there is

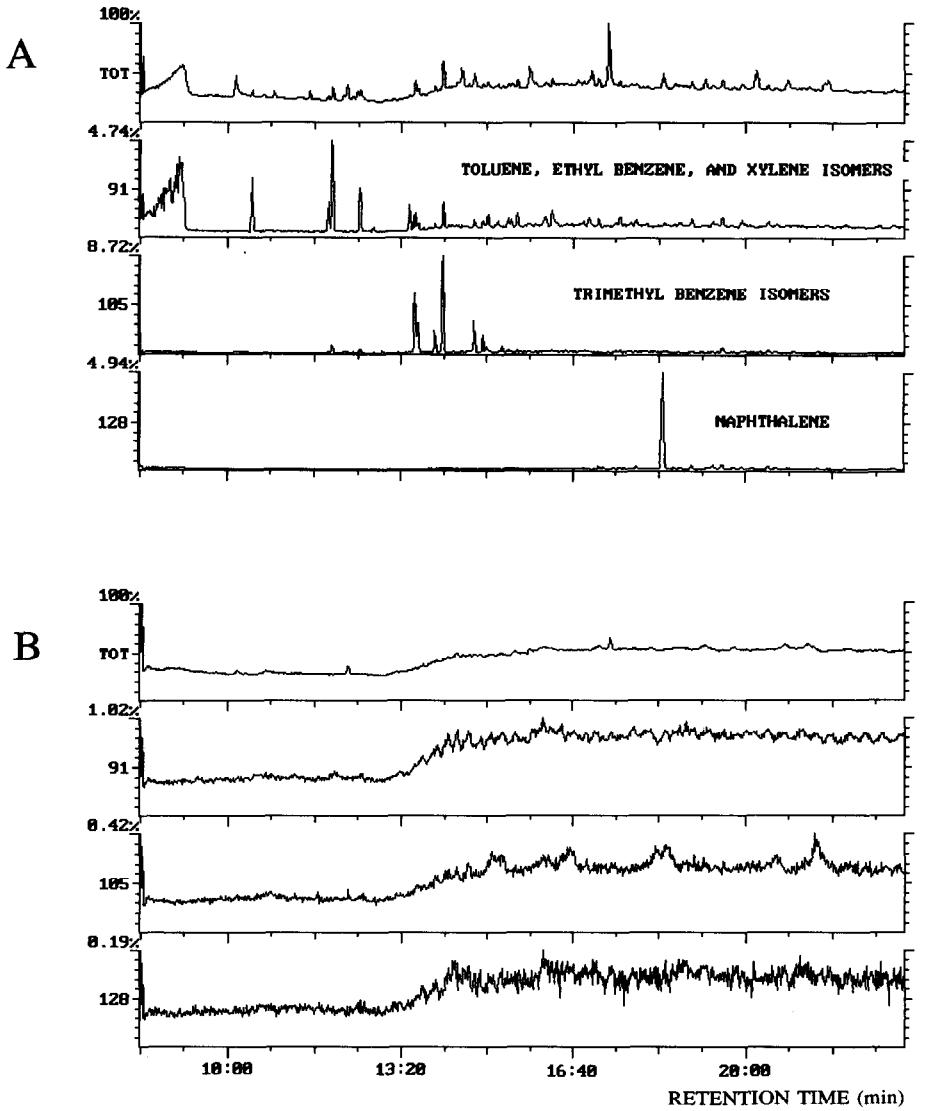

Fig. 5. (A) BTEX is present at the ng/ml level in parking lot runoff water, (B) BTEX is undetected in a fiber blank analyzed immediately afterwards.

TABLE III

BTEX DETECTED IN PARKING LOT RUNOFF WATER (ng/ml)

n/d = Not detected.

	Benzene	Toluene	Ethyl benzene	*m*- and *p*-Xylene	*o*-Xylene
Bottle A	n/d	17.4	10.7	18.1	18.9
Bottle B	n/d	16.9	10.6	17.3	17.7
Bottle B (2nd injection)	n/d	18.1	11.4	18.7	18.5

contamination somewhere in the lines or ion-exchange resin.

Toluene is used in our laboratory as a solvent for silanizing glassware and in soxhlet extractions. It is also used extensively by other laboratories in the same building. Contamination by adsorption to glassware was prevented by heating glassware in a muffle furnace at 400°C for several h. The possibility of the fiber being contaminated with analytes in laboratory air was also examined. A fiber exposed to laboratory air for 1 h was equivalent to sampling water containing 7.0 ng/ml toluene. The exact volume of air sampled is not known, however the extreme sensitivity of this technique is shown as well as the possibility of using SPME for air sampling. It is not surprising that toluene showed significant contamination considering the problems encountered with this analyte in aqueous samples. During transfer of the fiber from the sample vessel to the injector the fiber is retracted into the needle of the syringe. Contamination during the transfer process (10 s) can be ruled out since even after several min

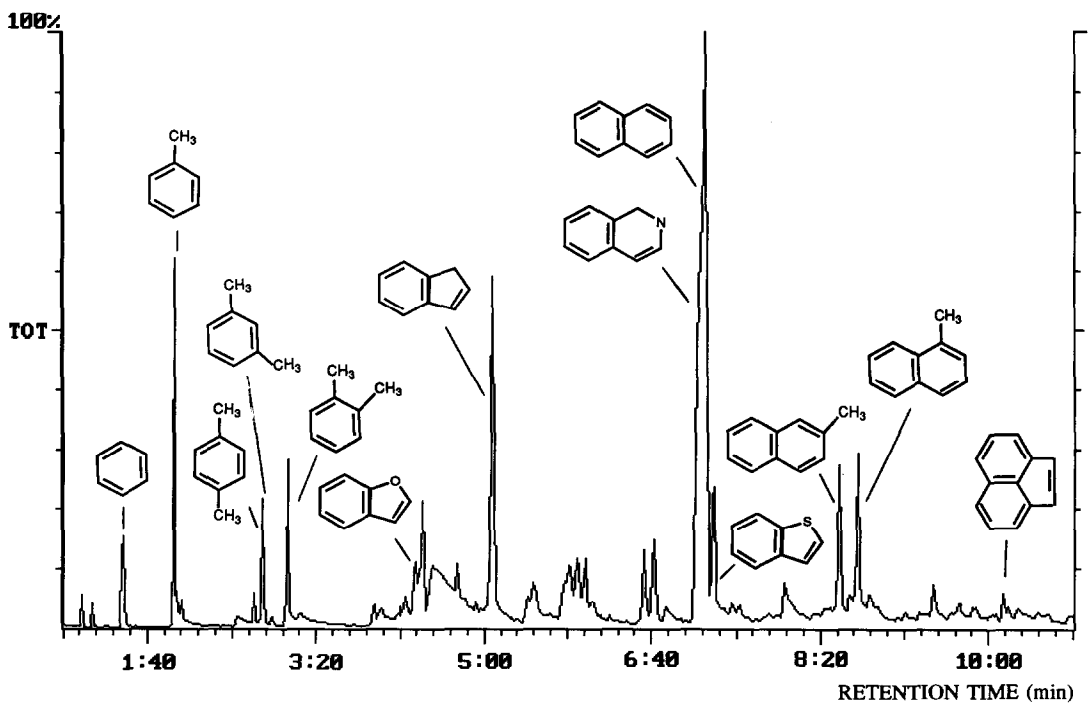

Fig. 6. Total ion chromatogram showing organics detected and identified in a coal gasification wastewater sample.

on a laboratory bench with the fiber sheathed no analytes were detected. However, the ultra sensitivity of this technique requires that extreme care be taken so that fibers are not inadvertently contaminated. In order to avoid contamination and incorrect results it is advisable to run a fiber blank before each sample when analyzing for ultra trace levels of pollutants.

In order to demonstrate the practicality of SPME–GC–IT-MS duplicate water samples were collected from parking lot runoff after a recent snowfall. A typical total ion and selected ion chromatograms for BTEX and other contaminants are shown in Fig. 5A, and can be compared to a fiber blank in Fig. 5B. Trimethyl benzenes and naphthalene were detected but not quantitated. The first sample collected was analyzed once while the second water sample was analyzed twice. The results are tabulated in Table III.

Detection limits required by USEPA and MISA for BTEX in water are easily obtainable in a full scan acquisition mode thus eliminating any ambiguity that may arise when acquiring data using selected ion monitoring. Since low pg/ml detection limits have been achieved for BTEX components with only one aromatic ring, pg/ml detection limits for compounds with larger K_{ow} values such as polynuclear aromatic hydrocarbons should be easily attainable. Polynuclear aromatic hydrocarbons have K_{ow} values up to several orders of magnitude larger than for the BTEX compounds studied in this paper. A total ion chromatogram showing many compounds with high K values extracted from a coal gasification wastewater sample is shown in Fig. 6. This was obtained with a relatively thick coating (56 μm) and only a 5-min absorption time so it does not necessarily reflect the relative concentrations of analytes in the sample, but rather constitutes qualitative information which can be used for screening purposes. It may be preferable to use a thinner stationary phase for quantitation. A thinner film will require shorter equilibration times and absorb fewer contaminants with low K values which could constitute interferences.

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