Determination of Volatile Organic Compounds in Biological Samples Using Headspace Solid-Phase Microextraction and Gas Chromatography: Toluene and Styrene

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Abstract

Epidemiological and laboratory investigations have shown that toluene and styrene are toxic compounds that lead to impairment of the nervous system. To quantitate toluene and styrene in biological samples, liquid–liquid phase, headspace (HS), and solid-phase microextraction (SPME) methods are generally used. Most of these methods are not sensitive enough for applications involving small sample volumes. Here, we present a method for quantitative analysis of low concentrations of styrene and toluene in very small volumes of biological samples using HS-SPME and gas chromatography (GC) equipped with a flame-ionization detector. The method was developed by optimizing operating parameters that affect the HS-SPME–GC process [i.e., desorption time (30 s), depth of the fiber in the GC injection port (3.7 cm), adsorption time (4 min), and adsorption temperature (room temperature)]. It has a wide range of linearity (0.5–500 ng/10 µL), high precision (coefficient of variation < 5%), good accuracy (deviation < 11%), and low detection limits of 0.13 and 0.08 ng/10 µL for styrene and toluene in serum, respectively. This analytical technique can be applied to the estimation of styrene and toluene in small volumes of biological fluids (blood, serum, and perilymph) and tissues of low lipid content (cochlea).

Introduction

Styrene and toluene are used extensively in the production of polymer surface coatings, resins, and synthetic rubber, where worker exposure can occur. The quantitative determination of these organic solvents in biological fluids is of importance as they are highly volatile and hazardous to human health. Human and animal studies suggest that chronic exposure to styrene and toluene are implicated primarily in central and peripheral nervous system disorders and loss in hearing activity (1–6).

Over the last few decades, several research groups have developed analytical methods for analyzing styrene and toluene in biological samples using liquid–liquid extraction (LLE), headspace (HS), and HS-solid-phase microextraction (SPME) techniques (7–20). Ramsey et al. (15) and Tornero-Velez et al. (7) extracted styrene from 5-mL homogenized whole blood samples by LLE and gas chromatography (GC), and Colin et al. (8) measured styrene from 100 µL of serum, liver, or kidney homogenate by LLE and reversed-phase high-pressure liquid chromatography (HPLC). Apostoli et al. (16) and Prieto et al. (9) measured toluene in 5 mL of whole blood samples using the purge-and-trap technique. Lee et al. (12) and Alegretti et al. (20) measured toluene in 0.5 and 2 mL of whole blood, respectively, using HS-SPME. Fustinoni et al. (11) assessed 2 mL of urine for toluene concentrations using HS-SPME. Campo et al. (18) attempted to quantitate styrene in organ of corti using HS-SPME, but were constrained by the small size of the sample (< 2 mg). Previously developed analytical techniques that are generally employed for the analysis of styrene and toluene in biological matrices use sample volumes ranging from 0.5 to 5 mL and are not sensitive enough for applications involving very small sample volumes.

The current paper describes an HS-SPME–GC method developed to analyze low concentrations of styrene and toluene in very small volumes of biological samples. The method is based on Markelov and Guzowski's full evaporation HS GC technique, which uses a very small sample size to eliminate the need for sample pretreatment and to overcome the interference of other compounds in the biological matrix (21). Our HS-SPME–GC method has been successfully used by Chen et al. (22) in a study aimed at determining the cellular mechanism underlying styrene ototoxicity.

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Methods and Materials

Chemicals and equipment

HPLC-grade styrene and toluene were obtained from Sigma-Aldrich (St. Louis, MO). Methanol, HPLC-grade, was procured from EM Science (Gibbstown, NJ). Sheep serum and whole blood were purchased from Quad Five (Ryegate, MO). For interference studies, lipid emulsions were prepared using linoleic acid (99% purity) obtained from Sigma-Aldrich. Standard solutions were prepared using 10- and 500-µL gas-tight syringes obtained from Hamilton (Reno, NV).

The SPME manual fiber holder and a carboxene™-polydimethylsiloxane microextraction fiber were purchased from Supelco (Bellefonte, PA). Before use, the fiber was conditioned by heating at 300°C in the GC injector for 2 h as per the manufacturer's instructions. Clear 10-mL glass vials and corresponding stainless steel screw-caps lined with PTFE-faced silicone septum were also purchased from Supelco.

The chromatographic analysis was performed using an HP 6890 GC (Agilent Technologies, Santa Clara, CA) equipped with an electronic pneumatically controlled split/splitless injector and a flame-ionization detector. Target analytes were resolved using an SPBTM-624 fused silica capillary column (30 m × 0.25-mm i.d. × 1.4-µm film thickness) purchased from Supelco. Chromatography conditions were as follows: injection port temperature 250°C, detector temperature 250°C, and an initial oven temperature of 35°C held for 1 min, followed by a 5°C/min ramp to 150°C, and held at 150°C for 2 min. Injections with the SPME fiber were made in the splitless mode using a narrow SPMEGC inlet liner (78.5 mm × 6.5 mm × 0.75 mm) purchased from Supelco. Ultra-high-purity helium was used as the carrier gas at constant flow rate of 1.5 mL/min.

Method development and analytical method validation

Method development. Optimization studies were conducted using serum as the sample matrix. The standard reference solutions were prepared by serial dilutions of a 10 mg/mL styrene and toluene standard in methanol. An intermediate standard solution of 10 µg/mL was prepared in blood and serum. The standard solutions for method optimization and validation studies were prepared by serial dilution of the 10 µg/mL intermediate standard into blood or serum. A 10-µL aliquot of these standard solutions was used in subsequent experiments.

For all experiments, samples were equilibrated in the HS vial for at least 15 min prior to sampling the HS with the SPME fiber. At the end of each run, the fiber was reinserted into the injection port for an additional 30 min to ensure that no carryover occurred in subsequent sampling. Five operating parameters were evaluated in the following order: desorption time, depth of the fiber in the GC injection port during desorption, adsorption time, adsorption temperature, and sample volume. During optimization, one parameter was varied at a time while keeping the other parameters constant. Once a parameter was optimized, its optimized condition was used in the evaluation of the remaining parameters and for subsequent analytical validation studies. The analytical performance of the method was studied with respect to linearity, precision, accuracy, recovery, and interference.

Method validation. Linearity studies were conducted in both serum and whole blood. The linearity of the method was determined for both styrene and toluene in the range of 0.5–500 ng/10 µL. From a stock of 10 mg/mL of styrene and toluene in methanol, standard concentrations of the analytes in serum and whole blood were prepared at room temperature by volumetric serial dilution. Linear regression analysis was performed to estimate the slope, intercept, and regression coefficient for the plots of peak area against analyte concentration.

The precision and accuracy of the method were studied by analyzing three standards: 1, 10, and 100 ng/10 µL. For each standard concentration, five aliquots of approximately 500 µL were prepared. The intraday precision and accuracy were determined by analyzing 1 aliquot 10 times on the same day (n = 10). Interday precision and accuracy were determined over a period of five days by thawing an aliquot each day and then analyzing it in triplicate (n = 3). The precision of the method was expressed as coefficient of variation (% CV). Accuracy was expressed as the percentage of deviation between the true and measured values.

The minimal detection limits (MDL) and minimal quantitation limits (MQL) of styrene and toluene in whole blood and serum were determined using the protocol outlined by U.S. Environmental Protection Agency (EPA) (23). Ten replicates of the lowest concentration (0.5 ng/10 µL) in the working curve were analyzed by HS-SPME–GC, and the absolute amount of styrene and toluene recovered from each run was calculated using the standard working curve. The MDL was calculated as: $MDL = SD \times t\,(n-1, \alpha = 0.99)$, where n = number of replicate analyses; t = value from Student's t value at the 99% confidence interval; and SD = standard deviation value for 10 repeat analyses of the lowest standard on the calibration curve (0.5 ng/10 µL). The MQL was calculated as 10 times the standard deviation of 10 repeat analyses of the 0.5 ng/10 µL standard.

Relative recovery was evaluated using two standard concentrations of styrene and toluene, 10 and 100 ng/10 µL, in various biological matrices. The absolute amount recovered from corresponding concentrations in serum, whole blood and phosphate buffer saline matrices were compared using Student's t-test. To evaluate the effect of interference of lipids in the sample matrix, a series of lipid emulsions of varying concentrations (1%, 5%, 10%, and 25%, w/w) were prepared by adding linoleic acid (99% purity) to serum. The lipid emulsions were homogenized by sonication at 20 MHz for 5 s. Concentrations of styrene and toluene emulsions at 10 and 100 ng in 10 µL were prepared at room temperature by volumetric serial dilution of the 10 µg/mL intermediate standard in serum. The amount of styrene and toluene recovered from each lipid matrix was then compared using one-way analysis of variance (ANOVA) and the Bonferronni post-hoc test.

Because styrene and toluene are volatile, the loss of analytes during sample manipulation and sample handling was a concern. The rate of loss of analytes in response to exposure time of the samples to the laboratory atmosphere at room temperature was evaluated at target analyte concentrations of 1, 10, and 100 ng/10 µL prepared in whole blood. A 10-µL sample of standard stock was placed in a 10-mL SPME vial and exposed

to the atmosphere for time intervals ranging from 10 to 120 s prior to closing the SPME vial. Triplicate determinations were made at each time point. Regression analysis was used to determine the rate of loss of analyte as a function of time.

Statistical analysis was performed using SPSS 15.0 for Windows (SPSS). A p value less than 0.05 was considered statistically significant.

Results

Method development

Desorption time. The kinetics of thermal desorption of styrene and toluene from the SPME fiber were evaluated using 10 µL of the 1 ng/µL standard in serum. During adsorption, the fiber was exposed to the HS of the vial for 4 min at room temperature. The depth of the fiber in the injection port of the GC was kept constant at 3.7 cm. Desorption times ranging from 15 to 120 s were investigated. As shown in Figure 1, at longer desorption times, larger amounts of styrene and toluene were detected. However, with an increase in desorption time, there was also a time-dependent peak broadening and tailing for toluene (Figure 2). Therefore, to avoid errors in peak estimation, a desorption time of 30 s was chosen for all subsequent experiments.

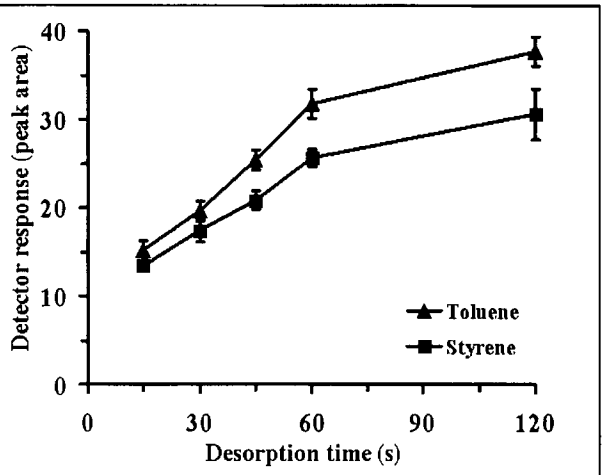

Figure 1. Effect of desorption time on the amount of styrene and toluene detected in serum. Values are means of three independent determinations, and error bars indicate standard deviation. Operating parameters: desorption fiber depth, 3.7 cm; sample volume, 10 µL; adsorption time, 4 min; and adsorption temperature, 23°C.

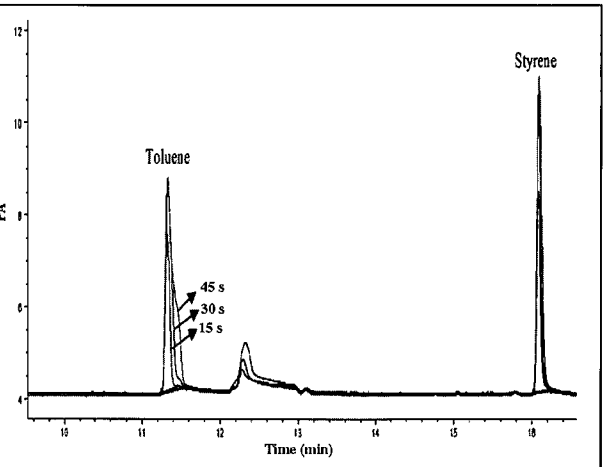

Figure 2. Overlaid chromatograms of three runs differing in time of desorption of the fiber.

Depth of the fiber in the injection port of the GC during desorption. The depth of the SPME fiber in the injection port of the GC during desorption was studied using 10 µL of the 1 ng/µL standard in serum. Adsorption was conducted at room temperature. The adsorption and desorption times were kept constant at 4 min and 30 s, respectively. The depth of the fiber was varied at 3.1, 3.3, 3.5, 3.7, and 3.9 cm using the depth adjustment gauge on the manual SPME fiber holder. As shown in Figure 3, beyond a depth of 3.5 cm, no significant increase was

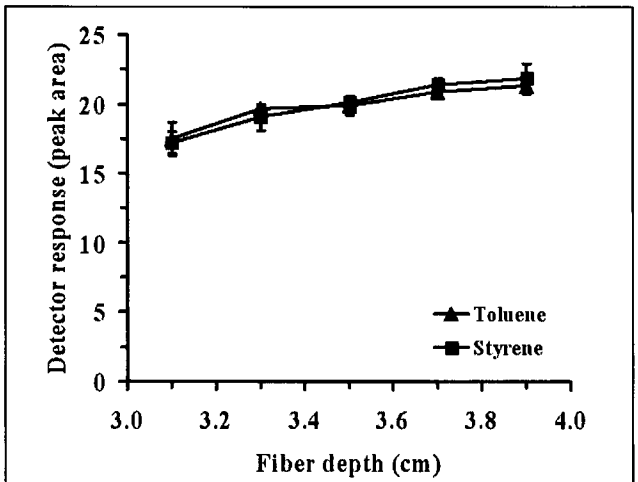

Figure 3. Effect of fiber depth in the injection port of the GC during desorption on the amount of styrene and toluene detected in serum. Values are means of three independent determinations, and error bars indicate standard deviation. Operating parameters: desorption time, 30 s; sample volume, 10 µL; adsorption time, 4 min; and adsorption temperature, 23°C.

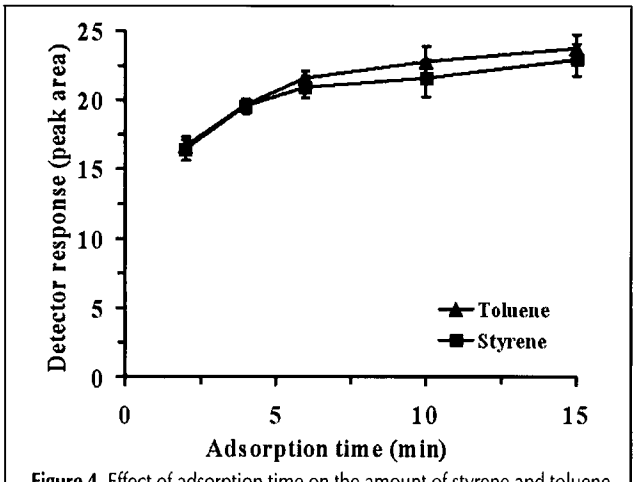

Figure 4. Effect of adsorption time on the amount of styrene and toluene detected in serum at a standard concentration of 10 ng/10 µL. Values are means of three independent determinations, and error bars indicate standard deviation. Operating parameters: desorption time, 30 s; sample volume, 10 µL; desorption fiber depth, 3.7 cm; and adsorption temperature, 23°C.

observed in the amount of analyte detected. Differences were evaluated statistically using the Student's t test. A p value less than 0.05 was considered statistically significant. Statistical comparison of styrene and toluene amounts recovered for the depth of the fiber pairs 3.1:3.3 cm and 3.3:3.5 cm yielded a p value of less than 0.05, and beyond 3.5 cm, the p value was greater than 0.05. The fiber depth of 3.7 cm in the injection port was chosen because this depth corresponds to the penetration depth of a 10-µL syringe needle when using the Agilent autosampler in the standard splitless mode.

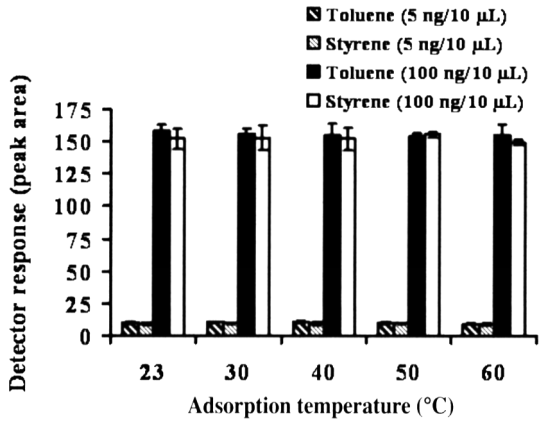

Figure 5. Effect of adsorption temperature on amount of styrene and toluene detected in serum at standard concentration levels of 5 and 10 ng/10 µL. Values are means of three independent determinations, and error bars indicate standard deviation. Operating parameters: desorption time, 30 s; sample volume, 10 µL; desorption fiber depth, 3.7 cm; and adsorption time, 4 min.

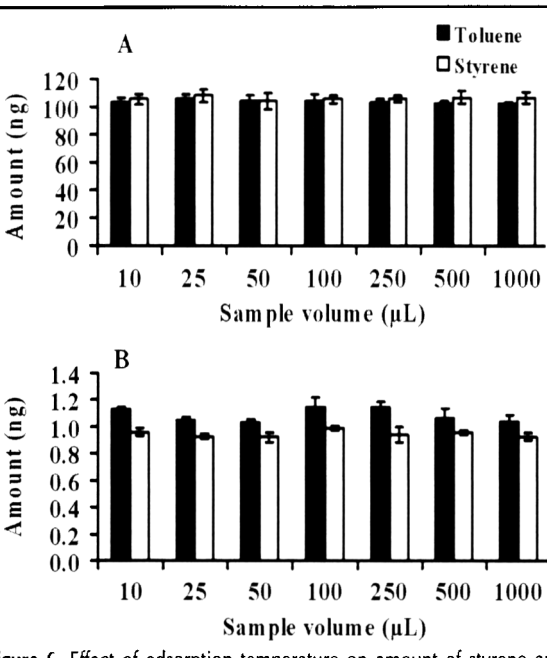

Figure 6. Effect of adsorption temperature on amount of styrene and toluene detected in serum at standard concentration levels of 100 ng/10 µL (A) and 10 ng/10 µL (B). Values are means of three independent determinations, and error bars indicate standard deviation. Operating parameters: desorption time, 30 s; desorption fiber depth, 3.7 cm; adsorption time, 4 min; and adsorption temperature, 23°C.

Adsorption time. The effect of adsorption time on detection of styrene and toluene in serum was studied using 10 µL of 1 ng/µL standard in serum. Desorption time and depth of the fiber in the injection port were kept constant at 30 s and 3.7 cm, respectively. At room temperature, the adsorption times investigated were 2, 4, 6, 10, and 15 min. Approximately 90% of the adsorption plateau value was reached at 4 min (Figure 4). An exposure time of 4 min was chosen for subsequent experiments.

Adsorption temperature. The effect of temperature on adsorption was studied using 10 µL of 10 and 0.5 ng/µL standards in serum. Temperatures ranging from room temperature (23°C) to 60°C were investigated. The temperature was controlled using a heat block (Barnstead/Thermolyne, Type 17600). The adsorption and desorption times and depth of the fiber in the injection port during desorption were kept constant at 4 min, 30 s, and 3.7 cm, respectively. As shown in Figure 5, no significant change in the amount of toluene or styrene recovered was observed with increase in temperature at either standard concentration. For subsequent studies, adsorption was performed at room temperature.

Sample volume. The effect of sample volume on recovery of styrene and toluene from serum was studied using sample concentrations 0.1 and 10 ng/µL. Ten microliters of the standard stock was placed in an SPME vial and the appropriate volume of serum was added to prepare samples with a total volume ranging from 10 to 1000 µL. The time of adsorption, desorption time, and depth of the fiber in the injection port during desorption were kept constant at 4 min, 30 s, and 3.7 cm, respectively. Adsorption was done at room temperature. The amount of toluene and styrene recovered was calculated using the standard curve. As seen in Figure 6, no significant change in the amount of toluene and styrene recovered was observed by increasing sample volume using a 10-mL SPME vial.

Analytical method validation

Linearity. Linearity studies were conducted in serum and whole blood. The calibration curves obtained for styrene and toluene in serum and whole blood were linear in the range 0.05–50 ng/µL. Because 10 µL of the standard concentration was used, the range of linearity can also be expressed as 0.5–500 ng/10 µL. Each standard concentration was analyzed in triplicate and the mean values were used for statistical analysis. Least square linear regression analysis was applied to estimate the slope, intercept, and regression coefficient of styrene and toluene in serum and whole blood. In spite of high regression coefficients (> 0.99), the whole range standard curve (0.5–500 ng/10 µL), owing to its high intercept, is unsuitable for measuring concentrations of styrene and toluene at the lower end of the curve. To ensure accuracy of quantitation at low concentrations, the 0.5–500 ng/10 µL standard curve was truncated at 25 ng/10 µL according to the Baadenhuijsen et al. method (24).

The standard working curves of styrene and toluene in serum are shown in Figure 7. The equation of the line for the whole range curve (0.5–500 ng/10 µL) was $y = 1.94x - 6.89$ and $y = 1.61x - 1.84$ for toluene and styrene, respectively. The equation of the line for the low range curve (0.5–25 ng/10 µL) was $y = 1.89x - 0.21$ and $y = 1.74x + 0.04$ for toluene and styrene, respectively. The standard working curves of styrene

and toluene in whole blood are shown in Figure 8. The equation of the line for the whole range curve (0.5–500 ng/10 µL) was $y = 1.89x + 3.42$ and $y = 1.75x - 2.24$ for toluene and styrene, respectively. The equation of the line for the low range curve (0.5–25 ng/10 µL) was $y = 1.97x + 1.23$ and $y = 1.63x + 0.49$ for toluene and styrene, respectively.

The calibration fitting (i.e., slope and intercept of styrene and toluene in both biological matrices) was similar. Paired t-tests between triplicate analysis of whole blood and serum at each standard curve concentration level indicates that there exists no significant difference between chromatographic peak areas obtained by HS-SPME–GC analysis of corresponding standard concentrations of styrene and toluene in whole blood and serum throughout the linear range (0.5–500 ng/10 µL). The % difference in slope for serum and whole blood was calculated as approximately 2% for toluene and 4.5% for styrene at the low

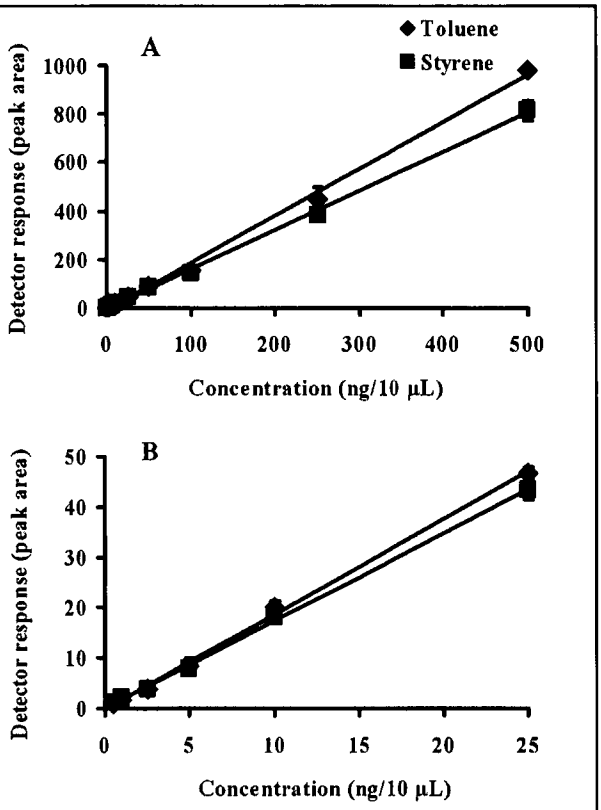

Figure 7. Whole range, 0.5–500 ng/10 µL, (A) and low range, 0.5–25 ng/10 µL, (B) standard working curves of styrene and toluene in serum. Whole range curve equations were $y = 1.94x - 6.89$, $R^2 = 0.99$ for toluene and $y = 1.61x - 1.84$, $R^2 = 0.99$ for styrene. Low range curve equations were $y = 1.89x - 0.21$, $R^2 = 0.99$ for toluene and $y = 1.74x + 0.04$, $R^2 = 0.99$ for styrene.

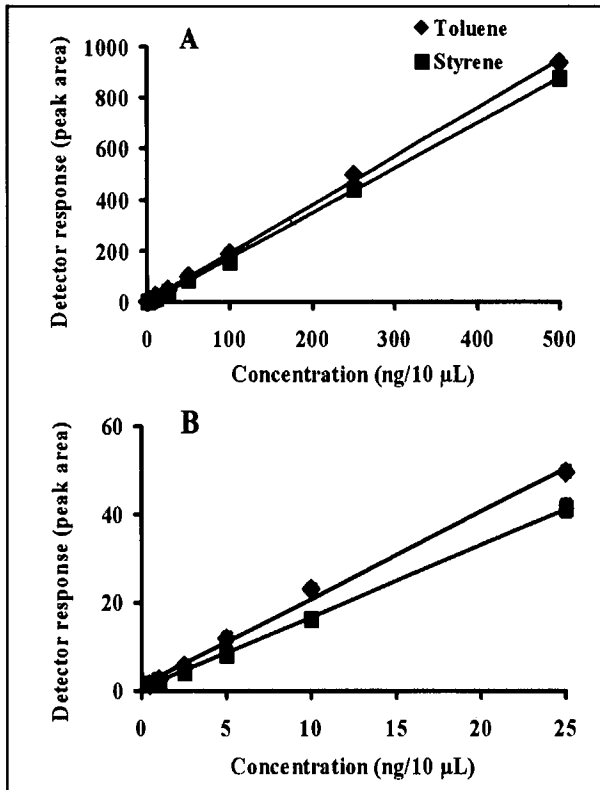

Figure 8. Whole range, 0.5–500 ng/10 µL, (A) and low range, 0.5–25 ng/10 µL, (B) standard working curves of styrene and toluene in whole blood. Whole range curve equations were $y = 1.89x + 3.42$, $R^2 = 0.99$ for toluene and $y = 1.75x - 2.24$, $R^2 = 0.99$ for styrene. Low range curve equations were $y = 1.97x + 1.23$, $R^2 = 0.99$ for toluene and $y = 1.63x + 0.49$, $R^2 = 0.99$ for styrene.

Table I. Intra- and Interday Precision and Accuracy of the Method

	Standard Concentration (ng/10 µL)					
	Toluene			Styrene		
	1	10	100	1	10	100
Intraday precision and accuracy* ($n = 10$)						
Mean ± SD	1.09 ± 0.05	10.52 ± 0.28	90.63 ± 4.54	1.05 ± 0.05	10.14 ± 0.35	102.31 ± 2.31
Precision (% C.V.)	4.59	2.81	5.01	4.70	3.46	3.22
Accuracy (%)	9.89	5.15	9.37	4.62	1.39	2.31
Interday precision and accuracy ($n = 3$, for 5 days)						
Mean ± SD	1.10 ± 0.01	10.32 ± 0.11	91.15 ± 1.21	1.05 ± 0.01	10.34 ± 0.13	101.25 ± 1.79
Precision (% C.V.)	3.61	3.91	2.65	3.49	4.04	3.11
Accuracy (%)	10.11	3.15	8.85	4.62	3.39	1.25

* Intraday precision and accuracy were determined over a period of five days.

concentration range (0.5–25 ng/10 µL).

Accuracy and precision. Intraday and interday precision and accuracy were calculated for three standard concentrations: 1, 10, and 100 ng/10 µL. According to the EPA guidelines (29), the precision and accuracy, expressed as %CV and % accuracy, should be 15% at all concentration levels, except at the MDL where it should not exceed 20%. The results are summarized in Table I. The mean values were within 10% of the expected values. For toluene and styrene, intraday and interday accuracy ranged from 3.15 to 10.11% and 1.25 to 4.62%, respectively. Intraday and interday precision expressed as % CV were less than 5% for both styrene and toluene. The method meets the criteria for both accuracy and precision as defined by the guidelines for bioanalytical method validation (29).

Minimal detection and quantitation limit. MDL and MQL for the analytes, styrene, and toluene in whole blood and serum were determined using the protocol outlined by EPA guidelines (23). Ten replicates of the lowest concentration (0.5 ng/10 µL) in the working curve were analyzed by HS-SPME–GC and the absolute amount of styrene and toluene recovered from each run was calculated using the standard working curve. The MDL was calculated as MDL = SD × t (n–1, α = 0.99), where n = number of replicate analyses; t = value from Student's t value at the 99% confidence interval; and SD = standard deviation value for 10 repeat analyses of the lowest standard on the calibration curve (0.5 ng/10 µL). The MQL was calculated as 10 times the standard deviation for 10 repeat analyses of the 0.5 ng/10 µL standard. The MDL and MQL of toluene in serum were 0.08 and 0.28 ng/10 µL, respectively, and the MDL and MQL of styrene were 0.13 and 0.46 ng/10 µL, respectively. The MDL and MQL of toluene in whole blood were 0.07 and 0.26 ng/10 µL, and the MDL and MQL of styrene were 0.14 and 0.49 ng/10 µL.

Relative recovery in different matrices and the interference of sample lipid content. The relative recoveries of styrene and toluene in whole blood, serum, saline, and a series of lipid emulsions of varying concentrations (1%, 5%, 10%, and 25%, w/w) at two standard concentrations, 100 and 10 ng/10 µL, were calculated using the standard working curve in serum. Recoveries were calculated relative to serum with no additional lipid added. Statistics was done using SPSS. The results were compared by one-way ANOVA and the Bonferronni post-hoc test (p = 0.05). As seen in Table II, recovery in serum, whole blood, and saline ranged from 98% to 102% for both standard concentrations. The addition of lipid to the serum matrix had no significant effect on toluene recovery up to a sample lipid concentration of 25%. For styrene, there was no significant effect on recovery up to a lipid content of 10%. There was a significant reduction in styrene recovery at sample lipid content of 25%.

Table II. Recovery Studies in Different Matrices

	Relative Recovery (%)			
	Toluene		Styrene*	
Matrix	10 ng	100 ng	10 ng	100 ng
Whole blood	101.04 ± 2.13	99.88 ± 4.08	101.09 ± 5.24	101.94 ± 3.12
Serum	98.90 ± 5.73	100.17 ± 3.53	99.37 ± 6.63	102.17 ± 4.27
Saline	99.09 ± 5.88	98.46 ± 2.78	98.98 ± 6.57	99.02 ± 6.5
1% lipids	100.51 ± 5.32	97.36 ± 3.65	100.52 ± 5.97	102.70 ± 5.51
5% lipids	101.76 ± 2.93	98.29 ± 2.93	100.54 ± 5.12	101.75 ± 5.7
10 % lipids	99.45 ± 5.50	97.60 ± 4.37	100.33 ± 4.87	96.33 ± 4.1
25% lipids	99.80 ± 2.18	95.49 ± 3.86	78.36 ± 3.72*	83.52 ± 4.95*

* There was a significant reduction in styrene recovery at sample lipid content of 25%. Recoveries were calculated relative to serum with no additional lipid added.

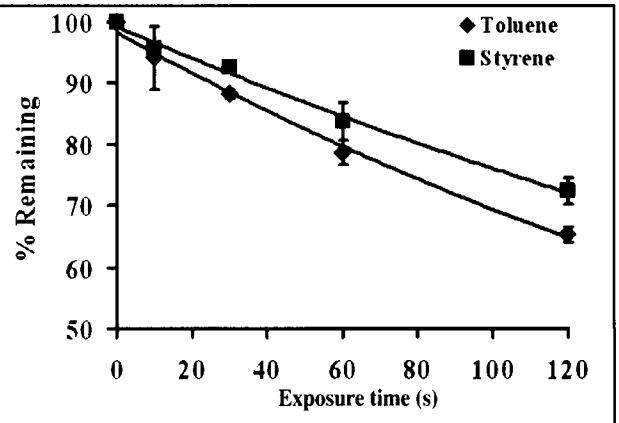

Figure 9. Effect of sample exposure on recovery of styrene and toluene. The loss of styrene and toluene in whole blood as a function of time from a 10-µL sample was determined at the concentration level of 1 ng/10 µL. The 100% reference indicates the amount of styrene and toluene recovered from stock solution before exposing the sample to the atmosphere. Values shown are means of three independent readings, and the error bars indicate standard deviation.

Loss of analytes from samples at room temperature. Because both toluene and styrene are volatile, loss from a sample during sample handling was a concern. The loss of styrene and toluene in whole blood as a function of time from a 10-µL sample in a 10-mL SPME vial at room temperature was determined at the three concentrations of 1, 10, and 100 ng/10 µL. A 10-µL aliquot of the whole blood standard was transferred to the SPME vial and exposed to the atmosphere for 0, 15, 30, 60, and 120 s prior to capping the vial. The amount of styrene and toluene recovered from the standard stock at the zero time exposure was used as the reference and percent recovery was calculated against it. The mean of triplicate analysis at each time point was used to construct the trend line. The trend line for the lowest concentration point studied 1 ng/10 µL is shown in Figure 9. Analyte half times were calculated by extrapolation of the trend line to 50% of the initial concentration. The styrene trend line equations were $y = 99.2e^{-0.00266x}$, $y = 99.5e^{-0.00221x}$, and $y = 100e^{-0.00217x}$ for 1, 10, and 100 ng/10 µL whole blood standard solutions, respectively. The toluene trend line equations were $y = 98.3e^{-0.00348x}$, $y = 98.3e^{-0.00356x}$, and $y = 99.6e^{-0.00308x}$ for 1, 10, and 100 ng/10 µL whole blood standard solutions, respectively. Analyte half-times varied less than 20% over the three concentration ranges (Table III).

Discussion

The apparent simplicity in sample preparation, applicability to small sample volumes, reliability, selectivity, sensitivity, and consequent reduction of cost and time of analysis has made this method an attractive tool for analyzing trace amounts of volatile organic compounds in biological samples. In this study, quantitative assessment of low concentrations of styrene and toluene in biological samples was carried out using HS-SPME–GC.

The adsorption and desorption kinetics were studied during method development. In the current method, exposure of the fiber to the HS of the sample vial for 4 min results in 90% recovery of the total amount of styrene and toluene that can be extracted from the sample matrix at equilibrium. Additional sensitivity can be achieved by increasing the adsorption time at the cost of longer analysis time. Although SPME has maximum sensitivity at the equilibrium point, full equilibration is not essential for accuracy and precision. (25). During optimization of desorption time, it was observed that although an increase in desorption time resulted in increased detection of styrene and toluene; it caused undesirable peak broadening and considerable tailing. Further enhancement of sensitivity might be achieved by modifying the method to include a low cryofocusing temperature during desorption. Watanabe-Suzuki et al. (26) developed a simple and sensitive method for the determination of styrene, toluene, ethyl benzene, and other volatile compounds in human body fluids using cryogenic oven trapping and capillary GC.

Pharmacokinetic studies have shown that following absorption, toluene and styrene are rapidly distributed in the body and the highest levels are observed in the adipose tissue (27,28). In the current study, the effect of increasing serum lipid levels on toluene and styrene recovery was evaluated. No adverse effect on toluene and styrene recovery was observed up to a serum lipid content of 25% and 10% for toluene and styrene, respectively. The study does suggest that for matrices with high lipid content, greater than 25% for toluene and 10% for styrene, recovery may be adversely affected. For fatty matrices, such as brain and adipose tissue, further investigation is required prior to application of this method to these matrices.

The volume of sample and sample surface area play important roles in evaporation kinetics during HS-SPME analysis. The rate of toluene and styrene loss determined in this study is applicable only to the 10-µL sample in a 10-mL SPME vial. As shown in Figure 9 and Table III, fairly rapid loss of analytes is observed in the 10-µL sample. Because the rate of analyte loss is fairly rapid, care must be taken to limit exposure time. In our laboratory, blood draws are made directly into capillary tubes and dissected tissue is transferred to capped SPME vials within 10 s. Exposure studies indicate that over 95% of the analytes were recovered from a 1 ng/10 µL whole blood standard after a 10 s exposure to the atmosphere (Figure 9).

The advantage of this method is in its applicability to small sample volumes. While previously developed methods using LLE, HS, and HS-SPME use sample volumes ranging from 0.5 to 5 mL (7–20), our method is applicable to sample volumes as low as 10 µL of whole blood, 2 µL of perilymph, and approximately 0.4 mg of cochlear tissue. In Table IV, the sample volume and minimal detection limits of previously developed HS-SPME methods are listed. An important factor driving the detection limit is the amount of sample available for analysis. In comparing the current method with previously established methods, we have normalized MDL for all methods to a 10-µL sample volume (Table IV). Most methods have considerable higher MDL than the current method, with the exception of the Fustinoni et al. (11) method, which uses a simpler biological matrix (namely, urine) and measures only toluene.

The method developed in this paper has been successfully used by Chen et al. (22) in studying the mechanism of hearing loss in rats exposed to styrene by gavage. In that study, HS-SPME–GC was used to determine the distribution of styrene in cochlea and the kinetics of styrene in blood as a function of time after exposure. The kinetics of styrene in blood samples was determined after as single oral gavage of 800 mg/kg. The styrene concentration in blood ranged from 18.2 to 22.9 µg/g over a period of 6 h after dosing. Similar levels were obtained with repeated daily dosing over 6 days. Blood levels returned to baseline within 24 h after dosing. Perilymph samples from isolated cochleae at this dose averaged 3.1 + 0.6 µg/g. Cochlear tissue exhibited decreasing styrene concentrations from the apical turn at 12.5 + 3.0 µg/g, to the middle turn at 7.4 + 1.6 µg/g to the basal turn at 4.8 + 1.0 µg/g in animals receiving the same dose.

Table III. Analyte Half Time

Standard Concentration (ng/10 µL)	Half Time (s)*	
	Toluene	Styrene
1	199	261
10	195	314
100	225	319

* Half times are calculated by extrapolation of the exposure trend line.

Table IV. Comparison of HS-SPME–GC Methods for the Analysis of Styrene and Toluene in Biological Samples

Analyte	Sample Matrix	Sample Volume Required	Sample Detection Limit	Normalized Detection Limit	Reference
Toluene	Whole blood & urine	0.5 mL	2.4 ng/0.5 mL	2.4 ng/10 µL	12
Toluene & styrene	Organ of corti	< 2 mg	Qualitative only		18
Toluene	Urine	2 mL	0.07 ng/2 mL	0.07 ng/10 µL	11
Toluene & styrene	Urine	5 mL	2.5 ng/5 mL	2.5 ng/10 µL	13
Toluene	Whole blood	2 mL	1 µg/2 mL	1000 ng/10 µL	20
Toluene	Whole Blood	10 µL	0.07 ng/ 10 µL	0.07 ng/10 µL	current method
Styrene	Whole Blood	10 µL	0.14 ng/ 10 µL	0.14 ng/10 µL	

Conclusions

The aim of this study was to develop and validate a method to determine low concentrations of styrene and toluene in very small volumes of biological fluids and tissues using HS-SPME–GC. The advantage of this method is its sensitivity (detection limits of 0.13 and 0.08 ng/10 µL for styrene and toluene in serum, respectively) and applicability to small sample volumes (approximately 10 µL). This method also showed a wide range of linearity (0.5–500 ng/10 µL), high precision (CV < 5%), and good accuracy (deviation < 11%). The use of small sample size also reduces matrix effects (21). Serum lipid levels as high as 10% had no adverse affect on toluene and styrene recovery. This analytical technique can be applied to the estimation of styrene and toluene in small volumes of biological fluids such as blood, serum, perilymph, and in tissue samples.

Acknowledgments

This work was supported in part by grants from the National Institute of Occupational Safety and Health (NIOSH R8113-01A1OSH) and by a student award from the Mark Diamond Research Fund (MDRF FC-0113), University at Buffalo.

References

1. S.Y. Tsai and J.D. Chen. Neurobehavioral effects of occupational exposure to low-level styrene. *Neurotoxicol. Teratol.* **18(4):** 463–469 (1996).
2. N. Cherry and D. Gautrin. Neurotoxic effects of styrene: further evidence. *Br. J. Ind. Med.* **47:** 29–37 (1990).
3. P. Campo, R. Lataye, B. Cossec, and V. Placidi. Toluene-induced hearing loss: a mid-frequency location of the cochlear lesions. *Neurotoxicol. Teratol.* **19(2):** 129–140 (1997).
4. F. Gagnaire and C. Langlais. Relative ototoxicity of 21 aromatic solvents. *Arch. Toxicol.* **79(6):** 346–354 (2005).
5. M. Greenberg. The central nervous system and exposure to toluene: a risk characterization. *Environ. Res.* **72(1):** 1–7 (1997).
6. C.A. Trenga, D.D. Kunkel, D.L. Eaton, and L.G. Costa. Effect of styrene oxide on rat brain glutathione. *Neurotoxicology* **12(2):** 165–178 (1991).
7. R. Tornero-Velez, S. Waidyanatha, H. Licea Pérez, S. Osterman-Golkar, D. Echeverria, and S. Rappaport. Determination of styrene and styrene-7,8-oxide in human blood by gas chromatography–mass spectrometry. *J. Chromatogr. B Biomed. Sci. Appl.* **757:** 59–68 (2001).
8. P. Colin, G. Sirois, and S. Chakrabarti, Determination of styrene in biological samples by reversed-phase liquid chromatography. *J. Chromatogr.* **375(2):** 431–437 (1986).
9. M.J. Prieto, V. Berenguer, D. Marhuenda, and A. Cardona. Purge-and-trap gas chromatographic determination of styrene in urine and blood: application to exposed workers. *J. Chromatogr. B Biomed. Sci. Appl.* **741(2):** 301–306 (2000).
10. R. Gill, S.E. Hatchett, M.D. Osselton, H.K. Wilson, and J.D. Ramsey. Sample handling and storage for the quantitative analysis of volatile compounds in blood: the determination of toluene by headspace gas chromatography. *J. Anal. Toxicol.* **12(3):** 141–146 (1988).
11. S. Fustinoni, R. Giampiccolo, S. Pulvirenti, M. Buratti, and A. Colombi. Headspace solid-phase microextraction for the determination of benzene, toluene, ethylbenzene and xylenes in urine. *J. Chromatogr. B* **723:** 105–115 (1999).
12. X. Lee, T. Kumazawa, and K. Sato. A simple analysis of 5 thinner components in human body fluids by headspace solid-phase microextraction (SPME). *Int. J. Legal Med.* **107(6):** 310–313 (1995).
13. F. Asakawa, F. Jitsunari, J. Choi, S. Suna, N. Takeda, and T. Kitamado. Method for analyzing urinary toluene and xylene by solid-phase microextraction (SPME), and its application to workers using organic solvents. *Bull. Environ. Contam. Toxicol.* **62(2):** 109–116 (1999).
14. N.Y. Kim and S.W. Park. The comparison of toluene determination between headspace-solid phase microextraction and headspace methods in glue-sniffer's blood and urine samples. *J. Forensic Sci.* **45(3):** 702–727 (2000).
15. J.C. Ramsey and M.E. Anderson. A physiologically based description of the inhalation pharmacokinetics of styrene in rats and humans. *Toxicol. Appl. Pharmacol.* **73:** 159–175 (1984).
16. P. Apostoli, F. Brugnone, L. Perbellini, V. Cocheo, M.L. Bellomo, and R. Silvestri. Biomonitoring of occupational toluene exposure. *Int. Arch. Occup. Environ. Health* **50:** 153–168 (1982).
17. P.H. Cramer, K.E. Boggess, J.M. Hosenfeld, J.C. Remmers, J.J. Breen, P.E. Robinson, and C. Stroup. Determination of organic chemicals in human whole blood: preliminary method development for volatile organics. *Bull. Environ. Contam. Toxicol.* **40(4):** 612–618 (1988).
18. P. Campo, G. Loquet, V. Blachere, and M. Roure. Toluene and styrene intoxication route in the rat cochlea. *Neurotoxicology and Tetralology* **21(4):** 427–434 (1999).
19. T.K. Alkalde, M. Peralba, C.A. Zini, and E.B. Caramao. Quantitative analysis of benzene, toluene and xylenes in urine by means of headspace solid-phase microextraction. *J. Chromatogr. A* **1027:** 37–40 (2004).
20. A.P. Alegretti, F.V. Theisen, and G.P. Maciel. Analytical method for evaluation of exposure to benzene, toluene, xylene in blood by gas chromatography preceded by solid phase microextraction. *J. Chromatogr. B* **809:** 183–187 (2004).
21. M. Markelov and J.P. Guzowski. Matrix independent headspace gas chromatographic analysis. The full evaporation technique. *Anal. Chim. Acta* **276(2):** 235–245 (1993).
22. G.D. Chen, L.H. Chi, P.J. Kostyniak, and D. Henderson. Styrene induced alterations in biomarkers of exposure and effects in the cochlea: mechanisms of hearing loss. *Toxicol. Sci.* **98(1):** 167–177 (2007).
23. U.S. Environmental Protection Agency Method Detection Limit (MDL), Title 40, Code of Federal Regulations, Part 136, Appendix B, Revision 1.11.
24. H. Baadenhuijsen, P.M. Bayer, H. Keller, M. Knedel, N. Montalbetti, S. Brenna, L. Prencipe, A. Vassault, M. Bailly, and H.T. Phung. Multicenter evaluation of the Boehringer Mannheim/Hitachi 717 analysis system. *J. Clin. Chem.* **28:** 261–269 (1990).
25. J. Ai. Solid-phase microextraction for quantitative analysis in non-equilibrium aituations. *Anal. Chem.* **69(6):** 1230–1236 (1997).
26. K. Watanabe-Suzuki, A. Ishii, and O. Suzuki. Cryogenic oven-trapping gas chromatography for analysis of volatile organic compounds in body. *Anal. Bioanal. Chem.* **373(1):** 75–80 (2002).
27. A. Carlsson and E. Ljungquist. Exposure to toluene: concentration in subcutaneous adipose tissue. *Scand. J. Work Environ. Health* **8(1):** 56–62 (1982).
28. J.R. Withey and P.G. Collins. The distribution and pharmacokinetics of styrene monomer in rats by the pulmonary route. *J. Environ. Pathol. Toxicol.* **2(6):** 1329–1342 (1979).
29. U.S. Food and Drug Administration, Guidance for Industry, Bioanalytical Method Validation, May 2001.

Manuscript received September 25, 2007;
revision received February 11, 2008.