The Use of Solid-Phase Microextraction in Conjunction with a Benchtop Quadrupole Mass Spectrometer for the Analysis of Volatile Organic Compounds in Human Blood at the Low Parts-Per-Trillion Level

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Abstract

The analysis of volatile organic compounds (VOCs) in whole human blood at the low parts-per-trillion level has until recently required the use of a high-resolution mass spectrometer to obtain the specificity and detection limits required for epidemiological studies of VOC exposure in the general public. Because of the expense and expertise required to operate and maintain a high-resolution instrument, the applicability of this method has been limited. These limitations are overcome in a new method using automated headspace solid-phase microextraction (SPME) in conjunction with a gas chromatograph and a benchtop quadrupole mass spectrometer. A combination of SPME and multiple single-ion monitoring minimizes the interferences and chemical noise associated with whole blood samples. This method permits the analysis of 10 VOCs in human blood while simplifying the sample preparation and reducing the possible exposure of the analyst to blood aerosols. Twelve samples can be run successively in a fully automated mode, thus eliminating the need for operator attention. Detection limits are below 50 ppt (pg/mL) for a majority of the VOCs tested with a 5-mL sample.

Introduction

In several studies, volatile organic compounds (VOCs) have been detected in the blood of people with no known occupational exposure (1,2). These compounds include 1,1,1-trichloroethane; benzene; toluene; tetrachloroethylene; ethylbenzene; *m*/*p*- and *o*-xylene; styrene; and 1,4-dichlorobenzene. The detection of these compounds has raised public health concerns, because a number of VOCs are known to be associated with health effects in people, such as cancer (3,4), neurological and reproductive problems (5), and birth defects (6). The Centers for Disease Control and Prevention (CDC) has conducted studies to monitor the magnitude of exposure to VOCs in the general population (2).

To support this effort, investigators within our laboratory developed a highly sensitive analytical method to detect and quantitate VOCs in whole human blood (7). With this previous method, VOCs were extracted from a 10-mL whole blood sample using helium as a purge gas, trapped on Tenax, cryofocused using liquid nitrogen, separated by capillary gas chromatography (GC), and measured using a high-resolution magnetic-sector mass spectrometer (MS). Although this method proved to be a sensitive and selective way of measuring VOCs in human blood, sample throughput was restricted by the need for operator attention and the time required for the complicated purge-and-trap manipulations.

Solid-phase microextraction (SPME) is a relatively new analytical separation technique developed in 1993 by Zhang and Pawliszyn (8). This technique presents a number of advantages over the previous purge-and-trap technique, including the choice of headspace sampling or immersion of the fiber, the ease of sample extraction, and the simplicity of the design. The technique also shows promise for easy automation. In this paper, the development of an automated SPME technique for the determination of parts-per-trillion levels of 10 VOCs in human blood is described.

Experimental

Materials

Descriptions of the preparation of blank water, standards, labeled isotope standards, and the source and purity of the reagents used to make these solutions have been previously published (7). The isotope composition of the labeled isotope

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standards used in this study are given in Table I.

Borosilicate glass SPME vials (12 mL, Supelco, Bellefonte, PA), along with the cap assemblies, were heated under vacuum for 24 h before use. After cooling under vacuum and restoring pressure with ultrapure helium, these sampling containers were assembled and stored in a desiccator containing activated charcoal until used.

Samples

Whole human blood samples were collected in specially prepared (9) 10-mL vacutainers (Becton Dickinson, Franklin Lakes, NJ) and shipped chilled to our laboratory. Upon receipt, the samples were transferred to a refrigerator and stored at 4°C until analysis. A 5-mL aliquot was removed from each vacutainer and then analyzed by SPME–GC–mass-selective detection (MSD) and/or an additional 5-mL aliquot was analyzed using purge-and-trap (P/T)–GC–high-resolution mass spectrometry (HRMS) in accordance with a previously published method (7) after the addition of an internal standard.

For both techniques discussed here, isotope-dilution MS was used to calculate analyte levels. In order to compensate for any matrix effects and losses during manipulation of the samples, isotopically labeled analogs of the 10 VOCs were added to all blanks, standards, quality control materials, and unknowns.

Table I. Analytical Parameters for the Determination of Selected VOCs using SPME–GC–MSD

Compound	Isotope composition	Internal standard mass (amu)	Analyte mass (amu)	Confirmation mass (amu)	Dwell time per ion (msec)	Number of dwell repeats
Methylene chloride	$-^{13}C$	85	84	86	15	6
1,1,1-Trichloroethane	$-D_3$	100	97	99	15	3
Benzene	$-^{13}C_6$	84	78	77	15	4
Toluene	$-D_8$	98	91	92	15	4
Tetrachloroethylene	$-^{13}C$	167	166	164	15	4
Ethylbenzene	$-D_{10}$	116	106	91	15	4
m/p-Xylene	$-D_{10}$	116	106	91	15	4
o-Xylene	$-^{13}C_2$	108	106	91	15	4
Styrene	$-D_8$	112	104	103	15	4
1,4-Dichlorobenzene	$-D_4$	152	146	148	100	1

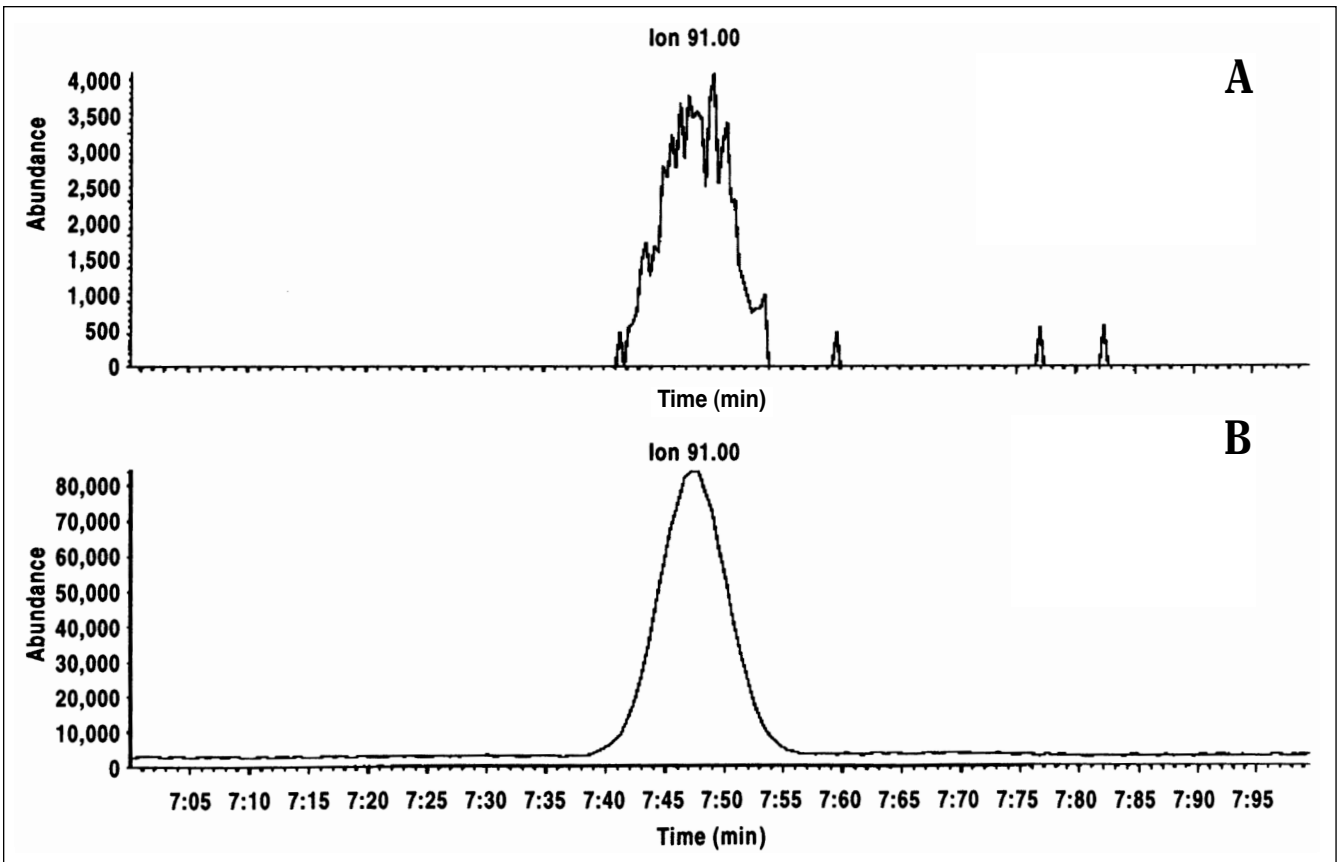

Figure 1. Comparison of the abundances and peak shapes of a limited full-scan acquisition (A) versus an MSIM (B) of the *m/z* 91 ion for toluene.

Instrumental analysis

A Hewlett-Packard (Palo Alto, CA) 5972 series MSD interfaced to an HP 5890 series II GC was used for all the experimental runs. The HP 5890 was equipped with electronic pressure control, cryogenic oven cooling with liquid nitrogen, and a split/splitless injector. A 0.75-mm-i.d. glass inlet (Supelco) replaced the 4.0-mm glass inlet supplied with the GC. A 10-m × 0.20-mm-i.d. (1.2-µm film thickness) VOCOL™ (Supelco) fused-silica capillary column was installed in the GC oven. The front of the column was threaded through a Cryo-Trap model 961 (Scientific Instrument Services, Ringoes, NJ) cryofocusing device that was attached to the bottom of the splitless injector. The Cryo-Trap was cooled with liquid nitrogen to –100°C.

The MS and GC were controlled with the MS ChemStation software supplied with the instrument. In order to maximize the area counts for the peaks generated by the various VOCs being investigated, the ChemStation integrator option was used. The injection of samples was automated by a Varian 8200 CX AutoSampler (Varian Instruments, Sugarland, TX) equipped with the SPME option and a 12-vial carrousel that was mounted directly over the injection port of the HP 5890. A 100-µm polydimethylsiloxane coated fiber assembly (Supelco) was installed on the SPME fiber-holding portion of the autosampler. The fiber was exposed to the headspace above the sample for 10 min. The 10-min sampling time was determined experimentally and found to provide the best combination of sensitivity and sample throughput. The robustness of the SPME fibers can

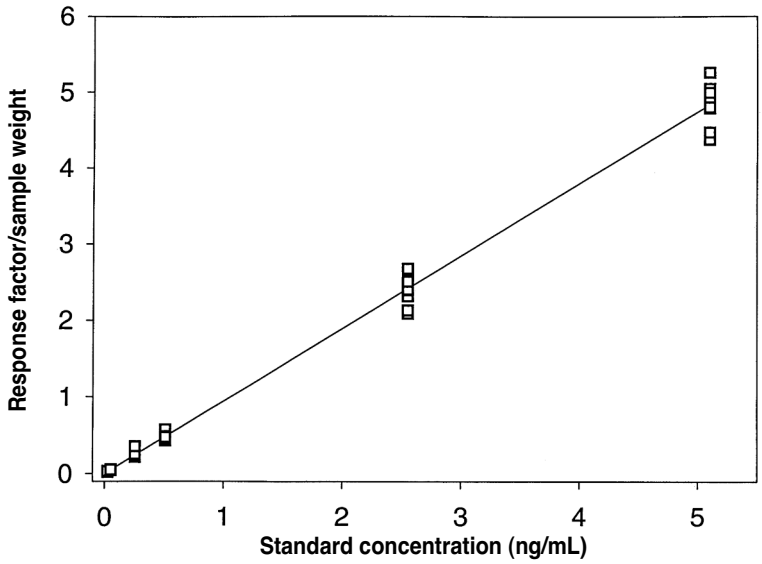

Figure 2. Calibration curve of the relative response divided by the sample weight versus standard concentration for tetrachloroethylene.

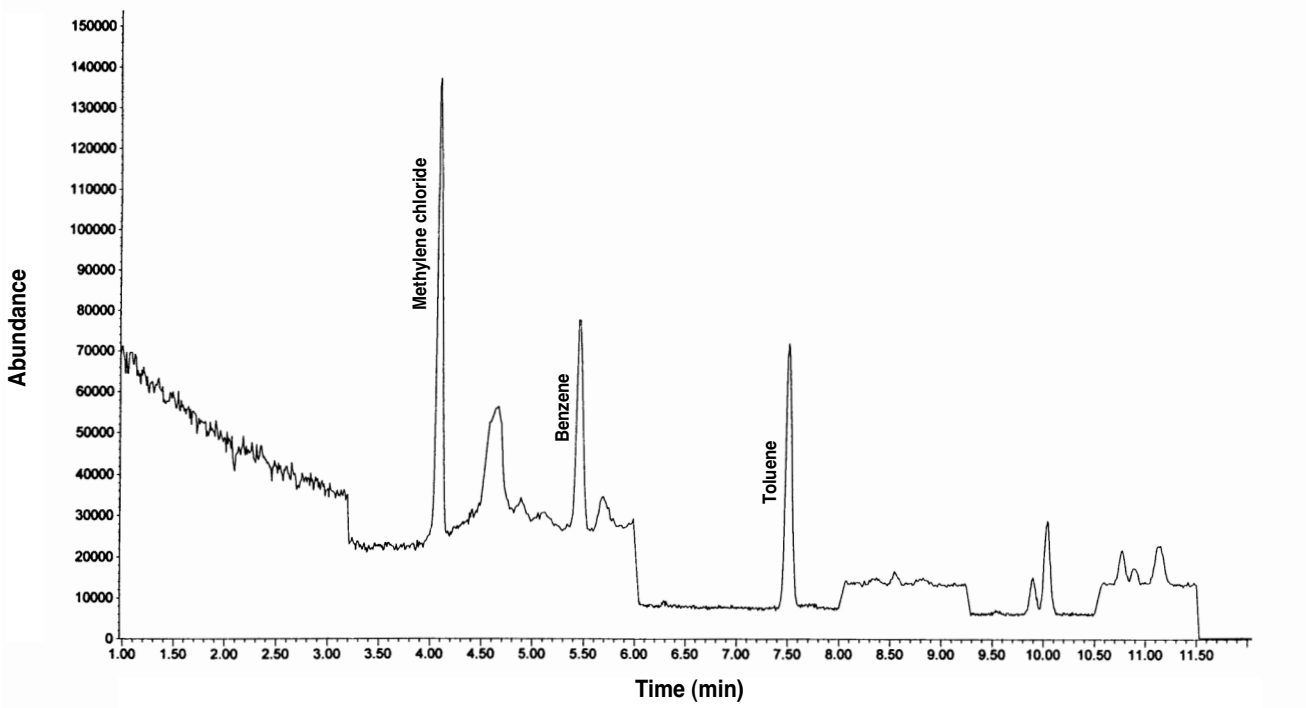

Figure 3. SIM of laboratory air sample showing contamination by methylene chloride, benzene, and toluene.

be seen in the fact that 300 or more samples per fiber were routinely run over a 4-month period. Replacement of the fiber usually occurred because of breakage rather than loss of adsorption capacity. The autosampler was controlled by a stand-alone program supplied by Varian (Varian Star 4.0) and installed on the same computer used to control the MSD and the HP 5890.

The GC injection port was set at 200°C, and the fiber was desorbed for 3 min. The cryofocusing device was at –100°C, and the GC oven temperature was maintained at 20°C during the desorb period. After 3 min, the cryofocusing device was ballistically desorbed to a final temperature of 250°C, and the GC temperature program began to ramp at 6.0°C/min to 110°C. After 1 min, the temperature program ramped at 10°C/min to a final temperature of 180°C and remained at this temperature for 1 min. The total run time was 27 min. The electronic pressure control was set to 1 psi at 20°C in the constant flow mode for the helium carrier gas.

Because whole human blood samples were involved, direct immersion of the fiber in the sample would have resulted in proteins coating the fiber surface and interfering or preventing adsorption of the VOCs onto the fiber coating. Therefore, the headspace above the blood sample was sampled throughout this investigation.

Table II. Standard Concentration Ranges and Detection Limits for Selected VOCs using SPME–GC–MSD

Compound	Standard concentration range (ng/mL)	Calculated detection limit (ng/mL)	Lowest reportable result (ng/mL)	P/T–GC–HRMS detection limit (ng/mL)*
Methylene chloride	0.063–6.3	0.319	0.319	0.080
1,1,1-Trichloroethane	0.025–4.9	0.002	0.025	0.049
Benzene	0.014–2.7	0.046	0.046	0.032
Toluene	0.016–3.2	0.098	0.098	0.088
Tetrachloroethylene	0.026–5.1	0.004	0.026	0.016
Ethylbenzene	0.014–2.8	0.0003	0.014	0.008
m/p-Xylene	0.022–4.4	0.041	0.041	0.010
Styrene	0.009–1.7	0.015	0.015	0.010
1,4-Dichlorobenzene	0.036–7.2	0.005	0.036	0.036

* Taken from Reference 7.

Table III. Recovery and Reproducibility of Selected VOCs in Spiked Human Blood Samples using SPME–GC–MSD

Compound	Spiking level (ng/mL)	Number of experiments	Recovery (%)	Coefficient of variation (%)
Methylene chloride	1.4	8	101	14
	2.8	7	89.3	13
1,1,1-Trichloroethane	1.1	8	124	11
	2.2	7	117	10
Benzene	0.6	8	106	3.1
	1.2	7	103	5.6
Toluene	0.7	4	94.7	2.2
	1.4	7	88.9	15
Tetrachloroethylene	1.1	8	114	5.9
	2.2	7	105	6.0
Ethylbenzene	0.6	8	93.6	6.4
	1.2	7	90.6	5.0
m/p-Xylene	1.0	8	111	12
	2.0	7	104	5.5
o-Xylene	1.0	8	115	6.3
	2.0	7	114	5.0
Styrene	0.4	8	78.8	6.6
	0.8	7	114	5.9
1,4-Dichlorobenzene	1.6	8	99.7	4.8
	3.2	7	93.9	4.6

Results and Discussion

Because of the low levels of VOCs in the blood samples and the lack of agitation or heat to enhance the partitioning of the VOCs between the blood sample and the vial headspace, multiple selected-ion monitoring (MSIM) was required for detecting and quantitating the VOCs. The advantage of this technique over a full-scan acquisition for parts-per-trillion analysis is illustrated in Figure 1. The comparison shown in Figure 1 was generated from a 5-mL blood sample of a person who smokes cigarettes. The blood sample was analyzed using both a full-scan method and MSIM method. Both ion chromatograms are for the *m/z* 91 quantitation ion for toluene. The upper chromatogram came from a limited full-scan acquisition (40–200 amu), and the lower chromatogram is a MSIM acquisition. As can be seen by comparing the two ion chromatograms, the MSIM acquisition gave a 20-fold increase in sensitivity and greatly improved the peak shape. Table I shows the MSIM acquisition parameters and ions monitored for the native and labeled VOCs.

A calibration curve of the relative response divided by the sample weight versus standard concentration for tetrachloroethylene is shown in Figure 2. At least 9 points are presented at each standard concentration. This figure illustrates the linearity of the detector response and the reproducibility of the measurement in water standards. The reproducibility for benzene, toluene, and methylene chloride at low concentrations were significantly poorer than for the other analytes because of uncontrollable sources of contamination within the laboratory air (Figure 3). Particular care was required to minimize these interferences, but even those efforts could not completely eliminate this source of contamination.

The effect of laboratory air contamination on the detection limits of benzene, toluene, and methylene chloride are seen in Table II, which presents detection limits for all of the analytes examined. Detection limits were calculated using 3 times the standard deviation at 0 concentration for multiple analyses of standards (10). This calculation technique combines the instrument detection limit together with the reproducibility of the method itself to get a realistic measure of the ability of the

method to detect low concentration levels. Thus, the effect of variable interference is seen by the higher detection limits for benzene, toluene, and methylene chloride. The lowest reportable value is the higher detection limit or the lowest linear standard concentration.

Table II also lists the limits of detection for the measurement of these VOCs by P/T–GC–HRMS. For all of the VOCs except methylene chloride, the limits of detection of the SPME–GC–MSD and P/T–GC–HRMS methods are comparable. The interference for methylene chloride appears to be significantly more pronounced when SPME is used than when purge-and-trap separation is used. The methylene chloride contamination in the laboratory air probably comes from a number of sources in our laboratory. Methylene chloride is used in maintenance procedures for high-resolution MS and in a number of extraction procedures used in other labs. Because a central heating and air conditioning system is used throughout our laboratory building, fugitive emissions of methylene chloride could be transported to other labs throughout the building.

Recovery studies were conducted at two spiked concentration levels using blood from one individual who was a nonsmoker. To correct for VOCs that might be present in the unspiked blood, a baseline measurement from each of the vacutainers that were used for the recovery experiments was run. Four 10-mL vacutainers of whole human blood were used for each concentration level. Because of the unique feature of SPME that allows multiple sampling from a single vial, all but one sample was run in duplicate.

Because the blood used for all recovery studies came from a single individual, it was anticipated that the background levels for all the VOCs would be fairly constant. However, this was not the case with toluene. The first set of two background blood samples showed an average toluene value of 0.11 ng/mL. The four blood samples associated with these background blood samples and which were spiked at the 0.7-ng/mL level had a 94.7% recovery, as noted in Table III. The second set of two background blood samples showed an average toluene value of 0.17 ng/mL. When the second set of four blood samples was spiked, the recovery was calculated as 152%, suggesting that contamination had occurred in this set of samples. Therefore, these four toluene results were rejected. Table III lists the results of the VOC recovery experiments. The spiking levels varied from 0.4 to 3.2 ng/mL. The percent recoveries ranged from a low of 79% for styrene to a high of 124% for 1,1,1-trichloroethane, with a mean for all of the analytes of 101%. The coefficient of variation for all VOCs was 15% or less, with most around 7%.

As mentioned previously, a unique feature of SPME is that it allows the analyst to make multiple injections from a single vial and reproducibly measure the analyte concentration. Table IV shows (for each individual VOC) the decrease in both the area of the label signal and the calculated concentration between the first and second SPME analysis of the same vial containing water standards. The elapsed time between the first and second injection was 1 h. The results show that even though there are appreciable decreases in the determined chromatogram peak areas, there are no real differences between the calculated concentrations determined from the first and second injections from the same SPME vial for the majority of VOCs. Even in the case of 1,4-dichlorobenzene, where the peak area for the labeled analog internal standard had decreased 33%, the mean

Table IV. Percent Decrease in the Area of the Labeled Ion and Calculated Concentration for Selected VOCs in Water Standards Between the First and Second Analysis of the Same Vial Using SPME–GC–MSD (*n* = 6)

	Area of labeled ion		Calculated concentration	
	Mean decrease (%) ± standard error	Prob > |T|	Mean decrease (%) ± standard error	Prob > |T|
Methylene chloride	4.3 ± 3.3	0.024	0.87 ± 5.2	0.70
1,1,1-Trichloroethane	5.6 ± 16.2	0.44	–0.15 ± 6.0	0.95
Benzene	8.6 ± 1.3	0.0001	–1.1 ± 3.8	0.52
Toluene	16.5 ± 0.66	0.0001	–0.27 ± 0.96	0.53
Tetrachloroethylene	25.0 ± 1.1	0.0001	0.14 ± 1.4	0.82
Ethylbenzene	28.0 ± 0.88	0.0001	1.9 ± 1.2	0.01
m/p-Xylene	30.3 ± 0.57	0.0001	0.09 ± 0.74	0.78
o-Xylene	28.8 ± 1.4	0.0001	–0.26 ± 1.6	0.70
Styrene	25.8 ± 1.3	0.0001	0.28 ± 4.0	0.87
1,4-Dichlorobenzene	32.6 ± 1.1	0.0001	0.97 ± 0.25	0.0002

Table V. Percent Decrease in the Area of the Labeled Ion and Calculated Concentration for Selected VOCs in Blood Between the First and Second Analysis of the Same Vial Using SPME–GC–MSD*

	Area of labeled ion (*n* = 15)		Calculated concentration (*n* = 7)	
	Mean decrease (%) ± standard error	Prob > |T|	Mean decrease (%) ± standard error	Prob > |T|
Methylene chloride	3.7 ± 1.2	0.009	4.4 ± 3.7	0.28
1,1,1-Trichloroethane	8.5 ± 1.9	0.0006	–0.90 ± 1.9	0.65
Benzene	8.8 ± 0.77	0.0001	–0.46 ± 1.2	0.71
Toluene	12.2 ± 0.72	0.0001	–0.92 ± 0.33	0.031
Tetrachloroethylene	16.5 ± 1.2	0.0001	–0.47 ± 0.74	0.55
Ethylbenzene	12.8 ± 0.79	0.0001	0.79 ± 0.91	0.42
m/p-Xylene	11.0 ± 0.84	0.0001	1.0 ± 0.82	0.27
o-Xylene	13.7 ± 1.2	0.0001	–1.5 ± 1.4	0.32
Styrene	11.4 ± 1.2	0.0001	–3.0 ± 3.1	0.37
1,4-Dichlorobenzene	7.5 ± 0.62	0.0001	–0.81 ± 0.17	0.004

* Mean concentrations are from spiked samples.

decrease in the concentration value was only 1%.

Table V shows the results of multiple injections from a spiked blood sample. Even though the labeled peak areas decreased significantly, the calculated concentration of most of the native VOCs did not show a significant change. These results illustrate a unique feature of the SPME method that is not shared by purge-and-trap. Analysts using SPME can make multiple injections from a single blood sample, thereby increasing the precision of the VOC measurements, and in the event of an autosampler or instrument failure, they can resample the blood samples and obtain valid data.

The analytes in Tables IV and V are listed in order of increasing retention time. A comparison of the decrease in labeled peak area in Tables IV and V shows that for both water standards and whole blood, the extraction efficiency increases as retention time increases, but to a different extent. For methylene chloride, the extraction efficiency in water standards is about 4% for both matrices, but for the substituted aromatics, the extraction efficiency is approximately 28% in water but only 12% in whole blood. This increased extraction efficiency in water is likely due to the lipid solubility of these latter compounds changing the blood–air partitioning. This finding is important for the analysis of VOCs, because if a labeled isotope is not used, the analyst must choose an internal standard with properties similar to those of the compounds for which concentrations are being reported.

Conclusion

Automated headspace SPME–GC–MSD is a viable alternative to P/T–GC–HRMS for the analysis of human whole blood samples. The limits of detection for the 10 VOCs tested were below 50 ppt (pg/mL) with the exception of methylene chloride and toluene. Both of these exceptions are attributable to contamination from the laboratory air. Because of the low limits of detection inherent in this method and its automation capability, this method is ideal for screening large numbers of nonoccupationally exposed individuals for possible VOC exposure. The simplicity of the MSD design and operation, the robustness of the SPME fiber, the minimal sample preparation, and the ease of automation makes VOC analysis of blood samples in the low part-per-trillion range available to both researchers and analytical laboratories that in the past could not afford a high-resolution MS.

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