TECHNICAL NOTE

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The Comparison of Toluene Determination Between Headspace-Solid Phase Microextraction and Headspace Methods in Glue-Sniffer's Blood and Urine Samples

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ABSTRACT: An accurate and simple method was developed to determine the level of toluene in urine and blood quantitatively by using the gas chromatography/mass spectrometry (GC/MS) with headspace—solid phase microextraction (HS-SPME) technique. An assembly of SPME with a replaceable extraction fiber, coated with 100 μm polydimethylsiloxane, was used. The detection limit of toluene in blood and urine with HS-SPME technique was 10 times higher than that with headspace (HS) technique. To compare the HS-SPME with HS technique for the determination of toluene in biological fluids, blood and urine samples from glue sniffers were analyzed by both methods. The level of toluene by the two techniques was highly correlated: the correlation coefficient (r^2) between the two sets of values were 0.98 and 0.96 in urine and blood, respectively.

KEYWORDS: forensic science, toluene, inhalant abuse, glue-sniffing, headspace-solid phase microextraction

Toluene has been widely used as industrial solvents as well as chemical intermediate in various chemical engineering processes. Especially, toluene is present in numerous paints, paint thinners, glues, and other products likely to be found in the household. However, it is a narcotic, and acute effect of toluene is similar to alcoholic intoxication in the sense of stimulating first and depressing a central nervous system later. Chronic abuse of toluene can lead to hepatomegaly and nephrotoxic symptoms (1–3).

Unfortunately, toluene has been frequently abused for its intoxicating effects by teenagers or adults who inhale the vapors of glue or varnish solvents intentionally (4,5).

In Korea, glues and varnishes are the most widely used inhalants, and forensic cases arise that a crime is committed while an individual is intoxicated after glue sniffing. In every year, about 10,000 teenagers have been arrested or about 20 persons died for its abuse in recent years. Therefore, it is very important to determine toluene and its metabolites in biological fluid samples for forensic purpose (6,7). However, Tawai T et al. reported that toluene itself in urine is the best marker of exposure to toluene vapor (8).

Analytical methods for qualitative and quantitative analysis of toluene in biological fluids are relatively simple to set up with the usual use of gas chromatography with headspace (HS) technique (9–11). However, the solid phase microextraction (SPME) technique has recently been introduced in the detection of volatile solvents in aqueous samples or solid matrices. Compared with direct SPME sampling from aqueous phase, the headspace SPME technique can be used to sample volatile organic compounds in very complex matrices such as oily or greasy water and human blood or urine. Especially, the HS-SPME technique is very efficient for analyzing volatile organic compounds such as toluene or benzene because they usually have large Henry's constant, K_H, thus a large gas/aqueous partition coefficient, K_2 (12–16).

This study analyzed toluene in blood or urine by using the SPME method combined with HS. The proposed HS-SPME method was successfully applied to the determination of toluene in glue sniffer's blood or urine, with results comparable to HS technique.

Materials and Methods

Materials

Toluene (5000 μg/mL in methanol) was purchased from Sigma Chemical Co. 1,4-Dioxane and *iso*-butanol as internal standards were obtained from Aldrich. An assembly of SPME with a replaceable extraction fiber, coated with 100 μm polydimethylsiloxane, was purchased from Supelco Inc. Whole blood which was obtained from the Red Cross Blood Bank, and urine which was obtained from a health volunteer were used after confirmation of their freedom from volatile solvents and used as control blood and urine.

Blood or urine samples were collected from autopsies or those who were suspected of glue sniffing.

Instrumentation

Gas Chromatography—The gas chromatograph (GC) used was a Hewlett Packard 5890 series II equipped with a flame ionization

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detector (FID) and a HP-7694 automatic headspace sampler. The capillary column of HP-INNOwax (INNO phase™ bondable PEG, 30 m × 0.25 mm (ID) × 0.25 μm (film thickness), Hewlett Packard) was used. The column temperature was programmed as a manner of the follow: the temperature was set for 60°C initially and maintained for 6 min, then increased to 140°C at the rate of 10°C/min and kept for 3 min. The injection port and detector were set at 250°C and 240°C, respectively. The flow rate of carrier helium gas was 1 mL/min and split ratio was 30:1.

Gas Chromatography/Mass Spectrometry—A Hewlett Packard gas chromatograph/mass selective spectrometer (GC/MSD, 6890 GC, and 5972 MSD) was used. The capillary column of HP-5MS (5% diphenyl-95% dimethylsiloxane copolymer, 30 m × 0.25 mm (ID) × 0.25 μm (film thickness), Hewlett Packard) was used. The temperatures of the column, injection port, and ion source were set at 60°C, 250°C, and 280°C, respectively. The flow rate of carrier helium gas was 1.0 mL/min. Target ions used by selected ion monitoring (SIM) were m/z 91, 65 for toluene and m/z 88, 58 for dioxane as an internal standard.

Preparation for Analysis of Toluene with HS Method and HS-SPME Method

HS Method—Headspace autosampler vial (12 mL volume) was filled with 2.0 mL sodium citrate solution (4.8 g citric acid, 13.2 g sodium citrate, and 14.7 g dextrose/L), 1.0 mL of blood or urine and 50 μL of internal standard (0.5% *iso*-butanol), and was sealed rapidly with rubber septum and an aluminum cap (6). Sample vials were heated and shaken at 60°C in headspace analyzer; valve and transfer line temperature were 140°C. Equilibrium time was 20 min and the injection sample size was 1.0 mL.

HS-SPME Method—1.0 mL of blood or urine sample, 100 μL of 0.1%-dioxane as an internal standard and 2 mL of 0.1% sodium azide solution were put into a vial (12 mL volume), and sealed rapidly with rubber septum and an aluminum cap. The vial was heated at 60°C for 20 min in a shaking water bath. The needle of a solid phase microextraction device was passed through the septum, and the extraction fiber in the needle was exposed for 1 min in the headspace of the vial by sonication in a sonicator at 60°C. The needle was removed from the vial and inserted into the injection port at 250°C of GC/MSD.

Optimal Condition of HS-SPME Method—In order to give an optimal condition, the effect of heating time, adsorption time, and desorption time were determined. The standard sample containing 1.0 μg/mL of toluene in urine was prepared and analyzed (n = 3).

To determine a heating time, the vial was heated and shaken at 60°C for three different lengths of time (10, 20, and 30 min). The fiber was then absorbed for 1 min and analyzed.

The vial was heated and shaken at 60°C for 20 min to determine the adsorption time. The fiber was adsorbed for five different lengths of time (10, 30, 60, 180, and 300 s) with sonication in a sonicator at 60°C.

In order to determine desorption time, the standard sample containing 10.0 μg/mL of toluene was prepared following the described procedure. The fiber was inserted into the injection port for 1 min at 250°C of GC/MSD and analyzed. After analysis of toluene, the fiber was inserted into the injection port at 250°C for 1 min again, we confirmed whether there is toluene peak or not.

Calibration Curves—In order to determine calibration curves, standard blood and urine samples containing toluene at concentrations ranging from 0.01 to 10.0 μg/mL were prepared and analyzed by using the described procedure.

To examine the precision with HS-SPME, control blood and urine samples added to levels of 0.1, 0.5, and 5.0 μg/mL of toluene were analyzed by using the described procedure by the six replicates.

Results and Discussion

This HS-SPME is a very useful technique for analysis of toluene in biological fluid samples because toluene is volatile compound which has large gas/aqueous partition coefficient, and SPME is simple, fast, sensitive, and solvent-free. For equilibrium and well-agitated aqueous phase (urine or blood phase), sampling vial was shaken (rpm:85) in the shaking water bath at 60°C for 20 min and was sonicated for 1 min instead of technique with stirred aqueous phase. In case we have lots of samples, it is troublesome to use stirrer bar for well-agitation because a stirrer bar is not disposable. As shown in Fig. 1, however, this procedure is very simple and comfortable.

Heating Time—As shown in Fig. 2, each peak area was almost the same, when the vial was heated for 10, 20, or 30 min. Therefore we decided to heat and shake the sample vial for 20 min at 60°C to ensure complete vapor equilibrium.

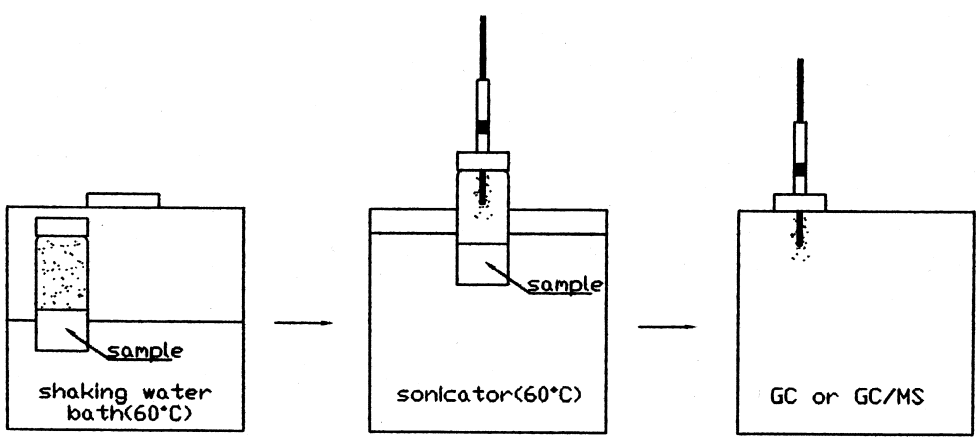

FIG. 1—*Schematic of Headspace-Solid Phase Microextraction (HS-SPME) method.*

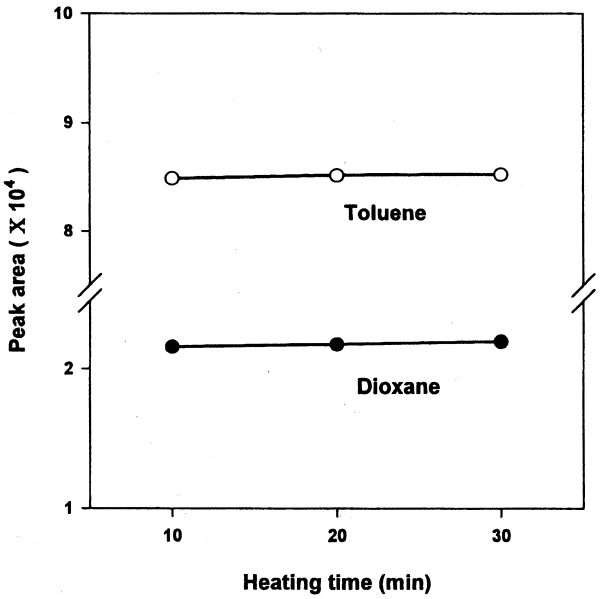

FIG. 2—*Correlation of the adsorbed amount of toluene and dioxane with heating time at 60°C; adsorption time: 1 min, desorption time: 1 min.*

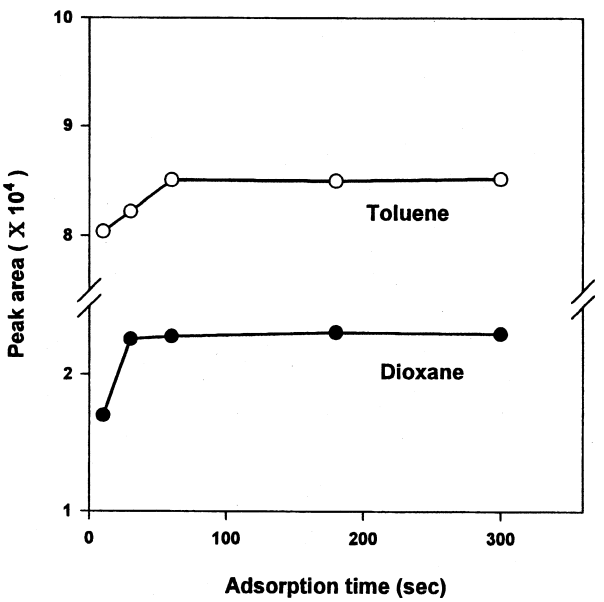

FIG. 3—*Correlation of the adsorbed amount of toluene and dioxane with the exposed time; heating time: 20 min, desorption time: 1 min.*

Adsorption Time—The times for exposing the fiber in the headspace of the sample vial were examined. Figure 3 showed the time profile of toluene and dioxane as an internal standard. In case of toluene, the amount of peak area was increased up to 1 min. In case of dioxane, it was increased up to 30 s. However, the amount of toluene and dioxane peak areas are almost the same when the exposing time was from 1 min to 5 min and from 30 s to 5 min, respectively. Therefore we decided the optimal adsorption time was 1 min.

Desorption Time—As shown in Fig. 4, there is no toluene and dioxane peaks after desorption for 1 min at 250°C. Therefore we decided the optimal desorption time was 1 min.

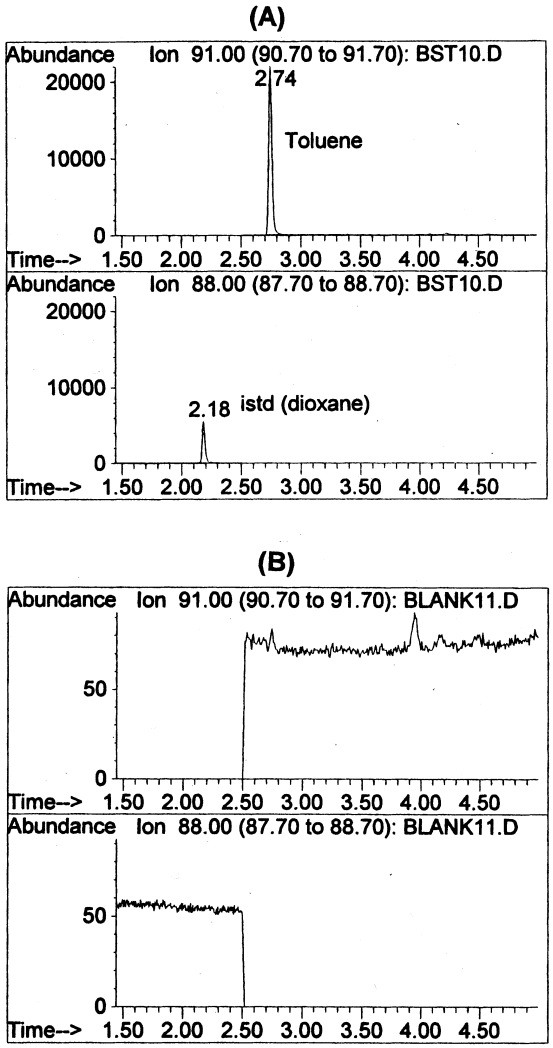

FIG. 4—*Selected ion chromatograms of toluene and dioxane. (A) Standard blood sample (1.0 μg/mL toluene) was analyzed by HS-SPME method. (B) After analysis by HS-SPME, fiber coating in the needle was analyzed again.*

Calibration Curves and Detection Limit—There was a linear relationship at concentration ranges from 0.01 to 10.0 μg/mL for toluene with both methods. As shown in Fig. 5, the correlation coefficients of the standard calibration curves were all 0.99 in both methods. In HS-SPME method, the precision (rsd) were 0.56 ~ 5.66% and 2.19 ~ 5.42% at the various levels in urine and blood, respectively. In static HS method, they were 2.21 ~ 4.57% and 1.33 ~ 4.92% at the various levels in urine and blood, respectively. The detection limit of toluene by this procedure with HS-SPME technique was 1.0 ng/mL, and it was 0.01 μg/mL by HS technique in blood and urine. This detection limit was 10 times higher than that of HS technique. However, we determined that the cut off level of toluene was 0.1 μg/mL as positive meaning.

Comparison of Toluene Concentration Between HS-SPME Method and HS Method—In order to compare the HS-SPME method with HS method (NIOSH method), the determination of toluene in biological fluids, blood and urine samples from glue sniffers were analyzed by both methods using the described procedure (Fig. 6). As shown in Fig. 7, the level of toluene by the two

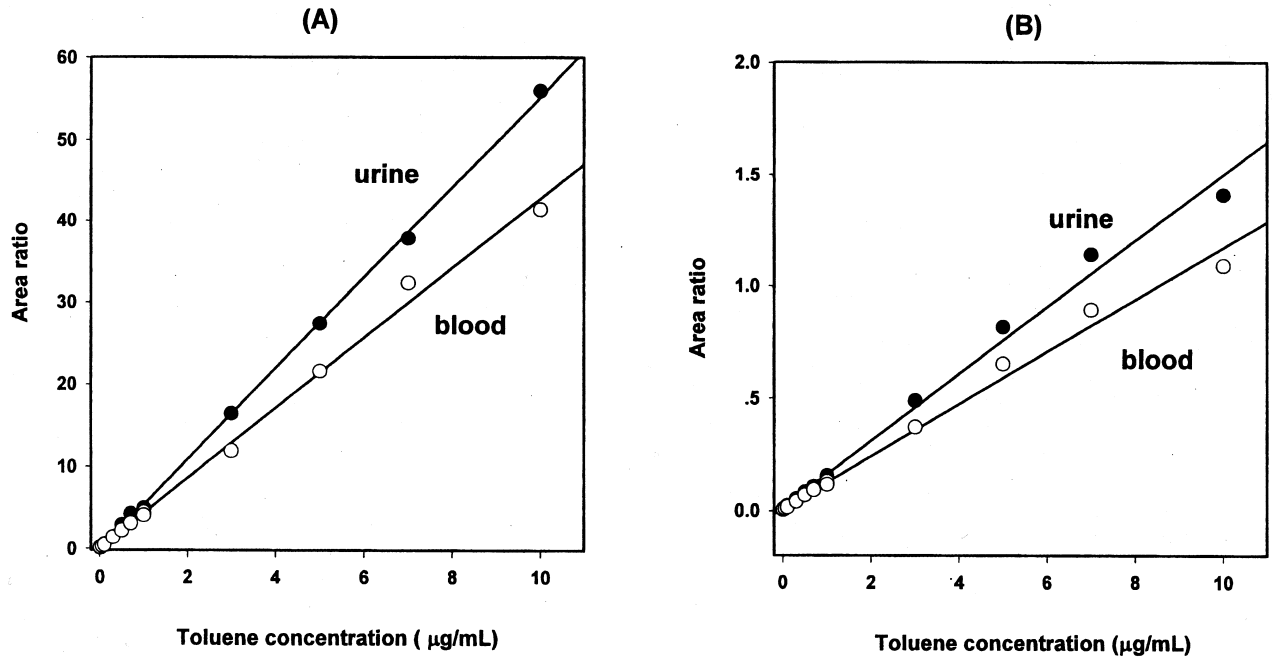

FIG. 5—*Toluene standard calibration curve. (A) With HS-SPME method. (B) With automated HS method.*

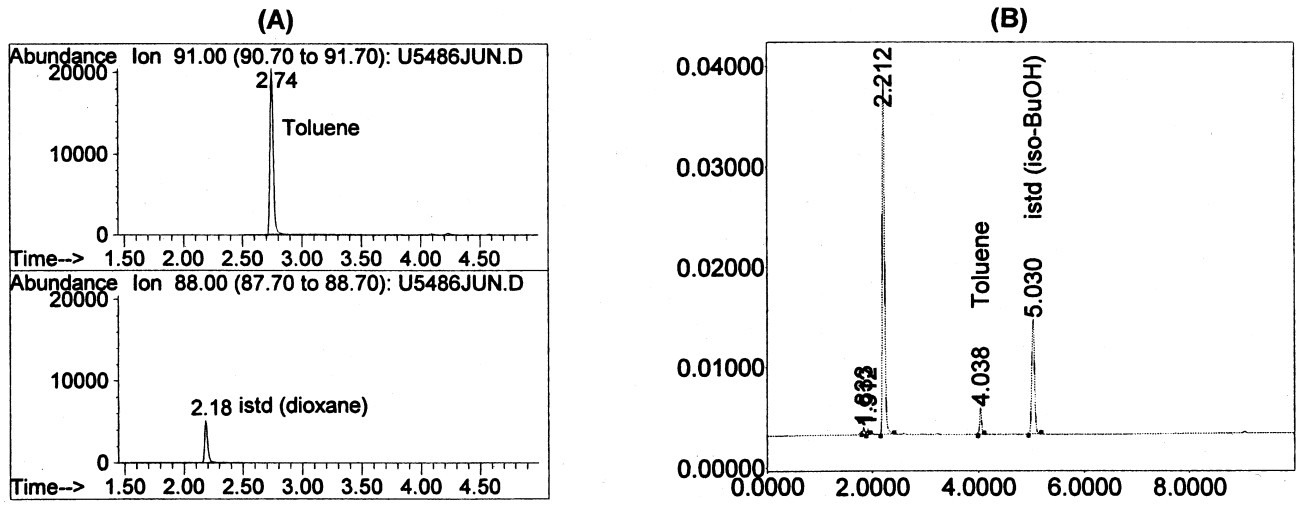

FIG. 6—*Total ion chromatogram of toluene in glue-sniffer's urine. (A) With HS-SPME method. (B) With automated HS method.*

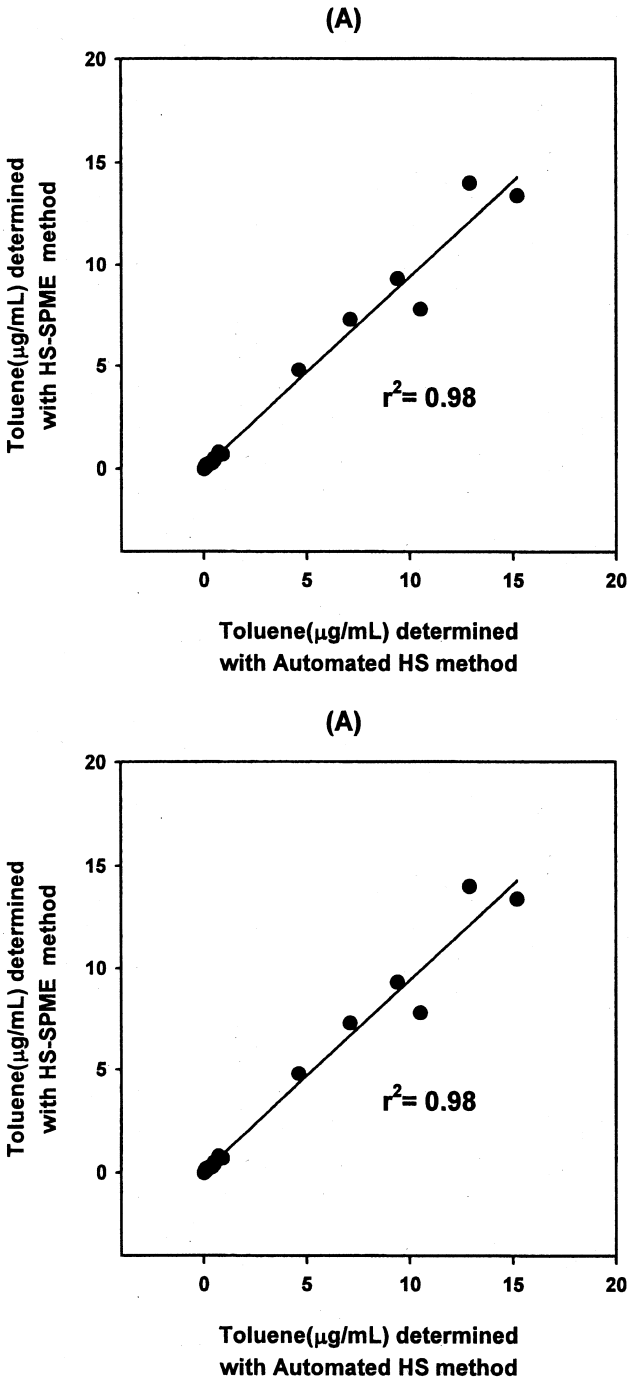

FIG. 7—*Linear regression for toluene in urine and whole blood samples with HS-SPME versus automated HS method. (A) In case of urine samples. (B) In case of whole blood samples.*

techniques showed high correlation, or the correlation coefficients (r^2) between the two sets of values were 0.98 and 0.96 in urine and blood, respectively. It was shown that correlation coefficient in urine is better than that in blood because of blood matrix effect. In glue sniffers blood and urine samples (urine: 25 samples, blood: 49 samples), the range of toluene concentration as positive meaning were 0.1 ~ 14.0 μg/mL and 0.1 ~ 17.6 μg/mL in urine and blood, respectively (Table 1).

TABLE 1—*Comparison of toluene concentration in urine and whole blood samples from glue sniffers by HS-SPME and HS method.*

	Toluene (μg/mL)			Toluene (μg/mL)	
Sample	HS-SPME	HS	Sample	HS-SPME	HS
Urine	0.3	0.4	Blood	0.4	0.5
Urine	0.3	0.3	Blood	12.6	9.8
Urine	9.3	9.4	Blood	2.5	2.5
Urine	0.4	0.5	Blood	3.2	3.4
Urine	0.2	0.1	Blood	0.2	0.3
Urine	0.2	0.2	Blood	4.7	4.7
Urine	0.2	0.2	Blood	0.9	0.8
Urine	0.2	0.2	Blood	1.4	1.2
Urine	0.6	0.6	Blood	0.4	0.2
Urine	0.3	0.3	Blood	13.9	12.4
Urine	0.2	0.2	Blood	1.7	1.4
Urine	Neg	N.D.	Blood	1.1	0.9
Urine	7.8	10.5	Blood	1.1	1.5
Urine	13.4	15.2	Blood	0.8	0.8
Urine	0.7	0.8	Blood	1.7	2.9
Urine	0.7	0.9	Blood	2.1	1.5
Urine	Neg	Neg	Blood	1.3	1.3
Urine	0.1	0.1	Blood	4.9	4.0
Urine	0.2	0.2	Blood	1.0	1.2
Urine	Neg	Neg	Blood	5.4	5.4
Urine	4.8	4.6	Blood	4.7	4.9
Urine	7.3	7.1	Blood	1.9	1.5
Urine	14.0	12.9	Blood	17.6	18.8
Urine	0.5	0.5	Blood	Neg	N.D.
Urine	0.8	0.7	Blood	2.2	2.3
Blood	1.0	1.3	Blood	3.2	2.6
Blood	2.7	1.6	Blood	4.0	4.0
Blood	0.4	0.5	Blood	1.8	1.8
Blood	12.9	11.8	Blood	3.4	2.5
Blood	6.4	4.8	Blood	7.2	8.6
Blood	3.9	2.9	Blood	3.0	2.1
Blood	0.7	0.6	Blood	3.4	2.9
Blood	0.2	0.2	Blood	4.4	3.2
Blood	1.4	1.0	Blood	4.1	5.9
Blood	1.8	1.6	Blood	0.4	0.4
Blood	0.2	0.3	Blood	N.D.	N.D.
Blood	0.4	0.4	Blood	1.8	1.6

N.D.: not detected. Neg: negative. Toluene cut off value: 0.1 μg/mL.

Conclusion

The concentrations of toluene with HS-SPME method in the blood and urine samples were similar to results obtained with HS technique. The HS-SPME technique was showed to be satisfactory as a practical technique for the determination of toluene in blood and urine. The minimum detection limit of toluene was improved. Especially, it is possible to analyze toluene in blood or urine by using GC/MSD with a non-polar capillary column without interference of moisture.

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