Evaluation of matrix effects in the analysis of volatile organic compounds in whole blood with solid-phase microextraction

Monica Alonso[1], Mar Castellanos[2,3], Juan M. Sanchez[1,3,*]

[1] Department of Chemistry, University of Girona, Campus Montilivi s/n, 17071- Girona, Spain
[2] Department of Neurology, Dr. Josep Trueta University Hospital, 17007- Girona, Spain
[3] Girona Biomedical Research Institute (IdIBGi), 17007- Girona, Spain

* Corresponding author:
Juan M. Sanchez
Department of Chemistry, University of Girona
Campus Montilivi s/n, 17071-Girona (Spain)
Phone: (+34) 972418276
FAX: (+34) 972418150
E-mail: juanma.sanchez@udg.edu

Non-standard abbreviations:

CAR/PDMS – Carboxen/polydimethylsiloxane
HS – headspace
IS – internal standard
NT – needle trap
PT – purge and trap
SPME - solid phase microextraction
WHO – World Health Organization
VOC – volatile organic compound

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Abstract

The complexity and matrix variability of biological samples requires an accurate evaluation of matrix effects. The dilution of the biological sample is the simplest way to reduce or avoid the matrix effect. In the present study, a set of volatile organic compounds with different volatilities was used to assess the effect of the dilution of blood samples on the extraction efficiency by headspace solid-phase microextraction. It was found that there was a significant matrix effect but that this effect differs significantly depending on the volatility of the compound. A 1:2 (blood/water) dilution was enough to allow quantitative recoveries of those compounds with boiling points <100ºC. For compounds with boiling points between 100–150ºC, the matrix effect was stronger and a 1:5 dilution was required. The dilution of blood samples proved to be inefficient for quantitative recovery of compounds with boiling points >150ºC. A 1:5 dilution of the sample allowed detection limits in the range of $ng \cdot L^{-1}$ to be obtained. This was sufficient to detect the main volatile compounds present in blood and contamination after exposure.

1. Introduction

The general public is continuously exposed to many different volatile organic compounds (VOCs) as they are encountered in the workplace, in daily routines, widely-used consumer products, and are ubiquitous in both outdoor and indoor air. It emerged in epidemiological studies published in the 1990s that there are significant associations between the daily average concentrations of pollutants in air and certain adverse health effects [1]. The World Health Organization (WHO) believes there is little doubt that exposure to indoor air pollution in developing countries is a major threat to public health [2]. Given this situation, there is an increasing interest in being able to determine VOCs in biological fluids at sub-trace levels.

Low concentrations ($\mu g \cdot L^{-1}$ to $ng \cdot L^{-1}$ levels) of many different VOCs are present in the blood of non-exposed healthy people [3-6]. Accurately measuring such low levels in human biological fluids in a reproducible manner presents a complex analytical problem that requires special techniques and great care [7]. Static headspace (HS) analysis is the routine technique for the determination of alcohols (mainly ethanol and methanol) in biological fluids [8-13]. However, this technique has the disadvantage of having high limits of detection (LODs in the $mg \cdot L^{-1}$ level), which are sufficient for the analysis of ethanol and other VOCs in blood just after a large exposure but are insufficient for the evaluation of these compounds in people who have not been submitted to large-scale or direct sources of exposure. The use of HS-GC with large volume collection (up to 5 mL) from the headspace [14-16], with cryogenic oven trapping [17,18] and cryo-focusing [19] has helped to improve sensitivity. However, very large injection volumes can only be applied if non-MS detection is used [15,17-19]. MS detectors have been described as automatically shutting down due to the impaired degree of vacuum of the ionization chamber when injection volumes >1.0 mL are introduced in the GC system [14,16]. Recently, post-column switching has been proposed to allow volumes of up to 5 mL in a GC–MS system [16]. Unfortunately, this system requires complex modification of the instrumentation that is beyond the reach of many conventional laboratories.

Active HS (purge-and-trap, PT) followed by GC–MS is probably the most sensitive technique for the analysis of VOCs from an aqueous solution. When this concentration technique has been applied to the analysis of blood samples, LODs in the $ng \cdot L^{-1}$ level have been obtained [3,4,20-22]. PT has some important technical limitations in the analysis of

blood as it causes serious foaming and clogging of the gas flow routes. The use of an antifoaming agent, added at a level that is high enough as to prevent foaming, is essential in the PT analysis of blood samples [7] but can lead to sample contamination unless the antifoaming agent is heated under vacuum [3,23]. Moreover, the method is not recommended for large epidemiologic studies due to poor throughput, relative imprecision and high cost [24].

Solid-phase microextraction (SPME) is a solvent-free extraction technique that includes single preparation and fast analysis of multiple substances using minimal sample volume. This technique has great potential for use in clinical medicine [25]. HS-SPME followed by GC–MS is currently the most common method for the analysis of VOCs in blood samples and LODs at $ng \cdot L^{-1}$ levels are obtained without foaming problems [5,12,24,26-37]. SPME is highly efficient in the determination of organic analytes in relatively simple aqueous matrices such as natural waters and wastewaters in both immersion and HS sampling mode. In more complex matrices, such as body fluids, the fiber can be fouled by protein molecules and changes in the surface area and nature of the SPME stationary phase can be produced [38]. Therefore, direct immersion SPME becomes ineffective in such matrices. HS-SPME sampling partially overcomes such problems but it still has significant matrix effects.

It is known that all HS sampling methodologies are heavily dependent on the sample matrix and so results can vary significantly [10,11,32,33,39,40]. Blood has a strong matrix effect as it consists of complicated components such as proteins, lipids, saccharides, and salts, and its composition varies significantly from one person to another [10,39].

Absolute recoveries usually obtained with SPME from biological matrices are much lower than those obtained from aqueous solutions [30,32,33,41]. Therefore, this matrix effect must be compensated for in order to obtain reliable quantitative results. A well-established and accepted method in forensic medicine for this is to use an internal standard (IS) calibration method [10-13], although it has been found that the use of an IS in itself does not generally eliminate the matrix effect and systematic errors may still occur [10,42]. The selection of the IS is crucial to obtain quantitative results. In order to compensate the matrix effects, the IS must be characterized by a chemical structure and chromatographic retention that is as similar as possible to those of the target analyte. The use of isotopically labeled ISs is the most powerful strategy to improve the accuracy of quantification [43]. To simplify experimental

procedures, many studies only use one or two ISs, depending on the number of target compounds [12,28,35,37] but only those studies with a very limited number of target analytes have obtained quantitative recoveries using this procedure [28]. In the case of multicomponent analysis, it is necessary to use one isotope-labeled standard for each individual compound in order to obtain quantitative recoveries [24,34,36]. However, this procedure is expensive and not all compounds have a labeled standard commercially available.

The dilution of blood with water is the simplest method to reduce matrix effects as it minimizes the effect of proteins and reduces the viscosity, which increases the diffusion coefficient so allowing greater extraction efficiency [39,44]. This procedure is effective when matrix effects decrease at a rate that is higher than the analyte response as the sample is diluted. It is not useful when the dilution of the samples leads to levels of the target compounds that are below the LODs of the method.

In this study, we have evaluated the matrix effects of blood samples for VOC analysis by SPME. Different dilution ratios have been evaluated to determine their effect on the extraction efficiency of a group of VOCs with different volatilities that are commonly detected in blood samples at sub-trace levels.

2. Materials and methods

2.1. Materials

HS vials and Teflon/silicone septum and caps were purchased from Fisher Scientific España (Madrid, Spain). Reagents (purity >97%, Table 1) were supplied by Sigma–Aldrich (Steinheim, Germany). Stock solutions were freshly prepared daily by spiking milli-Q water (Millipore Iberica, Barcelona, Spain) with 50 μL of a methanolic solution containing the compounds at 320–590 $mg \cdot L^{-1}$. These solutions were transferred to HS vials, which were filled to avoid any remaining headspace. The vials were then closed and stored at 4ºC. Working solutions were prepared by the appropriate dilution of the stock solution in milli-Q water. In order to prevent VOC losses during the preparation of the solutions and samples, glass syringes (Hamilton Bonaduz, Switzerland) were used for sample transfer avoiding the formation of any gas space in the syringe.

2.2. SPME procedure

SPME experiments were performed with a manual fiber holder (Supelco, Bellefonte, PA, USA). A fiber coated with 75 μm Carboxen/polydimethylsiloxane (CAR/PDMS) was used (Supelco). The fiber was previously conditioned following the manufacturer's instructions to stabilize the solid phase and remove contaminants. The extracted VOCs were desorbed in the injection port of the GC in splitless mode for 1 min using a 0.8 mm i.d. liner. The fiber was left in the injector for a further 10 min to ensure that other less volatile compounds were all completely removed from it. Carry-over experiments confirmed that the fiber had been successfully cleaned.

0.5 mL blood samples were introduced in 20 mL crimp-cap HS vials. 2.5 mL of water was then added to the vial to dilute the samples to compensate for matrix effects. A weighed amount of NaCl was added to the mixture to obtain 0.16 $g \cdot mL^{-1}$ content inside the vial. The vial was then closed, stirred and heated at 30±1ºC for 1 min in a dry heating block (FB15101, Fisher Scientific, Loughborough, UK). The fiber was then exposed to the HS phase for 30 min at 30ºC.

2.3. GC–MS analysis

Component separation was achieved by the use of a 30 m long TR-Meta.VOC column with an 0.25 mm id and 1.5 μm film thickness (Teknokroma, Barcelona, Spain). A Focus GC (Thermo Scientific, Waltham, MA, USA) with a mass spectrometer detector (DSQ II, Thermo Scientific) was used.

The injector (desorption) temperature was maintained at 280ºC. Injection was performed in the splitless mode and the split valve was opened after 1 min. The oven temperature program was 40ºC for 4 min, then ramped at $5ºC \cdot min^{-1}$ to 150ºC, followed by a ramp at $10ºC \cdot min^{-1}$ to 225ºC and held for 2 min. Helium carrier gas was used with a constant inlet flow of 0.8 $mL \cdot min^{-1}$ after purification for water vapor, hydrocarbons and oxygen. MS analyses were carried out in full-scan mode, with a scan range of 40–250 uma, electron impact ionization was applied at 70 eV, and the transfer line was maintained at 230ºC. Chromatographic data was acquired by means of Xcalibur software (v. 1.4, Thermo Electron).

2.4. Blood samples

Thirty-two whole blood samples were collected by venipuncture in vacutainers containing EDTA as the anticoagulant (BD Vacutainer, Trenton, NJ, USA) from volunteers attending the hospital for regular analyses. All volunteers were over 60 years old and no current exposure to volatile substances was declared other than smoking habits. Whole blood samples were refrigerated at 4ºC within 10 min of collection [3,7,31] and all measurements were performed within two weeks [3,31].

3. Results and discussion

3.1. Fiber selection

The CAR/PDMS coating was selected as it is the most common fiber coating used for the determination of VOCs at sub-trace levels in blood samples [24,28,31,34,35,37,45]. Moreover, comparison of different fiber coatings has shown that porous polymer coatings gave much higher extraction efficiencies for VOCs from blood samples than absorption-type coatings [26].

3.2. Salting-out effect

It is essential to compensate for the salting-out effect in biological fluids where the ionic strength, which influences the solubility of compounds in the liquid phase, may vary considerably from one sample to another [46]. It is recommended that salt be added at levels close to saturation in the analysis of VOCs in biological fluids to compensate for any ionic-strength effect and to improve the partition of volatile compounds towards the gas phase [26-28,37,47]. However, even with the addition of a salting-out agent, the partition of some compounds is still influenced by the type of biological specimen [48].

In this study, we added sodium chloride to the samples in order to reach a salt content of 0.16 $g \cdot mL^{-1}$. This amount of salt allowed values close to saturation to be reached for all the blood samples evaluated. Some samples became oversaturated when higher levels of salt were used and lower values were discarded as they did not ensure effective compensation of matrix ionic-strength differences between samples.

3.3. Extraction temperature

Temperature has a significant effect on the sorption mechanism of the volatile compounds when SPME is used [46]. Although the distribution coefficient of hydrophilic compounds towards the headspace phase is usually favored at increased temperatures, raising the temperature in blood samples can increase the formation of artifacts due to enhanced chemical reactions and clot formation [26-28,32,39]. Protein denaturation takes place at >43ºC and HS temperatures below this value are recommended for VOC analysis in blood samples [28]. A temperature of 30±1ºC was chosen for the extraction.

3.4. Assessment of matrix effects

A pooled blood sample obtained from different individual samples was used as a matrix solution for recovery studies. Recoveries were calculated by analyzing both the pooled blood sample and the same sample fortified at a fixed mass for each compound. The recovery achieved was calculated as the ratio between the mass difference obtained between fortified and non-fortified samples and the spiked mass in the fortified sample.

The complexity and matrix variability of biological samples requires an accurate evaluation of matrix effects [39]. It is very difficult to prepare a matrix that is identical to unknown samples when working with biological fluids. An established and accepted method in forensic medicine is to use ISs [10-13]. As indicated in the introduction section, the selection of an appropriate IS is especially complicated when a large number of target compounds with different volatilities are evaluated.

The evaluation of d-furan as the IS showed that the matrix effect was only compensated in the determination of furan when whole blood samples without dilution were analyzed. The other compounds did not show significant improvements in their recovery efficiencies. This confirms that the use of a limited set of ISs is not recommendable for multicomponent analysis, and that the best procedure to obtain good recoveries without sample dilution is to use specific isotopic ISs for each analyte. However, this results in a methodology that is both complex and expensive.

In a previous study [47] using needle traps (NTs) as the preconcentration device, a significant matrix effect was found when VOCs were analyzed from blood samples. A 1:4 (blood/water) dilution improved the recovery efficiency and permitted quantitative extraction of the most volatile compounds. The sorption mechanism of an NT is similar to those of SPME when an adsorption fiber is used and, therefore, similar behavior could be expected for the CAR/PDMS fiber used here. In this study we evaluated different dilution ratios to assess whether the matrix effect is independent of the volatility of the compounds (Table 2). The results confirm the strong interactions between VOCs and the matrix components of blood samples. Moreover, the matrix effect is not constant and changes significantly depending on the volatility of the compound. Only furan, the most volatile compound evaluated, yielded quantitative recoveries without water dilution (the one-tailed Student's test was used to confirm whether the recoveries obtained were significantly smaller than a fixed value of 90%, α=0.05). As can be seen from the data in Table 2, the greater the volatility of the compound, the lower the interaction with the matrix, which also confirms the need for a large number of ISs to compensate for the different matrix effects. Carbon tetrachloride, benzene, 2,5-dimethylfuran, and 1,2-dichloropropane yielded quantitative recoveries with a 1:2 (blood/water) dilution. Toluene required a 1:4 dilution to yield quantitative recoveries, whereas ethylbenzene, xylene isomers and styrene required a 1:5 dilution. 1,2-dichlorobenzene, the least volatile compound evaluated, did not yield quantitative recoveries in any of the dilution ratios tested. These results are equivalent to those obtained using NTs, although the dilution ratio required with SPME (1:5) is slightly higher than with NT (1:4). This difference can be attributed to the mixed sorption mechanism of the CAR/PDMS fiber used in this study.

Different studies have obtained quantitative recoveries in analyzing VOCs in blood samples without sample dilution [24,31,34,36,45] but in all these cases they used a single isotope labeled standard for each target compound to overcome matrix interactions. When no specific IS for each analyte was used, the recovery was not evaluated and only qualitative results were shown [35]. In order to prevent matrix effects with sample dilution, different dilution ratios have been proposed. Miekisch et al. [28] found that 1:1 dilution was enough to obtain good recoveries and to avoid matrix interference with blood samples. In this case, a short list of highly volatile compounds was evaluated (five VOCs with boiling points ranging from 34–56ºC). The only compound tested in our study within this range was furan, which gave quantitative recoveries without dilution (89%). Gottzein et al. [30] obtained recoveries of

<62% when analyzing eight VOCs using 1:2 dilution (boiling points ranging from 49–144ºC). Spinosa et al. [12] used a 1:5 dilution to analyze ethanol in blood but no recovery rates are reported.

It is clear from the results obtained that the matrix effect correlates well with the volatility of the target compounds. The matrix effect is small for those compounds with boiling points similar to or below the temperature applied in the extraction process (furan in the present study, b.p. 31.3ºC), which allows quantitative recoveries to be obtained without dilution. A 1:2 dilution of blood samples can be used, with a limited loss of sensitivity, only for compounds with boiling points <100ºC (up to 1,2-dichloropropane, b.p. 95ºC, for the compounds evaluated in this study). Compounds with boiling points between 100–150ºC require significantly greater dilution to be able to compensate for matrix effects (1:5). When compounds with higher boiling points are evaluated (e.g., 1,2-dichlorobenzene, b.p. 180.5ºC), the matrix effect cannot be compensated by dilution alone (25% recovery with a 1:5 dilution).

3.5. Figures of merit of the HS-SPME methodology

We have validated the SPME method for VOC analysis with a 1:5 dilution of the blood samples in order to assess whether this simple matrix compensation can be used for the analysis of volatile compounds in blood samples with boiling points <150ºC.

Calibration standard mixtures (n=6, each measured twice) in the 0.1–10 $\mu g \cdot L^{-1}$ range were analyzed. These values correspond to concentrations in the undiluted blood samples in the 1–60 $\mu g \cdot L^{-1}$ range. Table 3 shows the figures of merit obtained in these experiments. The method based on standard deviation of the response and the slope measured was applied for LOD determination [49]. A standard at ~0.1 $\mu g \cdot L^{-1}$ (n=5) was evaluated and the SD obtained was taken as the SD of the blank (SD_{blank}). The $3SD_{blank}$ criterion was then applied to calculate LODs and the $10SD_{blank}$ criterion for LOQs. Positive detection was confirmed by preparing standards at the calculated values and measuring them. A signal-to-noise ratio of >3 was obtained for all the compounds at the LODs proposed. Blank analyses always gave values below the calculated LODs when this procedure was applied. Linearity was confirmed in the range proposed by evaluating the distribution of residuals of the calibration plots. Good fits were achieved for all compounds (R^2>0.98).

When fibers containing PDMS coatings are used there is a ubiquitous problem in blank analysis when benzene and toluene are target compounds in that the coating is continuously degraded [24]. In our study, blank analyses of milli·Q water samples consistently showed peaks for benzene and toluene with S/N>3. The method used for calculating the LODs gave limits one order of magnitude greater than those obtained applying the basic criteria of obtaining chromatograms with a S/N=3, but allowed limits above the concentrations associated with the signals found in the blanks to be obtained.

Recoveries were evaluated from blood samples fortified at the levels indicated in Table 4. The results obtained were quantitative for target compounds (≥75%), except for 1,2-dichlorobenzene, which yielded a ~30% recovery. Precision (intra-day repeatability) was determined at high and low levels. All compounds gave repeatability values within the precision limits suggested for bioanalytical methods (within-run and between-run precisions not to exceed 15% except for the LOQ level, where they should not exceed 20%) [50].

3.6. Analysis of blood samples

Thirty-two samples from different individuals were evaluated. Each sample was analyzed three times. Figure 1 shows an extracted chromatogram obtained for a blood sample.

One of the target compounds (furan) was not detected in any of the samples. Toluene was the only compound quantified in all samples. Benzene was detected in 30 samples (93.7%). This compound showed levels >LOQ in 53% of the samples. Ethylbenzene was detected in 28 samples (87.5%). All other compounds (2,5-dimethylfuran, dichloropropane, xylene isomers and styrene) were only detected in a few samples, but practically always at levels <LOQ. Table 5 summarizes the results obtained, which broadly agree with those found in previous studies [3,4,6,45,47].

The fact that all blood samples were obtained from non-exposed healthy volunteers resulted in most of the compounds being quantified at levels close to the corresponding LOQ. However, the dilution method gave detection limits low enough to be able to detect the presence of target VOCs in a large number of samples. This indicates that it should be possible to quantify any increase in the levels of VOCs due to exposure or metabolic processes.

4. Conclusions

The evaluation of matrix effects using different blood/water dilution ratios has shown that this biological fluid has a different matrix effect for each VOC analyzed. It has been found that there is a significant correlation between the boiling point of the target VOC and the intensity of the matrix effect: the effect is more significant when the volatility of the compound decreases. This fact also indicates that the use of a limited set of ISs is not appropriate when multicomponent analysis of VOCs in blood is performed. A specific isotopically labeled IS is needed for each target VOC if no dilution of the blood sample is used. A 1:5 dilution has proved to be effective to obtain quantitative recoveries for those compounds with boiling points <150ºC. However, the sample dilution is not adequate for semi-volatile compounds as their interaction with the blood matrix is too strong and does not allow quantitative recoveries by SPME for compounds with boiling points >150ºC. For those compounds that can be analyzed using the dilution process, the LODs obtained allow the detection of the most common VOCs present in blood samples from non-exposed individuals.

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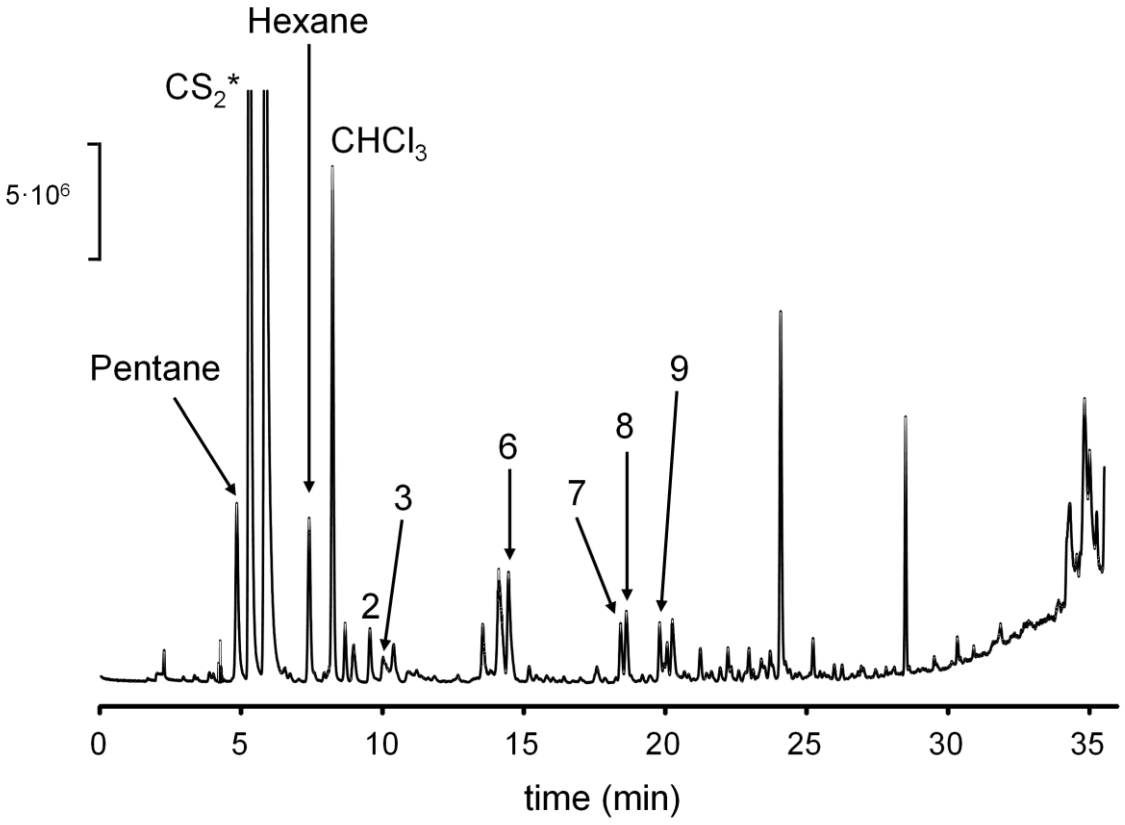
Figure 1. Extracted chromatograms (*m/z*=57,63,68,71,78,83,91,96,104,106,117,146) obtained in the analysis of a blood sample with the SPME method with a 1:5 dilution. Numbers by peaks correspond to the component numbers in Table 1. Mass/charge ratios corresponding to other common VOCs detected in blood (e.g., acetone, *m/z*=58, t_R=4.2 min) are not included in the extracted chromatogram for simplification.



* The peak indicated as carbon disulfide is a mixture of this and an unknown compound.

Table 1. List of volatile compounds evaluated.

	Compound name	Retention time (min)	Characteristic masses [a]
1	Furan	4.1	**68**
2	Carbon tetrachloride	9.6	117, 119
3	Benzene	10.0	77, **78**
4	2,5-Dimethylfuran	11.5	81, 95, **96**
5	1,2-Dichloropropane	11.8	**63**, 112
6	Toluene	14.4	**91**, 92
7	Ethylbenzene	18.4	**91**, 106
8	*m*-, *p*-Xylene	18.6	91, 105, **106**
9	*o*-Xylene	19.8	91, 105, **106**
10	Styrene	19.9	78, **104**
11	1,2-Dichlorobenzene	25.9	111, **146**, 148

[a] Quantification masses in bold

Table 2. Recoveries (*n*=3) obtained analyzing fortified blood samples without dilution of the sample and with different blood/water dilutions. Standard deviations are shown in brackets. Statistical values ≥90% are shown in bold (one-tailed Student's test, α=0.05).

	Recoveries (%)			
	No dilution	1:2	1:4	1:5
Furan	**89 (7)**	**119 (11)**	**125 (12)**	**117 (13)**
Carbon tetrachloride	57 (11)	**107 (6)**	**121 (8)**	**117 (14)**
Benzene	64 (6)	**102 (9)**	**119 (8)**	**114 (9)**
2,5-Dimethylfuran	52 (4)	**85 (4)**	**110 (14)**	**115 (10)**
1,2-Dichloropropane	55 (6)	**80 (12)**	**121 (8)**	**118 (11)**
Toluene	25 (3)	68 (13)	**84 (8)**	**104 (15)**
Ethylbenzene	22 (1)	38 (7)	64 (6)	**90 (5)**
m-, *p*-Xylene	16 (1)	32 (5)	59 (3)	**88 (6)**
o-Xylene	19 (3)	35 (4)	56 (4)	**80 (9)**
Styrene	18 (2)	37 (7)	56 (4)	**84 (8)**
1,2-Dichlorobenzene	2 (1)	15 (3)	19 (1)	25 (5)

Table 3. Linearity parameters, determination coefficients (R^2), LOD and LOQ for each target VOC with the HS-SPME method. None of the intercept values showed significant differences from 0.

Compound	**Slope (SD)** ($\cdot 10^6$)	$\boldsymbol{R^2}$	**LOD** ($\mu g \cdot L^{-1}$) [a]	**LOQ** ($\mu g \cdot L^{-1}$) [a]
Furan	31 (2)	0.989	0.02 (0.12)	0.07 (0.42)
Benzene	91 (9)	0.982	0.02 (0.12)	0.07 (0.42)
2,5-Dimethylfuran	21 (1)	0.997	0.01 (0.06)	0.07 (0.42)
1,2-Dichloropropane	21 (1)	0.992	0.02 (0.12)	0.10 (0.60)
Toluene	96 (11)	0.981	0.03 (0.24)	0.10 (0.60)
Ethylbenzene	59 (6)	0.984	0.03 (0.18)	0.10 (0.60)
m-, *p*-Xylene	17 (1)	0.999	0.03 (0.18)	0.10 (0.60)
o-Xylene	20 (2)	0.982	0.02 (0.12)	0.10 (0.60)
Styrene	25 (1)	0.996	0.04 (0.24)	0.15 (0.90)
1,2-Dichlorobenzene	16 (1)	0.988	0.04 (0.24)	0.15 (0.90)

[a] Values in undiluted blood samples are given in brackets

Table 4. Recoveries (*n*=3) and intra-day repeatability obtained with the HS-SPME methodology proposed (1:5 dilution, blood/water).

Compound	**Fortified level** ($\mu g \cdot L^{-1}$)	**Recovery** (%)	**Repeatability** (RSD, %)		
			high level[a] (*n*=3)	low level[b] (*n*=5)	blood samples[c]
Furan	5	123	13	24	–[d]
Benzene	4	136	9	11	3–18 (10)
2,5-Dimethylfuran	4	102	10	9	–[d]
1,2-Dichloropropane	4	116	11	18	–[d]
Toluene	4	106	15	17	2–23 (11)
Ethylbenzene	4	90	5	16	7–24 (17)
m-, *p*-Xylene	4	93	6	15	–[d]
o-Xylene	4	84	9	13	16–23 (19)
Styrene	4	80	8	16	–[d]
1,2-Dichlorobenzene	5	26	–	–	–[d]

[a] Repeatability obtained with a fortified sample at a concentration equal to the value indicated in the "fortified level" column

[b] Repeatability obtained with a fortified sample at a concentration around LOQ

[c] Range of repeatabilities obtained in the measure of blood samples. Mean RSD obtained from all quantified blood samples is indicated between brackets

[d] Compound not detected or detected below LOQ in all blood samples

Table 5. Main statistical parameters of blood concentrations of target VOCs ($\mu g \cdot L^{-1}$). For statistical analysis, a value of (LOD/$\sqrt{2}$) was used in the case of non-detected compounds (Q_1: 25th percentile; Q_3: 75th percentile).

Compound	Mean	Median	SD	Minimum	Maximum	Q_1	Q_3
Benzene	0.55	0.54	0.27	0.13[a]	1.29	0.33[a]	0.74
2,5-Dimethylfuran	<LOD	<LOD	–	<LOD	0.40[a]	<LOD	<LOD
1.2-Dichloropropane	<LOD	<LOD	–	<LOD	0.20[a]	<LOD	<LOD
Toluene	1.93	1.48	1.66	0.87	8.97	1.10	2.04
Ethylbenzene	0.49[a]	0.48[a]	0.20	<LOD	0.91	0.42[a]	0.61
m-, *p*-Xylene	0.18[a]	<LOD	0.10	<LOD	0.52[a]	<LOD	0.19[a]
o-Xylene	0.39[a]	0.41[a]	0.17	<LOD	0.80	0.31[a]	0.47[a]
Styrene	0.52[a]	0.55[a]	0.21	<LOD	0.87[a]	0.37[a]	0.68[a]

[a] <LOQ