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Determination of trichloroethylene in biological samples by headspace solid-phase microextraction gas chromatography/mass spectrometry

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Abstract

A simple, rapid and sensitive method for determination of trichloroethylene (TCE) in rat blood, liver, lung, kidney and brain, using headspace solid-phase microextraction (HS-SPME) and gas chromatography/mass spectrometry (GC/MS), is presented. A 100-μm polydimethylsiloxane (PDMS) fiber was selected for sampling. The major analytical parameters including extraction and desorption temperature, extraction and desorption time, salt addition, and sample preheating time were optimized for each of the biological matrices to enhance the extraction efficiency and sensitivity of the method. The lower limits of quantitation for TCE in blood and tissues were 0.25 ng/ml and 0.75 ng/g, respectively. The method showed good linearity over the range of 0.25–100 ng TCE/ml in blood and 0.75–300 ng TCE/g in tissues, with correlation coefficient (R^2) values higher than 0.994. The precision and accuracy for intra-day and inter-day measurements were less than 10%. The relative recoveries of TCE respect to deionized water from all matrices were greater than 55%. Stability tests including autosampler temperature and freeze and thaw of specimens were also investigated. This validated method was successfully applied to study the toxicokinetics of TCE following administration of a low oral dose.

Keywords: Trichloroethylene; GC–MS; EI; SPME

1. Introduction

Trichloroethylene (TCE) is a halogenated volatile organic compound (VOC) that has been used extensively as a metal degreaser, chemical intermediate, anesthetic and dry cleaning agent. The presence of TCE in the environment can be attributed to industrial discharge of the chemical to water and land and leaching from hazardous waste sites [1–3]. As a result of its widespread use and migration through soil, TCE can be found in the groundwater at more than 50% of the hazardous waste sites on the United States Environmental Protection Agency's (EPA) National Priorities List [4,5]. According to a survey by the National Water-Quality Assessment (NAWQA) program, 5% of wells throughout the United States have detectable levels of TCE, with concentrations ranging from 0.02 to 230 μg/l [6]. Exposure to high doses of TCE can result in central nervous system depression, liver changes and cardiac arrhythmias [2]. Exposure of the general population to very low levels of TCE in environmental media is of concern primarily because of potential carcinogenic risk [7–10].

The human body possesses a number of defense mechanisms to protect against low-level toxic and mutagenic insults. These mechanisms include presystemic elimination, metabolic detoxification, DNA repair, death of mutated cells, apoptosis, destruction of mutated cells by the immune system, and the action of tumor suppressor genes. The extremely high doses of TCE and other VOCs administered in rodent cancer studies "overwhelm" these protective processes, kill cells, exceed the capacity of tissue repair, and can then cause tumors in some strains of mice and rats.

A substantial amount of TCE and other VOCs may be removed from the portal venous blood by presystemic or first-pass elimination. Drugs and chemicals absorbed from the gastrointestinal tract must first pass though the portal blood into the liver and on to the lungs, before entering the arterial circulation and being transported to tissues throughout the body [11,12]. It has been theorized but not demonstrated experimentally, that low oral doses of VOCs are completely removed by presystemic elimination [13,14]. Lee et al. characterized the presystemic elimination of TCE in rats [15]. These experimen-

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tal results demonstrated that the liver and lungs can remove a significant proportion of moderate oral doses of TCE. Lack of analytical sensitivity, however, precluded working with more environmentally relevant levels. In order to directly measure the capacity of first-pass elimination of TCE under environmental exposure conditions, a valid, sensitive and rapid analytical method is needed to measure trace concentrations of TCE in multiple blood and tissues samples.

Methods that have been published to quantify TCE in biological samples include purge and trap, headspace, and liquid–liquid extraction [16–21]. Methods using purge and trap are extremely sensitive, but require large volume samples. The LOQ is usually in pg/ml levels [16,17]. Large volumes of blood (e.g. 10 ml) are not realistic for toxicokinetic studies in small rodents. Traditional headspace methods are less sensitive than purge and trap procedures because they do not involve sample preconcentration. A LOQ of 50 ng/ml was reported using headspace GC–ECD for the measurement of TCE and its metabolites in blood and tissues [18]. Other methods involving liquid–liquid extraction (LLE) with ether, followed by GC–MS analysis of TCE have been reported [19,20]. However, it has been found that many solvents used in LLE are contaminated with TCE [20]. These methods also involve a number of additional sample preparation steps. This results in increased cost, time, potential error and decreased sensitivity (LOQ = 5 ng/ml) in the analysis.

Solid-phase microextraction (SPME) is an innovative, solvent-free technology that is fast, economical, and versatile. With this method, a coated fiber is exposed to the volatilized chemical in the sample's headspace. The analyte partitions from the sample matrix to the coating. The fiber bearing the concentrated analyte is then transferred to the analytical instrument where desorption, separation, and quantification of the extracted analyte takes place [22]. SPME has several advantages over classical GC techniques. SPME can pre-concentrate the samples due to the high affinity of the analyte for the fiber coating. Potentially contaminated organic solvents commonly used for extracting volatile analytes from biological sample are not needed. Therefore with SPME, sample handling is minimized. When a biological sample is taken, it is simply placed into an autosampler vial and capped until analysis. This limited sample preparation helps to minimize the loss of volatile analytes and reduces potential error. Furthermore, headspace SPME methods can reduce matrix effects, because the biological macromolecules in the sample do not volatilize into the headspace. Several methods have been published describing the use of SPME for the analysis of TCE [23–30]. Some of these are focused on the determination of TCE in environmental media, such as air, drinking water and soil [23–26]. However, quantitation of chemicals in a biological matrix is much more difficult. Dehon et al. reported a SPME GC–MS method for determination of TCE in tissues [27]. Relatively large volume samples were used in their method. Optimization and validation of the method were not addressed in this paper. Poli developed a sensitive method for measurement of TCE in human urine using headspace SPME GC–MS with a detection limit of 0.01 μg/l [28]. But the sample volume used for their method was 2 ml. This large volume cannot be obtained in toxicokinetics studies with rodents and other small animals. Xu et al. reported a GC–MS method coupled with SPME for the determination of TCE in environmental biological samples, such as the microcosm of a cell [29]. Their method's detection limit was 5 μg/l, but complete immersion of the fiber in the biological samples substantially shortens the life of the fiber coating. In previous work in our laboratory, a SPME method for analysis of TCE in rat tissues with a LOQ of 5 ng/ml was developed [30], but not validated. Its sensitivity was not sufficient to study the absorption, disposition and excretion of environmentally relevant levels of the VOC.

Development and validation of physiologically based pharmacokinetic (PBPK) models for TCE require blood and tissue concentration time-course data for the four primary target organs (i.e. liver, kidney, lung and brain) [31–33]. We developed a HS-SPME GC–MS method for the determination of TCE in rats, in order to directly assess the extent of first-pass elimination of TCE in rats and to obtain data to develop and validate a PBPK model that can accurately forecast first-pass metabolism for any of a variety of exposure scenarios. This new method is quite sensitive and uses small sample sizes. The lower limit of quantitation (LLOQ) is 0.25 ng TCE/ml in blood and 0.75 ng/g in tissues.

2. Experimental

2.1. Chemicals and reagents

Analytical grade trichloroethylene (TCE) and ammonium sulfate were purchased from Sigma–Aldrich (St. Louis, MO, USA). Sodium chloride, sodium fluoride, sodium carbonate, sodium sulfate and sulfuric acid were purchased from J.T. Baker (Phillipsburg, NJ, USA). HPLC-grade acetonitrile was purchased from Fisher Scientific (Pittsburgh, PA, USA). Deionized water used in experiments was generated from a Continental deionized water system (Natick, MA, USA). Ultra-high-purity (UHP) helium was purchased from National Welders (Charlotte, NC, USA). Alkamuls, the emulsifying agent used in preparing doses for the animal study, was obtained from Rhone-Poulenc (Cranbury, NJ, USA).

2.2. Gas chromatography–mass spectrometry

The analyses were carried out on an Agilent 6890 gas chromatograph (GC) coupled with a model 5973 mass selective detector (Palo Alto, CA, USA). The GC was equipped with a 0.75 mm i.d. SPME liner. Separation of the analytes was obtained on a ZB-5MS column (Phenomenex, Torrance, CA, USA: 30 m × 0.25 mm i.d., 0.25 μm film thickness) using helium as a carrier gas (flow rate, 1 ml/min). The GC injection port and interface transfer line were maintained at 200 and 280 °C, respectively. During the fiber desorption process, the splitless mode of injection was operated. After 2.5 min, the split vent valve opened to sweep any residual vapors from the liner. The oven temperature was initially held at 35 °C for 3 min, then increased to 100 °C at 10 °C/min, and held for 2 min. The mass spectrometer was operated in positive electron ionization (EI)

mode with an electron energy of 70 eV. Quantitation of TCE was performed using selected-ion monitoring (SIM) mode by monitoring *m/z* 130 (quantitation ion), *m/z* 132 and *m/z* 134 (confirmation ions). A solvent delay of 1.5 min was set to protect the filament from oxidation.

2.3. Preparation of working standard and quality control solutions

A stock solution of TCE was prepared in acetonitrile to yield a final concentration of 100 mg/ml. Standard solutions for the calibration curve were prepared from the stock solution in the following concentrations: 2.5, 5, 10, 25, 50, 100, 250, 500 and 1000 ng/ml. Standards used to assess precision and accuracy were prepared in acetonitrile from the stock solution in concentrations of 2.5, 7.5, 75 and 750 ng/ml. All stock and standard solutions were refrigerated at 4 °C during the day of use and were prepared fresh daily.

2.4. Sample preparation

Prior to extraction, liver, kidney, lung and brain samples were homogenized with two volumes of deionized water (w/v) using a homogenizer (Polytron®, Brinkman, Switzerland). Samples for the calibration curves and quality control (QC) samples were prepared by adding 20 μl of the TCE standard into 200 μl of blank blood or blank tissue homogenate. This yielded calibration standard concentrations of 0.25, 0.5, 1.0, 2.5, 5.0, 10.0, 25.0, 50.0 and 100.0 ng/ml in blood and 0.75, 1.5, 3.0, 7.5, 15.0, 30.0, 75.0, 150.0 and 300.0 ng/g in tissues. The final concentrations of QCs were 0.25, 0.75, 7.5, 75.0 ng/ml in blood and 0.75, 2.25, 22.5, 225.0 ng/g in tissues. For blood samples, 200 μl of blood and 400 μl of sulfuric acid (1 mol/l) were added into a 2.0 ml SPME vial. For liver, kidney and lung tissues, 200 μl of tissue homogenate were transferred in 2-ml vials containing 200 μl of an ammonium sulfate solution (30%, w/v). Brain was treated in the same manner as liver, kidney and lung, but 200 μl of 5% NaCl solution (w/v) was used instead of 30% ammonium sulfate. All the autosampler vials were quickly sealed with PTFE-coated silicone septa and crimped aluminum caps. The vials were vortexed for 2 min and placed into the autosampler for analysis. Two blanks were run before each batch of samples to insure there was not a detectable background level of TCE before beginning.

2.5. Headspace SPME procedure

Headspace SPME sampling was performed using a 100-μm polydimethylsiloxane (PDMS) fiber (Sulpelco, Bellefonte, PA, USA) mounted on a Combi/Pal System autosampler (CTC Analytics, Zwingen, Switzerland). Fibers were conditioned at 250 °C for 30 min prior to use. Sample vials were preheated in the agitator for 5 min before analysis, and the SPME fiber was then exposed to the headspace by piercing the septum with the needle of the fiber assembly. After extraction for 5 min (blood and brain), 15 min (lung and kidney) or 20 min (liver) at 30 °C under agitation, the fiber was withdrawn into the needle and immediately desorbed at 200 °C for 2 min into the GC injection port.

2.6. Method validation

The methods were validated for linearity, recovery, accuracy and precision. Calibration curves were generated by linear regression analyses of the peak area of TCE against the concentration applying a weight ($1/x^2$). Precision (expressed as % relative standard deviation, R.S.D.) and accuracy (expressed as % error) were calculated for four QC samples. Five replicates of each QC point were analyzed to determine the intra-day accuracy and precision. This process was repeated three times over 3 days in order to determine the inter-day accuracy and precision. Relative recoveries from the blood method were calculated for spiked samples at 0.25, 0.75, 7.5 and 75 ng/ml ($n = 5$) by dividing the peak area for TCE by peak area for an equal concentration of TCE in deionized water. Relative recoveries from tissues were calculated for spiked samples at 0.75, 2.25, 22.5 and 225.0 ng/g ($n = 5$) in the same manner. Because TCE is volatile, every precaution was taken to ensure it was not lost during analysis. Analytes were considered stable if the relative error (%RE) of the mean test responses were within 15% of appropriate controls [34]. The autosampler stability was evaluated over a period of 24 h to determine if there was any loss of signal due to the time a sample spends in the autosampler prior to analysis. The freeze/thaw stability was investigated by comparing the stability of samples following three freeze/thaw cycles against freshly spiked samples. The stability testing was performed at 7.5 ng/ml in blood samples and 22.5 ng/g in tissue samples, respectively.

2.7. Sampling

Male Sprague–Dawley (S–D) rats were obtained from Charles River Laboratories (Raleigh, NC, USA). The rats were acclimated for at least 7 days in an AAALAC-approved animal care facility after arrival. All experimental protocols were approved by the Institutional Animal Care and Use Committee at the University of Georgia. Rats (264 ± 4 g, $n = 3$) were dosed orally with 1 mg TCE/kg body weight using Alkamulus as the vehicle and sacrificed by cervical dislocation 10 min postdosing. Blood samples were collected immediately via cardiac puncture. The liver, kidney, lungs and brain were perfused in situ with cold saline to remove as much as blood as possible. Each tissue specimen was weighed and homogenized with two volumes of cold deionized water. Blood samples were analyzed immediately, and tissue samples were stored at −20 °C until analysis.

3. Results and discussion

3.1. Optimization of the HS-SPME conditions

For the sampling of TCE from biological matrices, headspace (HS)-SPME was preferred to direct sampling for several reasons: (1) the headspace mode protected the fiber coating from damage by high molecular mass compounds present in the sample matrix, such as proteins; (2) the headspace allowed modifica-

tion of the matrix, such as changing the pH, without damaging the fiber; (3) the equilibrium times were generally shorter with the headspace mode than with direct extraction [35,36]. HS-SPME is an equilibrium technique. During extraction, the analyte migrates among the three phases (the aqueous matrix, the headspace above the sample and fiber coating) until equilibrium is reached. Therefore, optimization of several parameters that affect the equilibrium is of critical importance. These parameters include sample preheating time, extraction time and temperature, sample pH, salt concentrations and desorption time and temperature. All determinations were performed in duplicate, and the average values were reported. For the analysis of lower molecular weight non-polar analytes like TCE there are two choices for SPME fibers the 100-μm PDMS and 75-μm Carboxen-PDMS. The Carboxen fibers showed enhanced response for TCE (2–3× increase) but required a much higher desorption temperature (300 °C) when compared with PDMS fibers. In addition, the equilibration time with Carboxen fibers were nearly 3-fold longer and the repeatability was 20–30% lower than the PDMS fibers. Many of these same observations have been previously reported [37]. One additional note was that the peak shape for TCE showed tailing when using the Carboxen fiber even at high-desorption temperature of 300 °C. Therefore, the commercially available 100-μm PDMS fiber was chosen for this study, based upon its nonpolar properties, high affinity, higher precision and shorter equilibration times for TCE [29].

Extraction temperature and time are fundamental parameters for HS-SPME. According to SPME theory the fiber equilibration process is exothermic, and any increase in sampling temperature will decrease both analyte recovery and equilibrium extraction time [38,39]. The headspace/sample partition coefficient of the analyte increases with an increase in temperature, while at the same time the fiber coating/headspace partition coefficient decreases. Each biological matrix was tested over a range of temperatures (30–60 °C) ($n=4$) and extraction times (1–20 min) ($n=7$) to determine the optimal extraction conditions for TCE. Fig. 1 shows the effect of extraction temperature and time on peak areas of TCE in liver homogenate. Comparison of the extraction time profiles obtained at different temperatures reveals that higher sampling temperature will increase the speed of equilibrium but decrease the amount of extracted analyte on the fiber, due to the decreased fiber coating/headspace partition coefficient. The TCE extraction process is exothermic as demonstrated by the corresponding ΔH value reported in the literature. Therefore, by increasing extraction temperature there is a depletion in extraction capacity of the fiber for TCE as reported by Pawliszyn [39]. For liver homogenate, the equilibrium status can be reached within 20 min at 30 °C. For kidney and lung tissues, 30 °C was also the optimal temperature for SPME extraction, but a shorter extraction time (15 min) was required to reach equilibrium. The liver contains high levels of cytochrome P450s and other heme-containing proteins, so relatively high binding of TCE would be expected. A longer extraction time was therefore needed for TCE to be transferred from the liver matrix to the headspace. For the blood samples, the addition of sulfuric acid lysed the blood cells and lowered the binding of TCE to plasma proteins. Thus 5 min at 30 °C was found to be long enough to reach equilibration. Brain tissue behaves similarly to blood. Ionic solutes present in brain tissue help to decrease the equilibrium time and increase the extraction efficiency. Therefore, an extraction time of just 5 min was needed.

As it is known that salting can increase or decrease the amount of analyte extracted, the influence of salt on the extraction efficiency of SPME for TCE in different matrices was studied. The presence of salt increases the ionic strength of a solution and often affects the solubility of analytes in biological samples. The effect of five types of salt solutions was studied (Fig. 2), including sodium fluoride, sodium chloride, sodium sulfate, sodium carbonate and ammonium sulfate. With liver homogenate as an example, optimization results revealed that a different concentration of each salt was required for the highest sensitivity: 10% for NaCl, 30% for $(NH_4)_2SO_4$, 2% for NaF, 15% for Na_2SO_4 and 2% for Na_2CO_3. When the salt concentration exceeded the optimum value, a decrease in sample response was observed, because analytes contributing to electrostatic interactions in the aqueous phase lose their mobility and mass transfer towards the extracting phase. When the solution is saturated by salt, the presence of a solid-phase could interact with the analytes and consequently decrease the amount extracted. In the liver tissues, the use of NaCl resulted in the lowest signal. Both $(NH_4)_2SO_4$ and NaF produced high responses due to their increased ionic strength. The F^- ion enhances the ionic strength of the solu-

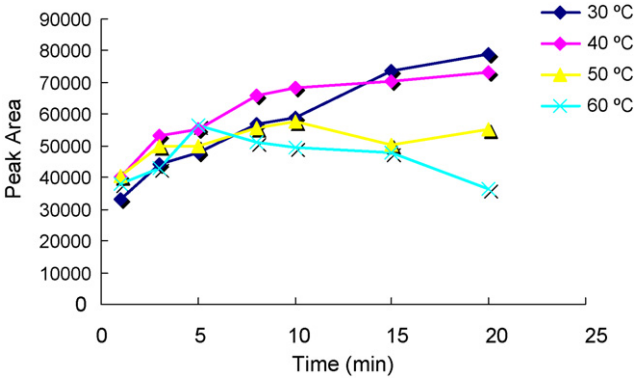

Fig. 1. Plot of TCE peak area vs. extraction time obtained on different extraction temperature (30, 40, 50 and 60 °C) in liver tissues. Conditions: preheat 5 min, 200 μl of $(NH_4)_2SO_4$ solution added.

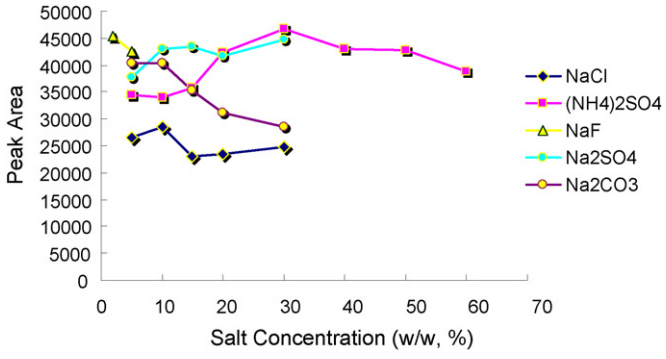

Fig. 2. Plot of TCE peak area vs. salt concentrations added into liver homogenate. Conditions: preheat 5 min, extraction time 20 min, 30 °C.

Table 1
Summary of HS-SPME conditions for determination of TCE from rat blood, liver, kidney, lung and brain

	Preheat time (min)	Incubation temperature (°C)	Extraction time (min)	Desorption Temperature (°C)	Desorption time (min)	Salt effects
Blood	5	30	5	200	2	1 M H_2SO_4 solution, 400 μl
Liver	5	30	20	200	2	30% $(NH_4)_2SO_4$ solution, 200 μl
Kidney	5	30	15	200	2	30% $(NH_4)_2SO_4$ solution, 200 μl
Lung	5	30	15	200	2	30% $(NH_4)_2SO_4$ solution, 200 μl
Brain	5	30	5	200	2	5% NaCl solution, 200 μl

tion due to the much lower dimension of F^- ion with respect to the Cl^- ion. The consequence is an increase in the salting-out effect because the water is coordinated stronger by F^- ion versus Cl^-. The effect of $(NH_4)_2SO_4$ addition can be explained by the fact that NH_4^+ is a weak base and therefore the ionic strength of the solution is increased due to hydrolysis effects. Furthermore, $(NH_4)_2SO_4$ is a divalent ion which are usually more effective than univalent ions (e.g., NaCl) for salting-out. Similarly, CO_3^{2-} is also an ion resulting from dissociation of a weak acid. However, the addition of Na_2CO_3 does not provide higher responses versus $(NH_4)_2SO_4$ due to its production of CO_2 bubbles in the matrices which lowers the mass transfer of TCE towards the fiber. In liver, lung and kidney homogenates, the highest sensitivity was obtained following addition of 30% ammonium sulfate solution. For brain, lower concentrations of salt (e.g. 5% of sodium chloride solution) worked better, because more ionic solutes are already present in brain tissue. Therefore, 30% $(NH_4)_2SO_4$ solution was selected for liver, kidney and lung, and 5% NaCl solution was chosen for brain. For blood samples, salt solutions did increase the sensitivity to some extent, but the sample response was not linear. It is likely this phenomenon resulted from partitioning of TCE into erythrocytes [40]. Salt solutions are not strong enough to lyse these cells. Sulfuric acid (1 mol/l) was therefore selected to release TCE from erythrocytes and thus increase the extraction efficiency.

Preheating time was another parameter that affected the equilibrium conditions. At low extraction temperatures, samples need to be preheated for some time before the fiber was exposed to the headspace for extraction. This preheating process improved the mass transfer kinetics of the analyte from the liquid sample to the headspace and shortened the equilibration time. Following addition of the optimized concentration of salts, each matrix was preincubated at 30 °C in the agitator for 0, 1, 3, 5, 8, 10, 15, 20 min and extracted using the optimized extraction conditions. From 0 to 5 min, an increase in preincubation time resulted in increased TCE response. Longer preheating times did not appear to improve the recovery. Thus, the shortest preheating time of 5 min was selected for this study.

Finally, the desorption time and temperature were optimized. Ideally the time interval required for desorption should be as short as possible and carryover effects should be avoided. This effect is normally obtained by applying the highest possible temperature that does not damage the fiber. The desorption temperature was examined over a range from 100 to 270 °C and the desorption time recorded from 15 to 120 s. When the injection port was maintained at 150 °C or less, a split or tailing of the chromatographic peak was observed. When higher temperatures (e.g. 250 or 270 °C) were applied, the sensitivity no longer increased, but coatings on the fiber were removed, resulting in shorter fiber life times. Therefore, 200 °C was selected as the optimum desorption temperature. At this temperature, 120 s were found to be sufficient for optimum recovery and complete analyte desorption. No carry-over was found, even following analysis of blood and tissue samples spiked with large amounts of TCE (1 μg/ml). Table 1 summarizes the HS-SPME conditions for each matrix.

3.2. Validation of the method

After optimization the methodology was validated according to internationally accepted criteria [34]. The parameters validated were selectivity, calibration curves, precision and accuracy, limits of quantitation, recovery and stability.

The selectivity of the method was evaluated by analysis of blank matrices and matrices spiked with TCE standards. Fig. 3 shows representative chromatograms obtained from each blank matrix and matrix spiked with the LLOQ standard (0.25 ng/ml in blood or 0.75 ng/g in tissues). No interfering peaks from endogenous compounds were observed at the retention time of TCE. Utilization of selected-ion monitoring (SIM) mode enhanced the mass spectrometric selectivity by eliminating the need to scan a large range of masses.

Results of statistical analyses of the calibration curves for linearity are shown in Table 2 for different matrices. The curves were linear ($R^2 > 0.994$) over the range of 0.25 to 100 ng/ml in blood samples or 0.75 to 300 ng/g in tissue samples. SAS

Table 2
Statistical data for linearity assessment including standard deviation (S.D.) for TCE from each matrix over 3 days

	Blood	Liver homogenate	Kidney homogenate	Lung homogenate	Brain homogenate
R^2	0.9947 ± 0.0034	0.9955 ± 0.0036	0.9982 ± 0.0008	0.9975 ± 0.0024	0.9954 ± 0.0019
Slope	2890 ± 330	1010 ± 220	1130 ± 160	1700 ± 60	1770 ± 200

Concentrations of calibration curves in blood: 0.25, 0.5, 1.0, 2.5, 5.0, 10.0, 25.0, 50.0 and 100.0 ng/ml; concentrations of calibration curves in tissues: 0.75, 1.5, 3.0, 7.5, 15.0, 30.0, 75.0, 150.0 and 300.0 ng/g.).

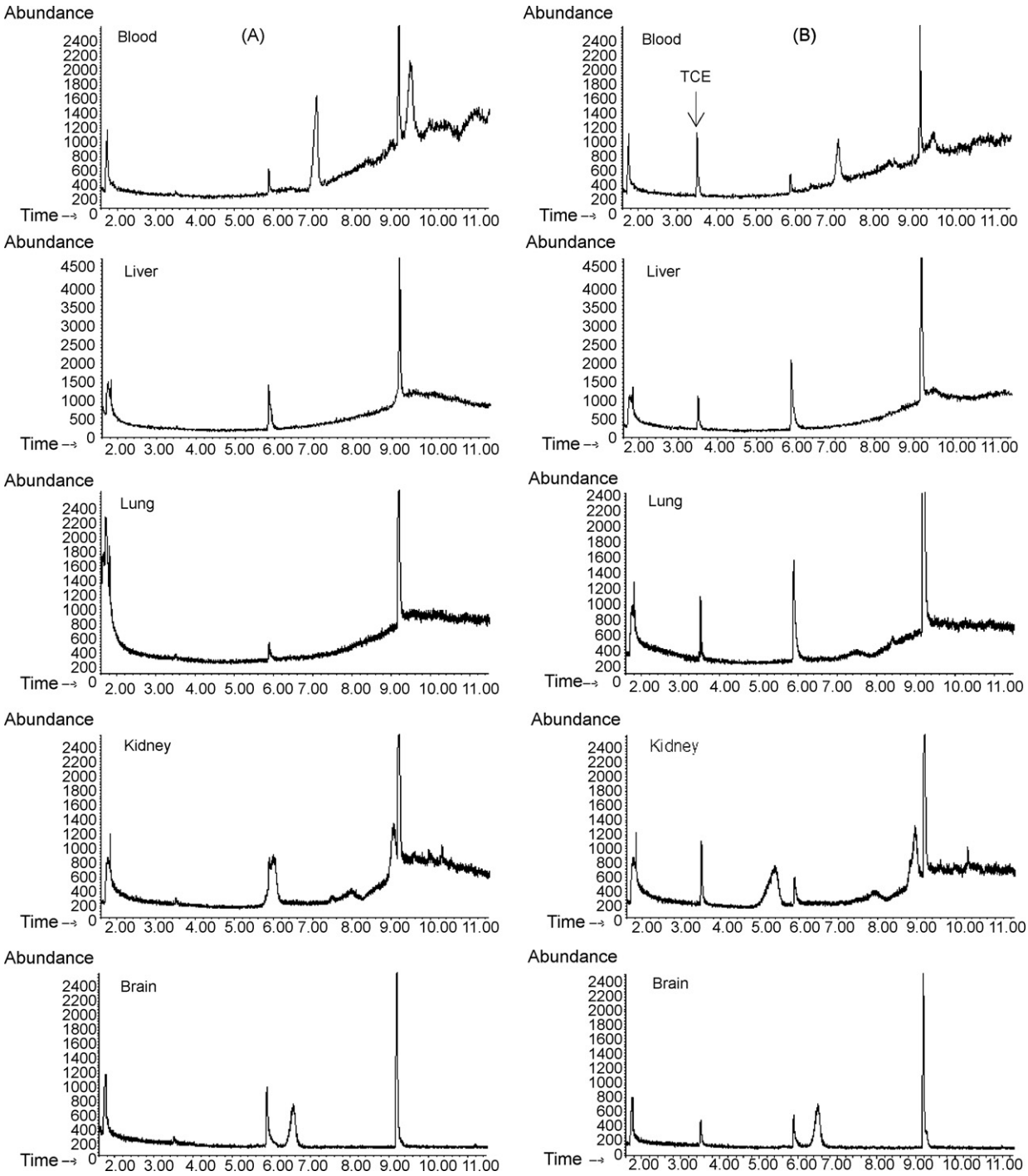

Fig. 3. Representative chromatograms using selected-ion monitoring (SIM) mode by monitoring *m/z* 130 obtained from (A) blank blood, liver, lung, kidney, brain; (B) blood, liver, lung, kidney, brain spiked with the LLOQ (0.25 ng/ml in blood or 0.75 ng/g in tissues) concentration of TCE.

JMPIN statistical software was used to generate linear regression equations for all calibration curves. A $1/x^2$-weighting scheme was used for each day of the validation and analysis for each matrix. The lower limit of quantitation, LLOQ, defined as the lowest concentration of analyte with an accuracy within 20% and a precision <20%, was 0.25 ng/ml for TCE in blood and 0.75 ng/g for liver, kidney, lung and brain, as shown in Table 3. A signal-to-noise (S/N) ratio >10 at the LLOQ was observed for TCE in all matrices. These limits were substantially lower than published values for TCE determination by SPME or by liquid–liquid extraction from blood, liver, kidney and lung [19,30]. These LLOQs were similar to the values obtained when purge and trap method were utilized, or TCE levels were measured in other matrices such as urine. However, our results were obtained using a considerably smaller sample volume [16,28] (e.g. 200 μl versus 2–10 ml). A procedure for determination of TCE in the brain has not previously been published.

Table 3
The intra-day ($n = 5$) and inter-day ($n = 15$) precision (%R.S.D.) and accuracy (%error) of the HS-SPME-GC/MS method used to quantitate TCE in rat blood, liver, kidney, lung and brain

Concentration TCE added	Intra-day			Inter-day		
	Concentration TCE found ± S.D.	R.S.D. (%)	Error (%)	Concentration TCE found ± S.D.	R.S.D. (%)	Error (%)
Blood (ng/ml)						
0.25	0.26 ± 0.01	1.62	2.48	0.25 ± 0.01	3.57	2.87
0.75	0.72 ± 0.03	3.54	4.22	0.73 ± 0.03	3.92	3.29
7.5	7.22 ± 0.34	4.70	4.02	7.52 ± 0.36	4.79	3.44
75	70.56 ± 2.32	3.29	5.92	76.23 ± 5.48	7.18	5.78
Liver homogenate (ng/g)						
0.75	0.73 ± 0.04	5.67	5.71	0.74 ± 0.07	9.21	7.22
2.25	2.04 ± 0.06	3.08	9.36	2.04 ± 0.10	4.87	9.52
22.5	20.83 ± 0.64	3.07	7.42	21.14 ± 1.24	5.88	7.19
225	211.20 ± 6.63	3.14	6.12	227.72 ± 11.06	5.06	4.30
Kidney homogenate (ng/g)						
0.75	0.75 ± 0.05	6.54	4.86	0.77 ± 0.05	6.19	5.45
2.25	2.20 ± 0.07	3.01	3.09	2.20 ± 0.07	2.96	2.95
22.5	23.15 ± 0.90	3.90	2.98	22.51 ± 0.90	4.00	2.91
225	238.10 ± 10.71	4.50	5.82	230.21 ± 9.46	3.15	3.30
Lung homogenate (ng/g)						
0.75	0.74 ± 0.05	6.36	4.48	0.77 ± 0.05	5.96	4.76
2.25	2.24 ± 0.16	6.08	4.30	2.23 ± 0.13	5.88	4.88
22.5	21.23 ± 0.78	3.66	5.75	21.95 ± 1.70	7.73	6.86
225	207.71 ± 1.71	0.82	7.69	222.43 ± 15.28	6.87	6.20
Brain homogenate (ng/g)						
0.75	0.79 ± 0.02	1.98	5.69	0.78 ± 0.05	7.07	6.47
2.25	2.23 ± 0.13	5.68	4.95	2.19 ± 0.12	5.67	4.47
22.5	21.33 ± 0.89	4.15	5.18	23.20 ± 1.81	7.78	7.52
225	205.32 ± 7.39	3.60	8.74	225.41 ± 20.51	9.10	7.74

Table 4
The relative recovery (%) (mean ± S.D.) respect to deionized water of TCE from rat blood, liver, kidney, lung and brain homogenates ($n = 5$)

Concentration (ng/ml or ng/g)	Blood	Liver homogenate	Kidney homogenate	Lung homogenate	Brain homogenate
0.25 or 0.75	54.5 ± 1.23	63.4 ± 2.71	65.5 ± 1.47	62.3 ± 3.26	65.5 ± 1.00
0.75 or 2.25	61.0 ± 2.68	59.8 ± 5.81	61.9 ± 2.17	57.3 ± 1.30	61.7 ± 0.63
7.50 or 22.5	56.5 ± 1.51	58.5 ± 4.69	58.4 ± 3.77	55.1 ± 0.78	64.5 ± 1.17
75.0 or 225	62.3 ± 3.96	55.4 ± 3.77	55.0 ± 1.31	58.2 ± 0.62	57.4 ± 2.27

Table 5
Stability testing of TCE in rat blood, liver, kidney, lung and brain homogenates ($n = 5$)

Stability	Spiked conc. (ng/ml or ng/g)	Observed conc. ± S.D. (ng/ml or ng/g)	R.S.D. (%)	Relative error (%)
Blood				
Autosampler stability (8 h)	7.5	7.03 ± 0.40	5.71	−6.32
Liver homogenate				
Three freeze-thaw cycle	22.5	23.46 ± 1.11	4.78	4.26
Autosampler stability (24 h)	22.5	20.07 ± 0.60	2.96	−10.79
Kidney homogenate				
Three freeze-thaw cycle	22.5	23.85 ± 1.47	6.11	6.03
Autosampler stability (24 h)	22.5	20.31 ± 0.93	4.59	−9.78
Lung homogenate				
Three freeze-thaw cycle	22.5	25.41 ± 2.10	8.22	12.92
Autosampler stability (24 h)	22.5	19.29 ± 1.98	6.64	−14.22
Brain homogenate				
Three freeze-thaw cycle	22.5	20.70 ± 1.26	6.05	−8.05
Autosampler stability (24 h)	22.5	19.74 ± 0.99	5.04	−12.23

Assay precision and accuracy for TCE in each matrix were established at the LLOQ, low, medium and high concentrations over 3 days. Table 3 summarizes the accuracy and precision data that were collected. The intra-day precision and accuracy ($n = 5$) were less than 6.54 and 9.36% for TCE in all matrices. The inter-day precision and accuracy were determined by pooling all of the validation assay ($n = 15$) QC samples. The values for the inter-day precision and accuracy were less than 9.21 and 9.52%.

While recovery studies are normally performed in bioanalytical method validation the values are not normally reported for SPME studies. However, relative recovery is a good parameter for evaluating the matrix effect during method validation. The relative recoveries for TCE were calculated by comparing the amount extracted by HS-SPME from different biological matrices with the amount extracted from water. The values obtained are summarized in Table 4. Relative recoveries for TCE from all matrices ranged from 54.5 to 65.5%. Biological matrices are complex. They are rich in proteins and other biomacromolecules. The mass transfer of TCE from the aqueous phase to the headspace may be hindered from such matrices. Interestingly, relative recoveries did not vary significantly among matrices. For TCE, the viscosity of the matrices was a key point for extraction recovery and sensitivity. When the salt solutions were added to the tissue homogenates, the viscosities of different matrices were almost the same. TCE is not a highly protein bound compound, so liver and blood, which contain high concentrations of proteins, do not demonstrate lower recoveries than other tissues.

Stability testing is very important for validated methods for analysis of biological samples. A sample may remain in the autosampler for hours. TCE is a volatile compound, so examining its loss during the sample analysis is critical. Autosampler stability was evaluated at 7.5 ng TCE/ml in blood and 22.5 ng TCE/g in tissue samples. Blank blood and tissue homogenates were spiked with TCE and left in the autosampler at room temperature for 8–24 h. These samples were compared with samples prepared freshly. The values obtained for precision were less than 6.64%, and the relative error was less than 14.22% (see Table 5). All matrices were stable in the autosampler for 24 h, except blood which was only stable for 8 h due to coagulation. Freeze and thaw stability was also evaluated for tissue homogenates at the same concentration ($n = 5$). Blood samples were analyzed immediately, so freeze and thaw stability testing was not necessary. Tissue homogenates were spiked with 0.75 ng/g of TCE, and these aliquots were stored at −20 °C for

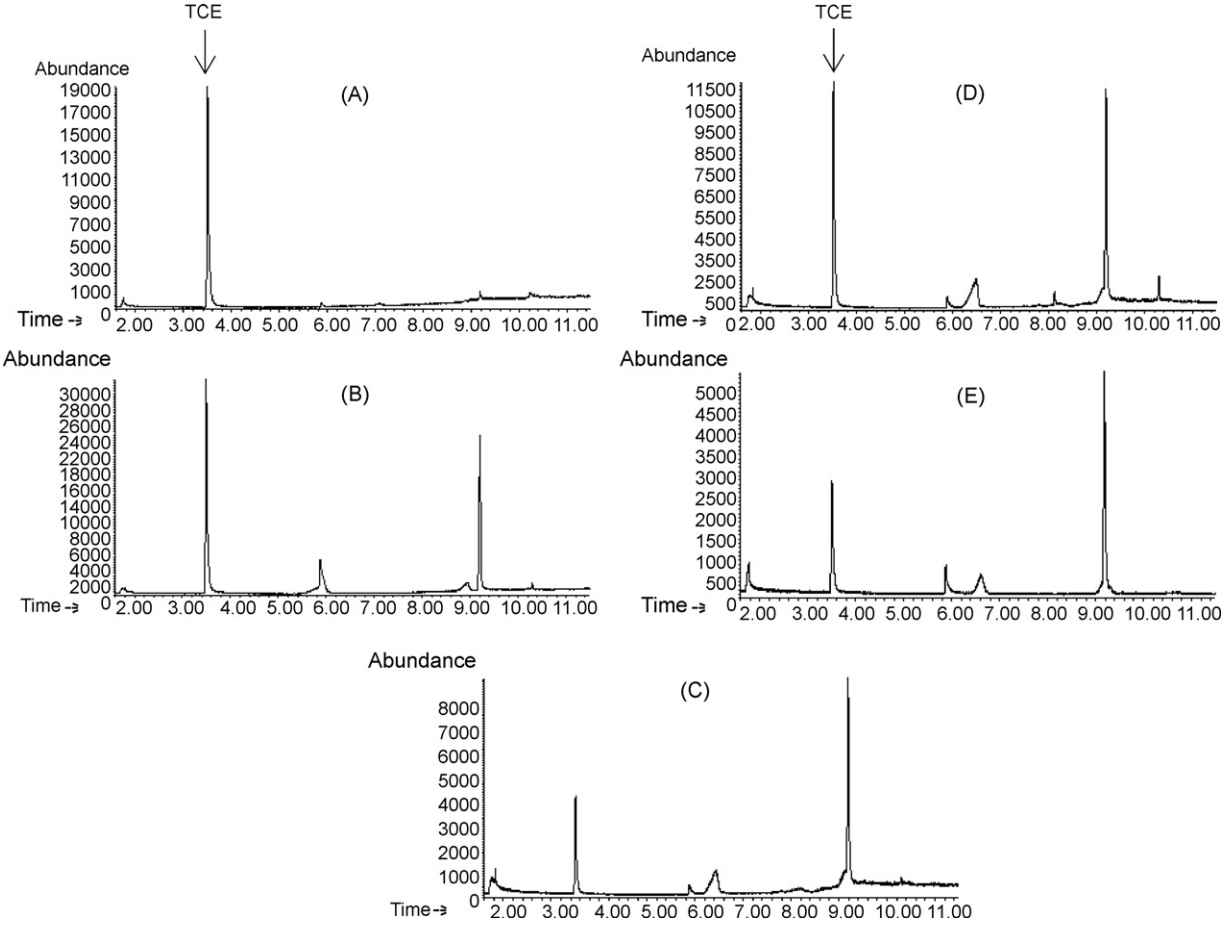

Fig. 4. Representative chromatograms of (A) blood; (B) liver; (C) lung; (D) kidney; (E) brain samples from rats dosed with 1 mg/kg TCE orally (blood and tissues were taken at 10 min post-dosing.).

Table 6
TCE concentrations 10 min postdosing in tissues of S–D rats dosed orally with 1 mg TCE/kg body weight

	Concentration in rat A	Concentration in rat B	Concentration in rat C	Average concentrations (ng/ml ± S.D.)
Blood	1.60	2.79	6.74	3.71 ± 2.69
Liver	11.67	16.73	6.54	11.65 ± 5.09
Lung	0.40	2.62	0.93	1.32 ± 1.16
Kidney	0.35	3.94	0.26	1.52 ± 2.10
Brain	0.99	2.08	2.67	1.91 ± 0.85

24 h. After three complete freeze and thaw cycles, the samples were compared to those prepared freshly. The values obtained for precision and relative error were less than 8.22 and 12.92%, respectively.

3.3. Applications

To demonstrate the applicability of this HS-SPME method to toxicokinetic studies, blood and tissue samples from TCE-dosed rats were analyzed and the TCE concentration data presented in Table 6. Representative chromatograms from analysis of TCE in blood, liver, kidney, lung and brain 10 min after rats dosed orally with 1 mg/kg of TCE are shown in Fig. 4. The highest TCE concentrations were found in liver, due to first-pass uptake of the chemical by the liver. TCE concentrations in kidney, lung, and brain were lower than in blood, as not enough time has elapsed for much of the lipophilic chemical to be taken up by tissues. This was similar to previously reported data for TCE in different tissues [31].

4. Conclusions

A simple, specific, rapid and very sensitive SPME-GC/MS method for the determination of TCE in various biological matrices (blood, liver, lung, kidney and brain) has been developed and validated. The technique overcomes limitations and obstacles of conventional methods such as the use of expensive and frequently contaminated organic solvents and of tedious and time-consuming sample preparation. During the SPME process, several influential parameters such as extraction time, extraction temperature, salt effect and desorption conditions were investigated and optimized for each matrix. It was clear that systematic optimization was necessary for each different biological matrix in order to enhance the extraction efficiency. This validated method yields excellent linearity, precision and accuracy over a wide calibration range and only requires small sample volumes. The limits of quantitation for this SPME-GC/MS method are 0.25 ng/ml in blood and 0.75 ng/g in tissues. To our knowledge, this is the first validated and the most sensitive SPME-GC/MS method for determination of TCE in blood and tissues of laboratory animals. This method was successfully used to quantify the blood and tissue distribution of TCE following administration of the lowest oral dose of the VOC for which the toxicokinetics have been characterized.

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