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# Determination of dichloromethane, trichloroethylene and perchloroethylene in urine samples by headspace solid phase microextraction gas chromatography–mass spectrometry

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**Abstract**

A method for the determination of volatile chlorinated hydrocarbons, namely dichloromethane (DCM), trichloroethylene (TCE), and perchloroethylene (PCE), in urine samples was developed using headspace solid phase microextraction (HS-SPME) gas chromatography–mass spectrometry (GC–MS). HS-SPME was performed using a 75 μm Carboxen-polydimethylsiloxane fiber. Factors, which affect the HS-SPME process, such as adsorption and desorption times, stirring, salting-out effect, and temperature of sampling have been evaluated and optimized. The highest extraction efficiency was obtained when sampling was performed at room temperature (22 °C), from samples saturated with salt and under agitation. Linearity of the HS-SPME-GC–MS method was established over four orders of magnitude and the limit of detection was 0.005 μg/l for all the compounds. Precision, calculated as %R.S.D. at three different concentration levels, was within 1–8% for all intra- and inter-day determinations. The method was applied to the quantitative determination of TCE and PCE in human urine samples from exposed (TCE, $n = 5$; median, 9.32 μg/l and PCE, $n = 39$; median, 0.58 μg/l) and non-exposed individuals ($n = 120$; median concentrations, 0.64, 0.22 and 0.11 μg/l for DCM, TCE and PCE, respectively. In addition, two cases of acute accidental exposure to DCM are reported, and the elimination kinetics in blood and urine was followed up. The calculated half-lives of urinary and blood DCM were, respectively, 7.5 and 8.1 h for one subject and 3.8 and 4.3 h for the other.


*Keywords:* Gas chromatography; Solid phase microextraction; Dichloromethane; Trichloroethylene; Perchloroethylene; Urine analysis; Biomonitoring

## 1. Introduction

Solid phase microextraction (SPME) is a solvent-free sampling technique [1], suitable for both qualitative and quantitative analysis of volatile and semivolatile compounds in aqueous and solid matrices. Since the introduction of SPME [2,3], numerous manuscripts have been published on its use in analytical sciences. SPME was also used in biological monitoring, e.g. to the determination of unmetabolized compounds, like monoaromatic hydrocarbons (benzene, toluene, ethylbenzene and xylene, BTEX) [4,5], polycylic aromatic hydrocarbons (PAHs) [6], chlorophenols [7], and anaesthetics [8] in human urine and blood. In the present study, headspace (HS)-SPME was applied to the determination of chlorinated compounds of industrial interest, namely dichloromethane (DCM), trichloroethylene (TCE) and tetrachloroethylene (or perchloroethylene, PCE) in urine samples obtained from occupationally exposed workers and from the general population.

DCM is commonly used as solvent in paint removers, aerosol propellant, degreaser agent, flammability depressant (hair sprays, room deodorants, herbicides and insecticides), and in the manufacture of foam polymers. Widespread

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exposure occurs during the production and the use of a variety of consumer products containing DCM. Substantial losses to the environment lead to ubiquitous low-level exposures from ambient air, surface and ground water [9]. Most of toxic effects of DCM have been observed after acute exposures and are reversible central nervous system depression, narcosis, irritation of eyes and respiratory tract, lung edema, and the production of elevated carboxyhemoglobin (COHb) levels in the blood. In fact, about 40% of absorbed DCM is retained and a portion of this is metabolized to carbon monoxide (CO) in the liver, kidneys and lungs, with a half-life of approximately 13 h [10]. DCM is classified in the 2B class ("possible" human carcinogen) by the International Agency for Research on Cancer (IARC) [9].

PCE and TCE have been extensively employed in the dry cleaning industry; currently, they are used for metal cleaning and degreasing, as extraction solvents, in chemical processing, as heat-exchange fluid, and in typewritter corrector fluid. Both have been detected in air, water, soil, food and animal tissues and the most heavily exposed people are those working in the degreasing of metals, for which exposure occurs by inhalation [11]. Toxicokinetic studies showed that part of inhaled PCE (38%) is eliminated by the lungs [12], whereas only a small percentage of the dose (2%) is excreted as metabolites, mainly trichloroacetic acid (TCAA), and trichloroethanol. PCE tends to distribute to body fat and its biological lifetime is about 65 h. For its low biotransformation and the aspecificity of its metabolites, PCE concentrations in exhaled air, blood and urine have been proposed as biomarkers of exposure [13]. Unlike PCE, TCE is rapidly and efficiently metabolized. In fact, only the 10% is eliminated via the lungs, more than 50% of the absorbed dose being biotransformed to trichloroethanol, which is partly excreted as a glucuronide, and TCAA [14,15]. Due to its relatively slow elimination, TCAA is detected in the urine of exposed individuals up to 12 days post exposure, suggesting a cumulative process probably related to TCE storage in fatty tissue. Both PCE and TCE are classified in the 2A ("probably" carcinogenic to humans) class by the IARC [11].

The currently adopted permissible exposure limits (PELs) as 8-h time-weighted average concentrations (8-h TWA) recommended by the US Occupational Safety and Health Administration (US-OSHA) are 25 ppm for DCM, and 100 ppm for TCE and PCE [16]. The biological exposure index (BEI®) proposed as guideline by the American Conference of Governmental Industrial Hygienists (ACGIH, 2002) is 200 μg/l for urine DCM [17]. For TCE [18] and PCE [19], urine concentrations of 26 and 100 μg/l have been detected after exposures to 50 and 25 ppm, respectively. Besides to be industrial chemicals, these compounds became ubiquitous environmental pollutants, and measurable concentrations were detected in the urine of the unexposed general population [20].

Aim of this paper was to optimize SPME sampling conditions and to develop of a highly sensitive HS-SPME-GC–MS method for the quantitative analysis of chlorinated solvents in urine, suitable not only for the routine monitoring of workers exposed to these substances, but also for their determination in samples from unexposed subjects.

## 2. Experimental

### 2.1. Chemicals and reagents

HPLC-grade DCM, 1-chlorobutane (used as internal standard, IS), and methanol were purchased from LabScan (Dublin, Ireland). TCE, PCE and sodium chloride (NaCl, 99+%) were from Sigma-Aldrich (Milan, Italy). Before the use, NaCl was dried in the oven at 80 °C to avoid contamination from laboratory air. Standard stock solutions (10 mg/ml) prepared in HPLC-grade methanol and stored at −20 °C were stable for at least 1 month.

### 2.2. Subjects

#### 2.2.1. Control population

DCM, TCE and PCE were analyzed in one analytical run in urine samples of 120 healthy subjects (65 males, 36% smokers; mean age, 38.6 ± 6.6 years) without known exposure to chlorinated hydrocarbons. The control population and workers were of the same geographical area (Parma, Italy). Spot urine samples were collected in the morning between 08:00 and 09:00 a.m.

#### 2.2.2. Workers

Urine samples were obtained from 39 workers (25 males, 45% smokers; mean age, 37.2 ± 7.3 years) exposed to PCE in the dry cleaning industry, and from five male workers (three smokers; mean age, 40.8 ± 8.7 years) exposed to TCE and styrene in the buttons manufacture. Samples were collected at the end of a 4-h work-shift; the subjects had to urinate before the shift. Two workers (subject A, age 34, BMI 23.7 and subject B, age 51, BMI 30.4) were acutely exposed to a solvent mixture including DCM [21]. Following DCM exposure, COHb levels were 13.7% and 9.7% for subjects A and B, respectively. Urine and heparinated full blood samples were collected starting from 12 h after the accident at approximately 12-h intervals for 2 days.

### 2.3. Sample preparation and HS-SPME procedure

In order to avoid loss of analytes during collection and storage [22], urine samples (2 ml) were immediately transferred in 4.0-ml SPME glass vials containing 1.0 g of NaCl. IS (final concentration, 2 μg/l) was added. The samples were shaken and stored at −20 °C until analysis. Blood samples were analyzed without the addition of salt. Headspace sampling was performed using a 75 μm Carboxen/PDMS fiber (Supelco, Bellefonte, PA, USA) mounted on a Combi/Pal System autosampler (CTC Analytics, Zwingen, Switzerland). Samples were thawed 10 min before analysis and extracted for 30 min at room temperature (22 °C) under stirring. Then, the fiber

was immediately desorbed at 280 °C for 5 min into the GC injection port, equipped with a 0.75-mm inlet liner for SPME. New fibers were conditioned for about 2 h at 300 °C using a stream of hydrogen in the GC injector. Optimization of the HS-SPME conditions was performed in urine at three different analyte concentrations (0.01, 1.0 and 100 μg/l), by varying the following parameters: amount of salt added to samples (0, 0.5, and 1 g of NaCl), stirring (yes and no), sampling temperature (22, 40, and 60 °C).

### 2.4. Calibrations

For quantitative analyses, calibrating standards were prepared by spiking a pool of urine samples from unexposed subjects with freshly prepared standard solutions containing all three analytes in the appropriated range of concentrations, i.e., 0.01–5 μg/l for all analytes in the case of controls, 0.1–10 μg/l for PCE-exposed workers, and 0.2–25 μg/l for TCE-exposed workers. In the case of DCM poisoning, a single analyte calibration was performed for both urine and blood and the concentration interval was split into two subranges, i.e., 0.02–100 and 100–1500 μg/l for low and high DCM concentrations, respectively. An analytical blank (water) and an internal quality control sample (1 μg/l) were included in every analytical series.

### 2.5. Gas chromatography–mass spectrometry

The analyses were carried out on a Hewlett Packard HP 6890 gas chromatograph coupled with a HP 5973 mass selective detector (Hewlett Packard, Palo Alto, CA, USA). Separation of the analytes was obtained on a HP-5MS column (30 m × 0.25 mm i.d., 0.25 μm film) using hydrogen as carrier gas (flow-rate, 1 ml/min). The GC conditions were: 45 °C hold for 5 min, 10 °C/min to 100 °C, hold for 2 min; injector temperature, 280 °C; detector temperature, 280 °C. Qualitative analysis was performed in the scanning mode (*m/z* 30–300), whereas for quantitative analysis, it was operated in selected-ion monitoring (SIM) by acquiring the signals of the following ions (dwell time in parentheses, Q indicates the ion chosen as quantifier): at 1.0 min, *m/z* 49 (60, Q), 84 (90) and 86 (120) for DCM; at 1.8 min, *m/z* 41 (90), 43 (120) and 56 (60, Q) for IS; at 2.35 min, *m/z* 95 (60), 97 (90), 130 (50, Q) and 132 (60) for TCE; and at 4.0 min, *m/z* 129 (60), 131 (60), 164 (60), 166 (50, Q) for PCE. A solvent delay of 1.0 min was set to protect the filament from oxidation. The chromatographic run was complete in 6 min.

### 2.6. Determination of urinary trichloroacetic acid

Trichloroacetic acid (TCAA) was determined using a colorimetric assay. Briefly, 0.5 ml of urine were added with 2.5 ml of KOH, 5 ml of pyridine and 0.5 ml of toluene. Samples were vortexed and warmed at 65 °C for 50 min. Then, samples were cooled with ice, and the supernatant (1.5 ml) was added with cool water (0.3 ml). Samples were mildly shaken before spectrophotometric determination (λ = 530 nm).

## 3. Results and discussion

### 3.1. Optimization of the HS-SPME conditions

For the sampling of chlorinated hydrocarbons from biological matrices, HS-SPME was preferred to direct sampling for several reasons: equilibrium times are generally much faster in the gas than in the liquid phase [23]; the direct contact of the fiber with the sample is avoided, providing cleaner extracts, greater selectivity and longer fiber lifetime [1]. For this study, a commercially available 75 μm Carboxen/PDMS fiber was chosen, based on the better affinity shown by this coating material for halides [24] as compared to PDMS alone.

To optimize HS-SPME sampling conditions, we evaluated the effect of several parameters, which are known to affect the extraction efficiency, i.e., adsorption and desorption time, addition of salt, stirring, and temperature of sampling. Extraction efficiency was calculated at three different analyte levels (0.01, 1.0 and 100 μg/l) from the comparison of the areas obtained by direct GC injections with the results of the HS-SPME sampling of the same analyte amounts added to urine. Since the percentage (%) of analyte extracted was not influenced by concentration, saturation of the fiber at the highest concentration level (100 μg/l) was excluded (data not shown). When the extraction time profile was studied at room temperature (22 °C), the equilibrium was reached in 30 min for all three compounds. After the sampling, the fiber was immediately transferred into the GC injector in order to avoid analyte losses and poor reproducibility. To this regard, previous studies reported that volatile chlorinated hydrocarbons are stable on the PDMS fiber at room temperature for about 2 min [25], and that the time elapsed after the sampling could be a source of analytical variability due to the evaporation of the analytes from the fiber [23]. When desorption in the GC injector was performed at 280 °C for 5 min, no carryover was observed.

As it is known that salting can increase or decrease the amount of analyte extracted, the extraction efficiency was then calculated for different amounts of salt (sodium chloride, NaCl) added to samples (0, 0.5, and 1.0 g), both without and with stirring. The results summarized in Table 1 show the effect of salt addition combined with agitation. The addition of salt always increased the yield of extraction, but for TCE and PCE the amount extracted without stirring was lower in the case of the highest (1.0 g) salt addition. This behaviour was probably due to a clot of substances normally present in urine over the NaCl unsolved, which lessen the transfer of analytes in gas phase. Above saturated salt conditions (36%, w/v, NaCl in water), stirring mainly influenced the time necessary to reach the equilibrium. Conversely, in correspondence of saturation stirring became essential, resulting in a two-fold increase of the concentration of chlorinated volatiles

Table 1
Percent amount extracted of chlorinated solvents using a 75 μm Carboxen/PDMS fiber: dichloromethane (DCM), tri- (TCE), and tetrachloroethylene (PCE)

| | DCM (%) | TCE (%) | PCE (%) |
|---|---|---|---|
| Without NaCl | 13.9 ± 1.8 | 21.8 ± 2.5 | 20.9 ± 1.9 |
| 0.5 g of NaCl | 19.4 ± 4.5 | 29.0 ± 4.7 | 31.9 ± 1.4 |
| 1 g NaCl | 22.9 ± 5.3 | 24.3 ± 4.0 | 23.8 ± 3.5 |
| 1 g NaCl and stirring | 38.6 ± 4.9 | 41.1 ± 1.9 | 42.1 ± 3.2 |

Experiments were performed at three concentration levels (0.01, 1 and 100 μg/l) of analytes in urine, by sampling at room temperature, without and with salt and stirring. Values are mean (±standard deviation) of the extraction efficiencies calculated at the different concentrations ($n = 9$).

in the gas phase. Since the use of inorganic chloride in the determination of chlorinated compounds could be questionable, preliminary experiments were performed to exclude any sample contamination or de novo production of analytes arising from added NaCl. The addition of NaCl was also used to normalize random natural salt concentrations and the ionic strength of the different urine samples.

By increasing the temperature of sampling (to 40 and 60 °C), it was found that the salting-out effect became less important (Fig. 1). In fact, if raising the temperature increased the number of molecules into the gas phase, it also lowered the absorption capacity of the fiber, which started releasing the analytes much faster than adsorbing. To support these results, we calculated some thermodynamic parameters, i.e., partition coefficients and heats of adsorption, at the different temperatures, as previously described [8]. The fiber–urine heat of adsorption ($\Delta H_{fu}$) values were negative for all the analytes (−31.6, −14.8 and −10.8 kJ/mol for DCM, TCE and PCE, respectively), thus confirming the exothermic nature of the adsorption process. As a conclusion of this experimental set aimed at optimizing HS-SPME conditions, the highest extraction efficiency was obtained when sampling was performed at room temperature (22 °C), from samples saturated with salt and under agitation.

### 3.2. Validation of the method

The GC run time was 6 min, whereas the total analysis time including HS-SPME sampling was about 36 min. The linear ranges, the equations, the correlation coefficients ($r^2$), and the limits of detection (LODs) are reported Table 2. Since calibrating standards were prepared using a pool of urine samples from unexposed subjects without detectable analyte concentrations, experimental data fitted a linear model, $y = ax$, where $y$ is the chromatographic peak areas to IS area ratio and $x$ is the concentration of analyte (expressed in μg/l). For all the compounds, the linear dynamic range was established over four orders of magnitude ($r^2 > 0.995$) and the LODs (calculated as S/N of about 3, using two ions) were in the low ppt-range. According to the literature, both the fiber coating and the detection system used are relevant factors in determining the sensitivity of the HS-SPME-GC method. When a PDMS 100 or 95 μm fiber was used for sampling, the LODs for halogenated compounds in aqueous samples were reported to be 20–50 μg/l using flame ionization detector (FID) [26], or in the range 20–200 ng/l with MS detection [27], or even lower (1–130 ng/l) when an electron-capture detector (ECD) was used [25]. In addition, the Carboxen/PDMS coating material showed a better affinity towards these chlorinated compounds as compared to PDMS alone [24]. The LODs we obtained by

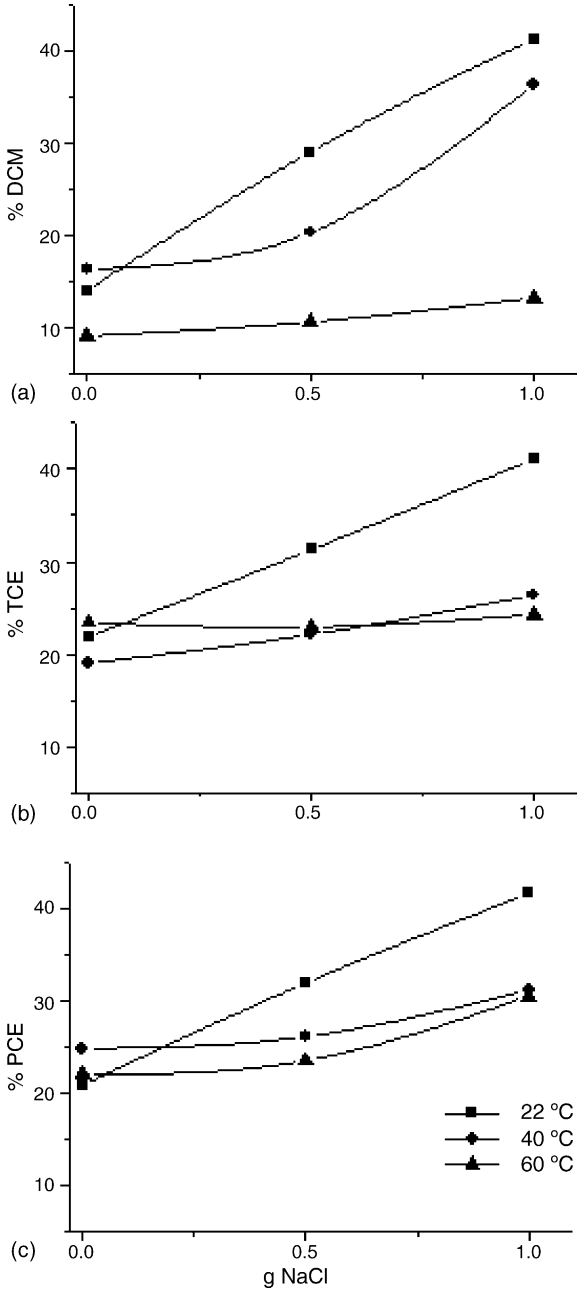


Fig. 1. Effect of the interaction between the addition of salt and the temperature of sampling on the extraction efficiency of dichloromethane (DCM), tri- (TCE) and tetrachloroethylene (PCE): (a) DCM, (b) TCE and (c) PCE, determined using a 75 μm Carboxen/PDMS fiber. An increase in the sampling temperature was found to reduce the mass of analyte extracted. For DCM, this effect was negligible at 40 °C and relevant only at 60 °C, due to its higher solubility in urine.

Table 2
Linear ranges, slopes, correlation coefficients ($r^2$) and limits of detection (LODs) of the HS-SPME-GC–MS method for the determination of chlorinated solvents in urine[a]: dichloromethane (DCM), tri- (TCE) and tetrachloroethylene (PCE), calculated under SIM conditions using two ions

| Compound | Ions, *m/z* Q, q (ratio)[a] | Range (μg/l) | a[b] | $r^2$ | LODs (μg/l)[c] |
|---|---|---|---|---|---|
| DCM | 49, 84 (1.5) | 0.01–100 | 0.46 ± 0.08 | 0.995 | 0.005 |
| TCE | 130, 132 (1.2) | 0.01–100 | 1.12 ± 0.02 | 0.997 | 0.005 |
| PCE | 166, 164 (1.2) | 0.01–100 | 1.20 ± 0.02 | 0.995 | 0.005 |

[a] Q, quantifier; q, qualifier; ratio, Q/q.
[b] Calibration fitting: $y = ax$ ($n = 12$); ±values are confidence intervals for 95% probability level.
[c] Limits of detection (S/N = 3) calculated under SIM conditions.

Table 3
Intra- and inter-day precision ($n = 6$) of the SPME-GC–MS method calculated at three concentration levels for dichloromethane (DCM), tri- (TCE) and perchloroethylene (PCE)

| Compound | Concentration (μg/l) | Precision (%) | |
|---|---|---|---|
| | | Intra-day | Inter-day |
| DCM | 0.01 | 3.8 | 6.5 |
| | 1 | 4.6 | 6.4 |
| | 100 | 2.3 | 6.1 |
| TCE | 0.01 | 7.9 | 6.1 |
| | 1 | 5.1 | 7.2 |
| | 100 | 6.0 | 7.0 |
| PCE | 0.01 | 7.9 | 6.1 |
| | 1 | 5.1 | 7.2 |
| | 100 | 6.0 | 7.0 |

Values are expressed as %R.S.D.

applying optimized HS-SPME conditions were much lower than those indicated in previous studies dealing with biological samples [28,29]. The precision of the method, calculated as intra- and inter-day reproducibility at three different levels of concentration (0.01, 1.0 and 100 μg/l) was between 1% and 8% (Table 3).

Table 4
Urinary concentration (μg/l) of DCM, TCE and PCE determined by SPME-GC–MS in the unexposed general population (120 subjects; mean age, 38.6 ± 6.6 years)

| Compound | Percentage of positive samples | Mean | S.D. | Median | Range |
|---|---|---|---|---|---|
| DCM | 88 | 0.78 | 0.44 | 0.64 | 0.27–2.22 |
| TCE | 72 | 0.41 | 0.58 | 0.22 | 0.02–3.64 |
| PCE | 68 | 0.08 | 0.11 | 0.05 | 0.01–0.70 |

### 3.3. Applications

The present method was developed for the simultaneous determination of DCM, TCE and PCE, although co-exposures to mixtures of these substances are rather uncommon in occupational toxicology. Nevertheless, due to the high sensitivity and its wide linear dynamic range, the method was suitable for the determination of background urinary levels of halogenates in the general population and for the characterization of both chronic and acute occupational exposures, as demonstrated by the casuistics reported below.

The results of the analytical determinations in urine samples from the general population are reported in Table 4.

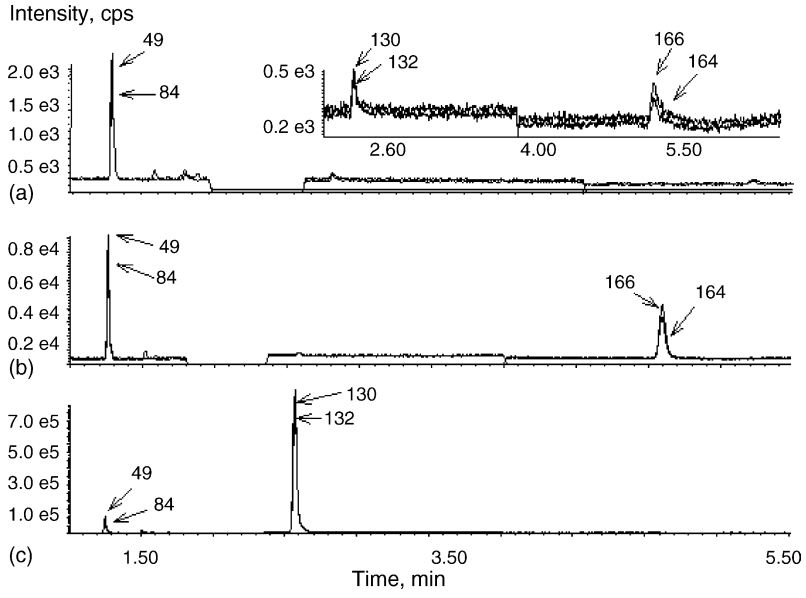


Fig. 2. HS-SPME-GC–MS SIM chromatograms of urine samples from a non-exposed person (a) and from workers exposed to PCE (b) and TCE (c). Ion used for quantitation: DCM, *m/z* 49 (quantifier, Q) and 84 (qualifier, q); TCE, *m/z* 130 (Q) and 132 (q); PCE, *m/z* 164 (q) and 166 (Q). Analyte concentrations: (a) DCM (0.35 μg/l), TCE (0.006 μg/l), PCE (0.007 μg/l); (b) DCM (1.08 μg/l), PCE (1.27 μg/l); (c) DCM (1.75 μg/l), TCE (8.34 μg/l).

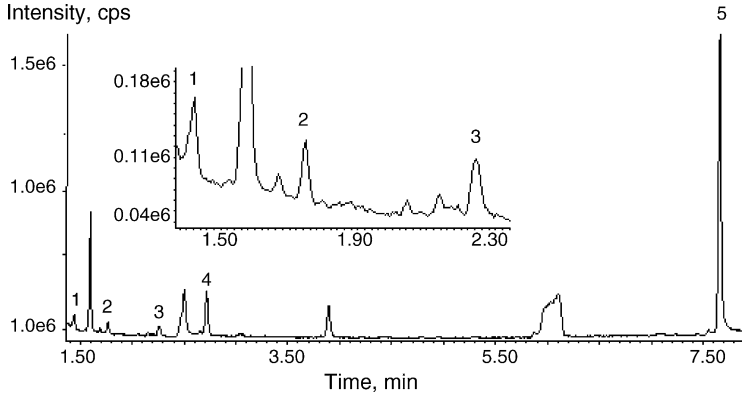


Fig. 3. HS-SPME-GC–MS chromatogram of a urine sample from a worker exposed to TCE acquired in the scanning mode (scan range, *m/z* 30–300). For other conditions: see the experimental section. Peak identification: (1) 1,2-dichloroethylene; (2) chloroform; (3) IS; (4) TCE; (5) trichloroethanol.

DCM, TCE and PCE were positively detected in the 83%, 72% and 68% of samples, respectively. Fig. 2a shows the chromatogram of a urine sample from a non-exposed subject. DCM concentration was 0.35 μg/l, while both TCE and PCE concentrations were close to the LODs (0.006 and 0.007 μg/l, respectively). Having excluded both in situ generation and contamination from reagents, our results show the uptake of small amounts of DCM, TCE and PCE in the general population. Detectable concentrations of TCE and halomethane, but not DCM and PCE were found in tap water [30,31]. Since chlorination is a widely used procedure for water disinfection in Italy, where the control population was recruited, laboratory experiments were performed to evaluate its possible contribution to the formation of organo-halogenates in tap water. Chlorination neither increased TCE levels nor led to de novo production of PCE and DCM, even when it was performed under "excessive" conditions (2 ppm of sodium hypochlorite added to water).

The HS-SPME-GC–MS method was then applied to the quantitative determination of unchanged PCE in the urine from 39 workers employed in the dry cleaning industry. The values we found were very low (median, 0.58 μg/l; range, 0.27–1.85 μg/l) and were consistent with very low exposure levels, although the median and the lower limit of the range were about 10-fold higher than those of the control group (Table 4). Fig. 2b shows the HS-SPME-GC–MS chromatogram of a urine sample of a worker exposed to PCE (PCE concentration, 1.27 μg/l; DCM, 1.08 μg/l).

For the group of workers exposed to TCE, the mean urinary concentration measured by SPME-GC–MS was $9.32 \pm 7.42$ μg/l ($n = 5$) and the corresponding TCAA concentration was $18.9 \pm 17.8$ mg/g creatinine. Airborne mean concentration of TCE, estimated using the regression equation proposed by Imbriani et al. [18], was 38.9 mg/m$^3$ (or 7.2 ppm). A representative chromatogram of a urine sample from a worker exposed to TCE is shown in Fig. 2c (TCE con-

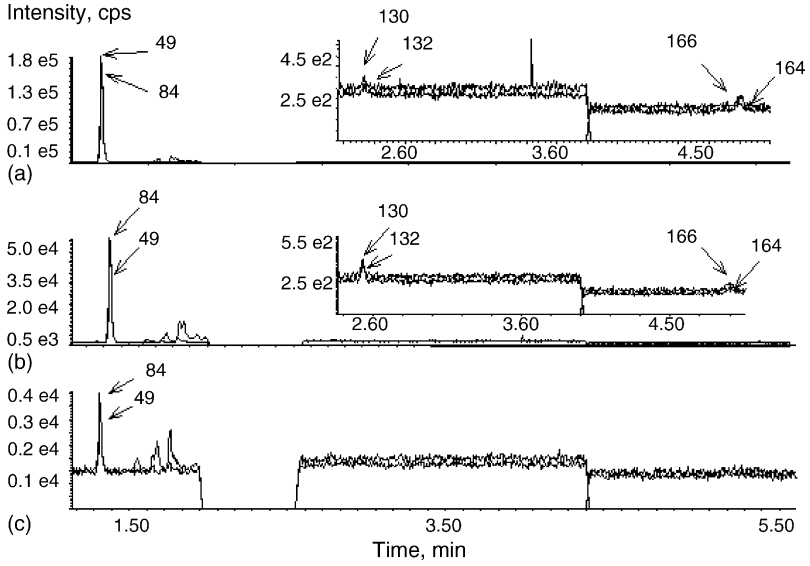


Fig. 4. HS-SPME-GC–MS SIM analysis of DCM in full blood samples from subject A (a), subject B (b) taken 36 h after the accident, and from a non-exposed person (c). Ion used for DCM quantitation: *m/z* 49 (Q) and 84 (q). DCM concentrations: (a) 47.2 μg/l, (b) 6.8 μg/l and (c) 0.5 μg/l.

centration, 8.34 μg/l; DCM, 1.75 μg/l; PCE, <0.005). Unlike PCE, TCE is rapidly and efficiently metabolized. Besides the unchanged substance, other chlorinated compounds, such as TCE metabolites and other contaminants present in the working environment were sampled by Carboxen/PDMS and identified when MS acquisition was performed in the scanning mode. As an example, Fig. 3 shows the HS-SPME-GC–MS chromatogram of a urine sample from a worker exposed to TCE, where TCE, trichloroethanol, and some known contaminants of industrial TCE, like 1,2-dichloroethylene and chloroform, were detectable.

Finally, we present the application of HS-SPME-GC–MS in a case of acute accidental exposure to large volumes of DCM and other solvents (acetone and styrene) involving two workers, subjects A and B, which were cleaning a tank normally used to contain polystyrene [21]. Urinary and blood concentrations of DCM were determined in samples collected starting from 12 h after the episode. As an example, the chromatograms of blood samples obtained 36 h after the accident from subjects A and B, together with a chromatogram of a blood sample from a non-exposed person are shown in Fig. 4. The corresponding blood DCM concentrations were 47.2, 6.8 and 0.5 μg/l. It should be noted that sampling conditions were not optimized for blood analysis, but simply adapted starting from those applied in the case of urine. Moreover, the efficiency of HS-SPME extraction of DCM from blood was lower, probably due to its higher lipid content. Fig. 5 shows the second part of the elimination kinetic of DCM in urine (a) and in blood (b). It should be noted that sampling conditions were not optimized for blood analysis, but simply adapted starting from those applied for urine. It is known that elimination of DCM through expiration is very fast [32]. The calculated half-times of urinary (7.5 and 3.8 h for subjects A and B, respectively) and blood (8.1 and 4.3 h) DCM clearly show that the first part was lost; nevertheless, high DCM concentrations were determined even 12 h after the accident. Owing to the peculiar characteristics of the HS-SPME-GC–MS method, e.g., the high sensitivity, the wide linear dynamic range and the large capability of the fiber, it was possible to monitor the follow-up of urine and blood DCM starting from the highest concentrations up to physiological DCM values, comparable to those found in the general unexposed population. The differences observed in the elimination kinetics of DCM for the two subjects were probably due to inter-individual differences in the BMI as well as in their different metabolic capacity.

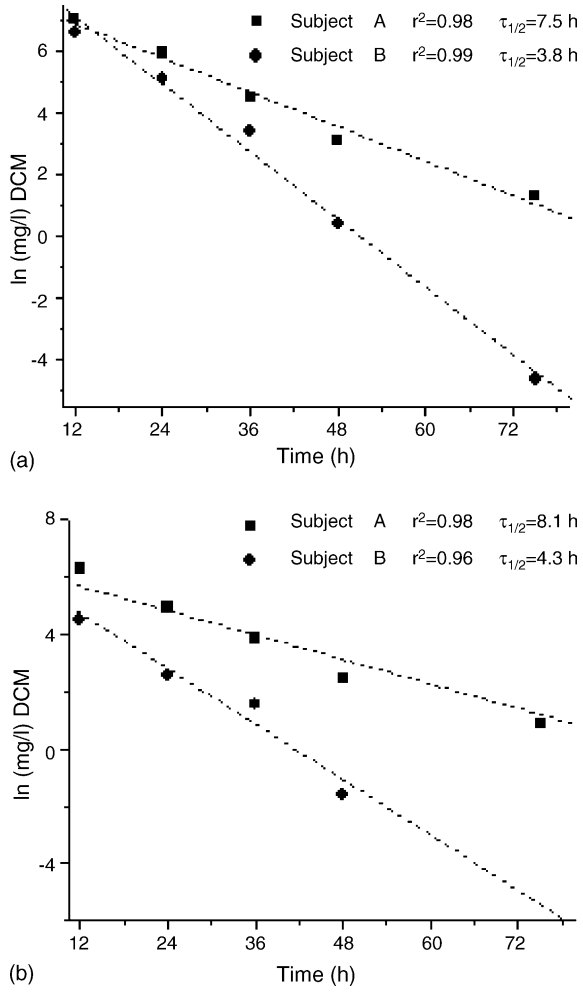


Fig. 5. Elimination curves of unchanged dichloromethane (DCM). Plot of urine (a) and blood concentrations (b) of DCM (ln μg/l) vs. time (h) occurred after the accident for subject A (■) and subject B (●). Quantitative blood analysis was performed by spiking a blank blood sample with DCM concentrations in the ranges 0.1–50 and 50–600 μg/l.

## 4. Conclusions

Our findings suggest that the HS-SPME-GC–MS method is suitable and reliable to detect the presence of DCM, TCE and PCE in human urine samples from occupationally exposed individuals, as well as from the general unexposed population. Background concentrations of these analytes were found in about the 70–80% of analyzed urine samples obtained from the general population.

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