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In matrix derivatization of trichloroethylene metabolites in human plasma with methyl chloroformate and their determination by solid-phase microextraction–gas chromatography-electron capture detector

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ABSTRACT

Trichloroethylene (TCE) is a common industrial chemical that has been widely used as metal degreaser and for many industrial purposes. In humans, TCE is metabolized into dichloroacetic acid (DCA), trichloroacetic acid (TCA) and trichloroethanol (TCOH). A simple and rapid method has been developed for the quantitative determination of TCE metabolites. The procedure involves the *in situ* derivatization of TCE metabolites with methyl chloroformate (MCF) directly in diluted plasma samples followed by extraction and analysis with solid-phase microextraction (SPME) coupled to gas chromatography-electron capture detector (GC-ECD). Factors which can influence the efficiency of derivatization such as amount of MCF and pyridine (PYR), ratio of water/methanol were optimized. The factors which can affect the extraction efficiencies of SPME were screened using 2^{7-4} Placket–Burman Design (PBD). A central composite design (CCD) was then applied to further optimize the most significant factors for optimum SPME extraction. The optimum factors for the SPME extraction were found to be 562.5 mg of NaCl, pH at 1 and an extraction time of 22 min. Recoveries and detection limits of all three analytes in plasma were found to be in the range of 92.69–97.55% and 0.036–0.068 $\mu g\,mL^{-1}$ of plasma, respectively. The correlation coefficients were found to be in the range of 0.990–0.995. The intra- and inter-day precisions for TCE metabolites were found to be in the range of 2.37–4.81% and 5.13–7.61%, respectively. The major advantage of this method is that MCF derivatization allows conversion of TCE metabolites into their methyl esters in very short time ($\leq$30 s) at room temperature directly in the plasma samples, thus makes it a solventless analysis. The method developed was successfully applied to the plasma samples of humans exposed to TCE.

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1. Introduction

Trichloroethylene (TCE) is a volatile organic chemical mainly used as industrial solvent in automotive and metal industries for vapor degreasing and cold cleaning of metal parts. The wide use of TCE by the workers of metal industries for cleaning purposes leads to high exposure of TCE. Dichloroacetic acid (DCA), trichloroacetic acid (TCA) and trichloroethanol (TCOH) are three major metabolites of TCE. DCA and TCA are additionally encountered in drinking water as chlorination disinfection by-products [1]. Animal studies demonstrated the carcinogenicity of TCE through its metabolite activation in mice liver [2,3].

Quantitative determination of TCE and its major metabolites in biological matrices will be used as a biomarker of TCE exposure which is necessary for medical and clinical monitoring and to evaluate their carcinogenicity. Several analytical methods have been reported for the determination of TCE metabolites. The first method was based on Fujiwara reaction and spectrophotometric determination [4]. Ion-exclusion chromatographic method was reported for the direct determination of TCOH in plasma and urine samples, however the LOQ of the method was

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very high (3 mg L^{-1}) [5]. Gas chromatographic determination of TCE metabolites requires derivatization due to their low volatility and polar nature. Derivatizing reagents such as BF_3–methanol, diazomethane, acidic methanol and ethanol [6–9] have been often used for the derivatization of TCE metabolites, but these reagents require longer reaction time and elevated reaction temperatures.

Alkyl chloroformates (ACF) are known for years as rapid esterification reagents in aqueous medium [10]. The remarkable advantages of ACF derivatizing reagent over the other derivatization techniques are, (a) the reaction can perform directly in aqueous medium, (b) derivatization is completed in less than a minute, (c) can occur at room temperature and (d) the non-polar derivatives formed can directly extracted with SPME [11]. ACF derivatization has been widely employed for the conversion of polar compounds to their non-polar derivatives. Analytes containing different polar functional groups such as phenolic hydroxy, carboxyl, amines and amino acids are derivatized with ACFs [12–14].

Determination of TCE metabolites in biological samples like plasma samples requires extensive sample preparation before analysis. Liquid–liquid extraction has been widely used for the extraction of derivatives of TCE metabolites [8]. In recent years, SPME has also been applied for the same purpose due to its advantages like solvent free system and requires very few amount of sample [9,15,16]. The extraction factors of SPME such as extraction temperature and time, desorption temperature and time, pH, ionic strength and head space volume were screened by multivariate strategy based on an experimental design using a Placket–Burman Design (PBD). The SPME factors screened were further optimized using central composite design (CCD) approach. So far, no analytical procedure based on MCF derivatization has been reported for the determination of TCE metabolites in human plasma samples. In the present study, we report a simple and rapid analytical method for the determination of TCE metabolites in human plasma samples based on *in matrix* derivatization with MCF followed by SPME extraction and GC-ECD analysis.

2. Experimental

2.1. Chemicals and reagents

All chemicals and reagents used in this study were of analytical grade unless otherwise stated. The standard DCA, TCA and TCOH were purchased from Sigma (St. Louis, MO, USA). MCF, pyridine and all other solvents used in this study were procured from Merck (Darmstadt, Germany). The SPME holder and polydimethylsiloxane (PDMS) fiber (100 μm film thickness), extraction vials, septa and aluminum caps were obtained from Supelco (Bellefonte, USA). The SPME–PDMS fiber was conditioned at 250 °C for 30 min according to the procedure recommended by the manufacturer. Plasma samples were collected from healthy volunteers for the purpose of method validation and application. Ultra pure water produced from Milli-Q water purification system (Millipore, Bedfore, MA, USA).

Stock solution of DCA, TCA and TCOH were prepared in ultra pure water at a concentration of 1 mg mL^{-1} and stored at 4 °C until analysis. Working standard solution was prepared every time before analysis by appropriate dilution of stock solution.

2.2. Subjects and blood collection

Blood was taken through venipuncture from trichloroethylene exposed subjects from a lock industry where trichloroethylene was used as a metal degreaser. The institutional ethical committee approval was obtained to conduct the study. A detailed informed consent was obtained from each subject. The exposure of subjects was for the duration of 4–20 years. Approximately 1 mL of blood was collected from each subject in EDTA coated vials (B.D. Vacutainer). These samples were placed on ice and plasma was separated through centrifugation at the speed of 2000 × g for 5 min. Plasma was aspirated to new collection tube and stored at −80 °C until use for processing.

2.3. Chromatographic conditions

The analysis of TCE metabolites were performed on Perkin Elmer Clarus 500 gas chromatograph equipped with DB-5 (5% phenyl methyl polysiloxane, 25 m × 0.25 mm I.D. × 0.25 μm film thickness) capillary column and an electron capture detector (ECD) operated at 375 °C. High purity nitrogen (99.999%) was used as carrier gas at a flow rate of 1 mL min^{-1} and also as a makeup gas for ECD at a flow rate of 30 mL min^{-1}. The oven temperature was programmed initially from 80 °C to 100 °C at a rate of 2 °C min^{-1} (hold for 3 min), it was further increased up to 280 °C at a rate of 45 °C min^{-1} and was held for 5 min (total run time 22 min). The GC injector port was held at 200 °C to allow complete vaporization of analytes and operated in split mode at split ration of 1:5 [9].

The confirmation of derivatization of TCE metabolites with MCF was achieved by analyzing the derivatives of TCE metabolites standard dissolved in Milli-Q water on Trace GC Ultra coupled to TSQ Quantum XLS mass spectrometer (Thermo Scientific, FL, USA) equipped with Elite-5MS capillary column (60 m × 0.25 mm i.d × 0.25 μm film thickness of 5% phenyl and 95% methylpolysiloxane). The GC oven was kept at an initial temperature of 50 °C for 5 min and increased at a rate of 10 °C min^{-1} up to 100 °C and held for 10 min. Helium was used as carrier gas at a flow rate of 1 mL min^{-1}. One μL of the derivatized sample was injected at an injector temperature of 200 °C. The mass spectrometer was operated using electron impact (EI) ionization mode (70 eV). Transfer line and source temperature were kept at 290 and 220 °C, respectively. The derivatization products were confirmed by comparing the mass spectra obtained from standard to their mass spectras available in instrument library i.e. NIST library.

2.4. Statistical data handling and processing

Designs of experiments (PBD and CCD) were constructed and the results were evaluated using the statsoft statistical software package "Statistica 10.0" (Tulsa, OK, USA). Calculations of optimized responses were based on the sum of the area of all the peaks obtained during GC–MS analysis [17,18].

2.5. Derivatization and extraction

The derivatization of TCE metabolites were carried directly in plasma samples after diluting with ultrapure water. An aliquot of plasma sample (100 μL) was mixed with 500 μL of methanol and 500 μL of water. To the resultant mixture, 200 μL of PYR was added as a catalyst followed by the addition of 150 μL of MCF twice (a total of 300 μL) in order to ensure complete derivatization of TCE metabolites and the reaction carried at room temperature for 30 s. The derivatized sample was then placed in 10 mL SPME vial and diluted with ultrapure water up to 3 mL to get the head space volume of 7 mL. The pH of the sample at this stage was 1 and adjusted with 5 M NaOH for optimization studies using design of experiments. To enhance the ionic strength of the sample 562.5 mg of NaCl was added. Then the sample was exposed to PDMS fiber for 22 min at 50 °C. After extraction of non-polar derivatives of TCE metabolites, the fiber was pulled back into the needle and inserted into the GC injector port for desorption of analytes for 1 min at 200 °C.

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