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Journal of Chromatography B, 783 (2003) 319–325

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Trace level determination of trichloroethylene from liver, lung and kidney tissues by gas chromatography–magnetic sector mass spectrometry

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Received 28 May 2002; received in revised form 30 July 2002; accepted 30 July 2002

Abstract

Trichloroethylene (TCE) is a common industrial chemical that has been heavily used as a metal degreaser and a solvent for the past 100 years. As a result of the extensive use and production of this compound, it has become prevalent in the environment, appearing at over 50% of the hazardous waste sites on the US EPA's National Priorities List (NPL). TCE exposure has been linked to neurological dysfunction as well as to several types of cancer in animals. This paper describes the development and validation of a gas chromatography–mass spectrometry (GC–MS) method for the quantitation of trace levels of TCE in its target tissues (i.e. liver, kidney and lungs). The limit of quantitation (5 ng/ml) is substantially lower than currently published methods for the analysis of TCE in tissues. The % RSD and % Error for the assay falls within the acceptable range (<15% for middle and high QC points and <20% for low QC points), and the recovery is high from all tissues (>79%).

Keywords: Trichloroethylene

1. Introduction

Trichloroethylene is most commonly used in industrial settings as a general-purpose solvent for lipophilic compounds and to remove grease from machinery. Known by the trade names of Vitran® and Triclene®, TCE also has many applications in household products, dry cleaning, taxidermy, and as a chemical intermediate [1,2]. Environmental releases of TCE are most commonly associated with vapor degreasing operations, but can also be linked to waste and water treatment facilities and landfills [2]. TCE contamination of ground and surface waters is a result of industrial discharge or leaching from hazardous waste sites [1]. According to the Third National Health and Nutrition Examination (NHANES III), an estimated 10% of the US population has detectable levels of TCE in their blood [3]. Pharmacokinetic models relating environmental concentrations of TCE to body burdens suggest that the prevalence of TCE in the general population is a result of multiple exposure routes including water

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1570-0232/02/$ – see front matter
PII: S1570-0232(02)00546-9

ingestion, inhalation, dermal absorption, and ingestion of TCE-contaminated food [4].

The main health risk associated with mild acute TCE exposure is central nervous system (CNS) depression. At vapor levels higher than 100 ppm, CNS effects such as sleepiness, headache, and dizziness can occur [1,5]. Coma, cardiac arrythmias, and even death are associated with very high acute TCE exposures [1]. Chronic rodent studies and epidemiological evidence suggests that chronic, high-level TCE exposures may cause liver, kidney, and lung cancer. There is more limited epidemiological evidence of increased incidences of non-Hodgkin's lymphoma, cervical cancer, testicular cancer, and multiple myeloma in humans [1,6]. Although TCE is a known carcinogen in rats and mice, it has been officially classified by the National Toxicology Program (NTP) and by the International Agency for Research on Cancer (IARC) as a ''probable carcinogen in humans'' because of the limited epidemiological data to support TCE as a cause of cancer in humans [6,8,9]. Although the subject is controversial, a number of leading authorities feel that environmentally-relevant concentrations of TCE are not likely to be a significant cancer risk [7,8,10].

Several analytical methods exist for the quantitation of TCE in water. The EPA has a GC–ECD method for determination of TCE and several other halogenated hydrocarbons that uses liquid–liquid extraction sample preparation [11]. The recommended extraction solvent for this method is methyl-*t*-butyl ether (MTBE), but this solvent is frequently contaminated with traces of TCE. Karp [12] describes a method with a TCE detection limit of 1 ng/ml in water, but there is no mention of validation or the type of instrument that was used. Zoccolillo and Rellori report quantitating TCE at levels below 1 ng/l, but their method is not validated and requires a sample of at least 1 l [13]. This far exceeds volumes of sample that can be secured for a bioanalytical assay. Purge-and-trap instrumentation has also been utilized to analyze trace levels of TCE and similar compounds, but these procedures involve time-consuming methods [14,15].

Quantitation of drugs or chemicals in a biological matrix is much more difficult than analysis in water. Chen et al. describe a GC–ECD method that is useful for analyzing TCE in several tissues including liver, kidney, and lungs. They indicate a limit of detection of 50 ng/ml, which is expressed as 1 ng on-column [16]. Muralidhara and Bruckner report a rapid assay for the measurement of TCE and its metabolites from blood [17]. Their LOQ is 50 ng/ml but it lacks complete validation data.

The ability to monitor the time-course of TCE in tissues is of particular importance to toxicologists and risk assessors. There is a limited amount of pharmacokinetic data generated from relatively high-level TCE exposures found in occupational settings. It has not been possible, however, to study the systemic uptake and disposition of trace levels of TCE typically encountered in environmental media (i.e. air and water). An assay that can accommodate the exposures at the lower end of the dose–response curve is needed to help provide more accurate information for cancer risk assessments. Recent papers on the development of physiologically-based pharmacokinetic (PBPK) models for TCE state that tissue concentration data for the three primary target organs (i.e. liver, kidney and lungs) would be necessary to develop and validate useful models [18,19]. The present paper describes an assay that may help meet the needs of the toxicologists and kineticists who struggle to obtain such data. This method has the potential for high throughput of samples with its simple extraction procedure and fast run-time. It is more sensitive than previously reported assays for quantitation of TCE from tissues and requires a very small sample size. Most importantly, this assay has been validated to measure TCE concentrations in three target tissues, thus guaranteeing precision and accuracy at environmentally-relevant exposure levels.

2. Experimental

2.1. Reagents and chemicals

Analytical grade trichloroethylene (TCE) was purchased from Aldrich (Milwaukee, WI, USA). Reagent grade anhydrous diethyl ether was obtained from J.T. Baker (Phillipsburg, NJ, USA). The perfluorokerosene used as a calibrant for the mass spectrometer was purchased from Sigma (St. Louis, MO, USA). The deionized water used was generated

from a Continental deionized water system (Natick, MA, USA). The helium used as a carrier gas for the GC was purchased from National Welders (Charlotte, NC, USA). Alkamus, the emulsifying agent used in preparing the doses for the animal study, was obtained from Rhone-Poulenc (Cranbury, NJ, USA).

2.2. Preparation of stock and standard solutions

A stock solution of TCE was prepared in deionized water to yield a final concentration of 10 μg/ml TCE. Standard solutions for the calibration curve were prepared from the stock solution in the following concentrations: 1 μg/ml, 600, 400, 200, 100, 50, 25, 10, 5 and 1 ng/ml. Standards used to assess precision and accuracy were prepared in deionized water from the 10 μg/ml stock solution in concentrations of 750, 75 and 7.5 ng/ml. All stock and standard solutions were refrigerated at 4 °C during the day of use and were prepared fresh daily.

2.3. GC–MS system and conditions

All GC experiments were conducted with the use of a Hewlett-Packard (Agilent) 5890 Series II gas chromatograph (Palo Alto, CA, USA) interfaced with a Micromass AutoSpec Magnetic Sector Mass Spectrometer (Manchester, UK). The electron energy in the electron ionization source of the mass spectrometer was set at 70 eV. A resolution of 1500 was used. The mass spectrometer was calibrated daily using perfluorokerosene (PFK). All samples were injected using a LEAP Technologies CTC-A200S Autosampler (Carrboro, NC, USA). Chromatographic separations were achieved on a DB-5ms capillary column (30 m×0.25 mm I.D., 0.25 μm film thickness) from J&W Scientific (Agilent). The temperature program for the GC was isothermal heating at 35 °C for 4 min. The injector temperature was set at a constant 100 °C. Helium was used as the carrier gas. The retention time for TCE was ~3.5 min.

2.4. Quantitation

TCE peaks (m/z 129.9144) were monitored using the SIR voltage experiment in the Micromass OPUS software (equivalent to selected ion monitoring or SIM) using a PFK peak of m/z 130.99202 as the lock mass. Concentrations of TCE in real samples were calculated using an external calibration curve prepared with spiked blank tissue homogenates. JMP statistical software was used to generate linear regression equations for all calibration curves. Each curve contained the following points ($n=9$): 1 μg/ml, 600, 400, 200, 100, 50, 25, 10 and 5 ng/ml.

2.5. Liquid–liquid extraction

All tissue samples were prepared using liquid–liquid extraction with anhydrous diethyl ether. Prior to extraction, tissues were homogenized with two volumes of deionized water (w/v) using a Tekmar tissue grinder (model SDT-1810, Cincinnati, OH, USA). A 100-μl volume of tissue homogenate plus 200 μl of ether (or 100 μl blank tissue homogenate plus spike solution plus 200 μl ether) were combined in a glass tube for extraction and sealed with parafilm. Plastic tubes were found to adsorb TCE to some extent; therefore glass tubes were used consistently throughout the experiments. The tissue/ether mixture was vortexed for 10 s using a Scientific Industries Vortex Genie 2 (Bohemia, NY, USA). The samples were then centrifuged at 2200 *g*, 4 °C for 15 min in a Jouan CR422 refrigerated centrifuge (Winchester, VA, USA). The ether layers were immediately transferred to autosampler vials and analyzed. The samples were always kept on ice during the physical transfer of sample vials due to the highly volatile nature of TCE.

2.6. Solvent selection

During the method development stage of this project, several solvents were investigated as potential liquid–liquid extractants. Initially methyl-*t*-butyl ether (MTBE) was used according to the EPA method 551.1 for drinking water analysis [10]. Upon observation of a high response for TCE from the ''blank'' solvent injections, we discovered that a majority of MTBE batches are highly contaminated with TCE. Multiple fractional distillations became necessary to prepare MTBE for use, and this was ultimately deemed unacceptable. A limited survey of solvents located in our laboratory showed that TCE contamination is not restricted to MTBE (see Table 1). Finally, diethyl ether was chosen as the best

Table 1
Estimated trichloroethylene levels found in various solvent types

	Concentration (ng/ml)	Manufacturer
Acetonitrile	1.21	J.T. Baker
Acetonitrile	1.80	J.T. Baker
Acetonitrile	2.11	Fisher
Acetonitrile.	1.97	Aldrich
Acetonitrile	1.46	Fisher
Methyl-*t*-butyl ether	730.6	Aldrich
Methyl-*t*-butyl ether	1.75	Aldrich
Diethyl ether	4.17	J.T. Baker
Diethyl ether	1.00	J.T. Baker
Diethyl ether	0.378	J.T. Baker
Heptane	3.39	E.M. Science
n-Hexane	2.38	J.T. Baker

extraction solvent. Not only does it provide acceptable recovery, but it also can be purified by a single distillation.

2.7. Sampling

Male Sprague–Dawley rats (Charles River Laboratories, Wilmington, NC, USA) weighing an average 277 ± 11 g ($n = 30$) were used for a tissue disposition study. An emulsion of 0.55 mg/ml TCE was prepared by combining 15.2 μl pure TCE with 2.0 ml Alkamus and 38.0 ml of physiological saline. An appropriate volume of the emulsion, based on the weight of each rat, was administered to yield a final dose of 2.0 mg/kg. The animals were divided into six groups of five rats each. Members of each group were dosed orally using a curved gavage needle. Groups were sacrificed by cervical dislocation at 2, 5, 10, 30, 60 and 120 min postdosing. The liver, kidney and lungs were perfused in situ with cold saline to remove as much blood as possible. Each tissue specimen was weighed and homogenized with two volumes of cold deionized water. All samples were analyzed immediately.

3. Results and discussion

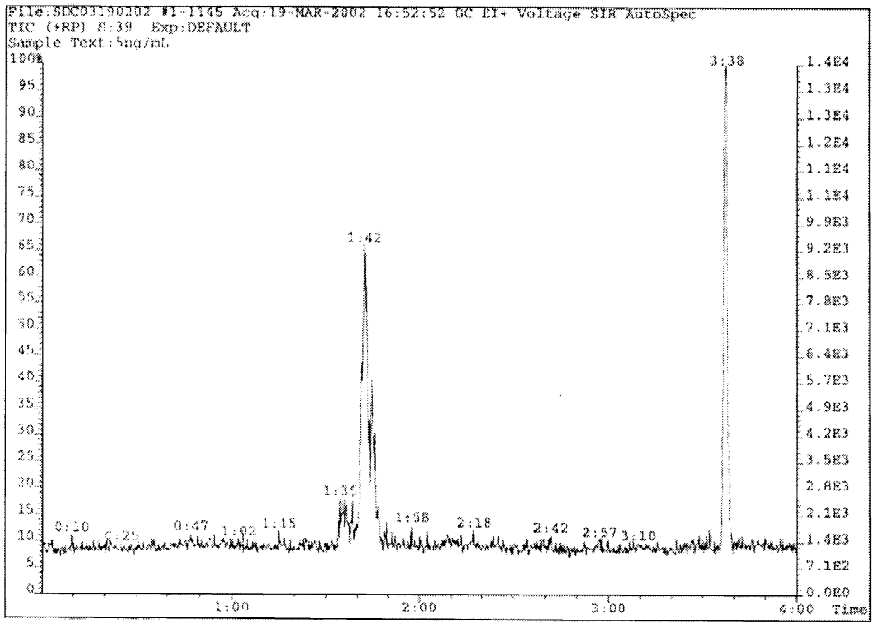
Fig. 1a and b shows a representative chromatogram of TCE at 5 ng/ml, the lowest point on the calibration curve (LOQ), extracted from a liver tissue homogenate and a chromatogram from a blank (liver) tissue extract. Because the experiments were done using the SIR voltage function, no interfering matrix peaks can be seen. This also helps maximize sensitivity of the assay by eliminating the need to scan a large range of masses.

Calibration curves were produced during each day of validation and during the analysis of the samples from the animal study. Since the calibration curve encompassed such a wide range (5 ng/ml–1 μg/ml), the points on the curves were weighted by a factor of $1/y$ using JMP statistical software to ensure that all points contributed equally to the slope of the regression line. The range of concentrations in the curve encompasses the range of concentrations present in the various tissues in a 2-h period following administration of the 2-mg/kg oral bolus dose.

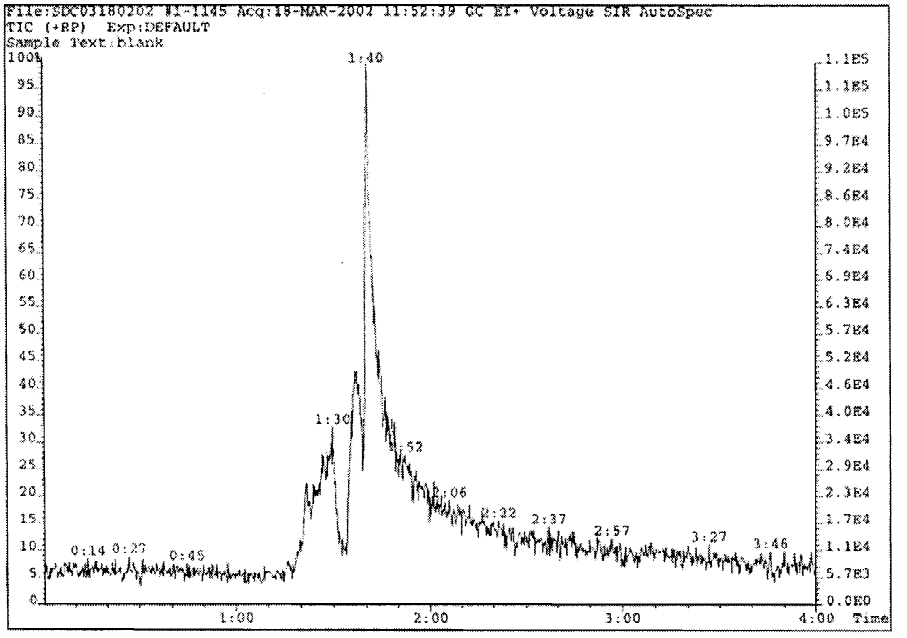
The limit of detection (LOD) for TCE in the tissue matrices was determined to be 1 ng/ml according to the 3:1 signal-to-noise ratio seen at this concentration. The assay was validated by analyzing five replicates of three different concentrations of TCE in spiked tissue over a period of 3 days. The concentrations of 7.5, 75 and 750 ng/ml were chosen to represent low, middle, and high portions of the curve. The precision (RSD) represents the reproduceability of the assay while the accuracy (error) shows how well the assay can predict concentrations correctly. Table 2 summarizes the validation data that were collected. All RSD and error values were $<15\%$ for the middle and high QC points and $<20\%$ for the lowest QC point for each day.

Recovery of TCE from the various tissues was measured by comparing the responses from spiked samples to the responses from ether standards. Five samples from each matrix homogenate were each spiked with 100 ng/ml TCE. The peak heights from each of these was compared to the peak heights of five ether standards. The recovery from lung was $79.20 \pm 10.8\%$, kidney was $79.93 \pm 14.2\%$, and liver was $87.23 \pm 2.78\%$.

The lung, liver and kidney tissues that were collected from the test rats were extracted and analyzed as described above. The peak heights of the TCE peaks from the real samples were compared to the calibration curve to calculate concentrations of TCE in these target tissues. Fig. 2 shows a concentration versus time profile of TCE in the three tissue matrices. The elimination phase in these tissues is rather lengthy compared to the distribution



(a)



(b)

Fig. 1. (a) Representative chromatogram of 5 ng/ml TCE (LOQ) from liver homogenate; (b) representative chromatogram of blank liver extract.

Table 2
Precision (RSD) and accuracy (error) of TCE in rat liver, kidney, and lung tissue

Concentration (ng/ml)		RSD (%)	Error (%)
TCE added	TCE found		
Liver tissue validation ($n=15$)			
7.5	7.93±1.6	19.9	18.6
75	75.7±8.7	11.4	9.83
750	766.2±110	14.4	12.3
Lung tissue validation ($n=15$)			
7.5	7.15±0.87	9.27	12.8
75	74.5±9.2	3.61	10.4
750	718.6±99	9.48	11.9
Kidney tissue validation ($n=15$)			
7.5	7.26±0.86	8.59	8.95
75	76.0±8.9	8.67	11.0
750	715.7±84	9.24	13.8

phase. Some time points in the latest group (120 min) approached the limit of quantitation for this assay. The profiles shown here are very similar to concentration–time profiles of TCE in blood reported previously [20].

4. Conclusions

A sensitive, efficient, and validated method for the extraction and analysis of TCE in liver, kidney, and lung tissues is described. This method yields acceptable recovery, precision, and accuracy over the calibration range of 5 ng/ml to 1 μg/ml. Liquid–liquid extraction is a quick, efficient way to minimize evaporation of the volatile TCE analyte during preparation of tissue samples for GC–MS analysis. The use of the SIR voltage function in the data acquisition capabilities of the mass spectrometer enables the quantitation of trace levels of TCE due to the low noise level and the absence of interfering matrix peaks. The most sensitive assay for quantitating TCE from biological matrices is the purge-and-trap MS method used by the CDC [15]. By starting with a 5-ml blood sample, the CDC assay reaches an LOD of 5 pg/ml. The method reported in this manuscript begins with a much smaller sample volume and is still capable of reaching an LOD of 1 ng/ml. Although slightly less sensitive than the CDC method, this assay is capable of much higher throughput. This assay can effectively be applied to the quantitiation of trace levels of TCE in tissue samples.

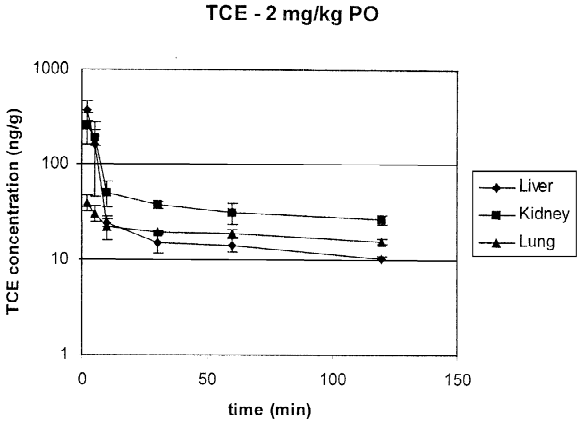


Fig. 2. Concentration versus time profile of liver, kidney, and lung concentrations of TCE from rats dosed with 2 mg/kg oral TCE (mean concentration±standard deviation, $n=5$ for each time point).

Acknowledgements

This work was supported by ATSDR Contract no. 0000068164 and DOE Cooperative Agreement no. DE-FC02-02CH11109.

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