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Evaluation of dynamic headspace with gas chromatography/mass spectrometry for the determination of 1,1,1-trichloroethane, trichloroethanol, and trichloroacetic acid in biological samples

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Abstract

A sensitive and reproducible method is described for the analysis of trichloroacetic acid in urine and 1,1,1-trichloroethane in blood using dynamic headspace GC/MS. Samples were analyzed using the soil module of a modified purge and trap autosampler to facilitate the use of disposable purging vessels. Coefficients of variation were below 3.5% for both analytes, and response was linear in the range of 0.01–7.0 μg/ml for trichloroacetic acid and 0.9 ng/ml–2.2 μg/ml for 1,1,1-trichloroethane. Attempts at using dynamic headspace for the analysis of trichloroethanol in urine were unsuccessful.

Keywords: Methyl chloroform; Trichloroacetic acid; Trichloroethanol; Dynamic headspace; Purge and trap

1. Introduction

Trichloroethylene (TCE), tetrachloroethylene, 1,1,1-trichloroethane (methyl chloroform or MC), and chloral hydrate are all biotransformed in the human body through pathways mediated by the cytochrome P-450 monooxygenase system, or by alcohol dehydrogenase and aldehyde dehydrogenase to form trichloroethanol (TCEOH) and trichloroacetic acid (TCA). TCE and tetrachloroethylene are widely used as metal degreasers, in the dry cleaning industry, and in the manufacture of a variety of products such as adhesives and paint removers. Methyl chloroform has been used extensively in industry over the past 40 years as a replacement for other more toxic chlorinated solvents, such as TCE and tetrachloroethylene. However, its production and use is now being phased out because of its involvement in the depletion of stratospheric ozone [1]. Chloral hydrate is a drug used clinically as a sedative prior to surgery for adults, and for children who are undergoing a procedure where they must remain still, such as magnetic resonance imaging (MRI). Small quantities of all of these compounds can be found in drinking water either through groundwater contamination or as a byproduct of water chlorination.

In studying an individual's exposure to these chemicals, it is useful to consider not only the parent compound in biological tissues, but its metabolites as well. Trichloroacetic acid has a relatively long biological half life (∼80 h), and therefore, levels of TCA in blood or urine can be used to assess exposure to one or more of these chlorinated compounds even if that exposure took place days before [2]. It is also of interest to assess the levels of TCA and TCEOH to study potential health effects of these metabolites.

Many different assays have been developed for the determination of TCA and TCEOH in biological samples. The first were spectrophotometric methods based on the Fujiwara reaction. However, these are limited by low sensitivity and specificity [3–5]. Several investigators have described methods for the analysis of these metabolites using organic extraction and analysis by GC/MS or GC with electron capture

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detection (ECD) [6–8]. These methods have the advantage of being more sensitive and specific than spectrophotometry, but sample preparation can be a tedious process involving many steps and consequent losses. In addition, injecting an organic extract onto a GC column may contaminate the liner and column, resulting in the need for frequent maintenance. This can be particularly problematic if a derivatizing agent is required in the analysis. Solid phase microextraction (SPME) is a relatively new sample preparation technique involving the injection of a sorbent-coated fiber into a liquid sample or sample headspace. Organic components of the sample partition onto the fiber and are then thermally desorbed and injected onto a GC column. SPME has recently been used by Dehon et al. [9] for the analysis of TCA and TCEOH in biological samples. SPME can be a rapid, sensitive, and inexpensive method for analyzing organic compounds in biological samples. Reported disadvantages of SPME include sample carryover and low recovery [10].

Static headspace methods have been developed by many investigators for the analysis of TCA and TCEOH in biological samples [11–14]. Sealed vials containing samples are held at a constant temperature for a known period, and after the volatile agent has reached equilibrium between the gas phase and the sample phase, a small volume of headspace (0.025–1.0 ml) is injected onto a GC column. This can be a very convenient method for the analysis of volatile compounds in biological samples that avoids contaminating the GC column. However, absolute recovery and sensitivity is normally low due to the small volume of headspace analyzed.

Similar to static headspace, dynamic headspace analysis allows a sample to be analyzed using the gas phase above the sample. However, with dynamic headspace, the sample is continuously purged with an inert gas (usually helium) and the volatile components collected on a sorbent trap. The purging gas can either be bubbled through, or swept over the sample. After the sample has been purged, the trap is thermally desorbed and the volatile components are injected onto a GC column. Due to the complete stripping of the volatile organic compound from the sample and trapping of the analytes in dynamic headspace analysis, there is the potential for increased sensitivity over static headspace, as a larger fraction of the analyte in the sample will reach the GC.

The primary goal of this study was to develop and evaluate a method for the analysis of TCA in urine and MC in blood using dynamic headspace analysis. It was our initial objective to use dynamic headspace for the analysis of both TCA and TCEOH in urine. However, our attempts to develop a dynamic headspace method for TCEOH were unsuccessful as described below. Therefore, a secondary objective of this study was to establish an assay using organic extraction for the analysis of TCEOH in urine. The assays developed were to be used to assess TCA and TCEOH excretion over time following controlled human exposures to low levels of MC. In this exposure study, blood samples are taken frequently during and following exposure to evaluate the washout of MC in blood. To minimize the amount of blood taken from each subject, it was necessary to develop a method for the analysis of MC in blood using small sample volumes.

2. Experimental

2.1. Reagents

All reagents used in this study were reagent grade or better. 1,1,1-Trichloroethane; 2,2,2-trichloroethanol; and 2,2-dichloroethanol were purchased from Aldrich Chemical (Milwaukee, WI). *n*-Butyric acid was obtained from Mallinckrodt Inc. (St. Louis, MO). Methanol was purchased from Fisher Scientific (Fair Lawn, NJ), and concentrated sulfuric acid was obtained from J.T. Baker (Phillipsburg, NJ). $^{2}H_{3}$-1,1,1-Trichloroethane was obtained from Cambridge Isotope Laboratories (Andover, MA). Deionized water was prepared using a Barnstead (Boston, MA) NANOpure II water system.

3. Calibrant and sample preparation

3.1. Methyl chloroform in blood

Individual stock solutions of MC (0.9 μg/ml–2.21 mg/ml) were prepared by serial dilution with methanol containing $^{2}H_{3}$-methyl chloroform as an internal standard. Working standards were prepared by 1:100 dilution of stock solutions in water to reduce the amount of methanol added to each sample. We found that increasing the concentration of methanol in the samples resulted in a decrease in response (Fig. 1), presumably by either altering the partitioning of MC between the sample and its headspace, or through interactions of methanol with analytes on the absorptive trap. The internal standard was effective in controlling for the effect of

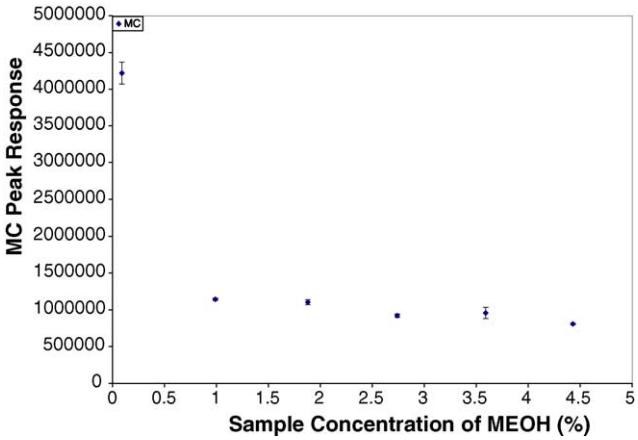

Fig. 1. Effect of adding increasing amounts of methanol on the response (mean ± S.D.; $n = 3$) of MC. MC concentration was approximately 1.0 μg/ml.

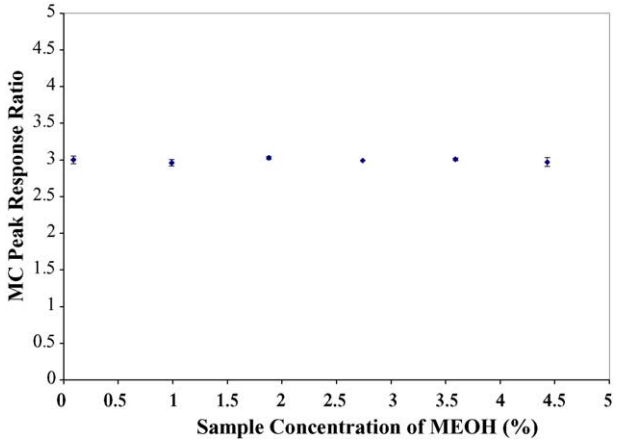

Fig. 2. Effect of adding additional methanol on the response ratio of methyl chloroform (mean ± S.D.; $n = 3$) using $^2H_3$-methyl chloroform as an internal standard.

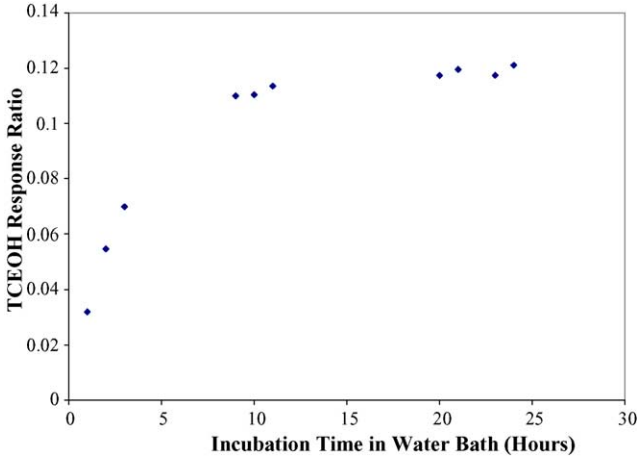

Fig. 3. TCEOH peak response ratio with dichloroethanol as an internal standard after incubation with β-glucuronidase in a 37 °C water bath; β-glucuronidase concentration = l100 U/sample; TCEOH concentration ∼ 1.5 μg/ml in urine; estimated original conjugated fraction > 0.90.

methanol on analytical response (Fig. 2). However, we chose to use an intermediate dilution in water to improve sensitivity.

To create standard curves, 30 ml of blood were collected in vacutainers (potassium oxalate added as an anticoagulant) from the subjects prior to each controlled exposure. One-millilitre aliquots of blood in 20 ml headspace vials were spiked with 100 μl of the working standard solutions. This resulted in final concentrations of MC in blood of 0.0009, 0.005, 0.03, 0.1, 0.3, 1.1, and 2.2 μg/ml, with an internal standard concentration of 0.35 μg/ml. Blood samples taken during and after exposure were refrigerated immediately after collection and prepared for analysis within 24 h. All standards and samples were analyzed in duplicate.

3.2. Trichloroacetic acid in urine

Following controlled exposures to MC, all voided urine was collected over a 4-day period, refrigerated prior to analysis, and analyzed at the end of the fourth day. Standard stock solutions of TCA (0.3–210 μg/ml) were prepared in methanol with butyric acid (analyzed as methyl butyrate) added as the internal standard. Urine samples were collected from each subject prior to exposure for preparation of standard curves. Aliquots of urine (300 μl) were spiked with 10 μl of the standard stock solutions in 20 ml headspace vials with resulting working standard concentrations of 0.01, 0.14, 0.4, 1.0, 2.1, and 7.0 μg/ml. All samples were spiked with 10 μl internal standard in methanol with a resulting concentration in urine of approximately 1.8 μg/ml. The derivatization of TCA to methyl trichloroacetate (M-TCA) was adapted from the methods of Ohara et al. [15] and Muralidhara and Bruckner [14]. The derivatizing reagent (600 μl) consisting of deionized water, concentrated sulfuric acid, and methanol (6:5:1, by volume) was added to each sample. Samples were then vortexed and placed in the autosampling tray. All samples were analyzed in duplicate.

3.3. Trichloroethanol in urine

In vivo, much of the TCEOH formed is conjugated to form trichloroethanol-glucuronide (urochloralic acid). In order to analyze total TCEOH in urine (free plus conjugated), the conjugate must first be hydrolyzed either enzymatically or by acid treatment. For the purposes of the MC exposure study, the conjugated TCEOH in urine was hydrolyzed enzymatically using β-glucuronidase (Sigma, Helix pomatia, type H-1). Solutions of β-glucuronidase (∼1100 U/ml) were prepared in an acetate buffer (pH 5). Stock standard solutions of TCEOH were prepared in methanol (∼5–1000 μg/ml) using dichloroethanol as an internal standard. Enzyme solution (500 μl) was added to urine (500 μl) spiked with 10 μl of the internal standard solution. Samples were incubated and gently rocked in a 37 °C water bath overnight, after which the total TCEOH was extracted by adding 1 ml methylene chloride, vortexing for 5 min, and centrifuging the samples for 15 min at 2390 × g. Christensen et al. [13] synthesized trichloroethanol-glucuronide in order to compare the efficiency of hydrolysis using β-glucuronidase to that of a strong acid (2 M sulfuric acid) in urine samples. They found that using β-glucuronidase resulted in complete hydrolysis of the glucuronide after an incubation period of 24 h in a 37 °C water bath. In contrast, only 5% of glucuronide was hydrolyzed in the presence of the strong acid. We conducted a time series analysis of enzymatic hydrolysis in a 37 °C water bath and found that the concentration of free TCEOH reached an asymptote after 20 h (Fig. 3).

4. Instrumentation

Dynamic headspace analysis for MC in blood and TCA in urine was performed using a Tekmar Precept II/Tekmar LSC 2000 purge and trap autosampler and concentrator (Teledyne

Tekmar; Mason, OH) containing a Vocarb 3000 trap (K) from Sigma-Aldrich (Milwaukee, WI). Samples were analyzed using the soil module with a purge flow of 65 ml/min and purge time of 16 min at 40 °C. The standard concentric needle was shortened to 10 cm so that the tip would not touch the liquid sample. To use the Precept autosampler with 20 ml vials, a 3.8 cm Teflon insert was placed in the soils cup, and the support rods were raised 3.8 cm. The trap was purged with helium without heating (dry purge) for 6 min to reduce moisture from the trap. The trap was then desorbed for 2 min at 250 °C at 76 ml/min. In order to improve peak shape, a Tekmar cryofocusing module was used with liquid nitrogen (−110 °C) to focus the sample in a pre-column (Rtx-624, 0.25 mm i.d., 1.4 μm film thickness, ∼1 m in length) before injection onto the GC column (Restek Rtx-5 Sil, 60 m × 0.25 mm i.d., with 1 μm film thickness). During the cryofocusing period the flow was split from the analytical column by means of a Y Press-Tight connector with one leg connected to a pneumatic valve controlled by the GC. A Hewlett Packard (Palo Alto, CA) 5890 gas chromatograph (GC) was used with the following oven temperature program: 35 °C for 1 min, 10 °C/min increase to 200 °C. The mass selective detector (Hewlett Packard 5971 A) was operated in selective ion monitoring (SIM) mode and was activated 8 min after injection to avoid the initial air and methanol peaks. Sample ionization was carried out in electron impact mode at 70 eV. Acquisition ions and retention times (rt) for analytes and standards were as follows (first listed *m*/*z* used for quantitation): $^2H_3$-methyl chloroform (*m*/*z* 100, 102; rt 9.59 min), MC (*m*/*z* 97, 99; rt 9.61 min), TCA as methyl trichloroacetate (*m*/*z* 59, 177; rt 14.88 min), and butyric acid as methyl butyrate (*m*/*z* 59; rt 10.74 min).

For TCEOH in urine, the sample extracts were analyzed using GC/MS run in splitless mode with an injection volume of 1 μl. A Hewlett Packard 5890 gas chromatograph was used with a Hewlett Packard 5989A mass selective detector operated in electron impact mode at 70 eV (column: Restek Rtx-5 Sil, 60 m × 0.25 mm i.d., with 0.25 μm film thickness). The injector and detector temperatures were 250 and 280 °C, respectively. The oven was cooled to 10 °C using liquid nitrogen prior to injection, held at 10 °C for 2 min following injection, heated at 10 °C/min until it reached 150 °C, heated at 50 °C/min to 200 °C and held for 1 min. The mass selective detector was operated in SIM mode acquiring the following ions (first listed *m*/*z* used for quantitation): dichloroethanol (*m*/*z* 31, 83; rt 6.71 min), and TCEOH (*m*/*z* 31,49, 77; rt 8.69 min).

5. Results

5.1. Methyl chloroform in blood

The method/detector response for MC in blood was linear between concentrations of 0.9 ng/ml and 2.2 μg/ml, with an R^2 value greater than 0.999 (Table 1). Precision was reflected in an average coefficient of variation of 1.5% (based on six different standard concentrations run in triplicate). The limit of quantitation (LOQ) was defined as 10σ above the mean response of four blank samples, and was found to be 0.8 ng/ml. Recovery was assessed by spiking blood with a known mass of MC (measured volumetrically) and comparing that concentration to the concentration determined from the standard curve (Table 2). A typical chromatogram is presented in Fig. 4.

Table 1
Regression parameters for calibration curves

Analyte/matrix	β	S.E.	*p*-Value	*N*	R^2
MC in blood					
Slope	2.66	0.008	<0.001	7	>0.999
Intercept	−0.002	0.007	0.76		
TCA in urine					
Slope	0.77	0.008	<0.001	7	0.999
Intercept	−0.0004	0.029	0.9		
TCEOH in urine					
Slope	0.072	0.001	<0.001	6	0.998
Intercept	0.0001	0.007	0.9		

Table 2
Method performance for MC in blood and TCA in urine

Analyte	Concentration added (μg/ml)	Concentration determined using standard curve (μg/ml): mean ± S.D. ($n = 3$)	Recovery (%)
MC	0.27	0.259 ± 0.004	96.0
	1.35	1.311 ± 0.023	97.1
TCA	0.33	0.334 ± 0.0016	101.2
	1.0	0.986 ± 0.029	98.6

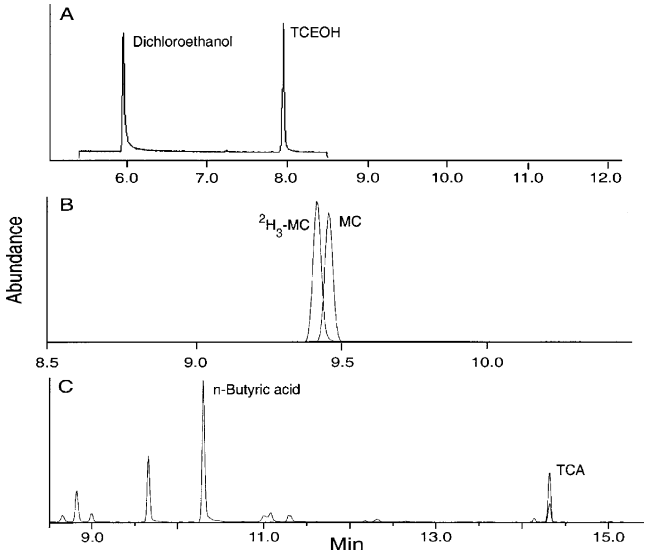

Fig. 4. Representative chromatograms using selected ion monitoring mode. (A) TCEOH (∼12 μg/ml in urine) with dichloroethanol as an internal standard. (B) MC (∼0.3 μg/ml in blood) with $^2H_3$-MC as an internal standard. (C) TCA (∼0.5 μg/ml in urine) analyzed as methyl trichloroacetate with *n*-butyric acid as an internal standard (analyzed as methyl butyrate).

Table 3
Stability of methyl chloroform in prepared blood samples

MC in blood	With internal standard correction			Without internal standard correction		
	Mean peak area response ratio ($n = 5$)	S.D.	p-Value	Mean peak area response, area units ($n = 5$)	S.D.	p-Value
Prepared and analyzed 8 h after collection	0.885	0.004	0.2713	1352475	18591	<0.001
Prepared 8 h after collection and analyzed 48 h later	0.880	0.008		1269044	30777	

As mentioned, blood samples were prepared for analysis within 24 h of collection. However, a set of 48 samples from a subject in the exposure study requires approximately 36 h for analysis. We assessed the stability of the samples over this period by spiking ten 1-ml aliquots of blood with MC (0.33 μg/ml in blood). Five were analyzed immediately after they were prepared, and the other five were analyzed approximately 48 h later. Using a two sided *t*-test for independent samples, no statistically significant difference was found between the two groups of samples using the internal standard correction. However, without correction using the internal standard, there was a significant difference in peak response (Table 3).

5.2. Trichloroacetic acid in urine

The response for TCA (analyzed as methyl trichloroacetate) was linear for concentrations in urine between 0.01 and 7 μg/ml with an $R^2 = 0.999$ (Table 1). The intrasample variability was reflected in an average coefficient of variation of 3.5% over the analytical concentration range. The LOQ for TCA was 0.009 μg/ml. Recovery was assessed by spiking urine with a known mass of TCA (measured gravimetrically) and comparing that concentration to the concentration determined from the standard curve (Table 2). A typical chromatogram is presented in Fig. 4.

A sample of urine taken from a subject exposed by inhalation to MC was used to assess the stability of TCA in urine both before and after sample preparation. This sample was collected 40 h after exposure and contained approximately 0.5 μg/ml TCA. Ten aliquots were taken from this sample and prepared for analysis using the method described above. Five of these aliquots were analyzed within 3 h of preparation, and the remaining aliquots were analyzed approximately 48 h later. The urine sample was then refrigerated for 3 weeks after which five additional aliquots were prepared and analyzed. ANOVA was applied to assess differences in observed TCA concentrations between the three sets of samples. As with MC in blood, there was a significant difference between the sets of samples without correction using the internal standard, but no significant difference was found when corrected for the internal standard (Table 4).

5.3. Trichloroethanol in urine

The LOQ for TCEOH was determined using a similar procedure to that used for MC and TCA in blood, and was found to be 0.06 μg/ml. The response was linear for concentrations between 0.1 and 15.5 μg/ml in urine (Table 1). The precision of the method was reflected in an average coefficient of variation of less than 1%. A typical chromatogram is presented in Fig. 4. The stability of trichloroethanol-glucuronide conjugate in urine over a 3-week period was also assessed. A sample collected from a subject 3 h after the end of exposure to MC was refrigerated and prepared for analysis 3 days after collection (five aliquots). The concentration of TCEOH in this urine sample was approximately 5 μg/ml. Five additional aliquots were prepared for analysis after the urine sample had been stored refrigerated for a 3-week period. Separate buffer solutions of β-glucuronidase were prepared before each set of aliquots were analyzed. No significant differences were found between the two sets of aliquots when corrected for the internal standard ($p < 0.001$).

We had initially attempted to use dynamic headspace for the analysis of TCEOH, but no response was seen at concentrations below 1 mg/ml in water. To increase the partitioning from the aqueous to gas phase, the vial was heated to 60 °C. At this temperature, we were only able to detect a concentration above 100 μg/ml in water. We next tried heating the moisture control trap to 90 °C during the desorption phase. While this resulted in an improvement in sensitivity (LOD ∼20 μg/ml), the limit of detection was still two orders of magnitude higher than what was required for our analysis (<1 μg/ml). In addition, at the elevated temperatures in the moisture control module and sample vials, the response was not reproducible from sample to sample (coefficient of variation = 140%).

Table 4
Stability of trichloroacetic acid in urine before and after sample preparation

TCA in urine	With internal standard correction			Without internal standard correction		
	Mean peak area response ratio ($n = 5$)	S.D.	p-Value	Mean peak area response, area units ($n = 5$)	S.D.	p-Value
Prepared and analyzed 3 days after collection	0.392	0.008	0.1737	64518	852	0.019
Prepared 3 days after collection and analyzed 48 h later	0.389	0.013		62021	1160	
Prepared and analyzed 21 days after collection	0.380	0.005		66828	3639	

Table 5
Trichloroacetic acid detection limits/quantitation limits in biological samples from various studies

Reference	Sample matrix	Method	Reported LOQ/LOD (μg/ml)
Humbert and Fernandez [16]	Urine	Derivatized with 3-methyl-1-*p*-tolyltriazene; extracted with diethylether; analyzed using GC/electron capture detection (ECD)	0.5 (LOQ)
O'Donnell et al. [6]	Urine	Derivatized with BF_3-methanol; extracted with toluene; analyzed using GC/ECD	0.05 (LOD)
Yan et al. [7]	Blood	Derivatized with BF_3-methanol; extracted with methylene chloride; anlalyzed using GC/MS	1.2 (LOQ)
Schmitt [8]	Blood	Derivatized with diazomethane; extracted with methyl tertiary butyl ether; analyzed using GC/ECD	0.1 (LOD)
Breimer et al. [11]	Blood/Urine	Derivatized with dimethyl sulphate; analyzed with static headspace, GC/ECD	0.1 (LOD)
Koppen et al. [17]	Liver Tissue	Thermal decarboxylation of TCA (and chloral hydrate) to chloroform; analyzed with static headspace, GC/ECD	0.02 (LOD)
Mizunuma et al. [18]	Urine	Derivatized with sulfuric acid/methanol; analyzed using static headspace, GC/ECD	0.005 (LOD)
Muralidhara and Bruckner [14]	Blood	Derivatized with sulfuric acid/methanol; analyzed using static headspace, GC/ECD	0.005 (LOD)
Current study (2004)	Urine	Derivatized with sulfuric acid/methanol; analyzed using dynamic headspace GC/MS	0.009 (LOQ)

Table 6
Methyl chloroform detection limits/quantitation limits in biological samples from various studies

Reference	Sample matrix	Method	Reported LOQ/LOD (ng/ml)
Nolan et al. [2]	Blood	Static headspace using a 5 ml sample loop; analyzed using GC/flame ionization detection (FID) and ECD	1.0 (LOQ)
Mizunuma et al. [18]	Urine	Static headspace using a 1 ml sample loop; analyzed using GC/ECD	0.5 (LOD)
Antoine et al. [19]	Blood	Purge and trap headspace using a liquid sample concentrator (6 ml sample)	0.5 (LOQ)
Ashley et al. [20]	Blood	Purge and trap headspace using a liquid sample concentrator (10 ml sample)	0.049 (LOD)
Current study (2004)	Blood	Dynamic headspace GC/MS using 20 ml vials as purging vessels (1 ml sample)	0.8 (LOQ)

6. Discussion

We found that MC and TCA can be effectively analyzed in biological samples using a sensitive and reproducible assay with dynamic headspace GC/MS. The sample preparation for the analysis of MC in blood requires only the dispensing of blood into headspace vials and the addition of internal standard. The sample preparation for TCA in urine requires only the additional steps of adding the derivatizing solution and briefly vortexting each sample.

We noted that the physical properties of TCEOH limit the utility of dynamic headspace in its analysis. Headspace analysis for a water soluble compound with a low vapor pressure often requires a relatively high equilibration temperature and a long equilibration time. In one recent study using static headspace to analyze TCEOH in blood, samples were heated for 30 min at 110 °C [14]. This temperature is not practical for use in a dynamic headspace system with a capillary column due to moisture accumulation on the purge trap. For our system, a moisture control trap was used to prevent water from being injected onto the column. When the moisture control trap was heated during desorption, the response to TCEOH increased slightly, but after a few samples had been run, the response decreased dramatically. It is likely that without the moisture control trap, excess water entered the pre-column and formed an ice plug during the cryogenic focusing.

For TCA, this technique has much improved sensitivity over spectrophotometric or organic extraction methods, and is comparable in sensitivity to static headspace analyses (Table 5). That we did not observe a significant increase in sensitivity using dynamic headspace over static headspace can most likely be explained by the lower sample vial temperatures used in dynamic headspace analysis (40 °C). The sensitivity of analysis of MC using this technique is also similar to static headspace or other purge and trap methods [19,20]. Detection/quantitation limits of MC in blood and urine from various studies are presented in Table 6. The other purge and trap methods developed for analysis of MC in blood have placed the sample in a non-disposable purge vessel and used sample volumes of at least 5 ml. This requires that the purging vessel be cleaned after analyzing each sample. Using the modified soil module, we were able to use separate headspace vials for each sample and we found good results with much smaller sample volumes (1 ml).

In summary, a sensitive, precise and reproducible dynamic headspace method was developed and validated for the analysis of trichloroacetic acid in urine and methyl chloroform in blood. We evaluated the dynamic headspace method for the analysis of trichloroethanol in biological samples, but found that it lacked the sensitivity and reproducibility required to accurately assess levels of trichloroethanol in urine following exposures to low concentrations of methyl chloroform.

Acknowledgments

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References

[1] A. McCulloch, P.M. Midgley, Atmos. Environ. 35 (2001) 5311.
[2] R.J. Nolan, N.L. Freshour, D.L. Rick, L.P. McCarty, J.H. Saunders, Fundam. Appl. Toxicol. 4 (1984) 654.
[3] P.J. Friedman, J.R. Cooper, Anal. Chem. 30 (1958) 1674.
[4] M. Ogata, Y. Takatsuka, K. Tomokuni, Br. J. Ind. Med. 27 (1970) 378.
[5] T.A. Seto, M.O. Schultze, Anal. Chem. 28 (1956) 1625.
[6] G.E. O'Donnell, A. Juska, R. Geyer, M. Faiz, S. Stalder, J. Chromatogr. A 709 (1995) 313.
[7] Z. Yan, G.N. Henderson, M.O. James, P.W. Stacpoole, J. Pharm. Biomed. Anal. 19 (1999) 309.
[8] T.C. Schmitt, J. Chromatogr. B 780 (2002) 217.
[9] B. Dehon, L. Humbert, L. Devisme, M. Stievenart, D. Mathieu, N. Houdret, M. Lhermitte, J. Anal. Toxicol. 24 (2000) 22.
[10] S. Ulrich, J. Chromatogr. A 902 (2000) 167.
[11] D.D. Breimer, H.C.J. Ketelaars, J.M.V. Rossum, J. Chromatogr. 88 (1974) 55.
[12] A.C. Monster, G. Boersma, Int. Arch. Occup. Environ. Health 35 (1975) 155.
[13] J.M. Christensen, K. Rasmussen, B. Koppen, J. Chromatogr. 42 (1988) 317.
[14] S. Muralidhara, J.V. Bruckner, J. Chromatogr. B 732 (1999) 145.
[15] A. Ohara, H. Michitsuji, Y. Jyoyama, K. Yamaguchi, Y. Fujiki, Sangyo Igaku 33 (1991) 94.
[16] B.E. Humbert, J.G. Fernandez, Int. Arch. Occup. Environ. Health 36 (1976) 235.
[17] B. Koppen, L. Dalgaard, J.M. Christensen, J. Chromatogr. 442 (1988) 325.
[18] K. Mizunuma, T. Kawai, S. Horiguchi, M. Ikeda, Int. Arch. Occup. Environ. Health 67 (1995) 19.
[19] S.R. Antoine, I.R. DeLeon, R.M. O'Dell-Smith, Bull. Environ. Contam. Toxicol. 36 (1986) 364.
[20] D.L. Ashley, M.A. Bonin, F.L. Cardinali, J.M. McCraw, J.S. Holler, L.L. Needham, D.G. Patterson Jr., Anal. Chem. 64 (1992) 1021.