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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

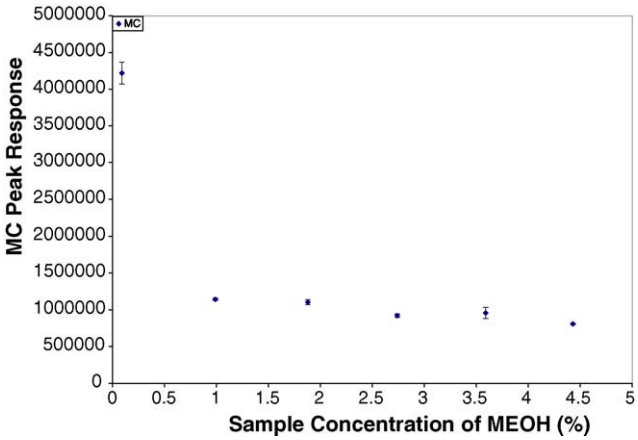

Fig. 1. Effect of adding increasing amounts of methanol on the response (mean ± S.D.; $n = 3$) of MC. MC concentration was approximately 1.0 μg/ml.