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ORIGINAL PAPER

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A comparison and evaluation of analysis procedures for the quantification of (2-methoxyethoxy)acetic acid in urine

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Abstract Several extraction and derivatization procedures were evaluated for the quantification of (2-methoxyethoxy) acetic acid (MEAA) in urine. MEAA is a metabolite and a biomarker for exposure to 2-(2-methoxyethoxy)ethanol, a glycol ether with widespread use in various industrial applications and the specific use as an anti-icing additive in the military jet fuel formulation JP-8. Quantification of glycol ether biomarkers is an active area of current analytical research. Various sample preparation procedures were evaluated; liquid-liquid extraction (LLE) using ethyl acetate yielded the highest recovery, and solid-phase extraction (SPE) gave low recovery of MEAA. Two derivatization procedures were thoroughly investigated and validated. Silylation of MEAA with *N*-methyl-*N*-[*tert*-butyldimethylsilyl]trifluoroacetamide (MTBSTFA) was one approach, and esterification of MEAA using ethanol was the other. Quantification was by means of a gas chromatograph (GC) equipped with a mass spectrometer for a detector and using a polydimethylsiloxane (HP-1) capillary column. Deuterated 2-butoxyacetic acid (d-BAA) was used as an internal standard. Recovery studies of spiked human urine demonstrated the accuracy and precision for both procedures. The limit of detection (LOD) and other figures of merit for both derivatization procedures will be discussed in detail. Applications of these analysis procedures are also discussed.

Keywords Glycol ethers · Alkoxyacetic acids · 2-(2-methoxyethoxy)ethanol · Urinary biomarkers

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Introduction

The toxicity of the class of compounds known as glycol ethers was studied as early as the 1930s [1] and has been reported extensively elsewhere in the literature [2–5]. The compound 2-(2-methoxyethoxy)ethanol [diethylene glycol monomethyl ether] has many industrial uses; these include its use in the formulation of inks and as an additive in the military jet fuel JP-8 as an anti-icing agent [6]. It is a health concern because of its chemical similarity to 2-methoxyethanol which has known and significant developmental toxicity [5, 7]. Many NATO military personnel are exposed to JP-8, and NATO uses five billion gallons of this fuel each year [6]. The toxicology data for 2-(2-methoxyethoxy)ethanol has been reviewed [2] and the compound has been the subject of numerous animal toxicity studies [8–10]. Therefore, dermal or vapor exposure to 2-(2-methoxyethoxy)ethanol represents a human work related health concern as well as an area of interest for supporting toxicological animal studies. (2-Methoxyethoxy)acetic acid (MEAA) has been shown to be the appropriate biomarker for exposure to 2-(2-methoxyethoxy)ethanol [11]. It was previously demonstrated by this laboratory that mice exposed to the jet fuel JP-8, which contains 0.1% 2-(2-methoxyethoxy)ethanol [6], have detectable quantities of urinary MEAA [12]. MEAA is formed from 2-(2-methoxyethoxy)ethanol by the metabolic path shown in Fig. 1 by alcohol dehydrogenase and aldehyde dehydrogenase. Glucuronyl transferases, dealkylases and sulfotransferases can also contribute to the metabolic conversion of 2-(2-methoxyethoxy)ethanol as is shown in Fig. 1. MEAA may be further metabolized by acyltransferase and dealkylase carboligase. The analysis work reported in this manuscript focused on the development of accurate, precise and sensitive procedures to be used in the detection and quantitation of MEAA in urine. Since urinary MEAA appeared to be a good biomarker for determining exposure

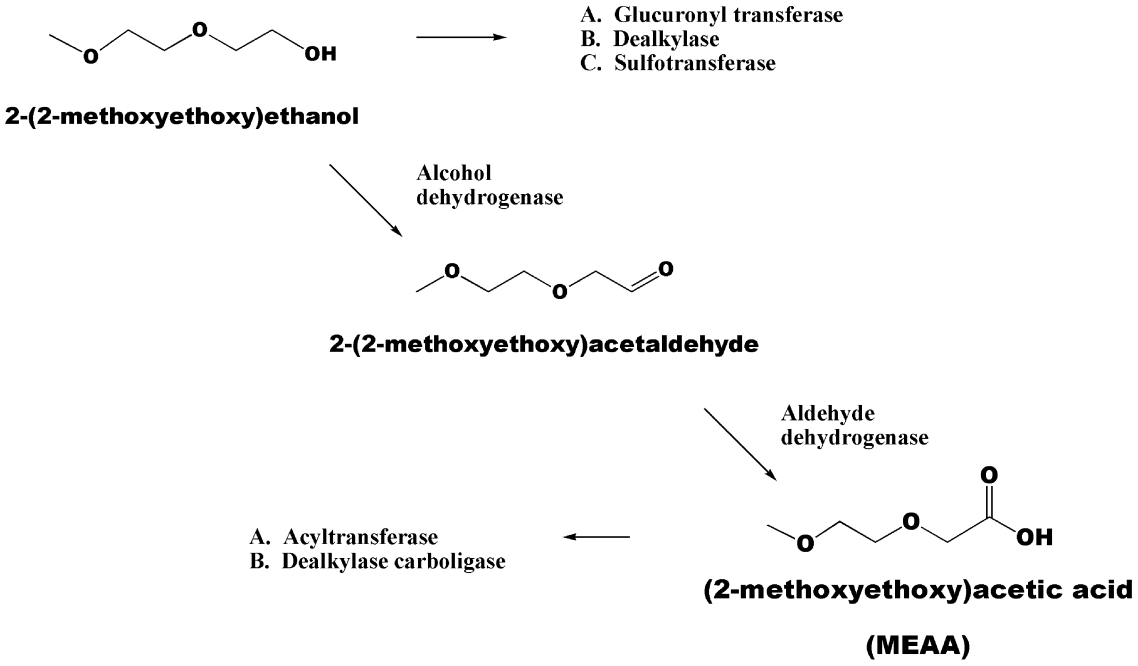

Fig. 1 The metabolic pathways for 2-(2-methoxyethoxy)ethanol leading to the formation of (2-methoxyethoxy)acetic acid, MEAA, a biomarker of exposure. MEAA can also be metabolized by glucuronyl transferase, dealkylase or sulfotransferase. MEAA can be further metabolized by acyltransferase or dealkylase carboligase. Evaluating methods to determine MEAA levels in urine was the focus of this study. (This metabolic pathway has been previously described in reference 36 and used with permission, Elsevier Science, 2003)

to 2-(2-methoxyethoxy)ethanol, the goal of this work was to develop and compare the best procedures to determine urinary MEAA levels.

Alkoxyacetic acid analysis represents an analytical challenge for analytical chemists, and numerous methodologies have been reported in the literature in recent years. Although the analysis of many urinary metabolites have utilized high-performance liquid chromatography (HPLC), the alkoxyacetic acids have generally been analyzed by GC [13–21] or by a radio labelling approach for metabolic studies. As for monitoring any biomarker of exposure, the lowest practical detection limit is desired for any quantitative test method as well as accurate results and the absence of interferences. Analyte extraction and cleanup are vital aspects for the test methodology for a urinary marker of exposure. Most reported analysis for alkoxyacetic acid metabolites from glycol ethers have utilized liquid-liquid extraction (LLE) followed by derivatization of the acid metabolite for analysis by gas chromatography. LLE has been noted in the literature for its inherent simplicity and ease of use over solid-phase extraction (SPE) and other enrichment techniques [22]; LLE was evaluated in this current study. Brown et al. [13, 14] reported the use of SPE for the extraction of butoxyacetic acid from urine, and SPE was also evaluated for MEAA in the current reported work. Other gas chromatographic alkoxyacetic acid urine analysis methods reported have followed extraction with derivatization using diazomethane [16], pentafluorobenzyl bromide [17–19] or trimethylsilyldiazomethane [20]. Shih et al. [21] first reported using direct GC analysis of butoxyacetic acid without the derivatization step and this option was also considered for the current work of urinary MEAA determination.

Two test methodologies evolved as being superior during this study and were directly compared for quantification of urinary MEAA and fully validated [23, 24]. LLE extraction followed by two derivatization procedures: ethyl esterification and silylation with *N*-methyl-*N*-[*tert*-butyldimethylsilyl]trifluoroacetamide (MTBSTFA). Laitinen [25] used an esterification approach in the analysis of butoxyacetic acid by derivatization to the methyl ester. Esterification has many advantageous and offers great simplicity and low cost reagents; esterification of organic acids with alcohols for GC analysis has been often reported in the literature [26–29]. *Tert*-butyldimethylsilane (TBDMS) derivatives have been reported to have high intensity ions for mass spectrometric detection [30] which is a positive attribute for a test method having high sensitivity and a low detection limit. TBDMS derivatives also have reasonable stability and are more stable to hydrolysis than trimethylsilane (TMS) derivatives. The use of TBDMS derivatives has been reported in the literature for the analysis of various alkyl acids [30–33]. It was, therefore, the objective of this study to evaluate a number of different analysis methods for the quantification of urinary MEAA and fully validate and compare those test procedures offering the best sensitivity, accuracy and method specificity. Applications of the two test procedures, as well as their advantageous and disadvantages, will be discussed.

Experimental

Instrumental and chromatographic conditions

The chromatographic analysis was conducted using an Agilent Technologies model 6890 gas chromatograph (Palo Alto, California, USA) equipped with a model 5973 MSD mass spectrometer and an autosampler. The detector output was evaluated and integrated using Chemstation software (Agilent Technologies). The column was a capillary HP-1 (Agilent Technologies) with a length of 50 m, internal diameter of 0.20 mm and film thickness of 0.33 μm. Chromatographic oven and injector temperature conditions were optimized for the type of MEAA derivative, either ethyl ester or *tert*-butyldimethylsilane, being analyzed (see Table 1). The carrier gas was helium used at a constant flow rate of 0.8 mL min^{-1} for all analysis. The mass selective detector was operated in electron impact mode with an electron energy of 70 eV. The detector source temperature was 230°C with the quadrupole set at 150°C, the recommended factory settings. Selective ions were monitored at *m/z* 59 for the MEAA derivatives and *m/z* 66 for the d-butoxyacetic acid derivatives. The injection volume of the final sample solution was 0.5 μL using splitless injection mode.

Chemical reagents

Standard compounds of (2-methoxyethoxy)acetic acid (MEAA, CAS no. 16024-56-9) and deuterated 2-butoxyacetic acid (d-BAA, $CD_3CD_2CD_2CD_2OCH_2COOH$) were synthesized and described previously by Cheever et al. [34] and by Brown et al.[13], respectively. Ethyl acetate used in the extractions was commonly available spectral grade (Burdick and Jackson, Muskegon, Michigan, USA). Methylene chloride used in the extractions was also spectral grade (Fisher Scientific, Fairlawn, New Jersey, USA). All stock standard solutions were prepared in deionized water (Barnstead NANOpure, Dubuque, Iowa, USA). The derivatizing reagent, *N*-methyl-*N*-[*tert*-butyldimethylsilyl]trifluoroacetamide (MTBSTFA) plus 1% *tert*-butyldimethylchlorosilane (TBDMCS) was also commercially available (Sigma-Aldrich Chemical Company, St. Louis, Missouri, USA). The pH 6.9 buffer solution was prepared using a Gram Pack (Fisher Scientific) buffer package of sodium phosphate. All other reagents were commonly available ACS reagent grade.

Urine samples used in this study were from various sources. Human urine collected from volunteers at this laboratory who were not exposed to 2-(2-methoxyethoxy) ethanol, MEAA or JP-8 was used for the preparation of standard and spiked samples. In addition, a human urine specimen was obtained from a fuel-tank-maintenance worker who was a participant in a US Air Force study to evaluate acute exposures to JP-8. This field study and collection has been described in detail by Serdar et al. [35]. The collection of mouse urine has been described previously by B'Hymer et al. [12].

General urine sample preparation

Urine sample preparation was performed either by liquid-liquid extraction or by solid-phase extraction and is described in detail as follows:

Liquid-liquid extraction LLE using ethyl acetate was conducted first by acidifying a 4.0 mL human urine sample [or smaller volume of animal urine, then diluting to 4.0 mL total volume with deionized water] with 20–40 μL of concentrated HCl. This solution was placed in a screw-capped vial. A 0.5-mL aliquot of a 40 μg mL^{-1} d-BAA internal standard solution was added (see Results and discussion). A 0.5-mL portion of deionized water for test samples or standard MEAA spiking solution was added. The urine sample was extracted four times with 5.0 mL of ethyl acetate using a vortex mixing for 1 minute during each extraction. The ethyl acetate layers were combined and reduced in volume to approximately 1 mL by evaporation under nitrogen gas sweep at room temperature. The concentrated extract was derivatized by one of two processes, to produce either the *tert*-butyldimethylsilane (TBDMS) derivative or ethyl ester of MEAA and d-BAA.

Solid-phase extraction Oasis Max (Waters Corporation, Milford, Massachusetts, USA) 1cc/30 mg and 6cc/500 mg cartridges were used for SPE. The extraction procedure used was that described by Brown et al. [14], a procedure used for the extraction of butoxyacetic acid from urine and will not be repeated in detail here. These extract solutions from the SPE eluant were combined and evaporated to dryness by nitrogen sweep at room temperature. A 1.0-mL portion of magnesium sulfate dried ethyl acetate was added, and the solution was silylated using TBDMS derivatization.

Table 1 Gas chromatographic conditions used for analysis

	t-Butyldimethylsilane derivative	Ethyl Ester derivative
Injector temperature	210°C	240°C
Column temperatures		
Initial	85°C	50°C with a 1 minute hold
Program ramp	2.5°C min^{-1} to 175°C	3°C min^{-1} to 140°C, then 15°C min^{-1} to 230°C
Post Run	225°C for 4 min	240°C for 5 min

Derivatization procedures

All urine extracts were derivatized by MTBSTFA reagent to form the corresponding *tert*-butyldimethylsilane (TBDMS) derivatives or esterification with ethanol to form ethyl esters of MEAA and d-BAA. Those procedures were as follows:

TBDMS derivatization The ethyl acetate/urine extract was dried with anhydrous magnesium sulfate and filtered using glass wool before being reduced to a final 1.0-mL volume by nitrogen gas sweep. This solution was placed into a 2-mL autosampler vial and 50 μL of the MTBSTFA with 1% TBDMCS reagent were added. The vial was quickly crimp-capped sealed and heated for 90 minutes at 70°C in a heating block. The solution was allowed to cool to room temperature and then was chromatographed according to the previously described conditions.

Esterification The ethyl acetate urine extract was mixed with 2.0 mL of ethanol and the slow addition of 0.4 mL concentrated sulfuric acid. This mixture was then heated for 16 hours at 50°C in a heating block. A 3.0-mL portion of methylene chloride was added to each esterified urine extract, followed by the addition of 5.0 mL of deionized water. Extraction was by use of vortex mixing for 1-minute time periods. The methylene chloride layer was collected, and 5.0 mL of deionized water were added to the original esterified sample tube. This mixture was extracted three more times with 3.0 mL of methylene chloride for each extraction. The extract solutions were combined and dried with anhydrous magnesium sulfate. After filtration, the methylene chloride solutions were reduced to 1-mL olume by evaporation by nitrogen gas sweep at room temperature. These concentrated solutions were placed in crimp-capped vials for GC analysis.

Standard sample preparation and recovery studies

MEAA calibration standards were prepared at the 0.1, 0.25, 0.5, 1, 2, 5, 10, 20, 30, 40 and 50 μg mL^{-1} levels in human urine plus a blank 0 μg mL^{-1} level for the ethyl esterification process. Because the TBDMS derivatization process appeared to be more sensitive, 0.025 and 0.05 μg mL^{-1} levels along with 0.1, 0.25, 0.5, 2, 5, 15, 35, 50 μg mL^{-1} levels in human urine plus a blank 0 μg mL^{-1} level were run.

Both derivatization procedures were used to generate recovery data for comparison. Blank urine was spiked at the 2, 10 and 20 μg mL^{-1} MEAA level for each experimental day of the primary recovery study. A secondary recovery study consisted of collecting urine from 20 non-exposed human volunteers. This volunteer urine was used and prepared as blanks with no spikes to check for possible interferences from individuals. Spiked samples were prepared using the individual volunteer urines containing 5 μg mL^{-1} MEAA plus 5 μg mL^{-1} d-BAA used as the internal standard.

Calculations

Calculations for all quantitations were based on peak area ratios of the specific derivatives of MEAA or d-BAA. Standard calibration curves were linear within the 0.025 to 50 μg mL^{-1} MEAA range used for the TBDMS derivatives; correlation coefficients were 0.97 or greater and y-intercepts approached zero for all curves generated using this method. Similar results were obtained with the ethyl ester procedure; standard calibration curves were linear within the 0.1 to 50 μg mL^{-1} MEAA range used, and correlation coefficients were 0.98 or greater with y-intercepts approaching zero for all curves generated. At least two calibration curves, at the beginning and end of each set of chromatographic runs, were collected for all the recovery data sets. Multiple run sets, including the use of different production lots of HP-1 columns, were used during this study. The calibration curve slope drift was minimal for both procedures; approximately 2% or less was observed within any batch run of samples for the ethyl ester process. Drift was slightly greater for the TBDMS process samples; the slope of calibration curves changed as much as 5% within a chromatographic sample sequence. Duplicate injections were performed for all spiked samples and average values were calculated for the recovery data.

The limit of detection (LOD) was calculated using a traditional approach based on three times the noise level [23] in a blank chromatogram. The average noise level was determined for each batch sample run using 100 data points to obtain an average standard deviation of the baseline by height. At least three chromatograms were randomly selected to produce an average standard deviation of the 100 data point noise instrumental noise levels. Peak height calibration curves were determined from the standard samples run during each sample set. The LOD was then calculated as three times the average standard deviation divided by the slope of the peak height calibration curves on the MEAA derivatives.

Results and discussion

Extraction recovery

Liquid-liquid extraction (LLE) using ethyl acetate proved to be the most efficient procedure in terms of the recovery of MEAA and d-BAA from spiked urine samples. Measuring extracts from spikes versus known concentrations of MEAA and d-BAA in ethyl acetate solutions, the recovery or extraction efficiency of MEAA was 95% (range was 91 to 99%, n=3) and d-BAA was 87% (range was 84 to 91%, n=3). The TBDMS derivatization procedure was used to measure the recovery of the analytes for all extraction recovery experiments. The recoveries for the solid-phase extraction procedure described by Brown et al. [14] were poor for both alkoxyacetic acids. Brown et al. [14] originally reported 20% yield for BAA and d-BAA for 1cc/60 mg bed and more when using a 6cc/500 mg bed

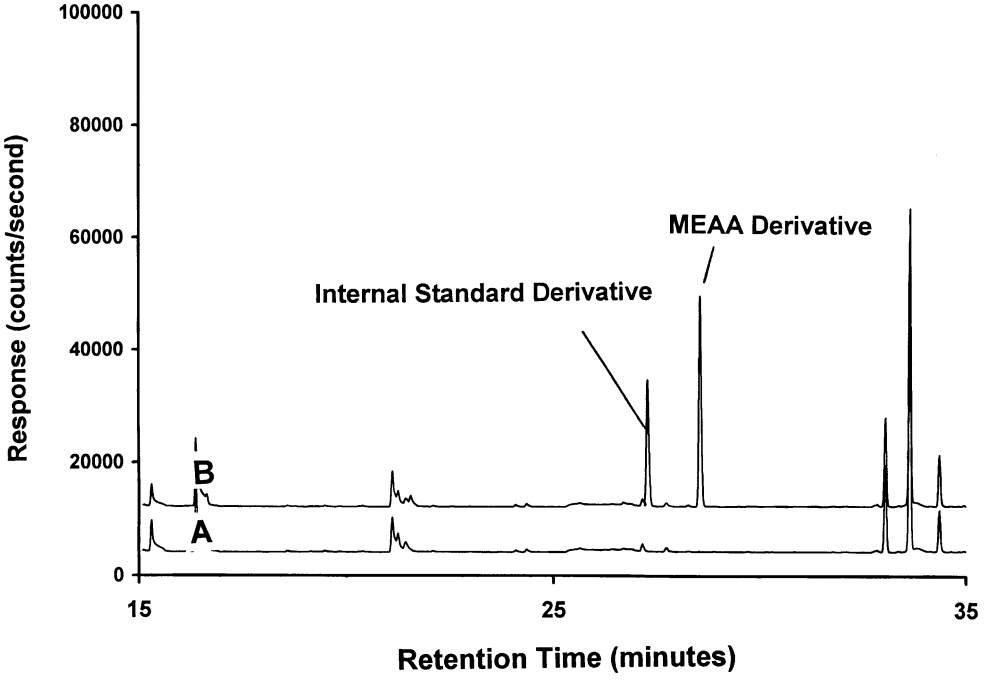

Fig. 2 **a**. A total ion chromatogram of a blank non-exposed volunteer urine sample analyzed by the described silylation procedure. **b**. A total ion chromatogram of a 5 μg mL^{-1} spiked MEAA and d-BAA human volunteer urine sample. d-BAA was used as the internal standard

SPE cartridge. The recovery of d-BAA using the 6cc/500 mg bed Oasis Max for the current study was 12% (range was 9 to 14%, n=3) for 3 μg mL^{-1} spiked urine. MEAA recovery was less which was not adequate to gain maximum sensitivity for the detection of a biomarker. Although SPE generally offers a less labor intensive alternative for extraction, MEAA extraction was not suited using the Oasis Max cartridge. Since it was the objective of this study to have the best sensitivity possible with a method having a low detection limit, ethyl acetate extraction offering 90% recoveries was the best analytical extraction procedure for urinary MEAA. Another advantage of LLE over SPE is that bed capacity is not a consideration. Given the results of extraction efficiencies, maximizing the recovery yield of MEAA and d-BAA was the logical analytical approach.

Chromatographic conditions

The optimized chromatographic conditions for each derivative of alkoxyacetic acid proved to be selective and have no major interferences within human urine. The unspiked urine samples extracted and chromatographed showed no interfering peaks for either the MEAA derivatives or the d-BAA derivatives. This was demonstrated for both deriv-

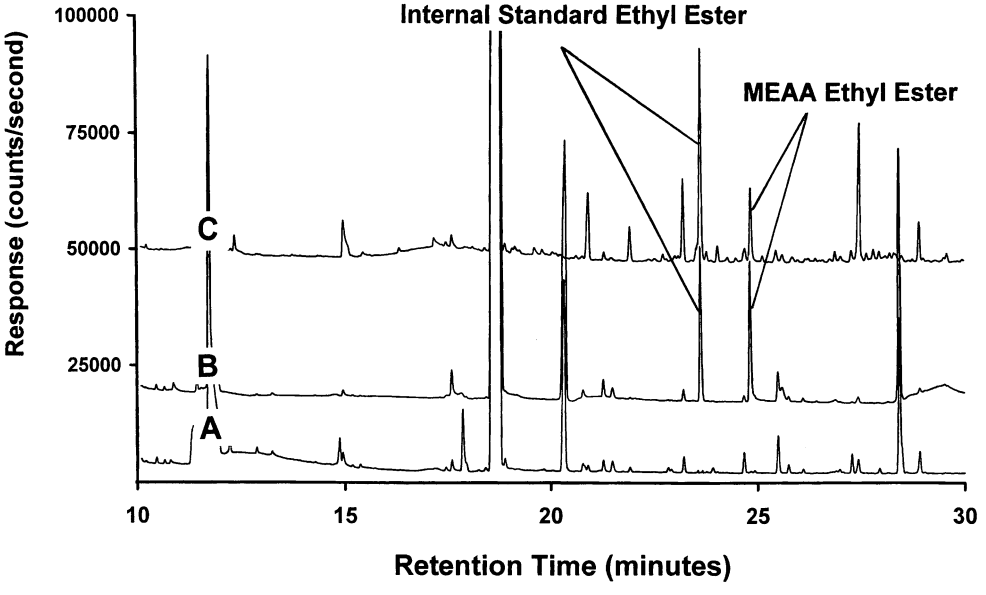

Fig. 3 **a**. A total ion chromatogram of a blank non-exposed volunteer urine sample analyzed by the described esterification procedure. The large peak observed at retention time 19 minutes was from the diethyl ester of sulfuric acid. **b**. A total ion chromatogram of a urine sample from a JP-8 exposed human using the esterification analysis procedure. MEAA was determined to be 8.4 μg mL^{-1} in this sample (4.0 mL aliquot) of urine. d-BAA was used as the internal standard. **c**. A total ion chromatogram of a pooled urine sample from JP-8 exposed mice using the esterification analysis procedure. MEAA was determined to be 8.5 μg mL^{-1} in this sample (2.0 mL aliquot) of urine

atization/chromatographic procedures using the urine from 20 individual volunteers. Therefore, both procedures were specific for MEAA in human urine. Typical total ion chromatograms from monitoring ions *m/z* 59 and 66 are shown in Figs. 2 and 3. Figure 2 shows chromatograms of the TBDMS derivatives of MEAA and d-BAA using the previously described chromatographic conditions (Experimental section and Table 1). The first chromatogram (Fig. 2a) shows an unspiked human urine sample; the second chromatogram (Fig. 2b) shows the TBDMS derivative peaks of MEAA and d-BAA at a 5 μg mL^{-1} spiked level in urine. Figure 3 shows chromatograms obtained using the ethyl ester derivative procedure (Experimental section and Table 1). The first chromatogram (Fig. 3a) shows an unspiked human urine sample. The second chromatogram (Fig. 3b) shows peaks of the ethyl esters of MEAA at 8.4 μg mL^{-1} level from actual exposure and d-BAA at a 5 μg mL^{-1} spiked in the field urine sample. Both chromatographic procedures displayed baselines with minimal drift, thus making peak integration easy. Both chromatographic procedures displayed symmetrical peaks with little peak tailing for their respective derivative analytes. Both chromatographic procedures had minimal signs of carry over between injections; however, the TBDMS derivatives did have some signs of peak carry over when employing well used injection port liners in the gas chromatograph. While running long chromatographic sequences, blank injections after high level spikes were used to verify the lack of carry-over of analytes for both chromatographic procedures. Frequent replacement of injection port liners when starting new sample sets appeared to eliminate any carry-over problem with the TBDMS procedure; the esterification procedure appeared to be more robust in this respect.

Shih et al. [21] and later Brown et al. [13] reported the analysis of alkoxyacetic acids directly by gas chromatography without a derivatization step using free fatty acid (FFAP) capillary GC columns. This chromatographic procedure was initially evaluated, but carry-over of both d-BAA and MEAA peaks was noticed. MEAA was found to have a significant carry-over/adsorption problem using the FFAP column and this column was dropped from further experimental consideration. Derivatization of the alkoxyacetic acids was the best approach to eliminate this problem associated with the acid metabolites of these specific glycol ethers.

The mass selective detector added a great degree of selectivity to these analysis procedures. The ion *m/z* 59 [$CH_3OCH_2CH_2-$] was chosen for monitoring and used in the calibration curve because of its greater abundance. As is shown in Fig. 4, ion *m/z* 59 was a characteristic of both the ethyl ester and the TBDMS derivative of MEAA. Ion *m/z* 66 [$CD_3CD_2CD_2CD_2-$] was monitored for the ethyl ester and the TBDMS derivative of d-BAA (Fig. 4) for the same reason; it was the most abundant ion detected. The use of ion *m/z* 58 [$-OCH_2C{=}O$] for a qualifying ion was considered initially for the derivatives of MEAA; however, no chromatographic interferences or co-eluting analytes were observed at any stage of the evaluation of these procedures.

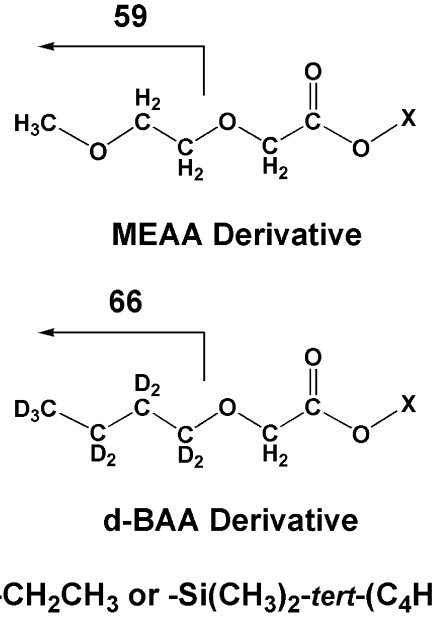

Fig. 4 Ion m/z 59 was the major ion for both derivatives of MEAA, and monitored for generating data for the estimation of MEAA. Ion m/z 66 was the major ion for both derivatives of d-BAA and was monitored for detection for the internal standard

Therefore, qualifying ions were not considered necessary for any of the target analytes in this study.

Selection of the internal standard

The use of 2-butoxyacetic acid, d-BAA, as an internal standard has been described previously [36]. It is unlikely that a worker or animal exposed to glycol ethers would have been exposed to the deuterated form of BAA. No major chromatographic interferences were identified during this study for either derivatization procedure. Generally, an internal standard added at the end of an extraction/derivatization procedure compensates for changes in solvent volume; the addition of d-BAA at the beginning of the procedure further reduces the analysis variation by accounting for any sample loss, differences in the extraction efficiency, and differences in the completeness of the derivatization reaction. All calibration curves obtained using d-BAA had a high correlation coefficient, 0.97 or greater, and appeared linear using both derivatization procedures. The recovery data discussed in the next section implies a reproducible extraction and derivatization of both d-BAA and MEAA. The precision of replicate injections was also increased by use of the internal standard.

Derivatization procedures—comparisons and analyte recovery

The two derivatization procedures described in detail within this work have numerous differences and advantages.

Table 2 Three level recovery studies of MEAA

MEAA Derivative	Spiked level (μg mL^{-1})	Mean MEAA recovered (n=9) (μg mL^{-1})	Average % recovery	SD (μg mL^{-1})	% RSD
t-Butyldimethylsilane	2	1.97	99	0.03	1.6
	10	9.5	95	0.28	2.9
	20	19.6	98	0.42	2.1
Ethyl ester	2	1.89	95	0.11	5.8
	10	10.0	100	0.78	7.8
	20	20.6	103	1.33	6.4

A total of nine separate spiked urine samples were prepared at each concentration level; three different urine spikes were chromatographed during each of three separate chromatographic sequence runs. SD is standard deviation. % RSD is percent relative standard deviation. Ethyl ester data has been previously reported in reference 36 and used with permission, Elsevier Science, 2003

TBDMS derivatives have been noted to be more stable to water hydrolysis over other silane derivatives. The TBDMS procedure was also more direct than the ethyl ester procedure, only the initial LLE was necessary to remove the alkoxyacetic acids from the urine. The ethyl esterification method had the advantage of using low cost reagents and being more rugged procedurally. The esterification process is minimally affected by the presence of water and is easily controlled by the use of anhydrous magnesium sulfate. Laitinen [25] had used methyl esterification which was initially investigated, but the cross reaction with remaining ethyl acetate produced both ethyl and methyl esters when attempting to reproduce his work; therefore, esterification using ethyl alcohol has an obvious advantage and inherent simplicity. Early experimentation using *N*, *N*-dimethylfomamide dimethyl acetal (Methyl-8) to derivatize MEAA failed to produce reasonable yields for analysis and was dropped from consideration.

The recovery studies of the TBDMS derivatization and esterification procedures are shown in Tables 2 and 3. The primary recovery study of urine spiked with MEAA was performed over three separate experimental batch runs to demonstrate the accuracy and precision of each procedure. This data is presented in Table 2; average recovery for the TBDMS procedure was between 95 and 99% for the three MEAA spiked sample levels studied. The three concentrations were 2, 10 and 20 μg mL^{-1} MEAA in urine. The ethyl ester procedure also had accurate average recoveries; 95 to 103% was recovered for the same three concentration levels. The differences of precision, percent relative standard deviation (% RSD), between the two procedures could be expected. The % RSD of each level was greater for the ethyl ester procedure as compared to the TBDMS derivative procedure owing to the variation introduced in having the second methylene chloride extraction used in the esterification analysis procedure. The ethyl ester procedure produced % RSDs in the range of 5.8 to 14.3 %, while the TBDMS procedure produced % RSDs in the range of 1.6 to 7.3%.

The results of a second recovery study at a single concentration level of 5 μg mL^{-1} MEAA spiked urine from 20 non-exposed humans are displayed in Table 3. Both procedures were reasonably accurate; TBDMS derivative resulted in 4.70 μg mL^{-1} average recovery (n=20) and ethyl ester resulted in 5.25 μg mL^{-1} average recovery (n=20). Precision showed the same trend as in the multi-level recovery; TBDMS was 7.3% RSD compared to ethyl ester's 14.3% RSD. The low and high value for this recovery study are also listed in Table 3. The chief difference between Tables 2 and 3 lies in the use of pooled or standard urine (Table 2) versus the individual volunteer urine (Table 3). The precision was generally less when using individual urine for both procedures owing to the wider range of variation among the 20 volunteers and possible matrix extraction effects.

Limit of detection, advantages and disadvantages

The limit of detection (LOD) shows that the TBDMS procedure has a distinct advantage over the esterification procedure. The LOD averaged 0.01 μg mL^{-1} MEAA in urine for the multiple trials and HP-1 columns used during this study. The LOD averaged 0.05 μg mL^{-1} MEAA for the esterification procedure. This is consistent for several reasons. As can be seen in Figs. 2 and 3, the TBDMS derivative peak of MEAA has slightly better symmetry and

Table 3 Recovery studies of MEAA

MEAA Derivative	(5 μg mL^{-1} spikes in volunteer urine)				
	Mean recovery (μg mL^{-1})	Average % Recovery	Lowest value (μg mL^{-1})	Highest value (μg mL^{-1})	% RSD
t-Butyldimethylsilane	4.70	94	3.9	5.4	7.3
Ethyl ester	5.25	105	3.8	6.5	14.3

These 20 volunteer urine samples were spiked at the 5 μg mL^{-1} level of MEAA. Interfering peaks and MEAA were not detected in any of the unspiked urine samples from the 20 volunteers

more theoretical plates (better peak efficiency) under these conditions than the corresponding ethyl ester peak. The esterification procedure required a second extraction to recover the ester, which would reduce the mass of analyte introduced into the detector. This second extraction, however, did have a positive effect. Urine is a complicated sample matrix, and a second LLE provided a more clean chromatographic sample matrix, less prone to possible interferences. This was especially true for animal urine samples which will be discussed in the next section. Ultimately, the need for the detection of low levels of MEAA would favor the silylation procedure with its 0.01 μg mL^{-1} LOD. Brown et al. [14] reported an LOD of 0.2 ppm (μg mL^{-1}) for butoxyacetic acid using 10 mL urine sample with SPE and direct detection of the analyte using a free fatty acid phase (FFAP) column with a MS detector. The silylation procedure used in this study is evidently more sensitive and has a lower LOD using a smaller urine sample (4 mL) for MEAA, a similar metabolic alkoxyacetic acid.

Applications and ongoing work

The two described analysis procedures have been applied to urine samples. Pilot studies of mice exposed to jet fuel JP-8, which contains 2-(2-methoxyethoxy)ethanol, have been conducted in this laboratory [12]. Animal urine can contain higher levels of various components compared to human urine and can cause additional chromatographic background peaks. This was the case in the analysis for MEAA in urine from exposed mice. Figure 3c shows an example chromatogram generated using the esterification procedure on urine collected from JP-8 exposed mice containing MEAA as a metabolized product from 2-(2-methoxyethoxy)ethanol. The esterification procedure was found to be the better chromatographic procedure for this animal study because of the additional minor background peaks found in mouse urine (see Fig. 3c). Initial human field samples have also been evaluated using the esterification procedure, and urine from JP-8 exposed humans have shown detectable levels of MEAA [37]. Figure 3b shows a urine sample from a human field subject with an 8.4 μg mL^{-1} MEAA level using the esterification procedure. The urine collected for this chromatogram was from a fuel-tank-maintenance worker exposed to JP-8. Proposed future work includes further analyses of urine field samples to be collected from the United States Air Force personnel exposed to JP-8 during their daily work activities along with other assessment tests for exposure. This field sample work is part of a larger comprehensive study which is beyond the scope of this manuscript; this manuscript only evaluates analytical procedures for the quantification of MEAA in urine.

Conclusions

Several extraction and derivatization procedures for the analysis of MEAA in urine were evaluated during this study. Liquid-liquid extraction proved to have higher analyte recovery than solid phase extraction. Silylation of MEAA to the TBDMS derivative was found to have better precision and a lower detection limit than esterification of the alkoxyacetic acid to the ethyl ester. The LOD for the silylation procedure was 0.01 μg mL^{-1} compared to 0.05 μg mL^{-1} for the esterification procedure. The accuracy of both procedures were equivalent; 94 to 105% recoveries for urine samples spiked at 2, 5, 10 and 20 μg mL^{-1} MEAA levels were obtained. Standard curves generated linear responses in the 0 to 50 μg mL^{-1} concentration range with correlation coefficients of 0.97 and greater for both procedures. Although the esterification procedure was more labor intensive, it was superior in providing additional sample clean-up which substantially reduced small chromatographic background peaks. These procedures have been demonstrated to be applicable for the quantification of MEAA in urine samples.

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