



High-throughput sample preparation and simultaneous column regeneration liquid chromatography–tandem mass spectrometry method for determination of nitrogen mustard metabolites in human urine

Muntha K. Reddy^{a,*}, Grier Mills^a, Christopher Nixon^a, Shane A. Wyatt^a, Timothy R. Croley^b

^a Division of Consolidated Laboratory Services, Commonwealth of Virginia, 600 N. 5th Street, Richmond, VA 23219, USA

^b U.S. Food and Drug Administration Center for Food Safety and Applied Nutrition, 5100 Paint Branch Parkway, HFS-707, College Park, MD 20740, USA

ARTICLE INFO

Article history:

Received 13 April 2011

Accepted 17 June 2011

Available online 26 June 2011

Keywords:

HTP-LC-MS/MS

LC-column switching

High-throughput extraction

N-ethyl-diethanolamine

N-methyl-diethanolamine

ABSTRACT

Nitrogen mustards (NMs) are known to have DNA alkylation and strong vesicant properties. Their availability to terrorist organizations makes them a potential choice for chemical attacks on civilian populations. After an exposure, it is difficult to measure NMs directly because of their rapid metabolism in the human body. Therefore to determine an individual's level of exposure to NMs, it is necessary to analyze for NM metabolites being excreted by the body. The metabolites of NMs are generated by a hydrolysis reaction, and are easily detectable by liquid chromatography tandem mass spectrometry (LC-MS/MS). This work is focused on the development of a high-throughput assay for the quantitation of N-ethyl-diethanolamine (EDEA) and N-methyl-diethanolamine (MDEA) metabolites of bis (2-chloroethyl) ethylethanamine (HN1) and bis (2-chloroethyl) methylethanamine (HN2), respectively. The method uses automated 96-well plate sample preparation of human urine samples and a 2-position 10-port switching valve to allow for simultaneous regeneration of the liquid chromatography (LC) columns. Using this method, over 18 h was saved through the reduction of sample preparation and analysis time when compared to a conventional method for 96 samples. The validated method provided excellent accuracy for both EDEA (100.9%) and MDEA (100.6%) with precision better than 5.27% for each analyte.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

The deliberate use of chemicals in terrorist activities is one of the greatest potential threats to human beings across the globe. Nitrogen mustards (NMs) are blister causing agents, which attack the central nervous system [1]. They are also bifunctional alkylating agents that may covalently bond to DNA and proteins, causing cytotoxicity [2–5]. The NMs, bis (2-chloroethyl) ethylethanamine (HN1), bis (2-chloroethyl) methylethanamine (HN2), and tris (2-chloroethyl) amine (HN3) are listed on the Chemical Weapons Convention Schedule of Chemicals [6].

The high reactivity and short lifetime of these compounds makes it difficult to directly measure NMs in exposed individuals. Hence the hydrolyzed metabolites of NMs are better biomarkers for assessing exposure to HN1, HN2, and HN3 [7]. However, as previously reported, the HN3 hydrolysis product N-triethanolamine (TEA) is a common ingredient in a wide variety of consumer products [8]. The prevalence of this compound in products like cosmetics and other personal care items has resulted in a significant

amount of TEA being detectable in the background population. Studies to evaluate its background level have found that as many as 47% of individuals tested for TEA show elevated levels [9]. To this end, TEA is not a reliable biomarker of HN3, and was not evaluated in this assay.

In previously reported methods for the detection of EDEA and MDEA in environmental samples, several gas chromatography–mass spectrometry (GC-MS) protocols were presented [10–13]. As part of the Chemical Weapons Convention, Kanaujia et al. performed a comparative SPE extraction using strong cation and mixed mode strong cation-exchange cartridges followed by GC-MS analysis of amino alcohols in water and human plasma [14]. The high polarity, basic nature, and low volatility of these analytes required derivatization and extensive sample preparation, as described by Black and Muir [15]. These prolonged sample preparation steps limit the application of GC-MS methods in the analysis of amino alcohols.

The LC-MS analysis of several NM degradation products, including EDEA and MDEA, in water and decontamination solutions has been reported [16]. The determination of ethanolamines in high salinity industrial wastewater was also reported using LC and tandem mass spectrometry [17]. Atmospheric pressure chemical ionization (APCI) in combination with liquid chromatog-

* Corresponding author. Tel.: +1 804 648 4480x377; fax: +1 804 225 3512.

E-mail address: kesava.muntha@dgs.virginia.gov (M.K. Reddy).

raphy tandem mass spectrometry (LC–APCI–MS/MS) methods have advantages over GC/MS methods in the quantitation of chemical warfare agents because of simple sample preparation steps and easy analysis procedure [18]. Finally, nitrogen mustard adducted with DNA, and depurination products such as the N-7-alkyl guanines (N-7-G) in urine, can also be used as biomarkers for the determination of NM's [5,19]. Still, each reported method has its limitations and none of the previously reported methods have ever been applied to determine EDEA and MDEA urinary metabolites after exposure to NMs [9].

A solid phase extraction (SPE), liquid chromatography electrospray–tandem mass spectrometry (LC–ESI–MS/MS) method using isotopically labeled internal standards of EDEA and MDEA in biological matrices was published by Lemire et al. [20]. Subsequently, further modifications were made by this same group to optimize the chromatographic separation of EDEA and MDEA [9]. A survey of the literature indicates that there is still great need for a robust and high-throughput method to detect NMs after a large-scale exposure event.

The present study describes an automated, high-throughput extraction, with alternating LC column regeneration by valve switching. A 2-position 10-port valve is utilized to switch flows between columns, allowing for regeneration of one column while separation is performed on the other. The detection and quantitation of EDEA and MDEA (Fig. 1) in urine is achieved using LC–ESI–MS/MS. In addition to higher analytical throughput, this method uses less sample volume (300 μ L), requiring fewer resources and labor, without sacrificing precision and/or accuracy.

2. Experimental

2.1. Chemicals and reagents

A mixture of EDEA and MDEA standards in urine were obtained from SPEX CertiPrep Group (Metuchen, NJ, USA) in concentrations of 1, 5, 10, 50, 100, 250, and 500 ng/mL in sealed ampoules. A certified urine blank (1.8 mL), and two quality-control standards (QC) were also provided. The nominal concentrations of the QC low and high materials were 25 and 300 ng/mL, respectively. An isotopically labeled internal standard solution of EDEA- $^{13}\text{C}_4$ and MDEA- $^{13}\text{C}_4$ at a concentration of 1000 ng/mL in 15 mM ammonium hydroxide solution was also obtained from SPEX CertiPrep in 1.8 mL flame sealed ampoules. Extraction was performed using Varian Bond Elut Certify (100 mg/well) solid phase extraction (SPE) 96-well plates (Lake Forest, CA, USA). Two Luna CN columns (100 mm $\times$ 2 mm, 3 μ m) from Phenomenex (Torrance, CA, USA) were used as column A and column B for liquid chromatography analysis. Strata 96 square well (2 mL/well) polypropylene plates (Phenomenex) were used for initial sample aliquots and collection after extractions. HPLC grade methanol, HPLC grade acetonitrile, ammonium hydroxide solution, and ammonium bicarbonate were obtained from ThermoFisher Scientific (Pittsburgh, PA, USA). Organic-free 18.3 M Ω cm $^{-1}$ ultra pure water was purified in-house using an E-pure system (Barnstead International, Dubuque, IA, USA). Pooled urine was donated by healthy laboratory personnel.

2.2. Instrumentation

A Tecan Freedom EVO $^{\text{®}}$ 200 (Research Triangle Park, NC, USA) liquid handling system equipped with an eight-channel liquid handling arm (LiHa) for pipetting tasks was used for high throughput extraction and sample preparation. The system also included a mounted Te-VacS plate vacuum manifold, Te-Shake plate vortexer, and robotic manipulator arm (RoMa) with gripper fingers for transporting plates around the work deck. Following SPE, sample

extracts were concentrated to dryness using a TurboVap $^{\text{®}}$ 96 evaporator (Caliper Life Sciences, Hopkinton, MA, USA) under N_2 . For comparison a Gilson 215 Liquid Handler (Middleton, WI, USA) was used for barrel SPE extraction and sample preparation. The sample extracts were concentrated to dryness under N_2 using a TurboVap $^{\text{®}}$ LV (Caliper Life Sciences). LC column switching was performed using a 2-position 10-port Valco valve (VICI $^{\text{®}}$ Valco Instruments Co. Inc., Houston, TX, USA). Liquid chromatography was performed using an Agilent 1200 HPLC system (Agilent Technologies, Wilmington, DE, USA). LC column regeneration was accomplished using an Agilent 1100 isocratic pump. Mass spectrometry analysis was performed with a 4000 QTRAP $^{\text{TM}}$ mass spectrometer (AB Sciex, Foster City, CA, USA). The LC–MS/MS system configuration, sample analysis, and data collection were performed using Analyst $^{\text{TM}}$ software version 1.4.2 (AB Sciex).

2.3. 96-well plate extraction and sample preparation

Three-hundred microliters of each calibration standard and QC standard was manually transferred into a clean 96-well plate. The 96-well plate with sample aliquots was placed in the Tecan for automated sample extraction. The LiHa arm was used to add 20 μ L of internal standard to each sample well. Each sample was diluted with 300 μ L of organic-free ultrapure H_2O , and vortex mixed for 35 s by the Te-shake at 1500 rpm. Each well of the SPE plate was conditioned with 1.0 mL of MeOH followed by vacuum. The plate was then equilibrated with 1.0 mL of 18.3 M Ω H_2O , and vacuum was applied. The LiHa arm was then used to transfer the total volume (620 μ L) of prepared sample to the SPE plate followed by vacuum. The SPE beds were then washed with 1.0 mL of acetonitrile under minimal vacuum to remove residual water from the stationary phase. Excess water present in the sample can result in inconsistent sample evaporation. Sample elution into a clean 96-well collection plate was performed with 2 cycles of 750 μ L of 10% NH_4OH in acetonitrile (v/v), each with the application of vacuum. The elution plate was evaporated to dryness using a TurboVap 96 under N_2 at 40 $^{\circ}\text{C}$. Finally, each sample was reconstituted with 100 μ L of 10 mM NH_4HCO_3 solution using the Tecan Freedom EVO. The 96-well plate extraction was accomplished using lower sample volume (300 μ L) with shorter sample preparation time. The automated system eliminated a number of manual transfer steps, thus minimizing errors, and the time required for extraction of 96 samples was 45 min.

2.3.1. Gilson sample preparation

For comparison, a Gilson 215 Liquid Handler extraction was performed employing a 3cc Varian Bond Elut Certify Tableless SPE cartridge (300 mg bed). A 1 mL sample aliquot was used for extraction, and the extract was concentrated to dryness under N_2 at 40 $^{\circ}\text{C}$ using a TurboVap. The sample was reconstituted into 150 μ L of 10 mM NH_4HCO_3 and transferred into 1.5 mL sample vials with inserts.

2.4. Validation

2.4.1. Linearity

The linearity of the method was determined by analysis of standard plots associated with a seven-point standard calibration curve within the range 1–500 ng/mL. The calculation was based on the peak area ratio of analyte versus the area of internal standard. Linearity was observed over the specified concentration range with $r = 0.9997$ and 0.9998 for MDEA and EDEA, respectively.

2.4.2. Limit of quantitation (LOQ)

The lowest concentration of calibration standard of MDEA and EDEA, 30 pg on-column, represented the limit of quantitation (LOQ)

Download English Version:

<https://daneshyari.com/en/article/1213315>

Download Persian Version:

<https://daneshyari.com/article/1213315>

[Daneshyari.com](https://daneshyari.com)