Journal of Chromatography B, 878 (2010) 2541–2547

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Quantitation of tetramethylene disulfotetramine in human urine using isotope dilution gas chromatography mass spectrometry (GC/MS and GC/MS/MS)☆

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ARTICLE INFO

Article history:
Received 2 December 2009
Accepted 19 March 2010
Available online 27 March 2010

Keywords:
Tetramethylene Disulfotetramine
Tetramine
Human urine

ABSTRACT

Tetramethylene disulfotetramine (tetramine) is a rodenticide associated with numerous poisonings was extracted and quantified in human urine using both gas chromatography/mass spectrometry (GC/MS) and GC/tandem mass spectrometry (MS/MS). 1200 μL samples were prepared using a $^{13}C_4$-labeled internal standard, a 96-well format, and a polydivinyl-benzene solid phase extraction sorbent bed. Relative extraction recovery was greater than 80% at 100 ng/mL. Following extraction, samples were preconcentrated by evaporation at 60 °C, and reconstituted in 50 μL acetonitrile. One-microliter was injected in a splitless mode on both instruments similarly equipped with 30 m × 0.25 mm × 25 μm, 5% phenylmethylpolysiloxane gas chromatography columns. A quantification ion and a confirmation ion (GC/MS) or analogous selected reaction monitoring transitions (GC/MS/MS) were integrated for all reported results. The method was characterized for precision (5.92–13.4%) and accuracy (96.4–111%) using tetramine-enriched human urine pools between 5 and 250 ng/mL. The method limit of detection was calculated to be 2.34 and 3.87 ng/mL for GC/MS and GC/MS/MS, respectively. A reference range of 100 unexposed human urine samples was analyzed for potential endogenous interferences on both instruments—none were detected. Based on previous literature values for tetramine poisonings, this urinary method should be suitable for measuring low, moderate, and severe tetramine exposures.

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1. Introduction

Tetramethylene disulfotetramine is a banned rodenticide in the United States and China; tetramine has been associated with numerous intentional and unintentional poisonings [1–3]. While these poisonings primarily have been reported in China, only one case has been reported in New York City [3]. The human LD_{50} has been reported to be as low as 0.1 mg/kg [3,4]. Tetramine is an odorless, tasteless white powder, with a molecular weight of 240.26 g/mol (see Fig. 1) that easily dissolves in water. Tetramine is quite stable [5,6], and destruction of the compound requires elevated temperatures and caustic conditions [5].

The most common route of tetramine exposure is by ingestion; tetramine is not absorbed through intact skin [3]. Symptoms associated with mild tetramine poisoning include headache, dizziness, fatigue, nausea, vomiting, perioral paresthesias, weakness, anorexia, and lethargy [3]. High-level poisonings are associated with seizures, coma and death [3]. The onset of symptoms occurs between 0.5 and 13 h post-exposure [3].

Most previously reported clinical results for tetramine were based on plasma measurements. Chau et al. quoted toxic (2–369 ng/mL plasma) and lethal concentrations of tetramine (640–5490 ng/mL plasma) [4]. Lu et al. divided these exposure levels into three ranges [7]: mild poisoning (<50 ng/mL plasma), moderate poisoning (50–100 ng/mL plasma) and severe poisonings (>100 ng/mL plasma). Seizures were associated with moderate to severe poisoning [7]. The ratio between urine and plasma (urine/plasma) tetramine concentrations was reported to be 1.299 ± 0.388 [8]. Some reports show that tetramine remains unchanged in the human body for up to 6 months after exposure [4] and is excreted intact in the urine and stool. In one case report, tetramine was monitored in urine for more than 100-h post-exposure [9] at concentrations greater than 50 ng/mL.

Analytical methods for the quantification of tetramine in urine and blood for sample analysis have included the use of gas chromatography coupled with nitrogen phosphorus, flame ionization, and mass selective detectors [9–12]. Solid phase microextraction (SPME) has been reported and offers unique advantages for reduced sample preparation [9–11]. Although these analytical methods

☆ This paper is part of the special issue "Biological Monitoring and Analytical Toxicology in Occupational and Environmental Medicine", Michael Bader and Thomas Göen (Guest Editors).

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1570-0232/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.jchromb.2010.03.038

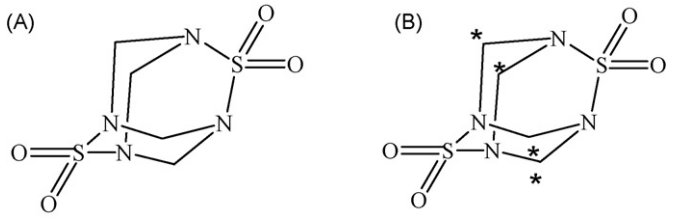

Fig. 1. Structure of (A) native and (B) $^{13}C_4$-labeled tetramethylene disulfotetramine (tetramine). *: positions of the ^{13}C-labels.

were useful for limited numbers of clinical samples, improvements were needed to increase method specificity and decrease possible carryover between samples.

More recent analytical methods have focused on food matrices and have included the use of direct insertion and headspace-SPME [13–15]. A comparison of three extraction techniques—membrane-assisted solvent extraction, stir bar sorbtive extraction, and SPME—was recently reported for food matrices [14]. The first instance in which LC/MS/MS was used for quantifying tetramine in beverages focused on a novel target molecule, which is the higher-molecular weight dimer of tetramine [15].

Limits of detection for both the clinical and food/beverage applications have generally been similar; they have ranged from low ng/mL or ng/g, depending on the matrix of interest. But the recovery and accuracy of these methods have been limited by two principal factors: (1) the difficulty of extracting the analyte, which primarily applies to food and beverage matrices and (2) lack of a commercially available, isotopically enriched internal standard.

The new analytical method reported here focuses on measuring tetramine in urine to, in turn, measure internal dose resulting from exposure. An efficient, 96-well format is used with a polymeric solid phase extraction sorbent and a commercially available $^{13}C_4$-labelled internal standard. An additional advantage of the method lies in its ability to process a large number of samples per day [16,17]. And to make the method more useful to the general laboratory community, extraction was characterized on both standard (GC/MS) and advanced (GC/MS/MS) instrumentation.

2. Experimental

All solvents were HPLC grade (Tedia, Fairfield, OH) and standard laboratory glassware was used to prepare standard solutions and extraction solvents. Native and labeled tetramine (see Fig. 1) were purchased from Cambridge Isotopes Laboratories (Boston, MA). Initial stock solutions were made by weighing 20.4 mg of tetramine into 250 mL of acetonitrile. The stock was then diluted into pooled urine (Tennessee Blood Services, Memphis, TN) for final concentrations of 5, 10, 25, 50, 100, and 250 ng/mL. Quality control (QC) solutions of 15, 35 and 75 ng/mL and a matrix blank were also made from the same pool of urine. All urinary samples, standards, calibrators, blanks and unknowns were stored at −20 °C until the day of testing.

Stability testing consisted of storing two stock solutions of tetramine in urine at −20 °C for 6 months. The stock solutions were 15 and 75 ng/mL and were analyzed using the method described here. The samples used for comparison were freshly prepared materials of the same concentrations.

An aliquot of 1200 μL of each blank, standard, quality control material—or unknown sample—was transferred to a polypropylene 2000-μL round-bottom 96-well plate (Nunc PN# 278752, Rochester, NY). A 50-μL aliquot of a 1000 ng/mL $^{13}C_4$-internal standard was added to each well, using a 12-port electronic pipettor (Rainin, PN# E12-300, Oakland, CA). The plate was mixed for 5 min using a plate shaker (Thermo-Electron, PN# 00509186, Bremen, Germany) and then covered with a standard plastic lid (ThermoFisher Scientific, PN# 07201731, Waltham, MA) to prevent environmental contamination and facilitate robotic handling.

A Caliper Life Sciences i1000 Liquid Handling Workstation (Hopkinton, MA) was used for the extraction. The i1000 was equipped with an SPE vacuum manifold, four bulk solvent dispensers, and a gripper for moving plates around the deck. A Twister II Microplate Handler with HyperStak Plate/Tip Dispenser was used for loading and unloading consumables from the deck of the i1000. At the beginning of the extraction, the HyperStaks were separately loaded with the covered sample plate, a 2000-μL Nunc covered receiving plate, a 96-well Strata-X SPE plate (60 mg, Phenomenex, PN# 8E-S100-UGB, Torrance, CA) and 200-μL Twister Stack tip racks (Caliper Life Sciences, PN# 78641, Hopkinton, MA).

The SPE plate was conditioned with 1125 μL of methanol followed by 1125 μL of deionized water. The sample volume (1250 μL with ISTD) was loaded onto the SPE plate and rinsed with 1125 μL of 5% methanol in water. Between each extraction step, vacuum was automatically applied; liquid detection assured the plate was empty. The i1000 gripper placed the 2000-μL Nunc receiving plate into the vacuum manifold, and the analyte was eluted with 1125 μL of 100% acetonitrile.

Samples were dried at 60 °C using a TurboVap 96 (Caliper, Hopkinton, MA) with nitrogen flow ranging from 20 to 50 L/min. The plate was initially dried at a low flow rate of 20 L/min for 15–20 min to prevent splashing, and then 50 L/min for 30 min. The dried sample was reconstituted with 50 μL of 100% acetonitrile. Samples were then transferred to conical 300-μL polypropylene vials (Lab Depot, PN# ARC-6026, Dawsonville, GA) for instrument analysis.

Relative recovery experiments were completed in duplicate over a range of loading volumes (see Table 1) and at a single concentration of 100 ng/mL. Two samples were extracted after the native and label compounds were added together (termed "normal-addition"). Two samples were also extracted before the internal standard was added (termed "post-addition"). The percent relative recovery was calculated using the following expression:

$$\frac{\text{Post-addition native area/ISTD area}}{\text{Normal-addition native area/ISTD area}} \times 100\%.$$

For method validation, prepared samples were analyzed on two different mass spectrometers. The first instrument used for this application was a gas chromatography–single stage mass spectrometer (GC/MS Agilent 6890N GC coupled with Agilent 5973 MS (Santa Clara, CA)) using ChemStation software complete with a HP5-MS 30 m × 0.25 mm, 0.25 μm film thickness GC column. One microliter of sample was injected into a 250 °C inlet using splitless injection. Before and after each injection, the injection syringe was cleaned immediately with two 5-μL washes of acetonitrile and two 5-μL washes of acetone. A constant flow of helium (99.9999%, research grade, Airgas, Atlanta, GA) was maintained throughout the analysis at a flow rate of 1 mL/min. The column oven was heated

Table 1
Relative recovery of tetramine spiked into pooled human urine and extracted by a polydivinyl-benzene solid phase extraction sorbent bed.

Load volume	Recovery
100 μL	92%
200 μL	81%
300 μL	94%
400 μL	82%
500 μL	98%
750 μL	97%
1000 μL	94%
1250 μL	95%
1500 μL	87%

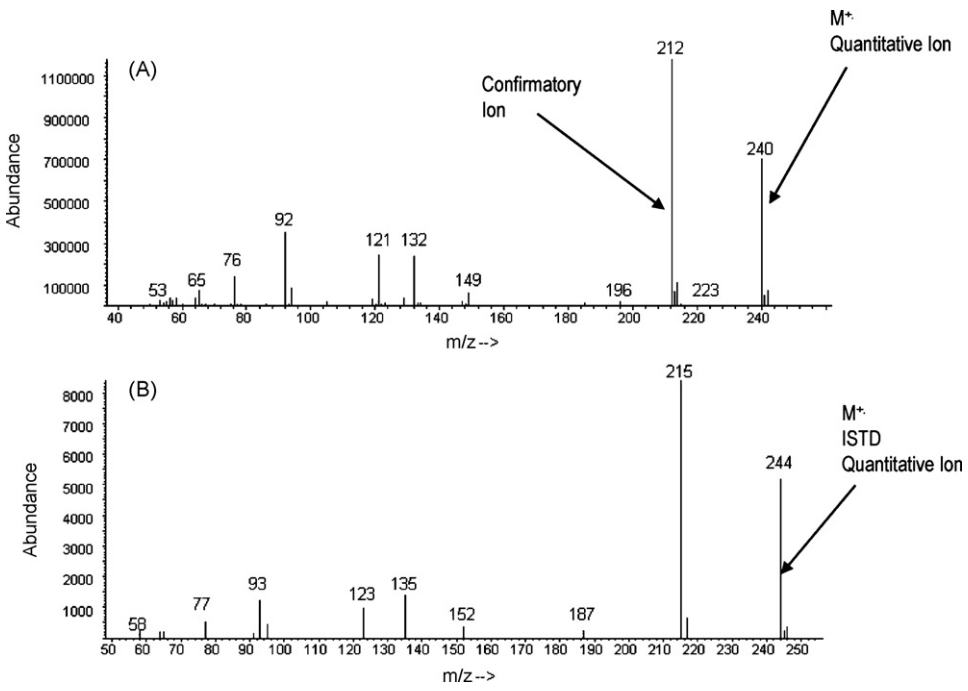

Fig. 2. Full scan electron impact mass spectrum of (A) native and (B) $^{13}C_4$-labeled tetramine using a gas chromatography–single quadrupole mass spectrometer.

from 100 to 200 °C at a rate of 8 °C/min followed by a rapid heating of 50 °C/min from 200 to 250 °C; the final temperature of 250 °C was held for 1 min to prepare the column for the next injection. This oven program resulted in a total runtime of 14.5 min. The following masses were monitored (see Fig. 2): 240, 212, and 244 *m*/*z*. Each mass was programmed for a dwell time of 100 ms. Acetonitrile, ethyl acetate, and methanol (HPLC grade, ThermoFisher, St Louis, MO) were all evaluated for use as the injection solvent.

The second instrument used was a gas chromatography–triple quadrupole mass spectrometer (GC/MS/MS) consisting of an Agilent 6890 GC coupled with an Agilent 5975 MS retrofitted with a Chromsys Evolution MS/MS (Chromsys, LLC, Alexandria, VA). This GC/MS/MS instrument was equipped with the same gas chromatograph, which was operated as described for the GC/MS experiments. The following selected reaction monitoring (SRM) transitions were monitored (see Fig. 3): 240–212 *m*/*z* and 212–92

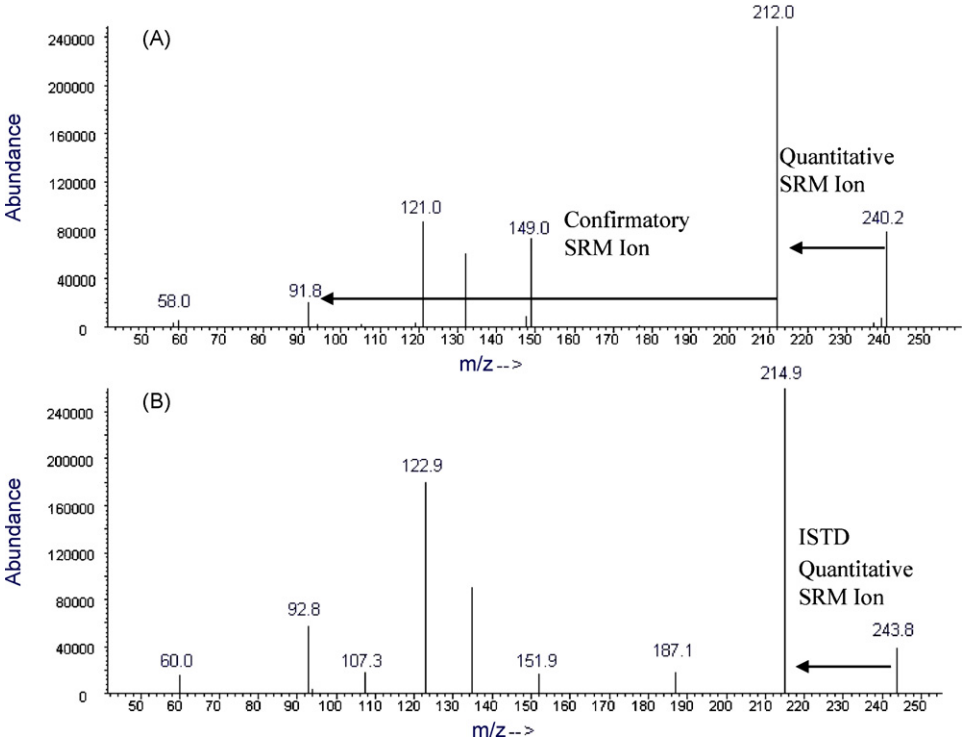

Fig. 3. M^+ product ion scan of (A) native and (B) $^{13}C_4$-labeled tetramine generated using selected reaction monitoring (SRM) on a gas chromatography–triple quadrupole mass spectrometer.

m/z for native tetramine and a single transition 244–215 m/z for the internal standard. The dwell time and collision energy were the same for all three transitions, and were optimized at 0.33 s and 16 eV, respectively. The collision gas pressure setting was 17 psi, with the electron multiplier set at 1500 V.

Each blank, standard, quality control sample or unknown sample was injected once. Calibrators were analyzed in order of increasing concentration. The quality control samples were analyzed after the highest calibrator, following a blank sample. Quantification of unknowns was performed using linear least-squares regression with no weighting. The response was linear, with a correlation coefficient of 0.99 or greater. Twenty sets of blanks, standards, and quality controls were used to characterize each method for accuracy, precision, and limit of detection [18]. All data were stored in an in-house database known as the Emergency Response Management System [17].

One hundred reference range samples were acquired from Tennessee Blood Services (Memphis, TN). Because no personal identifiers were available for these samples, they were exempt from human subjects research review. These samples were processed in a manner identical to the blank, standard, and quality control materials, except that they were initially aliquotted from a 10 mL cryovial and into a 96-well plate using a Tomtec Formatter (Hamden, CT).

3. Results

To optimize the analytical method for each instrument, we tuned the mass spectrometer in the selected ionization mode for optimum selectivity and sensitivity. Then, to provide sharp baseline-separated peaks, we evaluated the gas chromatography conditions, including injection solvent, injection mode, chromatography column composition, and column geometry. Solid phase extraction steps were selected that would best separate interferences from the tetramine while at the same time maximizing recovery. Finally, we characterized the method by analyzing tetramine-enriched human urine aliquots and 100 random, anonymous, and unexposed human reference samples.

Fig. 1 shows the structure of tetramine and the positions of the $^{13}C_4$-labels on the internal standard. The compound has been reported as stable [5] and was not found to be efficiently ionized by electrospray or atmospheric pressure chemical ionization. The use of electron ionization with tetramine had been previously cited [13–15] and was selected for use here. As noted in the full scan spectrum in Fig. 2A, the molecular ion (m/z 240) was selected as the quantitation ion in the GC/MS method, and the corresponding internal standard molecular ion (m/z 244, Fig. 2B) was offset by four mass units due to the $^{13}C_4$-labeling. The most abundant fragments of the native and the labeled compounds differed by 3 instead of 4 mass units, indicating that the molecular ion of the labeled compound had lost $^{13}C_1$. This further indicates that significant rearrangement had occurred during fragmentation, which had also been reported for compounds containing sulfones [19]. For the GC/MS/MS instrument, Fig. 3 shows that m/z 240 → 212 is the most abundant ion product and was used here as the quantitative SRM transition. The confirmation SRM transition was m/z 212 → 92, an MS^3 transition with the first neutral loss occurring in the instrument source and the second in the Q2 collision cell. However, this transition was not observed to offer any significant advantages in signal-to-noise ratio or in sensitivity compared to the quantitative MS^2 transition.

Isotopically-labeled reference material is key to developing precise and accurate methods in human clinical matrices such as urine, and compensating for analyte loss during extraction or manipulation (e.g., dry-down, reconstitution). The $^{13}C_4$-label positions were selected so that they were conserved during the fragmentation of the analyte and did not have any interferences in the single stage mass spectra generated in the GC/MS (see Fig. 2A and B). The conservation of the labeled carbons can also be observed in product ion spectra (Fig. 3A and B) where—even after the dissociation of more than 60% of the original molecular ion—the confirmatory ion still contains $^{13}C_1$.

Gas chromatography conditions were primarily selected to separate the tetramine signal from endogenous noise observed as a rise in baseline at the end of the analytical analysis. Peaks, believed to originate from the solvents used, were sporadically present near the tetramine peak at m/z 212 in the GC/MS instrument. Thus to ensure adequate separation from these potential interferences at low concentrations, the required runtime for this analyte was 14.5 min. Acetonitrile, ethyl acetate, and acetone, initially selected for reported tetramine solubility, were evaluated for use as injection solvents for the gas chromatography. When reconstituted in acetonitrile, tetramine produced the most linear response and consistent peak shape. Due to the calculated expansion of acetonitrile with an inlet and linear volume temperature of 250 °C, the maximum injection volume was limited to 1 μL with a splitless injection.

One of the primary issues associated with the gas chromatographic method on both instruments was tetramine carryover from sample to sample. This was resolved by cleaning the injector syringe with multiple solvents held in the autosampler. A rinse step consisted of inserting the instrument syringe into a solvent, drawing the solvent to full syringe capacity, and dispelling the solvent into a waste vial. Acetone was selected to dissolve tetramine, while acetonitrile matched both the injection solvent and also dissolved tetramine. To reduce the carryover from sample to sample, two rinses were required of each solvent before each injection, and two rinses of each solvent were required following each injection. To insure adequate solvent was available to operate the instrument for up to 16 h without refilling the solvent reservoirs, the syringe volume was reduced to 5 μL.

A 96-well sample preparation design allowed a single operator to dramatically increase sample throughput with minimal attention [16]. The maximum sample size was limited to 86% of the extraction plate volume—a necessary limitation to accommodate the additional internal standard volume (+50 μL) and pipette tip displacement (+300 μL). Primary adjustments to the use of the 96-well format from manual extraction cartridges were due to the limited size of the sample well and to prevent inadvertent contamination between the wells. Cross-contamination was not an issue unless an individual sample well clogged or was not clear before the subsequent rinse or elute step. This was resolved by using a liquid handler, which could verify each well was clear of sample or solvent prior to the next extraction step.

Because the method was intended to identify subjects who had both acute and subacute exposure to tetramine, we needed optimum recovery and method sensitivity. Relative recoveries for tetramine were greater than 80% (see Table 1) at a concentration of 100 ng/mL and a sample volume of 1200 μL. Increasing concentrations with a fixed sample volume of 1200 μL decreased the relative extraction efficiencies to as low as 60% at 180 ng/mL, while decreasing concentrations maintained or exceeded 80% relative recoveries. The use of the commercially available, isotopically labeled internal standard, compensated for any recovery variations or losses due to sample manipulation and allowed maintenance of high accuracy across the range of concentrations of interest.

Six calibrators were used with three quality control materials and a matrix blank, all derived from the same blank urine pool. Twenty analytical batches consisting of calibrators, quality control samples, and a blank were used to characterize the method for precision, accuracy, and limits of detection (see Table 2). Any positive response required the correct ratio of the quantitative and

Table 2
Characterization of quality control (QC) materials, characterized by 20 analyses using both a gas chromatography–single quadrupole mass spectrometer (GC/MS) and a gas chromatography–triple quadrupole mass spectrometer (GC/MS/MS).

Quality control name	Instrument	Expected concentration (ng/mL)	Measured concentration (ng/mL)	Accuracy (%)	Standard deviation (ng/mL)	Coefficient of variation (%)
QC-low	GC/MS	15	15.7	105	1.55	9.87
QC-low	GC/MS/MS	15	16.7	111	2.24	13.4
QC-medium	GC/MS	35	34.0	97.1	2.16	6.35
QC-medium	GC/MS/MS	35	34.3	98.0	3.76	11.0
QC-high	GC/MS	75	73.6	98.1	4.36	5.92
QC-high	GC/MS/MS	75	72.3	96.4	6.28	8.69

confirmatory ion response. This ratio was calculated from the calibrators used in each batch and was used to evaluate all positive responses from quality controls as well as any unknown responses with a tolerance of ±30%.

The GC/MS was slightly more sensitive than was the GC/MS/MS, as reflected by a lower percent coefficient of variation (%CV; −3.53%) and an accuracy closer to 100% when the lowest concentration material (15 ng/mL) was characterized. At higher concentrations, no substantial differences appeared between the accuracy of each instrument, but the CVs were higher in the triple quadrupole: +4.64% at 35 ng/mL and +2.77% at 75 ng/mL. The source of this increased instrument variation was not definitively identified. Because an isotopically labeled internal standard was used here, the variation source is probably not the autosampler or any differences in sample preparation recoveries. A more likely explanation is that the source of the variation included a general bias from the ion optics due to the need for more optimal tuning or the need for a longer settling time between each SRM transition.

The limits of detection for each instrumental method were calculated by plotting the absolute standard deviation of the lowest four standards versus their respective concentrations [18]. The *y*-intercept of the regression of these data, s_o, was calculated and multiplied by 3 to determine the limits of detection, which were 2.34 and 3.87 ng/mL for the GC/MS and GC/MS/MS, respectively. As expected, the higher variation of the triple quadrupole was translated into higher limits of detection. The lowest calibrator of the method, 5 ng/mL, was above the limit of detection and was referred to as the lowest reportable limit.

Stability testing of tetramine in urine was evaluated by using two quality control samples, enriched at 15 and 75 ng/mL. The samples were stored at −20 °C for 6 months and tested at the end of that period versus freshly prepared comparison samples. The values for the comparison and stored samples were (in ng/mL) 17.1 and 13.6 and 76.3 and 77.9. These concentrations were within with the 95% confidence limits of each QC pool. The stability of samples that had already been extracted was also evaluated to simulate a situation in which analysis was delayed due to instrument malfunction or a similar uncontrolled event. The extracted samples were stored at 5 °C for up to 2 weeks with no significant loss of signal or accuracy. Stability of the materials is a critical concern—the enriched urine pools (calibrators, quality controls, blanks) are stored for up to 2 years and are used to test operator proficiency. Storing the materials in a prediluted form also decreases response time and facilitates the rapid delivery of accurate and precise exposure data.

A reference range of 100 random urine samples was analyzed to measure any endogenous interferences present in the general population. This is a key part of developing a method for clinical samples. Sources of endogenous interferences can derive from diet, cosmetic products, or other environmental sources. For the 100 samples measured for the human reference range, which were quantified on both instruments, no interferences were observed. This indicates that the method is selective for tetramine; thus when analyzing unknown urine samples, no false positive results are anticipated.

4. Discussion

The goal of this method was to analyze clinically significant concentrations of tetramine in human urine. The derived data can aid in public health efforts to treat potentially exposed people. The method should have high sensitivity and few potential interferences to identify people who may have low or subacute exposure. Because symptoms of tetramine poisoning are severe and include possible death at high exposures, exposure assessment must be accurate and rapid.

The two instrumental methods developed here have shown good accuracy and precision. The limits of detection of the method are adequate to quantify low human internal dose if tetramine is ingested. The method has also been characterized using 20 experiments that included blanks, calibrators, and quality control materials. In a control group that had no known exposure to tetramine, no interferences were measured.

Previously reported tetramine exposures have been evaluated through analysis of plasma. Ranges of tetramine concentrations in plasma have been generically divided into three ranges of less than 50 ng/mL, 50–100 ng/mL, and greater than 100 ng/mL. These concentrations correlate to mild, moderate, and severe poisonings; seizures have been reported at moderate and severe poisonings. Few data are available on the use of urine as a matrix instead of plasma, but two key studies bear consideration. Tetramine was monitored in one patient in both urine and serum, and a correlation between the two matrices was established. The observable urinary concentration was 1.3-times higher (±30%) than in plasma. In another case study, tetramine was measured at concentrations above 50 ng/mL in urine for more than 100 h with little change in the overall concentration [9]. Whether the patient was exposed to low, moderate, or even severe tetramine concentrations was not clear. Combined, these two studies indicate that expected concentrations of urine should be equivalent to or greater than the three concentration ranges reported in plasma. In addition, tetramine should be excreted in urine for an extended period of time—up to 4 days. Urine samples are easily acquired and, typically, obtainable in larger volume than are most plasma samples.

With a lower reportable limit of 5 ng/mL (Figs. 4 and 5), both instrument methods described here are suitable for rapidly measuring urinary concentrations associated with mild tetramine poisoning, as well as more severe exposures. The accuracy and precision of the GC/MS method was consistently better than with GC/MS/MS. If we combine the 5% (GC/MS) or 11% (GC/MS/MS) error associated with a mild poisoning exposure with the limits of the 95% confidence interval of the method, an expected concentration of 15 ng/mL could be actually determined to be 18.9 ng/mL (GC/MS) or 21.2 ng/mL (GC/MS/MS). This bias however, would not, influence medical decisions regarding potential tetramine poisoning. Considering that the ranges of expected urinary concentrations following an exposure are not known and can vary by 30% compared to plasma levels, both instrumental methods are suitable for this application.

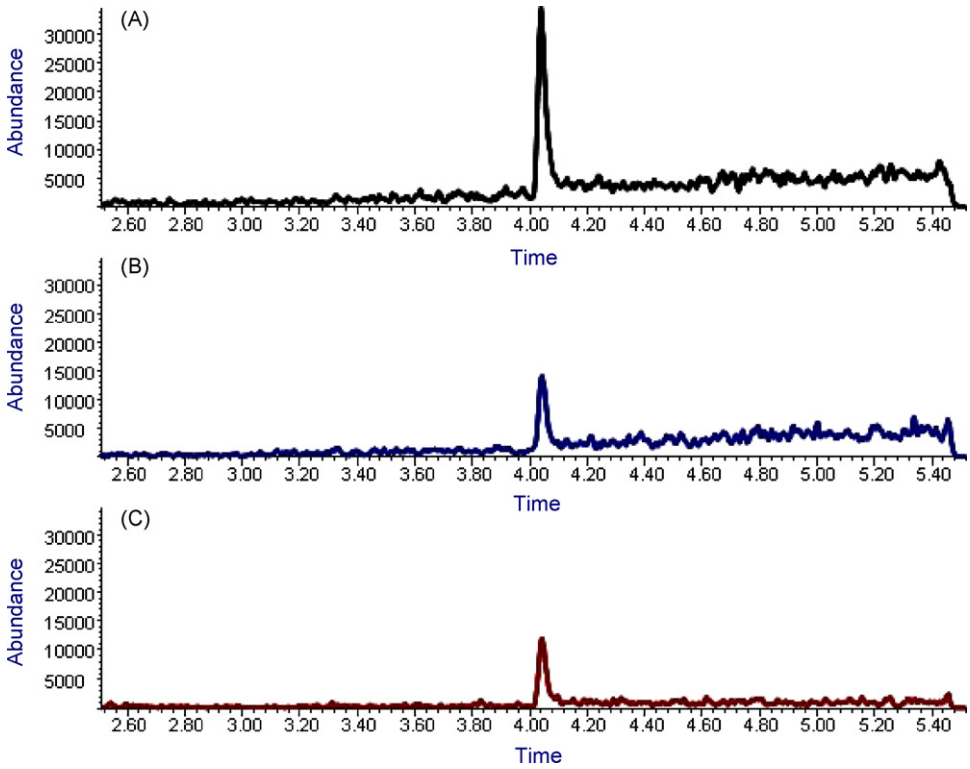

Fig. 4. Gas chromatography tandem mass spectrometry chromatogram at the method lowest reportable limit (5 ng/mL). The response of the (A) internal standard (B) quantitative ion, and (C) the confirmatory ion are shown here.

The primary advantage of using a GC/MS/MS instrument is that it can perform any experiment that a GC/MS can perform, but with additional scan functions that increase method selectivity. Tandem mass spectrometers have the ability to differentiate analyte interferences by using multiple stages of mass spectrometry, which include product ion scans and other linked scans (SRM, neutral loss, precursor scans). In discerning a false positive result, the additional selectivity of a triple quadrupole could be critical. This could be critical in patient care, especially if there were interferences in the urine, such as occur in blood or with an excessively high salt content. In this analytical method, however, those added capabilities were not required as no significant interferences occurred. The primary advantage of the GC/MS instrument is that it is more commonly used and is about 35% less expensive than the triple quadrupole instrument.

The speed of this method was also a consideration. It might be used following an event in which hundreds or thousands of people may have suspected exposure. Because samples were pro-

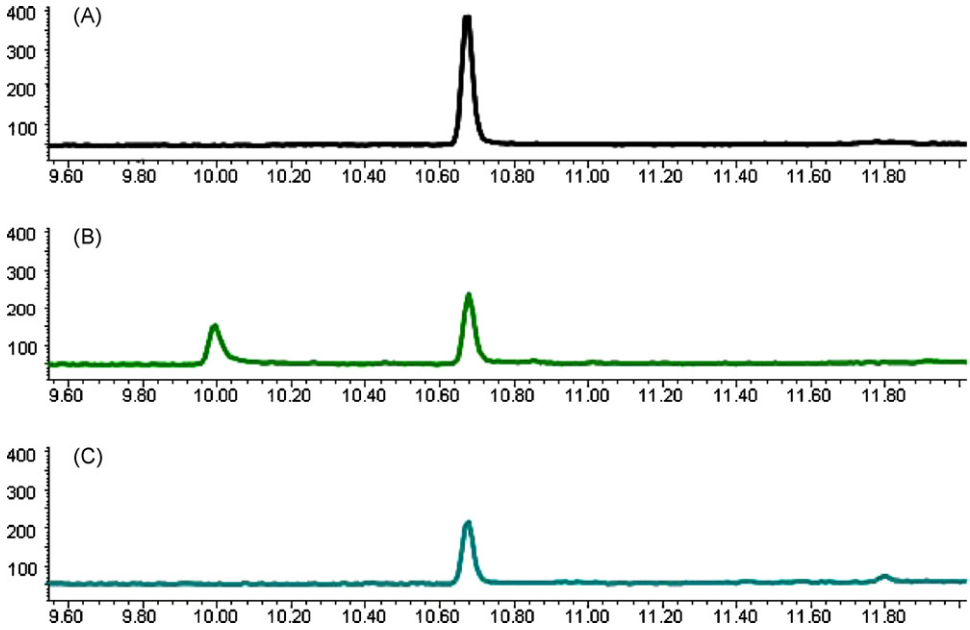

Fig. 5. Gas chromatography mass spectrometry chromatogram at the method lowest reportable limit (5 ng/mL). The response of the (A) internal standard, (B) quantitative ion, and (C) the confirmatory ion are shown here.

cessed in parallel versus sequentially as in a manual preparation, the 96-well format meant faster sample preparation. The time to extract 80 samples was 1.5 h. So, instead of the extraction step, the rate-limiting step was the gas chromatography analysis, in which samples required an analysis time of 14.5 min each, and analysis of 80 unknowns and 10 reference solutions (i.e., blanks, calibrators, and quality controls) required 21.8 h. If faster throughput were needed, future efforts could focus on decreasing the time of the chromatographic separation. An alternative approach would be to consider the use of liquid chromatography and mass spectrometry (LC/MS) by evaluating the tetramine dimer [15] or another derivative that would be amenable to softer ionization techniques. LC/MS analysis techniques for single, small alkaloids such as tetramine tend to be very rapid—on the order of 5 min or less [16].

There are limitations on the use of this method which including the inability to measure tetramine exposure effectively below the lowest reportable limit of 5 ng/mL. A more sensitive method with a lower reportable range would be useful for assuring worried-well patients who are not displaying symptoms and who need differentiation from those with subacute exposure. One approach to increase method sensitivity would be to increase sample size. Samples as large as 5 mL can be efficiently extracted in 48-well plates (6-mL volumes per well); even greater volumes can be extracted using manual, solid phase extraction cartridges. The method also does not differentiate any urinary tetramine adducts or other metabolic products that may be present in urine. A possible route to examining these metabolites may be to analyze the samples with an orthogonal detector, which would detect the expected nitrogen and sulfur atoms in the molecule (e.g. GC/NPD or LC/ICP/MS).

5. Conclusions

We have developed an analysis method for the determination of tetramine in urine that can be applied with either of two instrument platforms. The method has the sensitivity and selectivity that should be necessary for clinical analysis of urine from persons exposed to tetramine leading to low, moderate, or severe symptoms. The method allows for a quantification of tetramine from 5 to 250 ng/mL in urine that corresponds to these symptomatic levels. Sample preparation has been streamlined for throughput and sensitivity, but improvement is needed to decrease the gas chromatography analysis time. A possible future direction for this method is to examine tetramine dimers or other synthetic derivatives that may be amenable to faster LC/MS techniques using softer ionization techniques. The analysis of 100 random urine samples did not detect any endogenous interference from the population, and it is expected that this method will be selective for tetramine poisonings.

Acknowledgments

The authors would like to thank Chris Pittman (Battelle) for aliquotting reference range samples for this study, Chris Sandlin for assisting with the ERMS database, and David Ashley for proofreading of the document.

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