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ARTICLES

Diagnostic Determination of Melamine and Related Compounds in Kidney Tissue by Liquid Chromatography/Tandem Mass Spectrometry

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In 2007, it was determined that melamine, ammeline, ammelide, and cyanuric acid (abbreviated as MARC for melamine and related contaminants) had been added to wheat gluten and rice protein that were subsequently incorporated into pet food. The consumption of food tainted by MARC compounds was implicated in numerous instances of renal failure in cats and dogs. A method for the analysis of MARC compounds in kidney tissue using high-performance liquid chromatography/tandem mass spectrometry (HPLC/MS/MS) has been developed. MARC analytes were extracted by homogenization of kidney tissue in 50/40/10 acetonitrile/water/diethylamine. The homogenate was centrifuged, and an aliquot of supernatant was diluted with acetonitrile, concentrated, and fortified with a stable isotope-labeled analogue of melamine. Analytes were detected using atmospheric pressure chemical ionization and multiple reaction monitoring. Quantitation of positive samples was performed using the internal standard method and five-point calibration curves ranging between 50 and 1000 ng/mL of each analyte. The method was validated by analysis of replicate kidney tissue samples fortified with the individual analytes and by analysis of kidney samples fortified with melamine cyanurate powder at two different concentrations. This method was successfully used for routine postmortem diagnosis of melamine toxicosis in animals. Melamine was also detected by this method in paraffin-embedded tissue from animals suspected to have died of melamine toxicosis.

KEYWORDS: Melamine; cyanuric acid; melamine cyanurate; kidney; liquid chromatography-mass spectrometry

INTRODUCTION

In March of 2007, pet food ingredients contaminated with melamine and its analogues ammeline, ammelide, and cyanuric acid (abbreviated as MARC, for melamine and related contaminates) resulted in a major outbreak of renal disease and associated deaths in cats and dogs in the United States. There are limited data with regard to melamine or cyanuric acid kinetics or metabolism following ingestion. However, in rats, 90% of a dose of radiolabeled melamine was eliminated unchanged via the kidneys within 24 h (*1, 2*). Cyanuric acid elimination has not been investigated, but the structural similarity to melamine suggests substantial renal clearance as well. During the course of the investigation into the cause of this disease, it was noted histopathologically that kidneys from affected animals contained large numbers of melamine cyanurate crystals. The crystals are believed to obstruct and damage renal tubules leading to renal failure (*2, 3*). On the basis of this information, it was felt that kidney tissue would be an appropriate sample for postmortem detection of MARC to confirm exposure and possible intoxication.

Several methods have been developed for the analysis of MARC compounds. Melamine has been analyzed by gas chromatography/mass spectrometry (GC/MS) (*4*), liquid chromatography (*5–8*), and capillary electrophoresis (*9*). The presence of melamine in pet food was determined by direct analysis in real time (DART) mass spectrometry (*10*). Liquid chromatography/tandem mass spectrometry (LC/MS/MS) was used to determine melamine as a metabolite of the herbicide cyromazine (*11*) and in porcine muscle tissue (*12*). Cyanuric acid has been analyzed by a variety of methods including derivatization and GC/MS (*13*), high-performance liquid chromatography (HPLC) with UV detection (*14–16*), and LC/MS (*17*). The four MARC

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compounds have been analyzed in liquid solutions and in flour by HPLC (*7, 18*) and in pet food and its components by GC/MS (*19*).

This study was designed to develop a sensitive and highly specific analytical method for the extraction and quantitative determination of MARC compounds in tissue using a triple stage quadrupole mass spectrometer. Method performance was evaluated at concentrations consistent with those found in positive kidney samples. This method was used for analysis of kidney tissue taken during postmortem examination of animals suspected to have died from MARC intoxication.

MATERIALS AND METHODS

Reagents. Water, methanol, and acetonitrile were of HPLC grade, and diethylamine was of reagent grade (Fisher Scientific, Fair Lawn NJ). All HPLC running solvents were filtered through 0.45 μM nylon filters (Gelman Sciences, Ann Arbor, MI).

Preparation of Standard Solutions. Melamine, melamine cyanurate (cyanuric acid compound with melamine), and cyanuric acid were purchased from Sigma Chemical Co. (St. Louis, MO) (99% purity). Ammeline and ammelide were kind gifts of Dr. Fred Fricke (Forensic Chemistry Center, U.S. FDA, Cincinnati, OH). $^{15}N_3$-Melamine, ring-labeled, was purchased from ICON Services (Summit, NJ) for use as an internal standard. All standard solutions with the exception of melamine cyanurate were prepared and diluted in 1:1 acetonitrile water. Melamine cyanurate solutions were prepared in 50/40/10 acetonitrile/water/diethylamine.

Separate stock solutions of 1000 μg/mL of each MARC analyte and the labeled melamine were prepared, and aliquots of these solutions were combined to produce a 10 μg/mL mixed intermediate standard solution. Five-point calibration curves of combined MARC analytes were prepared weekly at 50, 100, 250, 500, and 1000 ng/mL by adding aliquots of 10 μg/mL intermediate standard solution to vials containing 100 μL of 10 μg/mL $^{15}N_3$-melamine. Sufficient dilution solvent was added to each vial to bring the final volume to 1 mL.

To establish the method's ability to detect MARC analytes present in the form of combined crystals, it was necessary to fortify tissue with crystalline melamine cyanurate. Accordingly, a solid melamine cyanurate fortification standard was prepared by weighing 10 mg of melamine cyanurate powder into a dry aluminum container and adding 35−70 mesh silica gel (Fisher Scientific, Fair Lawn, NJ) to make a total weight of 10 g. The container was shaken and placed on a test tube rotator for 10 min to ensure even distribution of the melamine cyanurate in the silica gel. Subsequent 1−10 g serial dilutions were made to produce the 10 μg/g melamine cyanurate fortification standard.

The storage stability of calibration solutions was evaluated by comparing analyses of calibration standards stored at room temperature for 1 month to freshly made calibration standards. A dissolved melamine cyanurate solution was also analyzed before and after 1 month of storage at room temperature to determine stability.

Sample Preparation. Bovine kidneys purchased from local grocery stores were used as control samples. These were fortified as necessary with the materials described above to provide positive control samples. Diagnostic analysis was performed on canine and feline kidney samples submitted to the laboratory for such analysis. A horizontal section of each kidney, including capsule, cortex, and medulla, was removed and chopped finely with a knife. One gram of chopped kidney tissue was weighed into a 60 mL round glass jar (Fisher Scientific). The sample was homogenized with 25 mL of acetonitrile:water:diethylamine (50:40:10, v/v) for 1 min with an Ultra-Turrax T-25 tissue homogenizer (IKA-Labortechnik/Tekmar Company, Cincinnati, OH) and centrifuged at 800 rpm (65 *g*) for 5 min using an Avanti J-E centrifuge (Beckman Coulter, Fullerton, CA). An aliquot (2.5 mL) of the extract was transferred into a 15 mL glass screw-cap test tube, 5.5 mL of acetonitrile was added, and the sample was vortexed briefly. The tube was then centrifuged for 5 min at 1500 rpm, and an aliquot (4 mL) of supernatant was filtered through a 0.8 μM Acrodisc syringe filter (Pall Corp., Ann Arbor, MI) into a separate tube and evaporated dry under nitrogen using an N-Evap evaporator (Organomation Assoc. Inc., Berlin, MA) set at 60 °C. The dried extract was reconstituted in 490 μL of 80:20 water:acetonitrile. Ten microliters of 10 μg/mL ^{15}N-melamine in acetonitrile:water (1:1) was added to the extract, resulting in a sample concentration of 0.10 g/mL. The mixture was vortexed for 10 s, sonicated for 2 min, and filtered through a 0.45 μM HPLC filter (Millipore Corp., Milford, MA) into a 2 mL autosampler vial fitted with a 250 μL insert (Restek, Bellefonte, PA). All control and fortified samples were prepared in the same manner.

Table 1. MRM Conditions for Each Analyte

analyte	ionization mode	precursor ion (*m/z*)	product ions (*m/z*)	collision energy (V)	collision exit potential (V)
melamine	+	127	85[a], 68	27, 46	17, 14
ammeline	−	126	83[a], 41	−18, −37	−15, −5
ammelide	−	127	84[a], 42	−15, −30	−15, −5
cyanuric acid	−	128	85[a], 42	−14, −30	−15, −5
$^{15}N_3$-melamine	+	130	87[a]	28	17

[a] Denotes quantitation ions.

LC-MS/MS Analysis. A HPLC (Microm BioResources Inc., Auburn, CA) coupled with a hybrid triple stage quadrupole/linear ion trap mass spectrometer, model 4000 Q TRAP with Analyst version 1.4.2 software (Applied Biosystems/MDS SCIEX, Concord, Canada), was used for all analyses. The analytical column was a 150 mm × 4.6 mm i.d., 4 μM Synergi Polar-RP (Phenomenex Inc., Torrance, CA), with a Polar-RP guard column cartridge. The HPLC was fitted with a 20 μL injection loop. The mobile phase consisted of: (A) 10 mM ammonium acetate in water, (B) acetonitrile, and (C) 0.1% formic acid in water at a flow rate of 500 μL/min. Gradient elution was utilized, with the initial mobile phase at 80% A, 15% B, 5% C held for 1 min, ramped to 30% A, 65% B, 5% C at 5 min. At 5.1 min, the mobile phase was ramped to 5% A, 90% B, 5% C and held until 7 min, at which time it was ramped back to the initial conditions and held for 5 min to re-equilibrate the column. Mass spectrometric data were acquired in positive ion atmospheric pressure chemical ionization (APCI) mode, using the multiple reaction monitoring (MRM) scan function. Instrument parameters were determined by direct infusion of a 1 μg/mL solution of melamine. The heated nebulizer source was run at a temperature of 450 °C with the following settings: curtain gas, 30 (manufacturer's units); source gas 1, 60; source gas 2, 60; CAD gas pressure high; and nebulizer current, 3.0. Two SRM transitions were monitored for each of the analytes with a single transition monitored for the internal standard. Collision energy and collision exit potential settings were optimized for each transition during infusion of the individual analytes. These precursor/product ion transitions and their associated collision energy and collision exit potential values are listed in **Table 1**. The scan time for each SRM event was 100 ms. Quantification of all analytes was performed using the internal standard method. Standard solutions of the analytes in 80:20 water:acetonitrile at levels of 50, 100, 200, 500, and 1000 ng/mL were analyzed to generate calibration curves. ^{15}N-Melamine was included in each standard at 200 ng/mL.

Method Performance. Method performance was measured through analysis of fortified control samples prepared and analyzed with each batch of diagnostic samples. A method validation set of seven replicates of kidney tissue fortified with each analyte at 0.50 μg/g was analyzed to determine method detection limits and single day method performance. Method detection limits for each analyte were calculated by multiplying Student's *t* value by the standard deviation values determined in the seven replicate analyses. A method validation set of five control samples fortified with melamine cyanurate at 250 μg/g was also analyzed as a single day validation. Method performance was also measured on an ongoing basis. For each batch of 10 or fewer tissue samples, a control sample fortified with melamine cyanurate at 5 μg/g was analyzed. Recoveries for these samples were calculated by adding the measured concentrations of melamine and cyanuric acid together and dividing the total by the 5 μg/g fortification level. Additionally, a second control sample fortified with each of the individual MARC analytes at 2.5 μg/g was analyzed with all but one of these batches. For melamine cyanurate, a total of 12 fortified control samples were extracted and analyzed between May 25, 2007, and November 29, 2007.

Table 2. MARC Recovery Data from Fortified Control Kidney Samples

Individual MARC Analytes (0.50 μg/g Fortifications)
Single Day

analyte	*n*	mean recovery (%)	SD	% RSD	minimum detection limit (μg/g)
ammeline	7	103	5.9	5.7	0.092
ammelide	7	120	8.9	7.6	0.14
cyanuric acid	7	110	8.8	7.8	0.14

Individual MARC Analytes (2.5 μg/g Fortifications)
Multiple Days, June−September 2007

analyte	*n*	mean recovery (%)	SD	% RSD
melamine	11	86	13	15
ammeline	11	75	19	25
ammelide	11	87	26	30
cyanuric acid	11	79	19	24

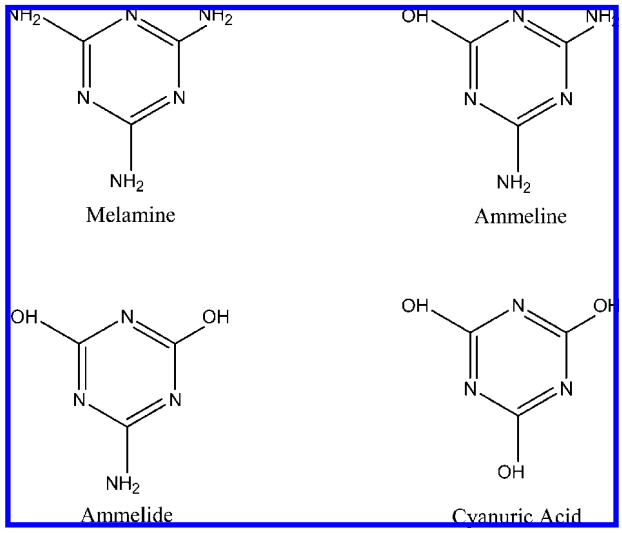

Figure 1. Structures of melamine and related compounds.

For the individual MARC analytes, 11 fortified control samples were analyzed over that same time period. A summary of these results is presented in **Table 2**.

RESULTS AND DISCUSSION

The chemical structures of the MARC compounds are shown in **Figure 1**. Upon direct infusion of 1 μg/mL solutions of each of the analytes, it was determined that melamine and the $^{15}N_3$-melamine internal standard responded best in positive ion mode, while the other three analytes responded best in negative ion mode. For each analyte, a single mass spectral peak corresponding to the $[M + H]^+$ ion or $[M - H]^-$ ion was observed with no detectible adduct formation. Accordingly, the $[M + H]^+$ ions were used as precursor ions for melamine and the internal standard, while the $[M - H]^-$ ions were used as precursor ions for ammeline, ammelide, and cyanuric acid. It was also determined by infusion that APCI using the heated nebulizer probe provided a higher level of sensitivity for several of the compounds than electrospray ionization. Product ion spectra for each of the analytes are shown in **Figure 2**.

The chromatographic system gave adequate separation for the four analytes. In particular, there was sufficient separation between the melamine and the other three analytes to allow for

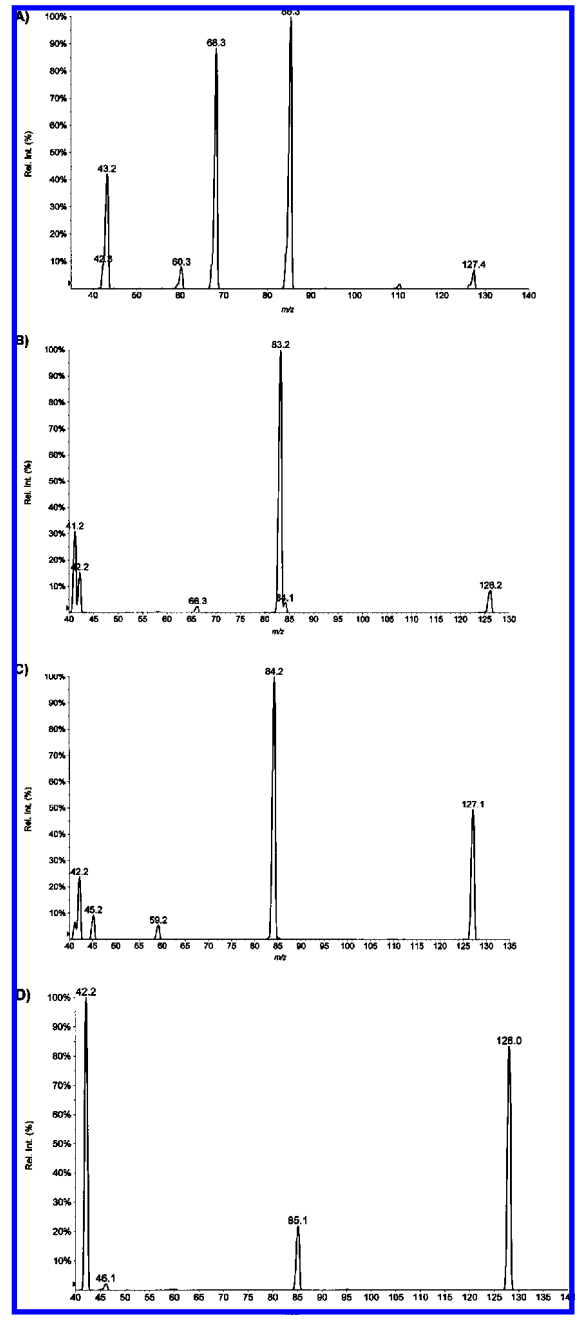

Figure 2. Product ion spectra. (**A**) Melamine, product ions of *m*/*z* 127; (**B**) ammeline, product ions of *m*/*z* 126; (**C**) ammelide, product ions of *m*/*z* 127; and (**D**) cyanuric acid, product ions of *m*/*z* 128.

polarity switching from negative to positive ion mode. **Figure 3** shows selected ion chromatograms for each of the analytes from the analysis of a 50 ng/mL standard solution.

Melamine contamination in blank analyses was a recurring problem during the development of this method. This appeared to be due to carryover of melamine in the HPLC/MS system. Previous work done by other investigators (*20*) had demonstrated that a mobile phase with higher ionic strength resulted in no carryover of melamine into blank analyses (control sample extracts and reagent blanks) when analyzing for melamine only. The mobile phase used for that analysis did not give separation adequate for analysis of all four MARC compounds. We therefore accepted the presence of low concentrations of melamine in blank samples to achieve analysis of all four analytes in a single run. The melamine level in the blank

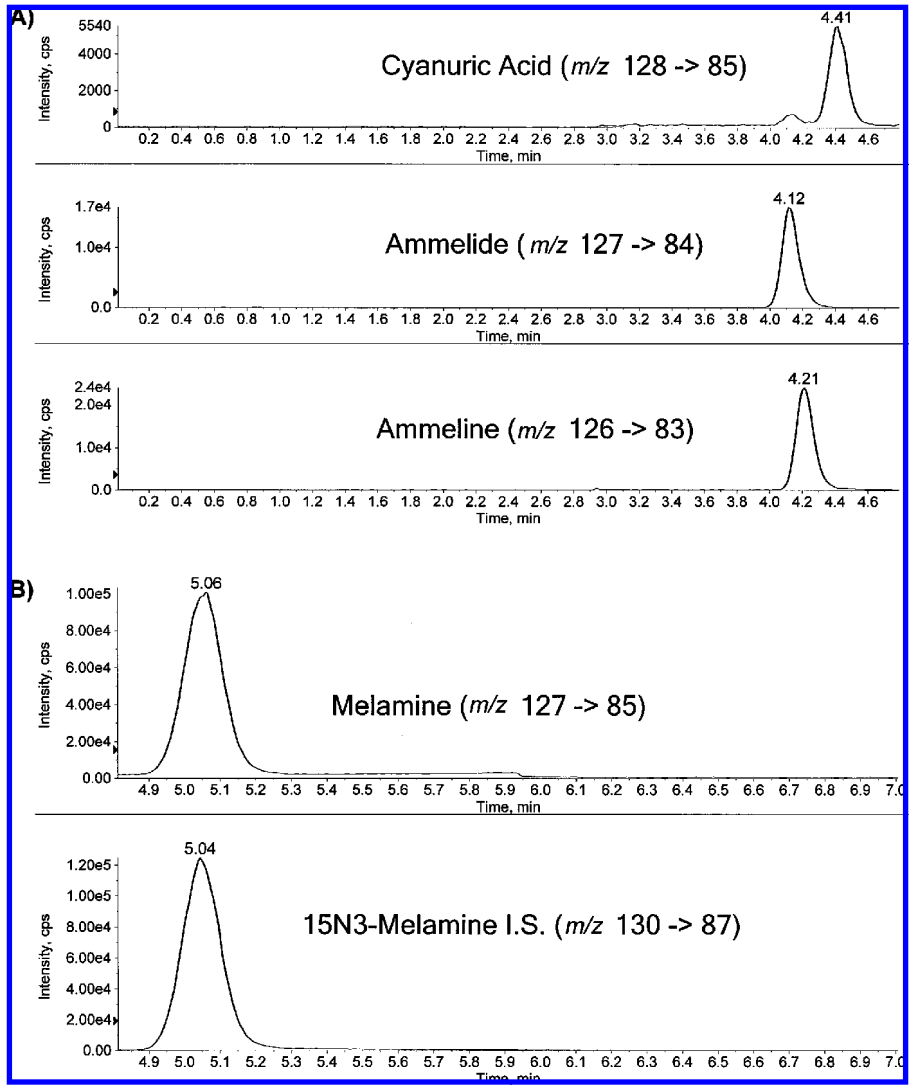

Figure 3. Ion chromatograms for a 50 ng/mL standard. (**A**) Cyanuric acid, ammelide, and ammeline. (**B**) Melamine and $^{15}N_3$-melamine.

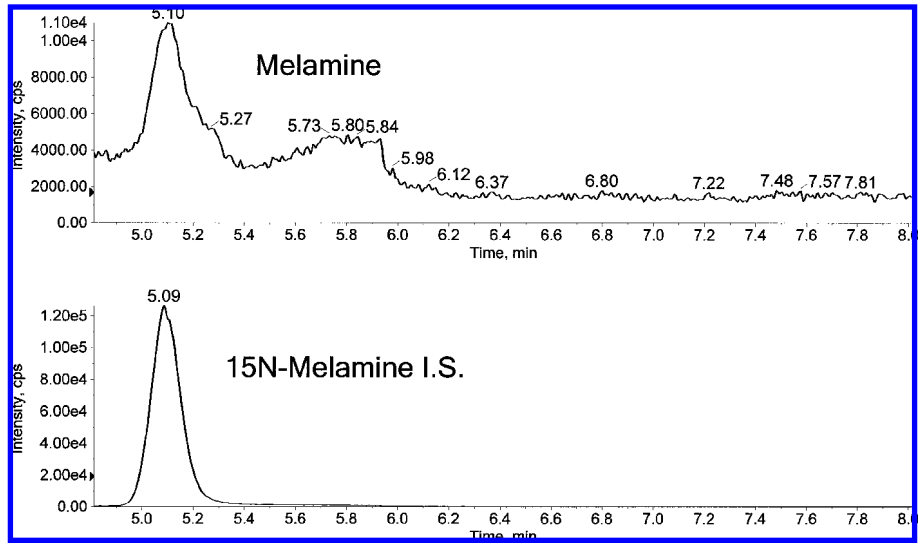

Figure 4. Ion chromatograms for melamine and $^{15}N_3$-melamine from analysis of a negative control kidney sample.

analyses was consistently at a concentration equivalent to 50−100 parts per billion in a sample. No other MARC analyte was routinely detected in blank analyses. **Figure 4** shows a selected ion chromatogram for melamine and the internal standard in a control kidney extract. Although instrument sensitivity allowed for detection limits in the low ng/g range, the melamine carryover required that we not report concentrations below 1 μg/g for that compound.

The extraction procedure was developed in anticipation of high concentrations of the MARC compounds in kidney tissue.

Table 3. Melamine Cyanurate Recovery Data from Fortified Control Kidney Samples

n	mean recovery (%)	SD	% RSD
	melamine cyanurate (50 μg/g;fortifications); single		
5	110	9.7	9.2
	melamine cyanurate (100 μg/g fortifications); single day		
5	100	9.8	9.8
	melamine cyanurate (250 μg/g fortifications); single day		
5	76	20	26
	melamine cyanurate (5 μg/g fortifications); multiple days, June−September 2007		
12	86	7.6	8.8

Crystals suspected to consist of melamine cyanurate with other MARC compounds were prevalent throughout the kidneys of affected animals when those kidneys were evaluated histopathologically. This was in accordance with the observation that melamine and cyanuric acid are known to combine to form hydrogen-bonded polymeric aggregates (*21*). Because it was likely that melamine cyanurate concentrations were high and because we initially did not know whether free MARC analytes remained in the tissue, we considered it important to dissolve any melamine cyanurate present in the tissue into free melamine and cyanuric acid during the sample preparation process. Although each of the individual MARC compounds was soluble in 1:1 acetonitrile:water, melamine cyanurate was not. Dissolution experiments involving a number of solvents were performed, and it was determined that the solvents diethylamine and dimethylformamide effectively dissolved melamine cyanurate. Upon further experimentation, it was determined that 10 mg of melamine cyanurate could be dissolved in 10 mL of the 50/40/10 mixture of acetonitrile/water/diethylamine used in the FDA's method for the analysis of MARC compounds in feed samples (*19*). When a 1 mg/mL solution of melamine cyanurate in this solvent mixture was diluted to 500 μg/mL in 1:1 acetonitrile water and analyzed, the measured concentrations of melamine and cyanuric acid were within 5% of the expected concentrations (250 μg/mL each) as compared to standards composed of the individual analytes. Multiple analyses of dissolved melamine cyanurate as well as the mixed standard solutions of the four MARC analytes showed that these solutions were stable for at least 1 month at room temperature. This indicates that the dissociated melamine and cyanuric acid do not recombine in acetonitrile/water to form melamine cyanurate.

The sample preparation procedure involved homogenization of tissue in the 50/40/10 acetonitrile/water/diethylamine mixture

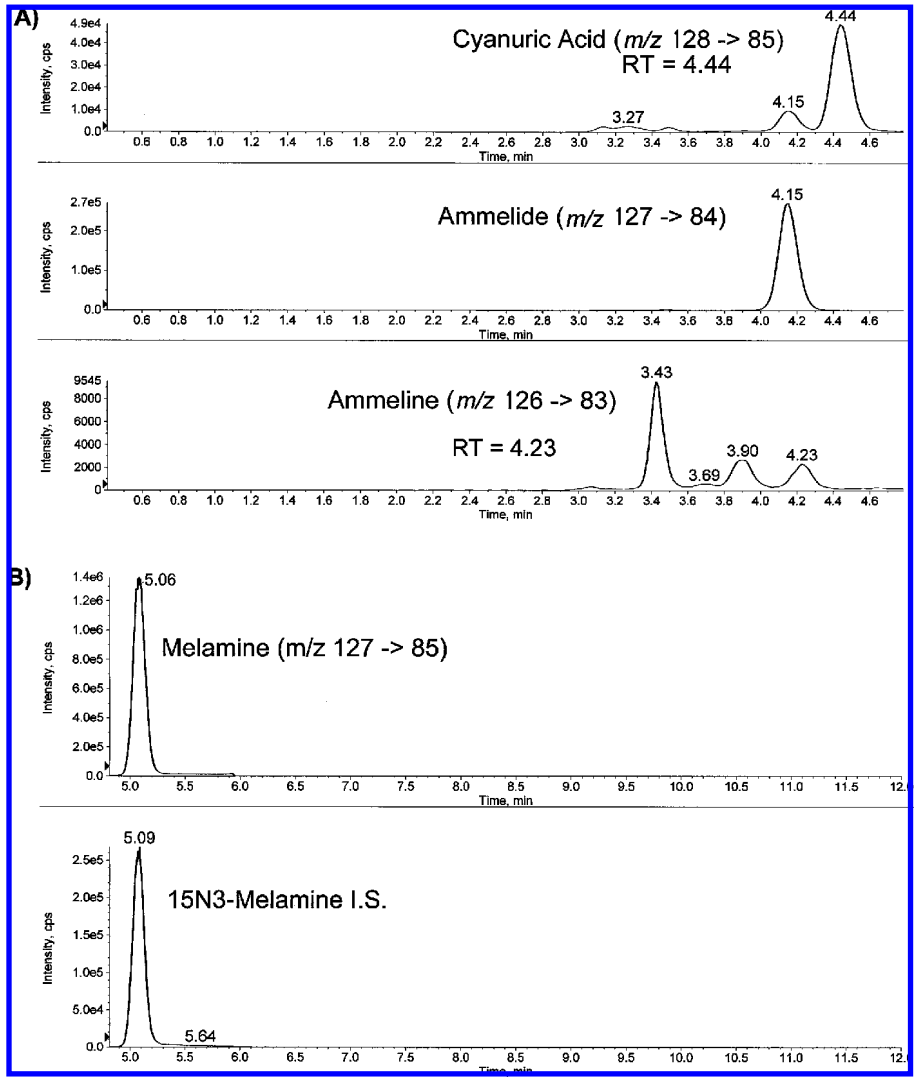

Figure 5. Ion chromatograms from analysis of kidney tissue taken from an animal suspected to have been exposed to MARC-contaminated feed. (**A**) Chromatograms for cyanuric acid, ammelide, and ammeline. (**B**) Chromatograms for melamine and $^{15}N_3$-melamine.

and dilution of the homogenate in acetonitrile. Although the dilution in acetonitrile resulted in the precipitation of much of the protein fraction of the extract, we initially encountered some difficulty in filtering the final extracts immediately prior to LC/MS analysis. Filtering the diluted extract prior to concentration and solvent exchange alleviated this problem.

Calibration curves were linear, with r^2 values greater than 0.995 for all analytes. A method validation set of seven replicate control tissue samples fortified with 0.50 μg/g of the MARC analytes demonstrated adequate precision and accuracy for ammeline, ammelide, and cyanuric acid. Minimum detection limits for these compounds ranged from 0.092 to 0.14 μg/g. (Melamine was not evaluated as part of this exercise as it could not be evaluated at this level due to the blank contamination mentioned above.) Ongoing precision and accuracy were measured by analysis of fortified control kidney samples. Each set of samples was analyzed with one control sample fortified with a mixture of each of the four analytes in solution at a level of 2.5 μg/g. **Table 2** summarizes the results of these analyses.

In addition to the fortified samples discussed above, evaluations of melamine cyanurate recovery at higher concentrations were conducted by analyzing control kidney fortified at 50, 100, and 250 μg/g. In the case of these samples as well as others with concentrations determined to be above the linear range of the mass spectrometer, a reduced volume of the initial 1 g → 25 mL kidney extract (typically 25−250 μL) was diluted into acetonitrile and then treated as per the extraction method. **Table 3** shows the results of these analyses. Although these data indicate that the method is adequate for diagnostic purposes at this level, it also shows that quantitative precision and accuracy at 250 μg/mL were lower than what was achieved at the lower concentration ranges. Interestingly, although cyanuric acid concentrations were close to those expected (125 μg/g), the melamine concentrations measured were significantly lower. We have not yet evaluated the reason for the discrepancy between the two compounds, and further work is needed to determine any changes in the method necessary to improve quantitative performance at these concentrations.

Instrument performance was routinely adequate for diagnostic purposes. Standard curves for all analytes typically gave r^2 values >0.990 for ammelide, with the other three analytes consistently >0.995. Signal to noise levels for 50 ng/mL standards were typically 20:1 or greater for all analytes. Ion ratios of quantitation vs qualifier ions varied by less than 10% within a day. Signal suppression by matrix components, as measured by comparison of internal standard peak areas in sample extracts vs standard solutions, was not encountered at significant levels except in cases in which melamine was detected at concentrations well above the standard curves. It is possible that some suppression occurred with the other three analytes, and this may be reflected in the higher variance in recoveries for these; however, the routinely high recoveries of these analytes in fortified samples indicate that this effect was minimal. This was expected with the use of APCI, which has been shown to be less prone to signal suppression than electrospray ionization (*22*).

This method was used for the diagnostic analysis of approximately 60 canine and feline kidney samples between April and October of 2007. Of the 60 samples submitted, 18 (including three of the samples from a feeding study (*2*)) contained melamine and cyanuric acid at concentrations above 1 μg/g. **Figure 5** shows chromatograms from a typical sample that tested positive for all four analytes. Measured concentrations in all samples ranged from 7.5 μg/g melamine with 3.9 μg/g of cyanuric acid up to 730 μg/g melamine with 690 μg/g of cyanuric acid for the intact tissue. Ammelide was also detected in all positive samples at concentrations comparable to those of melamine and cyanuric acid. Ammeline was detected in most of the positive samples at concentrations approximately 5−10% of the melamine concentrations. No samples were analyzed in which the MARC analytes were detected below these levels. Because the levels detected in diagnostic samples were well above the method detection limits, we believe that these detection limits are adequate for diagnosing melamine-related renal failure. In the absence of studies linking toxicosis with MARC levels in kidney tissue, this can not be stated with certainty and further studies are required to establish the minimum detectible levels required to definitively diagnose toxicosis.

Analysis of Tissue Preserved in Paraffin. During the course of these analyses, our laboratory received four samples of kidney tissue that had been formalin-fixed and embedded in paraffin for histopathological examination. One of these samples had initially been prepared in 2004 as part of an investigation into the deaths of a number of animals due to kidney failure (*3*). The samples were deparaffinized by melting the paraffin and washing the tissue with xylene prior to their receipt in our laboratory. The tissue was removed from each sample cassette, and a 10−20 mg subsample was analyzed as described above. Although the small amount of tissue available for analysis resulted in a much higher limit of detection than that achieved in fresh tissue, all of the MARC compounds were detected in the sample from the 2004 outbreak. Measured concentrations in this sample ranged from 60 μg/g for ammeline to 2500 μg/g for melamine. This indicates that this procedure can be useful in retrospective analysis of stored samples of fixed and paraffin-embedded tissue. Further investigation is required to determine limits of detection, accuracy of quantification, and effects of the deparaffinizing process on the samples.

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LITERATURE CITED

(1) Mast, R. W.; Jeffcoat, A. R.; Sadler, B. M.; Kraska, R. C.; Friedman, M. A. Metabolism, disposition and excretion of [14C]melamine in male Fischer 344 rats. *Food Chem. Toxicol.* **1983**, *21*, 807–810.

(2) Puschner, B.; Poppenga, R. H.; Lowenstine, L. J.; Filigenzi, M. S.; Pesavento, P. A. Assessment of melamine and cyanuric acid toxicity in cats. *J. Vet. Diagn. Invest.* **2007**, *19*, 616–624.

(3) Brown, C. A.; Jeong, K. S.; Poppenga, R. H.; Puschner, B.; Miller, D. M.; Ellis, A. E.; Kang, K. I.; Sum, S.; Cistola, A. M.; Brown, S. A. Outbreaks of renal failure associated with melamine and cyanuric acid in dogs and cats in 2004 and 2007. *J. Vet. Diagn. Invest.* **2007**, *19*, 525–531.

(4) Toth, J. P.; Bardalaye, P. C. Capillary gas chromatographic separation and mass spectrometric detection of cyromazine and its metabolite melamine. *J. Chromatogr.* **1987**, *408*, 335–340.

(5) Cabras, P.; Meloni, M.; Spanedda, L. High-performance liquid

chromatographic separation of cyromazine and its metabolite melamine. *J. Chromatogr.* **1990**, *505*, 413–416.
(6) Pukkila, J.; Peltonen, K.; Savolainen, T. Determination of melamine in air by high-performance liquid chromatography with ultraviolet detection. *J. Chromatogr.* **1987**, *411*, 409–414.
(7) Sugita, T.; Ishiwata, H.; Yoshihira, K.; Maekawa, A. Determination of melamine and three hydrolytic products by liquid chromatography. *Bull. Environ. Contam. Toxicol.* **1990**, *44*, 567–571.
(8) Yokley, R. A.; Mayer, L. C.; Rezaaiyan, R.; Manuli, M. E.; Cheung, M. W. Analytical method for the determination of cyromazine and melamine residues in soil using LC-UV and GC-MSD. *J. Agric. Food Chem.* **2000**, *48*, 3352–3358.
(9) Cook, H. A.; Klampfl, C. W.; Buchberger, W. Analysis of melamine resins by capillary zone electrophoresis with electrospray ionization-mass spectrometric detection. *Electrophoresis* **2005**, *26*, 1576–1583.
(10) Vail, T.; Jones, P. R.; Sparkman, O. D. Rapid and unambiguous identification of melamine in contaminated pet food based on mass spectrometry with four degrees of confirmation. *J. Anal. Toxicol.* **2007**, *31*, 304–312.
(11) Sancho, J. V.; Ibanez, M.; Grimalt, S.; Pozo, O. J.; Hernandez, F. Residue determination of cyromazine and its metabolite melamine in chard samples by ion-pair liquid chromatography coupled to electrospray tandem mass spectrometry. *Anal. Chim. Acta* **2005**, *530*, 237–243.
(12) Filigenzi, M. S.; Tor, E. R.; Poppenga, R. H.; Aston, L. A.; Puschner, B. The determination of melamine in muscle tissue by liquid chromatography/tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* **2007**, *21*, 4027–4032.
(13) Fiamegos, Y. C.; Konidari, C. N.; Stalikas, C. D. Cyanuric acid trace analysis by extractive methylation via phase-transfer catalysis and capillary gas chromatography coupled with flame thermoionic and mass-selective detection. Process parameter studies and kinetics. *Anal. Chem.* **2003**, *75*, 4034–4042.
(14) Briggle, T. V.; Allen, L. M.; Duncan, R. C.; Pfaffenberger, C. D. High performance liquid chromatographic determination of cyanuric acid in human urine and pool water. *J. Assoc. Off. Anal. Chem.* **1981**, *64*, 1222–1226.
(15) Cantu, R.; Evans, O.; Kawahara, F. K.; Shoemaker, J. A.; Dufour, A. P. An HPLC method with UV detection, pH control, and reductive ascorbic acid for cyanuric acid analysis in water. *Anal. Chem.* **2000**, *72*, 5820–5828.
(16) Cantu, R.; Evans, O.; Kawahara, F. K.; Wymer, L. J.; Dufour, A. P. HPLC determination of cyanuric acid in swimming pool waters using phenyl and confirmatory porous graphitic carbon columns. *Anal. Chem.* **2001**, *73*, 3358–3364.
(17) Patel, K.; Jones, K. Analytical method for the quantitative determination of cyanuric acid as the degradation product of sodium dichloroisocyanurate in urine by liquid chromatography mass spectrometry. *J. Chromatogr. B* **2007**, *853*, 360–363.
(18) Ehling, S.; Tefera, S.; Ho, I. P. High-performance liquid chromatographic method for the simultaneous detection of the adulteration of cereal flours with melamine and related triazine by-products ammeline, ammelide, and cyanuric acid. *Food Addit. Contam.* **2007**, *24*, 1319–1325.
(19) Litzau, J.; Mercer, G.; Mulligan, K. GC-MS Screen for the Presence of Melamine, Ammeline, Ammelide and Cyanuric Acid (version 2.1); http://www.fda.gov/cvm/GCMSMelamine.htm, 2007.
(20) Martos, P. Personal communication, 2007.
(21) Bielejewska, A. G.; Marjo, C. E.; Prins, L. J.; Timmerman, P.; de Jong, F.; Reinhoudt, D. N. Thermodynamic stabilities of linear and crinkled tapes and cyclic rosettes in melamine—Cyanurate assemblies: A model description. *J. Am. Chem. Soc.* **2001**, *123*, 7518–7533.
(22) Schuhmacher, J.; Zimmer, D.; Tesche, F.; Pickard, V. Matrix effects during analysis of plasma samples by electrospray and atmospheric pressure chemical ionization mass spectrometry: practical approaches to their elimination. *Rapid Commun. Mass Spectrom.* **2003**, *17*, 1950–1957.

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