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Determination of melamine in rat plasma, liver, kidney, spleen, bladder and brain by liquid chromatography–tandem mass spectrometry

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ABSTRACT

In this study, we describe a method for the analysis of melamine in rat plasma, liver, kidney, spleen, bladder, and brain using trichloroacetic acid precipitation with mixed-mode cation-exchange solid-phase extraction and hydrophilic interaction chromatography coupled to tandem mass spectrometry detection. Method validation was investigated completely, including linearity, precision, accuracy, matrix effect, extraction recovery, and carryover for the determination of melamine. The method exhibited a good linear range covering 20–500 ng/mL, and the overall precision ranged from 1.6 to 16.3%, with the accuracy varying from −7.9 to 15.1%. The mean matrix effects of melamine in rat plasma, liver, kidney, spleen, bladder, and brain ranged from 66.2 ± 6.7 to 95.5 ± 13.2%, and the mean recoveries for melamine varied from 79.8 ± 8.2 to 113.0 ± 9.6%. Rat kidney showed the highest level among the organs (192.5% of the plasma melamine level), and the average concentration of melamine in the brain was only 7.5% of the plasma melamine concentration. This work has pointed out that even with the application of two popular preparation procedures (acid precipitation and solid-phase extraction) of melamine, the matrix effect in analyzing biological samples still exists in certain kinds of matrices.

1. Introduction

Melamine (2,4,6-triamino-*s*-triazine) is a chemical intermediate originally used to manufacture amino resins and plastics [1], but it has been shown that melamine has been deliberately added to raw milk in order to increase the apparent protein content. As a result, an increased incidence of renal failure in infants was reported in China in 2008, and it is believed to be associated with the consumption of infant foods contaminated with melamine [2].

Quantitative methods for melamine detection include enzyme immunoassay [3], capillary zone electrophoresis with mass spectrometry [1], GC–MS [4], and HPLC with UV detection [4–6]. Recently, several HPLC technique with tandem mass spectrometric methods have been proposed for the determination of melamine in the pork [7], edible tissue from several species of fish [8], bovine kidney [9] or in cat and rat kidney tissue [10].

Simple precipitation using hydrochloric acid [10] and formic acid [11] has commonly been utilized to extract melamine from dairy and other food products. In addition, a liquid–liquid extraction method has been applied to extract melamine from bovine kidney tissue [9]. Furthermore, combined steps including precipitation and solid-phase extraction (SPE) have been used to remove melamine from edible tissues of fish and shrimp [8] and porcine muscle [7].

However, the early and still common perception that using HPLC–MS/MS practically guarantees selectivity has been challenged by a number of reported examples of lack of selectivity due to ion suppression or enhancement caused by the sample matrix and interferences from metabolites [12]. Experience has demonstrated that matrix effects are the scourge of HPLC–MS/MS and may lead to the generation of completely invalid data [13]. The aim of this work is to validate a HPLC–MS/MS method for the determination of melamine in biological samples, especially in evaluating the matrix effects of rat plasma, liver, kidney, spleen, bladder, and brain, after

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trichloroacetic acid (TCA) precipitation or with additional SPE. This is important because the method of sample preparation is directly related to the matrix effect. Therefore, this work investigates the sample preparation for melamine in different biological matrices and the organ distribution of melamine in Sprague–Dawley rats.

2. Experimental

2.1. Reagents and materials

Melamine (99% purity) was obtained from Alfa Aesar (MA, USA). Carboxymethylcellulose sodium, and heparin sodium was purchased from Sigma–Aldrich (St. Louis, MO, USA). Ammonium hydroxide (aq), formic acid, acetonitrile, and sodium chloride were of HPLC grade or GR for analysis grade from E. Merck (Darmstadt, Germany). Pure water for all preparations was prepared by the Milli-Q system (Millipore, Milford, MA, USA).

2.2. Experimental animal

Animal experimental protocols were reviewed and approved by the Institutional Animal Experimentation Committee of the National Yang-Ming University. Male Sprague–Dawley rats weighing 230 ± 20 g (National Yang-Ming University Animal Center, Taipei, Taiwan) were housed with a 12-h light and 12-h dark cycle. Free access to food (Laboratory rodent diet 5001, PMI Feeds, Richmond, IN, USA) and water was allowed at all times. Rats were anesthetized with a mixed solution of urethane (1 g/mL) and α-chloralose (0.1 g/mL) with a dosage of 1 mL/kg by intraperitoneal administration. The rat's body temperature was maintained by a heating pad. Surgical sites were shaved, cleaned with 70% ethanol solution, and the femoral vein was canulated for drug administration.

To assess melamine biodistribution, melamine solution (concentration: 3 mg/mL) was prepared in 0.9% sodium chloride solution following 30 min sonication for complete dissolution and was administered via femoral vein by bolus injection (10 mg/kg). A 3-mL volume of blood was taken by cardiac puncture and the rat was sacrificed by decapitation at 30 min after administration. Liver, kidney, spleen, urinary bladder, and brain were taken off and weighted immediately, and the brain cortex, striatum, hippocampus, cerebellum, stem, and the rest of the brain were further dissected and weighed for the determination of regional distribution of melamine. Each tissue sample was stored at −20 °C before sample preparation.

2.3. Sample preparation

2.3.1. Plasma samples

Extraction procedures were modified from a previous report [5], and acetonitrile instead of methanol was used for the extraction. Plasma samples (sample collection was described in Section 2.2) were obtained by centrifuging collected blood at $6000 \times g$ for 10 min. An aliquot of 100 μL plasma was mixed well with 900 μL pure water, and the mixture was centrifuged at $10{,}000 \times g$ before extraction. The supernatant was introduced into the SPE cartridge (Oasis MCX 60 μm, 1 mL, 30 mg) conditioned and acidified previously with 1 mL of acetonitrile and then 1 mL of 2% (v/v) formic acid to obtain better retention and recovery of melamine. Cartridges were washed with 1 mL of 1% (v/v) formic acid in water, followed by 1 mL of acetonitrile. Finally, the analyte was eluted with 1 mL acetonitrile alkalized by ammonium hydroxide (5%, v/v) and evaporated to dryness at 60 °C with a centrifugation evaporator. The dried plasma extract was reconstituted in 200 μL of 90% (v/v) acetonitrile before analysis.

2.3.2. Organ samples

One part (g) chopped organs (kidney, spleen, urinary bladder, and brain regions) not including the liver was mixed with 5 times volume of homogenizing solvent (mL), which was 1% (w/v) TCA solution. One part chopped liver was mixed with only 3 times volume of 1% TCA. Each mixture was homogenized to an even suspension by a Polytron PT 2100 homogenizer (Kinematica, Lucerne, Switzerland). The suspension was further sonicated for 30 min and then centrifuged at $10{,}000 \times g$ for 5 min. Part of the organ homogenate supernatant was analyzed by HPLC–MS/MS, and part of the organ homogenate supernatant was further extracted by SPE to assess the clean-up efficiency of SPE on matrix effect. For the preparation of calibrators and evaluation of matrix effect, whole rat brain was homogenated without further dissection.

Organ homogenates (500-μL volume of liver, kidney, and spleen, and 100-μL of urinary bladder, and 200-μL of brain homogenate) were diluted to 1 mL with pure water and loaded into a SPE cartridge (Oasis MCX 60 μm, 1 mL, 30 mg), which had been pre-conditioned and equilibrated with 1 mL of acetonitrile and then 1 mL of 2% (v/v) formic acid. Cartridges was washed with 1 mL of 1% formic acid in water, then followed by 1 mL of acetonitrile, and the analyte was eluted with 1 mL of 5% (v/v) ammonium hydroxide in acetonitrile. The eluent was evaporated to dryness by a centrifuge evaporator in 60 °C. The dried residues of organs, urinary bladder, and brain homogenate were reconstituted in 500, 200, and 200 μL of 90% (v/v) acetonitrile, respectively.

2.4. HPLC–MS/MS

Liquid chromatography was performed on a Waters 2690 separation module (Waters, MA, USA) consisting of a quaternary pump, autosampler and a column oven. The method used an Xbridge hydrophilic interaction chromatography (HILIC) column (100 mm × 2.1 mm, 3.5 μm) with a mobile phase composition of acetonitrile (A) and 10 mM ammonium acetate containing 0.1% acetic acid (B) (pH 4.5). The isocratic composition was 90% A and 10% B for 8 min. The flow rate was 200 μL/min and the injection volume 5 μL.

The mass spectrometer coupled to the HPLC system was a triple quadrupole Quattro Ultima MS from Micromass (Manchester, UK). A pneumatically assisted Z-spray electrospray ionization (ESI) ion source in the positive ionization mode was used throughout the experiments. Mass spectrometer conditions were optimized by injecting melamine standard solution (100 ng/mL, diluted in 90% acetonitrile) via HPLC into the MS. The optimized instrument settings were: capillary voltage 3.0 kV, cone voltage 40 V, collision energy 17 eV, source temperature 100 °C, desolvation temperature 300 °C, cone gas 100 L/h, desolvation gas 500 L/h. The multiple reaction monitoring (MRM) transition for melamine was m/z $127 \rightarrow 85$ under the positive ion mode. The MassLynx 3.5 (Micromass) software was used for data processing.

2.5. Method validation

Melamine powder was prepared in 50% (v/v) acetonitrile to make a 1 mg/mL stock solution, and this solution was further diluted to give a serial of working standard solutions. Calibration standards were prepared by adding melamine working standard solutions into blank plasma or organ homogenate to construct concentrations of 20, 50, 100, 250, and 500 ng/mL, and then processed according to the procedures described in Section 2.3. Briefly, melamine was spiked with different blank matrices (plasma or homogenate) before extraction, and these samples went through SPE, drying, and reconstitution in 90% acetonitrile. The calibration standard samples were prepared in triplicate and the standard

curves were obtained by least-square linear regression of the peak area versus the concentrations of melamine. All calibration curves were required to have a correlation value of at least 0.995. The concentration of each sample was derived from the calibration curve and corrected by respective dilution volume.

The quality control (QC) samples at low (20 ng/mL), middle (125 ng/mL), and high (200 ng/mL) concentrations in three replicates on the same day and on three successive days were prepared in the same manner to verify the precision and accuracy of the analytical method. The accuracy was calculated from the nominal concentration (C_{nom}) and the mean value of observed concentration (C_{obs}) as follows: accuracy (bias, %) = $[(C_{nom} - C_{obs})/C_{nom}] \times 100$. The precision (relative standard deviation, RSD) was calculated from the standard deviation and observed concentration as follows: precision (RSD, %) = [standard deviation (SD)/C_{obs}] × 100.

2.6. Matrix effect and recovery

Three sets of the samples were prepared to evaluate the matrix effect and recovery with the quantitative bioanalytical method.

Set 1. Melamine stock solution was diluted to 50, 100, and 500 ng/mL by 90% (v/v) acetonitrile ($n = 3$ for each group). After vortex, these samples were transferred into autosampler vials, and 5 μL was injected directly into the HPLC–MS/MS system.

Set 2. Post-extraction fortification. Samples of set 2 were prepared by adding 10 μL melamine standard solution into 90 μL reconstituted extract solutions from three different lots of blank matrices to give three concentrations of melamine (50, 100, and 500 ng/mL, $n = 3$ for each group). After vortex and filtration (0.45 μm), these samples were transferred into autosampler vials, and 5 μL was injected into the HPLC–MS/MS system.

Set 3. Pre-extraction fortification. In this set, samples were prepared by the same procedures of calibration standards as described in Section 2.5. In brief, melamine was first added into matrices to give three concentrations (50, 100, and 500 ng/mL), then extracted by SPE, and reconstituted in 90% (v/v) acetonitrile ($n = 3$ for each group). After vortex and filtration (0.45 μm), these samples were transferred

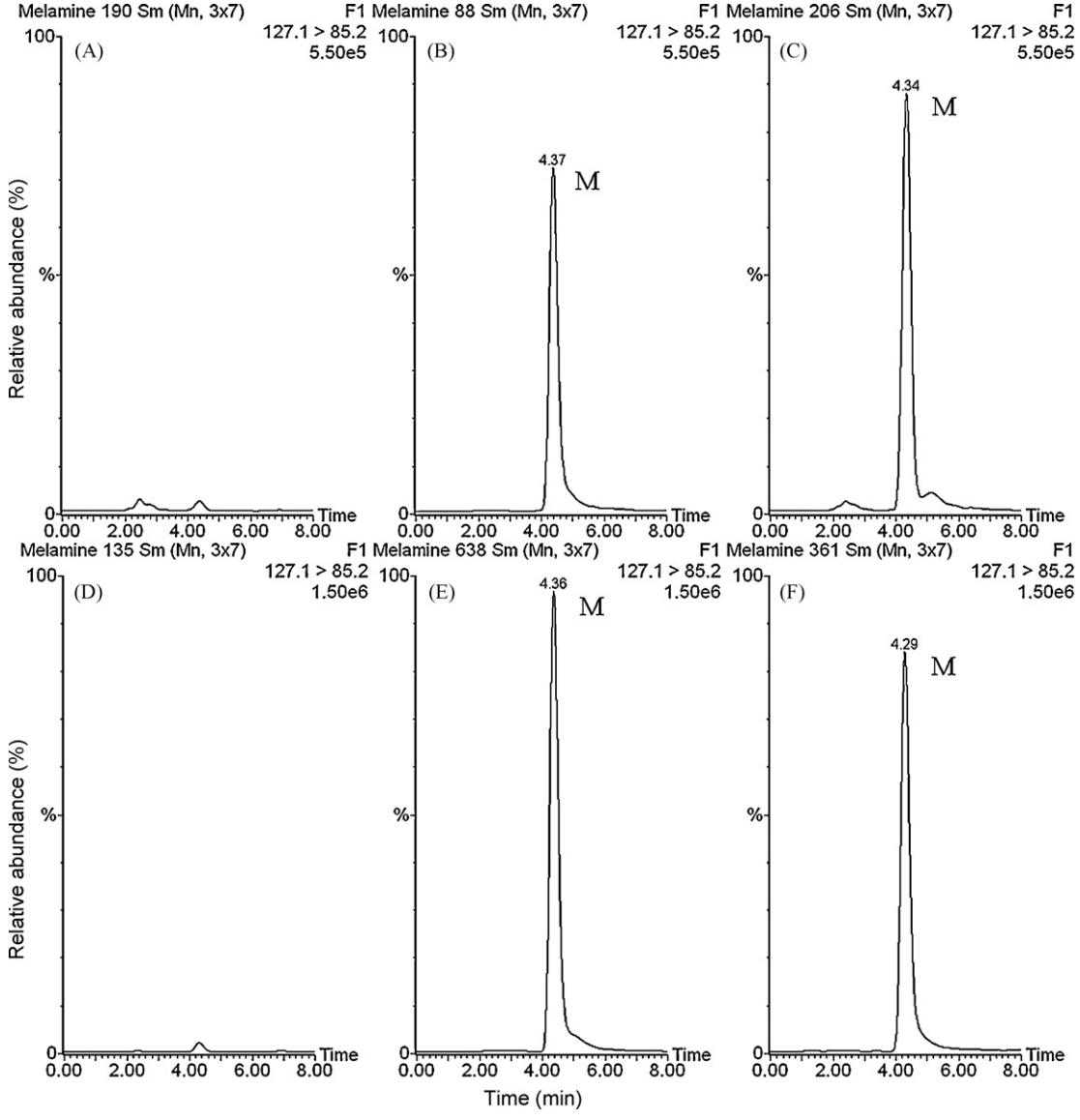

Fig. 1. HPLC–MS/MS chromatograms. (A) Blank plasma, (B) blank plasma spiked with melamine (100 ng/mL), (C) plasma sample (118.0 ng/mL) collected at 30 min after melamine administration (10 mg/kg), (D) blank liver homogenate, (E) blank liver homogenate spiked with melamine (500 ng/mL), (F) liver sample (450.2 ng/mL) collected at 30 min after melamine administration (10 mg/kg). M: melamine.

into autosampler vials, and 5 μL was injected into the HPLC–MS/MS system.

The results obtained in this way allow assessment of the matrix effect (ME) and recovery (RE). Matrix effect expressed as the ratio of the mean peak area of an analyte spiked post-extraction (set 2) to the mean peak area of the same analyte standards (set 1) multiplied by 100. A value of >100% indicates ionization enhancement, and a value of <100% indicates ionization suppression. Recovery calculated as the ratio of the mean peak area of an analyte spiked before extraction (set 3) to the mean peak area of an analyte spiked post-TCA treatment followed by SPE extraction (set 2) and multiplied by 100. The ME and RE values can be calculated according to previous reports [12,14].

3. Results and discussion

3.1. HPLC–MS/MS

The mass spectrum revealed a peak of melamine at *m*/*z* 127 corresponding to $[M+H]^+$, and its product ion *m*/*z* 85 is selected for quantification. The mass transition of *m*/*z* 127 → 85 was used for the quantification of melamine and this fragmentation pathway was in accordance with previous reports [1,7]. Two different types of columns were considered in the method development. The Luna C18 (50 × 4.6 mm, Phenomenex) column with a mobile phase consisting of 0.1% trifluoroacetic acid (TFA) and acetonitrile gave a tailing peak and did not provide sufficient retention for melamine, so an Xbridge HILIC was selected for the final analytical condition. Different injection solvents were also tested, such as acetonitrile/water (1:1, v/v) and acetonitrile/water (9:1, v/v). The results indicated that the ratio of acetonitrile/water (9:1, v/v) gave the sharpest peaks and it was therefore used in the final method. Figs. 1–3 represent the HPLC–MS/MS chromatograms for plasma, liver, kidney, spleen, bladder, and brain with their respective blank matrix, blank matrix spiked with melamine, and samples collected after melamine administration. The interference peaks in blank matrices appearing at 4.4 min in Figs. 1A, 1D, 2A, 2D, and 3A, 3D might come from environmental background levels of melamine from pollution due to worldwide use of triazine (1,3,5-triazines or *s*-triazines) derivatives in fertilizer [15], herbicides and pesticides [16–18] as well as from discharges of melamine from manufactur-

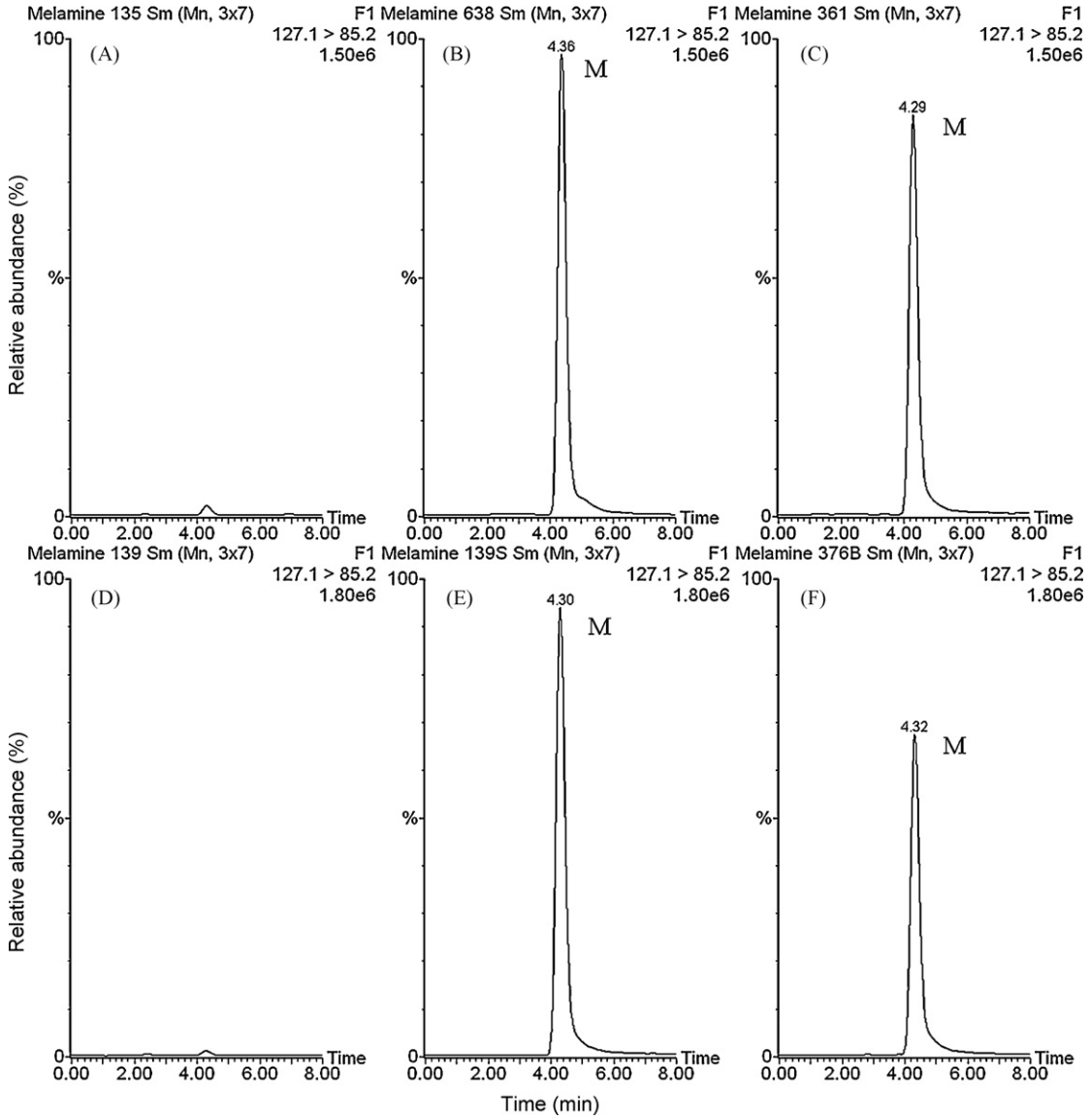

Fig. 2. HPLC–MS/MS chromatograms. (A) Blank kidney, (B) blank kidney spiked with melamine (500 ng/mL), (C) kidney sample (420.4 ng/mL) collected at 30 min after melamine administration (10 mg/kg), (D) blank spleen homogenate, (E) blank spleen homogenate spiked with melamine (500 ng/mL), (F) spleen sample (352.7 ng/mL) collected at 30 min after melamine administration (10 mg/kg). M: melamine.

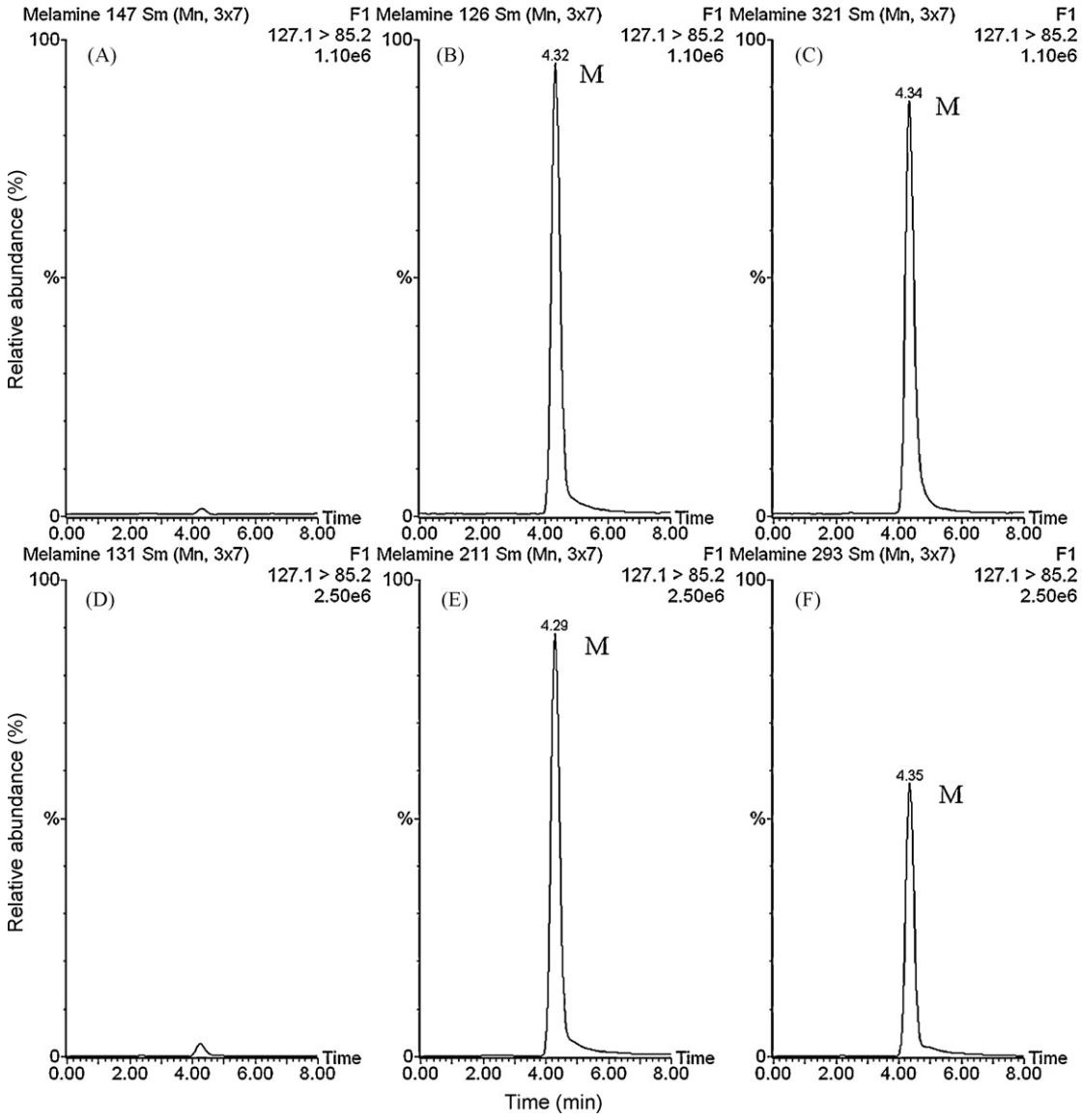

Fig. 3. HPLC–MS/MS chromatograms. (A) Blank bladder, (B) blank bladder spiked with melamine (500 ng/mL), (C) bladder sample (489.3 ng/mL) collected at 30 min after melamine administration (10 mg/kg), (D) blank cerebellum homogenate, (E) blank cerebellum homogenate spiked with melamine (500 ng/mL), (F) cerebellum sample (281.2 ng/mL) collected at 30 min after melamine administration (10 mg/kg). M: melamine.

ing and processing facilities [19]. In addition, in previous reports melamine contamination in blank analyses was also observed, resulting from the carryover effect [7,9]. Carryover effects were also observed in the current study, and the evaluated carryover ranged from 0.6 to 6.8 ng/mL.

3.2. Method validation

The linearity of the calibration curves was checked by the coefficients of determination (r^2) obtained for the regression line. Good linearity was achieved over the 20–500 ng/mL range, with all coefficients of correlation greater than 0.995. The limit of quantification (LOQ) was 20 ng/mL, based on the deviation of the response of the linear range (≤20%). The limit of detection (LOD) was approximately 5.6, 8.9, 7.2, 4.2, 5.4, and 8.3 ng/mL for plasma, liver, kidney, spleen, bladder, and brain, respectively, according to the equation: LOD = 3.3 × SD/S, where SD represents the standard deviation of the response and S = the slope of the calibration curve.

For plasma analysis, overall precision defined by the RSD ranged from 1.9 to 9.0%, and the accuracy expressed as the percent difference of the mean observed values compared with known concentration varied from −2.6 to 4.6% (Table 1). For organ analysis, method precision ranged from 1.6 to 16.3%, and the RSD for accuracy varied from −7.9 to 15.1% (Table 1). These results indicated both precision and accuracy were within ≤20% at the level of lower LOQ, indicating the method was reproducible [20].

A 90% (v/v) acetonitrile solution was injected after each sample run to remove any residual compounds from the injector and to evaluate the carryover effect. In addition, a wash program using gradient elution was applied to minimize the carryover effect after a batch (10 samples) of samples. Briefly, an injection of 50% acetonitrile was performed after each sample analysis, and the column was flushed by 40 times column volume 50% acetonitrile in 0.2% formic acid solution after batch analysis. The S/N ratios for the residue melamine detected in the wash samples varied from 1.9 to 4.8, and their approximate concentrations were much lower than 20 ng/mL.

3.3. Matrix effect and recovery

A stable isotope-labeled $^{15}N_3$-melamine was utilized as an internal standard to compensate for the matrix effects for the analysis of melamine in pork [7], fish tissue [8] and bovine kidney [9]. The use

Table 1
Intra- and inter-assay accuracy and precision values of the HPLC–MS/MS method for the determination of melamine in rat plasma and organ homogenate.

Nominal concentration (ng/mL)	Intra-assay		Inter-assay	
	Precision (%)	Accuracy (%)	Precision (%)	Accuracy (%)
Plasma				
20	5.9	0.4	7.8	3.4
125	2.2	−0.2	9.0	1.5
200	1.9	4.6	6.8	−2.6
Liver				
20	11.4	3.8	13.5	5.3
125	2.7	1.9	3.4	6.0
200	6.6	−7.9	16.3	−3.0
Kidney				
20	5.7	−0.2	7.0	−6.7
125	9.4	−0.9	9.8	5.0
200	11.2	−1.3	13.3	−1.9
Spleen				
20	3.6	15.0	4.0	15.1
125	1.6	3.5	5.6	−1.1
200	5.1	−1.3	7.4	1.4
Bladder				
20	6.2	−3.3	15.0	1.5
125	5.1	−5.8	7.8	1.1
200	5.3	−0.8	6.6	−0.6
Brain				
20	3.8	0.4	7.6	3.4
125	6.3	−0.2	8.7	1.5
200	7.3	4.6	11.7	−2.6

of a stable isotope-labeled analyte as internal standard is one way to compensate for matrix effects, but an isotope internal standard may require special handling for a common laboratory. Using a post-extraction fortification method, it is much easier to observe the matrix effects for the analysis of melamine in animal feed [11]. The results by the post-extraction fortification method indicate that the mean matrix effects of melamine in plasma, liver, kidney, spleen, bladder, and brain were 76.2 ± 2.9, 66.2 ± 6.7, 80.0 ± 1.3, 70.2 ± 6.9, 95.5 ± 13.2, and 92.9 ± 8.8%, respectively (Table 2). It was clear that the liver sample showed the most severe ion suppression. Therefore, the matrix effect in the quantitative analysis could not be ignored because of the evident difference between sets 1 and 2, so we diluted the samples which were higher than the calibration range by the extracted blank matrices to maintain the same matrix effect as the original samples and accurately quantitate melamine concentrations.

To determine the clean-up efficiency of the SPE step on the elimination of signal suppression, a set (set 4) of different blank homogenate without further SPE (chopped organ homogenized in 1% TCA) was spiked with melamine at 50, 100, and 500 ng/mL. Dividing the mean peak area of set 4 by the mean peak area of set 2 (melamine spiked with blank homogenate with SPE) revealed that there was only 26–59% signal intensity in set 4. In contrast, mean signal enhancement ranged from 173 to 427% when dividing set 2 by set 4, which indicates the clean-up efficiency of SPE on the elimination of signal suppression.

The mean recoveries for melamine were 113.0 ± 9.6, 98.3 ± 16.3, 79.8 ± 8.2, 111.6 ± 2.1, 83.4 ± 23.4, and 99.9 ±± 11.5% for plasma, liver, kidney, spleen, bladder, and brain samples, respectively (Table 2). The average recovery was better than 80% which is comparable with previous reports, but recovery variations were

Table 2
Matrix effect (ME) and recovery (RE) Data for melamine in rat plasma and organ homogenate[a].

Nominal concentration (ng/mL)	Set 1	Set 2	Set 3	ME (%)[b]	RE (%)[c]
Plasma					
50	75,900 ± 1100	59,200 ± 4600	73,400 ± 2700	78.0	124.0
100	169,000 ± 24,000	123,000 ± 5000	131,000 ± 7000	72.8	106.5
500	703,000 ± 8700	547,000 ± 18,000	5930,00 ± 32,000	77.8	108.4
Mean				76.2 ± 2.9	113.0 ± 9.6
Liver					
50	75,900 ± 1100	53,900 ± 3300	57,800 ± 4000	71.0	107.2
100	169,000 ± 24,000	98,900 ± 8100	107,000 ± 3000	58.5	108.2
500	703,000 ± 8700	4850,00 ± 60,000	385,000 ± 6000	69.0	79.4
Mean				66.2 ± 6.7	98.3 ± 16.3
Kidney					
50	75,900 ± 1100	61,700 ± 5700	55,000 ± 10,600	81.3	89.1
100	169,000 ± 24,000	1350,00 ± 14,000	104,000 ± 24,000	79.9	77.0
500	703,000 ± 8700	5530,00 ± 43,000	4060,00 ± 43,000	78.7	73.4
Mean				80.0 ± 1.3	79.8 ± 8.2
Spleen					
50	75,900 ± 1100	54,300 ± 3600	61,700 ± 1400	71.5	113.6
100	169,000 ± 24,000	106,000 ± 20,000	116,000 ± 1000	62.7	109.4
500	703,000 ± 8700	5370,00 ± 30,000	600,000 ± 4000	76.4	111.7
Mean				70.2 ± 6.9	111.6 ± 2.1
Bladder					
50	75,900 ± 1100	83,500 ± 12,800	88,200 ± 3200	110.0	105.6
100	169,000 ± 24,000	1560,00 ± 14,000	133,000 ± 4000	92.3	85.3
500	703,000 ± 8700	5920,00 ± 77,000	349,000 ± 2000	84.2	59.0
Mean				95.5 ± 13.2	83.3 ± 23.4
Brain					
50	75,900 ± 1100	63,000 ± 6000	56,500 ± 3000	83.0	89.7
100	169,000 ± 24,000	1690,00 ± 18,000	165,000 ± 2000	100.0	97.6
500	703,000 ± 8700	6720,00 ± 37,000	7550,00 ± 14,000	95.6	112.4
Mean				92.9 ± 8.8	99.9 ± 11.5

[a] Response was expressed as mean peak area ($n = 3$).
[b] ME (%) = (mean area of set 2/mean area of set 1) × 100.
[c] RE (%) = (mean area of set 3/mean area of set 2) × 100.

very obvious among different matrices. This situation has also been found in the analysis of wheat flour [11], and concentration-dependent recoveries have been found in the extraction of melamine from catfish and shrimp [8].

3.4. Application

The developed analytical method was applied to determine the plasma and organ distribution of melamine after intravenous administration. The plasma, liver, kidney, spleen, and bladder levels of melamine were 10.12 ± 1.68 μg/mL, 4.18 ± 0.61, 19.48 ± 2.75, 4.89 ± 0.78, and 1.74 ± 1.16 μg/g (expressed as mean ± SD, $n = 5$), respectively. Liver, kidney, spleen, and bladder concentrations were 41.3, 192.5, 48.4, and 17.2% of the plasma melamine level, respectively. The kidney had the highest melamine level, which is in agreement with a report that the kidney/plasma ratios of melamine was two- to threefold based on the result of a single oral dose of ^{14}C-melamine in Fischer 344 rats [21]. This phenomenon may be due to the fact that kidney is the organ primarily responsible the clearance of melamine [5].

For brain regional distribution, concentrations of melamine for cortex, striatum, hippocampus, cerebellum, brain stem, and the rest of the brain were 0.47 ± 0.20, 0.75 ± 0.11, 0.79 ± 0.10, 0.77 ± 0.09, 1.18 ± 0.25, 0.60 ± 0.40 μg/g (expressed as mean ± SD, $n = 5$), respectively, and they were only 4.6, 7.4, 7.8, 7.6, 11.7, and 6.0% of the plasma melamine level, respectively. These average low melamine levels in the rat brain might be because it is highly polar and its volume of distribution is close to that of total body water, suggesting that distribution of this compound may be virtually limited to the extracellular fluid compartment [5].

4. Conclusion

In this work, combined TCA precipitation with cation-exchange SPE with HILIC HPLC–MS/MS was used to determine six different biological matrices, including rat plasma, liver, kidney, spleen, bladder, and brain. The mean matrix effects of melamine in rat plasma, liver, kidney, spleen, bladder, and brain ranged from 66.2 ± 6.7 to 95.5 ± 13.2%, and the mean recoveries for melamine were all better than 80%. Though ion suppression was greatly eliminated after SPE treatment, the matrix effects still existed. To accurately quantitate melamine concentrations, samples that were higher than the calibration range were diluted with the extracted blank matrices. This method was successfully applied to evaluate the organ distribution of melamine in rats. The results showed that the kidney concentration was the double of the plasma melamine concentration, but melamine distributed to the brain was much lower than the amount in plasma.

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