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Use of Methanol for the Efficient Extraction and Analysis of Melamine and Cyanuric Acid Residues in Dairy Products and Pet Foods

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The recent worldwide shortage of acetonitrile has prompted the development of a new method using methanol as an alternative organic solvent in the extraction and liquid chromatographic analysis of melamine and cyanuric acid that may be present as contaminants in dairy products and pet foods. A simple extraction of melamine and cyanuric acid residues in fortified samples was successfully achieved, using a methanol−water mixture and analysis by isotopic dilution high-performance liquid chromatography−triple-quadrupole mass spectrometry (HPLC-MS/MS). A two-step centrifugation procedure was employed to remove matrix components from extracts. The separation of melamine and cyanuric acid was carried out on a Dionex Acclaim Trinity P1 column, with a methanol and ammonium acetate buffer used as the mobile phase. Excellent linearity was achieved for both the melamine and cyanuric acid calibrations. A variety of dairy products and pet foods were fortified with melamine and cyanuric acid at three levels, 1, 2.5, and 10 μg/g, producing recovery yields of 101−119% for melamine and 84−123% for cyanuric acid. The lower limit of quantification (LLOQ) of melamine was 0.03 μg/g for liquid milk and 0.05 μg/g for dry infant milk formula. The quantitative results were comparable with those derived from previous methods that have been proposed by the U.S. Food and Drug Administration for the screening of melamine and its analogues in foods.

KEYWORDS: Melamine; cyanuric acid; infant milk formula; pet foods; methanol extraction; mixed-mode ion exchange chromatography; HPLC-MS/MS

1. INTRODUCTION

In 2007, contaminated pet foods made from wheat gluten and rice protein imported from China were found to contain melamine. It is assumed that some manufacturers added the nitrogen-rich melamine to boost the apparent protein content of a diluted product. The combination of melamine and its analogue, cyanuric acid, has been linked to acute renal failure in pets; the contaminated foods caused the deaths of a large number of dogs and cats in the United States (*1*). In 2008, in China, the contamination of infant milk formulas by melamine caused thousands of children to be hospitalized due to kidney damage (*2*) and raised severe concerns about the safety of the human food supply.

Methods for the analysis of melamine and its analogue residues, including cyanuric acid, ammelide, and ammeline, in foods have been developed using trimethylsilyl derivatization−gas chromatography−mass spectrometry (GC-MS) (*3*), high-performance liquid chromatography with UV detection (HPLC-UV) (*4*), enzyme immunoassay, HPLC with diode array detection (HPLC-DAD) (*5*), and HPLC with tandem mass spectrometry (HPLC-MS/MS) (*5*−*13*).

U.S. FDA field laboratories are using LC-MS/MS methods capable of determining melamine and cyanuric acid at levels down to 0.25 ppm in powdered infant formula and other dairy-containing food products or ingredients (*14*−*16*). In one of these methods, melamine and its analogues are extracted by an acetonitrile/water mixture and cleaned up by solid phase extraction (SPE) before being analyzed by HPLC-MS/MS (*16*). The separation of melamine and cyanuric acid is performed on a zwitterionic HILIC column, with an acetonitrile/ammonium acetate buffer mobile phase (*16*).

Motivated by the recent worldwide shortage of acetonitrile, the present study was undertaken to develop a method that uses methanol as an effective long-term alternative solvent, for the extraction and analysis of melamine and its analogues in foods.

A number of matrices, including liquid soy milk, cow's milk, powdered infant formula, and some pet foods, were used for method development. The matrix samples were spiked with melamine and cyanuric acid each at three levels: 1, 2.5, and 10 μg/g. Isotopically labeled melamine and cyanuric acid, used as internal standards (ISs), were also added prior to the extraction. The samples were extracted in a methanol/water 3/1 (v/v) solution. A two-step centrifugation procedure was used to remove matrix components from extracts, prior to analysis by HPLC-MS/MS. The separation of melamine and cyanuric acid was achieved on a

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Dionex mixed-mode ion exchange column using a methanol and ammonium acetate buffer mobile phase, adjusted to pH 5. The MS/MS detector was operated in negative mode for cyanuric acid and in positive mode for melamine.

To meet the quality control (QC) requirements for such method development, we took steps to demonstrate low system background, high accuracy, and high precision, and we established acceptance criteria for the results.

2. EXPERIMENTAL PROCEDURES

2.1. Reagents. Melamine (purity = 99%), cyanuric acid (purity = 98%), and formic acid (88% concentration), ACS reagent grade, were purchased from Sigma-Aldrich (St. Louis, MO). Isotopically labeled melamine, $^{13}C_3H_6{}^{15}N_3N_3$ (purity = 98%), and isotopically labeled cyanuric acid, $^{13}C_3H_3{}^{15}N_3O_3$ (purity > 90%), were obtained from Cambridge Isotope Laboratories, Inc. (Andover, MA). Ammonium acetate (purity = 99.99%) was purchased from Mallinckrodt Baker Inc. (Phillipsburg, NJ). Methanol, high-purity chromatographic grade, was supplied by Burdick and Jackson (Muskegon, MI). Deionized (DI) water, purified to 18.0 MΩ cm resistivity, was prepared in our laboratory with a Nanopure Diamond water system (Barnstead International, Inc., Dubuque, IA).

2.2. HPLC-MS/MS. The HPLC-MS/MS system consisted of an Agilent Technologies 1200 series HPLC (Wilmington, DE) coupled to an API-2000 triple-quadrupole mass spectrometer (Applied Biosystems/MDS SCIEX, Toronto, Canada). The HPLC was equipped with a vacuum degasser, a binary pump, a thermostated column compartment, and an autosampler. The MS/MS system was equipped with a turbo electrospray ionization (ESI) source, capable of operation in both negative and positive modes; nitrogen was used as nebulizer gas. The ESI sheath gas was set at 50 psi.

HPLC separation was performed on a Dionex (Sunnyvale, CA) 100 × 2.1 mm (length × i.d.), 3 μm (particle size) Acclaim Trinity P1 column thermostated at 40 °C. A binary gradient of methanol (A) and 5 mM ammonium acetate adjusted to pH 5 (B) by formic acid at a flow rate of 0.2 mL/min was applied, with a sample injection volume of 10 μL. Separation was carried out according to the following gradient program: the initial mobile phase started at 10% B for 3 min, was ramped to 60% B over 2 min, and was then kept isocratic at 60% B for 8 min. After both cyanuric acid and melamine had eluted, the mobile phase was returned to 10% B and allowed to re-equilibrate for 17 min. Flow was diverted to waste 12 min after sample injection.

There were two acquisition experiments for MS/MS: the negative mode was applied from 0 to 3 min for cyanuric acid detection and the positive mode was applied from 3 to 13 min for melamine detection. The retention time was 2 min for cyanuric acid and 8−10 min for melamine.

2.3. Preparation of Standard Solutions. *Stock Solutions*. Five milligrams of neat melamine or cyanuric acid was added to a 50 mL volumetric flask and diluted with about 30 mL of 3/1 methanol/water. The solution was sonicated for 10−15 min until crystals of the neat material were no longer visible. The solution was then diluted to the mark with 3/1 methanol/water, to produce a 100 μg/mL stock solution. The stock solutions were stable over a study period of 6 months.

Intermediate Standard Solutions. Three intermediate standard mixtures, at 1, 2.5, and 10 μg/mL, were prepared by combining appropriate aliquots of melamine and cyanuric acid standard stock solution to a 10 mL volumetric flask and diluting to the mark with 3/1 methanol/water.

Internal Standard (IS) Solutions. The intermediate solutions of isotopically labeled melamine and isotopically labeled cyanuric acid at 4 μg/mL each were prepared individually by diluting the corresponding stock solution in DI water.

Calibration Standard. Calibration curves of melamine and cyanuric acid were constructed from standard solution mixtures at 1, 5, 20, 50, 200, and 500 ng/mL with both isotopically labeled IS solutions at 10 ng/mL each.

2.4. Sample Extraction. A 0.1 g food sample was weighed out into a 1.5 mL polypropylene microcentrifuge tube. For a solid sample, 100 μL of DI water was added, and the tube was vortexed for 20 s. For a liquid sample, this step was omitted. The sample was then spiked with 25 μL each of 4 μg/mL isotopically labeled melamine and cyanuric acid IS solutions. For matrix spike samples, 100 μL of an intermediate standard mixture of 1, 2.5, or 10 μg/mL was added to the sample, to give the desired spiking level. For an unspiked matrix sample, 100 μL of DI water was added, to produce a 250 μL aqueous suspension. Finally, 750 μL of methanol was added to each sample. The tubes were vortexed for 20 s between successive steps. Samples were then sonicated in a Branson ultrasonic water bath (Danbury, CT) for 30 min.

Samples were centrifuged at 10000 rpm (approximately 8944*g*) for 10 min at room temperature in a National Labnet microcentrifuge (Woodbridge, NJ). A 750 μL aliquot of supernatant was transferred to another microcentrifuge tube and centrifuged at 12000 rpm (approximately 12900*g*) for 15 min. A 100 μL aliquot of the second supernatant was transferred to an autosampler vial and diluted with 900 μL of 3/1 methanol/water. The concentration in the final extract of a sample fortified with residues at 2.5 μg/g is 25 ng/mL, a dilution factor of 100.

2.5. Method Development and Quality Control. Method development was performed in accordance with the guidelines found in Guidance for Industry: Bioanalytical Method Validation (*17*). Liquid soy milk, cow's milk, powdered infant milk formula, and several pet foods purchased from a local market were chosen as matrices for the method development. The matrix samples were spiked with the melamine/cyanuric acid mixtures at three levels, 1, 2.5, and 10 μg/g. Isotopically labeled melamine and cyanuric acid IS solutions were added to the matrix spiked samples at a fixed concentration of 1 μg/g each.

Calibration. At least five calibration concentrations are required to prepare the calibration curve spanning a 100-fold concentration range. The lowest standard concentration should be at or below the lower limit of quantification (LLOQ). Established acceptance limits are a relative standard deviation (RSD) < 20% for all response factors and a correlation coefficient of 0.995 or greater for the calibration curve of each analyte. A mid-level calibration standard is analyzed at both the beginning and the end of a daily analysis batch, to verify the acceptability of an existing calibration. The calculated concentration of each analyte at the mid-level should be within ±20% of the true value.

Initial Demonstration of Low System Background. The quality control requirement for the method development consists of an initial demonstration of low system background in a method blank (MB) for each matrix to identify whether the matrix is contaminated by melamine and/or cyanuric acid. If any analyte (melamine or cyanuric acid) is found in the MB at a value greater than triple the method detection limit (MDL), the matrix is considered to be contaminated.

Initial Demonstration of Precision and Accuracy. The precision, expressed as the RSD, as well as the recoveries (quantified value/spiked value), were evaluated at each of the three spiked levels with at least four replicates for each level and one matrix blank. Accuracy was acceptable when the recovery was within ±30% of the nominal concentration for the 1 μg/g spiking level and within ±20% of the nominal concentration for the spiking level of 2.5 or 10 μg/g. The acceptance criterion for precision was an RSD value within ≤20%.

Method Detection Limit. The MDL was calculated with 99% confidence level as 3 times the standard deviation of the calculated concentration, in eight replicate matrix spiked samples at 1 μg/g. The LLOQ of each analyte was set to 5 times the MDL.

3. RESULTS AND DISCUSSION

3.1. MS/MS Analysis. Analyses of melamine and cyanuric acid using HPLC-MS/MS and isotopic dilution methods have been previously reported (*6*, *7*, *13*, *16*). Here, we optimized the operating parameters for the ESI source to yield the best mass spectrometric performance for both melamine and cyanuric acid. The molecular ion and product ions of melamine and cyanuric acid were observed through continuous infusion of each compound at the concentration of 1 μg/mL in methanol, with the ESI source operating in positive mode for melamine and in negative mode for cyanuric acid. Melamine generated the precursor ion at *m*/*z* 127 in full scan mode and ions at *m*/*z* 85 and 68 as the most abundant product ions in product-ion scan mode. Cyanuric acid generated the precursor ion at *m*/*z* 128 and product ions at *m*/*z* 85

and 42. Isotopically labeled melamine ($^{13}C_3H_6{}^{15}N_3N_3$) generated the precursor ion at m/z 133 in full scan mode and product ions at m/z 89 and 72. Isotopically labeled cyanuric acid ($^{13}C_3H_3{}^{15}N_3O_3$) generated the precursor ion at m/z 134 and product ions at m/z 89 and 44 (**Table 1**).

Table 1. MS/MS Operating Parameters for Melamine, Cyanuric Acid, and Corresponding Labeled ISs

MS/MS parameter	melamine/ labeled IS	cyanuric acid/labeled IS
polarity	positive	negative
precursor ion (m/z)	127/133	128/134
product ion (m/z)	85/89	85/89
	68/72	42/44
collision energy (eV)	22 and 40	10 and 12
declustering potential (V)	5	25
ion spray voltage (V)	5500	3500
ion source temperature (°C)	550	550

MS/MS parameters, including ionization energies, temperature, and the voltages applied to the ESI source, were optimized in multiple-reaction monitoring (MRM) mode. Melamine and cyanuric acid each was injected at 1 μg/mL in 50/50 methanol/ammonium acetate (5 mM) at 0.2 mL/min, using flow injection analysis (FIA) optimization (**Table 1**). For optimum MS/MS precision, there must be at least 20 scans across the peak. Optimization of the flow rates for the nebulizing gas, auxiliary gas, and curtain gas was also performed by FIA, to maximize the sensitivity of the MRM signals for cyanuric acid and melamine.

Quantitation of melamine and cyanuric acid was performed in MRM mode with the combination of two mass transitions of m/z 127 → 85 at collision energy (CE) of 22 eV and m/z 127 → 68 at CE of 40 eV for melamine and m/z128 → 85 at CE of 10 eV and m/z128 → 42 at CE of 12 eV for cyanuric acid to maximize the signal sensitivity. Two mass transitions of m/z 133 → 89 and 133 → 72 were used for the labeled melamine IS, and m/z 134 → 89 and 134 → 44 were used for the labeled cyanuric acid IS (**Table 1**). The dwell time was set at 800 ms for both compounds. HPLC-MS/MS

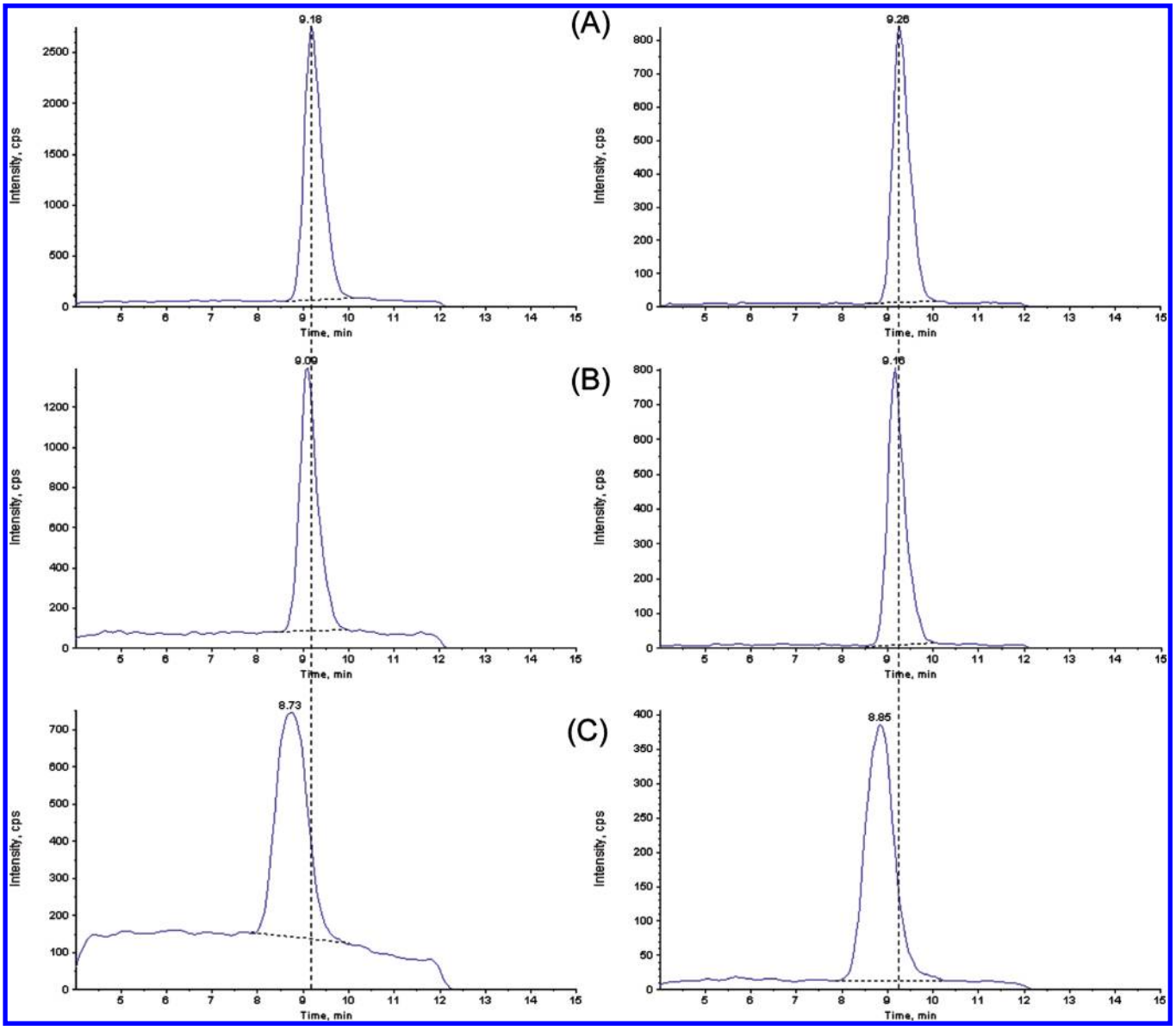

Figure 1. LC-MS/MS chromatograms of melamine (left) and labeled melamine IS (right) for the standard (**A**) and the extracts of liquid milk (**B**) and infant formula powder (**C**), both fortified at 1 μg/g. The observed signal is a combination of two transitions of m/z 127 → 85 and 127 → 68 for melamine and m/z 133 → 89 and 133 → 72 for labeled melamine IS. A shift in the retention time for melamine was observed for infant formula powder (**C**).

data were collected and processed by Analyst software, version 1.4.2 (Applied BioSystems/MDS SCIEX).

The relative signal ratio between two transitions was used to confirm the identification of each analyte and its IS for quality control purposes. The relative abundance ratio between two transitions for melamine and cyanuric acid and their ISs should match the comparison standard within ±10% following FDA/CVM guidance (*18*).

3.2. HPLC Analysis. Because the MS/MS detector in MRM mode for melamine and cyanuric acid is operated in inverse polarity, the HPLC-MS/MS analysis requires a complete separation of the two compounds. The goal of this portion of the analytical method was to separate melamine and cyanuric acid, using methanol as an alternate organic solvent. Several stationary phases with different polarities, such as HILIC and reverse phase (RP) C18 columns, were tested for this study.

The separation of melamine and cyanuric acid was initially performed on a Waters Atlantis HILIC column (50 × 3.0 mm length × i.d.; 3 μm particle size). A complete separation of the two analytes was achieved when acetonitrile and 5 mM ammonium acetate as binary eluent solvents were used. The mechanism of separation of melamine and cyanuric acid in this condition was previously described by Heller and Nochetto (*15*). However, when we replaced acetonitrile with methanol, we found that both melamine and cyanuric acid were poorly retained on the HILIC column; they coeluted at 1.5 min. Neither adjustment of the pH of the ammonium acetate buffer nor alteration of the methanol percentage in the binary solvent system served to improve the separation. A similar phenomenon was observed when we used a nonpolar Agilent Zorbax Eclipse Plus-C18 column (100 mm × 2.1 mm length × i.d.; 3.5 μm particle size). The poor retention can be explained by the hydrophilic nature of both melamine and cyanuric acid: they are easily eluted by a strong polar solvent such as methanol.

We searched for a stationary phase with a strong ion force capable of retaining either melamine or cyanuric acid, while eluting the other. An LC ion exchange condition was chosen. The separation of melamine and cyanuric acid was achieved with a Dionex Acclaim Trinity P1 column (100 × 2.1 mm length × i.d., 3 μm particle size), eluted with a mixture of methanol and 5 mM

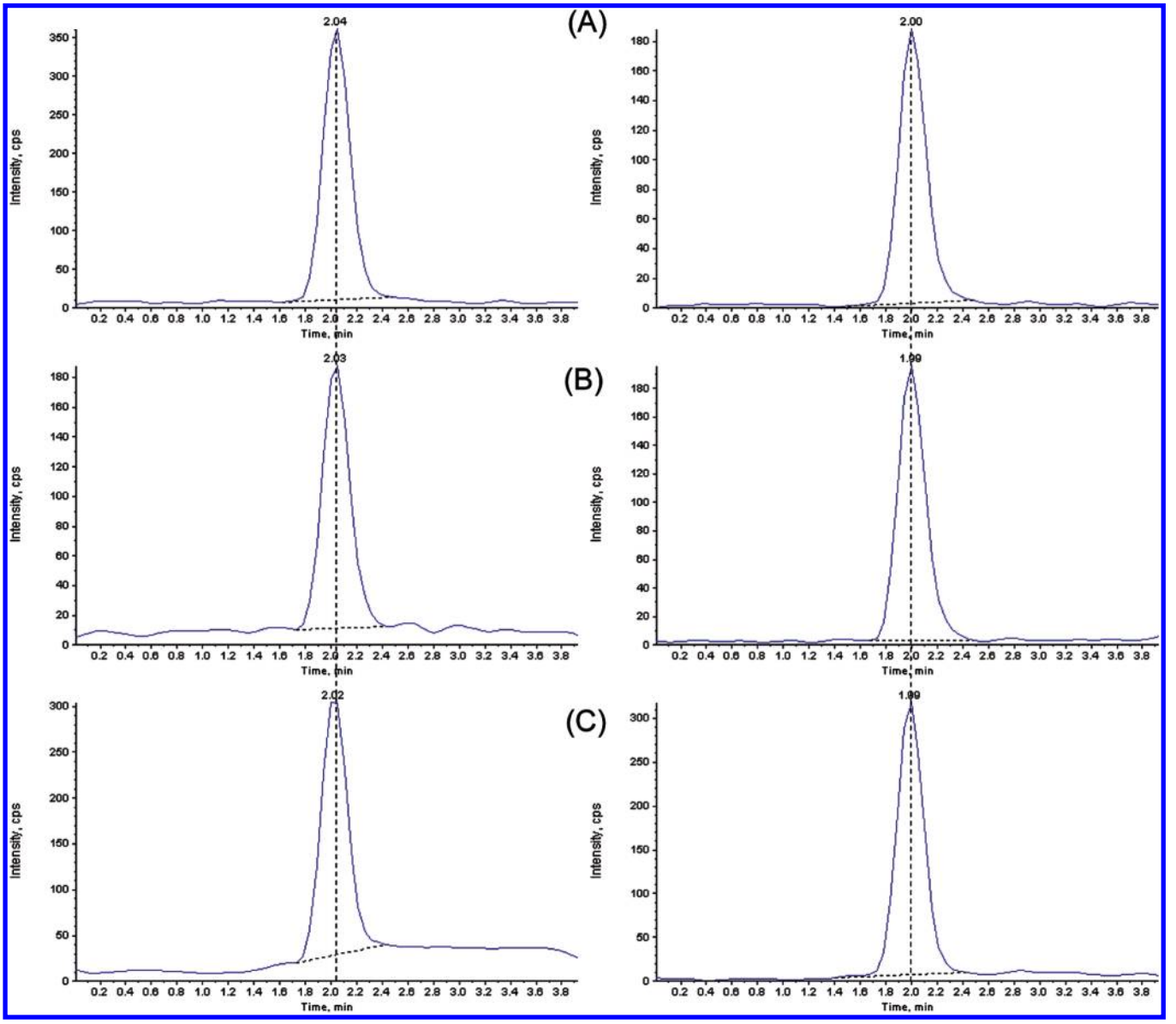

Figure 2. LC-MS/MS chromatograms of cyanuric acid (left) and labeled cyanuric acid IS (right) for the standard (**A**) and the extracts of liquid milk (**B**) and infant formula powder (**C**), both fortified at 1 μg/g. The observed signal is a combination of two transitions of *m*/*z* 128 → 85 and 128 → 42 for cyanuric acid and *m*/*z* 134 → 89 and 134 → 44 for labeled cyanuric acid IS.

aqueous ammonium acetate adjusted to pH 5. The Acclaim Trinity P1 column, which uses a mixed-mode silica gel coated with charged nanopolymer particles, can provide reverse phase, anion exchange, and cation exchange properties (*19*, *20*). Its chemistry ensures distinct spatial separation of the anion exchange and cation exchange regions, such that all three retention mechanisms can function simultaneously and can be controlled independently (*19*).

The separation of melamine and cyanuric acid was strongly affected by the pH of the aqueous solution. The pK_a values of melamine and cyanuric acid are 5.0 and 6.9 (*21*, *22*), respectively. At pH 5, cyanuric acid was a neutral form, which eluted at 2 min in a 90/10 methanol/5 mM ammonium acetate solution, whereas melamine was a cationic form and was retained on the stationary phase. Melamine was eluted only when a higher proportion of aqueous phase (60%) was used, as a result of a cation exchange process that occurred on the silica bead surface of the nanopolymeric particles (*19*). The retention time of melamine ranged from 8 to 10 min depending on the column age and on the proportion of aqueous buffer in the solvent mixture. The column was re-equilibrated for 17 min with a 90/10 methanol/5 mM ammonium acetate solution before the next run. An increase of flow rate in this step would help to accelerate the equilibrium process. The LC conditions are detailed in section 2.2. Typical LC-MS/MS chromatograms of melamine and cyanuric acid and their labeled IS solutions are shown in **Figures 1** and **2**, respectively. No interference was observed in the analyte or IS retention windows.

We assessed the effect of temperature on the separation of melamine and cyanuric acid on the Acclaim Trinity P1 column by performing the separation at 30, 40, and 50 °C. Because no significant improvement was obtained in these trials, the column was thermostated at 40 °C to reduce the LC pump back pressure.

3.3. Evaluation of Liquid Extraction. Several dairy products and pet foods, in either liquid or solid form, were used for the method development. They were fortified with both cyanuric acid and melamine and then tested under the same conditions to reveal matrix effects and possible interferences.

The method for the extraction of melamine and cyanuric acid reported previously by Fremlin and Pelzing (*23*) was followed in the present work with some modifications, most notably, the replacement of acetonitrile by methanol as the extraction solvent. Soy milk, the simplest liquid matrix with less protein content, was chosen as matrix for evaluation of the solvent extraction. Several mixing ratios (3/1, 2/1, 1/1, and 1/2) of methanol/water were used to test soy milk samples, which were spiked with the middle level (2.5 μg/g each) of melamine and cyanuric acid. The recoveries, defined as the relative ratio between absolute areas of the peaks for the ISs after extraction and those of the control samples, were then compared to the recoveries from the 3/1 acetonitrile/water extraction. The control samples were soy milk extracts, to which were added labeled melamine and cyanuric acid ISs, just before injection. The detailed extraction process was described in section 2.4.

The recoveries for the labeled melamine IS in 3/1 and 2/1 methanol/water mixing ratios were found to be 52.4 and 55.3% (data not shown), respectively, and those for the labeled cyanuric acid IS were 43.2 and 38.3%, respectively. Lower methanol percentage mixtures yielded recoveries below 40%. Moreover, the extracts obtained from 1/1 and 1/2 methanol/water mixtures were not transparent, indicating matrix contamination. Therefore, the 3/1 methanol/water mixture was selected for use as the extraction solvent through the remainder of the method development, because it is close to the initial mixing ratio of the liquid chromatographic mobile phase. The recoveries of the melamine and cyanuric acid ISs obtained from 3/1 methanol/water mixture were higher for extracted melamine compared to those obtained from 3/1 acetonitrile/water mixture, the latter yielded 38.5 and 59% recoveries for the labeled melamine and cyanuric acid ISs, respectively.

Table 2. Method Validation: Precision and Accuracy Values for Matrix-Spiked Melamine and Cyanuric Acid at Three Spiking Levels (1, 2.5, and 10 μg/g)

			melamine		cyanuric acid	
matrix	spike level (μg/g)	no. of replicates	mean recovery (%)	RSD (%)	mean recovery (%)	RSD (%)
soy milk	1	8	110	2.3	102	4.6
	2.5	4	114	2.8	97	2.7
	10	4	107	2.7	93	6.9
cow's milk	1	8	108	1.7	101	2.8
	2.5	4	109	1.8	101	3.7
	10	4	105	2.0	93	2.6
powdered infant formula	1	8	111	3.0	108	8.3
	2.5	4	105	1.1	97	3.3
	10	4	107	1.2	95	3.9
dry cat food	1	8	110	3.6	122	5.2
	2.5	4	110	0.6	111	4.1
	10	4	107	1.2	97	7.6
wet cat food	1	8	119	2.5	102	3.2
	2.5	4	109	0.9	96	5.2
	10	4	105	0.8	96	3.5
wheat gluten	1	8	107	2.0	123	6.7
	2.5	4	108	2.7	95	8.1
	10	4	101	0.5	84	5.5

The infant formula powder, which has a higher content of protein, fat, mineral salts, and carbohydrate than the others, exhibited the strongest matrix effect. The presence of mineral salts up to approximately 2% by weight in infant formula powder might cause broadening of the peak, and a shift of it toward a shorter retention time, for extracted melamine (**Figure 1**). Cyanuric acid was less affected by the mineral salts in this matrix, it was eluted as a neutral form in a predominantly organic solvent of 90/10 methanol/ammonium acetate, pH 5, buffer mixture, as detailed in section 3.2 (**Figure 2**). The high carbohydrate content of the infant formula powder samples resulted in a rapid contamination of the ESI source and MS interface. The curtain plate turned dark yellow or brown after a dozen powdered infant milk samples had been analyzed. The higher the sugar content of the sample that was injected, the darker the resultant color of the curtain plate. However, the sugar residue did not affect the analytical results, and the mid-level calibration standard analyzed at the end of the analysis batch was still within ±20% of the true value. Accordingly, to lower system background, we cleaned the curtain plate interface of the HPLC-MS/MS instrument before every analysis batch.

3.4. Quality Control and Method Validation. The linear regression correlation coefficients for melamine and cyanuric acid were > 0.995. The relative abundance ratio between two MS/MS transitions, 127 → 85 and 127 → 68 for melamine and 133 → 89 and 133 → 72 for its labeled IS, was found to match the criteria set within ±10%. Similar results were also found for cyanuric acid and its IS. Minimal background interferences were observed in the method blank (MB) for all matrices tested. The respective

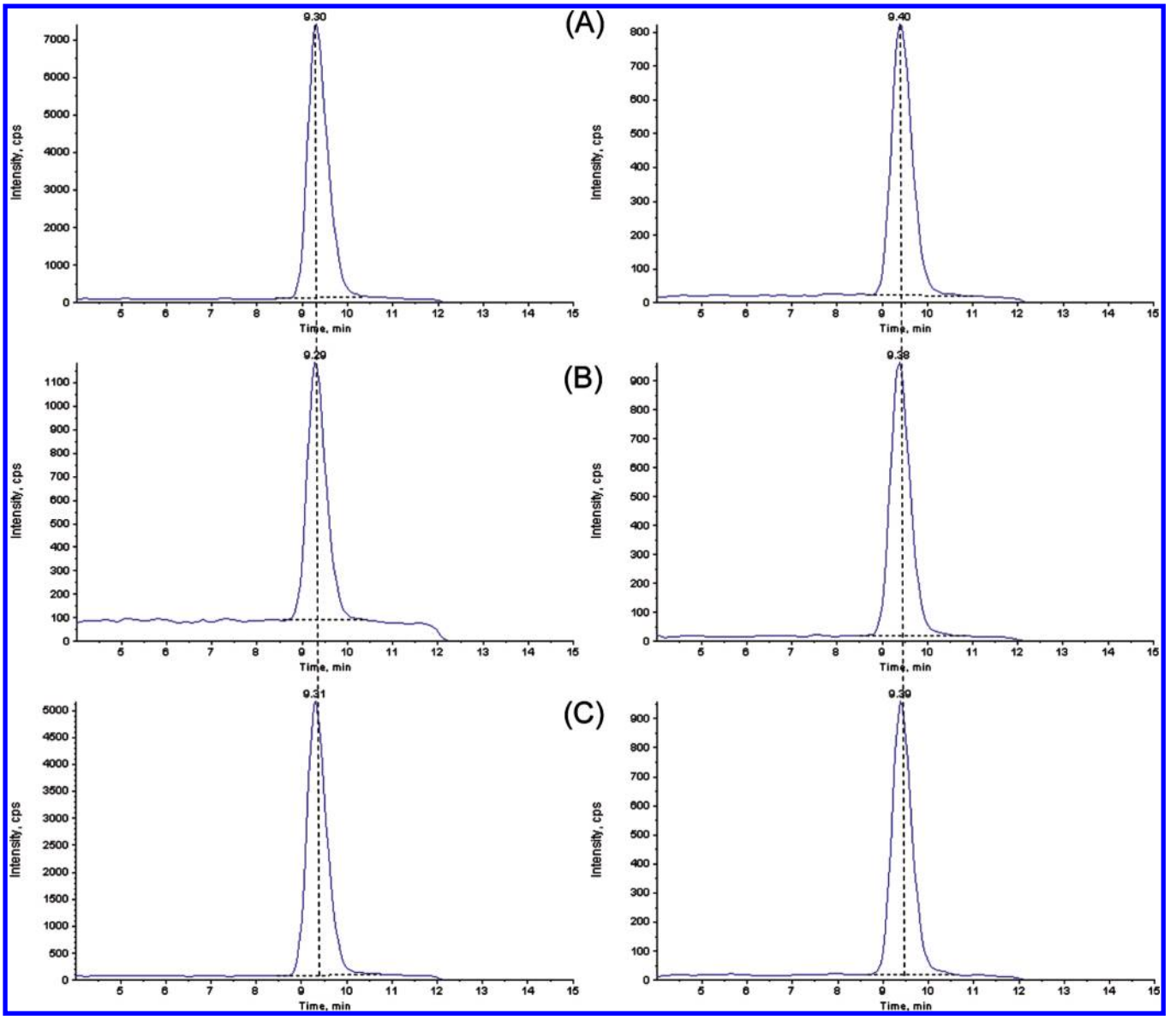

Figure 3. LC-MS/MS chromatogram of melamine (left) and labeled melamine IS (right) for the 50 ppb standard (**A**), imported coconut milk juice (**B**), and imported coconut milk juice sample fortified with melamine at 2.5 μg/g (**C**).

overall recoveries for melamine and cyanuric acid were found to be between 101 and 119% and between 84 and 123% across the spiking levels (1, 2.5, and 10 μg/g) in different matrices. The precision (RSD) values ranged from 0.5 to 3.6% for melamine and from 2.6 to 8.3% for cyanuric acid (**Table 2**).

The average MDL, determined in eight replicates at a spiking level of 1 μg/g, was 8.2 ng/g for melamine and 17 ng/g for cyanuric acid, across the matrices tested. Liquid cow's milks yielded the lowest MDLs for both melamine and cyanuric acid (**Table 3**), whereas powdered infant formula, which contained higher mineral salts and carbohydrates, yielded higher MDLs of 10 and 27 ng/g for melamine and cyanuric acid, respectively, resulting in high values of corresponding LLOQs (**Table 3**).

3.5. Application to the Analysis of Commercial Dairy Products. Several imported commercial dairy products were collected for melanine and cyanuric acid examination. The control and fortified samples were also prepared for quality assurance purposes. The examined diary samples were fortified with standard mixture at levels of 1 and 2.5 μg/g. The samples were extracted and analyzed under the same conditions as described for the method development. If a sample contained melamine at a concentration above the calibrated linear range, then the extract was diluted with 3/1 methanol/water at an appropriate ratio and reanalyzed.

Table 3. MDL and LLOQ Values for Matrix-Spiked Melamine and Cyanuric Acid, Determined at 1 μg/g (Eight Replicates)

	melamine		cyanuric acid	
matrix	MDL (ng/g)	LLOQ (ng/g)	MDL (ng/g)	LLOQ (ng/g)
soy milk	7	37	14	69
cow's milk	5	27	8	42
powdered infant formula	10	49	27	134
dry cat food	12	59	19	95
wet cat food	9	45	10	49
wheat gluten	6	30	25	124

A sample extract containing 0.63 μg/g melamine is shown in **Figure 3**. The presence of melamine in the sample extract was confirmed by characteristic MS/MS transitions of m/z 127 → 85 and 127 → 68. No trace amount of cyanuric acid was found in those examined diary samples.

4. CONCLUSION

A simple, accurate, and sensitive method for the analysis of melamine and cyanuric acid in dairy products and pet foods has been developed, with the substitution of methanol for the more costly acetonitrile used in existing methods. The method was validated by the labeled isotopic dilution method. The results obtained from the extraction method and the data from the quantitative analysis were comparable to those published by the FDA, demonstrating that the new method can be used to monitor melamine and its analogues in dairy products and pet foods in the market.

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