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ARTICLE

Liquid Chromatography−Tandem Mass Spectrometry (LC−MS/MS) Method Extension To Quantify Simultaneously Melamine and Cyanuric Acid in Egg Powder and Soy Protein in Addition to Milk Products

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As a consequence of the adulteration of infant formulas and milk powders with melamine (MEL) in China in 2008, much attention has been devoted to the analysis of MEL [and cyanuric acid (CA)] in dairy products. Several methods based on high-performance liquid chromatography (HPLC), liquid chromatography−tandem mass spectrometry (LC−MS/MS), nuclear magnetic resonance (NMR), or Raman spectroscopy have been described in the literature. However, no method is available for the simultaneous determination of MEL and CA in other raw materials, which are considered as high-risk materials for economically motivated adulteration. The present paper reports the results of an interlaboratory-based performance evaluation conducted with seven laboratories worldwide. The purpose was to demonstrate the ability of a cleanup-free LC−MS/MS method, originally developed for cow's milk and milk-powdered infant formula, to quantify MEL and CA in egg powder and soy protein. Limit of detection (LOD) and limit of quantification (LOQ) were 0.02 and 0.05 mg/kg for MEL in egg powder and soy protein, respectively. For CA, LOD and LOQ were 0.05 and 0.10 mg/kg in egg powder and 1.0 and 1.50 mg/kg in soy protein, respectively. Recoveries ranged within a 97−113% range for both MEL and CA in egg powder and soy protein. Reproducibility values (RSD_R) from seven laboratories were within a 5.4−11.7% range for both analytes in the considered matrices. Horwitz ratio (HorRat) values between 0.4 and 0.7 indicate acceptable among-laboratory precision for the method described.

KEYWORDS: Melamine; cyanuric acid; egg; soy; LC−MS/MS

INTRODUCTION

Melamine (MEL), chemically known as 2,4,6-triamino-1,3,5-triazine, is produced in large amounts (1.2 million tons in 2007) (*1*) primarily for use in the synthesis of MEL formaldehyde resins for the production of laminates, plastics, coatings, commercial filters, glues, or adhesives, as well as for dishware and kitchenware (*2*−*4*). MEL has recently become infamous as an adulterant to simulate protein content in food commodities. Because of the high nitrogen content of MEL (66.7%), it is an effective compound to mimic proteins when testing is based on the Kjeldahl method (*5*). The first cases of MEL adulteration were observed in Italy with fish-based meals in the late 1970s (*6*). In 2004 and 2007, MEL was found in pet food, causing renal failure in dogs and cats (*7*−*9*). A major MEL case occurred in 2008 when the media revealed severe kidney damage induced by urinary tract stone formation in Chinese infants fed with infant formulas and other milk powders tainted with MEL (*1*−*10*).

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MEL is considered relatively nontoxic, although chronic administration of high concentrations can induce renal pathology (*11*). Several studies in rat and mouse have been reported using doses up to 18 000 mg of MEL/kg of feed. Most consistent and dose-related effects were the formation of bladder stones and the development of hyperplasia of the bladder epithelium (*12*, *13*). However, the strong affinity between MEL and cyanuric acid (CA, 1,3,5-triazine-2,4,6-triol) for one another was described to form the low-soluble MEL−cyanurate complex through hydrogen bonding, which is considered responsible for kidney stones (*14*, *15*). CA can be produced either as a byproduct during the manufacturing process of MEL or by bacteria-mediated metabolism of MEL (*1*). CA is commonly used as a disinfectant, particularly for the treatment of water (*16*).

Hence, there is a need for effective and reliable methods to monitor MEL and CA in dairy products, as well as in food commodities considered as high-risk materials for MEL- and/or CA-mediated economically motivated adulteration. Egg powder and soy protein are considered as high-risk materials because they are a major source of proteins for the food industry. Several methods have been reported for the determination of MEL (*17*−*19*) or MEL and CA simultaneously (*20*−*22*) in food materials. However, they

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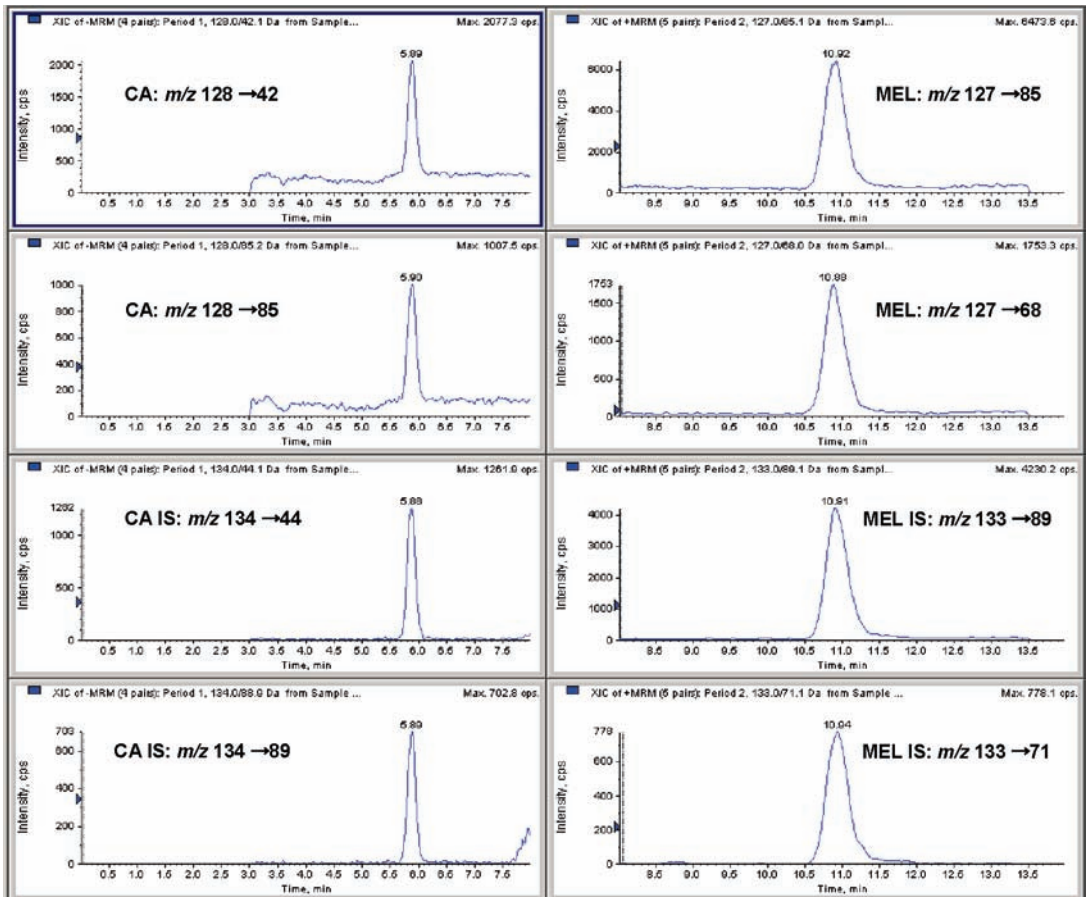

Figure 1. LC−MS/MS chromatogram of MEL and CA from an extract of spiked egg powder. Spiking levels: MEL, 0.1 mg/kg (IS, 0.1 mg/kg); CA, 0.15 mg/kg (IS, 0.1 mg/kg).

do not always provide a sufficient level of performance, in terms of selectivity and sensitivity, to be considered for routine work to demonstrate product compliance with regard to World Health Organization/Food and Agriculture Organization (WHO/FAO) (*1*) and Codex Alimentarius Commission (*23*) recommendations (for MEL, maximum limits at 1 mg/kg in milk-powdered infant formula and 2.5 mg/kg in other foods have been proposed as sufficient margins of safety). To circumvent these limitations, we recently developed liquid chromatography−tandem mass spectrometry (LC−MS/MS) for the simultaneous quantitative determination of MEL and CA in cow's milk and milk-based powder infant formula, designed and validated to accurately quantify MEL and CA at 1 mg/kg in milk-based powder infant formula (*24*). The method was successfully implemented worldwide in Nestlé Quality Assurance Laboratories (NQAL).

The present study reports the results obtained with the above-mentioned LC−MS/MS method (*24*) for the extension to two matrixes, namely, egg powder and soy protein. Single-laboratory validation data as well as interlaboratory test results for these new matrixes are shown and discussed.

MATERIALS AND METHODS

Chemicals and Reagents. MEL (2,4,6-triamino-1,3,5-triazine) and CA (2,4,6-triol-1,3,5-triazine) were obtained from Sigma (Buchs, Switzerland). Their respective isotopically labeled homologues, i.e., ($^{13}C_3$,$^{15}N_3$)-MEL [isotopic purity: $^{13}C_3$, 99%; amino-$^{15}N_3$, 98%; chemical purity, ≥98%] and ($^{13}C_3$,$^{15}N_3$)-CA (isotopic purity: $^{13}C_3$, 99%; $^{15}N_3$, > 98%; chemical purity, 90%), were supplied by Cambridge Isotope Laboratories (Andover, MA). Ammonium acetate, acetonitrile, and LiChrosolv water were from Merck (Darmstadt, Germany).

Standard Solutions. Stock solutions (250 μg/mL) of the unlabeled analytes MEL and CA were prepared separately by dissolving each compound in water by means of an ultrasonic bath for 15 min. Further separate working solutions in water at 100 μg/mL (to prepare the in-house reference materials) and at 20, 2, and 0.2 μg/mL (for analysis) were obtained by successive dilutions. Working standard solutions of the labeled homologues (used as internal standards, ISs) at 20, 2, and 0.2 μg/mL were similarly obtained from 100 μg/mL stock standard solutions available in ready-to-use ampules.

Sample Materials. To prepare in-house reference materials, egg powder and soy protein materials were obtained from a local retailer and were first checked for their absence of MEL and CA. The egg powder was fortified with MEL and CA at levels of 0.1 and 0.15 mg/kg, respectively, while the soy protein was supplemented with MEL and CA at concentration levels of 0.50 and 1.50 mg/kg, respectively, according to the following procedures.

Egg Powder and Soy Protein Material. For both materials, a well-homogenized test portion was weighed (150.0 ± 0.1 g) into a 1000 mL Erlenmeyer flask and suspended in water (340.0 ± 0.1 g for egg powder and 750.0 ± 0.1 g for soy protein).

The resulting slurries of egg powder and soy protein were fortified with 2.0 ± 0.1 and 10.0 ± 0.1 g of the 100 μg/mL unlabeled working standard solution of MEL and with 3.0 ± 0.1 and 30.0 ± 0.1 g of the 100 μg/mL unlabeled working standard solution of CA, respectively. The samples were mixed and freeze-dried overnight. The resulting powder was homogenized for 2 h in a turbula mixer (Willy A. Bachofen AG Maschinenfabrik, Basel, Switzerland) with either 1.850 kg of egg powder or soy protein previously checked to be free from MEL and CA. Concentrations of both analytes in the mixtures of egg powder and soy protein were, for MEL, 0.1 and 0.5 mg/kg, respectively, and, for CA, 0.15 and 1.5 mg/kg, respectively. Portions of the test materials (around 50 g) were packed into foil pouches and stored at room temperature.

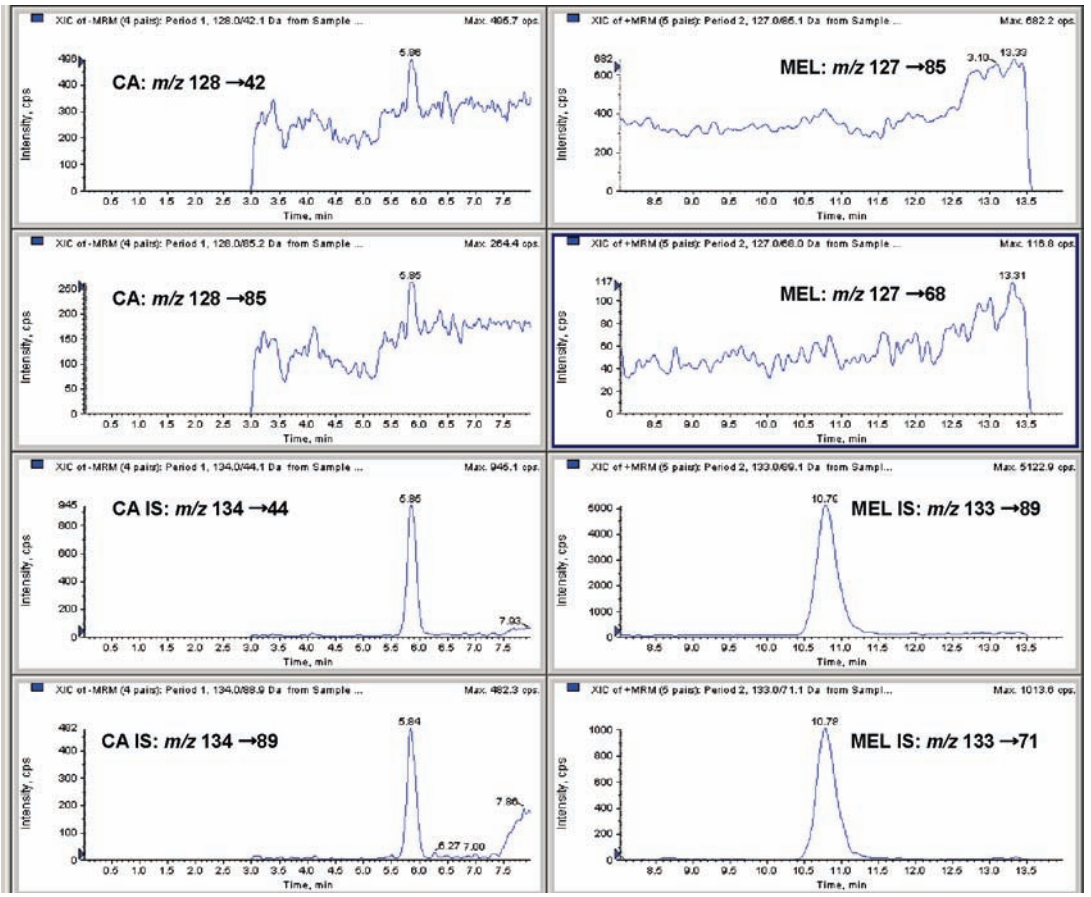

Figure 2. LC−MS/MS chromatogram of MEL and CA from an extract of blank egg powder. Spiking levels: MEL IS, 0.1 mg/kg; CA IS, 0.1 mg/kg.

Analysis. *Sample Preparation*. A well-homogenized test portion (1.0 g) of egg powder or soy protein was weighed into a 50 mL Falcon polypropylene tube and fortified at 0.10 mg/kg with MEL and CA IS working standard solutions (50 μL of an aqueous 2 μg/mL solution) in the case of egg powder or 1.0 mg/kg with MEL and CA IS working solutions (50 μL of an aqueous 20 μg/mL solution) in the case of soy protein. Water (5 mL) and acetonitrile (5 mL) were then added successively, and the resulting slurry was thoroughly mixed after each solvent addition, ensuring that there were no lumps in the sample. The slurry was further diluted with acetonitrile (30 mL) and water (10 mL) and placed onto an automated shaker for 5 min. The tube was then centrifuged at 4000*g* at room temperature for 10 min. The supernatant (ca. 1 mL) was then transferred to a high-performance liquid chromatography (HPLC) vial for further LC−MS/MS analysis.

LC−MS/MS. Detection was performed with a QTrap 4000 LC−MS/MS system (Applied Biosystems, Foster City, CA) equipped with a TurboIonSpray electrospray ionization (ESI) source and an 1100 series HPLC system (Agilent, Geneva, Switzerland). Chromatographic and mass spectrometric parameters of MEL and CA are described by Desmarchelier et al. (*24*).

Quantification. MEL and CA were quantified by means of external calibration curves [analyte/IS area ratio (y) versus analyte/IS concentration ratio (x)] constructed in acetonitrile/water (70:30, v/v) at six calibration levels. These levels ranged for egg powder from 0 to 30 pg injected on column (thus covering a 0−0.3 mg/kg range, concentration equivalent in the sample), with the concentration of ISs fixed at 10 pg injected on column (0.1 mg/kg in sample). For soy protein, 0−200 pg was injected on column (thus covering a 0−2.0 mg/kg range, concentration equivalent in sample), with the concentration of ISs fixed at 100 pg injected (1.0 mg/kg in sample). The linearity of MS responses was checked by calculating the relative standard deviation of repeatability (RSD_r) of the average of response factors (RFs, y/x), which should be below 15% (*25*).

Validation. *Limit of Detection* (*LOD*) *and Limit of Quantification* (*LOQ*). Values for LOD and LOQ for MEL and CA were determined on the basis of the blank egg powder and soy protein materials. LOD and LOQ were defined when a chromatographic peak producing a signal-to-noise (S/N) ratio > 3 and > 10 was found, respectively, for the transition reaction used for quantification.

Recovery. Recoveries were calculated by comparing the several CA and MEL concentrations measured with the reference amounts added to the blank egg powder and soy protein matrixes.

Precision. Within-laboratory precision was tested by analyzing six portions of fortified egg powder and soy protein for their CA and MEL concentrations. From these data, the RSD_r values were calculated. Between-laboratory precision was tested by sending a blank egg powder, the fortified egg powder, and the fortified soy protein for analysis to five NQAL worldwide and one external laboratory. In addition, analyses were carried out at the Nestlé Research Centre in Switzerland. Laboratories were asked to analyze samples in duplicate. This study was conducted between September and October 2009. From these data, the relative standard deviations of reproducibility (RSD_R) were calculated.

Horwitz Ratio (*HorRat*). The predicted reproducibility relative standard deviation ($PRSD_R$) for the levels analyzed was calculated according to the Horwitz formula: $PRSD_R = 2C^{-0.15}$, where C is expressed as a mass fraction. The ratio of the RSD_R calculated from the data to the $PRSD_R$ calculated from the Horwitz formula results in the HorRat value: HorRat = $RSD_R/PRSD_R$. In 1980, Horwitz et al. published an evaluation of 1000 interlaboratory comparisons (*26*). From these studies, it was concluded that a HorRat value of 1, with limits of acceptability between 0.5 and 2.0, indicates satisfactory interlaboratory precision. The corresponding within-laboratory relative standard deviations were found to be typically one-half to two-thirds the among-laboratory relative standard deviations. Consistent deviations from the ratio on the low side (values < 0.3 or 0.5) may indicate unreported averaging or excellent training and experience (*27*, *28*).

RESULTS AND DISCUSSION

The method described in this paper was originally developed for the simultaneous quantitative determination of MEL and CA

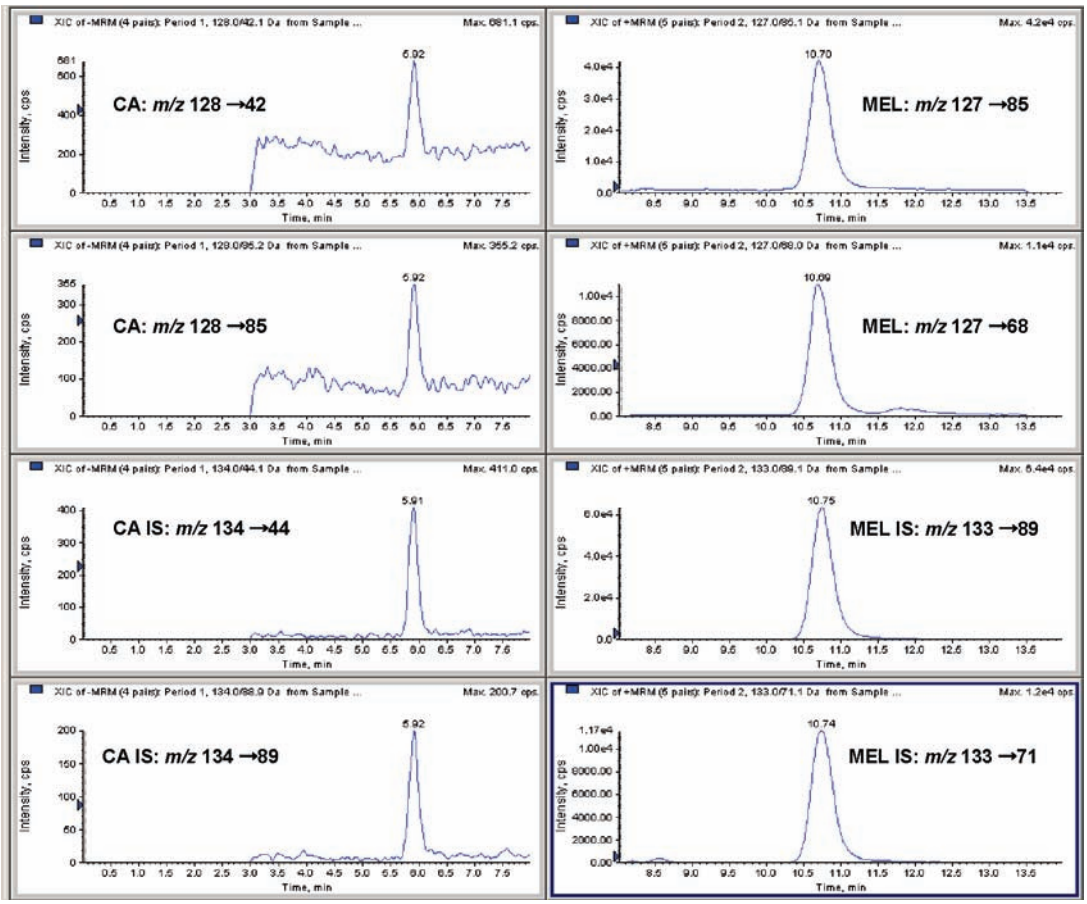

Figure 3. LC−MS/MS chromatogram of MEL and CA from an extract of spiked soy protein. Spiking levels: MEL, 0.5 mg/kg (IS, 1 mg/kg); CA, 1.5 mg/kg (IS, 1 mg/kg).

in cow's milk and milk-based infant formula (*24*), with the ultimate goal to both prevent potential adulteration in cow's milk and demonstrate the compliance of infant formulas with respect to the safety limit recommended by the World Health Organization (WHO) at 1 mg/kg (for MEL). For both matrices, the LOQ was found to be 0.05 mg/kg for MEL and 0.1 mg/kg in the case of CA. Excellent intermediate reproducibility was found for MEL within a 3.8−14.7% range, while an acceptable range (6.4−31.2%) was obtained for CA. When full validation had been completed, the method was rolled out in several NQALs worldwide to control products on a routine basis. The NQALs have extended the scope of application of the method to be able to determine MEL and CA in various food materials. The method was shown to be robust and versatile, as evidenced by the number of analyses. Since MEL was discovered in milk powders and infant formulas in September 2008, about 18 000 analyses have been carried out in various food categories (mainly dairy products, eggs, meat, seafood, cereals, fruits, vegetables, and seeds). The chromatographic profiles obtained in the frame of the current study with egg powder and soy protein show that the method is applicable for nondairy food materials as well (**Figures 1−3**).

The results reported in **Table 1** demonstrate that both MEL and CA can be quantitatively determined in egg powder and soy protein by isotope-dilution LC−MS/MS with appropriate performance. Recoveries were found in the range of 97−113%. An acceptable recovery requirement as function of the concentration determined ranges between 80 and 110% according to Codex Alimentarius Commission guidelines for single-laboratory validation (*29*). RSD_r values were less than 10%, which are acceptable because $HorRat_r$ values ranged between 0.1 and 0.5. Good performance was also obtained under reproducibility conditions, with seven laboratories involved in the analysis of the samples. MEL and CA were not detected by the participants in the blank egg powder sample, while the two analytes were quantified in the spiked egg powder and soy proteins samples (**Table 2**). Only laboratory 7 was not able to quantify CA in the soy protein matrix. They indicated that recovery of the IS was not possible. With regard to the precision, RSD_r was found below 7%, and RSD_R was less than 12%; these values, for which the RSD_r is comparable to the RSD_r obtained under single-laboratory conditions, demonstrate the good precision of the method. This is confirmed by acceptable HorRat values, which range between 0.4 and 0.7 (**Table 3**).

Table 1. Single-Laboratory Repeatability (Within-Laboratory Precision) Data for MEL and CA in Egg Powder and Soy Protein

			egg powder		soy protein	
		n	MEL	CA	MEL	CA
LOD[a]	mg/kg		0.02	0.05	0.02	1.0
LOQ[b]	mg/kg		0.05	0.10	0.05	1.50
fortification level	mg/kg		0.10	0.15	0.50	1.50
median	mg/kg	6	0.111	0.169	0.485	1.500
recovery	%	6	111	113	97	100
SD_r[c]	mg/kg		0.009	0.005	0.012	0.107
RSD_r[d]	%		8.5	2.9	2.5	7.2
$HorRat_r$[e]			0.4	0.1	0.1	0.5

[a] LOD = limit of detection. [b] LOQ = limit of quantification. [c] SD_r = standard deviation of repeatability. [d] RSD_r = repeatability relative standard deviation. [e] $HorRat_r$ = ratio of the within-laboratory repeatability relative standard deviation calculated from the data to the predicted reproducibility relative standard deviation (*27*, *28*).

Table 2. Between-Laboratory Study Results for MEL and CA in Egg Powder and Soy Protein

	egg powder				soy protein			
	MEL		CA		MEL		CA	
lab number	replicate 1	replicate 2	replicate 1	replicate 2	replicate 1	replicate 2	replicate 1	replicate 2
1	0.102	0.100	0.159	0.153	0.444	0.436	1.480	1.500
2	0.115	0.115	0.170	0.187	0.478	0.493	1.471	1.408
3	0.100	0.100	0.170	0.210	0.440	0.420	1.470	1.570
4	0.095	0.096	0.177	0.186	0.492	0.500	1.540	1.570
5	0.092	0.093	0.192	0.180	0.509	0.457	1.630	1.540
6	0.068	0.069	0.192	0.207	0.429	0.411	1.280	1.830
7	0.095	0.095	0.144	0.133	0.448	0.451	*a*	*a*

[a] Not quantified.

Table 3. Between-Laboratory Performance Data for MEL and CA in Egg Powder and Soy Protein

			egg powder		soy protein	
		n	MEL	CA	MEL	CA
fortification level	mg/kg		0.10	0.15	0.50	1.50
median	mg/kg	7[a]	0.096	0.182	0.450	1.538
SD_r[b]	mg/kg		0.001	0.013	0.016	0.080
RSD_r[c]	%		1.1	6.9	3.5	5.2
SD_R[d]	mg/kg		0.009	0.021	0.047	0.082
RSD_R[e]	%		9.1	11.7	10.4	5.4
HorRat[f]			0.4	0.7	0.6	0.4

[a] CA medians for soy protein are based on six values. [b] SD_r = standard deviation of repeatability. [c] RSD_r = repeatability relative standard deviation. [d] SD_R = standard deviation of reproducibility. [e] RSD_R = reproducibility relative standard deviation. [f] HorRat = ratio of the reproducibility relative standard deviation calculated from the data to the predicted reproducibility relative standard deviation (*28*).

In conclusion, the performance criteria of the method described demonstrate that the method is fit for the purpose to analyze ME and CA in egg powder and soy protein at suitable levels to enforce safety limits as recommended by the WHO.

LITERATURE CITED

(1) World Health Organization/Food and Agriculture Organization (WHO/FAO) of the United Nations. *Expert Meeting To Review Toxicological Aspects of Melamine and Cyanuric Acid*; WHO/FAO: Geneva, Switzerland, **2008**; pp 1−10.

(2) Martin, R. E.; Hizo, C. B.; Ong, A. M.; Alba, O. M.; Ishiwata, H. Release of formaldehyde and melamine from melamine tableware manufactured in the Philippines. *J. Food Prot.* **1992**, *55*, 632−635.

(3) Bradley, E. L.; Boughflower, T. L.; Smith, D. R.; Speck, D. R.; Castle, L. Survey of the migration of melamine and formaldehyde from melamine food contact articles available on the UK market. *Food Addit. Contam.* **2005**, *22*, 597−606.

(4) Lund, K. H.; Petersen, J. H. Migration of formaldehyde and melamine monomers from kitchen and tableware made of melamine plastic. *Food Addit. Contam.* **2006**, *23*, 948−955.

(5) Lynch, J. M.; Barbano, D. M. Kjeldahl nitrogen analysis as a reference method for protein determination in dairy products. *J. AOAC Int.* **1999**, *82*, 1389−1398.

(6) Cattaneo, P.; Cantoni, C. On the presence of melamine in fish meals. *Tec. Molitoria* **1982**, *33*, 17−18.

(7) 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.

(8) Burns, K. Recall of pet food. Witnesses at congressional hearing talk about timing, imports, and surveillance. *J. Am. Vet. Med. Assoc.* **2007**, *230*, 1601−1602.

(9) Dobson, R. L.; Motlagh, S.; Quijano, M.; Cambron, R. T.; Baker, T. R.; Pullen, A. M.; Regg, B. T.; Bigalow-Kern, A. S.; Vennard, T.; Fix, A.; Reimschuessel, R.; Overmann, G.; Shan, Y.; Daston, G. P. Identification and characterization of toxicity of contaminants in pet food leading to an outbreak of renal toxicity in cats and dogs. *Toxicol. Sci.* **2008**, *106*, 251−262.

(10) Ingelfinger, J. R. Melamine and the global implications of food contamination. *N. Engl. J. Med.* **2008**, *359*, 2745−2748.

(11) Cianciolo, R. E.; Bischoff, K.; Ebel, J. G.; Van Winkle, T. J.; Goldstein, R. E.; Serfilippi, L. M. Clinicopathologic, histologic, and toxicologic findings in 70 cats inadvertently exposed to pet food contaminated with melamine and cyanuric acid. *J. Am. Vet. Med. Assoc.* **2008**, *233*, 729−737.

(12) Heck, H. D. A.; Tyl, R. W. The induction of bladder stones by terephthalic acid, dimethly terephthalate, and melamine (2,4,6-triamino-1,3,5-triazine) and its relevance to risk assessment. *Regul. Toxicol. Pharmacol.* **1985**, *5*, 294−313.

(13) National Toxicological Program (NTP). *Carcinogenesis Bioassay of Melamine* (*CAS No. 108-78-1*) *in F344/N Rats and B6C3F1 Mice* (*Feed Study*); NTP: Research Triangle Park, NC, **1983**.

(14) Ranganathan, A.; Pedireddi, V. R.; Rao, C. N. R. Hydrothermal synthesis of organic channel structures: 1:1 hydrogen-bonded adducts of melamine with cyanuric acid and trithiocyanuric acids. *J. Am. Chem. Soc.* **1999**, *121*, 1752−1753.

(15) 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.

(16) Cantú, 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) Ishiwata, H.; Inoue, T.; Yamazaki, T.; Yoshihira, K. Liquid chromatographic determination of melamine in beverages. *J. Assoc. Off. Anal. Chem.* **1987**, *70*, 457−460.

(18) Wu, Q. Q.; Fan, K. X.; Sha, W.; Ruan, H. Q.; Zeng, R.; Shieh, C. H. Highly sensitive detection of melamine based on reversed phase liquid chromatography mass spectrometry. *Chin. Bull. Sci.* **2009**, *54*, 732−737.

(19) Yang, S.; Ding, J.; Zheng, J.; Hu, B.; Li, J.; Chen, H.; Zhou, Z.; Qiao, X. Detection of melamine in milk products by surface desorption atmospheric pressure chemical ionization mass spectrometry. *Anal. Chem.* **2009**, *81*, 2426−2436.

(20) Ehling, S.; Tefera, S.; Ho, I. P. High-performance liquid chromatography method for the simultaneous detection of the adulteration of cereals flours with melamine and related triazine by-products ammeline, ammelide, and cyanuric acid. *Food Addit. Contam.* **2007**, *24*, 1319−1325.

(21) Andersen, W. C.; Turnispeed, S. B.; Karbiwnyk, C. M.; Clark, S. B.; Madson, M. R.; Gieseker, C. M.; Miller, R. A.; Rummel, N. G.; Reimschuessel, R. Determination and confirmation of melamine residues in catfish, trout, tilapia, salmon, and shrimp by liquid chromatography with tandem mass spectrometry. *J. Agric. Food Chem.* **2008**, *56*, 4340−4347.

(22) He, L.; Liu, Y.; Lin, M.; Awika, J.; Ledoux, D. R.; Li, H.; Mustapha, A. A new approach to measure melamine, cyanuric acid, and melamine cyanurate using surface enhanced Raman spectroscopy coupled with gold nanosubstrates. *Sens. Instrum. Food Qual.* **2008**, *2*, 66−71.

(23) World Health Organization/Food and Agriculture Organization (WHO/FAO) of the United Nations. *Report of the 3rd Session of the Codex Committee of Contaminants in Foods*; Joint FAO/WHO

Food Standards Programme, Codex Alimentarius Commission: Rome, Italy, **2009**; pp 1−92.
(24) Desmarchelier, A.; Guillamon Cuadra, M.; Delatour, T.; Mottier, P. Simultaneous quantitative determination of melamine and cyanuric acid in cow's milk and milk-based infant formula by liquid chromatography−electrospray ionization tandem mass spectrometry. *J. Agric. Food Chem.* **2009**, *57*, 7186−7193.
(25) Rodriguez, M.; Orescan, D. B. Confirmation and quantitation of selected sulfonylurea, imidazolinone, and sulfonamide herbicides in surface water using electrospray LC/MS. *Anal. Chem.* **1998**, *70*, 2710−2717.
(26) Horwitz, W.; Kamps, L. R.; Boyer, K. W. Quality assurance in the analysis of foods and trace constituents. *J. AOAC Int.* **1980**, *63*, 1344−1354.
(27) Horwitz, W.; Albert, R. The Horwitz ratio (HorRat): A useful index for method performance with respect to precision. *J. AOAC. Int.* **2006**, *89* (4), 1095−1109.
(28) Definitions and calculations of HorRat values from interlaboratory data. www.AOAC.org (accessed on Jan 18, 2004).
(29) World Health Organization/Food and Agriculture Organization (WHO/FAO) of the United Nations. General criteria for the selection of methods of analysis. *Procedural Manual*; 19th ed.; Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission: Rome, Italy, 2010; p 53.

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