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Investigation of the Cyanuric Acid Contamination Caused by Acetonitrile and Alkali Detergent for the LC-ESI MS/MS Analysis

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Abstract During the replicate analysis of melamine and cyanuric acid by LC-ESI MS/MS for the method development, recurring cyanuric acid contamination was observed. The high concentration (6,950 mg/kg) of cyanuric acid was found in the strong alkali detergent used in a glassware washing machine. However, only small concentration of cyanuric acid (0.002–0.004 mg/kg) was detected in the glassware washed with the above alkali detergent. The major contamination source of cyanuric acid was some specific lot of acetonitrile (0.093 mg/kg) that increased the background level of the ion chromatogram.

Keywords Cyanuric acid · LC-ESI MS/MS · Contamination · Acetonitrile · Alkali detergent

The melamine (MEL) contamination crisis of milk in China has had such an impact on world-wide food safety concerns. Melamine is a chemical compound that has a number of industrial uses, including the production of laminates, glues, dinnerware, adhesives, molding compounds, coatings and flame retardants (WHO 2008a). Melamine is gradually hydrolyzed to ammeline (AMN), ammelide (AML), and cyanuric acid (CYA) under acidic conditions. MEL and CYA are absorbed into the bloodstream, concentrate and interact in the urine-filled renal microtubules. Then, they crystallize and form round, yellow crystals, MEL-cyanurate, which in turn block and damage the renal cell that lines the tubes, causing the kidneys to malfunction (Sugita et al. 1990; Dobson et al. 2008). MEL's co-exposure with CYA can induce acute MEL-cyanurate crystal nephropathy, which can lead to renal failure at much lower doses than either compound was ingested alone (WHO 2008b). Several methods have been developed for the analysis of MEL and related compounds (Michael et al. 2008). This study was performed by highly sensitive method for characterization and quantification of MEL and CYA adequately resolved in a run time of only 3 min. MEL and CYA in foodstuffs were analyzed by using ultra-performance liquid chromatograph (UPLC)—electrospray ionization (ESI) MS/MS in multiple reactions monitoring (MRM) mode by the modified method of US FDA (LIB No. 4422) (Michael and Alexander 2008). During the replicate analysis of MEL and CYA for the method development, recurring CYA contamination was observed even in blank samples. Contamination, carryover, or blank response from matrix or reagents can affect the accuracy and precision of quantitation at all concentrations (Viswanathan et al. 2007). Especially the carryover is a major problem that can influence the accuracy and precision of high performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS), and liquid chromatography-tandem mass spectrometry (LC-MS/MS) bioanalysis, with the consequence being more pronounced at lower concentrations (Hughes et al. 2007). This study reports the carryover (and/or contamination) factors to increase CYA background level during the LC-ESI MS/MS analysis. They were provoked by the cleaning detergent and the poor quality HPLC grade acetonitrile.

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Materials and Methods

De-ionized water with 18.2 MΩ-cm resistivity was produced by Millipore (Molsheim, France) equipment. All acetonitrile were of HPLC grade. Nylon filter (0.22 μm pore size) was from Millipore (Bedford. MA, USA). All HPLC mobile phase solvents were filtered through 0.22 μm Millipore nylon filters (Bedford, MA, USA). CYA (98% purity) was purchased from Sigma–Aldrich (St Louis, MO, USA). CYA standard solution was prepared in 50% (v/v) acetonitrile to make a 1 mg/mL stock solution that was further diluted to give a serial of working standard solutions. Five-point calibration curves of CYA was prepared at 1, 5, 10, 25 and 50 μg/L. The correlation value of the CYA standard curve was 0.9979.

Samples were collected from the acetonitrile, detergents and glassware of affected cleaning procedure. Acetonitrile samples were taken from the each bottle of 1 gallon HPLC grade acetonitrile of Merck (Lot No. I481030, Germany), Burdick & Jackson (Two lots such as Lot No. J4UM1H and J1OA1H, Korea) and Duksan (Lot No. 96G311, Korea). The alkali detergents of Regomat 6AL-P (Lot No. UBA07150281, Remsgold, Germany) and Extran MA01 (Lot No. 107555, Merck, Germany) and acid detergent of Regomat AC-L (Lot No. MHD-F, Remsgold, Germany) were diluted 2,500 times with acetonitrile/water (1/1) and filtered before the analysis. The 50 mL of 50% acetonitrile was added to 0.2 g detergent and stirred for 10 min. After 1 mL of the resulting mixture was transferred into separate tube, 9 mL of 50% acetonitrile was added and stirred for 1 min. The collected samples such as acetonitrile and diluted detergents were analyzed after filtration without any further treatment. For the sampling of CYA from the glassware washed with alkali detergent, 500 mL of 50% acetonitrile was added to the dried laboratory glassware (500 mL bottle) after washing with the glassware washing machine LA180 (Bellimed, Germany) using the alkali detergent (Regomat 6AL-P) and sonicated (or not) for 10 min. All samples were filtered through 0.22 μm Millipore nylon filter (Bedford. MA, USA). Then, they went into a small volume autosampler vial for the analysis by LC-MS/MS.

A UPLC (Waters, Milford, MA) coupled with a model Quattro Premier tandem mass spectrometer (Micromass, Manchester, UK) was used. A pneumatically assisted Z-spray ESI ion source in the negative ionization mode was adopted. Mass spectrometer conditions were optimized by injecting CYA standard solution (100 ng/mL, diluted in 50% acetonitrile) via UPLC into the MS. The analytical column was 150 mm × 2.1 mm × 1.7 μm BEH Hillic (Waters, Milford, MA) at a column temperature of 35°C. The injection volume was 4 μL. The mobile phase consisted of: (A) 95% acetonitrile (B) water at a flow rate of 0.5 mL/min. Gradient elution was utilized, with the initial mobile phase at 100% A, ramped for 100% B at 2 min, at which time it was ramped back to the initial conditions and held for 2 min to re-equilibrate the column. Mass spectral data were acquired by using the MRM scan function. The optimized instrument settings were: capillary voltage 3.8 kV, cone voltage 22 V, source temperature 150°C, desolvation temperature 400°C, desolvation gas 800 L/h, cone gas flow 50 L/h, collision pressure 4.23×10^{-3} mbar, collision energy 20 V. The MRM transition for CYA was *m/z* 128 → 85, 42 under the negative ion mode. The MassLynx 4.1 (Micromass) software was used for data processing.

Results and Discussion

MEL and CYA in foodstuffs were analyzed through LC-ESI MS/MS in multiple reactions monitoring mode. The mass spectrum revealed a peak of CYA at *m/z* 128 corresponding to $[M-H]^-$, and its product ions *m/z* 85 and 42 were selected for qualitative analysis. The mass transition of *m/z* 128 → 85 was used for the quantification.

Previous works done by other investigators had demonstrated that MEL contamination in the blank analyses were observed, resulting from the carryover effect and the interference peaks in blank analyses that might come from environmental background levels of MEL from pollution (Arcement and Levy 1998; Lim et al. 1990). In the replicate analysis of MEL and CYA for the method development, a suspicious peak having same mass spectrometric property and retention time (0.73 min) with CYA was observed in the blank samples constantly. Finally the suspicious peak was confirmed as CYA with co-injection of the CYA standard. Carryover and/or contamination from the chromatographic system can be caused by residues of a previously injected sample that are absorbed on, or trapped within the autosampler and column. It does not necessarily involve only the next sample in the sequence and can affect several samples in a sequence. The risk of cross-contamination is also very high during the vigorous mixing of organic solvents, supernatant transfer, and evaporation steps for liquid–liquid extraction (Hughes et al. 2007). However, if the chromatographic peak caused by the carryover and/or contamination of the target material constantly appeared in all chromatographic running, it might came from other sources such as the solvent used for the sample preparation and the mobile phase for the liquid chromatography and the glassware constantly contaminated with the target compounds. CYA (1,3,5-triazine-2,4,6-triol) is a chemical compound with the formula $(CNOH)_3$. This white, odorless solid finds use as a precursor or a component of bleaches, disinfectants, and

herbicides. In 1997, worldwide production was 160 million kilograms (LRC Ltd 2009). A variety of the chlorinated CYA may be used in the field of sanitation by themselves or formulated into various products. The carefully formulated products based on chloroisocyanurates may be used as (a) household and commercial laundry dry bleaches, (b) machine dishwashing compounds, (c) scouring powders, and (d) industrial sanitizing compounds (food, dairy, restaurant, swimming pool). When chloroisocyanurates are added to aqueous solution, they hydrolyze to form HOCl and CYA (Block 2001). Therefore, a strong suspect might be a detergent used for the glassware washing machine. And the other strong candidate for the CYA contamination might be acetonitrile used for the mobile phase. The blank baseline of LC-ESI MS/MS fluctuated a lot depending on the solvent proportion of the gradient elution system. The baseline was quite high until 0.4 min that is the position of the highest acetonitrile concentration in the mobile phase and it started to drop down until 1.8 min that is almost the close position of the lowest acetonitrile concentration. Additionally the baseline returned back to original highest position with increasing the proportion of acetonitrile in the mobile phase (Fig. 1).

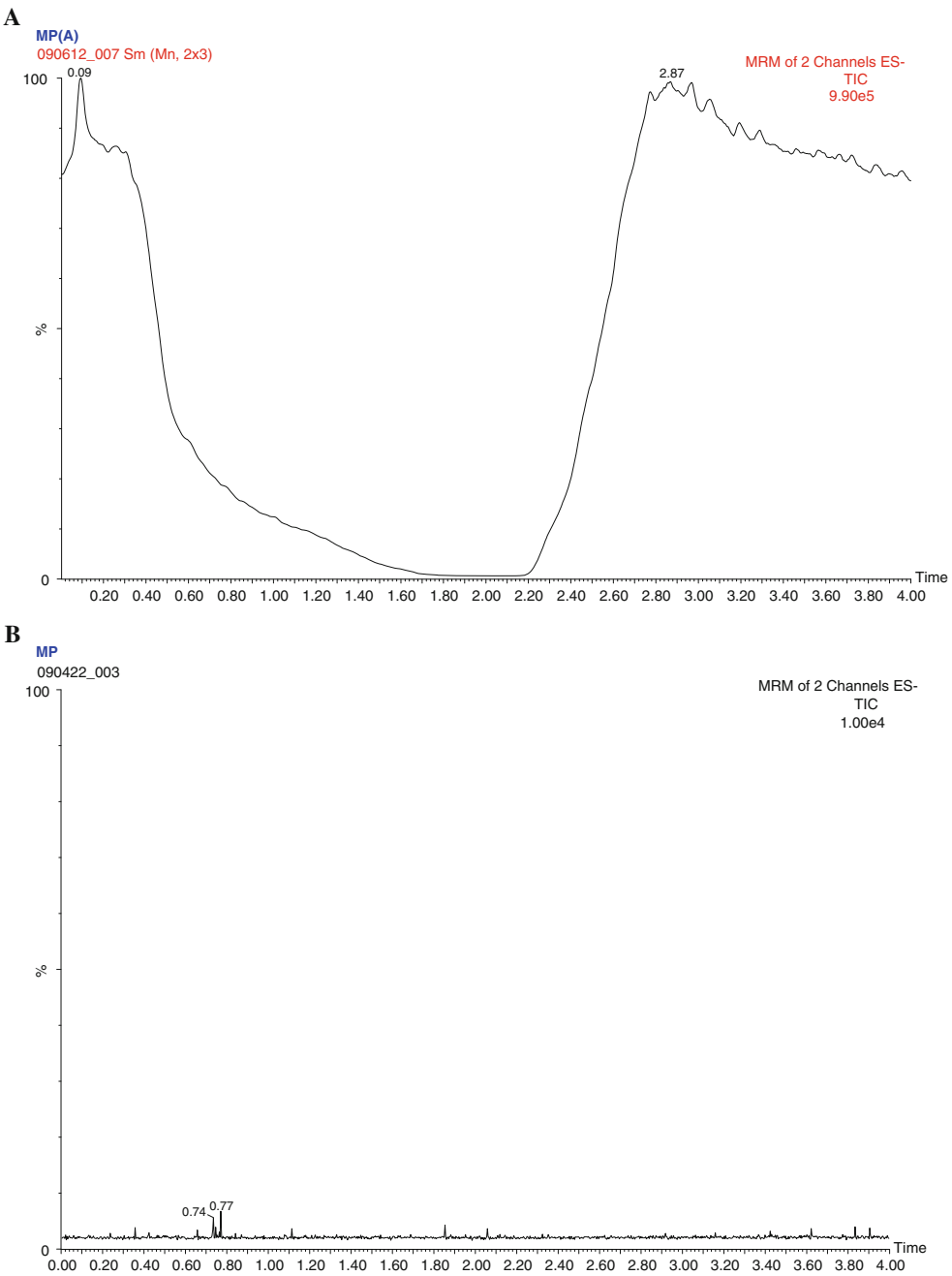

Fig. 1 The ion chromatogram monitored for cyanuric acid with the mobile phase including **a** the contaminated acetonitrile (Lot No. J10A1H, Burdick & Jackson, Korea) and **b** the clean acetonitrile (Lot. No. 96G311, Duksan, Korea)

Table 1 Cyanuric acid concentration detected in the suspects of the cyanuric acid contamination

Sample	Cyanuric acid (mg/kg)
Alkali detergent (Regomat 6AL-P, Remsgold, Germany, powder)	6,950
Alkali detergent (Extran MA 01, Merck, Germany, liquid)	N.D.*
Acid detergent (Regomat AC-L, Remsgold, Germany,liquid)	N.D.
Acetonitrile (Lot No. J10A1H, Burdick & Jackson, Korea)	0.093
Acetonitrile (Lot No. J4UM1H, Burdick & Jackson, Korea)	N.D.
Acetonitrile (Lot No. 107555, Merck, Germany)	N.D.
Acetonitrile (Lot. No. 96G311, Duksan, Korea)	N.D.
50% acetonitrile washed solution of the glassware**	0.002
50% acetonitrile sonicated of the glassware**	0.004

* N.D. means that cyanuric acid was not detected over LOQ

** The glassware was washed with the alkali detergent (Regomat 6AL-P)

Therefore it was possible that the acetonitile used for the mobile phase might be severely contaminated with CYA. To check out the potent contamination (and/or carryover) sources of CYA, the following samples were analyzed for CYA: the four acetonitrile (the one used for mobile phase and the three from different producers), the three detergents (two alkali and one acidic detergent) and the two washed solutions of glassware (after washing them with the alkali detergent 6 AL-P UBA07150281).

CYA was detected in an Alkali detergent (Regomat 6AL-P) as a very high concentration (6,950 mg/kg). A chloroisocyanurate might be used as an ingredient of the detergent and it must be hydrolyzed to CYA when the detergent was mixed with water. Also CYA was found in the specific lot of acetonitrile (Lot J10A1H, Burdick & Jackson, Korea) (0.093 mg/kg) and the glassware washed with the detergent (Regomat 6AL-P) (0.002–0.004 mg/kg) not like other samples where no CYA was detected (LOQ 1 μg/kg). Among the two acetonitriles having the different lot numbers from one producer, only one lot was contaminated with CYA. That means the more than one of the processing steps to generate the HPLC grade acetonitrile may have a high potential for the CYA contamination. No MEL, ammeline and ammelide were routinely detected in the blank analyses. A summary of these results is presented in Table 1. No other contamination and/or carryover source of CYA was found during the sample preparation and instrumental analysis.

After confirming the contamination (and/or carryover) sources of CYA, the detergent and acetonitrile was replaced by the pre-checked clean stuffs. After removing the contamination sources of CYA during the whole analysis procedure, no CYA peak was detected and the LC-ESI MS/MS baseline was stabilized without high fluctuation depending on the gradient elution of mobile phase (Fig. 1). Carryover and contamination can affect both the accuracy and precision of a method. Therefore, it should be investigated and minimized or eliminated during method development, assessed during method validation, and monitored routinely in study sample analysis. Authors recommend the analyst to check the LC-MS mobile phase solvent purity before using even from the same producer of the solvent that was checked before. And it might be required to get the information of the detergent ingredients which may include the chlorinated cyanurates. When unexpected occurrences of carryover and contamination do occur, the analytical scientist must interpret the impact on the results and carry out appropriate and corrective actions to eliminate further occurrences.

References

Arcement BP, Levy NH (1998) Liquid chromatographic determination of triamino-s-triazine in fertilizer mixes: collaborative study. J Assoc Off Anal Chem 71:611–613

Block SS (ed) (2001) Disinfection, sterilization, and preservation. Lippincott Williams & Wilkins, Philadelphia

Dobson RLM, Motlagh S, Quijano M, Cambron RT, Baker TR, Pullen AM, Regg BT, Bigalow-Kern AS, Vennard T, Fix A, Reimschuessel R, Overmann G, Shan Y, Daston GP (2008) Identification and characterization of toxicity of contaminants in pet food leading to an outbreak of renal toxicity in cats and dogs. Toxicol Sci 106:251–262

Hughes NC, Wong EY, Fan J, Bajaj N (2007) Determination of carryover and contamination for mass spectrometry-based chromatographic assays. AAPS J. doi:10.1208/aapsj0903042

Lim LO, Scherer SJ, Shuler KD, Toth JP (1990) Disposition of cyromazine in plants under environmental conditions. J Agric Food Chem 38:860–865

LRC Ltd (Liaocheng RongHeng Chemicals Ltd.) (2009) Cyanuric acid (CA), http://www.bikudo.com/buy/details/20645/cyanuric_acid_ca.html#desc. Accessed 18 May 2010

Michael S, Alexander JK (2008) Interim Method for Determination of Melamine and Cyanuric Acid Residues In Foods using LC-MS/MS: Version 1.0, U.S. FDA LIB 4422 http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/DrugChemicalResiduesMethodology/ucm071673.htm. Accessed 18 May 2010

Michael SF, Birgit P, Linda SA, Robert HP (2008) Diagnostic determination of melamine and related compounds in kidney tissue by liquid chromatography/tandem mass spectrometry. J Agric Food Chem 56:7593–7599

Sugita T, Ishiwata H, Yoshihira K, Maekawa A (1990) Determination of melamine and three hydrolytic products by liquid chromatography. Bull Environ Contam Toxicol 44:567–571

Viswanathan CT, Bansal S,Booth B, DeStefano AJ, Rose MJ, Sailstad J, Shah VP, Skelly JP, Swann PG, Weiner R (2007) Quantitative

bioanalytical methods validation and implementation: best practices for chromatographic and ligand binding assays. AAPS J. doi:10.1208/aapsj0901004

WHO (2008a) Expert meeting to review toxicological aspects of melamine and cyanuric acid, held in collaboration with FAO and supported by Health Canada, Ottawa, Canada, 1–4 December. http://www.who.int/foodsafety/fs_management/infosan_events/en/index1.html. Accessed 18 May 2010

WHO (2008b) Melamine and cyanuric acid: toxicity, preliminary risk assessment and guidance on levels in food, World Health Organization (WHO), Geneva, 25 Sept., Updated 30 October 2008. http://www.who.int/foodsafety/fs_management/Melamine.pdf. Accessed 18 May 2010