Journal of Food and Drug Analysis, Vol. 21, No. 1, 2013, Pages 66-72 doi:10.6227/jfda.2013210108

Simultaneous Determination of Melamine, Ammelide, Ammeline and Cyanuric Acid in Milk Products by Micellar Electrokinetic Chromatography

NARUMOL VACHIRAPATAMA* AND SUTTINEE MAITRESORASUN

Department of Chemistry, Faculty of Science and Technology, Thammasat University, Rangsit, Pathumthani, 12121, Thailand

(Received: May 12, 2012; Accepted: October 3, 2012)

ABSTRACT

Micellar electrokinetic chromatography (MEKC) was developed for the simultaneous determination of melamine, ammeline, ammelide and cyanuric acid on a fused silica column coated with poly (diallyldimethylammonium chloride). Separation was performed using 5 mM boric acid, 5 mM sodium tetraborate, 10 mM sodium dodecylsulfate and 2 mM tetrabutylammonium hydroxide at pH 9.15 as the background electrolyte, an applied voltage of +20 kV and UV detection at 210 nm. The four compounds were completely separated within 5.2 min. The detection limits of melamine, ammeline, ammelide and cyanuric acid were 0.10, 0.04, 0.03 and 0.08 μg/mL, respectively. The relative standard deviations of retention times for melamine, ammeline, ammelide and cyanuric acid were 0.14, 0.29, 0.05 and 0.12%, and those of peak areas were 0.11, 0.36, 0.28 and 0.24%, respectively. The method was successfully applied to determine melamine and its derivative in milk samples. Before analysis, protein in milk sample was removed using HCl and the remaining matrix was cleaned up using Sep-Pak C18, which otherwise caused electrophoretic de-stacking. The results showed that melamine and cyanuric acid were found in UHT milk samples.

Key words: melamine, ammelide, ammeline, cyanuric acid, milk products, capillary electrophoresis

INTRODUCTION

Melamine by itself is non-toxic in low doses, but when combined with cyanuric acid, it can cause fatal kidney stones due to the formation of insoluble melamine cyanurate (Figure 1)[1]. The addition of melamine to increase the total nitrogen concentration in food products can increase the apparent protein content measured by Kjeldahl nitrogen analysis. In 2007, the U.S. Food and Drug Administration (USFDA) revealed that melamine and its derivatives (ammeline, ammelide, cyanuric acid) were present in pet food, and crystalline precipitates formed in the kidney caused an outbreak of deaths of pet animals[2,3]. In September 2008, melamine-tainted infant-milk caused more than 54,000 young Chinese children to suffer from renal failure[4].

Many methods for sample preparation and detection of melamine in dairy products have been published, including methods based on capillary electrophoresis (CE)[5-15], liquid chromatography with ultraviolet detection (HPLC)[16-19], liquid chromatography/tandem mass spectrometry (LC-MS)[7,20-28] and gas chromatography/ mass spectrometry (GC-MS)[29-32]. All these methods require sample pretreatments such as extraction, preconcentration and derivatization. Some methods (MEKC[6], CZE[7], HPLC[16,17,19], LC-MS[22] and GC-MS[30]) have been developed for the simultaneous determination of melamine, ammeline, ammelide and cyanuric acid. CE has advantages of high separation efficiency and low consumption of chemicals. However, matrix in milk samples has to be removed before injection into the CE, which otherwise would cause electrophoretic de-stacking. At present, there is no CE method for the analysis of melamine and cyanuric acid in milk samples compared to GC-MS[33].

The aim of this work was to develop a simple and fast method for the simultaneous determination of melamine and its derivatives by capillary electrophoresis. The developed method was applied to determine melamine and its derivatives in ultra high temperature (UHT) milk and milk wafers.

MATERIALS AND METHODS

I. *Chemicals and Samples*

Ammeline (97%) and ammelide (99%) were purchased

* Author for correspondence. Tel and Fax: +66-85-1510481; E-mail: nvachira@yahoo.com

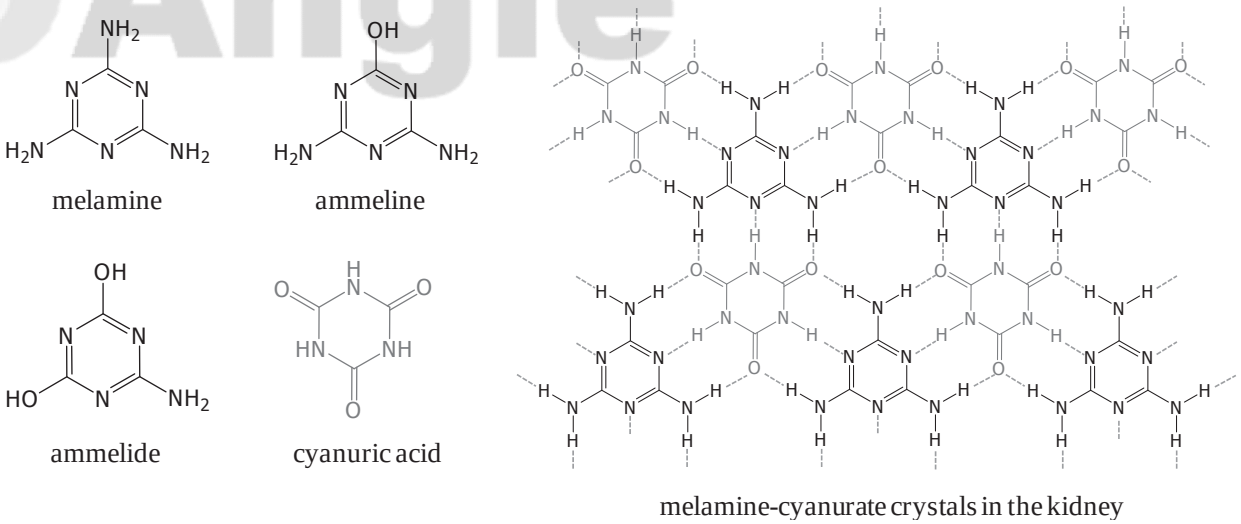

Figure 1. The chemical structures of melamine (MM), ammeline (AMN), ammelide (AMD), cyanuric acid (CA) and melamine cyanurate

from Dr. Ehrenstorfer GmbH (Augsberg, Germany). Cyanuric acid (> 98%) and melamine (99%) were purchased from Aldrich (Steiheim, Germany). The structures are shown in Figure 1. The melamine solution was prepared by dissolving accurately weighed amounts in hot water, whereas cyanuric acid, ammeline and ammelide solution were prepared by dissolving accurately weighed amounts in 3 mL of 4 M NaOH and diluting the solutions to volume with water. Others reagents used in this study were 20% (w/v) solution of poly (diallyldimethylammonium chloride) [PDDAC, molecular weight 400,000 - 500,000 (Aldrich)], tetrabutylammonium hydroxide (≥ 98% TBAOH; Fluka, Steiheim, Germany), AR grade hydrochloric acid (BDH, Poole, UK), sodium tetraborate (Aldrich), boric acid (Aldrich) and sodium dodecyl sulfate (SDS, Sigma-Aldrich, Steiheim, Germany). Milk samples were produced in China (2009, sold in Chiengrai market for 2 samples) and Thailand (2009, bought from supermarket in Prathumthani province for 2 samples).

II. *Instrumentation and Conditions*

Separations were performed in a 41 cm × 75 μm i.d. polyimide-coated fused-silica capillary (Polymicro Technology, Phoenix, AZ, USA) coated internally with 0.5% PDDAC for 60 min, and the distance from the point of injection to the detection window was 32.5 cm. The analyses were performed on a HP^{3D} CE system (Agilent Technologies, Bracknell, UK), equipped with a positive power supply. A separation potential of +25 kV was employed. Temperature was set to 25°C. Analyte injection was carried out by using a pressure of 50 mbar with an injection time of 4 s. The electrophoretic zones were detected at 210 nm with a photodiode array detector. The background electrolyte was a mixture of 5 mM boric acid, 5 mM $Na_2B_4O_7$, 10 mM SDS and 2 mM TBAOH at pH 9.15. Sep-Pak C18 cartridges (Waters) were used for removing the matrix from the milk samples.

III. *Sample Preparation*

As proteins and carbohydrates constitute a complex matrix in food samples, the isolation and extraction of melamine and its derivatives from the matrix are necessary prior to determination. UHT milk and milk wafers were purchased from the market. Milk wafers were homogenized before they were used. 10 mL of 0.01 M HCl was added to the sample (2 g of UHT milk or 1 g of milk wafer) for protein precipitation. After 10 min of sonication, the sample was centrifuged at 3,500 rpm for 10 min. The supernatant was passed through a SPE C18 cartridge previously conditioned with 3 mL of methanol followed by 10 mL of water. The eluted solution was collected after discarding the first 1 mL eluate and a dilution of 40% was made with water before injection into the CE.

For the recovery study of melamine and its derivatives, a milk sample spiked with standard melamine and its derivatives was prepared by adding 10 mL of 0.01 M HCl, followed by melamine (2 mg/L), ammeline (2 mg/L), ammelide (1 mg/L) and cyanuric acid (2 mg/L) into the milk sample. Then, extraction and matrix clean-up was performed. Recoveries were determined by comparing the peak areas between the samples spiked with known quantities of the standards.

RESULTS AND DISCUSSION

MEKC performed on a fused silica column coated with poly(diallyldimethylammonium chloride) was first used to improve the baseline for the separation of melamine and its derivatives. The effect of SDS concentration on the peak shapes of melamine and its derivatives was investigated and the results showed that good peak shape of all analytes was achieved at 9.4 - 12 mM SDS. However, the separation time was longer with higher SDS concentration.

I. *Selection of Extraction and Clean-up Procedure*

From the preliminary experiment, 10 mL of 1% trichloroacetic acid (TCA) was used to precipitate protein from samples, but the results indicated that a large peak of TCA overlapped with the cyanuric acid peak. Therefore, 10 mL of 0.01 M HCl was chosen to precipitate proteins from the samples, as there was no interference peak shown on the electropherogram. However, to clean-up the sample matrix remaining in the sample solution, the supernatant was passed through SPE after centrifugation. The matrix was bound on the SPE C18 cartridge, which would otherwise cause electrophoretic de-stacking. About 1 mL of the eluate from SPE was discarded before the collection of the sample solution. Dilution of the sample solution was made before injection into the CE instrument.

II. *Optimization of MEKC Conditions*

(I) *Separation Conditions*

The separation of melamine, ammeline, ammelide and cyanuric acid was performed with detection at the cathodic end of the capillary. The use of 5 mM borate buffer (5 mM boric acid and 5 mM sodium borate) at pH 9.15 as the background electrolyte on bare silica capillary was found to be unsuccessful, as the melamine and ammeline peaks were superimposed. 2 mM TBAOH was then added to the electrolyte and the electropherogram showed small split peaks of melamine and ammeline. The separation of these two peaks was successfully performed by adding SDS into the electrolyte, but the baseline was not smooth. To solve the unevenness of the baseline, a cationic polymer (PDDAC) was used to provide a semi-permanent coating on the capillary wall.

Preliminary experiments to determine the optimum electrolyte composition were conducted within the ranges 5 - 20 mM of borate buffer, pH 9 - 9.3, 8.2 - 12 mM of SDS and 2 - 5 mM of TBAOH. Increasing the concentration of borate in the electrolyte reduced the peak heights and 5 mM of borate buffer provided the highest peak heights for all analytes. Increasing the pH of the electrolyte increased the migration time and a pH of 9.15 provided good separation with a smooth baseline. Electrolyte containing 8.2 mM of SDS with 20 kV could elute only melamine, ammeline and ammelide from the CE column. Within the range 9 - 12 mM of SDS, melamine and its derivatives were successfully separated. Increasing SDS concentration increased the migration time but also resulted in increased peak height. The addition of 2 mM of TBAOH into the electrolyte reduced migration time but also caused increased peak heights for all analytes. The addition of more than 2 mM of TBAOH resulted in broad peak shapes. After consideration of these four factors, the best conditions for short analysis time and high detection sensitivity for melamine and its derivatives were to use a separation electrolyte of 5 mM boric acid, 5 mM sodium borate, 10 mM sodium dodecylsulfate and 2 mM tetrabutylammonium hydroxide at pH 9.15.

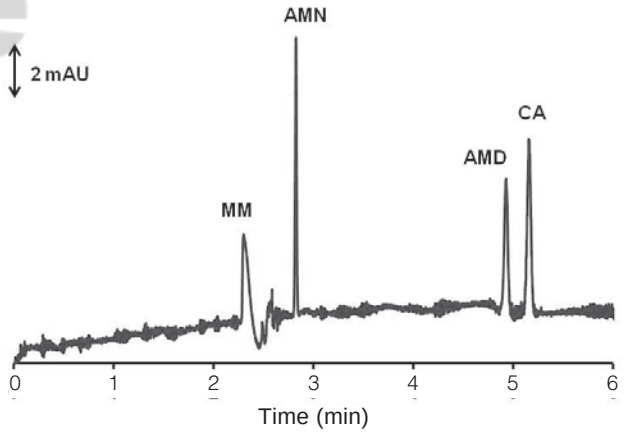

Figure 2. Electropherogram of standard melamine (2 mg/L), ammeline (2 mg/L), ammelide (2 mg/L) and cyanuric acid (5 mg/L). Separation conditions: electrolyte 5 mM boric acid, 5 mM sodium borate, 10 mM sodium dodecylsulfate and 2 mM tetrabutylammonium hydroxide at pH 9.15; separation voltage +20 kV, injection time 4 s.

The applied voltage and the injection time were optimized within the ranges 18 - 25 kV and 2 - 8 s, respectively, under a pressure of 50 mbar. Increasing the magnitude of the positive separation voltage resulted in reduced migration time and an increased peak height, but increasing the voltage to more than 20 kV caused a reduced peak height. Therefore, +20 kV was selected. Increasing the injection time to more than 4 s resulted in poor peak shape of melamine, hence 4 s was chosen. Figure 2 shows an electropherogram obtained from a standard mixture of melamine and its derivatives under the optimized conditions.

III. *Analytical Performance Characteristics*

Analytical performance characteristics were determined under the optimized conditions. The detection limits of melamine, ammeline, ammelide and cyanuric acid based on a signal-to-noise ratio of 3 are shown in Table 1. The linearity of an external calibration plot and precision values (expressed as percentage relative standard deviation) for migration, peak area and peak height of melamine and its derivatives are illustrated in Table 1. Recoveries of melamine and its derivatives from the extraction and clean-up step were determined by comparison of the peak areas between the samples spiked with standard known quantities and standard. The recoveries are shown in Table 2. The present method allowed the simultaneous determination of melamine and its derivatives in real samples with higher sensitivity and shorter analysis time, compared with previous capillary electrophoresis methods[5-8, 12-15] (Table 3).

IV. *Determination of Melamine and Its Derivatives in Milk Products*

Preliminary experiments with direct injection of sample solutions without passing through SPE C18 cartridge

Journal of Food and Drug Analysis, Vol. 21, No. 1, 2013

Table 1. Detection limits, precision and linearity for the determination of melamine, ammeline, ammelide and cyanuric acid (n = 5)

Standard	LOD (mg/L)	%RSD (Intra-day)			%RSD (Inter-day)			Linear range (mg/L)	Regression equation (established by peak area)	Correlation coefficient
		Migration time	Peak area	Peak height	Migration time	Peak area	Peak height			
Melamine	0.10	0.14	0.11	1.25	0.18	0.12	0.92	0.25 - 100	y = 10.3830x – 0.821	0.9998
Ammeline	0.04	0.29	0.36	2.74	0.33	0.31	2.92	0.25 - 200	y = 6.8202x + 6.3981	0.9992
Ammelide	0.03	0.05	0.28	1.83	0.17	0.22	1.51	0.25 - 80	y = 7.1587x + 5.1818	0.9996
Cyanuric acid	0.08	0.12	0.24	3.08	0.16	0.35	2.82	0.25 - 150	y = 5.7128x – 0.1789	0.9990

Table 2. Percentage recovery of melamine and its derivatives in milk samples (n = 3)

Sample	Melamine		Ammeline		Ammelide		Cyanuric acid	
	% Recovery	% RSD	% Recovery	% RSD	% Recovery	% RSD	% Recovery	% RSD
UHT milk	98.9	0.39	107.5	1.35	105.4	2.84	100.6	1.56
Milk chocolate wafer	95.4	4.79	105.9	3.63	98.0	6.65	102.9	0.76
Milk wafer	98.5	1.65	104.2	4.44	97.7	7.86	99.3	0.79

Recovery was obtained from the mean of three experiments with the same concentration of melamine, ammeline, ammelide and cyanuric acid (2 mg/L) added.
RSD was calculated from three experiments at the same concentration.

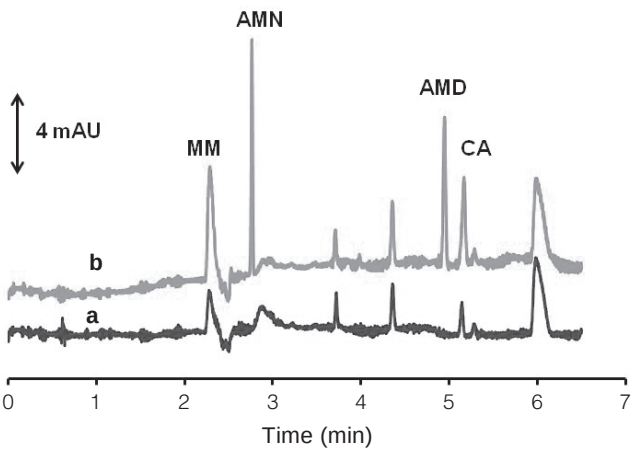

Figure 3. Electropherogram of (a) UHT milk, and (b) UHT milk spiked with melamine (2 mg/L), ammeline (2 mg/L), ammelide (1 mg/L) and cyanuric acid (2 mg/L). Other conditions were similar to Figure 2.

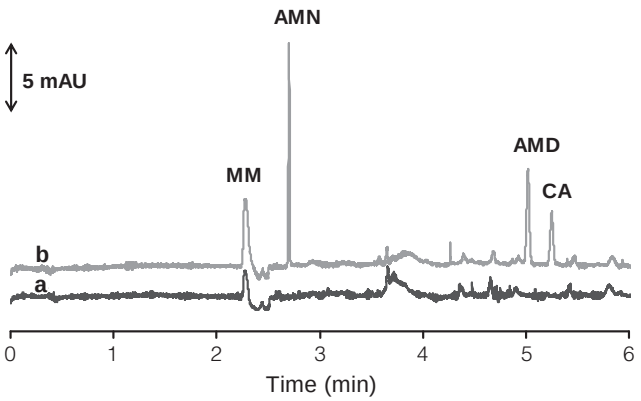

Figure 4. Electropherogram of (a) milk chocolate wafer, and (b) milk chocolate wafer spiked with melamine (2 mg/L), ammeline (2 mg/L), ammelide (1 mg/L) and cyanuric acid (2 mg/L). Other conditions were similar to Figure 2.

resulted in de-stacking of melamine and its derivative peaks. Electropherograms obtained from UHT milk and milk chocolate wafers before and after spiking with standard melamine, ammeline, ammelide and cyanuric acid are shown in Figures 3 and 4, respectively. The results showed that the developed MEKC method provided high sensitivity, shorter analysis time, lower running costs and lower usage of organic solvents, compared to HPLC[16,17,19], LC-MS[22] and GC-MS[30] methods (Table 3). With simple sample preparation, the developed method was successfully applied to milk product samples.

CONCLUSIONS

Simultaneous separation of melamine, ammeline, ammelide and cyanuric acid was successfully performed within 5.2 min by using an electrolyte consisting of 5 mM boric acid, 5 mM sodium borate, 10 mM sodium dodecylsulfate and 2 mM tetrabutylammonium hydroxide at pH 9.15 on a fused silica column coated with PDDAC. The results obtained from the MEKC method provided high sensitivity and reliability. The developed MEKC method can be applied to the analysis of milk samples with good recovery.

Table 3. Summary of published methods for measuring melamine in food products

Methods	Samples	Sample preparation	LOD				Separation time (min)	Ref.
			MM	AMN	AMD	CA		
MEKC	UHT milk, milk wafers	Extracted with HCl, sonication, SPE C18	0.10 mg/L	0.04 mg/L	0.03 mg/L	0.08 mg/L	5.2	Present work
Sweeping-MEKC	Infant formula	Dissolved in water, 1 M HCl, ACN, centrifuged and on-line SPE precon-centration quantified at 218 nm	10.9 ng/mL	-	-	-	5.5	[5]
MEKC	Flour products	Dissolved in water, HCl, ACN, vortex, centrifuged, load to SPE, evaporate the eluate to dryness and add 10 mM NaOH and 25 mM H_3PO_4, sonication detection at 200 nm	1.7 mg/kg	0.23 mg/kg	0.29 mg/kg	1.2 mg/kg	12	[6]
CZE	Milk, yoghurt, ice-cream, milk powder, egg, and pet feeds	Dissolved in ACN/water/diethylamine, sonication, centrifuged, detection at 214 nm	0.05 mg/L	0.26 mg/L	0.33 mg/L	0.02 mg/L	10	[7]
MEKC	Infant formula milk powder	Extracted with 8 mM SDS/20 mM borate buffer at pH 7.4, sonication, filter, amperometric detection	2.1 mg/L	-	-	-	8	[8]
CZE	Liquid milk, yogurt, whole milk powder, fish feed, fish	Extracted with 10% TCA, water, chloroform, sonication, centrifuged, filter, detection at 206 nm	1.2 mg/L	-	-	-	4	[12]
tITP-CE	Powder milk	Extracted with 50% ACN, sonication , centrifuged, evaporated ACN, dissolved residue in 80 mM phosphoric buffer, detection at 200 nm	6.3 ng/mL	-	-	-	9.3	[13]
CZE	Milk powder, milk, fish feed	Extracted with 1%TCA, ACN, sonication, centrifuged, filter, SPE, detection at 200 nm	0.08 mg/L	-	-	-	6.7	[14]
Sweeping-MEKC	Milk powder, gluten, cookie, chicken feed	Dissolved in water, 1 M HCl, vortex, ACN, vortex, centrifuged, SPE	5 ng/mL	-	-	-	13	[15]
HPLC	Cereal flours	Extracted with water, ethanol, , vortex, centrifuged, 0.01 M NaOH was added into residue, vortex, centrifuged, filter, detection at 200 and 220 nm	6 mg/kg	4 mg/kg	5 mg/kg	92 mg/kg	16	[16]
HPLC	Rice protein concentrate, animal feed	Dissolved in 5 mM sodium phosphate, sonication, filter, detection at 220 nm	65 μg/g	55 μg/g	60 μg/g	113 μg/g	8	[17]
HPLC	Liquid milk sample	Dissolved in 1% TCA and 2.2% lead acetate, sonication, , centrifuged, load supernatant to SPE	18 μg/kg	-	-	-	7	[18]
HPLC	Milk powder	Dissolved in 90% methanol/water, extraction in stainless-steel extraction cell, filter and dried at 40C, added 80% ACN, detection at 230 nm	10 μg/kg	10 μg/kg	10 μg/kg	10 μg/kg	12	[19]
LC-MS	Milk powder,	Extracted with 1%TCA, centrifuged	0.1 mg/kg	0.1 mg/kg	0.1 mg/kg	0.1 mg/kg	8	[22]
LC-MS	Royal jelly	Extracted with 1%TCA, ACN, vortex, sonication, centrifuge, load super-natant to SPE	0.01 μg/g	-	-	-	4.5	[20]
LC-MS	Food	Dissolved in ACN-water(1:1), HCl, sonication, centrifuged, add hexane to supernatant, centrifuge, dilute aqueous layer with ACN-water1:1), load to SPE	20 μg/kg	-	-	30 μg/kg	7	[23]
UPLC-MS	Milk products	Dissolved in ACN-water(1:1),vortex,sonication, centrifuged,add 1M HCl and dichloromethane to supernatant, rotary mixer, centrifuged, dilute upper aqueous layer with water, filter	0.2 mg/kg	-	-	-	2	[25]

Table 3 (continued)

Methods	Samples	Sample preparation	LOD				Separation time (min)	Ref.
			MM	AMN	AMD	CA		
GC-MS	Milk products	Zirconia hollow fiber sorptive microextraction	0.3 ng/mL	-	-	-	10	[29]
GC-MS	Milk products	Dissolved in ACN, water, diethylamine, sonication, centrifuged, filter, evaporated filtered solution, derivatization using pyridine, ACN, BSTFA with 1%TMCS	2 ng/g	2 ng/g	2 ng/g	2 ng/g	11.5	[30]
GC-MS	Egg	Extracted in 5% TCA, vortex, centrifuged, load supernatant on Oasis MCX cartridge, elute melamine with 5% ammonium hydroxide in methanol	10 ng/g	-	-	-	15	[32]
GC-MS	Powdered milk	Dissolved in ACN containg 5% DMSO, sonication, centrifuged, filter, extract filtrate with hexane, evaporated to dryness, redissolved in ACN containing 0.5% pyridine	0.2 ng/g	-	-	0.5 ng/g	19	[33]

MEKC=micellar electrokinetic chromatography, CZE=capillary zone electrophoresis, ACN=acetonitrile, MM=melamine, AMN, ammeline, AMD=ammelide, cyanuric acid (CA), tITP-CE=transient isotachophoretic capillary electrophoresis, TCA=trichloroacetic acid, DMSO=dimethyl sulfoxide

ACKNOWLEDGMENTS

The authors would like to thank the National Research University Project of Thailand, Office of Higher Education Commission for the financial support.

REFERENCES

1. File:Melamine-cyanuric acid complex color.png. http://en.wikipedia.org/wiki/Melamine_cyanurate/2008.
2. Kim, B., Perkins, L. B., Bushway, R. J., Nesbit, S., Fang, T., Sheridan, R. and Greene, V. 2008. Determination of melamine in pet food by enzyme immunoassay, high-performance liquid chromatography with diode array detection, and ultra-performance liquid chromatography with tandem mass spectrometry. J. AOAC Int. 91: 408-413.
3. US-FDA. 2007. Interim melamine and analogues safety/risk assessment. http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/2007/ucm108922.htm
4. FAO. 2008. Melamine milk crisis: Countries to ensure safe feeding for infants and increase vigilance. http//www.fao.org/newsroom/en/news/2008/1000926/Index.html.
5. Tsai, I. L., Sun, S. W., Liao, H. W., Lin, S. C. and Kuo, C. H. 2009. Rapid analysis of melamine in infant formula by sweeping-micellar electrokinetic chromatography. J. Chromatogr. A 1216: 8296-8303.
6. Hsu, Y. F., Chen, K. T., Liu, Y. W., Hsieh, S. H. and Huang, H. Y. 2010. Determination of melamine and related triazine by-products ammeline, ammelide, and cyanuric acid by micellar electrokinetic chromatography. Anal. Chim. Acta. 673: 206-211.
7. Xia, J., Zhou, N., Liu, Y., Chen, B., Wu, Y. and Yao, S. 2010. Simultaneous determination of melamine and related compounds by capillary zone electrophoresis. Food Control. 21: 912-918.
8. Wang, J., Jiang, L., Chu, Q. and Ye, J. 2010. Residue analysis of melamine in milk products by micellar electrokinetic capillary chromatography with amperometric detection. Food Chem. 121: 215-219.
9. Zhai, C., Qiang, W., Sheng, J., Lei, J. and Ju, H. 2010. Pretreatment-free fast ultraviolet detection of melamine in milk products with a disposable microfluidic device. J. Chromatogr. A 1217: 785-789.
10. Wang, X. and Chen, Y. 2009. Determination of aromatic amines in food products and composite food packaging bags by capillary electrophoresis coupled with transient isotachophoretic stacking. J. Chromatogr. A 1216: 7324-7328.
11. Cook, H. A., Klampfl, C. W. and Buchberger, W. 2005. Analysis of melamine resins by capillary zone electrophoresis with electrospray ionization-mass spectrometric detection. Electrophoresis. 26: 1576-1583.
12. Yan, N., Zhou, L., Zhu, Z. and Chen, X. 2009. Determination of melamine in dairy products, fish feed, and fish by capillary zone electrophoresis with diode array

detection. J. Agric. Food Chem. 57: 807-811.
13. Wang, X. and Chen, Y. 2009. Determination of aromatic amines in food products and composite food packaging bags by capillary electrophoresis coupled with transient isotachophoretic stacking. J. Chromatogr. A 1216: 7324-7328.
14. Wen, Y., Liu, H., Han, P., Gao, Y., Luan, F. and Li, X. 2010. Determination ofmelamine in milk powder, milk and fish feed by capillary electrophoresis: A good alternative to HPLC. J. Sci. Food Agric. 90: 2178-2182.
15. Wu, W. C., Tsai, I. L., Sun, S. W. and Kuo, C. H. 2011. Using sweeping-micellar electrokinetic chromatography to determine melamine in food. Food Chem. 128: 783-789
16. Ehling, S., Tefera, S. and Ho, I. P. 2007. High performance liquid chromatographic method for the simultaneous detection of the adulteration of cereal flours with melamine and related triazine by-products ammeline, ammelide and cyanuric acid. Food Addit. Contam. 24: 1319-1325.
17. Muñiz-Valencia, R., Ceballos-Magana, S. G. and Rosales-Martinez, D. *et al*. 2008. Method development and validation for melamine and its derivatives in rice concentrates by liquid chromatography. Application to animal feed samples. Anal. Bioanal. Chem. 392: 523-531.
18. Sun, H., Wang, L., Ai, L., Liang, S. and Wu, H. 2010. A sensitive and validated method for determination of melamine residue in liquid milk by reversed phase high-performance liquid chromatography with solid-phase extraction. Food Control. 21: 686-691.
19. Yu, H., Tao, Y., Chen, D., Wang, Y., Huang, L., Peng, D., Dai, M., Liu, Z. and Yuan, Z. 2010. Development of a high performance liquid chromatography method and a liquid chromatography–tandem mass spectrometry method with pressurized liquid extraction for simultaneos quantification and confirmation of cyromazine, melamine and its metabolites in foods of animal origin. Anal. Chim. Acta. 682: 48-58.
20. Zhou, J., Zhao, J., Xue, X., Zhang, J., Chen, F., Li, Y., Wu, L., Li, C. and Mi, J. 2009. Hydrophilic interaction chromatography/tandem mass spectrometry for the determination of melamine in royal jelly and royal jelly lyophilized powder. J. Chromatogr. B 877: 4164-4170.
21. Wu, Y. T., Huang, C. M. and Lin, C. C. *et al*. 2009. Determination of melamine in rat plasma, liver, kidney, spleen, bladder and brain by liquid chromatography-tandem mass spectrometry. J. Chromatogr. A 1216: 7595-7601.
22. Ibáñez, M., Sancho, J. V. and Hernández, F. 2009. Determination of melamine in milk-based products and other food and beverage products by ion-pair liquid chromatography-tandem mass spectrometry. Anal. Chim. Acta. 649: 91-97.
23. Vaclavik, L., Rosmus, J., Popping, B. and Hajslova, J. 2010. Rapid determination of melamine and cyanuric acid in milk powder using direct analysis in real time-time-of-flight mass spectrometry. J. Chromatogr. A 1217: 4204-4211.
24. Deng, X. J., Guo, D. H., Zhao, S. Z., Han, L., Sheng, Y. G., Yi, X. H., Zhou, Y. and Peng, T. 2010. A novel mixed-mode solid phase extraction for simultaneous determination of melamine and cyanuric acid in food by hydrophilic interaction chromatography coupled to tandem mass chromatography. J. Chromatogr. B 878: 2839-2844.
25. Goscinny, S., Hanot, V., Halbardier, J. F., Michelet, J. Y. and Van Loco, J. 2011. Rapid analysis of melamine residue in milk, milk products, bakery goods and flour by ultra-performance liquid chromatography/tandem mass spectrometry: From food crisis to accreditation. Food Control. 22: 226-230.
26. Jacob, C. C. and Comboa Costa, G. G. 2011. Low-level quantification of melamine and cyanuric acid in limited samples of rat serum by UPLC-electrospray tandem mass spectrometry. J. Chromatogr. B 879: 652-656.
27. Ge, J., Zhao, L. W., Liu, C. Y., Jiang, S., Lee, P. W. and Liu, F. 2011. Rapid determination of melamine in soil and strawberry by liquid chromatography-tandem mass spectrometry. Food Control. 22: 1629-1633.
28. Tran, B. N., Okoniewski, R., Storn, R. Jansing, R. and Aldous, K. M. 2010. Use of methanol for the efficient extraction and analysis of melamine and cyanuric acid residues in dairy products and pet foods. J. Agric. Food Chem. 58: 101-107.
29. Li, J., Qi, H. Y. and Shi, Y. P. 2009. Determination of melamine residues in milk products by zirconia hollow fiber sorptive microextraction and gas chromatography-mass spectrometry. J. Chromatogr. A 1216: 5467-5471.
30. Miao, H., Fan, S. and Wu, Y. N. *et al*. 2009. Simultaneous determination of melamine, ammelide, ammeline, and cyanuric acid in milk and milk products by gas chromatography-tandem mass spectrometry. Biomed. Environ. Sci. 22: 87-94.
31. Tzing, S. H. and Ding, W. H. 2010. Determination of melamine and cyanuric acid in powdered milk using injection-port derivatization and gas chromatography-tandem mass spectrometry with furan chemical ionization. J. Chromatogr. A 1217: 6267-6273.
32. Xia, X., Ding, S., Li, X., Gong, X., Zhang, S., Jiang, H., Li, J. and Shen, J. 2009. Validation of a confirmatory method for the determination of melamine in egg by gas chromatography-mass spectrometry and ultra-performance liquid chromatography-tandem mass spectrometry. Anal. Chim. Acta. 651: 196-200.
33. Tzing, S. H. and Ding, W. H. 2010. Determination of melamine and cyanuric acid in powdered milk using injection-port derivatization and gas chromatography-tandem mass spectrometry with furan chemical ionization. J. Chromatogr. A 1217: 6267-6273.