Application Note

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Abstract

The determination of Melamine and Cyanuric Acid go hand in hand with one another. Melamine is an industrial chemical used in the manufacturing of amino resins, plastics, and flame retardants. Cyanuric Acid is produced by the hydrolysis of raw or waste Melamine, followed by crystallization. Melamine is used as a profitable adulterant in milk based products to increase the protein content and thus increase the commercial value of the adulterated substance. In recent years Melamine and Cyanuric Acid has been found in infant formula and other milk based products. The presence of Melamine and Cyanuric Acid is highly regulated around the world.

In this study, the performance and versatility of the AutoMate-Q40, workstation platform, was evaluated for the extraction of Melamine and Cyanuric Acid in milk based products. A Liquid Chromatography (LC) coupled to a Triple-Quadrupole Mass Spectrometer (LC-MS/MS) was employed for the detection of these adulterants. Quantification was based on matrix match calibration curves. By using the AutoMate-Q40 to streamline this extraction, it provides us with appropriate analytical results, falling in the established method guidelines (recovery range of 70-120% and a %RSD <20%) for the target compounds.

Introduction

Since the late 2000's the United States Food and Drug Administration (US FDA) received reports of adulterated infant formula in China. It was determined that the infant formula had been contaminated with Melamine and related compounds such as Cyanuric Acid. This contaminated formula lead to at least 60,000 infants falling ill[1-4].

It was determined that water was added to the raw milk in order to increase the production volume. This resulted in diluted milk with a lower protein concentration. According to the World Health Organization (WHO), companies using this milk for further production, such as powdered infant formula, will check the protein concentration through a total determination test. This test does not identify the proteins, but reports nitrogen as a total. It was found that adding Melamine would increase the nitrogen content (67% by mass) and thus increase the commercial value of the adulterated substance[1].

Performance and versatility of the AutoMate-Q40, workstation platform, was evaluated for the extraction of Melamine and Cyanuric Acid in milk based products. A Liquid Chromatography (LC) coupled to a Triple-Quadrupole Mass Spectrometer (LC-MS/MS) was employed for the detection of these adulterants. Quantification was based on matrix matched calibration curves. By using the AutoMate-Q40 to streamline this extraction, it provides us with appropriate analytical results, falling in the established method guidelines (recovery range of 70-120% and a %RSD <20%) for the target compounds.

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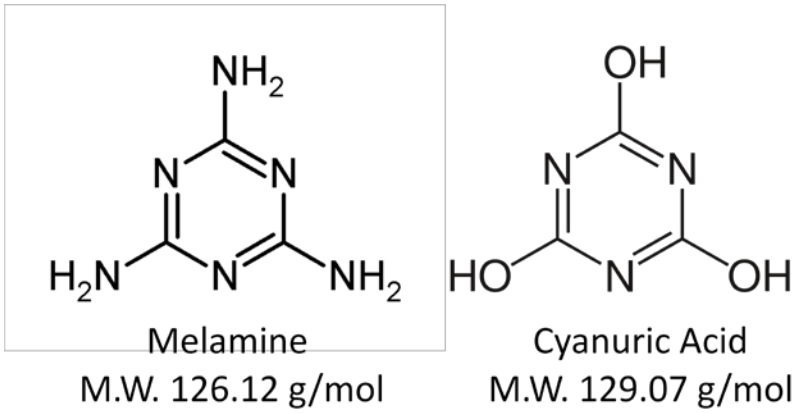
Experimental Instrument Conditions

Chemical Structures

See Figure 1 for the chemical structures and molecular weight for Melamine and cyanuric Acid.

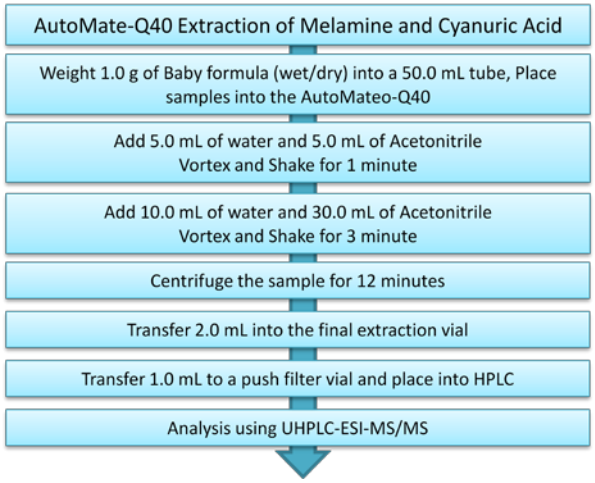
Figure 1 Melamine and Cyanuric Acid Structures



Sample Preparation/Extraction

Two kinds of infant formula (dry and pre-mixed) were purchased from the local grocery store. The appropriate sample amount of 1.0 g was chosen for both the dry and premade infant formula. These samples were then transferred into a 50.0 mL centrifuge tubes and placed into the AutoMate-Q40 sample prep workstation. The automated extraction for Melamine and Cyanuric Acid followed the manual sample preparation procedure presented in the 2009 NACRW poster in titled "LC-MS/MS method for the simultaneous Quantitative Determination of Melamine and cyanuric Acid in infant formula and raw cow's milk" (Figure 2).[5]

Figure 2 Automated Melamine and Cyanuric Acid Extraction



Instrumentation and Analytical Conditions

Sample analysis was conducted using a Shimadzu Nexera LC interfaced to an AB Sciex 4500 QTrap Triple-Quad Mass Spectrometer (LC-MS/MS). For separation of the compounds of interest, a Phenomenex Luna® 3.0 µm HILIC 200 Å (100.0 x 2.0 mm) column was used. Table I and Table II demonstrate the optimized LC-MS/MS analysis parameters for both the chromatographic separation and optimal analyte transitions. Figure 3 shows the scheduled MRM chromatogram spiked at 0.5 mg/kg.

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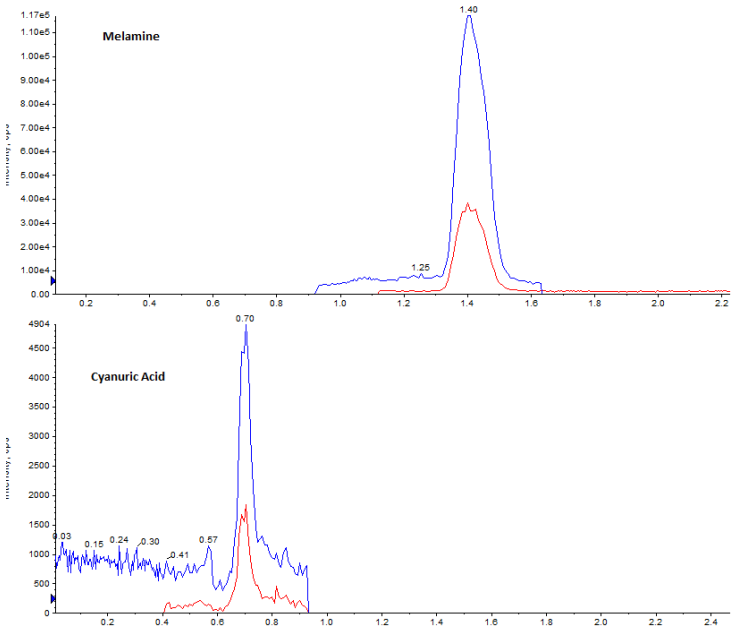
Table I Critical LC-MS/MS SRM Transitions and Parameters for AB Sciex 4500 QTrap			
Curtain Gas (CUR)			20
Ion Spray Voltage (IS)			+/-4500
Temperature (TEM)			500 ˚C
Collision Gas (CAD)			High
Analyte Transitions			
Compounds	RT (min)	Precursor Ion (m/z)	Quantization product Ion (m/z)
Melamine	1.2	127.0	85.0
Cyanuric Acid	0.67	128.0	42.0

Table II Shimadzu Nexera LC Parameters		
Column	Phenomenex Luna® 3.0 µm HILIC 200 Å	
Dimensions	100.0 X 2.00 mm	
Mobile Phase	A:10.0 mM Ammonium Acetate in H_2O	
	B:Acetonitrile	
Gradient	Time	%B
	0.0	90%
	8	90%
	13	35%
	14	10%
	15	10%
	15	90%
	25	90%
Flow Rate (mL/min)	0.600	
Column Temperature (°C)	Room Temperature	

Figure 3 Matrix Match Calibration Sample 10.0 µg/mL or 0.50 mg/kg Final Sample.



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Results

Automating the Melamine and Cyanuric Acid extraction enables fast, easy, reliable and highly reproducible extractions. The AutoMate-Q40 offers labor saving, and improves the repeatability and consistency between the extracted samples.

A precision and accuracy study was performed using the AutoMate-Q40 (Table III). The system was able to fortify the infant formula samples at 10.0 ng/mL; this translates to a final concentration of Melamine and Cyanuric Acid at 0.50 mg/kg. This is accomplished through the systems automated and selectable standard addition feature.

Table III shows that when using the AutoMate-Q40 to extract Melamine and Cyanuric Acid from infant formula (wet/dry) recoveries ranged from 96.6% to 104.3%. Table III also shows that the results have excellent precision, ranging from 3.0% to 8.6%RSD.

Table III. AutoMate-Q40 Extraction Results for Melamine and Cyanuric Acid

Dry Infant Formula Laboratory Spiked			Infant Formula Laboratory Spiked		
Sample Rep	Melamine 0.5 mg/kg	Cyanuric Acid 0.5 mg/kg	Sample Rep	Melamine 0.5 mg/kg	Cyanuric Acid 0.5 mg/kg
1	113.0	103.6	1	109.7	99.1
2	109.6	102.5	2	94.5	98.7
3	98.7	101.5	3	108.5	96.3
4	97.4	108.1	4	105.4	96.2
5	94.4	109.3	5	109.7	100.7
6	93.1	103.4	6	107.4	88.8
7	90.6	101.9	7	102.8	96.4
Avg	99.5	104.3	Avg	105.4	96.6
STDV	8.5	3.1	STDV	5.4	3.8
%RSD	8.6	3.0	%RSD	5.1	4.0

Conclusion

Automation of this Melamine and Cyanuric Acid extraction method produced reliable results for the spiked samples. Automating this extraction shows the versatility of the AutoMate-Q40 and how it can be adapted to other extractions. The AutoMate-Q40 led to improved repeatability, a reduction in the likelihood of human error and the potential for significant labor savings.

Precision and accuracy were assessed for the two commodities analyzed. Results for the automated procedure were well within the criteria set forth in this study: Average recoveries for the range of commodities were between 96.6% and 105.4% with good precision (ca. 5.2% RSD).

References

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3. A new, fast and sensitive LC/MS/MS method for the accurate quantitation and confirmation of Melamine and Cyanuric Acid in Pet food Samples http://www.lifescience.ca/DATA/CATALOGUE/202~v~Melamine-application-note.pdf
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5. LC-MS/MS method for the simultaneous Quantitative Determination of Melamine and cyanuric Acid in infant formula and raw cow's milk, NACRW poster 2009

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