

Comparing ESI and APCI Sources to Screen Dairy-Based Foods for Melamine by Rapid On-line Extraction with LC-MS/MS

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Overview

- A Thermo Scientific TurboFlow LC-MS/MS assay was developed to measure melamine in dairy-based foods with minimal sample preparation and high sample throughput. The four-minute method with a one-minute data window reliably detects at least 50 ppb and can be multiplexed across four channels to screen up to 60 samples per hour.
- Sample preparation involved mixing liquid or powdered food products with a diluent containing ammonium acetate in acetonitrile and water. The protein-precipitate mixtures were centrifuged and the supernatants were subjected to automated on-line extraction by a TurboFlow™ Cyclone MCX cation-exchange column.
- Extracted melamine was quickly eluted to either an electrospray ionization (ESI) or atmospheric-pressure chemical ionization (APCI) source of a tandem mass spectrometer and detected by positive-ion selective reaction monitoring (+SRM). Although recommended, a stable isotope internal standard was not used.
- When using APCI, recoveries of melamine spiked in various samples relative to injections of neat standards at the same concentration were typically above 90% and, for some samples, as much as 200%. The causes of these enhanced analyte signals were not investigated. When using ESI, relative recoveries were as low as 10%, typical of severe ion suppression by co-eluting sample components.
- APCI was favored over ESI to minimize false negatives during sample screening. False positives from the APCI method can be revealed by confirmatory tests¹.

Introduction

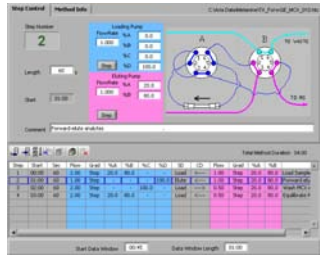
The need to efficiently detect melamine in various foods has become obvious during the past few years. Methods based on liquid chromatography with tandem mass spectrometry (LC-MS/MS) currently practiced by regulatory agencies¹ require extensive sample preparation to minimize matrix interferences. To permit a simpler sample preparation and achieve greater throughput while maintaining reasonable accuracy, sensitivity, and selectivity, we've developed a fast-flow cation-exchange LC method that can be multiplexed across up to four channels. We also compared matrix interferences in ESI and APCI sources.

Methods

Liquid and powdered milk products including infant formulas were mixed with a diluent of 70% acetonitrile and 30% water containing 50 mM ammonium acetate (3% acetic acid + 3% ammonium hydroxide). To test for matrix interferences, a duplicate set of samples was spiked with melamine (100 ng/mL). All samples were vortexed for 30 seconds and then centrifuged for 10 minutes at 10,000 RPM. After centrifugation, the supernatants were placed in plastic vials and capped immediately. Neat standards of melamine were prepared in diluent. Using a Thermo Scientific Aria TLX-4 (four-channel, two-dimensional LC) system configured for quick elute plumbing (Figure 1), 25 µL of standards and prepared samples were injected directly onto a TurboFlow Cyclone MCX mixed-phase cation-exchange column. From each 25 µL injection, the extracted melamine was quickly eluted into an APCI or heated electrospray ionization (H-ESI) source of a Thermo Scientific TSQ Quantum Ultra triple stage quadrupole mass spectrometer. Melamine was detected by positive-ion transitions 127-to-85 and 127-to-68. Confirmation of melamine peaks were based on the ratio of responses at the two transitions as well as retention time. Peak areas from neat standards, non-spiked, and spiked samples were compared to assess recovery and matrix interferences. Since a stable isotope of melamine was not used in this study, quantitation was based on external standardization.

FIGURE 1. Aria™ Quick Elute Method to Extract and Elute Melamine

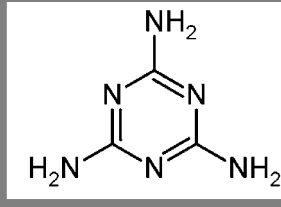
- Loading Solvent A: Water + 0.1% Formic Acid
 Eluting Solvent A: Water + 1% Formic Acid
 Loading & Eluting Solvent B: Methanol
 Loading Solvent C: 50% Isopropanol + 50% Acetone
 Loading Solvent D: Water + 100 mM Ammonium Acetate
- Load 25 µL of sample with 20% aqueous formic acid and 80% methanol into TurboFlow Cyclone MCX mixed-phase cation-exchange column
 - Forward-elute with 20% aqueous 1% ammonium hydroxide and 80% methanol to MS
 - Wash TurboFlow column with aqueous 100mM ammonium acetate pH 9, followed by 50% acetone and 50% isopropanol
 - Re-equilibrate TurboFlow column



Results

Figure 2 shows the structure of melamine. This compound is not very soluble in pure water, acetonitrile or methanol. Its solubility was improved by adding ammonium acetate to water-organic solutions. A diluent containing 70% acetonitrile, 30% water and 50 mM ammonium acetate provided good solubility of melamine while precipitating proteins from dairy-based food products.

FIGURE 2. Melamine Chemical Structure



The products tested were:

- | | |
|------------------------|--------------------------|
| <u>Liquid samples:</u> | <u>Powdered samples:</u> |
| Whole Milk | Infant Formula 3 |
| Reduced-Fat Milk | Instant Milk Shake |
| Fat Free Milk | Instant Breakfast |
| Condensed Milk | Instant Diet Meal |
| Evaporated Milk | Non-fat Dry Milk |
| Infant Formula 1 | |
| Infant Formula 2 | |

The selective reaction monitoring (SRM) transition of 127 to 85 gave the most reliable signals for estimating melamine concentrations in the various food products tested. For neat standards, both SRM transitions (127-to-85 and 127-to-68) gave similar calibration plots. Quantitation estimates were based on the 127-to-85 SRM transition. The 127-to-68 transition was used to assess peak identity.

Calibration plots of peak areas from neat standards were linear from 5 to 200 ng/mL with a slope of 7550 using the H-ESI source (Figure 3).

Using the APCI source resulted in a linear response from 5 to 500 ng/mL with a slope of 1500 (Figure 4).

FIGURE 3. Melamine Calibration with H-ESI

FIGURE 4. Melamine Calibration with APCI

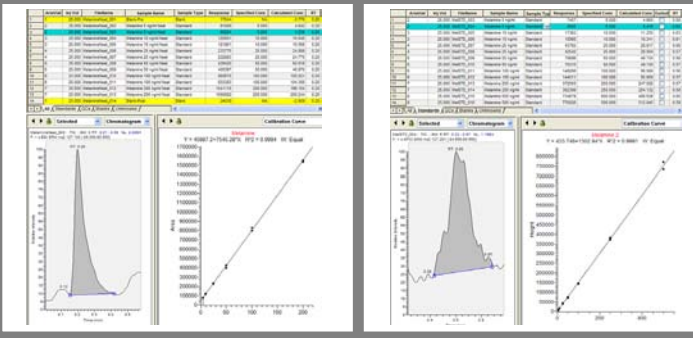


FIGURE 5. Melamine Detection with H-ESI

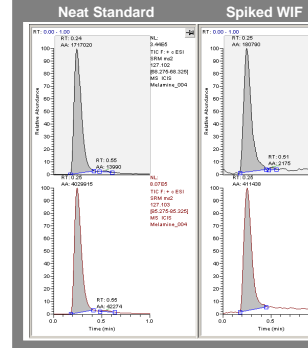
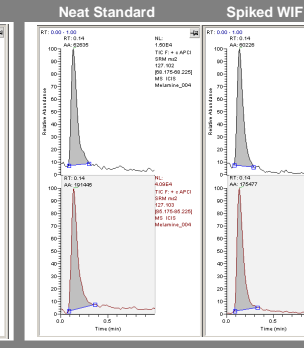


FIGURE 6. Melamine Detection with APCI



Infant Formula 3 (WIF) spiked with 100 ng/mL showed signals that were 10% of those from the 100 ng/mL Neat Standard when using the H-ESI source (Figure 5). Corresponding signals from the APCI source were 95% of those for the Neat Standard (Figure 6).

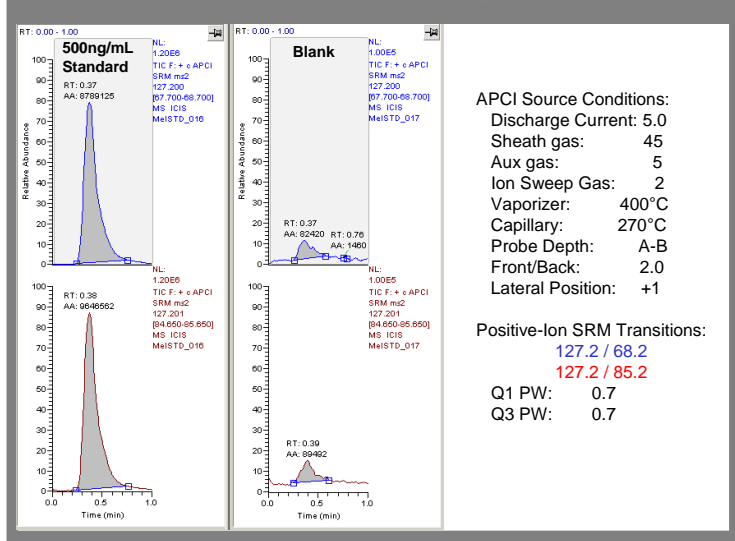
Although the H-ESI source gave better sensitivity, the APCI source showed less instances of ion suppression among spiked samples (Table 1). However, many samples showed enhanced signals for melamine. This may be due to ionization enhancement caused by co-eluting sample components and/or enhancement of solubility and extraction efficiency by sample components. Such possibilities were not investigated.

TABLE 1. Comparison of Melamine Peaks from Spiked Food Products Using H-ESI & APCI Sources

Product	Average Melamine Signal with HESI		Average Melamine Signal with APCI	
	Non-Spiked	Spiked (100 ng/mL)	Non-Spiked	Spiked (100 ng/mL)
Whole Milk	2	67	39	117
Reduced-Fat Milk	ND	87	38	125
Fat Free Milk	ND	86	41	116
Condensed Milk	2	20	ND	209
Evaporated Milk	ND	ND	49	120
Infant Formula 1	ND	25	49	133
Infant Formula 2	1	34	60	117
Infant Formula 3	ND	10	36	109
Instant Milk Shake	7	71	35	121
Instant Breakfast	ND	12	2	90
Instant Diet Meal	7	86	46	143
Non-fat Dry Milk	5	67	21	152

SRM transition 127-to-85 was used for quantitation. Average from duplicates runs from three different days (n=6)
 Coefficient of variation was less than 15% among averages

FIGURE 7. Melamine Detection by Tandem Mass Spectrometry using APCI Source



APCI Source Conditions:
 Discharge Current: 5.0
 Sheath gas: 45
 Aux gas: 5
 Ion Sweep Gas: 2
 Vaporizer: 400°C
 Capillary: 270°C
 Probe Depth: A-B
 Front/Back: 2.0
 Lateral Position: +1

Positive-Ion SRM Transitions:
 127.2 / 68.2
 127.2 / 85.2
 Q1 PW: 0.7
 Q3 PW: 0.7

The optimized APCI-based MS/MS method for detecting melamine is shown in Figure 7.

Carryover, as shown by a blank injection immediately following the highest standard, was less than 1%.

Conclusions

The system reliably extracted melamine from "crashed" samples while eliminating proteins, salts, and sugars. However, additional sample components that co-eluted with melamine caused as much as 90% ion suppression in the ESI source. Ion suppression was not observed in the APCI source. Although the ESI source provided sensitivity for pure melamine that was five times greater, the avoidance of ion suppression in the APCI source permitted the use of the simplified sample preparation with the on-line extraction and quick elution method. The four-minute method showed a functional sensitivity of 5 ng/mL, a linear response up to 500 ng/mL and carryover less than 1%. In practice, A maximum throughput of one sample per minute was achieved by multiplexing the method across four channels of the Aria TLX-4 system.

Reference

- Sherril Turnipseed, Christine Casey, Cristina Nochetto & David N. Heller. Determination of Melamine and Cyanuric Acid Residues in Infant Formula using LC-MS/MS. FDA Laboratory Information Bulletin No. 4421, Volume 24, October, 2008 (available at www.cfsan.fda.gov).

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