

Regarding the Melamine Crisis

2nd Method: For LC/MS/MS, Inertsil HILIC 5um 3.0X150mm

Experimental

Chemicals

Melamine (108-78-1), Melamine-¹⁵N₃ (98 atom % ¹⁵N), and Cyanuric Acid (108-80-5) standards were obtained from Sigma-Aldrich.

Sample Preparation

Liquid-Liquid-Extraction of pet food samples was performed using the following procedure:

1. Accurately weigh 5g of homogenized sample into a 250mL conical flask.
2. Add 1250μL Melamine-¹⁵N₃ stock solution (200μg/mL) to the sample and wait 15 minutes.
3. Add 100mL water and vortex rigorously to mix sample with water.
4. Connect a condenser apparatus to the top of the conical flask and boil the mixture for 10 minutes.
5. Remove the conical flask from the heat and leave on the bench for one minute.

6. Remove 100μL from the mixture and add to 9900μL water/acetonitrile (50:50) in a 15mL centrifuge tube and vortex.
7. Centrifuge sample at 5000rpm for 10 minutes at 4°C.
8. Filter the solution through 0.45μm PVDF syringe filter (Whatman).
9. Transfer to autosampler vial for LC/MS/MS analysis.

Further dilution of the extract with water/acetonitrile (50/50) might be necessary if the sample is heavily contaminated.

Regarding the Melamine Crisis

2nd Method: For LC/MS/MS, Inertsil HILIC 5µm 3.0X150mm

Liquid Chromatography

A Shimadzu Prominence LC system containing a CBM-20A system controller, two LC-20AD pumps, a semi-micro gradient mixer SUS-20A, and a SIL-20AC autosampler was used. Separation was performed on a GL Science Inertsil HILIC 5µm (150x3mm) column at a

temperature of 40°C with a mobile phase of (A) acetonitrile + 10mM ammonium acetate and (B) water + 10mM ammonium acetate at a flow of 0.5mL/min. The gradient using normal phase conditions is listed in Table 1. An injection volume of 5µL was used.

Mass Spectrometry

An API 3200™ LC/MS/MS System equipped with Turbo V™ source and Electrospray Ionization (ESI) probe was used to detect both targeted analytes in Multiple Reaction Monitoring (MRM) mode. Precursor and product ions with corresponding Declustering Potentials (DP) and Collision Energies (CE) of the detection of Cyanuric Acid in negative polarity and Melamine in positive polarity are given in Table 2.

Regarding the Melamine Crisis

2nd Method: For LC/MS/MS, Inertsil HILIC 5um 3.0X150mm

TABLE 1. LC CONDITIONS FOR THE ANALYSIS OF MELAMINE AND CYANURIC ACID ON AN INERTSIL HILIC 5 μ m (150x3mm) COLUMN

Time (min)	Flow (mL/min)	% (A)	% (B)
0.1	0.5	97	3
5.0	0.5	20	80
5.5	0.5	3	97
5.6	0.5	97	3
10.0	0.5	97	3

TABLE 2. MS PARAMETERS OF THE DETECTION OF MELAMINE AND CYANURIC ACID USING AN API 3200[®] LC/MS/MS SYSTEM

Compound	Retention time	Q1 (amu)	Q3 (amu)	DP (V)	CE (V)
Cyanuric Acid	3.6min	128	42	-30	-30
		128	85	-30	-13
Melamine	5.0min	127	85	44	26
		127	68	44	48
		127	60	44	27