

TN-0019

APPLICATIONS

SPE and HILIC HPLC Strategy for Determination of Melamine

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Introduction

As the world faces its second melamine scare in two years, there has been a global concern over international food product imports and food safety testing. The compound itself has a relatively low toxicity to humans. However, when melamine combines with cyanuric acid, it forms an insoluble salt crystal that causes kidney failure in animals and humans.

Nitrogen is a major component of amino acids, which are the building blocks for proteins. With 6 nitrogen atoms in its structure, melamine has been added to some foods to falsely increase their protein content. This manipulation is possible because the standard method to determine protein content in food is the Kjeldahl

method, which measures the amount of organic nitrogen in the sample. This method is non-specific, so any other nitrogen present in the sample will cause the test to show higher protein levels than are actually present. By adding melamine to their products, food producers have found a way to cover up diluting their products with the consumer suffering the consequences.

The determination of melamine in food products is of great importance, especially with the recent discovery of melamine in baby formula. The following methodology can be used to determine the levels of melamine in food products. In this method, SPE clean up of the sample is followed with analysis by LC/MS/MS.

SPE method - specific targeting using Strata[™]-X-C

SPE Method
Material: Strata-X-C, 100 mg/6mL
Part Number: 8B-S029-ECH
Condition: 2 mL Methanol
Equilibrate: 2 mL 0.1% TFA in water, pH<4
Load: Load sample and allow to slowly drip through cartridge at a rate of 1-2 dps (drops per second)
Wash A: 2 mL 0.1% TFA in Water, pH<4
Wash B: 2 mL Methanol
Elution: 2 mL Methanol / 5% NH ₄ OH. Use a 2 stage elution (2 x 1 mL)

HPLC method using Luna[®] HILIC

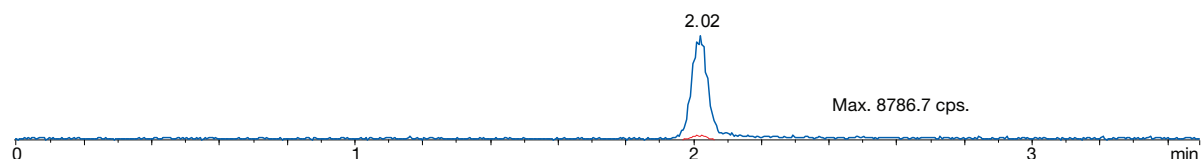
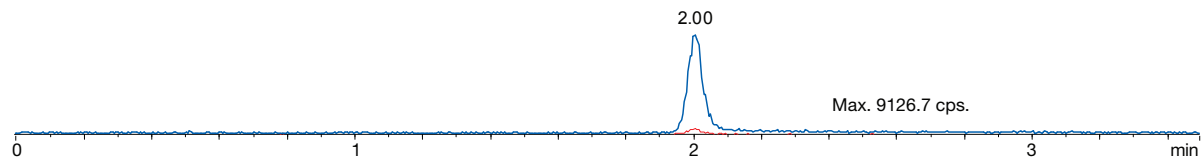
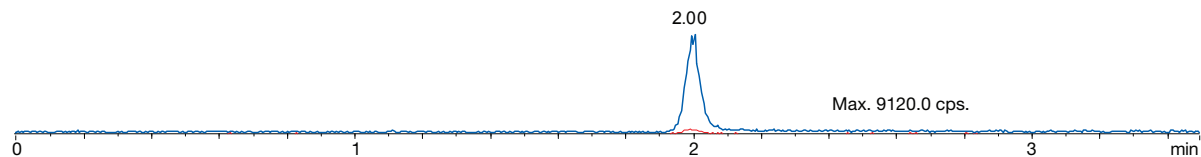
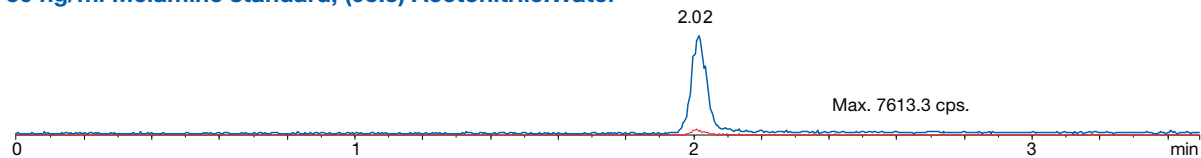
HPLC Method
Column: Luna 3 µm HILIC, 100x2.0 mm
Part Number: 00D-4449-B0
Flow rate: 0.4 mL/min
Mobile Phase: (90:10) Acetonitrile:100 mM Ammonium Formate, pH 3.2
Analysis: Isocratic
Run time: 3.5 minutes
Injection volume: 5 µL
Temperature: Ambient
MRM: m/z 127 – 85, 127 – 60 ESI Positive mode

For related information, see the FERN method for melamine analysis online at http://www.fsis.usda.gov/PDF/FERN_CHE_0003.pdf.

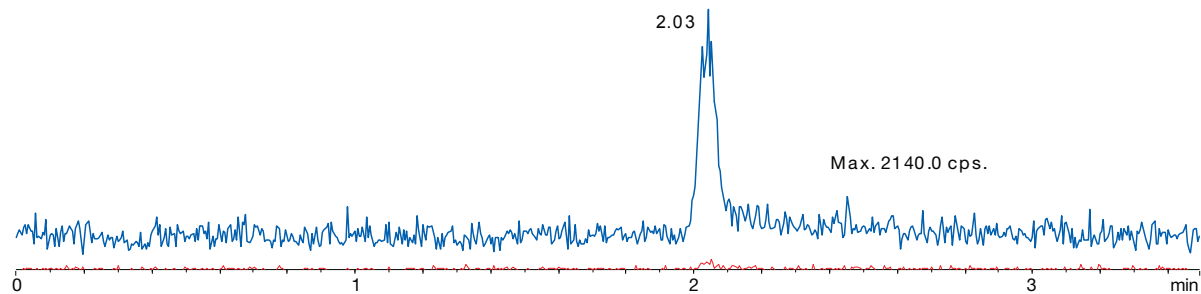
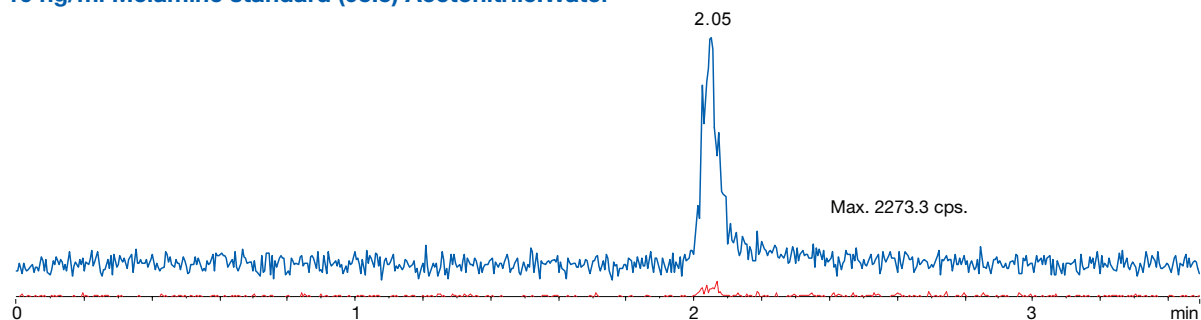
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50 ng/ml Melamine standard, (95:5) Acetonitrile:Water



10 ng/ml Melamine standard (95:5) Acetonitrile:Water



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