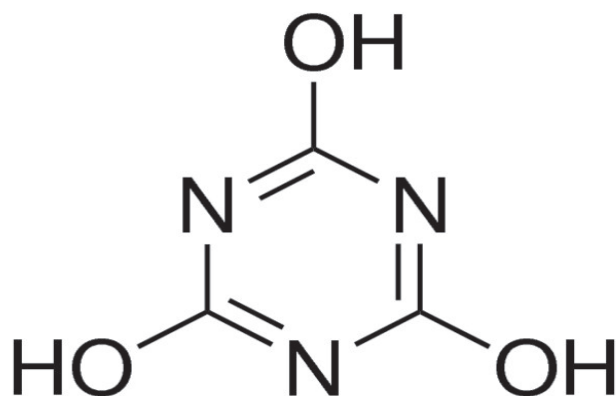


Cyanuric Acid and Melamine in Food Materials Using CSDAU206 and SSQAX



UCT Part Numbers

CSDAU206

Clean Screen® DAU
200 mg, 6 mL Column

SLGRDHLDR-HPOPT

Guard Column Holder

SSQAX056

Styre Screen®
SPE Column

Procedure

1. Prepare Sample

- To 1-5 g of sample add 10-25 mL of CH₃CN/ DI H₂O (50:50)
 - Shake for 5 minutes
- Centrifuge**
- Transfer 5 mL of supernatant to clean glass screw top tube
 - Add 1 mL of 100 mM HCl
 - Add 1 mL of CH₂Cl₂

- Shake for 5 minutes

Centrifuge

- Transfer upper layer to clean glass tube
- Add 2 mL of DI H₂O to CH₂Cl₂
- Shake for 5 minutes

Centrifuge

- Add upper layer to previous aqueous portion
- Apply to conditioned SPE (CSDAU206 (BCX) column

2. Condition Column CSDAU206

- 1 x 3 mL CH₃OH
- 1 x 3 mL DI H₂O

Note: aspirate at < 3 inches Hg to prevent sorbent drying out



3. Apply Sample

- a) Load sample at 1-2 mL / minute
- b) Collect effluent for use with SSQAX SPE

4. Wash Column: CSDAU206

- a) 1 x 1 mL DI H₂O
 - b) Collect wash for use with SSQAX
- Note:** Remove collection tubes from manifold and go to SSQAX section
- c) 1 x 3 mL 100 mM HCl
 - d) 1 x 1 mL CH₃OH
 - e) Dry column (5 minutes at > 10 inches Hg).

5. Elute Melamine

Note: Insert fresh collection tubes into manifold

- a) 1 x 2 mL of CH₃OH containing 5% NH₄OH
- b) 1 x 3 mL of CH₃OH containing 5% NH₄OH
- c) Collect eluate at 1-2 mL /minute

6. Evaporation

- a) Evaporate eluates under a gentle stream of nitrogen < 40°C

7. Reconstitute

- a) Sample in 1000 µL of CH₃CN (*Add External Standard)
- b) Inject 5 µL

SSQAX SPE Procedure

8. Adjust solution from Steps 4 +5 to pH 7 ***

9. Condition Column SSQAX

- a) 1 x 3 mL CH₃OH
 - b) 1 x 3 mL DI H₂O
- Note:** aspirate at < 3 inches Hg to prevent sorbent drying out

10. Apply Sample

- a) Load sample (from step 8) at 1-2 mL / minute.

11. Wash Column SSQAX

- a) 1 x 3 mL DI H₂O
- b) 1 x 1 mL CH₃OH
- c) Dry column (just enough to remove residual solvent)



12. Elute Cyanuric Acid

Note: Insert fresh collection tubes into manifold

- a) 1 x 3 mL of CH₃OH containing 1% HCl
- b) 1 x 2 mL of CH₃OH containing 1% HCl
- c) Collect eluate at 1-2 mL /minute.

13. Evaporation

- a) Evaporate eluates under a gentle stream of nitrogen < 40°C.

14. Reconstitute

- a) Sample in 100 µL of mobile phase (*Add External Standard)
- b) Inject 5 µL

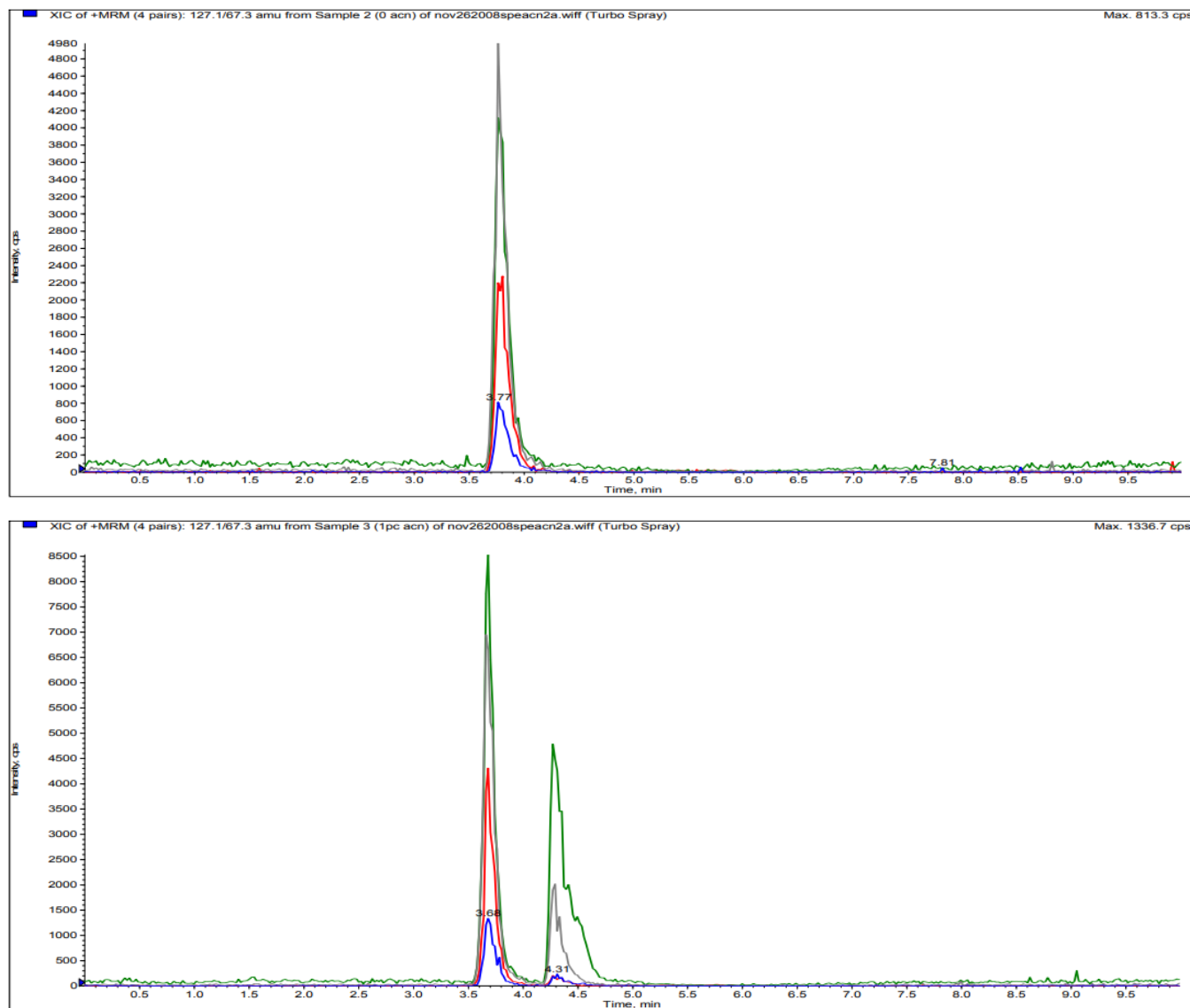
Instrument Conditions:

Column: 150 x 2.1 mm (4 µm)
Diamond Hydride (MicroSolv)

Mobile Phase		
Time	% Acetonitrile	0.1% Formic acid
0	90	10
3	20	80
3.5	90	10
10	90	10
Flowrate:		0.50 mL/ minute
Column Temperature:		ambient

Compound	MRM
Melamine	127.1/85.1
*2, 4 Diamino 6-hydroxy pyrimidine	127.1/ 67.0
Cyanuric Acid	127.8/84.9



CHROMATOGRAM OF: Blank Milk Powder (upper)**Spiked Milk Powder (lower)**

*** Adjust pH with 100 to 200 μ L of 5% (v/v) aqueous ammonium hydroxide.

Note: Melamine/ IS analyzed in Positive MRM mode: Cyanuric acid in Negative MRM mode.



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