

Rapid, Accurate Quantitation of Melamine by HPLC-UV using MycoSep 224 clean-up

Applicable for milk, milk powder, dry baby formula, liquid baby formula, white rabbit candy, dry and wet pet food samples

Melamine is an organic base with the chemical formula $C_3H_6N_6$. Melamine is used as a raw material for a range of products including glues, colorants, house wares and, due to its high nitrogen content, fertilizers. The use of melamine in food production is banned in most countries. Melamine by itself is nontoxic in low doses, but when combined with cyanuric acid it can cause fatal kidney stones.

Extraction:

Prepare the sample, as outlined below for the respective class of test material, and vortex for 1 min.

Ground samples:

Weigh out 1 g of ground sample and add 10 mL 50+50 acetonitrile + water in a 40 mL conical tube.

Wet pet food:

Weigh out 2 g of sample and add 10 mL 50+50 acetonitrile + water into a 40 mL conical tube.

Liquid samples:

Combine 2 mL samples, 2 mL DI H₂O, and 4 mL acetonitrile.

Extract the melamine from the sample by sonication for 30 min in an ultrasonic bath. Vortex for 1 min and finally centrifuge for 15 min at 10000 rpm. Filter the supernatant using a glass microfiber filter paper.

Clean-up & Purification:

Pipet 5 mL of sample extract into 15 x 85 mm test tube. Push 500 µL of sample through a MycoSep® 224 AflaZon column and transfer 500 µL of purified extract to a 12 x 75 mm cuvette. Evaporate to dryness. Reconstitute in 500 µL of melamine mobile phase and inject onto HPLC system.

Analytical Method:

HPLC: SPD-10A UV detector, SIL-10A autoinjector and LC-10AD pump.

Column: Zorbax Rx-C8 (4.6 x 150 mm, 5 µm)

Mobile phase: 75+5+25 Buffer: THF:0.1 M Ammonium Acetate

Flow rate: 0.5 mL/min

Injection volume: 50 µL

Detection wavelength: 240 nm

Method performance:

Limit of detection: 1.0 µg/g melamine

Recovery rates and variability for melamine:

Column Type	Average %Recovery	Average %RSD
224	80.0	13.6

Studies were performed with biopure standards



Prepare the sample and vortex for 1 min

Extract for 30 min by sonication, vortex for 1 min and centrifuge for 15 min at 10000 rpm

Filter the supernatant

Push 500 µl through MycoSep® 224 AflaZon

Remove 500 µL, evaporate to dryness

Reconstitute in 500 µL of mobile phase

Inject

References:

Dept. of Food Science and Human Nutrition Method – "Melamine Analysis of Pet Food by EIA, HPLC-DAD, and UPLC-MS-MS"

FCC Developmental Melamine Quantitation (HPLC-UV), April 2, 2007, US Food and Drug Administration

Further applications:

www.romerlabs.com

Or contact your local distributor



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Chemicals

- Acetonitrile, HPLC
- Tetrahydrofuran
- Deionized water
- Sodium 1- octanesulfonate, monohydrate
- Citric acid
- Ammonium acetate

Preparation of Reagents and Buffer

- Buffer:
10 mM citric acid and 10 mM sodium octanesulfonate. Add 2.1628 g sodium octanesulfonate to 1000 mL of 10 mM citric acid solution in DI H₂O.

Equipment

- Romer RAS Mill (EQMMR1010 (110V) & EQMMR1015 (220V))
- Romer Evap-System (EQQEV1030 (12 port) & EQQEV1040 (24 port))
- Balance, 400g (EQOLE1010)
- 40 mL conical tubes (LABSP1239)
- 100 mL graduated cylinders (EQOLE1050)
- Timer (EQOLE1300)
- Funnels (EQOLE1350)
- 50 µL glass syringe (EQOAS1030)
- Finnpiptette 1-5 mL (EQOLE1180)
- Finnpiptette 200-1000 µL (EQOLE1130)
- Test Tube Rack (EQOLE1210)
- Vortex mixer-110 volt (EQOLE1330)
- Sample containers
- Sonicator
- Centrifuge (10000 rpm)

Consumables

Clean-up column

MycoSep®224 AflaZon columns(COCMY2224, box of 25)

Liquid Calibrant

Melamine (CMT001, 5mL)



Calculations:

Samples Dry:

$$\frac{1 \text{ g sample}}{10 \text{ mL solution}} \times \frac{0.500 \text{ mL purified extract}}{0.500 \text{ mL mobile phase}} \times 0.050 \text{ mL injected sample} = 0.005 \text{ g sample equivalent}$$

Samples Wet :

$$\frac{2 \text{ g sample}}{10 \text{ mL solution}} \times \frac{0.500 \text{ mL purified extract}}{0.500 \text{ mL mobile phase}} \times 0.050 \text{ mL injected sample} = 0.01 \text{ g sample equivalent}$$