Determination of melamine in milk by HPTLC

A-88.1

Key words:

HPTLC, densitometry, melamine, milk, food analysis, contamination

Introduction:

In fall 2008 there was a scandal on milk products and infant formula adulterated with melamine, which caused kidney damage and several deaths among children. Melamine (1,3,5-triazine-2,4,6-triamine) may have been illegally added to mask low protein content in fraudulently diluted or low quality milk. Since then there has been great need for rapid and reliable methods of analysis.

Scope:

This HPTLC method is suitable for the screening and quantification of melamine in milk. The limit of detection is 20 mg/L.

Required or recommended CAMAG devices:

Automatic TLC Sampler 4 or Linomat 5
Automatic Developing Chamber ADC2 or
Twin Trough Chamber 10 x 10 cm or 20 x 10 cm
TLC Scanner 3 and winCATS software

Derivatization reagent (visualization only)

1. Chlorine chamber: Dissolve 0.3 g of $KMnO_4$ in 10 mL of water and add 10 mL of HCl 16%. Pour 10 mL of this solution in one trough of a twin-trough chamber, close the lid and wait 5 min.
2. Wurster's Blue reagent: Add 1 g of *N,N,N′,N′*-tetramethyl-*p*-phenylene diamine dihydrochloride to 200 mL of acetone. NOTE: solution is not clear.

Sample:

45 mL of milk are mixed with 105 mL of methanol. Then 0.05 mL of HCl 37 % are added. After standing for 30 min the mixture is centrifuged for 5 min at RCF 2700. The supernatant is used as test solution.

Standards:

10 mg of melamine are dissolved in 100 mL of water. 10 mL of this solution are diluted to 100 mL with methanol (concentration 0.01 mg/mL).

NOTE: The presented results are to be regarded as examples only!

Please contact CAMAG for more application notes and products for analysis of herbals!

Chromatography:

Stationary phase:	HPTLC Si 60 F_{254}, 10 x 10 cm or 20 x 10 cm (Merck).
Sample application:	1 or 2 µL of test solution and 0.5, 1, 2, 4, 6, 8 and 10 µL of standard are applied as 8 mm bands, min 2 mm apart, 8 mm from lower edge of plate.
Developing solvent:	Acetonitrile, water, ethyl acetate (3:1:1)
Development:	10 x 10 cm or 20 x 10 cm Twin Trough Chamber, saturated for 20 min (filter paper), 5 mL (respectively 10 mL) developing solvent per trough.
Developing distance:	55 mm from lower edge of plate.
Plate drying:	5 min in a stream of cold air.

Densitometry:

With CAMAG TLC Scanner 3 and winCATS software in absorption mode at 195 nm using a deuterium lamp; evaluation via peak height or area, polynomial regression.

Results:

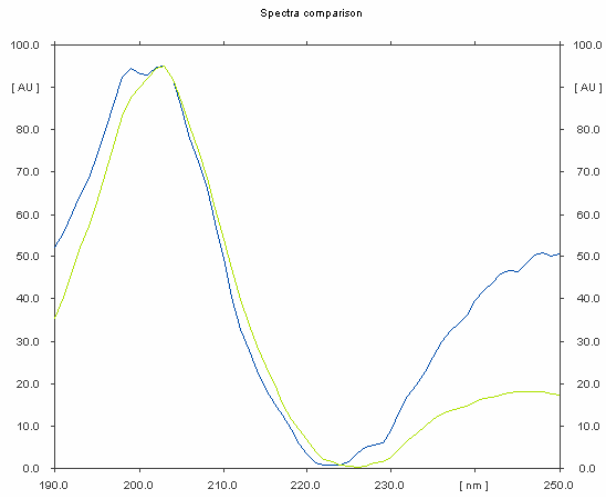

Fig. 1
Comparison of the UV spectra of melamine standard (blue) and of corresponding band in spiked sample (light green).

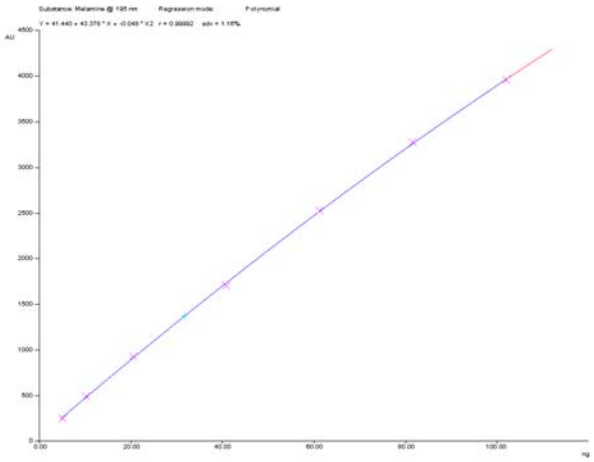

Fig. 2
Calibration curve for melamine measured at 195 nm via peak area.

NOTE: The presented results are to be regarded as examples only!

Please contact CAMAG for more application notes and products for analysis of herbals!

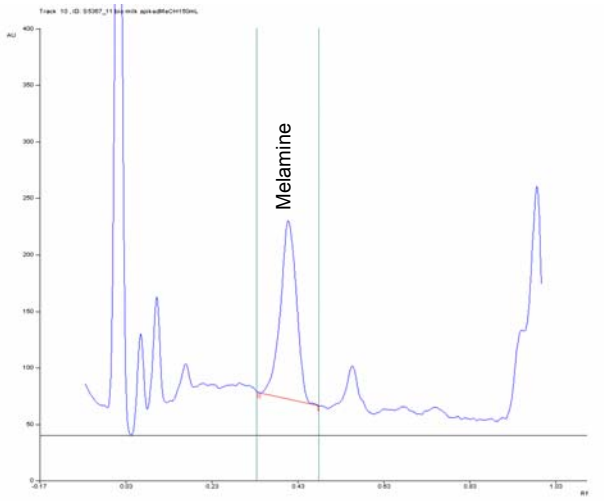

Fig. 3
Densitogram of a spiked milk sample.

Visual evaluation:

Derivatization: For chlorination place plate for 5 min in the empty trough of a chlorine chamber. Remove plate, wait for 10 sec, then dip plate for 2 sec in Wurster`s blue reagent and dry in a stream of cold air for 2 min.

Evaluation: Examination in white light.

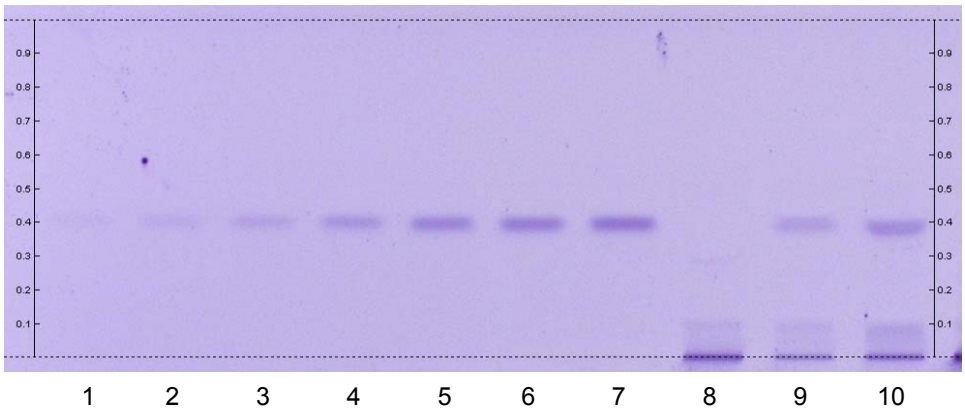

Fig. 4
Image of derivatized plate under white light WR

1-7: Melamine standard (5, 10, 20, 40, 60, 80, 100 ng absolute)
8: Milk
9: Milk spiked with melamine (spiking level 0.01 %), application volume 1 µL
10: Milk spiked with melamine (spiking level 0.01 %), application volume 2 µL

Literature

M. Broszat, R. Brämer, B. Spangenberg, J. Planar Chromatogr. 21 (2008) 6, 469–470