

# CHROMATOGRAPHY

Think about **food**  
Care about **safety**

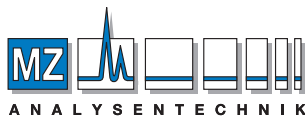


Vol. 1  
**Melamine**



**MACHEREY-NAGEL**

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*Since 1911*

# Focus on melamine

The global production and trading of food and feed products makes monitoring and control difficult.

Only analysis of food and raw materials for the production of food can ensure safe and edible food. Food safety is an essential part of our quality of life.

**MN shows examples for food analysis that will help you with your work and make our food safer!**

## Vol. 1: Melamine in dairy products

The most common analytical method for the determination of the protein content in dairy and other food products is the potentiometric titration of total nitrogen. However the procedure cannot distinguish between nitrogen originated from natural or other sources. A major food safety incident was the Chinese melamine scandal in 2008, when milk and other infant formula were adulterated with

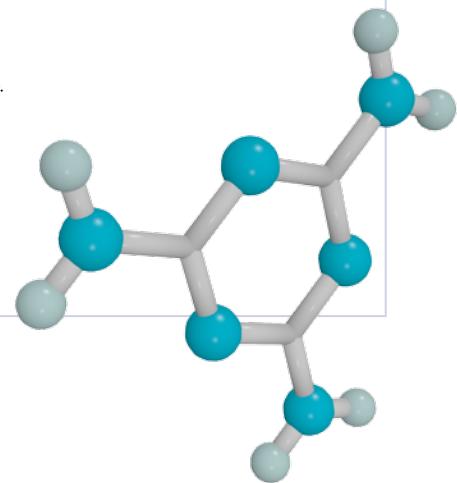
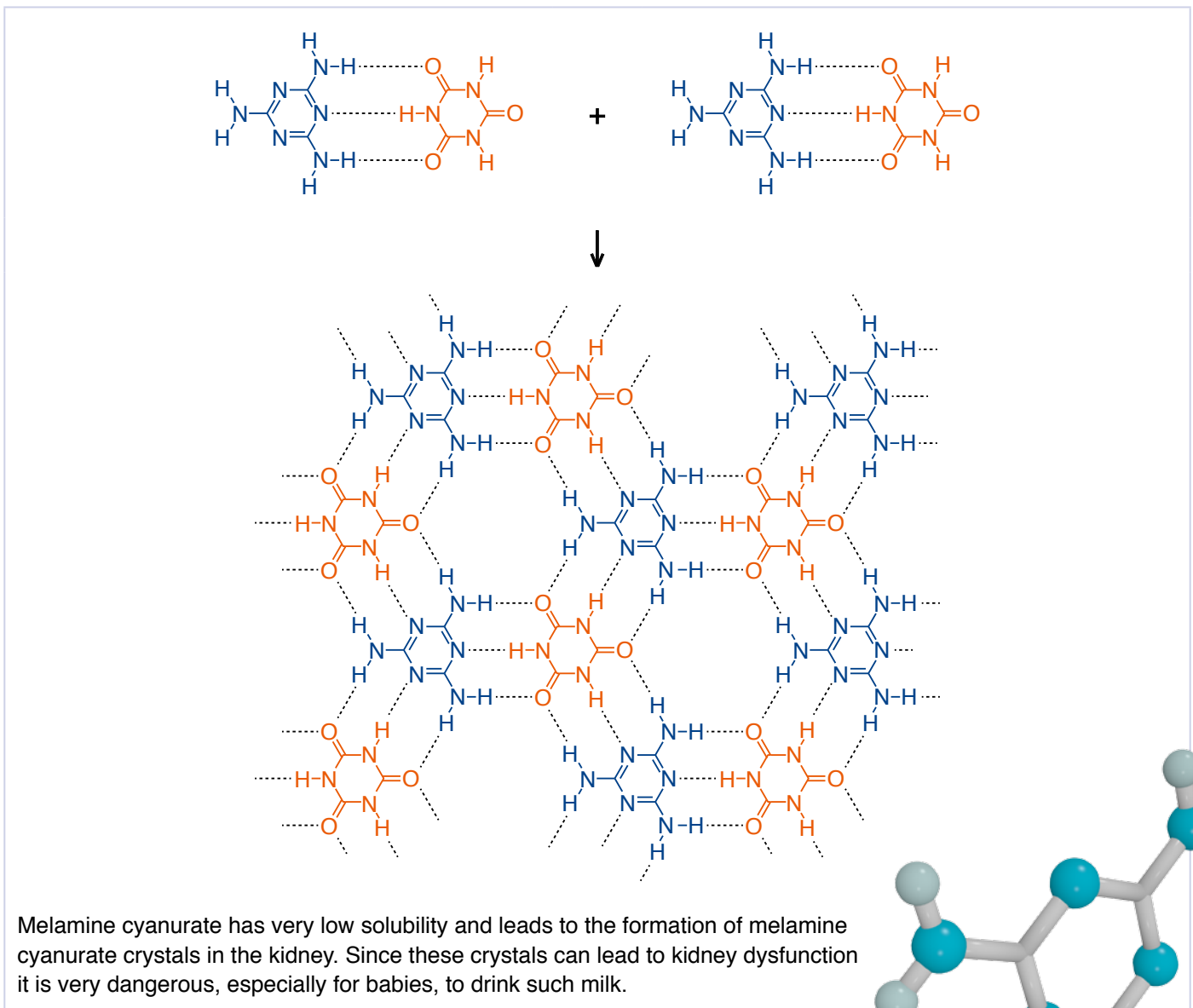
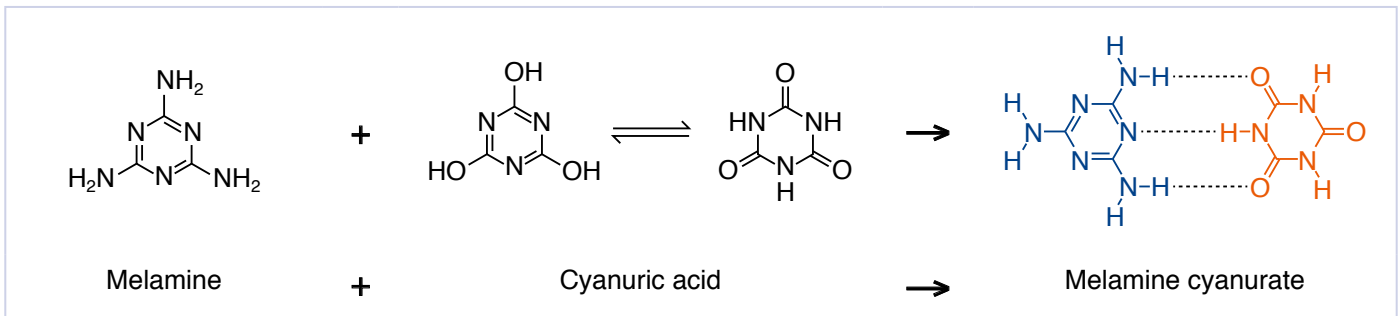
the nitrogen rich organic base melamine in order to feign a higher protein concentration. As reported by Chinese authorities almost 300,000 victims [Tania Branigan, on December 02, 2008 on [guardian.co.uk](http://guardian.co.uk)] were affected by consuming melamine-tainted foodstuff with a death toll of at least six infants because of kidney failure.



# Compounds of interest



## Formation of melamine cyanurate



# Typical samples, preparation

## Melamine in dairy products

### Sample pretreatment

- Add 1 mL of 1 mol/L HCl to 10 mL spiked milk (1 µg/mL or 0.1 µg/mL)
- Add 10 mL dichloromethane and vortex to mix
- Centrifuge for 15 min at 4500 rpm
- Remove and collect the aqueous layer
- Add 5 mL 0.1 mol/L HCl to the organic layer
- Vortex to mix for 1 min
- Centrifuge for 15 min at 4500 rpm
- Combine the aqueous phases
- Add once more 5 mL of 0.1 mol/L HCl to the organic layer, vortex and centrifuge
- Finally combine all aqueous phases for SPE

### Solid phase extraction (SPE)

**MN Appl. No. 304920**

**Column type:**  
CHROMABOND® HR-XC, 3 mL, 200 mg  
REF 730952

#### Column conditioning:

5 mL methanol, then 5 mL water  
(Do not let run the column dry.)

#### Sample aspiration:

The prepared sample is passed through the column by gravity or low vacuum

#### Washing:

5 mL of 0.1 mol/L HCl, then 5 mL methanol

#### Drying:

10 min by vacuum

#### Elution:

2 x 2.5 mL methanol with 5 % NH<sub>3</sub>

**CHROMABOND® vacuum manifold for 12 columns**  
REF 730150



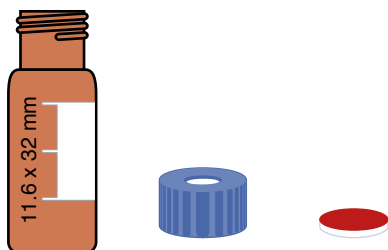
Evaporate in a nitrogen stream and reconstitute with a suitable solvent for subsequent analysis.

#### Recovery rates:

99 % for 1 µg melamine in 1 mL milk  
88 % for 0.1 µg melamine in 1 mL milk

## Sample storage and filtration

Transfer into a sample vial, e.g., 1.5 mL screw neck vials N 9, amber, flat bottom, label and scale, wide opening, REF 702284 and ready assembled screw closures N 9, blue, center hole with septum silicone / PTFE, REF 702287.1



It is recommended to filter the prepared sample through disposable filters **CHROMAFIL® Xtra PVDF-45/25** REF 729219



For other applications see [www.mn-net.com/apps](http://www.mn-net.com/apps)

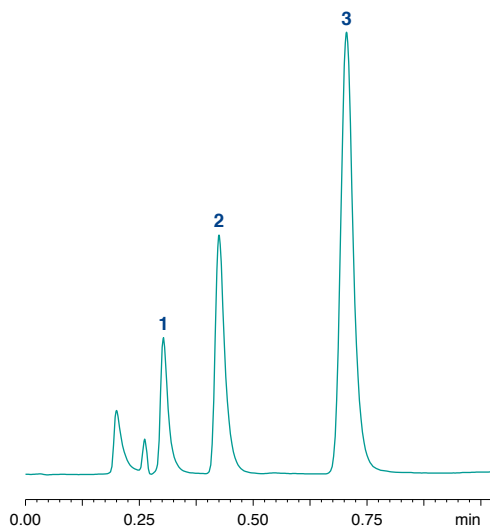


## Subsequent analysis: HPLC

### Analysis of melamine, dicyandiamide and metformin

*MN Appl. No. 125020*

Column: EC 50/4 NUCLEOSHELL® HILIC, 2.7 µm  
REF 763332.40



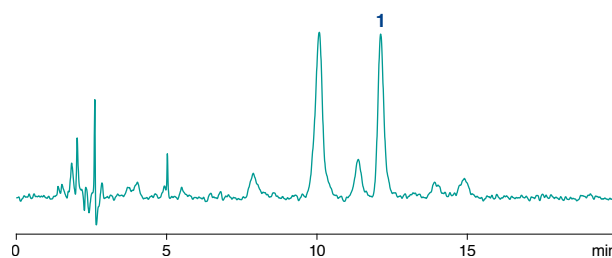
#### Peaks

1. Dicyandiamide
2. Melamine
3. Metformin

### Determination of melamine in milk

*MN Appl. No. 122670*

Column: EC 250/4 NUCLEODUR® 100-5 C<sub>8</sub> ec  
REF 760703.40



#### Peaks

1. Melamine

Detailed conditions are available online at  
[www.mn-net.com/apps](http://www.mn-net.com/apps)

MN suggests using guard columns to protect your main column and significantly increase column life.

The **Column Protection System** (REF 718966) is suitable for all analytical HPLC columns with 1/16" fittings!

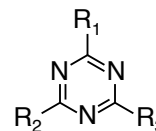
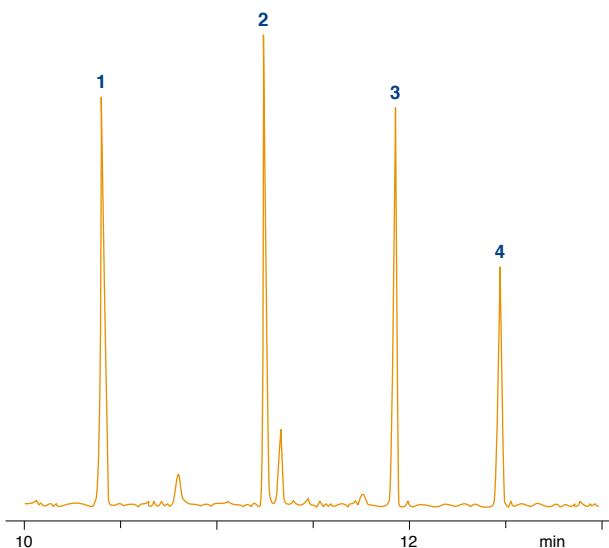


## Subsequent analysis: GC

Determination of melamine, ammeline, ammelide and cyanuric acid in accordance with FDA regulation

MN Appl. No. 213300

Column: OPTIMA® 5 MS, 30 m x 0.25 mm ID, 0.25 µm film  
REF 726220.30



### Peaks:

1. Cyanuric acid ( $R_1 = R_2 = R_3 = OH$ )
2. Ammelide ( $R_1 = NH_2, R_2 = R_3 = OH$ )
3. Ammeline ( $R_1 = R_2 = NH_2, R_3 = OH$ )
4. Melamine ( $R_1 = R_2 = R_3 = NH_2$ )



We recommend derivatization with our silylation reagent SILYL-991,  
REF 701490.201

Detailed conditions are available online at [www.mn-net.com/apps](http://www.mn-net.com/apps)





## Ordering information

Step	Product	Pack of	REF
<b>SPE</b>	CHROMABOND® HR-XC, 3 mL, 200 mg	30	730952
	CHROMABOND® vacuum manifold for 12 columns	1	730150
<b>Filtration</b>	Disposable filters CHROMAFIL® Xtra PVDF-45/25	100	729219
<b>Vials and caps</b>	1.5 mL screw neck vials N 9, amber, label and scale	100	702284
	Ready assembled screw closures N 9, blue, center hole with septum Silicone white / PTFE red	100	702287.1
<b>HPLC 1</b>			
Column	EC 50/4 NUCLEOSHELL® HILIC, 2.7 µm	1	763332.40
Guard column	EC 4/3 NUCLEOSHELL® HILIC, 2.7 µm	3	763338.30
Guard column holder	Column Protection System	1	718966
<b>HPLC 2</b>			
Column	EC 250/4 NUCLEODUR® 100-5 C <sub>8</sub> ec	1	760703.40
Guard column	EC 4/3 NUCLEODUR® 100-5 C <sub>8</sub> ec	3	761937.30
Guard column holder	Column Protection System	1	718966
<b>GC</b>			
Derivatization	Silylation reagent SILYL-991	20 x 1 mL	701490.201
Column	OPTIMA® 5 MS, 30 m x 0.25 mm ID, 0.25 µm film	1	726220.30

Visit us at [www.mn-net.com/chroma](http://www.mn-net.com/chroma) to get more helpful information

## Selection tools for

Vials and caps

Syringe filters

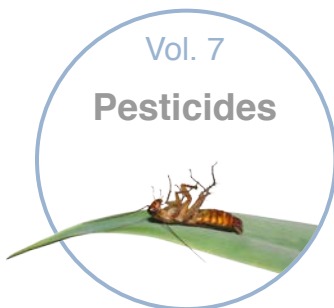
## Troubleshooting guides

GC and HPLC

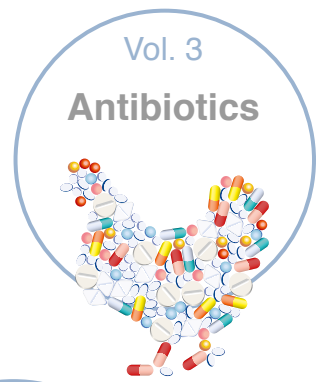
## Detailed product information

...and much more

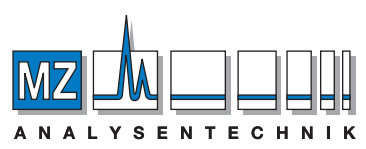




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