CHROMATOGRAPHY

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

Vol. 1

Melamine

MACHEREY-NAGEL
www.mn-net.com

ADIS INTERNATIONAL

MN Since 1911

Focus on melamine

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

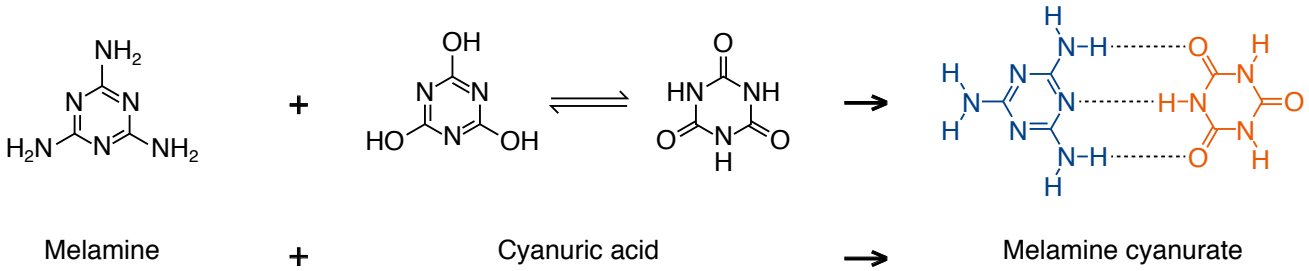
Only analysis of food and raw materials for the production of food can ensure safe and edible food. Food safety is an essential part of our quality of life.

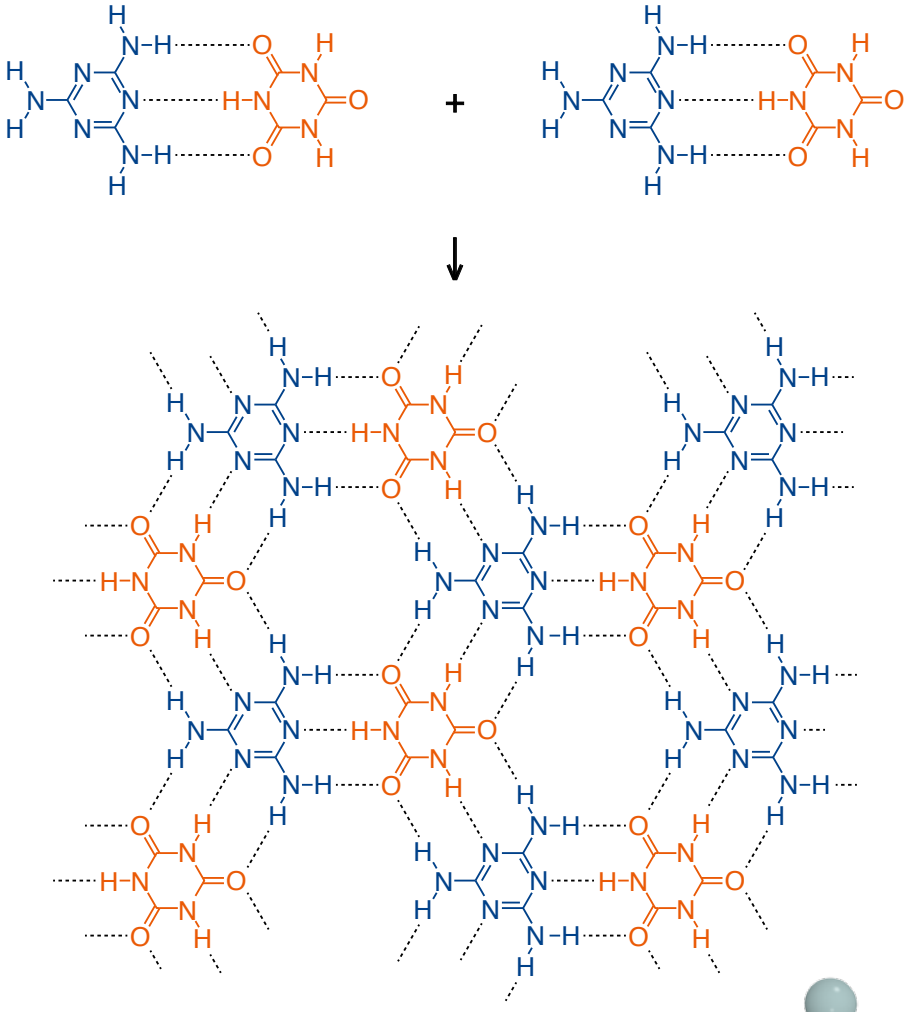
MN shows examples for food analysis that will help you with your work and make our food safer!

Vol. 1: **Melamine in dairy products**

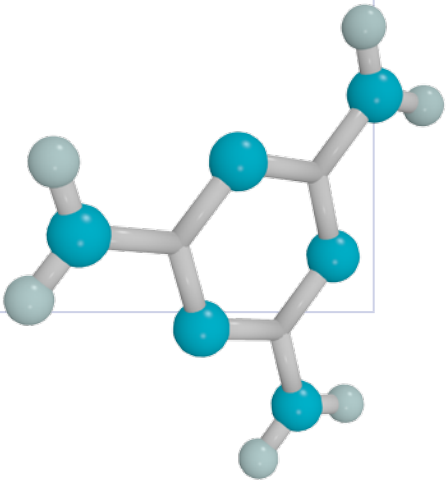
The most common analytical method for the determination of the protein content in dairy and other food products is the potentiometric titration of total nitrogen. However the procedure cannot distinguish between nitrogen originated from natural or other sources. A major food safety incident was the Chinese 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] were affected by consuming melamine-tainted foodstuff with a death toll of at least six infants because of kidney failure.

Formation of melamine cyanurate





Melamine cyanurate has very low solubility and leads to the formation of melamine cyanurate crystals in the kidney. Since these crystals can lead to kidney dysfunction it is very dangerous, especially for babies, to drink such milk.



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_3

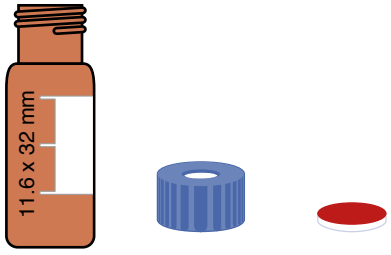
CHROMABOND® vacuum manifold for 12 columns
REF 730150

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

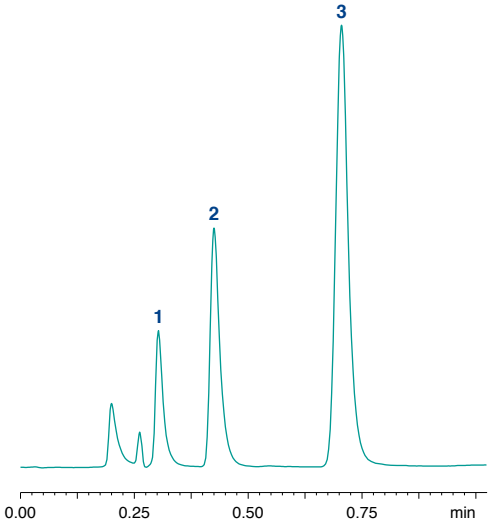
For other applications see *www.mn-net.com/apps*

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

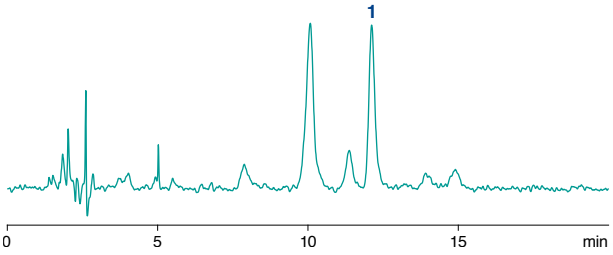


Peaks
1. Dicyandiamide
2. Melamine
3. Metformin

Determination of melamine in milk

MN Appl. No. 122670

Column: EC 250/4 NUCLEODUR® 100-5 C_8 ec
REF 760703.40

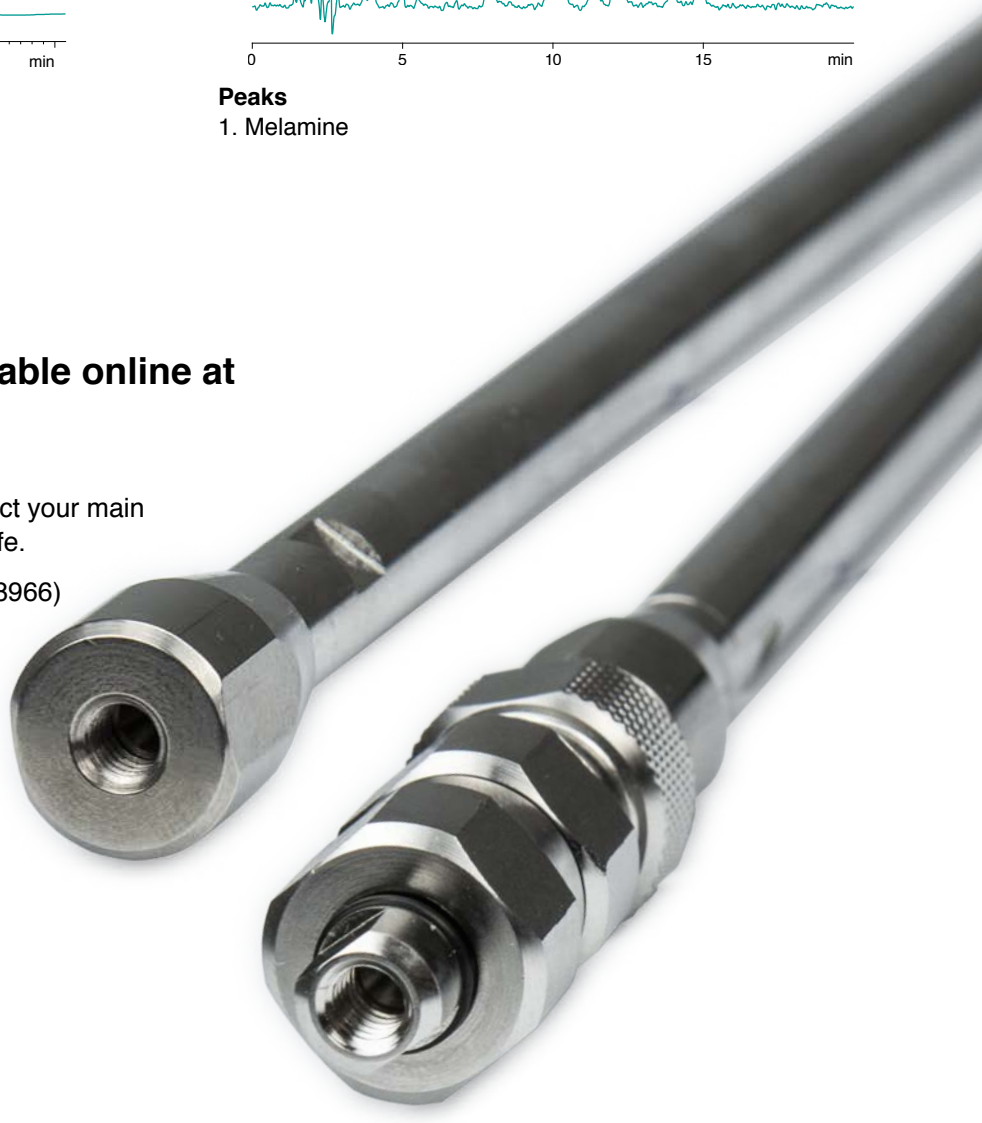


Peaks
1. Melamine

Detailed conditions are available online at *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!

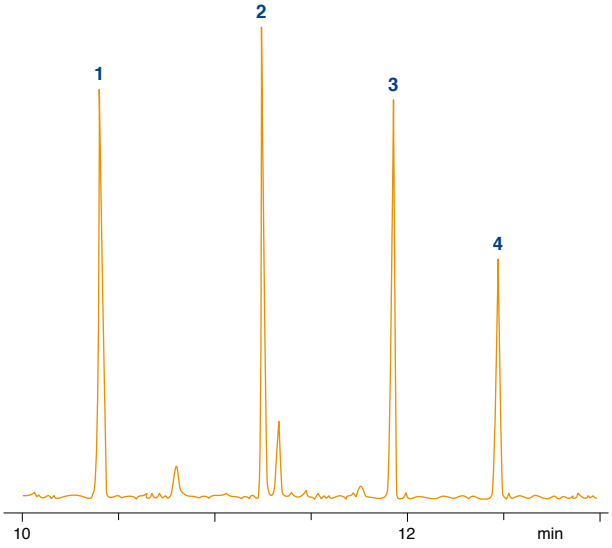


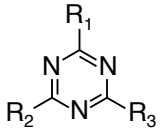
Subsequent analysis: GC

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*

Product information

Ordering information

Step	Product	Pack of	REF
SPE	CHROMABOND® HR-XC, 3 mL, 200 mg	30	730952
	CHROMABOND® vacuum manifold for 12 columns	1	730150
Filtration	Disposable filters CHROMAFIL® Xtra PVDF-45/25	100	729219
Vials and caps	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
HPLC 1			
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
HPLC 2			
Column	EC 250/4 NUCLEODUR® 100-5 C_8 ec	1	760703.40
Guard column	EC 4/3 NUCLEODUR® 100-5 C_8 ec	3	761937.30
Guard column holder	Column Protection System	1	718966
GC			
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* to get more helpful information

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local distributor

SC ADIS INTERNATIONAL SRL
Calea Crangasi Nr. 48, Bucuresti, Romania

T: 021.220.66.30 | M: +40773.74.23.20
office@adis-international.ro
www.adis-international.ro

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MACHEREY-NAGEL

MACHEREY-NAGEL GmbH & Co. KG · Neumann-Neander-Str. 6–8 · 52355 Düren · Germany

Germany and international:
Tel.: +49 24 21 969-0
Fax: +49 24 21 969-199
E-mail: info@mn-net.com

Switzerland:
MACHEREY-NAGEL AG
Tel.: +41 62 388 55 00
Fax: +41 62 388 55 05
E-mail: sales-ch@mn-net.com

France:
MACHEREY-NAGEL EURL
Tel.: +33 388 68 22 68
Fax: +33 388 51 76 88
E-mail: sales-fr@mn-net.com

USA:
MACHEREY-NAGEL Inc.
Tel.: +1 484 821 0984
Fax: +1 484 821 1272
E-mail: sales-us@mn-net.com

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