# **CHROMATOGRAPHY**

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



Vol. 2
Vitamins



**MACHEREY-NAGEL** 

www.mn-net.com



AUTHORIZED DISTRIBUTOR

MZ-Analysentechnik GmbH Barcelona-Allee 17 • D-55129 Mainz Tel +49 6131 880 96-0 Fax +49 6131 880 96-20 e-mail: info@mz-at.de www.mz-at.de



## Focus on vitamins

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. 2: Vitamins in food

They are not visible, they have no taste – but vitamins are essential for our health.

In small doses they display remarkable effects:

- They strengthen our immune system.
- They improve nervous system function.
- They are involved in our metabolism and much more.

A distinction is drawn between the 13 known vitamins. For example, vitamin C or ascorbic acid was discovered There are the fat-soluble and water-soluble vitamins. by Albert Szent-Györgyi in the early 20th century. He iso-Since fat-soluble vitamins A, D, E and K can be stored lated vitamin C from red peppers (paprika). The chemical in the fat deposits of the body, they need not be ingested structure was determined in 1933 by Norman Haworth. daily. This is different for the water-soluble vitamins. The Both received the 1937 Nobel Prize for medicine and eight B vitamins and vitamin C cannot be stored but must chemistry [Nobelprize.org – official web site of the Nobel Prize be regularly refilled. Only vitamin D can be produced by - http://www.nobelprize.org/nobel\_prizes/medithe human body. All other vitamins must be obtained from cine/laureates/1937/szent-gyorgyi-facts.html]. food [NIH - National Institutes of Health - http://ods.od.nih. gov/factsheets/list-VitaminsMinerals/].

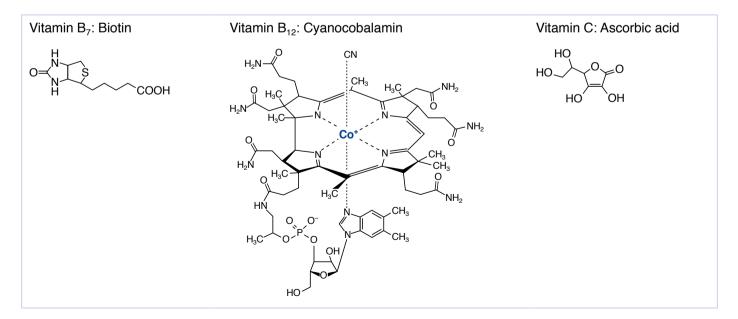
# Compounds of interest



### **Chemical structures of fat-soluble vitamins**

Vitamin A: all-trans-Retinol  CH <sub>3</sub> CH <sub>3</sub> CH <sub>2</sub> OH  CH <sub>3</sub> CH <sub>3</sub>	Vitamin E: alpha-Tocopherol  CH <sub>3</sub> HO CH <sub>3</sub> CH <sub>3</sub> CH <sub>3</sub> CH <sub>3</sub> CH <sub>3</sub> CH <sub>3</sub>
Vitamin D <sub>2</sub> : Ergocalciferol  H <sub>3</sub> C  CH <sub>3</sub> CH <sub>3</sub> CH <sub>3</sub> CH <sub>3</sub>	Vitamin K <sub>1</sub> : Phylloquinone  CH <sub>3</sub>
Vitamin D <sub>3</sub> : Cholecalciferol  H <sub>3</sub> C  CH <sub>3</sub> H <sub>3</sub> C  CH <sub>2</sub> HO	Vitamin K <sub>2</sub> : Menaquinone  O CH <sub>3</sub> CH <sub>3</sub> CH <sub>3</sub> CH <sub>3</sub> H n-1

### **Chemical structures of water-soluble vitamins**



## Typical samples, preparation

#### **Fat-soluble vitamins**

#### **Extraction of vitamins from food**

MN Appl. No. 303720

Column type:

CHROMABOND® XTR, 70 mL, 14.5 q REF 730507

Sample aspiration:

20 mL of the saponified sample solution are poured onto the CHROMABOND® column and allowed to adsorb for 15 min.

Column washing:

none

Elution:

100 mL of n-hexane (containing 5 mg BHT; 2,6-di-tert-butyl-pcresol).

Evaporation:

Eluates are evaporated to dryness in a stream of nitrogen, the residue is dissolved in 4 mL of HPLC mobile phase.

**Recovery rates in %** (n = 6):

Vitamin A 102  $\pm$  8 %, vitamin D<sub>3</sub> 105  $\pm$  7 %, vitamin E 96  $\pm$  9 %

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



#### Water-soluble vitamins

#### **Enrichment of ascorbic acid from urine**

MN Appl. No. 305600

Column type:

CHROMABOND® HILIC, 3 mL, 500 mg REF 730593

Sample pretreatment:

dilute 250 µL urine (10 µg/mL) with 750 µL THF or dioxane Column conditionina:

1 mL water

Equilibration:

6 mL THF or dioxane

Sample application:

dropwise pass 1 mL sample through the column

Column washing:

0.5 mL THF or dioxane

Elution:

2 mL water

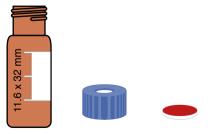
**Recovery rates:** 

Washing with THF: 97%,



An application of HILIC SPE is always worthwhile, if only moderate enrichment results are achieved with RP phases. This is usually the case for medium polar to polar analytes, i.e. substance groups like, e.g., amino acids, peptides, nucleotides, purine and pyrimidine bases. Also an enrichment of melamine, acrylamide, water-soluble vitamins and organic acids from food or the extraction of polar natural compounds from plant extracts, polar pesticides from environmental samples and polar active compounds from pharmaceuticals should be successful.

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



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

info@mn-net.com =

## **Analysis**



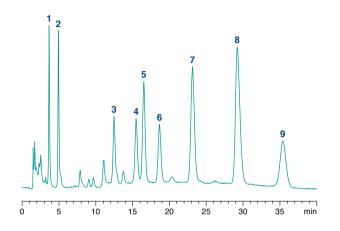
## Subsequent analysis: HPLC

**Fat-soluble vitamins** 

MN Appl. No. 121160

It is recommended to filter the prepared hydrophobic samples through disposable filters CHROMAFIL® Xtra PTFE-45/25 REF 729205

Column: EC 125/2 NUCLEODUR $^{\circ}$  C<sub>18</sub> Isis, 5  $\mu$ m REF 760412.20





#### Peaks

- 1. Vitamin A
- 2. Vitamin A acetate
- 3. Vitamin K<sub>2</sub>
- 4. Vitamin D<sub>2</sub>
- 5. Vitamin D<sub>3</sub>
- 6. Vitamin E (γ-tocopherol)
- 7. Vitamin E (a-tocopherol)
- 8. Vitamin E acetate (α-tocopherol acetate)
- 9. Vitamin K<sub>1</sub>

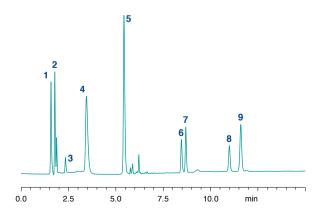


#### **Water-soluble vitamins**

MN Appl. No. 126730

It is recommended to filter the prepared hydrophilic samples through disposable filters CHROMAFIL® Xtra PET-45/25 REF 729220

Column: EC 100/3 NUCLEOSHELL $^{\circ}$  RP 18plus, 2.7  $\mu$ m REF 763234.30





#### **Peaks**

- 1. Vitamin B<sub>6</sub> (pyridoxamine)
- 2. Vitamin B<sub>1</sub> (thiamine)
- 3. Vitamin C (ascorbic acid)
- 4. Vitamin B<sub>6</sub> (pyridoxal)
- 5. Vitamin B<sub>6</sub> (pyridoxine)
- 6. Vitamin B<sub>9</sub> (folic acid)
- 7. Vitamin B<sub>12</sub> (cyanocobalamine)
- 8. Vitamin B<sub>2</sub> (riboflavin)
- 9. Vitamin B<sub>7</sub> (biotin)

Detailed conditions are available online at www.mn-net.com/apps

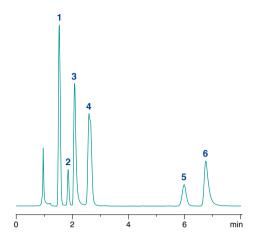
# Analysis -

## Subsequent analysis: HPLC

**Water-soluble vitamins** 

MN Appl. No. 122970

Column: EC 125/4 NUCLEODUR® HILIC, 3 μm REF 760531.40



#### Peaks:

- 1. Vitamin B<sub>3</sub> (nicotinamide)
- 2. Vitamin B<sub>7</sub> (vitamin B<sub>8</sub>, vitamin H, biotin)
- 3. Vitamin B<sub>6</sub> (pyridoxine)
- 4. Vitamin C (ascorbic acid)
- 5. Vitamin B<sub>12</sub> (cyanocobalamine)
- 6. Vitamin B<sub>1</sub> (thiamine)



## Product information -



## **Ordering information**

04	Duadwat	Daalaat	DEE
Step	Product	Pack of	REF
SPE	CHROMABOND® XTR, 70 mL, 14.5 g	30	730507
	Interesting alternative for mid-polar and polar analytes:	50	730593
	CHROMABOND® HILIC, 3 mL, 500 mg		
	CHROMABOND® vacuum manifold for 12 columns	1	730150
Filtration	Disposable filters CHROMAFIL® Xtra PTFE-45/25	100	729205
	Disposable filters CHROMAFIL® Xtra PET-45/25	100	729220
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	100	702287.1
	with septum Silicone white / PTFE red		
HPLC 1			
Column	EC 125/2 NUCLEODUR <sup>®</sup> C <sub>18</sub> Isis, 5 μm	1	760412.20
Guard column	EC 4/2 NUCLEODUR® C <sub>18</sub> Isis, 5 μm	3	761912.20
Guard column holder	Column Protection System	1	718966
HPLC 2			
Column	EC 100/3 NUCLEOSHELL® RP 18plus, 2.7 μm	1	763234.30
Guard column	EC 4/3 NUCLEOSHELL® RP 18plus, 2.7 μm	3	763238.30
Guard column holder	Column Protection System	1	718966
HPLC 3			
Column	EC 125/4 NUCLEODUR® HILIC, 3 μm	1	760531.40
Guard column	EC 4/3 NUCLEODUR <sup>®</sup> HILIC, 3 μm	3	761961.30
Guard column holder	Column Protection System	1	718966

## Visit us at www.mn-net.com/chroma to get more helpful information

#### **Selection tools for**

Vials and caps

Syringe filters

### **Troubleshooting guides**

GC and HPLC

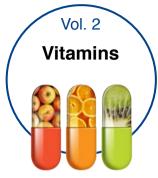
## **Detailed product information**

...and much more











**Food safety** made easy with products from **MACHEREY-NAGEL** 



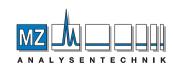








local distributor



**AUTHORIZED DISTRIBUTOR** 

MZ-Analysentechnik GmbH Barcelona-Allee 17 • D-55129 Mainz Tel +49 6131 880 96-0 Fax +49 6131 880 96-20 e-mail: info@mz-at.de www.mz-at.de

## MACHEREY-NAGEL

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

MACHEREY-NAGEL GmbH & Co. KG · Neumann-Neander-Str. 6-8 · 52355 Düren · Germany France: **MACHEREY-NAGEL EURL** 

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

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



www.mn-net.com