CHROMATOGRAPHY

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

Vol. 2

Vitamins

MACHEREY-NAGEL
www.mn-net.com

ADIS INTERNATIONAL

MN Since 1911

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**

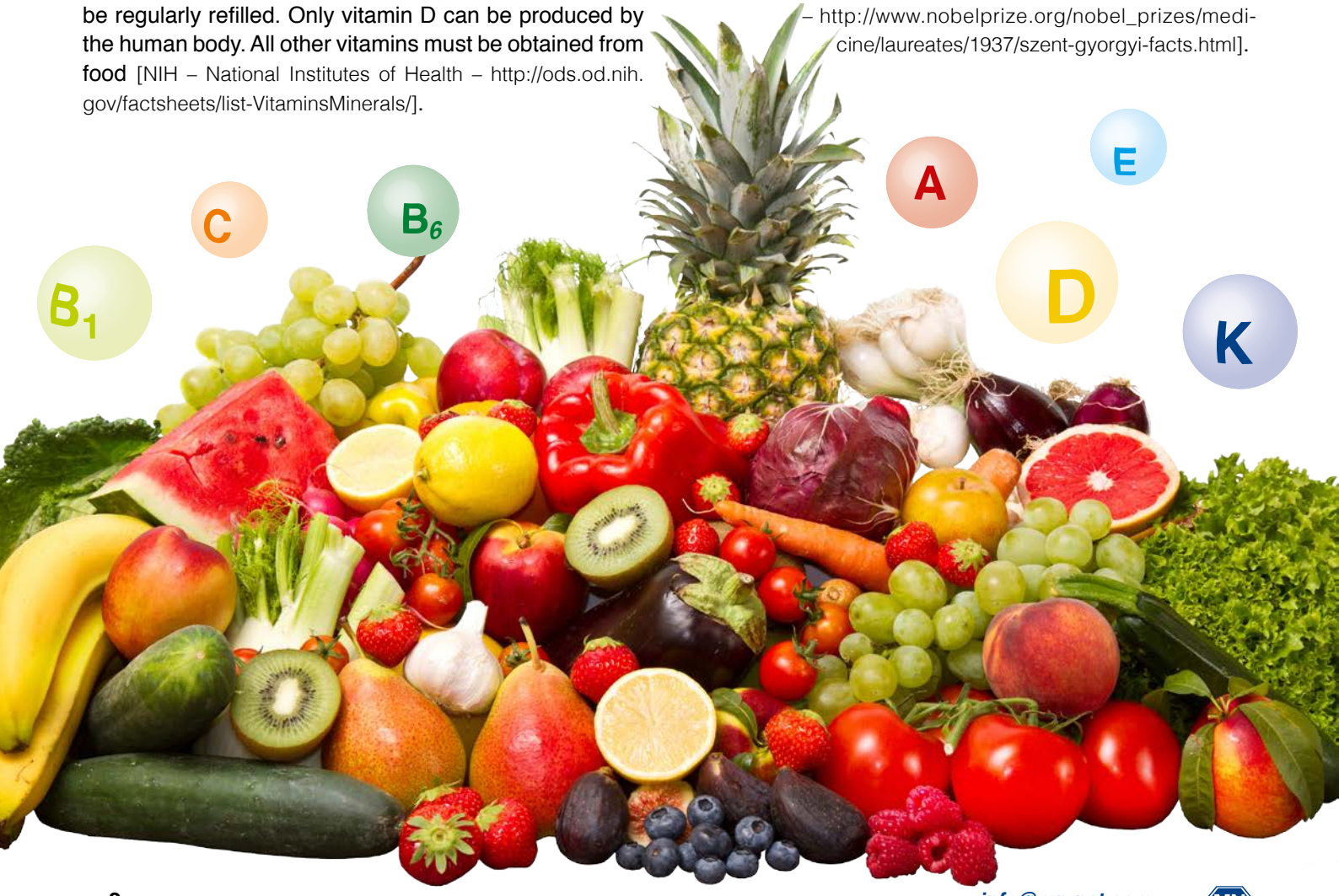
They are not visible, they have no taste – but vitamins are essential for our health.

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. There are the fat-soluble and water-soluble vitamins. Since fat-soluble vitamins A, D, E and K can be stored in the fat deposits of the body, they need not be ingested daily. This is different for the water-soluble vitamins. The eight B vitamins and vitamin C cannot be stored but must be regularly refilled. Only vitamin D can be produced by the human body. All other vitamins must be obtained from food [NIH – National Institutes of Health – http://ods.od.nih.gov/factsheets/list-VitaminsMinerals/].

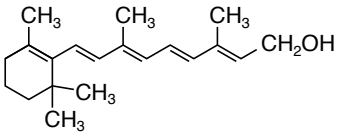
For example, vitamin C or ascorbic acid was discovered by Albert Szent-Györgyi in the early 20th century. He isolated vitamin C from red peppers (paprika). The chemical structure was determined in 1933 by Norman Haworth. Both received the 1937 Nobel Prize for medicine and chemistry [Nobelprize.org – official web site of the Nobel Prize – http://www.nobelprize.org/nobel_prizes/medicine/laureates/1937/szent-gyorgyi-facts.html].



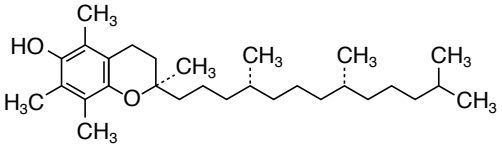
Compounds of interest

Chemical structures of fat-soluble vitamins

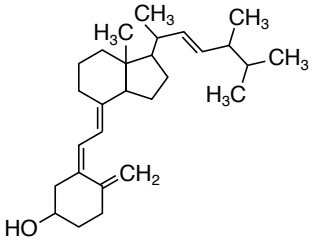
Vitamin A: all-*trans*-Retinol



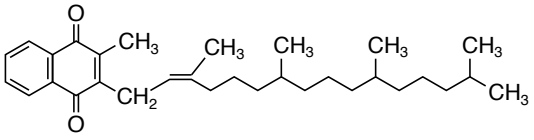
Vitamin E: alpha-Tocopherol



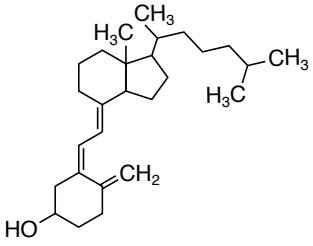
Vitamin D_2: Ergocalciferol



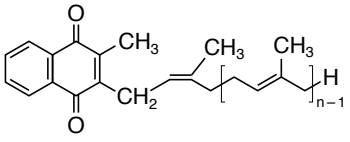
Vitamin K_1: Phylloquinone



Vitamin D_3: Cholecalciferol

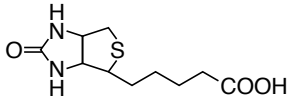


Vitamin K_2: Menaquinone

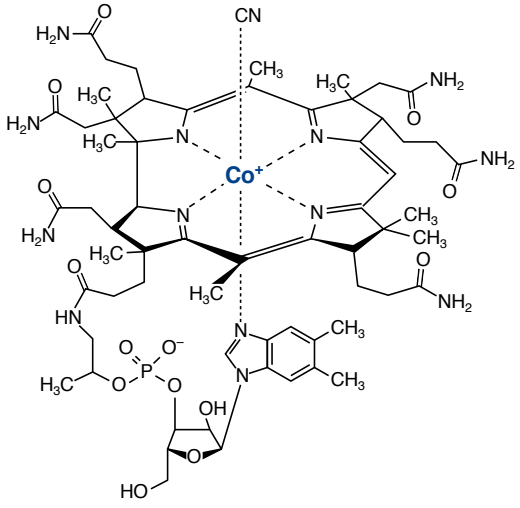


Chemical structures of water-soluble vitamins

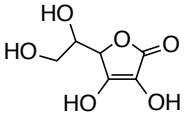
Vitamin B_7: Biotin



Vitamin B_{12}: Cyanocobalamin



Vitamin C: Ascorbic acid



Typical samples, preparation

Fat-soluble vitamins

Extraction of vitamins from food

MN Appl. No. 303720

Column type:
CHROMABOND® XTR, 70 mL, 14.5 g
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-*p*-cresol).

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 ± 8 %, vitamin D_3 105 ± 7 %, vitamin E 96 ± 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 conditioning:
1 mL water

Equilibration:
6 mL THF or dioxane

Sample application:
dropwise pass 1 mL sample through the column

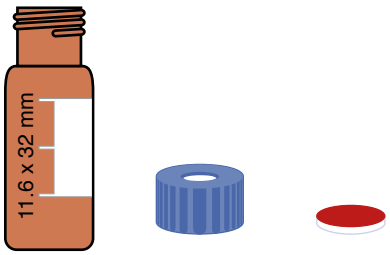
Column washing:
0.5 mL THF or dioxane

Elution:
2 mL water

Recovery rates:
Washing with THF: 97 %,
washing with dioxane: 93 %

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*

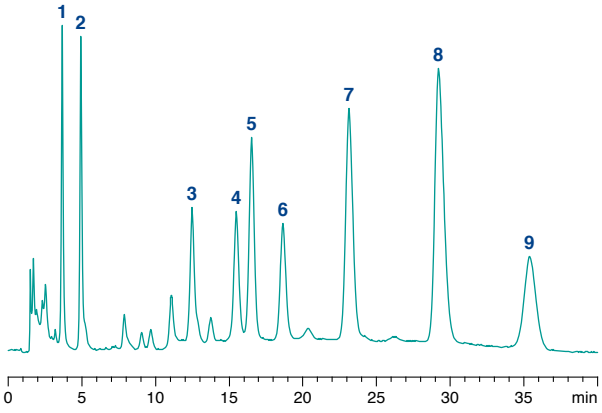
Subsequent analysis: HPLC

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® C_{18} Isis, 5 µm
REF 760412.20



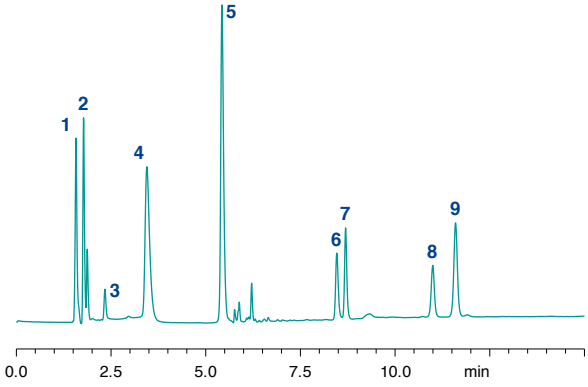
Peaks
1. Vitamin A
2. Vitamin A acetate
3. Vitamin K_2
4. Vitamin D_2
5. Vitamin D_3
6. Vitamin E (γ-tocopherol)
7. Vitamin E (α-tocopherol)
8. Vitamin E acetate (α-tocopherol acetate)
9. Vitamin K_1

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® RP 18plus, 2.7 µm
REF 763234.30



Peaks
1. Vitamin B_6 (pyridoxamine)
2. Vitamin B_1 (thiamine)
3. Vitamin C (ascorbic acid)
4. Vitamin B_6 (pyridoxal)
5. Vitamin B_6 (pyridoxine)
6. Vitamin B_9 (folic acid)
7. Vitamin B_{12} (cyanocobalamine)
8. Vitamin B_2 (riboflavin)
9. Vitamin B_7 (biotin)

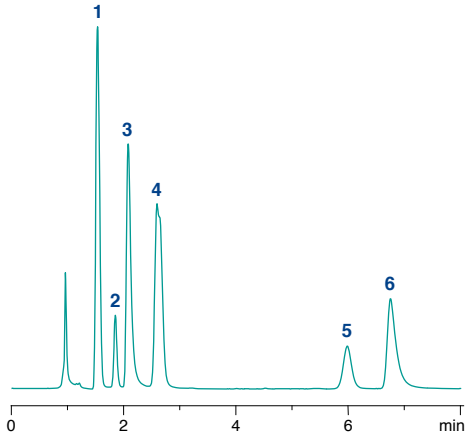
Detailed conditions are available online at *www.mn-net.com/apps*

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_3 (nicotinamide)
2. Vitamin B_7 (vitamin B_8, vitamin H, biotin)
3. Vitamin B_6 (pyridoxine)
4. Vitamin C (ascorbic acid)
5. Vitamin B_{12} (cyanocobalamine)
6. Vitamin B_1 (thiamine)

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!

Ordering information

Step	Product	Pack of	REF
SPE	CHROMABOND® XTR, 70 mL, 14.5 g	30	730507
	Interesting alternative for mid-polar and polar analytes: CHROMABOND® HILIC, 3 mL, 500 mg	50	730593
	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 with septum Silicone white / PTFE red	100	702287.1
HPLC 1			
Column	EC 125/2 NUCLEODUR® C_{18} Isis, 5 µm	1	760412.20
Guard column	EC 4/2 NUCLEODUR® C_{18} 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® HILIC, 3 µm	3	761961.30
Guard column holder	Column Protection System	1	718966

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