MACHEREY-NAGEL
Chromatography application note

MN application note 06/2017

Determination of water soluble vitamins in food supplements with HPLC-UV

MACHEREY-NAGEL application department · Dr. H. R. Wollseifen · T. Kretschmer

Abstract

This application note presents a method using high performance liquid chromatography for the determination of water soluble vitamins in food supplements. Using stationary phases with 5 µm particle size makes this method suitable for simple HPLC-systems.

Introduction

The supply of food supplements (NEM) in retail is very extensive, including many vitamin-containing NEMs, which are intended for different consumer groups to close nutritional gaps. Toxicological and nutritional physiological aspects are repeatedly discussed in the context of the use of vitamins in foodstuffs [1, 2]. The amount of vitamins in food supplements have to be labeled according to food law [3]. To avoid exceeding the safe quantities, a simple and reliable analysis is needed to determine and quantify the water soluble vitamins.

Compounds of interest

Analyte	Analyte
Pyridoxamine	Nicotinamide
Thiamine	Phanthothenic acid
Ascorbic acid	Cyanocobalamin
Pyridoxal	Folic acid
Pyridoxine	Riboflavin
Nicotinic acid	Biotin

Table 1: Overview of the analytes.

Subsequent analysis: HPLC-UV

Chromatographic conditions:

Column: EC 250/4.6 NUCLEODUR® C_{18} Gravity-SB, 5 µm, (REF 760619.46)

Eluent A: 20 mmol/L NaH_2PO_4 pH 3.0 (addition of 4.95 g NaH_2PO_4 and 565 µL concentrated phosphoric acid, mixture filled up to 2 L with water)

Eluent B: Methanol – acetonitrile (70:30, v/v)

Gradient: hold 0 % B for 0.5 min, 0-75 % B in 15 min, hold 75 % B for 5 min, 75-0 % B in 5 min, hold 0 % B for 5.5 min

Flow rate: 1.0 mL/min

Temperature: 35 °C

Injection volume: 10 µL

Detection wavelengths:

Analyte	Detection wavelengths in nm
Thiamine	246
Ascorbic acid	300 (optimum at 243)
Pyridoxine	291
Nicotinamide	261
Panthothemic acid	205
Cyanocobalamin	361
Folic acid	283
Riboflavin	268
Biotin	205

Table 2: Detection wave length for the analysis of water soluble vitamins.

Chromatogram of a mixture of 12 vitamins

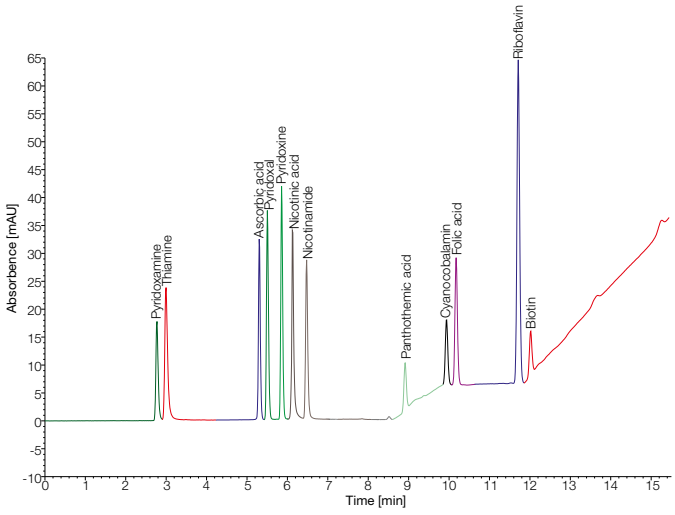

Figure 1: Chromatogram of HPLC-UV analysis of a mixture of 12 vitamins (each analyte β = 10 µg/ml, aqueous), each analyte is shown at detection wave length.

MACHEREY-NAGEL GmbH & Co. KG · Neumann-Neander Str. 6–8 · 52355 Düren · Germany
www.mn-net.com · info@mn-net.com

Determination of water soluble vitamins in food supplements with HPLC-UV

Analyte	Retention time (min)
Thiamine	3.00
Ascorbic acid	5.31
Pyridoxine	5.86
Nicotinamide	6.47
Panthothemic acid	8.91
Cyanocobalamin	9.34
Folic acid	10.17
Riboflavin	11.71
Biotin	12.27

Table 3: Retention times of HPLC analysis.

Chromatogramm of an extract of a vitamin pill

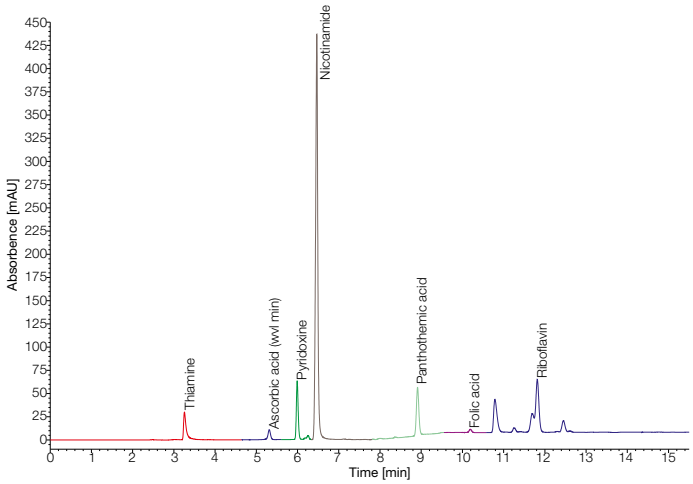

Figure 2: Chromatogram of HPLC-UV analysis of a vitamin pill, each analyte is shown at detection wave length.

Sample preparation

- Homogenize a vitamin pill
- Dissolve sample in 100 mL water
- Filter sample extract through a syringe filter (CHROMAFIL® PTFE-20/13 pore size 0.2 µm), (REF 729208)
- Sample extract ready for injection

Analyte	Amount of analyte in sample (µg/mL)	Recovery rate according to product labeling in %	Proof of labeling
Thiamine	9.6	105.7	✓
Pyridoxine	12.2	105.4	✓
Nicotinamide	157.2	108.9	✓
Pantothemic acid	54.2	118.5	✓
Folic acid	0.8	50.9	*
Riboflavin	20.2	174	✓
Ascorbic acid	786.2	95.3	✓

*(to proof, if the declared amount is calculated as folic acid or as another vitamin equivalent)

Table 4: Results of the analysis of a vitamin pill.

Chromatogram of an extract of a vitamin pill with extended-release function

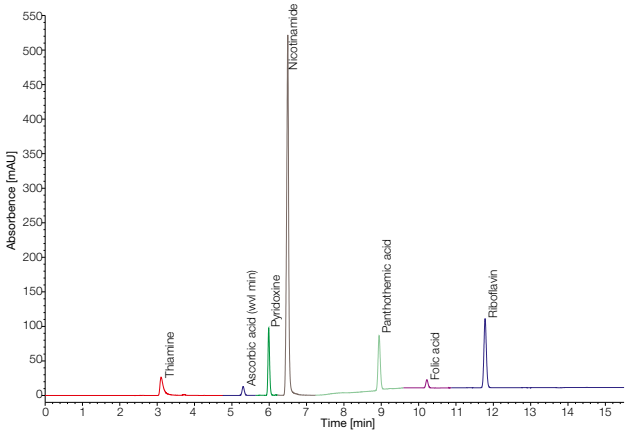

Figure 3: Chromatogram of HPLC-UV analysis of a vitamin pill with extended-release function, each analyte is shown at detection wave length.

Sample preparation

- Homogenize a vitamin pill
- Dissolve sample in 100 mL water
- Filter sample extract through a syringe filter (CHROMAFIL® PTFE-20/13 pore size 0.2 µm), (REF 729208))
- Sample extract ready for injection

Analyte	Amount of analyte in sample (µg/mL)	Recovery rate according to product labeling in %	Proof of labeling
Thiamine	12.2	111	✓
Pyridoxine	18.5	132	✓
Nicotinamide	196.6	123	✓
Pantothemic acid	86.6	144	✓
Folic acid	2.6	128	✓
Riboflavin	22.3	159	✓
Ascorbic acid	1098.8	137	✓

Table 5: Results of the analysis of a vitamin pill with extended-release function.

Determination of water soluble vitamins in food supplements with HPLC-UV

Chromatogram of an extract of a vitamin pill for children

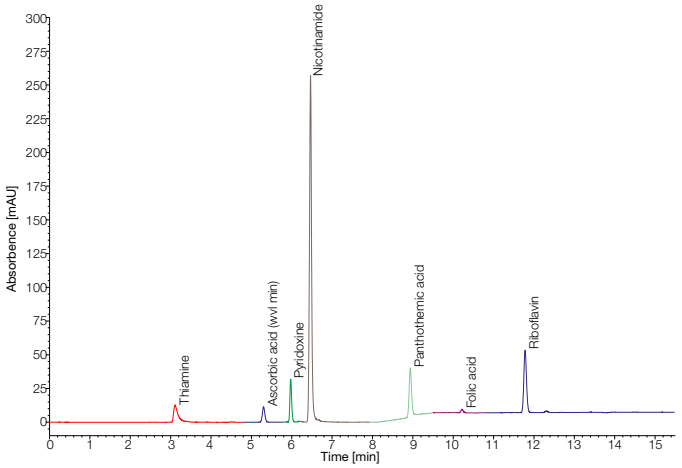

Figure 4: Chromatogram of HPLC-UV analysis of a vitamin pill for children, each analyte is shown at detection wave length.

Sample preparation

- Homogenize 2 vitamin pills
- Dissolve sample in 100 mL water
- Filter sample extract through a syringe filter (CHROMAFIL® PTFE-20/13 pore size 0.2 µm), REF 729208))
- Sample extract ready for injection

Analyte	Amount of analyte in sample (µg/mL)	Recovery rate according to product labeling in %	Proof of labeling
Thiamine	7.1	93	✓
Pyridoxine	6.3	132	✓
Nicotinamide	93.3	98	✓
Pantothemic acid	40.5	107	✓
Folic acid	0.6	44	*
Riboflavin	10.6	124	✓
Ascorbic acid	775.7	117	✓

*(to proof, if the declared amount is calculated as folic acid or as another vitamin equivalent)

Table 6: Results of the analysis of a vitamin pill for children.

Chromatogram of an extract of a vitamin pill containing vitamin B12 and B5

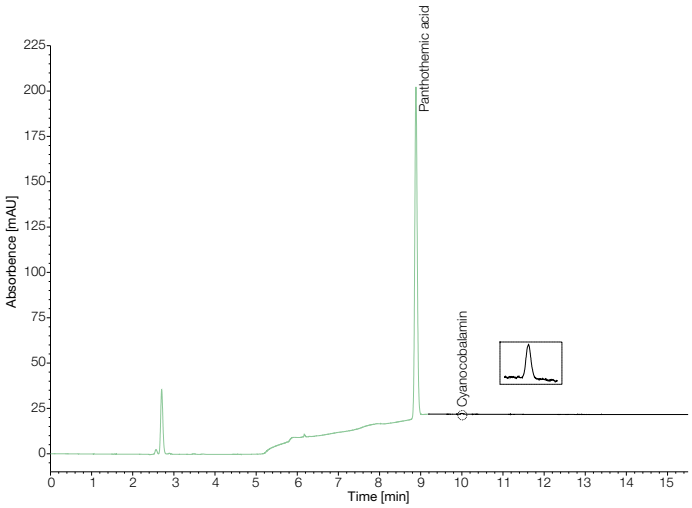

Figure 5: Chromatogram of HPLC-UV analysis of a vitamin pill containing vitamin B12 and B5, each analyte is shown at detection wave length.

Sample preparation

- Homogenize a vitamin pill
- Dissolve sample in 100 mL water
- Filter sample extract through a syringe filter (CHROMAFIL® PTFE-20/13 pore size 0.2 µm), (REF 729208))
- Sample extract ready for injection

Analyte	Amount of analyte in sample (µg/mL)	Recovery rate according to product labeling in %	Proof of labeling
Pantothemic acid	0.38	149.3	✓
Cyanocobalamin	197.23	109.2	✓

Table 7: Results of the analysis of a vitamin pill containing vitamin B12 and B5.

Conclusion

Basically, the determination of B vitamins by HPLC-UV is difficult because of the very different contents in food supplements. However, the results show that the proposed method for the determination of B vitamins for many food supplements is a suitable alternative to enzymatic methods. Sample preparation presented in this application note is fast and simple. In order to also detect ascorbic acid in one run, the quantification takes place with the minimum absorption wavelength of this compound. The quantification of the B vitamins must be at the absorption maximum, so multi-wavelength or diode array equipment is required for detection.

Determination of water soluble vitamins in food supplements with HPLC-UV

References

1. A. Domke, R. Großklaus, B. Niemann, H. Przyrembel, K. Richter, E. Schmidt, A. Weißenborn, B. Wörner, R. Ziegenhagen, Use of vitamins in foods -Toxicological and nutritional physiological aspects, BfR 2004.
2. M. Glanzig, ERNÄHRUNG/NUTRITION, 2006, 30/9, 363-368.
3. The European Union‘s Food Supplements Directive of 2002.

Additional information

The following applications regarding “Determination of water soluble vitamins in food supplements with HPLC-UV” and further applications can be found on our online application database at *www.mn-net.com/apps*:

MN Appl. No. 128040 (standard solution)
MN Appl. No. 128050 (vitamin pill)
MN Appl. No. 128060 (vitamin pill with extended-release function)
MN Appl. No. 128070 (vitamin pill for children)
MN Appl. No. 128080 (vitamin pill containing B12 and B5)

Product information

The following MACHEREY-NAGEL products have been used in this application note:

REF 729208, CHROMAFIL® Xtra PTFE-20/13
REF 760619.46, EC 250/4.6 NUCLEODUR® C_{18} Gravity-SB, 5 µm
REF 702293 Screw neck vials N 9, 1.5 mL
REF 702107 N 9 PP Screw cap, yellow, center hole,
silicone white / PTFE red