

## NIACIN (VITAMIN B3)

### HPLC Analysis via Photochemical Post-Column Derivatization

This application note is based on the EN 15652:2009 [1], nevertheless containing some optimizing changes.

Niacin (or Nicotinic acid) is a vitamin of the B-complex. The names vitamin B3, scarcely B5 or PP factor (pellagra-preventing factor), for nicotinic acid are obsolete today. Nicotinic acid was discovered in 1867 during the oxidation of nicotine; its physiological activity was detected in 1934.

Niacin can be found in all living cells and is stored in the liver. It is an important part of different coenzymes (NAD<sup>+</sup>, NADP<sup>+</sup>) and thus is of central importance for the metabolism of proteins, fats and carbohydrates.

Nicotinic acid is more tolerant against heat, light and aerial oxygen than other vitamins of the B-group.

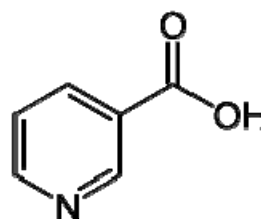


Fig. 1: Structure of niacin



Fig. 2: UVE, module for photochemical derivatization

### Method Description

There are three extraction procedures for the detection of niacin in food. The extraction can be done by acidic (option A), enzymatic (option B) or acidic/alkaline (option C) hydrolysis of food samples. Subsequently niacin is detected via HPLC with fluorimetric detection after post-column derivatization with UV radiation. Applying option A and B niacin is determined as the sum of nicotine amide and nicotinic acid. Niacin is expressed as nicotinic acid after correction of the molecular weight. Applying option C niacin is determined and expressed as nicotinic acid, because all nicotine amide is converted to nicotinic acid during the alkaline treatment.

# APPLICATION NOTE | AN0040

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## HPLC Conditions

HPLC	
Operating Mode	Isocratic
Eluant	KH <sub>2</sub> PO <sub>4</sub> 0.035 mol/L (4.77 g/L) <b>ATTENTION!</b> The eluant is purely aqueous, an appropriate RP phase has to be used!
Degassing	Helium or vacuum degassed
HPLC Column	Phenomenex Luna 5 µm C18 (2), 100 Å, 150 x 2.0 mm
Column Temperature	30 - 40 °C
Flow Rate	1.0 mL/min
Injection Volume	20 µL
Post Column Derivatization	
Photochemical Reactor UVE	254 nm
Reactor Volume	1 mL
Detection	
Measuring Mode	Fluorescence detection
Excitation Wavelength	322 nm
Emission Wavelength	380 nm
Cell	Analytical; tolerating pressure of up 7 bar

Ordering Information	
14368	Photochemical reactor UVE, 1 mL reactor volume
10563	Spare UVC lamp
10520	Spare reactor loop, 1 mL

## Literature

- 1) European Institute for Standardization: EN 15652:2009, *Detection of Vitamin B3 (Niacin) with HPLC*, 2009
- 2) Direct notice by Kantonales Laboratorium Thurgau, Switzerland