

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⁺, NADP⁺) 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.

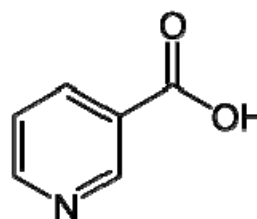


Fig. 1: Structure of niacin



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

HPLC Conditions

HPLC	
Operating Mode	Isocratic
Eluant	KH ₂ PO ₄ 0.035 mol/L (4.77 g/L) ATTENTION! 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 A, 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	
10519	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