APPLICATION NOTE

VPE184J046250

HPLC Analysis of the three Vitamins Thiamine (B_1), Niacin (B_3), and Pyridoxine (B_6) in Beverages and Concentrates

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Abstract

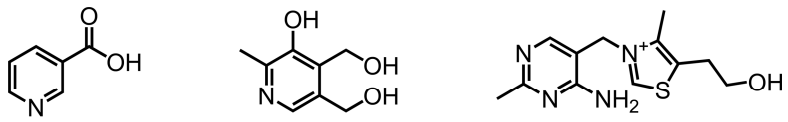
This application presents a fast and simple method for the determination of three B vitamins in beverages and concentrates. Ion-pair chromatography in reversed-phase mode is used to enable separation of these highly polar compounds from a standard solution and strawberry syrup. Detection is carried out by UV absorption at wavelengths of 260 nm (**NIACIN**), 290 nm (**PYRIDOXINE**), and 246 nm (**THIAMINE**).

Keywords

Water-soluble Vitamins
Reversed-Phase Ion-Pair Chromatography
Beverages and Food Analysis

Compound Information and Chemical Structures

COMPOUND NAME	CLASSIFICATION
NIACIN (B_3) / nicotinic acid	water-soluble vitamin / coenzyme precursor NAD and NADP
PYRIDOXINE (B_6)	water-soluble vitamin / coenzyme amino acid metabolism
THIAMINE (B_1)	water-soluble vitamin / coenzyme carbohydrate metabolism



NIACIN (B_3) **PYRIDOXINE (B_6)** **THIAMINE (B_1)**

Figure 1. Chemical structures of the three water-soluble B vitamins **NIACIN** (B_3), **PYRIDOXINE** (B_6), and **THIAMINE** (B_1).

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Chromatographic Conditions

COLUMN NAME / PARTICLE SIZE LENGTH × INNER DIAMETER	VDSpher PUR 100 C18-H 5 µm 250 × 4.6 mm	ORDER NUMBER VPE184J046250
SEPARATION MODE	reversed-phase ion-pair chromatography	
MOBILE PHASE	8% acetonitrile and 0.5% triethylamine in 10 mM KH_2PO_4 at pH 2.8 containing 5 mM 1-heptanesulfonic acid	
ELUTION CONDITIONS	isocratic elution	
FLOW RATE	1.0 mL·min^{-1}	
INJECTION VOLUME	20 µL	
TEMPERATURE	ambient	
DETECTION	UV 260 nm **NIACIN**, 290 nm **PYRIDOXINE**, 246 nm **THIAMINE**	
PRESSURE	120 bar	
HPLC SYSTEM	Perkin Elmer Series 200 System Thermo Scientific Accela PDA Detector	
SAMPLE PREPARATION	N/A	

Chromatographic Analysis

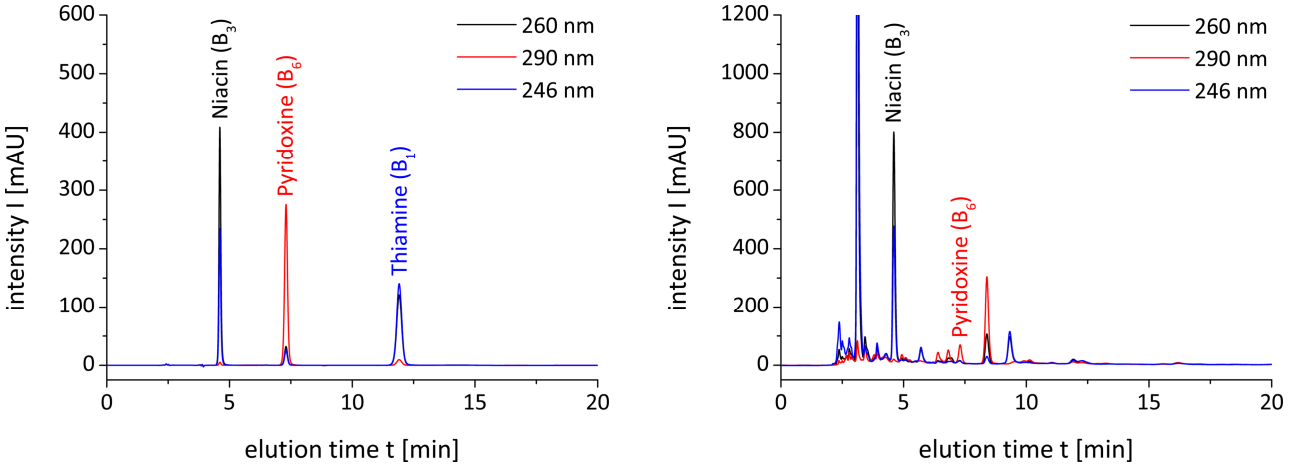

Figure 1. LEFT: HPLC chromatogram of a standard solution containing the three water-soluble B vitamins detected at 260 nm (**NIACIN**), 290 nm (**PYRIDOXINE**), and 246 nm (**THIAMINE**). RIGHT: HPLC chromatogram of a strawberry syrup sample containing two of three water-soluble B vitamins detected at 260 nm (**NIACIN**), and 290 nm (**PYRIDOXINE**).

References

Y. Maeda *et al.*, *J. Assoc. Off. Anal. Chem.* **1989**, *72*, 244 – 247.

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