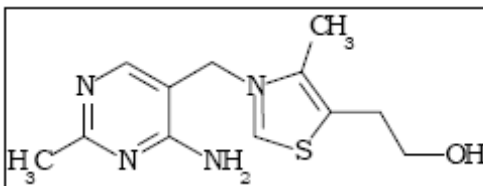


APPLICATION NOTE

VITAMIN B1 (THIAMIN)



Vitamin B1 (thiamine) belongs to the group of vitamins that are soluble in water. In food, thiamine can be detected in its free form as well as phosphoric acid ester and bound to proteins. Being one of the least stable vitamins, it is easily decomposed when food is produced or prepared. A permanent supply of thiamine is necessary, because it acts as a coenzyme in a number of enzymatic reactions, e.g. pyruvate decarboxylase. Excessive consumption of sugar therefore results in a thiamine deficiency.



Structure of Vitamin B1 (Thiamin)

Description of the Method

Thiamine is extracted from food with acid hydrolysis and enzymatically dephosphorylated. Quantification is performed with HPLC by means of post column derivatization. Vitamin B1 is derivatized in a one-step reaction with an alkaline potassium hexacyanoferrate-(III) solution to the fluorescent oxidation product thiochrome.

For the determination of thiamin with HPLC a single-pump Pickering system is used, which is up to the standard of the DIN EN 14122.

HPLC Conditions and Derivatization Parameters

HPLC	
Operation Mode	Isocratic
Eluent	MeOH/phosphate buffer pH 3.5 containing 1 g/L tetraethylammonium chloride and 5 mmol/L sodium heptansulfonate (35/65 v/v)
Degassing	Helium- or vacuum degassed
HPLC Column	RP phase, 5 µm particle size, 4.0 to 4.6 mm x 100 to 250 mm
Flow Rate	0.5 mL/min
Injection Volume	up to 200 µL
Post-Column Derivatization	
Pinnacle PCX	Single-pump; 500 µL reactor
Column Oven	40 °C
Reactor Volume	500 µL
Reactor Temperature	30 °C
Reagent	Alkaline* potassium hexacyanoferrat(III) solution
Reagent Flow	0.3 mL/min
Detection	
Detection Type	Fluorescence detection
Excitation Wavelength	368 nm
Emission Wavelength	440 nm
Flowcell	Analytic; pressure stable up to 7 bar

* The sodium hydroxide concentration of 200 g/L given in the norm may be significantly reduced to minimize viscosity!

APPLICATION NOTE

Order Information

Order Number	Description
1153-1022	PINNACLE PCX – Single-pump; 500 µL reactor

Literature

- 1) Deutsches Institut für Normung: DIN EN 14122, *Bestimmung von Vitamin B1 mit HPLC*, **2001**
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