Poster No. 3b

Simultaneous determination of vitamins B_1, B_2 and B_6 in whole blood by liquid chromatography-tandem mass spectrometry

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Background

Water-soluble vitamins B_1, B_2 and B_6 are involved in cell energy metabolism. They are chemically and functionally diverse.

Vitamin B_1 (thiamine diphosphate, **TDP**): Coenzyme in the catabolism of sugars and amino acids.

Vitamin B_2 (flavin adenine dinucleotide, **FAD**): Precursor of cofactors for flavoprotein enzyme reactions.

Vitamin B_6 (pyridoxal and pyridoxal phosphate, **PL** & **PLP**): Coenzymes in many metabolic reactions.

Vitamin B Deficiency

Possible Causes:

- Malnutrition
- Diabetes and other metabolic diseases
- Alcoholism
- Hemodialysis

Clinical manifestations:

- Alterations of the skin
- Neurological diseases
- Anemia (various forms)

Accurate diagnosis and treatment of the underlying disease requires simultaneous quantification of the most critical B vitamins in one assay.

- Whole blood – simple sample preparation
- LC-MS/MS for specificity and low-level detection

1-Step Sample Preparation

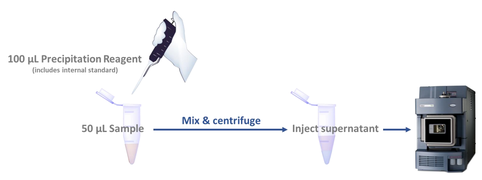


Fig. 1: The 1-step sample preparation consists of protein precipitation with an acidic reagent followed by mixing and centrifugation. The supernatant is then injected into the LC-MS/MS system.
Note that the internal standard for FAD is unstable under acidic conditions and must be reconstituted in water. All other standards can be reconstituted directly in the precipitation reagent.

Instrument Set-Up: UPLC-MS/MS

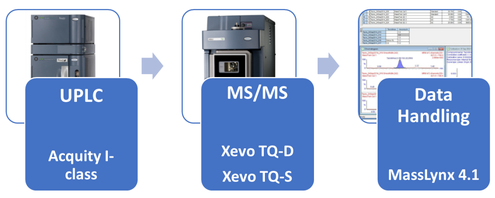


Fig. 2: The straightforward system setup includes a binary pump, analytical column and a tandem quadrupole mass spectrometer in positive MRM mode. The initial eluate (up until gradient time of 1.2 minutes) is diverted to waste using the built-in waste valve of the mass spectrometer. With this configuration, no on-line SPE is necessary.

Sample chromatogram

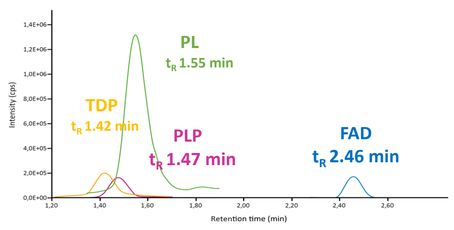


Fig. 3: All four analytes elute from the column within 3 minutes. The total gradient time is 4.5 minutes. Chromatogram shown was recorded with a TQ-D mass spectrometer.

Conclusions

The method facilitates the **simultaneous determination of vitamin B_1, B_2 and B_6** from 50 microliters of whole blood.

It **meets research laboratory requirements** in terms of precision, accuracy, linear range and detection limits.

RECIPE

New UPLC-MS-based method quantifies **vitamins B_1, B_2 and B_6 in whole blood** with 1-step sample preparation.

Method Information:

Methods:

- Quantification of TDP, FAD, PL and PLP in human whole blood.
- Protein precipitation followed by direct injection of the supernatant.
- Internal standards dissolved in water (FAD) or in precipitation solvent (all others).
- Chromatography:
  - Reversed-phase-type column
  - Binary gradient
  - Total run time of 4.5 minutes.
- Mass spectrometry:
  - Ionization: Electrospray, positive mode
  - Multiple reaction monitoring (MRM).
  - Quantification based on stable isotope-labeled internal standards
  - 4 levels of whole blood matrix calibrators.
- Method performance evaluation: ClinMass® LC-MS/MS **Complete Kit for the Determination of Vitamin B_1, B_2 and B_6 in Whole Blood** (RECIPE Chemicals + Instruments GmbH, Munich, Germany).
- Instrumentation:
  - Method development (research use only): ACQUITY I-class UPLC system (Binary Solvent Manager, Sample Manager with Flow-Through Needle, Column Manager); Xevo TQ-S tandem quadrupole mass spectrometer (Waters, Eschborn, Germany).
  - Method verification: Xevo TQ-D tandem quadrupole instrument coupled to the above UPLC system.
- Comments:

Fast processing of the samples was required due to the limited stability of B vitamins in biological matrix. Protein precipitation followed by mixing and centrifugation yielded a supernatant suitable for direct injection into the LC-MS/MS system.

ClinMass ® Kit for Vitamins B_1, B_2, B_6:

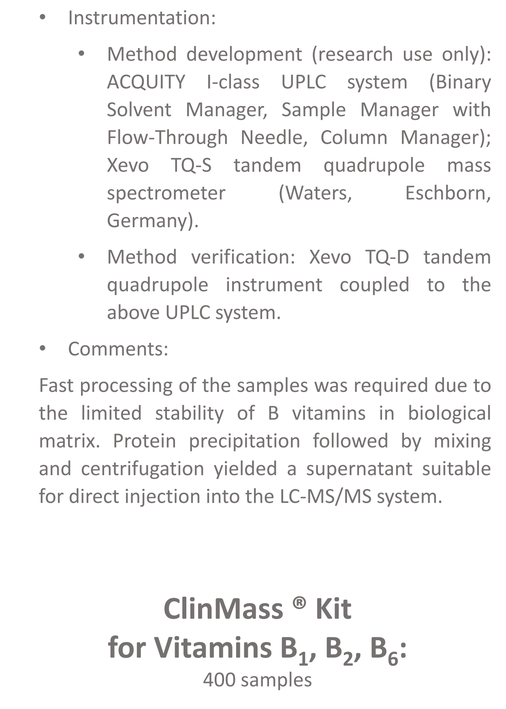
400 samples

Autosampler wash solution
Mobile phases A and B
Internal standards, lyophilized
Whole blood calibrators, level 0-3, lyophilized
Sample preparation vials
Precipitation reagent
Manual

Internal standards:
d_3-TDP $^{13}C_5$-FAD d_3-PLP d_3-PL

Calibration results

Cal 1 chromatograms & linearity



Analytical Performance

Xevo TQ-D and Xevo TQ-S

		TPP	FAD	PLP	PL
Linear range [µg/L] [a]		16.1 - 174	63.0 - 745	5.97 - 98.7	10.8 - 46.6
Target values [µg/L] [b]		43.6 / 111	187 / 195	14.5 / 28.7	15.9 / 23.2
LLoQ [µg/L] [c]	**Xevo TQ-D**	14.3	6.1	5.9	0.61
	Xevo TQ-S	3.6	0.51	0.96	0.10
Intraday precision: CV [%] [b]	**Xevo TQ-D**	3.7 / 8.2	8.2 / 4.6	7.2 / 9.8	5.1 / 5.9
	Xevo TQ-S	4.1 / 4.2	7.6 / 2.0	7.7 / 7.4	2.7 / 4.1
Accuracy [%] [b]	**Xevo TQ-D**	109 / 98	92 / 98	101 / 102	105 / 104
	Xevo TQ-S	97 / 95	95 / 93	98 / 105	100 /96

[a] Calibration range of ClinCal® samples MS13013 [b] ClinChek® samples MS13082, n = 5 [c] Calculated from S/N

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