

VITAMIN B1 IN WHOLE BLOOD BY FLUORIMETRY - FAST – Code Z85010

INTRODUCTION

Vitamin B1 is a water soluble vitamin that is part of the B group. Vitamins of this group involved in the metabolism of carbohydrates, fats and proteins and are thus essential for energy production and for the normal functioning of different organs and systems.

The most important sources of vitamin B1 include whole grains and meat, but it is also present in some pulses (chickpeas, white beans, Spanish beans), wheat germ, liver, beef, meat, pork, in salmon and dried sunflower seeds. The yeast contains significant quantities.

It is rapidly absorbed in the lower part of the small intestine and distributed to the liver, kidney and heart. The organism must be replenished daily, taking into account that it is rapidly destroyed by alcohol, and by excessive amounts of sugar and smoke.

Vitamin B1 is essential for the proper functioning of the nervous system, heart and muscles. It also contributes to the formation of red blood cells and plays an adjuvant in the treatment of herpes zoster. Among the symptoms that may indicate a deficiency of vitamin B1 are loss of appetite and fatigue and, in severe cases, nausea, vomiting, and a general weakening of the muscles.

Today, severe deficiency of this vitamin, is most common in alcoholics, leading to Beri-Beri. This is accompanied by damage to the nervous system which then extends to the cardiovascular and gastrointestinal systems. The most prominent symptoms are neurological ones: fatigue, irritability, poor memory, and later pain in the peripheral nerves (polyneuropathy).

Intoxication with this vitamin is very unlikely since the excess is usually excreted from the body through urine. The recommended daily intake of vitamin B1 is 1.2 mg for adult men (over 60 this drops to 0.8 mg) and for women aged 14 years and over 0.9 mg (pregnant women: 1 mg).

A supplement of vitamin B1 may be necessary for those suffering from cirrhosis or other shortages caused by infections or diseases such as hyperthyroidism or diabetes. Also those who are recovering from a surgery may need supplements of vitamin B1. The active form in the metabolism is thiamine pyrophosphate (TPP).

Bibliography: 1- J. Serb. Chem. Soc. 70 (10) 1229-1235 (2005)

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This product fulfills all the requirements of Directive 98/79/EC on in vitro diagnostic medical devices (IVD). The declaration of conformity is available upon request.

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TECHNICAL FEATURES

Principle of the Method : Vitamin B1 are separated treating the serum/plasma with a suitable precipitant and injected directly in the chromatographic system.

Recovery : n.a.

Sensitivity (LLOD): 10 ng/ml

Minimum concentration analyzable (LLOQ): 10 ng/ml

Linearity : 10 – 500 ng/ml

Accuracy intra serie (relative error %) :

C1	C3
75 ng/ml	125 ng/ml
5,14%	3,51%

Accuracy inter serie (relative error %) :

C1	C3
75 ng/ml	125 ng/ml
8,31%	5,13%

Reproducibility intra serie (coefficient of variation %) :

C1loq	C2	MAX C
10 ng/ml	100 ng/ml	150 ng/ml
5,05%	4,87%	3,54%

Riproducibility inter serie (coefficient of variation %) :

C1loq	C2	MAX C
10 ng/ml	100 ng/ml	150 ng/ml
9,74%	6,06%	8,00%

Coefficient of correlation R2 + Dev. std: 0,9922 ± 0,0009

Normal Range : Vitamin B1 32 – 95 ng/ml

Bibliography:

Herbeth B., J. Zittoun, L. Miravet, M. Boureay-Causse, G. Carre-Guery, E. Delacoux, C. Le Devehat, A. Lemoine, J.P. Mareschi, J. Martin, G. Portier de Courcy and J. Sancho (1986). Reference intervals for vitamin B1, B2, E, D, retinol, and folate in blood: Usefulness of dietary selection criteria. Clin. Chem. 32/9, 1756-1759.

Components of the kit :

All the components are ready-to-use and stable 3 years at 2–8 °C.

Reagent A – Deproteinization Solution + Internal Standard, **1 x 30 ml**

Reagent B – Derivatization Solution, **5 x 7,5 mg**

After reconstitution it is stable 15 days at 2-8 °C

Reagent C – Reconstitution Solution, **1 x 25 ml**

Reagent D – Diluting Solution, **1 x 10 ml**

Reagent E – Test Solution, **1 x 2 ml**

Calibrator in whole blood, **2 x 1 ml**

Code Z85016 (packed separately – see data sheet)

Reagent M – Mobile Phase, **4 x 500 ml**

100 pcs amber Eppendorf

Minimum Instrumental equipment required:

Isocratic HPLC System with loop of 10 μ l
Fluorimetric Detector $\lambda_{\text{EX}}=367 \text{ nm}$ $\lambda_{\text{EM}}=435 \text{ nm}$
Chromatograms Recorder

Optional Equipment:

Autosampler
Operational Computer

Blood Collection Procedure:

Take 3 ml of blood in a test tube with EDTA as an anticoagulant. The whole blood sample must be stored immediately in the dark in a closed container at a temperature of 2 - 8 ° C (stable for 1 day). Samples which are not analyzed immediately should be frozen at - 20 ° C. Avoid refreezing.

WARNING: VITAMIN B1 IS LIGHT SENSITIVE! USE ONLY **AMBER** VIALS AND TUBES.

PREANALYTICAL PROCEDURE

Reconstitute a bottle of **Reagent B – Derivatization Solution** with 5 mL of **Reagent C – Reconstitution Solution**. This solution is stable 15 days at 2-8 °C.

Pipette in a tube :

- 980 µl of H₂O HPLC grade
- 10 µl of **Reagent E – Test Solution**
- 10 µl of **Reagent B – Derivatization Solution RECONSTITUTED**

Vortex for 20 sec.

Verify that the Test Solution has retention time similar to fig. 1. If the Test is all right you can start with the analytical procedure; if not, check the functionality of the analytical system.

ANALYTICAL PROCEDURE

STEP 1 : Samples Preparation

Dispense in amber eppendorf of 1,5 ml with cap (included in the kit) :

	Blank	Calibrator	Sample
Water HPLC grade	100 µl		
Reagent F - Calibrator		100 µl	
Sample			100 µl
Reagent A – Deproteinization Solution	300 µl	300 µl	300 µl

Warning: deproteinize directly on Vortex for 20 sec.

STEP 2 : Centrifuge at 14.000 rpm for 10 minutes.

STEP 3 : Transfer 60 µl of supernatant in an amber glass vial with insert.

STEP 4 : Add at each vial 10 µl of **Reagent B – Derivatization Solution RECONSTITUTED**.

Vortex for 20 sec.

STEP 5 : Incubate at Room Temperature for 30 minutes at the dark

STEP 6 : Add at each vial 100 µl of **Reagent D – Diluting Solution**

Vortex for 20 sec.

N.B.: at this step, the sample is stable 1 day at 2-8 °

INJECTION :

- Inject 10 µl of the solution the chromatographic system.

VITAMIN B1 IN WHOLE BLOOD BY FLUORIMETRY – Warnings

REAGENT E: TEST SOLUTION

VITAMIN B1	1.000 ng/ml
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PARAMETERS OF THE FLUORIMETRIC DETECTOR

λ_{EX}	367 nm
λ_{EM}	435 nm
GAIN	0,001 AUFS

HPLC COLUMN PROTECTION

To save the analytical column Reverse Phase ZORBAX Eclipse Plus C18 (100 x 4,6 mm, 3,5 μ m), the use of Javelin Colfilters (1 x 10 pcs.) cod. S90199511 is obligatory.

HPLC COLUMN CONDITIONING

Install a new analytical C₁₈ Reversed-Phase column ZORBAX Eclipse Plus C18 (100 x 4,6 mm, 3,5 μ m).
Disconnect the detector and filter 30 ml of **H₂O : Acetonitrile (20 : 80 v / v)** set flow of 1,0 ml/minute. **Don't recycle the washing solutions.** Flux 30 ml of a solution of **H₂O : Acetonitrile (80 : 20 v / v)** at flow of 1,0 ml/minute. **Don't recycle the washing solutions.** Condition the column with the mobile phase at a flow of 1,0 ml/minute and discharge the first 20 ml. Finally inject the Chemical Standard and verify the quality of the HPLC run.
It's NOT possible to make analysis at recycling phase. If room temperature is > 20 °C store the Mobile Phase at 2-8 °C between an analytical session and another.

COLUMN CLEANING

Disconnect the detector. Flux 15 ml of **H₂O : Acetonitrile or Methanol (80 : 20 v / v)** set flow of 1,0 ml/minute and discharge. Flux a solution of **H₂O : Acetonitrile or Methanol (30 : 70 v / v)** for 30 minutes discharge it. **It is recommended to perform the washing of Analytical Column at the end of each analytical run.** Store the column in a solution of **H₂O : Acetonitrile or Methanol (30 : 70 v / v)**.

INJECTION NEEDLE WASHING

Wash with a solution of Water / Ethanol or Methanol or Acetonitrile (80 : 20 v/v).

HPLC PARAMETERS

LOOP	10 μ l
FLOW	0,7 – 1,8 ml/min
PRESSURE	About 110 bar

FLOW GRADIENT

Time (min)	Flow (ml/min)
0.0	0,7
4.5	0,7
4.51	1,8
10.0	1,8
10.01	0,7
12.0	0,7

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
Z85016	Calibrator in whole blood for Vitamin B1	4 x 1 ml
Z85019	Control in whole blood for Vitamin B1/B2/B6 – Levels 1 and 2	2 x 5 x 1 ml
Z85011	Mobile Phase	4 x 500 ml
Z959961902	Zorbax Eclipse Plus C 18 (100 x 4,6mm –3,5 um) Analytical Column	1 Pc
S90199511	Javelin Prefilters	1 x 10 Pcs
S29004U	Amber glass vials of 2 ml	1 x 100 Pcs
S51820717	Caps of amber glass vials of 2 ml and for amber vials	1 x 100 Pcs
S24717	Glass Insert 100 ul for vials of 2 ml	1 x 100 Pcs



VITAMIN B1 IN WHOLE BLOOD

(Reference Chromatograms)

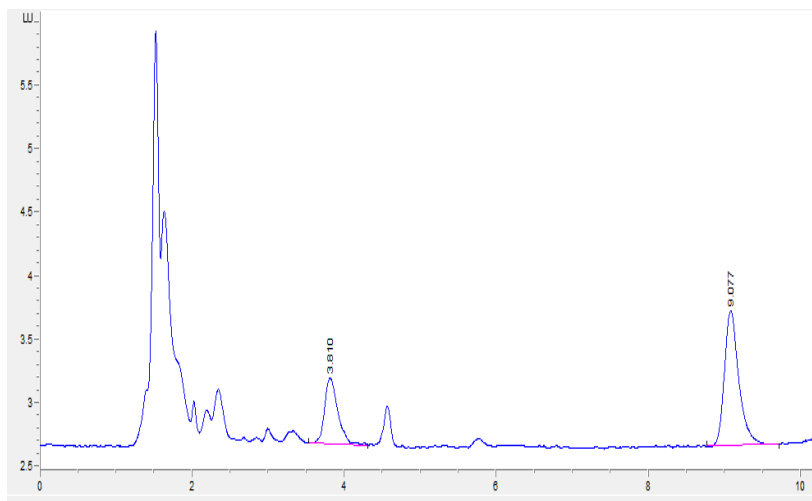


Fig. 1 :	Calibrator in whole blood
R.T. 3.8	Vitamin B1 (TPP) 100 ng/ml
R.T. 9.0	Internal Standard

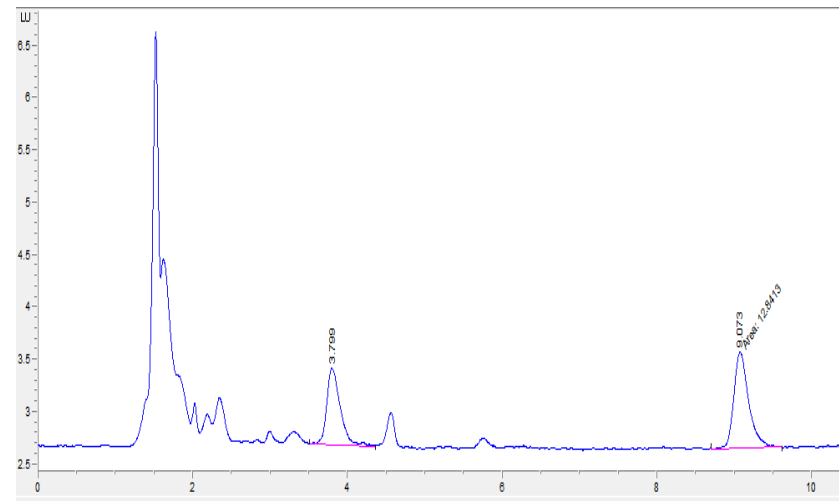


Fig. 2 :	Control L2 in whole blood
R.T. 3.7	Vitamin B1 (TPP) 125 ng/ml
R.T. 9.0	Internal Standard

VITAMIN B1 IN WHOLE BLOOD

(Reference Chromatograms)

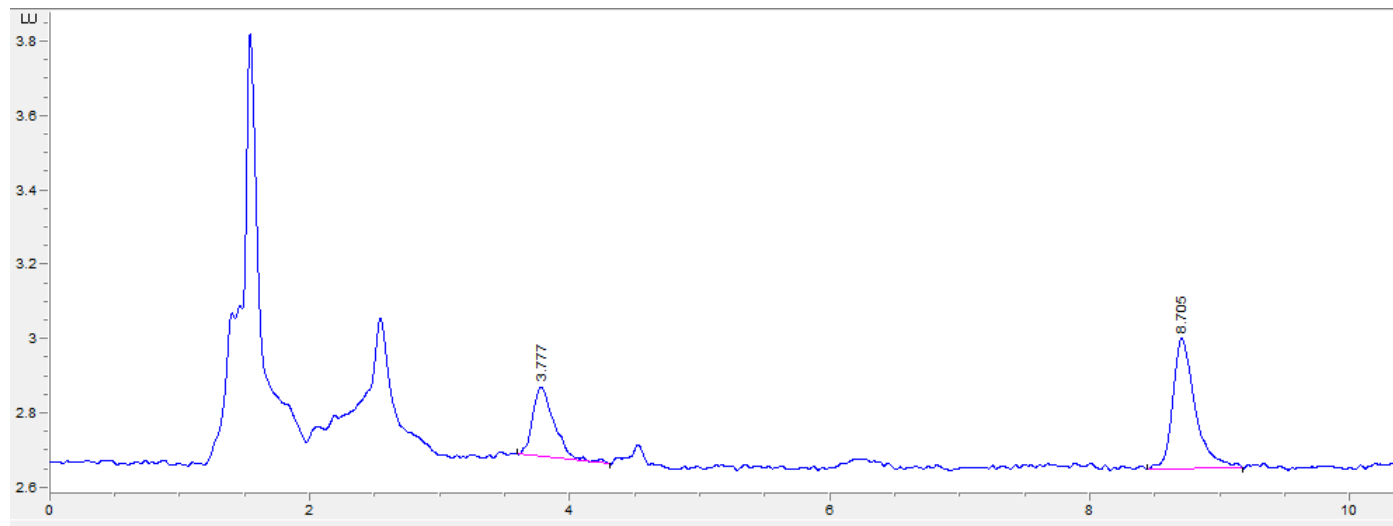


Fig. 3 :	Sample in whole blood
	R.T. 3.7 Vitamin B1 (TPP)
	R.T. 8.7 Internal Standard