

VITAMIN B6 IN WHOLE BLOOD by FLUORIMETRY - FAST – Code Z85610

INTRODUCTION

Vitamin B6 is a water soluble vitamin which forms part of the group B. The vitamins of this group involved in the metabolism of carbohydrates, fats and proteins and are thus essential for the production of energy and for the normal functioning of various organs and systems . The term vitamin B6 indicates three pyridine derivatives that occur in nature: pyridoxine, pyridoxamine and pyridoxal (PL), and their respective esters 5 'phosphate: pyridoxine-5'-phosphate, pyridoxamine-5'-phosphate and the active form pyridoxal -5'-phosphate (PLP). Vitamin B6 is present in both animal and vegetable products. Generally in the former there is a greater amount of pyridoxamine and pyridoxal phosphorylated while in the latter pyridoxine prevails. In plants, however, there may also be a portion of glycosylated pyridoxamine which is not hydrolyzed by intestinal enzymes and so is unusable. The forms of vitamin B6, also called adermine, are stable to heat, especially in acidic environment but are decomposed by light or by oxidizing substances. It appears that the absorption of vitamin B6 occurs while fasting by a process of passive diffusion. From the cells of the intestine, vitamin passes into the blood where it can be found bound to albumin or hemoglobin (in that case is set within red blood cells). Pyridoxine is converted into pyridoxal and / or pyridoxamine which, once phosphorylated, form the biologically active form of vitamin B6, acting as coenzymes. The reactions in which these two substances are involved, are concerned with the metabolism of amino acids and that of various other compounds. Given the direct involvement in the conversion of homocysteine, highly toxic amino acid, cysteine, deficiency of vitamin B6 favors an increase in the concentration of homocysteine and then a drastic increase in the risk of arteriosclerosis.

Among the symptoms that may indicate a lack of vitamin B6 are depression with nausea, vomiting, seborrheic dermatitis, mucosal lesions, glossitis and cheilosis and peripheral neuropathies.

As the proper utilization of protein ingested with the diet is very dependent on the presence of vitamin B6 it was decided to adjust the levels of recommended daily intake. Currently for adults the recommended quantity of vitamin is equal to 1.5 mg / 100 g of protein ingested.

For children, the recommended daily dose is between 0.3 to 0.8 mg. During pregnancy or while breastfeeding it is recommended to increase the dose of vitamin B6 by 20% and 30%, respectively. It is seen that amounts greater than 50 mg / day for long periods of time can cause peripheral neuropathies which is why you should not take such doses.

 EUREKA S.R.L. LAB DIVISION
Via M. D'Antona 28
60033 Chiaravalle (AN) - Italy
Tel. +39 071 7450790
eureka-support@sentinel.it
www.eurekakit.com



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.

N° 005	Vitamin B6 in whole blood by Fluorimetry - FAST	06/2024
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TECHNICAL FEATURES

Principle of the Method : The active form, Pyridoxal-5'-phosphate (PLP) is determined in blood after deproteinization, derivatization and injection of the supernatant properly treated.

Recovery : >99%

Sensitivity (LLOD): 0.33 ng/ml

Minimum concentration analyzable (LLOQ): 1.0 ng/ml

Linearity : 1 – 500 ng/ml

Accuracy intra serie (relative error %) :

Ci	Cs
34,65 ng/ml	84,6 ng/ml
17,8%	15,0%

Reproducibility intra serie (coefficient of variation %) :

C LLOQ	Cm	CA
18 ng/ml	51,3 ng/ml	351,3 ng/ml
7,4%	2,8%	4,1%

Reproducibility inter serie (coefficient of variation %) :

C LLOQ	Cm	CA
18 ng/ml	51,3 ng/ml	351,3 ng/ml
12,6%	8,6%	7,0%

Coefficient of correlation R2 + Dev. std: 0,9663 ± 0,0084

Normal range in blood : Pyridoxal-5'-phosphate (**PLP**) 8,7 – 27,2 ng/ml
G.J. den Ottolander, Diagnostisch Kompas 1997, S.441

Components of the kit :

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

Reagent A – Diluting Solution, 1 x 30 ml

Reagent B – Derivatization Solution, 1 x 4,5 ml

Reagent C – Deproteinization Solution, 1 x 5 ml

Reagent D – Stabilization Solution, 1 x 3 ml

Reagent E – Test Solution, 1 x 1 ml

Blood Calibrator, 4 x 1 ml

Code Z85616 (packed separately – see data sheet)

Reagent G – Internal Standard Solution, 1 x 6 ml

Reagent M – Mobile Phase, 4 x 500 ml

200 pcs amber Eppendorf

Minimum Instrumental equipment required:

Isocratic HPLC with loop of 10 µl
Fluorimetric Detector $\lambda_{EX}=380\text{ nm}$ $\lambda_{EM}=450\text{ nm}$

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.

Bibliography

1- G.J. den Ottolander, Diagnostisch Kompas 1997, S.441(*)

2- Piridoxal Phosphate decreases in plasma but not Erythrocytes during systemic inflammatory response.
Taiwar-Quasim-McMillan

3- A simple and effective Method for HPLC quantification of simultaneous vitamin B1 and vitamin B6 from whole blood. Wei-Orlowicz-Pike

WARNING: VITAMIN B6 IS LIGHT SENSITIVE! USE ONLY **AMBER** VIALS AND TUBES.

PREANALYTICAL PROCEDURE

Dispense in a tube :

- 900 µl of H₂O HPLC grade
- 100 µl of **Reagent E – Test Solution**
- 50 µl of **Reagent G – Internal Standard Solution**
- 40 µl of **Reagent B – Derivatization Solution**

Vortex for 20 sec.

Incubate at Room Temperature, at the dark for 30 min.

NOTE : Inject 10 µl of solution into HPLC system. 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 : Sample Preparation

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

	Blank	Calibrator	Sample
H₂O HPLC grade	300 µl		
Calibrator		300 µl	
Sample			300 µl
Reagent A – Diluting Solution	300 µl	300 µl	300 µl
Reagent G – Internal Standard Solution	50 µl	50 µl	50 µl

Vortex for 30 sec.

STEP 2 : Add at each vial 40 µl of **Reagent B – Derivatization Solution**

Vortex for 20 sec.

Incubate at Room Temperature, at the dark for 30 min.

STEP 3 : Add at each vial 50 µl of **Reagent C – Precipitant Solution**

Warning: deproteinize directly on Vortex for 20 sec.

Incubate at 2-8 °C, at the dark for 30 min.

STEP 4 : Centrifuge at 14.000 rpm for 10 min.

STEP 5 : Put 300 µl of supernatant in an amber eppendorf (if collecting the supernatant is taken of the precipitate, the eluate must be centrifuged again)

STEP 6 : Add at each vial 24 µl of **Reagent D – Stabilization Solution**

Vortex for 10 sec.

STEP 7 : Transfer about 200 µl of supernatant in a glass amber vial with insert

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

INJECTION :

- Inject 10 µl of solution into HPLC system.

VITAMIN B6 IN WHOLE BLOOD BY FLUORIMETRY – Warnings

REAGENT E: TEST SOLUTION

PYRIDOXAL-5-PHOSPHATE (PLP)	10.000 ng/ml
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PARAMETERS OF THE FLUORIMETRIC DETECTOR

λ_{EX}	380 nm
λ_{EM}	450 nm

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.
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HPLC COLUMN CONDITIONING

Install a <u>new</u> analytical C ₁₈ 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.
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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) .
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INJECTION NEEDLE WASHING

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

LOOP	10 μ l
RECOMMENDED FLOW	0,7/1,5 ml/min
PRESSURE	About 70-150 bar

FLOW SET

Time (min)	Flow (ml/min)
0	0.7
7.1	0.7
7.2	1.5
12.0	1.5
12.1	0.7
13.0	0.7

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
Z85616	Calibrator in whole blood for Vitamin B6	4 x 1 ml
Z85019	Control in whole blood for Vitamins B1/B2/B6 – Levels 1 and 2	2 x 5 x 1 ml
Z85611	Mobile Phase	4 x 500 ml
Z959961902	Zorbax Eclipse Plus C 18 (100 x 4,6mm –3,5 µm) Analytical Column	1 Pc
S90199511	Javelin Prefilters	1 x 10 Pcs
S29004U	Amber glass vials of 2 ml	1 x 100 Pcs
S24717	Glass Insert 100 ul for amber vials of 2 ml	1 x 100 Pcs
S51820717	Caps for Amber glass vials of 2 ml	1 x 100 Pcs



VITAMIN B6 IN WHOLE BLOOD

(Reference Chromatograms)

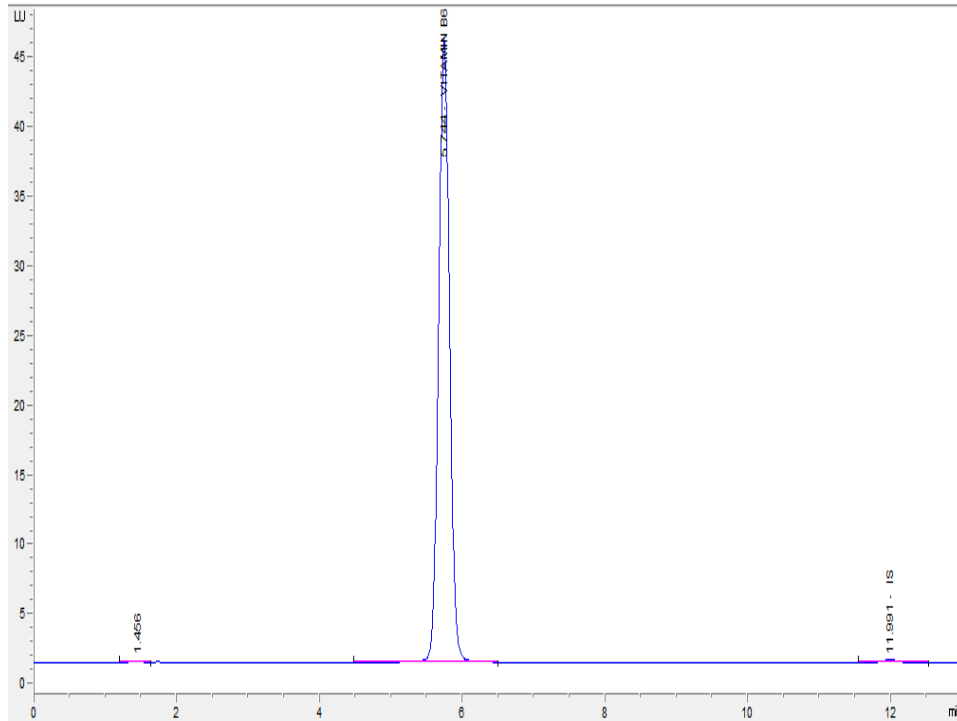


Fig. 1 :	Test Solution
	R.T. 5.7 Vitamin B6 (PLP)
	R.T. 11.9 Internal Standard

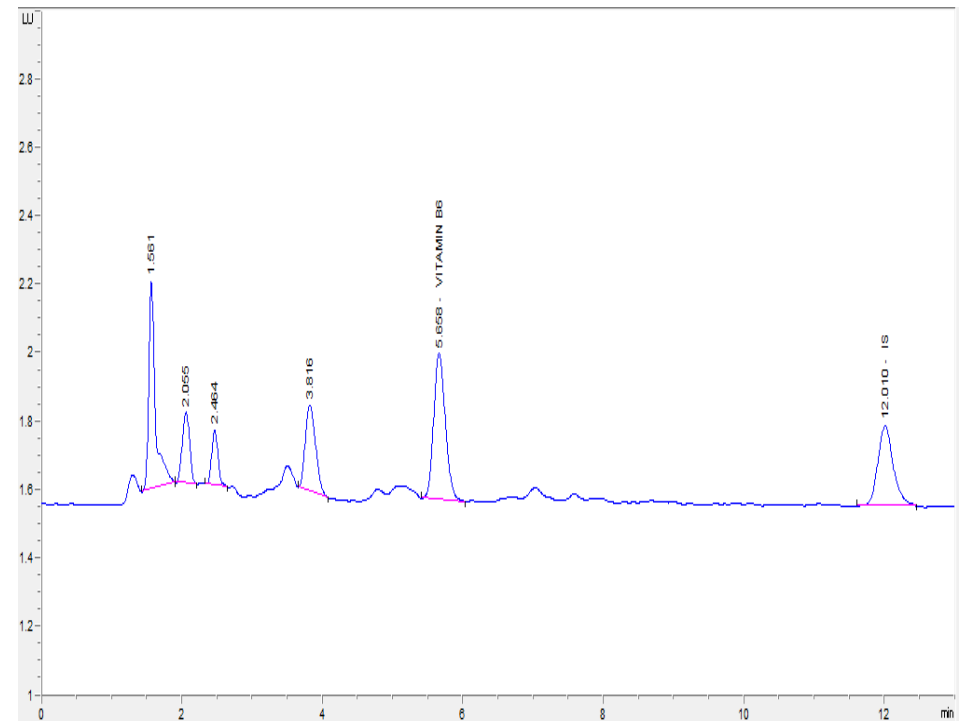


Fig. 2 :	Calibrator in whole blood
	R.T. 5.6 Vitamin B6 (PLP) 26 ng/ml
	R.T. 12.0 Internal Standard

VITAMIN B6 IN WHOLE BLOOD

(Reference Chromatograms)

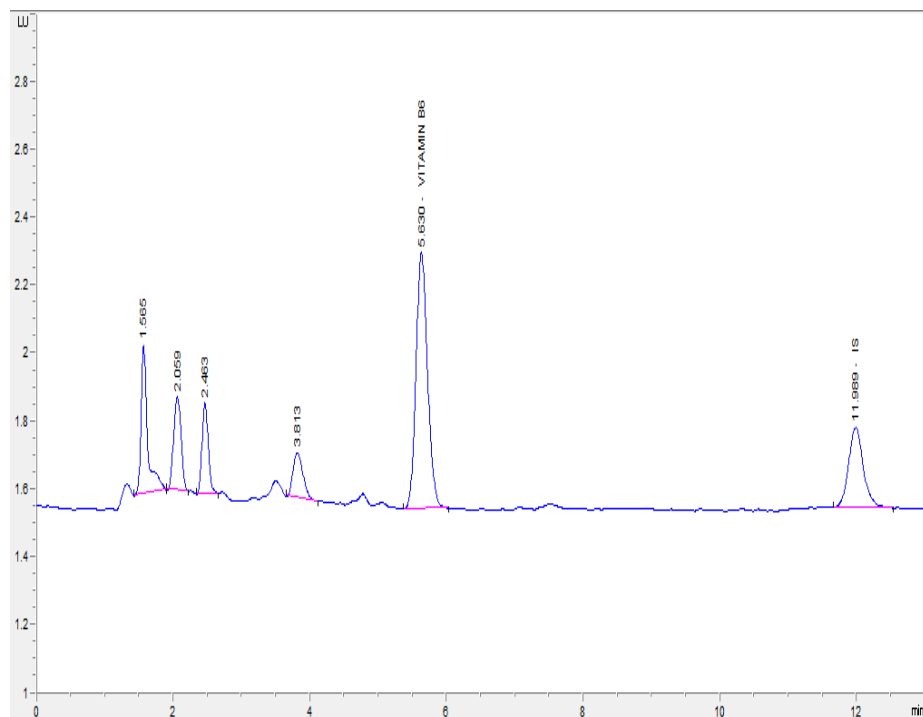


Fig. 3 : *Control in whole blood*

R.T.	5.6	Vitamin B6 (PLP)	50 ng/ml
R.T.	11.9	Internal Standard	

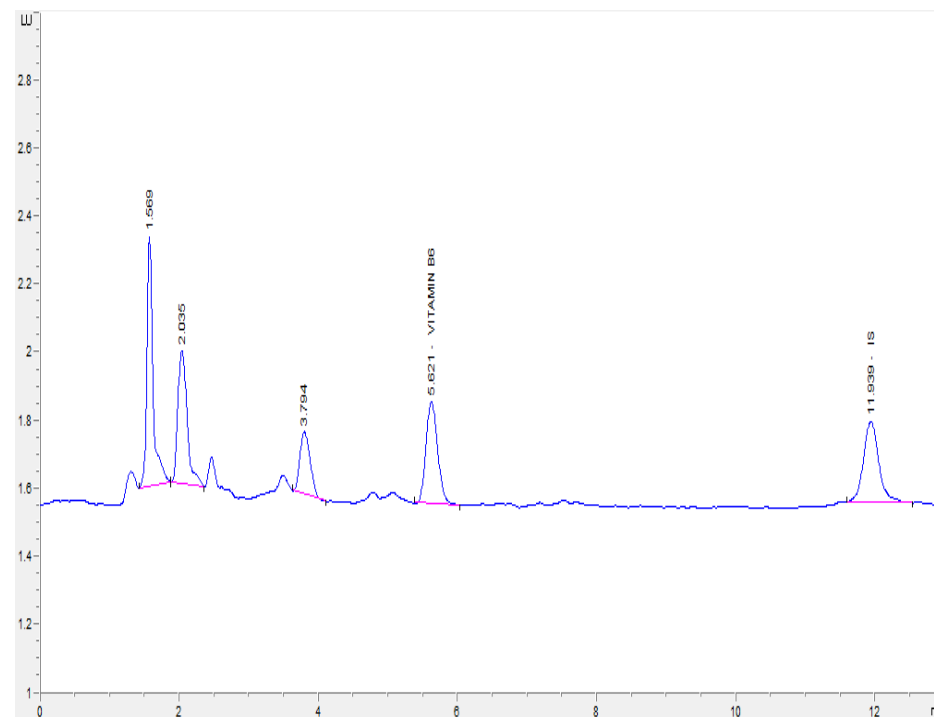


Fig. 4 : *Sample in whole blood*

R.T.	5.6	Vitamin B6 (PLP)	18 ng/ml
R.T.	11.9	Internal Standard	