

# Quantitative Determination of Pyridoxine on Pharmaceutical Forms and Vegetables

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*Pyridoxine plays an important role in maintaining the nervous and cardiovascular systems as well as the muscle tone of the gastrointestinal tract. The B vitamins are also vital for energy production. Vitamin B6 deficiency may arise from a deficient diet, or due to difficulty in absorbing it from food as a result of a stomach or intestinal disorder. The aim of this study is to quantify the pyridoxine from vegetables and pharmaceutical forms using volumetric and spectrophotometric methods. The results from the both methods are comparable. These methods ask usually reagents, classical apparatus being efficient from the economically point of view. The amount of B6 in peppers is in range of 0.14 mg/100g d.w. and 0.39 mg/100g d.w. The coefficient of variation (CV) is less than 5 that means two both analytical methods could be used successfully on determination of vitamin B6 from different samples.*

*Keywords: vitamin B6, volumetric and spectrophotometric analyses, peppers, pharmaceutical forms*

Vitamin B6 is one of the B complex vitamins. Pyridoxine, pyridoxamine and pyridoxal are three natural forms of this vitamin. Pyridoxine is mostly found in plants and seeds, while pyridoxamine and pyridoxal come mainly from animal foods. The foods richening vitamin B6 are: bananas, bell peppers, chick peas, potatoes (baked with skin), prune juice, spinach.

Nutritional supplements usually contain pyridoxine. Vitamin B6 is considered one of the more stable B vitamins and a large part of it ranging from 40 to 80% is lost during processing such as freezing, cooking, and canning of food.

Vitamin B6 plays an important biological role in the human body due to its metabolic process for carbohydrates.

Pyridoxal-5' -phosphate is the major phosphorylated bioactive form of vitamin B<sub>6</sub> that serves as a cofactor for more than 100 biochemical processes that have a wide impact on our health. Pyridoxal-5' -phosphate can be synthesized in the liver, from the nonphosphorylated pyridoxal by pyridoxine kinase or can be converted from pyridoxamine-5' -phosphate by pyridoxamine-5' -phosphate oxidase.

Vitamin B6 is administrated because it fights the anemia, neuropathy and depression. Patients under isoniazid therapy got this vitamin too in order to fight the toxic side effects of the drugs. It is needed for the synthesis of amino acids that the body uses to build proteins essential for cell formation, and the growth and repair of practically all body structures. Due to its role in the production of new cells, it is especially important to tissues that regenerate quickly, such as skin and mucous membranes.

Physico-chemical properties of pyridoxine are used in the quantitative analysis of pharmaceutical forms and food. The alkaline properties of vitamin B6 have been used in its acidimetric determination in an anhydrous environment consisting of a mixture of acetic acid and perchloric acid anhydride (FRX [1]).

According to literature, there are some spectrophotometric methods to analyze the vitamin B6. Therefore, by pyridoxine chlorhydrate reaction with 2,6-

dichlorochinoclorimide resulted a colored compound that can be measured by Vis spectrophotometric method [3, 5].

The presence of a chromophore group in a structure of pyridoxine is useful in the UV spectrophotometric determination of this substance in pharmaceutical preparations [6, 7].

The aim of this paper is to do quantitative determination of pyridoxine on vegetables and pharmaceutical forms.

The volumetric and also spectrophotometric procedures were applied in order to obtain quantitative results about the content of pyridoxine in yellow sweet pepper, red sweet pepper and green sweet pepper as vegetables and also pharmaceutical preparations.

The volumetric method uses the acido-basic reaction between pyridoxine and perchloric acid (FRX).

As spectrophotometric method we applied the colored reaction between pyridoxine chlorhydrate and ferric ion when results a red colored metal chelate with a maximum absorbance at 465 nm (NIEF RAHMAN AHMAD, OLA HAJUM [8]).

## Experimental part

All experiments were carried out in triplicates. All data are reported as means  $\pm$  S.D. Comparisons of means between the volumetric method and the spectrophotometric method were determined using correlation coefficient [2- 4].

## Materials and Methods

### Reagents, equipment and methods

Vitamin B6 (tablets and injections produced by Sicomed) was purchased from pharmacy. All reagents were of analytical reagent grade. In order to determine concentration of vitamin B6 on medicines and vegetables were used the spectrophotometric and volumetric methods (FR X, 2009) [8, 9].

An electronic balance type ABJ 220-4M (readibility 0.1 mg) KERN&Sohn GmbH was used. The pH measurements

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were done with the multiparameter Consort C – 862 calibrated with three standard buffer solutions (4.0, 7 and 8.0 value of pH). All measurements were being carried out at room temperature.

A spectrophotometer Spectro UV-Vis Double Beam PC 8 Auto Scanning cell UVD-3200, Lobomed, INC was utilized for data spectrum acquisition. A quartz cell of 1.00 cm optical path length was used for all measurements. For volumetric titration it was used a 5 mL microbiuret.

### Sample analysis

#### Volumetric method

Portions of 0.3 g of pyridoxine hydrochloride were dissolved in 15 mL of anhydrous acetic acid (R) by heating it in a water bath. It was cooled and 0.05 mL of crystal violet is added in anhydrous acetic acid (I) and titrated with perchloric acid 0.1 mol/L in acetic acid anhydride until blue-green color can be observed (FRX, 2009). A microbiuret of 5 mL was used for titration.

#### Spectrophotometric method

In order to obtain standard curve of ferric ion it was applied the general procedure: aliquots of standard pyridoxine hydrochloride solution equivalent 50-700 $\mu$ g (0.5 mL-7mL) were transferred into a series of 25 mL volumetric flasks, 0.5 mL of buffer solution pH 3, and 7mL of ferric ammonium sulfate solution were added. The content was mixed and then was diluted to the mark with distilled water and mixed well. The absorbance was measured at 465 nm against a blank reagent.

The ferric ammonium sulfate solution ( $\text{NH}_4\text{Fe}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$ ) in distilled water containing 3mL of concentrated  $\text{H}_2\text{SO}_4$  was prepared in 100 mL volumetric flask. Buffer solution (pH 3) was prepared by mixing of 22.3 mL of 0.1M

HCl with 50 mL of 0.1 M potassium hydrogen phthalate and dilute to 100 mL by distilled water in a volumetric flask.

#### Sample preparation

The mixed content of 10 tablets (0.4g/tb), (containing 250 mg of pyridoxine hydrochloride/tablet) were weighed and grounded, then the powder equivalent to 100 mg of pyridoxine hydrochloride in about 70mL of distilled water was stirred well for 30 min and then filtered through whatman no. 42 filter paper. The filtrate solution was diluted to 1 L by distilled water and 5 mL of this solution was treated as described above under general procedure.

The injections of B6 volume containing a concentration of 50mg/2 mL were used in analytical experiments. The content of injection was diluted to 100 mL volumetric flask and 1 mL was subject to general procedure.

#### Vegetables extracts

For this experiment we used vegetables as: yellow sweet pepper, red sweet pepper, green sweet pepper. Amounts of 10 g of each kind of pepper were weighed and grounded. In order to extract the vitamin B6, the vegetables were treated with ammonium acetate/methanol 50:50(v/v) and then filtered through cotton. The obtained solution was centrifugated 15 min at 1500 rpm. The supernatant was recuperated and diluted to 50 mL volumetric flask by acetic acid.

### Results and discussions

In order to study the stability and the interrelation between vitamin B6 and different pH medium, solutions of vitamin B6 at different pH levels were scanned separately in the range of 190- 400 nm to determine the wavelength of maximum absorption.

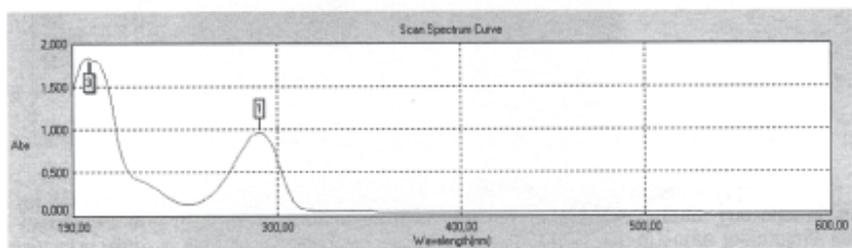


Fig. 1. Spectra of vitamin B6 at acidic medium (pH 2)

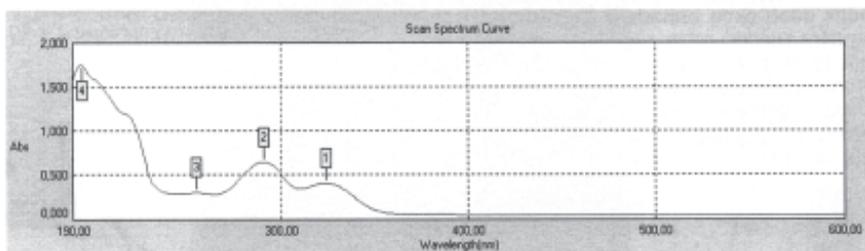


Fig. 2. Spectra of vitamin B6 at pH 4.4

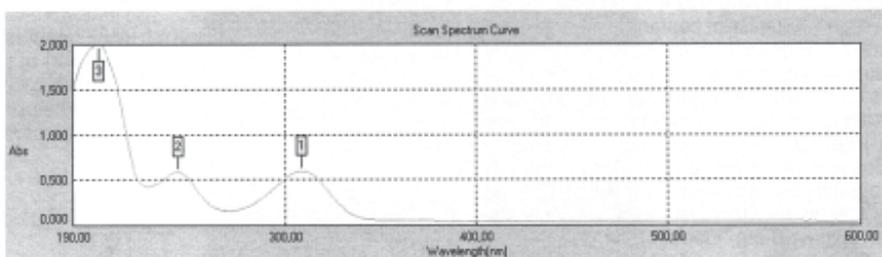


Fig. 3. Spectra of vitamin B6 at alkaline medium (pH 12)

Figure	pH	Peak	Wavelength, $\lambda$ (nm)	Absorbance
1	2	1	290.50	0.966
2	4.4	1	324.00	0.406
		2	291.00	0.647
3	12	1	309.00	0.599

**Table 1**  
VALUES OF ABSORBANCE AND  
WAVELENGTH (NM) FOR THE VITAMIN  
B6 IN DIFFERENT pH MEDIUM

Sample	Spectrophotometric Method	C.V. % (Spectropho- metric Method)	Volumetric method	C.V. % (Volumetric method)
Tablets(mg/tb)	250.146±0.570	0.228	256.79±9.289	3.617
Injections(mg/mL)	24.88±0.245	0.985	24.66±0.721	2.924

**Table 2**  
AMOUNT OF PYRIDOXINE IN  
TABLETS AND INJECTIONS

Figures from no. 1 to no. 3 show the spectra of pure solutions of vitamin B6 at different pH.

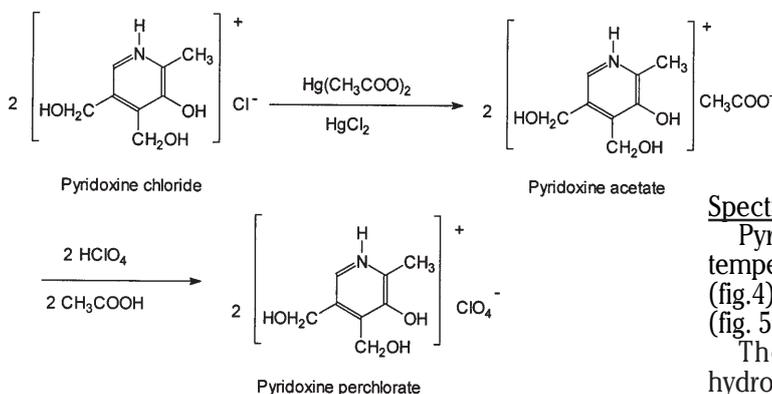
As can be observed in figure 1, vitamin B6 presents maximum of absorbance at  $\lambda_{\max} = 290$  nm in acidic solutions ( $pH < 2.5$ ).

The increase of pH levels (table 1), causing a considerable shift in  $\lambda_{\max}$  is an evidence of the presence of other deprotonated forms of this vitamin [7]. The presence of isosbestic point shows the variously protonated forms and they relate to each other with protonated/deprotonated equilibrium. The number of released protons in the indicated pH intervals is 2 for pyridoxal showing the distinct isosbestic points in a regular changing in pH.

Our all determinations of vitamin B6 were done in acidic medium at pH range 2÷3 which assures a maximum of stability of vitamin B6.

#### Dosage of vitamin B6 by volumetric method

Pyridoxine is a weak base and this fact makes more convenient dosing it in a non aqueous medium, using organic solvents which exalt basic character.



Titration is done in the presence of mercuric acetate linking ion  $\text{Cl}^-$  in the form of mercuric chloride, salt very little dissociated and resulting acetate pyridoxine to easily titrate with perchloric acid in acetic acid. Reactions occur as in above scheme.

The tablets and injections were used in volumetric determination of B6. All analyses were done in triplicate.

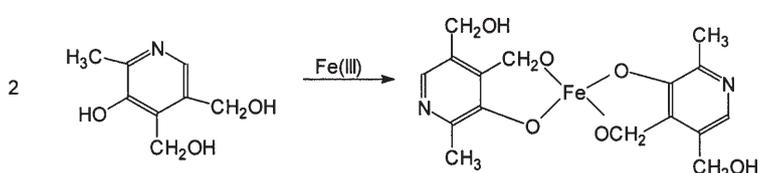


Fig. 4. Reaction between pyridoxine and Fe(III)

The results of spectrophotometric and volumetric determination of pyridoxine are presented in table 2. The values represent average of triplicate determination  $\pm$  standard deviation.

The tablets analyzed (0.4 g) have an amount of 250 mg active substance in agreement to prospect of instructions. The average level resulted in our determination is 256.8 mg active substance/tablet by volumetric method and 250.146 mg/tb by spectrophotometric method.

The injections have 50 mg/2 mL of vitamin B6. In this experiment by volumetric and spectrophotometric method was found a quantity of 24.66 mg/mL and 24.88 respectively.

The coefficient of variation (CV) indicating the statistical measure of the dispersion of data points in a data series around the average value is less than 5 in each situation. That means two the both analytical methods could be used successfully on determination of vitamin B6 from different samples.

#### Spectrophotometric method

Pyridoxine hydrochloride reacts with Fe(III) at room temperature resulting in formation of red coloured complex (fig.4) which has a maximum of absorbance at 465 nm (fig. 5).

The standard curve of ferric ion - pyridoxine hydrochloride solution was obtained by measuring the absorbance at 465 nm against a blank reagent.

The equation  $y = 0.026x + 0.037$  and correlation coefficient,  $R^2 = 0.996$  obtained from calibration linear curve of red complex Pyridoxine-Fe(III) show the linearity of selected range of the concentration of pyridoxine.

The spectrophotometric dosage of vitamin B6 on the peppers was done after standard procedure presented. We provided three kinds of peppers from supermarket: red sweet pepper, yellow sweet pepper, green sweet pepper.

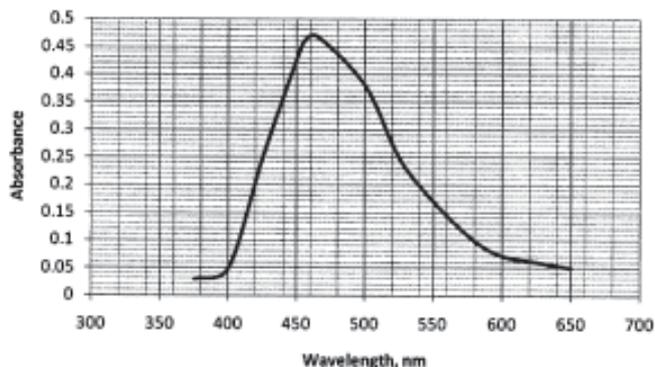


Fig. 5. Absorbance of Pyridoxine-Fe(III) complex versus wavelength

In the figure 6 the results are expressed as average of triplicate measurements for each kind of peppers.

The differences between the quantities of vitamin B6 obtained by the spectrophotometric and volumetric methods are insignificant as we can see on the figure 6. The differences between three kinds of peppers regarding the amount of vitamin B6 are significant.

Our results are in agreement with literature. In different internet references the amount of B6 expressed as mg/100g is in range of 0.27 - 1.5%. [10, 11]. The quantity of vitamin B6 is variable depending on species of peppers, cultivar group and environmental factors.

### Conclusions

In this work it was studied the dependence between pH and the spectral absorption of vitamin B6. The vitamin B6 shows the distinct isosbestic point in changing the pH levels.

A volumetric procedure and spectrophotometric method were applied for quantitative determination of pyridoxine.

In experimental determinations there were used pharmaceutical forms and red, yellow and green sweet peppers.

The spectrophotometric data show a correlation coefficient value less than 1.00 in the both samples of pharmaceutical forms. Also on pepper samples spectrophotometer analyses showed a CV value less than 5.

The both spectrophotometric and volumetric methods could be applied successfully for determination of pyridoxine hydrochloride in pure form as well as in different kind of vegetables. Red sweet peppers have a great quantity of vitamin B6 comparatively to green sweet peppers. The peppers are common vegetables in human diet and they could be a good source of vitamin B6.

### References

1.\*\*\* Farmacopeea Romana, Editia a X-a, Editura Medicala, Bucuresti, 2009.

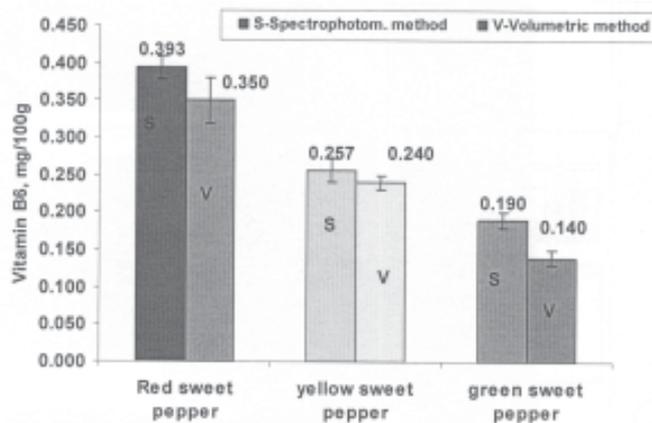


Fig. 6. Amounts of vitamin B6 in red, yellow and green sweet peppers

2.JAHANBAKSH GHASEMI, BAHMAN ABBASI, Simultaneous Spectrophotometric Determination of Group B Vitamins Using Parallel Factor Analysis: PARAFAC, *Journal of the Chinese Chemical Society*, 2005, 52, 1123-1129.

3.MUSZALSKA I., PUCHALSKA M., SOBCZAK, A., Determination of Vitamin B6 by Means of Differential Spectrophotometry in Pharmaceutical Preparations in the Presence of Magnesium Compounds, *Acta Poloniae Pharmaceutica - Drug Research*, Vol. 68 No. 6 pp. 845-851, 2011.

4. SURMEIAN, M.; CIOHODARU, G.; IONESCU, M. S.; COSOFRET, V. V., Derivative UV-spectrophotometric determination of binary mixtures of procaine hydrochloride with benzoic acid, pyridoxine hydrochloride and 4-aminobenzoic acid, from pharmaceutical preparations, *Revue Roum. Chim.*, 40, 2; 111, 1995.

5.PRATAP Y. PAWAR, ANJALI V. LAGAD, SANDHYA N. BAHIR, SUMEDHA R. RATHI, Simultaneous UV Spectrophotometric Method for Estimation of Isoniazid and Pyridoxine in Tablet Dosage Form, *Der Pharma Chemica*, 2012, 4 (2):749-754, (<http://derpharmachemica.com/archive.html>).

6.RAEED M. QADIR, AZZAM A. MOSA, Spectrophotometric Assay of Pyridoxine Hydrochloride (Vitamin B6) in Pharmaceutical Preparations and Serum Via Arsenazo III- Cerium (III) Reaction, *Raf. Jour. Sci.*, Vol. 19, No.2, p. 28 - 41, 2008.

7.STANCIU, G., CHIRILA, E., DOBRINAS, S., NEGREANU-PIRJOL, T., *Rev. Chim.(Bucharest)*, 61, no. 1, 2010, p. 41

8.NIEF RAHMAN AHMAD, OLA HAJUM, Spectrophotometric determination of pyridoxine hydrochloride via complexation with Fe( III )in pharmaceutical and environmental wastewater samples, *Iraqi National Journal of Chemistry*, 2012, volume 48, 413.

9.HSIN-YUEH CHANG, JASON T. C. TZEN, SU-JEN LIN, YI-TING WU AND, EN-PEI ISABEL CHIANG, Long-Term Prednisolone Treatments Increase Bioactive Vitamin B<sub>6</sub> Synthesis In Vivo, *J. Pharmacol. Exp. Ther.* 2011 337: 1 102-109.

10.\*\*\*[www.dietandfitnesstoday.com/vitamin-b6-in-peppers.php](http://www.dietandfitnesstoday.com/vitamin-b6-in-peppers.php)

11.\*\*\*"Vegetable and Fruit for Health: Bell Peppers." 2008. <http://umaine.edu/publications/4181e>

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