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## Validated Spectrophotometric Method to Assay of B<sub>6</sub> and B<sub>3</sub> Vitamins in Pharmaceutical Forms Using Potassium Iodide and Potassium Iodate

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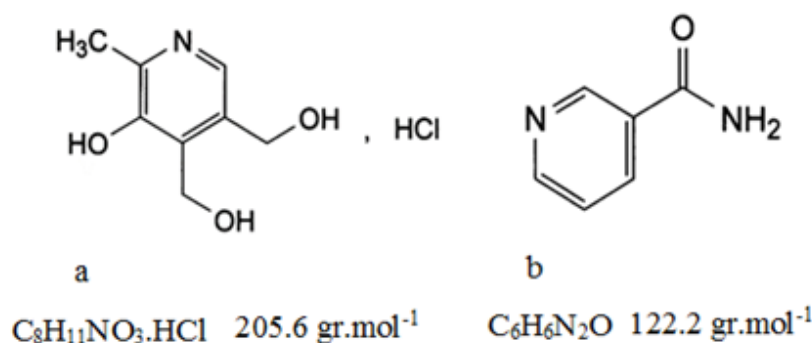
**Keywords:** Pyridoxine hydrochloride, Nicotinamide, Spectrophotometry; Potassium Iodide, Potassium Iodate; Pharmaceutical Formulations

**ABSTRACT:** A simple, precise and reliable spectrophotometric method for the determination of pyridoxine hydrochloride (vitB<sub>6</sub>) and nicotinamide (vitB<sub>3</sub>) in pure form and pharmaceutical formulations has been described. The method is based on the reaction of studied vitamin with a mixture of potassium iodide and potassium iodate in aqueous medium at (25 ± 0.5°C) to form yellow colored tri iodide ions (I<sub>3</sub><sup>-</sup>). The reaction is followed spectrophotometrically by measuring the absorbance at 290, 335 and 288, 350 nm for vitB<sub>6</sub> and vitB<sub>3</sub> respectively. Beer's law was obeyed in the range of 0.5-20.0 µg mL<sup>-1</sup> for all procedures. Molar absorptivities were found to be 1.49 × 10<sup>4</sup>, 1.93 × 10<sup>4</sup> and 0.89 × 10<sup>4</sup>, 1.25 × 10<sup>4</sup> L mol<sup>-1</sup> cm<sup>-1</sup>, for determination of vitB<sub>6</sub> and vitB<sub>3</sub> at 290, 335 and 288, 350 nm respectively. The proposed method has been applied to determine the components in commercial forms with no interference from the excipients. A comparative study between the suggested procedures and the official methods showed no significant difference between the two methods.

## 1. INTRODUCTION

Vitamin B<sub>6</sub>, also known as pyridoxine HCl is 3-hydroxy-2-methylpyridinine 4,5-dimethanol hydrochloride (Figure 1,a). VitB<sub>6</sub> is constituent of the enzyme system concerned with transamination and decarboxylation of amino acid. Its deficiency leads to neurological and neuromuscular disorders.

Vitamin B<sub>3</sub>, also known as (nicotinamide, nicotinic acid, niacin, or PP) is 3-Pyridinecarboxamide (Figure 1,b). VitB<sub>3</sub> is required for cell respiration, for proper circulation and healthy skin, for the functioning of the nervous system and for the normal secretion of bile and stomach fluids



**Figure 1:** Chemical structures of a) Vitamin B<sub>6</sub>, b) Vitamin B<sub>3</sub>. [1]

Many methods have been developed for the determination of vitB<sub>6</sub> in pharmaceutical formulations include spectrophotometry [2-6], spectrofluorimetry [7], voltammetry [8-12], potentiometry [13,14]. vitB<sub>3</sub> has been determined in pharmaceutical formulations by chemical luminescence [15], spectrophotometry [16]. Several methods have been developed for the

simultaneous determination of vitB<sub>6</sub> and vitB<sub>3</sub> in their combined formulations with other active ingredients include HPLC with UV detection [17-22], electrospray ionization-mass spectrometry (HPLC/ESI-MS) detection [23], UV spectrometry-tandem mass spectrometry (HPLC-UV-MS/MS) detection [24].

The aim of the present study was to report new spectrophotometric method that is simple, time-saving and accurate for the determination of vitB<sub>6</sub> and vitB<sub>3</sub> as a raw materials and in some pharmaceutical preparations with no interference of other constituents in their formulations.

## 2. EXPERIMENTAL

### 2.1. Apparatus

A Jasco V-530 UV-VIS spectrophotometer (Japan) with 1 cm quartz cells was used for all absorbance measurements under the following operating conditions: scan speed medium (400 nm/min), scan range 200–1100 nm and slit width 2 nm. Spectra were automatically obtained by Jasco system software. pH measurements were made with ORION 250A (USA) with combined glass pH electrode.

### 2.2. Reagents and materials

Pyridoxine hydrochloride (vitB<sub>6</sub>), and nicotinamide (vitB<sub>3</sub>) were obtained from Qualikems (India). with a purity 99.0% according to BP [1]. Pharmaceutical preparations containing vitB<sub>6</sub> and vitB<sub>3</sub> were purchased from commercial sources in the local market. Potassium iodate and potassium iodide were obtained from (Fluka Chemie AG, Switzerland).

Stock standard solution 100 µg mL<sup>-1</sup> of vitB<sub>6</sub> and vitB<sub>3</sub> were prepared by dissolving 10.1 mg from each of vitB<sub>6</sub> and vitB<sub>3</sub> in double distilled water and diluting to 100 mL with double distilled water. 0.01M of potassium iodate and 0.15M of potassium iodide solutions were prepared by dissolving the accurately weighed amount of the pure solid in double distilled water. All other chemicals and reagents were at analytical grade and all solutions were prepared with double distilled water. All solutions were stored at 4 °C, and protected from the light in dark bottles and kept in the refrigerator for not more than 3 days.

### 2.3. General Procedure

Aliquots of standard vitB<sub>6</sub> or vitB<sub>3</sub> (0.05-2.00mL, 100µg.mL<sup>-1</sup>) solutions were pipetted into series of 10mL calibrated volumetric flasks. Then 1.0mL of KIO<sub>3</sub> (0.10M), 2.0mL of KI (0.15M) and 1.0mL of KIO<sub>3</sub> (0.20M), 3.0mL of KI (0.20M) solutions were added for vitB<sub>6</sub> and vitB<sub>3</sub>, respectively. The volume was made up to the mark with distilled water and the absorbance was measured at 290, 335 and 288, 350 nm against a similar reagent blank for vitB<sub>6</sub> and vitB<sub>3</sub> respectively. The amount of vitB<sub>6</sub> and vitB<sub>3</sub> was computed from its Beer's law plot prepared with standard vitamin solution under identical conditions.

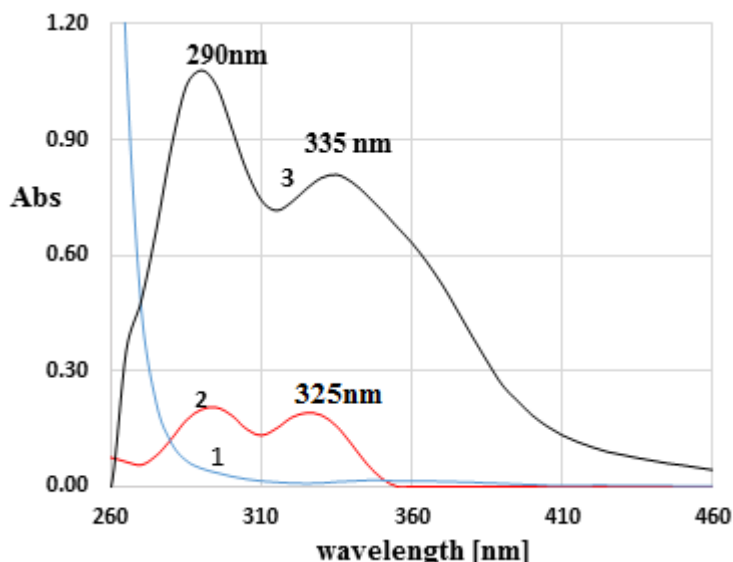
### 2.4. Procedure for pharmaceutical formulations

Twenty tablets of VITFOL 25mg/tab (vitB<sub>6</sub>) were powdered and mixed thoroughly. An amount corresponding to 10mg of vitB<sub>6</sub> was weighed, dissolved with 50 mL of methanol and mixed for about 30min, then filtered through Whatman filter paper (No. 1). The methanol was evaporated to the dryness. The remaining portion of solution was diluted in a 100mL volumetric flask to the volume with double distilled water. The resulting solution was used for analysis by the recommended procedures in the concentration range mentioned above. 0.5mL of FOLIN B<sub>12</sub> 20mg/10mL (vitB<sub>3</sub>) was diluted in a 10mL volumetric flask to the volume with double distilled water, mixed for about 5min. The resulting solution was used for analysis by the recommended procedures in the concentration range mentioned above.

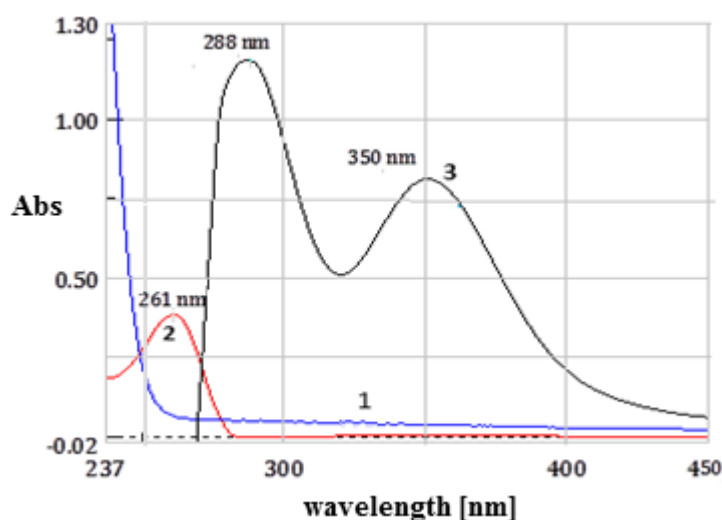
### 3. RESULTS AND DISCUSSION

#### 3.1. Absorption spectra

Iodide ions convert to free iodine in an acidic medium, then free iodine reacts with a surplus of iodide ions to form yellow complex from tri iodide ( $I_3^-$ ), with  $\lambda_{\max}$  at 290, 335 and 288, 350 nm for vitB<sub>6</sub> and vitB<sub>3</sub> respectively (Figure 2,3).



**Figure 2:** Absorption spectra of (1) reagent blank against distilled water, (2) 10 µg. mL<sup>-1</sup> vitB<sub>6</sub> against distilled water, (3) vit B<sub>6</sub>(10.0 µg mL<sup>-1</sup>) + 2.0 mL of 0.15 M KI + 1.0 mL of 0.1 M KIO<sub>3</sub> against reagent blank.



**Figure 3:** Absorption spectra of (1) reagent blank against distilled water, (2) 15 µg. mL<sup>-1</sup> vitB<sub>3</sub> against distilled water, (3) vit B<sub>3</sub>(15.0 µg mL<sup>-1</sup>) + 3.0 mL of 0.20 M KI + 1.0 mL of 0.20 M KIO<sub>3</sub> against reagent blank.

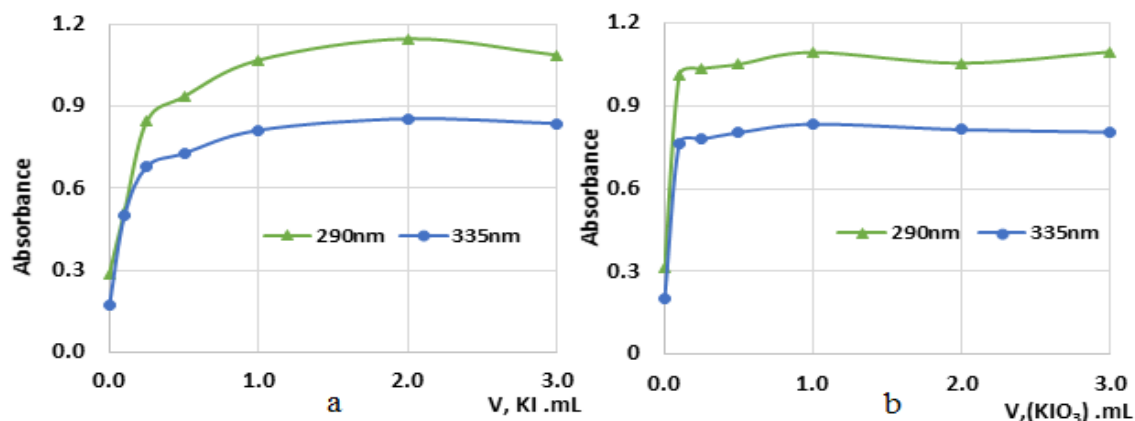
#### 3.2. Optimization of reaction conditions

The optimum conditions for the development of method were established by varying the parameters one at a time and keeping the others fixed and observing the effect produced on the absorbance of the colored products.

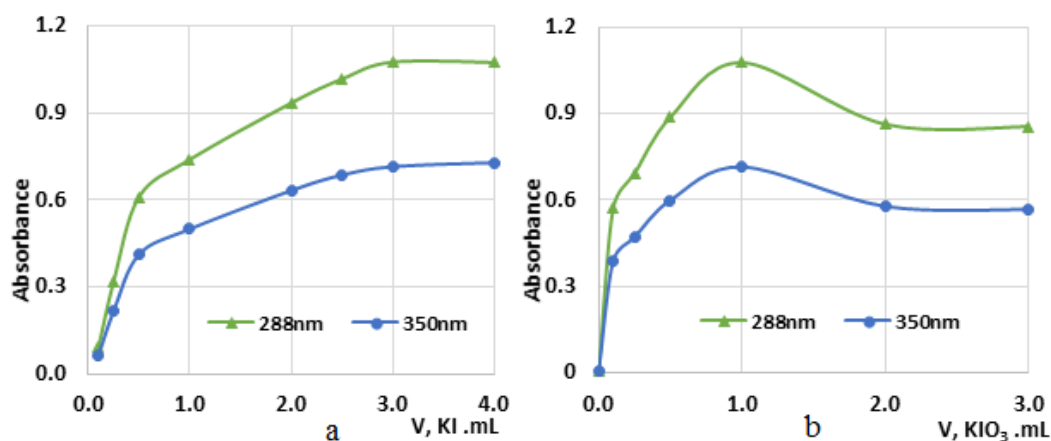
In order to establish experimental conditions, the effect of various parameters such as volumes of KIO<sub>3</sub>, KI addition of acidic or basic solutions, presence of vitB<sub>9</sub>, vitB<sub>12</sub>, and waiting time were studied at room temperature.

A volume of 2.0 mL of 0.15 M KI, 1.0 mL of 0.10 M KIO<sub>3</sub> and 3.0 mL of 0.20 M KI, 1.0 mL of 0.20 M KIO<sub>3</sub> were found to be optimum for maximum color development for determination of vitB<sub>6</sub>

and vitB<sub>3</sub> respectively, since the absorbances were found to be maxima at the mentioned volumes as shown in Figures 4 and 5. The laboratory temperature ( $25 \pm 0.5^\circ\text{C}$ ) and measurement of absorbance immediately were found to be optimum for all the experiments. Addition of acidic or basic solutions affects in a negative way on the analytical method. Both vitB<sub>9</sub>, vitB<sub>12</sub> present in the formulations do not interfere with the assay procedures.



**Figure 4:** Effect of **a)** volume of (0.15M)KI in the presence of 1.0mL of (0.10M)KIO<sub>3</sub> **b):** volume of (0.10M)KIO<sub>3</sub> in the presence of 2.0 mL of (0.15M)KI, on the formation of reaction product at 290, 335 nm, [vitB<sub>6</sub>]=10.0  $\mu\text{g. mL}^{-1}$



**Figure 5:** Effect of **a)** volume of (0.20M)KI in the presence of 1.0mL of (0.20M)KIO<sub>3</sub> **b):** volume of (0.20M)KIO<sub>3</sub> in the presence of 3.0 mL of (0.15M)KI, on the formation of reaction product at 288, 350 nm, [vitB<sub>3</sub>]=15.0  $\mu\text{g. mL}^{-1}$

### 3.3. Analytical Method Validation

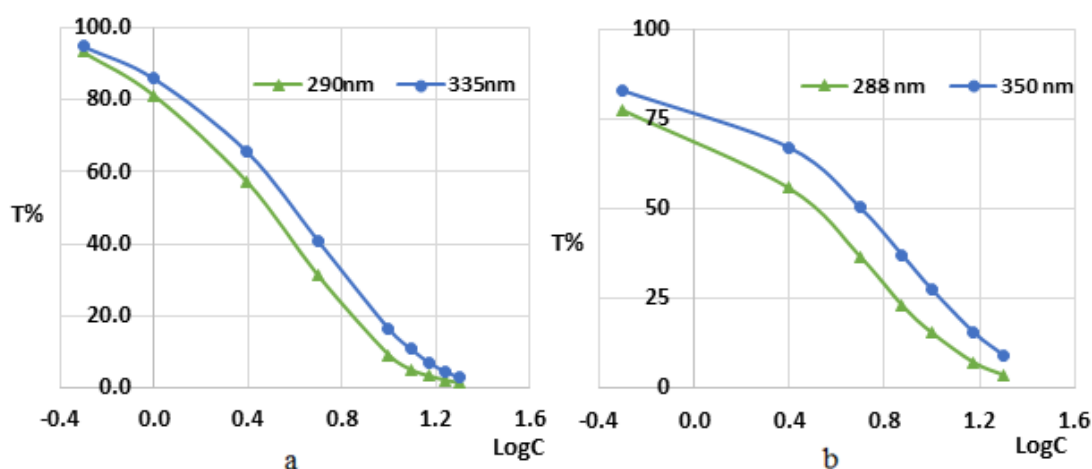
#### 3.3.1 Linearity

At described experimental conditions for determination of vitB<sub>6</sub> and vitB<sub>3</sub>, standard calibration curves with good linearity were obtained. The molar absorptivity, detection limit, limit of quantification and Sandell's sensitivity were calculated. The high molar absorptivity of the resulting colored product indicates the high sensitivity of the method. For more accurate analysis, Ringbom optimum concentration range was obtained by plotting the transmittance (T%) versus logarithm of vitamin concentration ( $\log C$ ), (figure 6) [25]. Values of some analytical characteristics for proposed procedures were shown in Table 1.

**Table 1:** The Analytical characteristics of Vit-KI-KIO<sub>3</sub>

parameters	vitB <sub>6</sub>		vitB <sub>3</sub>	
	335nm	290nm	350nm	288nm
Linear rang $\mu\text{g.mL}^{-1}$	0.5-20.0	0.5-20.0	0.5-20.0	0.5-20.0
Ringbom optimum concentration range $\mu\text{g.mL}^{-1}$	2.5-15.0	1.0-12.5	2.5-20.0	2.5-20.0
$\epsilon$ , $\text{L mol}^{-1} \text{cm}^{-1}$	$1.49 \times 10^4$	$1.93 \times 10^4$	$0.89 \times 10^4$	$1.25 \times 10^4$
Detection limit $\mu\text{g.mL}^{-1}$	0.09	0.14	0.05	0.04
Limit of quantification $\mu\text{g.mL}^{-1}$	0.50	0.50	0.50	0.50
Sandell's sensitivity	0.028	0.021	0.028	0.019
Regression equation	*( $A = m C + b$ )			
	m= 0.0782	m= 0.0957	m=0.0494	m=0.0685
	b= -0.0083	b= 0.0296	b=0.0554	b=0.0959
Correlation coefficient $R^2$	0.9998	0.9949	0.9995	0.9974

\*With respect to  $A=mC+b$ , where  $C$  is the concentration ( $\mu\text{g.mL}^{-1}$ ) and  $A$  is the absorbance.

**Figure 6:** Ringbom optimum concentration range a) Vitamin B<sub>6</sub>, b) Vitamin B<sub>3</sub>.

### 3.3.2 Accuracy and Precision

The accuracy and precision of the proposed procedures were carried out by five determinations at several different concentrations for vitamins. Percentage relative standard deviation (RSD %) as precision and percentage recovery as accuracy of the suggested procedures were calculated and showed in Table 2. The values of relative standard deviations for different concentrations of vitamins determined from the calibration curves. These results of accuracy and precision show that the proposed procedures have good repeatability and reproducibility. The proposed method was found to be selective to assay of vitamin in the presence of various excipients.

**Table 2:** Accuracy and precision for the determination of vitB<sub>6</sub> and vitB<sub>3</sub> in bulk powder by the proposed methods.

Vit	$\lambda_{\max}$	$\mu\text{g.mL}^{-1}$			RSD %	%Recovery
		Taken	Found	S.D*		
vitB <sub>6</sub>	335nm	5.00	4.84	0.12	2.48	96.80
		10.00	9.97	0.21	2.11	99.70
		15.00	15.05	0.41	2.72	100.33
		20.00	20.12	0.45	2.24	100.60
	290nm	5.00	4.90	0.21	0.21	98.00
		10.00	10.04	0.17	0.17	100.40
		15.00	14.60	0.13	0.13	97.33
		20.00	20.09	0.13	0.13	100.45
vitB <sub>3</sub>	350nm	5.00	5.11	0.15	2.93	102.20
		10.00	10.09	0.19	1.88	100.90
		15.00	15.25	0.09	0.59	101.67
	288nm	5.00	4.99	0.19	3.81	99.80
		10.00	10.05	0.14	1.39	100.50
		15.00	14.92	0.13	0.87	99.47

\*Average of five determinations.

### 3.4. Application to the pharmaceutical dosage forms

The proposed procedures were applied to determine the studied vitamins in their pharmaceutical formulations. The results in Table 3 indicate the high accuracy and precision. As can be seen from Table 3, the proposed method has the advantages of being virtually free from interferences by excipients and common degradation products. The results obtained were compared statistically by the student's *t*-test (for accuracy) and the variance ratio *F*-test (for precision) with those obtained by the pharmacopoeial methods [1] on samples of the same batch (Table 3). The values of *t*- and *F*-tests obtained at 95% confidence level and four degrees of freedom did not exceed the theoretical tabulated value indicating no significant difference between the methods compared.

**Table 3:** Determination of vitB<sub>6</sub> and vitB<sub>3</sub> in their pharmaceutical preparations using the proposed and pharmacopoeial methods

Formula	Vit	$\lambda_{\max}$	%Recovery $\pm$ S.D*	
			Proposed method	Pharmacopoeial method [1].
**VITFOL 25mg/tab	vitB <sub>6</sub>	335nm	103.34 $\pm$ 0.28 <i>t</i> =0.34 <i>F</i> =3.06	99.40 $\pm$ 0.16 <i>t</i> =0.87
		290nm	98.60 $\pm$ 0.24 <i>t</i> =1.30 <i>F</i> =2.25	
***FOLIN B <sub>12</sub> 20mg/10mL	vitB <sub>3</sub>	350nm	99.12 $\pm$ 0.28 <i>t</i> =0.12 <i>F</i> =1.62	97.78 $\pm$ 0.22 <i>t</i> =2.25
		288nm	100.96 $\pm$ 0.12 <i>t</i> =1.76 <i>F</i> =3.36	

\* Five independent analyses (four degrees of freedom). At 95% confidence level *t*-value is 2.776 and *F*-value is 6.26

\*\*Supplied by PHARMASYR products, Syria.

\*\*\*Supplied by IBN AL-HAYTHAM products, Syria

#### 4. CONCLUSION

The proposed analytical procedures were simple, rapid, accurate and precise, so it can be used for the routine analysis of vitB<sub>6</sub> and vitB<sub>3</sub> in bulk and pharmaceutical formulations. The sample recoveries from all formulations have good agreement with their respective label claims, which suggested non-interference of formulations excipients in the assay. Moreover, the present method is fast with respect to analysis time as compared to sophisticated chromatographic techniques

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