DEVELOPMENT AND VALIDATION OF STABILITY INDICATING SPECTROPHOTOMETRIC METHOD FOR PYRIDOXINE HYDROCHLORIDE IN BULK AND PHARMACEUTICAL FORMULATIONS

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ABSTRACT

Background: Pyridoxine Hydrochloride is a water soluble vitamin which is also known as Vitamin B_6. **Objectives:** In this recent research developed a very simple, least time consuming, inexpensive and efficient UV Spectrophotometric method for the assay of pyridoxine in bulk form and in its pharmaceutical dosage formulation. **Methods:** The assay is based on the Ultraviolet Spectroscopy (UV), measuring λ_{max} at 290 nm for pyridoxine. A sample of bulk was dissolved in 0.1 N HCl to produce a desired concentration of solution containing pyridoxine. Similarly, a sample of pharmaceutical formulation was grind and dissolved in 0.1 N HCl and various dilutions were made. **Results:** The absorbance of bulk, dosage formulation preparation was measured at 290nm against 0.1 N HCL (blank). Calibration curves were linear over the range of 2-14 μgmL^{-1} with a correlation coefficient is ≥ 0.9997 at λ_{max} 290 nm. **Conclusion**: The analysis shows a linear relationship between the absorbance and concentration. The proposed method was statistically validated and successfully applied for routine analysis of pyridoxine in bulk and tablet dosage forms.

Keywords: Pyridoxine, Bulk, Dosage Formulation, UV spectrophotometer.

1. INTRODUCTION

Pyridoxine hydrochloride (Figure 1) is (5- hydroxy- 6- methyl pyridine -3,4- diyl) dimethanol hydro-chloride [1]. It is also called vitamin B_6. Vitamin B_6 exists in seven forms: pyridoxine (PN), pyridoxine 5'-phosphate (PNP), pyridoxal (PL), pyridoxal 5'- phosphate (PLP), pyridoxamine (PM), pyridoxamine 5'-phosphate (PMP), and the catabolite, 4-pyridoxic acid. It is a water-soluble vitamin. It was discovered in 1934 by P. Gyorgy [2]. It may help balance hormonal changes in women and aid the immune system [3]. Lack of pyridoxine may cause anemia, nerve damage, seizures, skin problems, and sores in the mouth.

Literature survey revealed that there are many methods like Liquid chromatography with electrochemical detection [4], Spectrophotometric [5] HPLC [6], TLC [7], and mass spectroscopy [8] for determination of Pyridoxine. A spectrophotometric method is also described for the determination of pyridoxine hydrochloride by flow injection analysis depending on the reaction of pyridoxine with N,N-diethyl-p-phenylenediamine after oxidation by potassium hexacyanoferrate (III) [9]. Another Spectrophotometric method is also described for the determination of pyridoxine hydrochloride based on the reaction between pyridoxine hydrochloride and 1,10-phenanthroline in the presence of ferric nitrate, the reddish brown colored product shows maximum absorption at 510 nm [10]. A micellar reversed-phase liquid chromatographic procedure was also developed for the determination of B_6 group vitamins, i.e. pyridoxine, pyridoxal and pyridoxamine, in human serum [11]. The method is based on the oxidation-reduction reaction between vitamin B_6 and cerium (IV) ion (ceric ion), then the subsequent reaction of cerium (III) with arsenazo III reagent in acidic medium in the presence of the neutral surfactant (Triton X-100) to produce a green complex which is stable, water soluble and has a maximum absorption at 716 nm with a molar absorptivity of 1.12×105 $lmol^{-1}.cm^{-1}$ [8, 12].

The aim of the present work is to develop a simple, rapid, accurate, precise, reproducible and less time consuming Spectrophotometric method for the estimation of pyridoxine in bulk and pharmaceutical dosage formulation as per ICH guideline [13]. This method is preferred over other reported UV methods for routine analysis of pyridoxine in quality control. Spectrophotometers are easy and simple system, it takes less time to analyze as compare to others method. This study is very helpful for analyst to analyze the pyridoxine in bulk and dosage formulation.

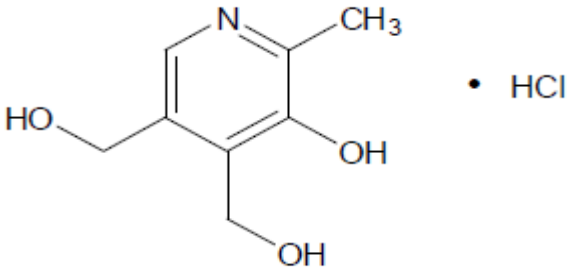

Figure 1: Chemical structures of Pyridoxine HCl

2. MATERIALS AND METHODS

2.1. Material and reagents

All chemicals and reagents were of analytical grade. Pyridoxine hydrochloride (purity 99.97 %) was a kind gift from Pfizer Pakistan (Pvt) Limited. Vita-6™ (Pyridoxine HCl 50 mg) tablets were purchased from local market. The expiry of tablets was not less than two year at the time of study.

2.2. Statistical study

Standard regression curve analysis was performed by use of STATISTICA version 7.0 (USA), without forcing through zero. Linearity graphs were obtained by use of Microsoft Excel 2007 software. SPSS software version 10.0 (Carry, NC, USA) was used for the calculation of means, standard deviations.

2.3. Apparatus

Instrument used in present study was double beam UV/Visible spectrophotometer with resolution of 1nm and 0.5 mm slit width (Model Shimadzu, 1800). Recording spectrophotometer by using 1cm quartz cells. Calibrated Pyrex glassware was used for the solution and sample preparation which was washed with chromic acid followed by a thorough washing with water and finally rinsed with deionized water, which was freshly prepared in the laboratory. In addition, Mettler Toledo electronic balance was also used in this study.

2.2. Methods

2.2.1 Preparation of standard stock solution:

Standard drug solution of Pyridoxine HCl was prepared by dissolving 10 mg of drug in 50 mL 0.1 N HCl and transfers it to 100ml of volumetric flask and volume was made up to mark with 0.1 N HCl which make the solution of 100 μgmL^{-1} concentration. For obtaining clear solution, solution was ultrasonicated.

2.2.2 Preparation of calibration curve:

Aliquots of 6 to 14 mL portion of standard stock solutions were transferred to 100 mL of volumetric flasks, and volume made up to mark with 0.1 N HCl. Solutions were scanned in the range of 200-400 nm against blank. The absorption maxima were found to be at 290 nm against blank.

2.2.3 Preparation of sample solution

The proposed method was applied to analyze commercially available Pyridoxine HCl tablet. Twenty tablets were weighed and powdered. The amount of tablet powder equivalent to 50 mg of Pyridoxine HCl was weighed accurately and transfer to 100ml volumetric flask then 50 mL of 0.1 N HCl was added and kept for 15-20 min with frequent shaking and volume was made up to mark with 0.1 N HCl. The solution was then filtered through Whattman filter paper. This filtrate was diluted suitably with 0.1 N HCl to get the solution of 10 μgmL^{-1} concentration. The absorbance was measured against blank solution. The drug content of the preparation was calculated using standard calibration curve. Amount of drug estimated by this method is given in (Table 4).

3. RESULT AND DISCUSSION

3.1 Precision

Assay of method precision (intra-day precision) was evaluated by carrying out six independent assays of test samples of Pyridoxine HCl. The intermediate precision (inter-day precision) of the method was also evaluated using two different

analysts and different days in the same laboratory. The Relative Standard Deviation (RSD) and average assay values obtained by two analysts were 0.61, 100.16% and 0.43, 100.44% respectively (Table 4).

Table 1: Calibration Data for the method development

S. No.	Concentration (μgmL^{-1})	Absorbance at 290 nm
1	0	0
2	2	0.088
3	4	0.175
4	6	0.258
5	8	0.345
6	10	0.434
8	12	0.520
9	14	0.595

Table 4: Precision data for the developed method.

Sample Number	Assay of Pyridoxine HCl as % of Labeled Amount	
	Analyst-I (Intra-day Precision)	Analyst-II (Inter-day Precision)
1	100.21	100.54
2	100.54	101.21
3	99.87	100.14
4	100.01	99.97
5	99.27	100.51
6	101.05	100.28
Average	100.16	100.44
R.S.D.	0.61	0.43

RSD: Relative Standard Deviation.

3.2 Accuracy (Recovery Test)

Accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts of the drugs in the placebo. The recovery was performed at three levels, 80,100 and 120% of Pyridoxine HCl standard concentration. The recovery samples were prepared in before mentioned procedure. Three samples were prepared for each recovery level. The solutions were then analyzed, and the percentage recoveries were calculated from the calibration curve. The recovery values for Pyridoxine HCl ranged from 99.79 to 100.06 % (Table 2).

Table 2: Determination of Accuracy by Percentage Recovery Method.

Ingredient	Tablet Amount (μgmL^{-1})	Level of Addition (%)	Amount Added (μgmL^{-1})	Amount Recovered (μgmL^{-1})	% Recovery	Average% Recovery
Pyridoxine HCl	10	80	4	13.97	99.79	99.86±0.3245
	10	100	6	16.01	100.06	
	10	120	8	17.95	99.72	

3.3 Linearity

The linearity of the response of the drug was obtained at 2 to 14 μgmL^{-1} concentrations. The calibration curve was obtained by plotting the absorbance versus the concentration data and was treated by linear regression analysis (Table 3). The equation of the calibration curve for Pyridoxine HCl obtained was $y = 0.0428x + 0.0023$, the calibration curve was found to be linear (Figure 2) in the aforementioned concentrations (The correlation coefficient (r^2) of determination was 0.9997).

Table 3: Validation Parameters for Pyridoxine HCl

Sr. No.	Parameters	Results
1	Absorption maxima (nm)	290
2	Linearity range (μgmL^{-1})	2-14
3	Standard Regression Equation	y = 0.0428x + 0.0023
4	Correlation coefficient (r^2)	0.9997
5	Accuracy (% recovery ±SD)	99.86 ± 0.3245
6	Specificity	A 4 μgmL^{-1} solution of candidate drug in 0.1N HCl at UV detection ƛ of 290 nm will show an absorbance value of 0.175
7	LOD (ngmL^{-1})	58
8	LOQ (ngmL^{-1})	177

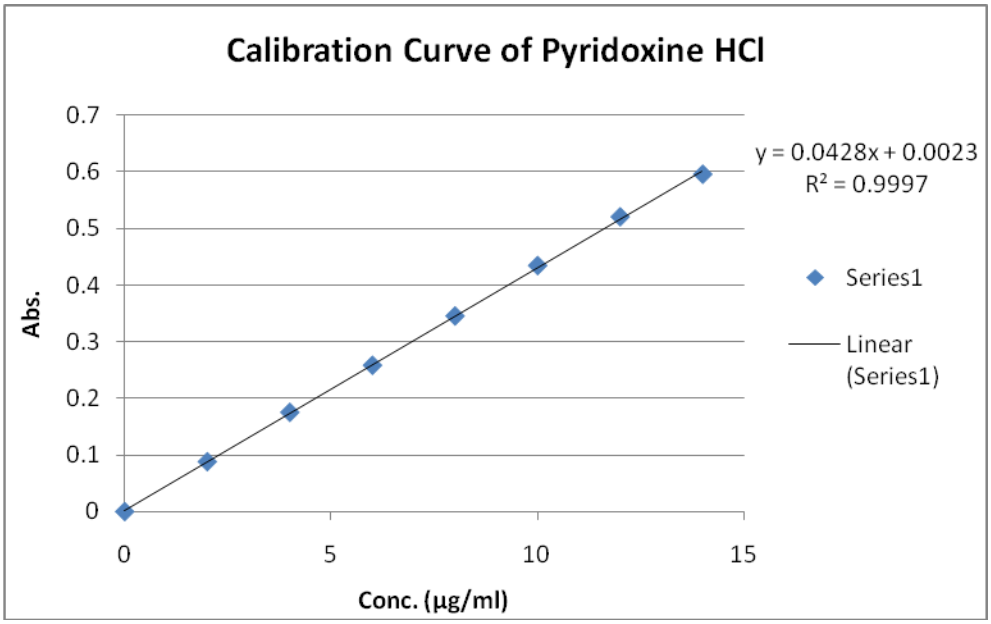

Figure 2: The Linearity Graph for Pyridoxine HCl (Absorption vs Concentration).

3.4 Limit of detection and quantification

The evaluated LOD and LOQ as three and ten times, respectively, the ratios between the standard deviation of regression and the slope of the calibration line have been evaluated as depicted in Table 3.

3.5 Determination of Active Ingredients in Tablets

The validated method was applied to the determination of Pyridoxine HCl in Tablet. Six tablets were assayed and the results are shown in (Table 4) indicating that the amount of drug in tablet samples met with requirements (98–102% of the label claim).

3.6 Long Term Stability

The long term stability was also evaluated for low 2.000 μgmL^{-1} and high concentrations of 14.000 μgmL^{-1}. The %CV of fresh samples for 2.014μgmL^{-1} was 0.045% and 0.014% for 14.010 μgmL^{-1}. After 2 and 3 weeks storage period at −20°C, %CV was determined to be 0.071% and 0.087% for low and 0.020% and 0.054% for high concentrations (Table 5).

Table 5: Long term stability of Pyridoxine HCl

Long Term Stability			
Low Concentration		**2.000 µgmL^{-1}**	
	Fresh Sample	**After 2 wk (at −20°C)**	**After 3 wk (at −20°C)**
Mean (n = 6)	2.014	2.001	1.957
SD	0.001	0.002	0.002
%CV	0.045	0.071	0.087
High concentration		14.000 µgmL^{-1}	
	Fresh sample	After 2 wk (at −20°C)	After 3 wk (at −20°C)
Mean (n = 6)	14.010	13.995	13.981
SD	0.002	0.004	0.005
%CV	0.014	0.020	0.054

4. CONCLUSIONS

The developed method was found to be simple, sensitive, accurate, precise, reproducible, and can be used for routine quality control analysis of Pyridoxine HCl in bulk and tablet dosage formulation and for stability studies.

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