

Derivative Spectrophotometric Method for The Estimation of Pyridoxine HCl in Bulk Drug & Dosage form

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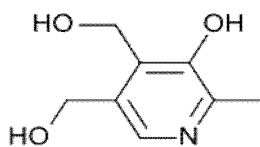
ABSTRACT

A simple, economic, sensitive, precise and accurate First and second derivative spectrophotometric method has been developed for determination of Pyridoxine hydrochloride in bulk and in tablet dosage form. The quantitative determination of the drug was carried out using the First derivative values measured at 302 nm and Second derivative values measured at 309nm. Calibration graph constructed at 302 and 309 nm was linear in concentration range of 2-12 µg/ml with correlation coefficient 0.998. The method was validated as per ICH guidelines and can be used for determination of Pyridoxine hydrochloride in tablet dosage form.

Keywords: Pyridoxine hydrochloride, Derivative spectrophotometry, Tablet, Validation.

INTRODUCTION

Pyridoxine is one of the compounds that can be called vitamin B₆, along with pyridoxal and pyridoxamine. It differs from pyridoxamine by the substituent at the '4' position. It is often used as 'pyridoxine hydrochloride'¹ It is based on a pyridine ring, with hydroxyl, methyl, and hydroxymethyl substituents. In present study, an attempt has been made to develop a simple, sensitive and efficient derivative spectrophotometric method for estimation of Pyridoxine hydrochloride in tablet dosage form.



4,5-

Bis(hydroxymethyl)-2-methylpyridin-3-ol

Chemicals and reagents

Pyridoxine Hydrochloride [Bulk Drug] used were of analytical reagent grade purchased from Zhejiang Tianxin

Pharmaceuticals Co Ltd, Double distilled water was used throughout the analysis.

Instrumentation

A JASCO V-530 UV/VIS double beam spectrophotometer with 1cm matched quartz cells was used for all spectral measurements.

Preparation of Standard Stock Solution

Accurately weighed Pyridoxine hydrochloride (10.0 mg) was transferred to 100 ml volumetric flask, dissolved in about 30 ml of distilled water and volume was up-to 100 ml with distilled water to obtain stock solution of drug concentration of 100µg/ml.

Preparation of calibration curve for Pyridoxine hydrochloride

From standard stock solution of Pyridoxine hydrochloride 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 ml solutions were pipette out in a series of 10 ml volumetric flasks. The volumes in each flask were made up to 10 ml with 0.1N hydrochloric acid, to obtain final solutions contained 2, 4, 6, 8, 10, and 12 µg/ml of drug. First derivative and Second derivative absorbance values were measured at 302 nm and 309 nm respectively.

Calibration curve was constructed from absorbance measure at 291 nm (against 0.1N hydrochloric acid as blank) for standard containing 2-12 µg/ml of Pyridoxine hydrochloride shown in Fig. 2

Estimation of Pyridoxine hydrochloride in tablets

The twenty tablets of Pyridoxine hydrochloride (label claim 100 mg) were triturated and mixed properly. Accurately weighed quantity equivalent to 100 mg of Pyridoxine hydrochloride was transferred in 100 ml volumetric flask containing small quantity of Distilled water. Then the mixture was ultrasonicated for complete dissolution. The solution were diluted to volume and filtered through whattman filter paper no. 40. Further suitable dilutions were made by 0.1N hydrochloric acid to obtain different concentrations (2-12 µg/ml). These solutions were analyzed and percent recovery of Pyridoxine hydrochloride tablet was determined.

Method Validation

Specificity

Commonly used excipients present in selected tablet formulation were spiked into a preweighed quantity of drug. The absorbance was measured and calculations determined the quantity of the drug.

Linearity

A calibration curve was constructed at optimum experimental conditions using First and Second derivative values versus concentration in the range of 2-12 µg/ml. Regression analysis using the method of least square was made for slope (0.003), intercept (0.000) and correlation coefficient (0.998). The regression equation ($y=0.003x+0.000$) was obtained, where 'y' is amplitude of the peak at 302 nm and 'x' is the concentration of the sample in µg/ml for First derivative. For Second derivative the regression equation obtained was ($0.000x+0.00005$) and correlation coefficient (0.998).

From calibration curve data, high value of the correlation coefficient (0.998) was found and the value of the intercept on ordinate, which is Zero, shows very good linearity of the calibration graph and adherence of the method to Beer's law.

Precision

For Intraday and Interday precisions of the method, solutions of Pyridoxine hydrochloride were prepared at three concentration levels 8 (µg/ml) each in triplicate. These solutions were analyzed respectively three times within one day and three consecutive days.

Accuracy

The accuracy of the method was assessed, based on recovery study. The technique of standard addition was used to assess accuracy of the method. For this purpose a concentration of 80%, 100%, 120% was selected. The absorbance of the sample after standard addition were measured in triplicate. The results are reported in terms of % recovery.

RESULTS AND DISCUSSION

According to the International Conference on Harmonization, the main objective of the validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose, and the parameters that need to be selected are the responsibility of the analyst. The solubility of Pyridoxine hydrochloride in distilled water, so it was used in this method. Pyridoxine hydrochloride in 0.1N HCl shows absorption maxima at 291 nm. The response for Pyridoxine hydrochloride was found to be linear in the concentration range of 2.0–12.0 µg/ml. The optical characteristics of the method and regression analysis of the calibration curve are shown in Table 1. The recovery of Pyridoxine hydrochloride was found to be satisfactory. The proposed spectrophotometric methods were applied to the determination of Pyridoxine hydrochloride in its pharmaceutical formulations.

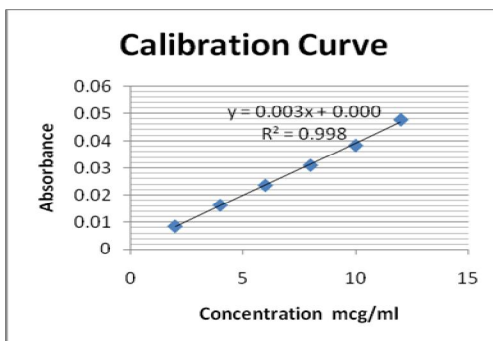


Fig. 1: It shows Calibration Curve of UV spectrum Pyridoxine Hydrochloride at 302 nm

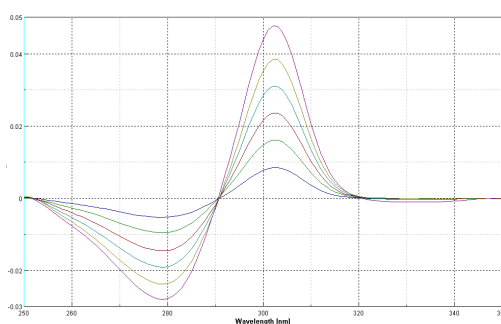


Fig. 2: It shows First derivative UV spectrum Pyridoxine Hydrochloride at 302 nm

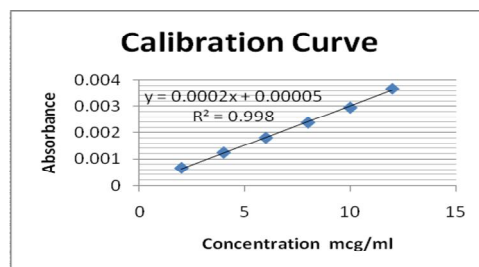


Fig. 3: It shows Calibration Curve of UV spectrum Pyridoxine Hydrochloride at 309 nm

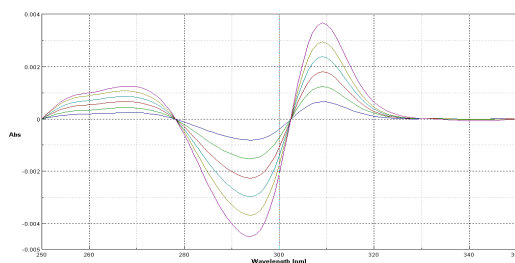


Fig. 4: It shows Second derivative UV spectrum Pyridoxine Hydrochloride at 309 nm

Table I: Optical characteristics and validation parameters of Pyridoxine hydrochloride

Parameter	Values	
	First Order Derivative	Second Order Derivative
Linearity	2-12 µg/ml	2-12 µg/ml
wavelength	302	309
Regression Equation Y=a+bc	Y=0.003x+0.000	Y= 0.0002x+0.00005
Correlation Coefficient r ²	0.998	0.998
Slope(b)	0.003	0.0002
Intercept (a)	0.000	0.00005
Precision	0.01188	0.01188
Accuracy (% recovery)	98.00-102.65	98.00-102.65

CONCLUSION

The method was validated and found to be sensitive, economic, accurate and precise. Hence, the method can be used successfully for routine analysis of pharmaceutical dosage form of Pyridoxine hydrochloride.

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REFERENCES

1. Beckett AH and Stenlake JB. Practical Pharmaceutical Chemistry, 4th edition, Part two, page no.293
2. Narender Sharma, Anurag Mishra, Ravinder Kumar, Sanjay Sharma and Anil Bhandari. second derivative spectrophotometric method for the estimatin of metformin Hydrochloride in bulk and in tablet dosage form, International journal of pharmacy and Pharmaceutical Sciences. 2011;3:4.
3. Kalpesh N Patel, Jayvadan K Patel, Ganesh C Rajput and Naresh B Rajgor. Derivative spectrometry method for chemical analysis: A review, Scholars Research Library Der Pharmacia Lettre. 2010;2(2):139-150.
4. Bosch Ojeda C and Sanchez Rojas F. Recent developments in derivative ultraviolet/visible absorption spectrophotometry.