Research Article

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Spectrophotometric Determination of Vitamin B6 in Pharmaceutical Formulations and Wastewater Samples

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ABSTRACT
A simple, accurate and rapid sensitive indirect method for determination of Vitamin B6 (pyridoxine hydrochloride) has been developed. The proposed method is based on formation of a colored complex by the reaction between released SCN ion from Hg $(SCN)_2$ and ferric ions to form red soluble product with maximum absorption at 460 nm. Beer's law is obeyed over the concentration range of 2-28µg/ml, with molar absorptivity and Sand ell s sensitivity of $0.729x10^4$l/mol.cm. and 0.0281µg.cm^{-2} respectively, relative standard deviation (RSD)is less than1.6 (n=10). The method is applied successfully for determination of Vitamin B6 in some pharmaceutical preparations (injection and tablets) and wastewater samples. The present method is considered to be simple because it does not need either heating or hydrolysis or solvent extraction steps. The ingredients often formulated with Vitamin B6 have been shown not to interfere, and the proposed method is suitable for the routine determination of Vitamin B6.

Keywords: Pyridoxine Hydrochloride (Vitamin B6), Hg $(SCN)_2$, Pharmaceutical Preparations, Wastewater Samples

Introduction

Vitamin B6 (Pyridoxine hydrochloride), chemically is (2-methyl-1- hydroxy-4,5 bis (hydroxyl-methyl) - pyridinum chloride Figure 1.

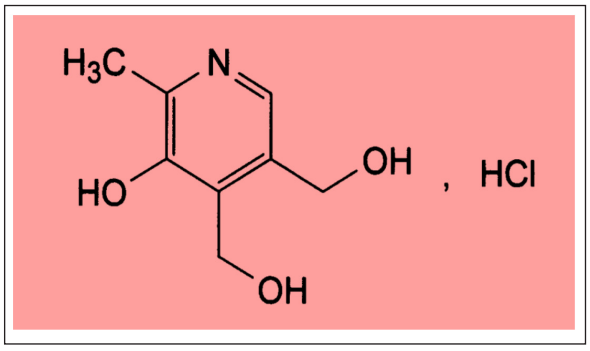

Figure 1: Chemical Structure of Pyridoxine hydrochloride

Is one of the members of the vitamin B6 group a water-soluble vitamin, is involved principally in amino acid metabolism, but is also involved in carbohydrate and fat metabolism. It is also essential for both protein and red blood cell metabolism. It is widely distributed in the plant and animal worlds, especially in yeast, liver, cereals and meat. In pharmaceutical formulations, vitamin B6 is usually found as the hydrochloride. Pyridoxine hydrochloride (Vitamin B6) is required for both mental and physical health, which has been used in the treatment of the nausea and vomiting of pregnancy and irradiation. The deficiency of pyridoxine hydrochloride has been suggested as the case of many types of illness and disease [1-4]. Several methods for the determination of pyridoxine hydrochloride have been described in the literature, including spectrophotometric methods, most of these methods use either diazotized reagents or indirect spectrophotometry methods, spectrofluorometry method,voltammetry methods, partial least-squares regression methods, non-aqueous titration method and HPLC method [5-16]. The official BP described potentiometric titration for pure drug and UV spectrophotometric for tablets and injections [17]. The present work describes a new, simple direct, spectrophotometric method for the determination of pyridoxine hydrochloride in pure form, pharmaceutical formulations and in industrial wastewater samples. The method is based on the reaction of drug with ferric ion at pH3 resulting in the formation of red complex which absorbs maximally at 460 nm. The present work describes a new, simple spectrophotometric method for the determination of pyridoxine hydrochloride in pure form,

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pharmaceutical formulations and in industrial wastewater samples. The method is based on reaction between chloride ion and Hg $(SCN)_2$, formation of a colored complex by the reaction between released SCN and ferric ion.

Experimental

Apparatus

Shimadzu UV-1700 pharmspec (double beam) spectrophotometer with 1.0 cm quartz cells was used for absorption measurements.

Reagents

All chemical used were of analytical or pharmaceutical grade and pyridoxine hydrochloride standard material was provided from AL-Hokamaa company for pharmaceutical industries (HPI) Mosul-Iraq.

Pyridoxine Hydrochloride Standard Solution: 0.01% (100µg/ml)

This solution was prepared by dissolve 0.01 gm. of pyridoxine hydrochloride in 100 mL of distilled water in volumetric flask.

Ferric Ammonium Sulfate Solution: 5%

5g of ferric ammonium sulfate $[FeNH_4(SO_4)_2.12H_2O]$ was dissolved in 50 ml double distilled water and 20ml of concentrated nitric acid was added and diluted with double distilled water to 100ml.

Hg $(SCN)_2$ Solution: 0.5%

0.5g of Hg $(SCN)_2$ was dissolved and diluted to 100 ml with ethanol. Mixed and filtered through filter paper.

General Procedure

Different aliquots of standard pyridoxine hydrochloride solution equivalent 50-700 µg (0.5-7 mL) were transferred into a series of 25ml volumetric flasks, and 2mL of ferric ammonium sulfate solution were added and 2ml of saturated solution of $Hg(SCN)_2$ were added to each flask and mixed well with occasional shaking. This was diluted to 25ml with double distilled water. and mixed well. Let stand for 5min, the absorbance of each solution was measured at 460 nm against a reagent blank.

Procedures for Pharmaceutical Preparations (Tablets)

To minimize a possible variation in the composition of the tablets(containing 25mg of pyridoxine hydrochloride/tablet were provided from AL-Hokamaa company for pharmaceutical industries (HPI) Mosul-Iraq).). The mixed content of 10 tablets were weighed and grounded, then the powder equivalent to 10 mg of pyridoxine hydrochloride in about 50 ml of distilled water was stirred well for 30 min and then filtered through Whitman No. 42 filter paper and the filtrate solution was diluted to 100ml by distilled water and different volume of this solution was treated as described above under general procedure.

Procedures for Pharmaceutical Preparations (Ampules)

Ampule containing 10mg of pyridoxine hydrochloride (were provided from state company of drug industries and medical appliance (NDI) Nineveh- Iraq.) was transferred into 100mL volumetric flask and diluted up to the mark with distilled water, The determination of pyridoxine hydrochloride was treated as described above under general procedure. and the concentration was calculated by using the calibration curve of this method.

Procedure for Industrial Wastewater Samples

To demonstrate the practical applicability of the proposed method, real industrial wastewater samples from Al-Hokamaa company for drug industries (HPI) Mosul-Iraq were analyzed by spiked with the concentrations ranging from 2-20 µg /ml of pyridoxine hydrochloride and aliquot of this solution was treated as described above under general procedure and the concentration was calculated by using the calibration curve of this method.

Results and Discussion

Spectrophotometry is a very valuable technique which has proven its worth in the field of pharmaceuticals analysis over the years. It is particularly popular because of its features like ruggedness, economical, suitable for wide range of pharmaceuticals by using different reagents [18-20]. The method depends upon the displacement of SCN ion from Hg $(SCN)_2$ by chloride ion in the Pyridoxine hydrochloride. The released SCN ion was found to react with Fe (III) at room temperature resulting in formation of red colored complex which absorbed at 460nm (Figure. 2).and the intensity of its color is proportional to the original chloride ion [21].

$$2Cl^- + Hg(SCN)_2 + 2Fe^{3+} \rightarrow HgCl_2 + 2[Fe(SCN)]_2^+$$

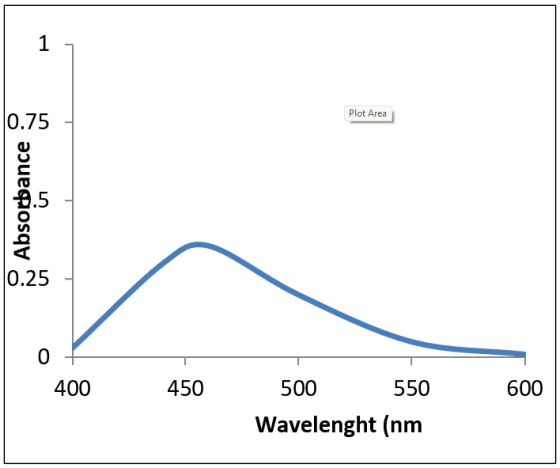

Figure 2: Absorption Spectra of 10µg /ml of Pyridoxine Hydrochloride

The various experimental affecting the development and stability of the reaction product was optimized by changing each variable in turn while keeping all other variables constant.

Effect of Ferric Ammonium Sulfate Solution

The amount of ferric ammonium sulfate solution (5%) for maximal color intensity was examined the maximum constant intensity was reached at 1 ml of reagent solution and remained constant up to 4ml. However 2 ml of the reagent solution was selected for the subsequent work.

Effect of Hg $(SCN)_2$ Solution

The amount of Hg $(SCN)_2$ solution (0.5%) for maximal color intensity was examined the maximum constant intensity was reached at 1 ml of reagent solution and remained constant up to 4ml. However 2 ml of the reagent solution was selected for the subsequent work.

Effect of Temperature and Time

The results obtained indicated that complete color formation occurred immediately and not effected by temperature therefAore, room temperature was selected as suitable temperature. The absorbance remained constant for 6 hours at least, and 5 min was selected as a suitable time.

Effect of Order of Addition

To test the effect of order of the addition of the reagents on the absorbance of the product, different order were tested. The selected order was sample solution, ferric ammonium sulfate followed by Hg $(SCN)_2$ solution which was gave high absorbance value.

Calibration Graph

Employing the conditions described in the general procedure a linear calibration graph of pyridoxine hydrochloride which obeys Beer's law in the concentration range of 2-28 µg/ml (Figure.3). Linear regression equation: Y= 0.0355X-0.0053 (r=0.997). Where Y is the absorbance and X is concentration in µg/ml. The apparent molar absorptivity was 0.729×104 l.mol-1. cm-1 and sandal's sensitivity was 0.0281µg.cm-2. The limit of detection and limit of quantification were evaluated as [22-24]: LOD = Intercept /Slopex10 and LOQ = 3.3LOD. The limit of detection was 0.36 µg/ml and the limit of quantification as the lowest standard concentration which could be determine with acceptable accuracy, and precision was 1.188µg/ml as the lowest standard concentration which could be determine with acceptable accuracy. The applied method can be used routinely for the estimation of pure drug salts through their chloride concentration.

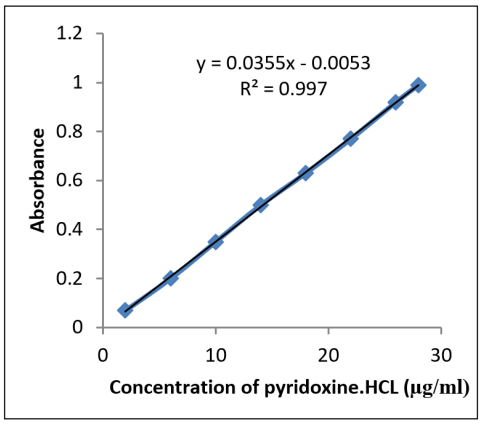

Figure 3: Calibration graph of pyridoxine hydrochloride

Accuracy and Precision

The accuracy and precision of the method were established by analyzing the pure drug solution at three different levels. The average recovery which is a measure of accuracy is 100 ±1% revealing high accuracy of the method. The relative standard deviation (RSD), which is an indicator of precision, is less than 1.6%, the result are compiled in (Table 1).

Table 1: optical characteristics and statistical data for regression equation of the proposed method

Parameters	Value
λ max (nm)	460
Beer's law limits (µg.ml^{-1})	2-28
Molar absorptivity (L.mol^{-1}.cm^{-1})	0.729×10^4
Limit of detection (µg.ml^{-1})	0.36
Limit of quantification (µg.ml^{-1})	1.188
Sandell's sensitivity (µg.cm^{-2})	0.0281
Correlation coefficient (r)	0.997
Regression equation (Y=C+bX)	
Intercept (C)	-0.0053
Slope (b)	0.0355
Recovery	100±1
Relative standard deviation (%)	< 1.6

Effect of Interferences

The interfering effect of foreign species often accompanied with pyridoxine hydrochloride in the pharmaceutical preparations were studied by adding different amounts of foreign species to 400µg/25ml of pyridoxine hydrochloride in solution and the recommended procedure for the determination of pyridoxine hydrochloride was followed. The species are considered to interfere seriously if the cause aching of more than 2% in the absorbance obtained for pyridoxine hydrochloride a lone [25]. Results of the recovery analysis are presented in (Table.2). Excipients at the concentration show in (Table.2). do not interfere with the assay. In addition recoveries in most cases were around 100%.

Table 2: Determination of pyridoxine hydrochloride in presence of excipients

Excipients	Amount taken, (µg/ml)	Average recovery, * %
Talc	500	99.95
	1000	100.08
Mannitole	600	100.05
	1000	99.98
Mg - stearate	600	100.07
	1000	100.07
Starch	500	100.06
	1000	100.08
Microcrystalline cellulose	500	99.96
	1000	99.97

* Average of five replicate determinations.

Application of the Proposed Method

The proposed method was successfully applied to the analysis of pyridoxine hydrochloride in pharmaceutical preparations [tablets and Ampules] and industrial waste water sample. The result of analysis for pharmaceutical formulations revels that there is close agreement between the results obtained by the proposed method and the label claim (Table. 3), And the results of water samples (Table.4) show that the recovery values obtained were close to 100%.

Table 3: Assay of pyridoxine hydrochloride in pharmaceutical formulations

Pharmaceutical formulation supplied by	Amount of pyridoxine hydrochloride * Proposed method	Label claim	%Recovery
Tablet 25mg/Table [HPI]	24.9 mg	25 mg	99.6
Ampules 10mg/Amp [NDI]	9.98	10mg	99.8

*Mean of ten determinations.

Table 4: Determination of pyridoxine. HCL in spiked industrial wastewater samples

Water samples	pyridoxine. HCL (µg/ml)*		Recovery %
	Taken	Found	
Industrial wastewater	4.0	4.01	100.25
	10	10.05	100.5
	16	16.08	100.5

*Mean of ten determinations

Acknowledgments

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Conclusions

The applied method was simple, rapid, accurate, precise, sensitive and low economical cost. Furthermore, the proposed method doesn't require elaboration of procedures, which are usually associated with chromatographic methods. The proposed method could be applied successfully for determination of pyridoxine hydrochloride in environmental water samples, pure form as well as in different pharmaceutical forms.

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