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Original Research Article

High Performance Liquid Chromatographic Method for the Analysis of Pyridoxine Hydrochloride in Liquid Dosage Form

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ABSTRACT

Vitamin B6 (pyridoxine hydrochloride) is available in the market as ampoule, but there is no simple method for its analysis. The aim of this study was to develop a relatively basic, very simple, fast, sensitive, accurate and less expensive HPLC method for the assessment of pyridoxine hydrochloride from vitamin B6 ampoule. The wavelength of absorbance of pyridoxine hydrochloride was measured at 280 nm. Mobile phase was prepared by dissolving 140 mg of sodium 1-hexanesulfonate per 100 mL of a mixture of methanol, glacial acetic acid and water (41:1:58). The mobile phase was transferred at a flow rate of 1.0 mL/min and the stationary phase used was a Waters Zorbax SB C18 column (5 µm pore size 250 * 4.6 mm). Injection volume was 20 µL/loop. The linearity was achieved with a linear regression (R^2) = 0.9994 over the concentration range of 0.25-1 mg/mL. Separation was performed within 4.4 min ± 0.10, no interference with any reported ingredients. Calculated percentage accuracy (recovery) was between 100.31 to 100.63%. The technique was discovered to be repeatable for inter- and intra-day precision level across the concentration of the sample with acceptable (RSD) NMT 2%. The technique have been verified and found suitable for determining pyridoxine hydrochloride in ampoule. This technique is useful in the quality control laboratory for routine use in the determination of pyridoxine hydrochloride in liquid dosage form.

Keywords: Pyridoxine hydrochloride, Reverse-phase HPLC, Stability, Verification.

Introduction

Vitamins play an important role in human wellbeing, but they constitute only a tiny portion in the diet.[1] Food not containing adequate amounts of vitamins may lead to deficiencies which frequently co-exist with mineral (zinc, iron, iodine) deficiencies. The groups most susceptible to vitamin deficiencies are pregnant and lactating women, and young children. Different types of vitamins play important roles in maintaining good health. For example, thiamine functions as a co-enzyme in the phosphogluconate process and structural part of the nervous system membrane, while it also plays a major role in the deamination and transamination of amino acids.[2,3] Sufficient consumption of vitamin B6 is known to prevent cardiovascular diseases, stroke, diabetes and cancer. Pyridoxal 5'-phosphate deficiency manifests as fatigue, nervousness, compromised immune system, and tiredness. Serious vitamin B6 deficiency due to impaired haemoglobin synthesis may be a causative factor for sideroblastic microcytic anaemia, convulsions refractory to traditional drugs, epilepsy and transient neuropathic pain.[4]
Recent research suggests that folate-based vitamin B6 can be effective against nasopharyngeal carcinoma.[5] Research has also shown that 16% of children with sickle cell disease have low levels of vitamin B6, and sickle cell disease is also associated with nutrient deficiencies.[6]
Pyridoxine hydrochloride is one of three forms of vitamin B6 that occurs in natural sources. The other two forms are known as pyridoxal and pyridoxamine, with all three forms differing based on the substituent on the carbon atom at position 4 of the pyridine molecule.[7] All three forms of vitamin B6 are converted in the body to pyridoxal phosphate, which is vital for metabolism and serves as a coenzyme for various metabolic transformation of amino acids (decarboxylation, transamination, and racemization) and metabolism of tryptophan. It is widely recognized as an integral component of human diet. If a certain member of the B vitamin family is found to be defective, so the inference is made that vitamin B6 is also defective. Therefore, it is important to give pyridoxine supplement as part of patients' medication.[8] It can be used for abortion prophylaxis or diagnosis of insomnia, nausea, and diarrhea, and as a vitamin B6 food additive.
Potentiometric titration and HPLC have been used for the analysis of Pyridoxine HCl in BP and USP, respectively. The analytical methods previously mentioned defined the quantity of pyridoxine HCl as a raw material.[9-11] Chemically, Pyridoxine hydrochloride is known as 2-methyl-3-hydroxy-4, 5-bis (hydroxymethyl) pyridine hydrochloride (Figure 1), and has a molecular weight of 205.4 g/mole. It is a white substance that is simply soluble in water at 1 g in 5 mL. It is stable in air, heat and acid solutions, whereas it is unstable in alkaline medium and light.[12]
An appropriate method for the analysis of vitamins B2, B3, B6, B12, E, K, D, A, C and β-carotene in okra utilizing HPLC has been described.[13]

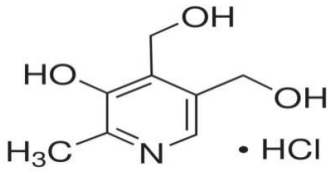

Figure 1: Structural formula of Pyridoxine hydrochloride

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Liquid chromatography-tandem mass spectrometry was found to be similar to HPLC for the analysis of vitamin B6. Moreover, acid phosphate has been used to dephosphorylate the vitamin sources of pyridoxal, pyroxamine and nicotinamide in processed extract solution prior to liquid chromatography–tandem mass spectrometry.[14] Additional approaches for the assessment of vitamin B6 include spectrofluorometric, and capillary zone electrophoresis techniques.[15] The present research describes the suitability and validation of reverse phase HPLC process, which can simultaneously measure pyridoxine hydrochloride in pharmaceutical solutions. According to the International Conference on Harmonization (ICH) Q2 (R1) confirmation of the applicability of this approach was validated.[16] Vitamins have been classified into water-soluble and fat-soluble vitamins, both of which can be easily measured utilizing reverse phase High-Performance Liquid Chromatography.[17]

For quantification from germinated chickpeas, water-soluble vitamins such as B1, B2, B3, and B6 can be identified. Liquid Chromatography-Tandem Mass Spectrometry for vitamin B6 assay has been shown to be equivalent to HPLC. [18] In some other analysis, acid phosphate has been used to dephosphorylate pyridoxal, pyroxamine, and nicotinamide vitamin forms in the digested test export before tandem mass spectrometry in liquid chromatography.[19] Other techniques for vitamin B6 determination include spectrofluorometric, spectrofluorometric-derivative and capillary zone electrophoresis techniques. In this study, a fairly easy, flexible, validated and effective HPLC system is being established for the analysis of pyridoxine hydrochloride injection.

Materials and Methods

Source of chemicals

Pyridoxine hydrochloride standard was obtained from Pioneer Company as a gift and all chemicals and reagents utilized were HPLC grade. Sodium 1-hexanesulfonate was obtained from AppliChem, Germany. Methanol, acetonitrile (CHROMASOLVE®, Sigma - Aldrich Chemise GmbH, Germany) and Glacial acetic acid (Merck Germany) are as described and -A Milli-Q Reagent Grade water system was used to deionize and purify the water.

Determination of pyridoxine hydrochloride by high performance liquid chromatography

HPLC analysis was performed using a Waters HPLC system fitted with a Waters 2487 UV vector absorbance detector and a Waters 2695 with auto sampler. For pyridoxine hydrochloride analysis, mobile phase with a flow rate of 1.0 mL/min was used, and the run time was 4.4 ± 0.2 min. The stationary phase was a Waters Symmetry C18 column (4.6 * 250 mm, 5 µm particle size). Degassing was accomplished by filtration with a 0.45 µm Millipore membrane filter and then sonication. The injected volume was 10 µL and the detection wavelength was at 280 nm with the HPLC system running at 25°C. The tests were carried out in an air-conditioned environment at a temperature of 25 ± 2°C.

Preparation of pyridoxine hydrochloride standard and sample solutions was done in a mixture of water, acetonitrile and glacial acetic acid at a ratio of 94:5:1 (v/v/v) to obtain a concentration of 5 mg/mL for both standard and sample solution. Thereafter, a calibration curve was obtained by diluting the stock concentration with the described diluent to achieve concentrations of 0.25, 0.37, 0.5, 0.75 and 1 mg/mL pyridoxine hydrochloride using same method as mentioned above.

Application of the HPLC method

The content of ten ampoules (10 STEROP 100 mg/2mL B.N 140219) was mixed and an appropriately determined amount of 5 mL equivalent to 250 mg Pyridoxine hydrochloride and 40 mL diluent was added to a 50 mL volumetric flask. These were dissolved and the flask was kept in an ultrasonic bath for 10 min and then made up to volume with the diluent to prepare sample stock solution. The working sample solution were prepared by transferring 1 mL of the sample stock solution, diluted with 100 mL of the diluent and passed through 0.45 Mille-Q filter paper before use. The Limit of Quantification and strength was calculated according to ICH Harmonized Tripartite method.[13] For the calibration curve, five different concentrations were prepared, and another three different separate concentrations were prepared in order to determine linearity, consistency, precision and stability.

System specificity was calculated through a comparison of standard substance with the test. Regular and functional solution with concentration of 0.5 mg/mL were prepared in six replicates and evaluated to determine specificity of sample. The ratio of Relative standard deviation of their peak area was determined. System performance monitoring was used to ensure that the system resolution and reproducibility is perfect for concentration of 5 mg/mL. Percentage Relative standard deviation for retention time and peak area of five replicates were calculated for the test sample and summarized in Table 1. For the calibration curve, five different solutions were prepared using the dilution from stock solution to achieve concentrations of 0.25, 0.37, 0.5, 0.7 and 1.0 mg /mL.

Recovery experiments were performed by applying regular standard product solution of pyridoxine hydrochloride to the sample placebo solution at three separate amounts (75, 100 and 125%) of the test concentration (3*3) to determine the efficiency of the process and to verify the impact of excipients. The recovery analysis was performed by applying precisely measured pyridoxine hydrochloride volumes to the combination of excipients and then by determining the rate of recovery for each concentration. (Table 2). Inter-day and intra-day precision were tested by six replicates measurements of 0.5 mg/mL pyridoxine hydrochloride for repeatability and accuracy, while three replicates measurements were done to determine the intermediate Precision (Table 4). The calibration curve approach was used to identify and measure pyridoxine hydrochloride. Pyridoxine hydrochloride solutions were formulated in 0.25–1 mg/mL concentration range and were administered in triplicate. Detection limit was determined by using the equation;[14]

Limit of detection = 3.3 x standard deviation of the response/Slope

Quantification limit was determined on the basis of the standard response and the slope variance.

Quantification limit = 10 x Standard deviation of the response/Slope of the calibration curve.

Statistical analysis

Data were recorded as mean ± Standard Deviation. Where applicable, data were analysed by one-way analysis of variance using the student t-test.

Results and Discussion

Vitamins are considered to be essential compounds for health and growth. They are required in limited amount but are typically available through fruits and vegetables. Vitamin B6 is critical for protein, carbohydrate and fat breakdown. Intravenous administration of vitamin B6 has also been used as an adjunctive treatment for acute cycloserine, hydrazine and Gyromitra (a fungus whose toxins release methyl hydrazine) poisoning.[20]

During trials with a range of mobile phase compositions, 140 mg of sodium1-hexanesulfonate per 100 mL (methanol: glacial acetic acid: water), v/v % was chosen as the appropriate mobile phase with a flow rate of 1.0 mL/min. Pyridoxine hydrochloride was observed to exhibit significant absorption at 280 nm, and therefore chosen as the wavelength of detection. Figure 3 shows an improved chromatogram for pyridoxine hydrochloride determination at a retention time of 4.4 min. Mobile phase requirements were streamlined, thereby reducing the ampoule components from solvent and excipient interferences. Certain parameters were also considered. For example, time needed for study, assay tolerance, solution related noise, and usage of the same solvent to remove compound from a product matrix. Octylsilane and Octadecylsilane columns were checked, including different stationary phases. A 250/4.6 mm I.D., 5 mm particle stain steel C18

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reversed phase column obtained adequate resolution and run time. Hence, this was chosen for further analysis. Pyridoxine hydrochloride eluted consistently at 4.4 min. The drug substances were analysed in HPLC-grade water directly injected at 20 µL and the resolution (peak areas) were recorded at 280 nm. It was observed that there was no interference from the mobile phase or baseline disturbance, and all the analytes absorbed well at 280 nm. For system validation, the process was tested as per the ICH standards with respect to linearity, consistency, precision, repeatability, selectivity and specificity.

Linearity of pyridoxine hydrochloride by high performance liquid chromatography

Validation experiments were performed by replicating sample treatments and standard solutions into the chromatograph. The linearity was determined by plotting peak area against standard pyridoxine hydrochloride concentration. The calibration graphs obtained were linear for the concentrations from 0.24 to 0.99 mg/mL of pyridoxine hydrochloride at five separate concentration levels (50, 75, 100, 125 and 150%), and then the average area was calculated by the average of three repeat measurements. The slope was 16872.59, intercept 51825.46, correlation (r) 0.999679 and coefficient of correlation (R^2) 0.9994 (Table 1). The maximum peak areas were plotted against the concentrations with the correlation coefficient (R^2) of 0.9994 and were viewed as proof of the appropriate fitness of the regression line (Figure 2).

Determination of recovery of pyridoxine hydrochloride

The accuracy of the model was carried out in accordance with ICH guidelines by studying the recovery at three concentrations: 75, 100 and 125%. The re-analysed sample solution was replaced with each concentration. All the results were within the acceptable limits, i.e. CV < 2.0%, and S.D. < 1.0[1].

The accuracy of an analytical method reflects the near consistency between a set of data obtained from various sampling of the same population under the conditions defined. Placebo of vitamin B6 STEROP was prepared by mixing excipients (0.5% anhydrous chlorobutanol [chlorine derivative], water for injection) without pyridoxine hydrochloride. The pH of placebo was kept between 2.0-3.8 with sodium hydroxide or hydrochloric acid because the pH of sample injection (STEROP) was 3.2. The result shows that in repeatability, the % RSD of peak area of pyridoxine hydrochloride at 75, 100, and 125% concentrations was found to be 0.29, 0.13, and 0.12%, respectively (Table 2). Therefore, the technique can be considered accurate and reliable.

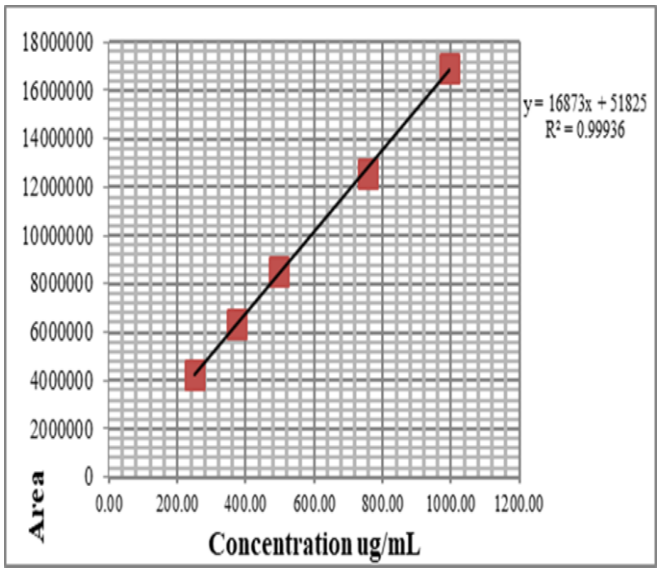

Figure 2: Representative Linearity Plot of Pyridoxine Hydrochloride

Analytical method validation is a mechanism for ensuring that an analytical technique is appropriate for its intended purpose. The data found from the validation study could be used to assess the quality, dependability and coherence of the experimental data. Different mobile phase components have been tested and mobile phase composition of water: glacial acetic acid: methanol (58:1:41) that included 0.14 g of sodium 1-hexanesulfonate per 100 mL was found to give the best quantitative separation. An optimized chromatogram of blank, the Retention Time of Standard Pyridoxine HCl peak was found to be at 4.39 min as shown in Figure 3. The chromatogram of the sample Vitamin B6 STEROP 100 mg/2 mL injection is presented in Figure 4, blank chromatogram is given in Figure 5 and the placebo chromatogram of Vitamin B 6 is shown in Figure 6.

Precision, detection limit and quantitation limit

It was also apparent that the system allowed rather precise quantitative assessment of pyridoxine hydrochloride in ampoule dosage form.

All intra-day and inter-day accuracies have been reported. The stock solution was prepared and five repeat sample solutions were prepared from each stock solution. Throughout the test of intra-day precision, three STEROP ampoule concentrations were calculated on the same day at time intervals of 1 h. The detection and quantitation limits (detection limit and quantification maximum) were determined respectively as highlighted in Table 3.

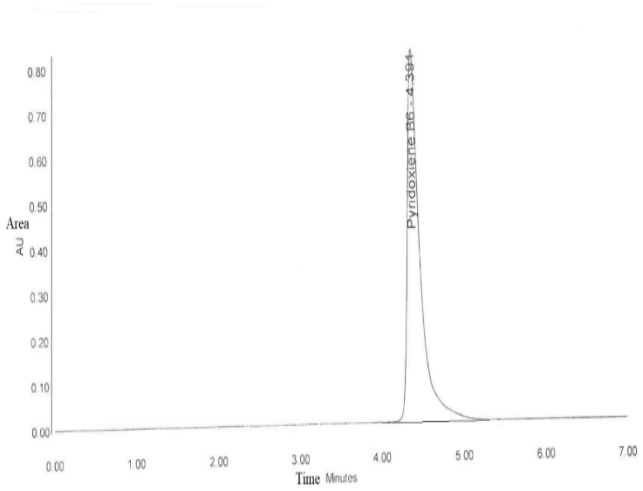

Figure 3: Chromatogram for Standard Pyridoxine Hydrochloride 497 µg/mL at 280 nm

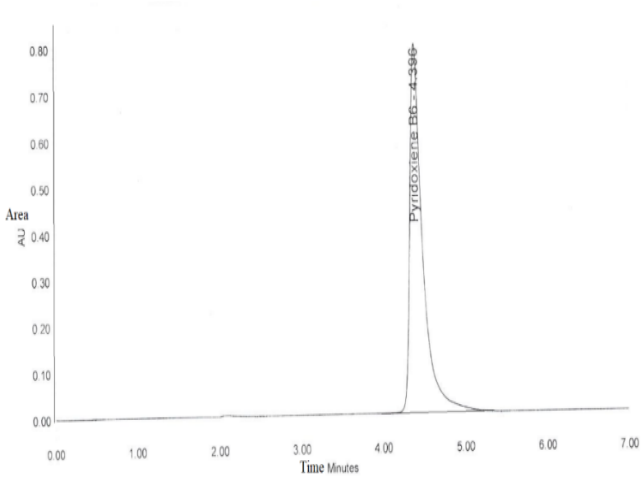

Figure 4: Chromatogram for Sample STEROP Ampoule 500µg/mL at 280 nm

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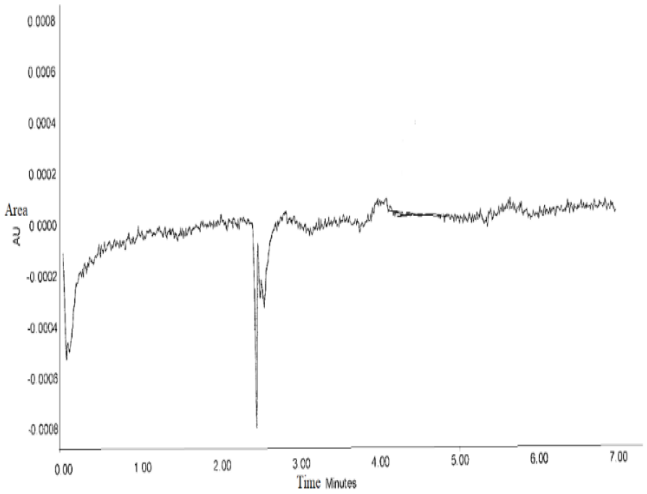

Figure 5: Chromatogram for Blank

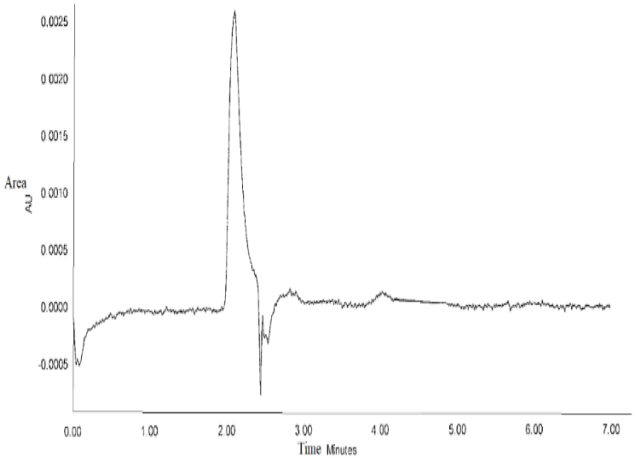

Figure 6: Placebo Chromatogram at 280 nm

Table 1: Linearity of Pyridoxine Hydrochloride and Level of Concentration

Level	Concentration ug/mL	Area	Average area		
		4224614.306		Slope	16872.59
50%	248.70	4228750.090	4227737.324	intercept	51825.46
		4229847.575		R	0.999679
		6391088.243		R^2	0.9994
75%	373.05	6384601.548	6386472.15		
		6383726.659			
		8530135.077			
100%	497.40	8524373.777	8526294.21		
		8524373.777			
		12587931.65			
125%	756.09	1604451.18	12596224.74		
		12596291.40			
		16951252.98			
150%	994.79	16948902.99	16947234.02		
		16941546.09			

Table 2: Determination and Recovery of Pyridoxine Hydrochloride

Standard data of pyridoxine hydrochloride				
Concentration µg/mL	**Area**	**Average Area**	**SD**	**RSD%**
	8517025.37300			
	8522279.61100			
497.396	8530135.07700	8523405.5642	4696.68	0.1%
	8524373.77700			
	8523213.98300			
Accuracy - recovery of pyridoxine HCl				

Nominal value	Amount spiked µg/mL	Area	Amount recovered µg/mL	% Recovery	Average Recovery	%RSD
		6450848	376.45	100.91%		
75%	373.05	6413997	374.30	100.34%	100.63%	0.29%
		6433667	375.45	100.64%		
		8557683	499.40	100.40%		
100%	497.40	8537329	498.21	100.16%	100.31%	0.13%
		8555221	499.25	100.37%		
		12843962	749.53	100.46%		
125%	746.09	12813374	747.74	100.22%	100.34%	0.12%
		12830044	748.72	100.35%		
		Average			100.43%	

Table 3: Intra-day and Inter-day Precision

Compound	Type of analysis	Level	Nominal value (mg/mL)	Found (mg/mL)	RSD%
Pyridoxine Hydrochloride	Within-day	75%	0.373	0.37645	0.29
		100 %	0.497	0.4994	0.13
		125%	0.746	0.74953	0.20
	Between-day	75%	0.373	0.37545	0.29
		100 %	0.497	0.49925	0.13
		125%	0.746	0.74872	0.13

Conclusion

A simple, rapid and sensitive analytical method was developed and validated for the analysis of pyridoxine hydrochloride. The chromatographic runtime was short. Statistical analysis proves that the method is suitable for the analysis of pyridoxine hydrochloride in pharmaceutical formulation without any interference from the excipients. The established validated process was discovered to be quick, sensitive, reliable, time-saving and less costly for quantification of several samples in ampoule formulations and suggest that the proposed approach will be very useful as a mechanism for quality control of pyridoxine hydrochloride.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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