Bulgarian Chemical Communications, Volume 47, Number 1 (pp. 131 – 134) 2015

New, simple and validated UV-spectrophotometric methods for the estimation of pyridoxine hydrochloride in bulk and formulation

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Received January 28, 2014; Revised June 30, 2014

In this study, two rapid, simple and accurate spectrophotometric methods for the determination of pyridoxine hydrochloride, in bulk and in pharmaceutical formulations are described. These methods are based on the reaction of pyridoxine hydrochloride with ferric salts, i.e. ferric nitrate and ferric ammonium citrate. The reaction produces a yellowish orange color which absorbs maximally at 445 and 450 nm for ferric nitrate and ferric ammonium citrate, respectively. Beer's law was obeyed in the range of 5-50 μg mL^{-1} for both reagents. The regression analysis of Beer's plot showed a good correlation coefficient (r^2= 0.9987, 0.9982). The results were validated analytically and statistically according to International Conference on Harmonization (ICH) guidelines. The proposed methods were applied to the determination of pyridoxine hydrochloride in bulk and pharmaceutical preparations with good results.

Keywords: Pyridoxine, ferric nitrate, ferric ammonium citrate, spectrophotometric methods.

INTRODUCTION

The importance of vitamins in one's diet can be ascertained by the fact that their deficiency leads to different diseases in humans. Therapeutic multivitamins are prescribed in such deficiencies as dietary supplements since the human body is unable to synthesize these vitamins [1], therefore a multivitamin complex is recommended to be used as a dietary supplementation. Pyridoxine hydrochloride (figure 1) is a well-known drug used for the treatment or prophylaxis of depression, pregnancy complications as nausea and vomiting and to overcome vitamin B6 deficiency [2, 3].

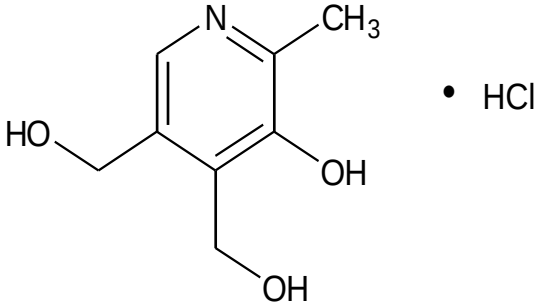

Fig. 1: Pyridoxine hydrochloride

After absorption in the gastrointestinal tract the two active forms, pyridoxal phosphate and pyridoxamine phosphate are released [4]. It helps in the metabolism of amino acids, carbohydrates and fats [5]. A number of methods for the determination of pyridoxine hydrochloride in combination with antihistamine drugs have been reported using HPLC [6-22], capillary electrophoresis [23], planar HPLC [24] and UV spectrophotometry [25-32].

However, all reported methods are time consuming with extensive use of chemicals. The economical significance of spectrophotometric methods over other methods cannot be overlooked. Several spectrophotometric methods for the estimation of pyridoxine hydrochloride using different complicated techniques and expensive reagents in tablets have been reported. Surmeian [25] reported a method using derivative UV spectrophotometric technique. Dinc and Baleanu [26] reported a method using a one-dimensional wavelet transform. Ozdemir and Dinc [27] reported the estimation of pyridoxine hydrochloride using a genetic algorithm based on multivariate calibration methods. El Gindy [30] estimated pyridoxine hydrochloride by using graphical (second derivative of the ratio spectra) and numerical spectrophotometric methods (principal component regression and partial least squares, applied to the zero order UV spectra of the mixture). Raza *et. al.*, [32] reported a method using chloranil, an expensive reagent.

The present study was designed to develop easy, economical accurate and least time-consuming spectrophotometric methods for the determination of pyridoxine hydrochloride in raw form and in pharmaceutical formulations. In the present methods, pyridoxine hydrochloride was reacted with ferric salts producing a yellow orange colored complex showing absorption in the visible region of the spectrum at 445 and 450 nm for the quantification. The proposed methods were validated according to International Conference on

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Harmonization (ICH) guidelines [33]. The two developed methods were found to be convenient, economical and easy for the routine analysis of the drug in laboratories and pharmaceutical industries.

EXPERIMENTAL

Apparatus

A double-beam Shimadzu (Japan) UV–Visible spectrophotometer, model UV-1601 was used. The software was UVPC personal spectroscopy software version 3.91 (Shimadzu) utilized for analysis.

Materials

Pyridoxine hydrochloride was kindly supplied by UCB (Belgium). Commercial pharmaceutical formulation named VITA-6® tablets from Chas. A. Mendoza Pharma Karachi containing 50 mg of pyridoxine was obtained from local pharmacies. Ferric salts were purchased from Merck Marker Pakistan. All other chemicals and reagents were of analytical grade and deionized water was used throughout the experiments.

Preparation of standard solutions

Standard stock solution of pyridoxine hydrochloride 100 µg mL^{-1} was prepared in deionized water and diluted to a working range of 5 to 50 µg mL^{-1} in 25 mL volumetric flasks. Then, 3 mL of 1% each of ferric salts solution was added to each flask. The volume was made up to the mark with deionized water and the yellowish orange complexes formed at room temperature were scanned on the spectrophotometer in the visible range of 400-700 nm against the reagent blank.

Analysis of tablets

Twenty tablets of weight equivalent to 100 mg of pyridoxine hydrochloride were crushed, and transferred to a 100 mL volumetric flask. The volume was adjusted to the mark with deionized water. Then, the solution was sonicated for 30 min, filtered and subjected to the proposed procedure for determination.

Study of interferences

Effect of excipients on the method was studied by mixing a known amount of pyridoxine (10 mg) with specified amounts of the excipients such as talc, magnesium stearate, aerosol, etc. in their recommended percentages [3].

RESULTS AND DISCUSSION

Numerous methods for the determination of pyridoxine and its mixtures have been reported in the literature using HPLC and spectrophotometry. It is reported that pyridoxine hydrochloride binds to ferric chloride [31] in which pyridoxal reacts with the –OH group following a second order rate constant. Ferric salts easily react with –OH groups and the complex thus formed, can be used for detection and quantification of -OH group containing drug molecules [28, 34, 35]. The iron forms a yellowish orange color complex which absorbs radiation in the visible range.

In the present work, the complexes formed by pyridoxine hydrochloride with ferric nitrate and ferric ammonium citrate, displayed the λmax of 445 and 450 nm, respectively (figure 2).

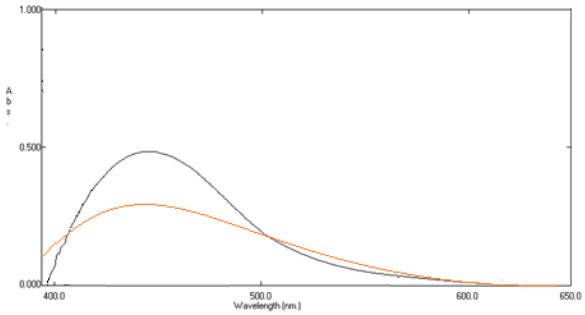

Fig. 2: UV-visible spectra of the complexes of pyridoxine with ferric nitrate (red) and ferric ammonium citrate (black)

These complexes followed Beer's law in the concentration range of 5-50 µg mL^{-1}. Regression analysis of pyridoxine hydrochloride-iron complexes formation and linearity of calibration graph was validated by the high value of the correlation coefficient (r^2 = 0.9987, 0.9982). Quantitative determination of pyridoxine hydrochloride in tablets using this method was performed according to ICH guidelines and the results were in good agreement with the labeled amount of pyridoxine hydrochloride. In addition, % RSD for the determination of pyridoxine hydrochloride and % recovery showed that the proposed method was accurate, precise and reliable. It was further observed that excipients of the tablet did not interfere with the reaction between pyridoxine hydrochloride and ferric salts. These proposed methods can be used for the determination of pyridoxine hydrochloride in pharmaceutical preparations.

METHOD VALIDATION

Validation of the method was carried out using International Conference on Harmonization Q2B guidelines for linearity, precision, accuracy, limits of detection and quantification [33].

Linearity

In developed UV method, calibration curves were linear in the range from 5-50 µgmL^{-1} for both

Table 1 Linear regression functions and their statistical parameters

Drug complex with	Ferric nitrate	Ferric ammonium citrate
Regression equations	y = 0.0364x-0.0094	y = 0.0133x+0.0001
Beer's law limits (μg mL^{-1})	5-50	5-50
λ_{max} (nm)	445	450
r^2	0.9987	0.9982
LOD (μg mL^{-1})	0.25	0.29
LOQ (μg mL^{-1})	1.10	1.17

complexes. Calibration curves were constructed with 9 different concentrations. Each concentration was analyzed 3 times. Statistical data (Table 1) showed that the methods were linear with correlation coefficient (R^2) 0.9987 and 0.9982 for complexes of pyridoxine with ferric nitrate and ferric ammonium citrate, respectively.

Accuracy and precision

Different levels of drug concentrations were prepared from the stock solution and were analyzed for accuracy determination. Accuracy was assessed as the mean percentage recovery of drug concentrations prepared from the stock solution and analyzed (Table 2).

Table 2 Accuracy of method

Drug with	Added Concentration (μg mL^{-1})	Measured Concentration (μg mL^{-1})	Accuracy %
Ferric nitrate ($FeNO_3$)	8	8.01	100.18
	10	10.11	101.10
	12	12.11	100.91
Ferric ammonium citrate ($C_6H_8O_7FeNH_3$)	8	8.19	102.37
	10	10.04	100.44
	12	11.98	99.83

Repeatability was determined by using different levels of drug concentrations prepared from the stock solution and analyzed. The relative standard deviation (in %) was taken as precision.

Statistical calculations for the above method are shown in Table 3 which proves the reliability of the method for the determination of pyridoxine hydrochloride in formulations, since no significant effect of excipients was observed.

Limit of detection and quantification

The LOD and LOQ of pyridoxine hydrochloride were determined and were calculated from the equations as 3.3 δ/S and 10 δ/S, respectively, where S is the slope of the calibration curve and δ is the standard deviation of y-intercept of the regression equation [33] (Table 1).

Application

The proposed methods were successfully applied to the assay of pyridoxine hydrochloride in their dosage forms. Excellent recoveries with low RSD (%) values were obtained. The results are tabulated in table 4. Tablet excipients did not interfere and therefore confirming that the developed methods are suitable for routine estimation of pyridoxine hydrochloride in their pharmaceutical preparation.

Table 3 Precision of method

Drug with	Measured concentration(μg mL^{-1})	RSD%
Ferric nitrate	50	1.08
Ferric ammonium citrate	50	1.17

Table 4 Recovery studies

Complex with:	Ferric nitrate		Ferric ammonium citrate	
Concentration (μg mL^{-1})	Found (μg mL^{-1})	Recovery %	Found (μg mL^{-1})	Recovery %
10	9.89	98.90	10.09	100.90
20	19.88	99.40	19.91	99.55
30	30.09	100.30	29.84	99.47
40	40.11	100.28	39.93	99.83
50	49.73	99.46	50.13	100.26

The methods are based on inexpensive chemicals and a very simple methodology using a common instrument. The sensitivity of the above methods is comparable to that of the HPLC technique and the validation results further prove its application for all drugs containing OH group.

CONCLUSION

Accurate, precise and convenient methods based on ultraviolet spectral data, were developed for the determination of pyridoxine hydrochloride in pharmaceutical dosage forms. The high recovery values of these drugs showed the good reproducibility of the methods. The methods were found to be easy, simple and quick with minimal sample preparation and simple instrumentation. The methods can be used for routine analysis in quality control laboratories. The methods can be applied for stock solution stability tests and for drug quantification in pharmaceutical formulations. Any of these methods may be adopted as an alternative to the existing time consuming methods.

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НОВИ, ПРОСТИ И ВАЛИДИРАНИ UV-СПЕКТРОФОТОМЕТРИЧНИ МЕТОДИ ЗА ОПРЕДЕЛЯНЕ НА ПИРИДОКСИН ХИДРОХЛОРИД В РАЗТВОРИ И ПРЕПАРАТИ

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Постъпила на 28 2014 г.; коригирана на 30 юки, 2014 г.

(Резюме)

В тази работа се описват два бързи, прости и точни спектрофотометрични методи за определянето на пиридоксин хидрохлорид в разтвори и фармацевтични препарати. Тези от реакцията на пиридоксин хидрохлорид с ферисоли, т.е. феринитрат и фери-амониев цитрат. Реакцията дава жълто-оранжево съединение, което максимално абсорбира светлина при 445 и 450 nm съответно за феринитрат и фериамониев цитрат. Законът на Beer се спазва в интервала 5-50 μg mL^{-1} за двата реагента.ргресионният анализ дава висок корелационен коефициент за калибровъчната права по закона на Beer (r^2= 0.9987, 0.9982). Резултатите са потвърдени аналитично и статистически по правилата на International Conference on Harmonization (ICH).