RESEARCH ARTICLE

Spectrophotometric Determination of Vitamin B6 in Pure and Pharmaceutical Formulations with Diazotized Metoclopramide Hydrochloride

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ABSTRACT

A rapid, simple, and spectrophotometric method has been worked out to determine pyridoxine hydrochloride (Vitamin B6) in pure form and in its pharmaceutical preparations. The primary reaction of the suggested method is the coupling reaction of pyridoxine hydrochloride with diazotized metoclopramide hydrochloride in an alkaline medium to form an intense yellowish-orange azo dye which is a water-soluble dye and has good stability. The maximum absorption of the dye is at 464 nm. A graph of the absorbance of formed azo dye versus concentration of pyridoxine hydrochloride demonstration that the linearity according to Beer's law is from 10 to 600 µg/25mL (0.4–24 ppm). The method's sensitivity was expressed by calculating the molar absorptivity (2.261×104 $L.mol^{-1}$ cm^{-1}) and Sandell's sensitivity index (0.009 $\mu g/cm^2$). The results of the latter two variables indicated that the suggested method could be considered a sensitive method. Pyridoxine hydrochloride has been presented in various pharmaceutical formulations, and the application part for determination of pyridoxine hydrochloride in tablet and injection as a formulation gave satisfactory results.

Keywords: Diazo-coupling, Diazotized metoclopramide hydrochloride, Spectrophotometric determination, Vitamin B6.

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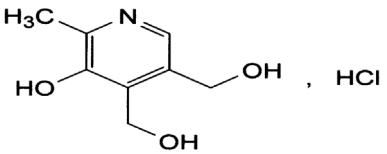
Conflict of interest: None

INTRODUCTION

Pyridoxine hydrochloride is the most important type of B group vitamin, a water-soluble vitamin, and plays important job in combining neurotransmitters hemoglobin, histamine, and amino acid metabolism. Their roles in cellular functioning, acting as co-enzymes. Plants classically synthesize the origins of the B vitamins.[1,2] Paul Gyorgy in 1934 was the first exposed it, and Harris and Folkers were synthesized it in 1939. The toxicity of pyridoxine is very low, and doses about 1000 mg /day for flexible periods might be correlated with neuropathy.[3]

B6 or Pyridoxine hydrochloride [(5-hydroxy-6-methylpyridine-3,4-diyl) dimethanol - HCl]. It is a crystalline powder, simply soluble in water, somewhat is soluble in ethanol. It has the chemical structure[4] as shown in Scheme 1.



Scheme 1: The chemical structure of pyridoxine hydrochloride**.**

Many instrumental procedures were employed for the determination of pyridoxine hydrochloride (PY-HCl) including HPLC,[5,6] ZIC-HILIC chromatography and their antioxidant interactions,[7] LC-MS/MS[8] differential pulse voltammetry by using unmodified boron-doped diamond electrode[9] electrochemical properties of the nanocomposite[10] and nanostructure electrochemical sensor for voltammetric determination.[11]

The spectrophotometric methods are also used for the determination of PY-HCl; these methods include: oxidative coupling reaction,[12] oxidation-reduction,[13] H-point standard addition,[14] ion pair, depending on the charge transfer reaction[15] and also the derivative spectrophotometry.[16]

The existing work means to present a spectrophotometric method to estimate pyridoxine hydrochloride, and applied this method to estimate PY-HCl in its pharmaceutical formulations.

EXPERIMENTAL

Apparatus

Shimadzu UV-Visible Recording Spectrophotometer UV-210 has been used in all measurements with 1-cm silica cells.

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Reagents and Materials

The chemicals used in the present investigation are analytical-grade reagents.

SOLUWTIONS

PY-HCl Working Solution, 100 µg/mL

A 0.0100 g of PY-HCl (NDI-Iraq) is dissolved using distilled water in 100 mL volumetric flask.

Diazotized Metoclopramide (DMCP), 0.004M Reagent Solution

The diazotized MCP reagent (DMCPR) solution is prepared by dissolving 0.3543 g of metoclopramide (Fluka) in 60 mL of distilled water (heat the solution to hasten dissolution), then 15 mL of 1M hydrochloric acid is added, the mixture is then let in an ice-bath (0–5°C), and a 0.0690 g of sodium nitrite is needed to produce the DMCPR. Then adding cooled distilled water to the mark of 250-mL volumetric flask.

Sodium Bicarbonate Solution, 0.1M

This solution is prepared by dissolving 2.100 g of sodium bicarbonate (Fluka) in 250 mL of distilled water.

PY-HCl Tablets Solution, 100 µg/mL

A weight equivalent to one tablet (40 mg/Tablet) from ten tablets powder was dissolved in a 100 mL calibrated flask with distilled water. A 100 (g/mL concentration solution was prepared by taking 25 mL of the above solution and diluting it with distilled water in a 100 mL-volumetric flask.

PY-HCl Injection Solution, 100 µg/mL

The contents of three ampoules of the vitamin B_6 injection (100 mg/2 mL) were mixed, and 2 mL was diluted with distilled water in a 100 mL volumetric flask and to prepare a solution of vitamin B_6 injection at a concentration of 100 µg/mL,10 mL of above solution diluted with distilled water to 100 mL in a calibrated flask.

Suggested Procedure and Linearity

To a set of 25 mL volumetric flasks 10−600 µg (0.4–24 µg/mL) of PY-HCl is added, followed by 1.0 mL of DMCP (0.004M), 2 mL of 0.1 M $NaHCO_3$, and 2 mL CTAB have been added, the mixture is let for 5 minutes, and the volumes are completed with distilled water to the mark. The absorbance is read against a reagent blank at 464 nm. The linearity is from 10 to 600 µg/25 mL (0.4–24 ppm) (Figure 1). The method is sensitive according to the value of molar absorptivity 2.2616×10^4 $L.mol^{-1}.cm^{-1}$.

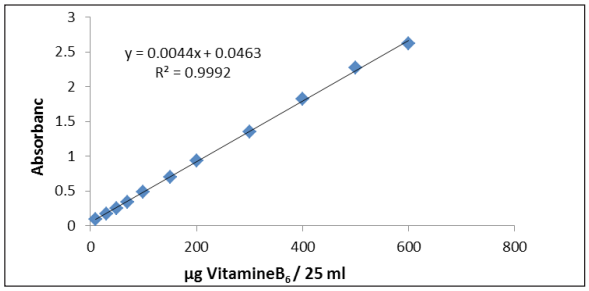


Figure 1: Calibration curve of PY-HCl determination

RESULTS AND DISCUSSION

In all experiments, 100 µg of PY-HCl is taken in 25 mL as a final volume using distilled water.

The Optimum Procedure Conditions

The below experiments included the effects of various parameters on the absorbance of the yellowish-orange azo dye, and the optimum results are followed.

Choice of the Base and its Amount

The first experiment shows that the azo dye develops only in an alkaline medium. Table1 contained the effect of using different strong and weak bases on the absorbance of the formed dye and the color contract (Δλ).

The results in Table 1 indicate that the formed azo dye needs weak alkaline medium and $NaHCO_3$ gives the highest color intensity and the best color contrast. The effect of different volumes (0.3–4.0 mL) of 0.1M$NaHCO_3$ solution on the color intensity is studied then, a 2.0 mL of 0.1M$NaHCO_3$ with a final solution pH of 8.75 is used in the next experiments (according to high absorbance) (Table 2).

Effect of DMCP Amount

Various volumes from 0.5 to 4.0 mL of 0.004M DMCP are tested; the results indicate that using 1.0 mL of DMCP solution gives maximum absorbance of the azo dye at 464 nm this volume is considered as an optimum value (Table 3).

Effect of Surfactant

As mentioned in the literature, the main importance of using surfactants is to increase the intensity of the product, increase stability, and shift the maximum wavelength used in determination to red shift. This effect has been studied by adding 2 mL of each type of surfactant with several orders of addition (Table 4).

From the results in Table 4,only the use of cetrimonium bromide (CTAB) increases the stability of azo dye with no effect on the intensity of the dye or the wavelength of determination.

Table 1: Selection of the base

Base used (1 mL of 0.1 M)	*Absorbance*	*$\Delta\lambda^*$*
NaOH	0.249	151.5
KOH	0.279	150.0
Na_2CO_3	0.413	194.5
$NaHCO_3$	0.438	201.0

$\Delta\lambda^*=\lambda^S{}_{max}-\lambda^B{}_{max}$; where S = the dye, B = blank

Table 2: The useful amount of base

mL of 0.1N $NaHCO_3$	*0.3*	*0.5*	*0.7*	*1.0*	*1.5*	*2.0*	*3.0*	*4.0*
Absorbance	0.054	0.142	0.427	0.435	0.442	0.456	0.446	0.373
pH	3.08	5.42	7.82	8.08	8.21	8.75	9.04	9.48

Order of Surfactant Addition

The three orders of CTAB addition are tried and results are given in Table 5.

The order I has been recommended for the next experiments. Also, the optimum volume of CTAB has been studied, 2 mL was the optimum volume and it has been selected for the following experiments.

The optimum conditions for good intensity of formed azo dye are listed in Table 6.

Final Absorption Spectrum

When a 100 µg of PY-HCl solution is treated according to the procedure mentioned before, the absorption spectrum demonstrates a maximum absorption at 464 nm. There is no absorption at 464 nm for the reagent blank (Figure 2).

The Stability of the Color

The color intensity is reached maximally directly after adding the components, and the absorbance of the azo dye stayed constant for at least 1 hour (in the presence of 2 mL of 1 x10^{-3} M CTAB), this stability period is enough for many measurements (Table 7).

Accuracy and Precision

Three different concentrations (50, 100, and 200 µg/25mL) of PY-HCl with 5 replicates have been determined, and the results in Table 8 indicated a good accuracy (express by relative error) and precision (express by relative standard deviation) for the suggested method.

The Nature of the Dye

Job's method[17] is indicated that the azo dye has a 1:1 PY-HCl to DMCP composition (Figure 3).

The coupling process depends on the nature of the groups present in the ring. In the present study, there are two possibilities of coupling: the coupling with nitrogen, also what will happen when inserting each indole18 or histidine19 as they are considered heterocyclic compounds in the same principle of the current reaction(diazo-coupling). This coupling is weak stability, and from the results of the first experiment of the present work, it was found that adding the surfactant CTAB increases stability, so that the presence of CTAB shift the coupling of DMCP with the carbon atom; according to above results, the azo dye must have the structure in Scheme 2.

Excipients Interfering

Some of the excipients were studied by adding them with different amounts (100, 500, and 1000 µg) to 100 µg PY-HCl (Table 9).

Analytical Application

The proposed method is positively applied for the determination of PY-HCl in its pharmaceutical preparation. The performance of the proposed method is judged by the application of t-test in comparison with the standard method.[4] At the 95% confidence level for eight degrees of freedom. The results in Table 10 show that the calculating t-values are less than the critical value in the standard table, indicating no significant difference between the proposed and standard methods for determining Vitamin B6.

Comparison with Other Methods

Table 11 gives the distinction between the suggested method and other two methods from literature in the same technique(spectroscopy).

Table 3: The optimum amount of DMCP reagent

Amount of DMCP solution (mL,0.004 M)	*Absorbance*
0.5	0.425
0.7	0.434
1.0	0.457
2.0	0.423
3.0	0.396

Table 4: The results of using surfactant

Surfactant solution	*λ max*	*Absorbance after dilution*	*Absorbance after 30 minutes*	*Absorbance after 1 hour*
*SDS, 1×10^{-3} M	464	0.387	0.389	0.391
**Tritonx-100, 1%	464	0.419	0.391	0.378
***CTAB, 1×10^{-3} M	464	0.454	0.455	0.456
Without	464	0.455	0.438	0.430

*Sodium dodecyl sulphate.
** Iso-Octylphenoxypolyethoxyethanol.
***Cetyltrimethylammonium bromide.

Table 5: The optimum order of CTAB addition

Order number	*Order of addition*	*Absorbance*
I	S+R+B+C	0.456
II	S+R+C+B	0.429
III	S+C+R+B	0.439

Assuming that: S = sample (PY-HCl),R = Reagent (DMCP), B = Base ($NaHCO_3$), C= CTAB

Table 6: Optimum condition of present method

Parameter	*The optimum*
Maximum wavelength of orang azo dye	464 nm
DMCP volume and conc.	1 mL, 0.004M
Base used, concentration and volume	$NaHCO_3$,1M, 2 mL
Surfactant used, conc., volume and order of addition	CTAB, 1×10^{-3} M, 2 mL, before dilution with DW

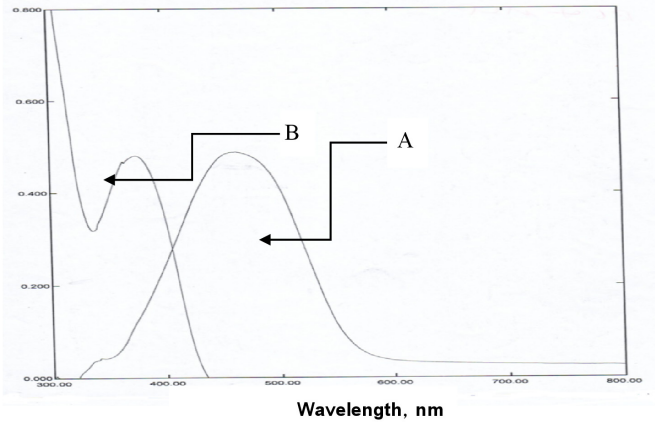


Figure 2: Absorption spectrum of 100 µg PY-HCl /25mL treated with the proposed procedure. (A) the azo dye against blank, (B) blank against distilled water.

Table 7: The stability of formed azo dye

	Absorbance/minute standing time						
µg of PY-HCl/ 25 mL	*0*	*10*	*20*	*30*	*40*	*50*	*60*
50	0.243	0.240	0.239	0.237	0.234	0.232	0.228
100	0.451	0.457	0.456	0.455	0.456	0.455	0.455
200	0.814	0.811	0.810	0.807	0.803	0.800	0.798

Table 8: Accuracy and precision.

PY-HCl (µg/ 25mL)	*Relative error* %*	*Relative standard deviation* %*	*Recovery*%*
50	-0.24	± 0.537	99.76
100	-0.33	± 0.345	99.66
200	-0.171	± 0.1794	99.82

*Average of 5 determinations.

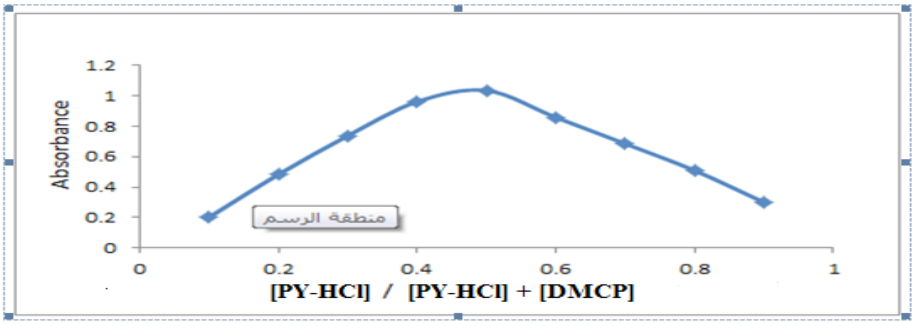


Figure 3: Job's plot for PY-HCl – DMCP azo dye.

Table 9: Effect of excipients on the analysis of PY-HCl.

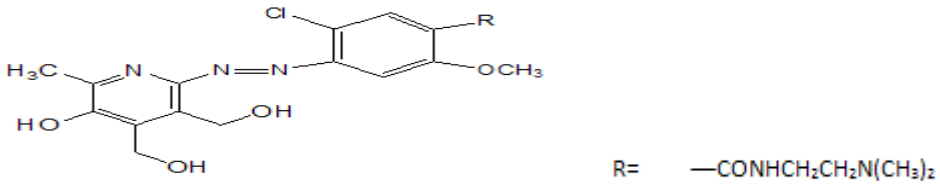
Foreign compound	*Recovery (%) of 100 µg pyridoxine hydrochloride per µg foreign compound added*		
	100	*500*	*1000*
Glucose	103.06	98.77	100.20
Fructose	101.43	100.61	99.18
Starch	101.22	101.43	102.65
Gum Arabic (Acacia)	101.22	103.06	99.79

The results above show no interference of excipients in the estimation of PY-HCl using the proposed method.

Table 10: Application of present method.

Drug	*µg PY-HCl present/25 mL*	*Recovery,% Presence method*	*Recovery, % Standard method*[4]	*t-exp.*
Samavit B_6 40 mg/Tablet SDI, Iraq	100	98.98	99.62	0.957
VitaminB_6 100 mg/2mL Injection Kontam,China	100	101.27	101.52	0.215

*Average for 5 determinations.



Scheme 2: The possible structure of the orange azo dye.

Table 11: The results of comparison

Analytical parameters	*Suggested method*	*Literature method*[20]	*Literature method*[13]
pH	8.75	9.84	3.02
Temperature (C°)	RT	RT	RT
$\lambda_{max\ (nm)}$	464	480	716
Reagent used	DMCP	Diazotized p-nitroaniline	Arsenazo III
Linearity (ppm)	0.4-24	0. 2-20	0.04-0.56
Molar absorptivity ($l.mol^{-1}.cm^{-1}$)	2.2616□10^4	2.70×10^4	1.12□10^5
Type of reaction	Diazo- coupling	Diazo- coupling	Redox reaction
Nature of the dye	1:1	1:1	1:1
Application of the method	Determination of PY-HCl in tablets and injection	Determination of PY-HCl in tablets and injection	Determination PY-HCl in tablets and injection ampoule and serum

CONCLUSION

The suggested method is sensitive, simple, and does not require temperature control or pre-conditioning as an extraction step. The method has been applied to the pyridoxine hydrochloride (vitamin B6) assay in pure Tablet and injection as the main product of its pharmaceutical formulations.

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