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Research Article

Simultaneous Spectrophotometric Determination of Vitamin B_6 by Coupling with *p*-Amino Phenol

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ABSTRACT

A simple, rapid and sensitive spectrophotmetic method for trace determination of pyridoxine hydrochloride (PYR) in aqueous solution and in pharmaceutical preparations is described. The method is based on the oxidative coupling reaction of the intended compound with p-aminophenol (PAP) to form an intense purpule, water soluble dye that is stable and shows maximum absorption at 534 nm. A graph of absorbance versus concentration indicates that Beer's law is obeyed over the concentration range of 50-1000 µg of PYR in a final volume of 25 mL (i.e. 2-40 ppm), with a molar absorbtivity 2.138×10^3 $L.mol^{-1}.cm^{-1}$, a sandell's sensitivity of 0.0962 $\mu g.cm^{-2}$ a relative standard deviation of 0.519-2.383 % depending on the concentration of PYR. The optimum conditions and stability of the colored product have been investigated and the method was applied successfully to the determination of PYR in dosage forms.

Keywords: Pyridoxine hydrochloride, oxidative coupling, spectophotometry.

INTRODUCTION

Pyridoxine hydrochloride (PYR) namely vitamin B6, is chemically 3-hydroxy-4,5-bis-(hydroxylmethyl)-2-picoline hydrochloride. It is a water soluble vitamin and involved principally in amino acid, carbohydrate and fat metabolism. It is required for the formation of haemoglobin. It is responsible mainly for the transference of amino acid groups and the maintenance of body cells, acting as a coenzyme[1]. Its lack results in skin and nervous system changes and certain types of anemia. A number of analytical methods in addition to microbiological assay have been reported for the determination of PYR, included spectrophotometric[2-5], flow injection-solid phase spectrophotometry[6], voltammetry[7,8] , flourimetric[9], and HPLC methods[10,11]. In this paper, we have reported the investigation and development of rapid analytical methodology for the simultaneous determination of PYR. The method is based on oxidative coupling reaction between PYR and PAP in the presence of sodium periodate in a neutral medium.The analytical procedure is simple, reproducible and accurate. It has been satisfactorily applied for the determination of PYR in pure and dosage forms. The reaction product has been spectrophotometrically measured at 534 nm.

Reaction mechanism of the method

The reaction between PYR and PAP in the presence of sodium periodate in neutral medium yield a purpul colored product (λ_{max} of 534 nm with a molar absorption coefficient of 2.138×10^3 $L.mol^{-1}.cm^{-1}$),the reaction given in scheme 1 was postulated. The absorption spectrum of the coloured product is given in Fig.1.Under the reaction conditions, PNP (I) upon oxidation with sodium periodate loses two proton forming a nucleophilic intermediate (II), which has been postulated to be an active coupling species. PYD has a free para position to the hydroxyl group, hence the intermediate of PAP (II) undergoes nucleophilic substitution with phenolic moieties (III) of PYD, to form a coloured quinonoid type product (IV)[12] according to scheme 1[2].

EXPERIMENTAL

Apparatus

All spectral and absorbance measurements were carried out on a Shimadzu UV-visible 260 digital double beam recording spectrophotometer using 1-cm silica cells.

Reagents

All chemicals used were of analytical reagent grade and pure pyridoxine hydrochloride (PYD) drug sample was provided from state company for Drug Industries and Medical Appliance,SDI,Samara.Iraq.Dosage forms were obtained from commercial sources.

Pyridoxine hydrochloride stock (1000 µg ml^{-1}) and working solution (500 µg ml^{-1})

The stock solution of PYD was prepared by dissolving 0.1000 g in distilled water and completed to 100 mL with the same solvent. Serial dilutions with distilled water were made to cover the working range.

p-Amino phenol reagent solution (BDH, 0.1%)

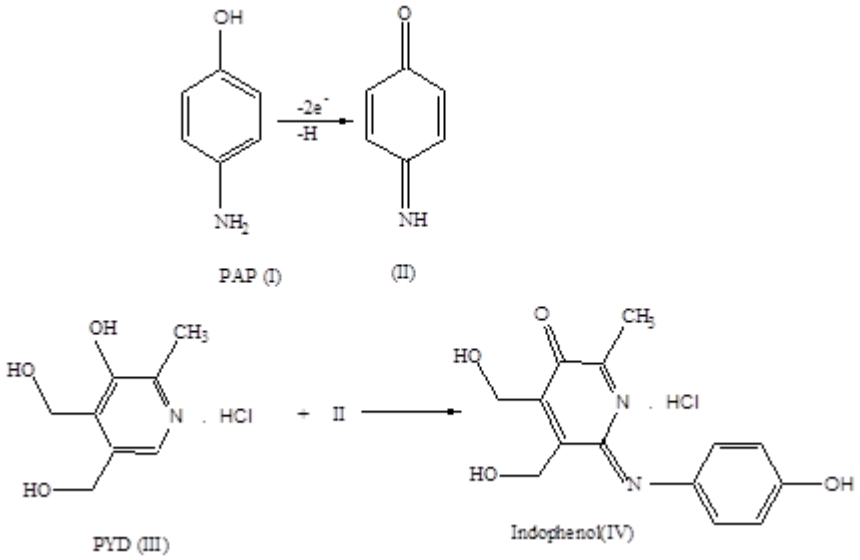
Prepared by dissolving 0.1000 g of reagent in 10 mL ethanol and completed to 100 mL volumetric flask with the distilled water, and stored in dark bottle.

Sodium periodate $NaIO_4$ (BDH,0.2%)

Prepared by dissolving 0.2000 g of reagent in distilled water and made up to 100 ml with distilled water.

Procedure of pure drug

**Author for Correspondence:*



Scheme 1: Proposed mechanism of the reaction between PYD and PAP

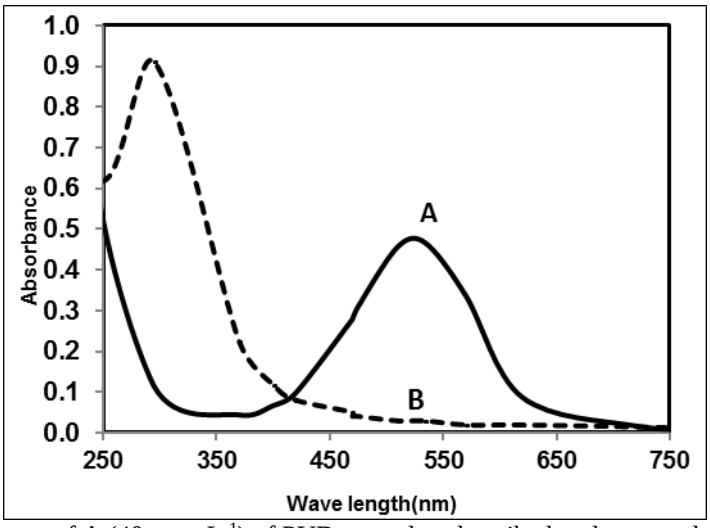


Figure 1: Absorption spectra of A (40 µg mL^{-1}) of PYD treated as described under procedure and measured against blank and B the reagent blank measured against distilled water.

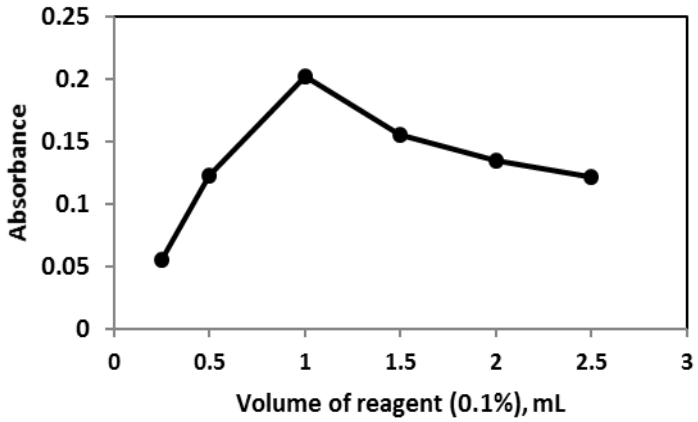


Figure 2: Effect of volume of reagent.

Into a series of 25 mL calibrated flask, transfer an increased volumes of PYD of 500 ppm to cover the range of calibration graph 50-1000 µg of PYD in final volume 25 ml , followed by 1 ml of PAP (0.1%) and 2 ml of sodium periodate (0.2%). The solutions are diluted to the mark with distilled water. The colour reach its maximum intensity and stability on standing for 35 min at room temperature then the absorbance is measured at 534 nm against the reagent blank prepared in the same way but containing no PYD. The colour of the formed product is

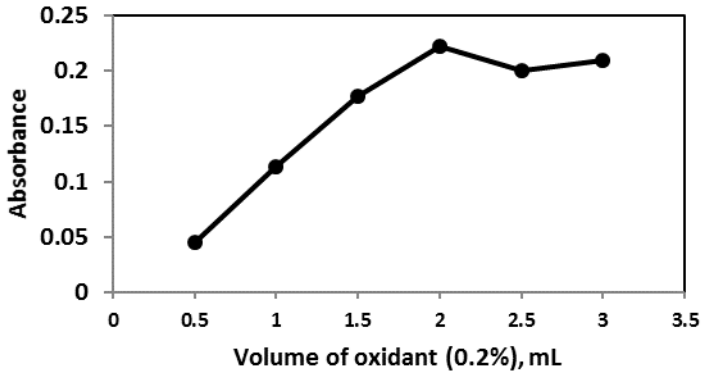


Figure 3: Effect of volume of oxidant.

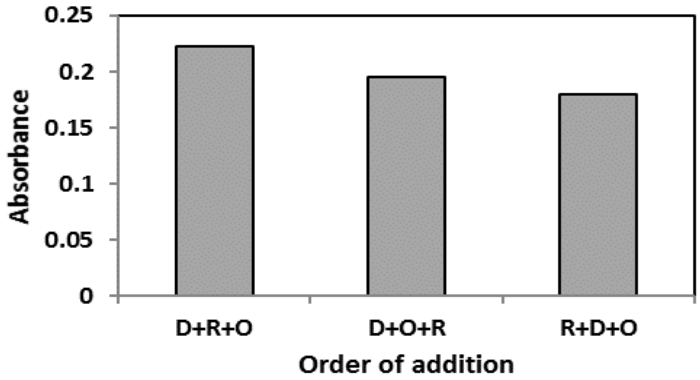


Figure 4: Effect of order of addition.

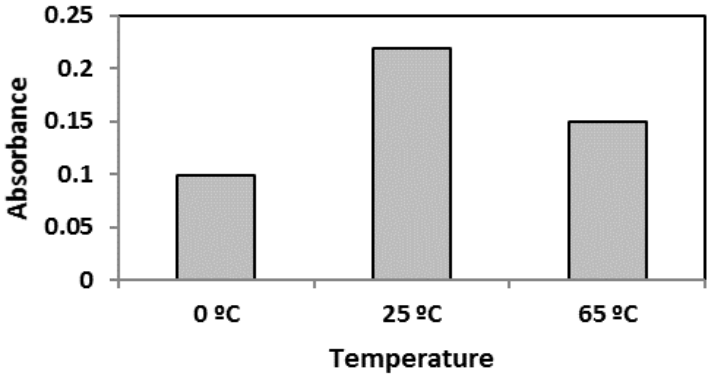


Figure 5: Effect of temperature.

stable for 15 min. For the optimization conditions and in all subsequent experiments a 500 µg in final volume 25 ml was used (i.e. 20 ppm).

Procedure of pharmaceutical forms

Twenty tablets of was weighed, powdered and mixed thoroughly. Similarly, content of 10 vials of PYD were also mixed. A quantity equivalent to 100 mg of the pure drug was transferred into a 100 mL volumetric flask. The drugs were dissolved in distilled water, shaken well and made up to the volume with distilled water. The resultant solutions were filtered to obtain 1000 ppm, dilute 50 ml of this solution to 100 ml by distilled water to prepared 500 ppm of PYD. The measurement was carried out as described earlier under general procedure using suitable volume of last solution.

RESULTS AND DISCUSSION

The purpul color which is obtained when dilute aqueous solution of PYD is mixed with solution of PAP in the presence of oxidizing agent (sodium periodate) in a neutral medium reach its maximum intensity and stability on standing for 35 min at room temperature. The colour have a maximum absorption at 534 nm, which is used in all subsequent experiments. The spectra of the bluish-green product formed and of reagent blank were show in Fig.1.

Study of the optimum reaction conditions:

The factors affecting on the sensitivity and stability of the coloured product resulting from the oxidative coupling reaction of PYD with PAP in the presence of oxidizing agent (sodium periodate) in a neutral medium were

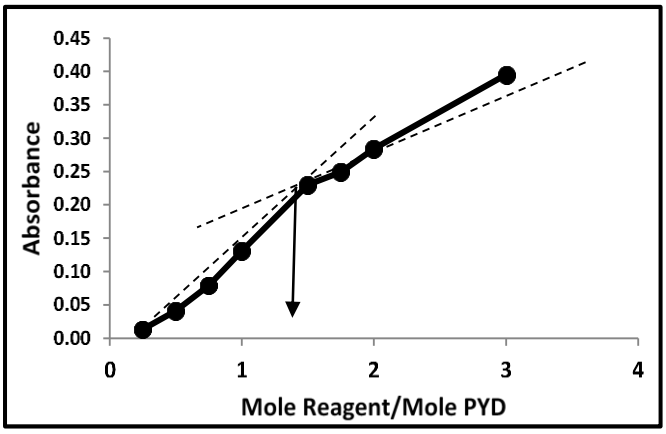


Figure 6: Mole ratio of the reaction.

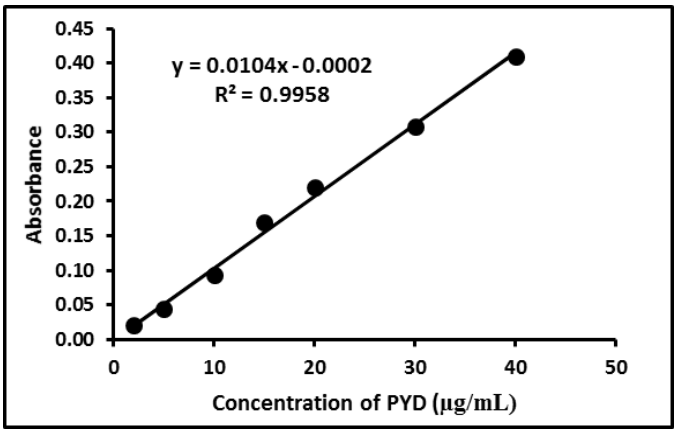


Figure 7: Calibration curve.

Table 1: Analytical data obtained from proposed method.

Parameter	value
Regression equation	y = 0.0104x-0.0002
Beer's Low limits(µg.ml^{-1})	2-40
Molar absorbativity(lit.mole^{-1}.cm^{-1})	2.138×10^3
Sandell's sensitivity(µg.cm^{-2})	0.0962
Slope(b)	0.0104
Intercept(a)	0.0002
Correlation coefficient (R^2)	0.9958
λ_{max} (nm)	534
R.S.D (%)	< 3.97
Average of recovery (%)	100.97

Table 2: Accuracy and precision of the proposed method for the determination of PYD.

Amount of PYD (µg.ml^{-1})		Recovery%*	R.S.D %*
Present	Found		
10.000	10.308	103.077	3.965
20.000	19.699	98.494	1.716
25.000	25.332	101.326	0.999

* Average of four determinations.

carefully studied. All experiments using 500 µg of PYD in final volume 25 ml (i.e. 20 ppm).

Effect of Reagent concentration

When various concentrations of PAP were added to a fixed amount of PYD solution, 1 mL (Fig.2) of 0.1% solution was found enough to develop the colour to its full intensity and give a minimum blank value, and was considered to be optimum for the concentration range of 50-1000 µg of PYR in a final volume of 25 mL.

Effect of oxidant concentration

When different volumes of sodium periodate ($NaIO_4$) solution (0.5-3.0 ml) were added (Fig. 3) to PYD solution, it was found that 2 ml of 0.2% is enough to give a maximum absorbance and full intensity.

Effect of order of addition

Different orders of addition (Fig. 4) of reagents were experimented and it was found that the order of addition of reagents cited under general procedure was used in all subsequent experiments.

Effect of temperature

The effect of temperature on the colour intensity of the dye was studied (Fig. 5). In practice, high absorbance was obtained when the colour was developed at room temperature (25°C) than when the calibration flasks were placed in an ice-bath at (0°C) or in water bath at (60°C).

Structure of the product

The stoichiometry of the formed colored complex between each PYD with PAP was investigated under the recommended optimum conditions by applying the molar ratio method (Fig. 6). The results obtained show that a 1:1 (drug to reagent) complex formed between PYD and PAP

Table 3: Application of the proposed method for the determination of PYD in pharmaceutical forms.

Drug Sample	PYD (µg.mL^{-1})		Error% *	Rec. % *	R.S.D%*
	Taken	Found			
Samavit B6 /SDI, Iraq	10.000	10.038	0.380	100.380	1.556
40mg/tablet	20.000	20.667	3.333	103.333	2.641
	30.000	30.199	0.664	100.664	1.764
Pyridoxine HCl,injections	10.000	10.067	0.672	100.672	1.216
MEHECOCorp.,China	20.000	20.193	0.965	100.965	0.514
100mg/2mL	30.000	29.729	-0.904	99.096	2.813

* Average of four determinations.

Table 4: Comparison of the proposed method with standard method to determination of PYD in pharmaceutical forms.

PYD preparations	Recovery%*	
	Proposed method	Standard method **
PYD pure	100.97	100.00
Samavit tablets	101.46	98.80
PyridoxineHCl injections	100.24	99.80
t (2.78)***		0.912
F(19.00)***		3.052

* Average of four determinations.
** British Pharmacopoeia standard method.
*** Theoritecal value.

reagent at 534 nm, therefore, the formation of product probably occurs as illustrated in Scheme 1.

Analytical data

Employing the conditions described under procedure, a linear calibration graph (Fig.7) for PYD and PAP was obtained. The optical characteristics, such as Beer's law, molar absorbativity, and correlation coefficient and other analytical data are summarized in Table (1).

Accuracy and precision

The accuracy and precision of the method was determined at three different concentrations. The results shown in Table (2) indicate that satisfactory precision and accuracy could be obtained with the proposed method.

Analytical Applications

The suggested method was applied to the quantitative determination of PYD in pharmaceutical formulations. Two types of tablets and one type of injections containing PYD have been analyzed and they gave a good accuracy and precision as shown in Table (3).

The proposed method was compared successfully with the British pharmacopeia's standard method, since F-test and T-test showed that there was no significant differences between the proposed and official methods[13] Table (4).

In conclusion, the proposed method, which is simple and selective, offers the advantage of a wide range of determination without the need for extraction or heating, or removal of excipients. The precision of the method evaluated by analyzing pure sample of PYD and a good recovery was obtained (Table (4)). In addition the proposed method was applied successfully to the analysis of some tablets and injections containing PYD, and finally statistical analysis F-test and T-test, reveals that no significant difference in accuracy and precision between the proposed and official methods.

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