Research Article

Simultaneous Estimation of Pyridoxine HCl, Folic Acid and Mecobalamine in Bulk Drugs and Marketed Formulation by Vierdott's Method

Kamepalli Sujana*, T. Pavani priyanka, P. Iswarya, K. Sai Tejaswini
*Department of Pharmaceutical Analysis, Acharya Nagarjuna University, Nagarjuna nagar, Guntur, Andhra Pradesh, India.
***Corresponding author's E-mail:** sujana_36@yahoo.co.in

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ABSTRACT

Pyridoxine HCl, Folic acid and Mecobalamine are the vitamins used to treat vitamin deficiency disorders. A simple, specific, accurate and precise UV spectroscopy method has been developed and validated for simultaneous determination of Pyridoxine HCl, Folic acid and Mecobalamine in bulk drugs and marketed formulation (capsules) using simultaneous equation method (vierdott's method). The developed method involves solving of simultaneous equations using 0.1N NaOH as solvent where absorbance maxima (λmax) for Pyridoxine HCl, Folic acid and Mecobalamine was found to be at 218nm, 256nm and 220nm respectively. All the drugs obeyed Beer's law in the concentration range of 4-32 µg/ ml, 2-16 µg/ ml & 4-32 µg/ ml. The method was validated as per ICH guidelines and the correlation coefficients (r^2) 0.999 indicated good linearity of Calibration curve for all the three drugs. The recovery of Pyridoxine HCl, Folic acid and Mecobalamine from the standard mixture solution was 97.8%, 97.7% and 91.76% respectively. The developed method was found to be accurate, reliable and robust showing LOD 0.016 µg/ml (Pyridoxine HCl), 0.012 µg/ml (Folic acid) and 0.017 µg/ml (Mecobalamine) and LOQ 0.059 µg/ml (Pyridoxine HCl), 0.056 µg/ml (Folic acid) and 0.053 µg/ml (Mecobalamine).

Keywords: Simultaneous Estimation method, UV spectroscopy, Pyridoxine HCl, Folic acid, Mecobalamine.

INTRODUCTION

Pyridoxine HCl (PDH) IUPAC Name is 5-hydroxy-6-methylpyridine-3, 4-dimethanol hydrochloride and Molecular formula is $C_8H_{11}NO_3$.HCl and Molecular weight is 205.6 g/mol. It is a white or almost white, crystalline powder. It is freely soluble in water, slightly soluble in ethanol (95%), practically insoluble in chloroform and in ether.[1]

It is official in IP 2007, BP 2009 and Martindale. It is a water soluble vitamin and involved principally in amino acid, fat and carbohydrate metabolism. It is also required for the formation of haemoglobin.[2]

Folic Acid (FOLI) IUPAC Name is (2S)-[4-[(2-amino-4-hydroroxypteridin-6-yl) methyl amino] benzamido] glutamic acid and Molecular formula is $C_{19}H_{19}N_7O_6$ and Molecular weight is 441.4 g/mol. It is a yellow to yellowish-orange in colour. It is practically insoluble in cold water, very slightly soluble in boiling water, soluble in dilute acids and alkalies.[1] It is official in IP 2007, BP 2009 and Martindale. Vitamin B_9 is essential for numerous bodily functions. Humans cannot synthesize folates de novo; therefore, folic acid has to be supplied through the diet to meet their daily requirements. The human body needs folate to synthesize DNA, repair DNA, and methylate DNA as well as to act as a cofactor in certain biological reactions.[3]

Mecobalamine (MECO) IUPAC Name (1R, 2R, 4S, 7S)-7-{[(2S)-3-hydroxy-2-phenylpropanol] oxy}-9, 9-dimethyl-3-oxa-9-azoniatricycle [3.3.1.02, 4] nonane, and Molecular formula $C_{63}H_{91}CoN_{13}O_{14}P$ and Molecular weight is 1344.38 g/mol. It is a dark red crystalline powder it is soluble in water and ethanol.[1] This water-soluble compound plays a role in neurological function, and is also involved in the process of producing blood. Mecobalamin can be used in the treatment of anemia.[4]

Literature survey reveals information that some of the methods like UV[5-8] individually and combination with other drugs. Hence it is necessary to develop a new method with less economical used for the routine analysis.

MATERIALS AND METHODS

Chemicals and Reagents

Pyridoxine HCl, Folic Acid, Mecobalamine standard drugs obtained as Gift samples from Cystron Labs Pvt Ltd. The marketed preparation (capsules) obtained from local pharmacy with brand name COGNISULES with label claim of PDH (20mg) **+** FOLI **(**5mg**)** **+** MECO (1.5mg) manufactured by Windus Health Care Pvt Ltd, Uttarakhand. All the chemicals and reagents used are of analytical grade.

Instrumentation

A Shimadzu UV – 1800 double beam spectrophotometer with 1 cm path length supported by Shimadzu UV – probe software, version 2.21 was used for spectral measurements with 1 cm matched quartz cells. Analytical balance Shimadzu (220h) was used for weighing purpose, volumetric glassware was used of class A.

Solubility studies of drug

Proper wavelength selection for the method depends upon the nature of sample and its solubility. 1mg of standard drug samples was taken separately and solubility was checked in various solvents like Distilled

water, Acetonitrile, 0.1N NaOH, 0.1N HCl, Phosphate Buffer, Acetone and these studies were carried out at 25 ± 2 °C. The drugs are freely soluble in 0.1N NaoH and it was selected as a solvent for the development of new method.

Preparation of Standard Stock Solutions

Preparation of PDH standard stock solution

Accurately weighed 10mg of Pure PDH (API) was taken in a 10ml volumetric flask, dissolved in 0.1N NaOH and made up to the mark to get a concentration of 1000 µg/ml, from above stock solution 1ml was transferred into 10 ml volumetric flask and volume was made up to the mark with 0.1N NaOH to get a concentration of 100 µg/ml, from above solution 1ml was transferred into 10 ml volumetric flask and volume was made up to the mark with 0.1N NaOH to get a concentration of 10 µg/ml. It was taken as working standard concentration.

Preparation of FOLI standard stock solution

Accurately weighed 10mg of Pure FOLI (API) was taken in a 10ml of volumetric flask, dissolved in 0.1N NaOH and made up to the mark to get a concentration of 1000 µg/ml, from above stock solution 1ml was transferred into 10 ml volumetric flask and volume was made up to the mark with 0.1N NaOH to get a concentration of 100 µg/ml, from above solution 1ml was transferred into 10 ml volumetric flask and volume was made up to the mark with 0.1N NaOH to get a concentration of 10 µg/ml. It was taken as working standard concentration.

Preparation of MECO standard stock solution

Accurately weighed 10mg of Pure MECO (API) was taken in a 10ml of volumetric flask, add 1 ml of distilled water and shake until it completely dissolved in distilled water, and made up to the mark with 0.1N NaOH get a concentration of 1000 µg/ml, from above stock solution 1ml was transferred into 10 ml volumetric flask and volume was made up to the mark with 0.1N NaOH to get a concentration of 100 µg/ml, from above solution 1ml was transferred into 10 ml volumetric flask and volume was made up to the mark with 0.1N NaOH to get a concentration of 10 µg/ml. It was taken as working standard concentration.

Procedure for selection of wave length

From working standard solution 10µg/ml solution of PDH, FOLI and MECO was transferred into three 10ml volumetric flasks separately and diluted to 10ml with 0.1N NaOH. The three solutions were taken and scanned between 200nm to 400nm on scan/spectrum mode using 0.1N NaOH as blank. As per spectra recorded PDH shows λ max at 218 nm (λ_1), FOLI shows λ max at 256nm (λ_2) and MECO shows λ max at 220 nm (λ_3) respectively.

Plotting of calibration curve

The calibration curves were plotted over a concentration range of 4-32 µg/ml for PDH, 2-18 µg/ml for FOLI and 4-36 µg/ml for MECO. The absorbance was measured at 218 nm, 256 nm and 220 nm for PDH, FOLI and MECO respectively and calibration curves were plotted. At the wavelengths, three equations were solved using the absorptivity values. The concentration of unknown sample were prepared from the capsule dosage form and calculated using the following simultaneous equations:

$Cx = A_1 / ax_1$ (Eq. 1)

$Cy = A_2 – ax_2\ cx/ay_2$ (Eq. 2)

$Cz = A_3 – (ax_2\ cx + ay_3 cy)/az_3$ (Eq. 3) Where, A_1, A_2 and A_3 are absorbance of sample solution at 218nm, 256nm and 220nm, respectively. ax_1, ax_2 and ax_3, absorptivity coefficients of PDH at 218nm, 256nm and 220nm, respectively.

ay_2 and ay_3, absorptivity coefficients of FOLI at 256nm and 220nm, respectively.

az_3, absorptivity coefficient of MECO at 220nm.

cx, cy and cz are concentrations PDH, FOLI and MECO, respectively in mixture.

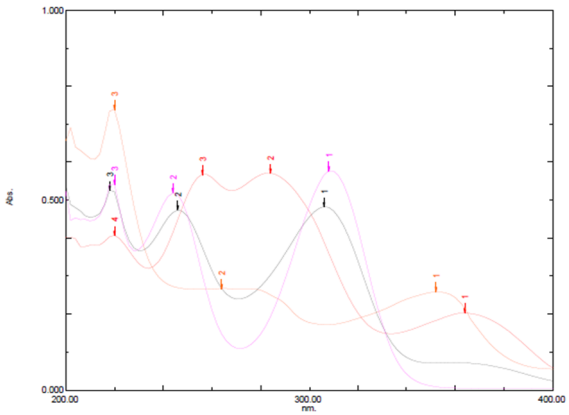

Figure 1: Overlay spectrum of Pyridoxine HCl, Folic acid, Mecobalamine (API) and Formulation

Method Validation Procedure

Validation of the developed method was carried out as per ICH guidelines. Parameters such as specificity, linearity, accuracy, precision, LOD and LOQ were taken up as tests for method validation.

Specificity

The UV graphs obtained depicts there is no interference of excipients, solvent with the absorbance of analyte which indicate that the method is specific for the analysis of analytes in their dosage form and it is shown in Fig 1.

Linearity and range

The linear response of samples was determined over a concentration range of 50-150 %. Accurately measured standard solutions of PDH, FOLI and MECO were transferred to a series of 10 ml of volumetric flask and diluted up to the mark with 0.1N NaOH. The absorbance of the solutions was measured at 218nm, 256nm and

220nm against 0.1N NaOH as blank. The calibration curve of absorbance vs respective concentration was plotted and a correlation coefficient (r^2) and regression line equation for PDH, FOLI and MECO was calculated. The results are shown in the Fig- 2, 3 & 4 and Table-1.

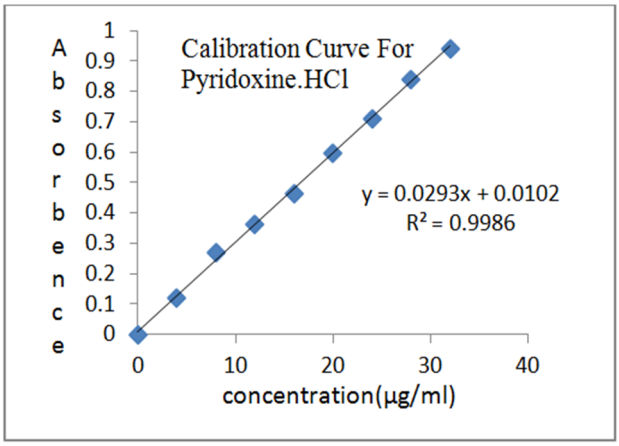

Figure 2: Calibration curve of Pyridoxine HCl

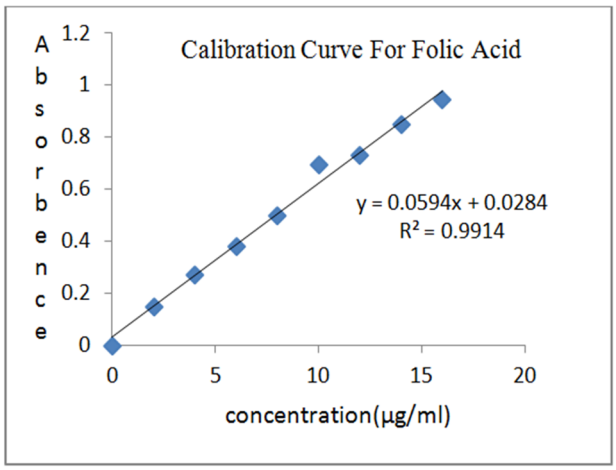

Figure 3: Calibration curve of Folic acid

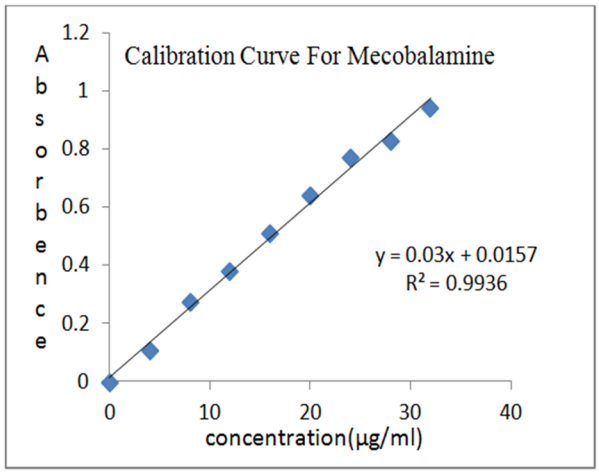

Figure 4: Calibration curve of Mecobalamine

Accuracy

Accuracy is determined by calculating percentage recovery. Recovery studies was carried by standard addition method, where to the formulation (pre analyzed sample), the reference standards of the PDH, FOLI and MECO were added at three concentration level of 50%, 100%, 150% of assay concentration and recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for all the drugs. The results are shown in the Table-2.

Precision

Method precision

Variation of results on same day analysed by actual determination of absorbance fixed concentration of the sample preparation (formulation) consisting of 8µg/ml for PDH, 8µg/ml for FOLI and 8µg/ml of MECO for six solutions on the same day within the Beer's range and measured the absorbance at three wave lengths. The results are shown in the Table-3.

System precision

The variation of results on same day analysed by actual determination of absorbance of fixed concentration of the standard preparation (API) consisting of 8µg/ml for PDH, 8µg/ml for FOLI and 8µg/ml of MECO for six times on the same day within the Beer's range and measured the absorbance at three wave lengths. The results are shown in the Table-3.

Acceptance criteria

%RSD should not be more than NMT 2.0% as per ICH guide lines

Limit of detection (LOD) & Limit of quantification (LOQ)

The LOD and LOQ of developed method were studied as per ICH guidelines. LOD and LOQ are calculated from the calibration curves. The results are shown in the Table-5.

Estimation of PDH, FOLI and MECO in capsule formulation (assay)

From calibration curve the concentration 100% is selected to perform assay.

Preparation of test solution (Capsules)

10 capsules were weighed without shell and powder equivalent to 100mg PDH, 25mg FOLI and 7.5mg MECO was weighed and taken into 100ml volumetric flask then 50ml 0.1N NaOH was added and shaken well to dissolve capsule powder completely and volume was made up to mark with diluent then solution as sonicated for about 20min and filtered with 0.45 µ whattman filter paper to remove particles if any. From the above stock solution 1ml of solution was withdrawn and taken in 10ml volumetric flask and volume was made up to mark with diluent.

Further dilution was prepared to obtain concentration of 10µg/ml solution. The concentration of PDH, FOLI and MECO obtained from simultaneous equations. The results are shown in the Table-4 & figure-1 overlay spectrum of PDH, FOLI, MECO (API) and Formulation.

Table 1: Linearity Results of PDH, FLA, MEC

S. No	Pyridoxine HCl		Folic acid		Mecobalamine	
	Conc. (µg/ml)	Abs. (µg/ml)	Conc. (µg/ml)	Abs. (µg/ml)	Conc. (µg/ml)	Abs. (µg/ml)
1	4	0.122	2	0.149	4	0.107
2	8	0.271	4	0.272	8	0.274
3	12	0.363	6	0.383	12	0.381
4	16	0.465	8	0.503	16	0.511
5	20	0.599	10	0.696	20	0.643
6	24	0.712	12	0.732	24	0.774
7	28	0.841	14	0.851	28	0.830
8	32	0.941	16	0.948	32	0.943
Regression equation	y = 0.0293x + 0.0102		y = 0.0594x + 0.0284		y = 0.03x + 0.0157	
Slope	0.0293		0.0594		0.03	
Intercept	0.0102		0.0284		0.0157	
R^2	0.9986		0.9914		0.9936	

Table 2: Recovery studies of proposed method for Pyridoxine HCl, Folic acid, Mecobalamine

S. No	Parameters	Pyridoxine HCl			Folic acid			Mecobalamine		
1.	Level of recovery (%)	50	100	150	50	100	150	50	100	150
2.	Pre analyzed conc.(µg/ml)	8	8	8	4	4	4	8	8	8
3.	Amount added (µg/ml)	4	8	12	2	4	6	4	8	12
4.	Amount found (µg/ml)	11.6	15.8	19.5	6.1	7.5	8.6	11.6	14.6	18.2
5.	% Recovery	96.9	98.9	97.7	103.1	103.1	86.9	93.05	91.25	91

Table 3: Method precision and System precision studies of proposed method for Pyridoxine HCl, Folic acid, Mecobalamine

S. No	Precision type	Pyridoxine HCl	Folic acid	Mecobalamine
1.	**Method Precision** Concentration Mean±SD %RSD	8µg/ml 0.271±0.0004 0.047	8µg/ml 0.391±0.0004 0.051	8µg/ml 0.173±0.0004 0.144
2.	**System Precision** Concentration Mean±SD %RSD	8µg/ml 0.271±0.0004 0.047	8µg/ml 0.391±0.0004 0.051	8µg/ml 0.173±0.0001 0.144

Table 4: Results of marketed formulation analysis

Brand Name	Drug	Label Claim (mg)	Test Concentration (µg/ml)	Amount Found (µg/ml)	% Assay	% RSD
COGNISULES	PDH FOLI MECO	1.5 20 25	10	0.73 9.7 2.23	97.3% 103% 89.2%	1.25%

Table 5: Summary of Optical and Validation characteristics

S. No	Parameter	Pyridoxine. HCl	Folic acid	Mecobalamine
1	Linearity(µg/ml)	4-32	2-16	4-32
2	Regression equation	y=0.0293x+0.01	Y=0.059x+0.02	Y=0.03x+0.015
3	Slope	0.0293	0.059	0.03
4	Intercept	0.012	0.028	0.015
5	Correlation coefficient	0.998	0.991	0.993
6	Precession indicated by %RSD			
7	Method precision	0.047	0.051	0.144
	System precision	0.047	0.051	0.144
	Accuracy indicated by %recovery	97.8	97.7	91.76
8	LOD(µg/ml)	0.016	0.012	0.017
9	LOQ(µg/ml)	0.059	0.056	0.053

RESULTS AND DISCUSSION

The conditions tested for method development indicates that all the system suitability parameters according to ICH guidelines were achieved by using Shimadzu UV – 1800 with a detection wavelengths of 218 nm for PDH, 256nm for FOLI and 220 nm for MECO.

The optical and validation characteristics of the proposed method results were shown in the table-5.

To validate the UV method, a series of tests were made using the most promising conditions.

A calibration curve was made and concentration examined within the detection range of 4-32µg/ml for PHD, 2-16µg/ml for FOLI and 4-32µg/ml for MECO and correlation coefficient was found to be 0.998 for PDH, 0.991 for FOLI and 0.993 for MECO respectively.

The precision (expressed as the percentage relative standard deviation) was determined for PDH, FOLI and MECO repeated analysis and the values are presented in Table-3. The assay values obtained by proposed method and recovery experiment values obtained were performed by adding different amounts to preanalysed concentration and summarized in Table-2.

CONCLUSION

The developed UV spectroscopy method for the determination of Pyridoxine HCl, Folic Acid and Mecobalamine was validated as per ICH guidelines. All the validation parameters like Specificity, accuracy, precision, linearity obtained results were within the limits. Hence from obtained data it is concluded that the developed method is simple, accurate, reliable, and economic and it can be employed for routine quality control analysis of Pyridoxine HCl, Folic Acid and Mecobalamine capsules in drug testing laboratories and pharmaceutical industries without any interference from excipients.

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