Available online at www.derpharmachemica.com

Scholars Research Library

Der Pharma Chemica, 2012, 4 (2):749-754
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Simultaneous UV Spectrophotometric Method for Estimation of Isoniazid and Pyridoxine in Tablet Dosage Form

Pratap Y. Pawar*, Anjali V. Lagad, Sandhya N. Bahir, Sumedha and R. Rathi

Department of Pharmaceutical Chemistry, P.D.V.V.P.F'S College of Pharmacy, viladghat, Ahmednagar, Maharashtra

---

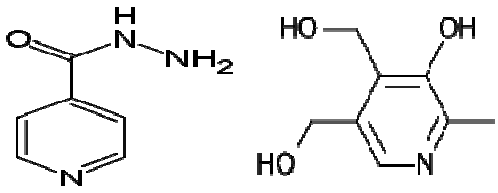
ABSTRACT

Tuberculosis (TB) is the world's second most common cause ofdeath from infectious disease, next to human immunodeficiency virus (HIV). TB has become an increasing health problem since emergence of HIV; TB/HIV co-infection adds further complications in treating the disease. It is also a major concern for industrialized nations because of emergence of drug resistance, alcohol / drug abuse, growth of immigrants and other factors.Method employs formation andsolving of simultaneous equation using 263 nm and 292 nm as two analytical wavelengths for both drugs inDistilled water. Isoniazidand Pyridoxine at their respective λmax 263 nm and 292 nm showslinearity in a concentration range of 1-11µg /mL and 5-30µg /mL. Recovery studies for Isoniazid 99.56 % and100.14% for Pyridoxine in case of simultaneous equation method confirming the accuracy of the proposed method. Theproposed method is recommended for routine analysis since it is rapid, simple, accurate and also sensitive and specific.

Keywords: Isoniazid, Pyridoxine, Simultaneous equation method.

INTRODUCTION

Pulmonary tuberculosis is an important public health concern in Mexico.Isoniazid (INH, isonicotinyl hydrazine) is the drug most widely used for treatment of tuber-culosis.Isoniazid (Pyridine-4-carboxilic acid hydrazide) is a synthetic antibacterial agent with bactericidal action against M. *tuberculosis*. It is the hydrazide of isonicotinic acid. Isoniazid is never used on its own to treat active tuberculosis because resistance quickly develops. Isoniazid is bactericidal to rapidly-dividing mycobacteria but is bacteriostatic if the mycobacterium is slow-growing.Pyridoxine is one of the compounds that can be called vitamin B_6.Pyridoxine assists in the balancing of sodium and potassium as well as promoting red blood cell production. Pyridoxine is given to patients taking Isoniazid (INH) to combat the toxic side effects of the drug. It is given 10–50 mg/day to patients on to prevent peripheral neuropathy and CNS effects that are associated with the use of INH.



Pyridine-4-carboxilic acid hydrazide4,5-Bis(hydroxymethyl)-2-methylpyridin-3-ol

MATERIALS AND METHODS

Materials:
A double-beam Jasco, V630 Visible spectrophotometer, spectral bandwidth of 2nm, wavelength accuracy ±0.5nm and a pair of 1-cm matched quartz cells was used to measure absorbance of the resulting solution

Fig.1 Linearity of Isoniazid in Distilled Water

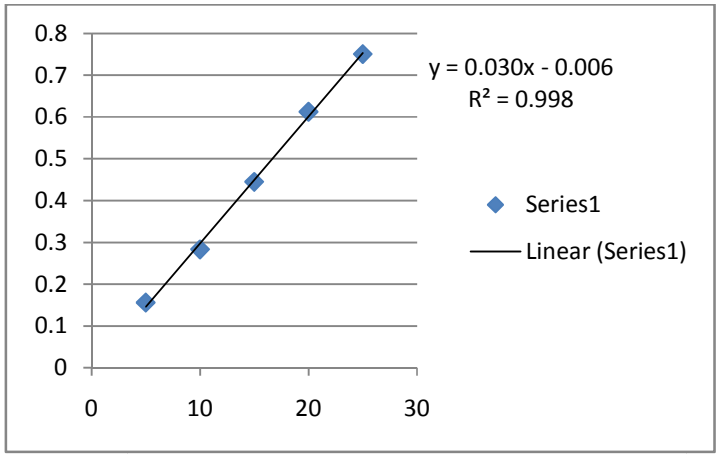
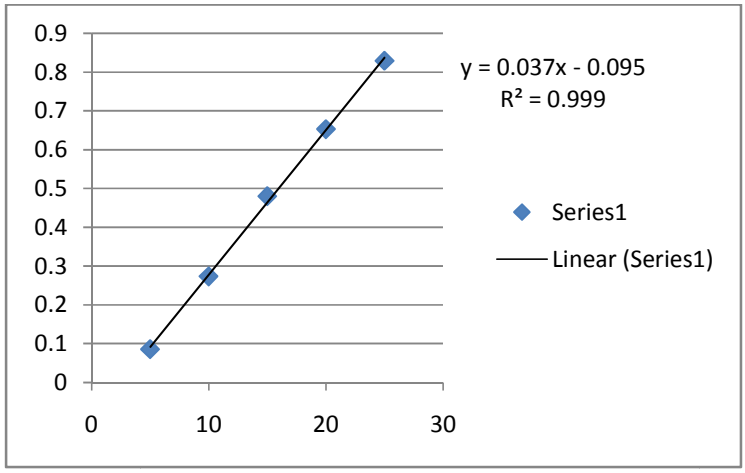


Fig.2 Linearity of Pyridoxine in Distilled Water



Preparation of standard drug solution:
Standard stock solutions containing isoniazid (INH) and pyridoxine ($VitB_6$) were prepared individually by dissolving 10 mg of Isoniazid and 10 mg of Pyridoxine separately in100 ml distilled water to get stock solutions containing 100mcg/ ml each of INH and $VitB_6$ in two different 100ml volumetric flasks.

Determination of absorption maxima:
By appropriate dilution of two standard drug solutions with distilled water, solutions containing 10 µg/ml of INH and 10 µg/ml of $VitB_6$ were scanned separately in the range of 200- 400 nm to determine the wavelength of maximum absorption for both the drugs. INH and $VitB_6$ absorbance maxima at 263 nm (λ1) and 292 nm (λ2) respectively. As shown in Fig.1 and 2

Fig.3 Spectra of Isoniazid in Distilled Water

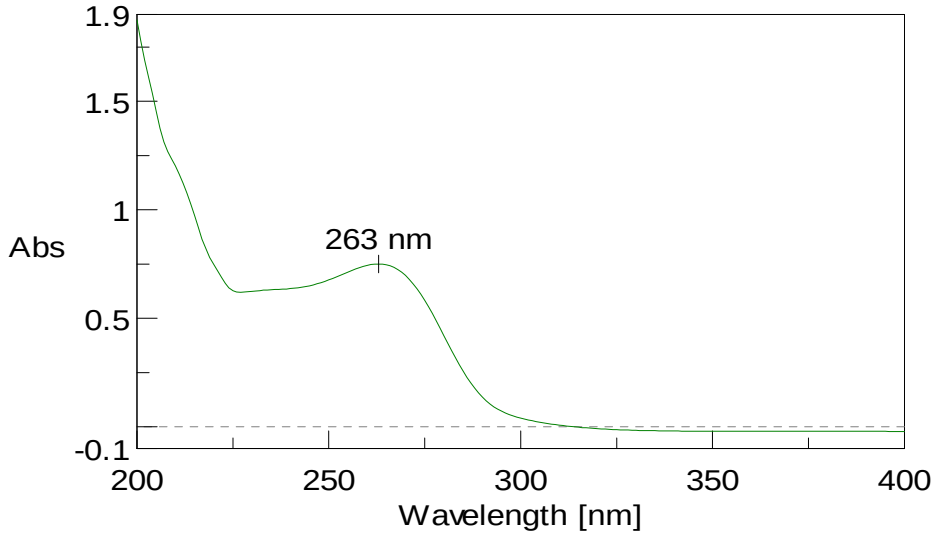
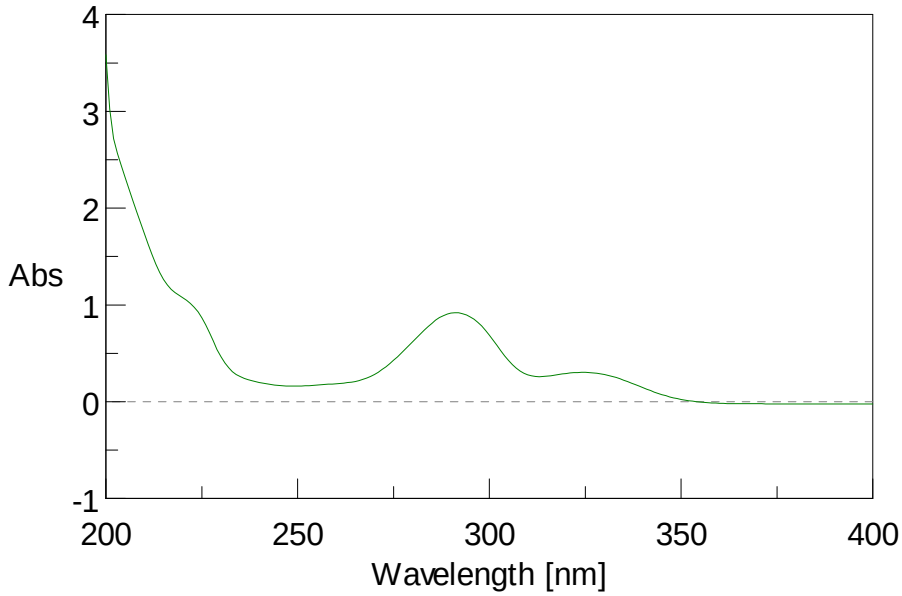


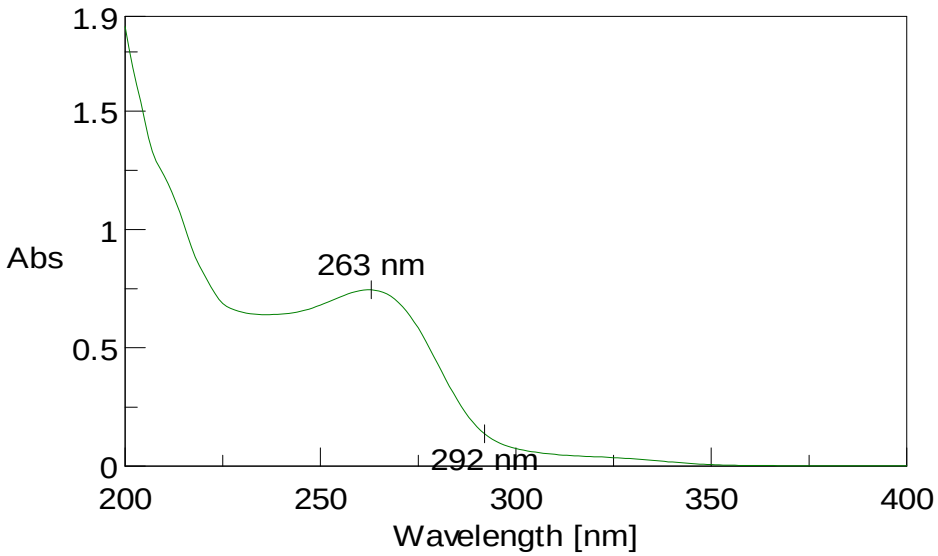
Fig.4 Spectra of Pyridoxin in Distilled Water



Simultaneous Equation Method:-
Two wavelengths selected for the method are 263 nm and 292 nm that are absorption maximas of INH and $VitB_6$ in Distilled water respectively. The stock solutions of both the drugs were further diluted separately with Distilled water to get a series of standard solutions of 5-30µg /ml concentrations of Isoniazid and 5-30µg /ml concentrations of Pyridoxine. The absorbance were measured at the selected wavelengths and absorptivities (A 1% ,1 cm) for both the drugs at both wavelengths were determined as mean of six independent determinations.

Application of the Proposed Method for the Determination of INH and Vit B_6 in Tablets:
Marketed tablet formulation containing Isoniazid 300 mg and Pyridoxine 10 mg was analyzed using this method. From the 20 tablets, an amount equivalent to 10 mg of Isoniazid was weighed and dissolved in 100ml with distilled water to get a stock solution containing 100mcg/ml of standard solution. Then the solution was filtered through Whatman filter paper. Appropriate aliquots of isoniazid and pyridoxine were made. The absorbance of resulting solutions were measured at 263 nm and 292nm.The concentration of isoniazid and pyridoxine present in the sample solution was calculated by using the equation generated from calibration curve of respective drugs..Values were substituted in the respective formula to obtain concentrations. As shown in table. no. 2

Fig.5 Spectra of tablet formulation



Validation parameters
The method was validated according to ICH Q2B guidelines for validation of analytical procedures in order to determine the linearity, sensitivity, precision and accuracy for the analyte.

Accuracy:
To ascertain the accuracy of the proposed methods, recovery studies were carried at three different levels (80%, 100% and 120%). Percent recovery for INH and Vit B_6 by this method. The results were reported in Table 3.

Linearity:
The linearity of measurement was evaluated by analyzing different concentration of the standard solution of INH and Vit B_6. For simultaneous equation method the Beer- Lambert's conc. range was found to be for5- 25µg/ml for INH and 5-25µg/ml VitB_6. The results were reported in Table 1.

Repeatability:
To check the degree of repeatability of the methods, suitable statistical evaluation was carried out. Five samples of the tablet formulations were analyzed for the repeatability study. The standard deviation, coefficient of variance and standard error was calculated. The results were reported in Table 3.

Intermediate precision (inter-day and intraday precision):
Intermediate precision of the method was checked by assay the sample solution on same day at an interval of one hour (intraday precision) for three hours and on three different days (interday precision) the result was reported in Table 3. This study indicates that the solutions can be analyzed within 48-72 h without having any bad effect on chemical stability of the drug in presence of urea. The results of the same were given in Table 3.

Limit of detection (LOD) and limit ofQuantitation (LOQ):
The LOD and LOQ were separately determined based on the standard deviation of response of the calibration curve. The standard deviation of Y-intercept and slope of the calibration curves were used to calculate the LOD and LOQ by using the equations 3.3σ/s for LOD and 10σ/s for LOQ, where σ stands for standard deviation of Y-intercept and S stands for slope of the calibration curve. The results of the same were given in Table 2.

Table (1) Optical Characteristics

Parameters	Simultaneous Equation method	
	Isoniazid	Pyridoxine
Working λ max in Water	263nm	290nm
Beer Lamberts Law range	5-25	5-25
Slope	0.0304	0.0373
Regression coefficient(r2)	0.998	0.999

Table (2) Results of analysis of tablet formulation

Drug	Label Claim (mg)	Amount Taken (mg/tab)	Amount Found (mg/tab	% Recovery	S.D	LOD (µg/ml)	LOQ (µg/ml)
Isoniazid	300	10	9.97	99.00	0.8	0.481	1.59
Pyridoxine	10	10	9.85	96.24	0.115	0.144	0.438

S.D: Standard Deviation, S.E: Standard Error, C.V: Coefficient Variation

Table (3) Validation Parameters

Parameters	Drug	Lable Claim	Amount Taken	Amount Found	S.D	C.O.V	S.E
Precision (Inter-day)	Isoniazid	300mg	10mg	9.88mg	0.02	1.1	0.02
	Pyridoxin	10mg	5mg	5.01mg	0.38	0.9	0.02
Precision (Intra-day)	Isoniazid	300mg	10mg	9.75mg	0.05	1.2	0.07
	Pyridoxin	10mg	5mg	4.92mg	0.24	0.9.	0.05
Accuracy	Isoniazid	80%	8mg	8.03	0.3	1.3	0.05
		100%	10mg	9.95	0.09	1.2	0.05
		120%	12mg	11.79	0.8	1.1	0.04
	Pyridoxin	80%	4mg	4.04	0.6	0.9	0.07
		100%	5mg	5.01	0.6	0.6	0.09
		120%	6mg	5.86	0.5	0.8	0.04

S.D- Standard Deviation; C.O.V-Coefficient of Variance; S.E- Standard Error

RESULTS AND DISCUSSION

The overlain spectra of INH and VitB_6 exhibit λ max of 263 nm and 292 nm respectively which are quite separated from each other. Standardcalibration curves for INH and VitB_6 were linear with correlation coefficients (r) values in the range of 0.998- 0.999 at all the selected wavelengths and the values were average of six readings with standard deviation in the range of . 0.02-0.8.. The accuracy of the method was confirmed by recovery studies from tablet at three different levels of 80 % , 100 %, 120 % .The most striking feature of this method is its simplicity, economy and rapidity, non- requiring consuming sample preparations such as extraction of solvents, heating, degassing which are needed for HPLC procedure. These are new and novel methods and can be employed for routine analysis in quality control analysis..

Acknowledgement
Authors are thankful to the Principal and Management of P.D.V.V.P.F's College of Pharmacy, Ahmednagar for providing the necessary facilities to carry out this work.

REFERENCES

[1] British pharmacopoeia, Vol II, London. **1998**,p.1854.
[2] Indian pharmacopoeisVol II, New Delhi, The controller of Publications.Govt of India, **1996**,554.
[3] Bhutani H, Singh S, Vir S, Bhutani KK, Kumar R, Chakraborti AK, Jindal KC (**2007**). *J. Pharm. Biomed. Anal.*, 43: 1213-1220.
[4] Butterfield AG, Lovering EG, Sears RW (**1980**). *J. Pharm. Sci*.,69(2): 222-224.

[5] Kompantseva E, Khalata A, Ovcharenko L, DukkardtL,Blagorazumnaya N (**2005**). *Pharm. Chem. J.*, 39: 441-443.
[6] International Pharmacopoeia, Fourth Edition (**2008**). Isoniazid and ethambutol hydrochloride tablets [cited 2011, May 12].