Research Article
AMERICAN JOURNAL OF PHARMACY AND HEALTH RESEARCH
www.ajphr.com
2014, Volume 2, Issue 6
ISSN:2321–3647(online)

Development and Validation of UV Visible Spectrophotometric Method for Simultaneous Estimation of Melatonin and Pyridoxine in Pharmaceutical Dosage Form

Sangeeta Rathod[1*], Ankita Bhavsar[1], Bhagirath Patel[1]
1.Department of Quality Assurance, Sat Kaival College of Pharmacy, Sarsa-388365, Gujarat, India.

ABSTRACT

A simple, accurate, precise and rapid UV spectrophotometric simultaneous equation method has been developed for the simultaneous estimation of Melatonin and Pyridoxine in tablet dosage form. The stock solution was prepared in methanol. 243 and 271nm, an absorbance maxima were selected for Melatonin and Pyridoxine respectively. Beer's law obeyed the concentration range of 3-9 µg/ ml and 10-30 µg/ml, for Melatonin and Pyridoxine. The results of analysis were validated statistically and by recovery studies. Accuracy and precision are within the limit. The % RSD for the recovery study was less than 2. The developed method was validated as per International conference Harmonization (ICH) guideline for its accuracy, precision, Limit of detection and Limit of quantitation. The proposed method can be effectively applied for the simultaneous estimation of both drugs in tablet dosage form.

Keywords: Melatonin, Pyridoxine**,** Simultaneous Equation Method Development, Validation

*Corresponding Author Email rathodsangeeta1990@gmail.com
Received 27 May 2014, Accepted 06 June 2014

Please cite this article in press as: Rathod S *et al.*, Development and Validation of UV Visible Spectrophotometric Method for Simultaneous Estimation of Melatonin and Pyridoxine in Pharmaceutical Dosage Form. American Journal of Pharmacy & Health Research 2014.

INTRODUCTION

Analytical methods are intended to establish the identity, purity, physical characteristics and potency of the drugs and to support drug testing against specifications during manufacturing and quality release operations as well as during long term stability studies.[1,2]

Method validation

Validation of an analytical method is the process by which it is established, by laboratory studies, that the performance characteristics of the method meet the requirements for the intended analytical applications.[3,4]

Simultaneous equation method (Vierodt's method)

Concentration of several components present in the same mixture can be determined by solving a set of simultaneous equation even if their spectra overlap. If Beer's law is followed, these equations are linear. If a sample contains two absorbing drugs (X and Y) each of which absorbs at the λ_{max} of other, this technique may be used to determine both the drugs. Let, C_X and C_Y are the concentrations of X and Y respectively in the diluted sample. Following equations are known as simultaneous equations and by solving these simultaneous equations we can determine the concentration of X and Y in the sample.

$C_x = (A_2\, a_{Y1} - A_1\, a_{Y2}) / (a_{Y1}\, a_{X2} - a_{Y2}\, a_{X1})$

$C_y = (A_1\, a_{X2} - A_2\, a_{X1}) / (a_{Y1}\, a_{X2} - a_{Y2}\, a_{X1})$

Criteria for obtaining maximum precision, based upon absorbance ratios, have been suggested that place limits on the relative concentrations of the components of the mixture.[8] The criteria are as follows;

1) The ratios $(A_2/A_1)/ (A_{X2}/A_{X1})$ and $(A_{Y2}/A_{Y1})/ (A_2/A_1)$ should lie outside the range 0.1 – 2 for the precise determination of Y and X respectively. These criteria are satisfied only when the λ_{max} of the two components are reasonably dissimilar.

2) Two components do not interact chemically, thereby negating the initial assumption that the total absorbance is the sum of the individual absorbance. The absorbance ratio and absorption factor method (Absorption correction method) are the modification of the simultaneous equation procedure.

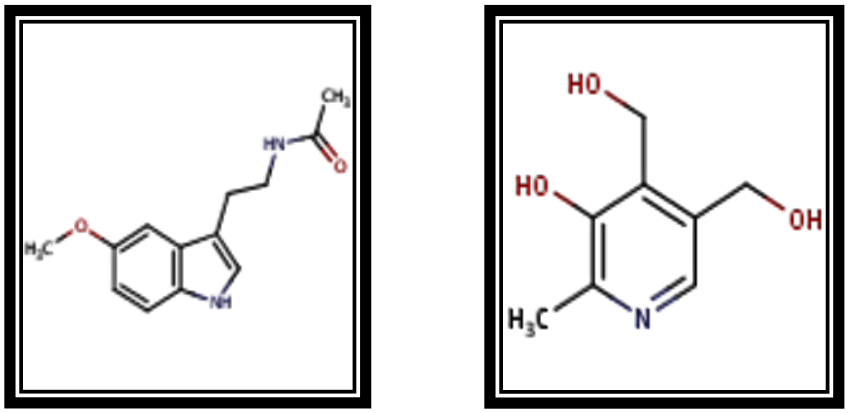
Melatonin

Chemically it is N- acetyl-5-methoxytryptamine ($C_{13}\, H_{16}\, H_2\, O_2$). It is a biogenic amine that is found in animals, plants and microbes. Melatonin regulates the sleep-wake cycle by chemically causing drowsiness and lowering the body temperature. Melatonin is also implicated in the

regulation of mood, learning and memory, immune activity, dreaming, fertility and reproduction. Melatonin is also an effective antioxidant.[5,6]

Pyridoxine

Chemically it is 4,5-bis (hydroxymethyl-2-methylpyridin-3-ol ($C_8H_{11}NO_3$). Pyridoxine is one of the compounds that can be called vitamin B6, along with pyridoxal and pyridoxamine. It is often used as 'pyridoxine hydrochloride.'[7] The chemical structure of Melatonin (A) and Pyridoxine (B) mentioned in Figure 1.



Melatonin **Pyridoxine**

Figure 1: Chemical structure of (A) Melatonin and (B) Pyridoxine

MATERIALS AND METHODS

Instruments

UV double beam spectrophotometer (Shimadzu Model 1800) was employed with automatic wavelength correction with a pair of 1 cm matched quartz cells. Electronic analytical balance (AX 200)

Reagents and material

Melatonin and Pyridoxine are procured from Merck Pharmaceutical Pvt. Ltd. as a gift sample. AR grade solvent Methanol was obtained from Gitar laboratory, Ahmedabad. Zytonin tablet (Melatonin 3 mg, Pyridoxine 10 mg) was gifted from Indon Zydus Cadila Health Care Ltd. Ahmedabad.

Preparation of standard stock solution and calibration curve

Standard stock solution of pure drug containing 6 mg/ml of Melatonin and 20 mg/ ml of Pyridoxine were prepared in methanol. The working standard solutions of these drugs were obtained by dilution of the stock solution in the series of solutions with conc. 2-12 µg/ ml of Melatonin and 5–35 µg/ ml of Pyridoxine respectively were used to prepare calibration curve. Solutions were scanned and proposed methods were applied. For determination of absorptivity

values, calibration curves using standard serial dilutions of individual drugs were plotted.

Selection of Analytical wavelength

To determine wavelength for measurement, standard spectra of Melatonin and Pyridoxine were scanned between 200-400 nm against methanol. Absorbance maxima were obtained at 243 nm and at 271 nm for Melatonin and Pyridoxine respectively. Overlay spectra of Melatonin and Pyridoxine is shown in Figure 2.

Simultaneous determination

The Simultaneous Equation Method of analysis based on the absorption of the drug Melatonin and Pyridoxine at their λ_{max}. Two wavelength selected for the development of Simultaneous Equation were 243 nm (λ1) and 271nm (λ2). Absorptivities of both the drugs at both the wavelengths were determined. Equations obtained for the estimation of concentration were,

Cx = (A1*y2) - (A2*y1) x1y2 - x2y1

Cy = (A2*x1) - (A1*x2) x1y2 - x2y1

Where A1 and A2 are absorbance of Sample solution at 243 and 271 nm respectively

x1= Absorptivity of Melatonin at 243 nm

x2 = Absorptivity of Melatonin at 243 nm

y1 = Absorptivity of Pyridoxine at 271 nm

y2= Absorptivity of Pyridoxine at 271nm

Cx and Cy are concentration of Melatonin and Pyridoxine in sample solution.

VALIDATION OF METHOD

Linearity and Range

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. For each drug, appropriate dilutions of standard stock solutions were assayed as per the developed methods. The Beer-Lambert's concentration range was 3-9 µg/ml for Melatonin and 10-30 µg/ml for Pyridoxine. The linearity data are shown in Table 1.

Sensitivity

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The sensitivity measurement of Melatonin and Pyridoxine by the use of proposed method was estimated in terms of (LOD) and (LOQ). The LOD and LOQ were calculated using following equations.

$$LOD = 3.3 \times \sigma/S \quad LOQ = 10 \times \sigma/S$$

Where, σ = the standard deviation of the response, S = slope of the calibration curve.

Precision

The precisions of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision of the method was determined by repeating assay 3 times. To study intraday precision, method was repeated 3 times in a day and the average % RSD was calculated. Similarly the method was repeated on three different days &average % RSD was calculated. The values for interday precision are reported in Table 2.

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. Accuracy was confirmed by recovery study as per ICH guidelines Q2R1 at three different concentration levels 80%, 100%, 120% by replicate analysis (n = 3). To a standard solution drug solutions were added and then percentage of drug content was calculated. From the recovery study it is clear that the method is accurate for quantitative estimation of Melatonin and Pyridoxine, as the statistical parameters are within the acceptance range. The results are shown in Table 3 and 4.

Quantitative estimation of pharmaceutical dosage form

Twenty tablets were weighed; their average weight was determined and finally powdered. An accurately weighed tablet powder equivalent to 6 mg of Melatonin and 20 mg Pyridoxine were then transferred to 10 ml volumetric flask containing 5 ml methanol and sonicated for 20 min. The solution was filtered through 0.45µm filter and the volume was adjusted up to mark with methanol. From the above solution 1 ml was taken into a 10 ml volumetric flask and the volume was adjusted up to mark with methanol to get a final concentration of 6 µg/ml of Melatonin and 20 µg/ml Pyridoxine. 20 µl of the test solution was injected and chromatogram was recorded for the same and the amount of the drug was calculated. The results are shown in Table 5.

RESULTS AND DISCUSSION

Overlay spectrum of Melatonin and Pyridoxine

The standard solutions of Melatonin and Pyridoxine were prepared separately in methanol. They were scanned in the wavelength range of 200-400 nm. Maximum absorbance was obtained at 243 nm and 271 nm for Melatonin and Pyridoxine, respectively. These two analytical

wavelengths were selected for determination of Melatonin and Pyridoxine, respectively as shown in Figure 2.

Further, it is observed that Melatonin gave highest absorbance at 318.95 nm and it is completely independent of Pyridoxine. Therefore, it can be estimated independently at 318.95 nm.

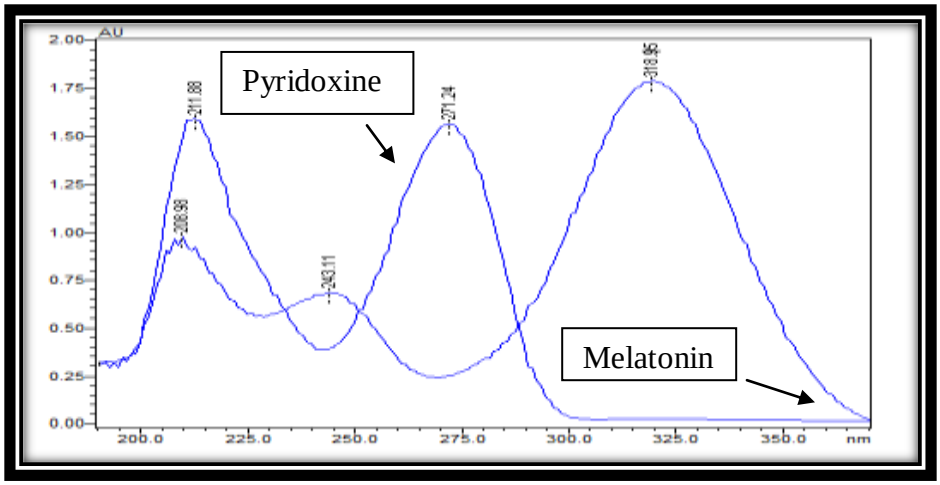


Figure 2: Overlay spectrum of Melatonin and Pyridoxine

Linearity

The calibration curves for linearity of this method were obtained by plotting the peak area against concentrations of Melatonin and Pyridoxine ranging between of 3-9 µg/ml and 10-30 µg/ml respectively. The calibration curves are shown in Figure 3 and 4.The results obtained for these calibration plots of Melatonin and Pyridoxine are provided in Table 1.

Table 1. Linearity for Melatonin and Pyridoxine

Melatonin	Pyridoxine
Correlation coefficient : 0.9980	Correlation coefficient : 0.9997
Slope : 0.065	Slope : 0.036
Regression Equation : y= 0.065x + 0.017	Regression Equation : y = 0.036x + 0.043
LOD: 0.5659 µg/ml	LOD: 0.7430 µg/ml
LOQ: 1.7149 µg/ml	LOQ: 2.2515 µg/ml

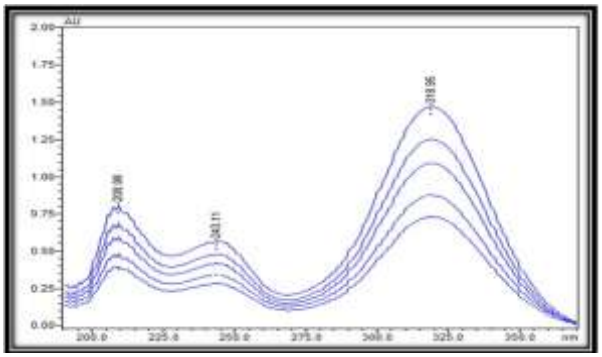


Figure 3: Overlay spectra of Melatonin in methanol (243 nm)

Precision

The repeatability of the analytical method was evaluated by assaying three sample solutions of Melatonin and Pyridoxine within the same day and under the same experimental conditions (Intra-day). Intermediate precision was evaluated by assaying similar solutions on three consecutive days. The peak areas were determined and compared. The precision was expressed as % Relative Standard Deviation (Acceptance range: RSD < 2). Results mentioned in Table 2.

Table 2. Precision for Melatonin and Pyridoxine

Drug	Conc. (µg/ml)	Intra-day area mean (n=3)	± SD and %RSD	Inter-day area mean (n=3)	±SD %RSD
	3	0.223	± 0.00372	0.237	± 0.00472
Melatonin	6	0.417	0.9221	0.419	1.1089
	9	0.615		0.610	
	10	0.777	± 0.01804	0.781	± 0.0190
Pyridoxine	20	0.977	1.6336	0.989	1.8985
	30	1.139		1.137	

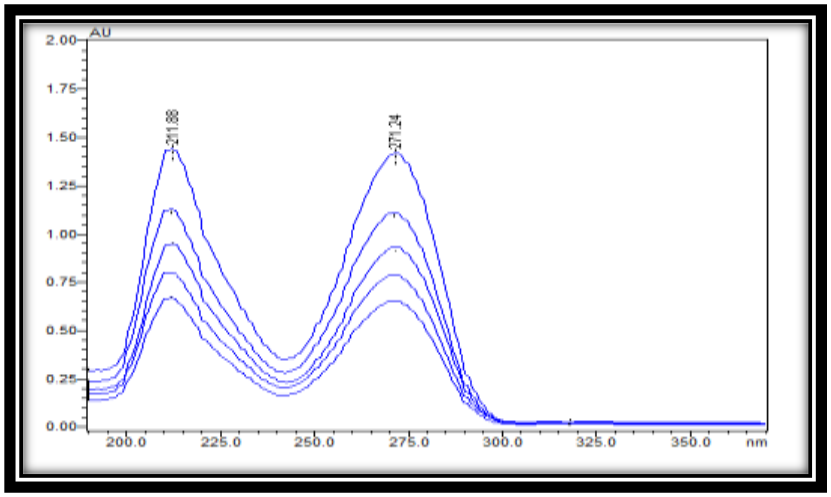


Figure 4: Overlay spectra of Pyridoxine in methanol (271 nm)

Accuracy

The accuracy of the method was determined by calculating the % recovery of Melatonin and Pyridoxine using the standard addition method. Accuracy for Melatonin and Pyridoxine using the proposed method are mentioned in table 3 and 4.

Table 3. Accuracy for Melatonin

% Level of Recovery	Conc. of sample solution (µg/ml)	Conc. of standard Solution (µg/ml)	Total conc. (µg/ml)	Conc. found (µg/ml)(n=3)	% RSD	% Recovery mean(n=3)
80	3	2.4	5.4	5.36	0.87	99.44
100	3	3	6	5.90	1.26	98.33
120	3	3.6	6.6	6.50	1.43	98.52

Table 4. Accuracy for Pyridoxine

% Level of Recovery	Conc. of sample solution (µg/ml)	Conc. of standard Solution (µg/ml)	Total conc. (µg/ml)	Conc. found (µg/ml)(n=3)	% RSD	% Recovery mean(n=3)
80	10	8	18	17.86	0.95	99.25
100	10	10	20	19.94	1.31	99.73
120	10	12	22	21.71	0.78	98.68

Table 5. Quantitative estimation of pharmaceutical dosage form

Zytonin Parameters	Melatonin	Pyridoxine
Actual Concentration (µg/ml)	3	10
Concentration obtained (µg/ml)	2.99	10.1
% Assay	2.99	101
Limit	98-102%	

CONCLUSION

Development and validation of UV –Visible simultaneous equation method was found to be simple, accurate, precise and economical. These methods can be applied for routine quantitative analysis of Melatonin and Pyridoxine in pharmaceutical dosage form. The lack of extraction procedures makes the method especially suitable for routine quality control analysis work particularly when large numbers of samples are encountered.

ACKNOWLEDGEMENTS

The authors would like to thank, Merck pharmaceutical Pvt. Ltd. India for providing a gift sample of standard Melatonin and Pyridoxine. The authors would like to thank to Department of Quality Assurance, Sat Kaival College of Pharmacy, Sarsa, Gujrat, India. For providing necessary facilities to carry out the work.

REFERENCES

1. Haghi AE. Analytical method validation: Razi vaccine and serum research Institute. J Pharm 2009; 3: 67-68.
2. ICH guidelines Validation of Analytical Procedure: Methodology Q2B. International conference on Harmonization, IFPMA. Geneva, Switzerland.
3. Sharma YR. Ultraviolet and visible spectroscopy in Elementary Organic Spectroscopy. 5th ed., New Delhi; S. Chand & Company Ltd; 2004: 9 -10.
4. Wang Q, Decheng M, Higgins and JP. Analytical method selection for drug product. Merck Res Lab Pharma Res & Develop 2008; 8: 5-7.
5. http:// www.drugs.com/melatonin.html

6. http://www.drugbank.ca/drugs/DB01065
7. http://www.drugbank.ca/drugs/DB01065
8. Conners KA. A Textbook of Pharmaceutical Analysis. 3rd ed., London: John Wiley and Sons; 1999: 221-23.