Pharmacophore 2014, Vol. 5 (2), 252-257 USA CODEN: PHARM7 ISSN 2229-5402

Pharmacophore

(An International Research Journal)

Available online at http://www.pharmacophorejournal.com/

Original Research Paper

SPECTROPHOTOMETRIC METHODS FOR SIMULTANEOUS ESTIMATION OF MELATONIN AND ZOLPIDEM FROM THE COMBINED TABLET DOSAGE FORM

Venkatachalam. T[1] and **Lalitha. KG**[2]*

[1]Department of Pharmaceutical Chemistry, JKKMMRF College of Pharmacy, B. Komarapalayam-638183, Tamil Nadu, India

[2]Department of Pharmaceutical Chemistry, ULTRA College of Pharmacy, Madurai-625020, Tamil Nadu, India

ABSTRACT

The present work aimed to develop and validate spectrophotometric methods for simultaneous estimation of melatonin and zolpidem in combined dosage form. Method is based on solving a simultaneous equation. Absorbance of melatonin and zolpidem were measured at the respective absorbance maximum (λ_{max}) at 277 and 311 nm. Methods are validated according to ICH guidelines. Linearity range for melatonin and zolpidem is 3-10.5 µg/ml and 5-17.5 µg/ml at respective selected wavelengths. The coefficient of correlation for melatonin at 277 nm and zolpidem at 311nm is 0.9940 and 0.9969, respectively. A percentage estimation of melatonin and zolpidem from the tablet dosage form is101.80 % and 101.70 % respectively, with standard deviation less than 2. The proposed method was simple, rapid, and validated and can be used successfully for the routine simultaneous estimation of melatonin and zolpidem combined tablet dosage form.

Keywords: Melatonin, Zolpidem, Simultaneous equation method, Method Validation, ICH guidelines.

INTRODUCTION

Melatonin (MEL) chemically is an N – [2-(5-methoxy-1H-indol-3-yl) ethyl] acetamide (figure 1A) (Merck index), clinically used in the treatment of cancer, immune disorder, cardiovascular diseases, depression and sexual dysfunction. Zolpidem (ZOL) chemically is a N, N-dimethyl-2-(6-methyl-2-p-tolylimidazo [1,2a] pyridin3yl) acetamide (figure 2B) (Merck index). It is used for short-term treatment of insomnia, as well as some brain disorders, short-acting non benzodiazepine hypnotic of the imidazopyridine class that potentiates gamma-amino butyric acid (GABA), an inhibitory neurotransmitter. Literature survey revealed that MEL is a estimated by, radioimmunoassay (Vakkuri, O *et al*., 1984) and RP-HPLC method for individually (Fumio, I *et al*., 1999). ZOL is estimated by spectrophotometric (Patil, KS *et al*.,2010; Chomwal R *et al*., 2010), stability indicating RP-HPLC method (Maharajan, MP *et al*., 2011) bulk drugs and human plasma (Nirogi, RV *et al*., 2004; Ring, PR *et al*., 2000), MEL and ZOL estimated by HPLC method (Lalitha, KG 2013). Aim attempt was made a simple, precise, accurate method for the simultaneous estimation of MEL and ZOL in combined tablet dosage form by UV spectrophotometry and to validate the developed method as per ICH guidelines (Q2,R1,1996; Q2B,R1,2005).

MATERIALS AND METHODS

Spectrophotometric analysis was carried out on a LAB INDIA3000+ Series double beam UV -

Visible spectrophotometer with fixed slit width 1nm attached to the computer with UV probe, version 5.20, UVWIN 5 spectrophotometer software for obtaining the spectra 1cm in matched quartz cells with the spectral bandwidth of 2 nm.

Reagents and Chemicals

Samples of MEL and ZOL standards were obtained from Reltfen Pharmaceutical Ltd, (Puducherry,India). The tablet formulation was obtained as gift sample from by Reltfen Pharmaceutical Ltd, (Puducherry, India). Methanol AR grade was procured from Qualigens Fine Chemical, (Mumbai, India).

Preparation of Standard Stock Solutions

An accurately weighed quantity of MEL (10 mg) and ZOL (10 mg) were transferred to a separate 100 ml volumetric flasks, dissolved well and diluted to the mark with methanol to obtain standard solution having concentration of MEL (100 µg/ml) and ZOL (100 µg/ml). A 1 ml of both the solutions were transferred into a separate 10 ml volumetric flasks and diluted to the mark with methanol to obtain the solutions having the concentrations of 10 µg/ml for MEL and ZOL.

Methods

The standard solutions of MEL (10 µg/ml) and ZOL (10 µg/ml) were scanned separately in the UV range of 200-400 nm and the spectrum were recorded. The λ max values of MEL and ZOL were found to be 277 nm and 311 nm, respectively. From the standard stock solutions having concentrations 3,4.5,6,7.5,9, and 10.5 µg/ml for MEL and 5,7.5,10,12.5,15 and 17.5 µg/ml of ZOL were prepared in methanol. The absorbance of resulting solutions was measured at 277 nm and 311 nm and the calibration curves were plotted at these wavelengths. The absorptivity coefficients of these two drugs were determined using the calibration curve equations. The concentration of MEL and ZOL in the sample solution was determined by solving the respective simultaneous equations generated by using absorptivity coefficients and absorbance values of MEL and ZOL at the selected wavelengths.

$$CX = \frac{A1ay2 - A2ay1}{Ax1ay2 - ax2ay1}$$

Where A1 and A2 are absorbance's of mixture at 277 and 311 nm respectively, ax1 and ay1 absorptivities of MEL at λ1 and λ2 respectively and ay1 and ay2 are absorptivities of ZOL at λ1 and λ2 respectively. Cx and Cy are concentration of MEL and ZOL respectively.

Determination of Absorptivity Value

The absorbance of each of the final dilution (10 µg/mL of MEL and 10 µg/mL ZOL) were measured in triplicate in 1.0 cm cell against solvent using methanol at 277 and 311 nm , respectively and A (1% 1cm) value were calculated using below formula.

Absorptivity,

$$A\ (1\%\ 1cm) = \frac{\text{Absorbance at selected wavelengths X 100}}{\text{Concentration in g/100 mL}}$$

Sensitivity

Absorbance of standard solutions of MEL and ZOL was taken at 277 and 311 nm. Sandell's sensitivity for drugs was calculated from the following formula, at both wavelengths

$$\text{Sensitivity (µg/cm3 AU)} = \frac{\text{Conc of drug (µg/100m-1) X0.001}}{\text{Absorbance}}$$

Method (Simultaneous Equation Method)

Two wavelengths selected for the method were 277 and 311 nm were the absorption maxima's of MEL and ZOL, respectively in methanol. The stock solutions of both the drugs were further diluted separately with methanol to get a series standard solution of 10 µg/ml. The absorbance's were measured at the selected wavelength and absorptivities (A 1%, 1 cm) for both the drugs at both wavelengths were determinations. Concentrations in the sample were obtained by using following equations.

$$CY = \frac{A1ax2 - A2ax1}{Ay1ax2 - ay2ax1}$$

Validation of the Proposed Method

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines.

Linearity (Calibration Curve)

The calibration curves were plotted over a concentration range of 3-10.5 µg/ml for MEL and 5-17.5 µg/ml for ZOL, respectively (figure 3 &4).

Method Precision (Repeatability)

The precision of the instrument was checked by repeated scanning and measurement of absorbance of solutions (n=6) for MEL and ZOL (10µg/ml for both MEL and ZOL) without changing the parameter of the proposed spectrophotometric method.

Intermediate Precision (Reproducibility)

The intra-day and inter-day precision of the proposed method was determined by analyzing the sample solutions for three times on the same day and one time for three successive days.

Accuracy (Recovery study)

The accuracy of the method was determined by calculating recovery of MEL and ZOL by the spiked method. To the sample solutions, known conc of was added in different level viz., 80,100 and 120% level. The amounts of MEL and ZOL were recorded and calculated. This procedure was repeated for three times.

Limit of Detection and Limit of Quantification

(LOD) and (LOQ) were calculated by constructing the calibration graph of MEL and ZOL at their selected wavelengths. LOD and LOQ were calculated from the slope and standard deviation of the response.

LOD=3.3X S/s

LOQ=10XS/s

Analysis of MEL and ZOL in A Combined Tablet Dosage Form

Twenty tablets were weighed and powdered. The powder equivalent to 10mg of MEL and ZOL was transferred into a 100 ml volumetric flask. Methanol was added to it and sonicated for 20 min .The solution was filtered through Whatman filter paper No.41, and the volume was adjusted up to the mark with methanol. The above solution was suitably diluted with methanol to get a final concentration of 10 µg/ml of MEL and ZOL. The absorbance's of the tablet sample solution, i.e. A1 and A2 were recorded at 277 nm and 311 nm and ratios of absorbance were calculated, i.e. A2/A1. Relative concentration of two drugs in the sample solution was calculated using respective simultaneous equations generated by using absorptivity coefficients and absorbance values of MEL and ZOL at these selected wavelengths.

RESULTS AND DISCUSSION

UV Spectrophotometric method for simultaneous equation method was selected for the simultaneous estimation of MEL and ZOL. 277 nm (λ max of MEL) and 311 nm (λ max of ZOL) were selected as analytical wavelengths at which calibration curves prepared for both the drugs. (Figure 2) The criteria for obtaining maximum precision by this method were calculated and found to be outside the range 0.1-2. Once the absorptivity values are determined, very little time is required for analysis, as would require determination of absorbance's of the sample solution at two selected wavelengths and few simple calculations. Linear correlation was obtained between absorbances and concentrations of MEL and ZOL in the concentration range 3-10.5 µg/ml and 5-17.5 µg/ml for drugs, respectively. (Figure 2a & 2b) The linearity of the calibration curve was validated by the high values of correlation coefficient of regression. LOD and LOQ values for MEL were found to be 0.118µg/ml and 0.359 µg/ml at 277 nm, respectively. LOD and LOQ values for ZOL were found to be 0.436 µg/ml and 1.32 µg/ml at 311 nm, respectively. These data show that the method is sensitive for the determination of MEL and ZOL. All the regression analysis data and the summary of validation parameters for the proposed method are reported in table 1.The recovery experiment was performed by the spiked method .The mean recoveries were 101.14±1.22 and 101.68±0.68 for MEL and ZOL, respectively, which indicates the accuracy of the proposed method table 2. The proposed validated method was successfully applied to determine MEL and ZOL in their combined tablet dosage form. The results obtained for MEL and ZOL were comparable with the corresponding labeled amounts (table 3). The relative standard derivation (% RSD) values for assay of MEL and ZOL were found to be 1.20 and 0.66, respectively. The

%RSD was found to be less than 2%, which indicates that the proposed method is repeatable (table 4).

CONCLUSION

No interference of the excipients with the absorbance of interest appeared, hence the proposed method is applicable for the routine simultaneous estimation of MEL and ZOL in pharmaceutical tablet dosage forms. The proposed spectrophotometric method was found to be simple, sensitive, accurate and precise for simultaneous determination of MEL and ZOL in tablet dosage form. The method utilizes easily available and low cost solvent like methanol for analysis of MEL and ZOL. Hence, the method was also found to be economical for the estimation of MEL and ZOL from tablets.

ACKNOWLEDGMENTS

The authors are thankful to the Principal of JKKMMRF College of Pharmacy, Komarapalayam for providing necessary facilities to carry out the research work.

Table 1: Regression analysis data and summary of validation parameter of the calibration curves

Parameters	Melatonin	Zolpidem
Wavelength(nm)	277	311
Beer's law limit(µg/ml)	3-10.5	5-17.5
Regression equation	0.0446X+0.0111	0.03777X-0.028
Slope	0.0446	0.03777
Intercept	0.0111	0.028
Correlation coefficient(r2)	0.9995	0.9964
LOD(µg/ml)	0.118	0.359
LOQ(µg/ml)	0.436	1.32

Table 2: Results of the recovery study

Level of recovery	Amount of pure drug is added (µg/ml)	Melatonin			Zolpidem		
		percentage	Mean±SD	% RSD	Percentage	Mean±SD	% RSD
80%	8(µg/ml)	100.66 98.33 100.41	99.80±1.2	1.27	101.25 102.50 103.20	102.31±0.98	0.95
100%	10(µg/ml)	102.3 103.3 100.41	102.20±1.15	1.12	102.80 100.80 101.80	101.80±1	0.98
120%	12(µg/ml)	101.66 101.33 101.33	101.44±0.19	0.18	101.4 101.66 99.80	100.95±1	0.99

Table 3: Results of analysis of tablets

Drug	Tab	Amount	Percentage	SD	% RSD
Melatonin	3	3.05	101.8	1.76	1.72
Zolpidem	5	5.08	101.7	0.68	0.66

Table 4: Results of intermediate precisions

Day	% Label claim estimated			
	Melatonin	% RSD	Zolpidem	% RSD
Intra- day	100.80±1.86	1.84	102.42±1.05	1.02
Inter- day	101.96±0.88	0.86	101.40±0.66	0.65

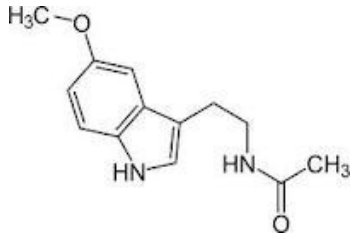

Figure 1 a: Melatonin

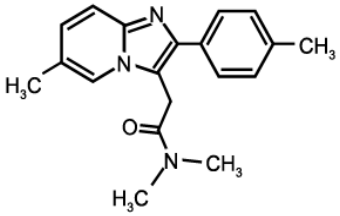

Figure 1 b: Zolpidem

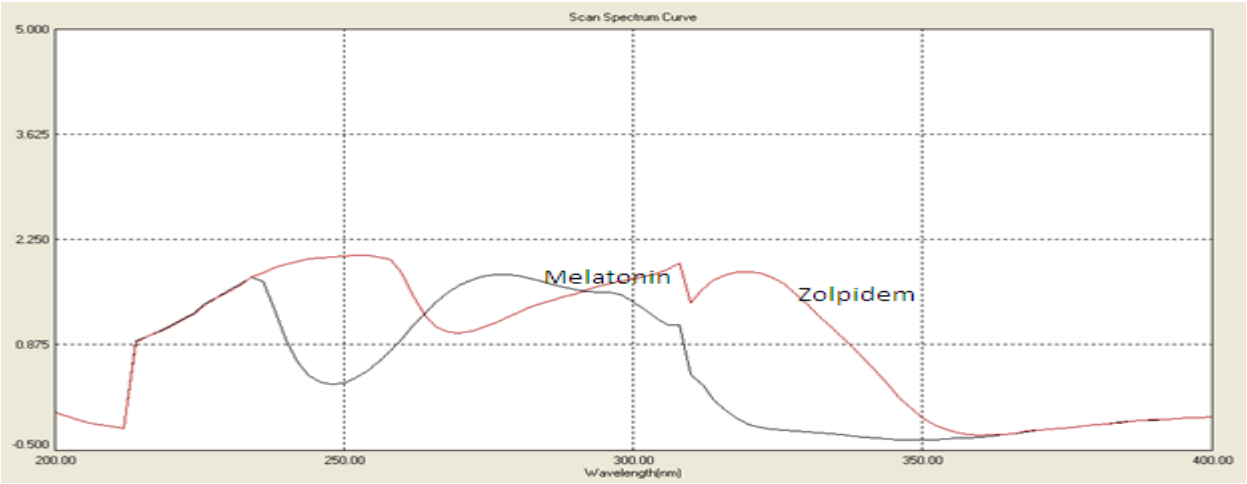

Figure 2: Overline spectra of Melatonin and Zolpidem

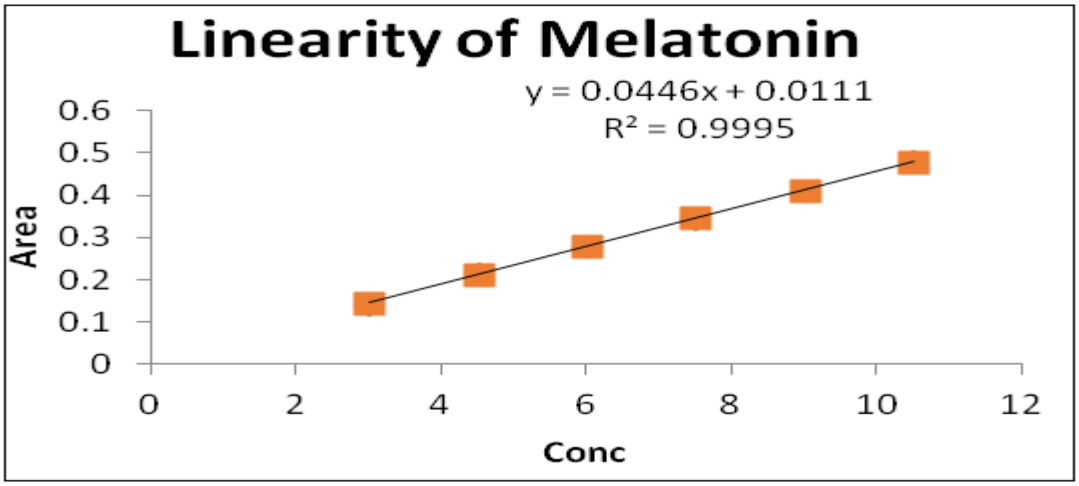

Figure: 3 Linearity of Melatonin

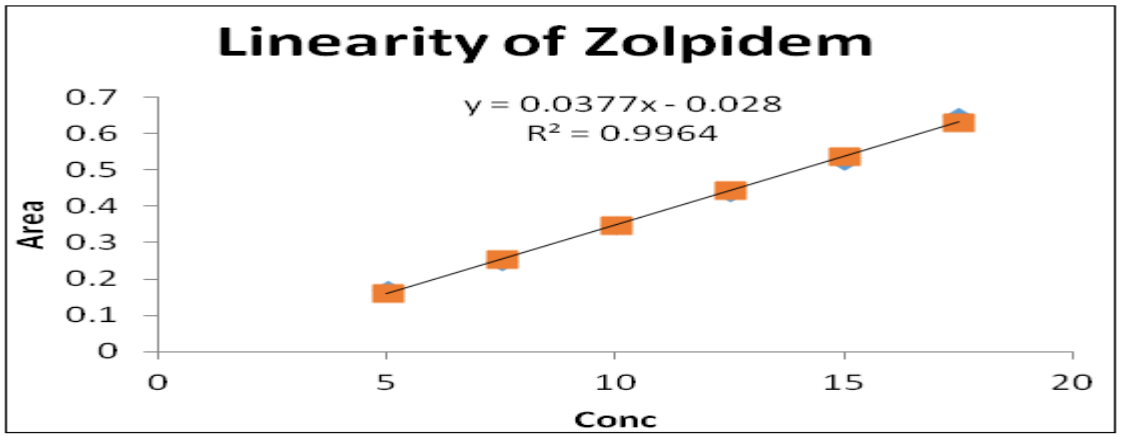

Figure: 4 Linearity of Zolpidem

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Correspondence Author:
K. G. Lalitha
Department of Pharmaceutical Chemistry, Ultra College of Pharmacy, Madurai – 625 020, Tamil Nadu, India
Email: kg.lalitha@gmail.com

Cite This Article: Venkatachalam, T and Lalith, KG (2014), "Spectrophotometric Methods for Simultaneous Estimation of Melatonin and Zolpidem from the Combined Tablet Dosage Form", ***Pharmacophore***, Vol. 5 (2), 252-257.