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Research Article

Method development and Validation for simultaneous estimation of Melatonin and Zolpidem tartrate by using RP-HPLC

Ganesh Akula[1*], Yadagiri Talari[2], S.S. Phanindra[2], Dr. A. Jaswanth[2]
[1*]Department of Pharmaceutical Chemistry, Procadence Institute of Pharmaceutical Sciences, Rimmanaguda, Gajwel. Medak(dt)-502312, Telangana, India.
[2]Procadence Institute of Pharmaceutical Sciences, Rimmanaguda, Gajwel. Medak(dt), Telangana, India.

***Corresponding author**
Ganesh Akula
Email: akula.ganesh@gmail.com

Abstract: A simple, accurate, economic, sensitive, rapid and robust reverse phase high performance liquid chromatography (RP-HPLC) method was developed for the estimation of melatonin and Zolpidem tartrate in pure and tablet dosage forms. Hypersil C18 ODS column (250x4.6mm,5µ) was used with a mobile phase containing a mixture of phosphate buffer and acetonitrile in the ratio of 55:45% v/v. The flow rate was maintained at 1.0 ml/min. Results were determined at 265 nm with a fixed wave length PDA detector. The linearity for melatonin was found between 6-42 µg/ml and between 10-70 µg/ml for zolpidem tartrate. The retention times were found as 2.517 and 3.630 for melatonin and zolpidem tartrate respectively. Validation parameters like accuracy, precision, robustness, LOD and LOQ, assay, system suitability parameters and stability studies were determined and examined by applying validated parameters.
Keywords: Zolpidem tartrate, Melatonin, RP-HPLC, Method development, Validation.

INTRODUCTION

Melatonin is a N-[2-(5-methoxy-1H-indol-3-yl) ethyl] ethanamide (Fig-1) an antioxidant, its actions are mediated through the binding and activation of melatonin receptors, while others are due to its role as a pervasive and powerful antioxidant, with a particular role in the protection of nuclear and mitochondrial DNA. It may also be produced by a variety of peripheral cells such as bone marrow cells, lymphocytes, and epithelial cells. Usually, the melatonin concentration in these cells is much higher than that found in the blood, but it does not seem to be regulated by the photoperiod.

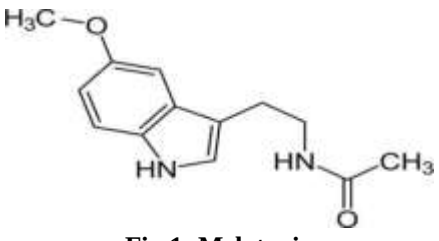

Fig.1: Melatonin

Zolpidemtartrate is aN,N,6-trimethyl-2-p-tolylimidazo[1,2-a] pyridine-3-acetamide-L-(+)-tartrate (Fig-2) and it is a prescription medication used for the 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, by binding to GABA-A receptors at the same location as benzodiazepines. It works quickly (usually within 15minutes) and has a short half-life (2–3 hours).

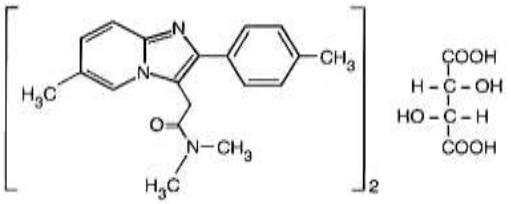

Fig.2: Zolpidem tartrate

The literature review reveals that there were methods available for the estimation of Melatonin [1-3] and Zolpidem tartrate [4-5] individually with the help of instruments like UV-Spectrophotometer, HPLC and for simultaneous determination there were methods found on UV- Spectrophotometer [6-7] and a very few on HPLC [5,8,9].an attempt was made to develop a method which is most precise, accurate, simple, robust and also most economic method so far for their determination.

MATERIALS AND METHODS:

All the reagents used in the experiment were HPLC grade solvents. After many trails it was observed

that a mixture of phosphate buffer and acetonitrile (55:45). Pure drugs of melatonin and zolpidem tartrate were obtained from Dr.Reddy's laboratories and the tablets were purchased from local pharmacy. The specifications of the instruments and all the conditions maintained were shown in Table 1.

Table-1: Optimized chromatographic conditions

Parameters	Method
Column	C_{18} ODS Hypersil (250 x 4.6mm, 5µ)
Mobile phase	phosphate buffer : Acetonitrile (55:45)
Flow rate (ml/min)	1.0
Pump	LC-20 AT Vp Series
Detector	PDA Detector
Operating temperature	Room Temperature
Selected wave length	265 nm.
Diluent	mobile phase
Injection volume	20 µl
Run time (min)	8

Preparation of buffer:

Accurately weighed quantities of 1.325 gm of dipotassium hydrogen phosphate and 0.3gm Potassium dihydrogen Phosphatewere dissolved in 1000ml of water.

Preparation of mobile phase:

450ml of acetonitrile and 550ml of buffer were mixed, sonicated for 30min and filtered through membrane filter.

Preparation of standard stock solution:

Accurately weighed quantity of 15 mg of Melatonin and 25 mg Zolpidem tartrate was transferred to a 50 ml volumetric flask, dissolved in 30 ml of mobile phase, sonicatedfor 15 mins and the volume was make up to 50ml with mobile phase.Concentration of stocksolution (Melatonin: 300µg/ml, ZolpidemTartrate:500µg/ml). This stock solution was used in the study after appropriate dilution.

Calibration of standards:

Based on the label claim of dosage form to be analyzed, different volumes of stock solutions of each drug were transferred accurately to 10ml volumetric flask and diluted to mark to give a series of concentrations of solution equal to 6, 12, 18, 24, 30, 36, 42 µg/ml of Melatonin and 10, 20, 30, 40, 50, 60, 70 µg/ml of Zolpidem tartrate. These solutions were injected into the optimized conditions. The calibration graph was plotted with mean peak area (n=6) on Y-axis and concentration of standard drug on X-axis. The degree of linearity was estimated by calculating the correlation coefficient, Y-intercept and slope of the regression line.

Sample preparation for Assay:

Locally available tablet dosage form (ZolsomaFC) pulse manufacturers contains Zolpidem Tartrate 5mg and melatonin 3 mg are taken . Accurately weighed 10 tablets, average weight is taken and powdered. Accurately weighed powder equivalent to 25 mg Zolpidemtartarate and 15 mg of Melatonin was accurately weighed and taken in a 50ml volumetric flask and 30 ml of mobile phase was added. The mixture was subjected to sonication for 15min with intermediate shaking for complete extraction of drugs. Filtered through a whatmann No.1 filter paper and cooled to room temperature and solution was made up to mark with mobile phase. The resulting solution was analyzed under optimized conditions. The results were shown in the Table 6.Chromatography was shown in Fig 3.

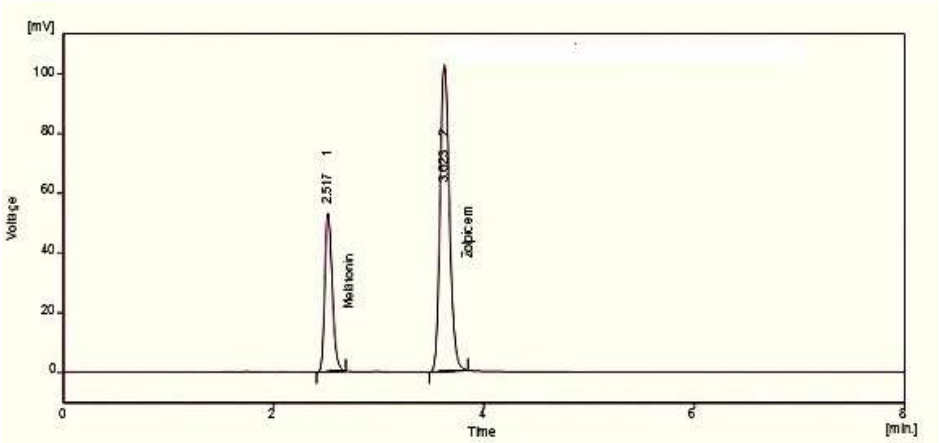

Fig 3: Chromatogram for Assay sample

Estimation Method:

The amount of Melatonin and Zolpidem tartrate present in each dosage form was calculated by using Linear line equation of calibration curve.

$$Y = mX \pm C$$

Where,

Y is Peak area
X is Concentration of the sample
m is Slope
C is Y- intercept
Amount found = Concentration x Dilution factor

Method Validation:

The developed method was validated as per ICH guidelines [15]. All the solutions were prepared according to the procedures given under preparation of standard and sample solutions.

RESULTS AND DISCUSSION

An attempt was made to develop a RP-HPLC method which is accurate, precise, economic and robust for the determination of Melatonin and Zolpidem tartrate in combined dosage form. The chromatographic conditions were optimized by changing the composition of mobile phase, pH, buffers and their concentration during many trails run on the instrument. Finally a mixture of 55 parts of phosphate buffer with 45 parts of acetonitrile at pH 6 was found suitable for best separation of two components. Different concentrations of standard solutions were injected to predict the linearity range for both drugs. Melatonin was found linear between the concentrations 6 to 42 µg/ml and 10 to 70 µg/ml for Zolpidem tartrate. Their correlation coefficients were found from the linear graph as 0.9999 for both the drugs. The retention times were found as 2.517 and 3.630 for melatonin and zolpiden tartrate respectively.

The assay (Table 6) was made for the combination tablets by preparing the solutions of concentrations from tablet powder which falls between the linear ranges of standard solutions. The accuracy (Table 2) of the method was checked by performing recovery studies. The recovery was determined at three levels, they are 80, 100 and 120% of the selected concentrations and performing three replicates at each recovery level. The precision (Table 3) of the method was determined from one lot of combined dosage forms. To determine the robustness (Table 4) of the developed method, experimental conditions were purposefully altered and the assay was performed. LOD & LOQ also determined for the developed method, results were showed in table-7.

Table-2: Accuracy studies of Melatonin and Zolpidem Tartrate

Recovery Level	MELATONIN				ZOLPIDM TARTRATE			
	Amount spiked (µg)	Amount recovered (µg)	% Recovery	% RSD	Amount spiked (µg)	Amount recovered (µg)	% Recovery	% RSD
80%	12	11.94	99.5	0.19	20	19.9	99.5	0.27
	12	11.98	99.8		20	19.8	99.0	
	12	11.96	99.6		20	19.9	99.5	
100%	15	14.98	99.8	0.14	25	24.8	99.2	0.39
	15	14.97	99.8		25	24.9	99.6	
	15	14.94	99.6		25	24.7	98.8	
120%	18	17.92	99.5	0.09	30	29.8	99.3	0.16
	18	17.96	99.7		30	29.9	99.6	
	18	17.94	99.6		30	29.8	99.3	
Mean % recovery				99.6	Mean % recovery			99.3
Overall %RSD				0.12	Overall %RSD			0.27

Table-3: Precision studies of Melatonin and Zolpidem Tartrate

S. No	System Precision studies		Method Precision studies	
	Melatonin Area (mv)	Zolpidem Area (mv)	Melatonin Area (mv)	Zolpidem Area (mv)
1.	351.700	751.896	99.30	99.98
2.	349.651	750.669	99.42	99.18
3.	350.232	752.235	99.33	99.89
4.	352.312	750.152	99.61	100.21
5.	351.450	751.682	99.32	99.96
Mean	351.069	751.326	99.14	99.83
%RSD	0.32	0.11	0.11	0.34

Table-4: Robustness data for Melatonin and Zolpidem Tartrate

Parameter	Variation	t_R of Melatonin (min)	t_R of Zolpidem tartrate (min)
Flow Rate (ml/min)	0.9	2.720	3.917
	1.1	2.337	3.367
Wave length (nm)	263	2.517	3.625
	267	2.517	3.623

Table-5: System Suitability parameters of Melatonin and Zolpidem Tartrate

Parameters	Melatonin	Zolpidem	Acceptance Criteria
Retention time (min)	2.517	3.623	-
No. of theoretical plates	5893	7473	NLT 2000
Tailing factor	1.464	1.352	NMT 2.0
Resolution	-	7.168	NLT 2.0

Table-6: Assay data for Melatonin and Zolpidem Tartrate

Drug	Amount found (mg)	Labeled amount (mg)	% Assay
Melatonin	3	2.99	99.64
Zolpidem tartrate	5	4.99	99.73

Table-7: LOD & LOQ for Melatonin and Zolpidem Tartrate

Parameter	Measured Value (µg/mL)	
	Melatonin	Zolpidem tartrate
Limit of detection (LOD)	0.031	0.212
Limit of quantification (LOQ)	0.108	0.641

All the validated parameters were checked by applying statistical formulas such as standard and relative standard deviation. The results were found to fall within the prescribed limits.

CONCLUSION

The present combination of Melatonin and Zolpidem tartrate is marketed as one formulation (Zolsoma FC) Melatonin -3mg/tablet and Zolpidem tartrate -5mg/tablet. The fixed dose combination tablet of Melatonin and Zolpidem tartrate was subjected to simultaneous estimation by reverse phase HPLC method. The proposed HPLC method was validated by evaluation of the validation parameters. The relative standard deviation of slope, correlation coefficient, within and between day repeatability, resolution and tailing factors for this techniques were obtained.Assay parameters used in this study reduced tailing for all peaks and improved the resolution.

Highly reliable and cost efficient HPLC method was developed for the quantitative estimation of melatonin and zolpidem tartrate in combined tablet dosage form. The results obtained were reproducible and reliable. The validity and precision of the methods were evident from the statistical and analytical parameters obtained.

From the forgoing it is concluded that the method developed is accurate, simple, rapid, specific and precise hence suitable for application in routine analysis of pharmaceutical preparations.

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