



## Research Article

### A VALIDATED RP-HPLC METHOD FOR THE DETERMINATION OF MELATONIN AND ZOLPIDEM TARTARATE IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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#### ABSTRACT

A new method was established for simultaneous estimation of Melatonin and Zolpidem Tartarate and by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Melatonin and Zolpidem Tartarate by using Hypersil BDS C18 column (250×4.6mm) 5.0µm, flow rate was 1.0ml/min, mobile phase ratio was (50:50 v/v) Potassium dihydrogen o-phosphate Buffer: Acetonitrile pH 4.3 (pH was adjusted with Ortho-phosphoric acid), detection wavelength was 235nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2690, photo diode array detector 996, Empower-software version-2. The retention times of Melatonin 2.60 mins and Zolpidem were found to be 3.31 mins. The % purity of Melatonin and Zolpidem Tartarate and was found to be 99.66% and 99.94% respectively. The system suitability parameters for Melatonin and Zolpidem Tartarate such as theoretical plates and tailing factor were found to be 3817 and 1.3, 4492, 1.1 and the resolution was found to be 6.0. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study Melatonin and Zolpidem Tartarate was found in concentration range of 12µg-72µg and 20µg-120µg and correlation coefficient ( $r^2$ ) was found to be 0.999 and 0.999, % recovery was found to be 95.0% and 105.0%, %RSD for repeatability was 0.095 and 0.087, % RSD for intermediate precision was 0.095 and 0.087 respectively. The precision study was precise, robust, and repeatable. LOD value was 0.7729 and 1.947, and LOQ value was 2.3422 and 5.9026 respectively. Hence the suggested RP-HPLC.

**KEYWORDS:** Hypersil BDS C18 column, Melatonin, Zolpidem Tartarate, RP-HPLC

#### INTRODUCTION

Melatonin is N-[2-(5-methoxy-1H-indol-3-yl) Ethyl] ethanamide, Antioxidant, Insomnia treatment, mild diuretic and soluble in water and ethanol and Melting point 117 °C. Melatonin<sup>1,2</sup> is a derivative of tryptophan. It binds to melatonin receptor type IA, which then acts on adenylate cyclase and the inhibition of a cAMP signal transduction pathway. Melatonin not only inhibits adenylate cyclase, but it also activates phospholipase C. This potentiates the release of arachidonate. By binding to melatonin receptors 1 and 2, the downstream signaling cascades have various effects in the body. The melatonin receptors<sup>3</sup> are G protein-coupled receptors and are expressed in various tissues of the body.

Zolpidem Tartarate N,N,6-Trimethyl-2-(4-methylphenyl)-imidazo[1,2-a]pyridine-3-acetamide tartarate and is used GABA Agonists, Hypnotics and Sedatives and very slightly soluble in water, soluble in methanol, acetone and sparingly soluble in ethanol 95%. Zolpidem<sup>4</sup> binds with high affinity and acts as a full agonist at the  $\alpha_1$  containing GABA<sub>A</sub> receptors, about 10-fold lower affinity for those containing the  $\alpha_2$  - and  $\alpha_3$  - GABA<sub>A</sub> receptor subunits, and with no appreciable affinity for  $\alpha_5$  subunit containing receptors.  $\omega_1$  type GABA<sub>A</sub> receptors are the  $\alpha_1$  containing GABA<sub>A</sub> receptors and  $\omega_2$  GABA<sub>A</sub> receptors are the  $\alpha_2$ ,  $\alpha_3$ ,  $\alpha_4$ ,  $\alpha_5$  and  $\alpha_6$  containing GABA<sub>A</sub> receptors.  $\omega_1$  GABA<sub>A</sub> receptors<sup>5</sup> are primarily found in the brain whereas  $\omega_2$  receptors<sup>6</sup> are primarily found in the spine.

#### MATERIALS AND METHODS

##### Instruments used

WATERS HPLC Auto Sampler, Separation module 2690, photodiode array detector 996, Empower-software version-2, pump (LC-10AT) and (LC-10ATVP), EV-100 UV-Visible spectrophotometer. Electronic balance and Ultrasonicator, Hypersil pack BDS c18 RP column, 250mm\*5mm. PH analyzer (ELICO). HPLC injecting syringe (25µg) HAMILTON.

##### Chemicals and reagents

Melatonin and Zolpidem Tartrate were supplied from Mylan Laboratories, Hyderabad and Potassium dihydrogen o-phosphate and Acetonitrile (MOLY CHEM, HPLC GRADE), Double distilled water and o-phosphoric acid (MERCK) were employed in the present work.

##### SELECTION OF WAVELENGTH

UV scan of the Melatonin and Zolpidem tartrate was done individually and both were overlaid upon each other to get the required wavelength. The wavelength of 235nm was found to be effective in determination of both the drugs at a time.

## OPTIMIZED METHOD

Buffer preparation: weigh accurately 1.36g of potassium dihydrogen O-phosphate and dissolve it in 1000ml of Milli-Q water. Adjust the pH to 4.3 with orthophosphoric acid, filter through 0.45µm nylon membrane filter and degas.

Mobile phase: Buffer and Acetonitrile were mixed in the ratio of 50:50 and sonicated to degas.

### Chromatographic conditions

Flow rate: 1.0 ml/min

Column: Hypersil BDS C-18, 250 x 4.6 mm, 5µ

Detector wave length: 235nm

Column temperature: Ambient

Injection volume: 10µl

Run time: 7 mins

Diluent: Buffer: Acetonitrile (50:50)

### Isocratic programme

Name of the peak	Retention time(min)
Melatonin	2.60
Zolpidem Tartrate	3.31

### Standard stock Preparation

Weigh and transfer accurately about 15.0 mg of Melatonin and 25mg of Zolpidem tartarate Working Standard into a 25 ml clean dry volumetric flask, add about 15 ml of the mobile phase, sonicate for 5 minutes, and dilute to volume with mobile phase.

### Diluted Standard

Pipette out 1ml from the standard stock solution, into a 25 ml clean dry volumetric flask, and dilute to the mark with 25 ml of diluent.

### Sample preparation

Weigh and powder about ten tablets in a neat clean and dry mortar and pestle. weigh and transfer accurately about 0.9211gm of the tablet powder into the 25ml clean dry volumetric flask, add about 15ml of the mobile phase, sonicate for 5 minutes, and dilute to volume with mobile phase. Filter the solution through the What Mann filter paper, from the filtrate pipette out 1ml of the sample solution into a 25ml volumetric flask, make up the volume of diluent (mobile phase).

## RESULTS AND DISCUSSION

### SYSTEM SUITABILITY

A Standard solution was prepared by using melatonin and zolpidem tartarate working standards as per test method and was injected five times into the HPLC system. The system suitability parameters were evaluated from standard chromatograms by calculating the % RSD from five replicate injections for melatonin and zolpidem tartarate retention times and peak areas. The % RSD for the retention times of principal peak from 5 replicate injections of each Standard solution should be not more than 2.0 % The number of theoretical plates (N) for the Melatonin and Zolpidem tartarate peaks is NLT

2000. The Tailing factor (T) for the Melatonin and Zolpidem tartarate peaks is NMT 1.5.

### PRECISION

Method precision: prepare five replicate injection of the standard solution of the same concentration and inject five times one after the other.

### ACCURACY (RECOVERY)

A study of accuracy was conducted by preparing three different concentrations of the working standards of melatonin and zolpidem tartarate i.e. 80%, 100% and 120%. inject them into the HPLC and the obtained parameters are considered to be standard. Later inject each concentration three times and compare the parameters with that of the standard. The average % recovery of melatonin and zolpidem tartarate was calculated. The mean % recovery of the melatonin and Zolpidem tartarate at each level should be not less than 95.0% and not more than 105.0%.

### LINEARITY OF TEST METHOD

Preparation of linearity stock solution: Transfer an accurately weighed quantity of about 15mg of melatonin and 25mg of zolpidem tartarate into a 25ml volumetric flask. Add about 15ml of the diluent and sonicate to dissolve. Make the volume up to the mark with the diluent. From the stock serial dilutions were made by taking 0.2, 0.4, 0.6, 0.8, 1.0ml and 1.2ml into the 10ml volumetric flask and diluted with the diluent up to the mark. Inject these solutions into the HPLC system and record the area of analyte peaks. Plot a graph of concentration (in x-axis) vs. analyte peak area (in y-axis). evaluate the correlation coefficient between concentration and peak area on y-intercept of the correlation plot. Correlation Coefficient should be not less than 0.9990.

% of y-Intercept should be  $\pm 2.0$ . % of RSD for level 1 and Level 6 should be not more than 2.0%.

### RUGGEDNESS OF TEST METHOD

Analyst to Analyst variable:

System to system /Analyst to Analyst/column to Column variability study was conducted on different HPLC systems, different columns and different analysts under similar conditions at different times. Six samples were prepared and each was analysed as per test method. The relative standard deviation for Melatonin and Zolpidem tartarate were found to be below 2 % on the columns, systems and Analysts. Comparison of both the results obtained on two different HPLC systems, different column and different analysts shows that the assay test method is rugged for System to system /Analyst to Analyst/column to Column variability.

### ROBUSTNESS

#### 1. Effect of variation of flow rate

A study was conducted to determine the effect of variation in flow rate. Standard solution prepared as per the test method was injected into the HPLC system using flow rates, 0.9ml/min and 1.1ml/min. The system suitability parameters were evaluated and found to be within the limits for 0.9ml/min and 1.1ml/min flow.

Melatonin and Zolpidem tartarate were resolved from all other peaks the retention times were comparable with those obtained for mobile phase having flow rates 1.0ml/min.

From the above study it was established that the allowable variation in flow rates is 0.9ml/min and 1.1ml/min.

## 2. Effect of variation of wavelength

A study was conducted to determine the effect of variation in wavelength. Standard and sample solutions were prepared as per the test method and injected into the HPLC system using wavelength 232nm and 237nm. The system suitability parameters were evaluated and found to be with in the limits for wavelength 232nm and 237nm.

Melatonin and Zolpidem tartarate were resolved from all other peaks and the retention times were comparable with those obtained for mobile phase having wavelength. From the above study it was established that the allowable variation in wavelength 232nm and 237nm.

## LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ)

From the linearity data. Calculate the limit of detection and quantification, using the following formula

$$LOD = 3.3\sigma/S$$

$$\text{Melatonin} = 3.3 * 7.5162 / 32.09 = 0.7729342 \mu\text{g/mL}$$

$$\text{Zolpidem tartarate} = 3.3 * 26.52995 / 44.946 = 1.9478671 \mu\text{g/mL}$$

Where,

$\sigma$  = standard deviation of the response.

S = slope of the calibration curve of the analyte.

$$LOQ = 10 \sigma/S$$

$$\text{Melatonin} = 10 * 7.5162 / 32.09 = 2.3422249 \mu\text{g/mL}$$

$$\text{Zolpidem tartarate} = 10 * 26.52995 / 44.946 = 5.90262759 \mu\text{g/mL}$$

## ASSAY OF TABLET DOSAGE FORM

Applicability of the proposed method of the simultaneous estimation of Melatonin and Zolpidem tartarate was studied by assay of commercial tablets Zolsoma label to contain Melatonin 3 mg and Zolpidem tartarate 5 mg. The results indicate that the amount of each drug in the tablets is with in the requirements of 98-102% of the label claim

## SPECIFICITY

## INTERFERENCE FROM DEGRADATION PRODUCTS

A study was conducted to demonstrate the effective separation of degradants from Melatonin and Zolpidem tartarate .Separate portions of Drug product exposed to following stress conditions to induce degradation.

- Acid degradation
- Base degradation
- Peroxide degradation
- Thermal degradation

Stressed samples were injected into the HPLC system with photo UV- detector by following test method conditions. All degradant peaks were resolved from Melatonin and Zolpidem tartarate peaks in the chromatograms of all samples and did not shown any considerable peaks under the above conditions. The chromatograms of stressed samples were evaluated for peak purity of Melatonin and Zolpidem tartarate using Spinchrom software. For all forced degradation samples the degradants should not interference in quantitating the Melatonin and Zolpidem tartarate.

TABLE 1 SYSTEM SUITABILITY FOR MELATONIN

Injection	Retention time	Peak Area	USP Plate count	USP Tailing
1	2.607	2053.695	3764	1.3
2	2.607	2032.791	4028	1.3
3	2.603	2041.007	3755	1.4
4	2.607	2043.002	3764	1.3
5	2.610	2044.85	3774	1.3
<b>Mean</b>	2.6068	2043.069	3817	1.32
<b>SD</b>	0.00249	7.516278	118.1439	---
<b>%RSD</b>	0.095519	0.367892	---	---

TABLE 2 SYSTEM SUITABILITY FOR ZOLPIDEM TARTARATE

Injection	Retention time	Peak Area	USP Plate count	USP Tailing
1	3.303	4792.44	4441	1.3
2	3.303	4721.838	4441	1.2
3	3.3	4745.158	4697	1.3
4	3.3	4770.05	4432	1.3
5	3.307	4761.404	4450	1.3
<b>Mean</b>	3.3026	4758.178	4492.2	1.28
<b>SD</b>	0.002881	26.52995	114.6634	---
<b>% RSD</b>	0.087233	0.557565	---	---

TABLE 3 PRECISION DATA FOR MELATONIN

Concentration 100%	Injection	Retention time	Peak Areas
	1	2.607	2056.182
	2	2.607	2047.687
	3	2.603	2046.484
	4	2.607	2043.00
5	2.610	2044.85	
Statistical Analysis	Mean	2.6068	2043.069
	SD	0.00249	7.516278
	% RSD	0.095519	0.367892

TABLE 4: PRECISION DATA FOR ZOLPIDEM TARTARATE

Concentration 100%	Injection	Retention time	Peak Areas
	1	3.303	4784.662
	2	3.303	4776.91
	3	3.300	4777.279
	4	3.300	4773.459
5	3.307	4775.943	
Statistical Analysis	Mean	3.3026	4777.651
	SD	0.002881	4.1932
	% RSD	0.087233	0.087767

TABLE 5 ACCURACY (RECOVERY) DATA FOR MELATONIN

Concentration of Melatonin	Peak Area	Amount found	%recovery		
<b>standard 60mcg</b>	2143.997				
54mcg injection 1	1890.612	52.9092	97.9801		
54mcg injection 2	1898.698	53.2354	98.3988	<b>SD</b>	4.2697
54mcg injection 3	1892.277	52.9545	98.0663	<b>%RSD</b>	0.23
<b>Average</b>	1927.196	53.0330	<b>99.87554</b>		
66mcg injection 1	2348.157	65.7132	99.5652		
66mcg injection 2	2341.570	65.5297	99.2864	<b>SD</b>	9.1723
66mcg injection 3	2330.036	65.2062	98.7970	<b>%RSD</b>	0.39
<b>Average</b>	2356.588	65.94937	<b>99.2160</b>		
78mcg injection 1	2773.142	77.6064	99.4951		
78mcg injection 2	2749.729	76.9512	98.6556	<b>SD</b>	12.510
78mcg injection 3	2769.076	77.4930	99.3501	<b>%RSD</b>	0.45
<b>Average</b>	2763.982	77.27573	<b>99.07145</b>		

TABLE 6: ACCURACY (RECOVERY) DATA FOR ZOLPIDEM TARTARATE

Concentration of Zolpidem tartarate	Peak area	Amount found	%recovery		
<b>standard 100mcg</b>	5067.553				
90mcg injection 1	4526.282	89.3190	99.243		
90mcg injection2	4561.378	90.0112	100.012	<b>SD</b>	34.599
90mcg injection 3	4492.181	88.6464	98.496	<b>%RSD</b>	0.76
<b>Average</b>	4526.614	89.32543	<b>99.25048</b>		
110mcg injection 1	5528.526	109.0978	99.179	<b>MEAN</b>	
110mcg injection 2	5552.49	109.5692	99.608	<b>SD</b>	30.5906
110mcg injection 3	5491.757	108.3710	98.519	<b>%RSD</b>	0.55
<b>Average</b>	5524.258	109.0123	<b>99.10212</b>		
130mcg injection 1	6528.526	132.777	102.136		
130mcg injection 2	6652.49	131.276	100.982	<b>SD</b>	85.1991
130mcg injection 3	6691.757	131.051	100.808	<b>%RSD</b>	1.29
<b>Average</b>	6624.257	132.034	<b>101.565</b>		

TABLE 7: LINERITY DATA FOR MELATONIN

Concentration Of Melatonin	Average area	Statistical Analysis	
12	517.00	Slope	32.09
24	829.106	y-Intercept	107.93
36	1271.69	Limit of detection	2.3968396
48	1685.658	Limit of quantification	7.2631503
60	2008.96	r <sup>2</sup> (coefficient of determination)	0.9981
72	2421.872		
		Correlation coefficient (r)	0.9994

TABLE 8: LINERITY DATA FOR ZOLPIDEM TARTARATE

Concentration Of Zolpidem	Average area	Statistical Analysis	
20	1012.992	Slope	44.946
40	1881.68	y-Intercept	128.99
60	2816.713	Limit of detection	1.75143
80	3836.228	Limit of quantification	5.30736
100	4682.996	r <sup>2</sup> coefficient of determination	0.998
120	5420.79		
		Correlation coefficient r	0.99919

TABLE 9: RUGGEDNESS DATA FOR MELATONIN AND ZOLPIDEM TARTARATE

S.No		Area of Melatonin	Area of Zolpidem tartarate
1	Analyst 1	2145.100	5068.467
2	Analyst 2	2138.949	5069.079
	% RSD	0.203051	0.0193666

TABLE 10: ROBUSTNESS DATA FOR MELATONIN AND ZOLPIDEM TARTARATE

System suitability parameters	Flow rate ml/min			Acceptance criteria
	0.9	1.0	1.1	
Tailing factor of Melatonin peak	1.2	1.3	1.3	NMT1.5
Tailing factor of Zolpidem tartarate peak	1.2	1.2	1.1	NMT1.5

TABLE 11 ASSAY DATA FOR MELATONIN AND ZOLPIDEM TARTARATE

Drug	Amount		% label claim
	Labeled	Measured	
Melatonin	3 mg	2.98mg	99.66
Zolpidem tartarate	5 mg	4.99mg	99.94

TABLE 12: STABILITY INDICATING DATA

Degradation mechanism / condition	Observation
Protected sample	No interference at Retention time of analyte peak
Acid degradation 0.1 N HCl Reflux – 30.0 min	No interference at Retention time of analyte peak
Base degradation 0.01 N NaOH Reflux 30.0min	No interference at Retention time of analyte peak
Peroxide degradation 3.0% H <sub>2</sub> O <sub>2</sub> Reflux – 30.0min	No interference at Retention time of analyte peak
Thermal degradation At 105°C - 48 Hrs	No interference at Retention time of analyte peak

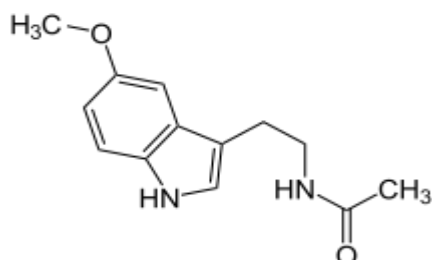


FIGURE 1: MELATONIN

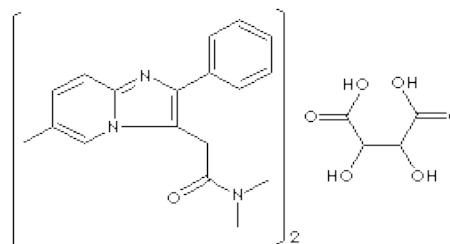


FIGURE 2: ZOLPIDEM TARTARATE

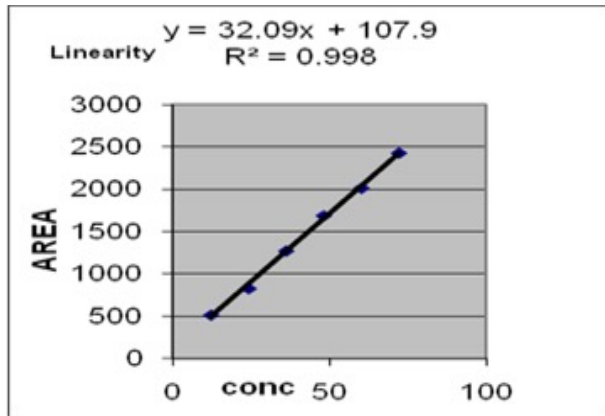


FIGURE 3 LINIRITY FOR MELATONIN

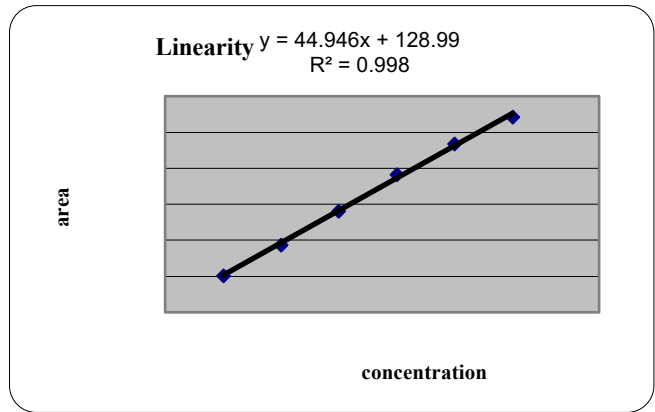


FIGURE 4 LINIRITY FOR ZOLPIDEM TARTARATE

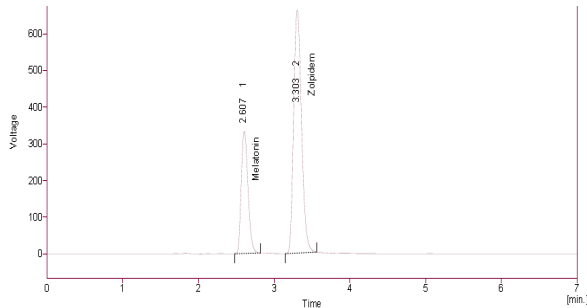


FIGURE 5 CHROMATOGRAM OF SYSTEM SUITABILITY

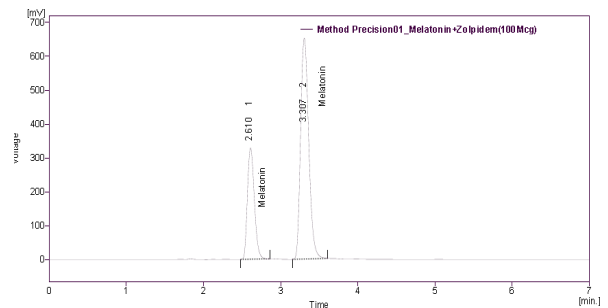


FIGURE 6 CHROMATOGRAM OF PRECISION

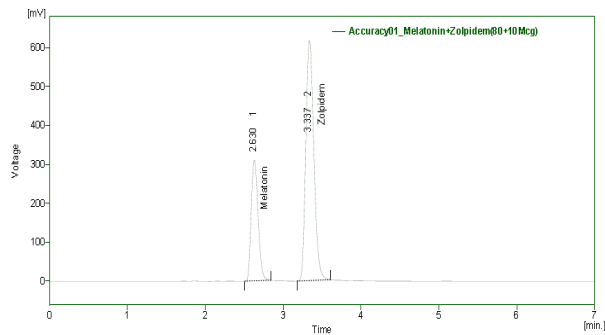


FIGURE 7 CHROMATOGRAM OF ACCURACY

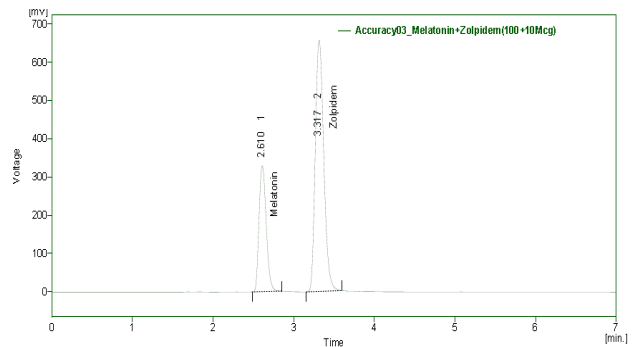


FIGURE 8 CHROMATOGRAM OF ACCURACY

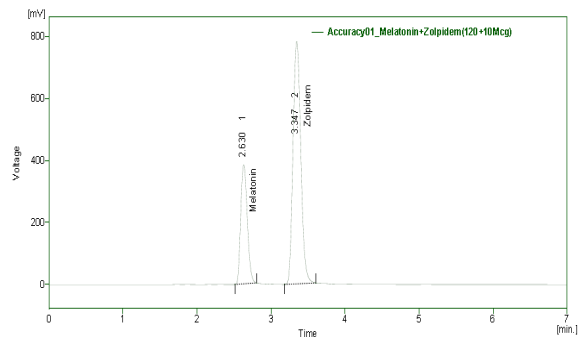


FIGURE 9 CHROMATOGRAM OF ACCURACY

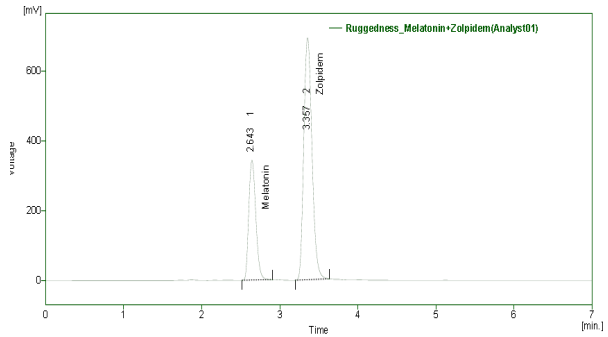


FIGURE 10 CHROMATOGRAM OF RUGGEDNESS

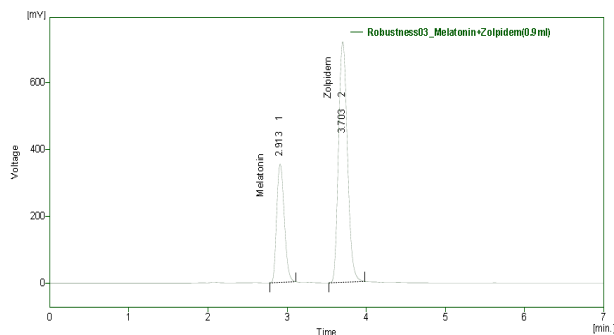


FIGURE 11 CHROMATOGRAM OF ROBUSTNESS

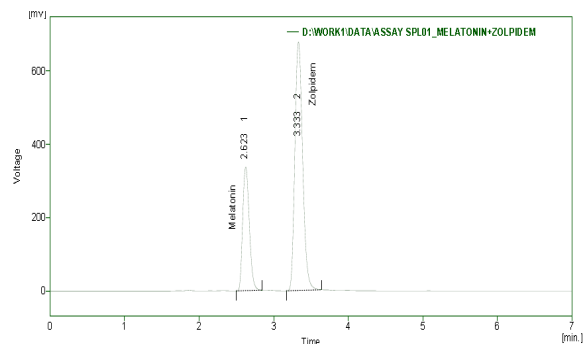


FIGURE 12 CHROMATOGRAM OF ASSAY

**SUMMARY AND CONCLUSION**

Development of new analytical methods for the determination of drugs in pharmaceutical dosage forms is more important in pharmacokinetic, toxicological and biological studies. Today pharmaceutical analysis entails much more than the analysis of active pharmaceutical ingredients or the formulated product. Analytical testing is one of the more interesting ways for scientists to take part in quality process by providing actual data on the identity, content and purity of the drug products.. As a result, new products can be assured to have comparable quality and can be brought to international markets faster.

A simple reverse phase HPLC method was developed for the simultaneous determination of Melatonin and Zolpidem tartarate in pharmaceutical dosage form. A Hypersil BDS RP-c18 (250 × 4.6 mm), 5µ column from Thermo in isocratic mode, with mobile phases pH 4.3 phosphate buffer and acetonitrile was used. The flow rate was 1.0-ml/ min and effluent was monitored at 235 nm. The retention times were 2.60 and 3.31 min for Melatonin and Zolpidem tartarate respectively. As per ICH guide lines the method was validated over the range of 10–1000 µg/mL for the three analytes and precise. The method was completely validated showing satisfactory data for all the method validation parameters tested. Hence this method can be introduced into routine use for determination of Melotonin and Zolpidem Tartarate.

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