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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION AND STABILITY STUDIES OF THE ESTIMATION OF AGOMELATINE IN TABLET DOSAGE FORM BY RP-HPLC

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ABSTRACT

Agomelatine is a new melatonergic antidepressant with a unique pharmacological action. A stability indicating RP-HPLC method was developed and validated for the determination of agomelatine in active pharmaceutical ingredient using enable thermo hypersil C18 column (250×4.6mm, 5µm) in isocratic mode. The mobile phase consisted of phosphate buffer: methanol (60:40, v/v) with a flow rate of 1.0 ml/min (PDA detection- 232nm).The Retention time was found to be 3.3 min. Linearity was observed over the concentration range of 25 µg/ml to 75µg/ml and the correlation coefficient R2value was found to be 0.999. The method is accurate and recovery was found to be in the range of 98.91-99.18%. The limit of detection of agomelatine was found to be 2.8µg/ml and limit of quantitation was found to be 9.4µg/ml. Agomelatine was subjected to stress conditions including acidic, alkaline, oxidation, photolysis and thermal degradation. Agomelatine is more sensitive to heat and oxidative degradation. The method was validated according to ICH guidelines.

Keywords: Agomelatine, HPLC method, Validation, Stability-indicating.

INTRODUCTION

Agomelatine is a new melatonergic antidepressant with a unique pharmacological action. It has potential role in the treatment of patients with major depressive disorder (MDD). Agomelatine is a chemical compound that is structurally closely related to melatonin [1].

Agomelatine has a new pharmacological mechanism of action, which combines melatonin MT1 and MTagonist properties with a serotonin 5-HT2C antagonist effect. Agomelatine was rapidly and well (≥80%) absorbed after oral administration. Because of its action upon the melatonin receptors, agomelatine shows a marked improvement in sleep quality [2-4].

Agomelatine (N-[2-(7-methoxynaphthalen-1-yl) ethyl] acetamide), its antidepressant efficacy has been verified in the treatment of major depressive disorder (MDD).Agomelatine showed significant benefits over paroxetine due to the complete absence of side effects including the associated sexual side effects that are troublesome with some antidepressants [4-5] monument.

Agomelatine has also proven to have anxiolytic properties and thus may prove to be very useful in the treatment of anxiety disorders [6-8].

Literature survey reveals that very few analytical methods reported for the estimation agomalatin in API, pharmaceutical formulations and biological samples. None of the literature review indicates stability studies for agomelatine. Therefore, an attempt has been made to develop a method for stability studies on agomelatine in pharmaceutical dosage form [9-11].

MATERIALS AND METHODS

Instrumentation and analytical conditions

The analysis of the drug was carried out on Waters e2695 with PDA waters 2998 detector with Empower software and hamilton syringe with 20 µl

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capacity. Chromatographic analysis was performed by using enable C18 G column (250×4.6mm, 5µ). Sartorius electronic balance was used for weighing. Isocratic elution was performed by using a mobile phase Phosphate Buffer : methanol (60:40) at a flow rate of 1.0 ml/min. Detection was carried out at 232 nm with a run time of 10 min. The mobile phase was prepared freshly and it was sonicated to degas the solvent for 5 min. The column and HPLC system were maintained at ambient temperature.

Chemicals and Reagents

All the solvents used like methanol and phosphoric acid which are of HPLC grade were purchased from Fisher Scientific Chemicals. The standard drug agomelatine is gifted from LARA Drugs Pvtlmt, Hyderabad.

Preparation of stock, working standard and sample solutions

The standard stock solution was prepared by transferring 25 mg of agomelatine into a 100 ml volumetric flask. To this, few ml of methanol was added and sonicated to dissolve the drug and the volume was made up with methanol. 5 ml of standard stock solution was taken in a 25 ml volumetric flask and the volume was made up with methanol and sonicated.

An accurate quantity of powder equivalent to 25 mg of agomelatine was weighed and transferred to a 100 ml volumetric flask. 100 ml of methanol was added, shaken for 5 min, sonicated for 15 min and filtered through 0.45 µ membrane filter to obtain a clear solution. From the primary stock solution, 5 ml was taken in a 25 ml volumetric flask and diluted with methanol and sonicated. This stock sample solution was diluted quantitatively with methanol to obtain suitable working sample solutions for chromatographic measurements.

RESULTS AND DISCUSSION

Method Development and Optimization

Proper selection of the method depends upon the nature of the sample (ionic/ionisable/neutral molecule), its molecular weight and solubility. The drug selected in the present study is polar in nature. The reversed phase HPLC was selected for the separation because of its simplicity and suitability. The sensitivity of HPLC method which uses PDA detector for the proper selection of wavelength. An ideal wavelength is one that gives good response for all the drugs to be detected. The λmax was obtained at 232 nm. Different mobile phases were tried but satisfactory separation and symmetrical peaks were obtained by using a mobile phase consisting of phosphate buffer : methanol in the ratio 60 : 40. The retention time for agomelatine was found to be 3.3±0.5min.The %RSD values obtained were found to be< 2% which revealed that the developed method was precise.

Method validation

The method was validated for Accuracy, linearity, precision, specificity, limit of detection, limit of quantification and robustness

Accuracy

The closeness of agreement between the true value which is accepted either conventional new value or an accepted reference value and the value found. The above prepared solutions of , Accuracy -50%, Accuracy -100% and Accuracy -150% solutions were injected. The Amount found and Amount added for Agomelatine individual recovery and mean recovery values were calculated.

Linearity

The linearity of this method was evaluated by Linear Regression Analysis, which was calculated by Least Square method and The calibration curve for agomelatine [Fig 1] was linear over the concentration range of 25-75µg/ml. The correlation coefficient was found to be 0.999.

Precision

The precision of an analytical method is a measure of the random error and is defined as the agreement between replicate measurements of the same sample. It is expressed as the percentage coefficient of variation (%CV) or relative standard deviation (RSD) of the replicate measurements.

$$\% \text{ RSD} = \frac{\text{Standard deviation}}{\text{Mean}} \times 100$$

Sensitivity

The Sensitivity of measurement of agomelatine by use of the proposed method was estimated in terms of the Limit of Detection (LOD) and the Limit of Quantitation (LOQ).

Limit of detection (LOD) and Limit of quantification (LOQ) were estimated from the signal-to-noise ratio. The detection limit was defined as the lowest concentration level resulting in a peak height of three times the baseline noise. The quantification limit was defined as the lowest concentration level that provided a peak height with a signal -to noise ratio higher than 10. The LOD and LOQ values for agomelatine were reported in the Table 3.

Robustness

The Robustness of the method was determined under different conditions including change in flow rate, temperature. The chromatograms were recorded and the results of the chromatograms are given in Table 4.

Force Degradation Studies

Stress testing of the drug substance can help identify the likely degradation products, which can in turn help establish the degradation pathways and the intrinsic stability of the molecule and/or validate the stability indicating power of the analytical procedures used.

Forced degradation is a powerful tool used routinely in pharmaceutical development in order to develop stability indicating methods that lead to quality stability data and to understand the degradation pathways of the drug substances and drug products. Forced degradation studies are indispensable in the development of stability-indicating and degradants monitoring methods as part of a validation protocol.In general, values anywhere between 5% to 20% degradation of the drug substance have been considered as reasonable and acceptable for validation of chromatographic assays.

In order to establish whether the developed method is stability indicating both the drugs were stressed under various conditions (acid, base, oxidation and thermal) to perform forced degradation studies. Agomelatine is more sensitive to acidic and oxidative degradation. The peaks of degraded products were well separated from the analyte peak with good resolution which indicates that the developed method is stability indicating. The forced degradation studies conditions and results summarized in Table 6 and 7.

Table 1. Method accuracy from recovery assays

Spiked Level	Sample Area	µg/ml added	µg/ml found	% Recovery	% Mean
50%	1532382	24.775	24.76	99.95	99.67
	1522694	24.775	24.61	99.32	
	1530961	24.775	24.74	99.86	
	1528252	24.775	24.70	99.68	
	1526691	24.775	24.67	99.58	
	1527132	24.775	24.68	99.61	
100%	3069777	49.550	49.61	100.12	99.9
	3066656	49.550	49.56	100.01	
	3053059	49.550	49.34	99.57	
150%	4597421	74.325	74.29	99.96	99.89
	4598108	74.325	74.30	99.97	
	4590280	74.325	74.18	99.80	
	4592383	74.325	74.21	99.85	
	4590676	74.325	74.18	99.81	
	4595224	74.325	74.26	99.91	

Table 2. Represents the regression data including, linearity range, slope, correlation coefficient

S.No	Parameter	Values
1.	Linearity Range	25-75µg/ml
2.	Correlation coefficient(r^2)	0.999

Table 3. Precision of agomelatine

S.No	Sample Area-1	% Assay
1	3067665	99.15
2	3061482	98.95
3	3060365	98.91
4	3061218	98.94
5	3067460	99.14
6	3068851	99.18
Average Assay:		99.04
STD		0.12
%RSD		0.13

Table 4. LOD and LOQ results for agomelatine

Drug	LOD ((µg/mL))	LOQ ((µg/mL)
Agomelatine	2.830	9.434

Table 5. Robustness values of agomelatine

S.No	Sample Name	Inj	Rt	Area	USP Tailing	USP Plate count	s/n
1	Flow 1	1	4.178	1833563	1.601	7053	130.205
2	Flow 2	1	3.343	1437873	1.538	6698	136.587
3	Temp 1	1	3.323	1427503	1.591	6678	141.708
4	Temp 2	1	2.805	1197149	1.569	6280	122.069
Mean				1474022			
%RSD				17.9			

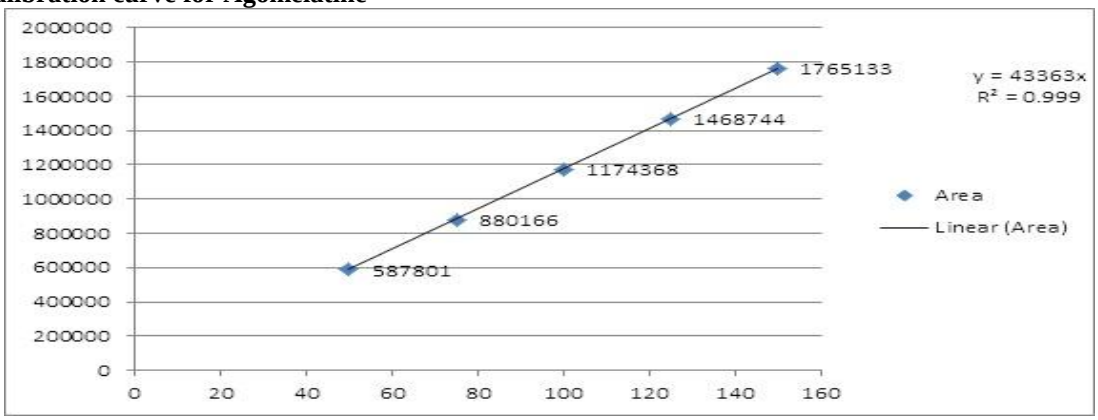
Table 6. Forced degradation conditions and parameters

Parameter	Condition	Time points
Acid/Solution	HCL (1.0N,RT,70C)	Initial-7days
Base/Solution	NAOH(1.0N,RT,70C)	Initial-7days
Oxidative/Solution	H2O2+INITIATOR	7-days
Thermal/Humidity	70C/75% RH	6weeks
Photo(UV light)	1,000 watt hrs/m2,RT	5 x ICH
Photo(Fluorescent light)	6 x 106 lux hrs, RT.	5 x ICH

Table 7. Results for degradation studies

Degradation mechanism	Degradation condition	Area	%Degradation
Bulk drug	Undegraded	3067665	0%
Acid degradation	1N HCL	2807863	8%
Base degradation	1N NaOH	2886757	6%
Peroxide degradation	30%H2O2/	2759459	10%
Thermal degradation	60C	2638329	3%
Photo degradation	6 x 106 lux hrs	2966757	14%

Figure 1. Calibration curve for Agomelatine



CONCLUSION

A new precise, accurate, robust, stability indicating RP-HPLC method has been developed for the estimation of agomelatine in active pharmaceutical ingridient.The intra-run and inter-run variability and accuracy results were found in acceptable limit. Simplicity of the method, economical nature and low limit of detection and quantitation makes the method superior to the other reported HPLC methods. The developed method was applied for the stability studies of agomelatine in bulk dosage form. The results offorced Degradation studies reveal that the method is stability indicating. The proposed method has the capability to separate the analyte from their degradation products obtained during forced degradation studies. The method can be employed for the routine analysis agomelatine.

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