

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF AGOMELATINE IN BULK AND TABLET DOSAGE FORM.

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ABSTRACT

A simple precise, accurate, sensitive and efficient reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for estimation of Agomelatine in its Tablet dosage form. Separation was done by using mobile phase consists of mixture of Acetonitrile and buffer in the ratios of 45:55% v/v. Chromatography separations were carried out on Athena C18 column (250X4.6mm; 5 μ m) at a flow rate of 1 mL/min and UV detection at 230 nm and the

retention time for Agomelatine is 2.76 min. The linear dynamic response was found to be in the concentration of 10-40 μ g/mL. The percentage recovery of was found to be 98.89%-100.50%. Proposed method was found to be simple, accurate, precise and rapid. The statistical parameters and recovery studies were carried out and reported. The results obtained were satisfactory and good in agreement as per the ICH guidelines.

KEYWORDS: Agomelatine, RP-HPLC, Validation, melatonin agonist.

INTRODUCTION

Agomelatine is structurally closely related to melatonin. It is a potent agonist at melatonin receptors and an antagonist at serotonin-2C (5-HT_{2c}) receptors, tested in an animal model of depression. Chemically it is N-[2-(7-methoxynaphthalen-1-yl) ethyl] acetamide. Agomelatine is a melatonergic agonist (MT₁ and MT₂ receptors) and 5-HT_{2c} antagonist. Binding studies indicate that agomelatine has no effect on monoamine uptake and no affinity for α , β

adrenergic, histaminergic, cholinergic, dopaminergic and benzodiazepine receptors. Agomelatine synchronises circadian rhythms in animal models of circadian rhythm disruption. Agomelatine increases noradrenaline and dopamine release specifically in the frontal cortex and has no influence on the extracellular levels of serotonin.^[1-3]

Common adverse effects of Agomelatine include diarrhea, headache and common cold symptoms. Additional adverse effects include blurred vision, eczema, agitation, restlessness. The recommended dose is 25 mg once daily taken orally at bedtime.^[4-10] After two weeks of treatment, if there is no improvement of symptoms, the dose may be increased to 50 mg once daily, i.e. two 25 mg tablets, taken together at bedtime. The literature survey reveals that few methods reported for the estimation of agomelatine and the previous methods are not that much of precise and sensitive.^[11-16] Therefore, present study is to develop a new, simple, efficient, reproducible RP-HPLC method for the analysis of Agomelatine in its bulk and formulation. To validate the developed method for estimation of drug in formulation using RP-HPLC method.

MATERIAL AND METHODS

Reagents and chemicals

Acetonitrile purchased from Merck, Mumbai. Ortho phosphoric acid purchased from Rankem, New Delhi. Ortho phosphoric acid AR grade purchased from Sdfine chemicals, Mumbai. Potassium dihydrogen phosphate for Chromatography purchased from Merck, Mumbai.

Chromatographic conditions

To optimize the developed HPLC method mixed phosphate buffer and Acetonitrile was used. Other parameter of chromatogram condition mentation in tabale-6.

Preparation of solutions

Preparation of standard stock solution

Agomelatine was accurately weighed 25mg and transferred to 25mL volumetric flask. It was dissolved properly and diluted up to the mark with diluent to obtain concentration of 1000 µg/mL.

Preparation of standard solution

The working standard solution of Agomelatine was prepared by taking 0.1mL of the above and diluted to 10 mL in volumetric flask to get concentration of 10µg/mL.

Preparation of buffer

136.09gms of 0.02M KH_2PO_4 and 174.18gms of 0.003M K_2HPO_4 were transferred into 1000ml of volumetric flask, dissolved and makeup to the mark with water and p^{H} was adjusted to 6 with 0.1% OPA.

Preparation of sample stock solution

Tablet powder equivalent to 25 mg of Agomelatine is weighed and transferred to 25ml Vol flask. Small amount of mobile phase is added to dissolve and the volume is made up to the mark. Then it is filtered with 0.45 μ filter paper and sonicated.

Preparation of sample solution

From the sample stock solution 0.1ml is transferred to the 10ml volumetric flask and volume is made up to 10ml to get 10 $\mu\text{g}/\text{ml}$ concentration.

METHOD VALIDATION

The developed analytical method was further subjected to validation in accordance to the ICH guidelines. The parameters evaluated were linearity, sensitivity, system suitability, precision, accuracy, robustness and stability. Co efficient of variation and relative errors <2% were considered acceptable.

Linearity

In order to check the linearity for the developed method, solutions of six different concentrations ranging from 25-150 $\mu\text{g}/\text{mL}$.

Sensitivity

The sensitivity of the proposed method estimated by Limit of Detection (LOD): The parameter LOD was determined by using formula i.e. $\text{LOD} = 3.3(\text{SD}/\text{S})$ and Limit of Quantification (LOQ): The parameter LOQ was determined by formula i.e. $\text{LOQ} = 10(\text{SD}/\text{S})$.

System suitability

The system suitability test is an integral part of chromatographic analysis .It is used to verify that the resolution and reproducibility of the system are adequate for the analysis. A system suitability test according to the United States Pharmacopeia Convention was performed on chromatograms obtained for standard and test solutions to check differences in the above mentioned parameters.

Precision

Precision was measured by the analysis of sample solutions of 6 replicates, to check the intraday and interday variations of the method.

Accuracy

The accuracy of the method was determined by the analysis of standard addition at three levels, that is, multiple-level recovery studies. The reference standard, at three different concentrations (50, 100, and 150%), was added to a fixed amount of the pre-analyzed sample and the amounts of the drug were analyzed by the proposed method.

Robustness

The robustness of the method was determined by making slight changes in the chromatographic conditions like flow rate (± 0.1), temperature (± 5), and organic phase of the mobile phase ($\pm 10\%$). It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed was robust.

RESULTS AND DISCUSSION**Calibration curve**

The coefficient correlation r , slope and intercept were 0.998, 23.55 and 22997 for AGOMELATINE with UV detection with absorbance maxima at 230 nm. The retention times were 2.76 min for AGOMELATINE. Linear regression of data from the calibration curve indicated a linear response over the concentration range of drug. The curve can be therefore be used for determination of Agomelatine.

Validation***Linearity***

The coefficient of determination (r^2) for Agomelatine was 0.998 (Table 1 and Figs. 1).

Sensitivity

The limit of detection and the limit of quantification were determined from the calibration curve, according to the formula $3.3 \times SD/slope$ and $10 \times SD/slope$, respectively. The LOD for this method was found to be $1.310 \mu\text{g/mL}$ and LOQ for this method was found to be $3.9 \mu\text{g/mL}$.

System suitability

System suitability was evaluated by six replicate injections of 100µg/mL standard solutions of Agomelatine. The parameters, such as tailing factor, per cent relative standard deviation (RSD) and theoretical plates were studied and found satisfactory (Table 2).

Precision

The relative standard deviation was found to be < 2.0% for Agomelatine indicating satisfactory precision Shown in Tables-3. The inter mediate precision of the expected results is expressed as a percentage.

Accuracy

Accuracy is the closeness of the test results to that of the true value, which can be determined in terms of percent recovery. To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to preanalyzed sample solution at three different levels 50, 100 and 150%. The results of recovery studies was tabulated and found satisfactory (Tables4).

Robustness

Robustness determines the reproducibility of the test result with small and deliberate variations in the method parameters. The experiment was carried out by slightly changing the chromatographic conditions like flow rate (± 0.1), temperature (± 5), and organic phase of the mobile phase ($\pm 10\%$). The retention times of the analytes did not change significantly when the flow rate, mobile phase ratio and temperature were changed. The statistical data (table-5) gives no significant variations in the above parameters indicating that the method is robust.

Table 1: Linearity range of Agomelatine.

S.no.	Concentration (µg/mL)	Area of the peak	Statistical analysis
1	5	3112.082	Slope=23.55 Correlation coefficient=0.998
2	10	3230.123	
3	15	3363.265	
4	20	3479.253	
5	25	3590.215	
6	30	3697.255	

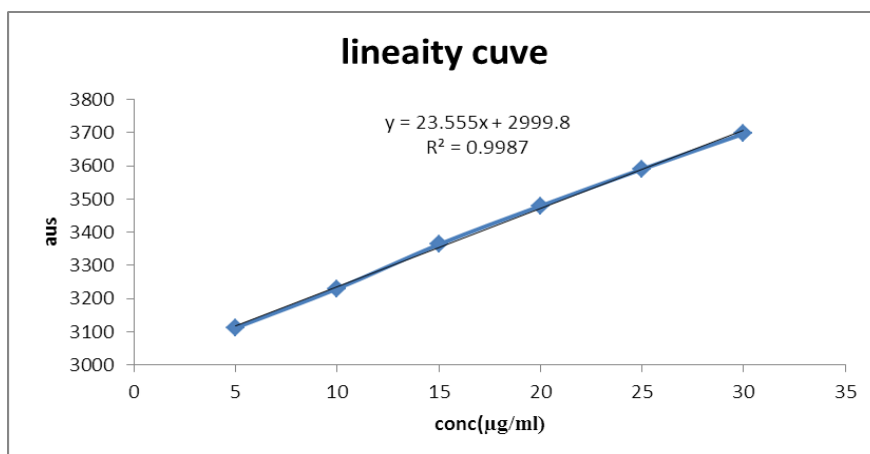


Figure 1: Linearity curve.

Table 2: System suitability data of the proposed method.

System suitability parameters	Results
Retention time	2.76
Area	3156.483
Theoretical plate number	3254
Tailing factor	1.17

Acceptance criteria: The %RSD should not be more than 2 %

Table 3: Method Precision.

S.No.	RT	Area
1	2.731	3105.075
2	2.750	3163.570
3	2.738	3164.751
4	2.731	3195.872
5	2.73	3267.703
6	2.741	3191.619
Avg	2.736	3102.419
Stdev	0.0078	53.2622
%RSD	0.04	0.12

Table 4: Accuracy data of the proposed method.

Spike level	Mixture of pure and formulation	Con. of pure drug, µg/ml	Conc. Of Formulation, µg/ml	% Recovery
50 %	50+50	50	49.44	98.89
100 %	50+100	100	100.5	100.5
150 %	50+150	150	150.74	100.49

Table 5: Robustness data of the proposed method.

S.no.	Variation	Chromatographic parameters	
		retention time (min)	Peak area
1.	a)Buffer 1%	3.417	3858.344
	b)Buffer 2%	2.300	2138.487
2.	a)Flow rate at 0.8mL/min	2.757	3691.640
	b)flow rate at 1.2 mL/min	2.747	2725.176

Table 6: Optimized chromatographic conditions of the proposed method.

S. No.	Parameter	Value
1.	Mobile phase	Acetonitrile: buffer (45:55v/v)
2.	Diluent	Mobile phase
3.	Stationary phase	Phenomenex Luna C ₁₈ , 250 X 4.6 mm 5µm
4.	Flow rate	1 mL/min
5.	Column temperature	Ambient
6.	Volume of injection	20µL
7.	Detection wavelength (λ_{\max})	230nm
8.	Run time	10 min
9.	Retention time	2.76 min

CONCLUSIONS

The proposed method reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for estimation of Agomelatine in its Tablet dosage form. Separation was done by using mobile phase consists of mixture of Acetonitrile and buffer in the ratios of 45:55% v/v. The percentage recovery of was found to be 98.89%-100.50%. Proposed method was found to be simple, accurate, precise and rapid. The statistical parameters and recovery studies were carried out and reported. The results obtained were satisfactory and good in agreement as per the ICH guidelines.

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