

**DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR  
THE ESTIMATION OF AGOMELATINE IN BULK AND TABLET  
DOSAGE FORM****A. Srinivas<sup>1</sup>\* G. Sai<sup>2</sup> and K. Sridhar Reddy<sup>2</sup>**

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**ABSTRACT**

Development an accurate, simple, precise and rapid method for the estimation of Agomelatine in bulk and Tablet dosage form. The method uses Reverse phase High performance Liquid Chromatography (RP-HPLC). PHENOMENEX Luna C<sub>18</sub>, (5µm, 250 x 4.6mm) column operated with a mixture of mixed phosphate buffer of pH 6 with ortho phosphoric acid and Acetonitrile(55:45) as mobile phase was found to be suitable for the estimation. The flow rate was maintained at 1ml/min. Detection was carried out at 230nm using a UV detector. The total run time was less than 10min the retention time of 2.7min for Agomelatine. Validation of the method was performed for precision, accuracy, linearity, ruggedness, specificity and sensitivity to confirm to

the ICH guidelines for validation of an analytical method.

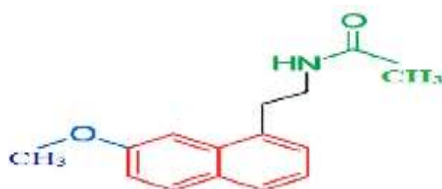
**KEYWORDS:** Agomelatine, RP-HPLC, Method development, Validation.

**INTRODUCTION**

Agomelatine is chemically N-[2-(7-methoxy naphthalen-1-yl) ethyl] acetamide (Fig.1). Its molecular formula is C<sub>15</sub> H<sub>17</sub> NO<sub>2</sub> and its molecular weight is 243.301gm/mol. Agomelatine, a sleep modulating antidepressant was approved by the European Medicines Agency for the treatment of major depressive disorder (MDD) in 2009.<sup>[1]</sup> The novel antidepressant agomelatine acts as a melatonergic receptor (MT<sub>1</sub> /MT<sub>2</sub>) agonist and serotonergic receptor (5-HT<sub>2C</sub>) antagonist. Binding studies indicate that it has no effect on

monoamine uptake and no affinity for  $\alpha$ ,  $\beta$  adrenergic, histaminergic, cholinergic, dopaminergic, and benzodiazepine receptors.<sup>[2-3]</sup> Agomelatine showed significant benefits over paroxetine due to the complete absence of side effects including the associated sexual effect that are troublesome with some antidepressant. Because of its action upon the melatonin receptors, agomelatine shows a marked improvement on sleep. Agomelatine has also proven to have anxiolytic properties and thus may prove to be very useful in the treatment of anxiety disorders.<sup>[4-5]</sup>

Methods reported in literature for analysis of Agomelatine include determination of agomelatine by RP-HPLC<sup>[6]</sup> and HPLC method for separating and analyzing agomelatine intermediate and final product thereof<sup>[7]</sup> and also Validated LC-MS/MS method for quantification of agomelatine in human plasma and its application in a pharmacokinetic study.<sup>[8]</sup>



## MATERIALS AND METHODS

### Materials

HPLC grade acetonitrile (Qualigens), HPLC grade Potassium dihydrogen ortho phosphate and Di potassium hydrogen phosphate were used. Deionised HPLC grade water was used to prepare mobile phase and diluents solutions. Agomelatine was purchased from servier laborateries India. Dosage form was purchased from local commercial sources.

### Equipments

High performance liquid chromatography SHIMADZULC 20AT reciprocating dual pump, UV-Visible Detector (SPD-20A), a reversed-phase PHENOMENEX Luna C<sub>18</sub>, 5 $\mu$ m, 250 x 4.6mm (size) used for separation. Chromatographic data was acquired using Spin Chrome software & Hamilton injector used.

### Preparation of Potassium dihydrogen orthophosphate buffer pH 6

Approximately weighed 3.16g of crystalline buffer and dissolve in 500 ml of distilled water to get 20mM buffer strength. Then pH of the buffer was adjusted to 6 using ortho phosphoric acid.

**Preparation of Mobile Phase**

The two components of mobile phase; Acetonitrile (HPLC grade) and Buffer (prepared previously) were separately filtered through a 0.45µm membrane filter. They were mixed respectively in the ratio of 45:55% v/v and sonicated for 15min.

**Preparation of Standard Solution**

Accurately weighed quantity of 10 mg Agomeiatine were transferred to 100 ml volumetric flask, dissolved in mobile phase and volume was made up to mark with same solvent. From stock solution suitable aliquot was transferred to 10 ml volumetric flask and diluted to mark with the mobile phase, to obtain the concentration of 10 µg/ml. A volume of 10 µl of solution was injected.

**Preparation of Sample (Tablet) Solution**

Twenty tablets were weighed accurately; the average weight was determined and then triturated to a fine powder. A quantity equivalent to 10 mg of agomelatine was weighed and transferred to a 100 ml volumetric flask. The contents were sonicated for 20 min with mobile phase to dissolve the active ingredients and the volume was made up to 100 ml with same solvent and filtered through a 0.45µm membrane filter. Suitable aliquots of the solution were further diluted with mobile phase, Then 10µl volume of each sample solutions were injected into sample injector of HPLC three times under the chromatographic conditions. The area under the curve of each peak was measured at 230nm.

**RESULT AND DISCUSSION**

Under the stated chromatographic conditions, the retention time of Agomelatine was 2.7min.

**METHOD VALIDATION**

The proposed method was validated as per ICH parameters and guidelines.<sup>[9]</sup>

**Linearity**

Appropriate aliquots of the standard stock solution of Agomelatine were pipette out and transferred to a series of 10 ml volumetric flasks respectively. The volume was made up to the mark with mobile phase to obtain working standard solutions of concentrations 10, 20, 30, 40, and 50µg/ml. From these solutions, 10 µl injections of each concentration of the drug were injected into the HPLC system repeate times separately and chromatogram was obtained

under the conditions as finalized by developed method. The peak areas were recorded. The standard calibration curves for Agomelatine were plotted.

### **Accuracy**

Accuracy of proposed method has been carried out by recovery studies. Recovery studies were carried out by applying the method to drug content analysis present in sample to which known amount of standard Agomelatine was added at 80%, 100% and 120% levels. The technique involves addition of standard drug to preanalysed sample solution. The resulting sample solutions were injected onto HPLC system and chromatogram was recorded.

### **Precision**

It is expressed as  $\pm$  S.D. of series of measurements. Precision was carried out by two methods according to ICH guidelines:

1. Repeatability studies
2. Intermediate precision – variation in days and analyst.

### **Repeatability**

It is measured by multiple injections of a homogenous sample of 10  $\mu\text{g/ml}$  of agomelatine that indicates the performance of the HPLC instrument under conditions.

### **Intermediate precision**

#### **1. Variation in days: Intra-day and Inter-day Precision.**

In the intra-day studies, 3 replicates of 3 different concentrations (10,15,20 $\mu\text{g/ml}$ ) of Agomelatine were analyzed in a day and percentage RSD was calculated. For the inter day variation studies, 3 replicates of different concentrations were analyzed on 3 consecutive days and percentage RSD was calculated.

#### **2. Variation in Analyst**

##### **Specificity and Selectivity**

The analytes should have no interference from other extraneous components and be well resolved from them. Specificity is a procedure to detect quantitatively the analyte in presence of component that may be expected to be present in the sample matrix, while selectivity is the procedure to detect qualitatively the analyte in presence of components that may be expected to be present in the sample matrix. The method is quite selective. There was no other interfering peak around the retention time of Agomeiatine.

### Sensitivity (LOD and LOQ)

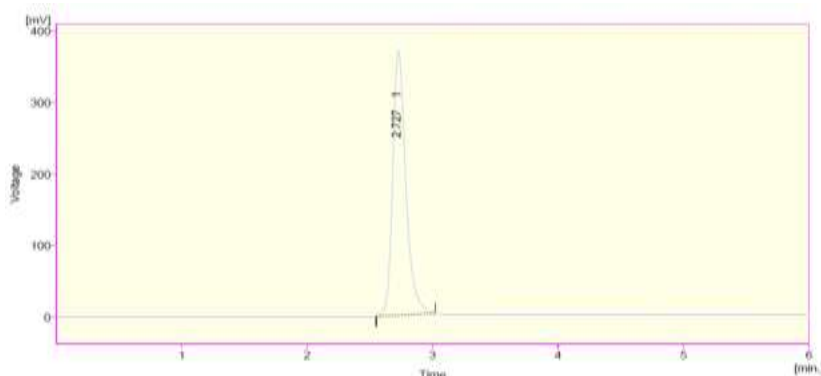
Sensitivity of the proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ). LOD was found to be 4.7 $\mu$ g/ml for agomelatine. LOQ was found to be 7.5 $\mu$ g/ml.

### Robustness

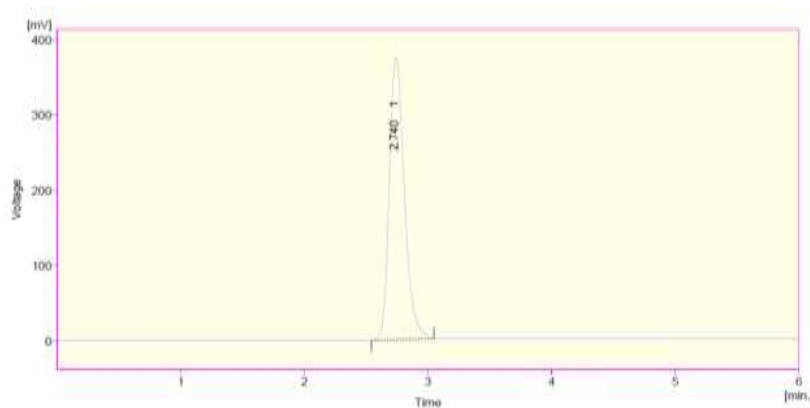
Robustness of the proposed method was assessed by making deliberate changes in flow rate, pH and proportion of mobile phase which was performed by injecting sample solution containing 10 $\mu$ g/ml of Agomelatine.

## RESULTS AND DISCUSSION

After several experiments with different mobile phase combination and flow rates, the optimised mobile phase composition and flow rate were determined as 45:55(ACN: mixed beffer with pH6) and 1ml/min. under these conditions Aomelatine eluted at 2.76mins. The typical chromatograms obtained using above mentioned mobile phase at 1ml/min are illustrated ain figures 1 and 2.



**Fig.1: Chromatogram of standard solution of Agomelatine**



**Fig.2: Chromatogram of sample solution of Agomelatine**

**Table 1: Results of three repetitions of the analysis**

Trail No	Agomelatine area in standard	Rt	Aaaaagomelatine area in sample	Rt
1	2721981	2.743	2685121	2.727
2	2747840	2.723	2578161	2.740
3	2708737	2.727	2701227	2.747
Average				
%RSD	0.039		0.032	

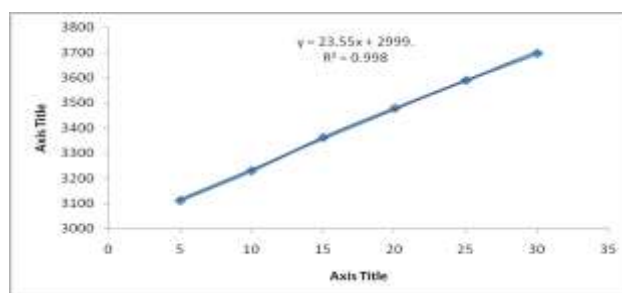
The lower percentage relative standard deviation of assay for test of accuracy (table 2) testifies the methods accuracy. From the mean recoveries, which were within the range of 98-102%. its clear that the excipients do no interfere with the method. The peak area concentration data of the agomelatine shown in figure.3.

**Table 2: Recovery studies of Agomelatine by standard addition method**

Concentration (% at specification Level)	Area	% recovery
50	2775309	98.89
100	3285082	100.5
150	356431	100.49
%Mean recovery		

The concentration range of agomelatine was 10-50µg/ml. the correlation coefficient values for agomelatine was found to be 0.998. The assay values obtained for the precision are shown in table 3. the relative standard deviation obtained for agomelatine was 0.13, thus, confirming the method's precision.

small changes were made in the flow rate and pH for the purpose of ascertaining robustness, Also, the flow rate was changed to 0.8ml/min and 1.2ml/min from the actual 1ml/min, The % RSD of retention time ,area and assay values of agomelatine was within the +\_2%. The LOQ of Agomelatine was found to be 0.09 and Te LOD was found to be 0. 102. The method developed here was utilized for estimation of agomelatine in tablet dosage form.

**Fig 3: Linearity graph of Agomelatine**

**Table 3: Results of precision**

S.No.	RT	Area
1	2.760	3119.741
2	2.740	3160.876
3	2.743	3178.176
4	2.737	3171.436
5	2.747	3251.471
6	2.730	3212.049
avg	2.7428	3182.292
stdev	0.0108	45.116
<b>%RSD</b>	<b>0.13</b>	<b>0.07</b>

## CONCLUSIONS

Hence, it may be concluded that a new RP-HPLC method for the estimation of Agomelatine in tablet dosage form has been developed. The accuracy, precision, sensitivity and robustness of method were confirmed. This method, which can be run 7mins, may be suitable for analysis in quality control units of pharmaceutical industries.

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