A new method Development and Validation of Agomelatine by RP-HPLC in its bulk and tablet dosage form

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Abstract: A novel and simple Reverse phase liquid chromatographic method was established for the determination of Agomelatine in presence of degradants. Agomelatine is a potent agonist at melatonin receptors and an antagonist at serotonin-2C (5-HT2C) receptors, tested in an animal model of depression. It is used for the treatment of major depressive disorder. The proposed work was performed on Thermo Hypersil extended C18 column (250 mm × 4.6 mm i.e., 5 µm particle size) using a mixture of Buffer (4.3pH) and methanol (60:40) as mobile phase with flow rate 1.0 ml/min. Water and Methanol (50:50) was used as diluent. The retention time was found to be 3.374 min. The linearity range used was 25-75µg/ml. The %RSD was found to be less than 2%. LOD & LOQ values were found to be 3.3002 and 10.0008µg/ml respectively.

Key words: Agomelatine, Antagonist, Chromatography, Buffer, Depressive Disorder.

INTRODUCTION

Agomelatine ($C_{15}H_{17}NO_2$) is structurally closely related to melatonin. A typical antidepressant that acts as a melatonergic anti-depressant. Agomelatine is a melatonin receptor agonist and a serotonin receptor antagonist with a favorable side effect profile. There is a lack of sexual dysfunction, sleep benefits, and no discontinuation symptoms – all of which confer valuable clinical benefits in the treatment of depression. Agomelatine exerts affinity for melatonin receptor subtypes, MT1 and MT2, and serotonergic 5-HT2C receptors. [(1)]

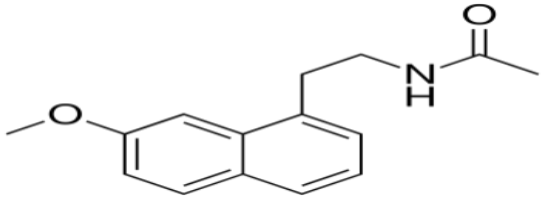

Fig 1: Structure of Agomelatine

According to our literature survey there are different methods for development and validation of Agomelatine such as RP-HPLC, UPLC, LC-MS, HPTLC. All the presented methods have retention time in the range of 5-7 min.

Here we have tried to develop a method having less retention time in the range of 3-4 minutes. [(2-22)]

MATERIALS AND METHOD

The present investigation was to develop a new method and validate and to study the stability of Agomelatine in pharmaceutical dosage form by RP-HPLC. From this experiment it was found that Agomelatine can effectively be analyzed by using the RP-HPLC method with buffer (pH 4.3) and methanol in the ratio 60:40 at a flow rate of 1ml/minute (Table.2) and detection wavelength of 232nm.The retention time of the drug was found to be 3.374 minutes (Table.1) (Fig.2).

Procedure:

Preparation of Buffer:

Weigh accurately 136mg of potassium dihydrogen phosphate into a 1000ml volumetric flask and dissolve with water sonicate for 20 minutes and make up to mark with water.

Preparation of Mobile Phase:

A mixture of above prepared buffer 600 mL (60%) and 400 mL of HPLC grade Methanol (40%) were mixed and degassed in ultrasonic water bath for 5 minutes. The mobile phase was filtered through 0.45 µ filter under vacuum.

Diluent Preparation:

A mixture of HPLC grade Methanol and water mixed in 50:50 ratio was prepared degassed in ultrasonic water bath for 5 minutes and filtered through 0.45 µ filter under vacuum.

Preparation of Agomelatine Standard & Sample Solution:

Preparation of Standard Solution:

Accurately weigh and transfer 25 mg of Agomelatine working standard into a 100mL clean dry volumetric flask and added about 70mL of diluent. It was sonicated to dissolve completely and made volume up to the mark with the same diluent. (Stock solution) (250µg/mL). From this, 5 ml of the solution was pipetted into another 25ml volumetric flask and diluted up to the mark with diluent (50µg/mL).

Preparation of Sample Solution:

Accurately weigh and transfer tablet powder equivalent to 25 mg of Agomelatine into a 100mL clean dry volumetric flask and added about 70mL of diluent. It was sonicated to dissolve completely and made volume up to the mark with the same diluent. (Stock solution) (250µg/mL) From this, 5 mL of the solution was pipetted into another 25ml volumetric flask and diluted up to the mark with diluent (50µg/mL).

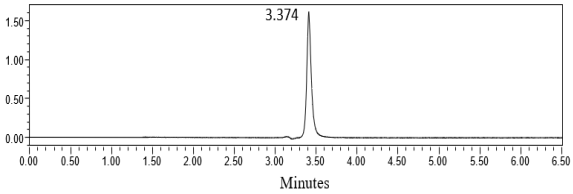

Fig.2: Optimization Chromatogram

ID	Name	Retention time (min)	Area (µg*sec)	USP Plate Count	USP Tailing
1	Agomelatine	3.374	8021057	6302	1.24

Table.1: Agomelatine Retention Time

PARAMETERS	CONDITIONS
Mobile Phase	Buffer (4.3 pH): Methanol (60:40)
Column (Stationary Phase)	Thermo Hypersil C18 (250x4.6mm, 5µ)
Flow Rate (ml/min)	1
Diluent	Water: Methanol (50:50)
Volume of injection loop (ml)	10
Detection wavelength (nm)	232
Column temperature (^{0}C)	30

Table.2: Optimized Method Parameters

Validation:

Establishing documentation evidence, which provides a high degree of assurance that specific process, will consistently produce a product meeting its predetermined specification and quality attributes.

A) 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.

Preparation of Standard Solution:

Accurately weighed and transferred 25 mg of Agomelatine into a 100mL clean dry volumetric flask and added about 70mL of diluent. It was sonicated to dissolve completely and made volume up to the mark with the same diluent. (Stock solution) (250µg/mL)

For preparation of 50% Solution:

From the above stock solution 2.5 mL was pipette into another 25mL volumetric flask and diluted up to the mark with diluent.

For preparation of 100% Solution:

From this above stock solution 5 mL was pipette into another 25mL volumetric flask and diluted up to the mark with diluent.

For preparation of 150% Solution:

From this above stock solution 7.5 mL was pipette into another 25mL volumetric flask and diluted up to the mark with diluent.

Procedure:

The above prepared solutions of Accuracy 50%, 100%, 150% solutions were injected. The Amount found and Amount added for Agomelatine individual recovery and mean recovery values were calculated.

B) 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.

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

Procedure for Precision:
Preparation of Stock Solution:
Accurately weighed and transferred tablet powder equivalent to 25 mg of Agomelatine working standard into a 100mL clean dry volumetric flask and added about 70mL of diluent. It was sonicated to dissolve completely and made up to mark.
From this, 5 ml of the solution was pipetted into another 25ml volumetric flask and diluted up to the mark with diluent.

Procedure:
The solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

C) Linearity:
It is an analytical procedure is its ability within a given range to obtain test results which are directly proportional to the concentration of analyte in the sample.
Accurately weighed and transferred tablet powder equivalent to 25 mg of Agomelatine working standard into a 100mL clean dry volumetric flask and added about70mL of diluent. It was sonicated to dissolve completely and made up to mark. (250ppm, stock solution)

Preparation of Level – I (25ppm):
2.5mL of the above stock solution has taken in 25ml of volumetric flask diluted up to the mark with diluent

Preparation of Level – II (37.5ppm):
3.75mL of the above stock solution has taken in 25ml of volumetric flask diluted up to the mark with diluent.

Preparation of Level – III (50ppm):
5mL of the above stock solution has taken in 25ml of volumetric flask diluted up to the mark with diluent.

Preparation of Level – IV (62.5ppm):
6.25 mL of the above stock solution has taken in 25ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V (75ppm):
7.5 mL of the above stock solution has taken in 25ml of volumetric flask diluted up to the mark with diluent.

Procedure:
Each level of solution was injected into the chromatographic system and the peak areas were measured. Plotted a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and the correlation coefficient was calculated.

Acceptance Criteria:
Correlation coefficient should be not less than 0.999

D) Limit of Detection:
The detection limit is determined by the analysis of samples with known concentration of analyte and by establishing that minimum level at which the analyte can reliably detected.

E) Limit of Quantification:
The quantification limit is generally determined by the analysis of sample with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision.

F) Robustness:
As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation were made to evaluate the impact on the method.

Preparation of Stock Solution:
Standard solution was prepared and analysed using the varied flow rates along with method flow rate.

Effect of Variation of Flow Rate:
On evaluation of the results, it can be concluded that the variation in flow rate has not affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±10 %.
The method is robust only in flow condition.

Effect of Variation of Temperature:
The temperature of the column oven is varied in order to determine the effect of variations in the temperature of more or less than 5ºC. Standard was prepared and analysed using the varied temperature in the method. On evaluation of the results, it can be concluded that the variation in 5ºC of temperature has not affected

the method significantly. Hence it indicates that the method is robust even by change in the temperature.

G) System Suitability Parameters:

System suitability is the evaluation of the components of an analytical system to show that the performance of a system meets the standards required by a method. A system suitability evaluation usually contains its own set of parameters. For chromatographic assays, these may include tailing factor, resolution, precision, capacity factor, retention time and theoretical plates.

Method Validation:

Accuracy:

S. No	Agomelatine Accuracy 50 %	Injection	Retention time	Area
1	Accuracy -1	1ml	3.322	1532382
2	Accuracy -2	1ml	3.332	1522694
3	Accuracy -3	1ml	3.337	1530961
4	Accuracy -4	1ml	3.340	1528253
5	Accuracy -5	1ml	3.343	1526691
6	Accuracy -6	1ml	3.342	1527132

Table.3: Observations of Accuracy 50%

S. No	Agomelatine Accuracy 100%	Injection	Retention time	Area
1	Accuracy -1	1ml	3.310	3069777
2	Accuracy -2	1ml	3.311	3066656
3	Accuracy -3	1ml	3.312	3053059
4	Accuracy -4	1ml	3.314	3054169
5	Accuracy -5	1ml	3.316	3043152
6	Accuracy -6	1ml	3.318	3042162

Table.4: Observations of Accuracy 100%

S. No	(Agomelatine) Accuracy 150%	Injection	Retention time	Area
1	Accuracy -1	1ml	3.295	4597421
2	Accuracy -2	1ml	3.396	4598108
3	Accuracy -3	1ml	3.298	4590280
4	Accuracy -4	1ml	3.309	4592383
5	Accuracy -5	1ml	3.313	4590676
6	Accuracy -6	1ml	3.317	4595224

Table.5: Observations of Accuracy 150%

The accuracy studies were shown as % recovery for Agomelatine at 50%,100% and 150%, the limits of % recovered should be in range of 98-102% the results obtained for Agomelatine were found to be within the limits. Hence the method was found to be accurate. The accuracy studies showed %recovery of the Agomelatine 99.973%. The limits of % recovery of drugs was 98-102% and from the above results it indicates that the method was accurate and also revealed that the commonly used excipients present in the pharmaceutical formulation do not interfere in the proposed method.

Precision:

S.no	Concentration(µg/ml)	Absorbance
1	50	1174056
2	50	1174004
3	50	1174039
4	50	1174028
65	50	1174017
6	50	1174012
MEAN		1174026
SD		19.15202
%RSD		0.001631

Table.6: Observations of Interday Precision

S.no	Concentration (µg/ml)	Absorbance
1	50	1174050
2	50	1174037
3	50	1174021
4	50	1174019
65	50	1174015
6	50	1174005
MEAN		1174025
SD		16.245
%RSD		0.001384

Table 7: Intraday precision

In the precision study, % RSD was found to be less than 2%. For Agomelatine 1.82% which indicates that the system has good reproducibility.

For precision studies 5 replicates injections of Agomelatine formulation (method precision) was performed. % RSD was determined for peak areas of Agomelatine. The acceptance limits should be not more than 2 % and the result was found to be with in the acceptance limit.

Linearity:

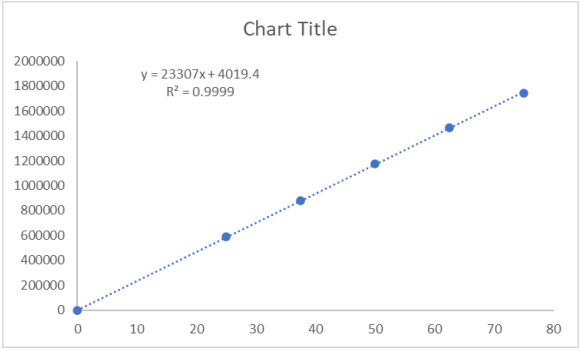

Fig 3: Linearity curve

Concentration %	Area	ug/ml
50%	587801	25
75%	880166	37.50
100%	1174368	50.00
125%	1468744	62.5

150%	1765133	75

Table.8: Observations of Linearity

The linearity range was found to be 25-75µg/ml for Agomelatine. Calibration curve plotted for drugs was found to be 0. 999.Hence the results obtained were within the limits.

Limit of Detection (LOD):

LOD was calculated by using standard deviation and slope values obtained from calibration curve.

$$LOD = 3.3 \times \sigma / S$$

Drug	Amount (µg/mL)
Agomelatine	3.3002

Table 9: LOD results of the Method

Limit of Quantification (LOQ):

LOQ was calculated by using standard deviation and slope values obtained from calibration curve.

$$LOQ = 10 \times \sigma / S$$

Drug	Amount (µg/mL)
Agomelatine	10.0008

Table 10: LOQ results of the Method

From the above, the LOD values of Agomelatine was found to be 0.88. The LOD values of Agomelatine was found to be 2.96. Thus, the method developed was found to be sensitive.

Robustness:

S. NO	Name	Injection	Area	Retention time	USP tailing	USP plate count
1.	Low flow rate	1ml	1833563	4.178	1.601	7053
2.	High flow rate	1ml	1197149	2.805	1.569	6280
3.	Low Temperature	1ml	1427503	3.323	1.591	6678
4.	High Temperature	1ml	1437873	3.343	1.538	6698

Table 11: Observations of Robustness

On evaluation of the above results, it can be concluded that the variation in temperature do not affect the method significantly. Hence it indicates that the method is robust even by change in the Temperature. For robustness studies the chromatograms were recorded for standard solutions of Agomelatine by changing flow rate ±0.1 and Temperature. Robustness studies reveal that the method was reliable.

Ruggedness:

S.no	Concentration (µg/ml)	Analysist -1	Analysist -2
1	50	1174015	1174052
2	50	1174011	1174027
3	50	1174055	1174014
	MEAN	1174027	1174031
	SD	24.33105	19.31321
	%RSD	0.002072	0.001645

Table 12: Ruggedness observations

System Suitability Parameters:

S. No	Parameter	Agomelatine
1	Retention time	3.374
2	Theoretical plates	6302
3	Tailing factor	1.24
4	Area	8021057

Table.13: Observations of System Suitability

The system suitability parameters were found to be within the specified limits for the proposed method.

Summary:

RP-HPLC method was developed for simultaneous estimation of Agomelatine in pharmaceutical dosage form. Chromatographic separation was performed on X-terra(C_8) (4.6mm x 250mm, 5µ)column, with mobile phase comprising of mixture of buffer (pH4.3, adjusted with potassium dihydrogen phosphate), and methanol in the ratio of 60:40v/v, at the flow rate 1ml/min. The detection was carried out at 232 nm.

S. No	Parameter	Acceptance criteria	Result obtained
1	System suitability	Theoretical plate – NLT 2500	6302
		Tailing factor - NMT 2	1.24
		Retention time	3.374
2	Precision	% RSD of Agomelatine – NL 22	0.13
3	Linearity	Correlation Coefficient NLT 0.999	0.999
4	Accuracy	Percentage recovery 98-102%	100
5	LOD		3.300284015
6	LOQ		10.00086065

Table 14: Summary for RP-HPLC Method

CONCLUSION

The proposed HPLC method was found to be precise, specific, accurate, rapid and economical for estimation Agomelatine in tablet dosage form. The sample recoveries in all formulations were in good agreement with their respective Label Claims and this method can be used for routine analysis. It can be applied for routine analysis in laboratories and is suitable for the quality control of the raw materials, formulations, dissolution studies and can be employed for bioequivalence studies for the same formulation.

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