Development and Validation of RP-HPLC Method for Assay of Agomelatine Drug in Tablet Dosage Form

[1]Ms. Rashmi Wani, [2]Ms. Bhagyashree Gaikwad, [3]Ms. Ashika Madhavi
[1]Assistant Professor, Department of Pharmaceutical Chemistry, Ideal College of Pharmacy & Research, Kalyan-421306, Maharashtra, India
[2]Assistant Professor, Department of Quality Assurance, Ideal College of Pharmacy & Research, Kalyan-421306, Maharashtra, India
[3] B. Pharm Student, Ideal College of Pharmacy & Research, Kalyan-421306, Maharashtra, India

ABSTRACT
A simple, efficient, and precise stability indicating RP-HPLC method has been developed and validated to measure Agomelatine at wavelength (230 nm) in order to assay. Agomelatine is used to trea Depression. Methanol was used as a solvent with λ_{max} of drug was found to be 230 nm. The samples were eluted in an isocratic method using Water symmetry (C_{18}, 5um, 4.6nm×250mm) with a mobile phase consisting of pH 5.0 Buffer Dipotassium Hydrogen Phosphate: ACN (50:50) using as diluents through ambient temperature delivered at a flow rate 1.2mL/min. Linearity was observed in the range of 20-120µg/ml with a regression coefficient (R^2) of 0.99. The method was quantitatively evaluated in terms of accuracy (recovery), linearity, precision, selectivity and robustness in accordance with standard ICH validation guidelines. The method is simple and suitable for analyzing Agomelatine in bulk and in pharmaceutical formulations.

Keywords: HPLC, Agomelatine, Validation, Buffer, Accuracy, Linearity

INTRODUCTION

In a quest to make available drugs for ever increasing diseases, disorders and ailments, new drugs, drug combinations and formulations are being introduced on regular interval. Due to this, analytical chemists are facing challenges for the scope of developing and validating a method to ensure a suitable strategy for a particular analyze which is more specific, accurate and precise. The main aim of this work was "to design, develop and validate a stable and highly effective analytical assay method for the estimation of Anti-depressant drug Agomelatine in tablet dosage form.

Agomelatine is a potent agonist of melatonin (MT_1 and MT_2) receptors with HT_2C antagonist properties. It is also a **5**-HT_2B antagonist. Agomelatine does not interact with adenosine, adrenergic, dopamine, GABA, muscarinic, nicotinic, histamine, excitatory amino acid, and benzodiazepine and sigma receptors, not with sodium, potassium or calcium channels. [1] [2] Through its **5**-HT_2C antagonist effect, Agomelatine increases dopamine and nor-adrenaline release specifically in the prefrontal cortex. [3] [4]

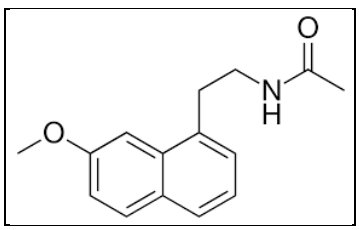

Figure 1: Chemical structure of Agomelatine

Table No.1: Properties of Agomelatine [18] [19]

IUPAC Name	N-[2-(7-methoxynaphthalen-1-yl) ethyl] Acetamide
Chemical Formula	C15H17NO2
Molecular Mass	243.301
Category	Anti-Depressant
pKa value	15.94
Potency of drug	99.09%
Physical State	White or White alike crystal powder
Melting Point	108 °C
Solubility	Organic solvents such as Ethanol, DMSO and Dimethyl Formamide

MATERIALS AND METHODS

2.1. *Chemicals*

Pure sample as well as capsule dosage form of Agomelatine was obtained from Watson Pharmaceutical Pvt Ltd Mumbai. All the chemicals were used of analytical grade Acetonitrile HPLC Grade Di-potassium hydrogen phosphate Acetic acid Hydrochloric acid. HPLC Grade Sodium Hydroxide pellets Hydrogen Peroxide (H_2O_2) were of Merck. The Methanol HPLC Grade methanol was used as a solvent throughout the studies. (Table 2 and 3)

Table No.2: List of chemicals

Chemicals	**Manufacturer**
Drug API	Watson (Mumbai)
Methanol	Rankem
Acetonitrile	Rankem
Di-potassium hydrogen phosphate	Merck
Acetic acid	Merck
Hydrochloric acid	Qualigens Fine Chemicals
MilliQ Water	Millipore Water

Table No.3: List of equipments/instruments

Name	**Brand Name**
HPLC	Waters HPLC 2489
Software	Empower
UV-visible Spectrophotometer	Shimadzu- 2010 A
pH Meter	Thermo Orion 3 Star
Electronic Balance	Mettler Toledo
Sonicator	Citizen CD-4820

An UV- visible Double Beam Spectrophotometer (Shimadzu Model No: 2010 A) with matched quartz cells corresponding to 1cm path length was used. [5-7]

2.2. Preparation of Solutions

Preparation of stock solution
Weighed accurately 10mg of drug and was dissolved in 10 ml of Methanol to give concentration of 1mg/ml (Stock Solution-A).
Preparation of Standard Stock solution
1ml of Solution (A) was diluted with methanol in 100ml volumetric flask to give concentration of 10µg/ml (Standard Stock Solution B) and series of 5-35 µg/ml of concentrations were prepared. UV spectrum was recorded using this solution in the range of 200-400 nm. Agomelatine showed absorbance maxima at 284nm. (Figure 2)

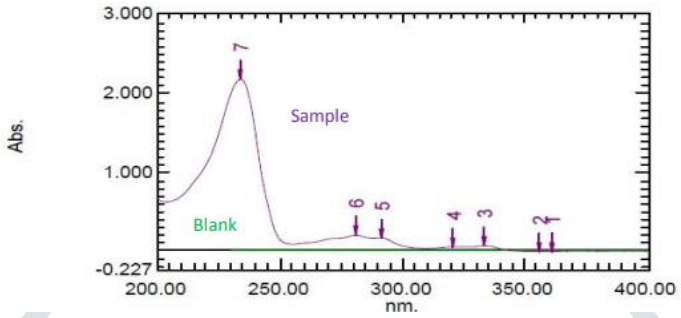

Figure 2: UV spectrum of Agomelatine USP

2.3. Instrument and Chromatographic Conditions

Preparation of Mobile phase
Mix buffer pH 5.0-0.05 and acetonitrile in the ratio 50:50 v/v and sonicate to degas.
Optimization of Chromatographic Method (Table 4)

Table 4: Optimization of chromatographic parameters

HPLC	Waters HPLC 2489
Mobile Phase	Buffer : Acetonitrile (50:50)
Column	Water Symmetry C18,5um,4.6nm×250mm
Diluents	Mobile Phase
Flow rate	1.2 ml /min
Detector	230 nm
Column Temperature	35º C
Injection Volume	20 µl
Run time	12 min

Proper selection of the method depends upon the nature of the sample (ionic/ionizable/neutral molecule, its molecular weight and solubility). The drug selected in the present study is polar in nature therefore; reverse phase or ion exchange or ion pair chromatography method can also be used. Here, reverse phase HPLC method was selected for the initial separation owing to its simplicity, suitability, ruggedness and its wider usage. In order to achieve the optimized chromatographic conditions to separate elute and quantify Agomelatin, one or two parameters were modified at each trial and chromatograms were recorded with all specified chromatographic conditions.
Preparation of Standard Solution:
Accurately weighed about 50 mg of Agomelatine standard and was transferred to a 100 ml volumetric flask. 70 ml of diluent was added and content was dissolved by sonication. The volume was made up to the mark with diluent and mixed well. Further 5.0 ml of this solution was diluted to 50 ml with diluent and was mixed well.
Preparation of Sample stock Solution:

Accurately weighed 5 intact tablets were transferred to a 250 ml volumetric flask. Then 50 ml of purified water was added and solution was sonicated to disintegrate tablets completely. Again 150 ml of methanol was added and sonicated for 30 minutes with intermittent swirling. The solution was then cooled and diluted up to mark with Methanol and mixed well. The above solution was centrifuged. The supernatant solution was filtered through 0.45 µ Nylon MDI filter discarding first 10 ml of the filtrate. Further 5 ml of the remaining filtrate was diluted to 50 ml with diluent and was mixed well. [8-11]

Assay Method

An HPLC method has been developed for the determination of the percentage assay of Agomelatin in its tablet dosage form.

2.4. Methodology Followed

Preparation of buffer

The Buffer was prepared by dissolving 1.74 g of di-potassium hydrogen phosphate into 1000 ml milli-Q water, was sonicated and mixed. pH was adjusted to 5.0 +- 0.05 with glacial acetic acid solution. Solution was filtered through 0.45µm Nylon membrane filter paper. [12-17]

Preparation of Diluent

Prepare a mixture of Methanol and Water in the proportion of 80:20

Preparation of Standard Solution

5 ml of standard stock solution was pipette out and transferred into 50 ml capacity volumetric flask and volume was made up to the mark with mobile phase and mixed properly.

2.5. Analytical method and Method Validation [20-25]

2.5.1. Specificity

Specificity is method of confirming no interference from blank and placebo at the maxima of the drug.

Sample Solution

The retention time of the Agomelatin peak in the chromatogram of the Sample corresponds to that of the peak in the chromatogram of the Standard. Retention time of peak in standard solution was 4.507 min. Retention time of Agomelatin peak in sample solution of tablet was 4.507 min. (Figure 3 and 4)

Blank and Placebo interference

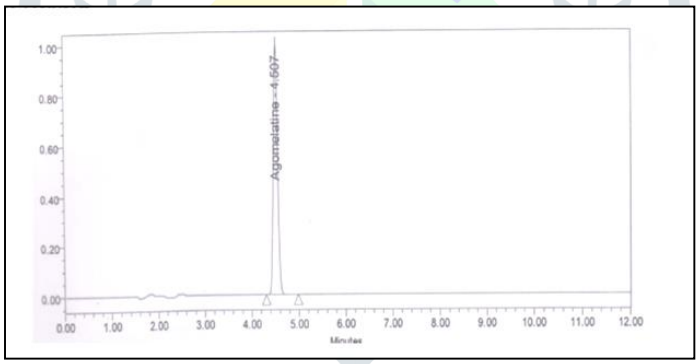

Figure 3: Specificity of Sample Solution Chromatogram

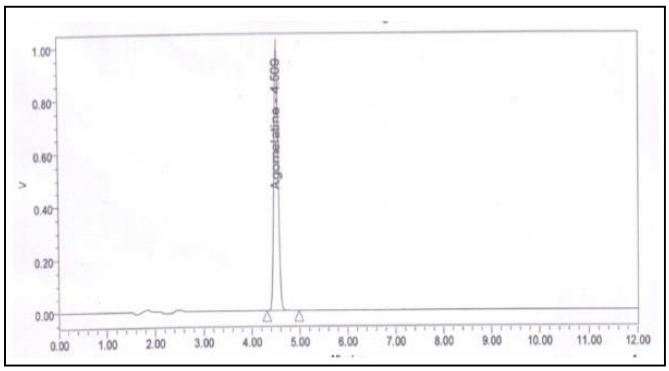

Figure 4: Specificity of Standard Solution Chromatogram

Table 5: Specificity of Agomelatine

Sr No.	Sample Name	Drug X	
		RT	Area
1	Agomelatine Standard	4.507	407530
2	Agomelatine Sample	4.509	407712
3	Placebo	-	-
4	Blank	-	-
	Average	407548	
	SD	704.98	
	%RSD	0.17%	

As per the method Blank, placebo solution, sample solution and standard solution was injected into HPLC system as per methodology. (Table 5)

2.5.2. Linearity and Range

Linearity was evaluated in the range of 20% to 120% of the working concentration level. (Figure 5) The Linearity of response was determined by preparing six different concentrations of standard stock solution ranging from 20%, 40%, 60%, 80%, 100% and 120%. (Table 6)

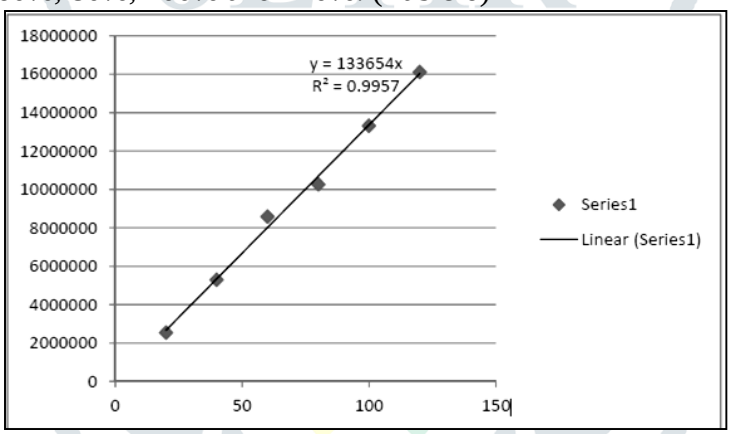

Figure 5: Linearity Graph of Agomelatine

Table 6: Linearity of Agomelatine

Sr. No.	Conc. (µg/ml)	Mean Peak Area
1	20	2538795
2	40	5295862
3	60	8588283
4	80	10266161
5	100	13316882
6	120	16117544

2.5.3. Accuracy (Recovery)

Placebo of capsule was spiked at three different levels: 50%, 100% and 150% of the label claim in triplicate. Each of the sample preparation was injected in triplicate and the average area count was taken for calculation.

Mean recovery should be in the range of 98.0% to 102.0%. The RSD should NMT than 2.0% (Table 7 & 8)

Table 7: Data sheet of Recovery

Level	Amount added in mg	Amount Recovered	% Recovery	Average % Recovery
50%	24.775	24.76	99.95	99.67%
	24.775	24.61	99.32	
	24.775	24.74	99.86	
100%	49.550	49.61	100.12	99.9%
	49.550	49.56	100.01	
	49.550	49.34	99.57	
150%	74.325	74.29	99.96	99.89%
	74.325	74.30	99.97	
	74.325	74.18	99.80	

Table 8: Statistical analysis for recovery data

Sr. No.	Level	% Recovery	SD	RSD
1	50	99.67	0.3407	0.34
2	100	99.9	0.2910	0.29
3	150	99.89	0.0953	0.095

2.5.4. Limit of Detection (LOD) and Limit of Quantitation (LOQ):-

LOD: It is the smallest amount of concentration of analyte in the sample than can detect which will be reliably distinguished by zero.

LOQ: It is the smallest amount of analyte in the sample which can be distinguished with suitable precision and accuracy

Limit of Detection and Limit of Quantitation were determined based on standard deviations of the response of calibration curve. (Table 9)

Table 9: LOD and LOQ

Sample	Parameter	
	LOD (µg/ml)	LOQ (µg/ml)
Agomelatine (µg/ml)	2.830	9.434

2.5.5. Precision

1. System Precision

System precision was evaluated by measuring of absorbance of drug from six replicate injection of standard preparation (10 ug/ml) were injected into UV and %RSD was calculated. (Table10)

Table 10: System Precision

Injection No	1	2	3	4	5	6
Area	407645	40921	409771	407413	409955	409982
Mean	408781.2					

SD	1221.87
%RSD	0.298

2. Method precision

System precision was evaluated by measuring of absorbance of drug from six replicate injection of standard preparation (10 µg/ml) were injected into UV and %RSD was calculated. (Table 11)

Table 11: Method Precision

Sample	**Mean peak area**	**% assay**
1	407142	99.15
2	407256	98.95
3	407622	98.91
4	407401	98.94
5	407340	99.14
6	407412	99.18
	Mean	99.04
% RSD		0.13

3. Intermediate precision (Ruggedness)

Six standard solutions and six sample solutions of the same lot of the capsule was made by a different analyst, using same column on a different day and injected in duplicate into different UV system. The mean and percent RSD values for area were calculated. (Table12)

Table 12: Intermediate precision

Sample	**Analyst-1 % Label claim**	**Analyst-2 % Label claim**
1	98.4	98.5
2	95.7	96.6
3	98.5	98.4
4	96.6	95.7
5	96.4	95.9
6	95.8	96.4
Mean	96.90	96.91
%RSD	1.28	1.28
Overall mean	96.9	
Overall %RSD	1.17	

RESULTS AND DISCUSSION

1. Specificity of the method was validated by the response shown by the peak, no interference from blank, impurity and placebo with the main peak, identification was done by the retention time.
2. Correlation of coefficient of Agomelatin was found to be 0.99. Therefore; the HPLC method for the determination of assay of Agomelatin was linear.
3. Mean recovery was found to be 100.33% and RSD was 0.09% Therefore, the HPLC method for the determination of assay of Agomelatin tablet was accurate.

4. The RSD of system precision was found to be 0.298%. Therefore, the UV method for the determination of X capsule was precise.
5. The RSD of method precision was 0.2%. Therefore, the HPLC method for the determination of Agomelatin was reproducible.
6. The RSD of Ruggedness was found to be 1.17%. Therefore, the HPLC method for the determination of Agomelatin tablet was rugged.

CONCLUSION

Under the conditions described the method is found to be specific, rugged, robust, accurate and linear. The method is suitable for the assay of Agomelatine.

Table 13: Validation report of a method for the assay of Drug X in capsule dosage form by UV

Sr. No.	Validation Parameter	Acceptance Criteria	Result
1	Specificity		Pass
1.1	Identification	Results should be comparable with respect to retention time.	Pass
1.2	Placebo Interference	Blank and Placebo should not shown any peak at the retention time of drug x Peak.	Pass (No interference)
2	Linearity & Range	Correlation Coefficient should not be less than 0.999	Pass (1.0)
3	Accuracy (Recovery)	Mean recovery should be in the range of 98.0% to 102%. The RSD should not be more than 2.0%.	Pass
4	Precision		
4.1	System Precision	RSD should not be more than 2.0%.	Pass
4.2	Method Precision	RSD should not be more than 2.0%.	Pass
5	Ruggedness	RSD should not be more than 2.0%.	Pass
6	Robustness		
6.1	Change in column Temperature (+5˚C)	RSD should not be more than 2.0%.	Pass
6.2	Change in flow rate (± 0.1mL/min)	RSD should not be more than 2.0%.	Pass
6.3	Change in pH (± 0.2)	RSD should not be more than 2.0%.	Pass

ACKNOWLEDGEMENT

I express my sincere thanks to Ms. Bhagyashree Gaikwad and Ms. Nikita Alange for their support and encouragement throughout my research work. I am also thankful to Watson Pharmaceuticals for providing the generous drug samples for research.

CONFLICT OF INTEREST

There is no conflict of interest. The authors alone are responsible for content and writing of this article.

REFERENCES

[1] Chung CC, Herman L, Lee YC, Xue-Ming Zang, 2004. Analytical Method Validation and Instrument Performance Verification, John Wiley & Sons, Inc., Publication, 11-12.

[2] Willard HH, Merritt, Jr. L.L., Dean, JA, Settle Jr. FA. 2001. 7th Edn, Instrumental Methods of Analysis, CBS Publishers and Distributors, Delhi, 1.

[3] Mendham J, Denney RC, Barnes JD, Thomas M, 2003. Vogel's Textbook of Quantitative Analysis. Pearson Education, Singapore, 8-9.

[4] Skoog A, Holler FJ, Timothy A, Nieman NW, 2004. Principle of Instrumental Analysis, 5th ed., Eastern Press, Banglore, 1-2:678-688, 695-696.

[5] Sethi P D, Quantitative Analysis of Drugs in Pharmaceutical Formulations, 2005. New Delhi CBS Publishers and Distributors, 52-53.

[6] Settle F. 2004. Handbook of Instrumental Techniques of Analytical Chemistry, 1sted, New Jersey, Upper Saddle River, 19-21, 609-617.

[7] Sharma B K. 2002. Instrumental Methods of Chemical Analysis, Meerut, Goel Publishing House, 3-5, 10

[8] Munson J W. 2001. Pharmaceutical Analysis, Modern Methods, Part-B, Mumbai, International Medical Book Distributors, 51-54.

[9] Christian G D. 2003. Analytical Chemistry, 6th ed, Singapore, John Wiley and Sons (Asia) Pvt. Ltd, 623-627. [10] Braun R D 2006. Introduction to Instrumental Analysis, 1st ed, Hyderabad, Pharma Book Syndicate, 839-865.

[11] Kar A. 2005. Pharmaceutical Drug Analysis, New Age International Pvt. Ltd, Revised 2nd ed, 455.

[12] Lee C D, Webb L M. 2003. Pharmaceutical Analysis, Blackwell Publishing, 44-45

[13] Snyder L R, Glajch J L, Kirkland J J. 1997. Practical HPLC Method Development, 2nd ed, USA, John Wiley & Sons, Inc., A Wiley-interscience Publication, 165-170.

[14] USP 24th revision / NF 19 ed 2000: Board of Trusties Asian edition. 605. [15] Cione P A, Edivan T, Paulo S, Stability Indicating Method. Brazil, Bioagri laboratories, 1-30

[16] Berry R I, Nash A R. 1993. Pharmaceutical Process Validation; Analytical method validation, New work, Marcel Dekker Inc. 57, 411-28

[17] Balamurali K K, Mahendra K, Syama Sunadar B A. 2011. A validated reverse phase HPLC method for the simultaneous estimation of Drug X and Drug Y in pharmaceutical dosage forms. *DerPharmaChemica*, 3-1.

[18] http://www.medicinet.com/drugx-oral/article.htm

[19] http://www.drugbank.ca/drugs/.Retrived

[20] ICH: Q3A (R2) (2006, 25 October) Text on Impurities in new drug substances.

[21] ICH: Q3B (R2) (2006, July). Text on Impurities in new drug substances

[22] Ermer J and JH. McB Miller, 2005. Method validation in Pharmaceutical Analysis, WILEY-VCH Verlag GbH & Co. KGaA

[23] Quality Assurance of Pharmaceuticals Geneva: World Health Organization, (1999).

[24] ICH: Q2 (R1) Text on Validation of Analytical Procedures, (2005, November)

[25] ICH: Q2 (B) (1996, November) Validation of Analytical Procedures-Methodology, 582-583.

(M. Pharm)

Mr. Shubham Dubey (B. Pharm Student)

Ideal College of Pharmacy & Research, Kalyan

(B. Pharm Student)

Ideal College of Pharmacy & Research, Kalyan

Email ID: ashikamadhavi27@gmail.com

Corresponding Author

Ms. Rashmi Darshan Wani (M. Pharm)

Department of Pharmaceutical Chemistry, Ideal College of Pharmacy & Research, Kalyan