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

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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 treat Depression. Methanol was used as a solvent with λmax of drug was found to be 230 nm. The samples were eluted in an isocratic method using Water symmetry (C18, 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 of 0.99. Stability of analytical solution was carried out at different time interval 0, 24, 36 and 48hr. The stress testing of the drugs was carried out under acidic, alkaline, photo-stability, neutral, oxidation and dry heat conditions. 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, Stability, Accuracy .

Abbreviations: UV Detector: Ultraviolet Detector; RSD: Relative Standard Deviation; HPLC: High Performance Liquid Chromatography; ICH: International Conference on Harmonization; GABA: Gamma Amino Butyric Acid; SD: Standard Deviation; PDA: Photodiode array; LOD: Limit of Detection; LOQ: Limit of Qualification; DNA: Deoxyribonucleic acid; RNA: Ribonucleic Acid; HIV: Human Immunodeficiency Virus; LC: Liquid Chromatography; LC-MS: Liquid Chromatography-Mass Spectroscopy; USP: United States Pharmacopeia; Rt: Retention time; RT: Room Temperature

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.

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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. [1] [2] [3] [4]

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. Through its **5**-HT_2C antagonist effect, Agomelatine increases dopamine and nor-adrenaline release specifically in the prefrontal cortex. [5] [6] [7] [8]

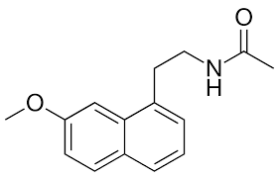


Fig. No.1: Chemical structure of Agomelatine [9]

Table No.1: Properties of Agomelatine

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

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 equipment/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

Materials and Methods [10] [11] [12] [13] [14] [15] [16]

1. Materials

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. The Methanol HPLC Grade methanol was used as a solvent throughout the studies.

2. Determination Of λ max

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. Agomelatine showed absorbance maxima at 284nm.

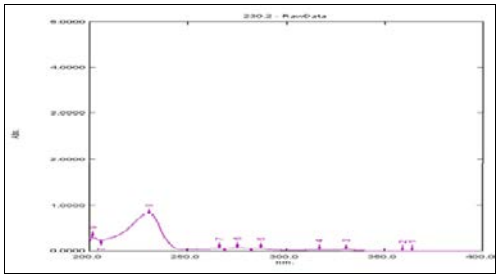


Fig.No.2: UV spectrum of Agomelatine USP

Preparation of Standard Solution

Accurately weighed 50 mg of Agomelatine standard, 70 ml of diluent was added and sonicated. The volume was made up to 100 ml with diluent. 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; 50 ml of purified water was added and sonicated to disintegrate tablets completely, 150 ml of methanol added and sonicated for 30 minutes with intermittent swirling, cooled and diluted up to mark with Methanol and mixed well and 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.

Table No.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	35° C
Injection Volume	20 µl
Run time	12 min

Assay Method [18]

Preparation of buffer: It was prepared by dissolving 1.74 g of di-potassium hydrogen phosphate into 1000 ml milli-Q water, 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.

Preparation of Mobile phase: Mix buffer pH 5.0+-0.05 and acetonitrile in the ratio 40:60 v/v and sonicate to degas.

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

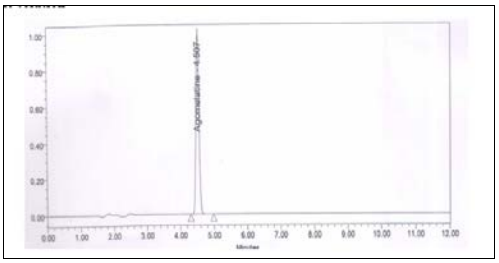
Preparation of Standard Solution

5 ml of stock solution pipette out and diluted up to 50 ml with mobile phase in volumetric flask.

Analytical method and Method Validation [19] [20]

Specificity Sample Solution

Retention time of peak in standard and sample solution was found to be 4.509 and 4.507 min.



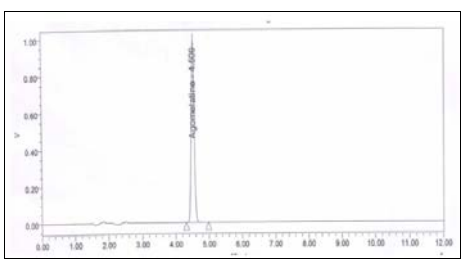


Fig. 3: Specificity of Sample Solution Fig. 4: Specificity of Standard Solution Chromatogram Chromatogram

Table No.5: Specificity of Agomelatine

Sr No.	Sample Name	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%	

Linearity and Range

Linearity was evaluated in the range of 20% to 120% of the working concentration level.

Correlation of coefficient of Agomelatin was found to be 0.99.

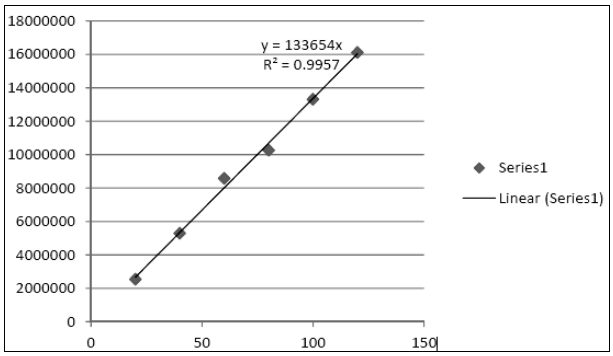


Fig.No.5: Linearity Graph of Agomelatine

Table No.6: Linearity of Agomelatine Accuracy (Recovery)

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

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.

Table No.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 No.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

Mean recovery was found to be 100.33% and RSD was 0.09%.

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.

Table No.9: LOD and LOQ

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

Precision [21] [22]

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.

Table No.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					

The RSD of system precision was found to be 0.298%. Therefore, the UV method for the determination of X capsule was precise.

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 No.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

The RSD of method precision was 0.2%. Therefore, the HPLC method for the determination of Agomelatin was reproducible.

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.

Table No.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	

The RSD of Ruggedness was found to be 1.17%. Therefore, the HPLC method for the determination of Agomelatin tablet was rugged.

System Suitability

Table No.13: System Suitability Parameters

Sr. No	Parameters	Drug X
1	Tailing factor (T)	0.99
2	Number of Theoretical plate (n)	34379
3	Retention time (RT)	4.159
4	% RSD	0.77
5	S/N ratio	23

Stability of Analytical Solution [23]

Stability of sample solution was determined by injecting (in triplicate) tablet sample solution at different time intervals.

Table No.14: Stability of Analytical solutions at room temperature

Sr. No	Name	% Content 0 Hr	% Content 24 Hr	% Content 36 Hr	% Content 48 Hr
1	Standard Solution	100.0	100.5	100.7	100.9
2	Sample Solution	100.0	100.4	100.7	100.9

Forced Degradation Studies [24] [25]

Accurately weighed quantity of tablet powder equivalent to about 30 mg of Agomelatin was transferred separately to six different 50.0 ml volumetric flask, (flask no. 1, 2, 3, 4, 5 and 6). To flask no. 1, 2 and 3, added 3.0 ml methanol as co-solvent followed by addition of 5.0 ml 5 M HCl, 0.1 M NaOH and 1 % H_2O_2 to flask no.1, 2, and 3, respectively.

For neutral hydrolysis, 5.0 ml of water was added to flask no.4. The content of flask no. 1 were heated on water bath at 80^{0}C for 6 hr. whereas content of flask no. 2, 3 and 4 were heated on water bath at 80^{0}C for 2 hr, Flask no. 5 containing tablet powder was kept in hot air oven at

80^{0}C for 24 hr to study the effect of heat on tablet sample (heat degradation). Flask no.6 containing tablet power was exposed to UV-radiations for 24 hr to study the effect of light on tablet sample (photo degradation). After stipulated time interval, the entire flasks were removed and cooled to room temperature. The samples were then treated and analyzed.

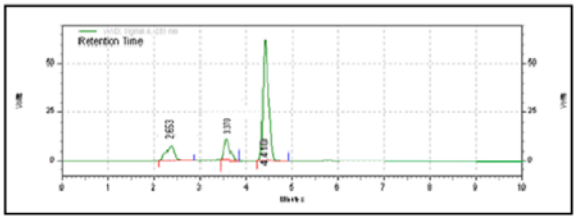


Fig.No.6: Acid Degradation of Sample

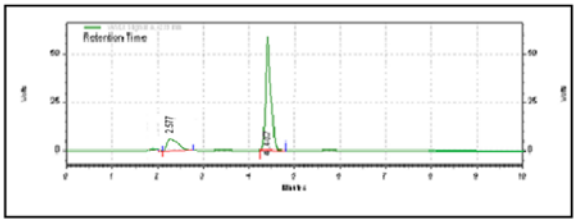


Fig.No.7: Base Degradation of Sample

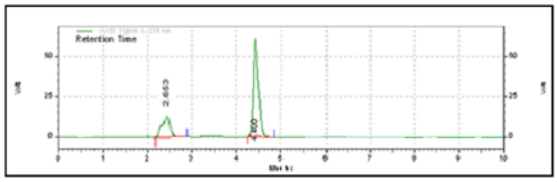


Fig.No.9: Thermal Degradation of Sample

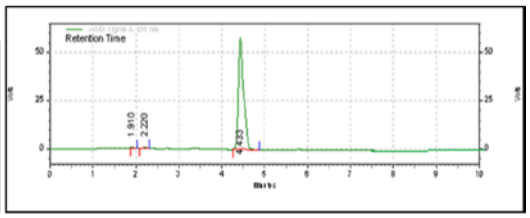


Fig.No.8: Neutral Hydrolysis of Sample

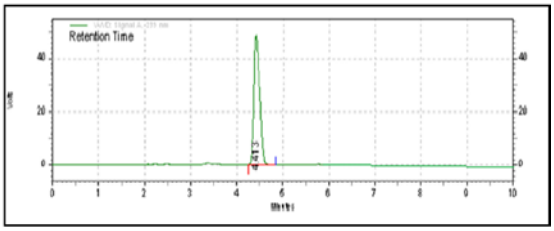


Fig.No.10: Peroxide Degradation of Sample

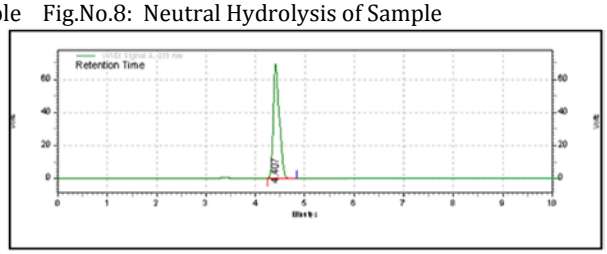


Fig.No.11: Photo Degradation of Sample

Table No.15: Forced Degradation Studies

Sr. No	Stress Conditions	Temperature and Time	% Assay of Active substance	Retention time of Degraded Produc	% Degradation
1	Acid (5 M HCl)	800C for 6 hr	73.43	2.66, 3.37	27%
2	Alkali (0.1 M NaOH)	800C for 2 hr	90.69	2.58	10%
3	Oxide (1 % H2O2)	800C for 2 hr	97.91	-	3%
4	Neutral	800C for 2 hr	84.31	2.65	16%
5	Heat	800C for 24 hr	99.39	-	1%
6	Photo degradation	24 Hr	99.07	-	1%

RESULT

Forced degradation studies for Agomelatine shows:

1) Acidic Degradation (5M HCl) at 80^{0}C for 6 hr Drug shows degradation 27%
2) Alkaline Degradation (0.1 M NaOH) at 80^{0}C for 2 hr Drug shows degradation 10%
3) Neutral Degradation at 80^{0}C for 2 hr Drug shows degradation 16%.

So it can be concluded that the parameters Accuracy, Precision, Specificity, LOD and LOQ are according to the standard limits. And the stress testing of the drugs was carried out under acidic, alkaline, photo-stability and dry heat conditions. Agomelatin was susceptible to acid and alkaline degradation than thermal and photo degradation conditions.

The method developed for quantitative determination of Agomelatin is rapid, precise, accurate and selective. The developed method can be conveniently used for the assay determination of Agomelatin in bulk drugs and pharmaceutical dosage form.

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 No.16: Validation report of the assay of Drug 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 show 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

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