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STABILITY INDICATING RP-HPLC METHOD FOR ESTIMATION OF AGOMELATINE IN TABLET DOSAGE FORM

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ABSTRACT

A simple reverse phase HPLC method was developed for the estimation of Agomelatine in tablet dosage form. Chromatography was performed by isocratic elution on a Stainless steel Thermo scientific C18 column with dimensions 4.6 x 250mm, packed with octadecylsilane bonded to porous silica (C18) with particle size 5 micron methanol and water in the ratio of 75:25 % v/v is used as mobile phase. The flow rate is 1.2 ml/ min and effluent is monitored at 236 nm. Agomelatine was eluted at a retention time of 4.53 minutes. The standard curve of Agomelatine was linear over a working range of 0.01– 200 µg/ml and gave an average correlation coefficient of 0.999. The limit of quantitation (LOQ) of the drug is 0.090 µg/ ml. Recovery studies were carried out by standard addition method and the recoveries are found satisfactory within the range of 99.24 to 99.6 %.The method is precise with % RSD below 2. The method is validated in terms of robustness and forced degradation studies were carried out.

KEY WORDS

Agomelatine RP HPLC, stability indicating assay, validation

1. INTRODUCTION:

Agomelatine (Figure1) N-[2-(7-methoxynaphthalenyl) ethyl] acetamide is a Melatonergic Antidepressant. The molecular formula is $C_{15}H_{17}NO_2$ and the molecular weight is 243.301 gm/mol. It is a Melatonergic agonist and 5-HT antagonist. It acts by increasing noradrenaline and dopamine release specially in frontal cortex and has no influence on the extracellular levels of serotonin. The drug is not official in any of the pharmacopoeia. Literature survey revealed that only one RP-HPLC method was reported, but till date there was no stability indicating RP-HPLC method for Agomelatine. The present study is undertaken in order to develop a new, simple, precise, accurate and specific stability indicating RP-HPLC method in tablet dosage form through stress studies.

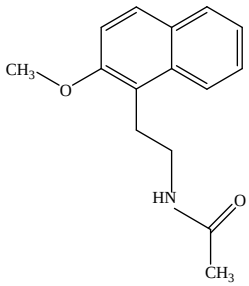


Figure.1: Structure of Armodafinil

2. MATERIALS AND METHODS:

Reagents and chemicals:

Agomelatine bulk drug and tablets were procured as gift sample form MSN MSN laboratories, Hyderabad, A.P, India. Methanol, Acetonitrile, Hydrochloric Acid, Sodium Hydroxide are purchased from MERCK.

Stock solutions and standards:

Standard solution of Agomelatine (1000µg/ml) was prepared by dissolving accurately weighed amount of Agomelatine (25mg) in sufficient quantity of diluent in a 25ml volumetric flask. Then the volume of the flask was made up to the mark with the same. Working standard solution of Agomelatine were prepared by pipetting 0.9 ml of the stock solution into a 10ml of volumetric flask and the volume was made up to the mark with the diluent.

Apparatus and chromatographic conditions:

Quantitative HPLC was performed on Waters HPLC system equipped with waters 515 pump and Waters 2489 dual wavelength UV detector. Empower2 software is used for data acquisition. A Stainless steel Thermo Scientific column with dimensions 4.6 x 250mm, packed with Octadecylsilane bonded to porous silica (C18) having particle size 5 micron.

Method development and optimization:

To develop a suitable HPLC method for the determination of Armodafinil, trials were made with different mobile phases, using methanol, water, buffer (0.5% w/v potassium dihydrogen phosphate in water) in different pH with different compositions of mobile phases (80: 20, 75:25). The method was optimized finally using combination of Methanol and water in the ratio of 75/25 % v/v with a flow rate of 1.2 ml/ min. The drug was eluted at retention time around 4.53 min with symmetric peak shape. The run time was set for 7 minutes. The detection is performed at wavelength 236 nm.

System suitability

For performing system suitability studies, 100% test concentration under degradation conditions was selected. System suitability test was performed by injecting blank solution once and standard solution of 100% test concentration six times in to stabilized HPLC system. The system suitability was established by evaluating the system suitability parameters from the last peak obtained. System suitability parameters include retention factor (k'), repeatability, resolution (R), tailing factor (T) and theoretical plates (N). It was performed by using the concentration of 90µg/ml. The system suitability data was given in the table 1

Assay of Armodafinil marketed formulation

Twenty tablets of Agomelatine were weighed, ground in to a fine powder and mixed thoroughly. A quantity of powder equivalent to 25 mg of Agomelatine was weighed and transferred in to a 25ml volumetric flask and was dissolved in the diluent. The volume was made up to the mark with the same and the resulting solution was labelled as sample stock solution. The solution obtained was diluted with mobile phase so as to obtain a required concentration. The solution thus prepared was filtered through 0.45µ membrane filter and the filtrate was sonicated for 5min. 20µl of working standard solution and test sample solution were injected six times at the optimized method conditions and the chromatograms were recorded and peak areas were calculated, and the % Assay was calculated by using the following formula:

$$\%ASSAY = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{AW}{LC} \times 100$$

Where,

AT - Average area counts of test sample preparation
AS - Average area counts of working standard preparation
WS - Weight of working standard taken in mg
DS - Dilution of standard preparation
DT - Dilution of sample preparation
WT - Weight of sample taken in mg
P - Percentage purity of working standard.
LC - Label claim
AW - Average weight of tablets

Validation of the assay method [7-9]:

Linearity:

Linearity solutions for assay method were prepared from stock solution at levels from 0.01 to 200µg/ml of analyte concentration. The graph of peak area versus concentration was plotted by least-squares linear regression analysis.

The linear fit of the system was illustrated graphically. The linearity range was found to be 0.01 - 200 µg/ml. The standard calibration curve for Agomelatine was constructed using the average peak-area versus the nominal concentrations of the analyte. Linear least-squares regression analysis was performed to assess the linearity.

Recovery and accuracy:

To evaluate the accuracy of the proposed method, recovery studies were carried out by standard addition method, where a known amount of different oncentrrations of pure drug at five levels of 50%, 75%, 100%, 125% and 150% were spiked with solution of pre analysed formulation of concentration 100 µg/ ml. At each level recovery studies were carried out in triplicate and expressed as % recoveries. The percentages of recoveries were calculated from the slope and *Y*-intercept of the calibration curve obtained. Accuracy/recovery experiments were performed in triplicate.

Precision:

The precision was carried out at three levels, intra assay precision of injection, intermediate precision and reproducibility.

Intra assay precision was assessed using 9 determinations covering the range of 50,100 and 150% concentration levels of drug solution.

Intermediate precision (inter day precision) was assessed by inducing typical variations like different days and different columns.

Reproducibility was assessed by different analysts.

Robustness:

Robustness of the method was studied under degradation conditions to study the effects of degradants on Armodafinil in changes method conditions. It was carried out by considering deliberate changes in detection wavelength, flow rate, mobile phase ratio. Robustness was carried out by changing detection wavelength by ±3 nm. Robustness was checked by changing the proportion of organic solvent in the mobile phase by ±4%. It was also checked for robustness by change in flow rate by ±0.2 ml/ min.

Forced degradation studies:

To study the specificity of the method, pure drug was stressed under different degradation conditions. Degradation studies were carried out by exposing drug for acid hydrolysis, alkali hydrolysis, oxidative degradation, thermal degradation and photolytic degradation. Mobile phase is used as solvent for all degradation studies. All the solutions for degradation studies were prepared by dissolving Armodafinil drug in little amount of mobile phase and the volume was made up to the mark with 0.1N HCl, 0.1N NaOH, 1% H_2O_2. Acid hydrolysis is carried out by exposing the drug to 0.1N HCl. Alkali hydrolysis is carried out by exposing the bulk drug and powdered sample to 0.1N NaOH. Oxidative degradation is carried out by exposing the bulk drug to 1% H_2O_2.Thermal degradation is carried out by exposing the bulk drug in Hot air oven at 50 ^{0}C. Photolytic degradation is carried out by exposing the bulk drug to sun light. The degradation studies were carried at a time interval of 15 minutes. The drug solution was prepared at a concentration of 100µg/ ml.

Acid degradation:

10mg of drug was dissolved in a few ml of mobile phase in a 10ml volumetric flask. The volume was made up to the mark with 0.1N HCl, mixed thoroughly and kept aside. After 15, 30 minutes, solution was mixed and 1ml of this solution was pipetted into another 10ml volumetric flask. To this 1ml solution, 1ml of 0.1N NaOH was added to neutralize the acid and final volume was made upto the mark with mobile phase and its peak area was observed by injecting into HPLC.

Alkali degradation:

10mg of drug was dissolved in a few ml of mobile phase in a 10ml volumetric flask. The volume was made up to the mark with 0.1N NaOH, mixed thoroughly and kept aside. After 15, 30 minutes, solution was mixed and 1ml of this solution was pipetted into another 10ml volumetric flask. To this 1ml solution, 1ml of 0.1N HCl was added to neutralize the alkali and volume was made up to the mark with mobile phase and its peak area was observed by injecting into HPLC.

Photo degradation:

Drug powder was exposed to sunlight. After 15, 30 minutes, 10mg of the exposed powder was dissolved in mobile phase in a 10ml volumetric flask. From this solution, 1ml was pippeted into another 10ml volumetric flask and its volume was made upto the mark with mobile phase. The peak area of this solution was observed.

Thermal degradation:

Drug powder was exposed to 50^0C in a hot air oven. After 15, 30 minutes, 10mg of the exposed powder was dissolved in mobile phase in a 10ml volumetric flask. From this solution, 1ml was pippeted into another 10ml volumetric flask and its volume was made up to the mark with mobile phase. The peak area of this solution was observed.

3. RESULTS:

System suitability

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. The System suitability Parameters were found to be within the specified limits for the proposed method. The results were given in table 1

Assay of Armodafinil marketed formulation

The representative chromatograms are shown in figure 1 & 2.the peak areas were mentioned in the table 2. The assay limits for Agomelatine was 90-110 % and the results obtained for Agomelatine was found to be 99.05. Hence the results were within the limits.

Validation of the assay method [7-9]:

Linearity:

The linearity range was found to be 25-75 µg/ml forAgomelatine. Calibration curve was plotted and correlation co-efficient for both the drugs found to be 0.999. Hence the results obtained were within the limits. The linearity curves were shown in figure 3**.** The linearity results were reported in table 3.

Recovery and accuracy:

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 results were shown in Table 4.

Precision:

In the precision study, % RSD was found to be 1.82% which indicates that the system has good reproducibility. % RSD was determined for peak areas ofAgomelatine. The acceptance limit should be not more than 2 % and the results were found to be within the acceptance limits. The Results were reported in Table 5.

Robustness:

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. The results were reported in Tables 6 &7.

Forced degradation studies:

Degradation studies were carried out by exposing drug for acid hydrolysis, alkali hydrolysis, oxidative degradation, thermal degradation and photolytic degradation. The results of forced degradation studies were shown in the table 8. Representative chromatograms were given in Figure 4.

Table 1: Observation of System Suitability Parameters

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

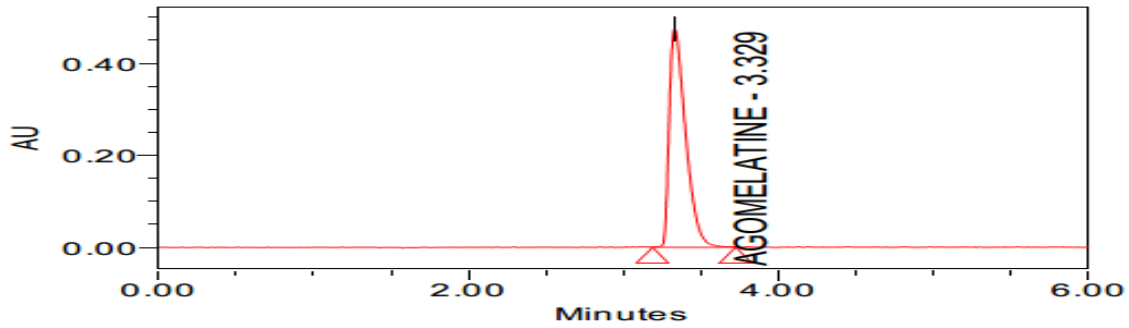


Fig. 1: Representative Chromatogram of standard Agomelatin

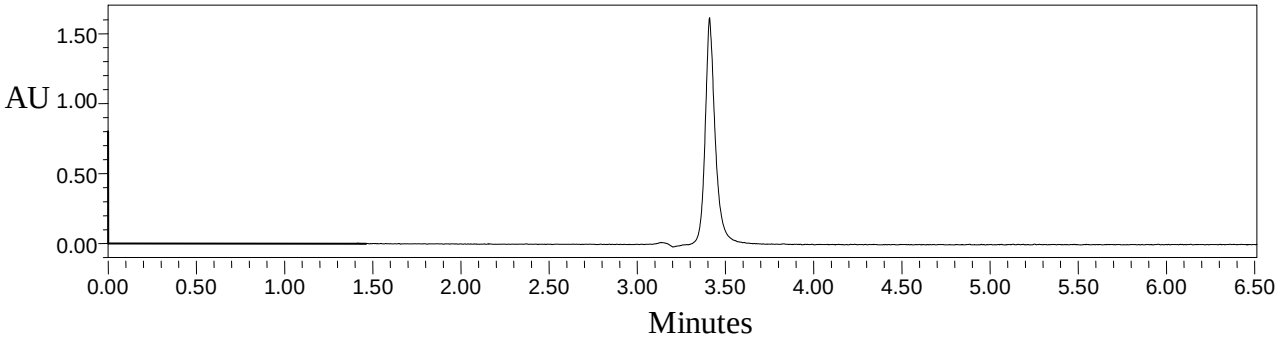


Fig. 2: Representative Chromatogram of Agomelatine test sample

Table 2: Assay Results: (Agomelatine)

PARAMETER	AGOMELATINE
Avg area of sample preparation	3067665
Avg area of standard preparation	3066234
Weight of standard taken	25 mg
Percentage purity	99.1
Avg weight of sample	153.95 mg
Avg weight of standard	153.95 mg
Label claim	25 mg
% Assay	99.05%

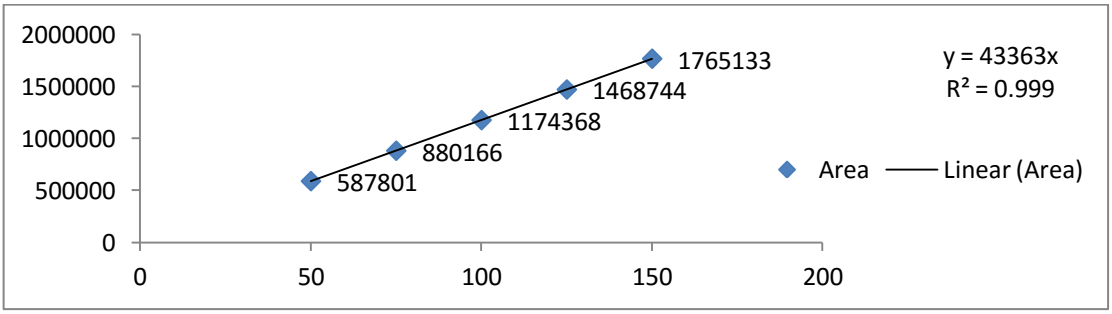


Fig. 3: Linearity curve

Table 3: Observations of Linearity

S.No	Conc %	Area	µg/ml
1	50	587801	25
2	75	880166	37.5
3	100	1174368	50
4	125	1468744	62.5
5	150	1765133	75

Table 4: Results of Accuracy

AGOMELATINE						
Spiked Level	**Sample Weight**	**Sample Area**	**µg/ml added**	**µg/ml found**	**% Recovery**	**% Mean**
50%	76.98	1532382	24.775	24.76	99.95	
50%	76.98	1522694	24.775	24.61	99.32	
50%	76.98	1530961	24.775	24.74	99.86	
50%	76.98	1528252	24.775	24.70	99.68	99.67
50%	76.98	1526691	24.775	24.67	99.58	
50%	76.98	1527132	24.775	24.68	99.61	
100%	153.95	3069777	49.550	49.61	100.12	
100%	153.95	3066656	49.550	49.56	100.01	99.9
100%	153.95	3053059	49.550	49.34	99.57	
150%	230.93	4597421	74.325	74.29	99.96	
150%	230.93	4598108	74.325	74.30	99.97	
150%	230.93	4590280	74.325	74.18	99.80	99.89
150%	230.93	4592383	74.325	74.21	99.85	
150%	230.93	4590676	74.325	74.18	99.81	
150%	230.93	4595224	74.325	74.26	99.91	

Table 5: Results of Precision

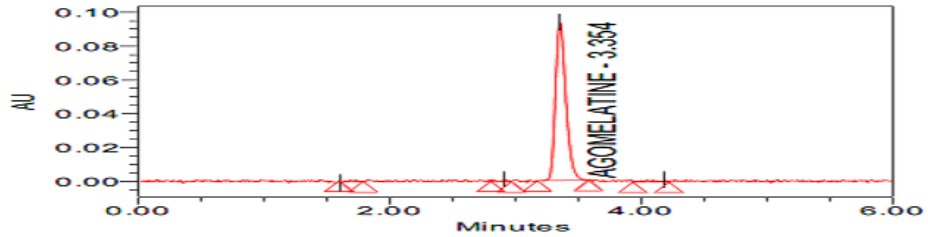
S.No	Sample Weight	Sample Area-1	% Assay
1	153.95	3067665	99.15
2	153.95	3061482	98.95
3	153.95	3060365	98.91
4	153.95	3061218	98.94
5	153.95	3067460	99.14
6	153.95	3068851	99.18
Average Assay:			99.04
STD			0.12
%RSD			0.13

Table 6: System suitability Results of Agomelatine for robustness

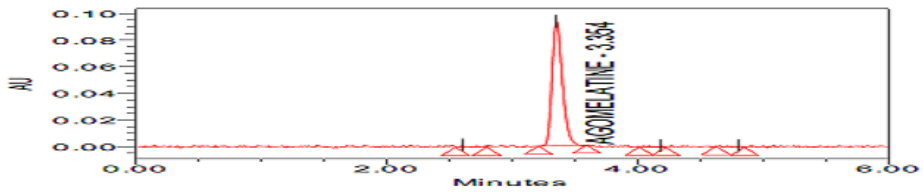
Change in Flow Rate (ml/min)		System Suitability Results		
		USP Plate Count	USP Tailing	Retention time(min)
Low	0.8	7053	1.601	4.178
Actual*	1.0	6302	1.24	3.374
High	1.2	6698	1.538	3.343
Change in Temperature		**System Suitability Results**		
		USP Plate Count	**USP Tailing**	**Retention time(min)**
5% more	6678	1.591	3.323	5% more
Actual*	6302	1.24	3.374	Actual*
5% less	6280	1.569	2.805	5% less

Figure 4: Representative chromatograms for forced degradation:

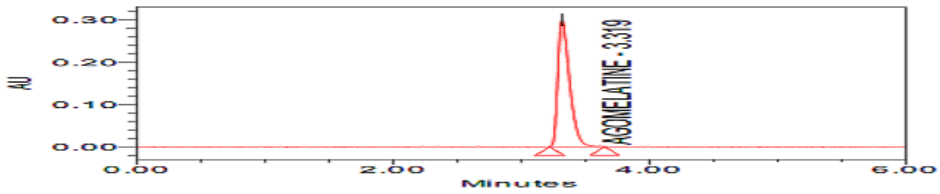
Hydrolytic condition:



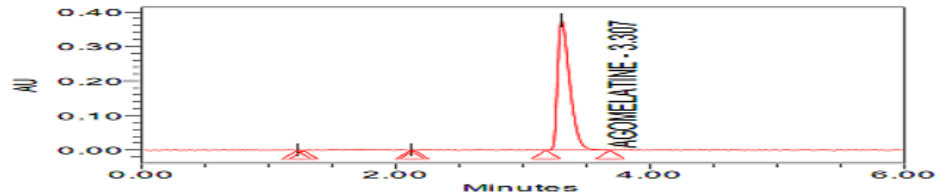
Oxidative condition:



Thermolytic condition:



Photolytic condition:



7: Stability indicating Parameters

S.No	Substance used for degradation	Sample Weight	Sample Area-1	% Assay	%DEG
1	ACID	153.95	2807863	90.75	8
2	BASE	153.95	2886757	93.30	6
3	PEROXIDE	153.95	2759459	89.19	10
4	LIGHT	153.95	2966757	95.88	3
5	HEAT	153.95	2638329	85.27	14
6	Average Assay:		2343194.2	-	-
7	STD		1153355.7	-	-
8	%RSD		49.2	-	-

4. CONCLUSION

The main objective of the present work is to develop a simple stability indicating RP-HPLC method in tablet dosage form. The optimum wavelength for the determination of Agomelatine was selected at 236 nm. Agomelatine peak was achieved with good, peak shape and symmetry at R_t - 4.227. The assay was performed on the tablet formulation and the % drug content for Agomelatine was found to be 98.86% which was within the acceptance limits. The system suitability of the proposed method was accomplished from the resolution, theoretical plate count and asymmetric factor of Agomelatine at the optimized conditions. The mean theoretical plate count was found to be 5643 and the mean asymmetric factor was found to be 1.12 which were in compliance with the acceptance specifications. The developed analytical method was validated according to ICH guidelines with respect to parameters such as precision, linearity, accuracy, limit of detection, limit of quantitation, robustness and specificity. From this forced degradation study it was found that the degradation products did not interfere with the analyte peak. Hence, the proposed method was found to be specific. The developed RP-HPLC method was used for quantitation of drug in the presence of degraded products.

5. REFERENCES

1. HE Xue-jun1, REN Zhein, WANG Juan, LIU Jing 2, DU YU1, QIAO Hong-qun, Determination of Agomelatine by RP-HPLC, West China Journal Of Pharmaceutical Sciences, 2010.
2. Wang Yujing, Zhang Ghen, Wang Decai, Tao Weibo, Ge Wen Lan, Determination of Agomelatine in tablets by HPLC, Modern Applied Pharmacy in China, 2012, 29(7), 652-655.

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