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Development and Validation of Stability- Indicating RP-HPLC Method for the Estimation of Agomelatine in API.

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ABSTRACT

Agomelatine is a new melatonergic antidepressant with a unique pharmacological action. A stability-indicating RP-HPLC method was developed and validated for the determination of agomelatine in active pharmaceutical ingredient using enable C18 column (150×4.6mm, 5µm) in isocratic mode. The mobile phase consisted of acetonitrile: methanol: water (55:25:20, v/v/v) with a flow rate of 1.0 ml/min (UV detection- 230nm). The Retention time was found to be 4.2 min. Linearity was observed over the concentration range of 19 ng/ml to 60µg/ml and the correlation coefficient R2value was found to be 0.9988. The method is accurate and recovery was found to be in the range of 98-100.7%. The limit of detection of agomelatine was found to be 4ng/ml and limit of quantitation was found to be 15 ng/ml. Agomelatine was subjected to stress conditions including acidic, alkaline, oxidation, photolysis and thermal degradation. Agomelatine is more sensitive to acidic and oxidative degradation. The method was validated according to ICH guidelines.

Key words: Agomelatine, HPLC method, Validation, Stability-indicating.

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INTRODUCTION

Agomelatine is a new melatonergic antidepressant with a unique pharmacological action. It has potential role in the treatment of patients with major depressive disorder (MDD). Agomelatine is a chemical compound that is structurally closely related to melatonin.[1] Agomelatine has a new pharmacological mechanism of action, which combines melatonin MT1 and MTagonist properties with a serotonin 5-HT2C antagonist effect. Agomelatine was rapidly and well (≥80%) absorbed after oral administration. Because of its action upon the melatonin receptors, agomelatine shows a marked improvement in sleep quality.[2]

Agomelatine (N-[2-(7-methoxynaphthalen-1-yl) ethyl] acetamide), its antidepressant efficacy has been verified in the treatment of major depressive disorder (MDD).Agomelatine showed significant benefits over paroxetine due to the complete absence of side effects including the associated sexual side effects that are troublesome with some antidepressants.[3] Agomelatine has also proven to have anxiolytic properties and thus may prove to be very useful in the treatment of anxiety disorders.[4,5]

Literature survey reveals that very few analytical methods reported for the estimation agomalatin in API, pharmaceutical formulations and biological samples.[6,7] None of the literature review indicates stability studies for agomelatine. Therefore, an attempt has been made to develop a method for stability studies on agomelatine.

MATERIALS AND METHODS

Instrumentation and analytical conditions

The analysis of the drug was carried out on Schimadzu HPLC model with LC-10 software containing LC-20AT pump, UV/Visible detector (SPD 20 A), and hamilton syringe with 20 µl capacity. Chromatographic analysis was performed by using enable C_{18} G column (250×4.6mm, 5µ). Schimadzu electronic balance was used for weighing. Isocratic elution was performed by using a mobile phase acetonitrile : methanol : water (55:25:20) at a flow rate of 1.0 ml/min. Detection was carried out at 230 nm with a run time of 10 min. The mobile phase was prepared freshly and it was sonicated to degas the solvent for 5 min. The column and HPLC system were maintained at ambient temperature.

Chemicals and Reagents

All the solvents used like methanol and acetonitrile which are of HPLC grade were purchased from SD Fine Chemicals. The standard drug agomelatine is gifted from MSN laboratories, Hyderabad.

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Preparation of stock, working standard and sample solutions

The standard stock solution was prepared by transferring 100 mg of agomelatine into a 100 ml volumetric flask. To this, few ml of methanol was added and sonicated to dissolve the drug and the volume was made up with methanol. 10 ml of standard stock solution was taken in a 100 ml volumetric flask and the volume was made up with methanol and sonicated.

An accurate quantity of powder equivalent to 100 mg of agomelatine was weighed and transferred to a 100 ml volumetric flask. 100 ml of methanol was added, shaken for 5 min, sonicated for 15 min and filtered through 0.45 µ membrane filter to obtain a clear solution. From the primary stock solution, 10 ml was taken in a 100 ml volumetric flask and diluted with methanol and sonicated. This secondary stock sample solution was diluted quantitatively with methanol to obtain suitable working sample solutions for chromatographic measurements.

RESULTS AND DISCUSSION

Method Development and Optimization:

Proper selection of the method depends upon the nature of the sample (ionic/ionisable/neutral molecule), its molecular weight and solubility. The drug selected in the present study is polar in nature. The reversed phase HPLC was selected for the separation because of its simplicity and suitability.The sensitivity of HPLC method which uses UV detection depends upon the proper selection of wavelength. An ideal wavelength is one that gives good response for all the drugs to be detected. A UV spectrum for methylphenidate was recorded between 200-400nm. The λ_{max} was obtained at 230 nm by using 1cm quartz cell. Different mobile phases were tried but satisfactory separation and symmetrical peaks were obtained by using a mobile phase consisting of acetonitrile : methanol : water in the ratio 55:25:20. The retention time for agomelatinewas found to be 4.2±0.5min.The %RSD values obtained were found to be< 2% which revealed that the developed method was precise.

Method validation

The method was validated for linearity, precision, specificity, limit of detection, limit of quantification and robustness

Linearity

The linearity of this method was evaluated by Linear Regression Analysis, which was calculated by Least Squaremethod and The calibration curve for agomelatine [**Fig 1**] was linear over the concentration range of 0.019-60µg/ml. The correlation coefficient was found to be 0.9988.

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Table 1 represents the regression data including, linearity range, slope, correlation coefficient.

S.NO	PARAMETER	VALUES
1.	Linearity range	0.019-60µg/ml
2.	Slope	269511
3.	Correlation coefficient(r^2)	0.9988

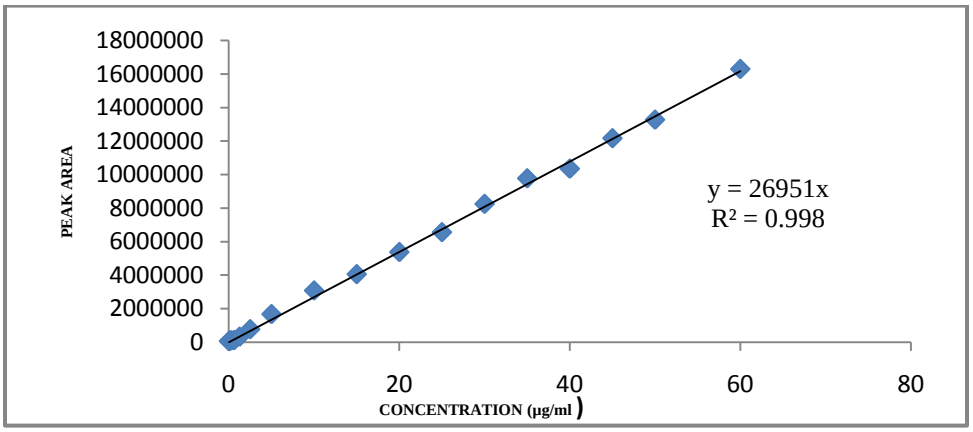

Fig: 1 Calibration curve for Agomelatine

Precision

Precision of the method was determined by performing intraday and interday precision. The % RSD obtained for intraday and interday precision was less than 2%. Intraday and interday precision values are presented in Table 2. The % RSD values were within 2 and the method was found to be precise.

Table 2: Intraday, inter day precision of agomelatine

Concentration (µg/ml)	Precision (%RSD)	
	Intraday	***Interday***
2.5	1.8	1.6
5	1.6	1.5
20	1.8	1.7
40	1.1	1.9
60	1.9	1.2

Sensitivity

The Sensitivity of measurement of agomelatineby use of the proposed method was estimated in terms of the Limit of Detection (LOD) and the Limit of Quantitation (LOQ).

Limit of detection (LOD) and Limit of quantification (LOQ) were estimated from the signal-to-noise ratio. The detection limit was defined as the lowest concentration level resulting

in a peak height of three times the baseline noise. The quantification limit was defined as the lowest concentration level that provided a peak height with a signal-to noise ratio higher than 10. The LOD and LOQ values for agomelatinewere reported in the Table 3.

Robustness

The Robustness of the method was determined under different conditions including change in flow rate, wave length.The chromatograms were recorded and the results of the chromatograms are given in Table 4.

Table 3: LOD and LOQ results for agomelatine

Sample	LOD (ng/ml)	LOQ (ng/ml)
Agomelatine	4.0	15

Table 4: Robustness values of agomelatine

Chromatographic changes		(% RSD)
Flow rate (mL/min)	0.6	1.6
	0.8	0.9
	1.0	1.3
Wavelength (nm)	230	1.2
	232	0.9
	234	0.8

Accuracy

Accuracy of the method was determined by recovery studies. Sample solutions were prepared in triplicate at levels 80%, 100% and 120% of standard concentration using agomelatinedrug substance as per the test method and injected each solution into HPLC as per methodology. The method is accurate and recovery was found to be in the range of 98-100.7% as shown in

Table 5: Method accuracy from recovery assays

Method	Concentration	Amount found	% Recovery
Percentage method	80%	78.5mcg	98.53
	100%	101.5mcg	100.18
	120%	109.56mcg	100.79

Force Degradation Studies

Stress testing of the drug substance can help identify the likely degradation products, which can in turn help establish the degradation pathways and the intrinsic stability of the molecule and/or validate the stability indicating power of the analytical procedures used.

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Forced degradation is a powerful tool used routinely in pharmaceutical development in order to develop stability indicating methods that lead to quality stability data and to understand the degradation pathways of the drug substances and drug products. Forced degradation studies are indispensable in the development of stability-indicating and degradant-monitoring methods as part of a validation protocol. In general, values anywhere between 5% to 20% degradation of the drug substance have been considered as reasonable and acceptable for validation of chromatographic assays.[8,9]

In order to establish whether the developed method is stability indicating both the drugs were stressed under various conditions (acid, base, oxidation and thermal) to perform forced degradation studies.Agomelatine is more sensitive to acidic and oxidative degradation. The peaks of degraded products were well separated from the analyte peak with good resolution [Fig 2 (a, b, c, d)] which indicates that the developed method is stability indicating. The forced degradation studies conditions and results summarized in Table 6 and 7.

Table 6: Forced degradation conditions and parameters

Parameter	Condition	Time points
Acidic/Solution	HCl (1.0N, RT, 70°C)	Initial –7 days
Basic/Solution	NaOH (1.0N, RT, 70°C)	Initial –7 days
Oxidative/Solution	H_2O_2+ Initiator	7 Days
Thermal/Humidity	70°C/75% RH	6 weeks
Photo (UV light)*	1,000 watt hrs/m2, RT	5 X ICH
Photo (Fluorescent light)*	6 x 106 lux hrs, R.T.	5 X ICH

Table 7: Results for degradation studies

Degradation mechanism	Degradation condition	Area	%Degradation
Bulk drug	Undegraded	6645683	-
Acid degradation	1N HCl	1733874	18%
Base degradation	1N NaOH	5874567	8%
Peroxide degradation	30%H_2O_2/85°C /120 min	5668227	12%
Thermal degradation	60^0C	6645683	Stable

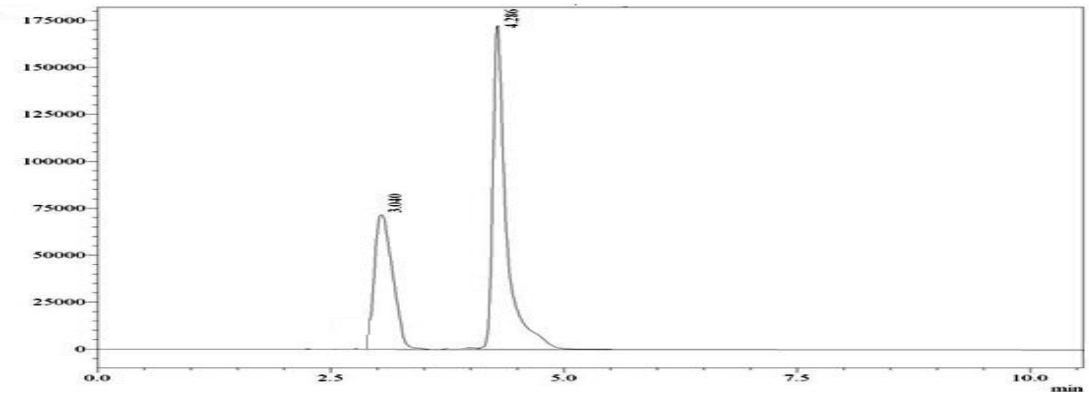

Fig 2a: Chromatogram for stability studies (acidic)

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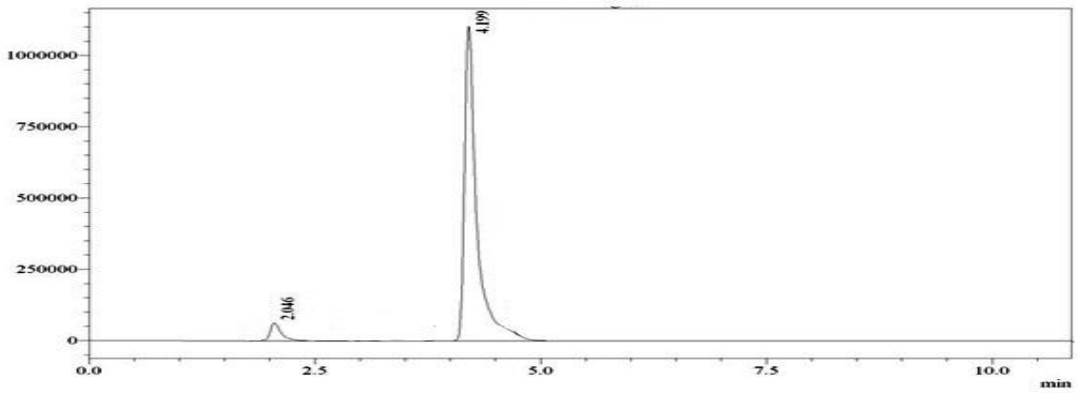

Fig 2b: Chromatogram for stability studies (Base)

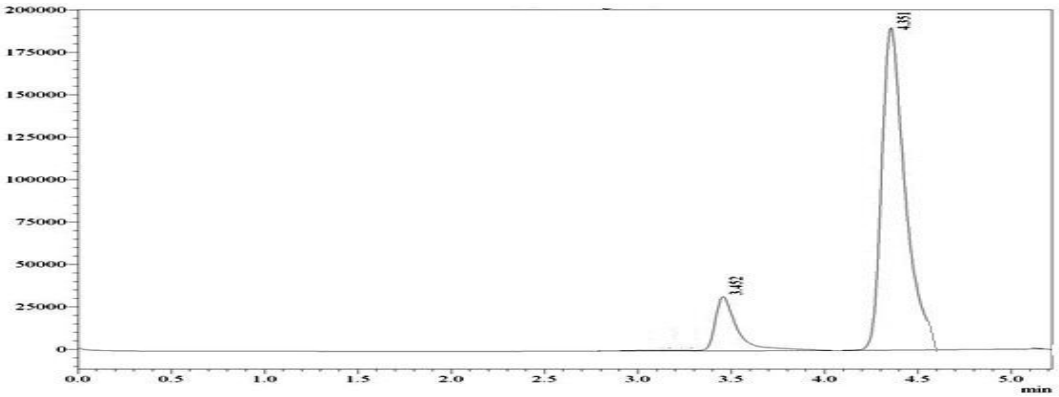

Fig 2c: Chromatogram for stability studies (Oxidation)

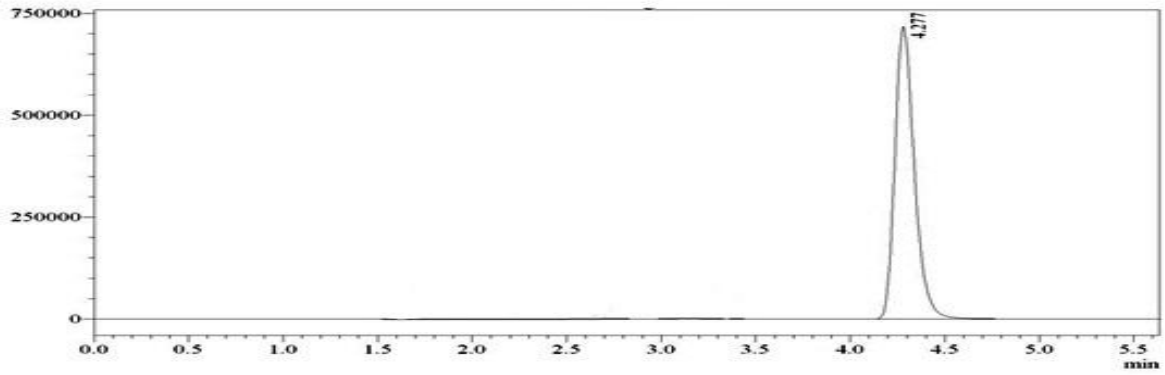

Fig 2d: Chromatogram for stability studies (Thermal degradation)

CONCLUSION

A new precise, accurate, robust, stability indicating RP-HPLC method has been developed for the estimation of agomelatine in active pharmaceutical ingridient.The intra-run and inter-run variability and accuracy results werefound in acceptable limit. Simplicity of the method, econamical nature and low limit of detection and quantitation makes the method superior to the other reported HPLC methods. The developed method was applied for the stability studies of agomelatine in bulk dosage form. The results of forced degradation studies reveal that the method is stability indicating. The proposed method has the capability to

separate the analyte from their degradation products obtained during forced degradation studies. The method can be employed for the routine analysis agomelatine.

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