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Development and Validation of UV Spectrophotometric Method for Estimation of Agomelatine in Bulk and Pharmaceutical Dosage Form

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ABSTRACT

A simple, rapid, accurate, precise, sensitive and economical UV Spectrophotometric method has been developed for estimation of Agomelatine from bulk and pharmaceutical formulation. The λ_{max} of Agomelatine in Acetonitrile was found to be 229 nm. The parameters linearity, precision, accuracy, robustness were studied according to International Conference on Harmonization guidelines. The drug follows linearity in the concentration range 2- 16 μ g/ml with correlation coefficient value 0.998. The accuracy of the method was checked by recovery experiment performed at three different levels i.e., 50%, 100% and 150 %. The % recovery was found to be in the range 98%– 101%. The low values of % R.S.D. are indicative of the accuracy and reproducibility of the method. The precision of the method was studied as an intra-day, inter-day variations and repeatability. The % R.S.D. value less than 2 indicate that the method is precise. The above method was a cost-effective quality- control tool for routine analysis of Agomelatine in bulk and in pharmaceutical dosage form.

Keywords: Agomelatine, Validation, UV Spectrophotometric method

INTRODUCTION

Agomelatine is chemically N-[2-(7-methoxy naphthalen-1-yl) ethyl] acetamide (Fig.1). Its molecular formula is $C_{15}H_{17}NO_2$ and its molecular weight is 243.301gm/mol. Agomelatine is a sleep modulating antidepressant was approved by the European Medicines Agency for the treatment of major depressive disorder (MDD) in 2009. The novel antidepressant agomelatine acts as a melatonergic receptor (MT_1/MT_2) agonist and serotonergic receptor ($5-HT_2C$) antagonist. Binding studies indicate that it has no effect on monoamine uptake and no affinity for α , β adrenergic, histaminergic, cholinergic, dopaminergic, and benzodiazepine receptors. Agomelatine showed significant benefits over paroxetine due to the complete absence of side effects including the associated sexual effect that are troublesome with some antidepressant. Because of its action upon the melatonin receptors, agomelatine shows a marked improvement on sleep. Agomelatine has also proven to have anxiolytic properties and thus may prove to be very useful in the treatment of anxiety disorders.

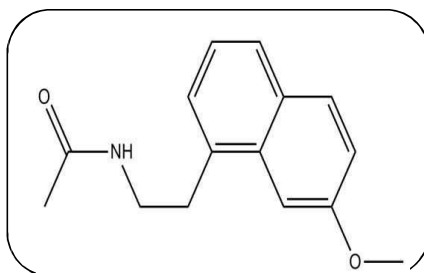


Figure: 1 Chemical structure of agomelatine

The parent drug stability test guideline Q1A (R2) issued by International Conference on Harmonization (ICH) suggests that stress testing is an essential part of development strategy and is carried out under more severe condition than accelerated conditions. These studies provide information to establish its inherent stability characteristics, leading to identification of degradation products and hence supporting the suitability of the proposed analytical methods. According to ICH guidelines stress testing should include the effect of temperature, light, oxidizing agents as well as susceptibility across a wide range of pH values and separation of drugs from degradation products. It is also suggested that analysis of stability sample should be done by using validated stability testing methods.

Methods reported in literature for analysis of agomelatine include determination of agomelatine by RP-HPLC and HPLC method for separating and analyzing agomelatine intermediate and final product thereof and also Validated LC-MS/MS method for quantification of agomelatine in human plasma and its application in a pharmacokinetic study. To our knowledge there is no validated UV Spectrophotometric method reported for agomelatine in pharmaceutical dosage form.

MATERIALS AND METHODS

UV-Visible Spectrophotometer with matched quartz cells (1cm)

- Model : UV-Visible Spectrophotometer T60
- Make : T60 LABINDIA

Reagents and Reference substance

Agomelatine standard was provided by Electron Inc.-Ahmadabad (India). Agomelatine tablets containing 25mg agomelatine and the inactive ingredient used in drug matrix were obtained from market. Analytical grade Acetonitrile and water were obtained from Merck Life Science Pvt. Ltd., Mumbai (India).

Diluents

Water is used as diluent.

Selection of Solvent and Detection wavelength:

Drug solution of 100 μ g/ml was scanned over the range of 200-400 nm in UV region using different solvents like water, acetonitrile and methanol. It was observed that the drug showed maximum absorbance in acetonitrile at 229 nm, hence acetonitrile was used as solvent and 229 nm was used as detection wavelength for Agomelatine.

Preparation of standard stock solution:

Accurately weighed 50mg of Agomelatine was dissolved in 50ml of acetonitrile to get 1000 μ g/ml stock solution. Working standard solutions were further diluted to get concentration range of 2-16 μ g/ml.

Determination of wavelength of maximum absorption:

A UV spectroscopic scanning (200-400 nm) was carried out with the standard solution to determine the λ_{max} for the detection of agomelatine using diluents as blank.

Specificity study:

The test for specificity was carried out using only excipients. Spectra for blank and sample were compared (Fig.2). Secondly the specificity was determined by subjecting the sample solution to accelerated degradation by heat (60 $^{\circ}$ C) for 72hrs in order to verify that none of the degradation products interfered with the quantification of the drug.

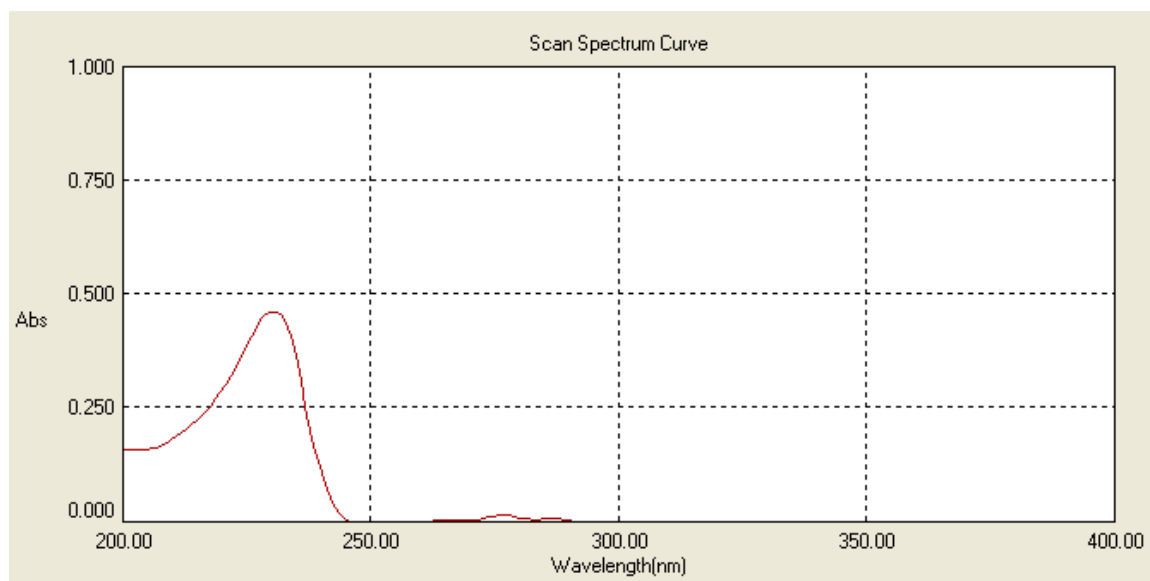


Fig. 2: UV spectrum of agomelatine

Linearity:

Linearity test solutions for the assay method were prepared at seven concentration levels from 40 to 160 % of assay analyte concentration (2, 4, 6, 8, 10, 14 and 16 $\mu\text{g/ml}$) with correlation coefficients of 0.998. The peak areas versus concentration data were evaluated by linear regression analysis.

Intra-day precision and Inter-day precision:

The precision of the assay method was evaluated in terms of repeatability by carrying out six independent assays of agomelatine test sample preparation and calculated the % RSD of assay (intra-day). Intermediate precision of the method was checked by performing same procedure on the different day (inter-day) by another person under experimental condition.

Accuracy:

An accuracy study was performed by adding known amounts of agomelatine to the placebo Preparation. The actual and measured concentrations were compared. Recovery of the method was evaluated at three different concentration levels (corresponding to 50, 100 and 150 % of test preparation concentration).

Robustness:

The robustness of study was carried out to evaluate the influence of small but deliberate Variations in the Spectrophotometric method. The factors chosen for this study were the diluents change and analyst change.

Solution stability:

The stability study of solution for test preparation was carried out. The solution was preserved at ambient temperature and 2-5°C and tested at interval of 12, 24, 36 and 48 hr. The responses for the aged solution were evaluated using a freshly prepared standard solution.

RESULTS AND DISCUSSION

Method development and optimization

Agomelatine is slightly soluble in aqueous medium and freely soluble in organic solvents like methanol and acetonitrile. During the development and optimization phase standard solution of agomelatine was prepared using Acetonitrile and then further dilution was given by using water to obtain sample solution. The pre-determined wavelength of maximum absorption (λ_{max}) was 229 nm.

Method validation

After development of the analytical method, it was validated in accordance with ICH and USP guidelines. This furnished evidence the method was suitable for its intended purpose. The intensive approach described in this manuscript was used to develop and validate a UV Spectrophotometric method that can be used for assay validation of agomelatine in a pharmaceutical dosage form.

The specificity of the method was evaluated by checking the interference of placebo with analyte and the proposed method was evaluated by checking the peak purity of agomelatine during the force degradation study. There was no interference of any peak of degradation product with drug peak.

To determine linearity a calibration graph was obtained by plotting agomelatine concentration against peak area. Linearity was good in the concentration range 2 to 16 $\mu\text{g/ml}$. The response of the drug was found to be linear regression equation for agomelatine was $y = 0.0476x - 0.041$ with correlation coefficient 0.9982 where x is the concentration in $\mu\text{g/ml}$ and y is the peak area in absorbance units (AU) (Fig.3).

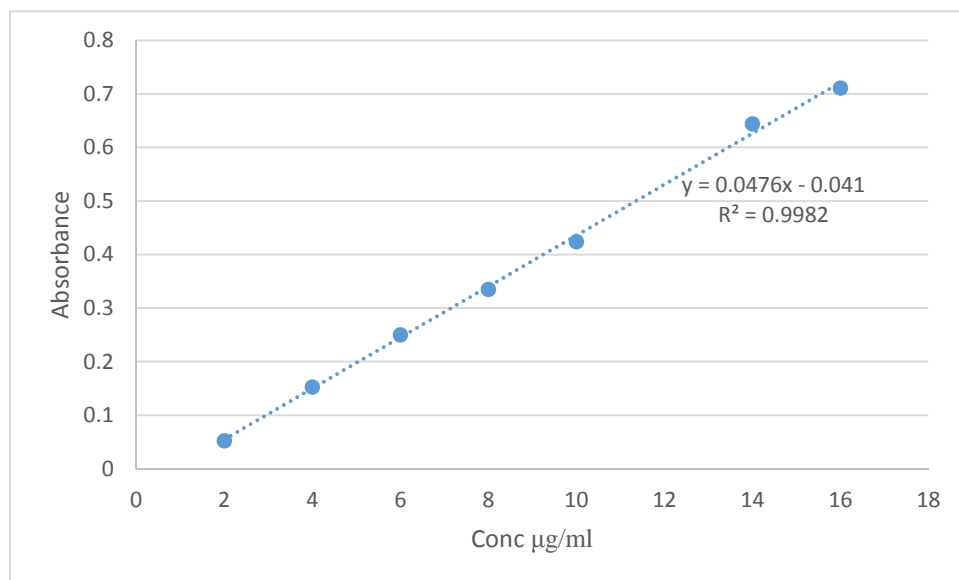


Fig.3 Linearity of Agomelatine

For assay of agomelatine ($n=6$), RSD of system precision was 0.26% on the same day (intra- day) and 0.41% on different days (inter-day). The mean values of % assay and % RSD for method precision (repeatability) were 99.95% and 0.24% respectively for assay on same day (intra-day) while, 99.98% and 0.31% respectively for assay on different days (inter day).

Intermediate precision was established by determining the overall (intraday and inter day) method precision for assay. For intermediate precision, overall assay value ($n=12$) was 99.96 %, and RSD was 0.26%. The precise result for content uniformity was indicative of uniform distribution of the drug in the tablets without significant variation; this is accordance with the USP, which stipulates acceptance limits for drug content uniformity and RSD as 85 - 115 % and < 6% respectively [13].

The accuracy of the method was assessed by determination of recovery for three concentrations covering the range of the method. Known amounts (2.5, 5.0, 7.5 $\mu\text{g/ml}$) for Agomelatine was added to a placebo preparation and the amount of Agomelatine recovered, in the presences of placebo interface was calculated. The mean recovery of Agomelatine was 100.05%, 99.98% and 98.94% respectively (Table I).

Table I: Evaluation data of accuracy study

Level (%)	Amount added concentration ^a (mg/ml)	Amount found concentration ^a (mg/ml)	% Recovery	% RSD
50	4.96	4.98	0.99	1.01
	5.01		1.00	
	5.02		0.98	
100	10.02	10.01	1.00	0.58
	9.96		1.00	
	10.10		0.99	
150	14.98	15.00	0.99	1.00
	15.02		1.01	
	15.08		1.00	

* Each value corresponds to the mean of three determination.

The robustness of the method was assessed by assaying test solutions under different analytical conditions deliberately changed from the original conditions. For each different analytical condition the standard solution and test solution were prepared separately. The result obtained from assay of the test solution was not affected by varying the conditions and was in accordance with the true value. System suitability data were also found to be satisfactory during variation of the analytical conditions (Table II). The analytical method therefore remains unaffected by slight but deliberate changes in the analytical conditions

Table II: Evaluation data of robustness study

Robust conditions	% Assay
Acetonitrile : Water(100:00,v/v)	99.95
Acetonitrile : Water(50:50,v/v)	100.07
Analyst change	99.93

During study of the stability of stored solutions of standards and test preparations for assay determination the solutions were found to be stable for up to 48hr. Assay values obtained after 48hr were statistically identical with the initial value without measurable loss (Table III).

Table III: Evaluation data of solution stability study

Intervals	% Assay for test preparation solution stored at 2-8 °C	% Assay for test preparation solution stored at ambient temperature
Initial	99.97	99.91
12 h	100.30	100.16
24 h	100.04	99.94
36 h	99.87	9.91
48 h	100.08	100.05

Before each measurement of validation data a system suitability test was performed by measurement of general characteristics such as peak asymmetry, number of theoretical plates and RSD (%) of peak area observed for a standard solution. The values obtained were satisfactory and in accordance with in-house limits (Table IV).

Table IV: Evaluation data of system suitability study

System suitability data	RSD (%)
In-House limit	NMT 2.0
Specificity Linearity Precision	0.40
Intermediate Precision	0.66
Accuracy Solution stability Robustness	0.25
	0.31
	0.23
	1.07
	0.56

*NMT: not more than

CONCLUSION

This UV method for assay validation of Agomelatine a tablet formulation was successfully developed and validated for its intended purpose. In this study, stability of Agomelatine in present dosage form was established through employment of ICH recommended stress condition. The developed procedure has been evaluated over the specificity, linearity, accuracy, precision and robustness in order to ascertain the stability of the analytical method. It has been proved that it was specific, linear, precise, accurate and robust and stability indicating. Hence, the method is recommended for routine quality control analysis and also stability sample analysis.

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