



DEVELOPMENT AND VALIDATION OF A UV SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF AGOMELATINE IN BULK AND A TABLET DOSAGE FORM

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Abstract

UV spectrophotometry is an analytical technique used routinely for qualitative and quantitative assay due the low cost and reliability during analysis. An simple, efficient, rapid, sensitive, precise and economical UV Spectrophotometric method has been developed for estimation of agomelatine from bulk and pharmaceutical formulation. The method was developed and validated according to International Conference on Harmonization (ICH Q2 R1) guidelines. The λ_{max} of agomelatine in acetonitrile was found to be 229.6 nm. The analytical method validation parameters linearity, precision, accuracy, robustness were studied according to International Conference on Harmonization guidelines. Pure drug concentration was prepared in the range of 1-10 $\mu\text{g/ml}$ and the linear regression analysis data showed good linear relationship with correlation coefficient value 0.9937. The precision of the method was studied as an intra-day, inter-day variations with value less than 2 % RSD. The limit of detection and limit of quantitation were found to be 0.577 and 1.248 $\mu\text{g/ml}$, respectively. Recoveries were found to be in the range of 100.815 to 101.744 % and % RSD was less than 2 %. This proposed UV spectroscopic method is simple and suitable for routine analysis.

Keywords: Keywords: Agomelatine, Validation, UV Spectrophotometric method

Introduction

Agomelatine (AGM) is chemically N- [2-(7-methoxy naphthalen-1-yl) ethyl] acetamide (Fig.1). Its molecular formula is $\text{C}_{15}\text{H}_{17}\text{NO}_2$ and its molecular weight is 243.301gm/mol. AGM is an acetamide naphthalene analogue of melatonin. AGM is a novel melatonergic antidepressant agent. It is a potential and well-tolerated medication for the treatment of major depressive disorder. AGM acts as a melatonergic receptor (MT_1/MT_2) agonist and serotonergic receptor ($5\text{-HT}_2\text{C}$) antagonist. AGM works by restoring the balance of the circadian rhythm. AGM has also proven to have anxiolytic properties and thus may prove to be very useful in the treatment of anxiety disorders. Because of its action upon the melatonin receptors, AGM shows a marked improvement on sleep. Bioavailability is less than 5%. AGM is absorbed

quickly in humans after oral administration. The mean half-life of AGM is 2.3 hours. AGM was bound to plasma proteins at 95% mainly to serum albumin (about 35%) and alpha1-acid glycoprotein (about 36%). The metabolism of AGM is almost completely hepatic. An extensive first pass hepatic effect is observed. It is practically insoluble in water and very soluble in organic solvents such as ethanol, methanol and dichloromethane.

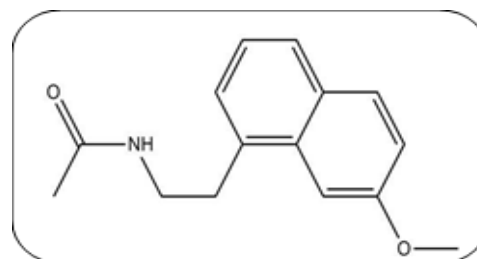


Figure 1: Chemical structure of Agomelatine (AGM)

The literature reports some analytical assays applied to AGM in different matrices. Among them, we highlighted HPLC chromatographic methods for quantitation in pharmaceuticals⁴⁻¹¹, LC-MS-MS method¹². Being an alternative to chromatographic assay, in the present study we aimed to develop an UV spectrophotometric method for quantitative analysis of agomelatine in commercial sample, applying validation protocols.

Materials and Methods

Instrumentation:

A double beam Shimadzu UV/Vis spectrophotometer, model 1800 (Japan) having a spectral bandwidth of 1 nm, wavelength accuracy of ± 0.5 nm and a pair of 1 cm quartz cells was used.

Reagents and Reference substance:

A generous gift sample of standard AGM was from Sun Pharma Laboratories Ltd, Sikkim (India). The marketed AGM tablets (Agoprex) containing 25 mg of AGM, manufactured by Sun Pharma Laboratories Ltd, Sikkim, (India) were purchased from market. All other chemicals used were of analytical grade.

Standard Preparation:

Accurately weighed quantity of 25.00 mg of AGM was transferred into 50 ml volumetric flask containing 25 ml acetonitrile. The volume was made up with acetonitrile with a strength of 500 $\mu\text{g/ml}$ (stock solution).

Further 1 ml of this stock solution was taken in 100 ml volumetric flask and make up to mark with using water. (Final concentration of standard solution obtained was 5 $\mu\text{g/ml}$).

Test Preparation:

Twenty tablets were weighed and powdered. The average weight of tablet was determined. From these, weighed amount of the powder, equivalent to 25 mg of AGM was transferred into the 50 ml volumetric flask. About 25 ml acetonitrile was added and sonicated for a minimum 30 min. with intermittent shaking. Then content was brought back to room temperature and diluted to volume with acetonitrile. The sample was filtered through 0.45 μm Whatman filter paper. The concentration obtained was 500 $\mu\text{g/ml}$ of AGM.

Further take 1 ml of this filtrate solution in 100 ml volumetric flask and make up to mark with using water. The concentration obtained was 5 $\mu\text{g/ml}$ of AGM.

Determination of wavelength of maximum absorption:

Determination the λ_{max} for AGM detection using diluents was carried out by UV spectroscopic scanning (400 - 200 nm) with the prepared standard solution (Fig. 2).

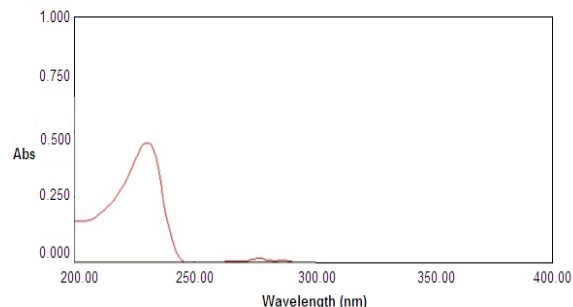


Figure 2: UV spectrum of AGM

Linearity:

AGM test solutions for the assay method were prepared at analyte concentration (1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 $\mu\text{g/ml}$). The linearity of test solutions for the assay method was found to be in the range of 1-10 $\mu\text{g/ml}$ with linear correlation coefficient 0.9937. Beer-Lambert law was obeyed in the range.

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 (intraday). Intermediate precision of the method was checked by performing same procedure on the different day (inter day) by another person under experimental condition.

Limit of detection (LOD) and limit of quantification (LOQ):

ICH defines the limit of detection of an analytical method as the lowest amount of analyte in a sample, which can be detected but not necessarily quantitated as an exact value whereas limit of quantitation of an analytical procedure is the lowest amount of analyte in a sample, which can be determined quantitatively with suitable precision and accuracy. LOD and LOQ were calculated by using following formula: $\text{LOD} = 3.3 \times \sigma / S$ and $\text{LOQ} = 10 \times \sigma / S$, where, σ is the standard deviation of y-intercepts of regression line, S is slope of the calibration curve.

Accuracy/Recovery:

Accuracy of the method was checked by the recovery studies at three different levels, i.e., 50, 100 and 150 %.

Robustness:

The study of robustness was carried out to evaluate the influence of slightly changed conditions 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:**

The standard solution of AGM was prepared in acetonitrile during development and optimization phase, as AGM is freely soluble in organic solvents like acetonitrile. And further dilutions were made using distilled water. The λ_{max} in acetonitrile was found to be 229.6 nm.

Method Validation:

The analytical method was validated as per ICH guidelines.

Linearity:

A calibration graph was obtained by plotting AGM concentrations against their corresponding absorbance values. Linearity was good in concentration range 1 to 10 $\mu\text{g/ml}$. The response of drug was found to be linear regression equation $Y = 0.0987x + 0.2373$ with correlation coefficient 0.9937 (Fig. 3). All the quantitative parameters were estimated is listed in **Table 1**.

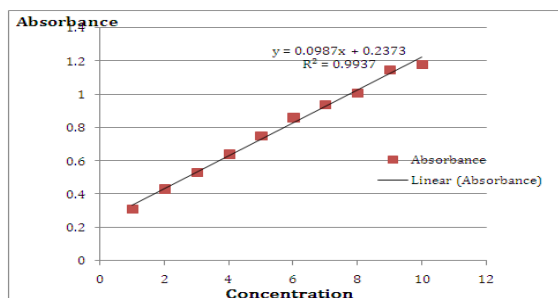


Figure 3: Calibration curve of pure AGM

Table 1: Quantitative parameters of UV spectrophotometric method

Parameter	Result
λ_{max} (nm)	229.6
Beer's law limits ($\mu\text{g/ml}$)	1-10
Regression equation	$Y = 0.0987x + 0.2373$
Slope	0.0987
Intercept	0.2373
Correlation coefficient (R^2)	0.9937

Precision:

Here, number of measurements made [n] are 6. The % RSD was found to be in the range of 0.614 - 1.053 for intra-day precision and 0.796 - 1.126 for inter-day precision (**Table 2**).

Table 2: Results of intra-day and inter-day precision

Concentration ($\mu\text{g/ml}$)	Intra-day precision		Inter-day precision	
	SD	%RSD	SD	%RSD
2	0.022	0.649	0.033	0.796
4	0.068	1.053	0.072	1.126
6	0.041	0.614	0.144	0.873

Limit of detection (LOD) and limit of quantification (LOQ):

The sensitivity of the method was assessed by determining the LOD and LOQ. The LOD and LOQ for AGM were found to be 0.577 and 1.248 $\mu\text{g/ml}$, respectively.

Accuracy:

Accuracy of the method was checked by the recovery studies at three different levels, i.e., 50, 100 and 150 %. The mean of the recovery for AGM was found to be 101.238 % (**Table 3**).

Table 3: Results of recovery studies

Amount of sample ($\mu\text{g/ml}$)	Amount of drug added ($\mu\text{g/ml}$)	Percent of spiked sample	Amount recovered ($\mu\text{g/ml}$)	Percent recovery
2	1	50 %	3.299	101.156
2	2	100 %	4.320	101.744
2	3	150 %	5.428	100.815

Robustness:

This method was assessed by assaying test solutions under different analytical condition. For each analytical condition standard and test solutions were prepared separately. The result obtained from test solution was not affected by varying condition.

System suitability data was found to be satisfactory during analytical conditions (**Table 4**).

Analytical method remains affected with slight changes but deliberately changes in the analytical conditions.

Table 4: Results of robustness studies

Robust conditions	% Assay
Acetonitrile : Water (75 : 25, v/v)	99.75
Acetonitrile : Water (50 : 50, v/v)	100.37
Analyst change	99.96

Stability of stored solutions:

Both standard and test preparations for assay determination the solutions were stable for up to 48hrs (**Table 5**)

Table 5: Results of stability studies

Intervals	% assay for test preparation solution stored at 2-8 degree Celsius	% assay for test preparation solution stored at ambient temperature
Initial	99.54	99.54
12h	100.41	100.23
24h	100.03	99.52
36h	99.32	99.91
48h	100.58	100.32

Conclusion

The UV spectrophotometric method for estimation of AGM in bulk and pharmaceutical dosage form was successfully developed validated and. The developed method has been evaluated through specificity, linearity, robustness, accuracy, precision and stability.

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