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Original Article

Validated UV-Spectrophotometric Method for Estimation of Agomelatine in Bulk Powder

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ABSTRACT

In the present research attempt has been to develop and validate new UV-Spectrophotometric method for estimation of agomelatine in bulk powder. UV-Spectrophotometric method was developed by utilizing solvent system composed of methanol: phosphate buffer (50:50 v/v). Agomelatine showed maximum absorbance wavelength at 229 nm. The method was optimized and validated in terms of specificity, selectivity, linearity and range, precision, robustness, ruggedness and solutions stability as per ICH guidelines. Agomelatine showed linearity between the concentration ranges of 0.5-4µg/mL. Newly developed method was found to be specific, selective linear, precise, robust, rugged and reproducible for estimation of agomelatine with %RSD values less than 2%. Hence newly developed and validated UV-Spectrophotometric method can be used for determination of agomelatine in bulk drug powder.

Keywords: Agomelatine, Spectrophotometric, Stability, Robust, Estimation.

1. INTRODUCTION

Depression is a devastating disorder, the treatment of which includes pharmacotherapy as well as psychotherapy. Mild depression can be treated with psychotherapy whereas moderate to severe depression requires pharmacotherapy. Despite the better understanding of the etiopathogenesis and availability of many antidepressants, treatment outcomes are not rewarding. Many patients either do not respond to

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treatment or have residual symptoms. Few others have intolerable adverse effects and become non-compliant or withdraw from therapy. Agomelatine is the latest in this category and belongs to an entirely new class [1].

Agomelatine, chemically (N-[7-methoxy-1-napthyl] acetamide) is potent melatonergic agonist and 5-hydrotryptamine 2C antagonist properties. It found to show good antidepressant activity with better tolerability and improved sleep quality compared to other antidepressants [2, 3]. Many pharmaceutical formulations containing agomelatine were marketed for health care delivery. Hence the quality control of formulations containing agomelatine is important in the pharmaceutical industries.

Literature survey revealed that analytical methods such as Spectrophotometric [4-7] HPLC [8-10] HPTLC [11] were reported for the estimation of agomelatine in its bulk and dosage forms. The reported methods were more time consuming, costly, involves the use of hazardous solvent. As per our knowledge through literature review concludes that there was no UV-Spectrophotometric method for estimation of agomelatine reported by using phosphate buffer and methanol as solvent. Hence there is need to develop and validate UV-Spectrophotometric method estimation of agomelatine using new solvent system. In the present research an attempt has been made to develop and validate new UV-Spectrophotometric method for estimation of agomelatine in bulk powder.

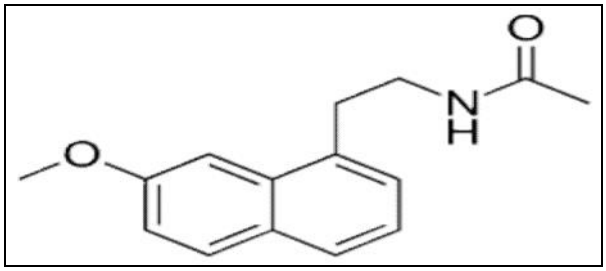

Fig 1: Chemical structure of agomelatine

2. MATERIAL AND METHODS

2.1 Drug samples

Agomelatine was obtained as gift sample (Batch No. 2AGM002079) from Symed Labs Ltd (unit-II) Hyderabad.

2.2 Reagents and chemicals

All the chemicals and reagents used for the analysis were pure and analytical grade and obtained from the store house of KLE College of Pharmacy, Belagavi.

2.3 Instruments and apparatus

UV-Spectrophotometer of Shimadzu make and model UV-1900having UV probe software were used for analysis. Calibrated weighing balance with Sartorius make used.

2.4 Method Development

Development of UV spectrophotometric method was started with the selection of solvent system and determination of maximum wavelength of absorption. Solubility of agomelatine was screened in various solvents by performing practically and also through the literature survey. In order to select solvent system few trials were carried out and finally mixture of solvents containing methanol: phosphate buffer (50:50% v/v) was chosen and then solution containing agomelatine was scanned between range of 400-200 nm and UV spectrum was obtained. Agomelatine showed maximum absorbance wavelength of 229 and it was selected for further analysis.

2.5. Method Validation

In order to validate newly developed method parameters ICH guidelines (ICH guidance Q2A; Q2B): were followed [11, 12].

2.5.1 Specificity and selectivity

Working standard solution containing 2 µg/mL agomelatine was scanned between the range of 400-200 nm and spectrum was obtained and also spectrum of solvent as blank was obtained and analyzed for any interference of solvent at maximum wavelength of absorbance [13].

2.5.2 Linearity and Range

In order to evaluate linearity, 10 mg of agomelatine was weighed and transferred into 100 mL of volumetric flask and volume was made up to the mark using solvent to obtained 100µg/ mL solution of agomelatine. From this stock serial dilutions were made to prepare 0.5, 1, 2, 3 and 4µg/mL solutions of agomelatine. The resulting solutions were prepared in triplicates and absorbance's was measured at 229 nm.

2.5.3 Limit of Detection and Limit of Quantification

Limit of detection and quantification was calculated by using statistical calculations using following formulas.

$$LOD = \frac{3.3 \times \text{standard deviation of y-intercept}}{\text{Slope of the calibration curve}}$$

$$LOQ = \frac{10 \times \text{standard deviation of y-intercept}}{\text{Slope of the calibration curve}}$$

2.5.4 Precision

Six replicates of solution containing agomelatine was prepared and absorbance of each solution was measured at 229 nm on same day at different time intervals and on different days to obtained system precision, intraday precision and interday precision data and absorbance were measured and % RSD was calculated.

2.5.5 Robustness

Solvent system composed of methanol: phosphate buffer (50:50% v/v) was used for analysis. For the robustness determination solvent system containing methanol: phosphate buffer (48:52% v/v) as composition-1 and methanol: phosphate buffer (52:48% v/v) as composition-2 were prepared and six replicates of solutions containing agomelatine was prepared by using each solvent system and absorbance's was measured and % RSD was calculated.

2.5.6 Ruggedness and reproducibility

In order to prove ruggedness of method six replicates of solutions containing agomelatine was prepared and absorbances of each replicate were measured by different analyst and also by using different instruments and %RSD was calculated for absorbances.

2.5.7 Solution and standard stock solution stability

In order to obtained the stability of solvent and stock solutions, fresh stock was prepared and dilutions were made using fresh solvent and absorbance's of each dilutions containing agomelatine was compared with that of old stock dilutions and % RSD for absorbance's was calculated.

3. RESULTS AND DISCUSSION

3.1 Development

Solvent development step involves the use of methanol: phosphate buffer (50:50%) in which agomelatine showed spectrum with maximum absorbance at 229 nm. Developed method parameters are presented in **Table 1.**

Table 1: Developed method parameters

Sr. No.	Parameters	Specifications
1	Analyte	Agomelatine
2	Solvent	Methanol: Phosphate Buffer (50:50% v/v)
3	Max Absorbance wavelength	229 nm

3.2 Validation

3.2.1 Specificity and selectivity

Solvent spectrum obtained showed no interference of absorbance229 nm which showed the specificity and selectivity of method. UV spectrum of solvent and agomelatine was showed in **Fig. 2** and **Fig. 3** respectively.

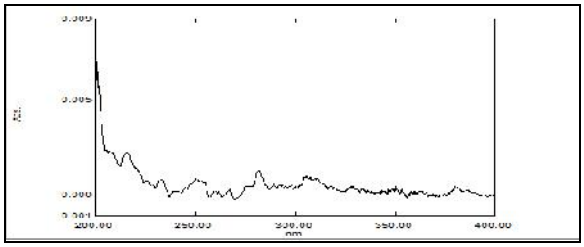

Fig 2: UV-Spectrum of Phosphate Buffer: Methanol

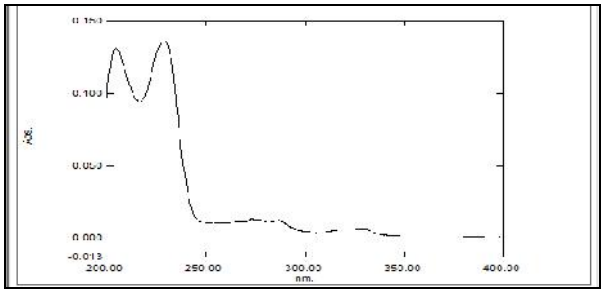

Fig 3: UV-Spectrum of Agomelatine

3.2.2 Linearity and Range:

Standard calibration curve was plotted using concentration vs absorbance's obtained by linear dilution of agomelatin. Each concentration showed linear absorbance's range between the concentration range of 0.5,1,2,3,4 µg/mL with regression equation of 0.999 for agomelatine. Linearity range data was presented in **Table 2.** Overlay spectrum of linearity of agomelatine was showed in **Fig. 4** and standard calibration curve was presented in **Fig.5**.

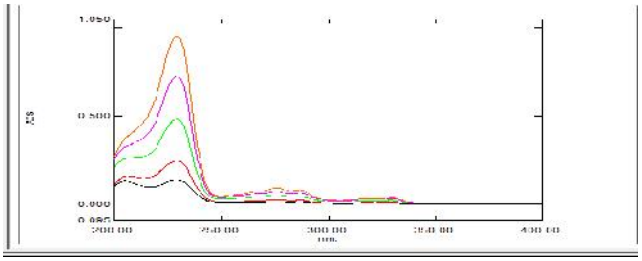

Fig. 4: Linearity overlay spectrum of Agomelatine

Table 2: Linearity and range data of agomelatine

Sr. No.	Concentration	Absorbance at 229 nm
1	0.5 µg/mL	0.134
2	1 µg/mL	0.247
3	2 µg/mL	0.472
4	3 µg/mL	0.694
5	4 µg/mL	0.908
r^2		0.999
% Curve fitting		99.90%
LOD		0.15 µg/mL
LOQ		0.47 µg/mL

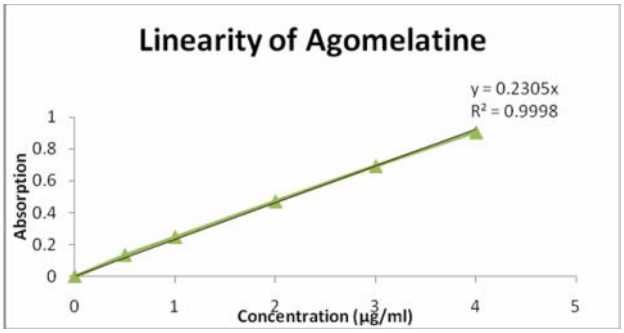

Fig 5: Standard calibration plot of Agomelatine

3.2.3 Limit of Detection and Limit of Quantification

By the statistical calculation Limit of Detection and Limit of Quantification was found to be 0.15 µg/mL and 0.47 µg/mL respectively.

3.2.4 Precision

Method was found to be precise as the %RSD calculated for six replicates solution of agomelatine at each precision level was found to be less than 2% **(Table 3 and 4).**

Table 3: System and intraday precision data of agomelatine

Precision		Intraday Precision		
Concentration	System Precision	Morning	Afternoon	Evening
2 µg/mL	0.472	0.546	0.508	0.489
2 µg/mL	0.458	0.556	0.511	0.483
2 µg/mL	0.461	0.540	0.510	0.498
2 µg/mL	0.466	0.547	0.530	0.495
2 µg/mL	0.473	0.538	0.513	0.508
2 µg/mL	0.480	0.530	0.502	0.494
%RSD	1.75%	1.64%	1.84%	1.71%

Table 4: Interday precision data of agomelatine

Conc.	Interday-1 Precision	Interday-2 Precision	Interday-3 Precision
3 µg/mL	0.693	0.717	0.774
3 µg/mL	0.714	0.740	0.759
3 µg/mL	0.688	0.726	0.754
3 µg/mL	0.684	0.755	0.751
3 µg/mL	0.714	0.729	0.757
3 µg/mL	0.691	0.743	0.758
%RSD	1.90%	1.86%	1.05%

3.2.5 Robustness, ruggedness and reproducibility

% RSD values calculated for agomelatine was found to be less than 2% which indicates method was robust with slight change in the % composition of solvent system and also found to be rugged and reproducible as %RSD obtained for absorbance's of each replicate of solutions was within the acceptance by change in the analyst and instrument **(Table 5).**

Table 5: Robustness data of Agomelatine

Robustness Ruggedness		Solvent Composition 1	Solvent Composition 2	By change in Analyst	By change in the Instrument
Replicates	Concentration				
1	3 µg/mL	0.695	0.665	0.739	0.794
2	3 µg/mL	0.721	0.643	0.753	0.797
3	3 µg/mL	0.716	0.653	0.761	0.789
4	3 µg/mL	0.720	0.665	0.757	0.799
5	3 µg/mL	0.705	0.656	0.754	0.6795
6	3 µg/mL	0.705	0.635	0.742	0.785
%RSD		1.45%	0.66%	1.84%	1.27%

3.2.6 Solution and standard stock solution stability

The %RSD for absorbance's obtained by fresh and old dilutions containing agomelatine was found to be within the acceptance and data obtained showed the standard stock solution and solvent showed stability of 4 days **(Table 6).**

Table 6: Solution stability data of agomelatine

Solution stability		Fresh stock Dilutions	Old stock Dilutions
Replicates	Conc.	Agomelatine	Agomelatine
1	3 µg/mL	0.665	0.739
2	3 µg/mL	0.643	0.753
3	3 µg/mL	0.653	0.761
4	3 µg/mL	0.665	0.757
5	3 µg/mL	0.656	0.754
6	3 µg/mL	0.635	0.742
% RSD		1.84%	1.27%

4. CONCLUSION

The present research concluded that, newly developed UV-spectrophotometric method was found to be simple, specific, selective, linear, precise, robust, rugged and reproducible for the estimation of agomelatine in its bulk powder.

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