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Research Article

Validated UV spectrophotometric method for quantitative determination of agomelatine coated tablets dosage form

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ABSTRACT

Objective: To develop and validate simple, definite, stability indicating UV spectroscopic method for determination of Agomelatine coated tablet and pharmaceutical formulations as per ICH Q2 R1 Guidelines. **Methods**: Agomelatine was subjected to different stress conditions as per ICH guideline Q1A (R2). A UV spectroscopic method is validated with different parameters such as linearity, Precision, Repeatability, Limit of Detection (LOD) and Limit of Quantification (LOQ), Accuracy, Robustness, Ruggedness. It involved a 2-h study in which methanol were used as solvents. **Results**: Agomelatine in methanol shows maximum absorbance at 229 nm. Beer's law was obeyed in the concentration range of 0.2- 1.0 mcg/mL. The LOD and LOQ were found to be 0.00271 mcg/ml and 0.0082 mcg/ml respectively. A recovery of Agomelatine in tablet formulation was observed in the range of 98.00-102.00%. Percentage assay of Agomelatine was found to be more than 99.93 %. **Conclusion**: The proposed method is definite, meticulous, reproducible and can be used for routine analysis of Agomelatine in bulk and pharmaceutical dosage form.

Key words: Agomelatine, Method development, UV spectroscopy, Validation, ICH Q2 (R1)

INTRODUCTION

The chemical name for Agomelatine is ((N-[2-(7-methoxy-1- naphthyl)ethyl]acetamide). It is used for the treatment of major depressive disorder. Literature survey revealed that not many analytical methods published to describe the quantification of Agomelatine in biological fluids includes UV-Spectrophotometric, HPLC. The target of this study is to develop a new, simple and fast analytical method by UV spectrophotometric method to quantify Agomelatine coated tablet and pharmaceutical dosage forms. However the requirement of fast, precise, very simple, efficient, time saving and highly reliable analytical UV-Spectrophotometric method for routine quality control purpose always necessities to see a new and better method. Hence, it was proposed to develop a simple, trouble-free, fast, perfect, and sensitive UV method for the concurrent estimation of Agomelatine in tablet dosage forms. This work describes the validation parameters stated by the International Conference on Harmonization [ICH] guidelines Q2 (R1). Figure 1 shows chemical

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structure of Agomelatine. The aim and objective of the present work was to develop and validate a simple, precise, sensitive spectroscopy method for Agomelatine tablet dosage form.

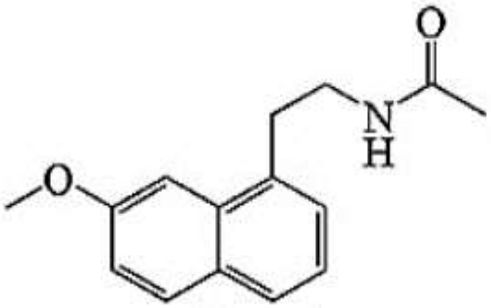

Fig 1: Chemical structure of Agomelatine

MATERIALS AND METHODS

Instruments used ELICO Double beam SL 210 UV-VIS spectrophotometer consisting two matched quartz cells with one cm light path was used for recording and measuring of spectra and absorbance of Agomelatine Essaevibra AJ (0.001g) analytical balance was used for weighing. Ultra sonicator bath Model no - 91250, PCI Ltd., Mumbai were used in this study.

Chemicals and Reagents: The marketed formulation Valdoxan® tablets containing 25mg of Agomelatine tablets were obtained from local market. Analytical grade methanol was procured from E. Merck specialties private Ltd., Mumbai, India.

Selection of solvent: Numerous trails were performed to find out the suitable solvent system for dissolving the drug. The solvents like methanol, double distilled water, dimethyl sulfoxide (DMSO) and acetonitrile were tried based on the solubility of the drug. Agomelatine is soluble in organic solvents such as methanol, acetonitrile and DMSO so methanol was selected throughout the experiment.

Selection of detection wavelength: Agomelatine 10 mcg /mL of working standard solution was prepared and scanned in UV range of 200 - 400 nm using as a blank. It was observed methanol that the drug showed maximum absorbance at 229 nm which was chosen as the detection wavelength for the estimation of Agomelatine.

Preparation of stock and working standard solution: Agomelatine 10 µg/ mL standard stock solution was prepared by transferring precisely weighed 10 mg of standard Agomelatine to 100 mL volumetric flask and dissolved in methanol. The volume was adjusted up to the mark with methanol. From this solution five mL was accurately transferred into a 50 mL volumetric flask and volume was made up to the mark with methanol. Working standard solutions of Agomelatine was prepared by suitable dilution of the stock solution (10 µg/mL) with the methanol.

Preparation of Calibration curve: A calibration curve was plotted over a concentration range of 2-10 µg/mL for Agomelatine. Precisely measured standard stock solution of Agomelatine (0.2, 0.4, 0.6, 0.8, and 1.0 mL) was transferred to a series of 10 mL volumetric flasks and the volume to each flask was adjusted to 10 mL with methanol. Calibration curve was prepared by plotting concentration of Agomelatine on X-axis and their respective absorbance's on Y-axis. Calibration data is presented in Table 1. The optical characteristics are presented in Table 3. Figure 2 shows the UV spectrum of Agomelatine at different concentrations. The calibration curve is shown in Figure 3.

Table 1: Calibration data of Agomelatine

S.no	Concentration	Absorbance
1	2	0.4167
2	4	0.7993
3	6	1.1347
4	8	1.4675
5	10	1.8993

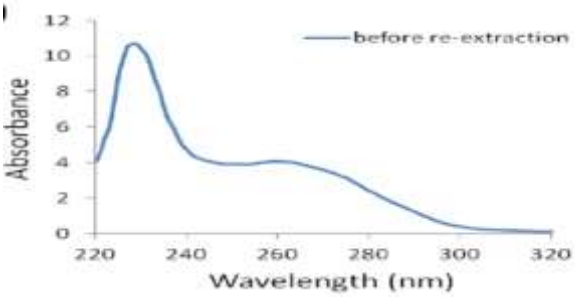

Fig 2: UV Spectrum of Agomelatine

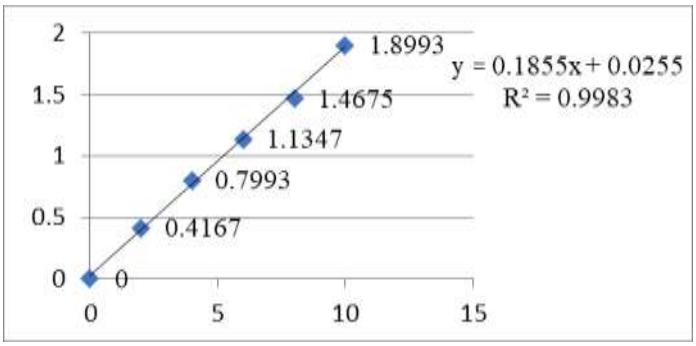

Fig 3: Calibration curve of Agomelatine

RESULTS AND DISCUSSION

Method development and Validation: In order to test the developed method to the pharmaceutical formulation, an assay of Valdoxan® 25 mg tablets

was used at working concentration. Assay for working concentration of sample at 229 nm was in limits of acceptance 98 to 102 %. According to ICH Q2 (R1) has provided guidelines for validation of analytical method which has defines this process as characteristic performance that is established by laboratory studies. UV spectrophotometric method developed according to guidelines for validation of analytical procedures. The method was validated for parameters like linearity, precision, accuracy, specificity, robustness, ruggedness, limit of detection (LOD) and limit of quantitation (LOQ).

Table 3: Summary of Optical characteristics and validation parameters

S.No	Parameters	Result
1	Detection wavelength	229
2	Beer's Law (µg/mL)	2-10 (µg/mL)
3	Regression equation (y = mx+c)	0.185x + 0.025
4	Correlation Coefficient (r2)	0.998
5	Slope	0.185
6	Intercept	0.025
7	Precision System precision Intra-day (n = 9) Inter-day (n = 9)	 0.010918 0.013466282 - 0.02971 0.008818 - 0.033105
8	Accuracy (% mean recovery) 80 % level 100 % level 120 % level	 96.80 97.68 98.28
9	Ruggedness 2 Analyst (% RSD) 2 instruments (% RSD)	 ≤ 2
10	Robustness Wavelength (± 2nm) (% RSD)	 ≤ 2
11	LOD and LOQ	0.93057208 and 2.819915395

Precision: The precision of an analytical procedure states the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under prescribed conditions. Precision was determined by system precision and method precision, intra-day and inter-day study were detected in method precision. In system precision 10µg/mL concentration of six replicate recordings of absorbance at 229 nm were observed. In method precision the repeatability (intra-day) of the method was evaluated by carrying out the assay 3 times on the same day and intermediate precision (inter day) was evaluated by carrying out the assay on three successive days for three times of the sample solution. The percent relative standard deviation (% RSD) was calculated. The results obtained are given in Table 4.

Table 4: Results of system precision

S.No	Absorbance
1	1.8994
2	1.8995
3	1.8992
4	1.8991
5	1.8996
6	1.8996
Mean	1.89936
Standard deviation	0.000207
% Relative Standard deviation	0.010918

Table 5: Results of method precision for intra - day precision

Concentration (µg)	Sample Absorbance	Mean Absorbance ± S.D	% RSD
4	0.7996 0.7997 0.7991	0.799466667 ± 0.000321455	0.040208684

6	1.134 1.1348 1.1349	1.134566667 ± 0.000493288	0.043478123
8	1.4674 1.467 1.4672	1.4672 ± 0.0002	0.01363141

Table 6: Results of method precision for inter - day precision

Concentration (µg)	Sample Absorbance	Mean Absorbance ± S.D	% RSD
4	0.7998 0.7991 0.7996	0.7995 ± 0.000360555	0.045097577
6	1.1342 1.134 1.1341	1.1341 ± 0.0001	0.008817565
8	1.4676 1.4678 1.467	1.467466667 ± 0.000416	0.02837088

Accuracy (Recovery studies): The accuracy of analytical method was determined by closeness of agreement between the value which is accepted either as a conventional true value or an accepted true value. Accuracy studies were performed at three different percentage determinations (80 %, 100 % and 120 %) by standard addition method. For each percentage level the analysis was repeated for three times (n = 3) for Agomelatine. The recovery studies were carried out by adding known amount of pure Agomelatine at 80 %, 100 % and 120 % of pre analyzed formulation. From the amount of Agomelatine found, % recovery was estimated. The results are presented in Table 7.

Table 7: Results of Accuracy

Recovery level %	Absorbance	% Recovery	Mean % Recovery	% RSD
80	83.77	97.4	96.80	1.81
80	82.44	94.75		
80	86.47	98.26		
100	107.49	101.4	97.68	1.45
100	104.466	97.63		
100	101.54	94.01		
120	127.47	101.16	98.28	1.73
120	124.17	97.17		

Ruggedness: Ruggedness is defined as the reproducibility of results when the method is performed under actual use conditions. This includes different analysts, laboratories, instruments, sources of reagents, chemicals, solvents and so on. Method ruggedness may not be known when a method is first developed, but insight is obtained during subsequent use of that method. Suggested % RSD less than 2 and indicates that the method developed is rugged. The results obtained are shown in Table 8.

Table 8: Results of Ruggedness

Parameter	Instrument-1 (Systronics model 2203)	Instrument-2 (Elico SL 159)	Analyst -1	Analyst -2
Mean	0.79936	0.79918	1.13464	1.13422
SD*	0.000477	0.000363	0.000329	0.000396
% RSD	0.059734	0.045461	0.028964	0.034934

Robustness: The most important aspect of robustness is to develop methods that allow for predictable variations in the separation parameters. For the determination of a method's robustness, parameters such as variation in detector wavelength are varied within an accurate range and the quantitative influence of the variables is determined. The analysis showed % RSD less than 2 and indicates that the method established is robust. The results obtained are shown in Table 9.

Table 9: Results of Robustness

Parameter	λ max 1	λ max 2
Mean	1.1343	1.13457
SD*	0.0002	0.00042
% RSD	0.00018	0.00037

LOD and LOQ: The limit of detection is the lowest amount of analyte in a sample which can be detected but not necessarily quantified as an exact value. The limit of quantitation of an individual analytical method is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

Limit of Detection and Limit of Quantitation were calculated using following formula LOD = 3.3*(SD) / S and LOQ = 10 *(SD) / S, where SD = standard deviation of response (absorbance) and S = slope of the calibration. The results of LOD and LOQ are shown in Table 10.

Table 10: Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Parameter	**Results**
Limit of Detection (LOD)	0.93057208
Limit of Quantification (LOQ)	2.819915395

Procedure for assay of pharmaceutical formulations: Twenty Aomelatine Valdoxan® tablets marketed tablets were accurately weighed, finely powdered in glass mortar and average weight of each tablet was determined. A sample of the tablet powder equivalent to 25 mg of Agomelatine was transferred to 25 mL volumetric flask and to this 10 mL of methanol was added. Then make up the volume with diluents. The solution was sonicated for 20 minutes and filtered through Whatmann filter paper no. 42 to remove insoluble materials. From the above prepared solution 1 mL is transferred to 100 mL volumetric flask to get eventual concentration of 10 µg/mL. Then analyzed following proposed procedure. The bulk and tablet contents were calculated by regression equation. Assay results are presented in Table 11.

Table 11: Result of Assay of Pharmaceutical Formulation

Concentration (µg/ml)	**Absorbance ± S.D.**	**% RSD**	**% Recovery* (Amount found)**
6	1.1347± 0.0001	0.013	99.93

*mean of three determinations

For the selection analytical wavelength, Agomelatine solution were prepared separately by appropriate dilution of standard stock solution and scanned in the spectrum mode from 200 - 400 nm by ELICO Double beam SL 210 UV- VIS spectrophotometer. The chemical structure of Agomelatine was shown in figure 1.The λmax of 229 nm was chosen for the determination of Agomelatine and the absorption maxima curve. Figure 2 illustrates the overlay spectrum of Agomelatine at different concentration. The calibration curve for Agomelatine was prepared in the concentration range of 0.2-1.0 µg/mL. The proposed method obeyed Beer's law in the concentration range of 0.2-1.0 µg/mL with a good correlation coefficient of r^2 = 0.9983. Beer's law range was confirmed by the linearity of the calibration curve of Agomelatine is shown in Figure 3. Calibration data is presented in Table 1. The optical characteristics and validation parameters of the proposed analytical method is represented in Table 3. The system precision and method precision of the method with inter-day and intra-day precision was found to be good with % RSD less than 2 which indicates that the method was precise and the results are presented in Table 4-6. Accuracy studies were carried out by recovery study using standard addition method at three different concentration levels (80, 100 and 120 %). The known amount of standard drug solution of Agomelatine was added to pre analyzed tablet sample solution at three different concentration levels. The resulting solutions were analyzed by the proposed methods. The recovery study results were found to be in the range of 99.93 percentages with percentage RSD less than 2. Results of accuracy study represented in Table 7. Ruggedness was performed by changing two different analysts and % RSD less than 2 which indicates that the method was two instruments, % RSD less than 2 which indicates that the method was rugged and the results are tabulated in Table 8. Robustness was performed by changing two different wavelengths and % RSD less than 2 which indicates that the method was robust results are tabulated in Table 9. For the determination of a method's robustness, ruggedness parameters such as detector wavelength are varied within a realistic range and the quantitative influences of the variables were determined. The LOD and LOQ were found to be 0.93057208 µg/mL and 2.819915395 µg/mL respectively which shows that this method was very sensitive as they were within the permitted levels. The LOD and LOQ results are shown in Table 10. The developed method was eventually utilized in analysis of tablet formulation and was found to be within the proposed limits and also the mean % assay value for bulk powder and tablet formulation were found to be 99.93 ± 0.0001 respectively. The assay results are shown in Table 11. The developed method has good linearity, accuracy and precision results indicates that the high quality of the method.

CONCLUSION

The established and validated UV spectrophotometric method was found to be reasonable due to the use of methanol as a solvent all the way through the experiment. None of the usual excipients employed in the formulation of Agomelatine dosage forms interfered in the analysis of Agomelatine by the developed method. The system suitability parameters and validation parameters are found within the limits. The plot is drawn between the concentration and absorbance which is found to be linear in the concentration range of 0.2-1.0 µg/mL with good correlation coefficient greater than r^2 = 0.9983. The results obtained by this method was precise and reproducible. The high percentage recovery and low percentage deviation were satisfactory and it shows the accuracy, reliability and suitability of the method. Thus, the developed method for Agomelatine was found to be simple, precise, accurate and cost effective and in actual fact feasible for routine sample analysis of Agomelatine in pharmaceutical dosage forms.

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