Validated Area Under Curve and Zero Order Spectroscopic Methods for Estimation of Agomelatine in Bulk and Pharmaceutical Tablet Dosage Form

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The objectives of present research work was to develop simple, precise, accurate, specific, cost effective, validated UV-spectrophotometric method for quantification of Agomelatin (AGM) in bulk and pharmaceutical tablets dosage form by Area Under Curve (AUC) and Zero Order Spectroscopic (ZOS) methods. Both methods involve the use methanol: millipore water (50:50 %v/v) as solvent for estimation. The area between 212-237 nm was used to measure the AUC for first method and 229 nm was used to measure the absorbance for second method. The developed technique was optimized and standardized as per ICH guidelines in terms of specificity, selectivity, linearity, ruggedness, solution stability, quantification and detection limits, precision, robustness, and accuracy. Both methods showed linearity between the amount ranges from $0.5 – 2.5 \mu g/mL$. The % RSD for all the validation parameters was found to be less than 2%. Both methods were found to accurate with recovery values. The results obtained by proposed method showed that method is linear, specific, selective, rugged, precise, stable, robust, and accurate and can be employed for the quality control of AGM in bulk and tablet formulation.

Keywords: Agomelatine; Area under curve; ICH guidelines; Validation; Zero Order Spectroscopy.

AGM, chemically "N-[2-(7-methoxy napthalen-1-yl) ethyl] acetamide" (Figure 1) and used as antidepressant agent which was approved in 2009 for treatment of depressive disorders by the European Medicines Agency. The molecular formula of AGM is $C_{15}H_{17}NO_2$ and molecular weight is 243.301gm/mol. AGO is a sleep modulating antidepressant and is a novel antidepressant, acts as melatonergic receptor agonist and serotonergic receptor antagonist.

AGM also used as anxiolytic agent and used in the treatment of anxiety[1,2]. The binding studies suggest that it has no effect on monoamine uptake and no affinity for histaminergic, dopaminergic, cholinergic, á, â adrenergic, and benzodiazepine receptors[3,4]. It showed significant advantages over paroxetine due to the complete absence of side effects including the associated sexual effect that are troublesome with some antidepressant. Due to its action on the melatonine receptors, it

gives a marked improvement on sleep. AGM has anxiolytic activity and hence it is very useful in the management of anxiety and related disorders[5,6].

Quality control of AGM is very much essential in the pharmaceutical industries as it is consumed by large number of population in the form of tablet dosage forms. In order to analyse the drug and formulations various methods have been reported by researchers[7-10] The scientific studies and literature reports few analytical methods used to estimate AGM in different forms. Few of these analytical methods were highlighted and reviewed. The AGM is estimated using chromatographic method and also impurities in its bulk and formulations were reported by few analytical methods[11-13]. The analytical method are reported for analysis of AGM include quantification by RP-HPLC[14] and the chromatographic methods also were reported for separating and analyzing AGM and its intermediates and final formulations[15]. The validated LC-MS/MS technique for quantification of AGM in human plasma and its application in a pharmacokinetic study also was reported[16].

Few of the other reports found in the literature for AGM determination discussed mainly on chromatographic techniques. The reported techniques used for quantification of AGM in bulk form and pharmaceutical formulations by HPLC with UV detection[17]. It has been also quantified along with some other antidepressants and also along with vitamin B12 in formulations and biological samples[18]. As we discussed earlier mass chromatographic assays were reported for determination of AGO and other antidepressants in biological samples[19, 20]. The UHPLC-DAD-MS/MS was also used to analyze the photostability of AGM[21]. The capillary liquid chromatography-mass spectrometry was also used for determination of AGM in blood plasma[22]. The stability indicating chromatographic assays also were reported for quantification of AGM and its degradation products[23, 24]. Literature survey also revealed very important techniques such as ultra-high performance super critical fluid chromatography and ultra-high performance liquid chromatography (UHPLC) were utilized successfully for quantification of AGM and its impurities[25]. Apart from these methods assessment of AGM stability under various stress conditions using HPLC with fluorescence detection was

reported[26]. The Gas chromatography–mass spectrometry quantification was used for assay of AGM and other antipsychotic drugs in biological fluids[27]. The UV Spectrophotometric estimation was carried out for determination of AGM at 299 nm in bulk and pharmaceutical formulations[28] and also the spectrofluorimetric analysis was reported for AGO quantification in marketed tablets[29]. AGM was also estimated by using assays based on the formation of a charge transfer complex methods and its estimation using spectroscopic methods[30].

Very few methods involve the use of UV spectroscopic principles and reported methods used of costly solvents in analysis. The literature review also revealed that AGM was analysed by measuring the absorbance no methods were reported based on measurement using area under curve spectroscopic method. Hence there is need for estimating the AGM by economic as well as by measuring the AUC method. In the present research we have made the attempt to develop, optimize and validate the AUC and ZOS methods using cost effective solvent system.

METHODS AND MATERIALS

Instruments, reagents and chemicals

Electronic analytical balance was used to weigh the drug; UV-Spectrophotometer was used to measure the AUC and absorbance, Ultrasonic bath Sonicator was used to sonicate and enhance the solubility of Agomelatine. Agomelatine was obtained as a gift sample from SYMED LABS LIMITED Hyderabad. The reagent solutions and chemicals used were of AR grade and collected from the store of KLE College of Pharmacy, Belgaum.

Optimization of Methods

Preparation of stock solution

AGM standard stock solution containing 500ìg/ml was prepared in 10ml volumetric flask (VF) by dissolving 5mg and then diluted to volume with methanol: water (50:50%v/v) as solvent system (SS). From this stock further 1ml was taken in 10mL VF and volume was made up to mark using SS. From this stock serial dilution were made to prepare 0.5 ìg/ml to 2.5 ìg/ml.

Selection of solvent and wavelength of analysis

The solubility analysis and literature survey revealed that the AGM was soluble in

methanol. Many trials were performed in different proportions of water and methanol. Finally SS composed of methanol: water (50:50%v/v) was chosen for UV analysis of AGM. In order to identify the wavelength for analysis, solution containing 0.5 ìg/ml was analysed in the UV region of 200 - 400nm and spectrum was obtained and wavelength for maximum absorbance was identified which was found to be 229nm.

Measurement of area under curve for AUC and absorbance for ZOS methods

For area under curve method the two wavelengths 212-237nm were selected and AUC between these two was used for measurement[31,32]. For ZOS method absorbance of solutions at 229 nm was measured.

Validation

The developed techniques were standardised as per ICH guidelines in terms of selectivity, precision, linearity, robustness, specificity, LOD, LOQ, ruggedness, solution stability, and accuracy[33, 34].

Selectivity and Specificity

It was performed to exclude the chances of interference of solvent in the region of maximum absorbance of AGM. The specificity and selectivity was evaluated by running the solvent and comparing the spectrum of AGM (35).

Standard calibration curve

The series of dilutions were made from the standard stock solution of AGM to obtain the amount of 0.5ìg/ml – 2.5ìg/ml. For AUC method the two wavelengths 212-237nm was selected for determination. The calibration plot was constructed as AUC v*s* concentration. For ZOS method Absorbance of the above solution was measured at 229nm and calibration curve of concentration *vs* absorbance was prepared and the r^2 was calculated[36].

Detection and Quantification Limits

The Detection and Quantification Limits of AGM by proposed method were estimated using calibration standard. Detection limit of AGM by AUC and ZOC method was found to 0.1457 ìg/ml and 0.4799 ìg/ml respectively. Quantification Limits of AGM by AUC and ZOC method was found to be0.0973 ìg/mland0.2951 ìg/ml respectively[37].

Precision

Precision was studied to evaluate the preciseness of methods. The system precision was evaluated by measuring AUC and Absorbance's of Agomelatine solution at three different concentrations. Same way intraday and interday precision was evaluated by performing analysis on same day at two different intervals and on three different days. After each analysis percentage "relative standard deviation" (% RSD) was calculated.

Ruggedness

The ruggedness presents the variation within the laboratory conditions (different analyst and different instrument). It was done by repeating the same analysis by different analyst on one more instrument. After analysis percentage relative standard deviation (% RSD) was calculated.

Robustness

Robustness was evaluated by measuring the absorbance's at different wavelengths and calculating the %RSD for ZOS.

Solution Stability

The solution was preserved at ambient room temperature and analysed at different day intervals .The responses for the older solutions were compared against freshly prepared standard solution.

Accuracy

The accuracy of techniques was estimated by recovery experiments at 3 different levels. The samples were spiked with 50%, 100% and 150% of mixed standard solution the mixture were analysed and recoveries were estimated.

Assay

Twenty marketed tablets of AGM were weighed and average weight was calculated. Tablets are made into powder form and then powder equivalent to 10 mg of AGM was weighed and transferred to 10 mL VF. Agomelatine was extracted from powder using SS and sonicated for 15 minutes. After extraction serial dilutions were made in beers range and absorbance was measured and used for calculating assay.

RESULTS

Development

Solvent development step involves the use of methanol: water (50:50) in which AGM showed spectrum with maximum absorbance at

229nm. Specifications of developed techniques were presented in Table 1.

Validation

Specificity Selectivity

Solvent spectrum obtained showed no interference of absorbance 229nm which show the specificity and selectivity of method. The spectrum of solvent and AGM were presenetd in Figure 2 and Figure 3.

Linearity and Range

Standard calibration curve was plotted using absorbance *vs* concentration obtained by linear dilution of AGM. Each concentration show linear absorbance range between the amounts of 0.5,1.0,1.5,2.0,2.5ìg/ml with regression equation of 0.9998 for AGM. The linearity data was presented in Table 2. Standard calibration curve was presented in Figure 2 and Figure 3.Overlay spectrum was showed in Figure 4 and AUC graphs was presented in Figure 5.

Precision

Method was found to be precise as the % RSD calculated for six replicates solution of AGM at each precision level was found to be less than 2%. Data of precision presented in Table 3.

Robustness

% RSD values calculated for AGM was found to be less than 2% which indicates method was robust with slight change in nm and also found to be rugged. The data of Robustness presented in Table 4.

Ruggedness

The % RSD obtained for absorbance of each replicate of solution was within the acceptance by change in the analyst and instrument. Ruggedness data is presented in Table 5.

Solution and Standard Stock Solution Stability

The % RSD for absorbance obtained by fresh and old dilution containing Agomelatine was found to be within the acceptance and data obtained show the standard stock solution and solvent shows stability of 3 days. The data is presented in Table 6.
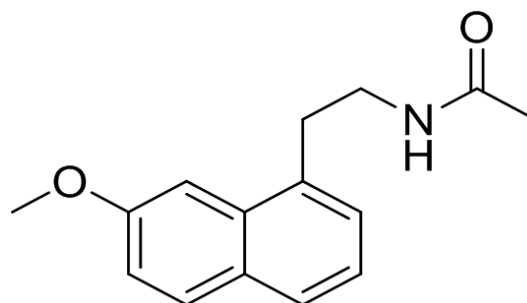
Table 1. Specifications of ZOS and AUC Spectroscopic Method

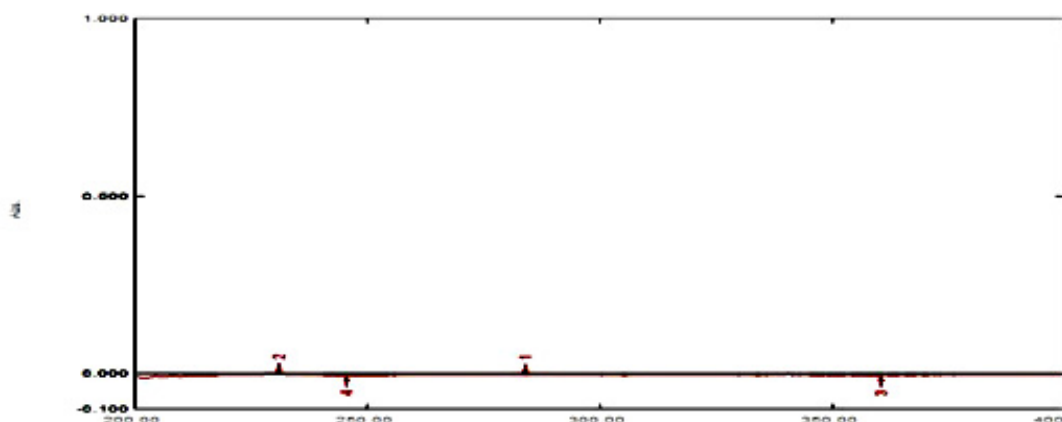| No.	Parameters	Specification
1	Method	Spectrophotometric
2	Instrument	UV-Spectrophotometer
3	Model	Shimadzu
4	Make	UV-1800
5	Software	UV-Probe
6	Analyte	Agomelatine
7	Solvent	Methanol :Water (50:50)
8	Lambda Max.	229nm

Fig. 1. Chemical Structure of AGM

**Fig. 2.** Spectrum of Blank Solvent

Accuracy

Accuracy of Agomelatine was found to be well within the acceptance for both methods and data was presented in Table7.

Assay

The assay values of Agomelatine by AUC and ZOC methods were found to be 96.37-97.64 and 98.23-102.25 % respectively.

Table 2. Linearity and range data of Agomelatine

No.	Concentration (µg/ml)	Area Under Curve(212 237)	Absorbance at 229 nm
1	0.5	1.482	0.205
2	1.0	3.139	0.412
3	1.5	4.444	0.622
4	2.0	6.127	0.821
5	2.5	7.584	1.018
r^2		0.999	0.9998
% Curve Fitting99.9%		99.98%	
LOD		0.1457 µg/ml	0.0973 µg/ml
LOQ		0.4799 µg/ml	0.2951 µg/ml
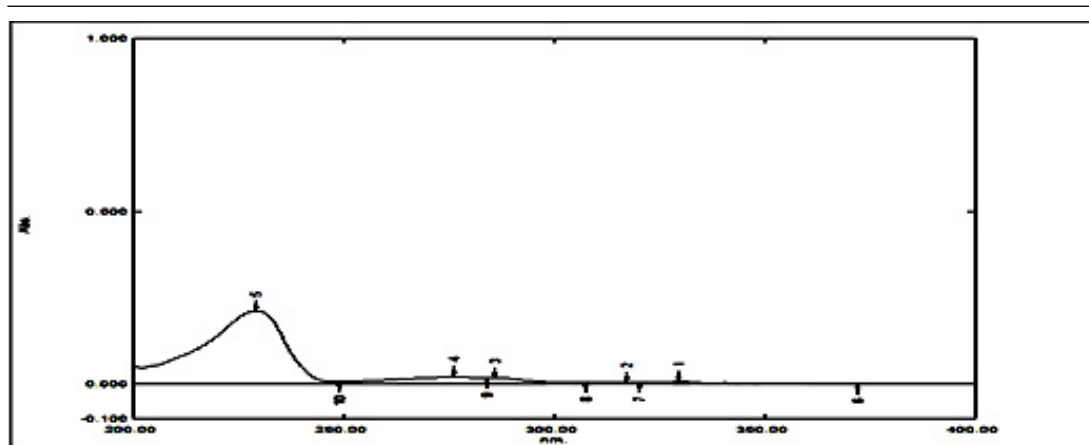
Fig. 3. Spectrum of AGM

Conc.	AUC
0	0
0.5	1.482
1	3.139
1.5	4.444
2	6.127
2.5	7.584

$y = 3.0371x$
$R^2 = 0.9993$
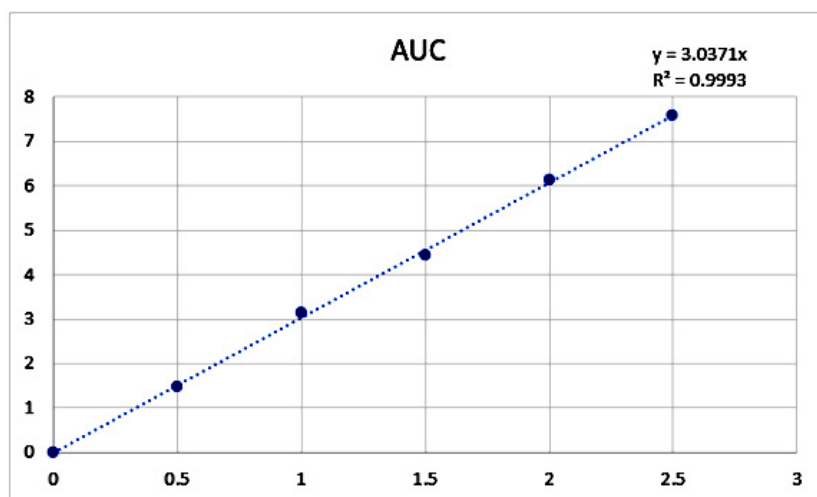
Fig. 4. Standard calibration curve for AUC method

DISCUSSION

The literatures reports that AGM was estimated by few chromatographic techniques and the reported techniques used for quantitative analysis of AGM in bulk form and pharmaceutical preparations with the help of HPLC. The UHPLC-DAD-MS/MS was also used to analyse the photo stability of AGM. The capillary liquid chromatography-mass spectrometry was also used for determination of AGM in blood plasma.

The stability indicating chromatographic assays also were reported for quantification of AGM and its degradation products. Literature survey also revealed very important techniques such as ultra-high performance super critical fluid chromatography and ultra-high performance liquid chromatography (UHPLC) were utilized successfully for quantification of AGM and its impurities. Apart from these methods, the assessment of AGM stability under various stress conditions using HPLC with fluorescence detection
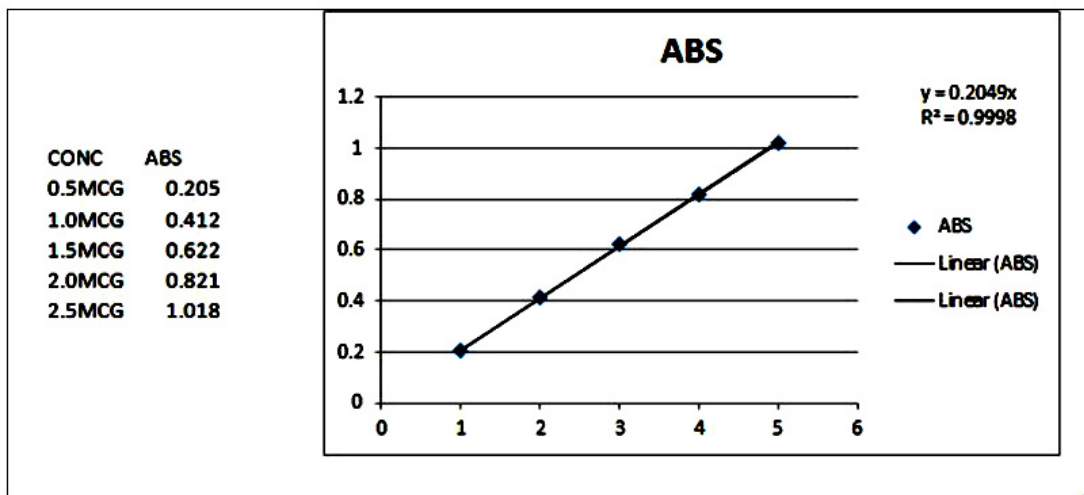
CONC	ABS
0.5MCG	0.205
1.0MCG	0.412
1.5MCG	0.622
2.0MCG	0.821
2.5MCG	1.018

$y = 0.2049x$
$R^2 = 0.9998$

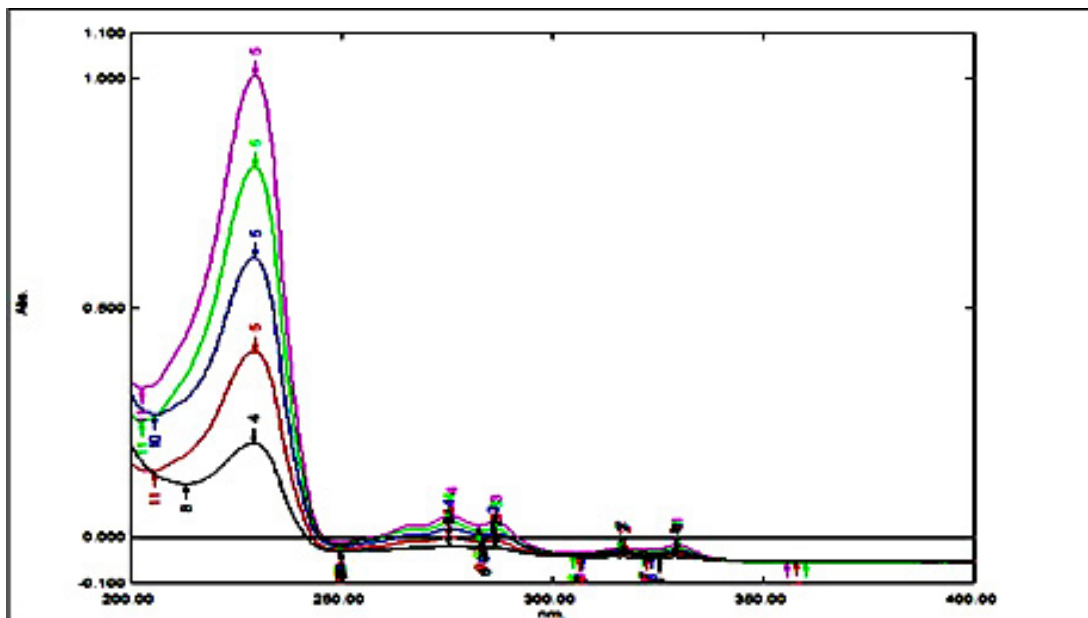**Fig. 5.** Standard calibration curve for ZOS method

Fig. 6. Linearity overlay spectrum of Agomelatine

was reported. The Gas chromatography–mass spectrometry quantification was used for assay of AGM and other antipsychotic drugs in biological fluids.

UV-Visible spectrophotometry is one of the commonly used analytical method in analysis pharmaceutical preparations. It involves measuring the amount of visible or ultraviolet light absorbed by analyte in suitable solution. The absorbance is quantitatively measured as it is based on amount of drug present in solution. Usually spectrum of analyte is taken and maximum wavelength of absorbance is measured for further quantitative estimation. The area under curve or spectrum also can be measured and used for the quantitative analysis of drugs, as it is very rarely used in most of reported literatures. The UV Spectrophotometric estimation was carried out for determination of AGM at 299 nm in bulk and pharmaceutical formulations. AGM was also estimated by
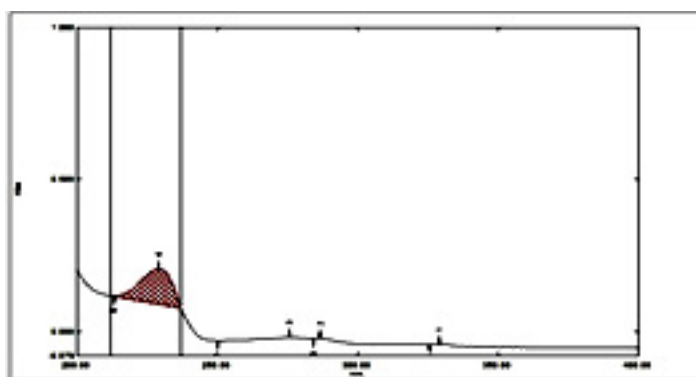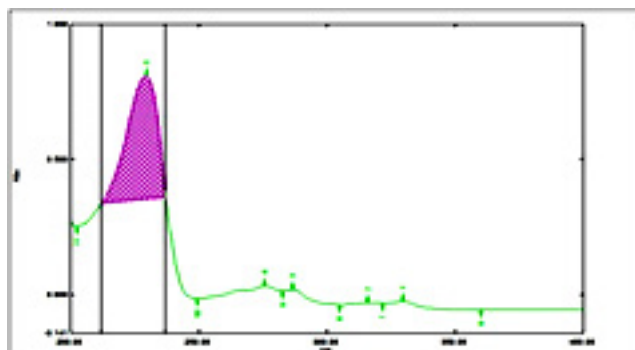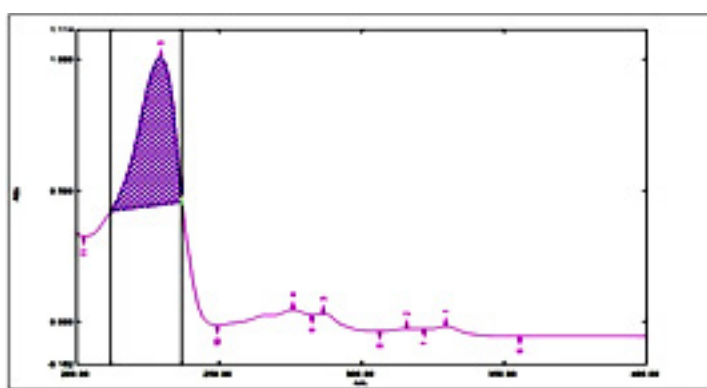

AUC of Agomelatine 0.5 µg/ml
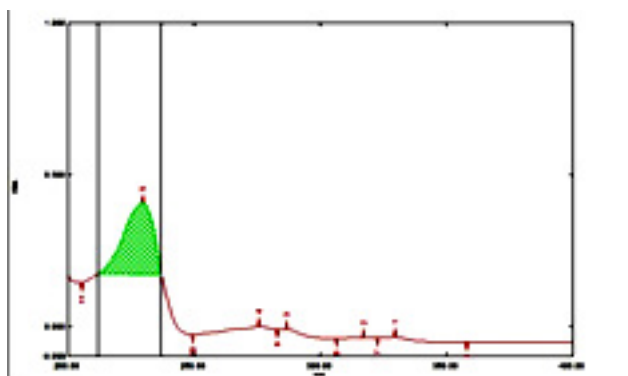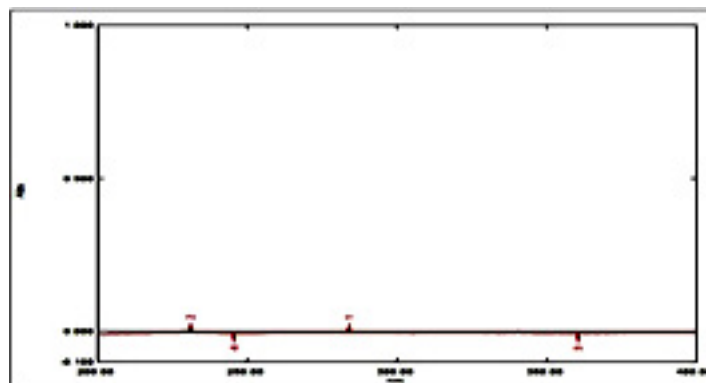

AUC of Agomelatine 1.0 µg/ml
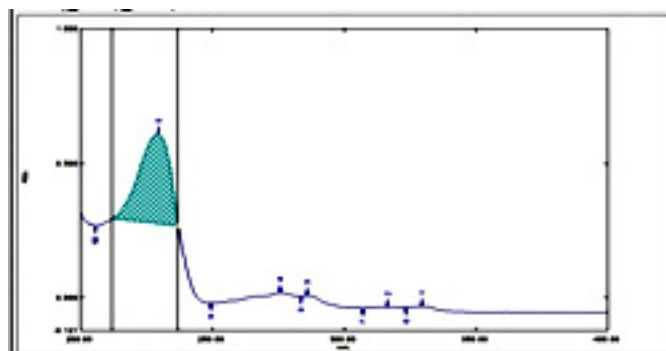
AUC of Agomelatine 1.5 µg/ml

AUC of Agomelatine 2.0 μg/ml

AUC of Agomelatine 2.5 μg/ml

UV-Spectrum of Blank

Fig. 7. Area Under Curve of Agomelatine with Different Concentrations

using assays based on the formation of a charge transfer complex methods and its estimation using spectroscopic methods. The literature review also revealed that AGM was analysed by measuring the absorbance no methods were reported based on measurement using area under curve spectroscopic method. In the present research we have made the attempt to develop, optimize and validate the AUC and ZOS methods using cost effective solvent system.

The present research was aimed to develop simple, precise, accurate, specific, cost effective, validated UV-spectrophotometric method for quantification of Agomelatin (AGM) in bulk

and pharmaceutical tablets dosage form by Area Under Curve (AUC) and Zero Order Spectroscopic (ZOS) methods. The area between 212-237 nm was used to measure the AUC for first method and 229 nm was used to measure the absorbance for second method. Both methods involve the use methanol: millipore water (50:50 %v/v) as solvent for estimation. The developed technique was

Table 3. Precision Data of Agomelatine

Precision	%RSD of AUC method			%RSD of ZOS method		
Concentration	0.5 μg/ml	1.5 μg/ml	2.5 μg/ml	0.5 μg/ml	1.5 μg/ml	2.5 μg/ml
System precision	0.651	0.444	0.293	0.48	0.09	0.15
Method Precision	0.825	0.231	0.086	0.744	0416	0.100
Inter day 1	1.940	1.643	0.504	0.740	0.415	0.317
Interday 2	0.806	0.690	0.634	0.739	0.593	0.199
Interday 3	0.917	0.359	0.692	0.736	0.342	0.250
Intraday-1	0.449	0.211	0.237	1.558	0.436	0.199
Intraday-2	0.953	0.041	0.304	1.214	0.592	0.263

Table 4. Robustness Data of Agomelatine

Robustness	%RSD of ZOS Method		
Conc (μg/ml)	0.5	1.5	2.5
227nm	1.012	0.167	0.270
228nm	0.998	0.094	0.251
229nm	0.952	0.162	0.248
230nm	0.732	0.093	0.261
231nm	0.746	0.095	0.209

optimized and standardized as per ICH guidelines in terms of specificity, selectivity, linearity, ruggedness, solution stability, quantification and detection limits, precision, robustness, and accuracy. Both methods showed linearity between the amount ranges from 0.5 – 2.5ig/mL. The % RSD for all the validation parameters was found to be less than 2%. Both methods were found to accurate with recovery values. The assay values

Table 5. Ruggedness data of Agomelatine

Ruggedness	% RSD of AUC Method			%RSD of ZOS method		
Concentration μg/ml	0.5	1.5	2.5	0.5	1.5	2.5
Change analyst	1.676	0.097	0.097	0.966	0.686	0.099
Change instrument	0.758	0.135	0.309	1.691	0.489	0.058

Table 6. Solution stability of Agomelatine

Solution stability	% RSD of AUC Method			%RSD of ZOS method		
Concentration (ig/ml)	0.5	1.5	2.5	0.5	1.5	2.5
Stability Day 1	1.368	0.041	0.259	1.214	0.592	0.263
Stability Day 2	0.421	0.108	0.165	0.744	0.416	0.100

Table 7. Accuracy of Agomelatine

Level	Standard added (ig/ml)	Sample added (ig/ml)	Total conc. (ig/ml)	% Recovery of AUC	% Recovery of ZOS
50%	0.5	0.25	0.75	98.37 -99.75%	93.71 – 95%
100%	0.5	1.0	1.5	99.13 – 99.68%	98.79-100.72%
150%	0.5	1.75	2.25	99.03 -99.32%	98.44-99.14%

of Agomelatine by AUC and ZOC methods were found to be 96.37-97.64 and 98.23-102.25 % respectively.

CONCLUSION

Quality control of AGM is very much essential in the pharmaceutical industries as it is consumed by large number of population in the form of tablet dosage forms. In order to analyse the drug and formulations various methods have been reported by researchers. The reported methods involve the use of UV spectroscopic methods and also the use of costly solvents such as methanol. Hence new UV-spectroscopic methods have been developed, optimized and validated in present research work. The proposed Area Under Curve and Zero Order Spectroscopic methods was found to be simple, precise, accurate and stable for the quantification of Agomelatine in bulk and its marketed tablet dosage forms. The reported method can be hence used for the quality control analysis of Agomelatine.

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