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Novel stability indicating RP-HPLC method for the determination of Agomelatine - A novel antidepressant

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ABSTRACT

A novel and simple reverse phase liquid chromatographic method was established for the determination of Agomelatine in presence of degradants. Agomelatine is used for the treatment of prostate cancer. The proposed work was performed on Shimadzu Model CBM-20A/20 Alite with Zorbax extended C_{18} column (150 mm × 4.6 mm i.d., 5 µm particle size) using a mixture of 0.05% formic acid and methanol as mobile phase with flow rate 1.0 ml/min (UV detection at 230 nm). The method was validated as per ICH guidelines and the regression equation was found to be y = 384117x + 59621. Agomelatine was subjected to acidic, alkaline, oxidation, photolytic and thermal stress degradations and the method was reported to be robust and specific and can be applied for the assay of pharmaceutical formulations.

KEY WORDS: Agomelatine, RP-HPLC, validation, stability-indicating.

1. INTRODUCTION

Agomelatine (AGM) is a melatonergic anti-depressent. Agomelatine is chemically N-[2-(7-methoxynaphthalen-1-yl) ethyl] acetamide ($C_{15}H_{17}NO_2$) with molecular weight 243.301 g/mol. Figure.1 represents the chemical structure of Agomelatine.

Agomelatine is a melatonin receptor agonist (Dridi, 2013). It is a novel anti-depressant approved in February 2009 for use in the European Union (European Medicines Agency, 2012). Agomelatine, a naphthalene analog of melatonin, is a newly developed selective agonist of the human melatonergic MT_1 and MT_2 receptors and also shows 5-HT_{2C} receptor antagonist activity (Milan, 2003). AGM has been reported to be an effective antidepressant therapy with an entirely new mechanism of action (Olie and Kasper, 2000).

Very few analytical methods have been reported for the determination of Agomelatine such as HPLC (Meghana, 2014; E.L.Shaheny, 2014; HE Xue et al., 2010; Harika, 2013; Nohas Rashed, 2014; Vineela P, 2014), LC-MS/MS in human plasma (Satish R.Patil, 2012; Xiaolin Wang, 2014) and HPTLC (Joshi Hitendra, 2013). So, at present the authors have developed a stability indicating RP-HPLC method for the determination of AGM in presence of its degradation products.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents: Reference standards of Agomelatine (purity >99%) was obtained from Sun Pharmaceuticals Industries Ltd (Hyderabad, India). Agomelatine is available as tablets with brand name AGOPREX® (Sun Pharmaceuticals Industries Ltd, Mumbai, India) and AGOVIZ® (Abbott India Limited, Mumbai) with label claim of 25 mg of drug.

Methanol, sodium hydroxide, hydrochloric acid, formic acid and hydrogen peroxide (H_2O_2) were purchased from Merck (India). All chemicals are of HPLC grade. All chemicals were of analytical grade and used as received.

2.2. Instrumentation: Chromatographic separation was achieved by using Shimadzu Model CBM-20A/20 Alite HPLC system, equipped with SPD M20A prominence photodiode array detector with Zorbax extended C18 column (150 mm × 4.6 mm i.d., 5 µm particle size) maintained at 25 ºC.

2.3. Chromatographic conditions: Isocratic elution was performed using 0.05% formic acid and methanol (35:65%, v/v) and the flow rate was 1.0 ml/min. The overall run time was 10 min. The detection was carried at 230nm. 20 µL of sample was injected into the HPLC system and all chromatographic conditions were performed at room temperature (25°C ± 2°C).

2.4. Preparation of 0.05% formic acid: 0.05% formic acid was prepared by transferring accurately 0.5ml of formic acid into a 1000ml volumetric flask and diluted with HPLC grade water. The resulting solution was sonicated for half an hour and filtered.

2.5. Preparation of stock solution: The stock solution was prepared by transferring accurately 25 mg of AGM in to a 25 ml volumetric flask and diluting with mobile phase (1000 µg/ml) and further dilutions were made on daily

basis from the stock solution with mobile phase as per the requirement and filtered through 0.45 µm membrane filter prior to injection.

2.6. Validation:

2.6a. Linearity: A series of solutions (0.01–100 µg/ml) were prepared from the Agomelatine stock solution and 20 µL of each solution was injected in to the HPLC system and the peak area of the chromatogram was noted. Calibration curve was plotted by taking the concentration of the solutions on the x-axis and the corresponding peak area values on the y-axis.

2.6b. Precision: The intra-day precision of the assay method was evaluated by carrying out 9 independent assays of a test sample of Agomelatine at three concentration levels (5, 10 and 20 µg/ml) (n=3) against a qualified reference standard. The %RSD of three obtained assay values at three different concentration levels was calculated. The inter-day precision study was performed on three different days i.e. day 1, day 2 and day 3 at three different concentration levels (5, 10 and 20 µg/ml) and each value is the average of three determinations (n=3). The % RSD of three obtained assay values on three different days was calculated.

2.6c. Accuracy: The accuracy of the assay method was evaluated in triplicate at three concentration levels (80, 100 and 120%), and the percentage recoveries were calculated. Standard addition and recovery experiments were conducted to determine the accuracy of the method for the quantification of Agomelatine in the drug product. The study was carried out in triplicate at 9, 10 and 11 µg/ml. The percentage recovery in each case was calculated.

2.6d. Robustness: The robustness of the assay method was established by introducing small changes in the HPLC conditions which included wavelength (228 and 232 nm), percentage of methanol in the mobile phase (63 and 67%) and flow rate (0.9 and 1.1 ml/min). Robustness of the method was studied using six replicates at a concentration level of 10 µg/ml of Agomelatine.

2.6e. Limit of quantification and Limit of detection: The limit of quantification and limit of detection were based on the standard deviation of the response and the slope of the constructed calibration curve (n=3), as described in ICH guidelines Q2 (R1) (ICH guidelines, 2005). Sensitivity of the method was established with respect to limit of detection LOD and LOQ for analytes.

2.6f. Forced degradation studies: Forced degradation studies were performed to evaluate the stability indicating properties and specificity of the method (ICH guidelines, 2003). All solutions for stress studies were prepared at an initial concentration of 1 mg/ml of AGM and refluxed for 20 min at 80 ºC and then diluted with mobile phase.

2.6g. Acidic degradation: Acidic degradation was performed by treating the drug solution (1.0 mg/ml) with 0.1 M HCl for 20 min in a thermostat maintained at 80 ºC. The stressed sample was cooled, neutralized with NaOH and then diluted with mobile phase as per the requirement. 20 µL of this solution was injected in to the HPLC system.

2.6h. Alkaline degradation: Alkaline degradation was performed by treating the drug solution (1.0 mg/ml) with 0.1 N sodium hydroxide for 20 min in a thermostat maintained at 80 ºC. The stressed sample was cooled, neutralized with HCl and then diluted with mobile phase as per the requirement and 20 µL of the solution was injected in to the HPLC system.

2.6i. Oxidation degradation: Oxidation degradation was performed by treating the drug solution (1.0 mg/ml) with 30% H_2O_2 for 20 min in a thermostat maintained at 80 ºC. The drug solution mixture was cooled and then diluted with mobile phase as per the requirement and 20 µl of the solution was injected in to the HPLC system.

2.6j. Photolytic degradation: Photolytic degradation was performed by exposing drug solution (1.0 mg/ml) to ultraviolet light (365 nm) for one hour and then diluted with mobile phase as per the requirement before injecting in to the HPLC system.

2.6k. Assay of marketed formulations (Tablet): Twenty tablets of Agomelatine from different brands (AGOPREX® and AGOVIZ®) were procured, weighed and crushed to a fine powder. Powder equivalent to 25 mg of Agomelatine was accurately weighed and transferred carefully into a 25 ml volumetric flask and made up to volume with mobile phase. The contents of the volumetric flask were sonicated for 30 min to enable complete dissolution. The solution was filtered and diluted with mobile phase as per the requirement. 20 µL of these solutions were injected into the system after filtering through 0.45 µm membrane and the peak area was recorded from the respective chromatogram.

3. RESULTS AND DISCUSSION

3.1. Method development and optimization: Initially the stressed samples were analyzed using a mixture of 0.05% formic acid: methanol (50: 50% v/v) with a flow rate of 1.0 ml/min in which the peak was obtained at R_t 17.27 mins

and also the resolution and peak symmetry were not satisfactory. The mobile phase ratio was changed to 45:55% v/v and the drug sample was injected in to the loop where a sharp peak was eluted at 12.11 mins with tailing. Finally the mobile phase composition was modified as 35:65% v/v and the drug peak eluted was sharp and symmetrical (UV detection at 230 nm) with retention time 5.20 ± 0.03 mins. The chromatogram of the mobile phase (blank) and the standard was shown in Fig. 2(A-B). A detailed comparative study of the previously published methods was extensively discussed with the present method in Table 1.

3.2. Method Validation: The method was validated for system suitability, linearity, limit of quantitation (LOQ), limit of detection (LOD), precision, accuracy, selectivity and robustness (ICH guidelines, 2005).

3.2a. Linearity: Agomelatine shows linearity over a concentration range 0.01–100 µg/ml (Table 2) with % RSD 0.12-0.60 and the chromatographic response was shown in Figure 3. The linear regression equations were found to be $y = 384117x + 59621$ ($r^2 = 0.9998$).

3.2b. Accuracy: The method accuracy was proved by the recovery test at three different concentrations (80, 100 and 120 %). A known amount of Agomelatine standard (5 µg/ml) were added to aliquots of sample solutions and then diluted to yield the total concentrations of 9, 10 and 11 µg/ml as described in Table 3. The % RSD was found to be 0.20- 0.62 (<2.0 %) with a recovery of 98.89 - 99.74 %.

3.2c. Precision: The intra-day precision of the method was determined by assaying three samples of each at three different concentration levels (5, 10 and 20 µg/ml) on the same day. The inter-day precision was calculated by assaying three samples of each at three different concentration levels (5, 10 and 20 µg/ml) on three different days. The % RSD for intra-day precision was found to be 0.23-0.56 whereas the inter-day precision was found to be 0.56-0.82 (Table 3).

3.2d. Robustness: Slight changes in flow rate, detection wavelength, mobile phase composition etc. affects the chromatographic response such as retention time, tailing factor and theoretical plates etc and the results were given in Table 4. The % RSD obtained was 0.70-1.16 for AGM (< 2.0%) indicating that the proposed method is robust.

The robustness of an analytical procedure refers to its ability to remain unaffected by small and deliberate variations in method parameters and provides an indication of its reliability for routine analysis. The robustness of the method was evaluated by assaying the same sample under different analytical conditions deliberately changing from the original condition. The detection wavelength was set at 228 and 232 nm (± 2 nm), the ratio of percentage of 0.05% formic acid: methanol in the mobile phase was applied as 33:67 and 37:63 (± 2, v/v), the flow rate was set at 1.1 and 0.9 $ml.min^{-1}$ (± 0.1 $ml.min^{-1}$). The results obtained (Table 4) from assay of the test solutions were not affected by varying the conditions and were in accordance with the results for original conditions. The % RSD value of assay determined for the same sample under original conditions and robustness conditions was less than 2.0% (0.70-1.16) indicating that the method is robust.

3.3. Stress degradation studies: The stability indicating capability of the method was established from the separation of Agomelatine peak from the degraded samples. The degradation of Agomelatine was found to be very similar for both the marketed formulation and standard. Typical chromatograms obtained following the assay of stressed samples are shown in Fig. 4(A-G). A slight decomposition (< 20 %) was observed when AGM drug was exposed to acidic (2.72 %), alkaline (7.80 %) thermal (6.80 %) and U.V (2.51 %) conditions whereas the drug was so sensitive towards the oxidative degradation (10.38 %) (Table 5).

The system suitability parameters for all the degradation studies were shown in Table 5. The number of theoretical plates (N) is used to determine the performance and effectiveness of the column. The efficiency of a column can be measured by the number of theoretical plates per meter. It is a measure of band spreading of a peak. Smaller the band spread, higher is the number of theoretical plates, indicating good column and system performance. Columns with theoretical plates ranging from 4,000 to 100,000 plates / meter are ideal for a good system. The theoretical plates were found to be more than 4000 and the tailing factor was less than <1.5–2 or <2 indicating that the method is more selective and specific.

3.4. Analysis of commercial formulations: The proposed method was applied for the determination of Agomelatine in marketed formulations available (AGOPREX® and AGOVIZ®). The % recovery was found to be 98.57-98.34 (Table 6). The resultant chromatograms obtained from the extraction of marketed formulations were shown in Figure 2(C-D).

Table. 1. Comparison of performance characteristics of the previously the published methods with the present method

Mobile phase/Reagent	λ (nm)	Linearity (µg/ml)	Method	Reference
Acetonitrile: methanol: water (55:25:20, v/v/v)	230	19-60	HPLC	Meghana, 2014
Methanol: 0.05 M phosphate buffer (pH 2.5) (35: 65, v/v)	230	(0.4-40) 10^{-3}	HPLC	Shaheny, 2014
Acetonitrile: water (30:70, v/v)	230	5-80	HPLC	HE Xue, 2010
Water: methanol (20:80, v/v)	230	10-50	HPLC	Harika, 2013
Ortho-phosphoric acid: triethylamine: methanol (18 : 22 : 60, v/v/v)	275	2-12	HPLC	Rashed, 2014
Phosphate buffer: methanol (60:40, v/v)	232	25-75	HPLC	Vineela, 2014
Methanol: 2 mM ammonium acetate (0.1% acetic acid) (80:20, v/v)	-	(0.05-8.069) 10^{-3}	LC – MS/MS	Patil, 2012
5 mM Ammonium acetate (0.1% formic acid): methanol (30:70, v/v)	-	(0.5-10) 10^{-3}	LC – MS/MS	Wang, 2014
Dichloro methane: methanol (95:05, v/v)	230	40-160	HPTLC	Hitendra, 2013
0.05% Formic acid : methanol (35:65, v/v)	230	0.01-100	RP-HPLC (stability indicating)	Present work

Table.2. Linearity of Agomelatine

Conc. (µg/ml)	*Mean peak area ± SD	RSD (%)
0.01	4091 ± 18.41	0.45
0.05	19432 ± 99.10	0.51
0.1	38425 ± 230.55	0.60
0.5	216564 ± 801.29	0.37
1	398459 ± 1753.22	0.44
5	1954415 ± 2345.30	0.12
10	3925235 ± 10598.13	0.27
20	7824514 ± 17213.93	0.22
50	19654487 ± 60928.91	0.31
100	38259877 ± 84171.73	0.22

*Mean of three replicates

Table.3. Precision and accuracy studies of Agomelatine

Conc. (µg/ml)	Intra-day precision * Mean peak area ± SD (%RSD)	Inter-day precision * Mean peak area ± SD (% RSD)
5	1952056.67 ± 5771.44 (0.30)	1936405.00 ± 15838.39 (0.82)
10	3917442.67 ± 22059.76 (0.56)	3900251.00 ± 21880.69 (0.56)
20	7820181.33 ± 18256.26 (0.23)	7768176.67 ± 51168.63 (0.66)

Accuracy

Spiked conc. (µg/ml)	Total conc. (µg/ml)	* Mean peak area ± SD (% RSD)	Drug Found (µg/ml)	% Recovery
4 (80 %)	9	3478413.67 ± 21141.51 (0.61)	8.90	98.89
5 (100 %)	10	3870527.67 ± 15781.98 (0.41)	9.92	99.21
6 (120 %)	11	4274057.67 ± 8617.56 (0.20)	10.97	99.74

*Mean of three replicates

Table.4. Robustness study of Agomelatine

Parameter	Condition	*Mean peak area	*Mean peak area ± SD (% RSD)	*Assay (%)
Flow rate (± 0.1 ml/min)	0.9	3875468	3894060.00 ± 27165.00 (0.70)	99.21
	1.0	3925235		
	1.1	3881477		
Detection wavelength (± 2 nm)	228	3885540	3890744.67 ± 32204.98 (0.83)	99.12
	230	3925235		
	232	3861459		
Mobile phase composition (TBAHS: methanol) (± 2, v/v)	33:67	3895448	3885777.67 ± 45077.29 (1.16)	98.99
	35:65	3925235		
	37:63	3836650		

*Mean of three replicates

Table.5. Stress degradation studies of Agomelatine

Stress Conditions	*Mean peak area	*Drug recovered (%)	*Drug decomposed (%)	Theoretical plates	Tailing factor
Standard drug (Untreated)	3925235	100	-	4869.732	1.122
Acidic degradation	3818346	97.28	2.72	4869.732	1.222
Alkaline degradation	3618897	92.20	7.80	4860.556	1.091
Oxidative degradation	3517606	89.62	10.38	4905.056	1.087
Thermal degradation	3658502	93.20	6.80	4938.208	1.101
Photolytic degradation	3826786	97.49	2.51	5056.946	1.095

*Mean of three replicates

Table.6. Analysis of Agomelatine in commercial formulation (Tablets)

Formulation	Labelled claim (mg)	Amount found* (mg)	Recovery* (%)
AGOPREX®	10	9.86	98.574
AGOVIZ®	10	9.83	98.339

* Mean of three replicates

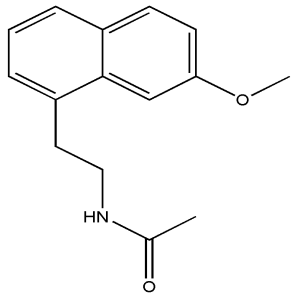

Figure.1. Chemical structure of Agomelatine (AGM)

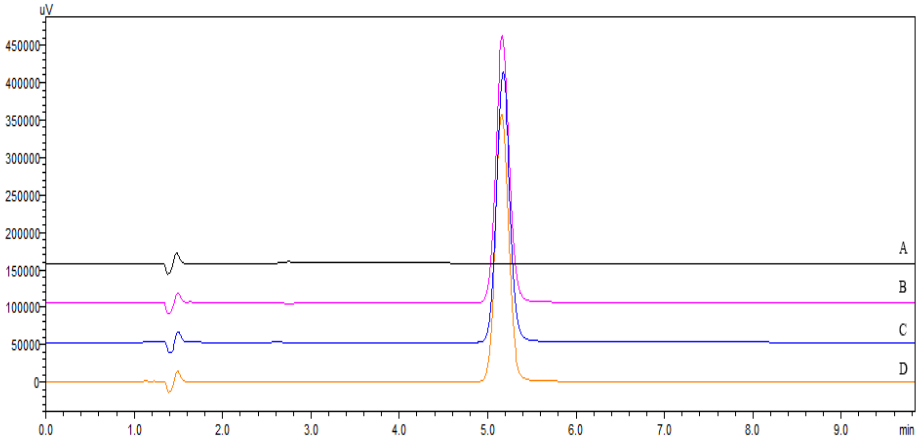

Figure.2. Typical chromatograms of blank [A], Agomelatine standard (10 µg/ml) [B], AGOPREX® (10 µg/ml) [C], AGOVIZ® (10 µg/ml) [D]

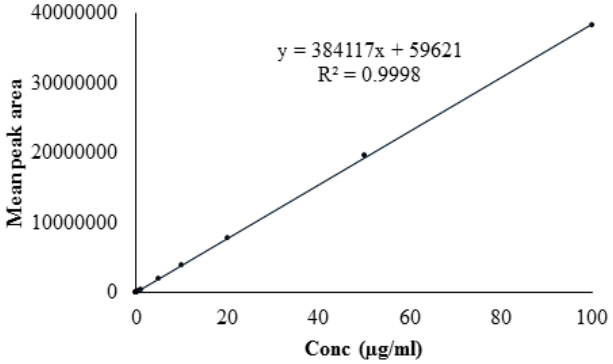

Figure.3. Calibration curve of Agomelatine

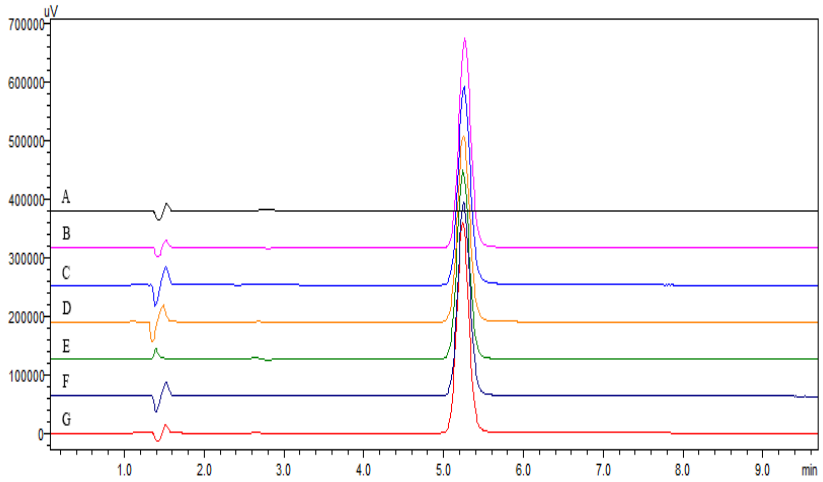

Figure.4.Typical chromatograms of blank [A], Agomelatine standard (10 µg/ml) [B], acidic [C], alkaline [D], oxidative [E], thermal [F], and photolytic [G] degradations

4. CONCLUSION

The proposed stability-indicating HPLC method was validated as per ICH guidelines and applied for the determination of Agomelatine in pharmaceutical dosage forms and can be successfully applied to perform long-term and accelerated stability studies of Agomelatine formulations. It was observed that Agomelatine is more sensitive towards the alkaline environment during the forced degradation studies.

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