

# A New Validated Stability Indicating Liquid Chromatographic Method for the Determination of Agomelatine

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## ABSTRACT

Agomelatine is a melatonergic anti-depressant. A stability-indicating high performance liquid chromatographic technique was developed for the determination of Agomelatine in pharmaceutical formulations. Chromatographic separation was achieved on Shimadzu Model CBM-20A/20 Alite, using Zorbax extended-C<sub>18</sub> column (150 × 4.6 mm i.d., 5 µm particle size) with a mixture of 0.1% ammonium formate and acetonitrile (40:60, v/v) as mobile phase with a flow rate of 0.8 ml/min. Agomelatine was subjected to stress conditions such as acidic, alkaline, oxidation photolytic and thermal degradations and the method was validated as per ICH guidelines.

**Key words:** Agomelatine, RP-HPLC, Validation, Stability-indicating.

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## INTRODUCTION

Agomelatine (AGM) is a naphthalene analog of melatonin. Agomelatine, a melatonergic novel anti-depressant with molecular formula C<sub>15</sub>H<sub>17</sub>NO<sub>2</sub> (243.301 g/mol) and chemically known as N-[2-(7-methoxynaphthalen-1-yl) ethyl] acetamide (Figure 1) has been approved for use by European Union<sup>1-2</sup> in February 2009. It is a selective agonist of the human melatonergic MT<sub>1</sub> and MT<sub>2</sub> receptors and also shows 5-HT<sub>2C</sub> receptor antagonist activity.<sup>3-4</sup>

Very few analytical methods have been reported for the determination of AGM such as HPLC,<sup>5-11</sup> LC-MS/MS in human plasma<sup>12-3</sup> and HPTLC.<sup>14</sup> So, at present the authors have developed a stability indicating RP-HPLC method for the determination of AGM in presence of its degradation products.

## MATERIALS AND METHODS

### Chemicals and reagents

Reference standards of AGM (purity >99%) was obtained from Sun Pharmaceuticals Industries Ltd (Hyderabad, India). Agomelatine is available as tablets with brand name AGOPREX<sup>®</sup> (Sun Pharmaceuticals Industries Ltd, Mumbai, India) and AGOVIZ<sup>®</sup> (Abbott India Limited, Mumbai) with label claim of 25 mg of AGM. Ammonium formate, acetonitrile, sodium hydroxide and hydrochloric acid, formic acid and Hydrogen peroxide were purchased from Merck (India). All chemicals are of HPLC grade. All chemicals were of analytical grade and used as received.

### 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 C<sub>18</sub> column (150 × 4.6 mm i.d., 5 µm particle size) maintained at 25°C.

### Preparation of 0.1% ammonium formate

Accurately 6.306 gm of ammonium formate was weighed and transferred into a 1000ml volumetric flask and diluted with HPLC grade water. The resulting solution was sonicated for half an hour and filtered.

### 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.

### Chromatographic conditions

Isocratic elution was performed using a mixture of 0.1% ammonium formate and acetonitrile (40:60%, v/v) as mobile phase with a flow rate of 0.8 ml/min. The overall run time was 10 min. and UV detection was carried out at 229 nm. 20 µL of sample was injected into the HPLC system and all chromatographic experiments were performed at room temperature (25°C ± 2°C).

### Validation

#### Linearity

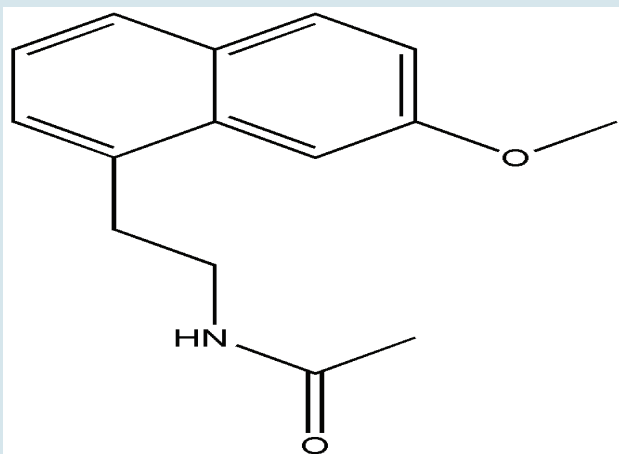
A series of solutions (0.1-100 µg/ml) were prepared from the AGM stock solution and 20 µL of each solution was injected in to the HPLC system and the peak area of the chromatogram was noted. Calibration curve was plotted by taking the concentration of the solutions on the x-axis and the corresponding peak area values on the y-axis.

#### 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).<sup>15</sup> Sensitivity of the method was established with respect to limit of detection (LOD) and LOQ for analytes.

### Precision

The intra-day precision of the assay method was evaluated by carrying out 9 independent assays of a test sample of AGM at three concentration levels (5, 10 and 15 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



**Figure 1:** Chemical structure of Agomelatine (AGM)

concentration levels (5, 10 and 15  $\mu\text{g ml}^{-1}$ ) and each value is the average of three determinations ( $n=3$ ). The % RSD of three obtained assay values on three different days was calculated.

#### 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 AGM in the drug product. The study was carried out in triplicate at 18, 20 and 22  $\mu\text{g/ml}$ . The percentage recovery in each case was calculated.

#### Robustness

The robustness of the assay method was established by introducing small changes in the HPLC conditions which included wavelength (227 and 231 nm), percentage of methanol in the mobile phase (62 and 58%) and flow rate (0.7 and 0.9 ml/min). Robustness of the method was studied using six replicates at a concentration level of 10  $\mu\text{g/ml}$  of AGM.

#### Forced degradation studies

Forced degradation studies were performed to evaluate the stability indicating properties and specificity of the method.<sup>16</sup> All solutions for stress studies were prepared at an initial concentration of 1 mg/ml of AGM and refluxed for 20 min at 80°C and then diluted with mobile phase.

#### Acidic degradation

Acidic degradation was performed by taking the drug solution mixture (1.0 mg/ml AGM) solution and exposed to acidic degradation with 0.1 M HCl for 30 min in a thermostat maintained at 80°C, the stressed sample was cooled, and then diluted with mobile phase as per the requirement and 20  $\mu\text{L}$  of the solution was injected in to the HPLC system.

#### Alkaline degradation

Alkaline degradation was performed by treating the drug solution (1.0 mg/ml AGM) with 0.1 N sodium hydroxide for 30 min in a thermostat maintained at 80°C. The drug solution was cooled, neutralized and then diluted with mobile phase as per the requirement and 20  $\mu\text{L}$  of the solution was injected in to the HPLC system.

#### Oxidative degradation

Oxidation degradation was performed by treating the drug solution (1.0 mg/ml AGM) with 30%  $\text{H}_2\text{O}_2$  for 30 min in a thermostat maintained at 80°C. The drug solution mixture was cooled and then diluted with mobile phase as per the requirement and 20  $\mu\text{L}$  of the solution was

injected in to the HPLC system.

#### Thermal degradation

Thermal degradation was performed by exposing drug solution (1.0 mg/ml AGM) to 80°C for 30min, cooled and then diluted with mobile phase as per the requirement and 20  $\mu\text{L}$  of the solution was injected in to the HPLC system.

#### Photolytic degradation

Photolytic degradation was performed by exposing drug (1.0 mg/ml AGM) to UV light (365 nm) in UV chamber for about 4 hours and then diluted with mobile phase as per the requirement before injecting in to the HPLC system.

#### Assay of marketed formulations

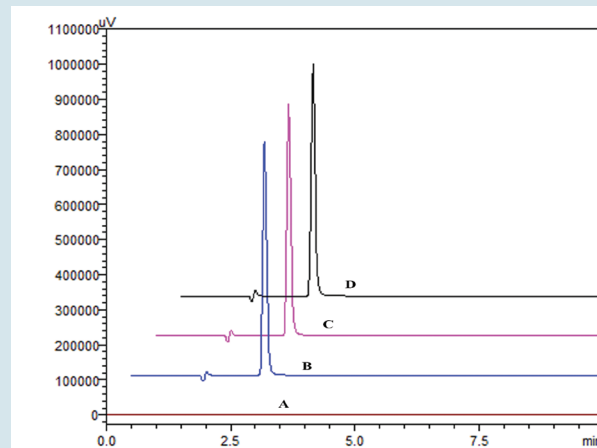
Twenty tablets of each brand of Agomelatine (AGOPREX<sup>®</sup> and AGOVIZ<sup>®</sup>) were procured from the local pharmacy store, weighed and crushed into fine powder. Powder equivalent to 25 mg of AGM was accurately weighed and transferred into a 25 ml volumetric flask and made up to volume with mobile phase. The contents of the volumetric flask were sonicated for 30 min to enable complete dissolution of both the drugs. The solution was filtered and diluted with mobile phase as per the requirement. 20  $\mu\text{L}$  of these solutions were injected into the system after filtering through 0.45  $\mu\text{m}$  membrane and the peak area was recorded from the respective chromatogram.

## RESULTS AND DISCUSSION

#### Method development and optimization

The authors have made an attempt to develop a stability indicating RP-HPLC method for the determination of Agomelatine in presence of its degradation products and also did a comparative study of the previously published methods with the present method in detail in Table 1.

Initially the drug samples were analyzed by keeping ammonium formate: acetonitrile (50:50 %, v/v) with a flow rate of 1.0 ml/min in which the peak was obtained at  $R_t$  10.215 mins and also the resolution and peak symmetry were not satisfactory. The mobile phase ratio was slightly changed (45:55%, v/v) and again the drug sample was injected in to the loop where a sharp peak was eluted at 8.754 mins with tailing. Therefore the mobile phase composition was modified as 40:60 %, v/v and then a sharp and symmetrical peak was eluted with retention time  $2.66 \pm 0.03$  mins (UV detection at 229 nm). The typical chromatograms obtained by injecting the mobile phase (blank) and the Agomelatine drug solution were shown in Figure. 2.



**Figure 2:** Typical chromatograms of Blank [A], Agomelatine (10  $\mu\text{g/ml}$ ) [B], AGOPREX<sup>®</sup> [C] AGOVIZ<sup>®</sup> [D]

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

Mobile phase/Reagent	$\lambda$ (nm)	Linearity (mg/ml)	Method	Reference
Methanol-0.05 M phosphate: buffer of pH 2.5 (35: 65, v/v)	230	(0.4-40)10 <sup>-3</sup>	HPLC (Fluorescence detection)	5
water: methanol (20:80%, v/v)	230	10-50	HPLC	6
acetonitrile and water (30:70, v/v)	230	5-80	HPLC	7
0.05% formic acid: methanol (35: 65, v/v)	230	0.01-100	HPLC	8
acetonitrile: methanol: water (55:25:20, v/v/v)	230	19-60	HPLC	9
0.16 % aqueous ortho-phosphoric acid – 0.14 % aqueous triethylamine - methanol (18: 22: 60, v/v/v)	275	2-12	HPLC	10
phosphate buffer: methanol (60:40, v/v)	232	25-75	HPLC	11
methanol and 0.1% acetic acid in 2 mM ammonium acetate solution (80:20, v/v)	-	(0.05-8.069)10 <sup>-3</sup>	LC – MS/MS	12
5 mM ammonium acetate solution (0.1% formic acid) and methanol (30:70, v/v)	-	(0.5-10)10 <sup>-3</sup>	LC – MS/MS	13
Dichloro methane and methanol in the ratio of (95:5v/v)	230	40-160	HPTLC	14
Ammonium formate: acetonitrile (40:60, v/v)	229	0.1-100	RP-HPLC (stability indicating)	Present work

**Table 2: Linearity of Agomelatine**

Conc. (mg/ml)	*Mean peak area $\pm$ SD	RSD (%)
0.1	38454 $\pm$ 99.98	0.26
0.5	214547 $\pm$ 729.46	0.34
1	405145 $\pm$ 1620.58	0.40
2	814547 $\pm$ 5213.10	0.64
5	1942456 $\pm$ 6798.60	0.35
10	3807831 $\pm$ 4188.61	0.11
15	6015464 $\pm$ 17444.85	0.29
20	7854113 $\pm$ 29060.22	0.37
50	20154364 $\pm$ 28216.11	0.14
100	41545766 $\pm$ 45700.34	0.11

\*Mean of three replicates.

#### 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).

#### Linearity

AGM shows linearity over a concentration range of 0.1-100  $\mu$ g/ml (Table 2) with %RSD 0.11-0.64 and the chromatographic response was shown in Figure 3. The linear regression equations were found to be  $y = 413957x + 135353$  ( $r^2 = 0.9997$ ). The limit of quantitation (LOQ), limit of detection (LOD) were found to be 0.0831 and 0.0274  $\mu$ g/ml respectively.

#### Accuracy

The method accuracy was proved by the recovery test at three different concentrations (80, 100 and 120%). A known amount of AGM standard (10  $\mu$ g/ml) were added to aliquots of sample solutions and then diluted

to yield the total concentrations of 18, 20 and 22  $\mu$ g/ml as described in Table 3. The % RSD was found to be 0.33-0.48 (<2.0 %) with a recovery of 98.95-99.57%.

#### Precision

The intra-day precision of the method was determined by assaying three samples of each at three different concentration levels (5, 10 and 15  $\mu$ g/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 15  $\mu$ g/ml) on three different days. The % RSD for intra-day precision was found to be 0.62-0.80 whereas the inter-day precision was found to be 0.24-0.77 (Table 3).

#### Robustness

The robustness of an analytical procedure refers to its ability to remain unaffected by small and deliberate variations in method parameters and

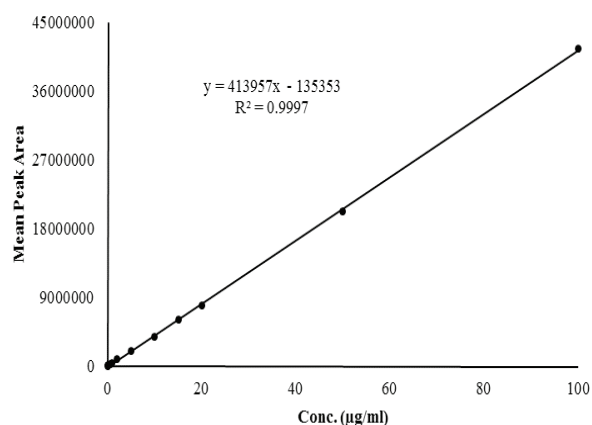


Figure 3: Calibration curve of Agomelatine

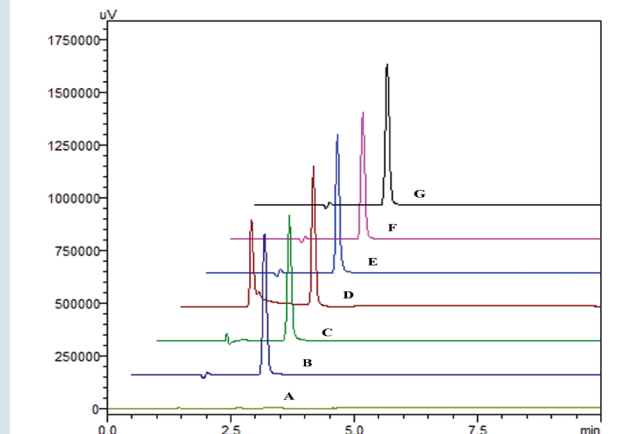


Figure 4: Typical chromatograms of blank [A] Agomelatine (10 µg/ml) [B] acidic [C] alkaline [D] oxidative [E] thermal [F] and photolytic [G] degradations

Table 3: Precision and accuracy studies of Agomelatine

Conc. (µg/ml)	Intra-day precision		Inter-day precision	
	* Mean peak area ± SD (%RSD)		* Mean peak area ± SD (% RSD)	
5	378117.33 ± 3020.31 (0.80)		374929.33 ± 2891.90 (0.77)	
10	1951887.33 ± 12039.30 (0.62)		1946558.67 ± 4627.76 (0.24)	
15	3814913.67 ± 28397.46 (0.74)		3788286.67 ± 25312.72 (0.67)	
Accuracy				
Spiked conc. (µg/ml)	Total conc. (µg/ml)	* Mean peak area ± SD (% RSD)	Drug Found (µg/ml) ± SD (% RSD)	% Recovery
8 (80 %)	18	7554413.7 ± 26402.25 (0.35)	17.92 ± 0.06 (0.36)	99.57
10 (100 %)	20	8355696.67 ± 27187.67 (0.33)	19.86 ± 0.07 (0.33)	99.29
12 (120 %)	22	9146639.33± 42819.46 (0.47)	21.77 ± 0.10 (0.48)	98.95

\*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)	0.7	3849183	3826519.33 ± 20960.66 (0.55)	100.49
	0.8	3807831		
	0.9	3822544		
Detection wavelength (± 2 nm)	227	3752114	3800797.67 ± 45575.86 (0.20)	99.82
	229	3807831		
	231	3842448		
Mobile phase composition (TBAHS: methanol) (± 2, v/v)	38-62	3854215	3819172.67 ± 30970.31 (0.81)	100.30
	40-60	3807831		
	42-58	3795472		

\*Mean of three replicates.

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 227 and 231 nm (± 2 nm), the ratio of percentage of 0.05% formic acid: methanol in the mobile phase was applied as 38:62 and 42:58 (± 2, v/v), the flow rate was set at 0.9 and 0.7 ml/min (± 0.1 ml/min). 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% (0.20-0.81) indicating that the method is robust.

### Stress degradation studies

The stability indicating capability of the method was established from the separation of AGM peak from the degradation peaks of degraded samples. Typical chromatograms obtained following the assay of stressed samples are shown in Figure. 4(A-G). A slight decomposition (< 20 %) was observed.

**Table 5: Forced degradation studies of Agomelatine**

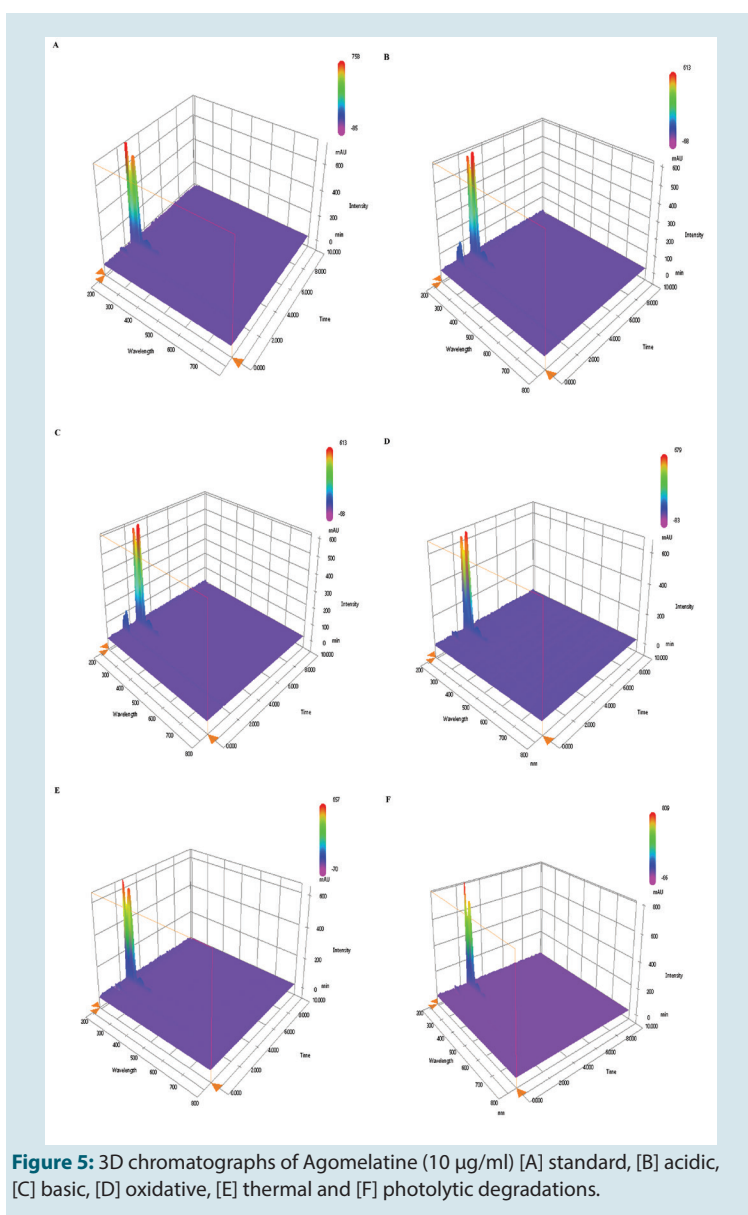
Stress Conditions	*Mean peak area	*Drug recovered (%)	*Drug decomposed (%)	Theoretical plates	Tailing factor
Standard drug (Untreated)	3807831	100	-	4219.329	1.284
Acidic degradation	3375418	88.64	11.36	4224.056	1.165
Alkaline degradation	3543742	93.06	6.94	4870.905	1.311
Oxidative degradation	3726760	97.87	2.13	4307.304	1.332
Thermal degradation	3326921	87.37	12.63	4283.98	1.288
Photolytic degradation	3749883	98.48	1.52	4044.089	1.286

\*Mean of three replicates

**Table 6: Analysis of Agomelatine in commercial formulations**

Formulation	Labelled claim (mg)	Amount found* (mg)	% Recovery*
AGOPREX®	10	9.93	99.275
AGOVIZ®	10	9.89	98.854

\* Mean of three replicates

**Figure 5:** 3D chromatograms of Agomelatine (10 µg/ml) [A] standard, [B] acidic, [C] basic, [D] oxidative, [E] thermal and [F] photolytic degradations.



was observed when AGM drug was exposed to acidic (11.36 %), alkaline (6.94 %), thermal (12.63 %), photolytic (1.52 %) and oxidative degradation (2.13 %) (Table 5).

The performance, efficiency and effectiveness of the column was determined by number of theoretical plates (N). 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 2,000 to 100,000 plates/meter are ideal for a good system. The theoretical plates were found to be more than 2000 and the tailing factor was less than  $<1.5$ -2 or  $<2$  indicating that the method is more selective and specific. The system suitability parameters for all the degradation studies were shown in Table 5. The 3D chromatographs for the degradation studies were obtained from the PDA data which shows the selectivity of the wavelength and the degradation peaks at the wide range of wavelength (Figure 5).

### Analysis of commercial formulations

The proposed method was applied to the available marketed formulations (AGOPREX® and AGOVIZ®) for the determination of AGM. The % recovery was found to be 98.85-99.28 (Table 6). The resultant chromatograms obtained from the extraction of marketed formulations were shown in Figure 2.

### CONCLUSION

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

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### CONFLICT OF INTEREST

The authors do not have conflict of interest.

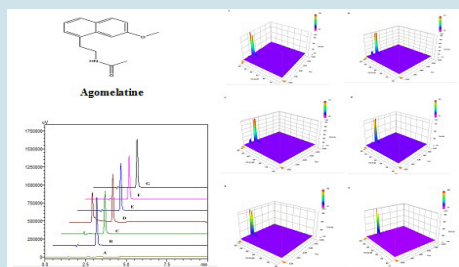
### ABBREVIATION USED

**RP-HPLC:** Reverse phase high performance Liquid chromatography; **ICH:** International conference on harmonization; **AGM:** Agomelatine; **HPTLC:** High performance thin layer chromatography; **HPLC:** High performance liquid chromatography; **LC-MS:** Liquid chromatography-Mass spectrometry; **RSD:** Relation standard deviation; **LOD:** Limit of detection; **LOQ:** Limit of quantification.

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### PICTORIAL ABSTRACT



### SUMMARY

- A stability-indicating liquid chromatographic method was developed for the determination of Agomelatine in pharmaceutical dosage forms which was validated using a mixture of mobile phase containing ammonium formate and acetonitrile.
- Agomelatine is found to be more sensitive towards the alkaline conditions during forced degradation studies.



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