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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING LIQUID CHROMATOGRAPHIC METHOD FOR THE QUANTIFICATION OF AGOMELATINE

Mukthinuthalapati Mathrusri Annapurna* and Bukkapatnam Venkatesh
Department of Pharmaceutical Analysis & Quality Assurance, GITAM Institute of Pharmacy,
GITAM University, Visakhapatnam-530045, India.
Email: mathrusri2000@yahoo.com

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Abstract

Agomelatine is relatively a new anti-depressant in comparison to other selective serotonin reuptake inhibitors used in medicine. 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_{18} column (150 mm × 4.6 mm i.d., 5 µm particle size) with a mixture of 10mM Tetra butyl ammonium hydrogen sulphate and acetonitrile (25:75, 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.

Keywords: Agomelatine, RP-HPLC, validation, stability-indicating.

Introduction:

Agomelatine (AGM) is a naphthalene analog of melatonin. Agomelatine, a melatonergic novel anti-depressant with molecular formula $C_{15}H_{17}NO_2$ (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[1-2] in February 2009. It is a selective agonist of the human melatonergic (MT1 and MT2) receptors and shows 5-HT2C receptor antagonist activity [3-4].

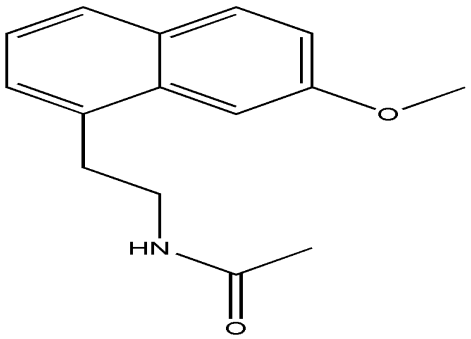


Figure 1: Chemical structure of Agomelatine (AGM)

Very few analytical methods have been reported for the determination of AGM such as HPLC [5-12], LC-MS/MS in human plasma [13-14] and HPTLC [15]. So, at present the authors have developed a stability indicating RP-HPLC method for the determination of Agomelatine in presence of its degradation products.

Materials and Methods

Chemicals and reagents:

Agomelatine (purity >99%) was obtained from Sun Pharmaceuticals Industries Ltd, India and it is available as tablets with brand names AGOPREX® (Sun Pharmaceuticals Industries Ltd, Mumbai, India) and AGOVIZ® (Abbott India Limited, Mumbai) with label claim of 25 mg of Agomelatine. Tetra butyl ammonium hydrogen sulphate, acetonitrile, sodium hydroxide and hydrochloric acid, formic acid and Hydrogen peroxide were purchased from Merck (India) and all chemicals are of HPLC 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 C18 column (150 mm × 4.6 mm i.d., 5 µm particle size) maintained at 25 ºC.

Preparation of 10mM Tetra butyl ammonium hydrogen sulphate buffer (pH 3.4):

The mobile phase was prepared by accurately transferring 3.3954 g of TBAHS in to a 1000 mL volumetric flask and dissolved with HPLC grade water.

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 10mM Tetra butyl ammonium hydrogen sulphate buffer and acetonitrile (25:75%, 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 230 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 (0.05–60 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.

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)[16]. 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 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^{-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 µg/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 (228 and 232 nm), percentage of acetonitrile in the mobile phase (73 and 77%) 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 µg/ml of AGM.

Forced degradation studies

Forced degradation studies were performed to evaluate the stability indicating properties and specificity of the method[17]. 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.

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 ºC, the stressed sample 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.

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 ºC. The drug solution was cooled, neutralized and then diluted with mobile phase as per the requirement and 20 µL 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% H_2O_2 for 30 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.

Thermal degradation:

Thermal degradation was performed by exposing drug solution (1.0 mg/ml AGM) to 80^0 C for 30min, cooled and then diluted with mobile phase as per the requirement and 20 µL 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® and AGOVIZ®) 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 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.

Results and Discussion

Method development and optimization:

An attempt has been made to develop a stability indicating RP-HPLC method for the assay of Agomelatine in pharmaceutical products. Table 1 describes a detailed comparative study of the previously published methods with the present method. The drug samples were analyzed using different mobile phase compositions and flow rates but the peak symmetry was not satisfactory and finally a mixture of TBAHS: acetonitrile (25:75%, v/v) with a flow rate of 0.8 ml/min has produced a sharp peak of the drug without tailing. The typical chromatogram of Agomelatine was shown in Figure 2A.

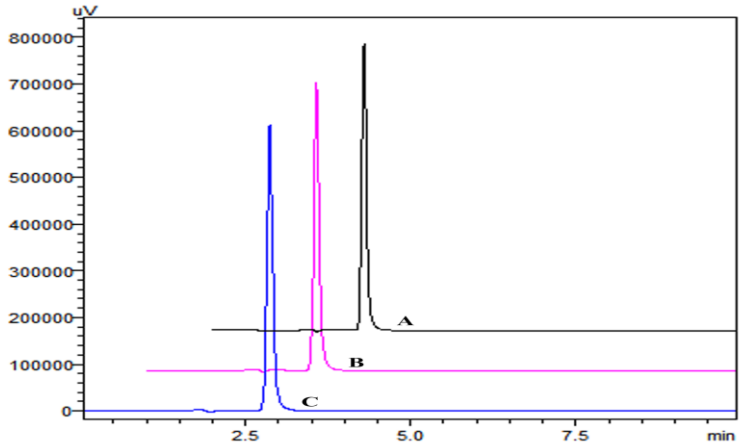


Figure 2: Typical chromatograms of Agomelatine (10 µg/ml) [A], AGOPREX® [B] and AGOVIZ® [C].

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

Mobile phase/Reagent	λ (nm)	Linearity (µg/ml)	Method	Reference
Methanol: phosphate: buffer (35: 65, v/v)	230	$(0.4\text{-}40)10^{-3}$	HPLC (Fluorescence detection)	5
Water: methanol (20:80%, v/v)	230	10-50	HPLC	6
Acetonitrile: 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
o-Phosphoric acid: 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
Ammonium formate: methanol (40:60, v/v)	229	0.1-100	HPLC (stability indicating)	12
Methanol: 0.1% acetic acid in ammonium acetate solution (80:20, v/v)	-	$(0.05\text{-}8.069)10^{-3}$	LC – MS/MS	13
Ammonium acetate solution (0.1% formic acid): methanol (30:70, v/v)	-	$(0.5\text{-}10)10^{-3}$	LC – MS/MS	14
Dichloro methane: methanol (95:5, v/v)	230	40-160	HPTLC	15
TBAHS: acetonitrile (25:75, v/v)	229	0.05-50	HPLC (Stability indicating)	Present work

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 0.05–50 µg/ml (Table 2) with % RSD 0.11-0.31 and the chromatographic response was shown in Figure 3. The linear regression equations were found to be y = 394083x + 46274 (r^2 = 0.9996). The limit of quantitation (LOQ), limit of detection (LOD) were found to be 0.0073 and 0.0024 µg/ml respectively.

Table 2: Linearity of Agomelatine.

Conc. (µg.mL^{-1})	*Mean peak area ± SD	RSD (%)	SEM
0.05	20158 ± 42.33	0.21	24.44027613
0.1	39547 ± 55.37	0.14	31.96545953
1	485153 ± 1309.91	0.27	756.2786809
5	2124886 ± 6587.15	0.31	3803.090863
10	4097588 ± 8604.93	0.21	4968.061423
20	7616778 ± 19041.95	0.25	10993.87207
30	11787476 ± 12966.22	0.11	7486.052686
40	15987530 ± 38370.07	0.24	22152.9714
50	19747589 ± 27646.62	0.14	15961.78615

*Mean of three replicates

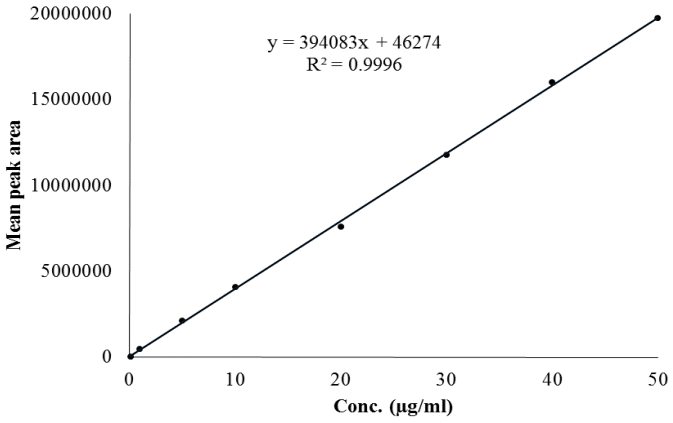


Figure 3: Calibration curve of Agomelatine.

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 µg/ml) were added to aliquots of sample solutions and then diluted to yield the total

concentrations of 18, 20 and 22 µg/ml as described in Table 3. The % RSD was found to be 0.23- 0.54 (<2.0 %) with a recovery of 98.21 – 98.98%.

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 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 15 µg/ml) on three different days. The % RSD for intra-day precision was found to be 0.34-1.36 whereas the inter-day precision was found to be 0.60-1.39 (Table 3).

Table 3: Precision and accuracy studies of Agomelatine.

Conc. ($\mu g.mL^{-1}$)	Intra-day precision			Inter-day precision		
	* measured concentration ($\mu g.mL^{-1}$) ± SD	%RSD	SEM	* measured concentration ($\mu g.mL^{-1}$) ± SD	%RSD	SEM
5	4.9447 ± 0.0674	1.36	0.0328	4.92 ± 0.0682	1.39	0.0394
10	9.9056 ± 0.0852	0.86	0.1307	9.92 ± 0.0967	0.97	0.0558
20	19.9445 ± 0.0675	0.34	0.1425	19.87 ± 0.1190	0.60	0.0687

Accuracy					
Spiked conc. ($\mu g.mL^{-1}$)	Total conc. ($\mu g.mL^{-1}$)	*Conc. found ($\mu g.mL^{-1}$) ± SD	%RSD	SEM	%Recovery
8 (80 %)	18	17.68±0.0948	0.54	0.3039	98.24
10 (100 %)	20	19.80±0.0524	0.26	0.1513	98.98
12 (120 %)	22	21.61±0.0504	0.23	0.1322	98.21

*Mean of three replicates

Robustness:

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 TBHS : acetonitrile in the mobile phase was applied as 23:77 and 27:73 (± 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 and retention time determined for the same sample under original conditions and robustness conditions was less than 2.0% (0.02-0.42) indicating that the method is robust.

Table 4: Robustness study of Agomelatine

Parameter (condition)	*%Assay ± SD	%RSD	SEM	*Retention time ± SD	%RSD	SEM
Mobile phase flow rate (± 0.1 mL.min^{-1})						
(0.7 mL.min^{-1})	100.66 ± 0.1011	0.1004	0.0584	2.983 ± 0.002	0.070	0.0012
(0.9 mL.min^{-1})	98.92 ± 0.0178	0.0180	0.0103	2.754 ± 0.004	0.128	0.0020
Detection wavelength (± 2 nm)						
(228 nm)	104.34 ± 0.0315	0.0302	0.0182	2.855 ± 0.002	0.073	0.0012
(232 nm)	98.33 ± 0.1811	0.1842	0.1045	2.857 ± 0.002	0.073	0.0012
Mobile phase composition (TBHS: acetonitrile) (±2, v/v)						
(27:73, v/v)	100.57 ± 0.4205	0.4181	0.2428	2.724 ± 0.002	0.056	0.0009
(23:77, v/v)	98.59 ± 0.0941	0.0955	0.0543	2.954 ± 0.004	0.122	0.0021

*Mean of three replicates

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. Figure. 4: has shown the evidence of chromatograms of the stressed samples. Extra peaks were observed during acidic and oxidation degradation study and the system suitability parameters 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). The efficiency of the column can be determined by the number of theoretical plates (N). Columns with theoretical plates ranging from 2,000 to 100,000 plates / meter are ideal for a good system. In the present proposed method the theoretical plates were found to be more than 2000 and that of tailing factor 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.

Table 5: Forced degradation studies of Agomelatine

Stress Conditions	Retention time (R_t)	peak area	*Drug recovered (%)	*Drug decomposed (%)	Extra peaks	Theoretical plates (N)	Tailing factor	Capacity Factor (k')	Resolution (R)
Standard drug (Untreated)	2.861	4097588	100	-	-	3810.498	1.304	-	-
Acidic degradation	2.866	3990501	97.39	2.61	-	3835.362	1.296	-	-
Alkaline degradation	2.864	4099215	100.04	-0.04	1.857, 3.563	3837.812	1.336	0.543	5.681
Oxidative degradation	2.865	4016866	98.03	1.97	1.677, 1.887	3673.561	1.398	0.709	5.434
Thermal degradation	2.862	4097432	100.01	-	-	3270.914	1.643	-	-

Photolytic degradation	2.868	4046892	98.76	1.24	-	3705.499	1.255	-	-

*Mean of three replicates

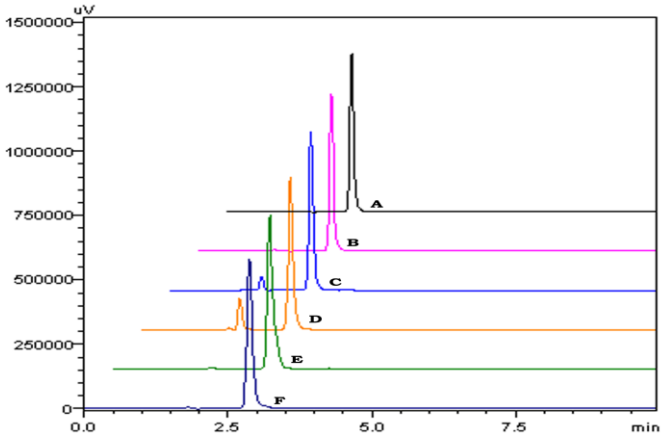
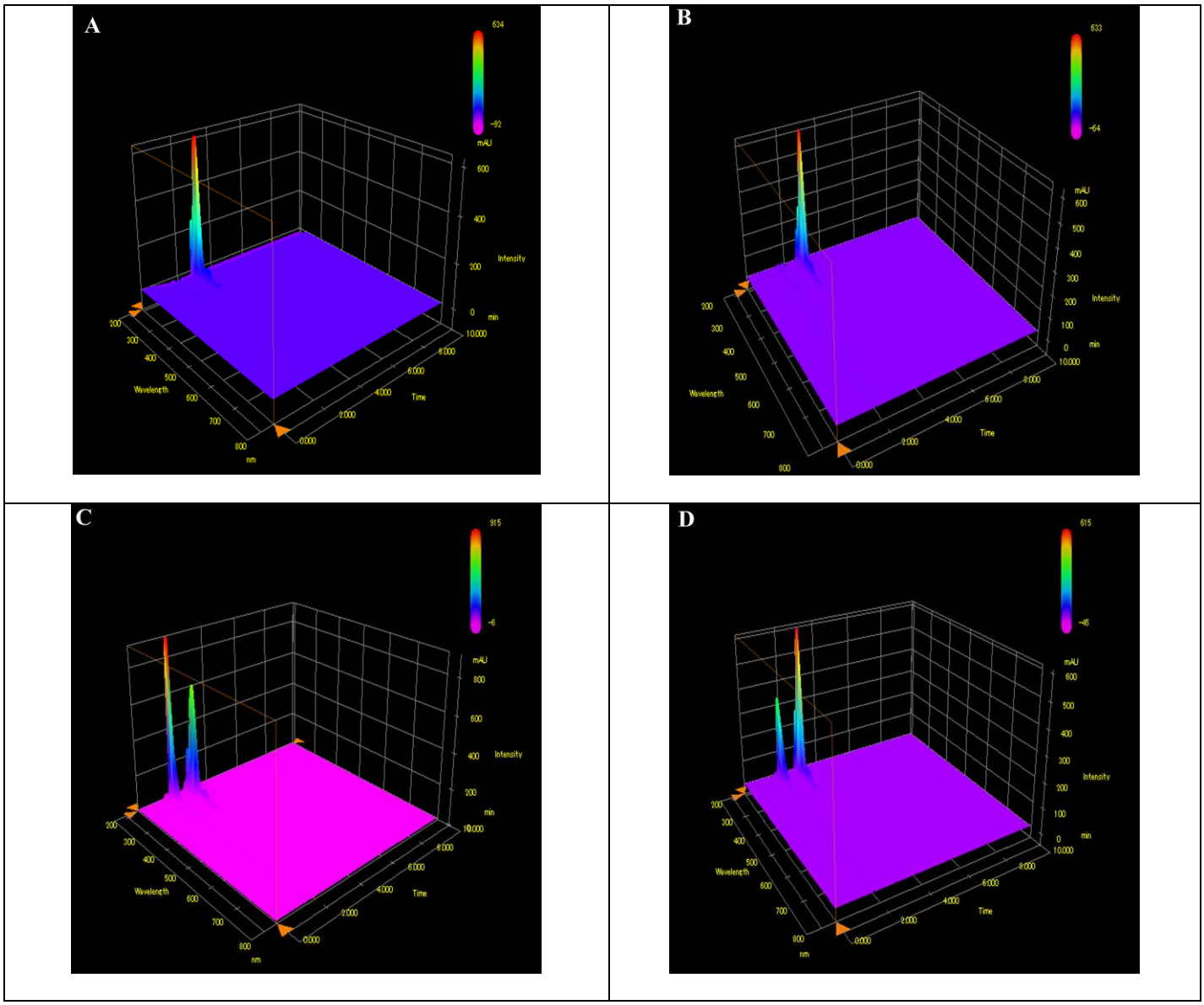


Figure 4: Typical chromatograms of Agomelatine (10 µg.mL^{-1}) [A], acidic [B], alkaline [C], oxidative [D], thermal [E] and photolytic [F] degradations.



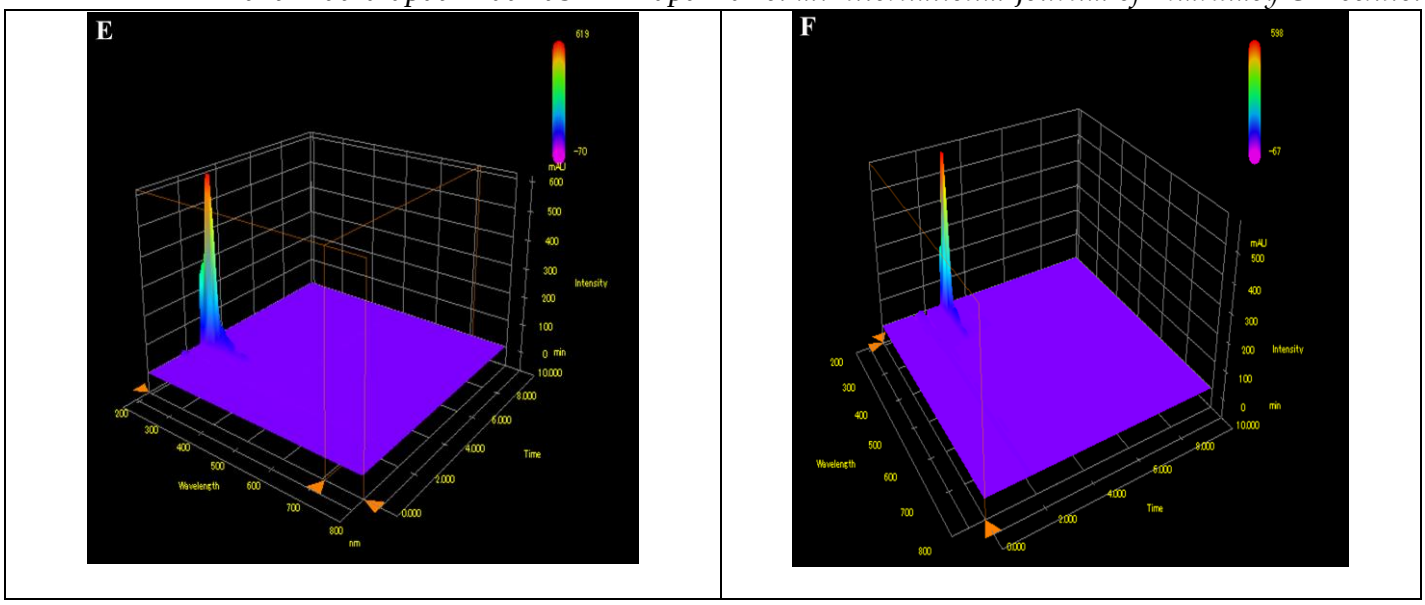


Figure 5: 3D chromatographs of Agomelatine (10 µg/ml) [A] standard, [B] acidic, [C] basic, [D] oxidative, [E] thermal and [F] photolytic degradations.

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 99.15-99.49 (Table 6). The resultant chromatograms obtained from the extraction of marketed formulations were shown in Figure 2.

Table 6: Analysis of Agomelatine in commercial formulations

Formulation	Labelled claim (mg)	Amount found* (mg)	% Recovery*
AGOPREX®	10	9.95	99.49
AGOVIZ®	10	9.91	99.15

* Mean of three replicates

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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Corresponding Author:

Mukthinuthalapati Mathrusri Annapurna*,

Email: *mathrusri2000@yahoo.com*