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Forced Degradation, Identification and Characterization of Impurities of Agomelatine Using Chromatographic and Spectroscopic Techniques.

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ABSTRACT

A High Performance Liquid Chromatography (HPLC) method was developed for determination of degradation impurities of Agomelatin drug substance. The chromatographic separation was achieved on Shimadzu LC-2010 with PDA system and C18 150 x 4.6mm, 5.0 µm column using gradient elution of mobile phase. The present study is aim to degrade the drug substance using different degradation conditions like acid, base, oxidative, thermal and light. The degraded products were subjected to LC-MS to find out the impurities mass. Based on the mass of impurities the structures were assigned. The proposed method was successfully employed for identification of Agomelatine impurities in pharmaceutical preparations.

Keywords: HPLC, Agomelatine, Degradation, LC-MS, Impurities and pharmaceutical preparations.

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INTRODUCTION

Agomelatine was discovered and developed by the European pharmaceuticalcompany Servier Laboratories Ltd. Agomelatine (BAN, rINN; trade names Valdoxan, Melitor, Thymanax) is a melatonergic antidepressant developed by the pharmaceutical company Servier. Each film coated tablet contains 25mg of Agomelatine. Agomelatine is indicated for the treatment of major depressive episodes in adults.[1] Ten placebo controlled trials have been performed to investigate the short term efficacy of agomelatine in major depressive disorder. At the end of treatment, significant efficacy was demonstrated in six of the ten short-term double-blind placebo-controlled studies [1]. The maintenance of antidepressant efficacy was demonstrated in a relapse prevention study. In patients with a greater baseline score (>30 on HAMD17 scale), the agomelatine-placebo difference was of 4.53 points [2].

Controlled studies in humans have shown that agomelatine is at least as effective as the SSRI antidepressants paroxetine, sertraline, escitalopram, venlafaxine and fluoxetine in the treatment of major depression [3-5]. Agomelatine is a substrate of CYP1A2, CYP2C9 and CYP2C19 and hence CYP1A2, CYP2C9 and CYP2C19 inhibitors (e.g. the SSRI antidepressant, fluvoxamine) reduce its clearance and can hence lead to an increase in agomelatine exposure [6,7]. A large meta-analysis of 20 trials with 7460 participants found agomelatine to be as effective as standard antidepressants [8]. A small open-label study has suggested efficacy in the treatment of atypical and melancholic depression [13]. Well-designed clinical trials have demonstrated efficacy in the treatment of anxious depression [9,10]. Agomelatine's onset of action has been reported to occur as early as the first week of treatment [11]. Additionally, possibly because of its action on melatonin receptors, agomelatine appears to improve sleep quality, with no reported daytime drowsiness [12]. Agomelatine has demonstrated anxiolytic properties in rodents [13]. It has been found significantly more effective than placebo in the treatment of generalised anxiety disorder [14].

A stability indicating method was developed and validated for drug substance of Agomelatine.[15] The aim of the study is to identification and characterization of the impurities which were formed during stress degradation.

Agomelatine and its impurities chemical structure are shown in Fig.1 [I-IV]. Agomelatine AGM-I undergoes degradation as per the degradation path shown in Fig-2.

In order to improve the sensitivity and selectivity of the chromatographic determination of Agomelatine impurities, a simple reversed-phase HPLC method with UV detection at 240nm is used, where all impurities have been separated in a single analytical column. In our study, Shimadzu HPLC has been successfully used for the determination of (AGM-II), (AGM-III) and (AGM-IV). A reduction in separation time has been achieved, without compromising separation quality compared to other traditional Liquid Chromatography (LC) methods.

MATERIALS AND METHODS

Agomelatine provided by SDS Labs Private Limited, Navi Mumbai, India. Acetonitrile (HPLC-grade from Merck), methanol (HPLC grade from Merck) and Ammonium acetate AR grade from Rankem, Hydrochloric Acid, Hydrogen Peroxide were from Merck (Darmstadt, Germany), Sodium hydroxide AR grade from Rankem. Water was purified by a water purifier (SG water purifier) and passed through a 0.45 µm membrane filter (Durapore) before use.

Standard and degradation samples were prepared in methanol as diluent.

Equipment

HPLC analysis was performed with a Shimadzu LC-2010 with PDA system consists of a Quaternary solvent manager, a sample manager, column-heating compartment, and Photodiode array detector. This system was controlled by Shimadzu LC solutions software. Hypersil BDS C_{18}, 150 x 4.6 mm, 5 µm employed as stationary phase for chromatographic separation. The wavelength was selected at 240 nm. The gradient method was employed as the ratio of Acetonitrile initially 5.0 % and 0 to 10 minutes 90.0 %, 10 to 30 minutes 90.0 % The flow rate was maintained at 1.0 mL per minute and the injection volume was 20.0 µL. All samples

were centrifuged by Thermo Scientific centrifuge. The thermal and photo degradation study was conducted by using hot air oven and photo stability chamber.

Standard and Sample Preparation

Weighed accurately 50 mg of sample and transferred into 100 mL volumetric flask. To this 35 mL of diluent is added and sonicated to dissolve the contents, and further diluted up to the volume with same diluent and mixed well.

Weighed accurately 50 mg of Standard and transferred into 100 mL volumetric flask. To this 35 mL of diluent is added and sonicated to dissolve the contents, and further diluted up to the volume with same diluent and mixed well.

Forced Degradation of Agomelatine by 1.0 N Hydrochloric acid

Weighed accurately 250 mg of Agomelatine Sample and transferred into 50 mL volumetric flask. To this 15 mL of 1.0 N hydrochloric acid is added and sonicated the contents and further diluted up to the volume with same Hydrochloric acid and mixed well, kept this solution for 24 hours for degradation.

After 24 hours transferred 1 mL of above solution into a 10 mL volumetric flask and neutralized with same amount of 1.0 N sodium hydroxide solution, then diluted up to the mark with diluent.

Forced Degradation of Agomelatine by 1.0 N Sodium hydroxide

Weighed accurately 250 mg of Fenoxazoline Sample and transferred into 50.0 mL volumetric flask. To this 5 mL of 1.0N Sodium Hydroxide is added, sonicated the contents and further diluted up to the volume with same Sodium Hydroxide and mixed well. Kept this solution for degradation.

After 24 hours transferred 1 mL degradation product into 10 mL volumetric flask and neutralized with same amount of 1.0N Hydrochloric acid, then diluted up to the volume with diluent.

Forced Degradation of Agomelatine by 5.0% Hydrogen peroxide

Weighed accurately 250 mg of Agomelatine sample and transferred into 50 mL volumetric flask. To this 5 mL of 5.0% hydrogen peroxide is added, sonicated the contents and further diluted up to the volume with same hydrogen peroxide and mixed well. This solution was kept for degradation.

After 24 hours transferred 1 mL of above solution into 10 mL volumetric flask and diluted up to the mark with diluent.

Forced Degradation by light

Weighed accurately about 250.0 mg of Agomelatine sample and kept for degradation in photostability chamber.

Transferred 50 mg of above degradation compound after 24 hours into a 100 mL volumetric flask and 35 mL of diluent is added, then sonicated to dissolve, finally diluted up to the mark with same diluent and mixed.

Forced Degradation by Thermal treatment

Weighed accurately about 250.0 mg of Agomelatine sample and transferred to petridish and kept for degradation in a calibrated oven at 70 °C for 24 hours.

Weighed accurately about 50 mg of above degradation product and transferred into 100.0 mL volumetric flask, added 35 mL of diluent and sonicated to dissolve. Then diluted up to the mark with diluent and mixed.

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RESULTS AND DISCUSSION

HPLC system has been proved to be a promising tool for separation of Agomelatine and its impurities. Agomelatine and its degradants were well separated with good peak shape and resolution. No interfering peaks were observed in blank.

The compound was degraded in acidic, basic and oxidative conditions and it was stable under thermal and sunlight conditions. The structure interpretation of Agomelatine and its impurities were as mentioned below.

Interpretation of different compounds

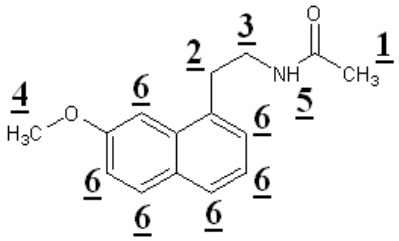
Agomelatine (AGM-I)

Molecular formula: $C_{15}H_{17}NO_2$

Formula weight: 243.30

^{1}H-NMR:

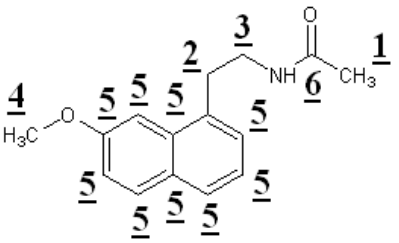
The ^{1}H-NMR of Agomelatine recorded on 400 MHz NMR in $CDCl_3$. Observed δ values and its interpretation are as follows.



S. No.	Chemical shift δ (In ppm)	Proton assignment
1.	1.947 (Singlet)	-CH_3 protons (3)
2.	3.288 – 3.264(Triplet)	–CH_2 protons (2) of attached to aromatic ring
3.	3.590 – 3.641 (Multiplet)	-CH_2 protons (2) in the vicinity of nitrogen atom.
4.	3.987 (Singlet)	-OCH_3 protons (3)
5.	5.597 (Broad singlet)	-NH proton (1)
6.	7.149 – 7.767	Aromatic protons (6)

^{13}C-NMR:

The ^{13}C-NMR of Agomelatine recorded on 400 MHz NMR in DMSO-d_6. Observed δ values and its interpretation are as follows.



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S. No.	Chemical shift δ (In ppm)	Carbon assignment
1.	22.656	-CH_3 Carbon
2.	33.153	–CH_2 carbon attached to aromatic ring
3.	39.632	-CH_2 carbon in the vicinity of nitrogen atom.
4.	55.249	-OCH_3 carbon
5.	102.653 – 157.499	Aromatic carbons
6.	169.511	Carbonyl carbon

Mass

The mass spectrum was obtained using mass spectrophotometer. Molecular ion peak of Agomelatine was observed m/z at 244.1 (M+H ion).

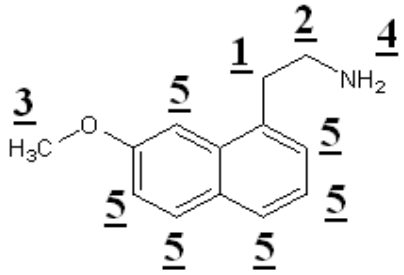
Agomelatine acid impurity (AGM-II)

Molecular formula: $C_{13}H_{15}NO$

Formula weight: 201.26

^{1}H-NMR

The ^{1}H-NMR of Agomelatine acid impurity recorded on 400 MHz NMR in $CDCl_3$. Observed δ values and its interpretation are as follows.



S. No.	Chemical shift δ (In ppm)	Proton assignment
1.	3.186 – 3.221(Triplet)	–CH_2 protons (2) of attached to aromatic ring
2.	3.546 – 3.598 (Multiplet)	-CH_2 protons (2) in the vicinity of nitrogen atom.
3.	3.968 (Singlet)	-OCH_3 protons (3)
4.	5.539 (Broad singlet)	-NH_2 protons (2)
5.	6.976 – 7.703	Aromatic protons (6)

^{13}C-NMR

The ^{13}C-NMR of Agomelatine acid impurity recorded on 400 MHz NMR in $CDCl_3$. Observed δ values and its interpretation are as follows.

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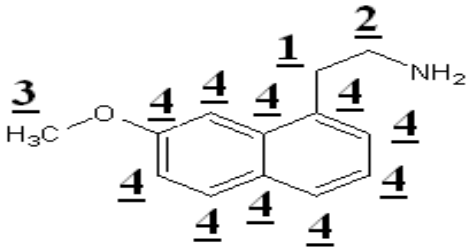


S. No.	Chemical shift δ (In ppm)	Carbon assignment
1.	33.129	–CH_2 carbon attached to aromatic ring
2.	39.628	-CH_2 carbon in the vicinity of nitrogen atom.
3.	55.215	-OCH_3 carbon
4.	102.637 – 157.389	Aromatic carbons

Mass:

The mass spectrum was obtained using mass spectrophotometer. Molecular ion peak of Agomelatine acid impurity was observed m/z at 202.1 (M+H ion).

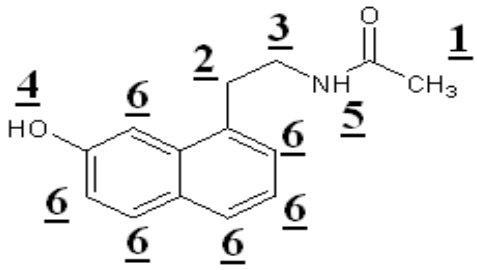
Agomelatine base impurity (AGM-III)

Molecular formula: $C_{14}H_{15}NO_2$

Formula weight: 229.27

^{1}H-NMR

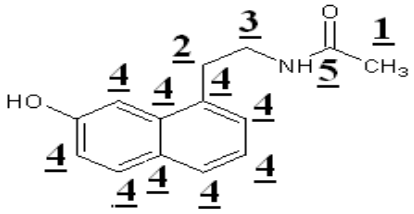
The ^{1}H-NMR of Agomelatine base impurity recorded on 400 MHz NMR in $CDCl_3$. Observed δ values and its interpretation are as follows.



S. No.	Chemical shift δ (In ppm)	Proton assignment
1.	1.924 (Singlet)	-CH_3 protons (3)
2.	3.185 – 3.224(Multiplet)	–CH_2 protons (2) of attached to aromatic ring
3.	3.456 – 3.495 (Multiplet)	-CH_2 protons (2) in the vicinity of nitrogen atom.
4.	4.995 (Broad Singlet)	-OH proton (1)
5.	5.549 (Broad singlet)	-NH proton (1)
6.	7.093 – 7.741	Aromatic protons (6)

^{13}C-NMR

The ^{13}C-NMR of Agomelatine base impurity recorded on 400 MHz NMR in $CDCl_3$. Observed δ values and its interpretation are as follows.



S. No.	Chemical shift δ (In ppm)	Carbon assignment
1.	22.589	-CH_3 Carbon
2.	34.041	–CH_2 carbon attached to aromatic ring
3.	41.220	-CH_2 carbon in the vicinity of nitrogen atom.
4.	103.212 – 159.154	Aromatic carbons
5.	173.149	Carbonyl carbon

Mass

The mass spectrum was obtained using mass spectrophotometer. Molecular ion peak of Agomelatine base impurity was observed m/z at 230.2 (M+H ion).

Agomelatine oxidative impurity (AGM-IV)

Molecular formula: $C_{15}H_{17}NO_3$

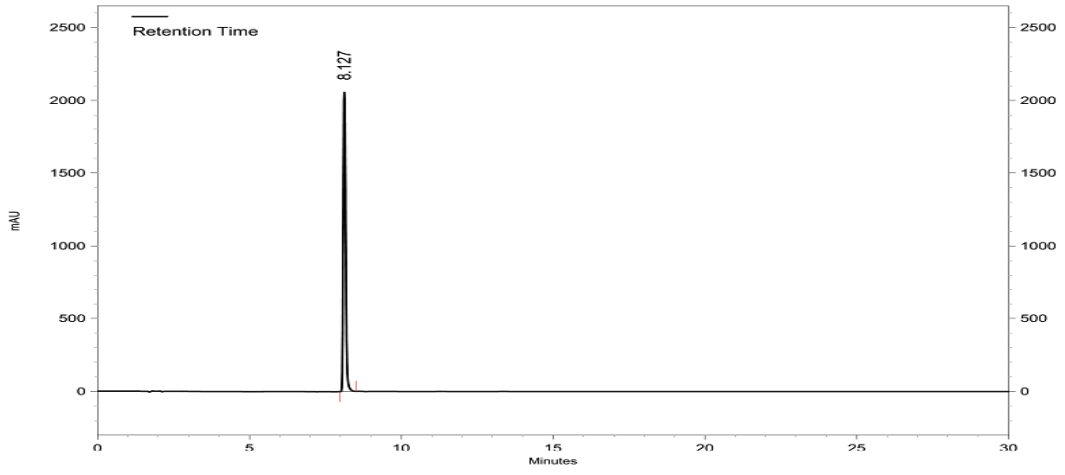
Formula weight: 259.30

Mass

The mass spectrum was obtained using mass spectrophotometer. Molecular ion peak of Agomelatine oxidative impurity was observed m/z at 260.2 (M+H ion).

Forced degradation HPLC chromatograms

Standard



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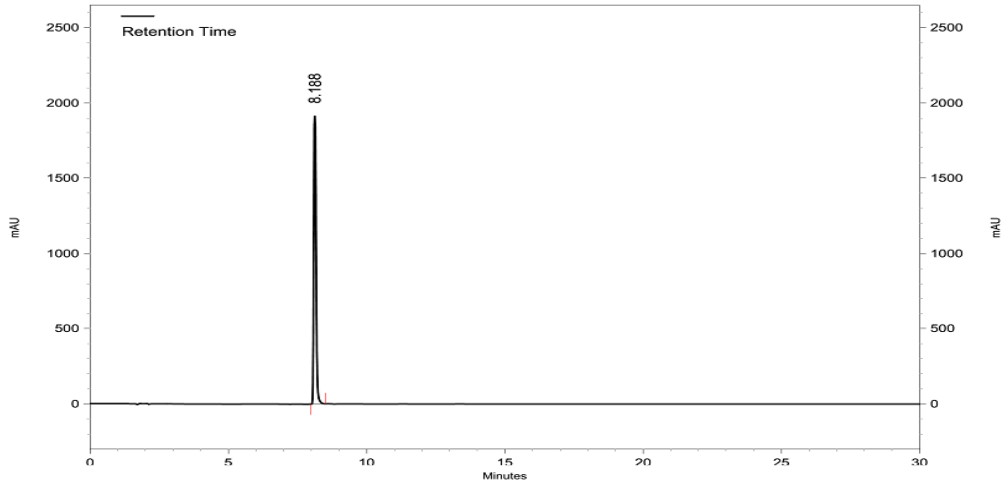
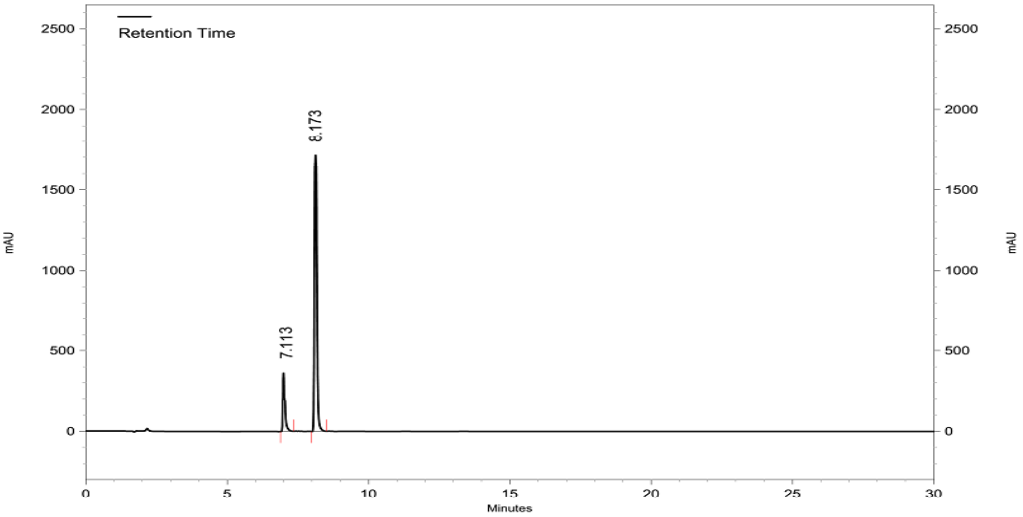
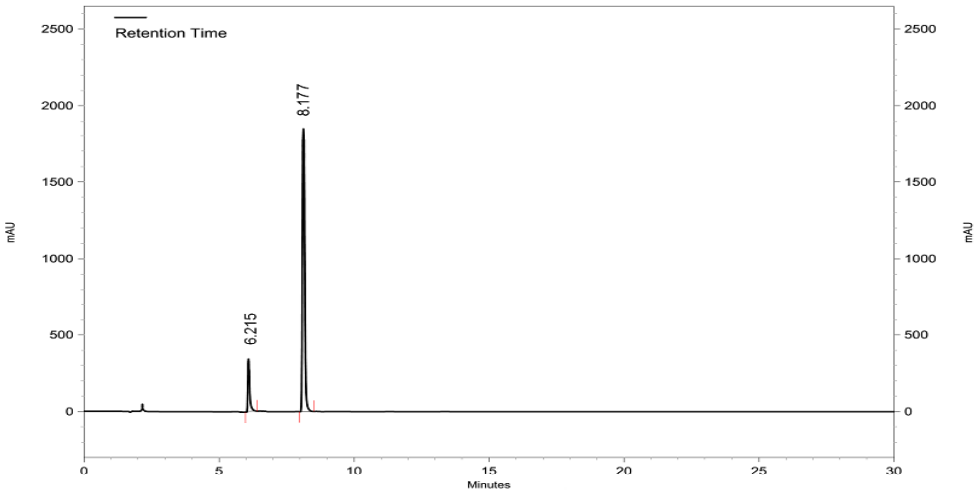
Sample



Acid degradation



Base degradation



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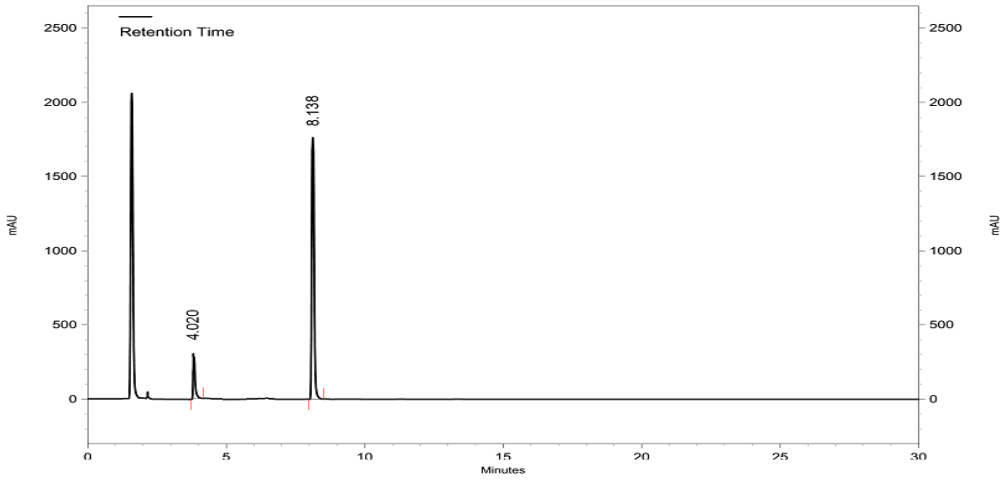
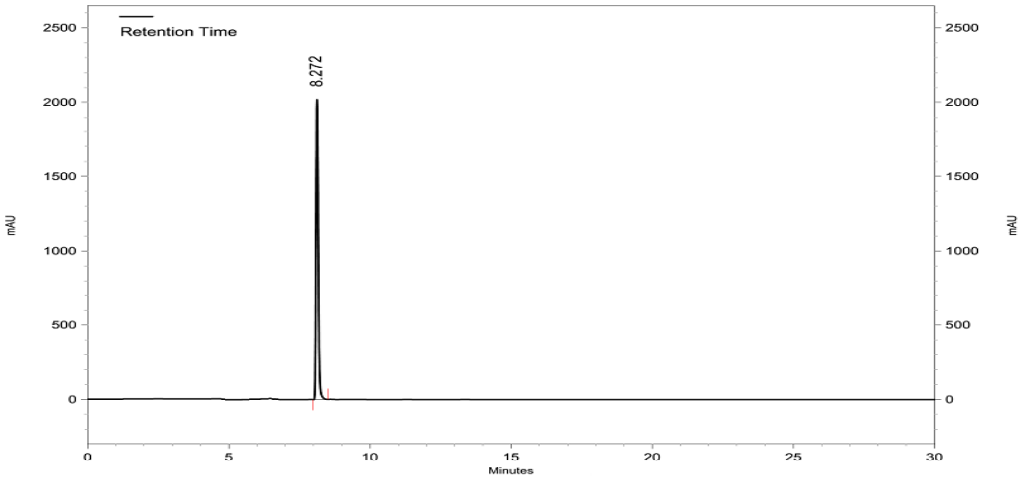
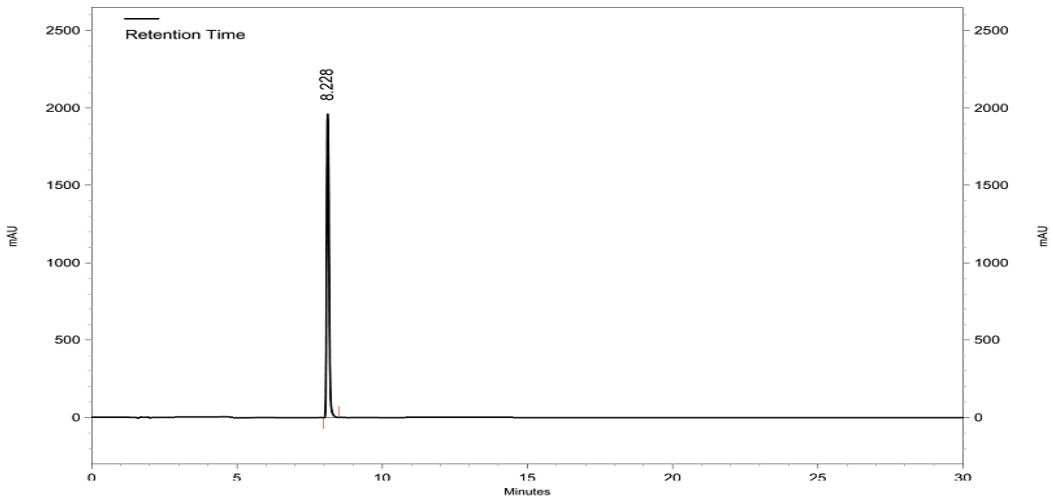
Oxidative degradation



Thermal degradation



Photodegradation



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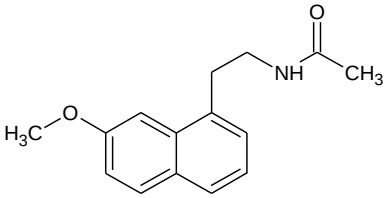
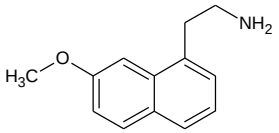
Agomelatine and its impurities structures

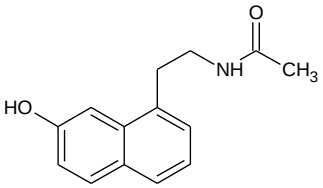
A). Agomelatine (AGM-I):



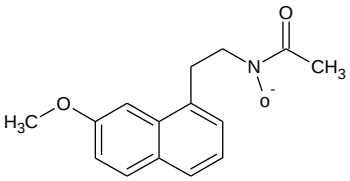
B). Impurity-1 (Acid degradation) (AGM-II):



C). Impurity-2 (Base degradation) (AGM-III):

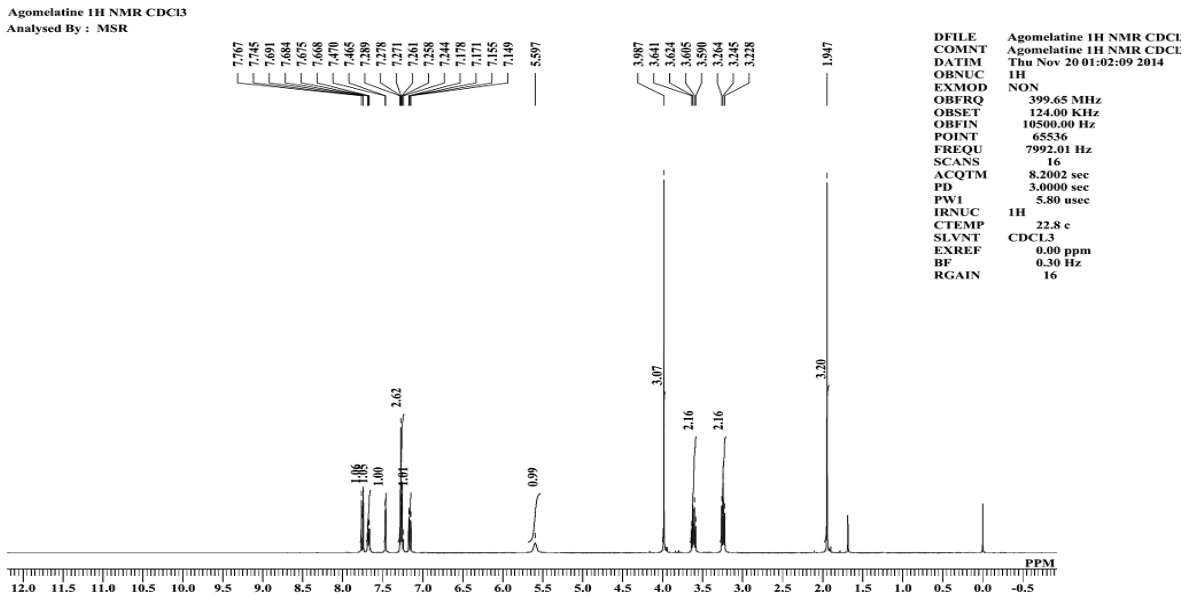


D). Impurity-3 (Oxidative degradation) (AGM-IV):



NMR and Mass spectra

Agomelatine (AGM-I):



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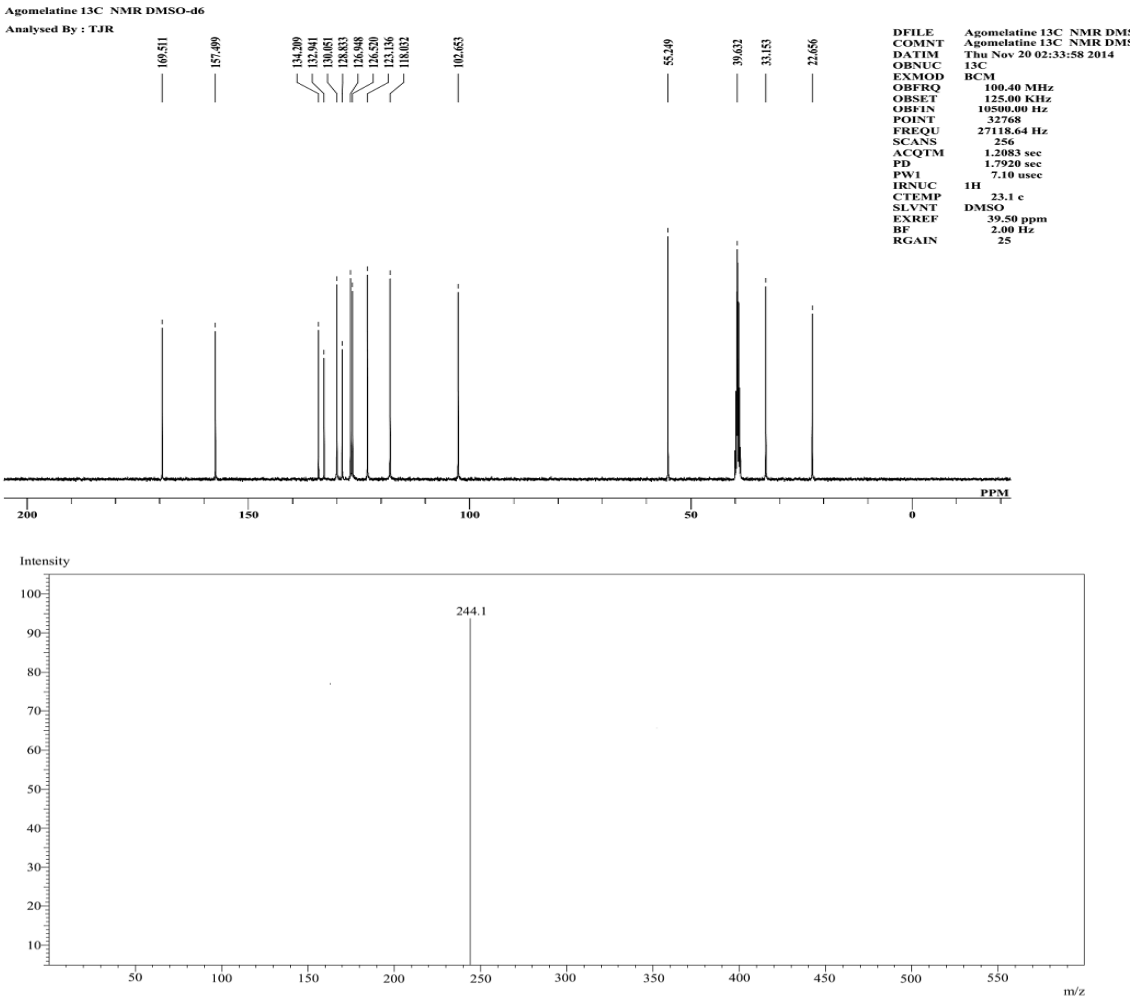
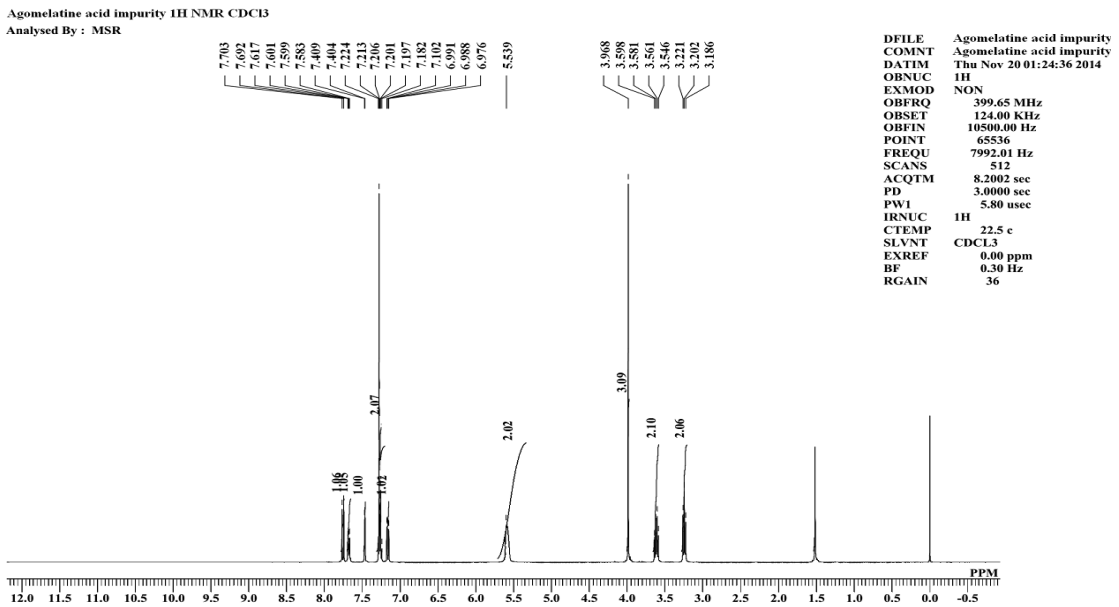


Impurity-1 (Acid degradation) (AGM-II):



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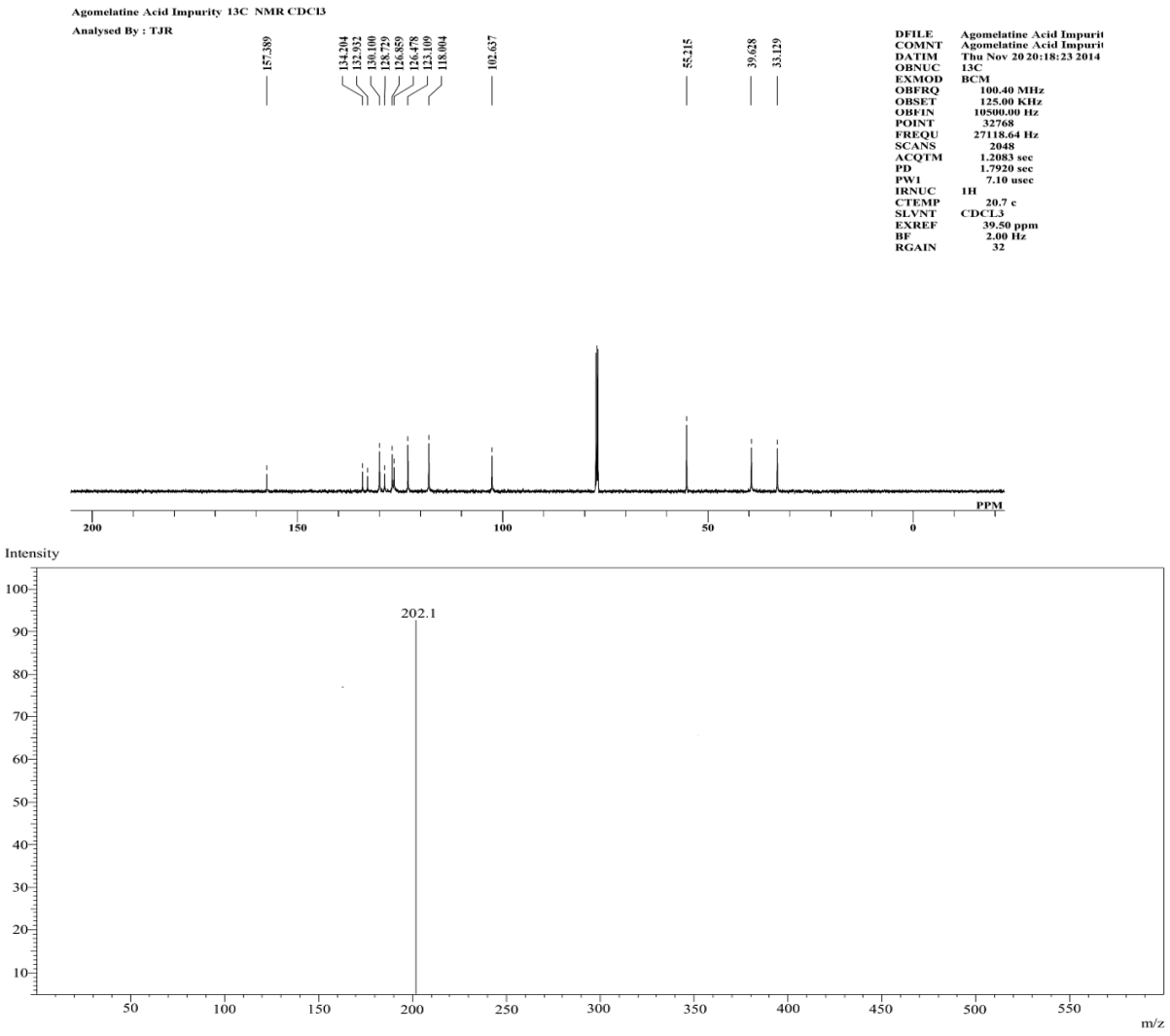
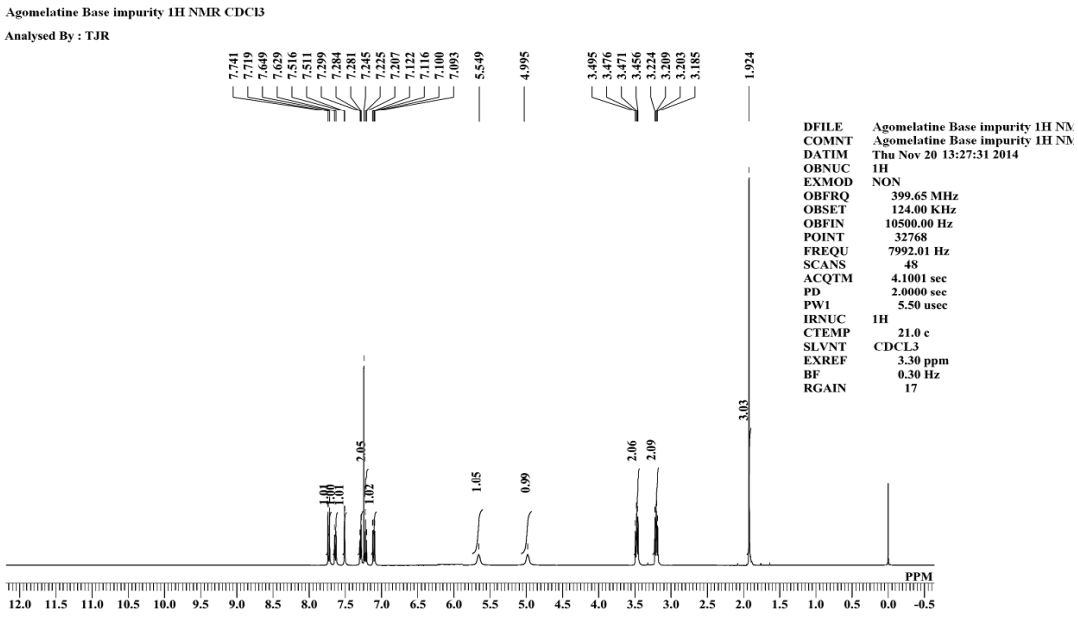


Impurity-2 (Base degradation) (AGM-III)



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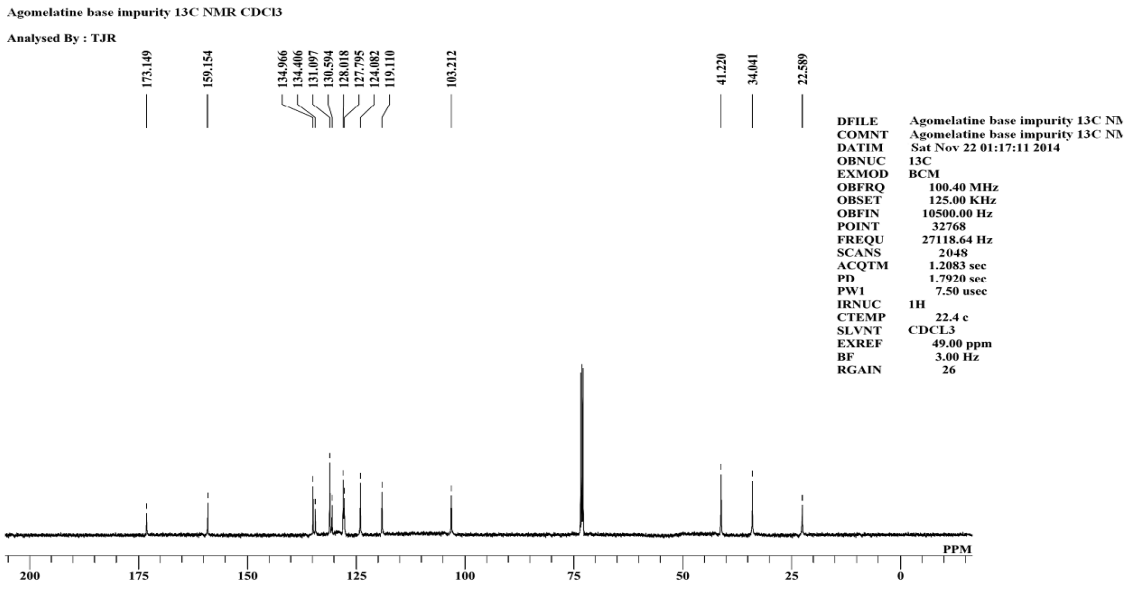
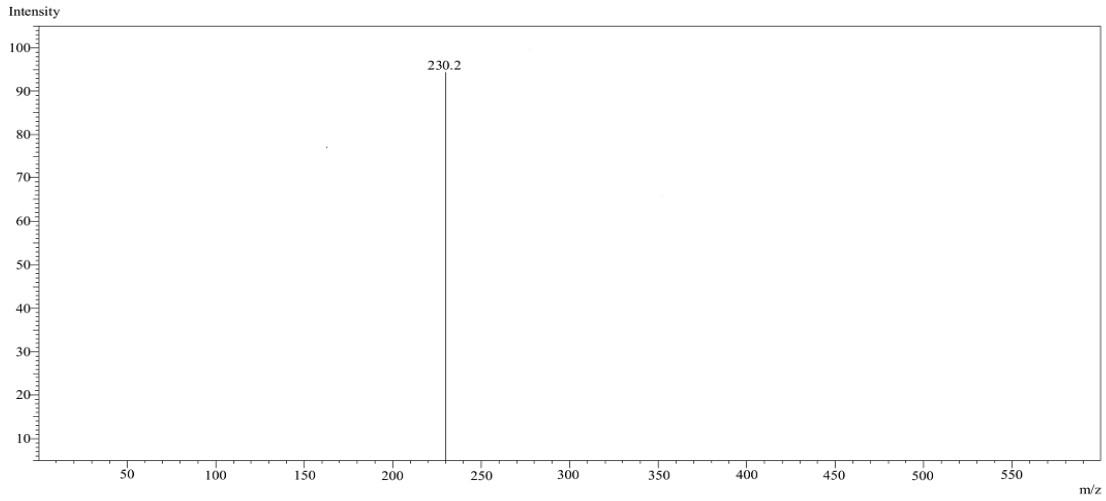
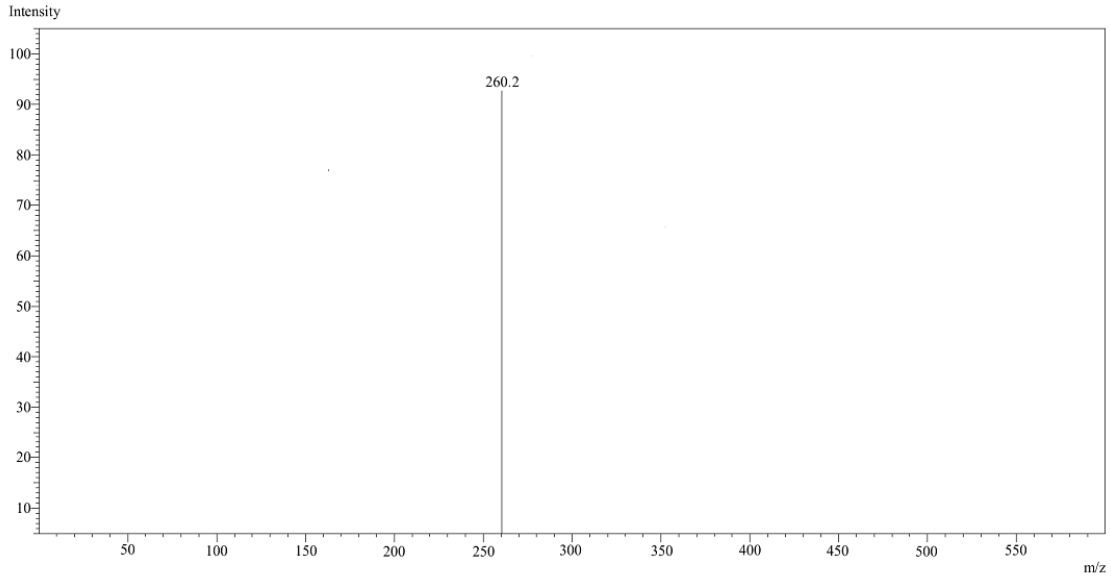




Impurity-3 (Oxidative degradation) (AGM-IV):



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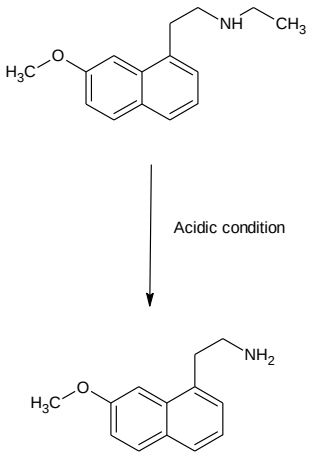
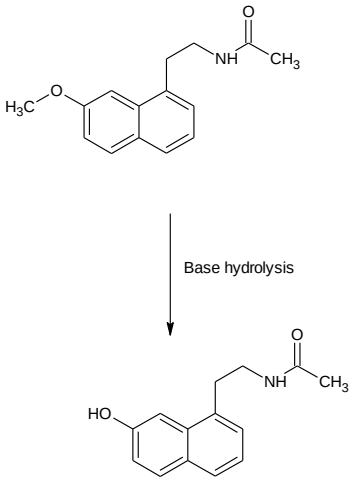
Probable Degradation pathway

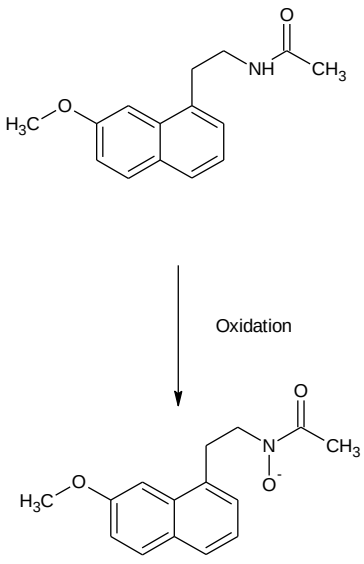
Acid degradation



Base degradation



Oxidative degradation



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CONCLUSION

In conclusion, the compound Agomelatine is stable under sunlight and thermal conditions. The compound degrades under Acidic, Basic and Oxidative conditions.

The degraded impurities were properly identified and characterized using chromatographic and spectroscopic techniques.

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