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Characterization of products formed by forced degradation of Amlodipine Besylate using LC/MS/MS

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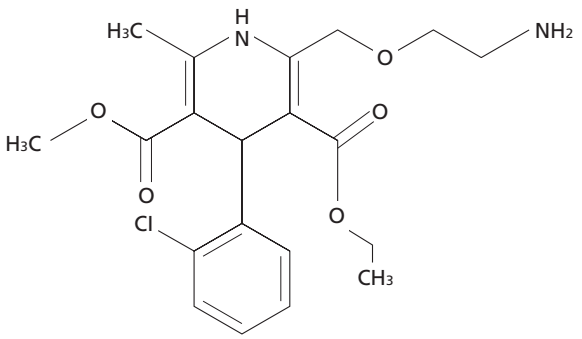
1. Introduction

Forced degradation is a process, whereby the natural degradation rate of a drug product or drug substance is accelerated by the application of an additional stress. Understanding of these products qualitatively and at times quantitatively, can assist in predicting toxicity of these degradation products and also in deciding shelf life of the drug[1].

The ICH guidelines indicate that stress testing is designed to determine the stability of the molecule by knowing degradation pathways in order to identify the likely degradation products. The degradation products are those formed under the different conditions like effect of temperature, humidity, oxidation, photolysis and susceptibility to hydrolysis across a wide range of pH value. Even though ICH and FDA ask to include this study at Phase III level, it is recommended to start this study as early as possible to be able to provide valuable information to assess inherent stability of a drug, and to improve formulation and the manufacturing process[2].

In this work, acid, base, peroxide and thermal degradation products of Amlodipine besylate were studied using a Triple Quadrupole Mass Spectrometer LCMS-8080. Ultra high sensitivity of LCMS-8080 system which gives clear background due to Hot Source Induced Desolvation (HSID) technology, this enables the fragmentation studies of degradation products present in low concentrations.

1-1. Amlodipine



3-ethyl 5-methyl 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate

Fig. 1 Structure of Amlodipine

Amlodipine (shown in Fig. 1) is a dihydropyridine calcium antagonist (calcium ion antagonist or slow-channel blocker) that inhibits the movement of calcium ions into vascular smooth muscle cells and cardiac muscle cells[3]. Experimental data suggest amlodipine binds to both dihydropyridine and nondihydropyridine binding sites. The contractile processes of cardiac muscle and vascular smooth muscle are dependent upon the movement of extracellular calcium ions into these cells through specific ion channels. Amlodipine inhibits calcium ion influx across cell membranes selectively, with a greater effect on vascular smooth muscle cells than on cardiac muscle cells. Amlodipine is a peripheral arterial vasodilator that acts directly on vascular smooth muscle to cause a reduction in peripheral vascular resistance and reduction in blood pressure.

2. Method of Analysis

2-1. Forced degradation

As per the ICH guidelines[4], the study of effect of temperature is suggested to be done in 10 °C increments above the accelerated temperature conditions and the humidity level of 75% or greater. No details are, however, provided for the study of oxidation, photolysis and hydrolysis at different pH. In absence of guidelines, it is difficult to decide on stress conditions to be employed for the study.

Amlodipine besylate was received from commercial sources in pure from and was used for the degradation study at different conditions.

Acid degradation	5 M HCl for 6 hrs at 80°C in water bath.
Base degradation	1 M NaOH for 2 hrs at 80°C in water bath.
Oxidative degradation	30% H_2O_2 for 80°C for 6 hrs in water bath.
Thermal degradation	100 mg of drug substance was placed in petridish a controlled temperature oven at 80°C for 48 hrs.

Amlodipine was subjected to degradation by above conditions and analyzed on LC/MS/MS with UV detector for determination of purity and mass of degradation samples.

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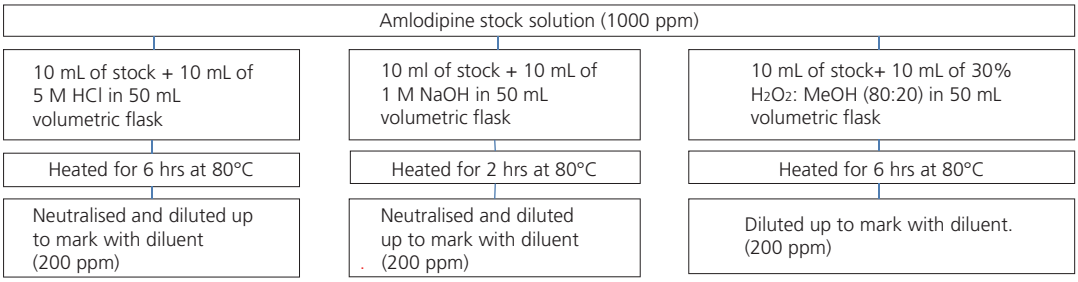
2-2. Sample Preparation

Standard Stock solution

Standard stock solutions were prepared by dissolving 50 mg of the drug in 50 mL volumetric flask and diluted up to mark with diluent.(Water : ACN 1:1)

Degradation samples



About 100 mg of Amlodipine besylate kept in petridish in a controlled temperature oven at 80 °C for 48 hrs and prepared 200 ppm solution from it.
All the degradation samples were injected on LCMS-8080 with UV detector to identify degradation products and extent of degradation.

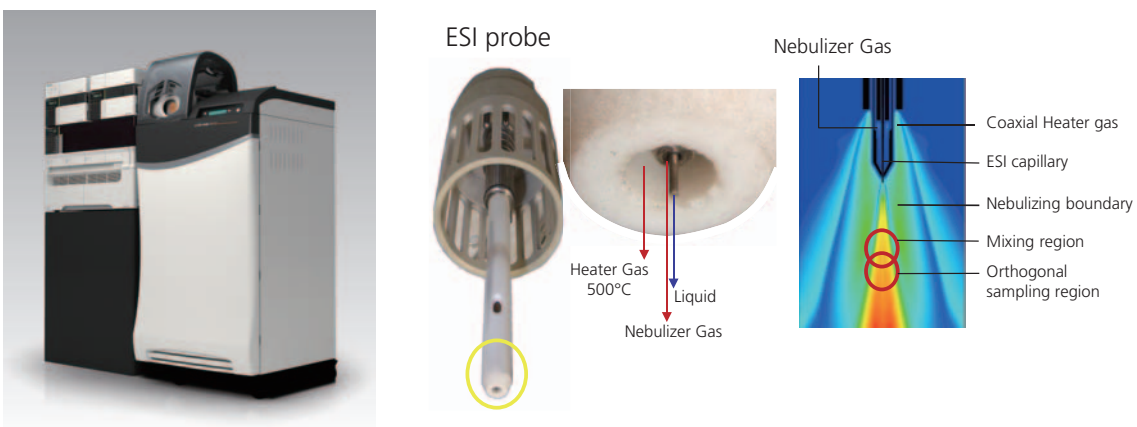


Fig. 2 LCMS-8080 triple quadrupole mass spectrometer by Shimadzu

2-3. LC/MS/MS analysis

Degradation samples as well as purified fractions were analyzed on LCMS-8080 (shown in Fig. 2) for identification and confirmation of degradation products. The analytical conditions are as follows:

Column	: Shim-pack XR ODS (75 mm L × 3 mm I.D. × 2.2 µm)
Flow rate	: 0.5 mL/min
Oven temperature	: 40°C
Detector	: UV detector at 238 nm
Mobile phase	: A:10 mM Ammonium Acetate with 0.4% Ammonia and pH adjusted to 7.0 with Glacial acetic acid B: Acetonitrile
Gradient program (%B)	: 0 – 1.0 min → 40 (%); 1.0 – 3.0 min → 40 -100 (%); 3.0 – 5.0 min → 100 (%); 5.0 – 5.5 min →100 - 40(%)
Injection volume	: 5 µL
MS interface	: Electro spray Ionization (ESI)
Nitrogen gas flow	: Nebulizing gas 2 L/min; Heating gas 15 L/min Curtain gas 2.4 L/min
MS temperature	: Probe temp.150°C HSID 150°C

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3. Results

3-1. LC/MS/MS Analysis

TIC of Q3 scan of Base degradation

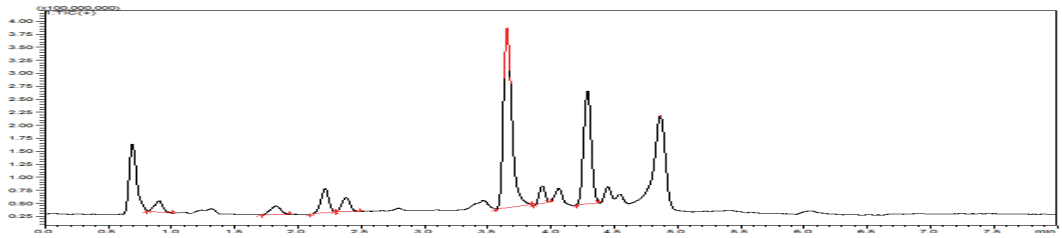

Fig. 3 TIC of Product ion scan of different precursors

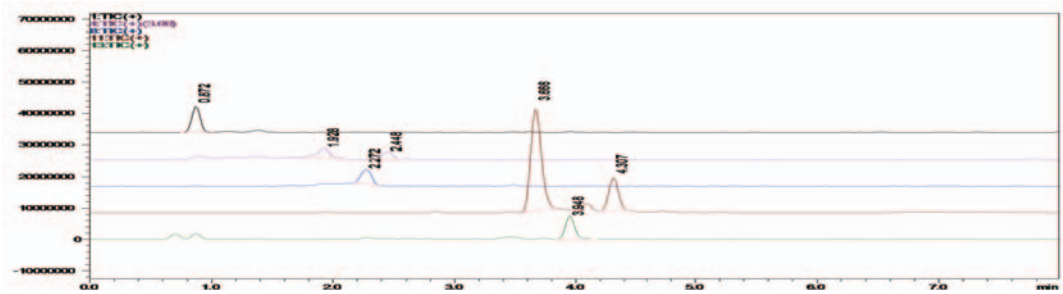

Fig. 4 TIC of Precursor ion scan

3-2. Forced degradation products

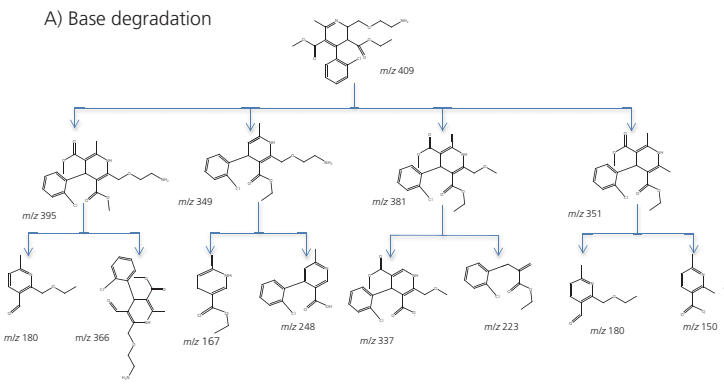

Fig. 5 Base degradation products

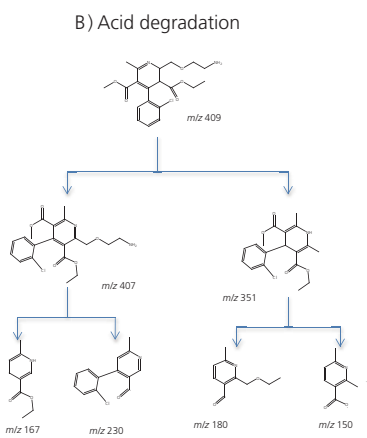

Fig. 6 Acid degradation products

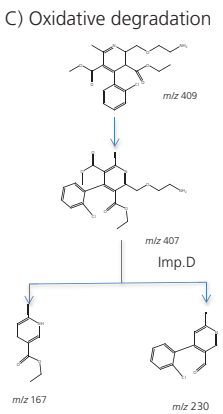

Fig. 7 Oxidative degradation products

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3-3. Precursor ion spectra

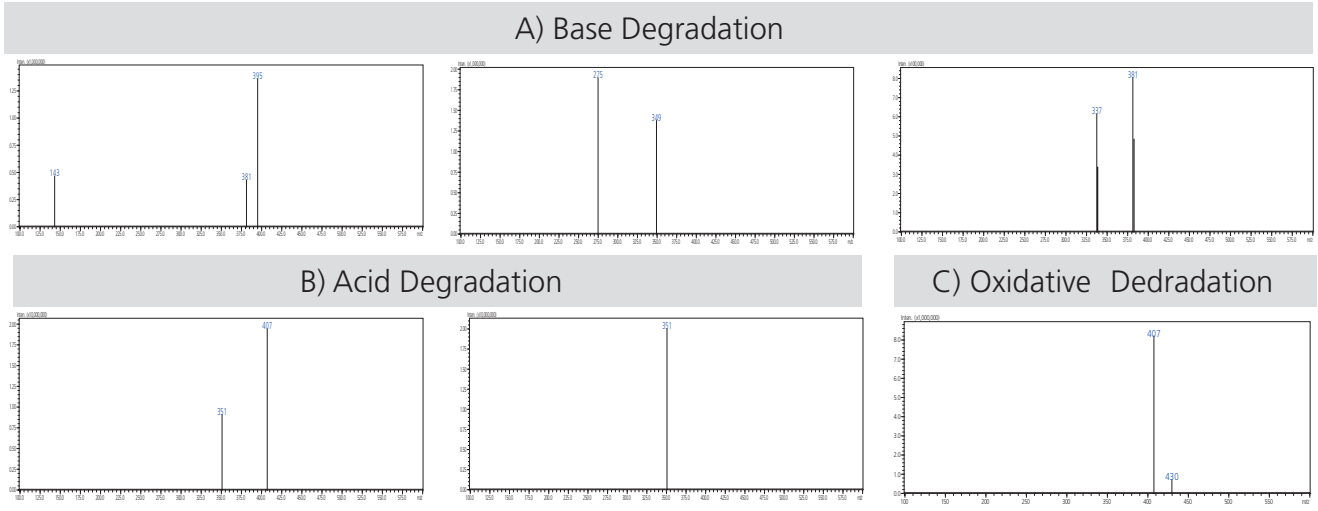

Fig. 8A,8B and 8C TIC of Precursor ions spectra of different degradents

Table 1 Degradation process and percentage of degradation observed

NO	Degradation Type	Experimental Conditions	Degradation observed
1	Acid Hydrolysis	5 MHCl, 80°C for 6 hrs	60%
2	Base Hydrolysis	1 MNaOH, 80°C for 2 hrs	25%
3	Oxidation	30% H_2O_2 80°C for 6 hrs	20%
4	Thermal	80°C for 48 hrs	0.25%

Amlodipine was subjected to degradation under different conditions to achieve maximum degradation. The main purpose of this study was to identify degradation products by LC/MS/MS.

Tough conditions used for forced degradation were attenuated to achieve degradation in the range of 10-80%, this could not be achieved in the case of thermal degradation even after exposure for prolonged duration. Oxidative degradation selectively gives an impurity which was identified as Imp D with *m/z* 407 (European Pharmacopeia), (shown in Fig. 7). The same impurity was found in acid degradation.

The identification of degradation products was also very effective for knowing the pathways of degradation of drug substances or drug product. (shown in Fig. 5,6 and 7). Use of LC/MS/MS technique made it possible to obtain detailed structural information rapidly on small quantities of substances without isolation of impurities (shown in Fig. 4). Degradation products with *m/z* 395,349,381 and 351 in base degradation (shown in Fig. 8) were selected and fragmented at different collision energies and product ions were scanned. For Acid degradation products with *m/z* 407 and 351 were selected and *m/z* 407 was selected for the oxidative degradation and product ions were monitored. Probable structures of product ions were drawn to confirm the degradation products.

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4. Conclusion

- A fast LC/MS/MS method was developed to identify degradation impurities in various stress conditions. Amlodipine was found to degrade 25% in basic and 60% in Acidic medium. Amlodipine showed 20% degradation in 30% H_2O_2 and no major impurities were found in thermal degradation.
- Identified the major degradation products formed under different stress conditions using LCMS-8080 and product ions were scanned after fragmentation to get structural information of degradation products.
- Product ions with *m/z* 180 and 167 were common in all degradation conditions while 230 was found in oxidation and acid hydrolysis. The method was fast and reliable to identify different products at the same time even at low concentration levels.

5. References

[1] Abela, D., Schembri O. and Farrugia, C.: A Chromatographic Determination of the Stability of Solutions of Amlodipine Benazepril and Amlodipine Besilate. (Presented at the Seventh World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology, Malta, 2010)

[2] Aneesh TP and Rajasekaran A: Forced degradation studies- A tool for determination of stability in pharmaceutical dosage form. Karpagam University, Coimbatore, Tamilnadu, India. International Journal of Biological & Pharmaceutical Research. 3(5): 699-702 ; 2012

[3] K. R Patil, V P Rane, J N Sangshetti, R D Yeole, D B Shinde:Stability indicating LC method for the simultaneous determination of Amlodipine and Olmesartan in dosage form. Department of Chemical Technology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad-431004 (MS), India Journal of chromatographic science 08/2010

[4] ICH guidelines (Q1A9R2)- Stability Testing of New Drug substances and Products (rev 2), Nov.2003

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