Journal of Pharmaceutical Analysis 2015;5(1):33–42

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Journal of Pharmaceutical Analysis

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ORIGINAL ARTICLE

LC, MS^n and LC–MS/MS studies for the characterization of degradation products of amlodipine

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Received 12 March 2014; revised 25 June 2014; accepted 7 July 2014
Available online 7 August 2014

KEYWORDS

Amlodipine;
LC–MS/MS;
Characterization;
Degradation pathway

Abstract In the present study, comprehensive stress testing of amlodipine (AM) was carried out according to International Conference on Harmonization (ICH) Q1A(R2) guideline. AM was subjected to acidic, neutral and alkaline hydrolysis, oxidation, photolysis and thermal stress conditions. The drug showed instability in acidic and alkaline conditions, while it remained stable to neutral, oxidative, light and thermal stress. A total of nine degradation products (DPs) were formed from AM, which could be separated by the developed gradient LC method on a C_{18} column. The products formed under various stress conditions were investigated by LC–MS/MS analysis. The previously developed LC method was suitably modified for LC–MS/MS studies by replacing phosphate buffer with ammonium acetate buffer of the same concentration (pH 5.0). A complete fragmentation pathway of the drug was first established to characterize all the degradation products using LC–MS/MS and multi-stage mass (MS^n) fragmentation studies. The obtained mass values were used to study elemental compositions, and the total information helped with the identification of DPs, along with its degradation pathway.

1. Introduction

The stability testing of drugs under different stress conditions is indispensable during the drug development process. In addition, stability testing guidelines stated by International Conference on Harmonization (ICH) and other international agencies [1,2] require the reporting, identification and characterization of degradation products (DPs). But DPs generated during storage may be in very low levels; therefore, stress studies are suggested to generate them in higher amounts [3]. Still sometimes it is very difficult to identify these DPs from the generated stressed mixture due to their lower amounts. As a result, hyphenated techniques like LC–MS are

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Peer review under responsibility of Xi'an Jiaotong University.

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http://dx.doi.org/10.1016/j.jpha.2014.07.005

currently extensively used for this purpose [4,5]. Amlodipine (AM) (Fig. 1) is chemically 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5 pyridinedicarboxylic acid 3-ethyl 5-methyl ester. It acts as a calcium channel blocker and inhibits the transmembrane influx of calcium ions into vascular smooth muscles and cardiac muscle; hence, it used as an antihypertensive for the treatment of angina.

A thorough literature review revealed that there exist several reports on bioanalytical method of development and pharmacokinetic studies of AM [6,7]. A wide array of articles are reported on stability-indicating HPLC method for the estimation of a single drug amlodipine in pure bulk samples, dosage forms as well as in combination with other drugs such as atorvastatin, benazepril, perindopril, olmesartan, valsartan, and hydrochlorthiazide [8–16]. A few articles are available on photostability studies of AM, where the thermal degradation kinetics are reported from 0 h to 108 h [17–20]. There exists report on isolation and characterization of three thermal degradation products of AM, which were formed due to intramolecular reactions and cyclization [21]. Moreover, there are few reports on isolation and characterization of process-related impurities of AM and accelerated stability studies. From the above literature, the reported masses and fragmentation pattern of all the degradation products/impurities of AM are quite different from those of our degradation products of AM formed under different stress conditions. In fact, the reported masses of impurities and their fragment ions were compared with the masses of DPs proposed in this article and they were all found non-resembling with each other. Moreover, as per previous reports [22–26] the masses of six different process-related impurities such as *m/z* 538, 569, 406, 394, 408 and 422 did not match with the masses of any of the DPs of AM. Hence, the endeavor of our present study was to: (i) carry out the stress studies on AM under the ICH-defined conditions; (ii) separate the degradation products by HPLC; and (iii) characterize and establish the degradation pathway of all the degradation products with the help of LC–MS/MS.

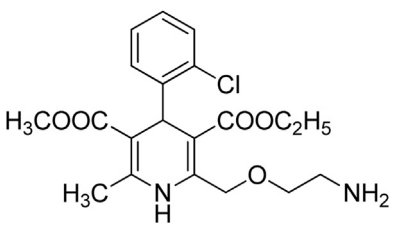

Fig. 1 Structure of amlodipine.

2. Experimental

2.1. Drug and reagents

Pure AM was obtained as gratis sample from Osaka Pharmaceuticals Pvt. Ltd. (Vadodara, India). Analytical reagent (AR) grade formic acid and sodium hydroxide (NaOH) were purchased from S.D. Fine-Chem Ltd. (Mumbai, India), hydrochloric acid (HCl) and HPLC grade methanol (MeOH) from Merck Specialities Pvt. Ltd. (Mumbai, India), and hydrogen peroxide (H_2O_2) from Qualigens Fine Chemicals Pvt. Ltd. (Mumbai, India). Ultra-pure water obtained from Millipore water purification system (Molsheim, France) was used throughout the studies.

2.2. Equipment

A high-performance liquid chromatography (HPLC) system from PerkinElmer (Shelton, CT, USA) used for the LC studies consisted of an on-line degasser, a sample injector (Rheodyne sample loop 20 mL), a UV–visible detector (Series 200), a pump (Reciprocating, series 200), and a computer system loaded with Total Chrome Navigator (version 6.3.1) software. The LC–MS system was controlled by Xcalibur software (version 2.0) consisting of LCQ Fleet and TSQ Quantum Access with Surveyor Plus HPLC System (Thermo, San Jose, USA). Precision water baths equipped with MV controller (Thermostatic Classic Scientific India Ltd. Mumbai, India) 90 were used for stress studies. Degradation experiments in acid, base, and neutral conditions were performed using a dry-bath (Labline Sun Scientifics Ltd. New Delhi, India). The solid state thermal stress studies were carried out in a dry-air oven (NSW Limited, New Delhi, India). Other equipment used was a pH meter (Labindia, Mumbai, India), a weighing balance (Shimadzu, AUX220, Kyoto, Japan), and a micro-pipette (Erba Biohit, Mannheim, Germany). In all studies, separations were achieved on a C_{18} column (250 mm × 4.6 mm i.d., particle size 5 µm; Kromasil (Eka Chemicals AB, Bohus, Sweden)). Photolytic studies were carried out in a photostability chamber (Thermolab, 95 Th-400G Mumbai, India), set at 40 ± 1 °C/75 ± 3% RH in accordance with option two of the ICH guideline Q1B [27].

2.3. Stress degradation studies

Stress degradation studies were carried out on AM as per ICH Q1A(R2)-prescribed stress conditions. As per ICH the stress degradation studies are conducted to determine the stability of drug substances or drug products by knowing the degradation

Table 1 Interpretation of MS data of fragments of amlodipine.

Peak No.	Experimental mass	Best possible molecular formulae	RDB	Possible parent fragment	Difference from parent ion	Possible losses corresponding to difference
1	408.90	$C_{20}H_{26}N_2O_5Cl^+$	8.5			
2	392.23	$C_{20}H_{23}NO_5Cl^+$	9.5	1	16.67	NH_3
3	294.13	$C_{15}H_{17}NO_3Cl^+$	7.5	2	98.10	$C_5H_6O_2$
4	238.10	$C_{12}H_{13}NO_2Cl^+$	6.5	3	56.03	C_3H_4O
5	208.14	$C_{12}H_{18}NO_2^+$	4.5	4	29.96	HCl
6	102.13	$C_5H_{12}NO^+$	0.5	2	290.10	$C_{15}H_{11}O_4Cl$

RDB: ring plus double bonds.

Table 2 LC–MS/MS data of DPs of amlodipine (AM) along with their possible molecular formulae and major fragments.

DPs	Experimental mass	Best possible molecular formula	RDB	Major fragments (chemical formula)
AM1	363.08	$C_{19}H_{27}N_2O_5^+$	8.5	292.92 ($C_{16}H_{22}NO_4^+$), 226.83 ($C_{14}H_{12}NO_2^+$), 157.00 ($C_8H_{15}NO_2^+$), 112.92 ($C_6H_8O_2^+$)
AM2	392.67	$C_{20}H_{23}NO_5Cl^+$	9.5	347.08 ($C_{18}H_{18}NO_4Cl^+$), 305.92 ($C_{16}H_{16}NO_3Cl^+$), 158.92 ($C_8H_{16}NO_2^+$), 113.08 ($C_6H_{11}NO^+$)
AM4	399.92	$C_{19}H_{28}N_2O_5Cl^+$	7.5	363.83 ($C_{19}H_{27}N_2O_5^+$), 331.92 ($C_{17}H_{16}N_2O_3Cl^+$), 263.08 ($C_{14}H_{16}N_2OCl^+$), 158.58 ($C_8H_{16}NO_2^+$), 106.92 ($C_7H_6O^+$), 90.75 ($C_4H_{12}NO^+$)
AM5	380.83	$C_{19}H_{23}NO_5Cl^+$	9.5	302.25 ($C_{17}H_{20}NO_4^+$), 284.83 ($C_{16}H_{14}NO_4^+$), 222.00 ($C_{13}H_{20}NO_2^+$), 192.00 ($C_{11}H_{14}NO_2^+$), 175.08 ($C_{11}H_{11}O_2^+$), 142.92 ($C_7H_{12}NO_2^+$)
AM6	393.00	$C_{19}H_{22}N_2O_5Cl^+$	9.5	306.42 ($C_{16}H_{17}NO_3Cl^+$), 218.17 ($C_{13}H_{16}NO_2^+$), 158.75 ($C_{11}H_{12}N^+$), 112.75 ($C_6H_{10}NO^+$), 90.92 ($C_4H_{12}NO^+$)
AM7	380.75	$C_{18}H_{21}N_2O_5Cl^+$	9.5	352.83 ($C_{18}H_{13}NO_4Cl^+$), 335.92 ($C_{17}H_{18}NO_4Cl^+$), 230.83 ($C_{14}H_{16}NO_2^+$), 163.17 ($C_9H_9NO_2^+$)
AM8	407.17	$C_{20}H_{24}N_2O_5Cl^+$	10.5	286.08 ($C_{17}H_{20}NO_3^+$), 231.25 ($C_{15}H_{21}NO^+$), 123.92 ($C_8H_{11}O^+$)
AM9	395.00	$C_{19}H_{24}N_2O_5Cl^+$	9.5	366.75 ($C_{18}H_{21}NO_5Cl^+$), 350.08 ($C_{17}H_{17}NO_5Cl^+$), 302.17 ($C_{16}H_{13}NO_3Cl^+$), 244.17 ($C_{14}H_{14}NO_3^+$), 226.25 ($C_{14}H_{12}NO_2^+$), 102.25 ($C_4H_8NO_2^+$)

RDB: ring plus double bonds.

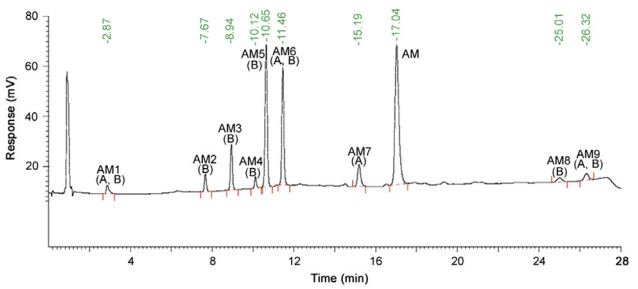

Fig. 2 Chromatogram showing separation of degradation products of amlodipine (AM) in the mixture of stress sample. A: acid; B: base; N: neutral; RT: retention time.

pathways to identify the likely degradation products. Moreover, the guideline explicitly requires the conduct of forced degradation studies under a variety of conditions like a wide range of pH, light, oxidation and dry heat. AM along with various stressors was exposed to different temperatures with the objective to achieve 15–20% degradation and for this the stress conditions were systematically optimized in the initial stages. AM was subjected to acidic (1 M HCl, 30 min, 80 °C), basic (1 M NaOH, 1 h, 80 °C), neutral (H_2O, 2 h, 80 °C), oxidative (H_2O_2, 15%, 48 h) at room temperature, thermal (50 °C, 48 h) and photolytic (1.2×10^6 lx h of fluorescent light and 200 Wh/m^2 UV-A light, 14 days) stress conditions.

2.4. *Sample preparation for HPLC and LC–MS analysis*

The stressed samples of AM collected during the desired time intervals were adequately diluted 10 times with water before injection into the HPLC. Moreover, the samples were filtered through a 0.22 µm membrane filter before making injections. A mixture containing all the degradation products was also prepared for final HPLC separation and LC–MS analysis. In total 100 µg/mL samples were prepared and injected so as to adequately compare the percentage degradation with the standard unstressed drug sample of the same concentration.

2.5. *Separation studies*

A concentration of 10 µg/mL of AM was scanned from 200 to 400 nm in the ultraviolet spectrophotometer to select UV wavelength suitable for HPLC analysis. From the spectra, 240 nm wavelength was found to show maximum absorbance. First separation of AM was attempted by varying the relative ratio of methanol to phosphate buffer, along with the variation in buffer pH. All the separations were carried out using a C_{18} column. Separation studies were first carried out on all reaction solutions individually, and then on a mixture of degraded drug solutions.

2.6. *MS, MS^n and LC–MS studies*

The fragmentation profile of the drug was established by carrying out mass spectral studies on AM, while multi-stage (MS^n) mass studies were carried out up to MS^6 to determine the origin of each fragment. AM was subjected to MS system at a concentration of 10 µg/mL prepared in methanol in positive electrospray ionization (ESI) mode in the mass range of 50–800 Da. High-purity nitrogen was used as the nebulizer and auxiliary gas. The interpretation data of fragments obtained in MS studies for AM are listed in Table 1. A previously developed LC method was used to analyze degraded drug samples on the LC–MS system, and provided phosphate buffer was replaced by 10 mM ammonium acetate buffer of pH 5.0. The mass parameters were properly tuned to obtain high-intensity peaks of molecular ions and daughter ions of degradation products. The fragments of all DPs obtained in LC–MS studies along with the best possible molecular formula, ring plus double bonds (RDB) and chemical formula of major fragments are shown in Table 2.

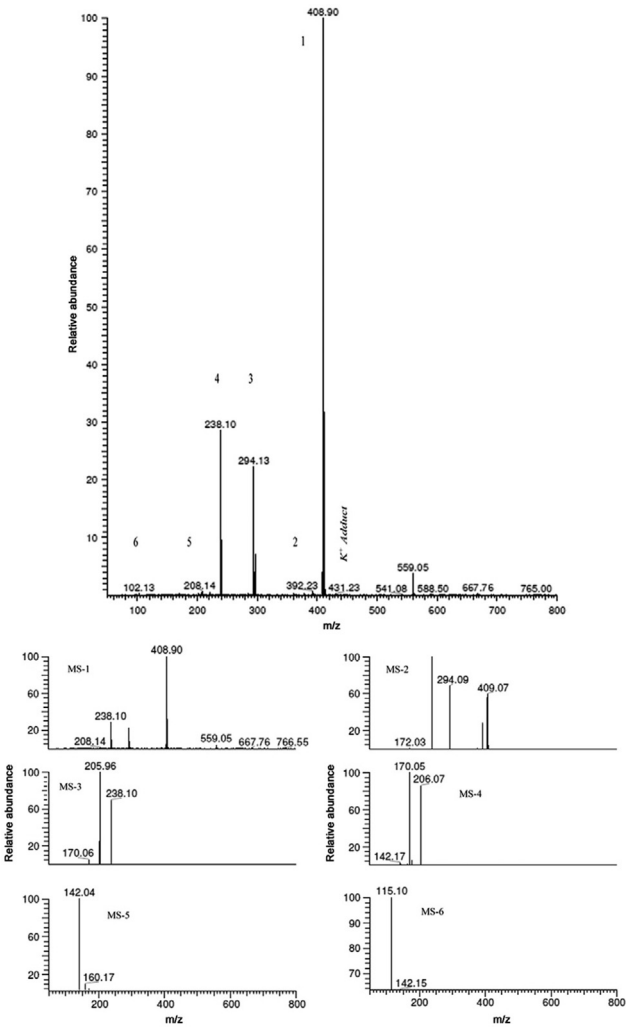

Fig. 3 Line spectra of amlodipine obtained in MS and MS^n studies.

3. Results and discussion

3.1. HPLC analysis

During the initial separation trials, methanol and water were adopted as a mobile phase on different gradient LC modes, but the separation of drugs and degraded products was not optimum; this might be due to the poor buffering capacity of water. Then in the later trials water was replaced with phosphate buffer. Stronger organic modifier viz., acetonitrile, was not utilized during the HPLC analysis, since methanol showed satisfactory resolution between drugs and degraded products, and hence it was kept unchanged. Logical modifications like change in pH and gradient program were made to improve the resolution between drug and degradation products and also between the degradation products. Finally an acceptable separation was achieved using methanol and phosphate buffer (10 mM, pH 5.0) in a gradient mode (T_{min}/A:B; T_0/10:90; T_8/50:50; T_{13}/60:40; T_{25}/75:25; T_{27}/10:90;T_{30}/10:90) on C_{18} column at room temperature. The detection wavelength was 240 nm, flow rate was 1 mL/min, and injection volume was 20 μL.

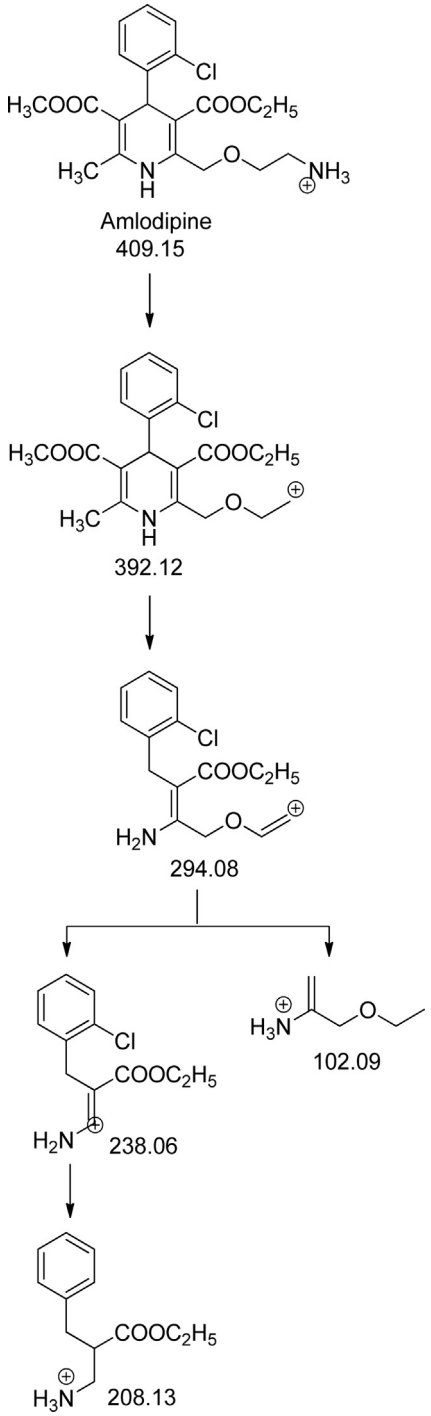

Fig. 4 Mass fragmentation pattern of amlodipine.

3.2. Stress decomposition behavior

A total of nine DPs AM1–AM9 were formed from AM during the stress degradation study. Out of these AM2, AM3, AM5, AM6 and AM7 were formed as major DPs, while AM1, AM4, AM8 and AM9 were minor DPs. Among all the DPs AM1, AM6 and AM9 were formed in both acidic and alkaline conditions, while the products AM2, AM3, AM4 and AM5 were formed only under alkaline stress condition, and AM7 was the product of acidic stress. A total of 16.41% degradation was observed in acid stress, while in

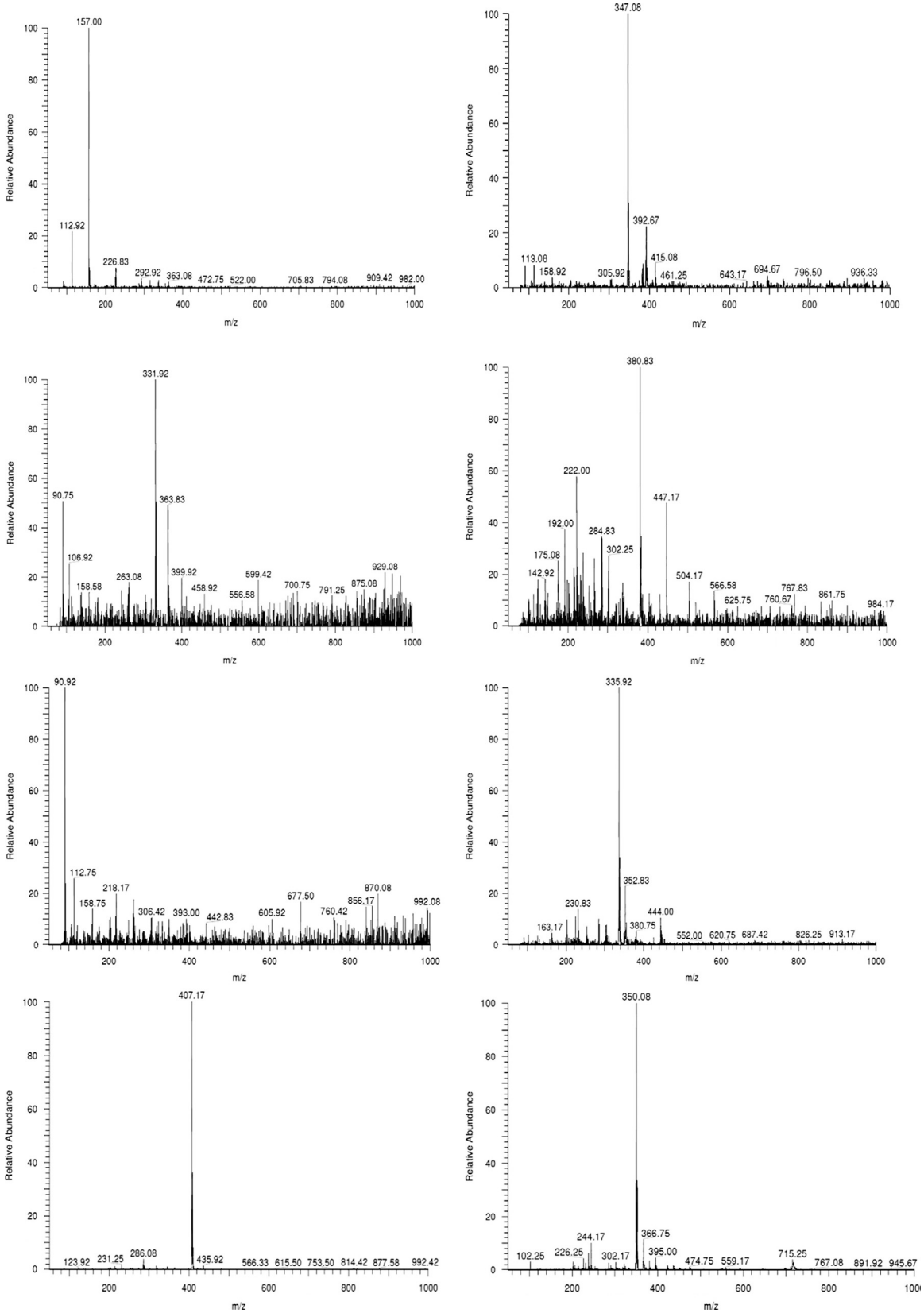

Fig. 5 Line spectra of degradation products of amlodipine obtained in LC–MS/MS studies.

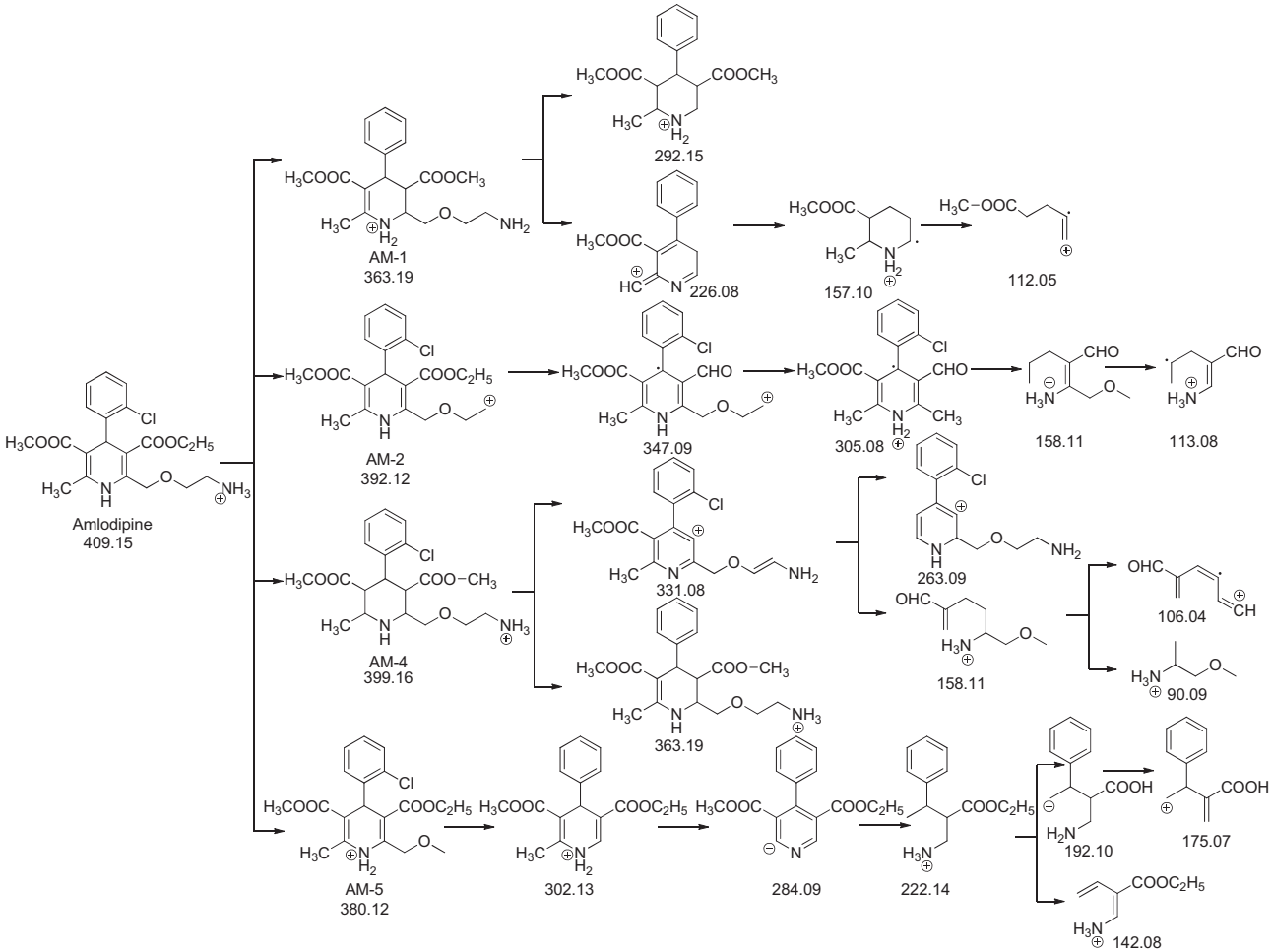

Fig. 6 Degradation products of amlodipine formed under acidic and alkaline stress conditions (AM1, AM2, AM4 and AM5).

alkaline stress 27.53% degradation was recorded. The chromatogram showing separation of AM and all the DPs is shown in Fig. 2. The degradation products of AM are denoted as AM1 to AM9 in accordance with the sequence in which the peak appeared from left to right in the chromatogram. From the chromatogram AM was found to be more susceptible to alkaline stress.

3.3. *Mass fragmentation pattern of AM*

A total of six fragments were formed from AM during its MS studies. A multi-stage (MS^n) mass fragmentation study was also carried out up to MS^6, to determine the origin of each fragment, which could help propose the fragmentation pathway of AM. Line spectra of AM, obtained in MS and MS^n studies, are shown in Fig. 3. The fragment with *m/z* 431.23 was formed as a potassium adduct, since its mass was ~28 Da higher than the molecular ion peak of AM (*m/z* 408.90). The fragments with *m/z* 392.23 were formed on loss of ammonia (NH_3) from AM, while in the subsequent step the fragment with *m/z* 392.23 on cleavage of methyl but-2-enoate moiety resulted in the formation of *m/z* 294.13. There onwards a parallel pathway was initiated from the fragment with *m/z* 294.13; here a direct loss of methoxy ethane entity led to the formation of a daughter ion with *m/z* 238.10, while loss of 1-chloro-2-methyl benzene and propionic acid moieties resulted in the formation of the last daughter ion with *m/z* 102.13. Finally the fragment with *m/z* 238.10 underwent cleavage of hydrogen chloride entity and resulted in the formation of ion with *m/z* 208.14. The mass fragmentation pattern of AM is shown in Fig. 4.

3.4. *Characterization of degradation products*

The data obtained in MS, MS^n and LC–MS/MS studies were systematically utilized for the structure elucidation of degradation products of AM.

3.4.1. *AM1 (m/z 363.08)*

As shown in Fig. 2, AM1 was formed as the first degradation product and the most polar DP among all because of its immediate elution after the solvent front at 2.87 min of retention time. LC–MS/MS line spectra of AM1 in Fig. 5 show the formation of fragments with *m/z* 363.08, *m/z* 292.92, *m/z* 226.83, *m/z* 157.00 and *m/z* 112.92, and among these the *m/z* 363.08 and *m/z* 157.00 were formed as molecular ion and base ion peaks, respectively, since 472.75 cannot be considered as the molecular ion peak of AM1 because of its huge difference in the molecular mass compared to the molecular mass of AM (*m/z* 409). Hence, AM1 was obtained from the drug by the loss of hydrogen chloride entity of benzene ring and a methyl group from

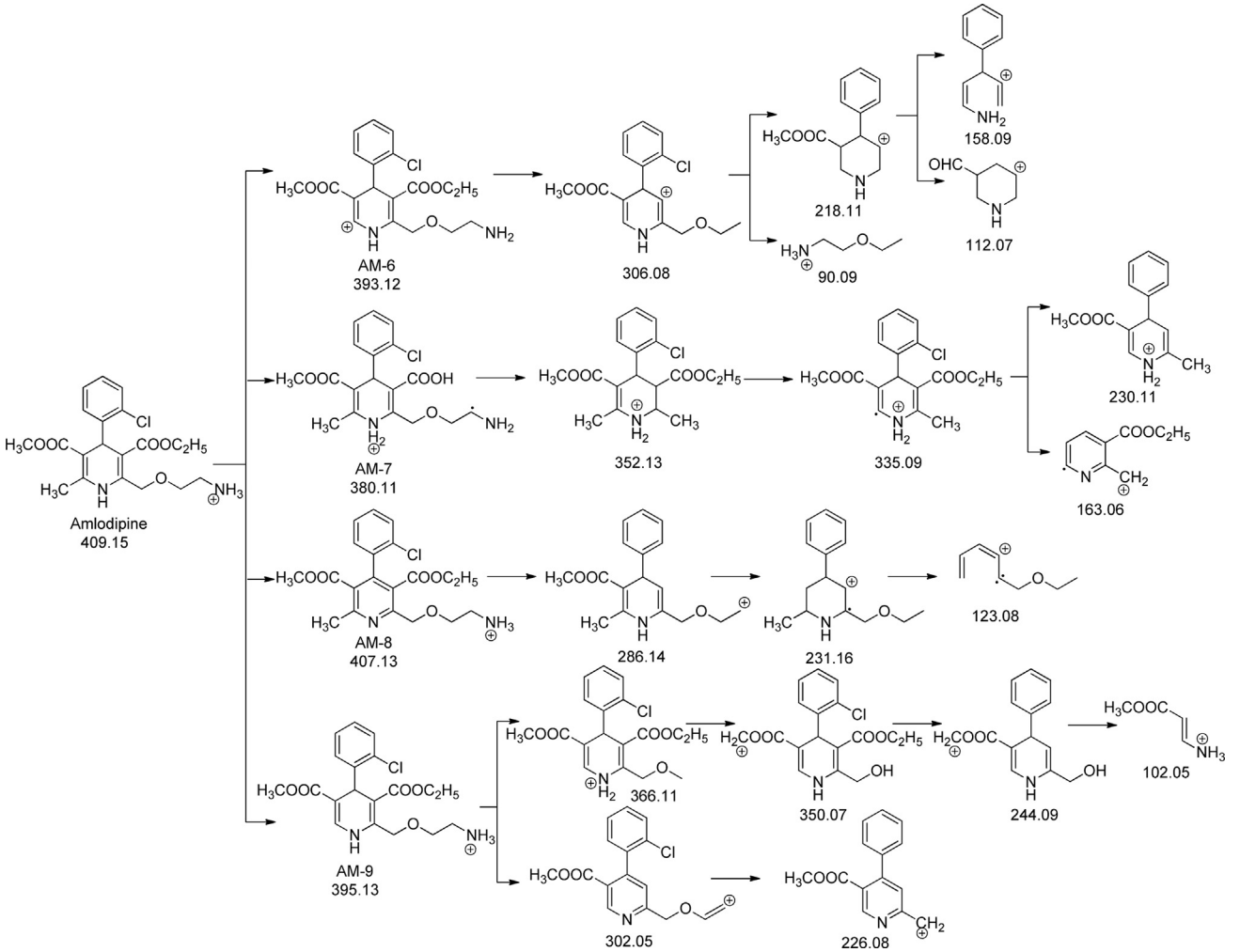

Fig. 7 Degradation products of amlodipine formed under acidic and alkaline stress conditions (AM6, AM7 AM8 and AM9).

the ethyl ester moiety at the third position of 1,4-dihydropyridine ring. The fragmentation pattern of AM1 is shown in Fig. 6.

3.4.2. *AM2 (m/z 392.67)*

A total of five line fragments were formed from AM2, viz., *m/z* 392.67, *m/z* 347.08, *m/z* 305.92, *m/z* 158.92 and *m/z* 113.08. Among these the fragment with *m/z* 392.67 was the molecular ion peak, while the one with *m/z* 347.08 was the base peak. AM2 was formed on loss of ammonia (NH_3) from terminal position of the side chain attached at the second position of 1,4-dihydropyridine ring. The subsequent steps in the fragmentation pathway of AM2 are shown in Fig. 6, which involved conversion of ester to aldehyde (*m/z* 347.08) intermediate, followed by the formation of fragments with *m/z* 305.08, *m/z* 158.11 and *m/z* 113.08, due to losses such as ethanol, methyl formate, chlorobenzene and methoxymethane entities. The impurity with *m/z* 392.2 reported by Reddy et al. [22] was formed during accelerated stability studies. Although this impurity had the same mass as that of AM2 but showed different MS/MS fragmentation pattern, the proposed structure of AM2 was different. The major ions formed during MS/MS of this impurity (*m/z* 392.2) were *m/z* 360.2, 346.5, 318.1 and 286.2, while the fragment ions formed from AM2 were *m/z* 347.08, 305.92, 158.92 and 113.08. The fragmentation pattern of AM2 is shown in Fig. 6.

3.4.3. *AM3*

The structure of AM3 degradation product was not elucidated, probably due to its poor ionization behavior during LC–MS/MS analysis in both ESI positive and ESI negative ionization modes.

3.4.4. *AM4 (m/z 399.92)*

As shown in Fig. 5, AM4 had the molecular ion peak of 399.92, with the base peak of 331.92. The drug (AM) underwent loss of a methyl group from ethyl 1,4-dihydropyridine-3-carboxylate and resulted in the formation of AM4. There onwards AM4 followed a parallel fragmentation pathway involving loss of hydrogen chloride and acetate moiety to form ions with *m/z* 363.83 and *m/z* 331.92, respectively. A loss of another acetate moiety and a methyl group from *m/z* 331.92 resulted in the formation of fragment with *m/z* 263.08. The formation of other minor fragments along with their structures and masses is depicted in Fig. 6.

3.4.5. *AM5 (m/z 380.83)*

A total of seven fragments were formed from AM5, where a fragment with *m/z* 380.83 was formed as the base peak as well as molecular ion peak. A direct cleavage of terminal methamine entity of the side chain attached to the second position of 1,4-dihydropyridine ring resulted in the formation of AM5. Ion with

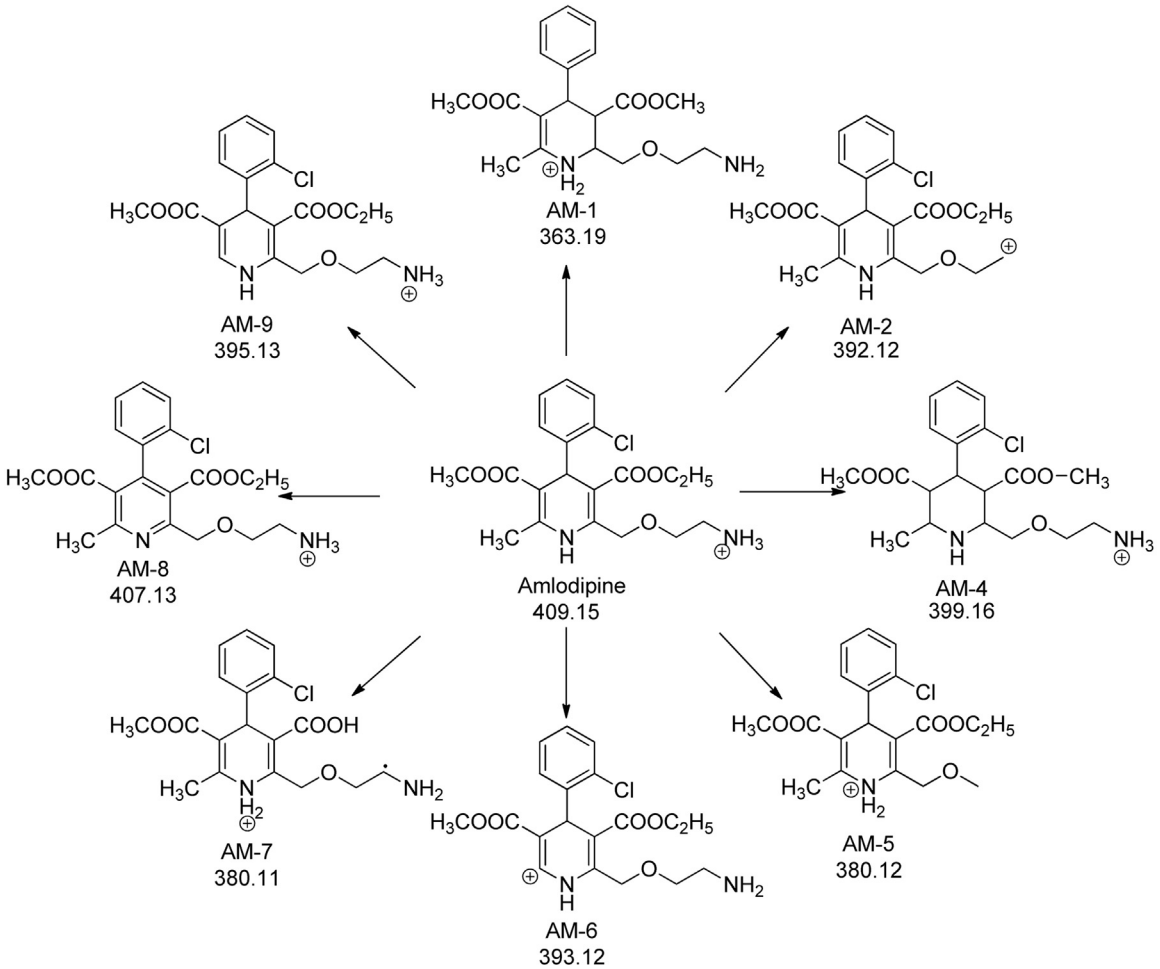

Fig. 8 Degradation pathway of amlodipine.

m/z 302.25 was formed from AM5 on loss of hydrogen chloride and dimethyl ether entities. Ion with *m/z* 302.25 underwent loss of two ring protons and a methyl group attached to the sixth position of 1,4-dihydropyridine ring and led to the formation of fragment with *m/z* 284.09. Subsequent losses such as acetate and methylene entity resulted in dihydropyridine ring opening to form an ion with *m/z* 222.00. The structures and masses of daughter ions with *m/z* 192.00, *m/z* 175.08 and *m/z* 142.92 formed in the same pathway are shown in Fig. 6.

3.4.6. AM6 (*m/z* 393.00)

The mass of AM6 was found to be *m/z* 393.00, which suggests loss of a methyl group from AM along with the charge migration from terminal amine to the sixth carbon of 1,4-dihydropyridine ring. AM6 in its subsequent steps lost the ethyl carboxylate entity to form a daughter ion with *m/z* 306.42, which on ring opening and cleavage of a larger fragment of around 216 Da resulted in the formation of ion with *m/z* 90.92. On the other hand, fragment with *m/z* 306.42 on loss of methoxyethane and hydrogen chloride led to the formation of fragment ion with *m/z* 218.17, which followed a minor parallel pathway with the formation of daughter ions with *m/z* 158.75 and *m/z* 112.75 on loss of methylcarboxylate and benzene moieties, respectively. The proposed structures of all the fragments of AM6 are shown in Fig. 7.

3.4.7. AM7 (*m/z* 380.75)

The ester functional group at the third position of 1,4-dihydropyridine underwent hydrolysis with the loss of ethyl group and resulted in the formation of AM7 with *m/z* 380.75 The loss of 2-aminoethanol from the second position of 1,4-dihydropyridine along with two ring protons resulted in the formation of fragment with *m/z* 352.83, which on loss of a methyl group led to the formation of a fragment with *m/z* 335.92. Then *m/z* 335.92 followed a parallel fragmentation to form an ion with *m/z* 230.83 on loss of hydrogen chloride and ethylcarboxylate entities, while ion of *m/z* 163.17 was formed on loss of chlorobenzene, methylcarboxylate and two ring protons. The proposed structures of all the fragments of AM7 are shown in Fig. 7.

3.4.8. AM8 (*m/z* 407.17)

As shown in Fig. 5, AM8 had the mass of *m/z* 407.17, which indicates around mass of 2 Da lesser (around two protons) than the mass of drug (AM). AM8 would be the result of one of the main degradation pathways of AM such as oxidative aromatization of dihydropyridine fragment to the pyridine moiety, which takes place in solution and solid states as well as under photolytic conditions [28]. One of the degradation products reported by Damale et al. [29] had the same mass of *m/z* 407 formed under oxidative and acidic stress conditions. The first fragment of AM8 had the mass of *m/z* 286.08 formed on losses of ammonia,

ethylcarboxylate and hydrogen chloride. Then the fragment with *m/z* 286.08 on loss of methylcarboxylate resulted in the formation of ion with *m/z* 231.25, which in its subsequent step lost ring nitrogen, methyl group and a benzene entity to form daughter ion with *m/z* 123.92. The proposed structures of all the fragments of AM8 are shown in Fig. 7.

3.4.9. AM9 (m/z 395.00)

A direct cleavage of methyl group from the sixth position of 1,4-dihydropyridine ring of the drug (AM) resulted in the formation of AM9 with *m/z* 395.00. There onwards AM9 followed a parallel fragmentation pathway. In its subsequent steps loss of methanamine and o-demethylation of the side chain resulted in the formation of fragments with *m/z* 366.75 and *m/z* 350.08, respectively. On the other hand, AM9 on loss of ethylcarboxylate and ammonia from the side chain resulted in the formation of ion with *m/z* 302.17. The same degradation product with *m/z* 395 reported by Damale et al. [29] was formed under alkaline stress, while in our case this DP was formed in acidic and alkaline conditions. The proposed structures of all the fragments of AM9 are shown in Fig. 7.

3.5. Degradation pathway of AM

Based on the data obtained from the line spectra of LC–MS/MS studies of each degradation product, a final fragmentation pathway of AM was established. The degradation pathway of AM is shown in Fig. 8 along with the proposed structures and masses of all the DPs.

4. Conclusion

Stress degradation studies were carried out on AM as per mentioned ICH guidelines, which provided information on the degradation behavior of AM under the acidic, basic, neutral, oxidative, photolytic and thermal stress conditions. HPLC analysis revealed that a total of nine degradation products were formed from AM, among these AM3 remained unidentified probably due to its poor ionization behavior, while all the remaining DPs were successfully characterized with the help of LC–MS/MS analysis. The above-stated study was able to explore various useful information which is not yet reported in the literature on AM such as (a) sensitivity of AM to different stress conditions, (b) the total number of degradation products of AM along with the nature of DPs, (c) mass fragmentation pathway of AM as well as its DPs, (d) MS^n study of the drug to determine the origin of each mass fragment, and (e) degradation pathway of the drug.

References

[1] ICH, Stability testing of new drug substances and products Q1A(R2), in: Proceedings of the International Conference on Harmonisation, IFPMA, Geneva, 2003.

[2] WHO, Stability Testing of Active Pharmaceutical Ingredients and Pharmaceutical Products, World Health Organization, Geneva, 2007.

[3] M. Bakshi, S. Singh, Development of validated stability-indicating assay methods critical review, J. Pharm. Biomed. Anal. 28 (2002) 1011–1040.

[4] S. Görög, The importance and the challenges of impurity profiling in modern pharmaceutical analysis, TrAC 25 (2006) 755–757.

[5] T. Murakami, N. Fukutsu, J. Kondo, et al., Application of liquid chromatography-two-dimensional nuclear magnetic resonance spectroscopy using pre-concentration column trapping and liquid chromatography–mass spectrometry for the identification of degradation products in stressed commercial amlodipine maleate tablets, J. Chromatogr. A 1181 (2008) 67–76.

[6] A. Zarghi, S.M. Foroutan, A. Shafaati, et al., Validated HPLC method for determination of Amlodipine in human plasma and its application to pharmacokinetic studies, Farmaco 60 (2005) 789–792.

[7] Y. Ma, F. Qin, X. Sun, et al., Determination and pharmacokinetic study of amlodipine in human plasma by ultra performance liquid chromatography–electrospray ionization mass spectrometry, J. Pharm. Biomed. Anal. 43 (2007) 1540–1545.

[8] B.G. Chaudhari, N.M. Patel, P.B. Sham, Stability indicating RP-HPLC for simultaneous determination of Atorvastatin Calcium and Amlodipine Besylate from their combination drug products, Chem. Pharm. Bull. 55 (2007) 241–246.

[9] K.R. Naidu, U.N. Kale, M.S. Shingare, Stability indicating RP-HPLC method for simultaneous determination of amlodipine and benzapril hydrochloride from their combination drug product, J. Pharm. Biomed. Anal. 39 (2005) 147–155.

[10] M.C. Nahata, R.S. Morosco, T.F. Hipple, Stability of amlodipine besylate in two liquid dosage forms, J. Am. Pharm. Assoc. 39 (1999) 375–377.

[11] M. Gumustas, S.A. Ozkan, A validated stability-indicating RP-LC method for the simultaneous determination of amlodipine and perindopril in tablet dosage form and their stress degradation behavior under ICH-recommended stress conditions, J. AOAC Int. 96 (2013) 751–757.

[12] R.A. Shaalan, T.S. Belal, F.A. El Yazbi, et al., Validated stability-indicating HPLC-DAD method of analysis for the antihypertensive triple mixture of amlodipine besylate, valsartan and hydrochlorothiazide in their tablets, Arab. J. Chem. (2013), http://dx.doi.org/10.1016/j.arabjc.2013.04.012.

[13] K.R. Patil, V.P. Rane, J.N. Sangshetti, et al., Stability indicating LC method for the simultaneous determination of amlodipine and olmesartan in dosage form, J. Chromatogr. Sci. 48 (2010) 601–606.

[14] A.R. Fakhari, S. Nojavan, S. Haghgoo, et al., Development of a stability-indicating CE assay for the determination of amlodipine enantiomers in commerical tablets, Electrophoresis 29 (2008) 4583–4592.

[15] A. Mohammadi, N. Rezanour, M. Ansari Dogaheh, et al., A stability-indicating high performance liquid chromatographic (HPLC) assay for the simultaneous determination of atorvastatin and amlodipine in commercial tablets, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 846 (2007) 215–221.

[16] B. Marciniec, M. Ogrodowczyk, Thermal stability of 1,4-dihydropyridine derivatives in solid state, Acta Pol. Pharm. 63 (2006) 477–484.

[17] G. Ragno, E. Cione, A. Garofalo, et al., Design and monitoring of photostability systems for amlodipine dosage forms, Int. J. Pharm. 265 (2003) 125–132.

[18] Y. Kawabe, H. Nakamura, S. Suzuki, et al., Photochemical stabilities of some dihydropyridine calcium-channel blockers in powdered pharmaceutical tablets, J. Pharm. Biomed. Anal. 47 (2008) 618–624.

[19] G. Ragno, A. Garofalo, C. Vetuschi, Photodegradation monitoring of amlodipine by derivative spectrophotometry, J. Pharm. Biomed. Anal. 27 (2002) 19–24.

[20] R.J.V. Eranki, G. Inti, V. Jayaraman, et al., New stability indicating method for quantification of impurities in amlodipine and benazepril capsules by validated HPLC, Am. J. Anal. Chem. 4 (2013) 715–724.

[21] A.S.L. Devi, Y.S. Rao, M. Satish, et al., Structure elucidation of thermal degradation products of amlodipine, Magn. Reson. Chem. 45 (2007) 688–691.

[22] G.V.R. Reddy, A.P. Kumar, B.V. Reddy, et al., Separation, identification and structural elucidation of a new impurity in the drug substance of amlodipine maleate using LC–MS/MS, NMR and IR, Croat. Chem. Acta. 83 (2010) 443–449.

[23] British Pharmacopoeia, British Pharmacopoeial Commission, London, 2010, pp. 138–140.

[24] P. Sudhakar, M. Nirmala, J. Moses Babu, et al., Identification and characterization of potential impurities of amlodipine maleate, J. Pharm. Biomed. Anal. 40 (2006) 605–613.

[25] G. Ananchenko, J. Novakovic, J. Lewis, Amlodipine Besylate, Profiles Drug Subst. Excip. Relat. Methodol. 37 (2012) 31–77.
[26] B. Suchanova, L. Sispera, V. Wsol, Liquid chromatography-tandem mass spectrometry in chiral study of amlodipine biotransformation in rat hepatocytes, Anal. Chem. Acta 573–574 (2006) 273–283.
[27] ICH, Stability testing: photostability testing of new drug substances and productsQ1B, in: Proceedings of International Conference on Harmonisation, IFPMA, Geneva, 1996.
[28] M.M.M. Sadeghi, H.R. Memarian, A.R. Momeni, Aromatization of 1,4-dihydropyridines with free radical reagents, J. Sci. Islam. Repub. Iran 12 (2001) 141–143.
[29] S. Damale, D. Bhandarkar, S. Raju, et al., Characterization of products formed by forced degradation of Amlodipine Besylate using LC/MS/MS. Shimadzu Excellence in Science, ASMS 2013-MP06-112.