

Identification and Characterization of Forced Degradation Products of Moexipril Using LC-ESI-MS/Q-TOF

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Abstract

A forced degradation study on drug moexipril has been conducted to provide an insight into degradation products and degradation pathways as per International Conference on harmonization (ICH) Q1A (R2) guideline. Moexipril was subjected to stress conditions by hydrolysis (acidic, alkaline and neutral), oxidation, photolysis and thermal. The drug degraded in the stress conditions of hydrolysis, oxidation and photolysis but remained stable in conditions of thermal stress. A total of 5 degradation products were formed from the drug, which were separated by developed gradient liquid chromatographic method on a C-18 column (4.6 × 150 mm, 5 µm) by gradient elution technique using ammonium acetate buffer and methanol at mobile phase flow rate of 1.0 mL/min at 25°C. The degradation products were identified and structurally elucidated by using liquid chromatography with quadrupole time of flight electrospray ionization tandem mass spectrometer combined accurate mass measurements. The most probable structures for the observed degradation products were assigned by comparing the product ions of all the protonated degradation products with the product ions of protonated moexipril.

Keywords: Moexipril; LC-ESI-MS; Degradation products; Characterization

Abbreviations

MOX: Moexipril; DP: Degradation Product

Introduction

Forced degradation studies on drug substances and drug products helps to provide insight into the degradation products (DPs) and degradation pathways. Structural elucidation of degradation products is essential during the stage of pharmaceutical drug discovery and development. Therefore it is indispensable to carry out stability testing of drugs under different stress conditions as per guidelines of International Conference on harmonization (ICH) and other International Agencies [1-3].

Moexipril (MOX) is an Antihypertensive drug belongs to a category of Angiotensin converting enzyme (ACE) inhibitor. MOX is a prodrug of Moexiprilat, which acts by inhibiting Angiotensin converting enzyme in humans and animals. MOX is metabolized in the liver to form pharmacologically active compound Moexiprilat, which blocks the conversion of Angiotensin I to Angiotensin II. Blockage of Angiotensin II limits hypertension within the vasculature. Additionally, MOX has been found to possess cardioprotective properties [4,5]. According to IUPAC, nomenclature of MOX is (3S)-2-[(2S)-2-[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino]propanoyl]-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (Figure 1).

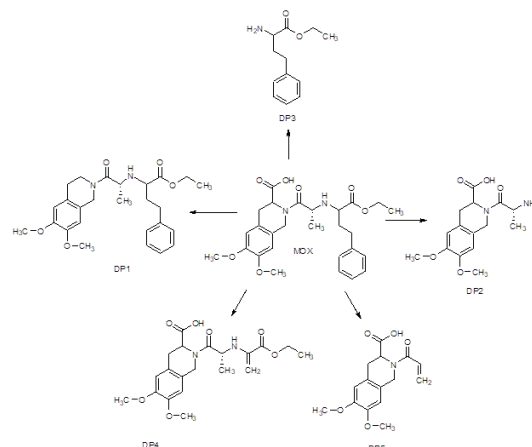


Figure 1: Proposed structures of DPs formed under various stress conditions.

A.A. Elshanawane et al. have reported a stability indicating reversed-phase liquid chromatographic method for determination of ramipril and MOX in the presence of their degradation products [6]. Koti et al. reported a liquid chromatography-electrospray ionization mass spectrometry (LC-ESI-MS) method for simultaneous analysis of MOX and Moexiprilat in biological samples [7]. Hammes et al. also reported a gas chromatography-negative Ion chemical ionization mass spectrometry (GC-NICI-MS) for simultaneous analysis of MOX and

Moexiprilat, in human plasma [8]. Karraa et al. developed a rapid and sensitive liquid chromatography-tandem mass spectrometric assay for MOX in human plasma [9]. As there are no reports available on the degradation behavior, identification and characterization of Degradation Products (DPs) of MOX formed under various stress conditions, we have carried out a detailed study on stability indicating LC-MS/MS method for MOX.

Materials and Methods

Chemicals and reagents

MOX reference standard was obtained from pharmaceutical manufacturing unit, Baroda, India. HPLC grade methanol was purchased from Merck Specialities (Mumbai, India). Ultra-pure water was obtained by Millipore Milli-Q plus purification system. Analytical reagent grade ammonium acetate, formic acid, sodium hydroxide, hydrochloric acid and hydrogen peroxide were purchased from S.D Fine chemicals (Mumbai, India).

Equipments and apparatus

LC studies were performed on a high performance liquid chromatography from Agilent 1200 series (Agilent Technologies, USA) equipped with a Quaternary pump, an online degasser, a diode-array detector, an auto sampler, a column oven and a computer system under control of Chemstation software. The samples were separated on a Agilent XBD, C-18 column (150 mm × 4.6 mm × 5 µm particle size).

For LC-MS studies, an Agilent 1200 series HPLC instrument (Agilent Technologies, USA) coupled to a Quadrupole time-of-flight (Q-TOF) mass spectrometer (Q-TOF LC/MS 6510 series classic, Agilent Technologies, USA) equipped with ESI source was used. The software used was Mass Hunter workstation.

Hydrolytic degradation studies were performed in a oil bath equipped with temperature controller (Heidolph, Germany). Photolytic degradation studies were carried out in a photostability chamber (Mack equipment, MK-10-PH, 230 V Phase). Other equipments used were a pH-meter (Elico LI-120, Schwerzenbach, Switzerland), Analytical balance (Schimadzu, Kyoto, Japan) and micropipettes (Eppendorf, Hamburg, Germany).

Forced degradation studies

Forced degradation studies of MOX were carried out to determine stability by knowing degradation pathways for identification of likely degradation products as per ICH guidelines Q1A (R2).

Amount of about 10.0 mg of MOX was subjected to stress hydrolytic degradation under acidic, basic and neutral conditions by refluxing with 10.0 mL of 0.1 N HCl, 0.1 N NaOH, and H₂O at 80°C for 48 hours, 48 hours, and 72 hours, respectively. The oxidative degradation of the drug was carried out using 3%, 6%, 30% H₂O₂ for about 7 days. The thermal stress was carried out by spreading the drug to about 1.0 mm thickness over the petridish and kept for 3 days at 100°C. The photolytic stress studies were performed by exposing the solid and liquid forms of MOX to UV light at 320 nm for 10 days. All the obtained forced degraded samples are preserved in refrigerator at 5°C.

Preparation of sample for HPLC and LC-MS analysis

The stressed samples of MOX were diluted 10 times with mobile phase before injection into the HPLC. All the samples were purified by filtration through 0.22 µm membrane filter before HPLC and LC-MS analysis.

Results and Discussion

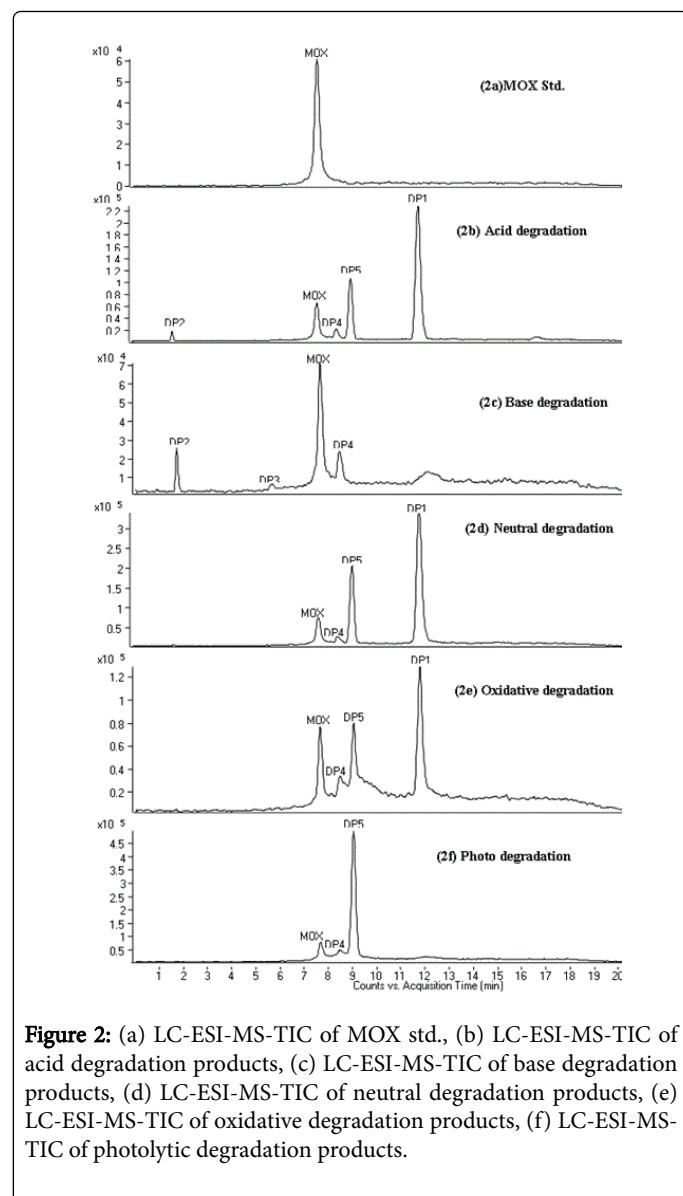
Optimization of LC-MS conditions

The main objective of this work was to develop liquid chromatography method to separate all degradation products from moexipril and degradation products from each other under various stress conditions. Agilent XBD, C-18 column (150 mm × 4.6 mm × 5 µm particle sizes) was found suitable for this chromatographic separation after tried with different columns. During initial separation trials methanol/water, methanol/ammonium acetate buffer solution (10 mM, pH adjusted to 6.0 ± 0.5) in different proportions were adopted as a mobile phase using isocratic modes, but the separation of MOX and its DPs were not optimum. The resolution of MOX and its DPs was influenced by the concentration of ammonium acetate buffer in the mobile phase. The peaks were merged at 10 mM ammonium acetate (pH 6.0). While there was improvement in the resolution at 20 mM ammonium acetate (pH 6.0). Finally we achieved a good resolution of peaks with acceptable shape with mobile phase consisting of 20 mM ammonium acetate buffer (A), pH adjusted to 6.0 using dilute formic acid and methanol (B) in a gradient elution program. The gradient program for mobile phase solvents was set as follows, (T_{min}/ %solution of B): 0-0/30, 0-5/65, 5-15/65, 15-20/30. The mobile phase flow rate of 1.0 ml/min, column temperature of 25°C and wavelength of 273 nm were found to be suitable for the chromatographic separation of MOX and its DPs.

The ESI source conditions were optimized for good signal and high sensitivity. The operating source conditions for MS scan of MOX in positive ESI mode were optimized as follows; the fragmentor voltage at 80 V; the capillary at 3000-3500 V; the skimmer at 60 V; high purity nitrogen was used as nebulizing (45 psi) and auxiliary gas; In experiments of collision induced dissociation (CID), MS1 was kept static, quadrupole analyzer was used selecting the precursor ion of interest and time-of-flight (TOF) analyzer was used for analyzing the product ions. Spectra were recorded with an average of 20-25 scans under identical experimental conditions.

Degradation behavior

The optimized LC-MS method is applicable to identify DPs. The LC-ESI-MS total ion chromatograms (TIC) of unreacted MOX are shown in (Figure 2a). The drug formed 4 DPs, DP1, DP2, DP4 and DP5 under acidic conditions (Figure 2b). Three DPs, DP2, DP3 and DP4 were formed under basic conditions (Figure 2c). The drug showed DP1, DP4 and DP5 under neutral and oxidative stress conditions (Figure 2d and 2e). Two DPs, DP4 and DP5 were formed under photolytic stress conditions (Figure 2f). No DPs were formed under thermal stress conditions. All these degradation products have been structurally elucidated by using LC-MS/MS combined with accurate mass measurements. The proposed structures of DPs and their elemental compositions are given in Figure 1 and Table 1, respectively.



MOX and DPs	Proposed formula	Observed mass (Da)	Calculated mass (Da)	Error (Da)
MOX	C ₂₇ H ₃₅ N ₂ O ₇	499.2434	499.2444	-0.0010
	C ₂₆ H ₃₅ N ₂ O ₅	455.2543	455.2546	-0.0003
	C ₁₅ H ₂₁ N ₂ O ₅	309.1441	309.1450	-0.0009
	C ₁₅ H ₁₈ NO ₅	292.1172	292.1185	-0.0013
	C ₁₄ H ₂₁ N ₂ O ₃	265.1542	265.1552	-0.0010
	C ₁₄ H ₁₈ NO ₃	248.1285	248.1287	-0.0002
	C ₁₂ H ₁₈ NO ₂	208.1338	208.1338	0.0000
	C ₁₁ H ₁₄ NO ₂	192.1014	192.1025	-0.0011
	C ₁₂ H ₁₅ O ₂	191.1063	191.1065	-0.0002
	C ₉ H ₁₂ N	134.0968	134.0970	-0.0002

DP1	C ₉ H ₉	117.0692	117.0695	-0.0003
	C ₂₆ H ₃₅ N ₂ O ₅	455.2543	455.2546	-0.0003
	C ₁₄ H ₂₁ N ₂ O ₃	265.1542	265.1552	-0.0010
	C ₁₄ H ₁₈ NO ₃	248.1285	248.1287	-0.0002
	C ₁₂ H ₁₈ NO ₂	208.1338	208.1338	0.0000
	C ₁₁ H ₁₄ NO ₂	192.1014	192.1025	-0.0011
	C ₁₂ H ₁₅ O ₂	191.1063	191.1065	-0.0002
	C ₉ H ₁₂ N	134.0968	134.0970	-0.0002
DP2	C ₉ H ₉	117.0692	117.0695	-0.0003
	C ₁₅ H ₂₁ N ₂ O ₅	309.1441	309.1450	-0.0009
	C ₁₅ H ₁₈ NO ₅	292.1172	292.1185	-0.0013
	C ₁₄ H ₂₁ N ₂ O ₃	265.1542	265.1552	-0.0010
	C ₁₄ H ₁₈ NO ₃	248.1285	248.1287	-0.0002
DP3	C ₁₁ H ₁₄ NO ₂	192.1014	192.1025	-0.0011
	C ₁₂ H ₁₈ NO ₂	208.1338	208.1338	0.0000
	C ₁₂ H ₁₅ O ₂	191.1063	191.1065	-0.0002
	C ₉ H ₁₂ N	134.0968	134.0970	-0.0002
DP4	C ₉ H ₉	117.0692	117.0695	-0.0003
	C ₂₀ H ₂₇ N ₂ O ₇	407.1819	407.1818	+0.0001
	C ₁₉ H ₂₇ N ₂ O ₅	363.1918	363.1920	-0.0002
	C ₁₄ H ₂₁ N ₂ O ₃	265.1542	265.1552	-0.0010
	C ₁₄ H ₁₈ NO ₃	248.1285	248.1287	-0.0002
DP5	C ₅ H ₁₀ NO ₂	116.0711	116.0712	-0.0001
	C ₁₅ H ₁₈ NO ₅	292.1172	292.1185	-0.0013
	C ₁₄ H ₁₈ NO ₃	248.1285	248.1287	-0.0002
	C ₁₁ H ₁₄ NO ₂	192.1014	192.1025	-0.0014

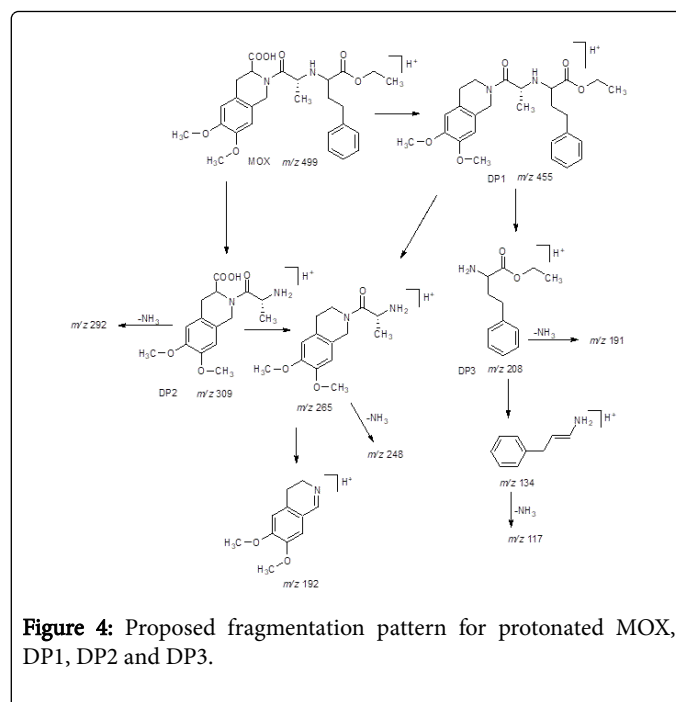
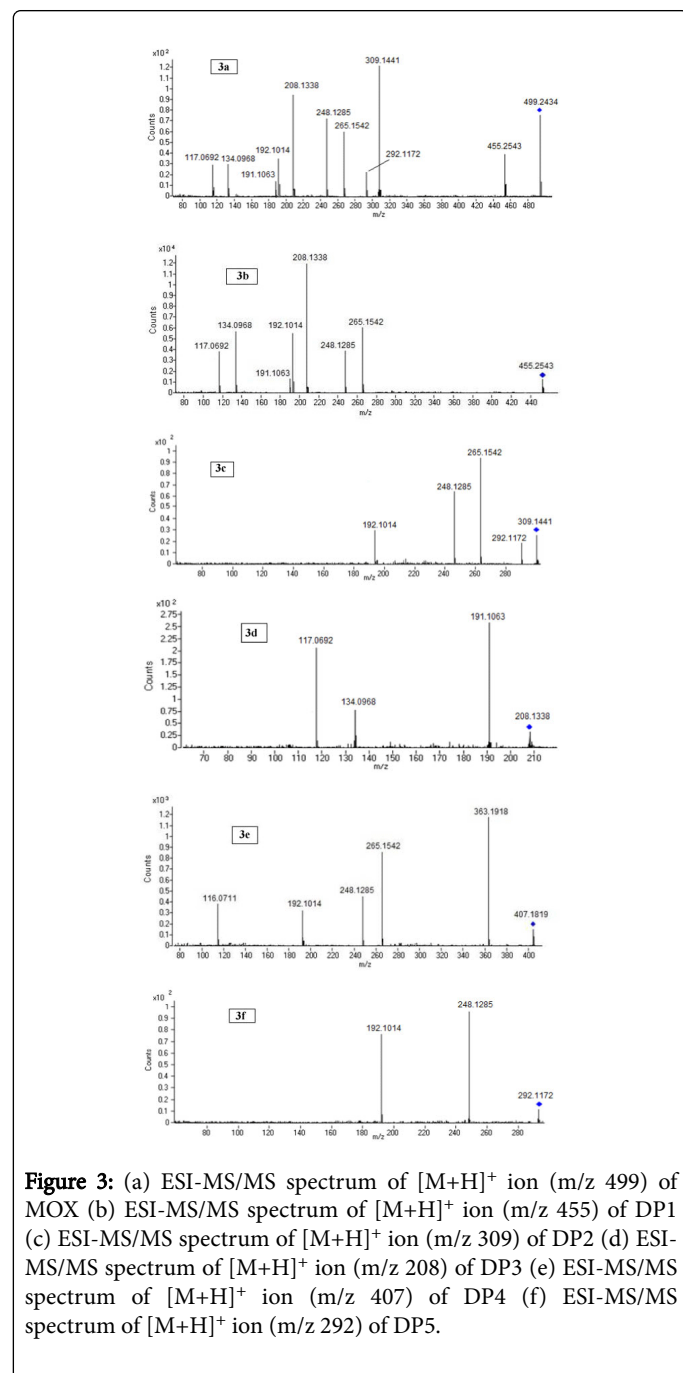
Table 1: Elemental compositions of daughter ions of MOX (m/z 499), DP1 (m/z 455), DP2 (m/z 309), DP3 (m/z 407), DP4 (m/z 407), DP5 (m/z 292).

Characterization of MOX and its DPs using LC-MS/MS

MS/MS of MOX

The positive ion ESI-MS of MOX shows an abundant [M+H]⁺ ion at m/z 499. Its MS/MS spectrum (Figure 3a) shows product ions at m/z 455 (loss of CO₂), m/z 309 (protonated 2-(2-aminopropanoyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid), m/z 292 (loss of NH₃ from m/z 309), m/z 265 (protonated 2-amino-1-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)propan-1-one), m/z 248 (loss of NH₃ from m/z 265), m/z 208 (protonated ethyl 2-amino-4-phenylbutanoate), m/z 192 (protonated 6,7-dimethoxy-1,4-dihydroisoquinoline), m/z 191 (loss of NH₃ from m/z 208), m/z 134 (protonated 3-phenylprop-1-en-1-amine) and m/z 117 (loss of NH₃).

from m/z 134). The proposed fragmentation mechanism for protonated MOX is shown in Figure 4. The most probable structures are proposed for the fragment ions based on accurate mass measurements (Table 1).



MS/MS of degradation products

All the DPs DP1-DP5 were eluted within 15 minutes on C-18 column. Table 2 provides information on the retention times (R_t) and elemental composition of these Dps. Figure 1 shows the proposed structures of all the Dps. The DPs were characterized by performing LC-ESI-MS/MS experiments. The elemental composition of all the DPs and their fragment ions were confirmed by accurate mass measurements.

DP1 (m/z 455): The degradation product DP1 at m/z 455.2543 ($[M+H]^+$; $C_{26}H_{35}N_2O_5$) was eluted at 11.9 min under acidic, neutral and oxidative stress conditions. The LC-ESI-MS/MS spectrum (Figure 3b) of protonated DP1 shows product ions at m/z 248 (protonated 1-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)prop-2-en-1-one), m/z 208 (protonated ethyl 2-amino-4-phenylbutanoate), m/z 192 (protonated 6,7-dimethoxy-3,4-dihydroisoquinoline), and m/z 191 (protonated ethyl 4-phenylbut-2-enoate). The proposed fragmentation mechanism for protonated DP1 is shown in Figure 4. From the structure of MOX, DP1 can be assigned the structure, ethyl 2-(1-(6,7-dimethoxy-3,4-dihydro iso-quinolin-2(1H)-yl)-1-oxopropan-2-ylamino)-4-phenyl butanoate. Table 1 gives the accurate mass measurements data which supports the fragmentation of protonated DP1.

MOX and DPs	R_t (min)	Proposed formula	Observed (Da)	mass	Calculated (Da)	mass	Error (Da)	Proposed elemental loss	MS/MS fragment ions
MOX	7.6	$C_{27}H_{35}N_2O_7$	499.2434		499.2444		-0.0010	---	455, 309, 292, 265, 248, 208, 192, 191, 134, 117
DP1	11.9	$C_{26}H_{35}N_2O_5$	455.2543		455.2546		-0.0003	CO_2	265, 248, 208, 192, 191, 134, 117

DP2	1.7	C ₁₅ H ₂₁ N ₂ O ₅	309.1441	309.1450	-0.0009	C ₁₂ H ₁₄ O ₂	292, 265, 248, 192
DP3	5.7	C ₁₂ H ₁₈ NO ₂	208.1338	208.1338	0.0000	C ₁₅ H ₁₇ NO ₅	191, 134, 117
DP4	8.6	C ₂₀ H ₂₇ N ₂ O ₇	407.1819	407.1818	0.0001	C ₇ H ₈	363, 265, 248, 192, 116
DP5	9.1	C ₁₅ H ₁₈ NO ₅	292.1171	292.1185	-0.0014	C ₁₂ H ₁₇ NO ₂	248, 192

Table 2: Elemental compositions of MOX and its DPs (DP1-DP5).

DP2 (m/z 309): The degradation product DP2 formed under acidic and basic conditions was eluted at 1.7 min. The LC-ESI-HRMS spectrum showed its [M+H]⁺ at m/z 309.1441 with an elemental composition of C₁₅H₂₁N₂O₅. Its MS/MS spectrum (Figure 3c) shows product ions at m/z 292 (loss of NH₃), m/z 265 (loss of CO₂), m/z 248 (loss of NH₃ from m/z 265), m/z 192 (protonated 6,7-dimethoxy-1,4-dihydroisoquinoline). The proposed fragmentation mechanism for protonated DP2 is shown in Figure 4. The fragmentation of DP2 giving rise to all the structure indicative ions, is highly compatible with the proposed structure, 2-(2-aminopropanoyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid.

DP3 (m/z 208): The degradation product DP3 at m/z 208.1338 ([M+H]⁺) formed under basic stress conditions, with an elemental composition of C₁₂H₁₈NO₂ was eluted at 5.7 min. The LC-ESI-MS/MS spectrum (Figure 3d) of protonated DP3 shows product ions at m/z 191 (loss of NH₃), m/z 134 (protonated 3-phenylprop-1-en-1-amine) and m/z 117 (loss of NH₃ from m/z 134). The proposed fragmentation mechanism for protonated DP3 is shown in Figure 4. The observed characteristic fragmentation is found to be consistent with the structure, ethyl 2-amino-4-phenylbutanoate.

DP4 (m/z 407): The degradation product DP4 at m/z 407.1819 ([M+H]⁺) formed under hydrolytic, oxidative and photolytic stress with an elemental composition of C₂₀H₂₇N₂O₇ was eluted at 8.6 min. The LC-ESI-MS/MS spectrum (Figure 3e) shows product ions at m/z 363 (loss of CO₂), m/z 265 (protonated 2-amino-1-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)propan-1-one), m/z 248 (loss of NH₃ from m/z 265), m/z 192 (protonated 6,7-dimethoxy-1,4-dihydroisoquinoline) and m/z 116 (protonated ethyl 2-aminoacrylate). The proposed fragmentation mechanism for protonated DP4 is shown in Figure 5. Based on accurate mass measurements (Table 1), the structure of DP4 was assigned as, 2-(3-ethoxy-3-oxoprop-1-en-2-ylamino)propanoyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid.

DP5 (m/z 292): The degradation product DP5 formed in acidic, neutral, oxidative and photolytic stress conditions was eluted at 9.1 min. The LC-ESI-HRMS spectrum showed its [M+H]⁺ at m/z 292.1171 with an elemental composition of C₁₅H₁₈NO₅. Its MS/MS spectrum (Figure 3f) shows product ions at m/z 248 (loss of CO₂) and m/z 192 (protonated 6,7-dimethoxy-1,4-dihydroisoquinoline). The proposed fragmentation mechanism for protonated DP5 is shown in Figure 6. The observed characteristic fragmentations are keeping in line with the proposed structure, 2-acryloyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid.

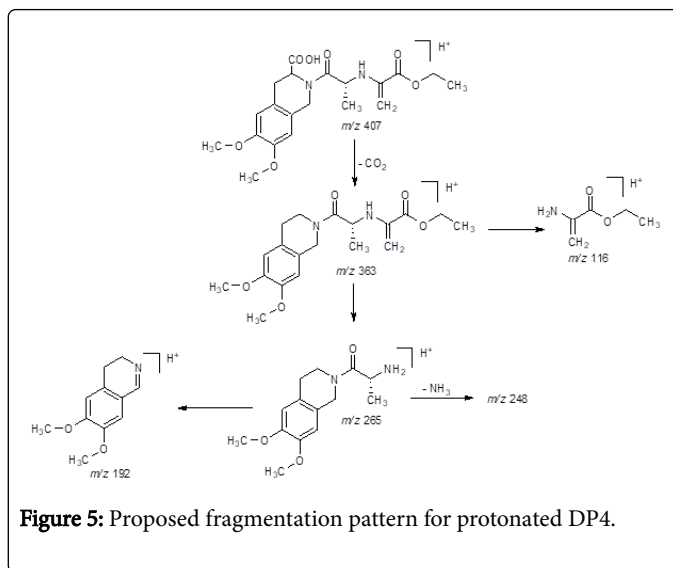


Figure 5: Proposed fragmentation pattern for protonated DP4.

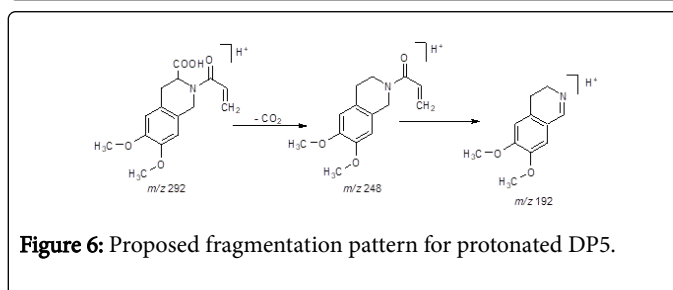


Figure 6: Proposed fragmentation pattern for protonated DP5.

Conclusions

Forced degradation studies were performed on moexipril as per ICH guidelines which provided information on the degradation products and degradation behavior of moexipril under the acidic, basic, neutral, oxidative and photolytic stress conditions. The developed liquid chromatography method separated all Degradation products from moexipril and degradation products from each other under various stress conditions. The drug showed degradation in hydrolytic, oxidative and photolytic stress conditions, it was stable in thermal stress conditions. A total of 5 Degradation products were characterized with the help of LC-MS/MS.

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