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Study of forced degradation behavior of enalapril maleate by LC and LC–MS and development of a validated stability-indicating assay method

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Abstract

In the present study, comprehensive stress testing of enalapril maleate was carried out according to ICH guideline Q1A(R2). The drug was subjected to acid (0.1N HCl), neutral and alkaline (0.1N NaOH) hydrolytic conditions at 80 °C, as well as to oxidative decomposition at room temperature. Photolysis was carried out in 0.1N HCl, water and 0.1N NaOH at 40 °C. Additionally, the solid drug was subjected to 50 °C for 60 days in a dri-bath, and to the combined effect of temperature and humidity, with and without light, at 40 °C/75% RH. The products formed under different stress conditions were investigated by LC and LC–MS. The LC method that could separate all degradation products formed under various stress conditions involved a C18 column and a mobile phase comprising of ACN and phosphate buffer (pH 3). The flow rate and detection wavelength were 1 ml min^{-1} and 210 nm, respectively. The developed method was found to be precise, accurate, specific and selective. It was suitably modified for LC–MS studies by replacing phosphate buffer with water, where pH was adjusted to 3.0 with formic acid. The drug showed instability in solution state (under acidic, neutral, alkaline and photolytic stress conditions), but was relatively stable in the solid-state, except formation of minor products under accelerated conditions. Primarily, maximum degradation products were formed in acid conditions, though the same were also produced variably under other stress conditions. The LC–MS *m/z* values and fragmentation patterns of two of the five products matched with enalaprilat and diketopiperazine derivative, previously known degradation products of enalapril. Also, *m/z* value of another product matched with an impurity listed in the drug monograph in European Pharmacopoeia. Rest two were hitherto unknown degradation products. The products were characterized through LC–MS fragmentation studies. Based on the results, a more complete degradation pathway for the drug could be proposed.

Keywords: Enalapril maleate; Stress testing; Stability-indicating assay method; Validation; LC–MS; Degradation pathway

1. Introduction

Angiotensin converting enzyme (ACE) inhibitors constitute one of the major classes of compounds used for the treatment of essential and renovascular hypertension and congestive heart failure. Enalapril is an ethyl ester of a long-acting ACE inhibitor, enalaprilat. It is basically a pro-drug, which after oral administration undergoes hydrolysis to yield enalaprilat and is used as its maleate salt in therapeutic indications. Enalapril maleate is chemically (2*S*)-1-[(2*S*)-2-[[(1*S*)-1-(ethoxycarbonyl)-3-phenylpropyl]amino]propanoyl]pyrrolidine-2-carboxylic acid (*Z*)-butenedioate (Fig. 1). It is a white to off-white crystalline, odourless powder which melts in the range of 143–144 °C [1].

The chemical and physical stability of enalapril has been studied by several workers. It is reported to be fairly stable when stored in closed containers, but shows instability in open containers and on exposure to high temperature and humidity. It degrades to two major degradation products, viz., enalaprilat and diketopiperazine derivative (Fig. 2), which are reported to be formed in solutions of pH above and below 3, respectively, and even in tablets [1,2]. Enalapril is also shown to be photolabile in solution, again yielding diketopiperazine derivative as the main degradation product [3]. Many ana-

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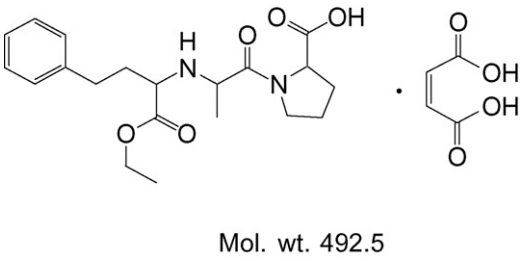


Fig. 1. Structure of enalapril maleate.

lytical methods have been reported in the literature for the analysis of enalapril maleate. The techniques include spectrophotometry [4], spectrofluorimetry [3], ^{1}H nuclear magnetic resonance (NMR) spectroscopy [5], atomic absorption spectroscopy (AAS) [4], etc. A number of high performance liquid chromatography (HPLC) methods have also been reported for this drug using ultraviolet (UV) as well as mass (MS) detectors [2,6–10].

A comprehensive LC and LC–MS study of the degradation behavior of enalapril under various ICH prescribed stress conditions has been lacking. So, it was decided to carry out forced decomposition studies according to the ICH requirements and develop a selective and validated stability-indicating HPLC method. An integral aim of the study was to identify new degradation products, if any, and to postulate complete degradation pathway of the drug.

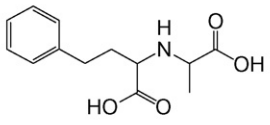
2. Experimental

2.1. Reagents

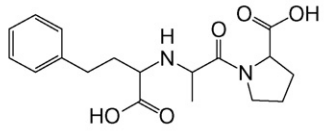
Pure enalapril maleate was obtained as gratis sample from Dr. Reddy's Laboratories Ltd. (Hyderabad, India) and was used without further purification. Analytical reagent (AR) grade sodium hydroxide (NaOH) was purchased from Ranbaxy Laboratories (SAS Nagar, India), hydrochloric acid (HCl) from LOBA Chemie Pvt. Ltd. (Mumbai, India) and hydrogen peroxide (H_2O_2) from S.D. Fine-Chem Ltd. (Boisar, India). HPLC grade acetonitrile (ACN) was procured from J.T. Baker (Mexico City, Mexico). Buffer salts and all other chemicals were of AR grade. Ultra pure water, obtained from ELGA water purification unit (Wycombe, Bucks, England), was used throughout.

2.2. Apparatus and equipment

For solution degradation studies, precision water baths equipped with MV controller (Julabo, Seelbach, Germany) were used. A Dri-Bath (Thermolyne, Iowa, USA) was used for solid-state thermal stress studies. Accelerated stability studies were carried out in humidity (KBF720, WTC Binder, Tuttlingen, Germany) and photostability (KBWF 240, WTC Binder, Tuttlingen, Germany) chambers, both set at $40 \pm 1\,^{\circ}C/75 \pm 3\%$ RH. The photostability chamber was equipped with an illumination bank on inside top consisting of a combination of two UV (OSRAM L18 W/73) and four white fluorescent (OSRAM L18 W/20)

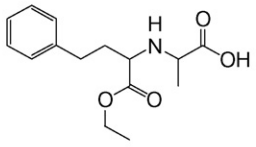


I (mol. wt. 251)
2-(1-Carboxy-ethylamino)-4-phenyl-butyric acid



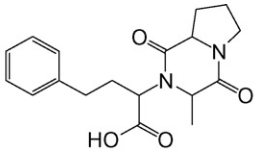
II (mol. wt. 348)
(2S)-1-[(2S)-2-[[(1S)-1-carboxy-3-phenylpropyl]amino] propanoyl]-pyrrolidine-2-carboxylic acid

(Enalaprilat, EP impurity C)

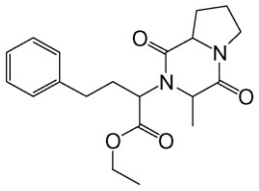


III (mol. wt. 279)
(2S)-2-[[(1S)-1-(ethoxycarbonyl)-3-phenylpropyl]amino] propanoic acid

(EP impurity B)



IV (mol. wt. 330)
2-(3-Methyl-1,4-dioxo-hexahydro-pyrrolo[1,2-*a*]pyrazin-2-yl) -4-phenyl-butyric acid



V (mol. wt. 358)
Ethyl (2S)-2-[(3S, 8aS)-3-methyl-1,4-dioxo-octahydropyrrolo [1,2-*a*]pyrazin-2-yl]-4-phenylbutanoate

(Diketopiperazine analogue, EP impurity D)

Fig. 2. Structures of known and unknown degradation products (I–V) of enalapril.

lamps, all in accordance with 'Option 2' of ICH guideline Q1B [11]. Both fluorescent and UV lamps were put on simultaneously. The samples were placed at a distance of 9 in. from the light bank. A calibrated lux meter (model ELM 201, Escorp, New Delhi, India) and a calibrated near UV radiometer (model 206, PRC Krochmann GmbH, Berlin, Germany) were used to measure visible illumination and near UV energy, respectively.

The HPLC system consisted of a system controller (SCL-$10A_{VP}$), on-line degasser (DGU-14A), low-pressure gradient flow control valve (FCV-$10AL_{VP}$), solvent delivery module (LC-$10AT_{VP}$), auto injector (SIL-$10AD_{VP}$), column oven (CTO-$10AS_{VP}$), photo-diode array (PDA) detector (SPD-$M10A_{VP}$) and CLASS-VP software version 6.13 (all from Shimadzu, Kyoto, Japan). A C18 (250 mm × 4.6 mm, particle size 5 μm) column (Zorbax Eclipse, Agilent Technologies, Wilmington, DE, USA) was used for LC studies and to develop the SIAM.

LC–MS studies were carried out on a system in which LC part consisted of 1100 series HPLC (Agilent Technologies, Waldbronn, Germany) comprising of an on-line degasser (G1379A), binary pump (G131A), auto injector (G1313A), column oven (G1316A) and PDA detector (G1315B). The MS system consisted of MicrOTOF-Q spectrometer (from Bruker Daltonik, Bremen, Germany). The whole system was operated using Hyphenation Star (version 3.1) and MicrOTOF Control (version 2.0) software. The separations were achieved on a C18 (250 mm × 4.6 mm, particle size 5 μm) column (Zorbax Eclipse, Agilent Technologies, Wilmington, DE, USA). The mass spectrometer was run in positive electron spray ionization (ESI) mode with mass/charge (*m/z*) ratio in the range of 50–3000 *m/z*.

pH/Ion analyzer (MA 235, Mettler Toledo, Schwerzenbach, Switzerland) was used to adjust and check the pH of buffers and other solutions. Other equipments used were sonicator (3210, Branson Ultrasonics Corporation, Danbury, CT, USA), precision analytical balance (AG 135, Mettler Toledo, Schwerzenbach, Switzerland) and auto pipettes (Eppendorf, Hamburg, Germany).

2.3. Stress studies

Stress studies were carried out under the conditions of dry heat, hydrolysis, oxidation and photolysis, as mentioned in ICH Q1A(R2) [12]. The approach suggested by Singh and Bakshi [13] was adopted for these studies. A minimum of four samples were generated for every stress condition, viz., the blank solution stored under normal conditions, the blank subjected to stress in the same manner as the drug (enalapril maleate), zero time sample containing the drug (which was stored under normal conditions), and the drug solution subjected to stress treatment. Hydrolytic decomposition of enalapril maleate was carried out in 0.1N HCl, water and 0.1N NaOH at a drug concentration of 2 mg ml^{-1} at 80 °C till sufficient degradation (~20% of initial amount) of the drug was attained. For oxidative stress studies, enalapril maleate was dissolved at a concentration of 2 mg ml^{-1} in 3% H_2O_2 and 20 mg ml^{-1} in 30% H_2O_2 and kept for 8 days at room temperature. Photolytic studies in solution were carried by dissolving enalapril maleate in 0.1N HCl, water and 0.1N NaOH, each at a concentration of 2 mg ml^{-1}, and exposing the solutions at 40 °C to 1.25×10^6 lx h of fluorescent light and 210 W h m^{-2} of UV light [11]. Studies were also conducted on solid drug, which was heated at 50 °C for 60 days in a dri-bath. The same was also exposed to ICH prescribed dose of light in a photostability chamber set at 40 °C/75% RH in 1 mm layer in a petri-plate. Dark control was run simultaneously.

2.4. Preparation of samples for HPLC analyses

The samples (50 μl) were diluted 10 times with water in case of acidic (0.1N HCl), neutral, alkaline (0.1N NaOH) and oxidative (3% H_2O_2) solutions. Samples from 30% H_2O_2 solution were diluted 100 times with water. The solid samples were suitably diluted in water. All the solutions were passed through 0.22 μ filter before making injections.

2.5. Separation studies and development of stability-indicating method

The initial literature search indicated that most of the reported HPLC methods for enalapril maleate, including official compendia, were developed either on C8 or polymeric columns, using harsh temperature conditions (70 °C or more). Despite the same, peak shapes were not good and there was considerable tailing. So attempts were made to develop a simple method on a C18 column, with possible lowering of column temperature.

First, HPLC studies were performed on all reaction solutions individually, and then on a mixture of degraded drug solutions. A C18 column was employed. Different logical modifications like change in pH, different mobile phase compositions and column temperature adjustment were tried to get good separation between the drug and the degradation products, as well as between the degradation products. Methanol was avoided during the study because of its significant absorption at the detection wavelength of the drug between 210 and 215 nm. Also, the use of acetate buffer was evaded as it completely suppressed the UV absorption of the drug.

2.6. Validation of the developed method

Validation was done with respect to various parameters, as required under ICH guideline Q2(R1) [14]. To establish linearity and range, a stock solution containing 0.5 mg ml^{-1} drug in water was diluted to yield solutions in the concentration range of 50–500 μg ml^{-1}. The solutions were prepared in triplicate and analyzed by injecting 15 μl into HPLC. The intra-day and inter-day precision was established by analyzing 100, 300 and 500 μg ml^{-1} drug solutions in triplicate on the same day and on consecutive days, respectively. To determine intermediate precision, the brand of the column was changed and also whole experiment was conducted by a different person. Accuracy was determined by spiking a mixture of stressed samples with three known concentrations of the drug, viz., 100, 300 and 500 μg ml^{-1} in triplicate and then determining the percent recovery of the added drug. Specificity of the method was established by determining the peak purity using a PDA detector.

Also, the resolution factor for the drug and nearest resolving peak was determined. In fact, both peak purity as well as the resolution was determined for all the degradation products' peaks, in addition to the drug peak, to prove that the developed method was selective in nature.

2.7. Development of LC–MS method and characterization of degradation products

To characterize degradation products by LC–MS studies, the developed method was modified by replacement of phosphate buffer with water and adjusting the pH to 3 by formic acid. Rest of the parameters were same. Satisfactory separation of degradation products was achieved using a C18 column (250 mm × 4.6 mm and particle size 5 μm). The obtained *m/z* values in positive ESI mode were compared to the molecular weights (mol. wts.) of the known degradation products and to the impurities listed in drug monograph in the European Pharmacopoeia (EP) [15]. The fragmentation pattern was also investigated. Based on the mol. wt. and the fragmentation pattern, the presence of known degradation products was confirmed and also, structures could be proposed for the unknowns. The degradation pathway was outlined based on the results.

3. Results and discussion

3.1. HPLC studies on the stressed solutions

Enalapril degraded to one or two degradation products in most of the stress conditions, except acidic hydrolysis, where a total of five degradation products were produced. The retention times (R_T) and relative retention times (RR_T) of the drug and the degradation products are given in Table 1. The degradation products carry the notations I, II, III, IV and V in accordance with the sequence in which the peaks appeared from left to right on the HPLC chromatogram. HPLC studies of the stressed samples showed the following degradation behavior:

3.1.1. Hydrolytic conditions

The drug showed ∼20% degradation in 0.1N HCl at 80 °C after 18 h. As mentioned above, in total five degradation products (I–V) were formed. In neutral condition in water, a similar degree of degradation was achieved after 2 days at 80 °C and as many as four peaks appeared at RR_T 0.54, 0.92, 1.12 and 1.43, with respect to peaks II, III, IV and V, respectively. The drug showed very susceptible behavior towards alkaline stress and was completely converted to a single product after exposure for 3 h in 0.1N NaOH at 80 °C. The product appeared at RR_T 0.54, pertaining to peak II, and was stable even after 24 h of exposure.

3.1.2. Oxidative studies

No change was observed even on exposure of the drug to 30% H_2O_2 for 5 days, showing that it was stable against oxidative stress.

3.1.3. Photolytic studies

Under light, enalapril degraded only in acidic conditions to give two peaks at RR_T 0.54 and 1.43 (peaks II and V).

3.1.4. Solid-state studies

There was no significant degradation of solid enalapril maleate on exposure to dry heat at 50 °C for 2 months, which indicated that the drug was stable against thermal stress. Similar stable behavior was observed on exposure of solid drug to the combined conditions of light (UV and visible light), temperature and humidity (40 °C/75% RH). However, the exposure of drug to 40 °C/75% RH for 3 months resulted in slight degradation, yielding two products resolving at RR_T of 0.54 and 1.43 (peaks II and V).

3.2. Development of validated stability-indicating HPLC assay method

3.2.1. Development and optimization of method

Initially, the drug was analyzed on a C18 column (250 mm × 4.6 mm, 5 μm) using ACN and water in the ratio of 50:50 at a flow rate of 1 ml min^{-1} and column temperature of 70 °C. Under these conditions, the shape of the drug peak was not good, perhaps due to low buffering capacity of water. Subsequent trials were made on stressed samples using phosphate buffers of various pH and at various temperatures. The peaks for the drug and degradation products were not well separated or did not have an acceptable shape at pH >3 and column temperature <60 °C. The best separation was achieved on the same column at 60 °C using a mobile phase composed of ACN and phosphate buffer (0.01 M, pH 3), which was run in a gradient mode, as outlined in Table 2. The flow rate was 1 ml min^{-1} and detection wavelength was 210 nm.

3.2.2. Stability-indicating nature of the developed method

As shown in Fig. 3, the method was able to resolve all the components in a mixture of stressed samples. The peaks of the

Table 1
Retention times and relative retention times of various peaks

Peak	Retention time (R_T)	Relative retention time (RR_T)
I	10.25	0.40
II	14.00	0.54
III	23.79	0.92
Enalapril	25.92	1
IV	28.95	1.12
V	37.04	1.43

Table 2
Gradient program for finally developed method of enalapril maleate

Time (min)	Acetonitrile (%)	Buffer (%)	Elution
0–1.00	2	98	Isocratic
1.00–10.00	2 → 24	98 → 76	Linear gradient
10.00–22.00	24	76	Isocratic
22.00–33.00	24 → 55	76 → 45	Linear gradient
33.00–35.00	55	45	Isocratic
35.00–35.01	55 → 2	45 → 98	Gradient
35.01–40.00	2	98	Re-equilibration

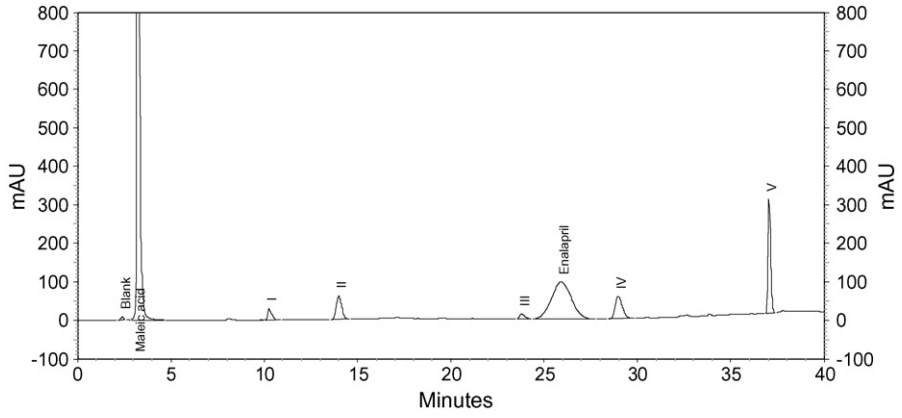


Fig. 3. Chromatogram showing separation of enalapril and its degradation products in a mixture of stressed samples.

Table 3
Data for enalapril maleate from linearity studies

Concentration ($\mu g\,ml^{-1}$)	Area under curve 1	Area under curve 2	Area under curve 3	Average area under curve	±S.D.	R.S.D. (%)
50	1,225,732	1,224,356	1,224,215	1,224,768	838.1076	0.06843
100	2,635,561	2,634,524	2,635,309	2,635,131	540.8478	0.020525
200	5,298,659	5,300,913	5,300,766	5,300,113	1261.056	0.023793
300	8,216,938	8,201,985	8,201,065	8,206,663	8910.581	0.108577
400	11,105,644	11,095,745	11,100,466	11,100,618	4951.258	0.044603
500	13,946,292	13,945,363	13,938,375	13,943,343	4327.703	0.031038

degradation products were not only well-resolved from the drug, but also from one another. The method thus proved to be selective and stability-indicating.

3.2.3. Validation of the developed stability-indicating method

The linearity study data are given in Table 3. The response for the drug was strictly linear in the investigated concentration range of 50–500 $\mu g\,ml^{-1}$ ($r^2 = 0.9998$).

The data for intra- and inter-day precision studies at three different concentrations (100, 300 and 500 $\mu g\,ml^{-1}$) in the linearity range are shown in Table 4. The %R.S.D. values for intra-day and inter-day precision were <1%, indicating that the method was sufficiently precise. As shown in Table 5, good recoveries were obtained when a mixture of stressed samples was spiked with the drug at the above given three concentrations (mean recovery = 100.18%).

The method (Fig. 3) proved to be specific to each peak, which was indicated through single point purity threshold and purity index values (Table 6), determined through use of a PDA detector. Also, the resolution factor among various peaks was found to be >2, proving good separation among all the peaks.

Table 4
Data of intra-day and inter-day precision studies ($n = 3$)

Concentration ($\mu g\,ml^{-1}$)	Intra-day precision	Inter-day precision
	Measured concentration ($\mu g\,ml^{-1}$) ± S.D., R.S.D. (%)	Measured concentration ($\mu g\,ml^{-1}$) ± S.D., R.S.D. (%)
100	101.6308 ± 0.02, 0.02	101.6186 ± 0.13, 0.13
300	298.5673 ± 0.10, 0.13	298.0189 ± 0.21, 0.25
500	501.3414 ± 0.03, 0.28	502.1604 ± 0.09, 0.11

Table 5
Recovery data for enalapril spiked into a mixture of stressed samples

Spiked concentration ($\mu g\,ml^{-1}$)	Calculated spiked concentration ($\mu g\,ml^{-1}$) ± S.D., R.S.D. (%)	Recovery (%)
100	100.443 ± 0.106106, 0.105638	100.4429667
300	299.909 ± 0.481925, 0.16069	99.96966667
500	500.676 ± 0.511298, 0.102122	100.1351067

3.3. LC–MS studies on forced decomposition samples of enalapril maleate

On modification of the method for its extension to LC–MS studies, a slight baseline shift and diminution in the magnitude of peaks were observed in the corresponding UV chromatogram. This was due to suppression of absorption of the drug by formic acid. Nevertheless, all the degradation products in the UV chro-

Table 6
Peak purity data

Peak (RR_T)	Peak purity threshold	Peak purity index
I (0.40)	0.982635	0.999837
II (0.54)	0.999875	0.999953
III (0.92)	0.999963	1.000000
Enalapril (1)	0.999957	1.000000
IV (1.12)	0.995252	0.999244
V (1.43)	0.992483	0.993542

Table 7
Observed *m/z* values for the $[M+H]^+$ ions and major fragments of enalapril and its different degradation products

Peak (RR_T)	Observed *m/z* value	Major fragments
I (0.40)	252	206, 160 and 102
II (0.54)	349	303, 206, 160 and 102
III (0.92)	280	206 and 234
Enalapril (1)	377	303, 234, 160 and 130
IV (1.12)	331	313, 285, 257, 169 and 117
V (1.43)	359	313, 285, 257, 169 and 117

matogram were present in the total-ion chromatogram, recorded by using the modified method.

The mass spectra of the drug and degradation products are shown in Fig. 4. The observed *m/z* values for molecular ion peak and major fragments of the drug and its degradation products are listed in Table 7. Of these, peaks II and V matched with the mol. wts. and fragmentation scheme of the two well-known degradation products of the drug [1,16], which were also listed in EP [15], viz., enalaprilat and diketopiperazine derivative, respectively. The *m/z* value of peak III matched to the mol. wt. of another impurity in EP list [15]. This product is not known otherwise as a degradation product in the literature. According to the *m/z* values and fragmentation pattern, the structures for the two remaining degradation products could be proposed, as shown in Fig. 2.

Fig. 5 gives the proposed scheme of fragmentation of the drug, and products I, II (enalaprilat) and III. The fragmentation pattern for drug and II is based on the behavior reported by Burinsky and Sides [16]. One major fragmentation route for these molecules includes elimination of ethylformate from enalapril and formic acid from II, to yield a common fragment of *m/z* 303, which does not seemingly fragments further. The other major route involves elimination of *N*-formylproline resulting in the formation of '*a*' ion ($[H_2NCH(R)]^+$) with *m/z* values of 234 and 206 for enalapril and product II, respectively. The '*a*' ion can further loose styrene in both drug and II, ethylformate in case of drug, and formic acid in case of II. The products I and III are expected to follow the same fragmentation pattern because of their structural similarity with enalapril and II, respectively. The proposed fragmentation scheme for I and III in Fig. 5 integrates well to the already known scheme for enalapril and II, thus lending support to their assigned structures.

The two products IV and V do not follow the above-mentioned general scheme and follow a separate fragmentation pattern, as outlined in Fig. 6. The scheme has been proposed based on the already reported fragmentation route of the drug to its diketopiperazine derivative and further on [1]. An additional

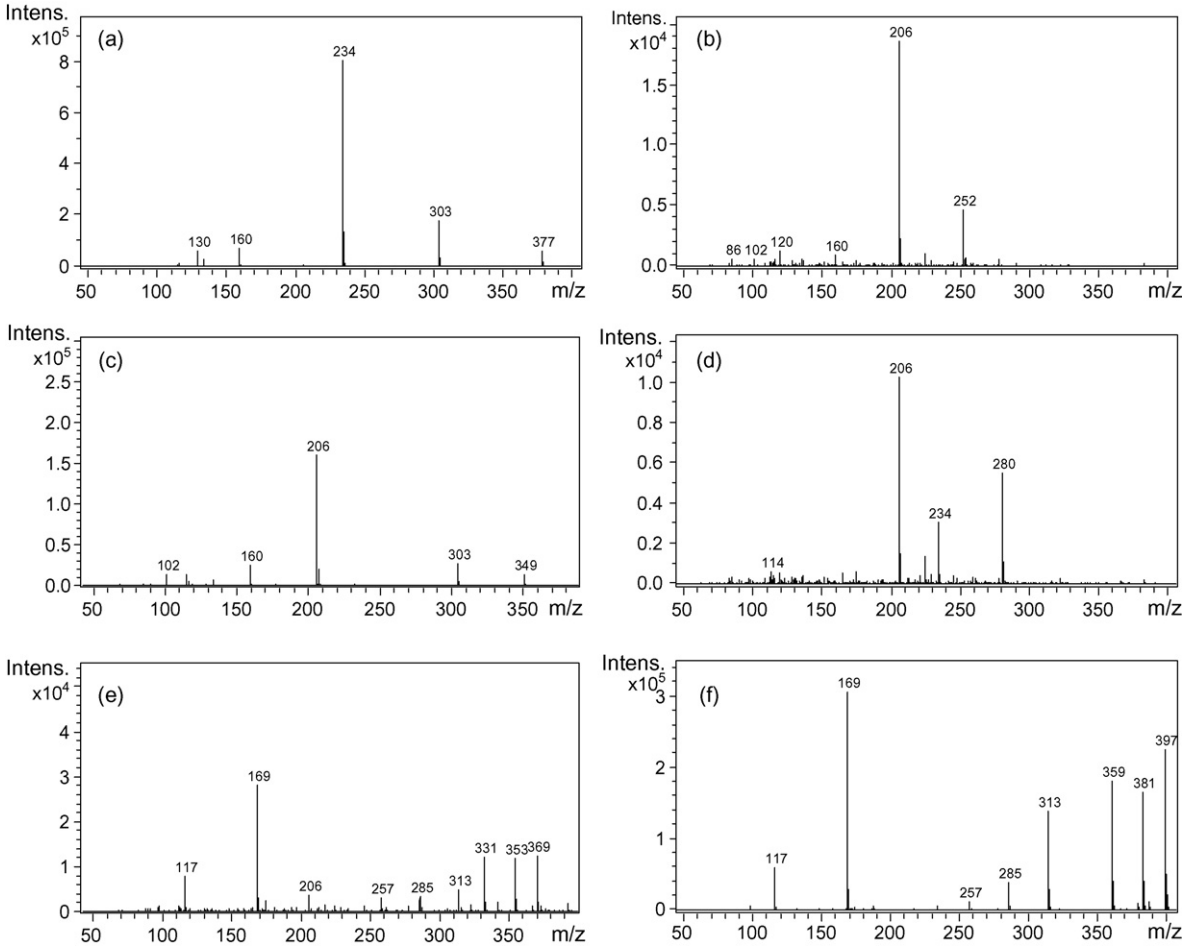


Fig. 4. Mass spectra of enalapril (a) and degradation products I (b), II (c), III (d), IV (e) and V (f).

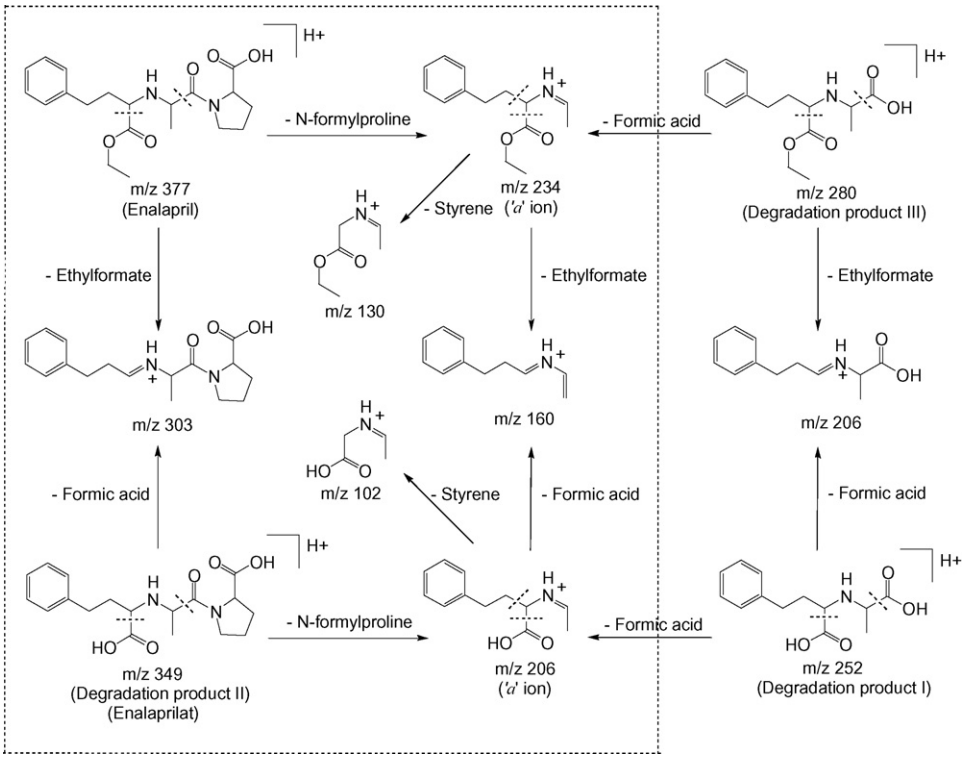


Fig. 5. Fragmentation pattern for enalapril and its degradation products I, II (enalaprilat) and III. The portion enclosed within the box is similar to what is reported earlier [15], while the one outside pertains to other degradation products identified in this study.

peculiar observation, seen in case of mass spectra of both IV and V, was the presence of $M+22$ and $M+38$ peaks for sodium and potassium adducts, respectively. Thus the parallelism of the fragmentation pathway between drug and IV provided support to the structure of the latter in Fig. 2.

3.4. Proposed degradation pathway of the drug

It is known that enalapril undergoes ester hydrolysis and cyclization to give enalaprilat (II) and diketopiperazine derivative (V), respectively [1]. The route of decomposition to these products is outlined in Fig. 7, which also includes the pathway for generation of products I, III and IV.

The decomposition route is explained on the basis that enalapril has two vulnerable bonds, which are susceptible to decomposition under different stress conditions, viz., an ester bond and an amide bond. The ester bond expectedly breaks under acidic, neutral as well as alkaline conditions, whereas amide bond has a tendency to cleave in acidic and neutral conditions. An added finding of this study is the proposed decomposition of

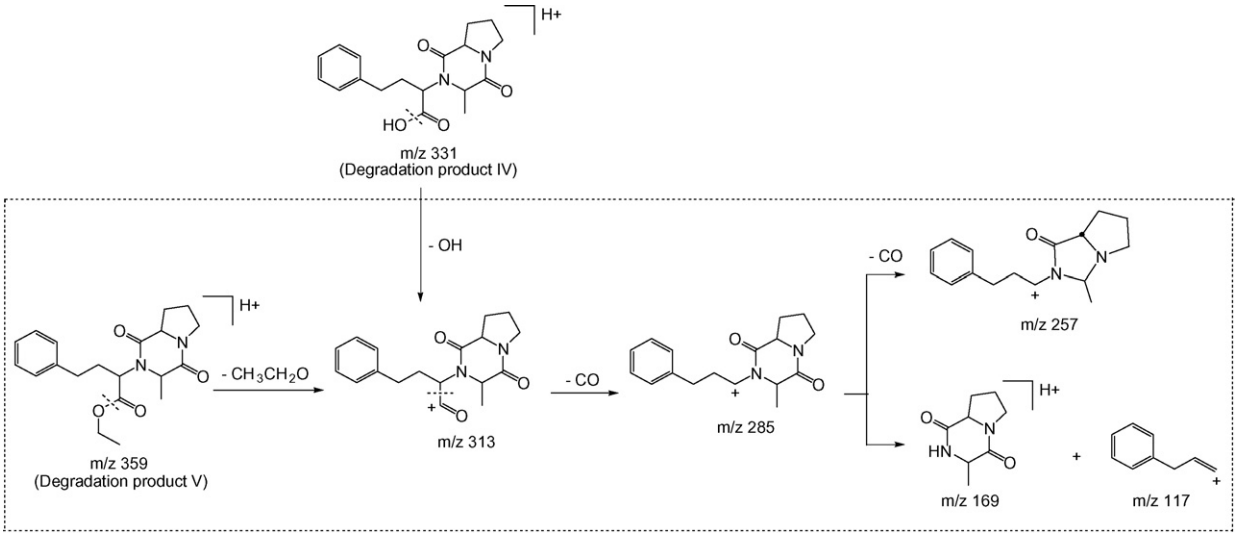


Fig. 6. Fragmentation pattern for degradation products IV and V (diketopiperazine derivatives). The portion enclosed within the box is similar to what is reported earlier [1], while the one outside pertains to new degradation product characterized in this study.

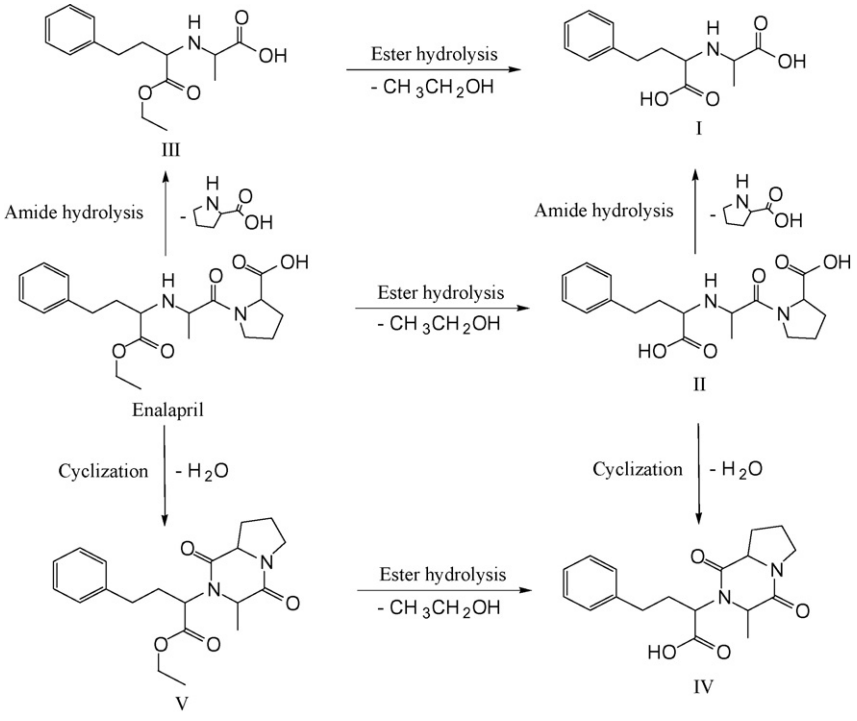


Fig. 7. A proposed pathway for the formation of degradation products I–V of enalapril.

drug through amide hydrolysis to III under the expected acidic and neutral conditions. The same undergoes ester hydrolysis to the new product I, parallel to the conversion of enalapril to II. It is also possible that product I is formed via an alternate route of amide bond hydrolysis of II. Similarly, the new product IV can be generated on ester hydrolysis of diketopiperazine derivative (V) and also upon cyclization of II. The whole scheme in Fig. 7 seems to be logical, as clearly the additional products are formed following a parallel reaction pathway to the already known degradation products of the drug.

4. Conclusions

In this study, it was possible to develop a selective and validated stability-indicating HPLC assay method for enalapril on a C18 column, which could separate the drug and its degradation products formed under a variety of stress conditions. Enalapril maleate was found to be unstable in solution state, whereas it was comparatively much stable in solid-state.

The *m*/*z* values and fragmentation patterns obtained for the degradation products through LC–MS studies helped to confirm the presence of known products and to propose the structures of unknown compounds. The results in totality helped to draw out a more extensive degradation route of the drug.

Indirectly, the study highlights the benefit of the use of ICH stress testing approach in establishment of complete degradation pathways of drugs. It is hoped that this report on stability-indicating method and degradation route of enalapril would be helpful for the multiple generic manufacturers of the drug around the globe by saving them from unnecessary repetition of same studies.

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