Journal of Pharmaceutical and Biomedical Analysis 52 (2010) 294–299

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Short communication

Identification of new impurities of enalapril maleate on oxidation in the presence of magnesium monoperoxyphthalate

Rebeka Toporišič[a,*], Anita Mlakar[a], Jernej Hvala[a], Iztok Prislan[b], Lucija Zupančič-Kralj[b]

[a] Krka, d.d., Šmarješka cesta 6, 8501 Novo mesto, Slovenia
[b] University of Ljubljana, Faculty of Chemistry and Chemical Technology, Aškerčeva 5, 1000 Ljubljana, Slovenia

ARTICLE INFO

Article history:
Received 15 July 2009
Received in revised form 6 December 2009
Accepted 18 December 2009
Available online 29 December 2009

Keywords:
Enalapril maleate
Forced degradation
Degradation impurities
HPLC
UPLC–MS
MMPP oxidation

ABSTRACT

Stress stability testing and forced degradation were used to determine the stability of enalapril maleate (EM) and to find a degradation pathway for the drug. The degradation impurities, formed under different stressed conditions, were investigated by HPLC and UPLC–MS methods. HPLC analysis showed several degradation impurities of which several were already determined, but on oxidation in the presence of magnesium monoperoxyphthalate (MMPP) several impurities of EM were observed which were not yet characterized. The HPLC methods for determination of EM were validated. The linearity of HPLC method was established in the concentration range between 0.5 and 10 μg/mL with correlation coefficient greater than 0.99. The LOD of EM was 0.2 μg/mL and LOQ was 0.5 μg/mL. The validated HPLC method was used to determine the degradation impurities in samples after stress stability testing and forced degradation of EM. In order to identify new degradation impurities of EM after forced degradation UPLC–MS/MS^n, Orbitrap has been used. It was found that new impurities are oxidation products: (S)-1-((S)-2-((S)-1-ethoxy-4-(o,m,p-hydroxyphenyl)-1-oxobutan-2-ylamino)propanoyl)pyrrolidine-2-carboxylic acid, (2S)-1-((2S)-2-((2S)-1-ethoxy-4-hydroxy-1-oxo-4-phenylbutan-2-ylamino)propanoyl)pyrrolidine-2-carboxylic acid. (S)-2-(3-phenylpropylamino)-1-(pyrrolidin-1-yl)propan-1-one was identified as a new degradation impurity.

1. Introduction

Enalapril maleate (EM) is a salt of enalapril and maleic acid, with a chemical name 1-{N-[(s)-1-carboxyl-3-phenyl-propyl]-L-alanyl-}-L-proline 1-ethyl ester maleate (Fig. 1) [1]. It is the first nonsulfhydryl angiotensin-converting enzyme inhibitor [2] and was discovered and developed by Patchett [3].

EM is indicated for the treatment of arterial hypertension [4,5], rennin-dependent hypertension [6] and congestive cardiac insufficiency [4], either alone or in combination with other drugs [7]. In the latter disease, the substance improves the symptoms, decreases the mortality and diminishes the frequency of patient hospitalizations. It is a very powerful drug at low therapeutic doses [8] and has minimal side effects [5].

The EM is a prodrug and as such is not manifested by a direct biological activity. It has a 60% absorption that is unaffected by concurrent food intake. Enalapril is absorbed after oral ingestion and hydrolysed in the liver to its active diacid, enalaprilat (impurity I – Fig. 1), which is responsible for the pharmacological action [5]. The absorption of enalapril is weaker than that of enalaprilat [3].

According to International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines [9], impurities associated with active pharmaceutical ingredients (API) are classified into the organic and inorganic impurities, and residual solvents. Organic impurities may arise during the manufacturing process (from the starting material or intermediates in the multi-step analysis) and/or during the storage of the drug substance (degradation impurities). Accelerated stability testing has to be carried out to prove the stability of the drug substance and its shelf life. The most commonly used analytical technique for impurity determination in drug substances and drug products is high performance liquid chromatography (HPLC). Mass spectrometry is often used for identification of unknown impurities.

Because of the importance of the drug, the synthesis of enalapril has been studied. A new process for preparing L-alanyl-L-proline derivates [10,11] and alkyl-L-alanyl-L-proline derivates [12] has been developed by KRKA, Slovenia.

Impurities of enalapril have been studied extensively since 1987 [4]. EM and its degradation impurities which are the result of degradation processes can be determined by several analytical methods

* Corresponding author at: Analytical Research, Krka, d.d., Šmarješka cesta 6, 8501 Novo mesto, Slovenia. Tel.: +386 07 3313 528.
E-mail address: rebeka.toporisic@krka.biz (R. Toporišič).

0731-7085/$ – see front matter © 2010 Published by Elsevier B.V.
doi:10.1016/j.jpba.2009.12.018

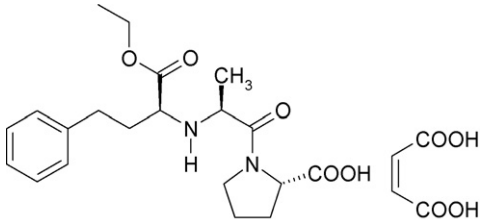


Fig. 1. Structure of EM.

such as HPLC [1,2,6,13,14], nuclear magnetic resonance [15], spectroscopy [16], spectrofluorometry [8], potentiometric method with enantioselective membrane electrode [6], capillary electrophoretic separation [7], thermal Fourier transform infrared microspectroscope [17,18], polarographic determination [19] and isothermal microcalorimetry [20]. Main degradation impurities and impurities from the synthesis of EM are described in the European Pharmacopoeia (Ph Eur) and can be determined by HPLC [21]. In the last couple of years two new synthesis impurities and two new degradation impurities have been discovered (Table 1).

Degradation of EM in solid-state occurs simultaneously and the rate of degradation is increased with temperature and time of heating. It has been shown that the rate and pathways of EM degradation in solutions are pH-dependant [1,21]. At pH below 5, the major degradation product is diketopiperazine derivative (impurity D from Ph Eur), and at pH above 5, the major degradation product is enalaprilat (impurity C from Ph Eur).

Degradation impurities of EM and its stability after exposing it to different temperatures and/or humidity have already been explored by Bhardwaj and Singh [14]. They have also performed forced degradation with different chemicals and identified two new impurities (Table 1). When exposing EM to different temperatures and/or humidity and after forced degradation with NaOH, HCl and H_2O_2 we have obtained similar results but several new impurities were observed after exposing EM to magnesium monoperoxyphthalate (MMPP).

Oxidation is the most important pathway of drug decomposition. MMPP and H_2O_2 were used to predict oxidation since they form sufficiently stable radicals. Hence our research was focused on quantifying and identifying new impurities. A novel UPLC analytical method has been developed for faster identification of new impurities. Identification of unknown degradation impurities of EM was performed by Orbitrap mass spectrometer. Results of our analysis can be used to better understand the degradation pathway of EM and thus improve the selection of excipients for the pharmaceutical formulation of the drug.

2. Experimental

2.1. Samples and chemicals

All of the chemicals, including samples of EM, secondary standard of EM and standards of impurity of enalapril from Ph Eur were supplied by Krka, and were either synthesized or purchased from commercial market. Used chemicals were analytical grade reagents for HPLC or ultra grade for UPLC–MS/MS.

Table 1
Published impurities from Al-Omari et al. [1] and Bhardwaj and Singh [14].

Impurity	Reference
Enalapril-lactone I (S)-synthesis impurity	Al-Omari et al. [1]
Enalapril-lactone II (R)-synthesis impurity	Al-Omari et al. [1]
Molecular ion *m*/*z* 252-degradation impurity	Bhardwaj and Singh [14]
Molecular ion *m*/*z* 331-degradation impurity	Bhardwaj and Singh [14]

Standard solutions were prepared in the solvent (sodium dihydrogen phosphate (pH 5.0; 0.02 M)–acetonitrile (95:5, v/v)) at concentration 1 μg/mL.

2.2. Stability testing

EM powder was kept in vials incubated at 40, 50, 80 °C and at 40 °C/75% relative humidity (RH), for 1, 2, 3 and 4 weeks. Afterwards, the sample solutions were prepared at concentration 1 mg/mL.

2.3. Forced degradation

Forced degradation of EM was performed in mixture of water:reagent:solvent = 50:10:40 (v/v/v) where either 1 M NaOH, 1 M HCl, 3% H_2O_2, 10 mM MMPP, H_2O or the solvent was used as reagent. The final concentration of EM in each case was 1 mg/mL. Solutions were exposed to different temperatures, 10 min at 100 °C, 60 min at 60 °C, 60 min at room temperature and 60 min at 4 °C (only for solutions where 3% H_2O_2 or 10 mM MMPP were used as reagent).

2.4. High performance liquid chromatography (HPLC)

HPLC analyses were performed on an HP-1100 liquid chromatograph with PLRP-S, 250 mm × 4.6 mm, 5 μm column. Analyses were performed by a gradient separation with two mobile phases. Mobile phase A was a mixture of phosphate buffer (pH 6.8; 0.02 M)–acetonitrile (95:5, v/v) and phase B was a mixture of phosphate buffer (pH 6.8; 0.02 M)–acetonitrile (34:66, v/v). The starting condition was 95% of phase A and 5% of phase B, then the amount of mobile phase B was linearly increased to 60% in 20 min and held at 60% for the next 5 min. Mobile phase A was linearly increased to 95% in 1 min and held at 95% for the next 4 min. The flow rate was 1.4 mL/min and separation was performed at 70 °C. The injection volume was 50 μl. The UV detector was operated at 215 nm.

HPLC method was validated for the determination of degradation products of EM. Linear relationship of the peak area of EM was within the concentration range from 0.5 to 10 μg/mL. The correlation coefficient (r^2) was more than 0.99 and relative standard deviation (RSD) was less than 2.0%. The precision of the method and stability of standard and sample solutions were also examined. The results showed that solutions were stable for at least 16 h at room temperatures. LOD of EM was 0.2 μg/mL and LOQ was 0.5 μg/mL.

Related substances of EM, formed during stability testing, were evaluated as relative peak area according to EM (normalization). Impurities, with exception of impurity H from Ph Eur, have similar absorption coefficient as enalapril, therefore no correlation factor was needed.

2.5. Ultra high performance liquid chromatography–mass spectrometry (UPLC–MS (Orbitrap))

The chromatographic separation was performed on the Thermo UPLC. Hypersil Gold, 100 mm × 2.0 mm, 1.9 μm column was used. Separation was carried out with gradient elution of two mobile phases; A was ammonium acetate (pH 6.8; 0.02 M), and B was 100% acetonitrile. The starting condition was 90% A and 10% B, then the amount of mobile phase A was linearly decreased to 57% in a period of 10 min, and held at 57% for the next 5 min. At the end of a run, the mobile phase composition was reverted to the initial conditions and kept for 4 min. The flow rate was constant at 0.3 mL/min, and the column temperature was 60 °C. Injection volume was 4 μl. High-resolution accurate mass measurements were performed on the LTQ-Orbitrap (Thermo Fisher Scientific) equipment with an electrospray ionization (ESI) probe. MS identification

Table 2
Levels of degradation impurities with RRT to EM exposed to different chemicals.

Forced degradation conditions	RRT and labels of degradation impurities									
	0.29 I	0.60 II	0.72 IIIa	0.78 IIIb	0.82 IV	0.86 IIIc	1.08 IIId	1.33 V	1.78 VIa	2.12 VIb
Forced degradation at room temperature										
1 M NaOH	65.90									
1 M HCl	0.14									
3% H_2O_2	0.13								1.00	
10 mM MMPP	0.70				2.23	0.06	1.58	0.30		0.14
Water	0.14							0.32		1.90
Solvent	0.14							0.32		
Forced degradation for 60 min at 60 °C										
1 M NaOH	74.60									
1 M HCl	0.46									
3% H_2O_2 [a]	0.15							0.34	1.05	
10 mM MMPP[a]	0.69				2.29	0.05	1.63	0.29		
Water	0.24							0.30		1.00
Solvent	0.24							0.34		0.12
Forced degradation for 10 min at 100 °C										
1 M NaOH	71.74								0.05	
1 M HCl	0.65							0.32		0.71
3% H_2O_2	0.27		0.18	0.18	0.18	0.20			0.71	
10 mM MMPP	1.47	0.08	0.09	0.05	0.55	0.14	6.21	0.30	0.17	0.43
Water	0.38							0.32		0.32
Solvent	0.30							0.32		

[a] 60 min at 4 °C.

of degradation impurities of EM was carried out in positive electrospray ionization (ESI) mode. The source temperature was 80 °C, and ionization discharge voltage was 3.0 kV. Sheath gas, auxiliary and sweep gas flow rate were 30, 15 and 1 AU, respectively. Capillary temperature was 275 °C and the relative collision energy was 20%.

3. Results and discussion

3.1. HPLC analysis of EM after stress stability testing

EM was exposed to 40, 50 and 40 °C at 75% RH for different time periods in accordance with stability tests. The samples were analyzed with validated HPLC method. The results were compared with previously published results [1,4,6,14,22]. Relative retention time (RRT) for each impurity of enalapril was determined and impurities were quantified using normalization according to EM. Exposure of EM to 40, 50 and 40 °C/75% RH for different time periods resulted in three major degradation impurities. Regardless of conditions the sum of all degradation impurities was slightly above 0.5% which is in good agreement with previously published results concluding that EM is very stable in the solid state, if kept at room temperature in an amber glass container [22] or under dry and humid conditions [1].

It was also confirmed that degradation of EM in solid state increased when kept for more than 1 week at 80 °C [1]. The continuous exposure to temperature of 80 °C for 2 weeks or more caused the formation of new types of degradation impurities and increased the level of degradation by one order of magnitude (4 weeks exposure).

To better understand the degradation pathway of EM forced degradation with different chemicals and at different temperatures was also studied. Results of the HPLC analysis of solutions after forced degradation are presented in Table 2. When treating EM with 1 M NaOH, only the degradation impurity with RRT 0.29 is formed. It appears that the 1 M HCl, 3% H_2O_2, water and solvent solutions do not have effect on degradation of EM. The level of impurities is a bit higher when the temperature is increased but the level of all impurities stays below 2% meaning that acid, peroxide, water and solvent are not particularly good agents for forced degradation of EM. When 10 mM MMPP was used for forced degradation, the increased level of degradation impurities and the formation of several already observed and also new impurities were observed, especially when the sample was treated for 10 min at 100 °C. Table 2

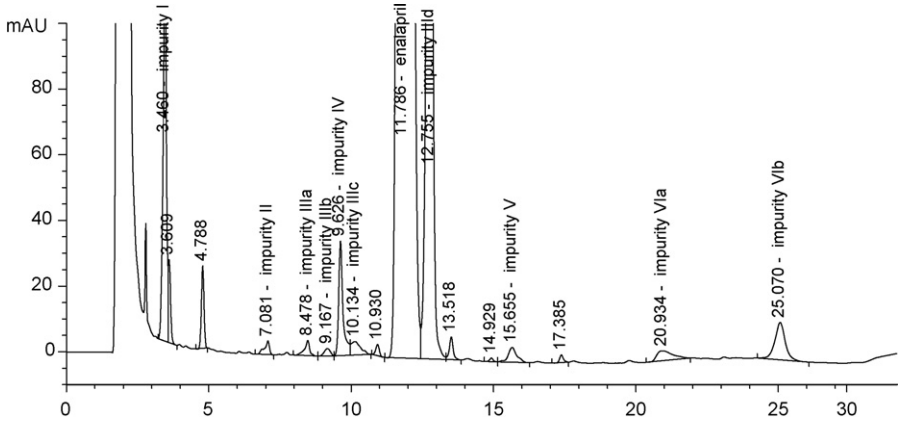


Fig. 2. HPLC chromatogram of EM treated with MMPP, with known impurities marked as impurity I, II, V, VIa and VIb and several new impurities: impurity IIIa, b, c, d and IV.

Table 3
m/z of $(M+H)^+$ for known and unknown degradation impurities of EM.

Degradation impurity	I	II	IIIa	IIIb	IV	IIIc	IIId	V	VIa	VIb
m/z of molecular ion	349	331	393	393	261	393	393	383	359	359

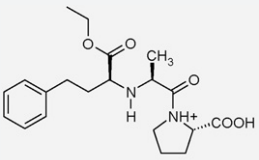
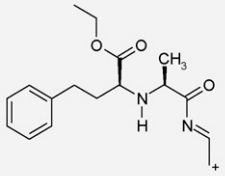
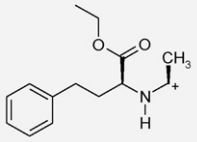
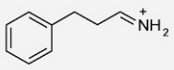
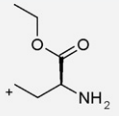
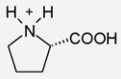
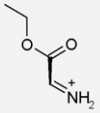
shows all of our results so a direct comparison between already known and new impurities can be made. The chromatogram of such solution is presented in Fig. 2.

3.2. Identification of impurities with UPLC–MS/MS^n Orbitrap

EM treated with MMPP gave the highest amount of new degradation impurities. In the chromatogram of this solution, known and unknown degradation impurities can be seen and are marked as impurity I, II, III, IV, V and VI (Fig. 2). UPLC–MS/MS^n-LTQ-Orbitrap was used for identification of selected peaks. m/z values of $(M+H)^+$ of EM degradation impurities marked in Fig. 2 are presented in Table 3.

The major degradation impurities of EM (impurities I, II, V and VI) are already known [14,21]. However, with forced degradations used in this study, several new oxidation impurities marked as IIIa, b, c, d and a degradation impurity, marked as IV have been characterized. In this work only MS^2 spectrum of enalapril (m/z 377) and

Table 4
Fragment ions used to identify EM and impurities IIIa, b, c and d with the same m/z $(M+H)^+$.

Fragment ions	m/z for molecular ion and major fragments of enalapril and impurities III				
	enalapril	Imp IIIa	Imp IIIb	Imp IIIc	Imp IIId
 377 (enalapril)	377	393 (M+16)	393 (M+16)	393 (M+16)	393 (M+16)
 303 (fragment ion 1); MS^2 mode	303	319 (M+16)	319 (M+16)	319 (M+16)	319 (M+16)
 234 (fragment ion 2); MS^2 mode	234	250 (M+16)	250 (M+16)	250 (M+16)	250 (M+16)
 134 (fragment ion 3); MS^3 mode	134	150 (M+16)	150 (M+16)	150 (M+16)	150 (M+16)
 130 (fragment ion 4); MS^3 mode	130	130	130	130	146 (M+16)
 116 (fragment ion 5); MS^3 mode	116	116	116	116	116
 102 (fragment ion 6); MS^3 mode	102	102	102	102	102

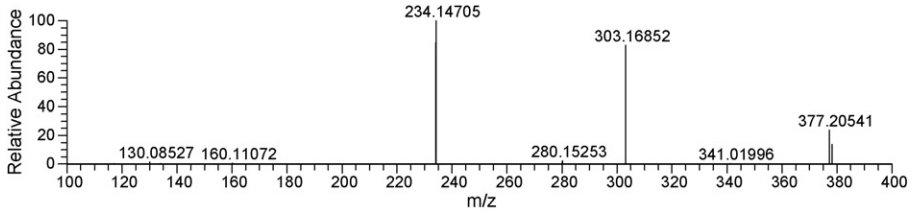


Fig. 3. MS^2 spectrum of EM.

product ion spectra for ll of the impurities of group III (*m*/*z* 393) are discussed. When comparing *m*/*z* for molecular ion of degradation impurities IIIa, b, c and d (Table 3) to *m*/*z* for molecular ion of EM, it can be seen that the difference equals 16, which corresponds to the mass of oxygen atom.

MS^2 of EM is shown in Fig. 3. All fragment ions used for identification of EM and impurities IIIa, b, c and d are shown in Table 4. The mass spectra of the four oxidation impurities of group III have protonated molecular ion $(M+H)^+$ with *m*/*z* 393. Therefore, they can only be distinguished by their retention times. The MS^2 spectrum of the $(M+H)^+$ shows peaks at *m*/*z* 319 and *m*/*z* 250 due to the loss of $C_3H_5O_2$ and $C_6H_8NO_3$, respectively. With additional fragmentation of ion with *m*/*z* 250, the impurity IIId could be distinguished from impurities IIIa, b and c. In the case of impurity IIId the oxygen is still bonded to the fragment ion 4.

MS^2 and MS^3 mass spectra of impurities IIIa, b and c showed the peaks at *m*/*z* 319 (fragment ion 1 with added oxygen), 250 (fragment ion 2 with added oxygen) and 150 (fragment ion 3 with added oxygen). With detailed analysis of mass spectrum and comparison to EM, we noticed that spectral data obtained from MS/MS^n studies show that the oxygen atom in impurities IIIa, b and c is situated on the phenyl ring in either the ortho, meta or para position.

MS^2 spectrum of the impurity IIId shows a fragment ion at *m*/*z* 375 corresponding to the loss of one molecule of water (results are not shown). In the case of impurity III d after the MS^2 experiment it cannot be determined whether an oxygen atom is situated on the carbon chain or on the phenyl ring. The presence of the peak at *m*/*z* 146 (fragment ion 4 with added oxygen) in the MS^3 spectrum suggests that an atom of oxygen is present on the carboxylic chain of the EM. Furthermore, the presence of peaks at *m*/*z* 116 (fragment ion 5) and 102 (fragment ion 6) in the mass spectrum indicate that the position of oxygen is between the phenyl ring and nitrogen group.

Impurity IV, eluted at RRT 0.82 on the HPLC chromatogram (Table 2 and Fig. 2), and the MS spectrum showed peak at *m*/*z* 261, which corresponds to the adduct ion $(M-COOC_2H_5+H)^+$. This might imply that the impurity IV is actually EM without a $COOC_2H_5$ group.

To confirm predicted structures of molecular ions the chemical formulas of degradation impurities were determined (Table 5). The measured masses of $(M+H)^+$ for EM and new impurities were compared to calculated masses, thus Δm (measured-calculated mass).

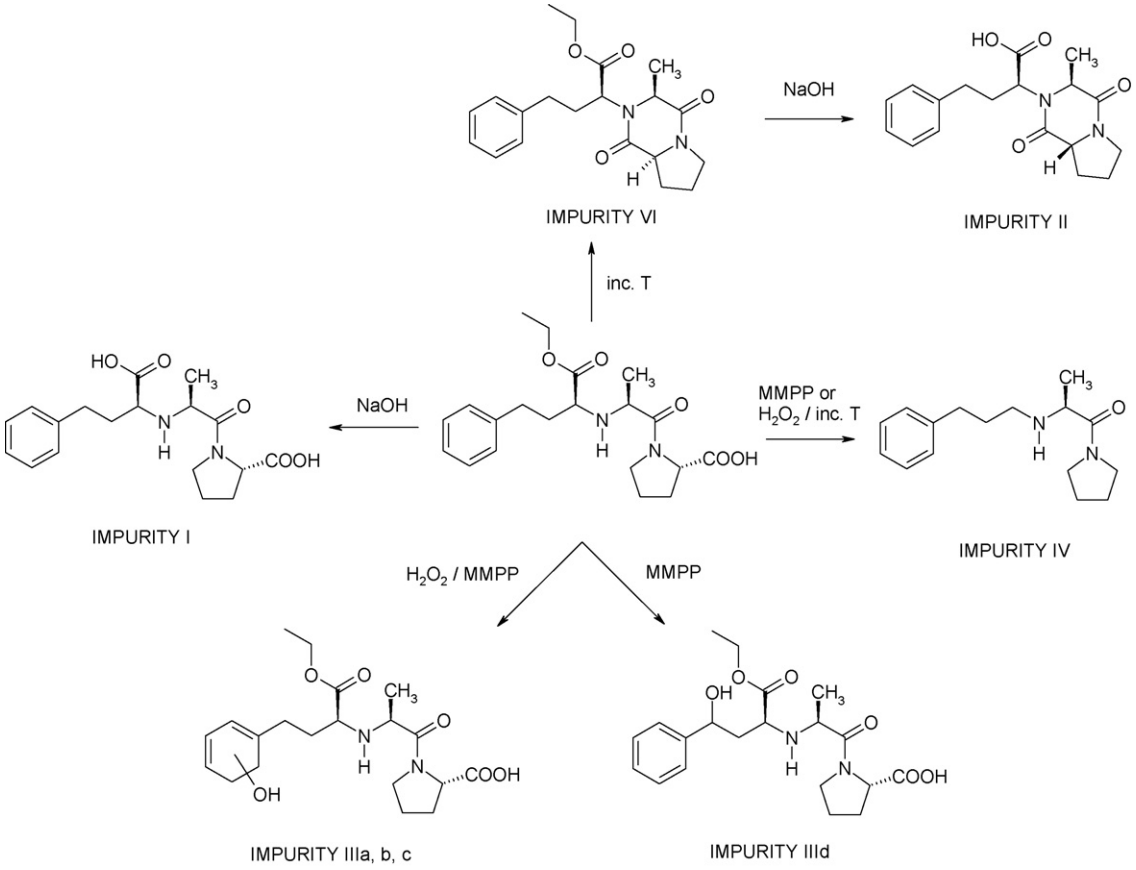


Fig. 4. Degradation pathways of identified degradation impurities of EM.

Table 5
Differences between measured and calculated masses for m/z of molecular ion of the degradation impurities of EM. The names of degradation impurities according to IUPAC nomenclature can be found below the table.

Impurity	m/z of $(M+H)^+$	Chemical formula	Δm (ppm)
EM	377	$C_{20}H_{28}O_5N_2$ (EM)	0.3
I	349	$C_{18}H_{24}O_5N_2$ [a]	0.2
II	331	$C_{18}H_{22}O_4N_2$ [b]	0.2
III	393	$C_{20}H_{28}O_6N_2$ [c]	0.9
IV	261	$C_{16}H_{24}O_1N_2$ [d]	1.3
V	383	$C_{20}H_{34}O_5N_2$ [e]	0.2
VI	359	$C_{20}H_{26}O_4N_2$ [f]	0.2

[a] Impurity I (S)-1-((S)-2-((S)-1-carboxy-3-phenylpropylamino)propanoyl) pyrrolidine-2-carboxylic acid.
[b] Impurity II (S)-2-((3S,8aR)-3-methyl-1,4-dioxohexahydropyrrolo[1,2-a]pyrazin-2(1H)-yl)-4-phenylbutanoic acid.
[c] Impurity IIIa, b, c, (S)-1-((S)-2-((S)-1-ethoxy-4-(m,o,p-hydroxyphenyl)-1-oxobutan-2-ylamino)propanoyl)pyrrolidine-2-carboxylic acid. Impurity IIId (2S)-1-((2S)-2-((2S)-1-ethoxy-4-hydroxy-1-oxo-4-phenylbutan-2-ylamino) propanoyl)pyrrolidine-2-carboxylic acid.
[d] Impurity IV (S)-2-(3-phenylpropylamino)-1-(pyrrolidin-1-yl)propan-1-one.
[e] Impurity V (S)-1-((S)-2-((S)-4-cyclohexyl-1-ethoxy-1-oxobutan-2-ylamino)propanoyl)pyrrolidine-2-carboxylic acid.
[f] Impurity VI (S)-ethyl 2-((3S,8aS)-3-methyl-1,4-dioxohexahydropyrrolo[1,2-a]pyrazin-2(1H)-yl)-4-phenylbutanoate.

Measured m/z of molecular ions was also confirmed with Mass Frontier Software.

3.3. Proposed degradation pathway of EM

The degradation of EM is an autocatalytic reaction of first order kinetics with respect to substrate concentration. Enalapril has two major ways of degradation in solid state: hydrolysis of ethylic ester to enalaprilat (degradation impurity I) and intermolecular cyclization to a diketopiperazine derivative (degradation impurity VI) [4,22].

By forced degradation of EM (Table 2) under alkali conditions mainly degradation impurity I is formed. Even at room temperature, 66% of impurity I was detected. Impurities IIIa, b, and c were formed in oxidation media at increased temperature (in H_2O_2 with heating to 0.2% and less in MMPP, to maximum 0.1%) and impurity IIId was formed after addition of MMPP with or without heating (from 1.6% to 6.2% at room temperature). Impurity IV is formed at increased temperature (0.5%) and after addition of oxidation chemicals (the amount of impurity increases from 0.6% to 2.2% after addition of MMPP). Level of impurities VI (a and b) is increased at higher temperature and after addition of water and H_2O_2 without heating or after addition of HCl, H_2O_2, MMPP and water with heating.

MS/MS^n analysis (Table 4) showed that impurities IIIa, b, c and impurity IIId are oxidation products of EM. Impurity V is formed during the synthesis of EM and is not a degradation impurity. Formation of other degradation impurities in MS/MS analysis can be explained by the mechanism shown in Fig. 4.

4. Conclusions

Stability test confirmed that EM is stable at 40 °C/75%RH, 40 and 50 °C for 4 weeks. The degradation of EM occurs after exposure to 80 °C for 3 weeks. Mainly, level of impurity IV and impurities VI (a and b) is increased. Forced degradation of EM using different chemicals shows that EM is stable in acid, peroxide, solvent and water solutions, less stable in the presence of oxidizing agent MMPP, and unstable in basic solutions. The degradation process was accelerated with the addition of MMPP, and five new degradation impurities (impurities IIIa, b, c, d and IV) were formed.

Impurities IIIa, b and c have additional oxygen atom on the phenyl ring in either the ortho, meta or para position. Impurity IIId showed added oxygen atom between the phenyl ring and nitrogen group. Impurity IV is formed when $COOC_2H_5$ group is branched off the enalapril. The structures of five new degradation impurities were confirmed with the determination of exact molecular mass.

Acknowledgement

We wish to thank KRKA, Slovenia, for supplying the samples, standards, reagents and instruments.

References

[1] M.M. Al-Omari, M.K. Abdelah, A.A. Badwan, A.M.Y. Jaber, Effect of the drug-matrix on the stability of enalapril maleate in tablet formulations, J. Pharm. Biomed. Anal. 25 (2001) 893–902.
[2] K. Ogawa, M. Matsunaga, H. Nagal, A. Hara, H. Koide, C.H. Pak, A. Hirakawa, C. Kawai, Effects of enalapril maleate (MK-421) on renovascular hypertension, Tohoku J. Exp. Med. 145 (1985) 125–135.
[3] A.A. Patchett, The chemistry of enalapril, Br. J. Clin. Pharmacal. 18 (1984) 201–207.
[4] D.P. Ip, G.S. Brenner, Enalapril maleate, Anal. Prof. Drug Sub. 16 (1987) 207–243.
[5] K. Ashok, Topics in clinical pharmacology: enalapril: a second-generation angiotensin converting enzyme inhibitor, Am. J. Med. Sci. 296 (1988) 332–335.
[6] B. Stanisz, Evaluation of stability of enalapril maleate in solid state, J. Pharm. Biomed. Anal. 31 (2003) 375–380.
[7] S. Hillaert, W. Van den Bossche, Optimization of capillary electrophoretic separation of several inhibitors of the angiotensin-converting enzyme, J. Chromatogr. A 895 (2000) 33–42.
[8] M.A. Oliva, L.L. Sombra, R.A. Olsina, A.N. Masi, A new flourescent assay for enalapril maleate, J. Fluoresc. 15 (2005) 723–728.
[9] ICH guidelines, http://www.ich.org/cache/compo/276-254-1.htmL.
[10] M. Merslavič, P. Zupet., V. Flego, M. Japelj, B. Stanovnik, J. Crinski, Postopek za pripravo derivatov L-alanil-L-prolina, Urad RS za intelektualno lastnino SI 8811355A, 1995-4-30.
[11] M. Merslavič, P. Zupet., V. Flego, M. Japelj, B. Stanovnik, J. Crinski, Verfahren zur Herstellung von L-Alanyl-L-prolin-Derivaten, Deutches Patentamt DD 284 028 B5, 1990-10-31.
[12] M. Merslavič, J. Crinski, Process for preparing alkyl-L-alanyl-L-proline derivates, United States Patent Office, US 5,359,086, 1994-10-25.
[13] A. Rotar, Application of spectroscopic, chromatographic and thermoanalytical methods for design and optimization of heterogeneous pharmaceutical systems, Doctor Dissertation, Ljubljana (2000)s.
[14] S.P. Bhardwaj, S. Singh, Study of forced degradation behavior of enalapril maleate by LC and LC–MS and development of a validated stability-indicating assay method, J. Pharm. Biomed. Anal. 46 (2008) 113–120.
[15] O.A. Zoppi, M. Linares, M. Longhi, Quantitative analysis of enalapril by ^{1}H NMR spectroscopy in tablets, J. Pharm. Biomed. Anal. 37 (2005) 627–630.
[16] B. Stanisz, The application of VIS spectrophotometric determination of enalapril maleate in substance, in tablets and estimation of ester group stability, Acta Pol. Pharm. Drug. Res. 56 (1999) 431–434.
[17] S.-L. Wang, S.-Y. Lin, T.-F. Chen, Reaction kinetics of solid-state cyclization of enalapril maleate investigated by isothermal FT-IR microscopic system, Chem. Pharm. Bull. 49 (2001) 402–406.
[18] E. Widjaja, G.H. Lim, P.S. Chow, S. Tan, Multivariate data analysis as a tool to investigate the reaction kinetics of intramolecular cyclization of enalapril maleate studied by isothermal and non-isothermal FT-IR microscopy, Eur. J. Pharm. Sci. 32 (2007) 349–356.
[19] O.A. Razak, S.F. Belal, M.M. Bedair, N.S. Barakat, R.S. Haggar, Spectrophotometric and polarographic determination of enalapril and lisinopril using 2,4-dinitrofluorobenzene, J. Pharm. Biomed. Anal. 31 (2003) 701–711.
[20] Z. Simončič, P. Zupančič, R. Roškar, A. Gartner, K. Kogej, V. Kmetec, Use of microcalometry in determination of stability of enalapril maleate and enalapril maleate in tablet formulations, Int. J. Pharm. 342 (2007) 145–151.
[21] European Pharmacopoeia, http://online.pheur.org/entry.htm.
[22] C. Pilatti, I. Ercolano, M.C. Torre, C. Chiale, M. Spinetto, Search for related substances in market products containing enalapril maleate as the active principle, Drug Dev. Ind. Pharm. 25 (1999) 807–811.