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Identification of losartan degradates in stressed tablets by LC-MS and LC-MS/MS

Zhongxi (Zack) Zhao *, Qingxi Wang, Eric W. Tsai, Xue-Zhi Qin, Dominic Ip

Merck Research Laboratories, PO Box 4, West Point, PA 19486, USA

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Abstract

Three unknown peaks were observed in the severely stressed losartan tablets (at 40°C and 75% relative humidity for 3 years) analyzed by a stability indicating HPLC method. The sample solutions were subjected to liquid chromatography-tandem mass spectrometric (LC-MS/MS) analysis to obtain the chemical identities of these potential degradates. High accuracy and sensitivity of losartan and its degradates were obtained by using atmospheric pressure chemical ionization (APCI) technique in the LC-MS/MS analysis. Three trace level degradates ($\leq 0.1\%$) were found to be the aldehyde and dimeric derivatives of losartan. The structural assignment was further confirmed by comparing the tandem mass spectra of the unknowns with those of the authentic materials of each corresponding degradate. Finally, the mechanistic pathways for the formation of the dimers are discussed.

Keywords: Losartan potassium; Degradate identification; HPLC-MS/MS; Degradation pathway

1. Introduction

It has been well documented that drug products may undergo physicochemical degradation during manufacturing processes and storage. The stability testing of drug products under various temperatures and levels of humidity is an essential part of drug development. The degradate and impurity profiles are critical to the safety and potency assessment of the drug products for clinical trials. Therefore, identification of impurities/degradates in drug formulations has been of increasing importance in drug product assessment. Since degradates/impurities are usually in very low levels (in the ng/ml range in the sample solutions) and since they are interfered by a large amount of excipients and/or active drugs, it is extremely difficult to isolate these species and to examine them subsequently by mass spectrometer and NMR to obtain structural information. On-line LC-MS and LC-MS/MS is an ideal choice for this purpose. The technique has been recently used in pharmaceutical industry [1–5].

* Corresponding author. Tel.: +1-215-652-9156; fax: +1-215-652-2835.

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Losartan (DuP 753, MK-954) is a potassium salt of 2-*n*-butyl-4-cholo-5-hydroxymethyl-1-[(2′-(1H-tetrazol-5-yl)biphenyl-4-yl)methyl) imidazole. It has been shown to be an orally active, highly specific non-peptide angiotensin II receptor blocker (Fig. 2) [6–8]. Losartan effectively reduces hypertension by suppressing the effects of angiotensin II at its receptors, thereby blocking the renin-angiotensin system. Losartan potassium has been formulated and marketed as tablet dosage forms. Although various analytical methods have been developed for the quantitative determination of losartan in losartan tablets [9,10] and biological samples [11], there has not been a report in literature on the identification and characterization of losartan degradates by HPLC-MS/MS.

In this work, on-line LC-MS and LC-MS/MS were used to study the losartan degradates in the severely stressed losartan tablets. During the method specificity test of the severely stressed losartan tablets (at 40°C/75% RH for 3 years), three major extra peaks which were believed to be the degradates of losartan were observed. These extra peaks observed in the stressed losartan tablets were identified to be an aldehyde and dimeric degradates by using LC-MS and LC-MS/MS techniques. The possible structures of the unknowns were proposed and then confirmed by comparison with the authentic materials basing on the molecular ion and the characteristic fragmentation obtained from LC-MS and LC-MS/MS. A reaction mechanism was also proposed for the dimer formation. The results suggest that dimers are formed via a general acid catalyzed S_N2 reaction.

2. Experimental

2.1. Materials

Losartan potassium (L-658,086) and Losartan stressed tablets were obtained from Merck Research Laboratories (West Point, PA, USA). The aldehyde and dimeric degradate standards were obtained from Du Pont-Merck Pharmaceutical Co. (Wilmington, DE, USA). All the standard solutions were prepared by dissolving the standards in an acetonitrile/water (1:1, v/v) diluent at

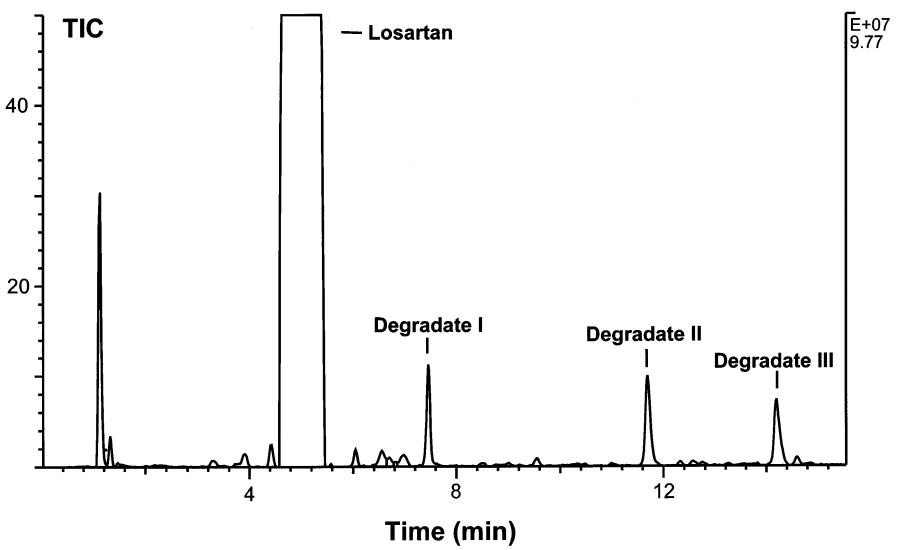

Fig. 1. Total ion chromatogram of severely stressed LOSARTAN® tablets (3 years at 40°C/75% RH). Chromatographic conditions: column: Spherisorb C-8, 4.6 × 250 mm; column temperature: 40°C; mobile phases: A, 0.1% TFA in water; B, acetonitrile; gradient, 25 min from 35% B to 75% B and 5 min at 75% B; flow rate, 1.5 ml/min; injection volume, 20 μl. Mass spectrometer: interface, Finnigan APCI source; heated capillary temperature, 200°C; evaporation temperature, 500°C; corona discharge current, 5 μA.

the concentration of about 0.05 mg ml^{-1} for the LC-UV and LC-MS studies. Triflouroacetic acid (99%) was purchased from Aldrich Chemical Co. (Milwaukee, WI, USA) and used without further purification. Acetonitrile and deionized water were obtained from Fisher Scientific (Philadelphia, PA, USA).

2.2. Sample preparation

A losartan tablet with 25 mg potency was transferred into a 250-ml volumetric flask, which was then filled up to volume with an acetonitrile-water (1:1, v/v) diluent. A stirring bar was inserted and the mixture was vigorously stirred for 30 min until the tablet was totally dispersed. An aliquot was centrifuged and clear supernatant was subjected to LC-MS and LC-MS/MS analysis. For HPLC-UV analysis, the stock solution described above was diluted twofold prior to analysis.

2.3. HPLC analysis of losartan tablets

HPLC-UV analyses were performed on a Dionex GP40 gradient pump (San Jose, CA, USA) equipped with a Thermal Separation AS3500 autosampler and a linear UVIS-205 UV detector. The chromatographic conditions were: a Spherisorb C-8 column (4.6×250 mm) thermostated at 40°C was eluted with a mobile phase consisted of acetonitrile-phosphate buffer (0.001 M, pH 2.3) (gradient: acetonitrile content increased from 40 to 75% in 25 min and hold for 5 min at 75%) at a flow rate of 1.5 m min^{-1}. The analytes were detected with a UV detection at 230 nm. Injection volume was 50 μl and run time was 30 min. Mulitchrom version 2.11 (Lab Systems, Beverly, MA) was used for the data acquisition.

2.4. LC-MS and LC-MS/MS analysis

The liquid chromatograph was a Hewlwett-Packard 1090L HPLC system equipped with an autosampler and an HP1050 UV detector. The HPLC column is a Spherisorb C-8 (4.6×250 mm, 5 μm particle). The mobile phases consisted of 0.1% trifluoroacetic acid in water (A) and acetonitrile (B) at a flow rate of 1.5 ml min^{-1}. The organic content in the gradient was increased from 35% B to 75% B over 25 min with an additional 5 min at 75% B. Injection volume was 20 μl and UV detection was set at 230 nm.

The mass spectrometer utilized in all studies was a Finnigan MAT TSQ 7000 (Finnigan Corp., San Jose, CA) triple quadrupole instrument equipped with a Finnigan atmospheric pressure ionization (APCI) and electrospray ionization (ESI) sources. An APCI positive ion mode was utilized in these experiments. The quadrupole manifold and metal ion inlet capillary were heated to 70 and 200°C, respectively, in order to desolvate the highly charged droplets formed by the ionization processes. Nitrogen generated from Whatman Nitrogen Generator (Haverhill, MA) was used for sheath and auxiliary gases, which were set at 50 and 30 units, respectively. The evaporation chamber temperature was heated up to 500°C and corona discharge current was set at 5 μA. For the MS/MS experiments, the collision chamber pressure was maintained at approximately 1 millitorr of argon. The collision energy for the monomers and dimers was set at 30 and 60 eV, respectively. Both first and third quadruples of the mass spectrometer were scanned at 2 s $scan^{-1}$ over the mass range of interest, and the mass spectrometer was tuned and calibrated by a Finnigan myoglobin/MRFA tuning solution.

2.5. Kinetic study of the dimerization of losartan potassium

In a 100 ml three-necked reaction flask, 50 ml reaction media (0.01 and 0.1 M phosphate buffers, adjust to pH 8) was added. Stirring was then commenced and the solution was equilibrated at 60°C for 30 min. Under nitrogen protection, 1 ml solution of 5 mg $/ml^{-1}$ Losartan Potassium in acetonitrile/water (70/30, v/v) was added rapidly through a syringe. The reaction mixture (1 ml) was sampled every hour and loss of losartan potassium and formation of the dimers were monitored by HPLC-UV.

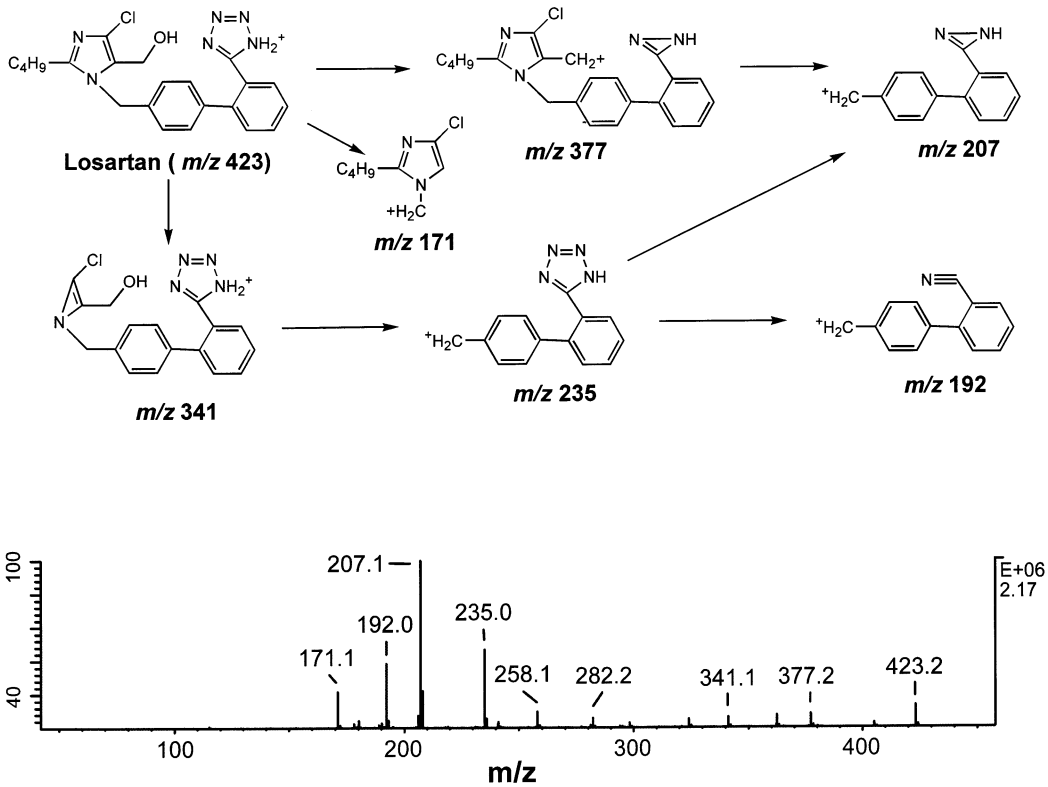

Fig. 2. Proposed fragmentation and tandem mass spectrum of Losartan. Chromatographic conditions are the same as in Fig. 1. Basic settings of mass spectrometer are the same as in Fig. 1 except: collision energy: 30 eV and collision chamber pressure: 1 mtorr of argon.

3. Results and discussion

3.1. Degradate structure characterization by LC-MS and LC-MS/MS

During the method development for a losartan potassium formulation, the severely stressed tablets were analyzed for method specificity tests. Three extra peaks which were believed to be the degradates of losartan at trace levels were detected by HPLC with described conditions. Although the HPLC methods can separate losartan and these three extra peaks, it can not provide any structural information. For the characterization of these degradates, on-line LC-MS and LC-MS/MS was preferred to isolation since isolating the very low level degradates in the dosage formulation was difficult and time consuming.

The stability-indicating HPLC assay method had to be modified by replacing the phosphate buffer with 0.1% trifluoroacetic acid since the use of volatile buffers is required for mass spectrometry. The gradient conditions were also adjusted to accommodate the buffer change. Fig. 1 shows a total ion chromatogram of severely stressed losartan tablets (at 40°C/75% RH for 3 years). Excellent separation between losartan and Degradates I–III was obtained within 15 min of running time. The molecular weights of Degradate I–III were found by HPLC-MS to be 420, 826, and 826, respectively. Then, HPLC-MS/MS had to be used to obtain further structural information.

A key step in elucidating the degradate structures is to understand the fragmentation pattern of drug substance (losartan). The tandem mass spectrum and proposed fragmentation of losartan are shown in Fig. 2. A protonated molecular ion

[MH^+] of Losartan is at m/z 423 (M_w 422). The characteristic fragment ions are at m/z 341, 235, 207 [235-N_2], 192 [235-HN_3] and 171, all of which should provide the base pattern for the degradate identification. Fig. 3 shows the tandem mass spectrum and proposed fragmentation of Degradate I. Molecular ion of Degradate I at m/z 421 is 2 amu less than losartan's, suggesting the possible oxidative degradate. Most intense fragment ions at m/z 235 and 207 in Degradate I are the same as those in losartan, indicating that the part of losartan at m/z 235 is intact and the oxidation should take place in another part of molecule. The fragment at m/z 187 [MH^+-235] containing chlorine clearly indicates that the hydroxyl group [CH–OH] in losartan is oxidized into keto [C=O] group. Also, the identical tandem mass spectra and chromatographic retention times between Degradate I and its authentic standard further confirm that Degradate I is an aldehyde degradate of losartan.

The HPLC-tandem mass spectrum and fragmentation pathway of Degradate II are shown in Fig. 4. The molecular weight of Degradate II obtained from the tandem mass spectrum is 826, indicating 18 amu less than the simple combination of two losartan monomers (M_w 422). Thus, Degradate II is formed by condensation of two monomers followed by the elimination of a water molecule. The characteristic fragment ions of Degradate II are at m/z 809 [MH^+-H_2O], 423 [losartan + H^+], 405 [losartan + H^+-H_2O], 377, 235 and 207 [235-N_2] (also see Fig. 2 for fragmentation pattern). The fragment ions at m/z 423 and 405 containing a chlorine atom clearly support the structure assignment (see Fig. 4). The proposed structure of Degradate II was also confirmed by the authentic degradate standard using both HPLC-UV and HPLC-MS/MS techniques. Degradate III was identified to be a dimer by using the same kind of characterization procedures. The structure of Degradate III is shown in

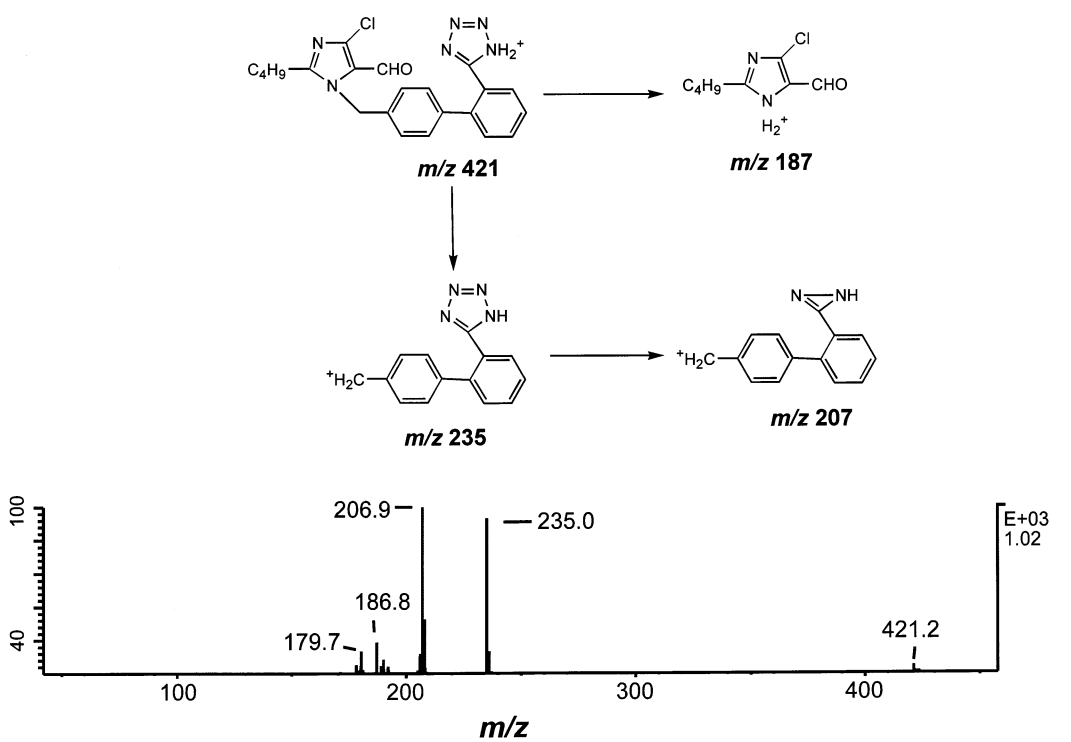

Fig. 3. Proposed fragmentation and tandem mass spectrum of Degradate I. Chromatographic and mass spectrometric conditions are the same as in Fig. 2.

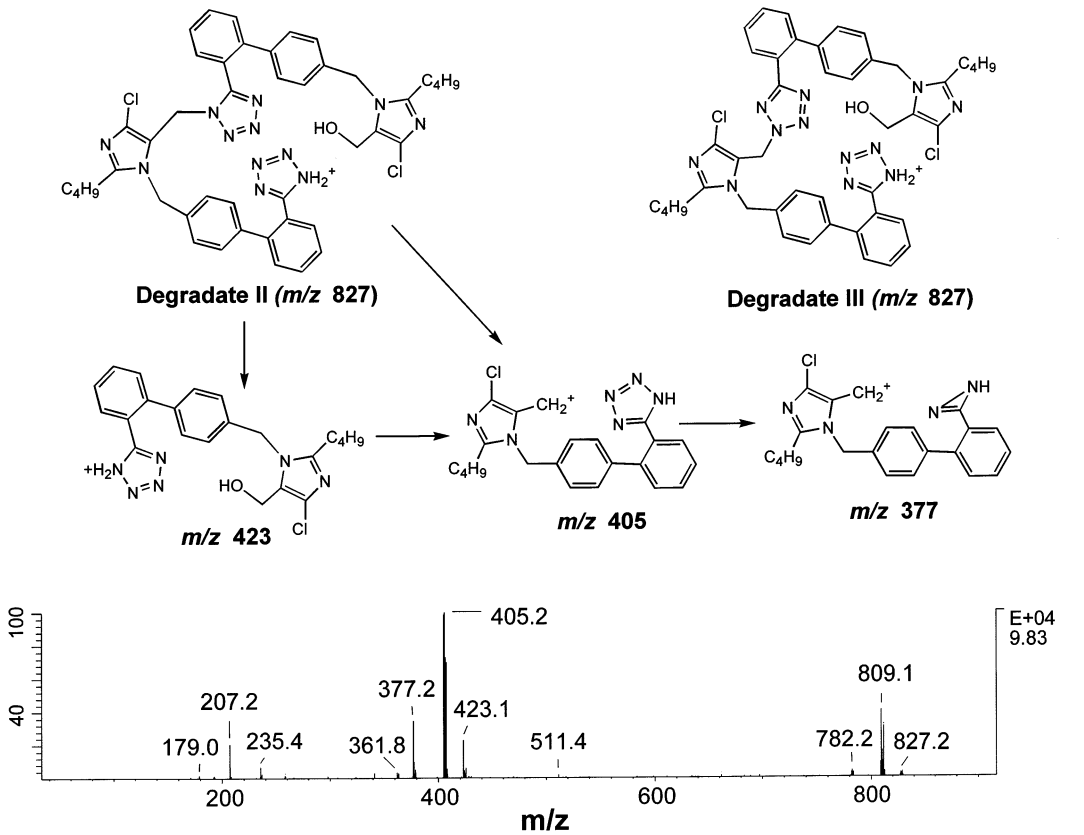

Fig. 4. Proposed fragmentation and tandem mass spectrum of Degradate III (IV). Chromatographic and mass spectrometric conditions are the same as in Fig. 2 except: collision energy, 60 eV.

Fig. 4. The tandem mass spectrum of Degradate III is the same as that of Degradate II although they have different structures. Degradates II and III were differentiated by using the corresponding authentic standards which had been confirmed by NMR.

3.2. Mechanistic study of dimeric formation

To better understand the formation of dimeric degradates in the tablet dosage form, mechanistic pathways in aqueous solution were performed. The dimerization of losartan was studied under both acid and base conditions. No dimers of losartan was observed under basic medium (0.1 N NaOH). The dimerization reaction did occur under acidic to neutral conditions. The pseudo-first order reaction rates in 0.01 M and 0.1 M phosphate buffers were determined to be 4.6×10^{-8} and 1.9×10^{-7} mol s^{-1}, respectively. These indicate that the reaction rate is proportional to buffer ionic strength (hydrogen ion concentration) of the reaction medium. The mechanistic pathway (Fig. 5) for the formation of dimeric degradates is proposed by interpreting the available experimental data. Under the acidic media, the alcoholic group on the imidazole moiety reacts with the amine functionality at the tetrazole through S_N2 nucleophilic substitution reaction.

As shown in Section 3.1, Degradate I is formed from the oxidation of alcoholic group on the imidazole moiety of losartan. The mechanistic study of Degradate I was not pursued in the work since this kind of oxidation has been well documented in the literature [12].

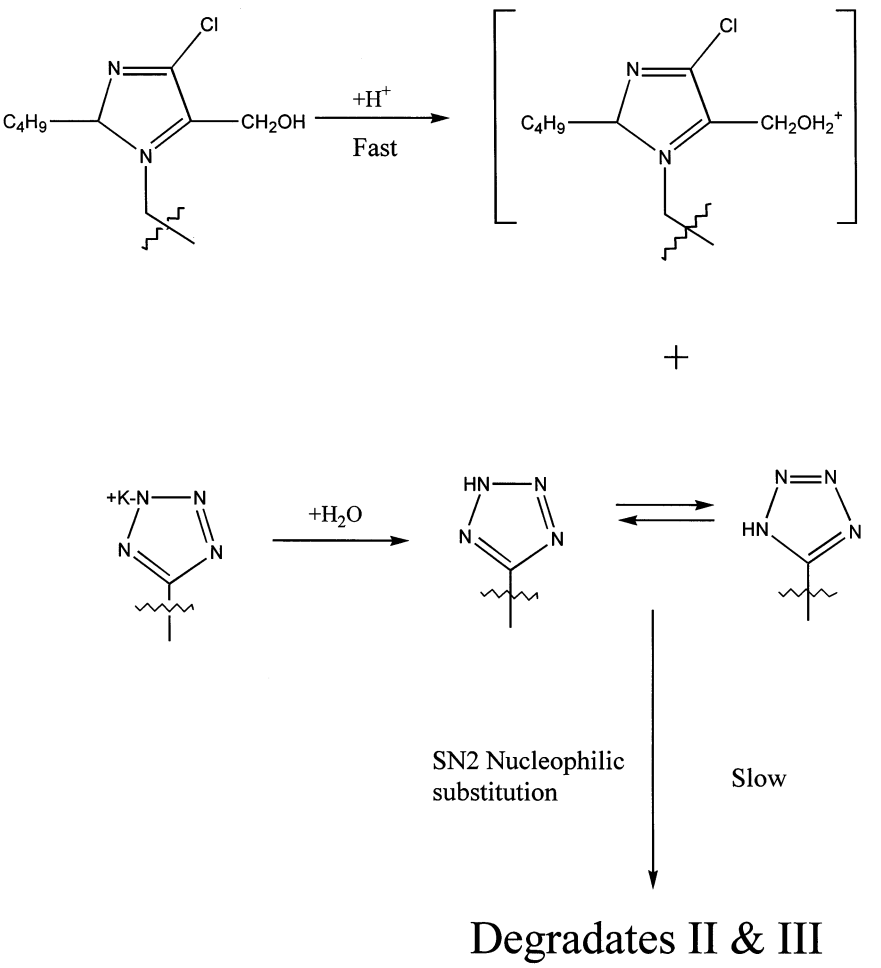

Fig. 5. Proposed formation mechanism of dimeric degradates.

4. Conclusions

Three losartan degradates have been identified in severely stressed losartan tablets by the use of HPLC-MS and HPLC-MS/MS. Degradate I was found to be aldehyde derivative of losartan, which is formed from the oxidation of hydroxyl group in losartan molecules. Degradates II and III were identified to be dimeric degradates of losartan, which are formed by the condensation of two losartan monomers with the elimination of a water molecule. The formation of losartan dimers (Degradates II and III) was determined to be via the acid-catalyzed S_N2 nucleophilic substitution reaction. This work provides another example that on-line HPLC-MS and HPLC-MS/MS are very effective techniques for the fast screening of drug impurities and degradates in the pharmaceutical industry.

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