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Impurity profiling of trandolapril under stress testing: Structure elucidation of by-products and development of degradation pathway

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ABSTRACT

Various regulatory authorities like International Conference on Harmonization (ICH), US Food and Drug Administration, Canadian Drug and Health Agency are emphasizing on the purity requirements and the identification of impurities in active pharmaceutical drugs. Qualification of the impurities is the process of acquiring and evaluating data that establishes biological safety of an individual impurity; thus, revealing the need and scope of impurity profiling of drugs in pharmaceutical research.

As no stability-indicating method is available for identification of degradation products of trandolapril, a new angiotensin converting enzyme inhibitor (ACEI), under stress testing, the development of an accurate method is needed for quantification and qualification of degradation products. Ultra high performance liquid chromatography (UPLC) coupled to electrospray tandem mass spectrometry was used for the rapid and simultaneous analysis of trandolapril and its degradation products. Chromatographic separation was achieved in less than 4 min, with improved peak resolution and sensitivity. Thanks to this method, the kinetics of trandolapril degradation under various operating conditions and the characterization of the structure of the by-products formed during stress testing have been determined. Thereafter, a mechanism of trandolapril degradation in acid and neutral conditions, including all the identified products, was then proposed.

1. Introduction

Angiotensin converting enzyme inhibitors (ACEI) constitute one of the major classes of compounds used for the treatment of essential renovascular hypertension and congestive heart failure (Materson et al., 1993; Weir et al., 1995); trandolapril (1-[2-[(1-ethoxycarbonyl-3-phenylpropyl) amino] propanoyl]-2,3,3a,4,5,6,7,7a octa hydroindole-2-carboxylic acid) (Conen and Brunner, 1993) is a new ACEI that is approved by the US Food and Drug Administration in 1996 (Guay, 2003). It is a long acting, highly lipophilic non-peptide, ACEI with a carboxyl group but without sulphydryl group (Weir et al., 1995). Trandolapril is a prodrug, which after oral administration undergoes hydrolysis to yield trandolaprilat which is the bioactive compound (Conen and Brunner, 1993; Guay, 2003). ACEI are divided into three groups based on their molecular structure: sulphydryl, phosphonate and carboxyl containing agents. Previous studies on the degradation of carboxylic ACEI indicate that these compounds degrade in general by two main mechanisms. The first is hydrolysis of ester function and the second is intramolecular cyclization (Beasley et al., 2005; Hanyšová et al., 2005; Freed et al., 2005; Bhardwaj and Singh, 2008; Elshanawane et al., 2008; Lima et al., 2008; Roškar et al., 2009). However, benazeprilat formed by cleavage of ester function is the major degradation product of benazepril both in acidic and basic media (Gana et al., 2002). The most important factors affecting the stability of ACEI in solutions are pH and temperature. The rate of degradation and the nature of the by-products also depend on the structure of ACEI (Gu and Strickley, 1988; Gana et al., 2002; Bhardwaj and Singh, 2008; Roškar et al., 2009). Roškar et al. (2009) showed that degradation in acidic medium of xpril, enalapril maleate and perindopril is faster than in neutral media. Moreover, functional groups also affected the stability for given conditions. In the case of perindopril the presence of a perhydroindole group leads to an increase of the degradation rate in acidic conditions compared to enalapril and xpril which do not contain perhydroindole function. However, the aromatic phenyl group in enalapril maleate has the highest impact on the stabilization in neutral media (Roškar et al., 2009). According to the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines (ICH, 2003), impurities associated with active pharmaceutical ingredients (API) are classified into

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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 synthesis) and/or during the storage of the drug substance (degradation impurities) (Sándor, 2003). Accelerated stability testing has to be carried out to prove the stability of the drug substance and its shelf life (Steven, 2006). The most commonly used analytical technique for impurity determination in drugs is high performance liquid chromatography (HPLC) (Görög et al., 1995).

In the literature there are only few works dealing with the chemical stability of trandolapril which however do not include an identification of the degradation products. Vikas et al. (2010) have reported that this product undergoes hydrolysis after acidic and alkaline hydrolysis at 80 °C to give rise to two degradation products. Recently, Sahu et al. (2011) have proposed a chromatographic method in order to investigate the degradation of trandolapril in various conditions. However further studies are needed to characterize the by-products formed by trandolapril degradation and to elucidate its mechanism of degradation. Many analytical methods have been reported in the literature for the analysis of trandolapril and others ACEI such as gas chromatography with mass spectrometry (Pommier et al., 2003), high-performance thin layer chromatography (Vikas et al., 2010), (ultra) high-performance liquid chromatography with UV detection (Sahu et al., 2011) or tandem mass spectrometry (Pistos et al., 2005), and square wave voltammetry (Prieto et al., 2003). To the best of our knowledge only one study demonstrates the ability of ultra-performance liquid chromatography (UPLC) to resolve the separation of trandolapril and its degradation products; while no study has focused on the identifications of degradation products. In the following paper, in order to better understand the mechanism of trandolapril degradation a chromatographic method involving diode array detector (DAD) and tandem mass spectrometry (MS/MS) was developed. Degradation of trandolapril according to the ICH guidelines has been performed and a complete mechanism for the trandolapril degradation under neutral and acidic conditions has been proposed.

2. Experimental conditions

2.1. Chemical standards and solutions

Trandolapril was provided by the national laboratory of control of drugs and screening dopage (Tunisia). All mobile phases were prepared from reagent-grade chemicals and purified water (UPW) delivered by a Millipore system (MilliQ Elga, France). Acetonitrile (ACN) for UPLC-DAD and UPLC–MS/MS was purchased from Fisher Chemicals (HPLC grade, Loughborough, Leicestershire, UK) and JT Baker (LC–MS grade, United States), respectively. Aqueous solution of ammonium hydrogencarbonate (Prolabo, Paris, France), (10 mM) was adjusted at pH = 8 ± 0.1 by means of a model 911 pH meter from knick Potamess (Germany). The mobile phases were filtered through a 0.2 μm cellulose acetate membrane filter (Sartorius Stedim Biotech, Goettingen, Germany) before filling the eluent organizer.

2.2. Ultra performance liquid chromatography-diode array detector (UPLC-DAD)

Ultra performance liquid chromatography (UPLC) was performed using a Waters Acquity H-class system (Waters Corporation, Milford, MA). Samples and standard were maintained at 4 °C in the sampler manager prior to analyses. 5 μL of samples was then injected into an Acquity BEH C18 column (100 mm × 2.1 mm 1.7 μm, Waters) thermostated at 45 °C. Different compositions of mobile phases were evaluated to achieve the separation of trandolapril and its degradation products. General composition of eluent consisted of a UPW/ACN mixture, effect of the ratio UPW/ACN, pH and addition of buffering species (ammonium hydrogencarbonate, formic acid) on the chromatographic separation was evaluated. Flow rate was set at 0.4 mL min^{-1} and detection was made between 190 and 500 nm. Waters Empower™ chromatography software was used to control the chromatographic system and to record data.

2.3. Ultra performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS)

Liquid chromatography tandem mass spectrometry system consisted of an Acquity UPLC system (Waters Corporation, Milford, MA) coupled with a Triple quadrupole detector (Quattro premier, Micromass). 5 μL of samples from the hydrolytic degradation experiments was separated on an Acquity BEH C18 column (100 mm × 2.1 mm, 1.7 μm, Waters). Isocratic separation was carried out with a mixture of eluent A (68 vol.%): eluent B (32 vol.%) with a flow rate of 400 μL min^{-1}. Eluent A consisted of an aqueous solution of ammonium hydrogencarbonate (10 mM, pH = 8.14); eluent B was ACN. Sampler manager and column oven was kept at 4 °C and 45 °C, respectively. The MS analysis was performed with electrospray ionization (ESI) interface both in the positive or negative ion mode with a capillary voltage of ±3 kV. Product ion spectra of trandolapril and its degradation products were acquired using N_2 as nebulizer and drying gas. The cone gas flow and the desolvation gas flow were set to 50 L h^{-1} and 750 L h^{-1}, respectively. The source temperature and desolvation gas temperature were 120 °C and 350 °C, respectively. The mass range (m/z) was 50–600.

2.4. Stress study

Stress studies were carried out under the conditions of heat and hydrolysis as mentioned in ICH Q1A (R2) guideline (ICH, 2003). Hydrolytic decomposition of trandolapril was performed at 80 °C with 0.1 N HCl and water at an initial drug concentration of 0.5 mg mL^{-1}. The approach suggested by Bakshi and Singh (2002) was adopted for this study.

2.5. Separation study and development of stability-indicating method

UPLC-DAD experiments were performed on all reaction solutions individually, and then on a mixture of degraded drug solutions. In order to obtain acceptable separation between trandolapril and its degradation products, as well as between the different degradation products, different logical modifications like change in pH, mobile phase compositions and column temperature adjustment were tried. To allow the transposition of the chromatographic method from UPLC-DAD to UPLC–MS/MS simple rules should be respected; eluent composition must involve only volatile compounds to avoid salt deposit into the cone. In the first step, a mixture of UPW acidified at pH = 3 by formic acid and ACN was used as a mobile phase. Formic acid was selected because apparent pK_a of trandolapril is equal to 5.6 and the conventional degradation pathway of ACEI leads to the formation of carboxylic acid by the ester function cleavage. However non-reproducible results were obtained with formic acid in terms of retention time and number of signals. Trials with ammonium hydrogencarbonate as the buffering compound led to more relevant results for the separation of trandolapril from its degradation products; it also resulted in a retention time observed on the UPLC-DAD system comparable to that obtained in the UPLC–MS/MS system. The selected

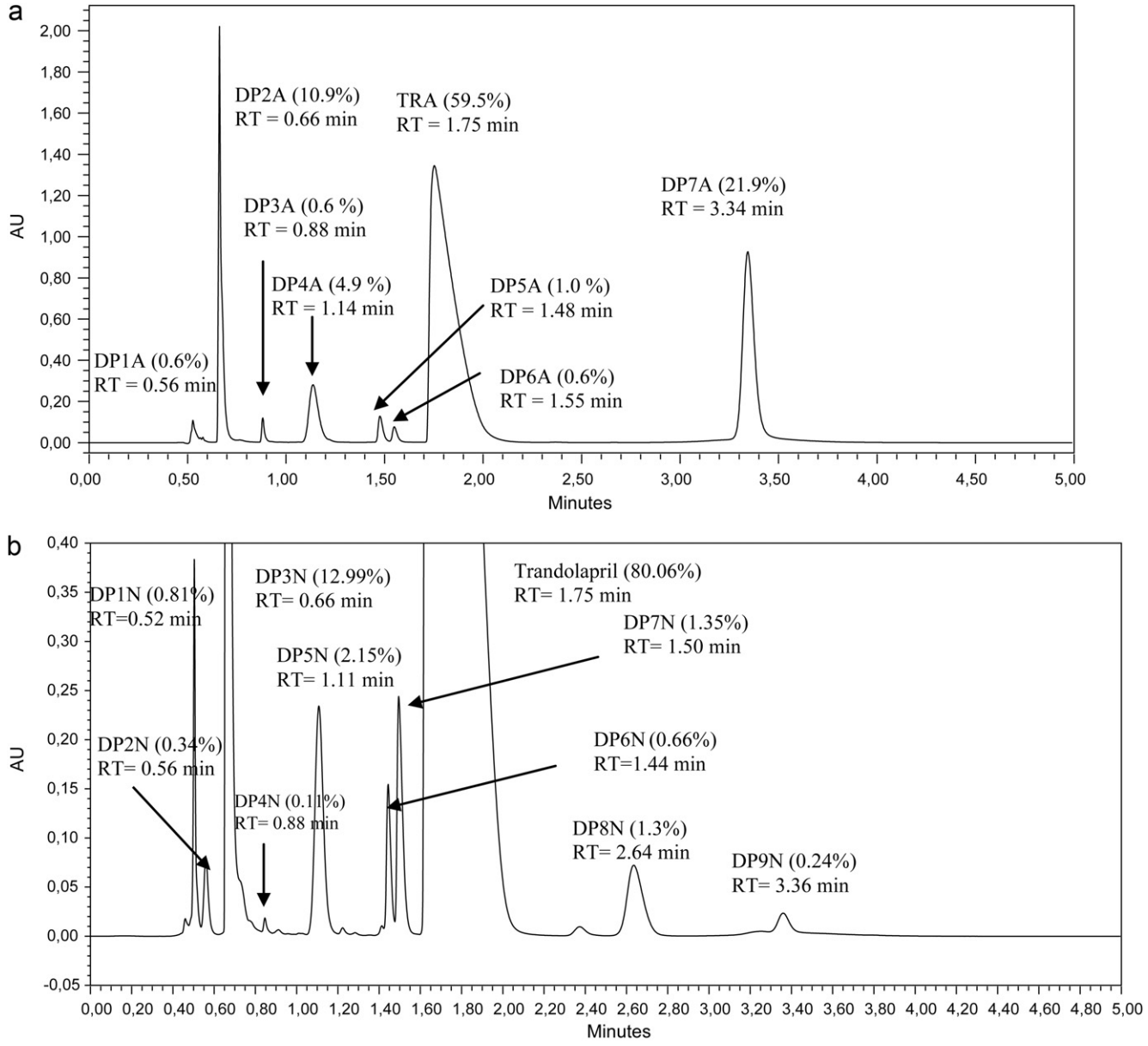

Fig. 1. Chromatograms of trandolapril and its degradation products obtained under acid (a) and neutral (b) conditions at $T = 80\,^{\circ}C$. ($[TRA]_0 = 500$ mg/L; after 23 h of treatment) with the UPLC/UV methods on the BEH C18 column (1.7 μm, 2.1 mm × 100 mm) (Waters). Mobile phases: 68% A: 10 mM ammonium hydrogencarbonate pH 8.2 and B: 32% acetonitrile. Flow: 0.4 mL/min. Wavelength: 211 nm. Column temperature: 45 °C.

composition of the mobile phase consisted of a mixture of 68% (vol.) ammonium hydrogencarbonate 10 mM in UPW and 32% (vol.) of ACN with a flow rate of 0.4 mL min^{-1}. From a practical standpoint, ammonium hydrogencarbonate is an ideal buffer for chromatographers since it provides excellent chromatographic behaviour and reproducible separation. This method was shown to provide fast and efficient separation of trandolapril from its degradation products (see examples of chromatograms Fig. A-4 in Supplementary Data). In addition, hydrogencarbonate buffer is thermally decomposed in CO_2 and NH_3 in the MS interface since 60 °C (Espada and Rivera-Sagredo, 2003).

3. Results and discussion

3.1. Degradation products formed from trandolapril hydrolysis

Stability of trandolapril in acidic ([HCl] = 0.1 M) and neutral media (UPW) was investigated at 80 °C. Kinetic of hydrolysis has been studied using the chromatographic method described above. The reactions of trandolapril degradation are assumed to follow first-order kinetics (see Fig. A-1 in Supplementary Data). As can be seen from Fig. A-1 trandolapril degraded faster under acidic conditions than under neutral conditions at $T = 80\,^{\circ}C$ with a first-order kinetic rate constants equal to $3.93 \times 10^{-2}\ h^{-1}$ and $1.18 \times 10^{-2}\ h^{-1}$ respectively. Half-life values of trandolapril at 80 °C under acidic and neutral conditions were found to be 17 h and 55 h, respectively. These results prove that trandolapril is more stable under neutral medium according to previous works on enalapril maleate, xpril, perindopril and benazepril (Gana et al., 2002; Simončič et al., 2007; Roškar et al., 2009; Simončič et al., 2008).

As can be seen from Fig. A-1, after a contact time of 23 h at $T = 80\,^{\circ}C$ the amount of trandolapril degraded under acidic and neutral conditions were 60% and 19%, respectively. Trandolapril degradation led to the formation of 7 degradation products well separated by means of the chromatographic method developed in this work (Fig. 1a). Comparison of the chromatograms obtained

under acidic and neutral conditions (Fig. 1a and b) shows, after 23 h of contact time, a higher number of degradation products was obtained under neutral conditions compared to acid conditions, 9 and 7 degradation products respectively. Some degradation products seem to be common to the two degradation procedures (same retention time and same UV spectrum). However, since diode array detector is not fully specific, these results should be confirmed by mass spectrometry. To easily differentiate between the two experimental conditions, the following notation has been considered thereafter, DPxA and DPxN for acidic and neutral conditions respectively.

As shown in Fig. 1 the chromatographic method was able to resolve all the components in a mixture of stressed sample. The peaks associated to degradation products were well-resolved, not only from trandolapril but also from one another. The method thus proved to be selective and stability-indicating either from acid and neutral hydrolysis. The chromatographic method was an interesting advance because it was isocratic and its run time was less than 4 min. On the other hand reversed phase UPLC conditions provided a general evaluation of the polarity of each compound, useful for interpretation of substructural differences between related compounds. The UPLC profile obtained for the acid degradation of trandolapril (Fig. 1a) revealed that six degradation products presenting a higher polarity were eluted before trandolapril. Whereas, only one product was eluted later showing that it was more apolar. Furthermore, Fig. 1a shows that three major degradation products (DP2A, DP4A and DP7A) were formed from trandolapril decomposition in acid aqueous solution and at 80 °C. The chromatogram, obtained on a C18 column (Fig. 1), shows a very broad peak relative to the trandolapril (RT = 1.75 min, $\omega_{½}$ = 0.125 min). This chromatographic phenomenon was attributable to an equilibrium between the cis- and the trans-conformer that arose from the hindered rotation around the amid bond having partial double bond character. A similar chromatographic phenomenon has been observed with other ACEI such as enalapril (Trabelsi et al., 2000), enalaprilat (Bouabdallah et al., 2003) and lisinopril (Bouabdallah et al., 2002).

In order to investigate structural differences between all products, UV spectra of trandolapril and its degradation products have been determined (Fig. S-3 in Supplementary Data). Except for the product eluted at 0.66 min, where a new absorption band at 257.8 nm was observed, UV spectra of trandolapril and its degradation products exhibit a similar shape with a broad absorption band at 206 nm. In spite of the similarity of UV visible absorption and response factor of related compounds, their MS ionization efficiencies can be significantly different.

3.2. Identification of degradation products of trandolapril by UPLC–MS/MS

UPLC–MS/MS has become a powerful technique to determine drugs in various matrix, specific fragmentation pattern give selectivity and sensitivity and allow the accurate determination of numerous drugs such as ACEI (Burinsky and Sides, 2004; Niessen, 2011). The use of UPLC–MS/MS is particularly relevant for the elucidation of impurities and degradation product structures and to propose mechanisms of degradation (Rourick et al., 1996; Marıín and Barbas, 2004; Gentili et al., 2008). In recent years, many studies have employed the LC–MS/MS technique in order to evaluate ACEI stability and to characterize their degradation products (Marıín and Barbas, 2004; Pérez et al., 2007; Bhardwaj and Singh, 2008; Toporišič et al., 2010). So, in order to elucidate structure of degradation products induced by acid and heat, LC–MS and LC–MS/MS substructural analysis method has been developed. This method includes information on molecular structures, chromatographic behaviour, molecular weight, and MS/MS substructural information.

Using ammonium hydrogencarbonate as mobile phase, the chromatographic method previously described was directly transferred from LC-DAD to LC–MS/MS. Due to its volatility, ammonium hydrogencarbonate is being an essential buffering specie for rapid LC–MS product identification (Espada and Rivera-Sagredo, 2003). However formation of ammonium adducts could make more complex the MS/MS interpretation (Niessen, 2011). In a first time, full-scan UPLC–MS/MS of the degradation mixture ($[TRA]_0$ = 500 mg/L; [HCl] = 0.1 M; contact time = 23 h; T = 80 °C) has been performed (Fig. B-1 in Supplementary Data). Retention time observed for trandolapril and the major degradation products are closed to those obtained by LC-DAD. MS spectra were extracted for each chromatographic peak and reported in Supplementary Data (Fig. B-2 and B-4). Single MS spectrum allowed the determination of molecular mass of each molecule; however this information was insufficient to determine the structure of degradation products. Moreover identical molecular mass was obtained from different chromatographic peaks; for example, trandolapril (RT = 1.75 min) and DP7A (RT = 3.34 min) show the same molecular mass (m/z 431). To further elucidate the structure of these degradation products, the MS/MS spectra (Fig. B-3 and B-5 in Supplementary Data) of these products were acquired in an additional run with a collision energy of 30 eV. This enabled to determine the elemental compositions for the product ions of degradation products.

The observed m/z values for molecular ion peak and major fragments of the drug and its degradation products in acidic and neutral media are listed in Table 1. It should be noted that ammonium adducts [M+NH_4^+] were not observed under the conditions used. These results confirmed that the number of degradation products in neutral medium was more than those obtained after acid hydrolysis. Moreover these data illustrated the presence of common products with m/z values equal to 403 (DP2A and DP3N), 385 (DP6A and DP7N) and 431 (DP7A and DP9N). In spite of the presence of common products, some molecules were specific of each medium, namely DP1A (RT = 0.56 min; m/z 170), DP3A (RT = 0.88 min; m/z 403), DP4A (RT = 1.14 min; m/z 280) and DP5A (RT = 1.48 min; m/z 403) for the acidic medium, and DP1N (RT = 0.52 min; m/z 252), DP2N (RT = 0.56 min; m/z 403), DP4N (RT = 0.88 min; m/z 280), DP5N (RT = 1.11 min; m/z 403), DP6N (RT = 1.44 min; m/z 385) and DP8N (RT = 2.64 min; m/z 431) for the neutral medium.

An initial step in elucidating structures of degradation products of trandolapril is to understand the fragmentation pattern of the parent-drug substance. Indeed, the detailed mass spectrometry analysis of the fragmentation pattern of trandolapril provides a basis for assessing structural assignment for the degradation products. As can be seen from Table 1 the fragmentation pattern for drug presented the same behaviour as reported by Burinsky and Sides (2004). Indeed, the (+) ESI-MS/MS spectrum of trandolapril (Fig. B-3 and B-5 in Supplementary Data) obtained by the fragmentation of the molecular mass (m/z 431) showed a series of fragment ions of m/z values 357, 234, 206, 170, 160, 134, 130, 117 and 102. The intense fragment-ion m/z 234 that was observed could be rationalized by cleavage of the bond between the carbon atom of the amide group and the α-carbon, followed by further loss of $CH_2{=}CH_2$ to yield m/z 206 and subsequent elimination of styrene to produce m/z 102. Another way of formation of the product-ion m/z 102 consisted of the styrene loss from the product-ion m/z 234, followed by further loss of $CH_2{=}CH_2$ from the intermediate m/z 130 (Fig. 2). Cleavage of the amide group led to the m/z 170 product-ion. The fragment-ion m/z 357 that was also observed for DP2N, DP2A and DP3N could be attributed to the loss of ethyl formate (74 Da) upon formation of a double bond in the backbone chain. The series ions m/z 160, 134 and 117 consisted of the aliphatic chain reduction from the product-ion m/z 206. Fragment-ion m/z 117 (C_9H_9) and 134 ($C_9H_{12}N$) were also commonly observed in the degradation products of trandolapril. This indicated that the left part of

Table 1
Observed *m*/*z* values for the $[M+H]^+$ ions and major fragments of trandolapril and its degradation products in acidic and neutral media.

Degradation products	Retention time (min)	$[M+H^+]$	Fragment ion	Fragment ion intensity (%)	Proposed elemental composition
DP1A	0.56	170	124	100	$C_8H_{14}N$
			107	8.57	C_8H_{11}
			81	38.57	C_6H_9
DP1N	0.52	252	206	82.85	$C_{12}H_{16}NO_2$
			160	54.28	$C_{11}H_{14}N$
			134	7.24	$C_9H_{12}N$
			117	100	C_9H_9
			102	20	$C_4H_8NO_2$
			91	31.42	C_7H_7
DP2N	0.56	403	357	3.71	$C_{21}H_{29}N_2O_3$
			206	80	$C_{12}H_{16}NO_2$
			170	100	$C_9H_{15}NO_2$
			160	8.57	$C_{11}H_{14}N$
			124	3.71	$C_8H_{14}N$
			102	12.85	$C_4H_8NO_2$
DP2A DP3N	0.66	403	357	7.14	$C_{13}H_{17}NO_4$
			206	62.85	$C_{12}H_{16}NO_2$
			170	100	$C_9H_{15}NO_2$
			160	2.85	$C_{11}H_{14}N$
			134	2.2	$C_9H_{12}N$
			124	4.28	$C_8H_{14}N$
			102	15.71	$C_4H_8NO_2$
DP4N	0.88	280	234	6.42	$C_{14}H_{20}NO_2$
			206	100	$C_{12}H_{16}NO_2$
			160	47.14	$C_{11}H_{14}N$
			134	12.85	$C_9H_{12}N$
			130	32.85	$C_6H_{12}NO_2$
			117	41.42	C_9H_9
DP3A	0.88	403	N.D.	–	N.D.
DP5N	1.11	403	252	3.05	$C_{13}H_{17}NO_4$
			206	2.5	$C_{12}H_{16}NO_2$
			124	100	$C_8H_{14}N$
DP4A	1.14	280	234	22.85	$C_{14}H_{20}NO_2$
			206	100	$C_{12}H_{16}NO_2$
			160	29.30	$C_{11}H_{14}N$
			134	11.42	$C_9H_{12}N$
			130	18.58	$C_6H_{12}NO_2$
			117	72.85	C_9H_9
			91	19	C_7H_7
DP6N	1.44	385	N.D.	–	N.D.
DP5A	1.48	403	N.D.	–	N.D.
DP6A DP7N	1.50	385	367	5	$C_{22}H_{27}N_2O_3$
			339	11.5	$C_{21}H_{27}N_2O_2$
			311	21.42	$C_{20}H_{27}N_2O$
			223	71.42	$C_{12}H_{18}N_2O_2$
			117	100	C_9H_9
Trandolapril	1.753	431	357	6	$C_{21}H_{29}N_2O_3$
			234	100	$C_{14}H_{20}NO_2$
			206	1.5	$C_{12}H_{16}NO_2$
			170	22	$C_9H_{15}NO_2$
			160	5	$C_{11}H_{14}N$
			134	5	$C_9H_{12}N$
			130	10	$C_6H_{12}NO_2$
			117	2	C_9H_9
			102	1	$C_4H_8NO_2$
DP8N	2.64	431	357	4	$C_{21}H_{29}N_2O_3$
			234	100	$C_{14}H_{20}NO_2$
			170	20	$C_9H_{15}NO_2$
			134	3.5	$C_9H_{12}N$
			130	11	$C_6H_{12}NO_2$
			124	7	–
			117	2	C_9H_9
DP7A DP9N	3.34	431	280	41.42	$C_{15}H_{21}NO_4$
			234	5.72	$C_{14}H_{20}NO_2$
			206	4.28	$C_{12}H_{16}NO_2$
			124	100	$C_8H_{14}N$

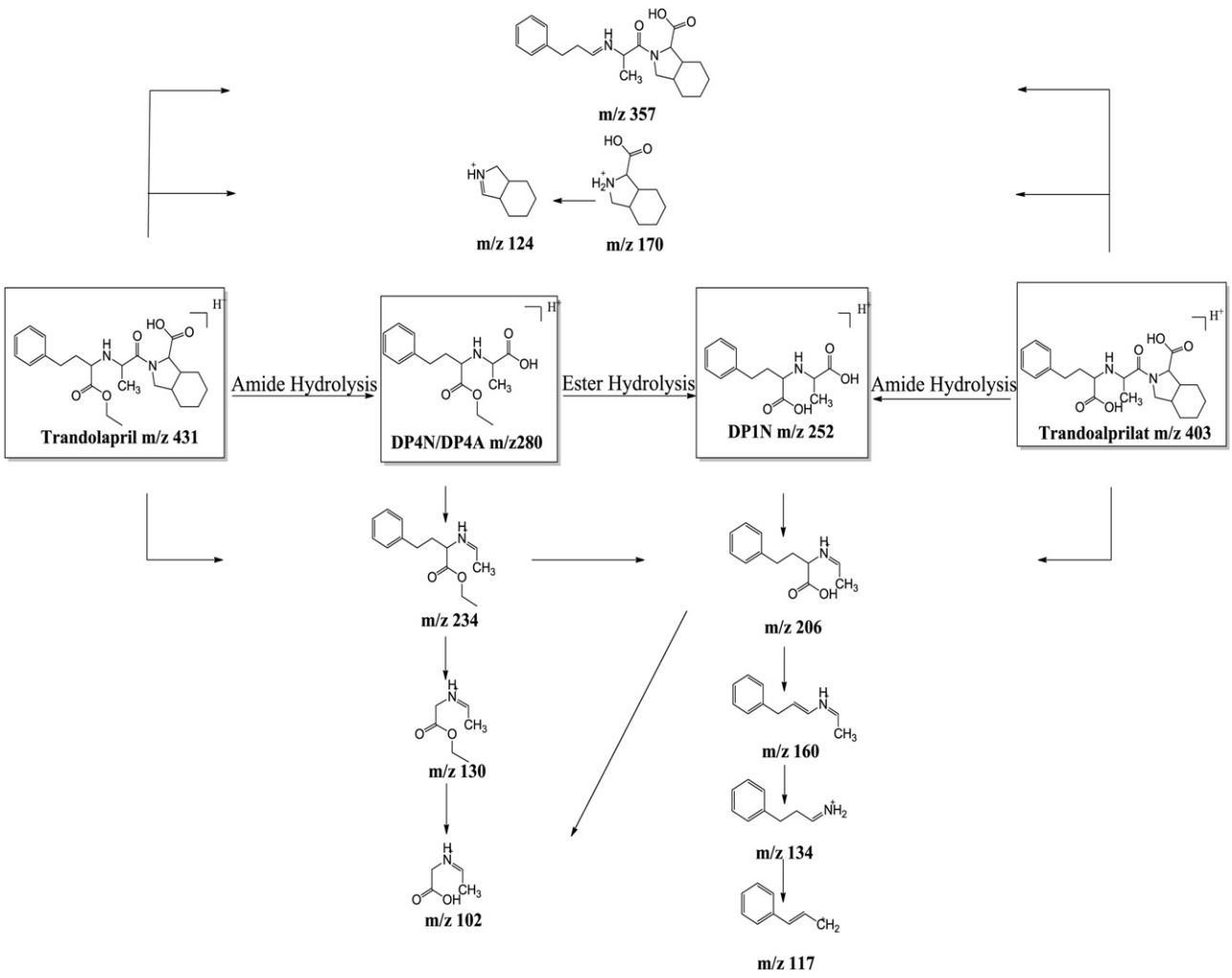

Fig. 2. Fragmentation pattern for trandolapril (*m*/*z* 431) and some degradation products; trandolaprilat (DP2A/DP3N)(*m*/*z* 403), DP4N/DP4A (*m*/*z* 280), and DP1N (*m*/*z* 252).

the trandolapril comprising the aromatic ring was intact in these degradation products.

From the MS^1 spectra of these products (Figs. B-4 and B-2 in Supplementary Data), the degradation products (DP1N) and (DP4N/DP4A) having respectively *m*/*z* 170 and 280 as molecular ion, have been identified. Based on the MS^2 and the most probable elemental composition for these products, $C_9H_{15}NO_2$ and $C_{15}H_{21}NO_4$ were the structures relative to *m*/*z* 170 and 280, respectively. Indeed, the two degradation products with protonated molecular ions ($[M+H]^+$), *m*/*z* 170 and 280 directly resulted from the cleavage of the amide bond of trandolapril.

The most probable elemental composition of the compound (DP1N) only formed under neutral conditions and eluted at a retention time of 0.52 min, was $C_{13}H_{17}NO_4$ ($[M+H^+]$ ion at *m*/*z* 252). MS^2 spectrum of this product showed two major product ions at *m*/*z* 206 and 160 indicating a loss of two HCOOH groups from the parent ion. As previously shown the other fragments at *m*/*z* 134, 117, 102 and 91 were typical of the intact part of the molecule ($C_9H_{12}N$). According to the elemental composition of this product it can be assumed that this diacid product derived from the cleavage of the amide bond of the active metabolite of trandolapril, trandolaprilat or even its isomers.

In addition to these products, three peaks presenting the same *m*/*z* value equal to 403 and eluted at retention times equal to 0.66 min (DP2A/DP3N), 0.88 min (DP3A) and 1.48 min (DP5A) were also observed. The mass spectrum of the degradation product (DP2A/DP3N), obtained both after acid and neutral hydrolysis, showed protonated molecular ion peak at *m*/*z* 403. This compound was identified as trandolaprilat, an active metabolite of trandolapril, listed in the European pharmacopeia. So the product DP2A/DP3N is named as trandolaprilat thereafter. Identification of this molecule was in agreement with the previous study of Burinsky and Sides (2004). In fact, the MS spectrum relative to this product was represented by the fragment ions at *m*/*z* 206, 170, 160, 134, 124 and 102 which are characteristic of trandolaprilat. The identical elemental composition suggested that the other degradation products (DP3A and DP5A) were conformational isomers of trandolaprilat.

The proposed scheme of fragmentation of the drug and the determined degradation products is given in Fig. 2. The fragmentation pattern for trandolapril and the diacid product is based on the behaviour reported by Burinsky and Sides (2004). One major fragmentation route for these molecules includes elimination of ethylformate from trandolapril and formic acid from trandolaprilat, to yield a common fragment of *m*/*z* 357, which does not seemingly further fragments. The other major route involves elimination of octahydro-isoindole carboxylic acid resulting in the formation of ion ($[H_2NCH(R)]^+$) with *m*/*z* values of 234 and 206 for trandolapril and (DP2A/DP3N), respectively. The products (DP4A/DP4N) and (DP1N) are expected to follow the same fragmentation pattern because of their structural similarity with trandolapril and trandolaprilat, respectively.

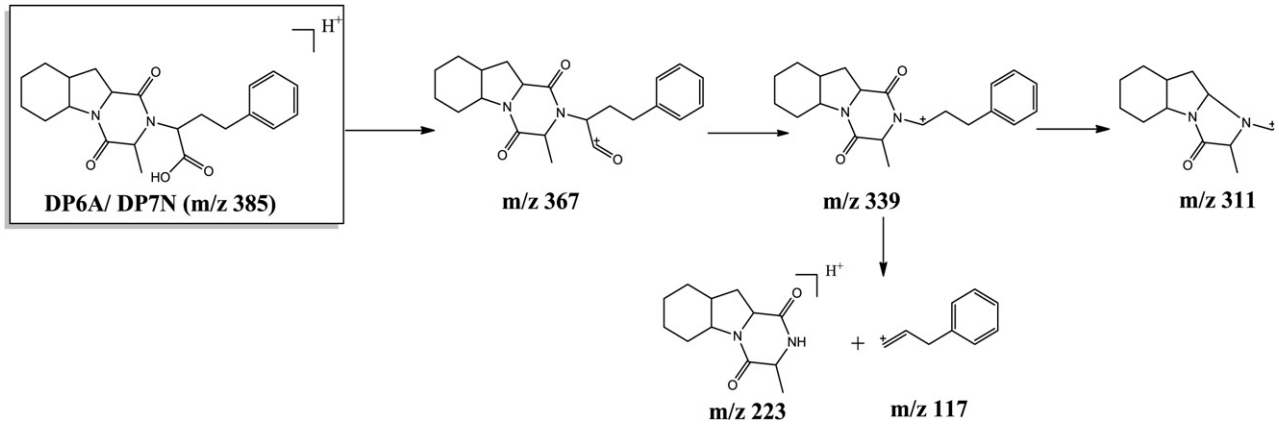

Fig. 3. Fragmentation pattern for the degradation product (DP6A/DP7N) with *m*/*z* 385.

Degradation products with a RT = 1.50 min formed both under acidic (DP6A) and neutral conditions (DP7N) exhibited the same MS^1 spectrum with a molecular ion at *m*/*z* 385. MS^2 spectrum obtained by fragmentation of the *m*/*z* 385 ion led to a series of atypical ions (*m*/*z* 367, 339, 311, 223, 117) compared to trandolapril fragmentation. With respect to the MS^2 spectrum, the mechanism presented in Fig. 3 can be suggested from the diketopiperazine product DP6A/DP7N. The formation of diketopiperazine requires deprotonation of the reacting amine followed by the addition of neutral nitrogen to the carbonyl of the neighboring carboxylic acid to form a tetrahedral intermediate. This intermediate then loses water to give the diketopiperazine product. On the other hand, compared to others ACEI such as moexipril (Elshanawane et al., 2008), enalapril (Lima et al., 2008), quinapril (Freed et al., 2005), lisinopril (Beasley et al., 2005) and ramipril (Hanysova et al., 2005; Elshanawane et al., 2008), trandolapril under acidic and neutral conditions does not yield to the diketopiperazine product with *m*/*z* value of 412 form, the cyclization of the ester form. Therefore, it

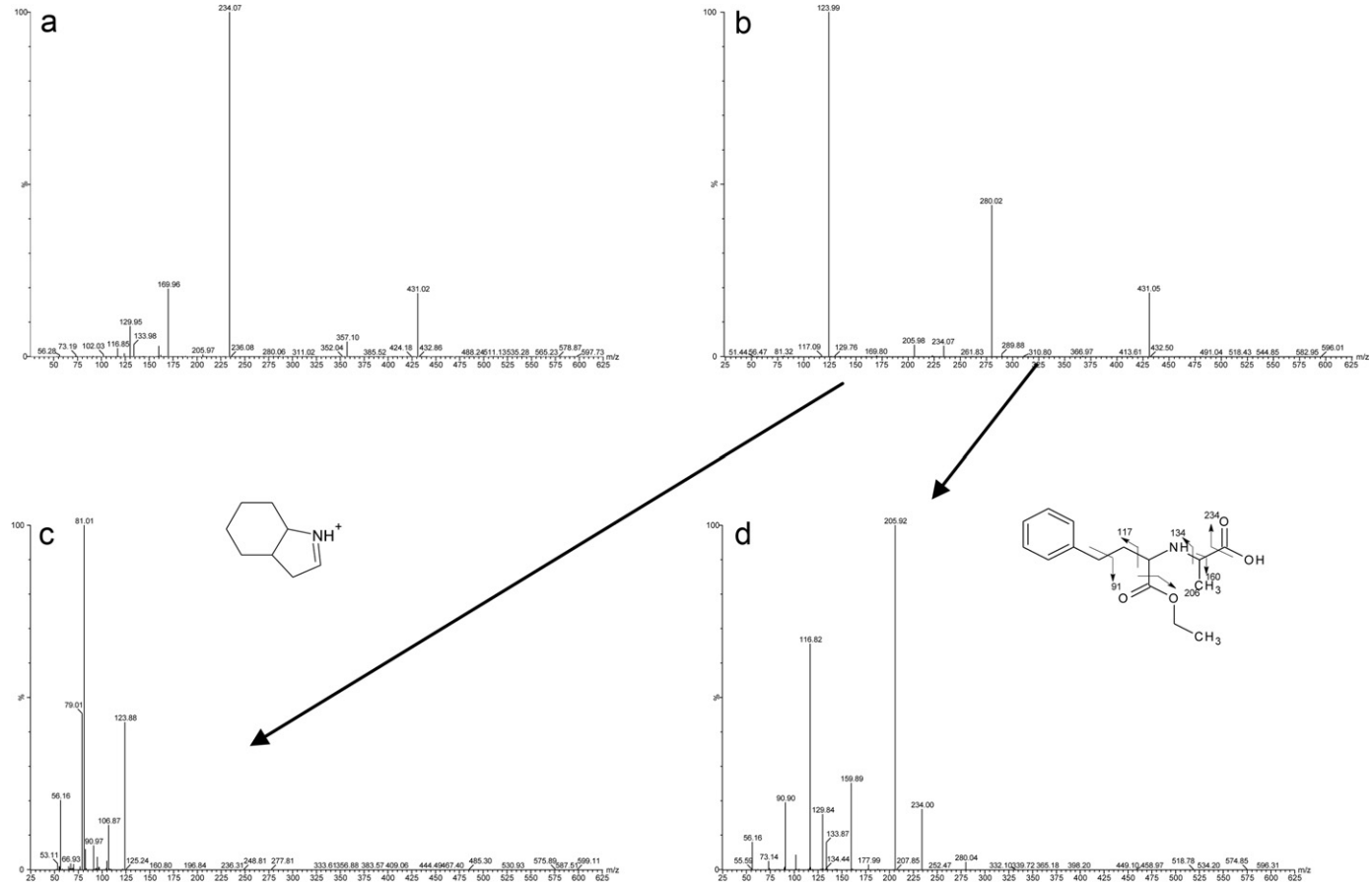

Fig. 4. (+) ESI MS^2 spectrum (daughter scan of $[M+H]^+$ = 431) of trandolapril (a) and DP7A/DP9N (b). (+) ESI MS^3 spectrum of DP7A/DP9N, daughter scan of 431 → 280 (b) and 431 → 124 (d).

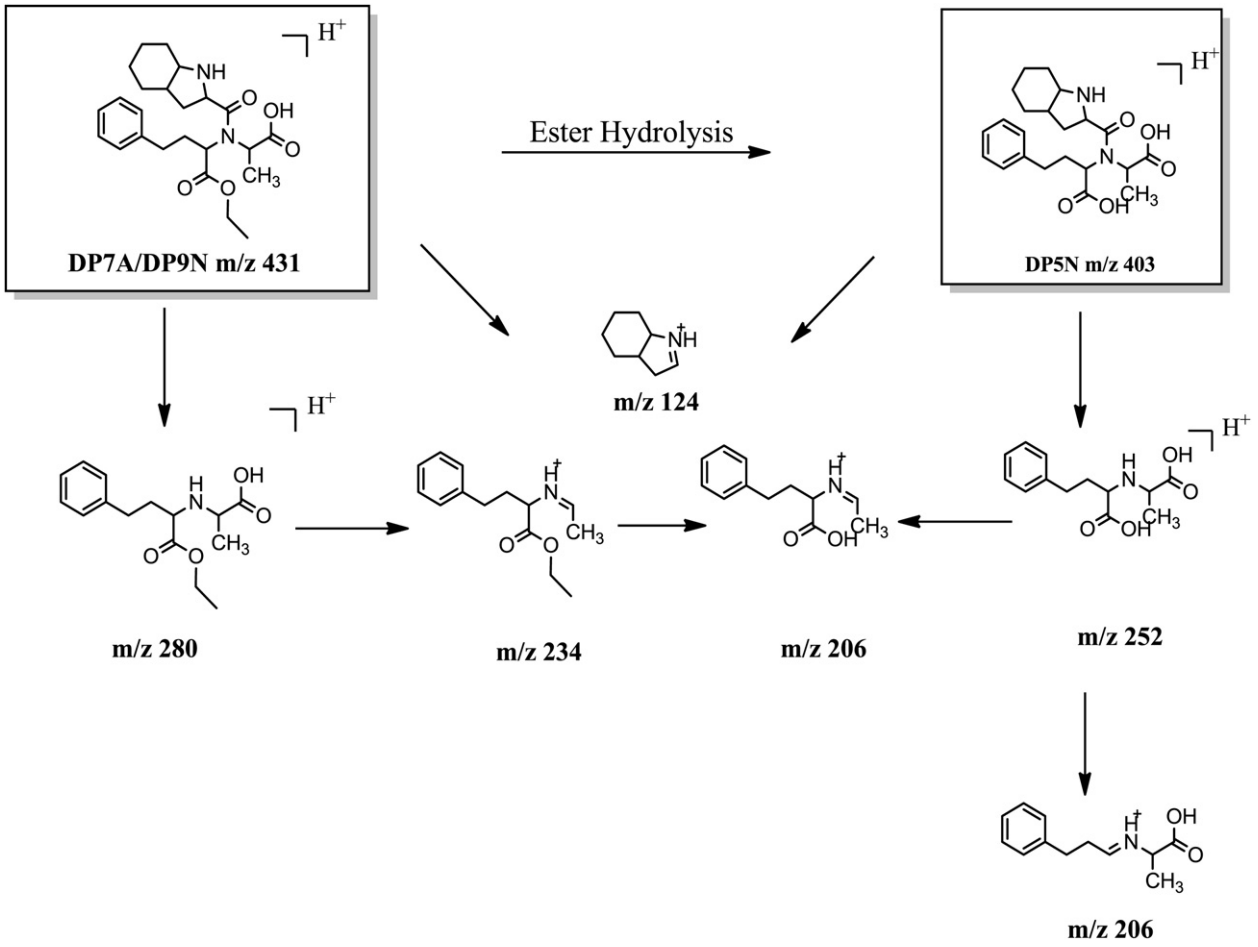

Fig. 5. Fragmentation pattern for DP7A/DP9N (*m*/*z* 431) and DP5N (*m*/*z* 403).

can be concluded that the cyclic product with protonated molecular, [M+H$^+$], *m*/*z* 385 resulted from the cyclization of the diacid product with *m*/*z* 403.

An unexpected product with a molecular mass similar to trandolapril ([M+H]$^+$, *m*/*z* 431) was detected both under acidic and neutral conditions (DP7A/DP9N).

In order to elucidate the structure of this new product, MS^2 spectra of trandolapril and of the molecular ion ([M+H]$^+$, *m*/*z* 431) of the degradation product named DP7A/DP9N have been determined (Fig. 4). So the sub-structural characterization of these fragments was required to determine the structure of DP7A/DP9N. The use of LC with multistage MS (LC–MS^n) is an essential tool of identification of by-products structures (Liu and Hop, 2005; Kostiainen et al., 2003). Sequential MS^n analysis ($n > 2$) can be only performed with ion trap instruments, nevertheless pseudo-MS^3 analysis are also feasible on triple quadrupole instruments. This pseudo-MS^3 approach results from combination of two-stage collision induced dissociation, first fragmentation stage was performed in the source on a non-mass-selective ion beam, whereas the second stage takes place in the collision cell on a specific ion selected by the first quadrupole (*m*/*z* 280 or 124) in the source and in the collision cell of triple quadrupole instrument (Gentili et al., 2008). This operating mode sacrifices the exact precursor/product relationship provided by a true MS^3 experiment, but it may give additional useful analytical information. The mass spectrometric characterisation of these two fragment ions *m*/*z* 280 and 124 are shown in Fig. 4c and d, respectively.

According to the fragmentation pattern mentioned above, only an intermolecular rearrangement could explain this fragmentation behaviour. The rearrangement, which is based on a conventional transamidation, consisted of (a) nucleophilic attack of the secondary-amine nitrogen on the carbonyl of the octahydroisoindole carboxylic group, (b) migration of the hydroxyl group to the amide carbonyl, and (c) subsequent hydrolysis of the amide bond with concomitant liberation of an amine in the octahydroisoindole moiety. This new structure was in agreement with the fragmentation pattern, the cleavage of the amide bond could explain the fragment ions *m*/*z* 280 and *m*/*z* 124 sought for. Moreover the elemental composition of this ion was identical to the fragment ions in MS^3 spectrum.

Both degradation products DP2N and DP5N which eluted at 0.56 and 1.11 min showed a protonated molecular ion similar to trandolaprilat at *m*/*z* 403. In spite of this similarity, MS^2 spectrum of DP2N differed from those obtained with DP5N; moreover DP2N MS^2 spectrum was close to that observed for trandolaprilat. This phenomenon can be explained by a possible epimerization as mentioned for moexiprilat (Gu and Strickley, 1988). However, additional experiments, which could be later considered, are needed to confirm this assumption. MS^2 spectrum of DP5N revealed the presence of two characteristic fragment ions at *m*/*z* 252 and 124.

Comparison of the (+) ESI-MS^2 spectra of degradation products DP7A/DP9N and DP5N (Fig. B-3 and B-4 in Supplementary Data) showed the presence of a common ion with *m*/*z* value equal to 124. This intense fragment could be rationalized by cleavage

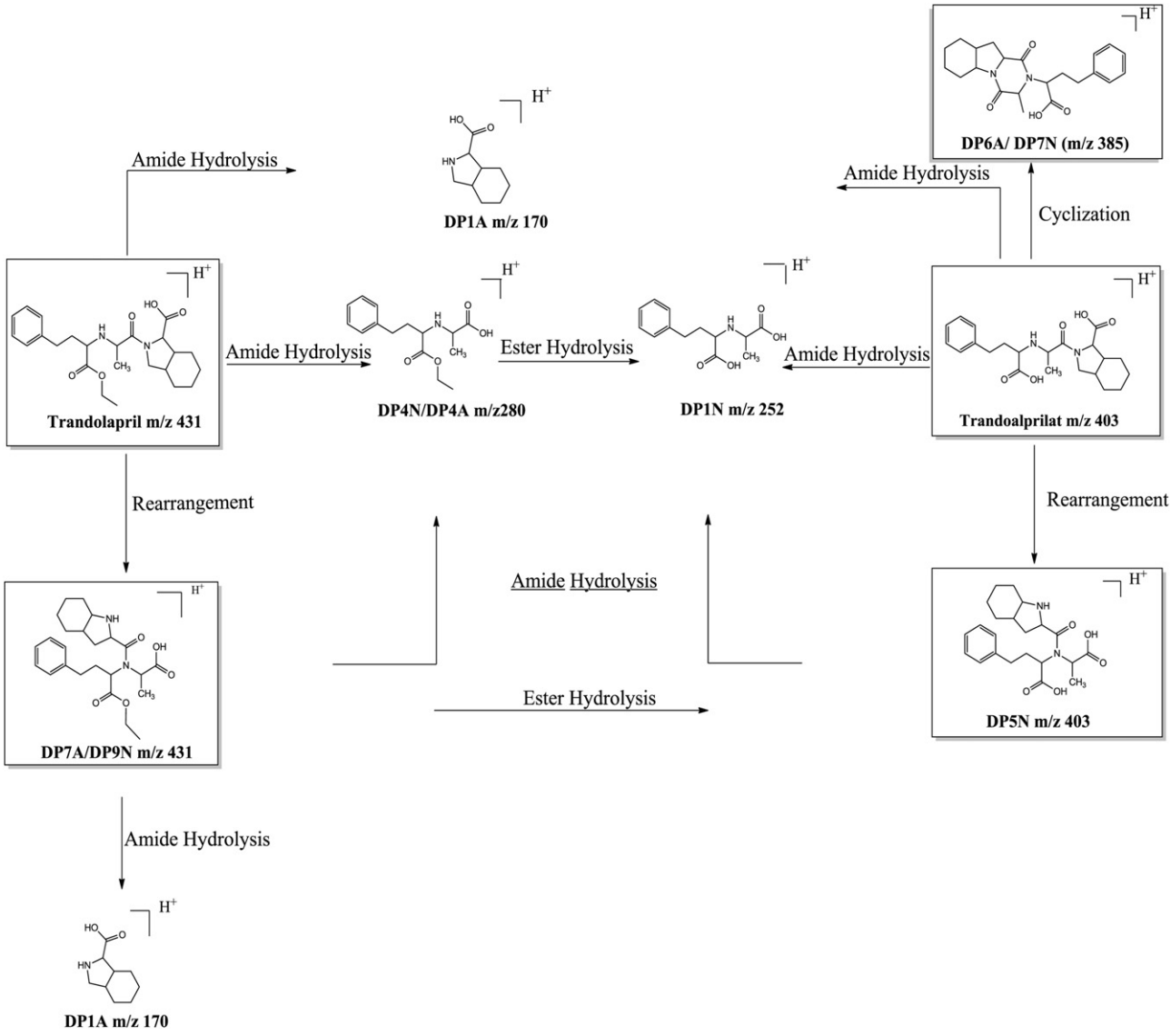

Fig. 6. A proposed pathway for the formation of degradation products of trandolapril.

of the bond between the carbon atom of the amide group and the α-carbon followed by elimination of carbon monoxide. These two products are characterized also by two pairs of analogous ions differing by *m*/*z* 28 (*m*/*z* 280/252) and (*m*/*z* 234/206); these sets belonged to fragment ions containing the ester (DP7A/DP9N: R = CH_3—CH_2) or the acid functionality (DP5N, R = H). These pairs of fragment ions are obtained by the cleavage of the amid bond (*m*/*z* 280/252), followed by further loss of formic acid (46 Da) to yield (*m*/*z* 234/206). As a result, fragmentation mechanisms can be suggested for DP7A/DP9N and DP5N formed by transamidation of trandolapril and trandolaprilat, respectively (Fig. 5).

In addition to the degradation product with *m*/*z* value 431, detected and identified in neutral medium, another degradation product with the same molecular mass ([M+H^+], *m*/*z* 431) was detected. The mass fragmentation for this compound eluted at a retention time of 2.64 min was almost the same to that of trandolapril, suggesting an identical structure. In fact, both compounds yielded the same product ions of *m*/*z* 357, 280, 234, 206, 170, 134, 130, 117 showing a possible epimerization of trandolapril. However, the potential formation of trandolapril isomer cannot be determined due to the lack of authentic isomers of these products.

3.3. Proposed degradation pathway of trandolapril

Several works have reported that trandolapril undergoes ester hydrolysis to give trandolaprilat (Gumieniczek and Hopkala, 2000). This paper demonstrates that others products are formed during trandolapril degradation both under acidic and neutral conditions. So a complete degradation pathway including the observed products is proposed (Fig. 6). Most of this mechanism is based on the hydrolysis of amide and ester functions, namely the most sensitive sites toward hydrolysis reaction. The various cleavage and consecutive hydrolysis reaction can explain the formation of DP1A, DP4N/DP4A and DP1N. In addition a new rearrangement mechanism has been described in order to explain the formation of DP7A/DP9N and DP5N from trandolapril and trandolaprilat, respectively. These new products may also decompose to form DP4A/DP4N, DP1N and DP1A via amide and/or ester hydrolysis. The degradation product DP6A/DP7N (diketopiperazine derivate) is formed by cyclization with a loss of water molecule from trandolaprilat. However, the analogous structure from cyclization of tandolapril has not been observed. Compared to other ACEI (enalapril maleate, xpril and perindopril) trandolapril cannot lead

to the formation of diketopiperazine product under acidic and neutral conditions.

4. Conclusion

Kinetic aspects of the degradation of trandolapril in neutral and acidic media have been developed in this study. Based on the kinetic results, it was concluded that like others ACEI, under acidic and neutral conditions trandolapril hydrolysis followed a first order kinetic.

In order to propose degradation pathways of trandolapril, a new analytical method for the rapid detection and structural characterization of degradation products under stressed conditions using two techniques chromatographic UPLC-DAD and UPLC–MS/MS has been investigated. This method proved to be convenient and effective since it provided fast and efficient separation of trandolapril from its degradation products. Structures of the detected degradation products were characterized on the basis of the mass shift from the drug, molecular formulae derived from the accurate mass measurements, and the interpretation of accurate MS^2 spectra.

These results showed the presence of common products detected in acidic and neutral media. Compared to some ACEI, such as enalpril maleate, xpril, and perindopril, trandolapril did not yield to the diketopiperazine product either in acidic or neutral media. The two main processes of degradation of trandolapril were rearrangement and hydrolysis of the ester function and the amide bond.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijpharm.2012.08.048.

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