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Polypill for the treatment of cardiovascular diseases Part 2. LC–MS/TOF characterization of interaction/degradation products of atenolol/lisinopril and aspirin, and mechanisms of formation thereof

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ABSTRACT

A polypill for cardiovascular diseases (CVD) is under development. It is proposed to contain a combination of antithrombotic agent (aspirin), low-dose blood pressure lowering agents, i.e., angiotensin-converting enzyme inhibitor (lisinopril), one among a β-blocker (atenolol) or diuretic (hydrochlorothiazide), and a statin (simvastatin/atorvastatin/pravatsatin, etc.). Due to the presence of multiple drugs in the same formulation, there is a strong likelihood of interaction among the drugs and/or their products. In a previous study, we observed formation of a number of interaction/degradation products from atenolol and lisinopril in the presence of aspirin. Accordingly, the purpose of this study was to characterize the resolved products using high resolution mass spectrometric and fragmentation analyses using a LC–MS/TOF system. Initially, studies were carried out on the drugs (atenolol, lisinopril and aspirin) to establish their complete fragmentation pattern. These studies were then extended to degraded samples to postulate the structures of interaction/degradation products. The characterized structures were justified through mechanistic explanations.

1. Introduction

The study of probable chemical incompatibilities and interactions amongst the drugs is considered to be a critical requirement during development of fixed-dose combinations (FDCs) [1–3]. In recent years, efforts are being made to develop a FDC for the management of cardiovascular diseases (CVDs) in the form of a 'polypill'. The same is proposed to contain a combination of an antithrombotic agent (aspirin), low-dose blood pressure lowering agents, i.e., angiotensin-converting enzyme inhibitor (lisinopril), one among a β-blocker (atenolol) or diuretic (hydrochlorothiazide), and a statin (simvastatin/atorvastatin/pravatsatin) [4]. Till date, there have been no reports in the literature on the possible interactions amongst the drugs being considered for addition to the polypill. Hence, studies directed to these were initiated in our laboratory.

In the first part of the study, which was published previously in this journal [5], we reported HPLC and LC–MS studies on various four-drug combinations stored under accelerated stability conditions for 3 months. In total, 17 products (10 interaction and 7 degradation products) were resolved in six different drug combinations. The products were classified in relation to atenolol, lisinopril and statins, based on their m/z values and comparison of major and common fragments with the drugs. The five products with m/z values of 309, 309, 351, 429 and 268 were related to atenolol, while five others with m/z values of 448, 448, 490, 388 and 430 were connected to lisinopril. The remaining seven were related to statins. It was indicated that all interaction products were formed typically in the presence of aspirin. It was also shown that hydrochlorothiazide was non-interacting and stable.

Overall, the interaction/degradation products, due to their multiplicity, were formed in relatively low amounts, between 0.2% and 3.6%. Therefore, efforts to characterize them were made using mass spectrometry, employing relative accurate mass and fragmentation analyses. The high resolution mass spectrometry (HRMS) studies, carried out using a LC–MS/TOF system, were extended to the drugs as well as the stability samples. The obtained experimental accurate mass values were used to generate molecular formula of the products, on which basis their structures were predicted. The postulated structures were justified through mechanisms of their formation. The characterization of 10 interaction/degradation products of atenolol/lisinopril and aspirin are described in this second paper in the series. Characterization of the remaining products (related to statins) will be reported in a future publication.

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2. Experimental

2.1. Chemicals and materials

Pure drugs were obtained as gift samples from Dr. Reddy's Laboratories Ltd., Hyderabad, India. HPLC grade acetonitrile was purchased from J.T. Baker (Mexico City, Mexico). Ultra pure water was obtained from a water purification unit (Elga Ltd., Bucks, England). Buffer materials and all other chemicals were of analytical-reagent grade.

2.2. Equipment

The LC–MS system consisted of an HPLC (1100 series, Agilent Technologies, Waldbronn, Germany) and MicrOTOF-Q mass spectrometer (Bruker Daltonics, Bremen, Germany) equipped with an ESI source (G-1948A). The LC part comprised of an on-line degasser (G1379A), binary pump (G131A), auto injector (G1313A), column oven (G1316A) and diode array detector (G1315B). The system was controlled by combination of Hyphenation Star (version 3.1) and MicrOTOF Control (version 2.0) software. In all studies, separations were achieved on a Discovery C-8 (250 mm × 4.6 mm i.d., 5 μm) column (Supelco, Bellefonte, PA, USA).

The samples were stored under accelerated conditions in a stability chamber (KBWF720, Binder, Tuttlingen, Germany) set at 40 ± 1 °C/75% RH ± 3% RH. Other equipment used were sonicator (Branson Ultra-sonic Corporation, Danbury, CT, USA), analytical balance (Mettler Toledo, Schwerzenbach, Switzerland) and auto pipettes (Eppendorf, Hamburg, Germany).

2.3. Degradation studies

Two-drug mixtures containing atenolol or lisinopril along with aspirin (50 mg each) were accurately weighed and transferred to 15 ml glass vials. The mixtures were thoroughly mixed using a spatula, and the open vials were exposed to accelerated conditions of temperature (40 °C) and humidity (75% RH) for 90 days to induce interaction and degradation amongst the drugs.

2.4. LC–MS/TOF studies

Pure drugs and the degraded samples were subjected to LC–MS/TOF studies using a previously reported LC–MS method [5]. MS analyses were performed in ESI positive and negative ionization modes in the mass range of 50–3000 amu. High purity nitrogen was used as the nebulizer and auxiliary gas. Mass parameters were optimized to the following values: hexapole Rf, 500.0 VPP; collision Rf, 200.0 VPP; pre-pulse storage, 4.0 μs; collision energy, 10.0 eV/Z; quadrupole ion energy, 5.0 eV/Z; nebulizer gas pressure, 1.2 bar; dry gas flow rate, 8.0 l min^{-1} and dry temperature, 200 °C. In some cases, quadrupole parameters were changed to get a more complete fragmentation pattern.

3. Results and discussion

3.1. LC–MS/TOF analyses of pure drugs

The line spectra for pure drugs obtained from LC–MS/TOF studies are shown in Fig. 1. The high-resolution mass spectrometry (HRMS) data of both molecular ion peaks and fragments are included in Table 1.

It is evident from Fig. 1a that the molecular ion peak $(M+H)^+$ of atenolol in positive ion mode appeared at *m*/*z* 267, while the fragments showed up at *m*/*z* 249, 225, 207, 190 and 145. An almost similar mass and fragmentation profile was observed by Johnson and Lewis [6]. It was postulated that fragment of *m*/*z* 249 arose from the loss of water from the drug; while the loss of propylamine moiety resulted in a fragment of *m*/*z* 190, which was converted further to fragment of *m*/*z* 145. The fragment of *m*/*z* 225 resulted from parallel loss of propene moiety from atenolol. The proposed fragmentation profile is drawn in Scheme 1.

Fig. 1b shows that $[M+H]^+$ parent ion peak for lisinopril was observed at anticipated *m*/*z* of 406, with fragments having *m*/*z*

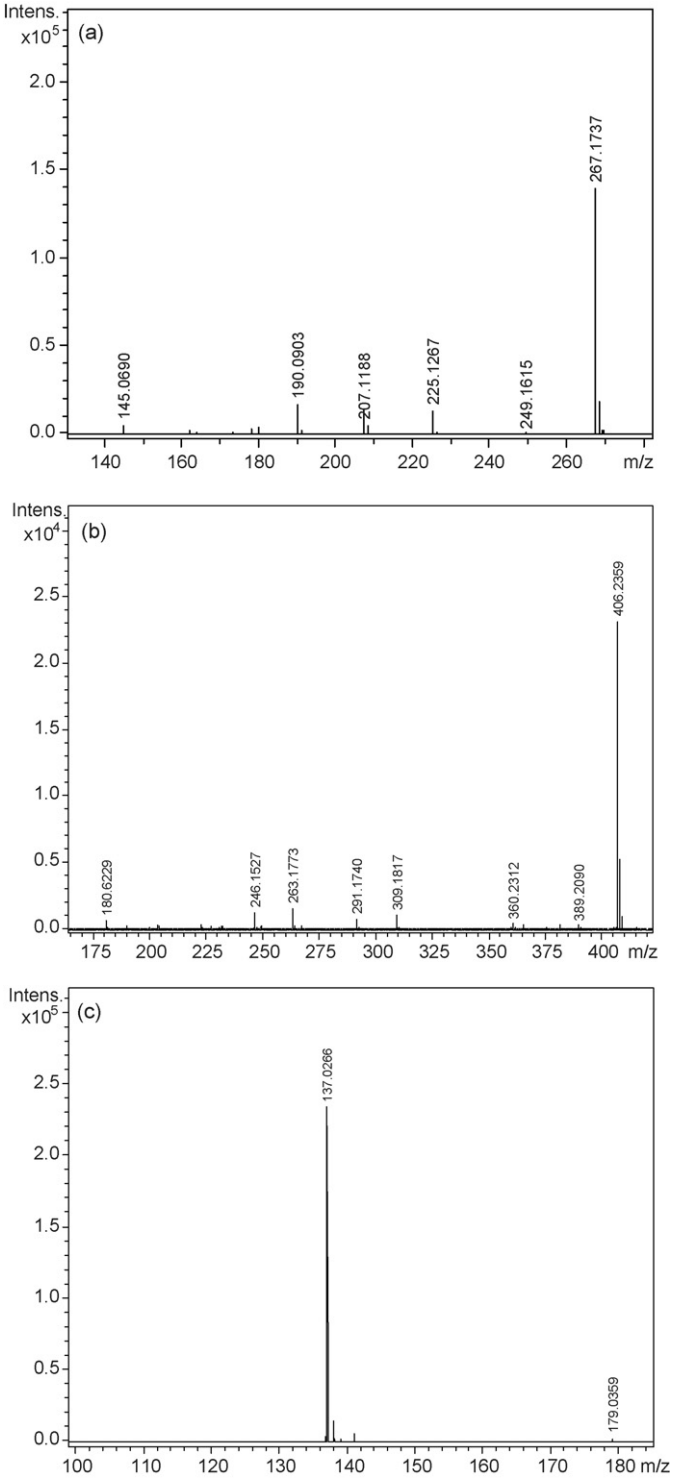
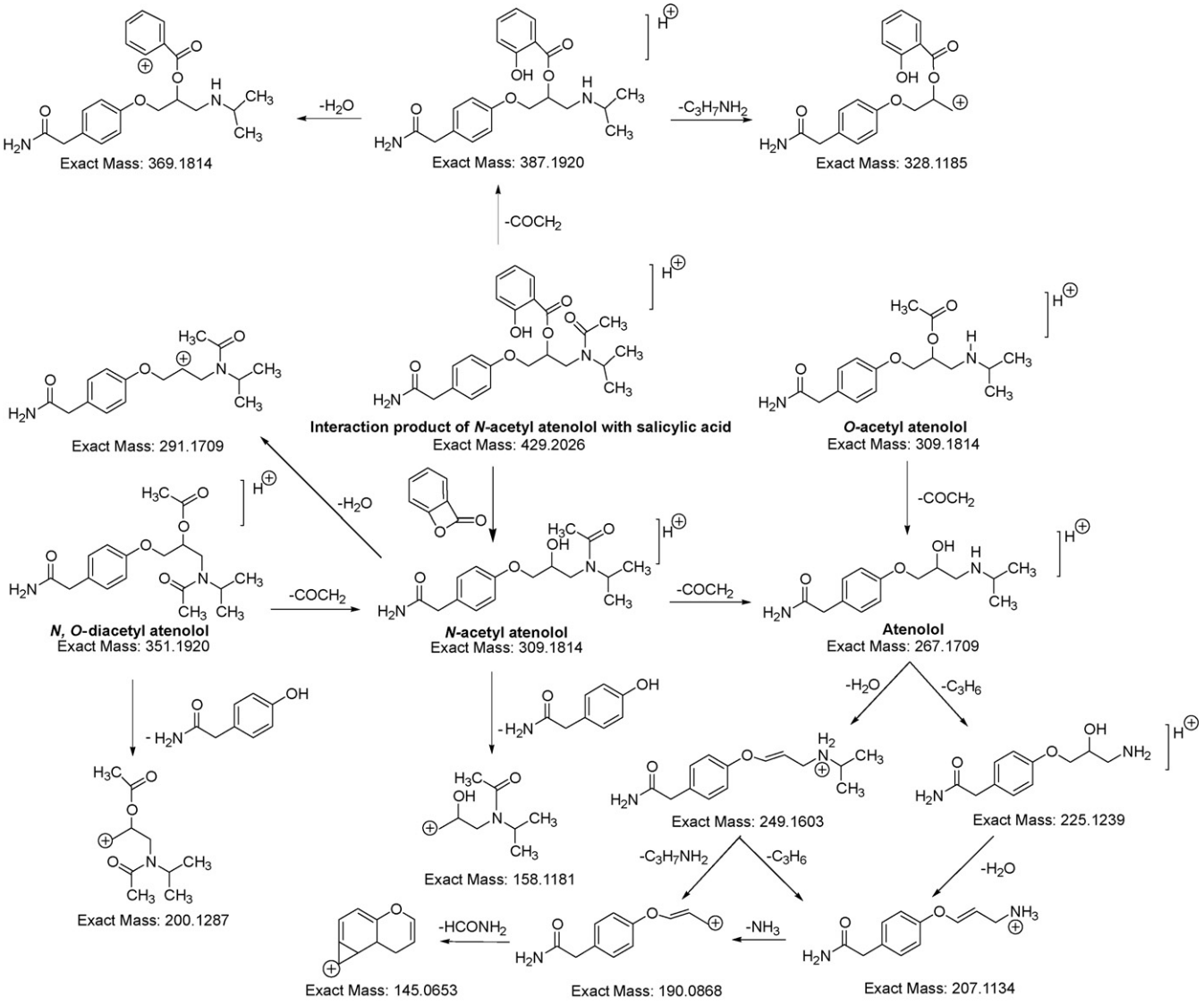


Fig. 1. Line spectra of atenolol (a), lisinopril (b) in positive ESI mode and aspirin (c) in negative ESI mode.

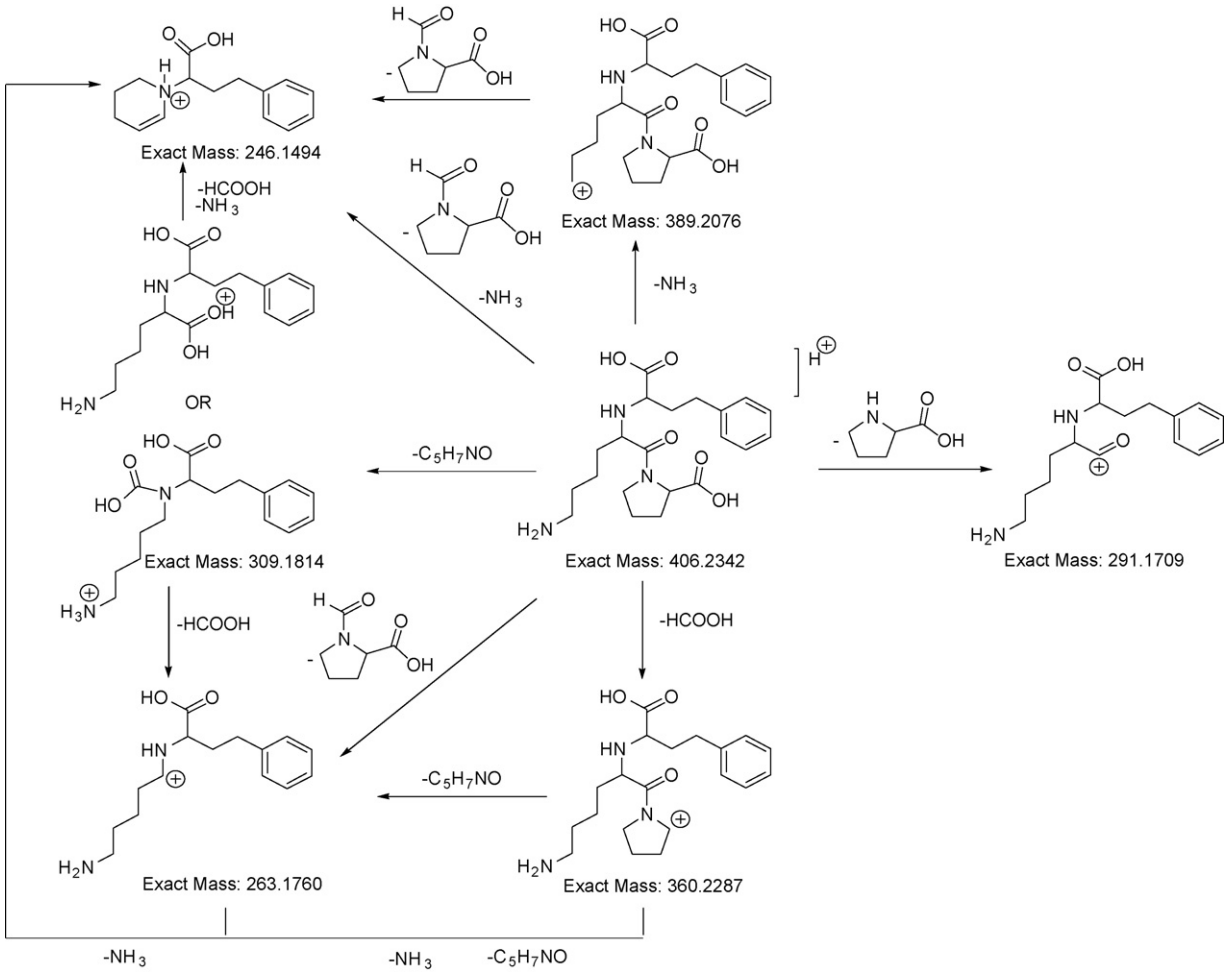
Table 1
HRMS data for molecular ion peaks and fragments of atenolol, lisinopril and aspirin

Name of drug	Molecular ion peak/fragment	Observed mass	Theoretical mass	Error (D)
Atenolol	$[M+H]^+$	267.1737	267.1709	0.0028
	$[M+H]^+$-H_2O	249.1615	249.1603	0.0012
	$[M+H]^+$-C_3H_6	225.1267	225.1239	0.0028
	249-C_3H_6, 225-H_2O	207.1188	207.1134	0.0054
	249-$C_3H_7NH_2$, 207-NH_3	190.0903	190.0868	0.0035
	190-$HCONH_2$	145.0690	145.0653	0.0037
Lisinopril	$[M+H]^+$	406.2359	406.2342	0.0017
	$[M+H]^+$-NH_3	389.2090	389.2076	0.0014
	$[M+H]^+$-HCOOH	360.2312	360.2287	0.0025
	$[M+H]^+$-C_5H_7NO	309.1817	309.1814	0.0003
	$[M+H]^+$-Proline	291.1740	291.1709	0.0031
	$[M+H]^+$-*N*-formylproline, 360-C_5H_7NO, 309-HCOOH	263.1773	263.1760	0.0013
	$[M+H]^+$-(*N*-formylproline + NH_3), 389-*N*-formylproline, 360-(C_5H_7NO + NH_3), 309-(HCOOH + NH_3), 263-NH_3	246.1527	246.1494	0.0033
Aspirin	$[M-H]^-$	179.0359	179.0344	0.0015
	$[M-H]$-$COCH_2$	137.0266	137.0239	0.0027

of 389, 360, 309, 291, 263 and 246. In this case, the fragmentation pattern was similar to that reported by Burinsky and Sides [7]. The authors indicated that most of the fragments were formed directly from the drug, and broke further to fragments of lower m/z values. The moieties lost were formic acid, ammonia, proline and *N*-formylproline, except in one case, where loss of dihydropyrrole and carbon monoxide was observed. The fragmentation profile in this case is proposed in Scheme 2.



Scheme 1. Fragmentation pattern of atenolol and its interaction products.



Scheme 2. Fragmentation pattern of lisinopril.

The parent ion peak for aspirin was recorded in negative ion mode, as the same did not appear in positive mode. As shown in Fig. 1c, $[M-H]^-$ peak appeared at m/z 179, along with a single fragment at m/z 137. The fragment, corresponding to salicylic acid, resulted from loss of ketene moiety from the drug molecule.

3.2. LC–MS/TOF studies on interaction/degradation products

The line spectra of interaction/degradation products of atenolol and lisinopril are shown in Figs. 2 and 3, respectively. The corresponding HRMS data are included in Tables 2 and 3, respectively.

3.2.1. Characterization of atenolol related products

Fig. 2 shows that $[M+H]^+$ peaks of the five interaction/degradation products of atenolol in the presence of aspirin appeared at m/z values of 309, 309, 351, 429 and 268. It is clear from the comparison of Fig. 2a and b that the two products had the same mass of 309, but somewhat different fragmentation pattern, indicating them to be non-stereo isomeric products. In both the cases, the mass value was 42 amu higher than that of the drug, which indicated possibility of acetylation of the molecule. The fragmentation pattern of the first among the two (Fig. 2a) showed characteristic peak at m/z 291 (loss of water), which was absent in the second (Fig. 2b). Considering the overall fragmentation pattern (Scheme 1), the first was postulated to be *N*-acetyl atenolol, while the second of equal mass was proposed to be *O*-acetyl atenolol. The same was justified because loss of water to a fragment of 291 was possible in the first and not in the latter due to acetylation at the same site. A typical fragment of m/z 158 observed in mass chromatogram of *N*-acetyl atenolol was explained through loss of 2-(4-hydroxy-phenyl)-acetamide, which was not seen in the case of *O*-acetyl atenolol. The third product (Fig. 2c) with m/z of 351 and a mass of 84 amu higher than atenolol was indicated to have two acetyl groups, probably involving both *N*- and *O*-sites. This product, *N*,*O*-diacetyl atenolol, was proposed to lose a ketene moiety at the *O*-site to yield *N*-acetyl atenolol (m/z 309), which further lost water to generate a fragment of m/z 291. The fragment of m/z 200 was generated through parallel fragmentation of the product involving loss of 2-(4-hydroxy-phenyl)-acetamide, similar to *N*-acetyl atenolol (Scheme 1).

The fourth product with m/z value of 429 (Fig. 2d) could be proposed as an interaction product of *N*-acetyl atenolol and salicylic acid (Scheme 1). The same is supported by the observation that during mass ionization, the product lost 2H-benzo[*b*]oxet-2-one to yield *N*-acetyl atenolol (m/z 309), which is sustained by the presence of typical daughter fragment of m/z 291 observed in the mass spectrum of the latter (Fig. 2a). Also, the remaining fragments of m/z 267 (atenolol), 249, 225, 207 and 190 were similar to those shown in mass spectra of *N*-acetyl atenolol (Fig. 2a). The product simultaneously lost ketene moiety, parallel to conversion of *N*-acetyl atenolol to atenolol, to form a fragment of m/z 387, which further lost water and propylamine to yield fragments of m/z 369 and 328, respectively (Fig. 2d; Scheme 1).

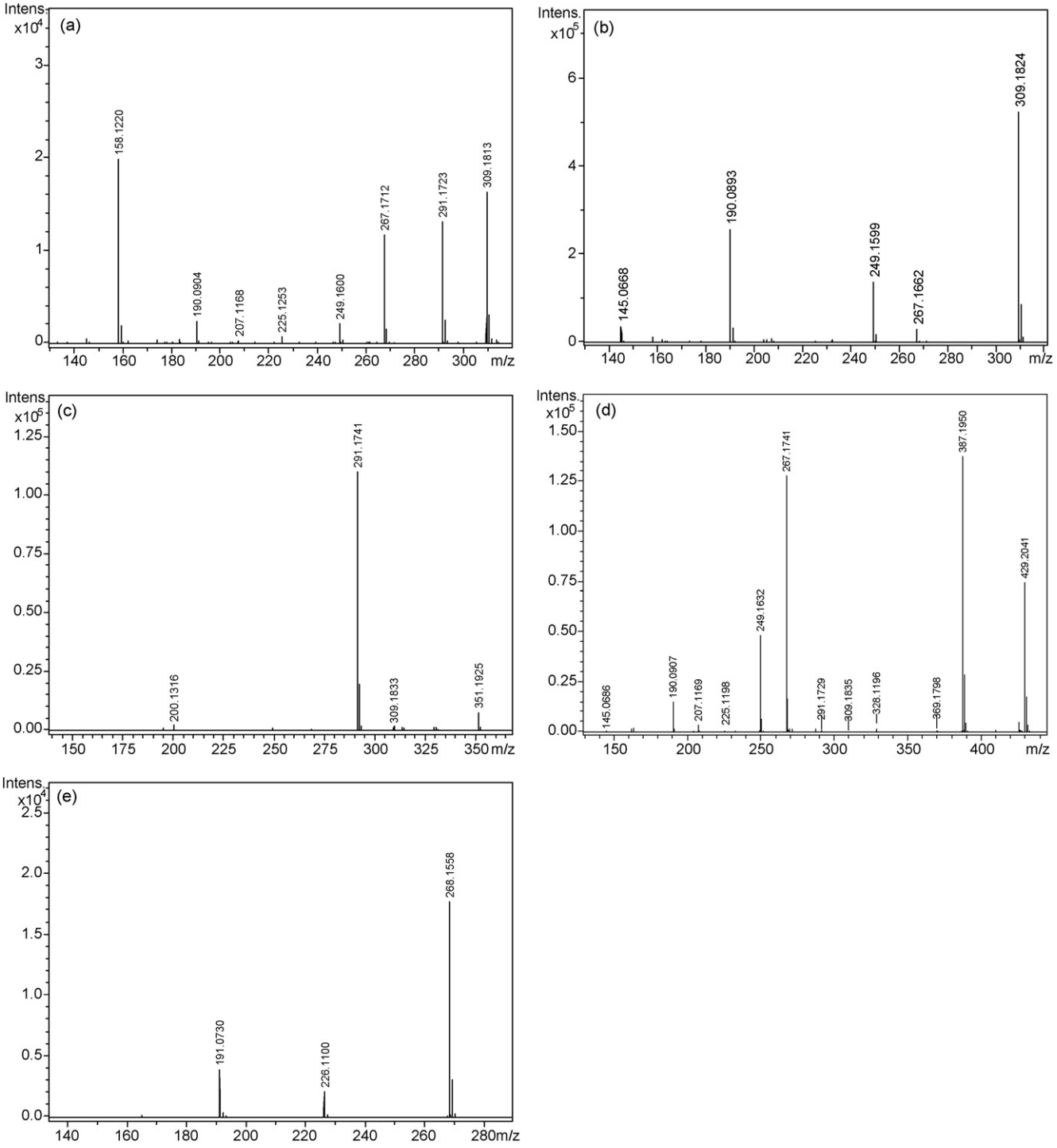


Fig. 2. Line spectra of interaction/degradation products of atenolol with *m*/*z* of 309 (a), 309 (b), 351 (c), 429 (d) and 268 (e) in positive ESI mode.

The fifth product with *m*/*z* 268 had a mass value one unit higher than that of the drug. The same was true even for its fragments, which otherwise were similar to the drug (Fig. 1a *versus* Fig. 2e). Based on the list provided in the European Pharmacopoeia (EP) [8], the product was indicated to be impurity G of atenolol. Its fragments of *m*/*z* 226 and 191 originated from the loss of propene, and combination of water and propylamine, respectively (Scheme 3).

The data in Table 2 clearly show that the error for difference between theoretical and experimental mass values of molecular ion peaks was <5.0 ppm in all cases, thus supporting the structures of postulated interaction/degradation products of atenolol.

3.2.2. *Characterization of lisinopril related products*

The five interaction/degradation products of lisinopril had *m*/*z* of 448, 448, 490, 388 and 430 (Fig. 3). Evidently, at least two products (Figs. 3a and b) had the same mass, higher by 42 amu than the drug. Also, a product with *m*/*z* 490 had a higher mass by 84 amu (Fig. 3c). These three had a parallel behaviour to atenolol, indicating that the first two products were perhaps monoacetylated drug

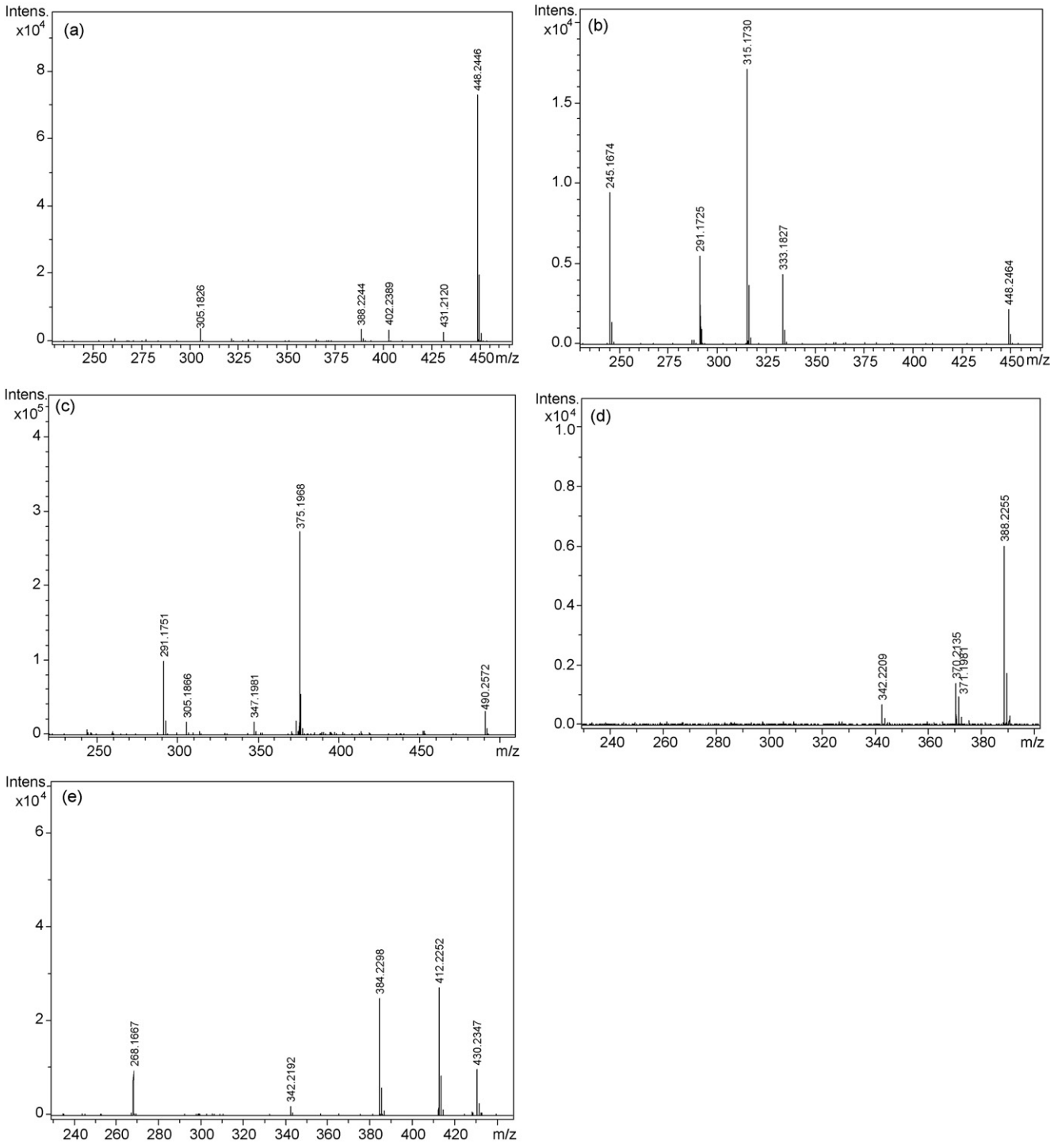


Fig. 3. Line spectra of interaction/degradation products of lisinopril with *m*/*z* of 448 (a), 448 (b), 490 (c), 388 (d) and 430 (e) in positive ESI mode.

derivatives, while the third was diacetylated lisinopril. To assign the structures to these three products, their fragmentation behaviour was compared to the drug (Scheme 2).

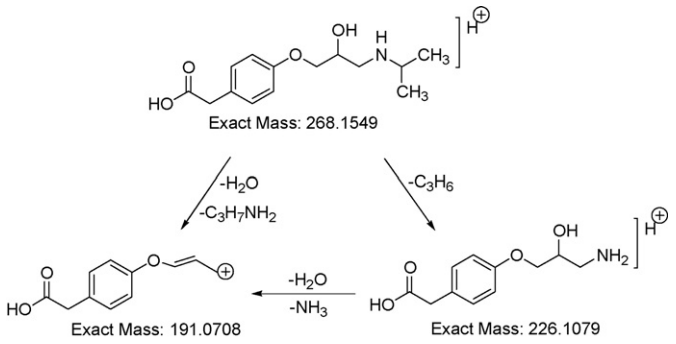
In case of the first product with *m*/*z* 448, the fragments observed were *m*/*z* 431, 402, 388 and 305 (Fig. 3a). The presence of a fragment of *m*/*z* 431, which was less by 17 amu than the product mass and could be attributed to the loss of ammonia, indicated presence of a free amine group in the molecule. Here possibility existed of two products satisfying the fragmentation pattern, *viz*., *N*-acetyl lisinopril (acetylation at secondary amine) and a carboxanhydride derivative at the carboxyl group. Because carboxanhydrides are unstable and their formation is unusual [9], this possibility was disregarded. Thus, the remaining masses of *m*/*z* 402, 388 and 305 were purported to arise from *N*-acetyl lisinopril. It lost formic acid from proline moiety, following the same pathway as the drug (Scheme 2), to yield a fragment of *m*/*z* 402 (Scheme 4). The latter further lost dihydropyrrole and carbon monoxide moieties to give the fragment of *m*/*z* 305. An alternate route for the same was the loss of *N*-formylproline directly from *N*-acetyl lisinopril. The fragment of *m*/*z* 388 was generated

Table 2
HRMS data for molecular ion peaks and fragments of degradation/interaction products of atenolol

Name of interaction/degradation product	Molecular ion peak/fragment	Observed mass	Theoretical mass	Error (*D*)	Error (ppm)
N-Acetyl atenolol	$[M+H]^+$	309.1813	309.1814	0.0001	−0.32
	$[M+H]^+$-H_2O	291.1723	291.1709	0.0014	4.81
	$[M+H]^+$-$COCH_2$	267.1712	267.1709	0.0003	1.12
	267-H_2O	249.1600	249.1603	−0.0003	−1.20
	267-C_3H_6	225.1253	225.1239	0.0014	6.22
	249-C_3H_6, 225-H_2O	207.1168	207.1134	0.0034	16.42
	249-$C_3H_7NH_2$, 207-NH_3	190.0904	190.0868	0.0036	18.94
	$[M+H]^+$-2-(4-Hydroxy-phenyl)-acetamide	158.1220	158.1181	0.0039	24.67
O-Acetyl atenolol	$[M+H]^+$	309.1824	309.1814	0.0010	3.23
	$[M+H]^+$-$COCH_2$	267.1662	267.1709	−0.0047	−17.59
	267-H_2O	249.1599	249.1603	−0.0004	−1.61
	249-$C_3H_7NH_2$	190.0893	190.0868	0.0025	13.15
	190-$HCONH_2$	145.0668	145.0653	0.0015	10.34
N,*O*-Di-acetyl atenolol	$[M+H]^+$	351.1925	351.1920	0.0005	1.42
	$[M+H]^+$-$COCH_2$	309.1833	309.1814	0.0019	6.15
	309-H_2O	291.1741	291.1709	0.0032	10.99
	$[M+H]^+$-2-(4-Hydroxy-phenyl)-acetamide	200.1316	200.1287	0.0029	14.49
Interaction product of *N*-acetyl atenolol with salicylic acid	$[M+H]^+$	429.2041	429.2026	0.0015	3.49
	$[M+H]^+$-$COCH_2$	387.1950	387.1920	0.0030	7.75
	387-H_2O	369.1798	369.1814	−0.0016	−4.33
	387-$C_3H_7NH_2$	328.1196	328.1185	0.0011	3.35
	$[M+H]^+$-$C_7H_4O_2$	309.1835	309.1814	0.0021	6.79
	309-H_2O	291.1729	291.1709	0.0020	6.87
	309-$COCH_2$	267.1741	267.1709	0.0032	11.98
	267-H_2O	249.1632	249.1603	0.0029	11.64
	267-C_3H_6	225.1198	225.1239	−0.0041	−18.21
	249-C_3H_6, 225-H_2O	207.1169	207.1134	0.0035	16.90
	249-$C_3H_7NH_2$, 207-NH_3	190.0907	190.0868	0.0039	20.52
	190-$HCONH_2$	145.0686	145.0653	0.0033	22.74
EP impurity G of atenolol	$[M+H]^+$	268.1558	268.1549	0.0009	3.36
	$[M+H]^+$-C_3H_6	226.1100	226.1079	0.0021	9.29
	$[M+H]^+$-($H_2O + C_3H_7NH_2$), 226-($H_2O + NH_3$)	191.0730	191.0708	0.0022	11.51

Table 3
HRMS data for molecular ion peaks and fragments of degradation/interaction products of lisinopril

Name of interaction/degradation product	Molecular ion peak/fragment	Observed mass	Theoretical mass	Error (*D*)	Error (ppm)
N-Acetyl lisinopril	$[M+H]^+$	448.2446	448.2448	−0.0002	−0.45
	$[M+H]^+$-NH_3	431.2120	431.2182	−0.0062	−14.38
	$[M+H]^+$-HCOOH	402.2389	402.2393	−0.0004	−0.99
	$[M+H]^+$-($COCH_2 + H_2O$)	388.2244	388.2236	0.0008	2.06
	$[M+H]^+$-*N*-formylproline, 402-C_5H_7NO	305.1826	305.1865	−0.0039	−12.78
N′-Acetyl lisinopril	$[M+H]^+$	448.2464	448.2448	0.0016	3.57
	$[M+H]^+$-Proline	333.1827	333.1814	0.0013	3.90
	333-H_2O	315.1730	315.1709	0.0021	6.66
	333-$COCH_2$	291.1725	291.1709	0.0016	5.50
	291-HCOOH	245.1674	245.1654	0.0020	8.16
N,*N*′-Diacetyl lisinopril	$[M+H]^+$	490.2572	490.2553	0.0019	3.88
	$[M+H]^+$-Proline	375.1968	375.1920	0.0048	12.79
	$[M+H]^+$-*N*-formylproline	347.1981	347.1971	0.0010	2.88
	347-$COCH_2$	305.1866	305.1865	0.0001	0.33
	375-2($COCH_2$)	291.1751	291.1709	0.0042	14.42
Diketopiperazine of lisinopril	$[M+H]^+$	388.2255	388.2236	0.0019	4.89
	$[M+H]^+$-NH_3	371.1981	371.1971	0.0010	2.69
	$[M+H]^+$-H_2O	370.2135	370.2131	0.0002	1.08
	370-CO	342.2209	342.2182	0.0028	7.89
N-Acetyl diketopiperazine of lisinopril	$[M+H]^+$	430.2347	430.2342	0.0005	1.16
	$[M+H]^+$-H_2O	412.2252	412.2236	0.0016	3.88
	412-CO	384.2298	384.2287	0.0011	2.86
	384-$COCH_2$	342.2192	342.2182	0.0010	2.92
	$[M+H]^+$-4-phenyl-but-2-enoic acid	268.1667	268.1661	0.0006	2.24

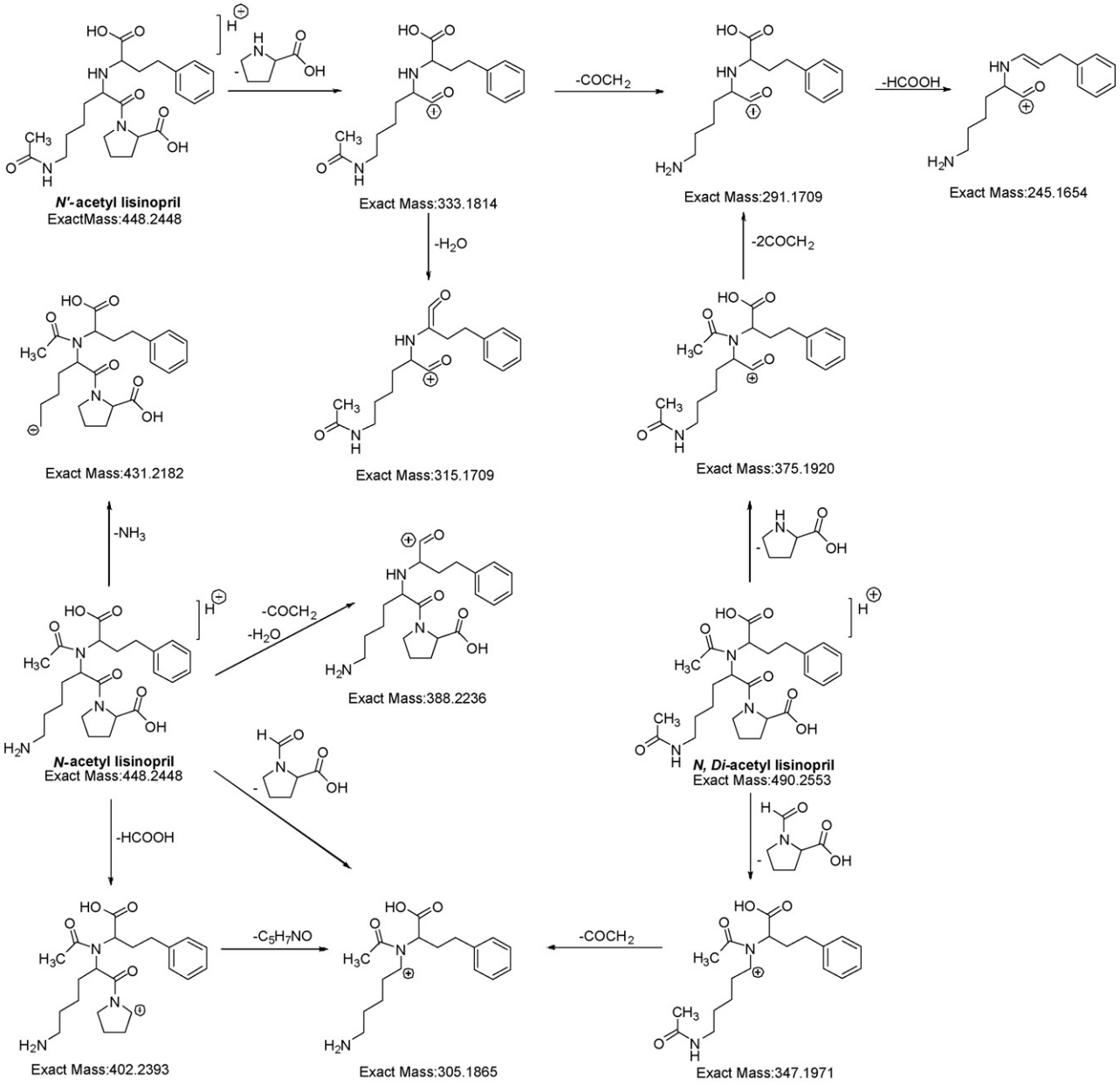


Scheme 3. Fragmentation pattern of EP impurity G of atenolol.

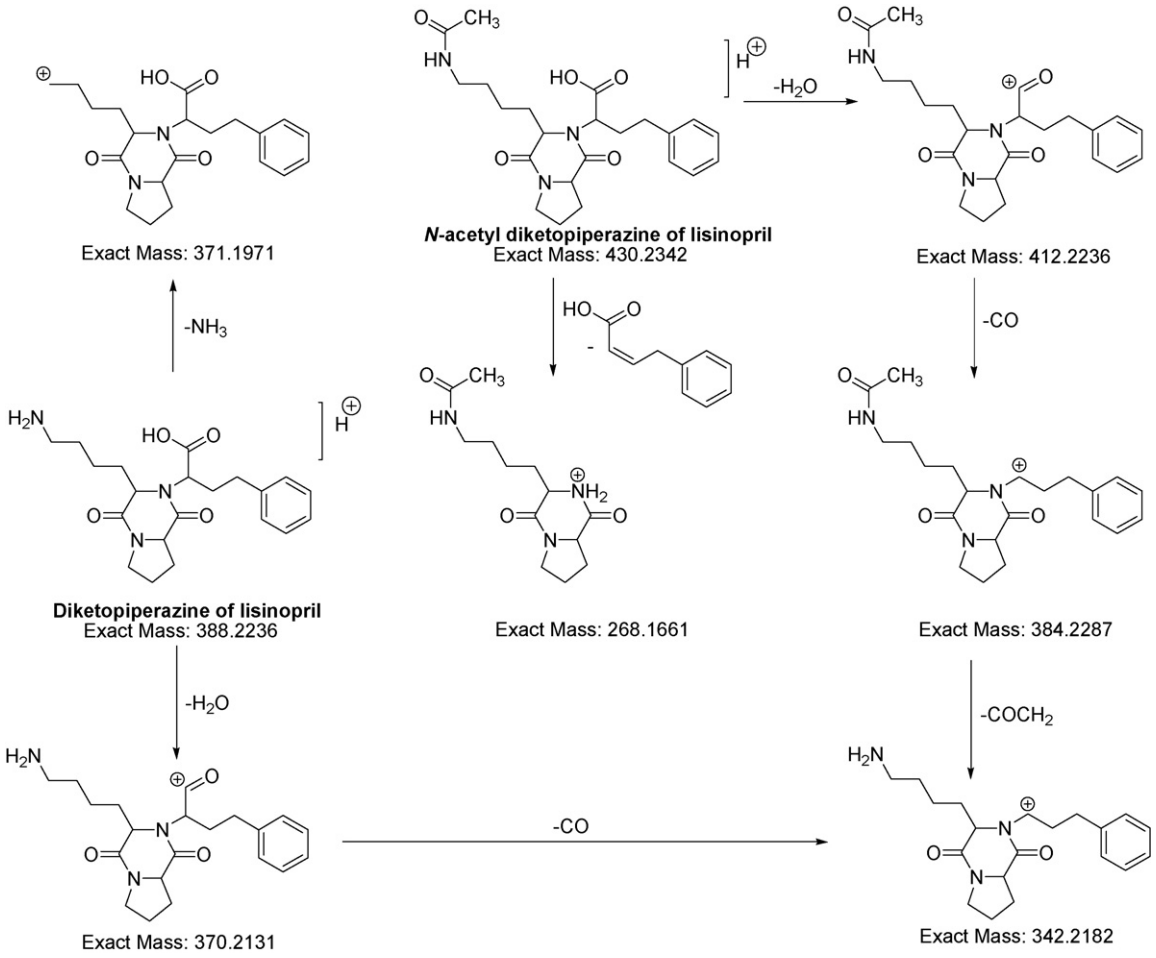
directly again from *N*-acetyl lisinopril upon loss of water and ketene together.

As evident in Fig. 3b, the second product, despite the same mass, showed an altogether different fragmentation pattern to the first (Fig. 3a). The fragments observed were at *m*/*z* 333, 315, 291 and 245. In this case, no fragment was observed at *m*/*z* 431, indicating the absence of free amine in the product. Hence, the product was postulated to be *N*′-acetyl lisinopril (acetylation at primary amine), which was justified even by its fragmentation pathway (Scheme 4). The fragment of *m*/*z* 333 was proposed to be formed from loss of proline moiety, which subsequently lost water and ketene to yield fragments of *m*/*z* 315 and 291, respectively. The latter further lost formic acid to generate the fragment ion of *m*/*z* 245.

The third, diacetylated product of lisinopril with *m*/*z* of 490 (Fig. 3c) and 84 amu higher than the drug, was perhaps acetylated at both *N*- and *N*′-sites. Its fragments appeared at *m*/*z* 375, 347, 305 and 291. Similar to *N*-acetyl lisinopril, the diacetylated product lost



Scheme 4. Fragmentation patterns of *N*-acetyl, *N*′-acetyl and Di-acetyl lisinopril.



Scheme 5. Fragmentation pattern of diketopiperazine and *N*-acetyl diketopiperzine of lisinopril.

N-formylproline to yield the fragment of *m*/*z* 347, thus supporting the involvement of secondary amine group in the acetylation (Scheme 4). The latter further lost ketene to yield the fragment of *m*/*z* 305. The diacetylated product, in parallel to *N*′-acetyl lisinopril, lost proline moiety to yield a fragment of *m*/*z* 375, which was further converted to fragment of *m*/*z* 291 through simultaneous loss of two ketene moieties. In this case also, no fragment was seen due to loss of ammonia, thus confirming the involvement of amine group in the acetylation.

The fourth product with *m*/*z* 388 (Fig. 3d) had a mass of 18 amu less than that of lisinopril. This indicated that the drug underwent dehydrative cyclization to form diketopiperazine derivative, which is a known degradation product of the drug [10–12]. The structure of diketopiperazine product was supported by its mass fragmentation pattern (Scheme 5). The compound lost ammonia to form a fragment with *m*/*z* value of 371. It also lost water to yield a fragment of *m*/*z* 370, which subsequently converted to fragment of *m*/*z* 342 on loss of carbon monoxide.

The fifth product (*m*/*z* 430, Fig. 3e) had a mass of 42 amu higher than the above-mentioned diketopiperazine product. Hence, it was indicated to be *N*-acetyl diketopiperazine derivative of lisinopril. The contention was supported by the absence of the peak due to loss of ammonia (17 amu), indicating substitution at the amine group. Scheme 5 shows that the product lost water to form fragment of *m*/*z* 412, which further lost carbon monoxide to yield fragment of *m*/*z* 384. The latter lost a ketene to form ion of *m*/*z* 342. The fragment of *m*/*z* 268 was formed from the product on loss of 4-phenyl-but-2-enoic acid.

In case of lisinopril interaction/degradation products also, the error for difference between theoretical and experimental mass values of molecular ion peaks was <5.0 ppm (Table 3), thus providing support to the envisaged structures.

3.3. Postulated mechanisms for the formation of interaction/degradation products

As discussed above, multiple interaction/degradation products of atenolol/lisinopril were formed in the presence of aspirin on exposure of drug combinations to accelerated stability conditions. This was different to aspirin alone, which showed no decomposition and remained stable (unpublished result). It was hence proposed that in the drug mixture, nucleophilic *N*- and/or *O*-centers of atenolol and *N*- and/or *N*′-centers of lisinopril attacked aspirin at electro-deficient carbonyl carbon to yield *N*-acetyl, *O*-acetyl, *N*′-acetyl and diacetyl products, releasing salicylic acid in the process. The free salicylic acid in turn esterified 2-OH of *N*-acetyl atenolol to yield a salicylated derivative (fourth atenolol product). The mechanism justified the absence of formation of similar derivatives in other products. The same was not possible with *O*-acetyl atenolol, as there was no free −OH group. The possible formation of amide of salicylic acid with *O*-acetyl atenolol was also ruled out due to apparent steric hindrance by the isopropyl moiety. Even, salicylic acid did not react with acetyl derivatives of lisinopril because of the absence of −OH group.

On the other hand, salicylic acid supposedly created an acidic microenvironment in the solid mixture because of its low pK_a of 3.0 [13]. It helped in the formation of EP impurity G of atenolol through SN^1 mechanism, involving amide hydrolysis of the drug in the acidic microenvironment.

The formation of diketopiperazine product of lisinopril was envisaged to occur through a known mechanism involving dehydrative cyclization [10–12]. The *N*-acetyl product of the same was then produced through simple acetylation.

4. Conclusions

The interaction/degradation products formed in case of atenolol and lisinopril in the presence of aspirin were characterized by employing LC–MS/TOF studies. The structures were delineated by comparison of fragmentation patterns of products with the drugs, and accurate mass analyses. Of five products each, three interaction products of atenolol were identified as *N*-acetyl, *O*-acetyl and *N*,*O*-diacetyl derivatives, and similar three of lisinopril were *N*-acetyl, *N*′-acetyl and *N*,*N*′-diacetyl derivatives. The remaining two products in atenolol were characterized as a salicylic acid derivative of *N*-acetyl atenolol and impurity G listed in EP [8]. Similarly, two other products of lisinopril were identified to be diketopiperazine and its *N*-acetyl derivative. The characterization of all the products was supported by the mechanism of their generation.

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