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Compatibility of atenolol with excipients: LC–MS/TOF characterization of degradation/interaction products, and mechanisms of their formation

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ABSTRACT

A study was carried out to investigate compatibility of atenolol, a β_1 blocker, with a variety of pharmaceutical excipients. The binary mixtures (1:1) of atenolol with the excipients were stored for 1 month at 40 °C/75% RH. The samples were directly observed for the physical changes, and also analyzed by a validated HPLC method to determine the chemical changes. The study revealed that atenolol was incompatible with ascorbic acid, citric acid and butylated hydroxyanisole. The degradation/interaction products formed in these mixtures were characterized by high resolution mass spectrometric and fragmentation analyses, using a LC–MS/TOF system. The identity of characterized structures was justified through mechanistic explanations.

1. Introduction

Drug–excipient compatibility studies play an important role in the development of pharmaceutical dosage forms. The incompatibility of a drug with one or more excipients in a formulation can alter the stability and/or bioavailability behavior of the active, thereby affecting its safety and/or efficacy [1]. The changes can be both physical and chemical. In literature, two types of chemical incompatibilities have been described: (i) excipient promoted intrinsic degradation of the drug, such as hydrolysis or oxidation, and/or (ii) covalent reaction between the drug and the excipient [2,3].

In the present study, the compatibility of atenolol was evaluated with pharmaceutical excipients of potential use in fixed-dose combinations being developed for cardiovascular diseases. The reason for the focus on atenolol was its facile reactivity shown during drug–drug interaction studies carried out previously in our laboratory [4]. The drug exhibited severe interaction even with some excipients. The degradation/interaction products formed were separated on a HPLC column and characterized by using liquid chromatography–mass spectrometry/time-of-flight (LC–MS/TOF), employing relative accurate mass, molecular formula, rings plus double bonds (RDB) and fragmentation analyses. The postulated structures were justified through mechanisms of their formation. The results are reported in this paper.

2. Experimental

2.1. Chemicals and materials

Pure drug and excipients 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 HPLC system used for analyses of drug–excipient mixtures consisted of an on-line degasser (DGU-14A), low-pressure gradient flow control valve (FCV-10ALVP), solvent delivery module (LC-10ATVP), auto injector (SIL-10ADVP), column oven (CTO-10ASVP), UV–visible dual-wavelength detector (SPD-10AVP), photo-diode array (PDA) detector (SPD-M10AVP), system controller (SCL-10AVP) and CLASS-VP software (all from Shimadzu, Kyoto, Japan). The LC–MS system used for mass studies 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),

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auto injector (G1313A), column oven (G1316A) and PDA (G1315B). The system was controlled by combination of Hyphenation Star (version 3.1) and MicrOTOF Control (version 2.0) software. In all the studies, separations were achieved on a Discovery C-8 (250 mm × 4.6 mm i.d., particle size 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 °C ± 1 °C/75% RH ± 3% RH. Other equipments used were sonicator (Branson Ultra-sonic Corporation, Danbury, CT, USA), analytical balance (Mettler Toledo, Schwerzenbach, Switzerland), pH/ion analyzer (Mettler Toledo, Schwerzenbach, Switzerland) and auto pipettes (Eppendorf, Hamburg, Germany).

2.3. Degradation studies

The drug–excipient mixtures were prepared in a ratio of 1:1 to accelerate the interaction process. 50 mg each of drug and excipients were accurately weighed and transferred to 15 ml glass vials in triplicate. The mixtures were thoroughly mixed using a capillary tube, which was broken inside the vial. The open vials were exposed to ICH recommended accelerated stability test conditions of 40 °C and 75% RH for 1 month. The vials were weighed initially and after 1 month to calculate the increase in weight (due to absorbed water) during the testing period. Parallel studies were also done on pure drug.

2.4. Physical changes and chemical analyses by HPLC

The samples were observed visually for any physical changes and also analyzed by a validated HPLC method to determine chemical changes [5]. The contents were dissolved in 50 ml methanol and filtered through 0.22 μ nylon filter before HPLC injection.

2.5. LC–MS/TOF studies

The drug–excipient mixtures, which showed severe interactions, were subjected to LC–MS/TOF studies using a reported LC–MS method [5]. MS analyses were performed in ESI positive 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: 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.

2.6. Estimation of micro-environmental pH

Micro-environmental pH of the drug–excipient blends showing incompatibility was determined by the method given by Serajuddin et al. [6]. For this, 0.5 ml water was added to the blend in a vial, the resultant suspension was mixed on a vortex mixer, followed by recording of the pH.

3. Results and discussion

3.1. Physical and chemical changes

Pure atenolol was almost stable on storage for 1 month under accelerated stability test conditions. There was no physical change, and chemical change was also very small (∼1.5%). The decrease

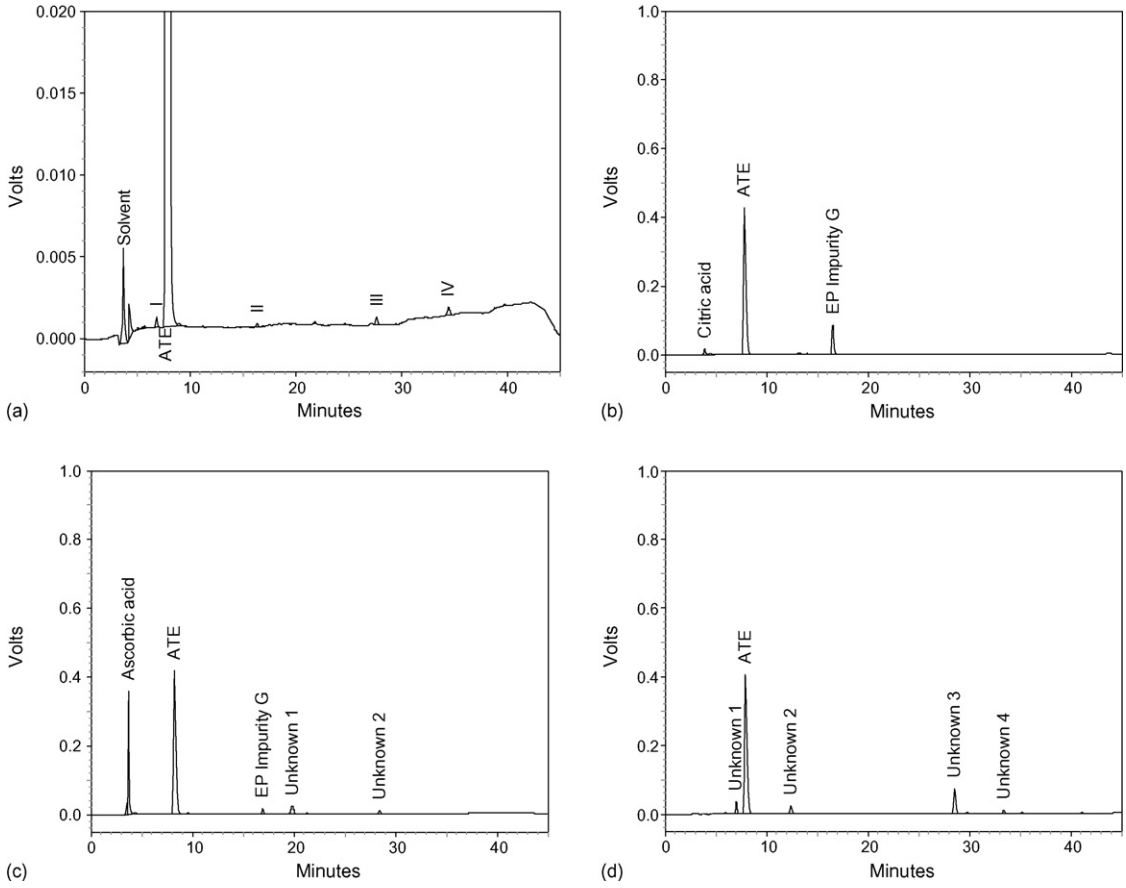


Fig. 1. Chromatograms showing pure atenolol (a), atenolol in the presence of citric acid (b), ascorbic acid (c) and butylated hydroxyanisole (d) after storage of 1 month at 40 °C and 75% RH. Key-ATE-atenolol.

Table 1
Physical and chemical changes of atenolol in the presence of excipients ($n = 3$).

S. no.	Name of excipient	Physical change	Chemical change (% degradation), R.S.D. (%)
1	Calcium carbonate	NC	1.36 (1.08)
2	Hydroxypropyl methyl cellulose	NC	1.24 (0.57)
3	Hydroxypropyl cellulose	NC	1.75 (0.03)
4	Sodium starch glycollate	NC	2.10 (0.01)
5	Starch	NC	2.79 (1.41)
6	Micro-crystalline cellulose	NC	2.86 (0.69)
7	Zinc stearate	NC	1.68 (1.36)
8	Ascorbic acid	Dark brown wet mass	11.75 (0.66)
9	Anhydrous citric acid	Wet mass	17.20 (0.35)
10	Povidone	NC	1.88 (0.60)
11	Dibasic calcium phosphate	NC	1.17 (0.78)
12	Butylated hydroxyanisole	Dark brown mass	21.30 (0.30)
13	Lactose	NC	2.37 (0.10)
14	Stearic acid	NC	2.04 (0.58)
15	Magnesium oxide	NC	2.87 (0.95)
16	Talc	NC	1.88 (0.23)
17	Mannitol	NC	1.54 (0.98)
18	Titanium dioxide	NC	2.71 (0.56)
19	Silicon dioxide	NC	2.66 (0.64)
20	Ponceau 4R	NC	2.68 (1.11)
21	Ferric oxide	NC	1.11 (0.36)
22	Allura red	NC	2.42 (0.81)

Key—NC: no change.

of peak area of atenolol was associated with appearance of very small peaks of degradation products, which were visible only on extensive enlargement of the chromatogram along the *y*-axis (Fig. 1a).

The physical and chemical changes observed in mixtures of atenolol with excipients are given in Table 1. As described, the drug was stable both physically and chemically in the presence of most of the excipients, other than those containing citric acid, ascorbic acid and butylated hydroxyanisole. In all the cases, there was no physical change. Also, the chemical decomposition was <3% and very small peaks due to degradation products were seen on enlargement of the chromatograms. In combinations of drug with citric acid, ascorbic acid and butylated hydroxyanisole, both physical and chemical changes were observed. The formation of wet mass in case of mixtures containing atenolol–citric acid and atenolol–ascorbic acid was due to moisture gain, which was verified through weight gain determinations, wherein an increase of ~17 mg and ~20 mg was observed for the two mixtures, respectively. The mixture containing butylated hydroxyanisole remained dry up to the end of the study, and also there was no increase in weight, though there was browning of color. Regarding the chemical change, the decrease in peak area for the three mixtures ranged between 11% and 21% (Table 1). As evident from Fig. 1b, atenolol in the presence of citric acid was converted to a single major product (~16%), which was indicated to be impurity G of the European Pharmacopoeia (EP impurity G, carboxylic acid hydrolysis product of atenolol) [4]. This was based on similar retention time observed for the same impurity in a previous study using the same method [5]. The said impurity was also formed in the mixture of atenolol and ascorbic acid to an extent of ~2% (Fig. 1c), wherein two other degradation/interaction products (unknown 1, ~6%; and unknown 2, ~2%) were also formed. In the mixture of atenolol and butylated hydroxyanisole, four degradation/interaction products (unknown 1, ~4%; unknown 2, ~3%; unknown 3, ~11%, and unknown 4, ~2%) were formed in total, as shown in Fig. 1d. The peak due to butylated hydroxyanisole was absent in Fig. 1d, because it eluted very late and to wash the column, the organic modifier was increased from 15% to 60% after 45 min.

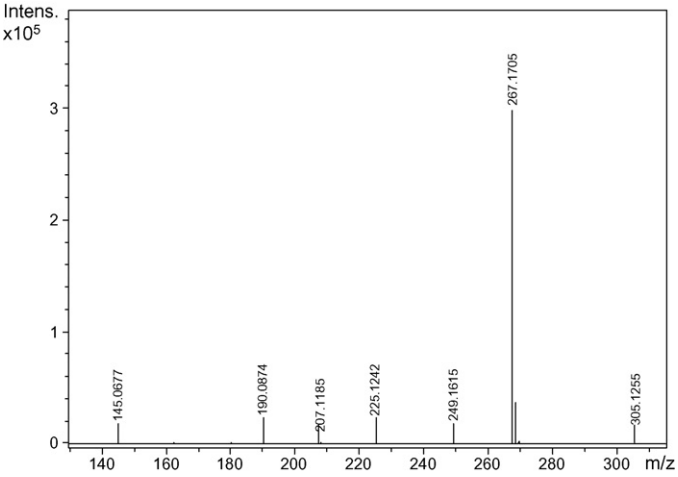


Fig. 2. Line spectrum of atenolol in positive ESI mode.

3.2. LC–MS/TOF studies

The LC–MS line spectrum for atenolol is shown in Fig. 2a. Its fragmentation profile is reproduced in Scheme 1 [4] to support the fragmentation pattern of the unknown drug–excipient degradation/interaction products. High resolution mass spectrometry (HRMS) data of molecular ion peak and fragments of the drug are given in Table 2.

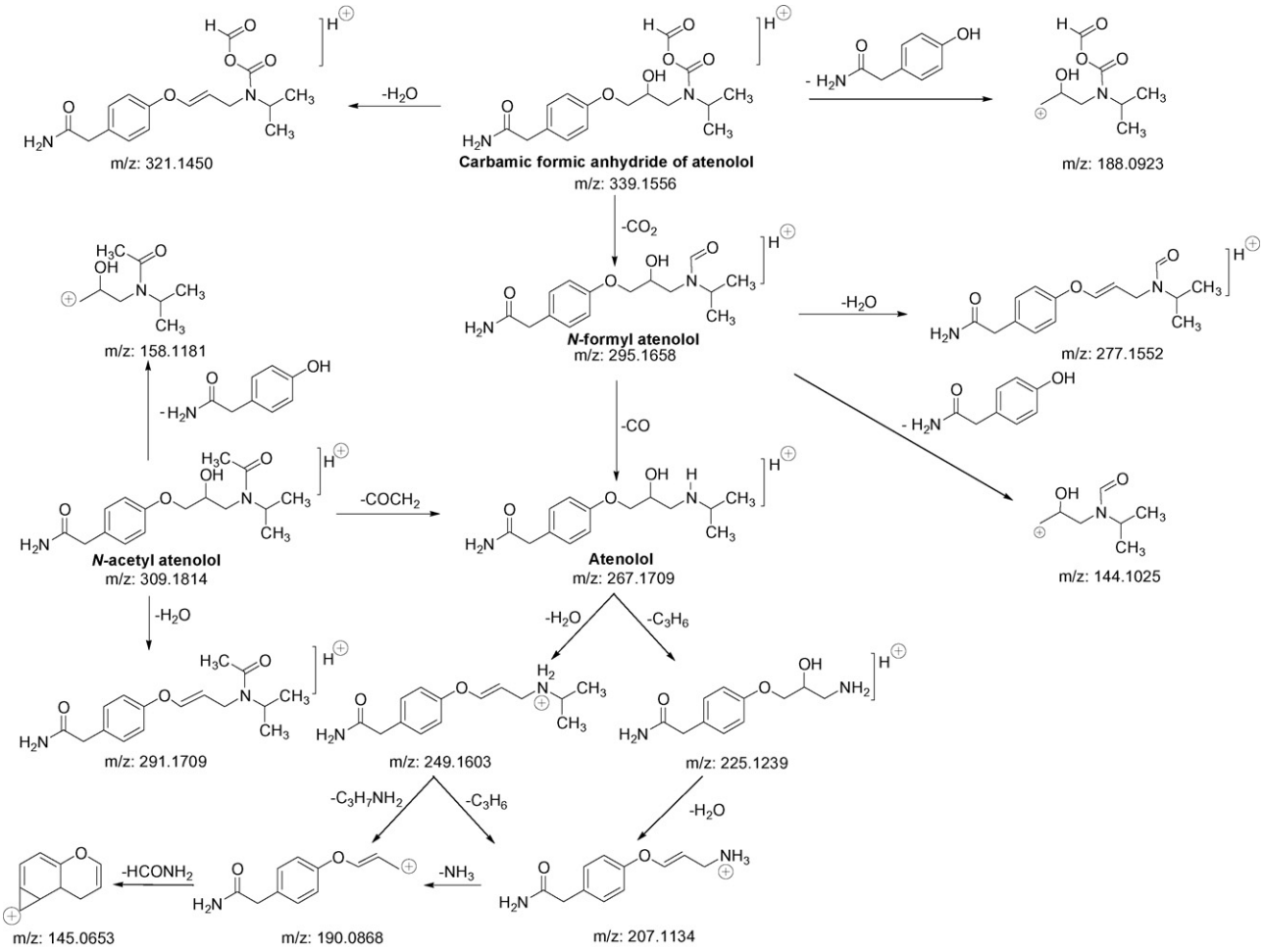
3.2.1. Atenolol–citric acid mixture

The LC–MS line spectrum of EP impurity G in the atenolol–citric acid mixture and its fragmentation profile was observed to be similar, as reported in an earlier study [4].

3.2.2. Atenolol–ascorbic acid mixture

The EP impurity G was also formed in atenolol–ascorbic acid mixture (Fig. 1c), which was confirmed in a similar manner, as discussed above. Regarding the other two unknown degradation/interaction products 1 and 2 (Fig. 1c), their line spectra are shown in Fig. 3a and b, respectively. Therein it could be observed that $[M+H]^+$ values of the two were *m*/*z* 339 and *m*/*z* 295, respectively. It was also shown that the fragments of atenolol existed in the line spectra of the two (Fig. 2 versus Fig. 3a and b) and furthermore, all fragments of product 2 were present in the line spectrum of product 1. This indicated possibility of both of them being derivatives of atenolol, with added moieties of *m*/*z* 72 and *m*/*z* 28, respectively.

In case of the unknown product 1, the accurate mass resulted in molecular formula $C_{16}H_{23}N_2O_6^+$, which indicated addition of $-C_2O_3$ to the drug. RDB studies indicated addition of $-CO_2$ and –CO groups to atenolol. Herein multiple possibilities existed, like addition of $-CO_2$ at –OH group and –CO at –NH group; addition of –CO and $-CO_2$ to –OH and –NH groups, respectively; and addition of $-CO_2$ followed by –CO on –NH group of atenolol. In the initial two cases, there were chances for the formation of carbonic acid (–OCOOH) and carbamic acid (–NCOOH), respectively, both of which were known to be highly unstable [7,8]. Hence, the last assumption was considered rational. It could be proposed that there was an initial addition of $-CO_2$ group to the –NH group of atenolol to form a carbamic acid, which was followed up by addition of –CO to yield a comparatively stable carbamic formic anhydride. The proposed structure was justified by the fragment pathway shown in Scheme 1, which is based on the line spectrum in Fig. 3a. The compound underwent simultaneous fragmentation losing water, carbon dioxide and 2-(4-hydroxy-phenyl)-acetamide to yield fragments of *m*/*z* 321, *m*/*z* 295 and *m*/*z* 188, respectively. The fragment of



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

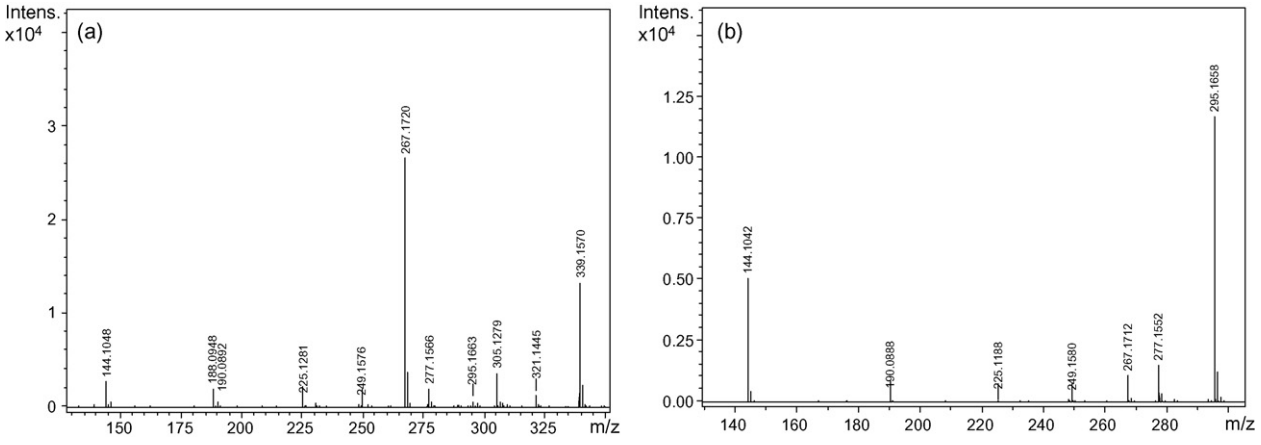


Fig. 3. Line spectra in positive ESI mode of unknown products of atenolol formed with ascorbic acid, product 1 (a) and product 2 (b).

Table 2
HRMS data for molecular ion peaks and fragments of atenolol.

Name of drug	Molecular ion peak/fragment	Observed mass	Theoretical mass	Molecular formula	RDB	Error (D)	Error (ppm)
Atenolol	$[M+H]^+$	267.1705	267.1709	$C_{14}H_{23}N_2O_3^+$	4.5	−0.0004	−1.50
	$[M+H]^+$-H_2O	249.1615	249.1603	$C_{14}H_{21}N_2O_2^+$	5.5	0.0012	4.82
	$[M+H]^+$-C_3H_6	225.1242	225.1239	$C_{11}H_{17}N_2O_3^+$	4.5	0.0003	1.33
	249-C_3H_6, 225-H_2O	207.1185	207.1134	$C_{11}H_{15}N_2O_2^+$	5.5	0.0051	24.62
	249-$C_3H_7NH_2$, 207-NH_3	190.0874	190.0868	$C_{11}H_{12}NO_2^+$	6.5	0.0006	3.16
	190-$HCONH_2$	145.0677	145.0653	$C_{10}H_9O^+$	6.5	0.0024	16.54

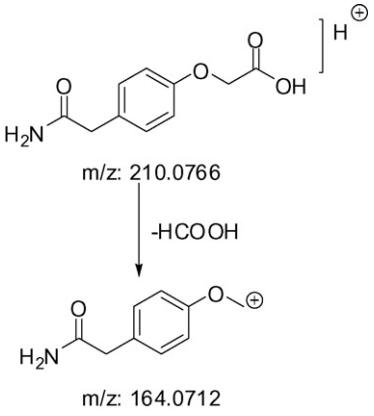
Table 3
HRMS data for molecular ion peaks and fragments of interaction products formed in atenolol–ascorbic acid mixture.

Name of interaction product	Molecular ion peak/fragment	Observed mass	Theoretical mass	Molecular formula	RDB	Error (D)	Error (ppm)
Carbamic formic anhydride of atenolol	$[M+H]^+$	339.1570	339.1556	$C_{16}H_{23}N_2O_6^+$	6.5	0.0014	4.13
	$[M+H]^+$-H_2O	321.1445	321.1450	$C_{16}H_{21}N_2O_5^+$	7.5	−0.0005	−1.56
	$[M+H]^+$-CO_2	295.1663	295.1658	$C_{15}H_{23}N_2O_4^+$	5.5	0.0005	1.69
	295-H_2O	277.1566	277.1552	$C_{15}H_{21}N_2O_3^+$	6.5	0.0014	5.05
	295-CO	267.1720	267.1709	$C_{14}H_{23}N_2O_3^+$	4.5	0.0011	4.12
	267-H_2O	249.1576	249.1603	$C_{14}H_{21}N_2O_2^+$	5.5	−0.0027	−10.84
	267-C_3H_6	225.1281	225.1239	$C_{11}H_{17}N_2O_3^+$	4.5	0.0042	18.66
	249-$C_3H_7NH_2$	190.0892	190.0868	$C_{11}H_{12}NO_2^+$	6.5	0.0024	12.63
	$[M+H]^+$-2-(4-hydroxy-phenyl)-acetamide	188.0948	188.0923	$C_8H_{14}NO_4^+$	2.5	0.0025	13.29
	295-2-(4-hydroxy-phenyl)-acetamide	144.1048	144.1025	$C_7H_{14}NO_2^+$	1.5	0.0023	15.96
N-formyl atenolol	$[M+H]^+$	295.1658	295.1658	$C_{15}H_{23}N_2O_4^+$	5.5	0.0000	0.00
	$[M+H]^+$-H_2O	277.1552	277.1552	$C_{15}H_{21}N_2O_3^+$	6.5	0.0000	0.00
	$[M+H]^+$-CO	267.1712	267.1709	$C_{14}H_{23}N_2O_3^+$	4.5	0.0003	1.12
	267-H_2O	249.1580	249.1603	$C_{14}H_{21}N_2O_2^+$	5.5	−0.0023	−9.23
	267-C_3H_6	225.1188	225.1239	$C_{11}H_{17}N_2O_3^+$	4.5	−0.0051	−22.65
	249-$C_3H_7NH_2$	190.0888	190.0868	$C_{11}H_{12}NO_2^+$	6.5	0.0020	10.52
	$[M+H]^+$-2-(4-hydroxy-phenyl)-acetamide	144.1042	144.1025	$C_7H_{14}NO_2^+$	1.5	0.0017	11.80

m/*z* 295 (apparently *N*-formyl atenolol) further lost water, carbon monoxide and 2-(4-hydroxy-phenyl)-acetamide to generate fragments of *m*/*z* 277, *m*/*z* 267 (atenolol) and *m*/*z* 144, respectively. The peak of *m*/*z* 305 could be attributed to potassium adduct of atenolol, which was also seen in the line spectrum of the drug (Fig. 2).

The product 2 could easily be characterized as *N*-formyl atenolol, based on its mass of *m*/*z* 295 and fragments of *m*/*z* 277, *m*/*z* 267 (atenolol) and *m*/*z* 144 (Fig. 3b), similar to those observed in case of product 1. A further confirmation was provided by the molecular formula, $C_{15}H_{23}N_2O_4^+$, which was in line with a structure having addition of –CO group to the drug.

The HRMS data in Table 3 clearly showed that the error for difference between theoretical and experimental mass values of molecular ion peaks was 4.1 ppm and 0.0 ppm in case of products 1 and 2, respectively, thus supporting the proposed structures.



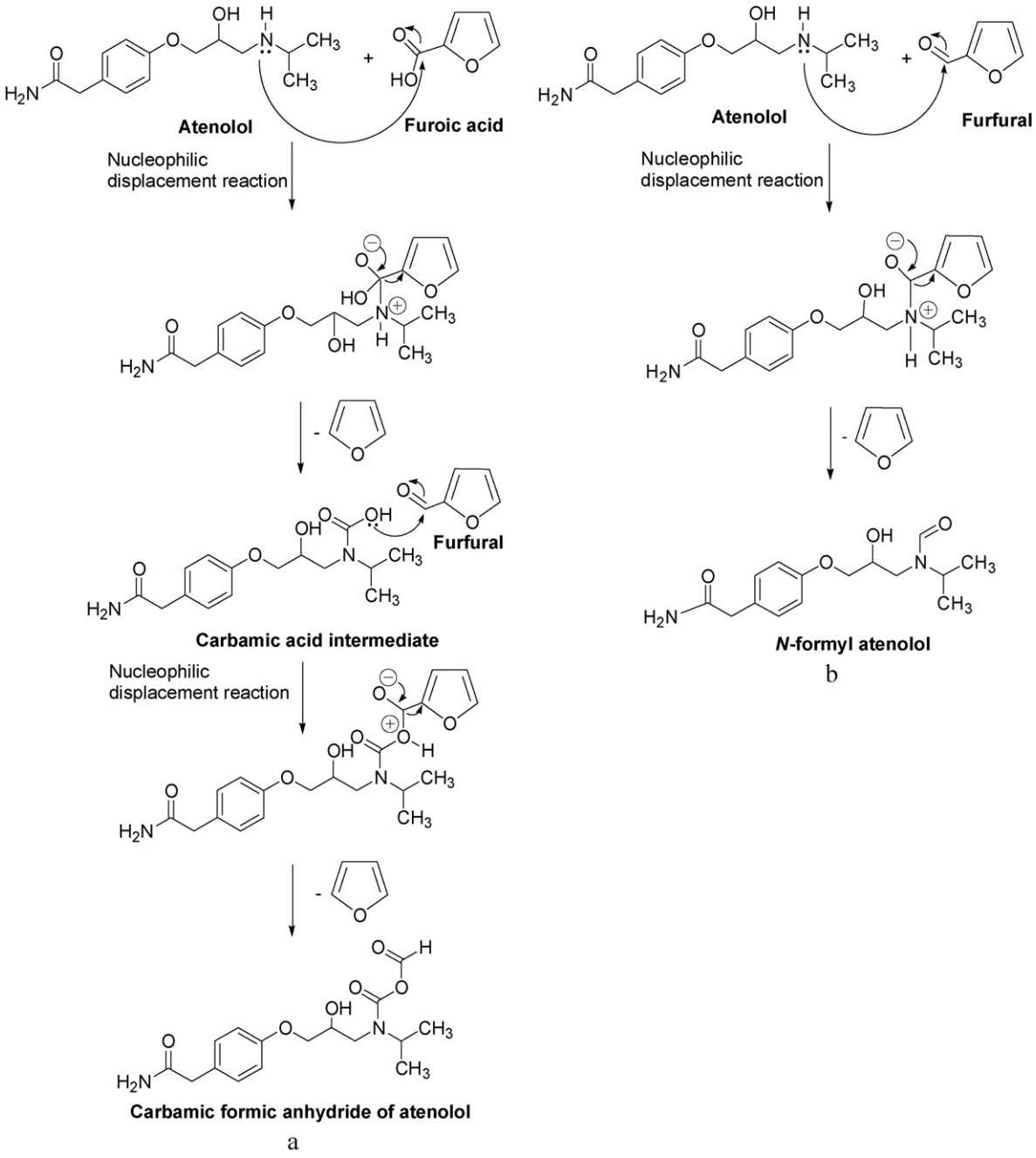
Scheme 2. Fragmentation pattern of product 2 formed in atenolol–butylated hydroxyanisole mixture.

3.2.3. *Atenolol–butylated hydroxyanisole mixture*

The line spectra of the four unknown degradation/interaction products (1–4) formed in the mixture of atenolol and butylated hydroxyanisole are shown in Fig. 4a–d, respectively. The four had *m*/*z* values of 152, 210, 295 and 309, respectively. Evidently, the first two products (Fig. 4a and b) had mass values less by *m*/*z* 115 and *m*/*z* 57 than atenolol, and the rest of the two (Fig. 4c and d) were higher by *m*/*z* 28 and *m*/*z* 42 than the drug. The assignment of structures to these products was also done through comparison of fragmentation behavior with respect to the drug (Scheme 1), accurate mass values, determination of molecular formula, and RDB calculations. The data are provided in Table 4.

Table 4
HRMS data for molecular ion peaks and fragments of degradation/interaction products formed in atenolol–butylated hydroxyanisole mixture.

Name of degradation/interaction product	Molecular ion peak/fragment	Observed mass	Theoretical mass	Molecular formula	RDB	Error (D)	Error (ppm)
2-(4-Hydroxyphenyl)-acetamide	$[M+H]^+$	152.0726	152.0712	$C_8H_{10}NO_2^+$	4.5	0.0014	9.21
2-(4-(2-Amino-2-oxoethyl)phenoxy)-acetic acid	$[M+H]^+$	210.0770	210.0766	$C_{10}H_{12}NO_4^+$	5.5	0.0004	1.90
	$[M+H]^+$-HCOOH	164.0737	164.0712	$C_9H_{10}NO_2^+$	4.5	0.0025	15.24
N-formyl atenolol	$[M+H]^+$	295.1656	295.1658	$C_{15}H_{23}N_2O_4^+$	5.5	-0.0002	−0.68
	$[M+H]^+$-H_2O	277.1552	277.1552	$C_{15}H_{21}N_2O_3^+$	6.5	0.0000	0.00
	$[M+H]^+$-CO	267.1713	267.1709	$C_{14}H_{23}N_2O_3^+$	4.5	0.0004	1.50
	267-H_2O	249.1580	249.1603	$C_{14}H_{21}N_2O_2^+$	5.5	−0.0023	−9.23
	267-C_3H_6	225.1284	225.1239	$C_{11}H_{17}N_2O_3^+$	4.5	0.0045	19.99
	249-$C_3H_7NH_2$	190.0891	190.0868	$C_{11}H_{12}NO_2^+$	6.5	0.0023	12.10
	$[M+H]^+$-2-(4-hydroxy-phenyl)-acetamide	144.1044	144.1025	$C_7H_{14}NO_2^+$	1.5	0.0019	13.19
N-acetyl atenolol	$[M+H]^+$	309.1816	309.1814	$C_{16}H_{25}N_2O_4^+$	5.5	0.0002	0.65
	$[M+H]^+$-H_2O	291.1712	291.1709	$C_{16}H_{23}N_2O_3^+$	6.5	0.0003	1.03
	$[M+H]^+$-$COCH_2$	267.1697	267.1709	$C_{14}H_{23}N_2O_3^+$	4.5	−0.0012	−4.49
	267-H_2O	249.1602	249.1603	$C_{14}H_{21}N_2O_2^+$	5.5	−0.0001	−0.40
	267-C_3H_6	225.1284	225.1239	$C_{11}H_{17}N_2O_3^+$	4.5	0.0045	19.99
	249-C_3H_6, 225-H_2O	207.1183	207.1134	$C_{11}H_{15}N_2O_2^+$	5.5	0.0049	23.66
	249-$C_3H_7NH_2$	190.0894	190.0868	$C_{11}H_{12}NO_2^+$	6.5	0.0026	13.68
	$[M+H]^+$-2-(4-hydroxy-phenyl)-acetamide	158.1204	158.1181	$C_8H_{16}NO_2^+$	1.5	0.0023	14.55
	190-$HCONH_2$	145.0701	145.0653	$C_{10}H_9O^+$	6.5	0.0048	33.09



Scheme 3. Postulated mechanisms of formation of carbamic formic anhydride of atenolol (a) and *N*-formyl atenolol (b) in atenolol–ascorbic acid mixture.

Product 1 had an accurate mass of *m*/*z* 152.0726 (Fig. 4a) and hence molecular formula $C_8H_{10}NO_2^+$. This along with RDB value (Table 4) indicated the compound to be 2-(4-hydroxyphenyl)-acetamide, a known degradation product of atenolol [9,10]. Fig. 4b shows that product 2 fragmented to an ion of *m*/*z* 164, involving release of formic acid (*m*/*z* 46). This hinted to the presence of a carboxyl group in the product structure. Based on the molecular formula $C_{10}H_{12}NO_4^+$ (accurate mass *m*/*z* 210.0770) and RDB value of 5.5, the structure was predicted to be 2-(4-(2-amino-2-oxoethyl)phenoxy)-acetic acid (Scheme 2). Product 3 showed the same RT value (Fig. 1c versus d), exactly similar line spectrum, and same fragmentation pattern as that of *N*-formyl atenolol (Fig. 4c versus Fig. 3b). Product 4 had *m*/*z* value of 309 along with a characteristic fragment of *m*/*z* 291 (Fig. 4d), attributed to loss of water. This indicated the product to be *N*-acetyl atenolol, as reported by us in a previous study [4].

The HRMS data in Table 4 showed that the error for difference between theoretical and experimental mass values of molecular ion peaks was <4.0 ppm in all the cases, except the small mass component, 2-(4-hydroxyphenyl)-acetamide, thus providing support to the envisaged structures even in this case.

3.2.4. PDA analyses of UV spectra

The PDA spectra for drug and all the degradation/interaction products showed λ_{max} at 235 nm and 273 nm. This indicated that all of them were related to atenolol, wherein the chromophore (4-alkyl substituted phenol ether) remained intact, while the changes occurred at other parts of the drug structure. A typical case was

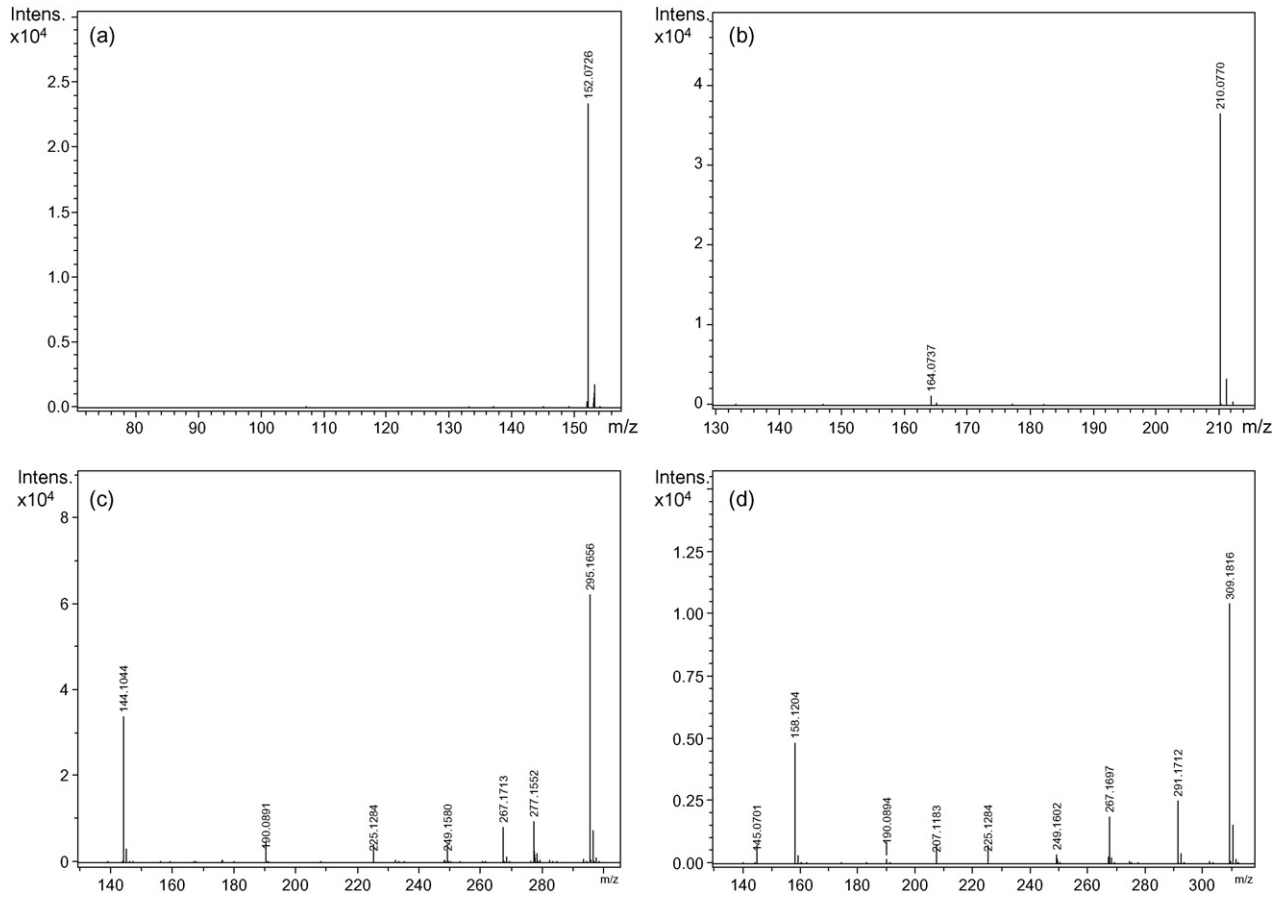
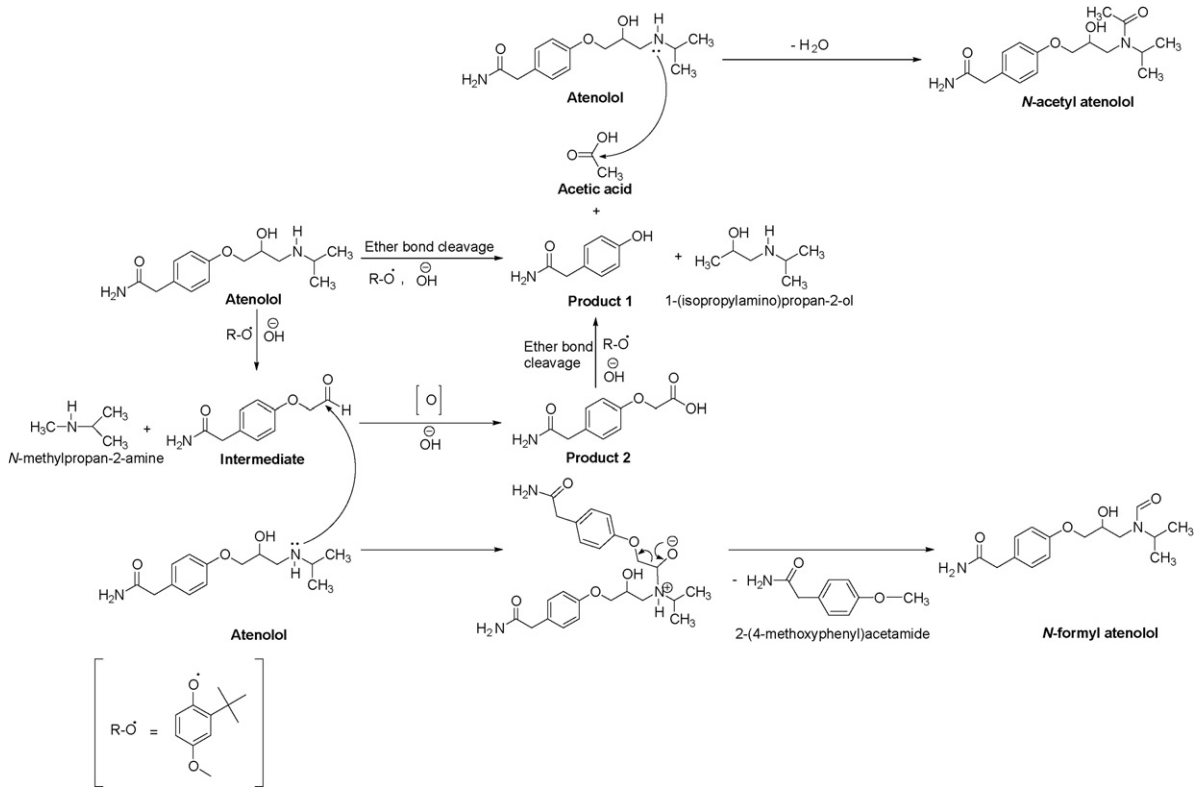


Fig. 4. Line spectra in positive ESI mode of unknown products of atenolol formed with butylated hydroxyanisole, product 1 (a), product 2 (b), product 3 (c) and product 4 (d).



Scheme 4. Postulated mechanism of formation of degradation/interaction products in atenolol–butylated hydroxyanisole mixture.

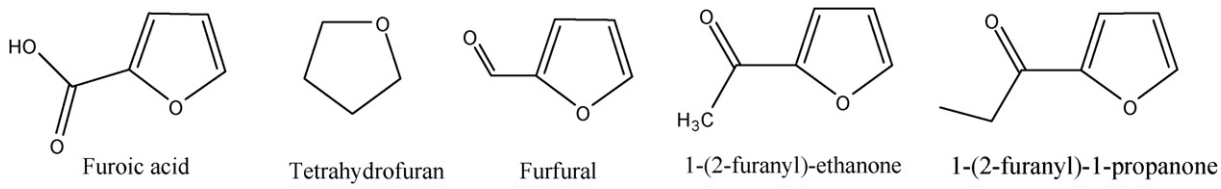


Fig. 5. Structures of degradation products of ascorbic acid formed in solid state [11].

that of 2-(4-hydroxyphenyl)-acetamide, which was formed in the presence of butylated hydroxyanisole, and despite being a small remnant of atenolol molecule, the spectrum was still alike atenolol, and similar to reported for this compound in the literature [10]. Thus indirectly this study also provided a good support to the characterized structures for the degradation/interaction products.

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

A critical study of the characterized structures, as discussed above, indicated that the products were either generated on degradation of the drug or formed on interaction of drug with degradation products of the excipients. The degradation of the drug or the excipient in a solid mixture could only be explained through predominant role of micro-environment pH. Hence the pH values were recorded, as discussed under Section 2.6, and they were found to be 3.34, 4.60 and 9.90 for the mixtures containing drug–citric acid, drug–ascorbic acid and drug–butylated hydroxyanisole, respectively. A simultaneous literature search revealed that ascorbic acid underwent solid state degradation to furoic acid, tetrahydrofuran, furfural, 1-(2-furanyl)-ethanone and 1-(2-furanyl)-1-propanone (Fig. 5) [11], while citric acid and butylated hydroxyanisole were reportedly stable [12,13].

Accordingly, it could be proposed that EP impurity G was formed in blends of atenolol and citric acid/ascorbic acid through amide hydrolysis [4,9] of the drug in acidic micro-environment provided by the latter. The only explanation that products 1 and 2 were formed in atenolol–ascorbic acid mixture was interaction of the drug with degradation products of ascorbic acid, *viz.* furoic acid and

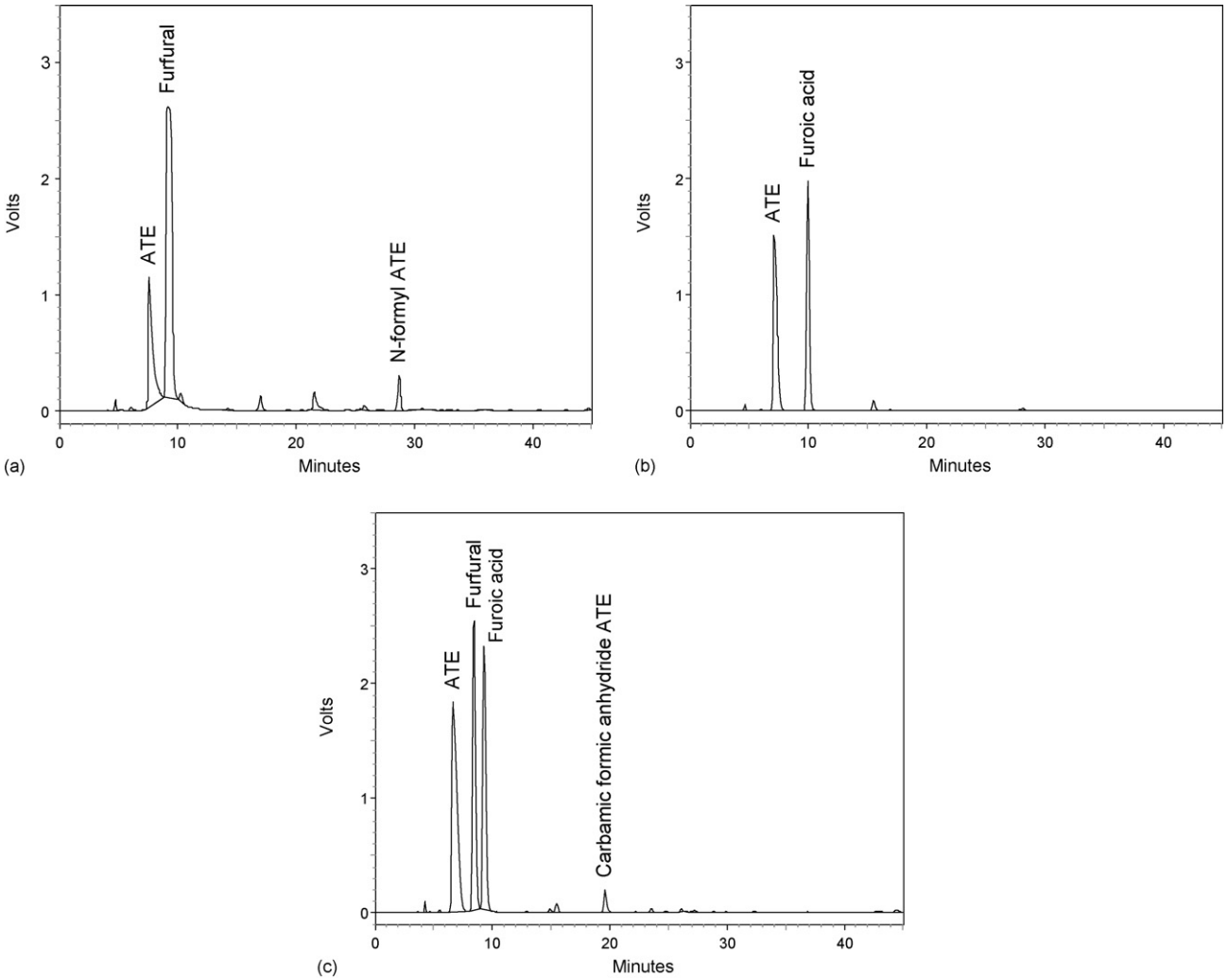


Fig. 6. Chromatograms for mixtures of atenolol–furfural (a), atenolol–furoic acid (b) and atenolol–furfural–furoic acid (c) after storage for 7 days at 40 °C and 75% RH. Key-same as Fig. 1.

furfural. An interesting case was that of product 1, where formation of a stable carbamic formic anhydride (Scheme 1) was possible only through involvement of both the said degradation products. The supposition was that atenolol underwent nucleophilic displacement reaction with furoic acid to yield an unstable carbamic acid, which reacted further with furfural to yield the stable anhydride. The mechanism for the same could also be outlined, as given in Scheme 3a. The formation of *N*-formyl atenolol (product 2) was explained through direct interaction between atenolol and furfural involving a nucleophilic displacement reaction (Scheme 3b). To confirm these postulations, atenolol was reacted independently with furfural and furoic acid, and also with combination of the two. The mixtures were stored for 7 days at 40 °C and 75% RH. The resultant chromatograms are shown in Fig. 6a–c. Evidently, the two products were formed as postulated, thus supporting the proposed mechanisms.

The four products 1–4 formed in a mixture of atenolol and butylated hydroxyanisole were proposed to result from a cascade of inter-related events, as outlined in Scheme 4. As butylated hydroxyanisole could form phenoxyl free radical under alkaline conditions [14,15], and atenolol in turn could degrade by a free radical mechanism [16–18], accordingly it was proposed that the drug, in the presence of phenoxyl free radical and alkaline pH, degraded to *N*-methylpropane-2-amine and an aldehydic intermediate by an oxidative mechanism, converting to 2-(4-(2-amino-2-oxoethyl)phenoxy)-acetic acid (product 2). This product and/or drug underwent ether bond cleavage by reductive mechanism in the presence of phenoxyl free radical to form 2-(4-hydroxyphenyl)-acetamide (product 1) along with acetic acid and/or 1-(isopropylamino)propan-2-ol, respectively. Atenolol further interacted with aldehydic intermediate and acetic acid formed in earlier reactions to yield *N*-formyl atenolol (product 3) and *N*-acetyl atenolol (product 4), respectively, as outlined in Scheme 4.

4. Conclusions

The degradation/interaction products formed in mixtures of atenolol with citric acid, ascorbic acid and butylated hydroxyanisole were characterized by employing LC–MS/TOF studies. The structures were delineated by comparison of fragmentation patterns of products with the drug, molecular formulae, RDB and accurate mass analyses. EP impurity G was detected to be formed with both citric acid and ascorbic acid. Two other products formed with ascorbic acid were *N*-formyl atenolol and carbamic formic anhydride of atenolol. Of the total four products generated in the presence of butylated hydroxyanisole, two were characterized as degradation products, 2-(4-hydroxyphenyl)-acetamide and 2-(4-(2-amino-2-oxoethyl)phenoxy)-acetic acid; while two others were interaction products, *N*-formyl atenolol and *N*-acetyl atenolol. The characterized structures were supported by appropriate mechanisms.

The study is a typical case of investigation of drug–excipient interactions using LC–MS tools.

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