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LC–MS analysis in the e-beam and gamma radiolysis of metoprolol tartrate in aqueous solution: Structure elucidation and formation mechanism of radiolytic products

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Abstract

E-beam and gamma products from the radiolysis of aqueous solutions of (±)-metoprolol tartrate, saturated in nitrogen, are analyzed by HPLC with on-line mass and UV detectors. The structures of 10 radiolytic products common to e-beam and gamma irradiations are elucidated by comparing their fragmentation pattern to that of (±)-metoprolol. Two of the radiolytic products are also metabolites. Different routes for the formation of the radiolytic products are proposed.

Keywords: (±)-Metoprolol tartrate; LC–MS; Radiolytic products; Identification; Radiolysis mechanism

1. Introduction

In a previous study, a quantitative analysis on the final products of e-beam and gamma irradiations of (±)-metoprolol tartrate in aqueous solution was performed by HPLC with ultraviolet detectors (Slegers and Tilquin, 2006). The diode-array detector failed to discriminate between radiolytic products because of their highly similar absorption spectra. Furthermore, the profile of the radiolytic products as a function of the absorbed dose showed many differences between e-beam and gamma irradiations of (±)-metoprolol tartrate in aqueous solution. Therefore, HPLC methods with on-line mass–UV detectors were developed to provide spectral, as well as, structural information on the radiolytic products detected. The on-line mass and UV detectors are essential to discriminate between radiolytic products and co-eluting peaks. Multiple tandem mass spectrometry was used to compare the fragmentation pattern of radiolytic products to that of (±)-metoprolol, with the aim of elucidating their structures and their mechanism of formation.

The chemical structures of (±)-metoprolol and the radiolytic products are presented in Fig. 1, and for clarity, they will be referred to by their compound number (1–11) throughout the text.

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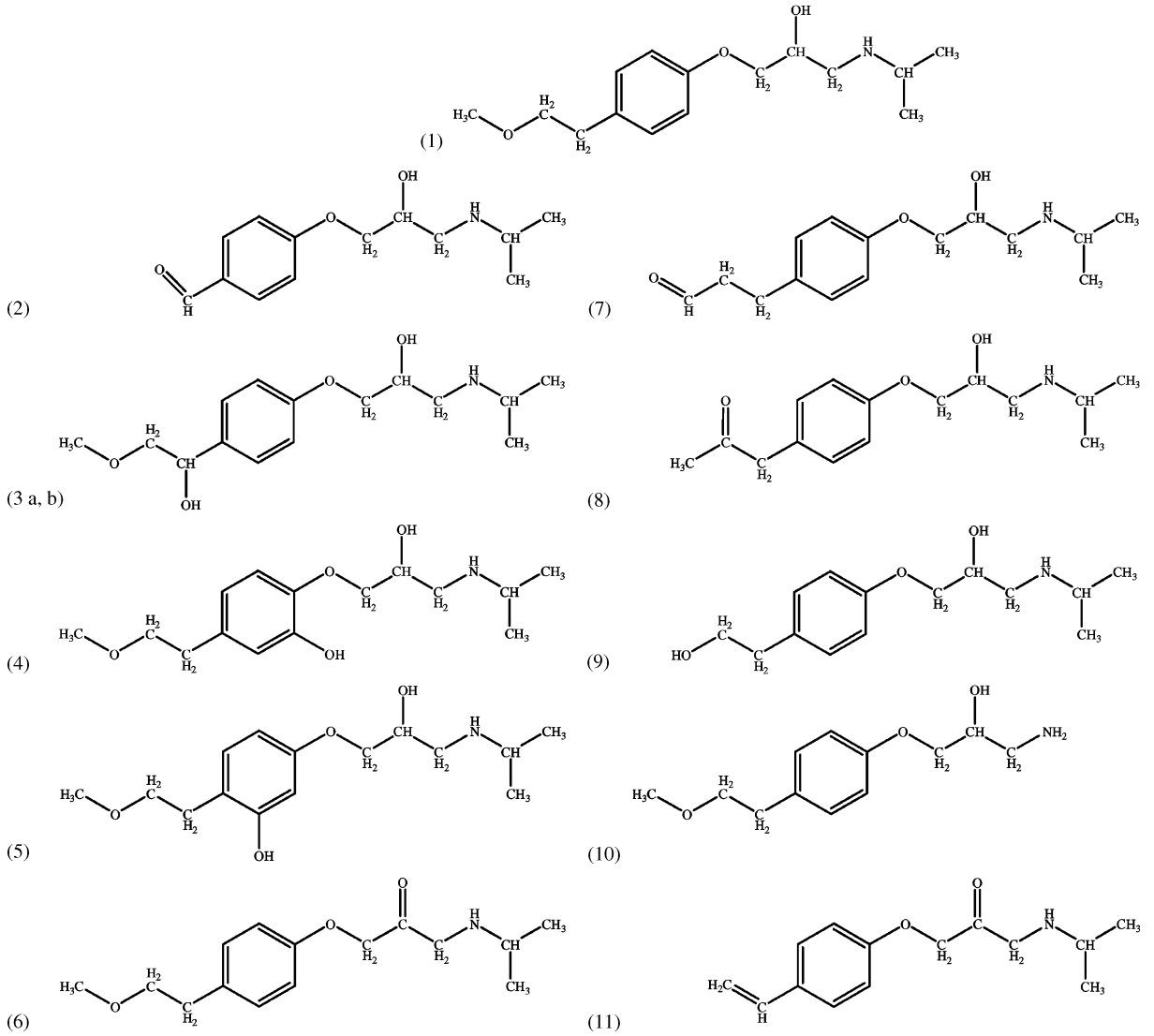


Fig. 1. Chemical structures of compounds referred to in the text: (1) (±)-metoprolol, (2) 4-[2-hydroxy-3-[(1-methylethyl)amino]propoxy]-benzaldehyde, (3 a, b) α-hydroxymetoprolol diastereoisomers (2R,1′R and 2S,1′S enantiomers) and (2R,1′S and 2S,1′R enantiomers), (4) 1-[2-hydroxy-4-(2-methoxyethyl)phenoxy]-3-[(1-methylethyl)amino]-propan-2-ol, (5) 1-[3-hydroxy-4-(2-methoxyethyl)phenoxy]-3-[(1-methylethyl)amino]-propan-2-ol, (6) 1-[4-(2-methoxyethyl)phenoxy]-3-[(1-methylethyl)amino]-propan-2-one, (7) 3-[4-[2-hydroxy-3-[(1-methylethyl)amino]propoxy]phenyl]-propanal, (8) 1-[4-[2-hydroxy-3-[(1-methylethyl)amino]propoxy]phenyl-propan-2-one, (9) O-demethylmetoprolol, (10) 1-amino-3-[4-(2-methoxyethyl)phenoxy]-propan-2-ol and (11) 1-[4-vinylphenoxy]-3-[(1-methylethyl)amino]-propan-2-one.

2. Materials and methods

2.1. E-beam and gamma irradiations

The preparation of the aqueous solutions of (±)-metoprolol tartrate 1 mg ml^{-1} and the details of the e-beam and gamma irradiations, performed under an atmosphere of nitrogen, may be found in the quantitative part of the study (Slegers and Tilquin, 2006).

2.2. LC–MS–UV analyses

The samples at all absorbed doses are each injected two times for the routine analyses. These analyses are performed with the following system: Merck Hitachi Intelligent L-6200 pump, a Spark Midas 830 NS70034 autosampler with a 10 μl sample loop and a Kontron Instruments 332 UV detector fixed at 223 nm. The system is coupled on-line to a ThermoFinnigan MAT

LCQ® Advantage mass detector with an atmospheric pressure chemical ionization (APCI) source in the positive ion polarity mode. The capillary temperature is 250 °C, the vaporizer temperature is 450 °C, the nitrogen sheath and auxiliary gases are 80 and 0 units, respectively, the source voltage is 6.00 kV and the discharge current is 5.00 μA. The mass detector is automatically tuned by direct injection of a 10 parts per million (ppm) (±)-metoprolol tartrate aqueous solution. Full mass scans of 100–700 units are performed, followed by selected ion monitoring (SIM) of the (±)-metoprolol and the main radiolytic products detected with isolation bandwidths of $2m/z$ (mass to charge ratio) and collision-induced dissociation (CID) spectra with 38% normalized fragmentation energy. ThermoFinnigan MAT Xcalibur® software version 1.3 is used to control the mass detector and for the data acquisition.

The results are confirmed with the 10 and 25 kGy samples at another laboratory equipped with a diode-array detector, as well as multiple mass tandem spectrometry to confirm the identification of some of the radiolytic products. This system is composed of a Spectra System P1000XR pump, a Spectra System TSP AS3000 autosampler with a 10 μl sample loop, a Spectra System UV6000LP diode-array detector, a ThermoFinnigan MAT LCQ® mass detector with an APCI source in the positive ion polarity mode. The capillary temperature is 170 °C, the vaporizer temperature is 400 °C, the nitrogen sheath and auxiliary gases are 37 and 0 units, respectively, the source voltage is 5.50 kV and the discharge current is 5.00 μA. The HPLC column thermostat is set at 30 °C. The mass detector is manually tuned by direct injection of a 10 ppm (±)-metoprolol tartrate aqueous solution. Full mass scans of 50–700 units are performed, followed by SIM of the main radiolytic products detected with isolation bandwidths of $3m/z$. CID spectra of main radiolytic products and most abundant fragments are recorded with 33% normalized fragmentation energy. CID spectra of (±)-metoprolol and main fragments are recorded by direct injection of a (±)-metoprolol tartrate methanol aqueous solution under the same conditions. ThermoFinnigan MAT Xcalibur® software version 1.1 is used to control the HPLC–DAD–MS system and for the data acquisition.

2.3. Chromatographic separation

A Merck 250 × 2 mm Lichrospher® RP select B column with 5 μm particle size is used. The mobile phase consists of 20% HPLC gradient grade acetonitrile and 80% water adjusted to pH 2.5 with formic acid. The flow rate is 0.2 ml min^{-1}.

Compared to the previous quantitative HPLC study (Slegers and Tilquin, 2006), the method has been adapted to an on-line LC–MS system and thus no ion-pairing reagent and no buffers are used, only acetonitrile of the same elution strength (20%) and water adjusted to the same pH with formic acid (2.5). The column is also adapted to the mass detector and thus the order of elution of some radiolytic products may change as the column is changed from a 250–4 mm C18 to a 250–2 mm C8.

2.4. Synthesis of compound (2)

Ten milligram of (±)-metoprolol tartrate is dissolved in 10 ml of HCl 0.1 M. The solution is placed in an open glass recipient of 10 cm diameter and exposed to UV radiation at 254 nm for 6 h. The recipient is positioned so that the solution is 5 cm below the lamp (European Pharmacopoeia, 2005). This solution is directly injected into the LC–MS system.

3. Results and discussion

3.1. Analysis of radiolytic products

3.1.1. UV and full mass scans

The chromatograms at 223 nm and the full mass scans (100–700m/z) of 12.8 kGy e-beam (EB) and 10 kGy gamma (γ) irradiations of (±)-metoprolol tartrate (1 mg ml^{-1}) solutions, saturated in nitrogen, are shown in Fig. 2. The chromatograms are zoomed in on the radiolytic products and thus the metoprolol peak, with retention time 14 min, saturates at such a scale of absorbance and relative ion abundance. The metoprolol peak shifted from a narrow symmetrical peak at 20 min to a large tailing peak of 12–16 min, compared to the previous quantitative HPLC study (Slegers and Tilquin, 2006) because no ion-pairing reagent was used, as it is not compatible with on-line LC–MS methods. No degradation products are detected in the non-irradiated solutions of (±)-metoprolol tartrate by the UV and MS detectors.

Two main e-beam radiolytic products with retention times 9.6 and 17.4 min are detected at 223 nm, as may be seen in Fig. 2. The full mass scan reveals that the peak at 223 nm with retention time 9.6 min is a co-elution of radiolytic products with m/z 284 and 266 and these correspond to the two main e-beam radiolytic products detected by mass spectrometry. Therefore, the main e-beam radiolytic products with retention times 13.1 and 13.7 min of the quantitative HPLC method (Slegers and Tilquin, 2006) co-elute at 9.6 min, in this LC–MS method. The other main e-beam radiolytic product at 223 nm does not shift and elutes at 17.4 min for both LC methods. However, it does not ionize by APCI and electrospray ionization (ESI) sources in the positive and negative ion modes. Many other radiolytic products

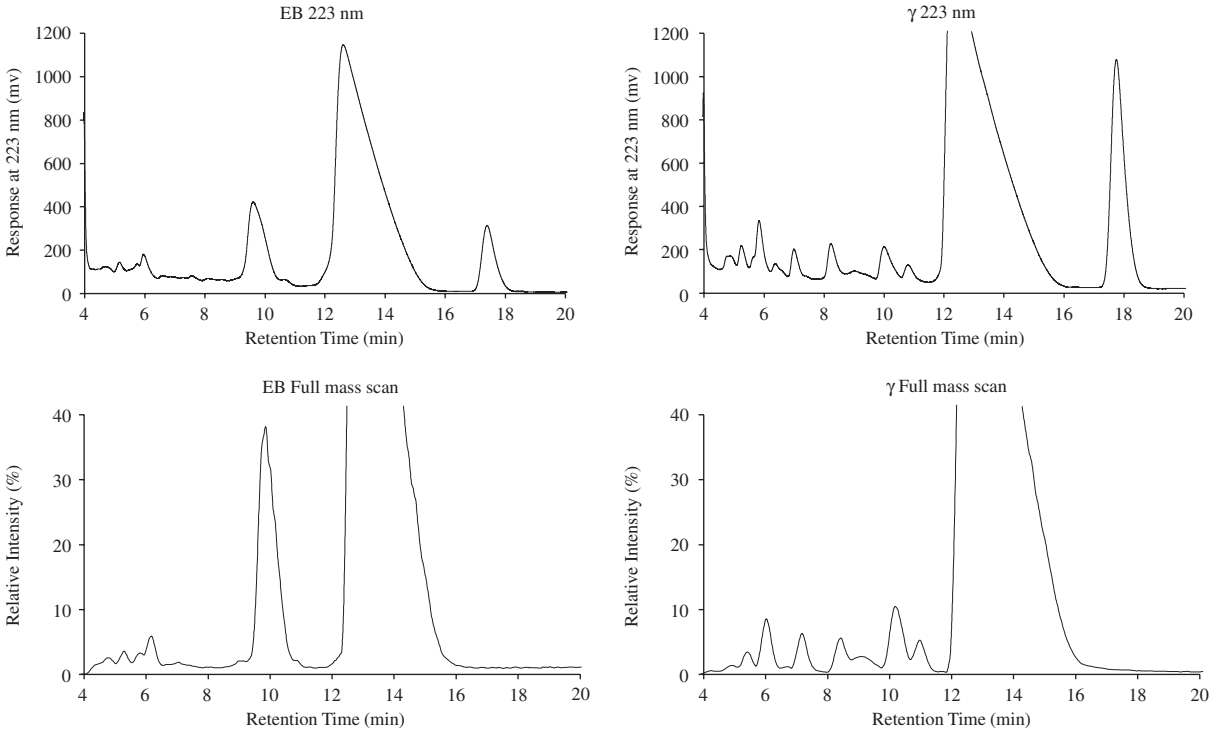


Fig. 2. Chromatograms at 223 nm and full mass scans from 100 to 700m/z, of a 12.8 kGy e-beam irradiation (EB) and a 10 kGy gamma irradiation (γ) of 1 mg ml^{-1} ($\pm$)-metoprolol tartrate aqueous solutions, saturated in nitrogen.

(m/z: 254, 226, 234, 238, 404, 369, 312, 289, 342, 387, 415, 270, 252, 553 and 551) are detected in traces after different e-beam irradiations. These have numerous mass isomers as they are detected at different retention times.

One main gamma radiolytic product with retention time 17.7 min is detected at 223 nm. This radiolytic product does not ionize by APCI and ESI sources in the positive and negative ion modes, as may be seen in Fig. 2. The main gamma radiolytic product also elutes at the same time in both LC methods. Five main gamma radiolytic products are detected by mass spectrometry with corresponding m/z of 284, 254, 238, 226 and 266. Many other radiolytic products (m/z: 240, 326, 318, 300, 270, 334, 256, 332, 316, 314, 312, 252, 296, 268, 224, 282, 242, 342, 368, 384, 234, 308, 324 and 404) are detected in traces after different gamma irradiations and these also show numerous mass isomers.

The LC–MS analysis confirms the quantitative study (Slegers and Tilquin, 2006) that the radiolytic products as a function of the absorbed dose are present in greater numbers for gamma than e-beam irradiations. The complexity of the radiolysis mechanism is also revealed by all the different m/z detected and their numerous mass isomers. Some of the radiolytic products have m/z ratios higher than ($\pm$)-metoprolol ($>300m/z$) and could result from coupling reactions between the radiolytic products.

Many radiolytic products are common to both e-beam and gamma irradiations; however, their distribution is very different. The e-beam and gamma radiolytic products detected at 223 nm, with retention times 17.4 and 17.7 min, respectively, are the same because they elute at the same time in both LC methods; they have similar UV–VIS spectra (not shown) and they fail to be detected by mass spectrometry with the use of different ionization sources and mass scans of up to 1500m/z units. The radiolytic products with m/z 284 and 266 are main products in both the e-beam and the gamma irradiations of ($\pm$)-metoprolol tartrate solutions. The other main gamma radiolytic products with m/z 254, 238 and 226 are also present in traces in the e-beam irradiations.

3.1.2. SIM and DAD scans

The main radiolytic products that are common to both e-beam and gamma irradiations are analyzed by SIM to improve the sensitivity of the method. The SIM chromatograms and the DAD spectra from 200 to 350 nm, of the e-beam (EB) radiolytic products with m/z 284 and 266 and the gamma (γ) radiolytic products with m/z 284, 266, 254, 238, 226, are shown in Fig. 3. The

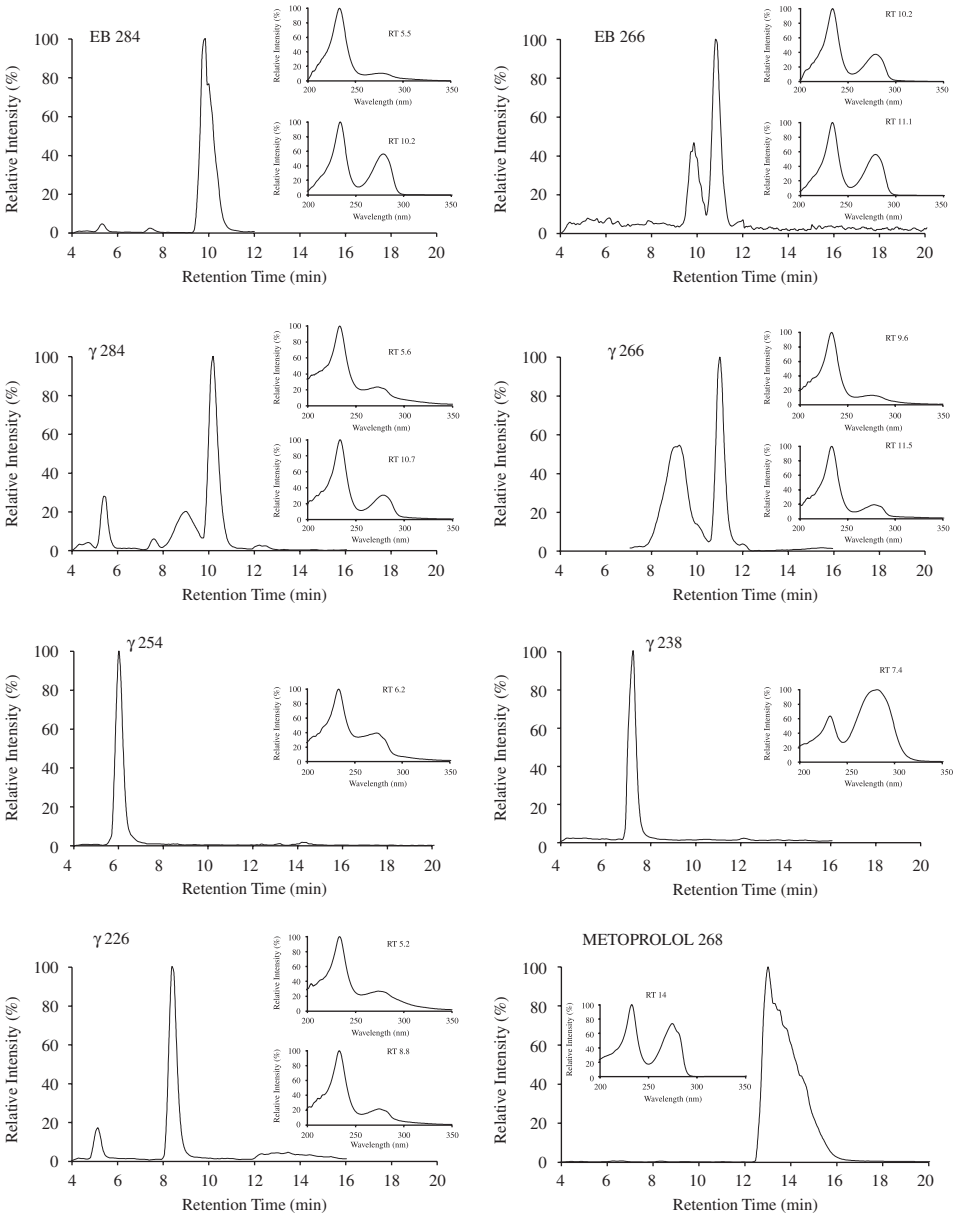


Fig. 3. Selected ion monitoring chromatograms and DAD scans from 200 to 350 nm, of the main e-beam (EB) radiolytic products (m/z 284 and 266), the main gamma (γ) radiolytic products (m/z 284, 266, 254, 238 and 226) and ($\pm$)-metoprolol (m/z 268).

SIM chromatogram and DAD spectrum of ($\pm$)-metoprolol are also shown for comparison. The SIM reveals many isobaric masses for both e-beam and gamma radiolytic products.

E-beam radiolytic products with m/z 284 are detected four times with the main isomer eluting at 10.2 min and corresponding to compound (4) or (5). E-beam products with m/z 266 are detected twice with compound (6) eluting at 10.2 min and compound (7) at 11.1 min.

Similarly, gamma radiolytic products with m/z 284 are detected seven times with the main isomers eluting at 10.7 min and 5.6 min and corresponding to compounds (4) or (5) and (3 a, b), respectively. Gamma products with m/z 266 are detected four times and the main mass isomers are compound (8) eluting at 9.6 min and compound (7) at 11.5 min. The main isomers of gamma products with m/z 254, 238 and 226 correspond to compounds (9), (2) and (10), respectively. These results are summarized in Table 1.

Table 1
Fragments from MS^2 scans of (±)-metoprolol, e-beam and gamma radiolytic products

m/z Ratio	Product	Average RT (min)	Compound	Fragments in order of intensity (*m/z* ratio)
268	Metoprolol	14	1	191, 218, 116, 226, 250, 159, 176, 121, 194, 98, 177, 133
238	Synthesized	6.9	2	196, 161, 220, 178, 116, 133, 149, 105, 123, 98, 74
238	EB	8.2	2	196, 161, 220, 178, 116, 133, 149, 105, 123, 98, 74
238	γ	7.4	2	196, 161, 220, 178, 116, 133, 149, 105, 123, 98, 74
284	EB	4.7		266, 207, 116, 248, 242, 234, 252, 175, 189, 133
		5.5[a]	3 (a, b)	116, 207, 175, 266, 224, 248, 242, 133, 189, 192, 98
		7.7	4 or 5	175, 207, 252, 242, 266, 116, 193, 234, 149, 210, 163
		10.2[b]	4 or 5	116, 266, 175, 234, 192, 207, 242, 252, 149, 98
284	γ	4.6		266, 207, 116, 242, 189, 234, 252, 248, 224, 145, 133, 98
		5.6[a]	3 (a, b)	116, 207, 175, 224, 248, 266, 133, 189, 242, 98
		7.0		116, 206, 241, 168
		8.0	4 or 5	175, 207, 266, 252, 242, 116, 132, 234, 193
		9.4		266, 248, 189, 116, 224
		10.7[b]	4 or 5	266, 116, 234, 175, 192, 207, 242, 252, 149, 98
		12.8		206, 241, 266, 116, 146, 248, 132
266	EB	10.2	6	234, 192, 175, 207, 224, 114
		11.1[b]	7	189, 224, 248, 116, 159, 177, 206, 218, 98
266	γ	4.3		224, 189, 248, 116
		5.8		224, 189, 248, 116, 177
		9.6[a]	8	189, 224, 204, 116, 248, 145, 162, 98, 133
		11.5[b]	7	189, 224, 116, 248, 159, 206, 177, 218, 98
254	EB	4.5		236, 212, 116, 195, 180, 204, 222
		6.0[b]	9	177, 212, 116, 236, 159, 218, 98, 194
		7.8[a]		236, 177, 116, 159, 212, 226, 195
254	γ	4.4[a]		212, 236, 177, 194
		6.2[b]	9	177, 212, 236, 116, 159, 218, 98
		8.2		177, 236, 116, 212
226	EB	5.1		149, 116, 208, 184, 194, 121
		8.4[b]	10	194, 121, 74, 176, 191, 159, 150, 133
226	γ	5.2		194, 149, 208, 116, 184
		8.8[b]	10	194, 121, 74, 176, 159, 150, 133
234	EB	10.2	11	192, 175, 149, 161, 133, 98, 145
234	γ	10.5	11	192, 175, 161, 149

RT = retention time.
[a]Second most abundant isobaric mass.
[b]Most abundant isobaric mass.

3.2. *Structure elucidation of radiolytic products*

The major e-beam and gamma radiolytic products are fragmented and their CID and DAD spectra are compared to that of (±)-metoprolol in order to elucidate their structures (Barbarin et al., 2001; Görög et al., 1997). The fragments in order of intensity are presented in Table 1 for the e-beam and gamma radiolytic products in common.

3.2.1. *(±)-Metoprolol (1)*

The CID spectrum and the simplified fragmentation pathway of (±)-metoprolol, with *m/z* 268, are shown in Fig. 4. The metoprolol molecule is divided into three parts: side chain B–phenoxy group–side chain A, in order to identify the key fragments that will reveal modifications in the radiolytic products.

The metoprolol molecule fragments on side chain A to give: a *m/z* of 250 which corresponds to the loss of water (18); a *m/z* of 226 corresponding to the loss of propene (42); a *m/z* of 191 corresponding to the loss of 77 (18 + 42 + 17) mass units, which corresponds to the loss of water, propene and ammonia.

The fragments characteristic of side chains A and B are: a *m/z* of 218 corresponding to a loss of 50 (18 + 32) mass units, i.e., water from side chain A and methanol from side chain B; a *m/z* of 194 corresponding to a loss of 74 (42 + 32) mass units, i.e., propene from side chain A and methanol from side chain B; a *m/z* of 176 corresponding to the loss of 92 (18 + 42 + 32) mass units: water and propene from side chain A as

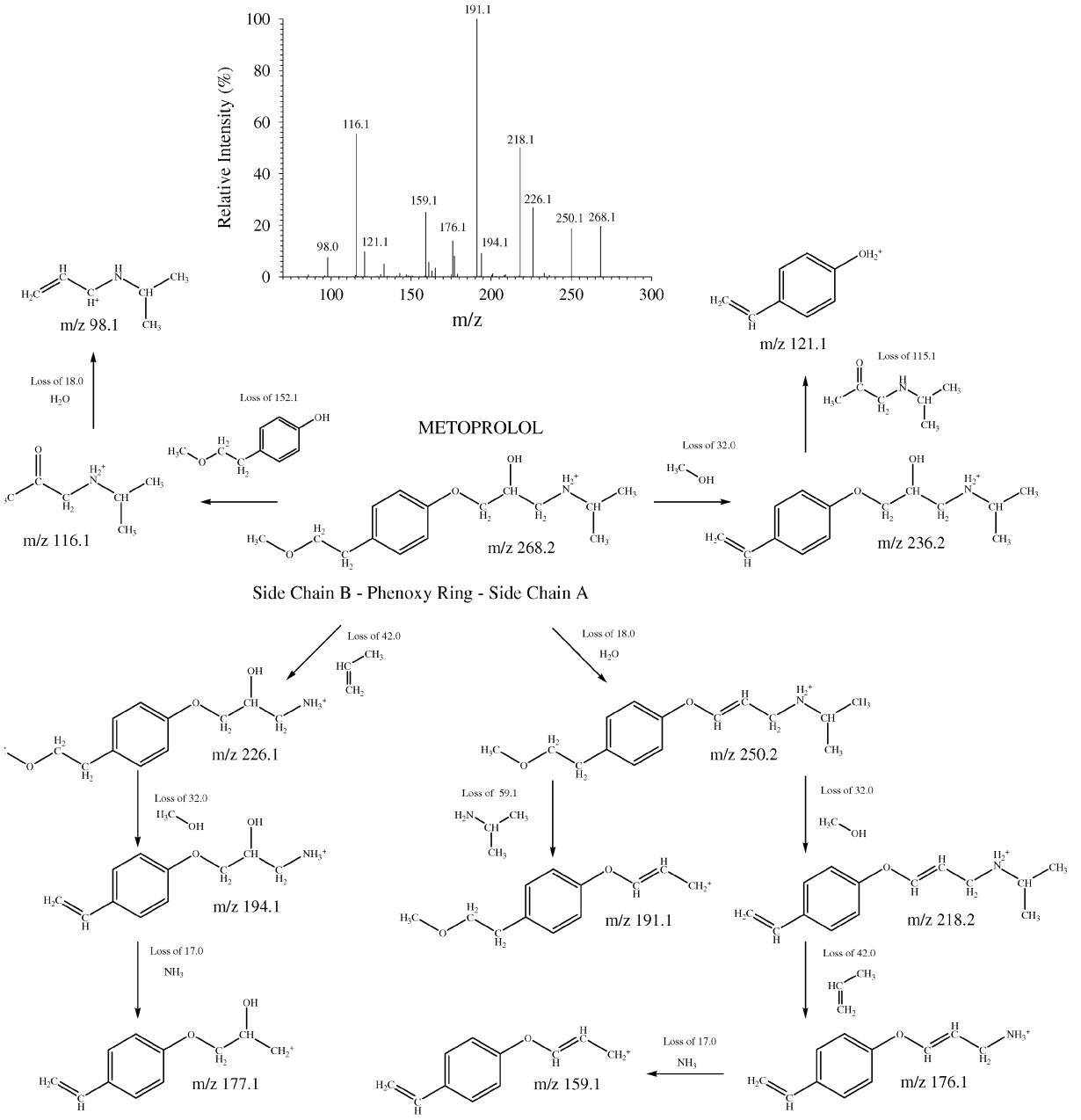


Fig. 4. Chemical-induced dissociation spectrum and fragmentation pathway of (±)-metoprolol (1).

well as the methanol from side chain B; a m/z of 159 corresponding to the loss of 109 (18 + 42 + 17 + 32) mass units: water, propene, ammonia from side chain A and methanol from side chain B; a m/z of 121 corresponding to a loss of 147 (115 + 32) mass units: 1-isopropylaminopropan-2-ol from side chain A and methanol from side chain B.

The fragments representative of side chain B and the phenoxy ring are: a m/z of 116 corresponding to the loss of a neutral fragment 152, i.e., 4-(2-methoxyethyl)-phenol; a m/z of 98 corresponds to the loss of 134 (18 + 152), i.e., water and the former fragment described.

3.2.2. Product (2)

Product (2), with m/z 238, is a main product in the gamma irradiations but is also present in the e-beam irradiations. The neutral fragment losses of 18, 42, 60

(18 + 42), 77 (18 + 42 + 17) and 115 mass units show that side chain A of (±)-metoprolol is intact. Compared to (±)-metoprolol (refer to Fig. 4), the m/z of 116 looses a neutral fragment of 122 instead of 152 mass units, the m/z of 98 looses a neutral fragment of 140 (122 + 18) instead of 170 (152 + 18) mass units. Since the loss of methanol is no longer observed and the fragmentation shows that the phenoxy group is intact, the modification of 30 mass units is on side chain B of (±)-metoprolol. The neutral fragment loss of 115 mass units gives a m/z of 123 instead of 121. Therefore, there is a modification of 2 mass units compared to the ethylene substituent ($C_2H_3 = 27 m/z$ units), i.e., an aldehyde substituent (CHO = 29 m/z units) to give compound (2) (Silverstein et al., 1991).

Compound (2) corresponds to an impurity that is sometimes found in (±)-metoprolol (Erickson et al., 1995) and it is synthesized so that its CID spectrum may serve as a reference to confirm the identity of the e-beam and gamma radiolytic products with m/z 238. The CID spectrum of compound (2) and its simplified fragmentation scheme are shown in Fig. 5. The radiolytic products with m/z 238 are confirmed to be compound (2) or [2-hydroxy-3-[(1-methylethyl)amino]propoxy]-benzaldehyde, since their CID spectra and retention times are identical.

3.2.3. Product (3 a, b)

This is the second most abundant isomer of the m/z 284 in the case of the gamma irradiations and it is also present in the e-beam irradiations. This product corresponds to the substitution of a hydrogen atom by a hydroxyl group (+16 mass units) on the metoprolol molecule, with m/z of 268. The CID spectra of the different mass isomers may reveal whether the hydroxyl group is on the phenoxy group, side chain A or side chain B of (±)-metoprolol.

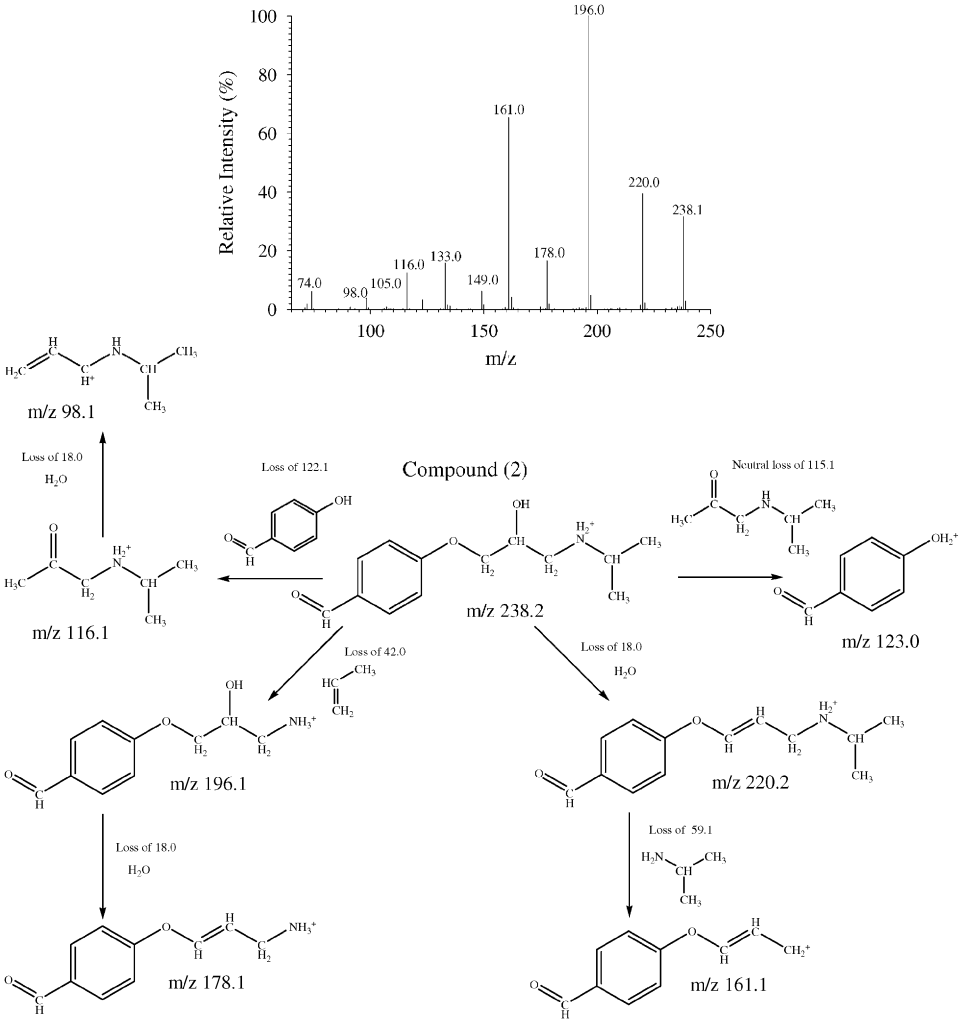


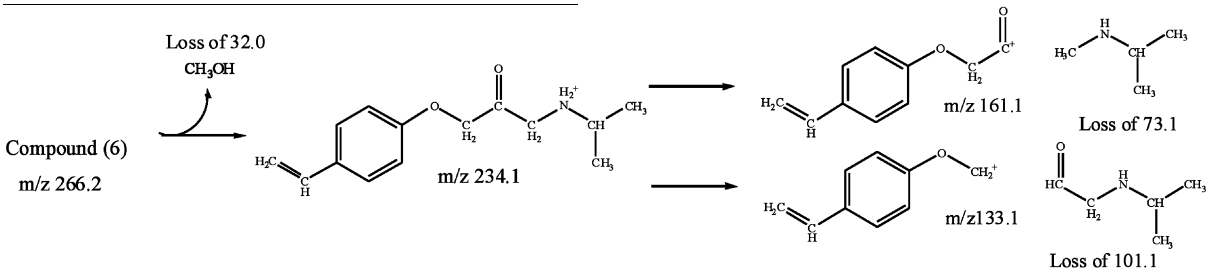
Fig. 5. Chemical-induced dissociation spectrum and fragmentation pathway of compound (2).

The neutral fragment losses of 18, 42 and 77 (18 + 42 + 17) mass units show that side chain A of (±)-metoprolol is intact. Compared to the fragmentation of (±)-metoprolol, the *m*/*z* of 116 has a neutral loss of 168 instead of 152 mass units, see Fig. 4. Therefore, the hydroxyl group is either on side chain B or on the phenoxy ring of (±)-metoprolol.

The *m*/*z* of 248 has a neutral loss of 36 (18 + 18) mass units and the *m*/*z* of 189 of has a neutral loss of 95 (18 + 42 + 17 + 18) mass units, and therefore, the product looses two water molecules which positions the hydroxyl group on side chain B of (±)-metoprolol.

The *m*/*z* of 175 has a neutral loss of 109 (18 + 42 + 17 + 32) mass units and this suggests the loss of methanol from side chain B. The MS^3 scan of the fragment that has lost water, with *m*/*z* 266, confirms the loss of methanol as it shows a fragment with *m*/*z* of 234. The substitution of a H-atom by a hydroxyl group is, therefore, on the α or β-carbon to the phenoxy group of (±)-metoprolol on side chain B.

A hydroxyl group on the β-carbon is highly improbable because it would form an unstable hemiacetal which hydrolyzes immediately into an alcohol and an aldehyde. Thus, the substitution of a H-atom by a hydroxyl group is more favorable on the α-carbon, forming α-hydroxymetoprolol or compound (3 a, b). Two diastereoisomers of α-hydroxymetoprolol are formed: a pair of enantiomers (2R,1′R and 2S,1′S) or (3 a) and a pair of enantiomers (2R,1′S and 2S,1′R) or (3 b). The separation of these diastereoisomers necessitates specific chiral stationary phases or special chiral derivatization techniques (Mistry et al., 2001).



3.2.4. Products (4) and (5)

These major radiolytic products are the same for both e-beam and gamma irradiations. Again, the CID spectra of the different mass isomers with *m*/*z* 284 may reveal whether the hydroxyl group is on the phenoxy group, side chain A or side chain B of (±)-metoprolol.

The neutral fragment losses of 18, 42 and 77 (18 + 42 + 17) mass units show that side chain A is intact. The neutral fragment losses of 32, 50 (18 + 32), 74 (42 + 32) and 109 (18 + 42 + 17 + 32) mass units reveal that the methoxy group is intact. The *m*/*z* of 116 looses a neutral fragment of 168 mass units, and thus the hydroxyl is either on side chain B or the phenoxy group. The fact that no further loss of water (18 mass units) is observed, i.e., the absence of the *m*/*z* 248, positions the hydroxyl group on the phenoxy ring of (±)-metoprolol as an ortho or meta substituent to give compounds (4) and (5), respectively.

3.2.5. Product (6)

This radiolytic product seems unique to e-beam irradiations as it is not detected in the gamma irradiations. Product (6) has a *m*/*z* of 266 which corresponds to the loss of 2 hydrogen atoms (−2 mass units) and its CID spectrum will reveal where the modification has taken place. The neutral losses of 32, 42, 59 (42 + 17), 74 (32 + 42) and 91 (32 + 59) mass units suggest that the methoxy and isopropylamino groups of (±)-metoprolol are intact.

The neutral loss of 152 mass units, i.e., 4-(2-methoxyethyl)-phenol, gives a *m*/*z* of 114 instead of 116 compared to (±)-metoprolol, refer to Fig. 4. Therefore, there is a loss of 2 mass units (H_2) on the 2-propanol of side chain A and thus the oxidation of the secondary alcohol into a propan-2-one to give compound (6). The presence of a ketone on side chain A is confirmed by the MS^3 spectrum of the fragment that has lost a methanol, with *m*/*z* 234. The MS^3 spectrum has two main fragments with *m*/*z* of 161 and 133 which may only result from the fragmentation of a ketone:

3.2.6. Product (7)

This is a major product in both e-beam and gamma irradiations and corresponds to a loss of 2 mass units (*m*/*z* 266) from (±)-metoprolol (*m*/*z* 268). Compared to the CID spectrum of (±)-metoprolol, shown in Fig. 4, the neutral loss of 18 mass units gives a *m*/*z* of 248 instead of 250. Similarly, the neutral loss of 42 mass units gives a *m*/*z* of 224 instead of 226; the neutral loss of 77 (18 + 42 + 17) mass units gives a *m*/*z* of 189 instead of 191. Therefore, side chain A is intact and the loss of 2 mass units is on side chain B.

The loss of 2 mass units on side chain B is confirmed by other fragments: the m/z of 116 has a neutral loss of 150 instead of 152 mass units; the m/z of 98 has a neutral loss of 168 (150 + 18) instead of 170 (152 + 18) mass units; the m/z of 159 has a neutral loss of 107 (18 + 42 + 17 + 30) instead of 109 (18 + 42 + 17 + 32) mass units; the m/z of 177 has a neutral loss of 89 (42 + 17 + 30) instead of 91 (42 + 17 + 32) mass units; the m/z of 218 has a neutral loss of 48 (18 + 30) instead of 50 (18 + 32) mass units. These fragments, plus the fact that the loss of methanol is no longer observed, reveal that the methoxy group is replaced by a fragment of 30 mass units (CH_2O) (Silverstein et al., 1991). The methoxyethyl on side chain B is replaced by a propanal group to give compound (7).

3.2.7. Product (8)

The product (8) with m/z 266 seems unique to gamma irradiations as it is not detected in the e-beam irradiations, see Table 1. In comparison to (±)-metoprolol, the neutral loss of 18 mass units gives a m/z of 248 instead of 250; the neutral loss of 42 mass units gives a m/z of 224 instead of 226; the neutral loss of 77 (18 + 42 + 17) mass units gives the m/z of 189 instead of 191. Moreover, the m/z of 116 has a neutral loss of 150 instead of 152 mass units and the m/z of 98 has a neutral loss of 168 (150 + 18) instead of 170 (152 + 18) mass units. Therefore, side chain A and the phenoxy ring of (±)-metoprolol are intact and the loss of 2 mass units is on side chain B.

The neutral losses characteristic of methanol, 32, 50 (18 + 32), 74 (42 + 32), 92 (18 + 32 + 42) and 109 (18 + 17 + 42 + 32) mass units, are no longer observed and this shows that the methoxy group is no longer present. A new neutral loss of 44 mass units is observed: the m/z of 204 has a neutral loss of 62 (18 + 44) mass units, the m/z of 162 has a neutral loss of 104 (18 + 42 + 44) mass units and the m/z of 145 has a neutral loss of 121 (18 + 17 + 42 + 44) mass units. The neutral loss of 44 mass units corresponds to ethanal (C_2H_4O) (Silverstein et al., 1991) and therefore the methoxyethyl group is replaced by a propane-2-one to give compound (8).

3.2.8. Product (9)

The product with m/z 254 corresponds to the loss of 14 mass units (CH_2) from (±)-metoprolol and could be O-demethylmetoprolol. This is a major product for gamma irradiations but it is also present in the e-beam irradiations.

The losses of 18, 42 and 77 (18 + 42 + 17) mass units show that side chain A of (±)-metoprolol is intact. The m/z of 116 corresponds to the loss of 138 instead of 152 mass units; the m/z of 98 has a loss of 156 (18 + 138) instead of 170 (18 + 152) mass units and this positions the loss of 14 mass units on side chain B. The absence of the neutral fragments, 32 and 50 (18 + 32) mass units, reveals that the methoxy group is no longer present. The radiolytic product looses two water molecules as shown by the loss of 36 (18 + 18) and 95 (18 + 18 + 42 + 17) mass units and thus has two hydroxyl groups. Compared to (±)-metoprolol, the m/z of 194 has a loss of 60 (42 + 18) instead of 74 (42 + 32) mass units and this reveals that the methoxy group is replaced by a hydroxyl group to give O-demethylmetoprolol or compound (9).

3.2.9. Product (10)

Product (10) is a major product in the gamma irradiations, but a lesser one in the e-beam irradiations. It has a m/z of 226 which corresponds to the loss of 42 mass units (C_3H_6) from (±)-metoprolol and could result from the loss of propene.

The fragment losses of 42 mass units are no longer observed. The neutral fragment losses of 32 and 50 (18 + 32) mass units show that the methoxy group of side chain B is intact. The m/z of 121 has a neutral loss of 105 instead of 147 mass units; the m/z of 74 has a neutral loss of 152 instead of 194 mass units; the m/z of 191 has a neutral loss of 35 instead of 77 mass units; the m/z of 159 has a loss of 67 instead of 109 mass units; and the m/z of 150 has a loss of 76 instead of 118 mass units. The fragmentation is consistent with the structure of compound (10).

3.2.10. Product (11)

Product (11) with m/z of 234 is present in both e-beam and gamma irradiations and could correspond to the loss of methanol (−32 mass units) from the e-beam radiolytic product with m/z of 266. The loss of 42 and 59 mass units show that the isopropylamino group from side chain A is intact. The MS^2 spectrum of the radiolytic product with m/z 234 is the same as the MS^3 spectrum of the fragment 234 from the m/z 266, see reaction (12) and Table 1. Therefore, this radiolytic product is compound (11).

The fact that product (11) is detected also in the gamma irradiations suggests that the radiolytic product from which it derives, i.e. product (6), is also present, but below the limit of detection.

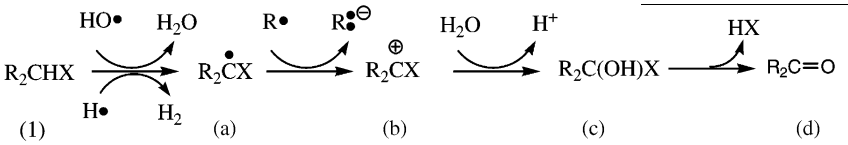
3.3. Formation mechanism of radiolytic products

When deaerated water absorbs ionizing radiations, it decomposes according to the following overall reaction: $H_2O \sim\!\!\rightarrow H^{\cdot}$, $HO^{\cdot}$, H_2, H_2O_2, e^-_{aq}, H_3O^+. The solute radiolysis is initiated by an attack of these primary species. Routes explaining the formation of compounds (2)–(11) in deaerated solution are proposed.

3.3.1. Routes to compounds (3 a, b), (6), (9) and (10)

These five compounds are obtained according to the same type of mechanism. (±)-Metoprolol (1) contains a

benzylic methylene, two ether functions, an alcohol and a secondary amine. These functions could be sketched as R_2CHX (1), X being an ARYL, OR, OH or NHR. In all cases, a carbon radical (a) is first obtained after hydrogen abstraction by the primary species $H^{\cdot}$ or $HO^{\cdot}$:



An oxidation of radical (a) then follows, by disproportionation with some other radical present in the solution during the continuous radiolysis. The resulting carbocation (b) reacts with water to give an alcohol (c). When X is an aryl, a stable compound (c) is obtained (3a, b). However, when X is OR, OH or NHR, compound (c) is a hemiacetal, a carbonyl hydrate or a hemiaminal, respectively. These organic functions are unstable in water and spontaneously decompose by loss of HX to give a carbonyl compound (d), e.g. (6).

Compound (9) originates from the modified metoprolol B chain: HO–CH_2–O–CH_2–CH_2–ARYL (c-type alcohol) by fragmentation in formaldehyde (d-type carbonyl compound) and HX = HO–CH_2–CH_2–ARYL (compound 9). Compound (10) originates from the modified metoprolol A chain: ARYL–O–CH_2–CH(OH)–CH_2–NH–$C(OH)(CH_3)_2$ (c-type alcohol) by fragmentation in acetone (d-type carbonyl compound) and HX = ARYL–O–CH_2–CH(OH)–CH_2–NH_2 (compound 10).

The carbon radical (a) oxidation/disproportionation giving a carbonyl derivative in the course of radiolysis of aqueous deaerated solutions has been amply documented in the case of alcohols (Awan et al., 1971; Castillo-Rojas et al., 1985; Naik et al., 2004), their phosphate esters (Kochetkov et al., 1974) and amines, notably amino acids (Anderson and Packer, 1974; Bhattacharyya and Saha, 1976; Bhattacharyya and Srisankar, 1976;

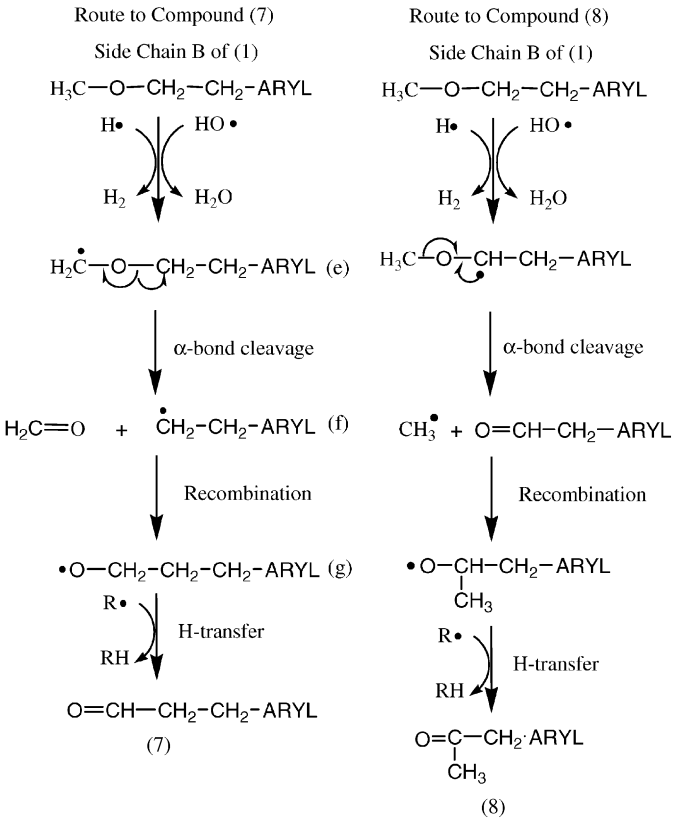


Fig. 6. Schemes for the formation of radiolytic products (7) and (8).

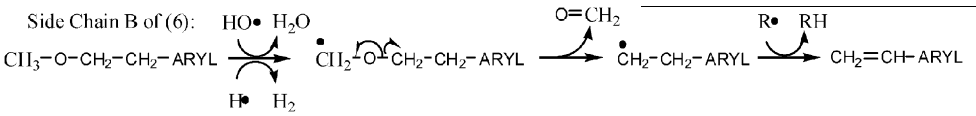
Willix and Garrison, 1965). Compounds (3 a, b), (6), (9) and (10) may be considered as primary radiolytic products.

3.3.2. Routes to compounds (4) and (5)

The reaction of the hydroxyl radical with benzene derivatives (Liu et al., 2005) and generally speaking aromatic compounds (Louit et al., 2005; Nicolaescu et al., 2005) to yield phenols has been intensively studied (Bhatia, 1974; Eberhardt, 1974; Klein and Schuler, 1978; Mantaka et al., 1971). The obtention of phenolic compounds (4) and (5) from (±)-metoprolol is a further example of this classical reaction. The hydroxyl radical adds to the benzene ring with poor positional selectivity (Albarran et al., 2003). The resulting hydroxycyclohexadienyl radicals are oxidized by disproportionation with some other radical present in the solution during the continuous radiolysis, to end up as phenols. The oxidation step may be interpreted as a hydrogen atom abstraction or alternatively as an electron transfer rapidly followed by a proton loss.

3.3.3. Route to compound (11) from (6)

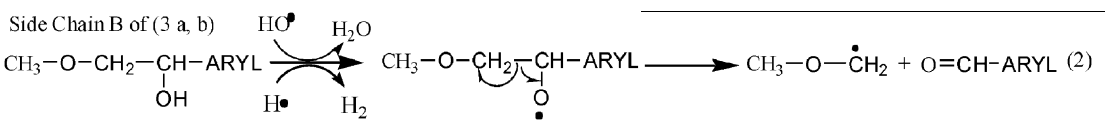
The usual hydrogen atom abstraction from the methoxy function of the primary radiolysis product (6) gives an alkoxy methyl radical. As also shown in Fig. 6, this type of radical may undergo an α-bond cleavage, followed here by an oxidation:



In this regard, compound (11) derives from the radiolysis of the radiolytic product (6) and the transformation is complete in the gamma irradiations since compound (6) is not detected. The difference in the profile of the radiolytic products between the e-beam and the gamma irradiations may be attributed to the reaction rate kinetics of the radiolysis of the radiolytic products which are affected by the dose-rate.

3.3.4. Route to compound (2) from (3 a, b)

The radiolysis product (2) also originates from the cleavage of a bond adjacent to a radical center, in this case the oxygen of the alcohol function of side chain B of compound (3 a, b):



Compound (2), therefore, derives from the radiolysis of the primary radiolytic product (3 a, b).

3.3.5. Route to compounds (7) and (8)

These two compounds are formed by a dramatic skeleton rearrangement as side chain B, initially a substituted ethyl group, becomes a propyl one: a propanal for compound (7) and a propanone for compound (8). This clearly points towards a radical recombination in the solvent cage. For compound (7), a hypothesis is as follows (see Fig. 6): the initially formed radical (e) cleaves to give formaldehyde and radical (f). These two fragments recombine to give radical (g). The aldehyde function of compound (7) may be formed from (g) by hydrogen abstraction on the methylene adjacent to the radical center. The same hypothesis holds for the route to compound (8). In this case, the skeleton rearrangement involves a transient methyl radical as may be seen in Fig. 6 (for theoretical calculations on this step, see Henry et al. (2004)).

Another fate for alkoxy radicals such as (g) could be a reduction to the corresponding alcohol by any suitable organic compound, RH, present in the solution ($RH + RO^{\cdot} \rightarrow R^{\cdot} + ROH$). A radiolytic product with 268m/z that is detected at a retention time of about 6.5 min at low doses of gamma radiation (500 Gy–10 kGy) could correspond to the alcohol related to (g).

4. Conclusion

LC–UV–MS^n techniques have proved to be very valuable in elucidating the structure and formation mechanism of 10 e-beam and gamma products, in the radiolysis of (±)-metoprolol tartrate aqueous solutions. A few primary radiolytic products are identified and the radiolysis of these is confirmed. The radiolysis mechanisms are highly complex but general trends appear as discussed for the formation of (3 a,b), (6), (9) and (10).

E-beam and gamma radiolytic products such as α-hydroxymetoprolol (3 a, b) and O-demethylmetoprolol (9) are also major metabolites of (±)-metoprolol formed in vivo, and this points towards similar oxidative

mechanisms between radiolysis and the cytochrome P-450. In both cases, a hydrogen abstraction is directly followed by an oxidation of the intermediate carbon radical (Jones et al., 1990; Sono et al., 1996).

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