Journal of Pharmaceutical and Biomedical Analysis 53 (2010) 755–761

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Short communication

Identification and characterization of a photolytic degradation product of telmisartan using LC–MS/TOF, LC–MS^n, LC–NMR and on-line H/D exchange mass studies

Ravi P. Shah, Saranjit Singh*

Department of Pharmaceutical Analysis, National Institute of Pharmaceutical Education and Research (NIPER), Sector 67, S.A.S. Nagar 160 062, Punjab, India

ARTICLE INFO

Article history:
Received 23 March 2010
Received in revised form 29 April 2010
Accepted 1 May 2010
Available online 8 May 2010

Keywords:
Telmisartan
Degradation product
LC–MS
LC–NMR
Hydrogen-deuterium exchange study

ABSTRACT

Telmisartan, an anti-hypertensive drug, was subjected to stress studies under ICH prescribed conditions of hydrolysis (acidic, neutral and basic), photolysis, oxidation and thermal stress. The drug showed labiality under only photo-acidic condition by forming a single degradation product. HPLC separation of the drug and the degradation product was achieved on C-8 column using gradient method. To characterize the product, a complete mass fragmentation pathway of the drug was initially established. Subsequently, the degradation product peak was subjected to LC–MS/TOF and on-line H/D exchange mass studies. Based on these studies, a tentative structure was assigned to the product as 3-((1,7′-dimethyl-2′-propyl-1H,3′H-2,5′-bibenzo[d]imidazol-3′-yl)methyl)-6H-benzo[c]chromen-6-one, which was verified through ^{1}H LC–NMR experiments.

1. Introduction

Telmisartan is an angiotensin II receptor antagonist used in the treatment of hypertension. Although, it was approved by US FDA in 2000 [1], no information exists in literature on identification and characterization of its degradation products. Only, a few analytical methods for the determination of drug in biological samples and in the presence of other drugs have been reported [2–5]. Hence, the purpose of the present study was to investigate the stress degradation behaviour of the drug, which was carried out by employing the following steps: (i) the drug was subjected to ICH prescribed hydrolysis, oxidative, photolytic and thermal stress [6,7], (ii) the stressed samples were analysed by HPLC, (iii) the mass fragmentation pattern of the drug was established using MS/TOF, MS^n and H/D exchange studies, (iv) the degradation product was characterized through LC–MS/TOF, on-line H/D exchange and LC–NMR data and comparison of the same with that of the drug, and (v) the elucidated structure of the degradation product was justified through mechanistic explanation.

2. Experimental

2.1. Drug and reagents

Pure telmisartan was obtained as gratis sample from Ranbaxy Research Laboratories (Gurgaon, India). Analytical reagent (AR) grade sodium hydroxide was purchased from Ranbaxy Laboratories (S.A.S. Nagar, India), hydrochloric acid from LOBA Chemie Pvt. Ltd. (Mumbai, India) and hydrogen peroxide from s.d. fine-chem Ltd. (Boisar, India). Buffer salts and all other chemicals of AR grade were bought from local suppliers. HPLC grade acetonitrile (ACN) was procured from J.T. Baker (Phillipsburg, NJ, USA). Deuterated acetonitrile (CD_3CN) and water (D_2O) of 99.9% purity were obtained from Aldrich (California, MO, USA). ES Tuning Mix solution (Agilent Technologies, USA) was used as a MS/TOF calibrant. Water for HPLC studies was obtained from ultrapure water purification unit (Elga, Wycombe, England).

2.2. Apparatus and equipment

The LC–UV system used for analyses of stressed samples consisted of basic modules and was equipped with a photo-diode array detector (all from Shimadzu, Kyoto, Japan). The system was controlled by Class-VP software (version 6.14 SP1). LC–MS/TOF studies were carried out on a system in which HPLC (1100, Agilent Technologies, Waldbronn, Germany) was hyphenated to MicrOTOF-Q spectrometer (Bruker Daltonik, Bremen, Germany). It was con-

* Corresponding author. Tel.: +91 172 2214682; fax: +91 172 2214692.
E-mail address: ssingh@niper.ac.in (S. Singh).

0731-7085/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2010.05.005

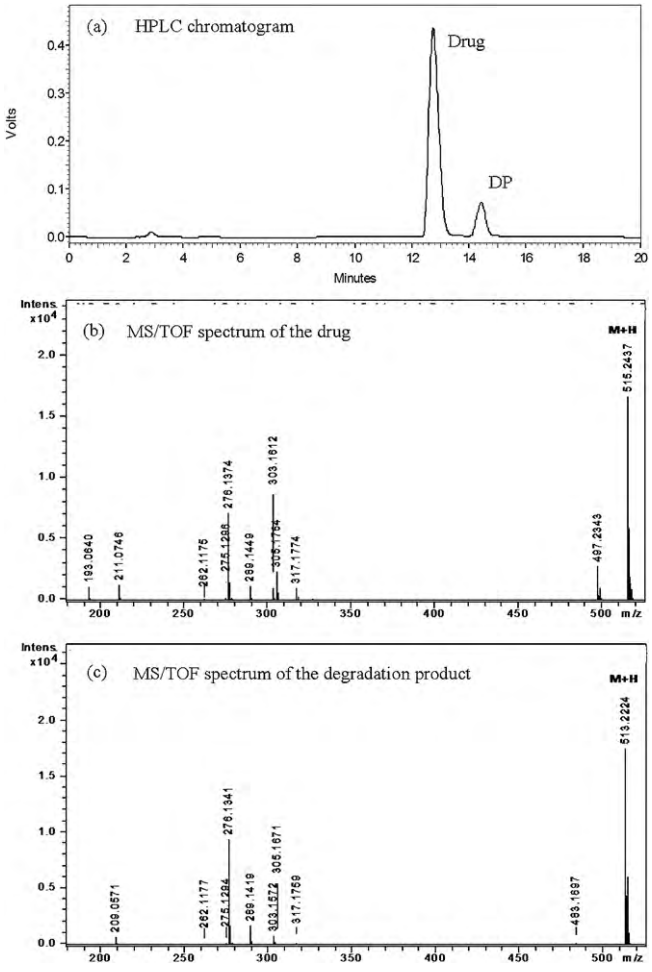


Fig. 1. HPLC and LC–MS studies: (a) HPLC chromatogram of the photo-acid stressed sample; (b) mass spectrum of the drug and (c) mass spectrum of the degradation product obtained in LC–MS/TOF.

trolled using Hyphenation Star (version 3.1) and MicrOTOF Control (version 2.0) software. On-line H/D exchange and multi stage mass studies (MS^n) were carried out on an LC–MS ion trap instrument (Thermo, San Jose, USA) wherein the LC system (Accela) was connected with MS (LTQ XL MS 2.5.0) via an ESI source and controlled by Xcalibur (version 2.0.7 SP1). LC–NMR measurements were done using JNM-ECA 500 MHz spectrometer (JEOL, Japan) coupled to Prominence HPLC system (Shimadzu, Kyoto, Japan), controlled by Delta (Version 4.3) and LC–NMR Dio (Version 2.0) software, respectively. In all the studies, separations were achieved on a Discovery C-8 column (250 mm × 4.6 mm i.d., particle size 5 μm) procured from Supelco (Bellefonate, PA, USA).

Table 2
MS^n fragmentation of the drug.

MS^n	Precursor ion	Product ions
MS^2	515	497, 305[a], 211
MS^3	497	317, 303, 289, 276, 262[a], 261[a], 193[a]
	211	193[a]
MS^4	317	302[a], 288[a], 274[a]
	303	288[a], 275[a], 262[a], 261[a]
	289	274[a], 261[a]
	276	262[a], 261[a]

[a] Fragments had low intensity, so could not be captured for further MS^n.

Precision water baths equipped with MV controller (Julabo, Seelbach, Germany) were used for solution degradation studies. A Dri-Bath (Thermolyne, IA, USA) was used for solid state thermal stress study. Photostability studies were carried out in photostability (KBWF 240, WTC Binder, Tuttlingen, Germany) chamber, set at 40 ± 1 °C/75 ± 3% RH. The latter was equipped with an illumination bank on inside top, consisting of a combination of two UV (OSRAM L18 W/73) and four white fluorescent (PHILIPS TRULITE 18W/86) lamps, in accordance with Option 2 of the ICH guideline Q1B [7]. Both fluorescent and UV lamps were put on simultaneously. The samples were placed at a distance of 0.23 m from the light bank. A lux meter (model ELM 201, Escorp, New Delhi, India) and a UV radiometer (model 206, PRC Krochmann GmbH, Berlin, Germany) were used to measure visible illumination and UV energy, respectively. A pH/Ion analyser (MA 235, Mettler Toledo, Schwerzenbach, Switzerland) was used to check and adjust the pH of buffer solutions. Other small equipments were sonicator (3210, Branson Ultrasoincs Corporation, Danbury, CT, USA), precision analytical balance (AG 135, Mettler Toledo, Schwerzenbach, Switzerland) and auto pipettes (Eppendorf, Hamburg, Germany).

2.3. Generation of stress samples

Stress studies were carried out under ICH recommended conditions of hydrolysis, photolysis, oxidation and dry heat. The stressors, choice of their concentration and preparation of samples were based on our previous publication [8]. As the drug was insoluble in water, it was dissolved in a mixture of ACN and water in a ratio of 50:50 (v/v) to a final concentration of 2 mg/ml. The stock was diluted 50:50 (v/v) with the stressor (e.g., HCl, NaOH, H_2O_2 and water). Hydrolytic decomposition of the drug was carried out in 2N HCl, water and 2N NaOH at 80 °C for 4 days. The oxidative study was carried out in 30% (v/v) H_2O_2 at room temperature for 5 days. For thermal stress testing, the drug was sealed in glass vials

Table 1
MS/TOF and on-line H/D exchange data of the drug and its fragments.

Accurate masses of the drug and its major fragments	Best possible molecular formulae	Exact mass of most probable structures	Error in mmu	RDB	Mass after H/D exchange in ESI +ve mode	Number of labile hydrogens
515.2437	$C_{33}H_{31}N_4O_2$	515.2442	−0.452	20.5	517	2
497.2343	$C_{33}H_{29}N_4O$	497.2336	0.712	14.5	497, 498	0, 1
317.1774	$C_{20}H_{21}N_4$	317.1761	1.327	12.5	317, 318	0, 1
305.1764	$C_{19}H_{21}N_4$	305.1761	0.327	11.5	307	2
303.1612	$C_{19}H_{19}N_4$	303.1604	0.777	12.5	303, 304	0, 1
289.1449	$C_{18}H_{17}N_4$	289.1448	0.127	12.5	289, 290, 291	0, 1, 2
276.1374	$C_{18}H_{18}N_3$	276.1495	−12.124	11.5	276, 277	0, 1
275.1296	$C_{17}H_{15}N_4$	275.1291	0.477	12.5	275, 276	0, 1
262.1175	$C_{17}H_{16}N_3$	262.1339	−16.374	11.5	263, 264	1, 2
211.0746	$C_{14}H_{11}O_2$	211.0754	−0.756	9.5	212	1
193.0640	$C_{14}H_9O$	193.0648	−0.791	10.5	193	0

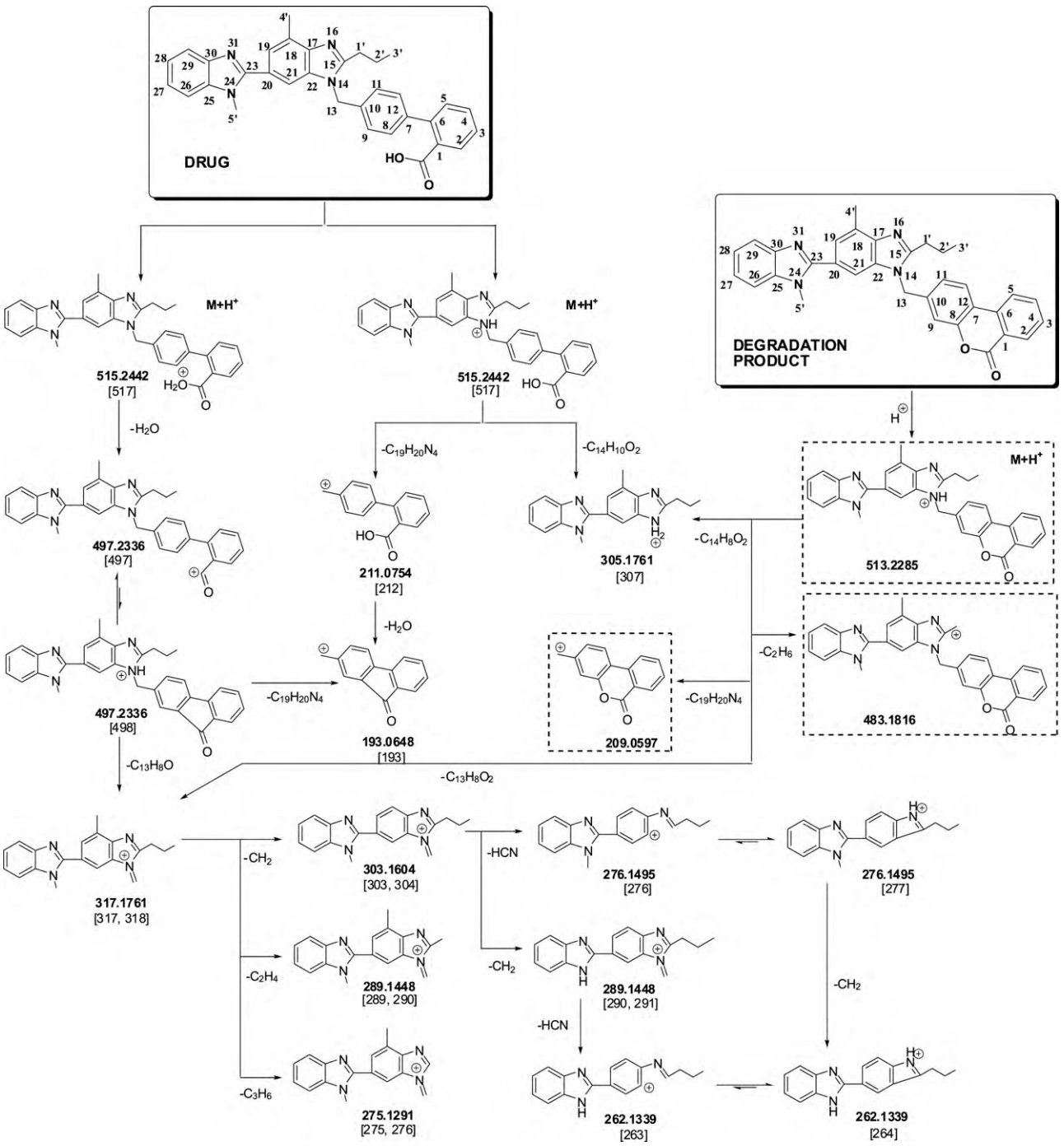


Fig. 2. Mass fragmentation pattern of the drug and the degradation product. The value of calculated accurate mass is shown below each structure, along with the mass obtained in H/D exchange study (in square brackets); the structures shown in dotted square were additional mass fragments formed from degradation product.

and placed in a thermostatic block at 50 °C for 21 days. Photolytic studies on the drug in the solution state were carried out in 0.1N HCl, water and 0.1N NaOH by exposing it for 13 days to a combination of fluorescent and UV light in a photostability chamber at 8500 lx and 0.05 W/m^2, respectively. Photolytic studies in the solid state were performed by exposing a thin layer of the drug to light under similar conditions as that of the solution state. Parallel blank sets were kept in the dark for 13 days. After subjecting to stress, samples were withdrawn at suitable time intervals and diluted four folds with ACN and water (50:50, v/v) before injection into HPLC.

2.4. Chromatographic conditions

The separation of components in stressed studies was achieved using mobile phase composed of ACN (A) and potassium dihydrogen phosphate buffer (0.01 M; pH 3.0) (B). The acceptable resolution was achieved in a gradient mode (T_{min}/A:B; T_0/35:65; T_7/40:60; T_{10}/40:60; T_{12}/35:65; T_{20}/35:65). The detection wavelength and flow rate were 250 nm and 1.0 ml/min, respectively. For LC–NMR investigations, the method was extended for 30 min at a flow rate of 0.5 ml/min. For LC–MS studies, the buffer component B was replaced by 0.01 M ammonium acetate of the same pH.

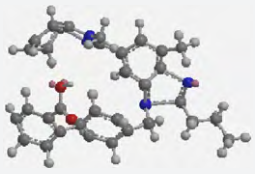
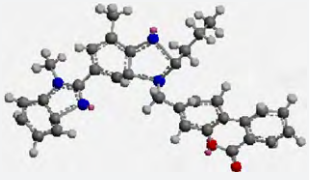
Table 3
MS/TOF and on-line H/D exchange data of the degradation product and its fragments.

Accurate mass of the degradation product	Molecular formula (exact mass; error in mmu; RDB)	Accurate mass of major fragments (chemical formula; error in mmu)	Mass of the degradation product after H/D exchange in ESI +ve mode	Number of labile hydrogens
513.2224	$C_{33}H_{29}N_4O_2^+$ (513.2285; −6.1; 21.5)	483.1697 ($C_{18}H_{20}N_5$; −2.9) 317.1759 ($C_{20}H_{21}N_4$; −0.2) 305.1671 ($C_{19}H_{21}N_4$; −8.9) 303.1572 ($C_{19}H_{19}N_4$; −3.2) 289.1419 ($C_{18}H_{17}N_4$; −2.8) 276.1341 ($C_{18}H_{18}N_3$; −15.4) 275.1294 ($C_{17}H_{15}N_4$; 0.3) 262.1177 ($C_{17}H_{16}N_3$; −16.2) 209.0571 ($C_{14}H_9O_2$; −2.6)	514	0

2.5. MS/TOF, MS^n and H/D exchange studies on drug

Mass fragmentation pattern of the drug was established with the help of MS/TOF, MS^n and H/D exchange mass data. MS/TOF studies were performed in ESI positive mode, and various parameters were suitably optimized in order to get clear information regarding the molecular ion peak of the drug. Mass parameters were optimized to the following values: end plate offset, −500 V; capillary voltage, −4500 V; nebuliser gas pressure, 1.2 bar; dry gas flow, 6.0 l/min; dry temperature, 200 °C; funnel 1 RF, 200 Vpp; funnel 2 RF, 200 Vpp; ISCID energy, 15.0 eV; hexapole RF, 280 Vpp; quadrupole ion energy, 17.0 eV/Z; collision energy, 15.0 eV/z; transfer time, 40 μs; collision RF, 300 Vpp; pre-pulse storage, 5.0 μs. Further, quadrupole ion energy, collision energy, transfer time, collision RF and pre-pulse storage were modified to 4.0 eV/Z, 32.0 eV/z, 38 μs, 280 Vpp and 4.0 μs, respectively, to get complete fragmentation of the drug. In the subsequent step, the information on the origin of each individual fragment was obtained from MS^n studies.

Table 4
Energy minimized 3D structures and ^{1}H peak assignments for the drug and the degradation product.

	Energy minimized 3D structure	
	Drug	Degradation product
		
Position	δ_H/ppm (multiplicity)	
2	7.862 (m)	8.310 (d)
3	7.717 (m)	7.711 (m)
4	7.717 (m)	7.981 (m)
5	7.862 (m)	8.325 (d)
8	7.346 (d)	–
9	7.406 (d)	7.788 (s)
11	7.406 (d)	7.354 (d)
12	7.346 (d)	8.287 (d)
13	5.810 (s)	5.878 (s)
19	7.793 (s)	7.283 (s)
21	7.906 (s)	7.839 (s)
26	7.862 (m)	7.878 (m)
27	7.509 (t)	7.711 (m)
28	7.620 (t)	7.711 (m)
29	7.369 (d)	7.878 (m)
1′	[a]	[a]
2′	1.858 (m)	1.880 (m)
3′	1.030 (t)	1.027 (t)
4′	2.755 (s)	2.763 (s)
5′	3.911 (s)	3.908 (s)

[a] ^{1}H peaks were not observed due to suppression region.

Fragmentation of various precursor ions was achieved at different normalized collision energies. Other parameters were optimized to the following values: capillary temperature, 325 °C; capillary voltage, 15 V; tube lens, 85 V; spray voltage, 4.75 kV; sheath gas flow rate, 25 arb. The structure of each fragment was further verified with the help of H/D exchange studies, which were carried out by injecting drug solution prepared in D_2O and CD_3CN (50:50%, v/v).

2.6. LC–MS/TOF and on-line H/D exchange studies on degradation product

The stressed sample containing degradation product was subjected to LC–MS/TOF analyses using the optimized MS/TOF parameters. For internal calibration, the ES Tuning Mix solution was injected through a diverter in a specific segment near the peak of interest. The on-line H/D exchange mass studies were carried out using the LTQ–MS^n system through injection of D_2O via an additional channel, just before the peak of interest started eluting from the column. The flow of D_2O was continued until the peak was completely eluted.

2.7. LC–NMR studies

The stressed sample containing the targeted degradation product was subjected to LC–NMR for recording ^{1}H NMR spectra of both the drug and the product. The resolved peaks of the drug and the degradation product were collected in the fraction loop using terminal cube and sent to inverse 3 mm flow probe equipped with ^{1}H $\{^{13}C\}$ channels and pulsed-field gradient along *z*-axis. The active sample volume of the probe was approximately 60 μl and the transfer time from the UV cell to the active volume was 45 s at a flow rate of 0.5 ml/min. One-dimensional ^{1}H NMR spectra were recorded using the WET pulse sequences, with attenuated power at 67.5 [dB] for solvent suppression of CH_3CN and HOD signal, which gave digital resolution of 0.76, and 0.95 Hz per point for drug and degradation product, respectively. Spectra were acquired with 15 kHz spectral width and 16 K data points. The chemical shifts were referenced to methyl signal of the residual CH_3OH at 3.30 ppm.

3. Results and discussion

3.1. Degradation behaviour

The drug showed degradation only in photo-acidic condition, while it was stable to all other studied conditions. The chromatogram of the photo-degraded sample is shown in Fig. 1a.

3.2. Mass fragmentation behaviour of the drug

Fig. 1b shows the mass spectrum of the drug obtained from MS/TOF studies. In total, 10 fragments were formed from the drug.

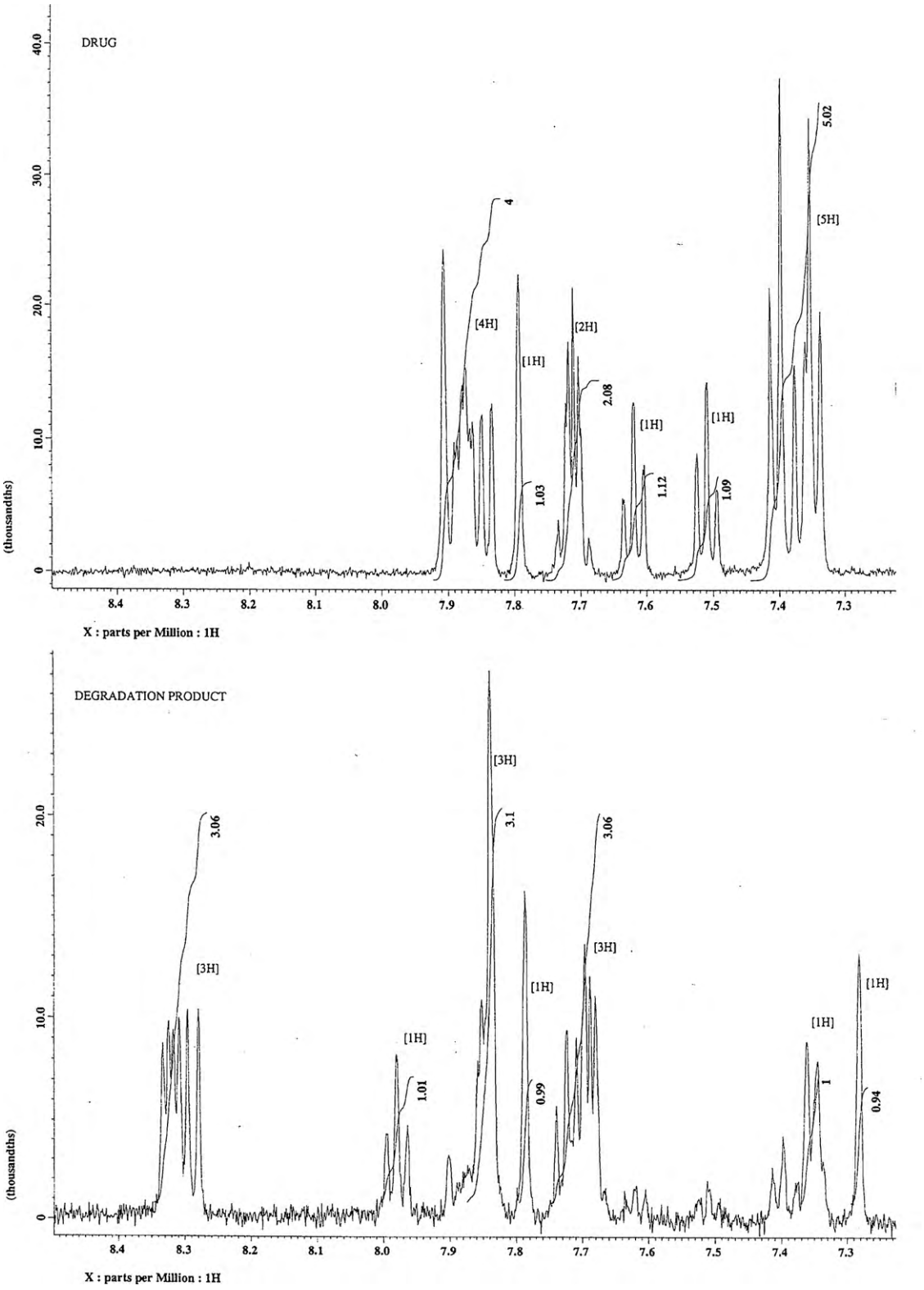


Fig. 3. ^{1}H LC–NMR spectra of aromatic region (δ 7.0–8.5 ppm) for the drug (upper) and the degradation product (lower). The figures show a total of 14 and 13 hydrogens in aromatic region, respectively.

The most probable molecular formula for each fragment, calculated with the help of Elemental Composition Calculator, along with errors in mmu, is shown in Table 1. The reason for high error in the case of *m*/*z* 262 and *m*/*z* 276 might be due to low intensity of fragment ion and co-appearance of isotopic peak of *m*/*z* 275, respectively. The H/D exchange data are also included in the table, in which some of the fragments showed two/three mass values (e.g., *m*/*z* 317 and 318; *m*/*z* 289, 290 and 291, etc.) instead of one fragment. These additional mass values were indicated to be due to incorporation of D^+ in the fragments from the surrounding deuterated solvent and/or possibility of two structures for the same mass. The data from MS^n studies (Table 2) showed one mass unit less than

the observed *m*/*z* of the fragments from MS/TOF. This could be due to formation of radical cations instead of positive ions in the ion trap at different collision energies. Further, the drug had four aromatic rings, which helped in stabilization of these radical cations by resonance phenomena. These data from MS^n studies were helpful in establishment of fragmentation pathway of the drug, which is outlined in Fig. 2.

The figure shows existence and involvement of two possible protonated forms of the precursor (*m*/*z* 515) in the drug's fragmentation pathway. The precursor with protonation at the carboxylic group produced a daughter ion of *m*/*z* 497 by losing water and the same could exist as non-cyclic and resonating cyclic structure, as shown in Fig. 2. This resonance phenomenon was confirmed through H/D exchange, as the fragment of *m*/*z* 497 corresponded to two fragments of *m*/*z* 497 and 498. The ion of *m*/*z* 497 was further fragmented to *m*/*z* 317 and 193 through cleavage of C–C and C–N bond, respectively. For *m*/*z* 317 as well, two corresponding fragments of *m*/*z* 317 and 318 were observed in H/D exchange study. The additional labile hydrogen of *m*/*z* 318 could be attributed to the incorporation of D^+ from the surrounding deuterated solvent. The ion of *m*/*z* 317 was fragmented to a number of other ions, i.e., *m*/*z* 303, 289, 276, 275 and 262 by removal of methyl, ethyl and propyl groups along with neutral loss of hydrogen cyanide. The drug (precursor) with protonation at the second site, i.e., imidazole nitrogen, reduced to parallel ions of *m*/*z* 305 and 211. The ion of *m*/*z* 211 was further fragmented into an ion of *m*/*z* 193 by losing water molecule. The best possible structures of all these fragments were elucidated based on available HRMS, MS^n and H/D exchange mass data.

3.3. LC–MS/TOF, on-line H/D exchange studies and ^{1}H LC–NMR studies on degradation product

The LC–MS/TOF analysis of the degradation product (Fig. 1c) showed molecular ion peak of *m*/*z* 513, two mass units less than the drug. The H/D exchange resulted in ion of *m*/*z* 514 indicating absence of labile hydrogen. The molecular formulae for the molecular ion peak as well as fragments were calculated from their accurate mass values, with the help of Elemental Composition Calculator. The same are included in Table 3.

^{1}H LC–NMR spectra of the drug showed similar peaks for both the drug and its degradation product except in the aromatic region. ^{1}H assignments of the drug and the degradation product are listed in Table 4. Further, the integration in aromatic region showed 14 and 13 hydrogens for the drug and the degradation product, respectively (Fig. 3).

3.4. Characterization of degradation product

The structure elucidation of the degradation product was achieved by systematic amalgamation of HRMS (MS/TOF), mass fragmentation, on-line H/D exchange and ^{1}H LC–NMR data.

The experimental mass (513.2224 Da) of this photolytic product was ~2 Da less than the drug. The only possible chemical formula that justified the nitrogen rule was $C_{33}H_{29}N_4O_2^+$, having two hydrogens less than the drug, suggesting the presence of an additional double bond or ring formation in the structure. The absence of any labile hydrogen, as observed through H/D exchange studies indirectly indicated that the lone labile hydrogen in the drug, i.e., –COOH attached to biphenyl ring was utilized in cyclization. The appearance of MS/TOF fragments of *m*/*z* 317, 305, 303, 289, 276, 275 and 262 in case of degradation product indicated the presence of 1,7′-dimethyl-2′-propyl-1H,3′H-2,5′-bibenzo[d]imidazole moiety, similar to the drug. However, the presence of an ion of *m*/*z* 209, with mass of 2 Da less than the corresponding fragment of the drug (*m*/*z* 211), showed that the change occurred in rest of the molecule. Taking into consideration these observations, the fragmentation pathway for the photolytic degradation product could be outlined, which is included in Fig. 2. According to it, one of the aromatic hydrogen was utilized in cyclization of the degradation product. This was confirmed through LC ^{1}H NMR study (Fig. 3, Table 4). The LC ^{1}H NMR data of the degradation product showed absence of *H*8 peak. Moreover, *H*2, *H*5, *H*9 and *H*12 were significantly downfielded compared to the drug indirectly indicating formation of a cyclic lactone. Though the drug and the degradation product had similarity in structure, but the same was not reflected in ^{1}H peak assignments (Fig. 3). These dissimilarities in chemical shifts could be due to different spatial non-bonding interactions. To confirm the same, 3D structures of the drug and the degradation product were derived through energy minimization by Chem3D MM2 and Sybyl programs. As shown from 3D structures in Table 4, there was significant difference in spatial conformations between the two.

3.5. Mechanistic explanation to the origin of the degradation product

The mechanism of formation of the degradation product from the drug is given in Fig. 4. As a matter of fact, aromatic cyclization is

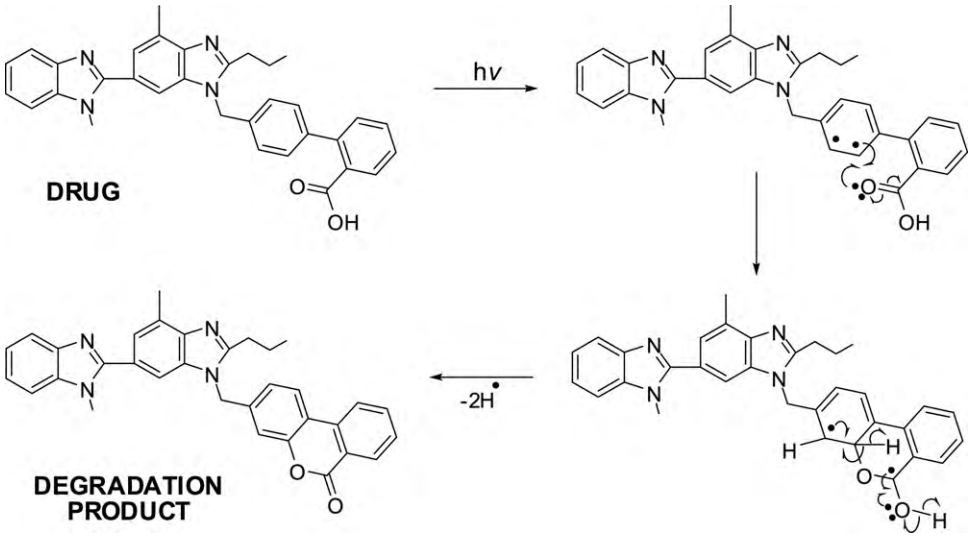


Fig. 4. Mechanism of formation of the degradation product from the drug.

a pre-known phenomenon under photolytic condition [9–11]. The exposure of the drug to UV radiations resulted in promotion of π-electrons to a non-bonding orbital yielding two aryl radicals [12], one of which attacked the adjacent carbonyl of the –COOH to result in a cyclized degradation product.

4. Conclusions

Stress degradation studies on telmisartan, carried out according to ICH guidelines, provided information regarding degradation behaviour of the drug. The drug was only susceptible to photo-acidic degradation, whereas it was stable in all other conditions. The degradation product formed in stressed sample was characterized using LC–MS, on-line H/D exchange mass and LC–NMR studies. It was elucidated as tricyclic lactone of the drug, *viz.*, 3-((1,7′-dimethyl-2′-propyl-1H,3′H-2,5′-bibenzo[d]imidazol-3′-yl)methyl)-6H-benzo[c]chromen-6-one. During the studies, a complete mass fragmentation pathway of the drug was established, which was not known earlier. The latter may be useful in future investigations on characterization of process related impurities, drug-excipient interaction products, and/or metabolites of the drug.

References

[1] Available at: http://www.medicinenet.com/telmisartan/article.htm (accessed on 10 January 2010).
[2] M.R. Brunetto, Y. Contreras, S. Clavijo, D. Torres, Y. Delgado, F. Ovalles, C. Ayala, M. Gallignani, J.M. Estela, V.C. Martin, Determination of losartan, telmisartan, and valsartan by direct injection of human urine into a column-switching liquid chromatographic system with fluorescence detection, J. Pharm. Biomed. Anal. 50 (2009) 194–199.
[3] B. Chen, Y. Liang, Y. Wang, F. Deng, P. Zhou, F. Guo, L. Huang, Development and validation of liquid chromatography–mass spectrometry method for the determination of telmisartan in human plasma, Anal. Chim. Acta 540 (2005) 367–373.
[4] L. Kristoffersen, E.L. Øiestad, M.S. Opdal, M. Krogh, E. Lundanes, A.S. Christophersen, Simultaneous determination of 6 beta-blockers, 3 calcium-channel antagonists, 4 angiotensin-II antagonists and 1 antiarrhytmic drug in post-mortem whole blood by automated solid phase extraction and liquid chromatography mass spectrometry: method development and robustness testing by experimental design, J. Chromatogr. B 850 (2007) 147–160.
[5] X. Zhu, J. Sun, H. Hao, G. Wang, X. Hu, H. Lv, S. Gu, X. Wu, J. Xu, Simultaneous determination of multiple angiotensin type 1 receptor antagonists and its application to high-throughput pharmacokinetic study: cassette dosing versus cassette analysis, Int. J. Mass Spectrom. 272 (2008) 127–136.
[6] ICH, Stability testing of new drug substances and products Q1A(R2), in: International Conference on Harmonisation, IFPMA, Geneva, 2003.
[7] ICH, Stability testing: photostability testing of new drug substances and products Q1B, in: International Conference on Harmonisation, IFPMA, Geneva, 1996.
[8] S. Singh, M. Bakshi, Guidance on the conduct of stress tests to determine inherent stability of drugs, Pharm. Tech. On-line (2000) 1–14.
[9] R.P. Shah, V. Kumar, S. Singh, Liquid chromatography/mass spectrometric studies on atorvastatin and its stress degradation products, Rapid Commun. Mass Spectrom. 22 (2008) 613–622.
[10] S. Mehta, R.P. Shah, S. Singh, Strategy for identification and characterization of small quantities of drug degradation products using LC and LC–MS: application to valsartan, a model drug, Drug Test Anal. 2 (2010) 82–90.
[11] R.P. Shah, A. Sahu, S. Singh, Identification and characterization of degradation products of irbesartan using LC–MS/TOF, MS^n, on-line H/D exchange and LC–NMR, J. Pharm. Biomed. Anal. 51 (2010) 1037–1046.
[12] D.E. Moore, in: H.H. Tønnesen (Ed.), The Photostability of Drugs and Drug Formulations, 2nd ed., Taylor and Francis, London, 2004, pp. 10–37.