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SEPARATION AND CHARACTERIZATION OF MAJOR OXIDATIVE IMPURITY IN FIMASARTAN DRUG SUBSTANCE

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ABSTRACT

In the stress degradation studies of Fimasartan, one major unknown oxidative degradation impurity was identified by LC-MS. This impurity was separated by preparative HPLC. By spectral data analysis (^{1}H NMR, ^{13}C NMR, DEPT, MS/MS and IR), this impurity is characterized as 2-(1-((2'-(1H-tetrazol-5-yl)-[1,1'-biphenyl]-4-yl)methyl)-2-butyl-1,6 –dihydro-4-methyl-6-oxo-pyrimidin-5-yl)-N,N-dimethylacetamide. The details of stress studies, identification, isolation, characterization, formation and mechanism of this impurity are discussed and presented here.

Keywords: Fimasartan, Degradation, Identification, Isolation, Characterization

INTRODUCTION

Fimasartan (FIMA) (Fig.-1) is an antihypertensive agent. It is ninth non-peptide angiotensin II receptor antagonist (ARB)[1]. It is also used for the treatment of heart failure. Fimasartan acts by blocking angiotensin II receptor type I (AT1 receptor). Fimasartan is pyrimidin-4(3H)-one derivative of losartan which is obtained by replacement of imidazole ring in losartan. Fimasartan has higher potency and longer duration than losartan. Fimasartan was approved in South Korea in September 9, 2010. It is marketed as Kanarb by Boryung Pharmaceuticals in Korea. It is available as a tablet for oral use which contains 60 mg or 120 mg of Fimasartan potassium trihydrate[2]. It is approved in India by CDSCO in 2016. HPLC method has been developed for evaluation of stability and simultaneous determination of fimasartan and amlodipine in tablet dosage form[3]. UPLC tandem mass chromatographic method has been reported for determination of fimasartan in human plasma[4]. Literature has been reported on LC-MS method development for the estimation of fimasartan in human plasma[5-7].Literature has been reported on pharmacokinetics and metabolite profiling of fimasartan[8].

Recently we have developed a stability indicating method development of Fimasartan[9]. Major degradation was observed in oxidative condition. The objective of this study was to identify the major degradation product in oxidative condition after its isolation and characterization by mass, NMR and IR.

EXPERIMENTAL

Chemical Reagents and Solutions

Fimasartan (FIMA) standard drug was obtained from Angene Chemical Ltd (China). HPLC grade Acetonitrile was purchased from Rankem Pvt. Ltd., Mumbai. Chemicals used in the analysis were potassium dihydrogen ortho phosphate(AR grade), ortho phosphoric acid, formic acid purchased from Loba Chemie Pvt. Ltd., Mumbai. Hydrogen peroxide (H_2O_2) 30% v/v was purchased from S.D. Fine Chemical Ltd, Mumbai.

Preparation of Mobile Phase

10 mm phosphate buffer (pH 3) was prepared by dissolving 1.37 g of potassium dihydrogen phosphate in sufficient double distilled water to produce 1000ml and then the pH of the buffer was adjusted to 3.0 with

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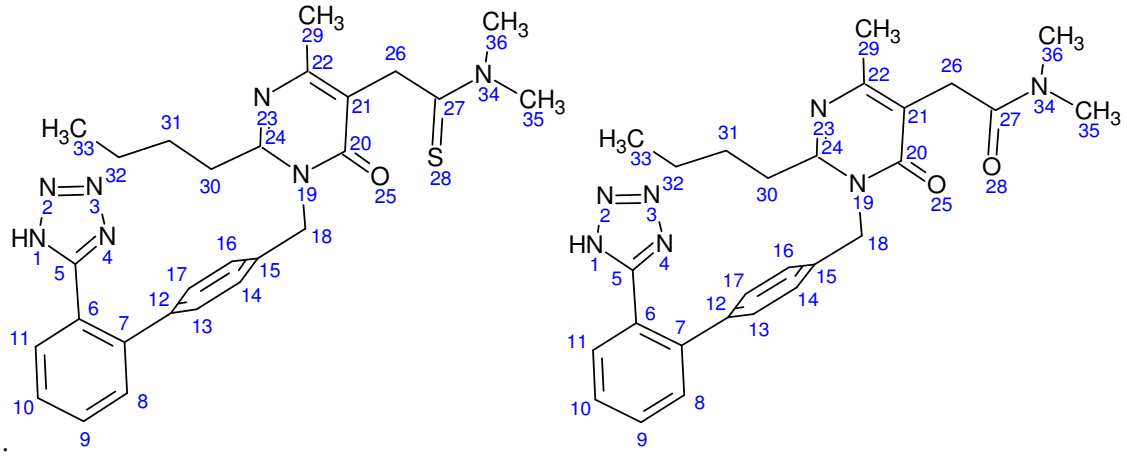
ortho phosphoric acid. Composition of mobile phase was having phosphate buffer and acetonitrile in the ratio of 50:50.



Fimasartan Degradation Product (DP)

Fig. -1: Structure of FIMASARTAN (FIMA) and Degradation Product(DP)

Instrumentation and Chromatographic Conditions

LC- PDA

The method was performed on a Shimadzu Prominence HPLC system containing an LC-20AD binary pump, Shimadzu SPD M-20A PDA detector. Samples were injected with a fixed loop of 20µL Rheodyne 7725 injector valve with a flow rate of 1ml/min. Data analysis was performed with LC solutions software (Shimadzu Corporation, Kyoto, Japan).The method was developed on a Hypersil BDS C-18 column (5µmX250mmX4.6 mm i.d.).The flow rate was 1ml/min. Detection was performed at 262nm. Before analysis, the mobile phase was filtered through 0.2 µ nylon N 6,6 membrane filter and sonicated for 5 min.

LC-UV (Preparative)

Chromatographic separation was performed on Shimadzu (Shimadzu Corporation, Kyoto, Japan), a chromatographic system equipped with Shimadzu LC-20 AP binary pump and Shimadzu SPD-20A detector. Samples were injected through Rheodyne 7725 injector valve. Data acquisition was performed with Class VP software. Daisogel-SP-100-10-ODS-P column was used for isolation. The flow rate kept at 5ml/min. Detection was performed at 262nm. The mobile phase in LC-PDA was replaced with 0.1% formic acid and acetonitrile in the ratio of 45:55.

LC-MS

LC-MS was performed on LCQ fleet (Thermo Fischer Scientific instrument).The system was coupled with quaternary system delivery module in positive and negative ESI (Electro Spray Ionization). The nebulizer pressure was set at 20 psi. The gas temperature was set at capillary voltage 5500V using nitrogen gas and gas temperature was set at 250ºC using drying gas nitrogen at 30 psi pressure. Data acquisition was done with Xcalibur software. The mobile phase containing 10 mm phosphate buffer pH 3 in LC-PDA was replaced by 0.1 % formic acid and acetonitrile in the ratio of 45: 55 in LC-MS.

NMR Spectroscopy

^{1}H and ^{13}C NMR spectra of FIMA and its degradation product were recorded by using Bruker Avance II 400 NMR spectrometer that consisted of dual broad band probe and z-axis gradients. DMSO-d_6 was used

as a solvent for analysis. ^{1}H and ^{13}C NMR chemical shifts were calculated on δ scale in ppm with respect to tetramethyl silane as internal standard (δ 0.00 ppm).

FT-IR Spectroscopy

FT-IR spectra were recorded as Schimadzu 8400s FT-IR spectrometer.

Isolation of Oxidative Degradation Sample

Our previous studies[9] have shown that FIMA undergoes extensive oxidative degradation. Efforts were made to use stronger conditions so as to achieve total degradation of FIMA and obtain the impurity in a pure state. For this, a solution of FIMA (500 mg) dissolved in 15mL of water and acetonitrile in 25mL of volumetric flask. To the solution was added 10 ml of 30% hydrogen peroxide. The solution for degradation was kept at room temperature for 48 hours.

RESULTS AND DISCUSSION

Confirmation of Full Degradation of FIMA

The oxidative degradation sample was diluted to the required concentration and analyzed with analytical high-performance liquid chromatography. The analytical HPLC chromatograms of FIMA is shown in Fig. 2. FIMA eluted about a retention time of 7.3 min. HPLC chromatogram of a stressed sample of oxidative degradation sample is shown in Fig.-3. Degradation product (DP) was eluted at 4.9 min and was found with a maximum degradation of 75.4%. The degradation product was purified by preparative HPLC. Fractions greater than 95% were collected together and concentrated on rotavapour to remove acetonitrile. The solution was kept in lyophilizer overnight. DP was obtained with colorless solid and was recrystallized with hot water. The isolated fraction was analyzed by analytical HPLC which shows complete removal of FIMA which confirms the presence of DP only which is shown in Fig.-4.

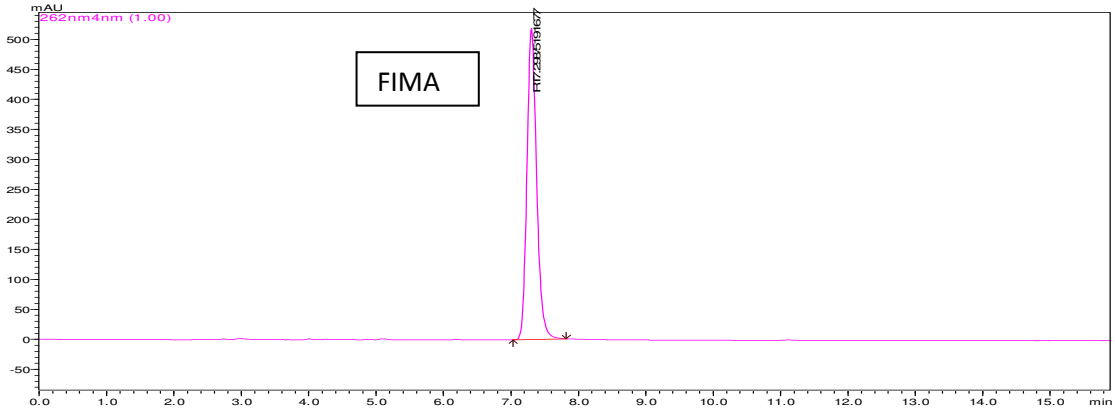


Fig. -2 : HPLC chromatogram of Fimasartan (FIMA)

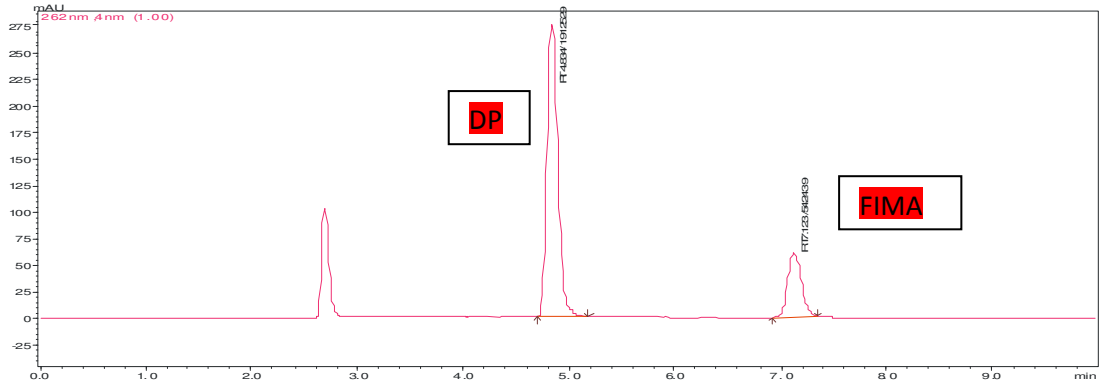


Fig.-3 : HPLC chromatogram of a stressed sample of oxidative degradation sample

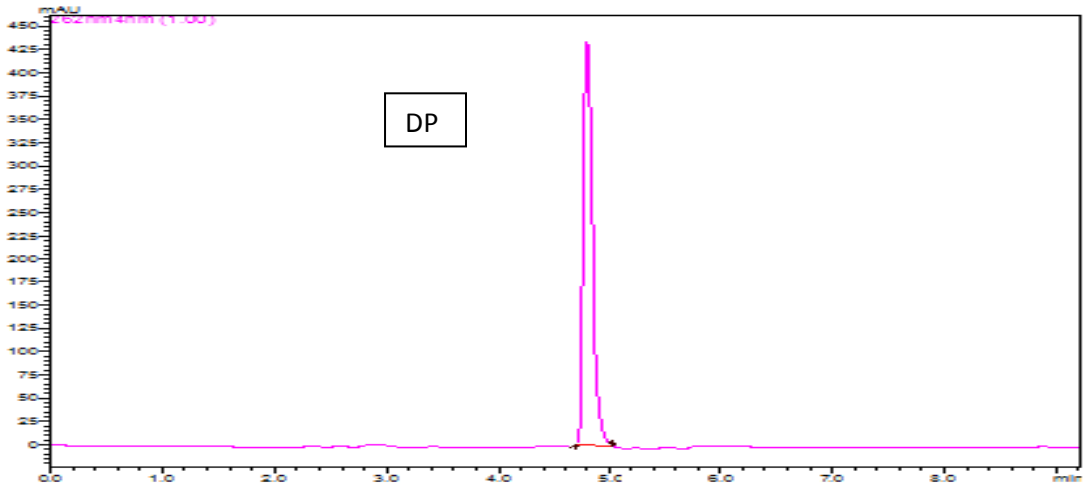


Fig. -4 : HPLC Chromatogram of isolated major oxidative degradation product

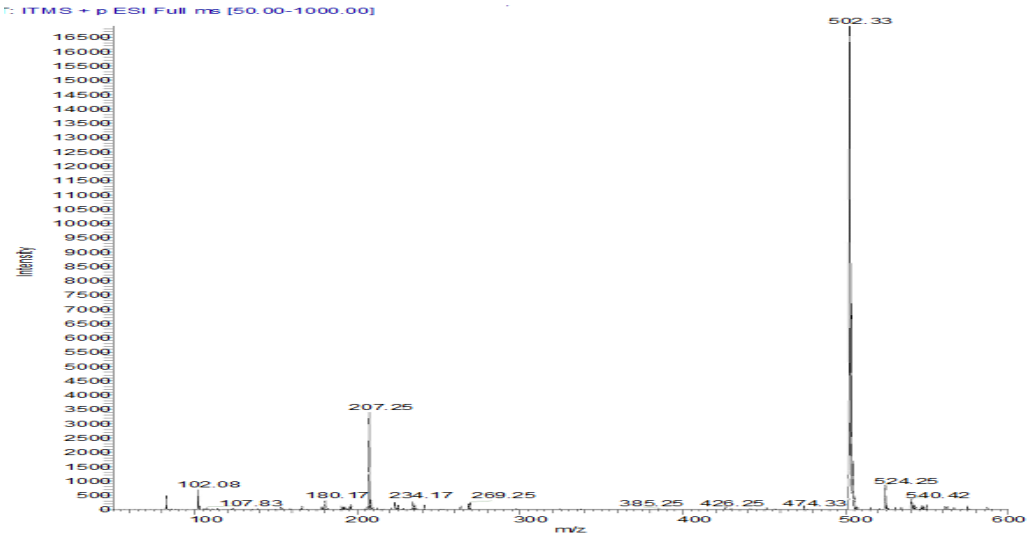


Fig.-5- Mass spectrum of FIMA

Identification of Oxidative Degradation Sample by LC-MS

To get structural insight, the LC-MS analysis was carried out on the oxidatively stressed sample. The mass spectrum thus obtained showed a protonated molecular ion of degradation impurity at m/z 486 (Fig.-6), whereas the FIMA displayed protonated molecular ion at m/z 502 (Fig.-5) Thus the degradation impurity DP has 16 amu less than the molecular ion of FIMA.

The ^{1}H NMR, ^{13}C NMR and DEPT spectral data of degradation product were compared with those of FIMA in Table-1. The numbering scheme for the NMR assignments is shown in Fig-1. In ^{1}H NMR, a number of protons in FIMA and its degradation product is the same.As in ^{13}C -NMR, the presence of –C=S (Thione) group carbon at 27th position in FIMA , is 199 ppm . In DP, there is the disappearance of -C=S (Thione) group and formation of carbonyl group at 27th position is observed, which is indicated by the chemical shift at 168.98 ppm. DEPT spectrum of FIMA and DP shows the presence of four methyl groups (positive). DEPT spectrum of FIMA and DP reveals the presence of five methylene groups (negative). Thus the degradation impurity structure can be rationalized in terms of substitution of thio group by a carbonyl group. The electrospray ionization (ESI) mass spectrum of the DP (Fig. 6) showed a molecular ion peak at m/z, 486.25 amu $[M+H]^{+}$ in positive ion mode, whereas the FIMA (Fig.5), exhibited protonated molecular ion at m/z 502. Thus the degradation impurity DP has 16 amu less than the molecular ion of FIMA indicating replacement of sulphur group by oxygen. IR absorption spectral

data of degradation impurity (Table-2) also supporting that formation of one carbonyl functional group at 1651cm^{-1}and disappearance of thione (-C=S) functional group at 1230 cm^{-1}. The IR (KBr) spectral data of the degradation impurity was compared with those of FIMA in Table-2. From the spectral data, the structure of this degradation impurity is characterized as 2-(1-((2'-(1H-tetrazol-5-yl)-[1,1'-biphenyl]-4-yl)methyl)-2-butyl-4-methyl-6-oxo-1,6-dihydropyrimidin-5-yl)-N,N-dimethylacetamide with molecular formula $C_{27}H_{31}N_7O_2$ and molecular weight 485.25.

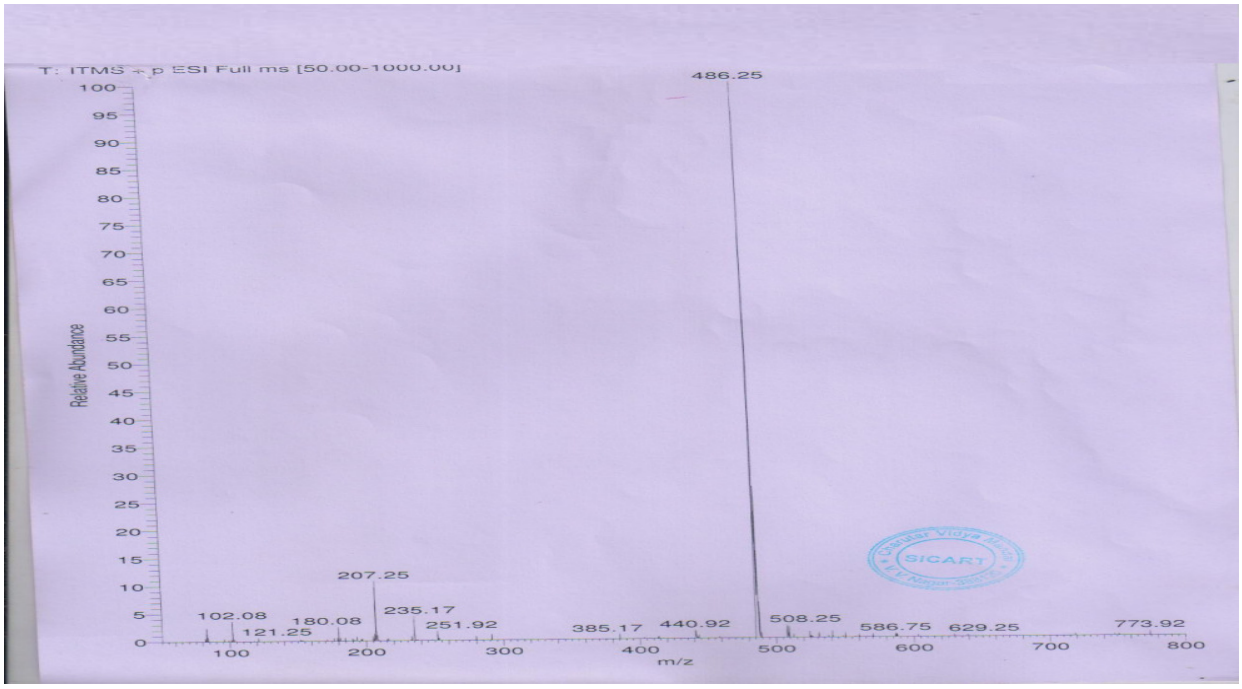


Fig.-6- : Mass spectrum of DP

Table-1: NMR Assignments of FIMA and DP

FIMA					DP				
Position	^{1}H	Chemical Shift(δ ppm)	^{13}C	DEPT	Position	^{1}H	Chemical Shift(δ ppm)	^{13}C	DEPT
1	--	--	--	---	1	---	----	--	---
2	--	--	--	---	2	--	--	--	--
3	--	--	--	--	3	--	--	--	--
4	--	--			4	--			
5	--	--	161.86	Quaternary Carbon	5	--	--	162	Quaternary Carbon
6	--	--	134.21	Quaternary Carbon	6	--		134	Quaternary Carbon
7	--	--	132.27	Quaternary Carbon	7			135	Quaternary Carbon
8	1H	7.3,m	125.40	-CH-	8	1H	7.58,m	122.25	-CH-
9	1H	7.36,m	130.45	-CH-	9	1H	7.57,m	131.03	-CH-
10	1H	7.4,m	130.00	-CH-	10	1H	7.65,m	130.54	-CH-
11	1H	7.5,m	129.26	-CH-	11	1H	7.65,m	129.09	-CH-
12	-	-	140.82	Quaternary Carbon	12	--	--	140.95	Quaternary Carbon
13	1H	7.06,dd	127.42	-CH-	13	1H	7.08,s	127.79	-CH-

14	1H	6.8,dd	127.42	-CH-	14	1H	7.08,s	127.79	-CH-
15	--	---	139.87	Quaternary Carbon	15	--		138.23	Quaternary Carbon
16	1H	6.9,dd	126.71	-CH-	16	1H	7.08,s	126.26	-CH-
17	1H	7.08,dd	126.71	-CH-	17	1H	7.08,s	126.26	-CH-
18	2H	5.2,s	32.13	$-CH_2$	18	2H	5.25,s	33.20	$-CH_2$
19	--	---	---	---	19	--	--	--	--
20	--	--	160.74	---	20	--	--	162.00	--
21	--	--	119.02	Quaternary Carbon	21	--	--	116.46	Quaternary Carbon
22	--	---	158.15	Quaternary Carbon	22	--	--	159.31	Quaternary Carbon
23	--	---	---	---	23	---	--	--	
24	---	---	159.17	Quaternary Carbon	24	--	--	155.01	Quaternary Carbon
25	---	---			25	--	--	--	
26	2H	3.79,s	32.18	$-CH_2$	26	2H	3.54,s	33.27	$-CH_2$
27	---	---	199 [20]		27	--	--	168.98	
28	--	--	--		28	--	--	--	
29	3H	2.15,s	20.32	$-CH_3$	29	3H	2.21,s	21.51	$-CH_3$
30	2H	2.63,t	20.5	$-CH_2$	30	2H	2.50,t	21.58	$-CH_2$
31	2H	1.5,q	27.05	$-CH_2$	31	2H	1.56,q	28.14	$-CH_2$
32	2H	1.3,q	29.26	$-CH_2$	32	2H	1.29,q	30.36	$-CH_2$
33	3H	0.824,t	12.45	$-CH_3$	33	3H	0.80,t	13.58	$-CH_3$
34	--	--	--	--	34	--	--	--	--
35	3H	3.44,s	33.68	$-CH_3$	35	3H	2.83,s	36.80	$-CH_3$
36	3H	3.45,s	33.92	$-CH_3$	36	3H	3.05,s	35.04	$-CH_3$

Refer Fig.-1 for numbering , s- singlet, m-multiplet, dd-double doublet, q-quartet

Table-2: FTIR Spectral Data of Fimasaration and Degradation Product

FIMA		DP	
Wave number(cm^{-1})	Functional group	Wave number (cm^{-1})	Functional group
2958	Broad peak covering NH, Aromatic C-H, CH_3(Stretching) and CH_2(Stretching)	3308	Tetrazole N-H
2931		3192	Aromatic C-H
2713		2967,2955	CH_3 Stretching
1741	Pyrimidine amide	2928	CH_2 Stretching
1616	Aromatic C=C (Stretching)	1740	Pyrimidine Amide
1537		1651	Tertiary Amide formation
1481		1537	Aromatic C=C
1481	Aromatic C=N	1460	Aromatic C=C
1356	Aromatic C-N	1402	Hetero Aromatic C=C
1230	C=S Stretching	1359	C-N Stretching
1185	C-N	1230	C=S disappeared
835	Out of plane C-H bending	1180	C-N Stretching
778		825	Out of plane C-H bending
600	Substituted Aromatic		

Formation of Degradation Impurity

The degradation impurity formed in the presence of oxidative stress degradation is due to the replacement of sulphur by oxygen and formation of the carbonyl group. The probable fragmentation pathway is shown in Fig.-7.

CONCLUSION

The major unknown oxidative degradation product was isolated and was characterized by using spectroscopic techniques namely NMR,ESI-MS and IR. The spectral studies indicate that C=S group is replaced by C=O during oxidative degradation. The hitherto unreported impurity has been identified as 2-(1-((2'-(1H-tetrazole-5-yl)-[1,1'-biphenyl]-4-yl)methyl)-2-butyl-1,6-dihydro-4-methyl-6-oxo-1,6-dihydropyrimidin-5-yl)-N,N-dimethylacetamide and its fragmentation route has been predicted.

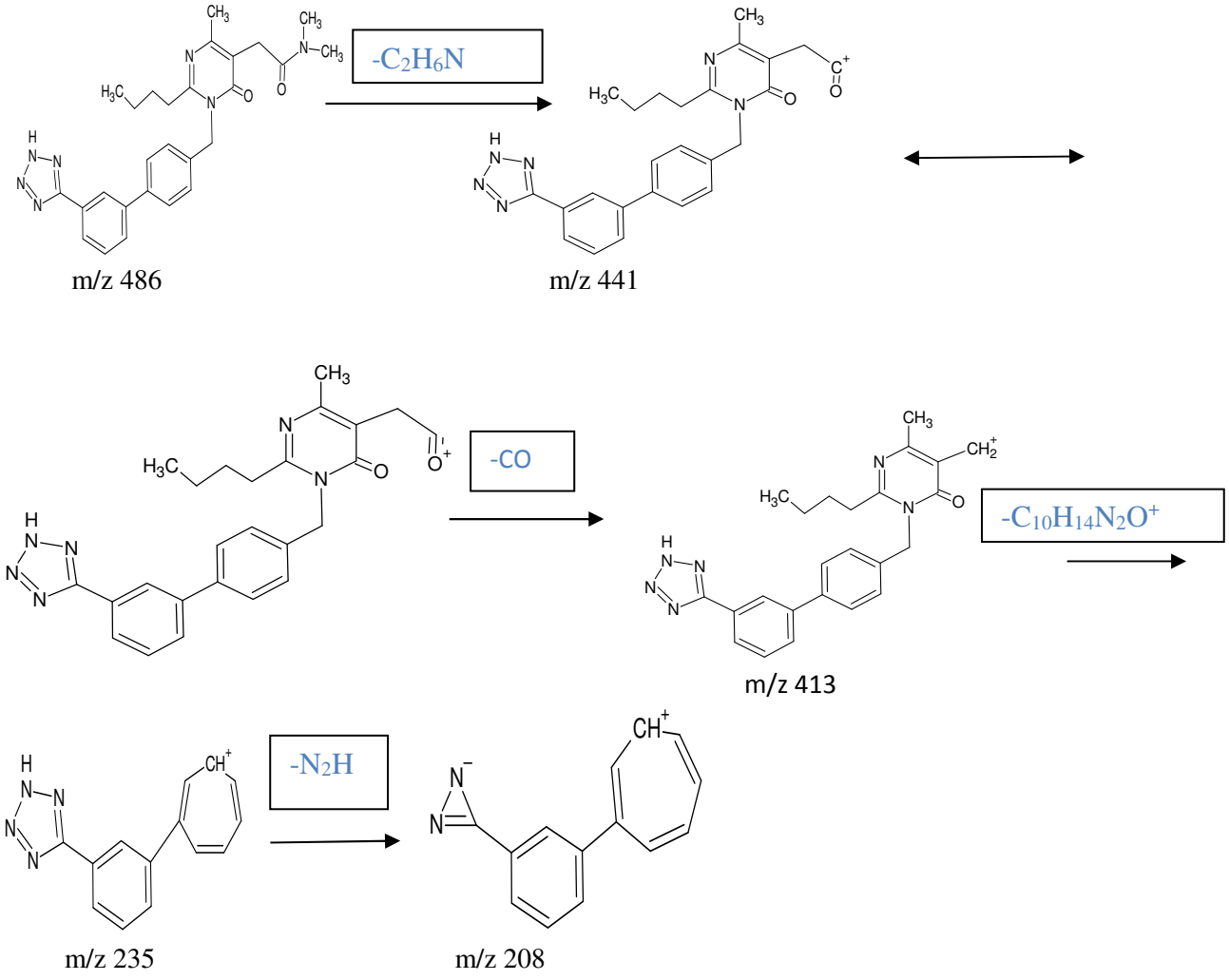


Fig.-7: Proposed Fragmentation Pathway of Degradation Product

REFERENCES

1. H.Y. Lee and B.H. Oh, *Drugs*, **76**, 1015 (2016), **DOI:** 10.1007/s40265-016-0592-1.
2. J. B. Park, K.C. Sung, S. M. Kang and E.J. Cho , *Amer. J of Cardiovas. Drugs*, **13, 47** (2013), **DOI:** 10.1007/s40256-013-0004-9.
3. H.W. Moon, A.M. Yousaf , K.H. Cho, C.S. Yong, J.O. Kim and H.G. Choi, *Asian J. of Pharma. Sci.*, **9(3)**, 123(2014), **DOI:** 10.1016/j.ajps.2014.04.002.
4. J.Y. Hyun, H.J. Kim, M.R. Gwon , H.W. Lee and S.J. Seong , *Ana. Sci.*, **31**, 1335(2015).

5. D-H Kim , J-H Lee ,W-S Shim and K.T. Lee, *J Pharma Care Health Sys*, **4**, 52(2017), **DOI:** 10.4172/2376-0419-C1-021.
6. S.H. Yoon, S. Oh, S.H.Kim , S.J. Yi , K.S. Yu, I.J. Jang and J.Y. Chu, *J of Chromato. Sci.*, **53(8)**, 1250 (2015), **DOI:** 10.1093/chromsci/bmu219
7. B.S. Shin, T.H. Kim, S.H. Paik,Y.H. Chi, J.H. Lee, H.K. Tan, Y. Choi, M. Kim and S.D. Yoo , *Biomed. Chromatography*,**25**, 1208 (2011), **DOI:** 10.1002/bmc.1592.
8. T.H. Kim ,S. Shin, M. Bashir ,Y.H. Chi, S.H. Paik, J.H. Lee, H.J. Choi, S.D. Yoo, J.B. Bulitta, E.Ma, S.H. Joo and B.S.Shin, *Xenobiotica*, **44**, 913(2014), **DOI:**10.3109/00498254.2014.915359.
9. C.P. Pandya and S.J.Rajput, *Indo American J. of Pharm. Research*, **7,** 929 (2017).
10. V. Yankov, D. Tsvetkova, D. Obreshkova , S. Ivanova and V. Petkova, *World J of Pharmacy and Pharm. Sci*,. **5**,18 (2016), **DOI:** 10.20959/wjpps20166-7105.
11. J. Mamta and N. Devanna, *Rasayan J. of Chem.*, **11(1)**,300(2018), **DOI:** 10.7324/RJC.2018.1112007.
12. P.A. Kumar, Y.R. Kumar and A. Jayshree , *Rasayan J. of Chem.*, **9 (2)**, 180 (2016).
13. V.V. Reddy, G. Gowardhan , K. Srinavasulu, G.M. Reddy, V. Himabindu, *Rasayan J. of Chem.*, **1(2)**, 301 (2008).
14. K. Ramulu, B.M. Rao and N. Someswara Rao, *Rasayan J. of Chem.*, **6(4)**, 274(2013).
15. D. Suneetha and A. Lakshamanarao, *Rasayan J. of Chem.*, **3(1)**, 117 (2010).
16. M. Bakshi and S. Singh, *J. Pharm Biomed Anal*., **28**,1011(2002), **DOI:** 10.1016/S0731-7085(02)00047-X.
17. M. Bakshi and S. Singh, *Pharma Tech On-line*, 1(2000).
18. ICH. Validation of analytical procedures: Text and methodology Q2(R1), International Conference on Harmonization,2005.
19. ICH. Stability testing of new drug substances and products Q1A(R2), International Conference on Harmonization,2003.
20. M. Badertscher, Structure Determination of Organic compound, Fourth Edition, Sprineger,p.127.

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