ISSN : 0974-7419 Volume 15 Issue 8

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 15(8) 2015 [352-363]

Generic workflow using advanced analysis and data interpretation tools for identification of Irbesartan degradation products by liquid chromatography coupled to high resolution mass spectrometry

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ABSTRACT

A high performance liquid chromatography (HPLC) coupled AB SCIEX TripleTOF™ 5600 high resolution mass spectrometer system was used for identification of degradation products (DPs) of Irbesartan using generic workflow. Degradation experiments were carried out under various conditions such as acid hydrolysis, acid photolysis, base hydrolysis and base photolysis. A simple reverse phase chromatography was developed to separate the degradation products or impurities on Phenomenex Kinetics C18 (50 x 2.10 mm, 1.7µ) column using 5mM ammonium acetate and methanol at 0.5 ml/min flow rate. Degradation products of Irbesartan were acquired using generic information dependent acquisition (IDA) with dynamic background substraction algorithm (DBS). The major degradation products were identified as 2-butyl-3-(tetrazole[1,5-f] phenanthridin-6-ylmethyl)-1,3-diazaspiro[4,4]non-1-en-4-one (m/z 427.2251; $C_{25}H_{27}N_6O^+$) and 1-(1-2-(1H-tetrazol-5-yl)biphenyl-4-yl) methylamino) pentylideneamino) cyclopentane carboxylic acid (m/z 447.2506; $C_{25}H_{31}N_6O_2^+$), acid photolysis and base hydrolysis stress condition, respectively. © 2015 Trade Science Inc. - INDIA

KEYWORDS

Irbesartan;
Degradation products;
Generic information dependent acquisition (IDA);
DBS;
TripleTOF™ 5600 mass spectrometer;
Advanced analysis and data interpretation tools.

INTRODUCTION

Degradation product analysis or Impurity profiling is an vital study in pharmaceutical analysis, particularly during the product development and quality control[1]. The safety of any drug product is determined by not only on the toxicological properties of the active drug substance, but also on the major and minor impurities that it having[2]. Monitoring of the degradation products or impurities in new drug substances is a key component of the guideline issued by the International conference in Harmonization (ICH)[3-6].

There are many analytical techniques which are being used for impurity profiling. High pressure liquid chromatography (HPLC) coupled with UV or PDA is the most common analytical technique to monitor the degradation kinetics but for identification and confirmation of the degradation products triple quadrupole,

quadrupole base linear ion trap (QTRAP), time of flight based high resolution mass spectrometer (QqTOF) mass spectrometer are being used[7-12].

The advents of hybrid mass spectrometer have made it possible to use combination scan functions for the identification and confirmation of structurally related molecules in complex samples. Selective scan functions in combination with unique software capabilities in the modern mass spectrometer provides very useful workflow based options for selective identification of low concentration of structurally related impurities efficiently.

Analysis of common degradation products can be carried out using generic information dependent acquisition (IDA) method followed by their product ion scan. In IDA acquisition method, full-scan MS acquisition is employed as a survey scan to search for predicted degradation products ion. Due to the complexity of the analysis of degradation products or impurity profiling, sufficient sensitivity and separation is difficult to achieve with HPLC. Using the hybrid quadrupole based time-of-flight mass spectrometer with HPLC provides new dimension to the sensitivity, sensitivity, resolution and mass accuracy[13]. Accurate mass and product ion spectra provide vital information for the identification and structure, confirmation of potential degradation products or impurities.

Irbesartan (2-butyl-3-({4-[2-(2*H*-1,2,3,4-tetrazol-5-yl)phenyl]phenyl}methyl)-1,3-diazaspiro [4.4] non-1-en-4-one) is an angiotensin II receptor used in the treatment of hypertension[14-15]. Hypertension is one of the most prevalent diseases with estimated one billion cases worldwide[16]. The therapeutic mechanism was significantly improved by discovering with losartan class of molecules[17,18]. Irbesartan is a nonpeptide angiotensin II type -1 (AT1) receptor antagonist. An entire therapeutic class of AT_1-receptor antagonists or sartans has been developed, among which Irbesartan has been marketed for over 10 years. Few studies on its stability and degradation have been reported by Singh and Mbah[19,20]. Singh et al. identify and characterize the degradation product of Irbesartan obtained using different stress conditions by LC-MS/TOF, MS^n, online exchange and LC-NMR. In his findings degradation of irbesartan was 4.5%, 51.4% and 48.7% under acid, base and photoacid conditions respectively. Hillaert et al. also reported the instability of irbesartan in basic conditions[21].

Therefore the objective of present study was to develop accurate, fast and sensitive generic workflow to identify degradation products of drug substance and drug product in single analytical run by using advanced analysis and data interpretation tools.

EXPERIMENTAL

Chemicals and reagents

HPLC grade methanol was purchased from J.T. Baker. Formic acid (MS grade) was obtained from Fluka. Ammonium acetate, Hydrochloric acid and sodium hydroxide solution were procured from Merck India. Ultrapure water (18.2 MÙ) was obtained using MilliQ apparatus from Millipore (Milford, USA)

Instrumentation

Shimadzu Prominance 20 AD (Kyto Japan) coupled with AB SCIEX TripleTOF™ 5600 mass spectrometer System (AB SCIEX, Concord, ON) equipped with dual ionization (electrospray ionization and atmospheric pressure chemical ionization) source was used for this analysis. External calibrant delivery system (CDS) was used to calibrate the TOF mass analyzer with APCI small molecules calibrant solution provided by instrument vendor.

Chromatographic and mass spectrometric conditions

Shimadzu Prominance 20AD HPLC with UV detector was connected with the AB SCIEX Triple TOF™ 5600 high resolution mass spectrometer. The separation of the drug and its degradation products were optimised using different mobile phase conditions. Isocratic and gradient flow systems were tried to achieve the acceptable resolution using organic solvent (methanol, acetonitrile) and volatile buffer (ammonium acetate and formate buffer) at different ratios. Best resolution between the drug and degradation products (DPs) was achieved using Phenomenex Kinetics C18 (50 x 2.10mm, 1.7µ, USA) column. The UV detector was optimised and set to 254 nm wavelength for better detection of degradation products.

Separation of the degradation products was achieved on Kinetics C18 (50 x 2.10mm, 1.7µ, Phenomenex USA) at the flow rate of 0.5 ml/min. The

sample tray temperature was 15 ^{0}C and column oven temperature was 50 ^{0}C. The chromatographic elution was carried out using 5mM ammonium acetate (mobile phase A) and methanol (moble phase B). The mobile phase gradient was started at 40 % of B, after 1 min it was increased to 90% within 5 min, held for 13min and, then brought bring back to 40% in 14 min. UV detector was set to 254 nm. Injection volume was kept 5 µL. Total run time was optimised to 15 min. The flow rate was set at 0.5 ml/min with a split after the HPLC column in the ratio of 1/20, producing an inlet flow into the mass spectrometer of 0.050 ml/min.

All the data acquisition was performed with a Triple TOF™ 5600 System (AB SCIEX, Concord, ON) coupled with dual ionization source (AB SCIEX, Concord, ON) which has electrospray and atmospheric pressure chemical ionization (APCI) probe for analysis. All the experiment were carried out in electrospray positive ionization mode in mass spectrometer. Data was acquired using an ion spray voltage of +5.5 kV, curtain gas of 30 PSI, nebulizer gas of 50 PSI, and an interface heater temperature of 600 ^{0}C with drying gas (GS2) 50 PSI. Generic method was setup using information dependent acquisition (IDA) survey scans for 100 ms and 8 product ion scans were collected for 50 ms with their threshold exceeding 200 counts per second (cps). Collision energy (CE) setting of 35 V with a spread of ±15V was applied to all parent ions for collisionally induced dissociation (CID). Dynamic background subtraction algorithm was switched on during the IDA acquisition to eliminate the background noise. All the acquired data were processed using Analyst TF® 1.5, PeakView® 1.1.1.

Stress condition

Hydrolysis (acidic and basic) and photolysis (acidic and basic) degradation experiment were carried out as per ICH recommendations. Stock solution of Irbesartan was prepared by dissolving 5mg Irbesartan (Figure 1), in acetonitrile-water (5ml; 50:50 v/v). The concentration of this stock solution obtained was 1000 µg/ml. For base hydrolysis the stock solution of Irbesartan (0.5 ml) was taken, to which sodium hydroxide solution (2N, 0.5ml) was added. Base hydrolysis was carried out at 80 ^{0}C. Similarly acidic hydrolysis experiment was setup with hydrochloric acid (1N, 0.5ml) at 80 ^{0}C. After subjecting to stress (acid and base), samples (100 µl) were taken out at different time points (0.0, 30.0 and 60.0 min) and were diluted five times with acetonitrile: water (50:50, v/v) before injecting into mass spectrometer. Similarly photo (acid and basic) degradation stress studies were carried out for

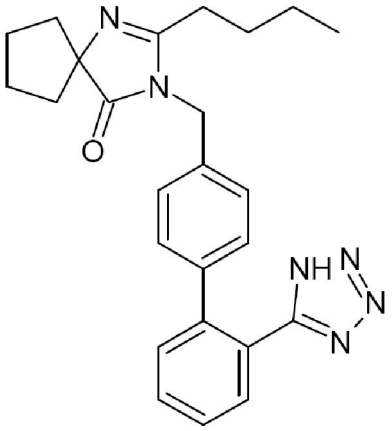

Figure 1 : Structure of Irbesartan

73 h under the natural sun light.

RESULTS AND DISCUSSION

Generic information dependent acquisition with real time dynamic background substraction method was developed to analyze the Irbesartan degradation products using high resolution mass spectrometry. Simultaneous acquisition for TOFMS and MS/MS data was developed using non targeted generic (IDA) method with real time DBS for this study at fast LC scale.

Non targeted IDA method was set to run this experiment in positive ionization mode to detect all the degradation product. The high resolution mass scan data of the sample (stressed) was compared with the control (unstressed) using PeakView® 1.1.1 software. The corresponding MS/MS data of the precursor ion was collected in the same IDA experiment using collision energy spread option (CES) and dynamic background substraction (DBS) in Analyst 1.5 TF® software. The advantage of CES is that less time and less optimization of collision energy are required to obtain complete distribution of fragments ion in MS/MS. These attributes are very useful when dealing with analysis of compound on LC time scale, especially for unknown analyte analysis. Information dependent information (IDA) offers the ability to simultaneously generate the MS and MS/MS

information collected from a single LC injection. As the sample complexity increases, ion selection criteria needs to carefully set so that the risk of missing ion of interest decreases significantly. Its main purpose is to get relevant information for product ion spectra in every injection. Real time dynamic background substraction (DBS) is useful triggering tool for MS/MS in IDA experiments, on peaks of interest not on the background ion. The concept of DBS is to subtract the previous scan from the current one, before applying any other IDA selection criteria[22,23].

This can eliminate the need for a second injection in order to acquire MS/MS. The full scan accurate mass and product ion spectra of Irbesartan were obtained from information dependent information (IDA) experiments (Figure 2 & 3). The most accurate molecular formula for each major and minor fragment was obtained with error (ppm and mDa) and RDB were calculated and tabulated. MS/MS information for all the degradation products helped to correlate their structural similarity with parent drug. The accurate mass spectrum with peak resolution of the irbesartan was obtained from the IDA experiments. Irbesartan was showing the peak eluting at 7.56 min (Figure 4). The experimental accurate m/z value for Irbesartan was 429.2398 (0.1 ppm error) having a resolution of 42754.1 at full width and half height (FWHH) obtained on fast LC scale. Using formula finder option in PeakView® software calculated the most probable formula of the corre-

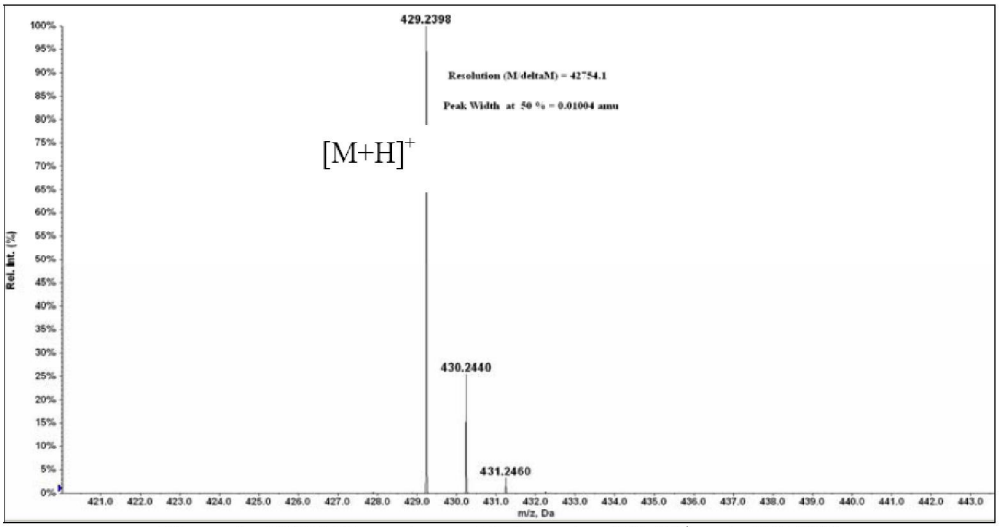

Figure 2 : TOFMS spectrum of Irbesartan $[M+H]^+$, m/z 429.2398

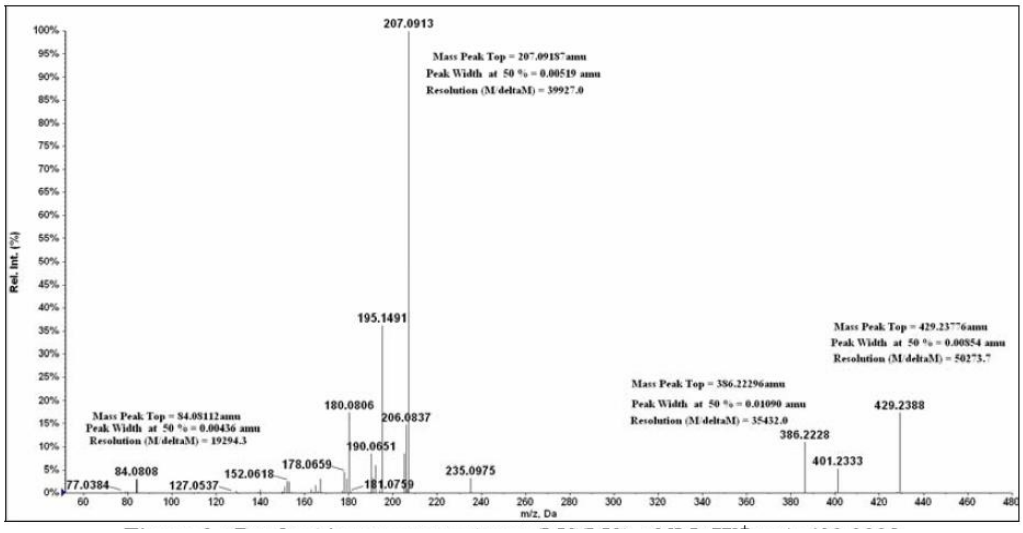

Figure 3 : Product ion mass spectrum (MS/MS) of $[M+H]^+$, m/z 429.2398

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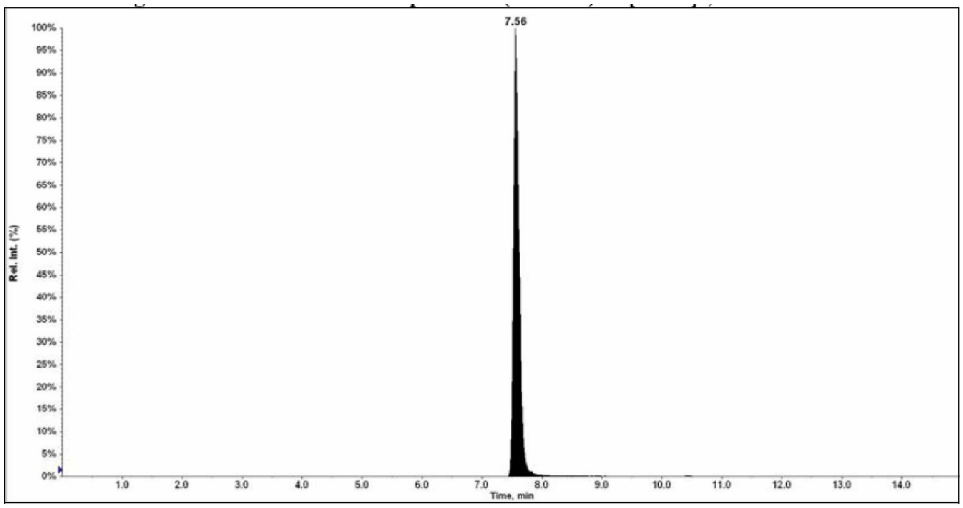

Figure 4 : Display the extracted ion chromatogram (XIC) of m/z 429.2398 using 20mDa XIC window

sponding m/z based on their mass accuracy in TOFMS and MS/MS. TOFMS data was used to generate the elemental formula using formula finding option in PeakView® software. This was also reconfirmed from the high resolution products ion spectra by selecting all the elemental formula for the fragments and the mass accuracy. The molecular peak of m/z 429.2398 fragmented in collision cell (Q2) produced twelve major fragments. The molecular formula, error (ppm and mDa) and ring to double bond information (RDB) information of the major products of m/z 429.2398 using the PeakView® software presented in (TABLE 1). TOF MS/MS spectrum of m/z 429.2398 shows the accurate masses and resolution of all the fragments. The m/z 84.0804, 152.0617, 180.0806, 190.0650, 195.1491, 205.0761, 206.0837, 207.0910, 235.0975, 386.2230 and 401.2339 are the products obtained from TOF MS/MS scan of m/z 429.2390.

TABLE 1 : Product ions of Irbesartan (m/z 429.2390) with their molecular formula, error and RDB

No.	m/z of Fragments	Molecular Formula	Error		RDB
			ppm	mDa	
1	84.0804	$C_5H_9N^+$	-4.2	-0.4	2.0
2	152.0617	$C_8H_{12}N_2O^+$	-2.6	-0.4	9.5
3	180.0806	$C_{11}H_{18}NO^+$	-0.8	-0.2	10.0
4	190.0650	$C_{14}H_8N_2O^+$	-0.7	-0.1	12.0
5	195.1491	$C_{11}H_{19}N_2O^+$	-0.5	-0.1	4.0
6	205.0761	$C_{14}H_{17}N_2O^+$	0.3	0.1	12.0
7	206.0837	$C_{14}H_{18}N_2O^+$	-0.7	-0.1	11.5
8	207.0910	$C_{14}H_{19}N_2O^+$	-3.1	-0.6	11.0
9	235.0975	$C_{14}H_{11}N_4^+$	-1.3	-0.3	11.5
10	386.2230	$C_{25}H_{28}N_3O^+$	0.8	0.3	14.0
11	401.2339	$C_{25}H_{29}N_4O^+$	0.8	0.3	14.0
12	429.2390	$C_{25}H_{29}N_6O^+$	-1.6	-0.7	15.0

Characterization of degradation products

The major degradation products under all stressed condition were characterized using high resolution mass spectra and their accurate mass product ion data at chromatography scale. The data was processed using Analyst TF®1.5 and PeakView® software to predict the structures of products and correlate if any structural similarities with the parent drug exist.

Acid hydrolysis

The irbesartan was found to be stable in acid hydrolysis conditions after 90 min hydrolysis. Exact mass of all the acid hydrolysis degradation products along with their UV and MS retention times, molecular formula, RDB, error (for TOFMS and TOF MS/MS) and major fragment ion were obtained (TABLE 2). HPLC UV spectra of acid hydrolysis products were also recorded (Figure 5a). The m/z 338.3420 eluting at 9.91 min in TOFMS was the major degradation product (>1.5e^{5}cps intensity) found in the 90 min degradation sample. PeakView® software calculated the accurate formula as $C_{22}H_{44}NO^+$ for m/z 338.3420. Sahu et al, 2010 reported m/z 252.1276 is the major hydrolysis product, which was not found in this study[19].

Acid photolysis

The UV spectrum of 48h acid photolysis sample

TABLE 2 : TOF MS data for acid hydrolysis degradation products along with their molecular formula, RBD, error and major fragments

Stressed Conditions	t_R (min)		Exact Mass for degradation products	Molecular Formula (RDB)	Error in ppm		Major Fragments Ion (MS/MS)
	UV	MS			MS	MS/MS	
Acid Hydrolysis	7.05	7.45	387.1924	$C_{21}H_{27}N_2O_5^+$ (10)	2.7	2.1	*331.1286, 275.0652, 231.0739, 175.0143, 147.1149, 129.0696*
		7.55	401.2346	$C_{25}H_{29}N_4O^+$ (14)	3.0	2.2	207.0912, 195.1483, 180.0797
		7.56	451.221	$C_{22}H_{27}N_8O_3^+$ (14)	1.6	2.2	423.2147, 408.2039, 394.2012, 207.0914,
		7.59	467.1934	$C_{25}H_{23}N_8O_2^+$ (19)	2.3	0.8	439.1896, 424.1746, 262.9191, 207.0903,
		7.63	473.2047	$C_{23}H_{29}N_4O_7^+$ (12)	-0.8	3.0	445.1965, 408.2040, 239.1126, 208.0943, 87.9876
		7.64	489.1765	$C_{25}H_{29}O_{10}^+$ (12)	2.4	1.7	408.2029, 103.9611
	7.61	7.94	235.1686	$C_{15}H_{23}O_2^+$ (5)	-2.8	4.0	235.1690, 217.1960, 179.1054, 147.1153, 133.1009, 119.0864, 115.0537, 105.0675, 91.0545, 79.0530, 57.0695
		8.88	437.1862	$C_{15}H_{33}O_{14}^+$ (0.0)	-2.0	2.9	369.1996, 301.2116, 226.9491, 158.9628, 90.9751
		9.84	637.3038	$C_{25}H_{45}N_6O_{13}^+$ (7.0)	0.9	4.2	581.2416, 525.1786, 469.1149, 393.0857, 337.0236, 141.0689, 83.0482
		9.88	360.3236	$C_{19}H_{42}N_3O_3^+$ (1.0)	3.1	4.5	292.8971, 224.9062
		9.91	338.3420	$C_{22}H_{44}NO^+$ (2.0)	0.8	4.6	321.3144, 303.3033, 296.3290, 282.2787, 268.2626, 226.2136, 83.0849
	10.25	10.45	413.2663	$C_{22}H_{33}N_6O_2^+$ (10.0)	-0.6	2.4	301.1412, 189.0150, 171.0053, 123.1145

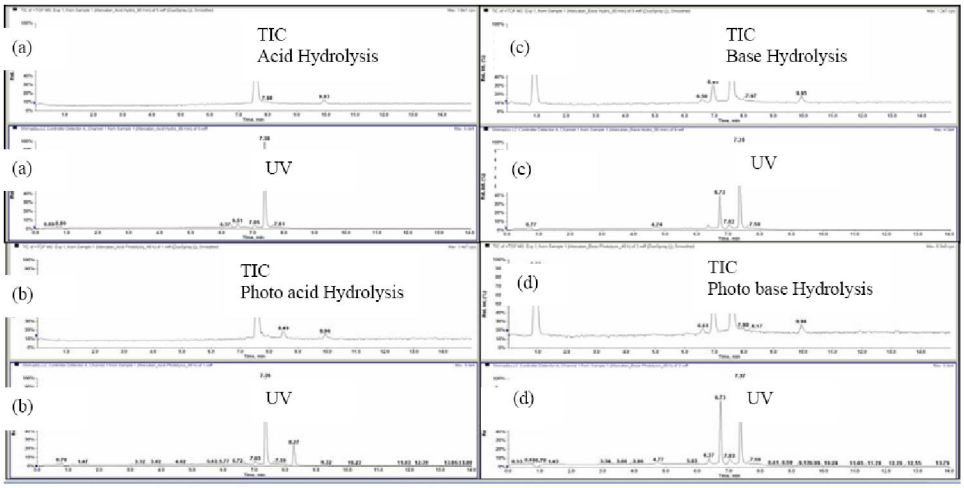

Figure 5 : Showing the total ion chromatogram and UV spectra of different stressed condition samples (a) Acid hydrolysis (b) Base hydrolysis (c) Photo acid hydrolysis (d) Photo base hydrolysis

is given in (Figure 5b). The major peak eluting at 8.49 min (Figure 6a), was identified as m/z 427.2251. High resolution data showed the isotopic pattern for this major peak of m/z 427.2551 (Figure 6b). The acid photolysis product was identified as 2-butyl-3-(tetrazole[1,5-f]phenanthridin-6-ylmethyl)-1,3-diazaspiro[4,4]non-1-en-4-one. Formula calculator option in PeakView® software calculated $C_{25}H_{27}N_6O^+$ formula based on TOF MS and MS/MS accuracy. Major degradation product, m/z 427.2251, is 2 amu less than the parent drug. The formula has two hydrogen atoms less than the parent compound which indicates the presence of an additional double bond or ring formation on the degradation product structure. The m/z 399.2189, 316.1451, 288.1500, 233.0822, 205.0764, 204.0680, 178.0649, 151.0539, 127.0536, 84.0797, 77.0376 were the accurate fragments of m/z 427.2251 (Figure 6c). Major product ion m/z 205.0764 and 84.0797 from m/z 427.2251 were common with

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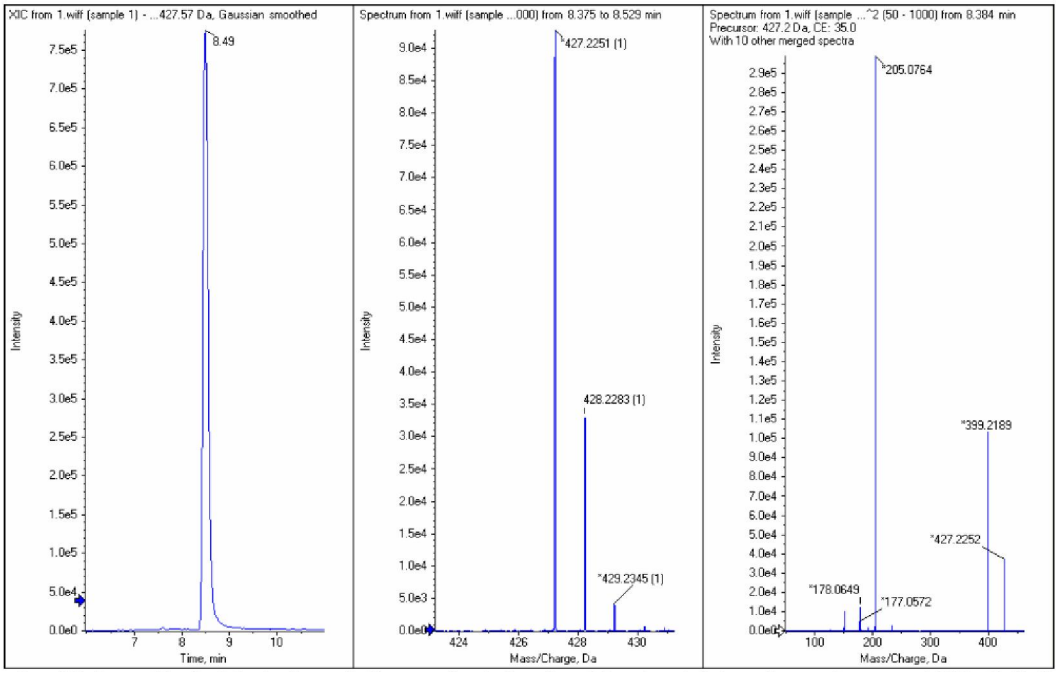

Figure 6 : Major acid photolysis degradation product showing (a) extracted ion chromatogram at RT 8.49 (b) high resolution data for base hydrolysis m/z 427.2251 (c) product ion spectra (TOFMSMS) of m/z 427.2251

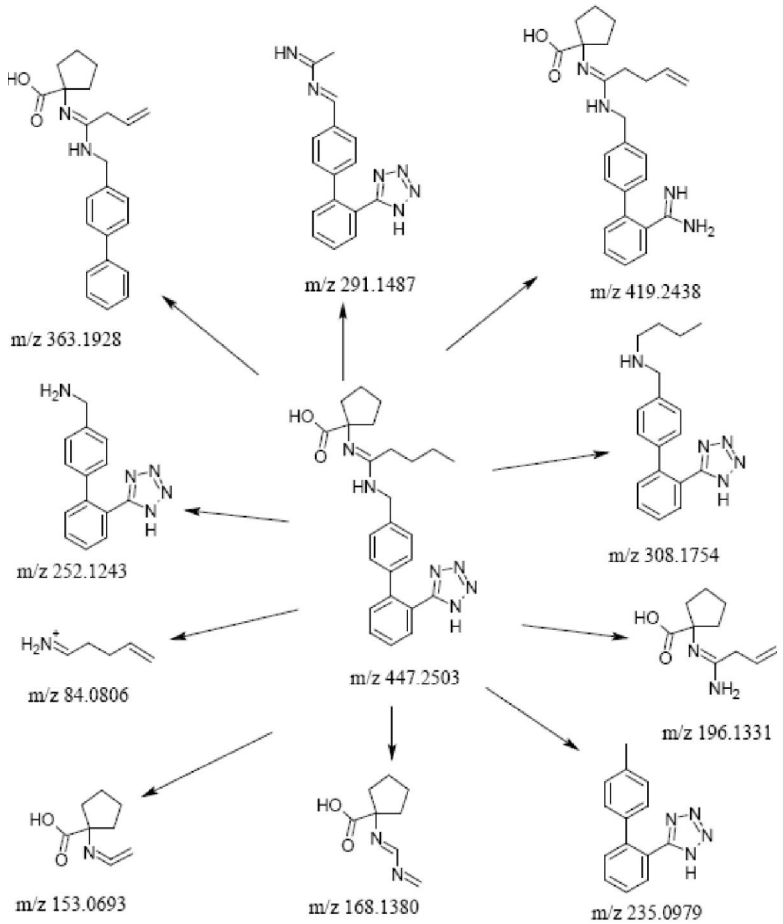

Figure 7 : Possible fragmentation pathway for major base hydrolysis product of Irbesartan (m/z447.2503) with their accurate masses and structure

TABLE 3 : TOF MS data for acid photolysis degradation products along with their molecular formula, RBD, error and major fragments

Stressed Conditions	t_R (min) UV	t_R (min) MS	Exact Mass for degradation products	Molecular Formula (RDB)	Error in ppm MS	Error in ppm MS/MS	Major Fragments Ion (MS/MS)
Acid photolysis		7.58	467.1960	$C_{25}H_{23}N_8O_2^+$ (19.0)	3.3	3.4	439.1921, 207.0914, 206.0836, 205.0763, 192.0767, 180.0804, 178.0668, 152.0612, 150.0486, 134.9987, 84.0803
		7.58	473.2029	$C_{24}H_{25}N_8O_3^+$(17.0)	-2.1	3.8	445.1965, 408.2045, 239.1133, 87.9879
	7.36	7.58	429.2404	$C_{25}H_{29}N_6O^+$ (18.0)	1.5	1.6	401.2340, 386.2231, 235.0978, 207.0913, 195.1494, 180.0807, 167.1542, 153.0695, 151.0540, 84.0807
		8.49	449.2064	$C_{26}H_{29}N_2O_5^+$ (14.0)	-0.7	0.8	421.2006, 205.0754, 178.0647, 151.0530
		8.50	465.2183	$C_{25}H_{21}N_8O_2^+$ (20.0)	4.7	0.0	432.0862, 344.0178, 329.2421, 260.9477, 180.0447, 147.0637
	8.27	8.49	427.2251	$C_{25}H_{27}N_6O^+$ (16.0)	1.7	2.0	399.2189, 316.1451, 288.1500, 233.0822, 205.0764, 204.0680, 178.0649, 151.0539, 127.0536, 84.0797, 77.0376
		8.84	437.1862	$C_{15}H_{33}O_{14}^+$(0.0)	-0.2	1.4	369.1998, 301.2110, 90.9758
		9.57	330.3373	$C_{20}H_{44}NO_2^+$(0.0)	0.4	0.9	312.3262, 286.3105, 286.3105, 257.2420, 150.0111, 106.0854, 88.0753
		9.58	465.2183	$C_{18}H_{33}N_4O_{10}^+$(5.0)	-2.1	3.9	432.0891, 344.0163, 329.2436, 265.0354, 158.9642, 90.9754,
		9.96	338.3422	$C_{22}H_{44}NO^+$ (2.0)	1.1	2.8	303.3044, 268.2639, 226.2169, 191.1790, 135.1159, 97.1004, 83.0849
	10.23	10.41	413.2653	$C_{21}H_{37}N_2O_6^+$ (5.0)	4.1	0.9	301.1415, 171.1170, 140.9590, 162.9366, 71.0862

irbesartan.

Resolutions (Full Width at Half Height) of 427.2251 in TOFMS and MS/MS mode were found to be 36852 and 36670, respectively at chromatographic run using Triple TOF™ instrument. Accurate mass, calculated formula, error and MS/MS of all the degradation products for 48h acid photolysis sample were also obtained (TABLE 3). Fragmentation pathway for major acid hydrolysis product (m/z 427.2251) was derived using software tool in PeakView® software.

Base hydrolysis

The major base hydrolysis product eluting at 6.7 min was showing accurate m/z 447.2503 which is addition of +18 Da to the parent drug (m/z 429.2398). The UV spectrum of 2h base hydrolysis sample is given in (Figure 5c). A spectrum was processed using PeakView® software to generate the accurate formula based on TOFMS and MS/MS accuracy. Formula finder option suggested $C_{25}H_{31}N_6O_2^+$ (m/z 447.2503) as the most probable formula based on TOFMS and MS/MS accuracy. Similar results were published by Sahu et al, 2010[10]. UV and MS retention times, most probable molecular formula, mass accuracy, and fragmentation ion of each degradation products formed during the base hydrolysis of irbesartan was obtained (TABLE 4). Product ion spectra of m/z 447.2503 exhibits m/z 419.2437, 363.1927, 291.1486, 252.1242, 235.0978, 207.0915, 196.1330, 190.0645, 180.0803, 168.1379, 153.0692, 140.0482, 85.0643, 84.0805 as its fragments using collision energy (CE) +35v and ±15V collision energy spread (CES). The fragments of m/z 447.2503 having m/z 235.0978, 207.0915, 190.0645, 180.0803 and 84.0805 were identical to fragments of Irbesartan (m/z 429.2398). Fragment of 447.2503 having m/z 235.0978 indicates the presence of tetrazole ring attached to biphenyl. Another characteristic fragment of 447.2503 having m/z 196.1330 ($C_{10}H_{18}NO_2^+$) was 1 Da higher than the irbesartan fragment having m/z 195.1941($C_{11}H_{19}N_2O^+$) which indicates the structural difference in the region of 2-butyl-1,3-diazaspiro [4,4] non-1-en-4-one part of Irbesartan[19]. Fragmentation pathway for major base hydrolysis product (m/z 447.2503) was given in Figure 7.

Base photolysis

The UV spectra of base photolysis products were showing the similar degradation pattern than hydrolysis (Figure 5d). The major peak identified was of m/z 447.2520 eluting at 6.95 min (Figure 8a). High resolution data showed the isotopic pattern for this

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TABLE 4 : TOF MS data for base hydrolysis degradation products along with their molecular formula, RBD, error and major fragments

Stressed Conditions	t_R (min)		Exact Mass for degradation products	Molecular Formula (RDB)	Error in ppm		Major Fragments Ion (MS/MS)
	UV	MS			MS	MS/MS	
Base Hydrolysis	4.75	4.87	274.1052	$C_{11}H_{12}N_7O_2^+$ (10.0)	1.8	2.5	246.0998
	6.35	6.51	336.1816	$C_{19}H_{22}N_5O^+$ (12.0)	-0.9	2.8	252.1236,235.0973,207.0913,180.0802, 153.0691
		6.93	469.2325	$C_{26}H_{33}N_2O_6^+$ (12.0)	-3.0	2.3	441.2256, 426.2149, 412.2116, 383.1727, 207.0909, 180.0801, 84.0792
		6.94	419.2434	$C_{25}H_{31}N_4O_2^+$ (13.0)	-0.8	2.1	402.2168, 308.1752, 291.1485, 235.0858, 224.1179, 207.0912, 192.0801, 194.0955, 180.0808, 84.0804
	6.72	6.96	447.2503	$C_{25}H_{31}N_6O_2^+$ (14.0)	0.7	1.3	419.2437, 363.1927, 291.1486, 252.1242, 235.0978, 207.0915, 196.1330, 190.0645, 180.0803, 168.1379, 153.0692, 140.0482, 85.0643, 84.0805
		6.97	485.2047	$C_{25}H_{25}N_8O_3^+$ (18.0)	3.3	2.7	457.1998, 442.1875, 428.1853, 426.2165, 399.1466, 383.1695, 329.1167, 280.9974, 222.9674, 176.9589, 149.0182
	7.35	7.49	415.2111	$C_{24}H_{31}O_6^+$ (10.0)	-0.3	4.6	207.0905, 133.0632, 119.0851, 117.0690, 91.0527
	7.58	7.58	451.2196	$C_{22}H_{27}N_8O_3^+$ (14.0)	-3.0	3.6	423.2152, 408.2039, 394.2016, 386.2229, 229.0731, 207.0906, 206.0833, 180.0802, 178.0658, 207.0906, 180.0802, 152.0609, 127.054, 84.0799
		9.96	338.3679	$C_{22}H_{44}NO^+$ (2.0)	-2.2	3.00	302.2151, 303.3045, 296.3301, 268.2625, 254.2472, 121.1005, 97.1002, 69.0695, 57.0695, 55.0538
		10.41	413.2661	$C_{22}H_{33}N_6O_2^+$ (10.0)	0.6	2.1	301.1402, 208.9478, 189.0138, 171.0055

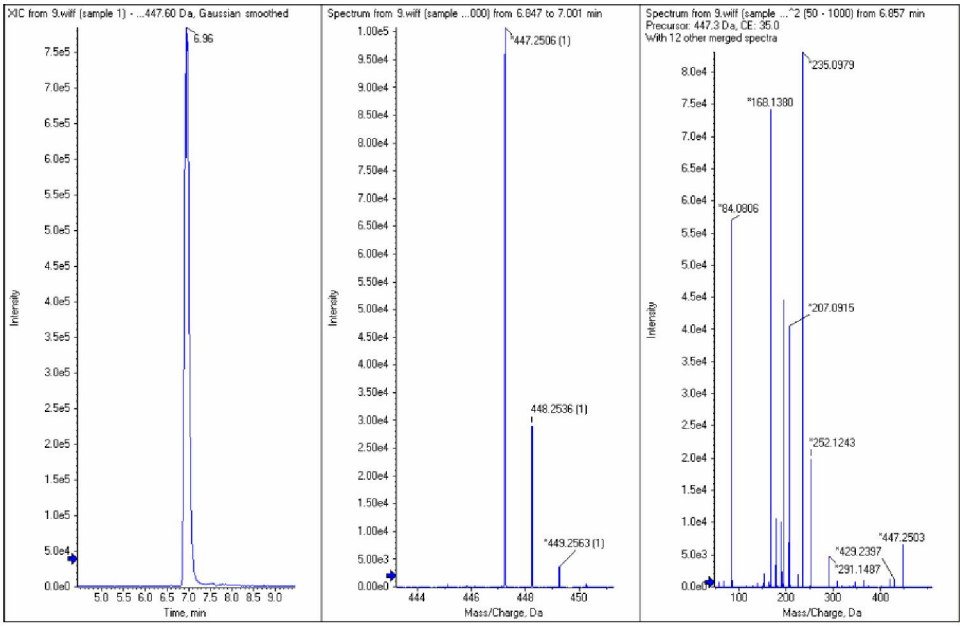

Figure 8 : Major base hydrolysis degradation product showing (a) extracted ion chromatogram at RT 6.96 (b) high resolution data for base hydrolysis m/z 447.2506 (c) product ion spectra (TOFMSMS) of m/z 449.2506

major peak of m/z 447.2520 (Figure 8b). The acid photolysis product was identified as 1-(1-2-(1H-tetrazol-5-yl)biphenyl-4-yl)methylamino) pentylideneamino)cyclopentane carboxylic acid. The fragment ions of m/z 447.2520 were similar as described in base hydrolysis section (Figure 8c). There were several other degradation products of base hydrolysis identified as m/z 336.1824 ($C_{19}H_{22}N_5O^+$), 358.1638 ($C_{20}H_{24}NO_5^+$), 419.2458 ($C_{25}H_{31}N_4O_2^+$), 485.2070 ($C_{25}H_{25}N_8O_3^+$), 469.2328 ($C_{26}H_{33}N_2O_6^+$), 451.2232 ($C_{22}H_{27}N_8O_3^+$). Other major identified base photolysis products were of m/z 328.0335 ($C_{18}H_6N_3O_4^+$), 312.0620 ($C_{15}H_{10}N_3O_5^+$), 412.0932 ($C_{23}H_{14}N_3O_5^+$), 380.1463 ($C_{18}H_{18}N_7O_3^+$), 374.1388

($C_{23}H_{20}NO_4^+$), 491.2154 ($C_{24}H_{27}N_8O_4^+$), 401.2328 ($C_{25}H_{29}N_4O^+$), 310.3106 ($C_{20}H_{40}NO^+$), 338.3421 ($C_{22}H_{44}NO^+$) and 321.3151 ($C_{22}H_{41}O^+$). They did not exhibit any characteristic product ion similar to product ions of m/z 429.2398 and 447.2520 (TABLE 5). Major degradation product m/z 447.2520 showed resolution (FWHH) 35390 and 35213 in TOFMS and TOF MS/MS mode, respectively.

Prediction of degradation pathway

Fragmentation option in Peakview® software predicts the possible fragments based on the structure of molecules. The software contains a simple fragment ion predictor that can generate the fragments, likely to be formed, by breaking bonds and adding or removing hydrogen atoms. Irbesartan structure was introduced into the software along with their accurate TOF MS/MS spectra[24]. Fragmentation option predicted the possible fragments structure of the molecule and calculated the formula. The major base hydrolysis product (m/z 447.2520) structure and fragments were predicted. Possible fragmentation pattern pathways of Irbesartan (m/z 429.2390) with their accurate masses are given (Figure 9).

TABLE 5 : TOF MS data for base photolysis degradation products along with their molecular formula, RBD, error and major fragments

Stressed Conditions	t_R (min)		Exact Mass for degradation products	Molecular Formula (RDB)	Error in ppm		Major Fragments Ion (MS/MS)
	UV	MS			MS	MS/MS	
Base Photolysis		4.95	312.062	$C_{15}H_{10}N_3O_5^+$ (13.0)	1.6	2.2	250.0815, 114.9606, 103.9620
		6.57	412.0932	$C_{23}H_{14}N_3O_5^+$ (19)	1.0	0.5	331.1200, 267.0912, 231.0492, 119.9348, 97.0994
		6.58	396.1198	$C_{21}H_{14}N_7O_2^+$ (19.0)	-1.4	1.6	368.1126, 315.1453, 103.9613, 101.0596
		6.59	336.1824	$C_{19}H_{22}N_5O^+$ (12.0)	1.2	1.0	252.1238, 235.0976, 207.0916, 180.0805, 153.0694, 115.0537,85.0639
		6.60	380.1463	$C_{18}H_{18}N_7O_3^+$ (14.0)	-0.7	6.1	352.1398, 315.1468, 251.0397, 146.0546, 87.9884
		6.61	374.1388	$C_{23}H_{20}NO_4^+$ (15.0)	-1.6	1.1	346.1316, 268.9971, 207.0910, 180.0803, 151.0542
		6.63	358.1638	$C_{20}H_{24}NO_5^+$ (10.0)	-2.5	4.9	315.1475, 301.1426, 207.0915, 190.0649, 180.0810, 178.0750, 152.0605
		6.92	491.2154	$C_{24}H_{27}N_8O_4^+$ (16.0)	-1.8	2.8	426.2151, 408.2056, 351.1442, 333.1117, 257.1235, 240.0752, 146.0547
	6.73	6.95	447.2520	$C_{25}H_{31}N_6O_2^+$ (14.0)	1.1	0.7	419.2442, 291.1489, 252.1244, 235.0980, 224.1182, 207.0917, 196.1332, 190.0650, 180.0805, 178.0775, 168.1382, 84.0807, 67.0539
		6.95	419.2458	$C_{25}H_{31}N_4O_2^+$ (13.0)	0.1	1.2	402.2175, 335.1866, 308.1756, 291.1491, 235.0866, 224.1181, 207.0912, 192.0805, 165.0690, 84.0802
		6.96	469.2328	$C_{26}H_{33}N_2O_6^+$ (12.0)	-1.9	2.1	441.2259, 426.2150, 423.2153, 413.2201, 412.2118, 408.2050, 383.1725, 370.1636, 256.0826, 246.0990, 235.1412, 229.0731, 217.1319, 207.0915, 206.0825, 190.0652, 180.0811, 168.1379,
	7.37	7.53	401.2328	$C_{25}H_{29}N_4O^+$ (14.0)	0.5	4.0	207.0913, 180.0786, 153.0665
		7.53	473.2038	$C_{23}H_{29}N_4O_7^+$ (12)	1.5	2.7	408.2031, 239.1107, 87.9880
		7.57	451.2232	$C_{22}H_{27}N_8O_3^+$ (14.0)	2.1	2.2	423.2151, 408.2034, 394.2011, 229.0740, 217.1308, 207.0923, 180.0810, 152.0627
		9.09	310.3106	$C_{20}H_{40}NO^+$ (2.0)	0.5	3.7	293.2847, 275.2722, 219.2120, 177.1621, 139.1112, 139.1112, 97.1004, 69.0703
		9.90	338.3421	$C_{22}H_{44}NO^+$ (2.0)	1.9	3.5	321.3162, 303.3050, 296.2979, 282.2789, 268.2639, 240.2328, 212.2025, 191.1790, 163.1474, 149.1321, 135.1156, 121.1010, 95.0854, 83.0853, 81.0694, 79.0537
		9.94	321.3151	$C_{22}H_{41}O^+$ (3.0)	-0.3	2.4	272.9669, 221.2240, 149.0209, 123.1171, 81.0687

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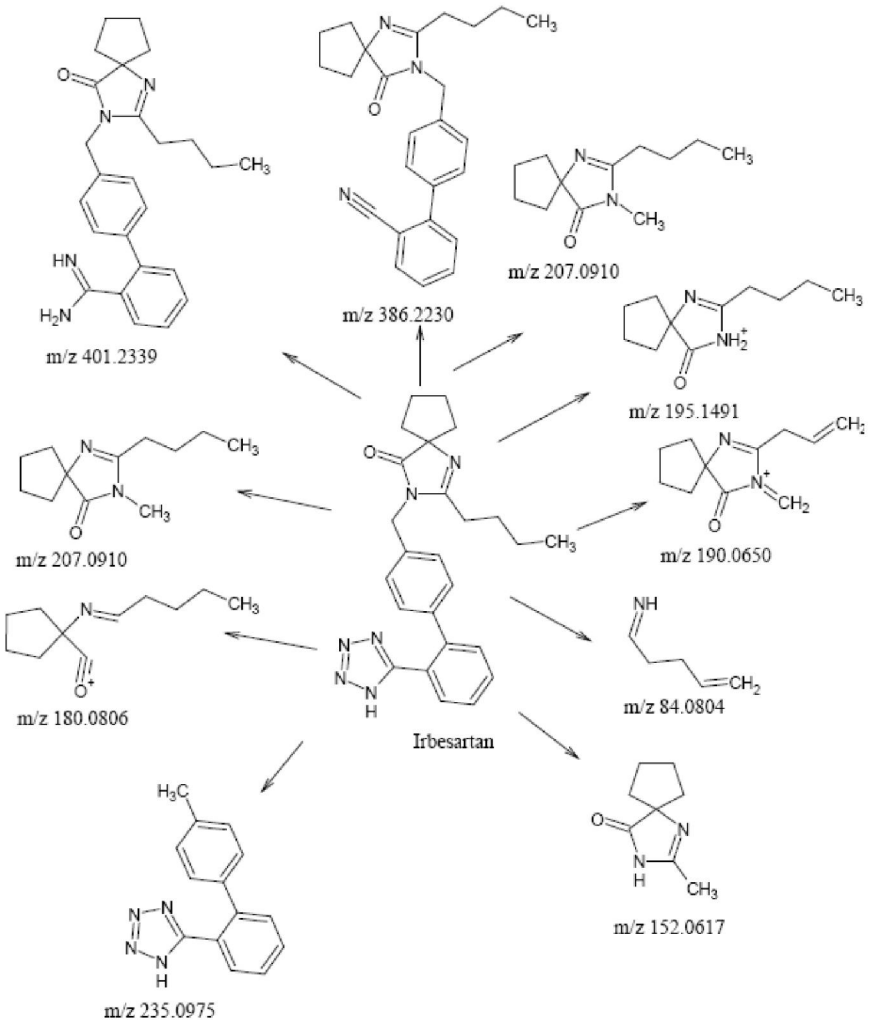

Figure 9 : Possible fragmentation prediction of Irbesartan (m/z 429.2390) with their accurate masses and structure using PeakViewTM software 1.1.1

CONCLUSIONS

In this study, generic IDA method was developed using high resolution systems to identify and confirm the Irbesartan degradation products in acidic and basic stressed conditions. The m/z 338.3420, 427.2251 and 447.2506 were major degradation products produced and identified in acid hydrolysis, acid photolysis and base hydrolysis stress condition, respectively. The irbesartan was found stable in acid hydrolysis conditions. Generic information dependent acquisition (IDA) method with unique dynamic background subtraction (DBS) shown the capabilities to identify and provide high number of relevant MS/MS to the real degradation product masses at fast chromatography run. This generic method will help to identify and confirm the low level degradation product in complex samples while maintaining the sensitivity, resolution and mass accuracy (in sub ppm level) in TOFMS and MS/MS mode at fast LC scale, which is imperative in impurity profiling work.

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