DEVELOPMENT OF STABILITY INDICATING METHOD OF FIMASARTAN POTASSIUM

5.1. SELECTION OF DRUG

Fimasartan (FIMA) is the ninth angiotensin II receptor blocker [1]. It was approved by Korean Food Drug and Administration in 2010. It is pyrimidine-4H (one) derivative of losartan by replacement of imidazole ring. It is selective angiotension II type I (AT1) receptor antagonist. Its affinity to AT1 receptor is 600 fold greater than that of losartan [2]. It is available under the brand name Kanarb through Boryung Pharmaceuticals. Daily dose of FIMA is 60-120 mg. It shows antihypertensive effect over 24 hr [3]. FIMA normalizes carbohydrate metabolism through AMPK pathway in diabetic condition [4]. FIMA causes reduction in diabetic chronic kidney disease with sustained proteinuria [5]. It has shown good efficacy in reduction of blood pressure [6], good safety profile since its effectiveness does not significantly differ according to age, sex and concomitant disease. When combined with hydrochlorothiazide/ amlodipine/ rosuvastatin [7, 8] it has well safety profile. It is approved by CDSCO in India in 2018. It is not official in any pharmacopoeia. There are no reports on stability indicating method development of FIMA, identification and isolation of degradation products.

5.2. DRUG PROFILE

General Properties

IUPAC name: 2-(1-((2'-(2H-Tetrazol-5-yl)-[1,1'-biphenyl]-4-yl)methyl)-2-butyl-4-methyl-6-oxo-1,6-dihydropyrimidin-5-yl)-N,N-dimethylethanethioamide

Molecular Formula: $C_{27}H_{31}N_7OS$

Molecular Weight: 501.653 g/mol

Log P: 4.03

pKa (*strongest Acidic*) : 4.23

pKb (*Strongest Basic*) : 1.34

Solubility: methanol, acetonitrile

Drug category: Antihypertensive

Mechanism of action:

Angiotensin II causes activation of AR1 as a result there is vasoconstriction and increase in release of nor adrenaline. Angiotensin II activates secretion of aldosterone there is increase in sodium and water reabsorption in the renal tubules. FIMA blocks Angiotensin II. It prevents vasoconstriction and cause reduction of aldosterone secretion thereby causing reduction in blood volume [9].

Uses: in the treatment of hypertension and heart failure

Marketed Formulation: As FIMA formulation Kanarab is not available in Indian market, synthetic mixture was prepared various studies as per the formula mentioned in patent [10] and is shown in Table 5.1.

Table 5. 1 - Composition of FIMA synthetic mixture

Composition	Quantity (mg)
Fimasartan	60 mg
Lactose	48.39 mg
Microcrystalline cellulose	10.6 mg
Cross caramellose sodium	10.2 mg
Magnesium stearate	2.04 mg

5.3. LITERATURE REVIEW

The literature already reported is summarized here:

- *Evaluation of stability and simultaneous determination of FIMA and amlodipine by HPLC method in combination tablets by Hyeon W M et.al.[11].*

Method was developed for simultaneous estimation of fimasartan and amlodipine in tablet dosage form by HPLC. Stability of fimasartan and amlodipine were estimated under normal and stress conditions. The method was developed in isocratic mode using Nucleosil C 18 column (250 x 4.6mm, 5µm) at 40˚C. The mobile phase composed of acetonitrile and 0.02 M monopotassium phosphate buffer (2.2) in the ratio 50: 50 with flow rate of 1mL/min. Detection was performed at 237 nm. The method was validated as per ICH guidelines.

- *Fully validated ultra-performance liquid chromatography-tandem mass spectrometry for the determination of Fimasartan in human plasma by Ji Y H et.al.[12].*

UPLC-MS/MS was developed for determination of FIMA in human plasma and validated. Analytes were quantified by MS/MS in a multiple reaction monitoring mode for FIMA and internal standard BR-A-563. The method was linear in the range of 3 to 1000 ng/mL with r^2 > 0.9950. The intra and inter day accuracy values at four concentration levels were 86.9-98.2 and 93.3-100.1% respectively. The intra- and inter-day precision values at four concentration levels were 2.0-3.1 and 0.8-8.0% respectively. The developed method was applied to pharmacokinetic study of FIMA in healthy volunteers.

- *Development and validation of a high-performance liquid-chromatographic tandem-tandem mass spectrometry for quantitative determination of FIMA and amlodipine in human plasma : Its application to pharmacokinetic study of 60 mg FIMA and 10 mg Amlodipine by Do-Hyung K et. al [13]*

HPLC-ESI –MS (LC-MS/MS) method was developed for the determination of FIMA and Amlodipine using BR-A-563 and Clarithromycin was used as an internal standard. Liquid – liquid extraction was performed on human plasma using tert-butyl ether and methyl chloride for amlodipine. Multiple reaction monitoring mode was performed for FIMA, BR-A-563 and clarithromycin. Method was developed on Kinetex C 18 (75 x 2.1mm, 2.6 µm) mobile phase composed of 0.05% formic acid and methanol in the ratio of 30: 70 at flow rate of 0.2 mL/min for FIMA and Luna C 18 (50 x 2.0 mm, 3.0 µm) with mobile phase composed of 0.1% acetic acid and methanol in the ratio of 30: 70 in the ratio of 0.2 mL/min for amlodipine. Retention time was about 1.6 min and 1.0 min for FIMA and amlodipine. The method was applied for pharmacokinetic study of complex tablet.

- *Validated LC-MS/MS assay for the quantitative determination of Fimasartan in human plasma: application to pharmacokinetic studies by Seo H Y et. al. [14]*

LC-MS/MS method was developed for quantification of FIMA in human plasma and internal standard BR-A563. Extraction was performed by protein precipitation with acetonitrile. Separation was developed on Phenyl-Hexyl Phenomenex Luna column (50 mm x 2.0 mm, 5µm). Elution was in gradient mode with mobile phase A (0.1 % formic acid in water) and mobile phase B (0.1 % formic acid in acetonitrile). Detection and quantification was performed by mass spectrometer using multiple reactions monitoring mode for FIMA and internal standard. The method was applied for determination of FIMA in Healthy Korean Male Volunteers in several drug-drug interaction studies.

- *Estimation of fimasartan and its active metabolite in rat plasma using liquid chromatography –tandem mass spectrometry by Beom S S et. al. [15]*

LC-MS method was developed for simultaneous determination of FIMA and its active metabolite BR-A-557 in rat plasma. The multiple reaction monitoring was performed for FIMA, BR-A-557 and BR-A-563 (internal standard). Precipitation was done with acetonitrile and isocratic elution. The developed method was used to study the metabolism and pharmacokinetics of FIMA in future studies.

- *Pharmacokinetics and metabolite profiling of fimasartan , a novel antihypertensive agent in rats by Tae H K et. Al.[16]*

Radiolabelled [(14) C] FIMA was dosed by intravenous injection or oral administration to rats. Concentration of unlabelled FIMA in biological samples was determined by LC/MS/MS assay. Metabolite identification was performed by product ion scanning using LC/MS/MS. Major metabolite desulpho fimasartan was identified with not more than 7.2% of the exposure of the parent drug.

- *Recent applications of spectrophotometric and thin layer chromatographic methods for quality control of fixed dosage combinations of sartans with hydrochlorothiazide by Vladimir P et. al. [17]*

The present study summarizes the application of spectrophotometric and thin layer chromatographic methods for simultaneous determination of sartans and thiazide diuretic

hydrochlorothiazide in fixed dosage preparations. Sartans in fixed dosage formulations with hydrochlorothiazide are estimated by different types of spectrophotometric methods : UV, first, second and fourth derivative and first derivative of ratio spectrophotometry, zero crossing difference spectrophotometric, simultaneous equation method, absorbance ratio and chemometric methods and HPTLC methods with different mobile and stationary phases.

5.4. SECTION - A

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING HPLC METHOD

5.4.1. EXPERIMENTAL

5.4.1.1. Chemicals and Reagents

- FIMA bulk drug was purchased from Angene Chemical Ltd, China.
- HPLC grade Acetonitrile was purchased from Rankem Pvt.Ltd. Mumbai.
- Potassium dihydrogen orthophosphate (AR grade), ortho phosphoric acid (HPLC grade), formic acid (HPLC grade) were procured from Loba Chemie Pvt. Ltd. Mumbai.
- Hydrochloric acid (HCl), sodium hydroxide (NaOH), hydrogen peroxide H_2O_2) were purchased from S.D. Fine Chemical Ltd. Mumbai.
- 0.22 µm Nylon 6, 6 membrane filter, Ultipore® N, 66® for filtration of mobile phase was procured from Pall Life Sciences, USA.
- 0.45 µm Nylon 6,6 syringe filter for sample filtration was procured from Pall Life Sciences ,USA.

5.4.1.2. Equipments and Instruments

The equipments utilized in the present experiment are same as those mentioned in section 3.4.1.2 and 4.4.1.2 (for NMR, Mass and IR studies).

5.4.1.3. Chromatographic conditions

Buffer used in the mobile phase was phosphate buffer (10 mM) which was prepared by dissolving 1.36 g of potassium dihydrogen orthophosphate (KH_2PO_4) in 1 L of double distilled water. The pH was adjusted to three with ortho phosphoric acid. The mobile phase consisted of mixture of 10 mM phosphate buffer (pH 3.0) and acetonitrile in the ratio of 50: 50. Analysis was

performed at ambient temperature with detection wavelength at 262 nm and flow rate of 1mL/min. The injection volume was 20µL. Analysis was performed on Thermo Hypersil BDS C-18 column (250 x 4.6 mm i.d. x 5 µm particle size).

5.4.1.4. Preparation of Standard solution

FIMA standard solution (1mg/mL) – 25 mg of FIMA was weighed accurately and transferred to 25 mL volumetric flask, dissolved in water and acetonitrile (50 :50) and volume was made up to the mark.

Working standard solution- Working standard solution was prepared in the concentration of 100µg/mL from stock solution in water and acetonitrile (50:50).

Calibration curves were prepared in the concentration ranging from 5 to 30µg/mL from working standard solution.

5.4.1.5. Preparation of forced degradation sample

For forced degradation study, stock solution of FIMA (1mg/mL) was prepared in water: acetonitrile (50:50). Diluent used was water and acetonitrile (50:50).

5.4.1.5.1. Acid degradation

2.5 mL of FIMA stock solution was transferred to 25 mL of volumetric flask, to this was added 1 mL of 1 M HCl. The solution was heated at 100ºC for 3 hrs. The solution was neutralized with 1mL of 1 M NaOH and volume was made up to 25 mL with diluent to make the concentration of 100µg/mL. The solution was filtered through 0.45 µ Nylon 6, 6 syringe filter before injecting into HPLC system.

5.4.1.5.2. Alkaline degradation

2.5 mL of FIMA stock solution was transferred to 25 mL of volumetric flask, to this was added 1 mL of 1 M NaOH. The solution was heated at 100ºC for 3 hrs. The solution was neutralized with 1 M HCl and volume was made up to 25 mL with diluent to make the concentration of 100µg/mL. The solution was filtered through 0.45 µ Nylon 6, 6 syringe filter before injecting into HPLC system.

5.4.1.5.3. Oxidative degradation

2.5 mL of FIMA stock solution was transferred to 25 mL of volumetric flask, to this was added 1 mL of 0.9% hydrogen peroxide. The solution was kept at room temperature (40ºC) for 30 min. The volume was made up to 25 mL with diluent to make the concentration of 100µg/mL. The solution was filtered through 0.45 µ Nylon 6, 6 syringe filter before injecting into HPLC system.

5.4.1.5.4. Neutral hydrolysis

2.5 mL of FIMA stock solution was transferred to 25 mL of volumetric flask, to this was added 1 mL of water. The solution was heated at 100ºC for 4 hrs. The volume was made up to 25 mL with diluent to make the concentration of 100µg/mL before injecting into HPLC system.

5.4.1.5.5. Dry heat degradation

For dry heat degradation, 25 mg of FIMA was kept in oven at 80ºC for 11 days. The sample was transferred to 25 mL of volumetric flask, dissolved in diluent. From this, concentration of 100 µg/mL of solution was prepared and injected into HPLC system.

5.4.1.5.6. Photolytic degradation (Dry)

For photolytic degradation, 25 mg of FIMA was spread in 1 mm thickness and was exposed in photolytic chamber for 11 days. Volume was made up to 25 mL and from this, concentration of 100µg/mL was prepared and injected into HPLC system.

5.4.1.5.7. Photolytic degradation (Solution)

FIMA solution (1mg/mL) in water and acetonitrile was exposed in photolytic chamber for 11 days. From this, concentration of 100µg/mL was prepared and injected into HPLC system.

5.4.1.6. HPLC method validation

The developed method was validated as per guidelines by ICH Q2B.

For linearity, standard dilutions of FIMA were prepared in the concentration ranging from 5 to 30µg/mL from FIMA working standard solution and were injected in triplicate. Linearity was determined by plotting peak area and concentration of solution. From the graph regression equation and regression coefficient was determined.

For precision, intra-day and inter-day precision were evaluated at six concentration levels (in triplicates). Peak areas corresponding to the concentration was calculated and % RSD was determined for intra-day and inter -day precision.

% Recovery was evaluated by standard addition method. To the sample solution of concentration 20µg/mL, standard solutions were added as 80%, 100% and 120% to get final concentrations of 36, 40, 44µg/mL. The concentrations were analysed in triplicates. % recovery and % RSD were calculated.

Limit of detection and limit of quantitation were calculated on the basis of standard deviation of the intercept and slope of the calibration curve. LOD and LOQ were calculated using equation 3.3*(σ/S) and 10*(σ/S), where σ is the standard deviation of intercept and S is the slope of the calibration curve.

For robustness, factors like pH of buffer (2.8, 3.0, and 3.2), percentage of acetonitrile in the mobile phase (48, 50 and 52) and flow rate (0.9, 1.0 and 1.1mL/min) were changed. Robustness of the method was evaluated at 10 µg/mL of concentration in triplicates.

The standard stock solution of FIMA was prepared in water and acetonitrile (50:50) and kept for 24 hours at room temperature. The stability of stock solution was determined.

Specificity of the method was evaluated by analysis of API as well as in synthetic mixture along with degradation products and to check the method for interference of any peaks affecting the estimation of FIMA.

System suitability tests were performed injecting six times of the concentration. The parameters retention time, asymmetry factor and theoretical plates were noted.

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5.4.2. RESULTS

5.4.2.1. Determination of suitable wavelength

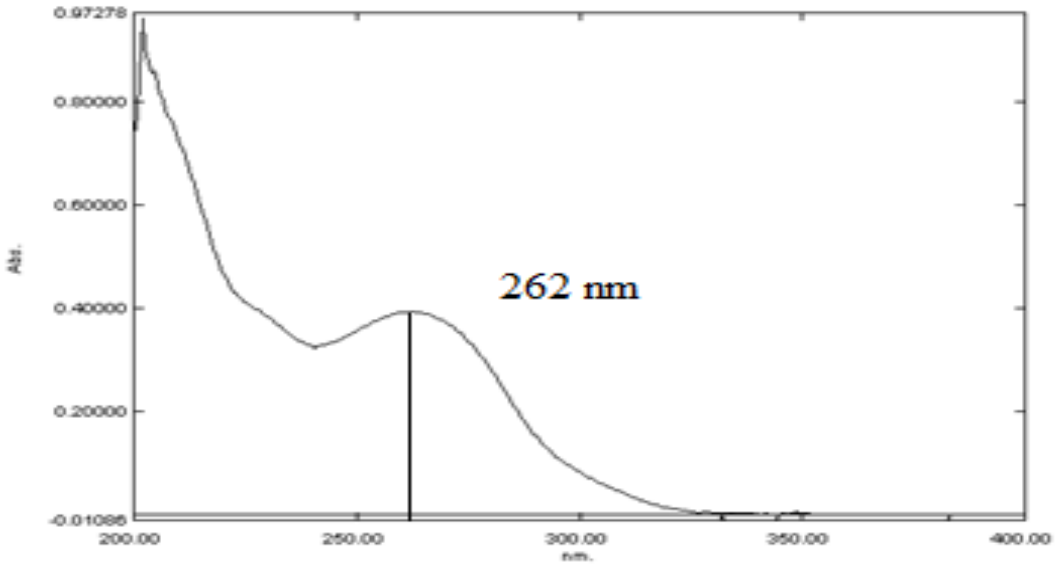


Fig. 5. 1 - Zero order spectra of FIMA (10 µg/mL)

FIMA in the concentration of 10µg/mL was scanned in the UV region of 200-400 nm and the spectrum was recorded. FIMA showed strong absorbance at 262 nm which was selected as the analytical wavelength (Fig. 5.1).

5.4.2.2. Method optimisation and development

For optimisation of chromatographic conditions, effect of chromatographic parameters like pH of mobile phase, composition of mobile phase and flow rate were studied. For optimization initial trials were taken with water: methanol, water: acetonitrile. With water: methanol elution was late and broad peak was observed. Phosphate buffer pH 3 was found to be suitable for stability, theoretical plates and reproducibility. Among the organic modifier, with acetonitrile good peak shape was observed and reduction in longer retention time. Method was developed on Hypersil BDS C 18 column with mobile phase 10 mM phosphate buffer pH 3 and acetonitrile in the ratio of 50:50. Trials for optimization are shown in Table 5.2.Optimised HPLC conditions are shown in Table 5.3. Chromatogram of standard solution of FIMA is shown in Fig. 5.2.

Table 5. 2 – Optimisation of HPLC conditions

Mobile phase	Rt (min)	Observation
Water : Methanol(50 :50)	16.23 min	Broad peak
Water : Methanol (30 : 70)	12.14 min	Broad peak
Water : Acetonitrile (50 :50)	13.23 min	Slightly broad
Phosphate buffer 10 mM pH 3 : Methanol (50 :50)	10.34 min	Broad peak
Phosphate buffer pH 3: ACN (45 :55)	5.610 min	Sharp and symmetrical peak
Phosphate buffer pH 3: ACN (50 :50)	**7.231 min**	**Sharp and symmetrical peak**
For LC-MS analysis 0.1 % Formic acid : ACN (50 :50)	9.012 min	Sharp and symmetrical Peak

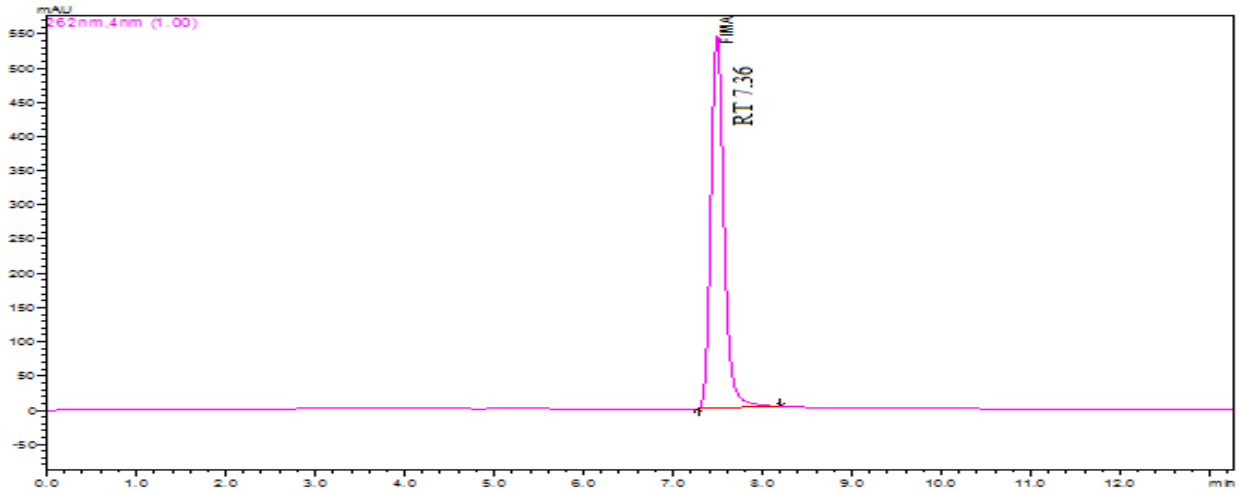


Fig. 5. 2 -Chromatogram of standard solution of FIMA (100µg/mL)

Table 5. 3 – Optimised HPLC conditions

Parameters	Optimised Value
Column	Thermo Hypersil BDS C 18 (250 x 4.6mm i.d. , 5µ particle size)
Mobile phase	Phosphate buffer 10 mM pH 3.0 and acetonitrile (50 : 50)
Flow rate	1.0 mL/min
Retention time	7.306 ± 0.052 min
Detection wavelength	262 nm
Needle wash	Mobile phase
Column temperature	Ambient

5.4.2.3. Method validation using ICH Q2(R1) guideline

5.4.2.3.1. *Linearity and range*

The calibration plotted for FIMA was found to be linear in the range of 5-30µg/mL. The regression equation was found to be y= 47916x+ 68343 with regression coefficient (r^2) of 0.999.The linearity data is shown in Table 5.4. and calibration curve is shown in Fig. 5.3.

Table 5. 4– Linearity Data of FIMA

Conc. (µg/mL)	Peak Area (Mean* ±%RSD)
5	288365.7± 0.80
10	573539±0.76
15	796531±0.97
20	1052270±0.81
25	1281521±0.68
30	1516550±0.80

*Average of three determinants

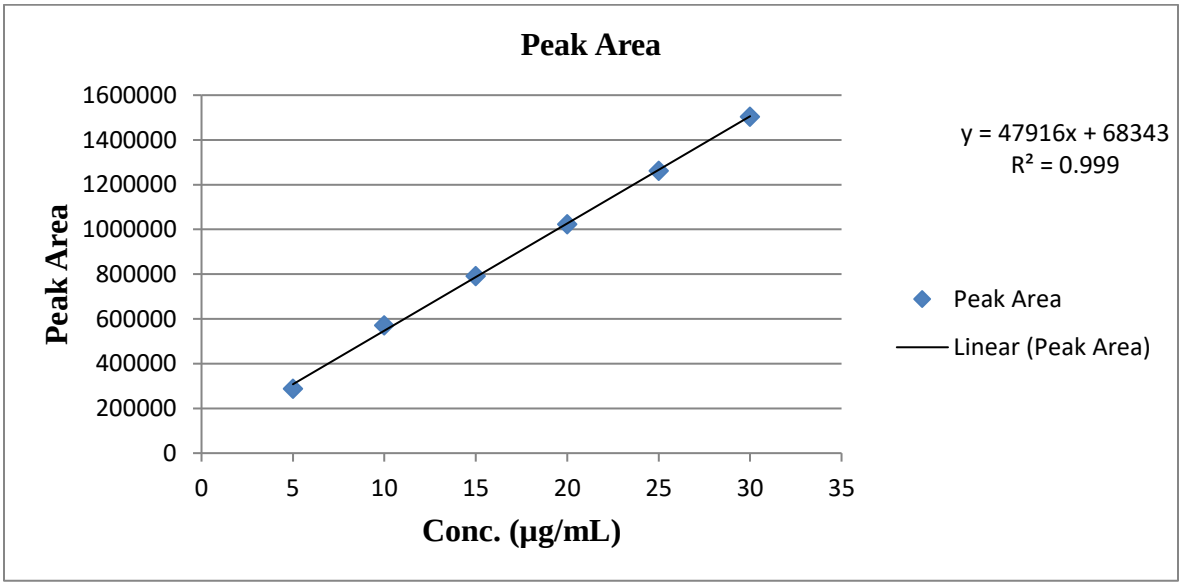


Fig. 5. 3 – Calibration curve of FIMA (Peak area versus concentration (µg/mL)

5.4.2.3.2. *Precision*

Intra-day precision was performed by repeating the experiment three times in a day and inter-day precision was performed by repeating the experiments on three consecutive days. The average %RSD of intra-day and inter-day were found to be 0.85 and 1.12. The developed method was found to be precise (Table 5.5 and 5.6).

Table 5. 5 - Intraday Precision of FIMA

Conc. (µg/mL)	Peak Area				
	Set 1	**Set 2**	**Set 3**	**Mean**	**%RSD**
5	288165	286762	282043	285656.7	1.12
10	570104	572092	562852	568349.3	0.85
15	802460	812578	810327	808455	0.65
20	1051365	1067476	1054634	1057825	0.80
25	1281873	1260741	1278207	1273607	0.88
30	1518083	1493457	1503145	1504895	0.82
			Average	%RSD	0.85

Table 5. 6 - Interday Precision of FIMA

Conc. (µg/mL)	Peak Area				
	Set 1	**Set 2**	**Set 3**	**Mean**	**%RSD**
5	288165	287134	280567	285289	1.44
10	570104	573789	561035	568309	1.15
15	802460	813567	803678	806568	0.75
20	1051365	1062836	1073214	1062472	1.02
25	1281873	1260584	1249064	1263840	1.31
30	1518083	1486788	1502345	1502405	1.04
			Average	%RSD	1.12

5.4.2.3.3. ***Accuracy***

Accuracy of method was determined by calculating % percent recovery of the analyte recovered. To the sample concentration of 20 µg/mL, standard solution of FIMA was added as 80%, 100% and 120% to give concentrations as 36, 40, 44 µg/mL. Recovery greater than 98% indicates the developed method was accurate (Table 5.7).

Table 5. 7– Accuracy data of FIMA

Excess drug added to analyte (%)	Theoretical Content (µg/mL)	*Amount Found (µg/mL)	Recovery (%)± SD
0	20	100.02	100.02 ± 0.04
80	36	35.81	98.75 ± 0.05
100	40	39.79	99.02 ± 0.06
120	44	44.19	100.80± 0.05

***Average of three determinants**

5.4.2.3.4. ***Limit of detection and limit of quantification***

LOD and LOQ were found to be 1.54 and 4.67 µg/mL.

5.4.2.3.5. ***Robustness***

For robustness study, slight changes were made in pH of buffer, % of organic in mobile phase and flow rate. The results were expressed as % RSD. % RSD less than 2 indicated that the developed method was robust (Table 5.8). Robustness was evaluated at concentration of 10µg/mL.

Table 5. 8 – Robustness data of FIMA

Parameter	Level	Area		Rt		Asymmetry		Theoretical Plates	
		Mean	%RSD	Mean	%RSD	Mean	%RSD	Mean	%RSD
pH	2.8	565131.7	1.24	7.32	0.30	1.22	0.53	10686.63	0.20
	3	572726	0.36	7.42	0.13	1.20	0.53	10666.13	0.22
	3.2	572847.3	0.30	7.32	0.12	1.21	0.48	10629.2	0.30
% Organic	48	569941.3	0.49	8.24	0.36	1.22	0.43	10712.07	0.15
	50	579613	0.09	7.36	0.25	1.20	0.53	10701.03	0.12

	52	572042.3	0.01	6.48	0.08	1.21	0.12	10678.37	0.04
Flow rate	0.9	568493.7	0.06	8.11	0.13	1.21	0.50	10636.4	0.32
	1	571116.3	0.26	7.30	0.54	1.21	0.12	10615.7	0.15
	1.1	561644.3	0.35	6.70	0.45	1.22	0.47	10637.6	0.09

% Organic and flow rate are the critical factor in the robustness method.

5.4.2.3.6. Specificity

The specificity was determined from the forced degradation studies as described in section 5.4.1.5. and 5.4.2.4. where Fig. 5.15 shows FIMA peak is well separated from all degradation products formed during different stress conditions with sufficient resolution. In the forced degradation studies, for all degradation products, peak purity index was greater than single point threshold , ensures degradation peaks are pure and peaks are not co-eluting. The specificity study ensures selectivity of the developed method which is able to separate and quantify FIMA in presence of degradation products. Peak purity data of FIMA and degradation products are shown in Table 5.9.

Table 5. 9 – Peak purity data of FIMA and degradation products

S.No.	Peaks	Rt	Peak Purity Index	Single Point threshold
1	FIMA	7.45 min	0.99999	0.999995
2	DP1	3.1 min	0.999921	0.995541
3	DP2	3.9 min	0.999973	0.994610
4	DP3	4.2 min	0.999798	0.978086
5	DP4	4.8 min	0.999891	0.995276
6	DP5	6.1 min	0.999999	0.999955

5.4.2.3.7. *Stability in sample solutions*

Stock solution of FIMA and stressed samples were prepared from standard stock solution and then stored at room temperature for 24 hrs. No additional peaks were observed which indicated stability of FIMA sample solution.

5.4.2.3.8. *System Suitability Parameters*

System suitability tests were performed on freshly prepared solution with n=6 containing FIMA. The results of system suitability parameters are shown in Table 5.10. Peak purity data of FIMA is shown in Table 5.9 and peak purity curve is shown in Fig.5.4.

Table 5. 10 – System suitability parameters of FIMA

Parameters	Data Obtained
Retention Time (min ± SD)	7.30 ± 0.05
Tailing Factor ± SD	1.22 ± 0.01
Theoretical Plate ± SD	10689.82 ± 123.79

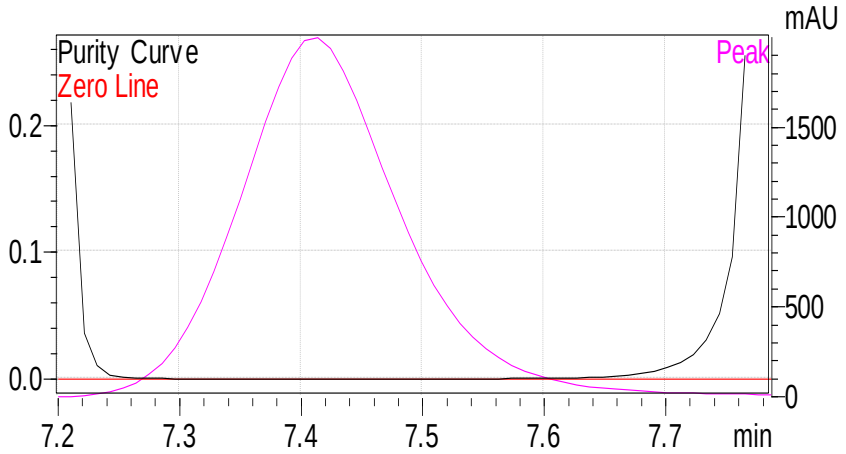


Fig. 5. 4 - Peak purity curve of FIMA

5.4.2.4. Stress Degradation studies

5.4.2.4.1. Alkaline degradation – Slight degradation (9.1%) was observed when FIMA was subjected to 1 M NaOH at 100 ºC for 4 hrs with the formation of degradation products DP1, DP2, DP3, DP4 and DP5 at retention time of 3.1, 3.9, 4.2, 4.8. and 6.10 min respectively(Fig. 5.5).

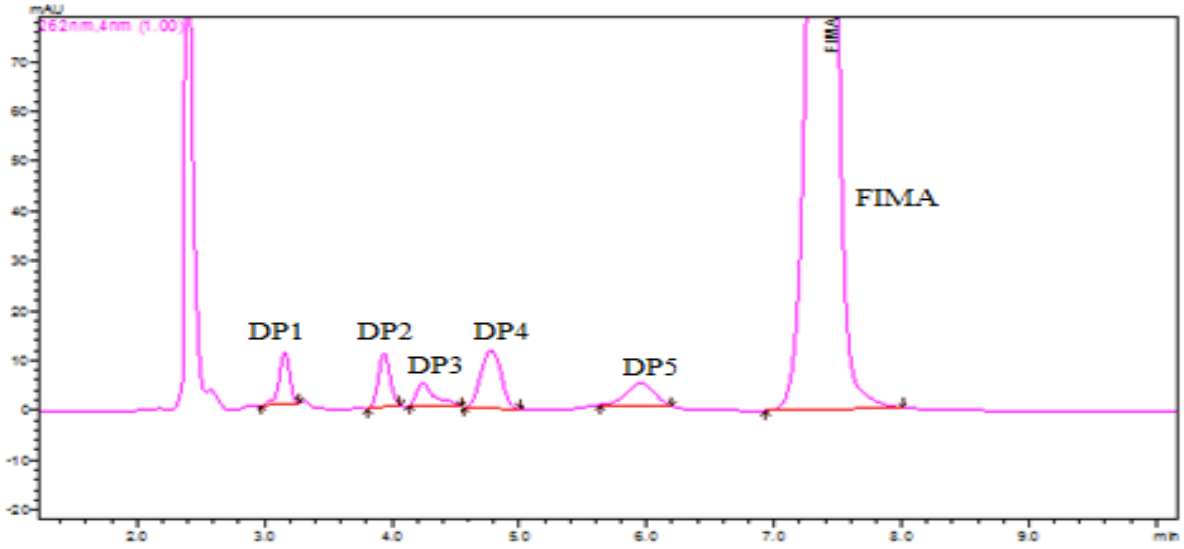


Fig. 5. 5- Chromatogram of alkaline degradation (API)

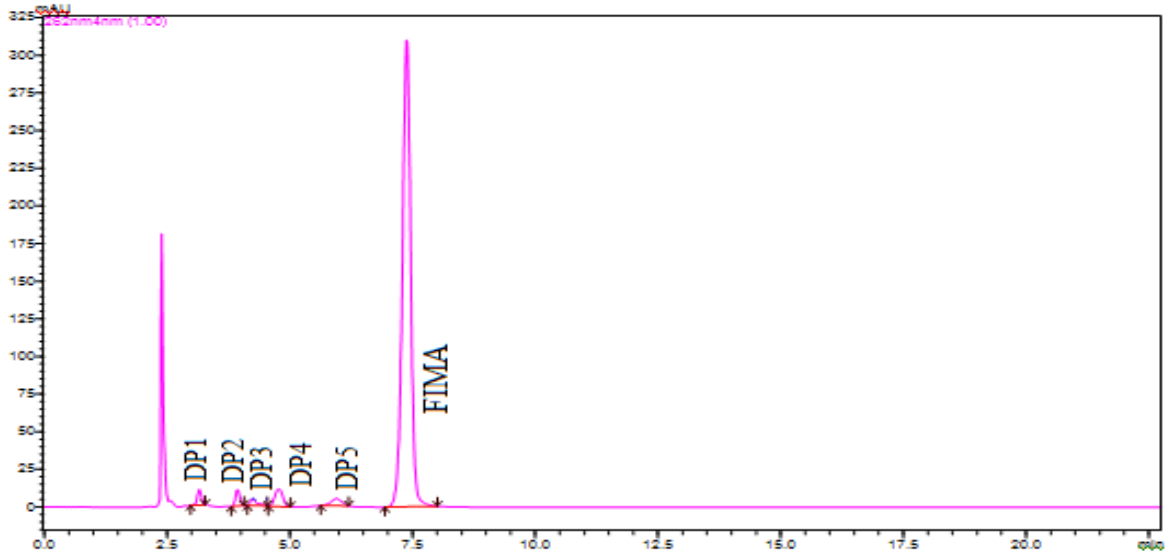


Fig. 5. 6 **- Chromatogram of alkaline degradation (synthetic mixture)**

5.4.2.4.2. Acid degradation – Slight degradation (0.5%) was observed when FIMA was subjected to 1 M HCl at 100ºC for 3 hrs with the formation of one degradation product DP4 at retention time of 4.9 min (Fig. 5.7).

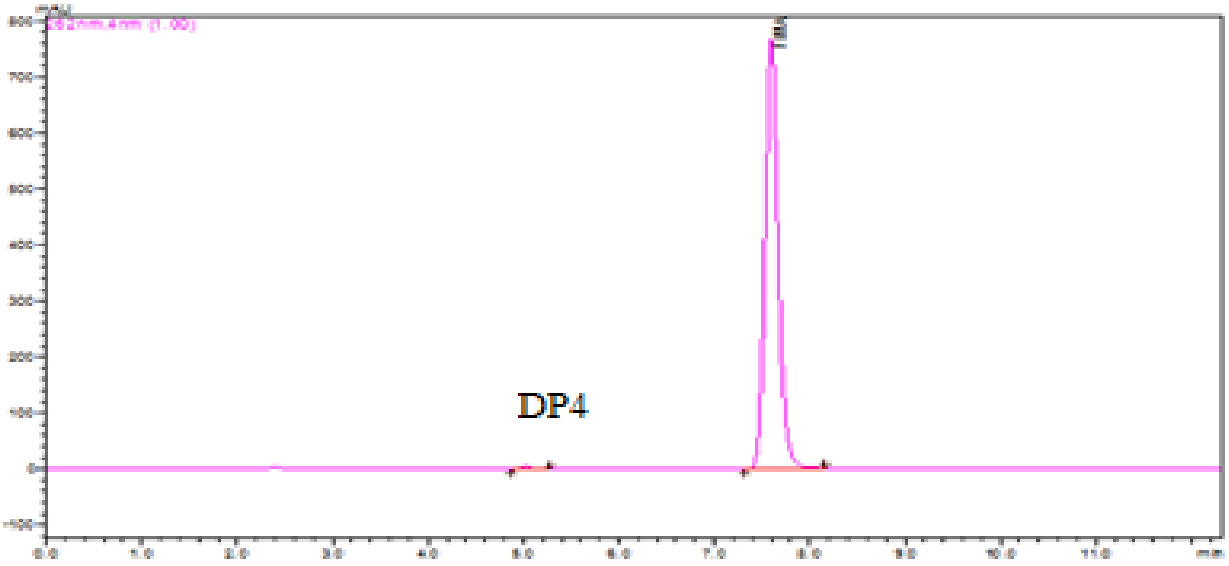


Fig. 5. 7- Chromatogram of acid degradation (API)

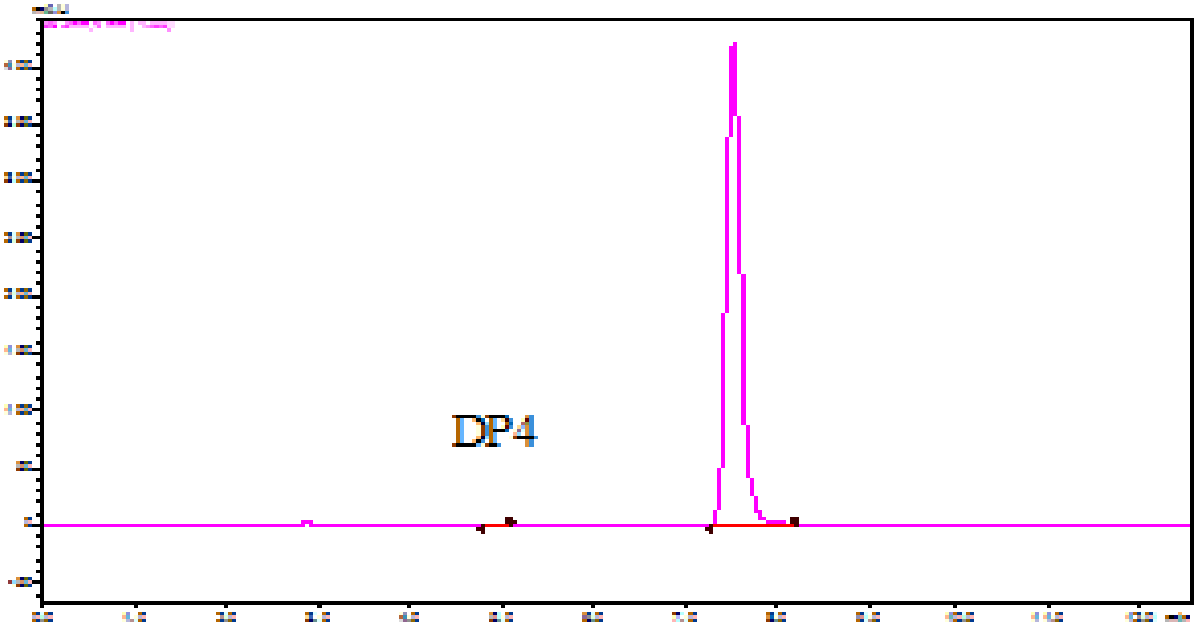


Fig. 5. 8 - Chromatogram of acid degradation (synthetic mixture)

5.4.2.4.3. Neutral hydrolysis degradation- No degradation was observed when FIMA was subjected to neutral hydrolysis at 100ºC for 4 hrs (Fig. 5.9).

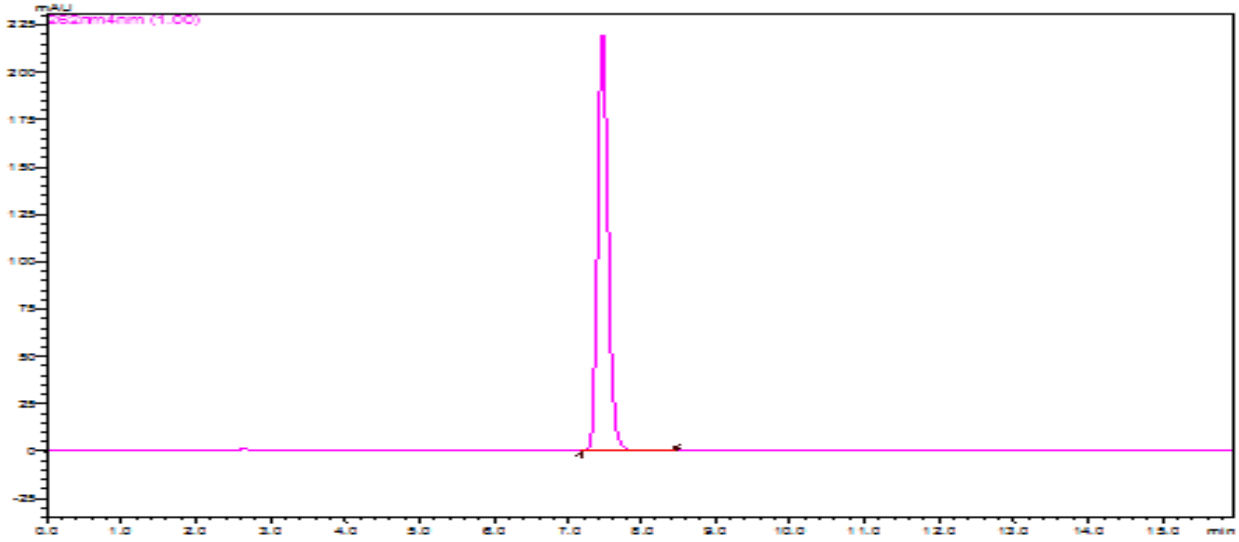


Fig. 5. 9 – Chromatogram of neutral hydrolytic degradation

5.4.2.4.4. Oxidative degradation- Significant degradation (27.9%) was observed when FIMA was subjected to 0.9 % hydrogen peroxide at room temperature for 30 min with the formation of degradation products DP2, DP4 at retention time of 3.9 and 4.9 min respectively (Fig. 5.10).

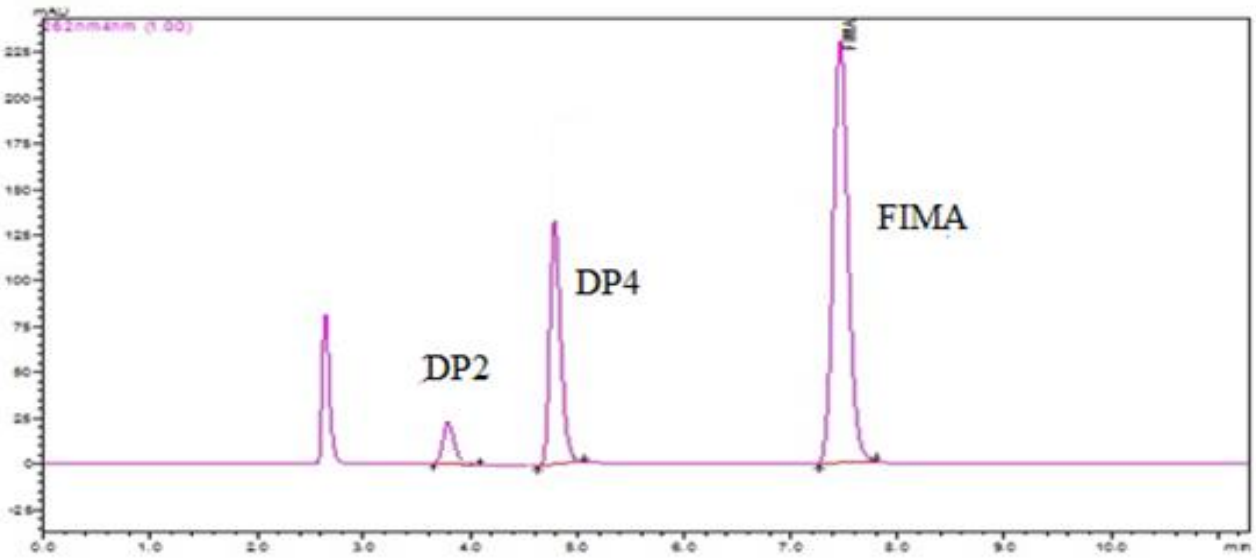


Fig. 5. 10 – Chromatogram of oxidative degradation (API)

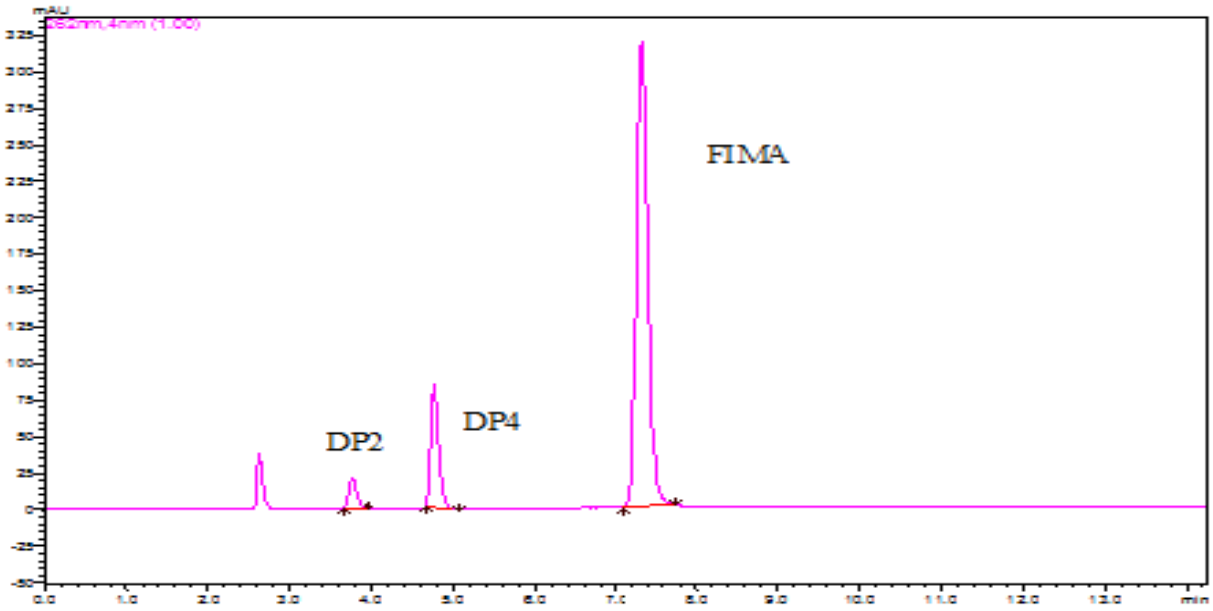


Fig. 5. 11 – Chromatogram of oxidative degradation (synthetic mixture)

5.4.2.4.5. Dry heat degradation

No degradation was observed when FIMA was subjected to thermal degradation at 80ºC for 11 days (Fig. 5.12).

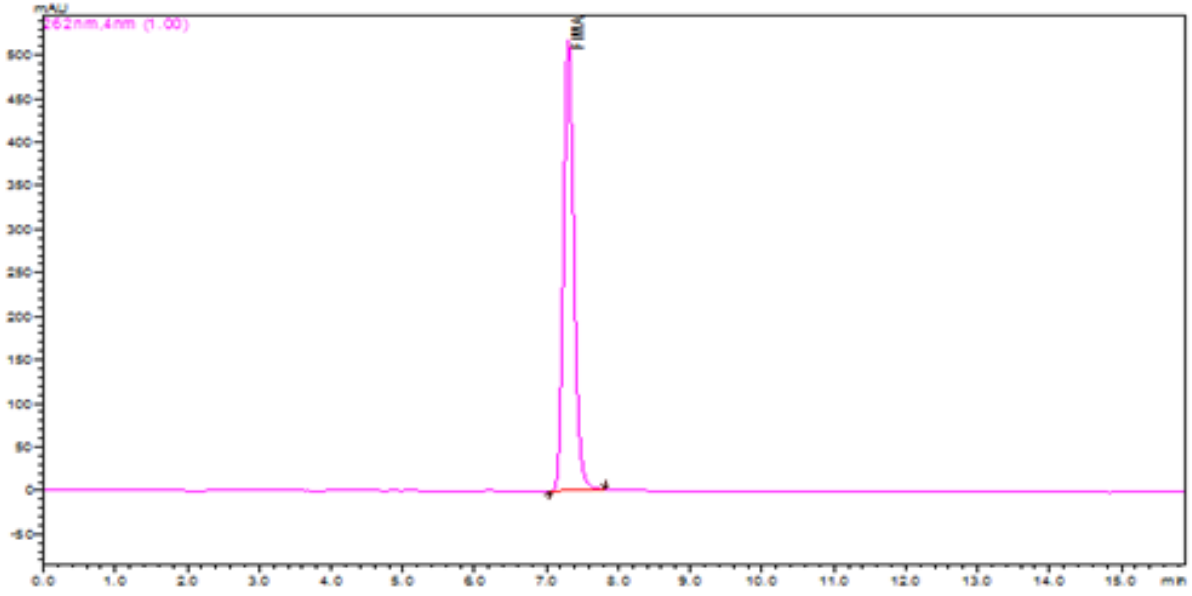


Fig. 5. 12 – Chromatogram of thermal degradation (API)

5.4.2.4.6. Photolytic degradation

No degradation was observed when FIMA was subjected to photolytic condition (dry) for 11days (Fig.5.13) . Slight degradation (0.5%) was observed when FIMA was subjected to photolytic condition (solution) for 11days with the formation of one degradation product DP4 at retention time of 4.9 min (Fig.5.14).

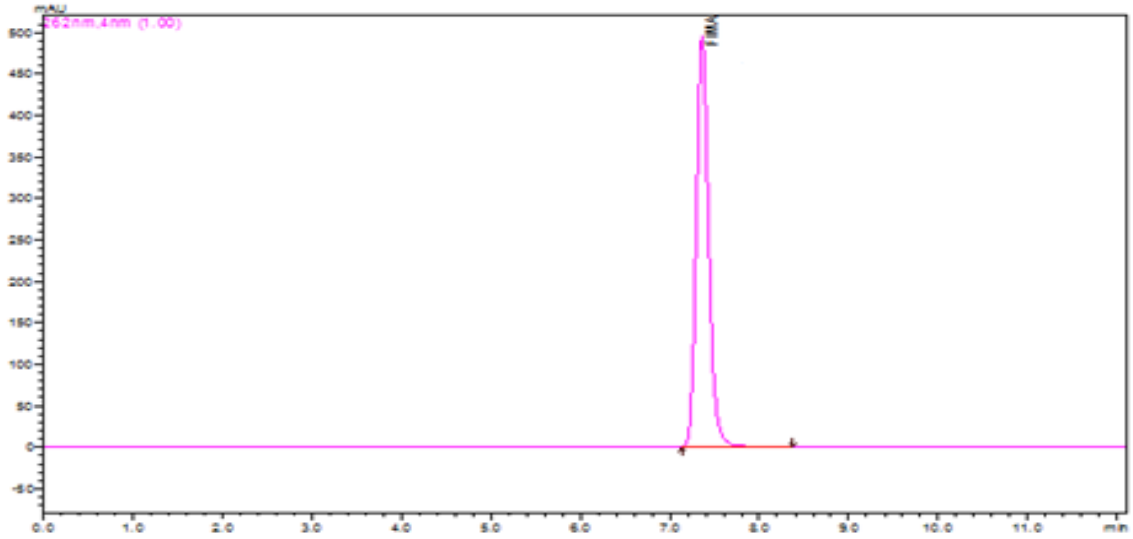


Fig. 5. 13 – Chromatogram of photolytic degradation (Dry) (API)

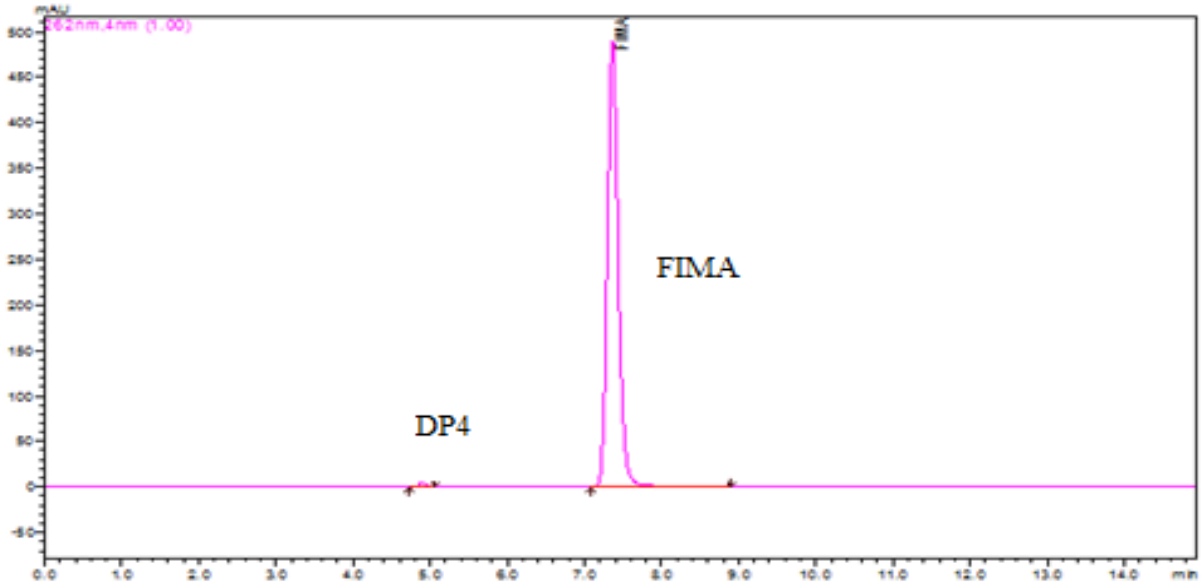


Fig. 5. 14- Chromatogram of photolytic degradation (solution) (API)

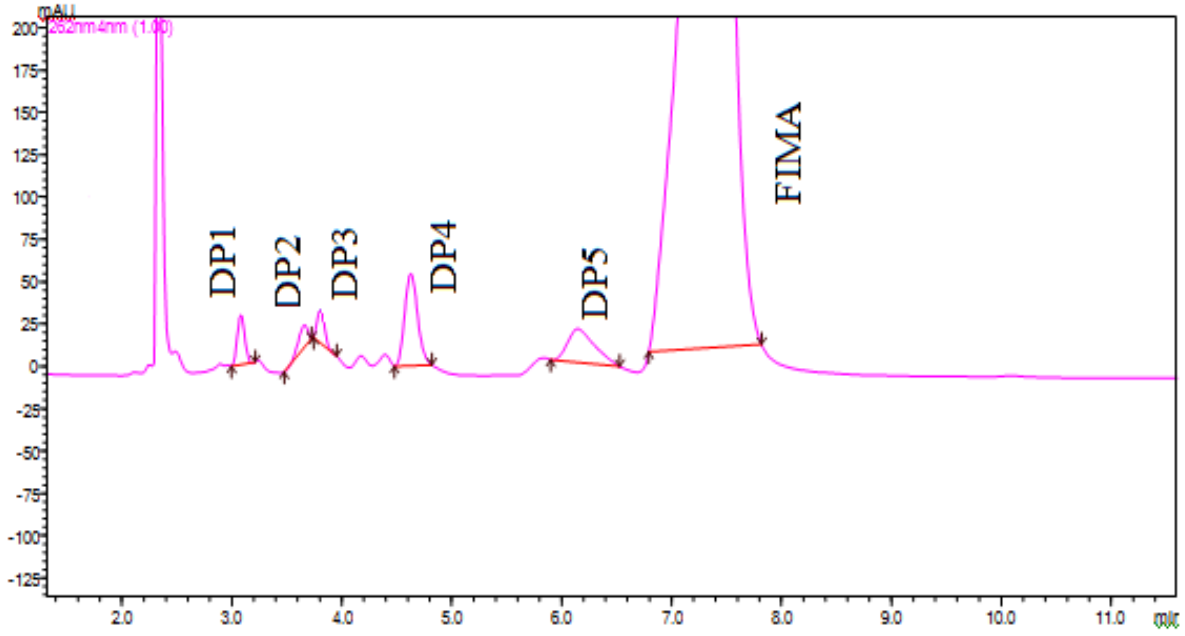


Fig. 5. 15- Chromatogram of combined degradation products of all stressors

Table 5. 11 - Summary of forced degradation study of FIMA

Stressor	**Conditions**	**RT of degradation products (min)**	**%Degradation (API)**	**% Degradation (Formulation)**
Acid	1 M HCl 100℃ for 3 hrs	4.9 (DP4)	0.5%	0.1%
Alkaline	1 M NaOH 100ºC for 3 hrs	3.1 (DP1) 3.9 (DP2) 4.2 (DP3) 4.8 (DP4) 6.1 (DP5)	9.1%	8.8%
Neutral hydrolysis	100ºc for 6 hrs	--	--	--

Oxidation	0.9% H_2O_2 RT (40ºC) for 30 min	3.9 (DP2) 4.9 (DP4)	27.9%	25.1%
Thermal	Dry at 80℃ for 11 days	--	--	--
Photolytic	Dry for 11 days	--	--	--
	Solution for 11 days	4.9 min (DP4)	0.5%	--

5.4.2.5. Applicability of the developed method for the analysis of synthetic mixture

Forced degradation study was performed on synthetic mixture. The conditions were same as mentioned for API and were analyzed in the same way as that of API. The degradation products were separated. Minor variation was observed in the degradation of API and synthetic mixture as shown in Table 5.11.

5.4.3. DISCUSSIONS

Maximum absorption wavelength of 262 nm was selected by scanning in the range from 400-200 nm. Various trials were optimized for determination. With mobile phase ratio of water and methanol, water: acetonitrile FIMA was eluting late. FIMA has pKb value 1.34, phosphate buffer 10 mM pH 3.0 was found to be suitable. Among organic modifier, sharp symmetrical peak was obtained with acetonitrile while with methanol late elution and broad peak was observed. In forced degradation study, significant degradation was observed in oxidative condition with the formation of formation of degradation products DP2 and DP4. Slight degradation was observed in alkaline conditions with the formation of five degradation products. DP2 and DP4 degradation products were formed in both alkaline and photolytic conditions and are confirmed by identification by LC-MS as mentioned in section 5.7.1.4. Degradation less than 1% was observed in acidic, photolytic condition (solution) with the formation of one degradation product DP4. No degradation was observed in neutral hydrolysis and thermal, photolytic condition (dry state). In wet photolytic condition, FIMA is not degraded due to neutral hydrolysis but degradation is because of photolysis i.e. aqueous medium is not affecting photolytic degradation.

5.5. SECTION - B

DEGRADATION KINETIC STUDY OF FIMASARTAN POTASSIUM BY HPLC METHOD

The degradation kinetics was studied for oxidative degradation only since FIMA was susceptible to oxidative condition, in all other conditions degradation was very low making it difficult to study its kinetics. Effect of H_2O_2 concentration, time and temperature was studied on the degradation of FIMA.

5.5.1. EXPERIMENTAL

5.5.1.1. Chemicals and Reagents

The chemicals and reagents used in the present section were same as those mentioned in section 5.4.1.1.

5.5.1.2. Equipments and Chromatographic Conditions

Equipments and chromatographic conditions were same as those mentioned in section 5.4.1.2.

5.5.1.3. Preparation of stock, sample and buffer solutions

Stock solution was same as those mentioned in section 5.4.1.5. To the 2.5 ml of stock solution of in FIMA in 25 ml of volumetric flask , 1 ml of reagent concentration 0.9% or 3% hydrogen peroxide was added. The solutions were kept at temperature ranging from 40ºC- 50˚C-60ºC and from time 1 hr to 5 hrs. The solution was made up to volume with diluent to make the concentration 100µg/mL and injected into the HPLC system.

5.5.2. RESULTS

Degradation rate kinetics was studied by % of drug remaining after degradation versus time (for zero order kinetics) using linear regression analysis, Log of % drug remaining after degradation (for first order process). Experiments were performed in triplicate and average values were taken for analysis. The rate constant (K), half-life ($t_{1/2}$) and activation energy (Ea) were calculated from slope of line at each temperature for oxidative degradation.

A regular decrease in concentration of FIMA was observed with increasing time intervals and with increase in temperature. Regression equation and regression coefficient was obtained for zero order and first order kinetics for two concentrations (0.9 and 3.0%) of hydrogen peroxide and at different temperatures. On the basis of regression, degradation follows first-order kinetics since r^2 values are highest (close to 1) (Table 5.12).

On the basis of first-order kinetics, further study was performed to study the effect of temperature on the rate constant the Arrhenius plots were plotted (log of rate constant versus reciprocal of temperature). Arrhenius equation as

$$\log K = \log A - Ea/2.303\ RT$$

where K is the rate constant, A is the frequency factor, Ea is the activation energy, R is the gas constant(1.987 cal/deg/mol) and T is the absolute temperature. Arrhenius plot was obtained by plotting lnK versus 1/T. Graph was linear in the temperature range. The first order kinetic plot and Arrhenius plot for oxidative degradation are shown in Fig 5.16-5.19. The values of degradation rate constant, half-life and activation energy are shown in Table 5.13.

Table 5. 12- r^2 value and Regression Equation for zero order, first order reaction for oxidative degradation

S.No.	Conc.H_2O_2	Temp(°C)	r^2		Regression Equation	
			Zero order	First order	Zero order	First order
1	0.9%	40	0.928	0.981	y=-11.6x+70.4	y=0.143x+1.932
		50	0.960	0.999	y=-11.89x+67.55	y=0.175x+1.961
		60	0.981	0.964	y=-12.19x+64.77	y=-0.229x+2.032
2	3.0%	40	0.778	0.99	y=-7.191x+30.63	y=-0.431x+2.120
		50	0.823	0.994	y=-8.691x+38.01	y=-0.473x+2.089
		60	0.853	0.993	y=-10.2x+45.44	y=-0.580x+2.121

Table 5. 13– Degradation rate constant, half-life and Activation Energy Ea for first order kinetic of oxidative degradation

S.No.	Conc. H_2O_2	Temp (°C)	K	$t_{(1/2)}$ hrs	E_a(KJ/mol)
1	0.90%	40	0.3293	2.104	16.71
		50	0.403	1.719	
		60	0.5273	1.314	
2	3%	40	0.9923	0.698	
		50	1.8093	0.636	
		60	1.3357	0.518	

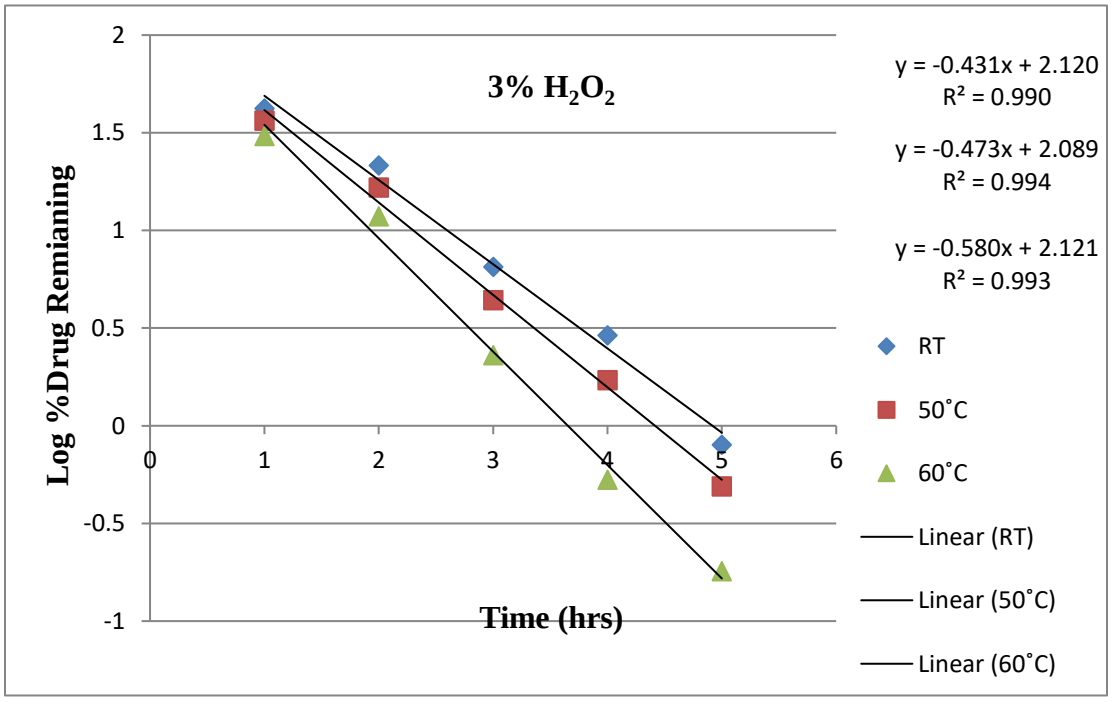


Fig. 5. 16 – First order reaction kinetics of 3% H_2O_2

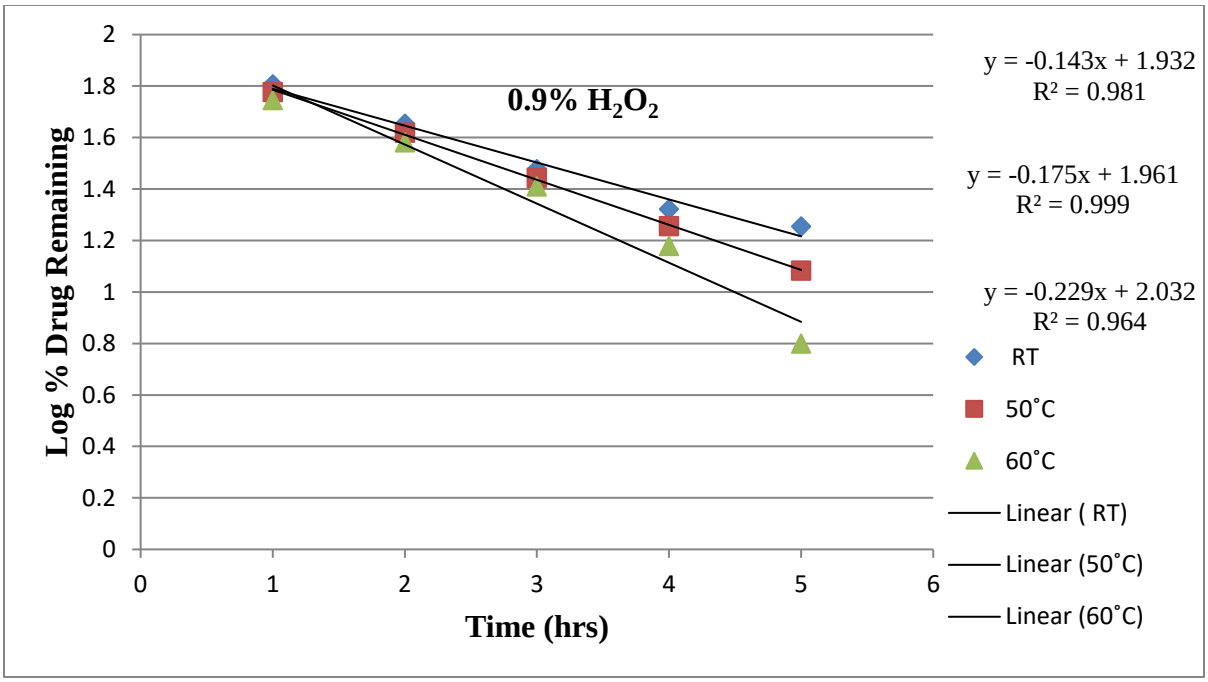


Fig. 5. 17 –First order reaction kinetics of 0.9% H_2O_2

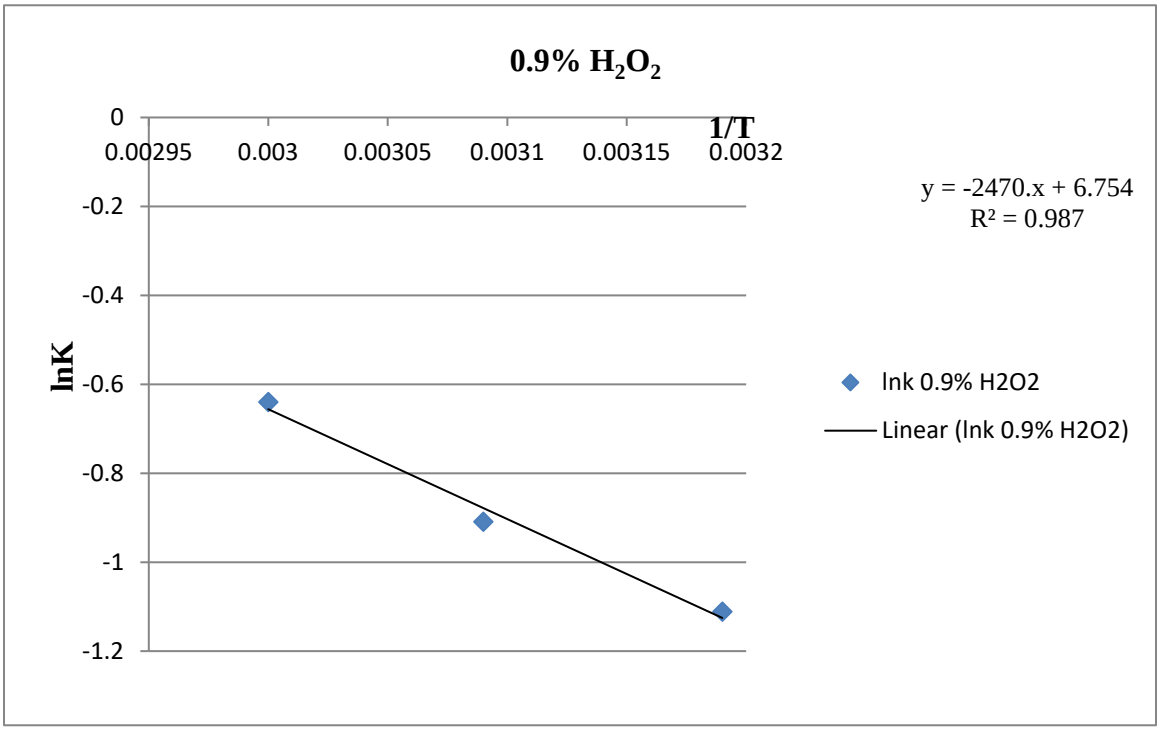


Fig. 5. 18 –Activation energy plot for 0.9% H_2O_2

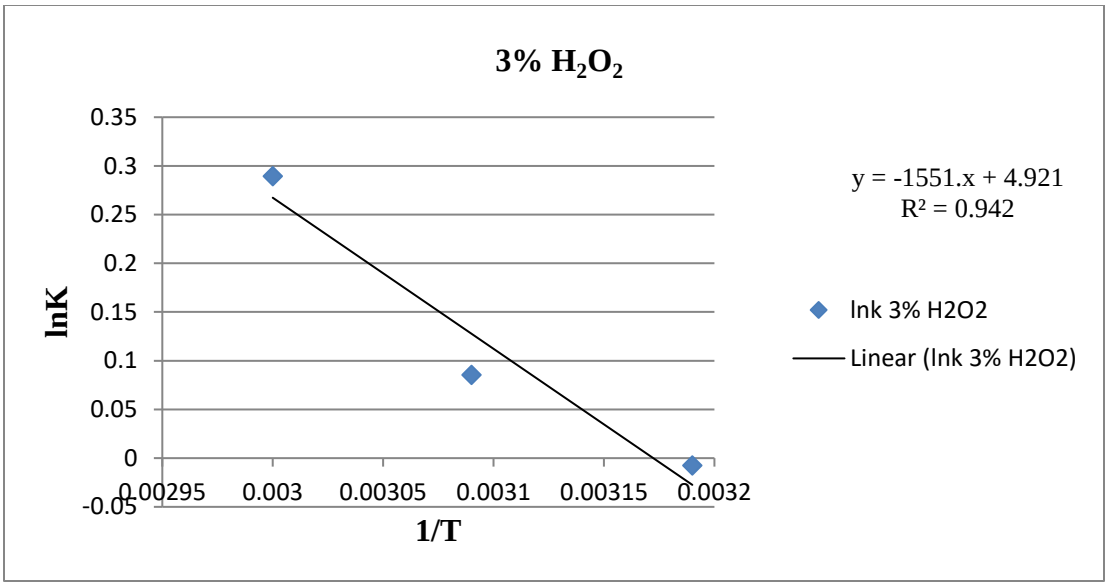


Fig. 5. 19 –Activation energy plot for 3% H_2O_2

5.5.3. DISCUSSIONS

Degradation kinetics was performed for oxidative conditions. Factors taken for kinetics study were: concentrations of hydrogen peroxide (0.9 and 3%), temperature (40º, 50ºand 60º C), time (1 to 5 hrs). Zero order kinetics study was performed by plotting graph between % drug remaining versus time and first order by plotting graph between log % Drug remaining versus time. Regression equation and regression coefficient were obtained for both zero and first order kinetics. Degradation follows first-order kinetics since regression coefficient r^2 *was* highest in first –order kinetics. Based on this degradation rate constant and half-life was calculated. On the basis of degradation rate constant by plotting lnk (rate constant) versus 1/T, activation energy was calculated and was found to be 16.71 KJ/mole.

5.6. SECTION - C

ISOLATION AND CHARACTERIZATION OF MAJOR DEGRADATION PRODUCT IN FIMASARTAN POTASSIUM

5.6.1. EXPERIMENTAL

5.6.1.1. Chemicals and Reagents

Chemicals and reagents used in the present section are same as those mentioned in 5.4.1.1.

5.6.1.2. Equipments and chromatographic conditions

Preparative High Performance Liquid Chromatography (HPLC):

Preparative HPLC system composed of Shimadzu LC-20 AP BINARY pump and Shimadzu SPD 20A detector.

Separation was performed on Daisogel-SP-100-10-ODS-P column with 250x50 mm, 100 Å, 10µ in diameter. Samples were injected through Rheodyne 7725 injector valve. Detection was performed at 262 nm. Flow rate was maintained at 55 mL/min. Class VP software was used for data processing. Mobile phase comprised of 0.1 % formic acid and acetonitrile in the ratio of 45:55.

5.6.1.3. Enrichment of oxidative degradation samples

500 mg of FIMA was weighed accurately and transferred to 25 mL of volumetric flask. To this was added 15 mL of water and acetonitrile followed by 10 mL of 30% hydrogen peroxide. The solution was kept at room temperature for 48 hrs.

5.6.1.4. Analysis of degradation samples by analytical HPLC

The oxidative degradation sample for separation was diluted to get the concentration of 100µg/mL and analyzed by analytical HPLC. Retention time of FIMA was 7.3 min. DP4 was formed with 75.4% degradation at retention time of 4.8 min (Fig. 5.20). Purification of DP4 was performed with preparative HPLC. DP4 with fractions of greater than 95% were pooled. Acetonitrile was removed by concentrating the solution on rotavapour. Remaining aqueous

solution was kept in lyophilizer overnight. DP4 was obtained as colourless solid. DP4 was recrystallized with hot water. % purity of DP4 was obtained as 99.4%.

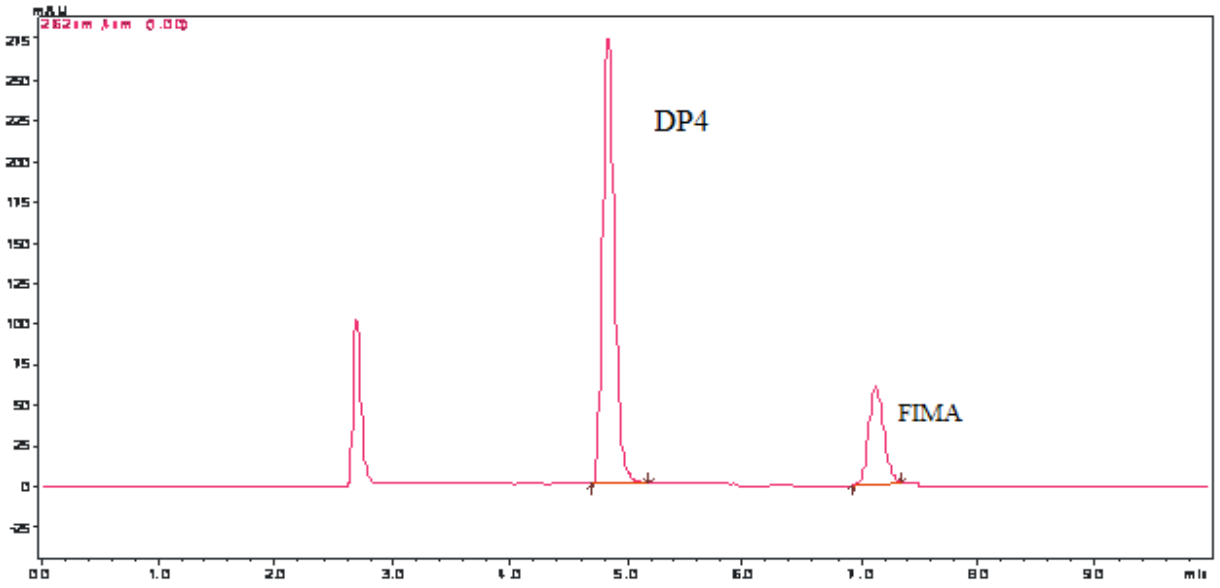


Fig. 5. 20 – Chromatogram of oxidative degradation for isolation

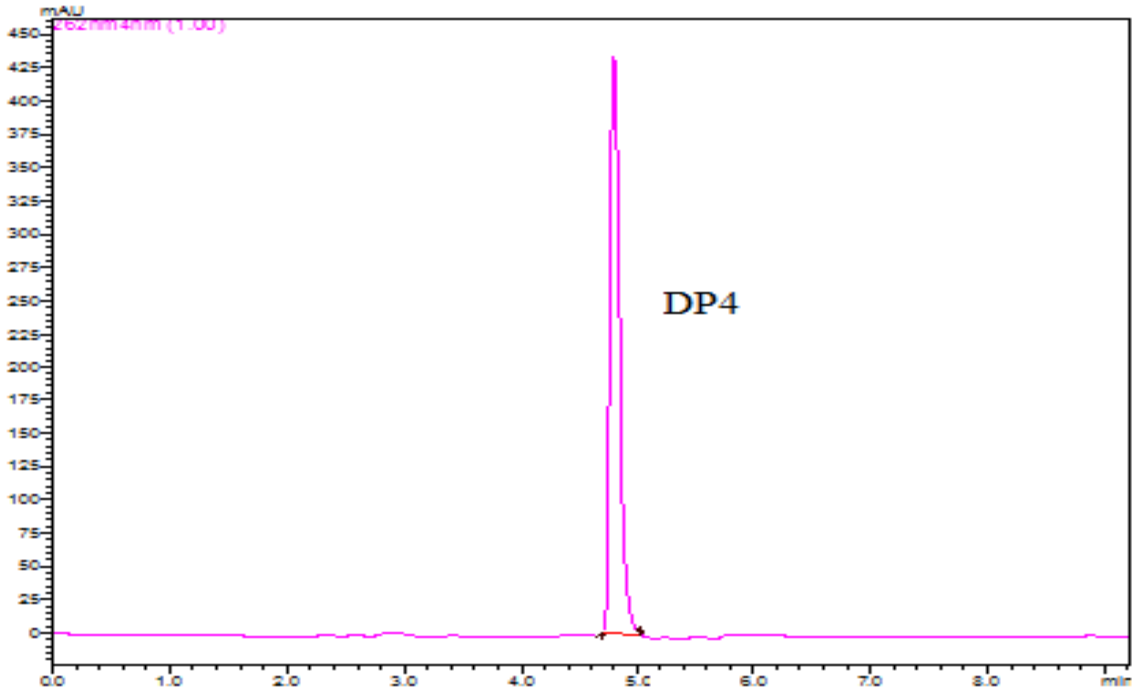


Fig. 5. 21- Analytical Chromatogram of DP4 after isolation

5.6.2. RESULTS

5.6.2.1. Identification of DP4

Spectral data for DP4 and FIMA was obtained and compared to get an insight into structure of DP4.

5.6.2.1.1. Spectral data of FIMA

Mass spectra

ESI-MS spectra of FIMA are provided in Fig.5.22. An ESI-MS spectrum of FIMA shows protonated m/z 502.33.

NMR spectra

^{1}H NMR spectra of FIMA (Fig. 5.24) indicates presence of methyl group at position 33 at 0.824 ppm. Methyl group at position 35 and 36 are indicated at 3.44, 3.45 ppm which are downfield since they are attached to nitrogen. Methyl group at position 29 is indicated at 2.15 ppm appears as singlet since attached to aromatic ring. Protons of aromatic ring (from 6 to 11) appear from 7.3 to 7.6 ppm. Protons of aromatic ring (from 13,14, 16,17) appears from 6.8 to 7.08 ppm. Presence of four methyl groups in FIMA is indicated with DEPT (Fig. 5.26) spectra as positive peaks. Presence of five methylene groups in FIMA is indicated in DEPT spectra as negative peaks. Thione group in FIMA is indicated ^{13}C NMR spectra (at 27 position) at 199 ppm. (Fig. 5.25).

IR spectra

IR spectra of FIMA indicates broad peak covering –NH, Aromatic C-H, CH_3 stretching and –CH_2 stretching in the region 2958 cm^{-1} to 2713cm^{-1}. Presence of pyrimidine amide is indicated at 1741 cm^{-1}. Aromatic C=C stretching in indicated in the region of 1616-1481 cm^{-1}. C=S stretching is indicated at 1230cm^{-1}. An I.R. spectrum of FIMA is shown in Fig. 5.27 and IR interpretation is shown in Table 5.15.

5.6.2.1.2. Characterization of DP4

Mass spectra

An ESI-MS/MS spectrum of DP4 is provided in Fig. 5.29. An ESI-MS/MS spectrum of DP4 indicates protonated m/z 486. It has 16 m/z less than FIMA. Fragmentation pathway of DP4 is shown in Fig. 5.30. DP4 undergoes fragmentation with the loss of dimethylamino group forms fragment ion at m/z 441. Further fragments are produced at m/z 413, 235 and 207 with the loss of –CO group, pyrimidinone moiety, $-N_2H$ from tetrazole respectively.

NMR spectra

Numbering assignments of DP4 are shown in Fig. 5.28. ^{1}H NMR spectra of DP4 (Fig. 5.31) indicates presence of methyl group at position 33 at 0.824 ppm. Methyl group at position 35 and 36 are indicated at 2.83 and 3.05 ppm. Methyl group at position 29 is indicated 2.21 ppm. Protons of aromatic ring (from 6 to 11) appears from 7.58 to 7.65 ppm. Protons of aromatic ring (from 13,14, 16,17) appears at 7.08 ppm. Presence of four methyl groups in DP4 is indicated with DEPT spectra (Fig.5.33) as positive peaks. Presence of four methylene groups in DP4 is indicated in DEPT spectra as negative peaks. Thione group in FIMA at 199 ppm is disappeared in DP4 and new peak is formed at 168 ppm (Fig.5.32) which indicates that oxidation has taken at thione group and ketonic group is formed which is indicated by formation of peak at 168 ppm (Table 5.16) .

IR spectra

IR spectra of DP4 indicates tetrazole peak at 3308 cm-1. Presence of aromatic C-H stretching is indicated at 3192 cm^{-1}. Methyl stretching is indicated at 2967-2955 cm^{-1}. Methylene stretching is indicated at 2928 cm^{-1}.There is formation of additional peak at 1651 cm^{-1} and peak of thione group at 1230 cm^{-1} is disappeared. Formation of peak at 1651 cm^{-1} may be due to ketonic group. An IR spectrum of DP4 is shown in Fig. 5.34 and IR interpretation is shown in Table 5.15.

Mechanism of formation of DP4 [18]

During oxidation, FIMA forms FIMA S, S–dioxide intermediate (II) on addition of oxygen. There is addition of hydroxide ion from water to thione and there is elimination of sulphoxylate

ion (HSO_2^-). Abstraction of proton from intermediate IV results in the formation of DP4 (Fig.5.34).

Based on the above, DP4 is characterised 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.

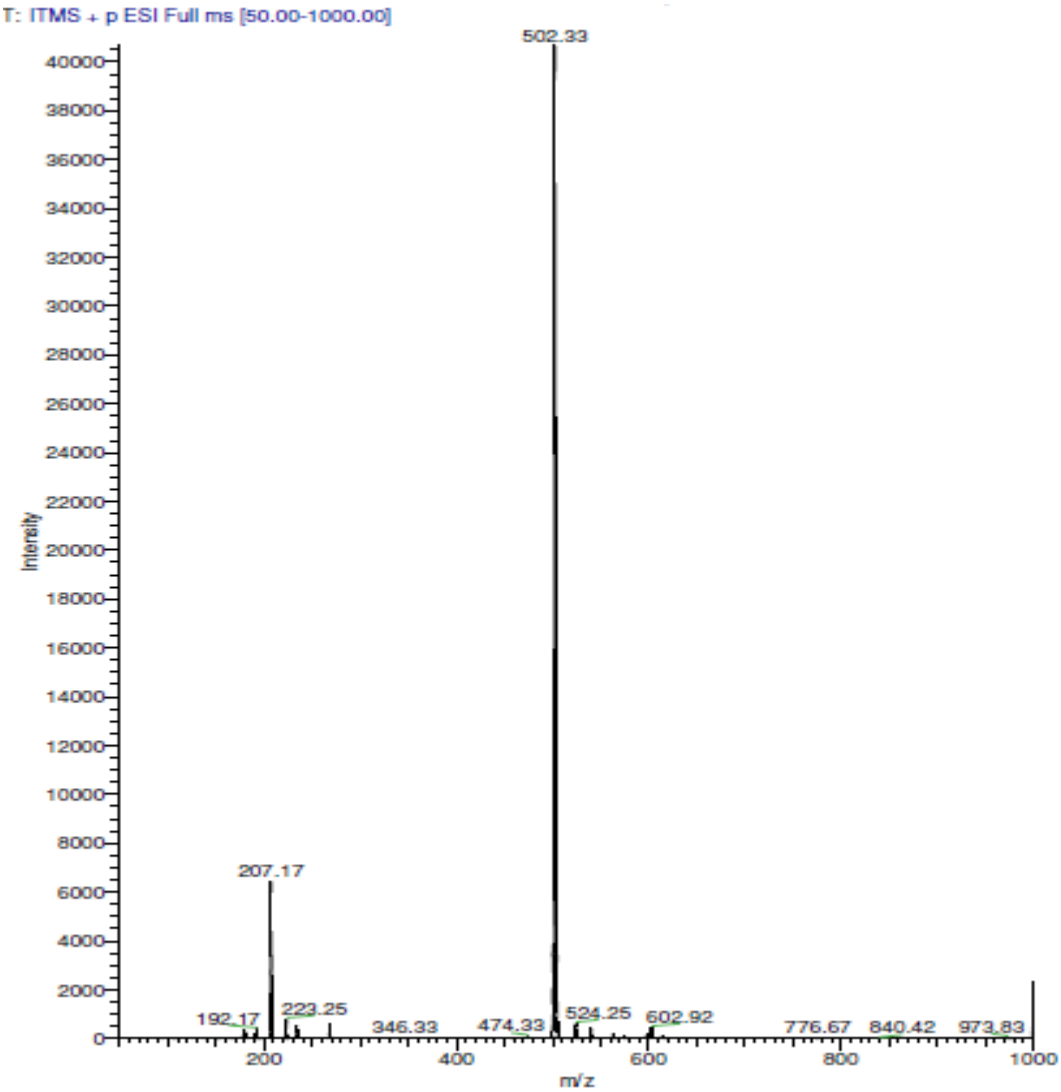


Fig. 5. 22- ESI-MS spectra of FIMA

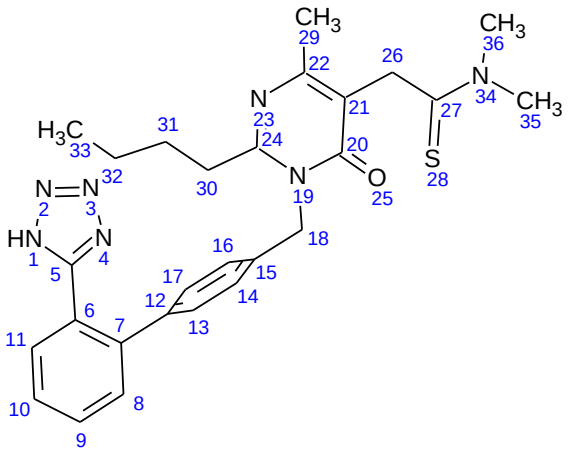


Fig. 5. 23 – Numbering assignment of FIMA

Table 5. 14- NMR assignments of FIMA

FIMA					
Position	^{1}H(Fig. 5.24)	Chemical Shift(δ ppm)	Position	^{13} C (Fig. 5.25)	DEPT (Fig. 5.26)
1	-NH	Absent	27	199 [20]	-C=S Thione
Aromatic 6 -11	4H	7.3- 7.5 ,m	Aromatic 5	161.86	Quaternary Carbon
Aromatic 12-17	4H	6.8- 7.08,dd	20	160.74	Ketonic group
18	2H	5.2, s	Aromatic 6-7	134.21, 132.27	Quaternary Carbon
26	2H	3.79,s	Aromatic 8-11	125.40, 129.26, 130, 130.45	-CH-

35	3H	3.44,s	Aromatic 12, 15	140.82, 139.87	Quaternary Carbon
36	3H	3.45,s	Aromatic 13,14,16,17	126.71, 127.42	-CH-
30	2H	2.63,t	18	32.13	
29	3H	2.15,s	24	159.17	Quaternary Carbon
31	2H	1.5,q	22	158.15	Quaternary Carbon
32	2H	1.3,q	21	119.02	Quaternary Carbon
33	3H	0.824,t	35,36	33.92,33.68	$-CH_3$
			26, 18	32.18, 32.13	$-CH_2$
			32	29.26	$-CH_2$
			31	27.05	$-CH_2$
			29	20.32	$-CH_3$
			30	20.5	$-CH_2$
			33	12.45	$-CH_3$

Table 5. 15– IR spectral interpretation of FIMA and DP4

FIMA		**DP4**	
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		

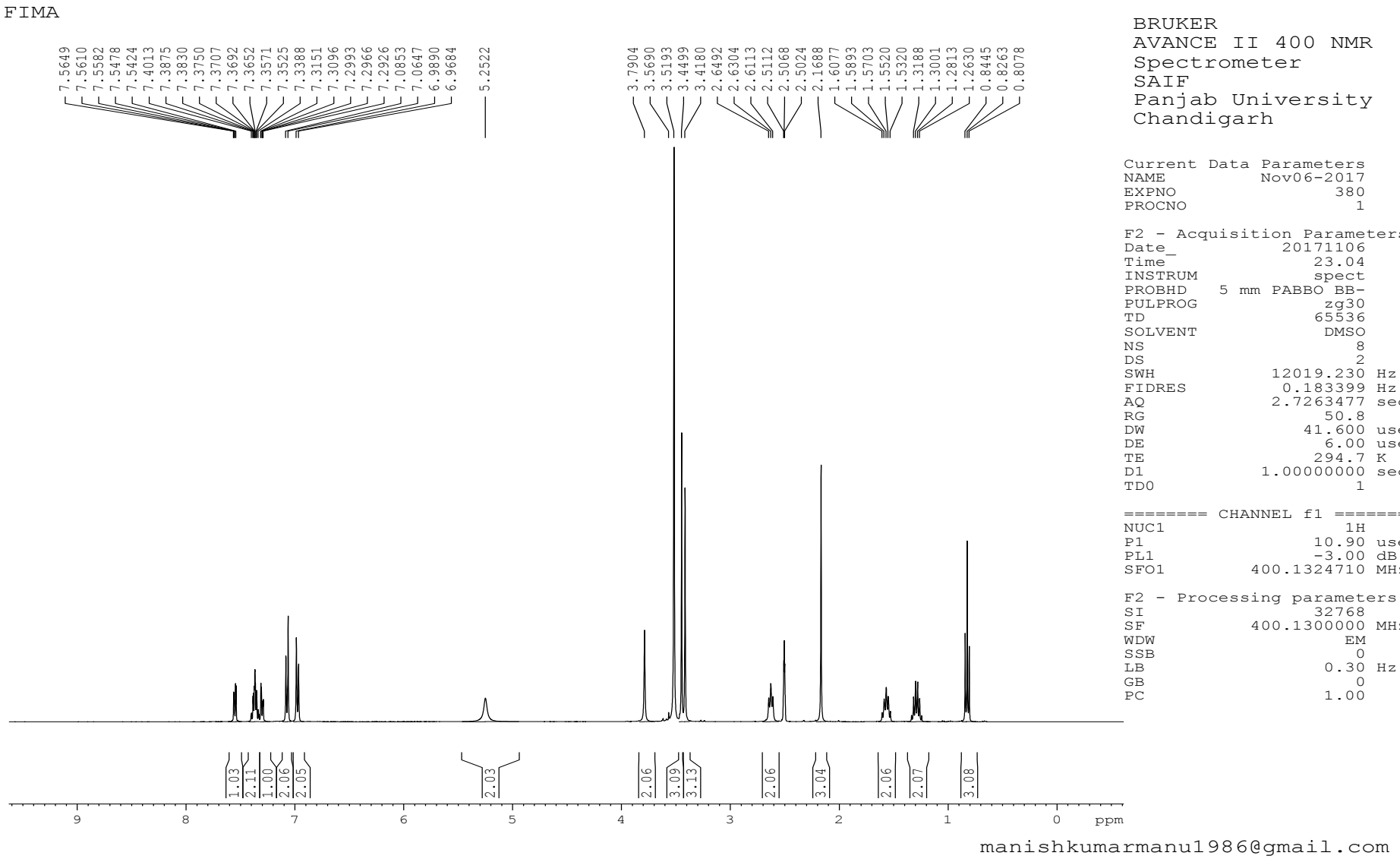


Fig. 5. 24 – ^{1}H NMR spectra of FIMA

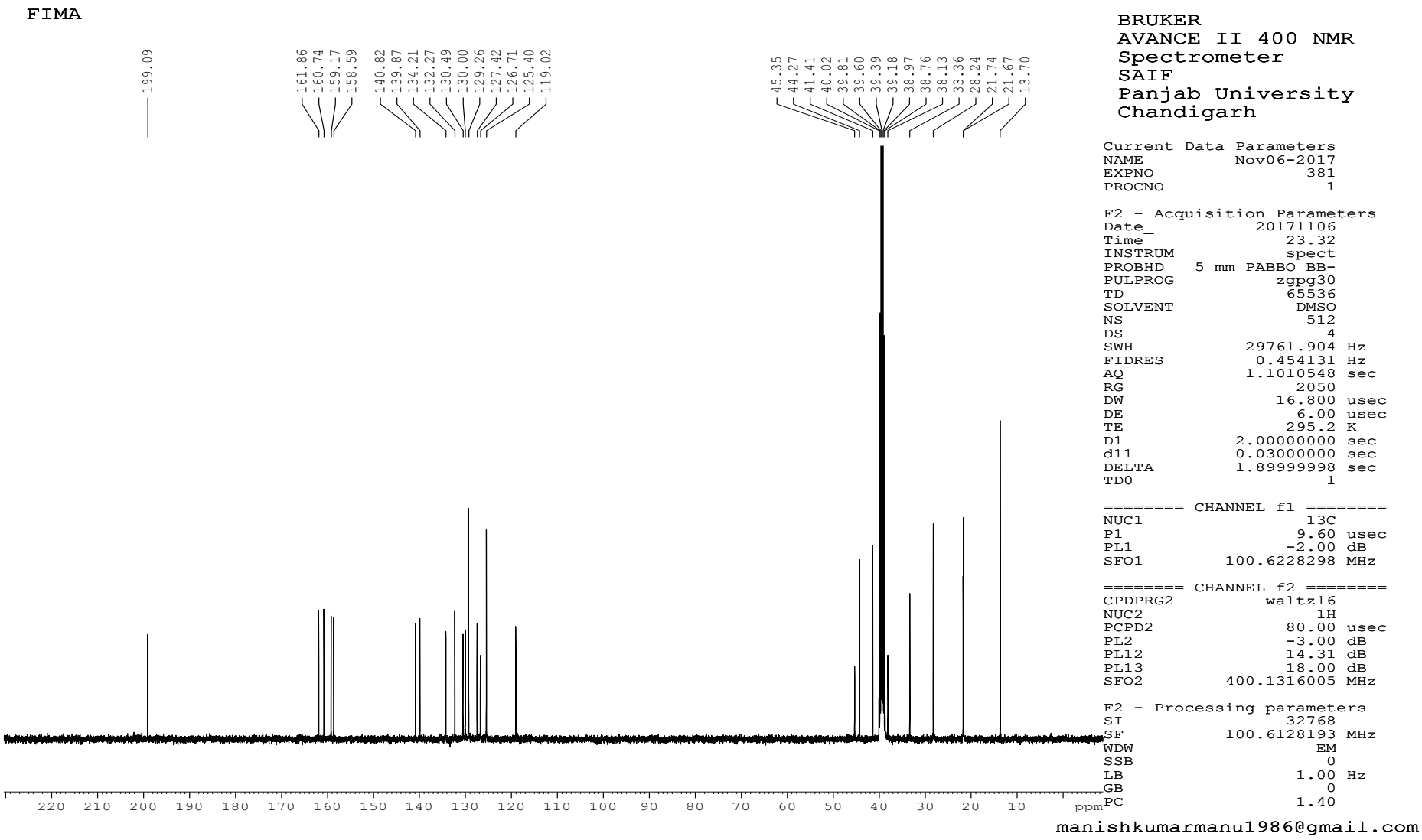


Fig. 5. 25– ^{13}C NMR spectra of FIMA

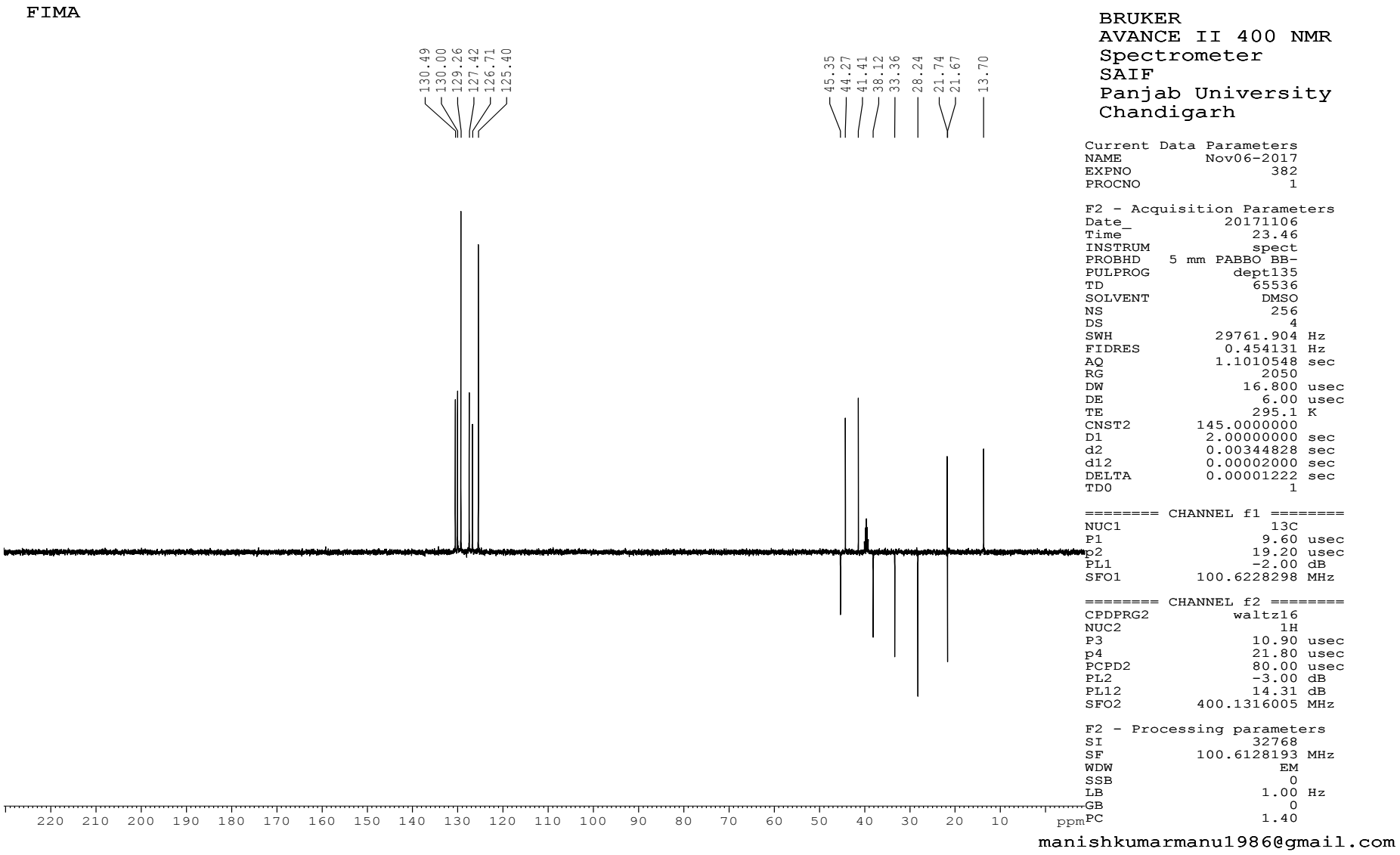


Fig. 5. 26– DEPT spectra of FIMA

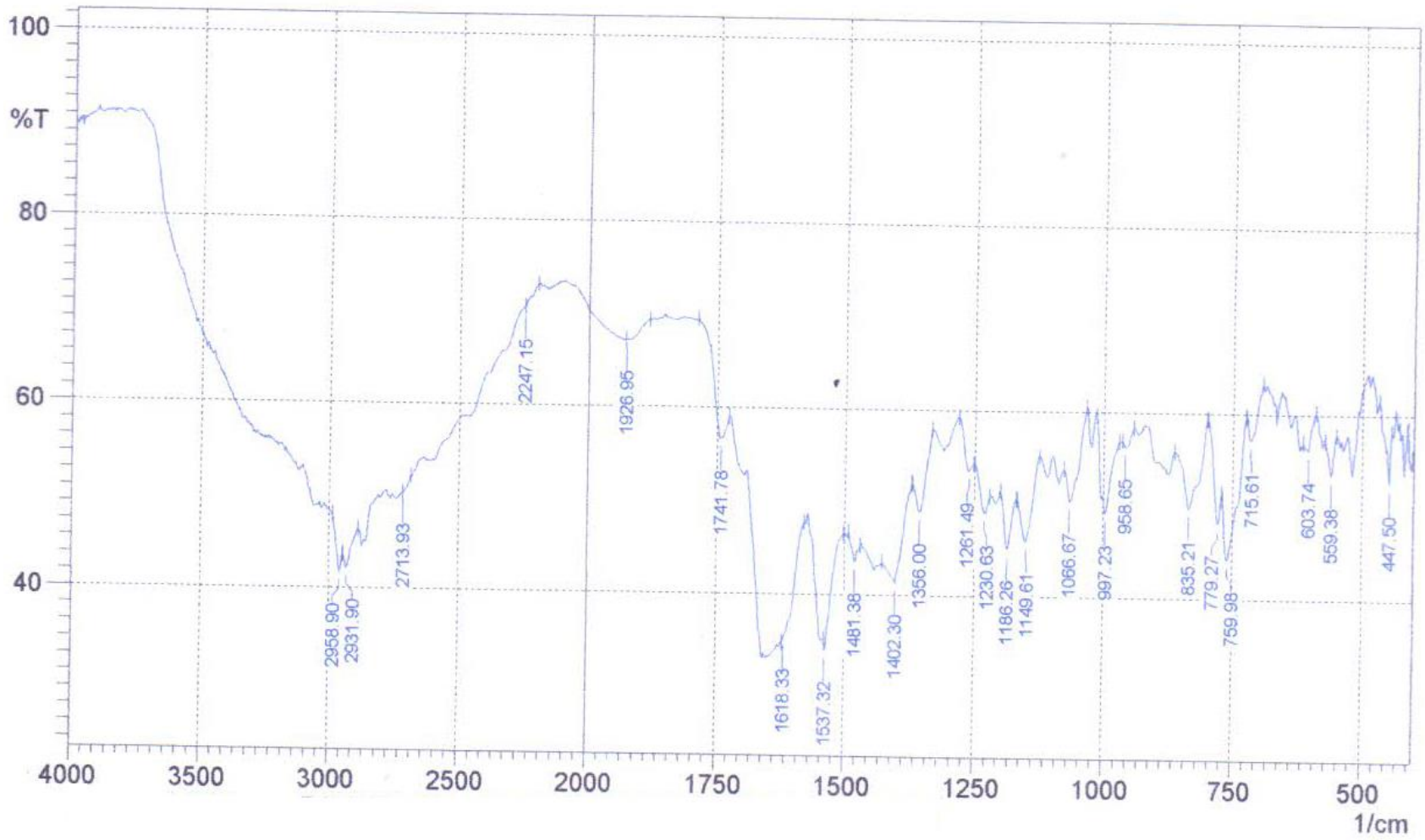


Fig. 5. 27- I.R. spectra of FIMA

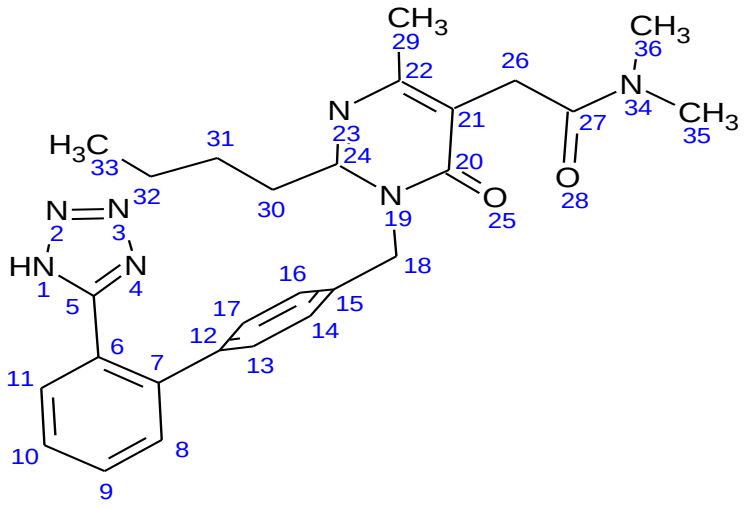


Fig. 5. 28– Numbering assignments of DP4

Table 5. 16 - NMR assignments of DP4

DP4					
Position	^{1}H (Fig. 5.31)	Chemical Shift(δ ppm)	Position	^{13} C (Fig. 5.32)	DEPT (Fig. 5.33)
1	NH	Absent	**27**	**168.98**	**Ketonic group**
Aromatic 6-11	4H	7.57,7.58,7.65, m	20	162.00	Ketonic group
Aromatic 12-16	4H	7.08, s	5	162	Quaternary Carbon
18	2H	5.25, s	22	159.31	Quaternary Carbon
26	2H	3.54,s	24	155.01	Quaternary Carbon
36	3H	3.05,s			

35	3H	2.83,s	12	140.95	Quaternary Carbon
30	2H	2.50,t	15	138.23	Quaternary Carbon
29	3H	2.21,s	Aromatic 6,7	134, 135	Quaternary Carbon
31	2H	1.56,q	Aromatic 8-11	131.03, 130.54, 129.09, 122.25,	-CH-
32	2H	1.29,q	Aromatic 13,14, 16, 17	127.79, 126.26	-CH-
33	3H	0.80,t	21	116.46	Quaternary Carbon
			35, 36	36.80, 35.04	$-CH_3$
			26	33.27	$-CH_2$
			18	33.20	$-CH_2$
			32	30.36	$-CH_2$
			31	28.14	$-CH_2$
			29, 30	21.51, 21.58	$-CH_3$
			33	13.58	$-CH_3$

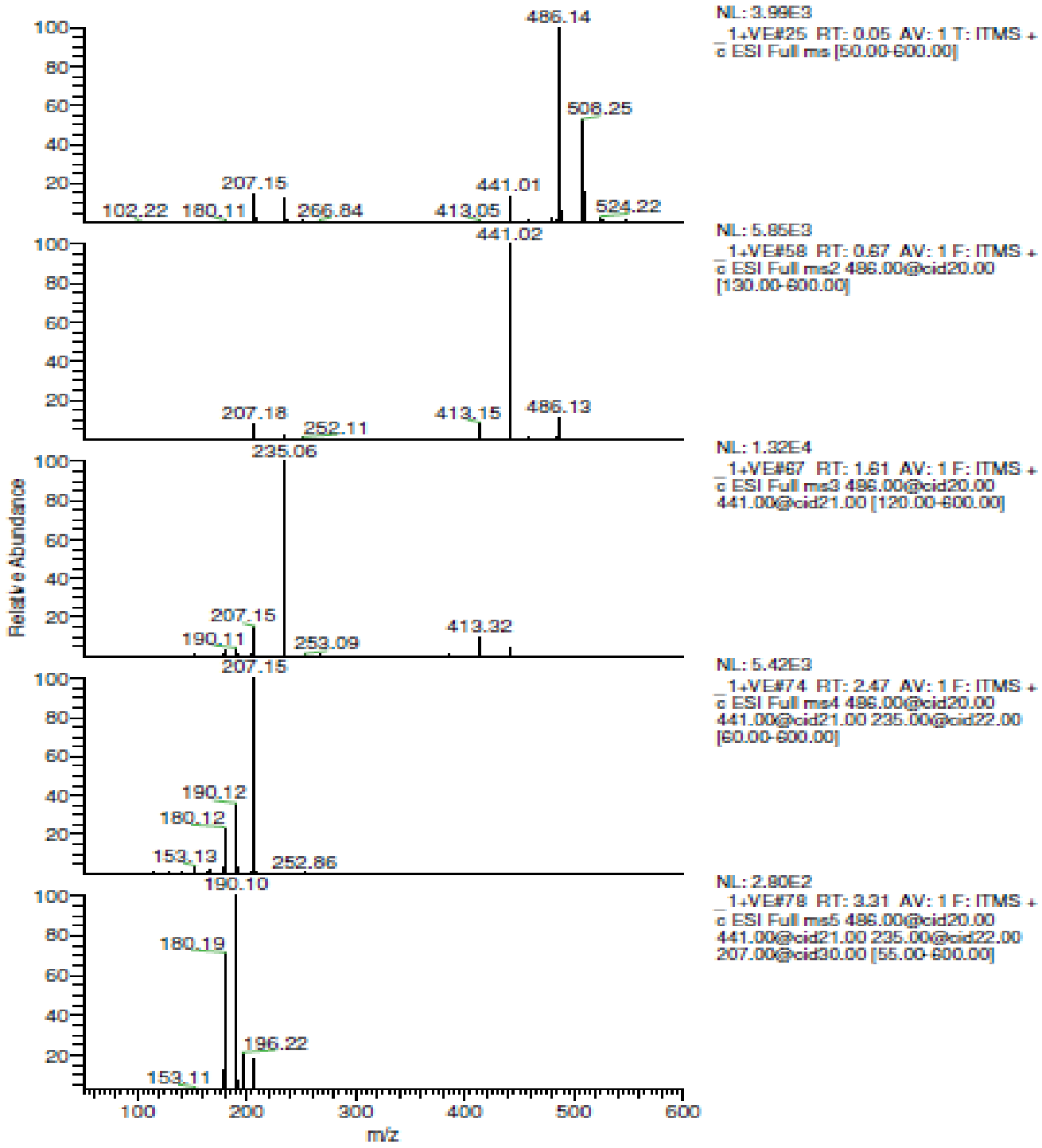


Fig. 5. 29- ESI-MS/MS spectra of DP4

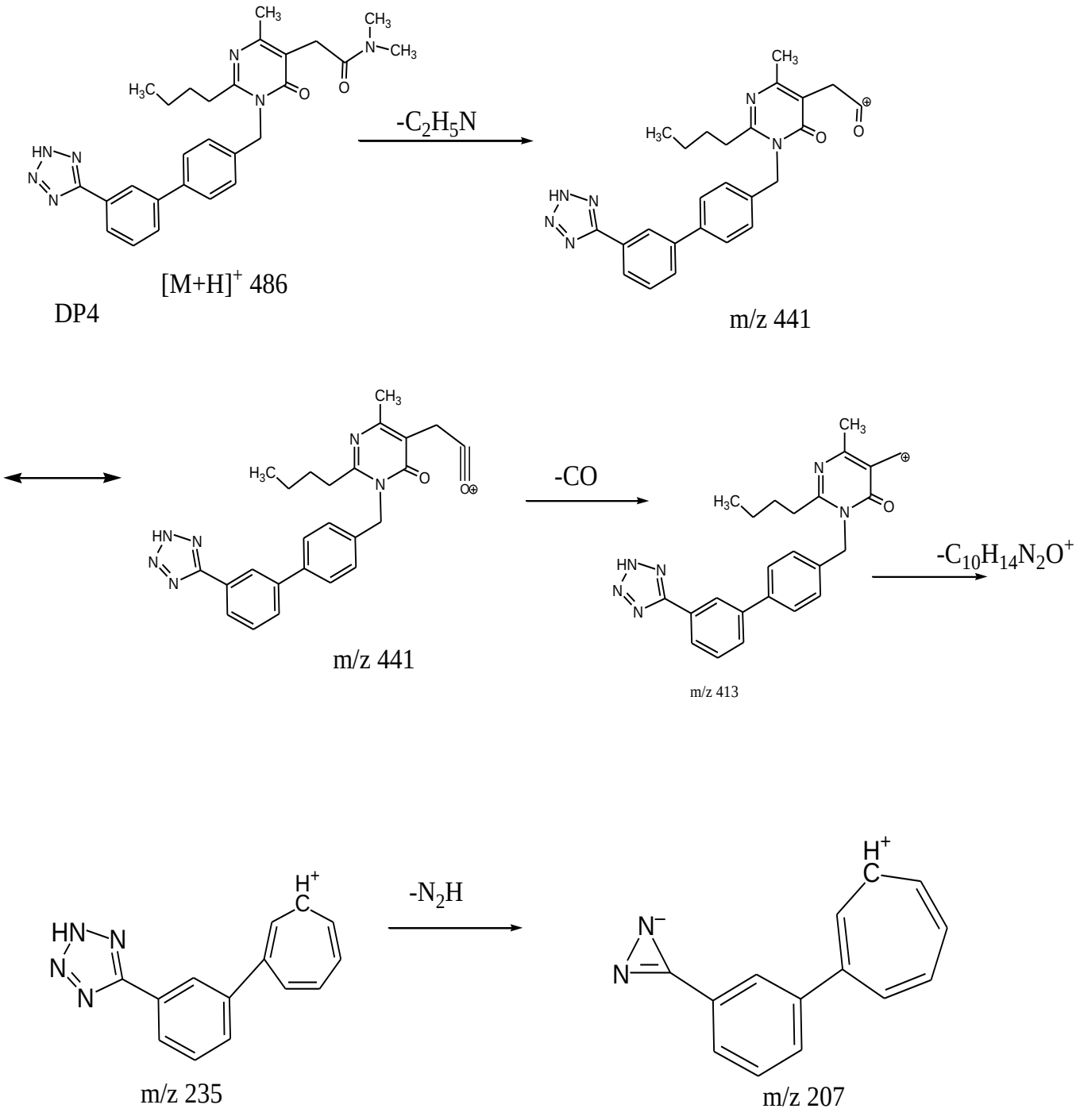


Fig. 5. 30– Fragmentation pathway of DP4

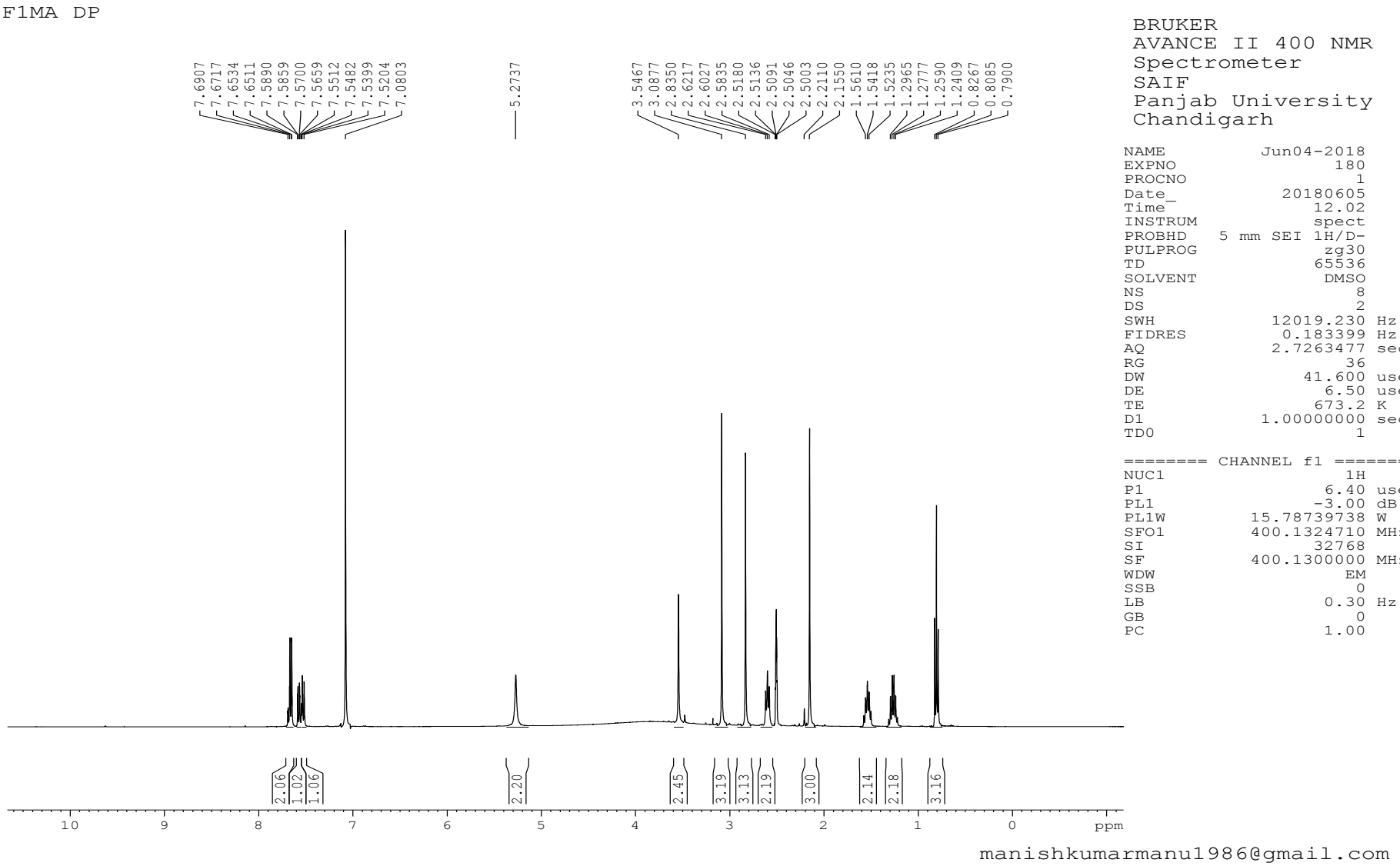


Fig. 5. 31 – ^{1}H NMR spectra of DP4

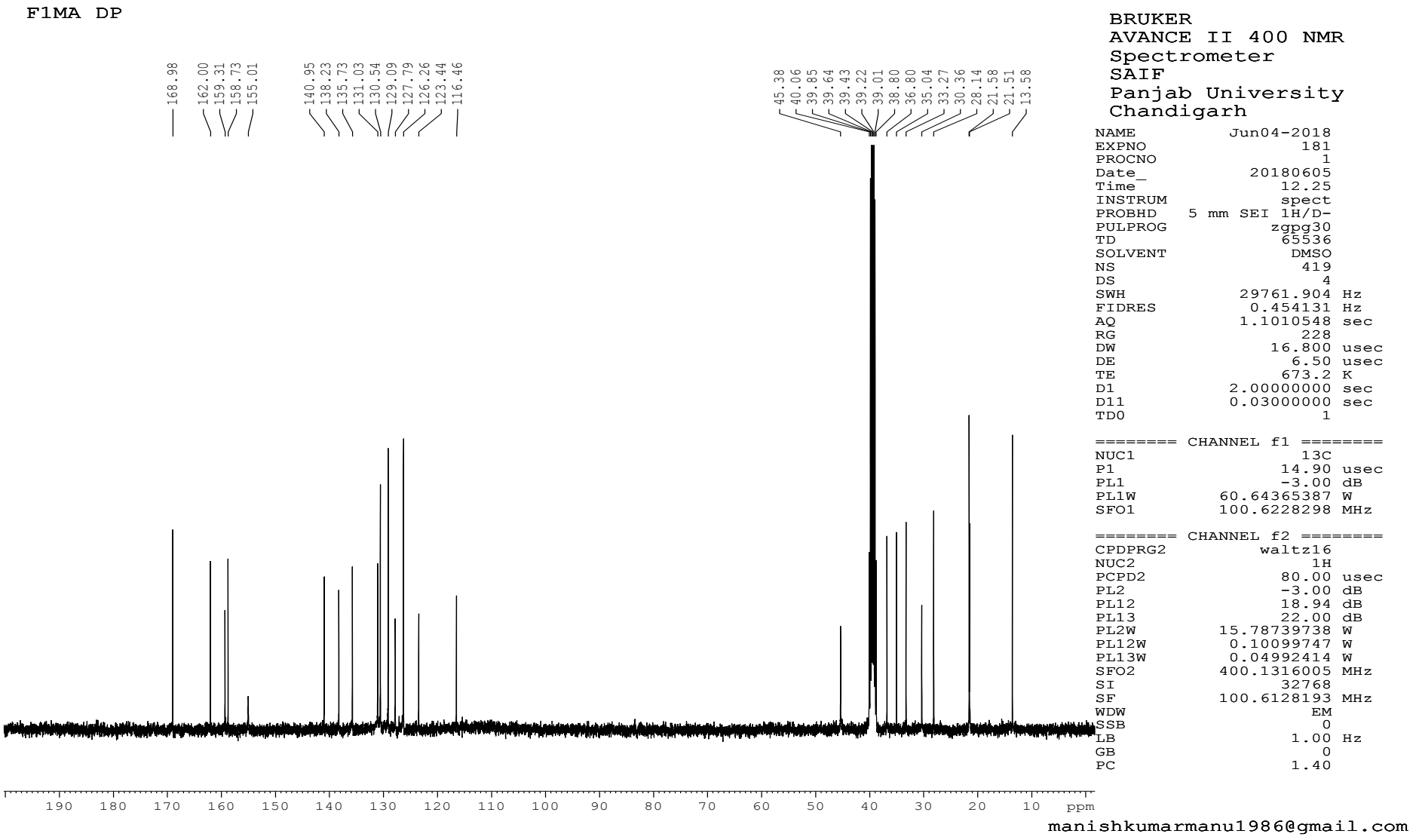


Fig. 5. 32 – ^{13}C NMR spectra of DP4

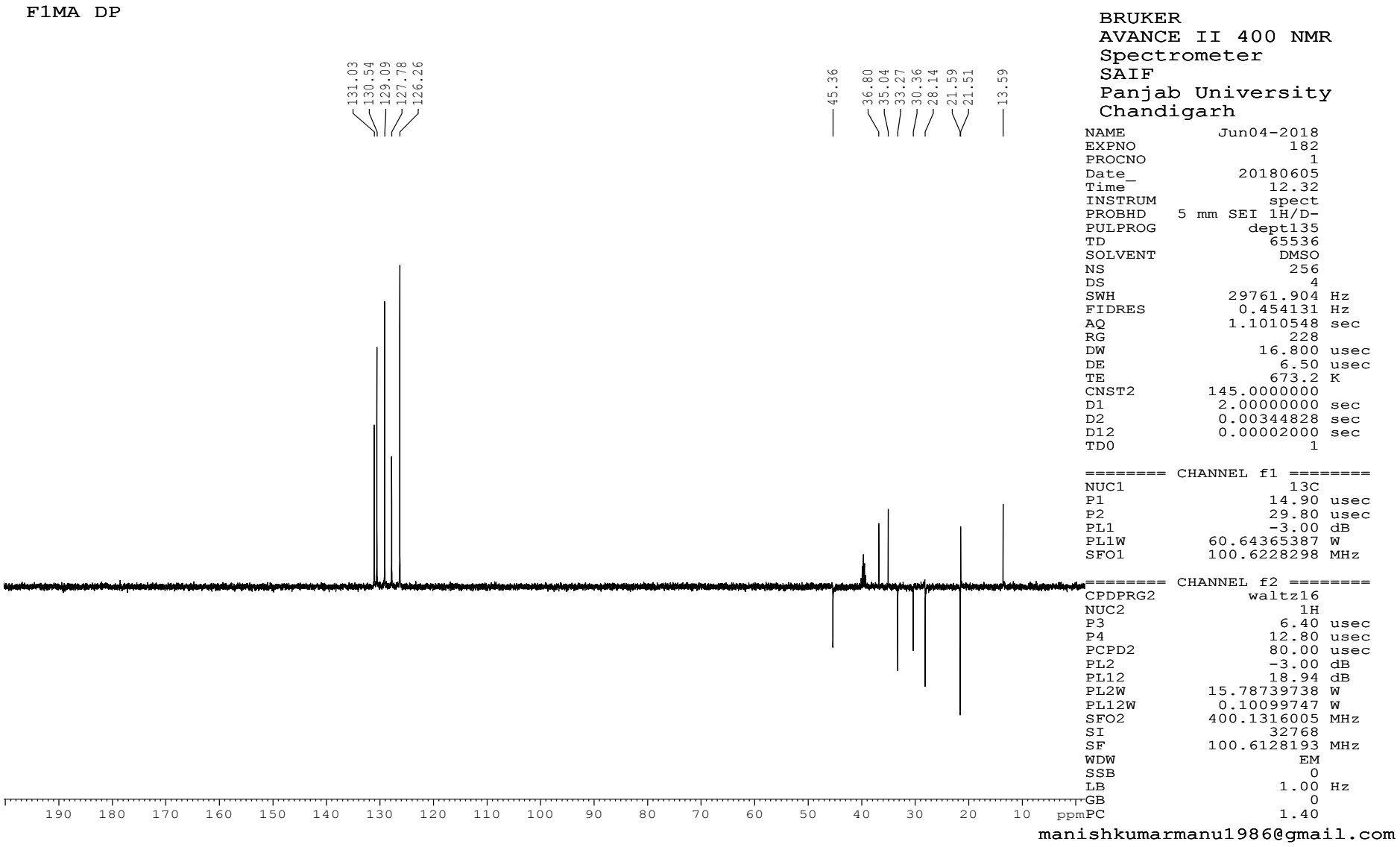


Fig. 5. 33– DEPT spectra of DP4

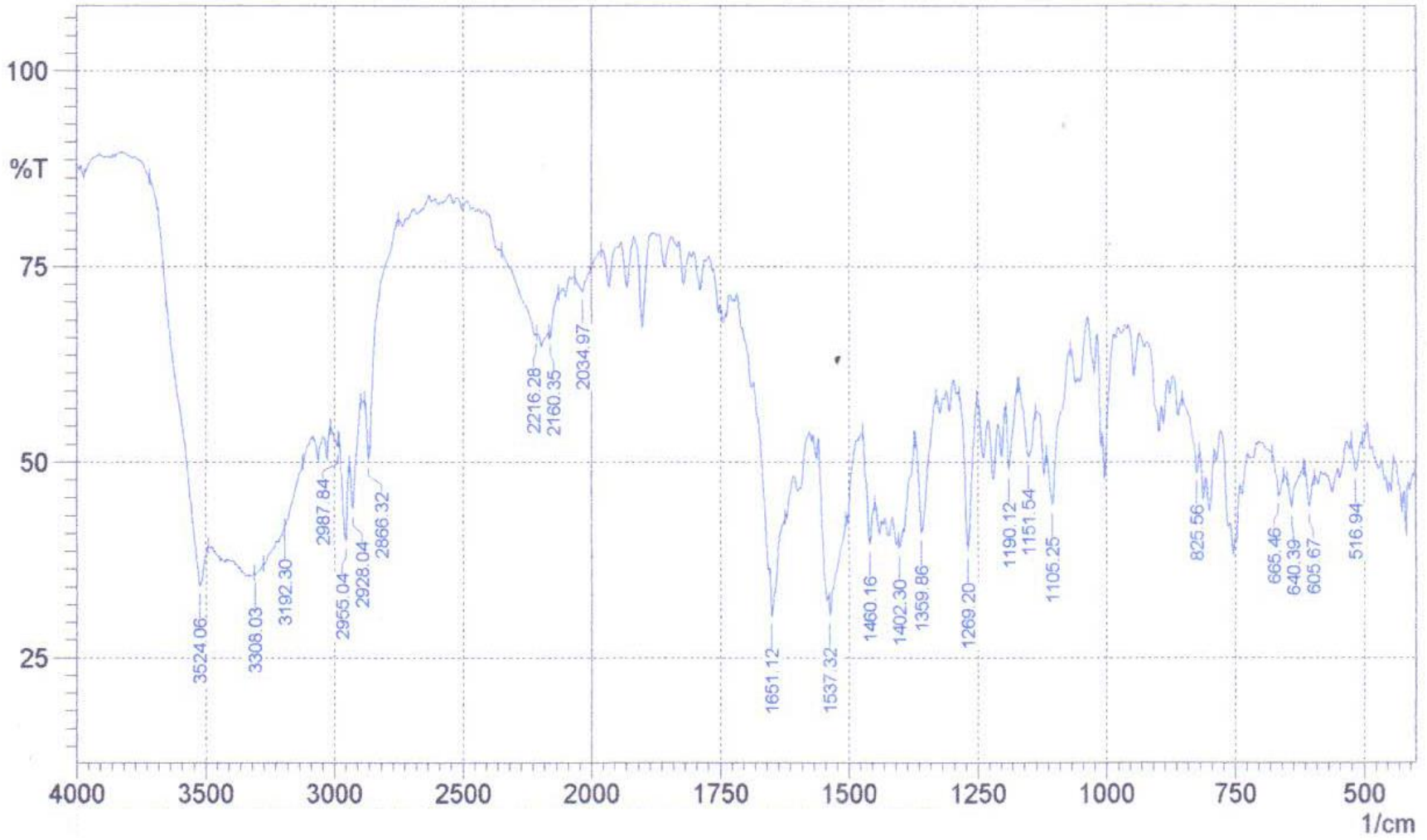


Fig. 5. 34- IR spectra of DP4

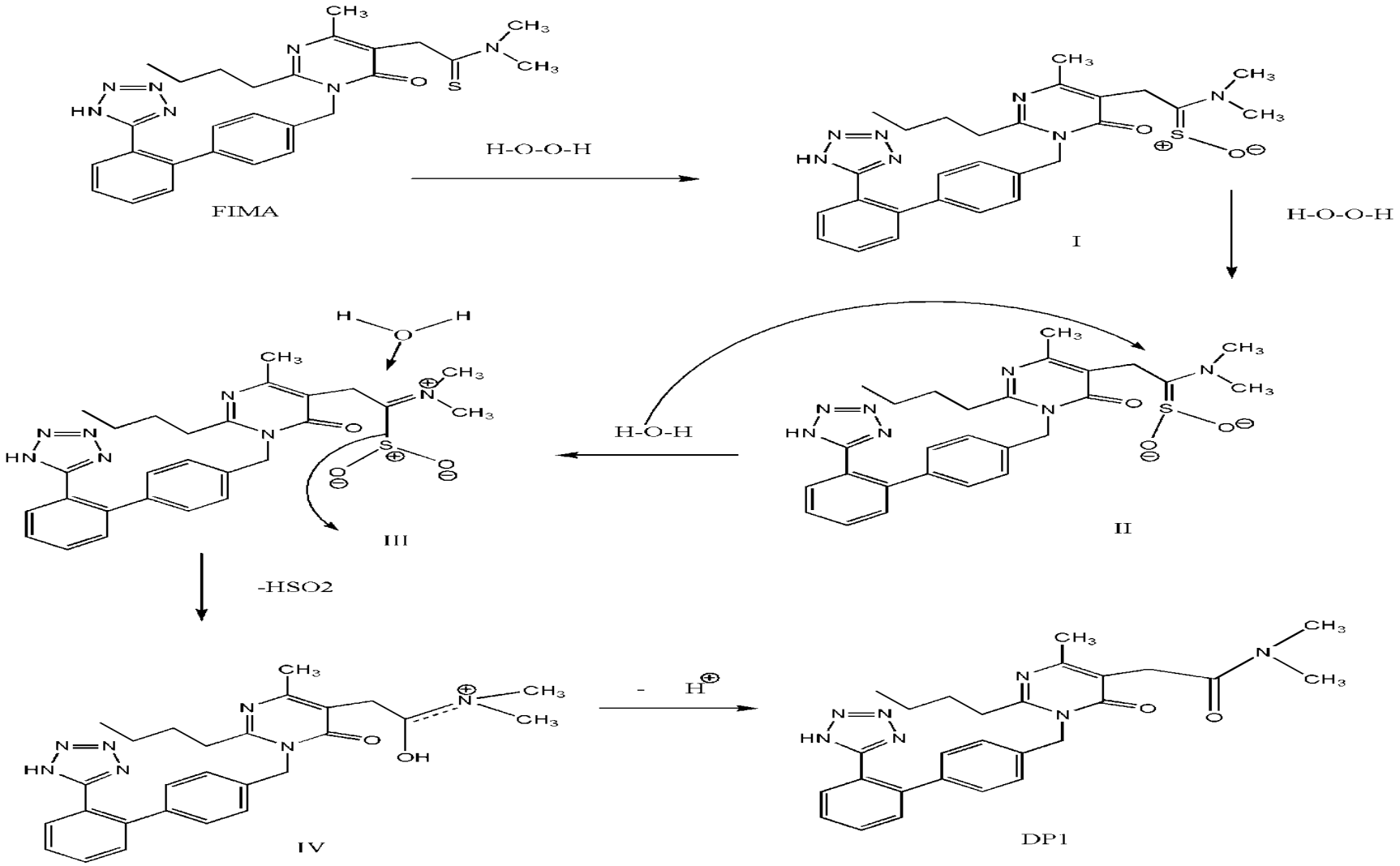


Fig. 5. 35- Mechanism of DP4

5.6.3. DISCUSSIONS

Major degradation product DP4 in oxidative condition was isolated and purified by preparative HPLC. By mass spectral analysis DP4 has 16 m/z less than FIMA. In ^{1}H NMR spectra, no. of protons in DP4 are same as that of FIMA. In ^{13}C NMR spectra of DP4, thione group which was present in FIMA at 199 ppm there is appearance of new peak at 168 ppm. This indicates the formation of carbonyl group. In IR spectra peak of thione group at 1230 cm^{-1} is disappeared and peak at 1651 $cm^{-1}$is formed may be due to presence of ketonic group. DP4 may be formed from FIMA by oxidation of thione group and formation of amide group in DP4.

5.7. SECTION - D

IMPURITY PROFILING AND DEGRADATION STUDY OF FIMASARTAN POTASSIUM

5.7.1. EXPERIMENTAL

5.7.1.1. Chemicals and Reagents

Chemicals and reagents used in the present section are same as those mentioned in section 5.4.1.1.

5.7.1.2. Equipments and Chromatographic conditions

Equipments used for impurity profiling and degradation study are same as those mentioned in 5.4.1.2.

Chromatographic conditions are same as those mentioned in section 5.4.1.3. The mobile phase composed of 0.1% formic acid and acetonitrile in the ratio of 45:55.

m/z values were determined both positive and negative ESI mode. On the basis of molecular weight, structures of DPs were proposed and degradation pathway was postulated.

5.7.1.3. Preparation of stock, sample and buffer solutions

Stock and sample solutions were in the same way as mentioned in section 5.4.1.5.

Buffer used in the LC-MS was 0.1 % formic acid which was prepared by dissolving 1 mL of formic acid in 1000 mL of double distilled water. Mobile phase composed of 0.1 % formic acid and acetonitrile in the ratio of 45 : 55.

5.7.2. RESULTS

5.7.2.1. LC-PDA study

Forced degradation study of FIMA showed formation of degradation products in LC-PDA are summarized in Table 5.17. Total 5 DPs were observed in LC-PDA.

Table 5. 17- Summary of forced degradation study of FIMA

Stressor	Conditions	RT of degradation products (min)	%Degradation (API)	% Degradation (Formulation)
Acid	1 M HCl 100℃ for 3 hrs	4.9 (DP4)	0.5%	0.1%
Alkaline	1 M NaOH 100ºC for 3 hrs	3.1 (DP1) 3.9 (DP2) 4.2 (DP3) 4.8 (DP4) 6.1 (DP5)	9.1%	8.8%
Neutral hydrolysis	100ºc for 6 hrs	--	--	--
Oxidation	0.9% H_2O_2 RT for 30 min	3.9 (DP2) 4.9 (DP4)	27.9%	25.1%
Thermal	Dry at 80℃ for 11 days	--	--	--
Photolytic	Dry for 11 days	--	--	--
	Solution for 11 days	4.9 min (DP4)	0.5%	--

5.7.2.2. LC-MS study and characterization of DPs

FIMA (m/z 502)

An ESI-MS spectrum is provided in Fig. 5. 22. An ESI-MS spectrum of FIMA shows protonated m/z 502. FIMA possess thione group. Thione group is susceptible to hydrolysis and oxidation and forms amide. The amide formed may undergo further hydrolysis and may forms corresponding acid.

DP1 (m/z 392)

An ESI –MS spectrum of DP1 is provided in Fig. 5.36. An ESI-MS spectrum of DP1 shows m/z value of 392. DP1has 109 m/z less than FIMA. DP1 may be formed from DP2 by elimination of tetrazole ring. DP1 undergoes fragmentation to give product ion at m/z 332 (Fig. 5.36, 5.37).

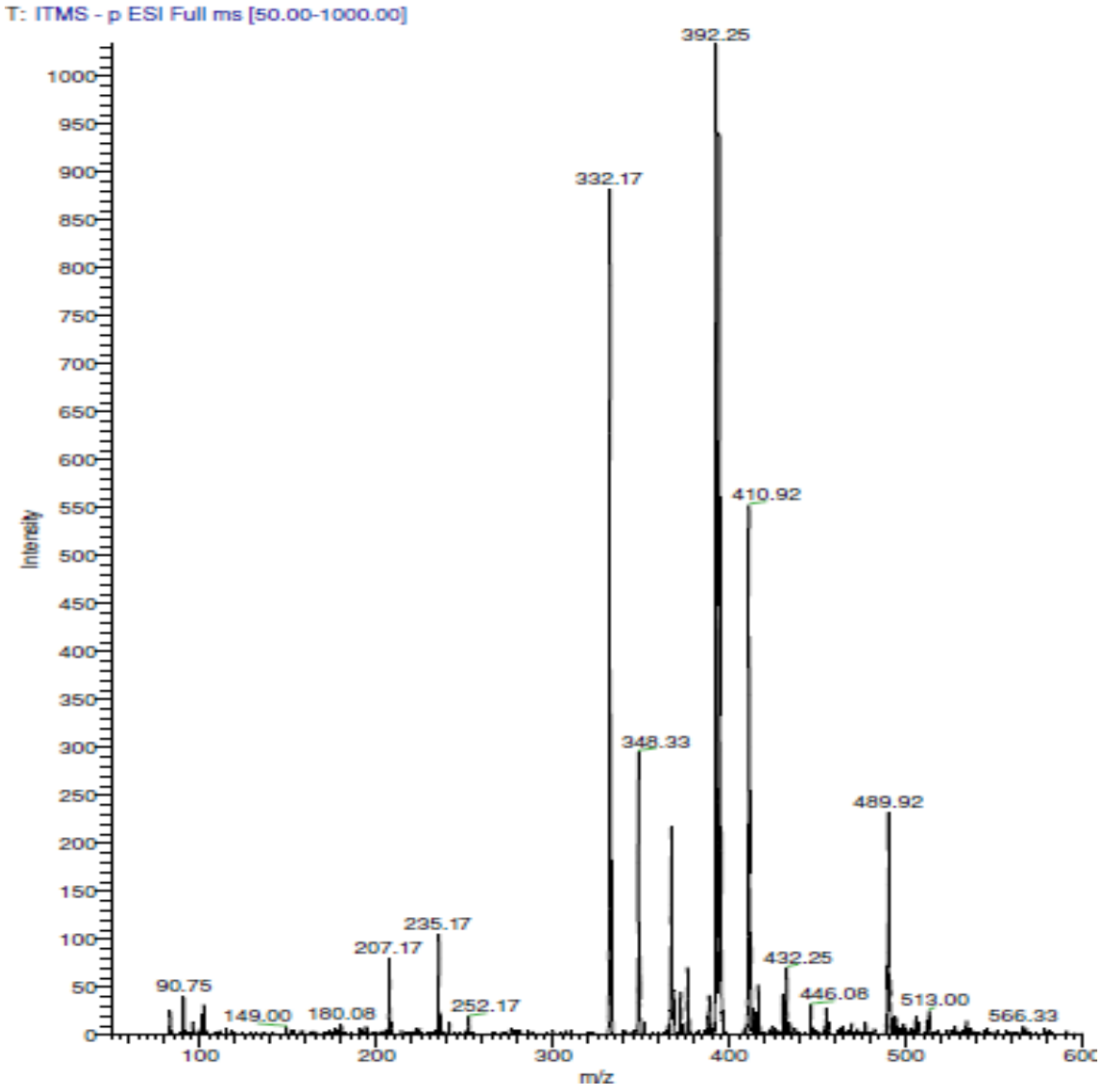


Fig. 5. 36- ESI-MS spectra of DP1

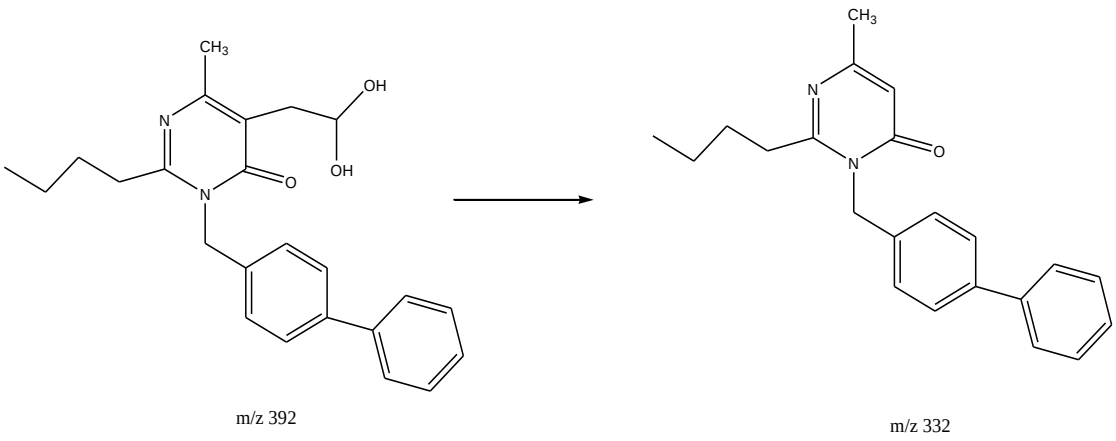


Fig. 5. 37 – Fragmentation pathway of DP1

DP2 (m/z 459)

An ESI-MS spectrum of DP2 is provided in Fig. 5.38. ESI-MS spectra of DP2 show protonated m/z 459. DP2 has less 43 m/z less than FIMA.DP2 may be formed from DP4 by hydrolysis of amide to carboxylic acid.

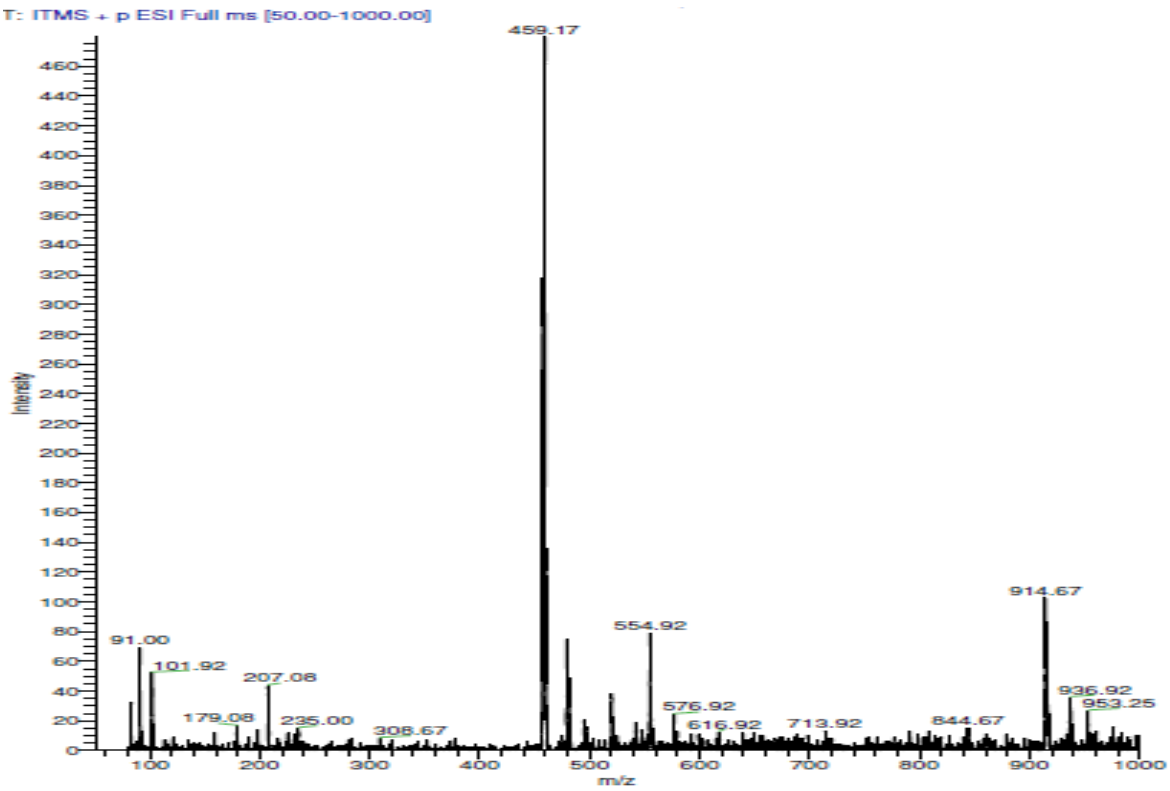


Fig. 5. 38- ESI-MS spectra of DP2

DP3

DP3 could not be confimed by LC-MS data.

DP4 (m/z 486)

An ESI-MS/MS spectrum of DP4 is provided in Fig.5.29 and mass spectra interpretation is mentioned in section 5.6.2.1.2. Fragmentation pathway of DP4 is provided in Fig. 5.30.

DP5 (m/z 475)

ESI-MS spectra of DP5 is provided in Fig. 5.39. It shows protonated m/z of 475. DP5 may be formed from FIMA by loss of two methyl groups from N,N-dimethylethanethioamide moiety.

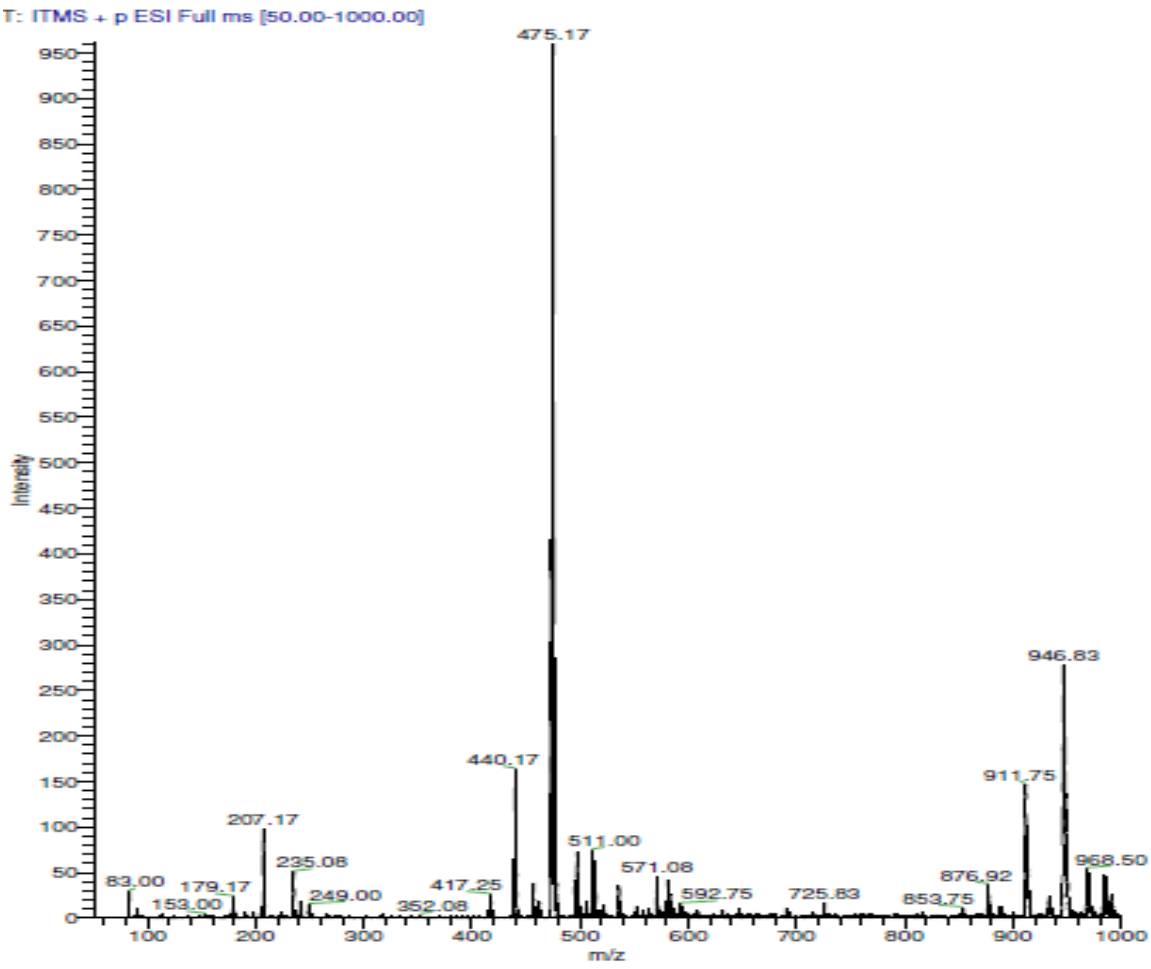


Fig. 5. 39- ESI-MS spectra of DP5

5.7.2.3. Degradation pathway of FIMA

FIMA contains 5 degradation products. These degradation products are formed in alkaline conditions. Among these, DP2 and DP4 are formed in oxidative conditions.

Degradation pathway of FIMA in alkaline condition is shown Fig. 5.40. Alkaline hydrolysis of FIMA takes place, there is formation of DP4. DP4 is formed by hydrolysis of thioamide to amide. Amide group in DP4 undergoes further hydrolysis to form DP2. DP1 may be formed from DP2 by the loss of tetrazole ring. DP5 is formed from FIMA by the loss of dimethyl group.

Degradation pathway of FIMA in oxidative conditions is shown in Fig. 5.41. Oxidation of FIMA takes place, there is formation of DP4. DP4 is formed by oxidation of thioamide to amide. Amide group in DP4 undergoes further oxidative hydrolysis to form DP2.

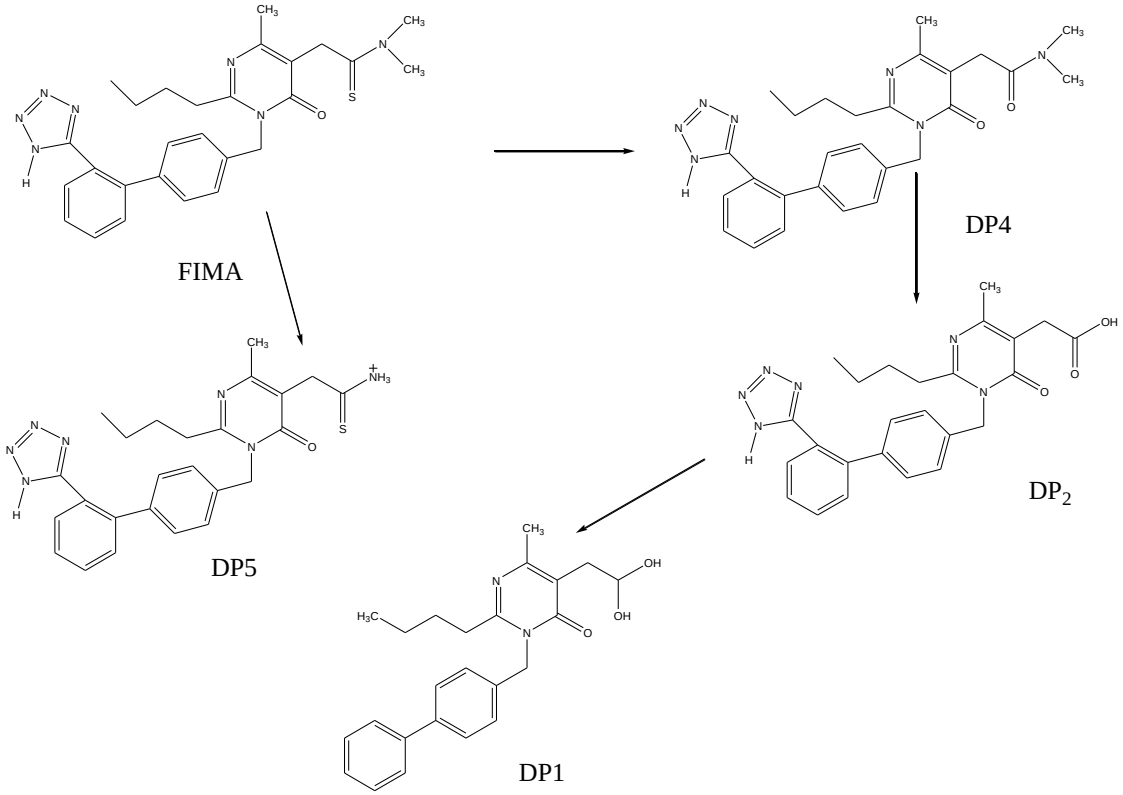


Fig. 5. 40 - Degradation pathway of FIMA in alkaline condition

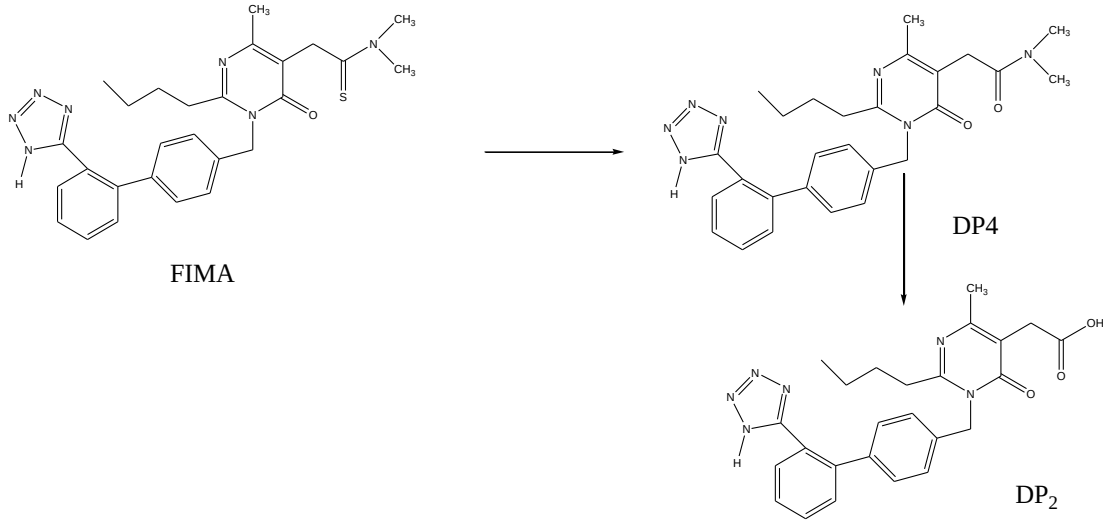
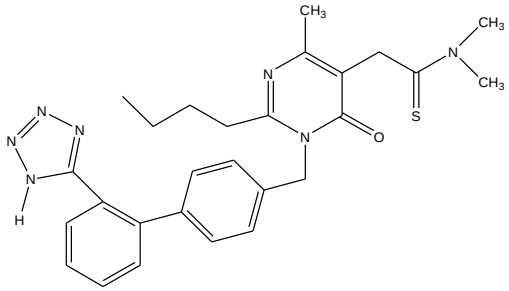
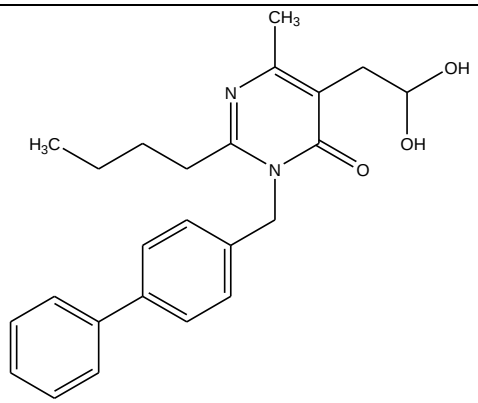
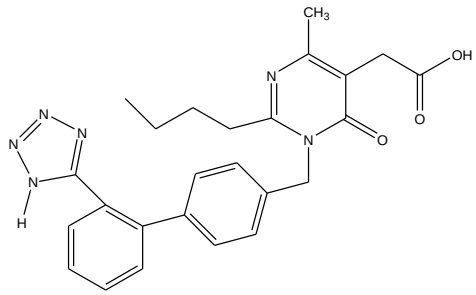
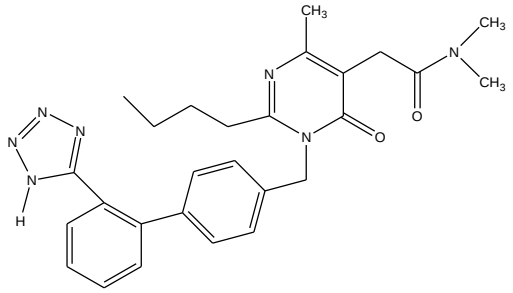
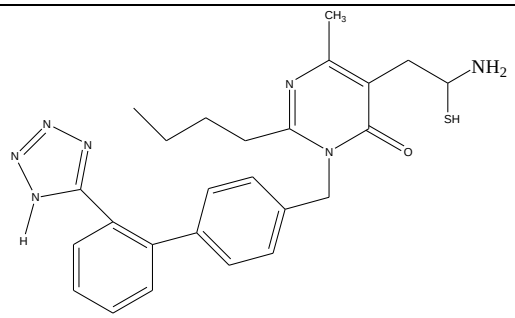


Fig. 5. 41- Degradation pathway of FIMA in oxidative condition

Table 5. 18- Chemical structures of FIMA and degradation products

Analyte	**Structure**	**Molecular Formula Molecular Weight Fragments(m/z)**	**Degradation Route**	**Rt (LC-PDA)**
FIMA	 2-(1-((2'-(2H-Tetrazol-5-yl)-[1,1'-biphenyl]-4-yl)methyl)-2-butyl-4-methyl-6-oxo-1,6-dihydropyrimidin-5-yl)-N,N-dimethylethanethioamide	501.65		7.32 min

DP1	 2-(1-[1,1'-biphenyl]-4-yl)methyl)-2-butyl-4-methyl-6-oxo-1,6-dihydropyrimidin-5-yl)-ethanediol	392.21	Alkaline	3.1 min
DP2	 2-(1-((2'-(1H-tetrazol-5-yl)-[1,1'-biphenyl]-4-yl)methyl)-2-butyl-4-methyl-6-oxo-1,6-dihydropyrimidin-5-yl)-acetic acid	458.20	Alkaline , Oxidative	3.9 min
DP4	 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	485.24	Alkaline , Oxidative	4.8 min

DP5	 2-(1-((2'-(1H-tetrazol-5-yl)-[1,1'-biphenyl]-4-yl)methyl)-2-butyl-4-methyl-6-oxo-1,6-dihydropyrimidin-5-yl)-1-amino-ethanethiol	475.21	Alkaline	6.1 min

5.7.3. DISCUSSIONS

Four degradation products in alkaline conditions and two degradation products in oxidative conditions were identified by LC-MS. DP2 and DP4 are formed in both alkaline and oxidative conditions. DP4 is formed from FIMA by alkaline hydrolysis/oxidation of FIMA due to conversion of thione group to carbonyl group. Amide group present in DP4 may further undergo further hydrolysis and forms DP2. DP1 may be formed from DP2 by the loss of tetrazole ring in alkaline condition. DP5 is formed from FIMA by the loss of dimethyl group in alkaline condition.

5.8. CONCLUSION

Stability indicating method was developed for determination of Fimasartan by HPLC. Significant degradation was observed in oxidative condition and slight degradation was observed in alkaline, acidic and photolytic condition in solution form. The method developed was validated as per guidelines by ICH. Degradation products in oxidative and alkaline conditions were identified by LC-MS. Oxidative degradation follows first-order kinetics. One degradation product in oxidative condition was isolated and characterized by mass, NMR and IR techniques. The degradation pathway in alkaline and oxidative condition was postulated. The developed method is simple, accurate and sensitive which are applicable for determination of Fimasartan and degradation products of Fimasartan. Structures of all these degradation products are not reported hitherto.

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