

## **Method Development and Validation of Degradation Studies of Imatinib Mesylate by UHPLC**

### **ABSTRACT**

**Background:** A simple, reliable and economical method was used for the study of imatinib mesylate. The optimized chromatographic conditions were determined by using a C18 intersil ODS (250 X 4.6 mm X 5 $\mu$ m) and a mobile phase containing phosphate buffer (pH 3.0): Acetonitrile: Methanol (40:30:30) v/v was pumped at 1 ml/min flow rate. The injected sample volume is 20  $\mu$ L and the analytes were eluted at 254 nm.

**Results:** The Retention time of imatinib mesylate was 3.503 minutes. The system suitability percentage RSD of imatinib mesylate is 0.27. The Assay of imatinib mesylate was found to be 99.37%. The imatinib mesylate LOD, LOQ values were found to be 0.901 and 2.73 $\mu$ g/ml. Regression equation was found to be  $y = 96.59x + 10.76$  from linearity calibration graph. Imatinib mesylate was degraded in acid and peroxide stress conditions, and no degradation was obtained in base, photolytic and thermal conditions.

**Conclusion:** The reliable UPLC method validation data observed that which can be used for analyzing routine quality control. The method is economical due to the run time is reduced, which can be used in regular quality control tests in the industry.

*Keywords: Imatinib mesylate, Retention time, Validation, Degradation studies, % RSD*

## 1. INTRODUCTION

Imatinib mesylate ( $C_{30}H_{35}N_7O_4S$ ) is anti cancer drug and authorized to use chronic myelogenous leukemia (CML). It is additional to cure gastro-intestinal stromal malignant tumor (GIST). Imatinib mesylate is a competitive inhibitor of Bcr-Abl tyrosine kinase, which is used for the administration of CML and GIST [1]. The oral route of administration can be taken imatinib mesylate and its works at the molecular level with high selectivity to get relieve of cancer by competitively slow down the binding of ATP to the kinase activation domain of Bcr-Abl [2]. Imatinib mesylate is metabolized mostly to N-desmethyl imatinib (CGP74588) ( $C_{28}H_{29}N_7O$ ), which exhibit same biological activity to imatinib [3]. The 400, 600 or 800 mg dose of imatinib mesylate is administered orally with a daily to patients in various stages of CML [4]. The adverse effects and hematologic response depends upon the dose [5]. The cancer cells selectively as alternative of destroying all other rapidly dividing cells targeted by imatinib mesylate. [6]. The chemical name of imatinib mesylate is 4-(4-methyl-piperazin-1-yl-methyl)-N-[4-methyl-3-(4-pyridin-3-yl-pyrimidin-2-yl-amino)-phenyl]-benzamide methane sulfonate. The imatinib mesylate chemical structure was shown in figure 1.

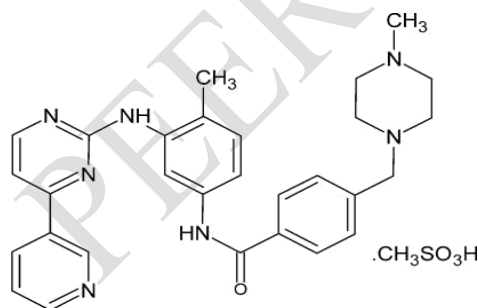


Fig. 1. Molecular structure of imatinib mesylate

## 2. MATERIALS AND METHOD

### 2.1 Chemicals and Reagents

Active pharmaceutical ingredient of imatinib mesylate was procured as gift sample from API industry and marketed formulation was procured from local pharmacy store. HPLC Grade methanol, Acetonitrile and di potassium hydrogen phosphate were procured from Avantor performance materials Ltd. HPLC water was procured from martin synges pharma science pvt ltd.

### 2.2 Instrumentation

UHPLC Agilent company model NO-1290 chromatographic method set with quaternary pumps with UV detector. Chromatograms were recorded by using open lab software.

### **2.3 Chromatographic conditions**

Mobile Phase: Phosphate Buffer (pH 3.0): Acetonitrile: Methanol (40:30:30 v/v)

Column: C18 intersil ODS (250 X 4.6 mm X 5 $\mu$ m)

Column temperature: 25°C

Detection wavelength: 254 nm

Run time: 10 min

Sample volume: 20  $\mu$ l

### **2.4 Solvents**

The chemical reagents methanol, acetonitrile (ACN), standard and sample solutions were filtered through 0.45  $\mu$ m. Phosphate Buffer (2.5 gr sodium di hydrogen phosphate dissolved in 1000 ml water and add 1ml of triethylamine (TEA), pH to 3.0 with ortho phosphoric acid)

### **2.5 Preparation of Standard Stock Solution**

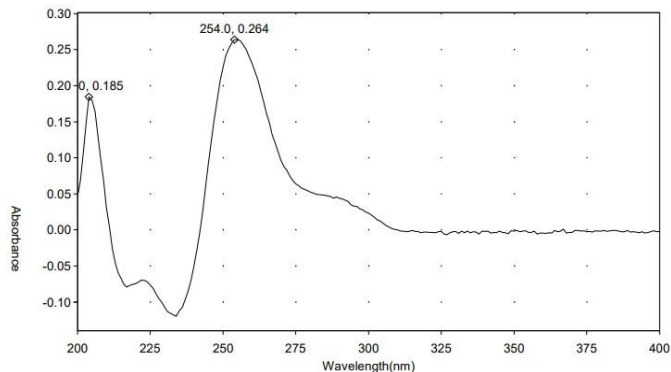
Weigh exactly 50 mg of imatinib mesylate standard sample transferred in 50 ml of volumetric flask and dissolved in methanol and make up to 50 ml with methanol. The resulting solution concentration is 1000  $\mu$ g/ml. This solution is considered as standard stock. From above standard stock solution transfer 10 ml solution in to 100 ml of volumetric flask and diluted with methanol up to the mark. The resulting solution concentration is 100  $\mu$ g/ml.

### **2.6 Preparation of Sample Stock Solution**

Weigh accurately 50 mg of imatinib mesylate API sample dissolved in methanol and make up to 50 ml with methanol. The resulting solution concentration is 1000  $\mu$ g/ml. This solution is considered as sample stock. From above sample stock solution transfer 10 ml solution in to 100 ml of volumetric flask and diluted with methanol up to the mark. The resulting solution concentration is 100  $\mu$ g/ml.

### **2.7 Selection of Analytical wavelength**

To analyte sample solution wavelength maximum was determined by ultraviolet spectroscopy over the range of 200-400 nm from resultant spectrum wavelength at 254 nm was chosen in this maximum absorption drug occurs. This wave length considered for analysis. The UV spectrum of imatinib mesylate was given in figure 2.



**Fig. 2 UV spectra of imatinib mesylate**

## 2.8 Method Validation

### 2.8.1 System Suitability

System suitability parameters were estimated by standard preparation replicates injected by instrument and to check the system performance. System suitability % RSD was determined by using standard solution of imatinib mesylate 6 replicate injections. (16 ppm).

### 2.8.2 Linearity

Linearity method was performed within the concentration range of 4-24  $\mu\text{g/mL}$  of imatinib mesylate. The regression coefficient was determined from linearity calibration graph.

### 2.8.3 Specificity / Selectivity

Specificity method was checked for the existence of probable interferences by estimation of chromatograms obtained from solvent blank, standard and placebo sample. There is no retention times data interference with blank, standard and placebo sample and the developed method is specific.

### 2.8.4 Precision

Analyzing 6 replicates of imatinib mesylate the concentration is 16  $\mu\text{g/ml}$  in method precision. For intraday & inter day precision is performed in different times & different days by using 12  $\mu\text{g/ml}$ , 16  $\mu\text{g/ml}$  & 20  $\mu\text{g/ml}$  for. Precision % RSD was calculated.

### 2.8.5 Accuracy

Accuracy was performed by spiking standard imatinib mesylate sample in suitable concentrations. The accuracy is done at intervals of 50%, 100% and 150%. The % recoveries and % RSD determined.

### 2.8.6 Robustness

Robustness was performed by small deliberate variations in the exploratory conditions. The chromatographic methods changes in flow rate ( $1.0 \pm 0.2\text{ml/min}$ ) and wavelength ( $254 \text{ nm} \pm 5\text{nm}$ ).

### 2.8.7 Assay

The label claim of imatinib mesylate 100 mg and marketed formulation % purity was determined.

## 2.9 Degradation Studies

The different degradation studies were performed in imatinib mesylate to get degradation products.

#### **2.9.1 Acid Degradation Study**

1.0 mg of imatinib mesylate was transferred in 10 ml of volumetric flask and dissolve in methanol and make up to mark with methanol. The resulting solution concentration is 100 µg/mL. Pipette out 0.3 ml from above solution taken in 10 ml volumetric flask. This solution subjected to acid hydrolytic condition in the presence of 0.1 N HCl concentration at 80°C for 5 hours. These solutions neutralized by 1ml of methanolic NaOH and make up to 10ml with methanol. The resulting solution was filtered through the 0.45µ membrane filter and injected in UHPLC.

#### **2.9.2 Base Degradation Study**

1.0 mg of imatinib mesylate was transferred in 10 ml of volumetric flask and dissolve in methanol and make up to mark with methanol. The resulting solution concentration is 100 µg/mL. Pipette out 0.3 ml from above solution transferred in three different 10ml volumetric flasks. These solutions subjected to alkali hydrolytic conditions in the presence of different concentrations of 0.1N NaOH at 80°C for 5 hours, 0.5N NaOH at 100°C for 8 hours and 1N NaOH at 120°C for 24 hours. These solutions neutralized by 1ml of methanolic HCl and diluted up to 10ml with methanol. The resulting solutions were filtered through the 0.45µ membrane filter and injected in UHPLC.

#### **2.9.3 Peroxide Degradation Study**

1.0 mg of imatinib mesylate was transferred in 10ml of volumetric flask and dissolve in methanol and make up to mark with methanol. The resulting solution concentration is 100 µg/mL. Transfer 0.3 ml from above solution taken in 10ml volumetric flasks diluted up to 10 ml with methanol. This solution subjected to of 3% H<sub>2</sub>O<sub>2</sub> at 80°C for 5 hours. The resulting solution was filtered through the 0.45µ membrane filter and injected in UHPLC.

#### **2.9.4 Photolytic degradation study**

1.0 mg of imatinib mesylate transferred in 10ml of volumetric flask and dissolves in methanol and make up to mark with methanol. The resulting solution concentration is 100 µg/mL. Pipette out 0.3 ml from above solution taken in four 10ml volumetric flasks and diluted with methanol. The photochemical stability of the drug was studied by exposing the solution to UV light by kept the beaker in UV chamber for 5 hours, 24 hours, 2 days and 5 days. The stability of sample evaluated by recorded chromatograms.

#### **2.9.5 Thermal degradation study**

The imatinib mesylate sample was spread uniformly in the petri dish and wrapped in aluminum foil placed in oven at 80°C for 5 hours, 100°C for 24 hours, and 120°C for 3 days and 150°C for 5 days in hot air oven. The sample was withdrawn on different time and different days and the resultant sample solution stability determined from recorded chromatograms.

#### **2.9.6 Degradation characterization:**

Isolation, characterization and structure elucidation of degradation products was performed by using mass spectroscopy.

### 3. RESULTS AND DISCUSSION

#### 3.1 Chromatography optimization method

The optimized chromatography conditions performed with C18 intersil ODS (250 X 4.6 mm X 5 $\mu$ m), mobile phase composition phosphate buffer (pH 3.0): acetonitrile: methanol (40:30:30 v/v) and flow rate 1 ml/min was selected as highly advanced technique. The different trials were performed using various columns and different proportion of mobile phase based on the reported methods and optimized chromatogram was shown in figure 3.

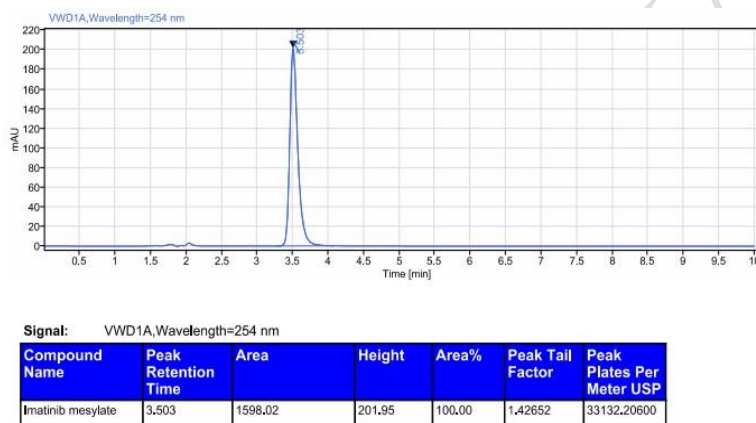


Fig. 3 Optimization method chromatogram of imatinib mesylate

#### 3.2 Method Validation

##### 3.2.1 System Suitability

System suitability parameters such as theoretical plates greater than 2000, tailing factor less than 2, resolution more than 2, % RSD of peak areas less than 2 % . In this present method, all parameters were conventional within acceptance range and results were given in table 1.

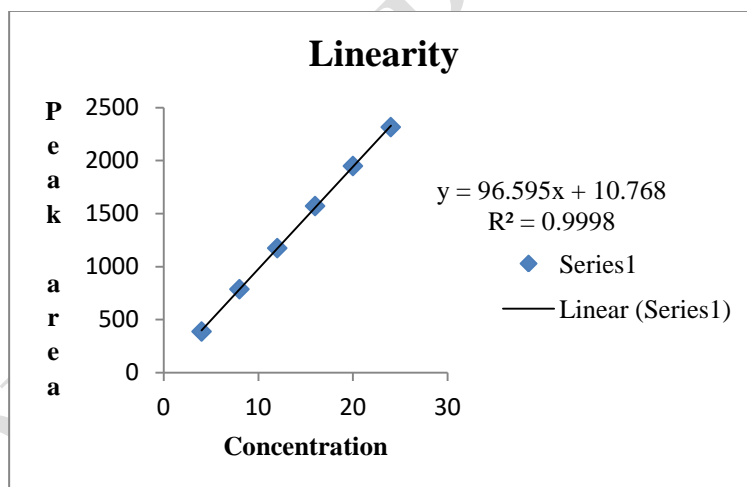
Table 1. System suitability data of imatinib mesylate

S.NO.	Rt	Peak Area	Theoretical Plates	Tailing factor
1	3.493	1615.39	31986.95970	1.45371
2	3.491	1608.98	32025.51006	1.44385
3	3.491	1613.14	32798.57352	1.44517
4	3.491	1612.38	32642.02000	1.45192
5	3.488	1616.40	31849.16083	1.48433
6	3.489	1622.22	33582.31260	1.45200
		<b>Avg:1614.752</b> <b>S.D: 4.482</b>		

		<b>% RSD: 0.278</b>	
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### 3.2.2 Linearity

Linearity was determined in the range of 4µg/mL to 24 µg/mL. Regression equation obtained for imatinib mesylate was found to be  $y = 96.59x + 10.76$  and correlation coefficient was found to be 0.999. The linearity graph is shown in figure 4. The linearity values data is given in table 2.



**Fig. 4. Linearity graph of imatinib mesylate**

**Table 2. Linearity data of imatinib mesylate**

S.NO.	Concentration (µg/mL)	Peak area
1	4	386.65
2	8	785.85
3	12	1173.37
4	16	1570.14
5	20	1947.75
6	24	2314.81

<b>Linearity Equation</b> $y = 96.59x + 10.76$
$r^2 = 0.999$

### 3.2.3 Specificity / Selectivity

Chromatograms of standard (16 µg/mL) and sample preparation (16 µg/mL) were given in figures 5 & 6 respectively. No interference was observed in blank solvent and excipients used in formulation of imatinib mesylate peak.

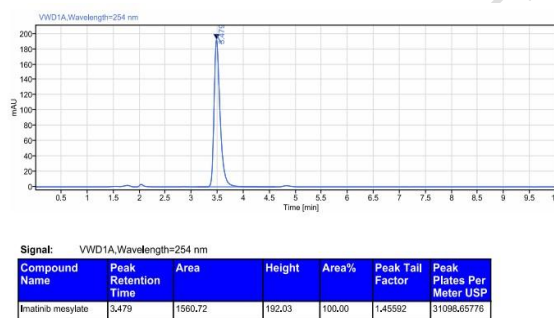


Fig. 5. Specificity chromatogram of imatinib mesylate standard

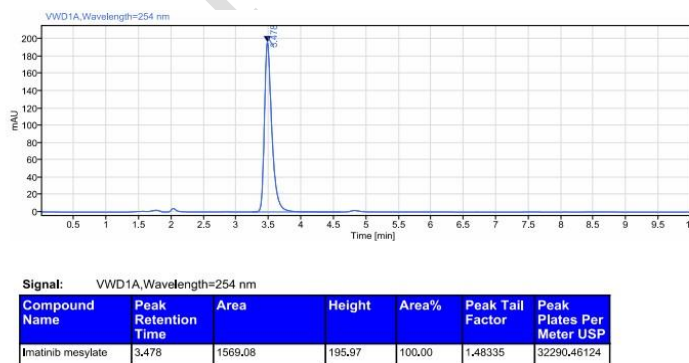


Fig. 6. Specificity chromatogram of imatinib mesylate sample

### 3.2.4 Precision

% RSD values of method precision, intraday and interday precision were obtained according to acceptance range that is less than 2. The results of method precision were shown in table 3 and intraday and interday precision results were shown in table 4.

S.NO.	Concentration (µg/ml)	Peak area
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Table 3.		1	16	1568.85	Method		
Level (%)	Sample conc. (µg/mL)	Standard Conc. (µg/mL)	Total conc. (µg/mL)	Peak area	Amount recovered (µg/mL)	Mean % Recovery ± S.D	% RSD
				SD = 3.069			
				% RSD = 0.195			

### precision of imatinib mesylate

Concentration (µg/mL)	Intraday Precision			Interday Precision		
	12	16	20	12	16	20
Peak area	1170.03	1561.55	1946.21	1217.85	1569.71	1963.66
	1174.23	1565.90	1962.97	1219.82	1568.77	1945.63
	1175.18	1568.75	1946.46	1180.45	1570.51	1962.25
Mean ± SD	1173.14 ± 2.74	1565.40 ± 3.62	1951.88 ± 9.60	1206.04 ± 22.18	1569.66 ± 0.87	1957.18 ± 10.02
% RSD	0.23	0.23	0.49	1.83	0.05	0.51

Table 4. Intraday and interday precision of imatinib mesylate

### 3.2.5 Accuracy

The % recoveries and % RSD values were obtained according to acceptance limits. The results of recovery studies are shown in table 5.

Table 5. Accuracy data of imatinib mesylate

				1178.78	11.68	0.30	
100%	8	8	16	1574.74	15.60	97.21 ± 0.25	0.26
				1566.98	15.52		
				1568.75	15.54		
150%	8	12	20	1946.21	19.28	96.73 ± 0.58	0.55
				1965.26	19.47		
				1947.75	19.29		

### 3.2.6 Robustness

% RSD of imatinib mesylate values were found to be below 2% at various conditions, so method was robust. The robustness values shown in table 6.

**Table 6. Robustness data of imatinib mesylate**

### 3.2.7 Assay

% purity of imatinib mesylate was found to be 99.37% and results were given in table 7.

**Table 7. Assay data of imatinib mesylate**

Parameter	Change in flow rate (1 ± 0.2 ml/min)		Change in wave length (254 ± 5 nm)	
	0.8 ml	1.2 ml	249 nm	259 nm
<b>Retention time</b>	5.632	5.637	3.475	3.469
<b>Tailing Factor</b>	1.51948	0.96902	1.46281	1.48278
<b>Theoretical plate</b>	37194.14398	33557.65011	31236.60780	30673.79046
<b>Peak area</b>	1612.25	1598.22	1600.65	155.75
<b>Mean</b>	1605.235		1598.200	
<b>S.D</b>	9.921		3.465	
<b>% RSD</b>	0.61		0.21	

3.3

S.NO.	Peak area	
	Standard	sample
1	1654.23	1647.60
2	1663.97	1655.52
3	1655.32	1647.44
4	1665.53	1656.29
5	1663.97	1643.62
<b>Mean</b>	1660.604	1650.094
<b>S.D</b>	5.373	5.545
<b>% RSD</b>	0.324	0.336
<b>% Purity</b>	99.37	

## Degradation studies

Degradation peaks were not observed in alkali, photolytic and thermal conditions. Degradation peaks were observed in acidic and peroxide conditions. The degradation chromatograms were shown in figure 7. The results of degradation methods data shown in table 8.

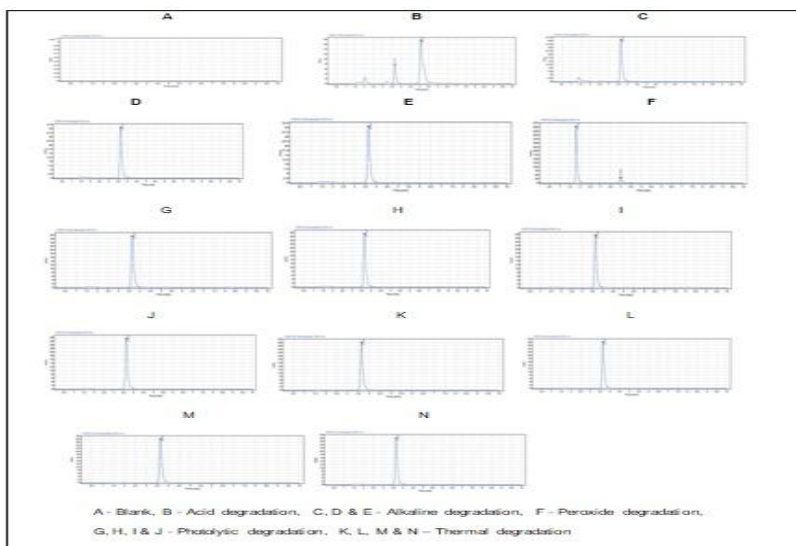


Fig. 7. Degradation chromatograms of imatinib mesylate

Table 8: Degradation methods data of Imatinib mesylate

Type of degradation	Condition for degradation			% Degradation
	Strength of solution	Temperature (°C)	Time period (hours/days)	
Acid	0.1 N HCl	80 °C	5 hours	79.03 %
Base	0.1 N NaOH	80 °C	5 hours	No Degradation
	0.5 N NaOH	100 °C	8 hours	No Degradation
	1 N NaOH	120 °C	12 hours	No Degradation
Oxidation	3 % H <sub>2</sub> O <sub>2</sub>	80 °C	5 hours	91.35 %
Photolytic	Instrument	Temperature	5 hours	No Degradation
			24 hours	No Degradation
			2 days	No Degradation
			5 days	No Degradation
			UV Chamber	-----
Thermal	Hot air oven	80 °C	5 hours	No Degradation
		100 °C	24 hours	No Degradation
		120 °C	3 days	No Degradation
		150 °C	5 days	No Degradation

### 3.3.1 Acidic Condition Degradation Peak Characterization

Characterization of isolated DP was performed using mass spectrum. The mass of the degradation product was acquired in the (+) ionization mode and it is shown in figure 8. The imatinib mesylate  $m/z$  is 495.28 (+) and actual mass is 494.28. The degradation product  $m/z$  is 395.05(+) and actual mass is 394.17. Mass spectra suggest the molecular weight of degradation product is 394.45, which was 100 mass units less than imatinib mesylate. The proposed degradation product molecular formula was found to be  $C_{24}H_{20}N_5O$  which matches with molecular weight of 394.45

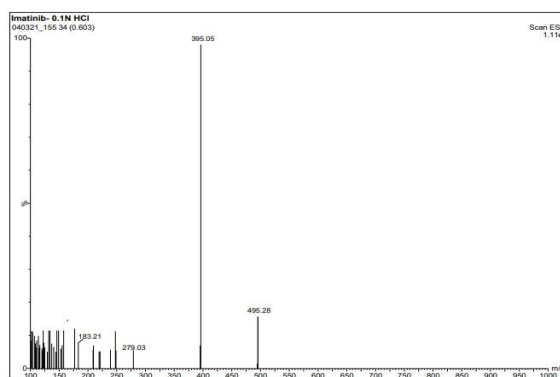


Fig. 8. Mass spectra of acidic condition degradation product

### 3.3.2 Peroxide Condition Degradation Characterization

Characterization of isolated DP was performed using mass spectrum. The mass of the degradation product was acquired in the (+) ionization mode and it is shown in figure 9. The imatinib mesylate  $m/z$  is 495.28 (+) and actual mass is 494.28. The degradation product  $m/z$  is 248.02(+) and actual mass is 247.10. Mass spectra suggest the molecular weight of degradation product is 247.27, which was 247 mass units less than imatinib mesylate. The proposed degradation product molecular formula was found to be  $C_{15}H_{11}N_4$  which matches with molecular weight of 247.27.

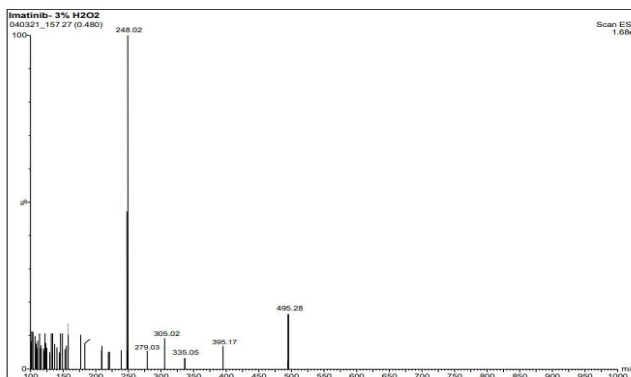


Fig. 9. Mass spectra of peroxide condition degradation product

#### 4. CONCLUSION

The UPLC method validation data show that this is a reliable method which can be used for analyzing regular quality control. The advanced UPLC method was determined to be specificity, linearity, precision, intermediate precision, accuracy, degradation studies and characterization by mass spectroscopy. This UPLC analytical method, the elution time and run time is reduced, which proves that the method is economical and widely acceptable, also simple and practical, which can be used in routine quality control tests in the industry.

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#### **COMPETING INTERESTS DISCLAIMER:**

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely

no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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