Method Development, Validation and Degradation Studies of Imatinib Mesylate by UPLC

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 5µm) 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 µL 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 of were found to be 0.901 and 2.73µg/ml. Regression equation was found to be y= 96.59x + 10.76 form 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 is a cancer medication prescribed to treat leukemia and gastrointestinal tumors. It operates by inhibiting proteins associated with cancer cell growth to relieve symptoms, prevent the spread of cancer cells, and aid other treatments. Imatinib is one of the newest anticancer drugs in the market and was one of the first drugs to be pushed through the Food and Drug Administration's (FDA) fast track designation for approval [1]. Imatinib is an antineoplastic drug used to treat leukemia, especially chronic myelogenous leukemia (CML). Imatinib is a tyrosine kinase inhibitor (TKI). A kinase is an enzyme that promotes cell growth. Imatinib mesylate is a small molecule that inhibits the c-Abl protein tyrosine kinase, a kinase specifically important for the proliferation of CML [2].

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 methanesulfonate. The imatinib mesylate chemical structure was shown in figure 1. Stability testing forms an important part of the process of drug product development. The purpose of stability testing is to provide evidence on how the quality of a drug substance varies with time under the influence of a variety of environmental factors such as temperature, oxidation, and light, which enables establishing shelf life recommended by the International Conference on Harmonization (ICH) guidelines [3]. Literature survey revealed for few analytical methods reported in the estimation of imatinib mesylate in bulk and pharmaceutical dosage form [4-12]. The current study aimed to develop a faster chromatographic technique a simple, specific, precise, and accurate reverse-phase ultra-performance liquid chromatography (UPLC) that can result in shorter analysis times without compromising on the resolution and sensitivity.The imatinib mesylate chemical structure was shown in figure 1.

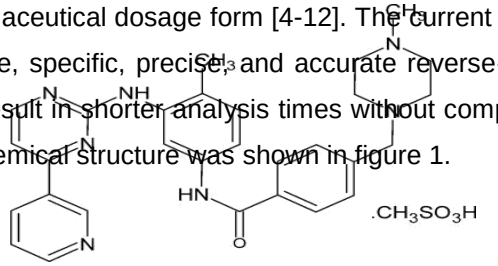


Fig. 1. Molecular structure of imatinib mesylate

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 synge pharma science pvt ltd.

2.2 Instrumentation

UPLC 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 5µm)

Column temperature: 25°C

Detection wavelength: 254 nm

Run time: 10 min

Sample volume: 20 µl

2.4 Solvents

The chemical reagents methanol, acetonitrile (ACN), standard and sample solutions were filtered through 0.45 µm. 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

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 µg/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 µg/ml.

2.6 Preparation of Sample Stock Solution

50 mg of imatinib mesylate API sample dissolved in methanol and make up to 50 ml with methanol. The resulting solution concentration is 1000 µg/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 µg/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.

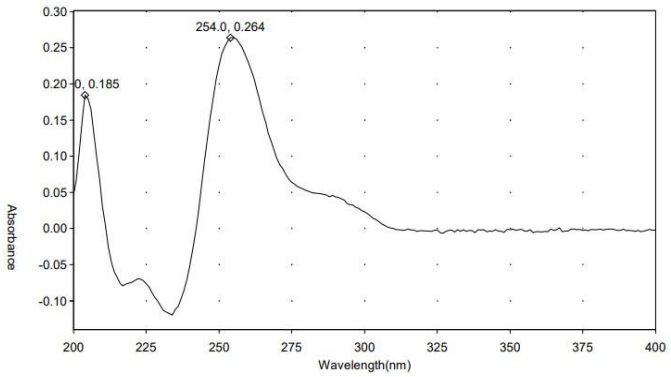


Fig. 2 UV spectra of imatinib mesylate

2.8 Method Validation

2.8.1 System Suitability

System suitability parameters were estimate 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 µg/mL of imatinib mesylate. The regression coefficient was determined form 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 µg/ml in method precision. For intraday & inter day precision is performed in different times & different days by using 12 µg/ml, 16 µg/ml & 20 µg/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 ± 0.2ml/min) and wavelength (254 nm ± 5nm).

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^{o}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 UPLC.

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^{o}C for 5 hours, 0.5N NaOH at 100^{o}C for 8 hours and 1N NaOH at 120^{o}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 UPLC.

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_2O_2 at 80^{o}C for 5 hours. The resulting solution was filtered through the 0.45µ membrane filter and injected in UPLC.

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^{0}C for 5 hours, 100^{0}C for 24 hours, and 120^{0}C for 3 days and 150^{0}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.

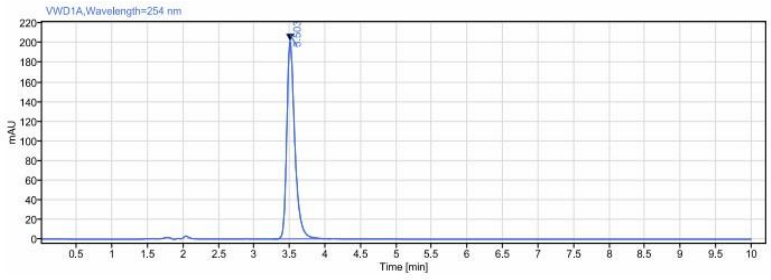
2.9.6 Degradation characterization:

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

3. RESULTS AND DISCUSSION

3.1 optimization chromatography method

The optimized chromatography conditions performed with C18 intersil ODS (250 X 4.6 mm X 5µm), 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.



Signal: VWD1A,Wavelength=254 nm

Compound Name	Peak Retention Time	Area	Height	Area%	Peak Tail Factor	Peak Plates Per Meter USP
Imatinib mesylate	3.503	1598.02	201.95	100.00	1.42652	33132.20600

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 **Avg:1614.752** **S.D: 4.482** **% RSD: 0.278**	33582.31260	1.45200

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.

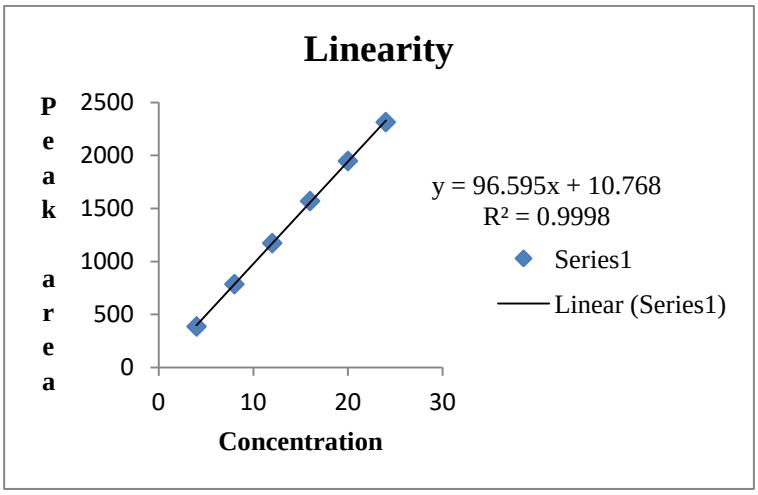


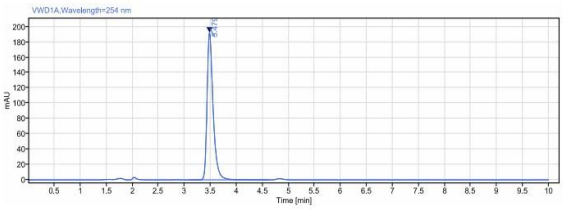
Fig. 4. Linearity graph of imatinib mesylate

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
Linearity Equation y= 96.59x + 10.76		
r^2 = 0.999		

3.2.3 Specificity / Selectivity

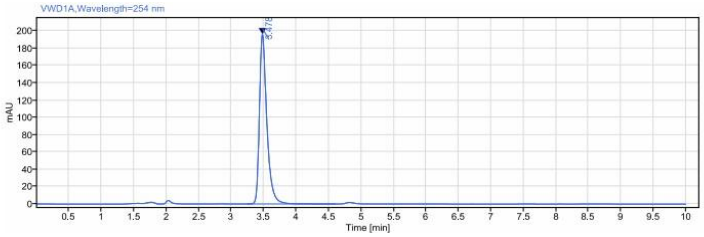
Chromatograms of standard (16 µg/mL) and sample preparation (16 µg/mL) were given in figures 5 & 6 respectively. No interference was observed in blank solvent and excipients used in formulation of imatinib mesylate peak.



Signal: VWD1A,Wavelength=254 nm

Compound Name	Peak Retention Time	Area	Height	Area%	Peak Tail Factor	Peak Plates Per Meter USP
Imatinib mesylate	3.479	1560.72	192.03	100.00	1.45592	31098.65776

Fig. 5. Specificity chromatogram of imatinib mesylate standard



Signal: VWD1A,Wavelength=254 nm

Compound Name	Peak Retention Time	Area	Height	Area%	Peak Tail Factor	Peak Plates Per Meter USP
Imatinib mesylate	3.478	1569.08	195.97	100.00	1.48335	32290.46124

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.

Table 3. Method precision of imatinib mesylate

S.NO.	Concentration (µg/ml)	Peak area
1	16	1568.85
2	16	1572.89
3	16	1575.65
4	16	1574.94
5	16	1574.08
6	16	1577.97
	Mean = 1574.063 **SD =** 3.069 **% RSD =** 0.195	

Table 4. Intraday and interday precision of imatinib mesylate

	Intraday Precision			Interday Precision		
Concentration (µg/mL)	**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

3.2.5 Accuracy

Level (%)	Sample conc. (µg/mL)	Standard Conc. (µg/mL	Total conc. (µg/mL)	Peak area	Amount recovered (µg/mL	Mean % Recovery ± S.D	% RSD
50%	8	4	12	1172.49	11.61	96.98 ± 0.30	0.31
				1173.37	11.62		
				1178.78	11.68		
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		

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

Parameter	Change in flow rate (1 ± 0 .2 ml/min)	Change in wave length (254 ± 5 nm)

3.2.6 Robustness

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

Table 6. Robustness data of imatinib mesylate

	0.8 ml	1.2 ml	249 nm	259 nm
Retention time	5.632	5.637	3.475	3.469
Tailing Factor	1.51948	0.96902	1.46281	1.48278
Theoretical plate	37194.14398	33557.65011	31236.60780	30673.79046
Peak area	1612.25	1598.22	1600.65	155.75
Mean	1605.235		1598.200	
S.D	9.921		3.465	
% RSD	0.61		0.21	

3.2.7 Assay

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

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
Mean	1660.604	1650.094
S.D	5.373	5.545
% RSD	0.324	0.336
% Purity	99.37	

Table 7. Assay data of imatinib mesylate

3.3 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.

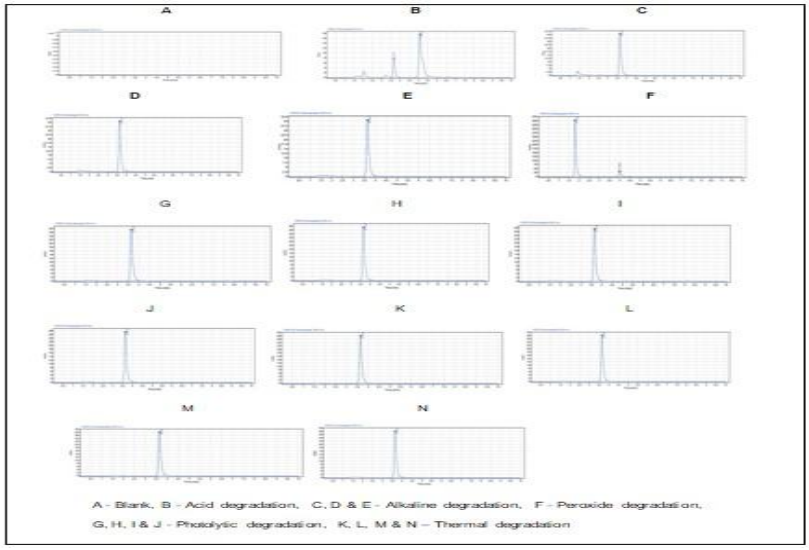


Fig. 7. Degradation chromatograms of imatinib mesylate

Table 8: Degradation methods data of Imatinib mesylate

Type of degradation	Condition for degradation			% Degradation
	Strength of solution	Temperature (^{0}C)	Time period (hours/days)	
Acid	0.1 N HCl	80 ^{0}C	5 hours	79.03 %
Base	0.1 N NaOH	80 ^{0}C	5 hours	No Degradation
	0.5 N NaOH	100 ^{0}C	8 hours	No Degradation
	1 N NaOH	120 ^{0}C	12 hours	No Degradation
Oxidation	3 % H_2O_2	80 ^{0}C	5 hours	91.35 %
	Instrument	**Temperature**	**Time period**	**% Degradation**
Photolytic	UV Chamber	---------	5 hours	No Degradation
			24 hours	No Degradation
			2 days	No Degradation
			5 days	No Degradation
Thermal	Hot air oven	80 ^{0}C	5 hours	No Degradation
		100 ^{0}C	24 hours	No Degradation
		120 ^{0}C	3 days	No Degradation
		150 ^{0}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 is matches with molecular weight of 394.45

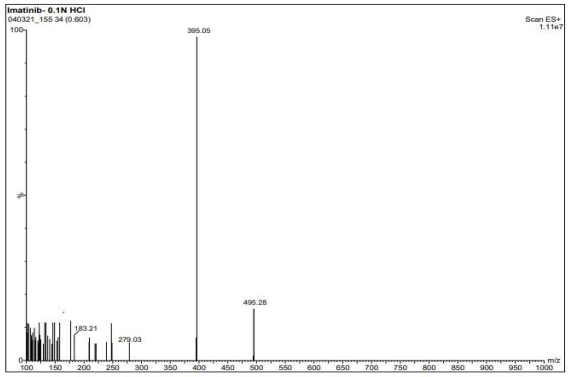


Fig. 8. Mass spectra of acidic condition degradation product

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 is matches with molecular weight of 247.27.

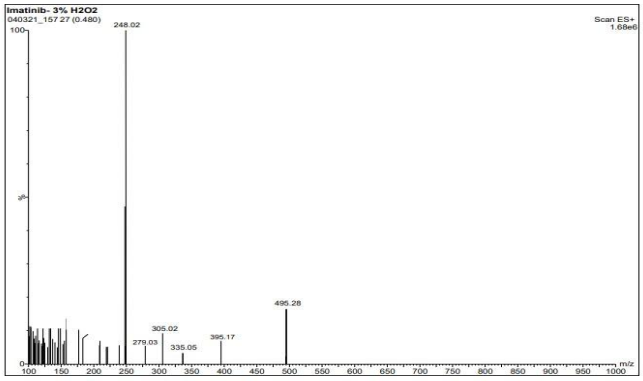


Fig. 9. Mass spectra of peroxide condition degradation product

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.

ACKNOWLEDGEMENT

The authors thankful to ramanbhai patel college of pharmacy, Charusat for providing research facilities.

CONFLICTS OF INTEREST

The authors are no conflicts of interest declared.

REFERENCES

1. Smita JP, Rajendra CD, Priya PD. Development of UV spectrophotometric method for the determination of imatinib mesylate (ITM) in bulk and formulation. Asian J Pharm Clin Res. 2013; 6(3):54-57. https://innovareacademics.in/journals/index.php/ajpcr/article/view/34.

2. Kuna A. K, Seru G, Radha G.V. Analytical method development and validation for the estimation of imatinib mesylate and its dimer impurity in pharmaceutical formulation by reverse-phase high-performance liquid chromatography. Asian J Pharm Clin Res.2018; 11(3):136-9. doi:10.22159/ajpcr.2018.v11i3.21073.

3. ICH Q1A (R2), Stability Testing of New Drug Substances and Products, 2003.

4. Yadav RR, Rokade MD, Salunke SA, Gangrade DM, Holkar GS, Daphal VN. Determination of potential genotoxic impurities in imatinib mesylate by RP-HPLC method. Biol Forum Int J. 2012; 4:15-8.

5. Madireddy V, Kondra SB, Narayanareddy P. Stability-indicative, validated, fast HPLC method for quantification of two genotoxic impurities in imatinib mesylate. Glob J Anal Chem. 2011; 2:198-207.

6. Ghrib F, Chtioui A, Saied T, Bellakhal N. Determination of imatinib and its genotoxic impurities in tablets. Biol Forum Int J. 2015; 7:1820-7.

7. Shah P, Shah R. A stability-indicating RP-HPLC method development and validation for the related substance determination of imatinib process impurities and their degradation products in tablet dosage form. Int J PharmTech Res. 2015; 8:128-46.

8. Jing Zeng, Hualin Cai, Zhiping Jiang, Qing Wang, Yan Zhu, Ping Xu, Xielan Zhao. A validated UPLC–MS/MS method for simultaneous determination of imatinib, dasatinib and nilotinib in human plasma. J Pharm Anal. 2017; 7:374–380. doi: 10.1016/j.jpha.2017.07.009. PMID: 29404062

9. Zhihui Ren, Xiaoxi Zhang, Haiyuan Wang and, Xinghua Jin. Using an innovative quality-by-design approach for the development of a stability-indicating UPLC/Q-TOF-ESI-MS/MS method for stressed degradation products of imatinib mesylate. RSC Adv. 2016; 16:13050-13062. https://doi.org/10.1039/C5RA26456A.

10. Xu RA, Lin Q, Qiu X, Chen J, Shao Y, Hu G, Lin G. UPLC-MS/MS method for the simultaneous determination of imatinib, voriconazole and their metabolites concentrations in rat plasma. J Pharm Biomed Anal. 2019; 166:6-12. doi: 10.1016/j.jpba.2018.12.036. Epub 2018 Dec 21. PMID: 30594035.

11. Gulen Melike Demir, Ismail Tuncer Degim. Determination of imatinib mesylate and erlotinib hydrochloride in mice plasma using ultrahigh performance liquid chromatography method. Kuwait J. Sci. 2018;

45(3):57-63.

12. Wolff FC, Dillenburg TL, Venzon Antunes M, Linden R, Comparsi Wagner S, Verza SG. Characterization of imatinib mesylate formulations distributed in South American countries: Determination of genotoxic impurities by UHPLC-MS/MS and dissolution profile. Biomed Chromatogr. 2018; 32(7):e4222. doi: 10.1002/bmc.4222. Epub 2018 Apr 6. PMID: 29488235.