Available online at www.icjpir.com ISSN: 2349-5448

INTERCONTINENTAL JOURNAL OF PHARMACEUTICAL INVESTIGATIONS AND RESEARCH

ICJPIR |Volume 3 | Issue 1 | Jan – Mar- 2016

Research Article

New RP HPLC method for the estimation of imatinib in pharmaceutical dosage form

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ABSTRACT

A simple and selective LC method is described for the determination of Imatinib dosage forms. Chromatographic separation was achieved on a c_{18} column using mobile phase consisting of a mixture of Water: Acetonitrile (30:70 v/v), with detection of 272nm. Linearity was observed in the range 50-150 µg /ml for Imatinib (r^2 =0.9976) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim. The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

Keywords: Water: Acetonitrile, %RSD and Imatinib.

INTRODUCTION

A drug includes all medicines intended for internal or external use for or in the diagnosis, treatment, mitigation or prevention of disease or disorder in human beings or animals, and manufactured exclusively in accordance with the formulae mentioned in authoritative books.[1]

Pharmaceutical analysis is a branch of chemistry involving a process of identification, determination, quantification, purification and separation of components in a mixture or determination of chemical structure of compounds. There are two main types of analysis – Qualitative and Quantitative analysis.

Qualitative analysis is performed to establish composition of a substance. It is done to determine the presence of a compound or substance in a given sample or not. The various qualitative tests are detection of evolved gas, limit tests, color change reactions, determination of melting point and boiling point, mass spectroscopy, determination of nuclear half-life etc.

Quantitative analysis techniques are mainly used to determine the amount or concentration of

analyte in a sample and expressed as a numerical value in appropriate units. These techniques are based on suitable chemical reaction and either measuring the amount of reagent added to complete the reaction or measuring the amount of reaction product obtained the characteristic movement of a substance through a defined medium under controlled conditions, electrical measurement or measurement of spectroscopic properties of the compound. [2]

AIM AND PLAN OF WORK

Aim

To develop new RP HPLC method for the estimation of **Imatinib** in pharmaceutical dosage form.

Plan of work

- Solubility determination of **Imatinib** various solvents and buffers.
- Determine the absorption maxima of the drug in UV–Visible region in different solvents/buffers and selecting the solvents for HPLC method development.
- Optimize the mobile phase and flow rates for proper resolution and retention times.
- Validate the developed method as per ICH guidelines.

METHODOLOGY

Mobile Phase

A mixture of 300 volumes of Water: 700 volumes of Acetonitrile were prepared. The mobile phase was sonicated for 10min to remove gases.

Determination of Working Wavelength (λmax)

In estimation of drug wavelength maxima is used.. So this wavelength is used in estimation to estimate drug accurately.

Preparation of standard stock solution of Imatinib

100mg of Imatinib was weighed and transferred in to 100ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 100 µg /ml of solution by diluting 1ml to 10ml with methanol.

RESULTS AND DISCUSSIONS

Solubility Studies

These studies are carried out at 25 ^{0} C

Imatinib

Soluble in water (200 mg/ml), ethanol (~0.2 mg/ml), DMSO (200 mg/ml), DMF (~10 mg/ml), and methanol (sparingly).

Wavelength determination

The wavelength of maximum absorption (λ_{max}) of the drug, 10 µg/ml solution of the drug in methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank. The resulting spectra are shown in the fig. no.1.and the absorption curve shows characteristic absorption maxima at 272nm for Imatinib.

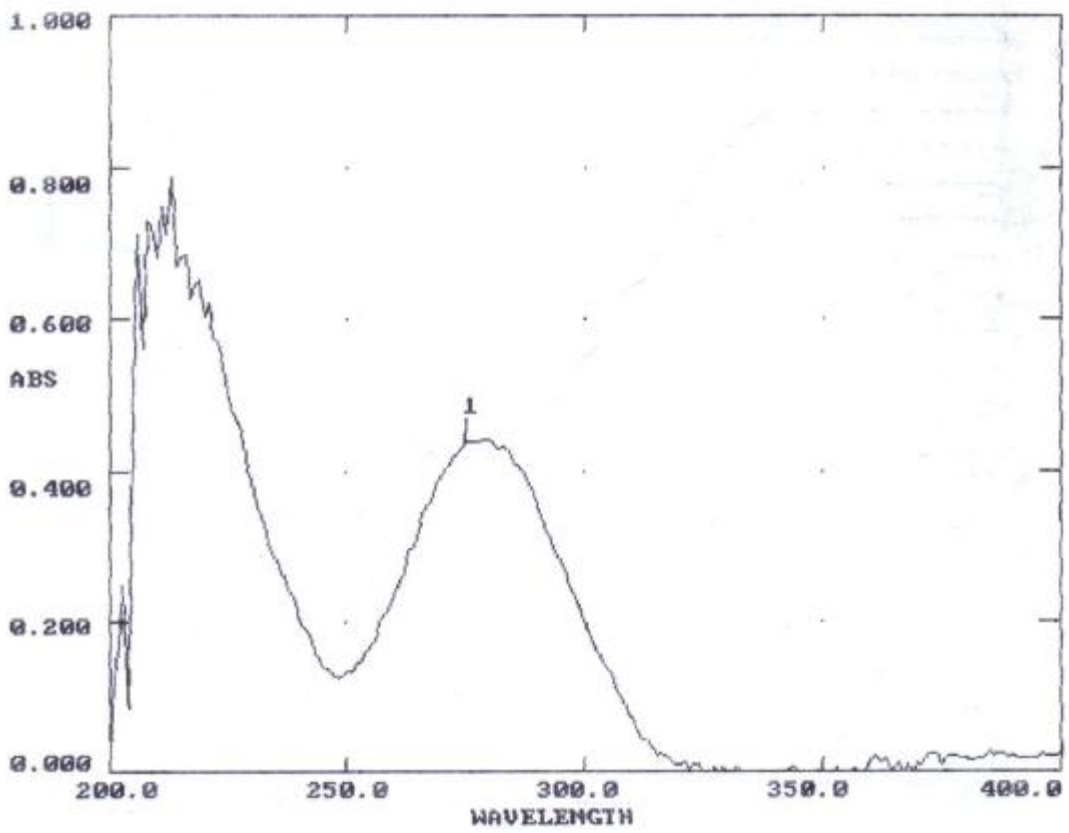

Fig. 1: UV-VIS spectrum of Imatinib

Obeservation

λ_{max} was found to be 272 nm for Imatinib shown in the figure 1

METHOD DEVELOPMENT OF IMATINIB

Trial- 6

Preparation of standard solution

100mg of Imatinib was weighed and transferred in to 100ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 100 µg /ml of solution by diluting 1ml to 10ml with methanol.

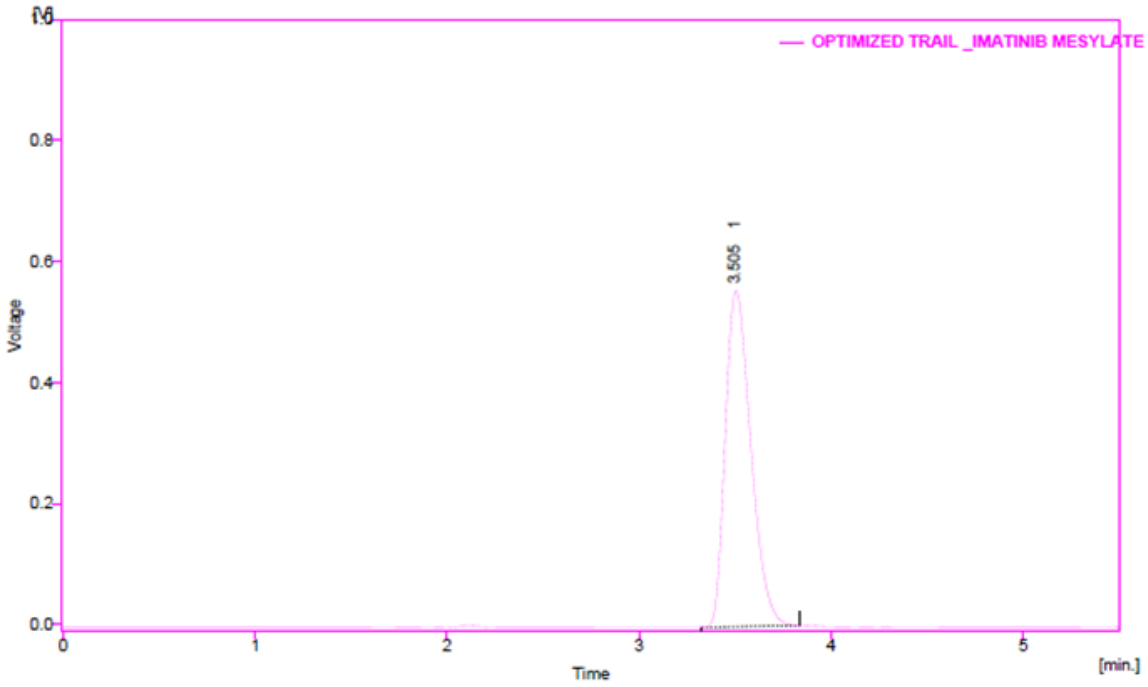

Fig. 2: Chromatogram of Imatinib

Observation

Efficiency was more and resolution was good. Asymmetry was good. Hence the method was optimized.

Table 1: Optimized chromatographic conditions

Mobile phase	Water: ACN (30:70)
pH	3.5
Column	INERTSIL column,C18(150x4.6 ID) 5µm
Flow rate	1.0 ml/min
Column temperature	Room temperature(20-25^{o}C)
Sample temperature	Room temperature(20-25^{o}C)
Wavelength	272nm
Injection volume	20 µl
Run time	6min
Retention time	About 3.505min for Imatinib

ASSAY

Preparation of samples for Assay

Preparation of standard solution

100mg of Imatinib was weighed and transferred in to 100ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 100 µg /ml of solution by diluting 1ml to 10ml with methanol.

Preparation of sample solution

5tablets (each tablet contains Imatinib 100mg) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of Imatinib (100µg/ml) and was prepared by dissolving weight equivalent to 100mg of Imatinib and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 100ml with mobile phase. Further dilutions are prepared in 5 replicates of 100µg/ml of Imatinib was made by adding 1ml of stock solution to 10 ml of mobile phase.

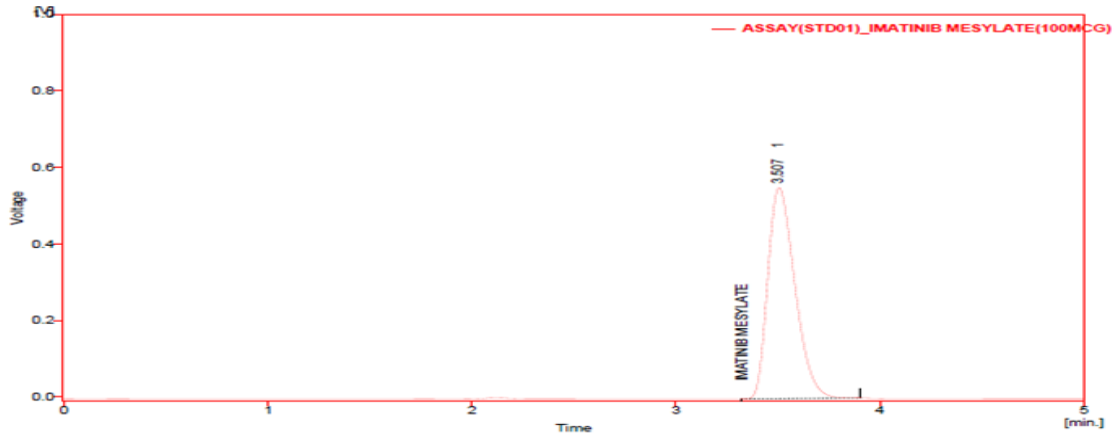

Fig. : 3 Chromatogram of Assay standard preparation-1

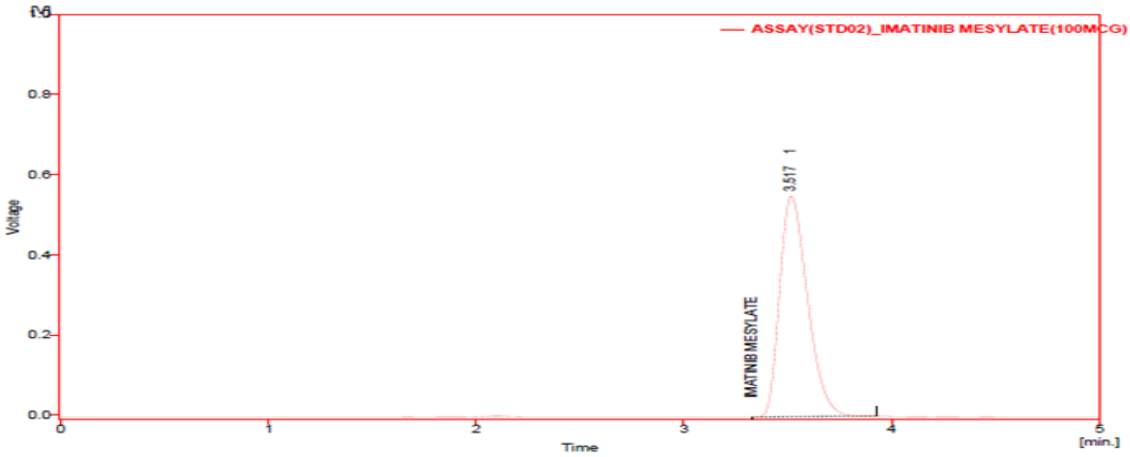

Fig. :4 Chromatogram of Assay standard preparation-2

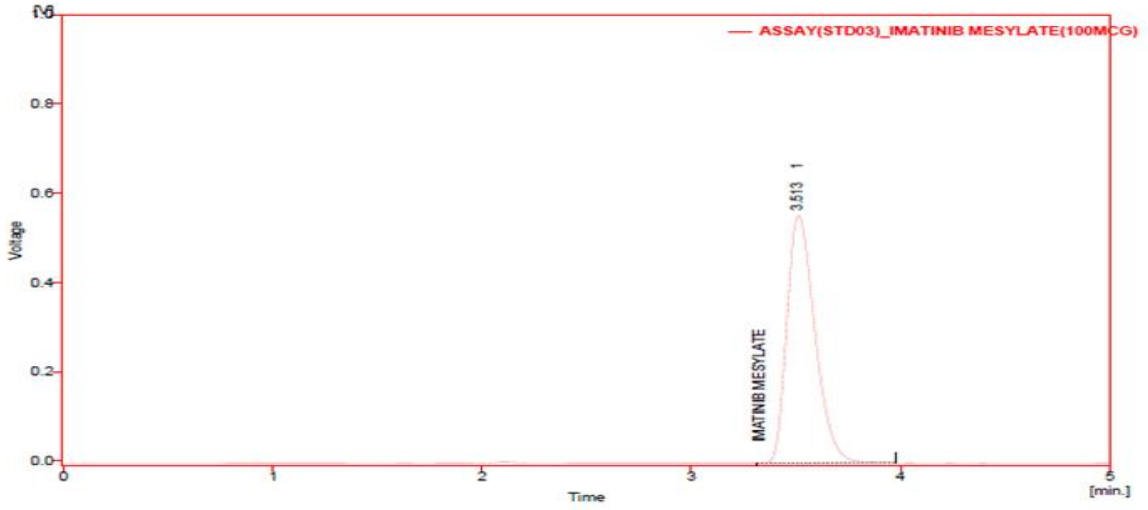

Fig. :5 Chromatogram of Assay standard preparation-3

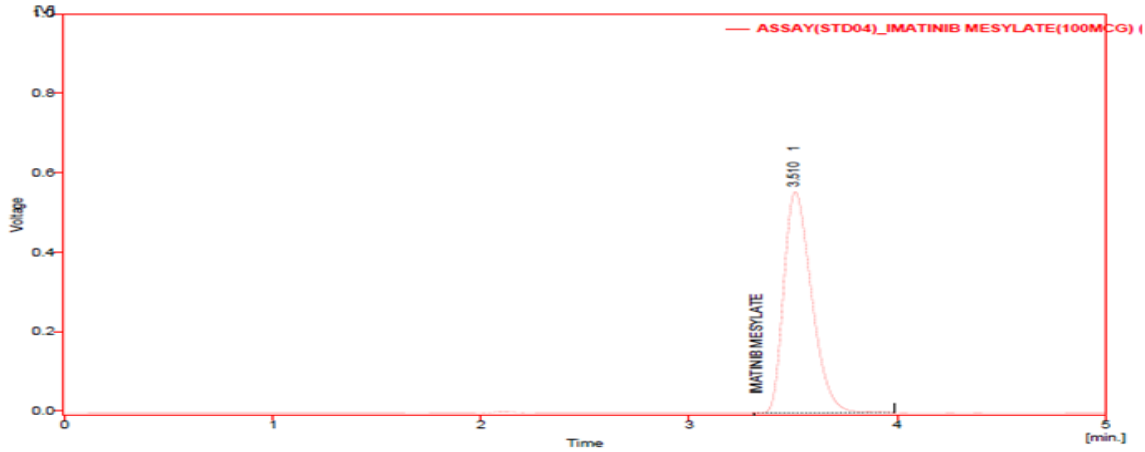

Fig.:6 Chromatogram of Assay standard preparation-4

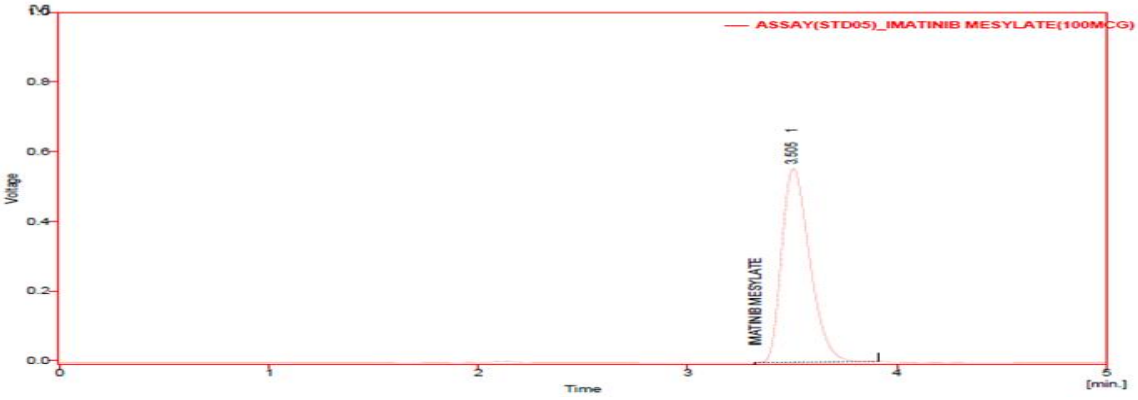

Fig. :7 Chromatogram of Assay standard preparation-5

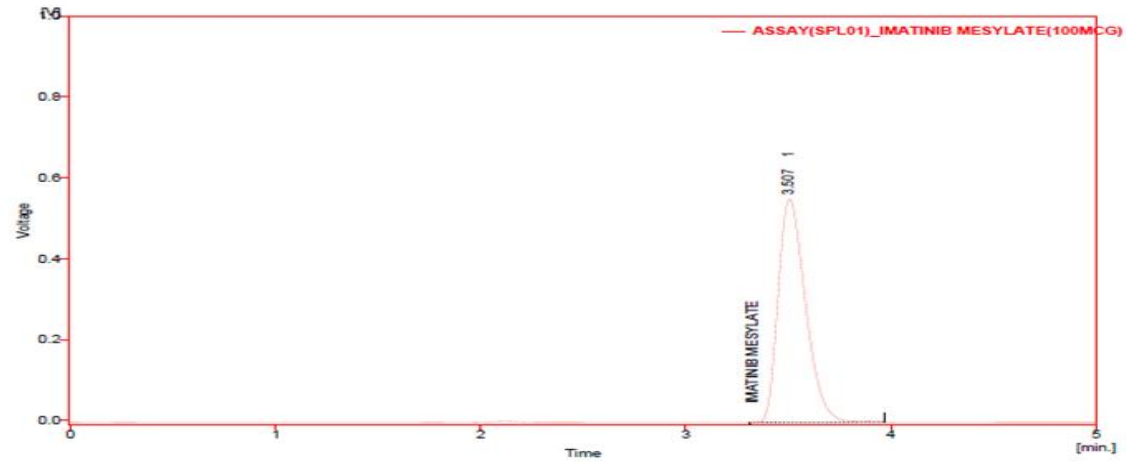

Fig. :8 Chromatogram of Assay sample preparation-1

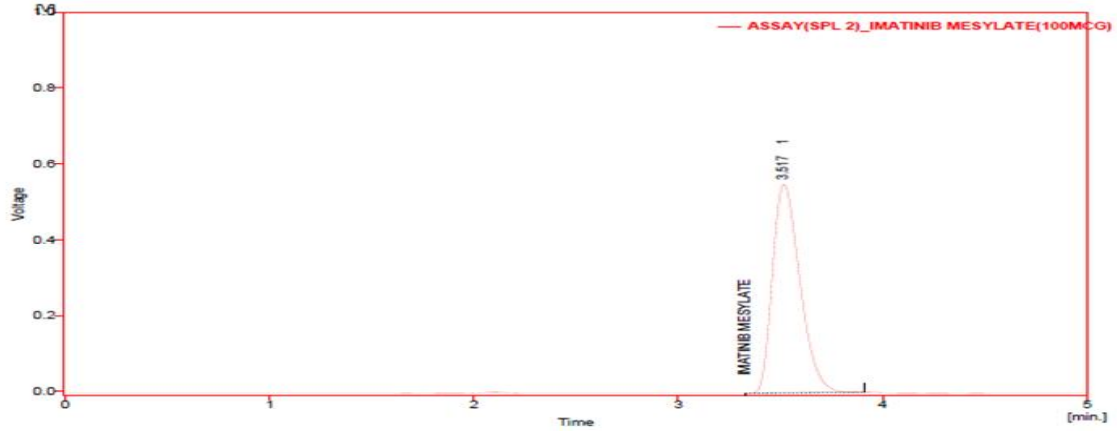

Fig. :9 Chromatogram of Assay sample preparation-2

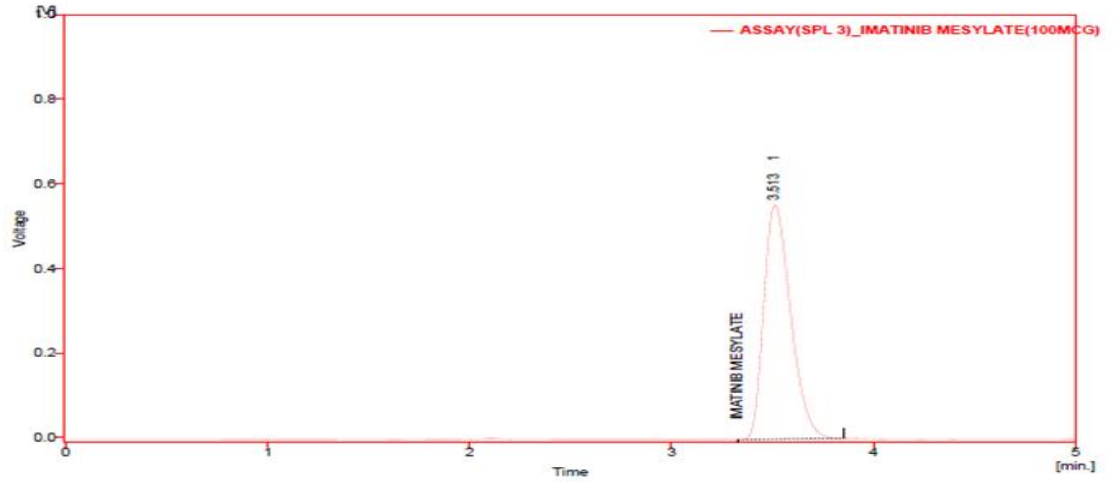

Fig. :10 Chromatogram of Assay sample preparation-3

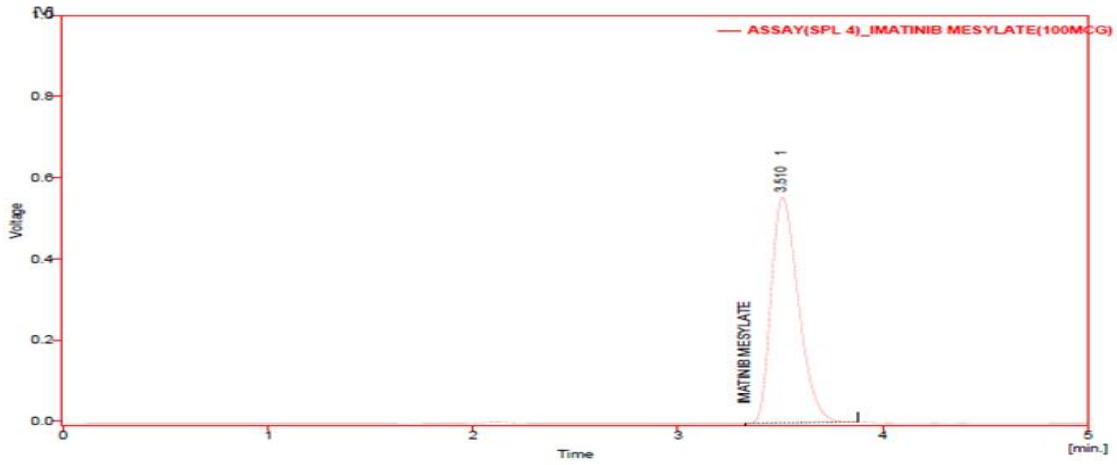

Fig. :11 Chromatogram of Assay sample preparation-4

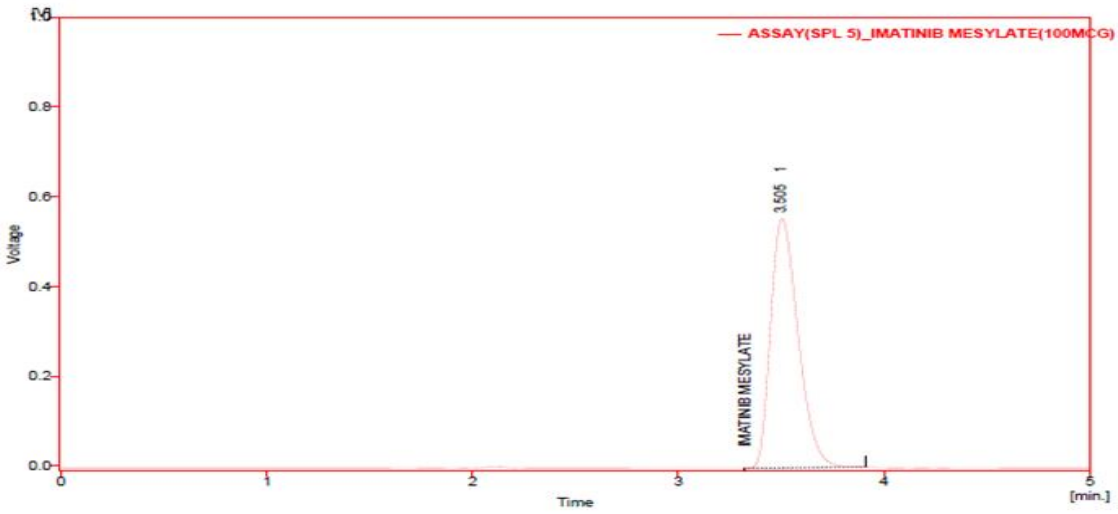

Fig. :12 Chromatogram of Assay sample preparation-5

Table No.:2 Assay Results

IMATINIB		
	Standard Area	**Sample Area**
Injection-1	5092.664	5172.331
Injection-2	5124.196	5120.732
Injection-3	5156.292	5116.770
Injection-4	5167.595	5130.174
Injection-5	5154.186	5154.186
Average Area	5124.384	5138.839
Tablet average weight	118.42	
Standard weight	100	
Sample weight	118.42	
Label amount	100	
std. purity	99.8%	
Amount found in mg	**100.08**	
Assay(%purity)	**100.08**	

Observation

The amount of Imatinib present in the taken dosage form was found to be 100.08%.

VALIDATION

Specificity by Direct comparison method

There is no interference of mobile phase, solvent and placebo with the analyte peak and also the peak purity of analyte peak which indicate that the method is specific for the analysis of analytes in their dosage form.

Preparation of samples for Assay

Preparation of standard solution

Prepared the standard as per assay method

Preparation of sample solution

Prepared the sample as per assay method

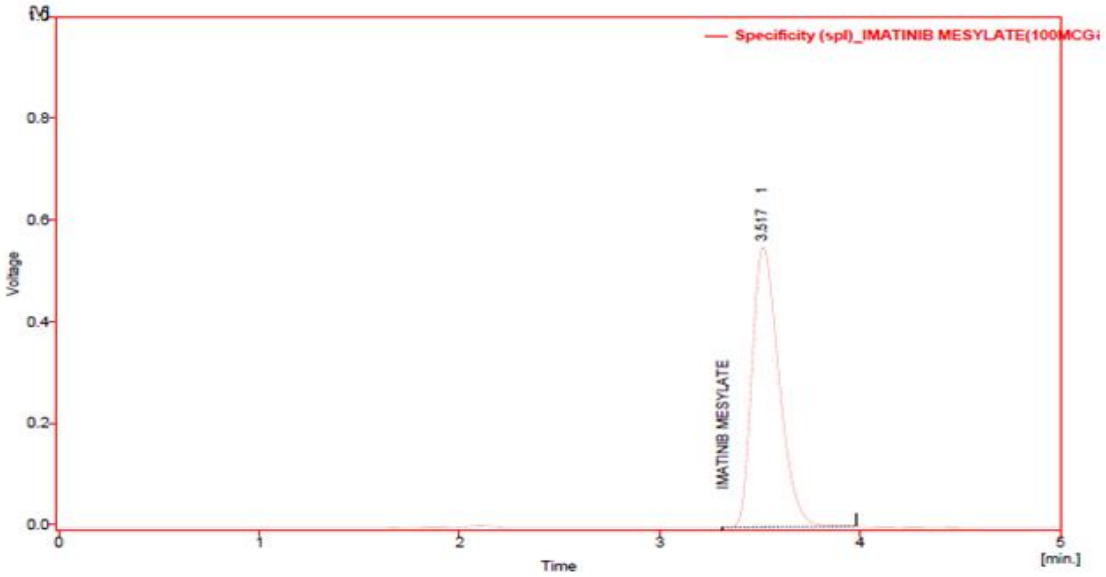

Fig. :13 Chromatogram for specificity of Imatinib sample

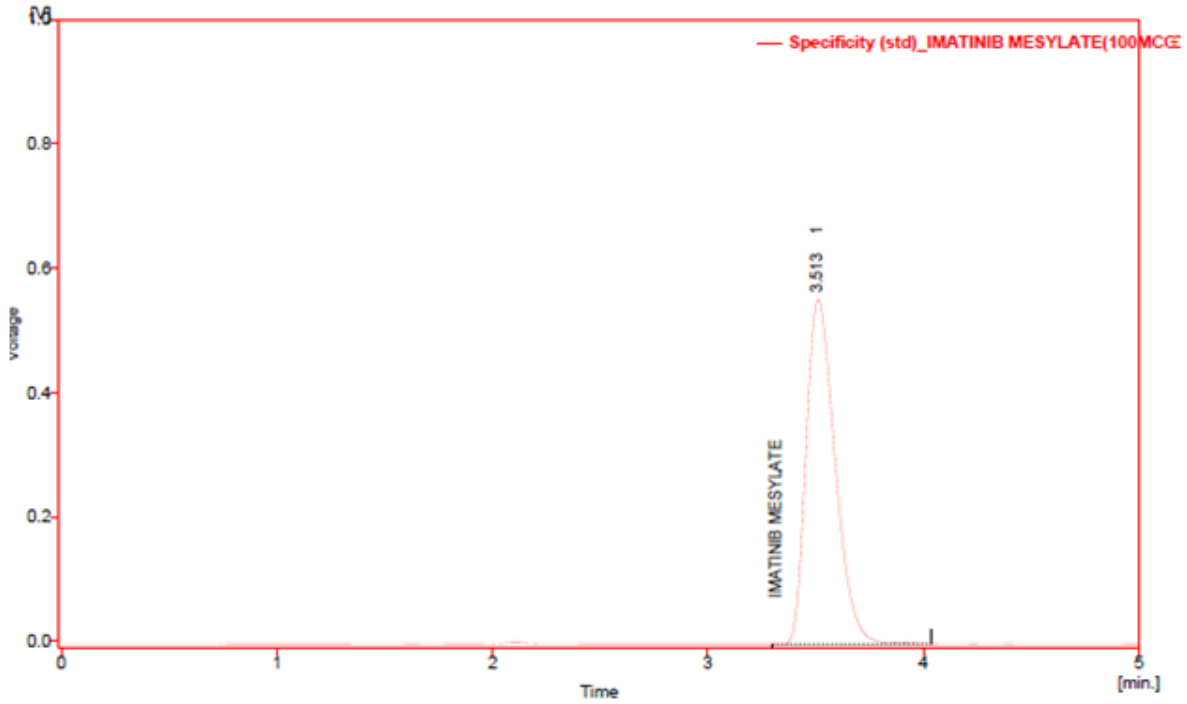

Fig. :14 Chromatogram for Specificity of Imatinib standard

Observation

It is observed from the above data, diluents or excipient peaks are not interfering with the Pioglitazone and Alogliptin peaks.

LINEARITY AND RANGE

Preparation of mixed standard solution

Weigh accurately 100mg of Imatinib in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase.

Table: 3 Linearity Preparations

Preparations	Volume from standard stock transferred in ml	Volume made up in ml (with mobile phase)	Concentration of solution(µg /ml)
			IMATINIB
Preparation 1	0.5	10	50
Preparation 2	0.75	10	75
Preparation 3	1	10	100
Preparation 4	1.25	10	125
Preparation 5	1.5	10	150

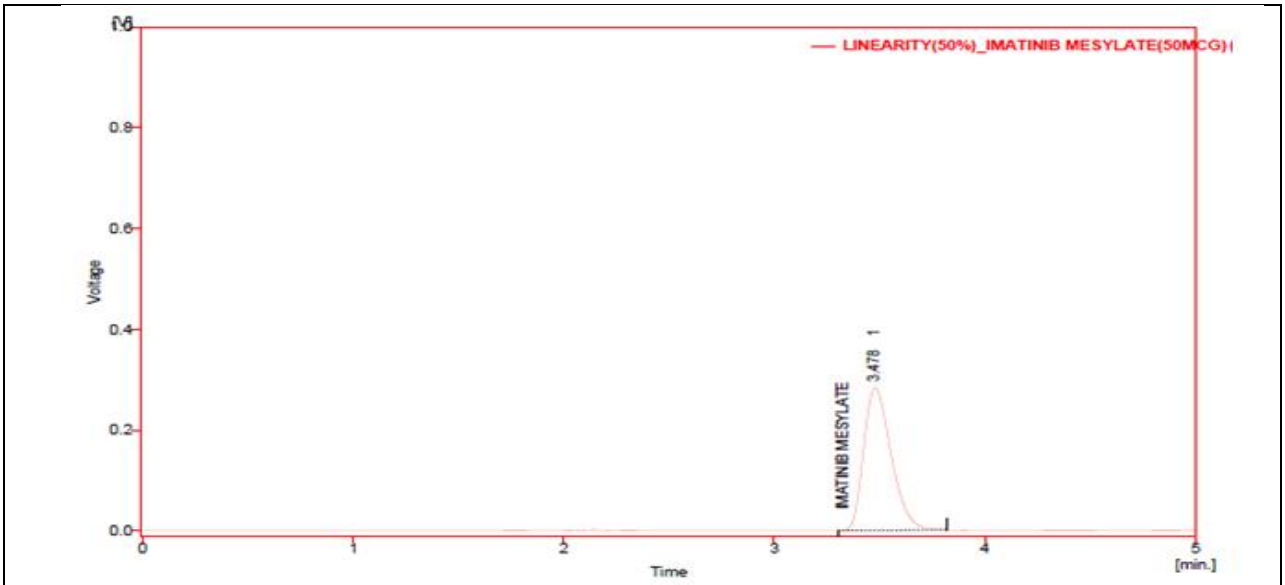

Fig. 15: Chromatogram of Imatinib preparation-1

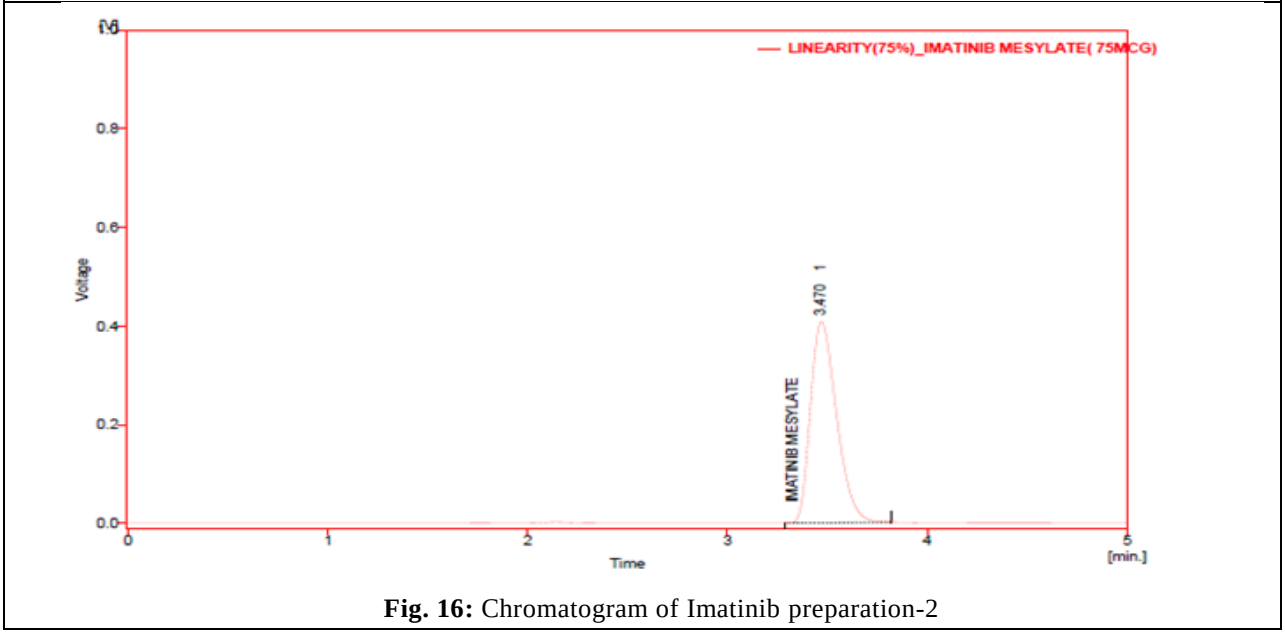

Fig. 16: Chromatogram of Imatinib preparation-2

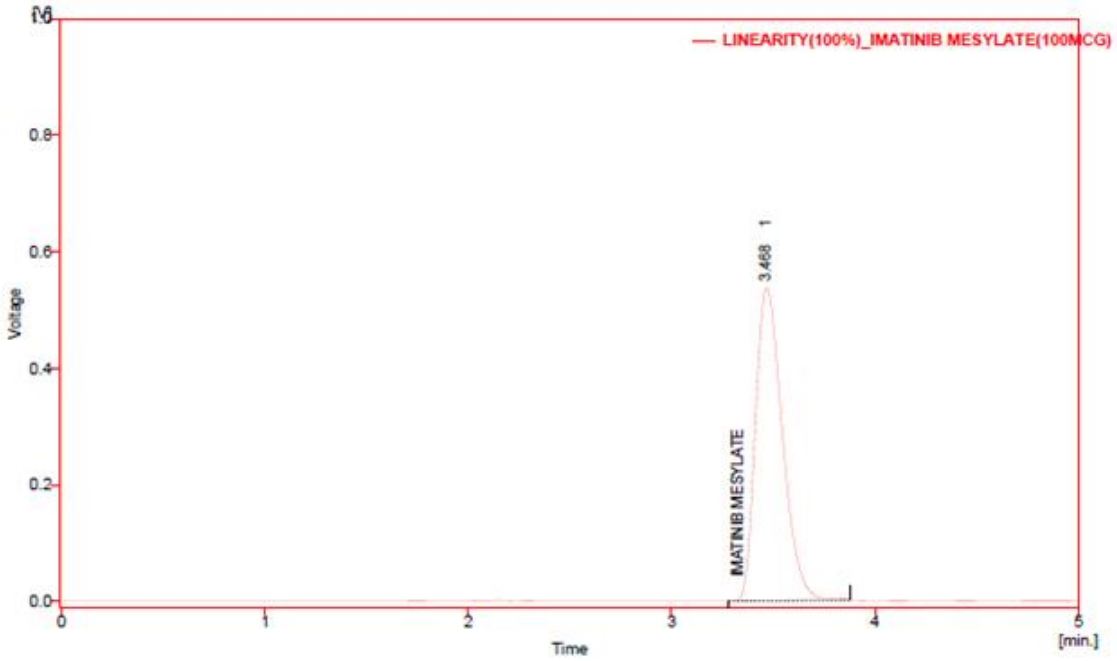

Fig. 17: Chromatogram of Imatinib preparation-3

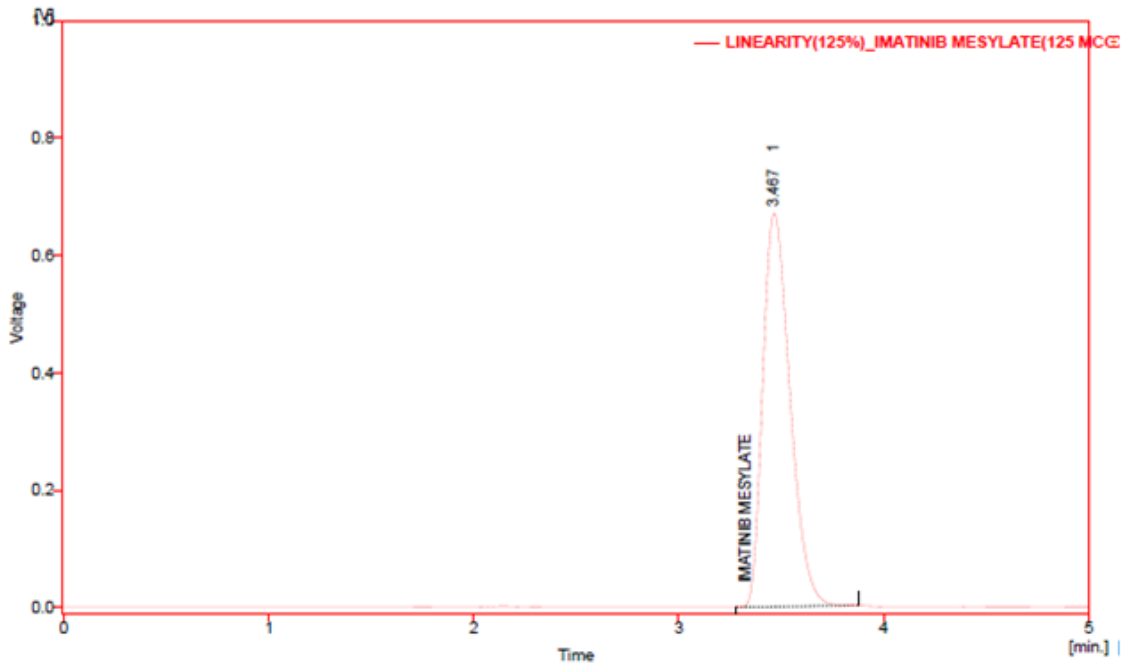

Fig.18: Chromatogram of Imatinib preparation-4

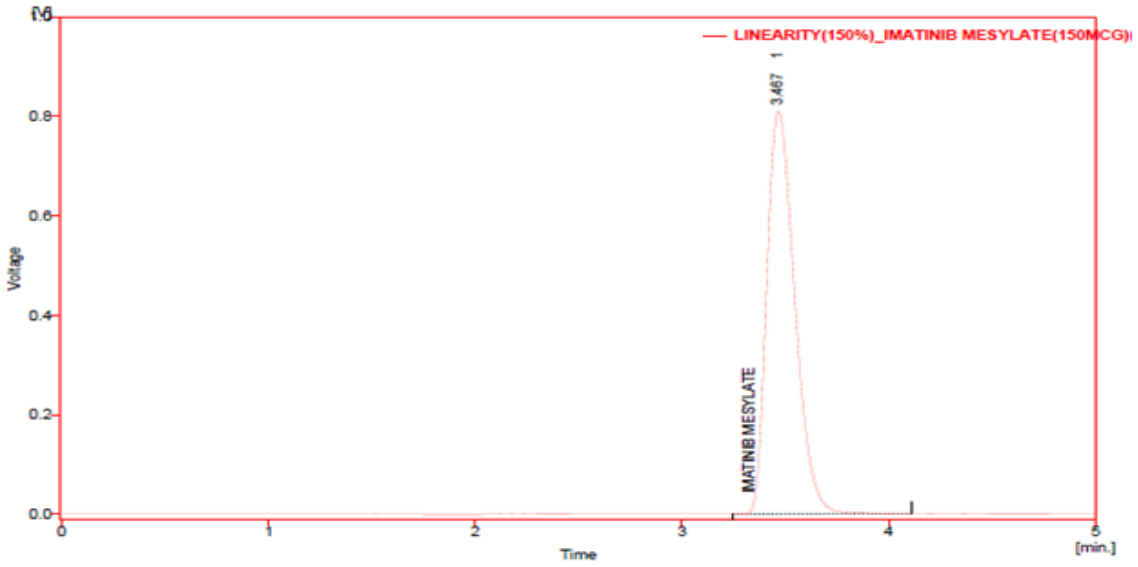

Fig. 19: Chromatogram of Imatinib preparation-5

Table 4: linearity of Imatinib

S.No.	Conc.(µg/ml)	Area
1	50	2556.673
2	75	3699.822
3	100	4920.949
4	125	6191.981
5	150	7622.845

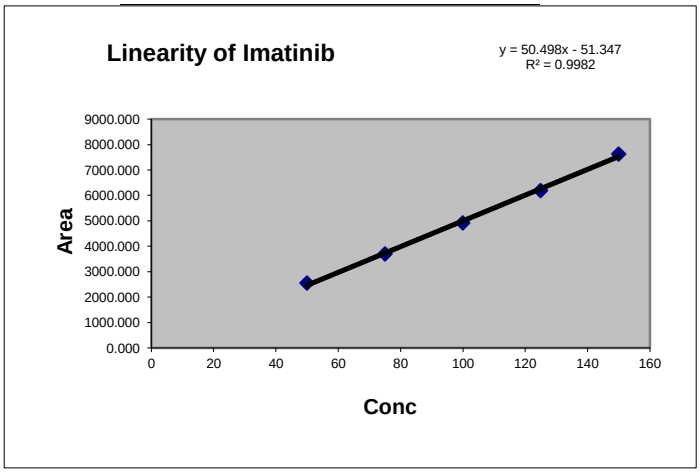

Fig. 20: Linearity graph of Imatinib

Acceptance criteria

The relationship between the concentration of Imatinib and area of Imatinib should be linear in the specified range and the correlation should not be less than 0.99.

Observation

The correlation coefficient for linear curve obtained between concentrations vs. Area for standard preparations of Imatinib is 0.998. The relationship between the concentration of Imatinib and area of Imatinib is linear in the range examined

since all points lie in a straight line and the correlation coefficient is well within limits.

ACCURACY

Accuracy of the method was determined by Recovery studies. To the formulation (pre analyzed sample), the reference standards of the drugs were added at the level of 50%, 100%, 150%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug is shown in table. To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 50%, 100% & 150%.

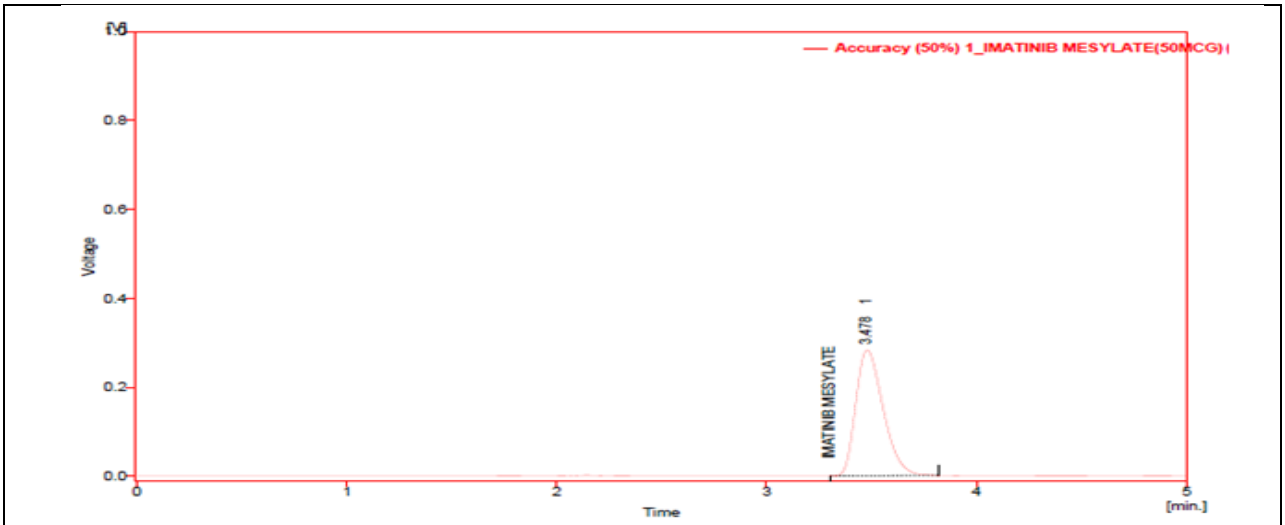

Fig. 21: Chromatogram of 50%recovery (injection 1)

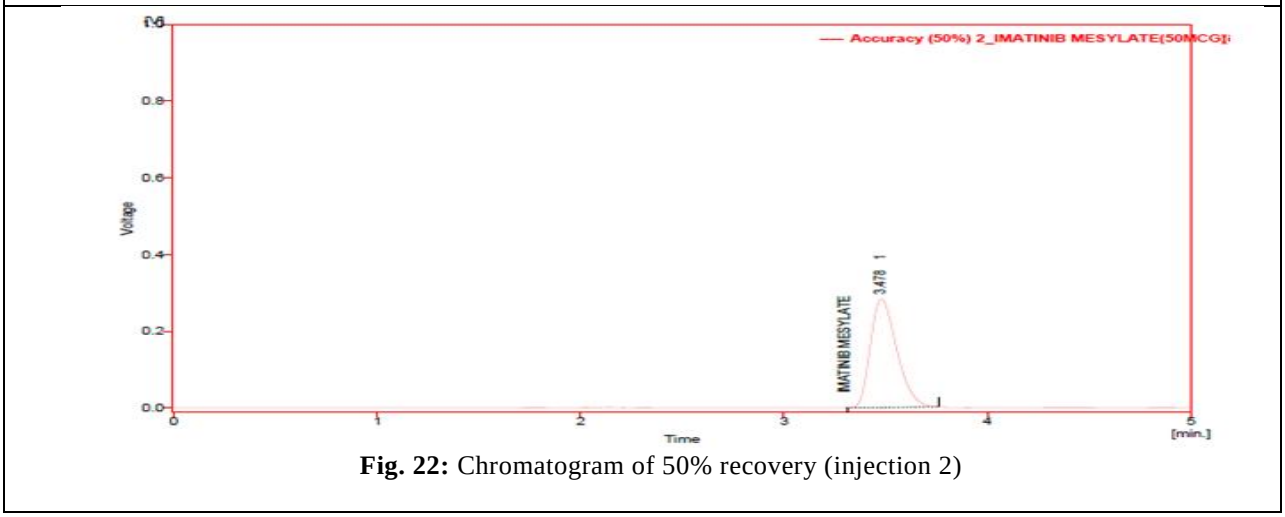

Fig. 22: Chromatogram of 50% recovery (injection 2)

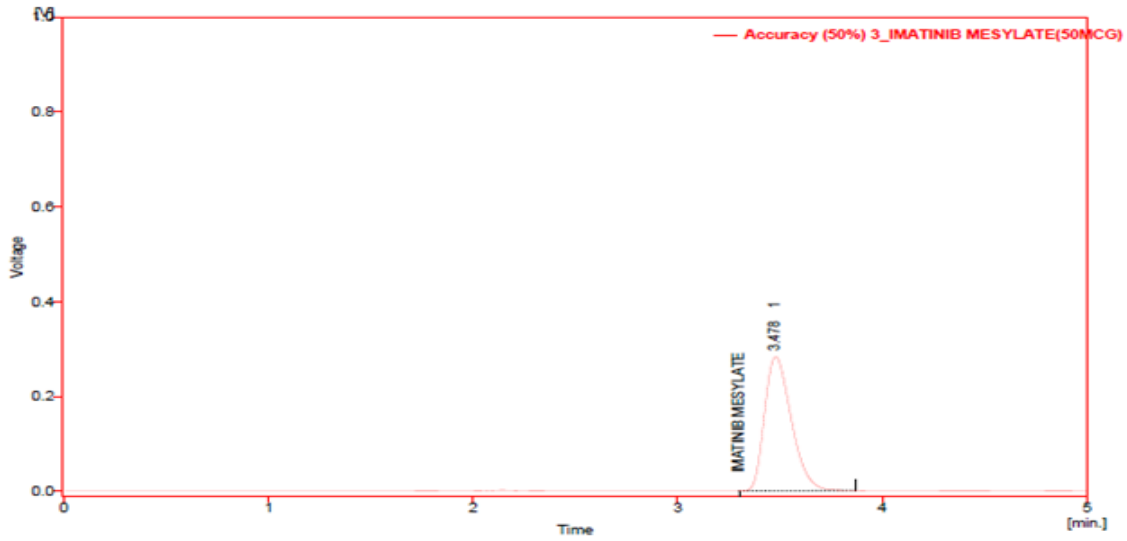

Fig. 23: Chromatogram of 50% recovery (injection 3)

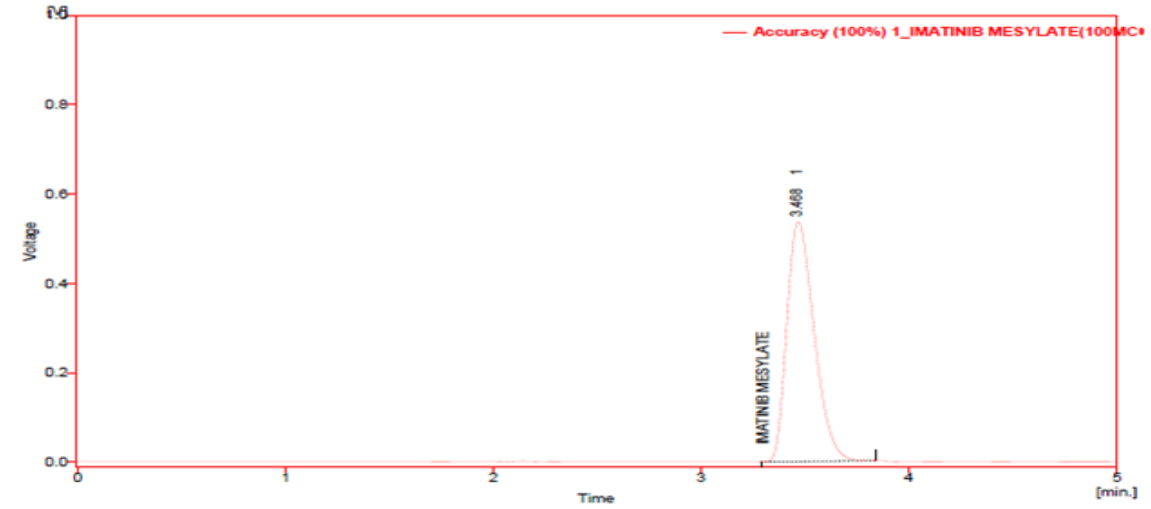

Fig. 24: Chromatogram of 100% recovery (injection 1)

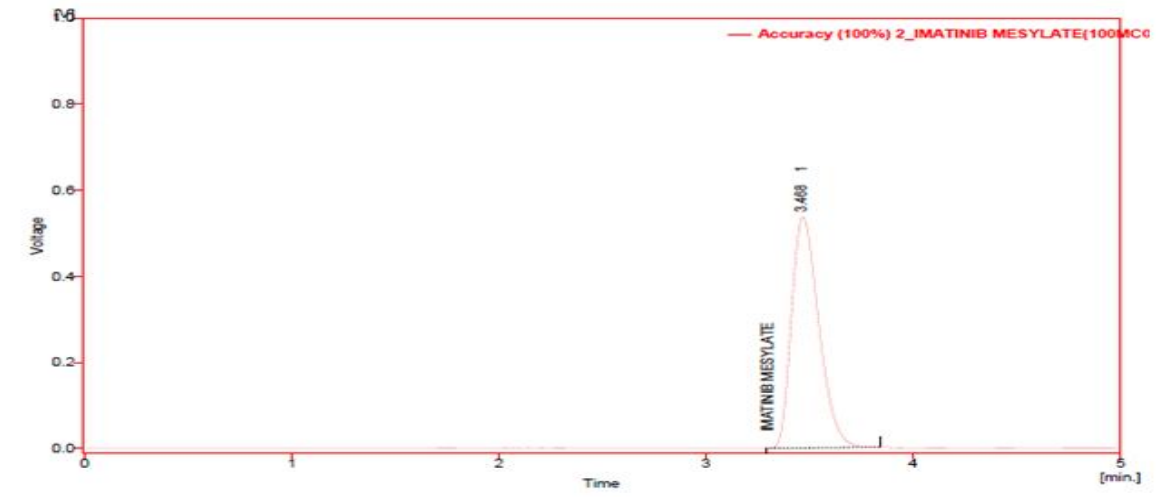

Fig. 25: Chromatogram of 100% recovery (injection 2)

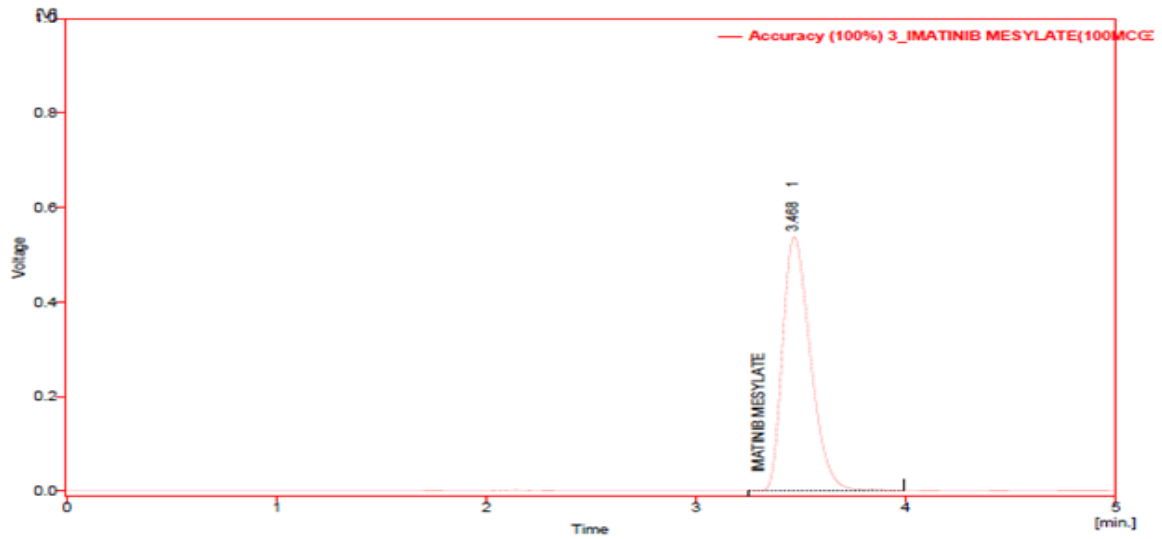

Fig. 26: Chromatogram of 100% recovery (injection 3)

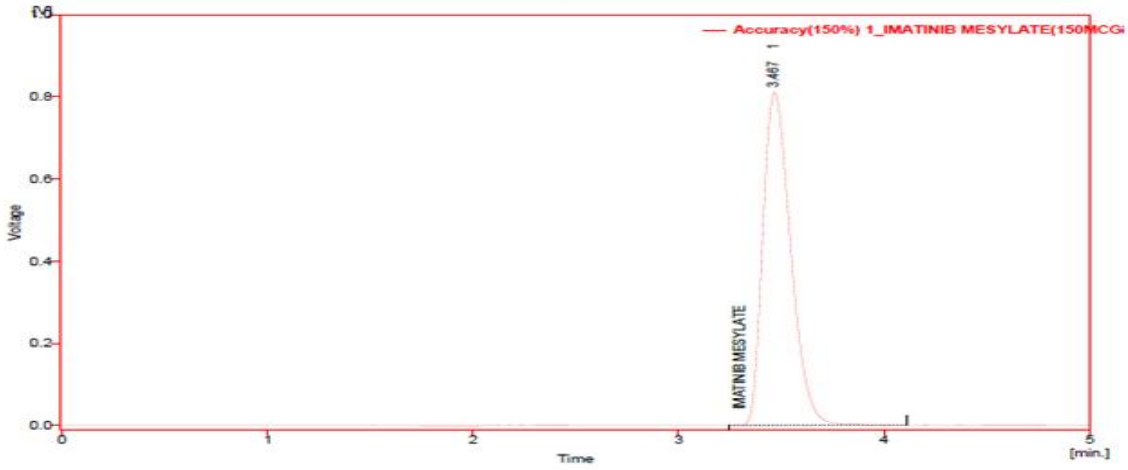

Fig. 27: Chromatogram of 150% recovery (injection 1)

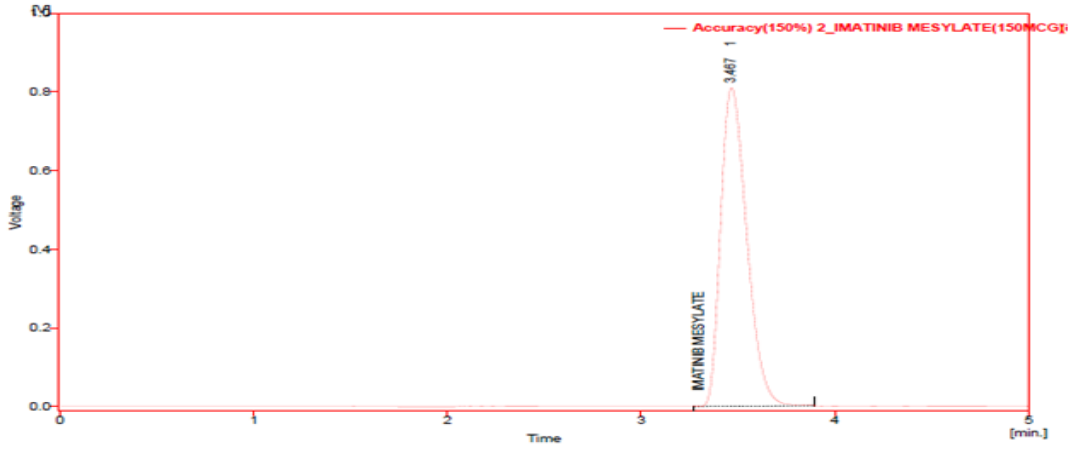

Fig. 28: Chromatogram of 150% recovery (injection 2)

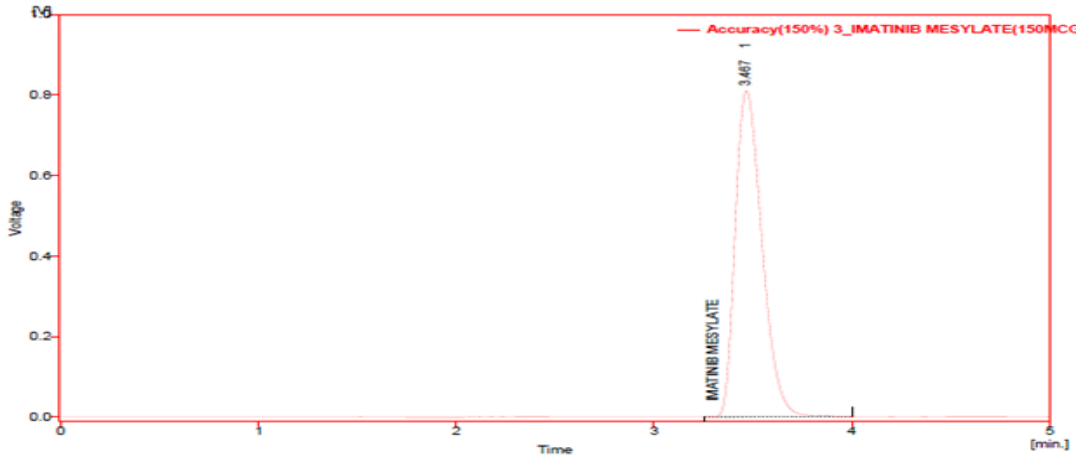

Fig. 29: Chromatogram of 150% recovery (injection 3)

Acceptance criteria

The % recovery of Imatinib should lie between 98% and 102%.

Table 5: Recovery results for Imatinib

Recovery level	Accuracy Imatinib					Average % Recovery
	Amount taken(mcg/ml)	Area	Average area	Amount recovered(mcg/ml)	%Recovery	
50%	50	2556.673	2554.563	49.37	98.75	
	50	2544.175				
	50	2562.841				
100%	100	4909.040	4922.828	98.80	98.80	98.99%
	100	4909.040				
	100	4950.404				
150%	150	7622.845	7605.162	149.13	99.42	
	150	7578.385				
	150	7614.255				

Observation

The percentage mean recovery of Imatinib is 98.99%.

PRECISION

Method precision

Prepared sample preparations of Imatinib as per test method and injected 5 times in to the column.

Acceptance criteria

The % Relative standard deviation of Assay preparations of Imatinib should be not more than 2.0%.

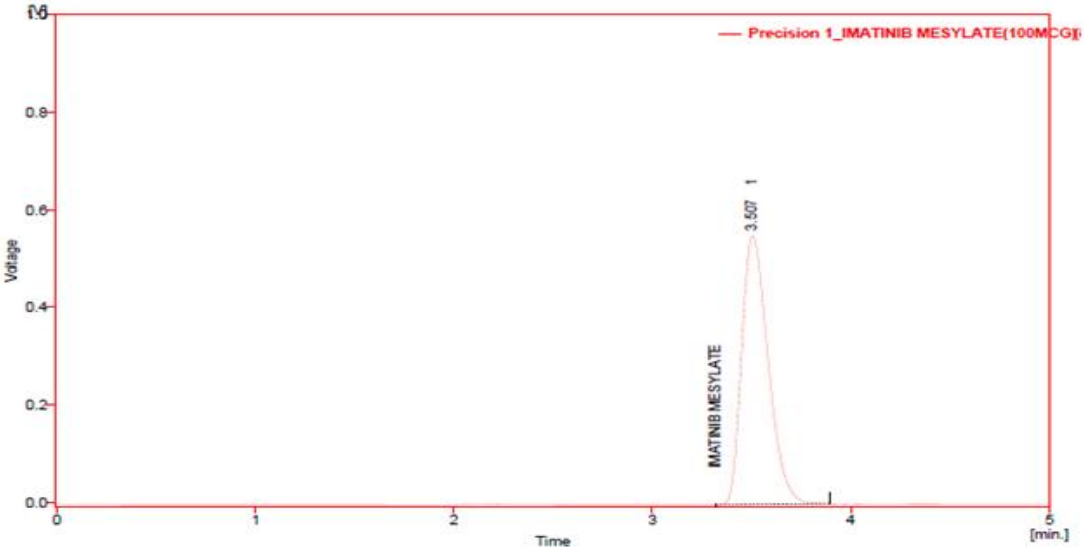

Fig. 30: Chromatogram of precision injection 1

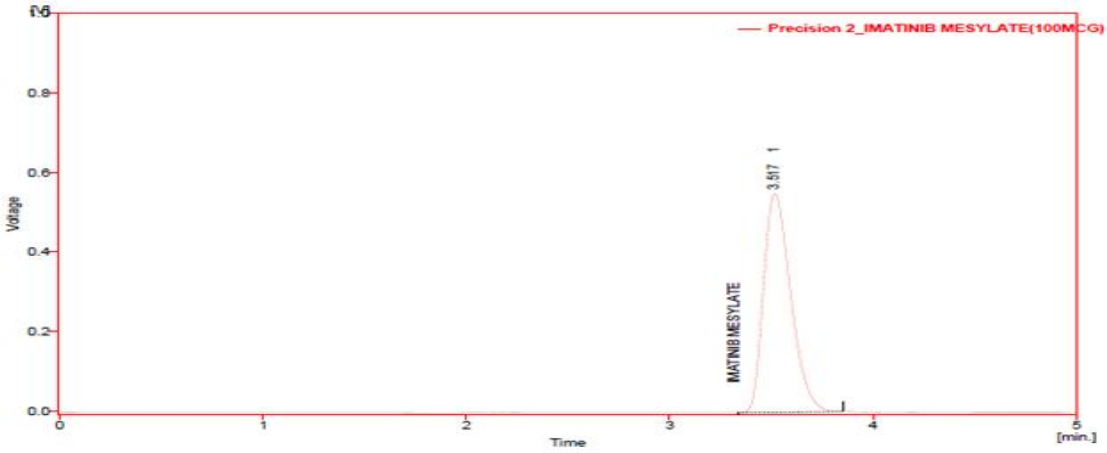

Fig. 31: Chromatogram of precision injection 2

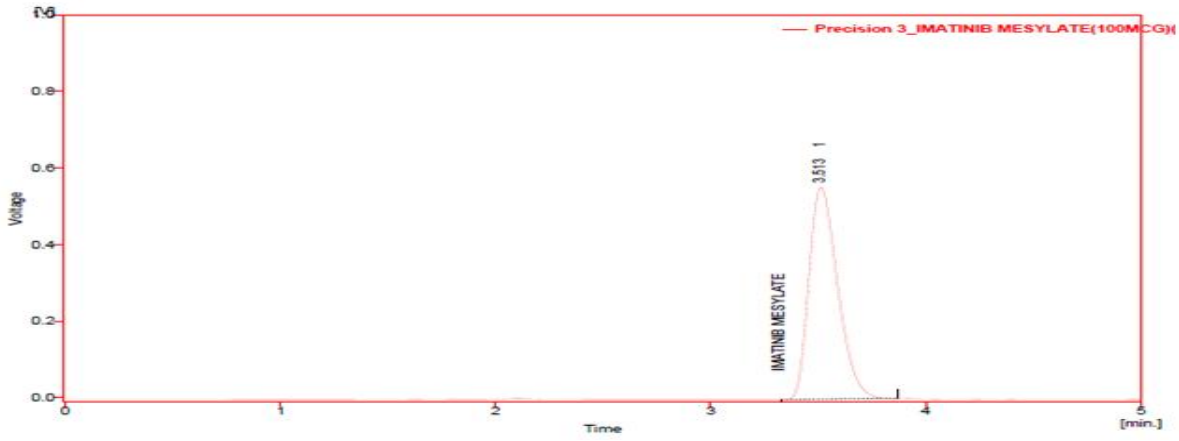

Fig. 32: Chromatogram of precision injection 3

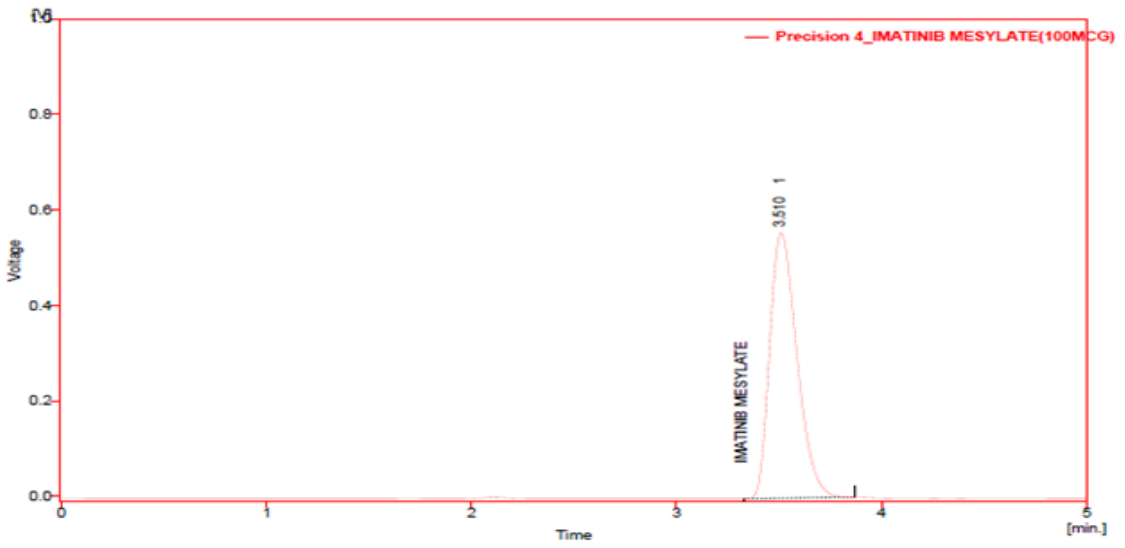

Fig. 33: Chromatogram of precision injection 4

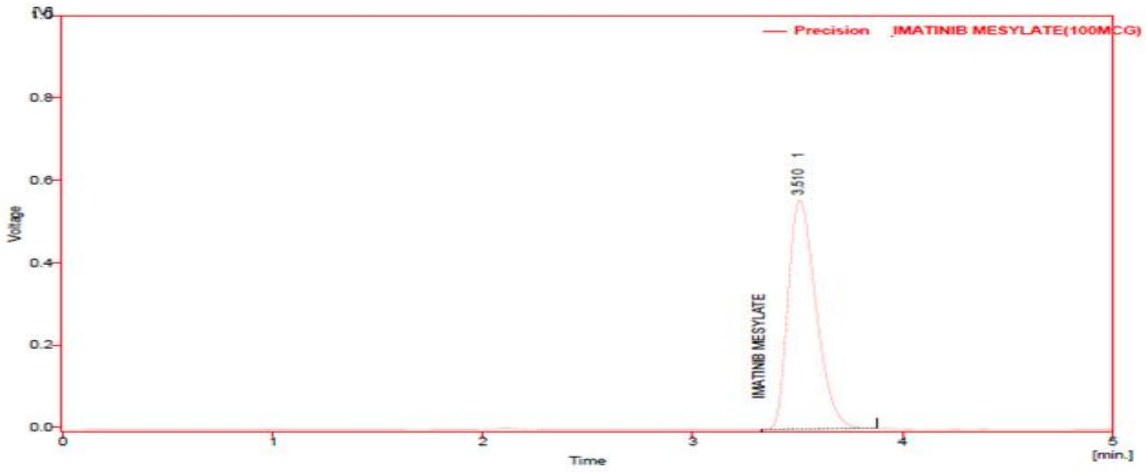

Fig. 34: Chromatogram of precision injection 5

Table 6: Results for Method precision of Imatinib

Imatinib		
S.No.	**Rt**	**Area**
1	3.507	5090.453
2	3.517	5107.271
3	3.513	5121.967
4	3.510	5128.367
5	3.510	5131.588
avg	3.5114	5115.929
stdev	0.0038	17.032
%RSD	**0.11**	**0.33**

Observation

Test results for Imatinib are showing that the %RSD of Assay results are within limits. The results were shown in table 8.5.7.

ROBUSTNESS

Chromatographic conditions variation

To demonstrate the robustness of the method, prepared solution as per test method and injected at different variable conditions like using different conditions like Temperature and wavelength. System suitability parameters were compared with that of method precision.

Acceptance criteria

The system suitability should pass as per the test method at variable conditions

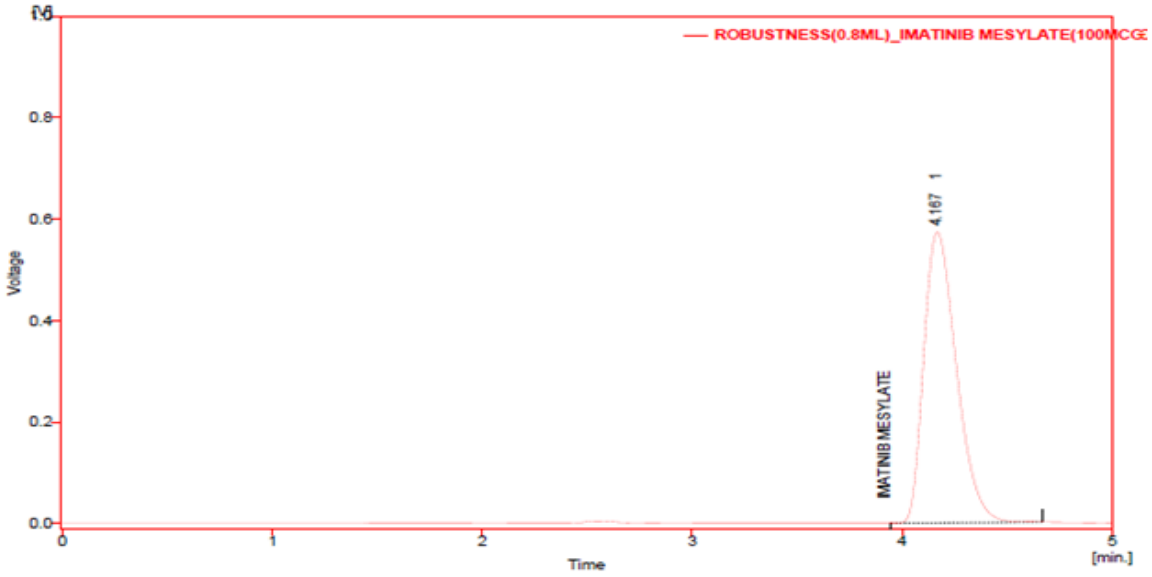

Fig. 35: Chromatogram of Imatinib Robustness (0.8 ml/min)

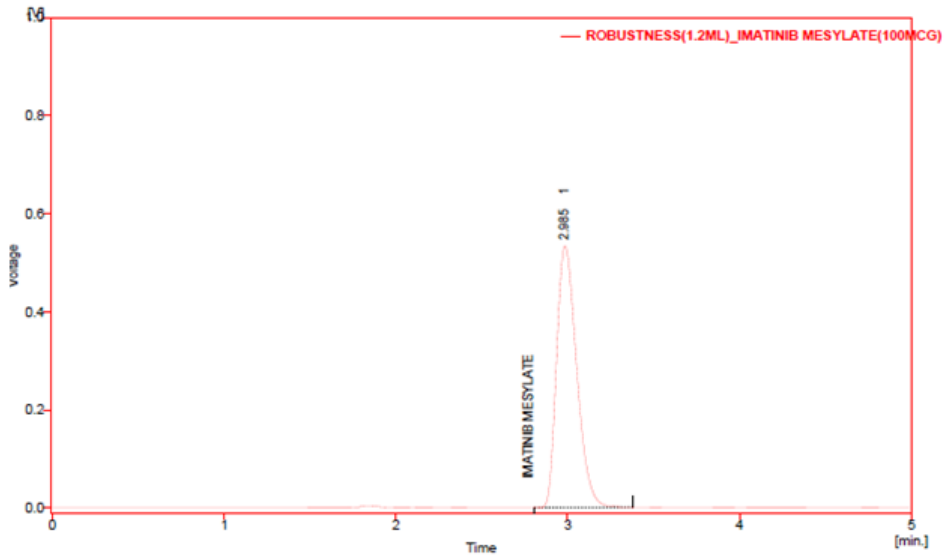

Fig. 36: Chromatogram of Imatinib for Robustness (1.2 ml/min)

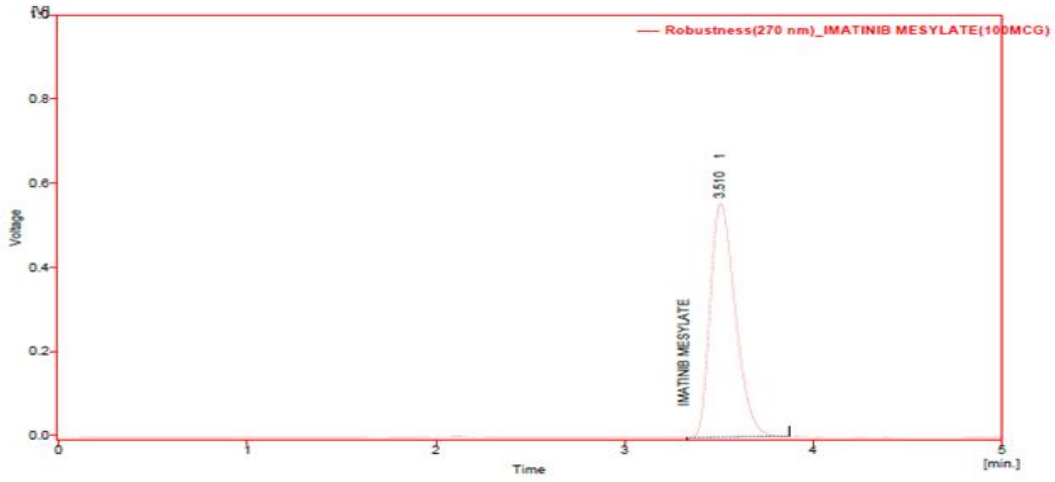

Fig. 37: Chromatogram of Imatinib for Robustness (270nm)

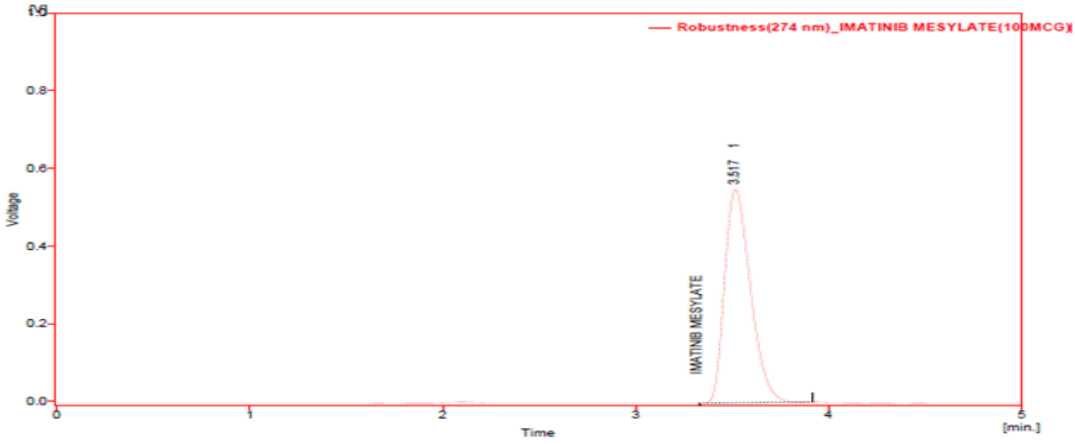

Fig. 38: Chromatogram of Imatinib for Robustness (274nm)

Table 7: Result of Robustness study

Parameter	IMATINIB	
	Retention time(min)	Tailing factor
Flow		
0.8ml/min	4.167	1.696
1.0 ml/min	3.507	1.607
1.2ml/min	2.985	1.593
Wavelength		
270nm	3.150	1.623
272nm	3.507	1.607
274nm	33.517	1.639

Observation

From the observation it was found that the system suitability parameters were within limit at all variable conditions.

Acceptance criteria

The % Relative standard deviation of Assay values between two analysts should be not more than 2.0%.

RUGGEDNESS

The ruggedness of the method was studied by the determining the analyst to analyst variation by performing the Assay by two different analysts

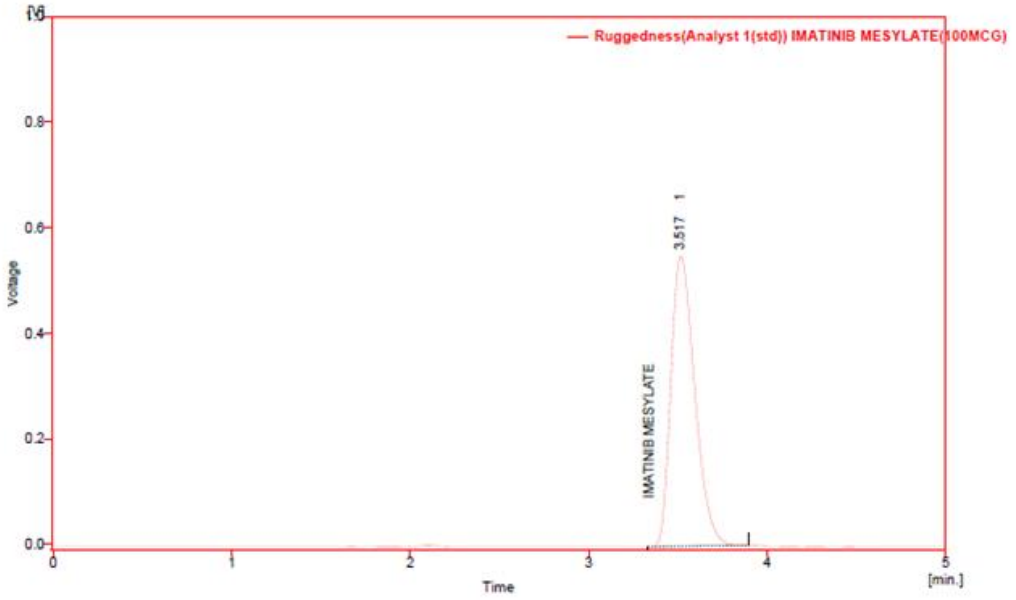

Fig. 39: Chromatogram of Analyst 01 standard preparation

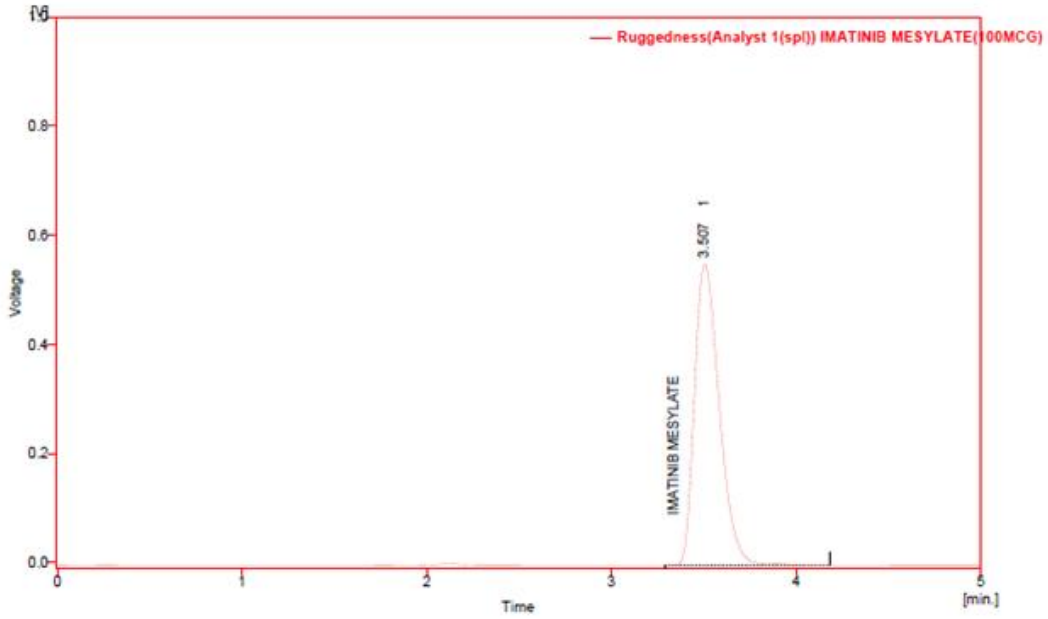

Fig. 40: Chromatogram of Analyst 01 sample preparation

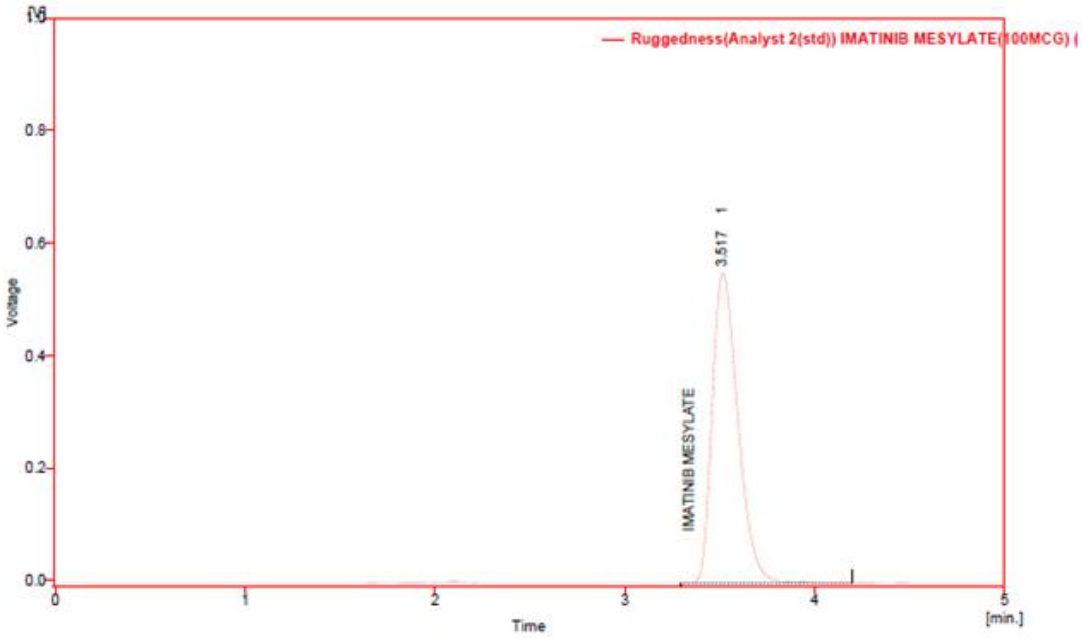

Fig. 41: Chromatogram of Analyst 02 standard preparation

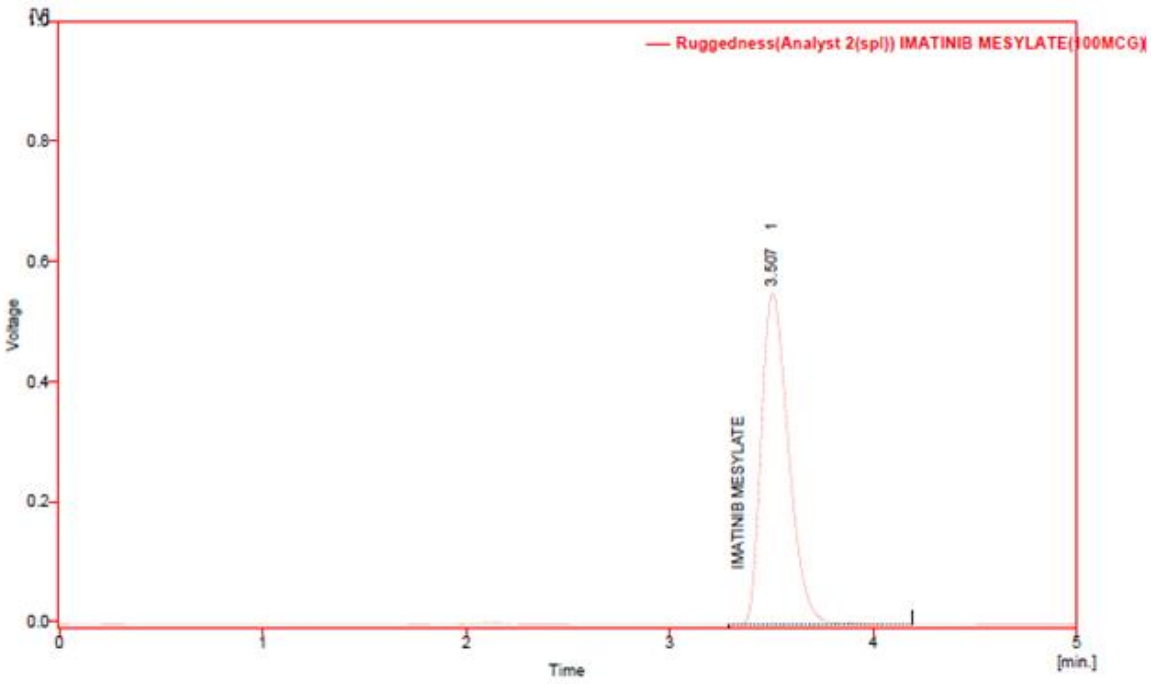

Fig. 42: Chromatogram of Analyst 02 sample preparation

Table 8: Results for Ruggedness

Imatinib	%Assay
Analyst 01	99.89
Analyst 02	100.56
%RSD	0.47

Observation

From the observation the %RSD between two analysts Assay values not greater than 2.0%, hence the method was rugged.

CONCLUSION

From the above experimental results and parameters it was concluded that, this newly developed method for the estimation of Imatinib was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories, bio-pharmaceutical and bio-equivalence studies and in clinical pharmacokinetic studies in near future.

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