ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF SOLID DOSAGE FORM OF ANTINEOPLASTIC DRUG IMATINIB MESILATE BY RP HPLC.

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ABSTRACT

A simple and sensitive High Performance Liquid Chromatographic method has been established and validated for Imatinib Mesylate in pharmaceutical dosage form, separation was performed on a C18, 150×4.6 mm, 5µ column in isocratic mode, with mobile phase containing a mixture of buffer: acetonitrile (72:28 v/v). The mobile phase was pumped at a flow rate of 1.0 ml/min and eluents were monitored at 265 nm. Linearity was found to be in the range of levels 80% to 120% and retention time was 3.63 min. The statistical validation parameters such as linearity, accuracy, precision, and specificity, limit of detection, limit of quantification were checked. The samples were prepared in water and the stability of Imatinib mesylate in aqueous solution at 30°C was studied. The results were satisfactory with good stability after 24 h at 30°C. The proposed method can be used for the related substances of Imatinib mesylate.

Key words: RP-HPLC, Validation, Imatinib mesylate, Acetonitrile

INTRODUCTION

Imatinib is an antineoplastic agent used to treat chronic myelogenous leukemia. Imatinib is a 2-phenylaminopyrimidine derivative that functions as a specific inhibitor of a number of tyrosine kinase (TK) enzymes[1, 2]. In chronic myelogenous leukemia, the Philadelphia chromosome leads to a fusion protein of Abl with Bcr (breakpoint cluster region) , termed Bcr-Abl. Imatinib is used to decrease Bcr-Abl activity[3-5]. It occupies the *TK* active site, leading to a decrease in activity. It inhibits proliferation and induces apoptosis in Bcr-Abl positive cell lines as well as fresh leukemic cells from Philadelphia chromosome positive chronic myeloid leukemia. It works by binding close to the ATP binding site of Bcr-Abl, locking it in a closed or self-inhibited conformation, and therefore inhibiting the enzyme activity of the protein semi-competitively [6-9].

Description

Imatinib Mesylate is a white to off-white to brownish or yellowish tinged crystalline powder with a molecular weight 589.7. It is soluble in aqueous buffers ≤ pH 5.5 but is very slightly soluble to insoluble in neutral/alkaline aqueous buffers. The drug substance is freely soluble to very slightly soluble in dimethyl sulfoxide, methanol and ethanol and is insoluble in n-octanol, acetone and acetonitrile[10].

Generic Name: Imatinib Mesylate

Brand Name: Gleevec

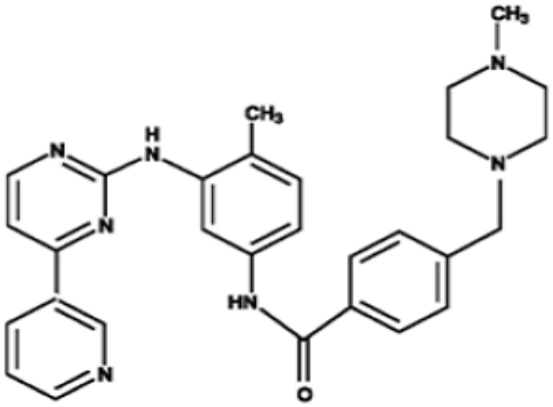

Fig 1: Chemical structure of Imatinib mesylate

MATERIALS AND METHODS

Chemicals and Reagents

Analytically pure sample of imatinib mesylate (GLEEVEC) with purity greater than 99% manufactured by Cipla Limited was used. Acetonitrile (HPLC grade) was obtained from Merck water (HPLC grade), potassium dihydrogen (AR grade), acetic acid (AR Grade) were obtained from SD Fine chemicals (Hyderabad, India).

Instrument

HPLC analysis was performed on Shimadzu LC-20AD Prominence Liquid Chromatograph comprising a LC-20AD pump, Shimadzu SPD- 20A Prominence UV-VISIBLE detector and a reverse phase C18 column, Enable C18G (250 X 4.6 mm; 5µ). A manually operating Rheodyne injector with 20 µL sample loop was equipped with the HPLC system. The HPLC system was controlled with "Lab solutions lite" software. An electronic analytical weighing balance (0.1 mg sensitivity, Shimadzu AY 220), digital pH meter (DELUX model 101), a sonicator (sonica, model 2200 MH) and UV-Visible Spectrophotometer (Shimadzu UV-1800 series, software-UV probe version 2.42) were used in this study.

Method Selection of Wavelength

Suitable wavelength for the HPLC analysis was determined by recording UV spectrums in the range of 200-400 nm for individual drug solutions of Imatinib Mesylate. Suitable wavelength selected was 269 nm (**Fig 2**).

Chromatographic conditions

The separation of the drugs was achieved on a reverse phase C18 column, Xterra C18 (100 X4.6 mm; 5µ), mobile phase consisting of a mixture of acetonitrile and phosphate buffer in the ratio of 80:20 v/v. The mobile phase was set at a flow rate of 0.6 ml/min and the volume injected was 20 µl for every injection. The detection wavelength was set at 269 nm.

Buffer Preparation The buffer solution was prepared by weighing 2.72 g of potassium dihydrogen phosphate and transferring to 1000 ml of HPLC water .The pH was adjusted to 3.0 with orthophosphoric acid. The buffer was then filtered through 0.45 µm filter under vacuum filtration.

Mobile phase Preparation The mobile phase was prepared by mixing 800 ml of acetonitrile HPLC (80%) and buffer 200ml (20%) and degassed in ultrasonic water bath sonicated for 5 minutes.

Diluent: Mobile phase

Preparation of the Imatinib Standard and Sample Solution:

Standard solution preparation:

Accurately 10 mg of Imatinib working standard was weighed and transferred into a 10 ml volumetric flask and about 7 ml of diluent was added and sonicated to dissolve it completely and made up to the volume mark with the same solvent. This was used as stock solution. Further 0.3 ml of the above stock solution was pipetted into a 10 ml volumetric flask and diluted up to the mark with diluent , mixed well and filtered through 0.45µm filter.

Sample Solution Preparation:

Five tablets of Imatinib were weighed and the average weight was calculated .The tablets were crushed and the sample equivalent to 10 mg of Imatinib was transferred into a 100 ml volumetric flask, 70 ml of diluent was added and sonicated to dissolve it completely and the volume was made up to the mark with diluent, mixed and filtered through 0.45µm filter. Further 0.3 ml of the above stock solution was pipetted into a 10 ml volumetric flask and diluted up to the mark with diluent, mixed well and filtered through 0.45 µm filter.

Procedure: 20 µl of the standard, sample was injected into the chromatographic system and the area for the Imatinib peak was measured and the %Assay was calculated using the formulae.

Assay % =

$$\frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{Avg\ Wt}{Label\ Claim} \times 100$$

Where:

AT = Peak Area of Imatinib obtained with test preparation

AS = Peak Area of Imatinib obtained with standard preparation

WS = Weight of working standard taken in mg

WT = Weight of sample taken in mg

DS = Dilution of Standard solution

DT = Dilution of sample solution

P = Percentage purity of working standard

RESULTS AND DISCUSSION

SYSTEM SUITABILITY

Preparation of Standard Solution

An accurate weighing of about 2.5 mg of the Imatinib and 25 mg of losartan potassium pure drug was done and transferred into two separate 25 ml clean, dry standard volumetric flask, 10ml of water was added, sonicated for 20 minutes, and the volume was made up with water. 5ml of the above solution was transferred into 100 ml clean, dry standard volumetric flask, and volume was made up with water (standard solution). This Standard solution was injected in six replicate injections and the suitability parameters were calculated which are given in **Table1**. The chromatograms were shown under the **Fig 3.1** and **Fig 3.2**

SYSTEM & METHOD PRECISION:

A. Preparation of stock solution:

Accurately 10 mg of Imatinib working standard was weighed and transferred into a 10 ml volumetric flask and about 7 ml of diluent was added and sonicated to dissolve it completely and volume was made up to the mark with the same solvent (Stock solution).

B. Preparation of 30 µg/ml solution:

Further 0.3 ml of the above stock solution was pipetted into a 10 ml volumetric flask and diluted up to the mark with diluent, mixed well and filtered through 0.45 µm filter.

Precision

The standard solution was injected for five times and the area for all six injections was measured in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

The results are summarized under the **Table 2.**

Accuracy

Accuracy was determined by means of recovery experiments, by addition of standard solution at different spiked levels (50-150%). At each level, three determinations were performed. The accuracy was calculated from the test results as the percentage of the analyte recovered by the assay. The amounts recovered, individual recovery and percent mean recovery were calculated. The accepted limits of recovery are 98% - 102% and all observed data are within the required range which indicates good recovery values and hence the accuracy of the method developed. The results are summarized under the **Tables 3.1-3.3**

Linearity

Standard solutions of Imatinib at different concentrations level (10µg/ml, 20 µg/ml 30 µg/ml 40 µg/ml and 50 µg/ml) were prepared. Calibration curves were constructed by plotting the concentration level of drugs versus corresponding mean peak area. The results show an excellent correlation between mean peak area and concentration level of drugs within the concentration range (10-50 µg/ml).The correlation coefficient was calculated, which meet the method validation acceptance criteria and hence the method is said to be linear in the range of 10-50 µg/ml. The represented data was shown in **Table 4** and the chromatograms were shown under the **Fig 5.**

Robustness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness of above system was tested and it was the variation in 10% organic composition in the mobile phase does not affect

the method significantly. The results are summarized under the **Table 5.1-5.2** and the chromatogram was shown in **Fig 6.1** and **Fig 6.2**

Limit of Detection (LOD) & Limit of Quantification (LOQ)

The LOD and LOQ for Imatinib was predicted based on the parameters of standard error of estimate and slope, which was calculated from linearity of the response data of Imatinib.Both LOD and LOQ values for Imatinib mesylate were calculated and shown in **Table 6** and the chromatograms were shown under the **Fig 7** and **Fig 8**

CONCLUSION

A reverse phase HPLC isocratic method developed has been validated as per ICH guidelines in terms of specificity, accuracy, precision, linearity, ruggedness, and robustness, limit of detection and limit of quantitation. The UV-Spectrophotometric method was rapid, simple and cost effective. A good linear relationship was observed for both the drugs between concentration ranges of 10 and 50 µg/ml with regression 0.999: 0.999, intercept 22201, slope 69828 for imatinib mesylate.However, RP-HPLC method may be considered more specific and sensitive than the Double Beam UV Spectrophotometric method, but RP HPLC method is more expensive requiring sophisticated chromatographic instrumentation for its performance. Both the developed methods may be recommended for routine and QC analysis of investigational drugs to provide simple, accurate and reproducible quantitative analysis.

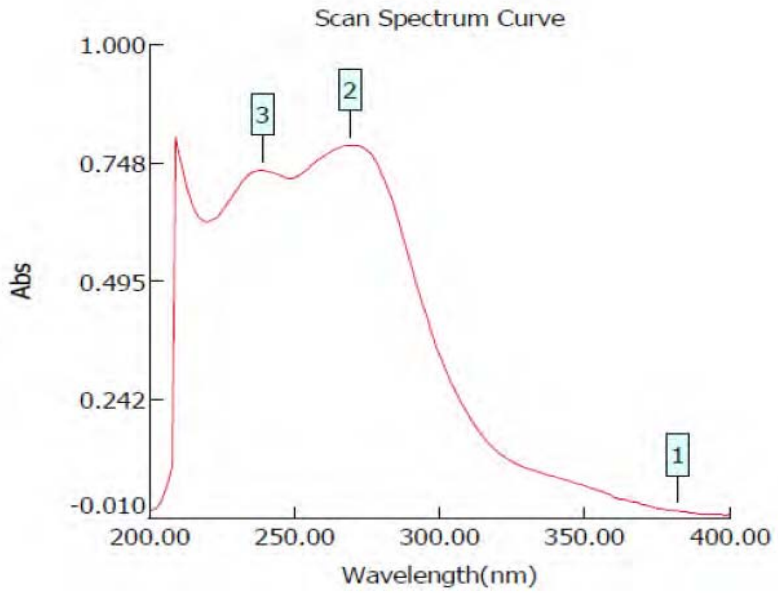

Fig 2: Spectra of Imatinib mesylate at 269 nm

Table1: Standard System Suitability

S.No	Name	RT	Area	%RSD	USP Plate Count	USP Tailing
1.	Imatinib mesylate(S1)	2.222	2121633	-	2801	1.75
2.	Imatinib mesylate(S2)	2.225	2103880	0.62	2831	1.75

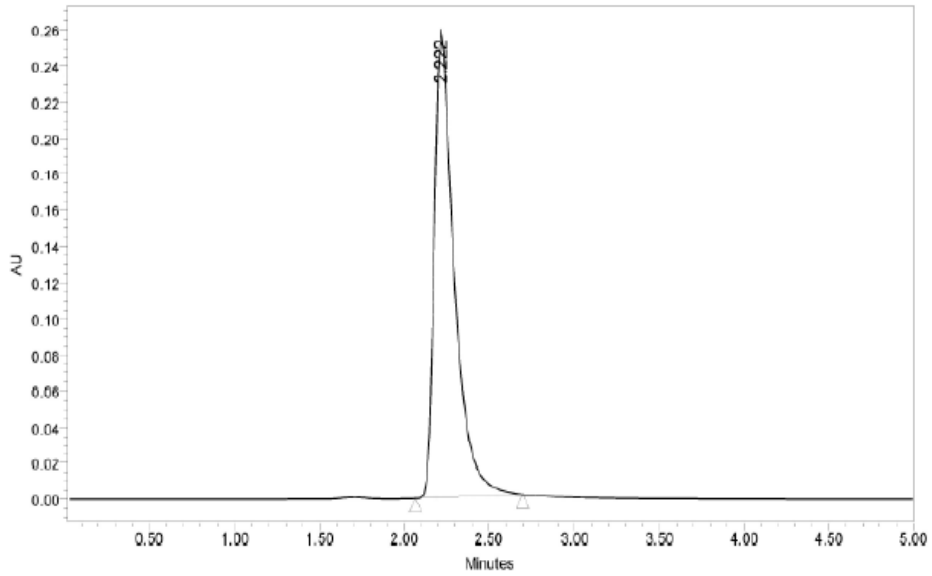

Fig3.1: Chromatogram of Imatinib mesylate [Standard 1]

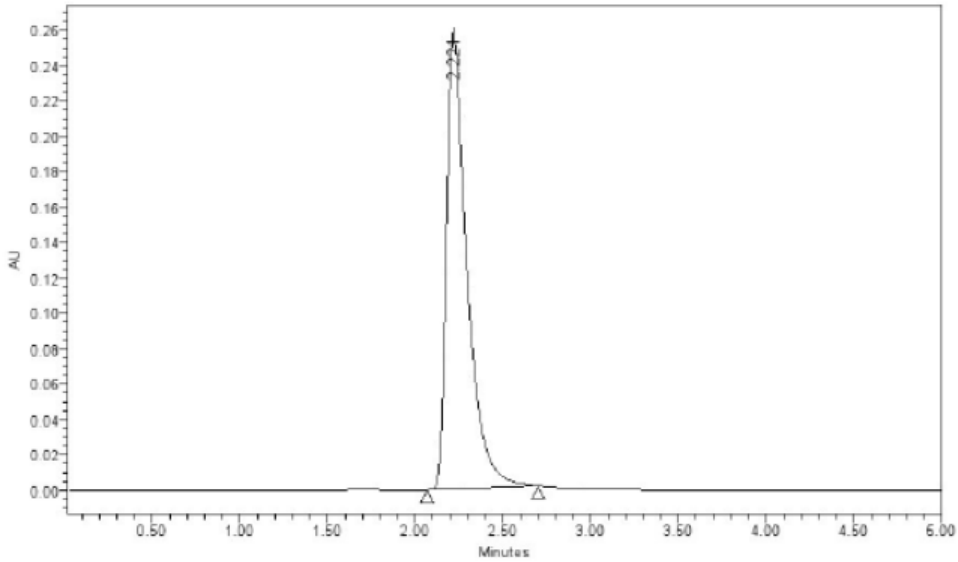

Fig 3.2: Chromatogram of Imatinib mesylate [Standard 2]

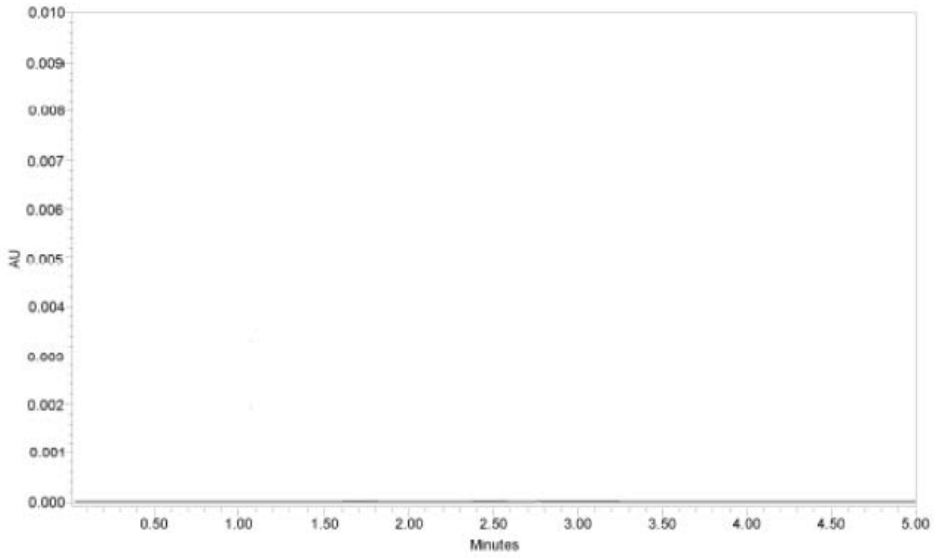

Fig 4: Chromatogram of Imatinib mesylate (Blank)

Table 2: Precision results for Imatinib mesylate

Injection	Area
P1	2116371
P2	2129623
P3	2131488
P4	2137589
P5	2146677
Average	2132350
Standard Deviation	11138.0
%RSD	0.52

Table 3.1: System Suitability data of Accuracy for Imatinibmesylate 50%

S. No	Accuracy Levels	Injection	RT	Area
1	50%	1	2.223	2136945
2	50%	1	2.224	2136941
3	50%	1	2.224	2152394
Mean			2142093	
Standard Deviation			7283.67	
%RSD			0.34	

Table 3.2: System Suitability data of Accuracy for Imatinibmesylate 100%

S.No	Accuracy Levels	Injection	RT	Area
1	100%	1	2.222	4119652
2	100%	1	2.222	4153490
3	100%	1	2.223	4135218
Mean			4136120	
Standard Deviation			13829.02	
%RSD			0.3343	

Table 3.3: System Suitability data of Accuracy for Imatinibmesylate 150%

S.No	Accuracy Levels	Injection	RT	Area
1	150%	1	2.221	6123856
2	150%	1	2.222	6159632
3	150%	1	2.222	6185317
Mean			6156268	
Standard Deviation			75611.4	
%RSD			1.22	

Table 4: System suitability data of Linearity for Imatinib mesylate

S.No	Vial	Sample Conc	Injection	RT	Area	Height(µV)
1	5	10µg/ml	1	2.227	777476	97036
2	6	20 µg/ml	1	2.225	1306651	161032
3	7	30 µg/ml	1	2.226	2155424	265481
4	8	40 µg/ml	1	2.227	2846823	355445
5	9	50 µg/ml	1	2.223	3498769	447164
Mean						2117029
Correlation Coefficient						0.999

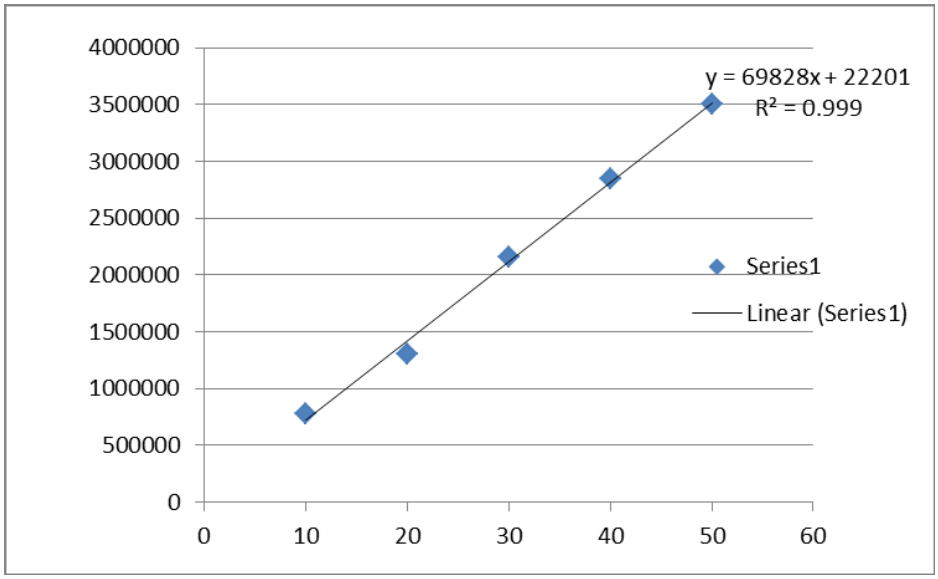

Fig 5: Calibration curve for Imatinib mesylate

Table 5.1: More Flow Variation

S.No	Flow Rate(ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.5	2889	1.65
2	0.6	2398	1.68
3	0.7	2808	1.75

Table 5.2: Flow variation when the Organic composition in the Mobile phase was varied from 90% to 70%

S.No	Change in Organic Composition in the Mobile phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10%	2534	1.60
2	*Actual	2398	1.68
3	10% more	2901	1.62

S.No	Peak name	RT	Area	Height	USP Plate Count	USP Tailing
1	Imatinib	2.016	1994666	258424	2651	1.58
2	Imatinib	2.203	2236098	285342	2901	1.62

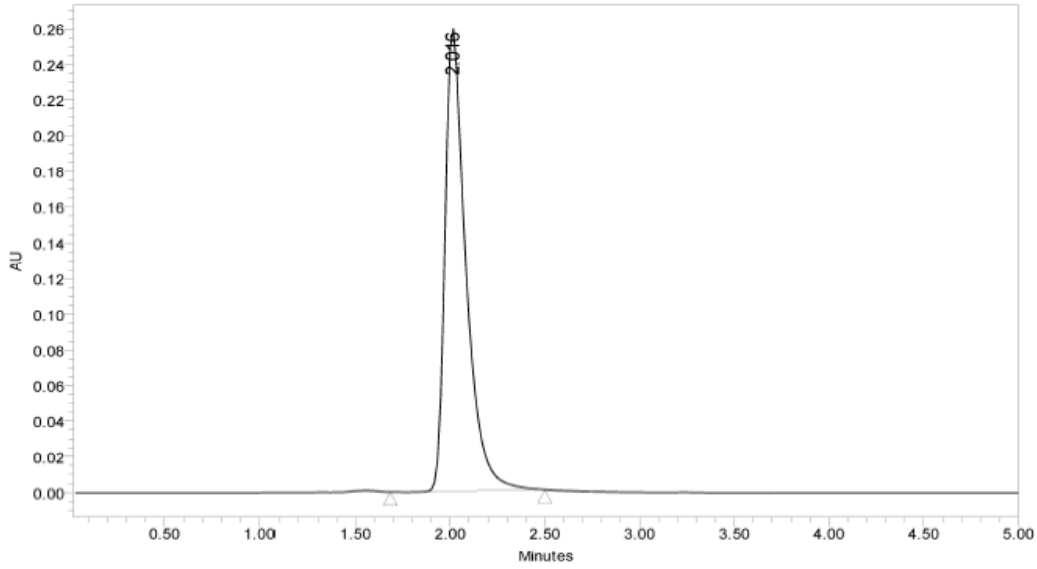

Fig 6.1: Chromatogram of Imatinibmesylate[more flow variation]

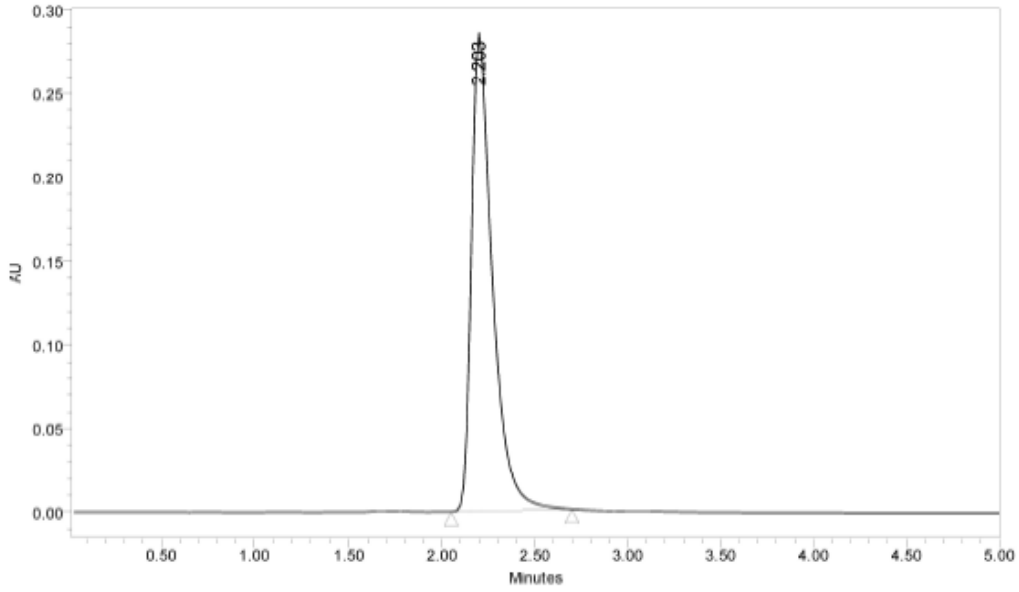

Fig 6.2: Chromatogram of Imatinibmesylate [Flow variation when the Organic composition in the Mobile phase was varied from90% to 70%]

Table 6: Limit of Quantification and Limit of Detection Values

S.No	Parameter	Peak Name	RT	Area	Height
1	LOD	Imatinib	2.224	1137	142
2	LOQ	Imatinib	2.224	3781	472

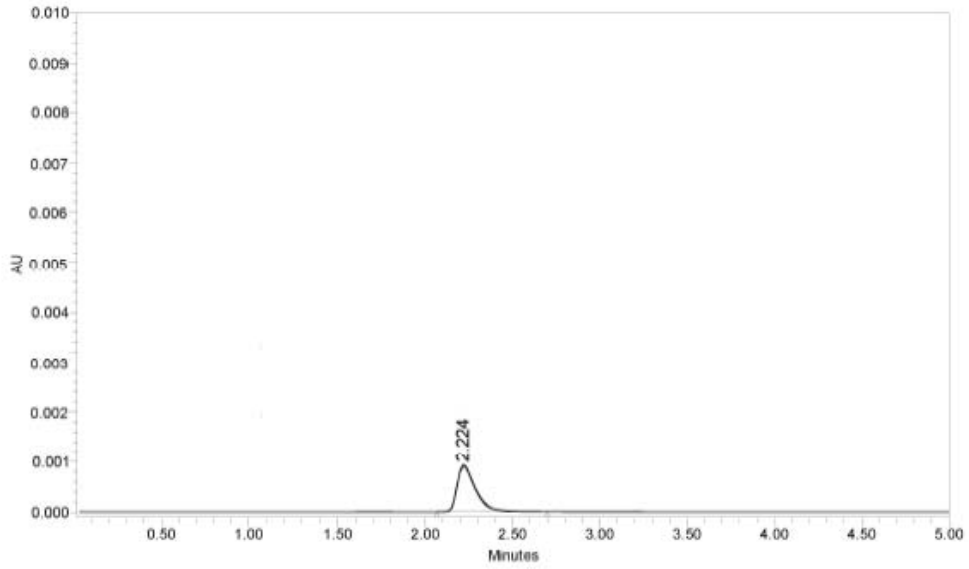

Fig7: Chromatogram of Imatinib mesylate[LOD]

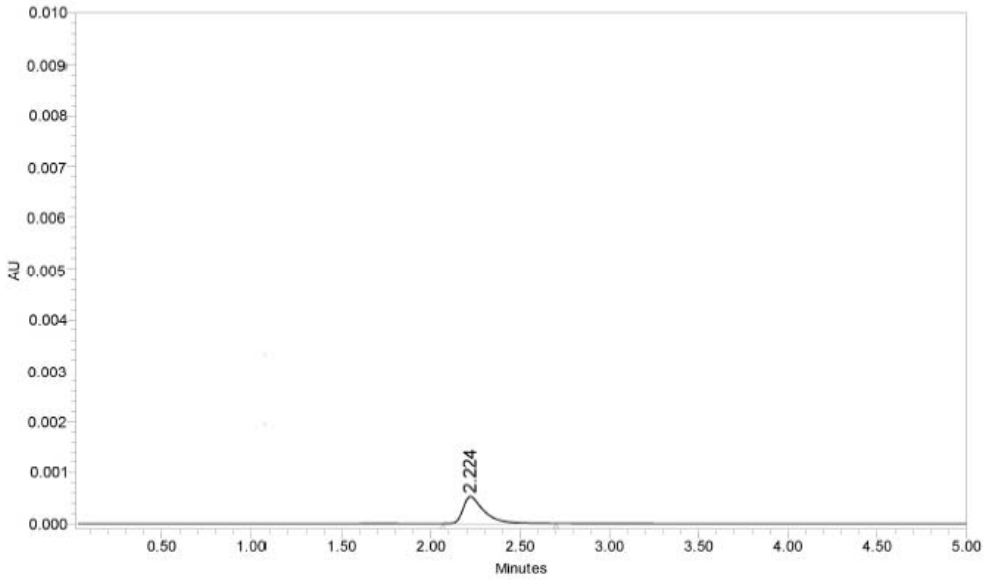

Fig 8: Chromatogram of Imatinib mesylate[LOQ]

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