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A Novel Rapid RP-HPLC Method Development and Validation of Imatinib Mesylate in Bulk and Pharmaceutical Dosage Form

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ABSTRACT

An accurate, simple, rapid, precise and economical RP- HPLC method has been developed for the rapid estimation of ImatinibMesylate in bulk and pharmaceutical formulation. The separation was achieved on Phenomenex C18 G column (250 x 4.6 mm i.d, 5 µm), using Methanol : 1-octanesulphonic acid (0.05M) at PH-8 with KOH 70:30 (v/v) as mobile phase, at a flow rate of 1.0 ml/min. Detection was carried out at 269 nm and drug eluted with a retention time of 5.548 min. Beer's law was obeyed in the concentration range of 2-12µg/ml with correlation coefficient 0.999. The method had been validated according to ICH guide lines for specificity, linearity, accuracy, precision, robustness, ruggedness, LOD and LOQ. The method was found to be specific, accurate, and precise, robust, rugged and sensitive. The proposed method was convenient for quantitative routine analysis and quality control of ImatinibMesylate in bulk and pharmaceutical dosage form.

Key words: Imatinib Mesylate , RP-HPLC, Validation, 1-Octanesulphonic acid.

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INTRODUCTION

ImatinibMesylateis a cancer medication prescribed to treat leukemia and gastrointestinal tumors. It operates by inhibiting proteins associated with cancer cell growth in order to relieve symptoms, prevent the spread of cancer cells, and aid other treatments. ImatinibMesylate is one of the newest anticancer drugs in the market and was one of the first drugs to be pushed through Food and Drug Administration's (FDA) fast track designation for approval. The drug is designed to inhibit tyrosine kinases such as Bcr-Abl and is used in the treatment of chronic myeloid leukemia (CML) and gastrointestinal stroma tumors**.**

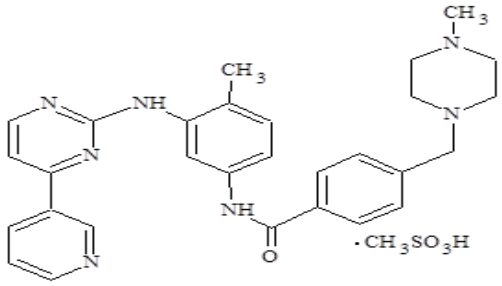

Figure 1: Chemical structure of ImatinibMesylate.

The Chemical name of ImatinibMesylate is 4-4[(4-methyl-1- piperazinyl) methyl]-N-[4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl] amino] phenyl] – benzamide mono methane sulfonate. It has a molecular formula of C29H31N7O.CH4O3S and a molecular weight of 589.71. It has the structural formula (Figure 1). ImatinibMesylate is a white crystalline powder which in freely soluble in distilled water, 0.1 NHcl, methanol and sparingly soluble in dimethyl ether [1.]

Literature Survey revealed that the drug has been estimated by UV spectrophotometric [2-6],HPLC methods[7-17], and HPTLC method[18] has been reported so far.

The aim of present work was to develop and validate a novel, rapid, simple, precise, sensitive and specific RP-HPLC method for estimation of ImatinibMesylate in its bulk and tablet dosage form.

MATERIALS AND METHOD

Instrument:

Chromatographic separation was performed on a Shimadzu LC-10ATVP HPLC system comprising a Shimadzu spd10A UV-Vis detector, Shimadzu LC-10ATVP pump and enable Phenomenex C18 G column (250 x 4.6 mm i d, 5µm particle). A manually operating Rheodyne injector 50µl (20µl injection valve) was used for injecting sample and standard solution. Baseline chromatography Data system N2000 software was used to collect and process the data.

Chemicals and Reagents:

ImatinibMesylate pure form was obtained as gifted sample from pharma industry and its pharmaceutical dosage form Imatib 100 Tablets labelled claim 100 mg were purchased from local pharmacy manufactured by CIPLA LTD. Methanol of HPLC grade(Merck India), Water of HPLC grade(Merck India) and 1-Octanesulphonic acid of Analytical grade (SD Fine Chemicals) were used.

Selection of mobile phase:

Based on sample solubility, stability and suitability various mobile phase compositions were tried to get a good resolution and sharp peaks. The standard solution was run in different mobile phases. From the various mobile phases, Methanol:1-Octanesulphonic acid (0.05M) at PH-8 with potassium hydroxide(70:30)was chosen with detection wavelength 269 nm, since it gave sharp peak with good symmetry within limits.

Buffer preparation: 1-octanesulphonic acid(0.05M)

0.117g 0f 1-Octanesulphonic acidis dissolved in 100ml of water and filtered through Millipore 4.5micron filter ,adjust the PH-8 by using KOH.

Preparation of mobile phase

Prepare a mixture of 300 volumes of buffer preparation and 700 volumes of methanol, and Sonicate for 10min.

Diluent: Methanol.

Chromatographic condition:

Table 1: The optimized chromatographic conditions of the developed method

Mobile phase	Methanol:1-Octanesulphonic acid at PH-8 with potassium hydroxide[70:30 v/v]
Stationary phase	PhenomenexC18GColumn dimension ID : 250× 4.6 mm,5µ
Wavelength	269 nm
Run time	10min
Injector	Rheodyne 50µl
Flow rate	1.0 ml per min
Injection volume	20 µl
Temperature	Ambient
Mode of operation	Isocratic elution

Preparation of standard stock solution

Weigh accurately about 25mg of ImatinibMesylate pure drug and then transferred into 25ml volumetric flask and diluted with methanol up to the mark and sonicated for 5 min to dissolve it completely (stock solution-1). From the above solution pipette out 2.5ml into 25ml volumetric flask add methanol and made up to the mark with methanol (stock solution-2),from this solution

pipette out 0.2, 0.4, 0.6 , 0.8, 1.0, and 1.2ml into 10ml individual volumetric flask and add methanol up to the mark , this gives 2, 4, 6, 8, 10, 12 µg/ml concentrations.

Preparation of sample solution

Twenty tablets were weighed and powdered, the tablet powder equivalent to 100mg of Imatinibmesylate was transferred into100ml volumetric flask then it was diluted with methanol and made upto mark and the solution was filtered through Whatman filter paper no.41. From this pipette out 10 ml in a 100ml volumetric flask and make up the volume up to the mark with methanol. From this solution pipette out 0.2 ml in 10ml volumetric flask and make up the volume with methanol, this gives 2µg/ml concentrations.

Flow rate selection

Different flow rates in between 0.50 to 1.50 ml /min were studied. A flow rate of 1.0 ml /min gave an optimal signal to noise ratio with a reasonable separation time.

Wavelength selection

Detection was carried out at 269nm it shows maximum absorbance in methanol, since it gave sharp peak with good symmetry within limits at 269nm, as shown in Figure 2.

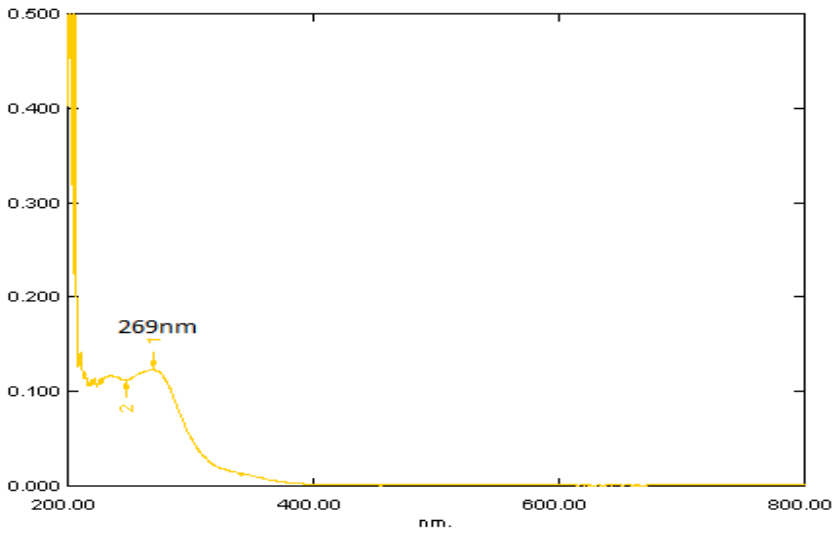

Figure 2: UV-absorbance of Imatinib Mesylate Shows maximum absorbance at269nmMethanol as a solvent

RESULTS AND DISCUSSION

System suitability

20µl of the standard solution was injected under optimized chromatographic conditions to evaluate the suitability of system. Parameters such as number of theoretical plates (N) ,tailing factor,

resolution(R), retention time(RT) were determined . The values of system suitability parameters were shown in Table- 2, it indicates good performance of system (Figure 4 and table 3).

Table 2: system suitability parameters

System suitability parameters	Results
Retention time	5.548
Area	3397380.500
Theoretical plate number	2522.83
Tailing factor	1.344

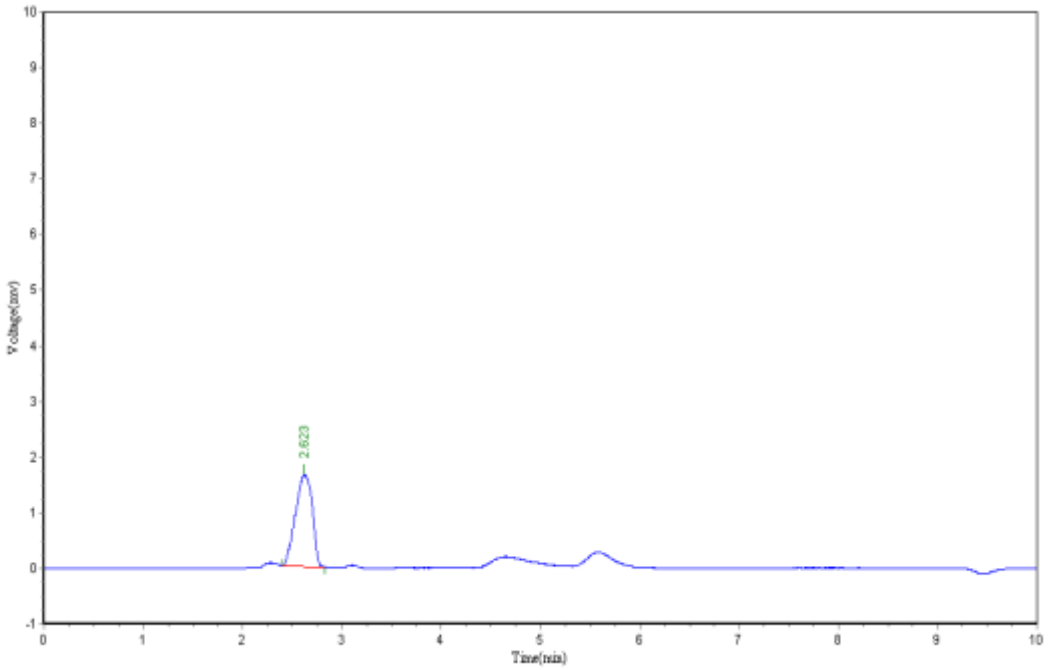

Figure 3: Chromatogram of blank (methanol)

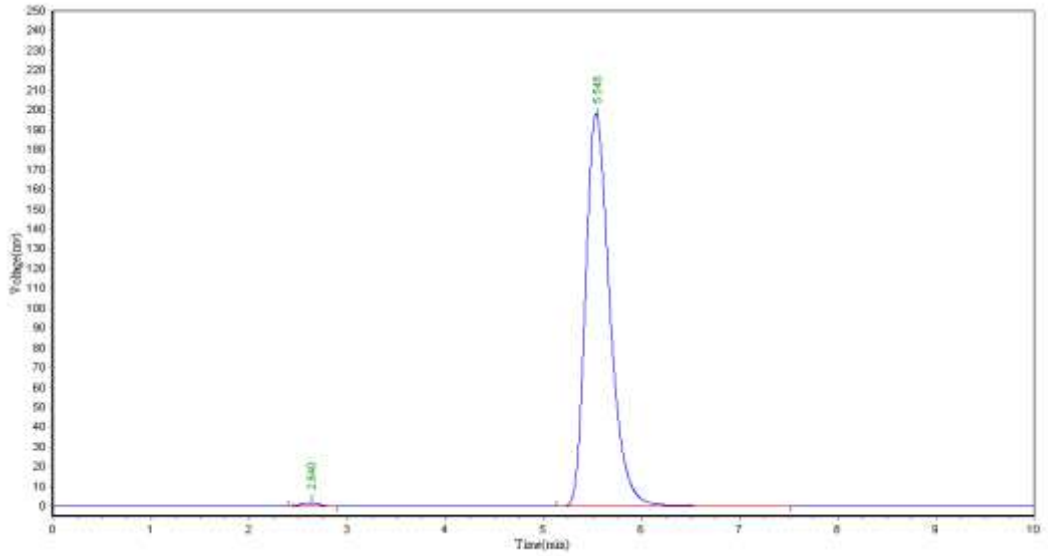

Figure 4: Chromatogram of Imatinibmesylate

Table 3: Results

Peak No.	Peak ID	Ret Time	Height	Area	Conc
1	Blank	2.640	1613.339	22312.100	0.6525
2	IM	5.548	197297.844	3397380.500	99.3475
Total	198911.183			3419692.600	100.0000

System Evaluation

Peak No.	Peak ID	Ret. Time	Half-Peak Width	Theoretical levels	Resolution	Tail Factor	Asymmetry
1	Blank	2.640	0.220	797.760	0.000	0.942	0.808
2	IM	5.548	0.260	2522.831	6.059	1.344	1.564

Method validation

The method is validated according to the ICH guidelines[19-21].

Specificity

Specificity was checked for the interference of excipients in the analysis of sample solution and was determined by injecting sample solution with added excipients under optimized chromatographic conditions to demonstrate separation of Imatinib Mesylate from excipients. There is no interference of excipient peak on the peak of Imatinib Mesylate indicating the high specificity of method.

Linearity and Range

Calibration curve was plotted for different concentrations of working standards prepared from standard drug solution of pure drug, shown in Figure 5 and showed linearity over a concentration range of 2-12µg/ml shown in Table 4, along with regression parameters in Table 5. Each calibration was injected three times. The calibration curve was performed in triplicate

Table 4: Calibration curve

Sl.no	Concentration(mcg/ml)	Peak Area
1	0	0
2	2	70303.383
3	4	139137.297
4	6	203656.135
5	8	269957.3126
6	10	335548.385
7	12	405611.437

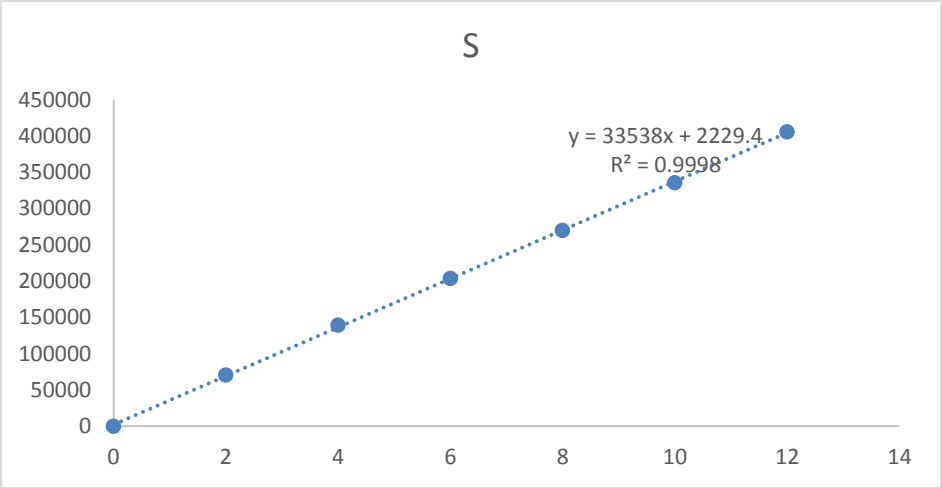

Figure 5: Linearity graph of Imatinib mesylate

Table 5: Regression parameters table for Imatinib Mesylate

Regression Parameter	Imatinib Mesylate
Regression Equation*	Y=33538x+2229.4
Slope (b)	33538
Intercept (a)	2229.4
Correlation Coefficient (r2)	0.999

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Six solutions of same concentrations were prepared and area was noted. The results were shown in terms of %RSD were within the limits, shown in Table 6.

Table 6: Precision data

Precision	Intra-day	Inter-day		
		Day-1	Day-2	Day-3
Mean area	203175.6736	202695.10	202405.7343	204296.8
Standard deviation	1907.585	1082.1	2236.65	1364.416
%RSD	0.9388848	0.533903	1.105034	0.66786

Each determination is average of six replicates, RSD indicates relative standard deviation

Accuracy

Accuracy (recovery) of the method was obtained by spiking 80, 100 and 120% of Imatinib Mesylate working standard concentrations, in which the amount of marketed formulation was kept constant and the amount of pure drug was varied. Solutions were prepared in triplicates and accuracy was indicated by % recovery which was between 99.53 to 100.82%. The results were shown in Table-7.

Table 7: Accuracy data

% Spiked levels	Amount of Formulated drug added In µg/ml	Amount of pure drug added in µg/ml	Amount found Inµg/ml	% recovery	Statistical parameters
80	2	2.8	4.81	100.2	Mean =100.82
	2	2.8	4.88	101.66	SD=0.75162
	2	2.8	4.83	100.62	%RSD=1.737
100	2	4	5.86	98.66	Mean =99.66
	2	4	5.92	98.65	SD=1.73201
	2	4	6.11	101.66	%RSD=1.0707
120	2	5.2	7.1	99.1	Mean =99.53
	2	5.2	7.13	99.07	SD=0.770909
	2	5.2	7.23	100.42	%RSD=0.774594

Robustness

Robustness was carried by varying two parameters deliberately from the optimized chromatographic conditions like mobile phase composition and flow rate and. The %RSD was found to be <2, shown in Table- 8.

Table 8: Robustness data

Parameters	Flow rate and Mobile phase 0.95ml 72:28	Flow rate and Mobile phase 1.05ml 68:32
Mean area	207181.849	208066.4
Standard deviation	3766.08568	2494.278
%RSD	1.817	1.198

Ruggedness

Ruggedness was determined between different analysts. The value of %RSD was found to be <2, showed ruggedness of developed analytical method. The values were shown in Table-9.

Table 9: Ruggedness data

Analyst	Analyst-1	Analyst-2
Mean area	207181.8177	202405.7343
Standard deviation	3766.386	2236.653
%RSD	1.817	1.105

RSD indicates relative standard deviation

Limit of detection and Limit of quantitation

The LOD and LOQ of the present method were calculated based on standard deviation of the response and slope of linearity curve. LOD and LOQ values of ImatinibMesylate were found to be 0.030154µg/ml and 0.30514µg/ml.

CONCLUSION

Thus, the developed method was found to easy, novel, simple, accurate, precise, selective and economical for the routine estimation of ImatinibMesylate in bulk and pharmaceutical dosage form.

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