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Method Development and Validation of Rp-Hplc Method for Estimation Of Imatinib Mesylate In Pure And Pharmaceutical Dosage Form

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ABSTRACT:*An accurate,precise,simple and economical RP-HPLC method has been developed for the rapid estimation of ImatinibMesylate in pure and pharmaceutical formulation. The separation was achieved on C_{18} (cosmosil) , column (250 × 4.6 mm i.d 5 µm) using Acetonitrile : O-Phosphoric acid (0.1 % v\v) 60:40 as mobile phase, at a flow rate of 1.0ml ,min.Detection was carried out at 264nm & drug eluted with a retention time of 6.08 min.Beer's law was obeyed in the concentration range of 10-50µg\ml. with correlation coefficient 0.999. The method has been validated according to ICH guidelines for linearity, accuracy, repetability,precision, robustness,ruddgedness, LOD & LOQ. The method was found to be specific,accurate & precise,robust & sensitive. The proposed method was convenient for quantitative routine analysis of ImatinibMesaylate in bulk & pharmaceutical dosage form.*
Keywords:*ImatinibMesylate, HPLC, Acetonitrile, Ortho phosphoric acid (OPA).*

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I. INTRODUCTION-

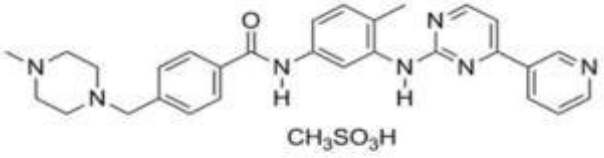

Figure 1:Chemical Structure of Imatinib

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[1].

It has a molecular formula of $C_{29}H_{31}N_7O.CH_4O_3S$ and a molecular weight of 589.71 g/mol. It has the structural formula (Fig.1). ImatinibMesylate is a white crystalline powder which in freely soluble is distilled water, 0.1 N HCl, methanol and sparingly soluble in dimethyl ether .

ImatinibMesylate is 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.

Objectives

To develop and validate a reverse phase high performance liquid chromatoghraphic method for stimultenouses estimation of imatinibdosageform.

II. MATERIALS AND METHODS

Instrument

Chromatographic separation was performed on a HPLCYounglin(S.K) isocratic System UV Detector C system equipped with a C18 G column (250 x 4.6 mm i.d, 5 µm particle), binary pumps, degasser, Variable

wave length detector and Rheodyne injector with 20 µl loop volume. 'LC solution' software was used to collect and process the data.

Chemicals & Reagents

ImatinibMesylate pure form was obtained as gifted sample from pharma industry and its pharmaceutical dosage form GLEEVAC Tablets labelled claim 400 mg were purchased from local pharmacy. Acetonitrile of HPLC grade(Merck India), Water of HPLC grade(Merck India) and o-Phosphoric acid of Analytical grade (SD Fine Chemicals) were used.

Preparation of mobile phase

o-Phosphoric acid (0.1% v/v) was prepared by taking 0.1 ml of analytical grade o-Phosphoric acid in 100 ml volumetric flask and the volume was made upto the mark with HPLC grade water.

The mobile phase Acetonitrile and o-Phosphoric acid (0.1% v/v) were taken in the ratio of 60 : 40 v/v separately and were filtered through membrane filter (Millipore Nylon disc filter of 0.45µ) using vacuum filter. This filtered mobile phase was sonicated for 15 min in ultrasonic bath before use.

Preparation of standard

1) Std. INITINIB10 MG in 10ml MeOH = 1000µgm/ml .--STOCK -I
2) Take 14.42mgs in 10 ml Methanol sonicate 10 min i.e. 1000 µgm/ml ----- STOCK –II

METHOD VALIDATION

The method was validated for the parameters like system specificity, linearity, accuracy, precision, robustness, and ruggedness, limit of detection (LOD) and limit of quantitiation (LOQ).

Linearity and Range

Calibration curve was plotted for different concentrations of working standards prepared from standard drug solution of pure drug, shown in and showed linearity over a concentration range of 10-50 µg/ml, along with regression parameters. Each calibration was injected three times. The calibration curve was performed in triplicate.

Table I: Linearity

Sr. NO.	Parameters	Values
1	Retention Time	6.08
2	Area %	100.00
3	Theoretical Plate	6105.4
4	Tailing Factor	1.3125

Repeatability

Repeatability is the closeness of agreement between mutually independent test results obtained with the same method on identical test material in the same laboratory by the same operator using the same equipment within short intervals of time.

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 absorbance was noted. The results were shown in terms of %RSD were within the limits.

Accuracy

Accuracy (recovery) of the method was obtained by spiking 80, 100 and 120% of ImatinibMesylate 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 double and accuracy was indicated by % recovery which was between 98.42 to 100.4%.

Ruggedness

Ruggedness was determined between different analysts. The value of %RSD was found to be <2, showed ruggedness of developed analytical method.

Robustness

Robustness was carried by varying three parameters deliberately from the optimized chromatographic conditions like mobile phase composition, flow rate and wave length.

LOD and LOQ

Limit of detection (LOD) and limit of quantification (LOQ) were calculated as 0.302 ∂/S and 1.009 ∂/S, respectively as per ICH guidelines, where ∂ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot. The results of validation parameters and System suitability parameters are discussed as follows.

III. RESULTS AND DISCUSSION

A Reverse phase HPLC method was developed keeping in mind the system suitability parameters i.e. tailing factor (T), number of theoretical plates (N), runtime and the cost effectiveness. The optimized method developed resulted in the elution of ImatinibMesylate at 3.7 min. fig 2 represents standard solution (100 µg/ml). The total run time is 5 minutes. System suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (Rt), number of theoretical plates (N) and peak asymmetric factor were evaluated for six replicate injections of the standard at working concentration.

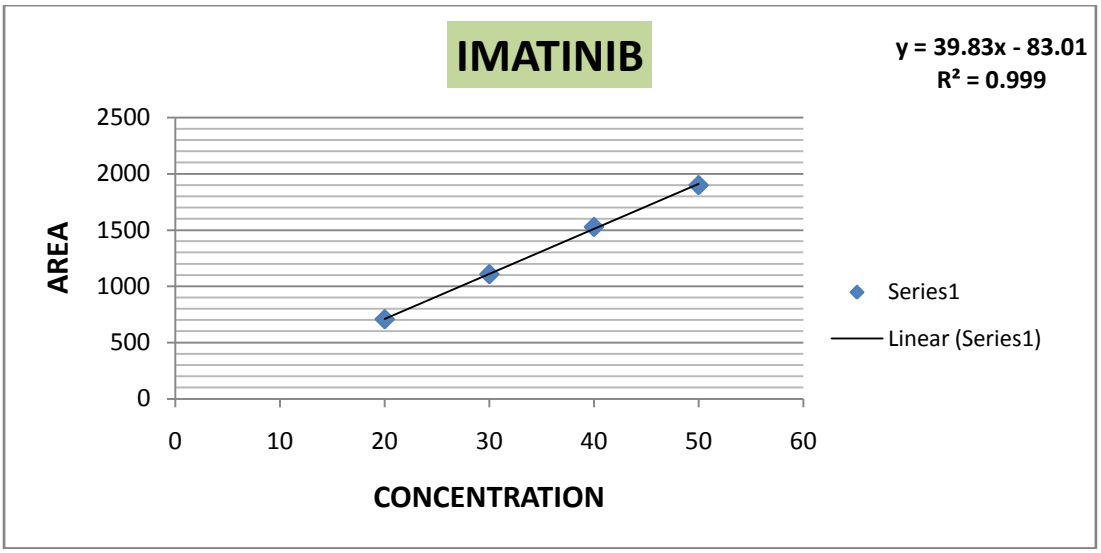

Figure 2:IMATINIB MESYLATE

We described more in detail tabulated format as follows

Table II:Linearity of Imatinib

Sr No.	Conc	Area I	II	III	IV	V	Mean	SD	%RSD
1	10	315.01	320.9	315.02	318.08	317.03	317.955	4.16	1.31
2	20	699.2	715.23	700.45	710.64	710.25	707.215	11.33	1.60
3	30	1104.89	1110.11	1109.45	1107.95	1106.08	1107.5	3.69	0.33
4	40	1534.39	1520.87	1525.87	1517.54	1533.23	1527.63	9.56	0.63
5	50	1897.32	1901.28	1899.65	1900.02	1898.25	1899.3	2.80	0.15
							Avrg SD	6.31	

Table III: Repeatability of Imatinib-

Sr No.	Conc	Area	II	III	Mean	Amt Found	% AmtFnd	SD	% RSD
1	20	710.51	718.55	719.1	716.05	19.88	99.40	4.81	0.67

TableIV:Presicion of Imatinib-

Concentration	%RSD			
	Intraday	Interday		
		Day1	Day2	Day3
10	0.43	0.50	0.59	0.63
30	0.19	0.20	0.31	0.28
50	1.24	1.19	1.26	1.30

Table V:Accuracy of Imatinib-

%spike level	sample µg/ml	stand.sample added	Standard amount found	% Recovery	stastastic Parameter
80	10	8	17.97	99.71	Mean-99.23
	10	8	17.90	98.75	SD-0.68 %RSD-0.68
100	10	10	19.86	98.61	Mean-99.51
	10	10	20.04	100.40	SD-1.27 %RSD-1.27
120	10	12	231.80	98.37	Mean-99.08
	10	12	21.97	99.80	SD-1.01 %RSD-1.02

Table VI: Ruggedness of Imatinib-

Sr No.	Conc	Area	II	III	Mean	Amtfnd	% AmtFnd	SD	RSD
Anal-i	30	1114.82	1122.84	1119.23	1118.96	30.17	100.57	4.02	0.36
Anal-ii	30	1109.54	1112.77	1117.95	1113.42	30.03	100.10	4.24	0.38

Table VII:Robustness of Imatinib-

PARAMETERS	IMITINIB	
Flow rate (ml/min)	0.9	1.1
% RSD	0.35	0.38
Wavelength	262	264
% RSD	0.43	0.45
Mobile phase	59+41	61+39
% RSD	0.43	0.21

Table VIII:LOD and LOQ of Imatinib-

Parameters	Measured Value
LOD	0.5227
LOQ	1.5842

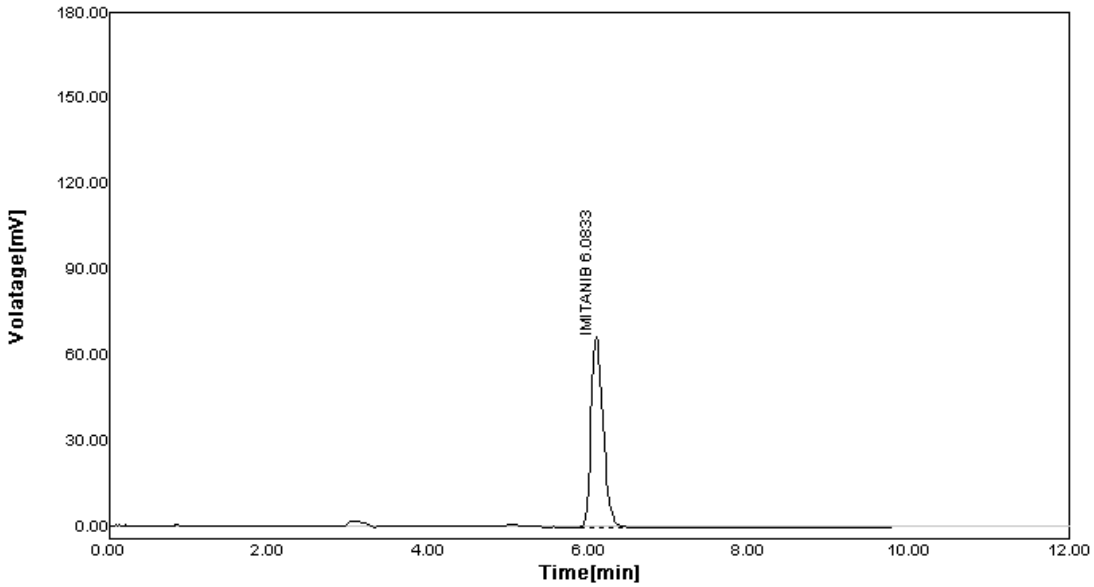

figure 3: Optimized Chromatogram for Imitinib

Assay for formulation of Imatanib

The validated method was applied for the determination of Imatinib in commercially available GLEEVAC Tablets. The results of the assay (n = 6) undertaken yielded 99.13% (%RSD = 0.37%) of label claimImatinib. The mean retention time of imatinib was 6.08 min. The results of the assay indicate that the method is selective for the analysis of imatinibwithout interference from the excipients used to formulate and produce these tablets.

Table IX: Analysis of Formulation-

Drugs	Labelled amount % Lable claim	Amount taken(mg)	Amount found for assay (µg/mL) (mg)	%
IMATINIB	400mg	40	9.987±0.371	99.13

IV. CONCLUSION:

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

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