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STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION OF IMATINIB MESYLATE BY RP-HPLC

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Abstract:

A simple,fast and precise reverse phase high performance liquid chromatographic method (Rp-HPLC) good sensitivity was determined for the development and validation of Imatinib Mesylate. A Elico,corp.Japan UV-Visible spectrophotometer was used. The chromatographic separation was achieved on a waters C18 column,5um,150X4.6mm as stationary phase with mobile phase Methanol, Acetonitrile, Dichloromathane, Water, 0.1N NaOH, 0.1N HCl. An isocratic elution mode at 1.0mi/min flow rate and maintaining column temperature at ambient. The detection was monitored at 200-250nm for Imatinib Mesylate. The linearity was found to be in the range of 0-35ug/ml. The calibration factor is 0.9963. Imatinib Mesylate was subjected to p acidic, basic, oxidative, thermal and photolytic degradation. The drug was found to be stable in all the conditions. The developed method was simple, accurate, precise, specific, sensitive & reproducible. Stress testing should be given importance for quantification of degraded products of drugs helps us to maintain the quality, safety & efficacy of drugs in formulations.

Keywords: *Imatinib mesylate, linearity, calibration factor, degradation.*

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INTRODUCTION:

Imatinib is a tyrosine kinase inhibitor used to treat a number of leukemias, myelodysplastic/myeloproliferative disease, systemic mastocytosis, hypereosinophilic syndrome, dermatofibrosarcoma protuberans, and gastrointestinal stromal tumors. Imatinib mesylate is a protein-tyrosine kinase inhibitor that inhibits the BCR-ABL tyrosine kinase, the constitutively active tyrosine kinase created by the Philadelphia chromosome abnormality in CML. Although the function of normal BCR is still unclear, ABL activation is overexpressed in various tumors and is heavily implicated in cancer cells growth and survival. Imatinib inhibits the BCR-ABL protein by binding to the ATP pocket in the active site, thus preventing downstream phosphorylation of target protein.[1-3] IUPAC name is N-(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)-4-[(4-methylpiperazin-1 yl)methyl]benzamide; methanesulfonic acid. Molecular formula $C_{30}H_{35}N_7O_4S$. Molecular Weight is 589.7.

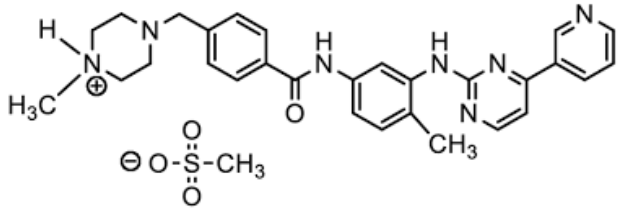

Figure 1: Structure of Imatinib mesylate

Literature survey reveals that the Imatinib Mesylate in both single and simultaneous with other drugs can be estimated by RPHPLC in pharmaceutical dosage forms. Several analytical methods like UVspectrophotometric [4-6] , HPTLC [7] and HPLC [8-13] have been reported in the literature for estimation of Imatinib mesylate in bulk drug, formulations, pure active pharmaceutical ingredient and Capsule dosage form. The objective of this study is to develop a simple, fast, economical, selective, accurate, precise and sensitive RP-HPLC method for the determination of Imatinib Mesylate in bulk and its pharmaceutical dosage forms suitable for routine quality control analysis. This work makes an attempt to develop a new sensitive and accurate RP-HPLC method for estimation in bulk and pharmaceutical dosage form and to validate the developed method in accordance with International Council on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines.[8]

MATERIALS AND METHODS:

Chemicals and Reagents: Imatinib Mesylate is obtained from Dr. Reddys laboratories Ltd., Hyderabad. NaH_2PO_4 was analytical grade supplied by Finerchem limited, Orthophosphoric acid (Merck), and Water and Methanol for HPLC (Lichrosolv (Merck).

Equipment and Chromatographic Conditions: The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, UV detector and Empower 2 software. Analysis was carried out at 268 nm with column Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm i.d. Particle size, dimensions at Ambient temperature. The optimized mobile phase consists of phosphate buffer (pH 3.4): acetonitrile (60:40). Flow rate was maintained at 1 ml/min.

Preparation of solutions:

Standard Solution Preparation:

25mg of Imatinib Mesylate Working standard was accurately weighed and transferred into a 25 mL volumetric flask and about 20 ml of diluent was added to it and sonicated to dissolve drug completely and volume was made up to the mark with the same solvent which gave Stock solution of 1000 ppm. 1 ml of the above stock solution was pippetted into a 10ml volumetric flask and was diluted up to the mark with diluents to prepare 100ppm solution. Further 1 ml of prepared 100 ppm solution was pippetted into a 10ml volumetric flask and was diluted up to the mark with diluents which gave 10ppm Imatinib Mesylate working standard solution. The solution was mixed well and filtered through 0.45µm membrane filter.

Sample Solution Preparation:

Four Imatinib Mesylate capsules were weighed and the average weight was calculated. The sample equivalent to 25 mg of Imatinib Mesylate was accurately Weighed and transferred into a 25 ml volumetric flask. About 20 ml of diluent was added and sonicated to dissolve drug completely and the volume was made up to the mark with diluent which gave stock solution of 1000ppm. The solution was mixed well and filtered through 0.45µm filter. 1 ml of the above stock solution was pippetted into a 10ml volumetric flask and diluted up to the mark with diluent to prepare 100ppm solution. Further 1 ml of prepared 100ppm solution was pippetted into a 10ml volumetric flask and diluted up to the mark with diluent which gave 10 ppm Imatinib Mesylate working standard solution. It was mixed well and filtered through 0.45µm membrane filter.

Preparation of Phosphate buffer:

About 6.8 grams of Potassium dihydrogen orthophosphate was weighed and transferred into a 1000ml beaker, dissolved in HPLC grade water and

diluted to 1000ml. The pH was adjusted to 2.5 with Orthophosphoric acid.

Preparation of mobile phase
700mL (70%) of above buffer and 300 mL of Acetonitrile HPLC (30%) were mixed well and degassed in ultrasonic water bath for 15 minutes. The solution was filtered through 0.45 µm filter under vacuum filtration.

Diluent Preparation:
Mobile phase as diluent.
Procedure:
20µL of the standard, sample are injected into the chromatographic system and the areas for peaks are measured and the %Assay are calculated by using the formulae.

METHOD:
The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.

System suitability parameters: To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 1.0 ml/min for 7 minutes to equilibrate the column at ambient temperature. Chromatographic separation was achieved by injecting a volume of 10 µL of standard into Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm i.d., the mobile phase of composition phosphate buffer (pH 3.4): acetonitrile (60:40) was allowed to flow through the column at a flow rate of 1.0 ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in table 1.

Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine Imatinib Mesylate in pharmaceutical dosage form. The result obtained for was comparable with the corresponding labeled amounts and they were shown in table-2.

Validation of Analytical method:
Linearity: Imatinib Mesylate working standard solutions were prepared across the range of the analytical method with a minimum of 5 concentrations that are within the specified range 5-35 µg/ml for 5 replicating injections were taken and calculated the %RSD. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The results are shown in table 3.

Accuracy studies: The accuracy was determined by help of recovery study. The recovery method carried out at three level 8%, 10%,12%. Inject the standard solutions into chromatographic system. Calculate the Amount found and Amount added for Imatinib Mesylate and calculate the individual recovery and mean recovery values. The results are shown in table 4.

Precision Studies: The system precision of the test method was performed by injecting 5 replicate determination of standard preparation injections were injected and the % RSD was calculated. The %RSD for the area of six replicate injections was found. The results are shown in table 5.

LOD and LOQ: The sensitivity of RP-HPLC was determined from LOD and LOQ. Which were calculated from the calibration curve using the following equations as per ICH guidelines. The results are shown in table 6.

$LOD = 3.3\sigma/S$ and
$LOQ = 10\ \sigma/S$, where
σ= Standard deviation of y intercept of regression line,
S = Slope of the calibration curve

Forced degradation studies
The forced degradation study is considered a vital analytical aspect of the drug development program for small molecules. Forced degradation, commonly known as stress testing, The ICH definition of stress testing for the drug product is “studies undertaken to assess the effect to severe conditions on the drug product. Such studies include photo stability testing and specific testing on certain products like metered dose inhalers, creams, emulsions etc. As per FDA guideline “Stability is defined as the capacity of a drug substance or drug product to remain within established specifications to maintain its identity, strength, quality, and purity throughout the retest or expiration dating periods”. The results are shown in table 7.

RESULTS AND DISCUSSION:

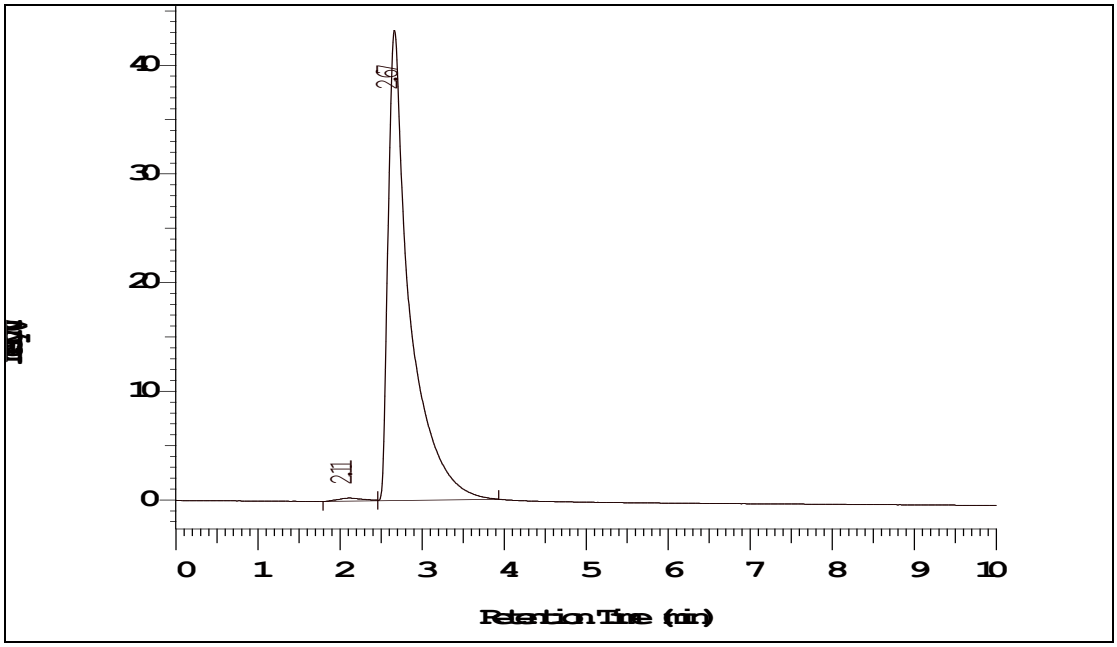

Figure 2: Standard chromatogram

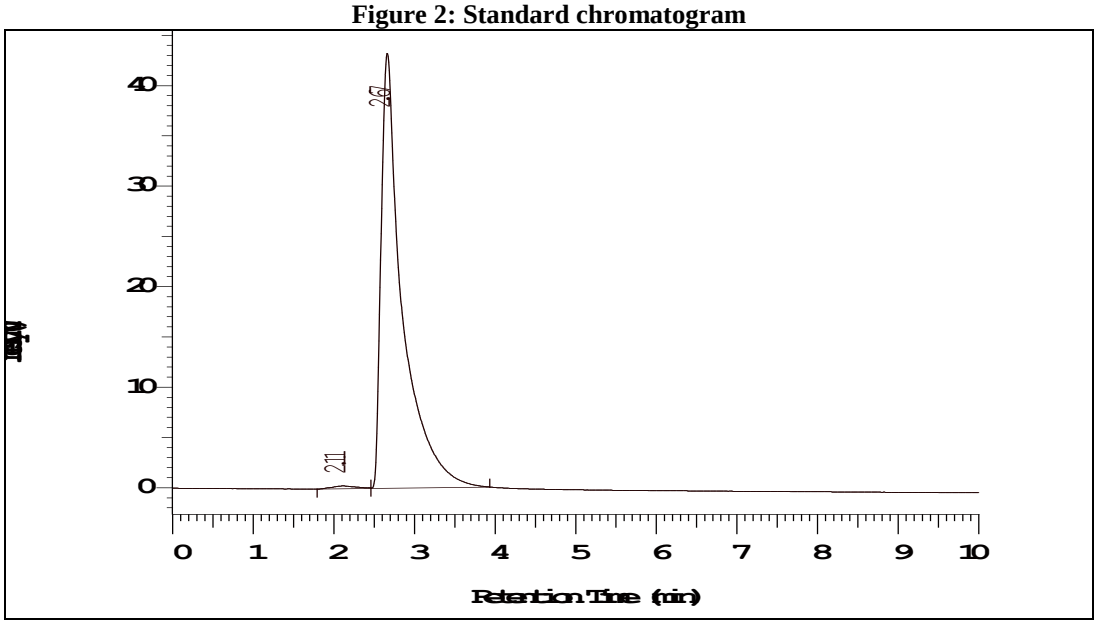

Figure 3: Sample chromatogram

Table 1: System suitability parameters

S.No.	Parameter	Limit	Result
1	Resolution	Rs > 2	9.15
2	Asymmetry	T ≤ 2	Imatinib=0.12
3	Theoretical plate	N > 2000	Imatinib=3246

Table 2: Assay results for Imatinib Mesylate

Brand name of tablets	Labeled amount of Drug (mg)	Mean (±SD) amount (mg) found by the proposed method (n=6)	Mean (± SD) Assay (n = 6)
Imatib Alpha (Cipla Limited)	100	100.13 (±0.06)	100.13 (±0.48)

Table 3: Linearity results of Imatinib Mesylate

Conc.	AUC (n -=6)
0	0
5	343726
10	801625
15	1064970
25	1811846
35	2721573

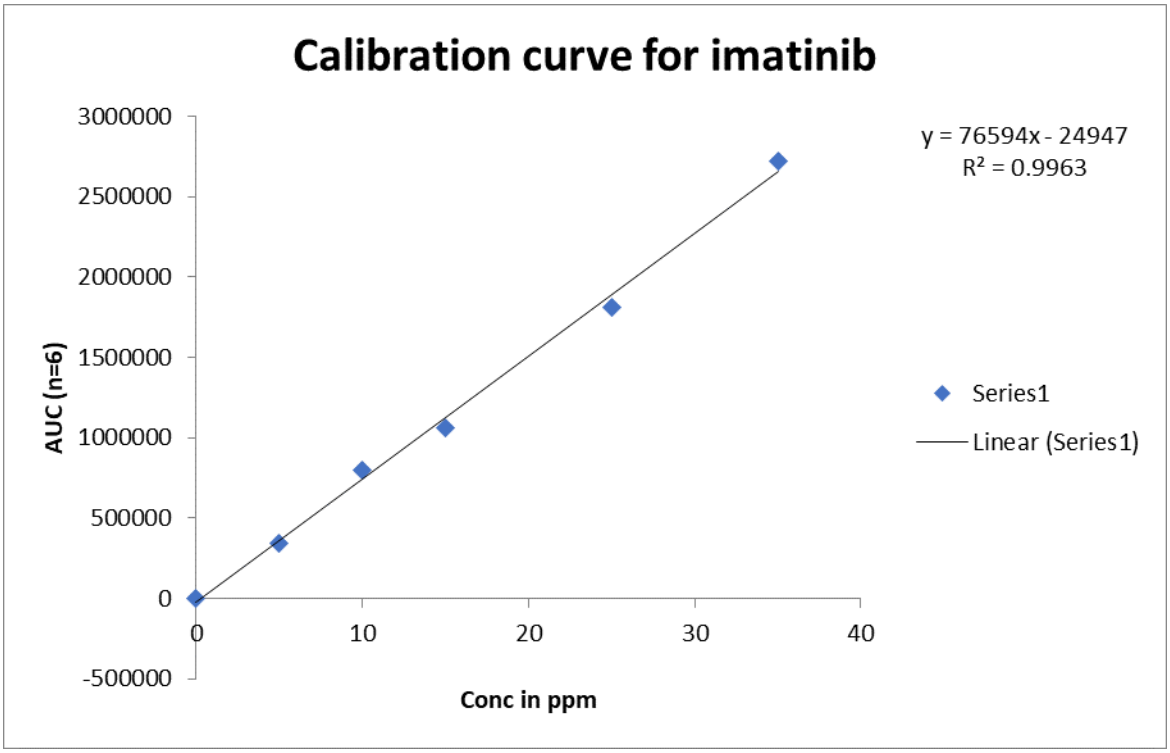

Figure 4: Linearity graph for Imatinib Mesylate

Table 4: Showing accuracy results for Imatinib Mesylate

STD			Spike-1				
Conc	AUC		Conc	AUC	Diff	% Recovery	
8	641300	1	18	1439576	633662	98.80898	
8	641300	2	18	1458471	652557	101.7553	
8	641300	3	18	1438963	633049	98.71339	
					Avg.	99.75924	
					SD	1.729335	
					% RSD	1.733509	

Table 5: Precision results for Imatinib Mesylate

HPLC Injection Replicates of Imatinib	Retention Time	Area
Replicate – 1	2.64	789939
Replicate – 2	2.67	790996
Replicate – 3	2.68	809774
Replicate – 4	2.67	796107
Replicate – 5	2.69	821313
Average	2.67	801625.8
Standard Deviation	0.018708287	13546.31
% RSD	0.700684904	1.689855

Table 6: LOD, LOQ of Imatinib Mesylate

Drug	**LOD**	**LOQ**
Imatinib Mesylate	0.341	1.023

Table 7: Forced degradation studies of Imatinib Mesylate

Stress condition	**Time**	**Assay of active substance**	**Assay of degraded products**	**Mass Balance (%)**	**No. Of impurity Peaks**
Acid Hydrolysis (0.1 M HCl)	24Hrs.	62.56	37.42	99.98	2
Basic Hydrolysis (0.I M NaOH)	24Hrs.	98.32	----------	98.32	0
Thermal Degradation (80 ^{0}C)	08Hrs.	98.36	----------	98.29	0
Oxidation	24Hrs.	98.79	----------	98.78	0
Sunlight	08Hrs.	98.79	----------	98.78	0

CONCLUSION:

From the above experimental results and parameters it was concluded that, this newly developed method for the estimation of Imatinib Mesylate 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 meant in industries, approved testing laboratories, bio-pharmaceutical and bio-equivalence studies and in clinical pharmacokinetic studies in near future.

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