Research Article

Analytical Method Development and Validation for the Estimation of Imatinib Mesylate and its Impurity in Pharmaceutical Formulation by RP-HPLC

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ABSTRACT

A simple, specific and accurate reverse phase high performance liquid chromatographic method was developed for the determination of Imatinib mesylate and its Amine Impurity in pharmaceutical dosage form. The column used was HiQSil C18 (250 x 4.6 mm, 5µm), with mobile phase containing methanol and Acetate Buffer pH 3.5 inthe ratio of 80: 20 v/v, the flow rate was 1.0 mL/ min and eluent was monitored at 273nm. The retention time for Imatinib mesylate was 8.071and for Amine Impurity it is 4.958. The proposed method was validated and successfully applied to the estimation of Imatinib mesylate and Amine Impurity in formulations.

Keywords: Imatinib mesylate, Drug Impurity, HPLC.

INTRODUCTION

Imatinib mesylate is a small molecule that inhibits the c-Abl protein-tyrosine kinase, a kinase specifically important for proliferation of chronic myelogenous leukemia (CML). A translocation event between chromosomes 9 and 22 generates the Philadelphia chromosome, which then produce the Bcr-Abl fusion protein with aberrant kinase activity that promotes rapid cell proliferation. Imatinib binds up this crucial kinase, halting CML related growth. Imatinib mesylate has further been displayed to inhibit PDGFR and tyrosine kinases associated with c-Kit. The structure of Imatinib mesylate is shown in Figure 1.

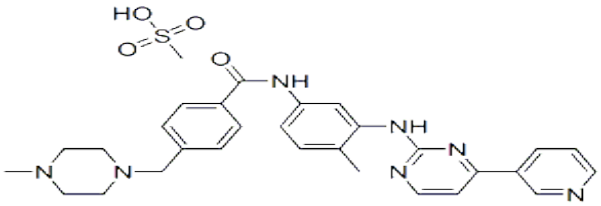

Figure 1: Structure of Imatinib mesylate

Imatinib Mesylate is chemically known as 4-[(4-Methyl-1-piperazinyl) methyl]-N-[4-methyl-3-[[4-(3-pyridinyl) - 2 -pyrimidinyl] amino] phenyl] benzamid methane sulfonate and has a chemical formula $C_{29}H_{31}N_7O{\bullet}CH_3SO_3H$. A molecular weight of Imatinib mesylate is 589.7 g/mol. Imatinib mesylate is a white to creamish yellow crystalline powder. It is freely soluble in distilled water, 0.1 N HCl and methanol. Regulatory requirements for the identification, qualification, and control of Impurities in drug substances and their formulated products are now being explicitly defined, particularly through the International Conference on Harmonization (ICH).[1-2]Several analytical methods have been reported in the literature for estimation of Imatinib mesylate and its Impurities in bulk drugs and formulations.[3-10]The aim of current study was to develop and validate HPLC method for the simultaneous determination of Imatinib mesylate and its Impurity i.e., Amine Impurity – (N-(5-Amino – 2-methylphenyl)-4-(3-pyridyl)-2-pyrimidine amine).

MATERIALS AND METHODS

Chemicals and Reagents

Imatinib mesylate- pure analytical sample was obtained as a gift sample from Nishka Labs, Hyderabad and its Amine Impurity was procured from Nishka Labs, Hyderabad. All chemicals and reagents used were of AR grade and all the solvents used were of HPLC grade. Methanol HPLC Grade, Acetonitrile HPLC Grade were of Merck Chemicals, Mumbai while Hydrochloric acid and Ammonium Acetate AR Grade was of S.D. Fine Chemical Industries, Mumbai. Double distilled water prepared at laboratory scale using ELGA water purification system, was used throughout the study. GLEEVAC film coated tablets (Novartis) were procured from the local pharmacy labeled to contain Imatinib mesylate equivalent to 100 mg of Imatinib free base.

Instrumentation

HPLC system used was JASCO system equipped with Model PU 2080 Plus pump, Rheodyne

Sample injection port (20 µL), MD 2010 PDA detector and Borwin- PDA software (version 1.5). A chromatographic column HiQSil C18 (250 x 4.6 mm, 5µm) was used for separation. All weighing were done on Shimadzu balance Model AY-120.

Chromatographic Conditions: Isocratic Procedure

Chromatographic separation carried using methanol and acetate buffer pH 3.5 in the ratio of 80:20 v/v as the mobile phase which gave good resolution and acceptable peak parameters. Separation was carried out at flow rate of 1 ml/min and detection at 273 nm. The peak purity was checked with the PDA detector. The mobile phase was

filtered through a 0.45 µ nylon membrane filter prior to use. The sample injection volume was 20 µl.

Preparation of Mobile Phase

Acetate Buffer pH 3.5 was prepared by dissolving 25 gm of ammonium acetate in 25 ml water. Added 38 ml of 7 M HCl adjusted the pH to 3.5 with 2 M HCl or 6 M ammonia and diluted with water to 100 ml. Mobile phase was prepared by mixing methanol and Acetate Buffer pH 3.5 in the ratio of 80: 20 v/v. It was then filtered through 0.45 µm membrane filter paper using filtration assembly and then sonicated on ultrasonic water bath for 15 min.

Preparation of Solution

Standard Solution

A standard stock solution 'A' containing 100 µg/ml of Amine Impurity was prepared by dissolving 10 mg of Amine Impurity in methanol in a 100 ml volumetric flask. The stock solution 'B' was further prepared by diluting appropriate amount with methanol to get 10 µg/ml solution of Amine Impurity. The Imatinib mesylate standard stock solution 'C' containing 1000 µg/ml was prepared using appropriate amount of Imatinib mesylate reference standard dissolved in methanol.

Resolution Solution

Resolution solution 'D' was 1 ml of Imatinib mesylate standard stock solution C and 5 ml of Impurity stock solution B, diluted up to 100 ml with methanol. This resolution solution was containing 10 µg/ml of Imatinib mesylate and 0.5 µg/ml of Impurity.

Sample Solution

To determine the content of Imatinib mesylate in conventional tablet, twenty tablets were weighed (each tablet containing Imatinib mesylate equivalent to 100 mg of Imatinib free base); their mean weight was determined and was finely powdered. The weight of the tablet powder equivalent to 100 mg of Imatinib mesylate was transferred into a 100 ml volumetric flask containing 80 ml methanol and sonicated for 5 min. to ensure complete dissolution of drug. The extract was filtered, residue was washed with methanol and volume was made up to the mark by adding washings to the flask. Appropriate volume of above solution was further diluted suitably with methanol to give 20 µg/ml of Imatinib mesylate and 20 µl was injected into HPLC system, under the conditions described above.

System Suitability

System suitability parameters were evaluated to verify that the analytical system is working properly and can give accurate and precise results using 20 µl resolution solutions 'D' (10 µg/ml of Imatinib mesylate and 0.5 µg/ml of Impurity). Parameters such as tailing factor, resolution, peak area were evaluated. The % RSD less than 2.0 for six replicate injections and theoretical plates not less than 2000 was set as acceptance criteria for system suitability of the proposed assay method.

Analytical Method Validation

The method was validated for linearity, range, accuracy, precision, sensitivity, specificity and robustness in accordance with ICH guidelines. [1-2]

Linearity and Range

Linearity of Imatinib mesylate was determined over a range of 10-60 µg/ml, while for the Amine Impurity a range of 0.5 µg/ml to 3 µg/ml was selected for linearity study. Calibration curve was drawn by plotting the peak areas of analyte versus their corresponding concentration. Values of coefficient of regression, slope and Y-intercept of the calibration curve were calculated.

Accuracy (recovery study)

To perform recovery studies tablet powder was first mixed with Impurity and appropriately diluted to get sample solution. Recovery studies were performed in triplicate at concentration levels i.e., 50, 100 and 150 % by standard addition method. Sample solutions were spiked with appropriate volume of standard solution of Impurity as well as Imatinib mesylate. The resulting mixtures were analysed by the developed method. Base level amount of Imatinib mesylate and its Impurity used for spiking were 20 µg/ml and 1 µg/ml, respectively. The % RSD and mean recovery was calculated.

Precision

The precision of the method was verified by repeatability (intraday) and intermediate precision studies. Repeatability was assessed by injecting six times 20 µl of standard solution containing Imatinib mesylate (20 µg/ml) spiked with Impurity solution (1 µg/ml) into HPLC system under stabilized chromatographic conditions and value of % RSD was calculated using peak area of Imatinib mesylate and Impurity. Further inter-day variation for the determination of Imatinib mesylate and Amine Impurity was carried out at three different concentration levels (20, 30 and40 µg/ml for Imatinib mesylate and 1, 1.5 and 2 µg/ml for Amine Impurity) on three consecutive days. The % RSD of the obtained assay values at three different concentration levels was calculated.

Method sensitivity (limit of detection and limit of quantitation)

The LOD and LOQ were calculated as per ICH guidelines, using equations, LOD =3.3 x σ/S; LOQ=10 x σ/S, respectively where σ is the standard deviation of the y-intercepts and S is the slope of the calibration curve. A series of standard preparation of Imatinib mesylate and Amine Impurity were prepared over different levels. Calibration graphs were plotted for the obtained area under the curve of each level against the concentration. Slope (S) and Standard Deviation were calculated to calculate LOD and LOQ.

Specificity

Specificity involved demonstration of the ability of the developed method for the separation and resolution of Imatinib mesylate and Amine Impurity. Further peak purity of all the analytes was measured to evaluate the specificity of the method. The sample and standard bands were scanned at three distinct levels, i.e., peak start (S), peak apex (M), and peak end (E) positions. The peak purity was determined by PDA detector.

Robustness

Robustness of the method was checked by carrying out the analysis under conditions during which little variations like changing detection wavelength, flow rate and mobile phase composition was done and the effects on the area were noted. The % RSD for each deliberate change was calculated.

RESULTS AND DISCUSSION

Development and optimization

The HPLC procedure was optimized with a target to achieve separation of Impurity and main component Imatinib mesylate. Based on literature survey and review of physico-chemical properties of analytes, preliminary chromatographic conditions were selected. Chromatographic separation studies were carried out on the resolution solution of Imatinib mesylate (10 µg/ml) and Amine Impurity (0.5 µg/ml). Initially, trials were carried out using methanol, water in various proportions along with buffer of varying pH, to obtain the desired system suitability parameters. After few trials, mixing methanol and Acetate Buffer pH 3.5 in the ratio of 80: 20 v/v was chosen as the mobile phase which gave good resolution and acceptable peak parameters. Wavelength for monitoring the eluent was selected by scanning standard solution of drug and Impurity within 200–400 nm. Separation was carried out at flow rate of 1ml/min and detection at 273 nm. The peak purity was checked with the PDA detector. The mobile phase was filtered through a 0.45 µ nylon membrane filter prior to use. The injection volume was 20 µl.

System Suitability

Chromatographic separation of resolution solution D was successfully achieved with the abovementioned chromatographic conditions. The representative chromatograms shown in Figure 2. Impurity was injected separately as well as by spiking into the Imatinib mesylate to check its interference with the main peak. The Impurities didn't interfere with Imatinib mesylate peak. The resolution between Imatinib mesylate and Amine Impurity was greater than 3.50. The retention times of the Imatinib mesylate and Amine Impurity were 8.071 and 4.958 respectively. The theoretical plates were above 2000 for both Imatinib mesylate and Amine Impurity. Table 1 contains system suitability parameter details of Imatinib mesylate and Amine Impurity.

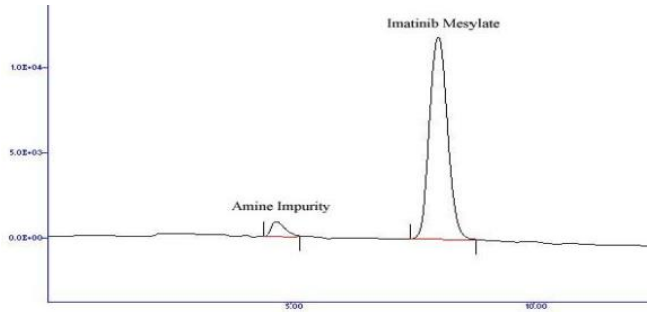

Figure 2(A): Chromatogram of Resolution Solution Imatinib mesylate (10 µg/ml) and Amine Impurity (0.5 µg/ml)

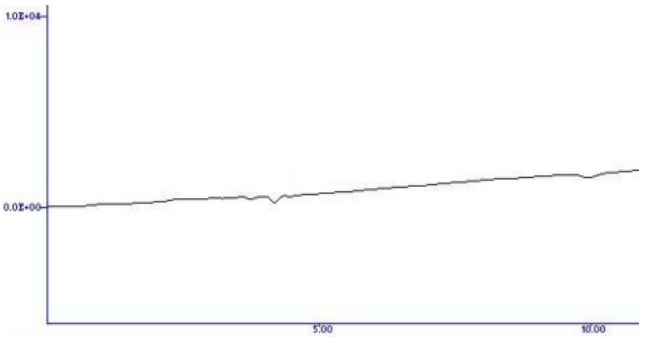

Figure 2(B): Mobile Phase Blank

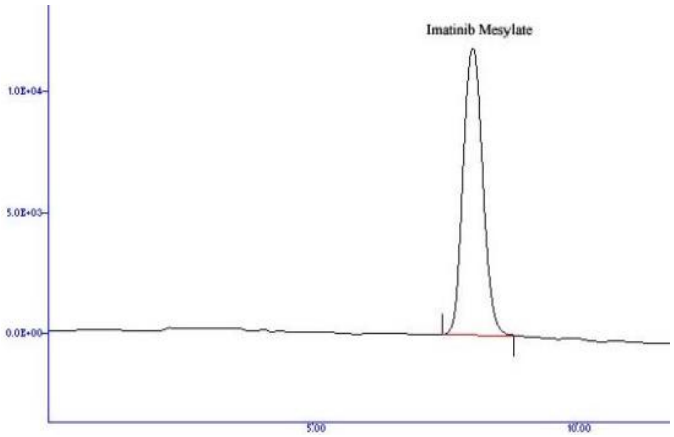

Figure 2(C): Chromatogram of Imatinibmesylate (10 µg/ml).

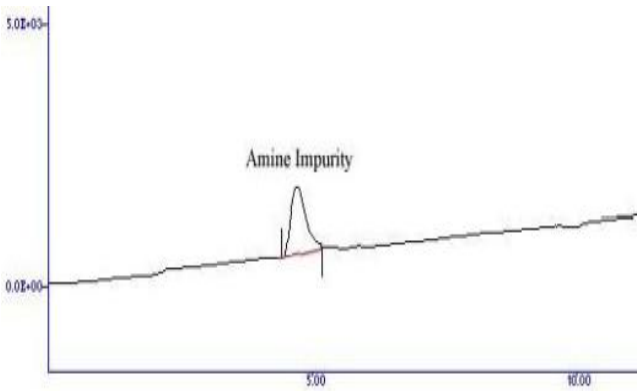

Figure 2(D): Chromatogram of Amine Impurity (0.5 µg/ml).

Table 1: System suitability parameters

Name	RT (Min) Mean ± % RSD	Concentration (µg/ml)	Avg. Area	Plates (N)	Tailing Factor	Resolution
Amine Impurity	4.958 ± 0.124	0.5	1552.130	3965.46	1.136	--
Imatinib mesylate	8.071 ± 0.547	10	116029.005	4238.78	1.024	3.6*

*with respect to previous peak

Linearity and Range

The method was found to be linear over the range; for Imatinib mesylate it was 10 µg/ml to 60µg/ml and for Amine Impurity 0.5 µg/ml to 3 µg/ml. The data generated was analysed by linear regression analysis shows the satisfactory result with correlation coefficient greater than 0.997. Linearity Curves of Imatinib mesylate and Amine Impurity are shown in Figure 3.

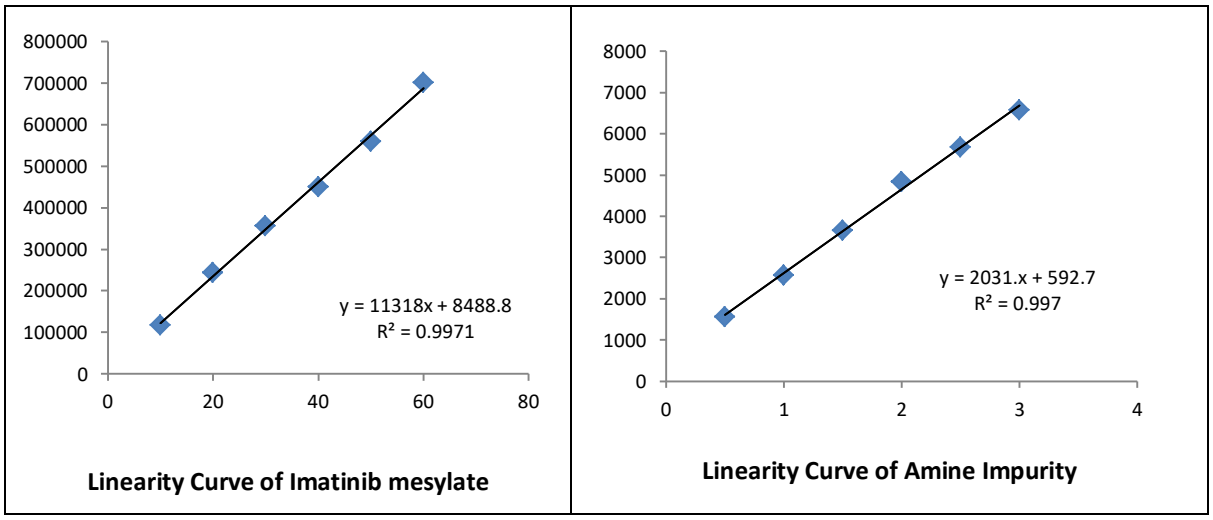

Figure 3: Linearity Curve of Imatinib mesylate and Amine Impurity.

Accuracy

The % mean recoveries for Imatinib mesylate and Impurity was in the range of 99.88-100.44and 99.49 – 100.96, respectively. The overall % RSD was observed as less than 2%. Accuracy details are given in Table 2.

Precision and Repeatability

The % RSD in precision studies was found to be 0.272 for repeatability and 0.34– 1.98 % Interday for Imatinib mesylate. The % RSD in precision studies was found to be 0.58 for repeatability and 0.38– 0.70 % Inter-day for amine Impurity. This indicates that the method is precise.

Method sensitivity

The LOD concentration was found to be 0.408, 0.062 µg/ml and LOQ concentration was 1.235, 0.188 µg/ml for Imatinib mesylate and Impurity, respectively.

Specificity

The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found to be more than 991, indicating the no interference of any other peak of degradation product, Impurity or matrix. Details about purity tail and purity front of Imatinib mesylate and Amine Impurity are given in Table 3.

Robustness

Small deliberate alteration in chromatographic conditions did not affect the assay and resolution significantly for the investigated compounds, % RSD was less than 2% indicates that the developed method was robust to minor changes in the experimental conditions. Robustness details are given in Table 4.

Table 2: Accuracy details of Imatinib mesylate and Amine Impurity.

Accuracy Results of Imatinib mesylate				
Accuracy Level	**Recovered (mg)**	**Recovered (%)**	**Average (%)**	**SD**
50%	9.984	99.84225	100.44	0.67
	10.116	101.1584		
	10.031	100.3063		
100%	20.182	100.9091	100.21	0.63
	19.941	99.7028		
	20.001	100.0063		
150%	29.837	99.45667	99.88	1.18
	29.689	98.96442		
	30.362	101.2077		
Accuracy Results of Amine Impurity				
Accuracy Level	**Recovered (mg)**	**Recovered (%)**	**Average (%)**	**SD**
50%	0.494619	98.924	99.49	0.58
	0.497385	99.477		
	0.500407	100.081		
100%	1.010942	101.094	100.96	0.12
	1.008668	100.867		
	1.009190	100.919		
150%	1.501363	100.091	100.05	0.41
	1.506589	100.439		
	1.494212	99.614		

Table 3: Details of Purity tail and Purity front.

Drug/Impurity	Purity tail (s, m)	Purity front (m, e)
Imatinib mesylate	995.75	999.26
Amine Impurity	997.50	992.003

Table 4: Robustness details of Imatinib mesylate and Amine Impurity.

Robustness for Imatinib mesylate				
Parameters	**Measure**	**Average**	**SD**	**%RSD**
Change in Wavelength	273nm	100.64	0.274	0.273
	272nm	100.26	0.177	0.177
	274nm	103.29	0.382	0.370
Change in flow rate	1ml/min	101.23	0.949	0.937
	0.95ml/min	101.51	1.874	1.846
	1.05ml/min	101.97	0.769	0.754
Change in Mobile Phase Composition*	80:20	100.93	0.074	0.073
	82-18	101.73	0.468	0.460
	78-22	101.06	0.294	0.291

Robustness for Amine Impurity				
Parameters	**Measure**	**Average**	**SD**	**%RSD**
Change in Wavelength	273nm	99.62	0.581	0.583
	272nm	97.66	1.360	1.393
	274nm	98.13	0.122	0.125
Change in flow rate	1ml/min	99.45	0.654	0.657
	0.95ml/min	98.41	0.348	0.353
	1.05ml/min	97.86	0.497	0.508
Change in Mobile Phase Composition*	80:20	100.10	0.520	0.519
	82-18	97.48	1.082	1.110
	78-22	97.91	0.660	0.674

*Methanol: Buffer

CONCLUSION

The proposed HPLC method for simultaneous estimation of Imatinib mesylate and its Impurity in bulk and Imatinib mesylate tablet was developed and validated as per ICH guidelines. The method was found to be linear in stated range. Statistical analysis proved that the method is specific, accurate, precise and sensitive. The method is suitable for simultaneous quantitative analysis of Imatinib mesylate and its amine Impurity in bulk drugs and formulations without anyinterference from the excipients. Thus, the proposed method can be successfully employed for routine quality control and Impurity profiling of Imatinib mesylate.

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