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Research Article

Development and Validation of a Stability Indicating RP-HPLC Method for the Determination of Imatinib Mesylate

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ABSTRACT

The aim of this paper was to develop and validate the stability indicating RP-HPLC method for the determination of Imatinib mesylate in bulk and pharmaceutical dosage forms. A simple, accurate, precise, sensitive and stability indicating RP-HPLC method has been developed for the determination of Imatinib mesylate in bulk drug and pharmaceutical dosage form, in which separations are done using develosil C_{18}, 5µm, 150 × 4.6mm i.d. column at a flow rate of 1.0mL/min with an injection volume of 20µL. The beer's law was obeyed over the concentration range of 5 - 35µg/mL. The correlation coefficient was found to be 0.996 and it showed good linearity, reproducibility, precision in this concentration range. The % recovery values were found to be within the limits, which showed that the method was accurate. The LOD and LOQ were calculated using statistical methods. The % RSD values were less than 2. The developed method was successfully applied for determination of Imatinib mesylate in pharmaceutical dosage form. The results obtained are in good agreement with those obtained by using the standard method.

Keywords: Imatinib mesylate, Develosil, Stability indicating, Method Development, Validation.

INTRODUCTION

Imatinib[1-3] (Fig. 1) is chemically designates as 4 - [(4 - methyl - 1 - piperazinyl) methyl] - N - [4 - methyl - 3 - [[4 - (3 - pyridinyl) - 2 - pyrimidinyl] amino phenyl] - benzamide methane sulfonate salt. It is a tyrosine kinase inhibitor used in the treatment of multiple cancers, most notably chronic myelogenous leukemia. It is also used to treat gastrointestinal stromal tumors and a number of other malignancies. According to the literature survey[4-7] it was found that few analytical methods on HPLC, HPTLC, UPLC and UV Spectrophotometer were reported for the estimation of Imatinib mesylate in bulk drug and formulations. Hence there is need to develop and validate an analytical method to estimate the drug. The objective of the proposed method is to develop simple, accurate, precise, stability indicating RP-HPLC method for the estimation of Imatinib mesylate in pharmaceutical dosage form.

MATERIAL AND METHODS

Reagents and Chemicals

Imatinib mesylate working standard was procured from Dr. Reddys Laboratories, Hyderabad, Telangana. Imatinib mesylate 100mg capsules (Imatib Alpha) were purchased from local pharmacy. Purified water was obtained from Millipore system. Acetonitrile (HPLC grade) and potassium dihydrogen orthophosphate were obtained from Loba Chem, Mumbai, and Sd fine-Chem ltd, Mumbai, respectively. All other chemicals used in the analysis were AR grade.

Instrumental and analytical conditions

The HPLC analysis was performed using a HITACHI L2130 with D Elite 2000 Software with Isocratic with UV-Visible Detector (L-2400). Column used was develosil C18, 5µm, 150 x 4.6 mm i.d. UV detection was performed at 268nm. The injection volume of sample was 20µL. An isocratic mobile phase containing Acetonitrile and 0.05M potassium dihydrogen orthophosphate buffer (30:70%v/v), at the pH 2.5 with O-phosphoric acid was carried out with the flow rate of 1.0mL/min. Column was maintained at room temperature.

Experimental

Mobile Phase Preparation

Mixture of Acetonitrile and Phosphate buffer, pH 2.5 in the ratio of 30:70(%v/v) respectively was used.

Preparation of Standard Solution

Accurately weighed 50mg of Imatinib mesylate working standard was transferred into 50mL volumetric flask. About 40mL of diluent (mobile phase) was added and

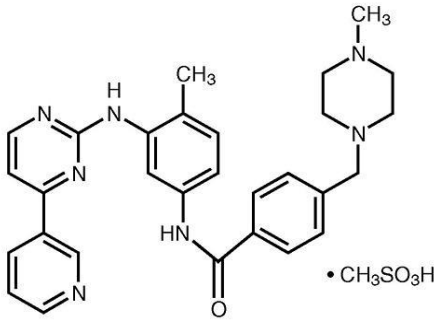

Figure 1: Chemical structure of Imatinib mesylate.

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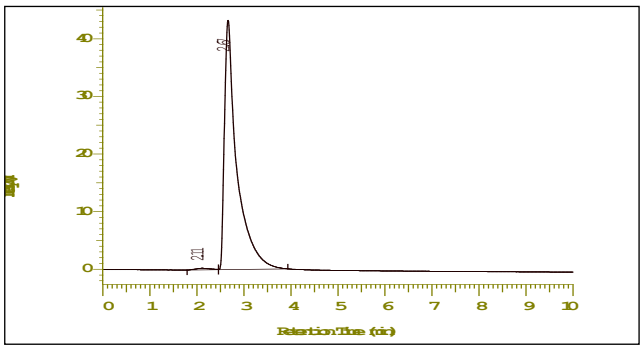

Figure 2: Standard Chromatogram.

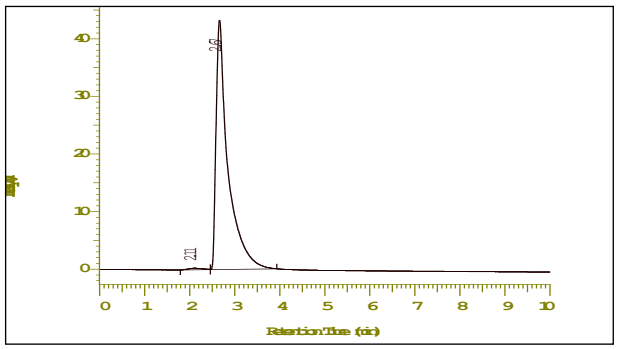

Figure 3: Sample Chromatogram.

Figure 4: UV spectrum of Imatinib mesylate.

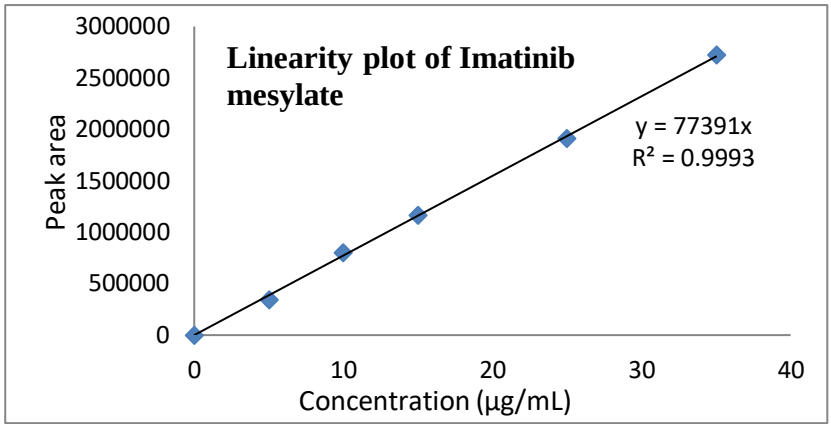

Figure 5: Linearity plot of Imatinib mesylate.

Table 1: Data of System Suitability Parameter.

S.No.	Parameter	Limit	Result
1	Resolution	Rs > 2	9.15
2	USP Tailing	T ≤2	0.12
3	USP plate count	N > 2000	3246

sonicated to dissolve. The volume was made up with diluent and mixed. 0.1mL of this solution was diluted to 10mL with mobile phase and mixed.

Preparation of Sample Solution

Four capsules were weighed accurately and the average weight was calculated.

Capsules were opened, fine powder was collected and equivalent to 50mg of Imatinib mesylate sample was weighed and transferred into 50mL volumetric flask. 40mL of diluent was added and sonicated for 30min with intermediate shaking. Volume was made up with diluent. The above solution was centrifuged for 10min at 8000rpm. 0.1mL of this solution was diluted to 10mL with mobile phase and mixed. The solution was filtered through 0.45 µm filter.

METHOD

Method Development

A develosil C18, 5µm, 150 x 4.6 mm i.d as a stationary phase with a mobile phase of Acetonitrile and Phosphate buffer, pH 2.5 (30:70) at a flow rate 1.0mL/min and a detection wavelength of 268nm afforded the best separation of drug. The standard solution and sample solution prepared as above were injected into the 20µL loop and the chromatograms were recorded as shown in the "Fig. 2" and "Fig. 3" respectively. The retention time of

Table 2: Result of different parameters.

Parameters		Imatinib mesylate
Specificity		Specific
Linearity	Regression equation, y=mx+c	y = 77391x
	Correlation coefficient (r)	0.999
Accuracy (recovery) n=3	Level I (80%)	99.76%
	Level II (100%)	100.54%
	Level III (120%)	98.91%
Precision (%RSD) n=5		0.76
Ruggedness (%RSD) n=5		0.56
Limit of Detection (LOD)		0.341µg/mL
Limit of Quantitation (LOQ)		1.023µg/mL
System Suitability	USP Plate Count	3246
	USP Tailing	0.12

Table 3: Forced degradation studies of Imatinib mesylate.

Stress conditions	Drug recovered (%)	Drug decomposed (%)
Standard drug	100.00	0.00
Acidic hydrolysis	62.56	37.44
Alkaline hydrolysis	98.32	1.68
Oxidative degradation	98.79	1.21
Thermal degradation	98.36	1.64

drug, Imatinib mesylate was found to be 2.67mins. The amount of drug present in sample was calculated.

Method Validation

The proposed stability indicating method has been developed and validated for the determination of Imatinib mesylate in pharmaceutical dosage forms. According to International Conference on Harmonization (ICH) guidelines[13-14,] validation of the method was carried out by using specificity, accuracy, linearity, suitability, LOD, LOQ, precision and stability studies.

System suitability

A standard solution was prepared by using Imatinib mesylate working standard as per test method and was injected 5 times into the HPLC system. The system suitability parameters were evaluated from the Resolution, USP tailing and USP plate count values obtained from standard chromatograms as shown in Table 1.

Specificity

Specificity was evaluated by injecting standard solution and placebo solution individually into HPLC system.

Linearity of test solution

A series of solutions are prepared from standard stock solution at concentration levels from 05-35µg/ml for Imatinib mesylate.

Accuracy

Drug assay was performed in triplicate as per test method with equivalent amount of drugs into each volumetric flask for each spike level to get the concentration of drugs equivalent to 80%, 100% and 120% of the labeled amount as the test method.

Precision

Repeatability

Repeatability of method was evaluated by calculating the %RSD of peak areas of five replicate injections for the standard concentration (10ppm) of drug.

Ruggudness

The ruggedness was also evaluated by analyzing five samples of drug by two analysts in the same laboratory using different HPLC systems.

Limit of Detection and Limit of Quantitation

The parameters LOD and LOQ were determined on the basis of standard deviation and slope of the regression equation.

Forced degradation studies

The forced degradation study was performed to determine the specificity and stability indicating property of developed method. The drug was deliberately subjected to stress conditions such as acidic condition, alkaline condition, oxidation condition and thermal condition. All the solutions for degradation were prepared by dissolving drug in diluent to get an initial concentration of 1mg/mL and filtered. Acid decomposition was carried out in 1N hydrochloric acid and alkaline degradation was conducted using 1N sodium hydroxide and kept aside for 24 hours. Solutions for oxidative degradation were prepared using 3% hydrogen peroxide at a concentration of 1mg/mL of Imatinib mesylate and kept aside for 24 hours. For thermal degradation study, the drug solution 1mg/mL was heated in calibrated oven at 80ºC for 8 hours, cooled and used. These solutions are injected into the HPLC system and values were noted.

RESULTS AND DISCUSSION

Selection of detection wavelength

From the UV spectrum, suitable wavelength considered for monitoring the drug was 268nm as shown in "Fig. 4". A stability indicating RP-HPLC method was developed by using an Develosil C_{18} (150mm × 4.6mm, 5µm particle size) as a stationary phase with a mobile phase of Acetonitrile and Phosphate buffer, pH 2.5 (30:70) at a flow rate 1.0mL/min and a detection wavelength of 268nm afforded the best separation of drug. The injection volume is 20µL and retention time for Imatinib mesylate is 2.67mins. The method was validated according to ICH guidelines for various parameters like accuracy, precision, linearity, specificity, ruggedness, LOD, LOQ and stability studies. Linearity was obtained in the concentration range of 05-35µg/mL with correlation coefficient (r) of 0.999. The %recovery was found to be 99.74%. The %RSD for precision was found to be 0.76 and for ruggedness it was found to be 0.53.

CONCLUSION

A simple, precise, accurate, rapid, economical, stability indicating RP-HPLC method for estimation of Imatinib mesylate has been developed and validated as per ICH guidelines. The proposed method shows good agreement

with all validation parameters. The optimized method is precise, accurate, specific, rugged, and a linear relation is observed between the concentration and the result. The developed method can be used for the analysis of routine quality control sample.

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