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RESEARCH ARTICLE

Analytical Method Development and Validation of Reverse-Phase HPLC for Quantitative Estimation of Imatinib Mesylate in Active Pharmaceutical Ingredient and Formulated Products

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Abstract: The goal of this work is to design and validate reverse-phase high-performance liquid chromatography (RP-HPLC) method for quantifying pharmaceutical formulations and bulk imatinib mesylate. A C18 column was used for the chromatographic analysis, and a mobile phase consisting of acetonitrile and phosphate buffer in equal amounts (50:50, v/v) was supplied with the flow rate of
1.0 mL/min. Imatinib mesylate exhibited a retention period of roughly 4.38 minutes when detected at a wavelength of 255.20 nm. By assessing criteria including linearity, precision, accuracy, specificity, robustness, and sensitivity (LOD and LOQ), the method was validated under ICH Q2 (R1) guidelines. A correlation coefficient (R2) of 0.996 indicated a linear response across the concentration range of 2–12 µg/ml. With percentage RSD values less than 2%, the approach showed excellent precision. Recovery trials, which varied from 98.91% to 99.7%, verified accuracy. It was found that the limits of quantification and detection were 0.80 µg/mL and 0.27 µg/mL, respectively. The approved RP-HPLC technique works well for routinely analyzing imatinib mesylate in bulk and tablet dosage forms and meets standard quality control requirements.

Keywords: Imatinib Mesylate; Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC); Method Validation; Analytical Method Development.

INTRODUCTION

Tyrosine kinase inhibitors like imatinib mesylate are frequently used to treat gastrointestinal stromal tumors (GIST) and chronic myeloid leukemia (CML). In order to relieve symptoms, stop cancer cells from spreading, and support alternate therapies, it works by blocking proteins linked to the proliferation of cancer cells1. Among the first anticancer drugs imatinib mesylate is one of the newest drugs in the market. that got authorization from the FDA under its fast track designation 2-3.

Chemically, it is designed as 4-[(4-methylpiperazin-1- yl) methyl]-N-[4-methyl-3-[(4 pyridin-3- ylpyrimidin- 2-yl) amino] phenyl] – benzamide4. For quality control and stability investigations of imatinib mesylate in pharmaceutical formulations, precise and trustworthy analytical techniques are crucial. Because of its great sensitivity, specificity, and reproducibility, reversed- phase high-performance liquid chromatography (RP- HPLC) is the preferred procedure 5-7. For the quantitative measurement of imatinib mesylate in pharmaceutical and pure dose forms, this work sought to design and validate a straightforward, cost-effective, and stability-indicating RP-HPLC method that complied with ICH Q2 (R1) guidelines8. According to the literature review, the drug has been assessed using liquid chromatographic techniques in biological fluids such as rat and human plasma, stability indicating RP-HPLC method, RP-HPLC method for pharmaceutical formulations, and UV spectrophotometric method 9 -11. Developing and validating a straightforward, specific sensitive, and accurate RP-HPLC method for estimating imatinib mesylate in bulk and marketed dosage form was the goal of the current study.

MATERIAL AND METHODS

Instruments

Chromatographic separation was conducted out on a Shimadzu LC-20AD HPLC system that contains a 20 µl loop volume Rheodyne injector, binary pumps, a degasser, a variable wave length detector, and a Phenomenex Luna C18 (250 mm x 4.6 mm, particle size: 5 µm). Data collection and processing were done using "LC solution" software.

Chemicals and Reagents

The pharmaceutical dosage form of imatinib mesylate, GLEEVAC tablets, with a claim of 100 mg, was purchased from a local pharmacy, whereas the pure form was obtained from Sun Pharma. o-Phosphoric acid of analytical grade (SD Fine Chemicals), HPLC standard water (Merck India), and acetonitrile of HPLC standard (Merck India) were utilized.

Preparation of Mobile Phase

In a 100 ml volumetric flask, 2.02 g of analytical-grade disodium hydrogen phosphate and 0.33 g of monosodium sodium phosphate were added together to produce phosphate buffer (0.1M). The volume was then adjusted with HPLC standard water to achieve the desired level. A membrane filter (Millipore Nylon disc filter of 0.45µ) was applied to filter the mobile phase, which comprised acetonitrile and phosphate buffer in a 50:50 v/v ratio, using a vacuum filter and sonicated in an ultrasonic bath for fifteen minutes.

Preparation of standard stock solution
Standard stock solution of Imatinib Mesylate: To create a stock solution of 100 ppm, 10 mg of imatinib mesylate, which had been precisely weighed, was transferred to a 100 ml volumetric flask and thoroughly dissolved in 50 ml of diluent. The volume was then made up of 100 ml of the same diluent. After passing the resultant solution through a 0.45µ µm membrane filter, it was ultrasonically sonicated for 10 minutes while being shaken repeatedly.

Making a Working Standard Solution
To produce a working solution of 25 ppm, pipette aside 2.5 ml of a standard stock solution and then dilute it with 10 ml of diluent.

Optimization of Analytical Wavelength
Imatinib Mesylate standard solution was scanned in the 200–400 nm range to optimize the analytical wave length. The spectra of standard solutions were examined, and the λmax at 255.20 nm was recorded for testing in order to establish the UV methods.

Optimized Chromatographic Conditions
Repeated trails were carried out by employing different mobile phases and varying the contents and flow rates in order to optimize the chromatographic conditions. A chromatogram having underwent optimization was eventually developed. The parameters for system suitability were
Retention time: 4.38 min Tailing factor: 1.29
Theoretical factor: 7106

The Calibration Curve Procedure
The calibration curve was constructed in a concentration range of 2-12 µg/ml by taking 0.2-1.2 ml of standard stock in a 10 ml volumetric flask and filling up to the mark with mobile phase. The solutions were filtered utilising a 0.45 µ membrane filter paper, and the filtrate was used for analysis.

Estimation of Imatinib Mesylate in tablet dosage Twenty Imatinib Mesylate tablets were weighed to determine their average weight. The tablets were then crushed to a powder. Imatinib Mesylate powder (10 mg) was dissolved in a 100 ml volumetric flask with some phase. The solution was sonicated for 15 minutes, then the volume was raised to 100 mL with mobile phase. The solution was filtered using a 0.2 µ membrane filter, and an appropriate aliquot was produced for analysis. The sample formulations were injected, and chromatograms were obtained at 250.20 nm. The amount of medication was estimated using the calibration curve.

RESULTS AND OBSERVATIONS:

A reverse phase HPLC method was developed with system appropriateness characteristics in mind, including tailing factor (T), run duration, number of theoretical plates (N) and cost effectiveness. The optimized technique eluted Imatinib Mesylate at 4.38 minutes. Figure 2 shows a standard solution (100µg/ml). The overall running time is 10 minutes. System suitability tests are a significant component of method development since they ensure the chromatographic system's proper performance. The retention time (Rt), peak asymmetric factor and number of theoretical plates (N) were determined for six replicate injections of the standard at the working concentration. The results are summarized in Table 1. In order to assess the method's applicability to commercial products "GLEEVAC" was chromatographed at a working concentration of 100µg/ml. The peak of sample was determined by comparing the retention time to the standard medication. System suitability parameters were within acceptable ranges for the chromatographed sample. The separated peak area was integrated, and the drug concentration was estimated using the peak area concentration relationship established during the standardization stage. The procedure provides a reliable test of the medication in the sample range from 98 to 102%, which is the usual threshold in any pharmaceutical quality control.
Figure 3 shows that the resultant chromatogram was free of excipient interference. The system suitability parameters were within their limitations. The developed approach was inexpensive and simple to do, and the retention time indicated that it was quick. The developed approach was validated based on ICH specifications.

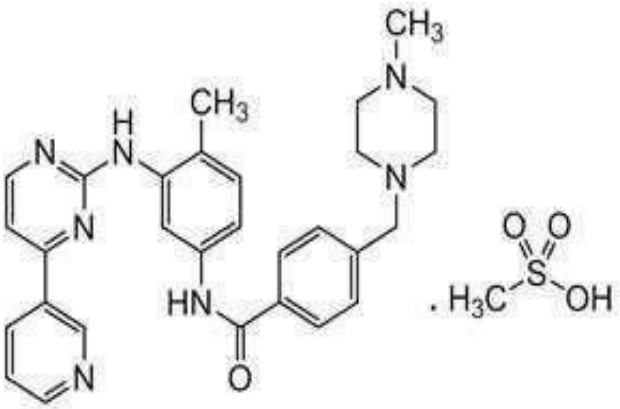


Figure 1: Structure of Imatinib Mesylate

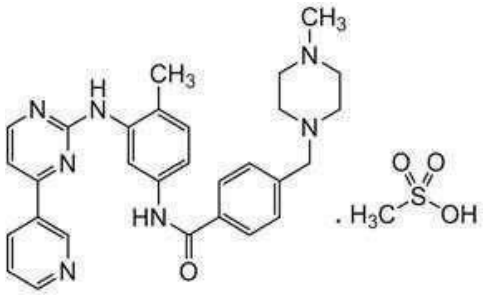


Figure 2: UV-Spectrum of Imatinib Mesylate

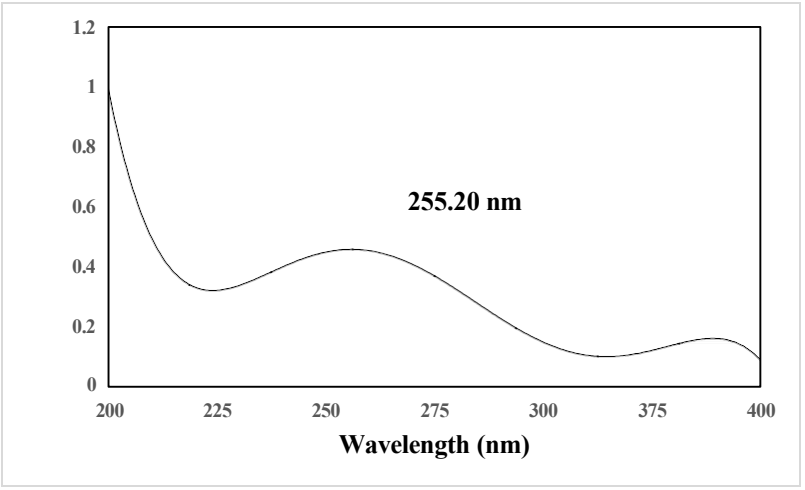
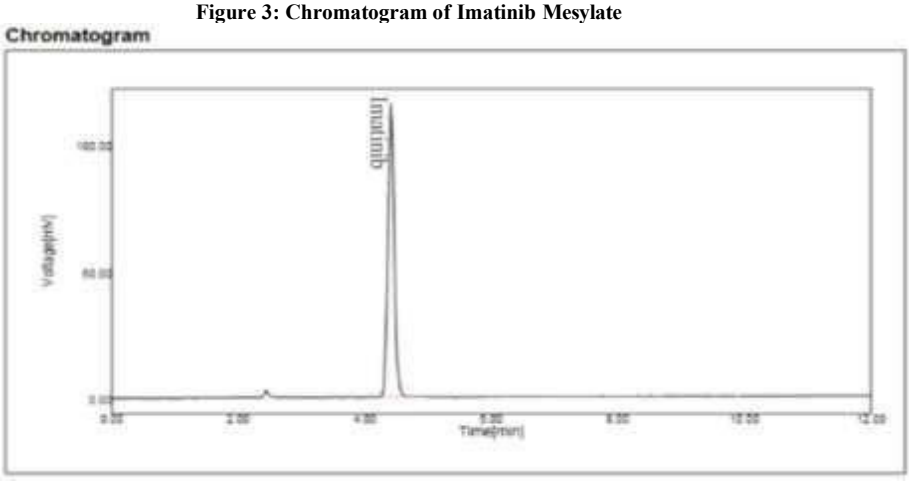


Figure 3: Chromatogram of Imatinib Mesylate



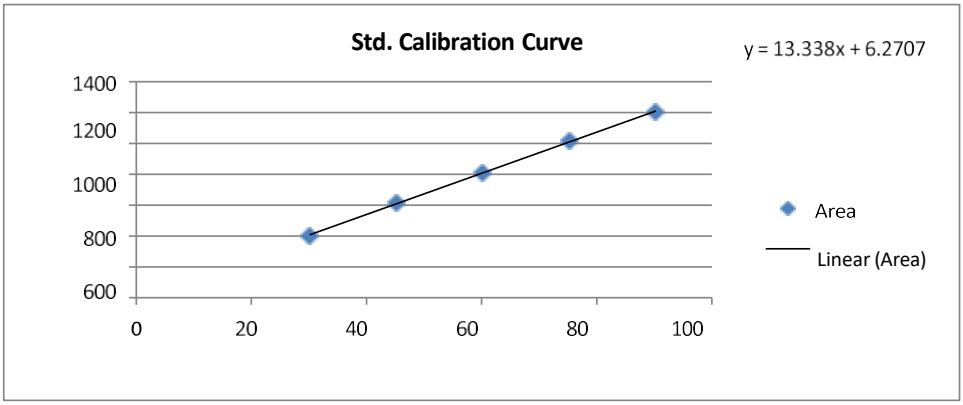


Figure 4: Calibration curve for Imatinib Mesylate Table 1: Results from system suitability studies

Properties	Value ± SD*	% RSD	Required Limits
Retention time (min)	0.0153	0.35	RSD < 1%
Theoretical plate (N)	835.3564	0.56	N > 2000
Tailing factor (T)	1.06	1.421	T < 2

**Average of six determinations*

Table 2: Linearity data for Imatinib Mesylate

S.N.	Concentration(µg/ml)	Peak area
1.	30	401.9248
2.	45	615.4218
3.	60	807.0649
4.	75	1016.8143
5.	90	1204.9289
Correlation coefficient		0.9998
Intercept		6.2707
Slope		13.338

Table 3: Precision Data of Imatinib Mesylate

Precision	day	Inter-day
Mean Area	524	799.4627
Standard Deviation	72	0.81
% RSD	89	0.71

Table 4: Imatinib Mesylate Recovery Data

Recovery level	Area	Amount added µg/ml	Amount found µg/ml	Amount received µg/ml	% Recovery
80%	1454.5264	48.0000	107.637	47.6037	99.17
80%	1442.5754	48.0000	106.7196	46.7196	97.33
80%	1459.4289	48.0000	107.9664	47.9664	99.93
100%	1620.5671	60.0000	119.8871	59.8871	99.81
100%	1607.2187	60.0000	118.8996	58.8996	98.17

100%	1631.0067	60.0000	120.6594	60.6594	101.10
120%	1787.2657	72.0000	132.2193	72.2193	100.30
120%	1791.9515	72.0000	132.5659	72.5659	100.79
120%	1766.0893	72.0000	131.6527	71.6527	99.13

Table 5: Robustness Result of Imatinib Mesylate

Parameters	Flow Rate		Wavelength (nm)		Mobile Phase	
	0.9 ml/min	1.1 ml/min	253.20	257.20	55:45	45:55
Mean Area	810.6330	818.1764	774.2195	769.4712	809.3237	797.9456
SD	7.5106	3.6848	10.5609	12.2741	7.8433	7.7453
%RSD	0.93	0.45	1.36	1.06	0.97	0.98

Table 6: Ruggedness Result of Imatinib Mesylate

Analyst	Analyst - 1	Analyst - 2
Mean	799.9524	797.4627
SD	6.4972	0.811
% RD	5.6789	0.712

DISCUSSION

Method validation

The process of validating an analytical technique involves conducting laboratory tests to determine whether the method's performance characteristics fulfil the requirements for the intended analytical application. The RP-HPLC method developed was verified in accordance with the International Conference on Harmonization (ICH) requirements for analytical process validation. The method was verified for system compatibility, specificity, linearity, accuracy, precision, robustness, and ruggedness, as well as the limit of detection (LOD) and limit of quantification (LOQ).

Specificity

Imatinib Mesylate separation from excipients was demonstrated by injecting sample solution containing excipients under ideal chromatographic conditions, which checked for excipient interference in the analysis of the sample solution. The method's great specificity is demonstrated by the absence of excipient peak interference with the imatinib mesylate peak.

Linearity and Range

The calibration curve, which showed linearity throughout a concentration range of 30-90 µg/ml and was plotted for multiple concentrations of working standards derived from standard drug solution of pure drug, is shown in Fig. 4 along with regression parameters. Three injections were made for each calibration.

Precision

An analytical procedure's precision is the degree of agreement (or scatter) between a set of measurements made by repeatedly sampling the same homogeneous sample under specified conditions 12. After preparing six solutions with identical concentrations, absorbance was measured. Table 3 displays the results in terms of the percentage RSD that fit within the constraints.

Accuracy

The accuracy was estimated by comparing the quantity to the average weight of marketed tablets using Imatinib Mesylate (equal to 25 mg Imatinib Mesylate, which is 80%, 100%, and 120% of the label claimed). Using the procedure indicated, chromatographic analysis was performed on this powder, which contained 25 mg of imatinib mesylate. Over three days, the resultant combinations were examined in triplicate. Accuracy was measured using the percentage recovery of added medication. Table 4 displays the findings.

Robustness

Small adjustments were made to several factors, including flow rate and wavelength, to demonstrate the method's robustness 13-14. Following wavelength adjustments, the flow rates were ± 2 nm and ± 1 ml/min, respectively. By computing the percentage RSD values, the method's robustness was assessed. Table 5 displays the findings.

Ruggedness

Various analysts' levels of ruggedness were assessed. The built analytical method was determined to be robust, as evidenced by the %RSD value of less than 2.

Table 6 reports the values.

Limits of Detection and Limits of Quantitation

The standard deviation of the response and the slope of the linearity curve were used to compute the LOD and LOQ of the current approach. Imatinib Mesylate LOD and LOQ values were determined to be 0.27 µg/ml and
0.80 µg/ml, respectively.

CONCLUSION

It was determined that the devised approach was easy, simple, accurate, exact, selective, and cost-effective for routinely estimating imatinib mesylate in pharmaceutical dosage form and bulk.

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