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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 Imatinib Mesylate in pure and pharmaceutical formulation. The separation was achieved on C18 G column (250 x 4.6 mm i.d, 5 µm), using o-Phosphoric acid (0.1% v/v): Acetonitrile 70:30 (v/v) as mobile phase, at a flow rate of 1.0 ml/min. Detection was carried out at 266 nm and drug eluted with a retention time of 3.25 min. Beer's law was obeyed in the concentration range of 5-30 µg/ml with correlation coefficient 0.999. The method had been validated according to ICH guide lines for specificity, linearity, accuracy, precision, robustness, ruggedness, LOD and LOQ. The method was found to be specific, accurate, and precise, robust, rugged and sensitive. The proposed method was convenient for quantitative routine analysis and quality control of Imatinib Mesylate in bulk and pharmaceutical dosage form.

Key words: Imatinib Mesylate, RP-HPLC, Validation.

INTRODUCTION

Imatinib Mesylate 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. Imatinib Mesylate 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. The drug is designed to inhibit tyrosine kinases such as Bcr-Abl and is used in the treatment of chronic myeloid leukemia (CML) and gastrointestinal stroma tumors.

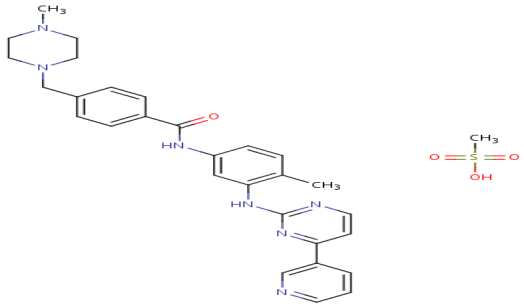

Figure 1. Structure of Imatinib Mesylate

The Chemical name of Imatinib Mesylate 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). Imatinib Mesylate

is a white crystalline powder which in freely soluble is distilled water, 0.1 N HCl, methanol and sparingly soluble in dimethyl ether .

Literature Survey revealed that the drug has been estimated by Liquid chromatograpic [2-9] methods in biological fluids like human plasma and rat plasma , Stability indicating RP-HPLC method[10], RP-HPLC method[11] for pharmaceutical formulations and UV Spectrophotometric [12] method has been reported so far.

The aim of present work was to develop and validate a simple, precise, sensitive and specific RP-HPLC method for estimation of Imatinib Mesylate in its bulk and tablet dosage form.

MATERIALS AND METHODS

Instrument

Chromatographic separation was performed on a Shimadzu LC-20AD HPLC 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

Imatinib Mesylate pure form was obtained as gifted sample from pharma industry and its pharmaceutical dosage form GLEEVAC Tablets labelled claim 100 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 70 : 30 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 stock solution

A stock solution was prepared by taking accurately weighed 10 mg of Imatinib Mesylate in 100 ml volumetric flask and initially dissolved in 50 ml mobile phase. Then the solution was made upto mark with mobile phase to obtain a concentration of 100 µg/ml and the resulting solution was sonicated for 15 min.

Optimization of Analytical Wave length

Optimization of analytical wave length was done by scanning Imatinib Mesylate standard solution in the range of 200-400 nm. By observing the spectra of standard solutions λ_{max} at 266 nm were taken for trials to develop UV method.

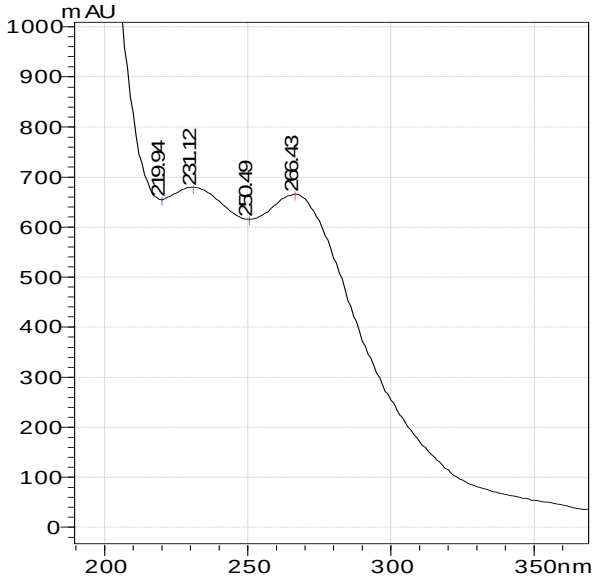

Figure 2. UV spectra of Imatinib Mesylate

Optimized chromatographic conditions

Optimizations of chromatographic conditions were done by performing different trails by taking different mobile phases and varying their compositions, flow rates. Finally an optimised chromatogram was obtained. The system suitability parameters were,

Retention time : 3.25 min
Tailing factor : 1.06
Theoretical factor: 3509.474

.**Procedure for calibration curve**

The calibration curve was prepared in a concentration range of 5-30 µg/ml by taking 0.5-3 ml of standard stock in 10 ml volumetric flask and the volume was made upto the mark with mobile phase. The resulting solutions were filtered through 0.45 µ membrane filter paper and the filtrate was used for analysis.

Estimation of Imatinib Mesylate in tablet dosage form

Twenty tablets of Imatinib Mesylate were weighed and their average weight was determined. Then the tablets were powdered. Powder equivalent to 10 mg of Imatinib Mesylate was taken in 100 ml volumetric flask and dissolved in some amount of mobile phase. The solution was sonicated for 15 min and the volume was made up to 100 ml with mobile phase. The resulting solution was filtered through 0.2 µ membrane filter from this solution suitable aliquot was prepared for analysis. The sample formulations were injected and chromatograms were recorded at 266 nm. The amount of drug was estimated from the calibration curve.

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 Imatinib Mesylate 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. The results are given in Table 1.

In order to test the applicability of the developed method to a commercial formulation, “GLEEVAC” was chromatographed at working concentration (100 µg/ml). The sample peak was identified by comparing the retention time with the standard drug. System suitability parameters were within the acceptance limits, ideal for the chromatographed sample. Integration of separated peak area was done and drug concentration was determined by using the peak area concentration relationship obtained in the standardization step. The protocol affords reproducible assay of the drug in the sample ranging between 98 and 102%, which is the standard level in any pharmaceutical quality control.

The obtained chromatogram showed no intereference from excipients, Fig. 3. The system sutibality parameters were within the limits. The developed method was economical, simple and the retention time showed that the method was rapid. The developed method was validated as per ICH guidelines.

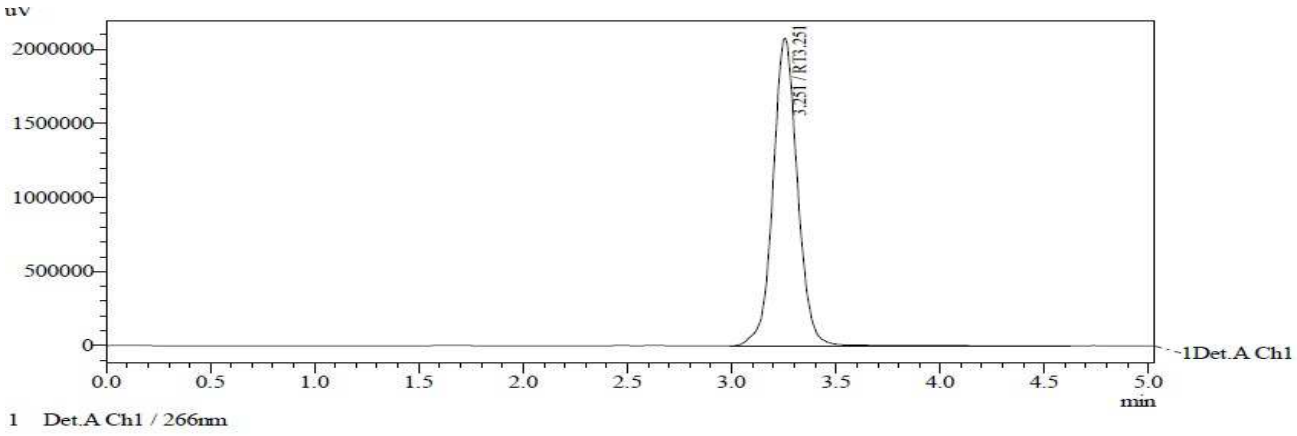

Figure 3. Chromatogram of Imatinib Mesylate

Table 1: Results from system suitability studies

Property	Values ± SD*	%RSD	Required Limits
Retention time (min)	3.251±0.0066	0.26	RSD<1%
Theoretical plates (N)	3509±14.88	0.56	N>2000
Tailing factor (T)	1.06±0.0223	1.421	T<2

**Average of six determinations*

Method validation[13]

Validation of the analytical method is the process that establishes by laboratory studies in which the performance characteristics of the method meet the requirements for the intended analytical application. RP-HPLC method developed was validated according to International Conference on Harmonization (ICH) guidelines for validation of analytical procedures. The method was validated for the parameters like system suitability, specificity, linearity, accuracy, precision, robustness, and ruggedness, limit of detection (LOD) and limit of quantitiation (LOQ).

Specificity

Specificity was checked for the interference of excipients in the analysis of sample solution and was determined by injecting sample solution with added excipients under optimized chromatographic conditions to demonstrate separation of Imatinib Mesylate from excipients. There is no interference of excipient peak on the peak of Imatinib Mesylate indicating the high specificity of method.

Linearity and Range

Calibration curve was plotted for different concentrations of working standards prepared from standard drug solution of pure drug, shown in Fig. 4 and showed linearity over a concentration range of 5-30 µg/ml, shown in Table 1, along with regression parameters in Table 2. Each calibration was injected three times. The calibration curve was performed in triplicate.

Table 1: Linearity data for Imatinib Mesylate

Concentration(µg/ml)	Peak Area
5	7936573
10	17038229
15	26354197
20	33741649
25	41943638
30	51762811

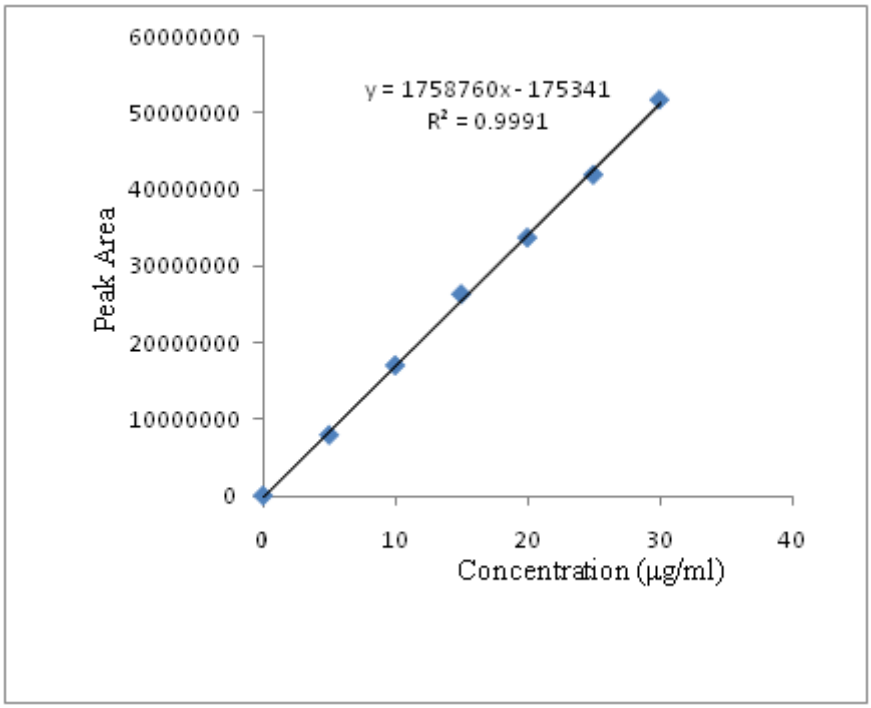

Figure 4. Calibration curve for Imatinib Mesylate

Table 2: Regression parameters table for Imatinib Mesylate

Regression Parameter	Imatinib Mesylate
Regression Equation*	Y=1758760x-175341
Slope (b)	1758760
Intercept (a)	175341
Correlation Coefficient (r^2)	0.9991

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, shown in Table 3.

Table 3: Precision for Imatinib Mesylate

Precision	Intra-day	Inter-day		
		Day 1	Day 2	Day 3
Mean Area	30800560	33438430	32942404	33724742
Standard deviation	158572.8	259271.2	206332.6	1955509.6
%RSD	0.51	0.77	0.62	0.57

Accuracy

Accuracy (recovery) of the method was obtained by spiking 80, 100 and 120% of Imatinib Mesylate 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 triplicates and accuracy was indicated by % recovery which was between 98.42 to 100.4%. The results were shown in Table 4.

Table 4: Accuracy of Imatinib Mesylate

% Spike level	Sample (µg/ml)	Std. Amount added (µg/ml)	Std. Amount found (µg/ml)	% Recovery	Statistical parameters
80	20	16	16.22	101.42	Mean=100.4
	20	16	16.11	100.72	SD=1.2
	20	16	15.85	99.08	%RSD=1
100	20	20	19.36	96.82	Mean=98.42
	20	20	19.85	99.25	SD=1.6
	20	20	19.23	96.19	%RSD=1.42
120	20	24	24.06	100.29	Mean=100.22
	20	24	24.05	100.22	SD=0.05
	20	24	24.04	100.19	%RSD=0.0432

Robustness

Robustness was carried by varying three parameters deliberately from the optimized chromatographic conditions like mobile phase composition, flow rate and wave length. The %RSD was found to be <2, shown in Table 5.

Table 5: Robustness results of Imatinib Mesylate

Parameters	Flow rate		Wave length		Mobile phase	
	0.9ml/min	1.1ml/min	264 nm	268 nm	80:20	60:40
Mean Area	33623081	32709781	32766070	32709783	52033621	51536456
SD	66417.55	135453.2	81615	135453	15357	140607.5
%RSD	0.2	0.41	0.25	0.42	0.3	0.27

Ruggedness

Ruggedness was determined between different analysts. The value of %RSD was found to be <2, showed ruggedness of developed analytical method. The values were shown in Table 6.

Table 6: Ruggedness results for Imatinib Mesylate

Analyst	Analyst-1	Analyst-2
Mean Area	33431727	33786502
SD	348181.5	206763.9
%RSD	1.0	0.61

Limits of detection and Limits of quantitation

The LOD and LOQ of the present method were calculated based on standard deviation of the response and slope of linearity curve. LOD and LOQ values of Imatinib Mesylate were found to be 1.16 µg/ml and 3.15 µg/ml.

CONCLUSION

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

REFERENCES

[1] Martindale: The complete drug reference. Pharmaceutical press, Lambeth High Street, London, 36th edition, **2009**; 773-774.
[2] Widmer et al, *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci*, **2004**, 803(2), 285-92.
[3] Thirumurthy et al, *J. of Chromatography B*, **2004**, 804, 431-434.
[4] Vivekanand et al, *Journal of pharmaceutical and biomedical analysis*, **2000**, 333(5), 879-889.
[5] D. Ivanovic, M. Medenica, B. Jancic and A. Malenovic, *J. chromatography B*, **2004**, 800 (1-2), 253-258.
[6] LO. Roos, HB. Jos, HM. Jan, T. Olaf van, *Biomedical chromatography*, **2007**, 21(7), 747-754.
[7] F. Solassol, L. Bressolle, V. Philibert, C. Charasson, F. Astre, *J. liquid chromatography and related technologies*, **2006,** 29 (20), 2957-2974.
[8] Rosasco et al, *J. liquid chromatography and related technologies*, **2005**, 28 (20): 3283-3292.
[9] Satyanarayana et al, *International Journal of Research in Pharmaceutical and Biomedical Sciences*, **2010,** Vol. 1 (1), 6-9.
[10] Bende G, Kollipara S, Kolachina V, Saha R. *Chromatographia* ,**2007**,66, 859–866.
[11] Arun Kumar Kuna, Jagadeesh Kumar Kuna. *Int J Pharm Pharm Sci*, **2011**,3(15),162-165.
[12] G. Bende, S. Kollipara, V. Sekar and R. Saha, *Die pharmazie*, **2008**, 63(9), 641-645.
[13] International conference on the harmonization of technical requirements for the registration of pharmaceuticals for human use (ICH) Q2 (R1), Validation of Analytical Procedures, Geneva, **2005.**