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Development and validation of RP-HPLC method for quantitative determination of imatinib mesylate in bulk drug and pharmaceutical dosage form

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ABSTRACT

The main aim of present work was to develop a simple, precise, rapid and reproducible isocratic reverse phase high performance liquid chromatographic (RP-HPLC) method for the estimation of Imatinib in pure and in tablet dosage form. An isocratic RP-HPLC was performed by utilizing Welchrom C_{18} column, (250 mm × 4.6 mm i.d., particle size 5 µm) maintained at ambient temperature and mobile phase composed of a mixture of 10 mM Phosphate Buffer (pH-3) Acetonitrile (50 : 50 v/v). The flow rate was adjusted 1.0 mL/min and UV detection was performed at 264 nm. The retention time of Imatinib peak was found at 3.053 minutes. The developed method was linear in the range of 2-10 µg/mL with correlation coefficient of 0.9999. The method was found to be specific and accurate with the overall mean % recovery of 99.85 %. The % RSD of intra and inter-day precision was found to be 0.833 and 0.877 respectively. The % RSD values were below two for intraday and inter- day precision indicated that the method was highly precise. The developed method was highly sensitive with LOD of 0.105 µg/mL and LOQ of 0.319 µg/mL. Assay content of Imatinib was determined and the mean % found for Imatinib was in good agreement with the label claim. The proposed method was statistically validated for its linearity, precision, accuracy, specificity, Robustness and ruggedness. The optimized methods proved to be specific, robust and accurate and can be used for quality control of Imatinib in bulk drug and pharmaceutical formulations.

Keywords: RP - HPLC, Imatinib mesylate, Validation, Quantitative determination, ICH guidelines.

INTRODUCTION

Imatinib (4-[(4-methylpiperazin-1-yl) methyl] -N-(4-methyl-3-{[4-(pyridin-3-yl) pyrimidin-2-yl] amino} phenyl) benzamide)[1] sometimes referred to by its investigational name STI-571, is a tyrosine-kinase inhibitor used in the treatment of multiple cancers, most notably *Philadelphia chromosome-positive* (Ph^+) chronic myelogenous leukemia (CML). Imatinib was one of the first cancer therapies to show the potential for such targeted action, and is often cited as a paradigm for research in cancer therapeutics. The developers of Imatinib were awarded the Lasker Award in 2009 and the Japan Prize in 2012. It is on the World Health Organization's List of Essential Medicines, a list of the most important medications needed in a basic health system.

Imatinib mesylate is not official in any pharmacopoeias. Literature survey revealed that there were few analytical methods have been reported for the estimation of above titled drug individually in biological samples as well as pharmaceutical dosage forms by spectrophotometry Spectrophotometry [2], LC-MS [3-7], HPLC [8-19]. However

most of the available methods have limitations such as long run time, poor resolution, uneconomical and low sensitivity. So based on the above mentioned reasons, infact an attempt has been made to develop a simple, precise, accurate, reproducible and robust RP-HPLC method for the simultaneous determination of Imatinib mesylate in pharmaceutical dosage form. Figure 1 shows chemical structure of Imatinib mesylate.

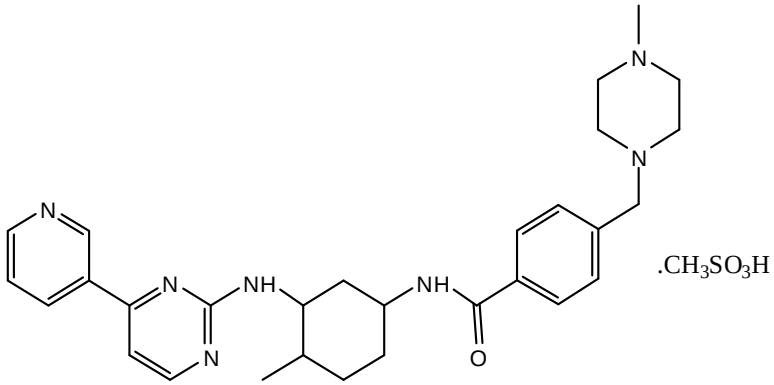

Figure 1. Chemical structure of Imatinib mesylate

MATERIALS AND METHODS

Materials used:
An analytically pure sample of Imatinib standard was gifted from Hetero Labs Ltd., Hyderabad India. All the chemicals were analytical grade. HPLC grade acetonitrile and triethylamine were obtained from Merck pharmaceuticals private Ltd., Mumbai, India. Methanol and water utilized were of HPLC grade and purchased from Merck specialties private Ltd., Mumbai, India. Commercial tablets of Imatinib formulation was procured from local pharmacy. Mitinab tablets containing Imatinib with labeled amount of 400 mg per tablet are manufactured by Glenmark Pharmaceuticals Ltd., Mumbai, India.

Instruments used:
Quantitative HPLC analysis was carried out on a isocratic reversed phase high performance liquid chromatography (Shimadzu LC-20AT Prominence Liquid Chromatograph) with a LC-20AT VP pump, manual injector with loop volume of 20 µL (Rheodyne), programmable variable wavelength Shimadzu SPD-20A Prominence UV-Vis detector and Welchrom C_{18} Column (4.6 X 250 mm, 5µm particle size). The HPLC system was equipped with "Spinchrome" software. In addition an electronic balance (Shimadzu TX223L), digital pH meter (Systronics model 802), a sonicator (spectra lab, model UCB 40), UV-Visible Spectrophotometer (Systronics model-2203) were utilized in this study.

Preparation of solutions and reagents:
Mobile phase:
10 mM of phosphate buffer was prepared by dissolving 6.056 gm of potassium di hydrogen orthophosphate 445 mL of HPLC grade water. To this 55 mL of 0.1M phosphoric acid was added. pH-3 with triethylamine. The above prepared phosphate buffer and acetonitrile mixed in the proportion of 50:50 v/v and was filtered through 0.45µm nylon member filter and degassed by sonication.

Preparation of standard stock and working standard of Drug Solution:
10 mg of Imatinib was accurately weighed and transferred to a 10 mL clean, dry volumetric flask with the addition of mobile phase, up to the mark and sonicate the solution to dissolve if necessary. This is primary stock standard solution of Imatinib 1000 µg/mL concentration. This stock solution was further diluted to obtain desired concentrations (linearity range solutions containing 2 µg/mL, 4 µg/mL, 6 µg/mL, 8 µg/mL and 10 µg/mL of Imatinib were prepared).

Preparation of stock solution for the commercially obtained tablets:
20 tablets of Mitinab (labeled claim 400 mg of Imatinib) were weighed and average weight was calculated. The tablets were crushed to get fine powder. Then a quantity of the powder equivalent to 100 mg of Imatinib was weighed in a 100 ml volumetric flask. The powder was then allowed to dissolve in mobile phase by sonication. Fill up the mark with mobile phase and the resulting solution was filtered through 0.45 µm membrane filters to remove insoluble materials. It was further diluted to obtain desired concentrations. Content of the tablets was calculated either from the previously plotted calibration graphs or by means of regression equations.

Optimization of mobile phase and method development:
Optimization of mobile phase was performed based on trial and error method. A series of trials were conducted in order to get proper optimized HPLC conditions. In the first instance a number of mobile phase compositions were tried such as methanol: water, acetonitrile: HPLC grade water, methanol: acetonitrile: water in different ratio without adjusting pH. Eventually the mobile phase comprising of 10 mM Phosphate Buffer (pH-3): Acetonitrile (50: 50 v/v) found to give best system suitability parameters and also obtained sharp, well-gaussian shape peak. This mobile phase was also selected as the diluent because the drug is freely soluble in the mobile phase. This mobile phase pH which is safe for column life and suitable for analyte stability. The stationary phase made up of Welchrom C_{18} column with 4.6 X 250 mm, 5 µm were observed and they are found to be utmost suitable for Imatinib. The ultra violet spectrum of diluted solutions of various concentrations of maximum absorption detection of Imatinib was recorded by utilizing UV Systronic double beam SL 2203. An absorption maximum was found to be 264 nm. From the spectrum λmax of Imatinib 264 nm was selected for the analysis. The developed method gave symmetric peak at retention time of 3.053 minutes and satisfied all the peak properties as pursuance of ICH guidelines.

Validation of analytical method:
The developed analytical method was further subjected to validation in pursuance of ICH Q2 (R1) guidelines [20]. The parameters evaluated were system suitability, specificity, linearity, precision, accuracy, robustness, limit of detection (LOD) and limit of quantitation (LOQ).

System suitability:
The chromatographic systems used for analysis must pass system suitability limits before sample analysis can commence. Set up the chromatographic system allow the HPLC system to stabilize for 40 minutes. Inject blank preparation (single injection) and standard preparation (six replicates) and record the chromatograms to evaluate the system suitability parameters like resolution (NLT 2.0), tailing factor (NMT 1.5), theoretical plate count (NLT 3000) and % RSD for peak area of six replicate injections of Imatinib standard (% RSD NMT 2.0).The parameters such as tailing factor, % RSD and theoretical plates were studied and found satisfactory. The system suitability data and the optimum chromatographic conditions are reported in Table 1.

Linearity:
Under proposed experimental conditions, the relationship between the area and concentration of Imatinib was studied. Linearity was checked by preparing standard solutions at 5 different concentration levels of each of Imatinib. Imatinib standard solutions (2, 4, 6, 8, 10 µg/mL) were injected into the HPLC system to get the chromatograms. The average peak area and retention time were recorded. The calibration curve was constructed between concentrations versus peak area by the prepared concentration of 2 -10 µg/mL of stock solution. The linearity range was found to be 2 -10 µg/mL and the results are presented in Table 2. The standard chromatograms of Imatinib calibration standards are depicted in Figure 2 to Figure 6. Results show that a phenomenal correlation exists between peak area and concentration of drug within the linearity range. The calibration graph of Imatinib is presented in Figure 7. Summary of validation parameters are shown in Table 3.

Specificity:
The specificity of the method was determined by the prepared standard, sample solutions and the blank solution were injected and check any other excipients interference occurs or not. It was shown that the excipients present in pharmaceutical tablets of Imatinib did not show any interference with Imatinib peak because no excipients peaks appear in the chromatogram of the prepared tablet. Furthermore the well-shaped peaks also indicate the specificity of the method. The specificity results are tabulated in Table 4.

Precision:
Precision of an analytical procedure is referred to as degree of scatterness between a series of observations obtained from multiple sampling of same homogenous sample in given conditions. The terms Intraday (repeatability) where as interday precision (intermediate precision) were investigated by replicating analysis for three concentrations (2 µg/mL, 4 µg/mL, 6 µg/mL) to the use of analytical procedure within same laboratory conditions over a short period of time by same analyst and same instrument. For interday precision, the analysis was carried out for three consecutive days at the same concentration levers as used in intraday precision. Regarding the intraday precision was carried out by using the 3 concentrations at different time interval in the day. The area was recorded as % RSD. The results of intraday and interday precision are shown in Table 5 and 6 respectively.

Accuracy/Recovery:
The accuracy of the method was found out by standard addition method. For the previously analyzed sample 6 µg/mL. A known amount of standard drug was added at 50 %, 100 % and 150 % level. The concentrations were re-analyzed with the above described procedure. The percent recovery of the triplicate solutions was determined and average of the percent recovery was calculated. The recovery results are presented in Table 7.

Robustness
Robustness of the method is its ability to remain unaffected by small changes in variety of parameters such as the slight variation in acetonitrile percentage composition of the mobile phase, flow rate, detection wavelength. The results of robustness study is shown in Table 8 indicated that the small change in the conditions did not significantly affect the determination of Imatinib.

LOD and LOQ:
Limit of detection is the lowest concentration in a sample that can be detected, but not necessarily quantified under the stated experimental conditions. The limit of quantitation is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy. Limit of detection and limit of quantitation were calculated using following formula LOD = 3.3 σ/S and LOQ = 10 σ/S, where, σ is the standard deviation of response and S is the slope of the calibration curve. The LOD and LOQ values are presented in Table 9. The results of LOD and LOQ supported the sensitivity of the developed method.

Application to commercial tablet
Using the developed RP-HPLC chromatographic method, assay of Imatinib in tablet was carried out as mention in the experimental section. Six replicate determinations were made. Satisfactory results were obtained and were good agreement with the label claim and assay results were shown in Table 10. The results were very close to the labeled value of commercial tablets. The representative sample chromatogram of Imatinib is shown in Figure 8.

RESULTS AND DISCUSSION

The present study was aimed at developing a precise, sensitive, rapid and accurate HPLC method for the analysis of Imatinib in bulk drug and in pharmaceutical dosage forms. In order to achieve extraordinary retention time and peak asymmetry, C_{18} stationary phase column (250 mm X 4.6 mm i.d, 5 µm particle size) and mobile phase composed of methanol a mixture of 10 mM Phosphate Buffer(pH-3): Acetonitrile (50 : 50 v/v) at a flow rate of 1mL/min was selected. The retention time for Imatinib was found to be 3.053 minutes. UV spectra of Imatinib showed that the drug absorbed maximum at 264 nm, so this wavelength was selected as the detection wavelength. The correlation coefficient (0.9999) of regression was found almost equal to one in the range of 2 - 10 µg/mL which states that the method was good linear to the concentration versus peak area responses. The comparison of chromatograms of placebo, standard and sample, there was no interference observed from the peaks of placebo, standard and sample. It shows that the method is specific. The precision studies were performed and the % RSD of the determinations was found to be 0.833 for intra-day precision and 0.877 for inter-day precision which are within the limits which indicates that the proposed method was found to be precise. The accuracy of the method was found to be good with the overall % RSD for recovery at 50 %, 100 % and 150 % levels were all within the limits which indicate that the proposed method was found to be accurate. Method validation following ICH guidelines indicated that the developed method had high sensitivity with LOD of 0.105µg/mL and LOQ of 0.319 µg/mL. The method was found to be robust even though on slight deliberate variation in the method conditions did have a tiny effect on the peak asymmetry, plate count and retention time and all are within the limits which indicated that the method is robust. Range is the minimum and maximum concentration of the sample at which the analytical procedure gives

reproducible results. Range can be determined by linearity, accuracy and precision studies. The method was found acceptable across wide range of concentration 2-10 µg/mL. The retention time of the sample solution of Imatinib tablet was found to be 3.053 minutes, which is similar to that of the standard solution of Imatinib. This indicates that there is no drug-excipient interference and the drug is decorously resolved by the developed method. Robustness determines the reproducibility of the test result with small and deliberate variations in the method parameters. The experiment was carried out by slightly changing the ratio of methanol in mobile phase, detection wavelength and flow rate. The effectiveness of the deliberate little variations was observed on the flow rate and mobile phase composition. The statistical data shows no significant variations in the above said parameters which indicate that the method is robust.

The developed method was successfully applied for the determination of Imatinib in bulk drug and tablet dosage form. The assay result was complied in Table 10 and also shows that there is no interference of the tablet matrix with the drug. The assay results satisfactory results were obtained and were in a good agreement with the label claim. Very low % relative standard deviation shows that this method can be easily utilized for the estimation of Imatinib in bulk drug and tablet dosage form.

Table 1. Optimum chromatographic conditions and system suitability data

Parameter	Chromatographic conditions
Instrument	SHIMADZU LC-20AT prominence liquid chromatograph
Column	WELCHROM C_{18} Column (4.6 mm i.d. X 250 mm, 5 µm particle size)
Detector	SHIMADZU SPD-20A prominence UV-Vis detector
Diluents	10 mM Phosphate Buffer(pH-3): Acetonitrile (50 : 50 v/v)
Mobile phase	10 mM Phosphate Buffer(pH-3): Acetonitrile (50 : 50 v/v)
Flow rate	1 mL/min.
Detection wave length	UV at 264 nm.
Run time	5 minutes
Temperature	Ambient temperature (25 °C)
Volume of injection loop	20 µL
Retention time (t_R)	3.053 min
Theoretical plates [th.pl] (Efficiency)	17,831
Theoretical plates per meter [t.p/m]	356629
Tailing factor (asymmetry)	1.115

Table 2. Calibration data of the proposed method for the estimation of Imatinib

S.No	Concentration, µg/mL.	Retention time, (t_R) min.	Peak area, mV.s.
1.	0	-	0
2.	2	3.053	303.88
3.	4	3.053	601.726
4.	6	3.053	903.498
5.	8	3.053	1211.212
6.	10	3.053	1498.801

Table 3. Summary of validation parameters

Parameter	Result
Linearity range (µg/mL)	2-10 µg/mL
Liner Regression equation (Y = a + bX)	Y = 150.25 x + 1.916
Intraday precision (% RSD) (n=3)	0.833
Interday precision (% RSD) (n=3)	0.877
% Recovery	99.85
LOD (µg/mL)	0.105
LOQ (µg/mL)	0.319
Robustness	Robust

Table 4. Specificity study for Imatinib

Name of the solution	Retention time, (t_R) min.
Mobile phase	No peaks
Placebo	No peaks
Imatinib 10 µg/mL	3.053 min.

Table 5. Results of precision study (intra-day) for Imatinib

Sample	Concentration (µg/mL)	Injection no.	Peak area (mV.s)	% RSD[#]
Imatinib	6	1	930.511	0.833
		2	931.312	
		3	929.514	
		4	930.311	
		5	931.413	
		6	930.611	

[#] *Acceptance criteria < 2.0.*

Table 6. Results of precision study (inter-day) for Imatinib

Sample	Concentration (µg/mL)	Injection no.	Peak area (mV.s)	% RSD[#]
Imatinib	6	1	931.411	0.877
		2	930.322	
		3	929.412	
		4	931.414	
		5	930.453	
		6	931.453	

[#] *Acceptance criteria < 2.0.*

Table 7. Recovery data for Imatinib

Type of recovery in % level	Sample conc. (µg/mL)	Amount of the standard added (µg/mL)	Total conc. (µg/mL)	Found conc. (µg/mL)	% recovery	% RSD
50	6	3	9	8.98	99.77	0.22
			9	8.99	99.88	
			9	8.97	99.66	
100	6	6	12	11.99	99.91	0.16
			12	11.98	99.83	
			12	11.97	99.75	
150	6	9	15	14.97	99.8	0.13
			15	14.98	99.86	
			15	14.99	99.93	

RSD: relative standard deviation.

Table 8. Robustness results of Imatinib.

S. no	Parameter	Optimized	Used	Retention time (t_R), min	Plate count[$]	Peak asymmetry[#]	Remark
1.	Flow rate (±0.2 mL/min)	1.0mL/min	0.8 L/min	3.089	17,890	1.112	*Robust
			1.0 L/min	3.053	17,831	1.115	*Robust
			1.2 L/min	3.002	17,812	1.114	*Robust
2.	Detection wavelength (±5 nm)	264 nm	259 nm	3.053	17,830	1.114	Robust
			264 nm	3.053	17,831	1.115	Robust
			269 nm	3.053	17,831	1.115	Robust
3.	Mobile phase composition (phosphate buffer: Acetonitrile)	50:50 v/v	55:45v/v	3.065	17,843	1.118	*Robust
			50:50 v/v	3.053	17,831	1.115	*Robust
			45:55v/v	3.019	17,820	1.110	*Robust

*Acceptance criteria (Limits):[#]Peak Asymmetry < 1.5, [$] Plate count > 3000, * Significant change in Retention time.*

Table 9. Limit of detection and Limit of quantitation

Limit of Detection(LOD)	0.105 µg/mL
Limit of Quantitation(LOQ)	0.319 µg/mL

Table 10. Assay results of Imatinib formulation

S. No	Formulation(tablets)	Labelled claim	Amount found (mg)	Assay ± SD*
1	Mitinab	400 mg/tablet	399.88 mg/tablet	99.94 ±0.41

*SD: standard deviation. *average of six determinations.*

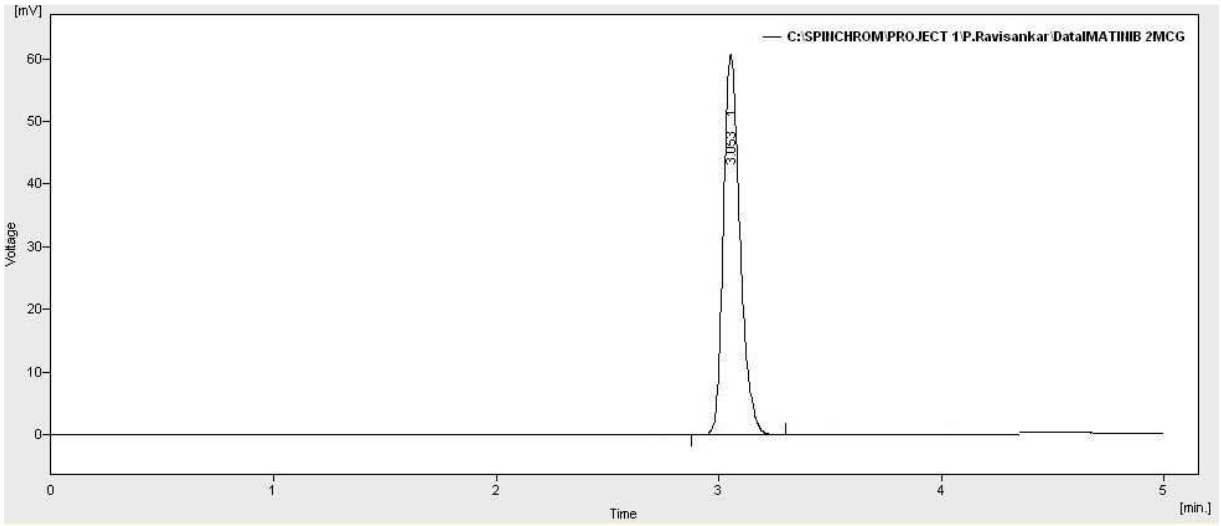

Figure 2. Standard chromatogram of Imatinib (2 µg/mL)

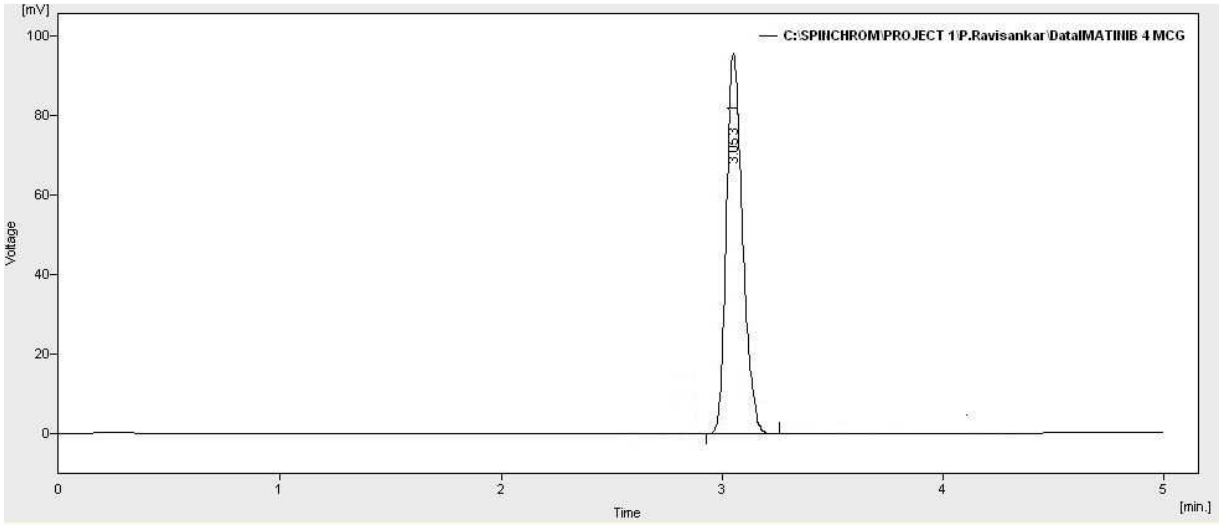

Figure 3. Standard chromatogram of Imatinib (4 µg/mL)

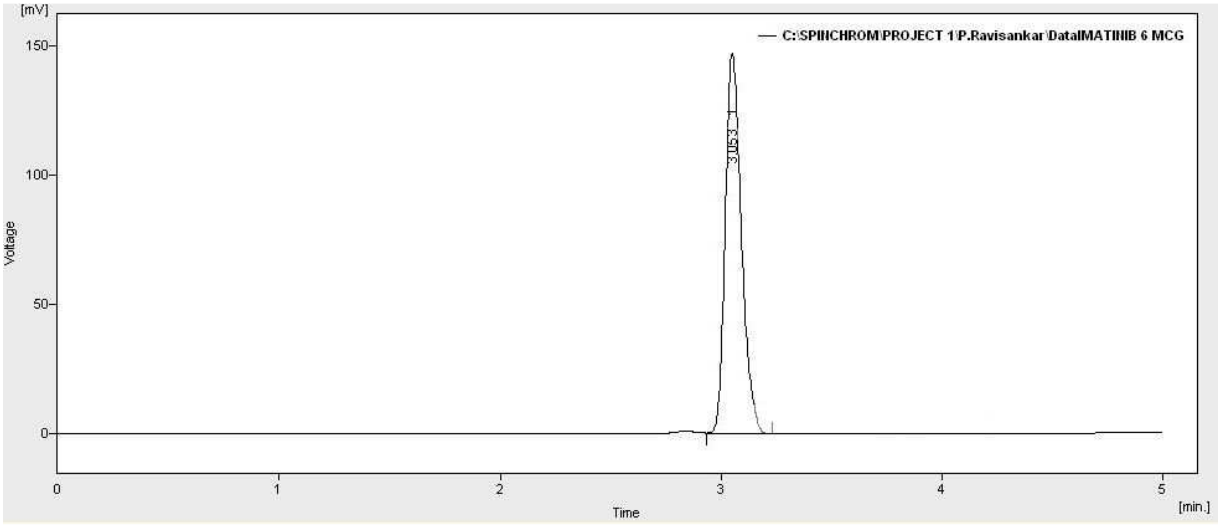

Figure 4. Standard chromatogram of Imatinib (6 µg/mL)

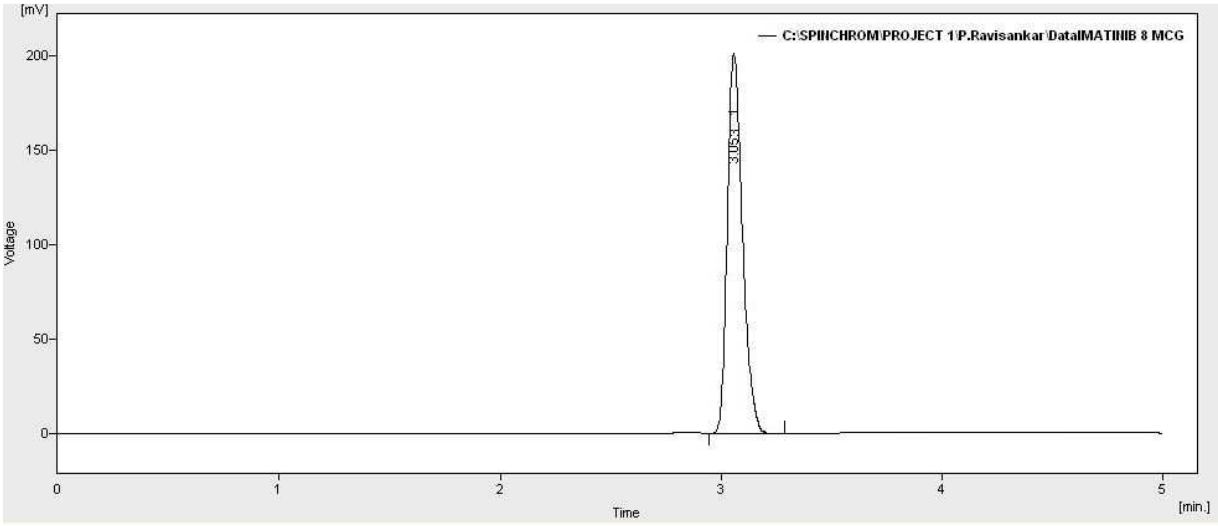

Figure 5. Standard chromatogram of Imatinib (8 µg/mL)

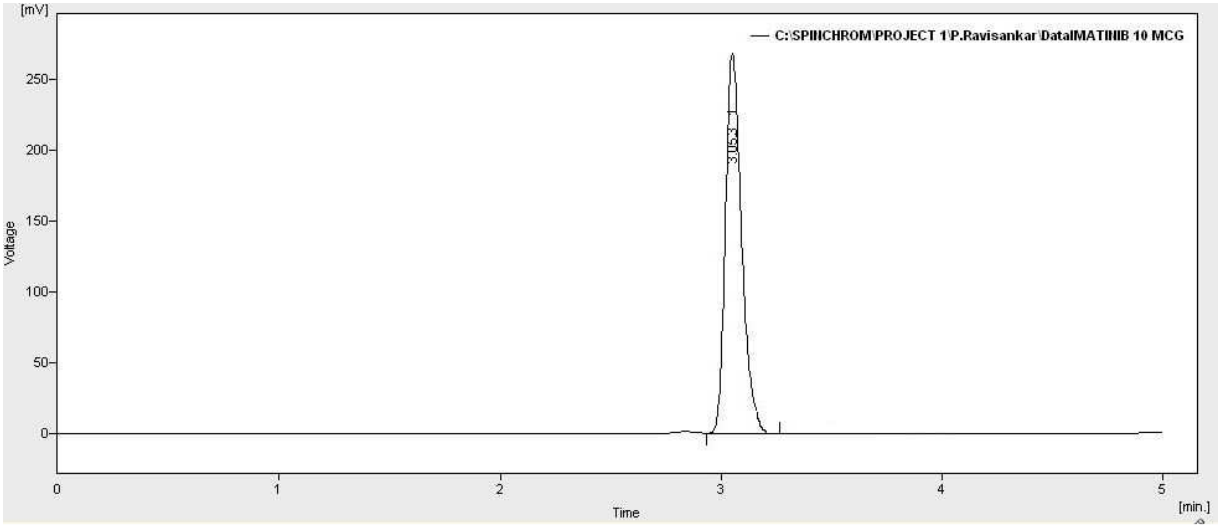

Figure 6. Standard chromatogram of Imatinib (10 µg/mL)

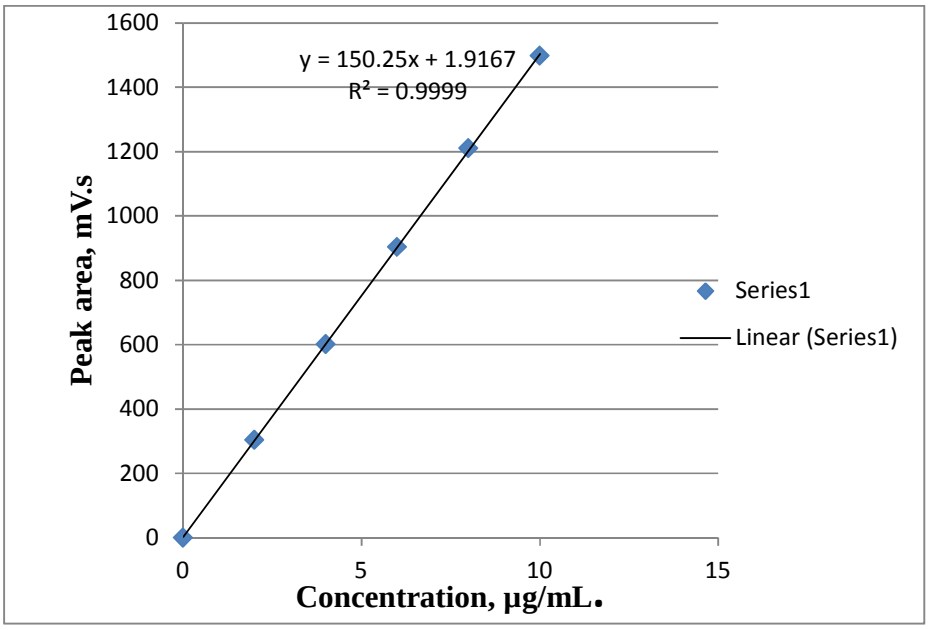

Figure 7. Calibration plot of Imatinib

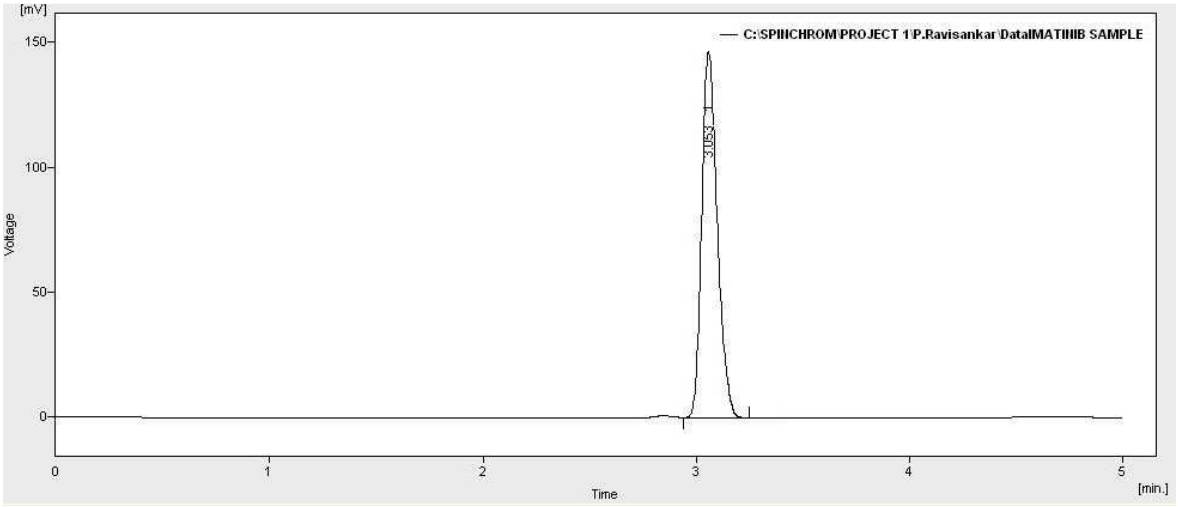

Figure 8. Chromatogram of marketed formulation Mitinab

CONCLUSION

The strange developed RP-HPLC method provides a convenient and efficient method for the quantitative estimation of Imatinib in bulk drug and pharmaceutical dosage form. The strange analytical method explained in this paper is validated subject to guide lines of ICH Q2 (R1). The Statistical analysis evolved in this research study denotes that the precision and accuracy found to be good. The validation of the method clearly indicates that the results obtained through the analysis are up to the mark and satisfactory. The results ultimately achieved through this experiment vividly explain that the analytical method was unambiguously highly sensitive, simple, specific, linear, precise, accurate and robust and reproducible with short run time. This method has various advantages like less retention time (retention time only 3.053 min), quick analysis time (runtime only 5 minutes), low solvent consumption, outstanding peak symmetry, user friendly and convenient approach and also shown to specific, linear, accurate, precise, phenomenal sensitive and robust. The mobile phase can be easily prepared and diluents are economical and readily available and it does not need sample preparation with sophisticated techniques. All these key features proposed that this method can be considered as advantageous over other methods. The drug solutions employed in the study were stable up to 48 hours. These attribute the high quality of the method. This developed method for quantitative estimation of Imatinib in bulk drug and tablet dosage form has been developed and found to be applicable for the routine analysis of Imatinib in bulk and tablet dosage forms without any interference from the excipients. Thus this method can be applied systematically for the estimation of Imatinib in the formulation of tablets and it is also highly useful to adopt for regular quality control analysis of Imatinib mesylate in API as well as pharmaceutical dosage forms.

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REFERENCES

[1] Current Index of Medical Specialities (CIMS-129, Apr-July [update 2]) UBM Medica India Private Limited; Bangalore: **2015**.p.356.
[2] Kumar Raja J, Sundar V.D; Magesh AR, Nandha Kumar S, Dhanaraju M.D. *International Journal of Pharmacy&Technology*, **2010**, 2, 490-495.
[3] Bakhtiar R, Khemani L, Hayes M, Tse F. *J.Pharm.Biomed.Anal*, **2002**, 28, 1183-1194.
[4] Bakhtiar R; Lohne J; Ramos L; Khemani L; Hayes M; Tse F. *J.Chromatogr. B*, **2002**, 768, 325-340.
[5] Parise RA, Ramanathan RK, Hayes MJ, Egorin MJ. *J. Chromatogr. B*, **2003**, 791, 39-44.

[6] D'Avolio A, Simiele M, De Francia S, Ariaudo A, Baietto L, Cusato J. *J Pharm Biomed Anal*, **2012**, 59, 243-248.
[7] Vivekanand VV, SreenivasRao D, Vaidyanathan G, Sekhar NM, Avijit Kelkar S, Ramachandra Puranik P. *J.Pharm.Biomed. Anal*, **2003**, 33, 879-889.
[8] Medenica M, Janic B, Ivanoic D, Malenovic A. *J. Chromatogr.A*, **2004**, 1031, 243-248.
[9] Ivanovic D, Medenica M, Janic B, Malenovic A. *J.Chromatogr. B*, **2004**, 800, 253-258.
[10] Mara A. Rosasco; Mara A. Moyano; Mara T. Pizzorno & Adriana. *Journal of Liquid Chromatography & Related Technologies*, **2007**, 28, 3283-3292.
[11] Bende G, Kollipara , Movva S, Moorthy G, Saha R. *J Chromatogr Sci*. **2010**, 48, 334-341.
[12] Aleti P, Venisetty RK, Kamarapu SK. *International Journal of Pharmacy and Biological Sciences*, **2011**, 1, 14-18.
[13] Arun Kumar K, Jagadeesh Kumar K. *International Journal of Pharmacy and Pharmaceutical Sciences*, **2011**, 3, 975-985.
[14] Venkataramanna M, SudhakarBabu K, Narayanareddy P. *Global Journal of Analytical Chemistry*, **2011**, 2, 198-207.
[15] Khalid Mohammed Alkharfy, Rao Muzaffer Ali Khan, Majed Al-Asmari, Baderelddin Hashim Alhadeyah. *Journal of pharmacy & BioAllied Sciences*, **2013**, 5, 49-52.
[16] Sandhya P, Vishnu Priya P, Shyamala, Anjali Devi N, Sharma JVC, WJPPS, **2013**, 3, 682-688.
[17] Ravi kanth Reddy. P, Rajavel P, Venkateswara Rao P. International Journal of Research in Pharmaceutical and Nano Sciences, **2013**, 2, 689-696.
[18] Alkharfy KM, Khan RM, Al-Asmari M, et al. *J Pharm Bioallied Sci*, **2013**, 5, 49-52.
[19] Naga Sindhu S, Srinivasa Rao Y, Hemant Kumar T, Vara Prasad Rao K. *Der Pharmacia Littre*, **2015**,7, 33-38.
[20] International Conference on Harmonization. Validation of Analytical Procedures: Text and Methodology Q2 (R1), **2005**.