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NEW ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR IMATINIB MESYLATE BY RP-HPLC METHOD

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ABSTRACT

A simple, accurate, precise, and, economical RP- HPLC method has been developed for the rapid estimation of Imatinib Mesylate. The separation was achieved on C18 column, X terr 18.5 µm, using Phosphate buffer (55%): Acetonitrile (45%) as mobile phase, with a injecting volume of 5 µl at a flow rate of 0.9 ml/min. Detection was carried out at 256 nm and drug eluted with a retention time of 3.648 min. The method had been validated according to ICH guide lines for accuracy, precision, linearity, robustness, ruggedness, LOD and LOQ. The method was found to be accurate, and precise, robust, rugged and sensitive. The proposed method was convenient for quantitative routine analysis and quality control of Imatinib Mesylate in tablets. The results of compound were good agreement with the label claims.

Keywords: *Imatinib Mesylate, Acetonitrile, Phosphate buffer.*

1. INTRODUCTION

Imatinib mesylate is a tyrosine-kinase inhibitor used in the treatment of multiple cancers, most notably Philadelphia chromosome-positive (Ph+) chronic myelogenous leukemia (CML)[1] Imatinib blocks this BCR-Abl enzyme, and stops it from adding phosphate groups. As a result, these cells stop growing, and even die by a process of cell death (apoptosis)[2]. Because the BCR-Abl tyrosine kinase enzyme exists only in cancer cells and not in healthy cells, imatinib works as a form of targeted therapy—only cancer cells are killed through the drug's action[3]. In this regard, 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[4-13]. The chemical structure of Imatinib mesylate is given in Fig 1. Chemically it is 4-[(4-Methyl-1-piperazinyl) methyl]-N-[4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl] amino]-phenyl] benzamide methane sulfonate with empirical formula $C_{29}H_{31}N_{7O} \bullet CH_4SO_3$. In the present work, were made to determine Imatinib mesylate in pure formulation by using RP-HPLC. The proposed method is simple and suitable for routine determination of Imatinib mesylate in tablet dosage form.

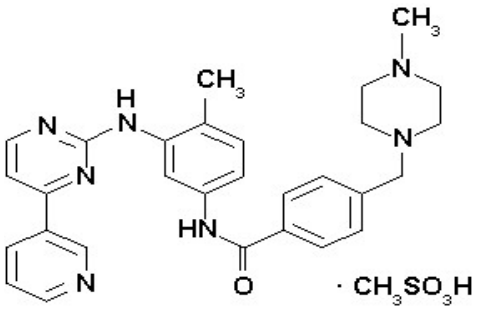

Fig. 1: Structure of Imatinib Mesylate

2. MATERIALS AND METHODS

2.1. Drugs and Chemicals: Imatinib Mesylate pure form was obtained as gifted sample from Strides Arco Labs, Bangalore. Phosphate buffer, Acetonitrile of HPLC grade (Merck India), Water of HPLC grade (Merck India) were used.

2.2 Instrument: Chromatographic separation was performed on a RP - HPLC system equipped with a C18 column (Xterr RP-18.5 µm), Quaternary Gradient pumps, G1379A and G1322A degasser, Diode array detector G 1314A, YL – Clarity software, PDA detector, Cooler G 1330B was used to collect and process the data.

2.3 Preparation of mobile phase

2.3.1 Preparation of Phosphate buffer: Weighed 7.0 grams of Potassium dihydrogen phosphate into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water. Adjusted the pH to 4.5 with ortho phosphoric Acid.

2.3.2 Preparation of mobile phase: Mixed above Phosphate buffer 60% and Acetonitrile 40% and degassed in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

2.4 Preparation of standard stock solution

2.4.1 Standard Stock solution I: Accurately weigh and transfer 10mg of Imatinib Mesylate Working standard into a 10 mL volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

2.4.2 Standard Stock solution II: Further pipette 0.7 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

2.5 Preparation of sample solution

2.5.1 Sample Stock solution I: Weighed 5 Imatinib Mesylate Tablets and calculated the average weight. Accurately weighed the sample equivalent to 10 mg of Imatinib Mesylate and transferred into a 10 mL volumetric flask. Added about 7 mL of diluent and sonicated to dissolve it completely and made volume up to the mark with diluent. Mixed well and filtered through 0.45µm filter.

2.5.2 Sample stock solution II: Further pipette 0.7 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

2.5.3 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 λmax at 256 nm were taken for trials to develop UV method.

2.6 Procedure: Method development of Imatinib Mesylate was carried out by using 45% Acetonitrile and 55% Phosphate buffer as Mobile phase by trial and error. The Chromatogram of Imatinib Mesylate and its symmetry was better with 45% Acetonitrile and 55% Phosphate buffer. Hence the method development and validation was done by using mobile phase as diluents.

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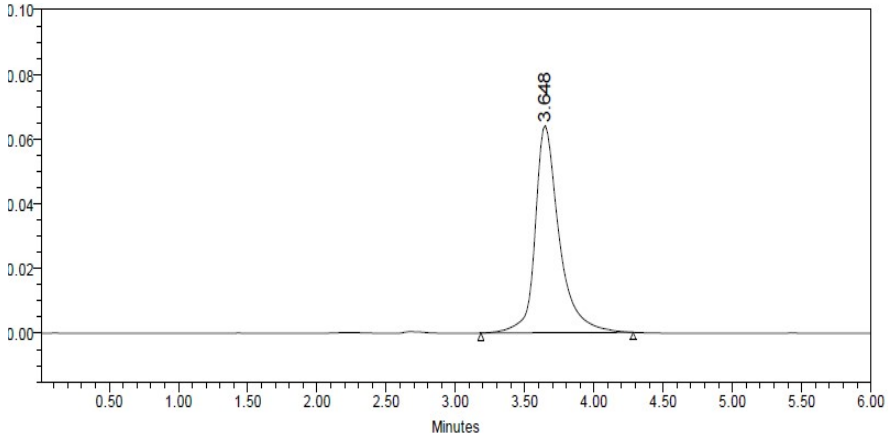

Fig. 2: Strategy for method development

3. RESULTS

3.1. System Suitability

To verify that the analytical system is working properly and can give accurate and precise results the system suitability parameters are to be set. Injected standard solution and recorded the chromatograms.

Typical chromatogram of standards (system suitability)

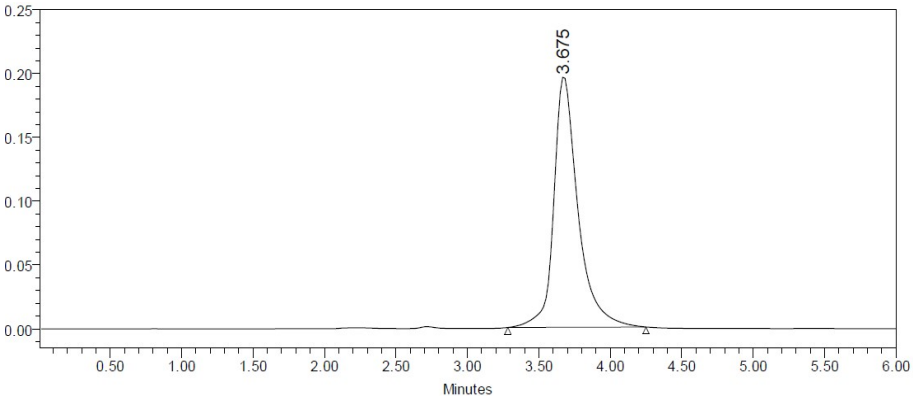

Fig.3: Typical chromatogram of standard

Acceptance criteria: Tailing factor for Imatinib Mesylate peaks should be NMT 1.2%**.** %Relative standard deviation for replicate injection for each peak should be NMT 2%

Table - 1: System Suitability Results

S. No.	System suitability parameters	Results
1	Tailing factor peak should be NMT 2%	**1.3%**
2	%Relative standard deviations should be not more than 2% for 5 replicate injections for each peak	**2%**

3.2. Initial Assay

Initial assay has been carried out 3 times by the same analyst with the same instrument and same column. The difference between individual assays was not more than 1.0 % (RSD). The average of three-assay values was taken for further validation process.

Repeatability was demonstrated by taking the RSD of retention time and AUC of chromatogram of 6 replicate injections of standard, which were computed and was within 1%. The average of three-assay values was taken for further validation process.

3.2.1 Standard Dilution

i) Preparation of stock solution-I: Accurately weigh and transfer 10 mg of Imatinib Mesylate Working standard into a 10 mL volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

ii) Preparation of stock solution-II: Further pipette 0.7 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

Table 2: Initial assay area and RSD

S. NO.	Imatinib Mesylate	
	Area	Retention Time
1	2306452	3.675
2	2304223	3.669
3	2309132	3.664
4	2309241	3.662
5	2306796	3.660
Avg	2307169	0.09

From Table 2, it can be seen that RSD of Retention Time and Area of six replicate is within 2%, which proves repeatability.

Results and Conclusion of Initial Assay for Three Trials

Table 3: Results and conclusion of Initial Assay

Assay	Imatinib Mesylate
1	99.44%
2	98.78%
3	99.65%
Avg	99.53
RSD	0.3914

From Table 3, we can see that RSD of 3 initial Assays performed in different timings are within 2%. This complies with the Initial assay of results

3.3. Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of homogeneous sample. The precision of analytical method is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) of series of measurement.

Table 4: Results obtained for system precision studies

S.No.	Injection No.	Imatinib Mesylate	
		Area	Retention Time
1	Injection-1	2306452	3.675
2	Injection-2	2304223	3.669
3	Injection-3	2309132	3.664
4	Injection-4	2309241	3.662
5	Injection-5	2306796	3.660
6	(%) RSD	2307169	0.09

Table 5: Results of method precision

S.No	% Imatinib
1	99.46
2	99.74
3	99.33
4	99.98
5	99.46
6	99.21
% RSD	0.375

Table 6: Results of intermediate precision studies

S.No	% Imatinib
1	99.46
2	99.74
3	99.33
4	99.98
5	99.46
6	99.21
% RSD	0.3599

Table 7: Results and conclusion of precision

S. No	Date	Time	Assay
1	13/01/2012	4:20PM	99.46
2	14/01/2012	5:14PM	99.74
3	14/01/2012	6:08PM	99.33
4	15/01/2012	10:29AM	99.98
5	15/01/2012	1:59 PM	99.46
6	16/02/2012	4:17 PM	99.21
RSD			*0.3752*

Table 8: Comparison of Precision with Initial Assay Value and Its RSD

Content	Initial Assay	Average Assay Value Obtained in Precision	Difference	RSD
Imatinib Mesylate	99.53	99.06	0.71	0.5175

3.4. Linearity

The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. Individually samples equivalent to 50%, 60%, 70%, 80%, 90%, of the stated amount of sample were weighed individually and the assay was carried out as described earlier. A graph of weight taken versus chromatographic area was plotted for Imatinib Mesylate peaks. The regression line obtained was linear. From the data obtained, co-relation coefficient, slope and y-intercept were calculated. Ideally co-relation coefficient should be around 1. Sample condition: 50%, 60%, 70%, 80%, 90%,

Table 9: Linearity Results

S. No	Linearity Level	Concentration	Area
1	I	50µg/ml	771444
2	II	60µg/ml	1551205
3	III	70µg/ml	2325365
4	IV	80µg/ml	3091676
5	V	90µg/ml	3856523
Correlation Coefficient			0.999

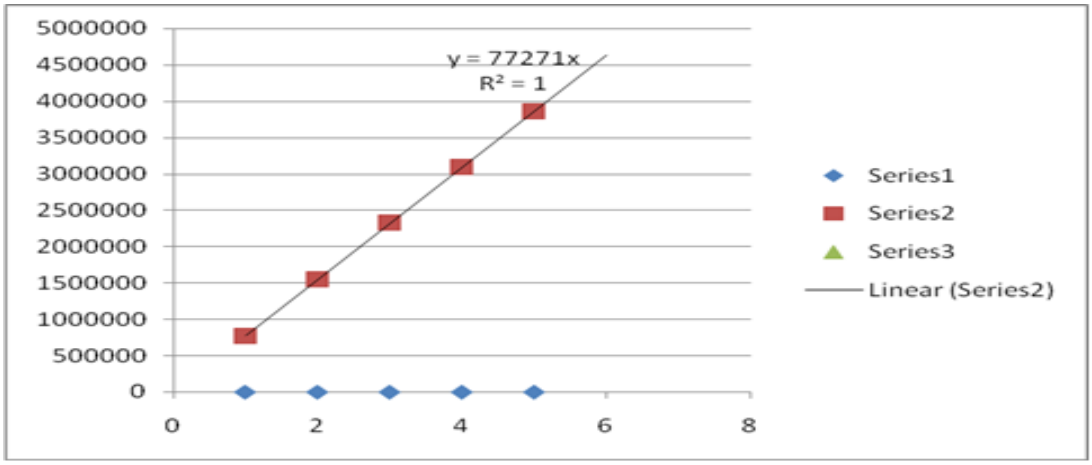

Fig. 4 : Calibration curve of Imatinib Mesylate

From the graph, it can be seen that the correlation co-efficient is almost equal to 1 and regression line obtained is linear.

Equation for the line y=77271x

Correlation coefficient R^2= 1

The percentage curve fitting is 99.7%

The method was found to be linear with the range 50 µgm/ml to 90 µg//ml, the correlation of coefficient of the plot for Imatinib Mesylate is 0.999

3.5. Quantitation Limit

This is carried out in order to determine that lowest concentration of analyte which it can be estimated with acceptable precision, accuracy under the stated experimental conditions. For this the sample solution can be further diluted and the minimum concentration at which the sample can be reliably quantified should be found.

Table 10: LOQ

Sl. No	Name	Retention time(min)	Area (µV*sec)	Height((µV)
1	Imatinib mesylate	3.498	7562316	519171

From the above data, it can be seen that sample recovery of Imatinib mesylate is 99.44%. Hence sample concentration up to 0.38µg/ml can be quantified with the acceptable accuracy.

3.6. Detection Limit

The limit of detection (LOD) is defined as the lowest concentration of an analyte in a sample that can be detected. The detection limit is determined by the analysis of sample with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected.

Table 11: LOD

S. No	Name	Retention time(min)	Area (µV*sec)	Height((µV)
1	Imatinib mesylate	3.675	5984	508

Table 12: Comparison of Quantitation with Initial Assay

Content	Initial Assay	Average Assay Value Obtained in Quantitation	Difference
Imatinib Mesylate	99.53	99-44	0-09

Acceptance criteria: The assay value obtained so should be within ± 1 of initial assay value.

3.7. Ruggedness

This is to prove the lack of influence of operational and environmental variables of the test results by using the method. Ruggedness is a measure of reproducibility of test results under the variation in conditions normally expected from laboratory to laboratory and from analyst to analyst.

Table 13: Different analyst

S. No	Peak name	Retention time(min)	Area (µV*sec)	Height((µV)	USP plate count	USP tailing
1	Imatinib mesylate	4.065	2620756	195957	2885.8	1.2

Table 14: Different instrument

S. No	Standard Imatinib Mesylate	Test Imatinib Mesylate	Average
1	2306452	2306454	100.8%
2	2304223	2304232	99.3%
Average	2309132	2304127	100%

Table 15: Different column

S. No	Standard Imatinib Mesylate	Test Imatinib Mesylate	Average
1	2306452	2306454	100%
2	2304223	2304232	99.7%
Average	2309132	2304127	99.8%

3.8. Robustness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Determination: using different column shall carry out this.

Table 16: Robustness different column

S. No	Standard Imatinib Mesylate	Test Imatinib Mesylate	Average
1	2306452	2306454	99.6%
2	2304223	2304232	99.3%
Average	2309132	2304127	99.5%

Table 17: Results and Conclusion of Ruggedness and Robustness

Condition	Assay	Difference with Assay obtained in Accuracy %	
	Imatinib Mesylate %		
Different Analyst	99.32	-1.67	-1.16
Different Instrument	99.73	-1.46	0.75
Different Column	99.31	-1.80	-1.17

Table 18: Comparison of initial Assay with Ruggedness

Content	Initial Assay	Avg Assay Valve Obtained in Ruggedness	Difference
Imatinib Mesylate	99.53	99.08	0.45

3.9. Accuracy

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The accuracy of an analytical method should be established across its range. Performed accuracy in three different levels for Imatinib Mesylate Spiked

known quantity of Imatinib Mesylate Standard at 50%, 100% and 150% level into the placebo. Analyze these samples in triplicate for each level. From the results, % recovery was calculated.

Table 19: Results of accuracy of Imatinib Mesylate

S. No	Level in %	Response Area	Mean % ± % RSD
1	Standard	343426	99.5±1.6
2	50	172403	
3	50	172201	
4	100	443907	99.7±0.6
5	100	452796	
6	150	521691	
7	150	521071	99.2±1.2
8	200	86892	
9	200	868948	
	Across all levels		100.1± 1.2

4. DISCUSSION

In HPLC method, the conditions were optimized to obtain an adequate separation of eluted compounds. Initially, various mobile phase compositions were tried, to separate title ingredients. Mobile phase and flow rate selection was based on peak parameters (height, tailing, theoretical plates, capacity or symmetry factor), run time, resolution. The system with Acetonitrile: Buffer with 0.9ml/min flow rate is quite robust. The optimum wave length for detection was 256nm at which better detector response for drug was obtained. The average retention time for Imatinib Mesylate was found to be 3.6. According to USP and IP, system suitability tests are an integral part of chromatographic method. They are used to verify the reproducibility of the chromatographic system. To ascertain its effectiveness, system suitability tests were carried out on freshly prepared stock solutions. The difference between individual assay values was less than 1.0%. RSD of retention time and area of six replicate injections of standard were taken and was found to be less than 1%. Precision (Repeatability)is the difference between individual assay values was within 1%. Relative standard deviation was found within 1%. Also, the average of the assay values of the precision were compared with the average initial assay values and found to be less than 1%. The calibration was linear in concentration range of 50µg/ml to 90µg/ml low values of %RSD indicate the method is precise and accurate. The mean recoveries were found in the range of 99-101%. Robustness of the proposed method was determined by varying various parameters the RSD reported was found to be less than 2%. Ruggedness is the assay was carried out as using different analyst, different instrument and different column. The assay values obtained in each of the variables did not deviate by more than ± 1.0% of initial assay. Quantitation limit is the lowest concentration for Imatinib Mesylate was found to be 0.38 µg/ml with which assay values can be obtained with acceptable precision and accuracy. The assay value obtained w less than ± 2% of initial assay values. Accuracy for Imatinib Mesylate was found to be 100.3%. The percent recovery should be between 98.0 to102.0%.

5. CONCLUSION

Since all the acceptance criteria of the parameters selected for validation are satisfied, the method stands validated. The method discussed is simple, precise, accurate, and rapid technique for determination of Imatinib Mesylate. The results for Imatinib Mesylate was good agreement with label claims. The method discussed is also easy, cost of the mobile phase is less when compared to costly solvents that has to be used like Dimethylformamide, Dimethylsulfoxide as described in IP and USP for the determination Imatinib Mesylate. The analytical method was developed based on HPLC. As the method has been validated according to USP and ICH guidelines. One can adopt the method in an industry confidently for routine analysis.

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