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Research Article

RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF IMATINIB MESYLATE IN TABLET DOSAGE FORM

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ABSTRACT

An accurate, Precise, Simple and Economical High Performance Liquid Chromatographic method for the estimation of Imatinb mesylate in its tablet dosage form has been developed. The method so developed is Reverse Phase High Performance Liquid Chromatographic method using Hypersil BDS C_{18} column (Length: 250nm, Diameter: 4.6nm, Particle size: 5µ) with a simple Ammonium Phosphate buffer and acetonitrile mixed in a proportion of 40:60v/v as mobile phase. The method so developed was validated in compliance with the regulatory guidelines by using well developed Analytical method validation tool which comprises with the analytical method validation parameters like Linearity, Accuracy, Method precision, Specificity, System suitability, Robustness and Ruggedness. The results obtained were well within the acceptance criteria.

Keywords: Imatinib mesylate, RP-HPLC, Method development, Validation.

INTRODUCTION

Imatinib mesylate is used in treating chronic myelogenous leukemia (CML), gastrointestinal stromal tumors (GISTs) and a number of other malignancies. It is the first member of a new class of agents that act by inhibiting particular tyrosine kinase enzymes, instead of non-specifically inhibiting rapidly dividing cells. It is a protein-tyrosine kinase created by the Philadelphia chromosome abnormality in chronic myeloid leukemia. It inhibits proliferation and induces apoptosis in Ber-Abl positive cell lines as well as fresh leukemic cells from Philadelphia chromosome positive chronic myeloid leukemias. The usual tablet dose is 100mg and 400mg. As a very novel and recently synthesized drug, there are only a few references for Imatinib mesylate. Most of the analytical techniques for Imatinb mesylate described in the literature are based on the liquid chromatographic determination of this drug in the monkey and human plasma[1-3], in bulk drugs[4], and pharmaceutical formulation like capsules[5-7]. 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, attempts were made to determine Imatinib mesylate in tablets by using RP-HPLC. The proposed method is simple and suitable for routine determination of Imatinib mesylate in tablet dosage form.

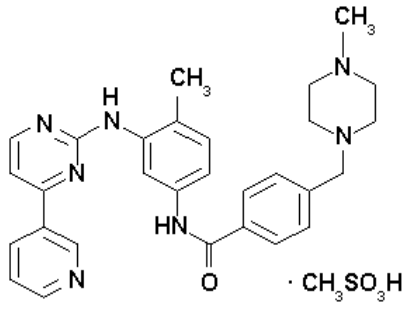

Fig. 1: Chemical structure of Imatinib mesylate

MATERIALS AND METHODS

Chemicals and reagents

All the chemicals and reagents used were of high purity procured from various sources. Imatinib mesylate (Active Pharmaceutical Ingredient, API) and reference material were procured from a reputed lab in India. A commercial local tablet formulation was used in this study. Its composition was: Imatinib mesylate 100mg (label claim) in a matrix of starlac, crospovidone, primellose, colloidal silicon dioxide, calcium phosphate dibasic, magnesium sterate and sodium stearyl fumarate.

Table 1: Chemicals and reagents used

Chemicals	Details
Imatinib mesylate reference standard	Chandra labs, Hyderabad
Imatinib mesylate tablets 100mg	Purchased commercial sample from market
Acetonitrile	HPLC grade, Merk chemicals
Ammonium phosphate	Merk chemicals
Orthophosphoric acid	Merk chemicals

Instrumentation

A Shimadzu HPLC separation module LC-20A equipped with SPD 20A UV-Vis detector was used for all the experiments. Data acquisition was performed by Spinchrom software. Analysis was carried out at 254nm with a Hypersil BDS C_{18} column (250x4.6mm, 5µ) at ambient temperature (25°C). The mobile phase was a mixture of Ammonium phosphate buffer (0.01M) and acetonitrile in the ratio of 40:60v/v. The buffer pH was adjusted with orthophosphoric acid to 4.0. The flow rate was 1.0ml/min and the retention time was 3.4 min. The mobile phase was degassed and filtered through 0.45µm membrane filter before pumping into the HPLC system.

Preparation of Solutions

Preparation of Mobile phase

The mobile phase was prepared by mixing Ammonium phosphate buffer and Acetonitrile in the ratio of 40:60 (pH adjusted 4.0 with Orthophosphoric acid). The mobile phase is then sonicated using Ultra-Sonicator to remove the impurities and dissolved gases, as they may lead to unwanted peaks in the chromatogram.

Preparation of standard stock solution

Stock solution of Imatinib mesylate (1mg/ml) was prepared by dissolving 100mg of Imatinib mesylate in 100ml of volumetric flask containing mobile phase. The solution was sonicated for about 10min and then made up to volume with mobile phase. Daily working standard solution of Imatinib mesylate was prepared by suitable dilution of stock solution with appropriate mobile phase.

Preparation of sample solution

20 tablets of Imatinib mesylate were weighed, the powder equivalent to 100mg of active ingredient present was transferred into a 100ml volumetric flask, 70ml of diluents was added to it and was shaken by mechanical stirrer, sonication for about 20minutes by shaking at intervals of five minutes each and was diluted up to the mark with mobile phase and the solution was filtered through 0.45µm filter before injecting into the HPLC system.

Experimental Procedure for Method Validation

The method was validated according to International Conference on Harmonization guidelines for validation of analytical procedures[8-9].

System suitability

System suitability was assessed by replicate analysis of 6 injections of the Imatinb mesylate standard solution at a concentration of 100µg/ml and the chromatogram was obtained. The system suitability parameters such as tailing factor, theoretical plate count and reproducibility (%RSD) of analyte retention time and area of the six replicated were calculated from the chromatogram. System suitability parameters are given in table 2.

Table 2: System suitability parameters

Parameters	Acceptance criteria
Tailing factor	should not be more than 2
No. of theoretical plates	should not be less than 2000
%RSD	should not be more than 2

Specificity

The analyte was subjected to forced degradation studies using photolytic, acid and alkali treatments for demonstration of specificity of the method. Imatinib mesylate was analyzed under these conditions for purity, indicating that the developed HPLC method effectively separated the degradation products from the Imatinib mesylate standard peak.

Linearity

A series of standard curves were prepared over a concentration range of 20-120µg/ml by diluting the standard stock solution of Imatinib mesylate (1mg/ml) in mobile phase. The data from peak area verses drug concentration plots were treated by linear least square regression analysis and r^2 was found to be 0.9994

Precision of the method

Precision was measured in accordance with ICH recommendations. The precision study was carried out by injecting sample preparation of 100µg/ml concentration six times.

Accuracy

Recovery studies by the standard addition method were performed with a view to justify the accuracy of the proposed method. Previously analyzed sample of Imatinib mesylate were spiked with known amount of standard so as to get three different levels (80%, 100%, 120%) and the mixture were analysed by the proposed method. The experiment was performed in triplicate % recovery, mean % recovery, RSD(%) were calculated for each concentration.

Robustness

The robustness of the method was determined to assess the effect of small but deliberate changes of the chromatographic conditions on the determination of Imatinib mesylate. The different variations are in flow rates by ±0.1ml/min, in mobile phase composition ±5ml, buffer pH ±0.5 and column temperature from developed HPLC conditions. The concentration of the solution analyzed was 100µg/ml.

Ruggedness

The ruggedness of the method was demonstrated by analysis of the sample as for precision study by a second analyst.

RESULTS AND DISCUSSION

Method development and optimization

Optimization of the method was done by fixing one parameter and changing the other parameter. Imatinib mesylate was analyzed by using different solvents and by changing the ratio of their composition. In all these cases Imatinib mesylate was analyzed using column (Hypersil BDS C_{18} column (250x4.6mm, 5µ)). Various buffer strengths with different pH ranges and different flow rates were examined by using different experimental conditions; different mobile phase compositions with isocratic elution were also used. Experimental were conducted to optimize the HPLC method for Imatinb mesylate in order to get reproducibility, better peak shape and rapid analysis. All the experiments were monitored using UV detector at a wavelength of 254nm.

Optimum mobile phase ratio for the analysis was found to be 40:60v/v of buffer and acetonitrile with a flow rate of 1ml/min. Experiments were conducted by changing the pH of the buffer. Best separation, good peak shape were observed at a pH of 4.0 with buffer strength of 0.01M.

Method Validation

System suitability

The %RSD of the peak area and retention time of Imatinib mesylate were within 2% indicating system suitability. The efficiency of the column is expressed by the number of theoretical plates for six replicate injections were found to be 3857 and the tailing factor was 1.42. Results are given in table 3.

Table 3: System suitability results

Injection	Retention time(Rt)	Area	Plate count	Tailing factor
1	3.433	6140	3810	1.42
2	3.427	6182	3876	1.43
3	3.431	6211	3798	1.42
4	3.436	6208	3894	1.41
5	3.432	6174	3916	1.44
6	3.429	6159	3847	1.42
Mean	3.431	6179	3857	1.42
SD	0.003	27.64	46.90	0.01
%RSD	0.08	0.44	1.21	0.70

Linearity

The calibration curve constructed was evaluated by using correlation coefficient. The peak area of the drug was linear in the range of 20-120µg/ml. The area for each of the concentration obtained was plotted against the concentration of the analyte. The correlation coefficient for the data was calculated as 0.9994 for Imatinib mesylate indicating a strong correlation between the concentration and the area under the curve. Concentration verses peak area results are given in table-4 and statistical data of calibration curves are given in table 5

Table 4: Concentration Vs mean peak area of Imatinib mesylate

Injection	Mean peak area	%RSD
1	1244.00	0.07
2	2371.69	0.04
3	3647.65	0.01
4	4977.04	0.01
5	6136.99	0.01
6	7299.14	0.01

Table 5: Statistical data of calibration curves

Parameters	Imatinib mesylate
Linearity	20-120µg/ml
r^2	0.9994
Slope	61.28
Intercept	-10.67

Accuracy

Accuracy of the method was expressed in terms of recovery of added compound. Percentage recovery was calculated by multiplying the ratio of the measured concentration with 100. Mean % recovery and %RSD were also calculated and were found to be within 99.12 to 100.19 and 0.33 to 0.49 respectively. It can be obtained from table 6 that the developed HPLC method is accurate.

Table 6: Results of recovery experiments

Inj.	%Conc	Amt present (mg)	%Recovery	SD*	RSD*
1	80	89.69	99.65		
2	80	90.09	100.11	0.49	0.49
3	80	89.20	99.12		
4	100	109.97	99.98		
5	100	110.20	100.19	0.40	0.40
6	100	109.41	99.47		
7	120	120.09	100.08		
8	120	119.30	99.41	0.33	0.33
9	120	119.78	99.82		

* SD & RSD was calculated for each (%Conc.)three values of %recovery

Assay

The assay for the marketed tablets was established with chromatographic condition developed and it was found to be more accurate and reliable. The %purity was found to be 99.90. No interfering peaks were found in chromatogram, indicating that the estimation of drug free from inference of excipients. The results were shown in table 7.

Table 7: **Estimation of amount present in tablet dosage form**

Name	Labeled	claim(mg)	Amount estimated(mg)	%Purity
Imatinib	mesylate	100	99.90	99.90

Precision

The precision of the method was calculated from the reproducibility of the area of standard solution and % assay of six Imatinib mesylate test samples. The results are given in table 8. The results showed that the precision of the method is good.

Table 8: Precision results

Injection	Retention time(Rt)	Plate count	%Assay
1	3.447	6244.491	100.49
2	3.447	6284.992	99.90
3	3.443	6263.454	100.19
4	3.443	6264.237	100.19
5	3.440	6283.937	99.90
Avg	3.444	6268.222	100.13
SD	0.00300	16.8075	0.246
%RSD	0.08710	0.26813	0.245

Specificity

Accelerated degradation studies under different conditions viz., acid treatment; base treatment were conducted to demonstrate the specificity. Photo stability test showed that the active substance is not light sensitive and no degradation products were formed during the oxidation and reduction treatment. Results are given in table 9.

Table 9: Specificity results

Treatment	Acid treatment	Base treatment	Photo degradation
Conc.	0.1N Hcl	0.1N NaoH	-
Time (min)	60	60	-
Rt of analyte	3.43	3.43	3.43
Rt of impurity(min)	4.21	3.10	-

Robustness

Robustness was performed by small variations in chromatographic conditions like volume of the mobile phase composition, flow rate, buffer pH and column temperature. The results are shown in table 10. It can be observed from table 10 that the method is unaffected by small variations in the chromatographic conditions.

Table 10: Robustness of Imatinib mesylate

Parameters	Variation	Tailing factor	Theoretical plate
Flow rate(ml)	1.2	1.4	3872
	0.8	1.5	3983
pH of buffer	3.5	1.5	3768
	4.5	1.4	3784
Temperature(^oC)	20	1.4	3747
	30	1.5	3742
Mobile phase	45:55	1.5	3782
composition(ml)	35:65	1.4	3794

Ruggedness

The results were well within acceptable limits of 98%-102% with %RSD less than 2.0% for the studied parameters. These results indicate that the developed HPLC method was rugged.

CONCLUSION

A rapid and accurate RP-HPLC method was developed for the determination of Imatinib mesylate in tablet dosage form. The method was evaluated for specificity, linearity, accuracy, precision, ruggedness and robustness as per ICH guidelines and proved to be economical and effective for the quality control of the drug in the given application. The measured signal was observed to be accurate, precise and linear with a correlation coefficient of 0.9994. Parameters included are given in table 11.

The proposed method was observed to be rapid and selective when compared to the method reported in the literature. The method is also cost effective with respect to solvent consumption.

Table 11: Parameters included

Parameters	Values
Standard solution concentration	100µg/ml
Test solution concentration	100µg/ml
Name of the column	Hypersil BDS C_{18} column (250x4.6mm, 5µ)
Mobile phase	Buffer: Acetonitrole (40:60v/v)
Buffer strength	0.01M Ammonium dihydrogen phosphate
pH (ortho-phosphoric acid)	4.0
Separation mode	Isocratic elution
Flow rate	1ml/min
Run time	8min
Retention time	3.4min
Absorbance (nm)	254nm with UV
Linearity concentration	20µg/ml to 120 µg/ml
Column temperature (^oC)	Ambient
Correlation coefficient (r^2)	0.9994

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