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July-September 2017

International Journal of CHEMICAL AND PHARMACEUTICAL ANALYSIS

eISSN: 2348-0726 ; pISSN : 2395-2466

DOI : http://dx.doi.org/10.21276/ijcpa

Research Article | Volume-4 | Issue-4 | Article ID: 1336

METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF IMATINIB MESYLATE IN TABLET DOSAGE FORM BY RP-HPLC METHOD

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Received: 13 June 2017 / Revised: 24 August 2017 / Accepted: 25 September 2017 / Available online 30 September 2017

ABSTRACT

RP-HPLC method was developed by Imatinib mesylate in bulk and pharmaceutical dosage form maximum absorbance was found at 230nm and peak purity was excellent. Imatinib mesylate is a protein-tyrosine kinase inhibitor, Inhibits the abnormally functioning Bcr-Ab1 tyrosine kinase which is produced by the Philadelphia chromosome abnormality found in chronic myeloid leukaemia (CML). It is found that the method of RP-HPLC with UV-detection system for the analysis of Imatinib mesylate is straight forward and applied in qualitative and quantitative analysis. This method is simple, rapid, selective and inexpensive. The percent recovery of drug ranged from 99.0 to 100.4. The proposed method for estimation of selected drug Imatinib mesylate was successfully applied in pharmaceutical formulation.

Keywords – RP-HPLC; Imatinib mesylate; Antineoplastic Agent

1. INTRODUCTION

Imatinib mesylate is Antineoplastic Agent, Tyrosine Kinase Inhibitor. Chemically it is 4-[(4-Methyl1-piperazinyl)methyl]-N-[4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-phenyl] benzamide methanesulfonate[1-6]. The chemical structure is shown in figure 1. Soluble in aqueous buffers ≤ pH 5.5 but it is very slightly soluble to insoluble in neutral/alkaline aqueous buffers. Freely soluble to very slightly soluble in dimethylsulfoxide, methanol and ethanol, but is insoluble in n-octanol, acetone and acetonitrile [4]. A protein-tyrosine kinase inhibitor, imatinib mesylate, inhibits the abnormally functioning Bcr-Abl tyrosine kinase which is produced by the Philadelphia chromosome abnormality found in chronic myeloid leukaemia (CML). Imatinib inhibits cell proliferation and induces apoptosis (programmed cell death) in the Bcr-Abl cell lines and in the leukemic cells generated by CML. Imatinib also inhibits proliferation and induces apoptosis in gastrointestinal stromal tumour (GIST) cells. Adverse effects like Bone marrow suppression, anemia, neutropenia, and thrombocytopenia. Median duration of neutropenia is 2-3 weeks, median duration of thrombocytopenia is 3-4 weeks.

2. EXPERIMENTAL WORK

2.1 Materials

Water (Milliq HPLC grade water) Ammonium phosphate buffer (HPLC grade)- Merck Acetonitrile (HPLC grade)

2.2 Instruments

Single pan balance (Mettler), Ph meter, Thermosonicator (Shimadzu HPLC – Waters, UV – 2489), BDS Hypersil C8 column (150 mm x 4.6 mm x 5µ), Membrane filter (0.45and 0.2 microns) and centrifuge.

2.3 Methodology

2.3.1 Preparation of mobile phase

Take 400ml HPLC Water and 4.52 gm of Ammonium phosphate to make the concentration of 0.1M dissolve in water. Adjust the P^H 3 in ortho phosphoric acid. Take 600 ml of Acetonitrile, prepare a filtered and degassed mixture of buffer and acetonitrile in the ratio of 40:60% v/v respectively.

2.3.2 Standard preparation

Accurately weigh and transfer about 100 mg of Imatinib mesylate working standard into a 100 ml volumetric flask. Add about 50mL of diluent and sonicate to dissolve. Cool the solution to room temperature and dilute to volume with diluents and mix. (Stock solution I) Transfer 5ml of the above solution into a 100mL volumetric flask and dilute to volume with diluents and mix well. (Stock solution II) inject this solution for 3 times. Calculate average area of standard from 3 injections and shown in figure 2.

2.3.3 Sample preparation

Weight and finely powder 10 tablets. Transfer accurately weighed portion of the powder equivalent to 100mg (122.7mg) into a 100ml volumetric flask Fill 2/3 volume of the flask with diluent and allow sonicate to dissolve for 5 to 10 minutes. Cool the solution to room temperature and dilute to volume with diluents and mix. Transfer 5ml of the above solution into a 100ml volumetric flask and dilute to volume with diluents and mix well. Now this solution is equivalent to 50mcg. Inject this solution for 3 times. Calculate average area of sample from 3 injections and shown in figure 3.

3. RESULTS AND DISCUSSION

3.1 System suitability

Stock solution-II of Imatinib mesylate standard was injected six times into HPLC system as per test procedure. The system suitability parameters were evaluated from standard Chromatograms obtained, by calculating the % RSD of peak areas from six replicate injections, tailing factor, and theoretical plates. From the system suitability studies, it was observed that all the parameters are within limit. Hence it was concluded that the instrument, reagents and column was suitable to perform the Assay and shown in table 3.

3.2 Linearity

The linearity of the method was demonstrated over the concentration range of 10-60 µg / ml. Aliquots of 10, 20, 30, 40, 50 and 60 µg / ml was prepared from stock solution-II and labeled as solution 1, 2, 3, 4, 5 and 6 respectively. The solutions were injected into HPLC system as per test procedure. The chromatogram was given in Figure 5; calibration curve was plotted for concentration v/s peak area.

3.3 Precision

Repeatability

Six sample solutions were prepared and injected into the HPLC system as per test procedure.

Intermediate precision (Analyst to Analyst variability)

Two analysts as per test method conducted the study. For analyst-1 refer precision (repeatability) results.

3.4 Accuracy

Assay was performed in triplicate for various concentrations of Imatinib mesylate equivalent to 50, 75, 100, 125 and 150 % of the standard amount was injected into the HPLC system as per the test procedure. The average % recovery of Imatinib mesylate was calculated and shown in table 6.

3.5 Specificity

A) Imatinib mesylate Identification

Solutions of Standard and Sample was prepared as per test procedure and injected into the HPLC system.

B) Placebo interference

A study to establish the interference of placebo was conducted. A sample of placebo was injected into the HPLC system as per the test procedure.

C) Blank interference

A study to establish the interference of blank was conducted. Diluent was injected into HPLC system as per the test procedure. The chromatogram of blank was shown.

Solutions of standard and sample was prepared as per the test method and injected into the chromatographic system The chromatograms of Standard and Sample were identical with nearly same Retention time, hence it is concluded that the standard and sample are same.

D) Placebo interference

Sample was prepared by taking the placebo equivalent to about the weight in portion of test preparation as per the test method and injected into the HPLC system. There is no interference due to Placebo and Sample at the retention time of analyte which indicates that the excipients and mobile phase used in the method have no interference, hence the method is specific.

3.6 Ruggedness

The assay of Imitinib was performed by different analysts on different days. The Chromatogram for Day-1, Analyst-1 was presented and the result was illustrated.

3.7 Robustness

The robustness of the proposed method was determined by analysis of aliquots from homogenous lots by differing physical parameters like flow rate and mobile phase composition which may differ but the responses were still within the specified limits of the assay.

3.8 Limit of detection (LOD)

Calibration curve was repeated for 5 times and the standard deviation (SD) of the intercepts was calculated. The LOD was determined by the formula:

$$LOD = 3.3\ \sigma / S$$

3.9 Limit of Quantitation (LOQ)

Calibration curve was repeated for 5 times and the standard deviation (SD) of the intercepts was calculated The LOQ was determined by the formula:

$$LOQ = 10\ \sigma / S$$

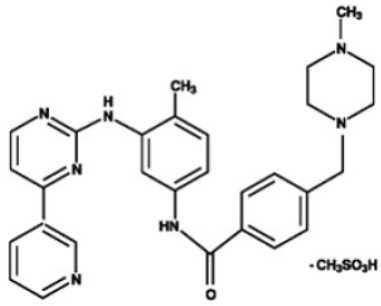

Fig. 1: Chemical structure of Imatinib mesylate

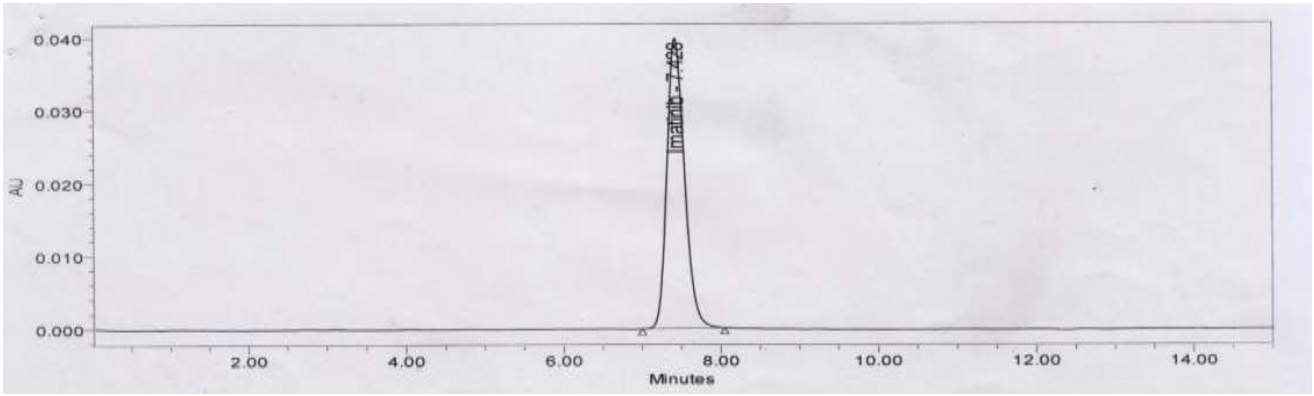

Fig. 2: A typical chromatogram of standard

Table 1: Column performance table for standard

Retention time	Area (m.Vs)	Height (mV)	Area (%)
7.428	292.186	59.480	100.00

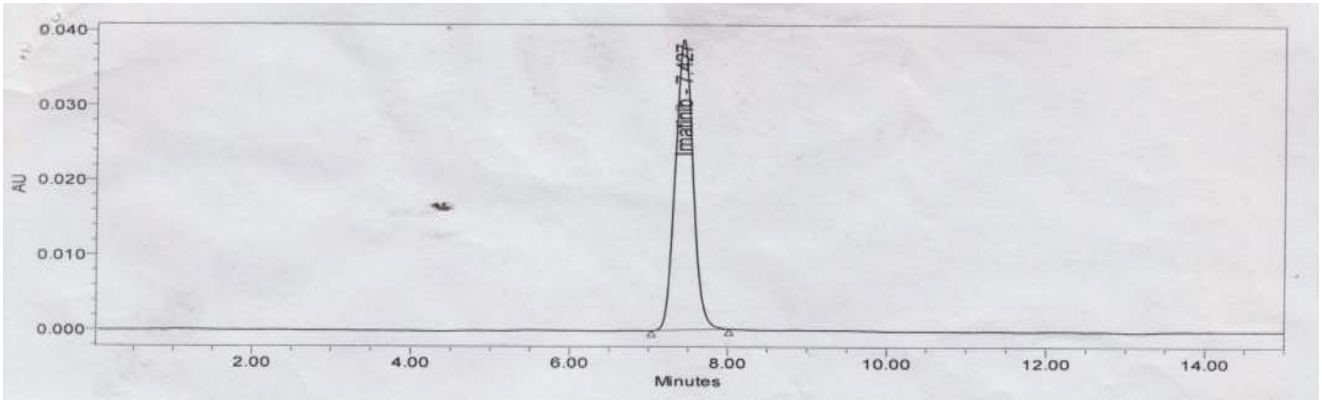

Fig.3: Sample Chromatogram

Table 2: Column performance table for sample

Retention time (min)	Area (m V.s)	Height (mV)	Area [%]
7.427	**292.186**	**59.480**	**100.000**

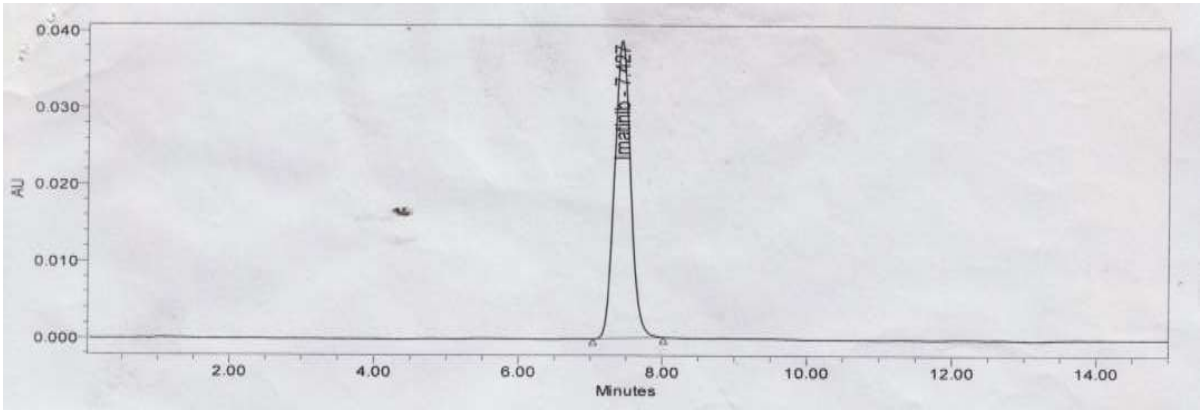

Fig. 4: A chromatogram for System suitability

Table 3: Standard Chromatogram values for System suitability

Injection	t_R	Peak Area	USP Plate count	USP Tailing
1	7.426	619368	5300.6	1.20
2	7.451	619333	5300.2	1.10
3	7.415	622042	5320.3	1.08
4	7.449	620281	5301.2	1.09
5	7.441	621079	5309.2	1.10
Mean	7.43	620420	5306.48	1.09
SD	0.015	5605.04	47.00	1.008
%RSD	0.16	0.43	0.42	0.77

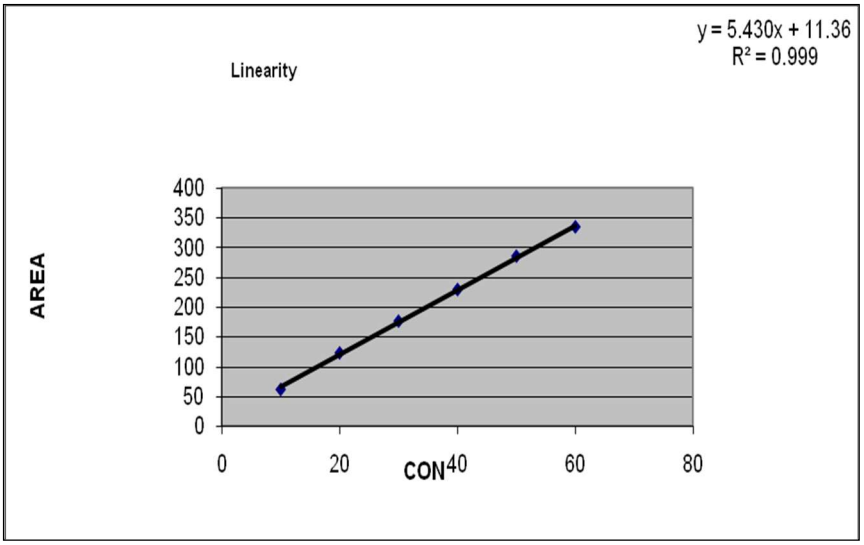

Fig. 5: Calibration curve

Table 4: Data of Linearity study

Linearity level	Conc(mg/ml)	Peak area	% of RSD	Statistical analysis
L1	4.8	131990	0.99	Slope=27537
L2	23.98	660130	0.80	
L3	38.36	1047634	0.95	Correlation co efficient=0.999
L4	43.16	116937	0.18	
L5	47.95	1309091	0.21	

Table 5: Results of Accuracy Study

Sample No:	Spike level	mg/ml added	mg/ml found	% of recovery	Mean % recover
1	5%	0.0048	0.0048	100.00	100.0
2	5%	0.0048	0.0048	100.00	
3	5%	0.0048	0.0048	100.00	
4	80%	0.0764	0.0771	100.92	100.2
5	80%	0.0765	0.0766	100.13	
6	80%	0.0766	0.0763	99.61	
7	100%	0.0940	0.0941	100.11	100.1
8	100%	0.0938	0.0940	100.21	
9	100%	0.0942	0.0941	99.89	
10	120%	0.1133	0.1131	99.82	100.2
11	120%	0.1127	0.1130	100.27	
12	120%	0.1124	0.1130	100.53	
13	150%	0.1416	0.1402	99.01	99.5

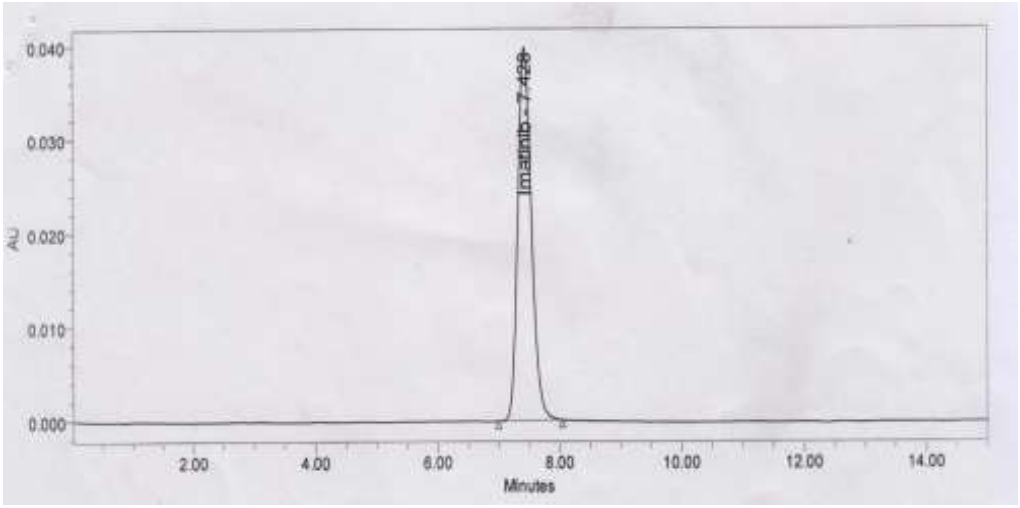

Fig.6: Chromatogram of Standard

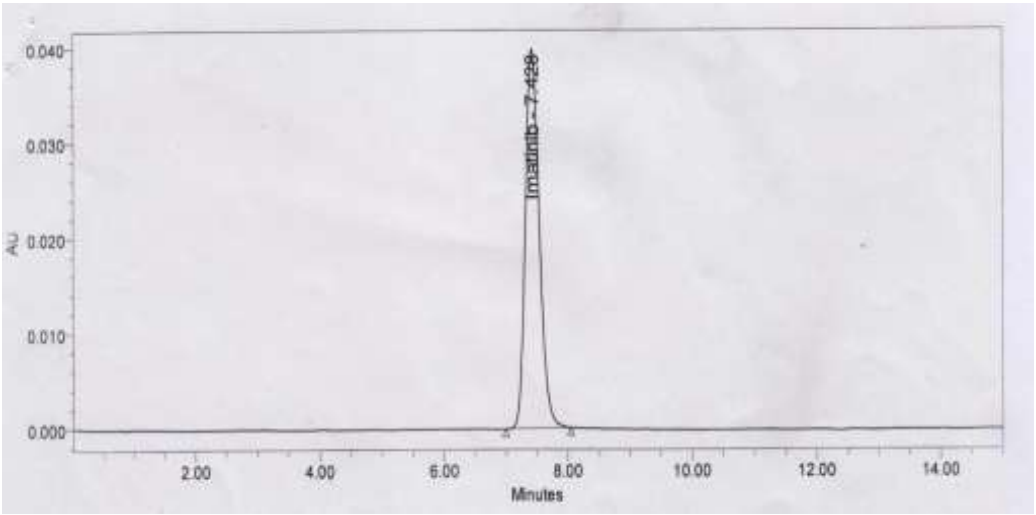

Fig. 7: Chromatogram of Sample

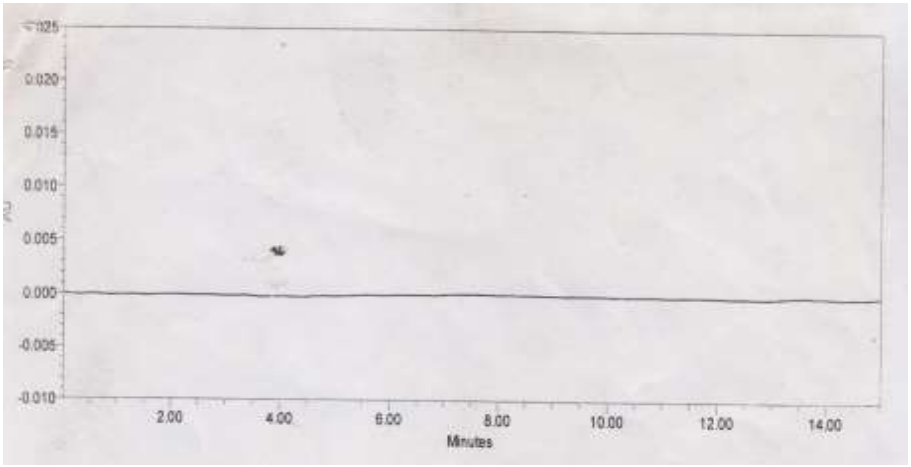

Fig.8: Chromatogram of Placebo

4. CONCLUSION

The analysis of Imatinib mesylate by improved High Performance liquid chromatography method with UV-detector and data handling system is investigated. Imatinib mesylate is a protein-tyrosine kinase inhibitor, Imatinib mesylate, inhibits the abnormally functioning Bcr-Abl tyrosine kinase which is produced by the Philadelphia chromosome abnormality found in chronic myeloid leukaemia (CML). Samples are analyzed by means of reverse phase HPLC using a BDS Hypersil C8 column (150*4.6*5µ) with sodium dihydrogen phosphate dehydrate (NaH_2PO_4).$2H_2O$ (adjust the solution to p^H 8.0 with Triethylamine) and acetonitrile (volume in the ratio of 55:45 v/v respectively) as mobile phase. And diluent is used as mobile phase. In which the flow rate is 1.0ml min^{-1}. And the column temperature is set at ambient temperature and wavelength fixed at 230nm UV-detection. This provides a complete separation and determination of Imatinib mesylate. It is found that the method of RP-HPLC with UV-detection system for the analysis of Imatinib mesylate is straight forward and applied in qualitative and quantitative analysis. And subsequently validations have to be study and compare as per ICH guideline. This method is simple rapid, selective, and inexpensive. The percent recovery of drug ranged from 99.0 to100.4. The proposed method for estimation of selected drug such as Imatinib mesylate was successfully applied in pharmaceutical formulation.

REFERENCES

1. Sundar BS, Balamuralikrishna K. Development and Validation of New Reversed Phase High Performance Liquid Chromatography Method for the Estimation of Efavirenz in Bulk and in Pharmaceutical Dosage Forms. Asian Journal of Research in Chemistry. 2011 Apr 28;4(4):555-7.
2. Willard HH, Merit LL, Dean JA, Settle FA. Instrumental methods of analysis, 7th ed. CBS publisher and distributors, New Delhi; 1986.
3. Chatwal G, Anand S. Instrumental methods of chemical analysis. 5th edition. Himalaya Publishing house. New Delhi: 2002.
4. Indian Pharmacopoeia, Vol. II, 4th Edn., The Controller of Publications, New Delhi, 1996.
5. Snyder LR, Kirkland JJ. Practical HPLC Method development, Wiley inter science publications. New York: John Wiley & Sons, Inc; 1997. Available from: http://doi.wiley.com/10.1002/9781118592014 doi: 10.1002/9781118592014.
6. Sudha T. Development and validation of RP-HPLC method for the estimation of rifaximin in bulk and in tablet dosage form. Asian Journal of Pharmaceutical and Clinical Research. 2009 Oct;2(4) :112-116.