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Research Article

Validation of Assay Indicating Method Development of Imatinib in Bulk and Its Capsule Dosage Form by Liquid Chromatography

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Abstract

A novel, simple and economic reverse phase High Performance Liquid Chromatography (RP-HPLC) method has been developed for the quantification of Imatinib in bulk and capsule dosage form with greater precision and accuracy. Separation was achieved on Analytical technologies, C-18, (250mm*4.6mm) column in isocratic mode with mobile phase consisting of acetonitrile: potassium dihydrogen phosphate buffer (pH 2.5) (30:70v/v) with a flow rate of 0.8 mL/min. The detection was carried out at 268 nm. The retention time of Imatinib was found to be 2.67 min. The method was validated as per ICH guidelines. Linearity was established for Imatinib in the range 5-35 µg / ml with r2 value 0.996. The percentage recovery of Imatinib was found to be in the range 99.49-99.67 %. The high recovery and low relative standard deviation confirm the suitability of the proposed method for the estimation of the drug in bulk and capsule dosage forms. Validation studies demonstrated that the proposed RP-HPLC method is simple, specific, rapid, reliable and reproducible for the determination of Imatinib for quality control level.

Introduction

Imatinib is chemically a N-(4-methyl-3-{[4-(pyridin-3-yl) pyrimidin-2-yl] amino}phenyl)-4-[(4-methylpiperazin-1-yl) methyl] benzamide. It is an anti neoplastic agent used to treat chronic myelogenous leukemia. Imatinib is used to treat Chronic Myelogenous Leukemia (CML), Gastrointestinal Stromal Tumors (GISTs) and a number of other malignancies. imatinib mesylate was effective in patients with systemic mastocytosis, including those who had the D816V mutation in c-Kit. However, since imatinib binds to tyrosine kinases when they are in the inactive configuration and the D816V mutant of c-Kit is constitutively active, imatinib does not inhibit the kinase activity of the D816V mutant of c-Kit.

Imatinib is a 2-phenylaminopyrimidine derivative that functions as a specific inhibitor of a number of tyrosine kinase enzymes. In chronic myelogenous leukemia, the Philadelphia chromosome leads to a fusion protein of Abl with Bcr (breakpoint cluster region), termed Bcr-Abl. As this is now a continuously active tyrosine kinase, Imatinib is used to decrease Bcr-Abl activity.

Imatinib mesylate is a protein-tyrosine kinase inhibitor that inhibits the Bcr-Abl tyrosine kinase, the constitutive abnormal tyrosine kinase created by the Philadelphia chromosome abnormality in Chronic Myeloid Leukemia (CML). It inhibits proliferation and induces apoptosis in Bcr-Abl positive cell lines as well as fresh leukemic cells from Philadelphia chromosome positive chronic myeloid leukemia. Imatinib also inhibits the receptor tyrosine kinases for Platelet Derived Growth Factor (PDGF) and Stem Cell Factor (SCF) - called c-kit. Imatinib was identified in the late 1990s by Dr Brian J. Druker. Its development is an excellent example of rational drug design. Soon after identification of the bcr-abl target, the search for an inhibitor began. Chemists used a high-throughput screen of chemical libraries to identify the molecule 2-phenylaminopyrimidine. This lead compound was then tested and modified by the introduction of methyl and benzamide groups to give it enhanced binding properties, resulting in imatinib [1].

Several analytical methods for the determination of imatinib by spectrophotometry [2,3], HPLC [4-12] and LC/MS [13] have been reported. The aim of the present work was to develop and validate a better sensitive RP-HPLC method that can be implemented for the quantification of imatinib in bulk as well as in its tablet dosage forms when compared to the data of previous established method [4-12].

Experimental

Materials

Imatinib Mesylate working standard was purchased from celon labs, Hyderabad,India. Imatinib Mesylate capsules containing 20 mg of Imatinib (Omef) were obtained from Apollo Pharmaceuticals Pvt. Ltd, Visakhapatnam. Acetonitrile (HPLC grade) were purchased from Qualigens, Potassium dihydrogen orthophosphate, Orthophosphoric acid are purchased from Sd fine-Chem ltd; Mumbai.

Instrumentation

Analytical technologies Alliance High Pressure Liquid Chromatograph installed with Empower software Model: e2487 dual absorbance, phenomenex, C-18, (250mm*4.6mm) column with UV – Vis spectophotometer model UV-2450 were used.

Chromatographic conditions

The High Performance Liquid Chromatographic (HPLC) system used was operated with the column temperature maintained at 30^0 C, using a mobile phase composition of acetonitrile and Potassium dihydrogen phosphate buffer (pH adjusted to 2.5 with O-Phosphoric acid) in the ratio of 30:70 v/v at a flow rate of 0.8 mL/min within a run time of 10 min. Prior to use, the mobile phase was degassed by an ultrasonic bath and filtered by a Millipore vacuum filter system equipped with a 0.45 µm high vacuum filter. The drug was detected and quantified at 268 nm.

Preparation of standard solutions

25mg of Imatinib Mesylate Working standard was accurately weighed and transferred into a 25 mL volumetric flask and about 20 ml of diluent was added to it and sonicated to dissolve drug completely and volume was made up to the mark with the same solvent which gave Stock solution of 1000 ppm. 1 ml of the above stock solution was pippetted into a 10ml volumetric flask and was diluted up to the mark with diluents to prepare 100ppm solution. Further 1 ml of prepared 100 ppm solution was pippetted into a 10ml volumetric flask and was diluted up to the mark with diluents which gave 10ppm Imatinib Mesylate working standard solution. The solution was mixed well and filtered through 0.45µm filter.

Assay of Imatinib from Marketed capsules

Twenty capsules (Omef capsules, Label claim: Each film-coated capsule contains: Imatinib 20 mg) were taken and the I.P. method was followed to determine the average weight. Above weighed capsules were finally powdered and triturated well. A quantity of powder equivalent to 82.5 mg of drugs were transferred to 100 ml volumetric flask, and 70 ml of HPLC grade methanol was added and solution was sonicated for 15 minutes, there after volume was made up to 100 ml with same solvent. Then 10 ml of the above solution was diluted to 100 ml with HPLC grade methanol. The solution was filtered through a membrane filter (0.45 mm) and sonicated to degas. From this stock solution (3.5 ml) was transferred to five different 10 ml volumetric flasks and volume was made up to 10 ml with same solvent system. The solution prepared was injected in five replicates into the HPLC system and the observations were recorded. A duplicate injection of the standard solution was also injected into the HPLC system and the peak areas were recorded.

Method validation

The method was validated in accordance with ICH guidelines [14]. The parameters assessed were linearity, accuracy, and Limit of Detection (LOD), limit of quantification (LOQ), precision, reproducibility, robustness and system suitability.

Accuracy

Accuracy was best determined by the standard addition method. Previously analyzed samples of Imanitib API were added with standard drug solutions and are analyzed by the proposed method. Recovery (%), RSD (%) and bias (%) were calculated for each concentration.

Precision

Precision was determined as both repeatability and intermediate precision, in accordance with ICH guidelines. Repeatability of sample injection was determined as intraday variation and intermediate variation. For these determinations, single concentration (10 µg/ml) at different time intervals and different days, of the solution of Imatinib API was used. The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of five replicates of a fixed amount of drug. Imatinib (API) The percent relative standard deviation was calculated for Imatinib.

Robustness

The concept of robustness of an analytical procedure has been defined by the ICH as "a measure of its capacity to remain unaffected by small but deliberate variations in method parameters". To determine the robustness of the method experimental conditions are purposely altered and chromatographic characters are evaluated. Influence of small changes in chromatographic conditions such as change in flow rate, wavelength of detection and acetonitrile content in mobile phase were studied to determine the robustness of the method

Limit of Detection (LOD)

The Limit of Detection (LOD) of an analytical method may be defined as the concentration, which gives rise to an instrument signal that is significantly different from the blank. For spectroscopic techniques or other methods that rely upon a calibration curve for quantitative measurements, the IUPAC approach employs the standard deviation of the intercept (Sa), which may be related to LOD and the slope of the calibration curve, b, by

$$LOD = 3\ Sa / b$$

Limit of Quantitation (LOQ)

The LOQ is the concentration that can be quantitated reliably with a specified level of accuracy and precision. The LOQ represent the concentration of analyte that would yield a signal-to-noise ratio of 10.

$$LOQ = 10\ Sa / b$$

Where, Sa is the standard deviation of the peak area ratio of analyte to IS (6 injections) of the drugs and b is slope of the corresponding calibration curve.

Table 1: Optimization of chromatographic conditions of Imatinib.

Trials	Column Used	Mobile Phase	Flow Rate	Wave length	Observation	Result
1	Hiq Sil, C-18, Vsize (250mm*4.6mm)	Potassium dihydrogen phosphate Buffer : ACN(50:50)	1 ml/min	268nm	Peak tailing and negative peak was found.	Method Rejected
2	Hiq Sil, C-18, V size (250mm*4.6mm)	Potassium dihydrogen phosphate Buffer: Methanol: ACN(50:30:20)	1 ml/min	268nm	Poor resolution. Peak fronting and a broad Peak were found.	Method Rejected
3	waters, C-18, (250mm*4.6mm)	Potassium dihydrogen phosphate Buffer : ACN (60:40)	1 ml/min	268nm	Peak shape was not good and also a tailing was found.	Method Rejected
4	waters,C-18, (250mm*4.6mm)	Potassium dihydrogen phosphate buffer: ACN (65:35)	1 ml/min	268nm	Poor resolution and peak tailing was also found.	Method Rejected
5	waters, C-18, (250mm*4.6mm)	Potassium dihydrogen phosphate buffer: acetonitrile (70:30)	0.8 ml/min	268nm	Good resolution, theoretical plate count and less tailing factor	Method Accepted

Table 2: Linearity data of Imatinib.

Conc.	AUC (n -=6)
0	0
5	343726
10	801625
15	1064970
25	1811846
35	2721573

Table 3: Precision data of Imatinib.

HPLC Injection Replicates of Imatinib	Retention Time	Area
Replicate – 1	2.64	789939
Replicate – 2	2.67	790996
Replicate – 3	2.68	809774
Replicate – 4	2.67	796107
Replicate – 5	2.69	821313
Average	2.67	801625.8

Results and Discussion

Optimization of chromatographic conditions

The chromatographic conditions were optimized by different means i.e. using different column, different mobile phase, different flow rate, different detection wavelength and different diluents for standard drug are summarized in Table 1&2 and the optimised chromatogram (Figure 1) is shown. Appreciable results were obtained by using mobile phase consisting of Potassium dihydrogen phosphate buffer: acetonitrile (70:30) on phenomenex C-18, (250mm*4.6mm) column with wavelength of detection of 268 nm. Flow rate was fixed at 0.8 ml/ min with a run time of 10 min.

A chromatogram of Imatinib standard was made to run by injecting the solution prepared in section 2.4 in to HPLC.

Specificity

Chromatogram obtained for the injection is shown figure 2 with Rt of 2.67 mins without the use of any internal standard.

Linearity & Range

The calibration curve showed in figure 3 has good linearity in the range of 5 – 35 µg/ml (Table 3), for Imatinib (API) with correlation coefficient (r^2) of 0.9963.The slope and intercept of the calibration graph was calculated by using linear regression analysis. The

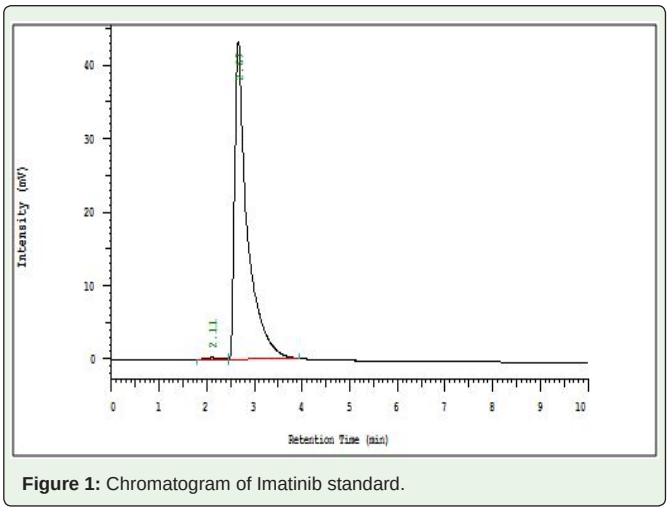

Figure 1: Chromatogram of Imatinib standard.

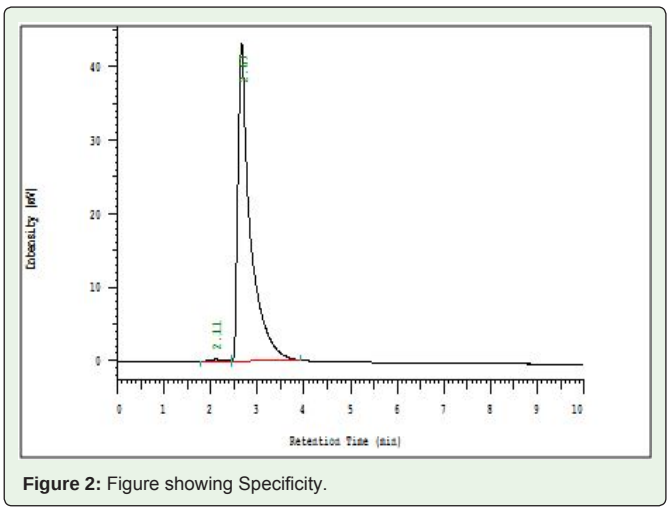

Figure 2: Figure showing Specificity.

Citation: Sahoo NK, Sahu M, Alagarsamy V, Srividya B and Sahoo CK. Current Status of Two-Dimensional Gel Validation of Assay Indicating Method Development of Imatinib in Bulk and Its Capsule Dosage Form by Liquid Chromatography. Ann Chromatogr Sep Tech. 2015;1(2):1010.

Table 4: Data for intra-day assay & inter-day assay.

Conc. of Imatinib (API) (µg/ml)	Observed Conc. of Imatinib (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=9)	% RSD	Mean (n=6)	% RSD
05	4.81	0.96	10.03	0.592193
10	20.04	0.40	30.03	01.074387
15	39.97	0.33	39.95	0.452849

Table 5: Data for Recovery of Imatinib.

Sample ID	Concentration (µg/ml)		%Recovery of	Statistical Analysis
	Pure drug	Formulation	Pure drug	
S_1 : 80 %	8	10	99.63	Mean= 99.67667%
S_2 : 80 %	8	10	99.92	S.D. = 0.223681
S_3 : 80 %	8	10	99.48	% R.S.D.= 0.224407
S_4 : 100 %	10	10	99.19	Mean= 99.19%
S_5 : 100 %	10	10	99.25	S.D. = 0.06
S_6 : 100 %	10	10	99.13	% R.S.D.= 0.06049
S_7 : 120 %	12	10	99.25	Mean= 99.49%
S_8 : 120 %	12	10	99.54	S.D. = 0.219317
S_9 : 120 %	12	10	99.68	% R.S.D. = 0.220441

regression equation of the calibration curve was: y = 76594x- 24947; r^2 = 0.996. A correlation coefficient of 0.996 suggests that the developed HPLC method had an excellent linearity over the investigated range.

Precision

Repeatability: The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of five replicates of a fixed amount of drug. Imatinib (API). The percent relative standard deviation was calculated for Imatinib are presented in the table 3 and Overall repeatability for Imatinib is shown in figure 4.

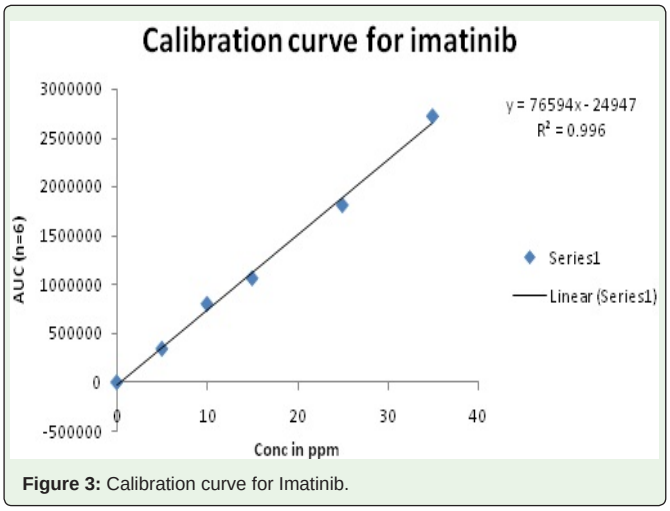

Figure 3: Calibration curve for Imatinib.

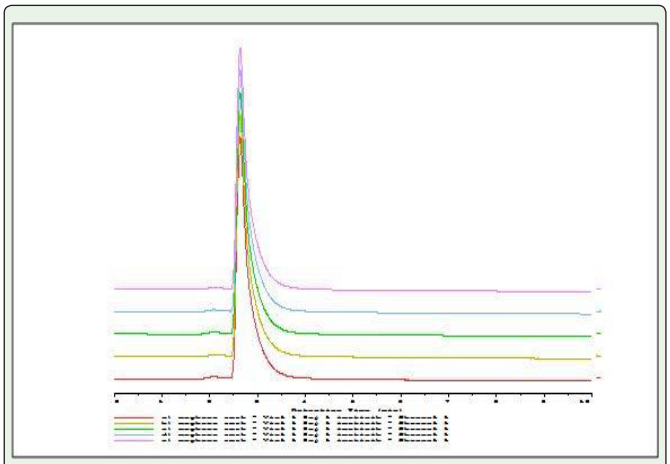

Figure 4: Overall repeatability for Imatinib.

Intra & inter day precision: The intra & inter day variation of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Imatinib revealed that the proposed method is precise and shown in table 4.

Accuracy: Recovery study

The recovery of the method, determined by adding a previously analyzed test solution with additional drug standard solution at three levels of concentration, was 99.99- 100.46 %. The values of recovery (%) and RSD (%) listed in Table 5 indicate the method is accurate.

Accuracy: The mean recovery was found to be 99.882% for Imatinib and shown in table 5. The limit for mean % recovery is 98-102% and as both the values are within the limit, hence it can be said that the proposed method was accurate.

Limit of detection and limit of quantification

The LOD was found to be 0.341µg/ml and LOQ was found to be 1.023 µg/ml for Imatinib which represents that sensitivity of the method is high.

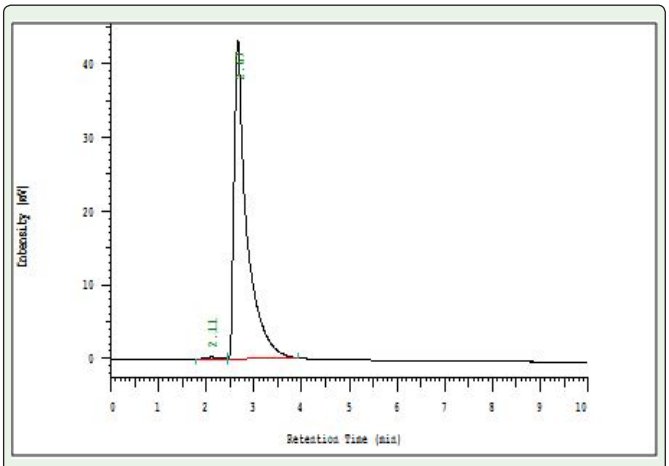

Figure 5: Chromatogram of Assay of Imatinib Capsule.

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Table 6: Data for system suitability parameters.

S. No.	Parameter	Limit	Result
1	Resolution	Rs > 2	9.15
2	Asymmetry	T ≤ 2	Imatinib=0.12
3	Theoretical plate	N > 2000	Imatinib=3246

Table 7: Data for Assay of Imatinib drug in marketed capsule.

Brand name of Capsules	Labelled amount of Drug (mg)	Mean (±SD) amount (mg) found by the proposed method (n=6)	% RSD
Omef	20	99.10 (±0.498)	0.494

System suitability

The system suitability parameters are shown in table 6.

Estimation of Imitanib in Capsule Dosage Form

Assay was performed by using the regression equation (y = 76594x- 24947; r^2 = 0.996) obtained from the standard curve of Imitanib API. Results obtained are given in table 7 and represented as chromatogram in figure 5.

The amount of drugs in omef capsule was found to be 99.10 (±0.498) mg/tab for Imatinib.

Conclusion

A New RP-HPLC method indicating assay of Imatinib in bulk and in pharmaceutical dosage form is established. This method is simple, reliable, linear, accurate, sensitive and reproducible as well as cost effective for the effective quantitative analysis of imatinib in bulk and capsule formulations. The method was completely validated showing satisfactory data for all the method validation parameters tested and method is free from interference of the other active ingredients and additives used in the formulations. Therefore the method is suitable for use of the routine quality control analysis of imatinib in API or in pharmaceutical dosage forms.

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