ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info/jasr

ISSN
0976-9595
Research Article

DEVELOPMENT AND VALIDATION OF HPTLC METHOD FOR DETERMINATION OF IMATINIB MESYLATE IN API AND TABLET DOSAGE FORM

Mahesh S. Wajurkar*, Manjusha N. Dole

Department Quality Assurance Techniques, STES's, Smt. Kashibai Navale College of Pharmacy, Kondhwa, Pune - 411048, Maharashtra, India.
**Corresponding author: mahiwajurkar07@gmail.com*

ABSTRACT

A simple, rapid, selective and quantitative HPTLC method has been developed and validated for determination of Imatinib Mesylate in bulk and tablet dosage form. The Imatinib Mesylate samples were applied on TLC Aluminium plate pre coated with Silica gel60 GF254 and developed using Methanol: Choloroform (8: 2) v/v as a mobile phase. The bands were scanned at λ=279 nm using Camag TLC scanner 3 and detection and quantification were carried out densitometrically using an UV detector. The Rf value was found to be 0.22. The linearity of the method was found to be within the concentration range of 50-300 ng/spot and its percentage recovery was found to 96.55 %. The limit of detection and the limit of quantification were found to be 16.36 ng/spot and 49.59 ng/spot respectively. The Coefficient of determination (r2) was 0.9996. The regression equation was found to be y = 17.209x + 63.798. The method was also validated for precision, specificity and recovery. This developed method was used to analyse marketed formulation.

Keywords: HPTLC, Imatinib Mesylate, Methanol, Choloroform, Rf Value

1. INTRODUCTION

Imatinib Mesylate is described chemically as the 4-[(4-methyl-1-piperazinenyl)methyl]-N-[4-methyl-3[4-(3-pyridinyl)-2-pyri -midinyl]amino] phenyl] benzamide methanesulphonate. Imatinib Mesylate is official in IP [1]. Imatinib is a cancer medication prescribed to treat leukemia and gastrointestinal tumors. It operates by inhibiting proteins associated with cancer cell growth in order to relieve symptoms, prevent the spread of cancer cells, and aid other treatments. Imatinib is one of the newest anticancer drugs in the market and was one of the first drugs to be pushed through Food and Drug Administration's (FDA) fast track designation for approval. The drug is designed to inhibit tyrosine kinases such as Bcr-Abl and is used in the treatment of chronic myeloid leukemia (CML) and gastrointestinal stroma tumors [2]. Literature survey reveals that some methods have been developed for their determination by HPLC [3-8] or spectrophotometry [9].

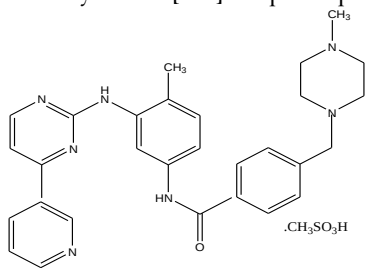

Fig. 1: Chemical Structure of Imatinib Mesylate

In this study, HPTLC method for the analysis of Imatinib Mesylate using a solvent system of methanol: chloroform (8:2 v/v) has been reported.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

Pure Imatinib Mesylate was obtained as gift sample from Neon Laboratories, Mumbai.India. Commercially available tablets manufactured by Cipla Imatib® 100 containing 100 mg of Imatinib Mesylate was procured from local pharmacy. Methanols, Chloroform used were of analytical grade (E. Merck, Mumbai, India). All the other chemicals used were also of analytical grade. (E. Merck, India).

2.2. Instrumentation and conditions

HPTLC plates (Merck) pre-coated with silica gel GF aluminium TLC plate; (10cm×10cm) were used. Densitometry was carried out with a CAMAG TLC Scanner 3, fitted with win-CATS 1.4.0 planar chromatography manager software. Sample were applied to the HPTLC plates using the spray on technique of CAMAG LINOMAT V under nitrogen gas, and developed in a CAMAG 10 cm×10 cm twin trough chambers.

2.3. Standard preparation

A standard stock solution of Imatinib Mesylate was prepared by dissolving 10 mg of standard API in 10 ml of methanol to get concentration of 1000 µg/ml. This solution was further diluted to get 100 µg/ml solution of Imatinib Mesylate as working standard.

2.4. Preparation of Sample solution

Twenty tablets of brand Imatib® 100 (Cipla Pharmaceuticals Ltd.) containing 100 mg of Imatinib mesylate were weighed, average weight determined and finely powdered. Appropriate quantity of powder equivalent to 100 mg of Imatinib mesylate was accurately weighed and transferred to a 100 ml volumetric flask and dissolved with methanol and shaken vigorously for 5 minutes. The solution was then sonicated for 20 minutes and volume was made up to 100 ml and filtered through the Whatman filter paper no. 41. Necessary dilutions of filtrate were made with methanol to get final concentration 10 µg/ml of Imatinib mesylate.

2.5. Selection of mobile phase

A trial and error method was used to select the optimised mobile phase. The solvent system of Methanol: Choloroform in the ratio 8: 2 (v/v) was the most appropriate mobile phase for the HPTLC analysis of Imatinib mesylate in methanol as solvent.

2.6. Application of standard solutions

Separate HPTLC pre-coated plate of silica gel G 60 F254 (10x10) was employed for the spotting of standard solutions. 10 µl of standard solutions of concentration 50, 100, 150, 200, 250 and 300 ng/spot were applied in the six tracks respectively in one plate.

2.7. Application of sample solution

10 µl of sample solution of 10 µg/ml for Imatinib mesylate was applied. The same procedure was repeated with the sample solution prepared from tablet dosage form. After application the position of spots were visualized and confirmed under UV cabinet at 279 nm.

2.8. Development of spot

Twin Trough chamber containing 10 ml of mobile phase system was used for developing the spotted plates and saturated for 15 minutes. The plates were dried after development and viewed under UV lamp to evaluate the spot obtained. The spots were uniform and there was no tailing.

2.9. Selection of wavelength for Detection

The working standard of Imatinib Mesylate in methanol was scanned by Camag TLC scanner 4 with UV visible detector over wavelength range 200 to 400 nm. Wavelength 279 nm was selected for detection of obtained spectrum (Fig. 2 &3).

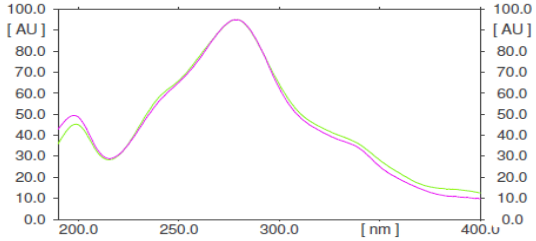

Fig. 2: The overlain UV spectra of Imatinib Mesylate (API and sample) between 200 and 400 nm

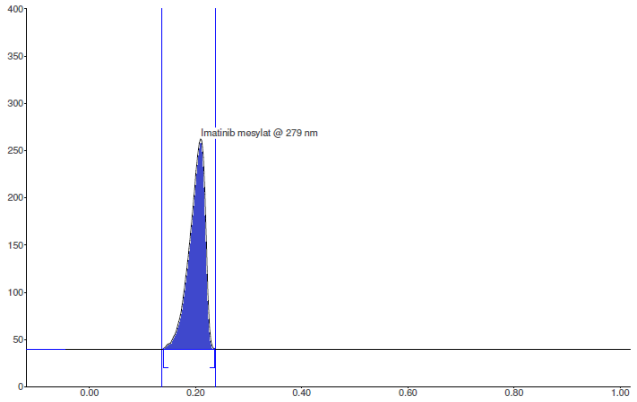

Fig. 3: Typical chromatograms obtained for Imatinib Mesylate

2.10. Chromatographic conditions

The analysis was performed on Camag HPTLC system (Switzerland). It is equipped with a Linomat-5 applicator, 100 µl sample syringe (Hamilton, Switzerland) and Camag TLC scanner-4. On the basis of trial and error method using different solvent system, following chromatographic conditions were chosen for analysis. Pre-coated silica gel 60 F_{254} TLC (E-Merck, Germany) plates (10x10 cm) were used as stationary phase. TLC plates were pre-washed with methanol and activated at 110 °C for 10 min prior to application. The standard samples of Imatinib Mesylate were spotted on pre-coated TLC plates in the form of bands of length 4 mm using 100 µl sample syringe with a Linomat-5 applicator. The chromatographic development was carried using Methanol : Chloroform (8:2 v/v) as mobile phase with chamber saturation time of 20 minutes and the migration distance of 80 mm. Densitometric scanning was performed using Camag TLC scanners at 279 nm, operated by win CATS Software (Version 1.4.3, Camag).

2.11. Assay for marketed preparation

Twenty tablets were weighed and average weight was calculated. These tablets were crushed and powdered in a glass mortar. The tablet powder equivalent to average weight of Imatinib Mesylate was accurately weighed, transferred to a 100 ml of volumetric flask dissolved in methanol then solution was ultrasonicated for 20 min and diluted up to mark with methanol then filtered with Whatmann filter paper No. 41 and the first 5 ml of filtrate was discarded. This solution was further diluted with same solvent and subjected for HPTLC study (Table1).

Table 1. Assay of marketed formulation of Imatinib Mesylate

Sample solution conc. (ng/spot)	Sample solution area	Mean Sample solution area	% Drug Found
200	3562.91		
200	3612.08	3569.14	101.84
200	3532.44		

The plate was developed under previously described chromatographic conditions.

3. METHOD VALIDATION

The objective of validation of an analytical procedure is to demonstrate whether the procedure is suitable for its intended purpose. The proposed method was validated for various parameters such as Linearity & Range, Precision, Limit of Detection (LOD) & Limit of Quantitation (LOQ) and Accuracy according to ICH Q2 (R1) guidelines [10].

3.1. Linearity and Range

The linearity was determined by using working standard solutions between 50-300ng/spot. The spectrums of these solutions were recorded and area in wavelength 279 nm. Calibration curve of peak area *v/s* concentration was plotted after suitable calculation and simple linear regression was performed [Table 2]. Regression equation and correlation coefficient were obtained. The regression equations for Imatinib Mesylate was y = 17.209x + 63.798, where y is response and x the concentration of drug. The correlation coefficients were 0.9996 [Fig. 4].

Table 2. Concentration, Rf and Area of Imatinib Mesylate

Amount ng/spot	Rf	Area
50	0.22	935.65
100	0.22	1766.60
150	0.22	2612.83
200	0.22	3549.51
250	0.22	4390.53
300	0.22	5197.10

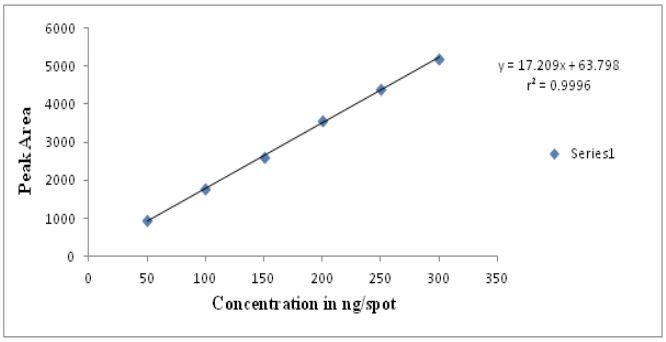

Fig. 4: Calibration Curve of Imatinib Mesylate

3.2. Precision Studies

The precision of the method was checked by repeatedly injecting (n= 8) standard solutions of Imatinib Mesylate (200 ng/spot). Area of each curve of these solutions was measured at the 279 nm. The intra-day and inter-day precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days of same concentrations of 200 ng/spot of Imatinib mesylate. The results were reported in terms of percentage relative standard deviation (%RSD). The RSD values were found to be below 2% which indicate that the proposed methods are precise. The results were tabulated in (Table 3).

Table 3: Intermediate Precision

Drug	Conc. (ng/band)	Intra-day precision (%RSD)	Inter-day precision (%RSD)
Imatinib Mesylate	200	1.2485	1.5917
	200	1.4753	1.3745

3.3. Limit of Detection (LOD) and Limit of Quantitation (LOQ)

LOD and the LOQ of the drug were calculated using the equations 3.3 σ/S and 10 σ /S respectively, where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot. The limit of detection was found to be 16.36 ng/spot. The limit of quantification was found to be 49.59 ng/spot.

3.4. Accuracy

To check the accuracy of the method, recovery studies were carried out by over spotting standard drug solution to pre-analyzed sample solution at three different levels 80 %, 100 % and 120 %. Basic concentration of sample chosen was 100 µg/ml. The areas were noted after development of plate. The drug concentration was calculated by using regression equation [Table 4].

Table 4. Recovery Studies

Level Recovery %	Amount taken (ng/spot)	Amount added (ng/spot)	% Recovery	% Mean Recovery	% RSD (All Conc. Level)
80	200	160	96.20		
100	200	200	96.14	96.55	0.6824
120	200	240	97.31		

3.5. Specificity

The specificity of the method was ascertained by analyzing standard drug and sample. The spot for drug in sample was confirmed by comparing the Rf and spectra of the spot with that of standard drug spot. The specificity of the method was also ascertained by peak purity profiling studies by analyzing the spectrum at peak start, middle and at peak end. The peak purity was determined on Win CATS software 5 [Fig. 5].

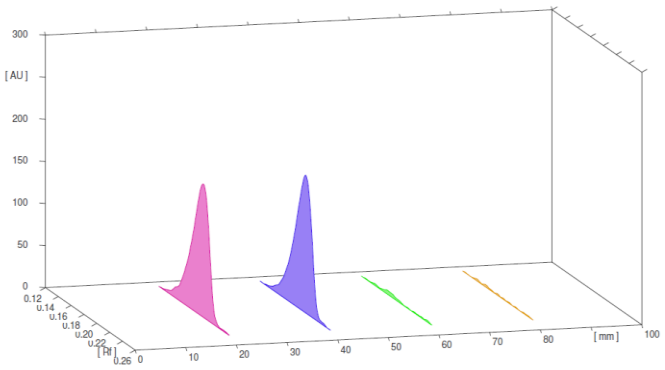

Fig. 5: Specificity curve of Imatinib Mesylate

4. RESULTS AND DISCUSSION

The Calibration curve was plotted of Imatinib Mesylate peak area v/s concentration. The generated regression equation was y = 17.209x + 63.798 (r^2= 0.9996). The r^2 value as 0.9996 indicates that developed method was linear. The calibration curve was obtained in the range of 50-300 ng/spot. The proposed method was found to be precise as % R.S.D values for intraday as well interday precision were satisfactory. The average percentage recovery at 80 %, 100 % and 120 % was found to be 96.55 %. Hence, it can be said that this method was accurate. The LOD and LOQ were calculated as 16.36 ng/spot and 49.59 ng/spot respectively. The result of the analysis of pharmaceutical formulation by the developed method was consistent with the label claim, highly reproducible and reliable. The method can be used for the routine analysis of the Imatinib Mesylate in tablet dosage form. The summary of validation parameters of proposed HPLC method is given in Table 5.

Table 5: Summary of validation parameters

Parameters	Results
Linearity range (ng/spot)	50-300
Correlation co-efficient	0.9996
Slope (m)	17.209
Intercept (C)	63.798
Precision (intraday) %RSD	1.2485
Precision (interday) %RSD	1.5917
Accurracy (mean)	96.55
LOD (ng/spot)	16.36
LOQ (ng/spot)	49.59

5. CONCLUSION

The developed and validated HPTLC method is found to be rapid, accurate, precise and economical, thus can be used for routine analysis of Imatinib mesylate in tablet dosage form.

6. ACKNOWLEDGEMENT

The authors are grateful to Neon Laboratories Mumbai, India for providing API of Imatinib Mesylate as gift sample. Authors also thanks to Anchrom Enterprises Pvt. Ltd. Mumbai, India and Smt. Kashibai Navale College of Pharmacy, Pune, India for providing necessary facilities to complete this project.

7. REFERENCES

1. Indian Pharmacopoeia, Published by the Controller of Publication , New Delhi, 2010, II, 1484-1485.
2. Patil S, Doijad R, Dhumal P. *Asian Journal of Pharmaceutical and Clinical Research*, 2013; **6(2)**:54-57.
3. Sandhya P, Vishnu Priya P, Shyamala, Anjali Devi N, Sharma JVC. *World Journal of Pharmacy and Pharmaceutical Sciences*, 2013; **3(1)**:682-688.
4. Kuna AR, Kuna JK. *International Journal of Pharmacy and Pharmaceutical Sciences*, 2011; **3(5)**:162-165.
5. Aleti P, Venisetty R, Kamarapu SK. *International Journal of Pharmacy and Biological Sciences*, 2011; **1(2)**:14-18.
6. Venkatramanna M, Kondrasudhakarbhau, Nrayanareddy P. *Global Journal of Analytical Chemistry*, 2011; **2(5)**:198-207.
7. Bende G, Kollipara S, Moorthy G, Saha R. *J Chromatogr Sci.*, 2010; **48(5)**:334-41.
8. Rosasco MA, Moyano MA, Pizzorno MT, Adriana. *Journal of liquid Chromatography and related technologies*, 2007; **28(20)**:3283-3292.
9. Kumar Raja J, Sunder VD, Magesh AR, Nandhakumar S, Dhanaraju MD. *International Journal of Pharmacy and Technology*, 2010; **2(3)**:490-495.
10. ICH Harmonized-Tripartite Guidelines. Validation of Analytical Procedure: Text and Methodology Q2 (R1), November, 2005.