RESEARCH ARTICLE

Method Development and Validation for Assay and Related Substance of Imatinib Mesilate in Bulk and Tablet Dosage form using RP-HPLC

Anand G Kshatriya[1], P Andal[1*], Ashok Mhaske[2]

[1]*Department of Chemistry, VISTAS, Pallavaram, Chennai, India.*
[2]*Scientia Qualiteck Laboratory, Navi Mumbai, Maharashtra, India.*

Received: 06th October 2023; Revised: 21st January, 2024; Accepted: 22nd February, 2024; Available Online: 25th March, 2024

ABSTRACT

A new method was developed and validated to assay imatinib mesilate and its impurities in drug substance and dosage forms. The developed method can be utilized to determine drug content and its related substances. The method validation study proves that the method is accurate, precise, specific and robust. The imatinib mesilate has five specified impurities and can be easily determined using this methodology. All impurities and imatinib peak are resolved using XB ridge C18, 250 mm x 4.6 mm, 5 µm column. A mixture of acetate buffer pH 9.5 and a mixture of methanol and acetonitrile in gradient mode are separated. The wavelength is selected at 264 nm with a column temperature of 30°C and a run time of 45 minutes. Linearity covered from 0.3 to 1985 µg/mL. The method has been validated as per ICH Q2 (R1) guidelines. Forced degradation study is performed using this method and proved that the method is stability-indicating and suitable for use.

Keywords: Impurities, Related substance, Imatinib mesilate, Validation, Assay.

International Journal of Pharmaceutical Quality Assurance (2024); DOI: 10.25258/ijpqa.15.1.11

How to cite this article: Kshatriya AG, Andal P, Mhaske A. Method Development and Validation for Assay and Related Substance of Imatinib Mesilate in Bulk and Tablet Dosage form using RP-HPLC. International Journal of Pharmaceutical Quality Assurance. 2024;15(1):76-82.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Imatinib mesilate is used to treat acute lymphoblastic leukemia in pediatrics and adults. It is also called as Philadelphia chromosome positive. Imatinib mesilate is used along with chemotherapy in adults it used in the treatment of hyper eosinophilic syndrome or chronic eosinophilic leukemia. It is preferably used in newly diagnosed chronic phase cancer in children and in adults.[1,2]

Reviewed number of literatures there are several methods available for the determination of imatinib mesilate. In these methods either only single or double impurities can be determined, some are only able to determinate the individual imatinib mesilate and some are using liquid chromatography–mass spectrometry (LCMS).[3-5] Reviewed number of literatures but no single method is available to separate five specified impurities in single chromatographic method.[6,7] Imatinib acid impurities, imatinib N-oxide impurity, imatinib impurity-A, imatinib impurity-B, and imatinib desmethyl impurity are well separated in this developed method. This is reverse phase UV detection method with very simple mobile phase and small run time method.

MATERIALS AND METHODS

Equipment used**:** Water HPLC 2695 equipped with UV and PDA detector 2998, Lab-India pH meter used for separation and XB ridge C18, 250 x 4.6 mm, 3.5 µm column used to achieve the separations, flow rate is 1.5 mL/min with gradient mode and Mettler Toledo balance is used for all method validation study.

Reagents and Chemicals

Ammonium acetate, ammonia, acetonitrile and methanol chemicals and reagent used for preparation buffer preparation.

Chromatographic conditions[9]: Separation was done by using mobile phases such as ammonium acetate, pH 9.5, and a 40:60 v/v combination of acetonitrile and methanol. The X-bridge C18 column (250 x 4.6 mm, 5 µm) was selected because to its 18% carbon weight, which helps in separation and gradient. The flow rate is 1.5 mL/min, and the selected spectrum range is 264 nm. The column temperature is 30°C, with an injection volume of 10 µL. The imatinib mesilate elutes at about 18 minutes, and the total run time is 45 minutes.

Preparation of Mobile Phase

Mobile phase-A[10]

Dissolve about 3.85 g/L of ammonium acetate in water. Adjust pH 9.5 ± 0.05 with ammonia solution. Filter through 0.45 µ filter.

Mobile phase-B

Prepare a mixture of acetonitrile and methanol in a ratio of 40:60, (% v/v)

**Author for Correspondence: andalprithu.sbs@velsuniv.ac.in*

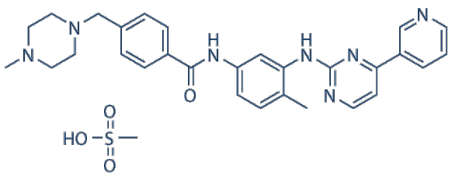


Figure 1: Imatinib mesilate: $C_{30}H_{35}N_7O_4S$[8]

Table 1: Gradient program: Mobile phase-A[9]

Time (Minutes)	0	25	35	40	45
Mobile phase-A (% v/v)	58	58	20	58	58
Mobile phase-B (% v/v)	42	42	80	42	42

Diluent

Prepare a mixture of water and acetonitrile in a ratio of 50:50 (% v/v) (Table 1).

System suitable solution

Prepared 0.5 mg/mL solution imatinib mesilate (Figure 1) system suitability CRS containing impurities.[11]

Preparation of standard solutions

Weigh an amount of 30 mg of imatinib mesilate standard and transfer to a 25 mL volumetric flask. Dissolve with 20 mL of diluent and dilute to volume with diluent, then mix.

Sample solution preparation[12]

Weigh exactly 20 tablets and crush into fine powder. From the fine powder 100.0 mg of imatinib powder was transferred in to a 100 mL volumetric flask. Add 70 mL of diluent and sonicate for 20 minutes while shaking intermittently. Cool the flask to room temperature, then dilute to volume with diluent and mix.

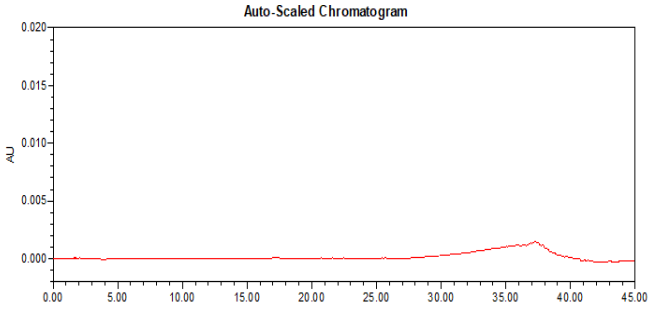


Figure 2(a): Chromatogram of blank

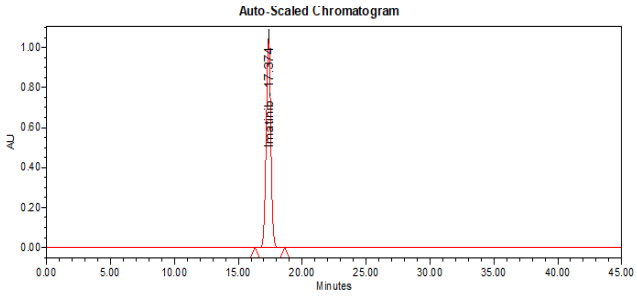


Figure 2(b): Chromatogram of standard

Table 2: System suitability of imatinib mesilate

Parameter	*Imatinib mesilate*
USP Tailing factor	1.1
All impurities peak resolution with respect to each other	More than 1.5
%RSD of Area	0.6

Table 3: RRT and RRF of impurities in system suitability of imatinib mesilate

Name of the component	*~ RRT*	*RRF*	*LoQ in %*	*LoD in %*
Imatinib	1.00	1.00	0.03	0.01
Imatinib acid impurity	0.10	0.04	0.05	0.02
Impurity-A	0.13	0.36	0.05	0.02
Impurity-B	0.15	0.85	0.05	0.02
N-Oxide impurity	0.37	0.33	0.03	0.01
Desmethyl impurity	0.58	1.03	0.03	0.01

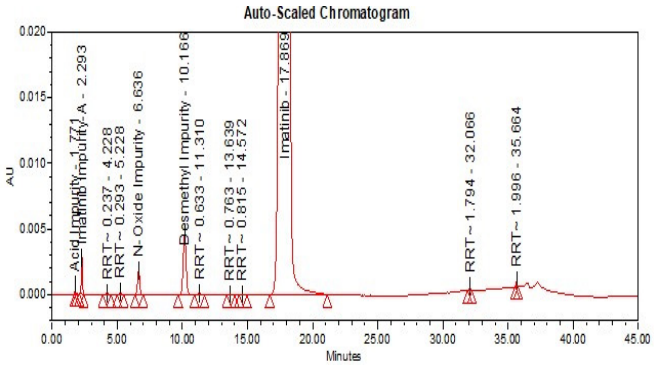
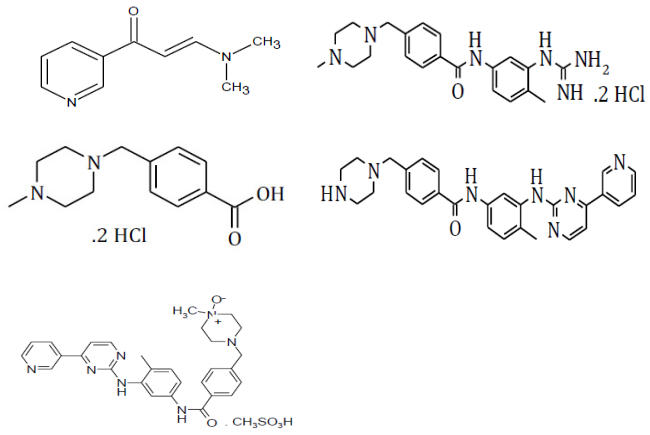


Figure 2(c): Chromatogram of spiked sample



(Name left to right, the name of impurities and impurity-A, B, Acid impurity. Desmethyl impurity & N-oxide impurity)

Figure 2(d): Structure and name of impurities

Table 4: Linearity results for imatinib mesilate

Parameters	*Impurity-A*	*Impurity-B*	*N-oxide impurity*	*Desmethyl impurity*	*Acid impurity*	*Imatinib assay*
Slope	8639.26	20552.40	38053.47	24839.74	1060.68	26179.66
Intercept	-141.11	-2819.30	-4243.45	-2252.95	89.47	211278.4
%Y-Intercept	-0.55	-4.73	-3.62	-1.92	2.60	0.80
Correlation (R)	1.000	0.999	0.996	0.996	0.997	1.000
R^2	1.000	0.999	0.996	0.996	0.997	1.000

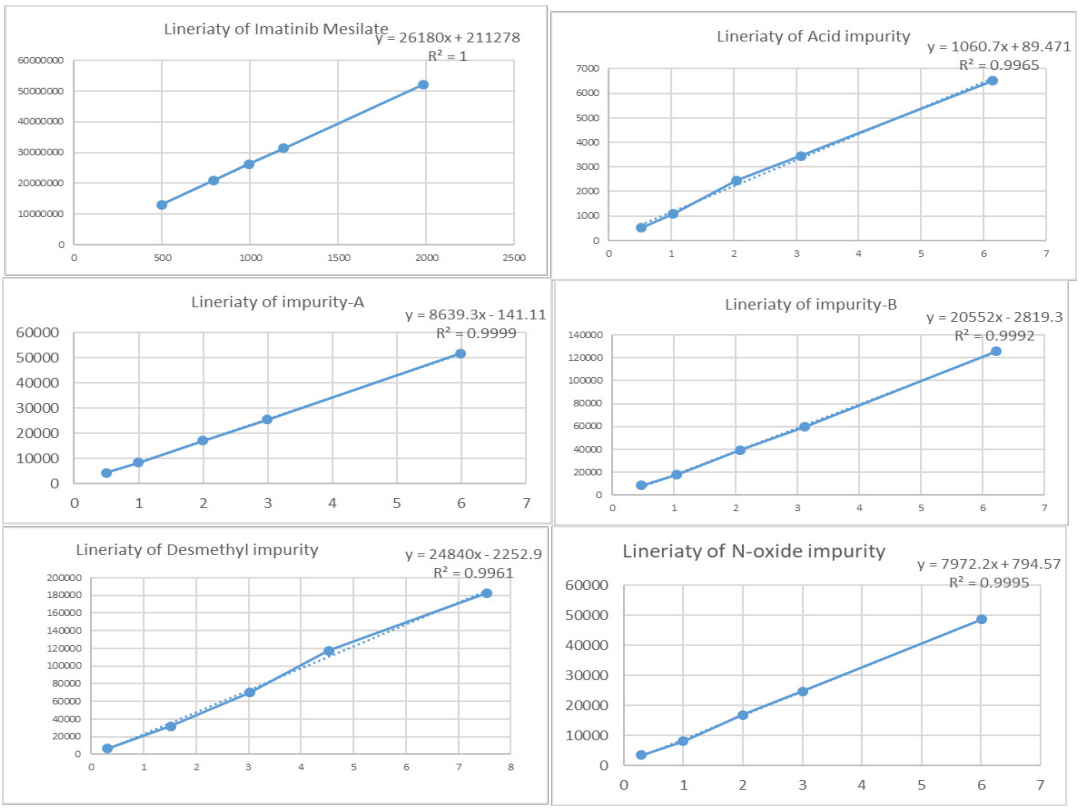


Figure 3: Linearity results for imatinib mesilate

Table 5: Result of precision study

S. No.	*Spl prep-1*	*Spl prep-2*	*Spl prep-3*	*Spl prep-4*	*Spl prep-5*	*Spl prep-6*	*%Average*	*Overall %RSD*
Acid Impurity	98.9	99.1	97.6	97.9	99.2	96.9	98.2	1.0
Impurity-A	101.9	103	100.8	100.2	102.5	99.4	101.3	1.4
Impurity-B	97.6	95.5	91.1	90.9	95.5	95.0	94.3	2.9
Desmethyl impurity	100.6	100.4	100.3	100.8	100.5	100.7	100.6	0.2
N-Oxide impurity	98.3	98.1	98.6	98.7	98.5	98.6	98.5	0.2

Enhancement of RP-HPLC method

This method developed considering imatinib acid impurity, impurity-A, impurity-B, N-oxide impurity and desmethyl impurity. The selection of the mobile phase's pH is considering the nature and elution of impurities based on the number of trials. The imatinib mesilate having four pka values, i.e., 1.52, 3.73, 2.56 and 8.07. Different pH ranges such as pH 3.0, 6.5 and mobile phase with ion pairs, buffers such as phosphate has been evaluated for the better separation of impurities but overall the pH 9.5 found more suitable. Different combination of buffer with acetonitrile has been evaluated, in this combination of methanol and acetonitrile plays important role. The ambient column temperature and 30°C have been evaluated, and 30°C was found to be more suitable than ambient temperature. The gradient plays an important role for the separation of all analytes with 18 minutes, however long eluting unknown peaks were observed hence the run time kept about 45 minutes. The method is suitable and the separation of above impurities is

Table 6: Result for accuracy

Acid impurity					
*% Level**	*Added qty in (µg/mL)*	*Found qty in (µg/mL)*	*Recovery in %*	*Average recovery %*	*%RSD*
LoQ	0.512	0.507	99.0	101.2	1.9
100%	2.048	2.025	98.9	98.2	1.0
150%	6.144	6.255	101.8	100.6	1.14
Impurity-A					
% Level	*Added qty in (µg/mL)*	*Found qty in (µg/mL)*	*Recovery in %*	*Average recovery %*	*%RSD*
LoQ	0.499	0.510	102.2	101.2	0.9
100%	1.996	2.033	101.9	101.3	1.4
150%	5.987	6.116	102.2	100.6	1.1
Impurity-B					
% Level	*Added qty in (µg/mL)*	*Found qty in (µg/mL)*	*Recovery in %*	*Average recovery %*	*%RSD*
LoQ	0.490	0.515	105.1	106.0	1.7
100%	2.073	2.024	97.6	94.3	2.9
150%	6.219	6.179	99.4	101.0	1.0
Desmethyl Impurity					
% Level	*Added qty in (µg/mL)*	*Found qty in (µg/mL)*	*Recovery in %*	*Average recovery %*	*%RSD*
LoQ Level-1	0.302	0.301	99.7	98.8	1.1
100% Level-1	3.020	3.038	100.6	100.6	0.2
150% Level-1	9.060	9.083	100.3	100.3	0.2
Desmethyl Impurity					
% Level	*Added qty in (µg/mL)*	*Found qty in (µg/mL)*	*Recovery in %*	*Average recovery %*	*%RSD*
LoQ Level-1	0.292	0.305	104.5	96.8	4.1
100% Level-1	1.834	1.802	98.3	98.5	0.2
150% Level-1	6.101	5.865	96.1	96.1	0.4

achieved in short run. This is unique method that separate the all impurities in single run. Further, forced degradation study performs to check the addition degradation peaks. Initially the European pharmacopeia method verified the above impurities however in this method the degradation impurities peak due to oxidation stress sample are found merged in N-oxide impurity. Various gradient, column temperatures, mobile phases, change in gradient has been evaluate. Finally, a suitable method is achieved with XBridge 250 x 4.6 mm, 5 µm column. The final mobile phase is pH 9.5 acetate buffer, and the mixture of acetonitrile and methanol is found most suitable.

Table 7: Robustness results for imatinib mesilate

Parameter	*Change*	*%Difference in assay of imatinib mesilate*
Wavelength (nm)	262	0.1
	266	0.1
Column temperature (°C)	25	0.7
	35	1.3
pH	9.3	1.1
	9.7	0.7

*Further there is no any significant variation observed in the impurities result as all result found below 0.05%

Validation Study

Validation of this method done using ICH Q2 (B) guidelines.[13]

System suitability

Blank (diluent) solution, standard solution five replicates injected as per above chromatography to establish the system suitability. Refer Tables 2 and 3 for results.

Specificity

The method's specificity was demonstrated by injecting blank, standard, and spiked sample solutions containing all contaminants as well as particular impurities. Placebo solution injected to prove the specificity of the method for dosage form. All imatinib peak and their impurities were resolved well with each other. there is any interference was not detected owing to the blank and placebo at the retention time of imatinib mesilate peak and specified impurities. The chromatograms of blank, standard solution and spiked sample is given in Figures 2a, 2b 2c and 2d. respectively.

Table 8: Forced degradation results for imatinib mesilate tablets

Name of the solution	*Finished sample*			*API*		
	Total impurities (%)	*%Assay*	*Mass balance*	*Total impurities (%)*	*%Assay*	*Mass balance*
Control sample	0.09	100.5	NA	0.06	99.9	NA
Base stress sample (2.0N NaOH/2 mL/80°C for 1-hour)	0.46	100.6	100.7	0.28	100.8	101.2
Acid stress sample (2.0N HCl/2 mL/80°C for 2 hours)	10.60	92.6	102.7	10.86	93.4	103.7
Oxidation stress sample ($KMNO_4$/2 mL/80°C for 1-hour)	0.90	98.3	98.7	0.47	99.8	100.4
Thermal stress sample (80°C/48 hours)	0.26	101.8	101.6	0.06	100.6	100.8
Photolytic stress sample (200-watt hours/m^2± 1.2 m Lux hour)	0.14	100.3	99.9	0.04	100.6	100.7
Water hydrolysis (H_2O/10 mL/80°C for 1-hour)	02	97.1	96.8	0.06	99.8	100.1

*Used empower software and PDA detector for determination of peak purity i.e Purity threshold should be greater than purity angle.

Table 9: Filter suitability study

Sample	*Centrifuged*	*spiked sample filtered with 0.45 µm PVDF filter 2 mL discarded volume*	*Spiked sample filtered with 0.45 µm nylon filter 2 mL discarded volume*
Imatinib acid impurity	0.203	0.211	0.205
Impurity-A	0.165	0.166	0.163
Impurity-B	0.087	0.085	0.090
N-oxide impurity	0.322	0.322	0.321
Desmethyl impurity	0.282	0.279	0.279
Single max unknown	0.012	0.012	0.012
Total impurity	1.10	1.10	1.10
Difference from Initial			
Imatinib acid impurity	NA	0.008	0.002
Impurity-A	NA	0.001	-0.002
Impurity-B	NA	-0.002	0.003
N-oxide impurity	NA	0.000	0.001
Desmethyl impurity	NA	0.003	0.003
Single max unknown	NA	0.00	0.00
Total impurity	NA	0.00	0.00
Assay	99.9	100.0	100.4
Difference of assay	NA	0.1	0.5

Linearity

Imatinib and its impurities linearity range covered from 0.3 to 6 ppm for impurities and for assay up to 1985 ppm. Calculated the correlation coefficient, slope and y-intercept for all imatinib and its impurities. Refer to Table 4 and Figure 3 for all the results.

Precision

The spiked sample was prepared by spiking all the specified impurities. Further intermediate precision performed by preparing similar preparation as precision. Precision and intermediate precision perform for impurities and for assay i.e. content of imatinib. All results found satisfactory. In Table 5 summarizes the results.

Accuracy

The accuracy study performed using individual impurity standards and against imatinib using RRF. Both the recoveries found well within acceptance criteria and comparable. The recovery was performed for the assay from 50 to 150% of the

Table 10: Solution stability study for RS

Sample	*Initial*	*After 27 hrs*	*After 37 hrs*	*After 47 hrs*
Imatinib acid impurity	0.193	0.188	0.197	0.199
Impurity-A	0.179	0.181	0.180	0.182
Impurity-B	0.189	0.179	0.181	0.181
N-oxide impurity	0.243	0.256	0.255	0.258
Desmethyl impurity	0.275	0.271	0.273	0.276
Single max unknown	0.02	0.02	0.02	0.02
Total impurities	1.11	1.15	1.16	1.17
Difference from Initial				
Imatinib acid impurity	NA	0.005	-0.004	-0.006
Impurity-A	NA	-0.002	-0.001	-0.003
Impurity-B	NA	0.010	0.008	0.008
N-oxide impurity	NA	-0.013	-0.012	-0.015
Desmethyl impurity	NA	0.004	0.002	-0.001
Single max Unknown	NA	0.00	0.00	0.00
Total impurities	NA	0.01	0.00	-0.01

Table 11: Solution stability study for assay

Assay sample solution at room temperature		
Time interval	*% of assay*	*%Difference*
Initial (hours)	100.7	NA
After 14	100.9	0.2
After 42	100.7	0.0
After 52	101.3	0.6
After 62	101.6	0.9

target concentration. The accuracy results are captured under Tables 6 and 7.

Robustness

The robustness study is performed by identifying the critical parameter during the development phase. Change in buffer pH, change in column temperature and change in wavelength are the identified parameters on which the robustness study was conducted by using spiked sample. The results are compared with controlled samples, i.e., unaltered condition spiked sample results. All results were found to be comparable with unaltered conditions. The results are captured in Table 8.

Forced degradation study

Acid stress, base stress, oxidation stress, thermal stress, photolytic stressed and water hydrolysis stressed condition blank, placebo, API and dosage form sample prepared using above sample preparation procedure and injected under the system. Calculated the %degradation, peak purity and mass balance. The degradation peaks from each known and unknown impurities are found well resolved. The peak purity found passes, which is verified by the empowering software. Refer Table 9 for result of forced degradation.[14-17]

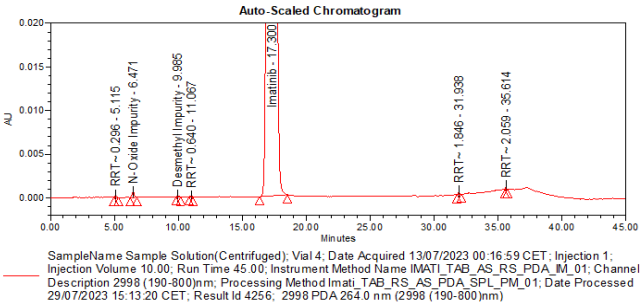


Figure 4: Chromatogram of sample

Table 12: Result of pharmacy market sample[11]

Product	*Strength/LC*	*Recovered in mg*	*%Assay*
GLEEVEC	400	396	99.0

Filter validation

Evaluated the 0.45 µ PVDF and nylon filter for both assay and impurities. The result compared against a centrifuged sample and the difference found well within acceptance criteria. The results are captured in Table 10.

Solution stability

The solution stability was established using a spiked sample solution and a controlled assay solution. Evaluated the system suitability followed by the %impurities and %assay with respect to initial results. For result refer Tables 11 and 12

Assay of marketed formulations

This method determined the assay and impurities of local pharmacy sample. The results found suitable. The chromatogram for results refer to Table 12 and Figure 4.

RESULTS

A high carbon load column with extended pH stability (Xbrigde C18, Wates) is selected for better stability and resolution of peaks (35–37). Mobile phase combination of acetate buffer pH 9.5 and mixture of methanol and acetonitrile with gradient elution plays important role in separation. The retention time 18.0 minutes is nominal considering the impurities separation and total run time 45 minutes. This method is capable of estimating the assay of imatinib and its related impurities. The chromatogram suitability parameters are summarized in tables. The sensitivity of method is less than 0.05% which is very good considering the 0.05% is disregard limit in many pharmacopoeial methods.

All method validation parameter results found well within pre-defined acceptance criteria. The developed method fully validated without any discrepancy. Further the forced degradation study and degradation peaks found well separate from known and unknown impurities, hence it can be concluded that the method is stability indicating method. The

acetate buffer pH 9.5 ± 0.05 and mixture of acetonitrile and methanol are found most suitable mobile phase with column XBridge C18, 250 X 4.6 mm, 5 µ in gradient mode separation. pH of mobile phase plays important role to separate all the impurities. Preferably acid impurity is highly polar and it elutes early. In this method the acid impurity peak is well resolved from other peaks. The method validation results proved that given method is selective, accurate, specific, precise and robust. Filter study and solution stability study performed. The nylon and PVDF both filters found suitable for sample filtration. This method is capable to quantify the five specified impurities and assay of imatinib within single method.

System suitability

Blank (diluent) solution, standard solution five replicates injected as per above chromatography to establish the system suitability. Refer Tables 2 and 3 for results.

CONCLUSION

The validation study was performed as per ICH guideline and All result found well within acceptance criteria. [9]. It is concluded that this method can be utilized to determine the assay and related impurities on imatinib mesilate from dosage form. The method accurate, precise, specific and robust. Further the methzod is stability indicating.

ACKNOWLEDGMENT

The authors are thankful to the Scientia Qualitek®, Navi Mumbai. for donating drug samples of imatinib mesilate to Chemistry Department of Vels Institute of Science Technology and Advanced Studies Velan Nagar. Lot of support received from Scientia Qualiteck and I am thankful for allowing me in their facilities to perform the activity.

REFERENCES

1. Biondi A, Schrappe M, De Lorenzo P, Castor A, Lucchini G, Gandemer V, Pieters R, Stary J, Escherich G, Campbell M, Li CK, Vora A, Aricò M, Röttgers S, Saha V, Valsecchi MG. Imatinib after induction for treatment of children and adolescents with Philadelphia-chromosome-positive acute lymphoblastic leukaemia: a randomised, open-label, intergroup study. Lancet Oncol. 2012 Sep;13(9):936-45.
2. Sacha T. Imatinib in chronic myeloid leukemia: an overview. Mediterr J Hematol Infect Dis. 2014 Jan 2;6(1): e2014007.
3. Ivanovic D, Medenica M, Jancic B, Malenovic A. Reversed-phase liquid chromatography analysis of imatinib mesylate and impurity product in Glivec capsules. J Chromatogr B Analyt Technol Biomed Life Sci. 2004 Feb 5;800(1-2):253-8.
4. Medenica M, Jancic B, Ivanovic D, Malenovic A. Experimental design in reversed-phase high-performance liquid chromatographic analysis of imatinib mesylate and its impurity. J Chromatogr A. 2004 Mar 26;1031(1-2):243-8.
5. Arun Kumar Kuna, Ganapaty Seru, GV Radha. Analytical method development and validation for the estimation of Imatinib mesylate and its acid impurity in pharmaceutical formulation by RP-HPLC. Pharma Innovation 2018;7(12):418-422.
6. Arun Kumar Kuna, Ganapaty Seru and Radha Gadela. Analytical method development and validation for the estimation of imatinib mesylate and its dimer impurity in pharmaceutical formulation by reverse-phase high-performance liquid chromatography,2018; Asian Journal of Pharmaceutical and Clinical Research 11(3):136.
7. Teoh M, Narayanan P, Moo KS, Radhakrisman S, Pillappan R, Bukhari NI, Segarra I. HPLC determination of imatinib in plasma and tissues after multiple oral dose administration to mice. Pak J Pharm Sci. 2010 Jan;23(1):35-41.
8. Badraddin M.H. Al-Hadiya, Ahmed H.H. Bakheit, Ahmed A. Abd-Elgalil. Imatinib Mesylate. Profiles of Drug Substances, Excipients and Related Methodology.2014, Vol. 39;265-297.
9. Kranthi Kumar Kotha. Process validation of citalopram hydrobromide tablets. International journal of research in pharmaceutical and biomedical sciences, 2010; Issue 1 (2); 109-123.
10. Kranthi Kumar Kotha. Rp-HPLC method for the simultaneous estimation of emtricitabine, tenofovir, and efavirenz in pharmaceutical dosage form, Journal of global trends in pharmaceutical sciences; 2011; Vol.2 (2); 177-186.
11. Gnana RPM, Devhare LD, Dharmamoorthy G, Khairnar MV, Prasidha R. Synthesis, Characterisation, Molecular Docking Studies and Biological Evaluation of Novel Benzothiazole Derivatives as EGFR Inhibitors for Anti-breast Cancer Agents. International Journal of Pharmaceutical Quality Assurance. 2023;14(3):475-480..
12. Kranthi Kumar Kotha. Formulation and Evaluation of Atorvastatin Calcium Immediate Release Tablets-20 mg, USP Indian Journal of Novel Drug delivery, 2013; 5(3); 130-141.
13. Devhare LD and Gokhale N. Antioxidant and antiulcer property of different solvent extracts of cassia tora linn. Research journal of pharmacy and technology. 2022, 15(3): 1109-1113
14. Kotta Kranthi Kumar, B Suma Padmaja, T Srikrishna Formulation and Evaluation of Atorvastatin Calcium Immediate Release Tablets-20 mg, USP Indian Journal of Novel Drug delivery, 2013; 5(3);130-141.
15. Sonule M, Devhare LD, Babu MN, Gunjal SD, Varalaxmi S. Microemulgel-based Hydrogel of Diclofenac Sodium using Lipidium sativum as a Gelling Agent. International Journal of Drug Delivery Technology. 2023;13(4):1235-1239.
16. Chawla A, Devhare LD, Dharmamoorthy G, Ritika, Tyagi S. Synthesis and In-vivo Anticancer Evaluation of N-(4-oxo-2-(4-((5-aryl-1,3,4 thiadiazole-2yl) amino) Phenyl thiazolidine-3-yl) Benzamide derivative. International Journal of Pharmaceutical Quality Assurance. 2023;14(3):470-474.
17. Bhakre H, Agrawal A, Chatap VK. Formulation, Development and Evaluation of Highly Oxidative Degradative Drug Molecule Injectable Dosage form by Lyophilisation Techniques. International Journal of Drug Delivery Technology. 2023;13(4):1378-1384.