METHOD DEVELOPMENT AND VALIDATION FOR QUANTIFICATION OF IMATINIB MESYLATE SPIKED *IN VITRO* SALIVA BY LC-MS/MS

Swetha Sri Remidicherla[a,b], Guntupalli Chakravarthi[b*], Pravallika S.[b], Alavala Rajasekhar Reddy[b] and GSN Koteswara Rao[b]

(Received 20 February 2023) (Accepted 16 March 2024)

ABSTRACT

A novel, sensible, rapid, reliable and economical analytical hyphenated LC-MS/MS method has been developed as a key for the safety surveillance in chronic leukemia patients, and as a part of therapeutic drug monitoring of imatinib mesylate in human saliva. Imatinib mesylate or imatinib methane sulfonate is a tyrosine kinase inhibitor, apoptosis inducer and also known to be an anticoronaviral agent. Imatinib mesylate is a monomesylate salt of imatinib used for the treatment of gastrointestinal tumors and chronic myelogenous leukemia, and also in other complex malignancies. The λmax of imatinib mesylate was observed at 258 nm by UV spectrometry, establishing a very good linearity along with sensitivity. The detection limit (LOD) =0.2925 µg mL^{-1} and quantitation limit (LOQ)= 0.8977 µg mL^{-1} were obtained from the linear concentrations taken in the range of 2-12 µg mL^{-1}. The correlation coefficient (r^2) found was 0.999. The method validation parameters according to ICH Q2 (R1) were performed. The developed method described here, UPLC-MS/MS, was found to be novel, sensitive and rapid with improved results when successfully tested for human saliva samples without significant differences in the steady state imatinib mesylate concentrations. Current method could overcome the safety issues during therapeutic drug monitoring and pharmacokinetic behavior of the drug when tested clinically.

Keywords: Imatinib mesylate , LOD, LOQ, ICH Q2 (R1), LC-MS/MS

ABBREVIATIONS

IMT-Imatinib mesylate, LOQ-Quantitation limit, LOD-Detection limit; ICH-International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, MRM-Multiple reaction monitoring, RE-Relative error; HQC-High quality control, MQC-Medium quality control, LQC-Low quality control

INTRODUCTION

Tyrosine kinase inhibitor and anti-cancer drug imatinib mesylate (IMT) is used to treat chronic myelogenous leukemia and other advanced cancers. Its formal chemical name is 4-[(4-methyl-1-piperazinyl)methyl]-*N*-[4-[4-(3-pyridinyl)-2-pyrimidinyl]amino]-3-[4-methyl]-phenyl] benzamide methane sulfonate[1]. Imatinib mesylate is a white, crystalline powder that is soluble in methanol and water. Imatinib mesylate typically works by preventing Bcr-Abl tyrosine kinase from autophosphorylation. Imatinib has strong oral absorption, and after 2-4 h of oral administration, C_{max} reaches the highest plasma concentration. It was revealed that the mean absolute bio-availability was 98 %. The elimination half-lives of imatinib and its *N*- demethyl derivative (CGP74588, a key active metabolite) after oral administration to healthy volunteers were found to be around 18 and 40 h, respectively. Imatinib mesylate's mean AUC was shown to rise proportionally when concentration increased from 25 to 1000 mg. The drug imatinib mesylate, which functions by binding to plasma proteins such albumin and 1-acid glycoprotein, may be estimated to reach 95 % clinically relevant doses in *in vitro* experiments. The primary factor in its metabolism is the enzyme CYP3A4. Other cytochrome P450 enzymes such CYP1A2, CYP2D6, CYP2C9 and CYPC19 have been discovered to slightly metabolise imatinib. The *N*-demethylated piperazine derivative was determined to be the primary active metabolite circulating in the body and was shown to have similar action to

[a] Department of Pharmaceutical Analysis, Sarojini Naidu Vanita Pharmacy Maha Vidyalaya, Vijayapuri Colony, Tarnaka – 500 017, Secunderabad, Telangana, India

[b] K L College of Pharmacy, Koneru Lakshmaiah Education Foundation, Vaddeswaram – 522 502, Guntur, Andhra Pradesh, India

*For Correspondence: E-mail: Chakra_varthi123@kluniversity.in

https://doi.org/10.53879/id.61.04.13917

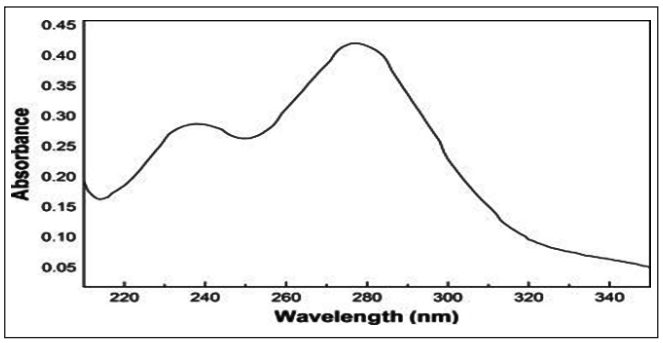

Fig. 1: UV-spectrum of imatinib mesylate in distilled water

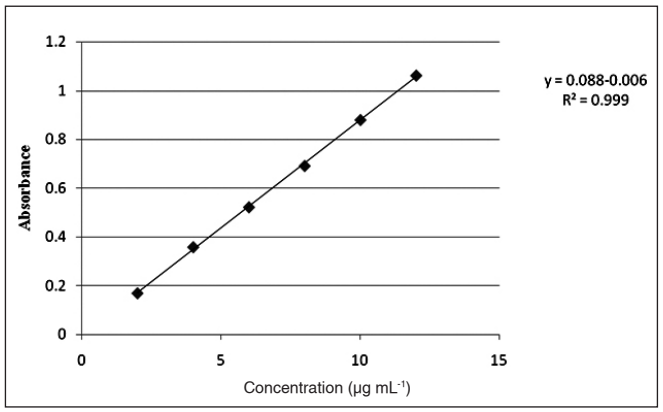

Fig. 2: UV calibration curve of imatinib mesylate at 258 nm

the parent drug. It is produced by the CYP3A4 enzyme. The demethylated metabolite plasma AUC was found to be 15 % of parent imatinib. It was discovered that the *N*-demethylated drug metabolite has similarities with the original medication in its affinity for plasma proteins such CGP74588. Imatinib that hasn't been metabolized may be accounted for to around 25 % of the dosage, or about 5 % in urine, 20 % in feces, and the rest as metabolites. According to the study, a 50-year-old patient weighing 50 kg might have an estimated imatinib clearance rate of 8 L h^{-1}, and a 50-year-old patient weighing 100 kg could have an increased imatinib clearance rate of 14 L h^{-1}. The first dosage modification based on body weight or age probably cannot account for the estimated 40 % variance in clearance across the individuals, which accelerates the requirement for vigilant monitoring for treatment-related toxicity.

Few UV spectroscopy methods have been reported[1-6]. Extensive literature is available on the liquid chromatographic determination as well as detection with hyphenated mass spectrometric method for simultaneous estimation of tyrosine kinase inhibitors[7-15]. The findings could suggest that the gradient elution and the simple, economical bio-analytical methods with lower costs, and high sensitivity which are validated are necessary to analyze several samples clinically in therapeutic drug monitoring. Current study involved developing a novel bio-analytical method to quantify the imatinib mesylate in human saliva by LC-MS/MS and UV spectrometry. Studies have shown a positive correlation between salivary and plasma IMT concentrations, suggesting saliva as a viable surrogate matrix for assessing systemic drug exposure. This simplifies monitoring and reduces costs compared to frequent blood draws. Stability indicating studies following ICH guidelines were executed[16-20].

MATERIALS AND METHODS

Instruments used

ELICO SL 210 UV-VIS spectrophotometer with two sample holders was employed. For recording the absorbance, samples filled in quartz cuvettes were taken. Samples were weighed on balance having readability of 0.0001 g. Acquity SDS Ultra Performance LC system (Waters, India) for liquid-liquid extraction method coupled with MASS spectrometer Mass Lynx version 4.1 SCN805 was used.

Standards and reagents

Imatinib mesylate reference standard was obtained as a gift sample from Hetero Labs Ltd., Sanathnagar, Hyderabad. Standard drug in its dosage of 400 mg was procured from a store located in Hyderabad, Telangana. The drug showed complete solubility in distilled water, which was used as a solvent throughout the experiment. Acetonitrile, formic acid and ammonium acetate were of HPLC grade. Other chemicals used in the study were of highest analytical grade.

UV-VIS METHOD

Standard stock solution of imatinib mesylate

IMT was precisely weighed at 10 mg, then taken into a flask with 10 mL capacity. A small amount of distilled water was added to dissolve, and the volume (1000 µg mL^{-1}) was made up with the same. 1.0 mL from the above solution was pipetted into a flask having 10 mL capacity and filled with water up to the mark, to get 100 µg mL^{-1} of concentration. In order to establish the lambda max and determine the linearity, the dilutions from the stock solutions were further processed to prepare a 10 µg mL^{-1} solution, which was then scanned by a UV-Vis spectrophotometer in the range of 180-380 nm. The results are presented in Fig. 1.

Table I: Linearity of imatinib by UV-visible spectrophotometer

Concentration[a]	Measured absorbance
2.0	0.1689
4.0	0.3578
6.0	0.5215
8.0	0.6908
10.0	0.8791
12.0	1.0612

[a] measured in µg mL^{-1}

Table II: Accuracy data obtained through UV-visible spectrophotometer

Accuracy level	Sample (bulk + formulation)	Absorb-ance	% Recovery	%RSD
50 %	2.5+5	0.5578	99.16	1.03 %
	2.5+5	0.5499	97.76	
	2.5+5	0.5611	99.75	
100 %	5+5	0.7834	97.05	0.903 %
	5+5	0.7953	98.52	
	5+5	0.7962	98.63	
150 %	7.5+5	1.0298	98.56	0.914 %
	7.5+5	1.0268	98.27	
	7.5+5	1.0123	96.88	

Table III: Precision data

Sr. No.	Concentration[a]	Measured absorbance
1.0	10	0.8832
2.0	10	0.8795
3.0	10	0.8815
4.0	10	0.8627
5.0	10	0.8842
6.0	10	0.8785
% RSD		0.89 %

[a] measured in µg mL^{-1}

Table IV: Robustness data

Sr. No.	Concentration (µg mL^{-1})	257 nm	259 nm
1	10	0.8512	0.8825
2	10	0.8493	0.8798
3	10	0.8515	0.8826
%RSD		0.12 %	0.16 %

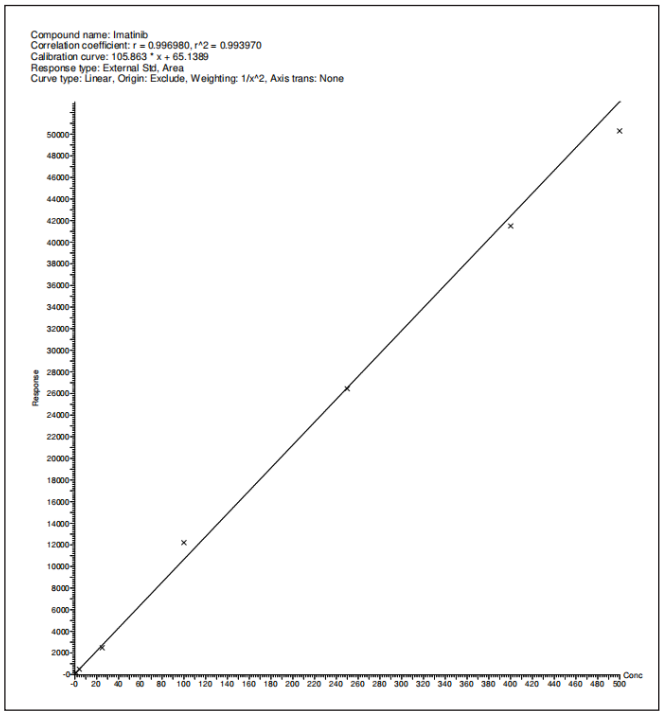

Fig. 3: Calibration curve obtained by LC detection of imatinib mesylate

METHOD VALIDATION

Precision

Both the intra-day and inter-day precision of the approach were investigated. It involved evaluating IMT (10 µg mL^{-1}) six times during the day. The results are shown in Table III. By measuring the same concentration of solution under several conditions such as a different day, analyst, and instrument inter day precision was ascertained. The data gathered are shown in Table V.

Study of linearity and range

Using an appropriate aliquot of the samples from the stock solution, a series of concentrations in the range of 1-12 µg mL^{-1}, as displayed in Fig. 2, were created. The absorbance of these solutions were measured at 258 nm after the volume was adjusted to the mark using

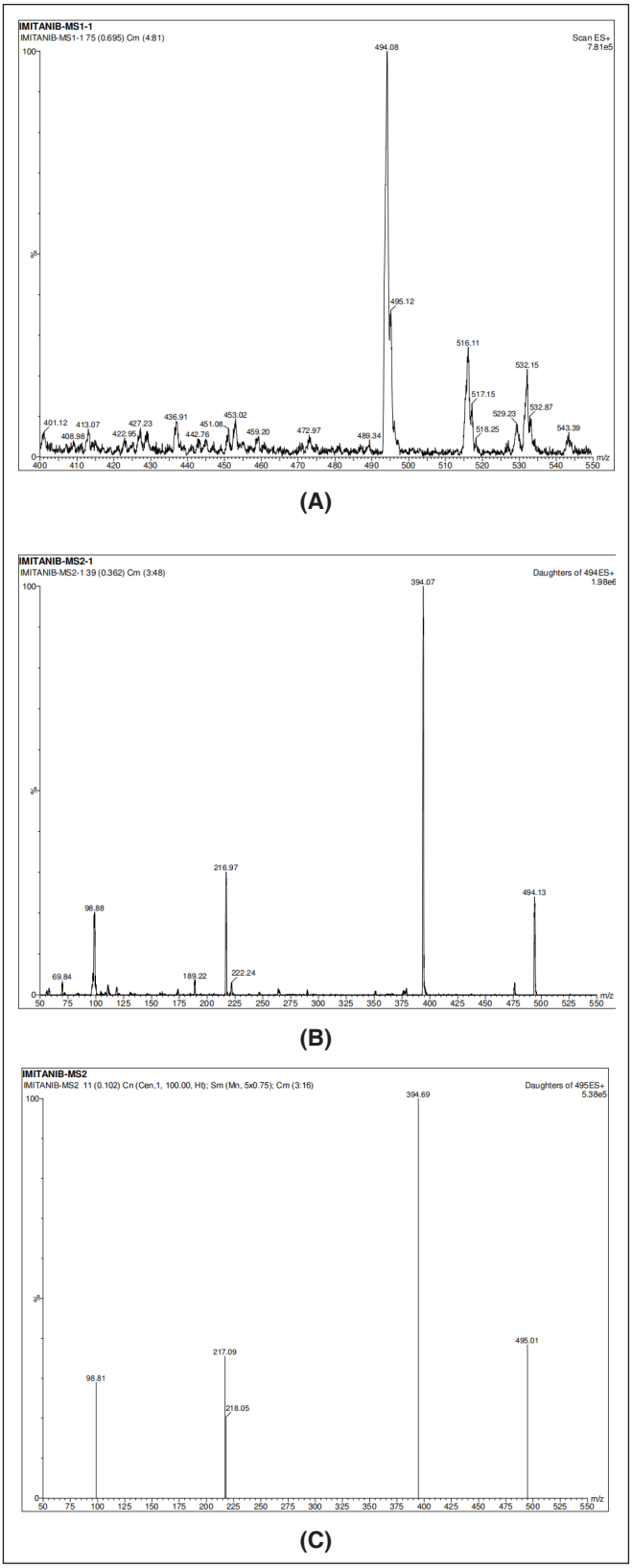

Fig. 4: A-Mass spectrum of IMT m/z values in its pure form, B-Mass spectrum of daughter ion peaks in its dosage form, C-MRM spectrum showing *in vitro* saliva spiked with IMT

diluent (Table I), and a curve plotting absorbance vs. concentration was created. The absorbance was found to be linear between 1 and 12 µg mL^{-1}.

Limit of detection and limit of quantification

According to ICH recommendations, the LOD and LOQ of IMT were calculated using the slope method and a standard error of response, LOD and LOQ were found to be 0.8977 and 0.2925 µg mL^{-1}, respectively.

Accuracy: Three distinct levels of triplicate absorbance, namely 50 %, 100 %, and 150 %, were measured. By adding a specified quantity of a standard solution to the optimized test concentration of sample solution, recovery tests were conducted. The percentage recovery was then determined using the above method.

Robustness: Three aliquots of 10 ppm test solution were prepared, and were scanned at wavelength of +1 nm of λ_{max}. The absorbance values are tabulated in Table IV.

Ruggedness: The reproducability was tested by two independent analysts on two different days, which shows that the procedure was built to withstand rugged conditions.

DRUG STABILITY STUDIES

Acid and base degradation

To a 25 mL volumetric flask, 2.5 mL of IMT and 5 mL of 0.1 N HCl/0.1 N NaOH were transferred from the aforementioned stock solution (100 µg mL^{-1}). After 24 h, 0.1 N NaOH/0.1 N HCl in a volume of 5 mL was used to neutralize the solution. The solution was left to stand at room temperature. With the goal of achieving a concentration of 10 µg mL^{-1}, the final volume was adjusted to the proper level. Each 25 mL volumetric flask contained 5 mL of 0.1 N HCl and 5 mL of 0.1 N NaOH, which were brought up to the specified level with distilled water (blank solutions), and then tested for absorbance.

Oxidation (hydrogen peroxide)

In a 25 mL volumetric flask, 2.5 mL of IMT and 5 mL of 0.3 % V/V H_2O_2 were added after 2.5 mL of the aforementioned stock solution (100 µg mL^{-1}) was transferred. The mixture was then left unattended for 24 h at room temperature. The solution is ultimately diluted with distilled water to a volume of 25 mL after 24 h. 25 mL capacity flask with 5 mL of 0.3 % H_2O_2, 20mL of water poured up to the lower meniscus (Blank). The absorbance was measured against this blank.

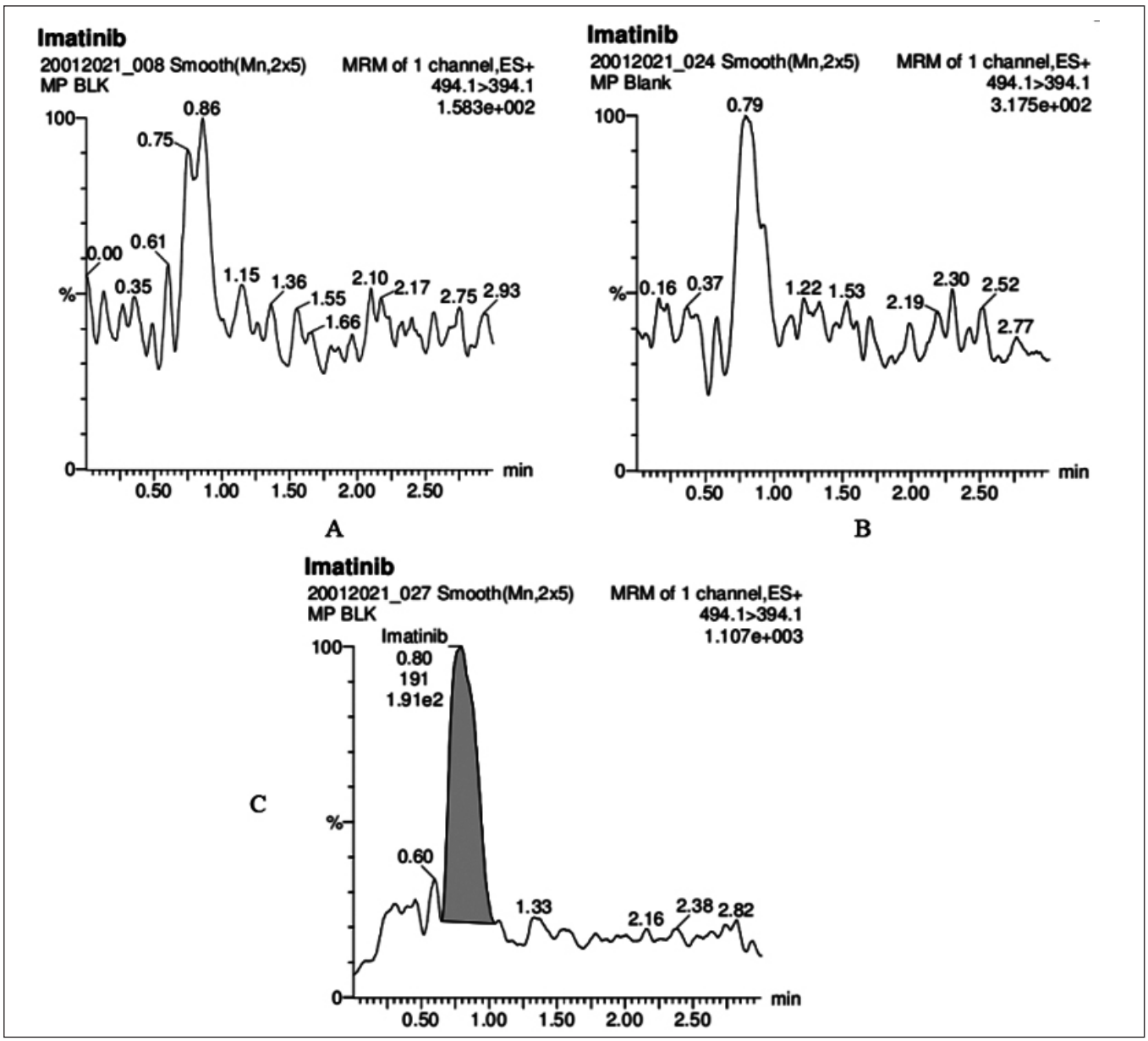

Fig. 5: UPLC chromatogram showing IMT (A-Blank, B, C-Inj-1,2)

Table V: Ruggedness data

Day 1			Day 2	
Analyst 1[a]		Analyst 2[b]	Analyst 1[a]	Analyst 2[b]
Concentration[c]	Absorbance	Absorbance	Absorbance	Absorbance
10	0.8832	0.8568	0.8793	0.8642
10	0.8795	0.8493	0.8548	0.8756
10	0.8815	0.8536	0.8675	0.8845
%RSD	0.17 %	0.13 %	0.14 %	0.15 %

[a] absorbance measured by first analyst [b] absorbance measured by second analyst [c] measured in µg mL^{-1}

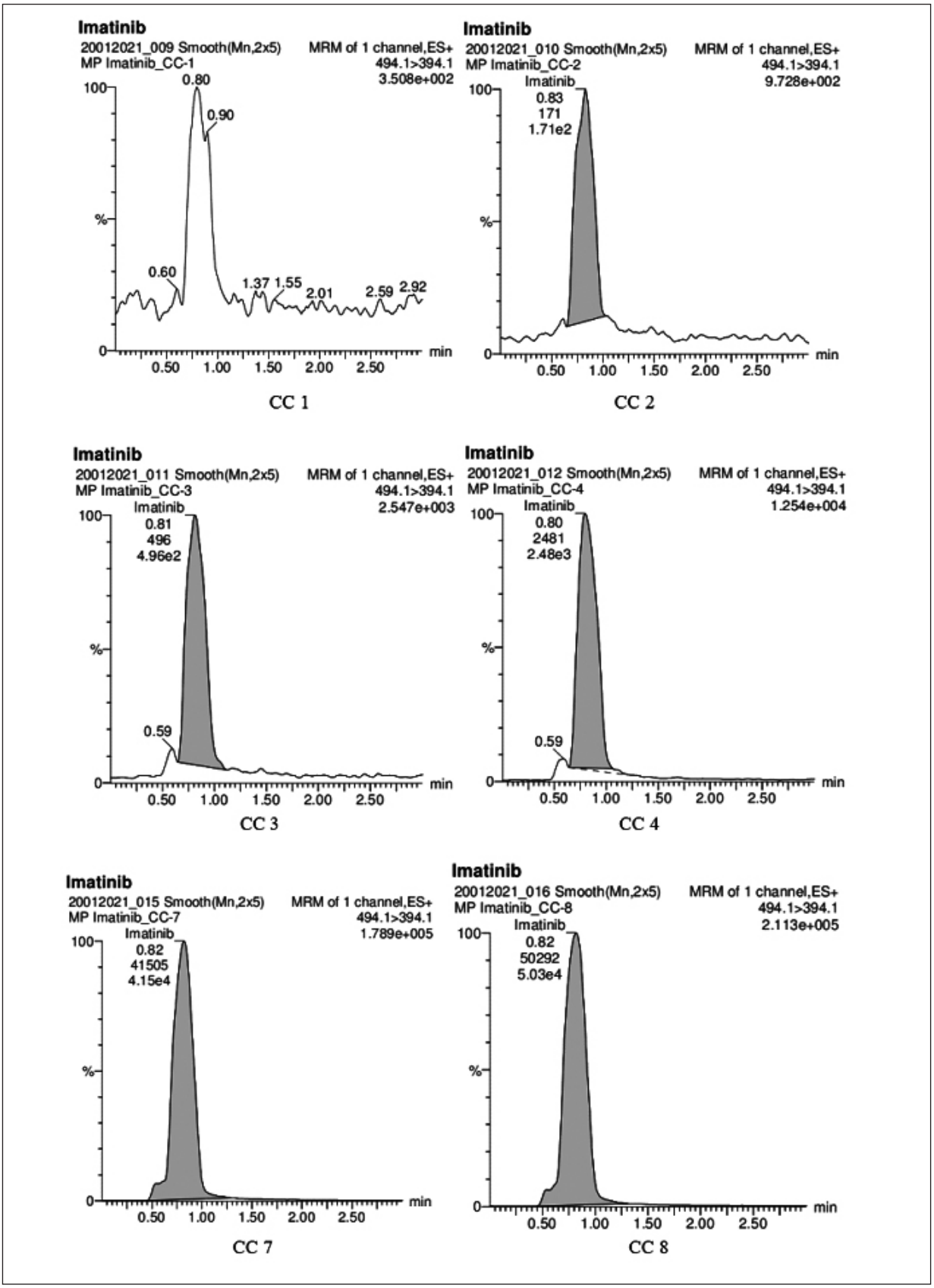

Fig. 6: Linearity chromatograms of imatinib mesylate CC 1-0.5 ng mL^{-1} to CC 8- 500 ng mL^{-1}

Photolytic conditions

For generating the major degradants found due to photolysis, photo stability experiments are carried out. Imatinib mesylate, weighing about 10 mg, was put in a petri dish, and subjected to UV light with a higher wavelength for 5 h. The drug component was then dissolved in diluent up to the volume to produce a 10 µg mL^{-1} solution.

Solution stability studies

Bench top and refrigerator: To determine the bench top solution stability up to 8 h, the test solution (10 µg mL^{-1}) was stored on the bench top under standard laboratory conditions, and tested at sufficient intervals. By keeping the solution in the freezer and refrigerator for three days each, the solution stability was assessed.

LC-MS/MS ANALYSIS

Sample preparation

Using the liquid-liquid extraction technique, a sample of human saliva was analyzed. A 100 mL sample of human saliva was mixed with 50 mL of the 0.1 M ammonium acetate solution and 60 mL of the IS working solution (50 µg mL^{-1}). A vortex mixer was used to combine the solutions. After that, 1.2 mL of formic acid was added, and it was gently blended for 5 minutes. For evenly mixing the solution, the centrifuge was run at 4 ℃ and 15,000 rpm for around 10 minutes. The extract-containing organic phase was poured into a clean, dry beaker, and the beaker was then dried at a temperature of 40 °C. The resulting residue was collected by centrifugation at 4 ℃ for 3 minutes after being dissolved in 100 mL of 60% acetonitrile. 20 µL of clear supernatant liquid was poured onto the column for examination.

Table VI: Extraction recovery of imatinib mesylate in bulk formulation

Drug	Sample name	Conc (ng mL^{-1})	Retention time	Extraction recovery
Imatinib mesylate	BLK	0.4	0.79	-
	BLK	1.2	0.80	-
	CC-1	-	-	-
	CC-2	1	0.83	99.3
	CC-3	4.1	0.81	101.8
	CC-4	22.8	0.80	91.3
	CC-5	114.7	0.81	114.7
	CC-6	249.2	0.80	99.3
	CC-7	391.4	0.82	97.9
	CC-8	474.5	0.82	94.9

Application of developed method

IMT concentrations at a steady state in a sample of human saliva were determined using the developed UPLC-MS/MS method, which was also used to precisely record the sampling time points. Then, the human saliva samples were centrifuged (4000 rpm for 5 min at 4 ℃). The saliva supernatant was stored until analysis at -70 ℃. The samples were processed after being freeze-thawed at room temperature.

Mass spectrophotometric and chromatographic conditions

An Acquity Ultra Performance LC system from Waters was used for the analysis. The separation was accomplished by chromatography using an X-Terra column (2.1 mm,100 mm, 3.5 µm, Sigma Aldrich, India). A and B, (40:60 V/V) were utilized in a binary gradient separation with A 0.1% formic acid was taken as organic phase and 0.05% ammonium acetate as aqueous phase. The mobile phase flow was 0.5 mL min^{-1} for 3 min run time. Column oven temperature, kept constant at not more than 30 ℃, the auto-sampler temperature was set to 5 ℃. The Mass Lynx version 4.1 SCN805 mass spectrometer was connected to the UPLC system. The electrospray ionization (ESI-positive ion ES+) source was used to run the mass spectrometer in MRM mode. The standard mass spectra were produced by injecting 1 µg mL^{-1} concentration during gradient elution in the appropriate mobile phase ratio.

To interpret the precursor and product ions, the imatinib sample that was run in human saliva that included IMT was analyzed using the mass scan function and daughter scan function. IMT peaks were most clearly seen at respective frequencies of 494.1 m/z and 394.1 m/z. As a result, the transition m/z 494.5,394.5 in MRM mode was created. The collision energy (CE), a factor that might influence the transition, was steadily optimized throughout several injections. To maintain the optimization of the collision energy for 494.5,394.5 m/z, the mode was operated between 10 and 30 V with a 5 V interval between each injection. The parameter optimization process was handled carefully to approach the maximum point. The strongest signal for the channel, 494.5, 394 m/z, was consequently acquired at 30 V. The optimal cone power supply was 30 V for imatinib and the internal standard, while maintaining a cone gas flow at 102 L h^{-1} and, desolvation gas flow at 850 L h^{-1}. Mass Lynx software was used for data processing and collecting.

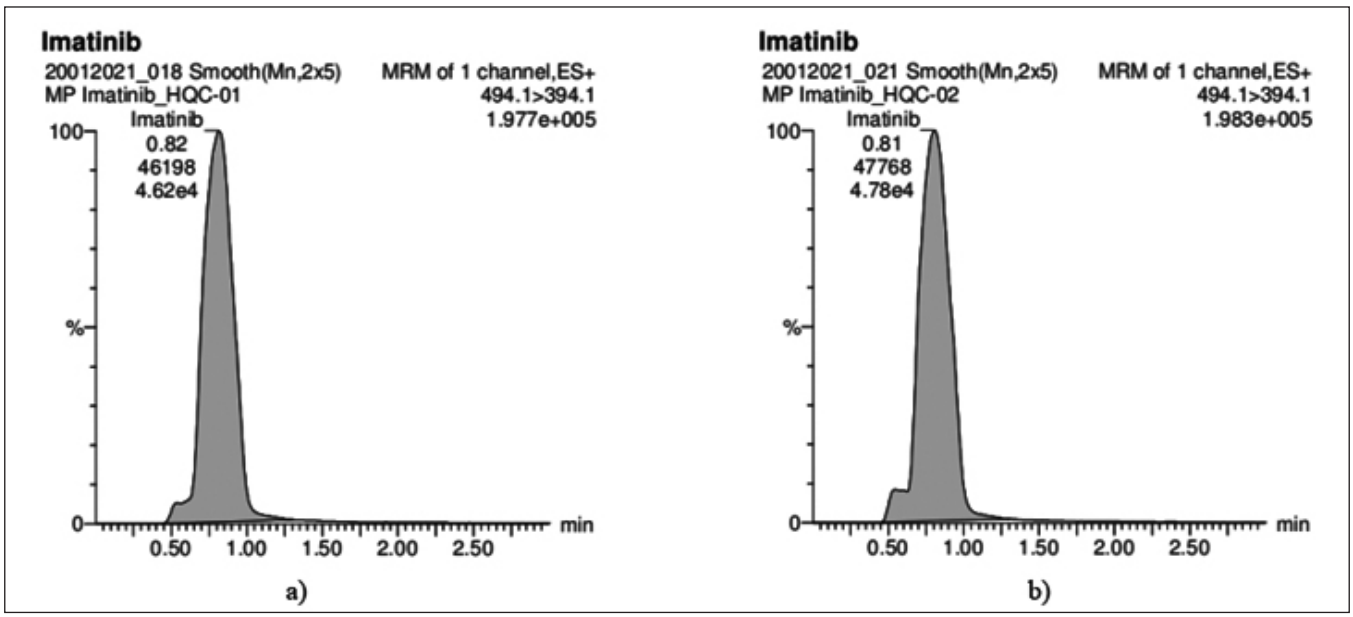

Fig. 7: LC chromatograms showing of IMT at HQC Level a) injection -1 b) injection-2

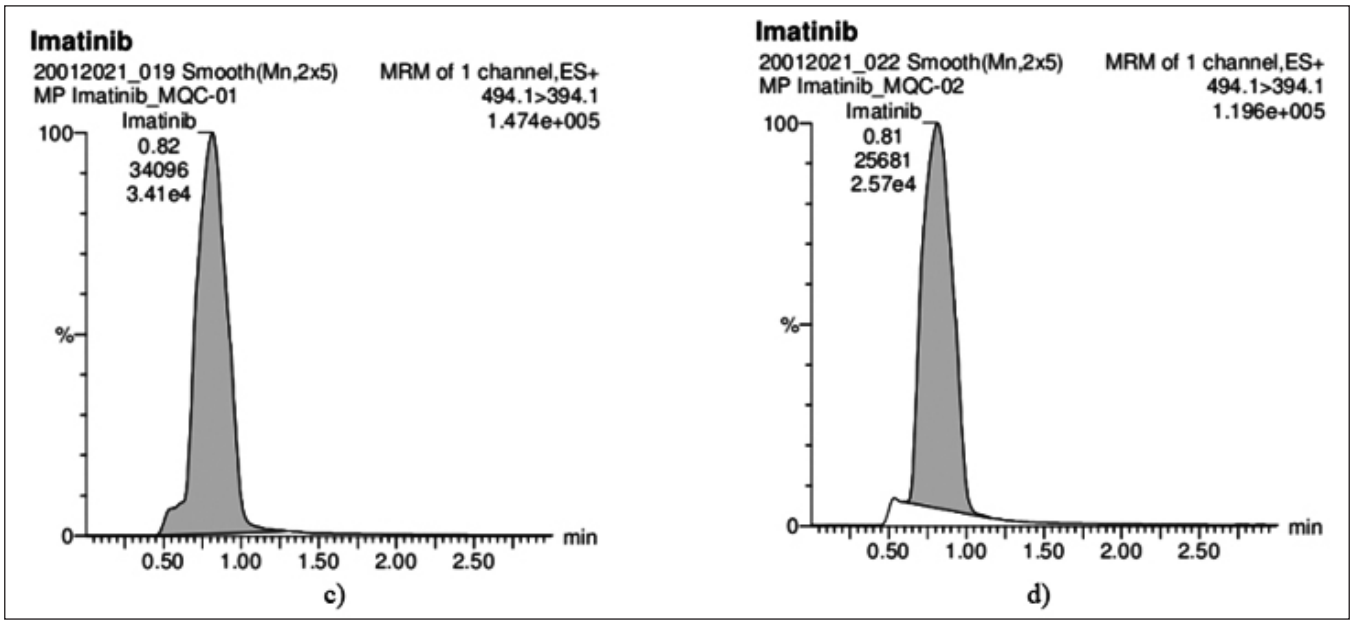

Fig. 8: LC chromatograms showing of IMT at MQC Level c) injection -1 d) injection-2

RESULTS AND DISCUSSION

Specificity and recovery

To confirm that no interfering substances were present, six samples of blank human saliva were administered. Fig. 3 presents the LC-derived calibration curve. Mass spectrum of IMT in its pure form is depicted in Fig. 4A, whereas the m/z values for daughter ion peaks and spiked human saliva are shown in Fig. 4B and Fig. 4C, respectively. Chromatograms made from blank are shown in Fig. 5A and spiked peaks were compared to determine the technique selectivity. The chromatogram of empty human saliva is shown in Fig. 5B and 5C. IMT had a retention period of around 0.82 minutes. There were no indications of interference in the retention periods of the analyte or the samples of blank saliva. At each stage of recovery, the test samples (n=5) at various concentration levels were analyzed. Illustrations are exhibited with the help of chromatograms in Fig. 7 at HQC, in Fig. 8 for MQC along with LQC level in Fig. 9.

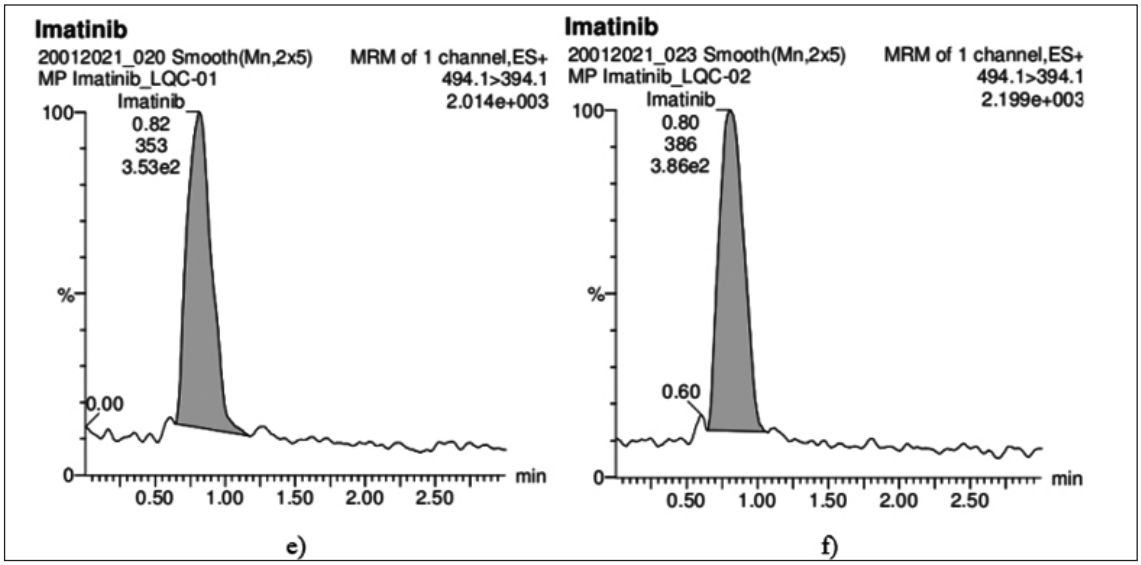

Fig. 9: LC chromatograms showing of IMT at LQC Level e) injection -1 f) injection-2

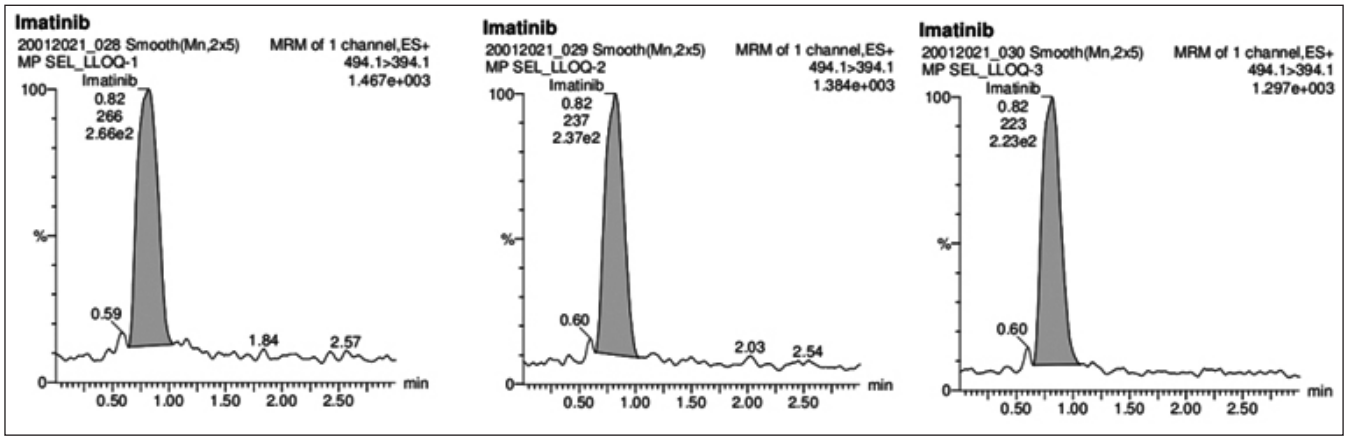

Fig. 10: Chromatograms showing triplicate injections of imatinib mesylate in human saliva at lower limit of quantification level

Table VII: *In vitro* saliva spiked with imatinib QC samples

Drug	Sample name	Concentration (ng mL^{-1})	Retention time	%Recovery
Imatinib mesylate	Blank			
	Saliva	136.1	0.72	-
	Saliva	120.6	0.73	-
	HQC-1	435.8	0.82	102.5
	HQC-2	450.6	0.81	450.6
	MQC-1	321.5	0.82	133.9
	MQC-2	242.0	0.81	100.8
	LQC-1	2.7	0.82	90.5
	LQC-2	3.0	0.80	100.9
	LLOQ-1	1.9	0.82	-
	LLOQ-2	1.6	0.82	-

Precision, linearity, LLOQ and accuracy

Concentrations ranging from 0.5 to 500 ng mL^{-1} were created. While the least squares linear regression (r^2) was determined to be 0.9939, the calibration curve was found to be linear as depicted in Fig. 6. The LLOQ is 1 ng mL^{-1}, while the LQC is 3 ng mL^{-1}.

UV-Vis chromatographic analysis

The estimated amount of imatinib mesylate in bulk and dosage form may be determined effectively using the established spectrophotometric approach. It is discovered that the relative standard deviation is well within the parameters. The accuracy was found to be statistically significant with RSD in the range of 0.9-1.03 % for 50 to 150 % (Table II). The precision was found to be validated (Table III) in the concentration range of 10-50 µg mL^{-1} with RSD 0.89 %. The robustness (Table IV) was found to be validated at 257 nm and 259 nm with RSD 0.12-0.16 %. The ruggedness (Table V) was calculated for two days with two analysts and the RSD was found to be in the range of 0.13-0.17 %.

UPLC coupled mass spectrometric analysis

Based on the molecule's chromatographic behavior (IMT), an ultra performance liquid chromatographic elution with MS/MS detection was used. This produced the ideal peak shape, and other suitability parameters were found to be well within the acceptable limits. The range of the mean recovery, which ranged from 90.3 % to 114.5% (RSD: 8.0 %), are shown in Tables VI and VII. With ideal MS/MS conditions, the parent and product ion peaks were at 494.1 and 394.1 m/z, respectively. Table VII lists the recovery of quality control samples. Extraction recovery was found to range from 99.3 % to 114.7 % for linear concentrations between 0.5 ng mL^{-1} and 500 ng mL^{-1}. Table VI lists the retention times corresponding to each concentration level with their respective chromatographic data supported in Fig. 10.

CONCLUSION

To determine IMT in human saliva and bulk formulations, a new, quick, selective, and highly sensitive UPLC MS/MS assay was developed and validated. This method allows for a more convenient and patient-friendly approach to monitoring the drug in cancer patients which eliminates the need for time-consuming and potentially discomforting blood collection procedures. The work has established a positive correlation between IMT levels in saliva and plasma, indicating that saliva holds potential as a reliable alternative matrix for assessing the drug's systemic exposure.

REFERENCES

1. Wajurkar M.S., Dole M.N. and Sawant S. D.: Development and validation of analytical methods for estimation of imatinib mesylate in bulk and solid dosage forms by UV Spectroscopy, **Der Pharmacia lettre.,** 2015, 7(3), 214-220.
2. Suresha D.N., Pramila. T. and Jose G. B.C.: Method development and validation of imatinib mesylate in bulk and tablet dosage forms by using UV-Spectrophotometric Method, **Imp. J. Int. Res.,** 2017, 3(3), 1283-1287.
3. Patil S. J., Dr. Doijad R.C. and Dhumalpriya P.: Development of UV- Spectrophotometric method for the determination of imatinib mesylate (IMT) in bulk and formulation, **Asian J. Pharm. Clin. Res.,** 2013, 6(3), 54-57.
4. Kumar R. J., Sundar V. D., Magesh A. R., Kumar S. N. and Dhanaraju M.D.: Validated spectrophotometric estimation of imatinib mesylate in pure and tablet dosage form, **Int. J. Pharm. Tech.**, 2010, 2(3), 490-495.
5. Haque M. A., Bakhshi V., Surekha D., Gouthami B., Reddy C. H. and Yakamma B.: Development of UV-Spectrophotometric method for the determination of imatinib mesylate (IMT) in bulk and formulation, **Int. J. Pharm. Res. Health. Sci.,**2016, 4 (2), 1130-1135.
6. Sreenivasulu B.V and Rambabu C.: New visible spectrophotometric assay of imatinib (β-form) in pure and formulations, **Int. J. Pharm. Pharm. Res.,** 2014, 2(1), 1-10.
7. Kumar A and Kumar J. K.: RP-HPLC method development and validation of imatinib mesylate in tablet dosage form, **Int. J. Pharm. Pharm. Sci.,** 2011, 3(5), 162-165.
8. Sankar R. P., Niharika A., Rani K. A., Neehaand S. M. and Pavan G.: Development and validation of RP-HPLC method for quantitative determination of imatinib mesylate in bulk drug and pharmaceutical dosage form, **Der Pharmacia Lettre.,** 2015, 7(7), 102-112.
9. Rele R.V. and Patil S.P.: Development of analytical method by RP-HPLC technique for determination of imatinib mesylate in bulk drug and pharmaceutical dosage form, **Asian J. Res. Chem.,** 2019, 12(2), 79-83.
10. Lankheet N.A.G., Hillebrand M.J.X. and Rosing H.: Method development and validation for the quantification of dasatinib, erlotinib, gefitinib, imatinib, lapatinib, nilotinib, sorafenib and sunitinib in human plasma by liquid chromatography coupled with tandem mass spectrometry, **Biomed. Chromatogr.,** 2013, 27 (4), 466–476.
11. Van Erp N. P., de Wit D. and Guchelaar H.: A validated assay for the simultaneous quantification of six tyrosine kinase inhibitors and two active metabolites in human serum using liquid chromatography coupled with tandem mass spectrometry, **J. Chromatogr. B Analyt Technol. Biomed. Life Sci.,** 2013, 937, 33–43.
12. L. Götze A., Hegele A. and Metzelder S. K.: Development and clinical application of a LC-MS/MS method for simultaneous determination of various tyrosine kinase inhibitors in human plasma, **Clin. Chim. Acta.** 2012, 413 (1-2), 143–149.
13. Andriamanana I., Gana H. and Duretz B.: Simultaneous analysis of anticancer agents bortezomib, imatinib, nilotinib, dasatinib, erlotinib, lapatinib, sorafenib, sunitinib and vandetanib in human plasma using LC/MS/MS, **J. Chromatogram. B,** 2013, 926, 83–91.
14. Bouchet S., Chauzit E. and Ducint D.: Simultaneous determination of nine tyrosine kinase inhibitors by 96-well solid-phase extraction and ultra-performance LC/MS-MS, **Clin. Chim. Acta,** 2011,412 (11-12), 1060–1067.

15. Couchman L., Birch M. and Ireland R.: An automated method for the measurement of a range of tyrosine kinase inhibitors in human plasma or serum using turbulent flow liquid chromatography-tandem mass spectrometry, **Anal. Bioanal. Chem.,** 2012, 403 (6), 1685–1695.
16. Reynolds JEF, Martindale- The extra Pharmacopoeia. 28th edition, The Pharmaceutical press, London, 1982.
17. Willard H., Merritt L., John A. Dean and Frank A.: Settle, Instrumental methods of analysis, 7th edition, CBS Publishers and Distributers, New Delhi, 1986, pp.356-375.
18. ICH HT. Validation of analytical procedures: text and methodology, Q2 (R1). Current Step 4 Version, Parent Guidelines on Methodology Dated November 6 1996. Incorporated in November 2005. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, Geneva, Switzerland, 2005.
19. Baertschi S.W, Alsante K.M, Reed R.A, editors. Pharmaceutical stress testing: predicting drug degradation. CRC Press; 2016.
20. Allwood M.C and Plane J.H.: The wavelength-dependent degradation of vitamin A exposed to ultraviolet radiation. **Int. J. Pharm.,** 1986, 31(1-2),1-7.