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Research Article

Development and Validation of a New Stability Indicating RP-UFLC Method for the Estimation of Imatinib Tablets Using an Ion Pairing Agent

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Abstract

Imatinib is a tyrokinase inhibitor used for the treatment of cancer. A new stability indicating RP-UFLC method has been developed and validated for the determination of Imatinib using an ion pairing agent, tetra butyl ammonium hydrogen sulphate in combination with methanol (45: 55) with flow rate 1.0 mL/min and PDA detection at 281 nm. Imatinib shows linearity over the concentration range 0.05-80 mg/ml and the linear regression equation is found to be y = 62542x + 9790.6 with correlation coefficient 0.9999. The LOD and LOQ were found to be 0.0138 mg/ml and 0.0418 mg/ml respectively. Imatinib was exposed to different stress conditions and results indicate that the method is specific and selective and the method was applied for the pharmaceutical formulations such as Imatinib tablets and was validated as per ICH guidelines.

Keywords: Imatinib; RP-UFLC; Stability Indicating; Validation; ICH Guidelines

Introduction

Imatinib (Figure 1) is an anti-neoplastic agent used for the gastrointestinal stromal tumors. It acts by competitive inhibition with the tyrokinase group of proteins [1,2]. Imatinib is chemically 4-[(4-methylpiperazin-1-yl) methyl]-*N*-[4-methyl-3-[(4-pyridin-3-ylpyrimidin-2-yl) amino] phenyl] benzamide. Imatinib is available as its methane sulphonate salt form and is used for the treatment of hematological malignancies [3].

Different analytical techniques were developed for the assay of Imatinib using liquid chromatography and spectrophotometry. Smita., *et al*. developed a spectrophotometric method [4] for the determination of Imatinib mesylate in distilled water and Beer-Lambert's law was obeyed over the concentration range 4-28 µg/ml for Imatinib mesylate. Nageswari., *et al*. have developed [5] a stability-indicating UPLC method for the quantitative determination of purity of Imatinib Mesylate in the presence of its 8 process related impurities, and degradation products using a mixture of 0.05 M ammonium acetate (pH 9.5), acetonitrile and methanol as mobile phase on gradient mode and LC–MS/MS system was used for the identification of degradants. María., *et al*. developed HPLC method [6] using a mobile phase mixture of 30 mM sodium heptane sulphonic acid in 0.01 M KH_2PO_4 (pH adjusted to 2.5) and methanol (42:58) and the linearity was 300-800 µg/ml. Naga Sindhu., *et al*. developed a RP- HPLC method [7] using 0.1% ortho phosphoric acid -acetonitrile mixture (70:30) as mobile phase and the linearity was followed over the concentration range 5-30 µg/ml. Lalit Mohan., *et al*. developed a stability indicating RP-HPLC method [8] using an

aq. phase mixture (sodium dihydrogen orthophosphate dihydrate (pH adjusted to 8.0 with triethyl amine)) and an organic phase mixture (methanol and acetonitrile) as mobile phase (55:45) and the linearity was 0.05-50 µg/ml. Pratik., *et al*. developed a stability indicating RP-HPLC method [9] for the assay of Imatinib tablets dosage forms using phosphate buffer (pH 8.0 adjusted with tri ethyl amine): methanol: acetonitrile (50: 30: 20) (UV detection 265 nm) and the linearity was shown over the concentration range 19.815-29.722 µg/ml. Arun Kumar., *et al*. developed a stability indicating RP-HPLC [10] method for the assay of Imatinib mesylate and its dimer impurity using a mobile phase mixture methanol and acetate buffer (pH 3.5) (80:20) and Beer-Lambert's law was obeyed over the concentration range 10-60 µg/ml for Imatinib mesylate and 0.4-2.4 µg/ml for its dimer impurity. Pratik Shah and Rutesh Shah developed a stability indicating HPLC method [11] for Imatinib, its process related substances and degradants on gradient mode using an ion pairing agent, 1-octane sulfonic acid sodium (pH adjusted to 3.0 with *o*-phosphoric acid) (Buffer) along with methanol.

Several analytical methods were also developed for the estimation of Imatinib using HPTLC [12], HPLC in biological samples [13-21] and also for the genotoxic impurities [22-24] and in the present study a new stability indicating RP-UFLC method has been proposed for the estimation of Imatinib and the method was validated as per ICH guidelines.

Figure 1: Structure of Imatinib.

Materials and Methods

HPLC grade methanol (Merck), Milli-Q water, tetra butyl ammonium hydrogen sulphate (TBHS) (Merck), sodium hydroxide (Qualigens), hydrochloric acid (S D Fine Chemicals) and hydrogen peroxide (30% w/v) (Merck) were procured for the present study.

Preparation of 10 mM Tetra butyl ammonium hydrogen sulphate solution (pH 3.4)

Tetra butyl ammonium hydrogen sulphate ($C_{16}H_{37}NO_4S$) is an ion pairing agent with molecular weight 339.54 grams/mole. 3.3954 grams Tetra butyl ammonium hydrogen sulphate was accurately weighed and transferred to a 1000 ml volumetric flask and dissolved in HPLC grade water to prepare 10 mM solution and the pH of the resulting solution is 3.4. This buffer solution was filtered through 0.42micron membrane filter and used for the preparation of mobile phase.

Preparation of imatinib stock and working standard solutions

25 mg of Imatinib was accurately weighed, transferred and dissolved in 25 mL volumetric flask in methanol and the volume was made up volume (1000 µg/mL) and this stock solution was further diluted with mobile phase to get working standard solution (100 µg/mL) and from which dilutions were made as per requirement.

Instrumentation and chromatographic conditions

Shimadzu Model UFLC system with Agilent C_{18} column and PDA detector was used for the chromatographic study. Mobile phase consisting of tetra butyl ammonium hydrogen sulphate in combination with methanol in 45: 55, v/v ratio with flow rate 1.0 mL/min with UV detection at 281 nm are the chromatographic conditions. The injection volume was 20 µl and the total run time was 10 minutes.

Method validation [25]

Linearity, precision, accuracy and robustness studies

A series of 0.05-80 µg/mL of Imatinib solutions were prepared from the stock and working standard solutions on dilution with the mobile phase and injected (n = 3) into the UFLC system. The peak area and thereby the mean peak area was calculated from the respective chromatograms and a calibration curve was drawn by plotting the concentration of Imatinib solutions on the x-axis and the corresponding mean peak area on the y-axis. The LOD and LOQ were calculated from the signal to noise ratio (S/N). The LOD is 3.3 times the signal to noise ratio and that of LOQ is 10 times the signal to noise ratio.

Intra-day and inter-day precision studies were performed on three different concentrations (10, 20 and 40 µg/mL) of Imatinib on the same day and on three consecutive days respectively and the chromatograms were recorded. The peak area as well as the mean peak area (n = 3) and the % RSD were calculated.

Accuracy of the method was measured by spiking the drug formulation (20 µg/mL) solution (50, 100, 150%) with a known concentration of standard drug (n = 3) where the final concentrations were found to be 30, 40 and 50 µg/mL. The mean peak area was calculated from the chromatograms obtained and finally the % RSD was calculated from the linear regression equation.

The robustness of the method was proved by incorporating a very small changes in the optimized chromatographic conditions such as pH (±0.1; 3.3 and 3.5), mobile phase composition (Tetra butyl ammonium hydrogen sulphate: Methanol 45: 55) (± 2%; 43:57 and 47:53), flow rate (± 0.1 mL; 1.1 and 0.9 mL/min) and detection wavelength (± 5 nm; 276 and 286 nm).

Assay of imatinib tablets

Imatinib tablets are available with brand names Mitinab-400 (Glenmark Pharmaceuticals Ltd), Imatib (Cipla Ltd), Imat (Zydus Lifesciences Ltd) etc. and Imatinib was obtained from Glenmark Pharmaceuticals Ltd as gift sample. Twenty tablets of Imatinib were weighed accurately and an amount of powder equivalent to 25 mg of Imatinib from two different brands was procured, powdered and powder equivalent to 25 mg of Imatinib was transferred in to two different 25 ml volumetric flasks and dissolved in HPLC grade methanol, sonicated and filtered. The resulting solution was diluted with the mobile phase as per the requirement and 20 µL of each of these solutions was injected in to the system (n = 3) and the peak area as well as the mean peak area were calculated from the respective chromatograms and the assay of Imatinib was calculated.

Stress degradation studies [26]

Stress degradation studies were performed to determine the stability of Imatinib towards stress conditions such as acidic hydrolysis, basic hydrolysis, oxidation and thermal degradation. The specificity of the method can be known from the stability studies and therefore Imatinib was exposed to the following stress conditions and the stability was studied.

Acidic hydrolysis was performed by heating Imatinib solution with 1 mL of 0.1 N HCl solution at 75^0 for 30 minutes on a water bath. The stressed sample was then cooled, neutralized with 1.0 mL 0.1N sodium hydroxide solution, diluted with mobile phase and then 20 µl of the solution was injected in to the UFLC system. Alkaline hydrolysis was performed by heating Imatinib solution with 1.0 mL 0.1N sodium hydroxide solution at 75^0 for 30 minutes on a water bath. The stressed sample was then cooled, neutralized with 1.0 mL of 0.1 N HCl solution, diluted with mobile phase and then 20 µl of the resulting solution was injected in to the UFLC system. Thermal degradation was performed by heating the Imatinib solution at 75^0 for 30 minutes on a water bath and then cooled, diluted with mobile phase and 20 µl of the resulting solution was injected in to the UFLC system. Oxidative degradation was performed by heating Imatinib solution with 1.0 mL 30% hydrogen peroxide solution at 75^0 for 30 minutes on a water bath. The stressed sample was then cooled, diluted with mobile phase and then 20 µl of the resulting solution was injected in to the UFLC system.

Results and Discussion

A new stability indicating RP-UFLC method has been developed and validated for the determination of Imatinib using an ion pairing agent, tetra butyl ammonium hydrogen sulphate in combination with methanol in 45: 55, v/v ratio with flow rate 1.0 mL/min and PDA detection at 281 nm. Some of the parameters of the previously published analytical methods for the estimation of Imatinib dosage forms were summarized and compared with the proposed method in table 1. The representative chromatograms obtained for the mobile phase and that of Imatinib were shown in figure 2 where Imatinib was eluted at 3.812 mins.

Method validation

Imatinib obeys Beer-Lambert's law over the concentration range of 0.05-80 µg/mL (Table 2) (% RSD 0.24-0.81) and the linear regression equation is found to be y = 62542x + 9790.6 with correlation coefficient 0.9999. The LOD and LOQ were found to be 0.0138 mg/ml and 0.0418 mg/ml respectively and the calibration curve was shown in figure 3. The % RSD was found to be 0.19-1.14

Reagent/Mobile phase (v/v)	λ (nm)	Linearity (mg/ml)	Method	Ref
Distilled water	281	4-28	Spectrophotometry	[4]
Ammonium acetate (pH 9.5): Acetonitrile: Methanol (Gradient mode)	237	-	UPLC and LC-MS/MS (8 process related impurities)	[5]
30 mM Sodium heptane sulphonic acid in 0.01 M KH_2PO_4 (pH adjusted to 2.5): Methanol (42:58)	237	300-800	HPLC	[6]
0.1% ortho Phosphoric acid: Acetonitrile (70:30)	266	5-30	HPLC	[7]
Methanol and Acetonitrile (6:4): Sodium dihydrogen orthophosphate dihydrate (pH 8.0 adjusted with TEA) (45:55)	267	0.05-50	HPLC	[8]
Phosphate buffer (pH 8.0 adjusted with TEA): Methanol: Acetonitrile (50: 30: 20)	265	19.815-29.722	HPLC	[9]
Methanol: Acetate buffer (pH 3.5) (80:20)	273	10-60 0.4-2.4	HPLC Dimer impurity	[10]
Buffer: 1-octane sulfonic acid sodium (pH adjusted to 3.0 with *o*-phosphoric acid) Mobile phase A: Buffer: Methanol (50: 50) Mobile phase B: Buffer: Methanol (4: 96)	240		HPLC Process impurities	[11]
Methanol: 10 mM Tetra butyl ammonium hydrogen sulphate: (55: 45) (pH 3.45)	281	0.05-80	UFLC Ion pairing agent Stability indicating	Present method

Table 1: Literature survey of Imatinib.

(Intraday) (Table 3) and 0.24-0.73 (Inter-day) (Table 4) in precision studies which is less than 2.0 indicating that the method is precise. The % recovery in accuracy studies was found to be 99.58-99.77 (Table 5) and % RSD was (0.71-1.07) less than 2% indicating that the method is accurate. The % RSD in robustness study was found to be 0.53-1.02 which was less than 2% indicating that the method is robust (Table 6).

Table 2: Linearity study of Imatinib.

Conc. (µg/mL)	*Mean peak area	% RSD
0	0	0
0.05	3412	0.25
0.1	65121	0.36
0.2	13698	0.24
0.5	33261	0.41
1	64625	0.45
2	127395	0.49
5	326504	0.51
10	639583	0.29
20	1262804	0.81
40	2512549	0.46
60	3729493	0.62
80	5036258	0.37

*Mean of three replicates.

Table 3: Intraday precision study of Imatinib.

Conc. (µg/mL)	*Mean peak area	Statistical Analysis
		*Mean ± SD (% RSD)
10	639583	636439.67 ± 7251.36 (1.14)
10	641589	
10	628147	
20	1262804	1258766.33 ± 4942.83 (0.39)
20	1253254	
20	1260241	
40	2512549	2517877.67 ± 4720.60 (0.19)
40	2521536	
40	2519548	

*Mean of three replicates.

Table 4: Inter day precision study of Imatinib.

Conc. (µg/mL)	*Mean peak area			*Mean ± SD (% RSD)
	Day 1	Day 2	Day 3	
10	639583	641254	632518	637785 ± 4637.24 (0.73)
20	1262804	1256984	1261327	1260371.67 ± 3025.33 (0.24)
40	2512549	2523547	2532587	2522894.33 ± 10034.93 (0.39)

*Mean of three replicates.

Table 5: Accuracy study of Imatinib.

Spiked Conc. (µg/mL)	Formulation (µg/mL)	Total Conc. (µg/mL)	*Conc. Obtained (µg/mL) ± SD (%RSD)	% Recovery
10 (50%)	20 20 20	30 30 30	29.93 ± 0.2125 (0.71)	99.77
20 (100%)	20 20 20	40 40 40	39.85 ± 0.4264 (1.07)	99.63
30 (150%)	20 20 20	50 50 50	49.79 ± 0.4879 (0.98)	99.58

*Mean of three replicates.

Table 6: Robustness study of Imatinib (20 µg/mL).

Parameter	Condition	*Mean peak area ± SD (% RSD)
Flow rate (± 0.1 ml/min)	1.1	1252987 ± 12153.97 (0.97)
	1.0	
	0.9	
Detection wavelength (± 5 nm)	276	1259768 ± 6676.77 (0.53)
	281	
	286	
Mobile phase composition Tetra butyl ammonium hydrogen sulphate: Methanol (± 2 %, v/v)	47:53	1254573 ± 8907.47 (0.71)
	45:55	
	43:57	
pH (± 0.1 unit)	3.5	1250598 ± 12756.09 (1.02)
	3.4	
	3.3	

*Mean of three replicates.

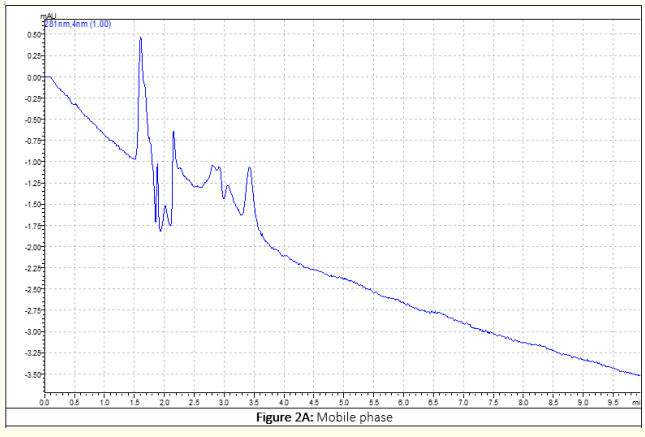


Figure 2A: Mobile phase

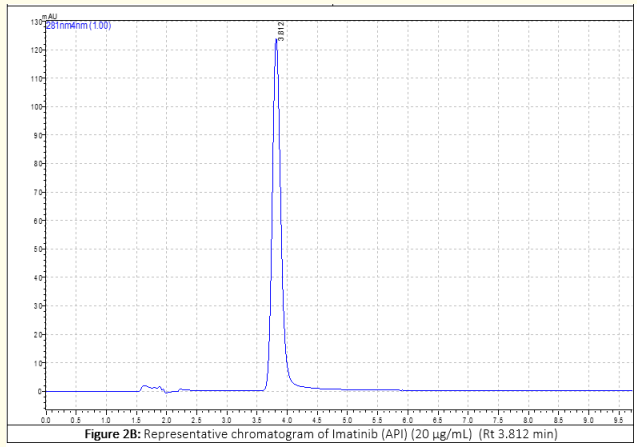


Figure 2B: Representative chromatogram of Imatinib (API) (20 µg/mL) (Rt 3.812 min)

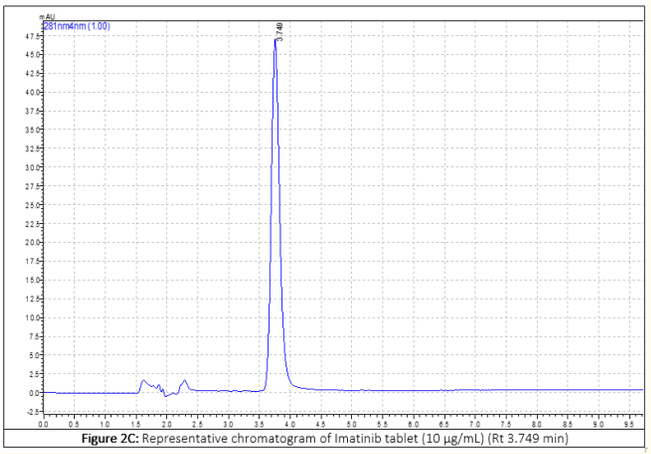


Figure 2C: Representative chromatogram of Imatinib tablet (10 µg/mL) (Rt 3.749 min)

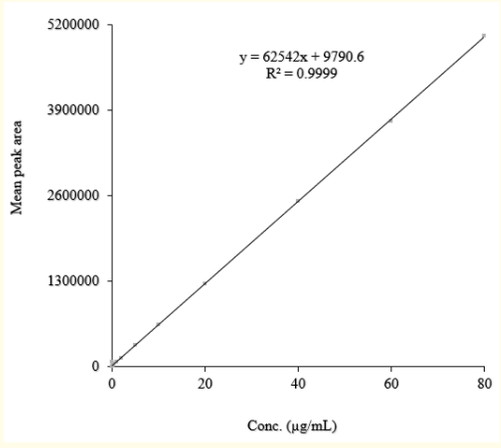


Figure 3: Calibration curve of Imatinib.

Stress degradation studies of imatinib

Imatinib was exposed to different stress conditions for evaluating the specificity of the proposed method. Imatinib was eluted at 3.812 min ± 0.1 as a sharp peak with theoretical plates 3228.317 (> 2000) and tailing factor 1.260 (< 1.5). During the acidic hydrolysis Imatinib was eluted at 3.818 mins and has shown 6.98% degradation with theoretical plates 3214.141 and tailing factor 1.273 respectively.

During the alkaline hydrolysis Imatinib was eluted at 3.558 mins and has shown 4.86% degradation with theoretical plates 3327.767 and tailing factor 1.298 respectively.

During the oxidative degradation Imatinib was eluted at 3.797 mins and has shown 10.36% degradation with theoretical plates 3272.222 and tailing factor 1.249 respectively with an extra peak at 1.904 mins with resolution 6.893 which is greater than 2. During the thermal degradation Imatinib was eluted at 3.773 mins and has shown 11.39% degradation with theoretical plates 3358.909 and tailing factor 1.260 respectively.

Imatinib drug peak has not at all interfering with any other degradant peak indicating that the method is specific and selective and less than 15% degradation was observed during the stress degradation studies (Table 8). The chromatograms obtained during the stress degradation studies were shown in figure 4.

Assay of imatinib tablets

Imatinib assay was performed by extracting Imatinib from the tablets of two marketed brands by applying the present proposed method and Imatinib was found to be 99.67-99.98 % (Table 7) in tablets. The chromatogram observed for one of the brands of Imatinib tablet formulation was shown in figure 3C. The excipients of the formulation have not interfered with the pure drug peak.

Table 7: Assay of Imatinib tablets.

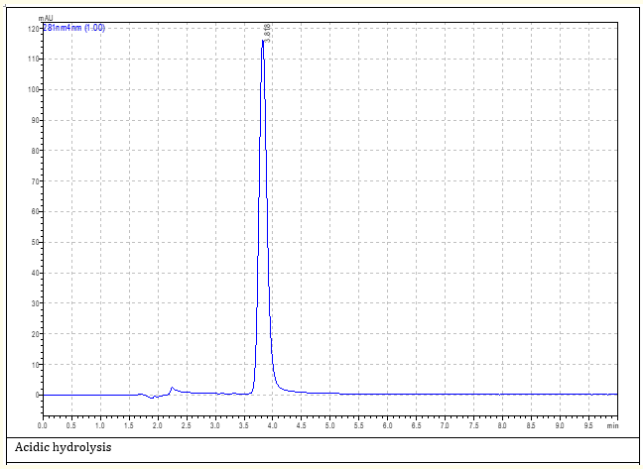
S. No.	Brand name	Label claim (mg)	*Observed amount (%w/w)	% Recovery*
1	Brand I	400	399.91	99.98
2	Brand II	400	398.67	99.67

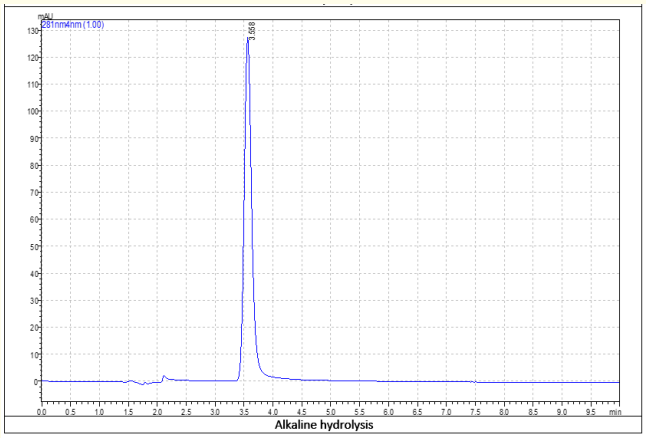
*Mean of three replicates.

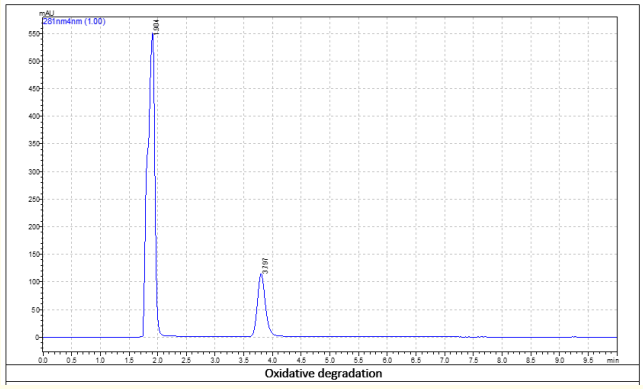
Table 8: Stress degradation studies of Imatinib.

Stress condition (Temp ^{0}C/ Time min)	Rt (min)	Mean peak area	% Recovery	% Drug degradation	Theoretical plates	Tailing factor	Resolution
Standard drug	3.812	1262804	100	-----	3228.317	1.260	-
Acidic hydrolysis 0.1N HCl/75^0/30 min	3.818	1174705	93.02	6.98	3214.141	1.273	-
Alkaline hydrolysis 0.1N NaOH/75^0/30 min	3.558	1201477	95.14	4.86	3327.767	1.298	-
Oxidative degradation 30% H_2O_2/75^0/30 min	3.797 1.904	1131948	89.64	10.36	3272.222	1.249	6.893
Thermal degradation 75^0/30 min	3.773	1118934	88.61	11.39	3358.909	1.260	-

*Mean of three replicates.







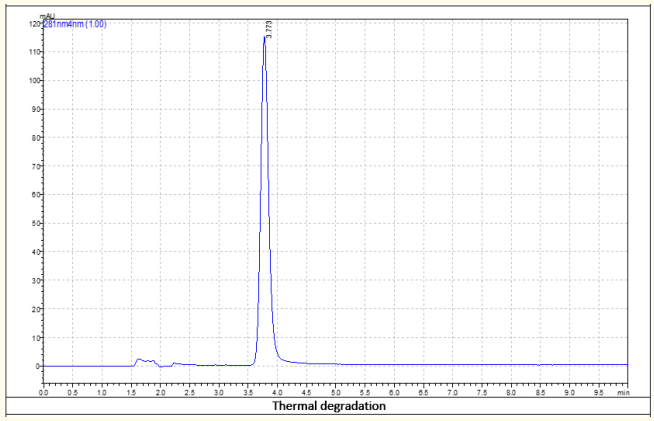


Figure 4: Representative chromatograms of Imatinib (20 µg/mL) during stress degradation studies.

Conclusions

A new stability indicating RP-UFLC was developed for the determination of Imatinib and validated as per ICH guidelines. It was observed that there was no interference of the degradants as well as the excipients during the study and there the method is said to be specific and selective. The proposed method is simple precise, accurate and robust and is suitable for the pharmacokinetic studies.

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Bibliography

1. Druker BJ., *et al*. "Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia". *The New England Journal of Medicine* 344.14 (2001): 1031-1037.

2. Gibbons J., *et al*. "Phase I and pharmacokinetic study of Imatinib mesylate in patients with advanced malignancies and varying degrees of renal dysfunction: a study by the National Cancer Institute Organ Dysfunction Working Group". *Journal of Clinical Oncology* 26.4 (2008): 570-576.

3. Druker BJ., *et al*. "Activity of a specific inhibitor of the BCR–ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome". *The New England Journal of Medicine* 344.14 (2001): 1038-1042.

4. Smita JP., *et al*. "Development of UV Spectrophotometric method for the determination of Imatinib mesylate (ITM) in bulk and formulation". *Asian Journal of Pharmaceutical and Clinical Research* 6 (2013): 54-57.

5. Nageswari A., *et al*. "Stability-indicating UPLC method for determination of Imatinib Mesylate and their degradation products in active pharmaceutical ingredient and pharmaceutical dosage forms". *Journal of Pharmaceutical and Biomedical Analysis* 66 (2012): 109-115.

6. María AR., *et al*. "Validation of an HPLC method for the determination of Imatinib mesylate in pharmaceutical dosage". *Journal of Liquid Chromatography and Related Technologies* 28.20 (2005): 3283-3292.

7. Naga Sindhu S., *et al*. "Method development and validation of RP-HPLC method for estimation of Imatinib mesylate in pure and pharmaceutical dosage form". *Der Pharmacia Lettre* 7.3 (2015): 33-38.

8. Lalit Mohan N., *et al*. "Development of a high sensitivity RP-HPLC method and stress testing of Imatinib mesylate". *Journal of Advanced Pharmacy Education and Research* 3.3 (2013): 238-243.

9. Pratik Shah*., et al. "*Method development and validation of a stability indicating RP-HPLC method for assay determination of Imatinib in Imatinib mesylate tablets dosage form". *International Journal of Pharmaceutical Sciences and Research* 6.10 (2015): 4453-4468.

10. Arun Kumar K., *et al*. "Analytical method development and validation for the estimation of Imatinib by reverse-phase high-performance liquid chromatography". *Asian Journal of Pharmaceutical and Clinical Research* 11.3 (2018): 136-139.

11. Shah P and Shah R. "A stability-indicating RP-HPLC method development and validation for the related substance determination of imatinib process impurities and their degradation products in tablet dosage form". *International Journal of PharmTech Research* 8 (2015): 128-146.

12. Vadera N., *et al*. "Stability-indicating HPTLC determination of imatinib mesylate in bulk drug and pharmaceutical dosage form". *Journal of Pharmaceutical and Biomedical Analysis* 43 (2007): 722-726.

13. Roth O., *et al*. "Imatinib assay by HPLC with photodiode-array UV detection in plasma from patients with chronic myeloid leukemia: Comparison with LC-MS/MS". *Clinica Chimica Acta* 411.3-4 (2010): 140-146.

14. Schleyer E., *et al*. "Liquid chromatographic method for detection and quantitation of STI-571 and its main metabolite N-desmethyl-STI in plasma, urine, cerebrospinal fluid, culture medium and cell preparations". *Journal of Chromatography B: Analytical Technologies in the Biomed Life Science* 799.1 (2004): 23-36.

15. Velpandian T., *et al*. "Development and validation of a simple liquid chromatographic method with ultraviolet detection for the determination of imatinib in biological samples". *Journal of Chromatography B: Analytical Technologies in the Biomed Life Science* 804 (2004): 431-434.

16. Widmer N., *et al*. "Determination of Imatinib (Gleevec) in human plasma by solid-phase extraction-liquid chromatography-ultraviolet absorbance detection". *Journal of Chromatography B: Analytical Technologies in the Biomed Life Science* 803.2 (2004): 285-292.

17. Oostendorp RL., *et al*. "Determination of imatinib mesylate and its main metabolite (CGP74588) in human plasma and murine specimens by ion-pairing reversed-phase high-performance liquid chromatography". *Biomed Chromatography* **21** (2007): 747-754.

18. Awidi A., *et al*. "Determination of imatinib plasma levels in patients with chronic myeloid leukemia by high performance liquid chromatography-ultraviolet detection and liquid chromatography-tandem mass spectrometry: Methods' comparison". *Leukemia Research* in press, (2010).

19. Masatomo Miura., *et al*. "Quantitative Determination of Imatinib in Human Plasma with High-Performance Liquid Chromatography and Ultraviolet Detection". *Journal of Chromatographic Science* 49 (2011): 412-415.

20. Le Coutre P., *et al*. "Pharmacokinetics and cellular uptake of imatinib and its main metabolite CGP74588". *Cancer Chemotherapy and Pharmacology* **53.4** (2004): 313-323.

21. Bende G., *et al*. "Validation of an HPLC method for determination of imatinib mesylate in rat serum and its application in a pharmacokinetic study". *Journal of Chromatography Science* 48.5 (2010): 334-341.

22. Yadav RR., *et al*. "Determination of potential genotoxic impurities in imatinib mesylate by RP-HPLC method". *Biological Forum-An International Journal* 4.2 (2012): 15-18.

23. Madireddy V., *et al*. "Stability-indicative, validated, fast HPLC method for quantification of two genotoxic impurities in imatinib mesylate". *Global Journal of Analytical Chemistry 2* (2011): 198-207.

24. Ghrib F., *et al*. "Determination of imatinib and its genotoxic impurities in tablets". *Biological Forum-An International Journal* 7 (2015): 1820-1827.

25. ICH Q2 (R1) Validation of analytical procedures: Text and methodology (2005).

26. ICH Q1A (R2) Stability testing of new drug substances and products (2003).