EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 4
Issue: 7
74-81
Year: 2017

VALIDATION OF ASSAY METHOD FOR THE ESTIMATION OF IMATINIB MESYLATE IN TABLET DOSAGE FORM BY HPLC

[1*]Farzana Hasin, [2]Md.Towhidul Islam, [3]Md.Forhad Ahmad, [4]Md.Mahadi Hasan Rakib

[1](Department of Pharmacy, University of Asia Pacific), [2,3,4](Department of Pharmacy, Stamford University Bangladesh)

***Corresponding Author: Farzana Hasin**
Bangladesh.

Article Received on 04/05/2017 **Article Revised on 24/05/2017** **Article Accepted on 14/06/2017**

ABSTRACT
A simple, sensitive high performance liquid chromatographic method was validated for the estimation of Imatinib Mesylate in pure and in pharmaceutical dosage forms. A 4.6-mm X 150-cm column that contains packing L1 (C18) was used with a mobile phase containing a mixture of Buffer: Aetonitrile: Methanol (50:25:25). The flow rate was 1.1 ml / minute and effluent was monitored at 230 nm. Calibration curve was plotted with a range from 40-160 mg/ml for Imatinib Mesylate. The assay was validated for the parameters like specificity, Linearity, Range, precision, accuracy & robustness parameters. The proposed method can be useful in the routine analysis for the determination on Imatinib Mesylate in pharmaceutical dosage form.

KEYWORDS: *Imatinib Mesylate, HPLC, Calibration curve, Validation.*

INTRODUCTION
Imatinib mesylate available in tablet dosage form. Chemically Imatinib mesylate is N-(4- methyl-3-{4-(pyridine-3- yl)pyrimidin-2-yl] amino}phenyl)-4-] (4-methylpiperizin-1yl) ethyl] benzamide. It is used in the treatment of chronic myeloid leukemia.[1,2,3,4,5] Imatinib mesylate is not official in any pharmacopoeias. Literature survey reveals, HPLC[6,7] methods for analysis of Imatinib mesylate as single component. The objective of the work is to develop RP-HPLC.[8,9] method for estimation of Imatinib mesylate in tablet dosage form with simple, rapid, accurate and economical methods and validated for system suitability, linearity, accuracy, precision, robustness and stability of sample solution as per ICH guideline.

MATERIALS AND METHOD
Subject: In-house developed tablets.

Working standard: Imatinib Mesylate, BN# 7SM103527960914, Valid up to: 30.09.18 Potency: 99.06%.

Buffer solution
Prepare a buffer solution dissolving 1.56 grams of sodium-dihydrogenphosphate dihydrate or 1.38 grams of sodium- dihydrogenphosphate monohydrate into a 1000 ml volumetric flask with HPLC grade water. Adjust pH 8.0 ± 0.2 with triethylamine.

Mobile phase
Buffer: Aetonitrile: Methanol (50:25:25).

Standard preparation
Weigh accurately about 24.0 mg working standard of Imatinib Mesylate in 100 volumetric flasks. Add 60 ml mobile phase sonicate few minutes to dissolve and then make volume up to the mark with Same solvent. Finally filter the solution with 0.45 micron disk filter.

Assay preparation
Weigh 10 tablets and determine the average weight. Take four tablets (about 1730.4 mg) into a 100 ml volumetric flask and add 60 ml of mobile phase and sonicate for 15 minutes, allow sample to cool to room temperature and add sufficient mobile phase to produce 100 ml and mix thoroughly. Filter the solution through Whatman filter paper size#41 and dilute 5 ml of this filtrate to 100 ml with mobile phase. Filter the solution through 0.45µ disk filter.

Chromatographic condition
Column	: 4.6-mm X 150-cm column that contains packing L1 (C18)
Detection	: 230 nm
Flow rate	: 1.1 ml / minute
Temperature	: 25°C
Injection volume	: 10 µl
Sampler temperature	: 10°C

Procedure
Inject 10 µl of standard solution one after another until

the relative standard deviation for replicate injections is not more than 2%. Inject the sample solution and obtain the chromatograms for the standard and the sample solution.

Calculation

Content of Imatinib/ Tablet

$$= \frac{Asam \times Wstd \times 100 \times 100 \times Pstd \times AWT}{Astd \times 100 \times Wsam \times 5 \times 100 \times 1.1947} \text{ mg}$$

Asam = Peak area of sample solution Astd = Peak area of standard solution Wstd = Weight of standard in mg Wsam = Weight of sample in mg

Pstd = Potency of standard in percentage AWT = Average weight of tablet.

1.1947 = Conversion factor of Imatinib to Imatinib Mesylate

METHOD VALIDATION

The method is validated according to ICH guidelines.[10,11]

RESULTS AND DISCUSSION

Specificity

The specificity of the method for identification is tested by injecting following solutions into the chromatographic system

- Mobile phase/ Diluents
- Placebo solution
- Reference solution
- Test solution

Preparation of Placebo solution

Weigh and take a quantity of powder about 1252.5 mg of formulation placebo in a 100 ml volumetric flask. Add about 60 ml mobile phase, sonicate for 15 minutes and dilute up to mark with mobile phase. Filter the solution through Whatman filter paper size# 41. Dilute 5 ml of this solution to 100 ml with mobile phase. Finally filter the solution with 0.45 micron disk filter.

Standard Preparation (Ref Solution)

Weigh accurately about 24.0 mg working standard of Imatinib Mesylate in 100 volumetric flasks. Add 60 ml mobile phase sonicate few minutes to dissolve and then make volume up to the mark with Same solvent. Finally filter the solution with 0.45 micron disk filter.

Sample Preparation (Test solution)

Weigh 10 tablets and determine the average weight. Take four tablets (about 1730.4 mg) into a 100 ml volumetric flask and add 60 ml of mobile phase and sonicate for 15 minutes, allow sample to cool to room temperature and add sufficient mobile phase to produce 100 ml and mix thoroughly. Filter the solution through Whatman filter paper size#41 and dilute 5 ml of this filtrate to 100 ml with mobile phase. Filter the solution through 0.45µ disk filter.

Observation

Sl. No.	Name of solution	Name of Peak	Retention time (minute)
01.	Diluents	-	-
02.	Placebo	-	-
03.	Reference solution	Imatinib	5.784
04.	Test solution	Imatinib	5.782
Remarks: By retention time analysis of diluents, placebo, reference and sample solution it is clear that there are no interfering peaks are observed from diluents, placebo at the retention time of Imatinib Mesylate reference/ working standard and retention time of standard & sample are 5.784 min & 5.782 min respectively which are within ± 0.2 minute of reference/ working standard's retention time.			

Linearity

To check the Linearity prepares a dilution series of standard solution from 40 to 160% of the nominal concentration. Inject separately 3 times each concentration level & calculate correlation coefficient, r^2 from the calibration curve from average area.

Linearity Stock Solution

Transfer 240 mg of Imatinib Mesylate working standard/API equivalent to 200 mg Imatinib to a 100 ml volumetric flask. Add 60 ml of mobile phase and sonicate for few minutes to dissolve and make volume with same diluents (2.0 mg/ml of Imatinib).

Concentration level in (%) of the active ingredients concentration	Volume of stock solution added (ml) in 50 ml volumetric flask with mobile phase	Approx. final concentration in (mg/ml)
		Imatinib
40	2	0.08
60	3	0.12
80	4	0.16
100	5	0.20
120	6	0.24
160	8	0.32

Acceptance Criteria

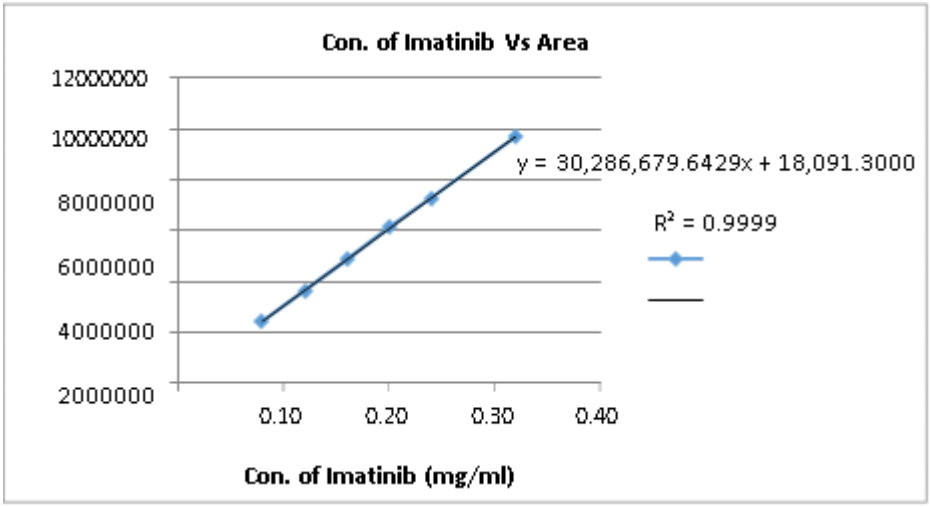
- Correlation coefficient: ≥ 0.995
- Intercept: To be reported

Slope regression line: To be reported

- Observation:

Different concentration of Imatinib and respective peak area:

Concentration level in (%) of the active ingredients concentration	Approx. final concentration in (mg/ml) of Imatinib	Peak area for Imatinib	
		Individual	Average
40	0.08	2431320	2427426
		2424603	
		2426354	
60	0.12	3646816	3644258
		3643571	
		3642389	
80	0.16	4851155	4860106
		4866613	
		4862550	
100	0.20	6131478	6121001
		6115909	
		6115616	
120	0.24	7293582	7287628
		7291072	
		7278230	
160	0.32	9710030	9689210
		9665024	
		9692577	



Graph-1: Different concentration of Imatinib VS Average peak area From Graph-1: Regression equation, y = 30,286,679.6429x + 18,091.3000, R^2 = 0.9999

Correlation coefficient, R^2	:	0.9999
Intercept	:	18,091.3000
Slope of regression line	:	30,286,679.6429

Range

Data taken from linearity studies to establish range.

Remarks: Based on the test results of linearity, accuracy and precision the range of method is established as 80 – 120% of the target concentration.

Precision

System precision (additional test for system suitability)

To check the repeatability of the system, inject the standard solution 6 times, immediate one after another, under conditions as similar as possible. Calculate the relative standard deviation.

Standard Preparation (Ref Solution)
Weigh accurately about 24.0 mg working standard of Imatinib Mesylate in 100 volumetric flasks. Add 60 ml mobile phase sonicate few minutes to dissolve and then make volume up to the mark with Same solvent. Finally filter the solution with 0.45 micron disk filter.

Procedure
Inject 10 µl of standard solution one after another until the relative standard deviation of retention time & peak area for six (06) replicate injections is not more than 2%.

Observation
Six injection reading of standard solution

No. of Sample	Retention time for Imatinib (min)	Average Retention time (min)	Relative standard deviation (%)	Peak area for Imatinib	Average Area	Relative standard deviation (%)
01.	5.792	5.789	0.0	6184453	6116365	0.6
02.	5.791			6114374		
03.	5.789			6085601		
04.	5.787			6109745		
05.	5.788			6101617		
06.	5.788			6102401		

Method precision (Repeatability)
To check the repeatability of the method, prepare separately the sample solution 6 times, immediately one after another, under conditions as similar as possible. Calculate the result for 6 determinations and calculate the Relative standard deviation.

Observation
Six injection reading of standard & sample solutions

No. of Sample	Peak area for Standard	Average Area	Relative standard deviation (%)	Peak area of Sample	Content of Imatinib in mg	Relative standard deviation (%)
01.	6184453	6116365	0.6	6105762	99.27	0.35
02.	6114374			6131311	99.74	
03.	6085601			6101399	99.27	
04.	6109745			6160314	100.10	
05.	6101617			6139158	99.85	
06.	6102401			6107953	99.35	
Remarks: Relative standard deviation for 6 replicate sample is 0.40%						

Intermediate precision (Reproducibility)
The intermediate precision for the assay of Imatinib Mesylate Tablet 100 mg should be determined by comparison of two independent repeatability experiments on 2 different days. The data of the 1st day can be taken from the analysis of 'Repeatability'. The second set of experiments (method precision for assay) is to be performed by a different analyst and different days. Calculate the relative standard deviation from the results on eachday.

Data for intermediate precision by two different analysts

Product: Imatinib Mesylate Tablet 100 mg						
Analyst Name: Jamal Hossain				**S.M. Jaman**		
Location: OQC lab				**OQC lab**		
Instrument used: HPLC with Empower Software (ID No.: HGER&D 045)				Instrument used: HPLC with Empower Software (ID No.: HGER&D 045)		
Date of analysis: 10.27.2016				10.28.2016		
SL No.	Average Area of Standard	Area of Samples	Assay (mg)	Average Area of Standard	Area of Samples	Assay (mg)
1.	6116365	6105762	99.27	6191426	6163527	99.02
2.		6131311	99.74		6162801	99.03
3.		6101399	99.27		6207857	99.76
4.		6160314	100.10		6330654	101.70
5.		6139158	99.85		6203145	99.65
6.		6107953	99.35		6171815	99.18
Mean Assay, n = 6			99.66	Mean Assay, n = 6		99.86

Standard deviation, n = 6	0.35%	Standard deviation, n = 6	1.07%
Relative standard deviation, n = 6	0.35%	Relative standard deviation, n = 6	1.07%
Mean value of assay difference between two analyst, Δ			0.20%
Remarks: Mean value of assay difference between two analyst, i.e. Δ is 0.20%			

Accuracy or Recovery

The accuracy of the method is evaluated by samples spiked with active ingredients. Data from triplicate determinations should be collected at 3 concentration levels i.e. 80%, 100% & 120% of the label claim of the active ingredient. The accuracy is expressed in recovery rates.

Accuracy Standard stock solution

Transfer 240 mg of Imatinib Mesylate working standard/API equivalent to 200 mg Imatinib to a 100 ml volumetric flask. Add 60 ml of mobile phase and sonicate for few minutes to dissolve and make volume with same diluents (2.0 mg/ml of Imatinib).

Concentration level in (%) of the active ingredients concentration	Volume of stock solution added (ml) in 50 ml volumetric flask with mobile phase	Approx. final concentration in (mg/ml)
		Imatinib
80	4	0.16
100	5	0.20
120	6	0.24

Preparation of Imatinib Mesylate Tablet 100 mg accuracy test solutions

Take three 100 ml volumetric flask and labeled it as 80%, 100% & 120%.Weigh and transfer placebo equivalent to 1 tablet (313.13 mg) into the marked volumetric flask each. Weigh 96 mg, 120 mg and 144 mg of Imatinib Mesylate API and add it into the 80%, 100%, 120% marked volumetric flask respectively. Add 60 ml of mobile phase into the each volumetric flask and sonicate for 15 minute to dissolve and make volume up to the mark at room temperature. Filter the solution through Whatman filter paper size# 41. Dilute 5 ml each of this above solution to 25 ml with mobile phase. Finally filter the solution through 0.45 micron disk filter.

Following table describe the concentration of sample at different level.

Concentration level in (%) of the active ingredients concentration	Approx. final concentration in (mg/ml)
	Imatinib
80 × 3 sample	0.16
100 × 3 sample	0.20
120 × 3 sample	0.24

Observation

The sample solution for evaluating the Accuracy / Recovery was prepared as 80% – 120% of nominal analyte of Imatinib.

Concentration of Imatinib (mg/ml)	% of nominal concentration	Average Peak area (Standard)	Average Peak area (Sample)	Recovery from sample in %
0.16	80	4823837	4859430	100.74
0.20	100	6074341	6099458	100.41
0.24	120	7270617	7331829	100.84
			Average	100.66
			Minimum	100.41
			Maximum	100.84

Remarks: Individual recovery for Imatinib is from 100.41– 100.84% and mean recovery is 100.66%.

Robustness

Stability of the analytical solutions

The stability of analytical solution is demonstrated by carrying out the analysis on the Reference and Test solution immediately after they are prepared and then at suitable intervals at room temperature.

The test solution to is be kept on bench top under normal laboratory conditions and to be analyzed at suitable time intervals to establish bench top solution stability up to 8 hrs.

Time program

Initial, After 4 hours, After 8 hours

In a table summarize the % change between the initial results and the results at each time point calculated with respect to the fresh standard where appropriate.

Standard and sample solutions are prepared as per test method and analyzed initially and at different time intervals by keeping the solution at room temperature (about 25 °C).

Standard Solution				Sample solution		
Time in Hours	**Area**	**% Results**	**% Change**	**Area**	**% Results**	**% Change**
Initial	5942163	-----	-----	5933573	99.22	-----
4th Hour	5939472	99.89	0.11	5939886	99.26	0.04
8th Hour	5934109	100.16	0.16	5934914	99.52	0.30

Remarks: From the above study, there is no significant change in % result of standard & sample solution a suitable interval after 4 hours & 8 hours.

Influence of the variation in test parameters

The influence of slightly changed parameters of the chromatographic conditions must be tested to demonstrate sufficient robustness of the method. In detail, effects of the change of flow rate, Column Temperature, composition of mobile phase and different brand of analytical column should be studied. The tests are carried out by using standard solution spiked and varying each of the parameters of chromatography mentioned above as follows:

Flow rate : 1.0 ml and 1.2 ml /min (required 1.1 ml/min) Ratio of organic phase in mobile phase : (Change 48 to 52%) required volume 50%

Analytical column : Column with same specifications of different brands or different lots of same manufacturer is considered as an additional part of robustness test and is recommended to verified or validated during routine work.

Temperature : 20°C & 30°C (required temperature 25°C)

Detection wavelength : As per the current guidelines published in USP and Ph. Eur. change in detection wavelength is not allowed.

Acceptance Criteria: In each experiment Tailing factor should be consistent with initial

Show the chromatograms of each run with individually changed parameters. A summary of the various conditions tested should be added.

If the results obtained for the proposed parameters are not meeting acceptance criteria, then the influence to be demonstrated at the intermediate level of the above proposed parameters.

Observation

Change in flow rate

Mobile Phase Composition	Flow Rate ml/min	Tailing Factor	Theoretical plates
Organic,50%	1.0	1.12	3538
Organic,50%	1.1	1.11	2979
Organic,50%	1.2	1.11	3186

Change amount of organic phase for mobile phase

Mobile Phase Composition	Flow Rate ml/min	Tailing Factor	Theoretical plates
Organic,48%	1.1	1.10	3481
Organic,50%	1.1	1.11	2979
Organic,52%	1.1	1.11	3344

Change of Column temperature

Mobile Phase Composition	Column temperature	Tailing Factor	Theoretical plates
Organic,50%	20°C	1.11	3050
Organic,50%	25°C	1.11	2979
Organic,50%	30°C	1.11	3799

Change of different column

Mobile Phase Composition	Different Column Lots	Tailing Factor	Theoretical plates	Column ID
Organic,50%	YMC-Basic; 3µ, 150 × 4.6 mm Lot: BA99S03-1546WT, Part No. 0415214732	1.18	4656	HPL/R&D-HPLC- 113

Organic,50%	150 ×4.6 mm, 5µ column that contains packaging C18. S/N: 739052-1	1.15	6343	HPL/R&D-HPLC- 104

Remarks
From the above data it is clear that change of flow rate, acetonitrile in mobile phase, column temperature, cooler temperature and different columns has no effect on tailing factor & theoretical plates.

Filter suitability test
Inject the following solutions into the chromatographic system.
- Test solution spiked with active ingredient at label claim concentration.
- Above solution filtered with different makes (0.45µ & 0.20 µ) of the disc filters. Calculate the % content in the above solutions.

Compare the observed results in a table and give a statement for the suitability of the filters.

0.45µ.disc filter		**0.20µ.disc filter**
Result in %		Result in %
Imatinib	99.27	99.03

Remarks
From the above study, we found no significant change % content in the above solutions by changing disc filter 0.45µ. & 0.20µ. We found 99.27% result through 0.45 micron disk filter and 99.03% through 0.20µ disk filter. So both disk filters is suitable for the product.

Analysis by Different Machine
Analysis on the Reference solution is to be performed by different HPLC system to demonstrate sufficient robustness of the method.

Acceptance Criteria: In each experiment
Tailing factor, theoretical plates and any undesired peak should be consistent Show the chromatograms of each run with Different HPLC system.

A summary of the various HPLC system tested should be added

Mobile phase stability study
Keep mobile phase under normal laboratory condition on bench for seven (07) days. The stability of mobile phase is demonstrated by carrying out the analysis on the freshly prepared reference and test solution the product with preserved mobile phase.

Observation	**Initial**	**After 07 days**
Appearance of mobile phase	Clear	Clear
Tailing Factor	1.18	1.23
Theoretical plates	4656	4999
Retention time	2.961	3.016
Assay result (Method precision)	98.84%	100.43%

Results summary

Sl. No.	**Validation Parameters**	**Acceptance Criteria**	**Results**	
			Imatinib	
1.0	Specificity	No peak co-elutes with main peak	Complies	
		No interfering peaks are observed from diluents, placebo at the retention time of Imatinib.	Complies	
		No interfering peaks are observed from diluents, placebo at the retention time of Imatinib Mesylate reference/ working standard and retention of sample will be within ± 0.2 minute of reference/ working standard's retention time.	Complies	
2.0	Linearity	Correlation coefficient : ≥ 0.995	0.9999	
		Intercept : To be reported	18,091.3000	
		Slope regression line : To be reported	30,286,679.6429	
3.0	Range	80 – 120 % of the limit concentration of active ingredient.	Complies	
4.0	Precision			
	4.1 System precision	Relative standard deviation is less than 2.0%.	Rt. Tm.	Area
			0.0%	0.6%
	4.2 Method precision (Repeatability)	The relative standard deviation for n ≥ 6, (6 determinations at 100 % concentration) should be ≤ 2.0 %	0.35%	

	4.3 Intermediate precision	RSD for n ≥ 6 is as per repeatability day 1 and mean values difference between day 1 and day 2 i.e. Δ < 2.0% absolute.	0.20 %	
5.0	Accuracy or Recovery	Individual recovery % must be between 97 - 103 %	100.41– 100.84%	
		Mean recovery % must be between 98 - 102 %	100.66%	
6.0	Robustness			
	6.1 Stability of the analytical solutions	Standard solution: ± 2.0% with regard to initial	4 Hr	0.11
			8 Hr	0.16
		Sample solution: ± 2.0% with regard to initial	4 Hr	0.04
			8 Hr	0.30
	6.2 Influence of the variation in test parameters	Tailing factor & theoretical plates should be consistent with initial.	Consistent	
	6.3 Filter Suitability	Content should be consistent through 0.45µ m & 0.20 µm disk filter.	Consistent	
	6.4 Mobile phase stability study	Tailing factor, theoretical plates, retention time of standard solution chromatogram and assay result should be consistent with initial.	Consistent	

CONCLUSION

The proposed HPLC method was found to be simple, specific, precise, accurate, rapid and economical for estimation and validation of Imatinib mesylate in pharmaceutical dosage form. Chromatographic separation of Imatinib mesylate was achieved on 4.6-mm X 150-cm column that contains packing L1 (C18). The mobile phase was Buffer: Aetonitrile: Methanol (50:25:25) ratio with flow rate of 1.1 ml/min. The chromatograms were recorded at 230nm. The developed method was validated in terms of specificity, Linearity, Range, precision, accuracy, robustness and results will be validated statistically according to ICH guidelines. The system suitability parameters were within limit, hence it was concluded that the system was suitable to perform the assay. Therefore the proposed method can be used for determination of Imatinib mesylate in pharmaceutical formulations.

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