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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF METHOD FOR QUANTITATIVE ESTIMATION OF IMATINIB MESYLATE IN FORMULATION AND BULK DRUG

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HPLC method has been developed for the estimation of Imatinib in pharmaceutical dosage form. Chromatography was performed by Isocratic reverse phase using Waters X Bridge C18 column 5µm 4.6x250mm with UV detection at 234 nm, Flow rate was set at.1.0ml/min with Tris and acetonitrile (40:60) as mobile phase. Validation parameters were performed to demonstrate linearity, accuracy, precision, LOD & LOQ in accordance to ICH guidelines. The current method demonstrates good linearity over the range of 2-24µg mL^{-1} for IMA with intra-day and inter-day precision, expressed as the relative standard deviation (RSD), of replicates is <2.0 and accuracy in the range of 98–102%. The developed RP-HPLC method was innovative, suitable for detecting IMA in pure form and in pharmaceutical preparation.

Key words: Imatinib; RP-HPLC; Tris and Acetonitril ; UV-visible spectroscopy.

INTRODUCTION:

Imatinib mesylate is used in treating chronic myelogenous leukemia (CML), gastrointestinal stromal tumors (GISTs) and a number of other malignancies. It is the first member of a new class of agents that act by inhibiting particular tyrosine kinase enzymes, instead of non-specifically inhibiting rapidly dividing cells. It is a protein-tyrosine kinase created by the Philadelphia chromosome abnormality in chronic myeloid leukemia. It inhibits proliferation and induces apoptosis in Ber-Abl positive cell lines as well as fresh leukemic cells from Philadelphia chromosome positive chronic myeloid leukaemia. Literature survey reveals that the drug has been estimated by Liquid chromatography and spectrophotometry methods in biological fluids like human plasma and rat plasma[4-24]and HPLC method in pharmaceutical formulations has been reported so far. The objective of this study is to develop a simple, fast, selective, accurate, precise and sensitive Rp HPLC method for the estimation of Imatinib in bulk and in pharmaceutical dosage forms (Tablets) suitable for routine quality control analysis. The chemical structure of Imatinib mesylate is given in Fig 1. Chemically it is 4-[(4-Methyl-1-piperazinyl) methyl]-N-[4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl] amino]-phenyl] benzamide methane sulfonate with empirical formula C29H31N7O •CH4SO3.

MATERIALS AND METHODS:

Chemicals and reagents

All the chemicals and reagents used were of high purity procured from various sources. Imatinib mesylate (Active Pharmaceutical Ingredient, API) and reference material were procured from a reputed lab in India. A commercial local tablet formulation was used in this study.

INSTRUMENTATION:

The Agilent LC 1120 HPLC system consisting of gradient pump (400 barr), auto injector, UV variable detector, was used. The separations were achieved on a Waters X Bridge C18 column 5µm 4.6x250mm with UV detection at 234 nm, Flow rate was set at.1.0ml/min. Analytical weighing balance (Shimadzu) was used for weighing, sonicator (Equitron), and vacuum pump (superfit), filtration kit (TARSONS) with millipore membrane for solvents and sample filtration were used throughout the experiment. The Spinchrome software was used for acquisition, evaluation and storage of chromatographic data.

Reagents and Pharmaceutical Preparations

Analytically pure sample of Imatinib procured as gift sample from Natco Laboratories, Hyderabad. The drug was used without further purification. HPLC grade Acetonitrile (Merck), Pharmaceutical formulation Imatinib tablets (label claim 400mg) batch no: HTF14004A, Mfg. Lic.no:22/RR/AP/2001/F&B/CC. Manufactured by Hetero Labs Ltd, Hyderabad. HPLC grade water obtained in-house by using Direct-Q3® with pump (Elec. Ratings: 100-230V of 50-60Hz 100VA) water purification system (made in France) was used in HPLC study.

Preparation of Mobile phase:
The mobile was prepared by mixing Tris and Acetonitrile in the ratio 40:60 pH 8.0. The mobile phase is then sonicated using Ultra-Sonicator to remove the impurities and dissolved gases, as they may lead to unwanted peaks in the chromatogram.

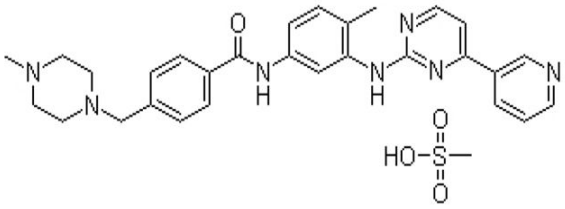

Fig. 1:Chemical structure of Imatinib mesylate

Chemicals	Details
Imatimib mesylate standard	Natco lab, Hyderabad
Imatinib mesylate tablets (400mg)	Purchased commercial sample
Acetonitrile	Merck
Tris	Merck

Table 1:Chemicals and reagents used

s.no	Imatinib	
	Concentration µg ml-1	Peak Area
1	4	835734
2	8	1843817
3	12	2905903
4	16	5286875
5	20	6502814
6	24	10411666

Table 3:Linearity data for Imatinib

Parameters	Imatinib mesylate
Linearity	4-24 µg/ ml
r^2	.9994
Slope	383840
Intercept	-992627

Table 4: Statistical data of calibration curves

Preparation of standard stock solution
Stock solution of Imatinib mesylate (1mg/ml) was prepared by dissolving 100mg of Imatinib mesylate in 100ml of volumetric flask containing mobile phase. The solution was sonicated for about 10min and then made up to volume with mobile phase. Daily working standard solution of Imatinib mesylate was prepared by suitable dilution of stock solution with appropriate mobile phase.

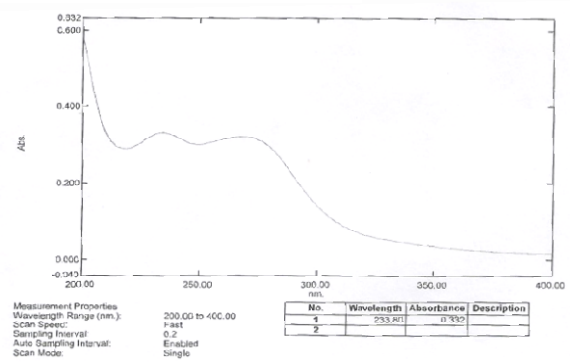

Fig 2: Uv spectrum of imatinib mesylate at 234 nm

Preparation of Standard Stock Solution of Imatinib:
400mg of Imatinib standard was accurately weighed and transferred to a 100ml volumetric flask and made up to the volume with acetonitrile and sonicated for 10 min and it results into 4000µg/ml (sol A). From the solution A 1 ml has taken and adjusted with acetonitrile to made up 100 ml which results in 40 µg/ml (sol B). From the solution B 1ml, 2ml, 3ml,4ml, 5ml, 6ml were pipetted out into separate 10 mL volumetric flasks and volume was made up to the mark with the diluent used. This gave the concentration of 4, 8, 12,16, 20,

24 µg/ ml These six dilutions of Imatinib were prepared and analysed by HPLC.

Specificity:
The instruments ability to measure or identify or measure the analyte without any interference from sample matrix, impurities, precursors or degradation products.

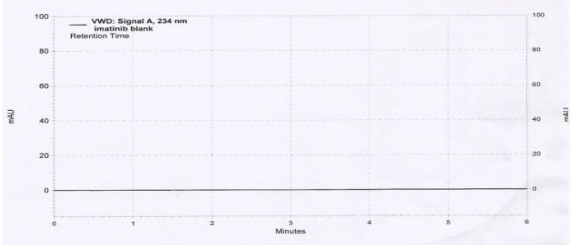

Fig: 3 Chromatogram of blank

Blank interference:
A study to establish the interference of blank was conducted. Mobile phase was injected as per the test method.

Chromatogram of blank should not show any peak at the retention time of analyte peak

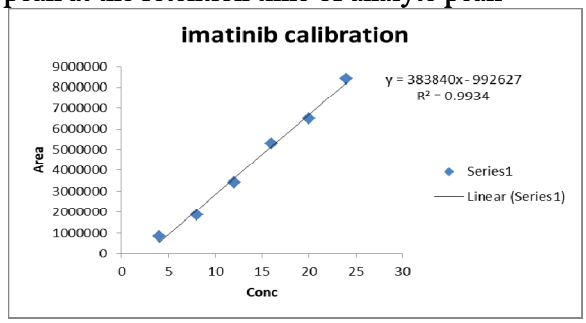

Fig 4: Linearity curve of imatinib

Linearity:
A series of standard curves were prepared over a concentration range of 4-24µg/ml by diluting the standard stock solution of Imatinib mesylate (1mg/ml) in mobile phase. The data from peak area verses drug concentration plots were treated by linear least square regression analysis and $r^2$was found to be 0.9994

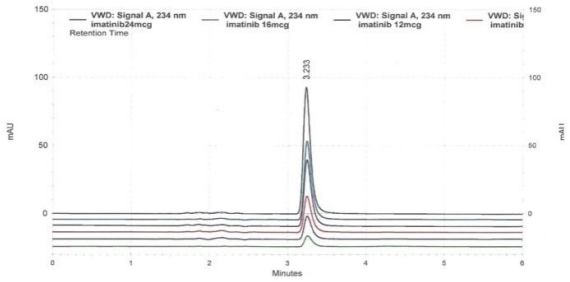

Fig 5: overlaylinearity chromatograms of imatinib mesylate

Precision of the method:
Precision was measured in accordance with ICH recommendations. The precision study was carried out by injecting sample preparation of 100µg/ml concentration six times. The obtained results within and between the days of trials were in acceptable range indicating good precision of the proposed methods.

Accuracy
Recovery studies by the standard addition method were performed with a view to justify the accuracy of the proposed method. Previously analyzed sample of Imatinib mesylate were spiked with known amount of

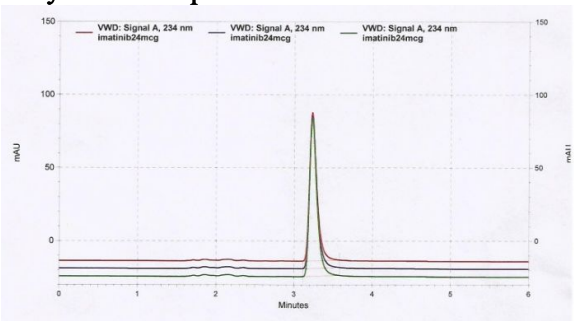

Fig 6: Chromatogram of Imaitinb showing 80% accuracy

standard so as to get three different levels (80%, 100%, 120%)and the mixture were analysed by the proposed method. The experiment was performed in triplicate % recovery, mean % recovery, RSD(%) were calculated for each concentration.

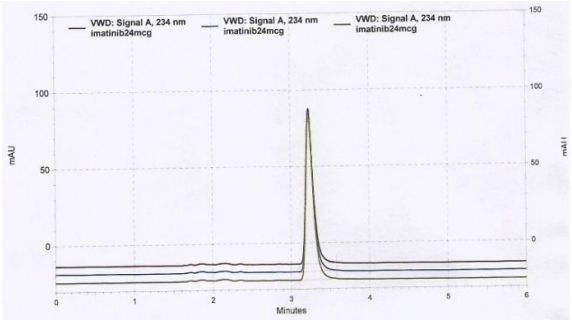

Fig 7: Chromatogram of Imaitinb showing 100% accuracy

METHOD VALIDATION:

Linearity

The calibration curve constructed was evaluated by using correlation coefficient. The peak area of the drug was linear in the range of 20-120µg/ml. The area for each of the concentration obtained was plotted against the concentration of the analyte. The correlation coefficient for the data was calculated as 0.9994 for Imatinib mesylate indicating a strong correlation between the concentration and the area under the curve. Concentration verses peak area results are given in table 3 and statistical data of calibration curves are given in table 4.

Table 5: Results of recovery of Imatinib

S.no	Level in %	Area Response	Mean % recovery
1	80	8432015	100.5
2	80	8434542	
3	80	8456721	
1	100	8432015	100.62
2	100	8443623	
3	100	84656734	
1	120	8454333	100.58
2	120	8467898	
3	120	8465423	

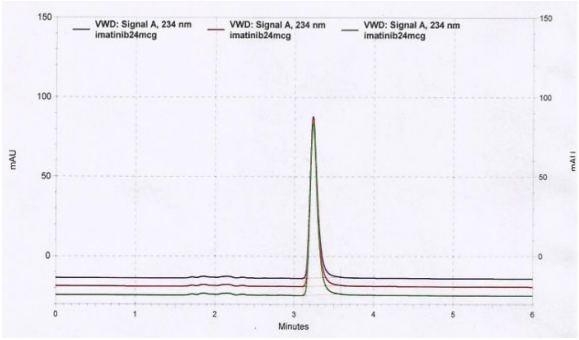

Fig 8: Chromatogram of Imaitinb showing 120% accuracy

Accuracy

Accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The accuracy of an analytical method should be established across its range. Accuracy is performed in three different levels, each level in triplicate for Imatinib using standards at 80%, 100% and 120%. Each sample was analysed in triplicate for each level. From the results, % recovery was calculated.

S.NO	MORNING		NOON	
	INJECTION	AREA	INJECTION	AREA
1	Injection 1	3450362	Injection 1	3410825
2	Injection 2	3427810	Injection 2	3456821
3	Injection 3	3434561	Injection 3	3467820
4	Injection 4	3445679	Injection 4	3486543
5	Injection 5	3432856	Injection 5	3493456
6	Injection 6	3422108	Injection 6	3435290
	Average	3435563	**Average**	3458459
	SD	10684.77	**SD**	31335.64
	%RSD	0.311	**%RSD**	0.906

Table 7: Intraday (Repeatability) study results by HPLC

S .no	Labelled amount (mg)	Imatinib mesylate		
		Amount added (mg)	Amount found (mg)**	% Recovery
1	400	720	402	100.5%
2	400	800	402.48	100.62%
3	400	480	403.8	100.58%

Table 6: Assay for pharmaceutical formulations

S.NO	DAY 1		DAY 2	
	INJECTION	AREA	INJECTION	AREA
1	Injection 1	3452018	Injection 1	3412345
2	Injection 2	3494571	Injection 2	3423451
3	Injection 3	3410298	Injection 3	3467891
4	Injection 4	3465432	Injection 4	3498765
5	Injection 5	3489765	Injection 5	3454320
6	Injection 6	3498765	Injection 6	3442311
	Average	3468475	**Average**	3449847
	SD	33812.44	**SD**	31300.17
	%RSD	0.974	**%RSD**	0.907

Table 8: Inter day (intermediate precision) study results by HPLC

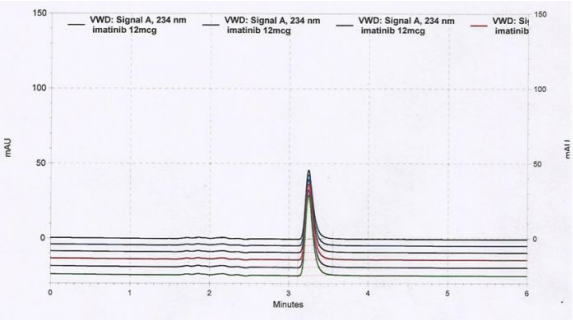

Fig 9: Chromatogram of Imatinib showing intraday (morning) precision

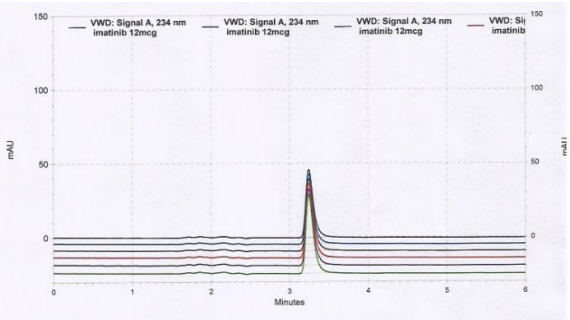

Fig 10: Chromatogram of Imatinib showing intraday (noon) precision

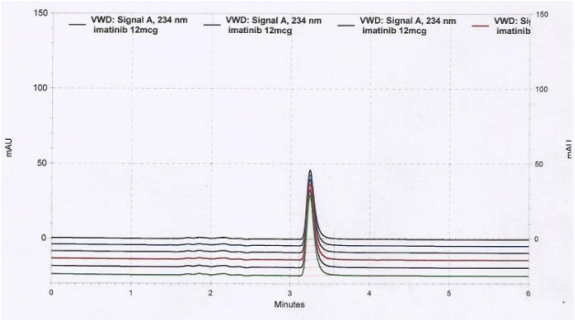

Fig 11: Chromatogram of imatinib showing Interday (day 1) Precision

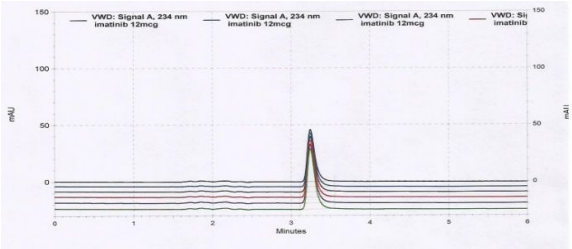

Fig 12: Chromatogram of imatinib showing Interday(day 2) Precision

Assay

The assay for the marketed tablets was established with chromatographic condition developed and it was found to be more accurate and reliable. The %purity was found to be 99.90. No interfering peaks were found in

chromatogram, indicating that the estimation of drug free from inference of excipients.

PARAMETERS	µg/ml
LOD	0.436
LOQ	1.454

Table 9: LOD & LOQ of Imatinib

Precision

Precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample. The precision expressed as % RSD

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

LOD and LOQ were calculated according to ICH recommendations where the approach is based on the signal-to-noise ratio. Chromatogram signals obtained with known low concentrations of analytes was compared with the signals of blank samples. A signal-to-noise ratio 3:1 and 10:1 was considered for calculating LOD and LOQ respectively. The values of LOD and LOQ were given in 9

RESULTS AND DISCUSSION:

Results of HPLC:

The RP-HPLC method was developed by using 250 X 4.6 mm, reverse phase column packed with Octadiacylsilane chemically bonded to porous silica or ceramic micro-particle, 5 µm (Waters X Bridge C18 column 5 X 4.6 X 250 mm) with mobile phase of 40 volumes of Tris buffer (pH 8.0) and 60 volumes of Acetonitrile as mobile phase and diluents is mobile phase, run as isocratic elution. Flow rate was 1 ml/min with UV detection at 234 nm and the injection volume was set at 20 µL with 6 minutes of run time. Mobile phase used has sufficient polarity to elute the drug. All system suitability parameters (theoretical plates, tailing factor) were optimal. The developed LC method was found to be specific for estimation of Imatinib in its tablet dosage form (400mg).
The developed method was validated by using following parameters as per ICH guidelines[3]

SUMMARY

On the basis of the experiments, we can conclude that the RP-HPLC & method developed for the Estimation of Imatinib can be used for routine analysis Q.C. Samples. Imatinib was determined by reverse phase HPLC using Tris buffer (pH 8.0): Acetonitrile (40:60v/v) as mobile phase, and Waters X Bridge C 18Column, 5µ 250×4.6mm as a stationary phase. Detection was carried out using UV detector at 234 nm. After development of the method, it was validated for system suitability, specificity and linearity, limit of detection and limit of quantification, precision, and accuracy.

- The system suitability was found to be within the limits. The limit was Not more than RSD <2. The retention time of Imatinib is 6.0 mins. The data regarding the system suitability is shown in table 7.
- The Specificity of Imatinib is shown in Chromatogram there was no interference. In this method it means no impurity was interfered and also reveals that commonly used excipients and additives present in the pharmaceutical formulations were not interfering in the proposed methods.

Validation parameters	Imatimib
Mobile phase	Tris buffer: Acetonitrile (40:60 v/v)
Flow rate	1.0 ml/min
Detection wavelength	234
Rt	3.2 min
Run time	6 min
Theoretical plates	5131
LOD	0.43 µg/ml
LOQ	1.45 µg/ml
Linearity	4-24 µg/ml
Precision	% RSD < 2

Table 9: Summary of the present study

- The precision was found to be within the limits. The limit were not more than RSD <2. This indicates that the method is precise. The data regarding the precision are shown in table 8 and 9.
- From the linearity table 5 it was found that, the drug obeys Beer's Law. For HPLC the calibration plot of Imatinib was observed as

linear in the range 4-24µg/ml and the correlation coefficients were found to be 0.999 respectively.

- From the results shown in the accuracy table 10 and 11, it was found that Recovery value of pure drug from the solution were between 100.22% to 100.66This indicates that the method is accurate.

CONCLUSION:

The proposed RP-HPLC method is suitable techniques for determination of Imatinib. All the parameters for analysing Imatinib met the criteria of ICH guidelines for method validation, RP-HPLC method may be considered more specific and sensitive. But also is more expensive requiring sophisticated chromatographic instrumentation for its performance. HPLC method was developed may be recommended for routine QC analysis of the investigated drugs to provide simple and accurate quantitative analysis for the determination of Imatinib.

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