

Original Article

METHOD DEVELOPMENT AND VALIDATION OF ANTINEOPLASTIC DRUG-IMATINIB BY UV-SPECTROSCOPY

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ABSTRACT

Objective: A simple, rapid, precise and highly selective spectrophotometric method was developed for estimation of Imatinib in tablet dosage form. **Method:** This method, involves the measurement of absorbances of Imatinib at the wavelength of 257nm. Distilled water was used as solvent. Linearity was observed in the concentration range of 10-50µg/ml for Imatinib. The accuracy of the method was confirmed by recovery studies of tablet dosage forms and was found to be 99.8% for Imatinib. The method showed good reproducibility and recovery with % RSD less than 4. The LOD of Imatinib was found to be 0.066µg/ml and LOQ of Imatinib was found to be 0.2µg /ml. Thus the proposed method was found to be rapid, specific, precise, accurate and cost effective quality control tool for the routine analysis of Imatinib in bulk and tablet dosage form. Drug stability studies have been determined for the formulation under specified conditions and it was found stable.

Keywords: Imatinib Mesylate, UV Spectroscopy, Method validation.

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1. INTRODUCTION

Imatinib is a cancer medication prescribed to treat leukemia and gastrointestinal tumors. It operates by inhibiting proteins associated with cancer cell growth in order to relieve symptoms¹, prevent the spread of cancer cells, and aid other treatments¹. Imatinib is one of the newest anticancer drugs in the market and was one of the first drugs to be pushed through Food and Drug Administration's (FDA) fast track designation for approval. The drug is designed to inhibit tyrosine kinases such as Bcr-Abl and is used in the treatment of chronic myeloid leukemia (CML) and gastrointestinal stroma tumors.

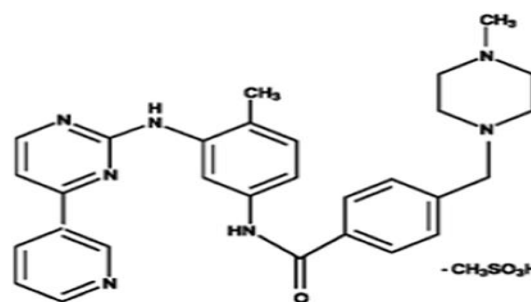


Figure 1: Structure of Imatinib Mesylate

The Chemical name of ImatinibMesylate is 4-4[(4-methyl-1- piperaziny)l methyl]-N-[4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl] amino] phenyl] – benzamide mono methane sulfonate. It has a molecular formula of C₂₉H₃₁N₇O.CH₄O₃S and a molecular weight of 589.71. It has the structural formula (Fig.1). Imatinib Mesylate is a white crystalline powder which in freely soluble is distilled

water, 0.1 N HCl, methanol and sparingly soluble in dimethyl ether. Literature Survey revealed that the drug has been estimated by Liquid chromatography²⁻⁹ and Spectrophotometry¹⁰ methods in biological fluids like human plasma and rat plasma and HPLC method in pharmaceutical formulations has been reported so far. But no UV-Spectroscopic method was reported for the estimation in bulk and pharmaceutical dosage forms. The aim of present work was to develop and validate a simple, precise, sensitive, specific spectroscopy method for Imatinib Mesylate in its bulk and tablet dosage form.

Selection of solvent

Solutions of Imatinib and Imatinib Mesylate were prepared by using distilled water, methanol, ethanol, DMSO and UV spectrum of each were recorded by scanning between 200-400 nm.

An overlain spectrum of Imatinib and Imatinib Mesylate were prepared in different solvents like distilled water, ethanol. Better absorbance were observed for both the API and Formulation when water is used as a solvent. Hence, water was selected as solvent for present study.

Different concentrations of Imatinib and Imatinib Mesylate were prepared in the range of 10-50 µg/ml and scanned and maximum absorbance was noted at the wavelength 257nm. From these data, it was noted that, at the wavelength 257nm, good linearity was observed and hence this wavelength was fixed for the study.^{2,3}

1.1. Linearity studies of IMATINIB (API)

Preparation of standard stock solutions of Imatinib:

Accurately weighed 10 mg of Imatinib transferred to 10 ml volumetric flasks. It was dissolved in Distilled Water and was shaken manually for 10 min. The volume was made up to the mark with same solvent to obtain final strength 1000 µg/ml.

Stock solution-2:

From the stock solutions, 1.0 ml of Imatinib was transferred to 10 ml volumetric flask and the volume was adjusted to the mark with same solvent to obtain strength 100 µg/ml.

Dilutions:

Appropriate known volumes of aliquots from 1st dilution were transferred to separate 10 ml volumetric flasks. The volume was adjusted to the mark with Distilled water to a series of concentration in the range of 10-50 µg/ml. The solution was scanned in the UV range 200-400 nm. Absorbance of these solutions were recorded at 257 nm and Calibration curve was plotted, absorbance vs. concentration

1.2. PRECISION:

Precision of the method was studied as repeatability, intra-day and inter-day precision.

1.2.1. INTRADAY:

Intra-day precision was determined by analyzing the 10, 20, 30 µg/ml for three times in the same day and the values are reported.

1.2.2. INTERDAY PRECISION:

Inter-day precision was determined by analyzing the same concentration of the solutions daily for three days.

1.3. SPECIFICITY

Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present. A major objective of determining specificity is to ensure "peak purity" of the main compound to be determined^{4, 5}

Specificity is determined by analyzing 10 µg/ml concentration repeatedly and measuring the absorbance at 257nm wavelength.

1.4. ACCURACY:

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an

accepted reference value and the value found. To determine the accuracy, measure the absorbance of all the concentrations at 257nm and calculate the concentration found and % concentration using the formula:

$$y=mx+c$$

1.5. %RECOVERY:

To determine the accuracy, recovery studies were carried out three different levels i.e. 80%, 100% and 120%. To the pre-analyzed sample solution a known amount standard drug solution was added at three different levels, absorbance was recorded.

2. RESULTS AND DISCUSSION

2.1 LOD and LOQ of Imatinib:

The LOD and LOQ of Imatinib were determined by using standard deviation of response and slope approach as defined by ICH guidelines.

2.2. Linearity Studies of Imatinib Mesylate (Itm):

For analysis of commercial formulation, twenty tablets were weighed, average weight determined and crushed into fine powder^{5,6}. An accurately weighed quantity of powder equivalent to 10 mg of ITM was transferred into 10ml volumetric flask containing 10 ml distilled Water, shaken manually for 10 min. and filtered through Whatmann filter paper no. 45. From the stock solutions, 1.0 ml of Imatinib was transferred to 10 ml volumetric flask and the volume was adjusted to the mark with same solvent to obtain strength 100µg/ml. An appropriate aliquots of 2-12µg/ml were prepared and absorbance was recorded at 257 nm.

Imatinib Mesylate was found to be linear in a concentration range of 10-50 µg/ml. The absorbance of these solutions was noted at wavelengths 257nm. Calibration curves were plotted using concentration Vs absorbance at wavelength of 257 nm and the slope and correlation coefficient values were found to be 0.05 and 0.996 respectively

2.3. DRUG STABILITY STUDIES:

2.3.1. Photo degradation:

Photo degradation should be carried out at more strenuous conditions than recommended ICH Q1A accelerated testing conditions. The Samples of Imatinibare exposed to dry heat. Studies may be conducted at higher temperatures for a shorter period and the values obtained is as shown in the table no-15

2.3.2. ACID Degradation:

Transfer 1ml.(1000µg/mL. ITM) of above stock solution to10mL.volumetric flask and add 1ml.of 1N HCL and finally make up the volume with distilled water and kept aside for 24hrs at room temperature. From this transfer 0.6mL.drug solution into 10ml. volumetric flask and neutralize with 1ml.of 1N NaOH, the final volume made up to with distilled water to get the concentration of 60µg/ml. The absorbance was measured using above developed methods against blank contain 0.5ml.of 1NHCl and 0.5ml.of 1NNaOH in 10ml.volumetric flask the final volume made up to the mark with distilled water^{12,13}.

2.3.3. BASIC Degradation:

Transfer 1ml.(1000µg/ml. ITM) of above stock solution to10ml.volumetric flask and add 1ml.of 1N NaOH and finally make up the volume with distilled water and kept aside for 24hrs at room temperature.^{7,8,9} From this transfer 0.6ml.drug solution into 10ml.volumetric flask and neutralize with 1ml.of 1N HCl, the final volume made up to the mark with distilled water to get the concentration of 60µg/ml. The absorbance was measured using above developed methods against blank contain 0.5ml.of 1NHCl and 0.5ml.of 1NNaOH in 10ml. volumetric flask the final volume made up to the mark with distilled water. The data obtained is as shown in the table-16

CONCLUSION

Thus, the developed method was found to easy, simple, accurate, precise, selective and economical for the routine estimation of Imatinib Mesylate in bulk and pharmaceutical dosage form. And the developed method has good linearity.^{10, 11}

ACKNOWLEDGEMENT

We like to thanks management, principal, teaching staff of School of pharmacy, Anurag group of institutions, Hyderabad for their continuous co-operation and support.

Table-1 Standard curve of Imatinib Mesylate

S.N	CONCENTRATION	λ_{max} (nm)	ABSORBANCE	STANDARD DEVIATION	RELATIVE STANDARD DEVIATION
1	10 μ g/ml	257	0.122	0.003	2.40%
2	20 μ g/ml	257	0.232	0.0007	0.30%
3	30 μ g/ml	257	0.336	0.001	0.20%
4	40 μ g/ml	257	0.428	0.0007	0.16%
5	50 μ g/ml	257	0.532	0.001	0.18%

Table-2 Intra-day precision of Imatinib Mesylate

S. N O	CONCENTRATION	λ_{max} (nm)	ABSORBANCE			STANDARD DEVIATION	RELATIVE STANDARD DEVIATION
			P1	P2	P3		
1	10 μ g/ml	257	0.249	0.243	0.246	0.003	1.21%
2	20 μ g/ml	257	0.336	0.331	0.325	0.005	1.60%
3	30 μ g/ml	257	0.532	0.526	0.522	0.005	0.95%

Table-2 Intra-day precision of Imatinib Mesylate

S. N O	CONCENTRATION	λ_{max} (nm)	ABSORBANCE			STANDARD DEVIATION	RELATIVE STANDARD DEVIATION
			P1	P2	P3		
1	10 μ g/ml	257	0.249	0.237	0.254	0.008	3.25%

2	20 μ g/ml	257	0.336	0.312	0.317	0.01	3.10%
3	30 μ g/ml	257	0.532	0.504	0.512	0.01	1.93%

Table-4 Specificity of Imatinib Mesylate

S. N O	CONC	λ_{max} (nm)	ABSORBANCE					STANDARD DEVIATION	RELATIVE STANDARD DEVIATION
1	10 μ g/ml	257	0.15	0.15	0.15	0.15	0.15	0.0004	0.02%

Table-5 Accuracy of Imatinib Mesylate

S. N O	CONCENTRATION	λ_{max} (nm)	ABSORBANCE	STANDARD DEVIATION	RELATIVE STANDARD DEVIATION	CONCENTRATION FOUND (μ g/ml)	% CONC
1	10 μ g/ml	257	0.125	0.0007	0.56%	2.5 μ g/ml	125%
2	20 μ g/ml	257	0.211	0	0%	4.22 μ g/ml	105.50%
3	30 μ g/ml	257	0.335	0	0%	6.7 μ g/ml	111.60%
4	40 μ g/ml	257	0.434	0.001	0.20%	8.6 μ g/ml	108.50%
5	50 μ g/ml	257	0.55	0.001	0.18%	11.02 μ g/ml	110.20%

Table-6 Percentage of Recovery of Imatinib Mesylate

S. N O	% TAKEN	CONC	λ_{max} (nm)	ABSORBANCE	SD	RS D	CONC FOUND (μ g/ml)	% CONC
1	80%	10 μ g/ml	257	0.325	0.001	0.30%	6.5 μ g/ml	101.50%
2	100%	20 μ g/ml	257	0.434	0.001	0.20%	8.6 μ g/ml	108.50%

3	120 %	30µ g/ml	257	0.492	0.0 01	0.2 0%	9.8µ g/ml	102. 50%
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Table-7 LOD and LOQ of Imatinib Mesylate

S.NO	Sample	LOQ(µg/ml)	LOD(µg/ml)
1	Imatinib	0.2	0.066

Table-8 Linearity study of Imatinib Mesylate

S. NO	CONCENTRATION	λmax(nm)	ABSORBANCE	STANDARD DEVIATION	RELATIVE STANDARD DEVIATION
1	10µg/ml	257	0.11	0.001	0.96%
2	20µg/ml	257	0.191	0.001	0.50%
3	30µg/ml	257	0.308	0.0007	0.20%
4	40µg/ml	257	0.375	0.001	0.20%
5	50µg/ml	257	0.475	0.001	0.20%

Table-9 Linearity study of Imatinib Mesylate

S.NO	CONCENTRATION	ABSORBANCE		
		257nm	250nm	260nm
1	10µg/ml	0.134	0.132	0.134
2	20µg/ml	0.179	0.177	0.179
3	30µg/ml	0.23	0.227	0.229
4	40µg/ml	0.289	0.285	0.288
5	50µg/ml	0.337	0.332	0.337

Table-10 Acid and Basic degradation study of Imatinib Mesylate

S.No:	Sample	Type of degradation	Absorbance
1	Imatinib	Acidic(HCl)	0.386
		Basic(NaOH)	0.301
2	ImatinibMesylate	Acidic(HCl)	0.184

	Basic(NaOH)	0.128
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Figure-1 Calibration graph of IMATINIB at 257 nm

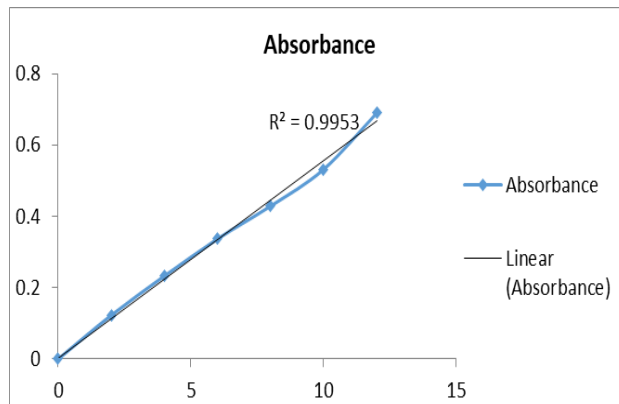


Figure-2 Overlain spectrum of Linearity studies

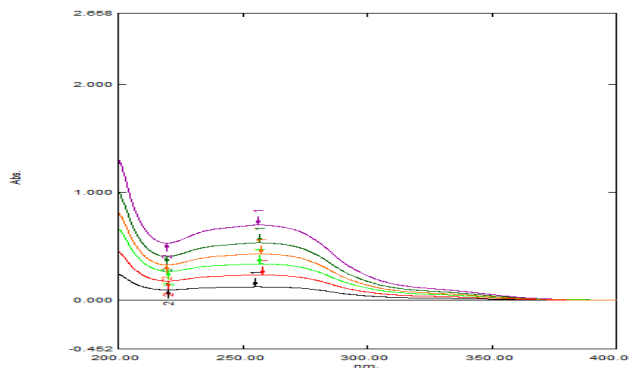


Figure-3 Overlain Spectrum Of 3 Intraday Precision's At 257nm :

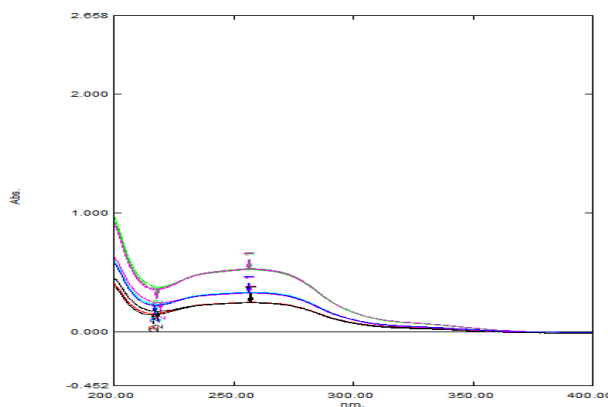


Figure-4 Overlain Spectrum Of 3 Interday Precision's At 257nm

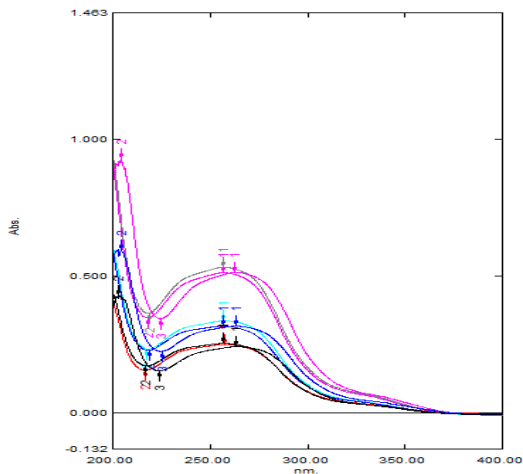


Figure-7 Overlain Spectrum of %Recovery at 257nm

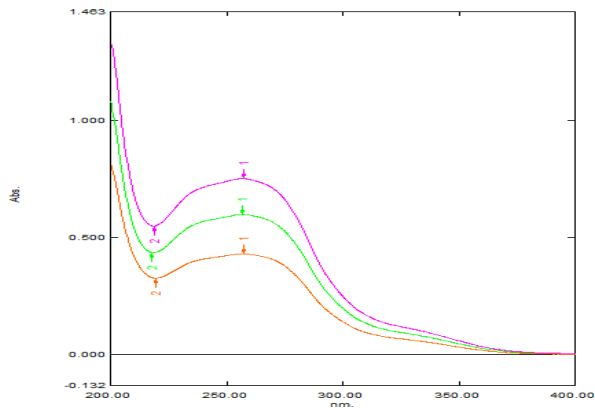


Figure-5 Spectrum of 10µg/ml at 257nm:

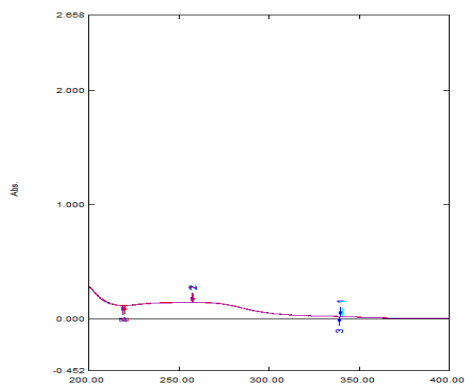


Figure-8 Calibration graph of Imatinibmesylate at 257 nm

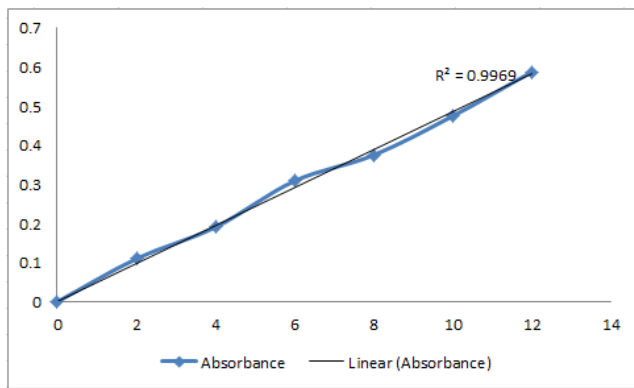


Figure-6 Overlain Spectrum of Accuracy At 257nm:

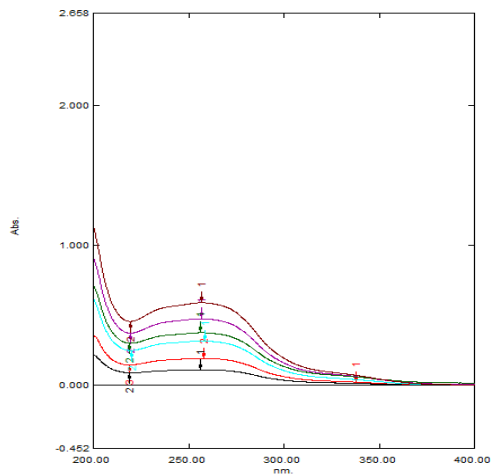


Figure-9 Overlain Spectrum of Linearity studies at 257nm:

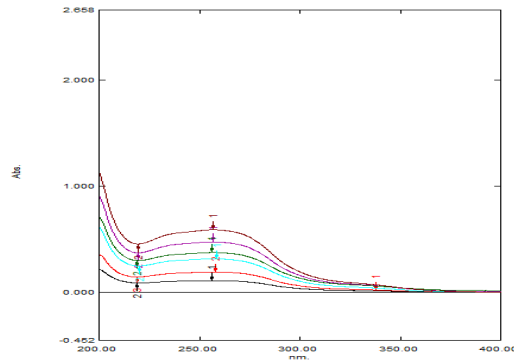


Figure-10 Overlain Spectrum of Photo degradation studies at 257nm:

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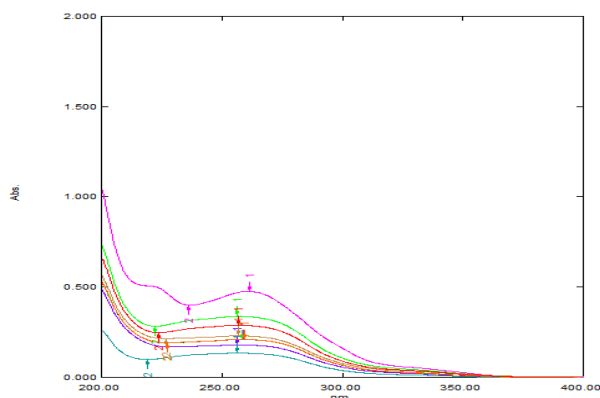


Figure-11 Overlain Spectrum of Acid degradation studies at 257nm:

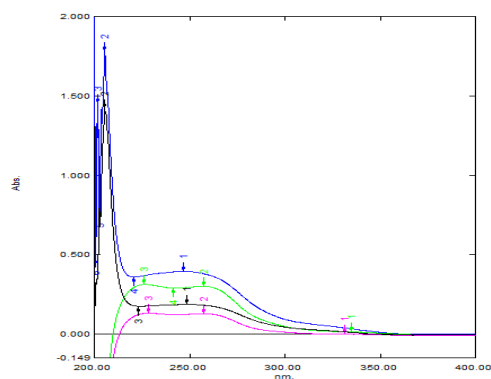
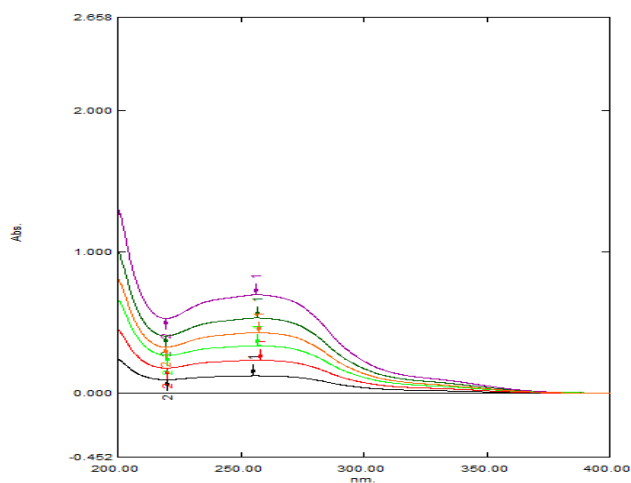


Figure-12 Overlain Spectrum of basic degradation studies at 257nm:



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