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Development and validation of uv-visible methods for Imatinib Mesylate in bulk and tablet dosage form

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Abstract : The present work deals with development of two rapid , precise and accurate spectrophotometric methods for the estimation of Imatinib Mesylate in bulk and solid dosage form . Method A is area under the curve in which wavelength range 237-277nm was selected for estimation of Imatinib Mesylate . Method B is area under the curve in which wavelength range 400-800nm was selected for estimation of Imatinib Mesylate . Linearity was observed in the concentration range 2-10µg/ml for both the methods (r2=0.9992 for method A and method B). The results of analysis have been validated statistically, which confirm the accuracy and reproducibility of the methods. All the methods were found to be simple , precise and accurate and can be employed for routine quality control analysis of ImatinibMesylate in bulk as well as in its solid dosage form.

Keywords : ImatinibMesylate, UV spectrophometry, visible spectroscopy , LOD, LOQ, Robustness.

Introduction:

Imatinib mesylate is an antineoplastic agent that is used for the treatment of chronic myelogenous leukemia. It is chemically 4-[(4-methyl-1-piperazinyl)methyl]-N-[4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl] amino]-phenyl]benzamzide methanesulfonate[1]. It is available in white crystalline powder form.It is Soluble in Water and Methanol.Imatinib is a protein tyrosine kinase inhibitor that inhibitsBcr-Abltyrosine kinase .It inhibits profiliration induces apoptosis in Bcr-Ablpositive cell lines as well as fresh leukemic cells from Philadelphia chromosome positive chronic myeloid leukemia. Imatinib is well absorbed after oral administration with Cmax achieved within 2-4 hours post-dose.Mean absolute bioavailability is 98%[2]. Following oral administration in healthy volunteers, the elimination half-lives of imatinib and its major active metabolite, the N-demethyl derivative (CGP74588), are approximately 18 and 40 hours, respectively. Mean Imatinib AUC increases proportionally with increasing doses ranging from 25 mg-1,000 mg. At clinically relevant concentrations of Imatinib, binding to plasma proteins in invitro experiments is approximately 95%, mostly toalbumin and α1-acid glycoprotein[3]. CYP3A4 is the major enzyme responsible for metabolism of Imatinib.

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Other cytochrome P450 enzymes, such as CYP1A2, CYP2D6, CYP2C9 and CYP2C19, play a minor role in its metabolism[4]. The main circulating active metabolite in humans is the N- demethylatedpiperazine derivative, formed predominantly by CYP3A4. It shows invitropotency similar to the parentImatinib. The plasma AUC for this metabolite is about 15% of the AUC for Imatinib[5]. The plasma protein binding of N-demethylatedmetabolite CGP74588 is similar to that of the parent compound. Human liver microsome studies demonstrated that Gleevec is a potent competitive inhibitor of CYP2C9, CYP2D6and CYP3A4/5 with Ki values of 27, 7.5 and 8μM respectively[6].Imatinib elimination is predominately in the faeces , mostly as metabolites. Based on the recovery of compound(s) after an oral ^{14}C-labeled dose of Imatinib, approximately 81% of the dose was eliminated within 7 days, in faeces (68% of dose) and urine (13% of dose). Unchanged Imatinib accounted for 25% of the dose (5% urine, 20% faeces)[7], the remainder being metabolites. Typically, clearance of Imatinib in a 50-year old patient weighing 50 kg is expected to be 8 L/h, while for a 50-year old patient weighing 100 kg the clearance will increase to 14 L/h[8]. The inter-patient variability of40% in clearance does not warrant initial dose adjustment based on body weight and or age but indicates the need for close monitoring for treatment related toxicity[9].

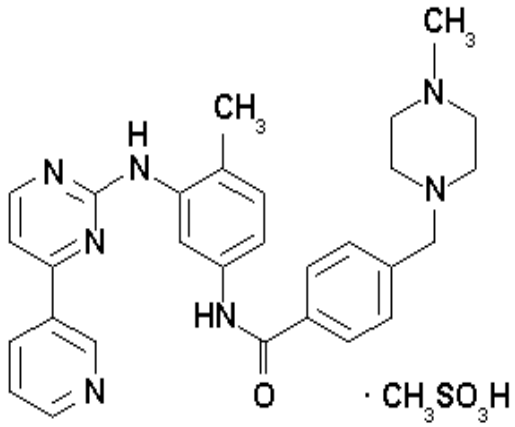

Fig.1 : Chemical structure of Imatinib Mesylate
Molecular formula:$C_{29}H_{31}N_7O$ Molecular weight:589.7gm

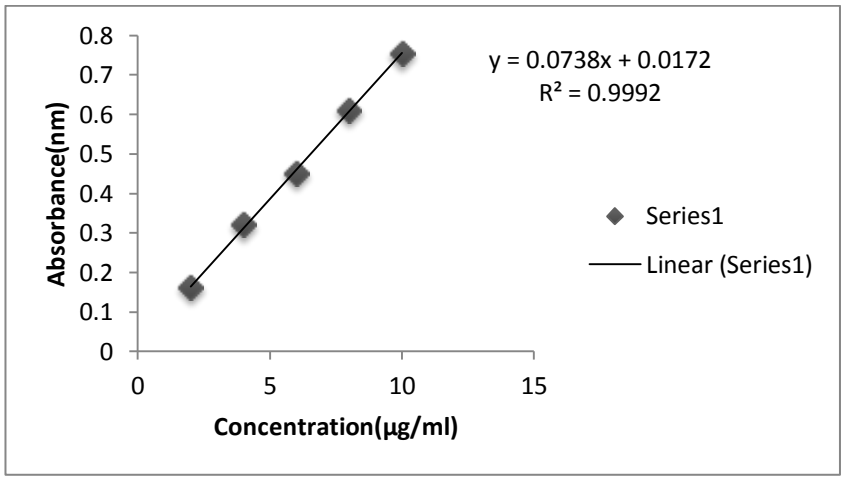

Fig no.2 Calibration Curve of ITM at 266 nm
% Purity=98.4%
Amount found=Standard sample weight×test absorbance/Standard absorbance=0.984gm.

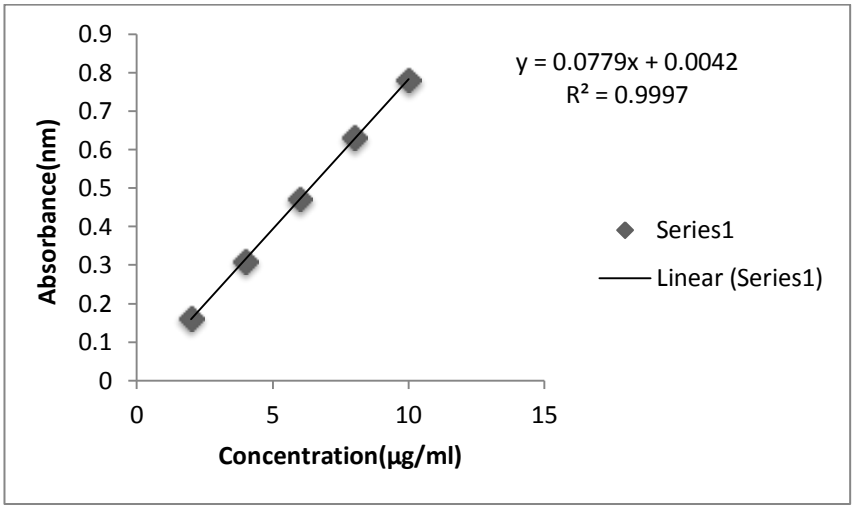

Fig no.3 Calibration Curve of ITM at 492 nm
% Purity=97.1%
Amount found=Standard sample weight×test absorbance/Standard absorbance=0.971mg

Materials and Methods:

Instruments used are UV-Spectrophotometer ELICO double beam SL-164 , UV thermoscientific , Ultra Sonicator: Cleaner-Cyberlab , Electronic balance/ shirmaDZU.

Reagents: For both UV and Visible methods are

- Methanol
- HCL
- Ninhydrin

Solvent: Water .

Determination of appropriate UV wavelength:

A suitable wavelength was required for the determination of Imatinib mesylate. The appropriate wavelength for the determination of Imatinib Mesylate was determined by wavelength scanning over the range from 200-400 nm and 400-800 nm with a Shimadzu UV/Visible 1601 Spectrophotometer.

Standard Imatinib Mesylate solution:

10mg of Imatinib Mesylate pure drug was accurately weighed .It is transferred into a 100ml volumetric flask and add little amount of water and sonicated for 10 minutes . The volume was made upto the mark with water to get the stock solution of 1mg/ml.The solution was diluted with same solvent to get the working standard solution.

Preparation of test solution for UV method:

20 tablets of Imatinib Mesylate were weighed accurately and powdered. A quantity of powder equivalent to 100mg was weighed and transferred into 100 ml volumetric flask and then dissolved in methanol to give 1mg/ml solution .From this 10ml was taken and made upto 100ml .This solution is called working standard. From this solution one unknown concentration was taken and measured at 266nm against standard. The results are given in the table no 1 .

Table no.1 Linearity study of ITM at 266 nm

S.NO	Concentration(µg/ml)	Absorbance(nm)
1	2	0.162
2	4	0.321
3	6	0.451
4	8	0.611
5	10	0.755

Preparation of test solution for visible method:

20 tablets of Imatinib Mesylate were weighed accurately and powdered. A quantity of powder equivalent to 100mg was weighed and transferred into 100 ml volumetric flask and then dissolved in water to give 1mg/ml solution .From this 10ml was taken and madeupto 100ml .This solution is called working standard .From this solution one unknown concentration was taken and measured at 492nm using Ninhydrin as reagent . The results are given in the table no 2 .

Table no.2Linearity study of ITM at 492 nm

S.NO	Concentration(µg/ml)	Absorbance(nm)
1	2	0.163
2	4	0.31
3	6	0.472
4	8	0.632
5	10	0.781

Validation of the Method :

The method was validated for selectivity, linearity, precision, accuracy, recovery and stability according to the principles of the Food and Drug Administration (FDA) industry guidance. Validation of analytical procedures is a vital aspect not just for regulatory purposes, but also for their efficient and reliable long - term application. The ICH guidelines achieved a great deal in harmonizing the definitions of required validation parameters, their calculation and interpretation. It is the responsibility of the analyst to identify parameters which are relevant to the performance of given analytical procedure as well as to design proper validation protocols including acceptance criteria and to perform an appropriate evaluation. The International Conference on the Harmonization of the Technical Requirements for Registration of Pharmaceuticals for Human Use has harmonized the requirements in two guidelines. The first one summarizes and defines the validation characteristics needed for various types of test procedures, the second one extends the previous test to include the experimental data required and some statistical interpretation. These guidelines serve as a basis worldwide both for regulatory authorities and industry and bring the importance of a proper validation to the attention of all those involved in the process of submission.

Table no 3 various parameters and their results:

S.No	Parameters	Experimental Values		Limits As Per Ich Guidelines
		UV-Method	Visible Method	
1.	λmax (nm)	266 nm	492 nm	-
2.	Regression Equation (y=mx+c)	Y=0.073x+0.017	Y=0.77x+0.004	-
3.	Linearity	0.999	0.999	0.999
4.	Accuracy	1.43	1.45	2
5.	Precision	1.42	1.27	2
6.	LOD	1.031mcg/ml	2.09mcg/ml	-
7.	LOQ	1.088mcg/ml	6.35mcg/ml	-
8.	Robustness	1.58%	1.62%	2
9.	Slope	0.073	0.077	-

The linearity of the proposed method was constructed for Imatinib Mesylate reference standard solution by plotting concentration of the compound versus the absorbance. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method. The parameters LOD and LOQ were determined on the basis of response and slope of the regression equation. The accuracy and precision of the method was evaluated within the linear range. Five independent analysis were performed at each concentrations level within one day as well as for five consecutive days. The accuracy was ascertained by recovery studies using the standard addition method. The amount of Imatinib Mesylate was determined from the regression equation. The above described parameters and their results are mentioned in table no 3 .

Conclusion:

The developed method was found to be simple, sensitive, accurate, precise, reproducibleand can be used for routine quality control analysis of Imatinib Mesylatein bulk and pharmaceutical formulation .

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