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Research Article

# DEVELOPMENT OF UV-SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF IMATINIB MESYLATE (ITM) IN BULK AND FORMULATION

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**ABSTRACT**

A simple and precise spectroscopic method for determination of Imatinib Mesylate in its bulk and tablet dosage forms has been developed and validated. This method based upon measurement of light absorption in UV region. The UV spectra of Imatinib Mesylate showed that maximum absorbance of light was observed at 281 nm and linearity was observed in the concentration range of 2-28ug/ml with correlation coefficient 0.999. The proposed method was validated as per ICH Q2 (R1) guidelines for linearity, accuracy, precision and recovery. The limit of detection (LOD) and limit of quantitation (LOQ) were found to be 0.040468 (µg/ml) and 0.122263 (µg/ml) respectively by simple UV Spectroscopy.

**Keywords:** Imatinib Mesylate, UV Spectroscopy, Method validation.

## 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 symptoms, prevent the spread of cancer cells, and aid other treatments. 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.

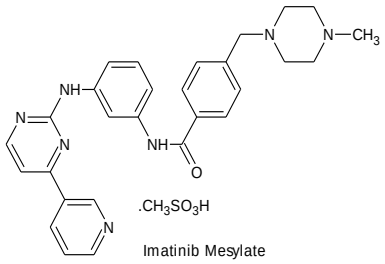


**Figure 1: Structure of Imatinib Mesylate**

The Chemical name of Imatinib Mesylate is 4-4[(4-methyl-1-piperazinyl) 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 [1].

Literature Survey revealed that the drug has been estimated by Liquid chromatography[2-9] and Spectrophotometry[10] 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.

## MATERIALS AND METHODS

### Instrument

A UV – Visible double beam spectrophotometer (JASCO), model no. V-530 with 10 mm matched quartz cells was used for experiment. All weights were taken on Analytical balance.

### Reagents and Standards

Imatinib Mesylate reference standard was obtained from Sun Pharmaceutical Industries Ltd , Mumbai, India. The tablets of brand Mitinab( Glenmark) of 100mg were obtained from local pharmacy. The double distilled water was used as solvent for the experiment.

### Experimental

### Preparation of standard stock solutions of ITM

Accurately weighed 100 mg of Imatinib Mesylate transferred to 100 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 100 µg/ml.

### Determination of λ max

From the stock solutions, 1.0 ml of Imatinib Mesylate was transferred to 10 ml volumetric flask and the volume was adjusted to the mark with same solvent to obtain Strength 10µg/ml. The solution was scanned in the UV range 200-400 nm. (Figure 2).

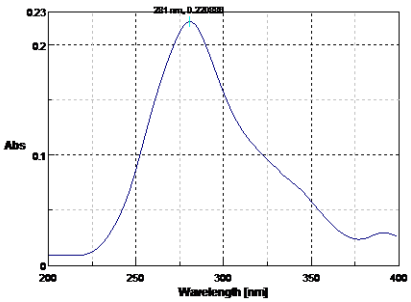


**Figure 2: UV Spectrum of Imatinib Mesylate in Distilled Water.**

### Study of Linearity

Appropriate known volumes of aliquots from standard stock Imatinib Mesylate solution 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 2-28 µg/ml (Fig.2). Absorbances of these solutions were recorded at 281.0 nm (shown in **Table 1**) and Calibration curve was plotted, absorbance *vs.* concentration (shown in **Fig. 3)**.

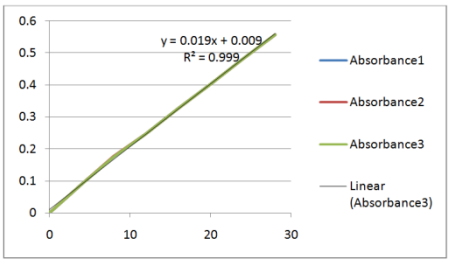


**Figure 3: Calibration Curve of ITM at 281.0.0 nm**

**Limit of Detection and Limit of Quantification**

The LOD and LOQ of Imatinib Mesylate were determined by using standard deviation of response and slope approach as defined by ICH guidelines. The LOD and LOQ were found to be 0.040468 and 0.122263 respectively (Shown in **Table 2**).

**Determination of E (1%, 1cm) of ITM**

Aliquot portions of Imatinib Mesylate from stock standard solution were transferred to five different 10 ml volumetric flasks; diluted with same solvent to obtain concentration of 20 µg/ml. The absorbance of each solution was measured at 281.0 nm.

A(1%,1cm) valuesof drugs were calculated using following formula –

A (1%, 1cm) = Absorbance/Concentration (g/100ml)

The data obtained for absorptivity values of drugs were as shown in Table 3.

**Analysis of bulk sample by proposed method**

In order to see the feasibility of proposed method for estimation of ITM in marketed pharmaceutical formulations, the method was first tried for estimation of drugs in standard laboratory mixture.

Accurately weighed quantities 10 mg ITM was taken in 100 ml volumetric flask and dissolved in distilled Water by vigorous shaking. The volume was made up to the mark by same solvent. The aliquot portions of this stock solution were further diluted with solvent to get final concentration of about 10 µg/ml ITM and the absorbance was measured at 281.0 nm against solvent as blank and data obtained were as shown in **Table 4.**

**Application of Proposed Method for Analysis of tablet formulation**

For analysis of commercial formulation, twenty tablets were weighed, average weight determined and crushed into fine powder. An accurately weighed quantity of powder equivalent to 10 mg of ITM was transferred into 100 ml volumetric flask containing 30 ml distilled Water, shaken manually for 10 min., volume was adjusted to mark with same solvent and filtered through Whatmann filter paper no. 45. An appropriate aliquot was transferred to 10 ml volumetric flask, volume was adjusted to the mark and absorbance was recorded at 281.0 nm. The data obtained were as shown in **Table 5.**

**Validation of proposed method**

The Proposed method was validated as per the ICH Q2 (R1) guidelines for linearity, range, accuracy, precision, ruggedness.

**Accuracy**

To assess the accuracy of the proposed method, recovery studies were carried out three different levels i.e. 50%, 100% and 150%. To the pre- analyzed sample solution a known amount standard drug solution was added at three different levels, absorbance was recorded. The % recovery was then calculated by using formula

**% Recovery = A – B/ C**

Where A = Total amount of drug estimated

B = Amount of drug found on preanalysed basis

C = Amount of Pure drug added

The data obtained were as shown in **Table 6.**

**Precision**

Precision of the method was studied as repeatability, intra-day and inter-day precision. Repeatability was determined by analyzing ITM (10 µg/ml) for six times and the results were as reported in **Table 7**. Intra-day precision was determined by analyzing the 8, 12, 16 µg/ml of LCD for three times in the same day. Inter-day precision was determined by analyzing the same concentration of the solutions daily for three days. The data obtained were as reported in **Table 8.**

**Ruggedness**

Ruggedness of the proposed method was determined by analysis of aliquots from homogenous slot by two analyst using same operational and environmental conditions. and the data obtained were as reported in **Table 9.**

**RESULTS AND DISCUSSION**

The development of a simple, rapid, sensitive and precise spectrophotometric method for the routine quantitative determination of samples will definitely reduce unnecessary tedious sample preparations and the cost of materials and labour. Imatinib Mesylate is an UV-absorbing molecule with specific chromophores in the structure that absorb at a particular wavelength and this fact was successfully employed for their quantitative determinations using the UV spectroscopic method. The spectral analysis showed the λ max of Imatinib mesylate to be 281 nm. The calibration curve was obtained for a series of concentration in the range of 2-28µg/ml. It was found to be linear and hence suitable for the estimation of the drug. The slope, intercept, correlation coefficient and optical characteristics were summarized in **Table 10.** Regression analysis of Beer's law plot revealed a good correlation. The effects of various excipients generally present in the tablet dosage form of Imatinib mesylate were investigated. The results indicated that they did not interfere in the assay. The proposed method was validated as per the ICH guidelines. The precision was measured in terms of repeatability, which was determined by sufficient number of aliquots of a homogeneous sample. The % RSD was found and lie within the range of ± 2.0. This showed that the precision of the method was satisfactory. The results showed that the recovery of Imatinib mesylate by the proposed method was satisfactory. Ruggedness and Robustness were also determined. Thus the developed UV Spectroscopic method for analysis of Imatinib Mesylate in pharmaceutical dosage form and use of spectroscopy enables analysis of several samples at the same time.

**Table: 1. Linearity study of ITM**

| S. No. | Concentration of ITM [µg/ml] | Absorbance Mean ± S.D. [n = 5] | %R.S.D. |
|---|---|---|---|
| 1 | 0 | 0.0000 | - |
| 2 | 4 | 0.08978 ± 0.000217 | 0.2417 |
| 3 | 8 | 0.17616 ± 0.000241 | 0.111 |
| 4 | 12 | 0.24828± 0.000259 | 0.1043 |
| 5 | 16 | 0.32676 ± 0.000207 | 0.0597 |
| 6 | 20 | 0.40376± 0.000241 | 0.0597 |

| | | | |
|---|---|---|---|
| 7 | 24 | 0.48136± 0.000207 | 0.0430 |
| 8 | 28 | 0.55688± 0.000259 | 0.1032± 0.0732 |

**Table 2: Lower Limit of Detection and Limit of Quantification**

| S. No. | Sample | LOD[µg/ml] | LOQ[µg/ml] |
|---|---|---|---|
| 1 | Imatinib Mesylate | 0.040468 | 0.122263 |

**Table No: 3. E (1%, 1cm) of ITM at 281.0 nm**

| Sr.No. | Concentration | Absorbance | E(1%,1cm) | Molar absorptivity |
|---|---|---|---|---|
| **1** | 20 | 0.4038 | 0.02019 | 11.9062 |
| **2** | 20 | 0.4029 | 0.020145 | 11.8791 |
| **3** | 20 | 0.4043 | 0.020215 | 11.92099 |
| **4** | 20 | 0.4035 | 0.020175 | 11.897 |
| **5** | 20 | 0.4045 | 0.020225 | 11.9269 |
| **6** | 20 | 0.409 | 0.02045 | 12.0596 |
| | **Mean ± S.D.** | | 0.0202± .00011 | 11.93163 ± 0.064988 |

**Table 4: Analysis of ITM in bulk sample**

| S. No. | Amount Taken [µg/ml] | Amount found* [µg/ml] | Amount found ± S.D. [%, n = 5] | % R.S.D. |
|---|---|---|---|---|
| 1 | 10 | 9.98 | 99.81 ± 0.26 | 0.26 |

**Table 5: Application of proposed method for analysis of tablet Dosage Form.**

| Sample | Label claimed | % Label claim ± SD | %RSD |
|---|---|---|---|
| **1** | 100 mg | 100.02 ± 0.47 | 0.47 |

**Table 6: Recovery study**

| Drug | Initial Amount [µg/ml] | Amount added [µg/ml] | Amount recovered [µg/ml, n = 3] | % Recovered | % R.S.D. |
|---|---|---|---|---|---|
| | 10 | 8 | 7.96 | 99.55 | 0.18 |
| **IMB** | 10 | 10 | 10.00 | 100.02 | 0.03 |
| | 10 | 12 | 12.01 | 100.05 | 0.13 |

**Table 7: Results of Repeatability Studies**

| Drug | Amount taken [µg/ml, n = 6] | Amount found(%) | %RSD |
|---|---|---|---|
| **IMB** | 10 | 99.92 ± 0.34 | 0.34 |

**Table 8: Intraday and Interday precision**

| Drug | Concentration [µg/ml] | Intra-day [n = 3] | % R.S.D | Inter-day [n = 3] | % R.S.D |
|---|---|---|---|---|---|
| | 8 | 99.79 | 0.31 | 99.60 | 0.41 |
| | 12 | 99.86 | 0.47 | 99.61 | 0.80 |
| **IMB** | 16 | 99.33 | 1.45 | 99.33 | 1.35 |

**Table 9: Ruggedness Data**

| Drug | Analyst II Amount found % ± S.D. [n = 3] | %R.S.D. | Analyst IIAmount found % ± S.D. [n = 3] | %R.S.D. |
|---|---|---|---|---|
| **ITM** | 100.04 ± 0.52 | 0.52 | 100.02 ± 0.23 | 0.23 |

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**REFERENCES**

1. Martindale: The complete drug reference. 36th edition, Pharmaceutical press, Lambeth High Street, London. 2009; 773-774.
2. Widmer et al. Determination of imatinib in human plasma by solid-phase extraction–liquid Chromatography–ultraviolet absorbance detection. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 2004; 803(2): 285-92.
3. Thirumurthy et al. Development and validation of a simple liquid chromatographic method with ultraviolet detection for the determination of Imatinib in biological samples. J. of Chromatography B. 2004; 804, 2(25):431-434.
4. Vivekanand et al. A validated LC method for imatinib mesylate Journal of pharmaceutical andbiomedical analysis, 200; 333(5): 879-889.
5. Ivanovic, D., Medenica, M., Jancic B. and Malenovic, A., Reversed-phase liquid chromatography analysis of imatinib mesylate and impurity product in Glive capsules. J. chromatography B. 2004; 800 (1-2): 253-258.
6. Roos, LO., Jos, HB., Jan HM.,Olaf van, T. Determination of imatinib mesylate and its main metabolite (CGP74588) in human plasma and murine specimens by ion-pairing reversed phase high-performance liquid chromatography. Biomedical chromatography 2007; 21(7):747-754.
7. Solassol, F., Bressolle, L., Philibert, V., Charasson, C., Astre, F.Liquid Chromatography- Electrospray Mass Spectrometry Determination of Imatinib and Its Main Metabolite, *N*-Desmethyl- Imatinib in Human Plasma. J. liquid chromatography and related technologies. 2006;29 (20): 2957-2974.
8. Rosasco et al. Validation of an HPLC Method for the Determination of Imatinib Mesylate in Pharmaceutical Dosage

forms. J. liquid chromatography and related technologies, 2005;28 (20): 3283-3292.

9. Satyanarayana et al. Development and Validation of New Reversed Phase High Performance Liquid Chromatography Method for the Estimation of Imatinib in Bulk and Pharmaceutical Dosage Forms. International Journal of Research in Pharmaceutical and Biomedical Sciences, 2010; Vol. 1 (1): 6-9.
10. Bende, G., Kollipara, S., Sekar, V. and Saha, R. UV-spectrophotometric determination of Imatinib mesylate and its application in solubility studies.Die pharmazie, 2008; 63(9): 641-645.