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# Development and validation of analytical methods for estimation of imatinib mesylate in bulk and solid dosage forms by UV spectroscopy

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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 first order derivative spectroscopy where derivative amplitudes were calculated by considering minima and maxima of the curve. Method B is area under the curve in which wavelength range 237-277nm was selected for estimation of Imatinib Mesylate. Linearity was observed in the concentration range 5-30µg/ml for both the methods ($r^2$=0.9992 for method A and $r^2$=0.9996 for 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 Imatinib Mesylate in bulk as well as in its solid dosage form.*

**Keywords:** Imatinib Mesylate, UV-spectrophometry, first order derivative spectroscopy, area under the curve.

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## INTRODUCTION

Imatinib Mesylate is described chemically as the 4-[(4-methyl-1-piperazinenyl)methyl]-N-[4-methyl-3[4-(3-pyridinyl)-2-pyrimidinyl]amino]phenyl]benzamide methanesulphonate. Imatinib Mesylate is official in IP[(1)]. 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.[(2)] Literature survey reveals that some methods have been developed for their determination by HPLC[(3-8)] or spectrophotometry [(9)]either alone or in combination by first order derivative spectroscopy and area under curve method. The purpose of this work is to develop a simple, accurate, precise, reproducible and economical UV spectrophotometric method for estimation of Imatinib Mesylate by first order derivative spectroscopy and area under curve method.

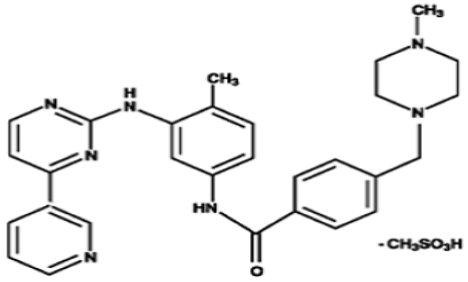


**Figure 1: Chemical Structure of Imatinib Mesylate**

## MATERIALS AND METHODS

### Apparatus and Instrumentation

Jasco V630 with Spectra manager software, was employed for this work. Single pan electronic balance (Mettler Toledo ME204) was used for weighing purpose. Sonication of the solutions was carried out using an Ultrasonicator (Spectra lab UCB 40, India). Calibrated volumetric glasswares (Borosil) were used in this study.

### Materials

Active pharmaceutical ingredient (API) of Imatinib Mesylate was procured from Neon Laboratories, Mumbai. Commercially available tablets manufactured by Cipla Imatib® 100 containing 100mg of Imatinib Mesylate was procured from local pharmacy.

## METHOD DEVELOPMENT

### Preparation of standard solution

The standard stock solution of Imatinib Mesylate was prepared by transferring, accurately weighed 100mg of Imatinb Mesylate to100ml of volumetric flask. The drug was dissolved with sonication in 50ml of distilled water and volume was made up to the mark by using distilled water. The standard stock solution (1000µg/ml) was further diluted with distilled water to get the concentration of 10µg/ml.

### Selection of Analytical wavelength

The standard solution of 10µg/ml was scanned between 400nm to 200nm in UV spectrophotometer against distilled water as blank after baseline correction. Wavelength range was selected around wavelength maxima (257nm). Different working standards were prepared between 5-30µg/ml (Figure2).

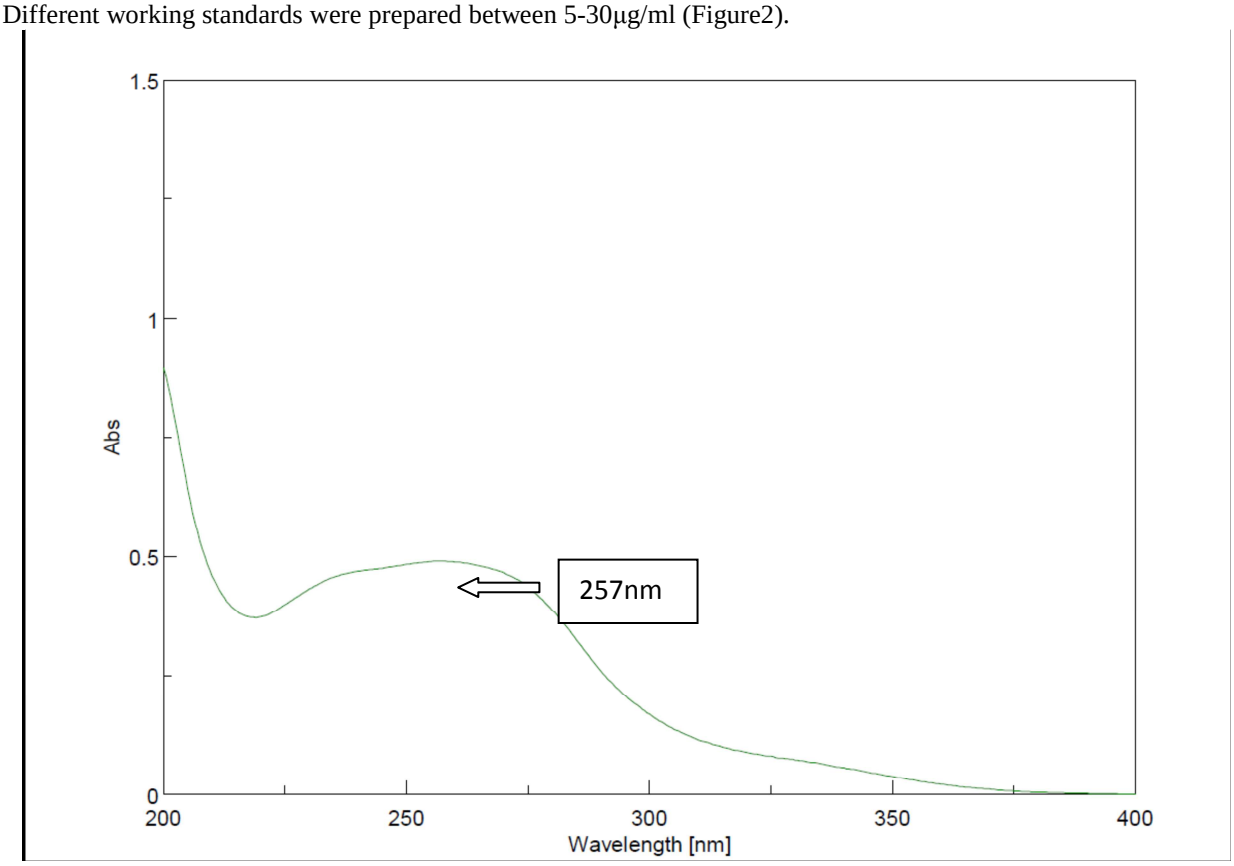


**Figure 2: UV Spectra of 10µg/ml Imatinib Mesylate solution**

## METHOD A -FIRST ORDER DERIVATIVE SPECTROSCOPY

### 1.Calibration curve for Imatinib Mesylate

From the standard stock solution, appropriate dilutions were made to obtain concentration in range of 5-30µg/ml. Absorbance measured at first order derivative spectrum is characterized by a maximum and minimum and a cross over point at λmax of absorption band. Further the absorbance difference at n=1 (dA/dλ) was calculated by the inbuilt software of the instrument which was directly proportional to the concentration of the standard solution. The calibration curve of dA/dλ versus concentration was plotted. (Figure 3)

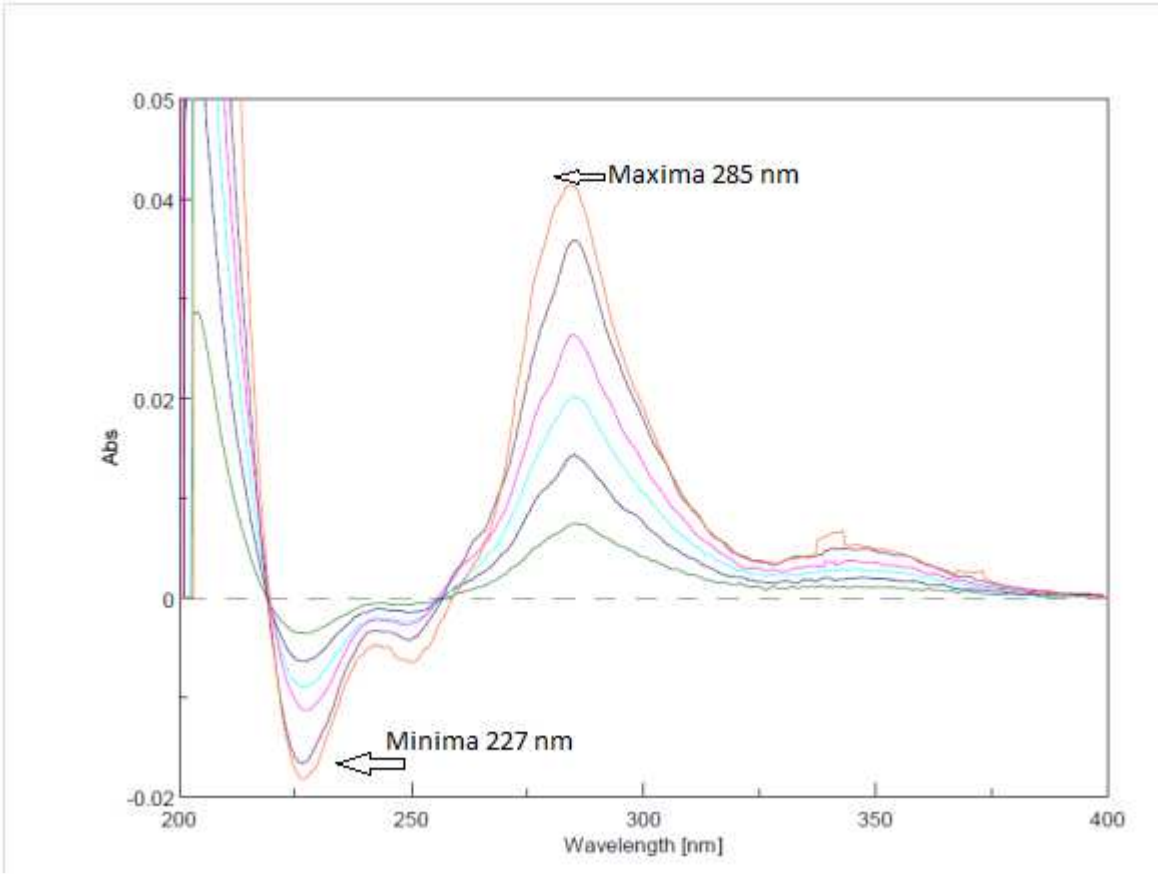


**Figure 3: Overlay spectra of Imatinib Mesylate for first order derivative method**

**2.Analysis of marketed formulation**

Twenty tablets were weighed and average weight was calculated. These tablets were crushed and powdered in a glass mortar. The tablet powder equivalent to 10 mg of Imatinib Mesylate was accurately weighed, transferred to 100 ml of volumetric flask and diluted up to mark with water. The solution was filtered with Whatmann filter paper No. 41 and the first 5 ml of filtrate was discarded. This solution was further diluted to obtain 10µg/ml solution with same solvent and subjected for UV analysis. In first order derivative spectroscopy, derivative amplitude was determined directly from calibration plot by measuring difference between absorbance maxima at 285 nm and minima at 227 nm (i.e. $dA/d\lambda$) (table no. 1).

**METHOD B -AREA UNDER CURVE**

**1) Calibration curve for Imatinib Mesylate**

For the selection of analytical wavelength standard solutions of Imatinib Mesylate were prepared and series of dilutions of standard solutions of Imatinib Mesylate were prepared by using distilled water and were scanned from 200 to 400nm. From the spectra of drug obtained after scanning of standard solution of Imatinib Mesylate area under the curve in the range of 237 nm-277 nm was selected for the analysis, the calibration curve was plotted with concentration versus area under the curve and regression equation was calculated. (Figure.4)

**2. Analysis of marketed formulation**

Tablets were procured from local market and average weight was determined. For the analysis of drugs, quantity of powder equivalent to 100mg of Imatinib Mesylate was transferred to 100ml volumetric flasks and dissolved in sufficient quantity of distilled water. It was sonicated for 45 minutes and then filtered through whatmann filter paper No 41. The filtrate was appropriately diluted with distilled water to give standard stock solution of 1000 µg/ml. Further dilutions were made using distilled water to get required concentration. Area in the range of 237 nm-277 nm was measured and content was calculated (table no. 1)

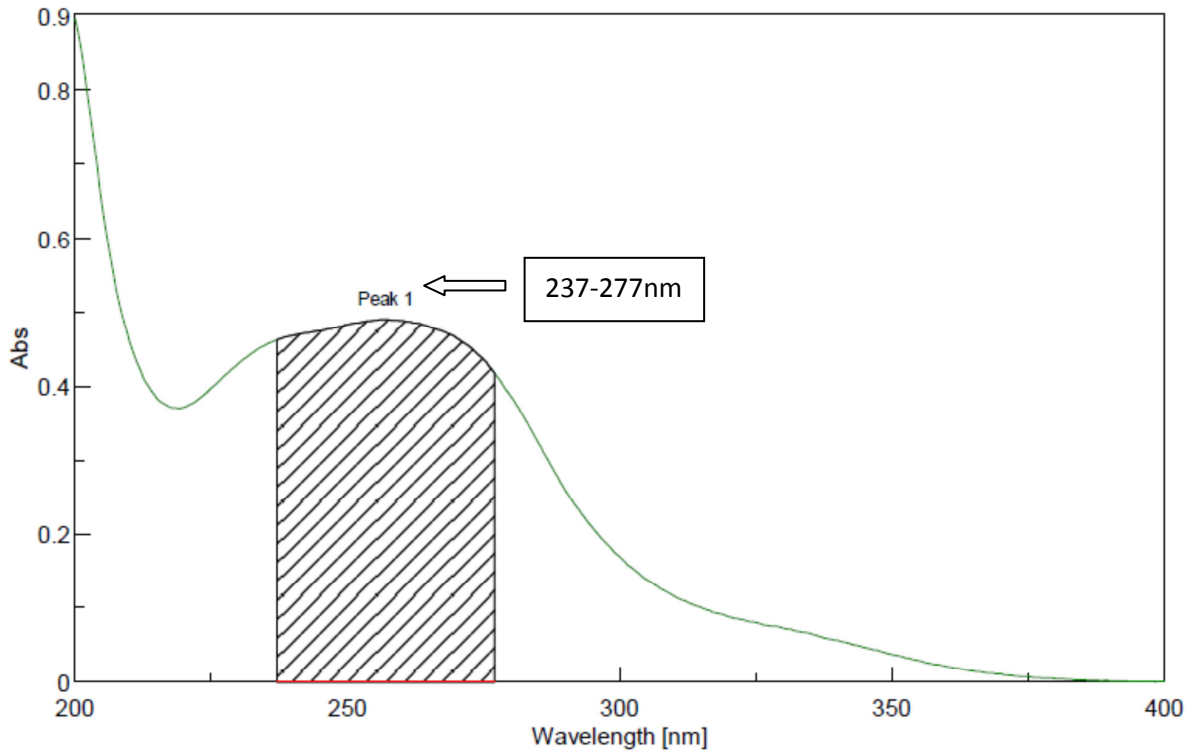


**Figure 4: Area Under Curve graph of 10µg/ml Imatinib Mesylate solution**

**Table No. 1: Result of marketed formulation analysis**

| Method | Label claim(mg) | %Label Claim* (Mean±SD) | %RSD* |
|---|---|---|---|
| First order derivative | 100 | 100.22±0.11 | 0.73 |
| Area under curve | 100 | 101.31± 0.008 | 0.05 |

**Accuracy studies:**

Accuracy studies were carried out by standard addition method. Pure Imatinib Mesylate was added at different levels i.e. 80%, 100% and 120% to pre analyzed sample solution. 100mg of Imatinib Mesylate was accurately weighed and transferred into a 100ml volumetric flask. This was dissolved in distilled water and volume was made up to 100ml to give standard stock solution of 1000µg/ml. To 1ml tablet stock solution in three different volumetric flask, aliquots of 0.8 ml, 1ml and 1.2ml of standard stock solution were added, volume was made up to 100ml with distilled water to give concentration of 18µg/ml (80%), 20µg/ml (100%) and 22µg/ml (120%).These were analyzed by first order derivative and area under the curve method. Procedure was repeated 3 times for 80%, 100% and 120% of level for recovery studies. The results are discussed in (table no. 2).

**Table No.2: Results of Accuracy studies**

| Level Recovery | Conc. Of Drug (µg/ml) | | Total Conc. (µg/ml) | Method A * | | | Method B* | | |
|---|---|---|---|---|---|---|---|---|---|
| | Drug taken | Std drug added | | % Recovery | SD | % RSD | % recovery | SD | % RSD |
| 80 | 10 | 8 | 18 | 99.29 | 0.031 | 0.1729 | 98.91 | 0.0153 | 0.085 |
| 100 | 10 | 10 | 20 | 99.42 | 0.087 | 0.4391 | 99.77 | 0.0152 | 0.076 |
| 120 | 10 | 12 | 22 | 99.94 | 0.073 | 0.3328 | 99.47 | 0.125 | 0.549 |

**Validation[10]**

The developed method was validated as per ICH (ICH Q2 R1) guidelines.

**Linearity:**

The linearity of measurement was evaluated by analyzing different concentrations of the standard solution of Imatinib Mesylate. The Beer's law was obeyed in the concentration range 5-30 µg/ml. The correlation coefficient was found to be 0.9992 and 0.9996 for method A and method B respectively (table no. 3 and 4).

**Table 3. Linearity and range of Imatinib Mesylate for Method A**

| Concentration | dA/d λ |
|---|---|
| 5 | 0.00378 |
| 10 | 0.00709 |
| 15 | 0.01113 |
| 20 | 0.01506 |
| 25 | 0.01920 |
| 30 | 0.02304 |

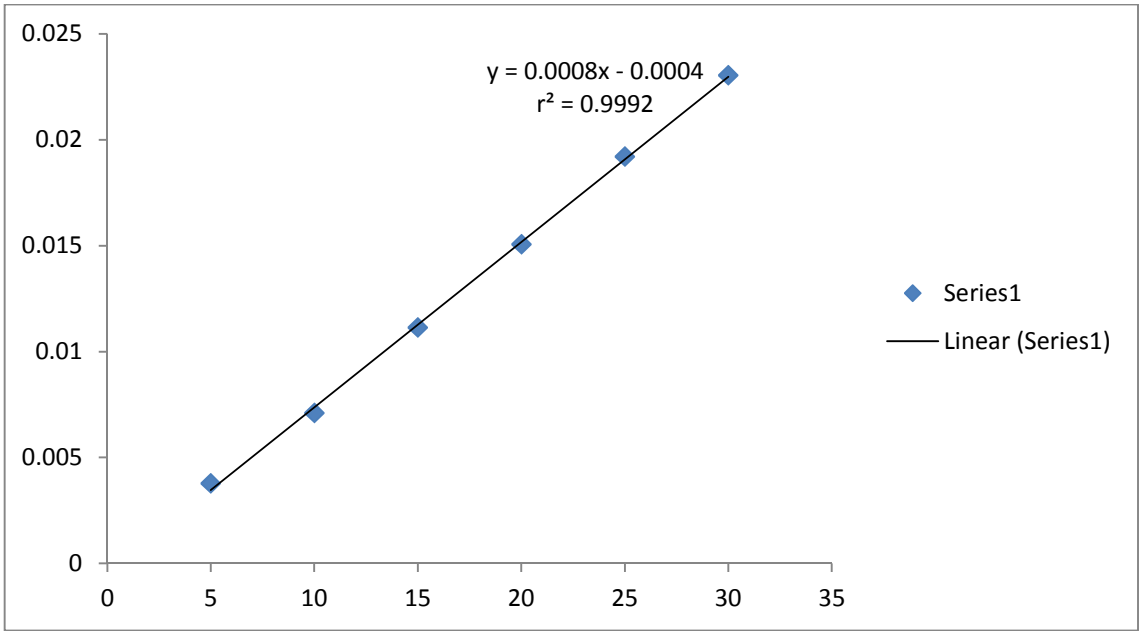


**Figure 5: Calibration curve for Frist order derivative of Imatinib Mesylate**

**Table 4. Linearity and range of Imatinib Mesylate for Method B**

| Concentration | Area |
|---|---|
| 5 | 10.7422 |
| 10 | 18.8933 |
| 15 | 28.2487 |
| 20 | 36.3656 |
| 25 | 45.8526 |
| 30 | 54.2113 |

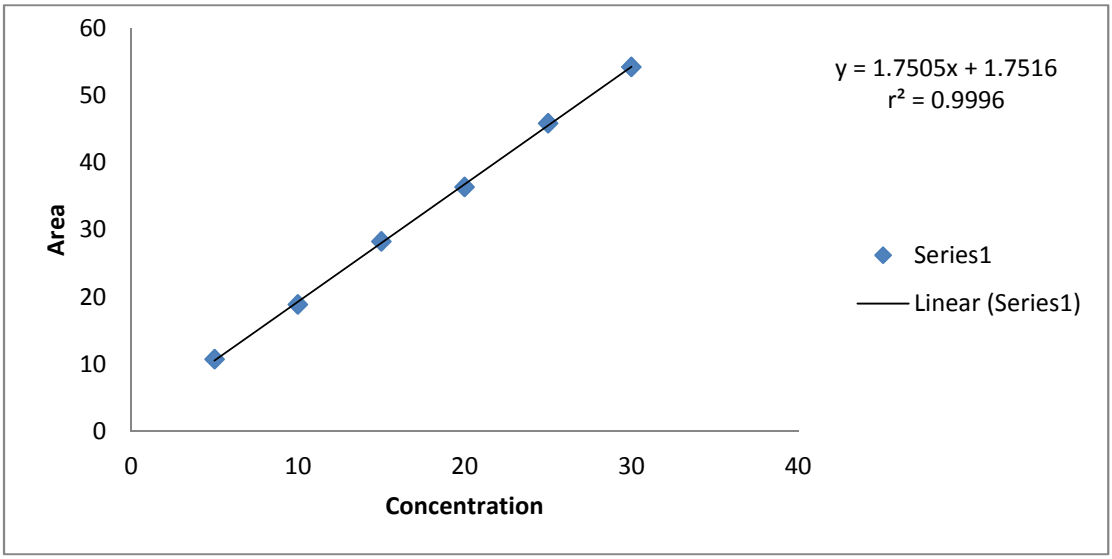


**Figure 6: Calibration curve for Area under curve method for Imatinib Mesylate**

**Limit of Detection (LOD) and Limit of Quantitation (LOQ)**
Known concentrations (5-30µg/ml) were prepared. Calibration curves were plotted for each set. LOD and LOQ were calculated using the regression equation and following formulae as:

LOD = 3.3 * SD/S
LOQ = 10 * SD/S
Where,
SD is standard deviation of y-intercept of the calibration curves
S is slope of calibration curves.

**Table 5. LOD and LOQ of Imatinib Mesylate**

| Paramters | Method A | Method B |
|---|---|---|
| LOD | 2.39 | 1.65 |
| LOQ | 5.25 | 5.02 |

**Precision:**
100 mg of Imatinib Mesylate was weighed accurately and dissolved in 100ml of distilled water to give concentration of 1000 µg/ml. From the standard stock solution appropriate quantity of solution was taken further dilutions were made with distilled water to give 10µg/ml. This procedure was repeated six times for each method individually (table no.6 & 7).

**Table 6. Intraday and Interday precision study of Imatinib Mesylate Method A**

| Parameter | %Mean | ±S.D. | %R.S.D. |
|---|---|---|---|
| Interday | 100.86 | 0.0094 | 1.2006 |
| Intraday | 100.35 | 0.0056 | 0.7333 |

**Average of six determinations*

**Table 7. Intraday and Interday precision study of Imatinib Mesylate Method B**

| Parameter | %Mean | ±S.D. | %R.S.D. |
|---|---|---|---|
| Interday | 98.15 | 0.0895 | 0.1229 |
| Intraday | 98.45 | 0.0214 | 0.1029 |

**Average of six determinations*

## RESULTS AND DISCUSSION

The present work describes the first order derivative method and area under curve method for the estimation of Imatinib Mesylate in bulk and dosage form. For both the methods linearity was observed in the concentration range of 5-30 µg/ml for Imatinib Mesylate and correlation coefficient was found to be greater than 0.999. Accuracy of proposed method was assessed by recovery studies. The results of marketed formulation analysis are found in range of 99-101%. Percent recovery for Imatinib Mesylate by both the methods was found in the range of 98.20% to 102 % (table no. 2). The % RSD for six determinations of sample by both the methods was found to be less than 2.0 indicating the good precision of both the methods. Hence, it can be concluded that the developed spectrophotometric methods are accurate, precise and can be employed successfully for the estimation of Imatinib Mesylate in bulk and formulation.

## CONCLUSION

The two spectrophotometric methods were developed for Imatinib Mesylate and validated as per ICH guidelines. The standard deviation and % RSD calculated for the proposed methods are within limits, indicating high degree of precision of the methods. The results of the recovery studies performed indicate the methods to be accurate. Hence, it can be concluded that the developed spectrophotometric methods are accurate, precise and can be employed successfully for the estimation of Imatinib Mesylate in bulk and dosage form.

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