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## **Estimation of Imatinib by Modern Analytical Techniques: A Review**

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### **ABSTRACT**

The introduction of imatinib, an oral tyrosine kinase inhibitor, as first-line standard therapy in patients with unresectable, metastatic, or recurrent gastro-intestinal stromal tumor (GIST), strongly improved their treatment outcomes. A fast and cheap method was developed and validated using high-performance liquid chromatography-mass spectrometry for quantification of imatinib in human serum and tamsulosin as the internal standard. Remarkable advantages of the method includes use of serum instead of plasma, less time spent on processing and analysis, simpler procedures, and requiring reduced amounts of biological material, solvents, and reagents. LC-MS, HPLC and UV Spectrophotometry validated methods have proved to be linear, accurate, precise, and robust, it is suitable for pharmacokinetic assays, such as bioavailability and bioequivalence, and is being successfully applied in routine therapeutic drug monitoring in the hospital service

**Keywords:** Imatinib, LC-MS, HPLC, UV Spectrophotometry.

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## INTRODUCTION

Imatinib a tyrosine kinase inhibitor, was called as “magical bullet,” when it revolutionized the treatment of chronic myeloid leukemia (CML) in 2001. Imatinib was invented in the late 1990s by biochemist Nicholas Lyndon its use to treat CML was driven by Brian Druker, an oncologist at the Dana-Farber Institute. The first clinical trial of Imatinib took place in 1998 and the drug received FDA approval in May 2001. Tyrosine kinases are important mediators of the signaling cascade, determining key roles in diverse biological processes like growth, differentiation, metabolism, and apoptosis in response to external and internal stimuli. Deregulation of protein kinase activity has been shown to play a central role in the pathogenesis of human cancers. Imatinib, a 2-phenyl amino pyrimidine derivative, is a tyrosine kinase inhibitor with activity against ABL, BCR-ABL, PDGFRA, and c-KIT. The active sites of tyrosine kinases each have a binding site for ATP. The enzymatic activity catalyzed by a tyrosine kinase is the transfer of the terminal phosphate from ATP to tyrosine residues on its substrates, a process known as protein tyrosine phosphorylation. Imatinib works by binding close to the ATP binding site, locking it in a closed or self-inhibited conformation, therefore inhibiting the enzyme activity of the protein semi competitively<sup>1</sup> Chronic myelogenous leukemia is a myeloproliferative disorder associated with an abnormal BCR-ABL tyrosine kinase. Imatinib, a synthetic phenylaminopyrimidine derivative, which inhibits the tyrosine kinase with high selectivity, has been established as a highly effective therapy for chronic myelogenous leukemia<sup>2</sup> and gastrointestinal stromal tumors . Imatinib is predominantly metabolized by CYP3A4 to *N*-desmethyl imatinib, which shows comparable biological activity to the parent drug<sup>3</sup>. The activity of CYP3A4 displays large inter-individual variability. Therefore, a given dose of imatinib can yield very different circulating concentrations of the parent drug and its metabolites.<sup>4</sup> Studies show that an adequate plasma concentration of imatinib is important for a good clinical response, which emphasizes the significance of therapeutic drug monitoring and pharmacokinetics investigation of imatinib. Evaluation of blood imatinib levels in patients with chronic myeloid leukemia has become a useful tool for achieving the optimum therapeutic level for patients who have experienced drug interactions or adverse side effects and for those who require dose adjustment. Several recently published studies have reported the validation of analytical methods for quantification of imatinib in human blood using chromatographic techniques coupled with ultraviolet or mass spectrometry detection. In this review we summarized various methods and techniques in the estimation imatinib viz LC-MS, HPLC and UV spectrometry.

## MATERIALS AND METHOD

### A: liquid chromatography– mass spectrometry (MS)<sup>5</sup>

All the LC-MS/MS measurements were made by an API-4000 QT (Sciex Brugherio, Italy) coupled to a LC Nexera system (Shimadzu Milano, Italy) in-house configured for the on-line cleanup. It included an autosampler, a binary pump, a column oven and an additional pump module for the trapping step. The chromatographic separation of the analytes (5  $\mu$ L injection volume) was conducted on a Synergi Fusion-RP column (4  $\mu$ m, 2x50 mm) from Phenomenex (Bologna, Italy) kept in the oven at 55 °C, while the trapping column was a POROS R1/20 (20  $\mu$ m, 2x30 mm) from Applied-Bio systems TF (Monza, Italy). The trapping step was conducted as follow: 1.5 min at 2 mL/min of an aqueous solution of methanol 10% containing formic acid 0.1% and 2 mM ammonium acetate. The subsequent elution step, conducted in forward flow, was obtained using the following gradient: at 0.45 mL/min starting with 10% of eluent B (mixture acetonitrile-isopropanol 80:20 containing formic acid 0.1%) and 90% of eluent A (aqueous solution of formic acid 0.1% containing 2 mM ammonium acetate). After 1.5 minute, eluent B moves up to 60% in 4 min. A cleaning step at 98% lasting 1 min is performed before re-equilibration. The total run time was 8.5 min. The retention times of IMA and norIMA were 5.50 and 5.39 min, respectively. The mass spectrometer worked in positive multi-reaction monitoring (MRM) mode and was equipped with a valve switching system and a Turbo Ion Spray source operating at 500°C. The ion spray voltage was set at 2200 V with curtain gas pressure at 25 psi, and both nebulizer gas and turbo gas pressure at 40 psi. The fragmentation patterns of each compound (Fig 1) were as follow: 494.4 > 394.3 *m/z* (quantifier, DP 110 volts, CE 40 V) and 494.4 > 217.2 *m/z* (qualifier, DP 110 volts, CE 35 V) for IMA; 480.4 > 394.3 *m/z* (quantifier, DP 110 volts, CE 35 V) and 480.4 > 203.2 *m/z* (qualifier, DP 110 volts, CE 35 V) for norIMA; 502.4 > 394.2 *m/z* (DP 110 volts; CE 40 V) for IMA-D8 used as IS.

### Intra- and inter-day precision and accuracy:

The precision and accuracy of the presented method were evaluated by analyzing six replicates of each QC sample (L, M, and H) within a single-run analysis for intra-day assessment and three replicates of each QC sample over five different working days for inter-day assessment, using standard calibration curves freshly prepared. The method precision, at each concentration, was reported as the coefficient of variation (CV %), expressing the standard deviation as a percentage of the mean calculated concentration. The accuracy of the method was determined by expressing the mean calculated concentration as a percentage of the nominal concentration. The measured

concentration for at least 2/3 of the QC samples had to be within 15% of the nominal value, in each run, and only one QC sample, at each concentration level, could be excluded.

### **Stability**

The stability of IMA and norIMA was assessed by analyzing QC DBS samples at the three concentrations L, M, and H during sample storage and handling procedures. The stability of the QC samples, processed as previously reported (“Preparation of standards and quality control DBS samples” section), was assessed in the auto sampler by repeatedly analyzing the extracts 24 and 48 h after the first injection. Long-term stability of DBS samples was assessed at the storage condition applied (in plastic envelopes containing a silica-gel drying bag at room temperature) at time intervals of 1, 2, 4 weeks and then months after preparation. Long-term stability of working solutions (methanol matrix) was assessed stored at approximately  $-80^{\circ}\text{C}$ . The two analytes were considered stable when the testing samples did not exceed 15% from the nominal concentrations at each QC concentration.

### **B High-Performance Liquid Chromatography-Mass Spectrometry <sup>6</sup>:**

The imatinib mesylate reference standard (lot 50325) was obtained from Bio vision (Milpitas, CA, USA). Tamsulosin hydrochloride (reference standard (lot F0H375) was obtained from the United States Pharmacopeia (Rockville, MD, USA). All of the solvents used were HPLC grade and provided by JT Baker Chemicals (Phillipsburg, NJ, USA). Ultrapure water (type I) was obtained from a Direct-Q3 UV water purification system (Millipore, Molsheim, France). Blank, normal, hemolyzed, and hyperlipemic human serum were provided by Fundação Pró-Sangue do Hemocentro de São Paulo (São Paulo, Brazil) from healthy and drug-free subjects. The choice of serum instead of plasma (the most widely used matrix) took into account the easiest collection procedure and whole blood availability in clinical centers during routine evaluation of patients.

### **Equipment:**

Automatic micropipettes (models P1000, P200, and P20, Gilson Inc, Villiers-le Bel, France) with disposable plastic tips provided by Axygen (MA, USA) as well as the Multi pipette Plus® with combitips (Eppendorf, Hamburg, Germany) were used. Weights were measured using an AY220 balance (Shimadzu Philippines Corporation, Rosario, Philippines), and the centrifuge used was an Eppendorf 5424. The analytical instrumentation included a Prominence UFLC® ultra-fast liquid chromatographic system (Shimadzu Company, Kyoto, Japan), composed of two LC-20 AD pumps, a DGU-20A3 degasser, an SIL-20AC HT autosampler, a CTO-20A column oven, an SPD-20A ultraviolet-visible detector, and a CBM-20A system controller. The chromatographic system was coupled to a Shimadzu LCMS-2020 MS with an electrospray ionization interface.

Preparation of calibration standards and quality controls: Standard stock solutions of imatinib and tamsulosin were prepared in pure methanol by dissolving a specified amount of material, weighed with a precision of 0.1 mg, in a suitable volume of solvent to provide a 1 mg/mL base equivalent clear solution after sonication. Working solutions were prepared by serial dilutions of the stock solutions to obtain final concentrations of 10, 20, 30, 40, 60, 80, 90, 120, 160, 162, and 200 µg/mL for imatinib and 5 µg/mL for tamsulosin. All stock solutions were stored at  $-20^{\circ}\text{C}$ , and working solutions were stored at  $2^{\circ}\text{C}$ – $8^{\circ}\text{C}$  until use. Serum calibrators and quality controls were prepared as 20-fold dilutions of the working solutions in blank (drug-free) serum to obtain a calibration curve at concentrations of 0.500, 1.00, 2.00, 3.00, 4.00, 6.00, 8.00, and 10.0 µg/mL. The quality controls were prepared at concentrations of 0.500 µg/mL for the lower limit of quantification (LLOQ), 1.50 µg/mL for the low level quality control, 4.50 µg/mL for the medium level quality control, and 8.1 µg/mL for the high level quality control. The highest concentration of the calibration curve, 10 µg/mL, also denominated the upper limit of quantification. Spiked serum for use as a calibrator and quality controls was freshly prepared prior to use. Analysis of calibrators was carried out in duplicate for the first and last levels, and unique replicates were prepared for the other levels. For quality control, at least three replicates for each concentration were used in every batch.

#### **Sample preparation:**

Blank serum samples were thawed at room temperature ( $22^{\circ}\text{C}$ ) and centrifuged at  $14,000 \times g$  for one minute prior to use as blank or spiked samples. An aliquot of 100 µL was transferred to 1.5 mL polypropylene centrifuge microtubes, and 50 µL of the internal standard solution was added. Next, 400 µL of cold methanol was added for protein precipitation. Cold methanol was obtained by immersing a cup of methanol in an ethanol/dry ice bath until the temperature reached  $-80^{\circ}\text{C}$ . Closed microtubes were manually shaken for 20 seconds and positioned on plastic plates. After mixing by shaking, the samples were centrifuged at  $14,000 \times g$  for 15 minutes, and the clear supernatant was transferred to a 1 mL borosilicate vial with a plastic cap and placed into an autosampler tray at  $8^{\circ}\text{C}$  until injection.

#### **Chromatographic conditions:**

After protein precipitation, 2 µL was injected into a 50 mm  $\times$  20 mm Luna C18 100 Å, 3 µm chromatography column preceded by a 4  $\times$  2 mm Security Guard Cartridge C18 (Phenomenex, Torrance, CA, USA). The column was operated at  $40^{\circ}\text{C}$ , and a gradient elution. The solvents pumped into the column consisted of methanol or water, each containing 10 mM of ammonium acetate and 0.1% formic acid.

**C: UV-Visible Methods<sup>7</sup>**

Instruments used were UV-Spectrophotometer ELICO double beam SL-164, UV thermo scientific, Ultra Sonicator: Cleaner-Cyberlab.

**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

**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.<sup>8</sup> 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<sup>9-12</sup>. 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<sup>13</sup>.

## CONCLUSION:

Assay methods are successfully developed and validated for the determination of Imatinib in Imatinib mesylate in blood serum and working standards. The methods is evaluated for all method validation parameters perform as per ICH guidelines. Method validation results have proven that the method is specific, rapid, precise, accurate, linear and robust. The LC MS method stability-indicating and can be used for routine analysis in quality control and stability of samples.

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