A REVIEW ON QUANTIFICATION OF AN ANTICANCER DRUG-IMATINIB BY LIQUID CHROMATOGRAPHY COUPLED TO MASS SPECTROMETRY (LC-MS) TECHNIQUE

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ABSTRACT
Imatinib mesylate is used in treating chronic myelogenous leukemia (CML), gastrointestinal stromal tumors (GISTs) and a number of other malignancies. It is the first member of a new class of agents that act by inhibiting particular tyrosine kinase enzymes, instead of non-specifically inhibiting rapidly dividing cells. Therapies with imatinib have shown significant inter-individual variability in pharmacokinetics, demanding a need for Therapeutic Drug Monitoring (TDM) in order to achieve an optimal response in CML therapy and to minimize adverse side effects. LC-MS has widely been applied as a reliable technique for quantification of imatinib in human plasma. The linearity of the LC-MS method was evaluated over the imatinib concentration range of 30 – 10000 ng/mL in human plasma with correlation coefficient (R²) of ≥ 0.999. The recoveries determined at low, medium and high concentration levels varied from 95 – 107 % and within the acceptable limits. Validated method has 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, Liquid Chromatography, Plasma, LC-MS/MS, Fragmentation.
INTRODUCTION
Imatinib mesylate is a protein-tyrosine Kinase (PTK) inhibitor and it is used for the treatment of chronic myeloid leukemia (CML) disease in adult patients. It is known as signal transduction inhibitor 571 (STI 571, formerly known as CGP 57148B). Chemical name of imatinib mesylate is benzamide, 4-[(methyl-1-piperazinyl) methyl]-N-[4-methyl-3-[(3-pyridinyl)-2-pyrimidinyl] amino] phenyl-, monomethanesulfonate. The structure of Imatinib is shown in Figure 1.

![Fig 1. Imatinib](image)

Trade name is Glivec, Novartis Pharma, Switzerland. Too low imatinib plasma levels could indicate an ineffective drug regimen, insufficient to achieve complete cytogenetic response (CCR) or major molecular response (MMR). Due in large part to the development of Gleevec and related drugs having a similar mechanism of action, the five year survival rate for people with chronic myeloid leukemia nearly doubled from 31% in 1993 (before Gleevec's 2001 FDA approval) to 59% for those diagnosed between 2003 and 2009. Compared to older drugs imatinib has a relatively benign side effect profile, allowing many patients to live a normal lifestyle. Median survival for imatinib-treated people with gastrointestinal stromal tumors is nearly 5 years compared to 9 to 20 months in the pre-imatinib-era.

Several analytical procedures have been developed to measure imatinib in biological fluids and pharmaceutical dosage forms using different detection techniques, including high-performance liquid chromatography (HPLC)UV and liquid chromatography and mass spectroscopy (LC-MS/MS) methods. Imatinib is mainly metabolised by CYP3A4/CYP3A5 to N-desmethyl imatinib (DMI) which exhibits in vitro activity comparable to that of the parent drug. Most of published analytical methods using liquid chromatography–mass spectrometry (LC-MS) or
liquid chromatography/tandem mass spectrometry (LC-MS/MS) have been for the quantification of imatinib without DMI.

**QUANTIFICATION STUDIES**

Zhang et al. 2012,\(^3\) developed a rapid, simple and sensitive liquid chromatography/tandem mass spectrometry assay for the simultaneous determination of imatinib and its metabolite N-desmethyl imatinib (DMI) in human plasma. After proteins were precipitated with acetonitrile, imatinib, DMI and the internal standard D8-imatinib were resolved on a Gemini-NX 3 μmC18 column using gradient elution of 0.05 % formic acid and methanol. The three compounds were detected using electrospray ionisation in the positive mode. Standard curves of imatinib and DMI were adequately fitted by quadratic equations (r>0.999) over the concentration range of 10 to 2,000 ng/mL which encompasses clinical concentrations. Bias was ≤±8.3 %, intra and inter-day coefficients of variation (imprecision) were ≤8.0 % and the limit of quantification was 10 ng/mL for both imatinib and DMI. The assay is being used successfully in clinical practice to enhance the safe and effective use of imatinib.

Marull et al. 2006,\(^4\) investigated the role of the activity of CYP1A1, 1A2, 1B1, 3A4, 4F2 and 4F3A/B on the fate of imatinib. Moreover, recent genomic studies have revealed that some isozymes of cytochrome P450 (CYP) are possibly associated with the treatment outcome. A study of imatinib fragmentation was affected using electrospray triple-quadrupole and linear ion trap tandem mass spectrometers (MS\( ^n \)). Accurate mass determinations were performed at enhanced mass resolution for the identification of some product ions that were not predicted by two fragmentation softwares. Imatinib metabolites were produced in microsomal incubations containing CYP isozymes. Imatinib and metabolites were extracted from incubation mixtures by protein precipitation, and supernatants were injected into a liquid chromatography equipment coupled with MS\( ^n \). Hydrophobic interaction liquid chromatography resolved one demethylated-, two hydroxy- and three N-oxide metabolites. Various rates of metabolite formation were observed between CYP isozymes. Liquid chromatography with deuterium oxide containing mobile phase (H/D exchange) or incorporation of \(^{18}\)O from H\(_2^{18}\)O added in the incubations was performed to elucidate the metabolite structure.

Various MS\( ^n \) product scans (n ≤ 4) were acquired on the linear ion trap or on the triple-quadrupole MS.
Austin et al. 2014,\textsuperscript{[5]} have reported that imatinib forms cyanide and methoxylamine adducts in vitro but without detail structural identification. The current work reports the identification of seven cyanide adducts that elucidate the bioactivation pathways and may provide hints for observed clinical adverse effects of the drug. Imatinib was incubated with human liver microsomal proteins in the presence of a NADPH-regeneration system and the trapping agents reduced GSH, potassium cyanide and methoxylamine. Samples were analyzed by high-performance liquid chromatography (HPLC) coupled with a LTQ-Orbitrap data collection system. Chemical structures were determined and/or postulated based on data-dependent high-resolution tandem mass spectrometric (MS\textsuperscript{n}) exact mass measurements in both positive and negative scan modes, as well as in combination with hydrogen deuterium exchange (HDX). GSH and methoxylamine conjugates were either not detected or were in insufficient quantities for characterization. However, seven cyanide conjugates were identified, indicating that the piperazine and p-toluidine partial structures in imatinib can become bioactivated and subsequently trapped by the nucleophile cyanide ion. The reactive intermediates were postulated as imine and imine-carbonyl conjugate (α,β-unsaturated) structures on the piperazine ring, and imine-methide on the p-toluidine partial structure. Chemical structures of seven cyanide adducts of imatinib have been identified or proposed based on high-resolution MS/MS data. Mechanisms for the formation of the conjugates were also proposed. The findings may help to understand the mechanism of hepatotoxicity of imatinib in humans.

Guetens et al. 2006,\textsuperscript{[6]} analyzed the signal transduction inhibitor imatinib in patient tumour tissue using LC and MS/MS is described. The anticancer agent is eluted over RP-C18 within 2 min together with its internal standard STI571-d8. Calibration curves were prepared in red blood cells (RBC). For quantitative isolation of the RBC, measurement of sediment was applied. There were no indications of signal suppression by substances originating in the biological matrix. The limit of determination in tumour tissue was in the range of those recorded for RBC and plasma. The assay is selective and sensitive, with its robustness favouring the experimental application in clinical oncology and its routine use in animal experiments. The LOD was 4.5 ng per gram in tumour tissue.

Marcondes et al. 2013,\textsuperscript{[7]} 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. Stability of the analyte was also studied. It also intended to drive the validation scheme in clinical centers. The method was validated according to the requirements of the US Food and Drug Administration and Brazilian National Health Surveillance Agency within the range of 0.500–10.0 µg/mL with a limit of detection of 0.155 µg/mL. Stability data for the analyte are also presented.

Bertrand et al. 2008,\(^\text{[8]}\) investigated the capability to analyze simultaneously the parent drug and newly identified metabolites in patients' plasma by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). The anticancer drug, imatinib, was chosen as a model drug because it has opened a new area in cancer therapy and is given orally and chronically. In addition, resistance and rare but sometimes severe side effects have been reported with this therapy. The quantification of imatinib and the profiling of its metabolites in plasma were established following three steps: (1) set-up of a generic sample extraction and LC-MS/MS conditions, (2) metabolite identification by LC-MS/MS using either in vitro incubations performed with human liver microsomes (HLMs) or patient plasma samples, (3) the simultaneous determination of plasma levels of imatinib and 14 metabolites in the plasma samples of 38 patients. Partial or cross method validation has been done and revealed that precise determinations of metabolite levels can be performed whereas pure standards are not available. Preliminary results indicate that the disposition of imatinib and its metabolites is related to interindividual variables and that outlier metabolite profiles can be revealed. This article underscores that, in addition to usual therapeutic drug monitoring (TDM), LC-MS/MS methods can simultaneously record a complete drug metabolic profile enabling various correlation studies of clinical interest.

Caterino et al. 2013,\(^\text{[9]}\) studied that measurement of imatinib plasma concentration can be useful to evaluate patient adherence to daily oral therapy, potential drug–drug interaction, treatment efficacy, and severe drug-related adverse events. The aim of the study was to correlate the two different blood level test methods, HPLC–UV and LC–MS/MS. 162 plasma samples were analyzed from patients treated with imatinib. They estimated the correlation between the two analytical methods on total data set and on five sets of patients grouped into different categories based on the drug-dose therapy. Finally, imatinib quantification was
correlated with genetic data on the molecular response in monitoring follow-up of CML patients.

Roth et al. 2010.,[10] developed a high-performance liquid chromatography (HPLC) method with UV/Diode Array Detection (DAD) for trough imatinib concentration determination in human plasma. Imatinib trough levels were measured in plasma from 65 CML patients using our method and LC-MS/MS as the reference method. Results with these two methods were compared using Deming regression, chi-square test, and sign test. The calibration curve was prepared in blank human plasma. HPLC-UV/DAD calibration curves were linear from 80 to 4000 ng/mL, and the limit of quantification was set at 80 ng/mL. The between-day variation was 6.1% with greater than 96% recovery after direct plasma deproteinization and greater than 98% recovery from the column. No significant differences in imatinib plasma levels were found between HPLC-UV/DAD and LC-MS/MS. This HPLC-UV/DAD method was sufficiently specific and sensitive for imatinib TDM, with no evidence of interference. Rapid inexpensive HPLC-UV/DAD method that requires only widely available equipment performs well for plasma imatinib assays.

Francia et al. 2009.,[11] described the quantification of plasma concentration of tyrosine kinase inhibitors imatinib, dasatinib and nilotinib by using new method high performance liquid chromatography coupled with electrospray mass spectrometry. A simple protein precipitation extraction procedure was applied on 250 µl of plasma aliquots. Chromatographic separation of drugs and Internal Standard (quinoxaline) was achieved with a gradient (acetonitrile and water + formic acid 0.05%) on a C18 reverse phase analytical column with 20 min of analytical run, at flow rate of 1ml/min. Mean intra-day and inter-day precision for all compounds were 4.3 and 11.4%; mean accuracy was 1.5%; extraction recovery ranged within 95 and 114%. Calibration curves ranged from 10,000 to 62.5 ng/ml. The limit of quantification was set at 78.1 ng/ml for imatinib and at 62.5 ng/ml for dasatinib and nilotinib. This novel developed methodology allows a specific, sensitive and reliable simultaneous determination of the three tyrosine kinase inhibitors imatinib, dasatinib and nilotinib in a single chromatographic run, useful for drugs estimation in plasma of patients affected by chronic myeloid leukemia.

Awidi et al. 2010.,[12] compared HPLC and LC-MSMS analytical methods and their applicability for the quantitation of imatinib in human plasma. A total of 50 patients with chronic myeloid leukemia (CML) in chronic phase (CP) receiving 400 mg/day imatinib were
enrolled in the study. Drug levels were determined by HPLC–UV and LCMSMS. HPLC intra-day accuracy ranged from 100.51 to 103.19%. LCMSMS accuracy ranged from 89.72 to 106.29%. The correlation coefficient between both methods was \( r^2 = 0.96 \). HPLC can be used for imatinib levels’ determinations in patients accurately and precisely.

Avolio et al. 2012.,\(^{[13]}\) described a new method using high performance liquid chromatography coupled with electrospray mass spectrometry for the quantification of PBMC concentration of tyrosine kinase inhibitors imatinib, dasatinib and nilotinib. Simple PBMC isolation and extraction procedures were applied on 10–14 mL of blood aliquots. Chromatographic separation of drugs and Internal Standard (quinoxaline) was achieved with a gradient (acetonitrile and water + formic acid 0.05%) on a C\(^{18}\) reverse phase analytical column with 25 min of analytical run, at flow rate of 0.25 mL/min. Mean intra- and inter-day precision for all compounds were 8.76 and 12.20%; mean accuracy was −3.86%; extraction recovery ranged within 79 and 91%. Calibration curves ranged from 50.0 to 0.25 ng. The limit of quantification was set at 0.25 ng for all the analyzed drugs. This novel developed methodology allows a specific, sensitive and reliable simultaneous intracellular determination of the three tyrosine kinase inhibitors imatinib, dasatinib and nilotinib in a single chromatographic run, useful for drugs estimation in PBMC of patients affected by chronic myeloid leukemia.

Arava et al. 2013.,\(^{[14]}\) developed LC-MS/MS method for the determination of Methyl-3-N [4-(3-Pyridinyl)-2-pyrimidinyl]-1,3-benzenediamine(PNMP) and 4-(4-Methylpiperazinomethyl) benzoic acid dihydrochloride(MPBA) content in Imatinib Mesylate on YMC –Basic, 250 X 4.6 mm 5µm column using a gradient mixture of solvent A (10mM ammonium formate, adjust the pH of the above solution to 7.0 using Aq.NH3) and solvent B (Methanol(70): Acetonotrile(30)). The flow rate is 1.0ml/min. Statistical analysis proved the method to repeatable, specific and accurate for estimation of PNMP and MPBA content. It can be used as a LC-MS/MS method due to effective quantification method for trace level potential genotoxic impurities.

Bhatt et al. 2013.,\(^{[15]}\) developed a sensitive and selective liquid chromatographic-tandem mass spectrometric (LC-MS/MS) method and validated for the trace analysis (> 1 ng/mL level) of 2-methyl-5-aminophenyl)-4-(3-pyridyl)-2-pyrimidine (Imp-A) a genotoxic impurity in imatinib mesylate drug substances. LC-MS/MS analysis of Imp-A was done on Inertsil C18 (150 mm × 4.6 mm) 5 µm column and 0.1% formic acid in 1000 mL of water was used
as buffer in mobile phase A and acetonitrile in mobile phase B. Gradient program was
developed for rapid analysis. The flow rate was 1.0 mL/min and elution was monitored by
mass spectrophotometer. The method was validated as per International Conference on
Harmonization (ICH) guidelines. LC-MS/MS is able to quantitate up to 1 ng/mL of Imp-A.

Yang et al. 2013.,[16] developed a simple, fast and robust analytical method to determine
imatinib in human plasma using liquid chromatography-tandem mass spectrometry with
electrospray ionization in the positive ion mode. Imatinib and labeled internal standard were
extracted from plasma with a simple protein precipitation. The chromatographic separation
was performed using an isocratic elution of mobile phase involving 5.0 mM ammonium
formate in water–5.0 mM ammonium formate in methanol (30:70, v/v) over 3.0 min on
reversed-stationary phase. The detection was performed using a triple-quadrupole tandem
mass spectrometer in multiple-reaction monitoring mode. The developed method was
validated with lower limit of quantification of 10 ng/mL. The calibration curve was linear
over 10-2000 ng/mL (R² > 0.99). The method validation parameters met the acceptance
criteria. The spiked samples and standard solutions were stable under conditions for storage
and handling. The reliable method was successfully applied to real sample analyses and thus
a pharmacokinetic study in 27 healthy Korean male volunteers.

CONCLUSION
A sensitive method for the analysis of imatinib in plasma is necessary in conducting
therapeutic drug monitoring and pharmacokinetic studies. The developed LC-MS methods
are robust and reached the level of sensitivity and reproducibility demanded by clinical
patient sample analysis. Hence, the method can be readily incorporated into the routine
testing of imatinib.

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