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DEVELOPMENT OF QUANTITATIVE PROTOCOL FOR IMATINIB IN HUMAN SERUM BY LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY FOR THERAPEUTIC DRUG MONITORING

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Abstract

Estimation of therapeutic levels of Imatinib and its correlation with clinical response has become important to monitor response of this drug in chronic myeloid leukemia (CML) patients. Various attempts have been made to develop quantitative protocols for imatinib in human serum using different analytical tools. A simple, sensitive and specific quantitative method was developed in the present study for monitoring of imatinib levels in human serum by applying liquid chromatography (LC) and QQQ tandem mass spectrometry. An efficient simple sample extraction method was developed using solid phase extraction (SPE) method for extraction of imatinib in human serum with a recovery of around 80-85%. The developed method was successfully tested on 30 human serum samples who have been receiving imatinib for CML.

Keywords: Imatinib, LCMS, MRM, CML, Serum

Introduction:

CML accounts for approximately 15% of adult leukemias. CML is a clonal myeloproliferative disorder of the primitive hematopoietic stem cell. It is characterized by presence of balanced genetic translocation of chromosomes 22 and 9, termed as Philadelphia chromosome. The resulting breakpoint cluster region-Abelson murine leukemia (BCR-ABL) fusion oncogene is translated into the BCR-ABL oncoprotein. BCR-ABL is a constitutively active tyrosine kinase that activates a number of signal transduction pathways that affect the growth and survival of hematopoietic cells. Imatinib is a tyrosine kinase inhibitor which is used in CML and has become first line choice and has revolutionized drug therapy of CML [1,2]. Monitoring of trough plasma concentration and clinical response in CML is mainstay of imatinib therapy. There are various studies which shows that there is correlation between trough plasma concentration and clinical response in CML.

Imatinib is an antineoplastic agent and the molecular weight of the compound is 493.6 g/mol. It is soluble in water. A trough level close to 1000ng/ml, appears to be correlated with better cytogenetic and molecular responses. Estimation of therapeutic levels of imatinib in CML has become investigation of choice for many clinicians. There are many articles published has mentioned about the development of protocols for imatinib by LCMS [3,4,5]. Kuna et al [6] developed the protocol for imatinib by using HPLC in which method was linear from 10 to 60 µg/mL which is low as in our method linearity was from 0.5 µg/mL to 8 µg/mL which is very well within the therapeutic range of imatinib. In the present study simple, specific and precise method was developed with simple sample preparation steps for extraction of imatinib from serum.

Materials and methods

Agilent 1260 infinity HPLC (Agilent technologies) along with Agilent triple quadrupole (QQQ) mass spectrometry was used for developing LCMS protocols for imatinib. Necessary approval from Institutional ethical committee was taken. The Vac Elut 12 manifold from Agilent (P/N : 5982 – 9110) was used for SPE during sample preparations. The

pure compound/standard for method development, Imatinib, Molcular weight 493.6 g/ mol of 25mg (Lot No CDS02217J) was procured from Sigma Aldrich USA. All solvents including methanol and formic acid used in the present study were at LCMS grade and purchased from Sigma Aldrich USA. The pure compounds was weighed and reconstituted in methanol. Stock solution of pure compound was 1 mg/ml. Further dilutions from the stock solutions were used for developing multiple reaction monitoring (MRM) transitions and method development on column. The electron spray ionization (ESI) in positive mode was used to identify the precursor ion, product ion and to standardize the different fragmentor voltage and the collision energy required for developing the MRM transitions. Accordingly MRM transitions were developed and tabulated (Table 1) before developing instrument method on column.

Table 1: MRM transitions developed for Imatinib.

Compound	Precursor ion	Product ion	Fragmentor Voltage	Collision energy
Imatinib	494.3	393.3	110v	22v

Liquid chromatography/Mass spectrometric conditions

The MRM transitions developed were used for developing instrument method. Briefly, the analyte separation was achieved using Agilent 1260 infinity HPLC (Agilent technologies) equipped with a RRHT Zorbax eclipse plus C18 (3.0 x 100mm , 1.8 µm P/N: 959964-30) analytical column from Agilent technologies.100ng of imatinib was injected using an Agilent G1367C autosampler. Identification and quantification of drugs were accomplished using a Agilent 6430 triple quadrupole mass spectrometer. For all determinations, the LC was operated in a gradient mode with a constant flow rate of 0.40 ml/min. However initially we tried in isocratic mode which was not successful and hence gradient mode was applied with slowly increasing the ratio of mobile phase A and mobile phase B till we get definite peak in chromatogram. The mobile phase employed consisted of water with 0.1% formic acid (A) and methanol (B). The method for setting up a gradient involved an initial mobile phase composition of 90% A and 10% B which changed to 10% of A and 90% of B in 2 min. The same composition was holded for 10 min. The mobile phase composition changed to initial composition in 10 min. Post run time was kept as 3 min. Total run time was 13 min.

Matrix match calibration.

A series of calibration standards were prepared using methanol:water mixture in 50:50 ratio by spiking 0.5, 1, 2,4 and 8 ppm of aqueous imatinib mix in a microfuge tube. Pure methanol was used as blank. In brief, 800 µL of methanol was added to 200 µL of methanol -water mixture spiked with known concentration of standard mix and vertexed for 30 s. The supernatant was transferred to Bond Elut Captiva ND Lipid cartridges after centrifuge at 7000rpm for 4 min at 8^0C. The vacuum was applied and the elute collected was injected into the system

Sample preparation: 200 µl of serum was mixed with 800 µl of chilled methanol to precipitate the protein, the mixture was vortexed and mixed well. The supernatant was transferred to Bond Elut Captiva ND Lipid cartridges after centrifuge at 7000rpm for 4 min at 8^0C. The vacuum was applied and the elute collected was injected into the system (Fig1).

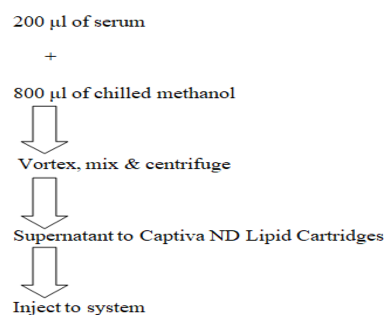

Figure 1: Scheme of the steps involved in extraction of drugs from serum

Preparation of quality control samples:

Quality control samples were prepared by spiking different serum with different and proper volume of the corresponding standards solution to make final concentration of 0.5, 2 and 4 ppm.

Results

MRM was developed using stock solution of 100ng/ml where compound was passed through mass spectrometer without passing it through the column under conditions (as mentioned in table 1). The total ion chromatogram was achieved is depicted in fig 2 and finally calibration curve was made from 0.5ppm, 1ppm, 2ppm,4ppm and 8 ppm standards as depicted in fig 3. Method validation was done by using selectivity, linearity, accuracy, precision, recovery, detection limit and quantitation limit.

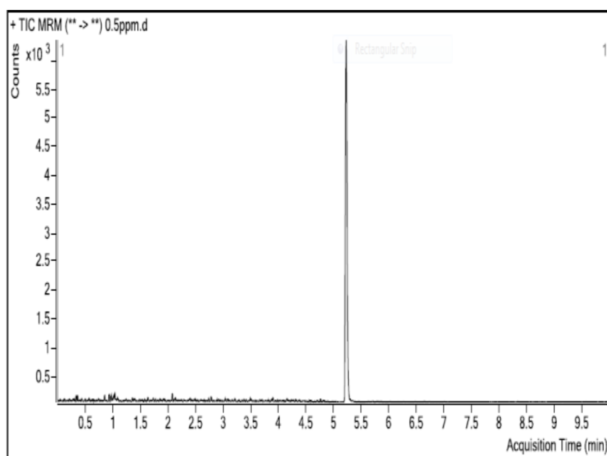

Figure 2: Total ion chromatogram of 0.5ppm imatinib

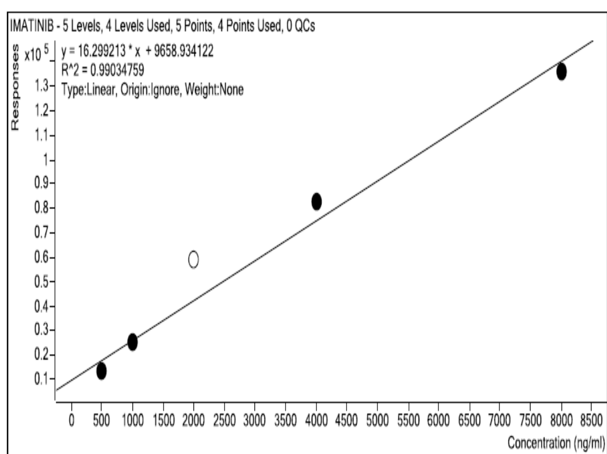

Figure 3: Calibration curve of imatinib

Selectivity: selectivity of the method was calculated by running four lots of blank serum samples from different individuals. Carry over was tested by running blank solvent immediately after the highest concentration. Further the samples were run using pure solvent. No carry over was noticed in our study.

Linearity: Calibration curve was made by using 0.5 ppm, 1 ppm, 2ppm, 4ppm and 8 ppm concentration of imatinib (Fig 3). Coefficient of correlation was 0.99 in our study.

Accuracy: it was calculated in lab by running known amount of in house made quality control samples five times and measures of accuracy was calculated. Accuracy of 80% to 85% was observed in our study.

Precision: it was determined by running known amount of sample five times at different days and CV% was calculated by following formula

$$\frac{\text{Standard deviation of observed value in lab}}{\text{Mean observed value in lab}} \times 100$$

CV% of less than 10% was acceptable.

Recovery: Recovery of any analyte response is obtained when an analyte is added to the biological matrix (serum) and this response is compared with the response obtained from the response obtained after analyte is added to pure solvent . Recover in our study was about 80-85%.

Limit of detection and limit of quantification: Limit of detection and limit of quantification in our study was determined using signal/noise ratio. Limit of detection was 0.2 ppm and limit of quantification was 0.5 ppm.

Discussion:

Therapeutic drug monitoring has become very important method for treatment of CML patients. The measurement of antileukemic drug plasma concentrations, in fact, can be useful to evaluate patient adherence to daily oral therapy, potential drugs interactions, treatment efficacy, and severe drug related adverse events [7-9]. Imatinib level in the plasma is most widely measured by trough plasma concentration. Trough concentration blood samples are collected before morning dosing at steady state and are typical determined using rapid, simple, sensitive and specific liquid chromatography-mass spectrometry (LC-MS) or high performance liquid chromatography (HPLC) coupled with tandem mass spectrometry protocols which have been validated very well [10-13]. Many studies have clearly reported that administration of an imatinib dose of 800 mg/day, titrated to the standard dose of 400 mg/day, might be more effective for patients in the chronic myeloid leukemia [14-15]. Rezende et al.[16] in their study where they developed the protocol for imatinib quantification, total analytical run time was 4 minutes compared to 13 minutes in our study. They used methanol and water, each containing 10 mM of ammonium acetate and 0.1% formic acid, whereas in our study methanol and water containing 0.1% formic acid was used. If we compare sample preparation of our study with the study mentioned above, sample preparation in our study is much easier. In another study by Rezende et al. [17] where they validated the imatinib protocol on ultrafast liquid chromatography coupled with mass spectrometry, they have used serum as a matrix which is similar to our study as processing of the sample with serum is easy and fast. They have used ammonium acetate as one of the constituents of mobile phase whereas we have tried during our method development but could not achieve better peak and we achieved better peak by

using methanol with 0.1% formic acid. There are many studies where they have employed different methods to develop and validate the imatinib protocol by using different equipments like High performance liquid chromatography and high performance liquid chromatography coupled with mass spectrometry but our method is easy and less cumbersome as mobile phase composition is easy as we have used only methanol and water with formic acid and also sample preparation is very easy which can be utilized in the lab.

Conclusion: Monitoring of therapeutic levels of imatinib in blood is mainstay of treatment of chronic myeloid leukemia. Development of protocol in LCMS for its therapeutic level monitoring is an important task. Our study mentions the protocol which is easy and less cumbersome with easy mobile phase composition.

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