ABSTRACT

In this review article determines the different analytical methods for the quantitative establishment of selected tyrosine kinase inhibitors (Imatinib mesylate, Nilotinib and Dasatinib) by using HPLC, HPLC-MS, HPLC-UV, LC-MS/MS. Pharmaceutical analytical method development of tyrosine kinase inhibitors (Imatinib Mesylate, Nilotinib and Dasatinib) requires valid analytical procedures for quantitative and qualitative analysis in Pharmaceuticals dosage formulations and human serum. An huge survey for determination of TKI (Imatinib Mesylate, Nilotinib and Dasatinib) follow from the research articles published in various pharmaceutical and analytical chemistry Journals. This assessment explain that the superiority of the HPLC/LC-MS methods reviewed is based on the quantitative analysis of drugs in formulations, (API), biological fluids such as serum and plasma.

KEYWORDS: Method development, High performance Liquid Chromatography (HPLC/LC-MS), (Imatinib Mesylate, Nilotinib and Dasatinib).

INTRODUCTION AND REPORTED ANALYTICAL METHODS FOR SELECTED ANTI CANCER DRUGS

IMATINIB MESYLA TE

This compound belongs to the class of organic compounds known as n-phenylbenzamides. These are benzamides that are N-linked to a phenyl group via the carboxamide group. N-(4-methyl-3-[[4-(pyridin-3-yl) pyrimidin-2-yl] amino} phenyl) – 4 - [(4-methylpiperazin-1-yl)
methyl] benzamide, C29H31N7O, 493.6027 gm/mol. Imatinib mesylate is a protein-tyrosine kinase inhibitor that inhibits the Bcr-Abl tyrosine kinase, the constitutive abnormal tyrosine kinase created by the Philadelphia chromosome abnormality in chronic myeloid leukemia (CML). It inhibits proliferation and induces apoptosis in Bcr-Abl positive cell lines as well as fresh leukemic cells from Philadelphia chromosome positive chronic myeloid leukemia.\(^{[1]}\)

Assay method for any drug is very notable for pharmaceutical industries and it is always advisable to select and develop simple, minutest time consuming, precise, accurate and inexpensive method for the estimation of drugs in API pharmaceutical dosage forms and biological samples like blood and serum. Analytical data are used to support formulation studies, assistance in the development of drug syntheses, monitor the (API) bulk pharmaceuticals and finished products.\(^{[2]}\)

Olivia Roth, et al.,\(^{[3]}\) This study indicates the reliability of simple, robust, rapid and inexpensive high performance liquid chromatography method was developed with DAD detection for through IMT concentration determination in human plasma from patients. The validation of the method was based on the guidance of the American Association for Clinical Chemistry and International Union of Pure and Applied Chemistry.\(^{[4-5]}\)

The main aim of this work to develop and validate a LC method for the simultaneous determination of Imatinib mesylate in the presence of its degradation products in pharmaceutical dosage forms. This method mainly used in a stability assay. The method was validated according with International Conference on Harmonization (ICH).\(^{[6]}\) the determination of the analytical parameters, it was shown that the method is linear (r ¼ 0.9994) at concentrations ranging from 0.3 mg/ml to 0.8 mg/ml. The RSD values for intra- and inter-day precision studies were 1.7 and 2.6 and Recoveries ranged between 96.2 and 101.4.

Masatomo Miura et al.,\(^{[7]}\) In this work HPLC-UV method was developed to quantitate Imatinib in human Plasma. The analysis required 100 μL of plasma and involved a solid phase extraction with an Oasis HLB cartridge, which gave recoveries of Imatinib from 73% to 76%. The lower limit of quantification for Imatinib was 10 ng/ml. The linear range of this assay was between 10 and 5000 ng/ml (regression line r\(^2\) > 0.9992). Inter- and intra-day coefficients of variation were less than 11.9%.
Liquid chromatography-tandem mass spectrometry (LC–MS) is used for most quantitative determinations of Imatinib in the plasma of CML patients. However, LC–MS equipment are not regularly available in standard hospital laboratories. On the other hand, several HPLC methods with ultraviolet (UV) detection for the estimation of Imatinib in plasma have been reported.[8-9]

ARUN KUMAR KUNA, et al.,[10] In this work a simple, rapid and economical method was developed by using RP-HPLC method for the estimation of imatinib mesylate in tablet dosage form. The method was validated as per ICH recommendations, the method was validated for specificity, linearity, accuracy, precision, ruggedness and robustness. The system suitability was assessed by six replicate injections of the IMT standard solution at a concentration of 100 mg/ml and chromatogram was obtained. The %RSD of the peak area and retention time of IMT were 2% indicating system suitability. The efficiency of the column is expressed by the number of theoretical plates were found to be 3857 and tailing factor was 1.42 and peak area of the drug linear in the range of 20-120 mg/ml. The correlation coefficient for the was calculated as 0.9994 for IMT indicating a strong correlation between the concentration and AUC and %RSD was 99.12 to 100.19 and 0.33 to 0.49 respectively and % purity is 99.90 and robustness was performed by small changes in chromatographic conditions like: mobile phase ratio, flow rate, change the pH of buffer and column temperature the method is unaffected by these changes in chromatographic condition.

S. Naga Sindhu et al.,[11]: In this developed method include method development and validation of RP-HPLC method for estimation of IMT in pure and pharmaceutical dosage form. The method is validated according to ICH guidelines for specificity, linearity, accuracy, precision, robust and ruggedness, LOD and LOQ. In this method the column is expressed by the number of theoretical plates were found to be 3509 and tailing factor was 1.06 and the correlation(r2) is 0.9991 obtained over a concentration range of 5-30 mg/ml. The limit of quantification and limit of detection were calculated based on standard deviation of the response and slope of linearity curve, the values are 1.16 mg/ml and 3.15 mg/ml.

Jose María Moreno et. al.,[12]. In this developed method include method development of imatinib assay by HPLC in tandem mass spectrometry with solid phase extraction in human plasma. The setting of mass spectrometer were the desolvation temperature was set at 350_C and flow is 10 L/min. Nebulizer pressure was 40 psi and capillary current was 10Na, fragmenter voltage was 150 V IMT and IS. SPE is a suitable technique for sample
preparation process by which compound that are dissolved or suspended in liquid mixture are separated from other compounds in the mixture according to their physical and chemical properties. In this developed method the calibration curve was linear in the range of 10-500ng/mL ($r^2 > 0.998$), the LOQ is 10ng/mL. This developed method is validated according to the recommendations of the FDA, the method is accurate 95-108%, recovery efficiency ( >80 and <120).

Mohammad-Reza Rouinia et al.,[13] This study include HPLC method development and validation of IMT and its major metabolite CGTP74588 in human plasma by applying D-optimal design and The optimization of HPLC procedure involved several variables like composition of mobile phase and pH of the added buffer. The method is proved to show good agreement between the experimental data and predictive values throughout the studied parameter. The method was validated according to FDA guidelines for specificity, linearity, accuracy and precision. The %RSD value is (<14.6), the accuracy ranged from 80.2 to 102.4 for both inter and intra day studies (n=5). The LOQ value for IMT and CGTP74588 were 62.5 ng mL$^{-1}$.

Table: 1 Estimation of Imatinibmesylate in different pharmaceutical formulations and biological samples by HPLC

<table>
<thead>
<tr>
<th>S. no</th>
<th>Drug name</th>
<th>Mobile phase ratio</th>
<th>Column details</th>
<th>Retention time(tr)</th>
<th>Detector</th>
<th>Pharmaceutical formulation/biological sample</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Imatinib mesylate</td>
<td>0.06M KH2PO4 and Acetonitrile(72:28 v/v)</td>
<td>Thermo 5µm hyper cell C18 column was used and associated with pre column silica high purity C18(10x4mm)</td>
<td>7.5min</td>
<td>265nm</td>
<td>Human Plasma</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>Imatinib mesylate</td>
<td>0.5/KH2PO4(PH-3.5) , Acetonitrile and methanol (55:25:20 v/v/v)</td>
<td>C18 (250mm×4.6m m)</td>
<td>12.3 min</td>
<td>265nm</td>
<td>Human plasma</td>
<td>7</td>
</tr>
<tr>
<td>3</td>
<td>Imatinib mesylate</td>
<td>Ammonium phosphate buffer and Acetonitrile (40:60 v/v)</td>
<td>Hypersil, BDS, C18 column (250x4.6mm, 5µ)</td>
<td>3.4min</td>
<td>254nm</td>
<td>Tablet dosage form</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>Imatinib mesylate</td>
<td>Orthophosphoric acid (0.1% v/v): Acetonitrile 70:30(v/v)</td>
<td>C18 G(250x4.6mm i.d, 5µm)</td>
<td>3.25min</td>
<td>266nm</td>
<td>Pharmaceutical dosage form</td>
<td>11</td>
</tr>
<tr>
<td>6</td>
<td>Imatinib mesylate</td>
<td>Methanol/Acetonitrile/triethyl amine/Diammonium phosphate(PH 6.25,0.048 mol$^{-1}$), 20:20:0.1:59.1 v/v/v/v</td>
<td>Chromolith™ performance RP-8e (100mmx4.6m m)</td>
<td>&gt;5min</td>
<td>261nm</td>
<td>Human Plasma</td>
<td>13</td>
</tr>
</tbody>
</table>
NILOTINIB
Nilotinib is chemically 4-methyl-N-[3-(4-methyl-1H-imidazol-1-yl)-5-(trifluoromethyl)phenyl]-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-benzamide, monohydrochloride, monohydrate is a white to slightly yellowish to slightly greenish yellow powder with molecular formula C28H22F3N7O•HCl•H2O and molecular weight 584. Nilotinib is an inhibitor of the BCR-ABL kinase. Nilotinib binds to and stabilizes the inactive conformation of the kinase domain of ABL protein. In vitro, nilotinib inhibited BCR-ABL mediated proliferation of murine leukemic cell lines and human cell lines derived from patients with Ph+ CML. Under the conditions of the assays.\[14\]

Robert A. Parise et. al.,[15]: This study introduced a HPLC-MS assay for determination of the tyrosine kinase inhibitor nilotinib. This method is developed and validated according to FDA guidelines, LC-MS assay is accurate and precise estimation of nilotinib in 0.2ml human plasma or serum. In this method chromatographic separation is achieved by with hydro-synergi column was used and gradient mode is used. This method leads to satisfactory accuracy (92.1-109.5%), intra-assay precision (2.5-7.8%) and inter-assay precision (0-5.6%) were within FDA limits.

Marek Dziadosz et al.,[16]: Have been reported HPLC-UV detection and protein precipitation as a method of quantitative determination of nilotinib with and without internal standard. The protein precipitation extraction method shows to adequate recoveries, accuracies and coefficient variations as 83.9-96.8%, 95.8-105.1% and 2.1-6.1% respectively.

Misato YUKI et al.,[17]: Numerous methods are established on solid phase extraction, it takes a great apportion of time to prepare the samples.[18-19] Furthermore, advanced equipment like that used for LC-MS/MS.[20] is not widely available due to its high maintenance cost. A more suitable method, using HPLC, may be useful for estimate plasma concentrations of Nilotinib in many institutions. We describe the development and validation of an alternative HPLC method for the determination of Nilotinib in human plasma. The method can be used to determine Nilotinib pharmacokinetics in clinical practice to help manage Nilotinib treatment.

The calibration curve be seen linearity at concentrations between 250-5000 ng/ml ($r^2_{2} 0.999$). The % recovery of Nilotinib from plasma was 99.2-3.3%. The coefficients of variation both inter- and intra-day precision were below 9.1%.
S. Pursche et al.,[21]: Have been reported HPLC-UV detection for the determination of BCR-ABL inhibitor nilotinib in plasma, urine, culture and cell preparations. The chromatographic separation was achieved by isocratic mode with C18 column and estimated by UV-detection at 258nm.

Masatomo Miura et al.:[22] This reported method include HPLC-UV detection was developed for the determination of nilotinib in human plasma. The linear range of this assay was between 10-5000 ng/ml (r2 0.9992), intra and inter day coefficient variation were >10.0% and accuracies were within 10.4% over the linear range.

G. Sowjanya et al.,[23] This reported research article include method development and validation of a SIM with RP-HPLC for the determination of nilotinib. Chromatographic separation achieved by isocratic mode on a C18 column, the method was validated according with ICH guidelines, linearity was observed in the concentration range of 0.4-150µg/ml (r2=0.9997), the % RSD range was 0.58-0.62 and 0.67-0.76 for intra-day and inter-day studies respectively and the LOD and LOQ was found to be 0.0785,0.2384.

Table: 2 Estimation of Nilotinib in different pharmaceutical formulations and biological samples by HPLC

<table>
<thead>
<tr>
<th>S.No</th>
<th>Drug name</th>
<th>Mobile phase ratio</th>
<th>Column details</th>
<th>Retention time(tr)</th>
<th>Detector</th>
<th>Pharmaceutical formulation/biological sample</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Nilotinib</td>
<td>Methanol:water:formic acid (50:50:0.1, v/v/v)</td>
<td>C18(5µm, 150mm x 2mm)</td>
<td>Approx 5.4min</td>
<td>270nm</td>
<td>Human plasma and serum</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>Nilotinib</td>
<td>0.05M H3PO4/KH2PO4-acetonitrile (7:3 v/v)</td>
<td>RP-8 250x 4mm</td>
<td>Not more than 10min.</td>
<td>265nm</td>
<td>Biological sample</td>
<td>16</td>
</tr>
<tr>
<td>3</td>
<td>Nilotinib</td>
<td>Acetonitrile:0.01M phosphate buffer(PH 3.0) 42:58 v/v</td>
<td>RP-18 250mm x 4.0mm x 5µm</td>
<td>19.8±0.4min.</td>
<td>266nm</td>
<td>Human plasma</td>
<td>17</td>
</tr>
<tr>
<td>4</td>
<td>Nilotinib</td>
<td>Acetonitrile :0.05M KH2PO4(37:63 v/v, PH 4.03) containing 0.5% acetic acid</td>
<td>C18</td>
<td>8.23min.</td>
<td>258nm</td>
<td>Plasma, urine, culture medium and cell preparation</td>
<td>21</td>
</tr>
<tr>
<td>5</td>
<td>Nilotinib</td>
<td>0.5%KH2PO4(PH 2.5)-acetonitrile-methanol(55:25:20 v/v/v)</td>
<td>Cap cell Pak MG II column(250x4.6 mm)</td>
<td>–</td>
<td>250nm</td>
<td>Human plasma</td>
<td>22</td>
</tr>
<tr>
<td>6</td>
<td>Nilotinib</td>
<td>Water:acetonitrile:acetic acid (20:80:0.3,v/v/v)</td>
<td>C18 (250mmx 4.6 mm i.d, 5µm in particle size)</td>
<td>3.87min.</td>
<td>254nm</td>
<td>Pharmaceutical dosage form</td>
<td>23</td>
</tr>
</tbody>
</table>
DASATINIB
N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxy-ethyl)-1-piperazinyl]-2-methyl-4 pyrimidinyl] amino]-5-thiazole carboxamide monohydrate. Used for the treatment of adults with chronic, accelerated, or myeloid or lymphoid blast phase chronic myeloid leukemia with resistance or intolerance to prior therapy. Also indicated for the treatment of adults with Philadelphia chromosome-positive acute lymphoblastic leukemia with resistance or intolerance to prior therapy. Dasatinib, at nanomolar concentrations, inhibits the following kinases: BCR-ABL, SRC family (SRC, LCK, YES, FYN), c-KIT, EPHA2, and PDGFRβ. Based on modeling studies, dasatinib is predicted to bind to multiple conformations of the ABL kinase. In vitro, dasatinib was active in leukemic cell lines representing variants of imatinib mesylate sensitive and resistant disease. Dasatinib inhibited the growth of chronic myeloid leukemia (CML) and acute lymphoblastic leukemia (ALL) cell lines overexpressing BCR-ABL. Under the conditions of the assays, dasatinib was able to overcome imatinib resistance resulting from BCR-ABL kinase domain mutations, activation of alternate signaling pathways involving the SRC family kinases (LYN, HCK), and multi-drug resistance gene overexpression.

Mohammed G. Kassema, et al.,[24] This reported article have been determined the dasatinib in rabit plasma by HPLC method using fluorescence detection and it is applied to pharmacokinetic study. The method was stastically validated according with FDA for linearity, accuracy, precision, selectivity and stability. The assay linear range of 50.0-3000ng/ml with LOD of 15.0ng/ml, accuracy of dasatinib was within ±15% of theoretical value. The assay of dasatinib is applied to pharmacokinetic study.

D.V. Mhaske et al.,[25]: Introduced a stability indicating HPTLC and LC estimation of dasatinib in pharmaceutical dosage form this method is very sensitive, accurate for quantitative determination of dasatinib in the prescence of degradation products. The frist method high performance thinlayer liquid chromatography used by densitometric measurement and second method is based on RP-HPLC using C18 column and this both method validated according with ICH guidelines, this method is effectively separate the drug from its degradation products and it is employed as stability indicating methods subjected to acid, alkali hydrolysis, oxidation, dry heat, photo-degradation.
Arun Kumar Kalekar et al.,\textsuperscript{[26]}: Introduced a method development and validation of dasatinib in bulk and pharmaceutical dosage form by RP-HPLC and separation was achieved by isocratic mode with C18 column. The % recoveries of API from dosage form ranged from 98.5-99.8%.

Bandi Ramachandra et al.,\textsuperscript{[27]}: Have been introduced analytical method validation of dasatinib in bulk and pharmaceutical dosage form by using RP-HPLC, the chromatographic separation was eluted through C18 column by isocratic mode. This method was validated as per ICH guidelines, parameters are specificity, linearity, accuracy, precision, robustness and ruggedness. The calibration curve was linear concentration range of 5-30µg/ml.

Thulase Nadh Reddi et al.,\textsuperscript{[28]}: This reported article consists mainly method development and validation of stability indicating by RP-HPLC method of dasatinib in tablet dosage form, chromatographic separation was done by isocratic mode by using C18 column. The method of dasatinib linear over a range of 5.0-30.0µg/ml, the correlation coefficient is 0.999, % RSD is >2 and % recovery of API from tablet dosage form ranged from 100.2-100.9 and the method validation is done by as per ICH guidelines.

Andrea Davies, et al.,\textsuperscript{[29]}: Have been reported simultaneous estimation of several tyrosine kinase inhibitors like imatinib and nilotinib. This developed method mainly determination of nilotinib, imatinib in human plasma by using ultra–voilet high performance liquid chromatography. This method sample preparation is carried with solid phase extraction process, separation of compounds through C6 column under isocratic mode by using methanol:50mM ammonium acetate as mobile phase in the ratio of (65:35v/v). Calibration curves \((n = 4)\) were linear in the range 12,000–100 ng/ml with lines of regression forced through the start with \(r^2\) values as follows (±standard deviation), CGP-74588, \(r^2 = 0.9999\) (SD±0.0001), Imatinib, \(r^2 = 0.9999\) (SD±0.0002), and Nilotinib, \(r^2 = 1.0000\) (SD±0.0001).

Antonio D’Avolioa, et al.,\textsuperscript{[30]}: This reported method is simultaneous estimation of tyrosine kinase inhibitors like imatinib, dasatinib and nilotinib in human peripheral mononuclear cell by using HPLC-MS method. Chromatographic separation of drugs and internal standard (quinoxaline) was achieved by C18 column and gradient elution mode is used for HPLC separation with the mobile phase of acetonitrile:water:formic acid.

MS conditions: Detector settings were ESI, positive polarity ionization; capillary voltage 3.5 KV; source temperature 110 °C; desolvation temperature 350 °C; nitrogen desolvation flow
800 L/h; and nitrogen cone flow 100 L/h. Ions detected, in single ion recording (SIR) mode, where m/z 493.8 with a cone voltage of 45 V for Imatinib, m/z 487.5 with a cone voltage of 35 V for Dasatinib, m/z 529.5 with a cone voltage of 35 V for Nilotinib and m/z 313.0 with a cone voltage of 50 V.

Eva Kral et al.,[31]: This reported method describes the simultaneous estimation of imatinib, nilotinib and dasatinib in dried blood spot by HPLC- MS. The chromatographic separation of drugs achieved by C18 column and eluted with gradient mode through the mobile phase A was 0.1% formic acid in milliQ water and mobile phase B was 100% acetonitrile. The MS was operated in positive ESI mode and the drying temperature, flow rate were 275 c and 5L/min. Mass spectrometer was operated in MRM mode. This method was validated in terms of linearity, selectivity, specificity, accuracy, precision and stability.

Table: 3 Estimation of Dasatinib in different pharmaceutical formulations and biological samples by HPLC

<table>
<thead>
<tr>
<th>S.NO.</th>
<th>Mobile phase</th>
<th>Column details</th>
<th>Retention time</th>
<th>detector</th>
<th>Pharmaceutical dosage form/biological sample</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.02M potassium dihydrogen phosphate:me thanol(10:90 v/v)</td>
<td>C18(260mmx2.4mm i.d x 5µm)</td>
<td>1.8min for Dasatinib, IS-8.1min.</td>
<td>340&amp;374nm</td>
<td>Rabbit plasma using fluorescence detection and its application to pharmacokinetic study</td>
<td>24</td>
</tr>
<tr>
<td>2</td>
<td>Methanol:20 mM ammonium acetate with acetic acid (40:55 v/v), PH:3.0</td>
<td>C18 5µm,25cm x 4.6mm,i.d</td>
<td>8.2 min±0.2min.</td>
<td>280nm</td>
<td>Pharmaceutical dosage form</td>
<td>25</td>
</tr>
<tr>
<td>3</td>
<td>Methanol:acetonitrile(50:50 v/v)</td>
<td>C18 5µm,25cm x 4.6mm,i.d</td>
<td>7.236min.</td>
<td>315nm</td>
<td>Bulk and its pharmaceutical dosage formulation</td>
<td>26</td>
</tr>
<tr>
<td>4</td>
<td>Sodium phosphate buffer: methanol (70:30v/v)</td>
<td>(100mmx4.6m,5µm C8</td>
<td>5.78±0.1min.</td>
<td>323 nm</td>
<td>Pharmaceutical dosage form</td>
<td>27</td>
</tr>
<tr>
<td>5</td>
<td>Buffer(triethyl)</td>
<td>C18 BDS</td>
<td>6.8min.</td>
<td>315nm</td>
<td>Tab let dosage</td>
<td>28</td>
</tr>
</tbody>
</table>
CONCLUSION: A sensitive and accurate RP-HPLC methods, stability-indicating HPLC, HPLC-PDA, HPLC-UV, stability indicating HPTLC and HPLC-MS, with solid phase extraction methods was developed for the estimation of the above selected anti-cancer drugs (Imatinib Mesylate, Nilotinib, Dasatinib) in pharmaceutical dosage forms, human plasma, Rabbit plasma, serum, urine, culture medium, cell preparations etc. The above methods was evaluated for Specificity, Linearity, Accuracy, Precision, Ruggedness and Robustness as per ICH&FDA guidelines. From this study it is clear that it is possible to develop a new sensitive and accurate HPLC method for anti-cancer drugs.

REFERENCES
26. Arun Kumar Kalekar1*, B Ananta Rao1, Yasawanth Allamneni1, P Dayananda Chary1, S Shanth Kumar1, Navya Allamneni2 Am, J. PharmTech Res. 2012; 2(4).