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A NEW ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF DASATINIB IN HUMAN PLASMA BY LC–ESI-MS/MS

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ABSTRACT

A specific and selective liquid chromatography/tandem mass spectrometry (LC–MS/MS) technique was desirable for the assessment of dasatinib in human plasma. Drug and internal standard were extracted utilizing liquid–liquid extraction technique using ethyl acetate and methanol in proportion of 4:2. Reversed phase high performance liquid chromatography (RP-HPLC) was carried out using Zorbax (50×4.6 mm i.d., 5 µm) C_{18} analytical column with a simple isocratic mobile phase composed of 0.1% formic acid, methanol and acetonitrile, (10:30:60, *v/v*). Detection was executed on a triple quadrupole mass spectrometer retaining electrospray ionization method, operating in multiple reaction monitoring (MRM), with the transitions of m/z 488.16/140.02 for dasatinib and m/z 496.15/144.2 for dasatinib-D8, respectively, in the positive ionization mode. The linearity was processed a concentration range of 1.0–1200.0 ng/mL for the analyte. All obtained recoveries were higher than 91.0% while the accuracy was in the range of 1.52% to 3.48% of relative error and the relative standard deviation was below 4.11% for all investigated drugs by the proposed method. The validated method has highly sensitive and nice recoveries values from plasma, utilized for the bioequivalence and pharmacokinetic studies.

Keywords: Dasatinib, Cancer, LC–MS/MS, Validation, Sensitivity and Accuracy

INTRODUCTION

Dasatinib is a tyrosine kinase inhibitor used for the treatment of lymphoblastic or chronic myeloid leukemia with resistance or intolerance to prior therapy. It is an oral dual BCR/ABL and SRC family tyrosine kinase inhibitor approved for use in patients with chronic myelogenous leukemia (CML) **[1-3]**. The main targets of Dasatinib, are BCRABL, SRC, Ephrins and GFR. 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 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 **[4-6]**. Dasatinib drug chemically titled as *N*-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl) -1-piperazinyl] -2-methyl -4-pyrimidinyl] amino] -5-thiazole carboxamide monohydrate. Its chemical formula and molecular mass are $C_{22}H_{26}ClN_7O_2S$ and 488.01 g/mol respectively **(Figure 1)**.

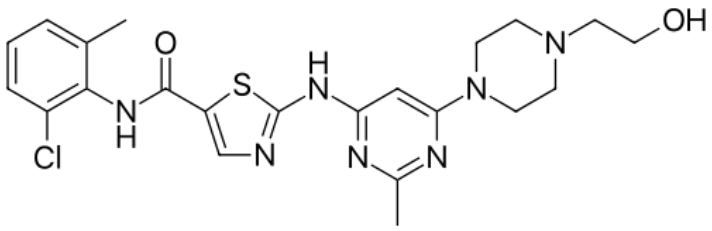

Figure 1: Structure of dasatinib

Literature review on Dasatinib reveals that a small number of analytical methods have been reported for dasatinib based on high-performance thin-layer chromatography (HPTLC) or high-performance liquid chromatography (HPLC) or radioactive labeling methods **[7–9]** and a liquid chromatography–mass spectrometry (LC–MS) method for plasma determination of this analyte **[10–13]**. Present work was aimed to develop a highly specific, selective and accurate LC–MS/MS technique was desirable for the assessment of dasatinib in human plasma.

MATERIALS AND METHODS

Reagents and chemicals

The standards of dasatinib (purity: 99.81%) and dasatinib-D8 (purity: 99.86%) used as internal standard (IS) were gained from the Hetero drugs, Hyderabad, India. Methanol and acetonitrile of HPLC purity were acquired from Merck, Mumbai, India. Deionized water was produced by a Milli-Q water system (Millipore, MA, USA).

LC–MS/MS system and its conditions

The LC–MS/MS system consisting of an Agilent/1200 liquid chromatographic instrument with a binary pump-SL and an Agilent/6460 triple-quadrupole mass spectrometer with electrospray ionization (ESI) source (CA, USA). Chromatographic data was processed by a MassHunter version B.01.04 software. Zorbax (50×4.6 mm i.d., 5 µm) C_{18} analytical column with a simple isocratic mobile phase composed of 0.1% formic acid, methanol and acetonitrile, (10:30:60, *v/v*). Detection was executed on a triple quadrupole mass spectrometer retaining electrospray ionization method, operating in multiple reaction monitoring (MRM), with the transitions of m/z 488.16/140.02 for dasatinib and m/z 496.15/144.2 for dasatinib-D8, respectively, in the positive ionization mode. The flow rate of 0.70 mL/min and collision energy of 20 eV were utilized in the chromatographic elution. The injection volume and auto-sampler temperatures were set to 10 µL and 5.0 ºC respectively. MS/MS analysis was controlled using multiple reaction monitoring (MRM) scan modes. The MS/MS setting parameters were set as follows: source temperature, 550 ºC; capillary voltage, 5.5.0 kV; nebulizer gas pressure, 45 psi and drying gas (N_2) flow, 10 L/min.

Protocol for standard and quality controls

1.0 mg/mL individual stock solutions of dasatinib and IS were prepared in 80% methanol in water (diluent) separately. The stock solution of dasatinib was then serially diluted with diluent to obtain the working solutions. The IS working solution of 200 ng/mL was also processed by diluting the IS stock solution with diluent. All the solutions were kept at -20 ºC and brought to room temperature before use.

Calibration standard standards of dasatinib (1, 2.8, 35, 120, 315, 600, 900 and 1200 ng/mL) were obtained by spiking the appropriate working solutions to blank plasma. Quality control (QC) samples at low, medium and high concentrations (2.8, 600 and 900 ng/mL) were prepared separately in the similar manner.

Protocol for sample preparation

A 250 µL aliquot of plasma sample was located in a 10 mL plastic tube followed by addition of 150 µL of IS working solution was then added to all samples except the blank samples. The mixture was extracted with 5.0 mL of ethyl acetate and methanol in proportion of 4:2 by vortex-mixing at a high speed for 5.0 min and shaking for 20 min. Thereafter, the samples were centrifuged at 5.0 ºC for 15 min at 5000 rpm. The upper organic layer was transferred to clean glass tubes and evaporated to dryness under a gentle stream of nitrogen. The dry residue was reconstituted with 100 µL of mobile phase and a 10µL aliquot was injected into the LC–MS/MS system for analysis.

Analytical method validation

The analytical method was validated to meet the acceptance criteria of the Food & Drug Administration (FDA) guidelines **[14, 15]**.

RESULTS AND DISCUSSION

Mass spectrometry

When the neat solution of dasatinib was infused a precursor ion of *m*/*z* 488.16 was observed in the positive ionization mode. Upon fragmentation of the precursor ion, fragments of *m*/*z* 321.4, 319.13 and 140.02 were detected. Fragment of dasatinib ion with *m*/*z* 140.02 was detected with the greatest intensity. Due to commercial availability of the dasatinib–D8 internal standard, it was uses as internal standard. Under the optimal conditions, the MRM transitions of *m*/*z* of m/z 488.16/140.02 for dasatinib and m/z 496.15/144.2 for dasatinib-D8 were monitored.

Selectivity and specificity

No interference peak was detected for dasatinib and IS from plasma samples. The typical chromatograms of blank plasma and plasma spiked with 1.0 ng/mL of dasatinib (LLOQ level) and IS were shown in **Figure 2**. The retention times of dasatinib and IS was 2.6 min.

Linearity of calibration curves and sensitivity

Calibration curves were processed for each batch analysis in the concentration ranges of 1.0–1200 ng/mL for dasatinib in plasma **(Table 1)**. The mean regression equation obtained for dasatinib was: y = 0.005x + 0.0088 (n = 6) for dasatinib, where y is the ratios of analytes to IS and x is the plasma concentrations **[17-19]**. The r^2 was found to be 0.9997. The LLOQ of the analyte was set to 1.0 ng/mL with precision and accuracy less 2.84% and the S/N values were more than 10.

Precision, accuracy and recovery

Intra-day and inter-day precision and accuracy are shown in **Table 2** and **Figure 3**. Intra-day precision ranged from 1.52% to

3.48% (RSD) for dasatinib, while the accuracy was within 1.32 to 3.93% of relative error. Similarly, for the inter-day experiments, the precision varied from 1.45% to 3.52% (RSD) for dasatinib, while the accuracy was within 1.49 to 4.11% of relative error. This proved that the method was accurate and precise over the range of the assay **[20]**.

The mean recoveries of dasatinib ranged from 94.62% to 101.85% at three QC levels **(Table 3)**. The simple liquid–liquid extraction procedure showed that dasatinib and the IS (98.46%) were excellently recovered in plasma **[18]**.

Matrix effects

The results of matrix effects are shown in **Table 4**. The corresponding peak area ratios of the analyte/IS dissolved with blank plasma extracts to those dissolved with mobile phase ranged from 94.92% to 102.31% for dasatinib at LQC level and 94.17% to 102.37% at HQC level. These results suggested that the matrix effects of the analytes were negligible under the present LC–MS/MS conditions **[19, 20, 21]**.

Stability tests

The stability of dasatinib was tested after subjecting the QC samples to different storage conditions. The applied conditions includes short term stability at room temperature for 8 h, long term stability after storage at −20 °C for 30 day, three complete freeze-thaw cycles (freezing at −20 °C for 12 h) and the processed sample (extract) stability after 24 h at 4 °C. The stability results of QC samples at plasma and processed sample are listed in **Table 5**. The calculated accuracies for dasatinib determination were within the range of 93.83%–103.64% of the nominal concentration which lies within the acceptable range. As a result, dasatinib was deemed to be stable under different studied storage conditions **[15-21]**.

Table 1: Calibration standards for dasatinib

CS-ID	Concentration (ng/mL)	Mean area[a]	Area ratio
CS-1	1	846	0.004999
CS-2	2.8	2368.8	0.013987
CS-3	35	29610	0.17447
CS-4	120	101520	0.6
CS-5	315	269490	1.592439
CS-6	600	527400	3.112955
CS-7	900	761400	4.496784
CS-8	1200	1015200	6.003229

a:6 replicates.

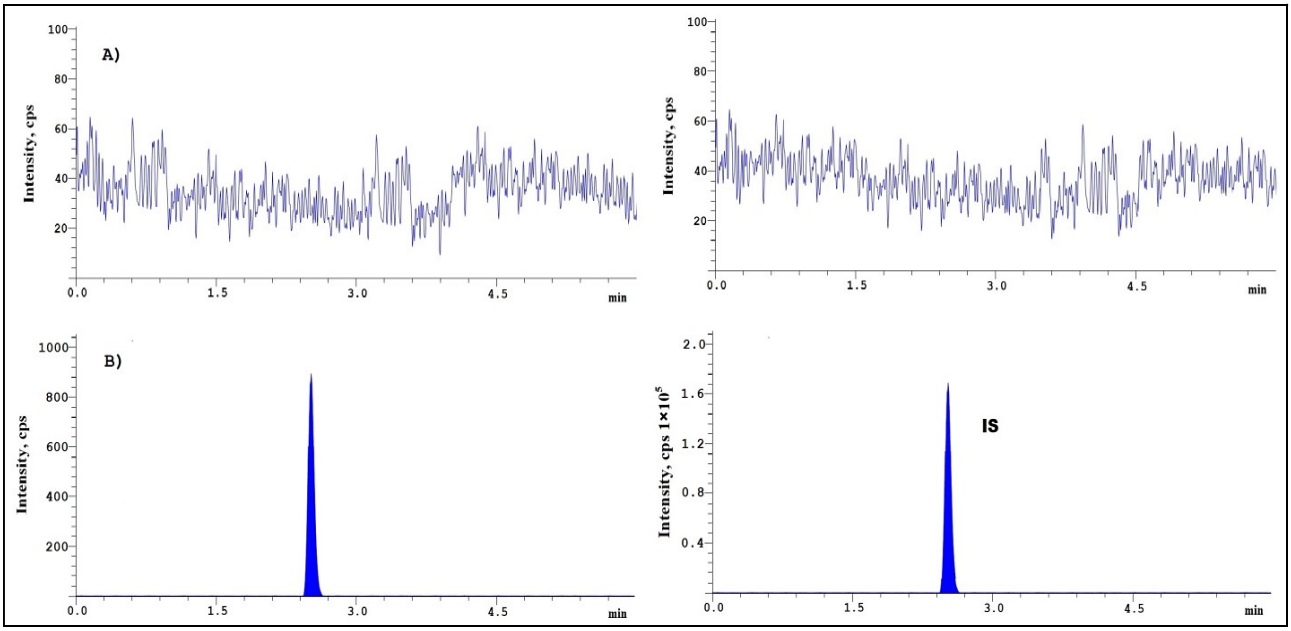

Figure 2: Representative chromatograms of Dasatinib A) Blank and B) LLOQ samples

Table 2: Intra- and inter-day precision and accuracy of the LC–MS/MS method to determine dasatinib in plasma (n = 3 days, 6 replicates per day)

Spiked conc. (ng/mL)	Intra-day (n = 6)			Inter-day (n = 6 × 3)		
	Measured conc. (mean ± SD: ng/mL)	Precision (RSD %)	Accuracy (RE %)	Measured conc. (mean ± SD: ng/mL)	Precision (RSD %)	Accuracy (RE %)
1	0.98±0.03	3.06	2.0	0.967±0.019	1.96	3.3
2.8	2.69±0.08	2.97	3.93	2.685±0.075	2.79	4.11
600	582.09±20.26	3.48	2.98	591.02±20.84	3.52	1.49
900	888.14±13.52	1.52	1.32	886.14±12.84	1.45	1.54

RSD: Relative standard deviation; RE: relative error

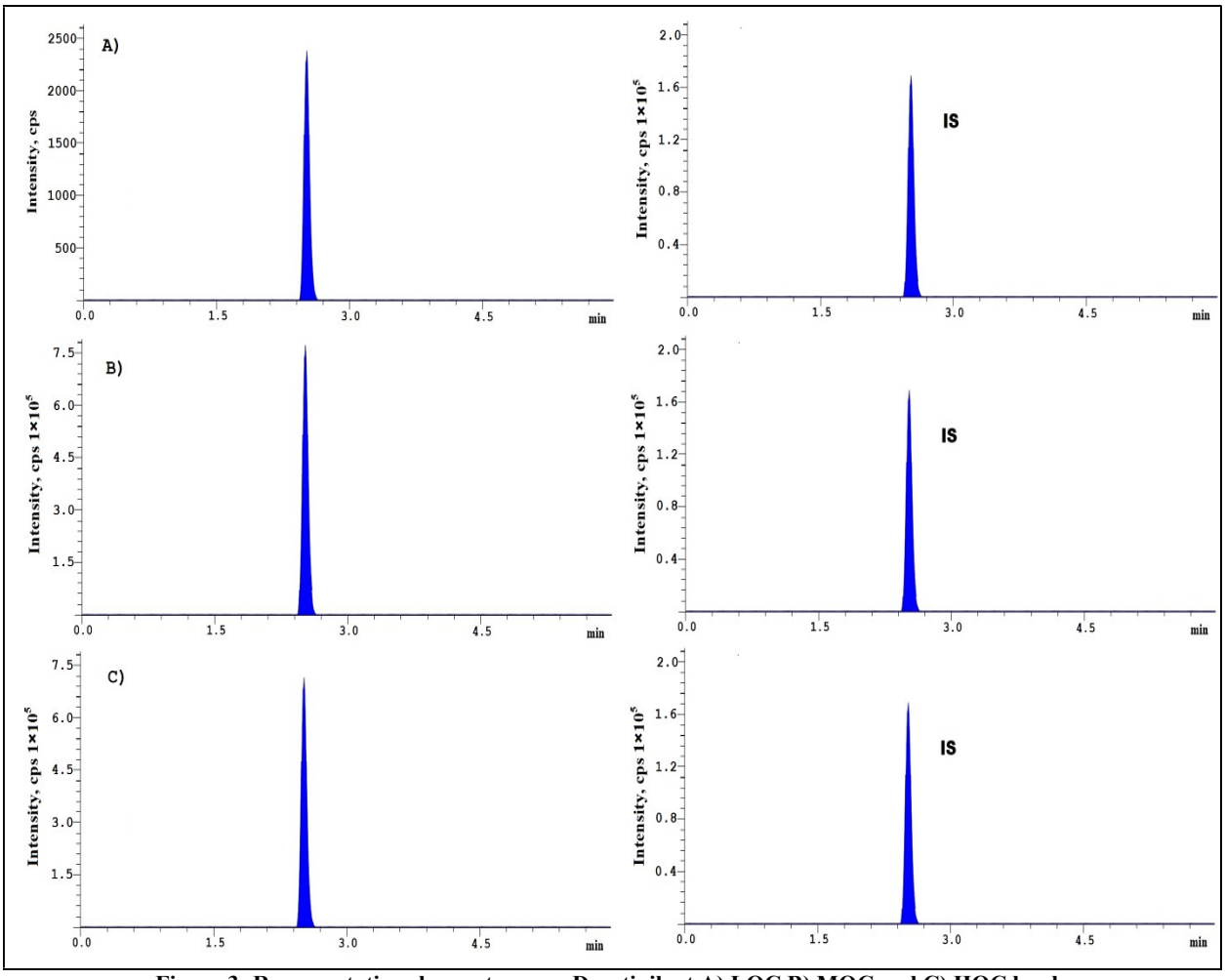

Figure 3: Representative chromatograms Dasatinib at A) LQC B) MQC and C) HQC levels

Table 3: Extraction recovery rates of analytes

Concentration level	X	Y	% Recovery	% Mean recovery	%RSD
LQC	2359	2232	94.62	97.31	3.32
MQC	507862	484855	95.47		
HQC	761534	775622	101.85		
IS	171385	168745	98.46		

X, mean recoveries of unextracted samples; Y, mean recoveries of extracted samples

Table 4: Matrix effect for dasatinib at LQC and HQC levels

	LQC			HQC		
S. No	Peak area in absence of matrix	Peak area in presence of matrix	Matrix factor	Peak area in absence of matrix	Peak area in presence of matrix	Matrix factor
1	2368	2326.56	98.25	761400	725690	95.31
2	2407	2291.7047	95.21	761395	717006	94.17
3	2359	2414.2006	102.31	761365	742635	97.54
4	2375	2321.325	97.74	761425	779471	102.37
5	2395	2273.334	94.92	761419	776343	101.96
6	2361	2277.8928	96.48	761391	725453	95.28
Mean			97.49			97.78
± SD			2.72			3.58
% RSD			2.79			3.66

RSD: Relative standard deviation; SD: standard deviation

Table 5: The stability data of dasatinib

Storage condition	LQC 2.8 ng/mL		MQC 600 ng/mL		HQC 900 ng/mL	
	Accuracy (Mean%)	Precision (RSD%)	Accuracy (Mean%)	Precision (RSD%)	Accuracy (Mean%)	Precision (RSD%)
30 day at −20 °C	94.75	2.65	95.28	2.95	102.38	1.85
Extract, 24 h at 4 °C	96.27	4.1	97.31	3.21	95.37	3.24
Room temp., 8 h	102.32	3.52	103.64	3.65	94.39	2.75
3 freeze-thaw cycles	95.34	2.94	93.83	31.68	97.42	3.72

RSD: Relative standard deviation

CONCLUSION

A specific and validated LC-MS/MS technique was developed for the estimation of dasatinib drug in human plasma. Validation process was in compliance with FDA guidelines and the procedure was Ecofriendly, precise and sensible with LLOQ at 1.0 ng/mL and rapid with total run time equals 2.6 min. The intra-day and inter-day accuracies were within 1.52% to 3.48% of relative error and the relative standard deviation of precision was less than 4.11%. The drug was sufficiently stable under different analytical conditions. LLE method was optimized for dasatinib extracting from plasma with mean percent recoveries of 94.62% by utilizing the dasatinib-D8 as an internal standard. The validated method has highly sensitive and nice recoveries values from plasma, utilized for the bioequivalence and pharmacokinetic studies.

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