Determination of Dasatinib in Human Plasma by Using Liquid Chromatography-Tandem Mass Spectrometry

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Abstract

A liquid chromatography tandem mass spectrometry (LC-MS/MS) method was developed and validated to determine dasatinib (DAS) in human plasma. Protein precipitation procedure was used in sample preparation. The analyte and internal standard (dasatinib-d8 (DASD8)) were separated on Waters Atlantis dC18 Column (75 × 4.6 mm, 3.5 μm). Multi reaction monitoring detection was performed by electrospray ionization in the positive ion mode. The MRM transitions of m/z 488.1 > 401.1 for DAS and 496.15 > 406.1 for DASD8 were used to quantify. The standard curve was found to be linear in the range of 1-400 ng/mL for dasatinib. The lower limit of quantification (LLOQ), using 200 μL human plasma, was 1 ng/mL. The developed and validated method could be applied to the bioequivalence study in human plasma samples.

Keywords

Dasatinib, Dasatinib-d8, Human plasma, LC-MS/MS

Introduction

Dasatinib is an anticancer tyrosine kinase inhibitor. Tyrosine kinase inhibitors are directed against tyrosine kinases, which play an essential role in the transduction of growth signals in cells [1]. Dasatinib is indicated for treatment of chronic myelogenous leukemia and philadelphia chromosome-positive acute lymphoblastic leukemia in chronic phase [2].

A number of methods have been developed for determination of DAS in human plasma individually or in combination with other drugs [3-10]. Common methods for the determination of dasatinib in human plasma are utilizing either protein precipitation (PP), solid phase extraction (SPE) or liquid-liquid extraction (LLE) for sample

preparation [3-10]. However, the procedures employed in these studies have relatively longer run times which are disadvantage to be employed for batches with large number of sample [3-6,8,9]; complicated and expensive sample preparation steps [7,10].

Development of simpler, inexpensive sample-preparation techniques is an important issue in chemical analysis, so that; protein precipitation technique is chosen. The aim of this present study was to develop a simple, rapid, cheap and sensitive method for DAS determination using protein precipitation in plasma samples. This method could be applied to the samples obtained from clinical trials.

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Table 1: MS parameters for DAS and DASD8.

Compound	Precursor	Product	Dwell time	Q1 pre-bias (V)	Collision energy (V)	Q3 pre-bias (V)
DAS	488.1	401.10	100	-17.0	-29.0	-27.0
DASD8	496.15	406.1	100	-23.0	-31.0	-27.0

Experimental

Chemicals and materials

Dasatinib, Dasatinib D8 were obtained from Clearysynth (Mumbai, India), respectively. HPLC grade methanol, formic acid and ethanol were purchased from Merck (Darmstadt, Germany). K₂EDTA drug free (blank) human plasma was obtained from Sera Laboratories International Ltd (UK). High purity water was prepared in-house using a Milli-Q water purification system obtained from Millipore (USA).

Stock solutions, calibration standards and QCs

Stock solutions were made by dissolving suitable amount of DAS in methanol. Further standard working solutions (0.02, 0.04, 0.2, 1, 2, 4, 7.2, 8 µg/mL for calibration standards and 0.02, 0.06, 0.8, 3.2, 6.4, 8 µg/mL for quality control samples) were obtained by serial dilution of the stock solutions with methanol. The working internal standard solution including DASD8 was prepared in methanol at concentration of 0.4 µg/mL (IS). Stock solutions of DAS and DASD8 were stored at -20 °C. For preparation of calibration standards and quality control samples, appropriate aliquots of the stock solution DAS or working solutions were added to drug-free human plasma. The calibration curve ranged from 1 to 400 ng/mL for DAS. The quality control (QC) samples were prepared at four concentration levels of 1 (LLOQ, the lower limit of quantification), 3 (QC Low), 40 (QC Medium 1), 160 (QC Medium 2), 320 (QC High) ng/mL for DAS in a manner similar to preparation of calibration curve samples from stock solutions.

Instrumentation

The LC-MS/MS system (Shimadzu, Japan) consisting of LC-20AD XR solvents pumps, SIL-20ACXR autosampler, CTO-10 AS VP column oven, FCV-20AH2 valve unit and Shimadzu 8040 Tandem Mass Spectrometer. Waters Atlantis dC18 (75 × 4.6 mm, 3.5 µm) column was used for separation with methanol, water (60:40 v/v) containing 0.1% v/v formic acid solution as a mobile phase at the flow rate of 0.7 mL/min. The total run time for

each analysis was 3 min. Shimadzu 8040 Tandem Mass Spectrometer was used equipped with electrospray ionization in the positive ion mode. The optimization of DAS and DASD8 in Shimadzu's LCMS systems was performed by using Shimadzu LabSolutions Software version 5.82 SP1 based on a rapid series of automated flow injection analyses. The multiple reaction monitoring (MRM) transitions were performed at m/z 488.1→401.10 and 496.15→406.1 for DAS and DASD8. The nebulizing gas and drying gas flow rates and the ESI voltage were 2.5 L/min, 15 L/min and 5500 V, respectively. The gas used for nebulizing and drying was high pure nitrogen generated by Peak Scientific NL-60. Detailed information for MS parameters is represented in [Table 1](#).

Sample preparation

Sample preparation (200 µL human plasma) resulted in a clear colorless supernatant by a short centrifugation step (5500 rpm for 10 min) after protein precipitation using 50 µL methanolic IS (0.4 µg/mL) and methanolic formic acid solution (0.1% v/v) as a precipitation reagent. An aliquot of 20 µL of the supernatant was injected into the LC-MS/MS system for analysis.

Results and Discussion

Method validation

The method was validated according to both US-FDA Bioanalytical Method Validation Guidance [11] and European Medicines Agency Guideline on Bioanalytical Method Validation [12] with respect to selectivity and linearity, calibration curve, precision and accuracy, recovery, matrix effect and stabilities. Method validation was performed with K₂EDTA human plasma.

Selectivity: [Figure 1](#) shows the chromatograms (a) Blank plasma spiked with IS and analytes at LLOQ; and (b) Drug-free human plasma. The method selectivity was determined by analyzing eight different sources of human plasma to demonstrate the lack of chromatographic interference at the retention time of DAS and DASD8.

Linearity: The calibration curves were created

Figure 1: MRM chromatograms of 1 ng/mL (LLOQ) of a) DAS Spiked with internal standard; b) Blank human plasma.

Table 2a: Within-batch precision and accuracy of the method for determining DAS in plasma samples.

Nominal Conc. (ng/mL)	Batch No: 1 (n = 6)			Batch No: 2 (n = 6)			Batch No: 3 (n = 6)		
	Conc. Found (mean $\pm$ SD; ng/mL)	RE (%)	CV (%)	Conc. Found (mean $\pm$ SD; ng/mL)	RE (%)	CV (%)	Conc. Found (mean $\pm$ SD; ng/ mL)	RE (%)	CV (%)
1 (QC1)	1.05 $\pm$ 0.02	4.65	2.10	1.00 $\pm$ 0.04	0.09	3.83	1.00 $\pm$ 0.05	0.05	5.08
3 (QC2)	3.06 $\pm$ 0.10	1.93	3.37	3.01 $\pm$ 0.06	0.39	2.11	3.07 $\pm$ 0.02	2.27	0.71
40 (QC31)	39.97 $\pm$ 0.36	-0.08	0.90	40.03 $\pm$ 0.46	0.07	1.14	40.05 $\pm$ 0.38	0.13	0.94
160 (QC32)	167.40 $\pm$ 1.96	4.63	1.17	163.03 $\pm$ 1.34	1.89	0.82	164.54 $\pm$ 0.75	2.84	0.45
320 (QC4)	323.66 $\pm$ 4.65	1.14	1.44	328.08 $\pm$ 3.12	2.52	0.95	328.79 $\pm$ 7.02	2.75	2.14

by plotting the peak area ratios of the various analytes to internal standard versus nominal concentration of the analytes standards. Both calibration curves were regressed using a linear equation with weighting factor of $1/X^2$. Calibration curves were linear with coefficient of correlation (r^2) values more than 0.9982. Range was 1-400 ng/mL for DAS including the LLOQ. Calibration curves were constructed from blank sample (processed matrix sample without analyte and without IS), a zero sample (processed matrix with IS) and eight

non-zero samples. The standard concentration must be within 15% deviation from the nominal value except at LLOQ. The LLOQ was defined as the lowest concentration yielding a signal to noise ratio of least 10 with a coefficient of variation (precision, CV) < 20% and accuracy of 80-120%. At least 75% of eight non-zero samples must be met exception criteria including acceptable LLOQ and ULOQ [11,12].

Accuracy and precision: The intra- and inter-day precisions and accuracies of LLOQ, QC

Table 2b: Between-batch precision and accuracy of the method for determining DAS in plasma samples.

Nominal Conc. (ng/mL)	Batch No: 1-3 (n = 18)		
	Conc. Found (mean $\pm$ SD; ng/mL)	RE (%)	CV (%)
1	1.02 $\pm$ 0.04	1.59	4.21
3	3.05 $\pm$ 0.07	1.53	2.34
40	40.02 $\pm$ 0.38	0.04	0.94
160	164.99 $\pm$ 2.30	3.12	1.34
320	326.84 $\pm$ 5.40	2.14	1.65

Conc: Concentration; n: Replicates at each concentrations; RE: Relative Error; CV: Coefficient of Variation; SD: Standard Deviation.

Low, QC Medium 1, QC Medium 2 and QC High were summarized in Table 2a and Table 2b. Six replicates of QC samples were analyzed at five concentration levels respectively to evaluate the intra-day precisions and accuracies. This process was repeated on three consecutive days in order to evaluate inter-day precisions and accuracies.

Matrix effect: Matrix effect was assayed at two concentration (QC Low and QC High) levels for DAS. Six different plasma lots (including one hemolytic and one lipemic plasma) free of any significant interference at the retention time (RT) of analytes and internal standards, were selected. Experiments were performed at low (QC2) and high (QC4) quality control levels in six replicates using the ratios of peak areas of the blank plasma samples spiked after extraction to those of pure standard solutions containing analyte at the same concentrations. The precision (%CV) of QC Low and QC High were 3.29% and 6.18% for DAS. The matrix effect results were summarized in Table 3.

Recovery: Recovery was determined by comparing the analytical results of extracted samples with those of extracts of control matrix spiked with analyte post extraction. Recovery was estimated at three concentration levels (3 ng/mL, 160 ng/mL and 320 ng/mL) by comparing the mean analytes responses of six extracted samples with those of appropriately diluted standard solutions. For three concentration levels; recovery was 113.08%, 105.66%, 108.37% respectively. So that for our validated method mean overall recovery for DAS was 109.04 $\pm$ 3.76%. The recovery of internal standard was measured in a similar manner using corresponding medium QC samples as reference.

Table 3: Results of matrix effects for dasatinib (n = 6).

	Plasma QC Low						Plasma QC High					
	Mean Peak Area	Matrix Factor	Mean Peak Area IS (n = 6)	IS Matrix Factor	IS Normalized Matrix Factor		Mean Peak Area	Matrix Factor	Mean Peak Area IS (n = 6)	IS Matrix Factor	IS Normalized Matrix Factor	
Pure Solution	21123.83	-	347032.50	-	-	Pure Solution	2389447.50	-	347032.50	-	-	-
Matrix 1	19708.33	0.93	365374.33	1.05	0.87	Matrix 1	2554665.50	1.07	365374.33	1.05	1.06	1.06
Matrix 2	20253.17	0.96	384316.00	1.11	0.87	Matrix 2	2936468.50	1.23	384316.00	1.11	1.11	1.11
Matrix 3	22970.17	1.09	437570.33	1.26	0.86	Matrix 3	3178715.17	1.33	437570.33	1.26	1.06	1.06
Matrix 4	22732.67	1.08	433044.33	1.25	0.86	Matrix 4	2841594.17	1.19	433044.33	1.25	0.95	0.95
Matrix 5	23380.50	1.11	434474.83	1.25	0.88	Matrix 5	2826883.17	1.18	434474.83	1.25	0.95	0.95
Matrix 6	23868.50	1.13	417903.33	1.2	0.94	Matrix 6	2870188.67	1.20	417903.33	1.20	1.00	1.00
				Mean IS Normalized Matrix Factor	0.88					Mean IS Normalized Matrix Factor	1.013	
				CV (%)	3.29					CV (%)	6.18	

Table 4: Results of stability of DAS in human plasma under different storage conditions.

DASATINIB				
Storage Condition	Nominal Conc. (ng/mL)	Conc. Found mean $\pm$ SD (ng/mL)	CV (%)	RE (%)
Autosampler stability ^a	3	3.17 $\pm$ 0.12	3.63	5.70
	320	337.06 $\pm$ 7.81	2.32	5.33
Short-term plasma stability ^b	3	3.20 $\pm$ 0.12	3.65	6.67
	320	341.87 $\pm$ 6.95	2.03	6.84
Long-term stability ^c	3	3.24 $\pm$ 0.12	3.63	8.18
	320	342.75 $\pm$ 9.09	2.65	7.11
Freeze-thaw stability ^c	3	3.05 $\pm$ 0.09	2.80	1.63
	320	325.64 $\pm$ 4.41	1.35	1.76

RE: Relative Error (Accuracy); CV: Coefficient of Variation (Precision); SD: Standard Deviation; ^aKept at autosampler temperature, 10 °C; ^bStored at room temperature; ^cStored at -70 °C.

Table 5: Results of long term stability of stock for dasatinib and dasatinib d8.

	Mean Peak Area at 0 hr	Mean Peak Area after 7 days stored at -20 °C	RE (%)
Dasatinib stock solution	4277389.17	4288089.33	0.250
Dasatinib D8 stock solution	742359.00	767394.67	3.372

Acceptance criteria: RE should be within $\pm$ 10% for the analyte.

Mean recovery of internal standard was 97.66% for DASD8.

Stability: The bench top stability was examined by keeping replicates of spiked plasma with low and high quality control samples at room temperature for approximately 5 hours for DAS. Freeze-thaw stability of samples were obtained over 3 cycles, by thawing at room temperature for 1 hour and refrozen for 12-24 h. The processed samples were stable up to 47.5 hours in autosampler at 10 °C. Long term plasma stability was evaluated at -70 °C over a period of 90 days. The stability results were summarized in Table 4. For DAS and DASD8 the stability of stock solutions at -20 °C was determined in triplicate. Analytes were considered to be stable in stock solutions if 90-100% of the initial concentration found. Long-term stock solution stability at -20 °C was established for stock solutions of DAS and DASD8 for 7 days (Table 5).

Conclusion

We developed and validated an LC-MS/MS method with ESI interface using positive ion mode for simultaneous determination of DAS in human plasma. Use of deuterated internal standards guaranteed the success of the assay by eliminating the impact of matrix effects. The developed

method was validated to have sufficient sensitivity, satisfactory selectivity and good reproducibility. Protein precipitation method was established to obtain the good extraction recovery and no obvious matrix effect. The validated method could be applied to evaluate the pharmacokinetics and to determine bioequivalence of DAS tablets after an oral administration.

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