Supplementary

Rapid Determination of Imatinib in Human Plasma by Liquid Chromatography-Tandem Mass Spectrometry: Application to a Pharmacokinetic Study

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Experimental

Method Validation. We have tested system suitability of LC-MS/MS by consecutively injecting high quality control sample in six replicates at the beginning of each analytical batch during the method validation. The system suitability was evaluated by intra- and inter-day precision of chromatographic retention time and peak area ratio of analyte to internal standard. In addition, peak shape was checked in terms of tailing and symmetry along with consistency of peak width.

To evaluate the specificity and selectivity of the method, double-blank plasma (free of analyte and internal standard), blank plasma spiked with internal standard and blank plasma spiked with analyte and internal standard at LLOQ concentration were prepared from six different batches of blank plasma. We compared the chromatograms between the batches to investigate the potential interferences around the chromatographic retention times of imatinib and internal standard.

The carry-over effect was evaluated by the consecutive analysis of LLOQ sample, upper limit of quantification (ULOQ) sample and double blank sample in five replicates. The responses of analyte and internal standard in double blank sample should be below 20% and 5%, respectively in comparison with those in LLOQ sample.

For the evaluation of linearity, eight calibration curve samples were prepared over a range of 10–2000 ng/mL for imatinib in plasma. The linearity of calibration curve, which was established by plotting peak-area ratios of analyte to internal standard *vs.* the nominal concentrations of analyte, was determined using weighted ($1/x^2$) linear least-squares regression analysis by which the regression parameters of intercept, slope and correlation coefficient were calculated. The lower limit of quantification (LLOQ) was determined as the lowest concentration of analyte with precision below 20% and accuracy within ± 20% on the calibration curve. The precision values were obtained by calculating percentage relative standard deviation (RSD), and the accuracy values were calculated by percentage relative error (RE).

The intra-day precision and accuracy were evaluated in five replicates (n=5) of quality control samples at four different concentrations of LLOQ, LQC, MQC and HQC within a day. To evaluate inter-day precision and accuracy, the run (within a day) was repeatedly done on five consecutive days (*totally* n=25). The acceptable limit of RSD and RE were below 15% and within ± 15% respectively for three QC samples, and below 20% and within ± 20% respectively for LLOQ sample.

The effect of plasma matrix on quantification of imatinib was evaluated by precision (RSD) of imatinib concentrations measured in blank plasma samples, spiked with imatinib and internal standard at a level of LLOQ after extraction, from six different batches of blank plasma.

To investigate extraction efficiency of analyte and internal standard in plasma sample, we have prepared quality control samples at three different concentrations of LQC, MQC and HQC as samples spiked before extraction, and blank samples spiked with analyte and internal standard, at equivalent concentration level to the quality control samples, after extraction.

The percent extraction efficiency was calculated by mean peak area ratio of samples spiked before extraction to samples spiked after extraction, and assessed by % relative standard deviation.

The stability of samples spiked with imatinib under storage and handling conditions was evaluated by preparing two QC plasma samples at concentration levels of 30 (LQC) and

1500 (HQC) ng/mL in five replicates. The concentrations of analyte in QC samples were calculated using the freshly prepared calibration curve samples. The freeze/thaw stability was assessed after three freeze-thaw cycles of samples, which were stored at 70 °C and thawed at room temperature every 24 h. The stabilities of short-term and long-term were determined by analyzing QC samples, which were stored at room temperature for 24 h and at 70 °C for 166 days, respectively. The stability of post-extracted (processed) samples was determined by analyzing extracted QC samples at four concentration levels of 10 (LLOQ), 30 (LQC), 250 (MQC) and 1500 (HQC) after 24 h-storage in autosampler at 10 °C.

During the method validation, we have assessed the short-term stabilities of imatinib and internal standard in prepared standard solutions that were stored and handled under two conditions: 5 °C for 5 days and room temperature for 6 h in triplicates. In addition, the long-term stability of the standard solution was evaluated in six replicates under the storage at 5 °C for 23 weeks covering the period of real sample analyses. The standard solution stabilities were estimated by calculating percentage relative error of mean peak area of stored solution from that of freshly prepared solution.

Results and Discussion

Table S1. Optimum operating mass spectrometric parameters for imatinib and d_8-imatinib (IS)

Parameter	Value
Source temperature, °C	120
Desolvation temperature, °C	350
Cone gas flow rate, L/h	50
Desolvation gas flow rate, L/h	650
Transition dwell time, s	0.2
Capillary voltage, kV	3.00
Extractor voltage, V	1.00
RF Lens voltage, V	0.1
Cone voltage, V	35.00
Collision energy voltage, eV	20 (imatinib), 25 (d_8-imatinib)
Collision gas, mbar	27
LM Resolution 1	15.0
HM Resolution 1	15.0
Ion Energy 1	0.4
LM Resolution 2	14.0
HM Resolution 2	14.0
Ion Energy 2	1.0
Multiplier	650
Mode of analysis	Positive ion
Ion transition for imatinib, *m/z*	494.4 → 394.2
Ion transition for d_8-imatinib (IS), *m/z*	502.3 → 394.4

Table S2. Precision and accuracy of imatinib in calibration samples (n = 5)

Nominal concentration (ng/mL)	Mean ± SD (ng/mL)	RSD (%)	RE (%)
10	10.06 ± 0.17	1.66	0.60
20	20.10 ± 0.58	2.90	0.50
50	48.82 ± 0.97	1.98	-2.36
100	95.96 ± 3.59	3.74	-4.04
200	195.54 ± 2.54	1.39	-2.23
500	493.04 ± 9.45	1.92	-1.39
1000	1027.44 ± 21.62	2.10	2.74
2000	2122.06 ± 42.78	2.02	6.10

Table S3. Precision and accuracy of imatinib concentration in calibration and QC samples used for determination of real plasma

Nominal concentration (ng/mL)	Calibration samples (n = 19)		
	Mean ± SD (ng/mL)	RSD (%)	RE (%)
10	10.18 ± 0.22	2.19	1.79
20	19.67 ± 0.80	4.07	-1.66
50	48.36 ± 1.61	3.33	-3.27
100	97.35 ± 2.63	2.70	-2.65
200	197.06 ± 7.05	3.58	-1.47
500	503.07 ± 10.66	2.12	0.61
1000	1024.98 ± 25.51	2.49	2.50
2000	2083.16 ± 58.70	2.82	4.16
	QC samples (n = 38)		
	Mean ± SD (ng/mL)	RSD (%)	RE (%)
30	30.33 ± 1.44	4.74	1.10
250	245.20 ± 8.39	3.42	-1.92
1500	1540.55 ± 47.67	3.09	2.70