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Simultaneous determination of flumatinib and its two major metabolites in plasma of chronic myelogenous leukemia patients by liquid chromatography–tandem mass spectrometry

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ABSTRACT

Flumatinib is an antineoplastic tyrosine kinase inhibitor used for the treatment of chronic myelogenous leukemia (CML). Its major metabolites in the circulation are *N*-desmethyl flumatinib (M1) and amide hydrolysis product (M3). To investigate the pharmacokinetics of flumatinib in CML patients, a simple, specific and rapid liquid chromatography–tandem mass spectrometry (LC–MS/MS) method was developed and validated for the simultaneous determination of flumatinib and its two major metabolites in patient plasma. After a simple, one-step protein precipitation with methanol, flumatinib, its two metabolites, and internal standard (HHGV-E) were separated on a C_{18} column using an isocratic mobile phase of methanol:5 mM ammonium acetate:formic acid (60:40:0.4, v/v/v). A total chromatographic run time of 4.2 min was achieved. The detection was performed in multiple reaction monitoring mode, using the transitions of m/z 563 → m/z 463 for flumatinib, m/z 549 → m/z 463 for M1, m/z 303 → m/z 175 for M3, and m/z 529 → m/z 429 for HHGV-E. The method was linear over the concentration ranges of 0.400–400 ng/mL for flumatinib, 0.100–100 ng/mL for M1, and 0.200–200 ng/mL for M3, using only 50 μL of plasma. The intra- and inter-day precisions were less than 8.5% for flumatinib, 9.8% for M1, and 10.6% for M3 in terms of the relative standard deviation. The accuracy was within ±2.2% for flumatinib, ±6.0% for M1, and ±9.9% for M3 in terms of relative error. The validated method was successfully applied to clinical pharmacokinetic studies of flumatinib mesylate in CML patients following oral administration at all dosage regimens.

1. Introduction

Chronic myelogenous leukemia (CML) is a myeloproliferative disorder caused by the neoplastic transformation of pluripotent stem cells. A reciprocal translocation between chromosomes 9 and 22 results in an abnormal chromosome, called the Philadelphia chromosome. The translocation generates the BCR-ABL oncogene which expresses protein with constitutively tyrosine kinase activity, causing the development of CML [1]. Imatinib mesylate, a selective inhibitor of BCR-ABL protein, is the first effective therapy for CML. For almost 10 years, it has become the standard therapy because of its high efficiency and mild toxicity. However, resistance to imatinib has been observed during CML treatment, which led to the development of other tyrosine kinase inhibitors of BCR-ABL, such as dasatinib and nilotinib.

Flumatinib (4-(4-methyl-piperazin-1-ylmethyl)-N-[6-methyl-5-(4-pyridin-3-yl- pyrimidin-2-yl-amino)-pyridin-3-yl]-3-trifluor omethyl-benzamide), a structural analogue of imatinib, has been shown to be slightly more potent than imatinib as a BCR-ABL inhibitor [2]. It is administrated orally as its mesylate salt and it is currently undergoing a phase II clinical trial as a treatment for CML. The metabolism of flumatinib in CML patients has been reported by our laboratory [3]. Base on the peak area ratios of the metabolites to the parent drug, the major metabolites in human plasma are identified as *N*-desmethyl flumatinib (M1) and amide hydrolysis product (M3). M1 has been demonstrated to exhibit similar pharmacodynamic activity with the parent drug. According to the USFDA guidelines issued in 2008, if the systemic exposure of any human drug metabolite reaches more than 10% of the parent drug systemic exposure at steady sate, a separate safety testing should be required [4]. Therefore, the simultaneous quantification of flumatinib, M1 and M3 in patient plasma was necessary to evaluate their circulating levels.

All the subjects participating in clinical studies are CML patients, and plasma samples are collected in low volumes. Hence, a high sensitive and reliable analytical method that is precise and accurate even at sample volumes below 100 μL is needed. In

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addition, high throughput analysis was also required since large numbers of plasma samples need to be analyzed in pharmacokinetic/pharmacodynamic studies. To the best of our knowledge, no study has been reported for the individual or simultaneous determination of flumatinib and its major metabolites in biological samples.

In the present study, a sensitive, simple, and rapid liquid chromatography–tandem mass spectrometry (LC–MS/MS) method was developed and validated for the simultaneous quantification of flumatinib, M1 and M3 in the plasma of CML patients, and it has been successfully applied to characterize the pharmacokinetic profiles of flumatinib, M1 and M3 after oral administration of flumatinib mesylate at all possible dosage regimens.

2. Experimental

2.1. Materials

Reference substances of flumatinib mesylate, M1, M3, and HHGV-E [4-(4-methyl-piperazin-1-ylmethyl)-N-[6-methyl-5-(4-pyridin-3-yl-pyrimidin-2-yl-amino)-pyridin-3-yl]-3-chlorobenzamide), internal standard (IS)] were provided by Jiangsu Hansoh Pharmaceutical Co., Ltd. (Lianyungang, China). HPLC grade methanol was purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). HPLC grade formic acid and ammonium acetate were supplied by Tedia (Fairfield, OH, USA). Deionized water (18.2 mΩ and TOC ≤ 50 ppb) was purified by a Millipore Milli-Q Gradient Water Purification System (Molsheim, France).

2.2. Preparation of calibration standards and quality control (QC) samples

Stock solutions of flumatinib, M1, and M3 at 1.00 mg/mL were prepared in duplicate by dissolving the accurately weighed reference substances in methanol for preparation of calibration standards and QC, respectively. The concentration of each analyte was calculated as free base. Standard combined dilutions were prepared in methanol at 20.0/5.00/10.0, 3.00/0.750/1.50, 0.300/0.075/0.150 and 0.040/0.010/0.020 μg/mL for flumatinib, M1 and M3. Calibration standards were prepared at 0.400/0.100/0.200, 1.00/0.250/0.500, 2.00/0.500/1.00, 5.00/1.25/2.50, 15.0/3.75/7.50, 50.0/12.5/25.0, 150/37.5/75.0 and 400/100/200 ng/mL by adding small volumes of standard combined dilutions (a maximum of 5% of the total volume) to analyte-free plasma. The QC samples were similarly prepared at concentrations of 0.400/0.100/0.200, 1.00/0.250/0.500, 15.0/3.75/7.50 and 320/80.0/160 ng/mL for lower limit of quantification (LLOQ), low QC (LQC), medium QC (MQC) and high QC (HQC), respectively. IS working solution at 20.0 ng/mL was prepared by diluting the HHGV-E stock solution (1.00 mg/mL) with methanol. All solutions were kept refrigerated (4 °C) and were brought to room temperature before use. Calibration standards and QC samples were prepared and dispatched in 500 μL aliquots and stored in plastic tubes at −20 °C until analysis.

2.3. Sample preparation

A 25 μL aliquot of IS solution (20.0 ng/mL HHGV-E, with final plasma concentration of 10.0 ng/mL) and 200 μL of methanol was added to 50 μL of plasma sample. The mixture was vortex-mixed for 1 min, and centrifugated at 11,000 × *g* for 5 min. A 120 μL aliquot of the supernatant was then transferred to another tube and mixed with 80 μL of 5 mM ammonium acetate. A 10 μL aliquot of the mixture was injected into the LC–MS/MS system for analysis.

2.4. LC–MS/MS instrumentation and analytical conditions

A Shimadzu LC-30AD HPLC system consisting of a DGU-20A_5 vacuum degasser, a LC-30AD binary pump, a CTO-30A column oven and a SIL-30AC autosampler (Shimadzu Corporation, Kyoto, Japan) was used for solvent and sample delivery. A Qtrap 5500 tandem mass spectrometer (AB Sciex, Forster City, CA, USA) equipped with a Turbolon Spray ionization (ESI) source was employed for mass analysis and detection. Data acquisition and processing were performed with Analyst 1.5.1 software (AB Sciex).

Chromatographic separation was carried out on a Zorbax SB-C_{18} column (150 mm × 4.6 mm i.d., 5 μm, Agilent, Santa Clara, CA, USA) using an isocratic condition. The mobile phase consisted of methanol–5 mM ammonium acetate–formic acid (60:40:0.4, v/v/v) and was delivered at a flow rate of 0.6 mL/min. The column temperature was maintained at 40 °C.

The mass spectrometer was operated in a positive ion mode. The temperature of the ionization source was maintained at 550 °C and the ion spray voltage was set at 5500 V. The nebulizer, heater, and curtain gas were set to 55, 50 and 35 psi, respectively. The collision activated dissociation (CAD) gas level was set at medium. Four MRM transitions (m/z 563 → m/z 463, flumatinib; m/z 549 → m/z 463, M1; m/z 303 → m/z 175, M3; m/z 529 → m/z 429, IS) were recorded and used for quantification. The optimized collision energies for the transitions of flumatinib, M1, M3, and IS were set at 32, 46, 50, and 39 eV, respectively. The dwell time for each transition was set at 100 ms.

2.5. Method validation

The method was validated for selectivity, linearity, precision and accuracy, matrix effect, recovery and stability according to the FDA guidelines [5].

To investigate the selectivity, blank plasma from six different donors were extracted and analyzed. The responses were compared with the LLOQ. The peak area of coeluting interferences should be less than 20% of the peak area of the LLOQ.

Calibration standards were prepared and analyzed in duplicate in three consecutive days. The peak area ratios (each analyte to IS) versus the nominal analyte concentrations were calculated to construct the calibration curves. The calibration curves were fitted via a $1/x^2$ weighted linear least-squares regression model.

The accuracy and precision of the method were determined by analyzing the QC samples at four concentrations in six replicates on three consecutive days. The accuracy and precision are expressed in terms of relative error (RE) and relative standard deviation (RSD), respectively. The intra and inter-run precision should not exceed 20% for LLOQ, and 15% for QC samples. Accuracy should be within ±20% for LLOQ, and ±15% for QC samples.

The recovery of flumatinib, M1 and M3 were determined at three QC levels by comparing the mean peak areas of QC samples (n = 6) with those of the blank plasma samples (n = 3) spiked with working solutions after extraction. The recovery of IS was determined using a similar method with the MQC level as a reference.

To evaluate the matrix effect in the experiment, six different lots of blank plasma were extracted and then spiked with QC solutions. Chromatographic peak areas of each analyte from the spike-after-extraction samples were compared to those of the solution standards at equivalent concentrations [6]. In the present study, the matrix effect was evaluated at two concentration levels (LQC and HQC) for each analyte. The matrix effect for IS was determined in a similar way at 10.0 ng/mL. Inter-subject variability at the matrix effect should be less than 15% [7].

The stability of flumatinib, M1 and M3 were investigated by analyzing replicates (n = 3) of plasma samples at two concentration levels (LQC and HQC), which were exposed to different conditions

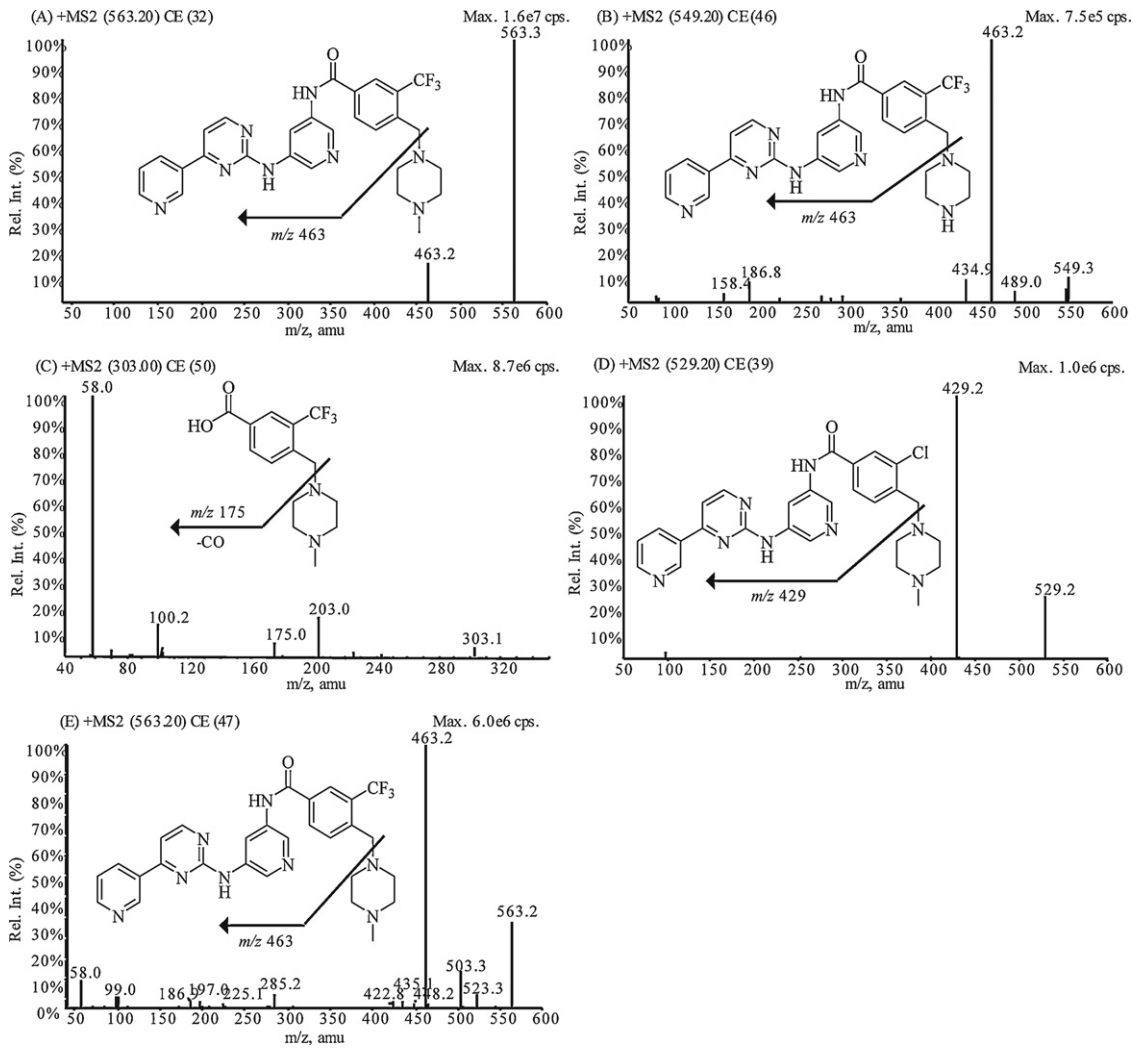

Fig. 1. Product ion spectra of $[M+H]^+$ ions: (A) flumatinib using a CE of 32 eV; (B) M1 using a CE of 46 eV; (C) M3 using a CE of 50 eV; (D) HHGV-E using a CE of 39 eV; (E) flumatinib using a CE of 47 eV.

(time and temperature). The analytes were considered stable when 85–115% of the nominal concentrations were found.

3. Results and discussion

3.1. Mass spectrometry

M3 has a basic piperazine ring and a carboxyl group in its structure. Hence, it can produce a mass spectrometric (MS) response in both positive and negative ionization mode. The signal intensity under different ionization polarity was compared. M3 showed similar MS intensity in both positive and negative ionization mode. However, the MS responses of flumatinib and M1 were too low to be recorded in negative mode. Finally, positive ion detection mode was chosen. In addition, each analyte exhibited higher MS responses under ESI source than under atmospheric pressure chemistry ionization (APCI) source. In the Q1 full scan experiment, distinct protonated molecules were observed for flumatinib (m/z 563), M1 (m/z 549), M3 (m/z 303), and IS (m/z 529), and no adductive ions were detected. In the product ion full scan experiment, flumatinib, M1 and IS have similar base peak of $[M+H-100]^+$, which was generated by the neutral loss of the *N*-methylpiperazine moiety from $[M+H]^+$. The most abundant fragment ion was selected in the MRM transitions. For M3, the most abundant ion was observed at m/z 58. However, the background noise of the transition (m/z 303 → m/z 58) was too high to satisfy the quantification requirement. Therefore, the MRM transition of m/z 303 → m/z 175 was chosen as it provided a better signal-to-noise ratio (S/N), reproducibility and response than the other transitions (Fig. 1). The highest MS response of flumatinib (m/z 563 → m/z 463) was achieved when the CE value was set at 47 eV. However, the MS response of flumatinib tended to be saturated when high concentrations of calibration standards were analyzed. Consequently, the CE value of flumatinib was finally set at 32 eV (Fig. 1) to achieve a similar MS response as those of M1 and M3.

3.2. Chromatography

The chromatographic behavior of M3 (t_R = 2.7 min) was considerably different from those of flumatinib (t_R > 10 min), M1 (t_R > 10 min) and IS (t_R > 10 min) when methanol: 5 mM ammonium acetate (60:40, v/v) was used as the mobile phase at a flow rate of 0.6 mL/min. Therefore, it is a challenge how to retain all analytes on the same HPLC stationary phase in a short chromatographic time.

During method development, when the proportion of acidic modifier increased in the mobile phase, the retention times of flumatinib, M1, and IS were distinctly shortened, whereas the retention time of M3 was slightly prolonged. These different changes in the retention behavior may be explained by the relationship between the reverse-phase chromatography (RPC) retention

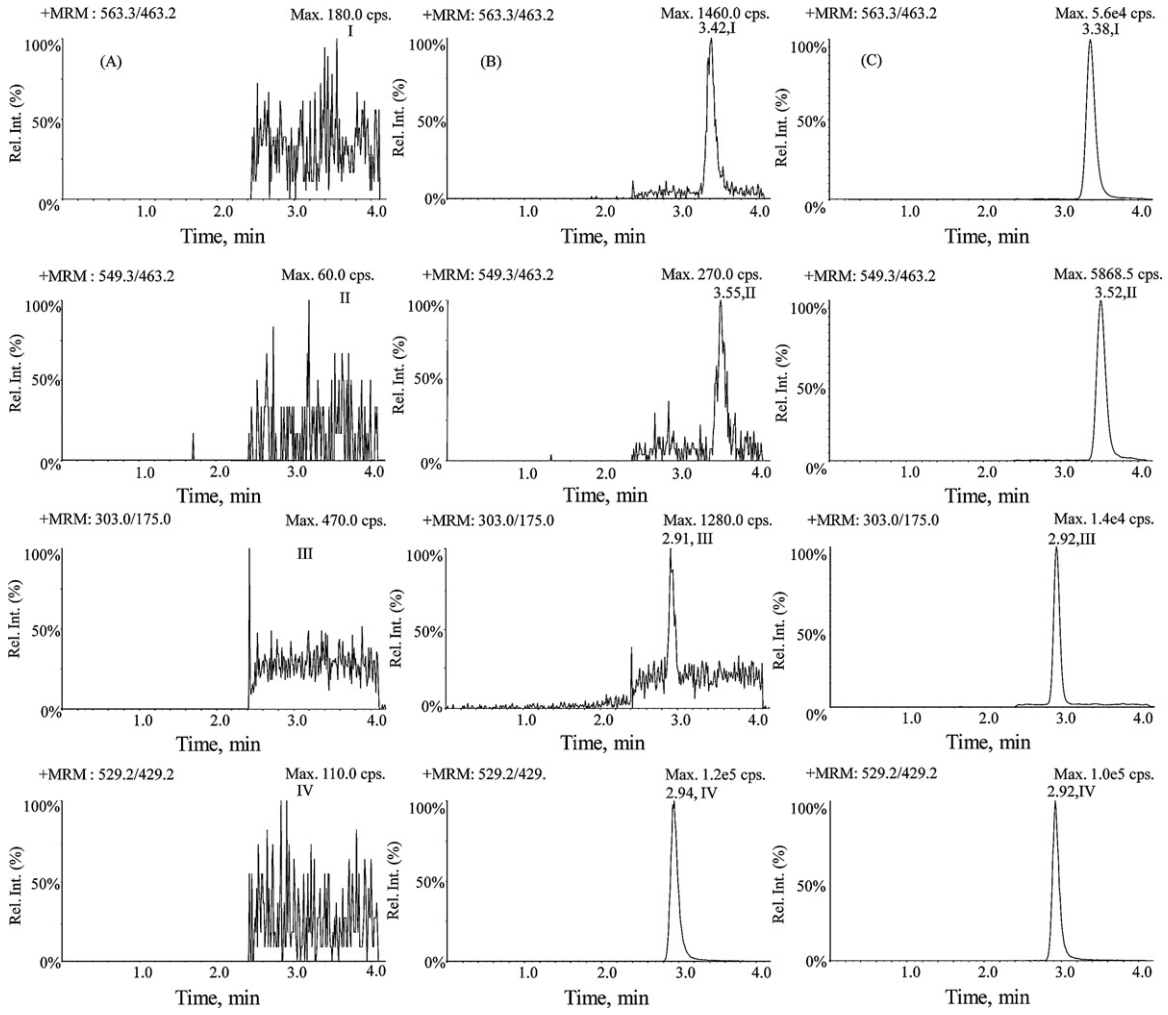

Fig. 2. Typical MRM chromatograms of flumatinib, M1, M3 and HHGV-E (IS) in the plasma of CML patients: (A) blank plasma sample; (B) blank plasma spiked with 0.400 ng/mL flumatinib, 0.200 ng/mL M1, 0.100 ng/mL M3 and 10.0 ng/mL HHGV-E (IS); (C) plasma sample 12 h after multiple administration of 400 mg of flumatinib mesylate once daily for 28 consecutive days. Peaks I, II, III, and IV correspond to flumatinib, M1, M3 and HHGV-E, respectively.

and the ionization of compounds. Flumatinib, M1, and IS all contain a basic piperazine ring in the structure. Their pKa values were calculated as 7.4 ± 0.4, 8.7 ± 0.4 and 7.5 ± 0.4, respectively, using the Advanced Chemistry Development software (ADME Suite, Version 5.0). When the mobile-phase pH decreases below the pKa of the analytes, piperazine rings gain a proton and become ionized and more hydrophilic. As a result, their retention in RPC significantly decreased. M3 is an amphoteric molecule that contains one acidic (pKa3.6 ± 0.4) and one basic (pKa7.5 ± 0.4) group. It could be ionized over a wide pH range and its retention in RPC does not change much with pH. At intermediate pH values ($3.6 < \text{pH} < 7.5$), the carboxyl and piperazine groups are ionized, and the molecule is maximally ionized. When the mobile-phase pH decreases below the pKa of the carboxyl group, basic piperazine ring remains ionized.

In the present study, the pH of the mobile phase was optimized by varying the proportion of the acidic modifier. The addition of 0.4% formic acid in mobile phase was found to be optimal. The pH of the optimized aqueous phase was 2.24. Under the optimized chromatographic condition, all analytes could be retained in the C18 column without sacrificing analysis time (Fig. 2). According to the pH of the mobile phase, a Zorbax SB-C_{18} column was chosen as the analytical column, and it presented better retention and peak symmetry for each analyte at 40 °C.

3.3. Sample preparation

Considering that large batches of plasma samples would be analyzed during the clinical trials, a simple sample preparation procedure was required. Protein precipitation (PPT) was firstly considered for its simplicity and efficiency. However, PPT is well known as a nonselective purification method, which may introduce high amounts of endogenous components and can cause signal suppression or enhancement, especially with an ESI ionization source. A post column infusion method was employed to evaluate the matrix effect [8]. Human blank plasma (50 μL) was precipitated with methanol and injected into the LC–MS/MS whereas a drug cocktail (flumatinib/M1/M3 at 10.0/2.50/5.00 ng/mL) in methanol: 5 mM ammonium acetate:formic acid (60:40:0.4, v/v/v) was introduced into the system by a post column syringe operated at 7 μL min^{-1}. No signal suppression or enhancement was observed at the retention time of each analyte (Fig. 3).

3.4. Selection of internal standard

Structure analogue was first considered because stable isotopically internal standard of flumatinib is difficult to be synthesized. Imatinib was firstly employed as the IS during the early stage of the study. The subjects participating in the current study were CML

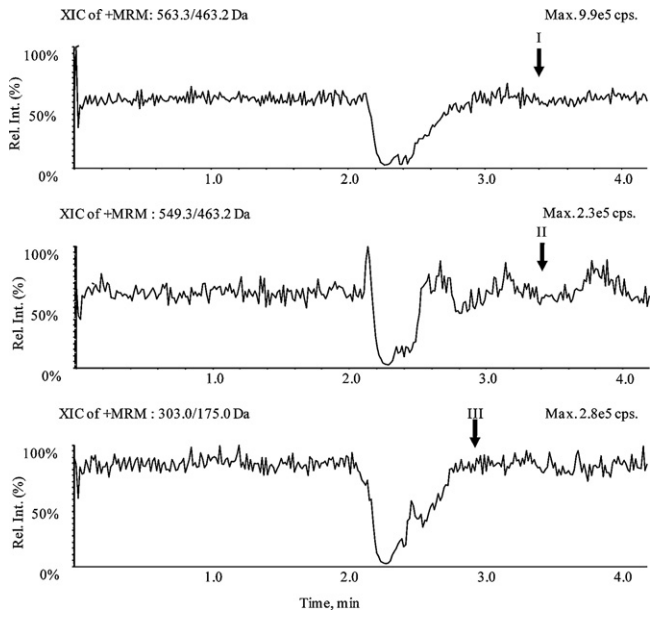

Fig. 3. MRM chromatograms of processed human blank plasma on the post-column infusion system. I, II and III mean the retention times of flumatinib, M1 and M3, respectively.

patients, and some of them have received and were resistant or intolerant to imatinib during the clinical treatment. If imatinib was chosen as the IS, the residual imatinib in the patient plasma may increase the peak area of the IS, producing a lower calculated concentration. Consequently, HHGV-E, another structural analogue of flumatinib, was finally chosen as the IS.

3.5. Validation

3.5.1. Assay selectivity

The validated method was high selective for each analyte because no significant interference was observed in the blank plasma samples from six different sources. Fig. 2 shows the typical chromatograms of flumatinib, M1, M3 and IS in a blank plasma sample, and blank plasma sample spiked with flumatinib, M1, and M3 at the LLOQ and IS, a plasma sample obtained 12 h after the last administration of 400 mg flumatinib mesylate to a CML patient. The retention times for flumatinib, M1, M3 and IS were 3.4, 3.5, 2.9 and 2.9 min, respectively.

3.5.2. Linearity of calibration curve and lower limit of quantification

The linear regression of the peak ratios versus concentrations were fitted over the plasma concentration ranges of 0.400–400 ng/mL for flumatinib, 0.100–100 ng/mL for M1, and 0.200–200 ng/mL for M3. The mean linear regression equations of the calibration curves generated during the validation were as follows:

$$\text{Flumatinib}: \quad y = (0.0298 \pm 0.0021)x + (0.000995 \pm 0.000401)\ (r = 0.9992 \pm 0.0004);$$
$$\text{M1}: \quad y = (0.0254 \pm 0.0034)x + (0.000450 \pm 0.000106)\ (r = 0.9979 \pm 0.0004);$$
$$\text{M3}: \quad y = (0.0284 \pm 0.0010)x + (0.000136 \pm 0.000044)\ (r = 0.9993 \pm 0.0001);$$

where y represents the peak area ratio of each analyte to the IS, and x is the nominal concentration of the analytes. The LLOQ of flumatinib, M1 and M3 were 0.400, 0.100, and 0.200 ng/mL, respectively, with acceptable accuracy and precision (Table 1).

Table 1
Accuracy and precision for the analysis of flumatinib, M1, and M3 in human plasma (n = 3 days, six replicates per day).

Analyte	Concentration (ng/mL)		RSD (%)		RE (%)
	Added	Found	Intra-day	Inter-day	
Flumatinib	0.400	0.406	4.8	14.3	1.4
	1.00	1.02	4.6	8.5	2.2
	15.0	15.0	5.2	7.6	−0.3
	320	321	3.3	7.3	0.4
M1	0.100	0.0944	11.6	5.7	−5.7
	0.250	0.248	9.8	2.6	−1.0
	3.75	3.52	7.2	10.3	−6.0
	80.0	78.2	4.0	5.5	−2.3
M3	0.200	0.209	10.0	15.4	4.7
	0.500	0.525	5.4	10.0	4.9
	7.50	7.71	4.6	10.6	2.8
	160	167	3.0	9.8	4.3

3.5.3. Precision and accuracy

Table 1 summarizes the intra and inter-day precision and accuracy values for the QC samples. The intra and inter-day precisions for flumatinib were less than 8.5%, while accuracy was within ±2.2%. The inter and intra-day precisions for M1 were less than 10.3%, while accuracy was within ±6.0%. For M3, inter and intra-day precisions were less than 10.6, while accuracy was within ±4.9%. The accuracy and precision data indicate that method is reliable and reproducible.

3.5.4. Recovery and matrix effect

The recovery levels of flumatinib at 1.00, 15.0, and 320 ng/mL were 90.2%, 102% and 99.8%, respectively. Recovery levels of M1 at 0.250, 3.75, and 80.0 ng/mL were 89.6%, 88.8%, and 93.7%, respectively. For M3, the recoveries at 0.250, 3.75 and 80.0 ng/mL were 99.1%, 101% and 102%, respectively. The recovery of IS at 10.0 ng/mL was 105%.

Table 2
Stability of flumatinib, M1 and M3 in human plasma (n = 3).

Conditions	Flumatinib			M1			M3		
	Conc. (ng/mL)		RE	Conc. (ng/mL)		RE	Conc. (ng/mL)		RE
	Nominal	Found	(%)	Nominal	Found	(%)	Nominal	Found	(%)
Ambient, 15 h	1.00	0.985	−1.5	0.250	0.234	−6.3	0.500	0.516	3.2
	320	307	−4.1	80.0	73.1	−8.7	160	167	4.2
Three freeze/thaw cycles	1.00	1.00	0.5	0.250	0.260	4.0	0.500	0.481	−3.8
	320	334	4.5	80.0	85.1	6.3	160	155	−3.3
Autosampler, 24 h	1.00	1.04	4.3	0.250	0.238	−4.9	0.500	0.563	12.5
	320	330	3.0	80.0	79.4	−0.8	160	168	5.0
−20 °C, 3 months	1.00	1.09	8.7	0.250	0.241	−3.5	0.500	0.492	−1.5
	320	322	0.5	80.0	80.1	0.1	160	157	−2.1

Conc., concentration.

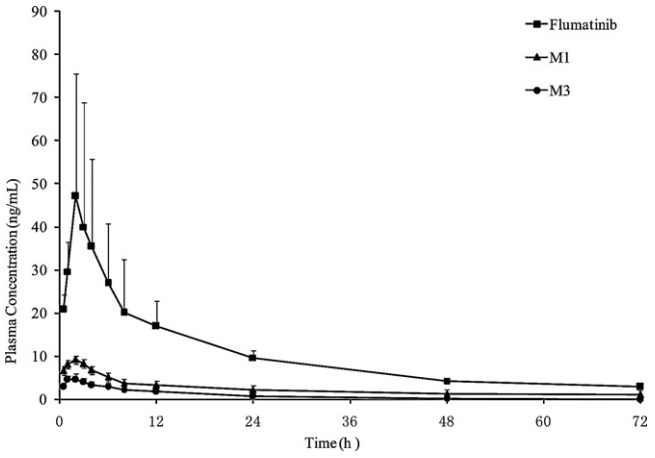

Fig. 4. Mean plasma concentration–time curves of flumatinib, M1, M3 at steady state in CML patients after multiple administration of 400 mg flumatinib mesylate once daily for 28 consecutive days. All data indicate mean ± SD.

The matrix effects for flumatinib determined at 1.00 and 320 ng/mL were 98.1% and 97.1%, respectively. The matrix effects for M1 determined at 0.250 and 80.0 ng/mL were 109% and 96.9%, respectively. The matrix effects for M3 determined at 0.500 and 160 ng/mL were 101% and 101%, respectively. The matrix effects for IS determined at 10.0 ng/mL was 103%. The inter-subject variability of matrix effects for each analyte was below 9.5%. As a result, the matrix effect for flumatinib, M1, M3, and IS was negligible in present conditions.

3.5.5. Stability

No significant degradation occurred during chromatography, extraction and sample storage processes for each analyte in human plasma samples. Stability data are shown in Table 2. Standard stock solutions of flumatinib, M1, and M3 were stable for at least 90 days at 4 °C.

3.6. Method application

This validated method was applied to the determination of the plasma concentrations of flumatinib, M1 and M3 in the CML patients. The clinical studies were approved by the Ethics Committees. Two groups of patients were evaluated: the first received once-daily flumatinib mesylate administration at three dose levels (200, 400 and 600 mg) for 28 days, the second received twice-daily administration at three dose levels (100, 200 and 300 mg) for 28 days. Blood samples were collected as the flowing times: pre-dose and at 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 h after the first dose; pre-dose and at 3 h after the 6th, 7th, 8th and 15th doses; pre-dose and at 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48 and 72 h after the last dose. Plasma samples were separated by centrifugation (2000 g for 10 min) and then stored at −20 °C until analysis. The mean plasma concentration–time curves of flumatinib, M1, and M3 at steady state after multiple administrations of 400 mg flumatinib mesylate to 3 CML patients are presented in Fig. 4. A significant inter-individual variation in plasma concentration of flumatinib, M1 and M3 was observed, which could be explained by the different physical state of CML patients and the small sample size ($n = 3$).

4. Conclusion

The optimized method was validated to guarantee the reliable determination of flumatinib and its two major metabolites in the plasma of CML patients. The LLOQ of the method are 0.400, 0.200, and 0.100 ng/mL for flumatinib, M1, and M3, using a 50 μL plasma sample. The strong acidic mobile phase enhanced the retention of M3 and decreased the retention of flumatinib, M1 and IS, which confirmed the retention of all analytes in a short chromatographic run time (4.2 min). A simple one-step protein precipitation procedure made the method easily applied to clinical trials.

References

[1] R. Ren, Nat. Rev. Cancer 5 (2005) 172.
[2] P. Sun, US Patent 0,312,251 (2008).
[3] A. Gong, X. Chen, P. Deng, D. Zhong, Drug Metab. Dispos. 38 (2010) 1328.
[4] USFDA, 2008, http://www.fda.gov/downloads/Drugs/GuidanceCompliance RegulatoryInformation/Guidances/ucm079266.pdf (accessed 19.06.11).
[5] USFDA, 2001, http://www.fda.gov/downloads/Drugs/GuidanceCompliance RegulatoryInformation/Guidances/ucm070107.pdf (accessed 19.06.11).
[6] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Anal. Chem. 75 (2003) 3019.
[7] C.T. Viswanathan, S. Bansal, B. Booth, A.J. DeStefano, M.J. Rose, J. Sailstad, V.P. Shah, J.P. Skelly, P.G. Swann, R. Weiner, Pharm. Res. 24 (2007) 1962.
[8] R. King, R. Bonfiglio, C. Fernandez-Metzler, C. Miller-Stein, T. Olah, J. Am. Soc. Mass Spectrom. 11 (2000) 942.