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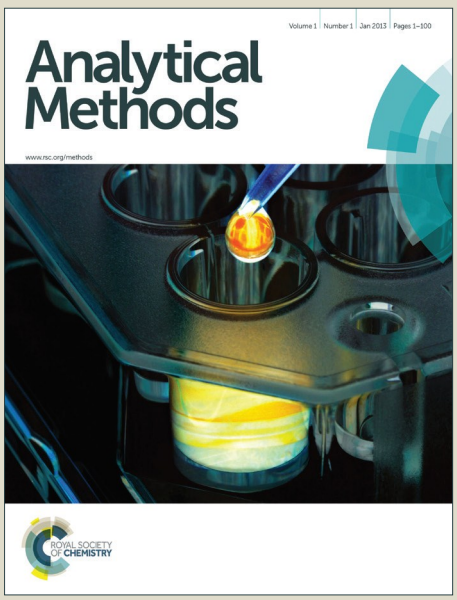

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ARTICLE

Simultaneous quantification of imatinib and CGP74588 in human plasma by liquid chromatography-time of flight mass spectrometry (LC-TOF-MS)

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Imatinib mesylate is widely used for the treatment of different types of cancer, such as chronic myelogenous leukemia and gastrointestinal stromal tumors. It is a tyrosine kinase inhibitor that binds to the active site of the enzyme inhibiting it. In clinical toxicology, a fast and sensitive quantification method for monitoring the imatinib concentration in blood can be very useful for personalized treatments. The aim of study was to propose an alternative novel analytical method (LC-MS-TOF) for the quantification of intracellular imatinib and its main metabolite in human plasma. The method is simple and fast. It has sufficient sensitivity for quantification of imatinib and its main metabolite CGP74588 in a smaller volume of plasma. The linearity of the method was evaluated over a range of concentrations (0.02 - 5 µg/mL) of imatinib and CGP74588 in human plasma. The correlation coefficients (r^2) were close to 1 for both analytes. The limit of quantification was 0.02 µg/mL for both imatinib and CGP74588. A number of (> 50) chronic myelogenous leukemia patient plasma samples have been analyzed with this method obtaining values ranging from 0.186 - 4.53 and 0.03 - 8.41 µg/mL for imatinib and CGP74588, respectively. This novel method allows a specific, sensitive and reliable simultaneous determination of intracellular imatinib and CGP74588 in a single chromatographic run, which is suitable for routine clinical practice.

Keywords: anticancer drug; desmethyl imatinib; imatinib; human plasma; liquid chromatography-mass spectrometry.

Introduction

Imatinib (Gleevec® or Glivec®) ($C_{29}H_{31}N_7O$) is approved to treat chronic myelogenous leukemia (CML) (Fig. 1). It is also known to cure gastro-intestinal stromal malignant tumor (GIST). Imatinib is a competitive inhibitor of *Bcr-Abl* tyrosine kinase, which is used for the treatment of CML and GIST.[1,2] Imatinib can be administered orally and works at the molecular level with high specificity to eliminate cancer by competitively inhibiting the binding of ATP to the kinase activation domain of *Bcr–Abl* (Fig. 2).[2] Imatinib is metabolized predominantly to *N*-desmethyl imatinib (CGP74588) ($C_{28}H_{29}N_{7O}$), which demonstrates comparable biological activity to imatinib (Fig. 1).[3] Imatinib is administered orally with a dose of 400, 600 or 800 mg daily to patients in different stages of CML. The dose determination was based on the adverse effects and hematologic response.[1] Imatinib is an ideal targeting anticancer drug which eliminates cancer cells selectively instead of destroying all other rapidly dividing cells.[1, 4, 5] Moreover, the clinical response can be correlated with imatinib concentration, thus a low concentration of imatinib in plasma could indicate an ineffective drug regimen, insufficient to achieve complete cytotoxic response or major molecular response. Measurement of the concentration of imatinib and its main metabolites in the plasma of patients is important due to the therapeutic outcome especially to find the variability in patients as the efficacy of the imatinib depends on individual physiology.[2, 5] Hence, the measurement of imatinib level in plasma is useful to determine the therapeutic dose to improve the response or to reduce adverse effects during imatinib treatment and therefore numerous imatinib/CGP74588 quantification methods have been reported.[3, 6-9]

Thus, several high throughput liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) methods have been established [3, 9, 10] but also LC separation system hyphenated to ultraviolet detection (LC-UV)[11], single quadrupole (LC-MS)[8], and capillary electrophoresis combined to time of flight mass spectrometry CE-TOF-MS[12] methods have been used for imatinib determination. LC-MS/MS technique is not always practical for

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routine analysis, thus the purpose of this study is to develop and validate a simple method adapted to routine application with a simple liquid extraction with protein precipitation, prior to LC-MS imatinib/CGP44588 analysis with relatively low volume plasma sample. According to our knowledge there is no LC-TOF-MS method for imatinib quantification. Therefore, we have developed a sensitive, rapid, in-house method for the simultaneous determination of imatinib and CGP74588 in human plasma or any other biological sample.

Experimental

Materials and Method

Imatinib and N-desmethyl imatinib (CGP44588) were kindly provided by Novartis (Basel, Switzerland) and the reference samples for validation tests were received from a reference laboratory in Bordeaux, France (http://www.eutos.org/content/home/). Human plasma samples from healthy blood donors were obtained from Academic Hospital, Uppsala, Sweden and the plasma samples of imatinib treated CML patients were obtained from Östergötland, Västervik, Jönköping, Örebro, Uppsala, and Lund Hospitals. All chemicals including internal standard and ultrapure solvents were purchased from Sigma Aldrich (Stockholm, Sweden), unless otherwise stated. Water was deionized and distilled with a Milli-Q purification system (Millipore, Bedford, MA, USA). The stock solutions of imatinib, CGP74588 and internal standard were prepared by dissolving an accurately weighed amount in methanol to obtain a final concentration of 1 mg/mL. Imatinib and CGP74588 stock solutions were diluted in methanol to obtain the working solutions. All stock and working solutions were stored at -20 °C.

Ethical approval

Participant recruitment for this collaborative research study was managed by clinicians from Linköping, and Uppsala Universities. The regional ethical review board in Linköping, Sweden (No: 02-221) approved the study protocols. Signed informed consent was obtained from all patients before participation. Blood was collected from each participant by venipuncture into EDTA vacutainer tubes. The blood was centrifuged at 3,500 g for 15 min; the plasma aliquoted and stored at -80 °C until further analysis.

Sample cohort

Patients with chronic-phase CML diagnosed and receiving first-line treatment with 400 mg/day imatinib were eligible for the study. A total of 55 patients were included in the study, 20 women and 35 men. The average age of patients when included in the study was 58 years (range 22-87), When sampled for imatinib plasma concentrations the median time from start of imatinib therapy were 52,5 months (range 1-132 months). All patients were sampled 24h =/- 2 hours after the last intake of imatinib.

Method validation

The performance of the method was validated using drug free human plasma. The method evaluation parameters were evaluated for selectivity, precision, accuracy, linearity and recovery.

Linearity

The linearity was determined by linear curves with different concentrations of analytes. The calibration curves were prepared daily for each experiment by spiking drug free plasma. The range of the calibration samples were 0.02-5 µg/mL for imatinib and CGP74588. These ranges were selected to cover the range of clinically relevant concentrations of imatinib and CGP74588 in CML patients. Plasma without spiking was considered as the basal level. Seven calibration standards were prepared and analyzed in duplicate. Standard curves were constructed by plotting the peak area ratio (analyte/internal standard) of imatinib and CGP74588 versus the corresponding concentrations. The calibration curves had determination coefficient < 0.99. The linearity was evaluated by means of back-calculated concentrations of the calibration standards, according to the EU Commission Decision/657/EEC.

Accuracy and precision

The intra-day accuracy and precision were established by analyzing six samples at three different concentrations of analytes (low; 0.1 µg/mL, medium; 1.0 µg/mL, and high; 2.0 µg/mL) on the same day. The inter-day accuracy and precision were calculated as the triplicate analysis of above samples at each concentration on six different days. The concentration in each sample was measured using the calibration curve prepared on the same day. The accuracy was determined as the percent deviation from the nominal concentration and was stated as bias (measured concentration minus the actual concentration, expressed as a percentage of the actual concentration). The intra-day and inter-day precision were expressed as the coefficient variance at each concentration. The standard deviation should not exceed 10 %

Limit of quantification (LOQ)

The limit of quantification was determined as the lowest concentration obtained with the percentage deviation from nominal concentration and relative standard deviation less than ±20 % was permissible.

Recovery

Recovery of the sample extraction efficiency was assessed by comparison of the concentrations of imatinib and CGP74588 obtained after replicate analysis of known concentrated samples (mobile phase) and spiked plasma with analytes.

The matrix effect was assessed by comparing the peak areas response of imatinib, CGP74588 and the IS from the spiked plasma after protein precipitation and the peak area response of standard analyte solution at the same concentration in the mobile phase (n=6). The percentage of area difference indicates the ionization behaviour of the analytes. If the

observed value is greater or lower than 100 % it indicates ionization enhancement or suppression, respectively.

Stability of the method

For the assessment of imatinib and the metabolite stability in plasma after storage, a fresh sample was drawn and separated into aliquots. One aliquot was analyzed at the time of collection. Aliquots were analyzed after one week, a month and three months. The stability of the working standard and quality control samples of imatinib and CGP74588 was also tested. We compared the stability of imatinib samples received 10 year ago from Novartis (Basel, Switzerland) and recently purchased from Sigma Aldrich (Stockholm, Sweden).

Extraction of Imatinib and CGP74588

Protein precipitation was applied as a sample pretreatment. 25 µL of methanol containing 1 µg/mL internal standard and 0.5 mL of methanol were added to 100 µL of plasma. The sample was shaken for 10 min and centrifuged at 4 °C for 10 min at 14,000 g. The resulting supernatant was transferred to 1.5 mL Eppendorf tubes and completely dried by vacuum centrifugation using an Eppendorf concentrator plus/vacufuge® plus (Becton Dickinson, NJ, USA) at room temperature. The residue was reconstituted in 100 µL of 5 % acetonitrile containing 0.1 % formic acid and 10 µL were injected into the LC-MS system.

Chromatographic and mass spectrometry conditions

The LC-TOF-MS instrument consisted of an Agilent 1260 Infinity HPLC binary pump system hyphenated with an Agilent TOF (Agilent Technologies, Santa, CA, USA) via electrospray ionization (ESI) at positive mode. Chromatographic separation was carried out at 40 °C on a Kinetex C_{18} (2.1 µm, 50 mm x 2.1 mm) (Phenomenex, Torrance, CA, USA) reversed phase analytical column protected by a Kinetex C_{18} (4 mm x 2.1 mm) (Phenomenex, Torrance, CA, USA) guard column. The chromatographic run was performed with a gradient and the mobile phase composed of 0.1 % formic acid in water (solvent A) and 0.1 % formic acid in acetonitrile (solvent B). The initial condition was 95 % solvent A and 5 % solvent B maintained for 0.1 min. Then, acetonitrile was increased from 5 % to 100 % over 4 min and the column was washed with 100 % acetonitrile for 1.5 min. Then, the system was returned to the initial condition and re-equilibrated for 1.5 min. The total run time of the LC program was 7 min at the flow rate of 0.8 mL/min. The drying gas temperature and the flow rate were 300 °C and 13 L/min. The nebulizer pressure and the capillary voltage were 45 psi and 3800 V. The voltages fixed at fragmentor, skimmer, and octopole guides were 150, 60, and 250 V, respectively. The ion pulsar at the TOF analyzer was set at the measurement frequency of four cycles/s. Instrument control and data acquisition were performed by Agilent MassHunter workstation software version B.02.00 (Agilent Technologies, Palo Alto, CA, USA). Quantification of imatinib and CGP74588 was performed by Agilent MassHunter quantitative analysis software version B.05.02 (Agilent Technologies, Santa, CA, USA). Extraction of total ion chromatogram for imatinib, CGP74588 and trazodone were done with m/z 494.20-494.35, 480.20-480.35 and 372.10-372.20, respectively.[7, 13]

Results and discussion

The metabolic rates of the individual patients display a large inter-individual variability for imatinib.[14] The optimization of the treatment efficiency depends on the quantification protocols of imatinib and its main metabolite, CGP74588. Therefore efficient and low cost single TOF-MS method is valuable, in the anticipated increase in the demand when therapeutic drug monitoring does become standard practice.

The very first LC-MS based analytical technique was developed and validated by Bakhtiar et al. in 2002.[3, 9] The analytical run time was 3 min and LOQ of the reported methods was 0.001 and 0.002 µg/mL for the drug and its main metabolite, respectively in plasma from both monkey and human. However, the method is rather quick and sensitive, but the applicability of the method in clinical research is low due to the use of atmospheric pressure chemical ionization (APCI) interface and tandem mass spectrometry. This technique is not commonly used in clinical labs, as it is expensive and required skilled labour (including on-line sample preparation) in comparison to electrospray ionization (ESI). Subsequently, numerous laboratories have reported the use of high throughput LC based analytical procedures with different detectors for the quantification of these analytes in human plasma or other biological samples since 2002 (Table 1).

Our method is based on LC-TOF-MS, mainly aimed for routine clinical in-house analysis available for quantification of imatinib/ CGP74588 in human plasma from CML patients. The novel method consists of two different critical steps prior to the analysis; extraction and enrichment of analytes from the plasma. The extraction protocol was optimized to be able to ensure high recovery from smaller volume of plasma. Sample preparation starts with protein precipitation, as imatinib is in complex with the protein.[15] The pH of the extraction solution is important for optimal extraction of imatinib due to the presence of functional groups which can be protonated (Fig. 1). The common protein precipitating solvents, such as methanol and acetonitrile, were tested at different pH.[1] The efficiency of the extraction was based on the pH of extraction solvent. We tested two solvent systems (100 % methanol and methanol/formic acid at pH 4.8) obtaining similar recoveries (data not shown). The 100% methanol was selected as the best extraction solvent for imatinib/CGP74588. The optimal volume of the plasma was 100 µL in this study after testing different volumes of plasma samples (50-500 µL) (Table 1). Nevertheless, the sensitivity is higher in tandem mass spectroscopy when compared to UV and single quadrupole detectors (Table 1). According to some previous reports, the extraction follows enrichment of analytes by solid phase extraction (SPE) to remove all endogenous interfering substances.[1, 2] Furthermore, it is important to get clean

extracts as it influences the total ion chromatogram (TIC). The clean extracts avoid the masking of the signal of analytes by impurities. The TIC obtained from this study is shown in Fig. 3, although the present method does not have any extra purification step. Therefore, the method is simple, not tedious, and has low variability of the results according to the recovery data. Recoveries in spiked plasma samples were above 95 % for both imatinib and CGP74588 (Table 2).

The analytes separation in the present study was performed with a Phenomenex Kinetex reverse phase (RP) C_{18} analytical LC column. RP-liquid chromatography (RP-LC) is a well-known technique for the determination of various tyrosine kinase inhibitors like imatinib (Table 1). In this work, several important chromatographic factors were evaluated, e.g. organic modifiers, pH, buffer molarity, temperature, and flow rates of mobile phases. The selected factors for optimization were mainly based on initial experiments, prior knowledge from literature, and certain instrumental limitations (See the method section and Table 1).

The quantification was based on internal standard (IS). The most appropriate IS for this analysis is isotope labelled analogs.[16] The isotope labelled imatinib is expensive. Therefore, trazodone ($C_{19}H_{22}ClN_5O$), a commercially available cheap compound with similar chemical and physical properties to imatinib was used as the IS. The IS was not naturally present in plasma but it was added to the samples to compensate for unavoidable assay variance owing to, for example, extraction efficiency, ionization effects and transfer losses.[13, 16] It is believed that trazodone behaves in a similar manner to the analytes.[13] We did not observe any suppressing effect on the analyte ions in the LC-TOF-MS analysis.[16] Mass spectrometric conditions were optimized to achieve maximum stable responses of the analytes in ESI. The analytes showed higher sensitivity at the positive mode.[13, 17] The extracted LC chromatograms and corresponding mass spectra are shown in Fig. 4. The analytes and IS provided singly charged protonated precursor molecular ion $[M+H]^+$. Usually, this molecular fragment is predominant in ESI (soft ionization) mode. However, in present method it is not predominant due to the low voltage (150 V) used in the MS, which hindered further fragmentation of molecular ions and gave m/z of 494, 480 and 372 for imatinib, CGP74885 and trazadone, respectively (Fig. 4 and 5).[7, 13, 18] Yet, imatinib MS spectra also showed the doubly charged ion of imatinib $[M+2H]^{++}$ at m/z of 247, and few other low intense fragments (m/z) such as 394, 476 and 217 (Fig. 4 and 5).[13, 18, 19] The imatinib fragmentation occurs due to single bond cleavages with charge migration of imatinib at the positive ESI mode. The m/z 394 was formed due to neutral losses of methylpiperazine and it was 100 amu less than the molecular ion of imatinib (Fig. 4 and 5).[18] The same fragment ion (m/z 394) is present in the metabolite of imatinib (Fig. 4 and 5). All fragmentation findings were compatible with Marull *et al*. and thereby identification of the analytes was confirmed.[18, 19]

After optimization of chromatographic and mass spectrometric conditions, the total chromatographic run time was 7 min and the retention times (RT) for imatinib and CGP74588 were 3.09 and 3.01 min, respectively. Thus, a large number of samples can be analyzed within a day. The identification of imatinib and CGP74588 was carried out by comparing the LC retention time and full scan mass spectra with the authentic standards. We have achieved baseline separation of imatinib and CGP74588 in plasma without any interference from endogenous compounds (Fig. 4). There is no reported study on LC-TOF-MS analysis of imatinib and CGP74588 to compare with our results. However, in a brief survey of published methods since 2002, the analytical time varies from 3 - 40 min, due to different chromatographic MS conditions and techniques (Table 1). LC separation column performances are specific, selective and efficient based on the analytes as well as column dimensions. According to Table 1, 70 % of published methods used RP-C_{18} analytical columns although different methods gave different analytical times to separate imatinib and CGP74588 (Table 1). The shorter columns elutes faster compared to longer columns except for Zhang, Y. et al. (Table1).[20] It is known that tandem mass spectrometry (MS/MS) methods allow shorter analytical times compared to single quadrupole MS or UV analysis, because MS/MS methods are more selective due to multiple reaction monitoring (MRM) tecnique.[3, 9, 20] The inner diameter (mm) and particle size (µm) of the LC-column are important factors determining the total analytical time of the LC analysis (Table 1). However, Bakhtiar *et al.* have used RP-LC-C_8 columns for the separation of the analytes.[3, 9] In his method, the total analytical time was 3 min and this might be due to the different column packing materials. The RP-C_8 column has eight carbon bonded silica particles where in RP-C_{18} it is eighteen carbon-bonded silica. Thus, polar analytes have less retention times in RP-C_8 column compared to RP-C_{18} column (Table 1).

The validation procedure as described in the EU Commission Decision/657/EEC was used as a guideline in this study. All the quantitative analyses were based on the calibration curves plotted using freshly prepared solutions daily. The calibration curve was prepared by plotting the peak area of imatinib and CGP74588, normalized to IS peak area, versus the nominal concentrations of analytes. The linearity was determined by linear regression analysis. The correlation coefficients (r^2) of imatinib and CGP74588 were always between 0.994 - 0.999 and 0.998 - 0.999, respectively. The therapeutic plasma concentration of imatinib is usually ranging between 0.5 and 1 µg/mL in CML patients. To ensure the highest treatment efficacy, it is necessary to determine the individual therapeutic variability of each patient. Yet, the imatinib concentration in circulation system can be very useful for personalized treatments.[4, 5, 12] It is reported that the bioavailability of CGP74588 in plasma is always 5 - 25 % of imatinib concentration. Consequently, the linearity of the calibration curve was established in order to cover the clinically relevant range of imatinib concentrations expected in most patients.[4, 5] The linearity range obtained from this study was comparable

to those already published (Table 1). The linearity responses (r^2) of the analytes are almost 1.00 for both imatinib and CGP74588. This shows that the signal generated for each analyte was linear within the selected mass range and no endogenous matrix interferences were observed.

The quantitative recovery of imatinib and CGP74588 in plasma was evaluated by comparing peak areas of the analytes in the reconstitution solvent with peak areas after extraction of analytes from plasma. Multiple aliquots (n=6) at each of the three different concentrations were assessed. The mean recovery of imatinib, CGP74588 and IS were ranged from 95 - 107 %. In this study, the peaks of analytes in the spiked samples were identified by LC-TOF-MS, and there were no noticeable co-eluting contaminants in plasma samples (Fig.4). The two analytes also did not influence each other (Fig. 5).

Precision and accuracy were assessed by replicate analysis (n = 6) of spiked plasma samples at three different concentrations and data were presented in Table 2. The intra and inter-day precisions were between 3.7 % and 7.7 % for all the compounds and the accuracy was within ±10 % (Table 2).

The lowest concentration levels that could be determined with a bias and CV% lower than ±20 % was considered as LOQ and found to be less than 0.02 µg/mL.

Already validated samples (n=15) from a reference laboratory in Bordeaux, France (see the material section) were analysed by our method. The data comparison of our results to those of Bordeaux was shown less than 10 % difference. Therefore, the method reported here is reliable (the data is not shown). Nevertheless, the data obtained from the study also showed higher recovery above 92 % which denoted minimized matrix effect with considerably low ionization suppression.

Stability of the imatinib and CGP74588

The recovery results of imatinib in human plasma indicate no significant degradation after 6 month storage at -70 °C. Re-injection of calibration and QC imatinib and CGP74588 samples after additional storage for 12 weeks at -20 °C showed no significant difference of the results. This showed the possibility of the long-term storage of diluted extracts before final analysis (data is not shown). Further, we have tested a 10-year old and a newly purchased (Sigma Aldrich (Stockholm, Sweden)) imatinib mesylate powder obtaining exactly the same results proving the stability of imatinib. Yet, we have prepared fresh solutions while analyzing GCP74588. The stability studies also concurred with former reports.[13, 22]

Sensitivity of the method

The LOQ was more sensitive or in the same range as previously published LC-MS analytical assays (Table 1). This method was already implemented for routine analysis and can be used easily in daily clinical practice. Assessed imatinib concentrations of 55 CML patient plasma samples varied in a range of 0.4-4.5 µg/mL (Fig. 6). Fig. 5 shows the typical chromatograms of imatinib and CGP74588 extracted from human plasma obtained from a patient.

Imatinib is commonly used to treat CML patients, and a target concentration of imatinib is 1 µg/mL as recommended elsewhere.[4] The variation of imatinib and CGP74588 concentrations in plasma of CML patients was possibly due to the individual pharmacokinetics.[4]

Conclusions

Here, we report the first validated LC-TOF-MS assay for the analysis of imatinib/CGP74588 in human plasma from CML patients. This method is accurate, simple and robust without extensive sample preparation thus ideal for routine analysis. This method can be a valuable tool for pharmacokinetic studies of imatinib and its main metabolite in the CML population.

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Figure Legends

Figure 1: Chemical structures of imatinib (A) and *N* desmethyl imatinib (B)

Figure 2: A simple illustration of the model mechanism. Imatinib (yellow) inhibits the binding of ATP to the *Bcr-Abl* kinase (blue).

Figure 3: Imatinib fragmentation in positive ESI ion mode.

Figure 4: The LC-MS-TOF extracted chromatograms and corresponding positive ion ESIMS-TOF spectra for CGP74588 (0.02 µg/mL) (top), imatinib (0.02 µg/mL) (middle) and trazadone (0.25µg/mL) (bottom).

Figure 5: Typical chromatograms of plasma obtained from a patient sample (A) CGP74588 (0.36 µg/mL) (B) imatinib (1.26 µg/mL).

Figure 6: The amount of imatinib and CGP74588 distribution in the circulation system of CML patients after intake of their daily dosage of 400 mg.

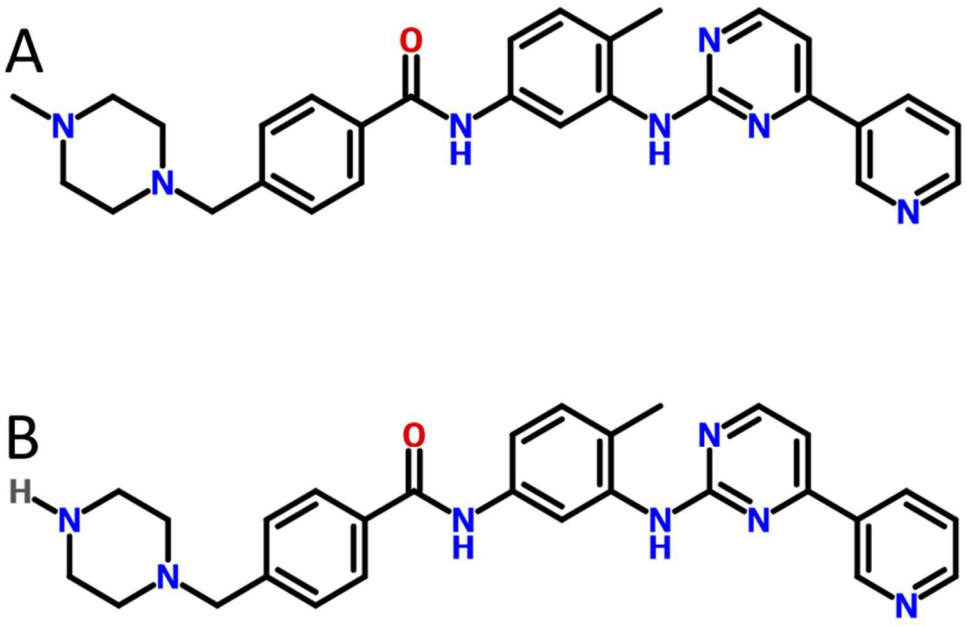

Figure 1: Chemical structures of imatinib (A) and N desmethyl imatinib (B)
81x54mm (300 x 300 DPI)

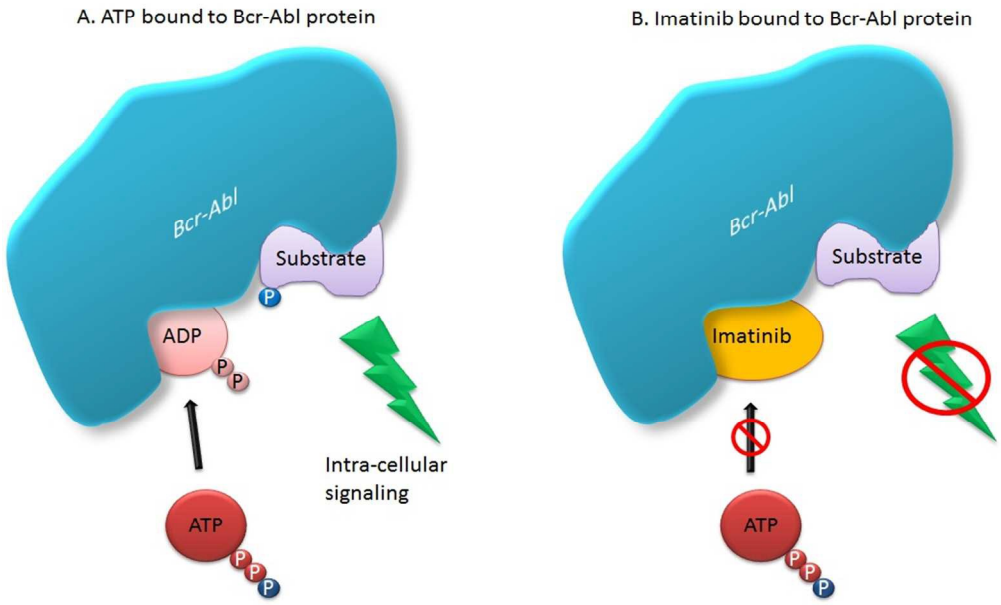

Figure 2: A simple illustration of the model mechanism. Imatinib (yellow) inhibits the binding of ATP to the Bcr-Abl kinase (blue).
275x194mm (96 x 96 DPI)

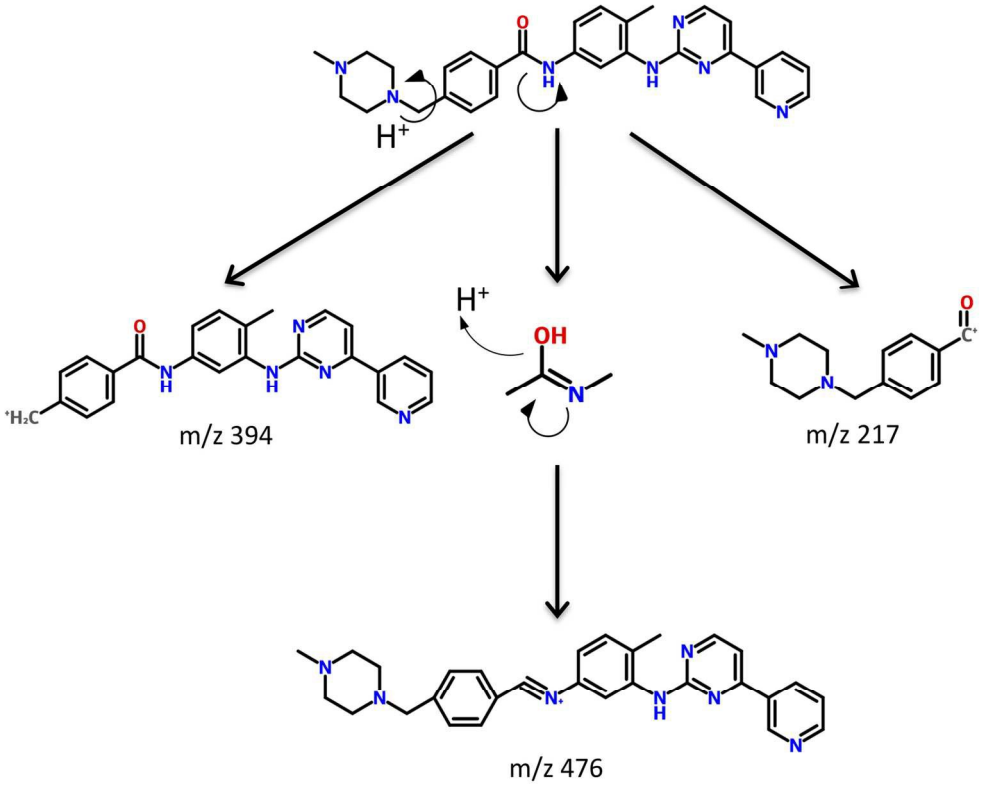

Figure 3: Imatinib fragmentation in positive ESI ion mode.
140x113mm (300 x 300 DPI)

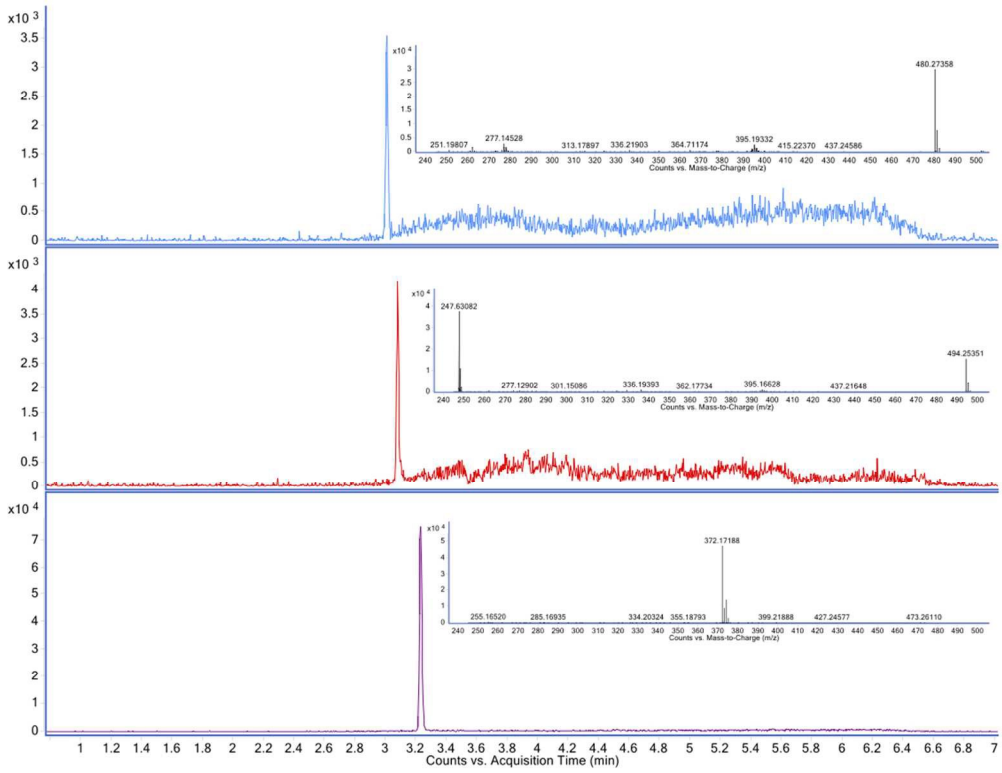

Figure 4: The LC-MS-TOF extracted chromatograms and corresponding positive ion ESIMS-TOF spectra for CGP74588 (0.02 µg/mL) (top), imatinib (0.02 µg/mL) (middle) and trazadone (0.25µg/mL) (bottom).
191x147mm (150 x 150 DPI)

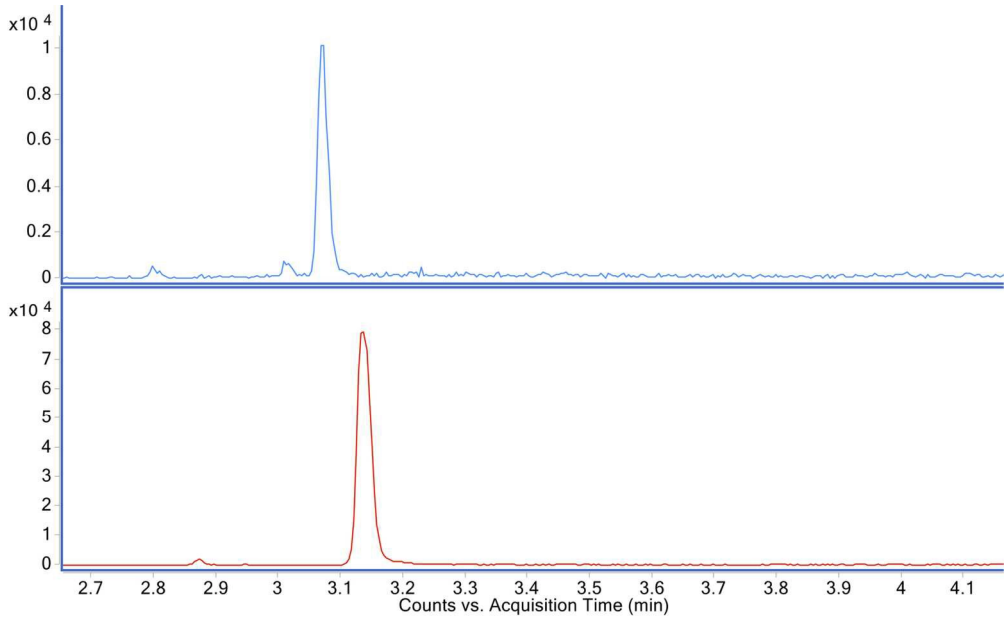

Figure 5: Typical chromatograms of plasma obtained from a patient sample (A) CGP74588 (0.36 µg/mL) (B) imatinib (1.26 µg/mL).
135x82mm (300 x 300 DPI)

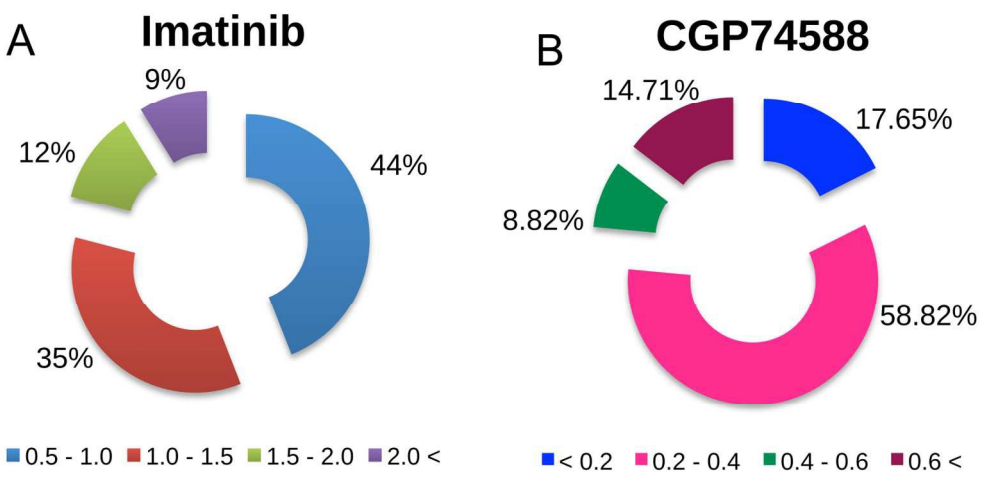

Figure 6: The amount of imatinib and CGP74588 distribution in the circulation system of CML patients after intake of their daily dosage of 400 mg.
147x73mm (300 x 300 DPI)

Table 1: Overview of the liquid chromatography (LC) based analysis of imatinib and CGP74588

Analytes	LOQ µg/mL	Sample type (µL)	Analytical method	Column	Mobile phases	Total run time/min	Linear range (µg/mL)	Ref
Imatinib CGP74588	0.0005 0.0005	K562 cells, 0.4×10^6/mL	LC-MS/MS	C_{18}, 250 x 2.0 mm, 5.0 µm	MeOH: H_2O (65:35) 7mmol/L NH_4Ac.	15.0	0.001-5.0 0.002-5.0	21
Imatinib CGP74588	0.0100 0.0100	Plasma:50	LC-MS/MS	C_{18}, 50 x 2.0 mm, 3.0 µm	H_2O (0.05 % HCOOH) : MeOH	6 .0	0.01-2.0	22
Imatinib CGP74588	0.0100 0.0100	Plasma:100 Tissues: 0.05-0.2 g per mL	LC-UV	C_{18}, 150 x2.1 mm, 3.5 µm	ACN (50 mM NH_4Ac): H_2O 0.005M octane sulfonic acid	30.0	0.01-10.0	11
Imatinib CGP74588	0.0100 0.0200	Plasma:200	UPLC-MS/MS	C_{18}, 50 x 2.1 mm, 1.7µm	H_2O(2 mM NH_4-Formate): CAN (0.1%FA)	5.5	1.0-7.5 1.0-2.5	10
Imatinib CGP74588	0.0080 0.0030	Plasma:400	LC-MS/MS	C_{18}, 100 x 4.6 mm, 2.4µm	MeOH (0.1% FA): H_2O (0.2%NH_4Ac)	4.0	0.008-5.0	20
Imatinib CGP74588	0.0100 0.0100	Plasma:500 Urine:500 CSF:500	LC-UV	PDB-ZrO_2, 50 x 4.6mm, 3µm	0.1M $KH_2PO_4H_2O$ +MeOH:0.01M KH_2PO_4/0.09M K_2HPO_4 + MeOH	40.0	0.01-20.0	23
Imatinib CGP74588	0.0010 0.0020	plasma:250	LC-MS/MS	C_8, 50 x 4.6 mm, 3.5 µm	MeOH (0.05% NH_4Ac):H_2O (0.05% NH_4Ac)	3.0	0.001-1.0 0.002-1.0	9
Imatinib CGP74588	0.0040 0.0040	Plasma:200	LC-MS/MS	C_8, 50 x 4.6 mm, 3.5 µm	MeOH (0.05% NH_4Ac):H_2O (0.05% NH_4Ac)	3.0	0.004-10.0	3
Imatinib CGP74588	0.0300 0.0300	Plasma:200	LC-MS	C_{18}, 50 x 4.6 mm, 5 µm	H_2O(0.1% FA) MeOH (0.1% FA)	14.0	0.03-10.0	6
Imatinib CGP74588	8.3500 8.3500	Plasma:500	LC-MS	C_8, 50 x 4.6 mm, 5 µm	H_2O(0.1% FA) ACN (0.1% FA)	10.0	Not presented	24
Imatinib CGP74588	0.0500 0.0500	Plasma:200	LC-UV	C_6 phenyl, 150 mm x 4.6 mm, 5 µm	MeOH: 50 mM NH_4Ac	35.0	0.1-12.0	25
Imatinib **CGP74588**	**0.02** **0.02**	**Plasma:100**	**LC-MS-TOF**	**C_{18}, 50 x 2.1 mm, 2.6 µm**	**ACN (1% FA) : Water (1% FA)**	**7.0**	**0.02-5.0**	**Present study**

Table 2: Inter- and intra-day accuracy and precision of the method

		Nominal conc. (µg/mL)	Mean conc.(ng/mL)	Precision CV (%)	Accuracy (% bias)
Imatinib	Intra-day	0.1	106	5.1	6.0
		1.0	1031	3.9	3.1
		2.0	1892	7.2	-5.4
	Inter-day	0.1	109	4.9	9
		1.0	944	4.2	-5.6
		2.0	2151	7.7	7.6
CGP74588	Intra-day	0.05	33	6.3	10.0
		0.1	103	4.4	3.0
		0.5	487	6.4	-2.6
	Inter-day	0.05	28	6.9	6.7
		0.1	105	4.7	5.0
		0.5	517	5.9	3.4