Quantitative Determination of Imatinib in Human Plasma with High-Performance Liquid Chromatography and Ultraviolet Detection

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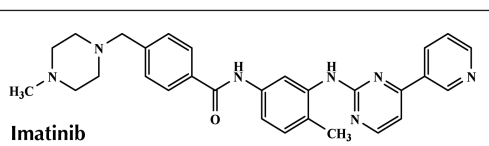
Abstract

A simple and sensitive high-performance liquid chromatography (HPLC) method was developed to quantitate imatinib in human plasma. Imatinib and the internal standard dasatinib were separated using a mobile phase of 0.5% KH_2PO_4 (pH3.5)–acetonitrile–methanol (55:25:20, v/v/v) on a CAPCELL PAK C18 MG II column (250 mm × 4.6 mm) at a flow rate of 0.5 mL/min and measurement at UV 265 nm. Analysis required 100 µL of plasma and involved a solid phase extraction with an Oasis HLB cartridge, which gave recoveries of imatinib from 73% to 76%. The lower limit of quantification for imatinib was 10 ng/mL. The linear range of this assay was between 10 and 5000 ng/mL (regression line $r^2 > 0.9992$). Inter- and intra-day coefficients of variation were less than 11.9% and accuracies were within 8.3% over the linear range. The plasma concentrations of imatinib obtained by our present method were almost the same as those assayed by an LC–MS–MS method at the Toray Research Center, Inc. This method can be applied effectively to measure imatinib concentrations in clinical samples.

Introduction

Imatinib mesylate (Figure 1) is a potent and selective inhibitor of protein tyrosine kinase Bcr-Abl, platelet derived growth factor receptors, and the receptor kinase c-KIT. It has been approved for the treatment of chronic myelogenous leukemia (CML) and gastrointestinal stromal (GIST) tumors (1–3). Cytogenetic and molecular responses to imatinib are correlated with the trough plasma concentration of imatinib for patients with CML (4,5). Therefore, imatinib requires therapeutic drug monitoring for optimal efficacy.

Liquid chromatography-tandem mass spectrometry (LC–MS) is used for most quantitative determinations of imatinib in the plasma of CML patients (6–9). However, LC–MS facilities are not always available in standard hospital laboratories. On the other hand, several high-performance liquid chromatography (HPLC) methods with ultraviolet (UV) detection for the quantitation of imatinib in plasma have been reported. Some methods allow quantitation only of imatinib concentration (10,11), while some methods using ion pairing reversed phase HPLC permit the simultaneous determination of imatinib and its main metabolite (12,13). The UV detection methods reported by Oostendorp et al. (12) and Schleyer et al. (13) required multi-step liquid–liquid extraction. The method reported by Schleyer et al. (13) required relatively large sample volumes (300 µL) to achieve sensitivity (10 ng/mL). On the other hand, although the method reported by Velpandian et al. (10) used methanol to extract 100 µL of plasma, the limit of quantitation for imatinib was 30 ng/mL. Although one method using solid phase extraction has been reported (11), that method required 750 µL of plasma and the limit of quantification for imatinib was 50 ng/mL. Thus, a simpler assay method would be beneficial. The method presented here is rapid and simple, and consists of one step solid-phase extraction followed by HPLC-UV, which allows the determination of imatinib concentration in human plasma. The extraction procedure used for the pretreatment of a plasma sample ensures high recovery from a relatively small amount of plasma (100 µL) for complete analysis. To validate this method, trough plasma concentrations of imatinib in CML patients was assessed.



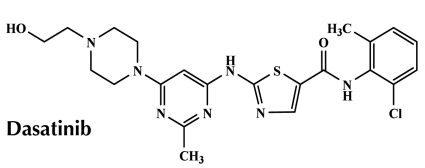


Figure 1. Chemical structures of imatinib and dasatinib.

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Experimental

Chemicals and reagents

Imatinib mesylate and dasatinib were purchased from Toronto Research Chemicals Inc. (Ontario, Canada). An Oasis hydrophilic lipophilic balance (HLB) extraction cartridge (1 mL, 30 mg) was purchased from Waters (Milford, MA). All other reagents were purchased from Nacalai Tesque (Kyoto, Japan). All solvents were HPLC grade.

Stock solutions for generating standard curves of imatinib and dasatinib were prepared by dissolving the dry reagents in methanol to yield concentrations of 1.0 mg/mL. Working standard solutions of imatinib (10, 100, 1000, 3000, and 5000 ng/mL) and dasatinib (10, 100, and 1000 ng/mL) were prepared by serial dilution with methanol.

Chromatographic conditions

A PU-2080 plus chromatography pump (JASCO, Tokyo, Japan) equipped with a UV-2075 plus ultraviolet detector (JASCO) was used. The HPLC column was a CAPCELL PAK C18 MG II (250 mm × 4.6 mm i.d., Shiseido, Tokyo, Japan). This column is provided with packing material made of totally porous spherical silica coated with a silicone polymer monolayer with octadecyl (C18) groups. The mobile phase was 0.5% KH_2PO_4 (pH 3.5), acetonitrile, and methanol (55:25:20, v/v/v), which was degassed in an ultrasonic bath prior to use. Before mixing with acetonitrile, the pH of the 0.5% KH_2PO_4 was adjusted with 50% phosphoric acid. The flow rate was 0.5 mL/min at ambient temperature and sample detection was carried out at 265 nm

Extraction method

A 10 µL solution of dasatinib (100 ng) was added as an internal standard to a 100 µL plasma sample and then the plasma sample was diluted with 900 µL water and vortexed for 30 s. This mixture was applied to an Oasis HLB extraction cartridge that had been activated previously with methanol and water (1.0 mL each). The cartridge was then washed with 1.0 mL water and 1.0 mL 60% methanol in water, and eluted with 1.0 mL 100% methanol. Eluates were evaporated to dryness in a vacuum at 40°C using a rotary evaporator (Iwaki, Tokyo, Japan). The residues were dissolved in 50 µL methanol (vortex mixed for 30 s) and then 50 µL mobile phase was added to each sample (vortex mixed for another 30 s). An aliquot of 50 µL of each sample was then processed on the HPLC apparatus.

Calibration curve

The calibration curve was obtained from spiked blank plasma samples in a concentration range of 10 to 5000 ng/mL for imatinib. The calibration curve consisted of a blank sample and five calibrator concentrations (10, 100, 1000, 3000, and 5000 ng/mL). Blank plasma samples were treated as described above. Calibration graphs were constructed from the peak height ratio of imatinib to the dasatinib internal standard from the HPLC chromatograms and the nominal concentration of imatinib. The calibration curves were calculated by the least squares method.

Recovery

Recovery following the extraction procedure was determined by comparing the peak height of blank plasma samples extracted according to the above procedure with those of non-extracted control samples. Control samples were prepared by mixing solutions containing the same amount of compound that was added to the plasma blank; however this compound was obtained by evaporating to dryness directly, rather than by extraction, and was then reconstituted in methanol.

Assay validation

Inter-day precision and accuracy were determined from the analyses of control samples done on five different days, whereas intra-day precision and accuracy were determined by analyzing spiked controls that were run in random order five times over the course of one day. The precision of the method at each concentration was determined by comparing the coefficient of variation (CV), obtained by calculating the standard deviation (SD) as a percentage of the calculated mean concentration. The accuracy estimated for each spiked control was obtained by comparing the nominal concentration with the assayed concentration. The limits of quantitation (LOQ) were determined as the lowest non-zero concentration measured with an intra-day CV of within 20% and an accuracy of less than ± 20% (14), and the limit of detection (LOD) was determined as the lowest concentration with a signal to noise ratio of 3.

Stability

Imatinib is stable in blood and plasma for at least 96 h at room temperature (11,12), while long term stability studies showed that imatinib is stable in plasma for at least 12 months when stored at –20°C (11).

Application to pharmacokinetic studies

The method was used to measure the steady state trough plasma concentrations of imatinib in eight patients. This study was approved by the Ethics Committee of Akita University School of Medicine, and all patients gave written informed consent. Patients were given a standard dose (400 mg or 600 mg) of Gleevec brand of imatinib (Novartis Pharma) for at least 12 months. Blood samples were collected by venipuncture 24 h after oral administration of imatinib. Plasma was isolated by centrifugation at 1900 × *g* for 15 min and stored at –40°C until analysis. Plasma samples (100 µL) were then extracted as described above. On the other hand, trough plasma concentrations of imatinib from eight CML patients were analyzed using HPLC equipped with a mass spectrometric detector by Toray Research Center, Inc. (8,9), the only assay system in Japan authorized by Novartis Global.

Results and Discussion

Chromatograms

Typical chromatograms obtained for blank plasma and for plasma samples spiked with imatinib and dasatinib are shown in Figure 2A–2C. The separation of imatinib and dasatinib was sat-

isfactory and free of interfering peaks in the biological matrix. The peaks of imatinib and dasatinib were clearly separated using a mobile phase of 0.5% KH_2PO_4 (pH 3.5), acetonitrile, and methanol (55:25:20, v/v/v) on a CAPCELL PAK C18 MG II column at a flow rate of 0.5 mL/min. Dasatinib was used as an internal standard for the method. The second generation Abl kinase inhibitor dasatinib has been developed to overcome imatinib-resistance in CML (15). Dasatinib is not used in combination with imatinib in clinical settings. In addition, the most important reason for selecting dasatinib was that it co-eluted with imatinib on the Oasis HLB extraction cartridge, and they are not eluted with methanol solutions up to 60% concentration. Furthermore, the retention times of imatinib and dasatinib are similar (12.3 and 14.5 min, respectively). It was found that the percentages of acetonitrile and methanol in the mobile phase were quite important. When acetonitrile alone was used as an organic solvent in the mobile phase, the peaks of imatinib and dasatinib could not be separated at baseline level. On the other hand, when methanol alone was used in the mobile phase, the retention times and the peak shapes of the two compounds were large and broad, and the analytical time was long. Eventually, a mixture of 0.5% KH_2PO_4 (pH 3.5), acetonitrile, and methanol (55:25:20, v/v/v) was adopted to achieve an efficient chromatographic separation of the analytes and no interference of endogenous peaks with imatinib or dasatinib at their respective retention times was observed. Recommendations state that the CAPCELL PAK C18 MG II analytical column should be washed in 60% methanol in water to ensure maximal column life. In our hands, it was washed for one hour after measuring about 20 plasma samples. More than 1000 samples were injected onto the same column without loss of resolution.

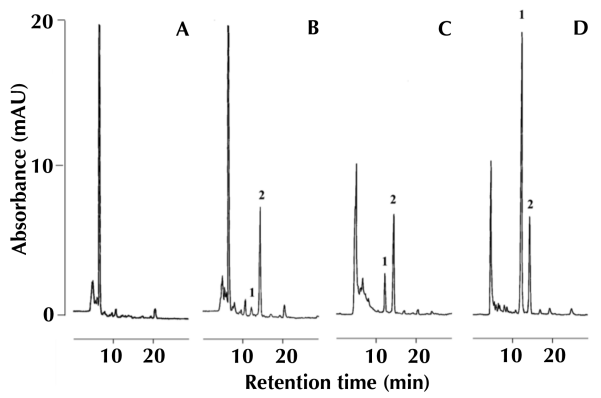

Figure 2. Typical chromatograms of A) plasma blank, B) 100 µL plasma spiked with imatinib (1 ng) and dasatinib (100 ng), C) 100 µL plasma spiked with imatinib (10 ng), and dasatinib (100 ng) and D) a plasma sample taken 24 h after oral administration of 400 or 600 mg imatinib. Calculated concentrations are 660 ng/mL of imatinib. Peaks: 1; imatinib, 2; dasatinib (IS).

Calibration curve

Calibration curves were analyzed by a peak height method. Calibration curves for imatinib in plasma were linear over the concentration range of 10–5000 ng/mL. Typical calibration curves (obtained using the least-squares method) could be expressed as $y = 0.0044x - 0.0075$ ($r^2 = 0.9992$), where y is the peak height ratio and x is the concentration in ng/mL.

Recovery

The results of recovery studies from human plasma are shown in Table I, with the recoveries of imatinib and dasatinib determined by adding five known imatinib concentrations (10, 100, 1000, 3000, and 5000 ng/mL) and three dasatinib concentration (10, 100, and 1000 ng/mL) to drug-free plasma. Solid-phase extraction using a Waters Oasis HLB cartridge was optimized to extract the analyte and internal standard from plasma samples. Use of water (1 mL) during the washing step gave consistent recovery with increased specificity, especially at the LOQ level with minimized polar matrix interference. In addition, imatinib and dasatinib were not extracted from an Oasis HLB extraction cartridge at methanol concentrations up to 60%. The extraction procedure described here offers a rapid way to isolate the analyte and internal standard from the plasma matrix. The extraction recovery values for imatinib and dasatinib were 73–76%, and 73–75%, respectively (Table I). Extraction using the Oasis HLB cartridge was simpler and faster than previously reported methods (11).

Table I. Accuracy and Precision of HPLC Assay for the Determination of Imatinib and Dasatinib in Human Plasma (n = 5)

	Intra-day			Inter-day			
Added (µg/mL)	Found mean ± SD	CV (%)	Accuracy (%)	Found mean ± SD	CV (%)	Accuracy (%)	Recovery (%)
Imatinib							
10	10.6 ± 1.1	10.4	6.0	10.9 ± 1.3	11.9	8.3	73
100	99.2 ± 3.4	3.4	–0.8	98.7 ± 3.8	3.9	–1.3	75
1000	986 ± 40	6.6	–4.0	1034 ± 68	6.6	3.3	76
3000	2967 ± 157	5.3	–1.1	2882 ± 223	7.7	–3.9	73
5000	5020± 287	5.7	0.4	5087 ± 263	5.2	1.7	76
Dasatinib							
10	10.3 ± 1.6	15.8	3.2	10.4 ± 1.9	18.3	3.8	74
100	98.0 ± 10	10.5	–2.0	100 ± 9.9	9.8	0.0	73
1000	1008 ± 92	9.1	0.8	1054 ± 99	9.4	5.1	75

Precision and accuracy

The CV and accuracy for intraday and inter-day assays were determined at concentrations of 10–5000 ng/mL for imatinib and 10–1000 ng/mL for dasatinib. CVs for intraday and inter-day assays were less than 11.9% for imatinib and 18.3% for dasatinib (Table I). Accuracies for intraday and inter-day assays were within 8.3% for imatinib and 3.8% for dasatinib (Table I). The precision and accuracy of this HPLC assay is suitable for both routine therapeutic drug monitoring applications and clinical pharmacokinetic studies.

Sensitivity

The value of the LOQ was 10 ng/mL and the LOD was 5 ng/mL for imatinib. The LOQ (or LOD) was more sensitive or in the same range as the LOQ of several LC–MS analytical assays (6–9) and HPLC-UV

analytical assays (10–13). This sensitivity was achieved using a limited sample volume of 100 µL plasma. Assays that require small sample volumes are useful for routine drug monitoring of patients.

Application

A typical chromatogram of a plasma sample collected from a subject 24 h after administration of a 400 or 600 mg oral dose of imatinib is shown in Figure 2D. The peaks corresponding to imatinib and dasatinib were separated, with no interfering peaks detected at the retention of each analyte. The plasma concentrations of imatinib obtained by our present method were almost the same as those assayed by LC–MS–MS of the Toray Research Center, Inc. (Figure 3). Thus, this developed HPLC-UV method can be considered a clear, low cost alternative to LC–MS–MS for therapeutic drug monitoring of imatinib. Picard et al. reported that the threshold for trough concentration of imatinib should be set above 1002 ng/mL, as this level was significantly associated with a major molecular response based on concentration-effect receiver operating characteristic curve analysis (5). Monitoring the trough concentration of imatinib would likely be useful in clinical practice when the expected response is not achieved during imatinib therapy. The simple and sensitive HPLC–UV method using a small amount of plasma (100 µL) would permit therapeutic drug monitoring of imatinib for CML patients in standard hospital laboratories which do not have LC–MS–MS facilities and represents a more practical platform to measure imatinib plasma concentration in clinical practice. Furthermore, our method requires only one hour between the sampling of blood to completing the calculation of imatinib plasma concentration. In clinical situations, convenience is often given priority over precision, but in the present method, both precision and convenience were acheived.

Conclusion

A new HPLC-UV method for quantitation of imatinib is presented in this paper. It is simple, highly sensitive and rapid considering the sample treatment procedure. The precision and accuracy of the HPLC assay is suitable for pharmacokinetic studies. This method can be useful for therapeutic drug monitoring of imatinib in a routine setting.

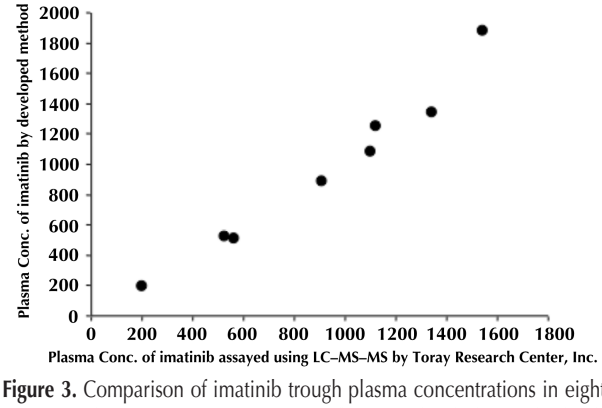

Figure 3. Comparison of imatinib trough plasma concentrations in eight patients with CML. Concentrations were determined with the newly developed HPLC-UV method and by an LC–MS–MS method at the Toray Research Center, Inc.

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