Imatinib assay by high-performance liquid chromatography in tandem mass spectrometry with solid-phase extraction in human plasma

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ABSTRACT: We have developed a method of liquid chromatography in tandem with mass spectrometry to monitor therapeutic levels of imatinib in plasma, a selective inhibitor of protein tyrosine kinase. After solid-phase extraction of plasma samples, imatinib and its internal standard, imatinib-D8, were eluted with Zorbax SB-C\textsubscript{18} at 60 °C, under isocratic conditions through a mobile phase consisting of 4 mM ammonium formate, pH: 3.2 (solution A) and acetonitrile solution B. The flow rate was 0.8 mL/min with 55% solution A + 45% solution B. Imatinib was detected and quantified by mass spectrometry with electrospray ionization operating in selected-reaction monitoring mode. The calibration curve was linear in the range 10–5000 ng/mL, the lower limit of quantitation being 10 ng/mL. The method was validated according to the recommendations of the Food and Drug Administration, including tests of matrix effect (bias <10%) and recovery efficiency (>80 and <120%). The method is precise (coefficient of variance intra-day <2% and inter-day <7%), accurate (95–108%), sensitive and specific. It is a simple method with very fast recording time (1.2 min) that is applicable to clinical practice. This will permit improvement of the pharmacological treatment of patients. Copyright © 2012 John Wiley & Sons, Ltd.

Keywords: imatinib; validation method; liquid chromatography in tandem with mass spectrometry; solid-phase extraction; isocratic; chronic myeloid leukemia

Introduction

Imatinib (Glivec\textsuperscript{*} or Gleevec\textsuperscript{*}) is an orphan drug that acts as a selective competitive inhibitor of protein tyrosine kinase (ITK), mainly used in the treatment of chronic myeloid leukemia (CML), as well as in gastrointestinal stromal tumors (GIST) and other types of cancers. CML is a rare disease that constitutes 15% of all leukemias with an incidence of 1–2 per 100,000 people per year. It is a myeloproliferative disease characterized by the production of a clone of hematopoietic cells that in 90% cases express a Phyladelphia positive (Ph+) chromosome. The Ph+ chromosome is the result of reciprocal translocation between chromosomes 9 and 22 (Rowley, 1973) that encodes an abnormal protein BCR-ABL tyrosine kinase that is constitutively active (Shittalman et al., 1985), thus transferring a phosphate group from ATP to tyrosine residues of protein target substrates. Imatinib binds to the ATP binding site of BCR-ABL enzyme and inhibits its activity in a competitive manner (Chen et al., 2010). Therefore, the substrate cannot be phosphorylated and the tumor cell cannot proliferate, stopping their growth.

For clinical purposes, trough concentration of imatinib before the morning dose has been the most accepted measure because it is easier to obtain and it has been reported in several studies (Larson et al., 2008; Picard et al., 2007) through peak before the morning dose has been stimated. Although there is little variability in plasma concentrations of imatinib to valley within each individual, the variability between patients is very large (Larson et al., 2008; Picard et al., 2007). This is partly explained by incomplete adherence to therapy (Noens et al., 2009; Yoshida et al., 2011), intrinsic variations in the metabolism of imatinib (Cortes et al., 2009) or drug interactions (Cortes et al., 2009). There is evidence from recent studies showing a correlation between plasma trough levels of imatinib and clinical response, suggesting that maintaining imatinib plasma concentrations above 1000 ng/mL may be associated with better therapeutic results (Awidi et al., 2010; Larson, 2009; Picard et al., 2007). There are studies that show that, in patients who do not respond well to initial treatment with imatinib, the measurement

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Abbreviations used: ACN, acetonitrile; CML, chronic myeloid leukemia; ESI, electrospray ionization; GIST, gastrointestinal stromal tumors; ITK, inhibitor of protein tyrosine kinase; SPE, solid-phase extraction; SRM, selected-reaction monitoring.
of trough plasma concentrations may help to adjust doses in order to optimize treatment (Cortes et al., 2009). This would suggest that the monitoring of imatinib levels would be required to adequately control the disease.

So far, the majority of the techniques describing the quantification of imatinib in human plasma have been performed in gradient conditions using high-performance liquid chromatography (HPLC) coupled to ultra-violet (UV) detection (Roth et al., 2010; Vivekanand et al., 2003), mass spectrometry (Parisé et al., 2003) or mass spectrometry/tandem mass spectrometry detection (Bouchet et al., 2011; De Francia et al., 2009; Haouala et al., 2009; Tittier et al., 2005). The method used in this work is based on the isocratic mode. The advantages of this method are the simplicity of technique and device, the fast resolution and the short time of recording, not needing time to established initial conditions. In contrast, the gradient elution mode requires longer recording times with longer post-time to recover the column, more complex apparatus needing regular maintenance, more variables that interfere with recording at elution onset, different steps and different times (Jandera, 1989) that increase the background noise (Boelens et al., 2004).

To date, several methods for quantification of imatinib in human plasma have been developed under isocratic conditions. Most of them have been determined by HPLC coupled with UV detection using protein precipitation for sample preparation (Schleyer et al., 2004; Tan et al., 2011; Velpandian et al., 2004). Only one method used solid-phase extraction (Davies et al., 2010), but with HPLC coupled with UV detection, which is less sensitive and selective than liquid chromatography in tandem with mass spectrometry (LC/MS-MS). Another method used LC/MS-MS with protein precipitation (Guettens et al., 2003). As described in the literature, matrix effect and ion suppression are major problems with LC/MS-MS and electrospray ionization (ESI) (King et al., 2000; Shen et al., 2005; Taylor, 2005). In that sense, solid-phase extraction (SPE) procedures minimize them compared with precipitation extraction procedures.

The aim of this study was to develop an LC/MS-MS method, with SPE under isocratic conditions to reduce matrix effect and recording time that can be used easily and rapidly for imatinib monitoring in clinical practice.

**Experimental**

**Chemicals and reagents**

Imatinib mesylate or \(4\{4[4\text{-methylpiperazin-1-yl}\text{methyl]N-[4\text{-methyl-3-}\{4\text{-pyridin-3-yl\text{pyrimidin-2-yl}\text{amino\text{phenyl}benzamide}\}\text{internal standard (IS) [2 H8]-Imatinib or Imatinib-D8} were provided by Selleckchem\text{Chemical structure of imatinib (a) and imatinib-D8, its internal standard (IS) (b). The fragmentation pattern of imatinib and its IS are indicated by an arrow.}

Figure 1. Chemical structure of imatinib (Gleevec, STI-571)

Table solutions, calibration standards and quality controls

Stock solutions of imatinib were prepared by dissolving an accurately weighed quantity in methanol to obtain a concentration of 1 mg/mL. The IS working solution was prepared by dissolving an exact amount in methanol to obtain a concentration of 1 mg/mL and was diluted 1000 times to give a working solution of 1 μg/mL. Stock solutions of imatinib and its IS were stored at \(-20^\circ\text{C}\) until use. Calibration standards were made to obtain concentrations of 10, 25, 100, 500, 1000, 3000 and 5000 ng/mL and the concentrations of quality control (QC) samples were 10, 50, 500 and 1500 ng/mL in plasma or methanol. The highest concentrations of the calibration standard and the three QCs were prepared by adding a certain volume of stock solution to the blank plasma. The other calibration standards were prepared by serial dilution of the highest to the lowest concentration, obtaining nine different concentrations to produce the calibration curve according to Food and Drug Administration (FDA) recommendations on bioanalytical methods validation (US Food and Drug Administration, 2001). A sample of blank drug-free plasma was included. Both calibration standards as QCs were stored at \(-20^\circ\text{C}\) until analysis to avoid more than one cycle of freezing for no longer than 6 months.

**Chromatographic conditions**

The HPLC system consisted of a 1200 Series separation module (Agilent Technologies) controlled by Agilent Mass Hunter Workstation Data Acquisition for programming samples and chromatographic conditions. Separations were carried out at 60°C on a Zorbax SB-C\(_18\) column (2.1 x 50 mm i.d., 1.8 μm particle size; Agilent Technologies). The mobile phase consisted of a combination of ammonium formate 4 m\(\text{mol}\), pH 3.2 (solution A) and ACN (solution B). The chromatographic run was performed under isocratic conditions at the flow rate of 0.8 mL/min with 55% solution A and 45% solution B. The elution time of each sample was 1.2 min, not requiring a post-time to return the column to initial conditions owing to isocratic conditions. At the end of every day, a
washing method of the column was applied consisting of increasing the percentage of ACN to 100% at 0.8 mL/min flow rate over 5 min, then continuing for 10 min more and back to initial conditions within 5 min. While validation experiments, we have never run more than 34 samples without washing column. This cleaning column protocol was enough because the column is very short and with rapid resolution. Supplementary Figure 1 and 2 can justify that is something is deposited inside the column is very little and do not disturb the quality of our recordings.

**Mass spectrometry**

The mass spectrometric detection system consisted of an Agilent Technologies 6410, triple quadrupole with ESI in positive-ion mode. The mass spectrometry was operated in selected-reaction monitoring (SRM) mode.

The settings of the mass spectrometer were as follows: The desolvation temperature was set at 350 °C and gas flow was set at 10 L/min. The nebulizer pressure was 40 psi and the capillary current was 10 nA. The fragmentor voltage was 150 V for imatinib and its IS. The MS collision gas was nitrogen of high purity (> 99.9995) at 1.5 mbar and the collision energy was set at 25 eV for the two compounds. After HPLC separation, a peak area corresponding to the transition m/z 494.2→394.1 for imatinib (scan time of 200 ms) was measured relative to that of transition m/z 502.2→394.1 of its IS (scan time of 200 ms).

**Sample preparation**

Sample preparation was carried out by SPE using Versaplate-SCX cation exchange cartridges (Agilent Technologies), 25 mg, with a vacuum pressure of about 5 bar. SCX with very low pH, has a unique nature that is useful to isolate both cationic and nonpolar characteristics, which is the case for imatinib. The benzene sulfonic acid sorbent produced ionic interaction with the sulfonic acid group and a nonpolar interaction with the benzene ring. The overall result of this dual character is a cleaner interaction with the sulfonic acid group and a nonpolar interaction with the benzene ring. The overall result of this dual character is a cleaner sample preparation was carried out by SPE using Versaplate-SCX cation exchange cartridges (Agilent Technologies), 25 mg, with a vacuum pressure of about 5 bar. SCX with very low pH, has a unique nature that is useful to isolate both cationic and nonpolar characteristics, which is the case for imatinib. The benzene sulfonic acid sorbent produced ionic interaction with the sulfonic acid group and a nonpolar interaction with the benzene ring. The overall result of this dual character is a cleaner sample preparation was carried out by SPE using Versaplate-SCX cation exchange cartridges (Agilent Technologies), 25 mg, with a vacuum pressure of about 5 bar. SCX with very low pH, has a unique nature that is useful to isolate both cationic and nonpolar characteristics, which is the case for imatinib. The benzene sulfonic acid sorbent produced ionic interaction with the sulfonic acid group and a nonpolar interaction with the benzene ring. The overall result of this dual character is a cleaner
dual character is a cleaner

**Assay validation procedures**

The validation of the method is based on the recommendations published online by the FDA (US Food and Drug Administration, 2001) and Matuszewski (2006).

**Calibration curve and lower limit of quantitation.** Quantitative analysis of imatinib in plasma was performed using an IS, Imatinib-D8. Eight calibration standards in duplicate were used. A linear regression model adjusted by least squares was used to calculate the equation relating the area ratio of imatinib vs IS, and the concentration of imatinib in the calibration standards. The standard curve was chosen to cover the range of clinically relevant concentrations of CML patients. To validate the curve, at least six of the eight calibration standards should be within ±20% of the coefficient of variation (CV).

The lower limit of quantitation (LLOQ) is the lowest calibration standard that meets the following two conditions:

- the response of the analyte is at least 5 times the blank response;
- the response of the analyte is identifiable and reproducible with an uncertainty of <20% and an accuracy between 80 and 120%.

**Accuracy and precision.** The accuracy of the method was developed by analyzing replicates of QC samples 10, 50, 500 and 1500 ng/mL of imatinib. These concentrations were chosen to cover the entire calibration range corresponding to different levels of drug in the majority of patient samples and to define the LLOQ as recommended by the FDA. The intra-day precision was evaluated by analyzing 15 replicates of each QC in three different runs. The inter-day variation was evaluated by injecting each QC sample for five consecutive days in duplicate. The accuracy was measured as the percentage difference between the theoretical and the measured value according to the following equation:

\[
\text{Bias} = \frac{\text{concentration}_{\text{measured}} - \text{concentration}_{\text{theoretical}}}{\text{concentration}_{\text{theoretical}}} \times 100\%
\]

To validate the accuracy, error must be <10% for all except the lowest LLOQ (<20% for LLOQ).

**Selectivity.** The specificity of the method was examined by analysis of six different targets from human plasma, with or without IS, according to the recommendations of the FDA. In addition to check the interference of other ITKs of similar chemical structure 1000 ng/mL of dasatinib and nilotinib were added to QC of 10, 50, 500 and 1500 ng/mL of imatinib and after that the concentration of imatinib was quantified.

**Extraction recovery and matrix effect.** The matrix effect of plasma was investigated through the addition of a known concentration of analyte with its IS to a blank plasma that had undergone SPE. This response was compared with the addition of the same amount of analyte and IS to 800 μL of MeOH–ACN (1:1 v/v) + 5% NH₃ (25%). Recovery was measured as the ratio between the concentration of imatinib in plasma following SPE compared with the same concentration dissolved directly in MeOH–ACN (1:1 v/v) + 5% NH₃ (25%). Any difference from 100% recovery could be attributed to matrix effect. Three concentrations (50, 500 and 1500 ng/mL) were analyzed in six different targets of human plasma. To be acceptable, recovery should be between 80 and 120% for QC samples.

The extraction recovery was determined in human plasma. The results of imatinib plasma samples 10, 50, 500 and 1500 ng/mL (n = 6) after extraction were compared with the response of extracted plasma blanks spiked with the same nominal concentration of imatinib just before injection into the LC/MS-MS. To validate the extraction recovery, it should be within ±20% of the nominal value.

**Results**

**Chromatography**

Figure 2 represents a typical chromatogram of plasma spiked with 500 ng/mL of imatinib and its IS under our working conditions at a flow rate of 0.8 mL/min. The LC/MS-MS system was operated in selected-reaction monitoring mode. The ionic transitions of each compound are listed in Table 1. The mass spectrometric detection system consisted of an Agilent Technologies 6410, triple quadrupole with ESI in positive-ion mode. The mass spectrometry was operated in selected-reaction monitoring (SRM) mode.

**Figure 2.** Representative extraction ion chromatogram of plasma spiked with 500 ng/mL of imatinib and its internal standard under selected-reaction monitoring mode. The ionic transitions of each compound are showing in the figure.
conditions in SRM. Thanks to the method of analysis of extracted ion chromatogram, we could separate imatinib and its IS, although their retention times (0.5 min) and elution times (1.2 min) were the same. This resolution was performed based on reconstructed ion currents. The recording time was too small owing to isocratic elution not requiring post time to reach basal conditions.

**Calibration curve and lower limit of quantitation**

The calibration curve obtained with the eight calibration standards was linear in the range 10–5000 ng/mL ($r^2 > 0.998$). The LLOQ of 10 ng/mL showed an identifiable and reproducible response with an uncertainty of <20%, an accuracy of 1.5% and a signal more than 5 times that of any blank plasma (6.4 ± 0.6 LLOQ signal/blank signal). The rest of the calibration standards showed a response that was very precise and accurate with CV <15%, allowing validation of the curve according to the recommendations of the FDA (US Food and Drug Administration, 2001).

**Accuracy and precision**

The results obtained for precision and accuracy are presented in Tables 1 and 2. The intra-day precision was optimal with CV of 1.3, 0.7, 0.2 and 0.8 for QCs of 10 (LLOQ), 50, 500 and 1500 ng/mL, respectively, showing accuracy of 95–108% in all cases (Table 1). The interday precision data also was optimal with CV of 7.7, 2.4, 2.8 and 2.8 for QCs of 10 (LLOQ), 50, 500 and 1500 ng/mL, respectively, showing accuracy of 98–108.5% in all cases (Table 1).

**Selectivity**

No peak of imatinib, or IS, was found by analyzing the different plasma blanks after SPE, its signal being much lower than that found in the LLOQ (Figure 3a). There are some beneficial effects of combining nilotinib and imatinib in preclinical models of BCR–ABL leukemias (Weisberg et al., 2007), suggesting clinical combinations of ITKs in the future. Therefore, we considered it relevant to determine the interfering effect of other ITKs such as nilotinib and dasatinib. The presence of dasatinib or nilotinib did not show any interference or cross-reacting targets in both plasma and QCs. Figure 3(b) represents a typical chromatogram of imatinib 10 ng/mL compared with imatinib in the presence of nilotinib and dasatinib 1000 ng/mL, not showing any differences.

**Extraction recovery and matrix effect**

The percentage recoveries and matrix effect obtained are presented in Table 3. The recovery was optimal for all QCs, being 84.1, 99.7, 106.1 and 92.1% for QCs of 10, 50, 500 and 1500 ng/mL, respectively (Table 2).

No matrix effect was observed after the tests performed over six different human plasma samples and three QCs. The average of blank human plasma spiked with a known concentration of imatinib after SPE, compared with QCs without SPE procedure, showed an acceptable recovery range of 80–120%. The average recoveries obtained in the test matrix effect were 95.2, 93.4 and 105.7% for QCs of 50, 500 and 1500 ng/mL, respectively (Table 3). In any case, the retention times of both imatinib and imatinib-D8 were modified.

**Discussion**

Imatinib is a new drug for the treatment of chronic myeloid leukemia that is associated with better therapeutic results at plasma

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**Table 1.** Precision and accuracy intra- and inter-day

<table>
<thead>
<tr>
<th>Concentration (ng/mL)</th>
<th>10 (LLOQ)</th>
<th>50</th>
<th>500</th>
<th>1500</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Precision intra-day</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD (n = 15)</td>
<td>9.7 ± 0.1</td>
<td>47.9 ± 0.3</td>
<td>536.6 ± 1.1</td>
<td>1513.6 ± 11.7</td>
</tr>
<tr>
<td>CV (%)</td>
<td>1.3</td>
<td>0.7</td>
<td>0.2</td>
<td>0.8</td>
</tr>
<tr>
<td>Accuracy (%)</td>
<td>−4.8</td>
<td>−4.7</td>
<td>7.3</td>
<td>0.7</td>
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<tr>
<td><strong>Precision inter-day</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD (n = 15)</td>
<td>10.2 ± 0.8</td>
<td>49.0 ± 1.2</td>
<td>542.2 ± 14.0</td>
<td>1429.5 ± 43.0</td>
</tr>
<tr>
<td>CV (%)</td>
<td>7.7</td>
<td>2.4</td>
<td>2.8</td>
<td>2.8</td>
</tr>
<tr>
<td>Accuracy (%)</td>
<td>−1.9</td>
<td>−2.0</td>
<td>8.5</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Precision and accuracy intra-day data were obtained from four quality controls (QCs; 10, 50, 500 and 1500 ng/mL) repeated five times in three different runs. The lower limit of quantification (LLOQ) was 10 ng/mL. The number of the total experiments is in parentheses. Precision and accuracy inter-day data were obtained from four QCs (10, 50, 500 and 1500 ng/mL) repeated on five consecutive days in duplicate. The LLOQ was 10 ng/mL. The number of the total experiments is in parentheses.

**Table 2.** Extraction recovery data

<table>
<thead>
<tr>
<th>Concentration (ng/mL)</th>
<th>Extraction recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD (n = 6)</td>
<td>CV (%)</td>
</tr>
<tr>
<td>10 (LLOQ)</td>
<td>84.1 ± 5.3</td>
</tr>
<tr>
<td>50</td>
<td>99.6 ± 8.4</td>
</tr>
<tr>
<td>500</td>
<td>106.1 ± 4.8</td>
</tr>
<tr>
<td>1500</td>
<td>92.1 ± 4.7</td>
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</tbody>
</table>

The average data are the means ± standard deviation of six different blank human plasma samples spiked with four QCs (10, 50, 500 and 1500 ng/mL) after solid-phase extraction (SPE) and compared with two blank plasma under SPE procedure and spiked with the same known concentrations of four QCs just before injection in HPLC. The LLOQ was 10 ng/mL. The data are given as percentages of recovery.
imatinib and clinical response (Larson et al., 2008; Picard et al., 2007). Based on these data, it is interesting to monitor the plasma levels in order to evaluate the effectiveness of treatment, patient adherence to its oral therapy, drug–drug interactions or clinical studies on pharmacokinetic–pharmacodynamic properties. In fact, studies have shown correlation between plasma trough levels of imatinib and clinical response (Larson et al., 2008; Picard et al., 2007). As a result of these studies there has emerged the need to confirm the relationship between plasma concentration and cytogenetic and molecular response. In this sense, the plasma level monitoring should be performed by LC/MS-MS, which allows the quantification of imatinib in a short time, at low cost and in a robust manner with high sensitivity and specificity (De Francia et al., 2009; Haouala et al., 2009; Titier et al., 2005), which was not achieved with other methods of HPLC with UV detection (Roth et al., 2010). Different methods have been described in the literature for the determination of imatinib or other ITKs in plasma samples by HPLC with UV (Roth et al., 2010; Vivekanand et al., 2003) or mass detection (Parise et al., 2003). More recently, several ITKs were simultaneously measured by LC/MS-MS (Titier et al., 2005, De Francia et al., 2009, Haouala et al., 2009, Bouchet et al., 2011). In all cases the elution was carried out by gradient instead of isocratic conditions. However, other authors (Davies et al., 2010; Tan et al., 2011; Velpandian et al., 2004) performed the method using HPLC with UV under isocratic conditions, obtaining an LLOQ of 50 ng/mL compared with our LLOQ of 10 ng/mL, which was similar to other authors (Haouala et al., 2009; Titier et al., 2005) who used LC/MS-MS. This demonstrates the greater sensitivity and selectivity of LC/MS-MS compared to HPLC coupled with UV detection.

The advantages of these isocratic conditions are the simplicity of the method and that the lifetime of the column is longer owing to the lack of pressure variations. The concentration gradient facilitates the separation of samples with very similar retention times, as in the case of ITKs. However, working with LC/MS-MS and extracting the ion chromatogram produced the same results, even working with an isocratic method, especially if we did not need to analyze many ITKs at the same time. Further studies with the same protocol with other ITKs such as nilotinib and dasatinib should be performed to corroborate the feasibility of this method with more drugs simultaneously.

Our chromatogram recording time is shorter (1.2 min) compared with other methods with times >20 min (Haouala et al., 2009) or even 4 min by means of ultrahigh-performance liquid chromatography (UHPLC) (Bouchet et al., 2011). To achieve these fast times we used a rapid resolution Zorbax SB-C18 column (HT 2.1 × 50 mm, 1.8 μm) with a shorter length and smaller internal diameter designed for high-performance, high-speed separations to improve analytical productivity. It is a stable column up to 90 °C, set at 60 °C to decrease the pressure from 390 (at 40 °C) to 290–300 bar owing to the flow rate of 0.8 mL/min. Thanks to this rapid resolution column and the flow employed, the recording time chromatograms were reduced, being similar to those obtained with UHPLC equipment (Bouchet et al., 2011). In our method, like Titier et al. (2005) and Bouchet et al. (2011), we used an isotopic IS that is much more specific than the broad-spectrum quinoline used by other authors (De Francia et al., 2009). Although determination of imatinib by HPLC coupled to UV detection has already been developed under isocratic conditions, retention times of 9 (Tan et al., 2011), 7.74 (Velpandian et al., 2004) and 10 min (Schleyer et al., 2004) were much longer than the 0.5 min in our method.

The disadvantages of the ESI source used in mass spectrometry are ion suppression and matrix effect. There are several mechanisms that occur during desolvation and ionization of the sample that could be responsible for the loss of analyte signal (King et al., 2000), thereby compromising the accuracy and precision. Greater efficiency in the elimination of matrix components can minimize ion suppression and matrix effect. Therefore, precipitation extraction procedures should be avoided, even though they are simple and inexpensive (Shen et al., 2005). Only one method under isocratic conditions has been performed by
LC/MS-MS, but the authors used precipitation for purification of the sample (Guetens et al., 2003). In this regard, we chose an SPE process for the extraction of the sample, thus reducing the matrix effect. The SPE procedure was improved by adding phosphoric acid at sample preparations, which lowers the pH solution, changing the bases and neutralizing the acids. This provides a better retention of imatinib by SCX sorbent, which requires opposite charges owing to electrostatic interactions. Furthermore, NH$_3$ was added to raise the pH to neutralize bases, facilitating the elution of the retained compound. SPE has the advantage of allowing small elution volumes with almost no limitations on the use of solvents, which allows little solvent consumption and little chance of contamination. Furthermore, this method can be automated and can be adapted for routine application. Recently, Bouchet et al. (2011) have described a method for monitoring of imatinib by SPE, but most methods described so far have been carried out using precipitation (De Francia et al., 2009; Haoaula et al., 2009) or liquid–liquid extraction (Titier et al., 2005). The linear range of the curve of our method, ranging from 10 ng/mL (LLOQ) to 5000 ng/mL, allows correct therapeutic monitoring of patients since plasma thresholds of about 1000 ng/mL have been described to positively correlate with a clinical response (Picard et al., 2007).

**Conclusion**

In summary, the LC/MS-MS described here shows the quantification of imatinib levels quickly and easily, with high sensitivity and specificity. The method has been validated according to FDA recommendations (US Food and Drug Administration, 2001) and Matuszewski (2006), including matrix effect (<10% of bias) and recovery efficiency tests (> 80 and <120%). The method is precise (intraday CV <2%, interday CV <7%), accurate (95–108%), sensitive and specific, not showing interferences on peaks or retention times with other drugs of similar chemical structures such as nilotinib or dasatinib. In addition, this method is actually being applied to follow up the treatment efficacy and safety of imatinib patients suffering from CML, indicating the applicability in clinical practice. It is a simple method that will permit assessment of adherence to treatment, therapeutic efficacy and drug resistance, improving the pharmacological treatment of CLM patients, either by adjusting doses or substituting new ITK drugs.

**SUPPORTING INFORMATION**

Supporting information can be found in the online version of this article.

**Acknowledgments**

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