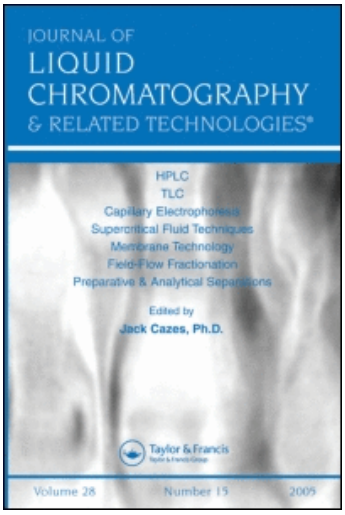


**Journal of Liquid Chromatography & Related Technologies**
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

# Liquid Chromatography-Electrospray Mass Spectrometry Determination of Imatinib and Its Main Metabolite, *N*-Desmethyl-Imatinib in Human Plasma

I. Solassol[a]; F. Bressolle[ab]; L. Philibert[ab]; V. Charasson[ac]; C. Astre[a]; F. Pinguet[a]
[a] Onco-pharmacology Department, Pharmacy Service, Val d'Aurelle Anticancer Centre, parc Euromédecine, Montpellier, France [b] Clinical Pharmacokinetic Laboratory, Faculty of Pharmacy, University Montpellier I, Montpellier, France [c] Department of Medical Pharmacology and Toxicology, Lapeyronie Hospital, Montpellier, France

**To cite this Article** Solassol, I. , Bressolle, F. , Philibert, L. , Charasson, V. , Astre, C. and Pinguet, F.(2006) 'Liquid Chromatography-Electrospray Mass Spectrometry Determination of Imatinib and Its Main Metabolite, *N*-Desmethyl-Imatinib in Human Plasma', Journal of Liquid Chromatography & Related Technologies, 29: 20, 2957 — 2974
**To link to this Article: DOI:** 10.1080/10826070600981058
**URL:** http://dx.doi.org/10.1080/10826070600981058

*Journal of Liquid Chromatography & Related Technologies*®, 29: 2957–2974, 2006

ISSN 1082-6076 print/1520-572X online
DOI: 10.1080/10826070600981058



# Liquid Chromatography-Electrospray Mass Spectrometry Determination of Imatinib and Its Main Metabolite, *N*-Desmethyl-Imatinib in Human Plasma

**I. Solassol**
Onco-pharmacology Department, Pharmacy Service, Val d'Aurelle Anticancer Centre, parc Euromédecine, Montpellier, France

**F. Bressolle and L. Philibert**
Onco-pharmacology Department, Pharmacy Service, Val d'Aurelle Anticancer Centre, parc Euromédecine, Montpellier, France and Clinical Pharmacokinetic Laboratory, Faculty of Pharmacy, University Montpellier I, Montpellier, France

**V. Charasson**
Onco-pharmacology Department, Pharmacy Service, Val d'Aurelle Anticancer Centre, parc Euromédecine, Montpellier, France and Department of Medical Pharmacology and Toxicology, Lapeyronie Hospital, Montpellier, France

**C. Astre and F. Pinguet**
Onco-pharmacology Department, Pharmacy Service, Val d'Aurelle Anticancer Centre, parc Euromédecine, Montpellier, France

**Abstract:** The aim of this paper was to develop a specific and sensitive liquid chromatography/electrospray ionization mass spectrometry method for the determination of imatinib and its metabolite in human plasma. The method involved a solid phase extraction of the compounds and internal standard (imatinib-$D_8$) from human plasma. LC separation was performed on a SymmetryShield™ RP8 column with a mobile phase of water:acetonitrile:formic acid. MS data were acquired in single ion

Address correspondence to Dr. F. Bressolle, Ph D, Laboratoire de Pharmacocinétique Clinique, Faculté de Pharmacie, B.P. 14491, 15 Avenue Ch. Flahault, 34093 Montpellier Cedex 5, France. E-mail: fbressolle@aol.com

monitoring mode at $m/z$ 494.4, $m/z$ 480.4 and $m/z$ 502.4 for imatinib, *N*-desmethyl-imatinib, and imatinib-$D_8$, respectively. The absence of ion suppression was demonstrated. The drug/internal standard peak area ratios were linked via a quadratic relationship to plasma concentrations (8.35–8350 μg/L for imatinib and *N*-desmethyl-imatinib). Precision was 2.8–10.8% and accuracy was 91.3–111.3%. Extraction recoveries were ≥70%. The lower limit of quantitation was 8.35 μg/L for both imatinib and *N*-desmethyl-imatinib.

**Keywords:** Imatinib, *N*-desmethyl-imatinib, Liquid-chromatography/electrospray ionization mass spectrometry method, Quantitation, Human plasma



## INTRODUCTION

Imatinib mesylate (4-[(4-methyl-1-piperazinyl)methym]-N-[4-methyl-3-[(3-pyridinyl)-2-pyrimidinyl]amino]-phenyl]benzamide methanesulfonate) is a 2-phenylaminopyrimidine derivative that functions as a specific inhibitor of a number of tyrosine kinase enzymes (TK). It occupies the *TK* domain, leading to a decrease in activity. There are a large number of *TK* enzymes in the body, including the insulin receptor. Imatinib is specific of the *TK* domain in the *abl* (the Abelson proto-oncogene), c-kit, and PDGF-R (platelet-derived growth factor receptor). Imatinib is currently marketed by Novartis as Gleevec®. This drug is used in treating chronic myelogenous leukemia (CML), gastrointestinal stromal tumors (GISTs), and a number of other malignancies in evaluation.

Metabolism of imatinib occurs in the liver and the main metabolite, *N*-demethylated piperazine derivative (or N-desmethyl-imatinib) has comparable intrinsic activity to that of imatinib regarding the bcr-abl, c-abl, PDGF-R, and c-kit tyrosine kinases.[1,2] Recently, a single oral dose of $^{14}$C-labeled imatinib was administered to healthy volunteers.[3] Drug disposition, as well as drug metabolism were investigated. *N*-demethylated piperazine derivative was found to be the major imatinib metabolite found in the plasma samples. CYP3A4 is the major enzyme responsible for the metabolism of imatinib, whereas other enzymes, such as CYP2D6, play a minor role.[4] The major route of elimination is in the bile, only a small portion is excreted in the urine. Most of the imatinib is eliminated as metabolites; only 25% is eliminated unchanged. The half-lives of imatinib and its main metabolite are 18 and 40 h, respectively.

Resistance to imatinib is observed in a variable proportion of patients, especially in leukaemia and GIST. This resistance has been found in patients with an acute-phase disease, but may also occur in patients with a chronic-phase disease. Two cellular mechanisms for resistance to imatinib have been identified: amplification of the BCR-ABL gene and mutations in the catalytic domain of the protein. In addition, suboptimum inhibition of BCR-ABL in vivo could contribute to the selection of resistant cells.[5]

To examine whether resistance to the imatinib treatment could be explained in some patients by alterations in the pharmacokinetics and/or

pharmacogenetics of imatinib, a clinical study was undertaken to assess the variability of plasma levels of both imatinib and its metabolite in GIST patients. For this purpose, we validated a robust and sensitive analytical method for the measurement of these two drugs on a semi routine basis.

To date, some high performance liquid chromatography (HPLC) methods with ultra-violet[6–8] or mass spectrometry (MS or MS/MS) detection with atmospheric pressure chemical ionisation (APCI)[9,10] or electrospray ionisation[11,12] have been published for the quantitation of imatinib in biological fluids (plasma, urine, and cerebrospinal fluid). Four of them allowed the quantitation of both imatinib and its main metabolite, the *N*-desmethyl-imatinib.[6,9–11] These methods were preceded either by protein precipitation,[7,11] solid-phase extraction,[7] liquid-liquid extraction,[12] or high throughput solid phase extraction.[8,9] In the method published by Schleyer et al.,[6] the sample cleanup procedure involved an online enrichment column (switching technique) after protein precipitation. Recently Marull and Rochat[13] characterized new imatinib metabolites produced by some CYP isozymes by LC-MS/MS and linear ion trap MS. However, some of these published methods reported assay validation, which was incomplete or demonstrated several interferences when applied to clinical samples.



The main objective of this study was to develop and validate a reliable, specific, and sensitive liquid chromatography-electrospray mass spectrometry (LC/ESI-MS) method for the simultaneous quantitation of imatinib and its main metabolite, *N*-desmethyl-imatinib (Figure 1) in human plasma. This method was validated according to validation procedures, parameters, and acceptance criteria based on USP XXIII guidelines and FDA guidance.[14–17] LC-MS and LC-MS-MS based procedures were very sensitive to matrix effects. This is why, in the present paper, we demonstrated the absence of ion suppression by the method of Matuszewski et al.[18] The HPLC assay that is described in the present article was developed based on the need i) to perform therapeutic drug monitoring in routine setting, on patients under imatinib treatment; ii) to develop a specific and sensitive assay to simultaneously quantify imatinib and its main metabolite in human plasma; and iii) to use a more widely available and less expensive instrumentation than that required in previously published methods.[9,10]

## EXPERIMENTAL

### Materials and Reagents

Imatinib mesylate, imatinib-$D_8$, and N-desmethyl-imatinib mesylate (CGP 74588) were kindly provided by Novartis Pharmaceuticals (Rueil Malmaison, France). Methanol and acetonitrile with high purity were purchased from Merck (Darmstadt, Germany). Formic acid was purchased from Sigma (St Louis, MO, USA).

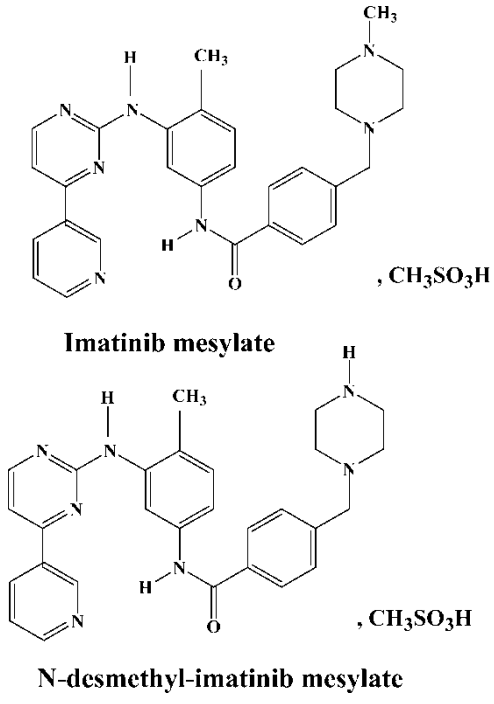


***Figure 1.*** Chemical structures of imatinib and *N*-desmethyl-imatinib.

Purified water was generated by a Milli-Q reagent water system (Millipore Corporation, Bedford, MA). $C_8$ solid phase extraction (SPE) cartridges (15 mg of sorbent, porosity 7 μm, pore diameter 70 Å) were supplied by Varian (Les Ulis, France).

For the validation of the method, blood samples from healthy volunteers (Etablissement Français du sang, Montpellier, France) were collected in heparinized tubes and plasma was obtained by centrifugation at 1000 × *g* for 10 min. Pooled drug free plasma samples were aliquotted, frozen at −20°C, and then used during the study in the preparation of standard and quality control (QC) samples. Each batch of pooled drug free plasma was obtained from ten volunteers.

Drug stock standard solutions were prepared by dissolving accurately weighed quantities of imatinib mesylate or N-desmethyl imatinib mesylate in methanol-purified water (50:50, v/v) to obtain solutions containing 0.835 g/L of free form equivalents of each compound. Working solutions of the two compounds were prepared daily by dilutions of the stock standard solutions with methanol-purified water (50:50, v/v) in ratios 1/10, 1/100, and 1/1000. The internal standard solution was prepared in methanol purified water (50:50, v/v) yielding a concentration of 1 g/L, then diluted 100-fold in the same mixture before use.

Stock solutions were stored at −20°C. For each compound, two separate stock standard solutions were prepared: one which was used for the preparation of the calibration curve standards, and the second, which was used for the preparation of quality control (QC) samples.

A reference standard solution (0.835 μg/L of imatinib, plus 0.835 μg/L of N-desmethyl-imatinib, plus 1 μg/L of internal standard) was prepared daily in methanol purified water (50:50, v/v) to check the resolution of the chromatographic system.

## Instrumentation and Chromatography

The LC-MS system consisted of a Hewlett Packard Agilent 1100 (Les Ulis, France) quadrupole mass spectrometer equipped with an electrospray interface and a data acquisition station (HPChem software, version 08.04). The mass spectrometer was coupled to a Hewlett Packard LC system equipped with a quaternary pumping unit (G1312A pump) and an autosampler (G1329A autosampler) with a loading valve fitted with a 100 μL sample loop (Interchim, Montluçon, France), and set at 4°C. Separation of the analytes was performed at room temperature (20°C) on a SymmetryShield ™ RP8 column (3.5 μm, 4.6 × 50 mm I.D., Waters, Saint Quentin, France). A 10 min mobile phase gradient was used. The mobile phase consisted of water containing 0.1% formic acid (v/v) (solvent A) and acetonitrile containing 0.1% formic acid (v/v) (solvent B). Table 1 shows the variations in proportions of solvents A and B. The mobile phase was deaerated ultrasonically prior to use, then by a stream of helium during use and was used at a flow rate of 0.5 mL/min. The injection volume was 10 μL.

The mass spectrometer was calibrated in the positive ion mode using a mixture of NaI and CsI (peak width of the mass: 0.6–0.7 amu). Voltages were set at +3.0 kV for the capillary and +0.5 kV for the skimmer lens. The source was heated at 100°C. Nitrogen was used as the nebulizing gas (0.25 MPa) and drying gas (720 L/h). The sampling cone voltage was set up at 80 V. The eluent was split prior to the mass spectrometer entrance to reach 10 μL/min of mobile phase infused in the source. During all experiments,

***Table 1.*** Variations in proportions of solvents A and B

| Time (min) | % Solvent A | % Solvent B |
|---|---|---|
| 0 | 95 | 5 |
| 5 | 0 | 100 |
| 6 | 0 | 100 |
| 7 | 95 | 5 |
| 10 | 95 | 5 |

mass spectra were obtained by scanning from m/z 200 to m/z 600. Mass spectrometric data were acquired in the single ion monitoring (SIM) mode (SIM dwell time: 98 ms). From the full-scan spectra (Figure 2), imatinib, N-desmethyl-imatinib, and imatinib-$D_8$ were characterized by the protonated molecules $(M + H)^+$ at $m/z$ 494.4, $m/z$ 480.4, and $m/z$ 502.4, respectively.

## Preparation of Standards and QC Samples

Calibrators were prepared by spiking appropriate volumes of working solutions into 0.5 mL of drug free plasma. The effective concentrations of

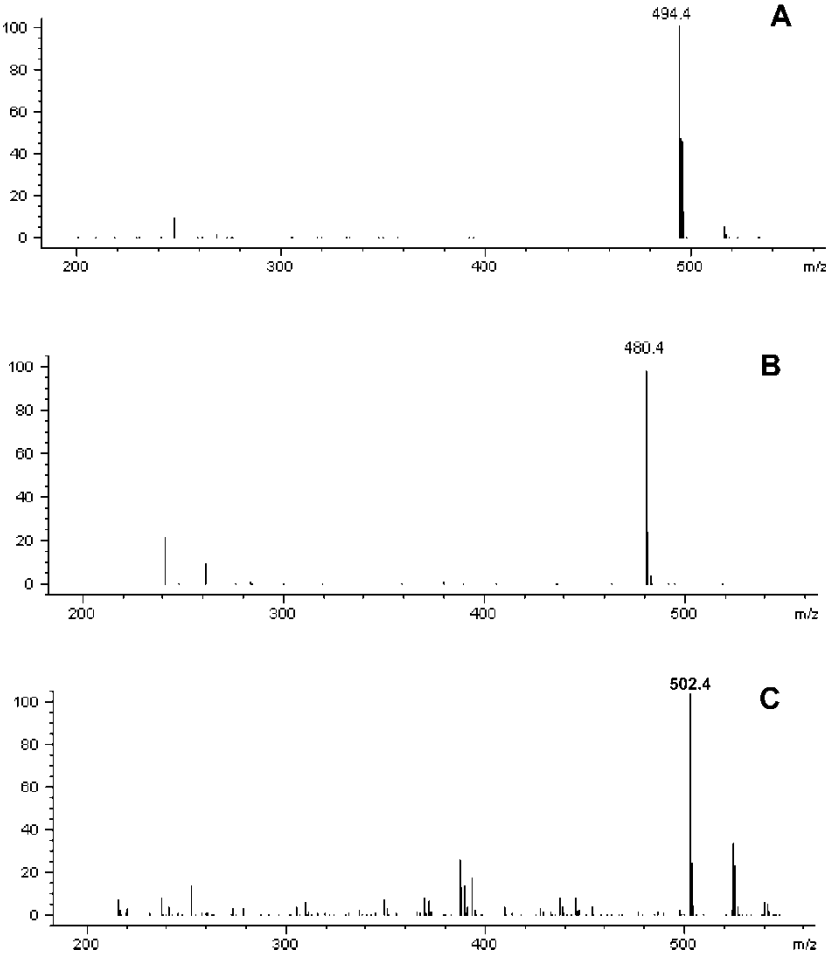


***Figure 2.*** Mass spectra (scan mode) of (A) imatinib, (B) *N*-desmethyl-imatinib and (C) imatinib-$D_8$.

imatinib and N-desmethyl-imatinib in plasma (expressed in free base) were 8.35, 20.9, 41.8, 83.5, 208.8, 417.5, 835, 1670, 4175, and 8350 μg/L.

QC samples used in the validation were prepared in the same way as the calibration standards, by mixing drug free plasma samples with appropriate volumes of working solutions to achieve five different concentrations 8.35, 66.8, 668, 2505, and 6680 μg/L.

## Sample Pretreatment Procedure

Sample pretreatment involved a solid phase extraction (SPE) procedure. The cartridges were conditioned with 250 μL of methanol followed by 250 μL of distilled water before use. In a 5 mL glass tube, a 0.5 mL aliquot of plasma was mixed with 10 μL of internal standard solution (10 mg/L). The mixture was vortex mixed for 15 s, then, loaded onto the conditioned extraction column under a light vacuum (approximately 86 kPa) using a Vac Elut 20® (Varian, Les Ulis, France). The column was then rinsed with $2 \times 200$ μL of 5% (v/v) methanol in purified water and was then dried for 1 min by vacuum aspiration (approximately 27 kPa). The elution was carried out with $2 \times 100$ μL of 1% (v/v) 1 M hydrochloric acid in methanol. The eluate fractions (130 μL) were collected in 200 μL vials and a 10 μL volume was injected onto the LC system for analysis.



## Data Analysis

The ratios of each analyte to internal standard were calculated from the recorded peak areas. The peak area ratios were linked to the concentrations of each analyte in plasma according to a quadratic process such as $Y = aX^2 + bX + c$. The regression curve was not forced through zero. Calibration curve parameters were used to calculate "back-calculated" concentrations for the calibrators. The "back-calculated" values were statistically evaluated. The normal distribution of the residuals (difference between nominal and back-calculated concentrations) was verified. Moreover, the mean residual values (mean predictor error) were computed and compared to zero (Student *t*-test); the 95% confidence interval was also computed.

## Ion Suppression Study

The absence of ion suppression was demonstrated by the method of Matuszewski et al.[18] To investigate potential ion suppression effects attributable to the matrix, six different batches of drug free human plasma were treated in duplicate as described above. The eluate fractions (130 μL of 1% (v/v) 1 M hydrochloric acid in methanol) were then enriched with the two drugs (at 2 different concentrations, 208.8 and 4175 μg/L) and with the internal

standard at 769 μg/L. A reference solution comprising 130 μL of 1% (v/v) 1 M hydrochloric acid in methanol was also enriched with the three drugs to the same nominal concentrations. The enriched eluate fractions (n = 12 per concentration studied) and reference solutions (n = 6 per concentration studied) were injected into the LC-MS system. Peak areas obtained from the enriched eluate fractions were compared with the corresponding mean peak areas of the reference solutions. The mean area ratios (enriched eluate fractions/reference solutions) were as follows: imatinib, 1.05 (R.S.D.: 0.80%); *N*-desmethyl-imatinib, 1.03 (R.S.D.: 0.40%); internal standard, 1.01 (R.S.D.: 2.4%). No ion suppression was observed.

## Validation

### Specificity

The specificity of the method was investigated by analyzing ten different batches of pooled, blank human plasma samples from healthy volunteers, to determine whether endogenous constituents coeluted with the different analytes. The retention times of endogenous compounds in the matrix were compared with that of the compounds of interest.

Plasma samples (n = 10) from patients receiving other drugs were analyzed for interference. The following drugs were checked: ondansetron, metoclopramide, loperamide, racecadotril, paracetamol, nonsteroidal, anti-inflammatory drugs, and corticosteroids.

### Precision and Accuracy

Within-day and between-day precision and accuracy of the assay were assessed by performing replicate analyses of QC samples in plasma against a calibration curve. The procedure was repeated on different days, on the same spiked standards, to determine between-day repeatability. Intra-day repeatability was determined by treating spiked samples in replicate the same day.

The accuracy was evaluated as [mean found concentration/theoretical concentration] × 100. Precision was given by the percent relative standard deviation (R.S.D.).

### Extraction Efficiency

Absolute recoveries of imatinib and *N*-desmethyl-imatinib were measured 3 times at two concentrations of calibration standards (20.9 and 835 μg/L); based on the comparison of the areas under the peaks of the extracted samples with those of unextracted reference standard solutions containing the corresponding concentrations prepared in the mobile phase. The extraction recovery was also computed for the internal standard. In all cases, the means and standard deviations (S.D.) were calculated.

Determination of the Lower Limit of Quantitation (LLOQ)

The LLOQ estimated on QC samples was defined as the lowest drug concentration, which can be determined with an R.S.D. $\leq$20% and an accuracy of 100 $\pm$ 20% on a day-to-day basis.[14–17]

## RESULTS

### Retention Times and Specificity

Representative chromatograms are shown in Figure 3. During the six months of validation, observed retention times were 4.01 min (R.S.D. = 0.44%, n = 20) for imatinib, 3.85 min (R.S.D. = 0.61%, n = 20) for *N*-desmethyl-imatinib, and 3.93 min (R.S.D. = 1.1%, n = 20) for the internal standard. Under the chromatographic conditions used, the number of theoretical plates (calculated from the imatinib peak) was approximately 7786. The column was replaced when the number of theoretical plates fell below 4900 (i.e., after 600 analyses).

As shown in Figure 4, no peaks due to the matrix interfered at the retention time of the analytes. No interference was found with all tested drugs.



### Drug/Detector Response Relationship

Quadratic regression indicated a mean coefficient of determination of 0.995. Mean parameters of the quadratic equation are given in Table 2.

For each point on the calibration curves, the concentrations were back-calculated from the corresponding quadratic equation parameters, and mean $\pm$ S.D. values were computed. Results are presented in Table 3. A linear regression of the back-calculated concentrations versus the nominal ones provided a unit slope and an intercept equal to 0 (Student *t*-test). The distribution of the residuals showed random variations, the number of positive and negative values being approximately equal. Moreover, they were normally distributed and centred around zero. The mean values of residuals were not statistically different from zero (12.8 and 22.3 for imatinib and its metabolite, respectively) and the 95% confidence intervals included the zero value ($-$16.4:42.4; $-$17.8:52.4, respectively).

### Precision, Accuracy, Extraction Efficiency, and LLOQ

The accuracy and precision results are shown in Table 4.

The mean (S.D.) extraction recoveries averaged 72.7 (3.7)% for imatinib and 71.0 (2.4)% for its metabolite (n = 6). These recoveries were not statistically different over the range of concentrations studied. We also determined

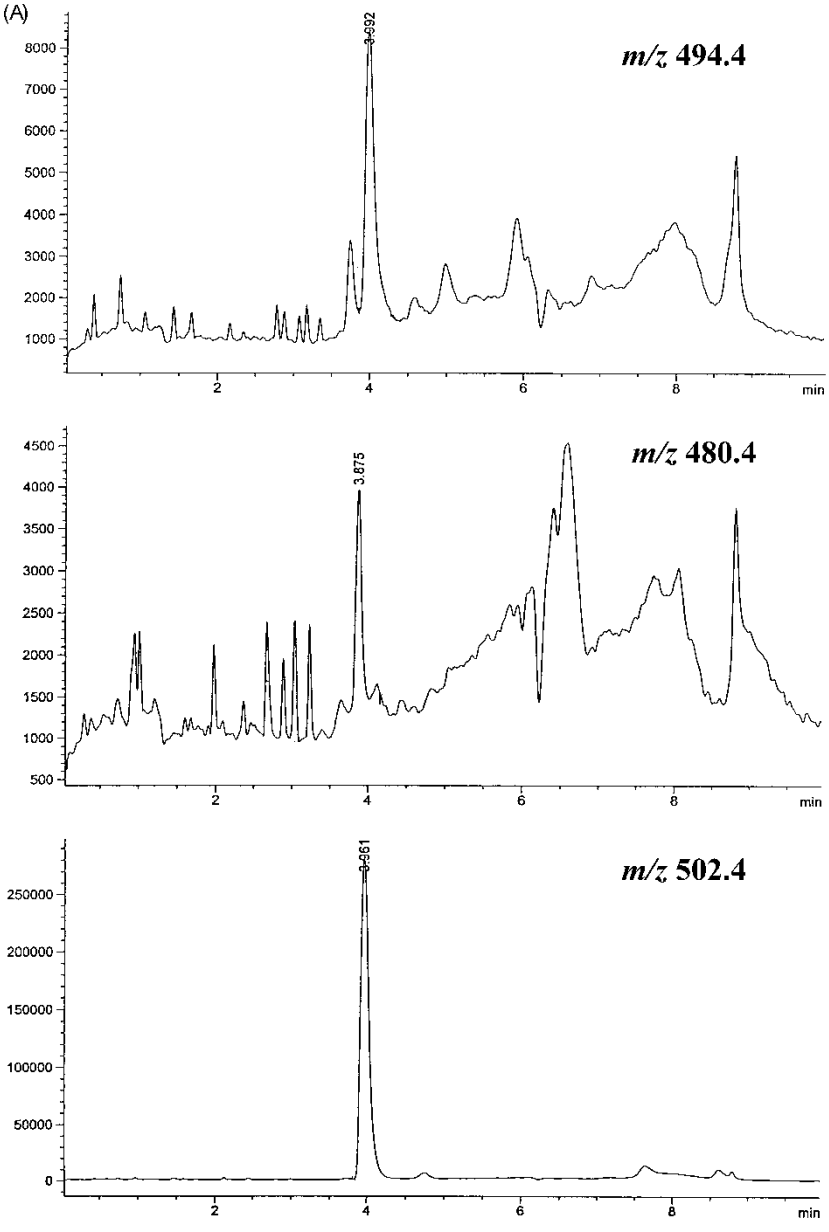


***Figure 3.*** Typical chromatograms (single ion monitoring mode) of drug free human plasma spiked with the two analytes at the following concentrations: (A) 8.35 ng/mL of imatinib and *N*-desmethyl-imatinib, (B) 0.835 μg/mL of imatinib, and *N*-desmethyl-imatinib. For LC-MS conditions see instrumentation section.

(*Continued*)

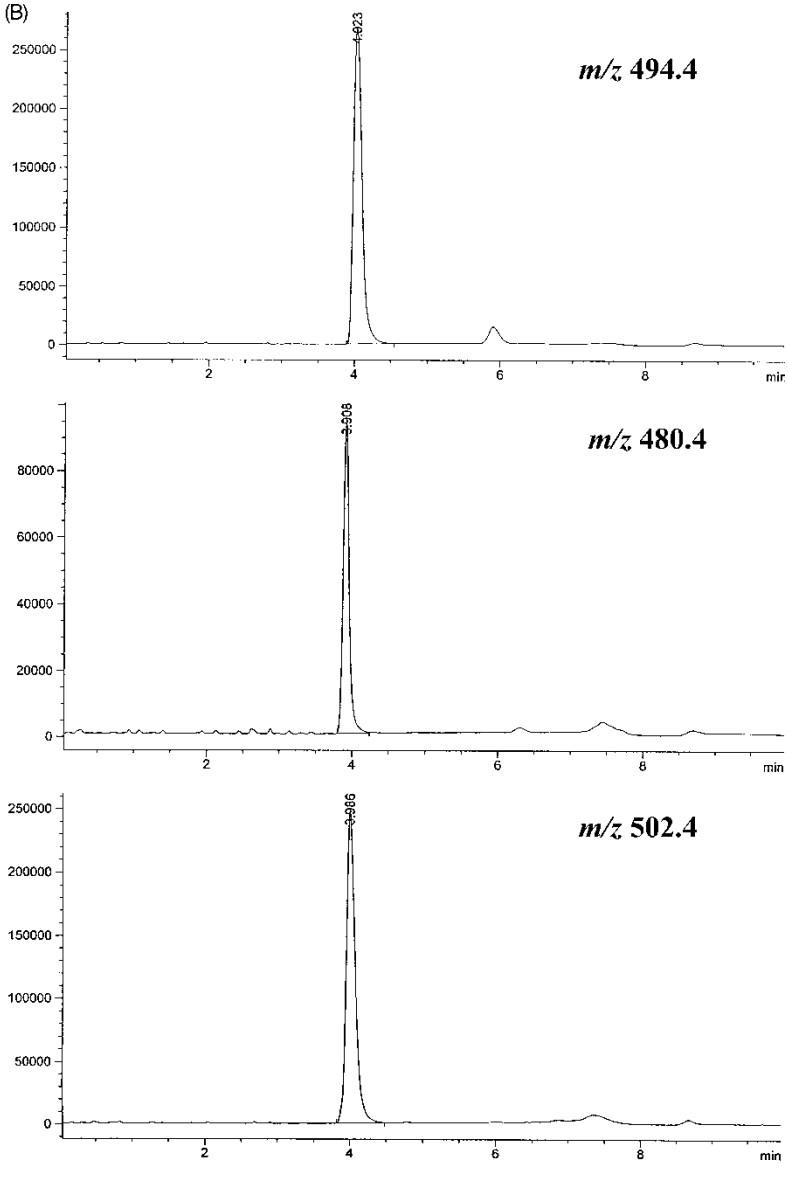


***Figure 3.*** Continued.

the extraction recovery of the internal standard, which was 87.3 (9.4)% (n = 3).

From 500 μL of plasma, the LLOQ was established at 8.35 μg/L for both imatinib and *N*-desmethyl-imatinib. At this concentration, inter-day accuracy

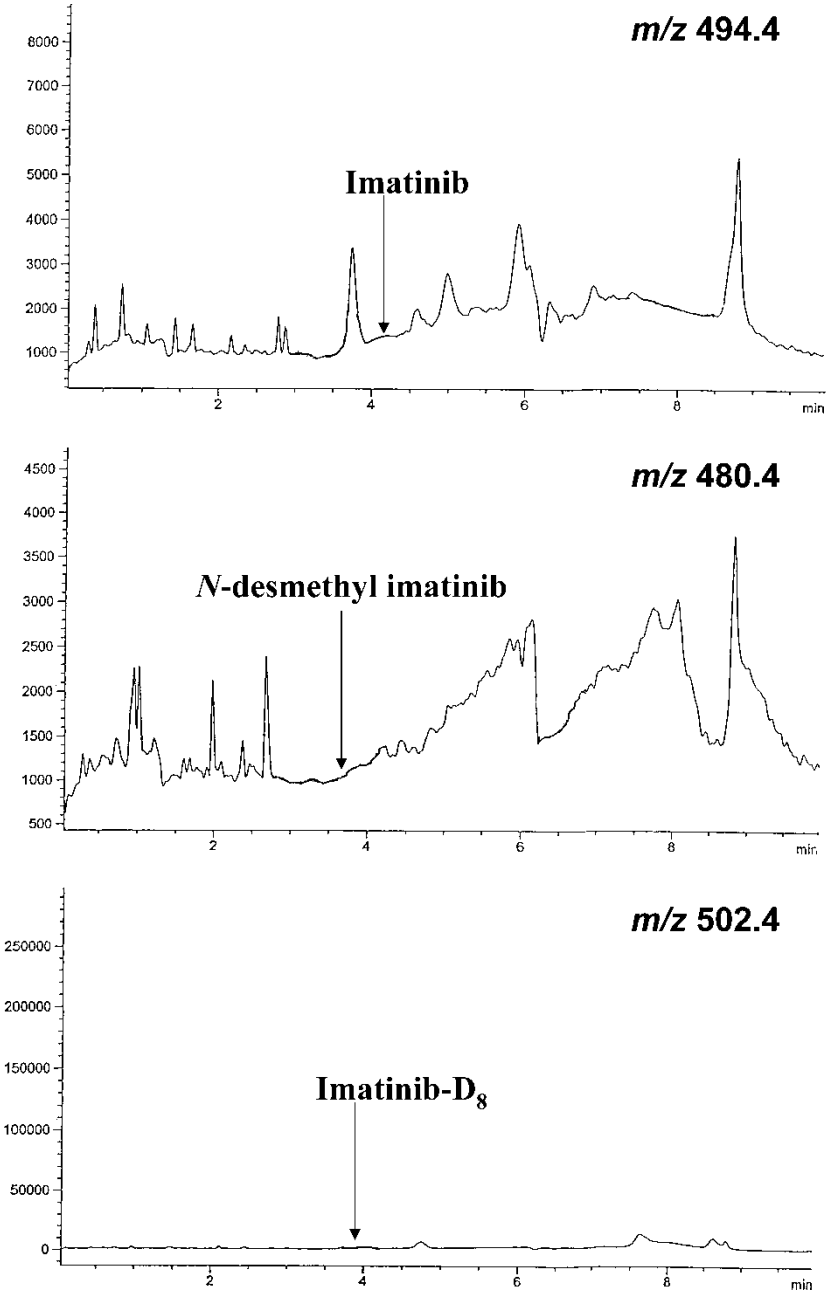


*Figure 4.* Mass chromatogram obtained from 0.5 mL of blank human plasma. For LC-MS conditions see instrumentation section.

***Table 2.*** Results of calibration curves[a]

| | a (Mean ± S.D.) | b (mean ± S.D.) | c (Mean) | $r^2$ (Mean ± S.D.) |
|---|---|---|---|---|
| Imatinib | | | | |
| Intra-assay reproducibility (n = 6) | $1.9 \times 10^{-2} \pm 1.32 \times 10^{-3}$ | 0.94 ± 0.046 (R.S.D. = 4.96%) | $-2.5 \times 10^{-3}$ | 0.995 ± $2.23 \times 10^{-3}$ (R.S.D. = 0.23%) |
| Inter-assay reproducibility (n = 9) | $1.2 \times 10^{-2} \pm 4.53 \times 10^{-3}$ | 0.98 ± 0.058 (R.S.D. = 6.0%) | $-1.2 \times 10^{-2}$ | 0.993 ± $4.53 \times 10^{-3}$ (R.S.D. = 0.45%) |
| *N*-desmethyl-imatinib | | | | |
| Intra-assay reproducibility (n = 6) | $7.2 \times 10^{-3} \pm 3.3 \times 10^{-3}$ | 0.56 ± 0.012 (R.S.D. = 11.6%) | $-3.5 \times 10^{-3}$ | 0.995 ± $3.60 \times 10^{-4}$ (R.S.D. = 0.094%) |
| Inter-assay reproducibility (n = 8) | $6.9 \times 10^{-3} \pm 2.5 \times 10^{-3}$ | 0.47 ± 0.033 (R.S.D. = 7.0%) | $-8.2 \times 10^{-3}$ | 0.997 ± $4.60 \times 10^{-4}$ (R.S.D. = 0.05%) |

[a]Unweighted regression, $y = ax^2 + bx + c$.

$r^2$ = determination coefficient; n = number of replicates.

S.D., standard deviation; R.S.D., relative standard deviation.

***Table 3.*** Back-calculated concentrations from calibration curves

| Theoretical concentration[a] (μg/L) | Imatinib | | | | *N*-desmethyl-imatinib | | | |
|---|---|---|---|---|---|---|---|---|
| | Intra-assay reproducibility | | Inter-assay reproducibility | | Intra-assay reproducibility | | Inter-assay reproducibility | |
| | R.S.D. (%) | Recovery (%) | R.S.D. (%) | Recovery (%) | R.S.D. (%) | Recovery (%) | R.S.D. (%) | Recovery (%) |
| 8.35 | 16.9 | 108 | 8.7 | 102 | 10.2 | 109 | 10.4 | 111 |
| 20.9 | 13.1 | 93.8 | 13.3 | 104 | 7.3 | 101 | 8.7 | 102 |
| 41.8 | 5.4 | 106 | 11.8 | 95.4 | 8.9 | 99.3 | 6.7 | 93.2 |
| 83.5 | 7.2 | 99.2 | 7.6 | 93.7 | 8.7 | 109 | 10.3 | 94.8 |
| 208.8 | 4.4 | 103 | 7.9 | 90.6 | 7.3 | 99.5 | 10.5 | 96.1 |
| 417.5 | 6.6 | 105 | 3.3 | 93.7 | 9.8 | 105 | 3.5 | 106 |
| 835 | 10.6 | 106 | 2.5 | 105 | 7.8 | 95.0 | 5.0 | 102 |
| 1670 | 8.3 | 108 | 9.5 | 104 | 7.8 | 103 | 7.2 | 99.1 |
| 4175 | 5.6 | 102 | 9.4 | 96.5 | 3.4 | 98.6 | 1.7 | 98.3 |
| 8350 | 2.2 | 98.9 | 1.3 | 99.5 | 3.9 | 105 | 2.5 | 100 |

[a]Expressed in free base equivalent.
R.S.D., relative standard deviation.

***Table 4.*** Accuracy and precision of the method

| Theoretical concentration[a] (μg/L) | Imatinib | | | | *N*-desmethyl-imatinib | | | |
|---|---|---|---|---|---|---|---|---|
| | Intra-assay reproducibility (n = 6) | | Inter-assay reproducibility (n = 8) | | Intra-assay reproducibility (n = 6) | | Inter-assay reproducibility (n = 8) | |
| | Precision (%) | Accuracy (%) | Precision (%) | Accuracy (%) | Precision (%) | Accuracy (%) | Precision (%) | Accuracy (%) |
| 8.35 | 10.8 | 113 | 10.2 | 105 | 5.4 | 91.3 | 7.5 | 97.3 |
| 66.8 | 4.9 | 105 | 5.4 | 106 | 4.9 | 104 | 1.9 | 111 |
| 668 | 9.3 | 103 | 6.5 | 92.9 | 5.3 | 97.5 | 6.3 | 98.1 |
| 2505 | 8.4 | 104 | 6.7 | 103 | 3.6 | 99.0 | 5.0 | 103 |
| 6680 | 3.7 | 99.2 | 3.7 | 102 | 2.8 | 103 | 4.7 | 103 |

[a]Expressed in free base equivalent.

n = number of replicates.

and precision values were 105 and 10.2% for the parent drug and 97.3 and 7.5% for its metabolite, respectively.

## DISCUSSION AND CONCLUSION

Two LC-MS-MS assays to quantify imatinib and its main metabolite have been previously published by Bakhtiar et al.,[9,10] the first one in monkey plasma and the second one in human plasma. However, their suitability for clinical pharmacology studies is reduced due to the usually low availability of the LC/APCI-MS-MS apparatus used by the authors. LC assays using a single-[11] and triple-quadrupole[12] operating in the electrospray mode have also been published. The LLOQs were 30 and 10 ng/mL, respectively. However, the first method allowing the simultaneous analysis of imatinib and its metabolite is not fully validated, and we observed interferences when applied to clinical samples. Moreover, the method published by Titier et al.[12] was validated for imatinib alone, and the possible interference with the metabolite was not studied by the authors. So, in the present paper, we described a specific and sensitive LC/ESI-MS method for the simultaneous determination of imatinib and its main metabolite, *N*-desmethyl-imatinib, which can be implemented in laboratories using standard LC-MS instrumentation. This method was developed to perform therapeutic drug monitoring of patients under imatinib treatment at our anticancer centre. Due to the high sensitivity of LC-MS and LC-MS-MS to matrix effects, the absence of ion suppression was demonstrated by the method of Matuszewski et al.[18] The sample pretreatment procedure involved an SPE. Protein precipitation is a commonly used procedure for the treatment of plasma samples prior to SPE. During the optimization step, we explored the utility of protein precipitation before loading the sample onto the conditioned extraction column. We also explored the utility of an evaporation step for the eluate fractions. Extensive cleanup procedure was not needed to ensure column longevity. Moreover, using an evaporation step did not increase the LLOQ. So, the present method requires no precipitation and centrifugation steps of the plasma samples prior to the SPE procedure, and no evaporation and reconstitution of the extracts prior to their injection into the LC system. With this SPE procedure, it was possible to treat about 20 plasma samples per hour. The LLOQ for both imatinib and *N*-desmethyl-imatinib was 8.35 μg/L. Higher LLOQ values were reported from published studies.[6–8,11,12] However, the LLOQ reported in the present paper was two times higher than that reported by Bakhtiar et al.[10] in human plasma using LC/APCI-MS-MS. This method is currently applied at our anticancer centre on a routine basis for the analysis of a large number of plasma samples collected from patients undergoing treatment, in order to better understand its efficacy and safety.

## ACKNOWLEDGMENTS

The authors gratefully acknowledge support of this work by the "Ligue Nationale de Lutte contre le Cancer", Montpellier, France. Special thanks are given to B. Hawkins, for his assistance in the preparation of this manuscript.

## REFERENCES

1. Novartis; Glivec. Summary of Product Characteristics **2001**.
2. Cohen, M.H.; Williams, G.; Johnson, J.R.; Duan, J.; Gobburu, J.; Rahman, A.; Benson, K.; Leighton, J.; Kim, S.K.; Wood, R.; Rothmann, M.; Chen, G.; KM, U.; Staten, A.M.; Pazdur, R. Approval summary for imatinib mesylate capsules in the treatment of chronic myelogenous leukemia. Clin. Cancer Res. **2002**, *8*, 935–942.
3. Gschind, H.P.; Pfaar, U.; Waldmeir, F.; Zollinger, M.; Sayer, C.; Zbinden, P.; Hayes, M.; Pkorny, R.; Seiberling, M.; Ben-Am, M.; Peng, B.; Gross, G. Metabolism and disposition of imatinib mesylate in healthy volunteers. Drug Metab. Dispo. **2005**, *6*, 1503–1512.
4. Peng, B.; Hayes, M.; Resta, D.; Racine-Poon, A.; Druker, B.J.; Talpaz, M.; Sawyers, C.L.; Rosamilia, M.; Ford, J.; Lloyd, P.; Capdeville, R. Pharmacokinetics and pharmacodynamics of imatinib in a phase I trial with chronic myeloid leukemia patients. J. Clin. Oncol. **2004**, *22*, 935–942.
5. Nimmanapalli, R.; Bhalla, K. Mechanisms of resistance to imatinib mesylate in Bcr-Abl-positive leukemias. Curr. Opin. Oncol. **2002**, *14*, 616–620.
6. Schleyer, E.; Pursche, S.; Kohne, C.H.; Schuler, U.; Renner, U.; Gschaidmeier, H.; Freiberg-Richter, J.; Leopold, T.; Jenke, A.; Bonin, M.; Bergemann, T.; le Coutre, P.; Gruner, M.; Bornhauser, M.; Ottmann, O.G.; Ehninger, G. Liquid chromatographihic method detection and quantification of STI-571 and its main metabolite N-desmethyl-STI in plasma, urine, cerebrospinal fluid, culture medium and cell preparations. J. Chromatogr. B **2004**, *799*, 23–36.
7. Widmer, N.; Beguin, A.; Rochat, B.; Buclin, T.; Kovacsovics, T.; Duchosal, M.A.; Leyvraz, S.; Rosselet, A.; Biollaz, J.; Decosterd, L.A. Determination of imatinib (Gleevec) in human plasma by solid-phase extraction-liquid chromatography-ultraviolet absorbance detection. J. Chromatogr. B **2004**, *803*, 285–292.
8. Velpandian, T.; Mathur, R.; Agarwal, N.K.; Arora, B.; Kumar, L.S.; Gupta, S.K. Development and validation of a single liquid chromatographic method with ultraviolet detection for the determination of imatinib in biological samples. J. Chromatogr. B **2004**, *804*, 431–434.
9. Bakhtiar, R.; Khemani, L.; Hayes, M.; Bedman, T.; Tse, F. Quantification of the anti-leukemia drug STI571 (Gleevec) and its metabolite (CGP 74588) in monkey plasma using a semi-automated solid phase extraction procedure and liquid chromatography-tandem mass spectrometry. J. Pharm. Biomed. Anal. **2002**, *28*, 1183–1194.
10. Bakhtiar, R.; Lohne, J.; Ramos, L.; Khemani, L.; Hayes, M.; Tse, F. High-throughput quantification of the anti-leukemia drug STI571 (Gleevec) and its main metabolite (cgp 74588) in human plasma using liquid chromatography-tandem mass spectrometry. J. Chromatogr. B **2002**, *768*, 325–340.
11. Parise, R.A.; Ramanathan, R.K.; Hayes, M.J.; Egorin, M.J. Liquid chromatographic-mass spectrometric assay for quantification of imatinib and its metabolite (CGP 74588) in plasma. J. Chromatogr. B **2003**, *791*, 39–44.

12. Titier, K.; Picard, S.; Ducint, D.; Teilhet, E.; Moore, N.; Berthaud, P.; Mahon, F.; Molimard, M. Quantification of imatinib in human plasma by high-performance liquid chromatography-tandem mass spectrometry. Ther. Drug Monit. **2005**, *27*, 634–640.
13. Murall, M.; Rochat, B. Fragmentation study of imatinib and characterization of new imatinib metabolites by liquid chromatography-triple quadrupole and linear ion trap mass spectrometers. J. Mass Spectrom. **2006**, *41*, 390–404.
14. US Food and Drug Administration, Guidance for industry, Bioanalytical Method Validation, http://www.fda.gov/cder/guidance/4252fnl.htm (accessed May 2004).
15. *United States Pharmacopoeia XXXIII*; The United States Pharmacopeia Convention: Rockville, Md, 2003; 2439.
16. Shah, V.P.; Midha, K.K.; Dighe, S.; McGilveray, I.J.; Skelly, J.P.; Yacobi, A.; Layloff, T.; Viswanathan, C.T.; Cook, C.E.; McDowall, R.D.; Pittman, K.A.; Spector, S. Analytical methods validation: bioavailability, bioequivalence, and pharmacokinetic studies. J. Pharm. Sci. **1992**, *81*, 309–312.
17. Bressolle, F.; Bromet-Petit, M.; Audran, M. Validation of liquid chromatographic and gas chromatographic methods. Applications in pharmacokinetics. J. Chromatogr. B **1996**, *686*, 3–10.
18. Matuszewski, B.K.; Constanzer, M.L.; Chavez-End, C.M. Matrrix effect in quantitative LC/MS/MS analyses of biological fluids: a method for determination of finasteride in human plasma at picogram per mililiter concentrations. Anal. Chem. **1998**, *70*, 882–889.

Received July 18, 2006
Accepted August 28, 2006
Manuscript 6917