

Short communication

Imatinib Quantification in Human Serum for Clinical Purposes using High-performance Liquid Chromatography with a Diode Array Detector

Marek Dziadosz* and Heidemarie Bartels

Institute of Forensic Medicine, Otto-von-Guericke-University, Leipziger Str. 44, 39120 Magdeburg, Germany

* Corresponding author: E-mail: marek.dziadosz@med.ovgu.de;

Tel: +49 391 67 17887

Received: 04-05-2010

Abstract

The popularity of imatinib in modern medicine has underscored the need for a fast and effective quantification method without the use of expensive instruments. The aim of this study was to develop and validate a relatively fast, cheap and effective HPLC method for the quantification of imatinib in human serum. This method uses a simple preparation step with an Extrelut® NT 3 extraction tube and commercial available solvents. A Marcherey-Nagel Lichrospher 100-5 RP8 250 × 4 mm column held at 30 °C, a mobile phase of 0.05 M H₃PO₄/KH₂PO₄-acetonitrile (7:3, v/v) at a flow rate of 1 mL/min and a diode array detector operated at a wavelength of 265 nm were used for the analysis of 50 µL prepared sample injected into the HPLC. A single run was completed in 15 min. The method presented here has a limit of quantification of 30 ng/mL and is linear between 0.1 and 10 µg/mL.

Keywords: Imatinib, Gleevec, Glivec, STI-571, tyrosine kinase

1. Introduction

Imatinib mesylate (STI-571, Gleevec®, Glivec®, imatinib, Fig. 1) is widely used for the treatment of different types of cancer, such as chronic myelogenous leukemia and gastrointestinal stromal tumors.¹⁻² It is a tyrosine kinase inhibitor which binds to the active side of the enzyme and prevents the binding of ATP.

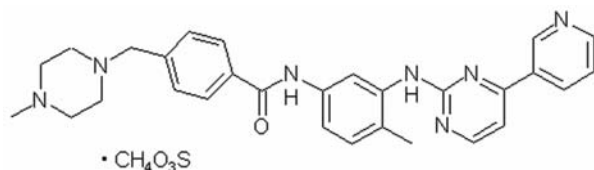


Figure 1. Imatinib mesylate

In clinical toxicology, a fast and sensitive quantification method for monitoring the imatinib blood concentration can be very useful. Bakhtiar et al. described an LC-MS/MS method with two different preparation steps:

SPE³ or protein precipitation.⁴ For the assay of imatinib in human plasma, Widmer et al.⁵ developed a sensitive HPLC/DAD method with an off-line solid phase extraction. An isocratic online-enrichment HPLC/DAD assay was developed by Schleyer et al.⁶ This method allows for a simple and fast separation, as well as for quantification of imatinib and its main metabolite N-desmethyl-STI in plasma, urine, cerebrospinal fluid, culture media and cell preparations in various concentrations. Parise et al. used an LC/MS system for quantifying imatinib and its main metabolite in plasma with a deproteination preparation step.⁷ Guetens et al. proposed a quantification of STI-571 in erythrocytes, plasma⁸ and patient tumor tissue⁹ by measuring sediment technology and LC-MS/MS. They also presented an HPLC/DAD method for a simultaneous determination of AMN107 and imatinib in cultured tumor cells with a liquid-liquid extraction with TOXI-TUBES® A.¹⁰ Oostendorp et al. developed an HPLC method for the determination of imatinib and its metabolite in plasma and murine matrices with a sample pretreatment procedure which involved liquid-liquid extraction with tert-butyl-methyl-ether.¹¹ Velpandian et al. presented a rapid and sensitive HPLC method with UV detection for the estima-

tion of imatinib of patients with chronic myeloid leukemia. They prepared the samples in a simple and single step by precipitating the plasma with methanol and injecting 50 μL aliquot from supernatant.¹²

The aim of this study was to propose an alternative analytical method for imatinib quantification in human serum for clinical purposes using only an Extrelut[®] NT 3 extraction tube, commercial available solvents and HPLC with a diode array detector. These tubes were used successfully in other applications,¹³ and we hoped to achieve good results with them.

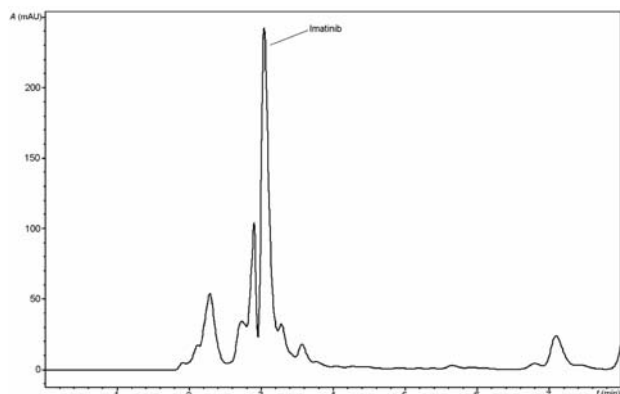


Figure 2. Imatinib in our patients serum

2. Experimental

2.1. Chemicals

Imatinib mesylate was purchased from LC Laboratories (Woburn, MA 01801 USA). All other chemicals and solvents used in this study were of analytical grade, and were purchased from Merck KGaA (Darmstadt, Germany). Blank serum was purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany).

2.2. Equipment

An Agilent HPLC 1200 Series system with a diode array detector was used for all purposes. Data acquisition and integration were performed by ChemStation for LC 3D Systems.

2.3. Chromatography Conditions

Imatinib was separated using a Lichrospher 100-5 RP8, 250 \times 4 mm column (Marcherey-Nagel GmbH & Co. KG, Düren, Germany) maintained at 30 ± 1 °C. The samples were eluted in a mobile phase of 0.05 M H_3PO_4 / KH_2PO_4 -acetonitrile (7:3, v/v) at a flow rate of 1 mL/min. The diode array detector was operating at a wavelength of 265 nm. 50 μL of a prepared sample was injected into the HPLC, and a single run was completed in 15 min.

2.4. Preparation of Calibration Standards

A stock solution of imatinib mesylate was prepared by dissolving 50 mg in 100 mL water. Its dilutions (each in blank serum) were prepared daily when experiments were being conducted (range 0.1–10 $\mu\text{g}/\text{mL}$).

2.5. Imatinib Extraction from Serum

1.5 mL of a prepared standard was mixed with 1.5 mL water and added to an Extrelut[®] NT 3 tube (Merck KGaA, Darmstadt, Germany) for the extraction. After 10 min, the tube was washed with 15 mL of diethyl ether/ethyl acetate (50:50, v/v) and then with 15 mL of chloroform/isopropanol/ammonia (84:15:1, v/v/v). The solvents were collected in a glass reaction tube and evaporated until dry. The residue was redissolved in 100 μL of the mobile phase.

2.6. Validation Parameters

2.6.1. Linearity

The linearity was examined by preparing dilutions of the stock solution in the range of 0.1–10 $\mu\text{g}/\text{mL}$ on each experimental day and by plotting the analytical signal against the concentration. This procedure made the calculation of the reproducibility possible.

2.6.2. Recovery

The percentage recovery was calculated by comparing the analytical signal of a sample prepared at a low, middle and high concentration (0.1; 1.0; 10 $\mu\text{g}/\text{mL}$) in the matrix (blank serum) and in the mobile phase. Imatinib in the matrix was extracted as described in 2.5., and in the mobile phase, it was directly injected (50 μL) into the HPLC. The analyses of all the samples were repeated five times, and the final recovery was calculated as an average value.

2.6.3. Determination of LOD and LOQ

The limit of detection (LOD) was calculated by the equation $\text{LOD} = 3 \cdot \text{S.D.}/S$ and the limit of quantification by the equation $\text{LOQ} = 10 \cdot \text{S.D.}/S$, where S.D. is the standard deviation of the blank and S the slope of the calibration curve.

2.6.4. Accuracy of Estimation

The accuracy of estimation was examined by the preparation of two quality control (QC) samples at a low (0.1 $\mu\text{g}/\text{mL}$) and a high concentration (10 $\mu\text{g}/\text{mL}$). Every QC sample was analyzed twice a day over a period of five days. The inter- and intra-day precision was also calculated.

2. 6. 5. Selectivity

The blank serum was analyzed ten times, and no interferences were observed at the retention time of imatinib. Other drugs for the determination of selectivity were not used, because the list of drugs that can affect imatinib is extensive, and a parallel medication is usually not recommended.

3. Results and Discussion

Under applied conditions, imatinib eluted as a well-resolved peak at a retention time of 3.0 min, and was recognized by the UV library. The LOD was calculated as 8 ng/mL and LOQ as 30 ng/mL. A perfect linearity was obtained at concentrations ranging from 0.1–10 µg/mL. The linearity of this calibration range was examined by measuring every standard at least five times. The relative standard deviation (RSD) of every calibration level was < 3%, and the Grubbs test applied for this calibration range did not detect any outlier. The correlation coefficient was found to be 0.999.

The recovery was 90%, 83% and 86% at 0.1; 1.0 and 10 µg/mL, respectively, against the mobile phase. For the low concentrated QC sample (0.1 µg/mL), the accuracy of estimation was 102%, the intra-day precision RSD was 7.59%, and the inter-day precision RSD was 7.75%. For the highly concentrated QC sample (10 µg/mL), the accuracy of estimation was 99.74%, the intra-day precision RSD was 0.80% and the inter-day precision RSD was 2.20%.

The described method was used to control the imatinib serum concentrations of our patients. In most cases, no interferences were observed, and the achieved results were satisfactory. Unfortunately, in very few HPLC runs, we had problems concerning the separation of the components at 265 nm (Fig. 2). Fortunately, the UV spectrum of imatinib allows for an effective detection at a wide range of wavelengths. A useful advantage of a diode array detector lies in the simple change of the wavelength and fast data reprocessing during the quantification. This proceeding can help to solve the separation problem.

In this study, we wanted to present a relatively fast, cheap and effective HPLC method for the estimation of imatinib, an anti leukemic agent which has reached great popularity in modern medicine. The validation of this method was based on the recommendations of the GTFCh (Society of Toxicological and Forensic Chemistry), and our aim was to present a comparison with other published methods.

The use of the Extrelut® NT 3 extraction tubes allows for an analysis that is faster compared to SPE. There is no need for an extra equipment, column conditioning and washing. This shortens sample preparation and makes it easier.⁵ In this way, modifications of the HPLC system⁶

are not necessary, and the analysis can be done in the standard configuration with a relatively fast elution program. The presented method reaches the required sensitivity for clinical applications and ensures a comparable LOD and LOQ with other more complicated and more time-consuming HPLC methods with UV detection.^{5–6, 10–11}

It has been shown that this method gives a relatively high recovery and leads to very good results without an internal standard. This fact, together with the circumvention of the use of a multi step extraction procedure, such as solid phase extraction, allows for an easier, cheaper and faster analysis. The use of a diode array detector makes a flexible wavelength change possible. This function can be very useful in HPLC runs with separation problems, which can sometimes appear in biological matrices.

In our laboratory, our work with a sample volume of 1.5 mL has turned out to be successful. Nevertheless, for some laboratories, such a volume could be too huge. In such cases, it is recommended to use 750 µL of human serum and an appropriate dilution factor in the method.

4. Conclusions

The method presented here reaches the required sensitivity for clinical applications. High recovery and the circumvention of a multi step extraction procedure allow for an easier and faster analysis. The use of an HPLC/ DAD system in the standard configuration with a relatively fast elution program makes the analysis cheaper compared to other expensive laboratory equipment, such as liquid chromatography coupled with mass spectrometry.^{3–4, 7–9} In comparison with other HPLC methods with ultraviolet detection, the sample preparation in our method is faster, simpler and ensures a comparable LOD and LOQ. Thus, the described imatinib quantification method is a very good alternative for clinical purposes.

5. References

1. A. T. van Oosterom, I. Judson, J. Verweij, S. Stroobants, D. Di Paola, S. Dimitrijevic, M. Martens, A. Webb, R. Scot, M. Van Glabbeke, S. Silberman, O.S. Nielsen, *Lancet* **2001**, 358, 1421–1423.
2. G. D. Demetri, M. Von Mehren, C. D. Blanke, A. D. Van den Abbeele, B. Eisenberg, P. J. Roberts, M. C. Heinrich, D. A. Tuveson, S. Singer, M. Janicek, J. A. Fletcher, S. G. Silverman, S. L. Silberman, R. Capdeville, B. Kiese, B. Peng, S. Dimitrijevic, B. J. Druker, C. Corless, C. D. Fletcher, H. Joensuu, *N. Engl. J. Med.* **2002**, 347, 472–480.
3. R. Bakhtiar, L. Khemani, M. Hayes, T. Bedman, F. Tse, *J. Pharm. Biomed. Anal.* **2002**, 28, 1183–1194.
4. R. Bakhtiar, J. Lohne, L. Ramos, L. Khemani, M. Hayes, F. Tse, *J. Chromatogr. B* **2002**, 768, 325–340.

5. N. Widmer, A. Béguin, B. Rochat, T. Buclin, T. Kovacsovic, M. A. Duchosal, S. Leyvraz, A. Rosselet, J. Biollaz, L. A. Decosterd, *J. Chromatogr. B* **2004**, *803*, 285–292.
6. E. Schleyer, S. Pursche, C. H. Köhne, U. Schuler, U. Renner, H. Gschaidmeier, J. Freiberg-Richter, T. Leopold, A. Jenke, M. Bonin, T. Bergemann, P. le Coutre, M. Gruner, M. Bornhäuser, O.G. Ottmann, G. Ehninger, *J. Chromatogr. B* **2004**, *799*, 23–36.
7. R. A. Parise, R. K. Ramanathan, M. J. Hayes, M. J. Egorin, *J. Chromatogr. B* **2003**, *791*, 39–44.
8. G. Guetens, G. De Boeck, M. Highley, H. Dumez, A. T. Van Oosterom, E. A. de Bruijn, *J. Chromatogr. A* **2003**, *1020*, 27–34.
9. G. Guetens, H. Prenen, G. De Boeck, M. Highley, I. de Wever, A. T. van Oosterom, E. A. de Bruijn, *J. Sep. Sci.* **2006**, *29*, 453–459.
10. G. Guetens, H. Prenen, G. De Boeck, A. van Oosterom, P. Schöffski, M. Highley, E. A. de Bruijn, *J. Chromatogr. B* **2007**, *846*, 341–345.
11. R. L. Oostendorp, J. H. Beijnen, J. H. M. Schellens, O. van Tellingen, *Biomed. Chromatogr.* **2007**, *21*, 747–754.
12. T. Velpandian, R. Mathur, N. K. Agarwal, B. Arora, L. Kumar, S.K. Gupta, *J. Chromatogr. B* **2004**, *804*, 431–434.
13. G. Lutze, W. Römhild, J. Elwert, J. Leppelt, K. Kutschmann, *Deutsche tierärztliche Wochenschrift* **2003**, *110*, 31–33.

Povzetek

Zaradi priljubljenosti imatiniba v sodobni medicini potrebujemo hitro in učinkovito kvantifikacijsko metodo brez drage inštrumentacije. Namen dela je bil, da razvijemo in validiramo relativno hitro, poceni in učinkovito HPLC metodo za kvantifikacijo imatiniba v človeškem serumu. Preprosto pripravo vzorca izvajamo z ekstrakcijskimi kolonami Extrelut[®] NT 3 in komercialno dosegljivimi topili. Za analizo smo uporabljali kolono Marcherey-Nagel Lichrospher 100-5 RP8 250 × 4 mm pri 30 °C, mobilna faza je bila 0,05 M H₃PO₄/KH₂PO₄-acetonitril (7:3, v/v) s pretokom 1 mL/min. Detektor na niz diod je bil nastavljen na 265 nm, injicirali smo 50 µL pripravljenega vzorca. Analiza je trajala 15 min. Predstavljena metoda ima mejo določljivosti 30 ng/mL, linearno območje je med 0,1 in 10 µg/mL.