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Development and clinical application of a LC-MS/MS method for simultaneous determination of various tyrosine kinase inhibitors in human plasma

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ABSTRACT

Background: Increasing numbers of tyrosine kinase inhibitors (TKIs) were studied and approved for therapy of malignancies and other diseases. The aim of this study was to develop and validate a specific, simple and rapid quantification method for various TKIs in human plasma.
Methods: A simultaneous test for six TKIs (erlotinib, imatinib, lapatinib, nilotinib, sorafenib, sunitinib) was developed using liquid chromatography tandem mass spectrometry in a multiple reaction monitoring mode. After protein precipitation the specimens were applied to the HPLC system and separated using a gradient of acetonitrile containing 1% formic acid with 10 mM ammoniumformiate on an analytic RP-C18 column.
Results: The calibration range was 10–1000 ng/mL for sunitinib and 50–5000 ng/mL for the other TKIs with coefficients of determination ≥0.99 for all analytes. The intra- and inter day coefficients of variation were ≤15% and the chromatographic run time was 12 min. Plasma specimens were stable for measurement for at least 1 week at 4 °C. Clinical applications of the assay are exemplarily discussed.
Conclusions: This novel high-throughput method is suitable for specific simultaneous determination of different TKIs in routine clinical practice.

1. Introduction

Tyrosine kinases are key regulators controlling many cellular pathways including growth and differentiation. Therefore, tyrosine kinase inhibitors (TKIs) are small drug molecules developed for the inhibition of biologic pathways for uncontrolled cell growth and cell proliferation caused by over-expression or increased activity of tyrosine kinases [1]. Today increasing numbers of TKIs are used for targeted therapy of various cancer diseases. The first approved TKI is imatinib which has revolutionized the therapy and prognosis of chronic myeloid leukemia (CML) and gastrointestinal stromal tumors (GIST) [2]. Drug targets are the Bcr-Abl kinase in CML and c-Kit in GIST [3]. Although there are excellent responses in the treatment with imatinib, resistance can occur due to a change of conformation of the Bcr-Abl kinase caused by mutations. Other Bcr-Abl inhibitors, which are registered in the second line therapy of CML are nilotinib and dasatinib showing activity against mutated Bcr-Abl kinase in cases of imatinib resistance.

In the last years several other TKIs were developed which have improved the treatment of various tumors. These are TKIs like sunitinib approved for the therapy of kidney cancer and GIST, sorafenib for the therapy of kidney cancer and hepatocellular carcinoma, erlotinib for the treatment of non small cell lung cancer and lapatinib for the treatment of breast cancer [4]. Moreover, other TKIs such as cediranib [5], motesanib [6], neratinib [7], telatinib[8] or vatalanib [9] are currently tested in clinical studies.

During anticancer therapy, TKIs were administered orally and must be taken for a prolonged time period, if not indefinitely. They are metabolized in the liver mostly by the enzyme cytochrome P450 3A4 pathway, whose activity shows a large inter-individual variability and is influenced by environmental factors, such as food [10] or other various drugs [11,12]. Some of them are activators or inhibitors of the cytochrome P450 3A4 system and thus can manipulate the clearance of TKIs when administered together. Other drugs are substrates of cellular drug transporters which mediate uptake or efflux of xenobiotics from the cell. Finally, as TKIs are extensively bound to plasma proteins (>95%) only a small fraction is free to enter the cells to exert its pharmacological action. The amount of circulating plasma proteins in turn depends on their hepatic synthesis as well as their retention during renal passage. Therefore, the concentrations of TKIs in the blood circulation are influenced by various physiological and pathophysiological effects in individual patients. A given dose of TKIs can result in different plasma concentrations which may lead to sub-therapeutic drug exposure or otherwise increased adverse drug reactions at excessive plasma concentrations [13, 14].

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Therapeutic Drug Monitoring (TDM) based on blood concentration measurements is used to optimize the therapeutic use of drugs. TDM can improve the treatment benefit, reduce adverse effects and has been shown to be cost-effective. Especially therapies with drugs showing larger inter-individual pharmacokinetic variability, such as TKIs, benefit from TDM. To date TDM has led to recommendations on target levels of imatinib for optimal response in CML therapy and preclinical data also suggest target plasma concentrations of sunitinib [15].

In the last years several conventional HPLC-methods with optical detection were developed for the quantification of TKIs [16]. A disadvantage of classic HPLC-methods is their restricted ability to distinguish between multiple components which cannot be separated chromatographically. Otherwise TKIs are ideal substances for LC-MS/MS measurements with positive ionization due to their chemical structures combined with their proton affinity. Therefore several LC-MS/MS tests were developed recently. Initially most of them were focused on the analysis of single a TKI while a few tests describe multiple-parameter analysis of these drugs [17, 18, 19]. The use of different assays for various TKI drugs is impracticable in the routine clinical laboratory. Moreover, as recent studies suggest that combination therapies of different TKIs may improve the clinical outcome in various cancer diseases [20, 21] a multicomponent assay would be favorable to monitor plasma concentrations during therapy. Quite recently also high sensitive simultaneous determination of nine TKIs by ultra performance LC/MS-MS was reported [22] while many clinical laboratories use a common liquid chromatography mass spectrometry device. Therefore we developed and validated a simple and robust method using standard LC-MS/MS for simultaneous analysis of six TKIs, erlotinib, imatinib, lapatinib, nilotinib, sorafenib and sunitinib which are presently used in our hospital.

2. Materials and methods

2.1. Patient samples

Plasma samples of the following patients were included in the study. Imatinib treatment: 11 patients (7 women, 4 men, mean age 55.5 years, range 22–72) with CML and 1 patient with GIST. Sunitinib treatment: 17 patients (2 women, 15 men, mean age 68.4 years, range 46–84) with kidney cancer. Sorafenib treatment: 5 patients (2 women, 3 men, mean age 68.6 years, range 50–85) with kidney cancer and a 68 year old male patient with AML. Additional measurements were performed during erlotinib and nilotinib treatment (data nor shown).

2.2. Chemicals and reagents

Water, methanol, and acetonitrile HPLC gradient grade were purchased from Fisher Scientific (Schwerte, Germany), formic acid 98–100% was purchased from Merck (Darmstadt, Germany), ammonium acetate p.a. was purchased from Sigma-Aldrich (St. Louis, MO, United States), erlotinib hydrochloride, imatinib methanesulfonate, lapatinib di-p-toluenesulfonate, nilotinib free base, sorafenib p-toluenesulfonate, and sunitinib malate were purchased from LC Laboratories (Woburn, MA, USA), imatinib-d8 was purchased from ALSA-Chim (Illkirch, France), and sorafenib-d3 was purchased from Synfine-research (Ontario, Canada).

2.3. Standard solutions and quality controls

Master stock solutions were prepared in methanol containing 1 mg/mL (erlotinib, imatinib, lapatinib, nilotinib, sorafenib) and 0.1 mg/mL (sunitinib).

Each master stock solution was pooled and diluted with methanol in order to obtain a stock solution of 20 μg/mL for sunitinib and 100 μg/mL for the other five TKIs respectively. The resulting multicomponent stock solution was further diluted to a 1000 (5000) ng/mL sunitinib (other TKIs) calibration sample with pooled human blank plasma and thoroughly vortexed. A part of the 1000 (5000) ng/mL sunitinib (other TKIs) calibration sample was further diluted with blank plasma to 200 (1000), 100 (500), 40 (200), 20 (100) and 10 (50) ng/mL for sunitinib (other TKIs). The resulting calibration solutions were vortexed and stored in 0.1 mL aliquots in polypropylene tubes at −30 °C.

2.4. Sample preparation

Methanol (400 μL) containing 200 ng/mL of sorafenib-d3 and imatinib-d8 as internal standard were added to the plasma (0.1 mL) and vortexed thoroughly. After centrifugation at 14,000 *g* (4 °C, 10 min), the supernatant (200 μL) was diluted with eluent A (600 μL) to obtain the solution for the HPLC injection (total dilution factor 20).

2.5. Chromatography and LC-MS/MS method

Chromatographic analysis was performed using a Waters Model 2795 separation system (Milford, MA, USA). After protein precipitation with methanol containing the deuterated internal standards and dilution with the eluent the samples were applied to a Kinetex C18 column (50×4.6 mm, 2.6 μ, Phenomenex) with acetonitrile-ammonium formiate buffer 10 mM pH 4.0 (buffer A). Separation of the analytes was achieved at a column temperature at 20 °C using a gradient elution profile with additional buffer B (acetonitrile with 1% formic acid) such as 5–50% B (0–6 min) 50–100% B (6–7 min) and 100% B (7–9 min) at a flow rate of 0.3 mL/min.

The TKIs are detected by electrospray ionization mass spectrometry with multiple reaction monitoring (MRM)-mode using a Micromass Quattro LC triple-quadrupole mass spectrometric detector (Beverly, MA, USA). The instrument was equipped with an electrospray interface, and controlled by the Masslynx Version 4.1 software (Micromass). The samples were analyzed using an electrospray probe operating in the positive ionization mode. The samples were introduced into the MS-interface through a heated nebulized probe. The MS conditions were as follows: the source temperature was set to 140 °C and the desolvation temperature was set to 350 °C with a desolvation gas flow at 600 L/h and a cone gas of 50 L/h. The capillary voltage was 3 kV. Argon was used as collision gas at 0.003 mBar. Each analyte was measured using a confirmation transition as qualifier ion to differentiate the analytes from other drugs which might have close or isobaric primary transitions. Compound dependent parameters are reported in table 1.

2.6. Analytical method validation

The method validation was based on the recommendations published by the Food and Drug Administration (FDA) [23] and the recommendations of the conference report of the "Analytical method validation: Bioavailability, bioequivalence and pharmacokinetic studies" [24].

Calibration curves were calculated using the ratio of the peak area of analyte and internal standard with a 1/X weighted quadratic regression. To study accuracy, precision and recovery, replicate analysis (n = 6) of plasma-samples spiked with three different concentrations was used for the intra-assay precision and accuracy determination (table 2). The three concentrations were chosen to cover the whole range of the calibration curve according the expected drug concentrations in patient samples. Inter-assay accuracy and precision were determined by repeated sample work-up and analysis performed on six different days. The concentration of each sample was determined using calibration standards prepared on the same day. Recovery was expressed as percentage of the target value. The precision was calculated as the coefficient of variation (CV %) within a single run (intra-