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Short communication

Development and validation of a method for gefitinib quantification in dried blood spots using liquid chromatography-tandem mass spectrometry: Application to finger-prick clinical blood samples of patients with non-small cell lung cancer

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A B S T R A C T

A liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed and validated for the quantification of gefitinib in dried blood spots (DBSs). Gefitinib was extracted with methanol from DBS of 3 mm in diameter and detected using a triple quadrupole mass spectrometer. The method was validated by evaluating its precision, accuracy, selectivity, carryover, matrix effect, recovery, and stability. For clinical validation, paired finger-prick DBS and plasma concentrations were compared for 10 patients with non-small cell lung cancer (NSCLC) taking gefitinib. The calibration linear range was 37.5–2400 ng/mL (coefficient of determination [R^2] = 0.99), encompassing the therapeutic concentrations of gefitinib. The accuracy and precision were within 15% of the quality control (QC) concentrations of 80, 200, and 2000 ng/mL. The lower limit of quantification was determined to be 40 ng/mL. Gefitinib was stable in DBSs for up to 5 months at room temperature and −20 °C, and at 40 °C for 24 h. A good correlation was observed between the gefitinib levels measured by the DBS method and plasma concentrations (R^2 = 0.99). This method provides a simple, fast, and accurate approach to the quantitative analysis of gefitinib in finger-prick DBSs. The method would be useful for minimally invasive evaluation of the clinical gefitinib blood concentration.

1. Introduction

Gefitinib is a molecular targeting drug that inhibits epidermal growth factor receptor (EGFR) tyrosine kinase and is used for the treatment of advanced non-small cell lung cancer (NSCLC) harboring an EGFR mutation. Some clinical studies have indicated a relationship between the pharmacokinetics of gefitinib and clinical outcomes or toxicity. Mizoguchi et al. [1] reported that a ratio of day 8 to day 2 plasma concentration higher than the median is a predictor of better progression-free survival in patients with NSCLC harboring EGFR mutations treated with oral gefitinib at 250 mg/day. Kobayashi et al. [2] reported that the area under the concentration-time curve and trough levels of gefitinib are significantly greater in patients with diarrhea or hepatotoxicity than in those without these conditions. However, the above-cited studies used small sample sizes (n < 40), and thus, further clinical studies would be needed to optimize the administration of gefitinib.

The dried blood spot (DBS) method is a breakthrough in pharmacokinetic studies, which often require accurate timing, multiple samplings, and multicenter recruitment of patients for specimens. The advantages of DBS sampling in pharmacokinetic studies are its minimally invasive procedure, easy sampling, the stability of the analyte during sample storage, and a low risk of infection [3].

Faivre et al. [4] reported a simple high-performance liquid chromatography with ultraviolet detection (HPLC-UV) method for gefitinib in plasma, and several liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods for gefitinib in plasma [5], serum, and whole blood [6] have been demonstrated. However, there are no

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reports of quantification methods for gefitinib in DBSs. It should be possible to conduct integrated, minimally invasive, and cost-effective multicenter clinical pharmacokinetic studies using DBS methods.

This article describes the development and validation of a quantification method suitable for clinical pharmacokinetic studies of gefitinib in finger-prick DBSs using LS-MS/MS.

2. Materials and methods

2.1. Materials

Gefitinib and erlotinib (both ≥98% purity) were purchased from Cayman Chemicals Co., (Ann Arbor, MI, USA). Qualitative filter paper No. 2, 125 g/m^2 (Advantec, Tokyo, Japan) was used for the analysis. Ultrapure water was obtained using an RFU554CA ultrapure water system (Advantec). Acetonitrile, methanol, and ammonium acetate were purchased from Nacalai Tesque (Kyoto, Japan). Drug-free heparinized whole blood and plasma were provided by the Institute of Biomedical Research and Innovation (Kobe, Japan). Finger-prick blood samples were obtained using a BD Microtainer® contact-activated lancet (BD Biosciences, San Jose, CA, USA). The DBS was punched out using an SK11 Leather Punch, hole diameter 2.02–4.5 mm (Fujiwara Sangyo Co., Ltd., Hyogo, Japan).

2.2. Chromatography and MS/MS conditions

All analyses were performed using a QTRAP 4500 triple quadrupole mass spectrometer (AB Sciex, Framingham, MA, USA) controlled using the Analyst 1.6.2 software. The mass spectrometer was operated in the multiple reaction monitoring (MRM) mode with positive ion electrospray ionization. The instrument was directly coupled to a series 1260 LC system (Agilent, Waldbronn, Germany). An isocratic mobile phase consisting of 10 mM ammonium acetate in water-acetonitrile (50:50, *v/v*) was used. The mobile phase flow rate was maintained at 0.2 mL/min, and the chromatography was performed at 40 °C, with a total run time of 3 min per injection using an InertSustainSwift C18 analytical column (3 μm, 2.1 × 50 mm, GL Sciences, Tokyo, Japan).

2.3. Standard solutions

Stock solutions of gefitinib and erlotinib (internal standard [IS]) were prepared at a concentration of 1 mg/mL in methanol. Working standard solutions of gefitinib were prepared by diluting the stock solution with acetonitrile to a concentration range of 0.375–24 μg/mL. The DBS extraction solution was prepared by diluting the erlotinib (IS) stock solution with methanol to a concentration of 1 ng/mL. The plasma extract solution was prepared by diluting the IS stock solution with acetonitrile to a concentration of 1 ng/mL. The stock, standard, and extract solutions were stored at −20 °C.

2.4. Calibration standards and quality control (QC) samples

Calibration standards for DBS were prepared by diluting the gefitinib working standard solutions 10-fold with drug-free whole blood to concentrations of 2400, 1200, 600, 300, 150, 75, and 37.5 ng/mL. Likewise, quality control (QC) samples for DBS were prepared by diluting the gefitinib working standard solutions with drug-free whole blood to concentrations of 2000, 200, 80, and 40 ng/mL as the high, medium, low, and lower limit of quantification (LLoQ), respectively. Calibration standard samples for plasma were prepared by diluting the gefitinib working standard solutions 10-fold with drug-free plasma to the same concentrations as those used for DBS samples.

2.5. Sample preparation

For DBS samples, 10 μL whole blood, which was enough to produce a 3 mm DBS, was pipetted onto filter paper and allowed to dry for 2 h. Then, a series of 3 mm punches were cut out, added to 500 μL DBS extract solution, and shaken at 240 spm using a KM Shaker Model V-S (Iwaki Sangyo Co., Ltd., Tokyo, Japan) for 30 min at room temperature in a 1.5 mL Microtube (WATSON Co., Ltd., Tokyo, Japan). The supernatant (5 μL) was analyzed using LC-MS/MS. For the plasma samples, 20 μL plasma was added to 40 μL of the plasma extract solution, vortexed, and centrifuged at 10,000 × *g* for 10 min. The supernatant (1 μL) was subsequently analyzed using LC-MS/MS.

2.6. Validation procedure

The validation procedure was carried out in accordance with the bioanalytical method validation (BMV) guidelines recommended in Japan [7].

Calibration samples were prepared on each validation day. Peak area ratios of gefitinib (analyte) to that of IS were used for all calculations. A least squares linear regression (1/x weighting factor) of seven non-zero samples was used to define the calibration curve.

The precision and accuracy of the method were evaluated by measuring the four QC samples (high, medium, low, and LLoQ concentrations), which were prepared on each validation day (n = 5 each). Inter-day precision was assessed on 3 separate days. The accuracy was expressed as the percentage relative error (RE%) of the measured and theoretical concentrations. The precision was expressed as the percentage relative standard deviation (RSD%).

The selectivity of the method was determined by determining the presence or absence of interfering peaks from six individual drug-free human DBS samples at the retention times of gefitinib and IS. The autoinjector carryover was determined by injecting the highest calibration standard, followed by injection of blank samples. The response of the blanks was then compared to that of the LLoQ.

To determine the recovery of gefitinib, the IS ratios of extracts of the QC samples (high, medium, and low, n = 3) equivalent to 10 μL whole blood were compared to blank DBS extracts spiked with standard solutions at the same concentration. To evaluate the matrix effect, peak areas of blank DBS extracts spiked with standard solutions were compared to matrix-free solutions of QC samples (high, medium, and low). For the matrix effect, extracts of six individual drug-free human DBSs were used.

The DBS stability was evaluated by comparing the concentrations of the QC samples stored at room temperature or −20 °C with theoretical values at the low, medium, and high levels (n = 3). The stability was assessed at 24 h, 1 month, and 5 months. The DBS stability at 40 °C for 24 h was also investigated to simulate a rise in temperature during sample transportation to the analytical laboratory. The plasma assay method was also validated in accordance with the BMV guidelines recommended in Japan [7].

2.7. Clinical validation

Gefitinib blood concentrations were evaluated using the DBS method in 10 patients with NSCLC administered gefitinib daily or every other day. DBS samples were obtained by puncturing their fingertips with a lancet immediately before gefitinib administration (trough level). Puncture site was cleaned with an alcohol swab and completely dried. The skin puncture was self-performed by the patient on a preferred finger. The area above the puncture site was gently squeezed to produce a large droplet of blood. The first drop of blood was directly absorbed on the filter paper, and the finger was not “milked” to reduce excess tissue fluid and avoid specimen hemolysis.