Brief Report

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Development of a simple high-performance liquid chromatography-ultraviolet detection method for selpercatinib determination in human plasma

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SUMMARY Selpercatinib is a selective *rearranged during transfection* (*RET*) kinase inhibitor effective for the treatment of *RET*-positive non-small cell lung cancer, thyroid cancer, and other cancers. However, its clinical use requires careful management because of dose-dependent adverse effects and pharmacokinetic interactions. Given the multiple factors influencing selpercatinib blood levels, we hypothesized that establishing a therapeutic drug monitoring system for selpercatinib could help reduce adverse events and optimize efficacy. Therefore, we herein developed a high-performance liquid chromatography-ultraviolet (HPLC-UV) method for measuring selpercatinib blood levels to facilitate therapeutic drug monitoring in clinical practice. Proteins were precipitated with acetonitrile, and selpercatinib and the internal standard (gefitinib) were separated *via* HPLC-UV. The calibration curve was linear over 0.5–8.0 μg/mL with a coefficient of determination (r^2) equaling 0.9996. Intra- and interday validation coefficients were both under 2.80%. The corresponding measurement precision ranged from − 1.50% to 12.60% and − 1.32% to 7.50%, respectively, with recoveries exceeding 94.43%. Thus, this study establishes a simple and sensitive method for quantifying selpercatinib in human plasma. Future studies will analyze plasma samples from patients treated with selpercatinib and utilize this method to explore the relationships among plasma concentration, efficacy, and adverse events to define the therapeutic concentration range.

Keywords targeted kinase inhibitor, clinical settings

1. Introduction

Selpercatinib is an adenosine triphosphate-competitive and highly selective oral small-molecule inhibitor of the *rearranged during transfection* (*RET*) kinase, demonstrating efficacy and safety in the treatment of *RET* fusion-positive lung and thyroid cancers and *RET*-mutant medullary thyroid cancer (*1-3*). In a phase-3 trial involving patients with advanced *RET*-mutant medullary thyroid cancer, the most common adverse events during selpercatinib treatment were hypertension (42.5%), dry mouth (31.6%), diarrhea, and elevated alanine aminotransferase levels. Adverse events led to dose reductions in 38.9% and dose interruptions in 56.0% of patients receiving selpercatinib (*3*). Additionally, chylous effusions, a newly identified treatment-related adverse event, have been reported to occur in a dose-dependent manner in patients with *RET*-mutant thyroid cancer receiving selpercatinib (*4*).

As selpercatinib is metabolized by cytochrome P450 (CYP)3A, its pharmacokinetics are affected by CYP3A inhibitors or inducers (*5,6*). Furthermore, the blood levels of selpercatinib decrease when it is coadministered with drugs that increase intragastric pH, such as proton pump inhibitors (*6*). Whereas the dosage of selpercatinib in children is based on body surface area, no such regimen exists for adults, which leads to possible variations in blood levels depending on body size (*6*). To the best of our knowledge, no studies have examined the association between the blood levels of selpercatinib and its efficacy or adverse events. Given the multiple factors influencing selpercatinib blood levels, we hypothesized that establishing a therapeutic drug monitoring (TDM) system for selpercatinib could help reduce adverse events and optimize efficacy. Therefore, we aimed to develop a method for measuring selpercatinib blood levels to enable TDM in clinical practice.

Currently, only liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods have been reported for selpercatinib quantitation in human plasma (*7*). LC-MS/MS enables rapid, accurate, and sensitive drug quantitation across various biological matrices; however, its high cost and maintenance requirements limit its accessibility in hospitals. High-performance liquid chromatography (HPLC) is more affordable than LC-MS/MS and therefore suitable for routine clinical use. Hence, we developed a high-performance liquid chromatography-ultraviolet (HPLC-UV)-based method for quantifying selpercatinib in human plasma.

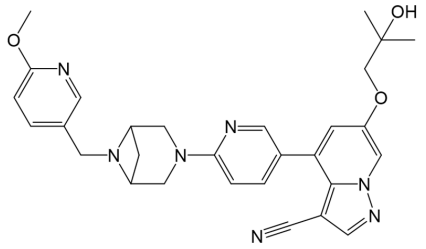
2. Materials and Methods

2.1. Reagents and chemicals

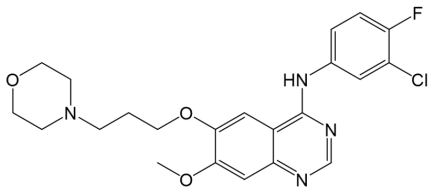
Selpercatinib and gefitinib (internal standard, IS) were obtained from MedChemExpress (Monmouth Junction, NJ, USA) and Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan), respectively (Figure 1). HPLC-grade acetonitrile, methanol, distilled water (Kanto Chemical Co., Inc., Tokyo, Japan), dimethyl sulfoxide (Fujifilm Wako, Osaka, Japan), and KH_2PO_4 (Fujifilm Wako, Osaka, Japan) were used to prepare the HPLC mobile phase and stock/working solutions. Human plasma (pooled) collected with ethylenediaminetetraacetic acid (EDTA)-2Na was purchased from Cosmo Bio Co., Ltd. (Tokyo, Japan).

2.2. Equipment and chromatographic conditions

The chromatography system consisted of a Jasco HPLC instrument (Tokyo, Japan) equipped with a pump (PU-4180), a UV detector (UV-4075), and an autosampler (AS-4550). Chromatographic separation was achieved on a Capcell Pak C18 MG II (Osaka Soda, Tokyo, Japan) reversed-phase column (250 mm × 4.6 mm i.d.) with a Capcell Pak C18 MG II guard column (10 mm × 4.0 mm; Osaka Soda, Tokyo, Japan) at ambient temperature. The mobile phase, a mixture of 0.5% KH_2PO_4 (pH 4.5) and acetonitrile (70:30, v/v), was delivered at a flow rate of 1.0 mL/min over a 15 min run. UV detection was performed at 240 nm.



Selpercatinib



Gefitinib

Figure 1. Chemical structures of selpercatinib and gefitinib.

2.3. Preparation of stock and working solutions

Selpercatinib and IS stock solutions were prepared in dimethyl sulfoxide at a concentration of 1 mg/mL. The selpercatinib stock solution was further diluted with acetonitrile to obtain working solutions with concentrations of 2.5, 5.0, 12.5, 20, 30, and 40 µg/mL. The IS stock solution was diluted with acetonitrile to a working concentration of 10 µg/mL. All stock and working solutions were aliquoted and stored at − 60°C in the dark.

2.4. Sample preparation

Prior to analysis, human plasma and working solutions were thawed and vortexed. A protein precipitation procedure was used to extract selpercatinib and the IS. Blank plasma (50 µL) was spiked with selpercatinib (10 µL), vortexed for 5 s, supplemented with the IS (10 µL) and acetonitrile (180 µL) at − 20°C, and revortexed for 1 min. Subsequently, the sample was centrifuged at 15,000 *g* for 10 min at 4°C, and 10 µL of the supernatant was directly injected into the HPLC system for analysis (Figure 2).

2.5. Specificity

Samples collected from six lots of human plasma were analyzed to determine whether the endogenous matrix of these plasmas eluted near the retention time of selpercatinib or the IS.

2.6. Calibration curves

Accuracy and linearity were evaluated by analyzing a set of standards with concentrations of 0.5-8.0 µg/mL. Precision and accuracy were assessed using samples spiked with selpercatinib at concentrations of 0.5, 1.0, 2.5, 4.0, 6.0, and 8.0 µg/mL.

2.7. Recovery

Recovery was evaluated by comparing the levels of selpercatinib extracted from control plasma samples of the six abovementioned concentrations with those obtained for saline.

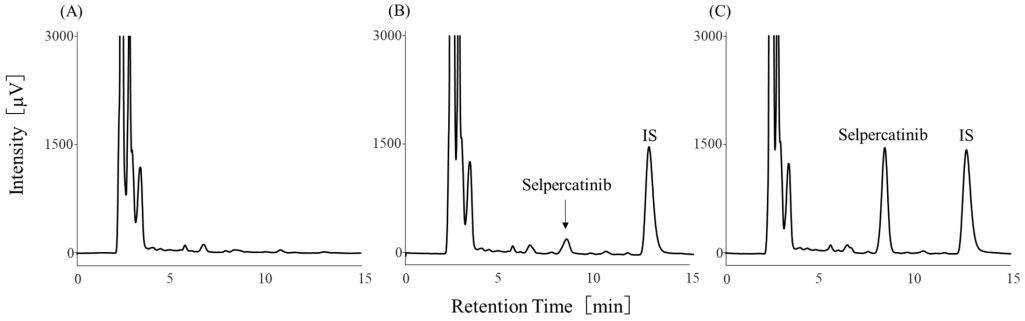


Figure 2. Chromatograms of (A) blank plasma, (B) plasma containing selpercatinib at 0.5 μg/mL, and (C) plasma containing selpercatinib at 4.0 μg/mL.

Table 1. Intra- and interday accuracy and precision of our method

Theoretical selpercatinib concentration (μg/mL)	Intraday (n = 5)			Interday (n = 5)			Recovery (%)
	Detected mean ± SD (μg/mL)	CV (%)	Accuracy (%)	Detected mean ± SD (μg/mL)	CV (%)	Accuracy (%)	
0.5	0.56 ± 0.01	2.08	12.60	0.54 ± 0.02	2.80	7.50	96.51
1.0	0.99 ± 0.02	2.24	−1.50	0.99 ± 0.02	1.53	−1.32	94.43
2.5	2.52 ± 0.06	2.42	0.67	2.48 ± 0.03	1.33	−0.90	96.80
4	4.00 ± 0.06	1.62	−0.02	3.99 ± 0.05	1.35	−0.30	97.30
6	5.94 ± 0.13	2.20	−1.05	5.95 ± 0.04	0.60	−0.87	96.71
8	8.07 ± 0.18	2.22	0.91	8.00 ± 0.11	1.33	−0.05	96.33

CV, coefficient of variation; SD, standard deviation.

Table 2. Stability analysis results (n = 5)

Theoretical selpercatinib concentration (μg/mL)	Stability condition (%)			
	Benchtop mean ± SD	Short-term (24 h) mean ± SD	Long-term (three months) mean ± SD	Freeze and thaw mean ± SD
0.5	99.13 ± 5.95	103.60 ± 5.96	104.71 ± 6.55	105.81 ± 4.29
4.0	95.84 ± 1.67	97.98 ± 1.41	100.26 ± 2.73	98.09 ± 1.13
8.0	97.45 ± 1.28	96.65 ± 0.81	101.12 ± 1.34	99.09± 0.93

2.8. Stability

Analyte stability in human plasma was tested at three different concentrations (0.5, 4.0, and 8.0 μg/mL). For (i) bench-top, (ii) short-term, (iii) long-term, and (iv) freeze-thaw stability evaluations, the samples were stored for (i) 6 h at 22°C, (ii) 4°C for 24 h, and (iii) one month at − 60°C and (iv) subjected to three cycles of freezing at − 60°C or below and thawing at 22°C. The samples were analyzed using a calibration curve prepared with a freshly spiked analyte, and the obtained concentrations were compared with the nominal values.

3. Results and Discussion

We developed an HPLC-UV method to quantify selpercatinib in human plasma, following the analytical validation guidelines of the US Food and Drug Administration. To the best of our knowledge, selpercatinib concentrations in human plasma have previously been determined only using LC-MS/MS (*7*), which is prone to ion suppression and may misinterpret samples containing multiple drugs with similar masses (*8*). Moreover, the high cost and limited availability of LC-MS/MS in general hospitals motivated us to establish an alternative HPLC-UV method.

In our method, linear calibration curves for selpercatinib were obtained over a range of 0.5-8.0 μg/mL. The six-point selpercatinib standard calibration curve was expressed as $y = 0.2464x + 0.0071$ (r^2 = 0.9996). Table 1 lists the intra- and interday coefficients of variation (CVs) and accuracies, with all CVs lying below 2.80%. The intra- and interday accuracies ranged from − 1.50% to 12.60% and − 1.32% to 7.50%, respectively. Protein precipitation with acetonitrile resulted in a high recovery of > 94.43%, whereas no recovery data were provided by Gulikers *et al.* (*7*). The results of stability testing (Table 2) demonstrated

quantifiable results for each concentration (0.5, 4.0, and 8.0 μg/mL) under various clinical conditions.

The representative chromatograms of blank human plasma (Figure 2A) indicated that selpercatinib and the IS were well separated from the coextracted materials under the employed chromatographic conditions, with the respective retention times equaling 8.3 and 12.6 min. No interference from endogenous plasma components was observed at these retention times (Figures 2B and 2C). Additionally, the analysis of six plasma lots confirmed the absence of matrix effects near selpercatinib and IS retention times. The analysis time (15 min) and plasma volume (50 μL) of our method minimally exceed those of a previously reported LC-MS/MS method (9.5 min and 20 μL, respectively) (*7*).

Selpercatinib is administered orally at 160 mg twice daily and has a half-life of 32 h, with the average steady-state C_{max} [coefficient of variation (CV%)] equaling 2,980 (53%) ng/mL (*6*). Unlike the LC-MS/MS method, which can measure plasma concentrations of 50-5,000 ng/mL (*7*), our method has a quantitation limit of 50 ng/mL but is still suitable for assessing plasma concentrations in patients receiving selpercatinib, as selpercatinib trough levels in clinical practice range from 1000 to 4000 ng/mL.

Medullary thyroid cancer is a malignant tumor primarily driven by *RET* gene mutations, particularly in advanced cases with the *RET* M918T mutation (*9*). The plasma inhibitory concentration (IC_{90}) of selpercatinib for the *RET* M918T mutation is 1.1 μg/mL (*10*), suggesting that a trough plasma concentration of ≥ 1.1 μg/mL could serve as a target therapeutic range for selpercatinib. However, the relationship between adverse events and trough or peak plasma concentrations remains unclear.

This study has certain limitations. *RET* fusions are present in a variety of malignancies, including 1-2% of lung cancers, 10-20% of papillary thyroid cancers, and rarely in other solid tumors (*11*). Consequently, our method was not be used to evaluate selpercatinib levels in patient samples. Additionally, we did not assess the selectivity of this method with respect to concomitant medications or their metabolites in patients on selpercatinib. Future studies should confirm selectivity in clinical samples from patients treated with selpercatinib.

In conclusion, we have developed a HPLC-UV method for determining selpercatinib in human plasma. Future studies will examine plasma samples from selpercatinib-treated patients and use the proposed method to investigate the relationships among plasma concentration, efficacy, and adverse events to define the therapeutic target concentration range.

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Conflict of Interest: The authors have no conflicts of interest to disclose.

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