Brief Report

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Solid phase extraction and high-performance liquid chromatographic determination of lazertinib in human plasma

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SUMMARY: Lazertinib is a novel third-generation tyrosine kinase inhibitor (TKI) developed for the treatment of epidermal growth factor receptor (EGFR) mutant non-small cell lung cancer (NSCLC). Reports on previous EGFR-TKIs have detailed significant associations between blood drug concentration and efficacy. In addition, significantly elevated blood concentrations of lazertinib have been observed in Asians compared to Caucasians, suggesting the influence of interethnic variability. In this study, we developed and validated a method to determine lazertinib concentrations in human plasma for therapeutic drug monitoring (TDM). Lazertinib and its internal standard, sotorasib, were extracted by solid-phase extraction using an Oasis hydrophilic lipophilic balance cartridge. Chromatographic separation was performed on a reversed-phase column with 0.5% KH_2PO_4 (pH 4.5) and acetonitrile (52:48, v/v) as the mobile phases in an isocratic elution mode with a flow rate of 1.0 mL/min. The detection wavelength was 296 nm. The calibration curves were linear in the range of 25–2,000 ng/mL, with a coefficient of determination (r^2) of 0.9997. The accuracy and precision of all validation experiments were within the criteria set by the Food and Drug Administration guidelines. This study represents the first development and validation of a method for quantifying lazertinib in human plasma. This study is expected to facilitate the widespread use of TDM in studies on lazertinib.

Keywords: lazertinib, high-performance liquid chromatography-ultraviolet, tyrosine kinase inhibitor, human plasma, therapeutic drug monitoring

1. Introduction

Lazertinib is a third-generation epidermal growth factor receptor-tyrosine kinase inhibitor (EGFR-TKI) that is used in combination with amivantamab to treat EGFR-mutated advanced non-small cell lung cancer (NSCLC) (*1*). The National Comprehensive Cancer Network (NCCN) guidelines recommend osimertinib as a first-line therapy for EGFR-mutated NSCLC (*2*). Recently, combination therapy with lazertinib and amivantamab has been shown to significantly prolong progression-free survival (PFS) compared to osimertinib therapy (*1*). Therefore, an increasing number of patients are expected to receive lazertinib instead of osimertinib.

Typical adverse events (all grades) associated with lazertinib are rash or acne (33%) and diarrhea (21%), with the dose being reduced or treatment discontinued at the approved dose (240 mg/d) in 17% and 4% of patients, respectively (*3*). Erlotinib and gefitinib, EGFR-TKIs similar to lazertinib, also show adverse reactions. There is a significant association between blood erlotinib concentration (*4*), various grades of skin rash, and diarrhea. Meanwhile, plasma gefitinib trough concentrations ≥ 200 ng/mL are significantly associated with prolonged overall survival (*5*). Therefore, therapeutic drug monitoring (TDM) of trough concentrations has been recommended for these drugs (*6*).

However, the relationship among blood lazertinib concentration, clinical efficacy, and safety has not been clarified. The approved dose of lazertinib is 240 mg/d worldwide (*7-9*), even though the maximum plasma concentration (C_{max}) and area under the curve (AUC) are significantly higher in Asians than in Caucasians owing to interethnic variation (*10*). Therefore, TDM may be necessary for implementing effective and safe lazertinib therapy. In clinical trials, plasma lazertinib concentrations have been measured using liquid chromatography-mass spectrometry (LC-MS); however, detailed LC-MS conditions have not been described (*10*).

To the best of our knowledge, there have been no reports on methods for quantifying lazertinib concentrations in human plasma. In addition, LC-MS is susceptible to ion suppression and may misinterpret samples containing multiple drugs with the same mass (*11*). Furthermore, LC-MS is expensive and has limited

application in general hospitals. Hence, we developed a method to determine the concentration of lazertinib in human plasma using high-performance liquid chromatography-ultraviolet (HPLC-UV), in accordance with the Food and Drug Administration (FDA) analytical validation guidelines (*12*).

2. Materials and Methods

2.1. Reagents and chemicals

Lazertinib and sotorasib (internal standard, IS) were obtained from MedChemExpress (Monmouth Junction, NJ, USA) and Toronto Research Chemicals, Inc. (Toronto, ON, Canada), respectively. Sotorasib is a Kirsten rat sarcoma viral oncogene (KRAS) inhibitor used to treat KRAS mutation-positive NSCLC that is not clinically concomitant with lazertinib (*13*). Acetaminophen, amlodipine, cimetidine, clarithromycin, droperidol, esomeprazole, fluconazole, furosemide, gabapentin, isavuconazole, lacosamide, loperamide, metformin, minocycline, nifedipine, omeprazole, posaconazole, and pregabalin were obtained from Tokyo Chemical Industry Co. (Tokyo, Japan). Arotinolol and tedizolid were obtained from Toronto Research Chemicals, Inc. (Toronto, ON, Canada) and ChemScene (Monmouth Junction, NJ, USA), respectively. HPLC-grade acetonitrile, methanol, distilled water (Kanto Chemical, Co., Inc., Tokyo, Japan), KH_2PO_4 (Fujifilm Wako, Osaka, Japan) were used in the HPLC mobile phase. Oasis hydrophilic-lipophilic balance (HLB) extraction cartridges were purchased from Waters Corp. (Milford, MA, USA). Human plasma (pooled) and ethylenediaminetetraacetic acid (EDTA)-2Na were purchased from Cosmo Bio Co., Ltd. (Tokyo, Japan).

2.2. Equipment and chromatographic conditions

The HPLC system consisted of pumps (PU-4180), a UV detector (UV-4075), and an autosampler (AS-4550; all from Jasco, Tokyo, Japan). The mobile phase consisted of 0.5% potassium dihydrogen phosphate (KH_2PO_4, pH 4.5) and acetonitrile (52:48, v/v). The flow rate was 1.0 mL/min and detected at 296 nm using Capcell Pak C18 MG II reversed phase (250 × 4.6 mm i.d., 5 μm) column (Osaka Soda, Tokyo, Japan). A rotary evaporator (CVE-2200) was purchased from Tokyo Rikakikai (Tokyo, Japan).

2.3. Preparation of stock solutions and working solutions

Stock solutions of lazertinib and the IS were prepared in methanol at a concentration of 1 mg/mL. The lazertinib stock solution was diluted further with methanol to obtain working solutions with concentrations of 0.5, 1, 2, 5, 20 and 40 μg/mL. The IS was diluted with methanol to obtain a working solution of 12.5 μg/mL.

2.4. Preparation of samples

A 10 μL lazertinib working solution was vortexed with 200 μL plasma for 60 s. Lazertinib-spiked plasma (210 μL), 10 μL of IS and 780 μL of HPLC-grade distilled water were added and vortexed for 30 s. This mixture was applied to an Oasis HLB extraction cartridge that had been activated previously with 1,000 μL methanol and then 1,000 μL distilled water. Following application of the sample, the cartridge was washed with 1,000 μL of distilled water and then 1,000 μL of 60% methanol in distilled water and was eluted with 1,000 μL of 100% acetonitrile. The eluates were dried by vortex-vacuum evaporation at 80°C using a rotary evaporator. The dried residues were reconstituted in 100 μL methanol, and vortexed for 60 s. After mixing well, the samples were sonicated for 60 s and 50 μL aliquots were injected into the HPLC system.

2.5. Selectivity and specificity assessments

Selectivity was evaluated using blank plasma samples from seven healthy volunteers without the addition of lazertinib or IS solution. Minocycline is often administered prophylactically to treat skin disorders, an adverse event of lazertinib, in clinical trials (*1*). Therefore, we tested the interference by 20 drugs (Table 1) that patients may receive concomitantly with lazertinib. Interference was defined as a retention time within a range of ± 1 min of the lazertinib and IS retention times.

2.6. Calibration curves and quantitation

Accuracy and linearity were evaluated by analyzing a set of standards ranging from 25 to 2,000 ng/mL. Intra- and inter-day precision and accuracy were determined by replicate analyses of five sets of samples spiked with six concentrations of lazertinib (25, 50, 100, 250, 1000, and 2,000 ng/mL) within the same day or on five consecutive days. The precision of the method for each concentration was determined by comparing the coefficient of variation (CV), obtained by calculating the standard deviation (SD) as a percentage of the calculated mean concentration. The limit of quantification (LOQ) was determined as the lowest nonzero concentration in the calibration curves. Methods were validated according to the Bioanalytical Method Validation Guidelines published by the FDA (*12*).

2.7. Stability studies

The stability of lazertinib was assessed using three different concentrations (25, 250, and 2000 ng/mL) in the evaluations of benchtop, short-term, long-term, freeze-thaw, and post-preparative stability. The bench-top stability samples were kept for 6 h at room temperature (22°C), short-term stability samples were stored at 4°C

for 24 h, long-term stability samples were stored for 4 weeks at -80°C, freeze-thaw samples underwent three cycles of freezing at -80°C or below in a freezer with thawing at room temperature, and post-preparative sample stability was analyzed after 24 h of storage in an autosampler at room temperature.

2.8. Recovery

Extraction recoveries from plasma were determined by comparing the peak height ratios of extracted plasma samples spiked with known amounts of lazertinib according to the above procedure with those of non-extracted quality control samples. Control samples were prepared by mixing solutions containing the same amount of compound that was added to the blank plasma samples; however, this compound was obtained by direct evaporation until dry, rather than by extraction. It was then reconstituted in methanol.

3. Results and Discussion

Table 1. Medications listed for the specificity evaluation

Medication	Retention times
Acetaminophen	< 3 min or none
Amlodipine	3.6 min
Arotinolol	< 3 min or none
Cimetidine	< 3 min or none
Clarithromycin	< 3 min or none
Droperidol	< 3 min or none
Esomeprazole	4.2 min
Fluconazole	< 3 min or none
Furosemide	3.4 min
Gabapentin	< 3 min or none
Isavuconazole	< 3 min or none
Lacosamide	< 3 min or none
Loperamide	< 3 min or none
Metformin	< 3 min or none
Minocycline	< 3 min or none
Nifedipine	11.0 min
Omeprazole	4.2 min
Posaconazole	< 3 min or none
Pregabalin	< 3 min or none
Tedizolid	3.6 min

We developed an HPLC-UV method for the determination of lazertinib concentrations in human plasma that meets the sensitivity and accuracy requirements for performing TDM according to the FDA's analytical validation guidelines. This assay can measure one sample in 8 min and is suitable for performing TDM in a clinical setting. In our method, linear calibration curves for lazertinib were obtained over a range of 25–2,000 ng/mL. In clinical trials, the geometric mean trough plasma concentration of lazertinib was reported to range from 195.0 to 211.4 ng/mL and was similar in cycles 2 to 13 (*14*). Additionally, lazertinib has a two-step dose reduction protocol for adverse events, allowing dose adjustments of 160 and 80 mg/d (*8*). The trough concentrations of lazertinib at 160 and 80 mg/d are approximately 110 and 60 ng/mL, respectively (*3*). Therefore, the quantitative range of this assay system is appropriate even after the lazertinib dose is reduced and can be applied to TDM in daily clinical practice.

The six-point lazertinib standard calibration curve was expressed as $y = 0.0053x + 0.0229$ ($r^2 = 0.9997$). Table 2 shows the intra- and inter-day CVs and accuracies, with all CVs lying below 8.24%. The intra- and inter-day accuracies ranged from −7.23% to −3.85% and −3.88% to 2.86%, respectively. Solid phase extraction with an Oasis HLB cartridge resulted in a high recovery of > 91.28% (Table 2). The results of stability testing (Table 3) demonstrated quantifiable results for each concentration (25, 250, and 2,000 ng/mL) under various clinical conditions. Figure 1 shows the chromatograms of blank human plasma samples, 25 ng/mL (LOQ), and 250 ng/mL lazertinib. The retention times of the lazertinib and IS were 7.0 and 4.9 min, respectively. The chromatograms were free of interfering peaks from the biological matrix, and no interfering peaks representing endogenous compounds were observed near the retention times of lazertinib or IS.

In addition, 20 drugs that could be used concomitantly in patients with NSCLC were tested for interference. Eighteen drugs showed no interference. The retention time of esomeprazole and omeprazole

Table 2. Intra- and inter-day precision and accuracy results of lazertinib in human plasma using the proposed high-performance liquid chromatography method (*n* = 5)

Added lazertinib concentration (ng/mL)	Intra-day (*n* = 5)			Inter-day (*n* = 5)			Recovery (%)
	Detected (ng/mL) mean ± SD	CV (%)	Accuracy (%)	Detected (ng/mL) mean ± SD	CV (%)	Accuracy (%)	
25	23.85 ± 1.25	5.24	-4.59	24.03 ± 1.92	7.99	-3.88	98.85
50	48.08 ± 2.80	5.83	-3.85	49.31 ± 2.91	5.90	-1.38	95.44
100	95.38 ± 7.65	8.02	-4.62	102.86 ± 6.33	6.15	2.86	91.28
250	231.92 ± 12.43	5.36	-7.23	256.21 ± 20.78	8.11	2.48	96.61
1000	958.70 ± 56.39	5.88	-4.13	987.24 ± 81.36	8.24	-1.28	99.60
2000	1898.82 ± 91.86	4.84	-5.06	2028.62 ± 166.34	8.20	1.43	98.63

CV, coefficient of variation; SD, standard deviation.

Table 3. Stability analysis

Stability test conditions	Ratio of plasma concentration to the spiked value (%)		
	25 ng/mL (mean ± SD)	250 ng/mL (mean ± SD)	2,000 ng/mL (mean ± SD)
Benchtop storage (24°C, 6 h)	93.98 ± 3.34	105.06 ± 8.49	100.94 ± 3.72
Short-term storage (4°C, 24 h)	102.02 ± 7.35	100.75 ± 4.46	100.70 ± 3.05
Long-term storage (−60°C, 4 weeks)	102.25 ± 9.08	92.44 ± 5.63	97.92 ± 1.61
Freeze–thaw, three cycles (−60°C to room temperature)	97.43 ± 6.98	94.63 ± 2.87	107.49 ± 9.68
Post-preparative (24°C, 24 h)	101.05 ± 3.64	100.13 ± 7.63	92.92 ± 2.83

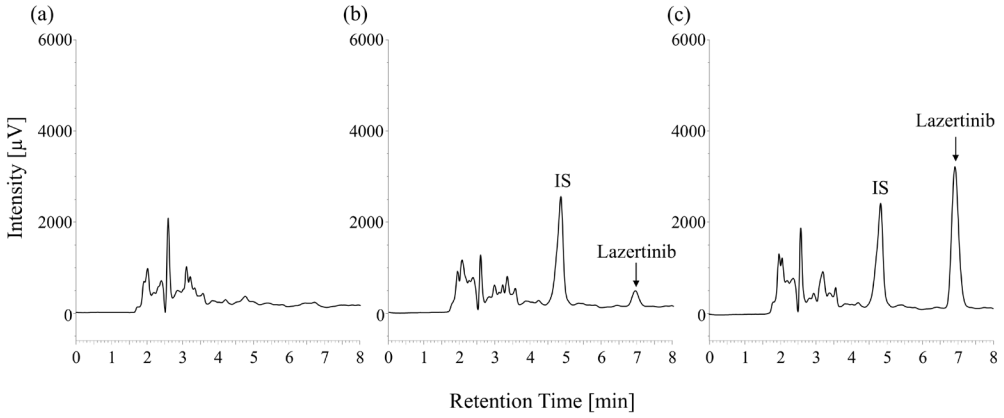

Figure 1. Chromatograms of (a) a blank plasma sample, (b) plasma sample containing lazertinib at 25 ng/mL, and (c) plasma sample containing lazertinib at 250 ng/mL.

was 4.2 min, indicating that they interfered with the IS. The C_{max} values for esomeprazole and omeprazole (both 20 mg/d) were 445 ng/mL and 430 ng/mL (*15,16*), respectively, with a half-life of < 1 h (*17*). Assuming a C_{max} of 500 ng/mL for esomeprazole and omeprazole, these drugs were spiked into plasma and analyzed after pretreatment using the present method. The peak heights of both drugs were 1.2- to 1.9-fold higher than that of the IS, indicating that accurately quantifying lazertinib may be difficult when blood samples are collected at C_{max}. In contrast, when the plasma concentration of esomeprazole and omeprazole was set to 125 ng/mL at 3 h post-administration (assuming at least two half-lives had elapsed), their peak heights were 0.32- to 0.35-fold lower than the IS peak height, confirming that lazertinib quantification was not affected. These findings suggest that accurate quantification of lazertinib is feasible when trough blood sampling is performed at least 3 h after administering esomeprazole or omeprazole. Therefore, when performing TDM of lazertinib by trough blood collection, we expect that esomeprazole and omeprazole will be below the detection limit and thus will not affect the quantitation of lazertinib in plasma when taken concurrently with lazertinib. Gefitinib and erlotinib show decreased absorption when co-administered with acid-reducing agents (*18*); however, lazertinib has no effect on absorption when co-administered with such agents and is not problematic from a pharmacokinetic perspective (*19*). Lazertinib should be administered orally with apixaban at a dose of 2.5 mg twice daily for the first 4 months of therapy (*7*). The trough concentration of apixaban at these doses was 21.0 ng/mL (*20*). We did not examine the interference with apixaban in this study. However, the low trough concentration of apixaban was difficult to quantify using HPLC-UV and was not expected to affect the quantification of lazertinib using this assay.

This study has several limitations. First, plasma lazertinib concentrations could not be determined in patients treated with lazertinib, as it has only been available on the market for a short period in Japan. Second, patients with NSCLC are often elderly and concomitantly take multiple medications to manage comorbidities and adverse events. Therefore, we could not evaluate the specificity of this method with respect to concomitant medications and their metabolites in patients receiving symptomatic therapy or medications for comorbidities. In the future, specificity should be confirmed using clinical specimens from patients receiving lazertinib.

In conclusion, we developed a novel method for determining lazertinib concentration in human plasma using HPLC-UV. Future studies should determine plasma samples from patients receiving lazertinib treatment and investigate the relationship between plasma concentrations, efficacy, and adverse events.

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

References

1. Cho BC, Lu S, Felip E, *et al*. Amivantamab plus Lazertinib in Previously Untreated *EGFR*-Mutated Advanced NSCLC. N Engl J Med. 2024; 391:1486-1498.
2. NCCN Guidelines - Non-Small Cell Lung Cancer Version 7.2025 *https://www.nccn.org/professionals/physician_gls/pdf/nscl.pdf* (accessed July 25, 2025).
3. Ahn MJ, Han JY, Lee KH, *et al*. Lazertinib in patients with EGFR mutation-positive advanced non-small-cell lung cancer: results from the dose escalation and dose expansion parts of a first-in-human, open-label, multicentre, phase 1-2 study. Lancet Oncol. 2019; 20:1681-1690.
4. Fiala O, Hosek P, Pesek M, Finek J, Racek J, Stehlik P, Sorejs O, Minarik M, Benesova L, Celer A, Nemcova I, Kucera R, Topolcan O. Serum Concentration of Erlotinib and its Correlation with Outcome and Toxicity in Patients with Advanced-stage NSCLC. Anticancer Res. 2017; 37:6469-6476.
5. Zhao YY, Li S, Zhang Y, Zhao HY, Zhao HY, Liao H, Guo Y, Shi YX, Jiang W, Xue C, Zhang L. The relationship between drug exposure and clinical outcomes of non-small cell lung cancer patients treated with gefitinib. Med Oncol. 2011; 28:697-702.
6. Mueller-Schoell A, Groenland SL, Scherf-Clavel O, van Dyk M, Huisinga W, Michelet R, Jaehde U, Steeghs N, Huitema ADR, Kloft C. Therapeutic drug monitoring of oral targeted antineoplastic drugs. Eur J Clin Pharmacol. 2021; 77:441-464.
7. Japanese Ministry of Health, Labour and Welfare, Pharmaceuticals and Medical Devices Agency (PMDA), package insert. *https://www.pmda.go.jp/PmdaSearch/iyakuDetail/ResultDataSetPDF/800155_4291091F1027_1_01* (accessed July 25, 2025).
8. US Food and Drug Administration (FDA). LAZCLUZE (lazertinib) tablets, for oral use. Prescribing information. *https://www.accessdata.fda.gov/drugsatfda_docs/label/2024/219008s000lbl.pdf* (accessed July 25, 2025).
9. European Medicines Agency (EMA). Lazcluze: Product information. *https://www.ema.europa.eu/en/documents/product-information/lazcluze-epar-product-information_en.pdf* (accessed July 25, 2025).
10. Huh KY, Lim Y, Yoon DY, Hwang JG, Sim S, Kang J, Wang J, Kim M, Jang SB, Shreeve SM, Mehta J, Haddish-Berhane N, Oh J, Lee S, Yu KS. Effects of food and race on the pharmacokinetics of lazertinib in healthy subjects and patients with EGFR mutation-positive advanced non-small cell lung cancer. Lung Cancer. 2023; 175:112-120.
11. Sauvage FL, Gaulier JM, Lachâtre G, Marquet P. Pitfalls and prevention strategies for liquid chromatography-tandem mass spectrometry in the selected reaction-monitoring mode for drug analysis. Clin Chem. 2008; 54:1519-1527.
12. US DHHS, FDA, CDER, CVM; Guidance for industry: Bioanalytical method validation. US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research and Center for Veterinary Medicine, Rockville, MD, (2018).
13. de Langen AJ, Johnson ML, Mazieres J, *et al.* Sotorasib versus docetaxel for previously treated non-small-cell lung cancer with $KRAS^{G12C}$ mutation: a randomised, open-label, phase 3 trial. Lancet. 2023; 401:733-746.
14. Lee KH, Cho BC, Ahn MJ, *et al*. Lazertinib versus Gefitinib as First-Line Treatment for EGFR-mutated Locally Advanced or Metastatic NSCLC: LASER301 Korean Subset. Cancer Res Treat. 2024; 56:48-60.
15. Choi HG, Jeon JY, Kwak SS, Kim H, Jin C, Im YJ, Kim EY, Wang HM, Kim Y, Lee SY, Kim MG. Pharmacokinetic comparison study of a combination containing 500 mg of Naproxen and 20 mg of Esomeprazole: a randomized, single-dose, 2-way crossover, open-label study in healthy Korean men. Clin Ther. 2015; 37:83-93.
16. Hassan-Alin M, Andersson T, Niazi M, Röhss K. A pharmacokinetic study comparing single and repeated oral doses of 20 mg and 40 mg omeprazole and its two optical isomers, S-omeprazole (esomeprazole) and R-omeprazole, in healthy subjects. Eur J Clin Pharmacol. 2005; 60:779-784.
17. Budha NR, Frymoyer A, Smelick GS, Jin JY, Yago MR, Dresser MJ, Holden SN, Benet LZ, Ware JA. Drug absorption interactions between oral targeted anticancer agents and PPIs: is pH-dependent solubility the Achilles heel of targeted therapy? Clin Pharmacol Ther. 2012; 92:203-213.
18. Kim B, Lee J, Jang H, Lee N, Mehta J, Jang SB. Effects of Acid-Reducing Agents on the Pharmacokinetics of Lazertinib in Patients with EGFR Mutation-Positive Advanced Non-Small-Cell Lung Cancer. Adv Ther. 2022; 39:4757-4771.
19. Byon W, Garonzik S, Boyd RA, Frost CE. Apixaban: A Clinical Pharmacokinetic and Pharmacodynamic Review. Clin Pharmacokinet. 2019; 58:1265-1279.

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