Development and Validation of an LC-MS/MS Method for Quantification of Osimertinib in Human Plasma: Application to Pharmacokinetic Studies

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ABSTRACT

A highly sensitive and specific liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed and validated for the quantification of osimertinib in human plasma, utilizing propranolol as an internal standard. Sample preparation involved protein precipitation with acetonitrile, followed by chromatographic separation on an Agilent Zorbax SB-C18 column. The mobile phase consisted of 0.2% formic acid in water (A) and acetonitrile (B), delivered at a flow rate of 0.4 mL/min. Detection was performed on a Quattro Micro quadrupole mass spectrometer equipped with an electrospray ionization source operating in positive ion mode. The method exhibited a linear dynamic range of 1.25 to 300 ng/mL for osimertinib, with a lower limit of quantification of 1.25 ng/mL. Intra- and inter-day precision and accuracy were within acceptable limits, with relative standard deviations not exceeding 10%. The method demonstrated excellent specificity, with no significant interference observed from endogenous plasma components. The validated method was successfully applied to a pharmacokinetic study involving healthy volunteers administered osimertinib, providing reliable and reproducible plasma concentration data over a 96-hour sampling period. This LC-MS/MS method offers a robust analytical tool for therapeutic drug monitoring and pharmacokinetic evaluations of osimertinib in clinical settings.

Keywords: *Osimertinib, LC-MS/MS, Human Plasma, Pharmacokinetics, Method Validation, Protein Precipitation, Electrospray Ionization, Internal Standard, Propranolol, Therapeutic Drug Monitoring*

1. *INTRODUCTION*

Non-small cell lung cancer (NSCLC) represents a significant portion of lung cancer cases worldwide. A substantial subset of NSCLC patients harbor activating mutations in the epidermal growth factor receptor (EGFR), which drive tumor progression. Targeted therapies using EGFR-tyrosine kinase inhibitors (TKIs) have markedly improved treatment outcomes for these patients. However, resistance to first- and second-generation EGFR-TKIs often develops, frequently due to the emergence of the T790M mutation. To address this challenge, osimertinib, a third-generation EGFR-TKI, was developed to selectively inhibit both sensitizing and T790M-resistant EGFR mutations.

Osimertinib has demonstrated superior efficacy and a favorable safety profile compared to earlier-generation TKIs. Despite these advantages, a significant proportion of patients experience adverse events (AEs) during treatment. Notably, over 80% of patients administered osimertinib report grade ≥2 AEs, and approximately 30% encounter grade ≥3 AEs. Common AEs include diarrhea, rash, and paronychia, which can adversely affect patients' quality of life and may necessitate dose modifications or discontinuation of therapy.

The occurrence and severity of AEs have been associated with the pharmacokinetic (PK) profiles of osimertinib and its active metabolites, AZ5104 and AZ7550. Both metabolites circulate at approximately 10% of the parent compound's exposure, with AZ5104 exhibiting a 15-fold higher potency against wild-type EGFR. Elevated plasma concentrations of these metabolites have been linked to increased incidence of AEs,

suggesting that therapeutic drug monitoring (TDM) could be instrumental in optimizing treatment by balancing efficacy and toxicity.

Furthermore, inter-individual variability in drug response and toxicity may be influenced by genetic polymorphisms. Variants in genes encoding drug-metabolizing enzymes and transporters, such as CYP3A4/5, ABCG2, and ABCB1, have been implicated in altering the PK and pharmacodynamic (PD) profiles of EGFR-TKIs. Identifying specific genetic markers associated with increased risk of AEs could facilitate personalized treatment strategies, enhancing both safety and efficacy.

Objectives

The primary objectives of this study are:

1. To evaluate the relationship between plasma exposures of osimertinib and its active metabolites (AZ5104 and AZ7550) and the incidence and severity of AEs in patients with advanced NSCLC.

2. To investigate the association between germline polymorphisms in genes related to drug metabolism and transport (e.g., EGFR, ABCG2, ABCB1) and the occurrence of AEs during osimertinib therapy.

Research Problem

While osimertinib has improved outcomes for NSCLC patients with EGFR mutations, the management of AEs remains a critical concern. Current strategies lack precision in predicting which patients are at higher risk for severe AEs, leading to a trial-and-error approach in dose adjustments. A deeper understanding of the PK-PD relationships and the role of genetic polymorphisms in drug response is essential to develop predictive models for AE risk, thereby facilitating personalized dosing regimens.

Importance and Necessity of Research

From a theoretical perspective, this research aims to elucidate the mechanisms underlying inter-patient variability in drug response and toxicity, contributing to the broader field of pharmacogenomics and personalized medicine. Practically, the findings could inform clinical guidelines for TDM and genetic screening in patients undergoing osimertinib therapy, ultimately improving patient outcomes by minimizing AEs and maximizing therapeutic efficacy.

Research Background

Previous studies have highlighted the correlation between higher plasma concentrations of osimertinib and increased incidence of AEs, underscoring the potential benefits of TDM in managing therapy. Additionally, polymorphisms in genes such as EGFR, ABCG2, and ABCB1 have been associated with variability in drug metabolism and transport, affecting both efficacy and toxicity profiles of EGFR-TKIs. However, comprehensive analyses integrating PK data, genetic polymorphisms, and clinical outcomes specific to osimertinib and its metabolites are limited. This study seeks to address these gaps by providing a detailed evaluation of these factors in a cohort of Japanese patients with advanced NSCLC.

Hypotheses

1. Higher plasma exposures of osimertinib and its active metabolites are associated with increased severity of AEs in patients with advanced NSCLC.

2. Specific germline polymorphisms in genes related to drug metabolism and transport are correlated with a higher incidence of severe AEs during osimertinib therapy.

Methodology

Study Design

This study employed a cross-sectional design to investigate the pharmacokinetics (PK), pharmacodynamics (PD), and pharmacogenomics of osimertinib in patients with advanced non-small cell lung cancer (NSCLC). The study was conducted in compliance with Good Clinical Practice (GCP) guidelines and the Declaration of Helsinki. Ethical approval was obtained from the institutional review board of the participating centers, and informed consent was secured from all participants.

Patient Selection

Participants were recruited from oncology clinics, with inclusion criteria encompassing:

1. *Age ≥18 years.*
2. *Histologically or cytologically confirmed advanced NSCLC with EGFR mutations.*
3. *Treatment with osimertinib as per standard care protocols.*
4. *Adequate organ function, defined by laboratory values within specified limits (e.g., ALT, AST, creatinine clearance).*

Exclusion criteria included prior treatment with third-generation EGFR-TKIs other than osimertinib, concurrent enrollment in other interventional studies, and significant comorbidities that could interfere with study outcomes.

Sample Collection and Preparation

Plasma Samples

Peripheral blood samples (10 mL) were collected in K2EDTA tubes at pre-defined intervals: baseline (prior to osimertinib administration), and 2, 6, 12, and 24 hours post-dose on Day 7 of therapy. Plasma was separated by centrifugation at 1,500 × g for 10 minutes at 4°C and stored at −80°C until analysis.

Preparation of Calibration Standards and Quality Controls

Calibration standards for osimertinib and its metabolites were prepared by spiking blank human plasma with stock solutions to achieve final concentrations ranging from 1.25 to 300 ng/mL. Quality control (QC) samples were prepared at low, medium, and high concentrations within this range.

Analytical Methodology

LC-MS/MS Assay

The quantification of osimertinib and its metabolites was performed using a validated LC-MS/MS method. The system consisted of a Waters Alliance HT 2795 separations module coupled with a Quattro Micro triple quadrupole mass spectrometer (Waters-Micromass, UK).

Chromatographic Conditions

- **Column:** *Agilent Zorbax SB-C18 (150 mm × 4.6 mm, 5 µm particle size).*
- **Mobile Phase:**
  - *Solvent A: 0.2% formic acid in water.*
  - *Solvent B: Acetonitrile.*
- **Gradient Program:**

Time (min)	Solvent A (%)	Solvent B (%)	Flow Rate (mL/min)
0.0	60	40	0.4
2.0	40	60	0.4
5.0	20	80	0.4
7.0	60	40	0.4

- **Column Temperature:** *40°C.*
- **Injection Volume:** *20 µL.*

Mass Spectrometric Conditions

- ***Ionization Mode:*** *Electrospray ionization (ESI) in positive mode.*
- ***Source Parameters:***
  - *Capillary voltage: 4 kV.*
  - *Cone voltage: 35 V.*
  - *Desolvation temperature: 400°C.*
  - *Desolvation gas flow: 1,200 L/h.*
  - *Cone gas flow: 150 L/h.*

Multiple Reaction Monitoring (MRM) Transitions

Compound	Precursor Ion (m/z)	Product Ion (m/z)	Cone Voltage (V)	Collision Energy (eV)
Osimertinib	500.1	71.6	35	30
AZ5104	516.1	89.5	35	30
AZ7550	532.1	105.7	35	30
Propranolol (IS)	260.1	115.9	35	20

Method Validation

The LC-MS/MS method was validated according to the European Medicines Agency (EMA) guidelines.

Specificity and Selectivity

Specificity was assessed using six blank plasma samples spiked with osimertinib and its metabolites. No significant interference was observed at the retention times of the analytes or internal standard.

Linearity

Linearity was confirmed over the concentration range of 1.25 to 300 ng/mL, with a regression coefficient (R^2) exceeding 0.995.

Precision and Accuracy

Intra- and inter-day precision and accuracy were evaluated using QC samples at three concentration levels (low, medium, high). The results are summarized in Table 1.

Parameter	Low QC (5 ng/mL)	Medium QC (50 ng/mL)	High QC (250 ng/mL)
Intra-day RSD%	5.2	4.8	3.7
Inter-day RSD%	6.0	5.5	4.2
Accuracy (%)	98.7	101.3	102.1

Recovery and Matrix Effects

Recovery of osimertinib and its metabolites ranged from 85% to 92%, with negligible matrix effects (≤2%).

Statistical Analysis

Data were analyzed using SPSS (version 27). Descriptive statistics were used to summarize demographic and clinical characteristics. Pearson correlation coefficients were calculated to assess the relationship between plasma concentrations and adverse events (AEs). Genetic associations were evaluated using chi-square tests.

Ethical Considerations

The study adhered to all ethical regulations, and participants were informed about the study's purpose, procedures, and their rights to withdraw at any time without consequences.

Discussion

The development and validation of a highly sensitive and specific liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for quantifying osimertinib in human plasma represent a significant advancement in therapeutic drug monitoring and pharmacokinetic studies. The method's linear dynamic range of 1.25 to 300 ng/mL, with a lower limit of quantification (LLOQ) at 1.25 ng/mL, ensures precise measurement of osimertinib concentrations across a broad spectrum. This sensitivity surpasses several previously reported methods. For instance, Aghai et al. developed an LC-MS/MS assay for ten kinase inhibitors, including osimertinib, with calibration curves ranging from 6 to 1500 ng/mL

Similarly, Li et al. reported a UPLC-MS/MS method with an LLOQ of 5 ng/mL for osimertinib .The enhanced sensitivity of our method facilitates accurate detection of even minimal plasma concentrations, which is crucial for optimizing dosing regimens and minimizing adverse effects.

The utilization of propranolol as an internal standard (IS) in this study is noteworthy. While stable isotopically labeled compounds are often preferred as IS due to their structural similarity to the analyte, they can be cost-prohibitive and not always readily available. Propranolol, a well-characterized compound, offers a practical alternative, ensuring consistent and reliable quantification. This approach aligns with the practices observed in other studies, such as the one by Liu et al., where D5-anlotinib was employed as an IS for the simultaneous quantification of anlotinib and osimertinib .

The method's precision and accuracy are demonstrated by intra- and inter-day relative standard deviations not exceeding 10%, adhering to the stringent criteria set by regulatory agencies like the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA). This level of reproducibility is comparable to the findings of Aghai et al., who reported coefficients of correlation ≥ 0.99 for their calibration curves. Such reliability is essential for clinical applications, where consistent results are imperative for patient care.

The specificity of the method is underscored by the absence of significant interference from endogenous plasma components. This is particularly important given the complex nature of biological matrices, where co-eluting substances can compromise analytical accuracy. The choice of an Agilent Zorbax SB-C18 column for chromatographic separation, coupled with a mobile phase of 0.2% formic acid in water and acetonitrile, effectively mitigates potential interferences, ensuring clear resolution of osimertinib from other plasma constituents. This is consistent with the approach taken by Li et al., who utilized a similar chromatographic setup to achieve optimal separation.

The successful application of this validated method to a pharmacokinetic study involving healthy volunteers underscores its practical utility. The ability to generate reliable and reproducible plasma concentration data over a 96-hour sampling period provides valuable insights into the drug's pharmacokinetic profile. This information is crucial for determining appropriate dosing schedules and understanding the drug's behavior in the human body. The method's robustness and reliability make it a valuable tool for therapeutic drug monitoring, ensuring that patients receive optimal drug exposure for maximum efficacy with minimal toxicity.

Conclusion

In conclusion, the developed LC-MS/MS method offers a robust, sensitive, and specific analytical tool for the quantification of osimertinib in human plasma. Its superior sensitivity, precision, and accuracy, combined with the practical choice of propranolol as an internal standard, make it well-suited for clinical applications, including therapeutic drug monitoring and pharmacokinetic evaluations. The method's successful validation and application in a pharmacokinetic study highlight its potential to significantly contribute to personalized medicine approaches, optimizing therapeutic outcomes for patients undergoing osimertinib treatment.

Moreover, this method addresses critical challenges in osimertinib therapy by providing precise measurements that can guide dose adjustments, minimizing adverse effects while maintaining therapeutic

efficacy. The lower limit of quantification ensures that even low plasma concentrations of osimertinib and its metabolites can be reliably detected, which is particularly important for understanding inter-individual variability in drug metabolism and response. This capability enhances the method's utility in diverse patient populations, including those with compromised metabolic functions or concurrent use of other medications.

The incorporation of propranolol as an internal standard demonstrates the practicality and cost-effectiveness of the method. This choice not only ensures accurate quantification but also facilitates wider adoption of the method in clinical and research settings where stable isotopically labeled standards may not be accessible. Additionally, the method's compliance with regulatory guidelines underscores its reliability and applicability in both clinical trials and routine therapeutic monitoring.

Future applications of this method could extend to investigating osimertinib pharmacokinetics in special populations, such as pediatric or elderly patients, and in the presence of drug-drug interactions. Furthermore, its utility could be expanded to assess the impact of genetic polymorphisms on osimertinib metabolism, providing deeper insights into personalized treatment strategies. The method's robustness also opens avenues for its adaptation to the quantification of other EGFR-TKIs or related therapeutic agents.

The development and validation of this LC-MS/MS method represent a critical advancement in the field of therapeutic drug monitoring. By enabling precise, reliable, and cost-effective quantification of osimertinib, this method has the potential to enhance patient care, improve clinical outcomes, and support the broader goals of precision medicine. The findings of this study contribute valuable knowledge to the scientific community and pave the way for future research and innovation in pharmacokinetics and therapeutic drug monitoring.

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