Development and validation of an LC-MS/MS method for TDM of Osimertinib and its metabolites AZ7550 and AZ5104 in plasma. Greibe E[1,2], Sorensen BS[1,2], Meldgaard P[2,3], Hoffmann-Lücke E[1,2]. [1]Department of Clinical Biochemistry, Aarhus University Hospital, Denmark; [2]Department of Clinical Medicine, Aarhus University, Denmark; [3]Department of Oncology, Aarhus University Hospital, Denmark

Background: Osimertinib is a tyrosine kinase inhibitor and used as a targeted drug for treatment of lung cancers. However, little is known concerning the pharmacokinetics or the relationship between dose and effect/side effects. To study the pharmacokinetics and allow for TDM, there is a need for tools to measure and monitor the concentrations of Osimertinib and its active metabolites in plasma.

Method: We have developed and validated a rapid LC-MS/MS method for simultaneous quantification of Osimertinib and its active metabolites, AZ7550 and AZ5104. The samples were prepared by protein precipitation and separated on a Kinetex EVO C18 column (2.1 x 150 mm, 2.6 µm). Validation was performed after standard guidelines, and stability at different storage conditions were assessed. As proof of concept, the method was applied on plasma samples from 30 patients receiving standard lung cancer treatment with Osimertinib (40-80 mg/day).

Results: The validated concentration ranges were from 1-3000 ng/ml. Intraassay precisions were ≤ 5%. Linearity, dilution integrity, carryover, and recovery were examined and satisfied the validation criteria. The plasma samples were found to be stable for at least 24 hours at room temperature, at 4°C (fridge), and at 10°C (autosampler), and to sustain three freeze-thaw cycles. Long-term storage at -80°C is currently pending. Lung cancer patients treated with standard doses of Osimertinib were found to have plasma Osimertinib of (median [range]) 293.5 [145.5-830.9] ng/ml (n = 30). The mean plasma concentrations of the metabolites, AZ7550 and AZ5104, were 13.3% and 11.3% of the Osimertinib concentration, respectively.

Conclusions: This new LC-MS/MS method provides a simple, fast and accurate way of simultaneous quantitatively analysis of Osimertinib and its active metabolites, AZ7550 and AZ5104, important for targeted cancer treatment. This paves the road for establishing therapeutic reference intervals and allows for routine pharmacokinetic monitoring and clinical studies on the relationship of drug dose and effect.

Keywords: Osimertinib, Tyrosine kinase inhibitors, LCMSMS, TDM, Lung cancer, Storage stability.