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DEVELOPMENT AND VALIDATION OF A SENSITIVE LC-MS/MS METHOD FOR THE SIMULTANEOUS DETERMINATION OF NIVOLUMAB AND RELATLIMAB IN RAT PLASMA

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ABSTRACT

Objective: This study aimed to develop and validate a sensitive, specific, and rapid **LC-MS/MS** method for the simultaneous quantification of n**ivolumab & relatlimab** in rat plasma using daclizumab as internal standard (IS)

Methods: Chromatographic separation was achieved using a Luna **C18 column (**250mm x 4.6mm, 5µm) with an isocratic mobile phase consisting of a**mmonium formate of pH-2.5 adjusted with formic acid (A) and acetonitrile (B) in 40:60 (v/v)** at a flow rate of **1.0mL/min**. Rat plasma samples were processed by **liquid-liquid extraction** with **acetonitrile**. Mass spectrometric detection was performed in **positive electro spray ionization (ESI) mode** using multiple reaction monitoring (MRM) for n**ivolumab and relatlimab**. The method was validated per **USFDA guidelines** for selectivity, sensitivity, linearity, precision, accuracy, matrix effect, and stability.

Results: The method demonstrated excellent linearity over the concentration ranges of 12-480 ng/mL for nivolumab and 4-160 ng/mL for relatlimab, with a correlation coefficient (R^2) of ≥0.999. The chromatographic separation was achieved with retention time of 3.35 min for nivolumab and 4.08 min for relatlimab. Accuracy, precision, recovery, matrix effect and stability results were found to be within the suitable limits. A MALDI-TOF mass spectrometry was used to study nivolumab, relatlimab at KDa 143.61→32.51, 145.27→21.76, and IS for m/z 144.04→ 35.61 which were ion pairs of mass analysis.

Conclusion: The developed **LC-MS/MS** method is **highly sensitive, selective, and robust** for the simultaneous estimation of n**ivolumab & relatlimab** in rat plasma, making it suitable for preclinical and clinical pharmacokinetic investigations.

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