

Analytical Development and Application of a Targeted LC-MS/MS Pharmacokinetics Assay for Chimeric Aducanumab

Overview

Quantifying therapeutic monoclonal antibodies in preclinical studies is essential for understanding pharmacokinetics (PK) and tissue exposure, yet it remains analytically challenging, particularly in complex matrices such as brain tissue. Aducanumab, an amyloid- β -targeting antibody used in Alzheimer's disease research, presents additional difficulties due to interference from endogenous immunoglobulins and transgene-derived amyloid proteins in mouse models.

To address limitations of ligand-binding assays, the authors developed and applied a targeted liquid chromatography–tandem mass spectrometry (LC-MS/MS) assay using parallel reaction monitoring (PRM) to quantify chimeric aducanumab (chAdu) in mouse plasma and cortex. The study describes assay development, validation, and application to a multi-dose pharmacokinetic study in a transgenic Alzheimer's disease mouse model.

Impact of the Evosep One on the LC-MS/MS PK workflow

The Evosep One LC was used as the chromatographic platform for peptide separation prior to MS detection on a Thermo Fisher Scientific Orbitrap Lumos Tribid MS. The authors employed the Evosep 100 samples-per-day method, corresponding to an approximately 11.5-minute gradient. During method development, Evotips were loaded manually, while pilot pharmacokinetic samples were loaded using an Agilent AssayMap Bravo system, exhibiting excellent sample stability over multi-day storage of the samples on the Evotips.

Within the overall workflow, sample preparation involved protein A enrichment, reduction, alkylation, and tryptic digestion, prior to the targeted LC-MS/MS acquisition of chAdu-specific peptides.

Gains enabled by the Evosep based workflow

The authors demonstrate several performance characteristics associated with the Evosep-based workflow. The 100 samples-per-day method enabled high analytical throughput, supporting analysis of large plasma and cortex sample sets generated during the 28-day pharmacokinetic study. Chromatographic performance was stable across biological matrices, with consistent peptide retention times observed between plasma and cortex samples.

Additionally, peptide stability on Evotips was assessed, demonstrating low intra-day variability for selected peptides when stored at room temperature for up to two days, while noting increased (negligible) oxidation with longer storage times. Compatibility with automated Evotip loading facilitated consistent sample handling during pilot studies.

Interpretation & Conclusions

The authors conclude that the targeted PRM LC-MS/MS assay provides a selective, reproducible, and sensitive method for quantifying chimeric aducanumab in murine plasma and brain tissue with varying levels of beta-amyloid expression. The assay offers a broad linear dynamic range and reliably distinguishes chAdu from endogenous antibodies and control IgG, overcoming limitations encountered with ELISA in matrices containing high concentrations of aggregated A β . The assay enabled dose-dependent and sex-specific differences in plasma pharmacokinetic profiling, detection of cortical antibody exposure, and differentiation between treatment groups. Overall, the study demonstrates the applicability of a targeted MS-based approach, incorporating Evosep chromatography, for preclinical quantification of therapeutic antibodies in complex biological matrices.

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