nibrt
National Institute for Bioprocessing Research and Training

Contract Research

CASE STUDY

Bridging data between CE-LIF and UPLC-FLR *N*-glycan profiling methods

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Challenge

A client requested NIBRT Contract Research to perform a comparability study of *N*-glycan profiling methods, performed using CE-LIF and UPLC-FLR platforms, for characterisation of a glycoprotein therapeutic. The client's standard *N*-glycan profiling method, which utilised a CE-LIF approach, presented limitations in extracted data and had prompted the change to a more sensitive and robust UPLC-FLR method involving use of the fluorescent RapiFluor-MS™ label. This action would require bridging *N*-glycan characterisation data (peak identification/relative peak abundance) between CE-LIF and UPLC-FLR methods.

Solution

The client provided their protocol detailing sample preparation and separation conditions for both the UPLC-FLR and CE-LIF approaches. According to the protocol provided, *N*-glycans were enzymatically cleaved from the glycoprotein and fluorescently labelled with RapiFluor-MS™ for UPLC-FLR analysis, or ATPS for CE-LIF analysis. RapiFluor-MS™ labelled *N*-glycans were separated using HILIC-UPLC with fluorescence detection, while ATPS labelled *N*-glycans were separated on CHO capillary using CE-LIF detection. Interrogation of results involved development of an optimal method for peak assignment and identification, based around NIBRT's characterisation experience and best practices. Exoglycosidase digestions were performed and analysed by both UPLC-FLR and CE-LIF. This enabled determination of sequence and linkage of the *N*-glycans present for abundances as low as 0.1%. LC-MS analysis of released and exoglycosidase digested *N*-glycans was also performed as an orthogonal approach to support structural assignments.

Outcome

A comprehensive report was provided to the client detailing all data, including peak identifications, for UPLC-FLR and CE-LIF analysis. Bridging data for the two analytical approaches allowed for the comparability between methods. These results provided the client with confidence that the *N*-glycan profiling method could be successfully transferred from CE-LIF to the new UPLC-FLR method.

Project Process

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Contact Us

Tel: +353 1 215 8100 Email: contract.research@nibrt.ie
Web: www.nibrt.ie/contract-research /
Foster Avenue, Mount Merrion, Blackrock, Co. Dublin, A94 X099, Ireland