Integrated SegFlow, μSIA and UPLC for online sialic acid quantitation of glycoproteins directly from bioreactors

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Abstract

Monitoring and controlling of sialic acid contents in glycoproteins such as erythropoietin (EPO), interferon-γ, Orencia, Enbrel and others are critical to achieve desired therapeutic benefits. The pharmacokinetics (PK) profile of asialoglycoprotein is known to impact protein clearance with its uptake by hepatic asialoglycoprotein receptors (ASGPR) and subsequent physiological clearance. The ASGPR recognizes and binds to glycoproteins with exposed terminal galactose or N-acetyl galactosamine residues to undergo receptor mediated endocytosis. Recent studies have demonstrated that sialylation of O-linked-glycan plays a role in protecting against macrophage galactose lectin (MGL) mediated clearance. In addition to the impact on serum half-life, sialylation can influence other clinical performances including immunogenicity, potency, and cytotoxicity. Therefore, the level of sialic acid is a critical quality attribute (CQA) and has become a regulatory requirement to monitor and regulate sialylation to ensure desired clinical performance. To achieve consistent levels of sialic acid in certain therapeutics, the harvest decision as well as the ionic strength of downstream process buffer composition is dependent upon the sialic acid content. Therefore, utilization of Process Analytical Technology (PAT) tools for generating real-time or near-real-time sialic acid content is a business-critical requirement. The work presented here demonstrating the utility of an integrated system consisting of a micro-sequential Injection Analyzer (μSIA) interfaced with SegFlow and a UPLC to enable near-real-time online sialic acid measurements. The fully automated architecture exemplifies the execution of online sampling, automatic sample preparation and subsequent online UPLC analysis. Carefully orchestrated such framework is in alignment with the requirements of PAT to support QbD-driven continuous bioprocessing.

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