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An alternate approach for sialic acid estimation in glycoproteins by HPAEC-PAD method

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High-pH anion exchange chromatography method coupled with pulsed amperometric detection (HPAEC-PAD) was developed as an alternative to calorimetric and flourometric based methods for estimation of sialic acid in highly sialylatedglycolproteins such as erythropoietin (EPO). N-acetylneuraminic acid (Neu5Ac) and N-glycolylneuraminic acid (Neu5Gc) are most commonly determined sialic acids in glycoproteins. Using this method, these two sialic acid moieties were successfully determined without derivatization in glycoprotein samples. The samples were treated with neuraminidase enzyme isolated from Arthobacterureafaciensfollowed by addition of internal standard 2-keto -3-deoxy-D-glycero-D-galacto-2 nonulosonicacid (KDN). Analysis was performed on Carbopac PA20 column with disposable gold electrode on PTFE using acetate buffer system in NaOH. USP RS materials for Neu5Ac and Neu5Gc were used as calibration standards. The responses of standards were found to be linear from 50 to 250 pmol for Neu5Ac and 2 to 10 pmol for Neu5Gc with r2 ≥ 0.99 respectively. This method was further evaluated for specificity, linearity, accuracy and precision to determine its suitability for routine analysis of sialic acid. Hence, this method can be used as an alternate method to colorimetric methods for quantifying the Sialic acid content. In this case study EPO is used as a model to evaluate the Sialic acid content.

Biography

Jayachandran R is currently working with USP as a senior manager and responsible for overseeing the development of documentary and reference standards for various biotherapeutic products such as Mabs, Proteins and Peptides. He has around 12 years of experience in the area of analytical method development, validation and physico-chemical characterization. He has explored high-end analytical techniques like Q-TOF, protein sequencing and gained in-depth understanding about the application of such tools in protein characterization. He has experience in setting up of laboratory and quality management system. Academically, he holds a Post Graduate degree in Pharmacy with specialization in biotechnology and worked with reputed biotech organizations like INTAS, NEKTAR and SANOFI.

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