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Comparison of different approaches for quantitative N-, O-linked glycan and monosaccharide composition analysis in biopharmaceutical production

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Abstract

Glycosylation of therapeutic recombinant proteins is of importance due to its potential impact on solubility, bioactivity, pharmacokinetics and immunogenicity of glycoprotein pharmaceuticals. Detailed characterization of glycans present on recombinant glycoprotein remains an important challenge in the development and production of biotherapeutics. Analytical strategies for characterization of N- and O- glycosylation and monosaccharides analysis will be presented. These include comparison of HILIC-FLR, MALDI-TOF MS and CE-LIF for N-glycan analysis, choice of a method for quantitative and non-selective release of O-linked glycans, and selection of a method for monosaccharide composition analysis. With few exceptions (e.g., deoxyribose), monosaccharides have this chemical formula: (CH2O)x, where conventionally x ≥ 3. Monosaccharides are often classified by the amount x of carbon atoms they contain: triose (3), tetrose (4), pentose (5), hexose (6), heptose (7), and so on.

Glucose, used as an energy source and for the synthesis of starch, glycogen and cellulose, may be a hexose. Ribose and deoxyribose (in RNA and DNA respectively) are pentose sugars. Samples of heptoses include the ketoses, mannoheptulose and sedoheptulose. Monosaccharides with eight or more carbons are rarely observed as they're quite unstable. In aqueous solutions monosaccharides exist as rings if they need quite four carbons. Two monosaccharides with equivalent molecular graphs (same chain length and same carbonyl position) should be distinct stereoisomers, whose molecules differ in spatial orientation.

This happens as long as the molecule contains a stereogenic center, specifically an atom that's chiral (connected to four distinct molecular sub-structures). Those four bonds can have any of two configurations in space distinguished by their handedness. During a simple open-chain monosaccharide, every carbon is chiral except the primary and therefore the last atoms of the chain, and (in ketoses) the carbon with the keto group. For instance, the triketose H(CHOH)(C=O)(CHOH)H (glycerone, dihydroxyacetone) has no stereogenic center, and thus exists as one stereoisomer. the opposite triose, the aldose H(C=O)(CHOH)2H (glyceraldehyde), has one chiral carbon — the central one, number 2 — which is bonded to groups −H, −OH, −C(OH)H2, and −(C=O)H. Therefore, it exists as two stereoisomers whose molecules are mirror images of every other (like a left and a right glove). Monosaccharides with four or more carbons may contain multiple chiral carbons, in order that they typically have quite two stereoisomers. The amount of distinct stereoisomers with an equivalent diagram is bounded by 2c, where c is that the total number of chiral carbons. The Fischer projection may be a systematic way of drawing the skeletal formula of an acyclic monosaccharide in order that the handedness of every chiral carbon is well specified. Each stereoisomer of an easy open-chain monosaccharide are often identified by the positions (right or left) within the Fischer diagram of the chiral hydroxyls (the hydroxyls attached to the chiral carbons).

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