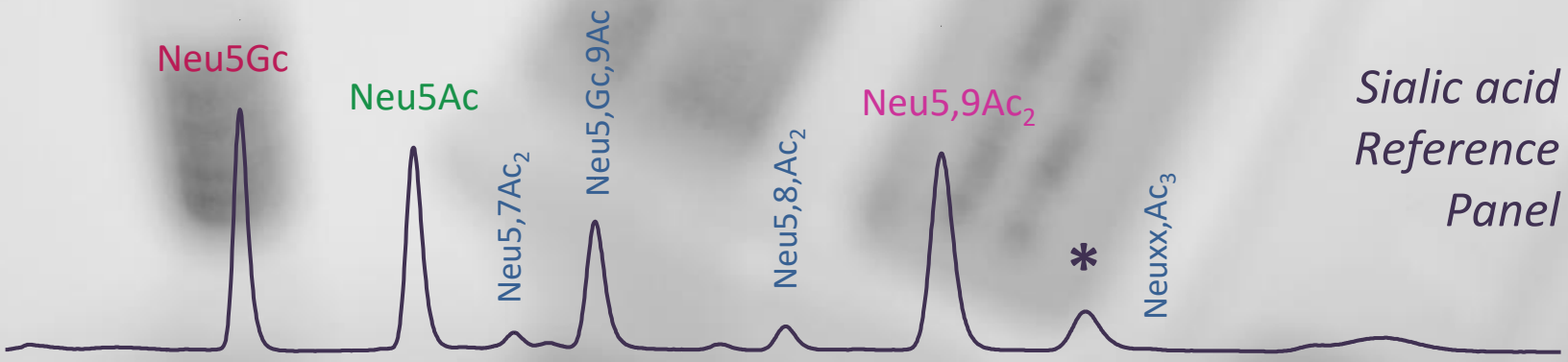
Glycan Analysis Services:

Quantitative Sialic Acid Analysis

Ludger



Sialic acid Reference Panel

Quantitative Sialic Acid Analysis *and why it is important*

Sialic acid analysis is a regulatory requirement laid out in the ICH Q6B guidelines for characterisation of biopharmaceuticals.

Sialic acids are negatively charged monosaccharides found on the non-reducing termini of glycans. They are important for the stability and 3D conformation of glycoproteins and are involved in many biological interactions. Sialic acids often have a pivotal functional impact: for example sialylation of the N-glycans on IgG increases anti-inflammatory activity; *O*-acetylated sialic acids can change ligand interactions and affect degradation (*O*-acetylated sialic acids including Neu5,9Ac_2 are found on EPO); and the presence of sialic acids also increases the serum half life of glycoproteins by preventing uptake by the asialoglycoprotein-receptor located on liver cells.

A diverse range of sialic acids are found in nature, but the two major sialic acids found on N-glycans and O-glycans in biopharmaceuticals are *N*-acetyl-neuraminic acid (Neu5Ac, or NANA) and *N*-glycolyl-neuraminic acid (Neu5Gc, or NAGA). Humans cannot synthesise Neu5Gc and its presence on a drug can lead to immune reactions such as chronic inflammation. Anti-Neu5Gc antibodies have been detected in normal human sera, and can neutralize any Neu5Gc containing biopharmaceutical, thus lowering the drug's efficacy. It is important to be aware that the choice of cell line can greatly influence the type of sialic acids present on a biopharmaceutical, for instance a large proportion of the sialic acids on mouse IgG are often Neu5Gc.

It is therefore imperative, for drug safety and efficacy, to monitor both the level and types of sialic acids during all stages of the product life cycle as well as QC batch to batch consistency.

Ludger's Method – using our LT-KDMB-A1 kit

Sialic acids are released from the glycoprotein by mild acid hydrolysis
(2 hours incubation at 80°C with 2M acetic acid)

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Samples are labelled with DMB
(reacts with the α-keto carboxylic acid which is not present on neutral monosaccharides)

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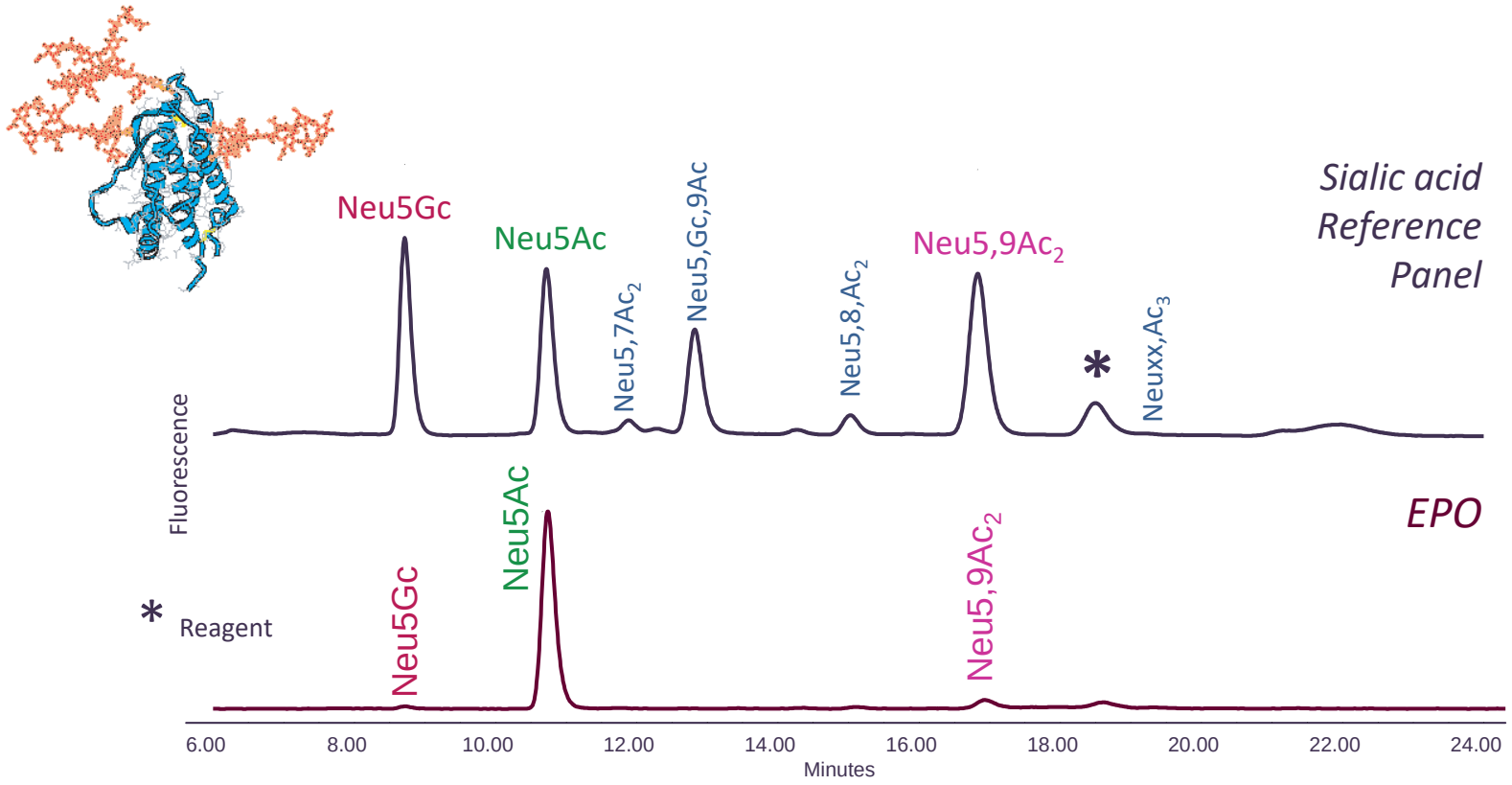
Samples are run on RP-LC

↓

Data analysis.
The absolute amounts of Neu5Ac & Neu5Gc are calculated by reference to standard curves from quantitative Neu5Ac & Neu5Gc standards. The presence of O-acetylated sialic acids is determined by comparison to a Neu5,9Ac_2 standard and to a reference panel containing Neu5Ac, Neu5Gc, Neu5,7Ac_2, Neu5,Gc9Ac, Neu5,9Ac_2 and Neu5,7,(8),9Ac_3Gc. The relative proportions of these different sialic acids are determined from the peak areas.

Quantitative Sialic Acid Analysis: EPO

- Quantitation of sialic acids (Neu5Ac & Neu5Gc) as nmol/mg protein
- Relative quantitation of the *O*-acetylated Neu5,9Ac$_2$



Neu5Gc & Neu5Ac quantified in nmol/mg protein by reference to standard curves

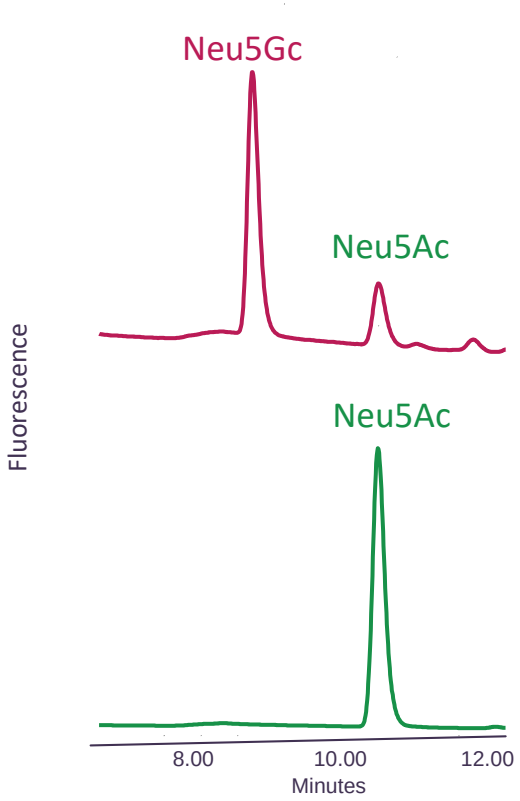
	nmol/mg protein	
Sialic Acid	**Average**	**%CV**
Neu5Gc	3.61	5.11
Neu5Ac	270.32	5.31

Relative proportions of Neu5Gc, Neu5Ac & Neu5,9Ac$_2$ calculated from % peak areas.

	Relative % Area	
Sialic Acid	**Average**	**%CV**
Neu5Gc	0.99	0.24
Neu5,Ac	93.91	0.02
Neu59Ac$_2$	5.09	0.39

Quantitative Sialic Acid Analysis: IgG

- Quantitation of sialic acids (Neu5Ac & Neu5Gc) as nmol/mg protein by reference to standard curves



Mouse IgG

Mouse IgG	nmol/mg protein	
Sialic Acid	Average	%CV
Neu5Gc	1.65	6.06
Neu5Ac	0.53	5.41

Human IgG

Human IgG	nmol/mg protein	
Sialic Acid	Average	%CV
Neu5Gc	0.00	-
Neu5Ac	8.90	2.81