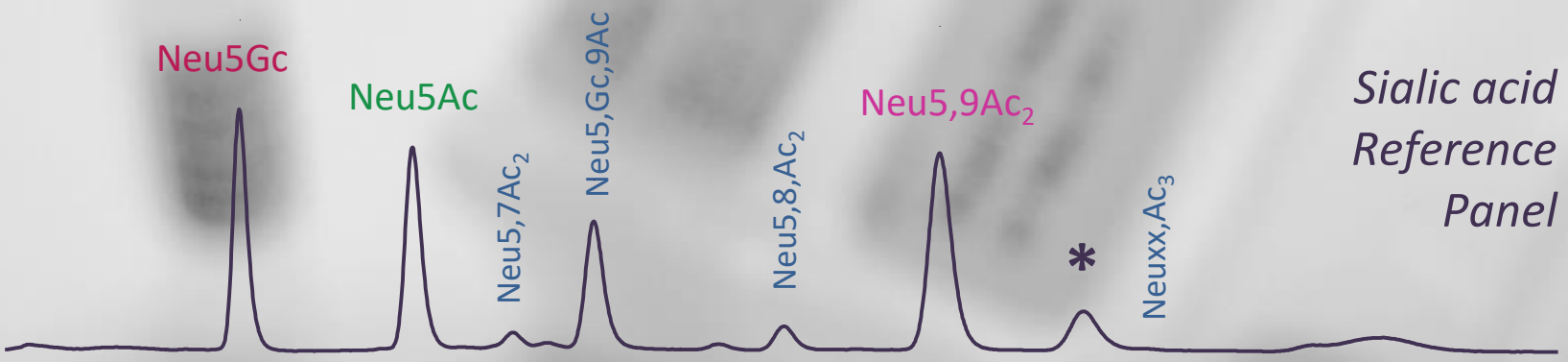
多糖分析服务 / Glycan Analysis Services:

唾液酸定量分析 / Quantitative Sialic Acid Analysis

Ludger



Sialic acid Reference Panel

唾液酸定量分析及其重要性

唾液酸分析是ICH Q6B《生物制药表征指南》中规定的一项监管要求。

唾液酸是一种带负电荷的单糖，存在于多糖的非还原性末端.它们对糖蛋白的稳定性和三维构象具有重要作用，并参与许多生物之间的相互作用。唾液酸通常有一个关键的功能影响:例如，唾液酸的N-多糖能增强IgG的抗炎活性; *O-乙酰化唾液酸可改变配体之间的相互作用，影响降解(EPO上发现有包括Neu5,9Ac2在内的O-乙酰化唾液酸);*唾液酸的存在也通过阻止位于肝细胞上的亚洲糖蛋白受体的摄取，增加了糖蛋白血清的半衰期。

自然界中存在着各种各样的唾液酸，但生物药中存在于N-糖基和O-糖基上的两种主要唾液酸是N-乙酰基神经氨酸(Neu5Ac，或NANA)和N-糖基神经氨酸(Neu5Gc，或NAGA)。人类无法合成Neu5Gc，它在药物上的存在可能导致免疫反应，如慢性炎症。抗Neu5Gc的抗体已在正常人血清中检测到，可中和任何包含生物药物在内的Neu5Gc，从而降低药物的疗效。重要的是要知道，细胞系的选择可以极大地影响生物药中唾液酸的类型，例如，大部分小鼠IgG上的唾液酸通常都是Neu5Gc。

因此，为了药物的安全性和有效性，必须在产品生命周期的所有阶段监测唾液酸的水平和类型，并对批次之间的一致性进行质量控制。

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的方法-使用我们的LT-KDMB-A1试剂盒

通过温和的酸水解从糖蛋白中释放出唾液酸
(在80°C，2M的醋酸中孵育2小时)

用DMB标记样品
(与中性单糖上不存在的a-酮羧酸反应)

在RP-LC上跑样

数据分析.
Neu5Ac、Neu5Gc的绝对含量参照Neu5Ac、Neu5Gc定量标准品的标准曲线计算。通过与Neu5,9Ac2的标准品和包含Neu5Ac, Neu5Gc, Neu5,7Ac2, Neu5,Gc9Ac, Neu5,9Ac2和Neu5,7，(8)，9Ac3Gc的参考面板进行比较，确定O -乙酰化唾液酸的存在。这些不同唾液酸的相对比例是由峰面积决定的。

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)

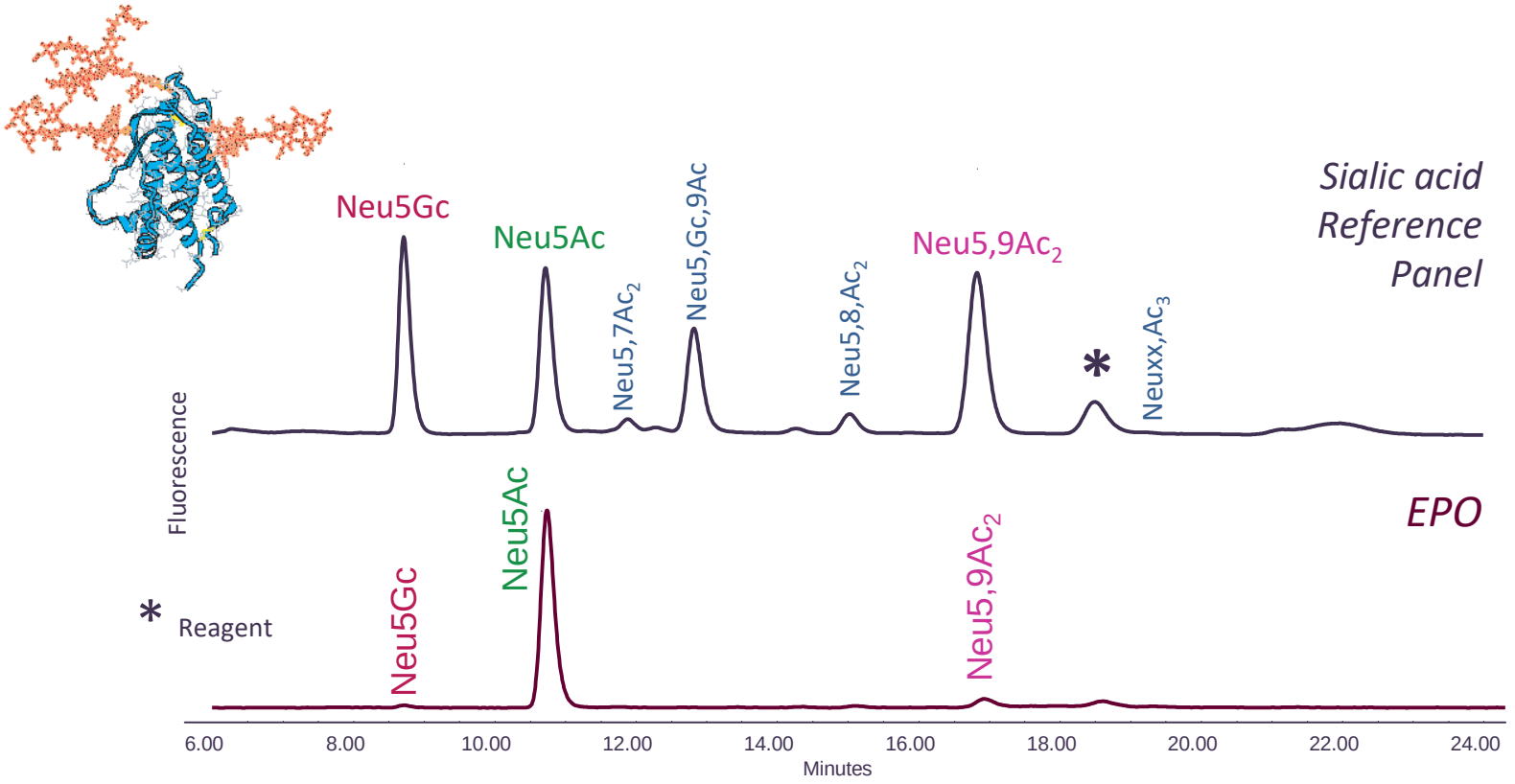
Samples are labelled with DMB
(reacts with the α-keto carboxylic acid which
is not present on neutral monosaccharides)

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.

唾液酸定量分析: EPO / Quantitative Sialic Acid Analysis: EPO

- 作为nmol/mg蛋白定量的唾液酸(Neu5Ac和Neu5Gc) / Quantitation of sialic acids (Neu5Ac & Neu5Gc) as nmol/mg protein
- 相对定量的O-乙酰化的Neu5,9Ac_2 / Relative quantitation of the *O*-acetylated Neu5,9Ac_2



参照标准曲线测定nmol/mg蛋白中Neu5Gc、Neu5Ac的含量 / Neu5Gc & Neu5Ac quantified in nmol/mg protein by reference to standard curves

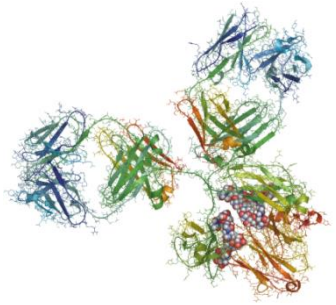
	nmol/mg protein	
Sialic Acid	**Average**	**%CV**
Neu5Gc	3.61	5.11
Neu5Ac	270.32	5.31

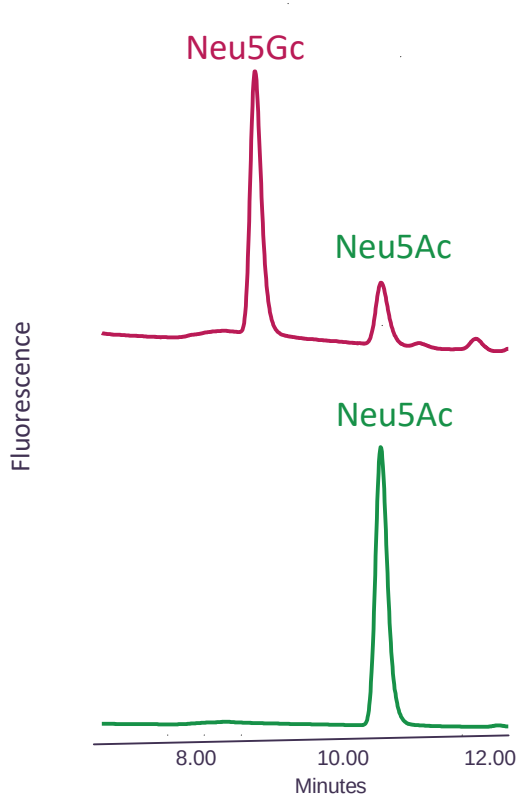
Neu5Gc、Neu5Ac、Neu5,9Ac_2的相对比例按%峰面积计算。/ Relative proportions of Neu5Gc, Neu5Ac & Neu5,9Ac_2 calculated from % peak areas.

	Relative % Area	
Sialic Acid	**Average**	**%CV**
Neu5Gc	0.99	0.24
Neu5,Ac	93.91	0.02
Neu59Ac_2	5.09	0.39

唾液酸定量分析: IgG / Quantitative Sialic Acid Analysis: IgG

- 参照标准曲线测定唾液酸(Neu5Ac和Neu5Gc)作为nmol/mg蛋白的含量 / 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