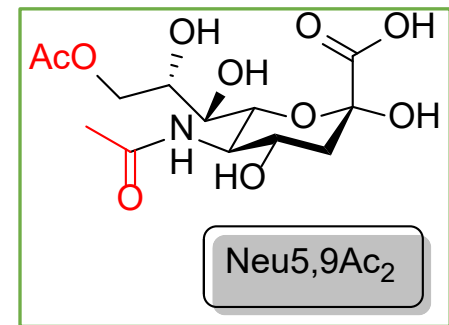
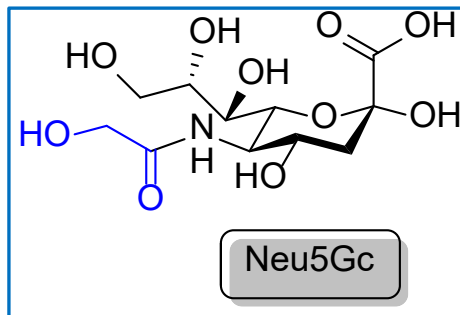
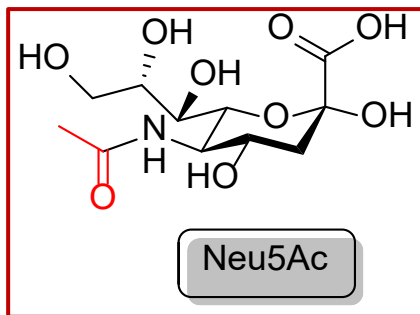


# Quantitative Analysis of Sialic Acids

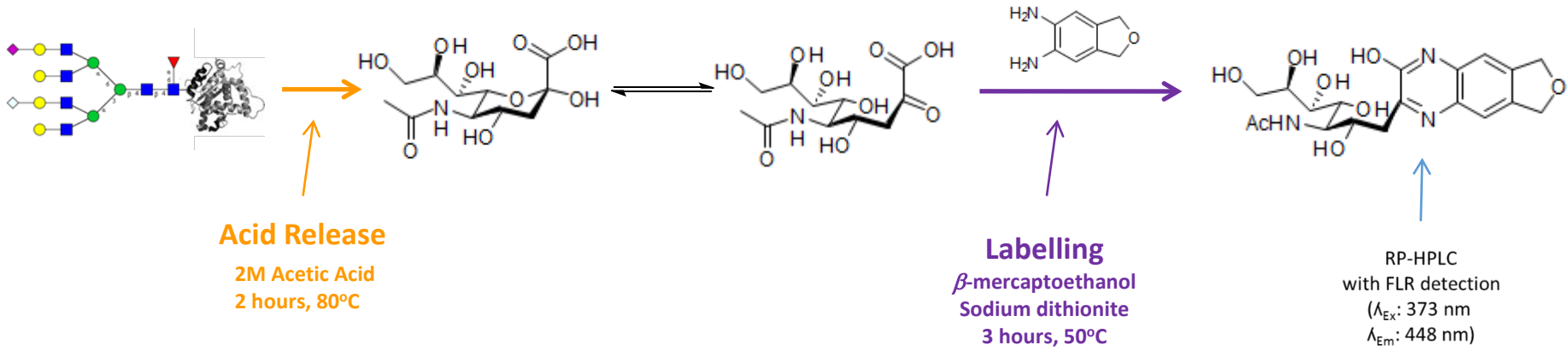


# Sialic Acid Analysis

- Sialic acids are terminal, negatively charged monosaccharides present on many N- and O-glycans
- Biopharmaceuticals often contain two main types of sialic acid; **N-acetyl-neuraminic acid (Neu5Ac)** and **N-glycolyl-neuraminic acid (Neu5Gc)**. **Neu5Ac** is found in both human and non-human cells, whereas **Neu5Gc** is not present on human glycoproteins and is immunogenic
- Sialic acids are important for the stability and 3D conformation of glycoproteins and are involved in many biological interactions. Sialic acids often have a key functional impact by both the abundance and the type of sialylation (including **O-acetylation**)
- Consequently, sialylation is a **glycosylation critical quality attribute** (GCQA). Sialic acid analysis is in the quality guidelines for registration of biopharmaceuticals (ICHQ6B) and should be performed throughout the product lifecycle



# Sialic Acid Analysis – Workflow



**Step 1.** Sialic acids are released from glycoproteins by mild acid hydrolysis (2M acetic acid) using conditions that preserve the N-acetyl, N-glycolyl and O-acetyl groups.

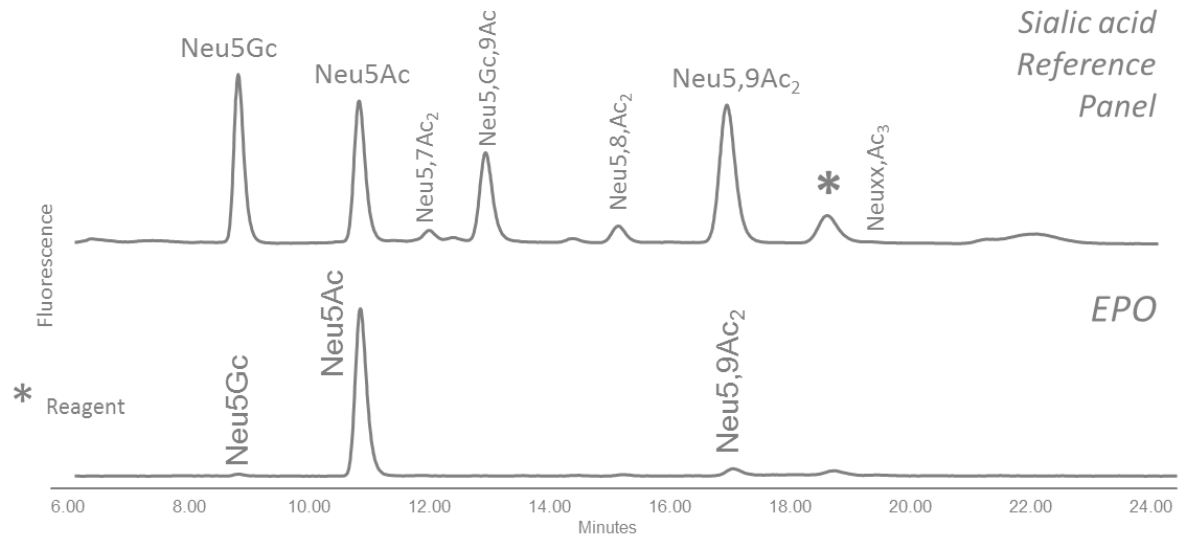
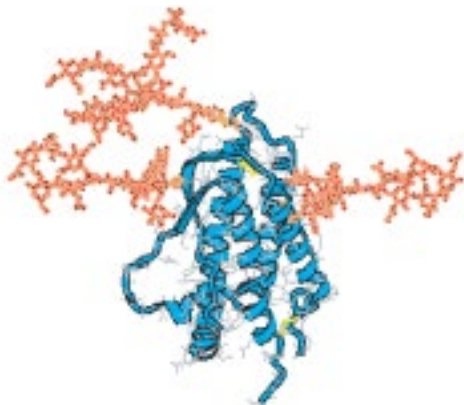
**Step 2.** The  $\alpha$ -keto acid of the free sialic acids are derivatised with 1,2- diamino-4,5-methylenedioxybenzene (DMB).

The DMB-labelled sialic acids are ready to be analysed by RP-HPLC.

# Sialic Acid Analysis – Benefits

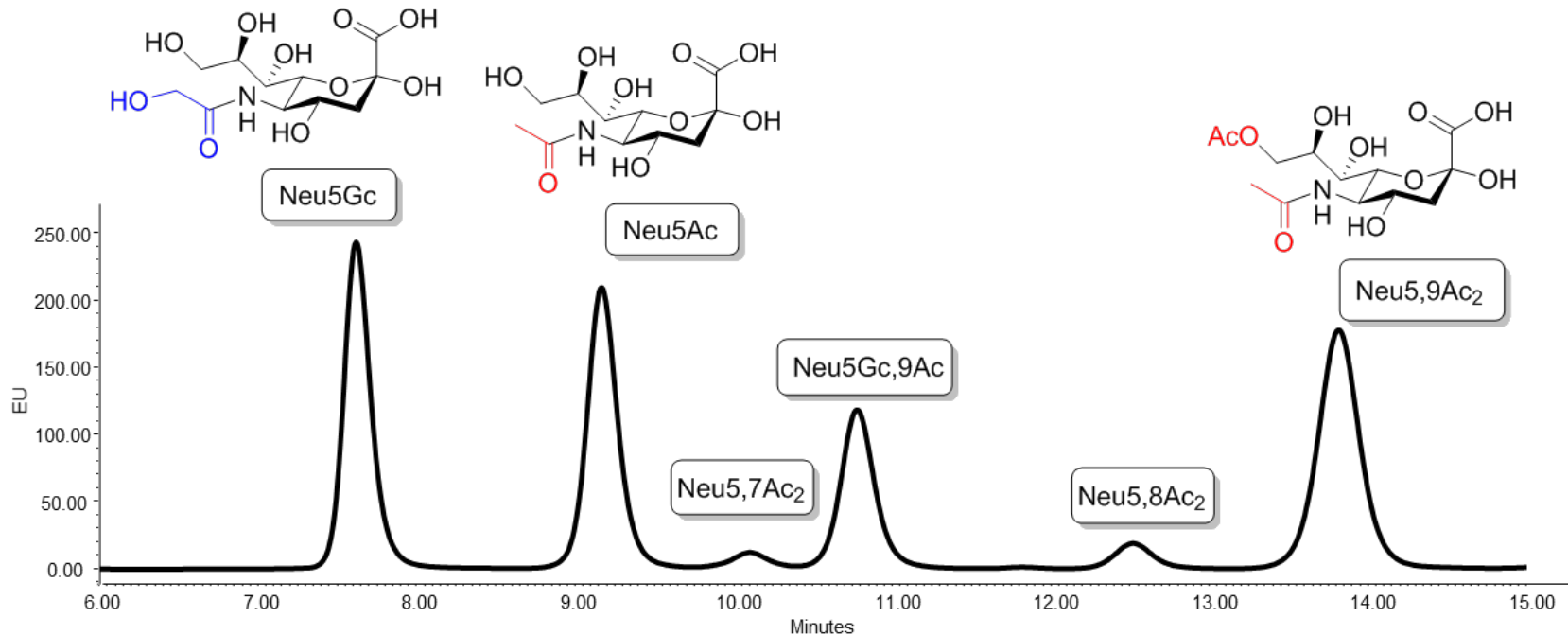
The benefits of using Ludger's sialic acid analysis methodology are:

- Utilizes **multiple standards** to provide a robust analysis method. The standards that are used in-house to ensure good data quality are: Sialic Acid Reference panel (**SRP**), quantitative **Neu5Ac** and **Neu5Gc**, quantitative **GPEP-A2G2S2**.
- Is **validated** to an ICHQ2 standard
- Satisfies the regulatory requirements for biopharmaceutical sialic acid content analysis, providing:
  - 1) **Absolute quantitation** of sialic acid residues per molecule
  - 2) **Relative quantities** of Neu5Ac:Neu5Gc
  - 3) **Relative percentages** and identity of O-acetylated sialic acids.



# Sialic Acid Analysis - Standards

1) **Sialic acid reference panel (SRP)** Contains a mixture of sialic acids found in humans and animals



- ✓ **System suitability Standard:** enable an analyst to test the holistic functionality of an analytical system
- ✓ **Reference Standard:** allow for characterisation by comparison
- ✓ **Process control:** are used to verify that part of or an entire process has worked correctly.

## Acceptance criteria:

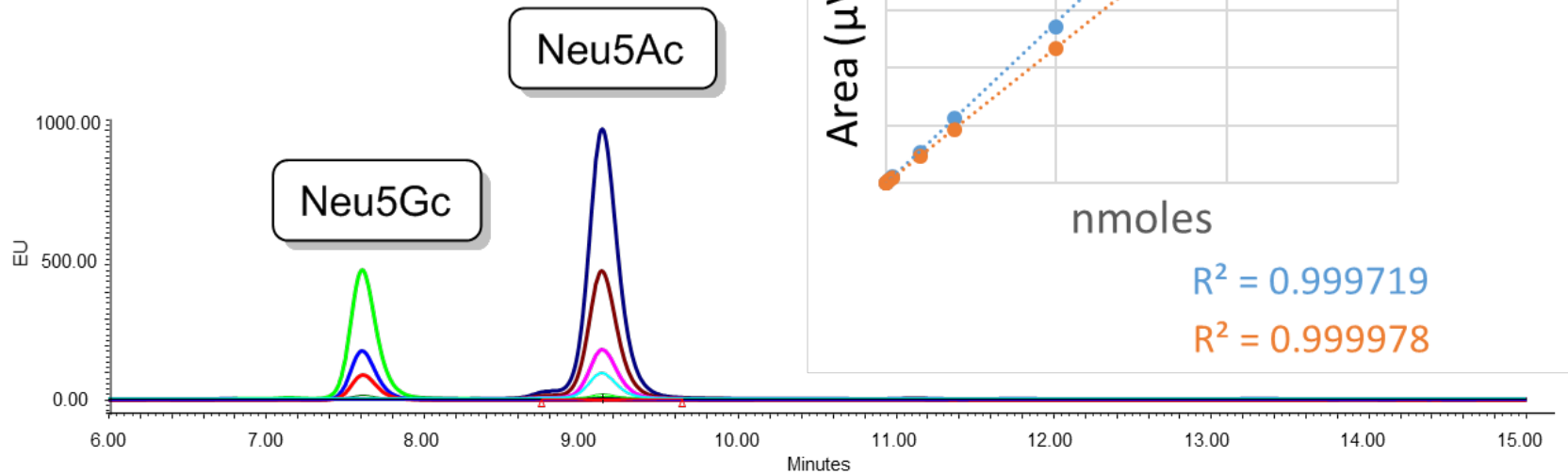
HPLC profiles at the start and end of the sample set should overlap with minimal drift in retention time (e.g.  $\pm 0.1$  min.)

# Sialic Acid Analysis - Standards

## 2) Neu5Gc and Neu5Ac (1 nmol)

Serial dilutions provide calibration curves

- ✓ **Quantitative Standards:** are used to determine the absolute amount of an analyte in a sample.



### Acceptance criteria:

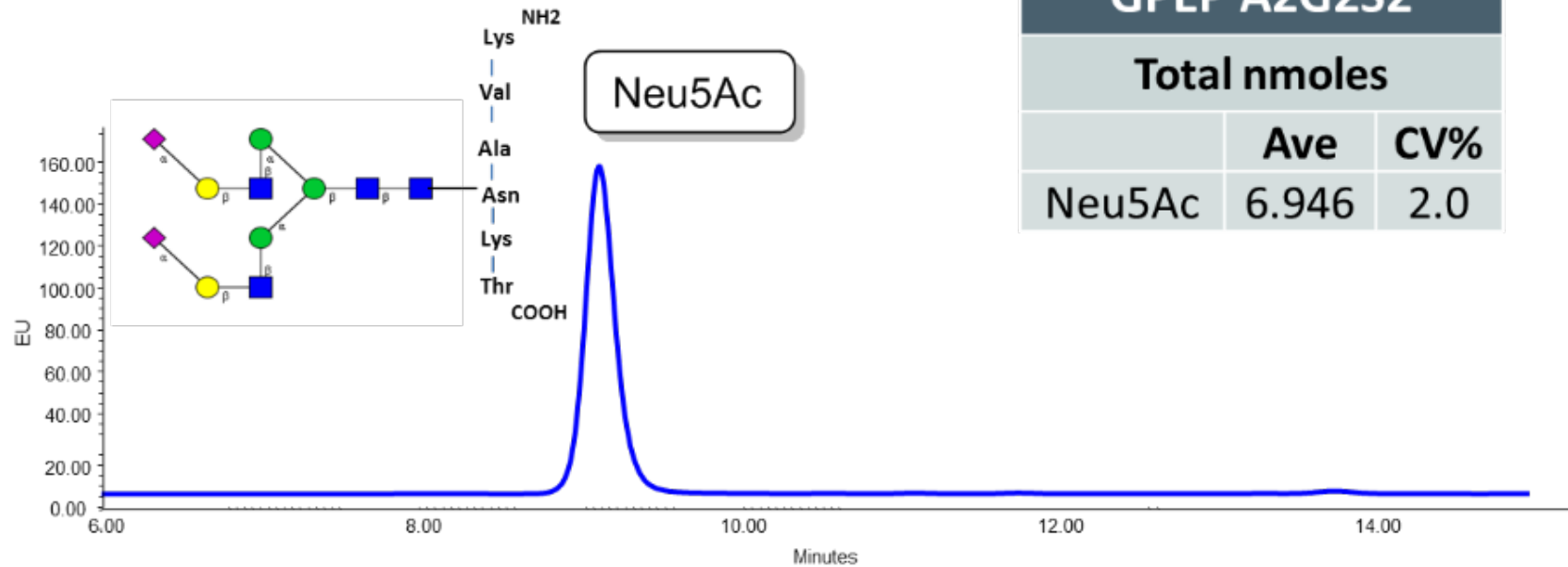
Calibration curves should give R2 values of >0.99

# Sialic Acid Analysis - Standards

## 3) GPEP-A2G2S2 glycopeptide

Biantennary *N*-linked glycan terminating in two sialic acids and has been quantified using qNMR

- ✓ **Quantitative Process Standard:** used to quantify the efficiency of a process



### Acceptance criteria:

Neu5Ac in the range of 5.6 to 8.4 nmol (which is the amount determined by quantitative NMR  $\pm$  20%)

# Sialic Acid Analysis – KITS

## LT-KDMB-A1

Quantity per Kit	Cat #	Component Name
2	LT-ACETIC2M-01	Acetic Acid 2Molar
1	LT-MERCAPTO-01	Mercaptoethanol
1	LT-DITHIO-01	Sodium Dithionite
1	LT-DMB-01	DMB Dye
1	CM-SRP-01	Sialic Acid Reference Panel
1	CM-NEUAC-01	N-Acetyl Neuraminic acid
1	CM-NEUGC-01	N-Glycolyl Neuraminic acid

The kit contains reagents and materials for up to 22 samples

1 set of labelling reagents per kit

## LT-KDMB-96 **NEW PRODUCT**

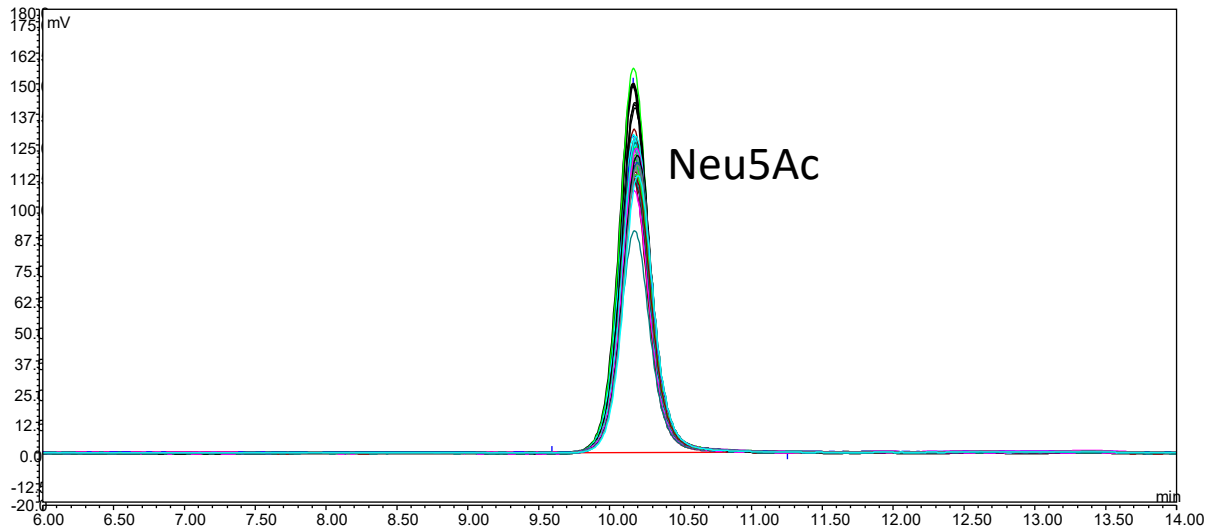
Quantity per Kit	Cat #	Component Name
1	LT-ACETIC2M-02	Acetic Acid 2Molar
1	LT-MERCAPTO-02	Mercaptoethanol
1	LT-DITHIO-02	Sodium Dithionite
1	LT-DMB-02	DMB Dye
1	CM-SRP-01	Sialic Acid Reference Panel
1	CM-NEUAC-01	N-Acetyl Neuraminic acid
1	CM-NEUGC-01	N-Glycolyl Neuraminic acid
1	PM-GLASSVIAL5MLCLEAN	5mL Glass vial pyrolyzed

The kit contains reagents and materials for up to 96 samples (+ soke dead volume if you want to automate the process)

1 set of labelling reagents per kit.



# Sialic Acid Analysis – LT-KDMB-96



Neu5Ac quantified in nmol/ $\mu$ l plasma by reference to standard curve. 48 samples of 3 $\mu$ l plasma analyses

	nmol/ $\mu$ l plasma	
Sialic Acid	Average	%CV
Neu5Ac	1.87	8.97

48 replicates of plasma analysed with **LT-KDMB-96**

New kit size ideal for larger number of samples

Automatable for High Throughput analysis<sup>1</sup>

Can be used for cohorts of biological samples such serum or plasma<sup>1</sup>

<sup>1</sup> [Neu5Ac and Neu5,9Ac2 in Human Plasma: Potential Biomarkers of Cardiovascular Disease \(ludger.com\)](https://www.ludger.com)