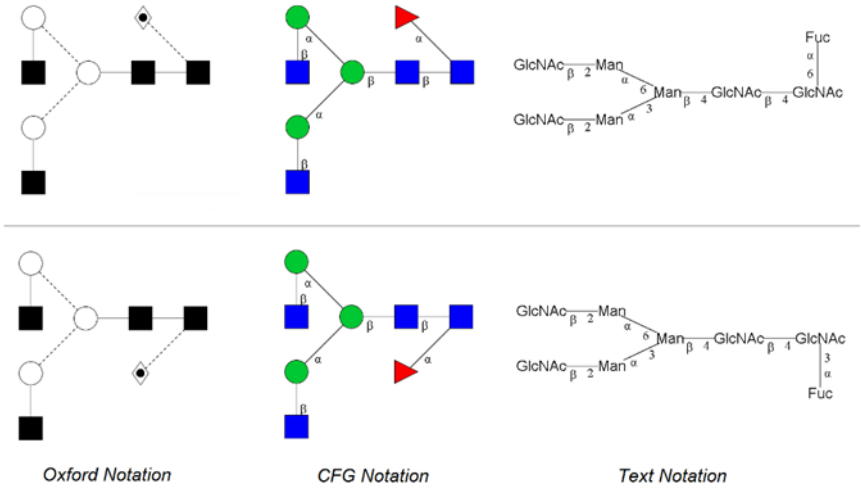
Certificate of Analysis

GPEP FA2 Glycopeptide Standard

Cat. #: GPEP-FA2-01 Batch: B7BM-04

Average Amount: 6.6 µg Expiry Date: 25 July 2028

Glycan Structures



The mixture is composed of two glycopeptides which are comprised of either an F(6)A2 glycan or a F(3)A2 glycan attached to the asparagine amino acid of a peptide with the sequence Lysine-Valine-Alanine-Asparagine-Lysine-Threonine (KVANKT).

Glycan Purity determined as approximately 90% by LC-MS

Monoisotopic mass: 2104.9411 $[M+H]^+$

Storage conditions: -20°C

Monosaccharide analysis of BQ-GPEP-A2G2S2-10U

Quantitative monosaccharide analysis using the Ludger LT-MONO-96 kit was performed on 5 replicates of GPEP-FA2-01 using 2M trifluoroacetic acid or 6M hydrochloric acid hydrolysis (HCl) to release the monosaccharide constituents of the glycopeptide. The data from HCl hydrolysis gives the most accurate results for quantitation of the core GlcNAc and GalNAc as they require stronger acid (HCl) to release them from the protein. On the other hand, the other monosaccharides (Gal, Man, Glc and Fuc) degrade under harsh acid conditions, and in this case the data from the TFA hydrolysis provides the most accurate quantitation. Following hydrolysis the monosaccharides were labelled with 2-aminobenzoic acid and chromatography was performed on a HPLC equipped with a LudgerSep R2 monosaccharide analysis column (LS-R2-4.6×150). The core GlcNAc requires stronger acid (HCl) to release it from the protein, hence the TFA data is always an

underestimate of the amount of GlcN. However, the other monosaccharides degrade under harsh acid conditions, so the TFA data is the most accurate.

Hydrochloric Acid Hydrolysis

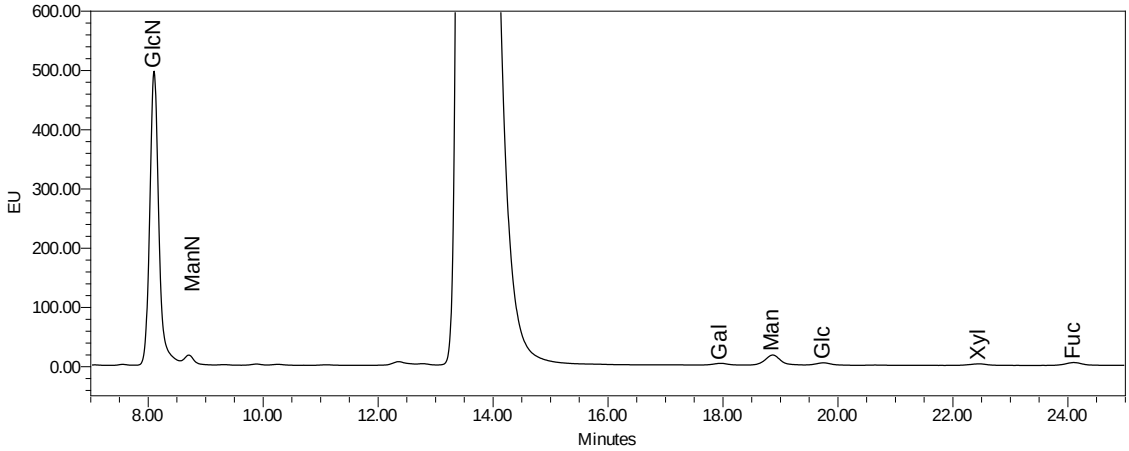


Figure 1. LudgerSep-R2 HPLC profile of 2-aminobenzoic acid (2-AA) labeled monosaccharides of HCl hydrolysed GPEP-FA2-01 (Batch B7BM-04). The ManN monosaccharide is due to epimerisation of the GlcN monosaccharide during sample processing.

Approximate amount of GPEP-FA2 using the GlcN value (HCl release):

Quantity of GlcN per vial = 12.84 ± 0.88 nmol

Quantity of GPEP-FA2-01 per vial (determined by GlcN content) = 6.76 ± 0.46µg (3.21 nmol)

Trifluoroacetic Acid Hydrolysis

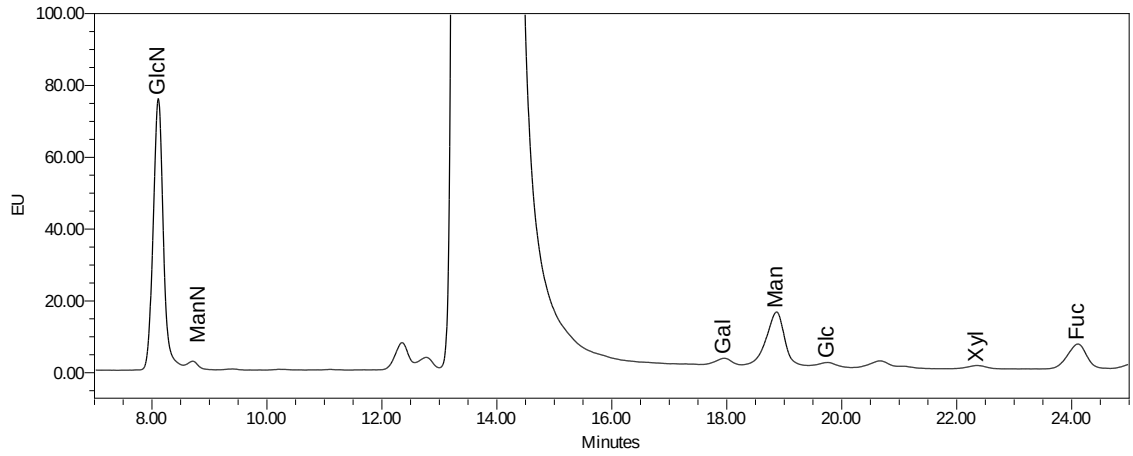


Figure 2. LudgerSep-R2 HPLC profile of 2-aminobenzoic acid (2-AA) labeled monosaccharides of TFA hydrolysed GPEP-FA2-01 (Batch B7BM-04). The ManN monosaccharide is due to epimerisation of the GlcN monosaccharide during sample processing.

Approximate amount of GPEP-FA2 using the Fuc value (TFA Value):

Quantity of Fuc per vial = 3.08 ± 0.10 nmol

Quantity of GPEP-FA2-01 per vial (determined by Fuc content) = 6.48 ± 0.21 µg (3.08 nmol)

Glycopeptide Purity and Confirmation of Identity of GPEP-FA2-01

LC-MS: Purity and Mass Information

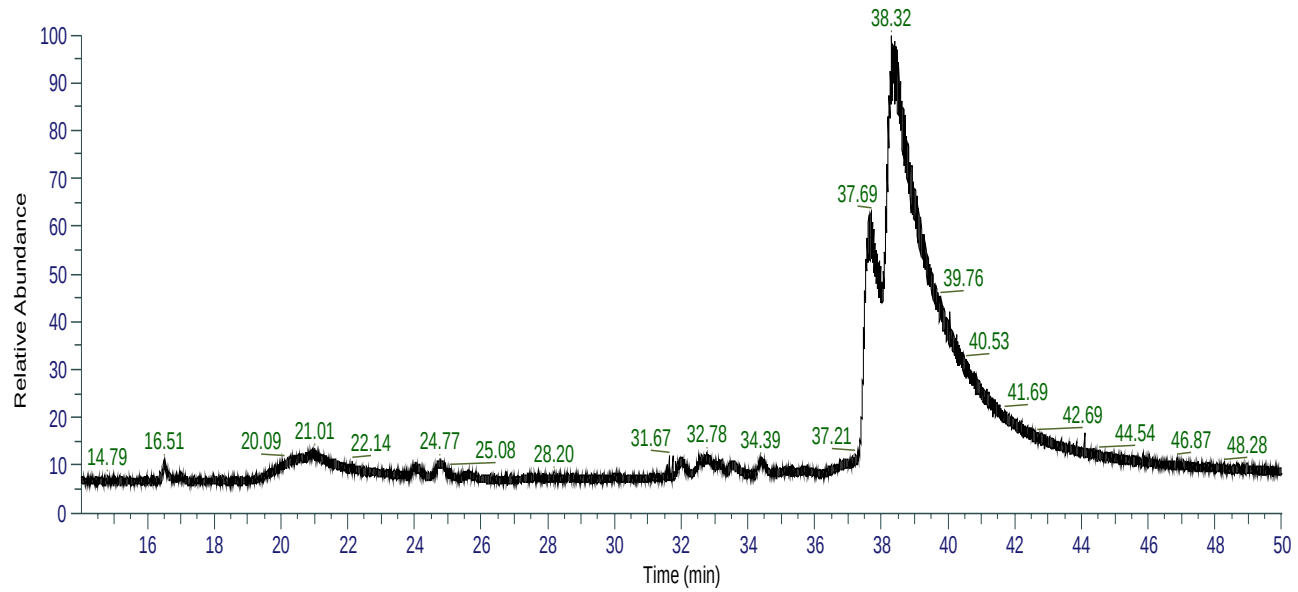


Figure 3: Base Peak Chromatogram – Positive ESI mass spectrum of GPEP-FA2-01 (Batch B7BM-04).

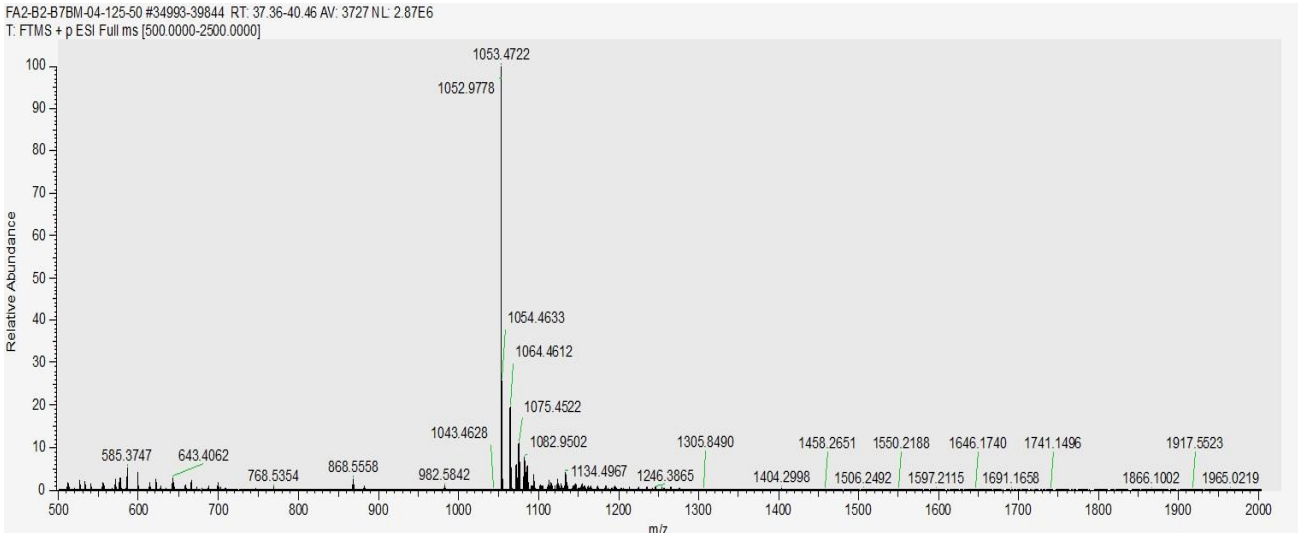


Figure 4. Positive ion ESI mass spectrum of GPEP-FA2-01 (Batch B7BM-04). theoretical mass: 2103.933 Da.

Enzyme digestion: Glycosidic Linkage Information

The gold standard method for release of N-linked glycans is peptide-N-glycosidase F (PNGase F), an endoglycosidase that cleaves the glycosidic bond between the Asn residue of the protein and the GlcNAc of the glycan. While PNGaseF is suitable for most therapeutic glycoprotiens, it cannot cleave N-glycans with a core α1-3 fucosylation. If core α1-3 fucosylation is present, which is commonly the case in plant and insect cells, peptide-N-glycosidase A (PNGase A) can be used to cleave the glycosidic bond between the Asn residue of the protein and the GlcNAc of both N-linked glycans with and without α(1,3)-linked core fucose residues. The released glycans were labelled by reduction amination with fluorescent label procainamide.

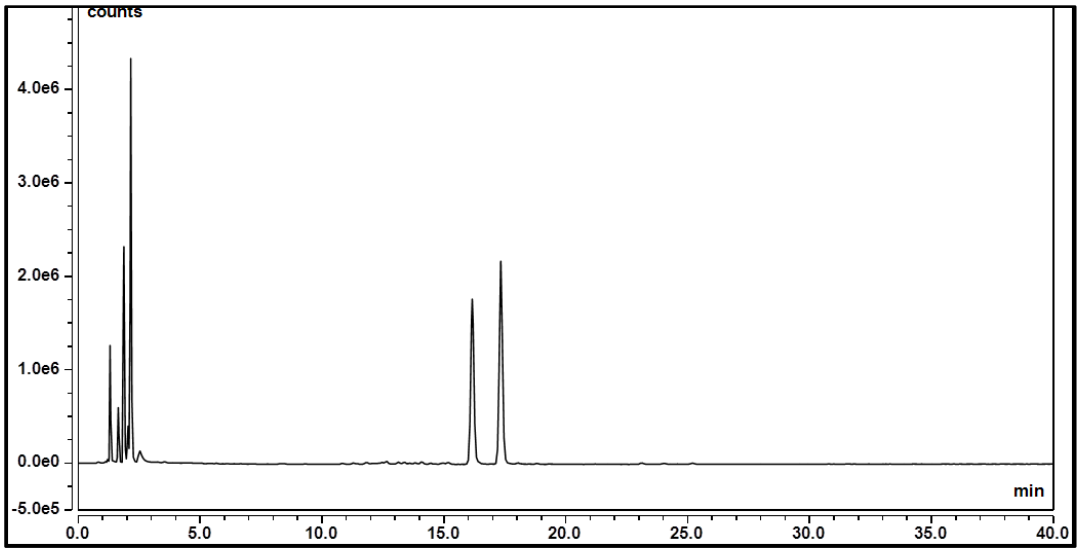


Figure 5: GPEP-FA2-01 B7BM-04 chromatogram following PNGaseA release. MS confirms peaks at 16 and 17 mins are FA2-Proc. PNGaseA releases all glycans, both 1,3 and 1,6 linkages are released. Ratio of 4.5:5.5 (1,6 and 1,3 GPEP-FA2-01 respectively)

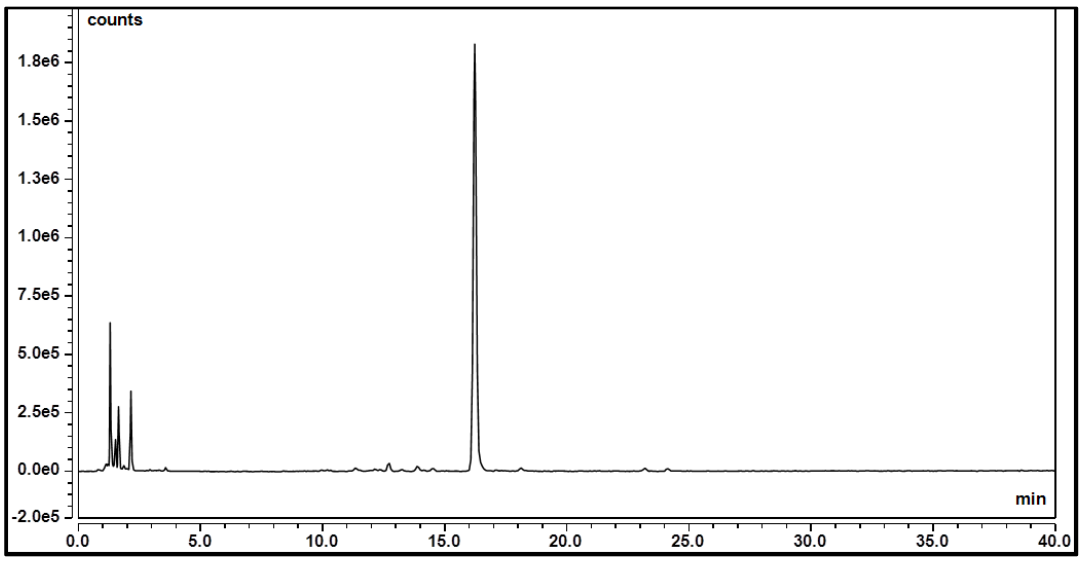


*Figure 6: GPEP-FA2-01 B7BM-04 chromatogram following PNGaseF release. MS confirms peak at 16 mins is FA2-Proc. PNGaseF is known to release glycans with alpha 1,6 linked core fucose residues but not 1,3 linked core fucose residues. (Reference: J.Q. Fan, Y.C. Lee. J Biol Chem, **1997**,272,* 27058*).*