Characterization of sialylated N-glycopeptide isomers on RP LC-EAD mass spectrometry

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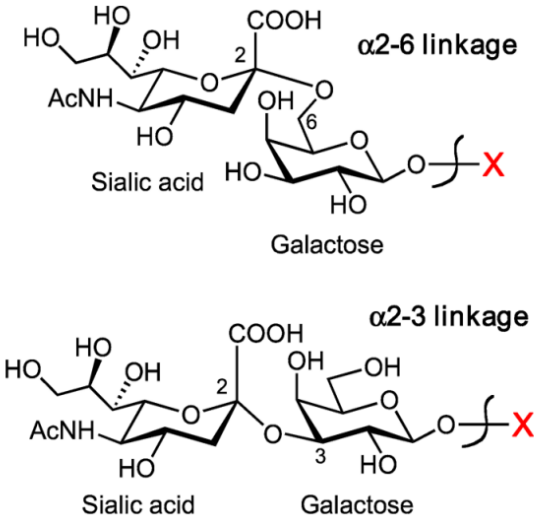
Biological significance of sialic acid

- Sialic acid has important biological functions
  - Cell–cell interaction
  - Immune reactions
  - Development processes
  - Virus host cell interaction
- Sialic acid linkage is also very important



Cited from Zhu, X.Y. et al, dx.doi.org/10.1021/cb5004114 | ACS Chem. Biol. 2014, 9, 1877−1884

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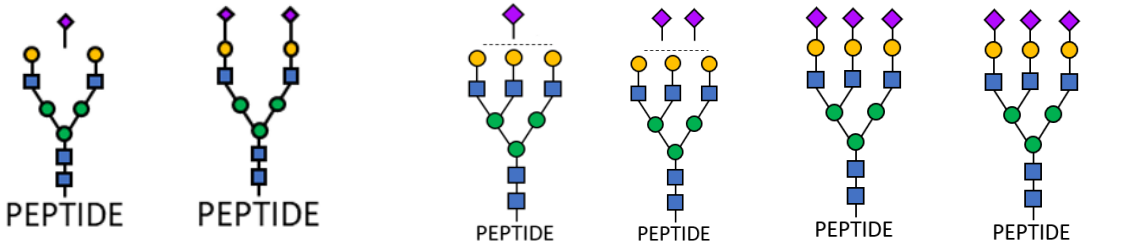
LC separation of sialylated N-glycopeptide isomers

MODEL GLYCOPEPTIDE FROM FETUIN DIGEST

- Current LC methods
  - HILIC, PGC, RP HPLC with high column temparature

- Sample: fetuin digest, LCPDCPLLAPLNDSR



– [A2Sa1] [A2Sa2] [A3Sa1] [A3Sa2] [A3Sa3] [A3Sa4]

– Sialic acid Gal GLcNac Man

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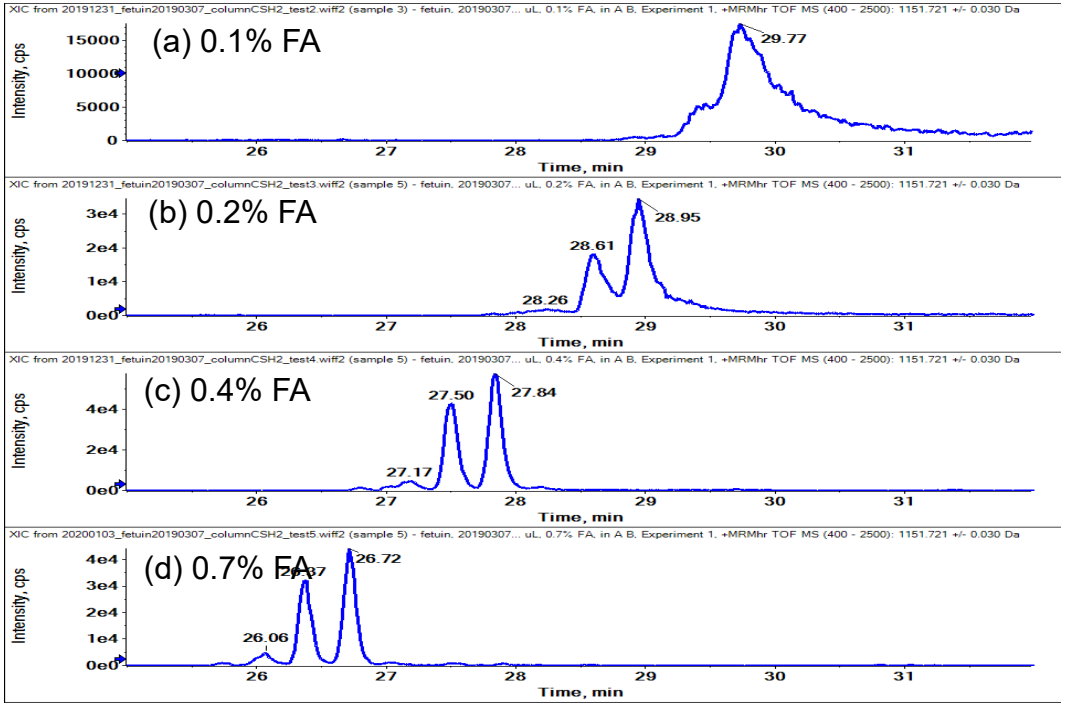
Optimization to achieve superior separation of sialylated N-glycopeptide isomers

LCPDCPLLAPLN[A3SA3]DSR, M/Z = 1151.215, +4



Shimadzu Prominence LC

ACQUITY UPLC

CSH C18 column (130 Å, 1.7 µm, 2.1 mm × 100 mm, 148 Waters)

A: 0.1 % FA water
B: 0.1% FA acetonitrile
Flow rate 0.15 mL/min

Zeno TOF 7600 system

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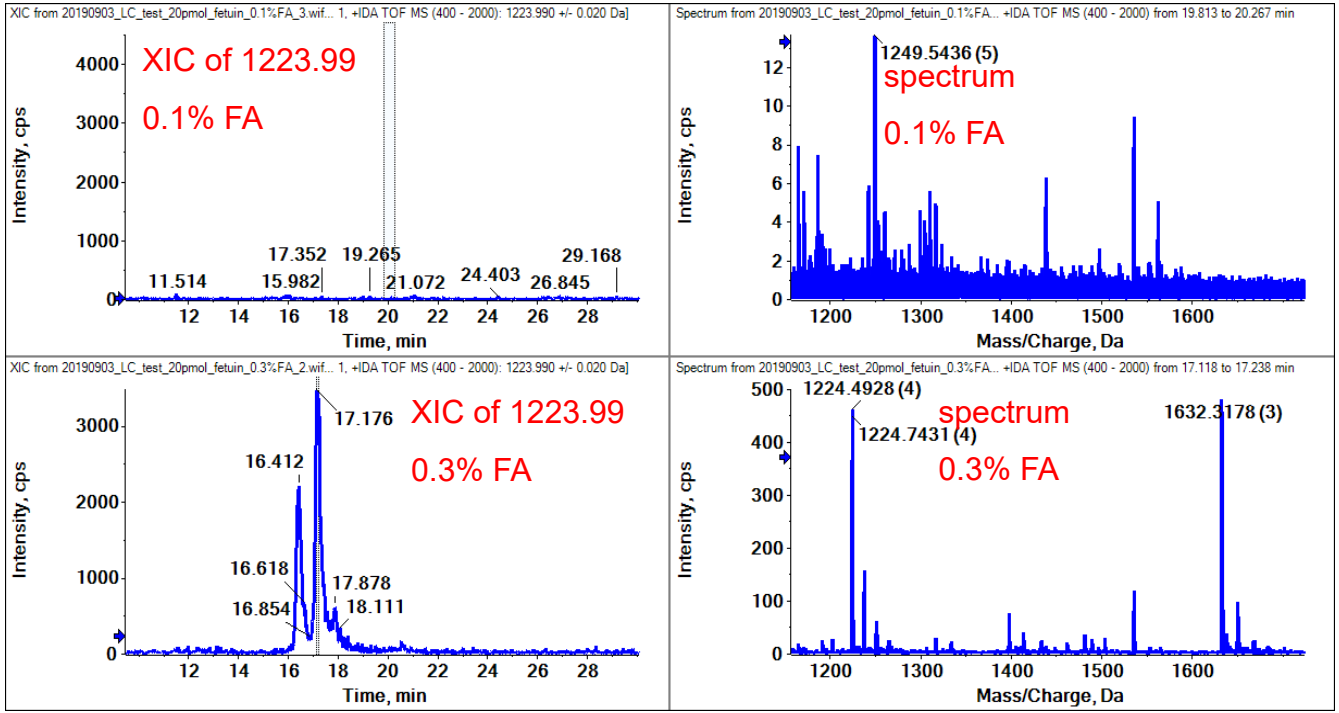
Optimization to achieve superior separation of sialylated N-glycopeptide isomers

LCPDCPLLAPLN[A3SA4]DSR, M/Z = 1223.99, +4



The glycopeptide was only detected when >0.3% FA in the mobile phase

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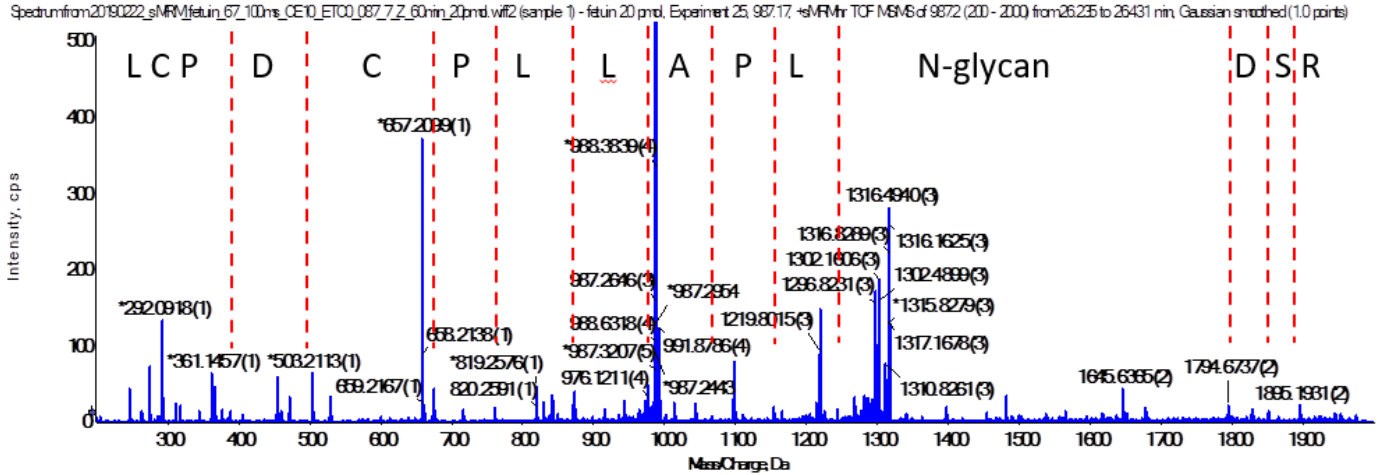
EAD of glycopeptide characterization

LCPDCPLLAPLN[A3SA2]DSR, M/Z = 987.17, +4

4585.8	4472.7	4312.7	4215.7	4100.6	3940.6	3843.5	3730.5	3617.4	3546.3	3449.3	3336.2	361.2	246.1	159.1
Z15	Z14	Z13	Z12	Z11	Z10	Z9	Z8	Z7	Z6	Z5	Z4	Z3	Z2	Z1
L	C	P	D	C	P	L	L	A	P	L	N-glycan	D	S	R
C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15
131.1	291.1	388.2	503.2	663.3	760.3	873.4	986.5	1057.5	1154.6	1267.7	3586.5	3701.5	3788.5	3944.5

LC-EAD on Zeno TOF 7600
KE 7,



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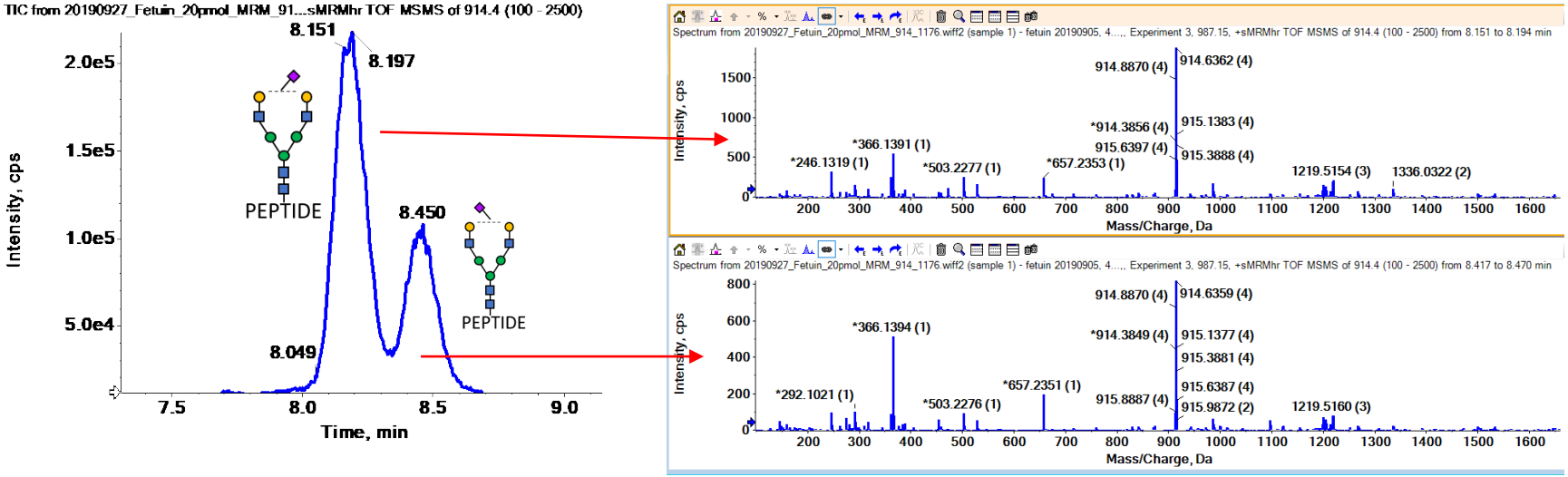
Identification of sialic acid linkage isomers

LCPDCPLLAPLN[A3SA]DSR, M/Z = 914.38, +4



The two isomers actually generated the same m/z fragment ions

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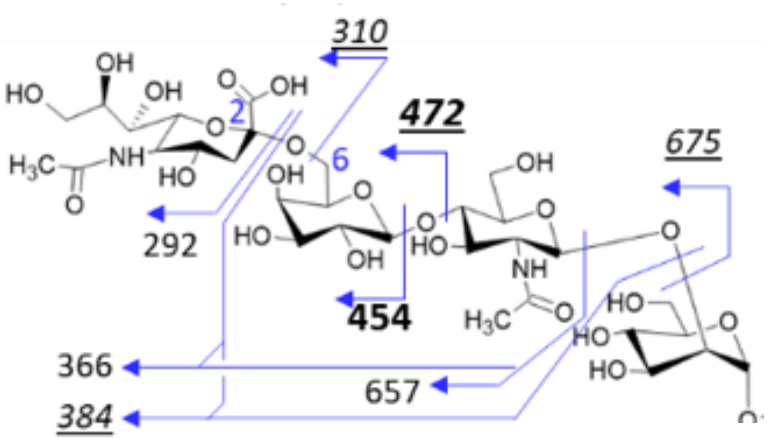
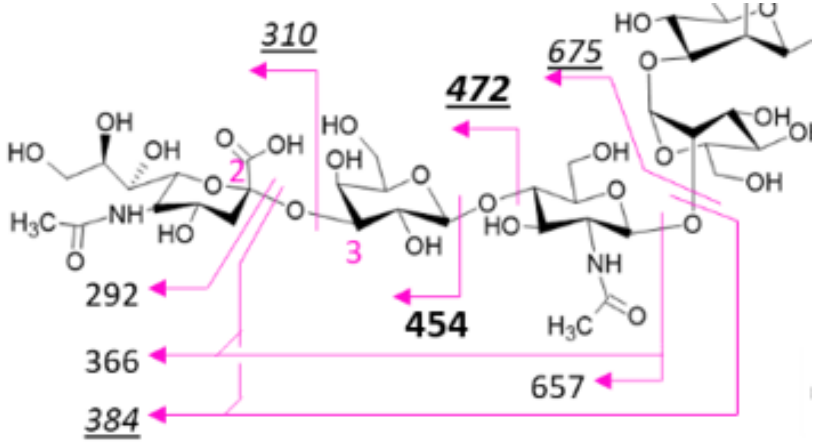
Identification of sialic acid linkage isomers

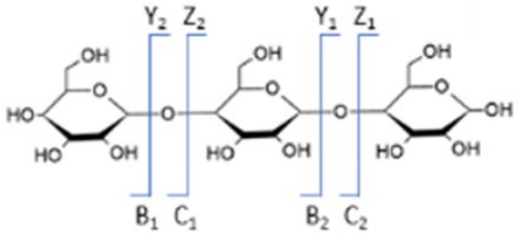
FRAGMENTATION OF SIALIC ACID LINKED GLYCANS



Neu5Ac - α(2-6) - Gal



Neu5Ac - α(2-3) - Gal



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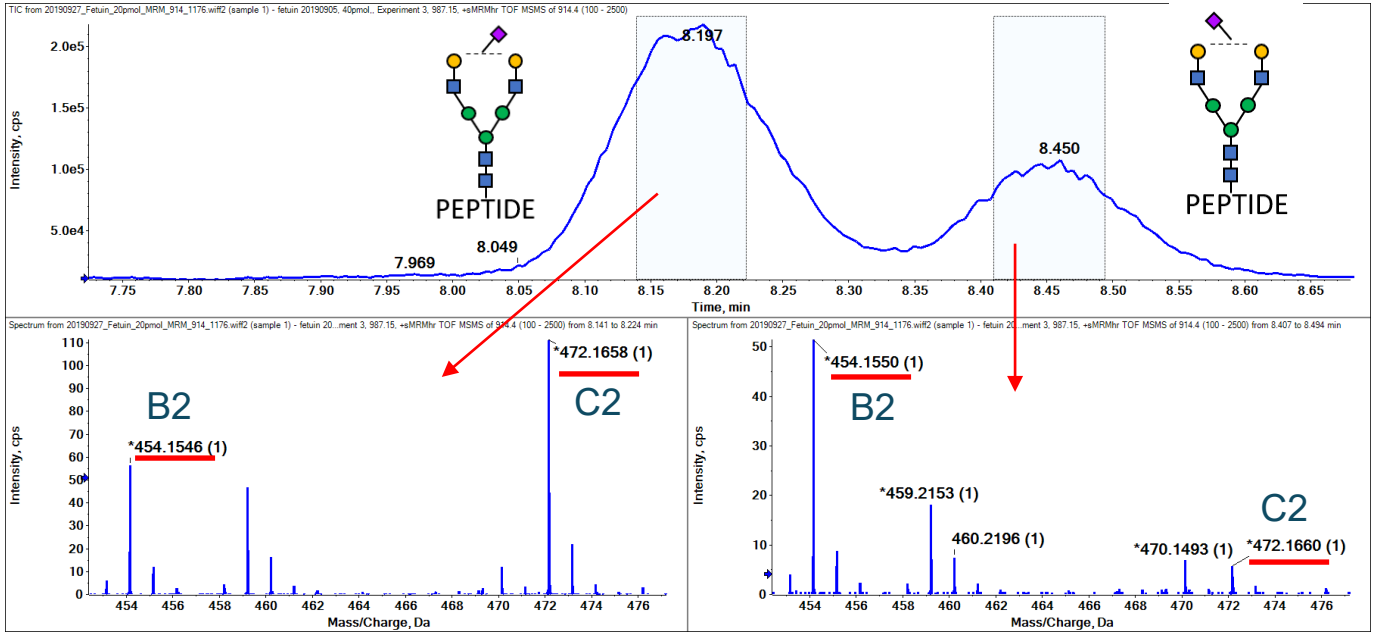
Identification of sialic acid linkage isomers

LCPDCPLLAPLN[A2SA]DSR M/Z = 914.38, +4



However, the fragment ion ratios showed difference between isomers

472/454 = 2 for α2-6
472/454 = 0.2 for α2-3

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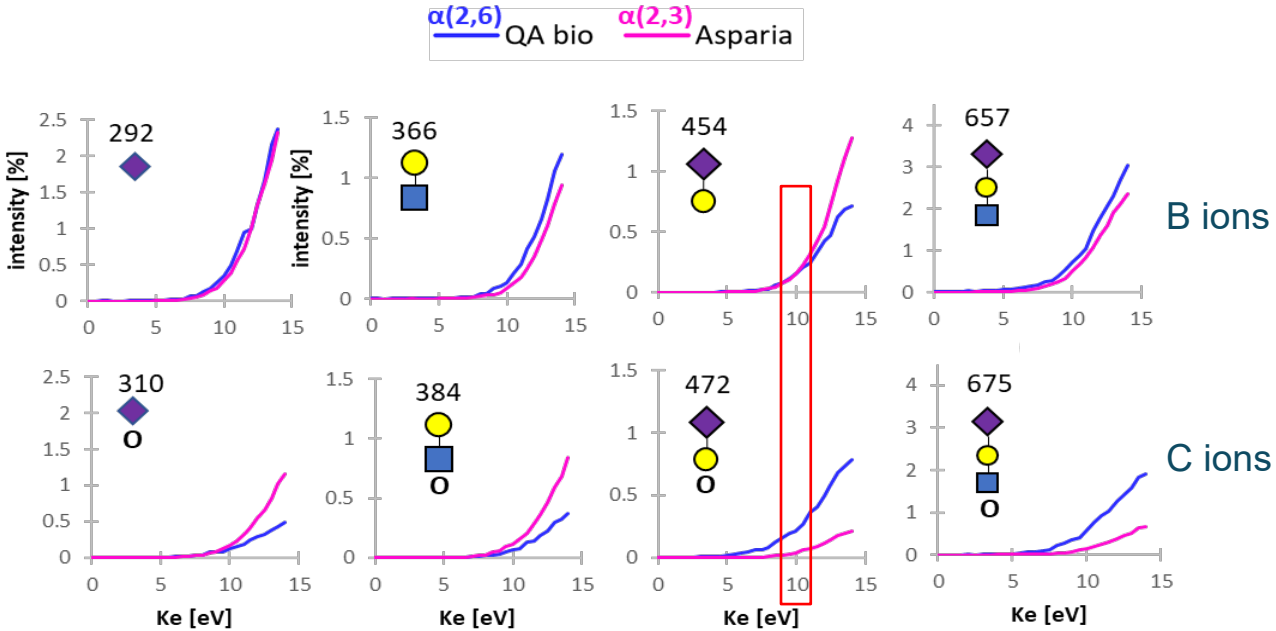
Identification of sialic acid linkage isomers

B AND C ION INTENSITY VS. KE



KVAN[A2Sa2]KT

QA Bio α2-6
Asparia α2-3

C and B ion intensity normalized to charge reduced precursor

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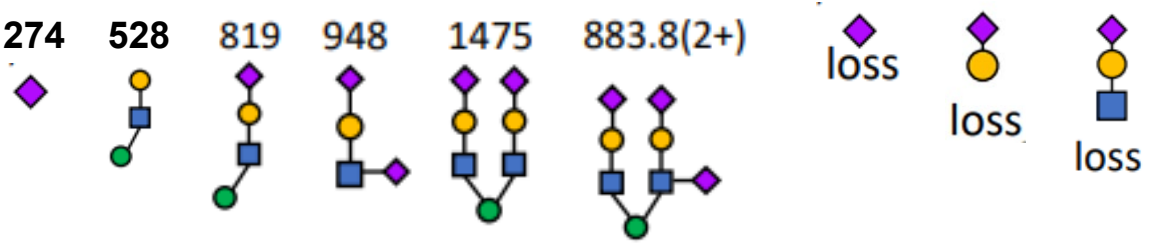
Identification of sialic acid linkage isomers

OPTIMIZATION OF ECD KE FOR SIALIC ACID LINKAGE ISOMERS

- Diagnostic parameter (DP) for linkage differentiation
  - $DP = \frac{\text{intensity of B2 ion at 454}}{\text{intensity of C2 ion at 472}}$
- Ke 10 V is optimal for the linkage differentiation of the glycopeptides studied
- Other glycan ions are helpful to assign the Sa linkage and position
  - Glycan specific fragment ions, precursor - sugar ion
  - **274** **528** 819 948 1475 883.8(2+) loss loss loss



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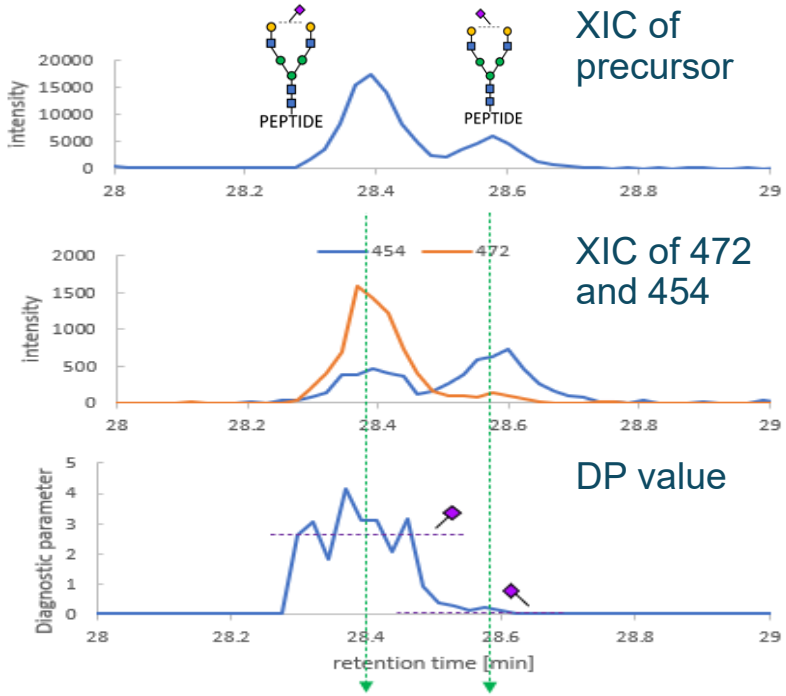
Identification of sialic acid linkage isomers

LCPDCPLLAPLN[A2SA]DSR, M/Z = 914,38, +4



For this single sialylated glycopeptide

the DP clearly assigned the α2-3 and α2-6 isomers

DP = intensity 472/ intensity 454

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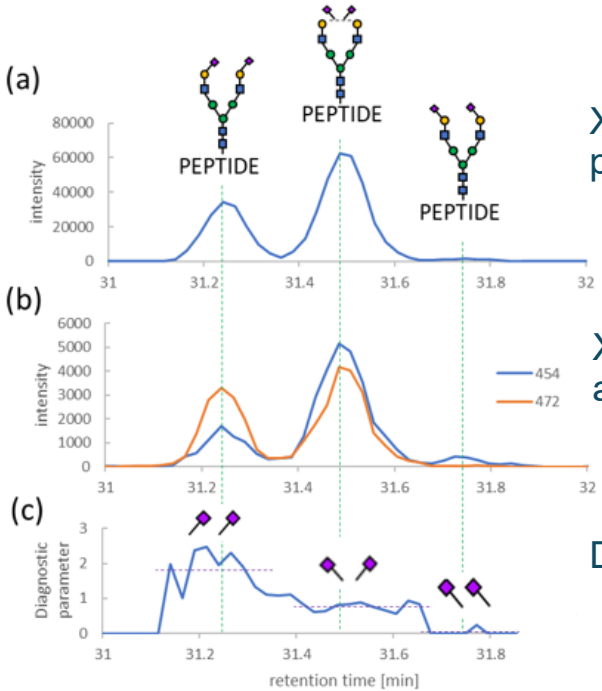
Identification of sialic acid linkage isomers

LCPDCPLLAPLN[A2SA2]DSR, M/Z = 987, 17, +4



XIC of precursor

XIC of 472 and 454

DP value

For this double sialylated glycopeptide the DP clearly assigned the α2-3 and α2-6 isomers

The DP value for the middle peak depends on the ratio of α2-3 and α2-6 linkage

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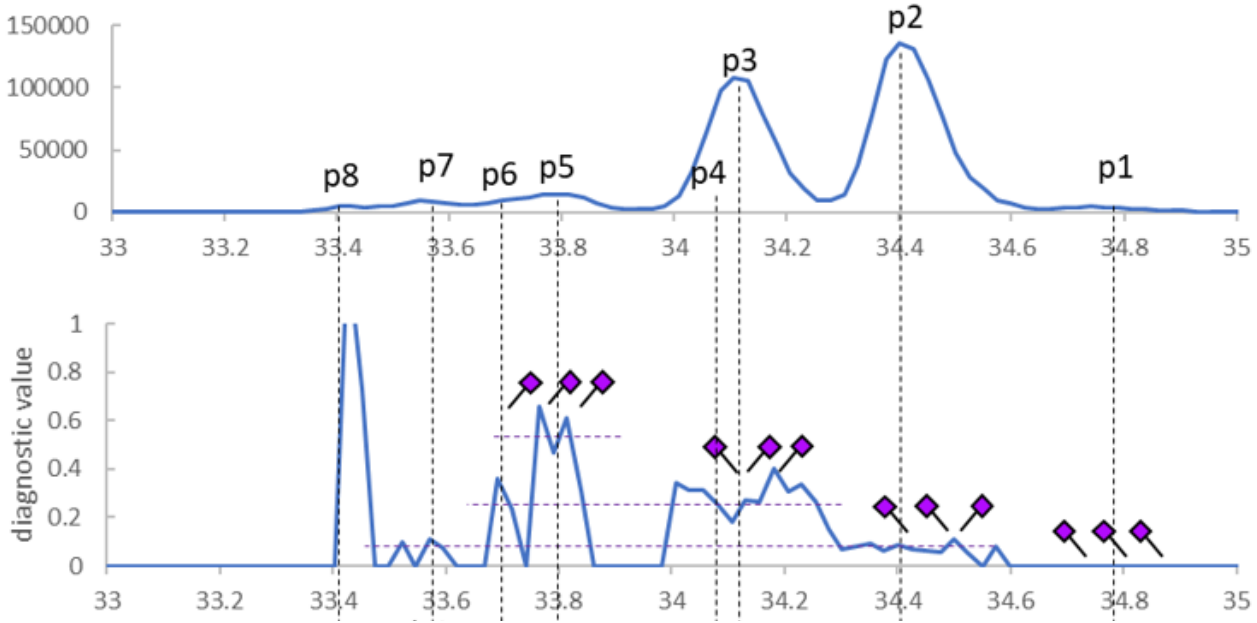
Identification of sialic acid linkage isomers

LCPDCPLLAPLN[A2SA3]DSR M/Z = 1151.21, +4



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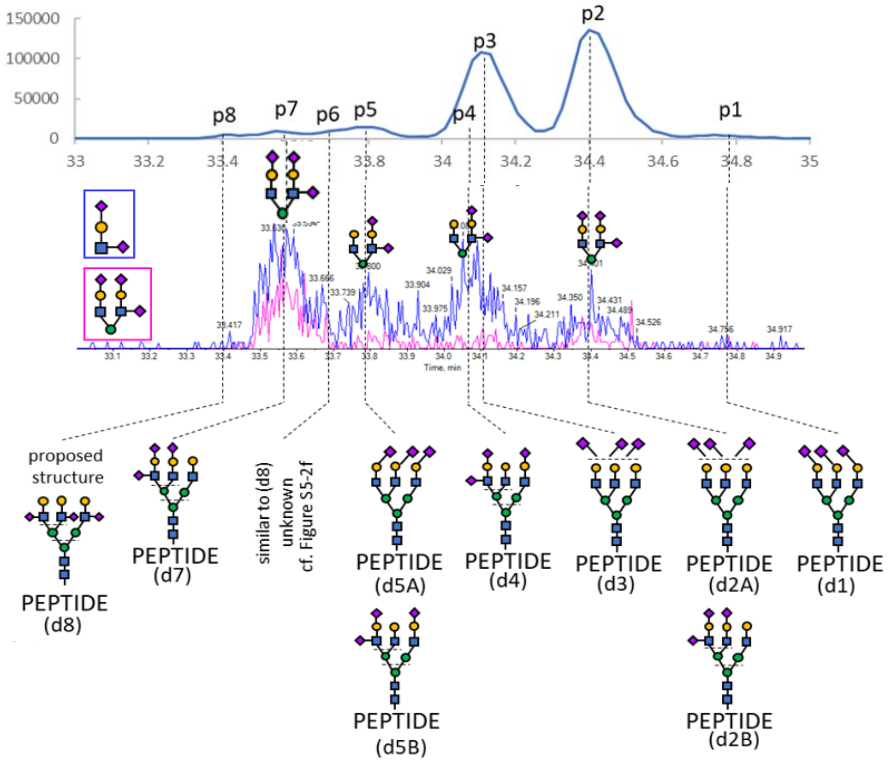
Identification of sialic acid linkage isomers

LCPDCPLLAPLN[A2SA3]DSR M/Z = 1151.21, +4



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Identification of sialic acid linkage isomers

CONCLUSION

- Single RP LC MS method for sialylated N-glycopeptide
  - 0.3-0.4 % FA in mobile phase
  - High sensitivity
  - Better separation of sialic acid position and linkage isomer
- EAD generated B and C glycan fragment ions, ratio can be used to differentiate Sa linkage and position isomer

Ref: Liu, S.; Simmons, D.; Ryumin, P.; Baba, T. 69th ASMS Conference on Mass Spectrometry and Allied Topics; ASMS, 2021; p 265

Ref: Liu, S.; Ryumin , P.; Albanese, J.; Zhang, Z.; Baba, T. *Analytical Chemistry* **Article ASAP ,** DOI: 10.1021/acs.analchem.2c04581

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Thank you!

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