nibrt
National Institute for Bioprocessing Research and Training

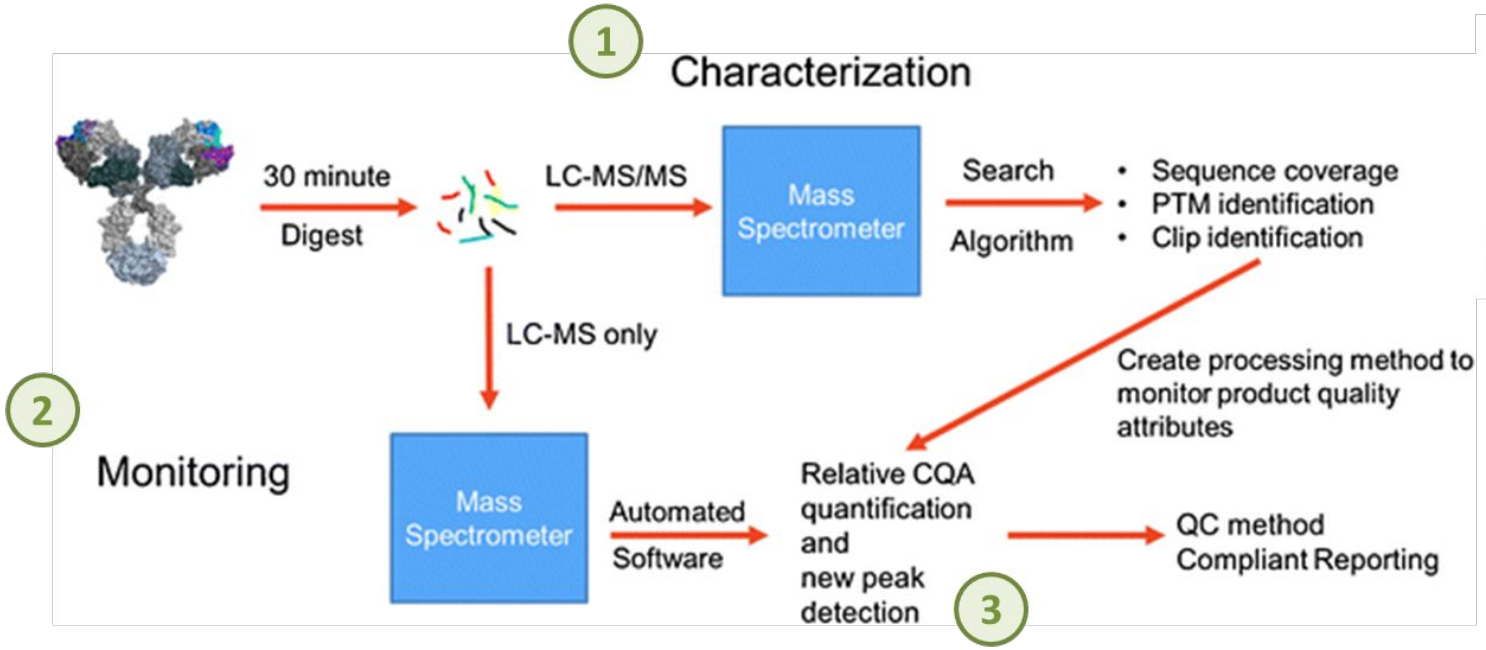
# *Expanding MAM horizons beyond mAb-based therapeutics*

**Sara Carillo[1], Silvia Millán-Martín[1], Corentin Beaumal[1], Anna Mulligan[1], Kai Scheffler[2], Cong Wang[3], Ulrik Mistarz[4], Ken Cook[5], Jonathan Bones[1,6]**

[1] National Institute for Bioprocessing Research & Training (NIBRT), Dublin, Ireland.
[2] Thermo Fisher Scientific, Germenring, Germany.
[3] Thermo Fisher Scientific, Bremen, Germany.
[4] Thermo Fisher Scientific, Allerod, Denmark.
[5] Thermo Fisher Scientific, Hemel Hempstead, United Kingdom.
[6] School of Chemical and Bioprocessing Engineering, University College Dublin, Dublin, Ireland

**CASSS Mass Spec, Costa Mesa, CA, September 26th 2025**

# Multi-Attribute Method




mam consortium

usp®

REPORT

mAbs 7:5, 881–890; September/October 2015; © 2015 Amgen Inc.

**Development of a quantitative mass spectrometry multi-attribute method for characterization, quality control testing and disposition of biologics**

Richard S Rogers[†,*], Nancy S Nightlinger, Brittney Livingston[†], Phil Campbell, Robert Bailey[#], and Alain Balland[§]

Analytical Sciences; Amgen Inc.; Seattle, WA USA
[†]Present affiliation: Just Biotherapeutics; Seattle, WA USA
[#]Present affiliation: Zymeworks; Seattle, WA USA
[§]Present affiliation: Boehringer Ingelheim Pharma GmbH & Co. KG; Biberach an der Riss, Germany

nature protocols

PROTOCOL

https://doi.org/10.1038/s41596-022-00785-5

Check for updates

**Comprehensive multi-attribute method workflow for biotherapeutic characterization and current good manufacturing practices testing**

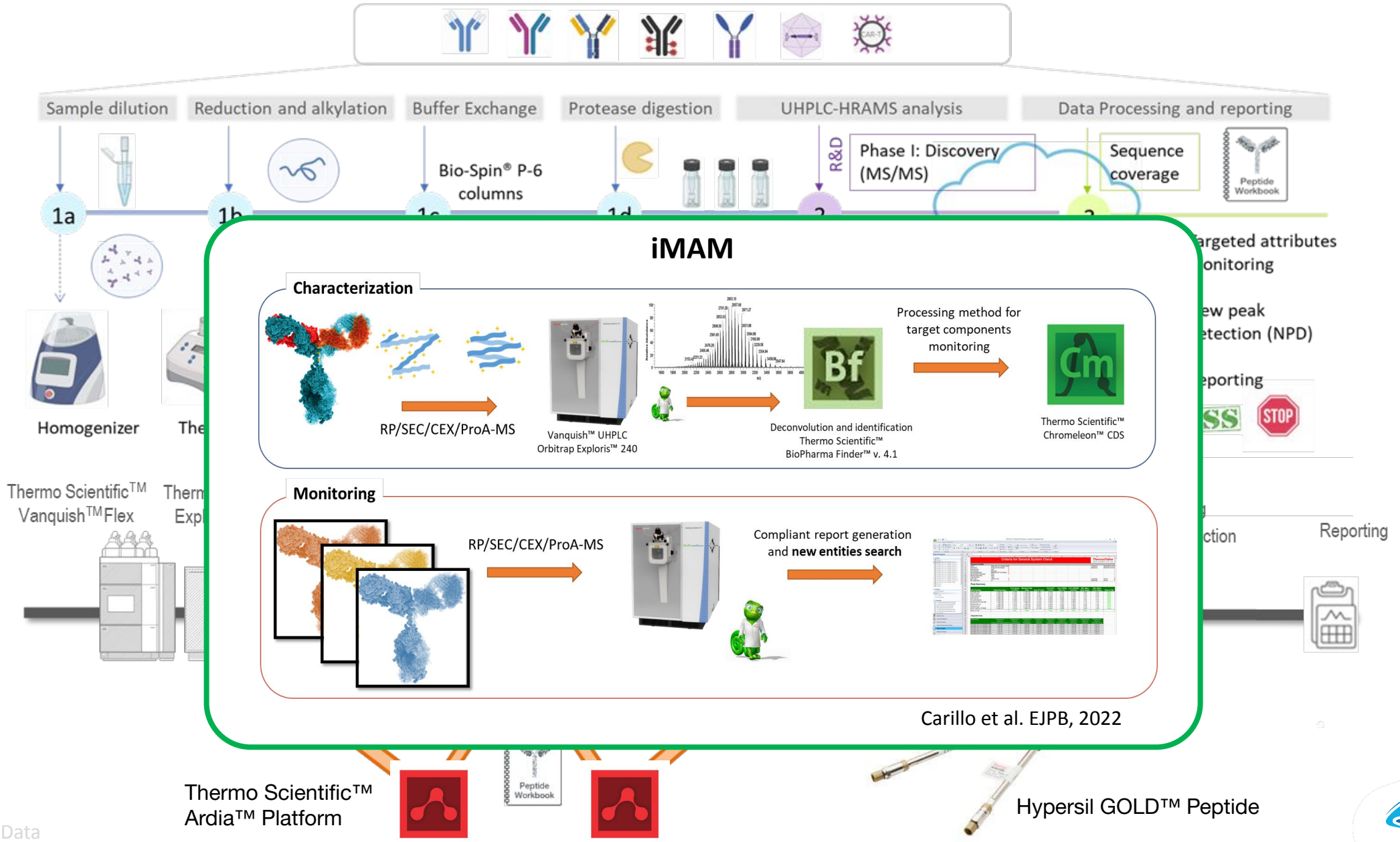
Silvia Millán-Martín[1], Craig Jakes[1,2], Sara Carillo[1], Richard Rogers[3], Da Ren[4] and Jonathan Bones[1,2]✉

nibrt

# Multi-Attribute Analysis vs Multi-Attribute Method

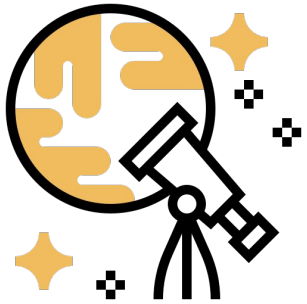
1. Deep knowledge of product under investigation
2. Tight control of analytical tools and sample preparation steps
3. Real-time, GxP compliant, reporting of selected PQAs
4. Confident detection of changes

# MAM Workflow @ NIBRT

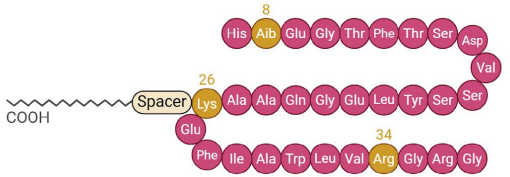


# New challenges in Biopharma

1. **Bringing bioanalysis into early-stage**



2. **New modalities**




3. **Sustainability goals (EU REACH Regulation)**

my green lab.

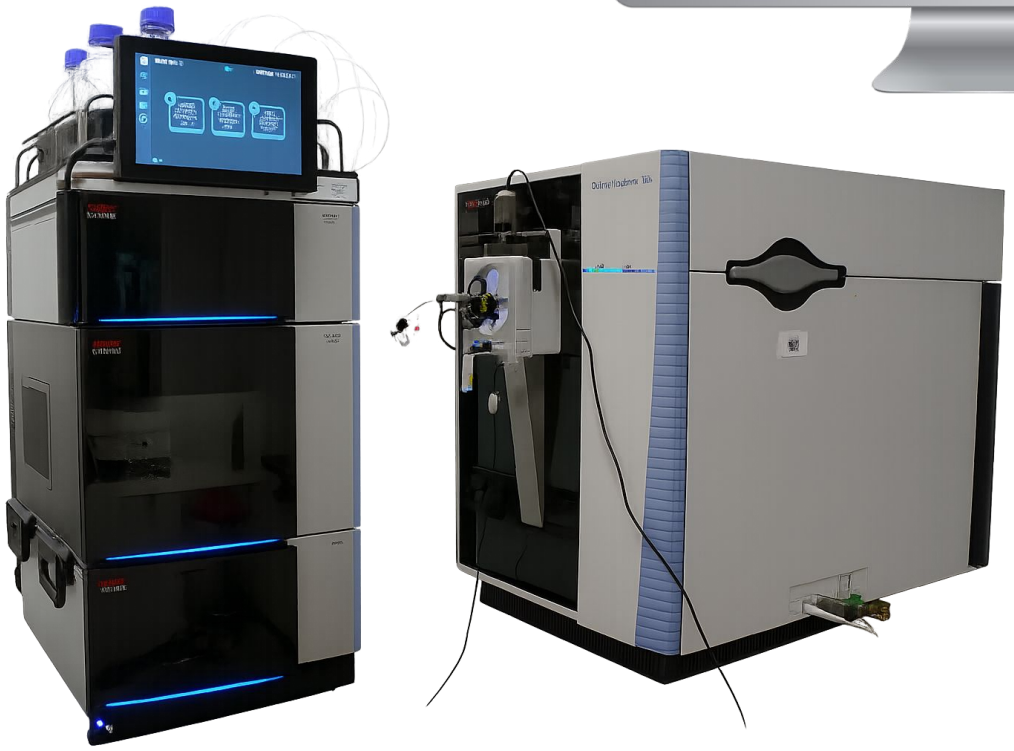


nibrt

# MAM in Early-Stage

**Analytics are the bottleneck to accelerate drug and process development**

- **Long analysis**
- **Low high-throughput**
- **High sample demand**



**Points of concern to scale-down**

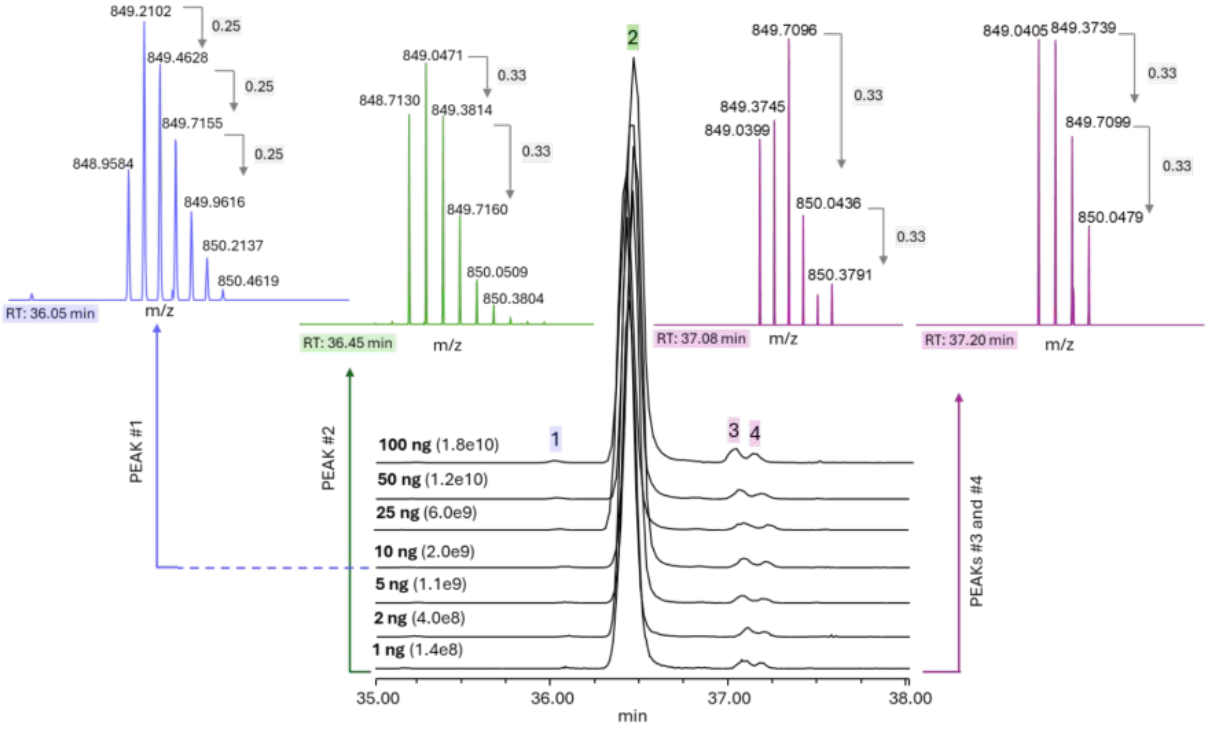
- **Dynamic range**
- **Sample loss**
- **Instrumentation**

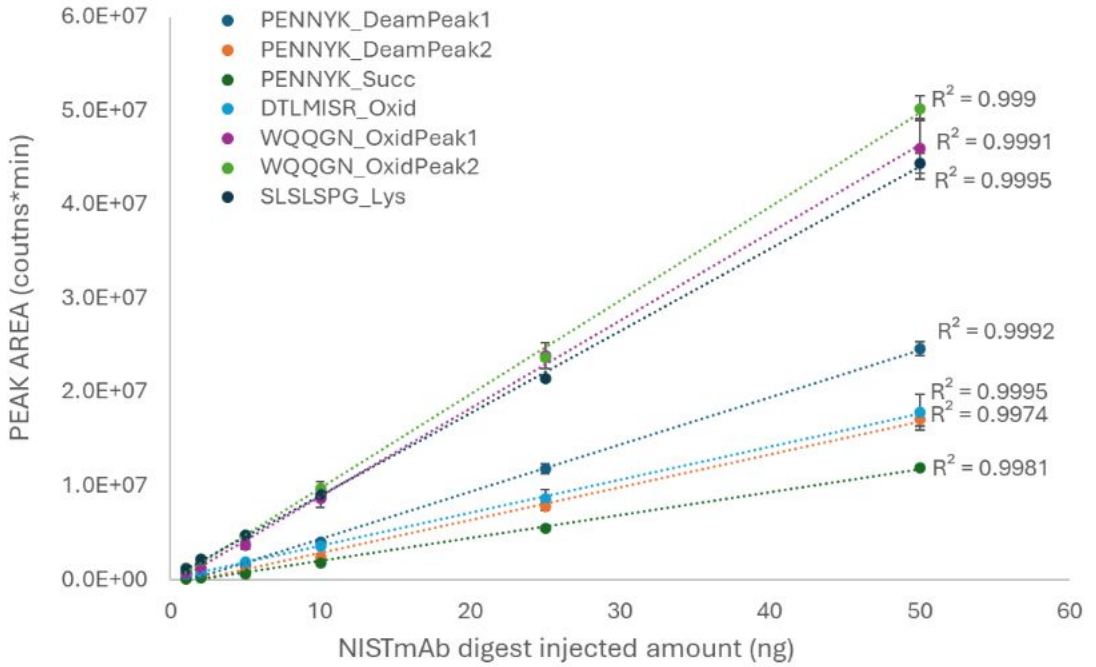
# Exploring Dynamic range


Lisa Strasser


Anna Mulligan


Silvia Millán-Martín

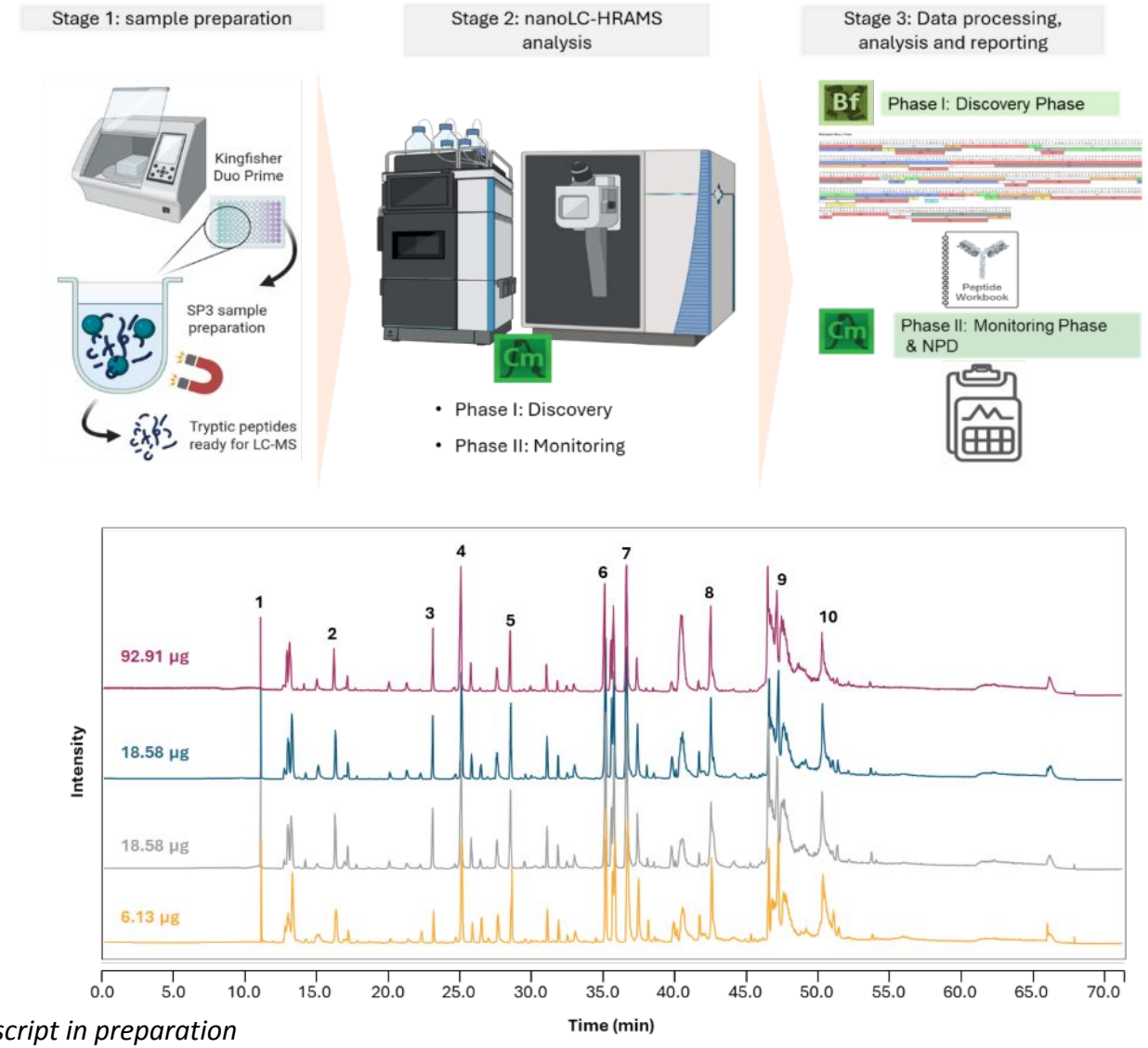



| | PENNYK Deam Peak1 | PENNYK Deam Peak2 | PENNYK Succ | DTLMISR Oxid | WQQGN Oxid Peak1 | WQQGN Oxid Peak2 | SLSLSP Lys |
|---|---|---|---|---|---|---|---|
| Slope (S) | 5.04E+05 | 3.51E+05 | 2.44E+05 | 3.53E+05 | 9.37E+05 | 9.97E+05 | 8.77E+05 |
| σ | 1.36E+05 | 1.44E+05 | 6.58E+04 | 2.48E+05 | 4.38E+05 | 2.60E+05 | 1.66E+05 |
| $R^2$ | 0.9979 | 0.9950 | 0.9979 | 0.9856 | 0.9936 | 0.9980 | 0.9989 |

*Manuscript in preparation*

# MAM to assist clone selection

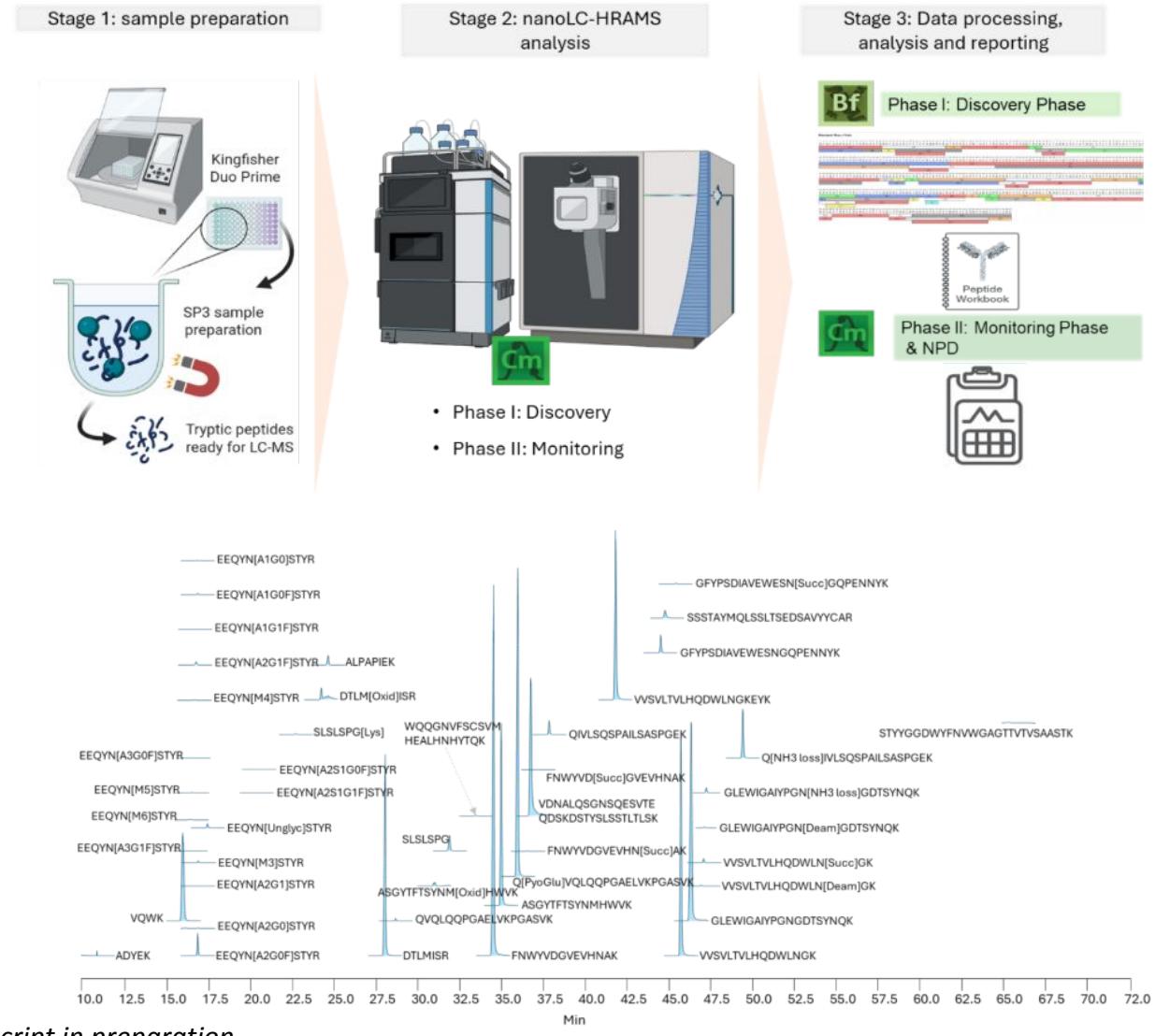



Based on data available in the lab, we investigated feasibility and transferability of MAM for IgG sample amounts produced by and high producing and low producing cell line cultured in 96-well plates.

Starting material was calculated based on specific productivity and cell densities at day 3 and day 5.

*Manuscript in preparation*

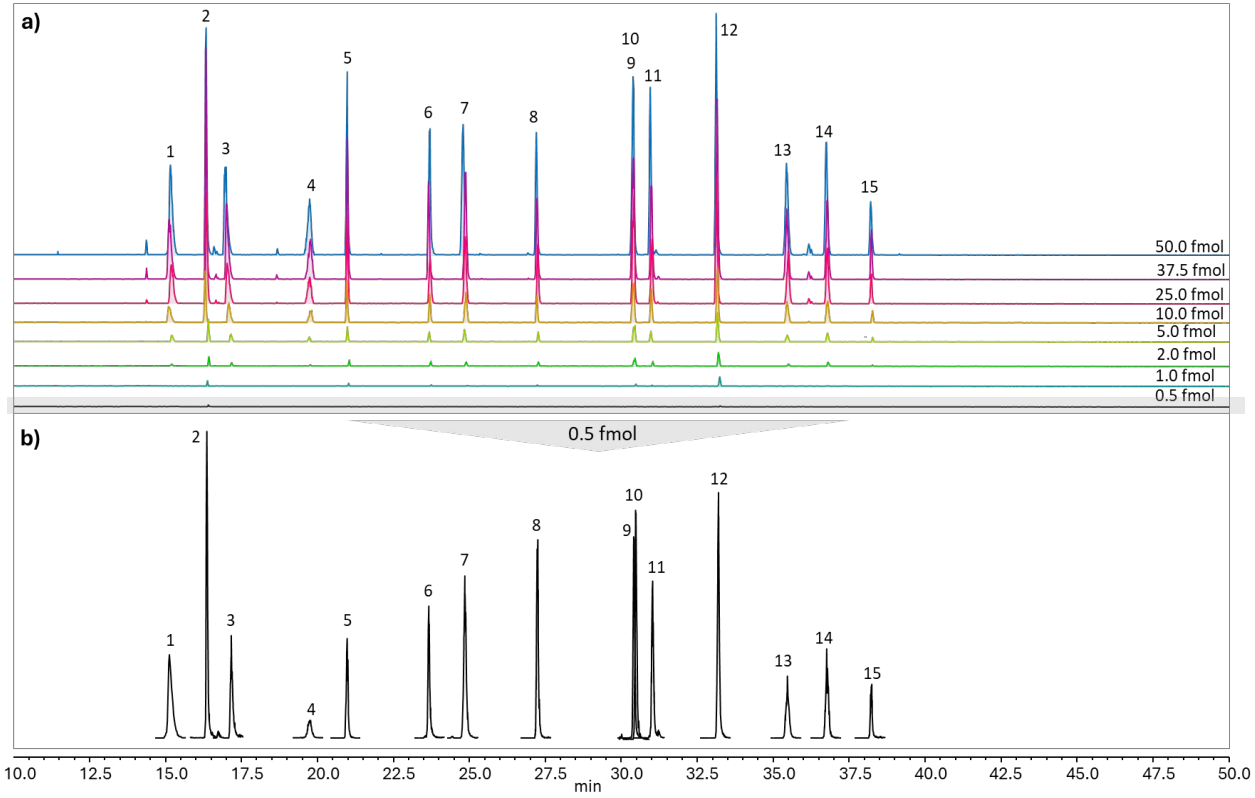
# MAM to assist clone selection

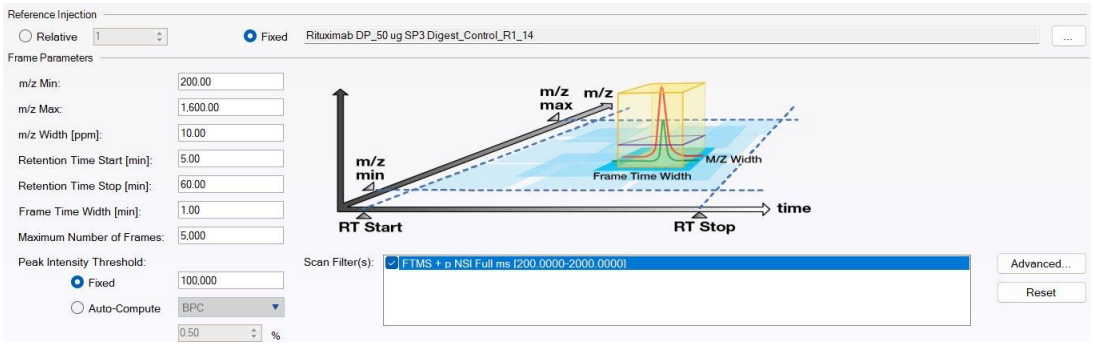


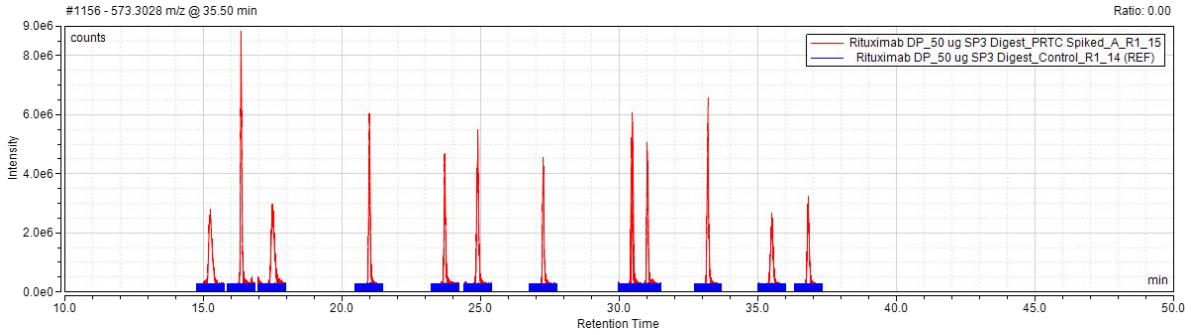

| Peptide | Description | % PQA (Avg.) | %CV (PQA) |
|---|---|---|---|
| DTL**M**[256]ISR | Oxidation | 4.49 | 5.08 |
| ASGYTFTSYN**M**[34]HWVK | Oxidation | 2.62 | 5.88 |
| GLEWIGAIYPGNGDTSY**N**[55]QK | Deamidation | 0.63 | 7.71 |
| GLEWIGAIYPGNGDTSY**N**[55]QK | Succinimide (N) | 1.65 | 5.91 |
| FNWYVDGVEVH**N**[290]AK | Succinimide (N) | 0.11 | 14.35 |
| FNWYV**D**[284]GVEVHNAK | Succinimide (D) | 0.22 | 11.89 |
| VVSVLTVLHQDWL**N**[319]GK | Deamidation | 0.46 | 11.79 |
| VVSVLTVLHQDWL**N**[319]GK | Succinimide | 1.50 | 11.45 |
| GFYPSDIAVEWES**N**GQP**ENN**YK | Succinimide | 0.94 | 7.01 |
| SLSLSP**G**[449] | C-term Lys | 7.31 | 4.04 |
| **Q**[1]VQLQQPGAELVKPGASVK | N-term PyroGlu (HC) | 99.31 | 0.03 |
| **Q**[1]IVLSQSPAILSASPGEK | N-term PyroGlu (LC) | 80.94 | 1.10 |
| EEQY**N**[301]STYR | A2G0F | 56.56 | 2.31 |
| EEQY**N**[301]STYR | A2G1F | 11.66 | 4.62 |
| EEQY**N**[301]STYR | A1G0F | 5.40 | 3.64 |
| EEQY**N**[301]STYR | A2G2F | 0.99 | 5.68 |
| EEQY**N**[301]STYR | A1G1F | 0.61 | 2.99 |
| EEQY**N**[301]STYR | A2G0 | 0.60 | 9.63 |
| EEQY**N**[301]STYR | A2G1 | 0.30 | 9.80 |
| EEQY**N**[301]STYR | A1G0 | 0.37 | 5.66 |
| EEQY**N**[301]STYR | M3 | 4.74 | 2.42 |
| EEQY**N**[301]STYR | M4 | 1.75 | 4.72 |
| EEQY**N**[301]STYR | M5 | 3.66 | 4.93 |
| EEQY**N**[301]STYR | M6 | 0.40 | 4.32 |
| EEQY**N**[301]STYR | A3G0F | 0.09 | 6.27 |
| EEQY**N**[301]STYR | A3G1F | 0.04 | 8.92 |
| EEQY**N**[301]STYR | A2S1G0F | 0.18 | 7.93 |
| EEQY**N**[301]STYR | A2G1S1F | 0.16 | 19.34 |
| EEQY**N**[301]STYR | Unglycosylated | 12.87 | 7.81 |

*Manuscript in preparation*

# MAM to assist clone selection



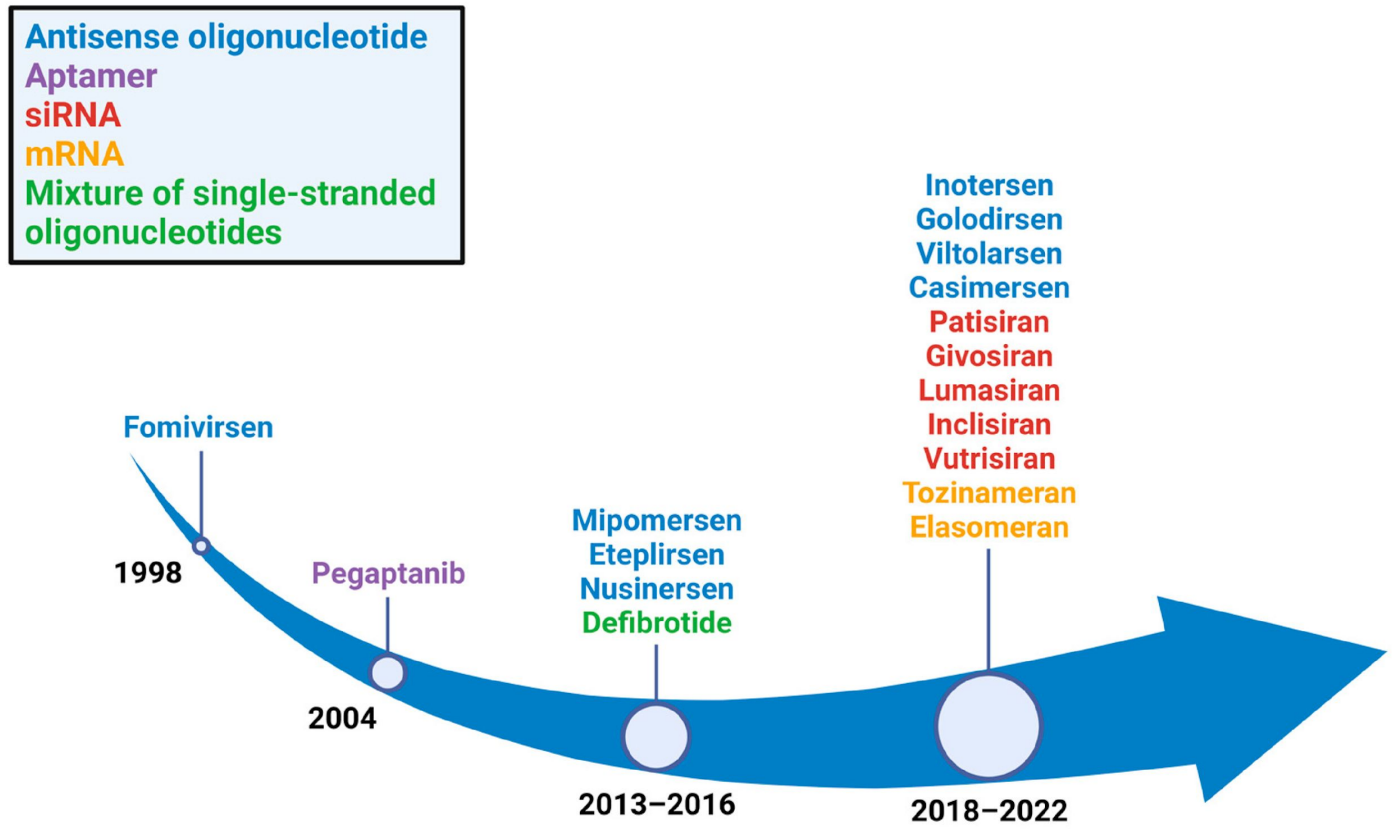




NPD was also explored spiking different amounts of PRTC mix. All 15 peptides were detected down to 0.5 fmol (in a 25 ng injection) by lowering intensity threshold for the non-targeted MS search.

*Manuscript in preparation*

# Antisense Oligonucleotide Therapeutics

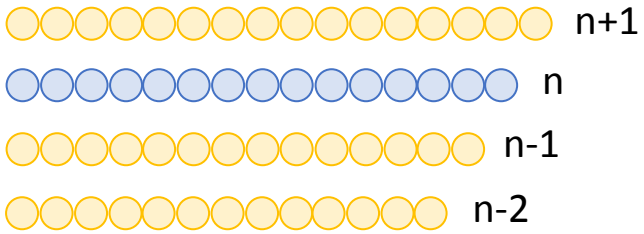
Antisense oligonucleotide (ASO) therapy uses short, synthetic strands of modified DNA-like molecules to target and alter messenger RNA (mRNA), thereby changing protein production to treat diseases

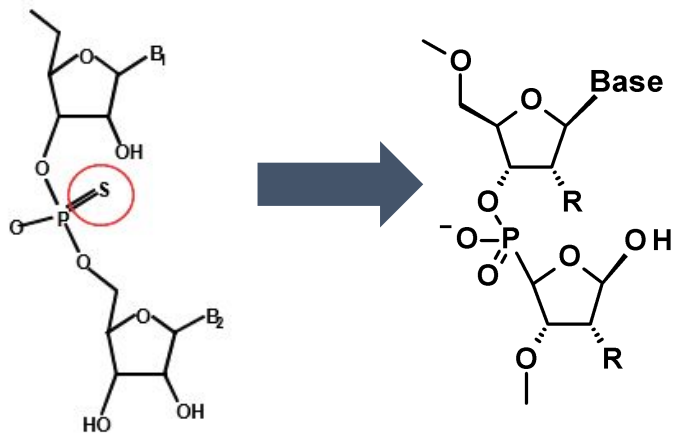


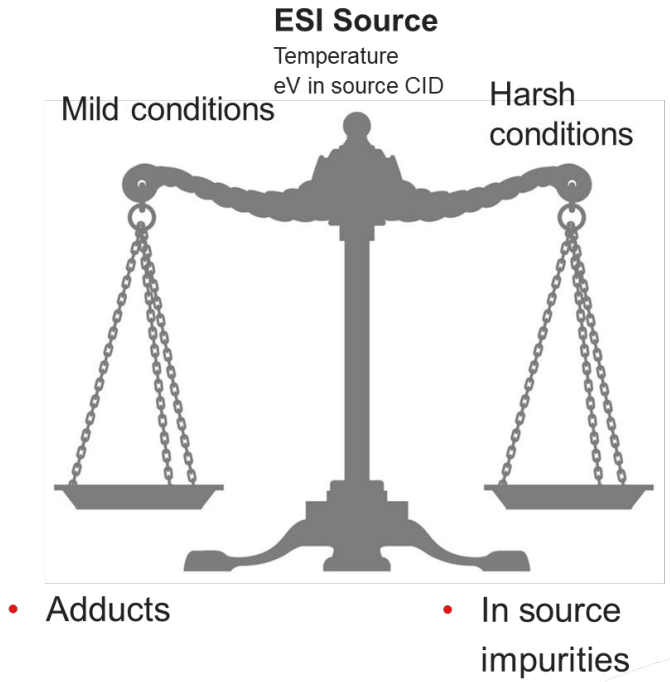

Adapted by Jun Jo et al. Int. J. Mol. Sci, 2023

# Antisense Oligonucleotide Therapeutics

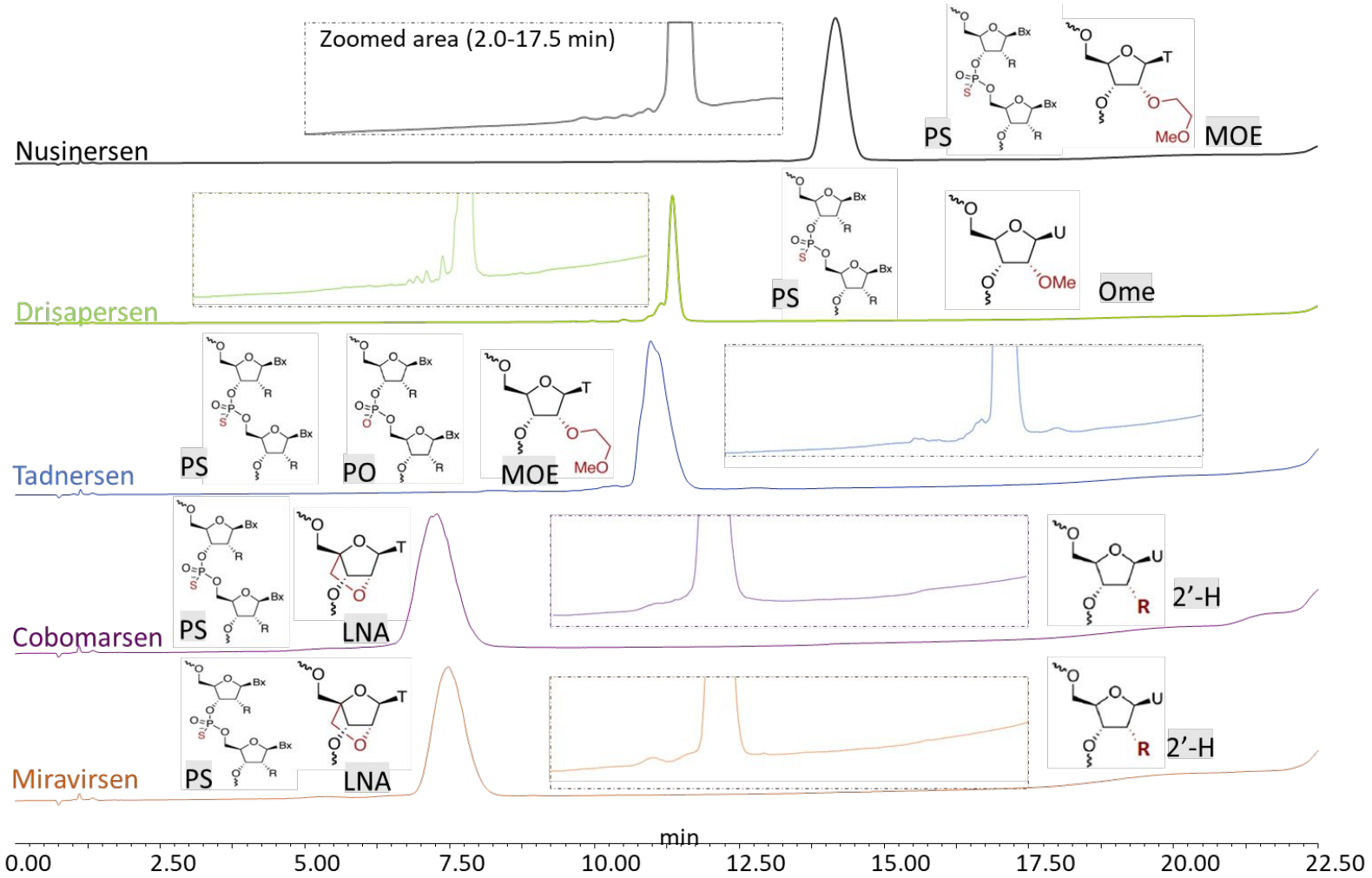
- Synthesis imperfections (n-1, n-2, n-x, n+1, n+2)
- Sequence verification with modifications
- PO impurity from PS modification – Could be any of the positions
- Deamidation
- Oxidation
- Depurination
- Protection impurities



# ASO Therapeutics




IP-RP-MS/MS analysis using HFIP and PA using DNAPac RP column and Orbitrap Exploris 480

Silvia Millán-Martín

Felipe Guapo Melo

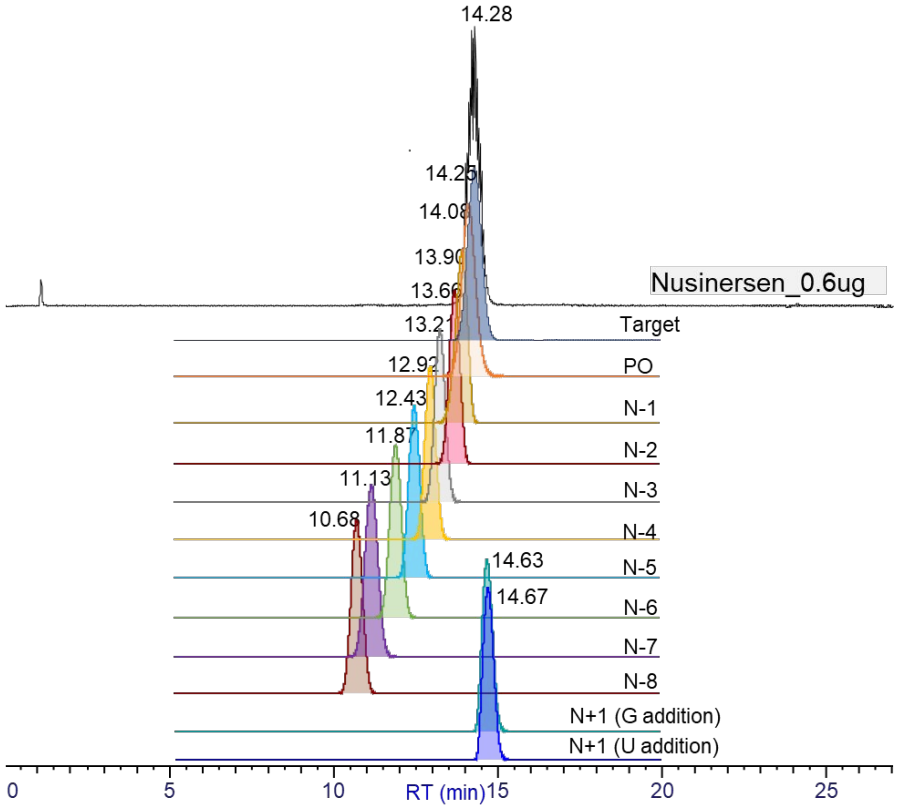
Ken Cook

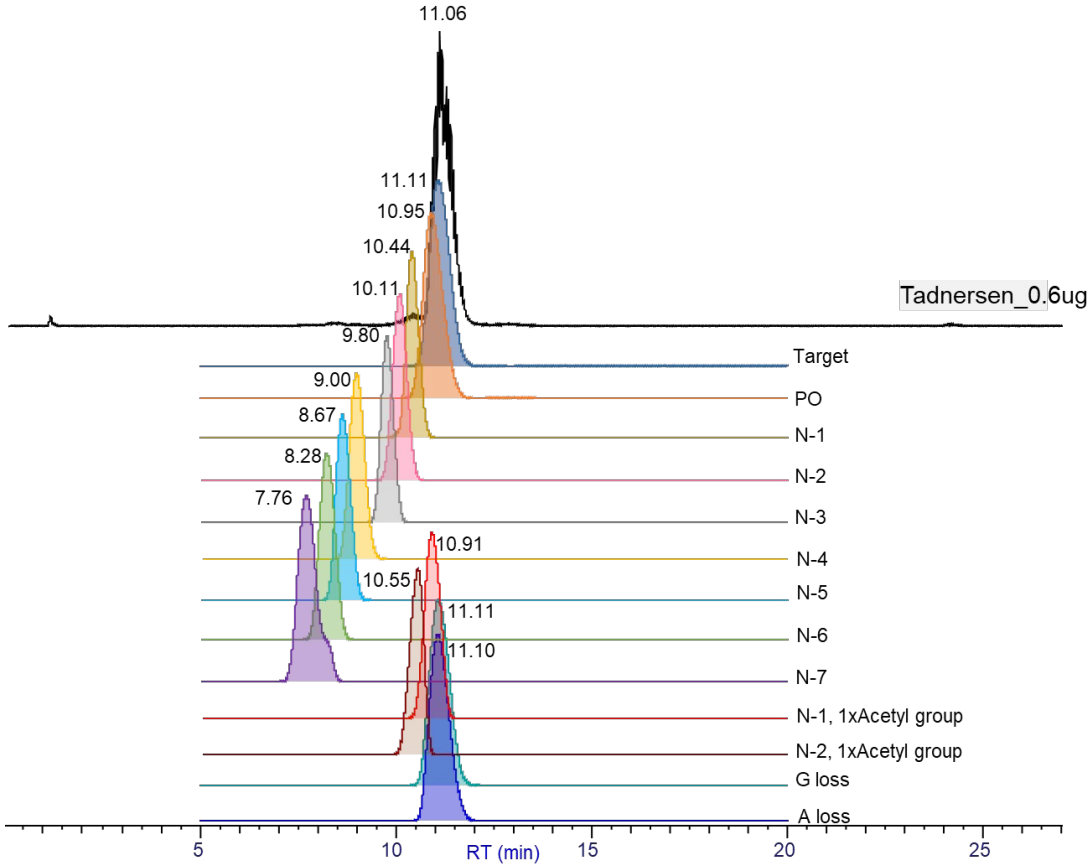
Ulrik Mistarz



*JPBA, 2025*

# ASO Therapeutics



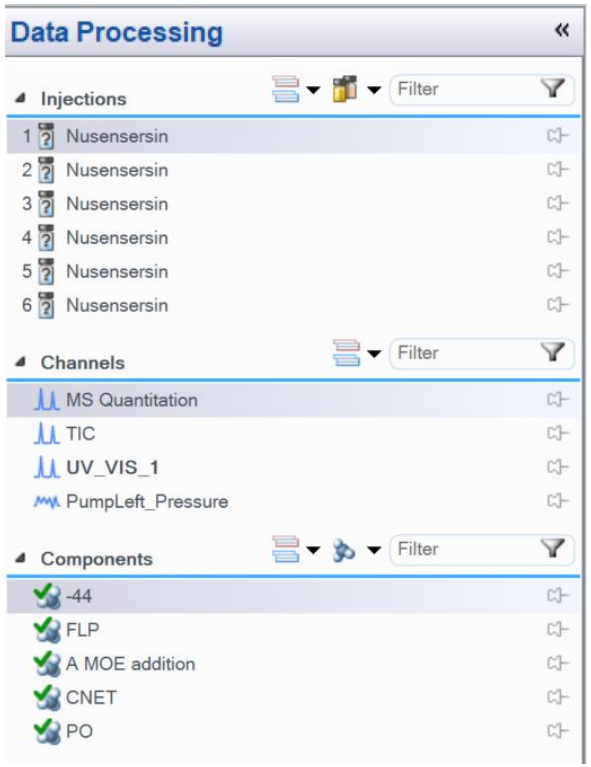



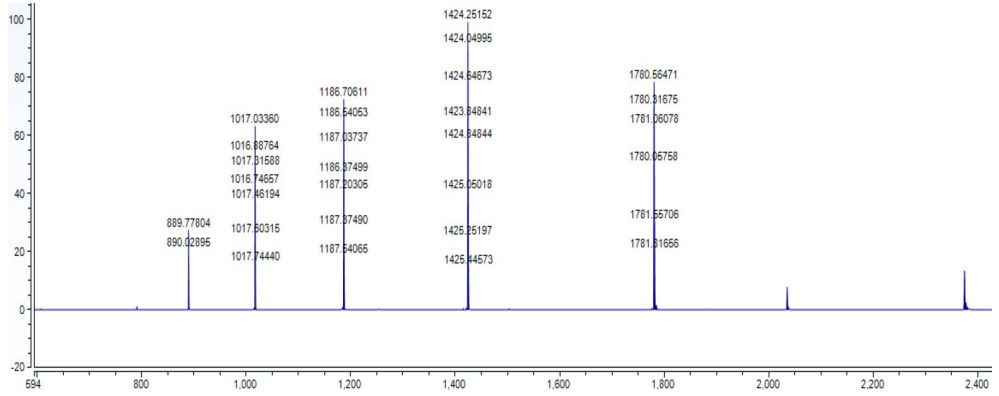

Deconvolution with Sliding Window allows to process Full MS data to obtain accurate quantitation of impurities with up to 0.1% abundance even for non-fully resolved peaks.

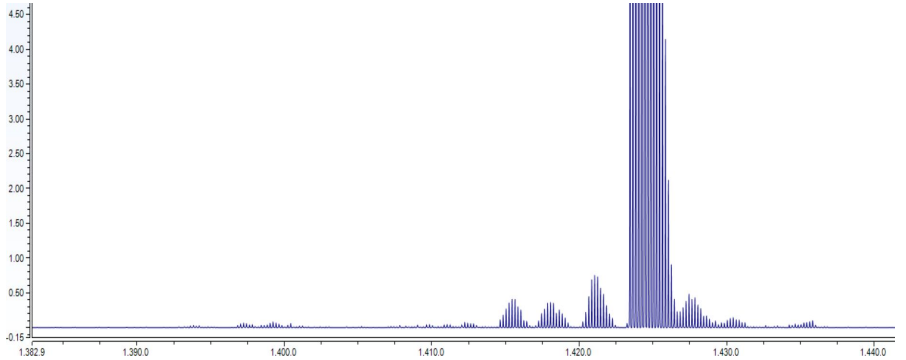


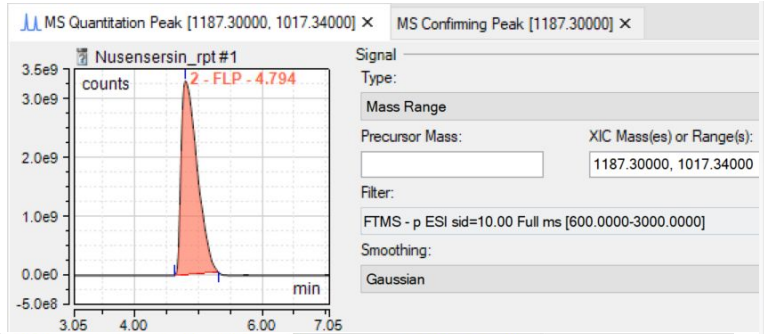
*JPBA, 2025*

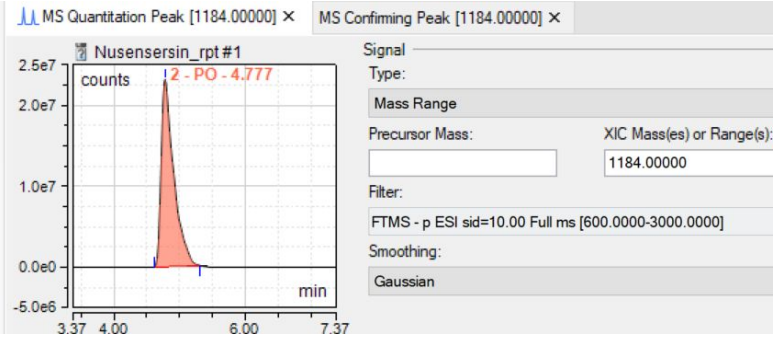
# ASO Therapeutics: Compliant reporting

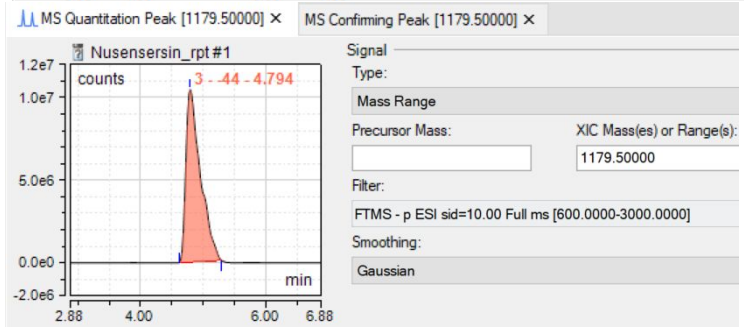


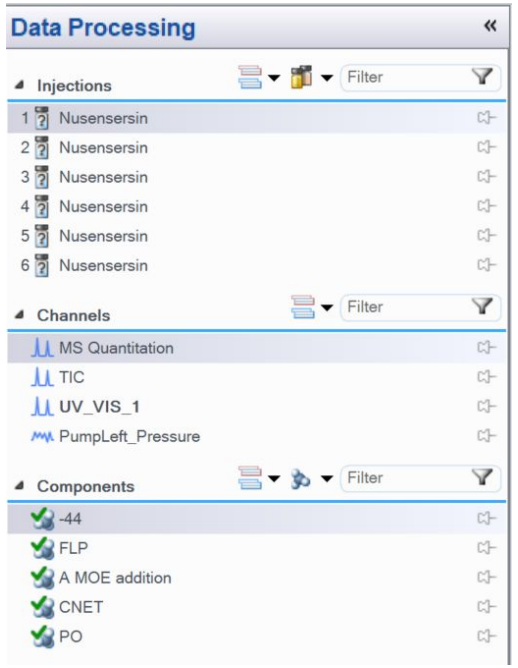


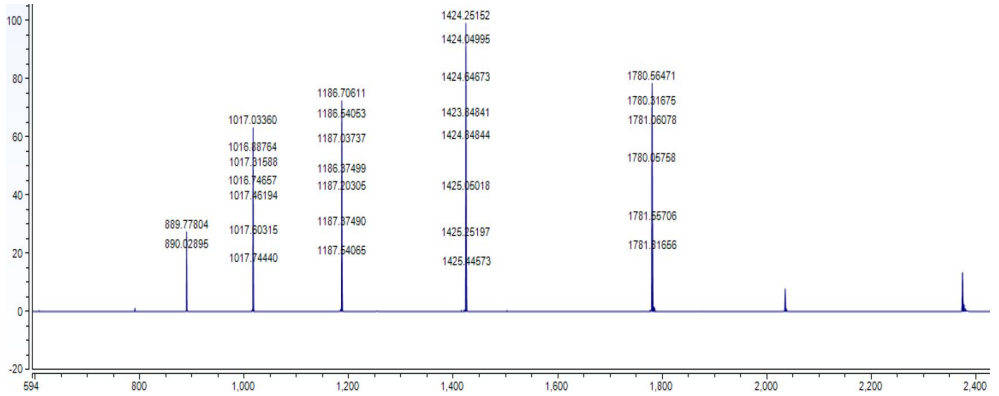



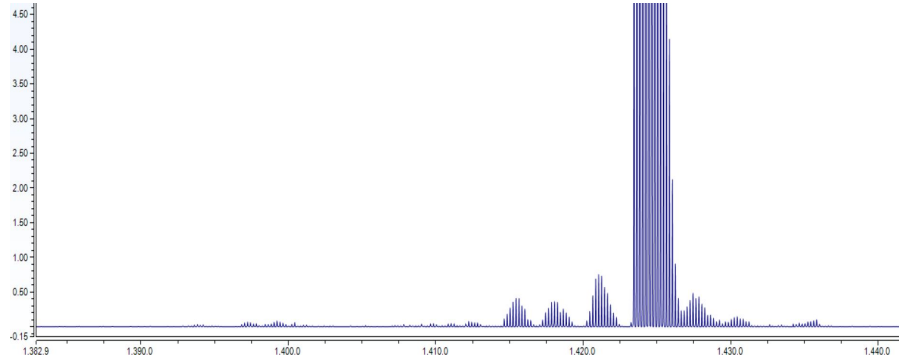






# ASO Therapeutics: Compliant reporting



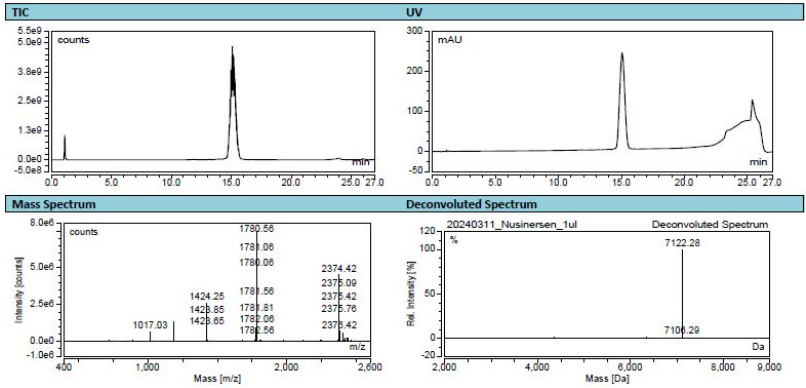
## Identification Summary

**Injection Details**

| | | | |
|---|---|---|---|
| Sequence Name: | 20250127_OligoFocusGroup_Report | Sequence created date: | 27-Jan-2025 14:19 |
| Sequence Directory: | Instrument Data\Oligo Focus Group | Sequence created operator: | ulrik.mistarz |
| Sequence Data Vault | ChromeleonLocal | Sequence updated date: | 12-Mar-2025 17:10 |
| Number of Injections: | 11 | Sequence updated operator: | ulrik.mistarz |

**Deconvoluted mass overview**

| No. | Injection Name | Inj. Pos. | Target Tolerance Da | Expected Mass Da | Identified | Measured Mass Da | Delta Mass Da | Mass Accuracy ppm | Abundance % |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 20240311_Inotersen_1ul | G:A1 | 0.100 | 7178.056 | ✓ | 7178.068 | 0.012 | -1.6 | 74.19 |
| 2 | 20240311_Nusinersen_1ul | G:A2 | 0.100 | 7122.276 | ✓ | 7122.283 | 0.007 | -0.9 | 88.54 |
| 3 | 20240311_Lumasiran_1ul | G:A3 | 0.100 | 7627.145 | ✓ | 7627.167 | 0.022 | -2.8 | 47.91 |
| 4 | 20240311_Mipomersen_1ul | G:A5 | 0.100 | 7172.092 | ✓ | 7172.105 | 0.014 | -1.9 | 80.51 |
| 5 | 20240311_Custirsen_1ul | G:A8 | 0.100 | 7340.993 | ✓ | 7341.002 | 0.009 | -1.3 | 77.19 |
| 6 | 20240311_IonisMAPTRx_1ul | G:A9 | 0.100 | 6426.056 | ✓ | 6426.060 | 0.005 | -0.7 | 80.50 |
| 7 | 20240311_GIVOSIRAN_1ul | G:B1 | 0.100 | 8732.021 | ✓ | 8732.050 | 0.029 | -3.3 | 38.12 |
| 8 | 20240311_Inclisiran_1ul | G:B2 | 0.100 | 7694.201 | ✓ | 7694.214 | 0.013 | -1.6 | 43.92 |
| 9 | 20240311_Eterplisen_pos3500 | G:A4 | 0.100 | 10300.589 | ✓ | 10300.588 | -0.001 | 0.1 | 58.40 |
| 10 | 20240311_Goladersen_pos3500 | G:A7 | 0.100 | 8642.963 | ✓ | 8642.977 | 0.014 | -1.6 | 48.21 |
| 11 | 20240311_Rovanersen_1ul_repeat | G:A6 | 0.100 | 6612.853 | ✓ | 6612.852 | -0.001 | 0.1 | 71.73 |

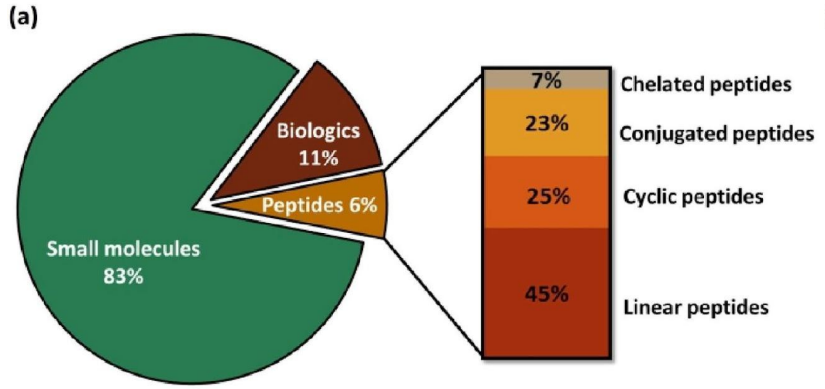


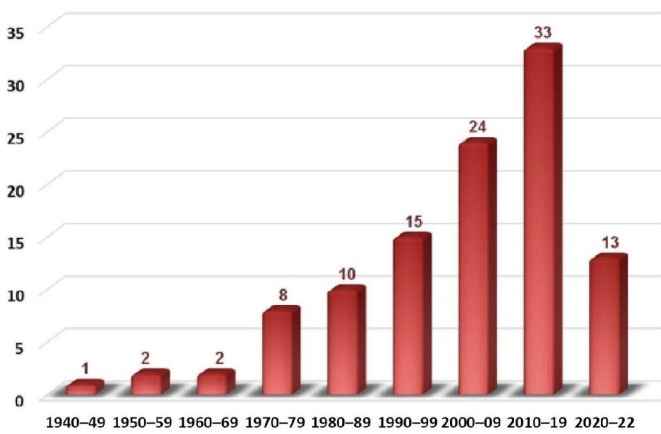

**Deconvolution Results**

| Component Number | Measured Mass Da | RT min | Delta Mass Da | Identification | Theoretical Mass Da | Mass Accuracy Da | Mass Accuracy ppm | Intensity counts | Fractional Abundance % | RRT min |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 7122.283 | 15.08 | 0.007 | Target | 7122.276 | -0.01 | -0.9 | 1.70E+7 | 88.54 | 0.00 |
| 2 | 7106.289 | 14.95 | -15.987 | PO | 7106.206 | -0.08 | -11.7 | 3.49E+5 | 1.82 | -0.13 |
| 3 | 6335.149 | 14.52 | -787.127 | N-2 (UC) | 6335.146 | 0.00 | -0.5 | 1.75E+5 | 0.91 | -0.56 |
| 4 | 4356.817 | 11.85 | -2765.460 | N-7 (UCACUUU) | 4356.806 | -0.01 | -2.4 | 1.44E+5 | 0.75 | -3.23 |
| 5 | 5932.077 | 14.02 | -1190.199 | N-3 (UCA) | 5932.066 | -0.01 | -1.8 | 1.28E+5 | 0.67 | -1.06 |
| 6 | 7250.409 | 15.35 | 128.133 | PA+ACN | 7250.492 | 0.08 | 11.4 | 1.24E+5 | 0.65 | 0.27 |
| 7 | 7078.262 | 15.05 | -44.014 | 2'-O-methyl loss | 7078.256 | -0.01 | -0.8 | 1.17E+5 | 0.61 | -0.03 |

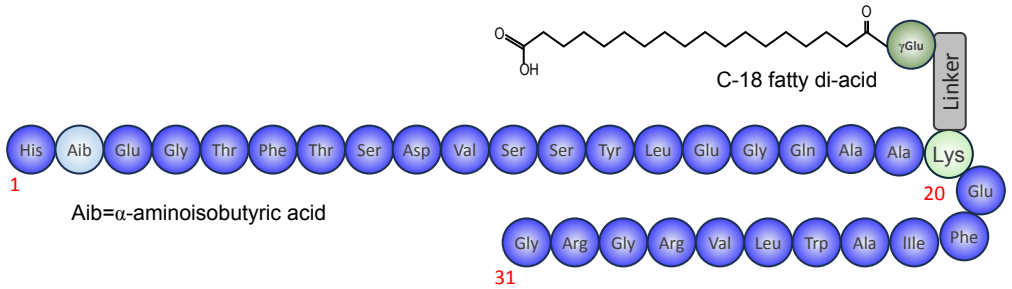
# Peptide Therapeutics: GLP-1 agonists

- GLP-1 receptor agonist therapeutic peptides have emerged to become some the world's largest selling drugs.
- Samples of GLP-1 peptides from different vendors analyzed by LC-MS using simple linear gradients of water and acetonitrile containing formic acid.

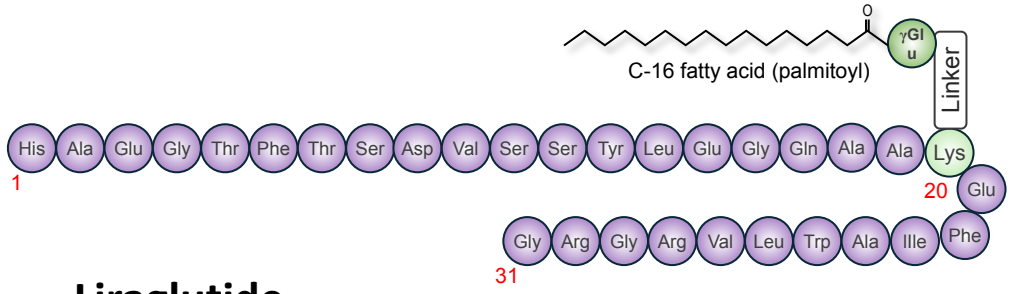



Drug Discovery Today 28, 103464 (2023)



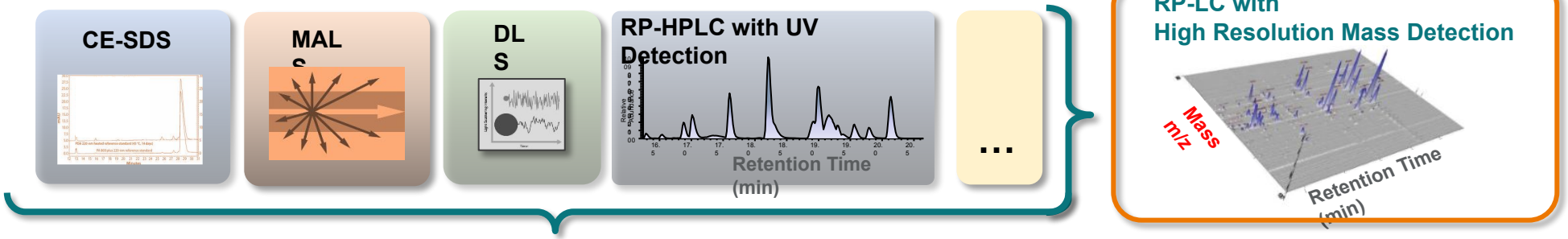

**Semaglutide**



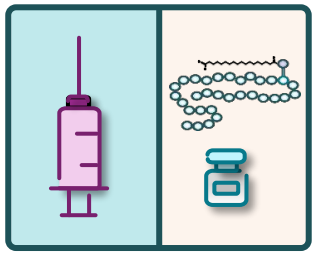

**Liraglutide**

| | Semaglutide | Liraglutide |
|---|---|---|
| M | 4111.1143 | 3748.9459 |
| *m/z*, *z*=+2 | 2056.5644 | 1878.4802 |
| *m/z*, *z*=+3 | 1372.3787 | 1250.6559 |
| *m/z*, *z*= +4 | 1028.7859 | 938.4898 |
| chemical formula | $C_{187}H_{291}O_{59}N_{45}$ | $C_{172}H_{365}O_{51}N_{43}$ |
| modifications | Lys20: C18 diacid γ-Glu $(AEEA)_2$ *AEEA: 2-(2-(2-aminoethoxy)ethoxy) acetic acid* | Lys20: C16 palmitoyl γ-Glu |

# MAM workflow for GLP-1 agonists




Multiple workflows are required for quality control of GLP-1 analogs



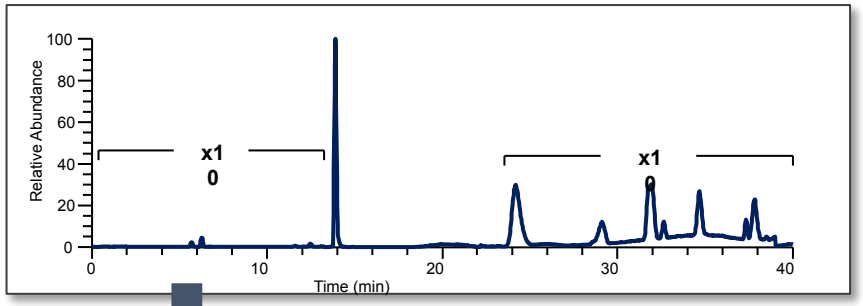
**GLP-1 analog**

**Digestion** (optional) → **LC-MS**

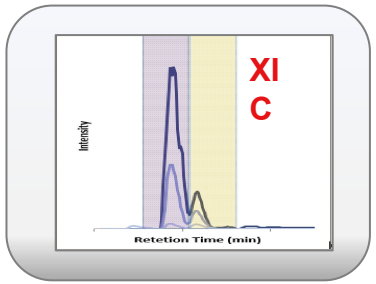
**Peptides with and without modifications**

Sum of all species = product quality attributes (PQA)

**Peptides are separated with LC and detected with MS**

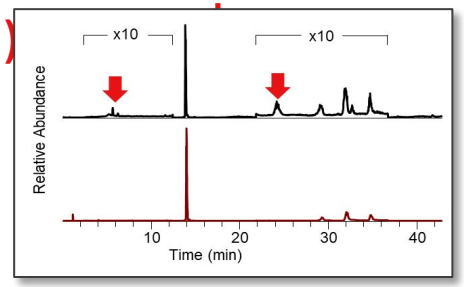



**1. Targeted approach**




**Impurity Quantitation**

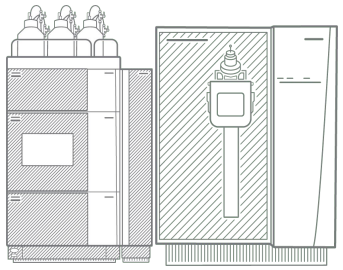
**2. Untargeted**




**New Peak Detection (NPD)**

# MAM workflow for GLP-1 agonists

**Characterization** → **Impurity Monitoring & New Peak Detection**

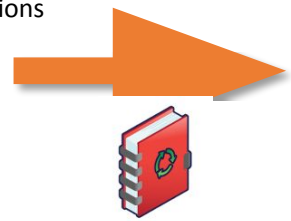


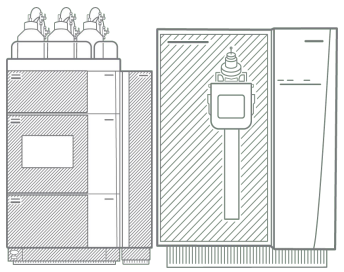
**STEP 1:**

Product characterization with MS/MS

- Unambiguous sequence confirmation
- Identification of amino acid modifications and sequence truncations

- **STEP 2:**
- Generation of target list in target workbook format





**STEP 3:**

Monitoring of known modifications

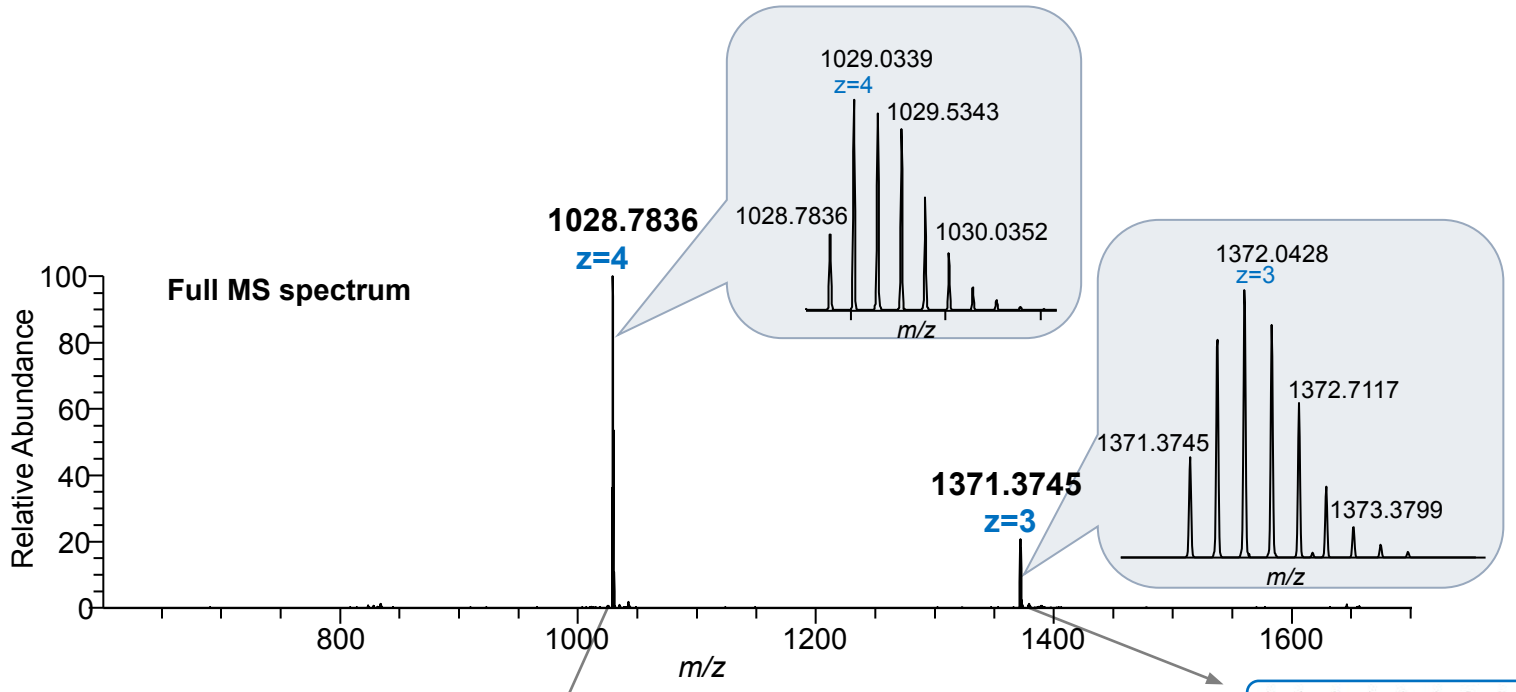
- Quantitative assessment

- **STEP 4:**

New Peak Detection

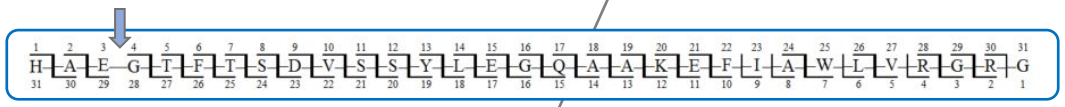
- Assessment for unkwnown/unanticipated species

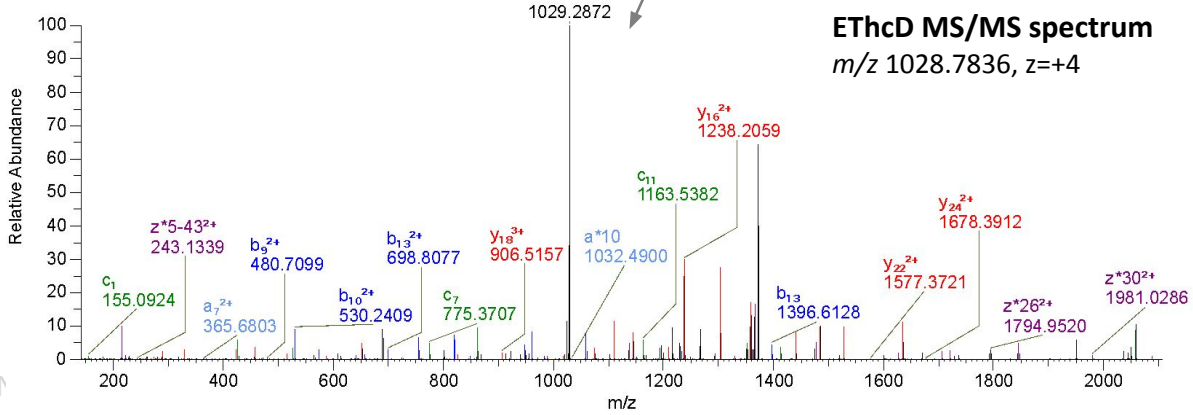
# MAM workflow for GLP-1 agonists

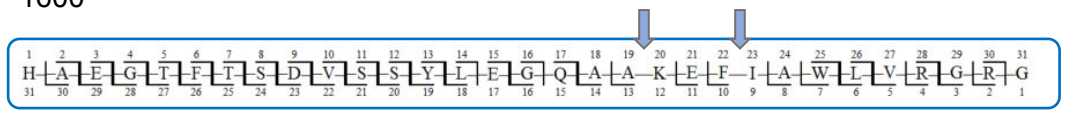


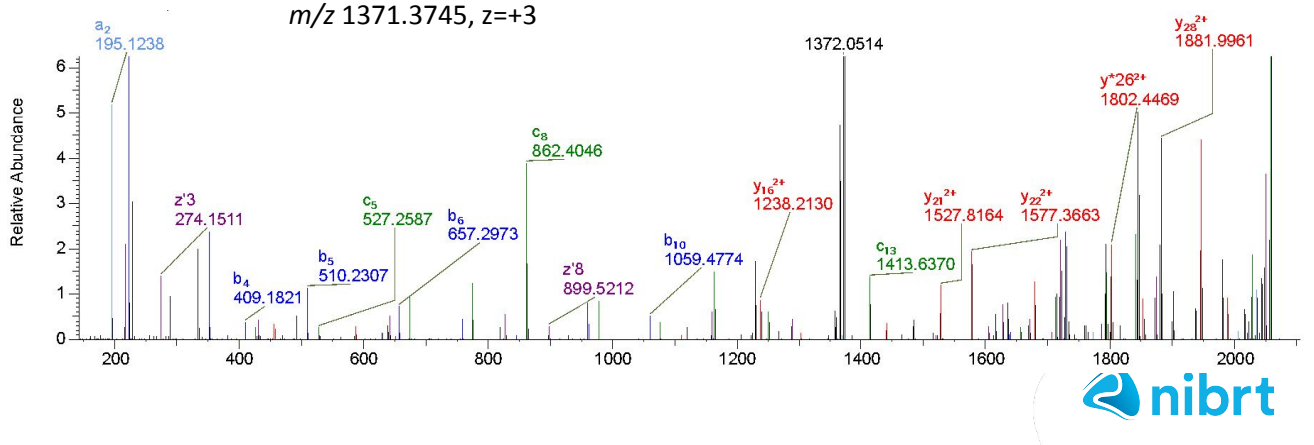

## Peptide characterization with EThcD

The predominant detected charge states for semaglutide are z=+4 and z=+3. Fragmentation using EThcD provides fragment ions supporting complete bond cleavages across the peptide supporting full sequence confirmation.

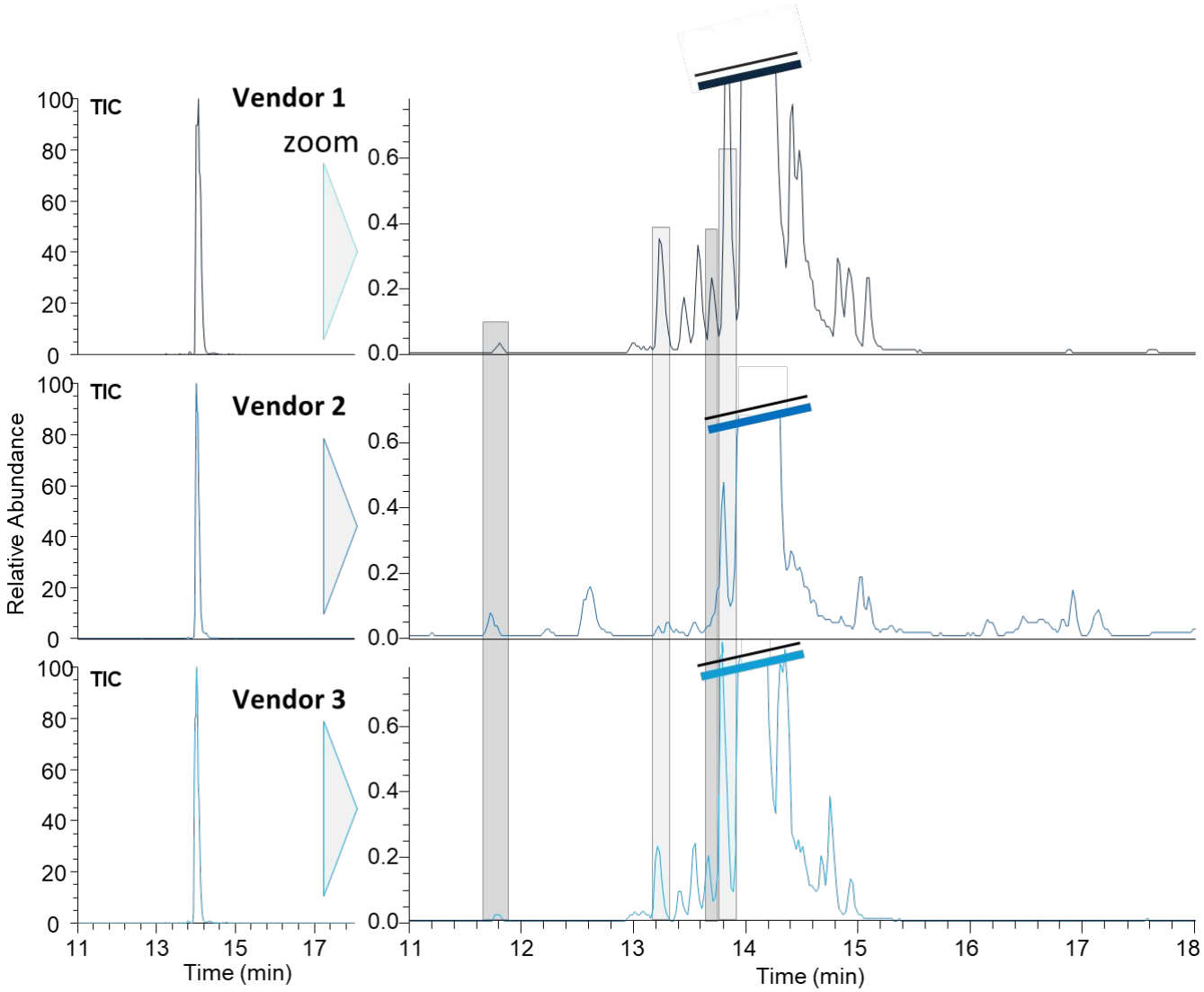


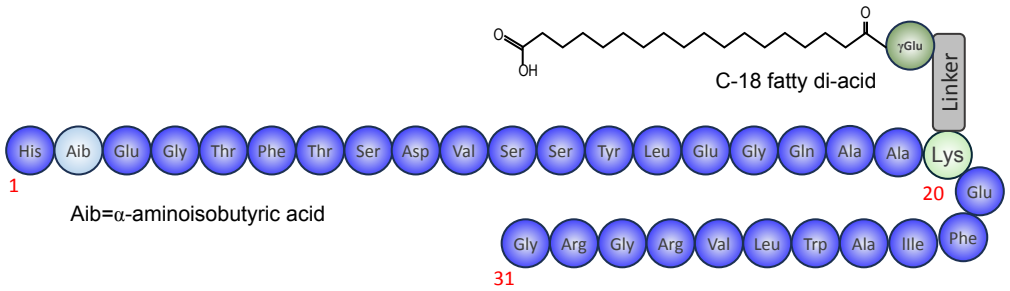


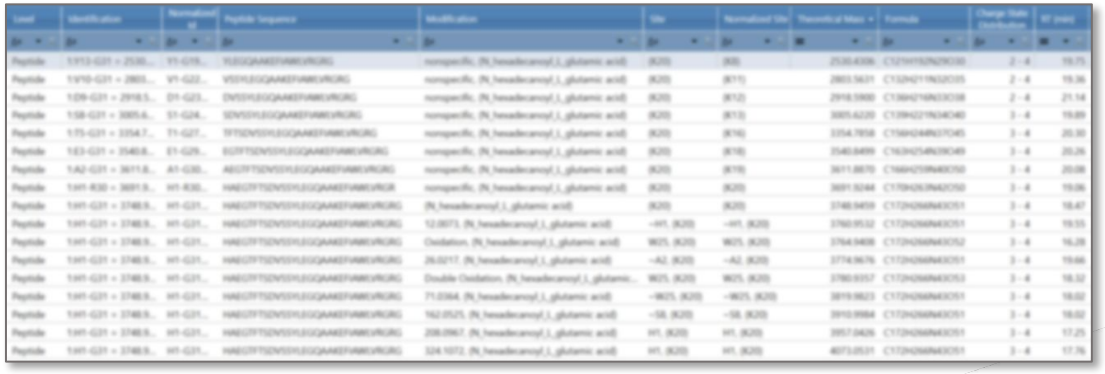


# MAM workflow for GLP-1 agonists

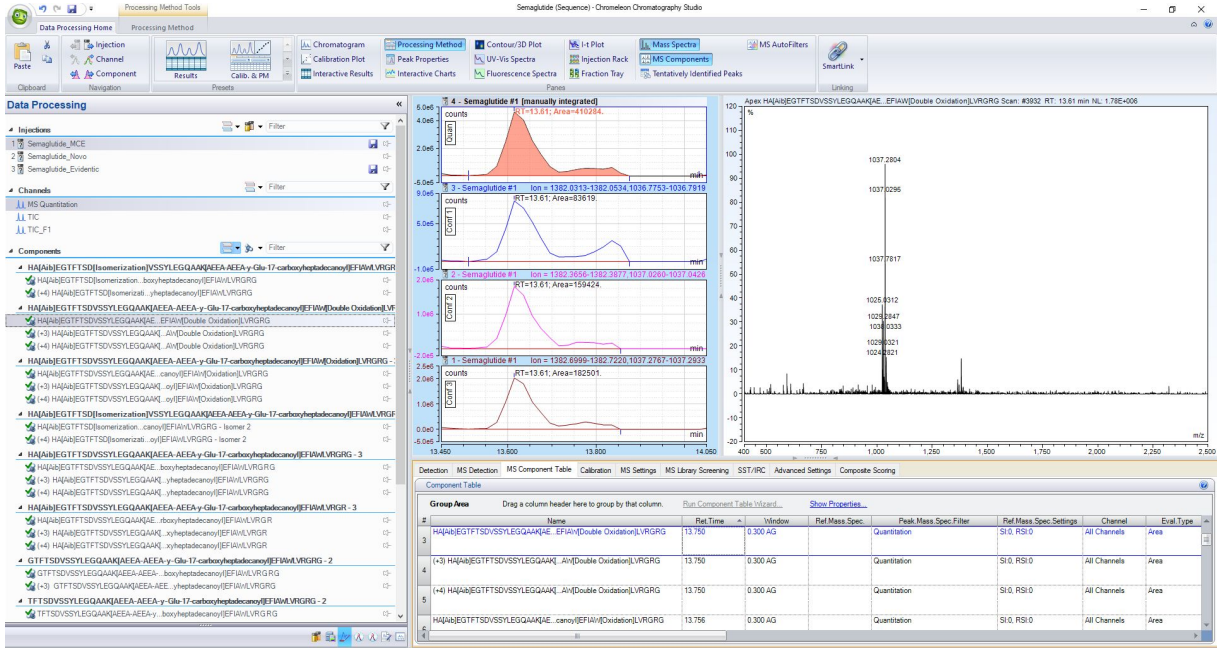



Simplified report output using targeted peptide workbook for data processing

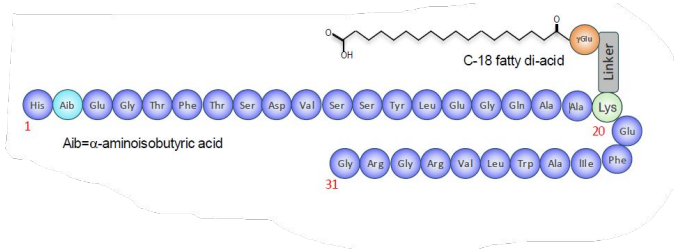



# MAM workflow for GLP-1 agonists




## MS Quantitation XIC traces

x10 zoomed for small impurities



# Conclusions

- MAM potential goes beyond QC environment and can facilitate biotherapeutics production for the whole life cycle.

- New modalities and sustainability goals are bringing new analytical challenges, but improved instruments and workflow robustness can accelerate method transfer in routine analysis to improve drug safety at any production scale and stage.

- MAM approach has huge potential for analysts with little MS expertise even outside industrial settings (regulatory agencies, law enforcement).

# Acknowledgments

- Silvia Millán Martín
- Corentin Beaumal
- Anna Mulligan
- Felipe Guapo Melo
- Jonathan Bones
- Kai Scheffler
- Cong Wang
- Catharina Crone
- Kelly Broster
- Ken Cook
- Ulrik Mistarz

nibrt
National Institute for Bioprocessing Research and Training

ThermoFisher SCIENTIFIC
The world leader in serving science