Comparison of MS platforms for quantitative analysis of peptides

Lieve Dillen

Drug Safety Sciences

Analytical Sciences/Non-regulated Bioanalysis

Acknowledgements:

Willy Cools
Liesbeth Vereyken
Willy Lorreyne
Tinne Huybrechts
Ronald De Vries
Russell Mortishire-Smith
Philip Timmerman
Hesham Ghobarah, AB Sciex
Filip Cuyckens

Drug Safety Sciences

Analytical Sciences/Non-regulated Bioanalysis

janssen | PHARMACEUTICAL COMPANIES OF Johnson & Johnson

Outline

- Rationale for quantitative MS analysis of peptides
- Evaluation of 6 peptides on different MS platforms
  - **Triple quadrupole instruments**:
    - MRM based approaches on QTRAP5500 and TQS
    - MRM^3 on QTRAP5500
  - **High resolution (HR) MS** on TripleTOF5600
- Comparison of platforms
  - Analysis of 3 calibration curves and QC
  - Evaluate sensitivity, accuracy and precision
- conclusions

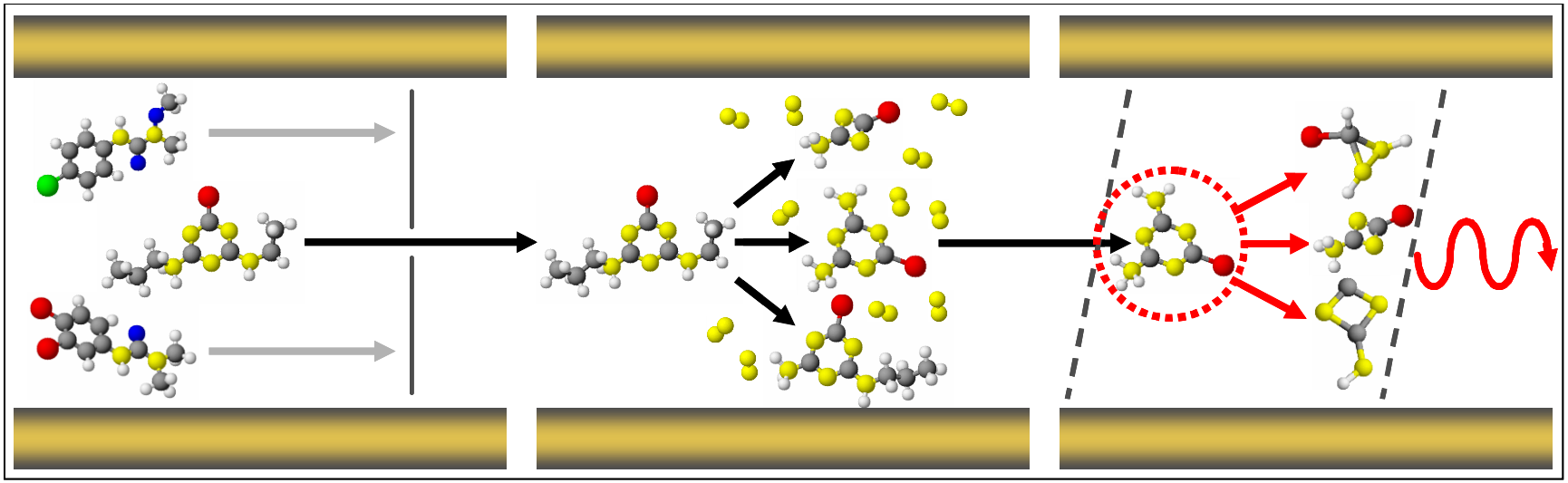
Rationale for quantitative MS analysis of peptides in biological matrices

- research programs introducing peptides as new drug entities increases
- Immunological methods not readily available in an early development phase
- LC-MS(MS) methods for ELISA validation
- Traditional and new MS approaches available

Traditional fragmentation approaches

- BA familiar with SRM/MRM based quantitation
  - Sensitivity and selectivity
  - On QQQ or ion trap instrumentation
- But for peptides …
  - Multiply charged peptides often show poor fragmentation efficiency
  - More elaborate method development (multiple charges, combination of multiple transitions)
- MS^3 on ion trap instruments
  - enhanced selectivity

MRM and MS^3 on QTRAP5500 for quantitative analysis



- better removal of endogenous interferences
- improved S/N (reduced background noise)

w/o fragmentation approaches

- SIM (and/or parent/parent SRM) not an option due to insufficient selectivity
- Targeted enhanced multiply charged scans (tEMC)
  - On hybrid quadrupole/linear ion trap
  - Eliminates singly charged ions (JASMS 2011,22:67-74)
- Targeted Ion parking (TIPing)
  - Peptide-specific charge state reduction through ion-ion reaction (JASMS 2010, 21:2011-2022)
- FAIMS-MS (pseudo SRM) (RCMS2009, 23:2301-23060)
- **High resolution/accurate mass**
  - **All available charge states detected – sum charges and isotopes**
  - **Specificity through narrow XIC (typically) 5-50 mDa**

Experimental set up – the peptides

Peptide name	# AA	Average Mr	modification
Glufibrinogen peptide	14	1570	none
somatostatin	**14**	**1638**	**none**
TRI1144	38	4499	Acetylation, amidation
exenatide	39	4187	Amidation
enfuvirtide	36	4492	Acetylation, amidation
C34	**34**	**4289**	**Acetylation, amidation**

Shimadzu LC (LC20) - QTRAP5500: MRM and MRM^3

NanoAcquity - TQS

Nexera - tripleTOF5600

Experimental set up – sample prep/LC

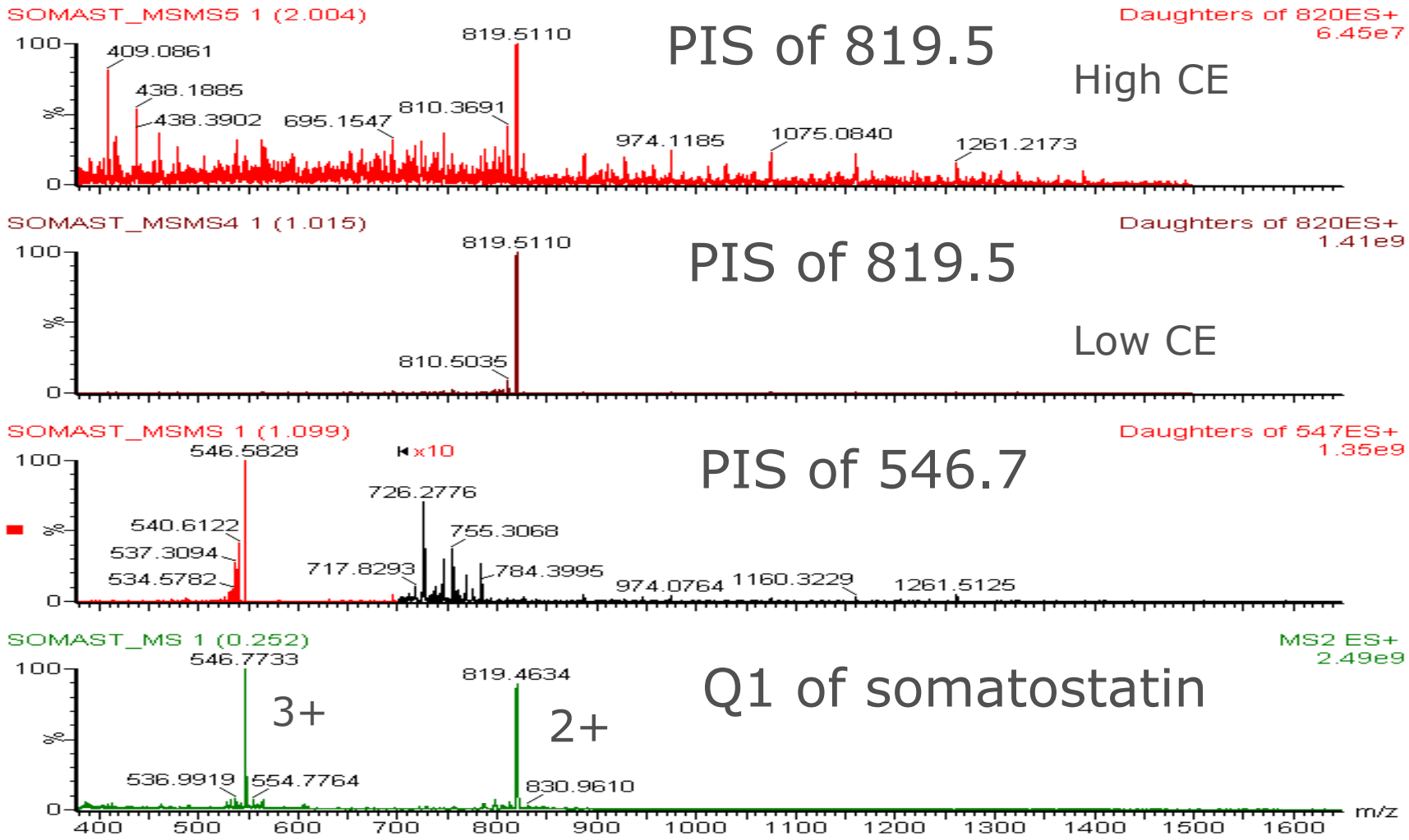
	Nexera-triple TOF 5600 Shimadzu –QTRAP5500	**nanoAcquity-TQS**
Column	2.1 x 50 mm, X-bridge C_{18} 300A 3.5 µ	X-bridge C_{18} **0.3 x 100 mm 3.5 µ**
Flow rate	**250 µl/min**	**15 µl/min**
Sample prep	Protein precipitated plasma Add 5 parts of ACN Diluted 1:1 with 0.2% formic acid Spike peptides – cassettes of peptides	idem
solvents	Solvent A: 0.1% Formic acid - solvent B: Acetonitrile	idem
Injection volume	**5 µl** (0.2 µl plasma equivalent on column)	**2 µl** (0.08 µl plasma equivalent on column)

Experimental set up – Quantification approach QQQ: QTRAP5500 and TQS

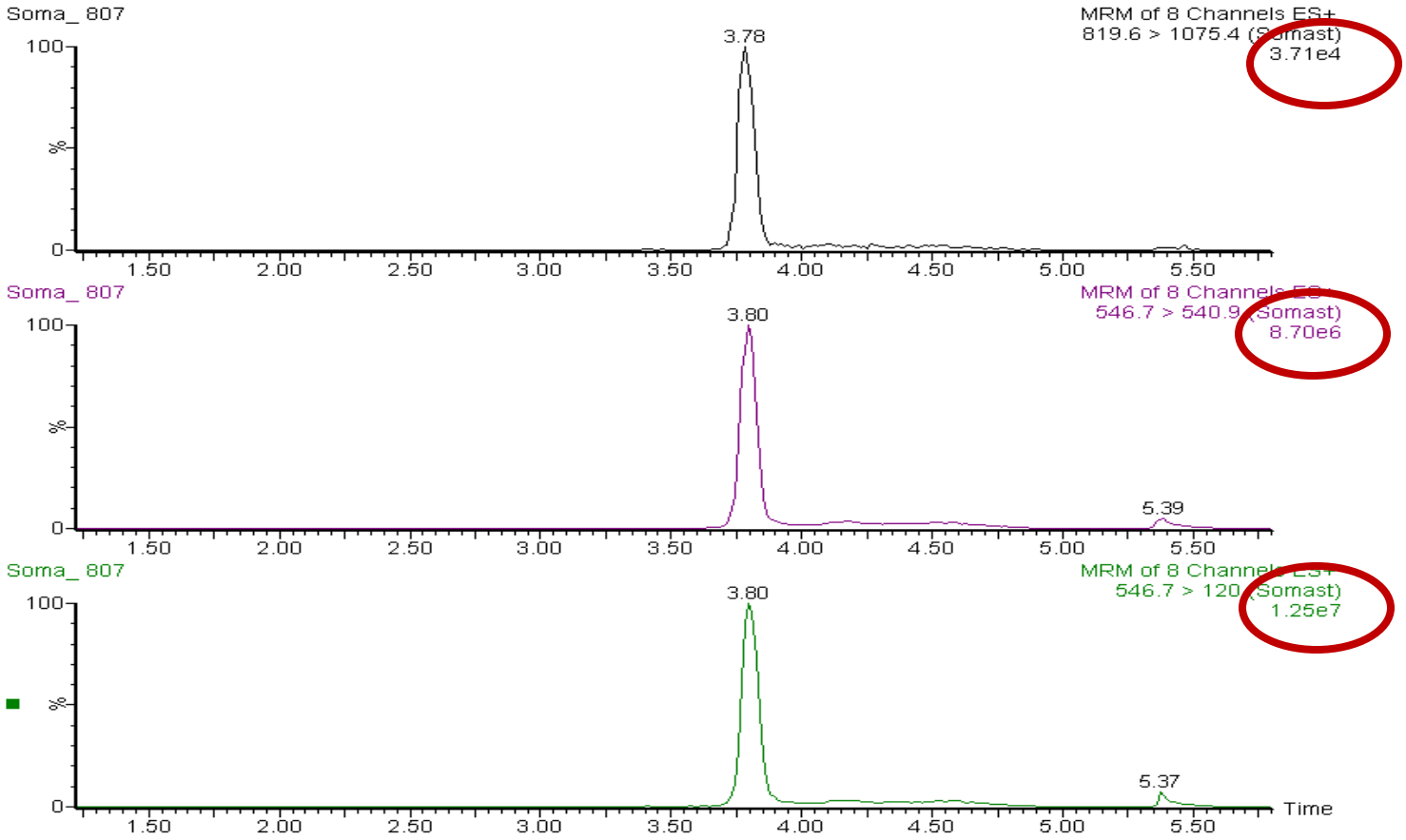
- 3 most abundant transitions summed – evaluate accuracy and precision
  different charge states preferred over different transitions from same charge state

Peptide name	# AA	QTRAP5500			TQS	
		charge	Q1 m/z	Q3 m/z	Q1 m/z	Q3 m/z
somatostatin	14	3	546.7	537.2 540.6 120.2	546.6	537.5 540.9 726.7
C34	34	4	1073.2	1068.7 1040.5 1012.3	1073.0	1068.6 1040.5 1012.2

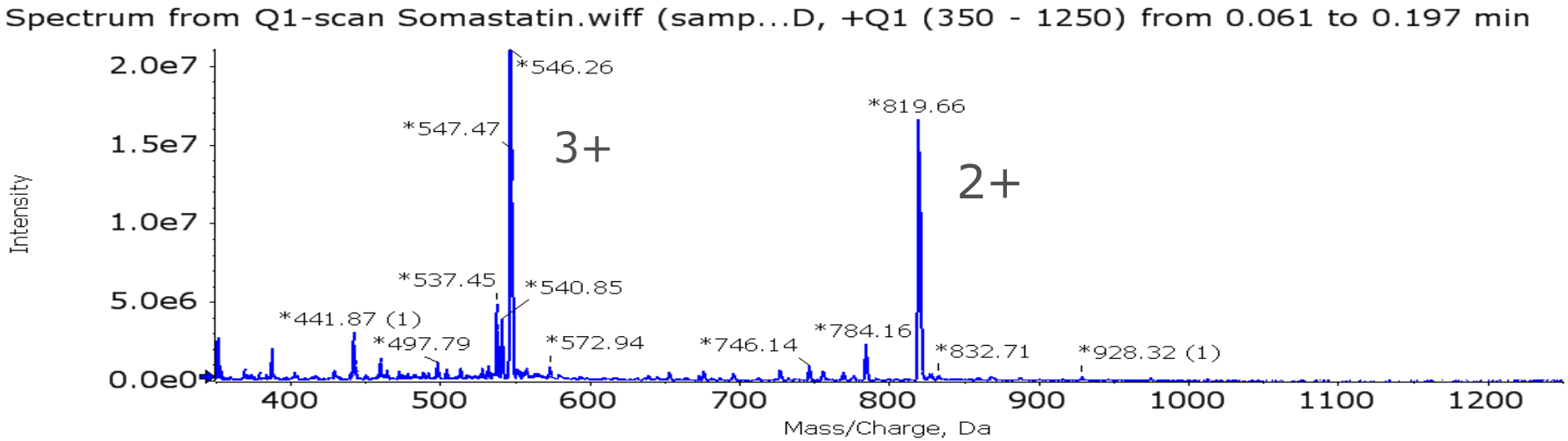
Somatostatin spectra on TQS

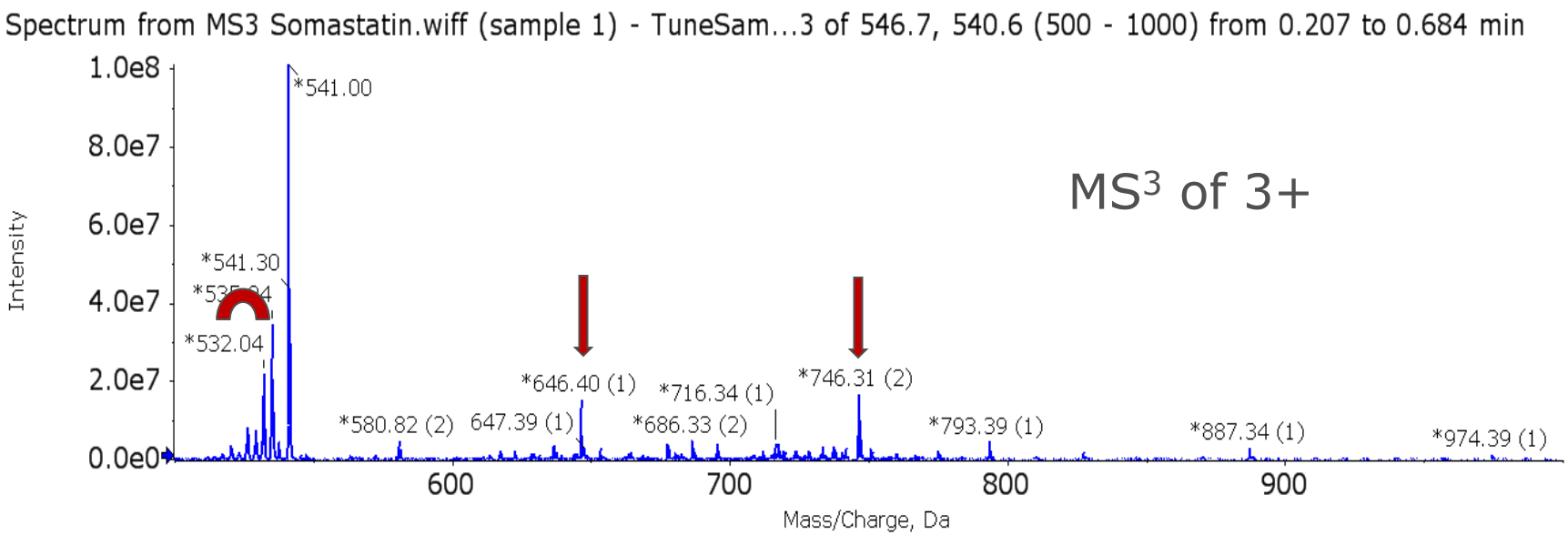


Somatostatin MRMs on TQS

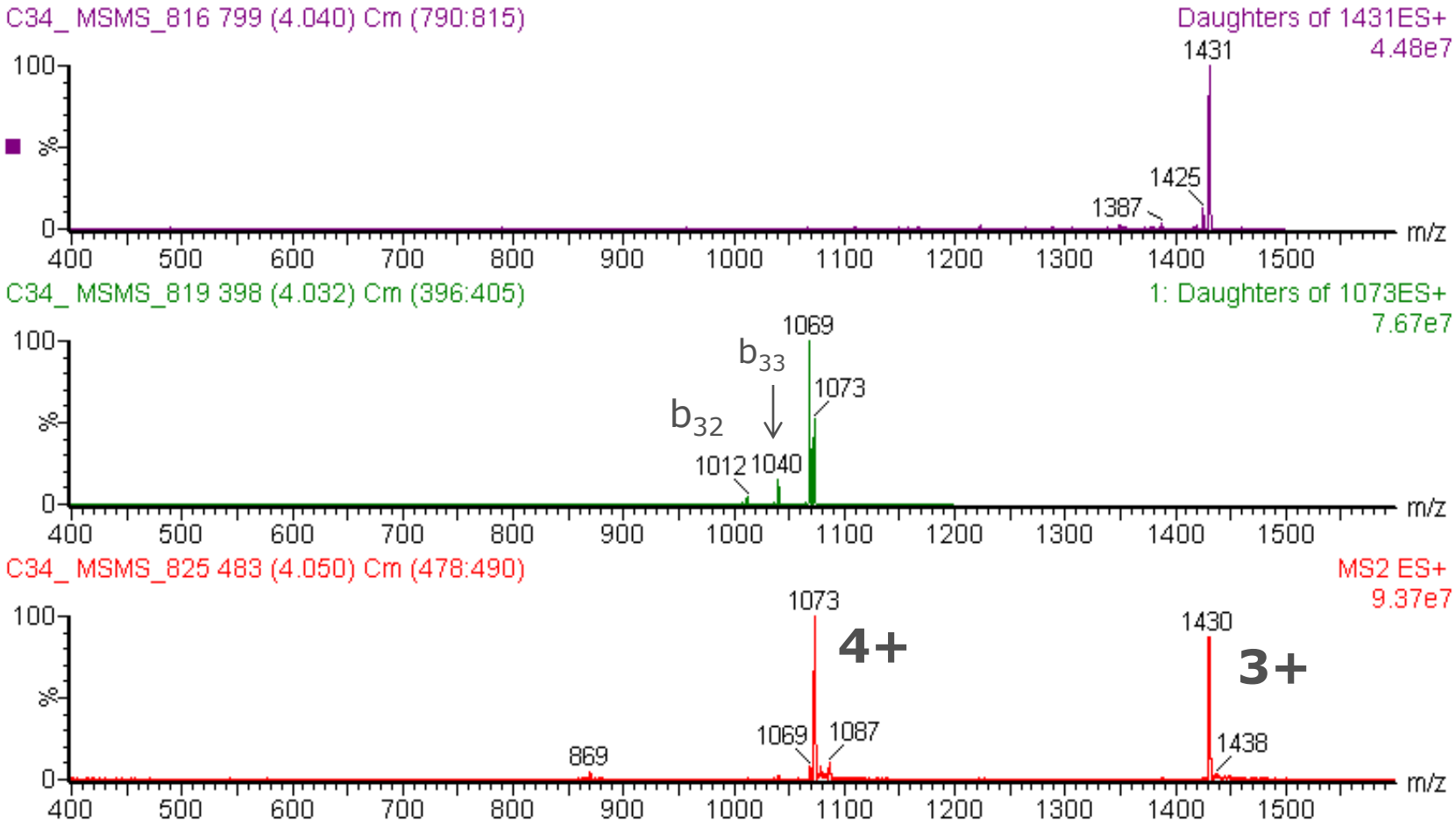


Q1 and MS^3 spectra for somatostatin on QTRAP5500

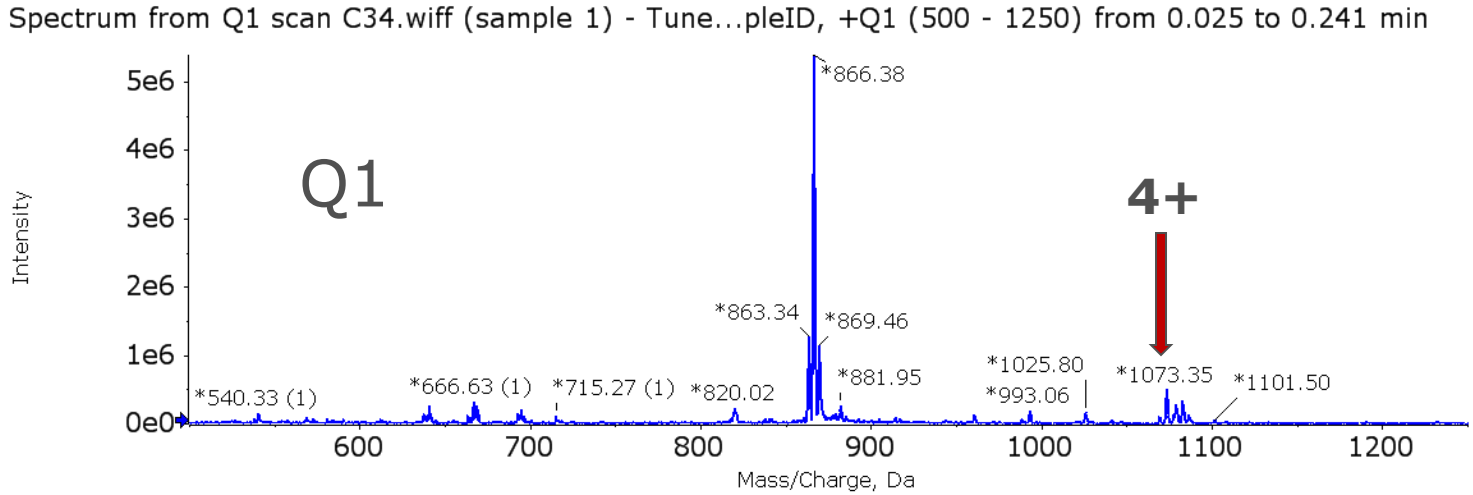


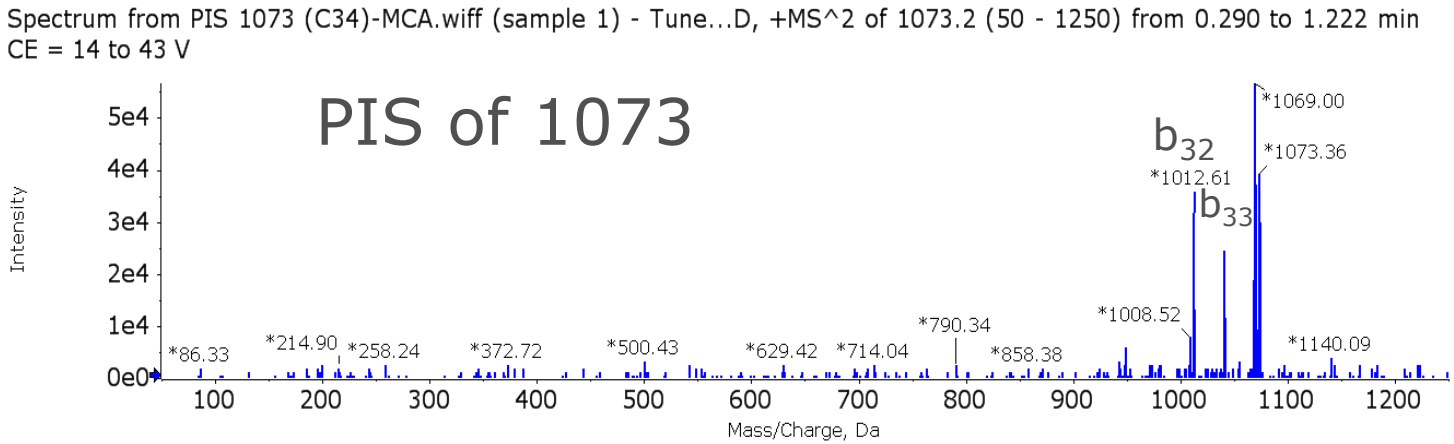


Q1 and Product ion spectra for C34 on TQS



Q1 and Product ion spectra for C34 on QTRAP5500





Conclusions part 1: optimizing QQQ methods

- QQQ: method development needs careful optimization
  - Multiple charge states
  - Fragmentation – peptide and charge state dependent
  - flow rate: lower flow rates offer better sensitivity for peptide quan
  - summing transitions can improve sensitivity and reproducibility

High Resolution Mass spectrometry

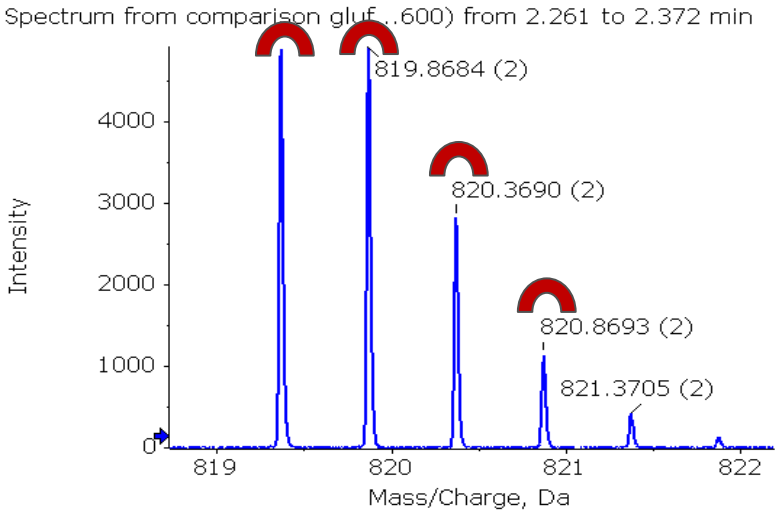
Experimental set up – HRMS – Triple TOF 5600

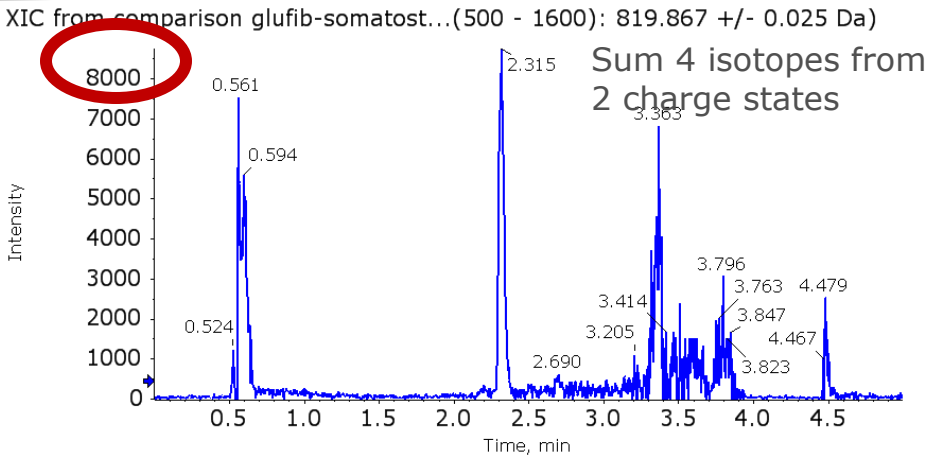
- XIC extraction 0.05 amu, use different charge states and 4 most abundant isotopes/charge state

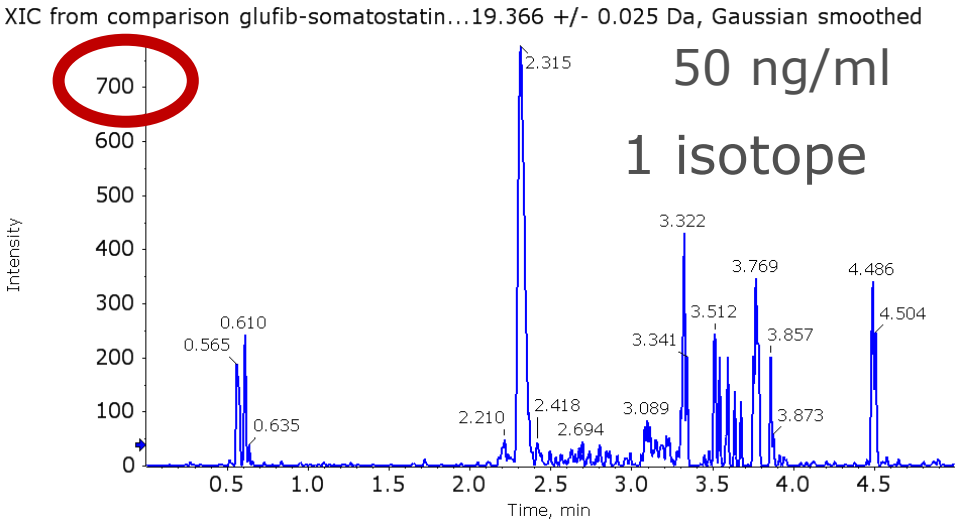
Peptide name	# AA	m/z
somatostatin	14	1637.7245 (1+) **819.3662 (2+)** **546.5800 (3+)**
C34	34	**1430.0138 (3+)** **1072.7623 (4+)** 858.4114 (5+)

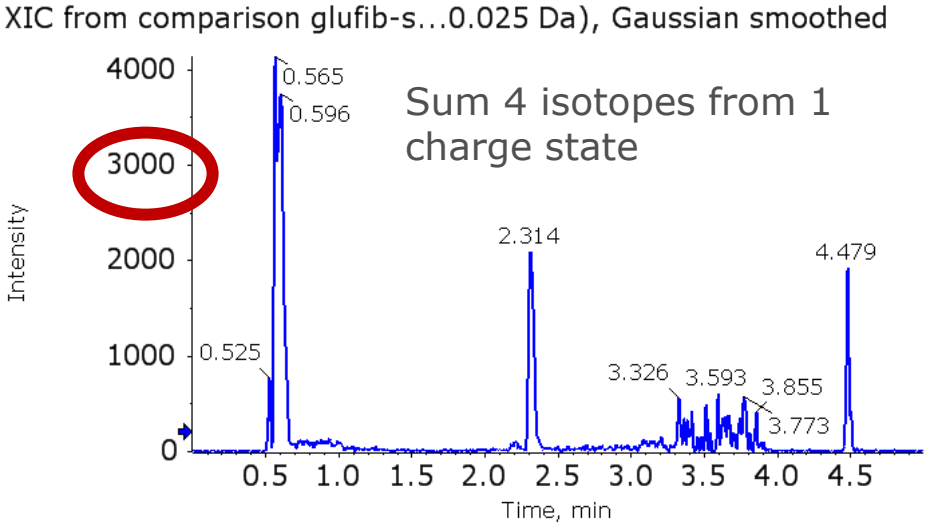
Somatostatin – HRMS

AGCKNFFWKTFTSC

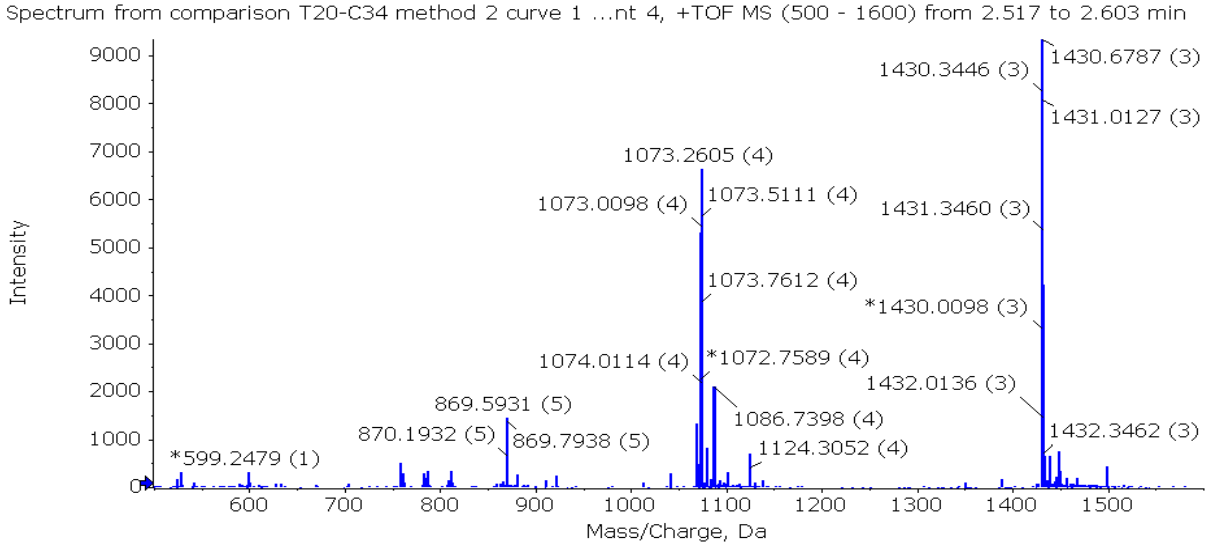


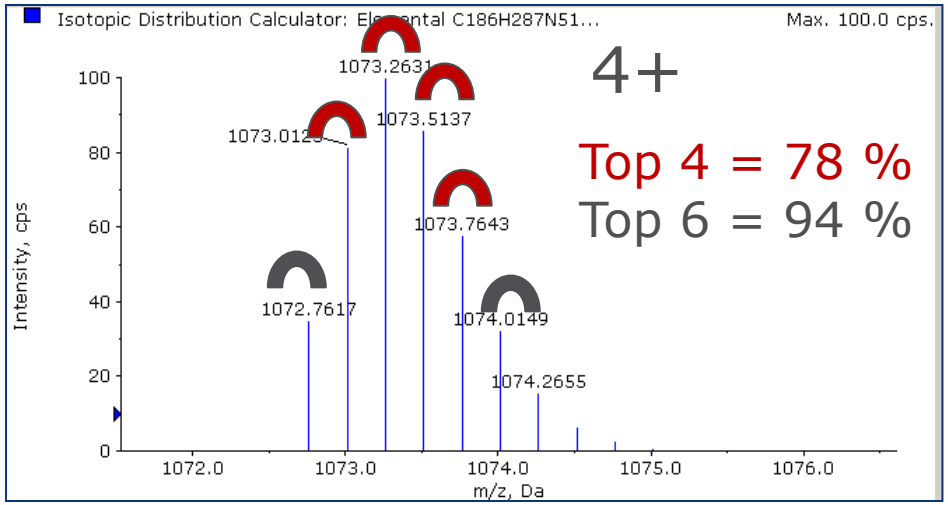


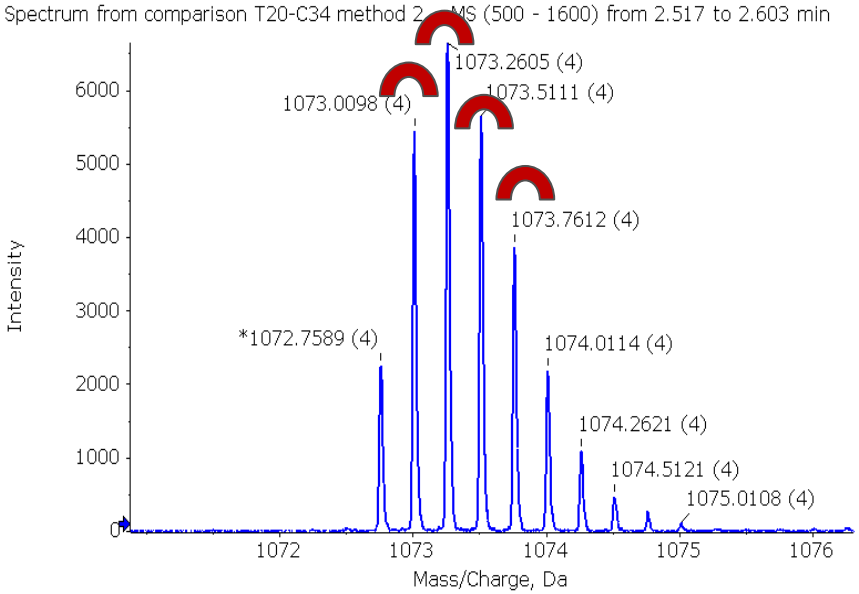




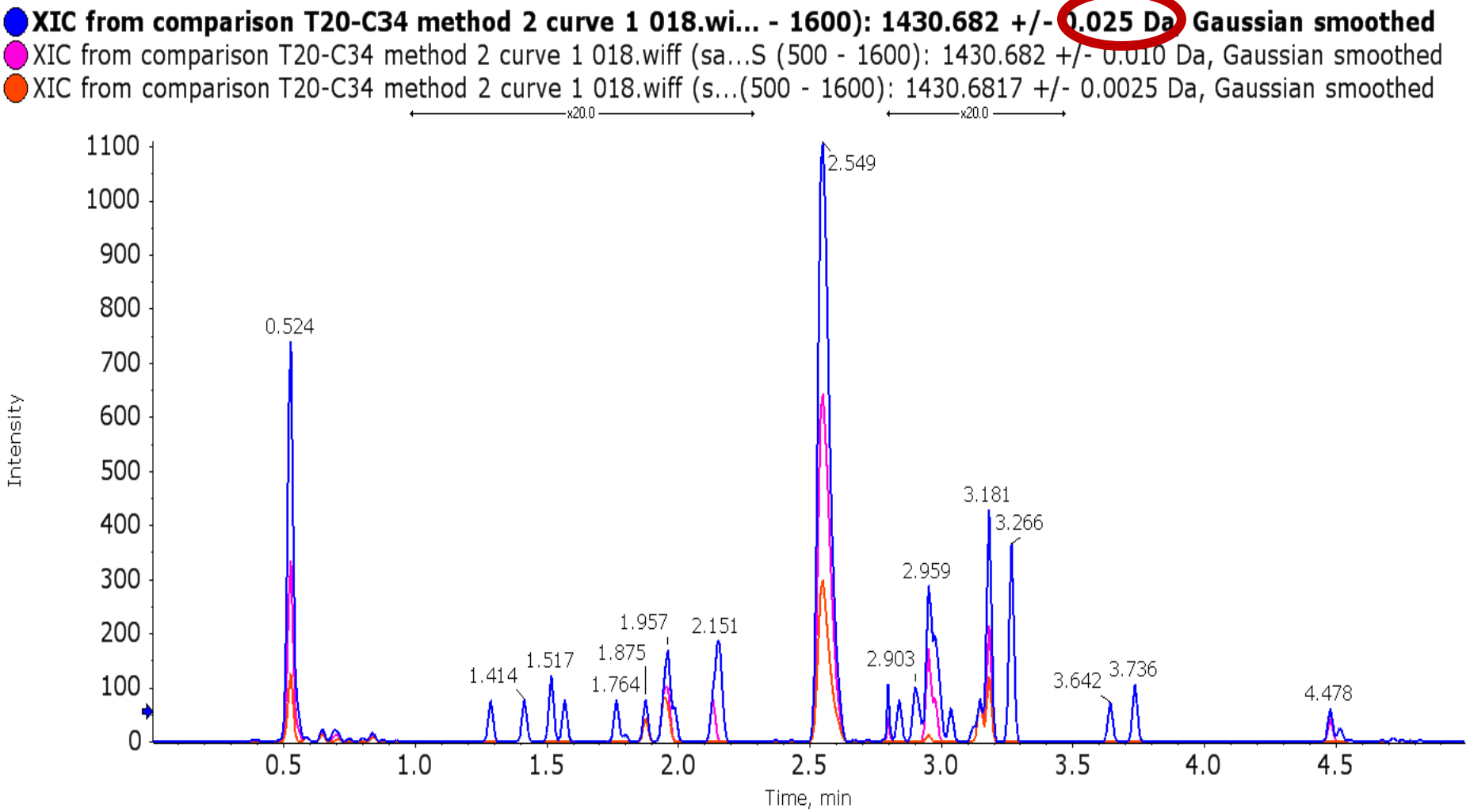
C34 - HRMS


Spectrum from comparison T20-C34 method 2 curve 1 ...nt 4, +TOF MS (500 - 1600) from 2.517 to 2.603 min
Intensity
Mass/Charge, Da
1430.6787 (3)
1430.3446 (3)
1431.0127 (3)
1073.2605 (4)
1073.0098 (4)
1073.5111 (4)
1431.3460 (3)
1073.7612 (4)
*1430.0098 (3)
1074.0114 (4)
*1072.7589 (4)
1432.0136 (3)
869.5931 (5)
1086.7398 (4)
870.1932 (5)
869.7938 (5)
1124.3052 (4)
1432.3462 (3)
*599.2479 (1)

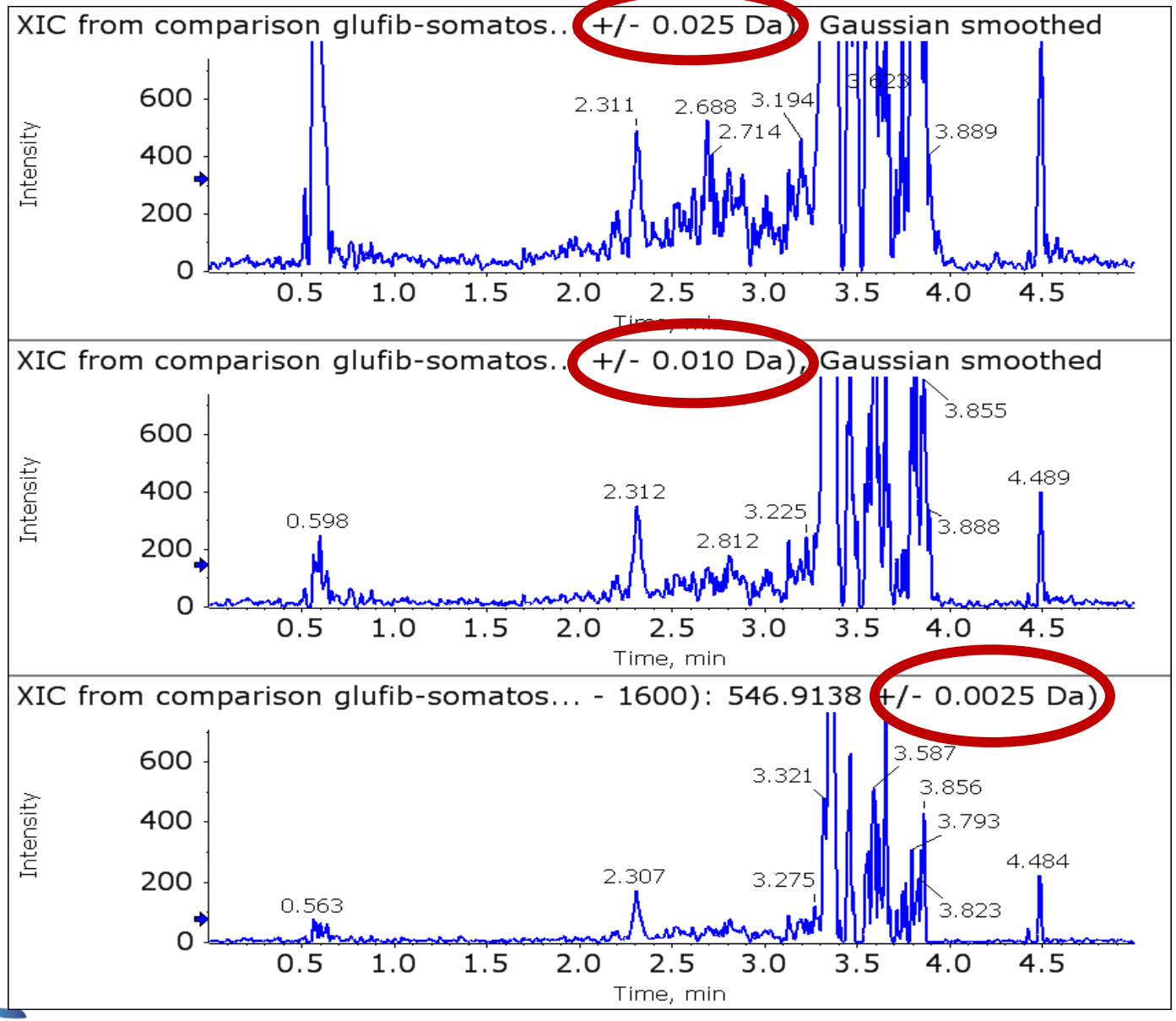

Isotopic Distribution Calculator: Elemental C186H287N51...
Max. 100.0 cps.
Intensity, cps
m/z, Da
1073.2631
1073.5137
1073.0123
1073.7643
1072.7617
1074.0149
1074.2655
4+
Top 4 = 78 %
Top 6 = 94 %


Spectrum from comparison T20-C34 method 2 MS (500 - 1600) from 2.517 to 2.603 min
Intensity
Mass/Charge, Da
1073.2605 (4)
1073.0098 (4)
1073.5111 (4)
1073.7612 (4)
*1072.7589 (4)
1074.0114 (4)
1074.2621 (4)
1074.5121 (4)
1075.0108 (4)

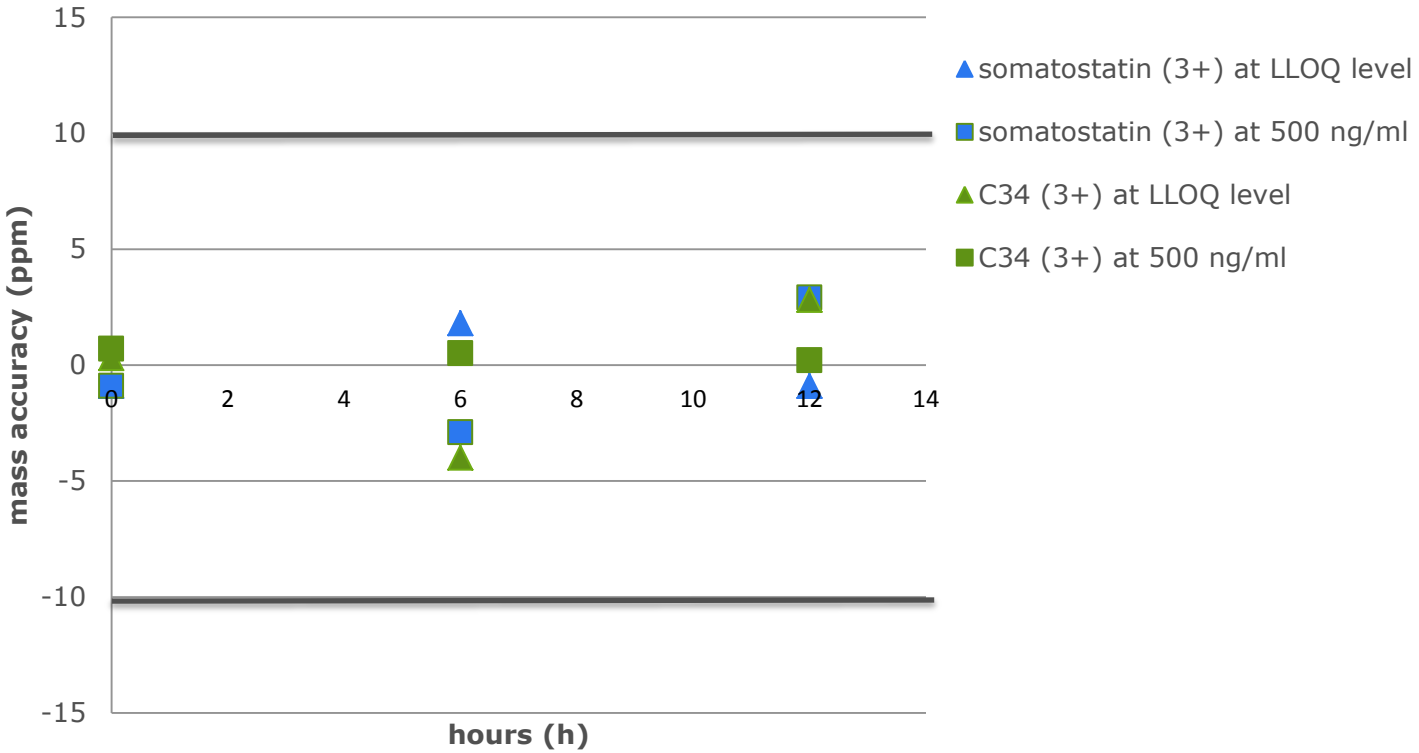
C34 – HRMS – effect of extraction window



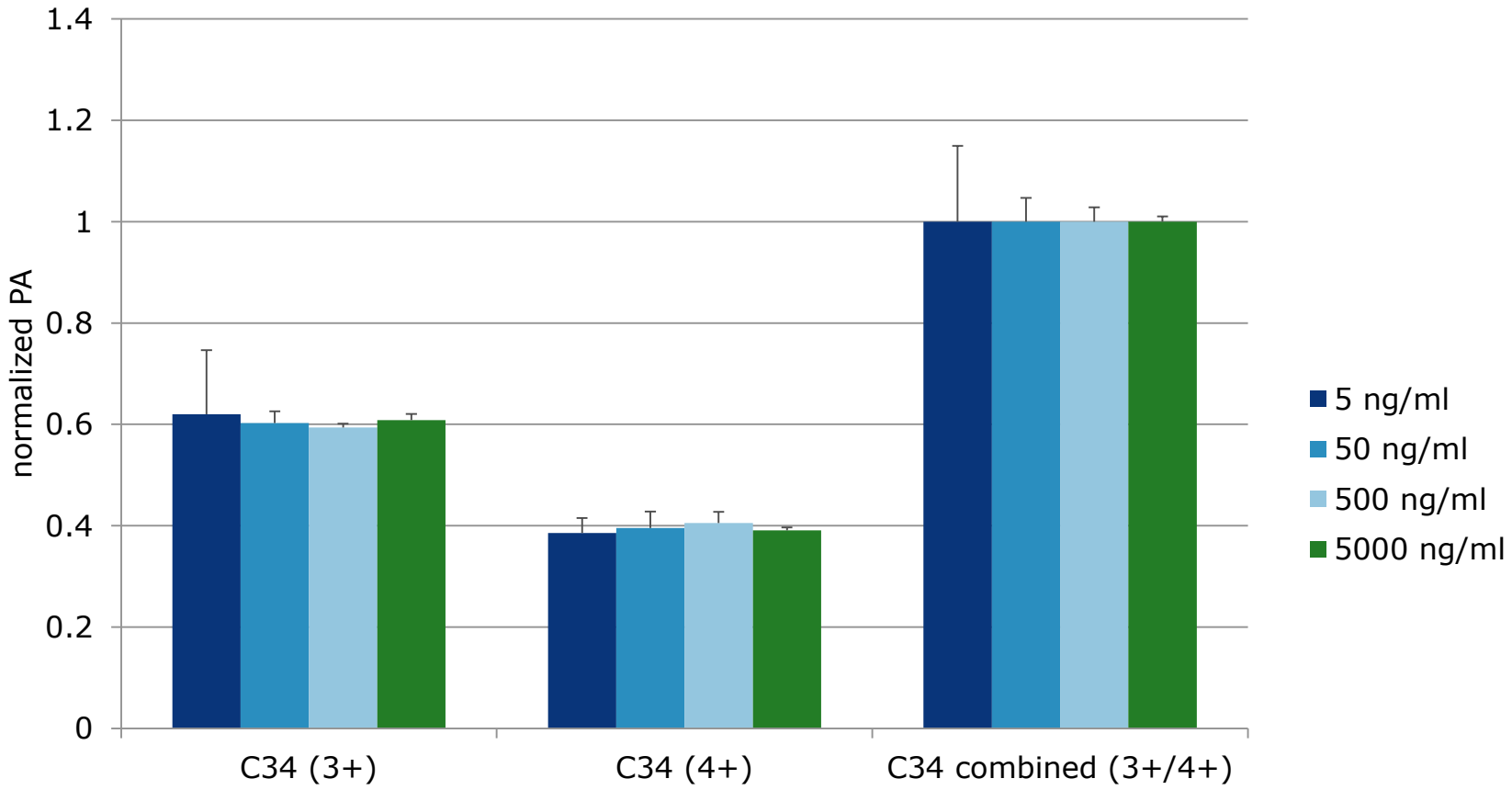
HRMS – effect of extraction window – LLOQ chromatograms



TripleTOF5600: mass accuracy over concentration and time range



TripleTOF5600: relative intensity of different charge states in function of concentration for C34



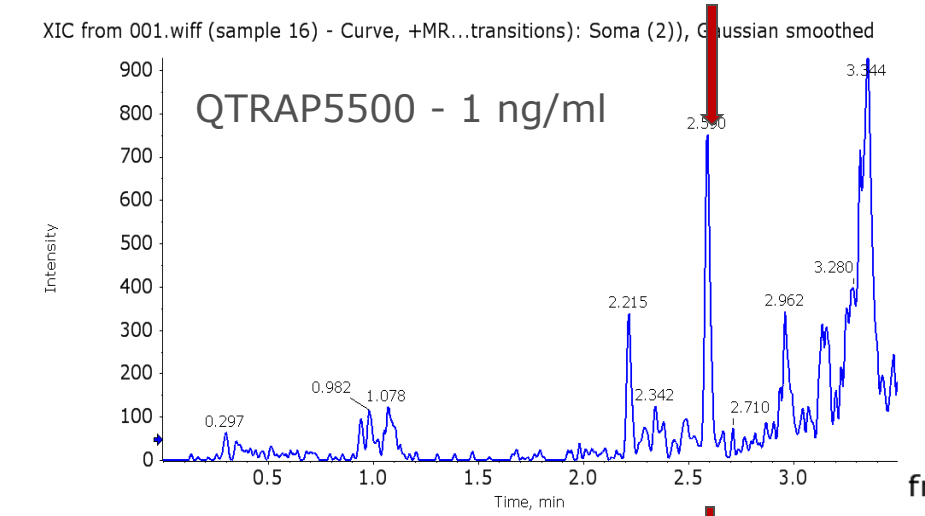
No change in charge state distribution in function of concentration

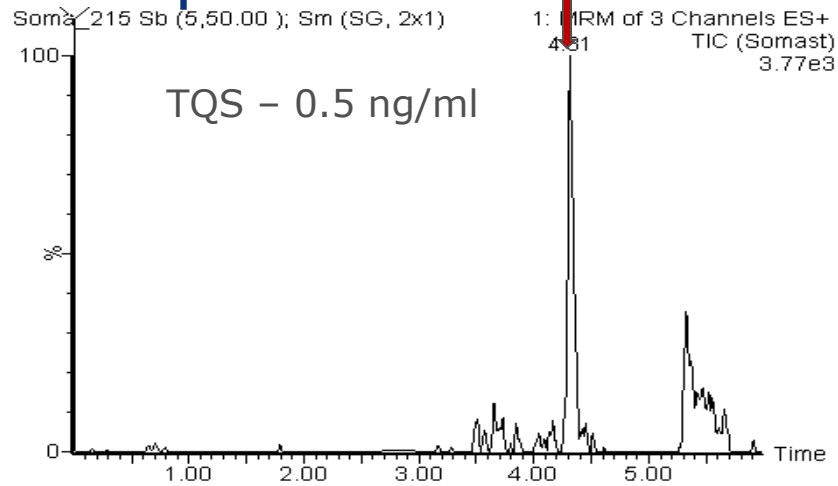
Conclusions part 2: optimizing HR methods on TripleTOF5600

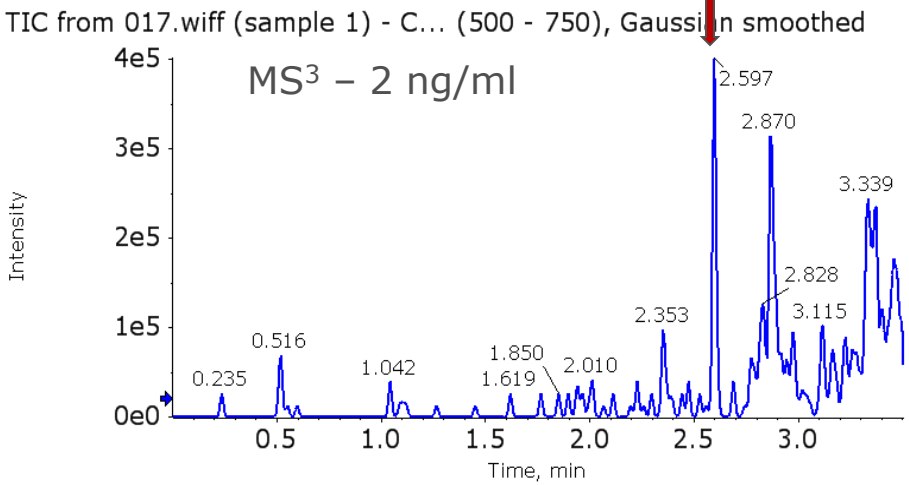
- Combine all available charge states
- Isotopic resolution: take into account a least 4 most abundant isotopes
- Extraction window:
  - Narrow XIC (5-10 mDa) offer specificity
  - Sensitivity dependent on width of window
- Mass accuracy over time (10 mDa extraction windows)
- No effect on charge state distribution with concentration
  - For our peptides and experimental conditions

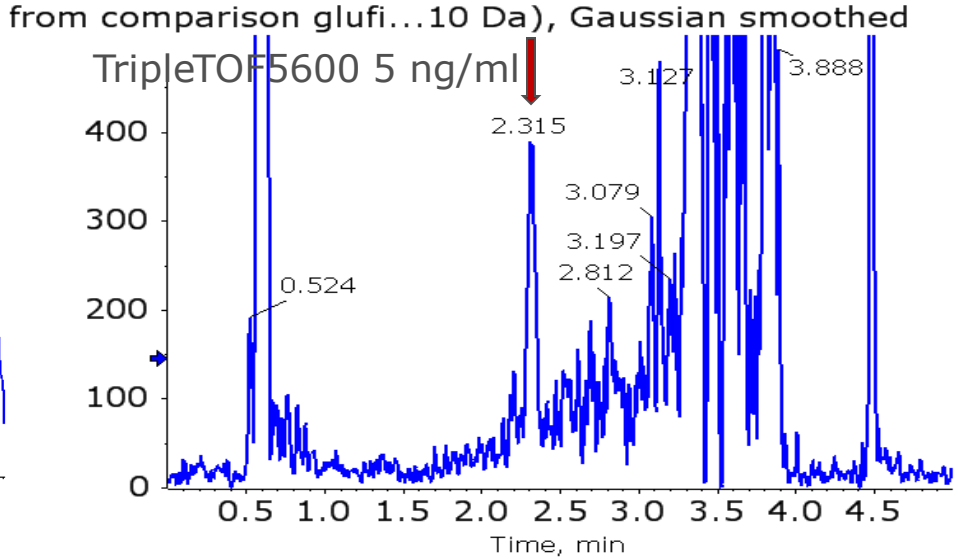
Comparison of the results on different platforms

LLOQ chromatograms of somatostatin on QTRAP5500, TQS and TripleTOF5600

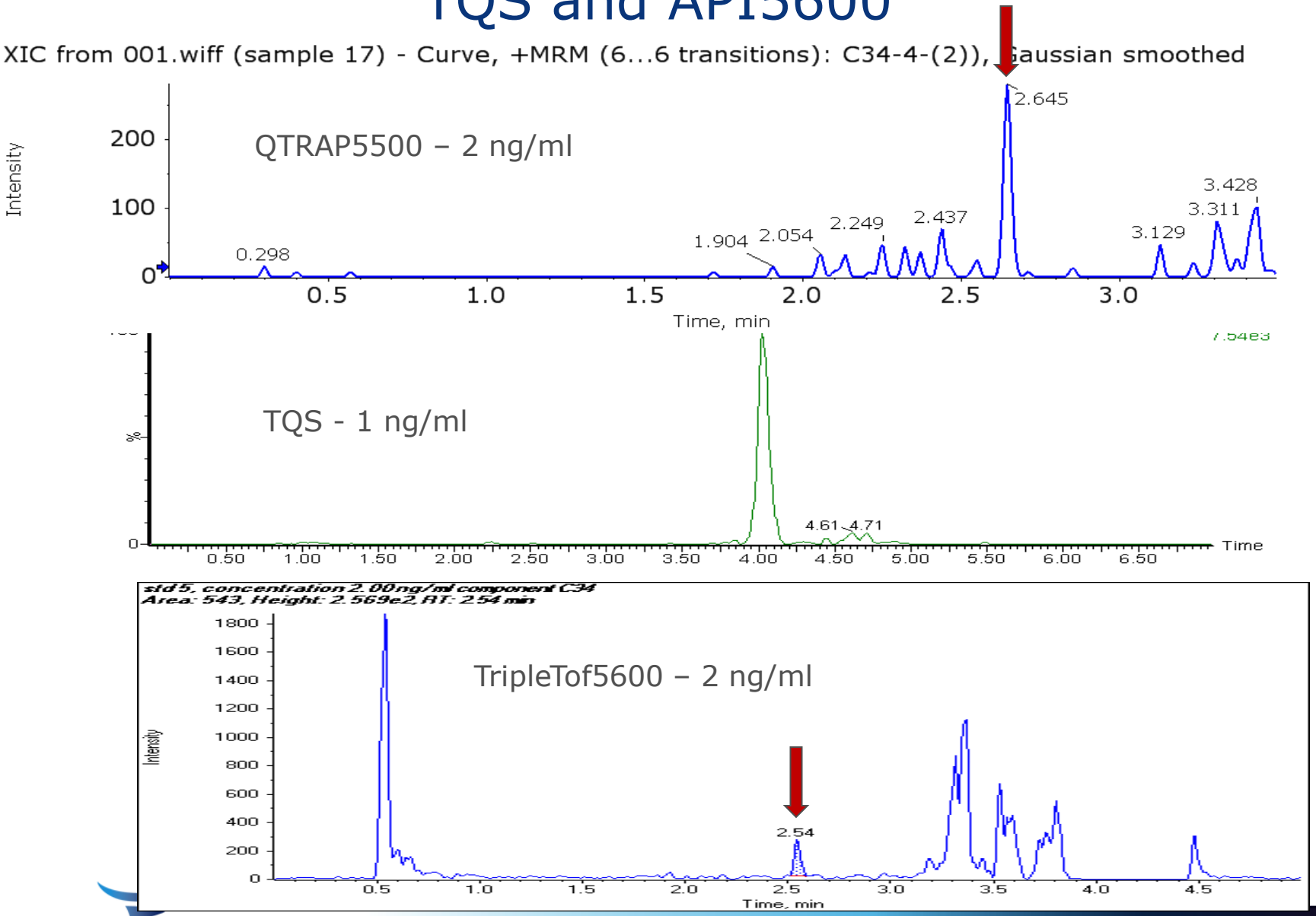






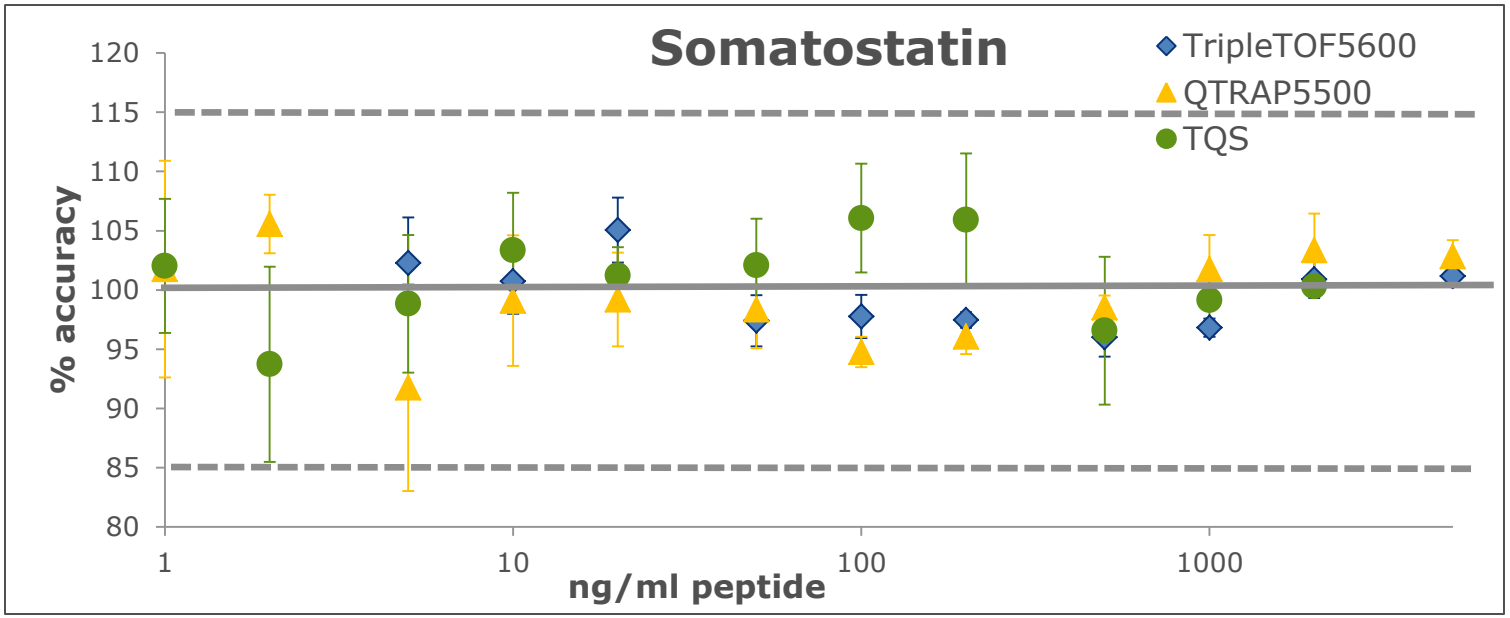


LLOQ chromatograms of C34 on API5500, TQS and API5600

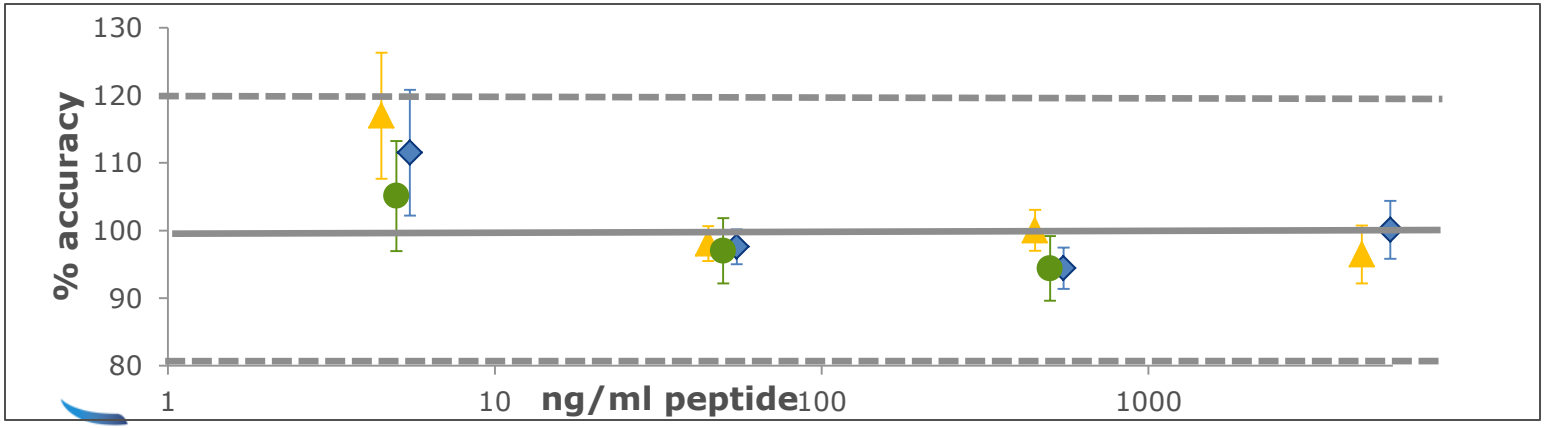


Comparison of results obtained on different instruments

mean of 3 calibration curves

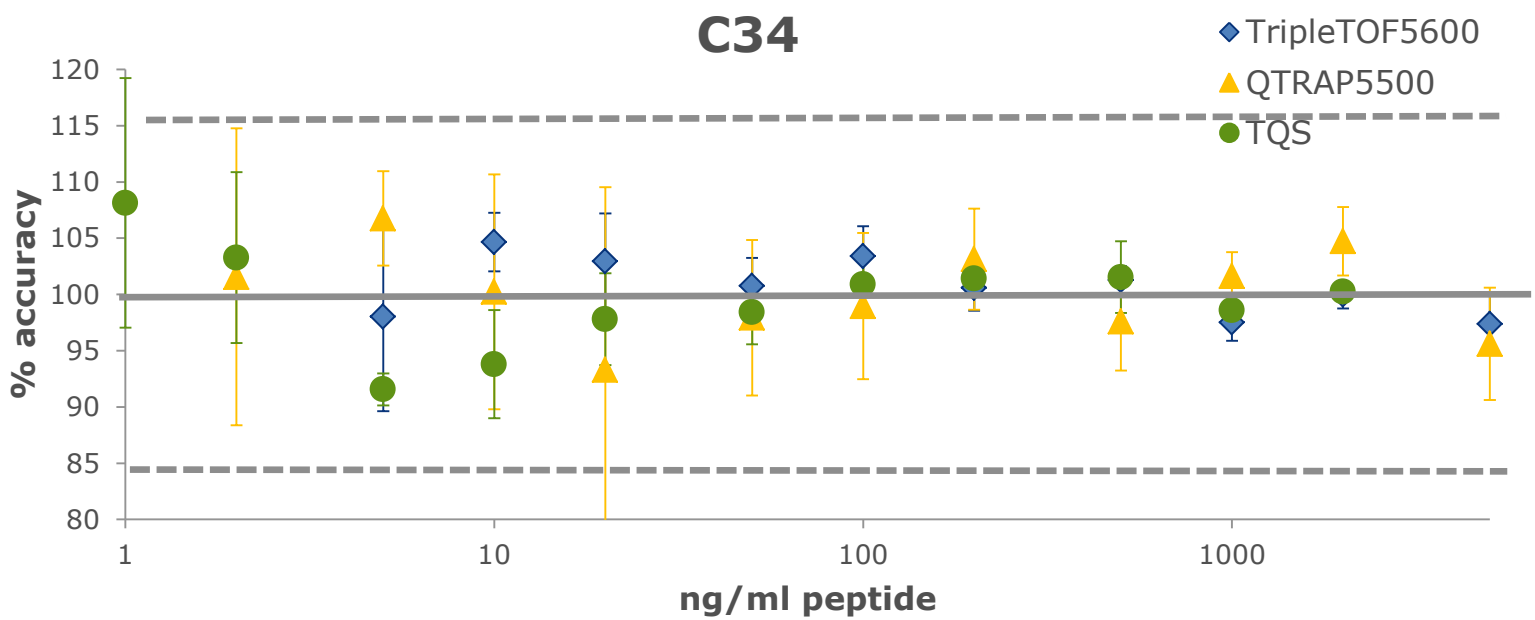


mean of 18 QC samples/concentration level

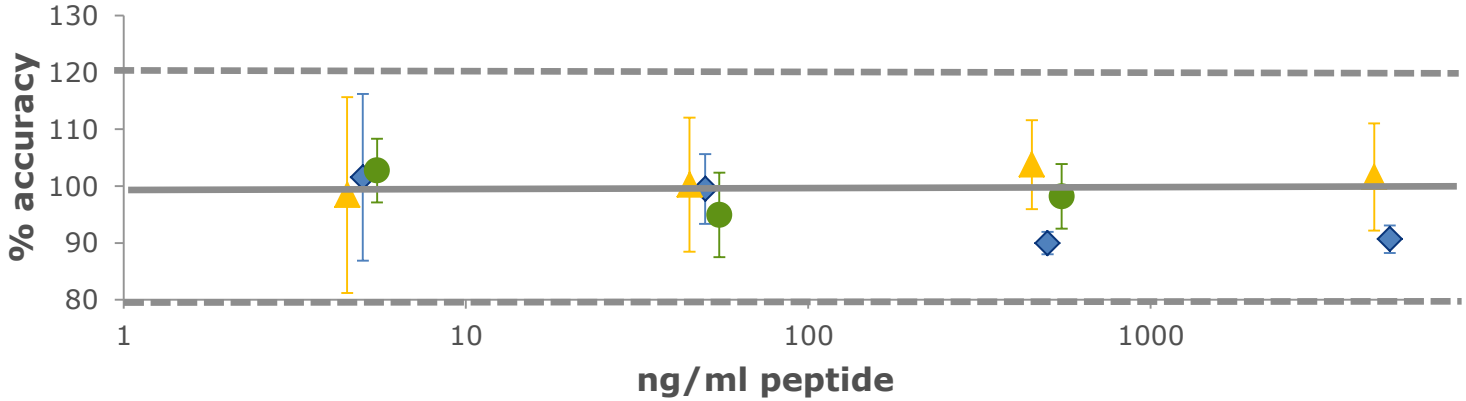


Comparison of results obtained on different instruments

mean of 3 calibration curves



mean of 18 QC samples/concentration level



Comparison LLOQ in PP plasma (n=3)

peptide	LC-QTRAP5500	micro LC-TQS	LC-TripleTOF5600
	LLOQ in ng/ml (%CV)		
glufibrinogen	1.00 (20.8)	1.00 (9.06)	5.00 (1.42)
somatostatin	1.00 (9.15)	0.500 (9.14)	5.00 (3.85)
TRI1144	2.00 (12.6)	5.00 (12.4)	5.00 (11.5)
exenatide	10.0 (6.43)	2.00 (6.44)	100 (2.60)
T20	5.00 (7.77)	2.00 (11.2)	5.00 (8.85)
C34	2.00 (13.2)	1.00 (11.1)	2.00 (8.41)

Impact of CO on the LLOQ

Conclusions Part 3: comparison

- All platforms : good performance for quantitative analysis for peptides
- Sensitivity gap between QQQ en HR (TripleTOF5600): +/- 5X
- Micro LC can offer better S/N chromatograms
- In discovery: ultimate sensitivity may not be required in first PK studies – HR might reduce MS-method development time