

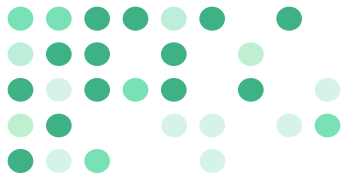
High Resolution and Accurate Mass Spectrometry for Specific and Sensitive Quantification of Target Compounds in Complex Matrices.

Dr. Steven Watt

A&M Labor für Analytik und Metabolismusforschung GmbH

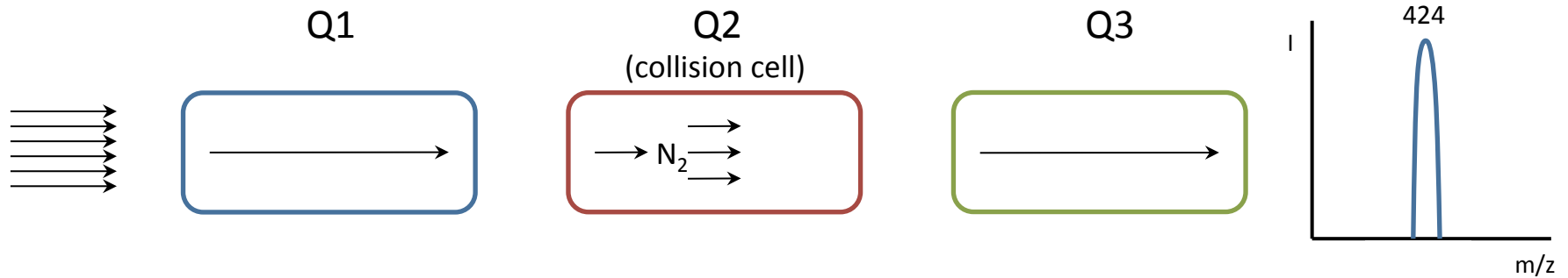
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A&M STABTEST Labor für Stabilitätsprüfung GmbH

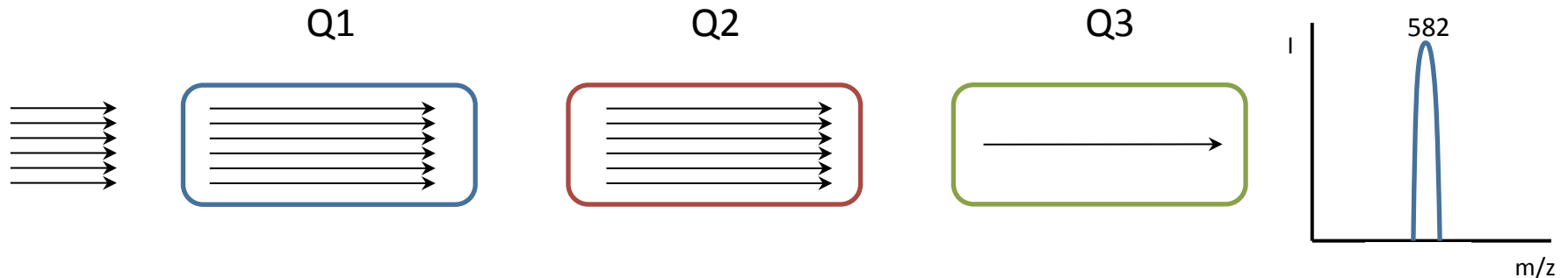


Measurement in SRM and SIM Mode

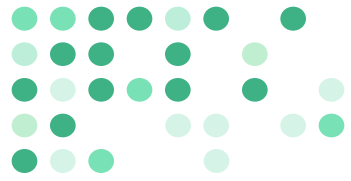
SRM:



SIM:

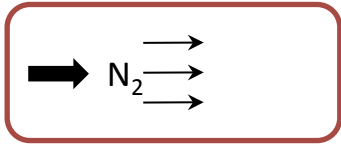


➔ Do we lose signal intensity by quantifying a fragment ion? *Of course!*

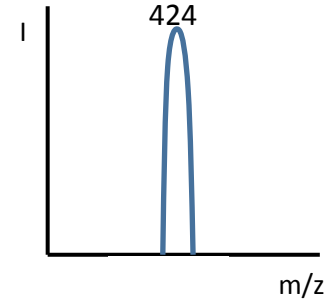
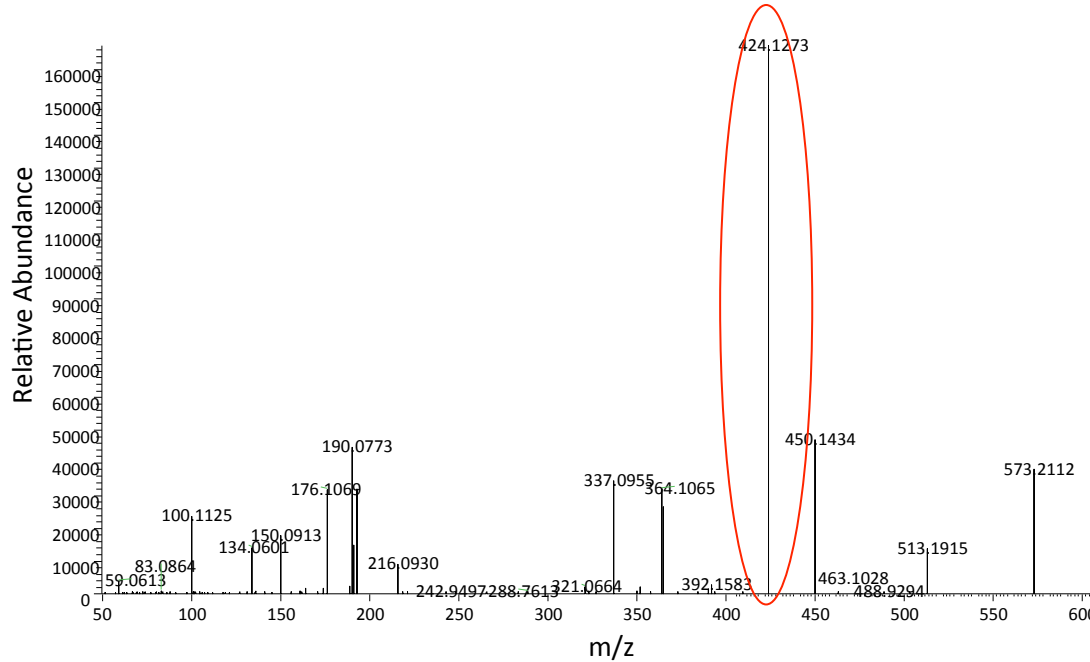


Product Ions Lost During Fragmentation

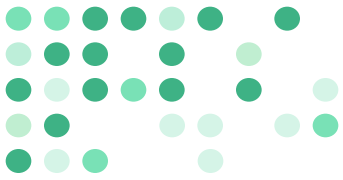
Q2
(collision cell)



MS² spectrum of the parent compound



➔ Several product ions are lost, because Q3 only selects one product ion.



SRM Mode vs. SIM

AB SCIEX 5500 QTRAP®

	Signal Intensity:
SIM:	$4.47 \cdot 10^5$
SRM:	$1.65 \cdot 10^4$

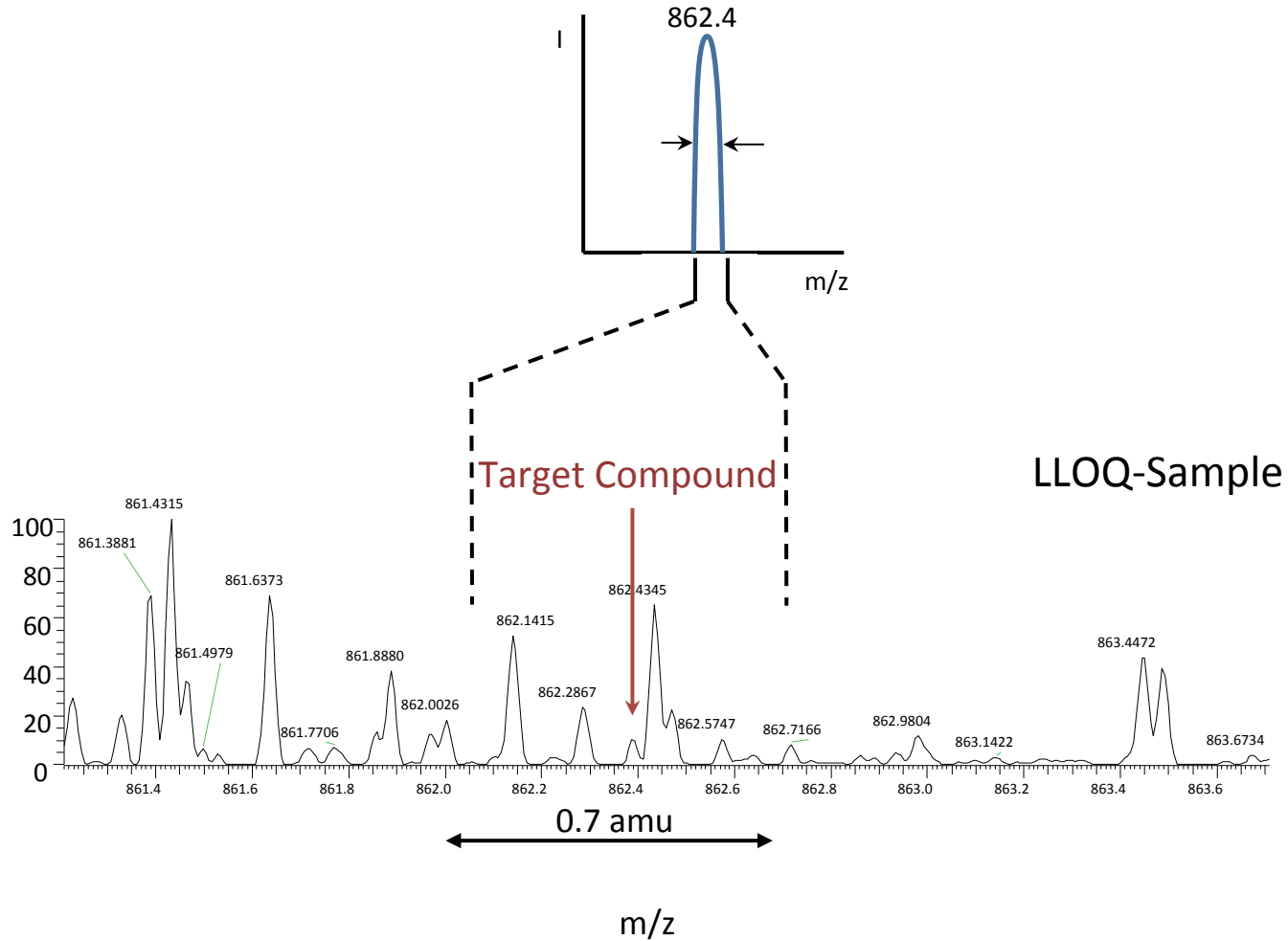
Remaining signal intensity: 4%

→ Why do we bother with SRM?



Selectivity!

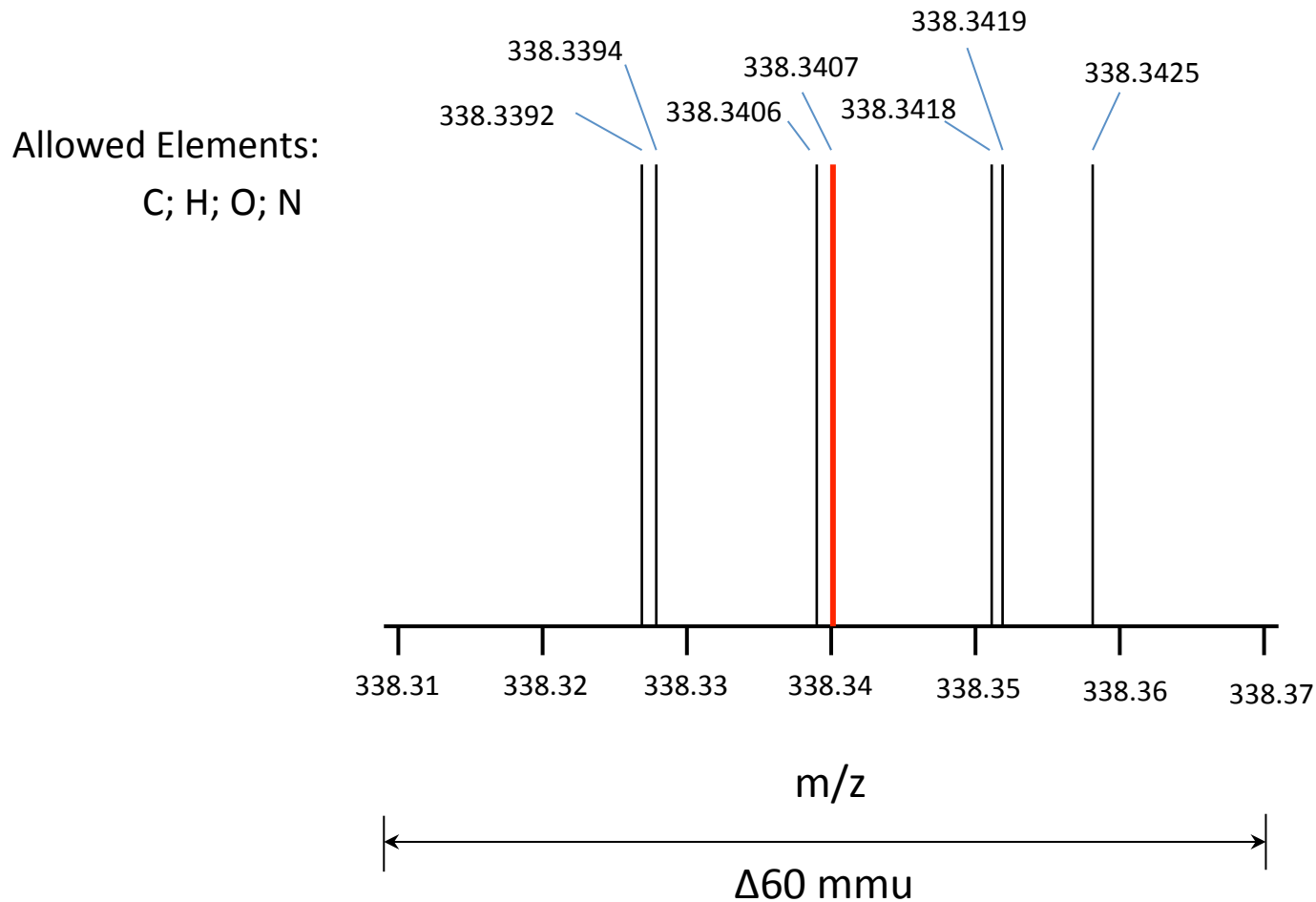
Unit resolution = 0.7 amu

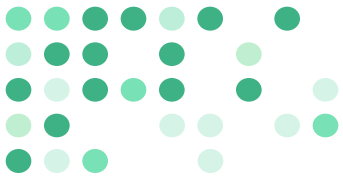




Selectivity by High Resolution SIM

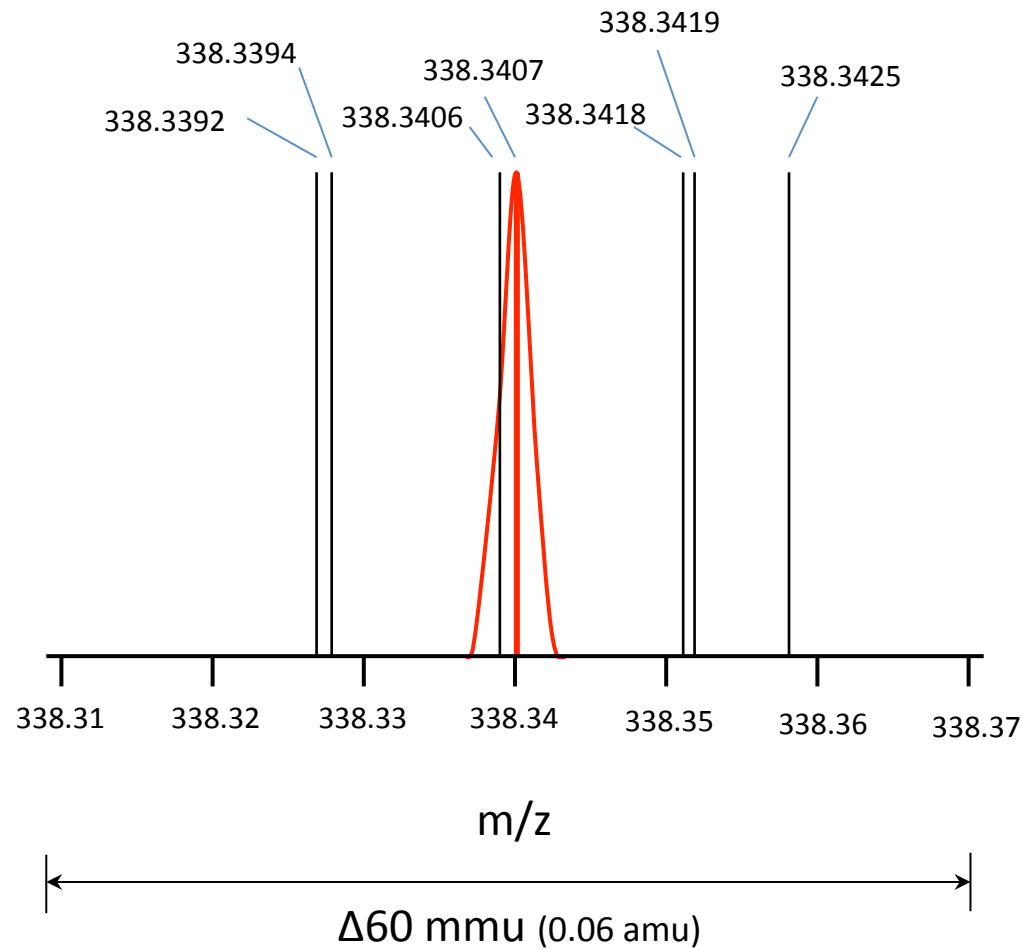
There are seven possible compounds within a 60 mmu range!





Selectivity by High Resolution SIM

With a resolution of 120.000 we can resolve all but two possible ions!



Quantitative Bioanalysis by High-Resolution-SIM

The Q-Exactive[®] was used to test the capabilities of high resolution SIM in quantitative bioanalytical assays.



Thermo Scientific Q-Exactive[®]

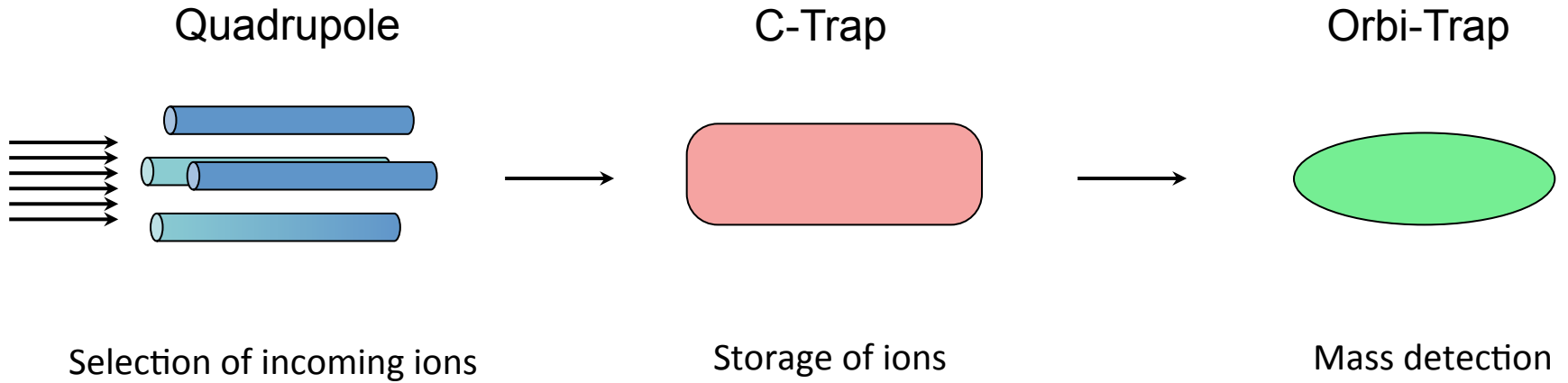
Resolution: max 140.000 (@ 200m/z)

Mass Accuracy: better than 3 ppm

Cycle time: is the Orbitrap fast enough?



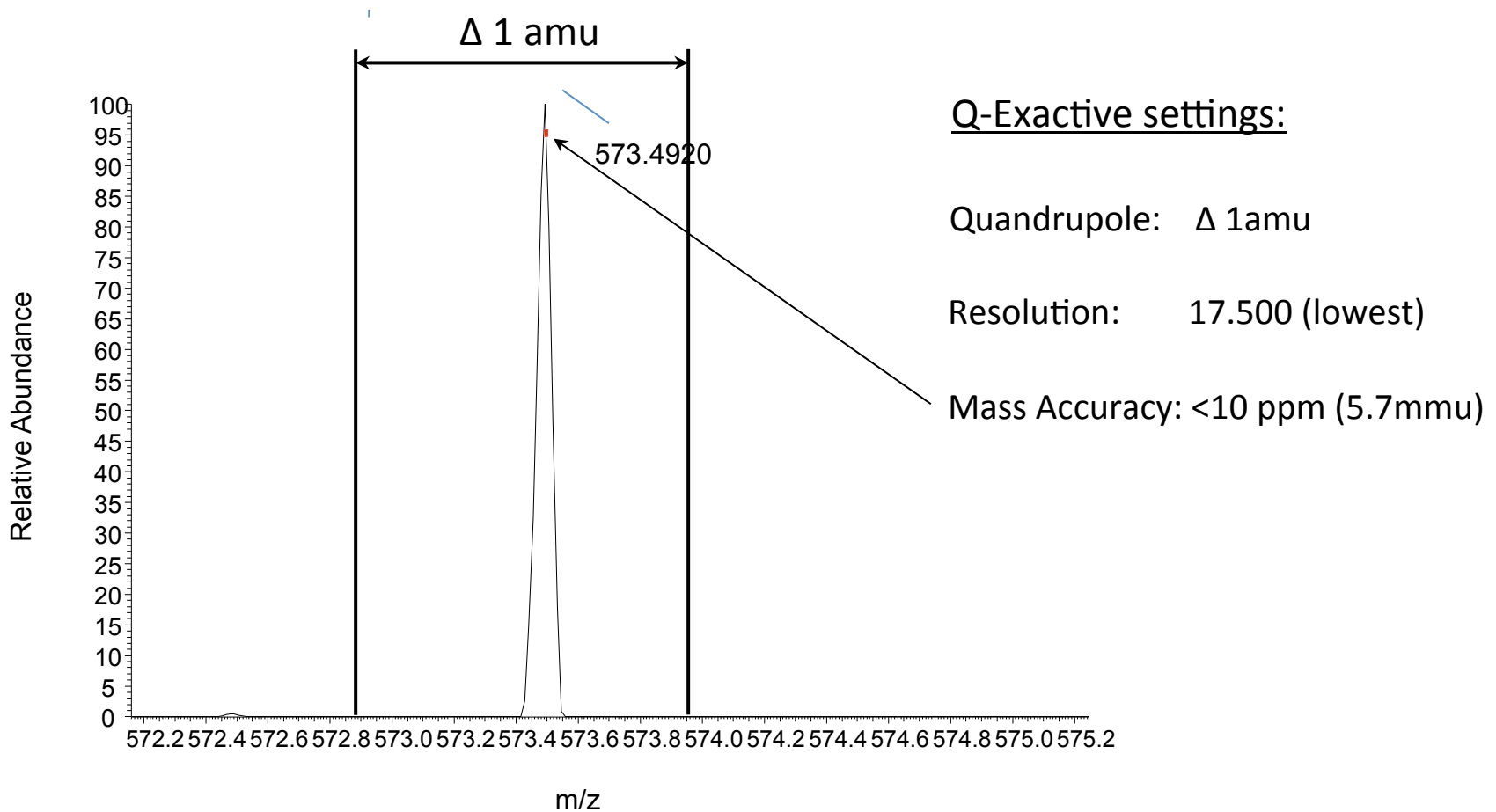
How does the Q-Exactive Work?





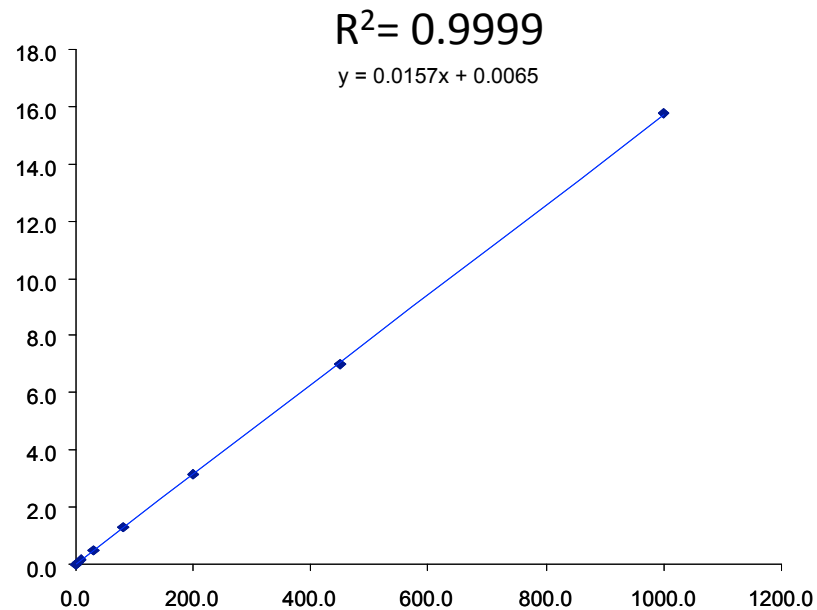
Quantification of a Small Molecule in Plasma

To test the high resolution SIM approach we took a method that was developed on a AB SCIEX 5500 QTRAP®



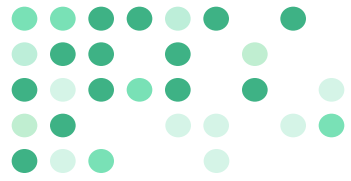


Quantification of a Small Molecule in Plasma

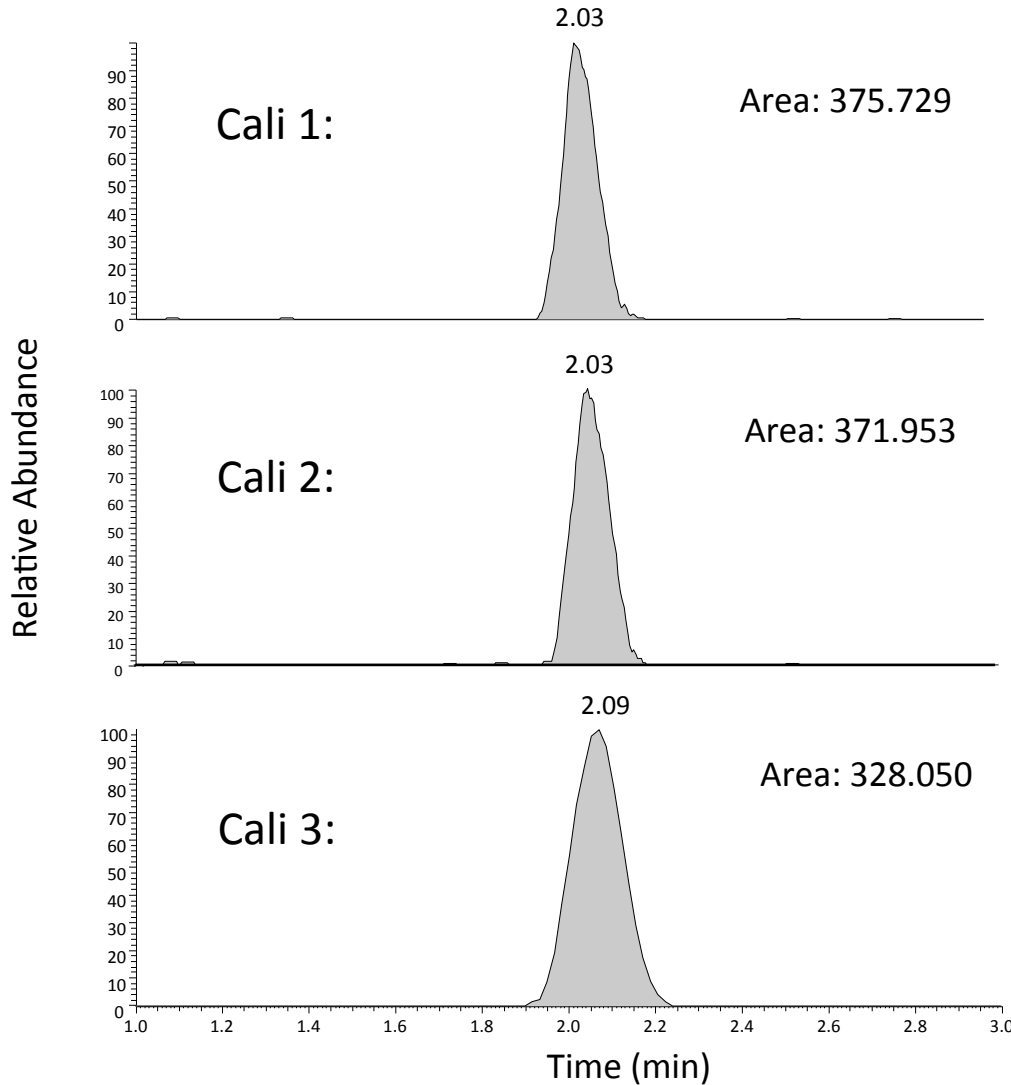


Concentration in plasma ranging from 1-1.000 ng/mL

➔ Setup time prior to measurement *15 min!*



Accuracy of the Measurement at LLOQ



% RSD at LLOQ: 7.4%

Peak width: 0.3 min

Average data points: 42

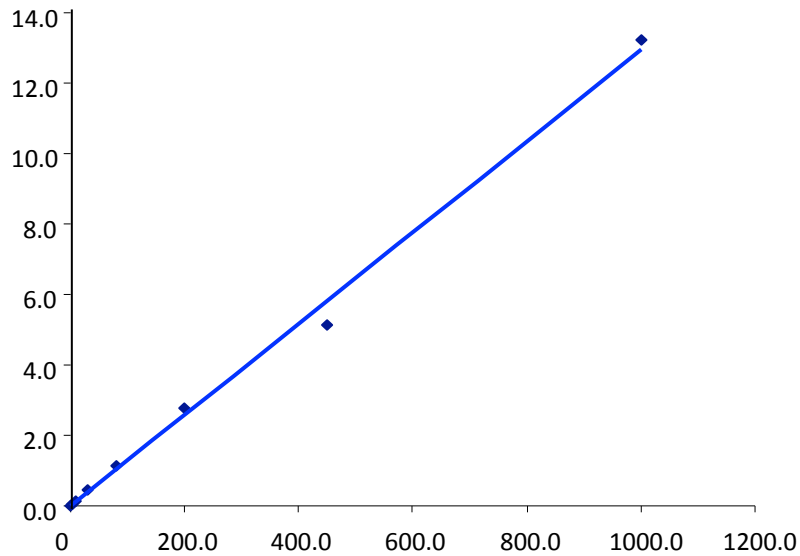


Comparison High-Res-SIM vs. SRM

Thermo Scientific *Q-Exactive*®

$y = 0.013x - 0.014$

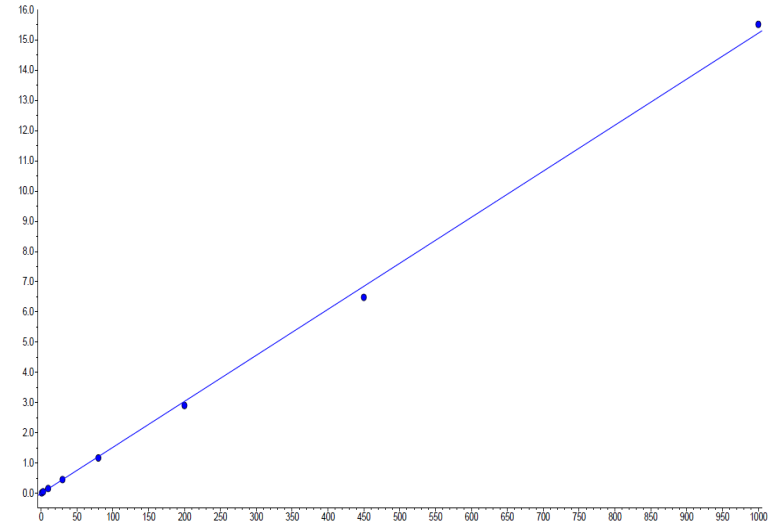
$R^2 = 0.9959$



AB SCIEX 5500 QTRAP®

$y = 0.0152x + 0.0015$

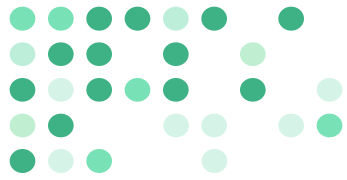
$R^2 = 0.9981$



Concentration in plasma ranging from 1-1.000 ng/mL

High-Resolution-SIM

SRM



High-Res-SIM Suitable for Quantification of Small Molecules

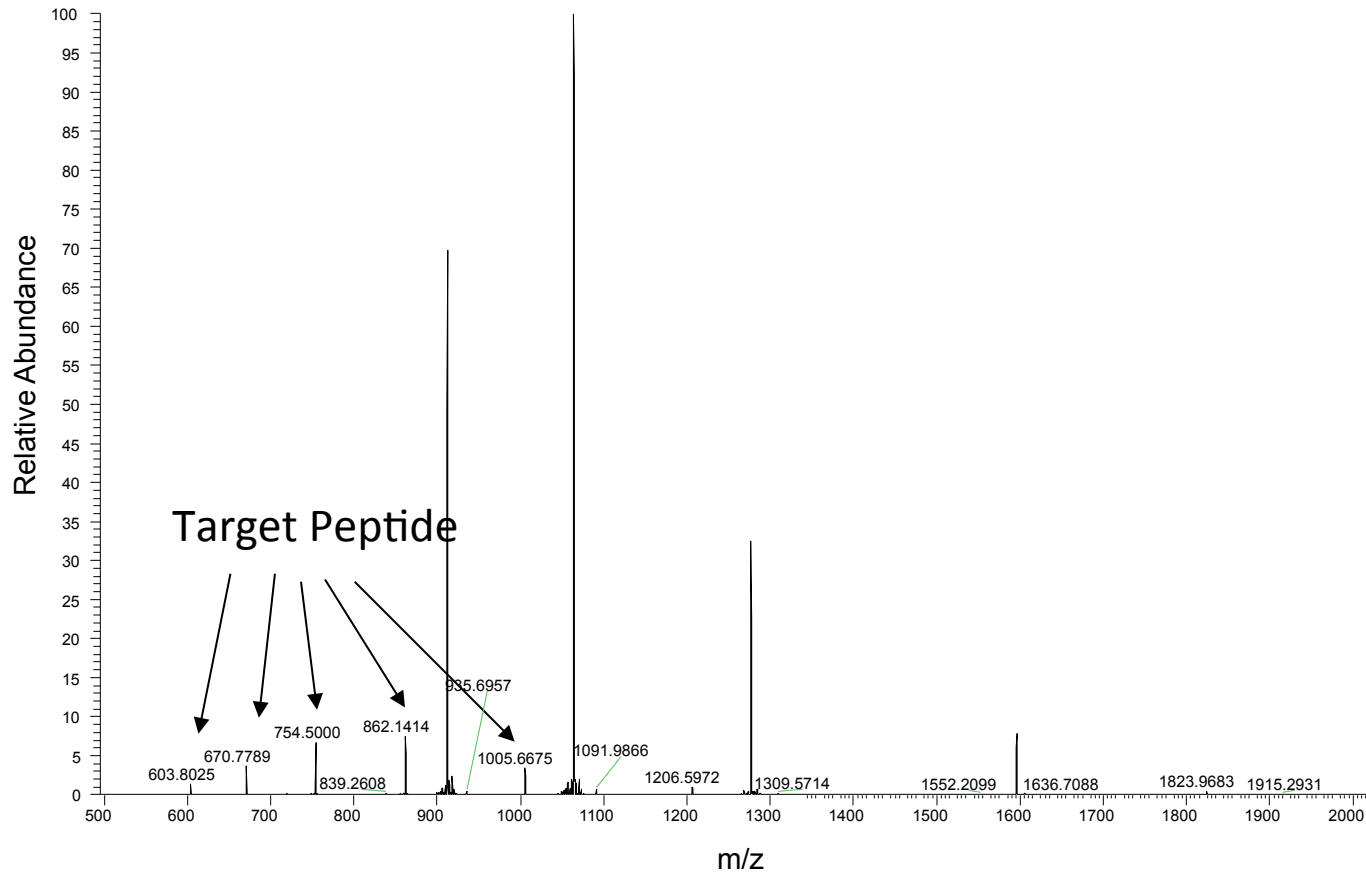
- High-resolution SIM can be used to quantify a small molecule in complex matrix (plasma).
- Easy setup!
- Sensitivity gained by high resolution. No need to identify a characteristic daughter ion.

High-resolution SIM should be an advantage for molecules that:

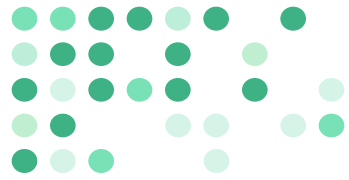
- ➡ do not yield characteristic daughter ions.
- ➡ proteins and peptides which have several charge states.



Full MS Spectrum of the Target Peptide

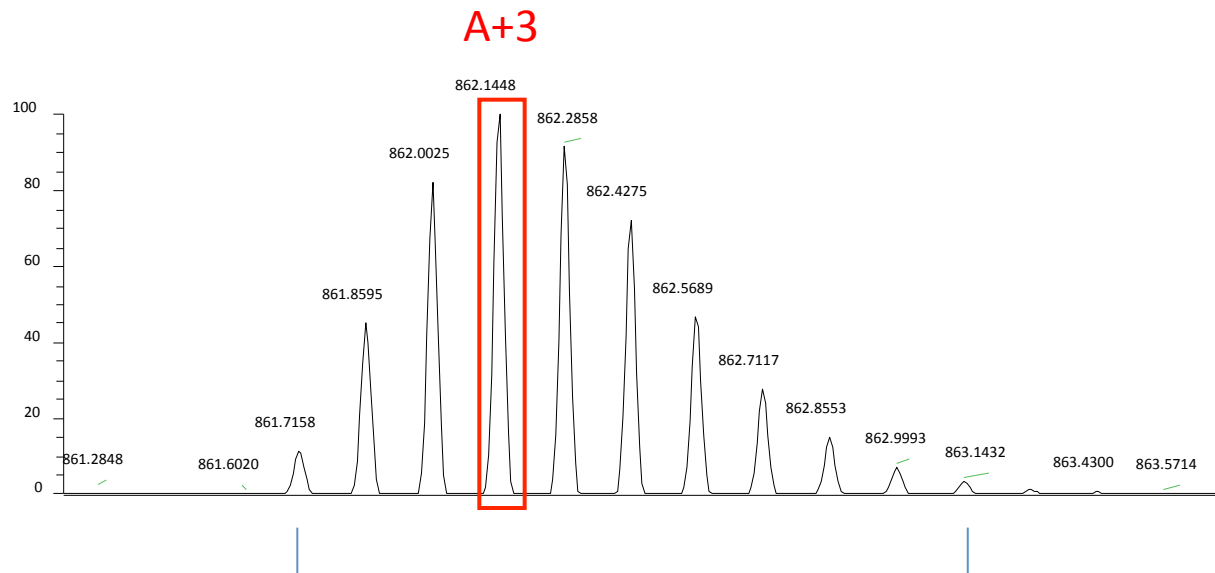


Problem: the overall intensity of the compound is distributed over five charge states.



The Most Intense Charge Stage is Used for Quantification

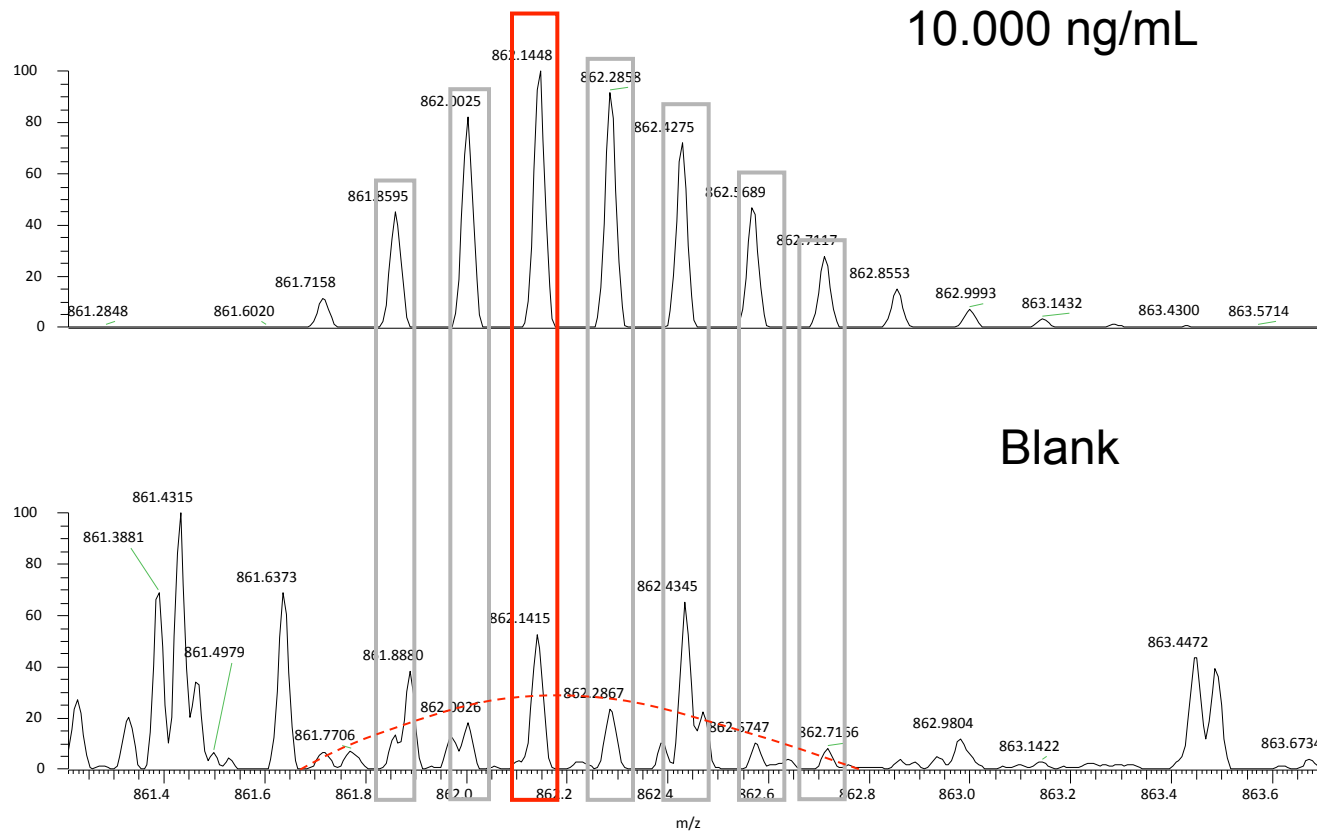
Isotopomers of the z=7 ion of the target peptide



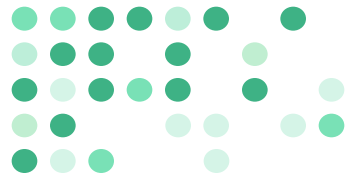
Spectrum of the 10.000 ng/mL plasma sample

The A+3 ion will be extracted with an 10 ppm (8.6 mmu) window for quantification.

Always Have a Careful Look at the Blank and LLOQ Sample!



- ➔ Obviously there is a carryover issue.
- ➔ These is an underlying signal in the blank for the A+3 ion.
- ➔ The A+4 ion is more suitable for quantification (not interfered).



Results from a Quantification Experiment in Plasma!

The results from three independent sample preparations.

Target in ng/mL	Area Cal 1	Area Cal 2	Area Cal 3	%RSD	Recovery
0	21217	64122	51643	48.3	
100	185728	223745	223151	10.3	24%
1000	1806498	1497201	1360484	14.6	10%
100000	12188088	10490679	10064451	10.3	10%

Target peptide concentrations on column.

Target in ng/mL	Column in ng/mL
0	
100	0.48
1000	2.00
100000	20.00



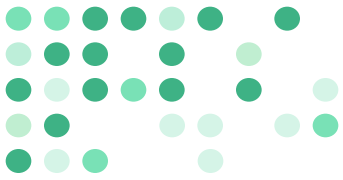
Conclusion

- ➔ High-Resolution-SIM is suitable for quantitative experiments in complex matrix:
 - Specificity gained by high resolution.
 - No need to identify specific daughter ions.
 - No loss of signal intensity through fragmentation.
 - If necessary quantification can be performed on a isotope of the target compound.
 - Ideal approach to quantify proteins and peptides.

- ➔ The accurate mass and high resolution Q-Exactive performs at least comparable to high-end triple-quadrupole instruments.



Thermo Scientific Q-Exactive[®]



Acknowledgement

Thanks to Thermo Fisher Scientific for letting us test the Q-Exactive and especially to:

Simon Szwandt

Simon Lauter

Markus Kellmann

...and of course thanks to all of you for your attention!