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The Comparison of High Resolution MS with Triple Quadrupole MS for the Analysis of Oligonucleotides

Mohammed Abrar Unilabs York Bioanalytical Solutions

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Outline

- Introduction
- Why LC-MS/MS?
- Limitations of LC-MS/MS
- Triple vs HRMS
- Case study
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Introduction

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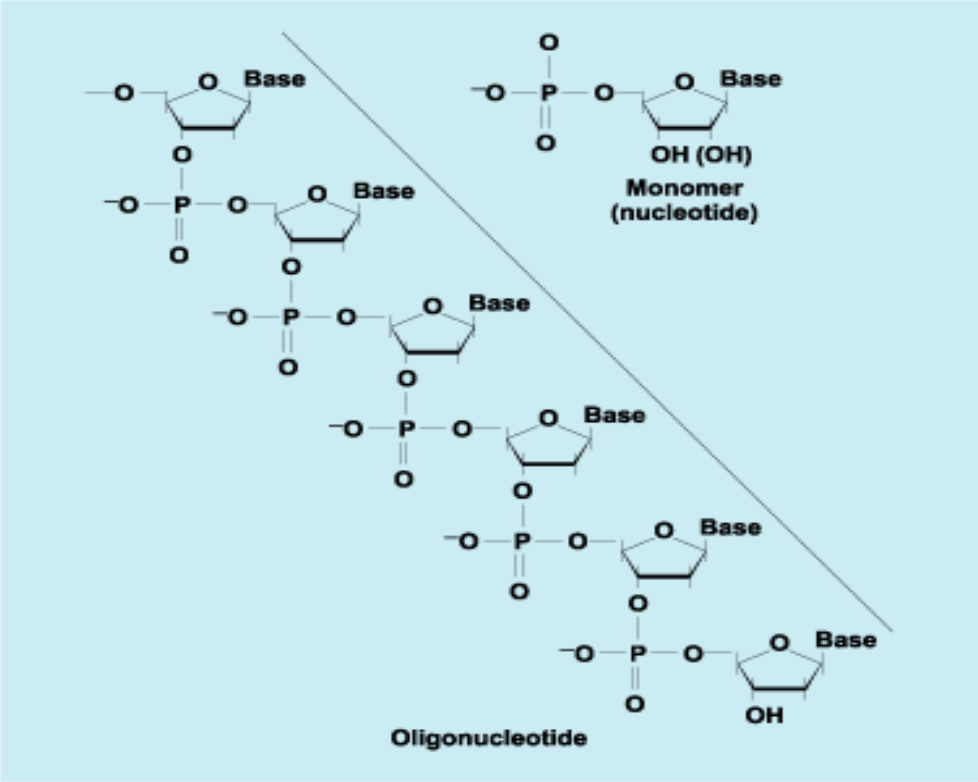
- Each nucleotide in the nucleic acid polymer comprises of 3 parts
  - 5 carbon sugar
  - Nitrogenous base
  - Phosphate group

Bases

- Cytosine
- Guanine
- Adenine
- Uracil or Thymine



Introduction

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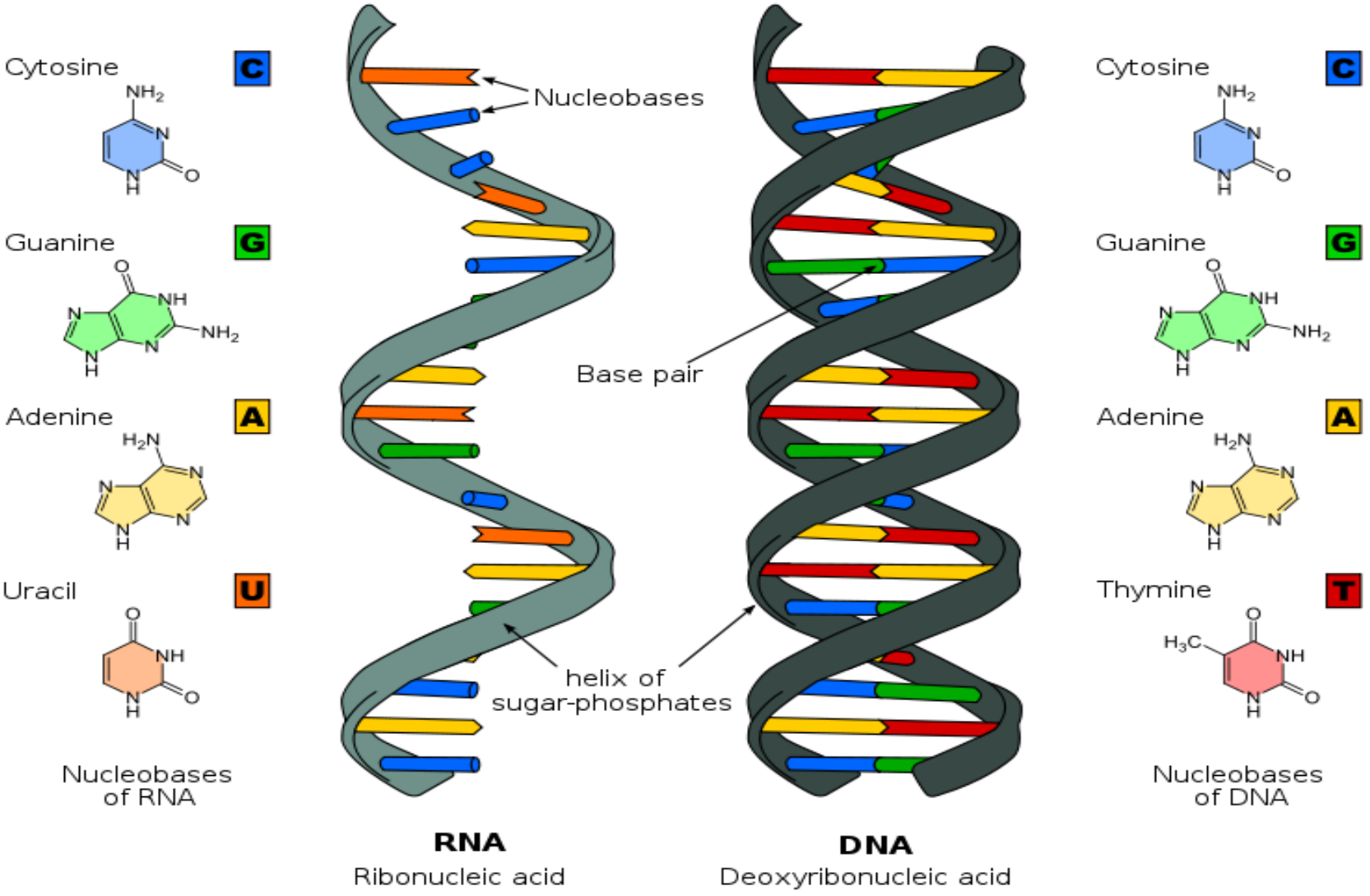

Why LC-MS/MS?

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When

- Elisa gives the highest sensitivity for SiRNA
- Reported sensitivity down to 25 pg/mL

But

- Problem is it cannot distinguish between different chain lengths ie parent from shorter metabolites
- Leads to over estimation of parent

Why LC-MS/MS?

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- Very high degree of selectivity
- Identification and quantification of parent and metabolites

But

- Best reported sensitivity of a validated method 4 ng/mL [Deng et al] 2010

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Limitations of LC-MS/MS

- Chromatography
  - Difficult due to the highly polar nature of the multiple Phosphate groups
  - Fine balance between ionisation and chromatographic retention
  - Ion pair chromatography uses mixture of TEA and HFIP
- Ionisation
  - Negative electrospray produces multiply charged states
  - Produces multiple adducts H^+, Na^+, K^+
  - Poor fragmentation of Oligos down to small common fragments

Triple vs HRMS

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- Triple
  - We have plenty of these in our bioanalytical labs
  - Very sensitive MS instruments Xevo TQ-S, API6500 etc
  - Give maximum sensitivity in MRM mode
  - Easy to use
- HRMS
  - We have very few high res instruments in our labs
  - Usually found in metabolism labs and used for qualitative analysis
  - High end machines (QTOF and Q-Exactive) now give similar sensitivity as API4000 in full scan
  - Q-Exactive is very easy to use (software & hardware)

How do the 2 instrument types compare for the analysis of Oligos?

Case study

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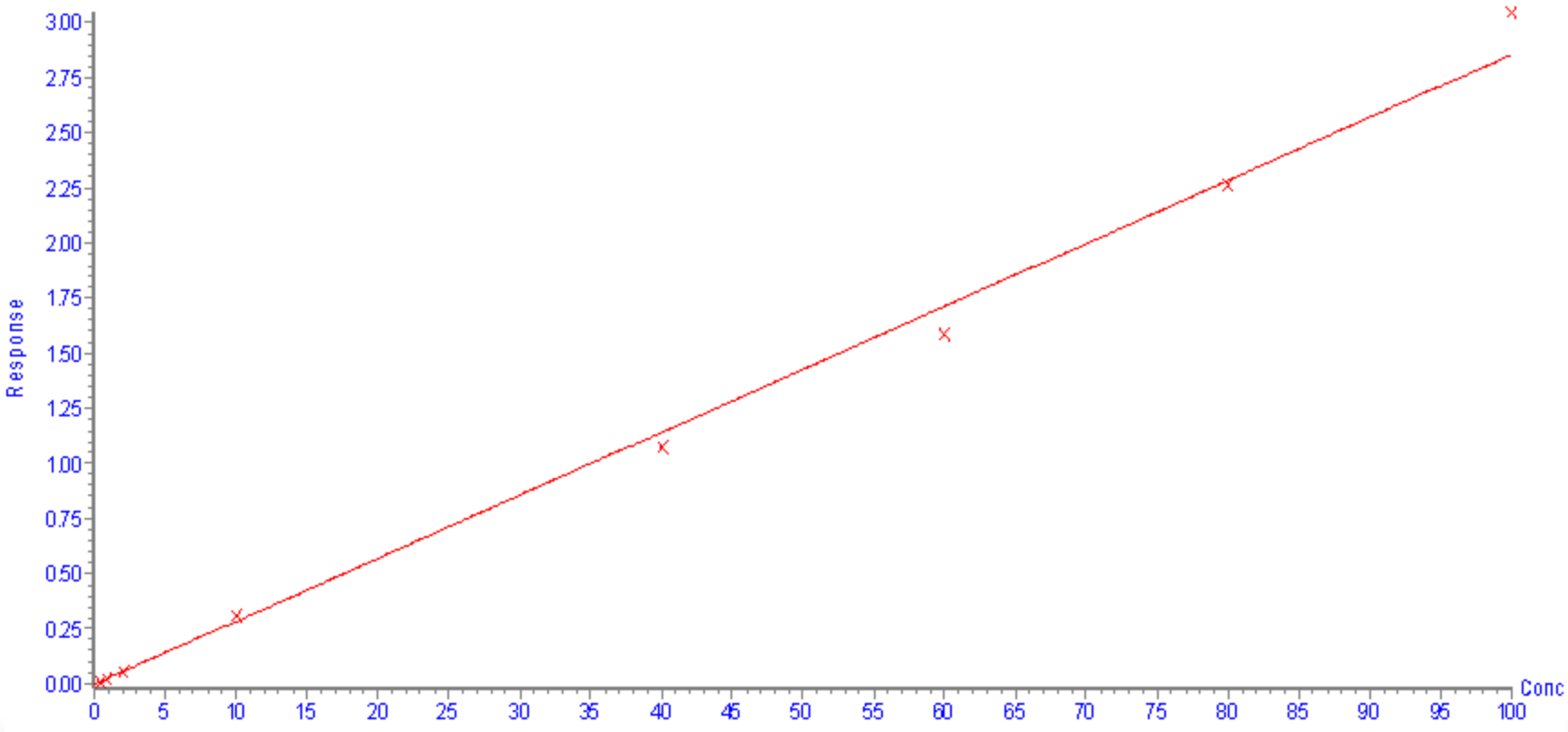
- Oligonucleotide Mwt ~5 kDa
- Assay range required 0.4 to 100ng/mL
- EDTA human plasma samples spiked with Oligo
- Samples extracted using waters mixed mode anion exchange SPE
- Chromatography consisted of C18 column using water/MeoH with TEA and HFIP modifiers
- Same samples run on both Waters Xevo TQ-S and Thermo Q-Exactive
- Samples were extracted and analysed on both instruments using same chromatographic conditions
- Analytical variables minimised to compare performance of the two instruments

Calibration curve (Triple)

Correlation coefficient: r = 0.998180, r^2 = 0.996362
Calibration curve: 0.0285598 * x + -0.001392
Response type: Internal Std (Ref 2), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



Calibration curve summary (Triple)

	#	Sample Text	Type	Std. Conc	RT	Area	IS Area	Response	Conc.	%Dev
1	1	cal 100	Standard	100.000	4.24	42066.828	13816.016	3.0448	107	6.7
2	2	cal 80	Standard	80.000	4.23	32002.691	14136.611	2.2638	79.3	-0.9
3	3	cal 60	Standard	60.000	4.24	19243.434	12153.964	1.5833	55.5	-7.5
4	4	cal 40	Standard	40.000	4.33	15695.229	14618.066	1.0737	37.6	-5.9
5	5	cal 10	Standard	10.000	4.25	4913.417	15870.658	0.3096	10.9	8.9
6	6	cal 2	Standard	2.000	4.25	965.542	16779.898	0.0575	2.06	3.2
7	7	cal 0.8	Standard	0.800	4.24	296.517	15425.008	0.0192	0.722	-9.8
8	8	cal 0.4	Standard	0.400	4.25	138.945	13059.384	0.0106	0.421	5.3
9	9	MB	Blank		4.33	35.956	15148.041	0.0024	0.132	
10	10	DB	Blank		4.38	141.447				

Cal 0.4 ng/mL (Triple)

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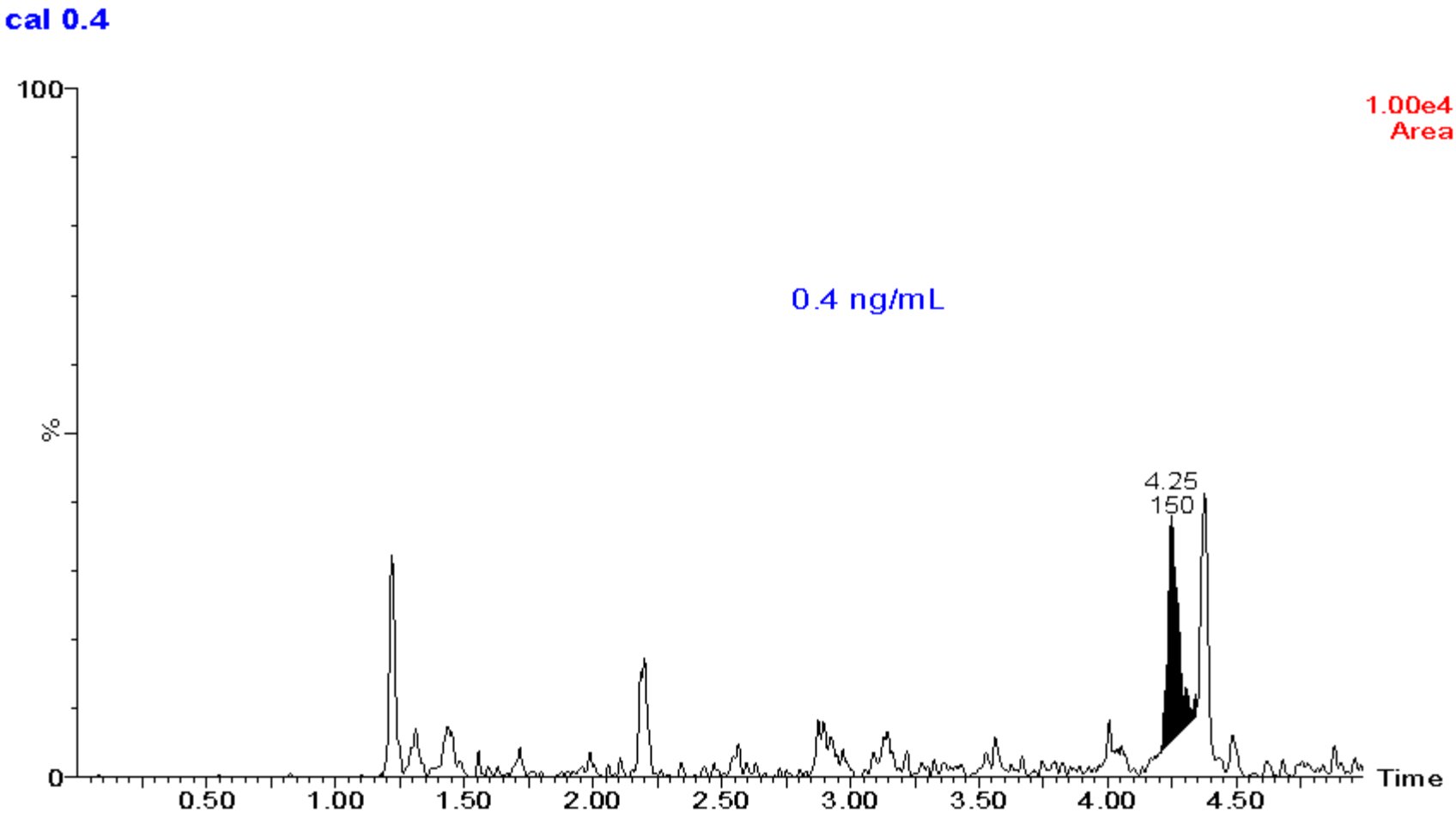

Cal 0.8 ng/mL (Triple)

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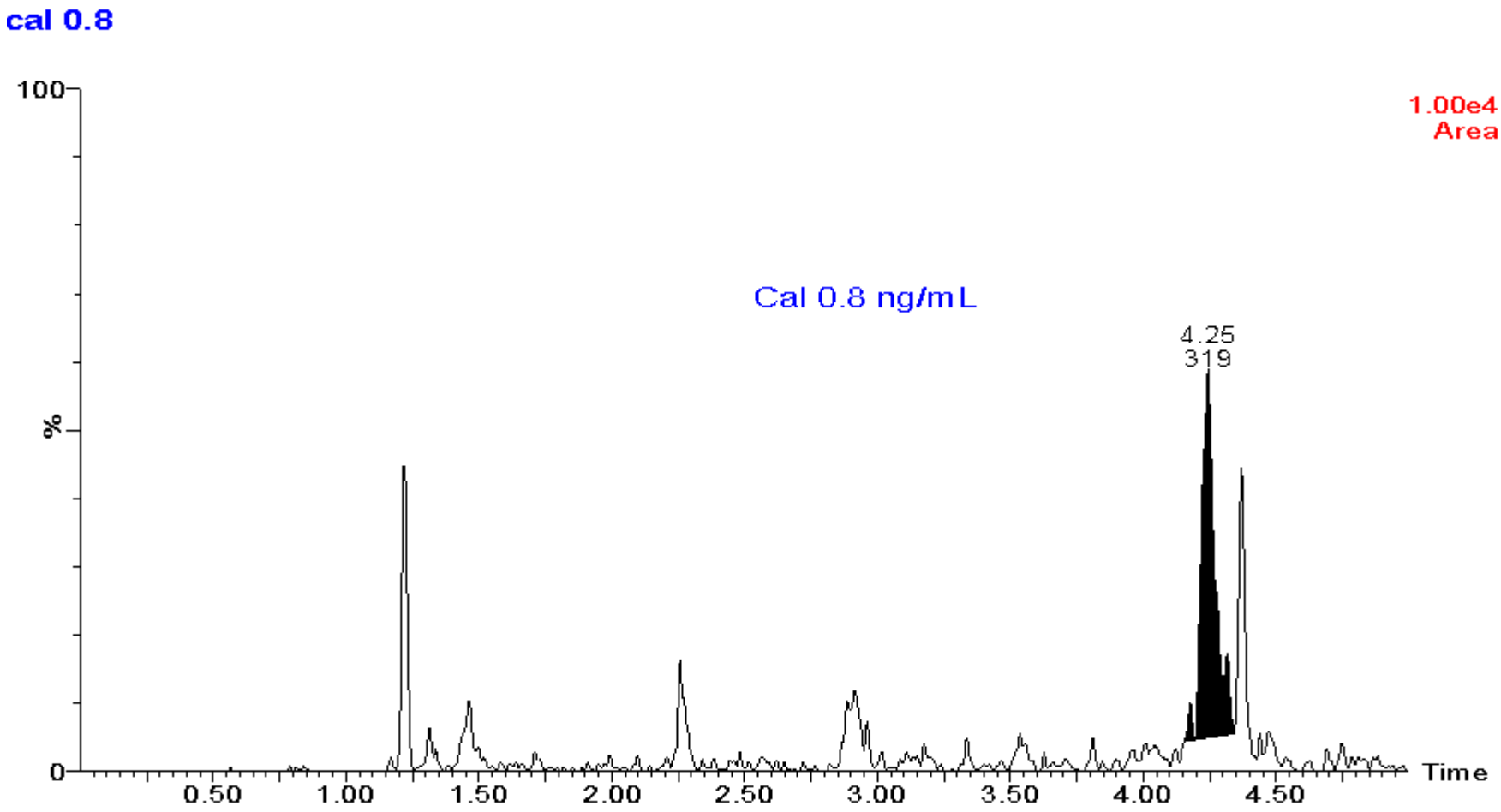

Matrix Blank (Triple)

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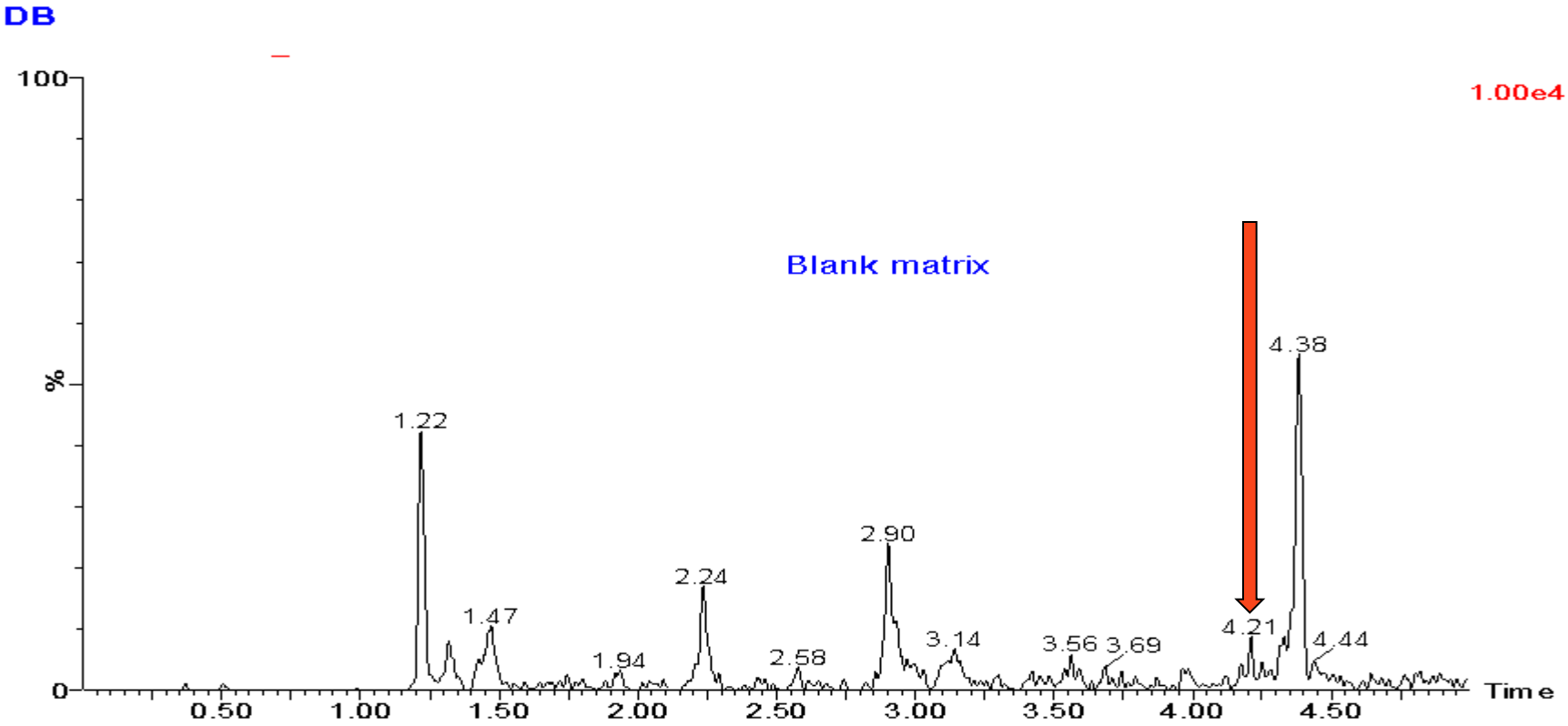

Q-Exactive

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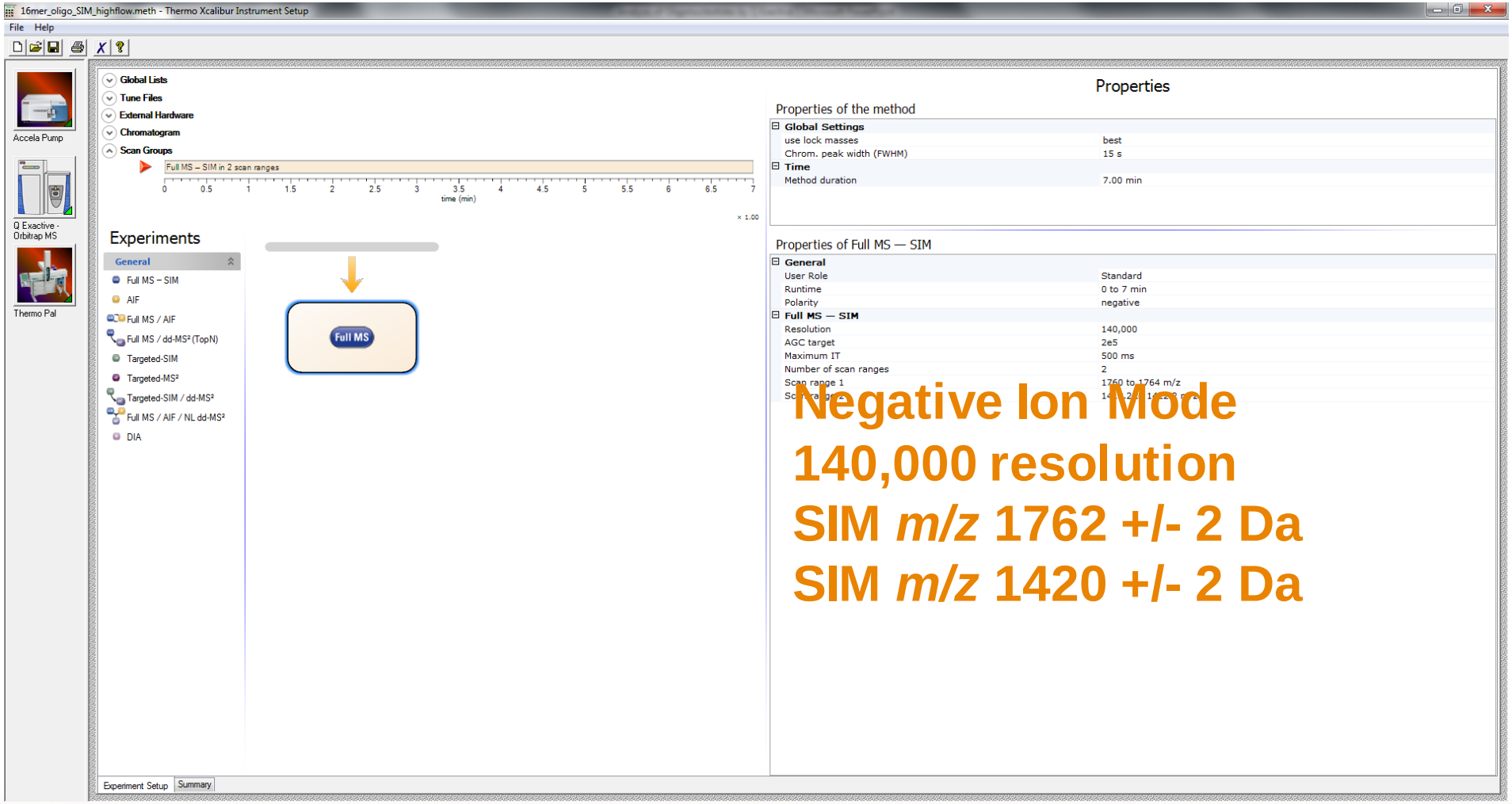

Negative Ion Mode
140,000 resolution
SIM *m/z* 1762 +/- 2 Da
SIM *m/z* 1420 +/- 2 Da

Cal 100 (Q-Exactive)

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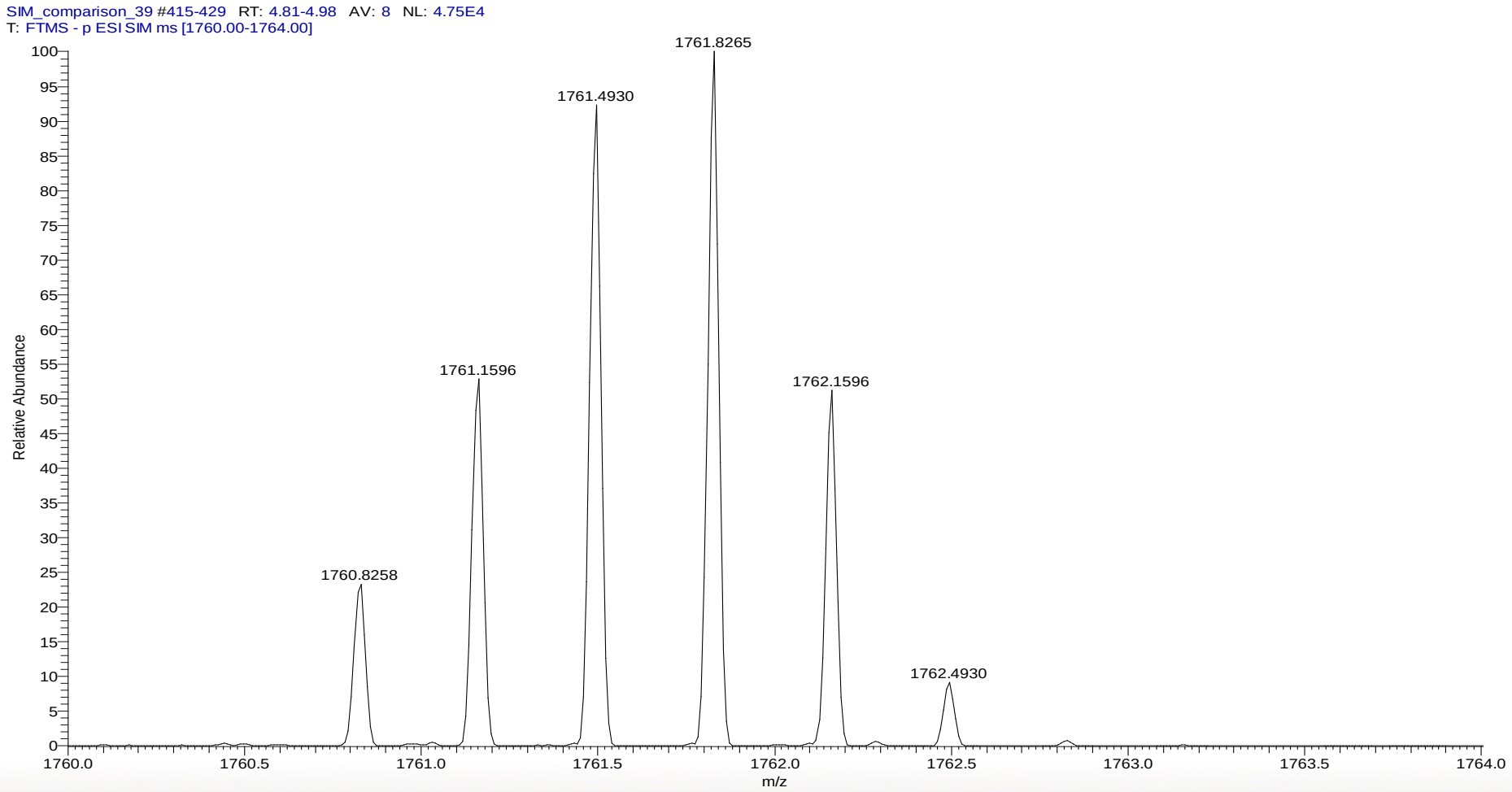

0.4 ng/ml (Q-Exactive)
Extracted Ion Chromatogram and Calibration Line

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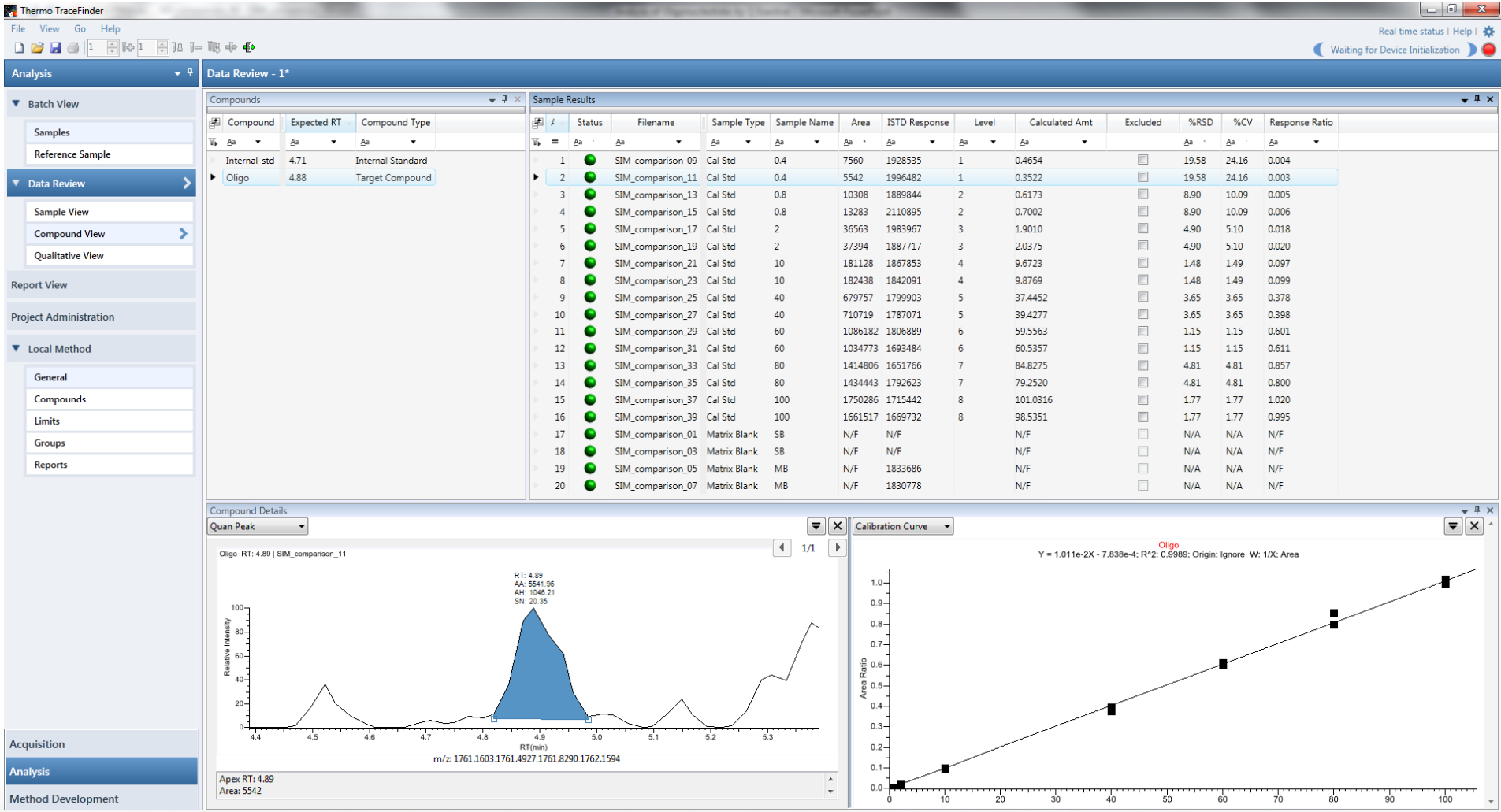

Matrix Blank (Q-Exactive)

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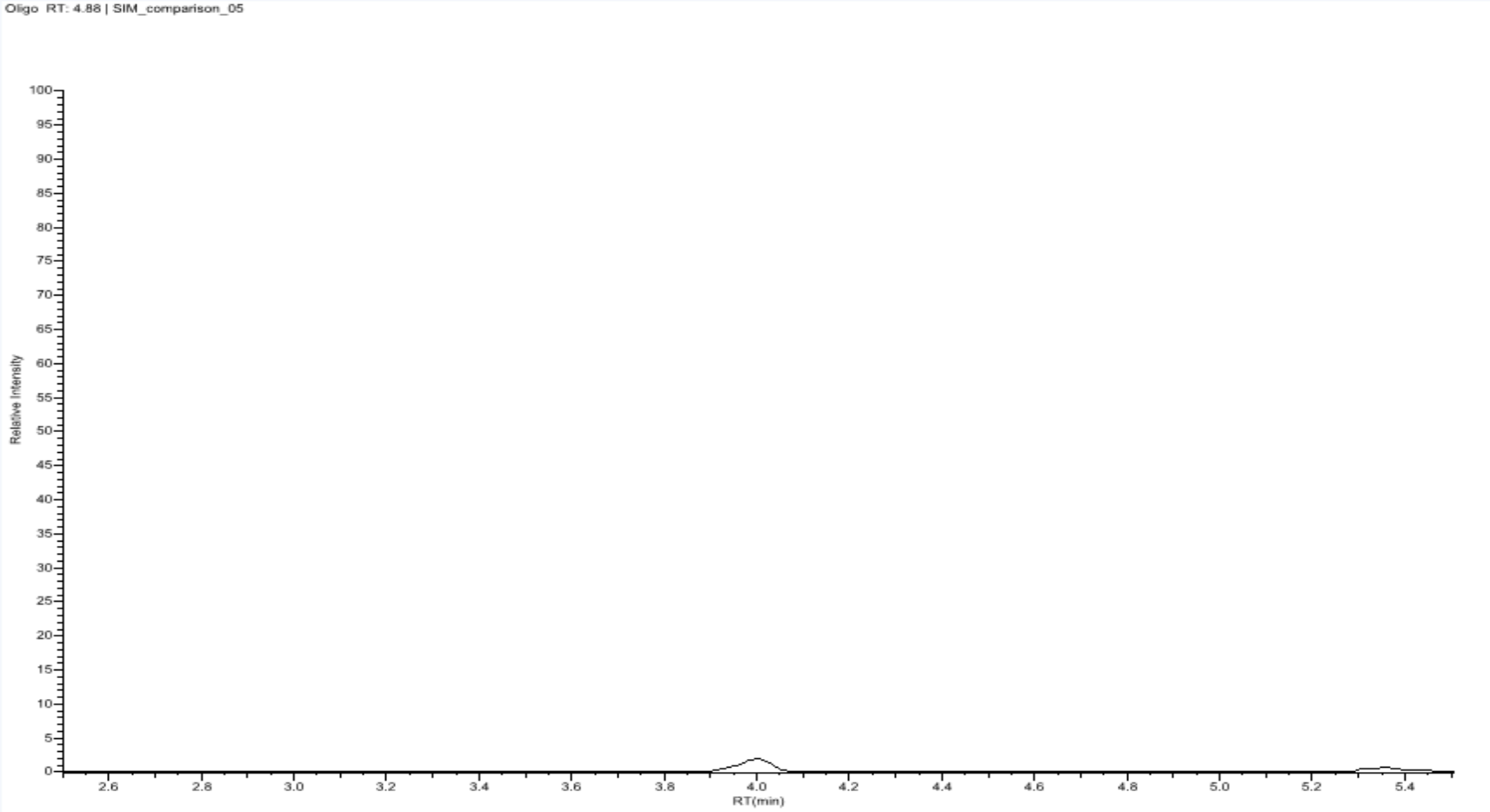

0.4 ng/ml (Q-Exactive)

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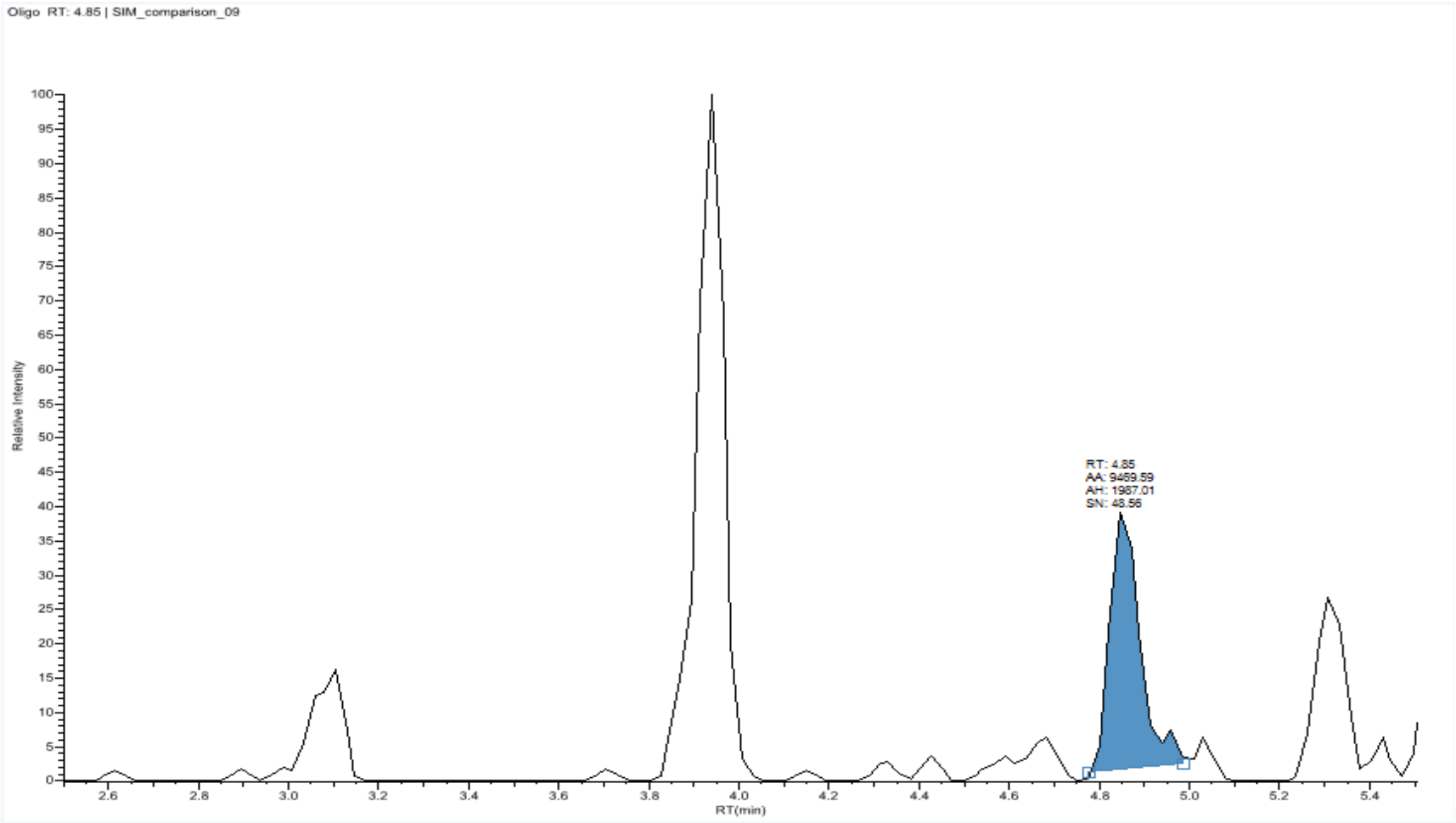

0.8 ng/ml (Q-Exactive)

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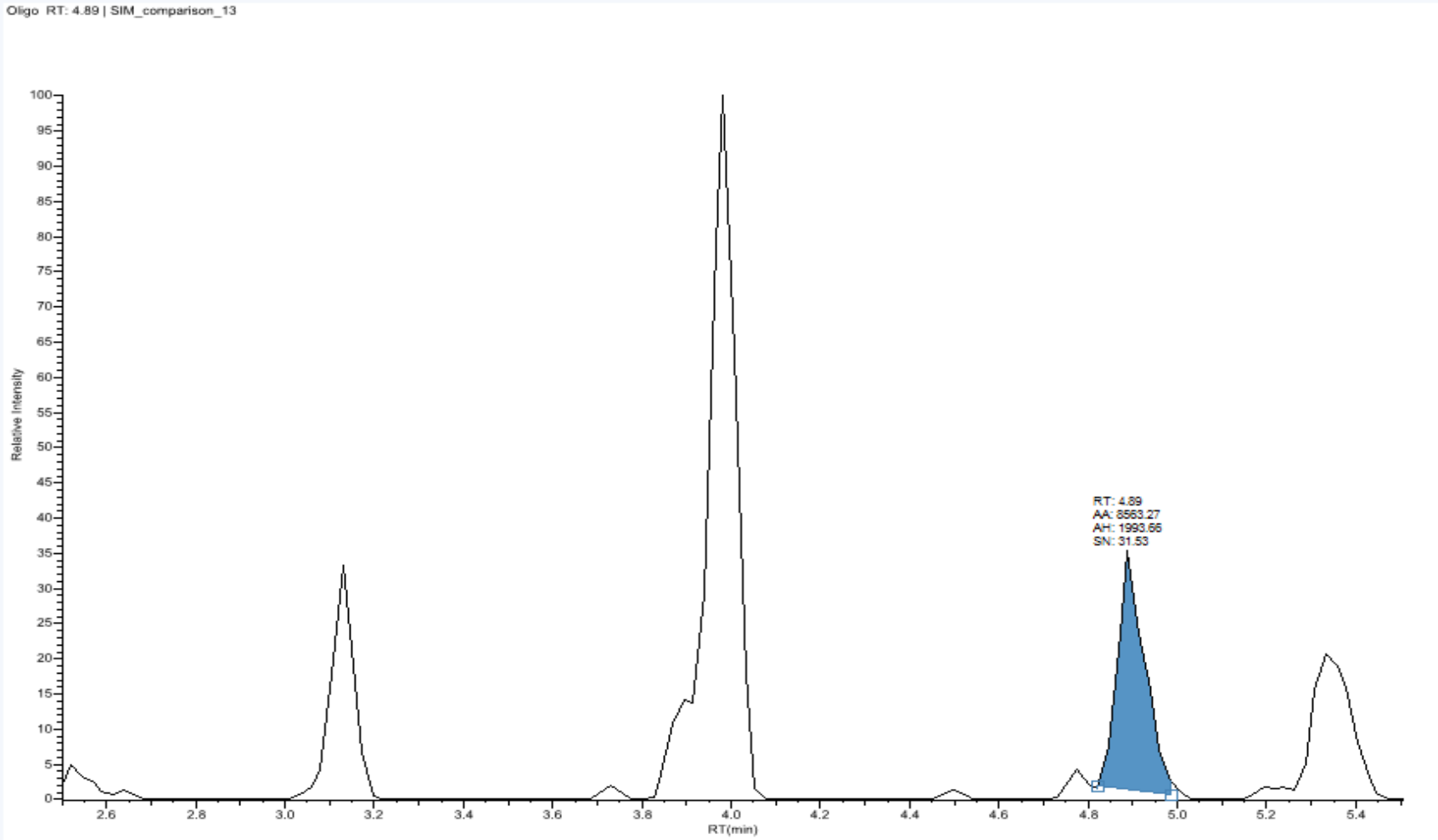

Triple vs HRMS

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- Sensitivity comparable on both instruments
- Selectivity much better on Q-Exactive
- HRMS SIM performed better than MRM on a Triple
- Slight issue with HRMS method not enough data points due to high scan time at 140,000 Res
- Both instruments very easy to setup

Summary

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- Oligos are analytically very challenging
  - Very polar and hence difficult to chromatograph without ion pairing
  - Chromatographic separation of Oligos difficult and based on mass ie no of mers
  - Multiply charged ions produced in negative ESI
  - MS/MS fragmentation poor
  - Non specific fragments produced under CID
  - No real advantage in running MRM in terms of sensitivity/selectivity
  - HRMS SIM gives both high sensitivity and selectivity by measuring individual isotope mass peaks

Conclusions

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- For targeted analysis
  - Compounds showing good CID fragmentation MRM triple
  - Compounds showing poor CID fragmentation SIM HRMS
  - Compounds showing non specific fragmentation MRM triple or HRMS SIM
- Will HRMS replace triples for bioanalysis? **NonSense**
- Do HRMS instruments give advantages for biotherapeutics? **Sense**
- Do HRMS instruments complement triples? **Sense**
- In the future 20% instrumentation HRMS (depending on work mix) **Sense**
- Screening + quantification in the same run ie chemical series by HRMS **Sense**
- HRMS an effective tool for bioanalysis **Sense**

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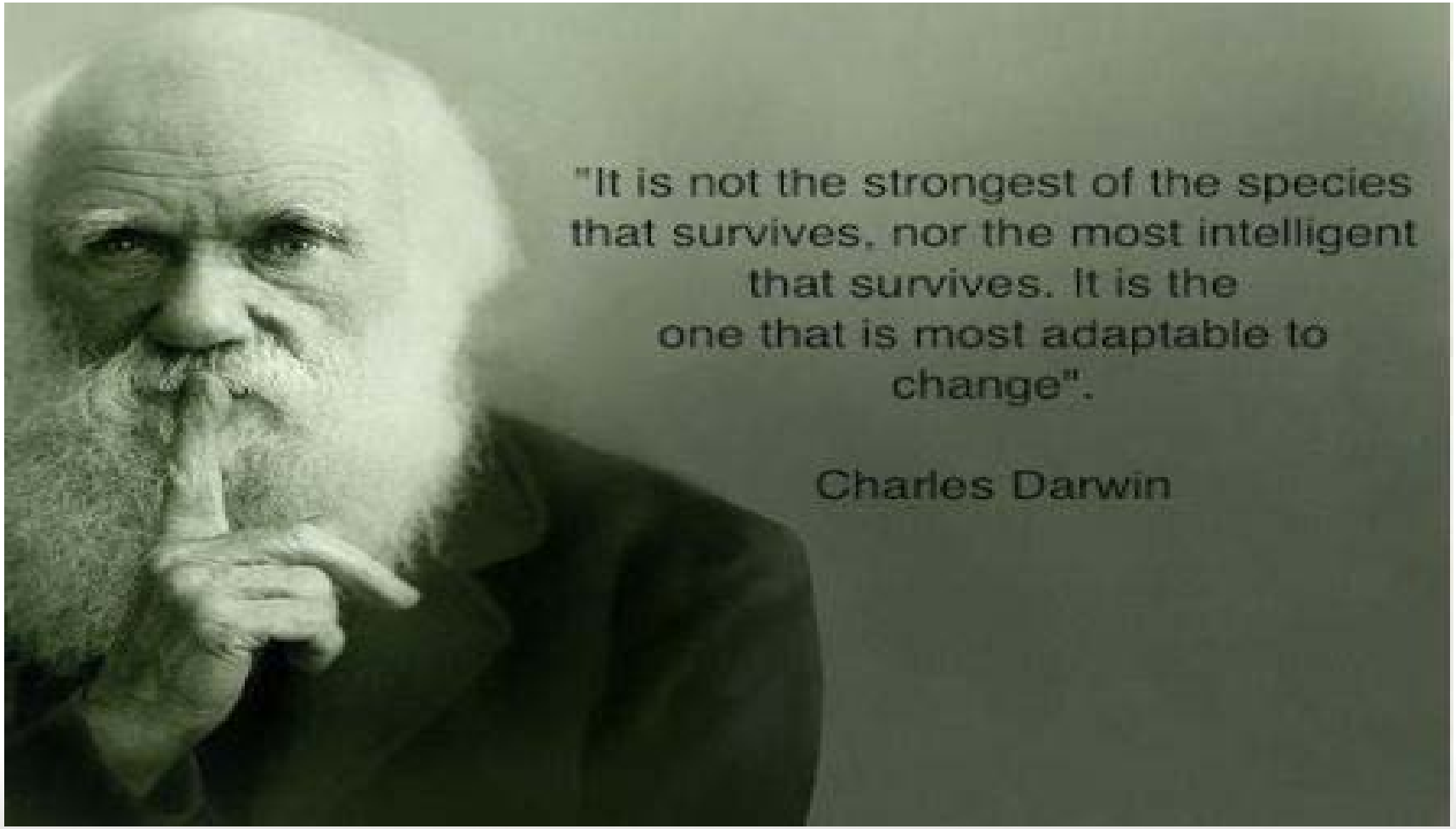
The drug development paradigm is changing!

Are we ready to adapt?



Acknowledgements

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