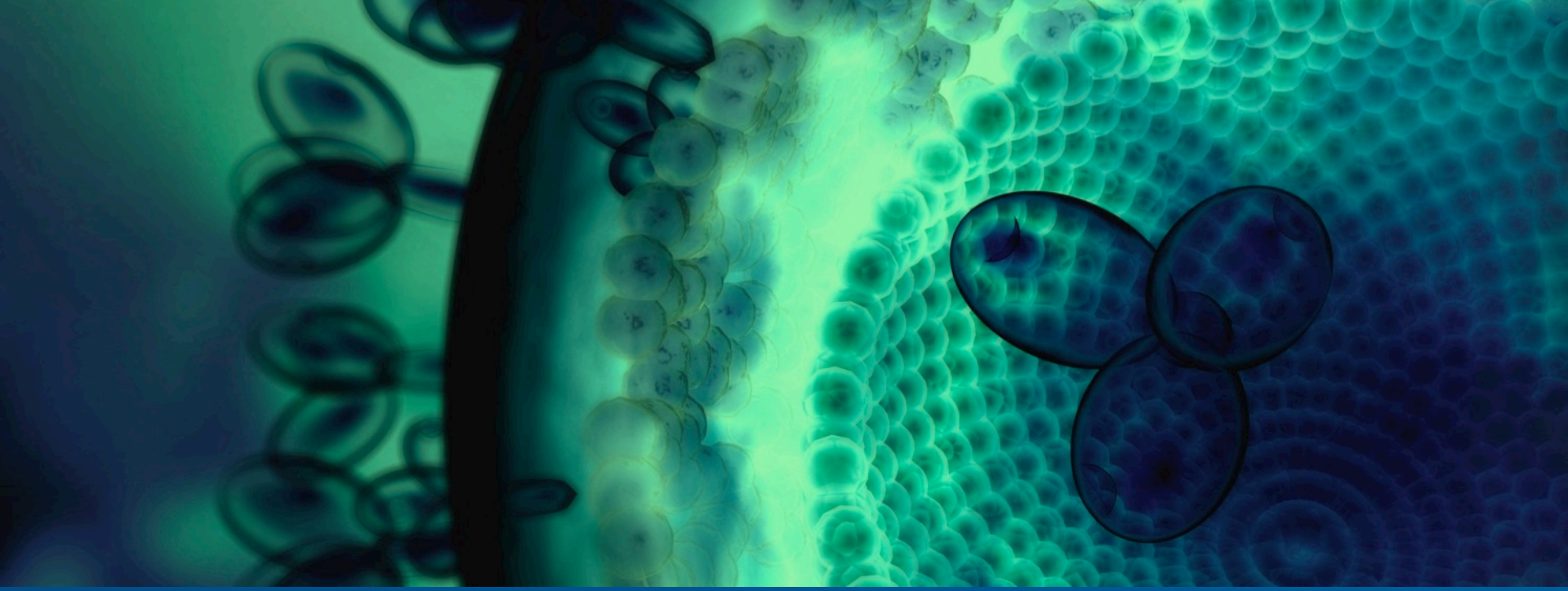

Combined use of LBA + LC-MS/MS in drug development of a 2 kDa peptide: 1+1=3 or where complementary data made a difference

Pictured above: The structure of HIV.

Ronald de Vries | EBF Meeting Barcelona | 19-21 November 2014

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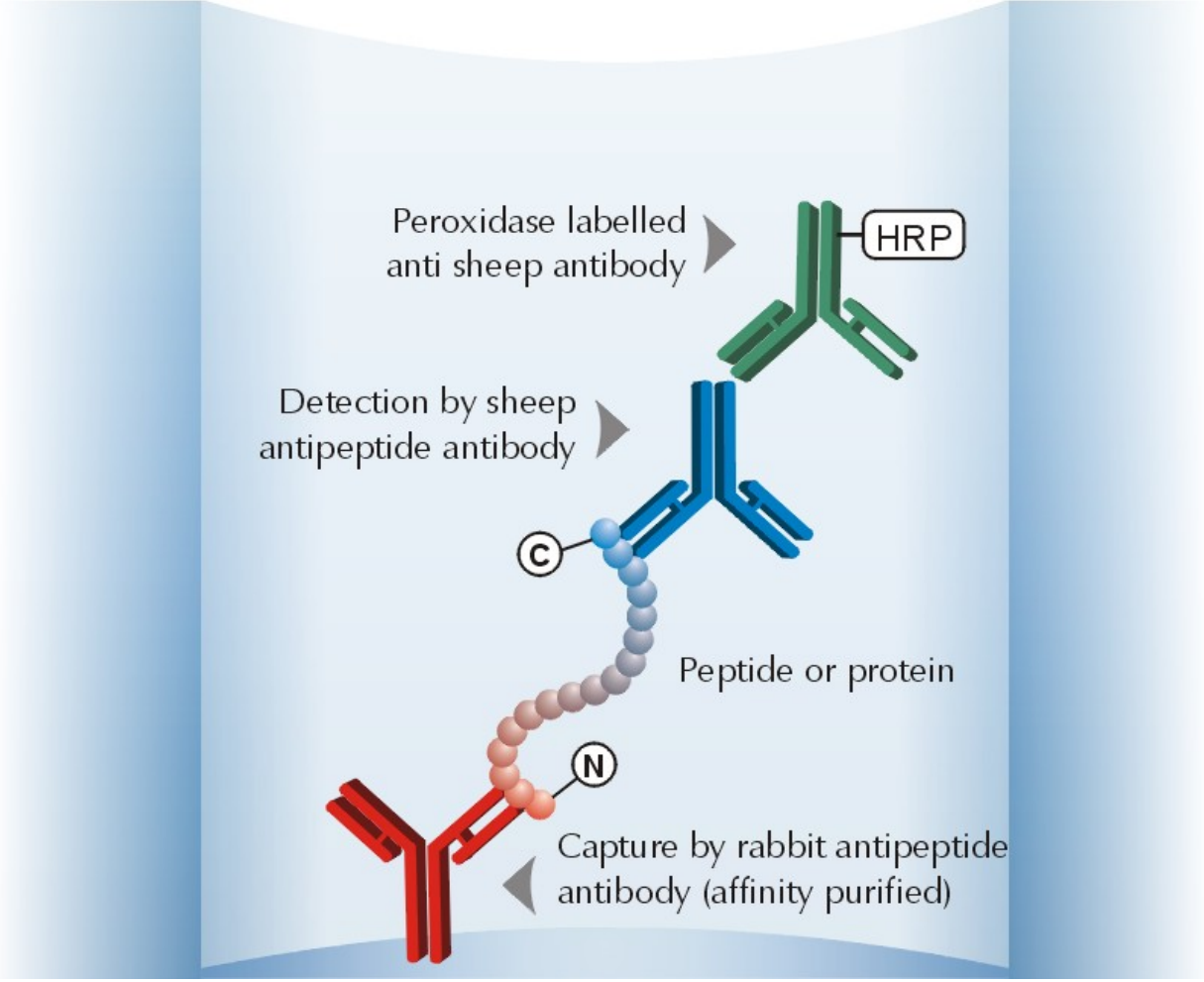
LBA versus LC-MS/MS assay

Basic principles of the analytical techniques differ significantly

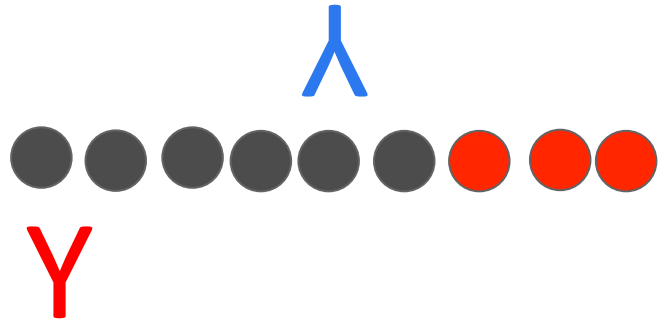
- LC-MS/MS tends to measure parent peptide drug only
  - active and inactive metabolites are missed unless intentionally added to the assay
- LBA measures
  - peptide drug only OR
  - peptide drug + active metabolites OR
  - peptide drug + active metabolites + inactive metabolites

Sandwich ELISA

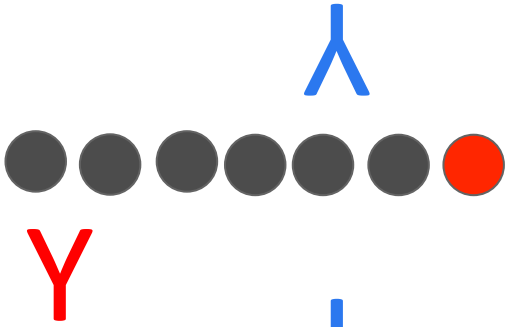


Most common Ligand Binding Assay conformation

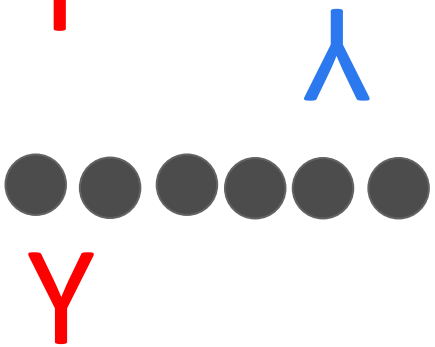
Sandwich ELISA measuring drug, active metabolite and inactive metabolite



Peptide drug - detected

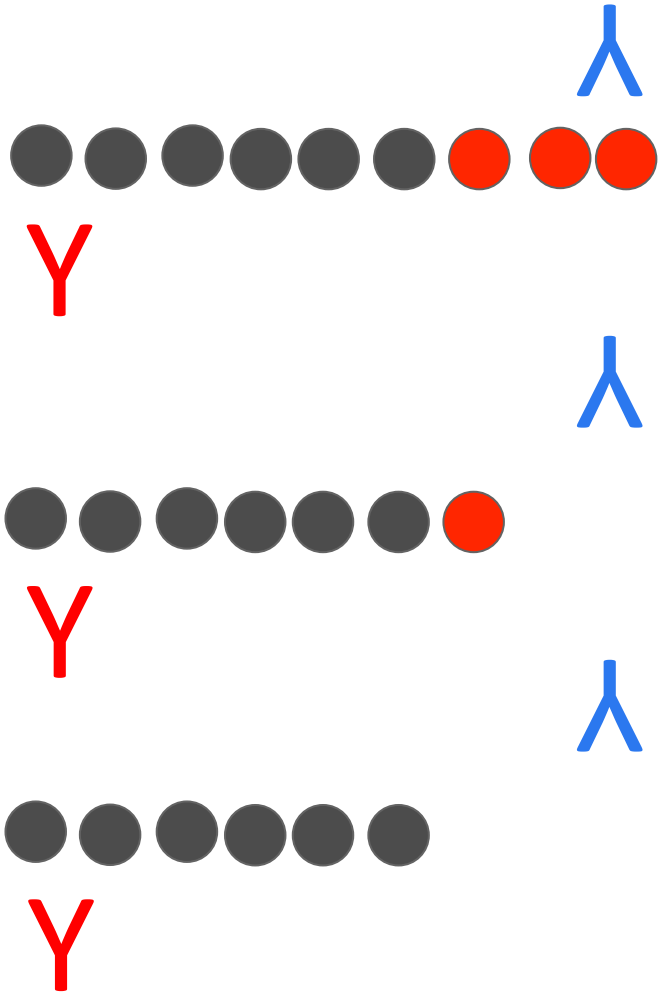


Active metabolite - detected



Inactive metabolite - detected

Sandwich ELISA measuring drug but no metabolites



Peptide drug - detected

Active metabolite – NOT detected

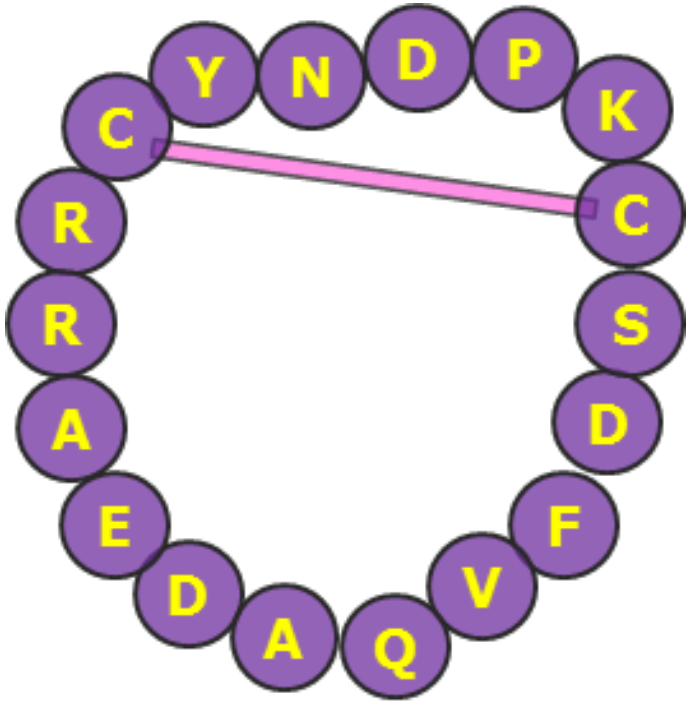
Inactive metabolite – NOT detected

The draft FDA Bioanalytical Method Validation Guidance (2013)

"LBA assays should be compared with a validated reference method (such as LC-MS) using incurred samples and predetermined criteria should be used to assess the accuracy of the LBA method".

- This text suggests that an LBA and an LC-MS/MS assay should have a comparable result in order to be valid.
- However, based on the difference in basic principles of both assay formats, the results from both assays can differ significantly …
- Difference in results LBA vs LC-MS/MS should NOT be an issue, but it is important to understand "what" each assay is measuring

An example from Janssen Portfolio



Different results from PK assays

- LBA (sandwich ELISA) used as bioanalytical method before in-licensing of this peptide drug
- When using LC-MS after in-licensing
  - concentrations in dog, rat and human plasma/serum by LCMS were much lower than by LBA
  - difference was larger at later time points
  - cross-reactivity LBA with metabolites suspected

- Samples from a Dog Study after single IV dosing, same samples analyzed by LCMS and ELISA

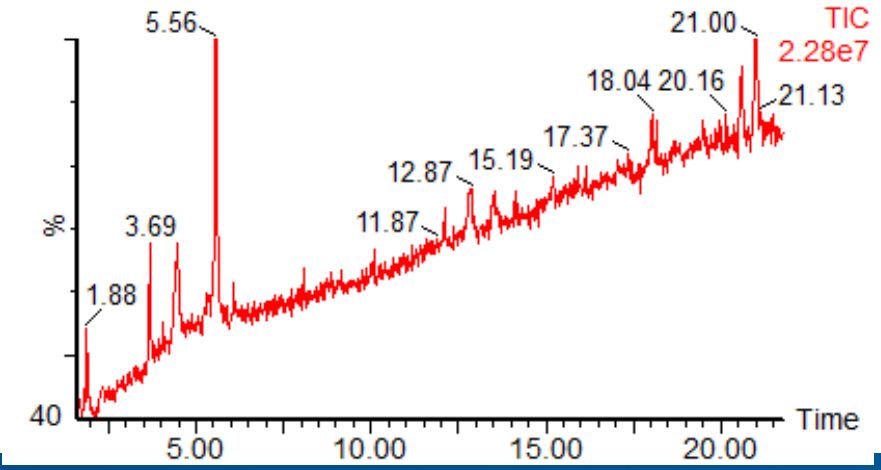
Hour	Minute	LCMS (ng/ml)	ECLIA (ng/ml)	ECLIA/LCMS
	2	42000	74470	1.8
	5	10700	28042	2.6
	15	647	4222	6.5
	30	87.0	1191	13.7
1		55.1	384	7.0
1.5		12.7	137	10.8
2		7.12	17.3	2.4
4		BQL	BQL	

Metabolite identification

- Process rat, dog and human plasma/serum samples using
  - Protein precipitation with acetonitrile
  - Incubation with capture antibody used in ELISA, followed by isolation of IgG's (including capture antibody) by protein G
- Analyze by LC-MS (Q-TOF, Synapt G2-S)
- Clean up spectra by ion extraction based on charge state (next slide)

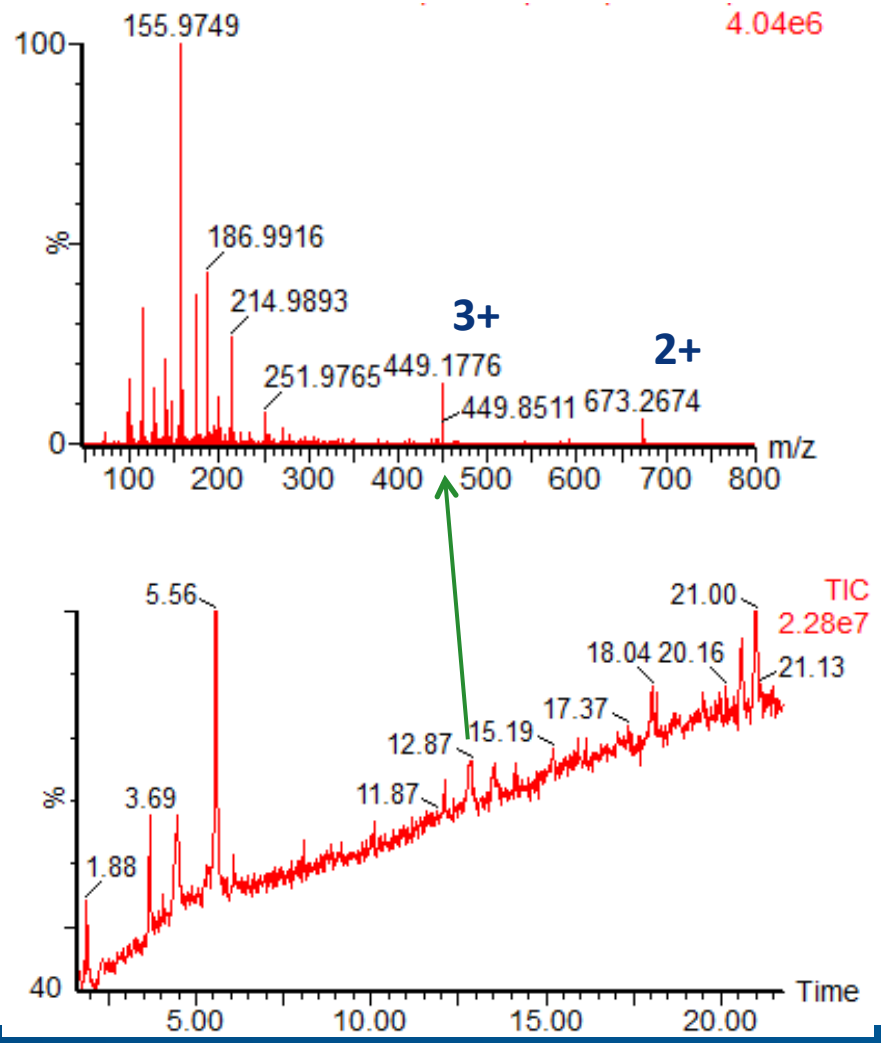
Ion extraction based on charge state

original chromatogram



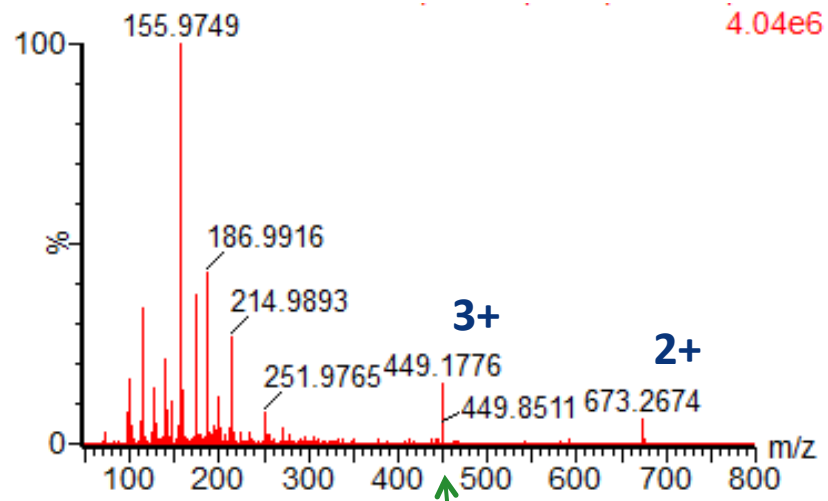
Ion extraction based on charge state

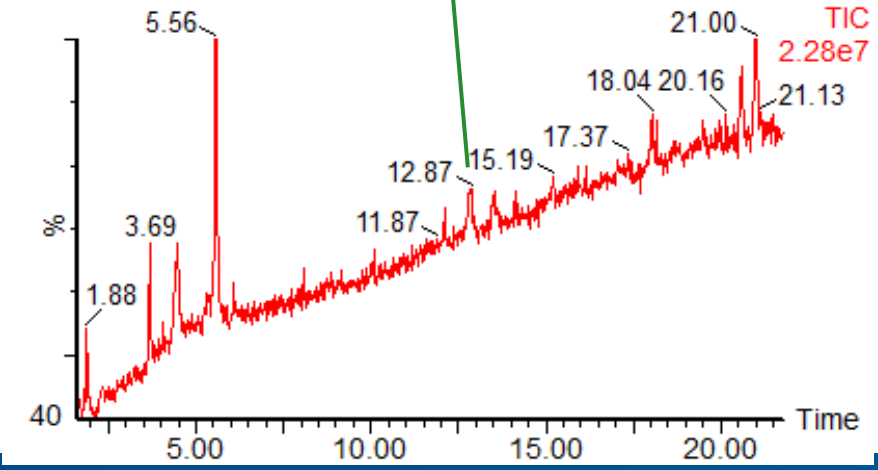
original chromatogram



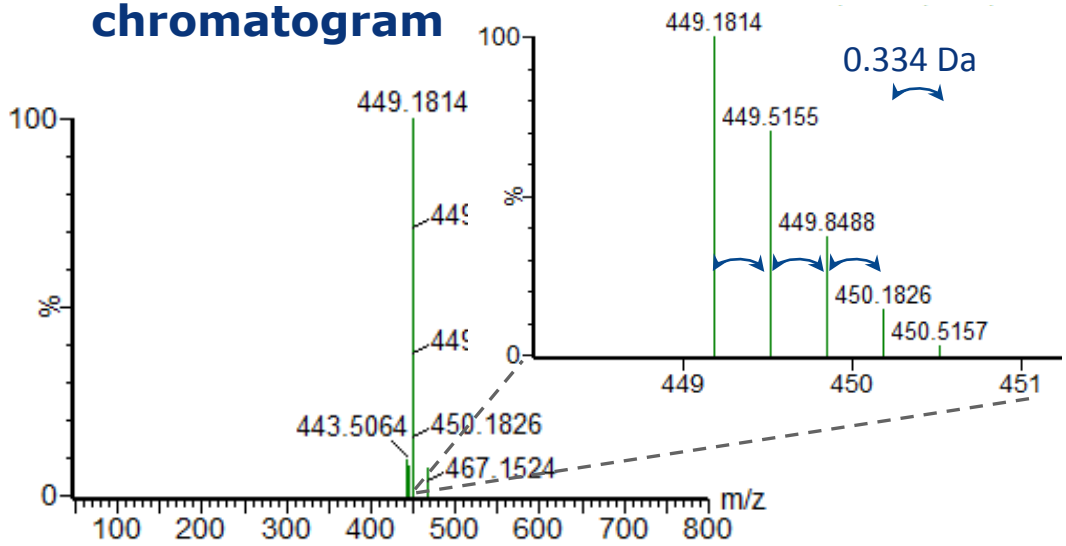
Ion extraction based on charge state

original chromatogram



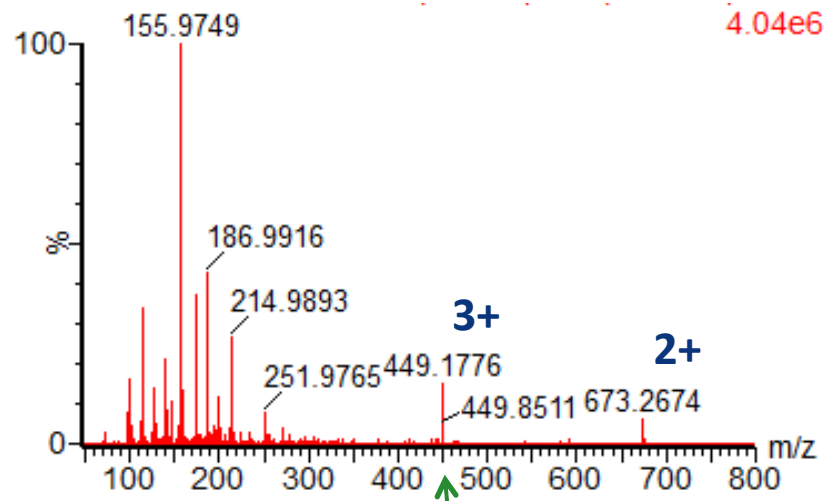


3+ charge state filtered chromatogram

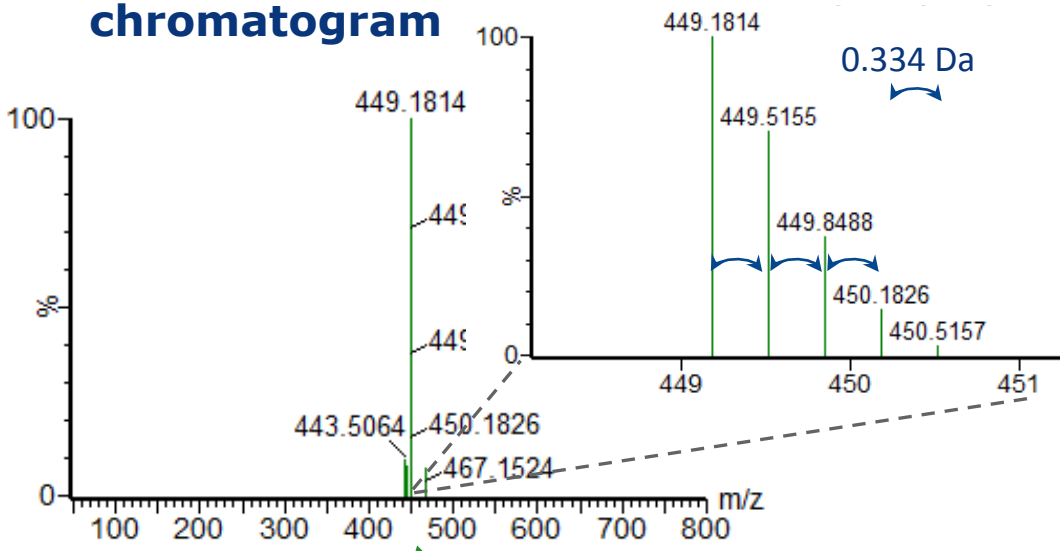


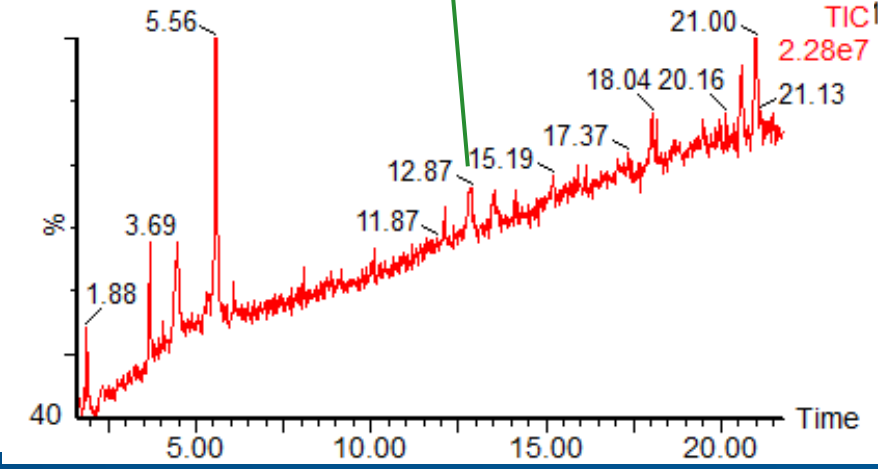
Ion extraction based on charge state

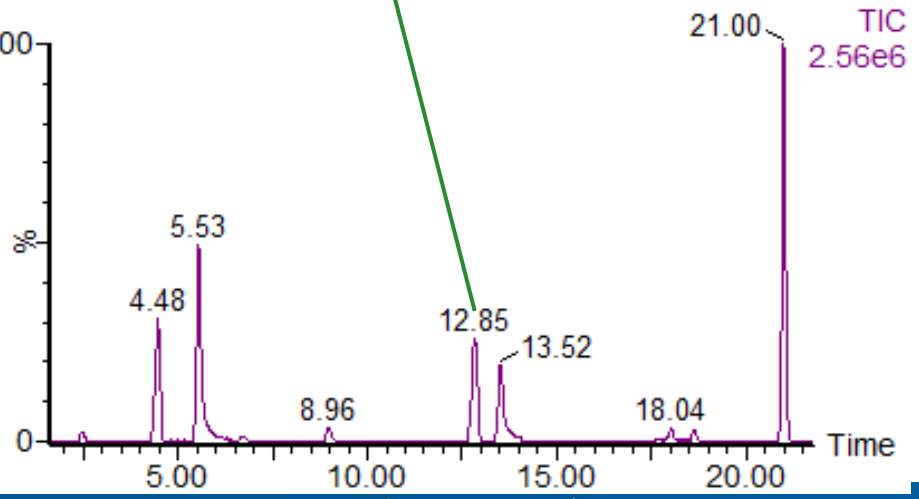
original chromatogram

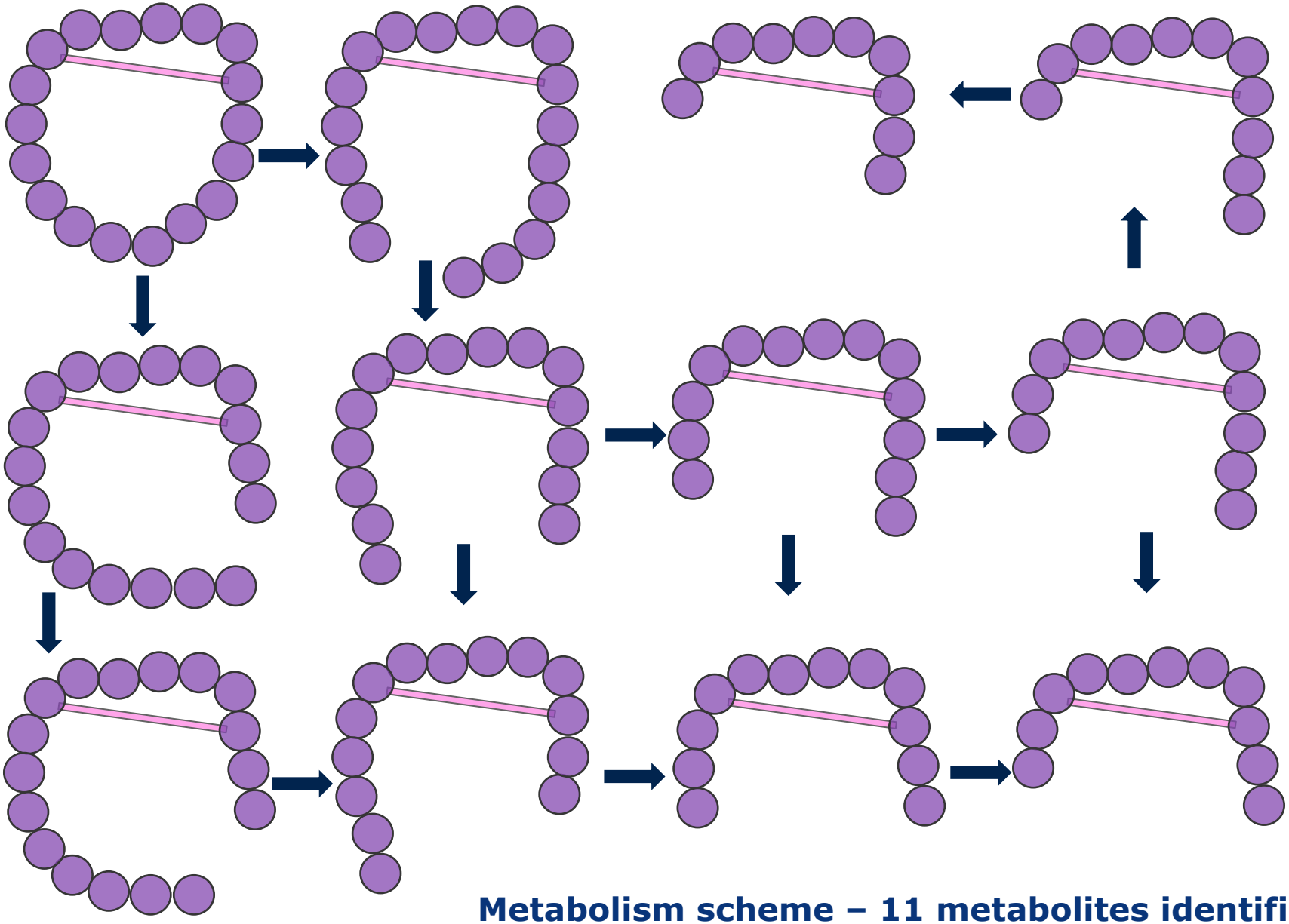


3+ charge state filtered chromatogram







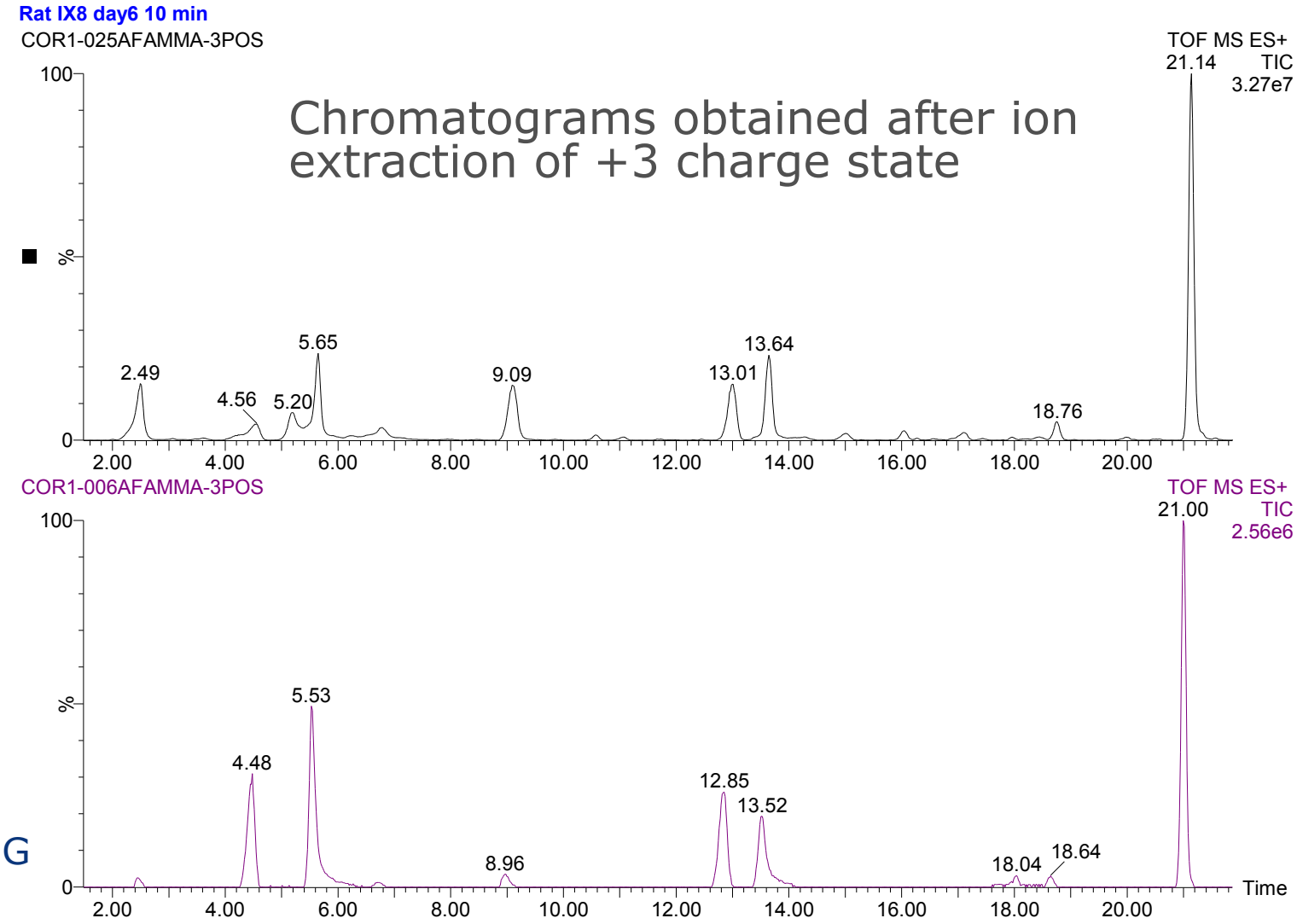


Metabolism scheme – 11 metabolites identified

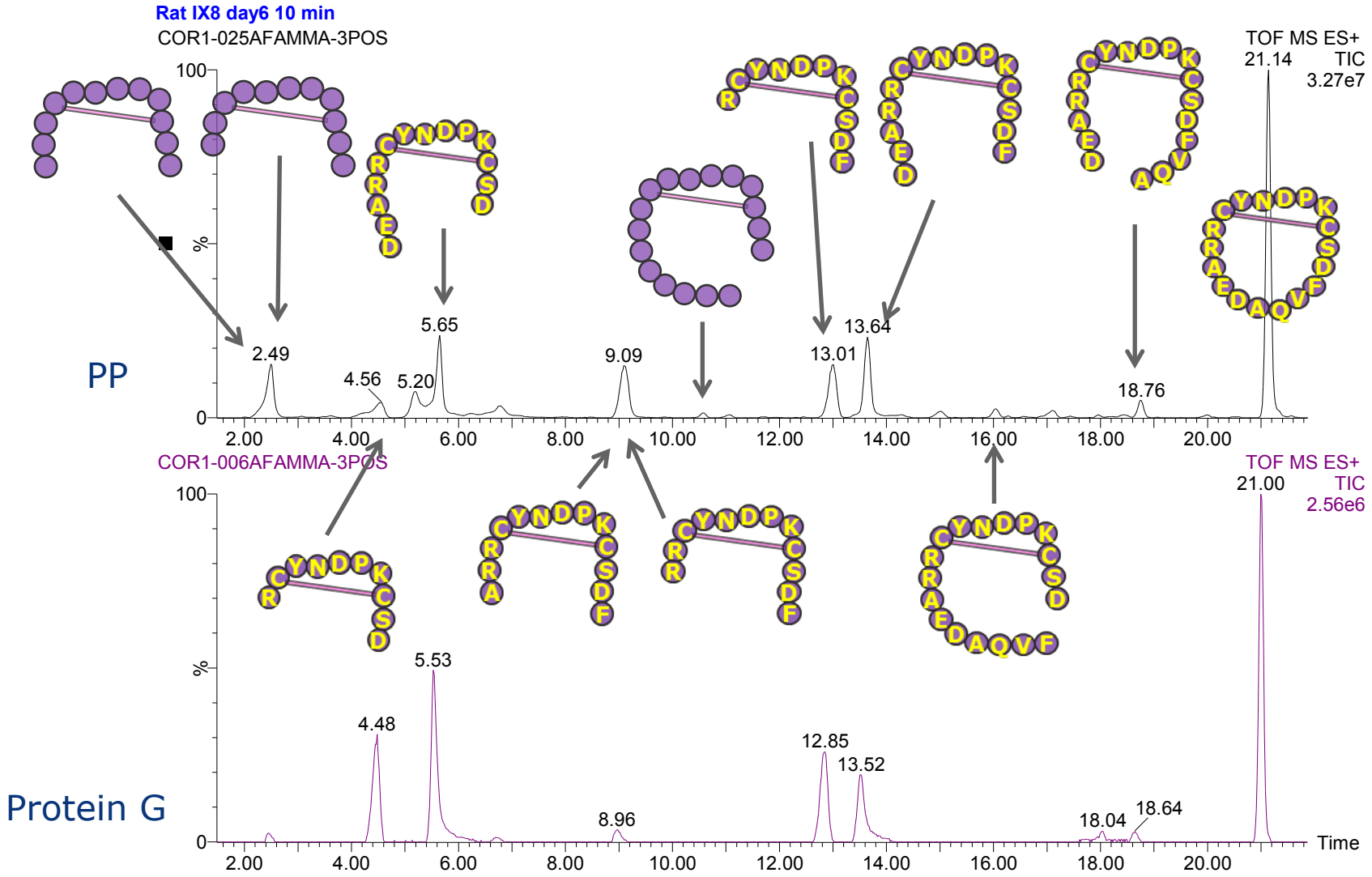
Protein precipitation vs Protein G

PP

Protein G



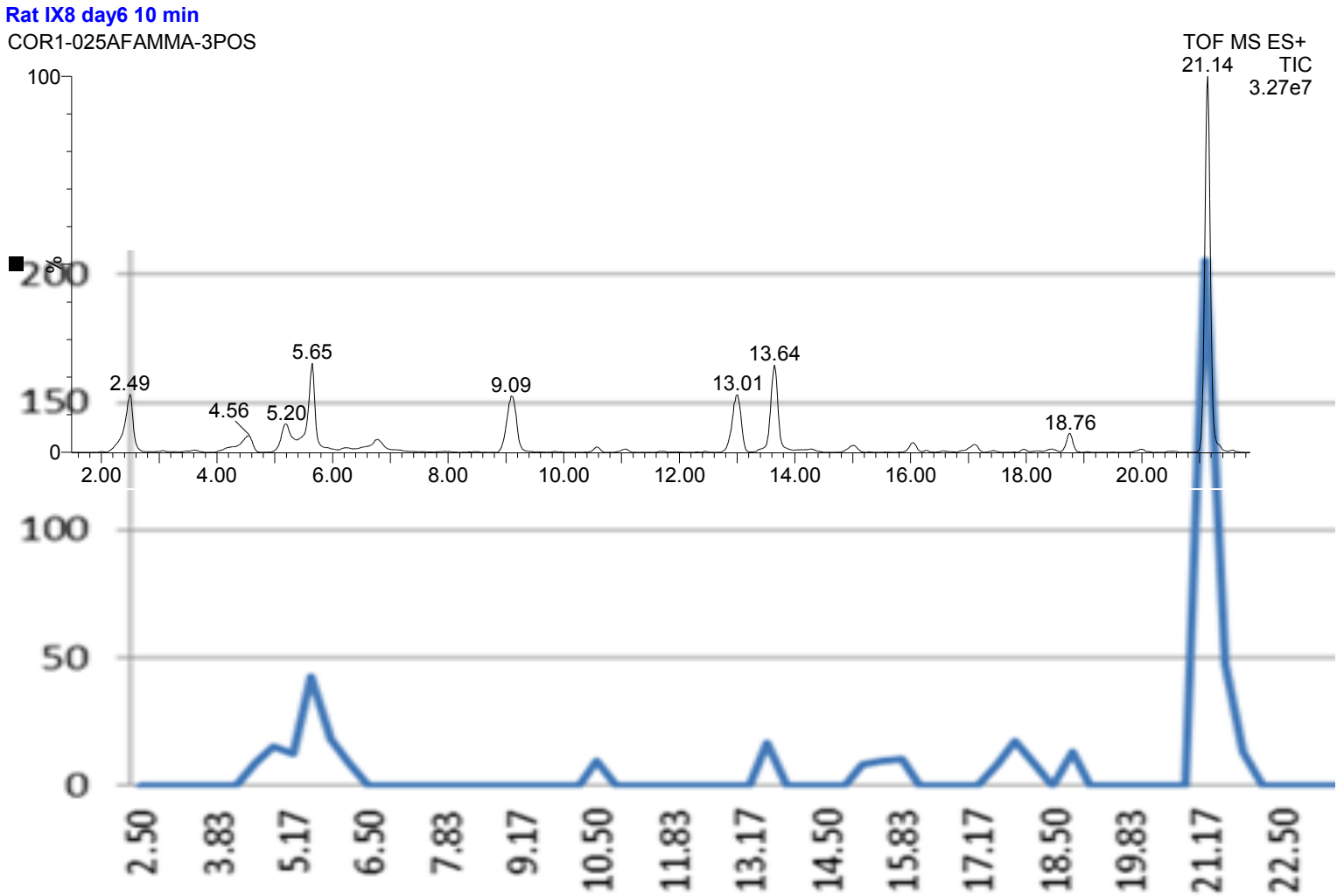
Identity of metabolites versus retention time



LBA on HPLC fractions

PP

LBA



Conclusions - Project

- For the peptide drug, a large difference between LBA and LC-MS/MS was observed
- It was shown that the difference was due to cross reactivity in the LBA with metabolites of the peptide drug
- Tools used to demonstrate cross-reactivity with metabolites in general and showing with which metabolites the cross-reactivity occurred were
  - Incubation with capture Ab followed by Protein G purification
  - LBA on HPLC fractions
- Metabolites of the peptide drug were identified, and ion extraction based on charge state was a very useful tool to clean up the spectra to aid in metabolite ID of the peptide

Conclusions - Strategic

- Bioanalysis using LBA and LC-MS/MS assays can have a significantly different result because of cross reactivity in LBA and/or because of LC-MS/MS only measuring the peptide drug and not active metabolites (unless added intentionally)
- The use of both platforms is very useful to gain a better understanding of the peptide drug and of the read-out of the assays – could be part of a company strategy to use both assays for this purpose
- In contrast with what the DRAFT FDA Guidance is suggesting, a difference in results LBA vs LC-MS/MS should NOT be an issue, but it is important to understand "what" each assay is measuring

Acknowledgements

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