Supercharging reagents: Revving up peptide LC-MS analyses

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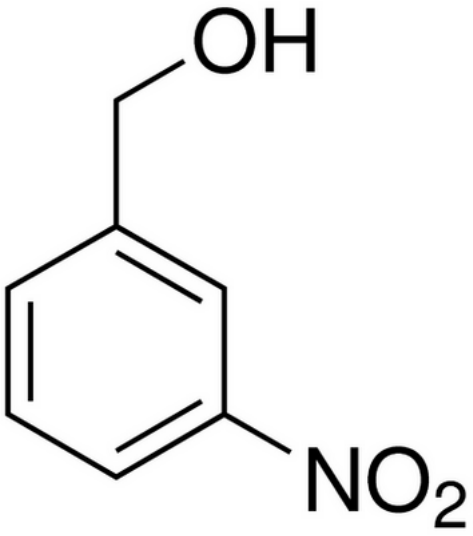
Science
for a safer world

Overview

- What are “superchargers”?
- How do they work?
- Case studies
  - 2 Positives
  - 1 Negative

What is a supercharger?

Superchargers: Mobile phase additives



m-NBA

How do you use m-NBA?

- Add it into mobile phases
  - Can also T-in
- m-NBA is an oily substance
  - Doesn’t dissolve in 100% aqueous
- Will dissolve in 5% ACN in water
  - This does affect retention time if m-NBA is in aqueous
- We initially used it in both aqueous and organic
  - Changed to adding only in organic
  - No shift in retention time

Historic use of superchargers

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J Am Soc Mass Spectrom. 2000 November ; 11(11): 976–985.

Effects of Solvent on the Maximum Charge State and Charge State Distribution of Protein Ions Produced by Electrospray Ionization

Anthony T. Iavarone, **John C. Jurchen**, and **Evan R. Williams**
Department of Chemistry, University of California, Berkeley, California, USA

Published in final edited form as:
Anal Chem. 2001 April 1; 73(7): 1455–1460.

Supercharged Protein and Peptide Ions Formed by Electrospray Ionization

Anthony T. Iavarone, **John C. Jurchen**, and **Evan R. Williams***
Department of Chemistry, University of California, Berkeley, California 94720-1460

J. Am. Soc. Mass Spectrom. (2012) 23:1390–1399
DOI: 10.1007/s13361-012-0404-0

RESEARCH ARTICLE

Charge State Coalescence During Electrospray Ionization Improves Peptide Identification by Tandem Mass Spectrometry

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In-Spray Supercharging of Peptides and Proteins in Electrospray Ionization Mass Spectrometry

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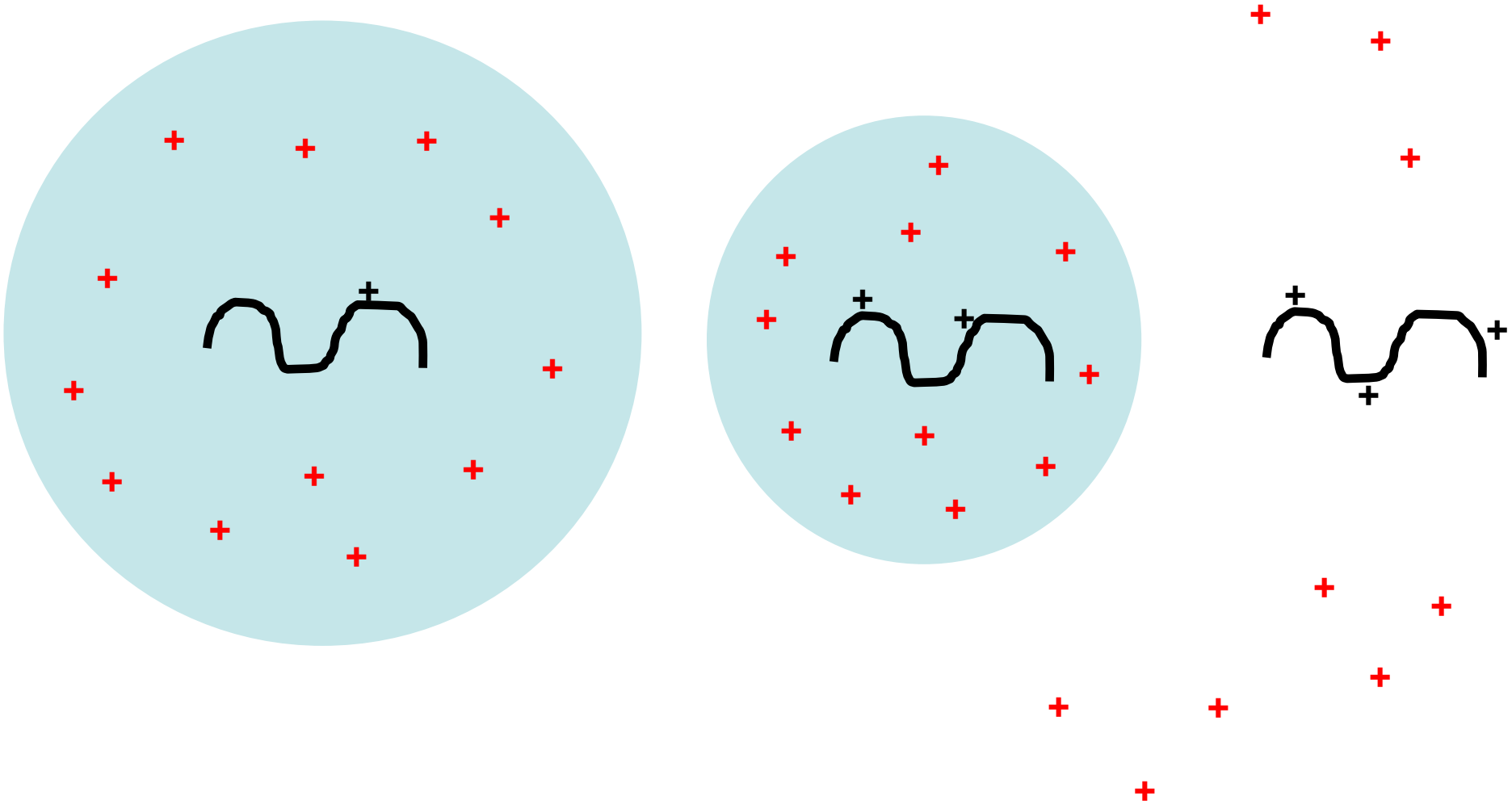
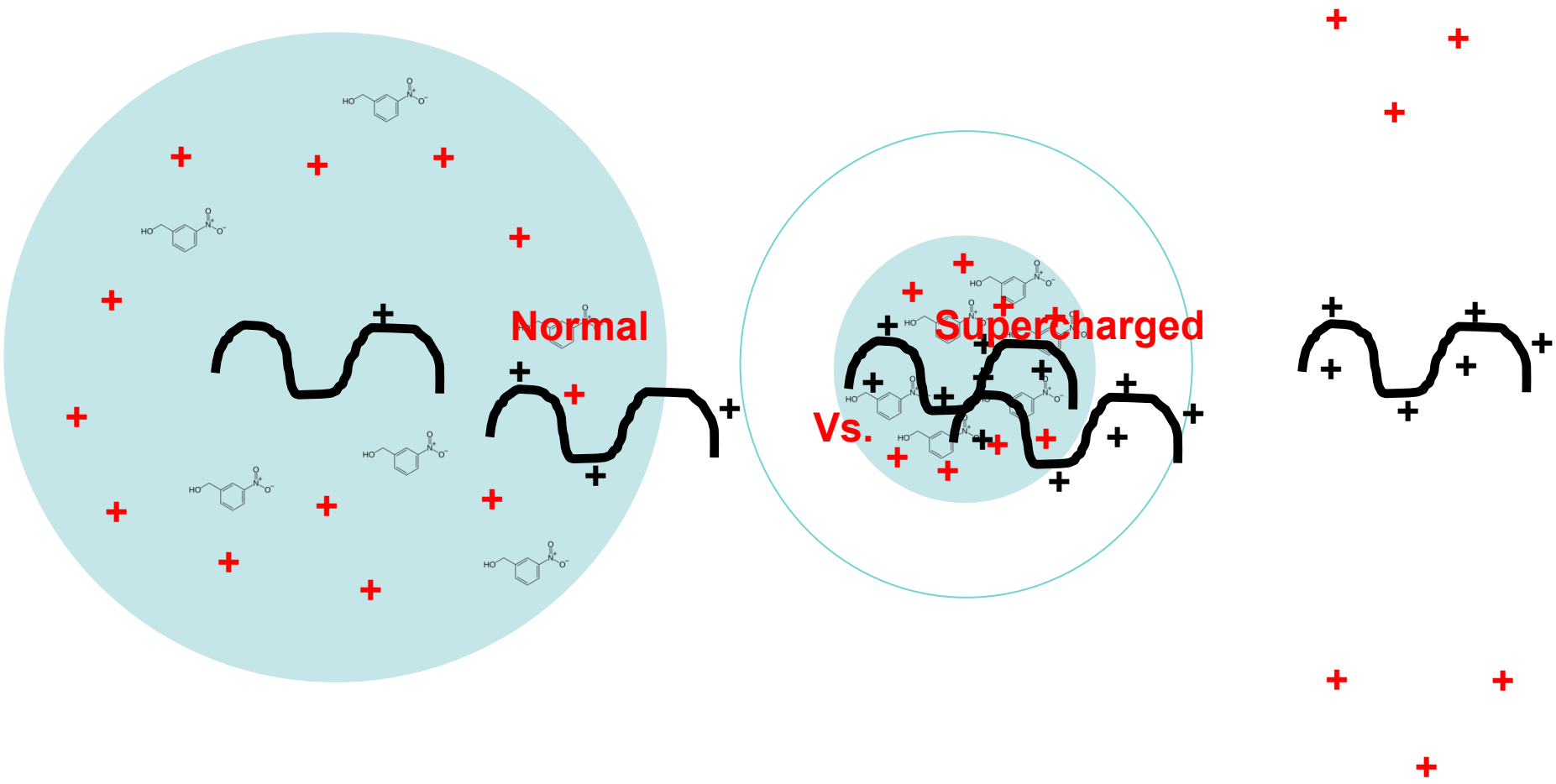
Electrospray ionisation (normal)

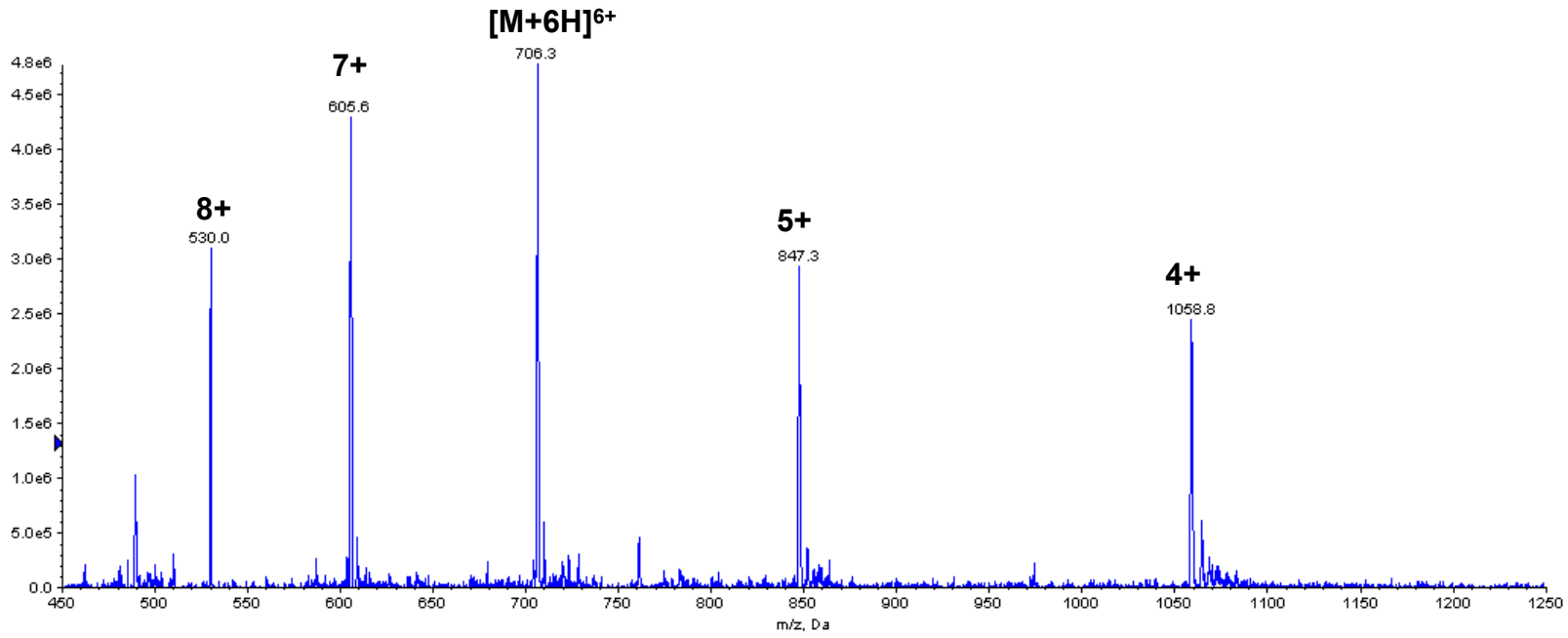


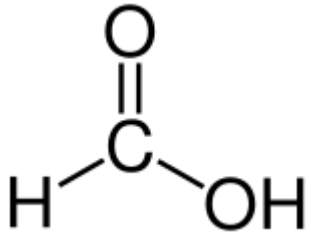
Electrospray ionisation (Supercharged)



Case study 1 : 4 kDa peptide

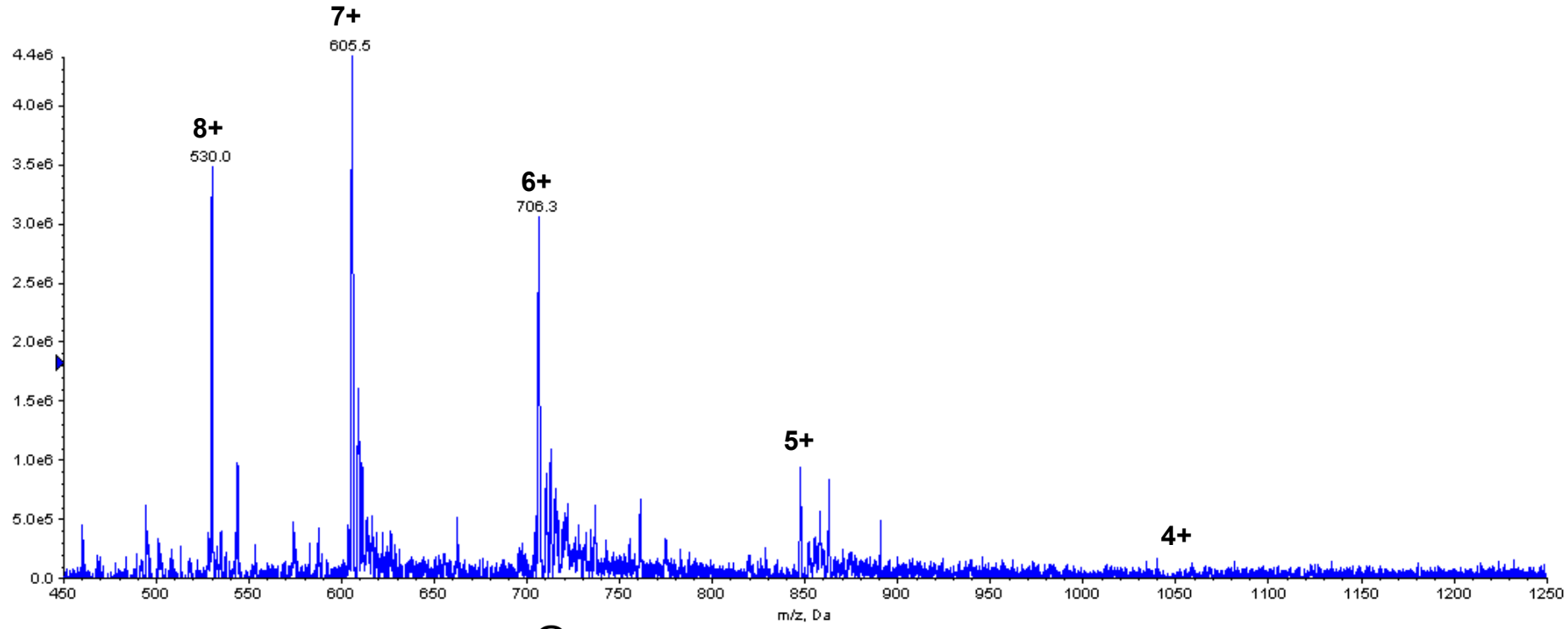
- Mass spectrum using only formic acid

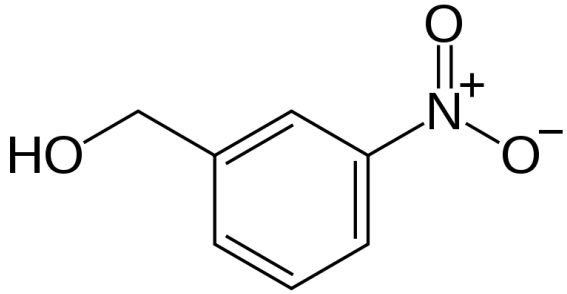


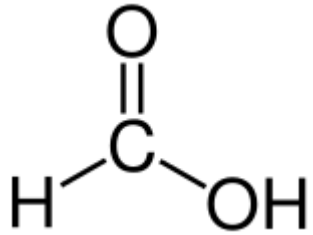


Case study 1 : 4 kDa peptide (+ mNBA)

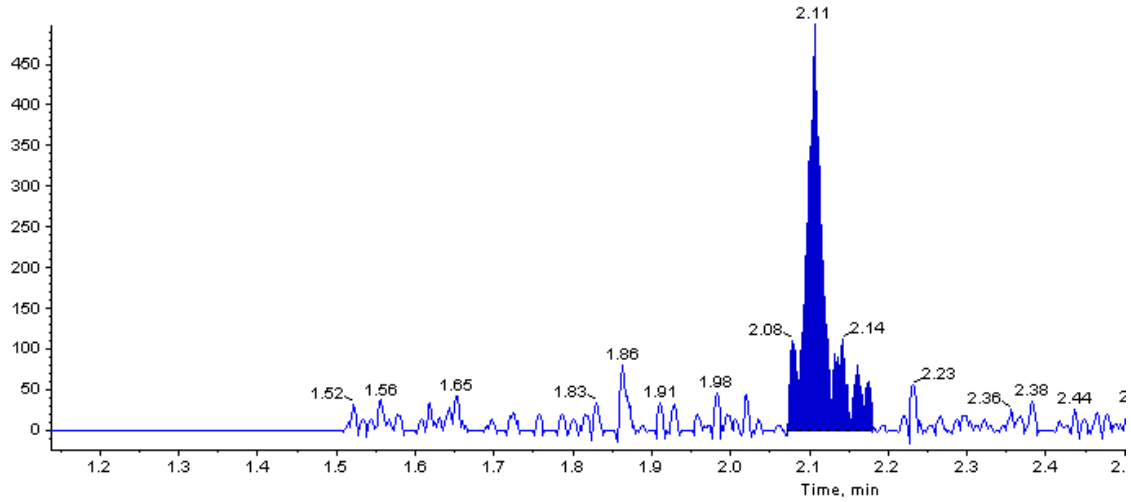
- Large shift in charge state envelope:



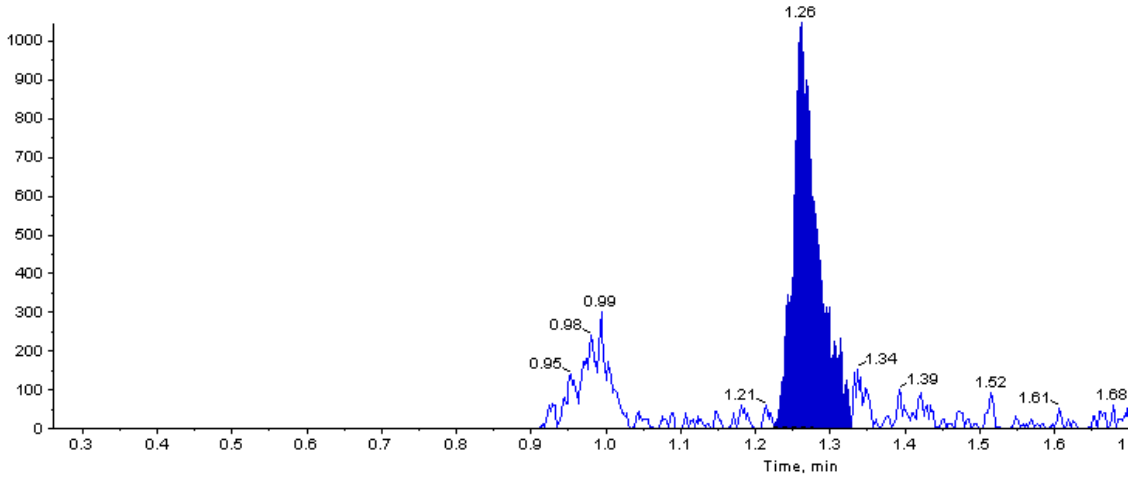




Comparison of 0.2 ng/mL LLOQ



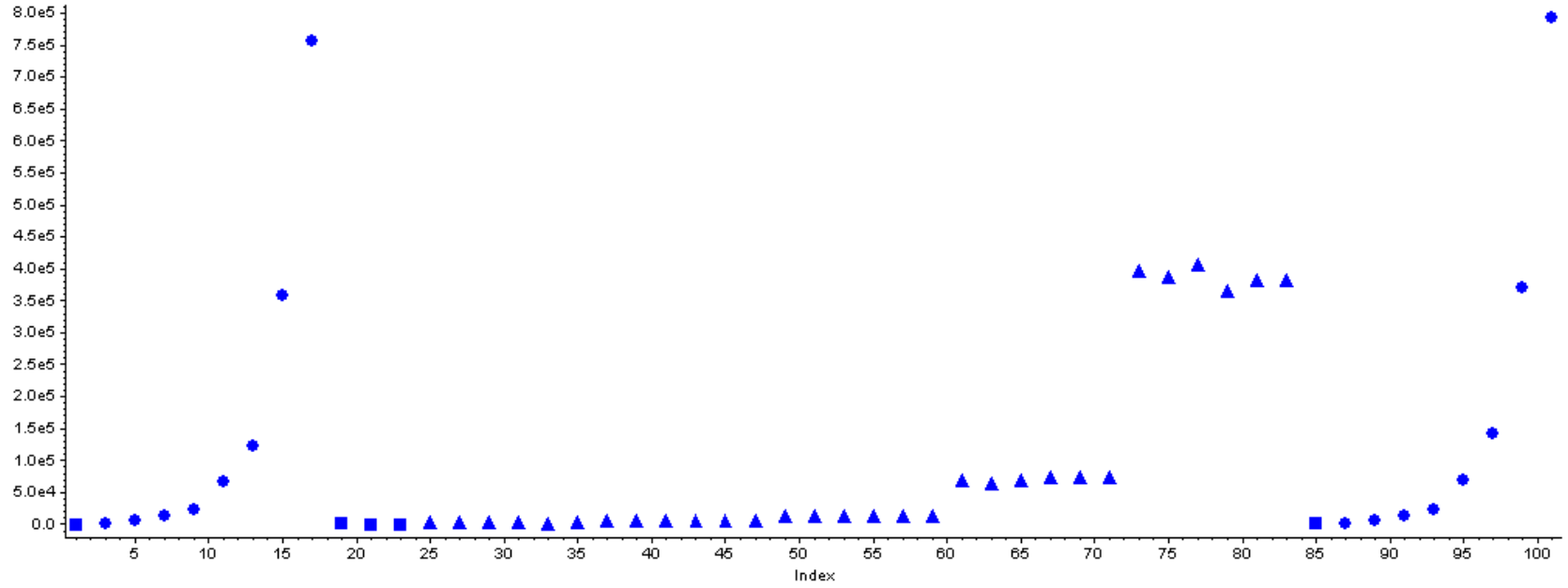
No m-NBA
7+



+ m-NBA
8+

Case study 1: Assay performance

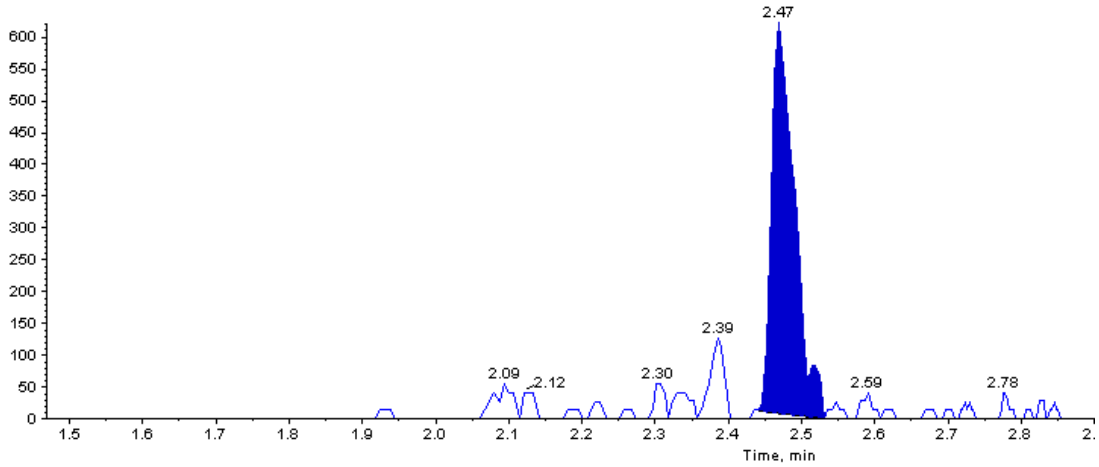
QC Level	Level (ng/mL)	Calculated conc.	%CV	Accuracy
LLOQ 1	0.200	0.234	8.9	117.0
LLOQ 2	0.500	0.549	4.1	109.9
LOW QC	1.00	1.02	2.9	102.4
MED QC	5.00	5.17	4.9	103.4
HIGH QC	25.0	28.1	3.6	112.4



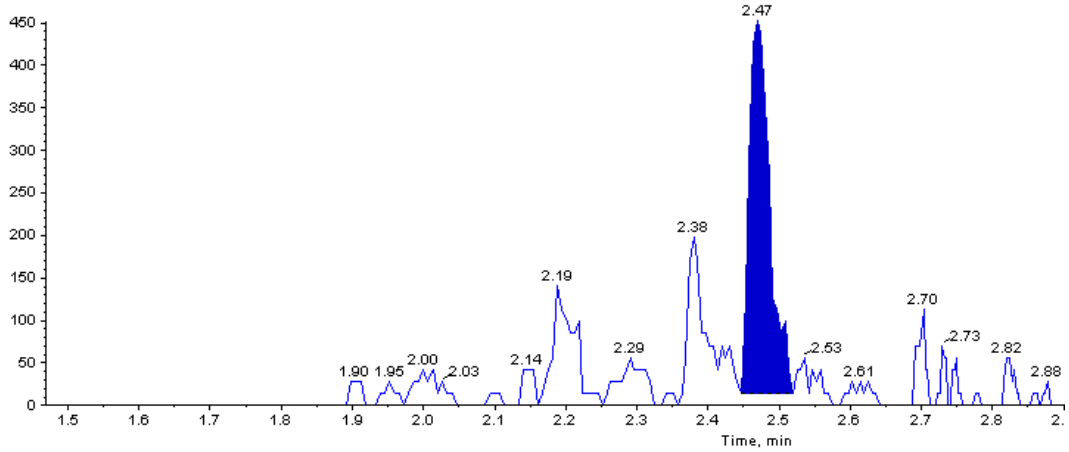
Case study 2: Protein digest

- Large protein (~70 Da)
- Study involved metabolite ID on the protein
  - required the use of a tryptic digest
- Large tryptic peptide was generated
  - Contained 67 amino acids
  - Critical for metabolite ID
- Peptide generated multiple charge states:
  - $[M+5H]^{5+}$
  - $[M+6H]^{6+}$

67 aa peptide 5+ charge state (SRM)

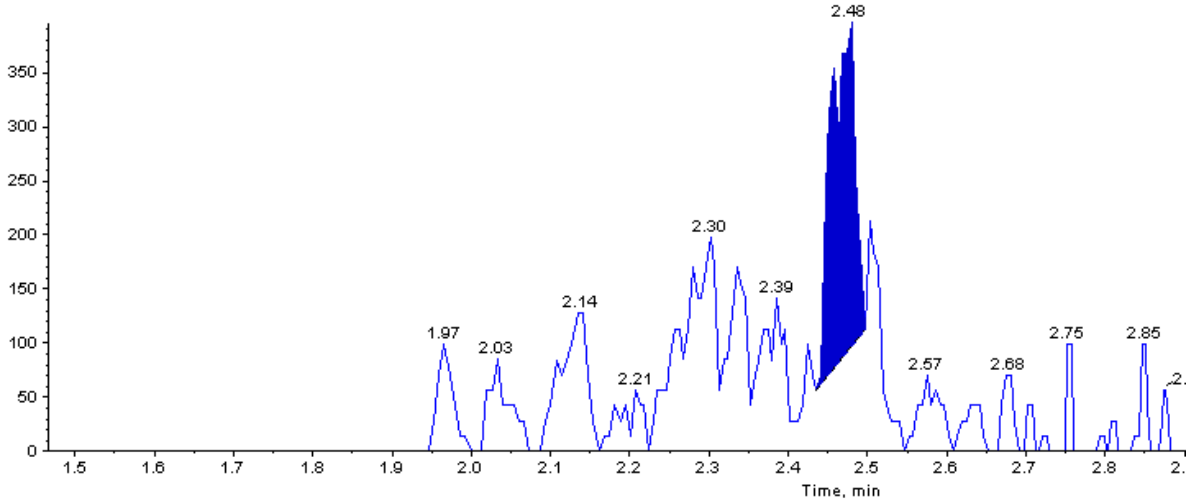


No m-NBA

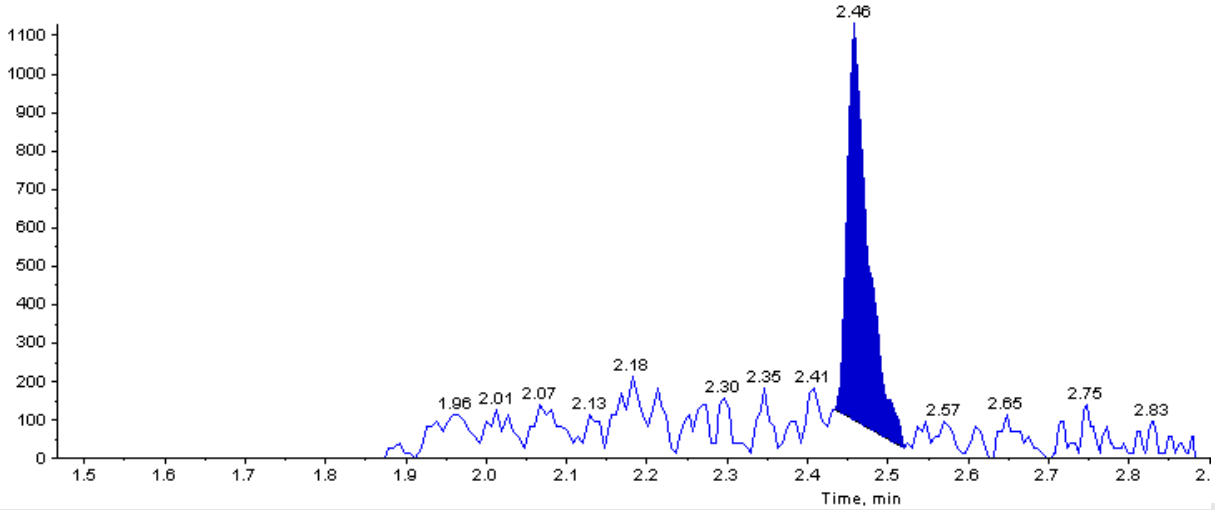


+ m-NBA

67 aa peptide 6+ charge state (SRM)



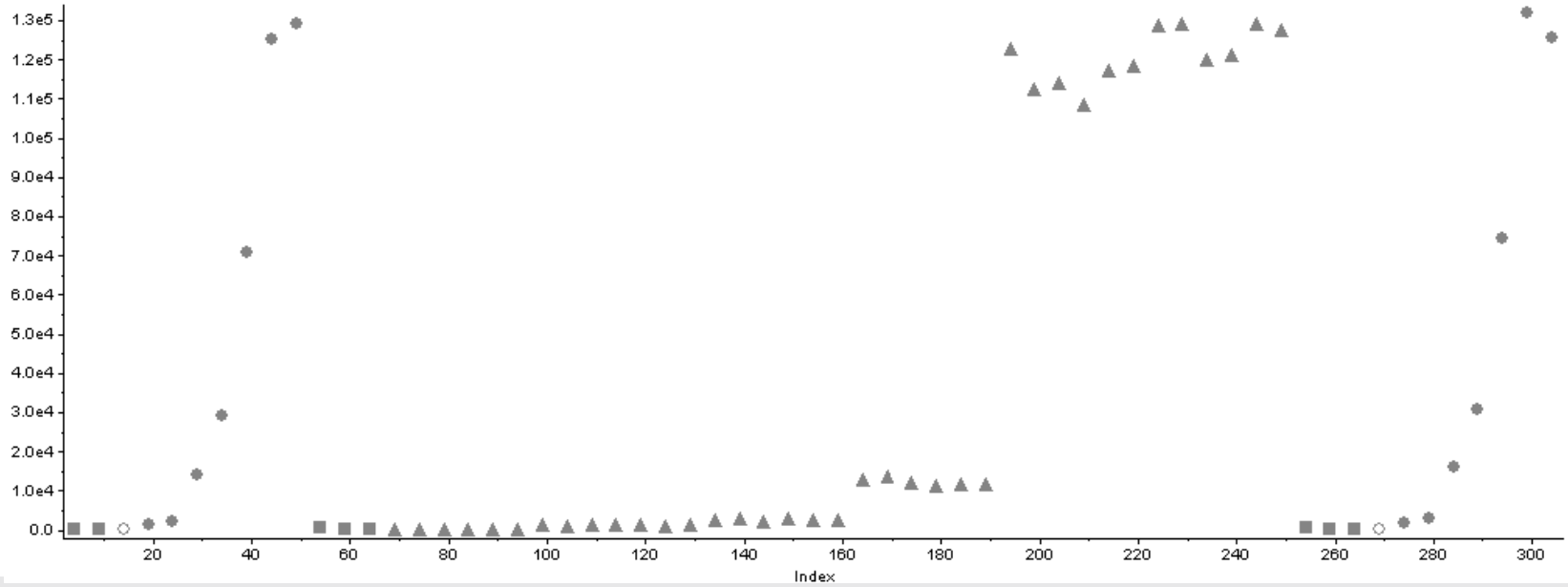
No m-NBA



+ m-NBA

Case study 2: Assay performance

QC Level	Level (µg/mL)	Calculated conc.	%CV	Accuracy
LLOQ	0.500	0.425	14.1	85.0
LOW QC	1.00	0.902	14.7	90.2
MED QC	4.00	4.28	7.3	107.1
HIGH QC	40.0	40.8	4.3	102.1



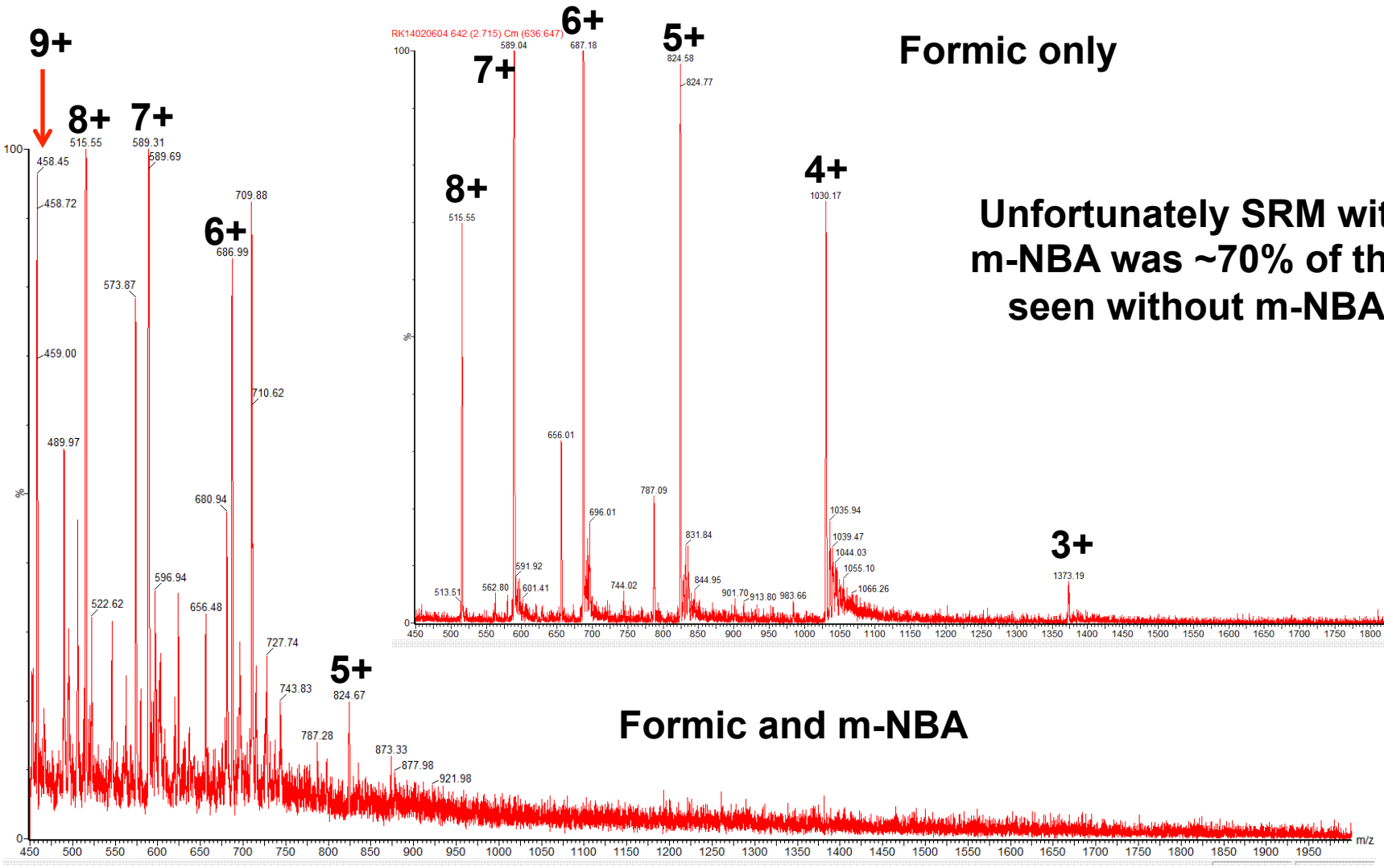
Case study 3: PTH 1-34

- Developed and validated a PTH 1-34 method in pig plasma
  - 15 pg/mL LLOQ
  - 3 pM concentration
  - 145 attomoles on column
  - 40,000,000 molecules!

- Enough sensitivity?

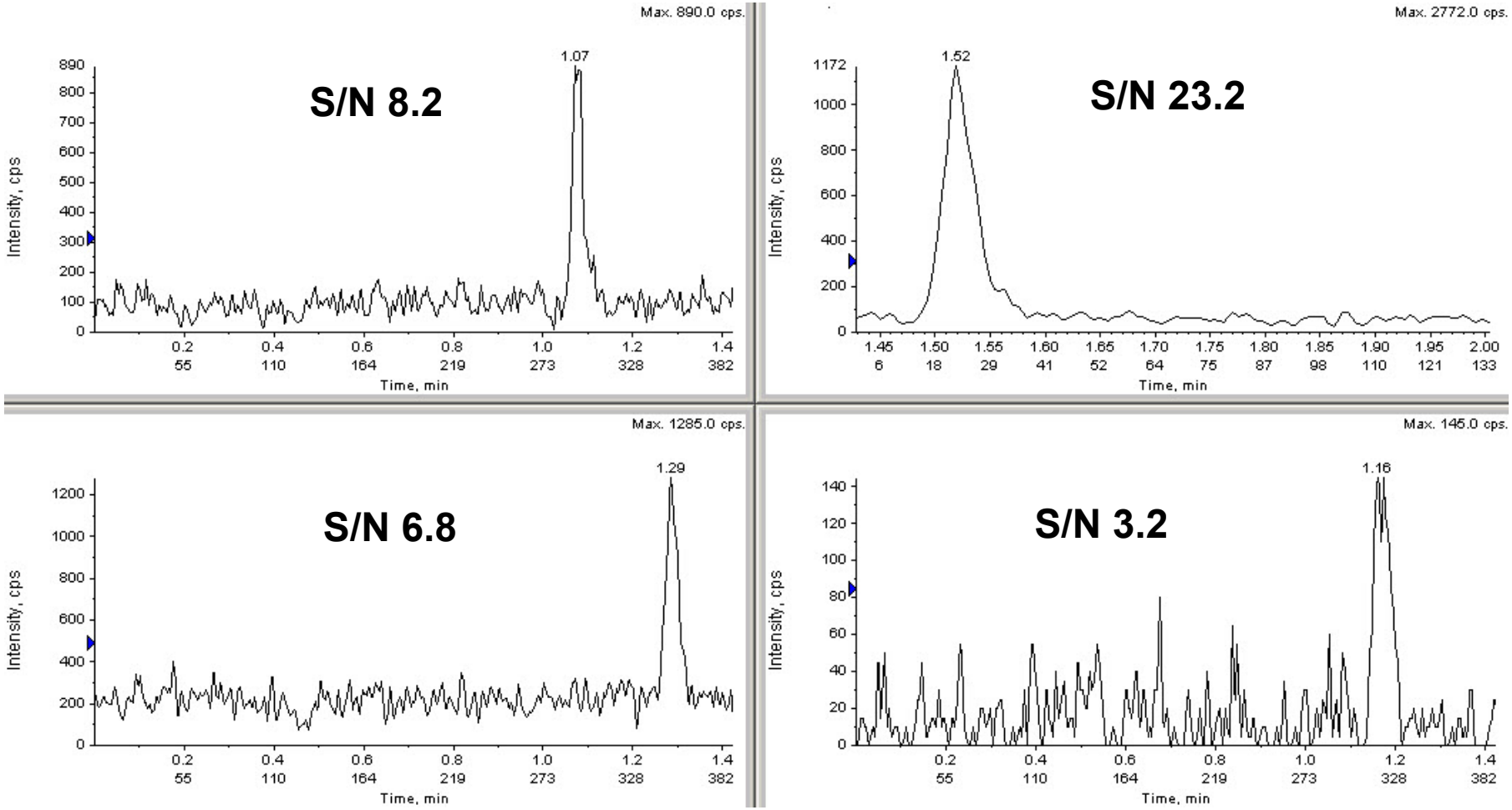
- Can m-NBA improve sensitivity
  - Can we get 10 pg/mL or lower?

PTH 1-34 full scan data



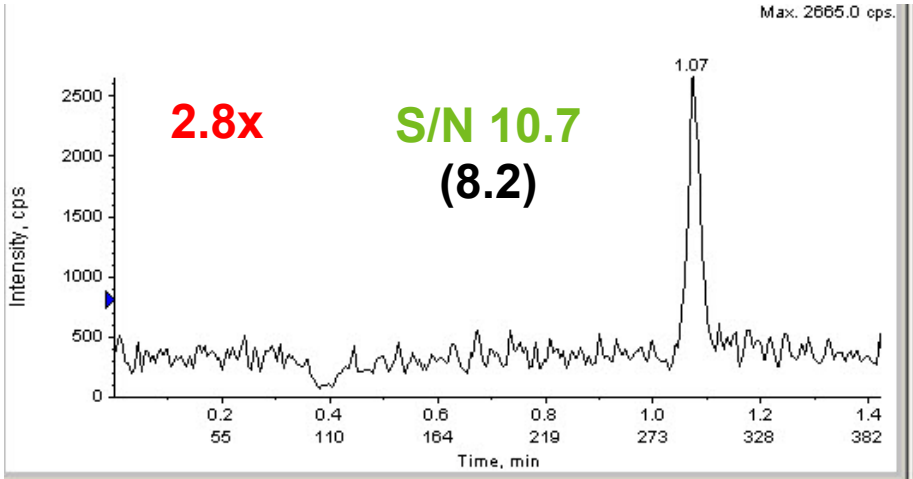
Stop the press..

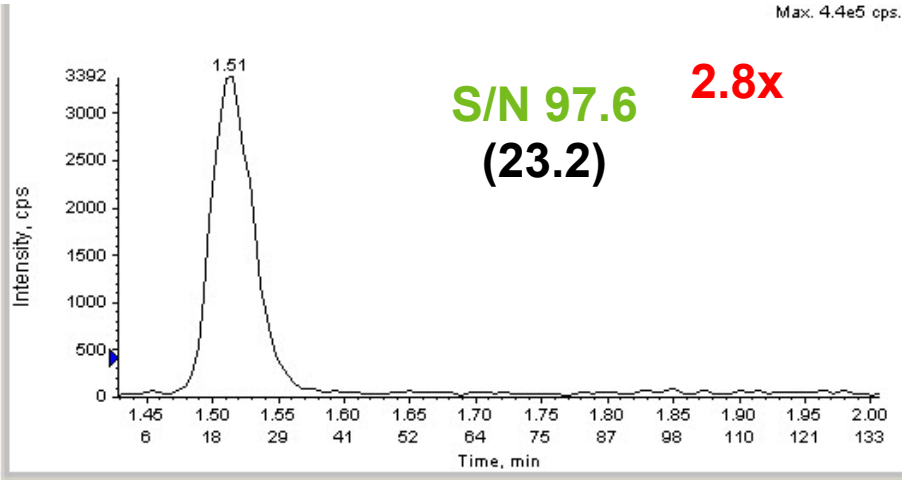
- It also works for small molecules:
  - Parent and 3 metabolites: No m-NBA

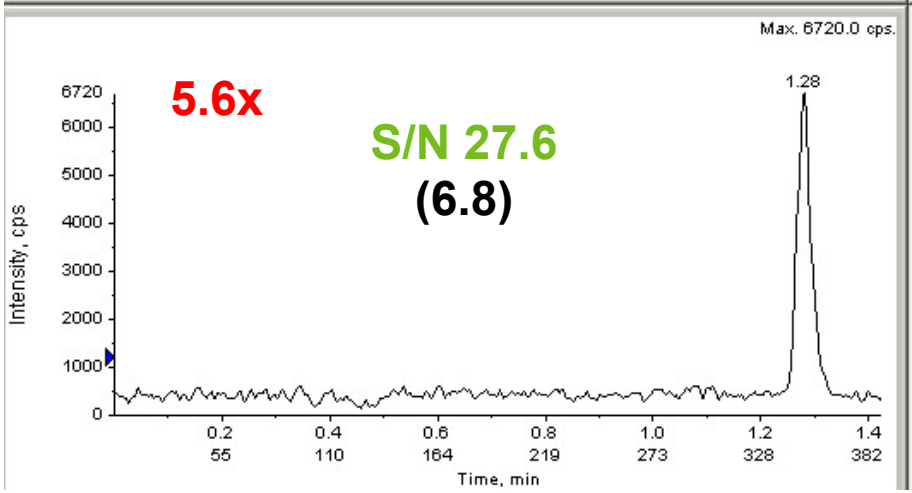


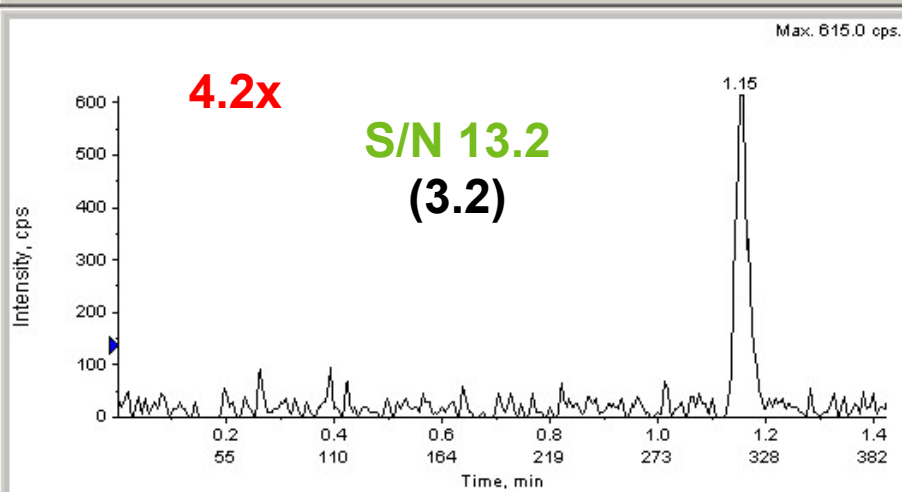
Stop the press..

- It also works for small molecules:
  - Parent and 3 metabolites: + m-NBA









Conclusions

- Does what it says on the tin!
  - For every peptide we have tested it on
  - Sometimes too much charge
- Average of ~2-4x sensitivity increase
- Still treating with kid gloves re. instrument stability
  - Using diverts where possible
  - Increased source temperature

Acknowledgements

Colleagues at LGC:

- David Neville
  - Sent me the original supercharger paper
- Jon Hands
  - PTH1-34 data
- Geoff Wallace
  - Small molecule data

Thank you for listening

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