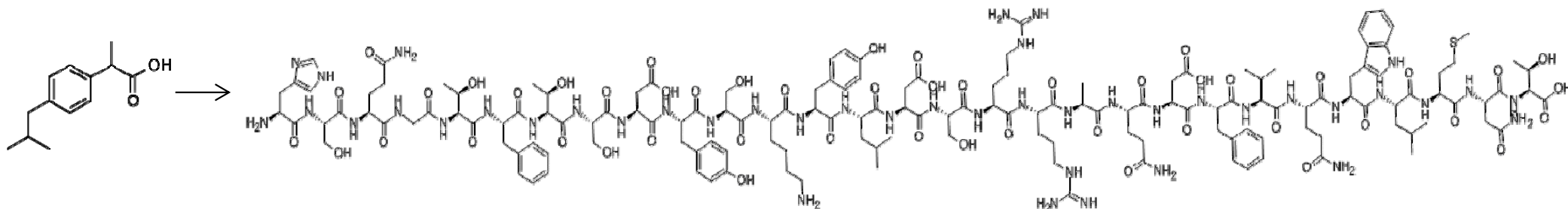


Little to LARGE



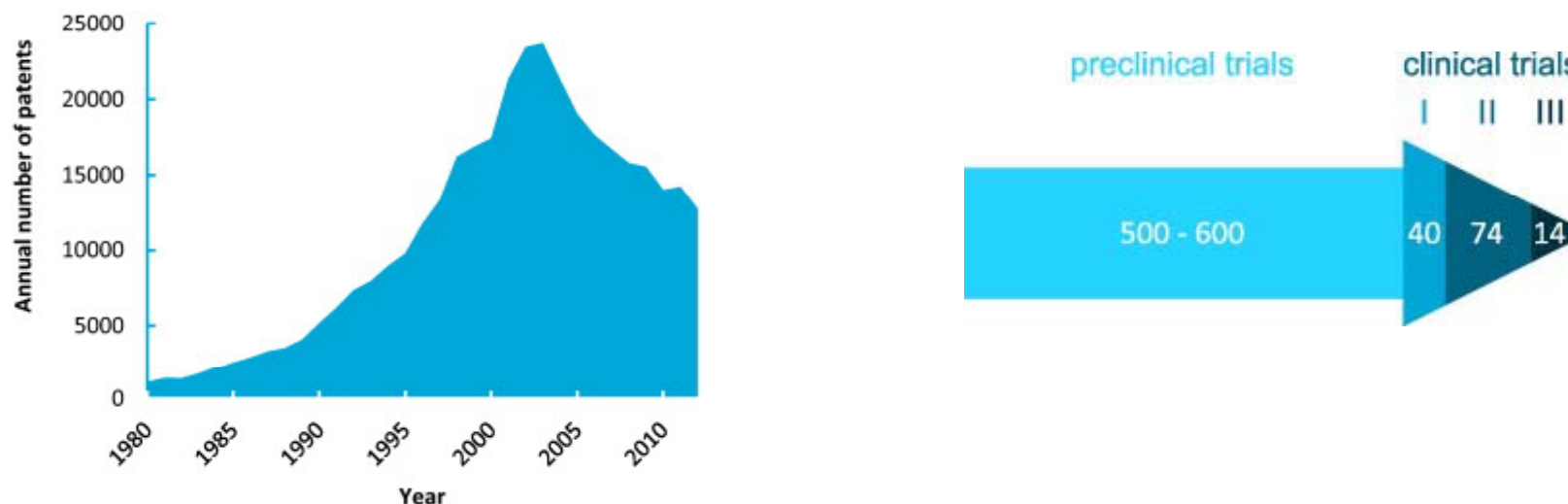
LC-MS/MS peptide method development for small molecule specialists

Science
for a safer world



Peptide Quantitation

- Peptide biomarkers
 - Diagnosis (e.g. cancers, diabetes, Alzheimer's, cardiovascular)
 - Measure effectiveness of novel treatments
- Peptide drugs
 - For the treatment of a wide variety of diseases (pain, cancer, CVD)



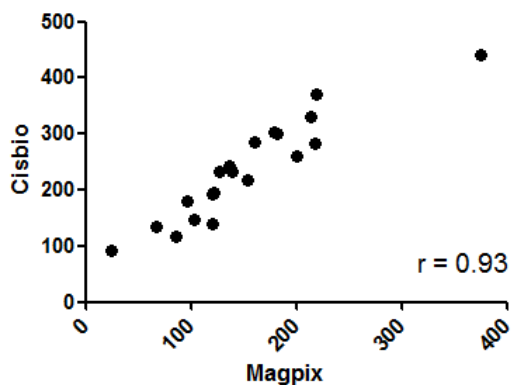
The emergence of peptides in the pharmaceutical business: From exploration to exploitation- EuPA Open Proteomics Volume 4, September 2014, Pages 58–69

- Peptide quantitation in blood plasma samples - Needed to support the pharmacodynamic (PD) characterisation of biomarkers and pharmacokinetic (PK) characterisation of drugs

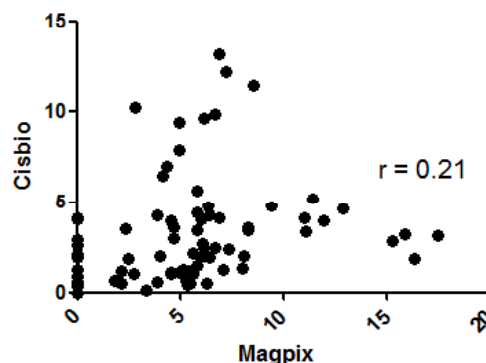
Peptide Quantitation

- Immunochemistry - e.g. RIAs, ELISAs
- However - antibodies may not be available, can overestimate concentrations (nonspecific antibody binding), can have long extractions, radioactivity precautions (RIA) or poor P&A

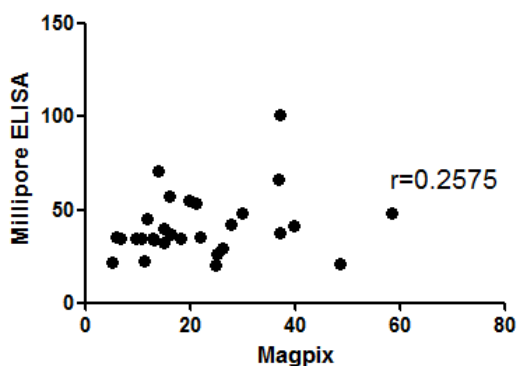
Correlation of glucagon levels (infusion)



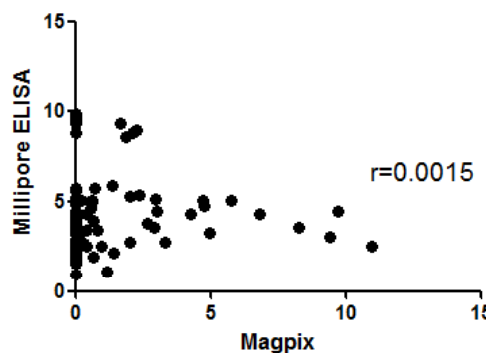
Correlation of glucagon levels (fasting)



Correlation of GLP-1 infusion



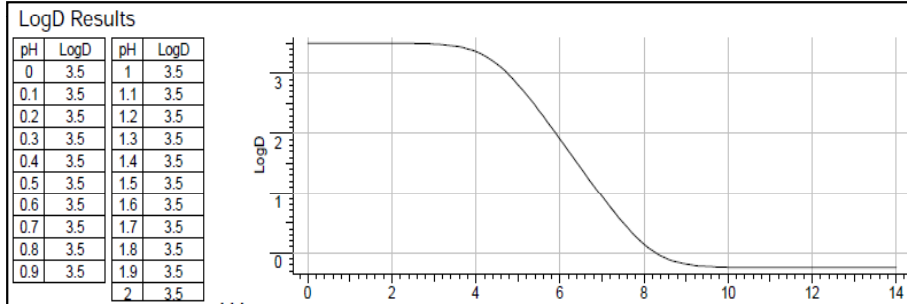
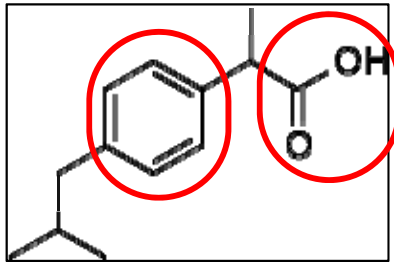
Correlation of GLP-1 fasting



- LC-MS/MS - as an alternative
- Method development is similar to small molecules, but is not the same

Research: Physicochemical Properties

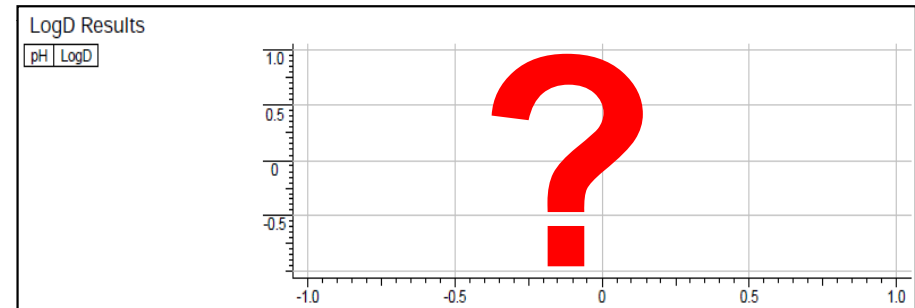
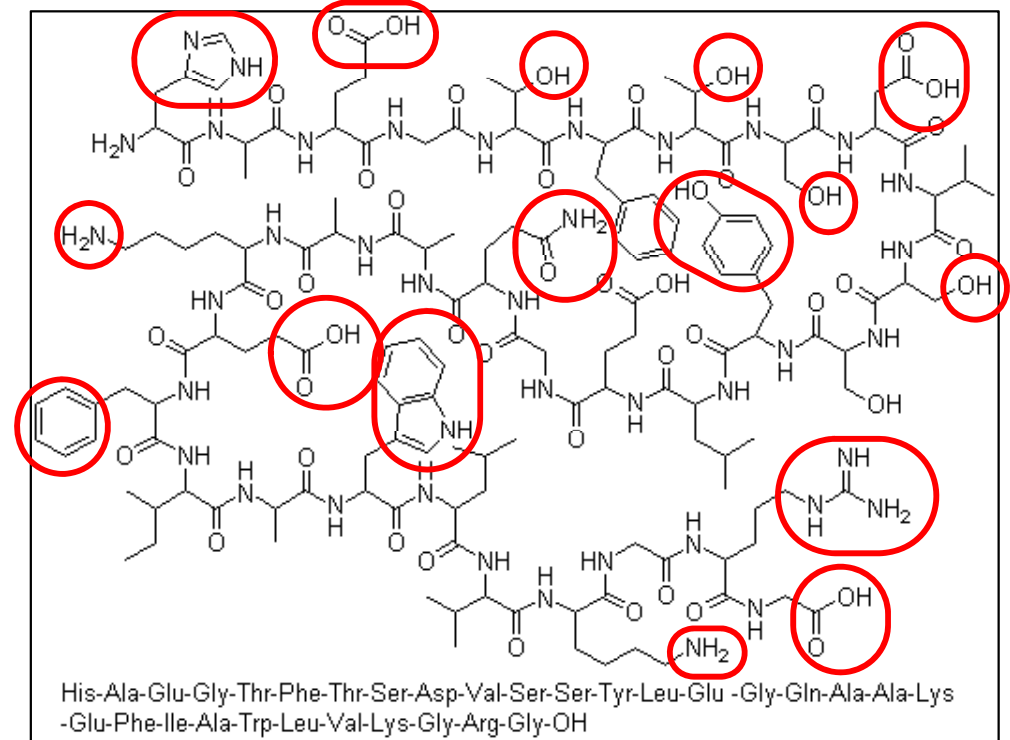
Ibuprofen, 206 Da



pKa Results

Diss. Atom	Acidic/Basic	Apparent pKa Value	Error
7	MA	4.41	0.1

Glucagon, 3483 Da



pKa Results

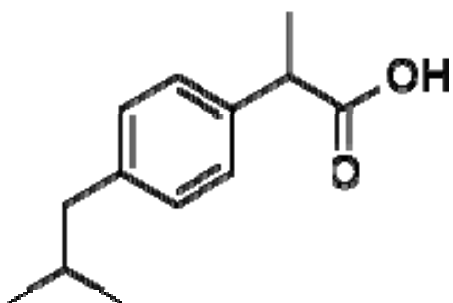
Diss. Atom	Acidic/Basic	Apparent pKa Value	Error

Sensitivity

Small Molecules	
Plasma LLOQ (pg/mL)	Approx. Mass (Da)
0.25	340
0.25	390
1	420
1	500
5	470

Ibuprofen- 206 Da

1pg = $2.92 \cdot 10^9$ molecules

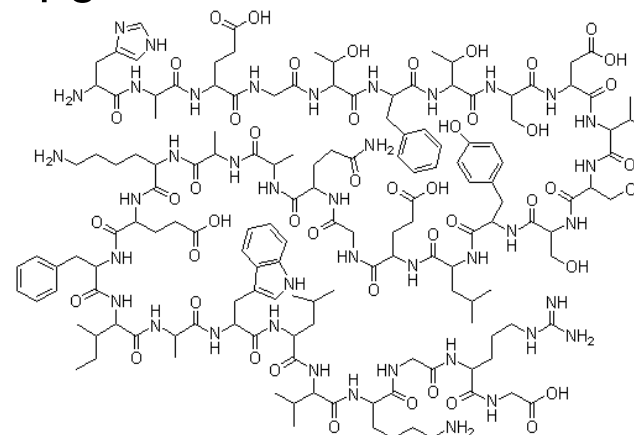


÷ **17**

Peptides	
Plasma LLOQ (pg/mL)	Approx. Mass (Da)
10	3,500
15	4,100
25	3,300
30	4,400
40	4,100

Glucagon- 3,483 Da

1pg = $1.72 \cdot 10^8$ molecules

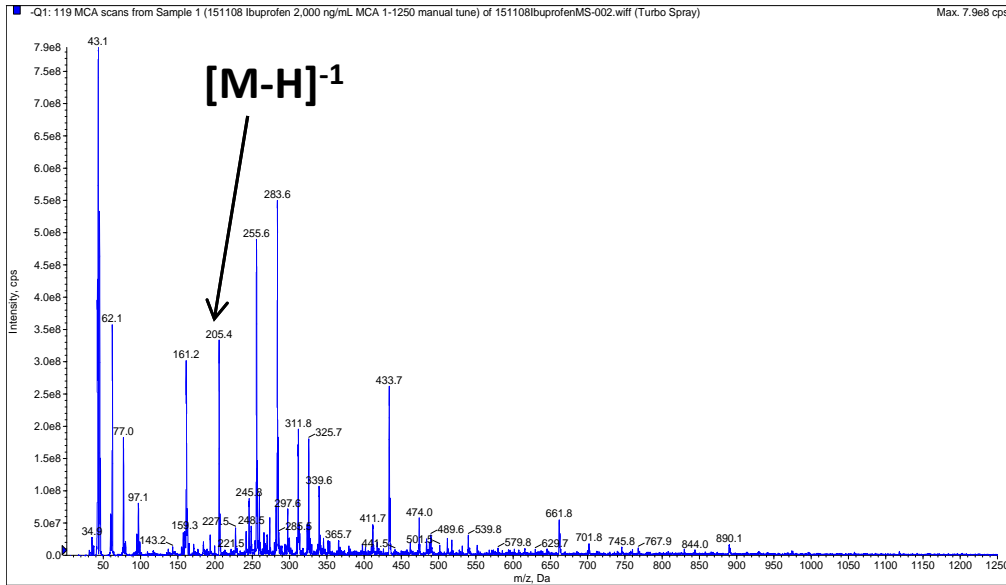


His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys
-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-Gly-OH

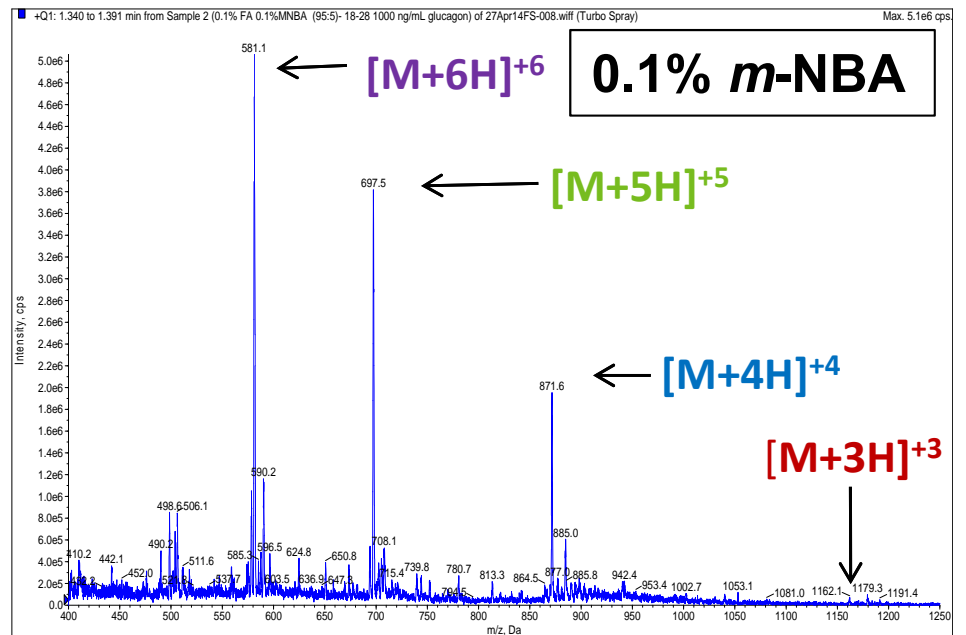
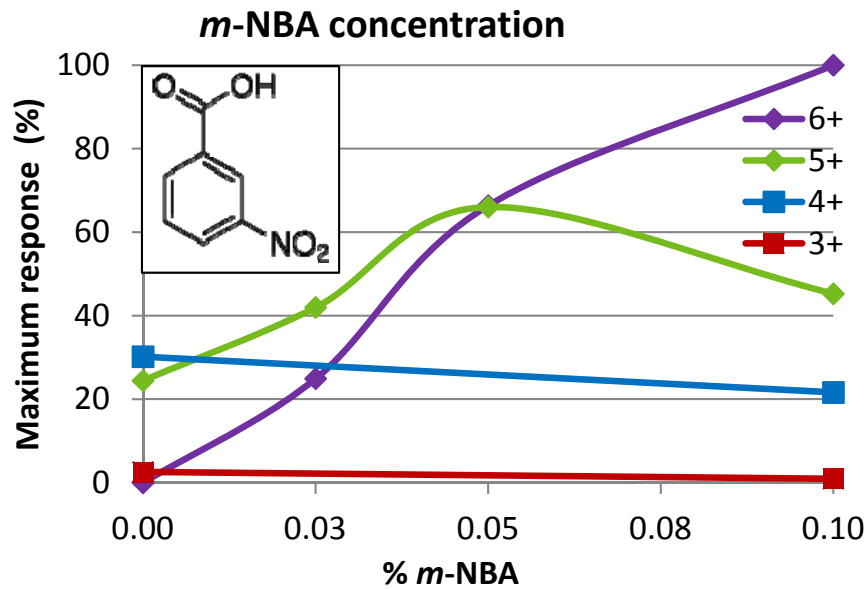
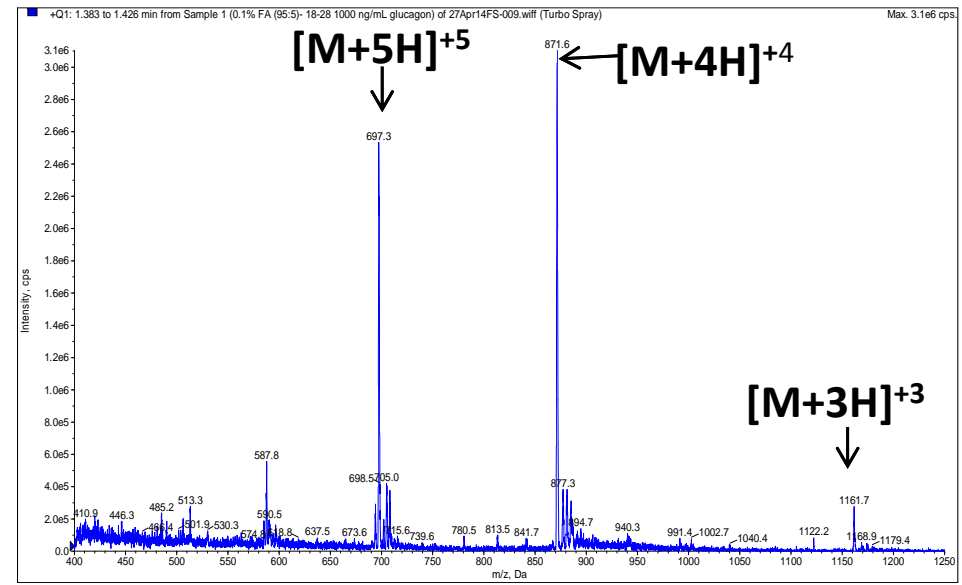
Sensitivity



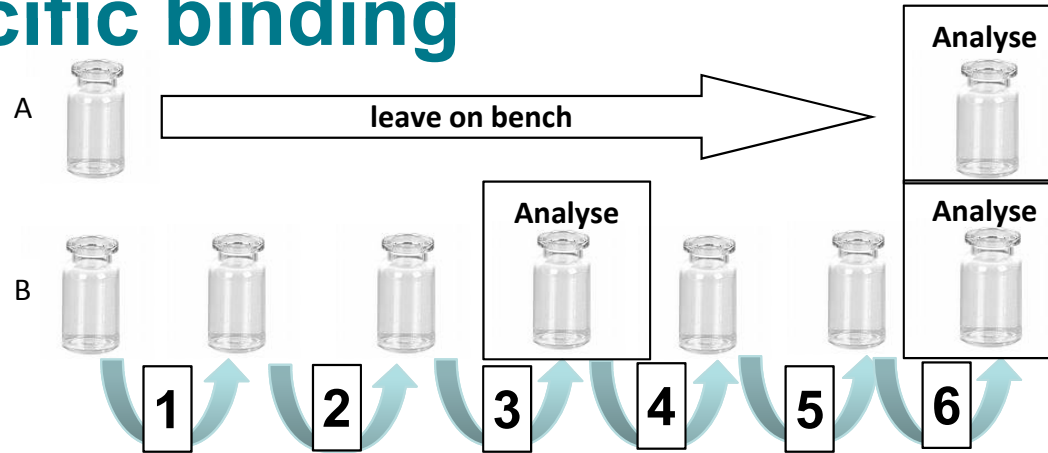
Ibuprofen



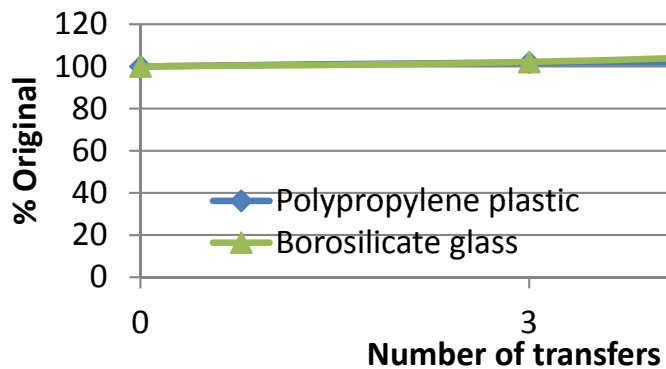
Glucagon



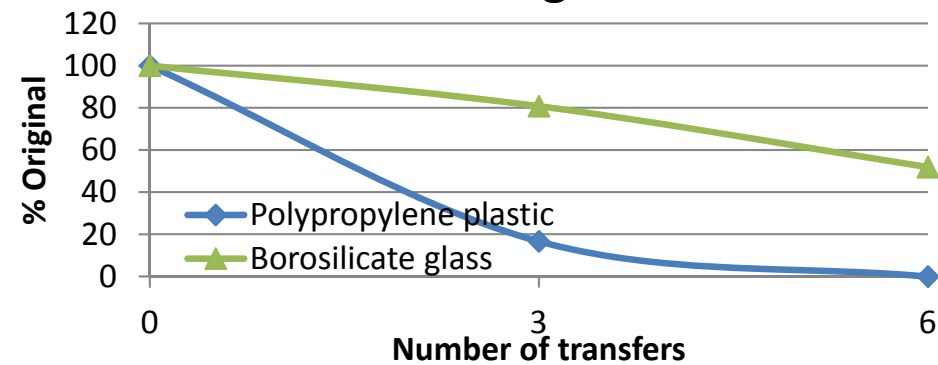
Non-specific binding



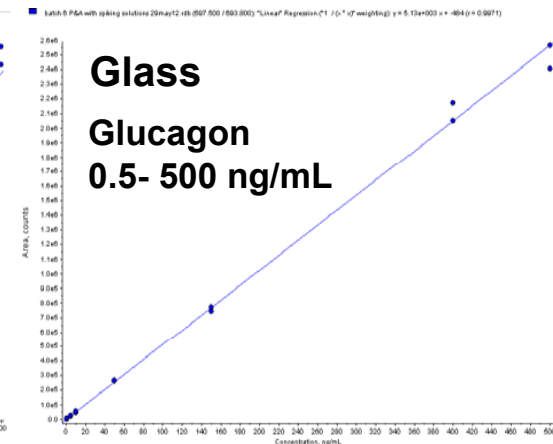
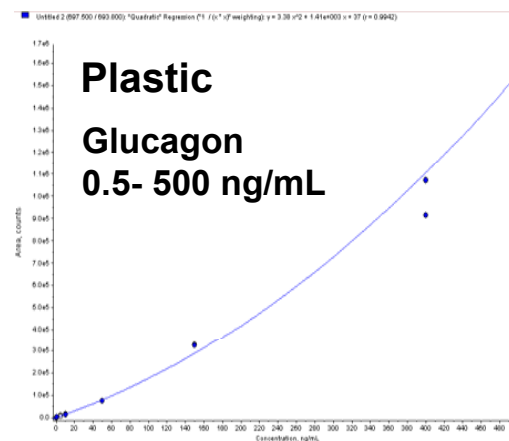
Ibuprofen



Glucagon



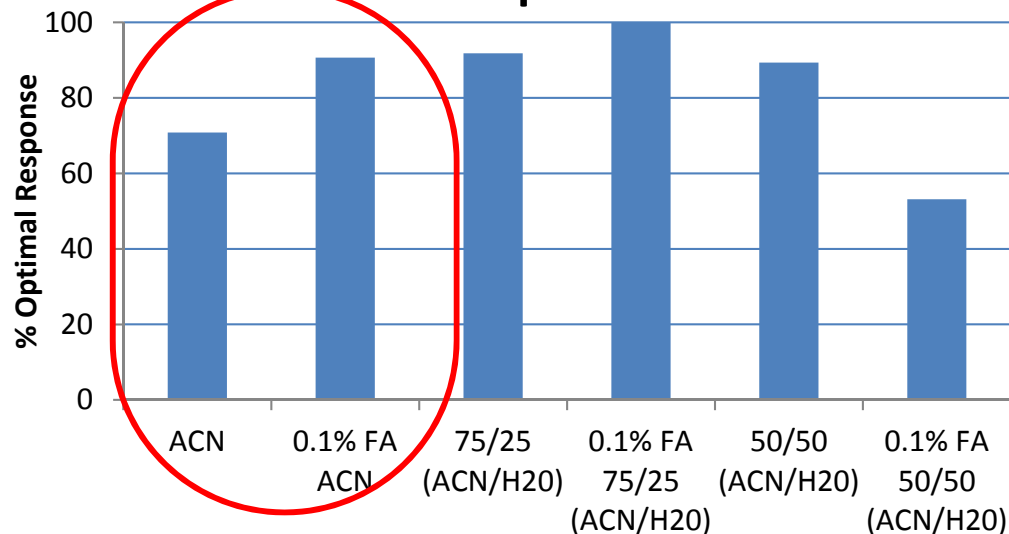
- Blocking buffers- e.g. BSA
- Optimising pH
- Serial dilution in plasma
- Selection of vials



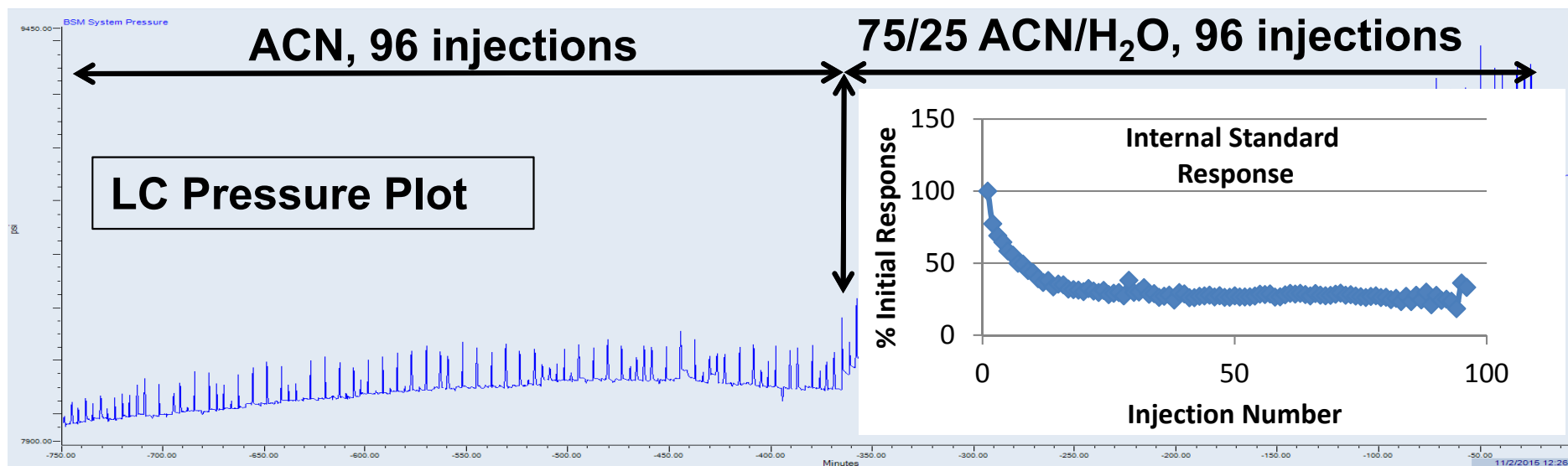
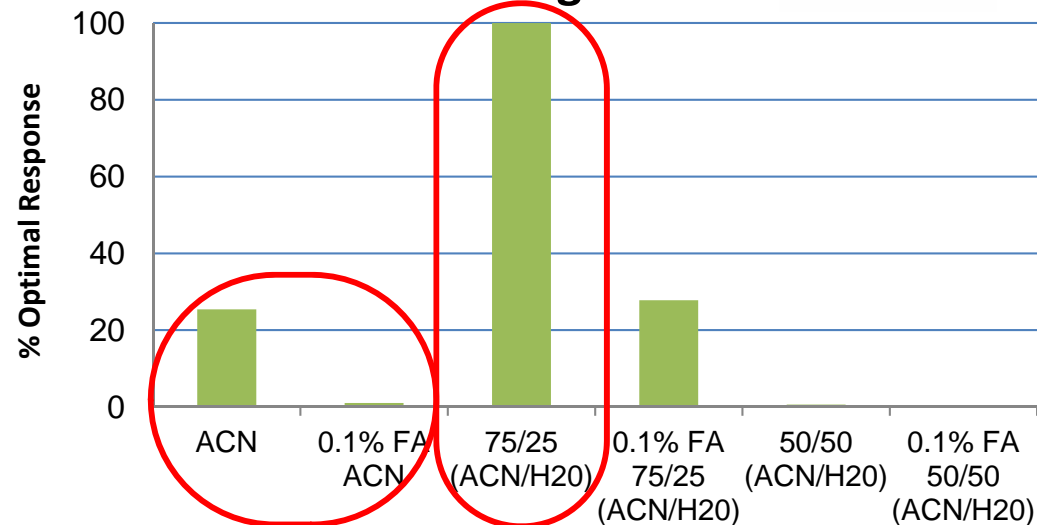


Plasma Extraction

Ibuprofen



Glucagon

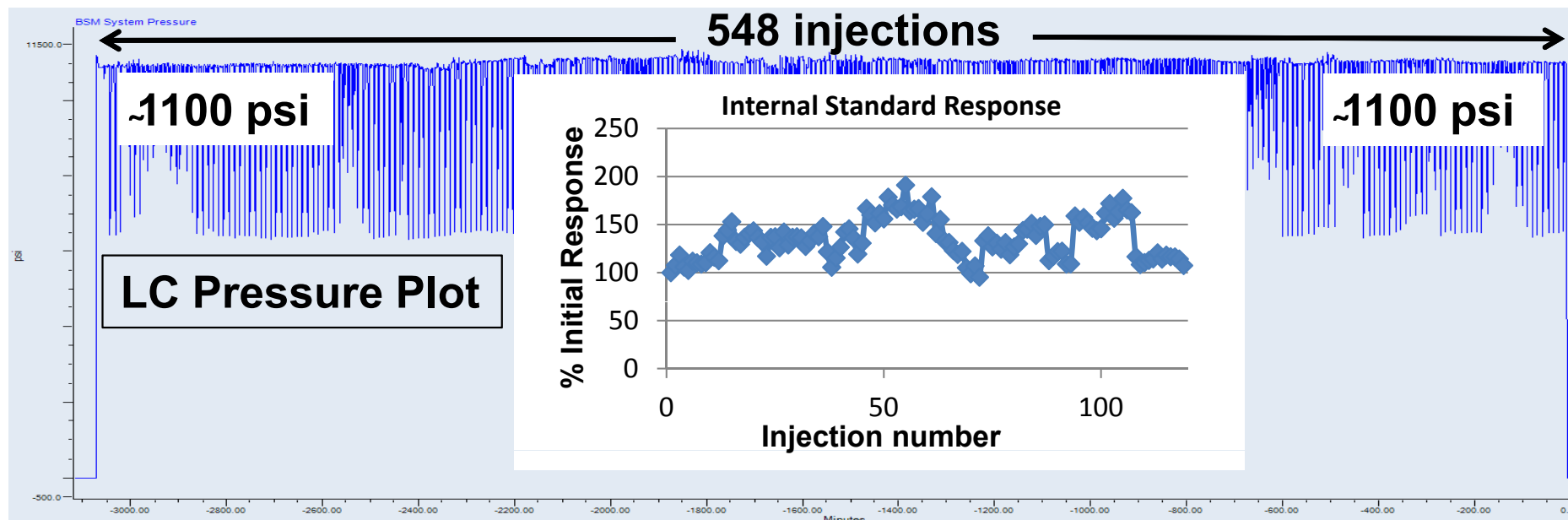


400 μ L plasma sample extracted

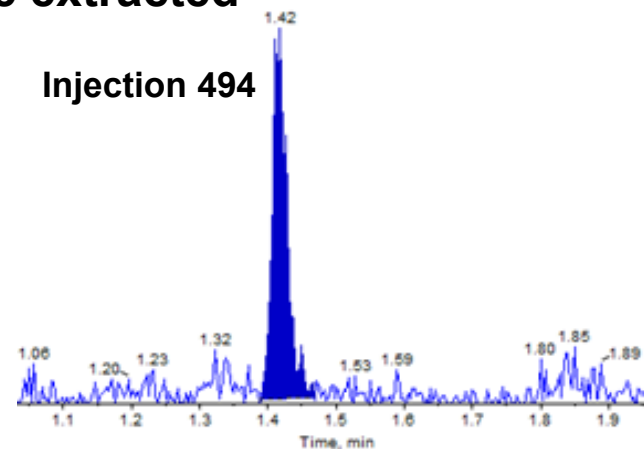
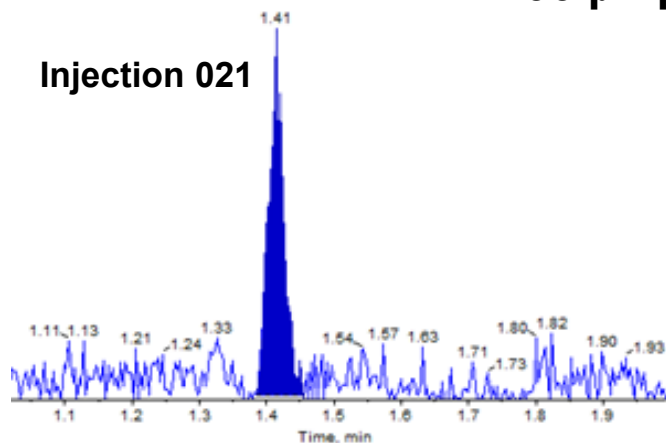


Plasma Extraction

2D Extraction: Protein Precipitation then SPE

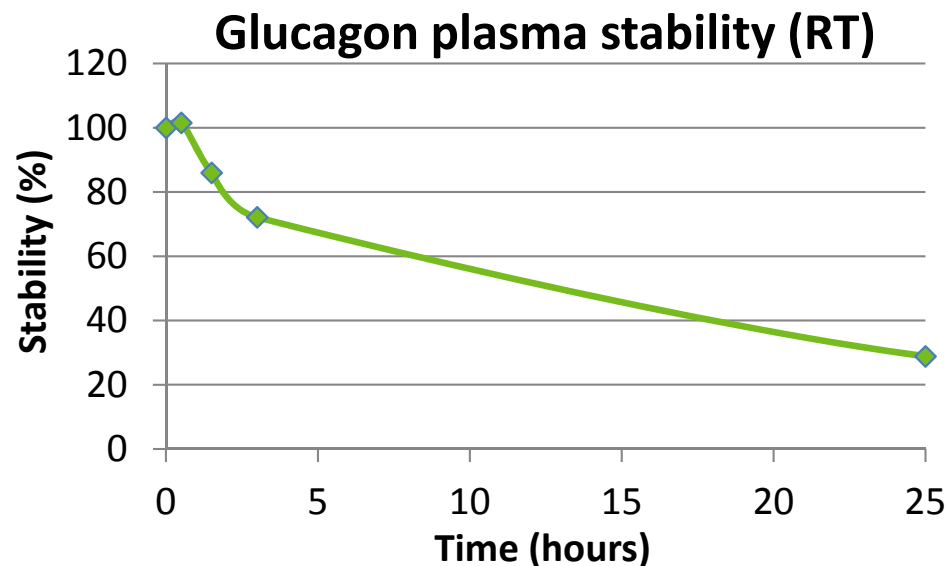
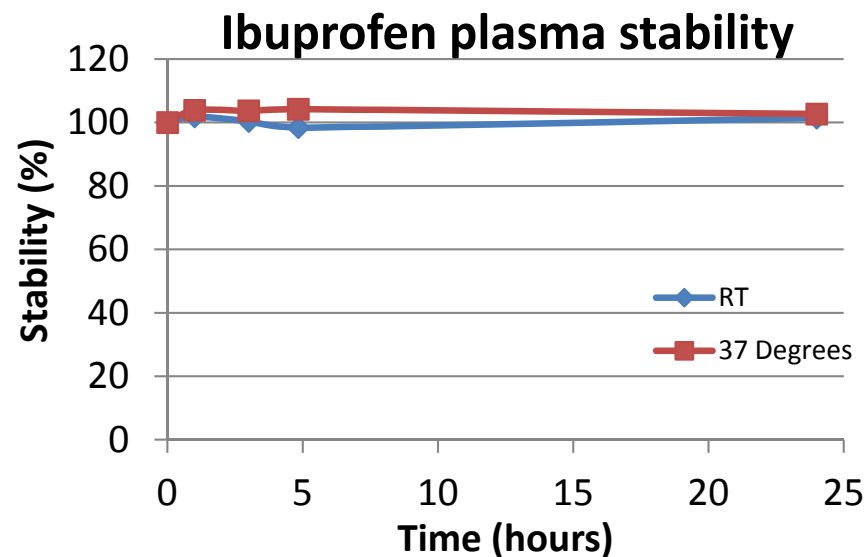


400 μ L plasma sample extracted



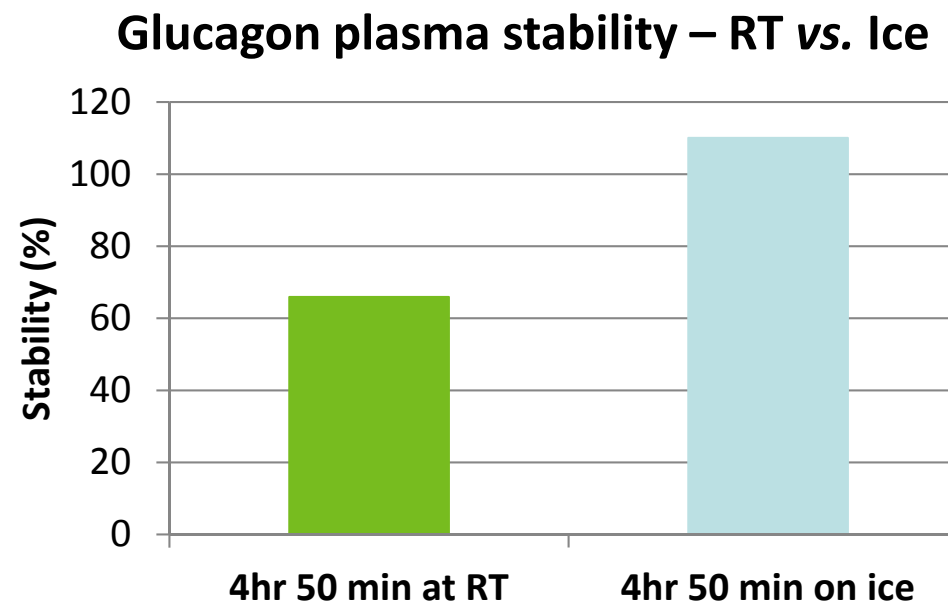


Stability in plasma



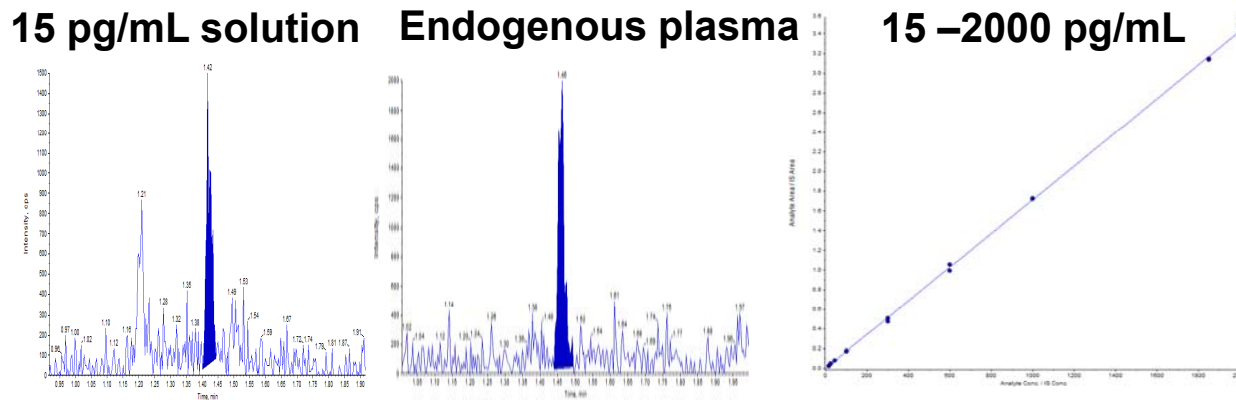
Stabilisers

- Acid denaturant
Citric acid
- Enzyme inhibitors
Aprotinin
DPP-IV inhibitor
P800 cocktail inhibitor
- Anticoagulant
Lith Hep vs. EDTA

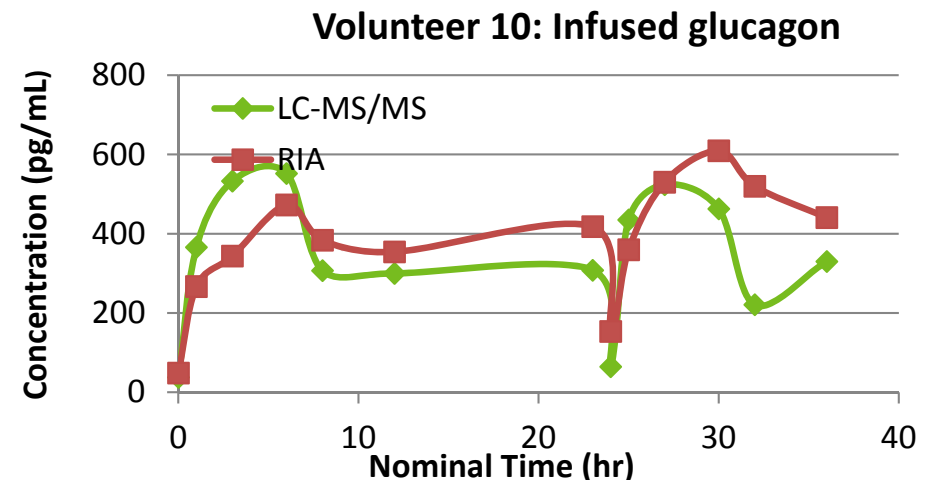
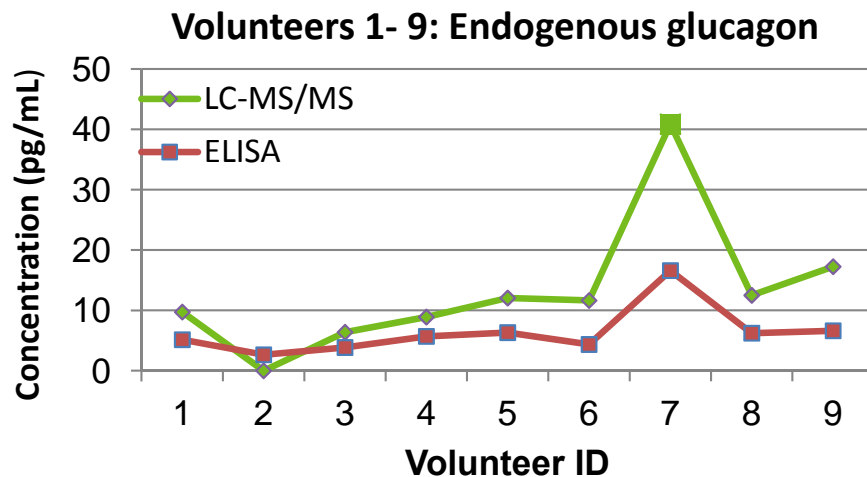


Established Glucagon Peptide Method

- Glass vials + BSA blocking buffer
- *m*-NBA and regular formic acid (FA) mobile phases gave equivalent sensitivity (SRM)
- 2D Extraction
- Performed on ice
- Surrogate matrix approach



QC Level	Matrix	n	% Nominal	%CV
15	Surrogate	6	110.3	11.9
25	Surrogate	6	114.0	10.0
45	Surrogate	6	113.8	6.2
56.9	Plasma	6	92.6	7.0
106.9	Plasma	6	92.4	11.3
231.9	Plasma	6	91.4	8.7
1781.9	Plasma	6	98.0	10.7
10	Surrogate	6	87.7	25.3
7.5	Surrogate	5	68.6	40.4





Conclusion

- Small molecule and peptide method development is similar but not the same
- Need to consider
 - Binding: Plastic v.s glass, blocking buffers
 - MS Sensitivity: Multiple charge states, supercharging agents
 - Extraction: Diluted organic precipitation solvents, 2D extractions
 - Stability: Stabilisers, extraction temperature
- Can routinely develop precise, accurate, and robust peptide methods using LC-MS/MS. These can be used as an alternative to immunoassays



Acknowledgements

LGC

- Richard Kay, Industrial Supervisor
- Godknows Hlatshwayo

Imperial College London

- Jaimini Cegla
- Tricia Tan
- James Minnion

Loughborough University

- Prof Colin Creaser, Academic Supervisor

Any Questions?

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