Optimized Microflow LC-MS/MS Methods for the Bioanalysis of Large Molecules

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The LC-MS Experts

Session Description and Objectives

- Here we report on methods that improve the selectivity and sensitivity for the analysis of peptides and large molecules from biological fluids by LC-MS/MS. In-silico peptide selection, immunocapture, micro-elution solid phase extraction and microflow LC-MS/MS (MFLC-MS/MS) with optimal ESI source design were used to improve the selectivity, sensitivity and productivity for the analysis of large molecules from biological fluids.

- Bioanalysis of ADC's, peptides and proteins
- Use of MFLC-MS
- Use of optimized ESI-MS sources for MFLC
- Use of Micro SPE in bioanalysis of Peptides

Shane Needham, Ph.D.

- Chief Scientific Officer and Co-Founder Alturas Analytics, Inc.
- More than 100 publications in the area of LC-MS bioanalysis
- Leaders in the field of bioanalytical research: Microsampling, Microflow LC-MS/MS, ADC bioanalysis, Macromolecule bioanalysis
- >100,000 samples per year, >10,000 samples from macromolecules
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What do you call 25 millionaires watching the World Series?

The New York Yankees

Go Nationals!

Humira (Adalimumab)

- Top selling prescription drug in the world (AbbVie)
- FDA approved for the treatment of ten autoimmune diseases
  - RA, juvenile arthritis, Crohn's disease, plaque psoriasis, ulcerative colitis…
- First fully human anti-TNFα monoclonal IgG1 antibody

Why Humira LC-MS/MS Research?

- Biosimilars (Bioequivalence studies)
  - Three already FDA approved waiting patent expiration many more waiting for expiry 2023
  - At least four on sale in Europe others on the way
- Increase of Biologics development
  - 2018 6/10 top sellers biologics
- Solution to ligand binding selectivity challenges

Humira LC-MS/MS Challenges

- **Selectivity**
  - Fully Human
  - NCBI BLAST Results
    - Light Chain: 97% amino acid sequence match
    - Heavy Chain: 94% amino acid sequence match
  - SKYLINE Peptide Predictions
    - Zero selective peptides predicted from trypsin digest
- **Sensitivity**
  - How low is good enough?

Research Goals

- Accurate/precise/selective method
- Method can be validated per BMV Guidance

GAINS IN SELECTIVITY AND SENSITIVITY

- ~~High resolution mass spectrometer~~

- Chromatographic improvements
  - Microflow LC-MS – optimized source
  - More resolution
  - Different stationary phase or orthogonal chromatography

- **Off-line sample preparation**

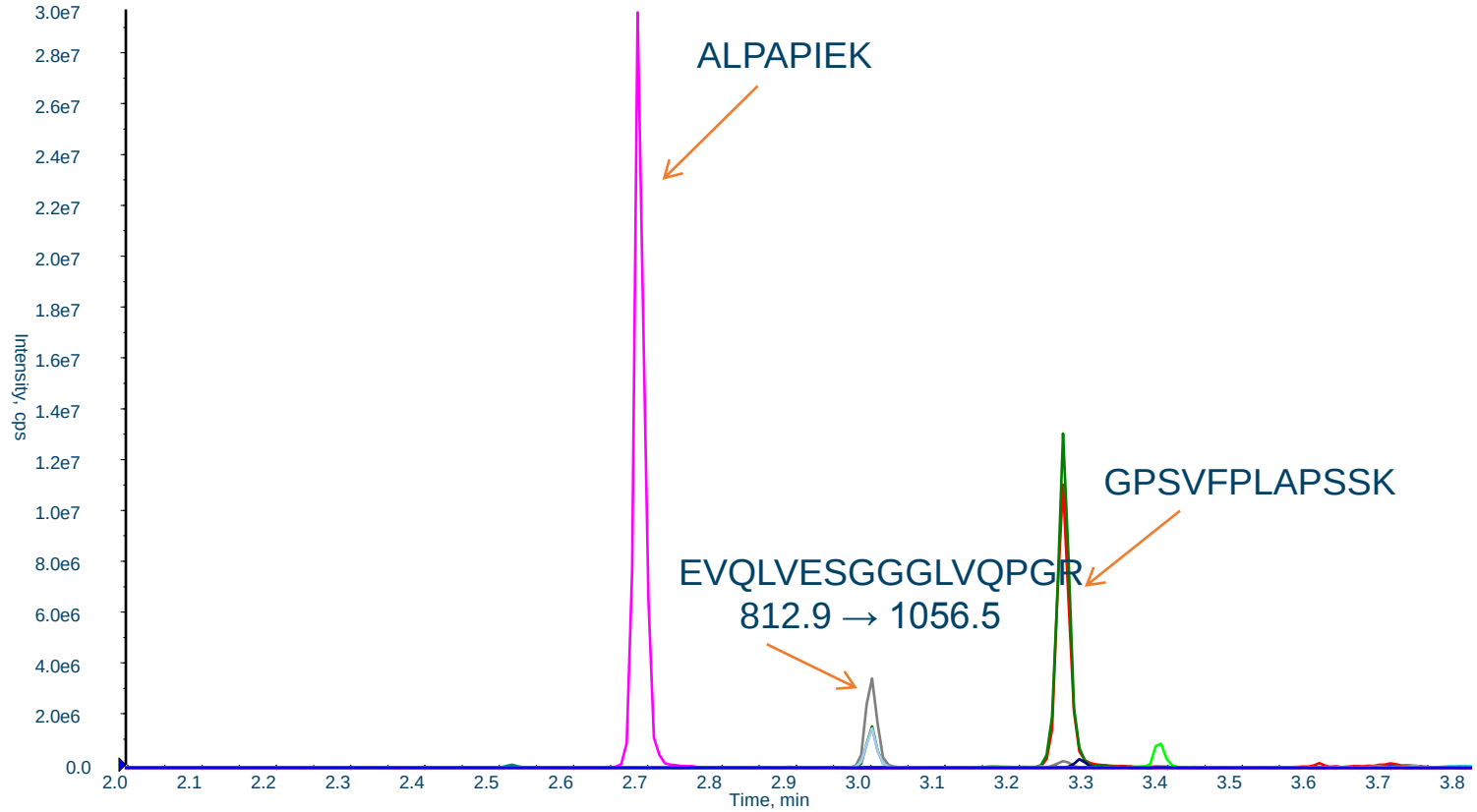
Heavy Chain Skyline Predictions

Parameters: 8-20 amino acids

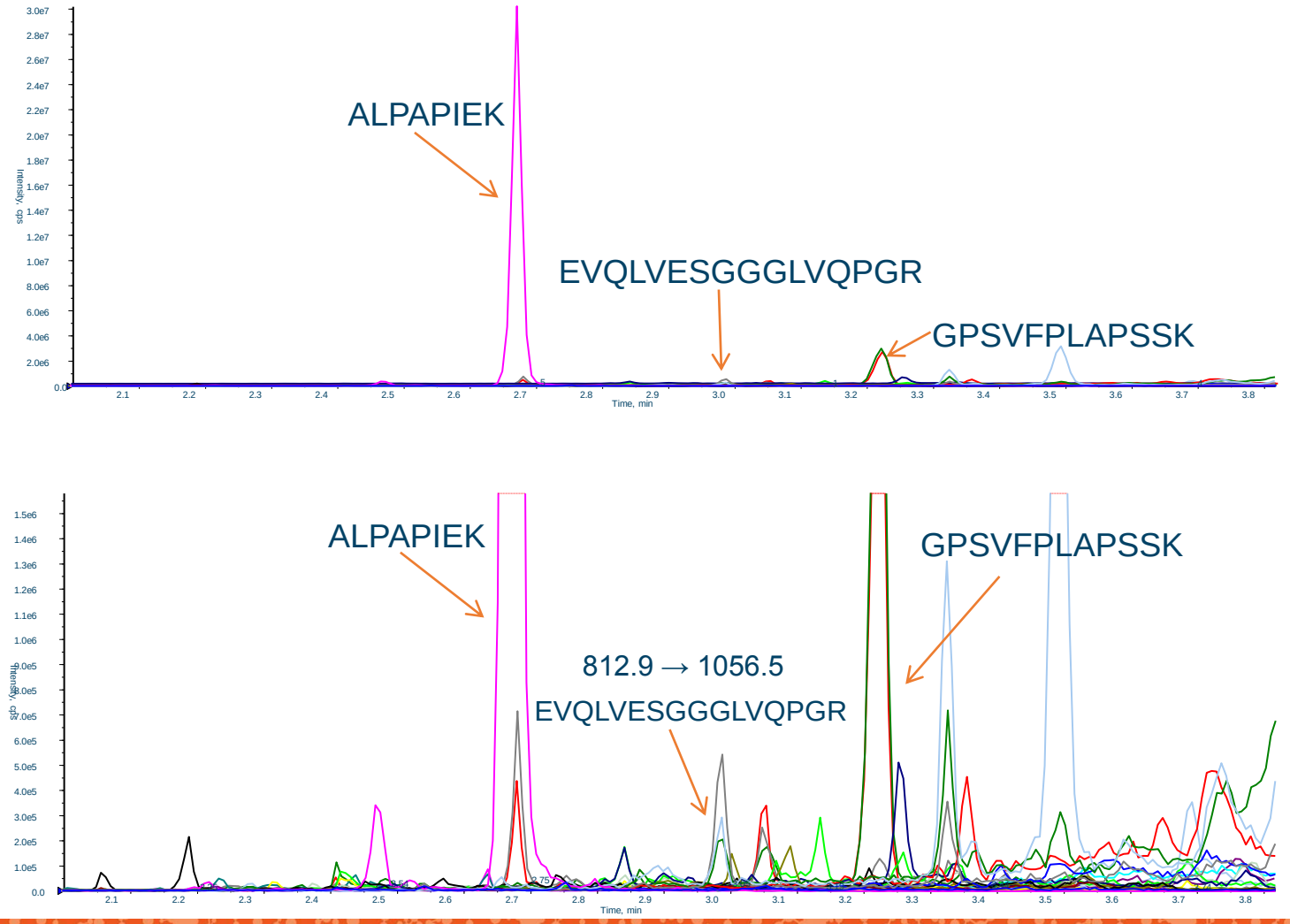
Sequence	Q1	Q3
GPSVFPLAPSSK	593.8	1129.6
GPSVFPLAPSSK	593.8	846.5
GPSVFPLAPSSK	593.8	699.4
GPSVFPLAPSSK	593.8	602.4
NSLYLQMNSLR	669.8	1024.5
SLSLSPGK	394.7	701.4
SLSLSPGK	394.7	588.3
VVSVLTVLHQDWLNGK	904.5	1209.6
VVSVLTVLHQDWLNGK	904.5	1110.6
EPQVYTLPPSR	643.8	1157.6
EPQVYTLPPSR	643.8	932.5
EPQVYTLPPSR	643.8	833.5
EPQVYTLPPSR	643.8	670.4
EEQYNSTYR	595.3	931.4
EEQYNSTYR	595.3	803.4
EEQYNSTYR	595.3	640.3
FNWYVDGVEVHNAK	893.4	1067.5
FNWYVDGVEVHNAK	893.4	968.5
EVQLVESGGGLVQPGR	812.9	840.5
EVQLVESGGGLVQPGR	812.9	1056.5
EVQLVESGGGLVQPGR	812.9	927.5
ALPAPIEK	419.8	654.4

Humira in Buffer

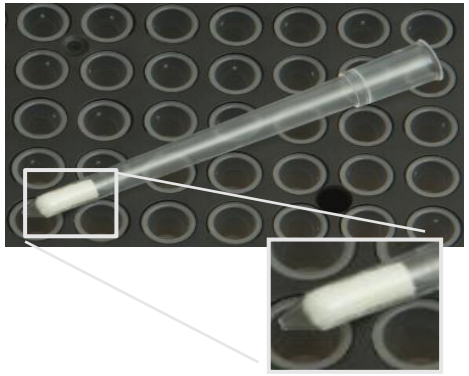
Skyline MRM Predictions



Pellet Digestion of Human Plasma

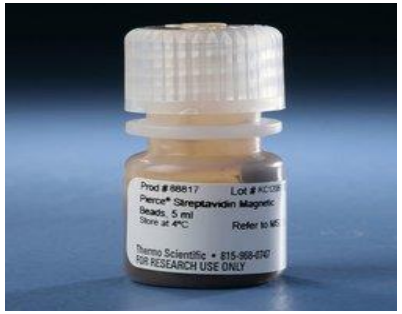


What is MSIA (Mass Spec Immuno Assay)?



- Monolithic microcolumn bonded with affinity ligand
- 96 well format for easy automation
- Compatible with Novus I, Tecan EVO and Versette
- Available in Streptavidin, Insulin, Protein A, G, A/G and Custom (direct attachment of an Ab, protein or peptide)

Magnetic Beads



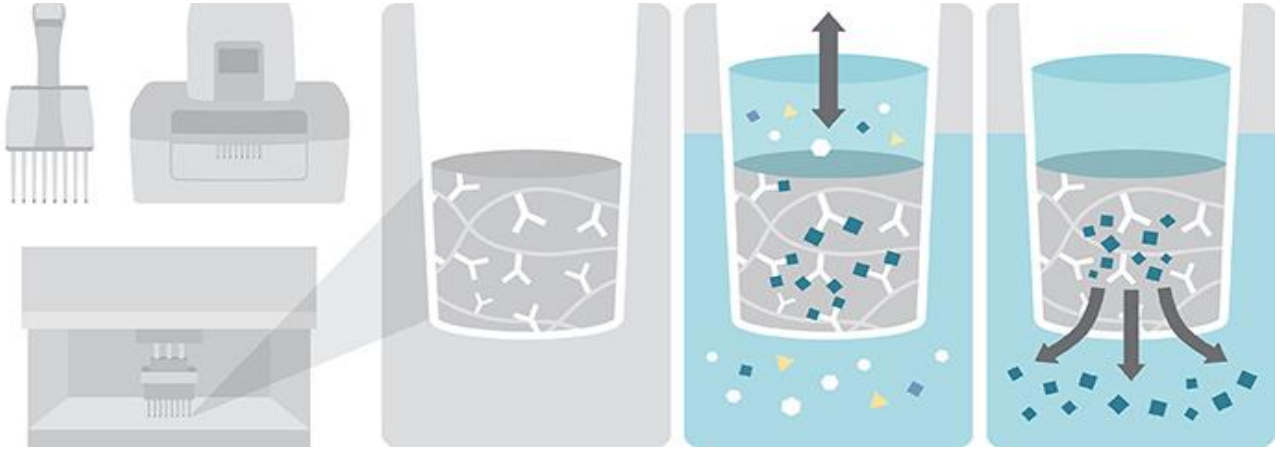
MSIA

Criteria	Magnetic Beads	MSIA
Automation	Difficult	Simple
Selective	Good	Great
Elution Volume	~100mL	30 mL
Capacity	Unlimited	Limited
Versatility	Flexible to any automation	Limited
Speed	Time/Labor Intensive	≥2X Faster

Materials

- SigmaMAb Adalimumab – Sigma
- Internal Standard: SILu™MAb Adalimumab Stable-Isotope Labeled Monoclonal Antibody [$^{13}C_6$, $^{15}N_4$]-Arginine and [$^{13}C_6$, $^{15}N_2$]-Lysine - Sigma
- 300 mL Mass Spectrometric Immunoassay (MSIA) Streptavidin D.A.R.T.S -Thermo Scientific
- Biotinylated Human TNFa Protein - ACROBiosystems
- Water, PBS Buffer, TCEP and Trypsin (Promega)

Preparation Procedure MSIA™



- Aliquot plasma
- IS
- Rinses
- Biotin-TNFa
- Elution Solvent

Capture Biotin-TNFa
Rinse

Capture
Humira
Rinse

Elute
Water 2% FA

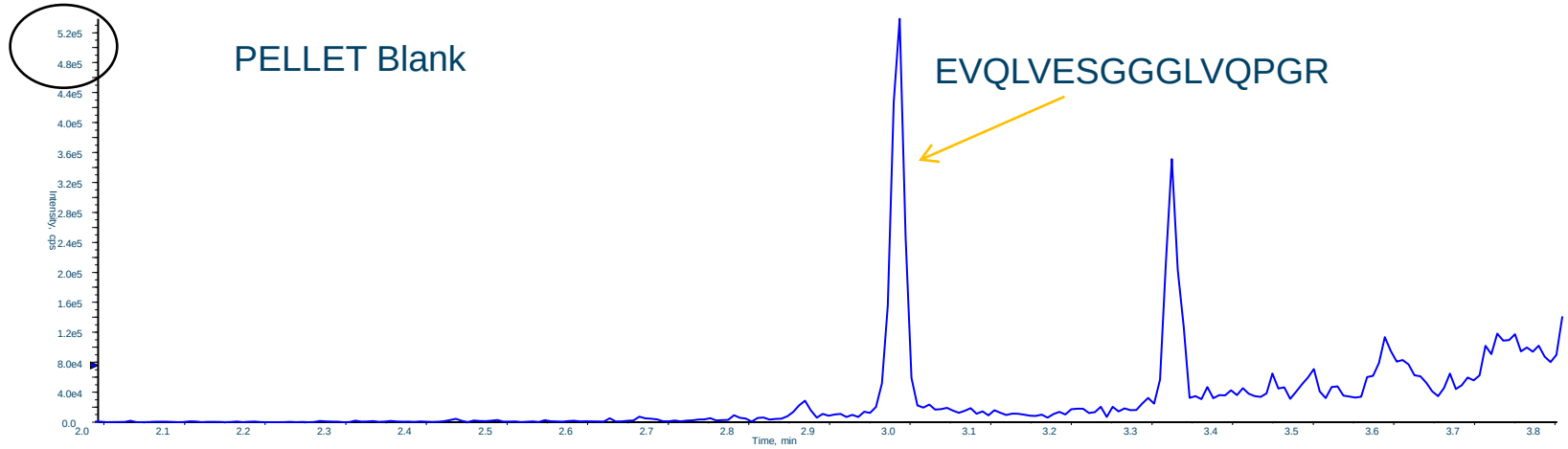
Denature, Reduce, Digest Eluent

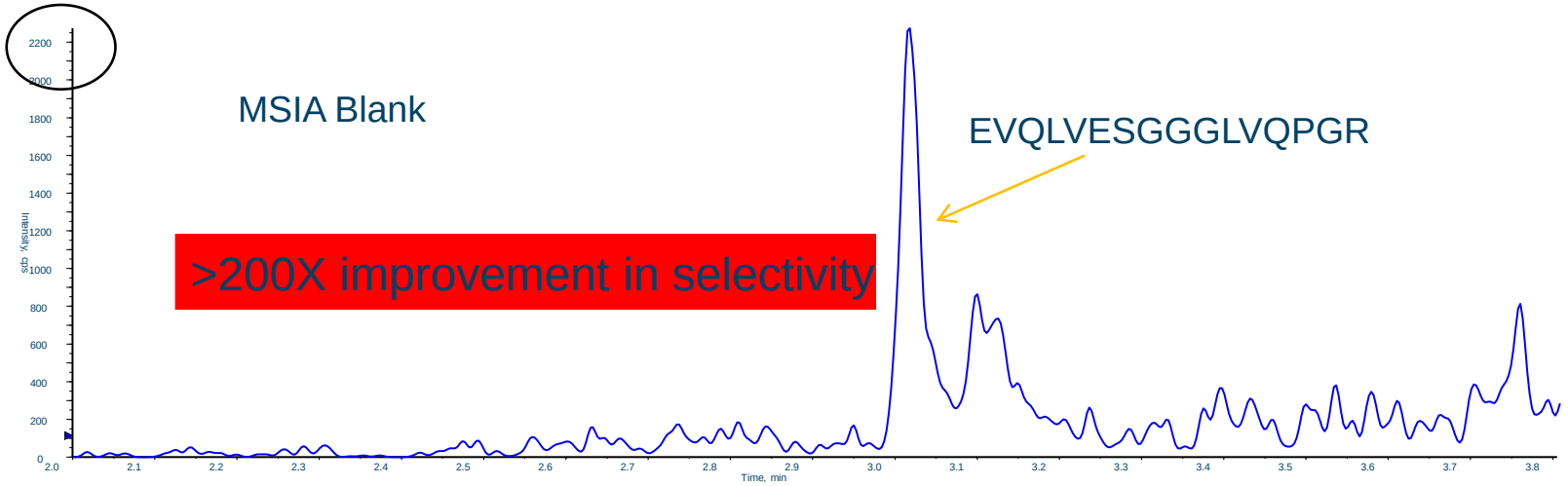
Methods

MFLC-MS/MS

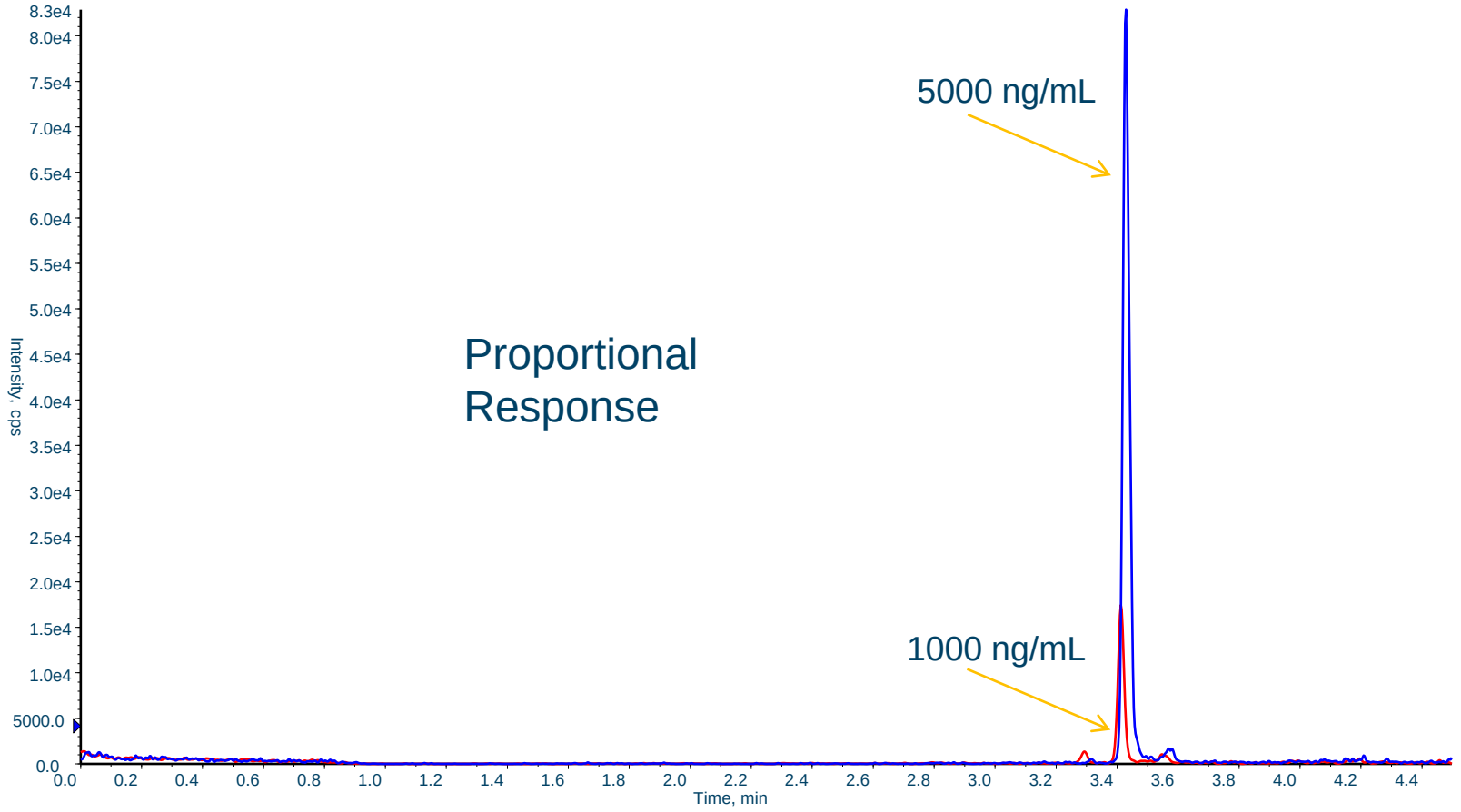
- Sciex 6500+ operating in MRM mode
- Optiflow Source (1-50 mL/min probe)
- Waters Acquity M-Class Binary LC Systems (10 µL/min)

Pellet Extraction vs MSIA (Blank Plasma)





1000 and 5,000 ng/mL Humira Extracted from Human Plasma



Analytical Results

- 1000-20,000 ng/mL dynamic range
- A/P 90% ± 5%
- Blanks <15% of LLOQ Response

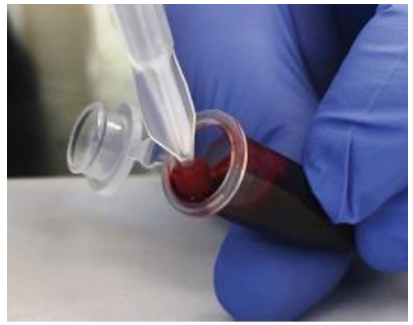
Conclusions

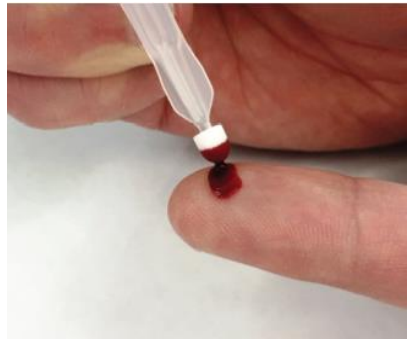
- Accurate/precise/selective method developed to analyze Humira using MSIA and MFLC-MS/MS
- Sample Preparation ~2X Faster than bead method
- Method can be validated to support clinical studies
- **Stay tuned!! – Trypsin embedded tips!**

Next Steps in Humira Research: Microsampling

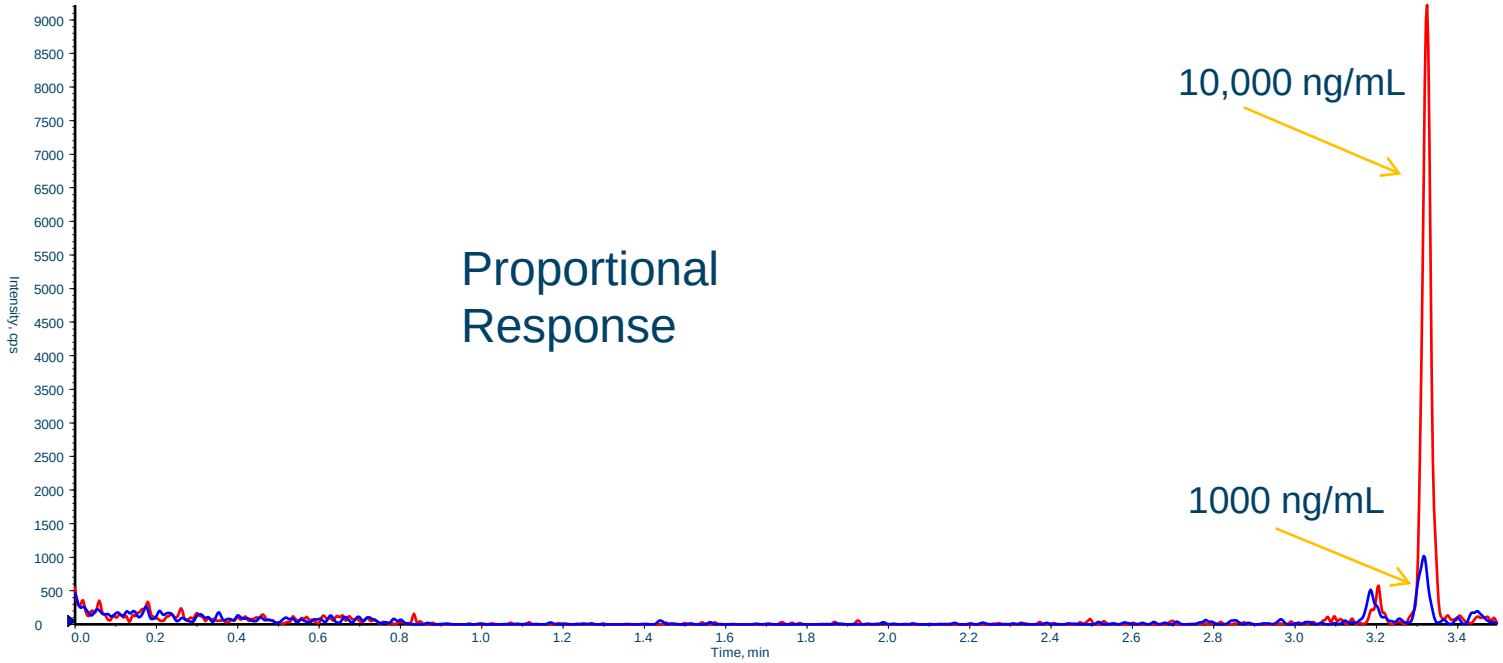
MITRA (Neoteryx) microsampling device

- Precise sample collection
- Ideal for pediatric or at home sampling
- Not impacted by hematocrit
- 10 or 20 µL sample volume
- Preparation Procedure
  - Allow to dry
  - Place tip in 100 µL water (1 hour)
  - Follow MSIA protocol





1000 and 10,000 ng/mL Humira Extracted from MITRA (20 µL whole blood sample volume)



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Questions

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