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# Critical evaluation of common sample preparation techniques for bioanalysis on microfluidic LC/MS performance

**Paul Rainville**
**Waters Corporation**

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# Capillary/nano LC/MS in DMPK

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Rapid Commun Mass Spectrom. 2009 Dec;23(23):3736-42. doi: 10.1002/rcm.4311.

## Online nanoelectrospray/high-field asymmetric waveform ion mobility spectrometry as a potential tool for discovery pharmaceutical bioanalysis.

Hatsis P, Valaskovic G, Wu JT.

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panos.hatsis@novartis.com

### Abstract

Nanoelectrospray ionization (nESI) coupled online with high-field asymmetric waveform ion mobility spectrometry (FAIMS) for small molecule analysis in a discovery pharmaceutical setting was examined. A conventional capillary pump, autosampler and nESI source were used to introduce samples directly into the FAIMS device. The FAIMS device was used to separate gas-phase ions on a timescale that was compatible with the mass spectrometer. The capability of the nESI-FAIMS combination to efficiently remove metabolite interferences from the parent drug, and reduce ion suppression effects, was demonstrated. On average, 85% of the signal intensity obtained from a neat sample was preserved in the extracted plasma samples. Standard curves were prepared for several compounds. Linearity was obtained over approximately 3 to 4 orders of magnitude. Comparison of results from nESI-FAIMS with those from conventional LC/MS for a mouse pharmacokinetic study yielded concentration values differing by no more than 30%.

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PMID: 19902415 [PubMed - indexed for MEDLINE]

# Capillary/nano-scale LC/MS in DMPK

- Why ?
  - Need for sensitivity
  - Limited sample volumes
  - Multiple injections/ same sample
  - Single model PK data
  - "Green" approach
- Why not ?
  - Plenty of sensitivity
  - Plenty sample available
  - Speed on analysis
  - Proteomics not DMPK platform
  - Chromatographic performance*
  - Robustness*

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# Signal Intensity Improvement in comparison to a 2.1mm format

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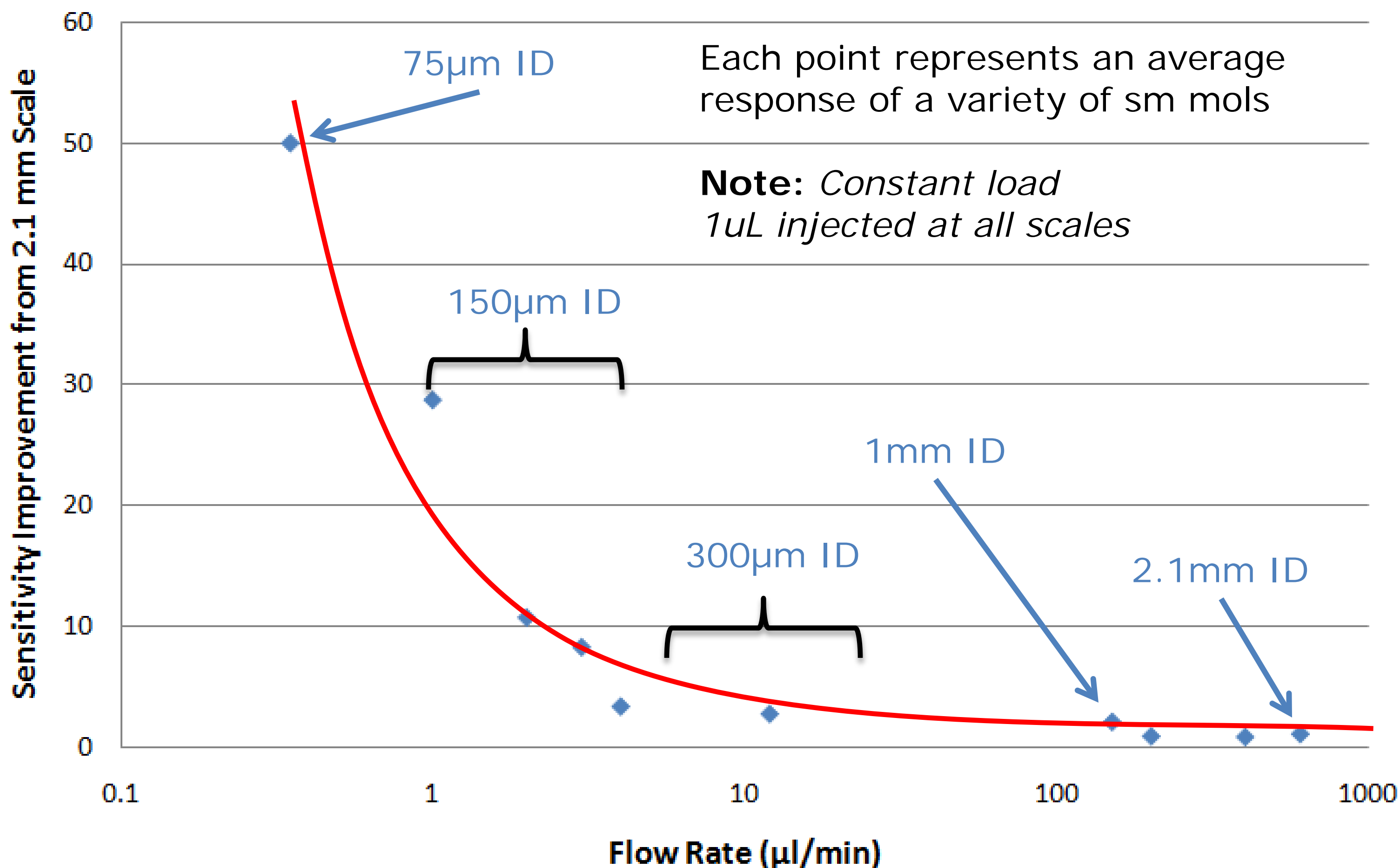


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# Separation Comparison

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RAPID COMMUNICATIONS IN MASS SPECTROMETRY
*Rapid Commun. Mass Spectrom.* 2008; 22: 1053–1061
Published online in Wiley InterScience (www.interscience.wiley.com) DOI: 10.1002/rcm.3472

RCM

**High-throughput, accurate mass liquid chromatography/tandem mass spectrometry on a quadrupole time-of-flight system as a 'first-line' approach for metabolite identification studies**

**Philip R. Tiller[1]*, Sean Yu[1], Jose Castro-Perez[2], Kerry L. Fillgrove[1] and Thomas A. Baillie[1]**
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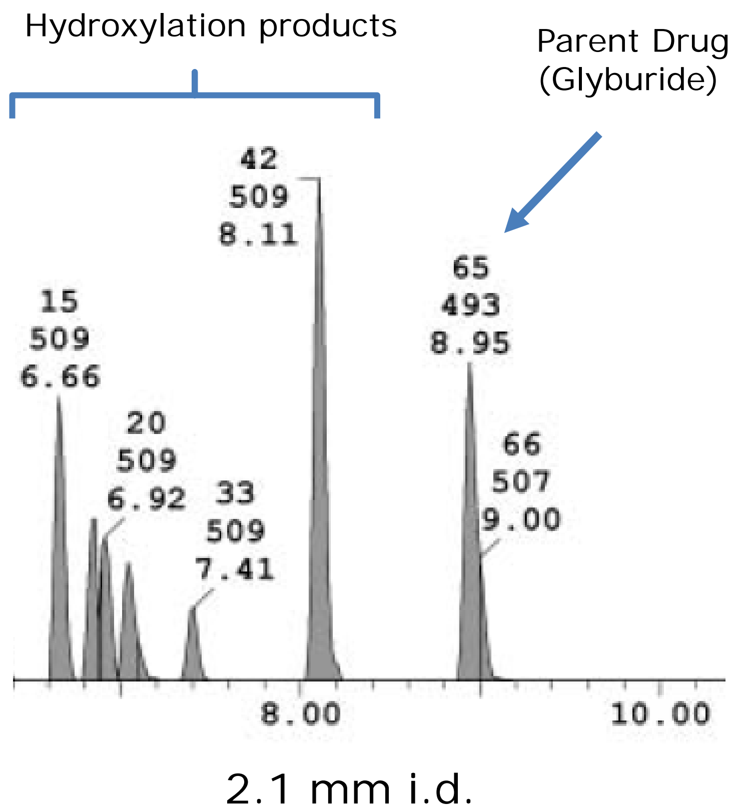
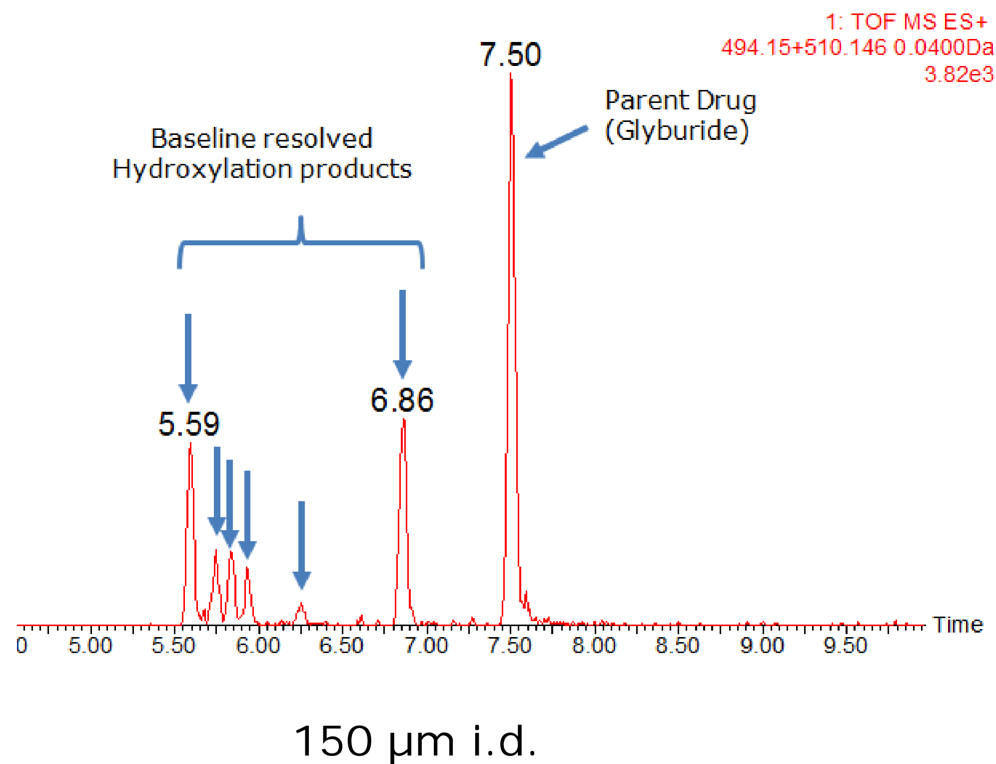



2.1 mm i.d.




150 µm i.d.

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# Sample Preparation Schemes

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- Place into format acceptable for injection onto LC/MS system.
- Various techniques depending on goal of analysis:
  - Protein precipitation*
  - Liquid-liquid extraction*
  - Solid-phase extraction
  - Solid-liquid extraction
  - Digestion*
  - Affinity
  - Filter
  - Centrifugation
  - Dilution

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# Methods

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- Various biological matrices tested with small and large molecules:
  - Plasma*
  - Urine
  - Bile
  - Microsomes
- Criteria: 1000 injections (approx. 5 days)
  - Chromatographic peak shape
  - System pressure
- Chromatographic conditions:
  - 150 µm X 50 mm prototype microfluidic device 1.7 µm BEH $C_{18}$, , temperature controlled, 3-4 µL/ min, gradient elution, formic acid/ MeCN , various injection volumes

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# Robustness Evaluation Test Case 1

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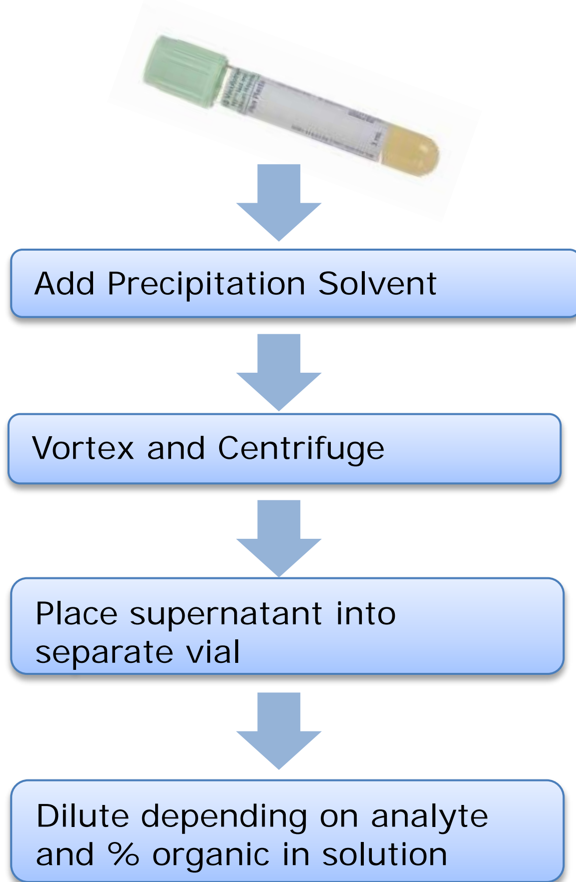
A typical Protein Precipitation workflow




- Protein precipitation (PPT)
  - 1000 1 µL injections of 2:1 crashed human plasma
  - 1000 3 µL injections of 2:1 crashed human plasma
  - 1000 5 µL injections of 2:1 crashed human plasma
  - a standard mixture of dextromethorphan and propranolol (critical pair) was injected every 50 plasma injections

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# Robustness Testing (PPT data)

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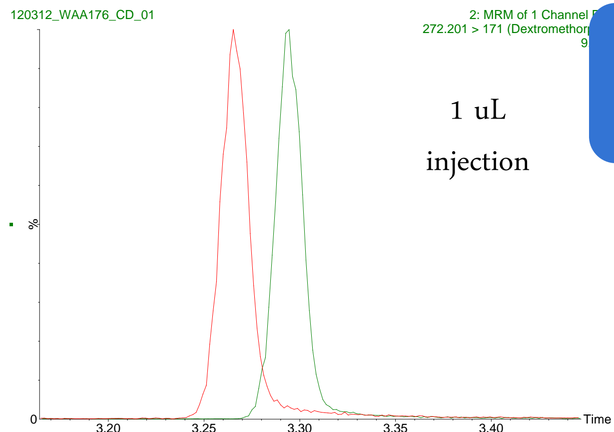
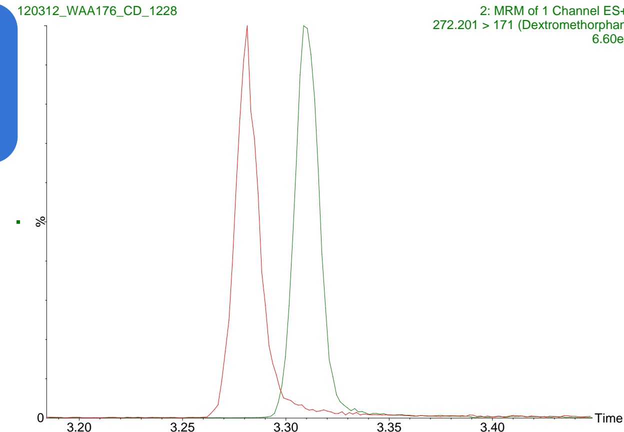
First injection

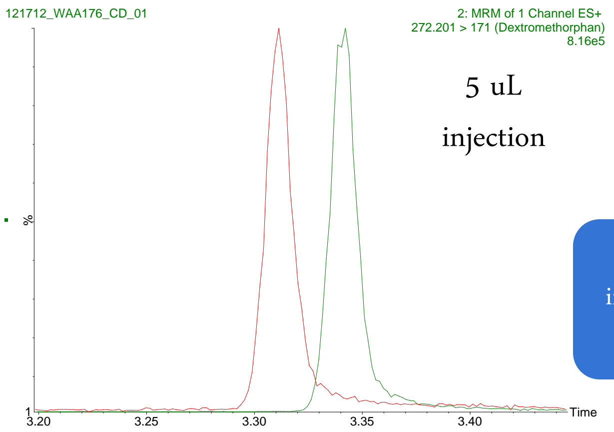
After 1000 injections of crashed plasma




1 uL injection

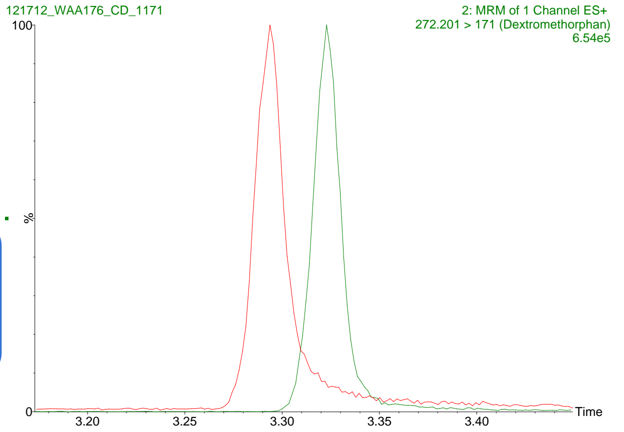
Equivalent to 200uL injection on 2.1mm scale!




5 uL injection

Equivalent to 1mL injection on 2.1mm scale!

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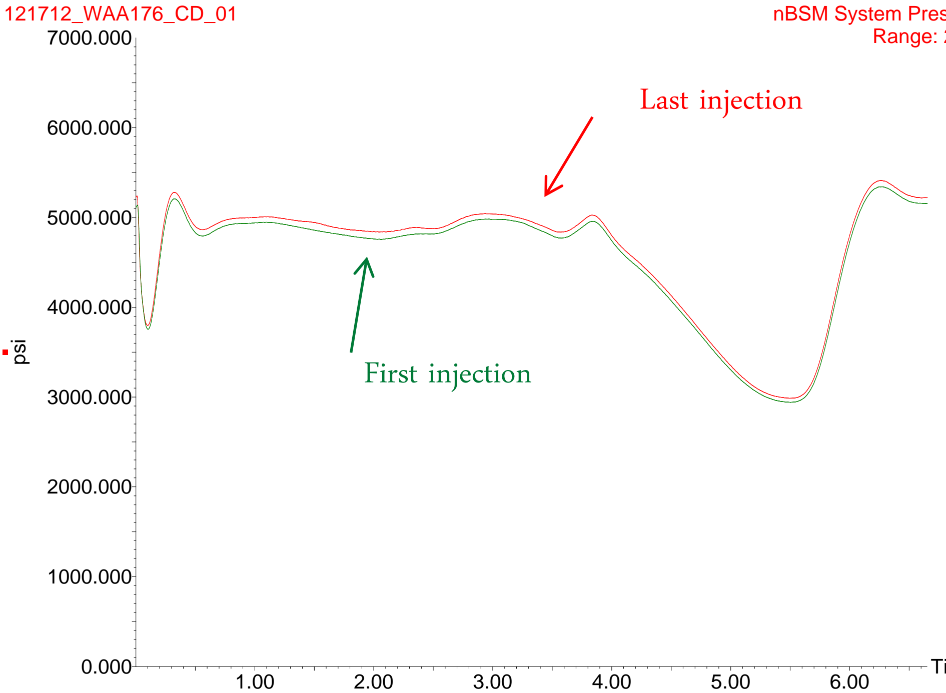
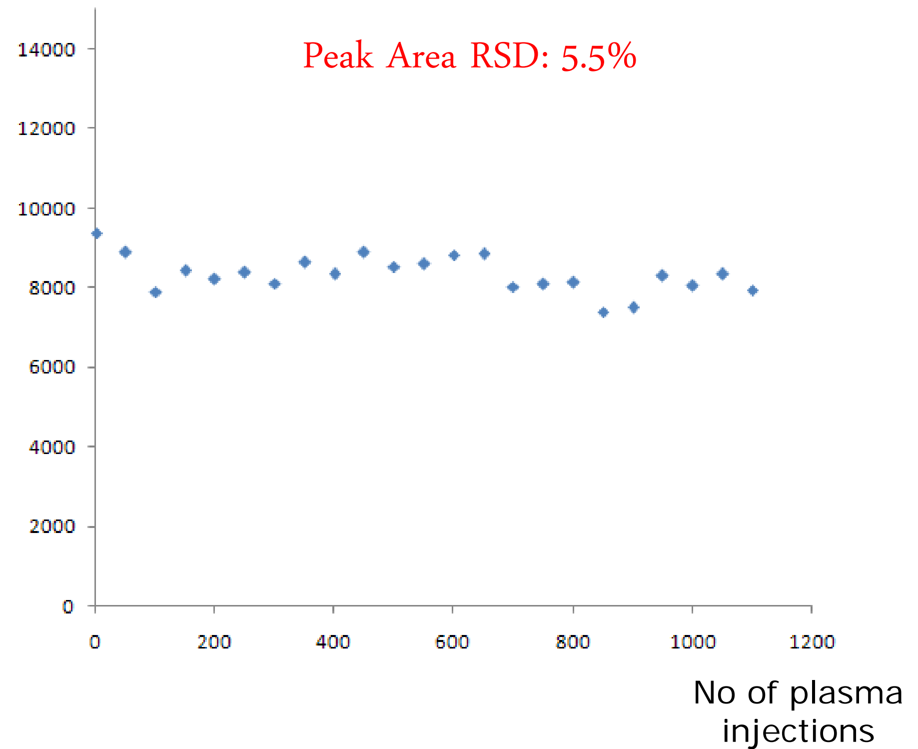
# Robustness Testing (PPT data)

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# Peptide Test (PPT data)

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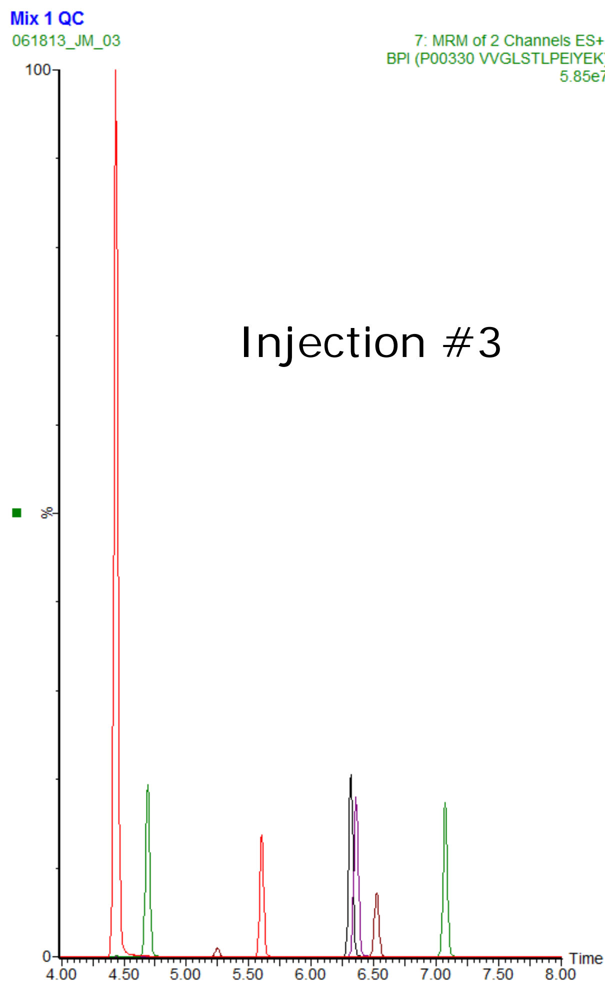


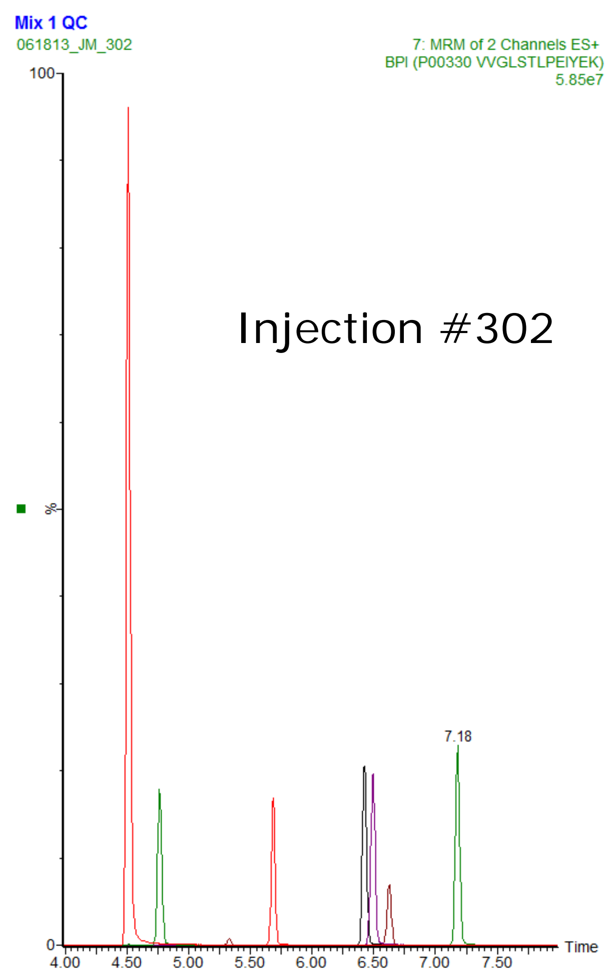


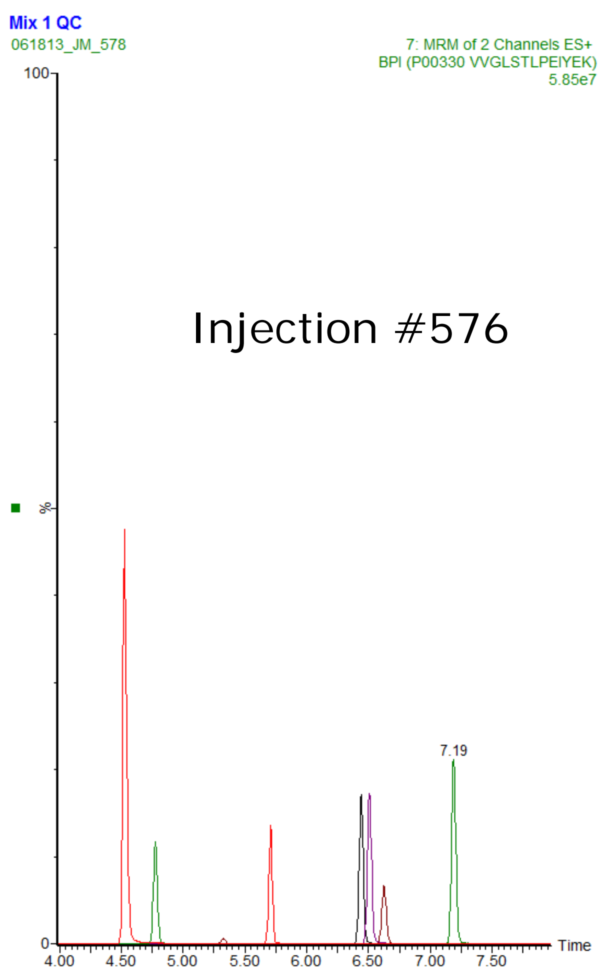


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# Different devices
# Different plasma sources (PPT data)

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| | Peptide P00924 |
|---|---|
| Retention Time | 6.64 min |
| SD of Retention Time | 0.09 |
| Average Peak Width at 10% | 2.64 s |

| | Average of all Peptides, all Tiles |
|---|---|
| Peak Width RSD | 4.16% |
| Retention Time RSD | 0.57% |
| Area RSD | 15.5% |

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# Robustness Evaluation Test Case 2

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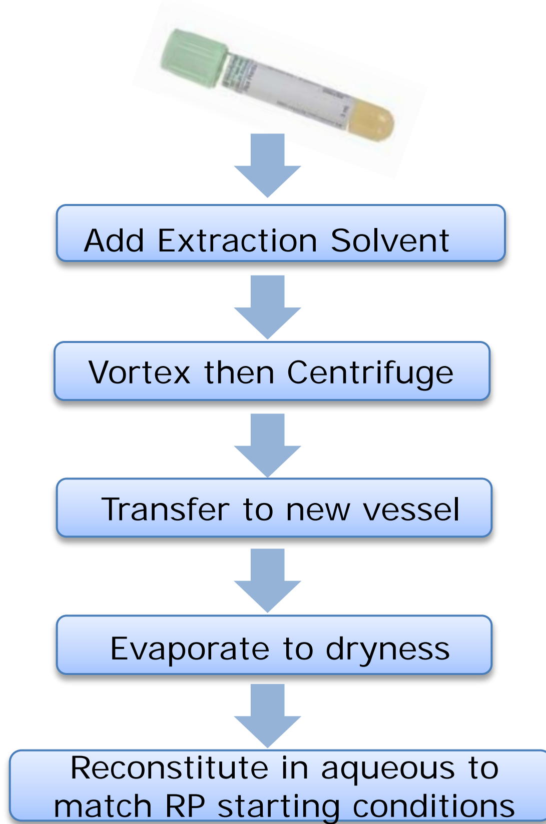
## A typical LLE workflow




Solvents Tested
Hexane
Ethyl Acetate
Methyl tert Butyl Ether

1:10 ratio plasma:solvent
Dry down
Recon in initial gradient conditions
X 5 enrichment

5 µL injection volume

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# Robustness Testing (LLE data)

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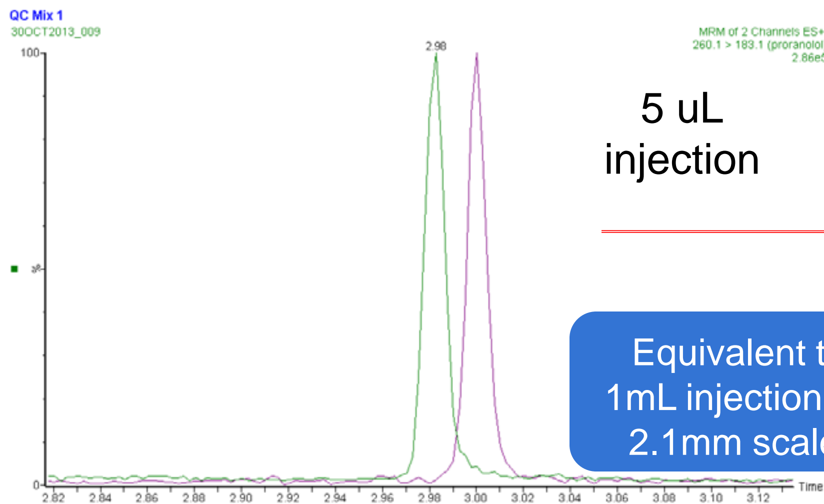
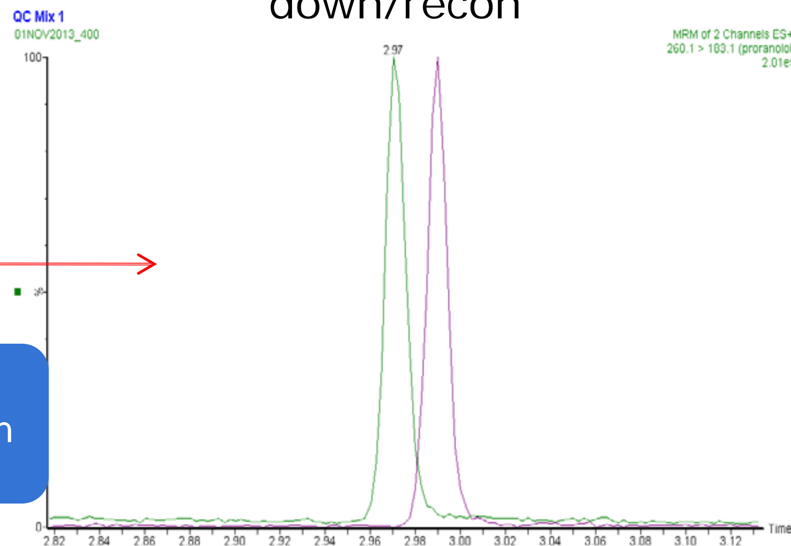
First injection

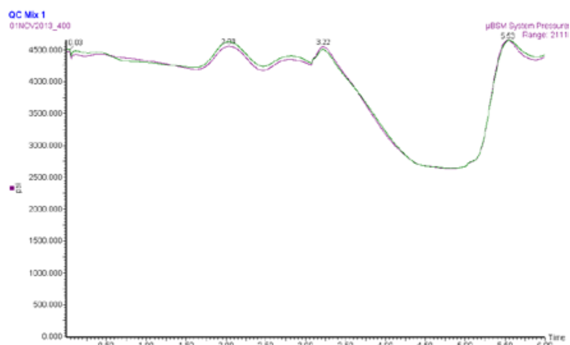



5 uL injection

Equivalent to 1mL injection on 2.1mm scale!

After 1000 injections of LLE/dry down/recon

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# Robustness Evaluation Test Case 3

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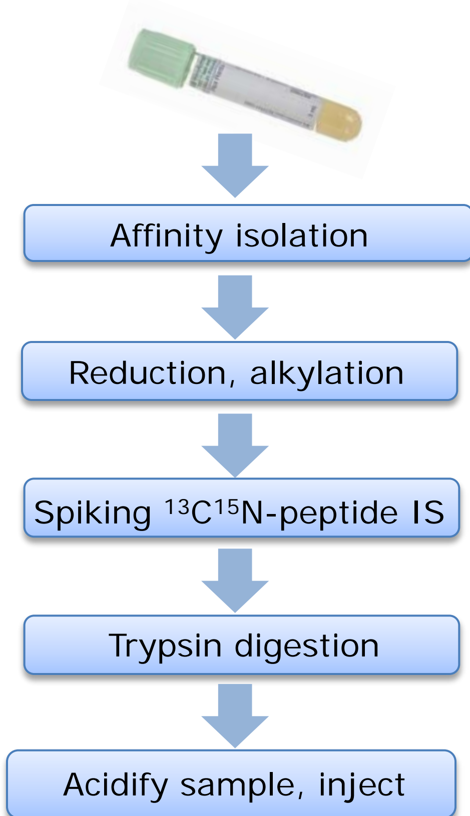
## A typical Protein BioA workflow



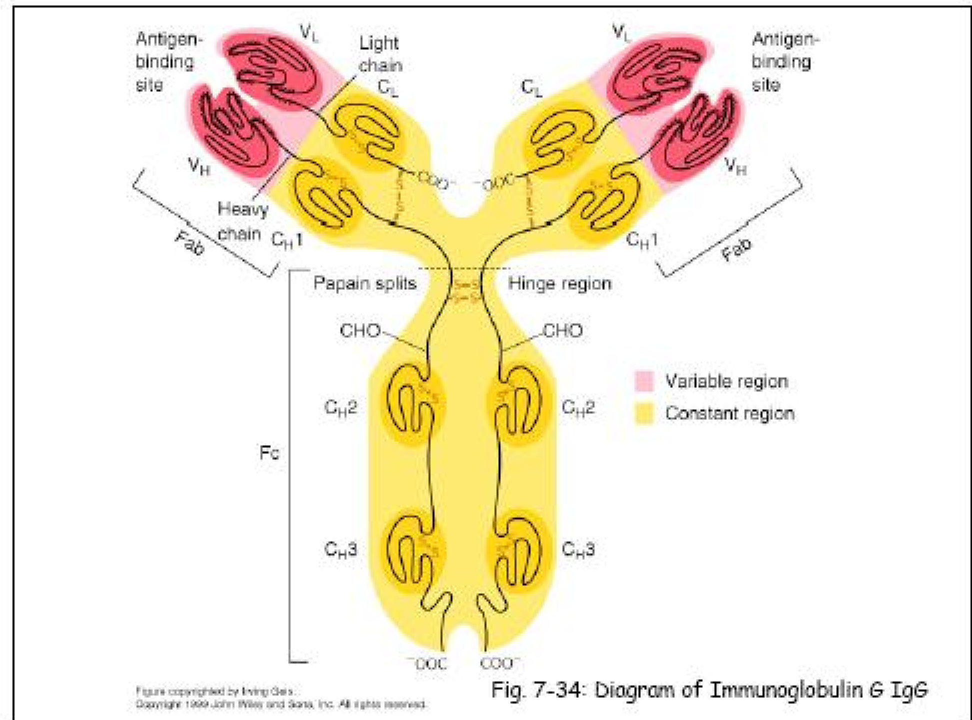




Fig. 7-34: Diagram of Immunoglobulin G IgG

https://www.google.com/search?q=mAb&source=lnms&tbm=isch&sa=X&ei=gwN5UsSWK_jfsASZg4DwBA&ved=0CAcQ_AUoAQ&biw=1024&bih=577

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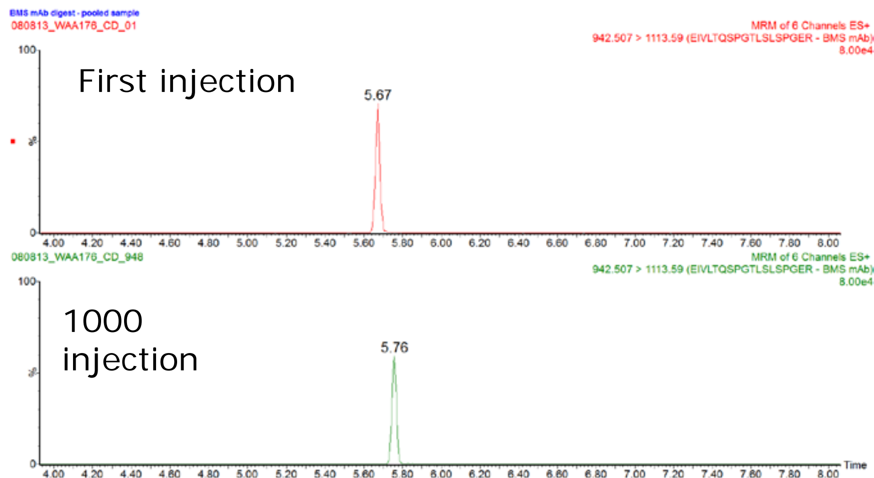
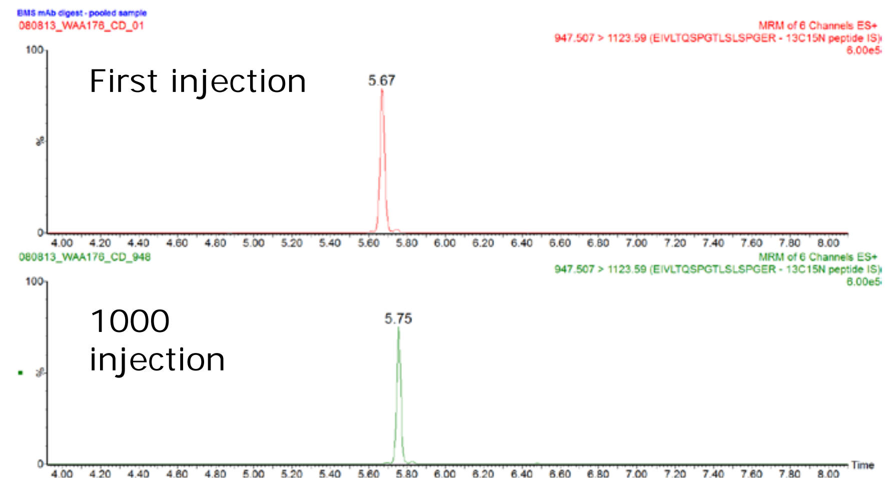
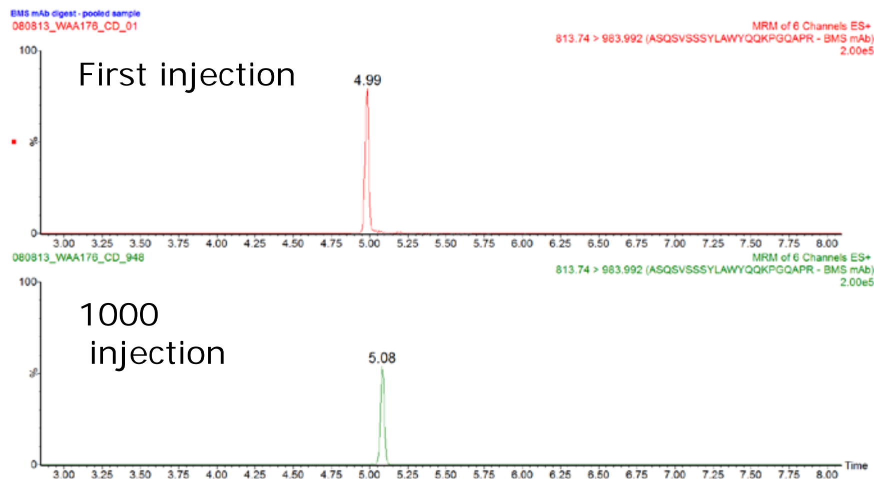
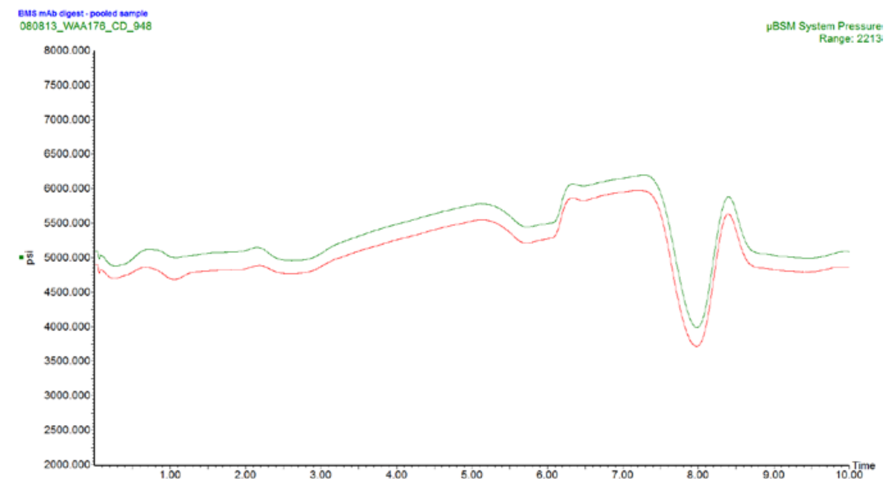
# Robustness Testing (mAb digest data)

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# Separations Device

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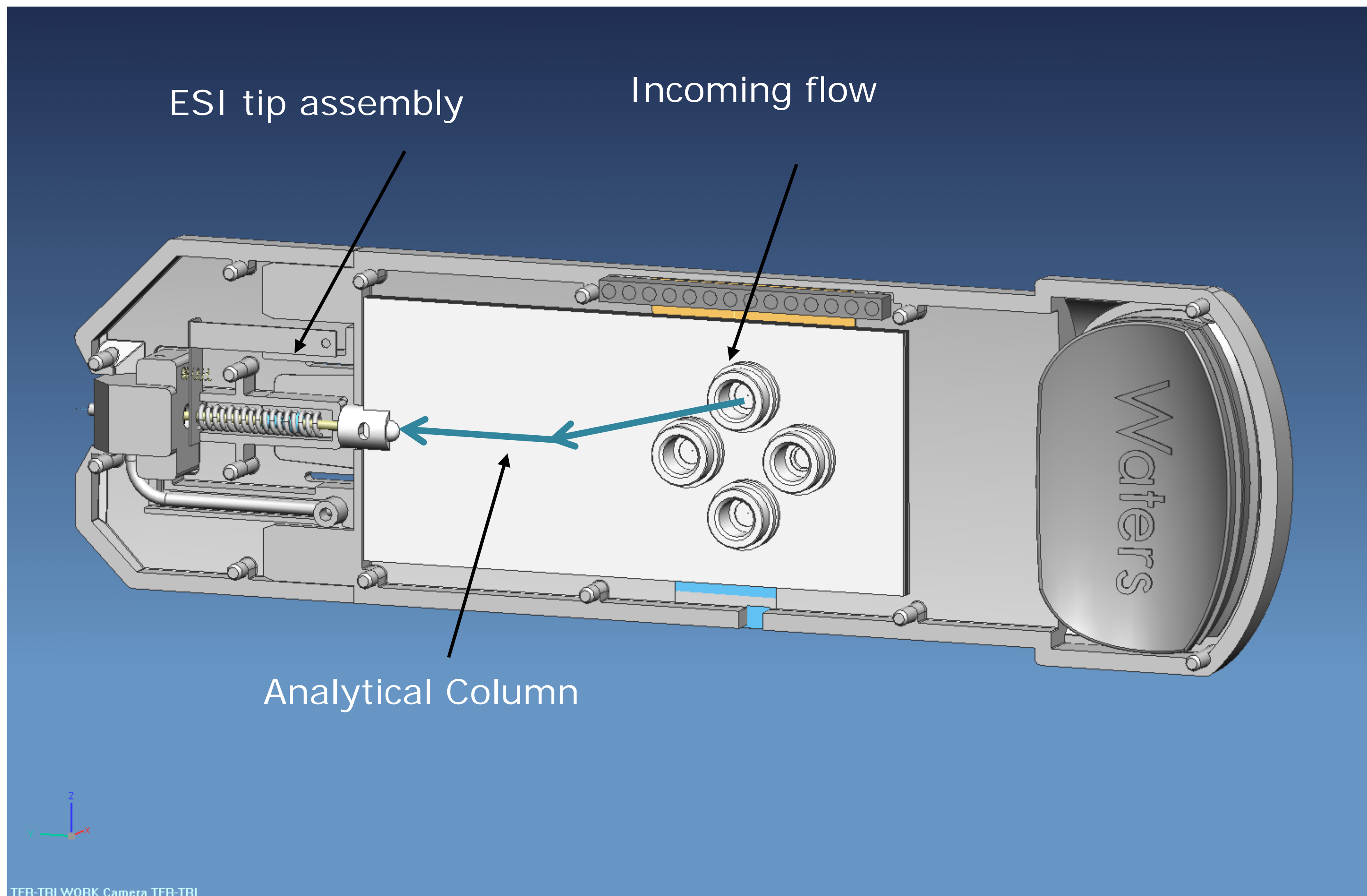


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# Ceramic Microfluidic Device

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# Peer-reviewed
# Beta collaborators

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*Rapid C...*
(wileyo...

# Me... ...human subjects using a mic... ...mass spectrometry

Haih... ...E. Lassman[2†], Tiffany Thomas[4], Wenyu Li[1],
Ther... ...s[3], John A. Wagner[3], David E. Gutstein[3],
Bria... ...Millar[5], Henry N. Ginsberg[4],
Giss... ...hen F. Previs[1*] and Thomas P. Roddy[1]

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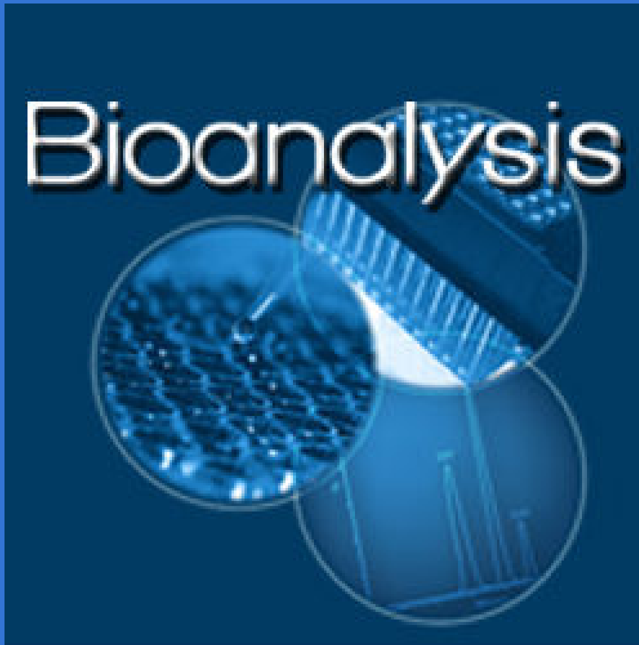
[2]Clinic... ...Whitehouse Station, NJ, USA
[3]Clinic... ...e Station, NJ, USA
[4]Colur...
[5]Divisi... ...n School of Medicine, University of Pennsylvania,
Philad...




M Lassman *et al.*

**RATI...** ...component of lipoprotein(a) [Lp(a)], an independent risk factor... ...s in blood is poorly understood in part due to technical challenges in measuring Lp(a) kinetics. Improvements in the ability to readily and reliably measure the kinetics of apo (a) using a stable isotope labeled tracer is expected to facilitate studies of the role of Lp(a) in cardiovascular disease. Since investigators typically determine the isotopic labeling of protein-bound amino acids following acid-catalyzed hydrolysis

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# Summary

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- A 150 µm i.d. ceramic micro-fluidics prototype device was successfully implemented in the analysis of biofluid samples with good chromatographic performance.

- Common sample preparation techniques already utilized in BioA workflows can be readily implemented when scaling down chromatography to 150 µm i.d.
  - PPT
  - LLE
  - IA/Digestion

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# Acknowledgements

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- Jim Murphy
- Jay Johnson
- Mark Wrona
- Catalin Doneanu
- Erin Chambers


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