

High Sensitivity Analysis of Biotherapeutics Using Capillary LC/MS/MS

Robert Plumb FRSC, CChem

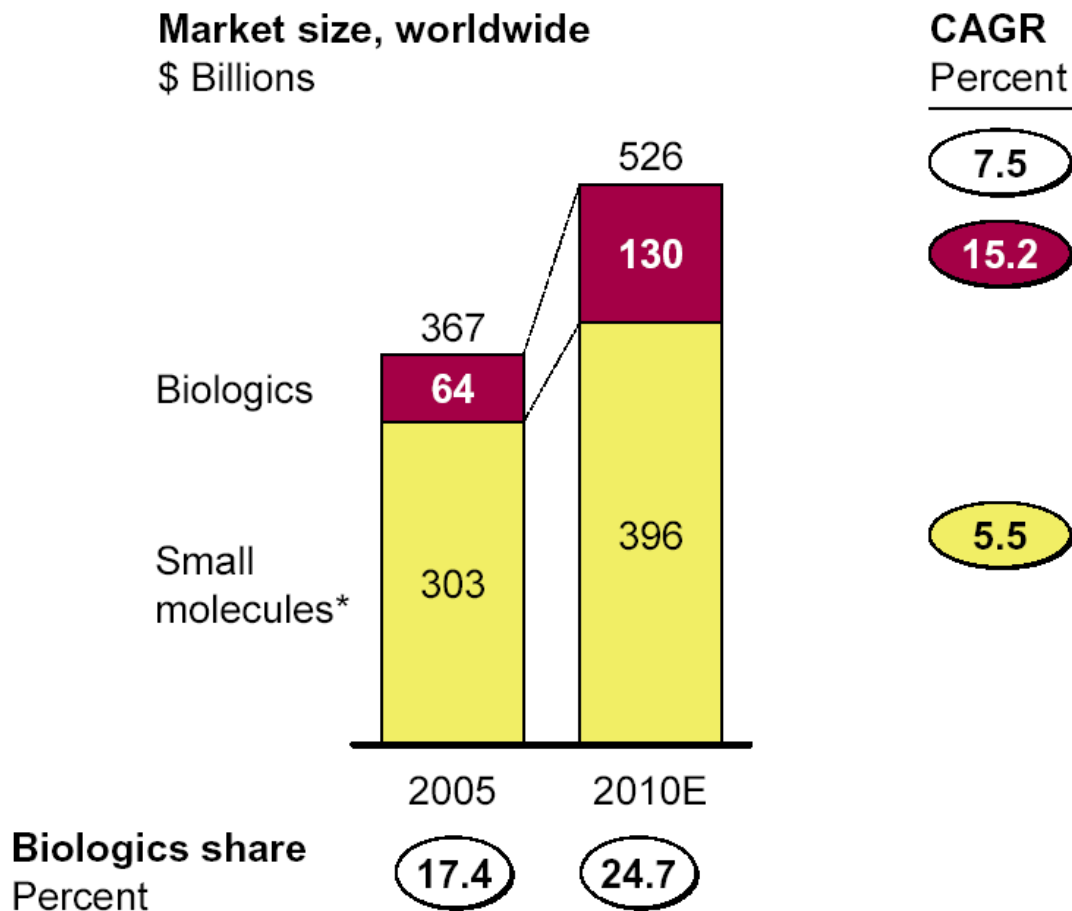
Acknowledgements

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- Paul Rainville Waters, MA, USA
- Mike Tomany Waters, MA, USA
- Gareth Booth Waters, Manchester, UK



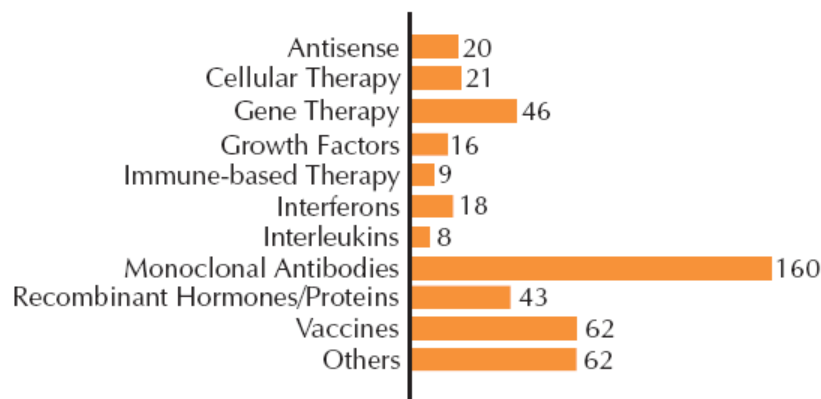
Pharmaceutical Growth

Biopharmaceuticals vs. Small molecules



* Includes small molecule generics

BIOTECHNOLOGY MEDICINES IN DEVELOPMENT—BY PRODUCT CATEGORY



- Antibodies: 34%
- Recombinant Proteins: 18%
- Vaccines: 12%
- **Proteins represent a total of >50%**
- Gene Therapy: 10%
- Cell Therapy: 5%
- Antisense: 5%
- Immune Therapy: 2%

2006
Report

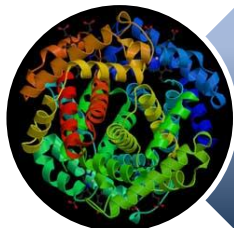
MEDICINES IN DEVELOPMENT

Biotechnology

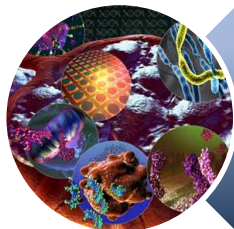
PRESENTED BY AMERICA'S PHARMACEUTICAL COMPANIES

418 Biotechnology Medicines in Testing Promise to Bolster the Arsenal Against Disease

Why are Proteins and Peptides Important



Proteins are well tolerated, specific, less susceptible to side effects, often more potent.



Biomarkers for many disease states, e.g. alzheimers



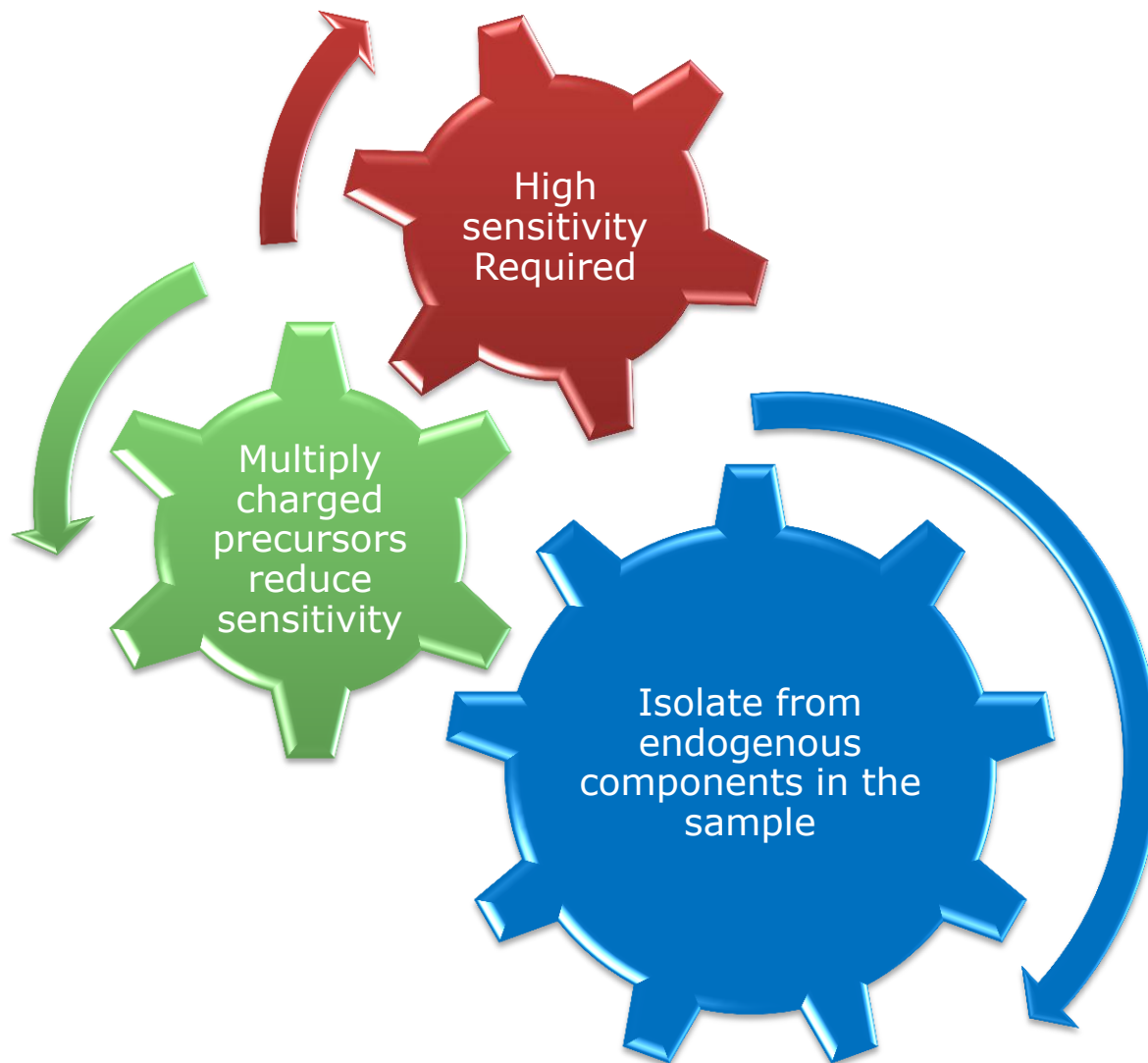
More successful in Phase II than small molecules. Reducing attrition in Phase I and II can reduce cost from \$1.8Bn per drug to \$1.2Bn*.



Protein based drugs account for 15-20% (on average) of the development pipeline.

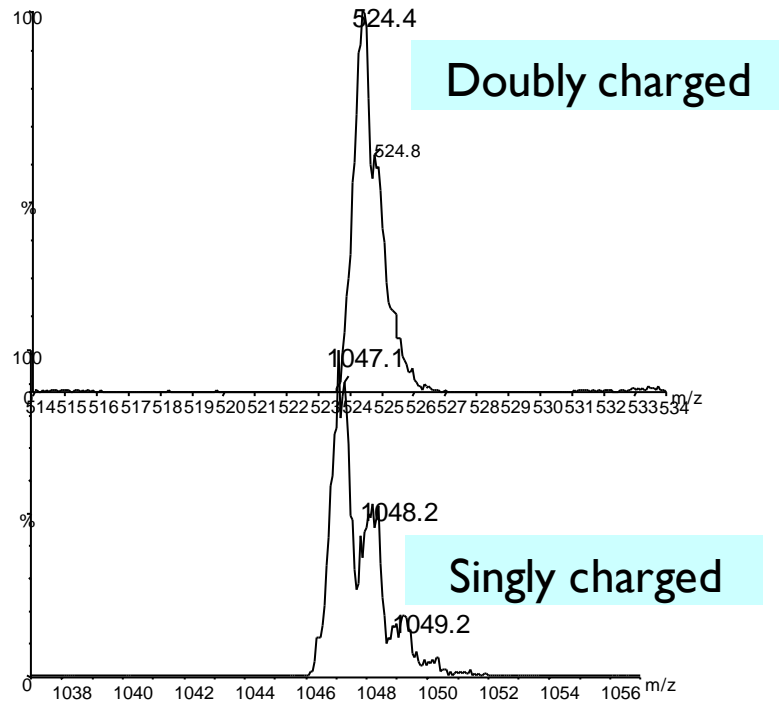
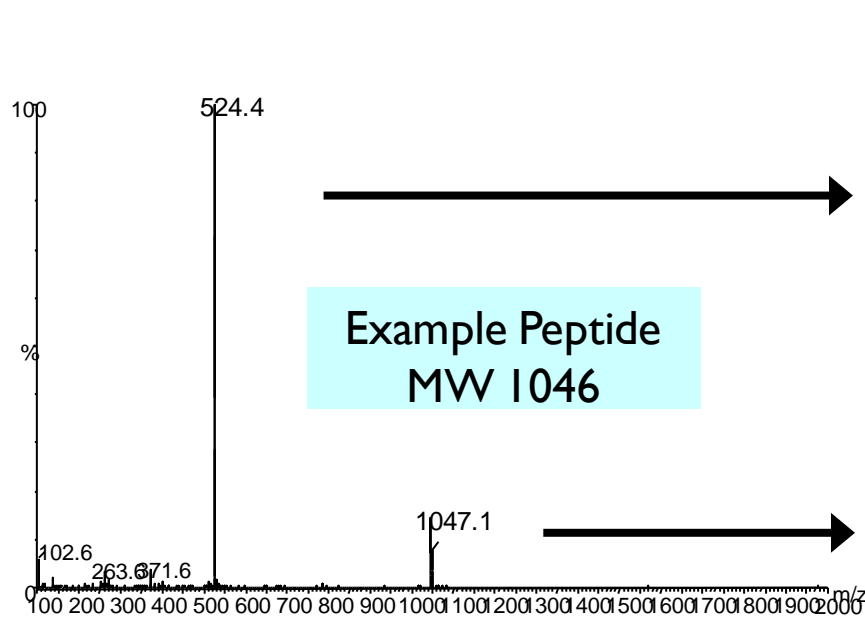
*Source. Eli Lilly, Nature Drug Discovery March 2010

Challenge For LC/MS/MS Analysis



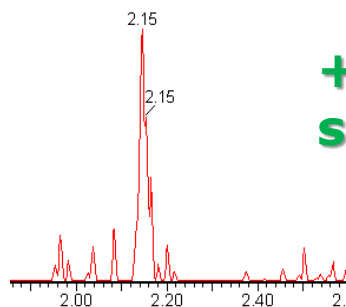
Multiply Charged Precursors

- Common to see 2⁺, 3⁺, 4⁺, 5⁺ depending on size of peptide
 - Occasionally 1⁺ for smaller peptides
 - Small molecules typically only 1⁺

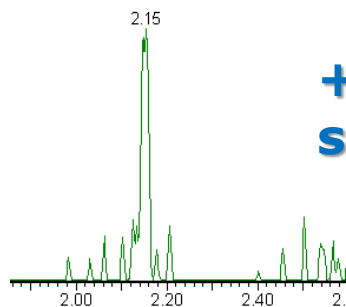


Multiple charging: detection of large peptides possible in lower m/z ranges

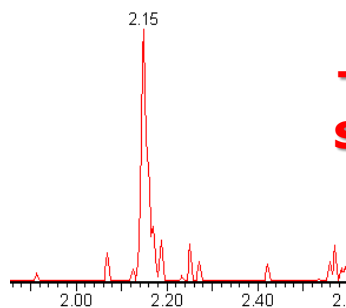
Summing the Charge States



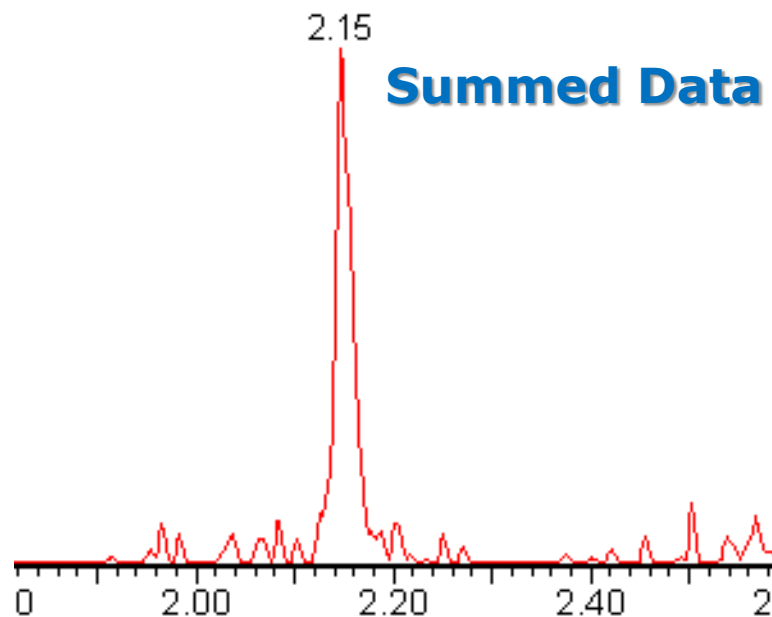
+3 Charge state



+4 Charge state



+5 Charge state



Summed Data

SPE Method Development: Using Hybrid Particle

Oasis® WCX
μElution

Oasis® MAX
μElution

Protocol

Dilute plasma with
4% H₃PO₄

Condition MeOH/Equilibrate H₂O

Load Diluted Plasma

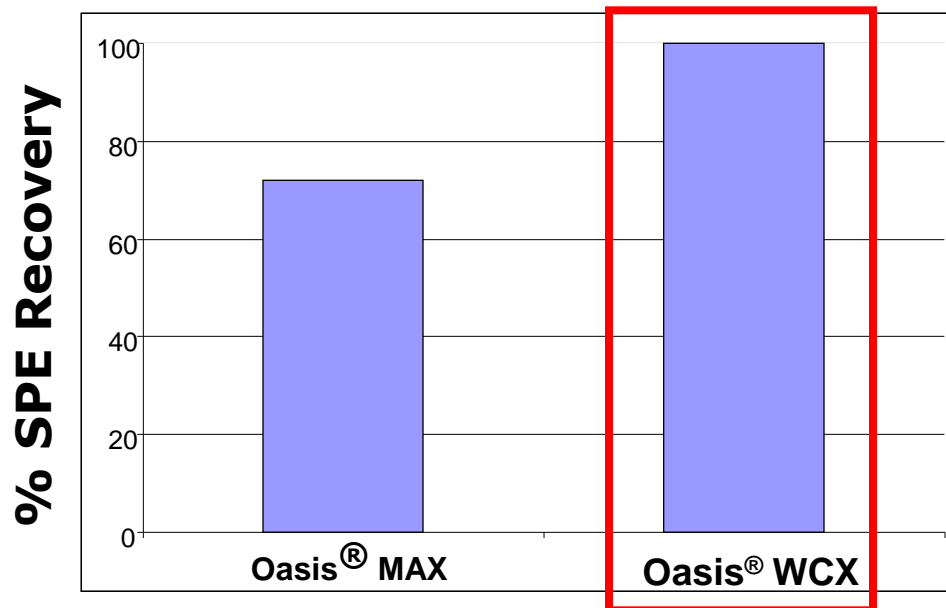
Wash 1:
5% NH₄OH

Wash 2:
20% ACN

Elution:
1% TFA in 75/25 ACN/H₂O

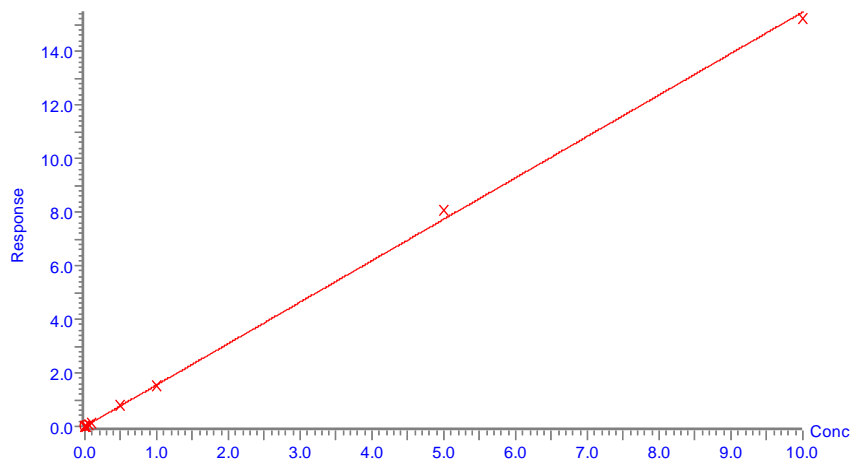
Dilute:
H₂O

- SPE screening method from used
- Analyte recovery best on WCX
- Matrix effects <10% on Oasis® WCX

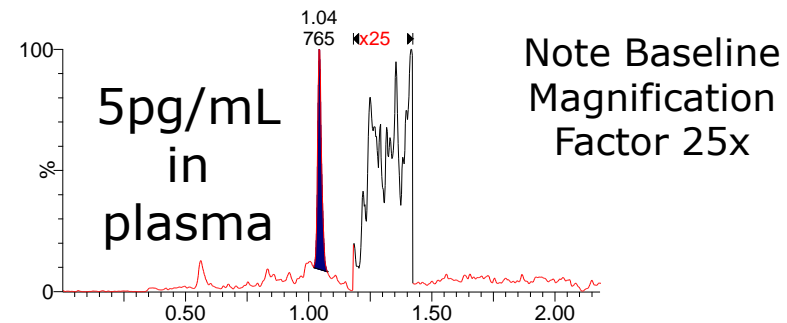


Extracted Plasma Curve For Desmopresin 1-10000pg/mL

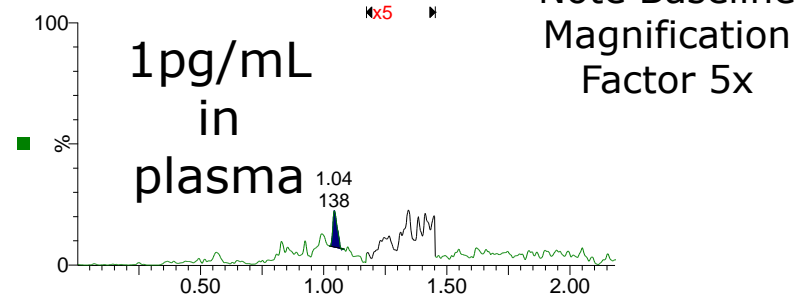
Compound name: Desmopressin (1)
Correlation coefficient: $r = 0.999622$, $r^2 = 0.999244$
Calibration curve: $1.5469 \cdot x + 0.000270265$
Response type: Internal Std (Ref 2), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



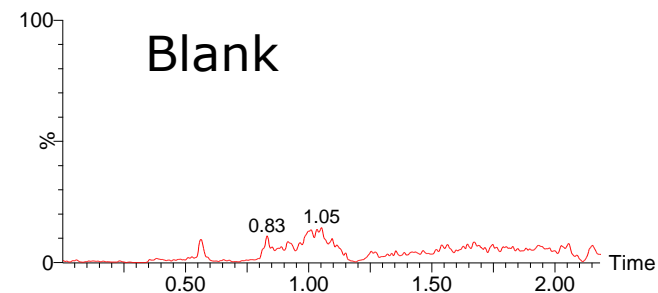
1 pg/mL



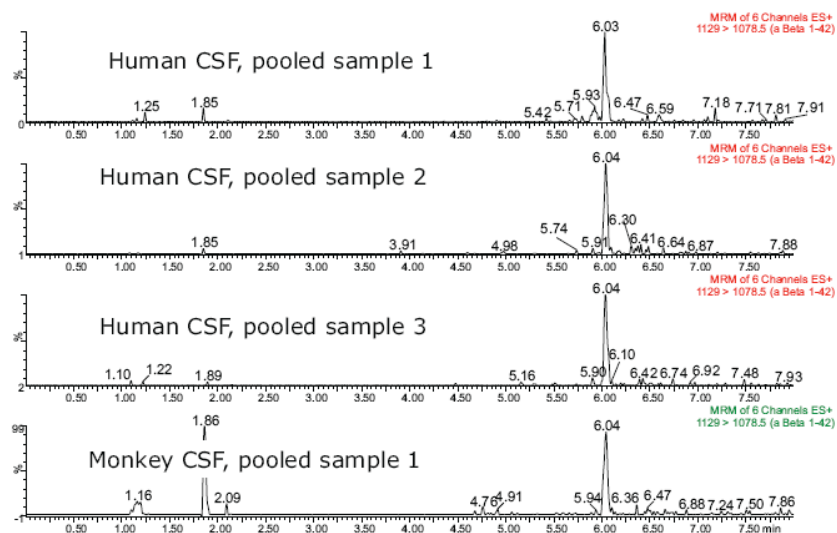
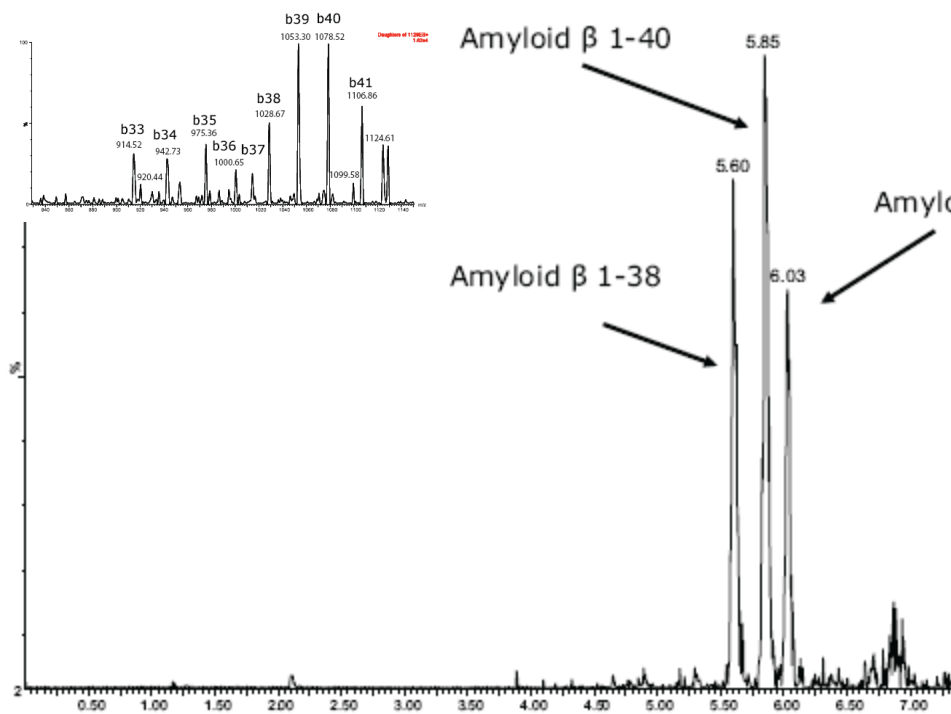
Note Baseline
Magnification
Factor 25x



Note Baseline
Magnification
Factor 5x



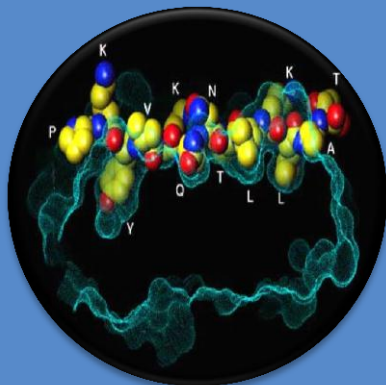
Amyloid β Analysis in CSF



Data courtesy of Erin Chambers, Waters

Reduced Sample Volume and Dried Blood Spot Samples

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Peptide quantification can be achieved using LC/MS/MS technology



Small sample volumes such as CSF and Dried Blood Spots provide a new challenge



Greater sensitivity is required to address these challenges



- **Can be accomplished utilizing different approaches**
 - More sensitive detection
 - Sample enrichment
 - *Capillary- and nano-scale chromatography*

- **Capillary / nano LC**
 - Elution of analytes of interest in smaller elution volumes increases concentration
 - Facilitates the utilization of small sample volumes

Can Chromatography Further Improve Sensitivity?

- Theoretical increase in sensitivity is inversely proportional to square of column radius for equal sample load

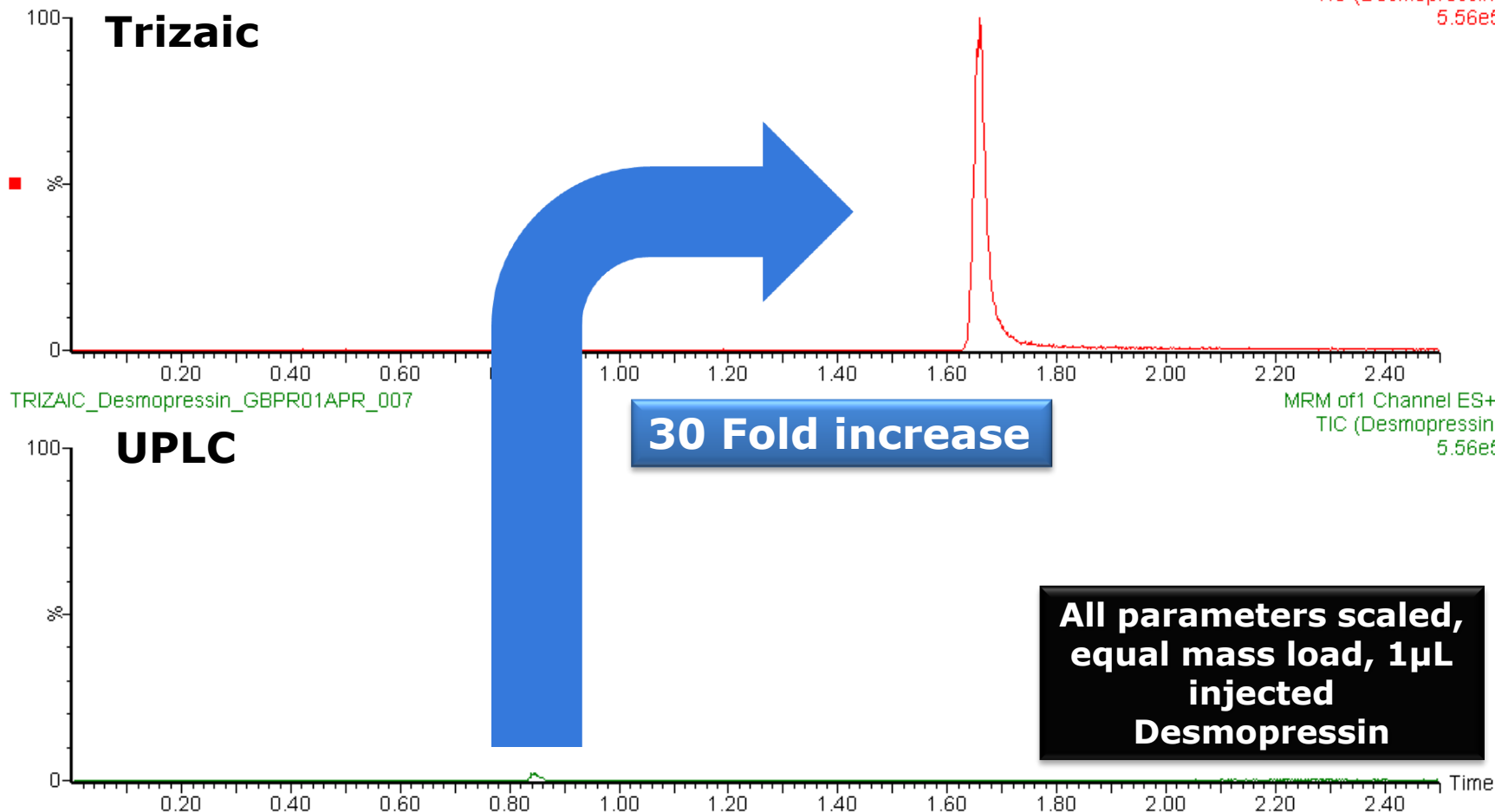
$$\frac{(1.05)^2}{(0.15)^2} = 49$$

- Moving from 2.1 mm (std column) to 300 μm theoretical gains – based on column diameter – could be up to:

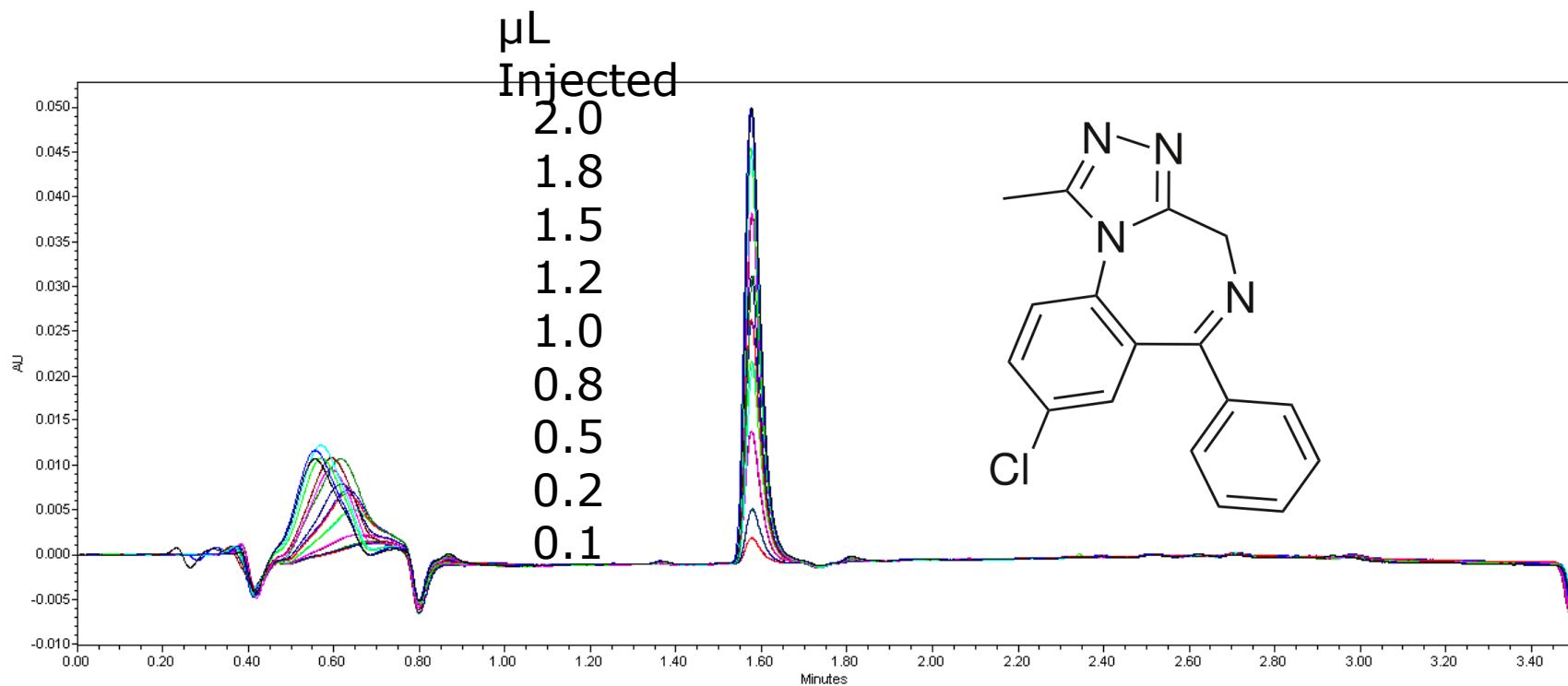
Comparing Sensitivities (Traditional vs. Micro-Fluidic)

TRIZAIC_Desmopressin_GBPR30MAR10_016

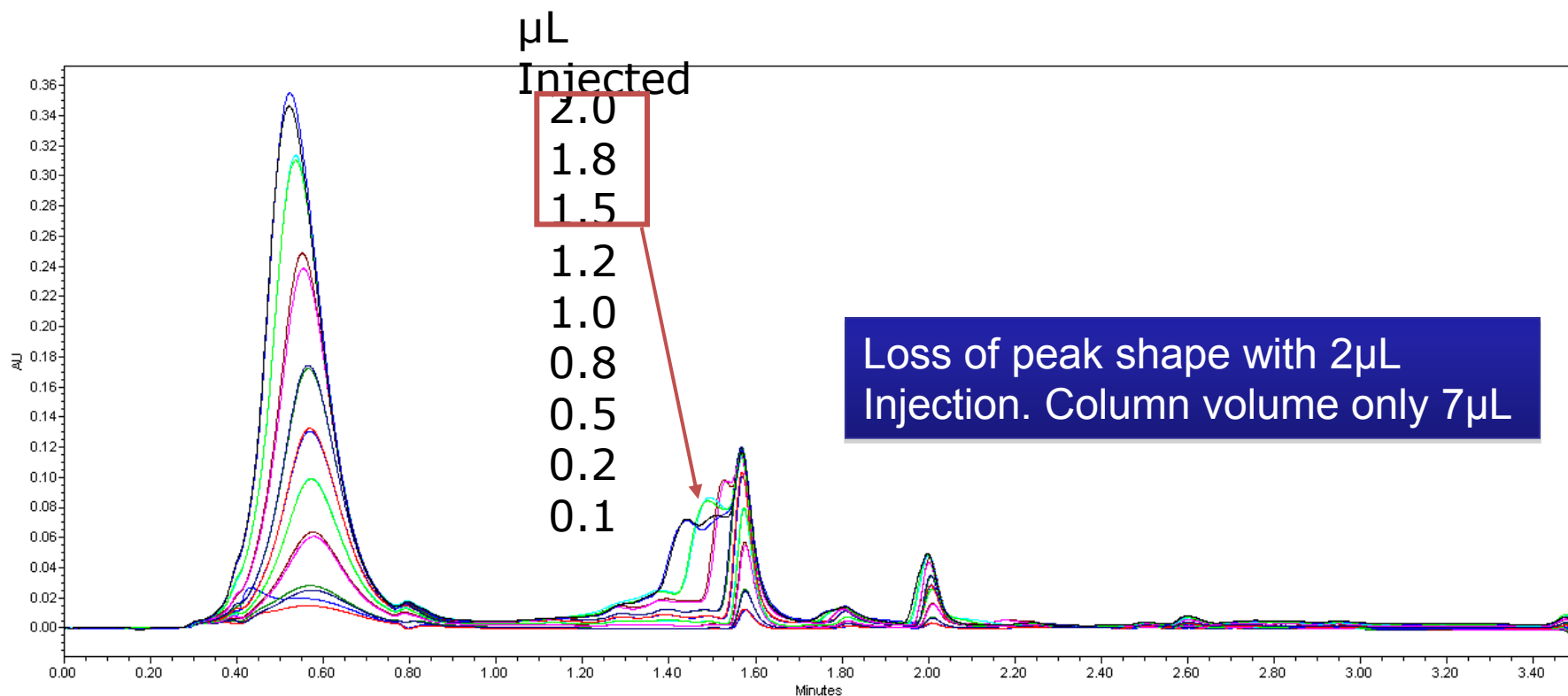
MRM of1 Channel ES+
TIC (Desmopressin)
5.56e5



Loading Study

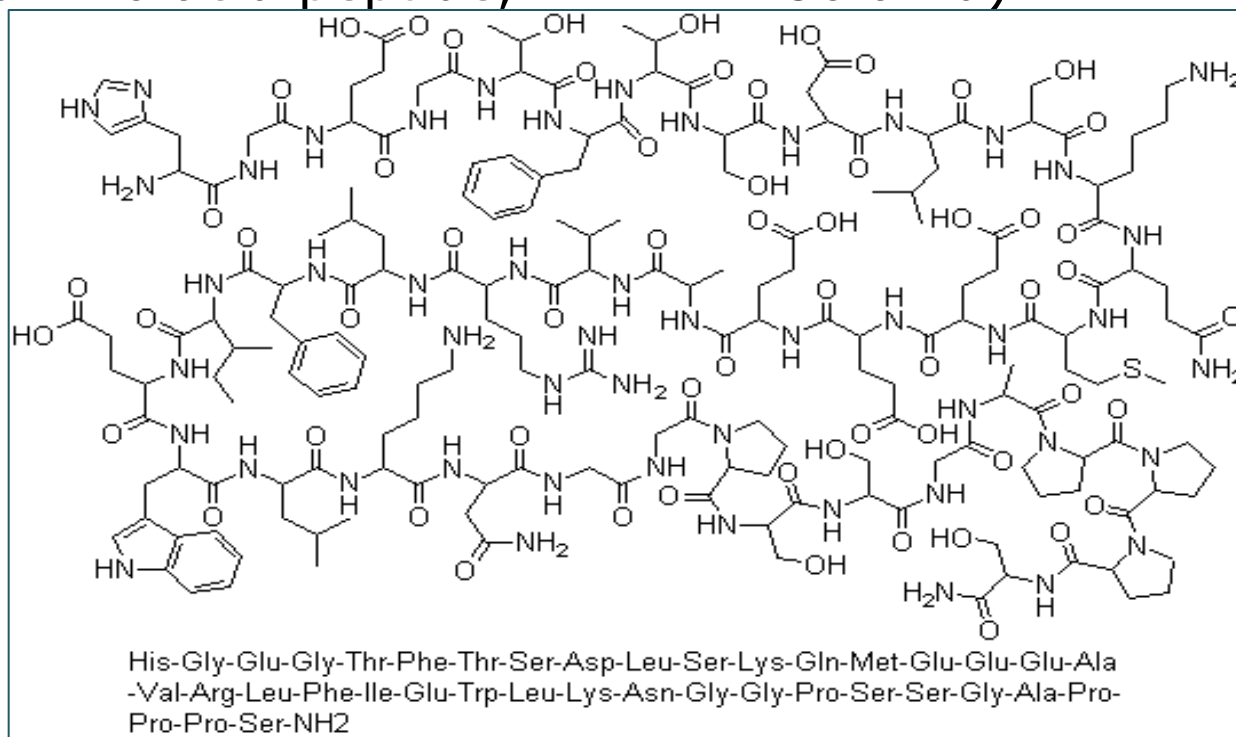


Loading Study Alprazolam in PPT Rat Plasma 2:1 (MeCN:Plasma)



Large Molecule DBS Example nano-UPLC / Trizaic / Xevo TQ-S

- Model compound: Exendin-4
(38 amino acid peptide, MW = 4186 amu)



Large Molecule DBS Example nano-UPLC / Trizaic / Xevo TQ-S

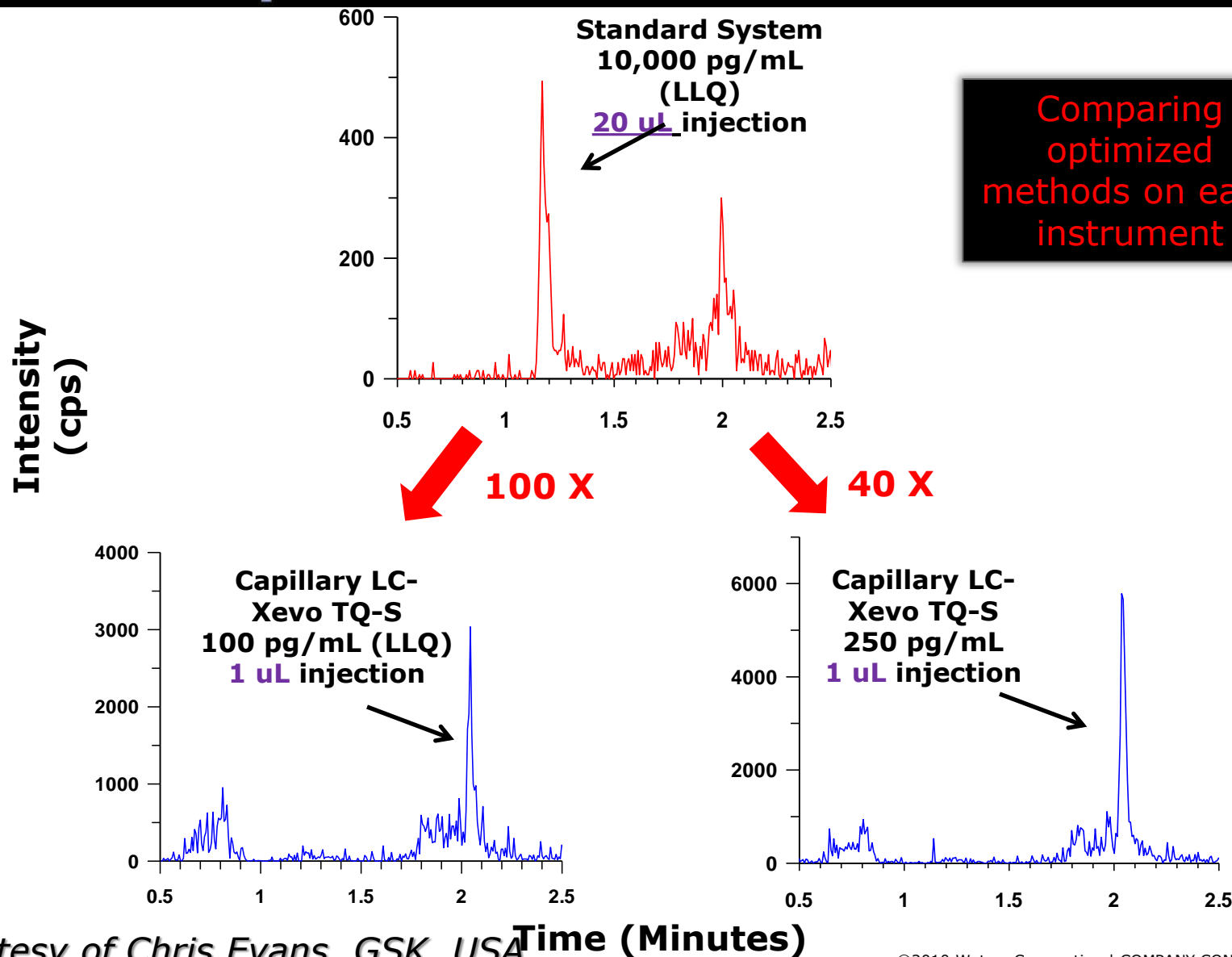
■ API 5000-UPLC Conditions:

- LC : Gradient (A) 0.1% Formic Acid in DI water; (B) Acetonitrile @ 1 mL/min.
- 2.1 x 50 Waters BEH C18, 1.7 μ m

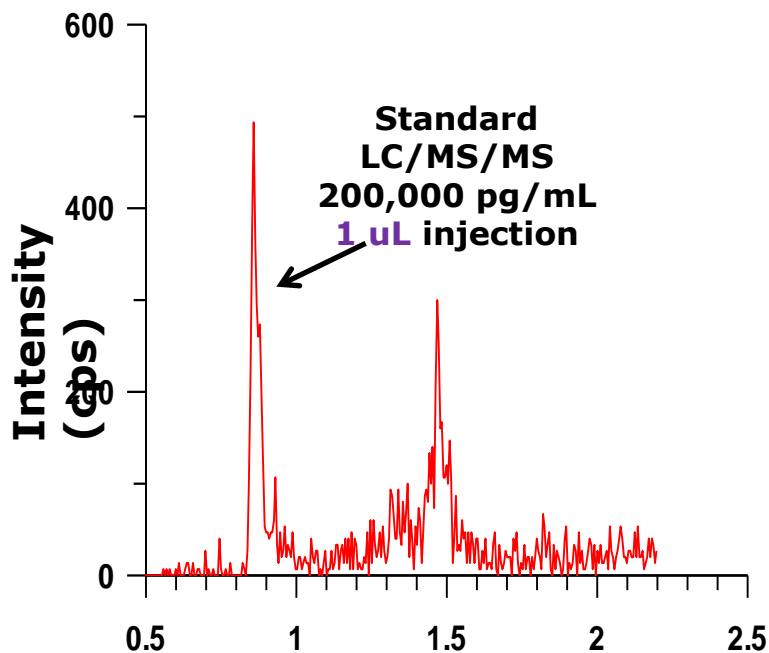
■ Xevo TQ-S Trizaic:

- LC: Gradient : (A) 0.1% Formic Acid in DI water; (B) Acetonitrile @ 12 μ L/min.
- Trizaic nano-tile: 300 μ m x 100 mm Waters BEH C18
- 4mm punch of a 20 μ L DBS; extracted in 75 μ L water presoak followed by 200 μ L MeOH with IS

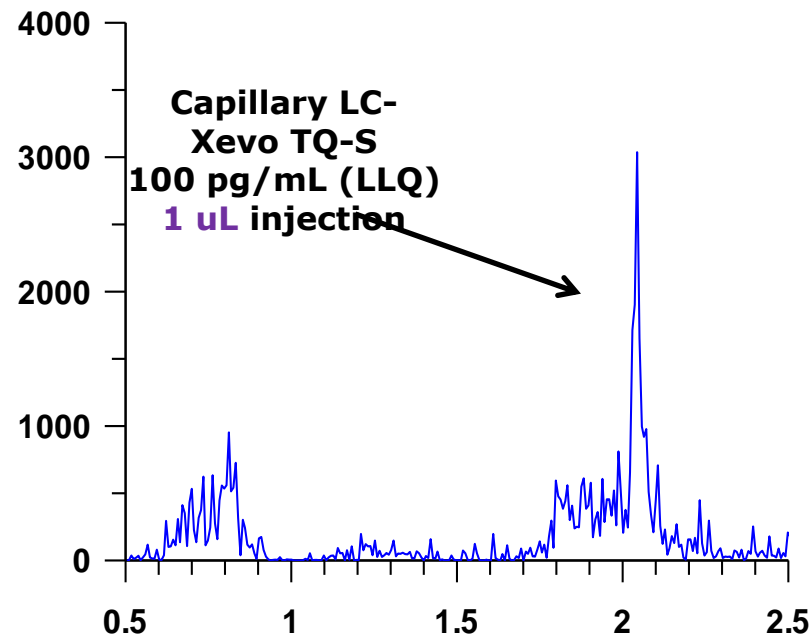
Large Molecule DBS Example Capillary-UPLC / Xevo TQ-S



Large Molecule DBS Example Capillary-UPLC / Xevo TQ-S



2000
X



Time (Minutes)

Comparing like
injections on
each instrument

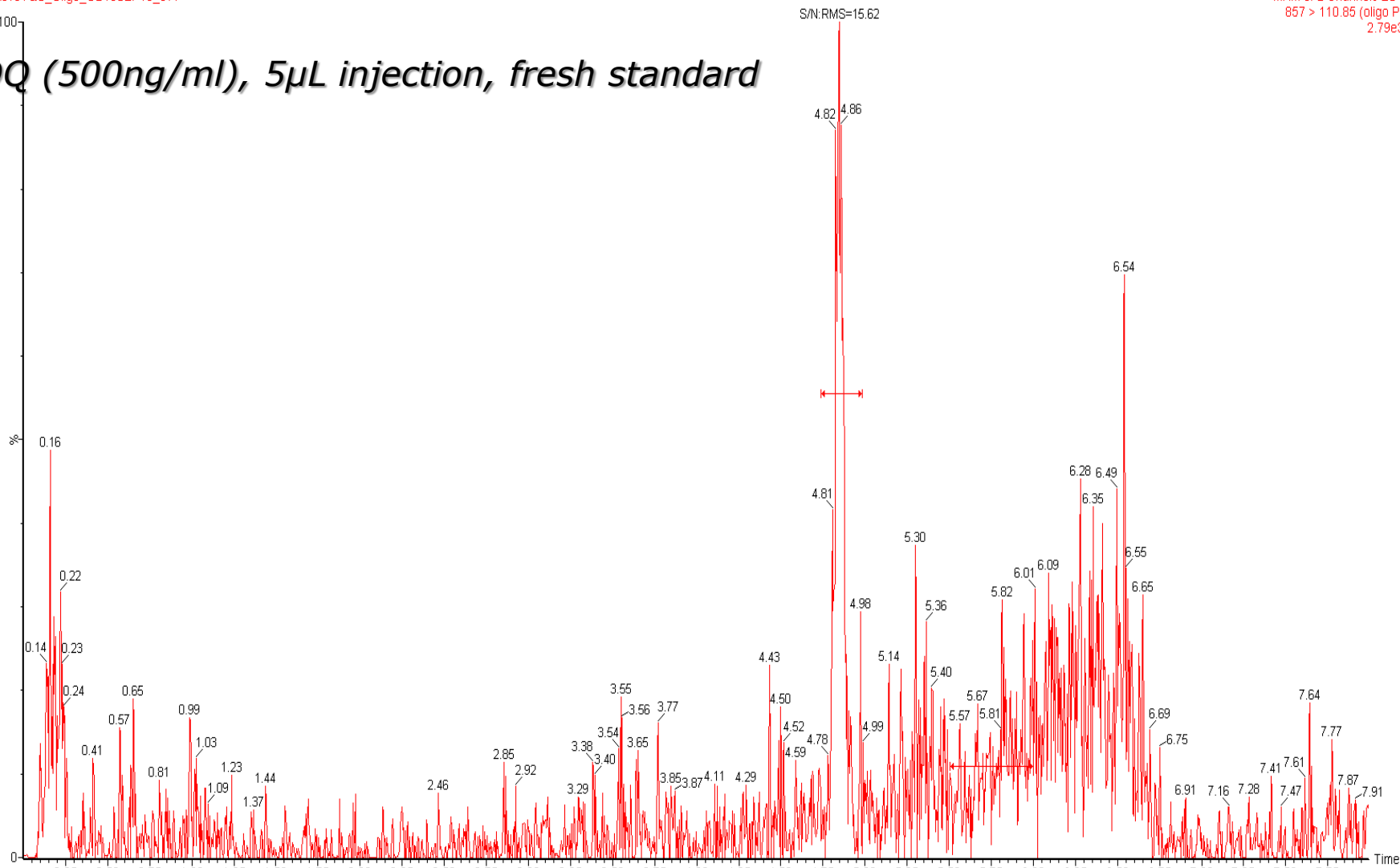
Oligo P – Standard source with Acquity UPLC

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500ng/ml
XevoTQS_Oligo_GB16SEP10_077

MRM of 2 Channels ES-
857 > 110.85 (oligo P)
2.79e3

LOQ (500ng/ml), 5µL injection, fresh standard



Oligo P – Trizaic source with Capillary Scale UPLC

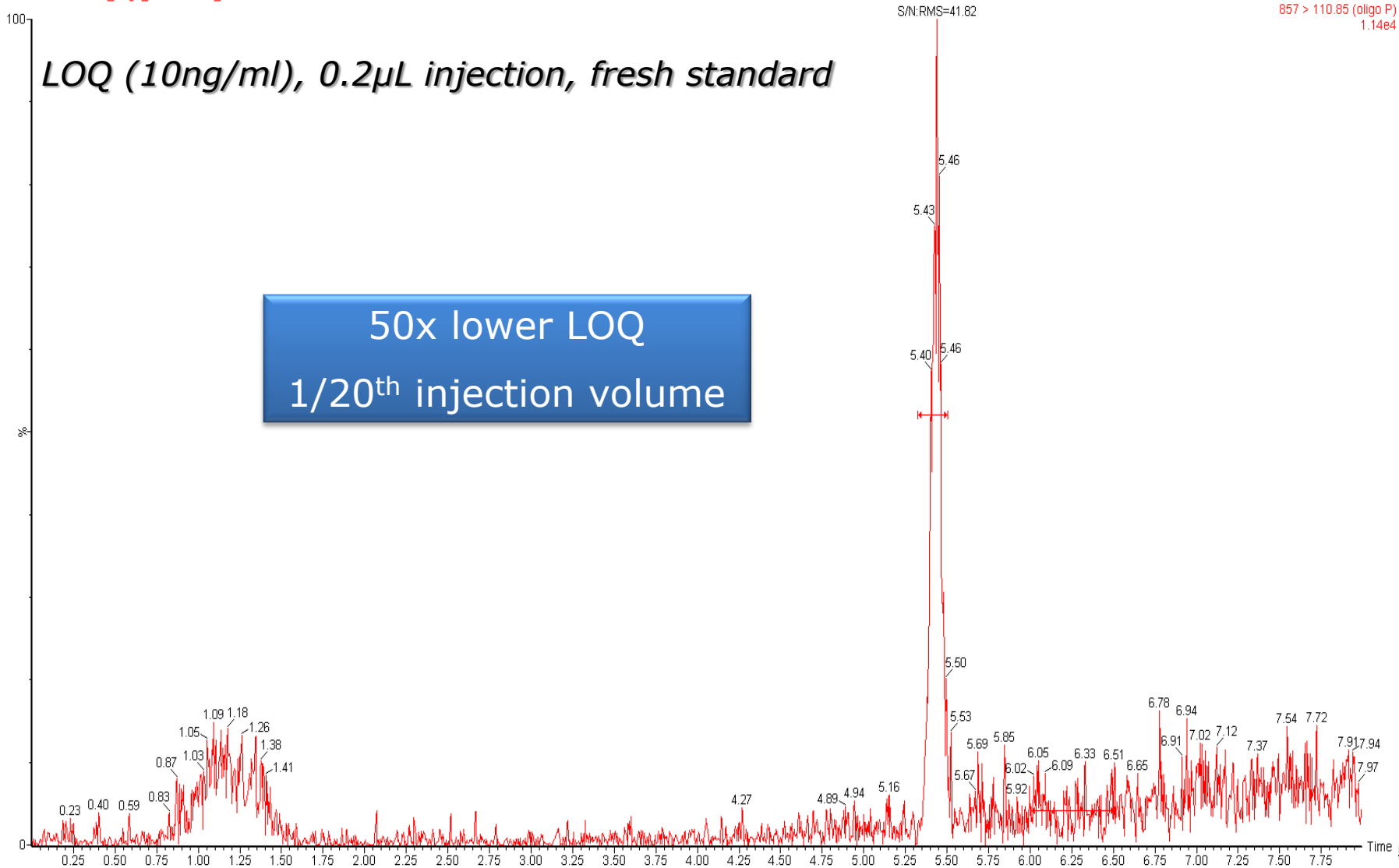
5ng/ml

XevoTQSwthTrizaic_Oligo_GB15SEP10_058

MRM of 2 Channels ES-
857 > 110.85 (oligo P)
1.14e4

LOQ (10ng/ml), 0.2μL injection, fresh standard

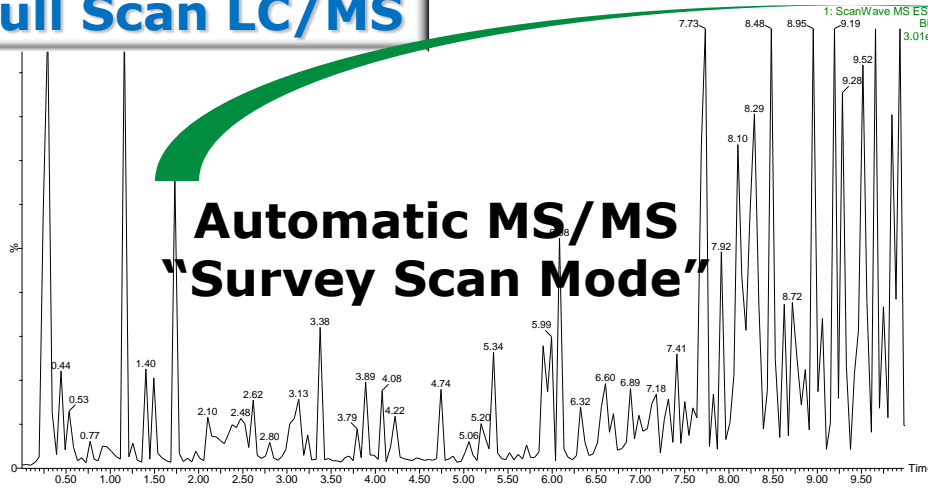
50x lower LOQ
1/20th injection volume



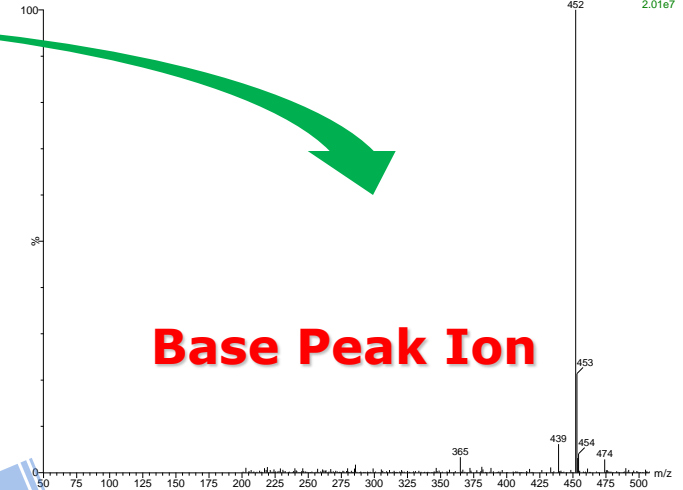
- With the challenge of dried blood spots there is an advantage to perform metabolite detection directly from the card.
- These small volumes require greater full scan sensitivity
- Limited sample volume means that MS and MS/MS data may need to be acquired in one run

Metabolite Detection With Capillary LC and Tandem MS/MS

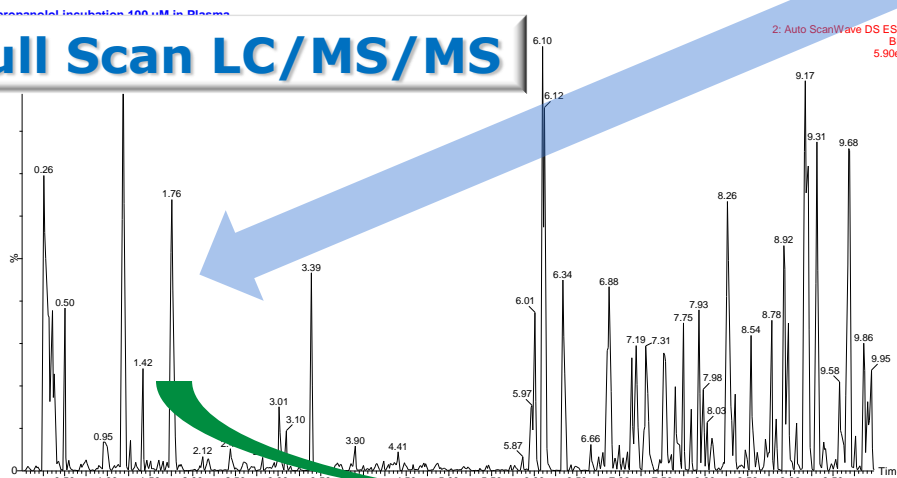
Full Scan LC/MS



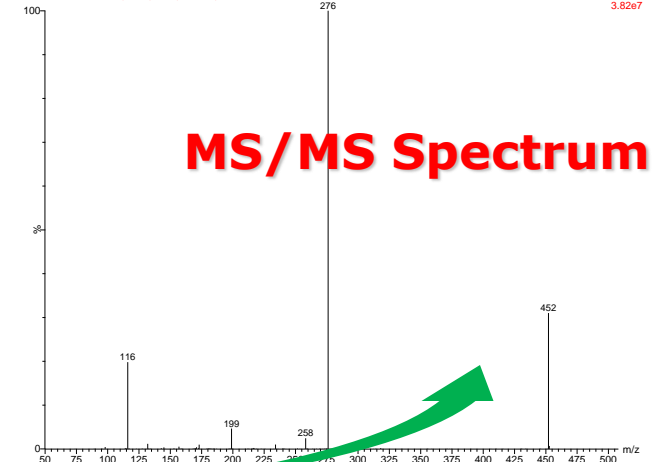
propranolol incubation 100 μ M in Plasma
041609_PR_018 37 (1.730) Cm (37)



Full Scan LC/MS/MS



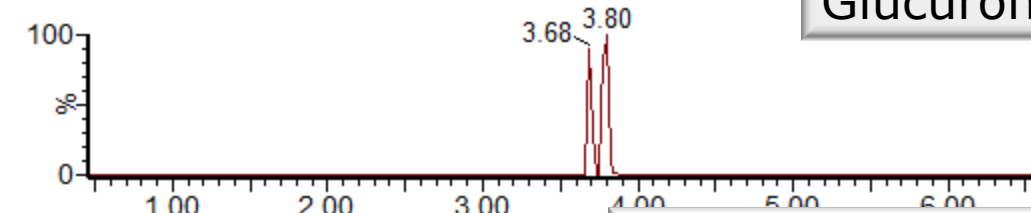
propranolol incubation 100 μ M in Plasma
041609_PR_018 109 (1.745) Cm (109.111)



MS/MS Data

Microsomal Incubation

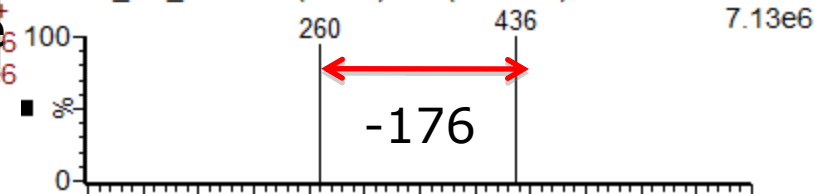
052709_PR_227



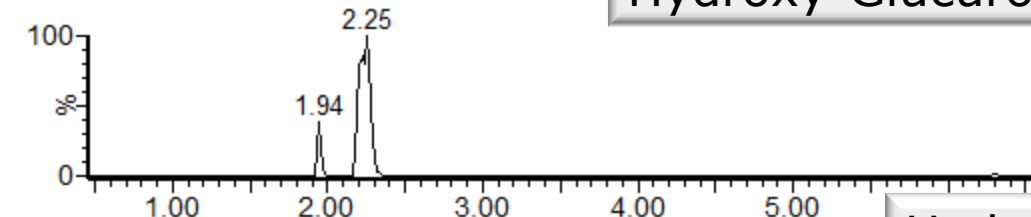
2: Glucuronide

Microsomal Incubation

052709_PR_227 224 (3.785) Cm (222:226)

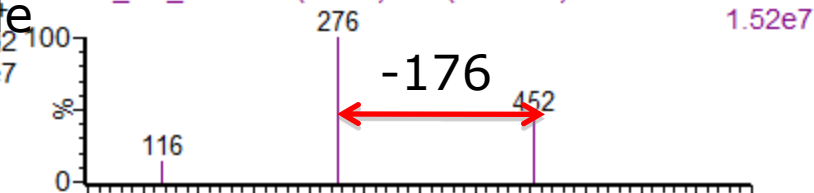


052709_PR_227

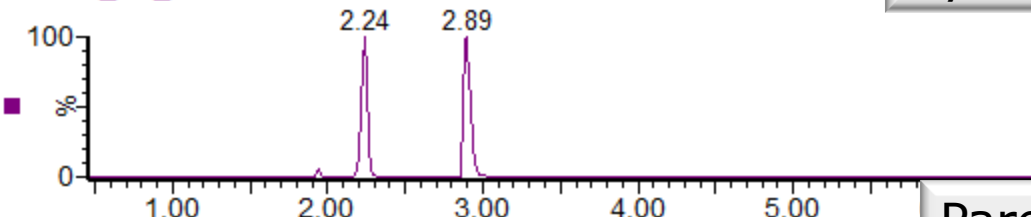


Hydroxy-Glucuronide

052709_PR_227 125 (2.236) Cm (121:131)

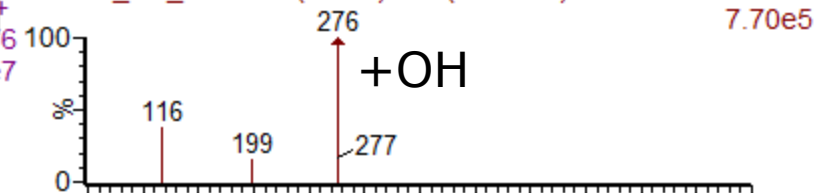


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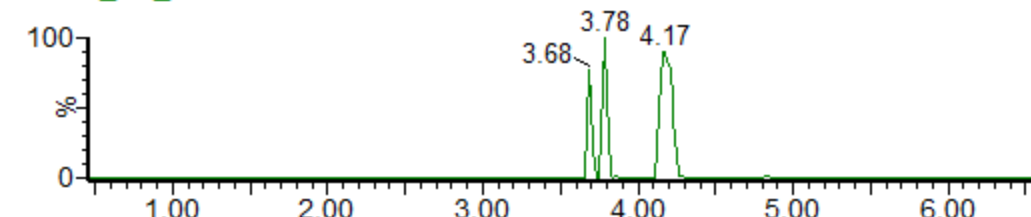


2: Auto S Hydroxy

052709_PR_227 167 (2.892) Cm (166:171)

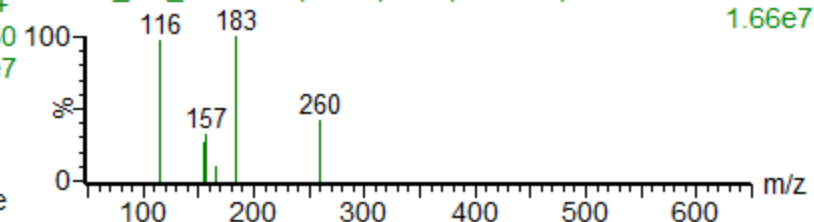


052709_PR_227



2: Auto Sca Parent

052709_PR_227 250 (4.169) Cm (247:255)



- Proteins, oligonucleotides and peptides offer a new class of therapeutics molecules.
- New analytical challenges in the bioanalysis of these molecules
- Microscale LC/MS can significantly increase the sensitivity of the analysis process, allowing desired sensitivities to be reached
- Micro fabricated device simplifies the the application of micro scale LC/MS

Thank You

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