

iFunnel Technology Revolutionizes Atmospheric Sampling

***High Sensitivity Determination
of Fluticasone Propionate in
Human Plasma Using the 6490
Triple Quadrupole LC/MS***

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Dec 2, 2010

EBF

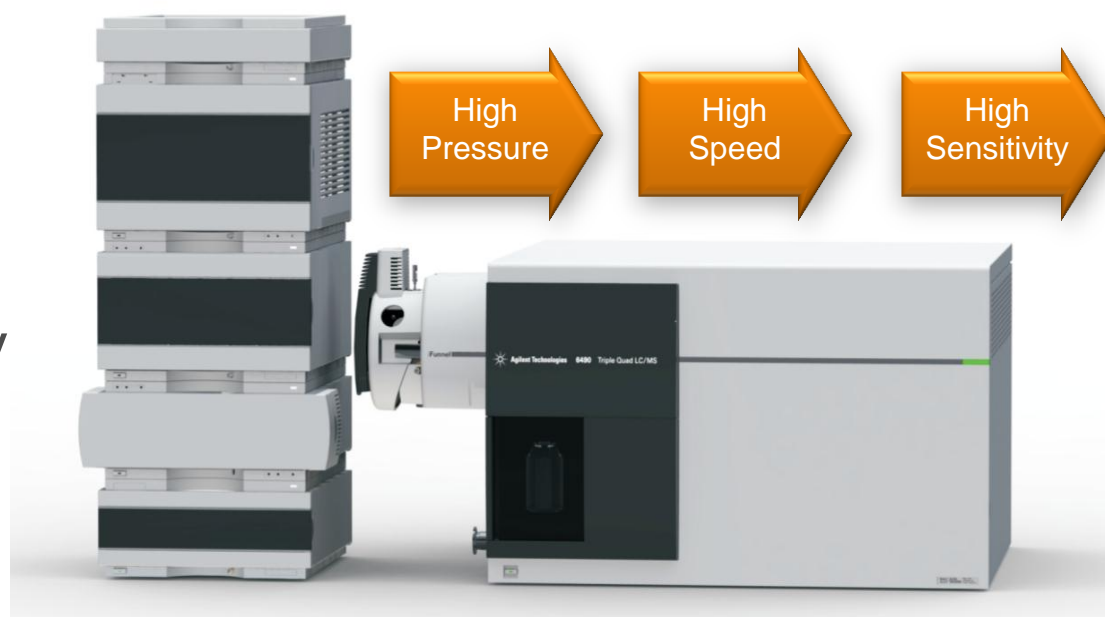
Barcelona, Spain



Agilent Technologies

Topics

- 6490 and iFunnel Overview
 - Sensitivity
 - Dynamic Range
 - Robustness
 - Precision & Accuracy
- Fluticasone



1290 Infinity LC/6490 Triple UHPLC and MS

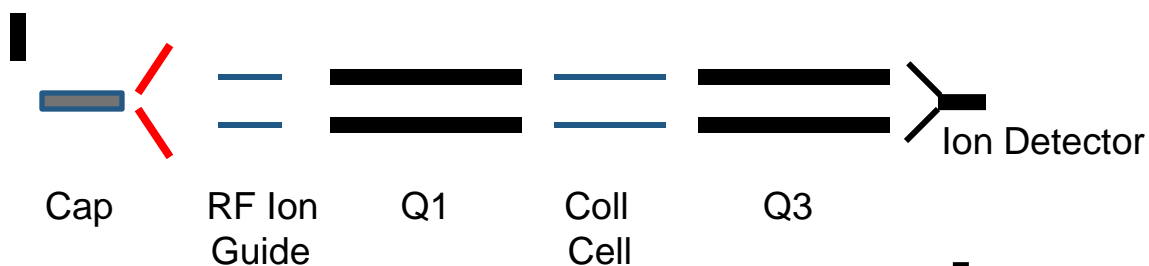
The 6490 High Sensitivity Triple Quadrupole

- Ionization and Ion Transfer Technology
- Collision Cell
 - Hexapole field axial focusing curved collision cell
 - Tapered cell structure for increased ion acceptance at entrance
 - Reduced noise
- New Quad Drive Electronics
 - Improved Quad DC frequency response
 - Higher RF power capability
 - Quad drive frequency increased to 1.4 MHz

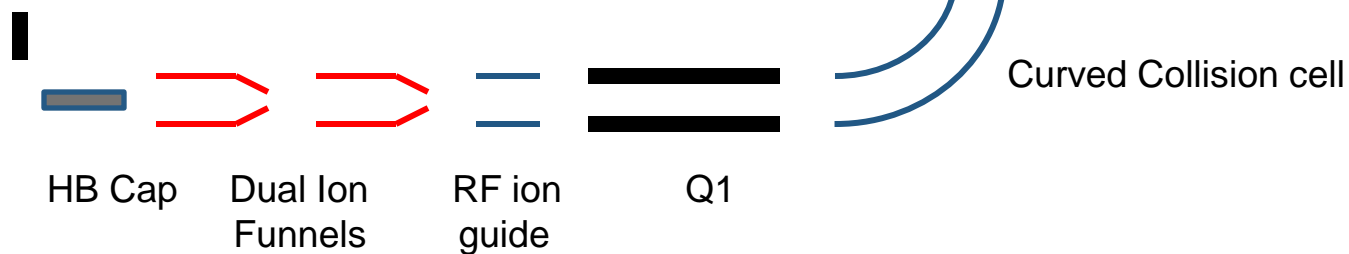


Quadrupole Instrument Configurations

6460 LC/MS (Skimmer Configuration)



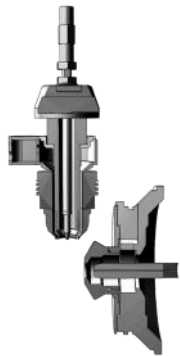
6490 LC/MS (iFunnel Configuration)



iFunnel Technology Captures 10 Times More Ions

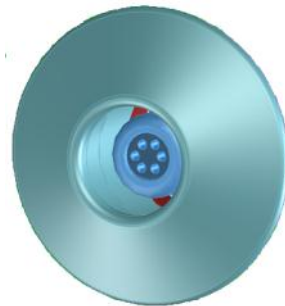
Agilent Jet Stream

- Thermal confinement of ESI plume
- Efficient desolvation to create gas phase ions
- Creates an ion rich zone



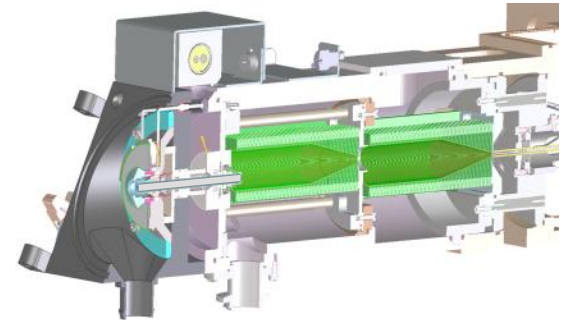
Hexabore Capillary

- 6 capillary inlets
- Samples 6 times more ion rich gas from the source
- Captures the majority of the gas from the source region



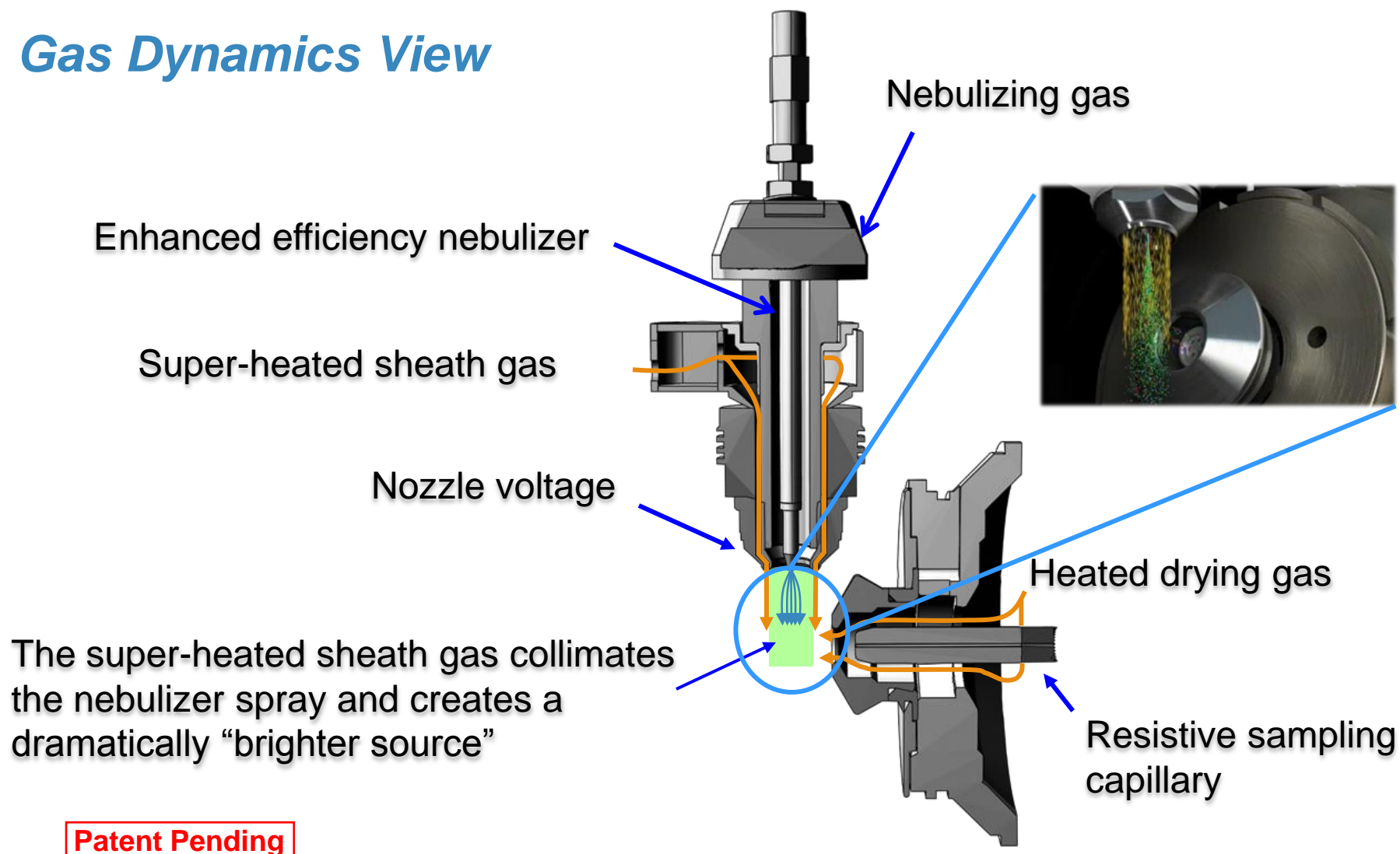
Dual Ion Funnel

- Removes the gas but captures the ions
- Removes neutral noise
- Extends turbo pump life



Agilent Jet Stream Ion Generation

Gas Dynamics View

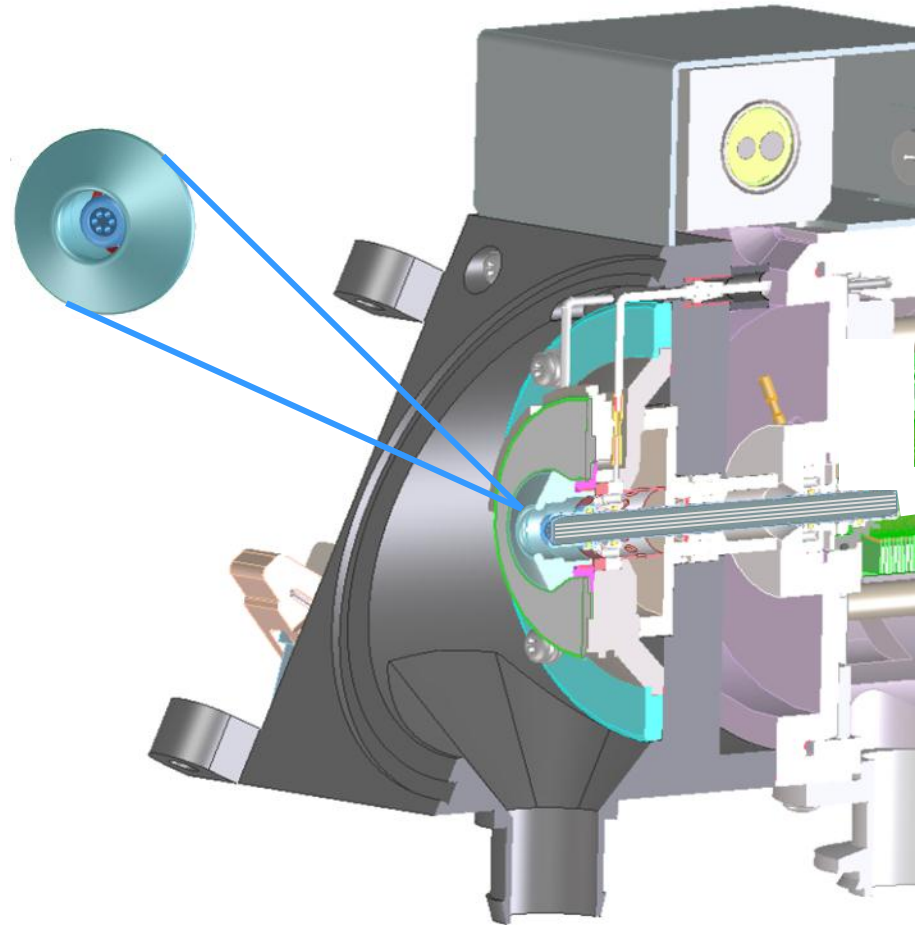


Hexabore Atmospheric Sampling....

- **SIX** bores
- **HALF** as long

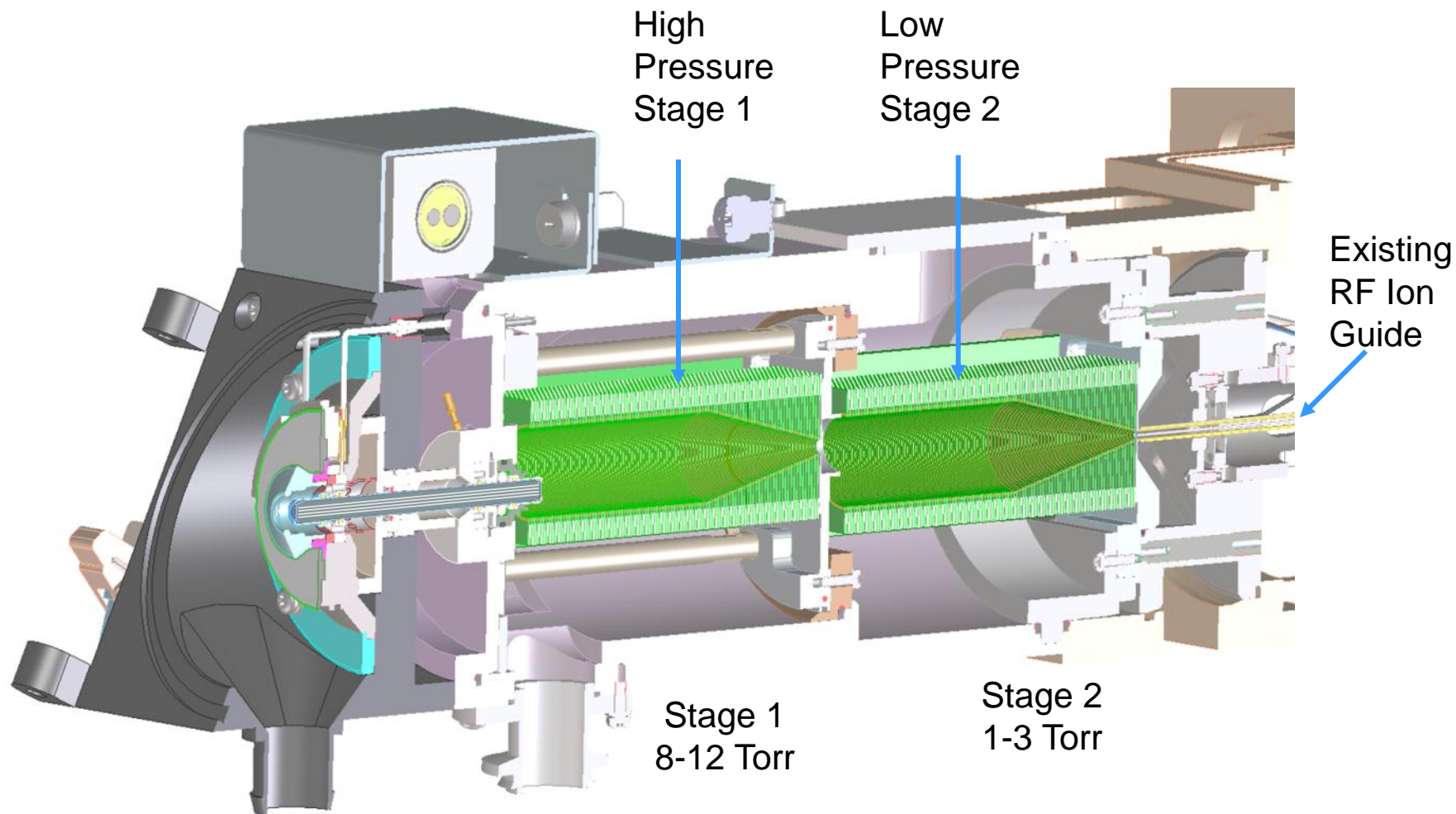
Six bores with half the restriction means...

- 6 times the amount of atmospheric gas sampled
- AND
- 5 – 10X the number of ions sampled over wide mass range.



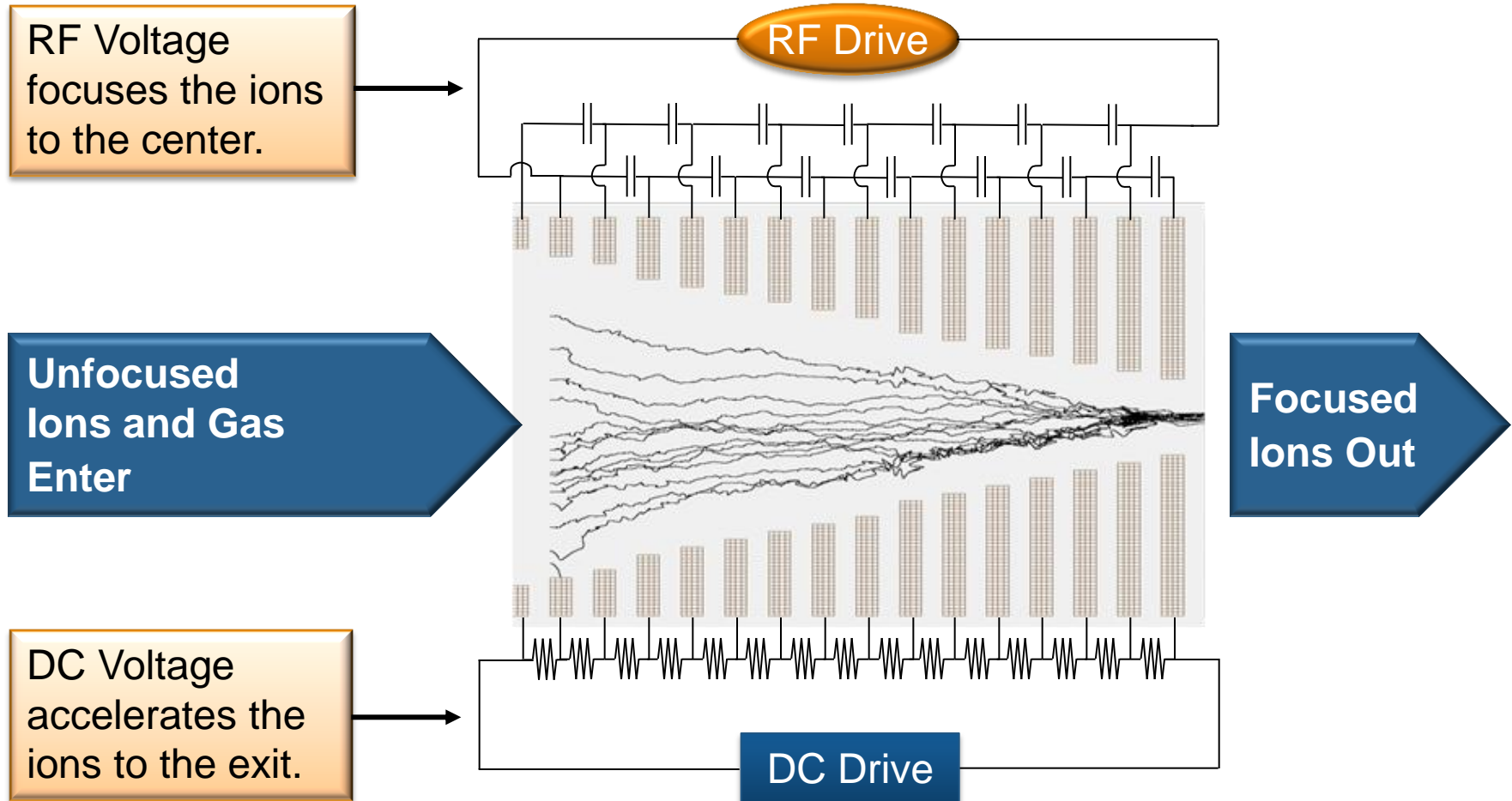
But how do we handle all the extra gas molecules?

Two Stage Ion Funnel Manages the Gas Load



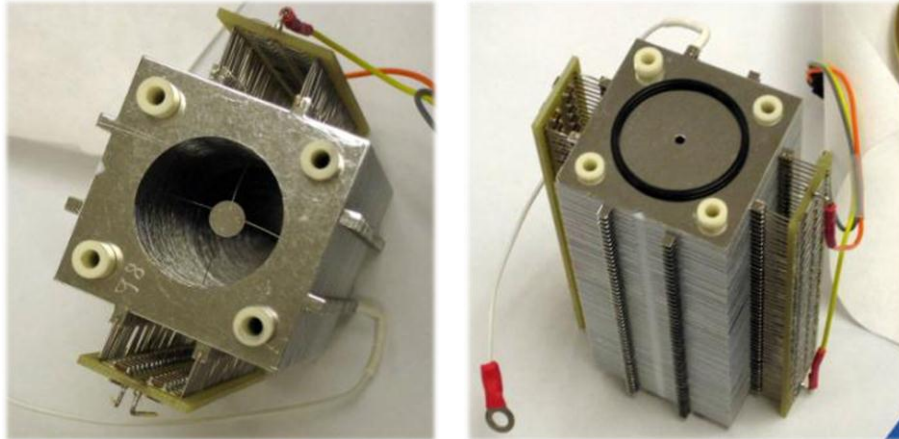
Stage 1 offset breaks up the high pressure gas exiting the Hexabore Capillary

Ion Funnel Operation



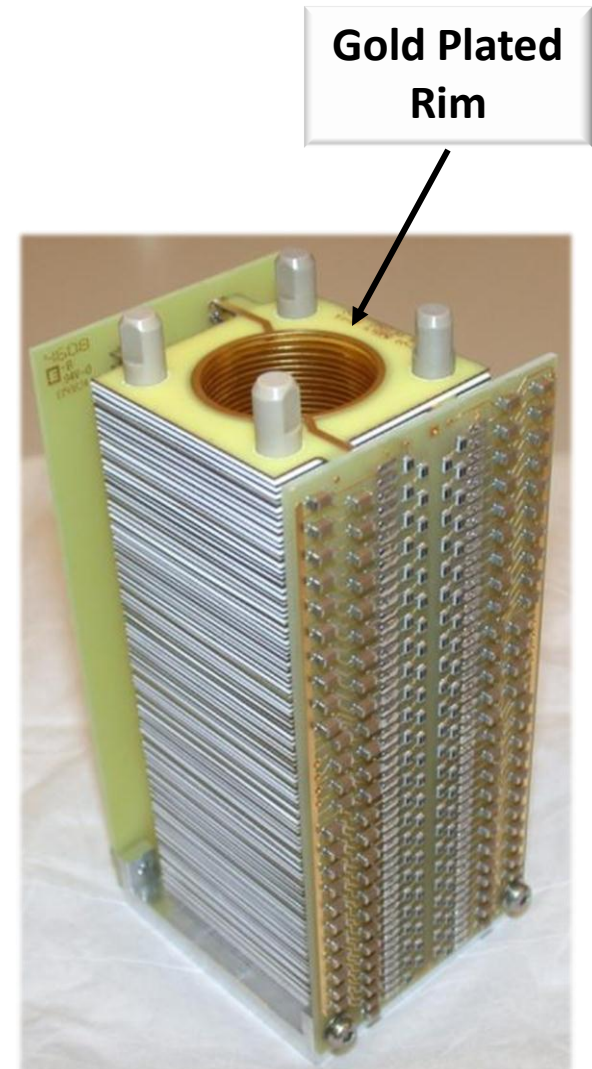
Ion Funnel Construction

Previously, with many metal plates....



... resulting in a large capacitive load and relatively large RF power electronics.

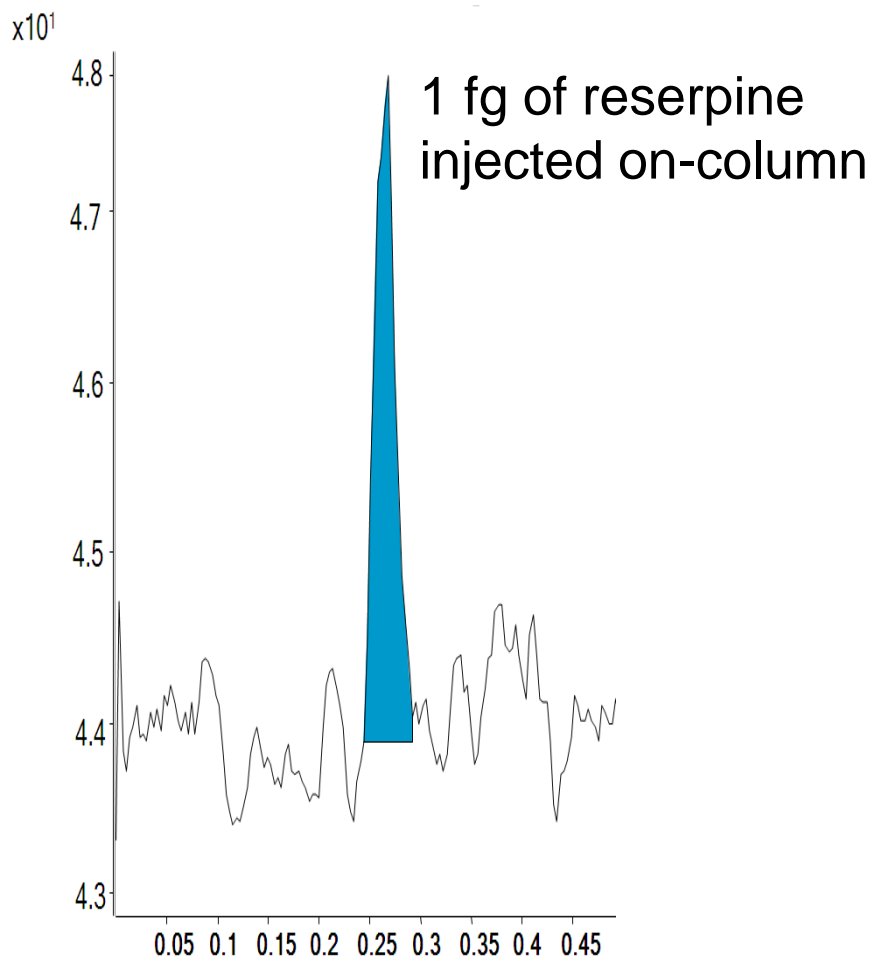
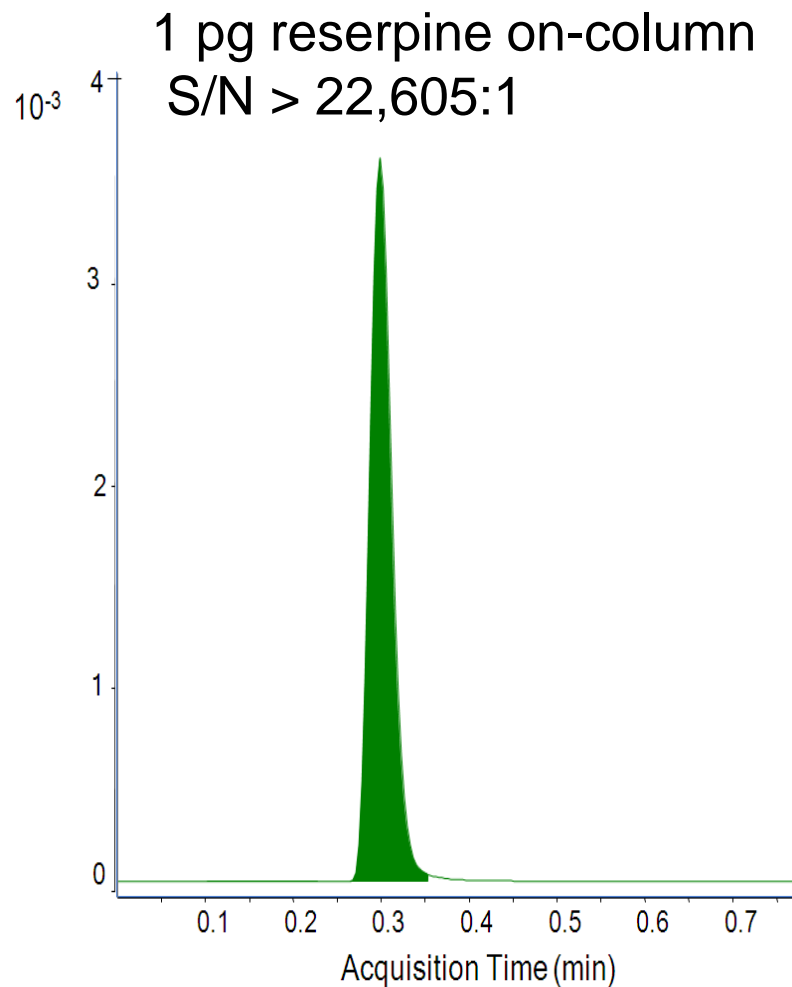
The 6490 design uses printed circuit board technology with only a small conductive rim resulting in a reduced capacitance load. This makes +/- ion switching possible.



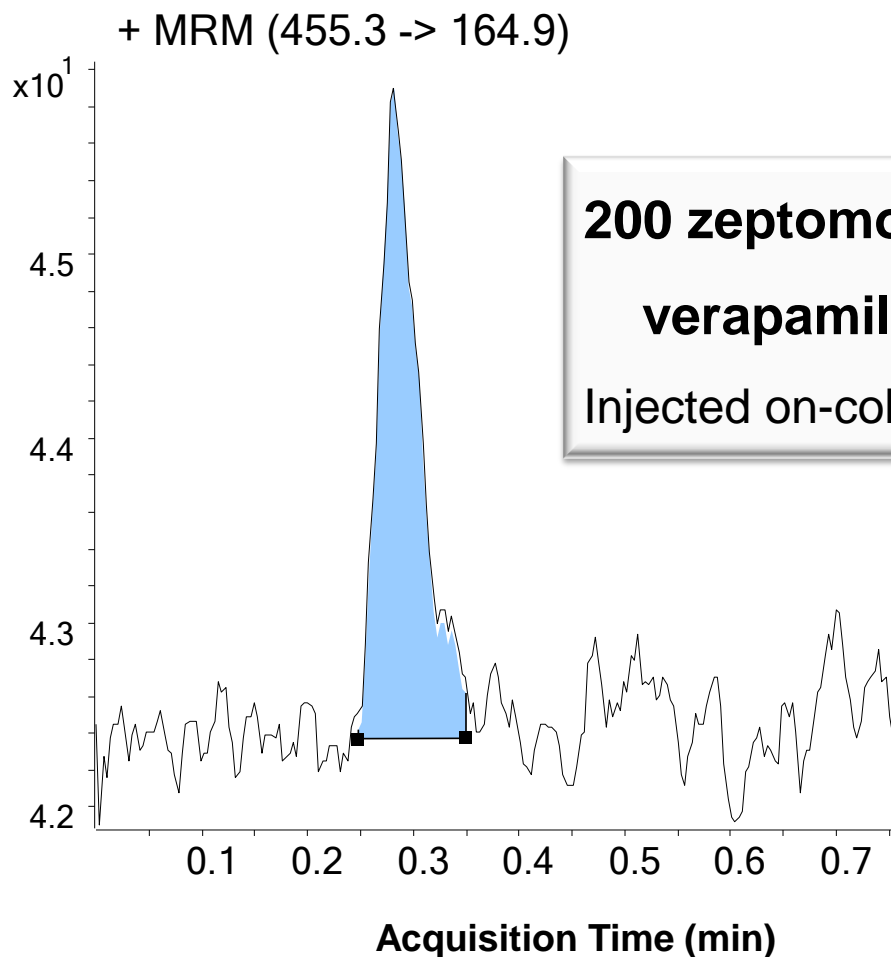
High and Low Pressure Funnel



6490 Specification: $S/N > 10,000:1$ so the 6490 should be able to detect 1 fg ?



Zeptomolar Detection Limits



Micro	10^{-6}
Nano	10^{-9}
Pico	10^{-12}
Femto	10^{-15}
Atto	10^{-18}
Zepto	10^{-21}

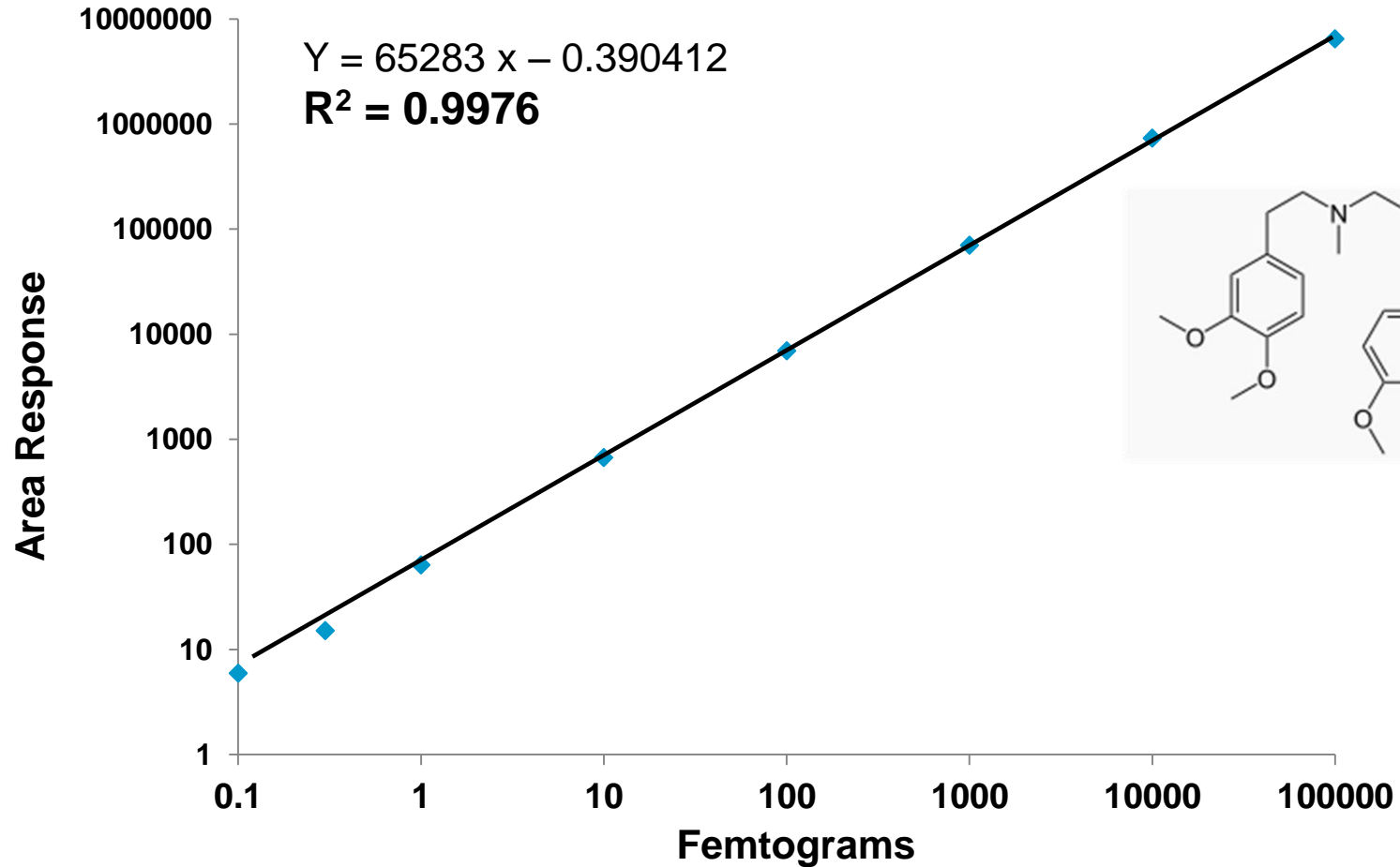
Avagadro's
Constant

6.02×10^{23}

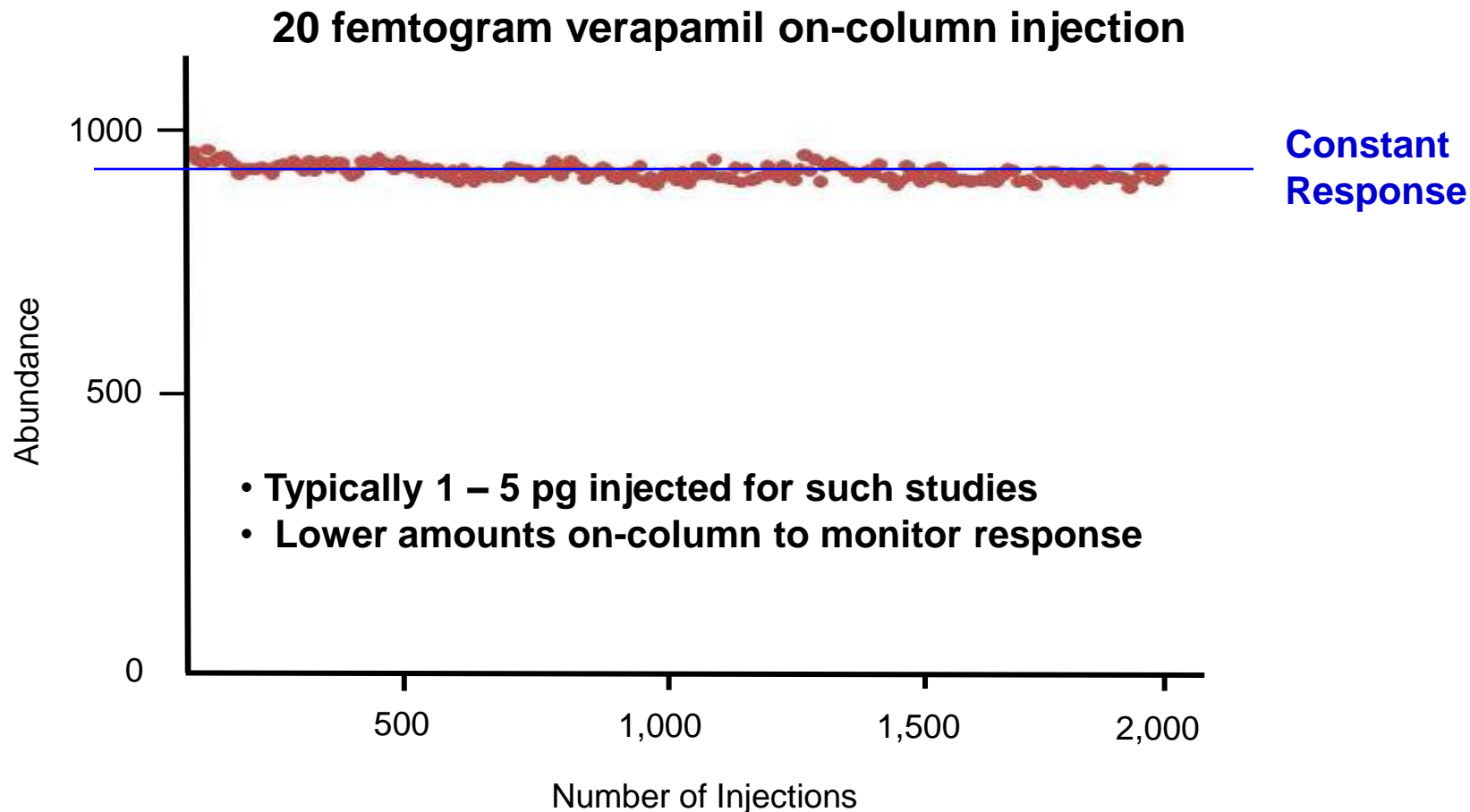
One zeptomole (zmol) contains 602 atoms.

Dynamic Range: 6 Orders of Linearity with 6490

100 Attogram to 100 Picogram verapamil on column



6490 Performance: For Over 2,000 Samples Protein Precipitated Plasma over Four Days with 5% RSDs



Standard Flow LC/MS on the 6490: Quantitation of SIS Peptides in Plasma

- Objectives:
 - To demonstrate the performance of the Agilent 6490 QQQ mass spectrometer for the analysis of 12 synthetic peptides in un-depleted human plasma digest
- Specifically:
 - MRM sensitivity
 - Linearity and range
 - Accuracy
 - Reproducibility
 - Precision

These samples were kindly provided by Derek Smith and Christoph H. Borchers from the UVic-Genome BC Proteomics Centre

Matrix Comparison: Plasma Digest 25 μg vs. 2.5 μg

Plasminogen: LFLEPTR

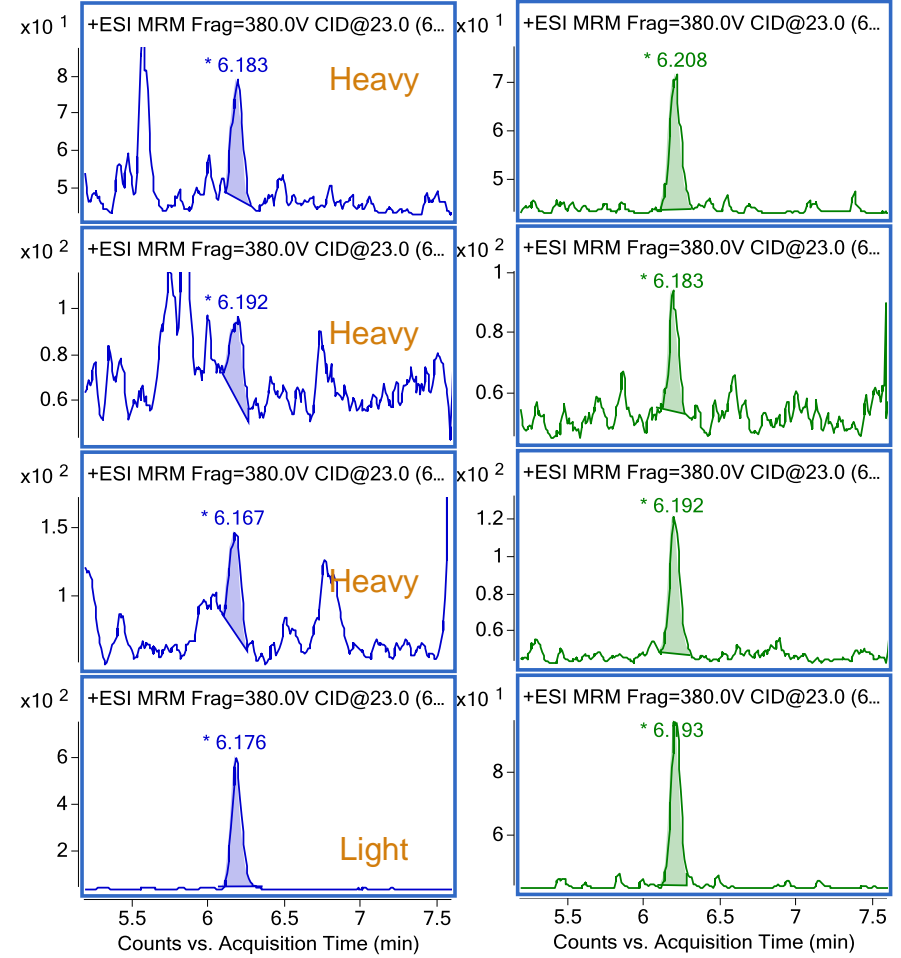
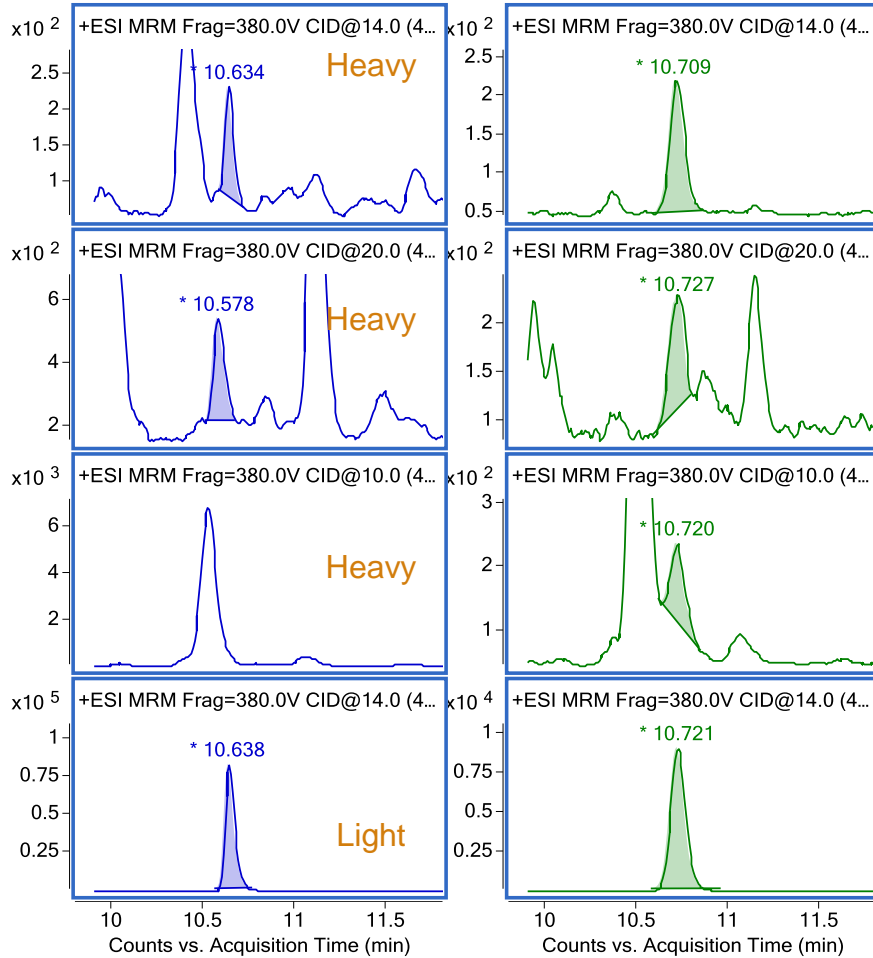
von Willebrand Factor: ILAGPAGDSNVVK

25 μg

2.5 μg

25 μg

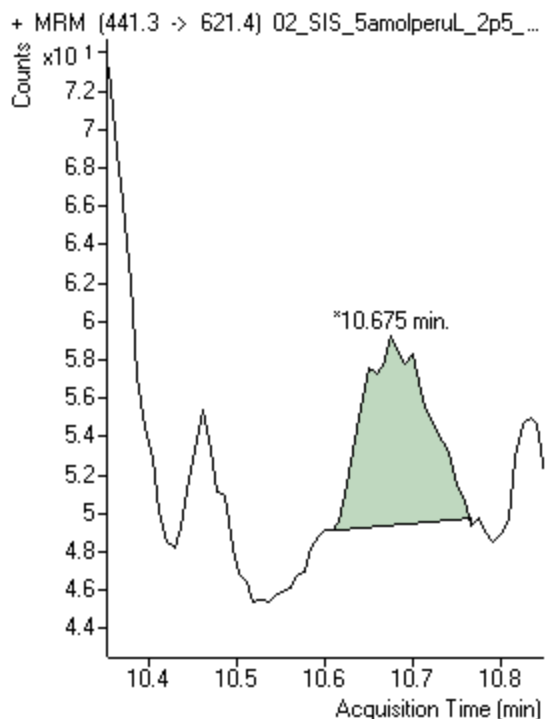
2.5 μg



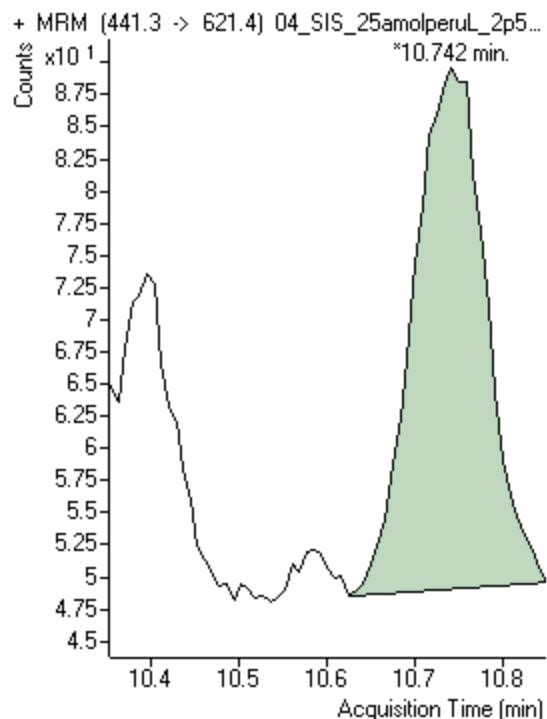
These samples were kindly provided by Derek Smith and Christoph H. Borchers from the UVic-Genome BC Proteomics Centre

Standard Flow LC/MS on the 6490: Plasminogen Peptide in 2.5 μ g of Plasma Digest Matrix

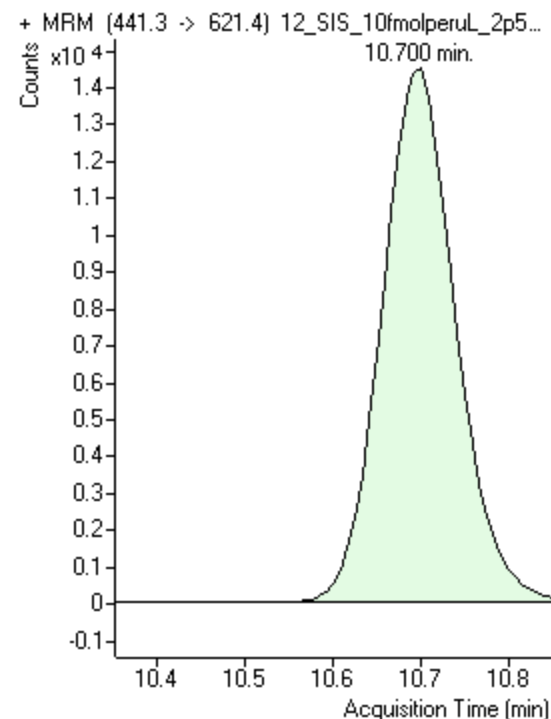
50 amol on-column



250 amol on-column



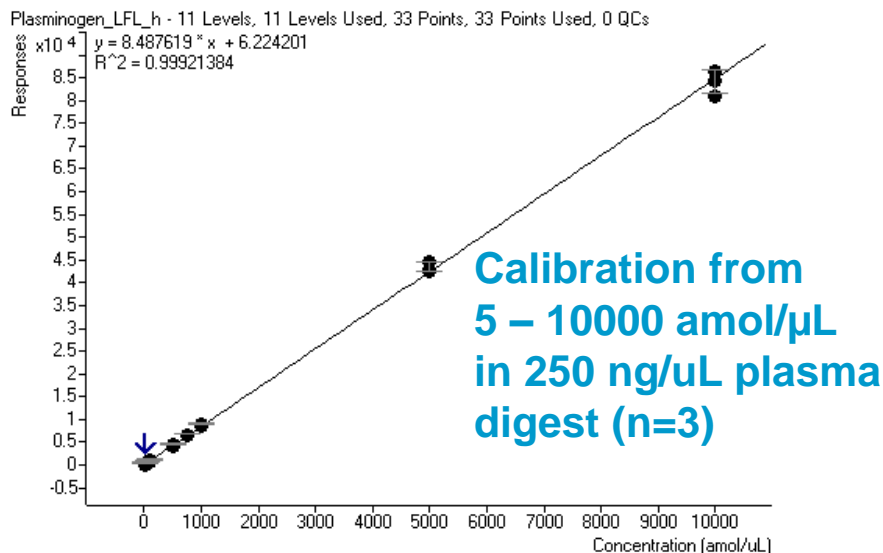
10 fmol on-column



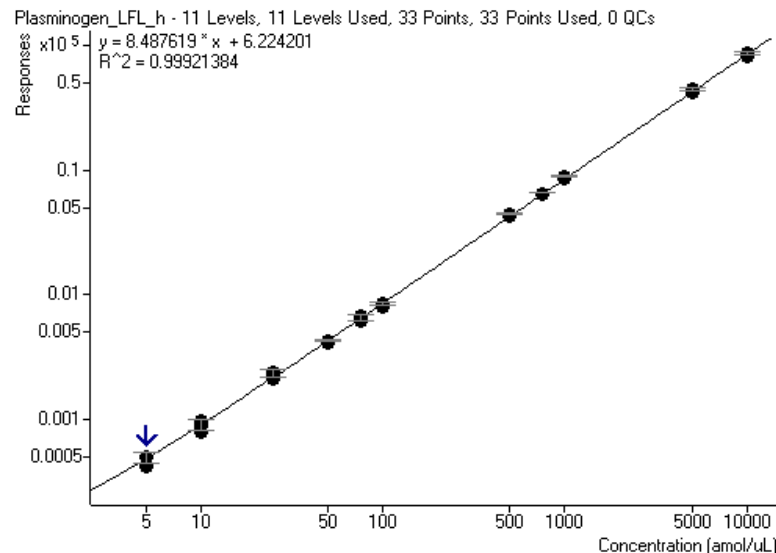
These samples were kindly provided by Derek Smith and Christoph H. Borchers from the UVic-Genome BC Proteomics Centre

Standard Flow LC/MS on the 6490: Quantitation of the Plasminogen Peptide in Plasma

Linear plot



Log-Log plot

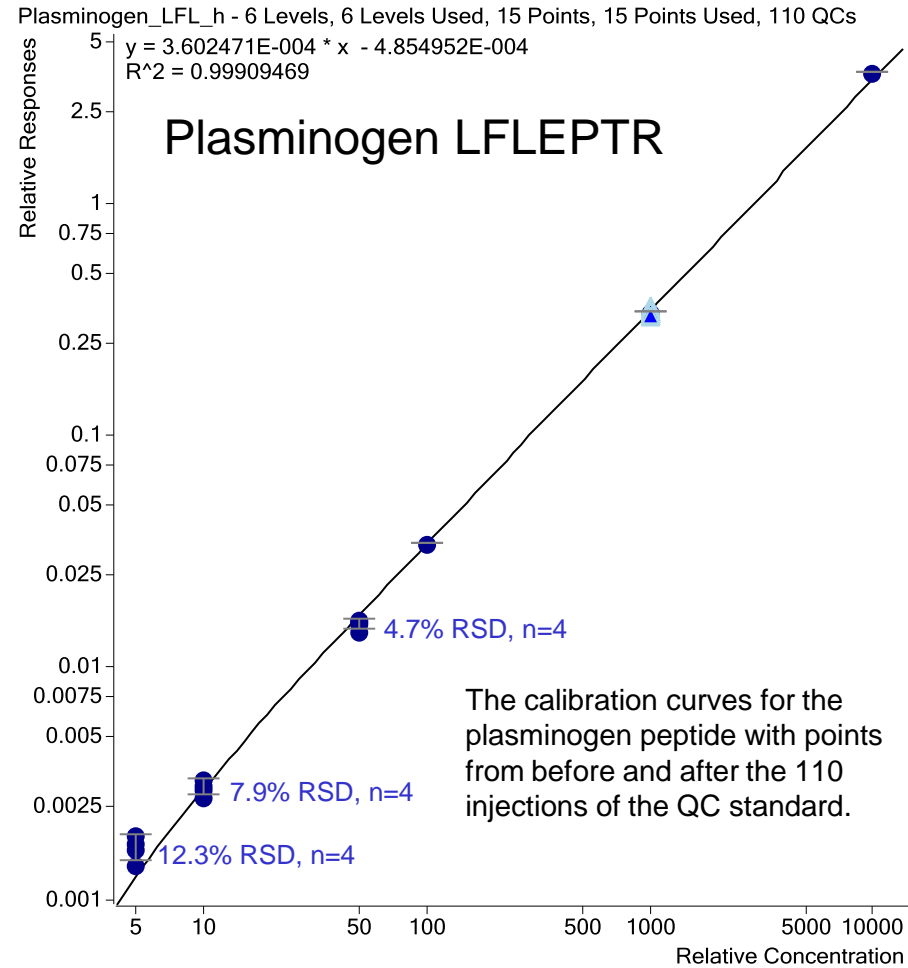


441.3>621.4	ESTD Calibration standards (amol/uL)										
	5	10	25	50	75	100	500	750	1000	5000	10000
%Accuracy (average, n =3)	98.2	97.5	104.6	98.1	99.2	97.4	101.3	101.8	101.3	101.8	98.8
Reproducibility (%RSD, n = 3)	11.85	10.78	7.21	1.17	6.22	2.74	1.41	0.48	1.52	2.58	3.24
Response factor	8.33	8.28	8.87	8.32	8.42	8.27	8.60	8.64	8.60	8.64	8.39
Precision (%RSD, n = 11)	2.30										

The samples were provided by Derek Smith and Christoph H. Borchers from the UVic-Genome BC Proteomics Centre

Reproducibility for 110 Injections (10 fmol SIS Peptides and 2.5 µg Plasma Digest On-column)

Protein	Response %RSD	Ret. Time %RSD
Adiponectin: IFYNQNHYDGSTGK	9.8	0.13
Antithrombin-III : DDLIVSDAFHK	4.7	0.16
Apolipoprotein A-II precursor: SPELQAEAK	6.7	0.12
Apolipoprotein C-III: GWVTDGFSSLK	2.3	0.08
Ceruloplasmin : EYTDASFTNR	9.6	0.14
Heparin cofactor II: TLEAQLTPR	6.1	0.15
Histidine-rich glycoprotein: DGYLFQLLR	3.4	0.02
Kininogen-1: TVGSDTFYSEK	3.3	0.13
L-selectin: AEIEYLEK	9.5	0.15
Plasminogen: LFLEPTR	2.2	0.13
Vitamin D-binding protein: THLPEVFLSK	3.0	0.12
von Willebrand Factor: ILAGPAGDSNVVK	9.5	0.15



The samples were provided by Derek Smith and Christoph H. Borchers from the UVic-Genome BC Proteomics Centre

Conclusions From Preliminary Studies

- The 6490 iFunnel system shows at least a 5x improvement in sensitivity compared to the 6460
- With the AJS source on the 6490 iFunnel system, there was no significant gain in sensitivity from going to smaller column id (lower flows)
- HPLC-Chip/MS on the 6490 shows that nanoflow is still significantly more sensitive than cap or standard flow.
- The 6490 iFunnel system demonstrated robust performance for peptide quantitation in plasma digests using standard flow LC/MS

Acknowledgements

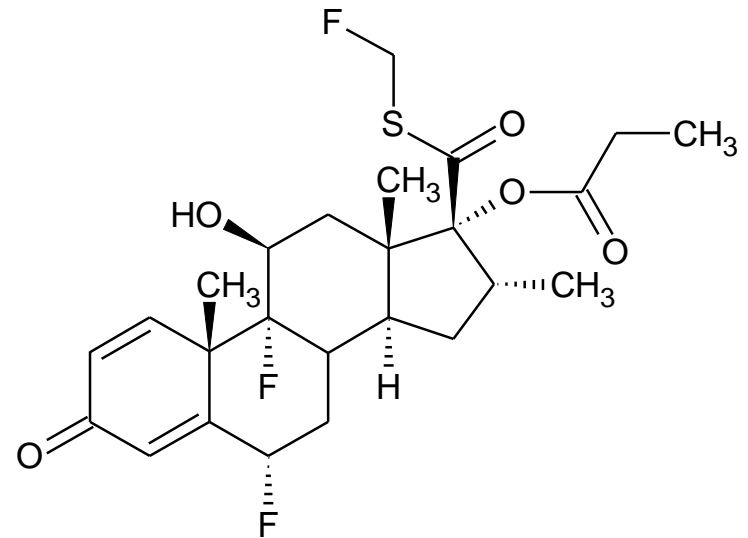
Agilent

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Quantitation of fluticasone propionate in Human Plasma Using the 1290 LC and 6490 Triple Quadrupole



Inhaled glucocorticoids for asthma control are efficiently metabolized in the liver after oral administration resulting in very low plasma levels.

LC/MS Analysis of Low Levels of Fluticasone

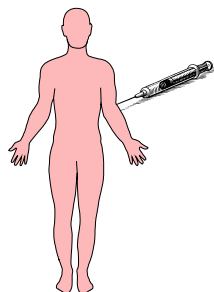
- LLOQ in plasma should be 5 -10 pg/mL
- Speed (high sample throughput): short LC/MS run times
- Chromatographic separation with good resolution
 - narrow peak widths
 - reduced ion suppression effects
- Precise and accurate quantitation
- Fast sample preparation

The Dosing, Sample Prep and Analysis Workflow

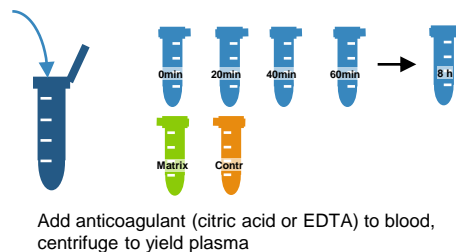
1 Drug Application



2 Blood sampling after certain time points



3 Blood workup to yield plasma samples



Add anticoagulant (citric acid or EDTA) to blood, centrifuge to yield plasma

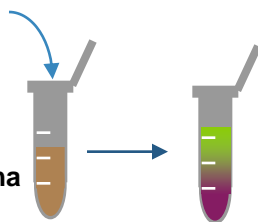
4 Calibration samples in blank plasma



5 Protein Precipitation

300 μ L Acetonitrile

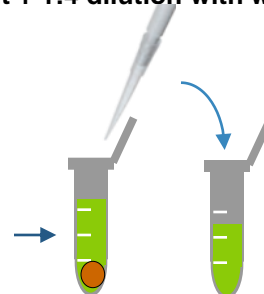
100 μ L Plasma Sample



6 Centrifugation and transfer of supernatant + 1:4 dilution with water



15min @
13200 rpm



7 UHPLC-MS/MS Analysis



6490 Linearity, Precision, Accuracy

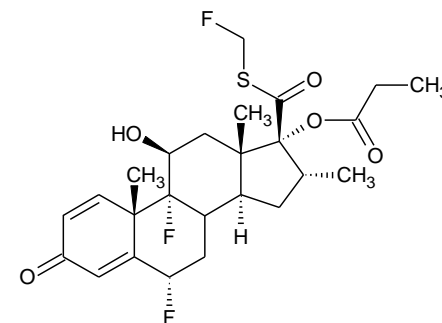
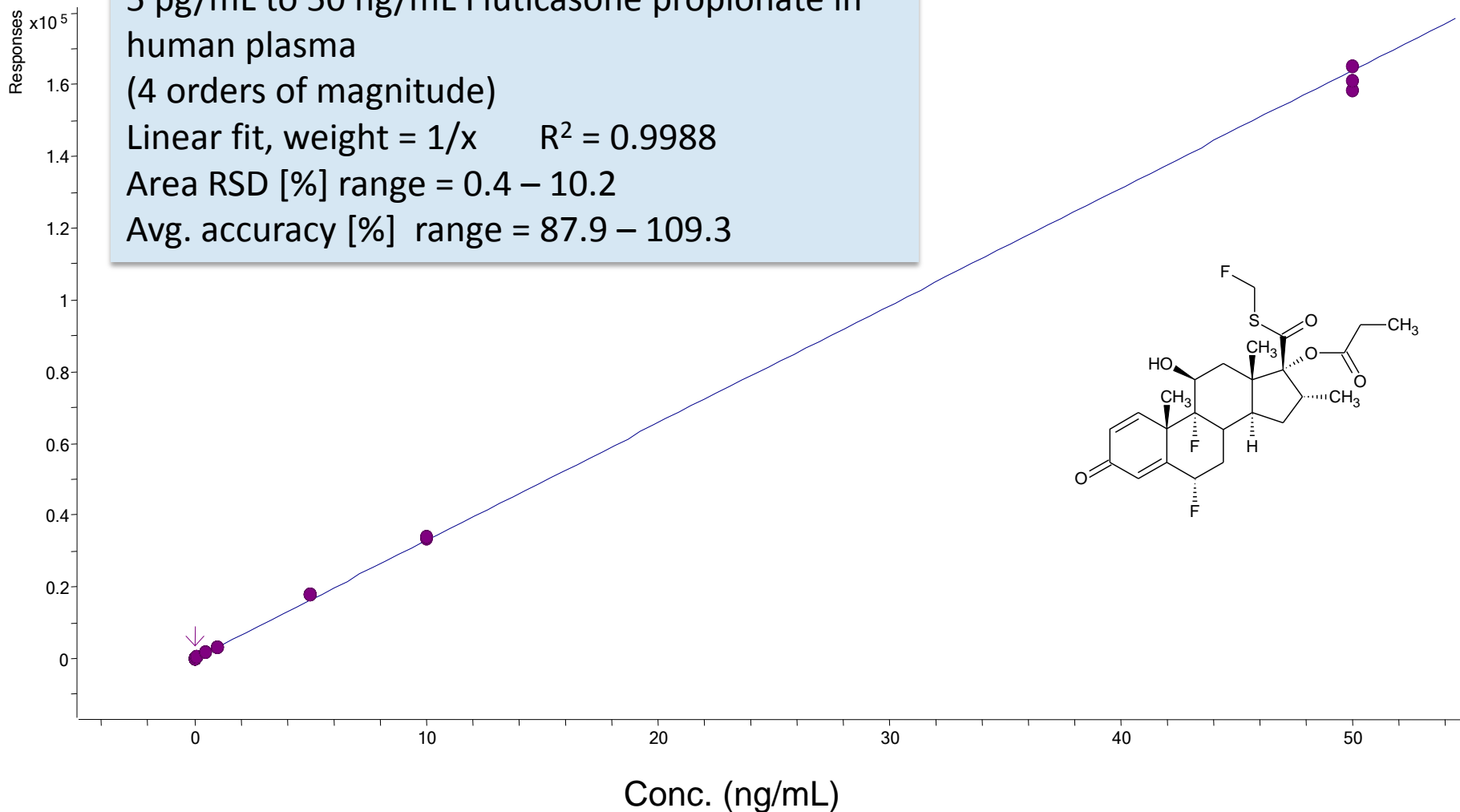
5 pg/mL to 50 ng/mL Fluticasone propionate in human plasma

(4 orders of magnitude)

Linear fit, weight = $1/x$ $R^2 = 0.9988$

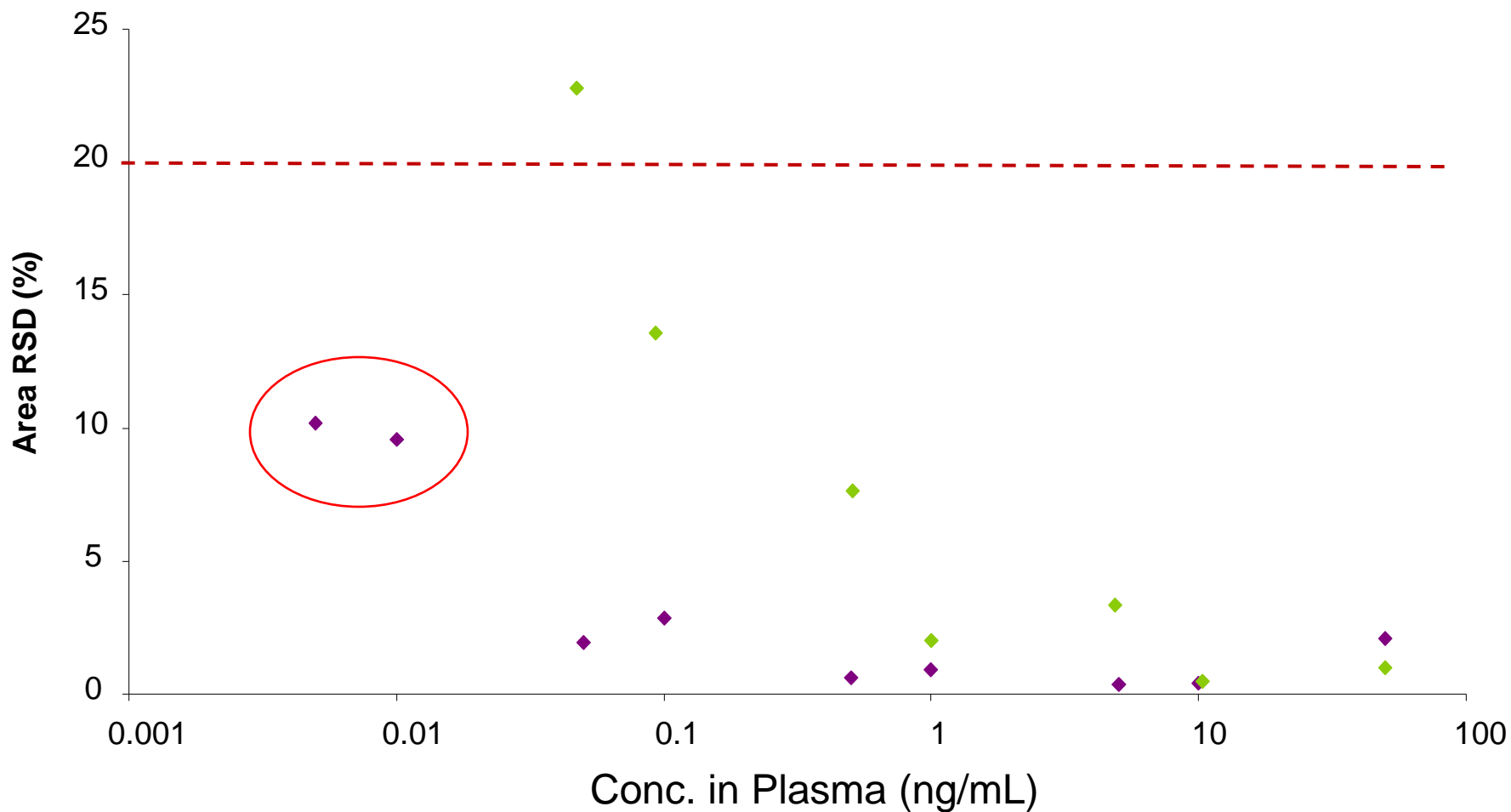
Area RSD [%] range = 0.4 – 10.2

Avg. accuracy [%] range = 87.9 – 109.3

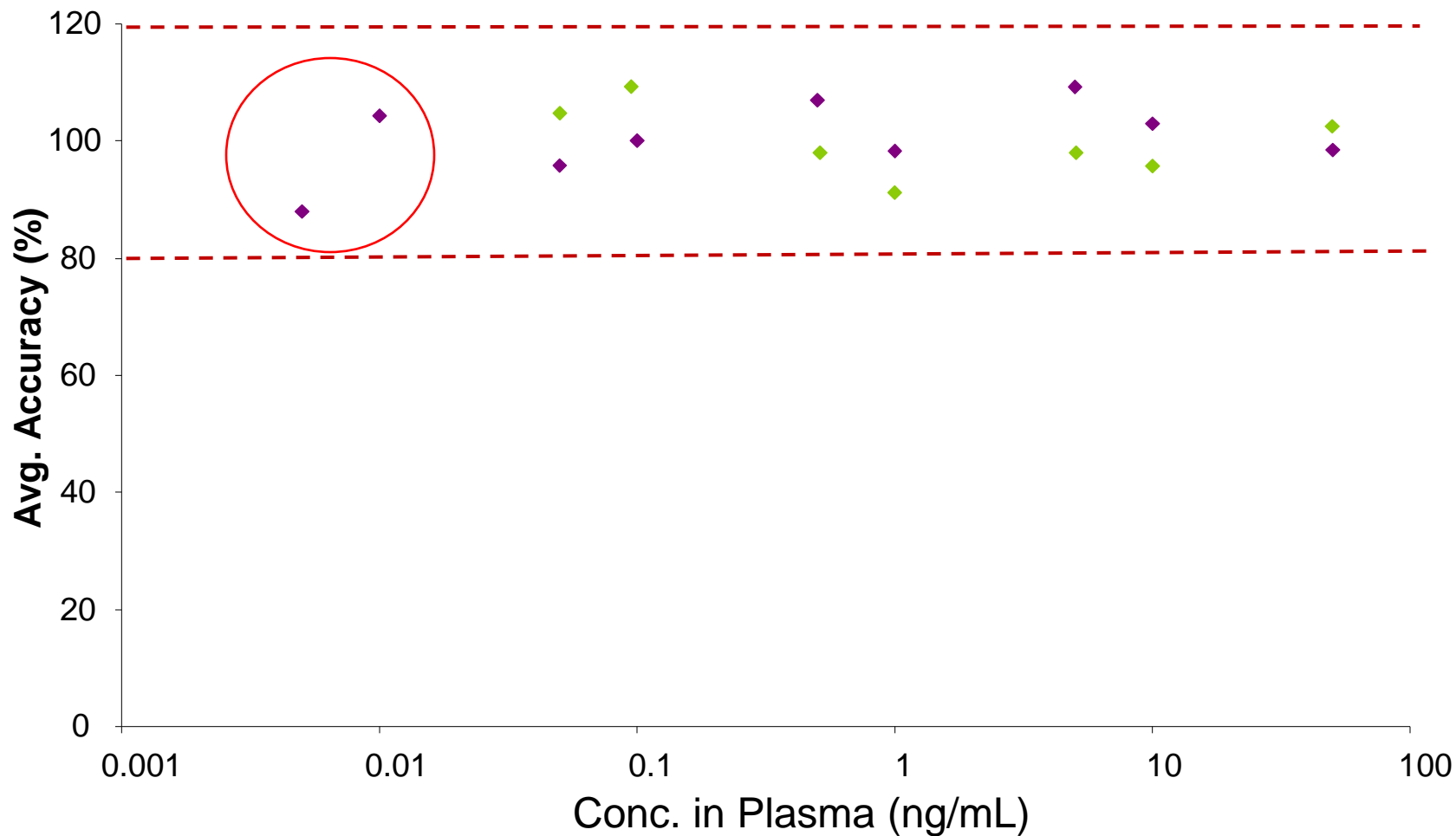


Fluticasone: Precision (6490 vs. 6460 QQQ)

Area RSD [%]: 0.005 ng/mL to 50 ng/mL in human plasma

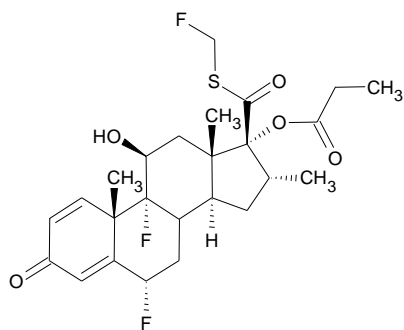


Fluticasone: Accuracy [%] (6490 vs. 6460 QQQ) 0.005 ng/mL to 50 ng/mL in human plasma



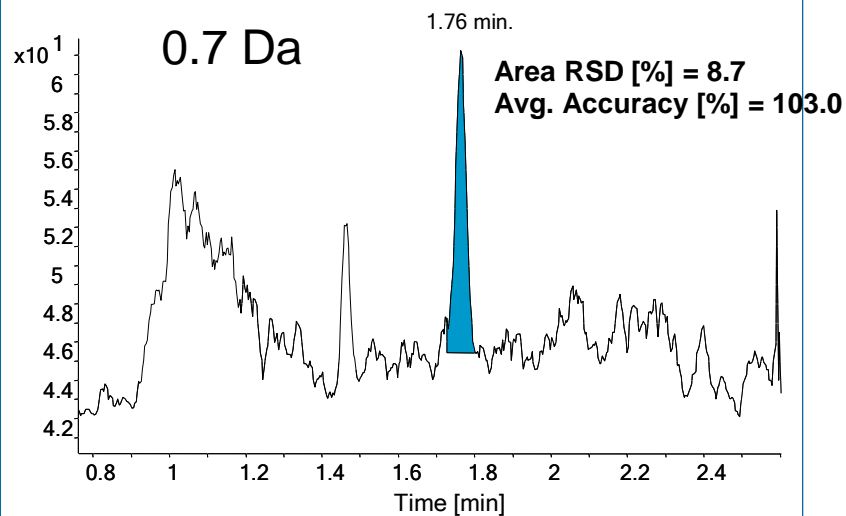
LLOQ: Fluticasone

- Assay LLOQ 5 pg/mL
- 2.5 fg on column
- 5 replicates
- Comparable Accuracy & RSDs



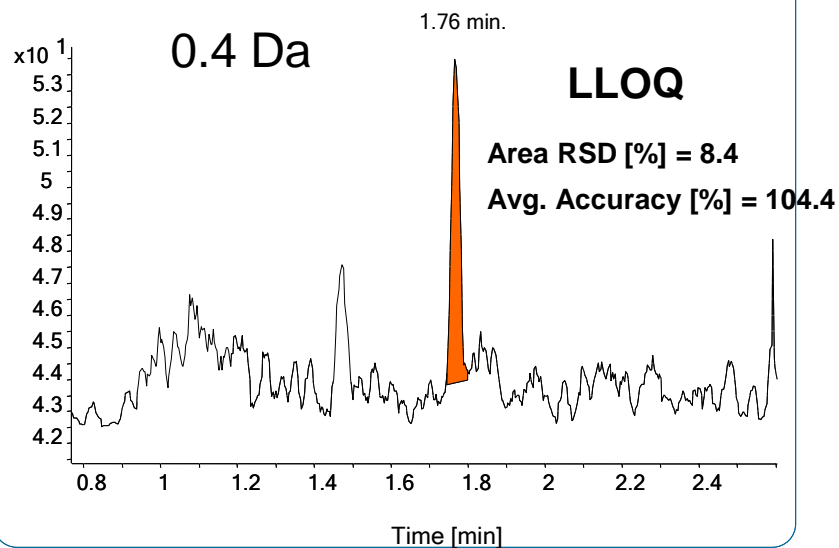
Unit/Unit resolution

LLOQ



Enhanced/Unit resolution

LLOQ



Summary: 6490 Fluticasone Propionate LC/MS

- Sensitivity of the 6490 triple quadrupole has allowed streamlining sample preparation by use of protein precipitation (instead of SPE) and matrix dilution
- LLOQ at 5 pg/mL, corresponding to 2.5 fg injected on column
- Enhanced/unit resolution gave better S/N due to the decrease in noise level.
- Assay performance within accepted guidelines
- Robust iFunnel operation

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