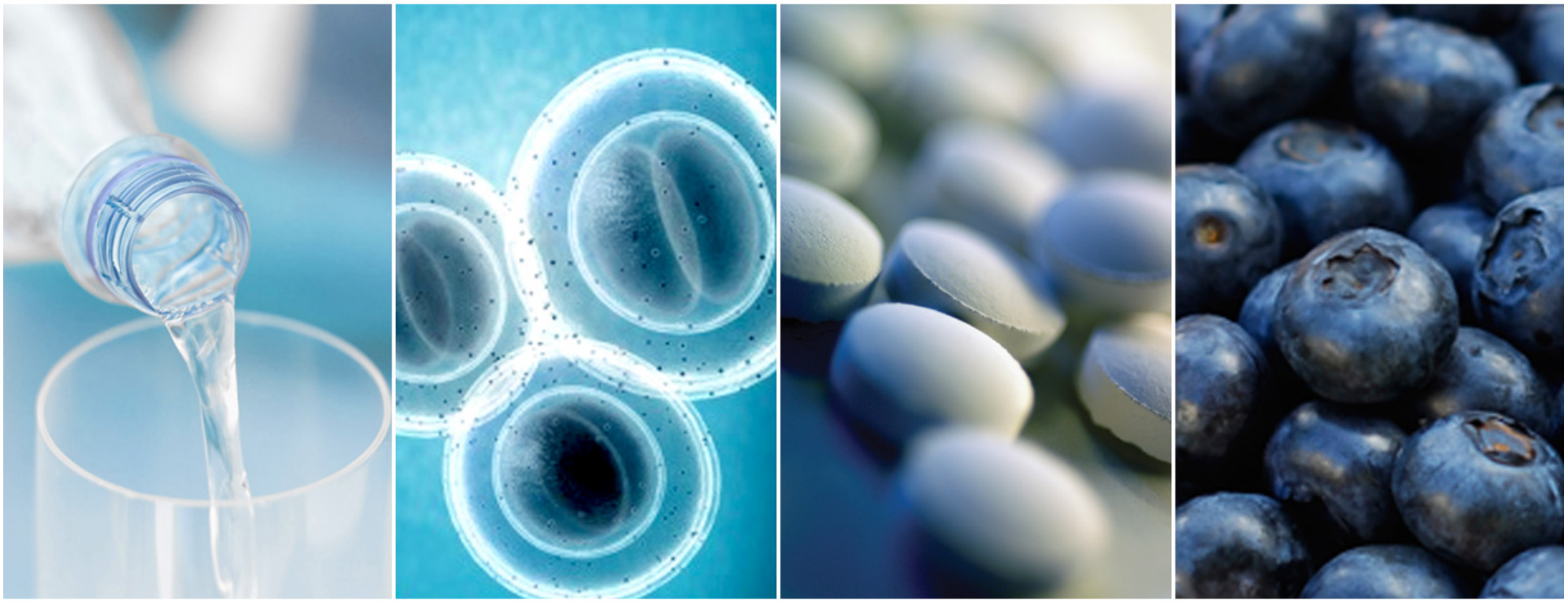


IonDrive Technology: A Diversified Approach to Peptide Quantitation

Steve Taylor

AB SCIEX

Agenda

- Achieving fg/mL LOQ's in bioanalysis with the new 6500 series
- IonDrive™ technology innovations enabling unprecedented sensitivity and ruggedness
- Bioanalytical assay examples
  - Precision at LOQ
  - Linear range
  - Robustness
- Benefits of combined sensitivity, selectivity, and dynamic range gains

Achieving LOQ's in the fg/mL Range

The Challenge

- Inhalation, topical, and ophthalmological drugs
  - Very low plasma levels (C_{max} ≤ 1 pg/mL)
- Non-traditional sampling formats
  - Dried blood spots
  - Microdialysis
- Challenging study types
  - Pediatric studies
  - Micro-dosing

A Major Step Forward in LC/MS/MS Sensitivity and Dynamic Range is Required

The New AB SCIEX 6500™ Series with IonDrive™ Technology

- ***IonDrive Technology***
  - More Efficient Ionization
  - Better Ion Capture and Transmission
  - More Sensitive Detection
  - Increased Dynamic Range
- Higher Selectivity with SelexION™ Technology

The New AB SCIEX 6500™ Series with IonDrive™ Technology

- Our most sensitive, selective, and fastest MS for highest performance targeted quantitation
- Up to 10X greater sensitivity
- Improved robustness
- Dynamic range – up to 20X increase
  - Linear count rates to >100x10^6 CPS
- Mass range 5 – 2000 m/z on LIT and quadrupole
  - Novel dual RF frequency power supply implementation
  - maximize low mass performance while increasing mass range
- Fast Scanning MRM^3 – up to 2X faster

IonDrive™ Technology Key Innovations

IonDrive™ Turbo V Source

IonDrive™ QJet Guide

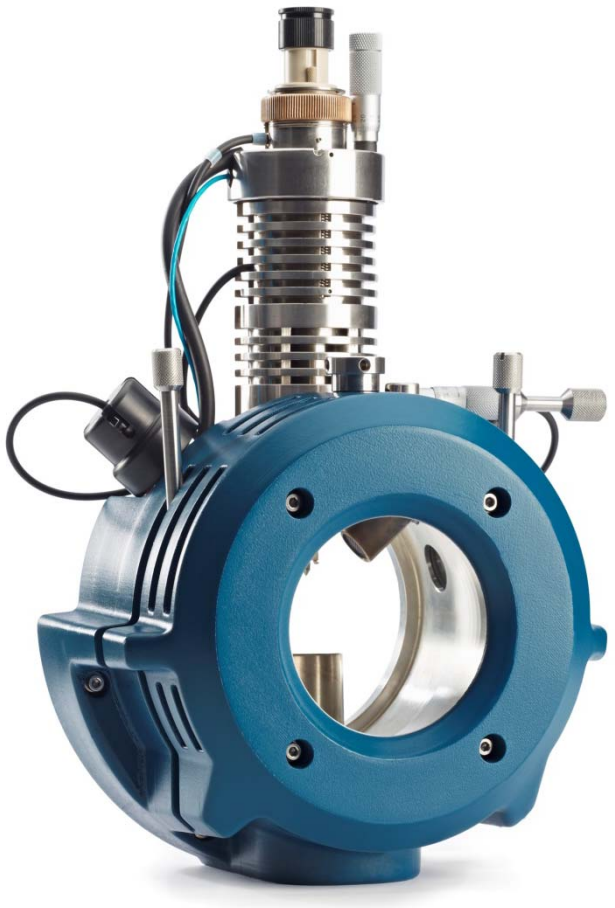
IonDrive™ HE Detector

Driving Performance by Driving More Ions While Maintaining Ruggedness

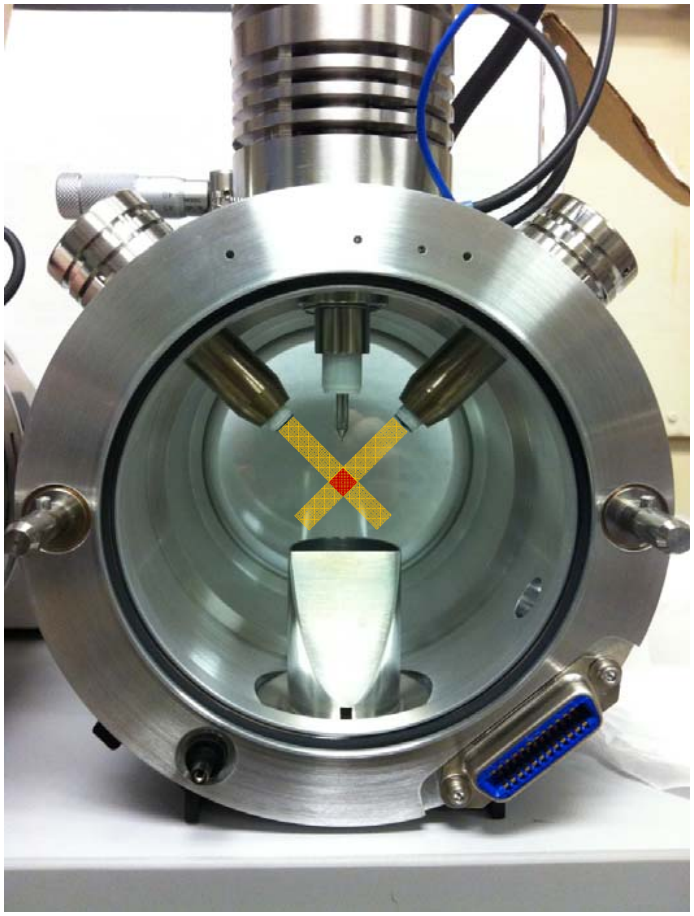
- Better Ionization
- Higher Transmission
- Extended dynamic range

IonDrive™ Turbo V Source – Produce More Ions

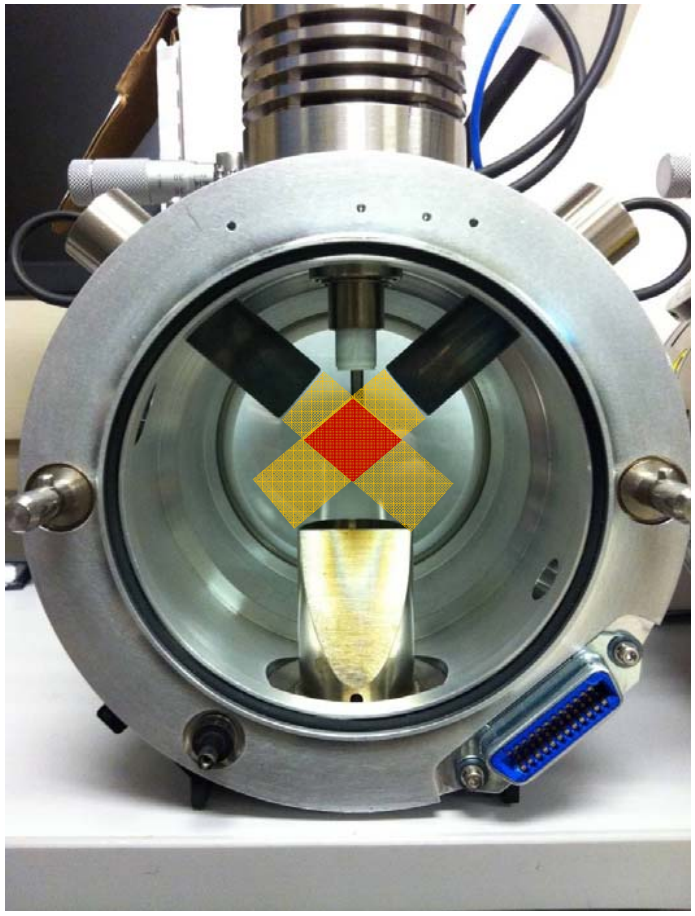
- Larger diameter (11 mm) heaters
- Optimized geometry
- More efficient heat transfer
- Covers a larger cross-section of the spray cone
- Wider “sweet spot” when optimizing probe position
- More robust against fluctuations in gas flow dynamics, and source to source differences



IonDrive™ Turbo V Source – Produce More Ions



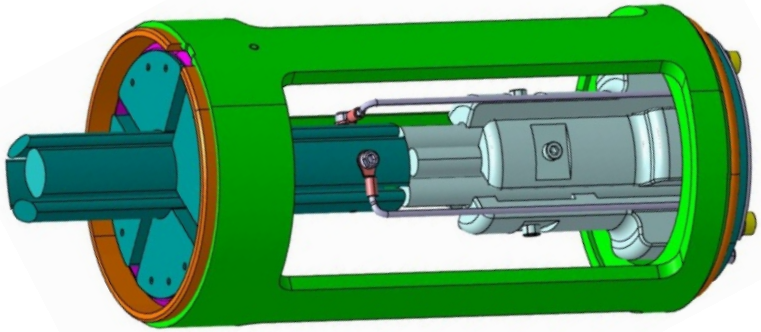
Original Turbo V™



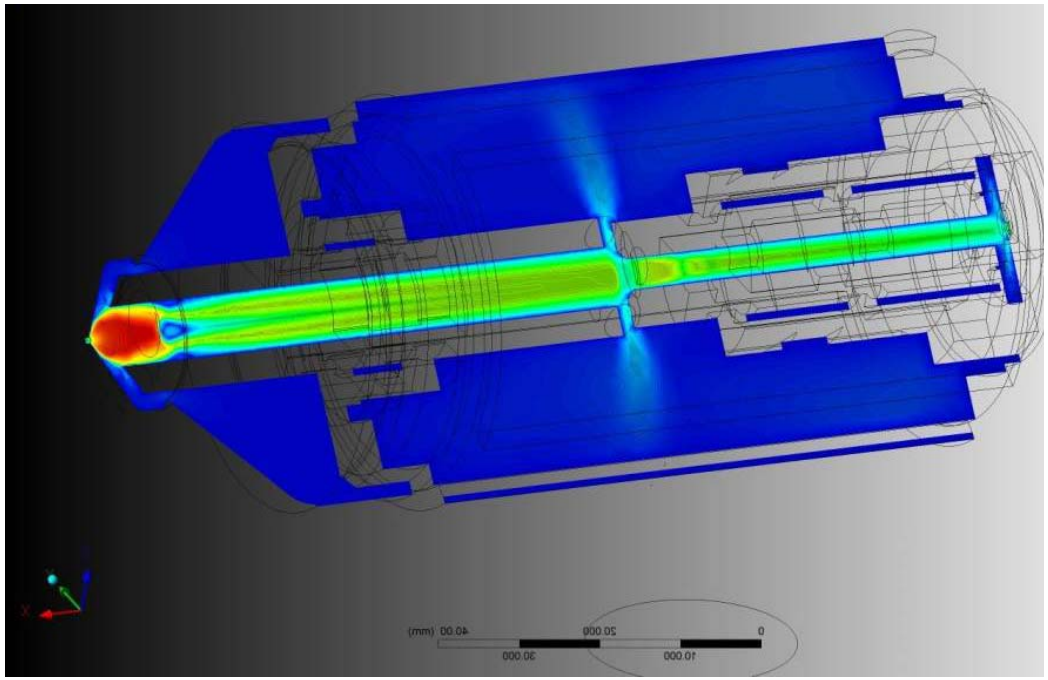
New IonDrive Turbo V

IonDrive™ QJet Ion Guide – A Dual Stage RF Ion Guide

- 1st Stage: Better ion capture
- 2nd Stage: Better ion transfer to Q0 region

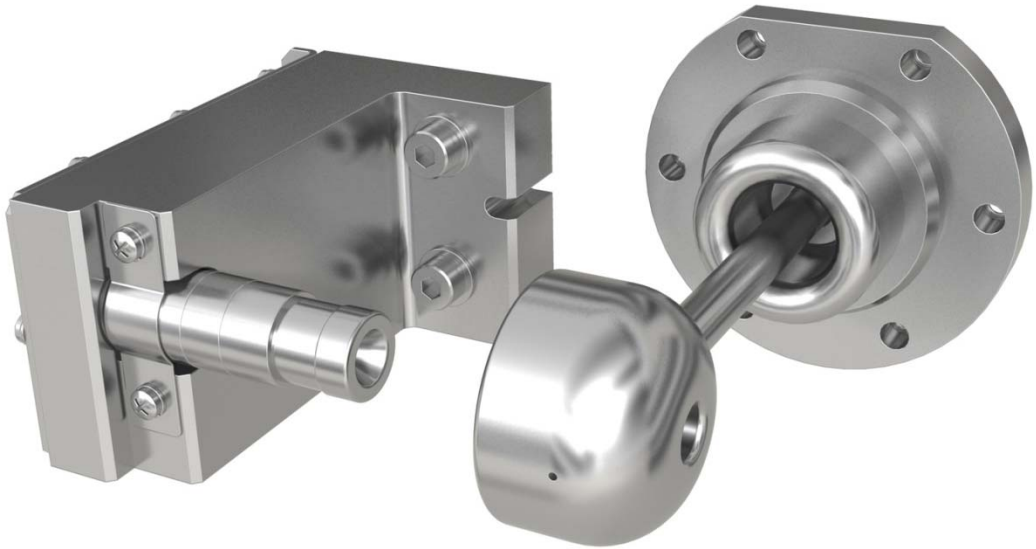


IonDrive™ QJet Ion Guide – A Dual Stage RF Ion Guide



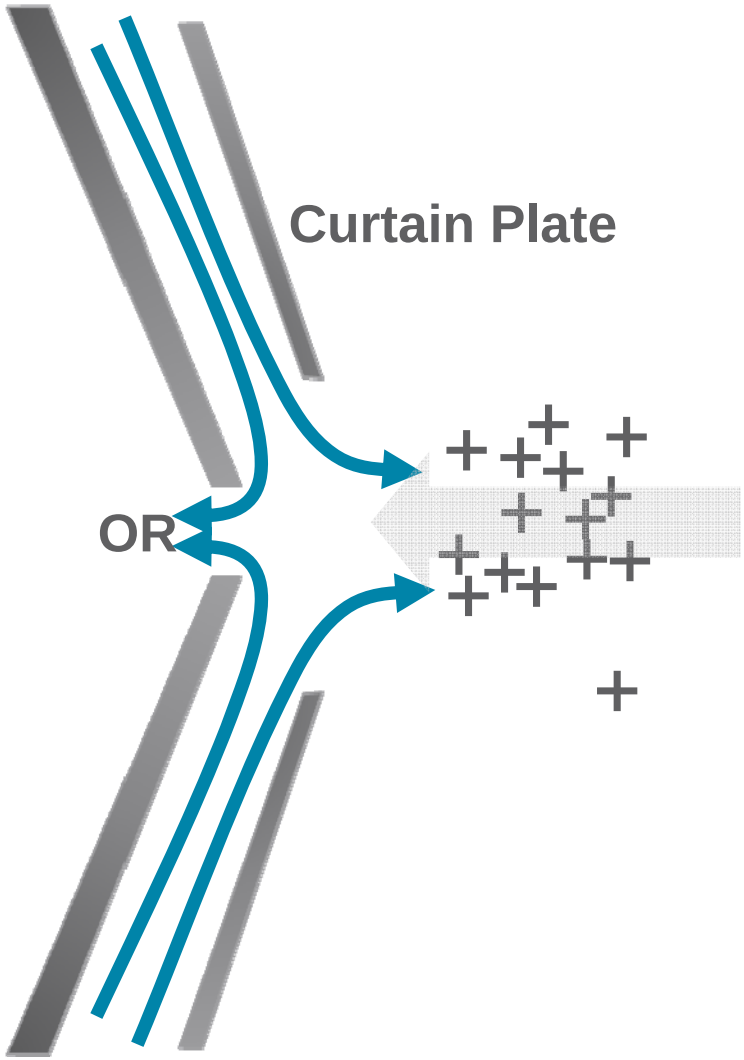
- Computed gas flow model showing flow through the ion guide
- Larger diameter of first stage → Better ion capture
- Smaller diameter of second stage → Better focusing into Q0
- End Result: More ions transmitted, fewer neutrals reaching Q0

IonDrive™ High Energy Detector



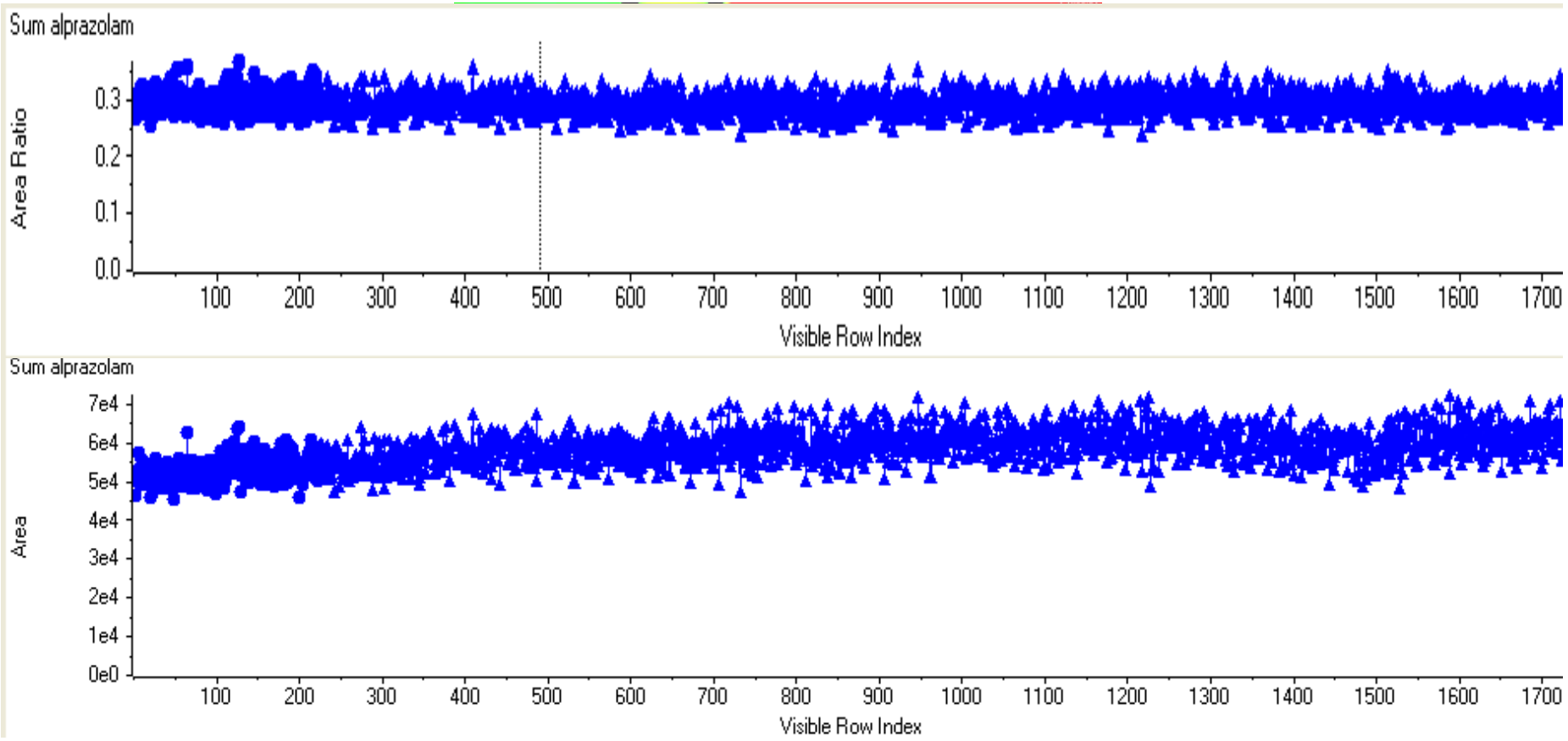
- Sensitivity gains are not at the expense of dynamic range
- Ultra-fast pulse counting – 10^8 cps
  - Up to 20x improvement
- Higher saturation point
- Pulse counting ensures low end sensitivity is not compromised

Curtain Gas™ interface is the best protection against contamination



Curtain Gas™ interface is the best protection against contamination

Alprazolam – 1700 crashed plasma samples with 1 minute gradient



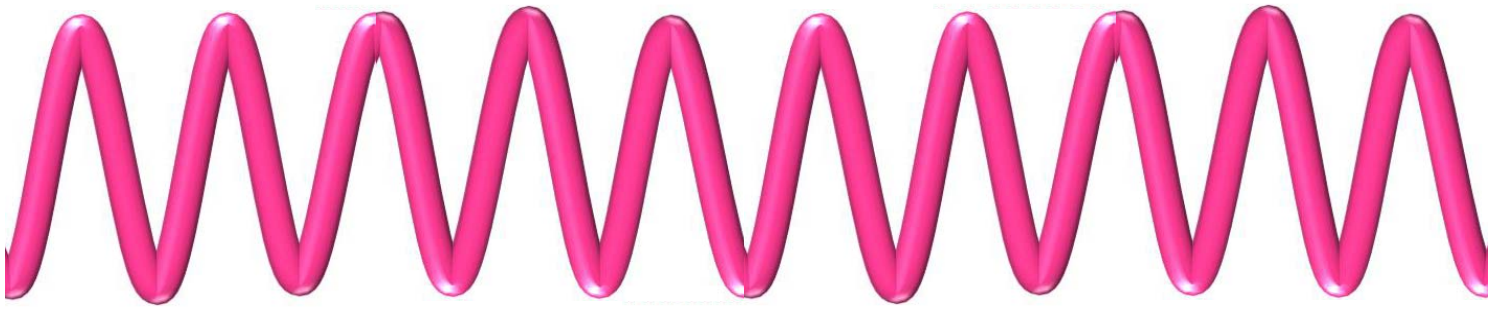
New Technology for Increased Mass Range

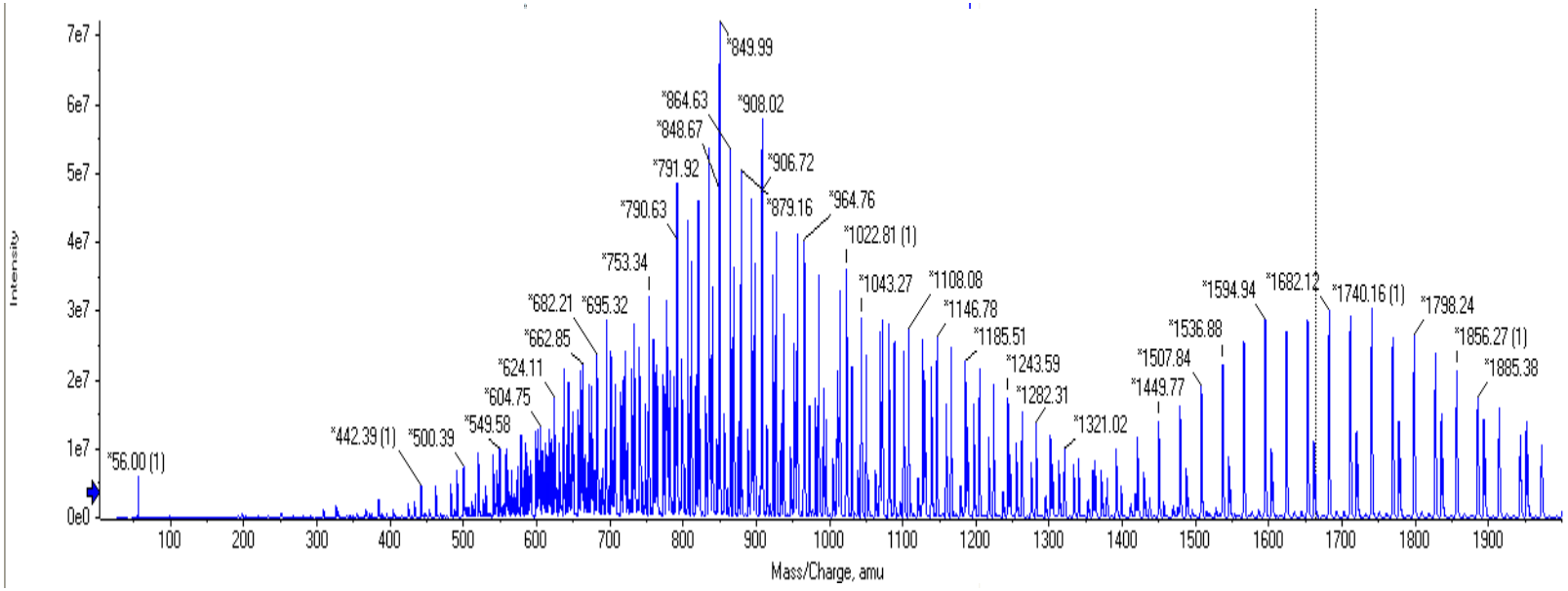
Novel Dual Frequency RF Drive

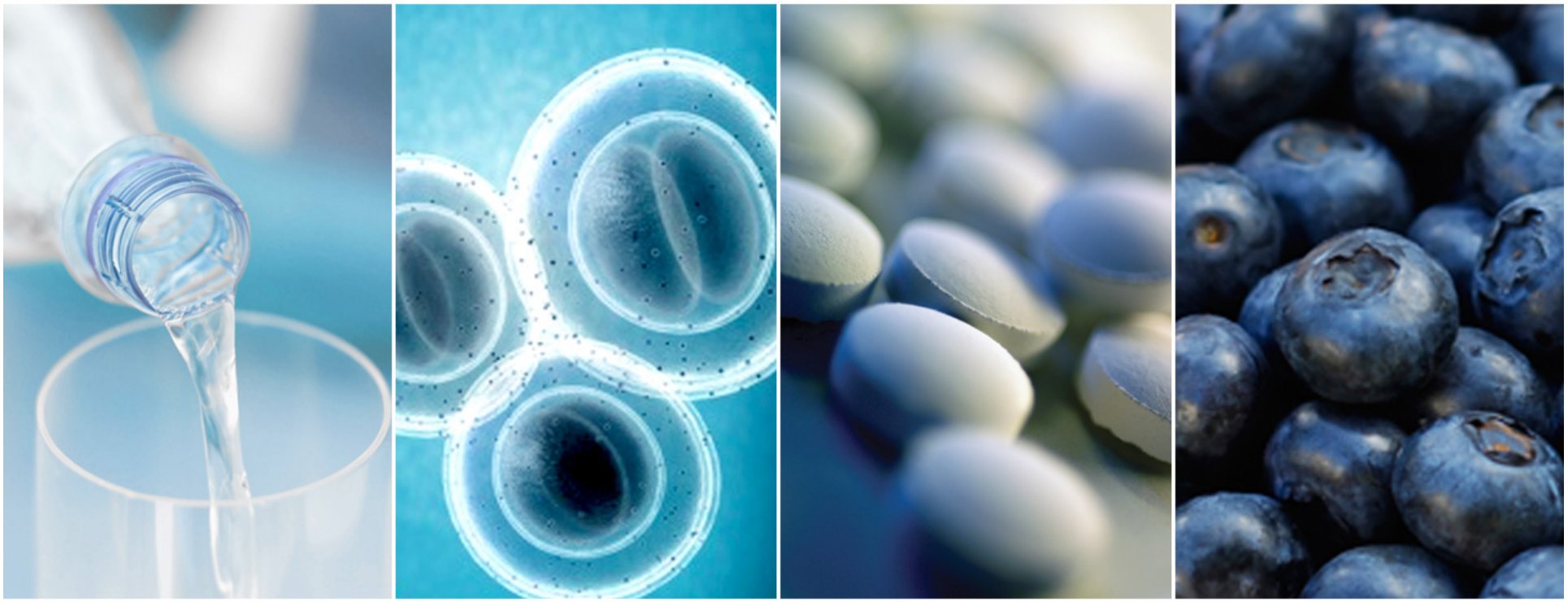
- No compromise between mass range and low mass sensitivity
- Switch between high mass to 2000 m/z and low mass to ~1250m/z
  - Switch made via software
- Allows beneficial transmission properties of high RF frequency to be used effectively while allowing a broader mass range on a single system

New Technology for Increased Mass Range

Two Software Selectable Mass Ranges



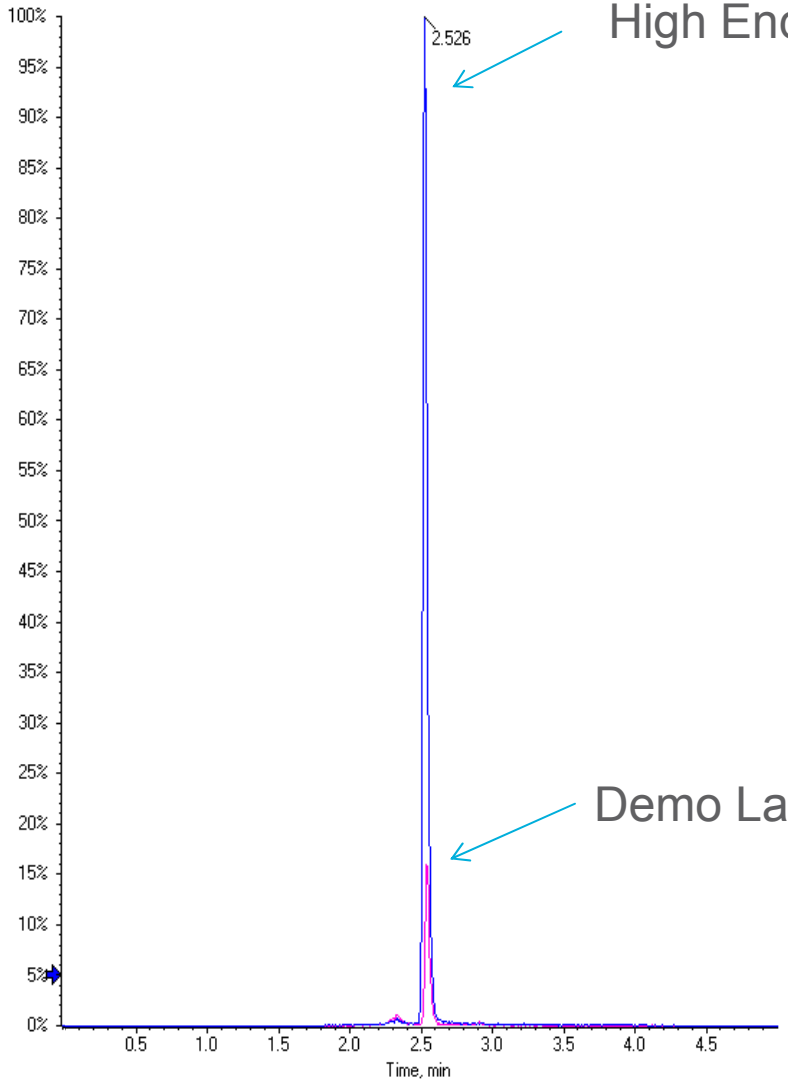




Applications Examples with the 6500 Series Instruments

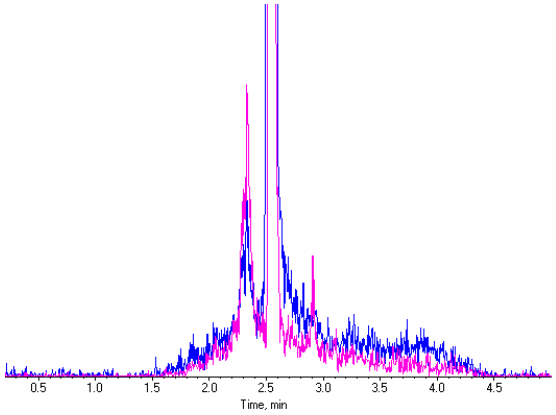
AB SCIEX

Actual MRM Sensitivity Gains - Verapamil



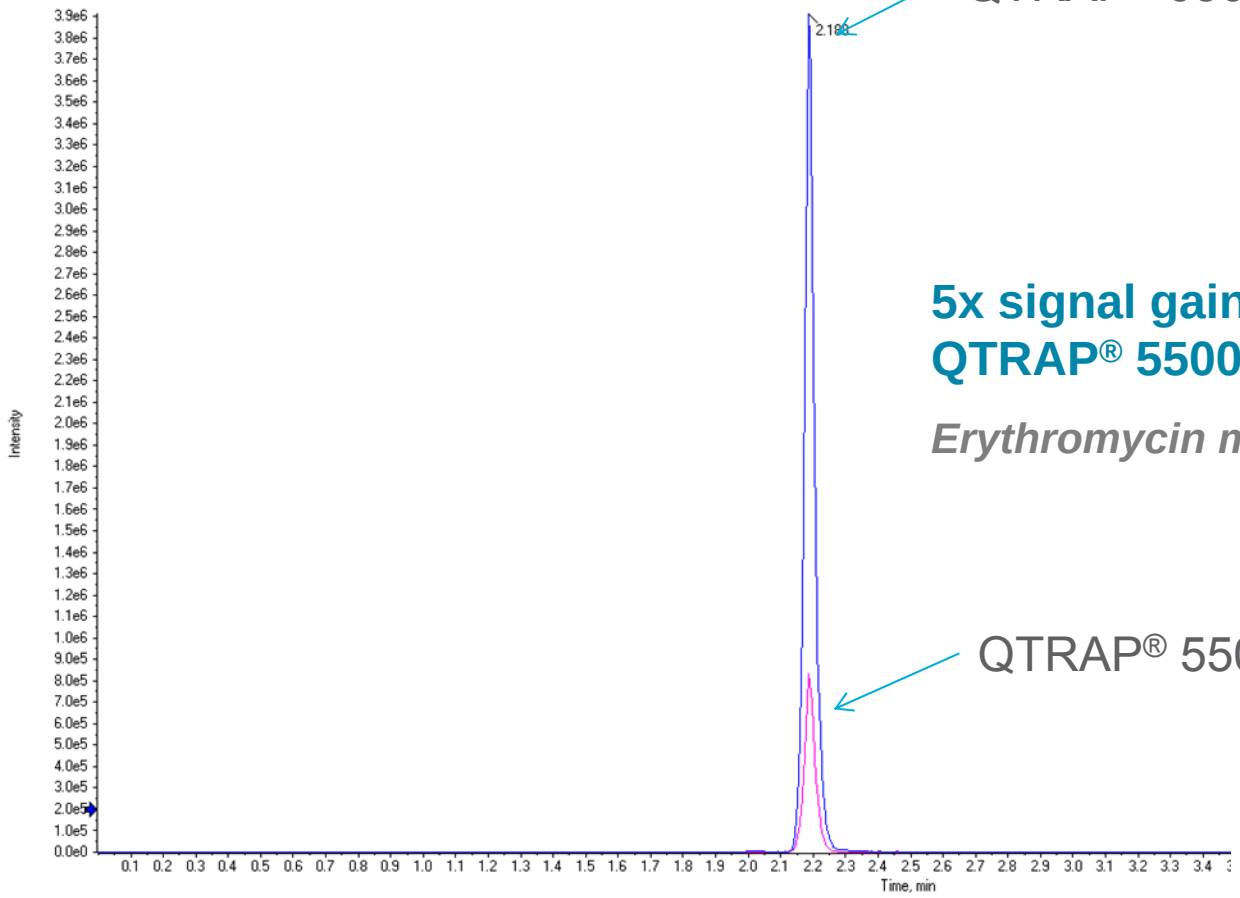
High End Triple/QTRAP® Prototype

6x signal gain >4x S/N gain vs. QTRAP® 5500 system



Demo Lab QTRAP® 5500 system

Actual MRM Sensitivity Gains - Erythromycin



Sensitivity Gains

Alprazolam and Verapamil in Rat Plasma

- Experiment Design
  - Direct back to back comparison with a QTRAP® 5500 system
  - Same samples, LC stack, column, mobile phases
- Sample Prep
  - Protein precipitation with 2:1 acetonitrile
  - Supernatant diluted 2:1 with water (Verapamil) and 3:1 (Alprazolam)
  - Injection volume: 1 µL
- HPLC
  - Shimadzu UFLC-XR
  - Kinetix C-18 column, 2.1 x 50 (2.6 µm)
  - ACN / Water / 0.1% formic acid with 2 mM ammonium formate
  - Flow rate: 0.8 mL/min
  - Total run time: 2 minutes

Instrument Conditions

- IonDrive™ Turbo V Conditions
  - Optimization performed using standard t-infusion and flow injections
  - CUR = 25, GS1 = 75, GS2 = 80
  - TEM = 650˚C, IS = 1800 V
- MS/MS Conditions
  - Q1 / Q3 Resolution: Unit / Unit

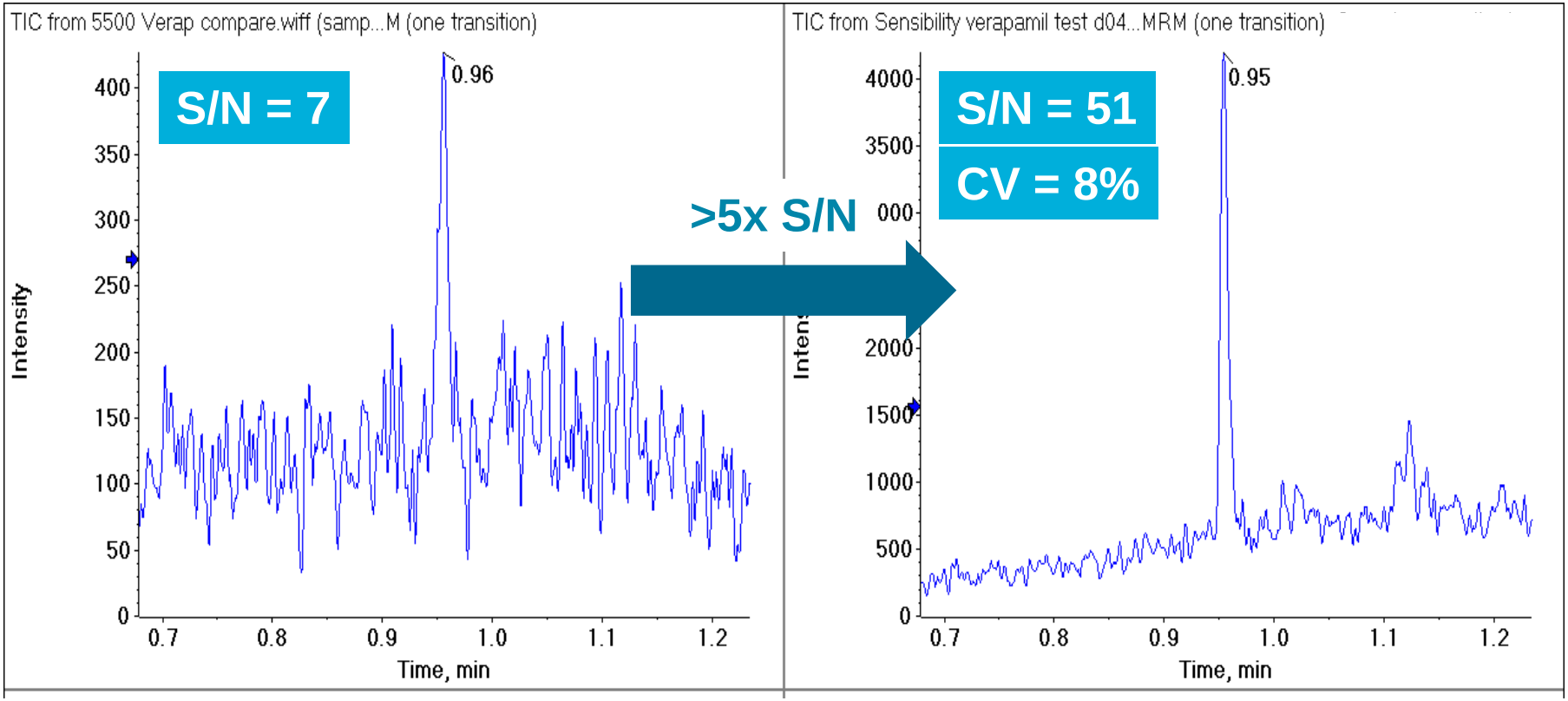
Name	Q1	Q3	Dwell (ms)	DP	CE	CXP
Alprazolam 205	309.1	205.1	20	80	55	10
Alprazolam 281	309.1	281.1	20	80	36	11
D5 Alprazolam	314.1	210.1	20	80	55	10
Verapamil	455.3	165.1	60	79	36	18

Verapamil Neat STD – Sensitivity Gains

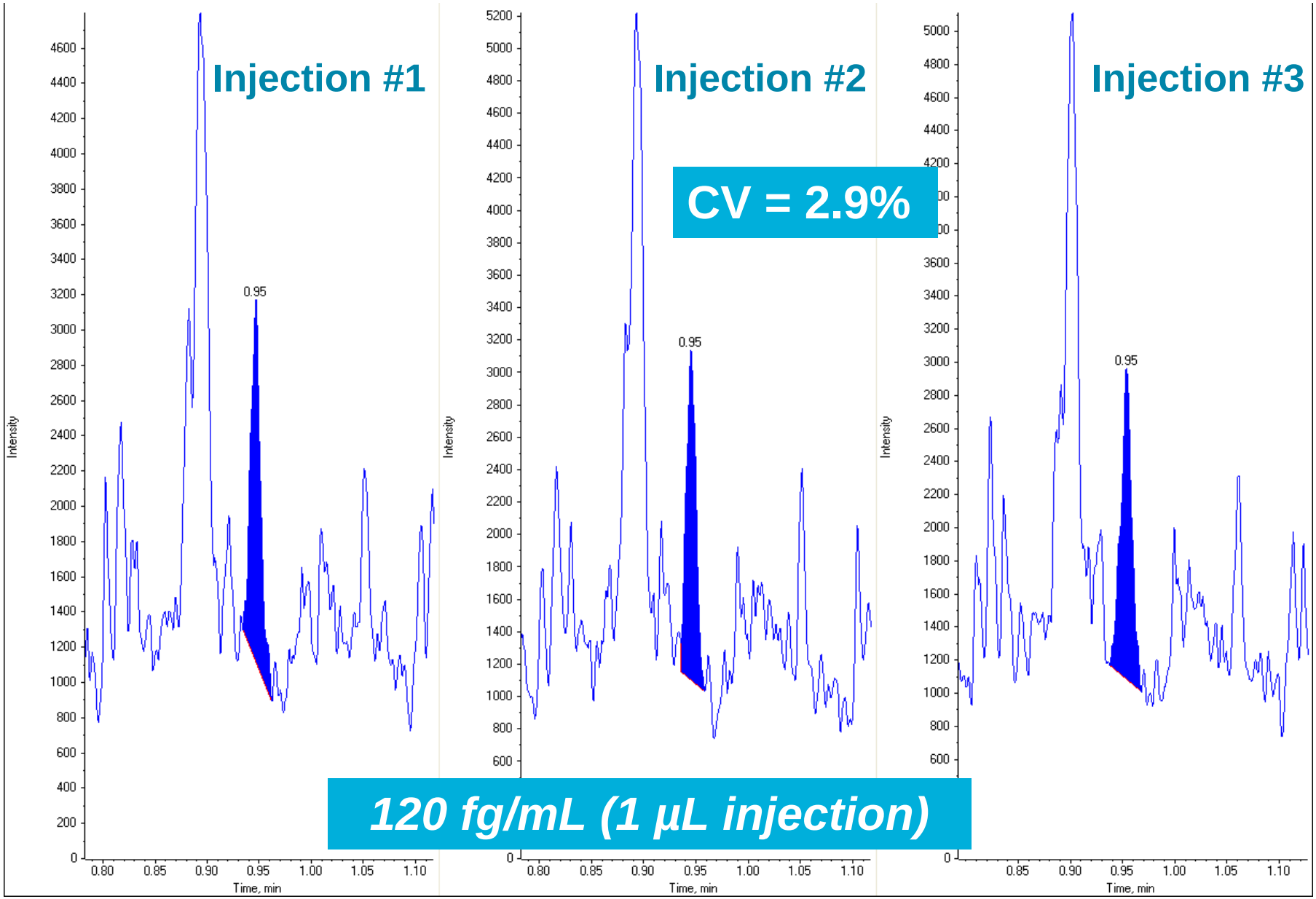
500 ag On Column

QTRAP® 5500 System

QTRAP® 6500 System



Verapamil in Plasma – 120 Attograms LOQ

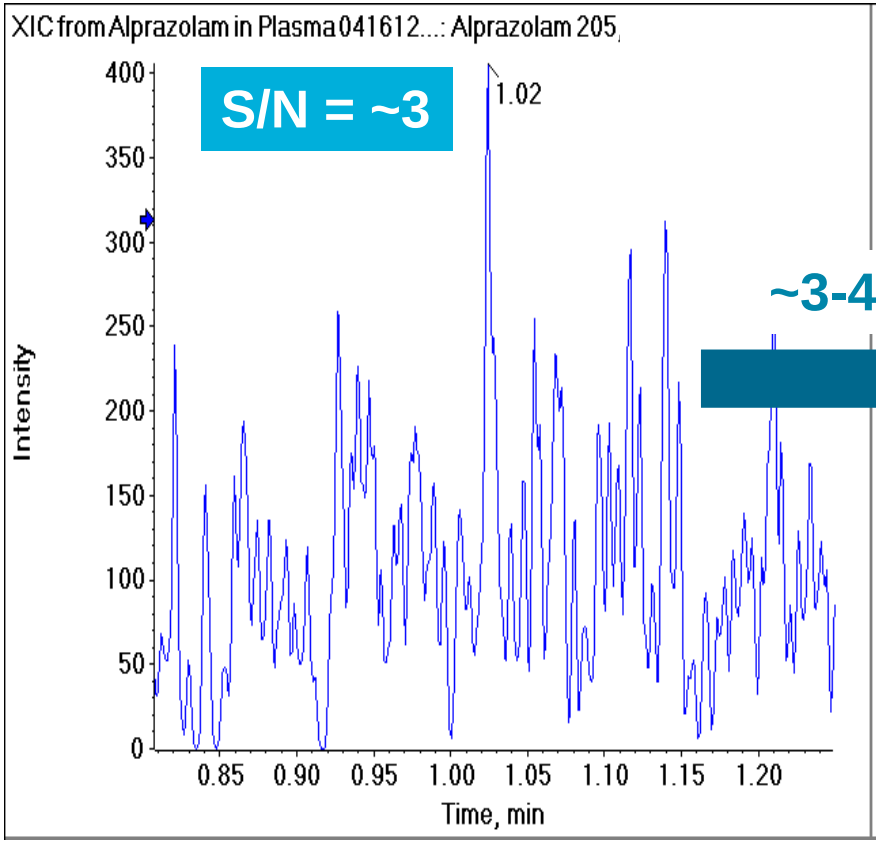


120 fg/mL (1 µL injection)

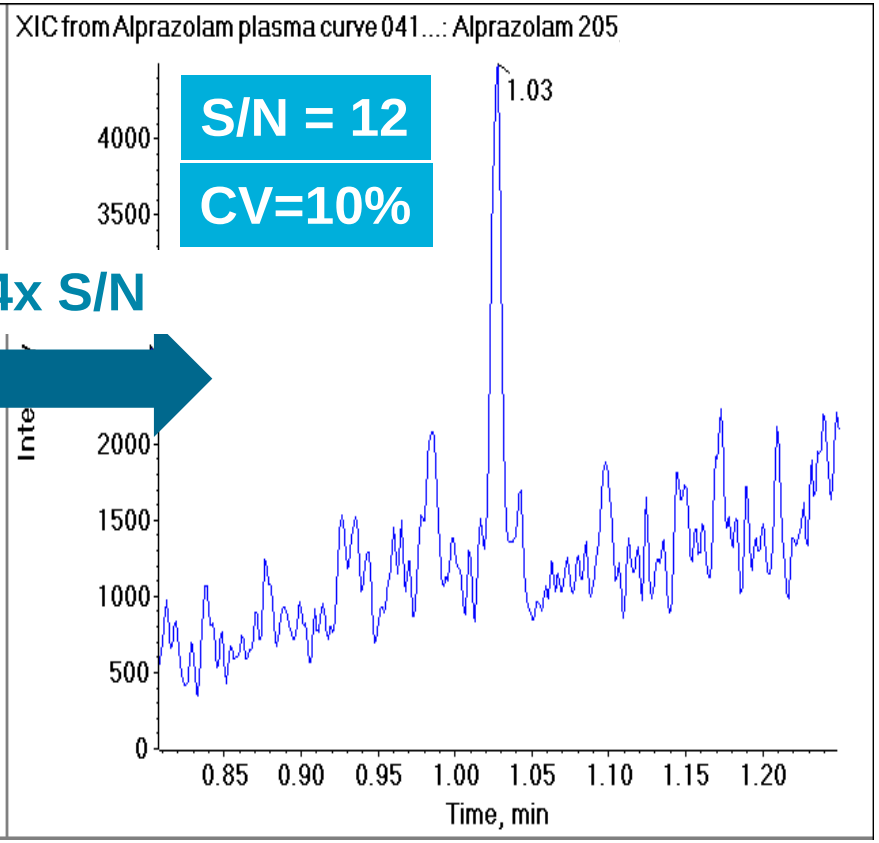
Alprazolam Neat STD – Sensitivity Gains

1.3 fg On Column

QTRAP® 5500 System

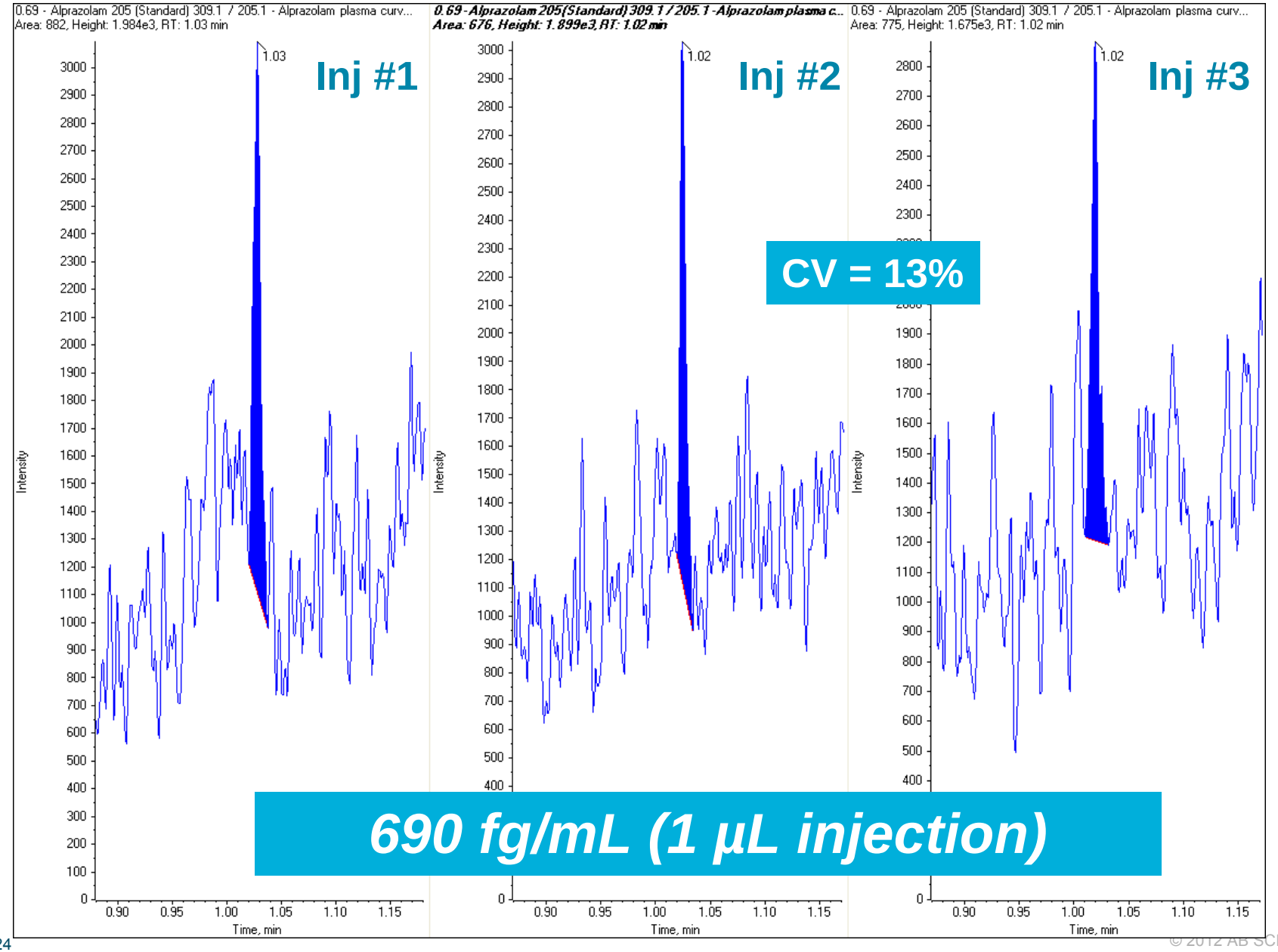


QTRAP® 6500 System

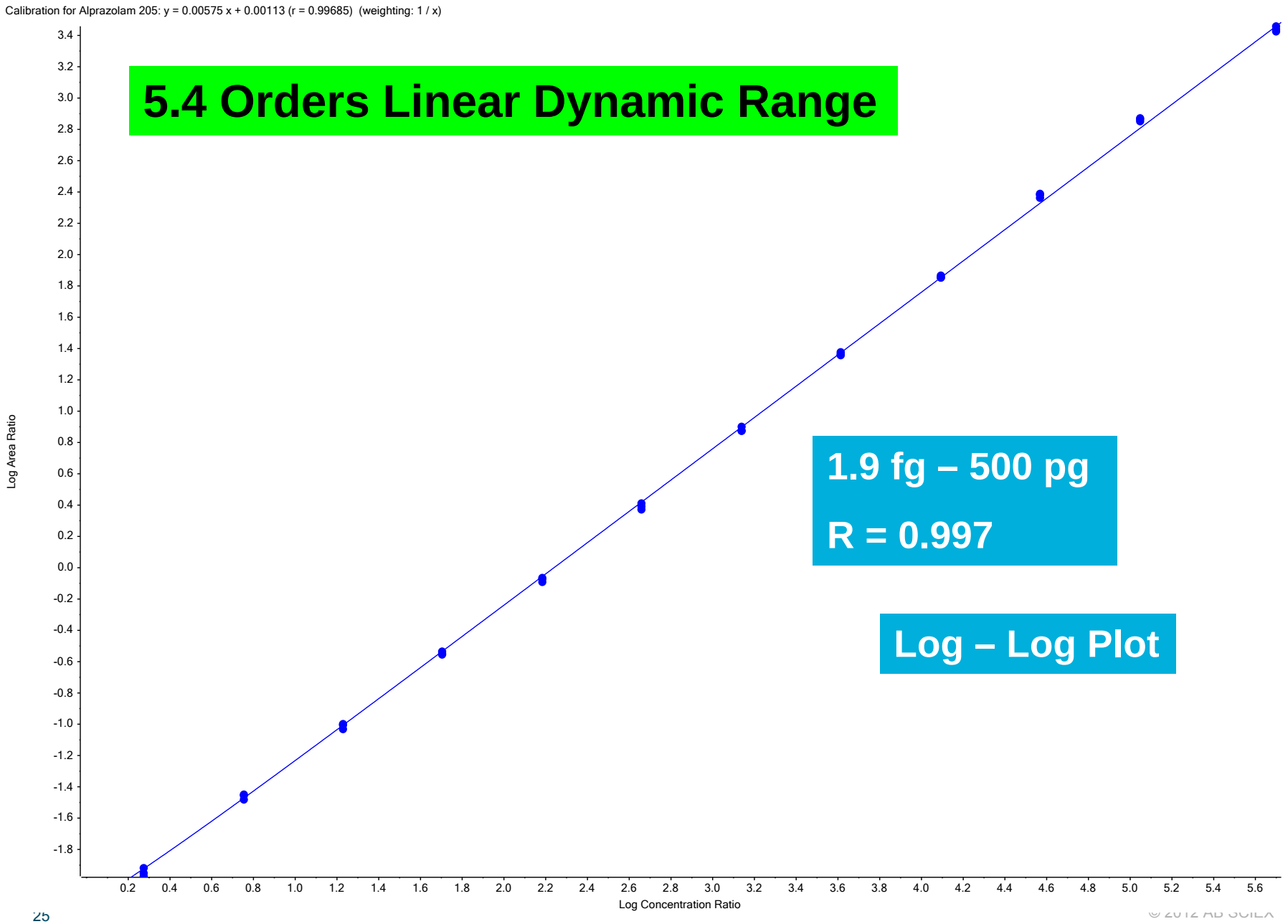


~3-4x S/N

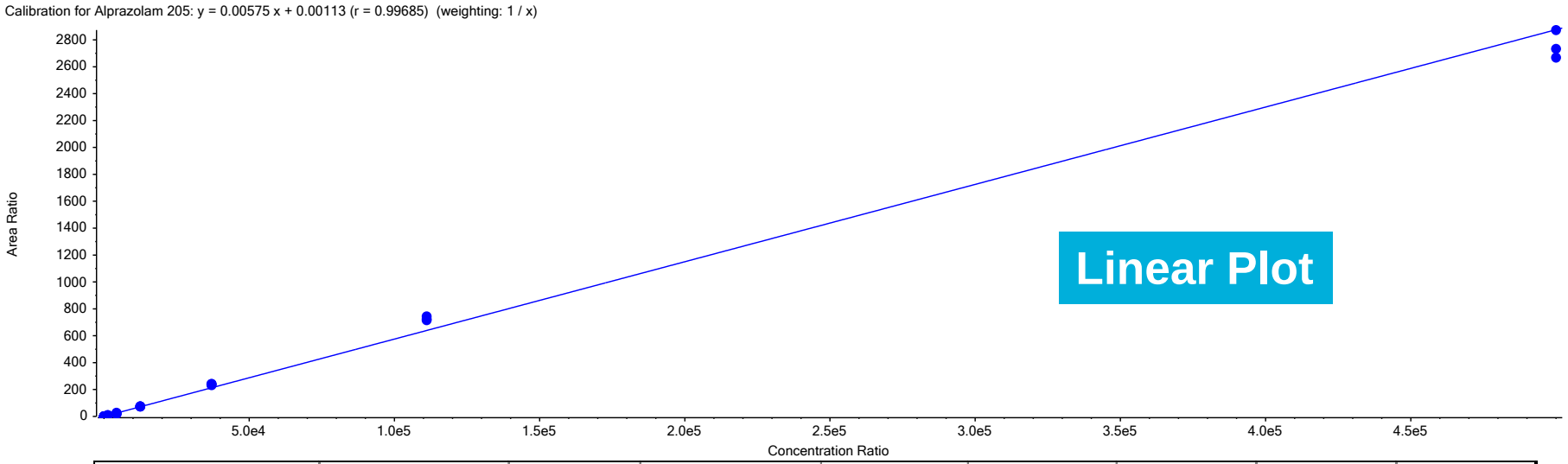
Alprazolam in Plasma – 690 Attograms LOQ



Dynamic Range – Alprazolam in Plasma



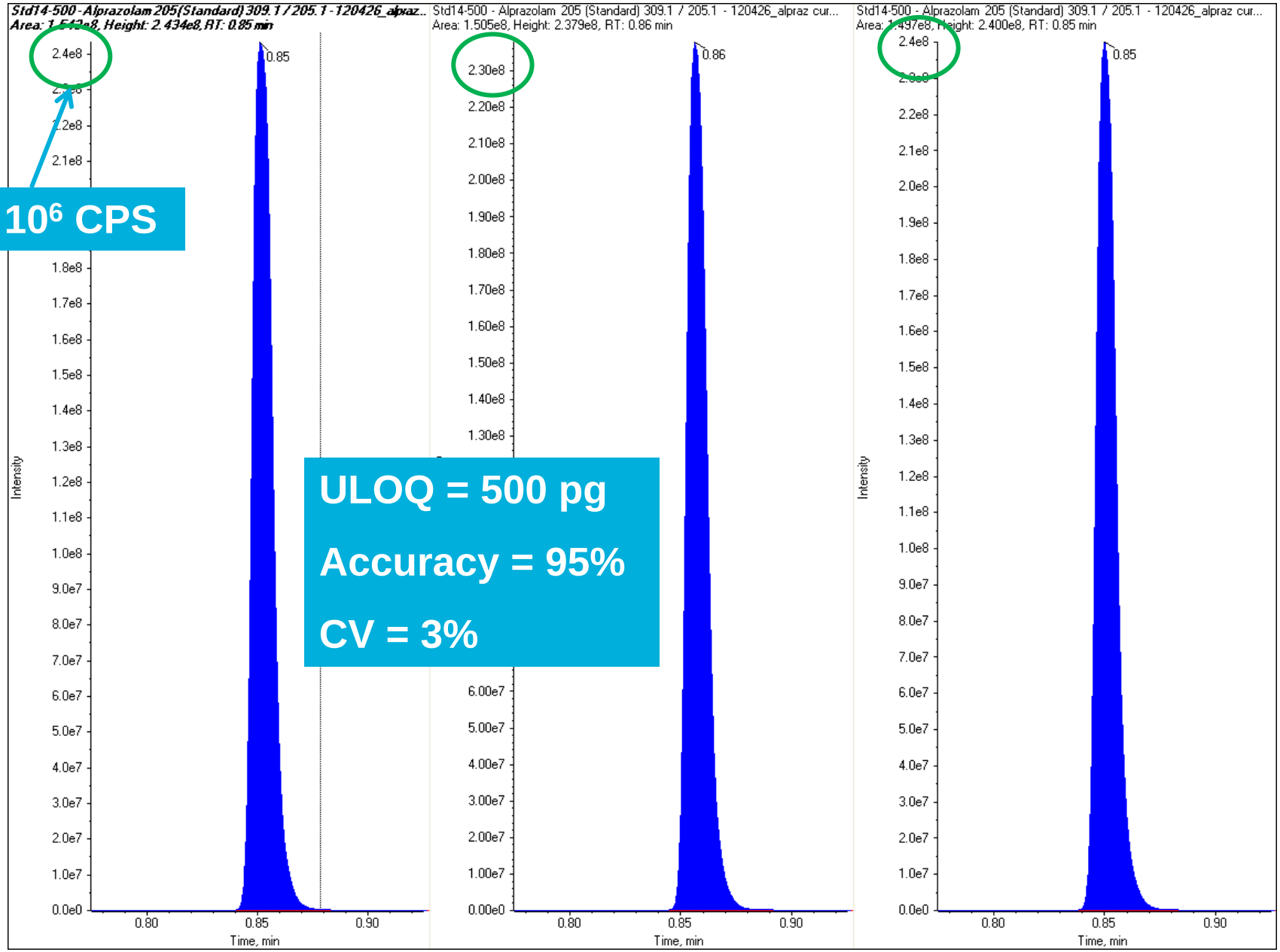
Dynamic Range – Alprazolam in Plasma



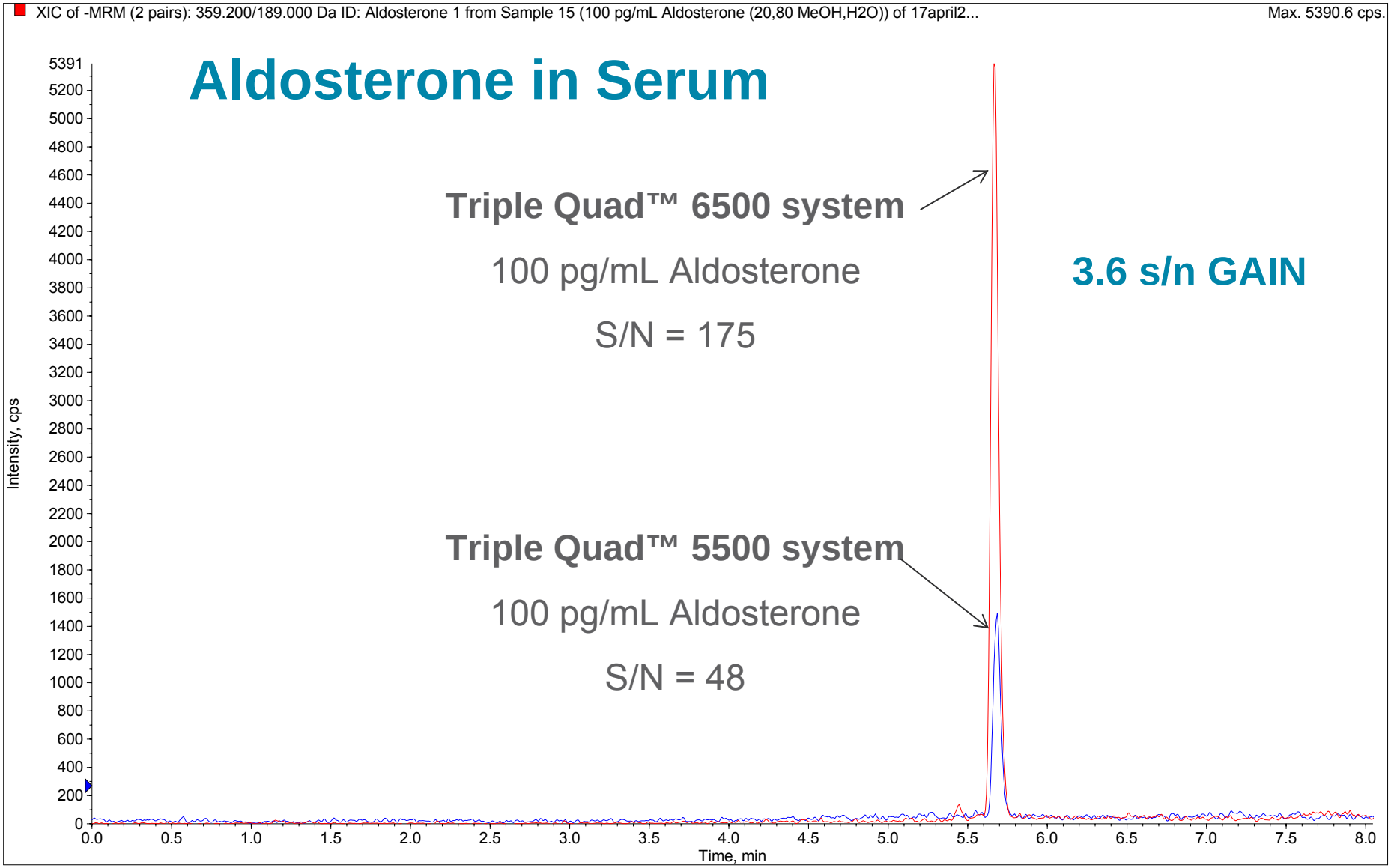
Linear Plot

	Actual Conc (fg on column)	Num. Values	Mean	Standard Deviation	%CV	Accuracy	Value #1	Value #2	Value #3
LLOQ	1.88	3 of 3	1.86	0.12	6.2	99.0	1.99	1.76	1.84
	5.65	3 of 3	5.89	0.23	3.9	104.3	6.08	5.64	5.96
	16.9	3 of 3	16.7	0.60	3.6	98.4	17.1	16.0	16.9
	50.8	3 of 3	49.0	1.07	2.2	96.3	48.4	48.2	50.2
	152	3 of 3	145	4.65	3.2	95.3	140	149	147
	457	3 of 3	423	20.0	4.7	92.5	422	403	443
	1372	3 of 3	1319	37.7	2.9	96.2	1294	1363	1301
	4115	3 of 3	4004	80.1	2.0	97.3	4080	3920	4011
	12346	3 of 3	12391	191.6	1.5	100.4	12283	12278	12612
	37037	3 of 3	41317	1311	3.2	111.6	39896	42479	41576
	111111	3 of 3	126138	2684	2.1	113.5	123526	125999	128888
ULOQ	500000	3 of 3	476169	18206	3.8	95.2	495981	460175	472349

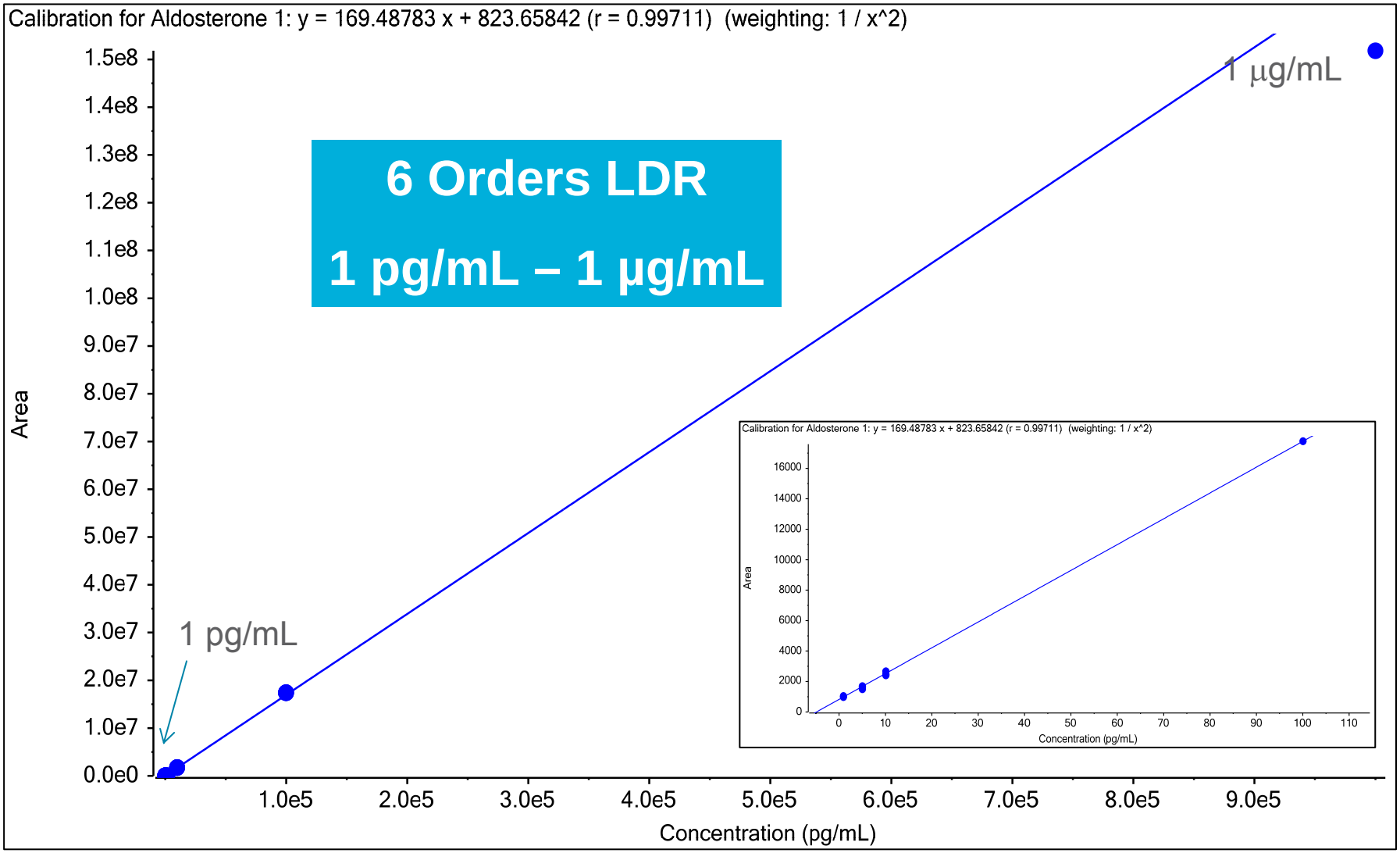
No Saturation at 200 x 10^6 Counts Per Second



Sensitivity Gains - Steroids



Aldosterone in Serum

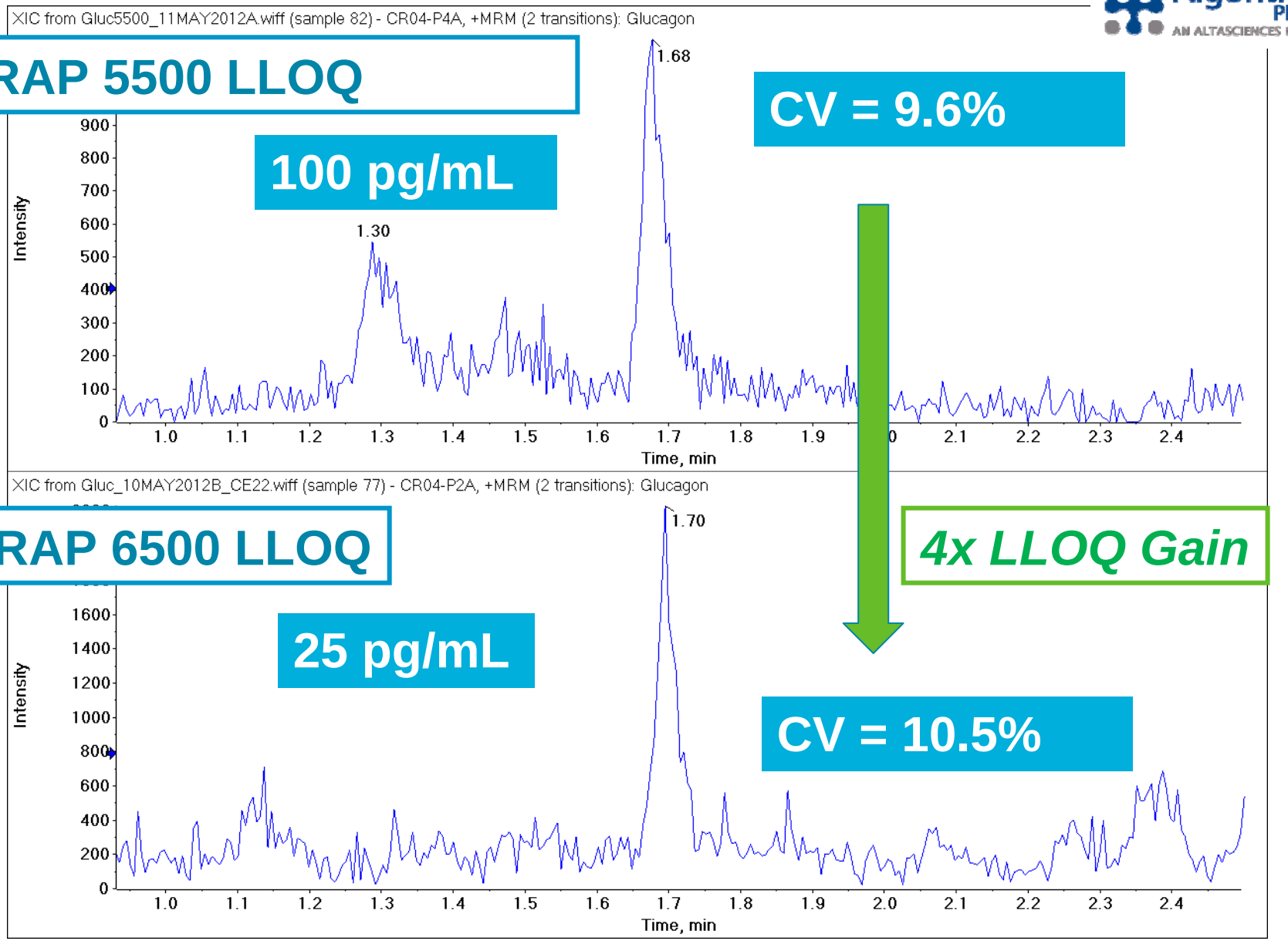


Sensitivity Gains: Large Molecules

- **Glucagon**
  (3485Da large therapeutic peptide, 5+ charge)
- Traditionally analyzed by LBA but presently LC-MS/MS is preferred
- Validated plasma assay on QTRAP 5500 system at Algorithme Pharma
- Current LLOQ: **100 pg/mL** in plasma
- **Novel formulation** of glucagon, which should be easier to administer, requires lower LLOQ
  (*http://clinicaltrials.gov/ct2/show/NCT01556594?term=Algorithme+Pharma&rank=8)*
- New LLOQ: **25 pg/mL** in plasma

"Sensitivity & Selectivity Challenge for Large Molecules Bioanalysis: Glucagon"
Algorithme Pharma / AB Sciex collaboration

Sensitivity Gain: Glucagon New formulation



QTRAP 5500 LLOQ

100 pg/mL

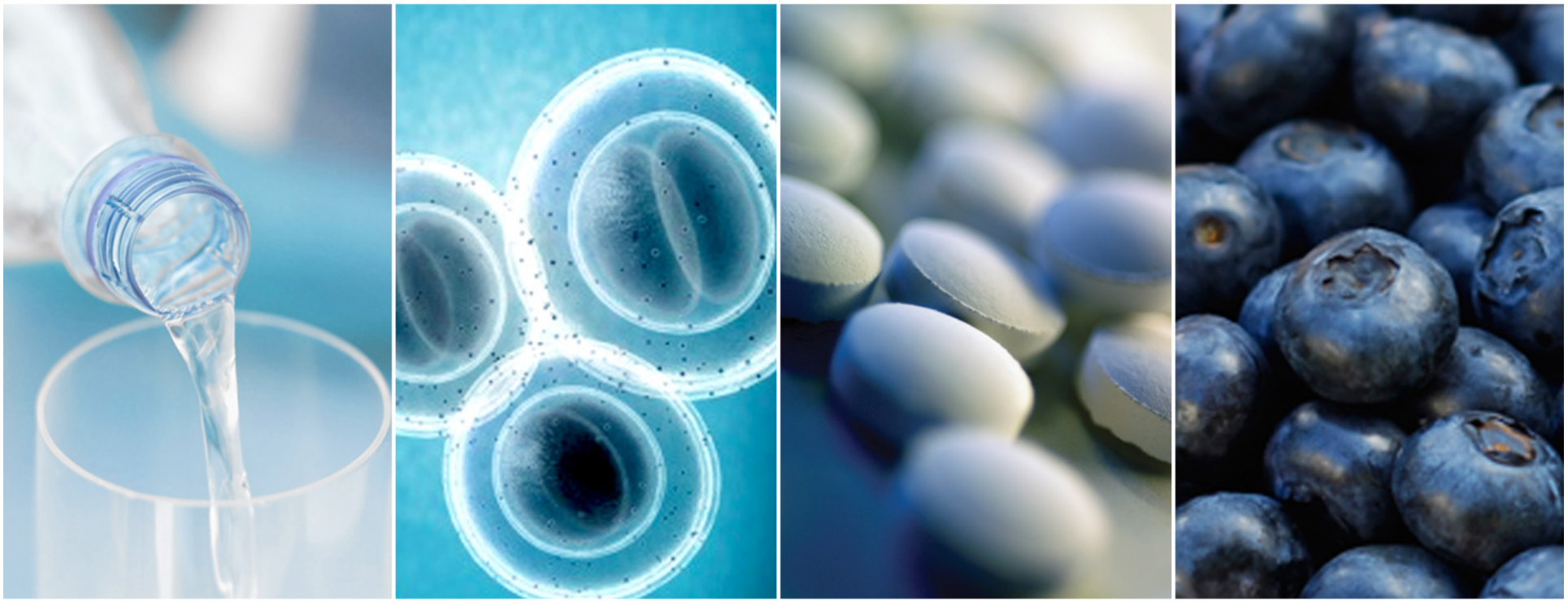
CV = 9.6%

QTRAP 6500 LLOQ

25 pg/mL

CV = 10.5%

4x LLOQ Gain



"Sensitivity & Selectivity Challenge for Large Molecules Bioanalysis: Desmopressin"

Kelli Jonakin[1], Louis-Philippe Morin[2], Jean-Nicholas Mess[2] and Fabio Garofolo[2]
[1]AB Sciex, Concord, ON, Canada; [2]Algorithme Pharma, Laval, QC, Canada

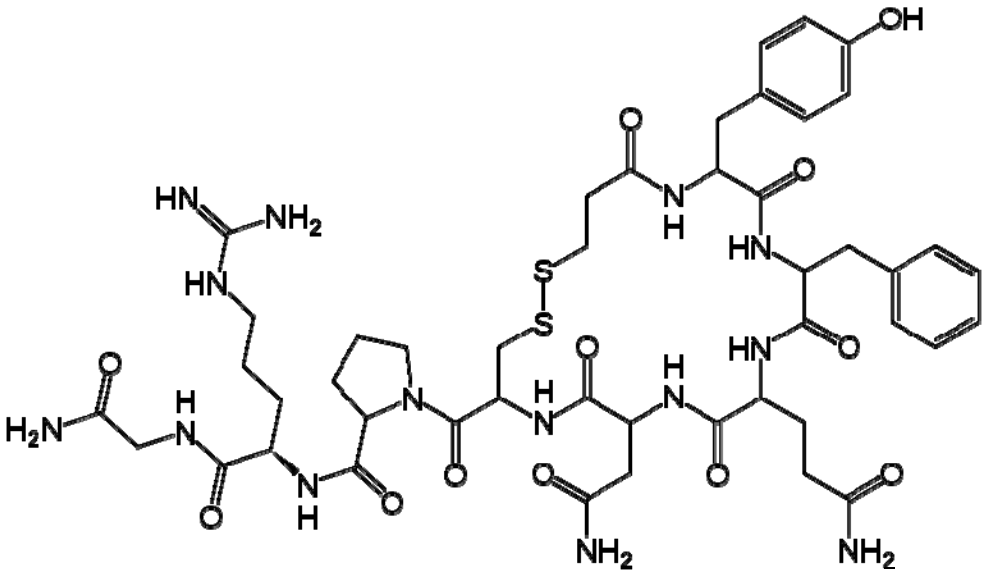
EBF Barcelona November, 2012

Algorithme Pharma
AN ALTASCIENCES COMPANY

AB SCIEX

Large Molecules Quantification

Desmopressin is a synthetic analogue used as an antidiuretic. Desmopressin can replace natural vasopressin hormone. It can be taken nasally, intravenously, or as an oral or sublingual tablet.



$C_{46}H_{65}N_{14}O_{12}S_2$

Molecular Mass: 1069.322 g/mL

Challenge → Very low limit of detection required (low to sub pg/mL)

Desmopressin: QTRAP® 5500

An assay was developed on the QTRAP® 5500 system to achieve the lowest limit of quantification possible (LLOQ)

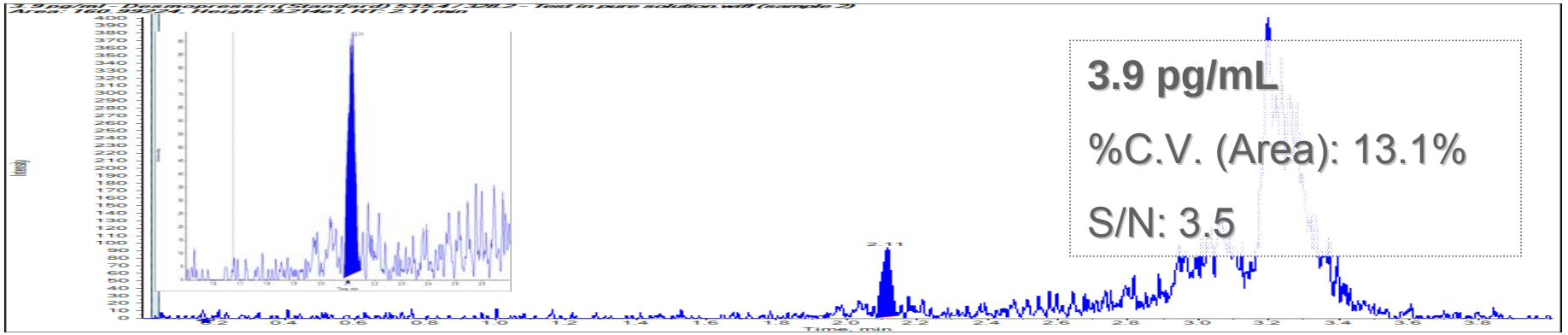
Chromatographic Conditions:

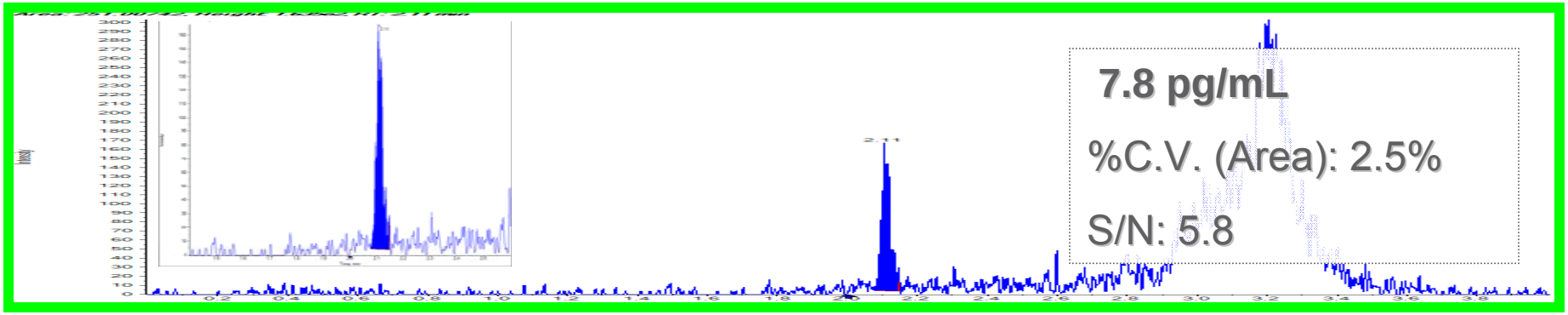
- HPLC Agilent 1100 series.
- Analytical Column: C18
- Channel A: 0.1% Formic Acid Channel B: MeOH
- Flow rate: 550uL/min
- Run time: 4 minutes
- Injection volume: 20µL

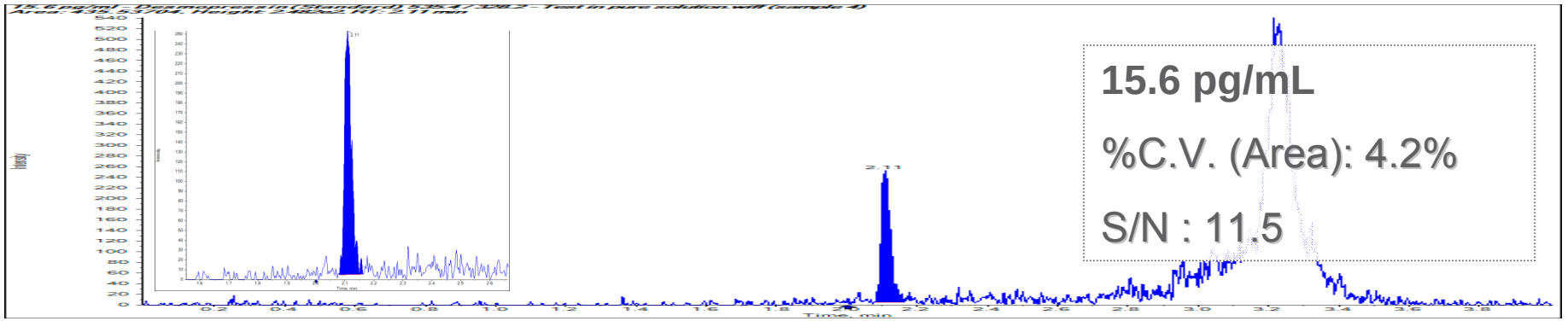
MS parameter:

- Positive ESI
- IS voltage: 5000 V
- Temperature: 750°C

Desmopressin: LLOQ QTRAP® 5500

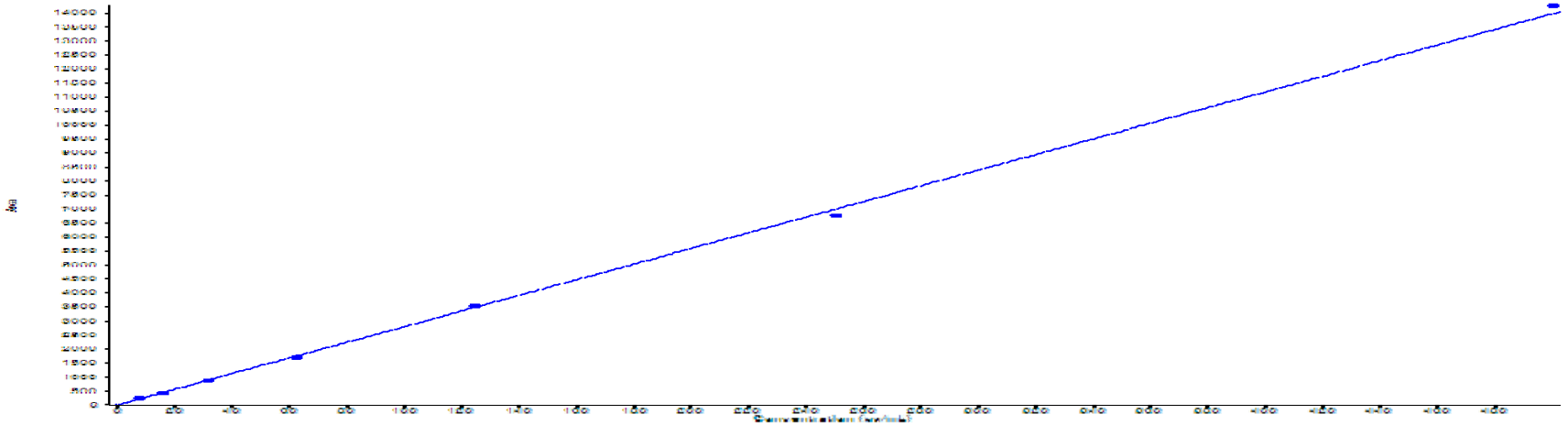






Desmopressin: QTRAP® 5500

With a signal to noise of 5.8 and a %C.V. of 2.4% the 7.80 pg/mL was found the most appropriate LLOQ for Desmopressin.



Concentration			Accuracy	%CV
Solution concentration (pg/mL)	Molar [ ] (pM)	On Column (fmol)	%	
0.98	0.913	0.018	NA	NA
1.95	1.826	0.037	NA	NA
3.91	3.652	0.073	NA	13.2
7.81	7.305	0.146	112.7	2.4
15.6	14.609	0.292	94.3	4.2
31.3	29.219	0.584	97.6	
62.5	58.438	1.169	95.7	
125.0	116.875	2.338	100.9	
250.0	233.750	4.675	96.7	
500.0	467.500	9.350	102.1	

% CV: Coefficient of correlation
NA: Not available
Correlation Coeffcient r = 0.99959
Regression: Linear weighted to 1/x, where x = analyte

Calibration range

7.8 to 500.0 pg/mL

Desmopressin: QTRAP® 6500

The assay was transferred on the QTRAP 6500 with minor difference (temperature) to achieve extreme level of sensitivity.

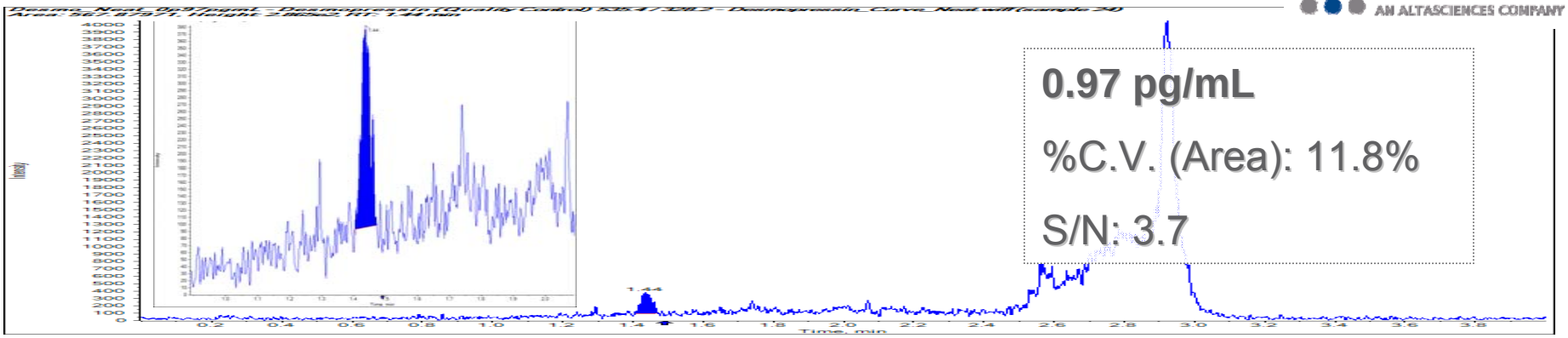
Chromatographic Conditions:

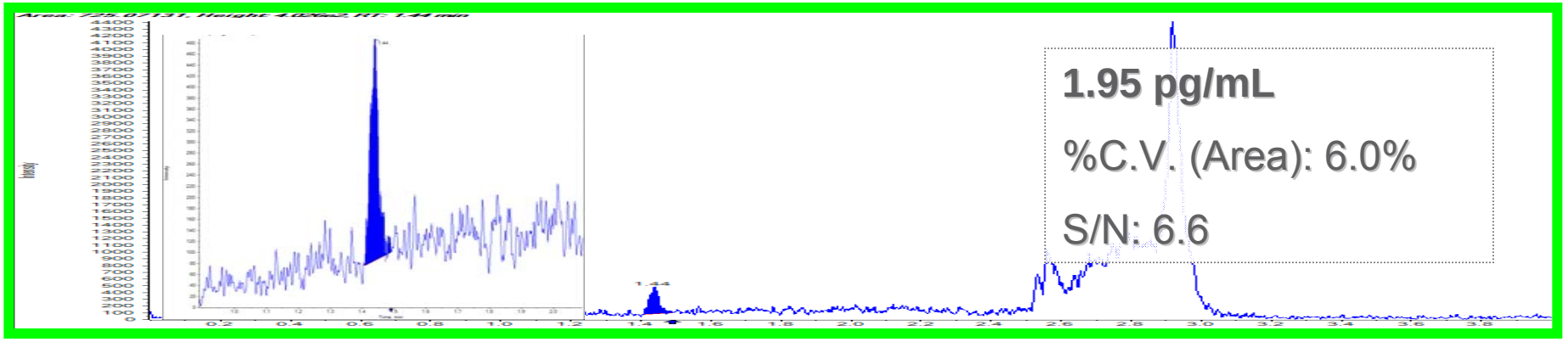
- Shimadzu LC20AD
- Analytical Column: C18
- Channel A: 0.1% Formic Acid Channel B: MeOH
- Flow rate: 550uL/min
- Run time: 4 minutes
- Injection volume: 20 uL

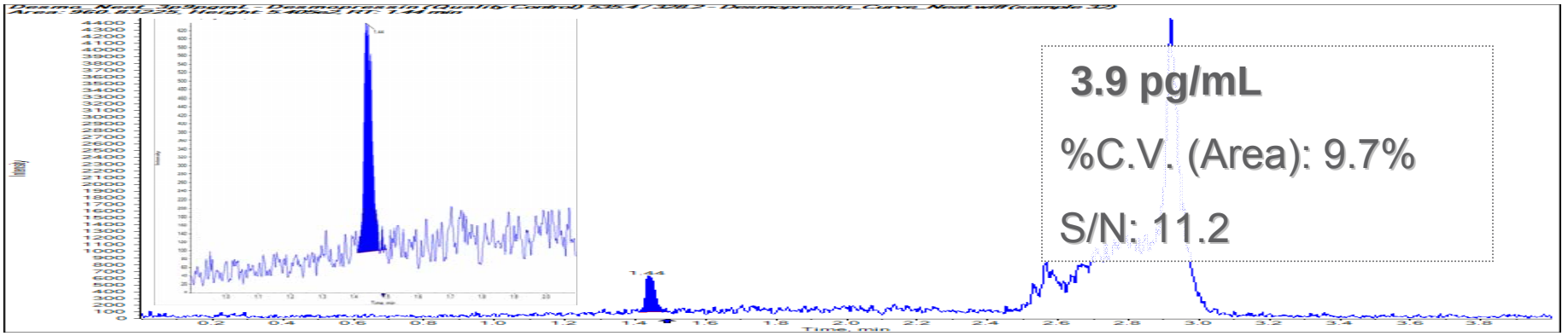
MS parameter:

- Positive ESI
- IS voltage: 5000 V
- Temperature: 500°C

Desmopressin: LLOQ QTRAP® 6500

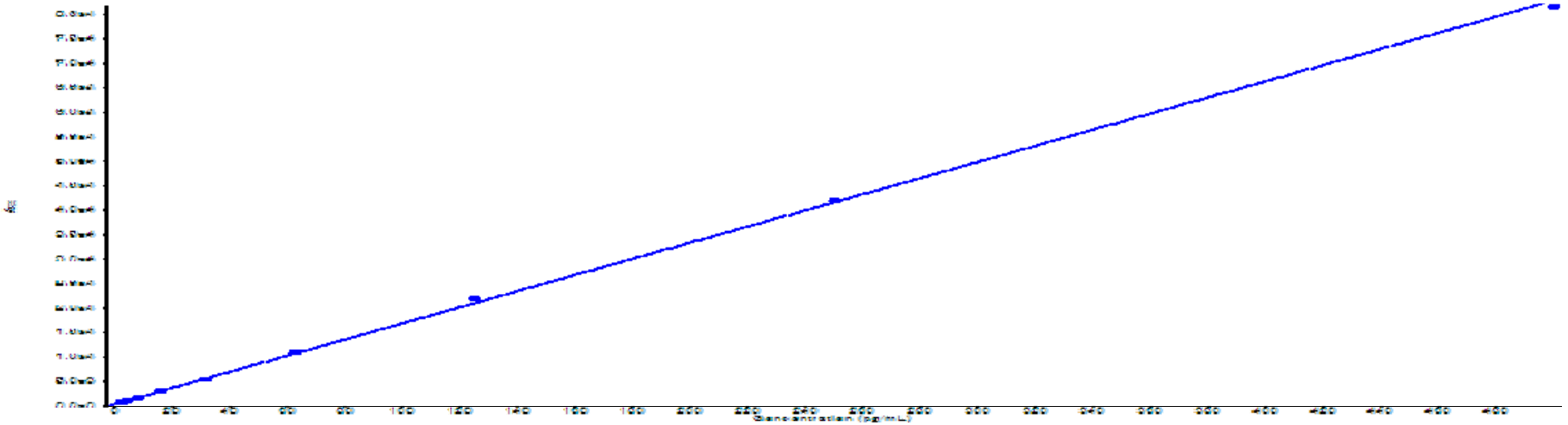






Desmopressin: QTRAP® 6500

With a signal to noise of 6.6 and a %C.V. of 6.0% the 1.95 pg/mL was found the most appropriate LLOQ for Desmopressin.



Concentration			Accuracy	%CV
Solution concentration (pg/mL)	Molar [ ] (pM)	On Column (fmol)	%	
0.98	0.913	0.018	NA	11.8
1.95	1.826	0.037	88.6	6.0
3.91	3.652	0.073	108.3	9.7
7.81	7.305	0.146	97.5	
15.6	14.609	0.292	103.3	
31.3	29.219	0.584	97.9	
62.5	58.438	1.169	100.8	
125.0	116.875	2.338	104.2	
250.0	233.750	4.675	100.9	
500.0	467.500	9.350	98.5	

% CV: Coefficient of correlation
NA: Not available
Correlation Coeffcient r = 0.99975
Regression: Linear weighted to 1/x, where x = analyte

Calibration range

1.95 to 500.0 pg/mL

Summary

- ✓ Large Molecule bioanalysis by LC-MS/MS requires very high sensitive mass spec to achieve low/sub pg/mL level.
- ✓ The QTRAP 6500 was able to decrease the LLOQ from 7.8 pg/mL (obtained on the QTRAP 5500) to **1.95 pg/mL** (by a factor of 4x).
- ✓ On-going work at Algorithme Pharma includes fine-tuning of the extraction method to achieve an LLOQ of **0.500 pg/mL** for human plasma.

Selectivity Gains

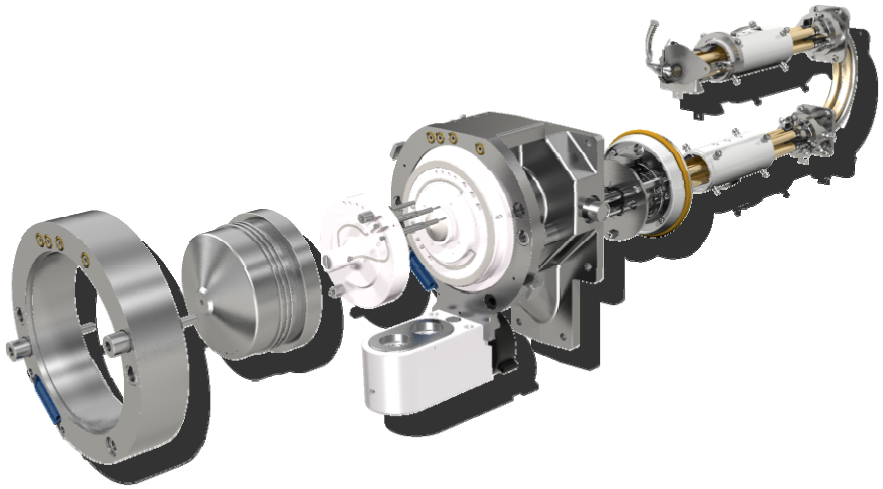
- Is increased sensitivity alone sufficient to consistently achieve fg/mL LOQ's ?
- With very low LOQ's, more likely to encounter matrix interferences:
  - Chromatographic interference
  - High baseline at analyte retention time

Solution: Selectivity beyond standard MS/MS

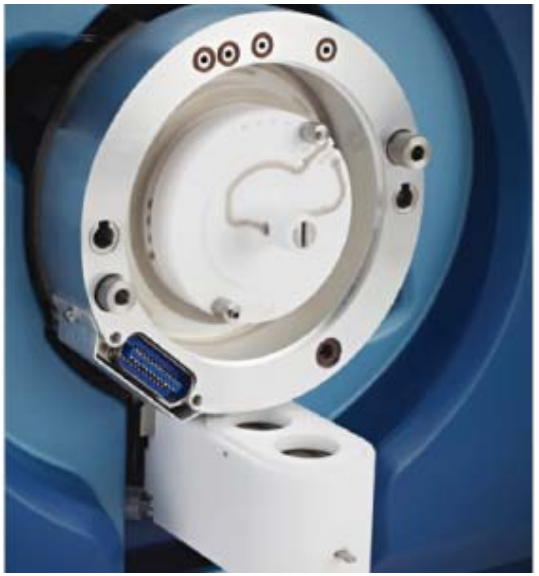
- SelexION™ Differential Ion Mobility Technology
- MRM^3

The 6500 Series with SelexION™ Technology

- Differential ion mobility provides true orthogonal selectivity to MS/MS
- Chemical modifiers increase separation capacity



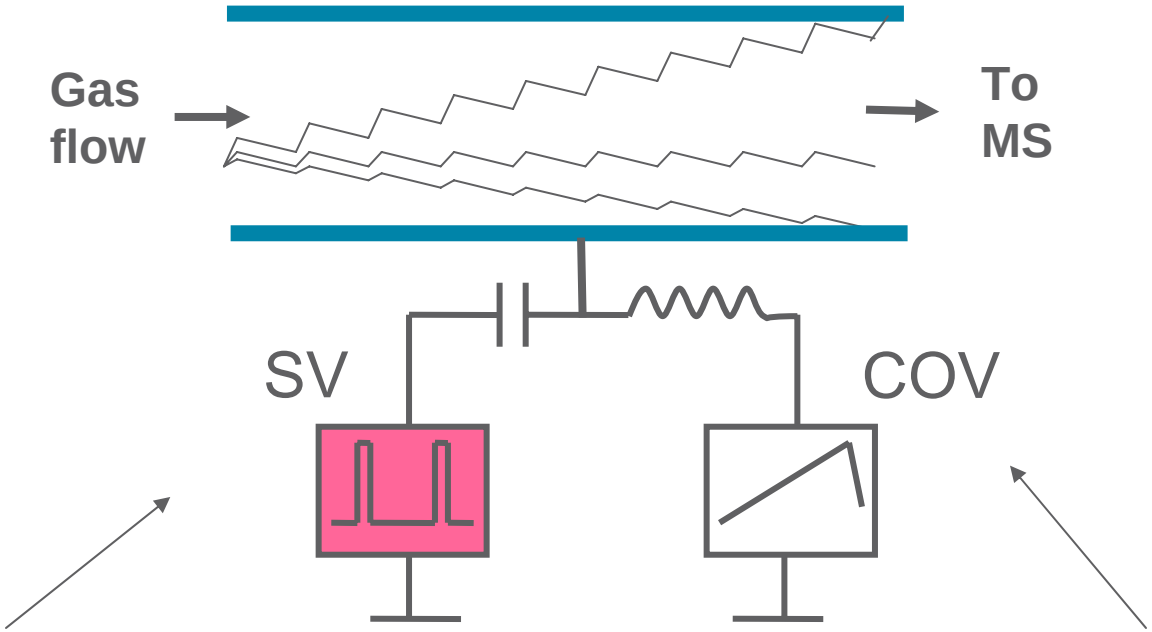
SelexION™ Technology with the 6500 Series


- Robust hardware designed for ease of use
- Few minutes to install/remove
- No tools required
- No need to break vacuum
- Fully compatible with the AB SCIEX Triple Quad™ and QTRAP® 6500 systems

How does SelexION™ Technology separate Ions?

•Differential Mobility Spectrometry (DMS) is the term used for planar geometry



Separation waveform (SV):

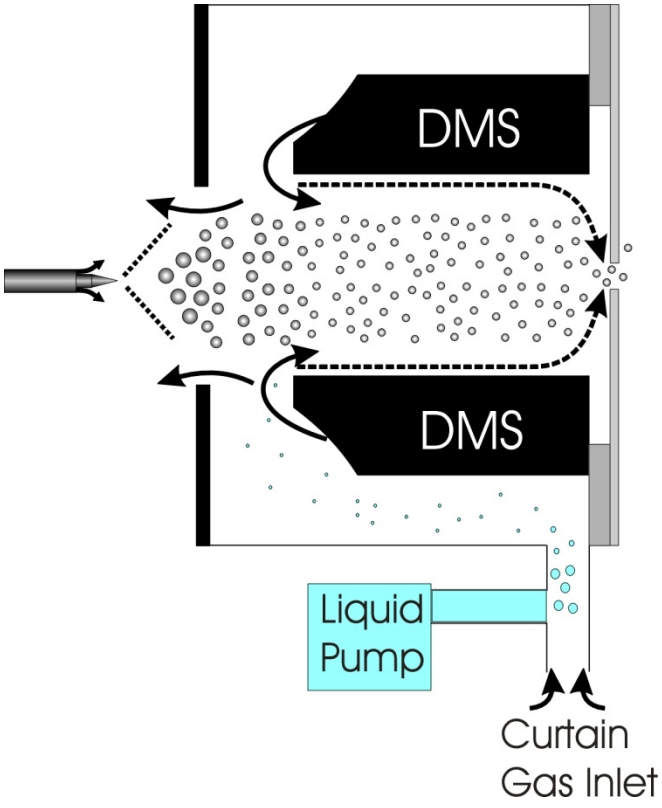
Radially displaces ions towards one or the other electrode, depending upon high and low field mobility characteristics

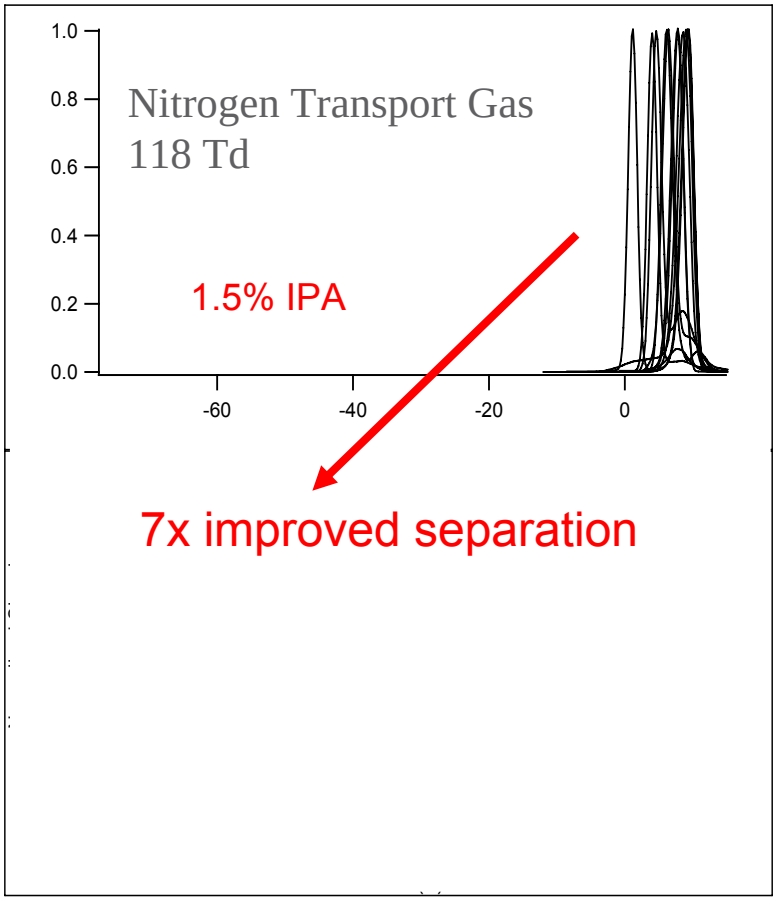
Compensation voltage (COV):

Restores the trajectory for a given ion to allow them to transmit through the DMS device and enter the mass spectrometer

Modified Transport Gases Improve Separations

- Liquid modifiers can be added to the curtain gas flow
- Improves separations
- More options for separation in difficult cases

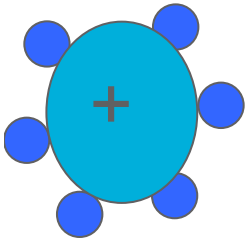


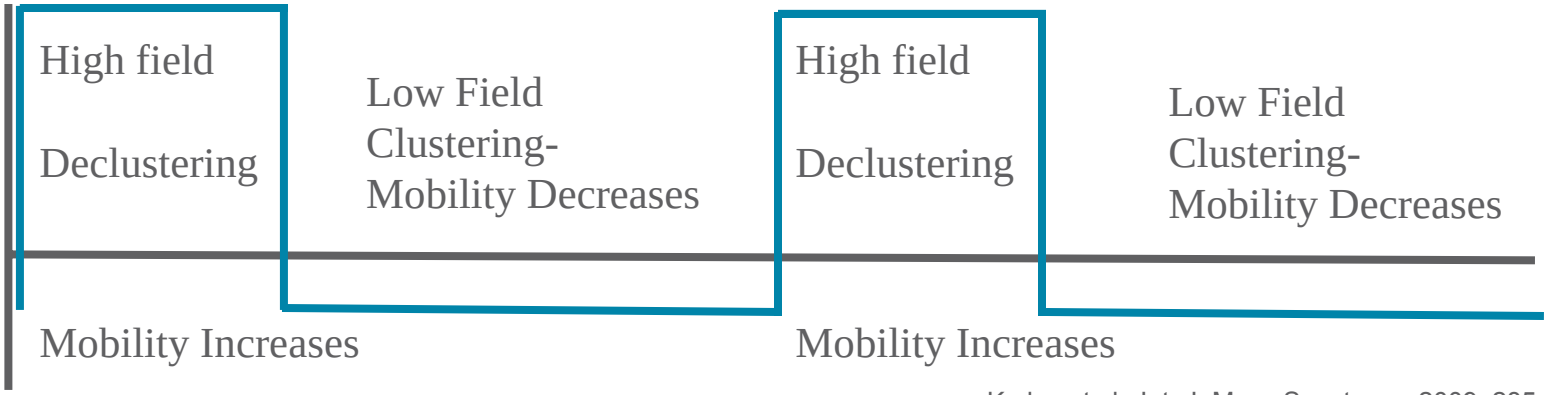


11 compounds: methylhistamine, minoxidil, ephedrine, norfentanyl, acyclovir, clenbuterol, tramadol, quinoxifen, pamaquin, fendiline, buscopan.

Dynamic Cluster/Decluster Model

Separations are Chemical in Nature



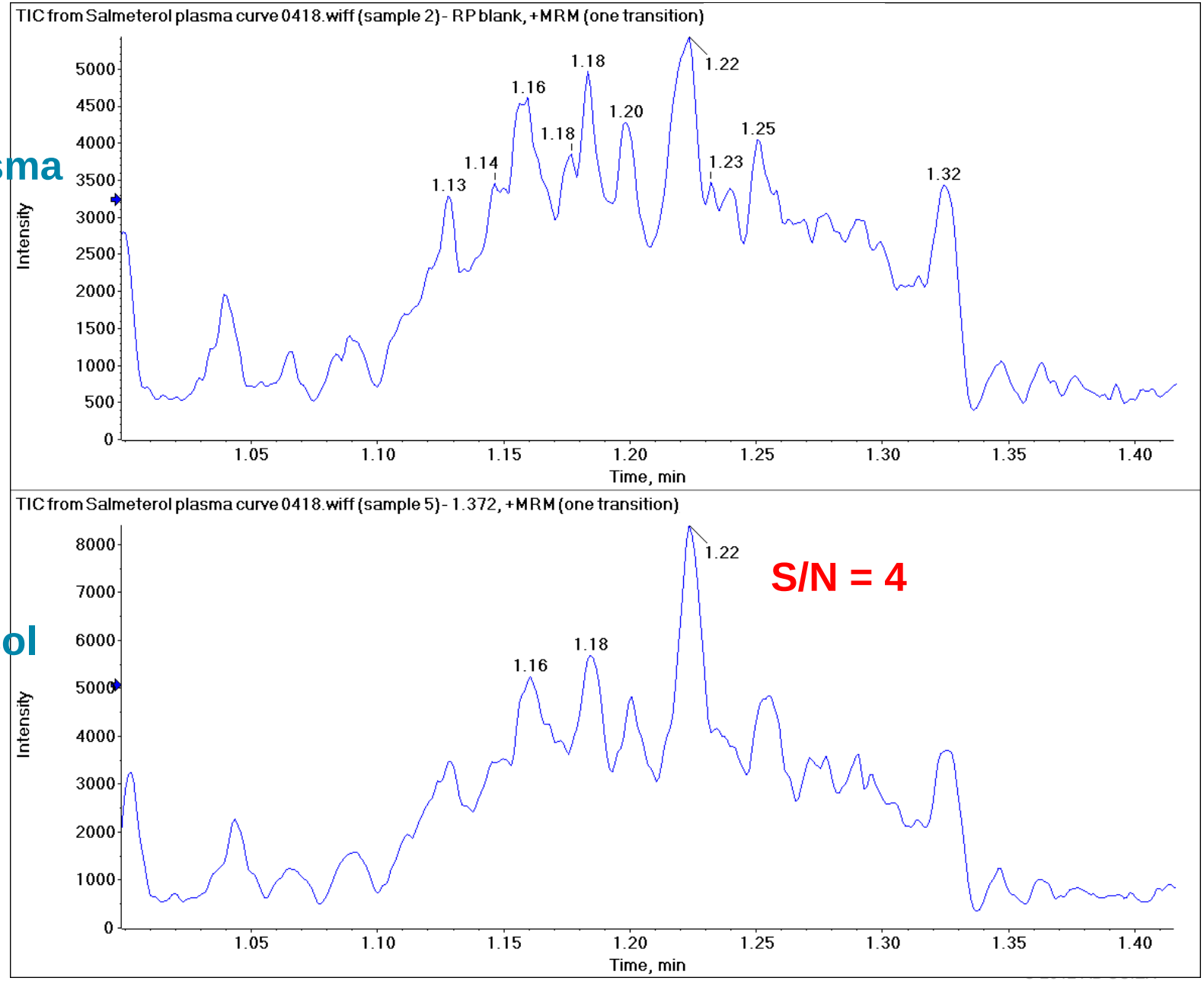


Krylov et al., Int. J. Mass Spectrom., 2009, 285, 149-156

Salmeterol in Plasma – Matrix Interference

Blank Plasma

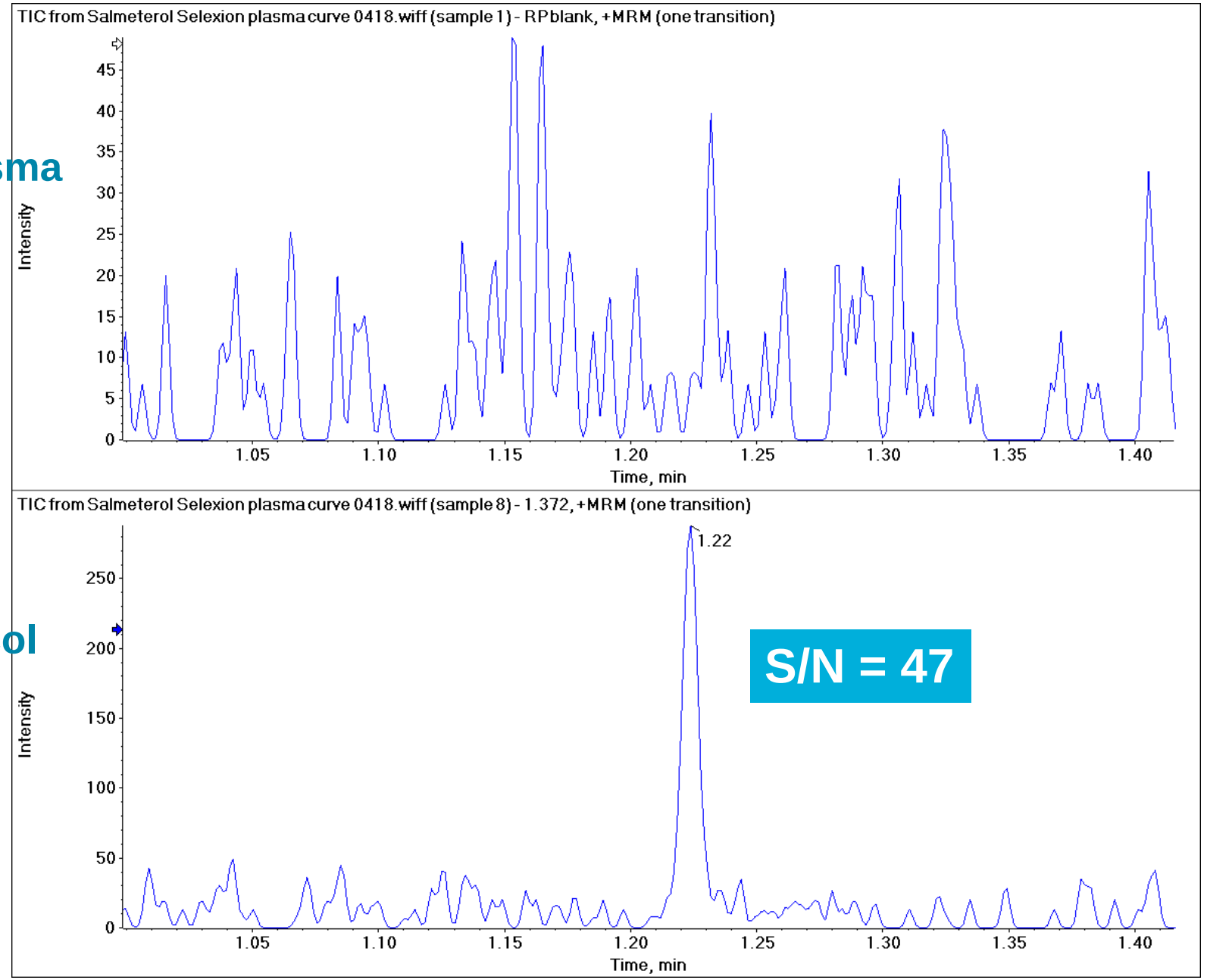
1.4 fg on-col



Salmeterol in Plasma – with SelexION

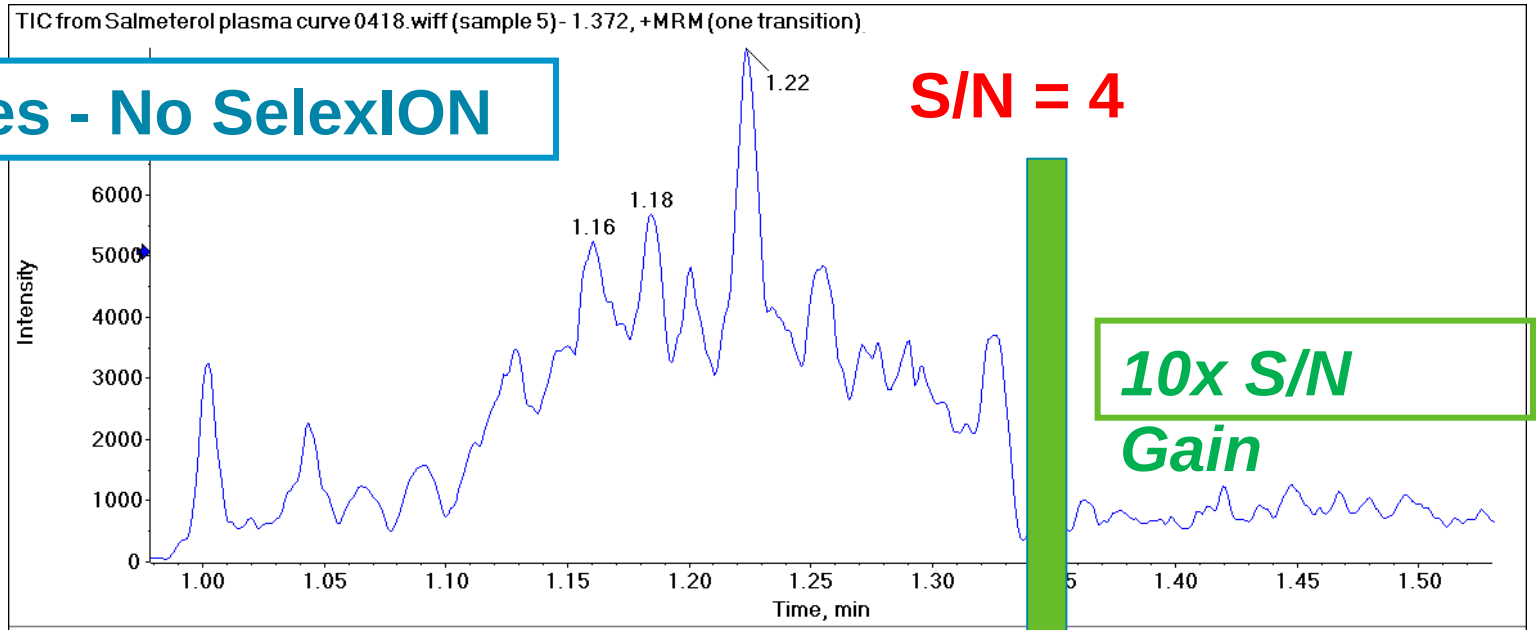
Blank Plasma

1.4 fg on-col



Selectivity Gains = LOQ Gains

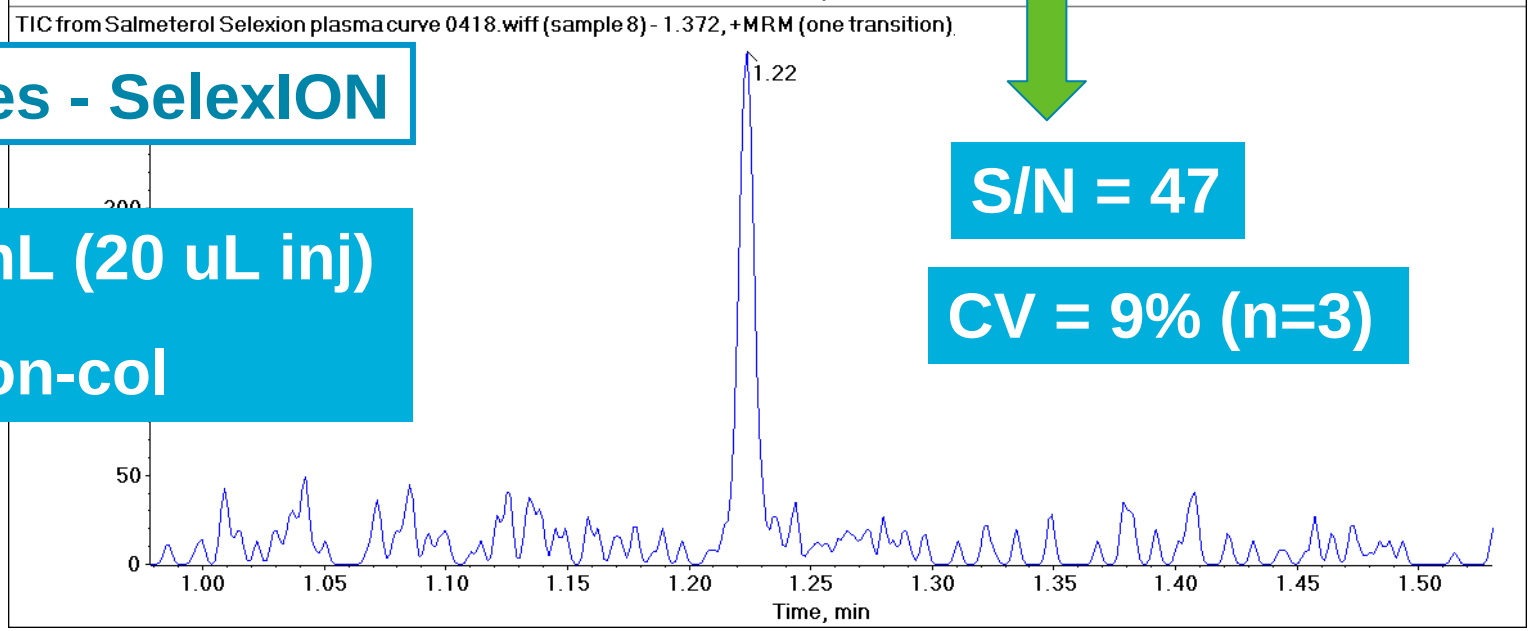
6500 Series - No SelexION



S/N = 4

10x S/N Gain

6500 Series - SelexION



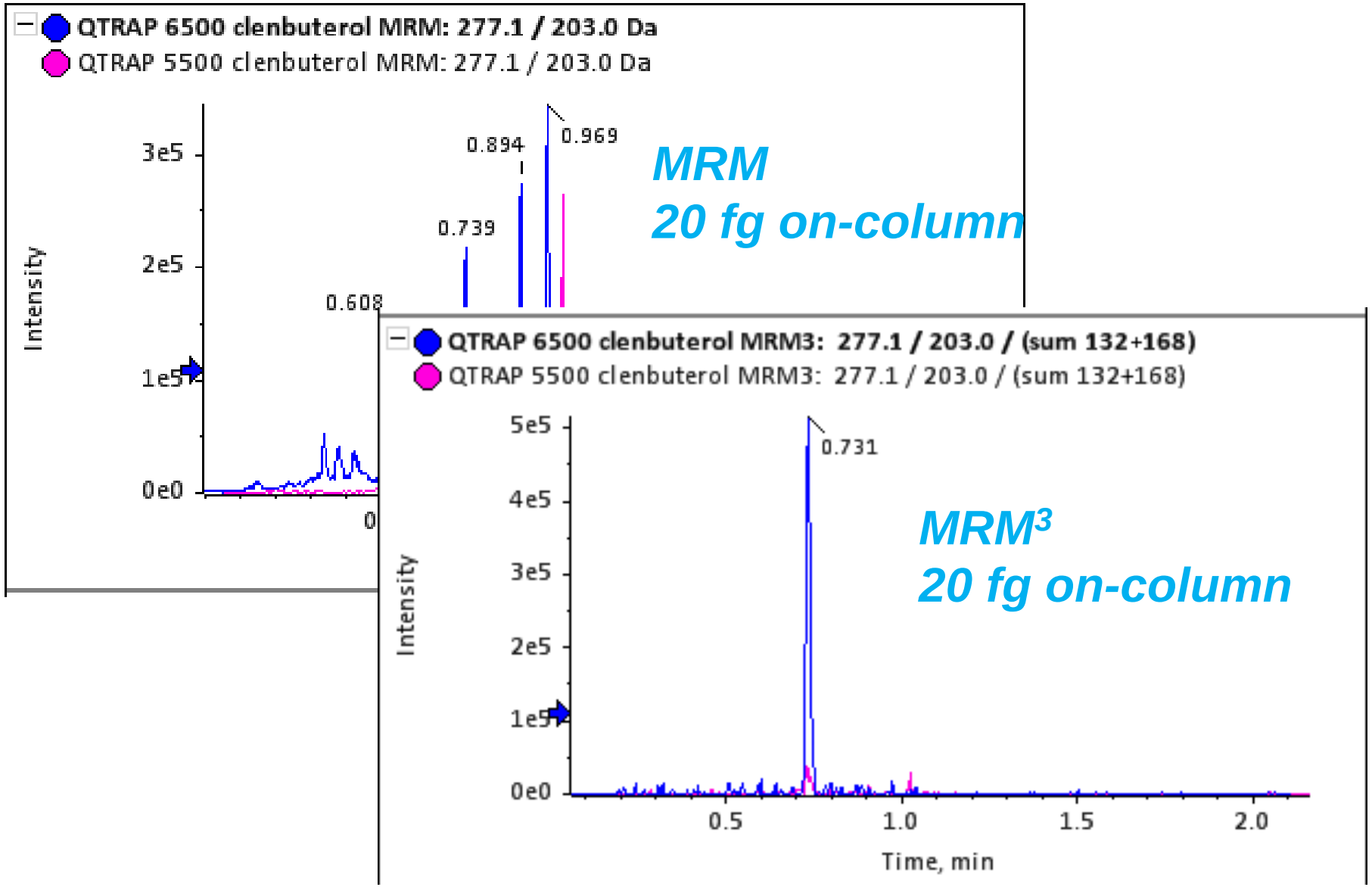
S/N = 47

CV = 9% (n=3)

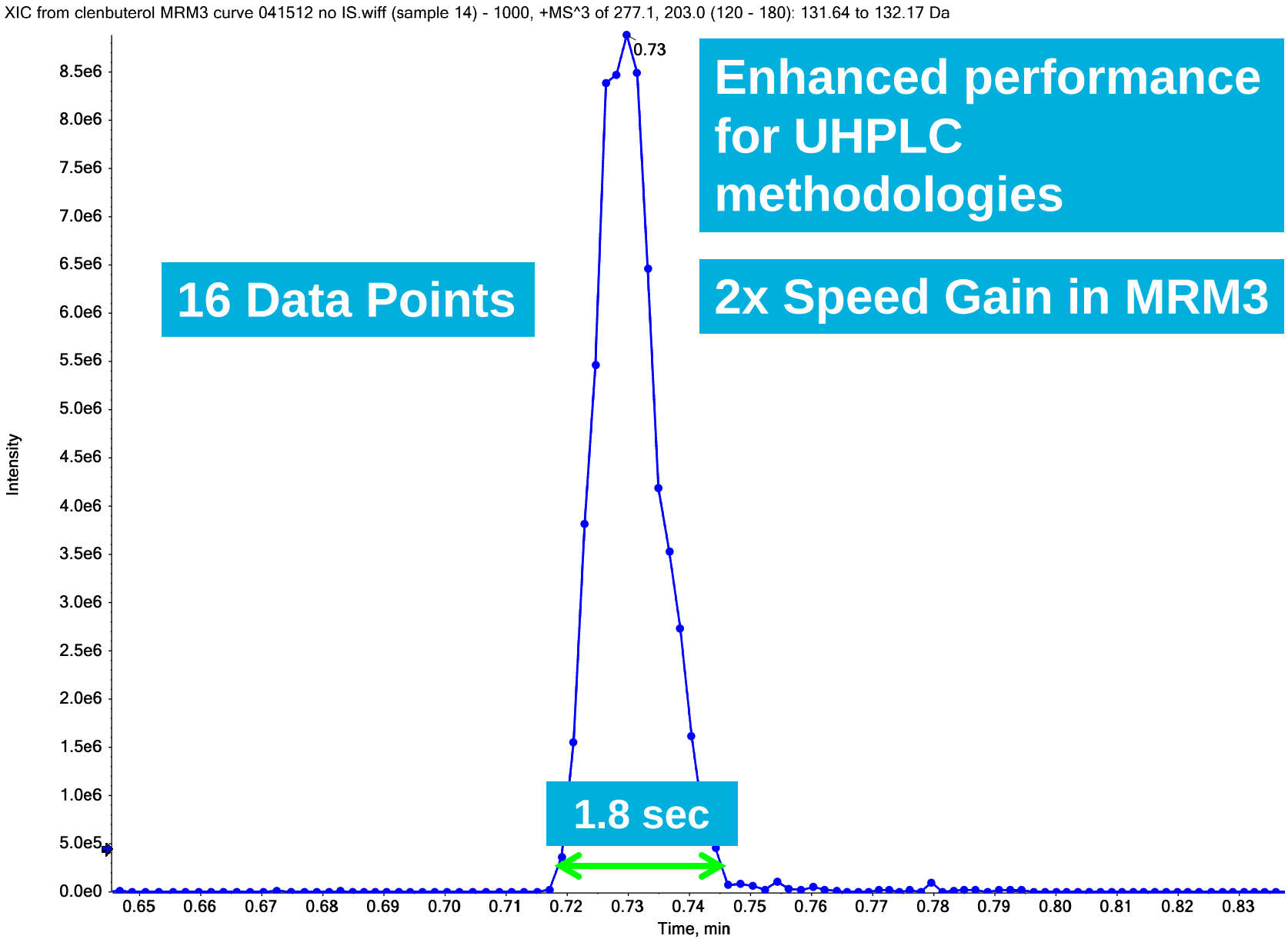
70 fg/mL (20 uL inj)

1.4 fg on-col

Selectivity and Sensitivity Gains - MRM³



MRM³ – Selectivity *and* Speed Gains



AB SCIEX Accelerated Lab Integration™ Package for BioAnalysis

AB SCIEX Triple Quad™ and QTRAP® 6500 systems

Best in class quantitative performance with highest confidence in compound identification

MultiQuant™ and Analyst® software

Next generation data processing software from AB SCIEX

Powerful small molecule *and* peptide quantitation capability

QTRAP® and Triple Quad Training

Training course for new system operators.

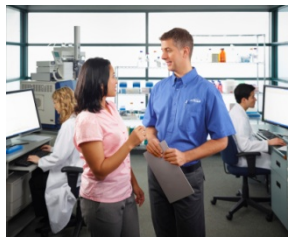
- Peptide quant track
- Small mol quant track
- Qualitative track

AB SCIEX Professional Validation Services

MS IQ/OQ

LC IQ/OQ

Analyst® and MultiQuant™ software validation



Comprehensive Range of Validation Services from AB SCIEX

HPLC IQ-OQ

MS IQ-OQ

SW Validation

LC MS PQ

Regulatory Compliance using an integrated approach for Hardware Qualification and Software Validation

The New AB SCIEX Triple Quad™ and QTRAP® 6500 Systems

- Unprecedented sensitivity, enabling fg/mL lower limits of quantitation in bioanalysis
  - 2-5x better LOQs vs 5500 Series
- Optimized geometry and new large diameter heaters in the **IonDrive™ Turbo V source** result in improved ionization efficiency at high flows and more robust source conditions
- Efficiency gains in ion sampling with the **IonDrive™ QJet ion guide** increase sensitivity without compromising robustness

The New AB SCIEX Triple Quad™ and QTRAP® 6500 Systems

- The remarkable sensitivity gains achieved are not at the expense of linear dynamic range. The new **IonDrive™ High Energy detector** increases ion counting capacity up to 20-fold, resulting in 5- 6 orders of magnitude LDR
- The **increased mass range** of m/z 5 – 2,000 provides versatility and high sensitivity for both small molecules and peptides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  - Jean-Nicholas Mess
  - Fabio Garofolo

Questions and Answers

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