

**ThermoFisher**  
SCIENTIFIC

*The world leader in serving science*

# TSQ Vantage

## Highest Sensitivity, Lowest Noise

**ASMS Workshop**

Rohan A. Thakur

# Introducing the *TSQ Vantage*

*Challenging conventional thinking for bioanalysis...*



SENSITIVITY

# Differentiating Features for Bioanalysis

**Highest Sensitivity, Lowest Noise**

**Wide Linear Dynamic Range**

**Superior ion source robustness**

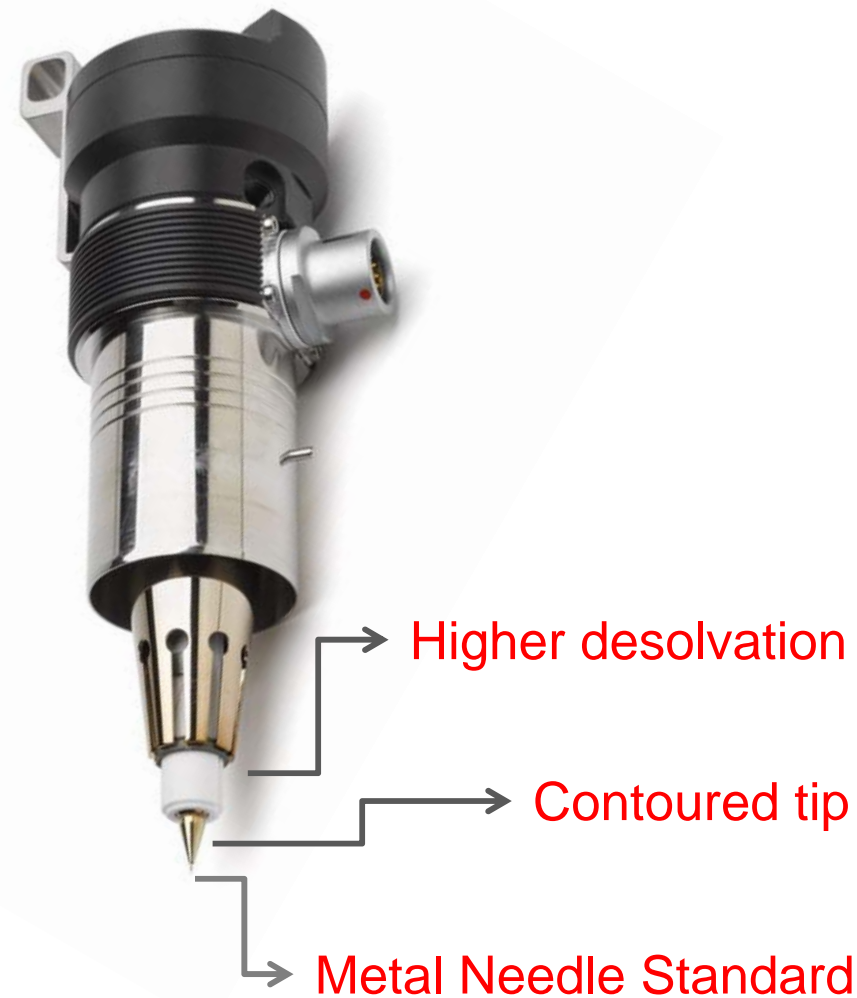
**All “digital” with Watson LIMS**



**SENSITIVITY**

# Technology Enhancements: HESI-II

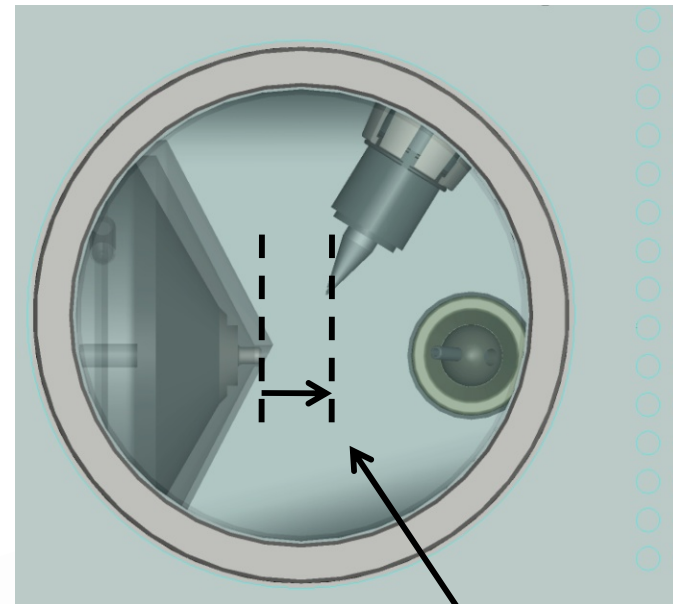
- **Benefits: Sensitivity across a wider range of flow rates**
  - Higher desolvation for  $>1\text{mL}/\text{min}$  flow rates
    - *More heated nitrogen auxiliary gas flow aids desolvation at high flow rates*
  - One piece metal needle allows for easy replacement
    - *Available as low flow and regular (high) flow*
  - Contoured tip for enhanced low flow (micro-spray;  $5\text{-}25\mu\text{L}$ ) stability
    - *Generates better electrostatic fields enabling spray stability for lower flow rates*



\* Backwards compatible

# Technology Enhancements: Ion Sweep Cap

- **Benefits: Maximum Robustness in real samples**
  - Fixed Probe Position
    - *Plug & play position ensures spray hits below the “hood”*
    - *Ensure ion transfer tube orifice is never obstructed*
  - Asymmetric Profile Below Orifice Hood
    - *Ensures spray is directed into the drain to reduce re-circulation in the ion source*
    - *Build-up of matrix always occurs below orifice*
  - **Run hundreds of matrix rich samples without compromising sensitivity!**



Fixed probe position

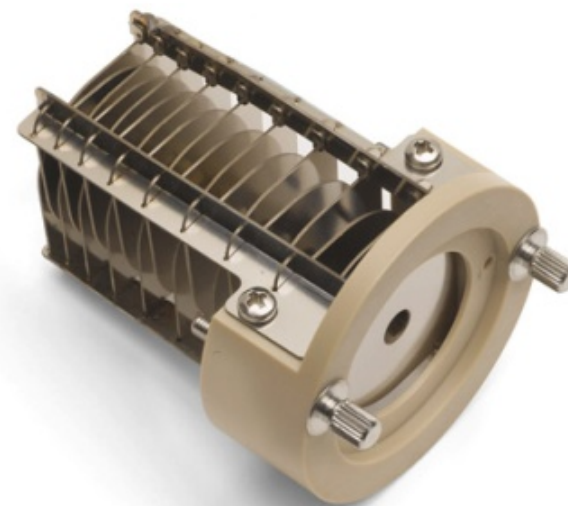
Special “hooded” shape & asymmetric profile

\* Backwards compatible

# Technology Enhancements: S-Lens

- **Benefits:** *Higher Signal and Less Noise*

1. The innovative **S-Lens** captures all the ions in the expanding gas plume and efficiently focuses the ions into a **tight beam**.
2. The ion beam from the source is then transmitted into Q00 with **maximum efficiency**.
3. The **Hyperbolic quadrupoles** then filter out the signal from the noise, delivering the lowest LOQ.
4. This leads to **highest sensitivity**, with the **lowest noise**

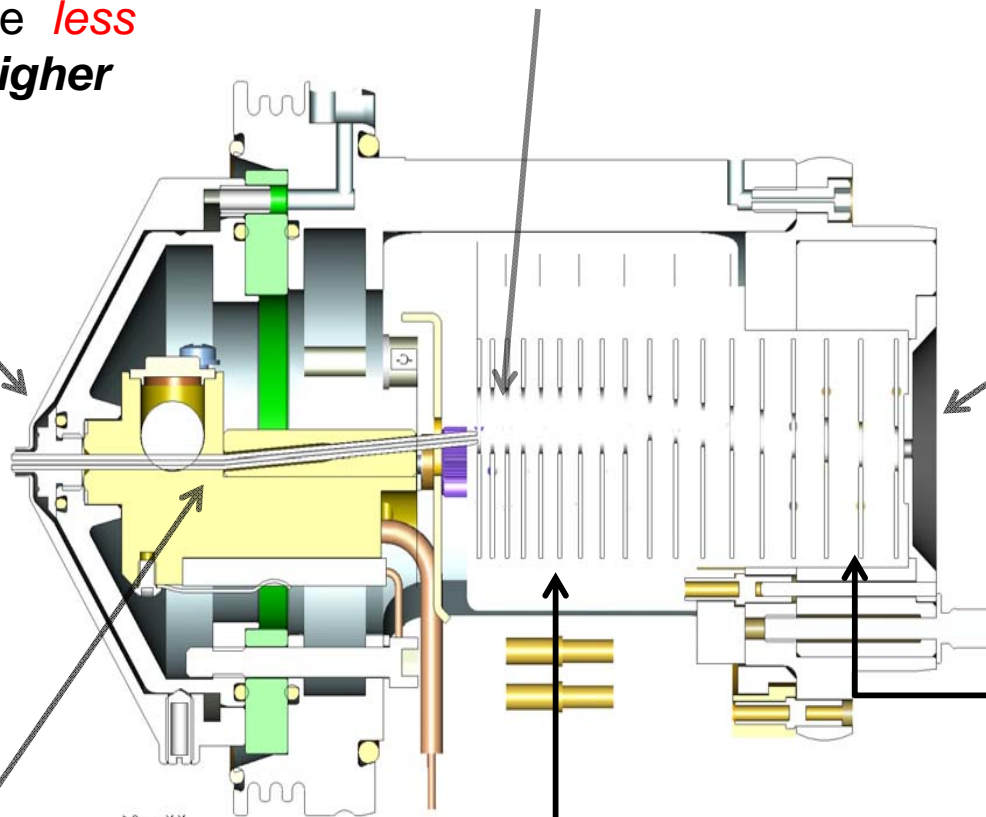


# S-Lens (More Signal, Less Noise)

Improved Ion Sweep Gas Cap  
(N<sub>2</sub> gas limits solvent i.e. **less chemical noise**) for **Higher Robustness**

Off-Axis to instrument axis =  
**Less Chemical Noise**

Optimum opening into high vacuum chamber  
**limits chemical noise = Long-life Turbo Pump**

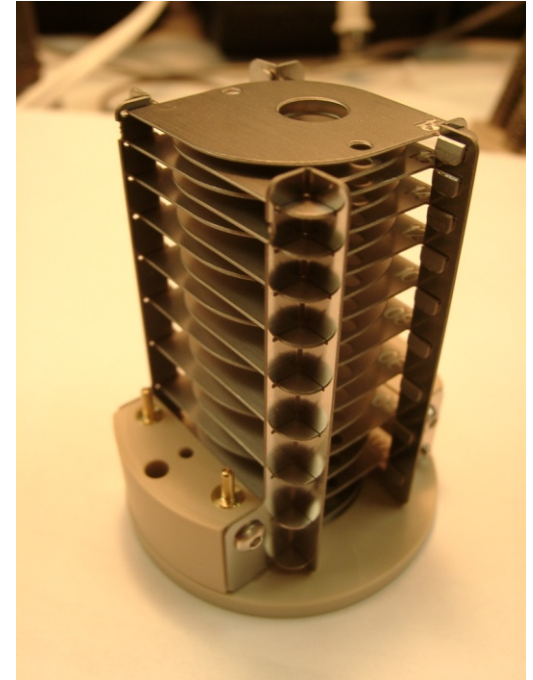


Shorter Ion Transfer Tube  
(larger ID) = **More gas in (more ions)**

Maximum efficiency for pumping gas  
(removes extraneous solvent laden gas  
i.e. **less chemical noise**) = **Better Vacuum**  
(note progressive spacing)

# S-Lens Ion Optics Design

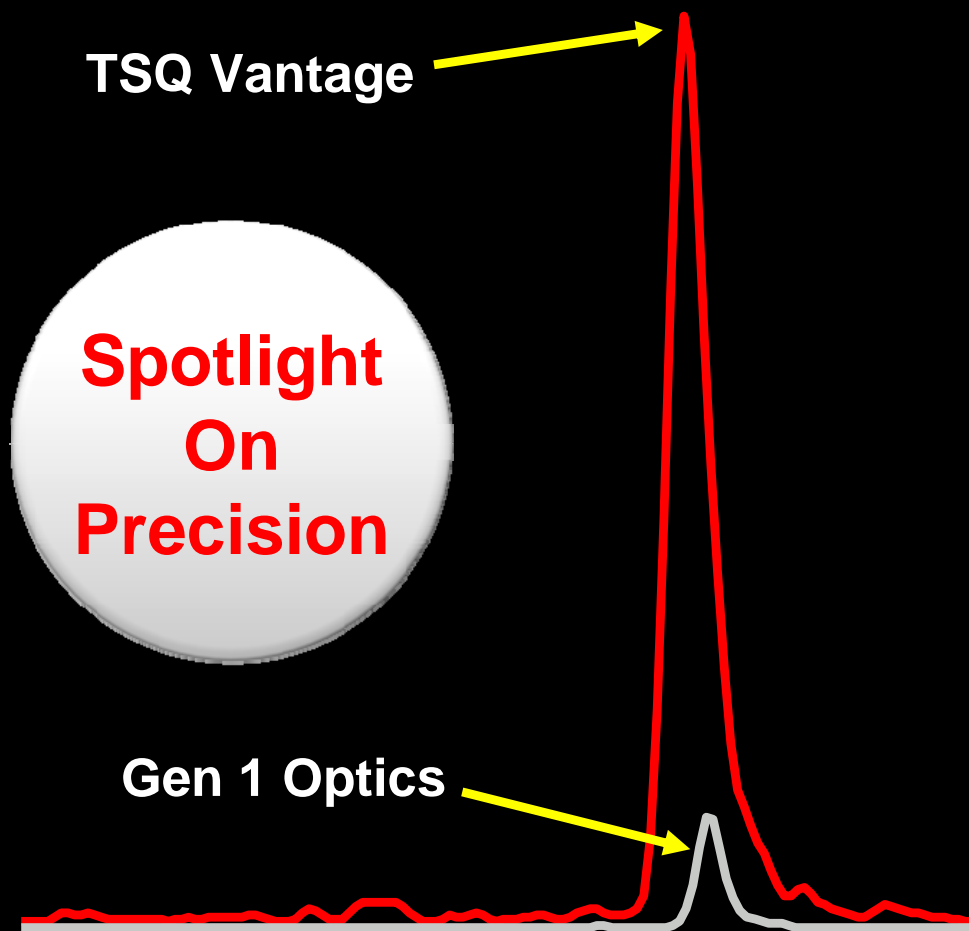
- Stainless steel electrodes and supports for ruggedness and easy cleaning
- Can sonicate entire S-lens assembly
- Efficient removal of solvent laden, extraneous gas to reduce chemical noise
- Patent pending



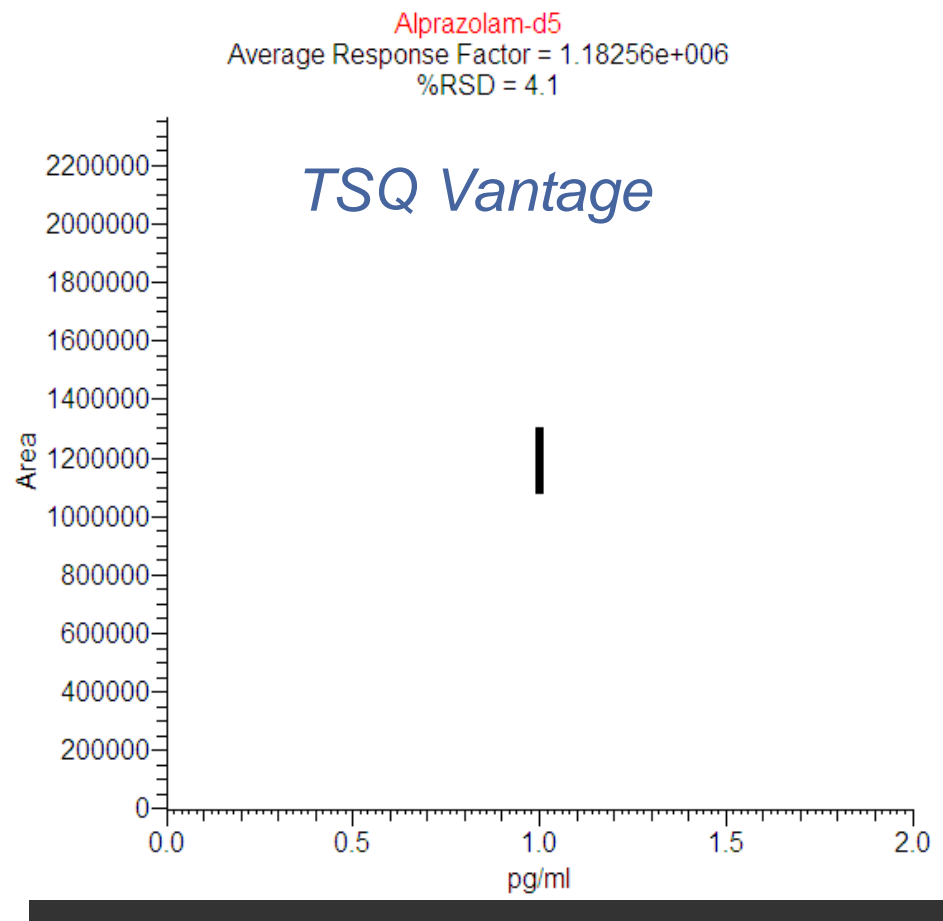
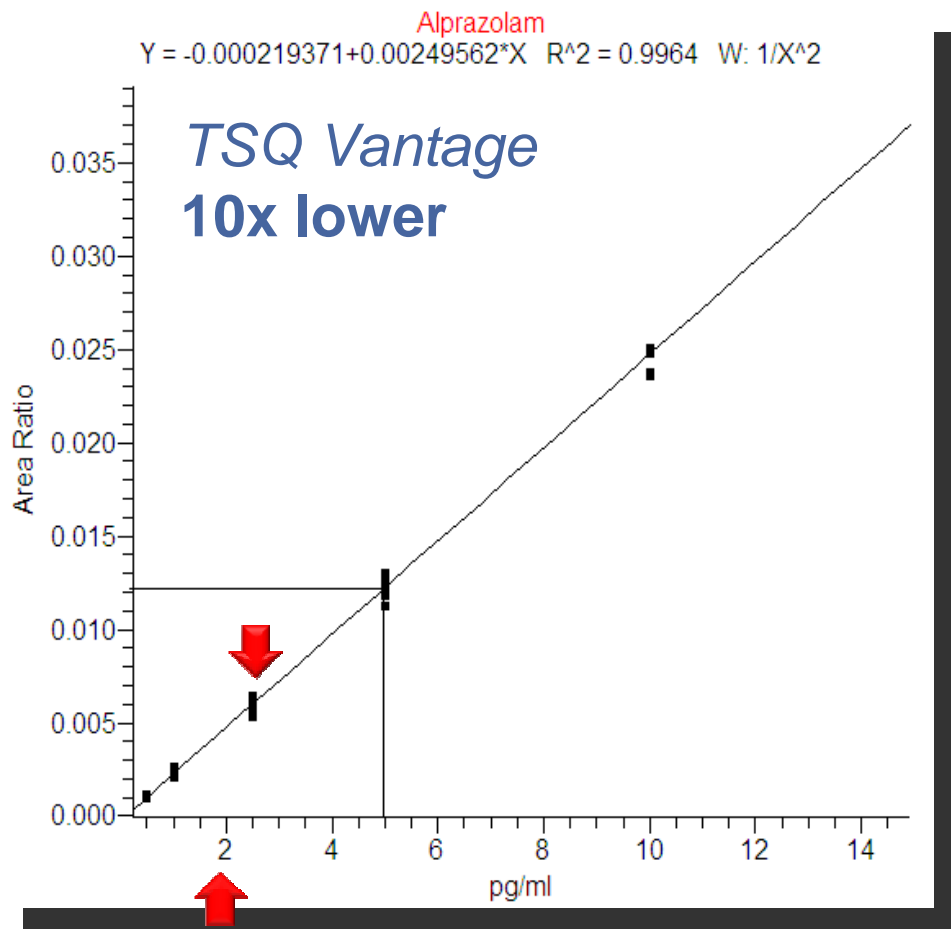
# S-Lens Animation



# TSQ Vantage: *Highest Sensitivity, Lowest Noise*



# TSQ Vantage (500attograms/uL – 10picograms/uL)



TSQ Vantage's superior "**Precision**" is evident at the low end of the std. curve

# Better %CV (Precision) as a result of *Sensitivity*

%CV at 5fg (7.66%) and 10fg (11.48%)

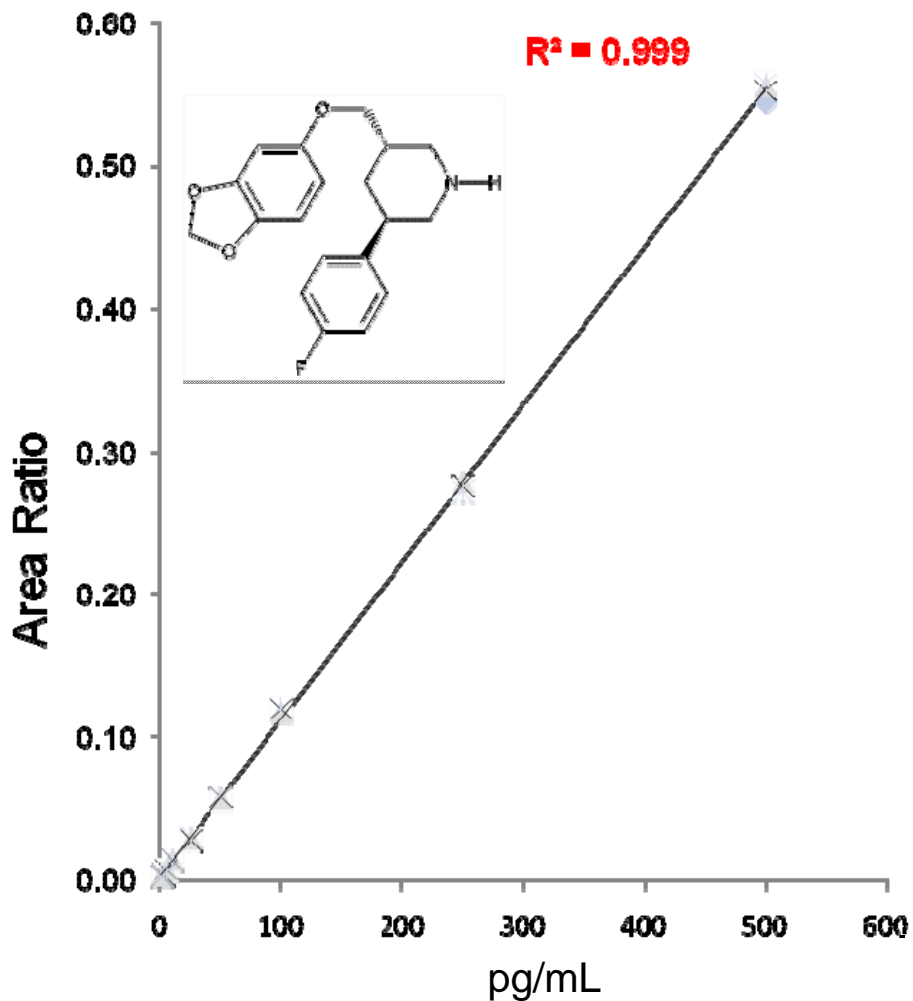


↑  
5fg  
↓  
↑  
10fg  
↓

	FileName	Integration Type	Response	ISTD Area	Specified C	Calculated	% CV	% Diff	Level	Units	Exclude
1	Alprazolam_5pgml_01	Method Settings	964	2313888	0.500	0.455	7.66	-8.98	0.5 pg/ml	pg/ml	<input type="checkbox"/>
2	Alprazolam_5pgml_02	Method Settings	931	2314825	0.500	0.437	7.66	-12.65	0.5 pg/ml	pg/ml	<input type="checkbox"/>
3	Alprazolam_5pgml_03	Method Settings	1251	2313708	0.500	0.606	NA	NA	0.5 pg/ml	pg/ml	<input checked="" type="checkbox"/>
4	Alprazolam_5pgml_04	Method Settings	934	2299934	0.500	0.443	7.66	-11.48	0.5 pg/ml	pg/ml	<input type="checkbox"/>
5	Alprazolam_5pgml_05	Method Settings	1089	2280200	0.500	0.539	7.66	7.71	0.5 pg/ml	pg/ml	<input type="checkbox"/>
6	Alprazolam_5pgml_06	Method Settings	988	2277779	0.500	0.481	7.66	-3.73	0.5 pg/ml	pg/ml	<input type="checkbox"/>
7	Alprazolam_5pgml_07	Method Settings	1095	2248250	0.500	0.540	7.66	8.02	0.5 pg/ml	pg/ml	<input type="checkbox"/>
8	Alprazolam_5pgml_08	Method Settings	1107	2221581	0.500	0.555	7.66	10.97	0.5 pg/ml	pg/ml	<input type="checkbox"/>
9	Alprazolam_5pgml_09	Method Settings	723	2219778	0.500	0.347	NA	NA	0.5 pg/ml	pg/ml	<input checked="" type="checkbox"/>
10	Alprazolam_5pgml_10	Method Settings	1097	2188238	0.500	0.555	7.66	10.95	0.5 pg/ml	pg/ml	<input type="checkbox"/>
11	Alprazolam_1pgml_01	Method Settings	2494	2468775	1.000	1.188	11.48	18.82	1 pg/ml	pg/ml	<input type="checkbox"/>
12	Alprazolam_1pgml_02	Method Settings	2238	2447054	1.000	1.064	11.48	6.37	1 pg/ml	pg/ml	<input type="checkbox"/>
13	Alprazolam_1pgml_03	Method Settings	1908	2459344	1.000	0.893	11.48	-10.66	1 pg/ml	pg/ml	<input type="checkbox"/>
14	Alprazolam_1pgml_04	Method Settings	1877	2414297	1.000	0.910	11.48	-9.05	1 pg/ml	pg/ml	<input type="checkbox"/>
15	Alprazolam_1pgml_05	Method Settings	2296	2399488	1.000	1.140	11.48	14.00	1 pg/ml	pg/ml	<input type="checkbox"/>
16	Alprazolam_1pgml_06	Method Settings	1749	2376780	1.000	0.847	11.48	-15.26	1 pg/ml	pg/ml	<input type="checkbox"/>
17	Alprazolam_1pgml_07	Method Settings	2008	2375708	1.000	0.978	11.48	-2.18	1 pg/ml	pg/ml	<input type="checkbox"/>
18	Alprazolam_1pgml_08	Method Settings	1801	2389371	1.000	0.866	11.48	-13.42	1 pg/ml	pg/ml	<input type="checkbox"/>
19	Alprazolam_1pgml_09	Method Settings	2087	2347034	1.000	1.033	11.48	3.31	1 pg/ml	pg/ml	<input type="checkbox"/>
20	Alprazolam_1pgml_10	Method Settings	2058	2364277	1.000	1.010	11.48	0.96	1 pg/ml	pg/ml	<input type="checkbox"/>
21	Alprazolam_2-5pgml_0	Method Settings	4912	2396032	2.500	2.482	9.11	-0.71	2.5 pg/ml	pg/ml	<input type="checkbox"/>
22	Alprazolam_2-5pgml_0	Method Settings	4960	2396636	2.500	2.494	9.11	0.25	2.5 pg/ml	pg/ml	<input type="checkbox"/>

n= 10; More signal = higher precision

# Reproducibility Study (Inter-day, Paroxetine in Rat Plasma)



*TSQ Vantage*

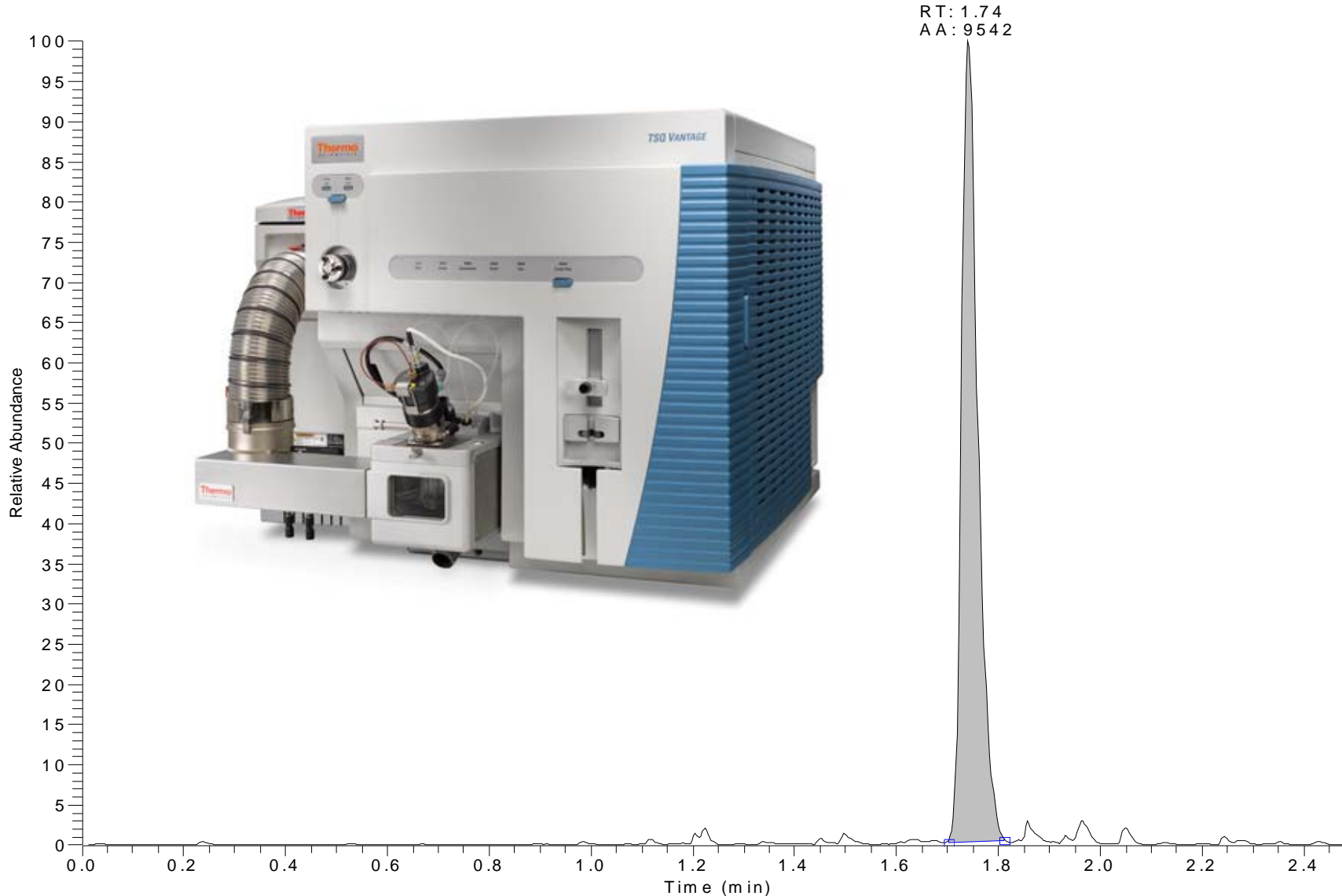
Conc (pg/mL)	Calculated Conc (pg/mL)					Avg Calc. Conc. ( 5d)	%CV (5d)
	Day 1	Day 2	Day 3	Day 4	Day 5		
1.0	1.163	1.170	1.063	0.925	0.962	1.057	6.62%
2.5	2.521	2.689	2.423	2.571	2.435	2.528	4.31%
5.0	5.084	5.062	5.194	5.249	4.977	5.113	2.12%
10.0	10.377	10.675	10.087	10.470	10.632	10.448	2.25%
25.0	25.540	24.929	24.831	24.982	24.627	24.982	1.36%
50.0	50.275	49.038	50.199	50.052	49.801	49.873	1.00%
100.0	108.333	105.863	104.106	108.246	105.643	106.438	1.71%
250.0	252.346	248.579	249.451	251.288	248.419	250.016	0.69%
500.0	498.107	499.085	497.290	503.946	499.066	499.499	0.52%

*\*data represents 1000 plasma samples*

Overlay of 5 std. curves, run once every day  
(200 plasma samples per day for 5d\*)

# 1 fg of Neat Paroxetine on column by FAIMS

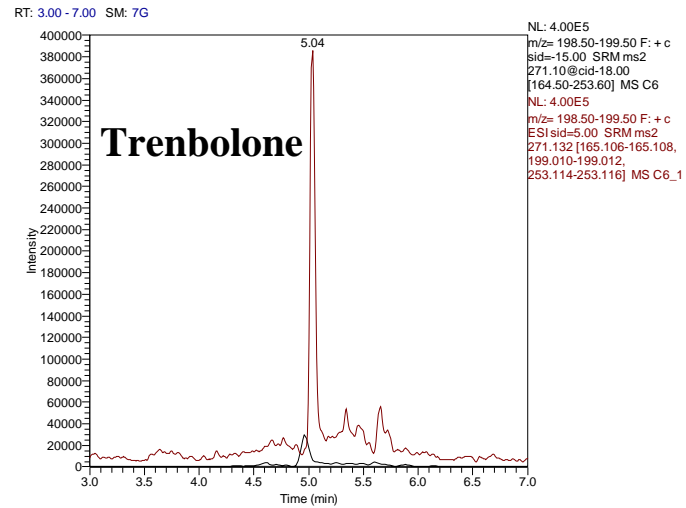
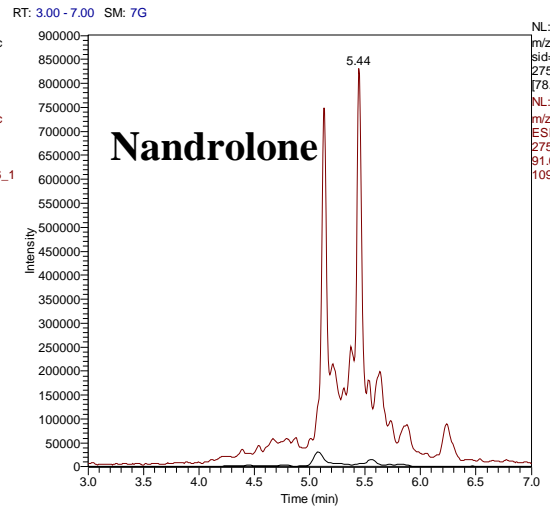
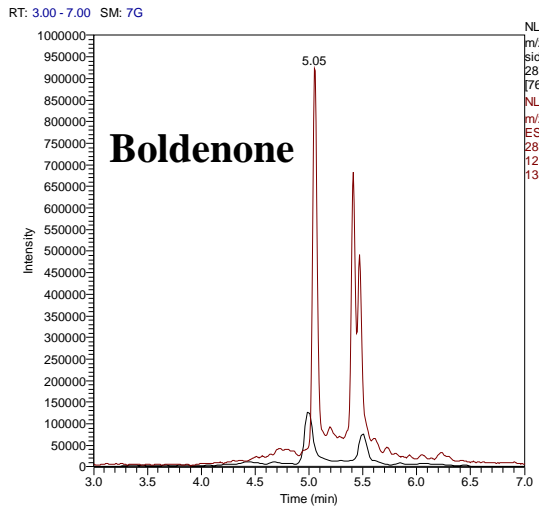
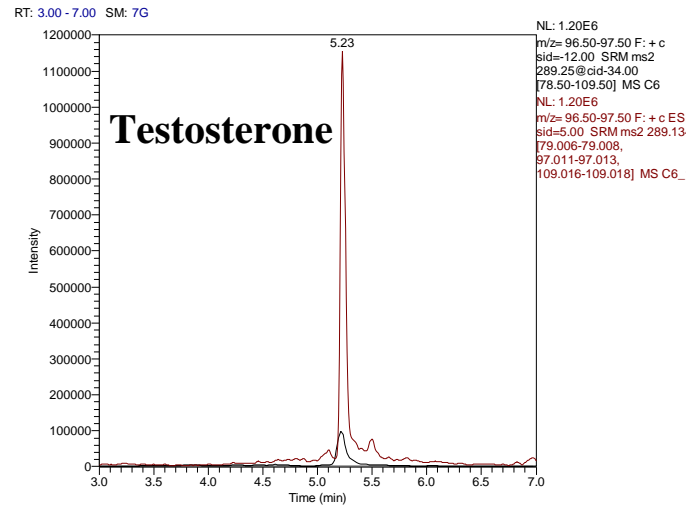
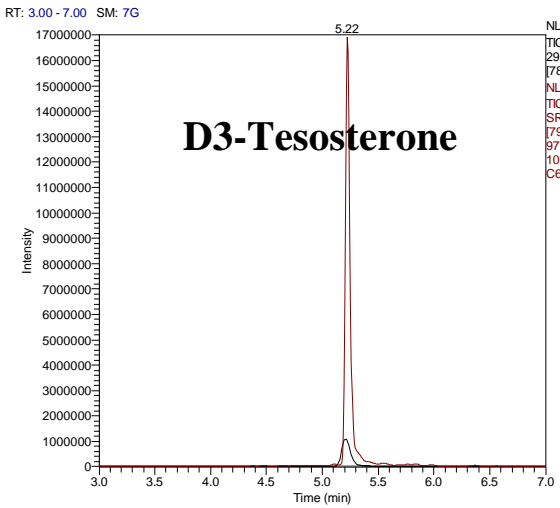
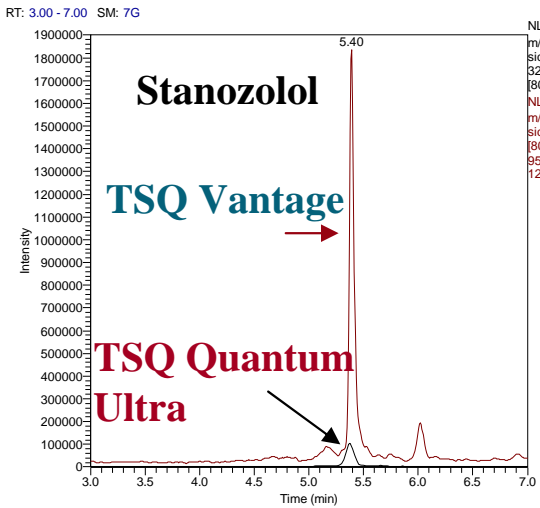
RT: 0.00 - 2.50



RT: 1.74  
AA: 9542

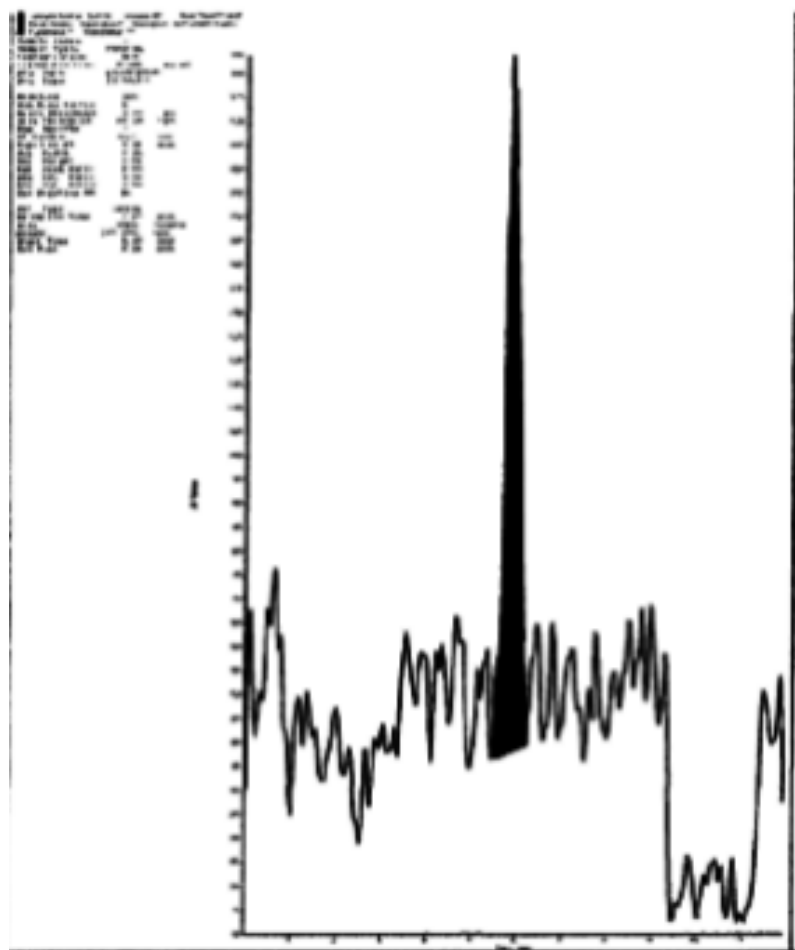
NL: 4.24E3  
TIC F: + c E S I S R M  
ms2 330.151  
[192.099-192.101]  
MS IC IS  
ParoxetineNeatSRM  
\_003

# TSQ Vantage vs TSQ Quantum Ultra – 50 pg/mL

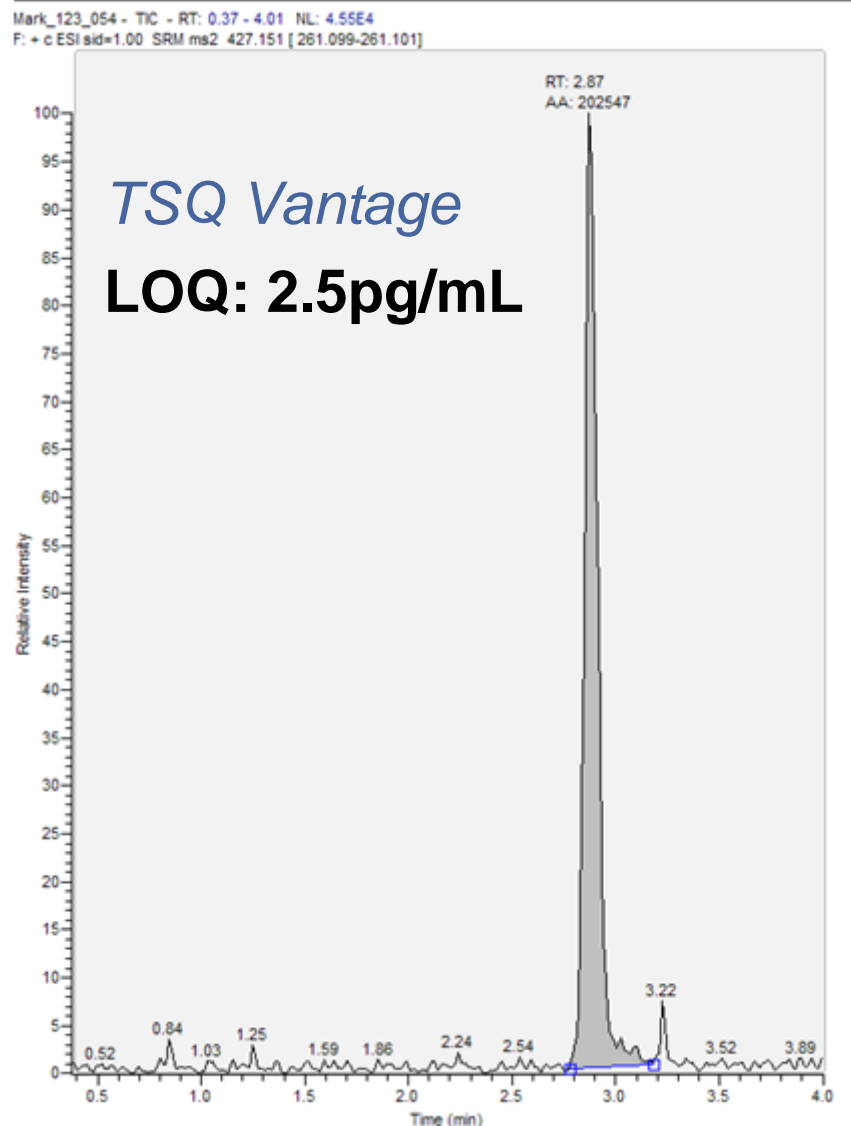


# TSQ Vantage vs Competitor A

## Sample Chromatograms (Current LLOQ)



Compound 1 (50 pg/mL)



*TSQ Vantage*

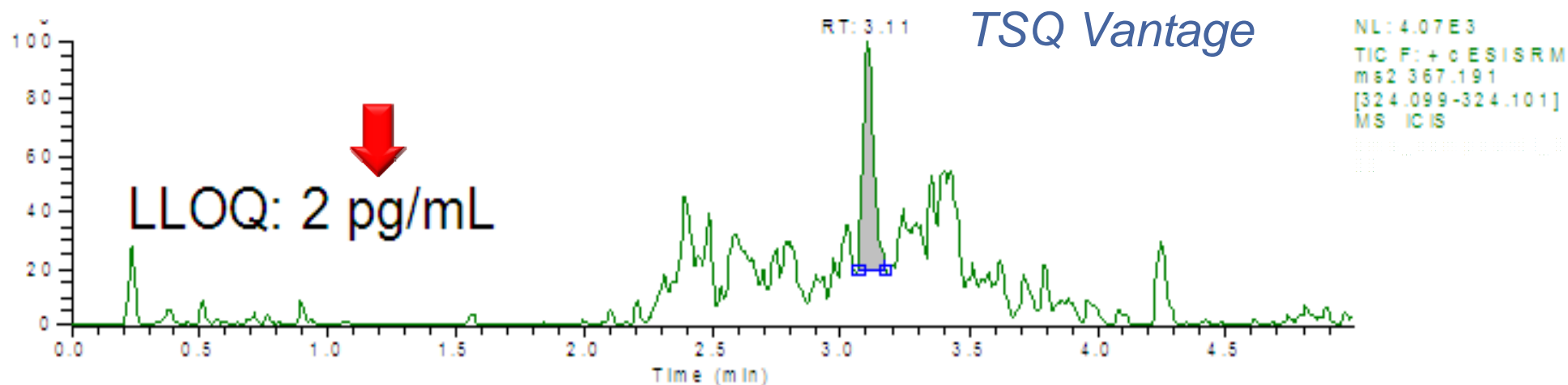
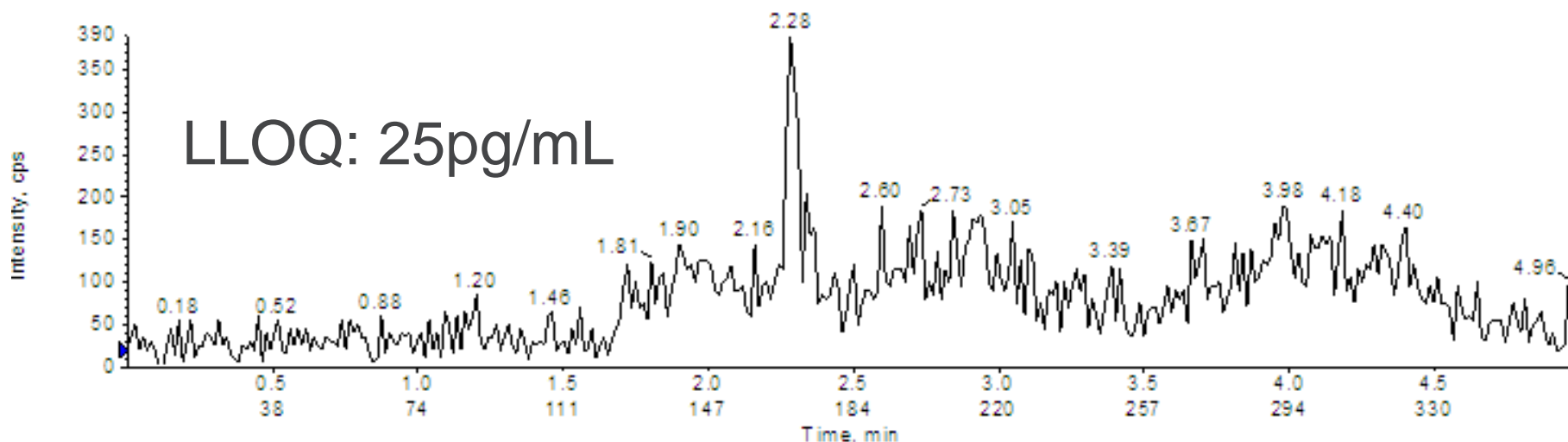
**LOQ: 2.5pg/mL**

**Data obtained at unit resolution (0.7FWHM) on TSQ Vantage**

# TSQ Vantage vs Competitor A (Peptide X in Rat Plasma)

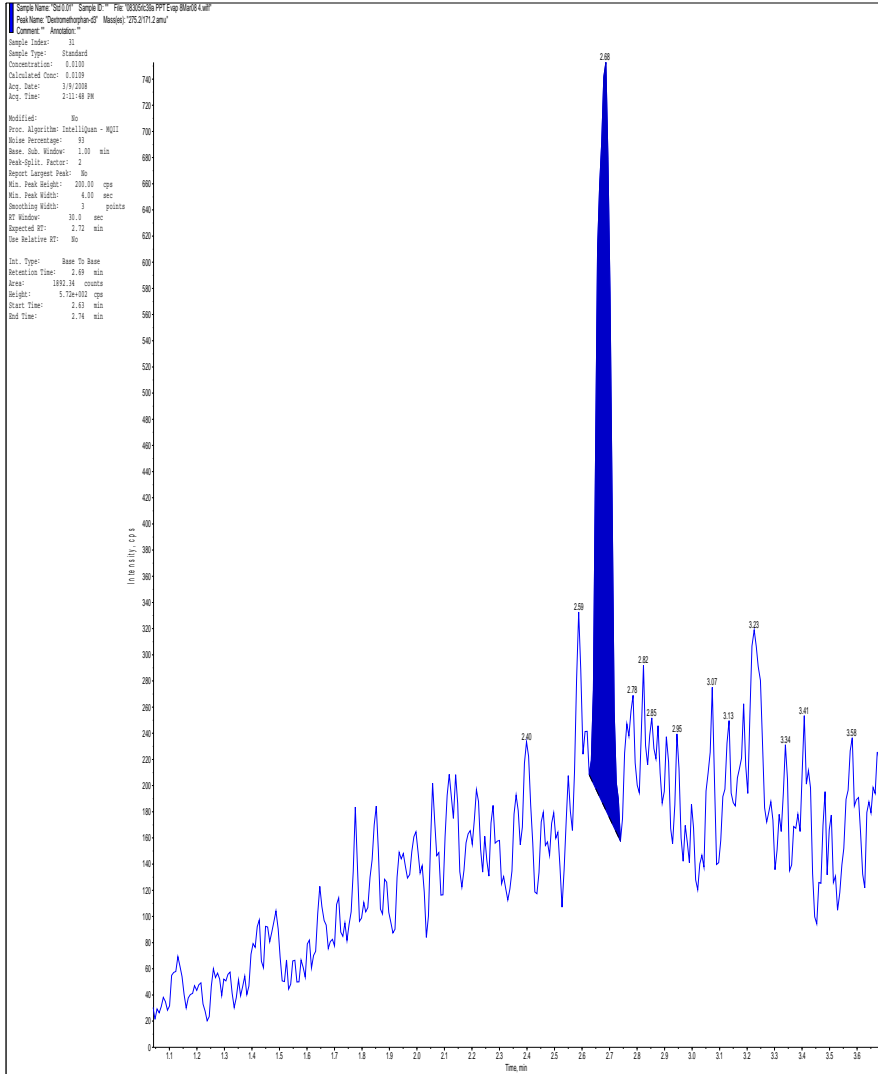
XIC of +MRM (4 pairs): 367.2/324.1 amu from Sample 2 (HCP\_10March08\_HP002) of HCP\_10March08\_...

Max. 390.0 cps.

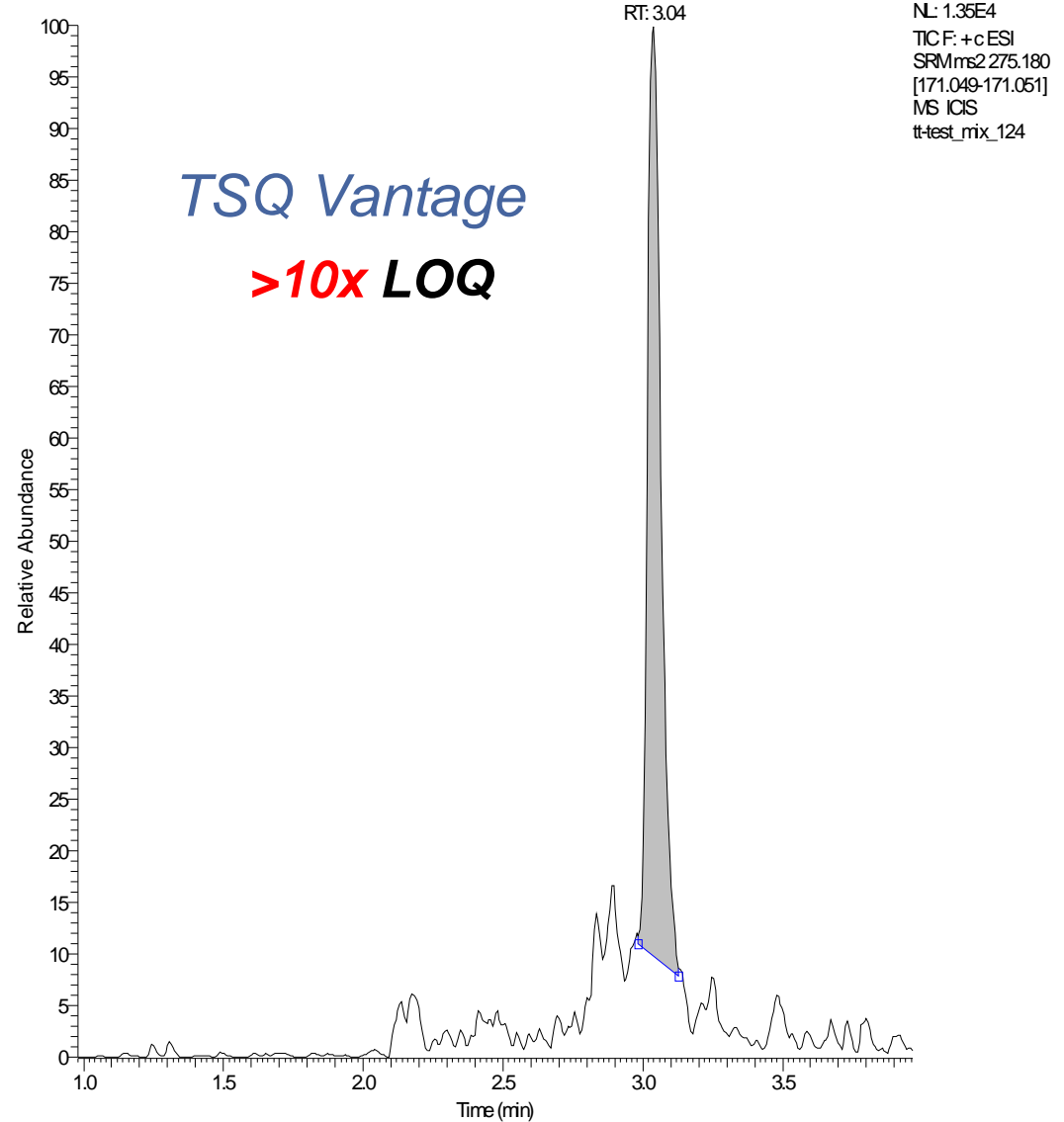


**Data obtained at unit resolution (0.7FWHM) on TSQ Vantage**

# Dextromethorphan-d3 in Rat Plasma (LLOQ Comparison at 0.01 ng/ml)

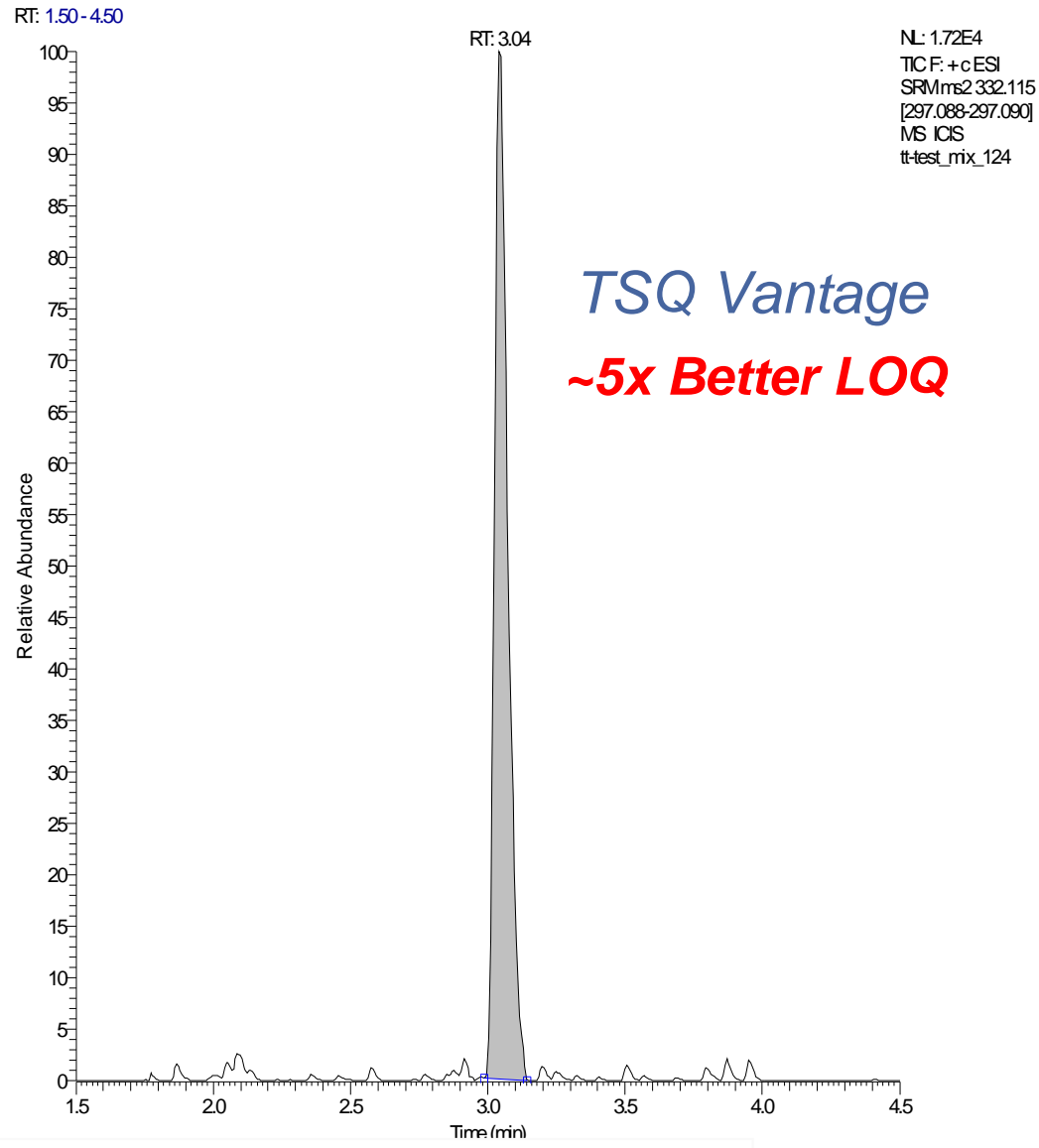
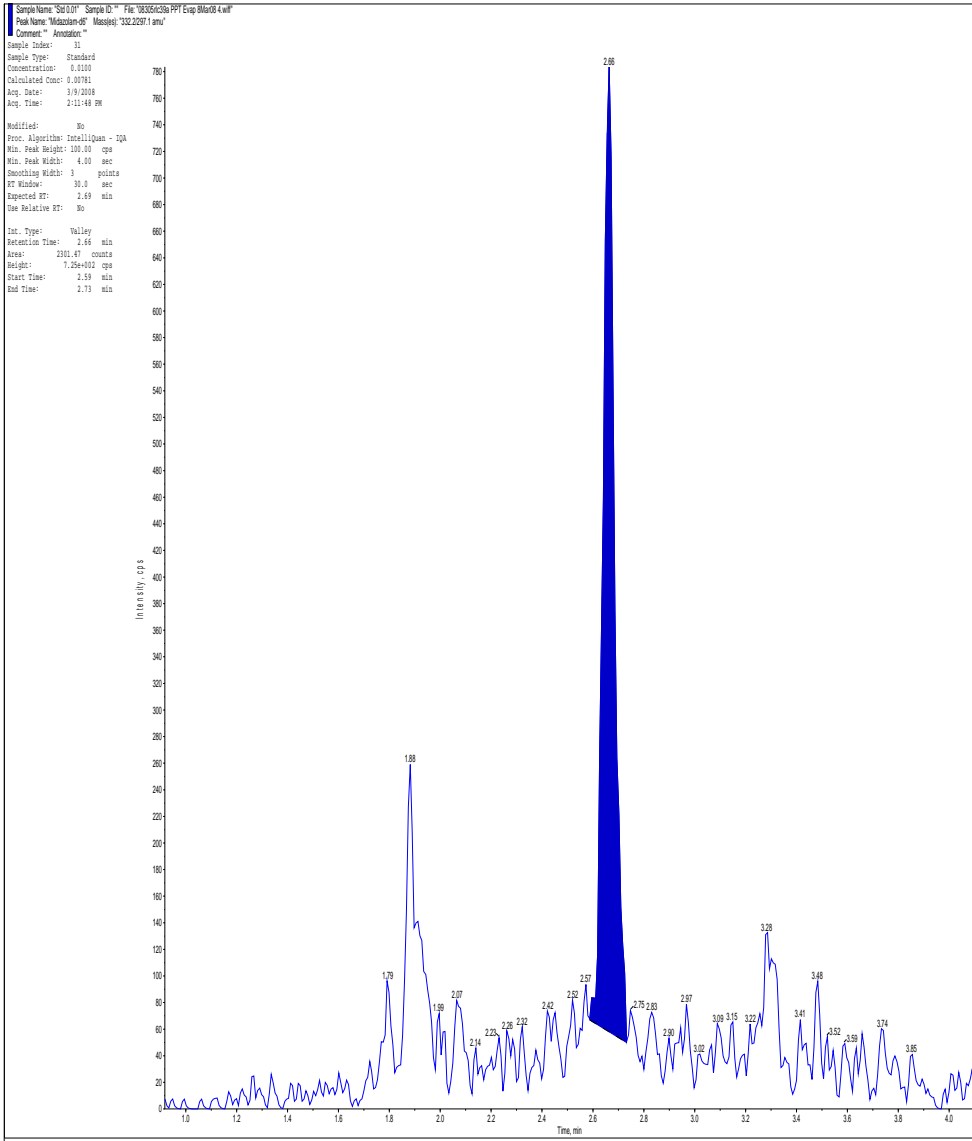


RT: 0.98-3.96



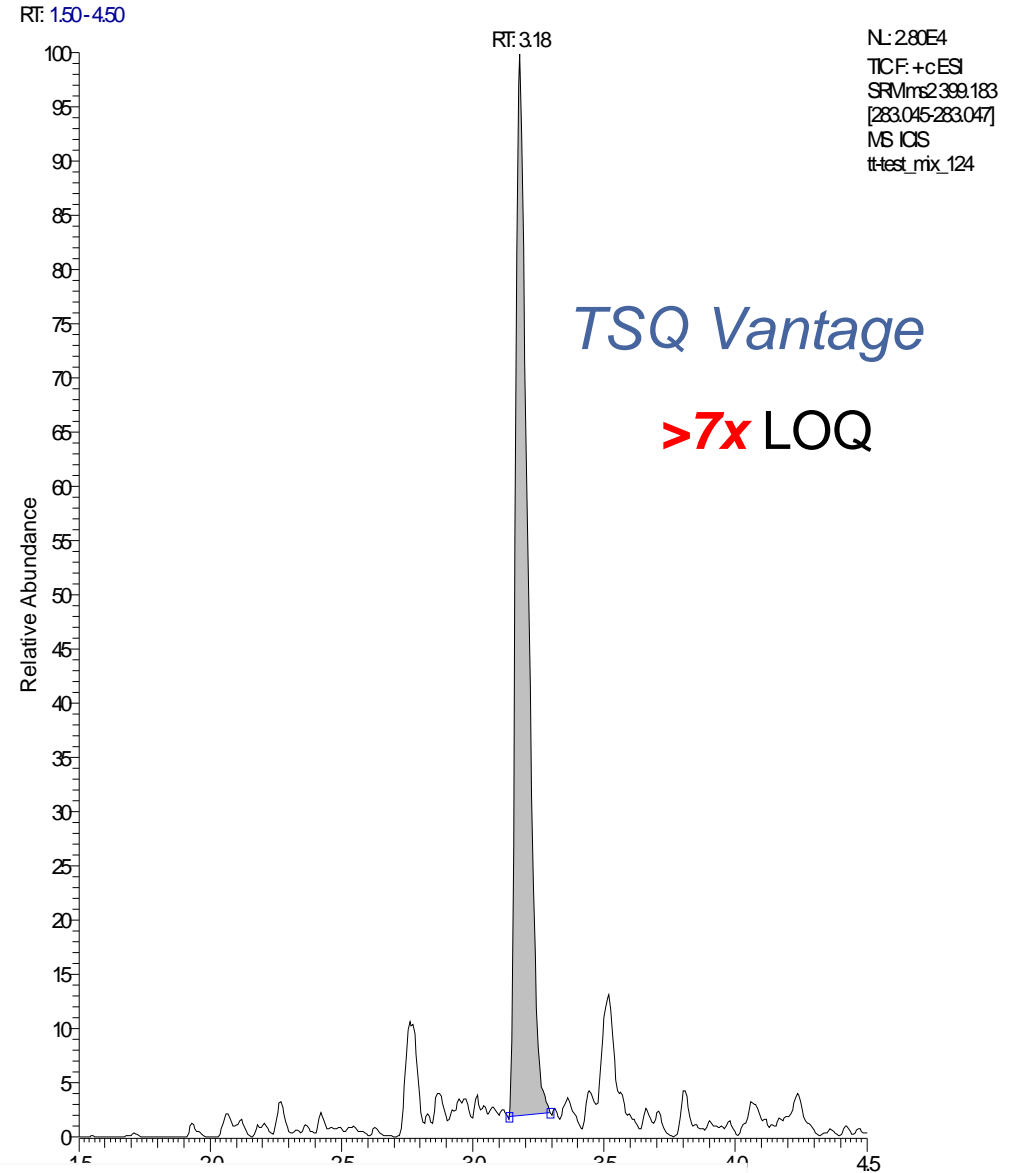
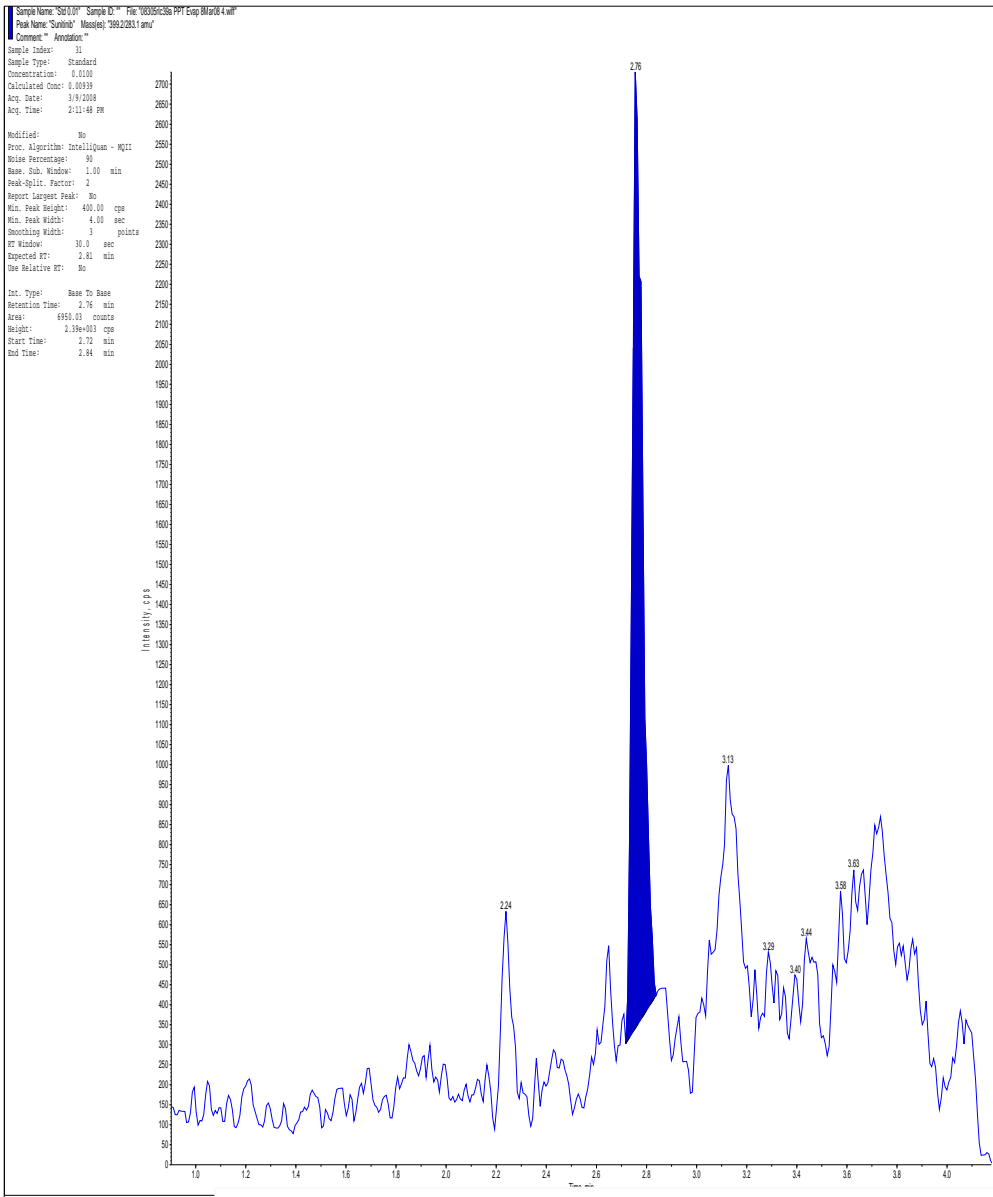
**Data obtained at unit resolution (0.7FWHM) on TSQ Vantage**

# Midazolam-d6 (LLOQ comparison at 0.01 ng/ml)



**Data obtained at unit resolution (0.7FWHM) on TSQ Vantage**

# Compound Z (LLOQ Comparison at 0.01 ng/ml)



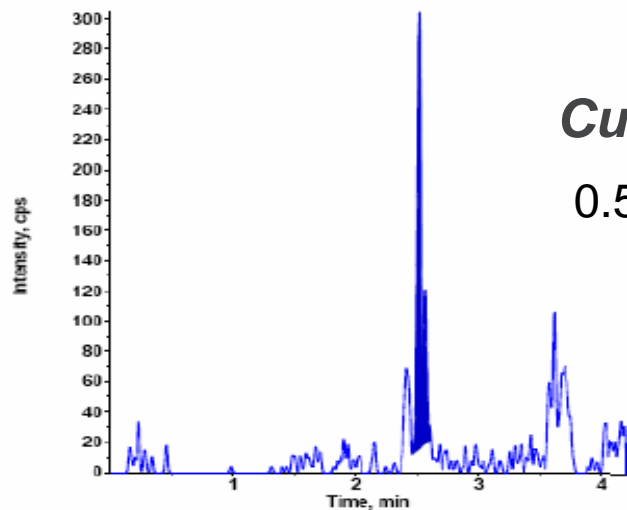
**Data obtained at unit resolution (0.7FWHM) on TSQ Vantage**

# TSQ Vantage vs Competitor (Split Sample- Pure Std)

```
Sample Name:
Sample Type: Standard
Concentration: 0.500 ng/mL
Calculated Conc: 0.509 ng/mL
Acq. Date: 5/25/2007
Acq. Time: 1:09:33 ET

Modified: No
Proc. Algorithm: Analyst Classic
Bunching Factor: 3
Noise Threshold: 25.00 cps
Area Threshold: 200.00 cps
Num. Smoother: 10
Sep. Width: 0.20
Sep. Height: 0.01
Exp. Peak Ratio: 5.00
Exp. Adj. Ratio: 4.00
Exp. Val. Ratio: 3.00
RT Window: 6.00 sec
Expected RT: 2.52 min
Use Relative RT: No

Int. Type: Base To Base
Retention Time: 2.52 min
Area: 876 counts
Height: 2.89e+002 cps
Start Time: 2.46 min
End Time: 2.62 min
```

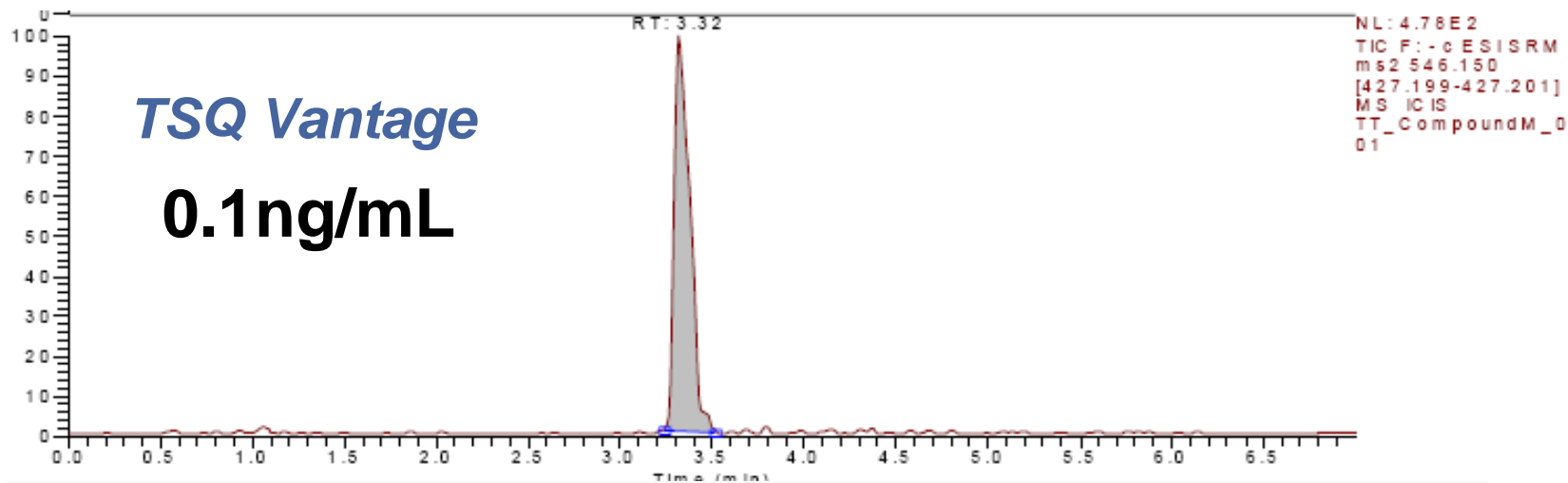


*Customer name with-held*

0.5ng/mL



Note: 0.1 ng/ml is indistinguishable from the control blank



**TSQ Vantage**

**0.1ng/mL**

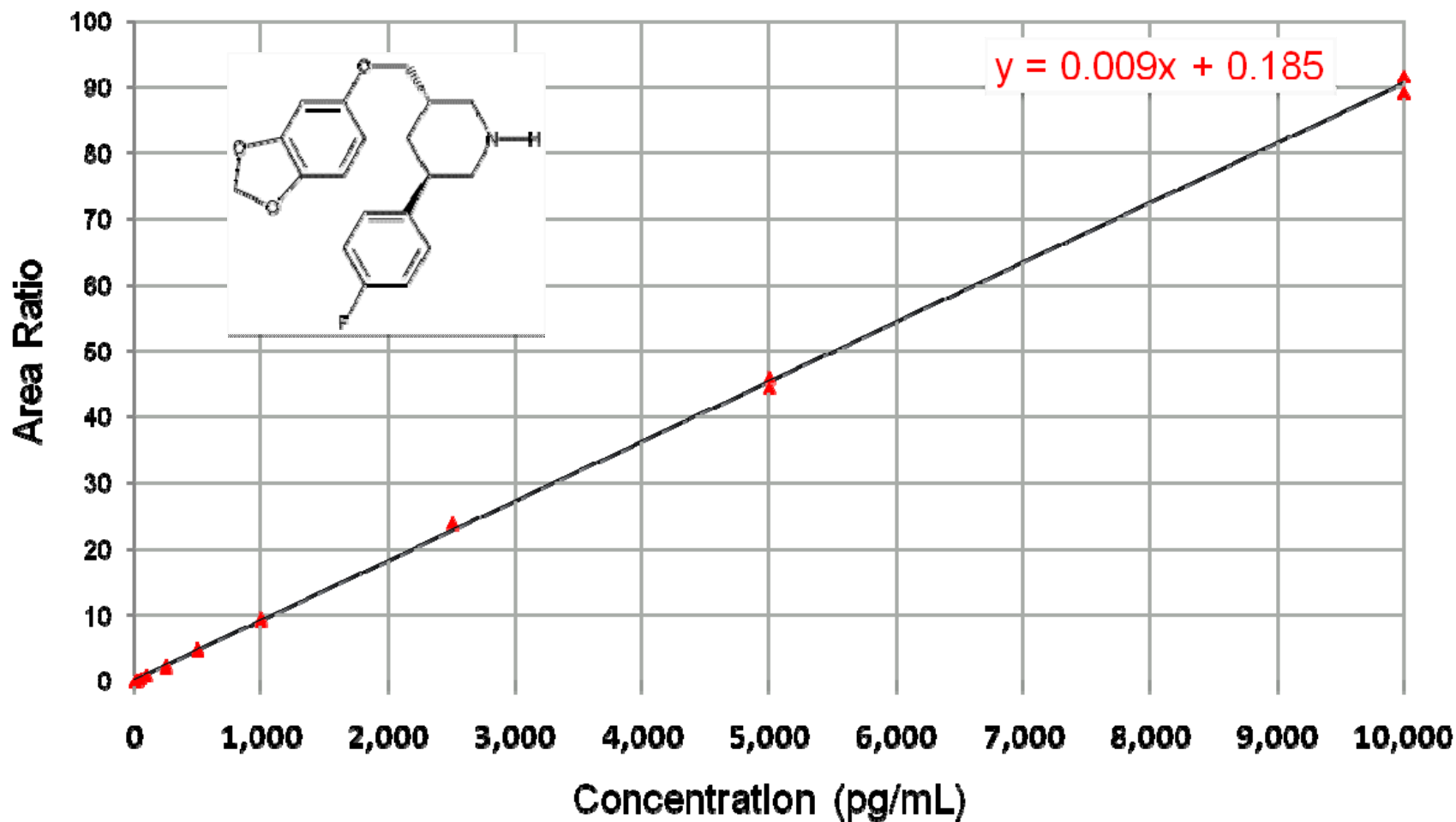
**Data obtained at unit resolution (0.7FWHM) on TSQ Vantage**

# TSQ Vantage: *Highest Sensitivity, Lowest Noise*

***Reproducibility  
&  
Linear Dynamic  
Range***

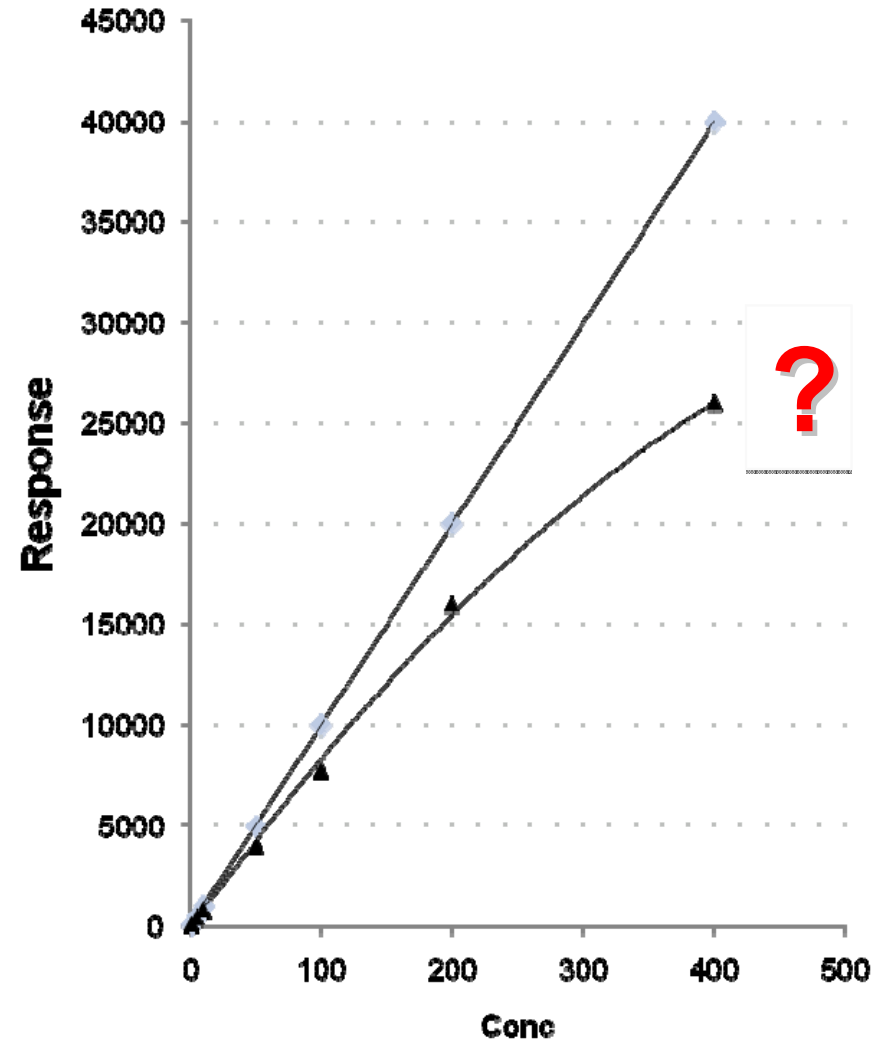


# Excellent Linear Dynamic Range (Small Molecules)



# Linearity & Dynamic Range

- Detector response should be linear
- What causes non-linearity?
  - Detector saturation (pulse counting issues)
  - ESI saturation
  - Matrix effects
- Having a wide dynamic range **saves time**
  1. One standard curve can cover a wider range
  2. No need to re-assay
  3. No dilutions needed
  4. Pre-Clinical & Targeted Peptide Quan



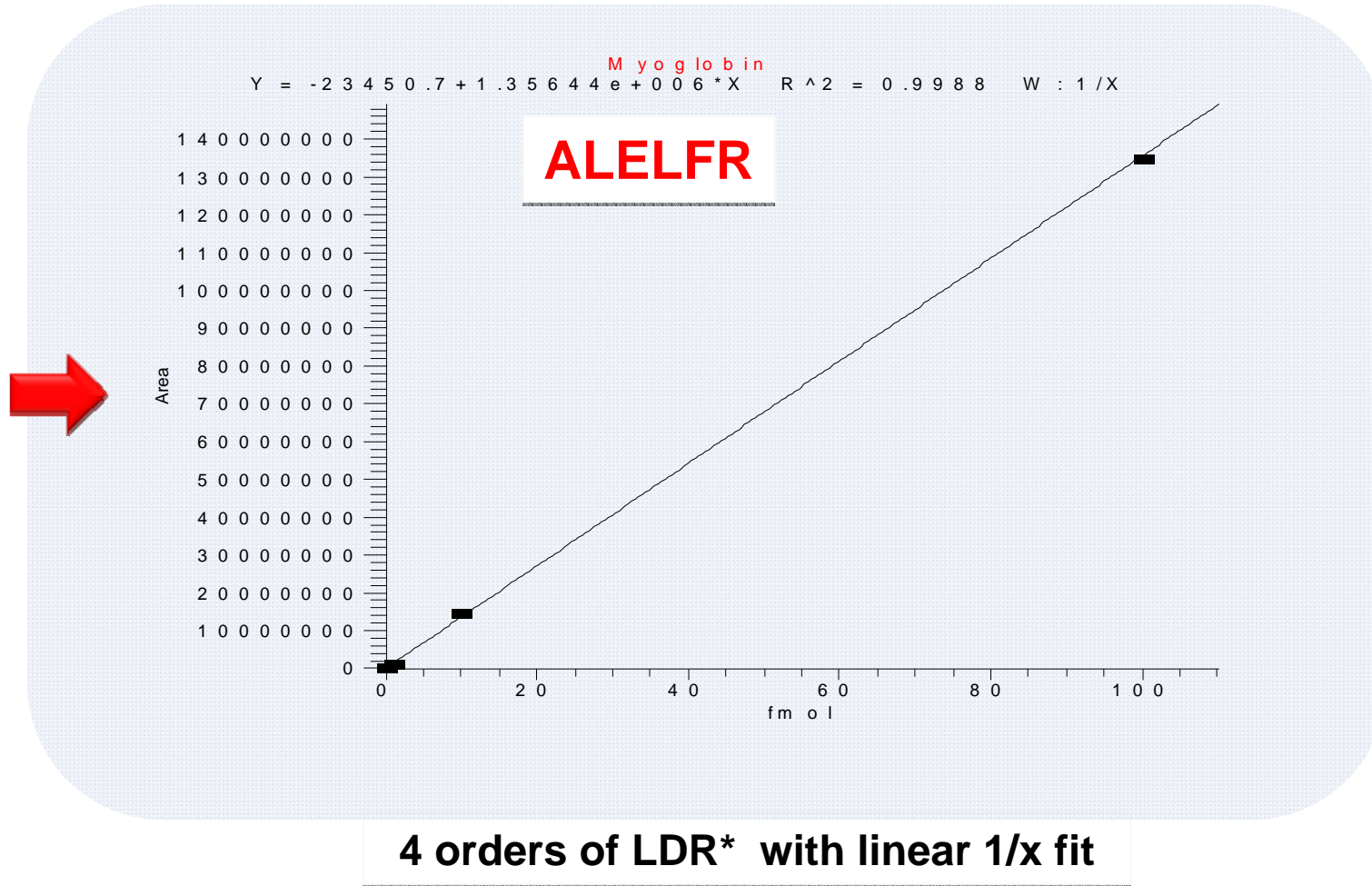
# Targeted Peptide Quantitation

## ■ Basic Challenges:

- Required: *High sensitivity with wide dynamic range*
- Complex mixture with wide dynamic range of proteins
- No internal standard – often dependent on absolute signal only!
- Low flow rates (nanospray / microspray applications)
  - *Fast scanning collision cell*
  - *No X-talk in collision cell*



# Proof Statement 1: Targeted Peptide Quantitation



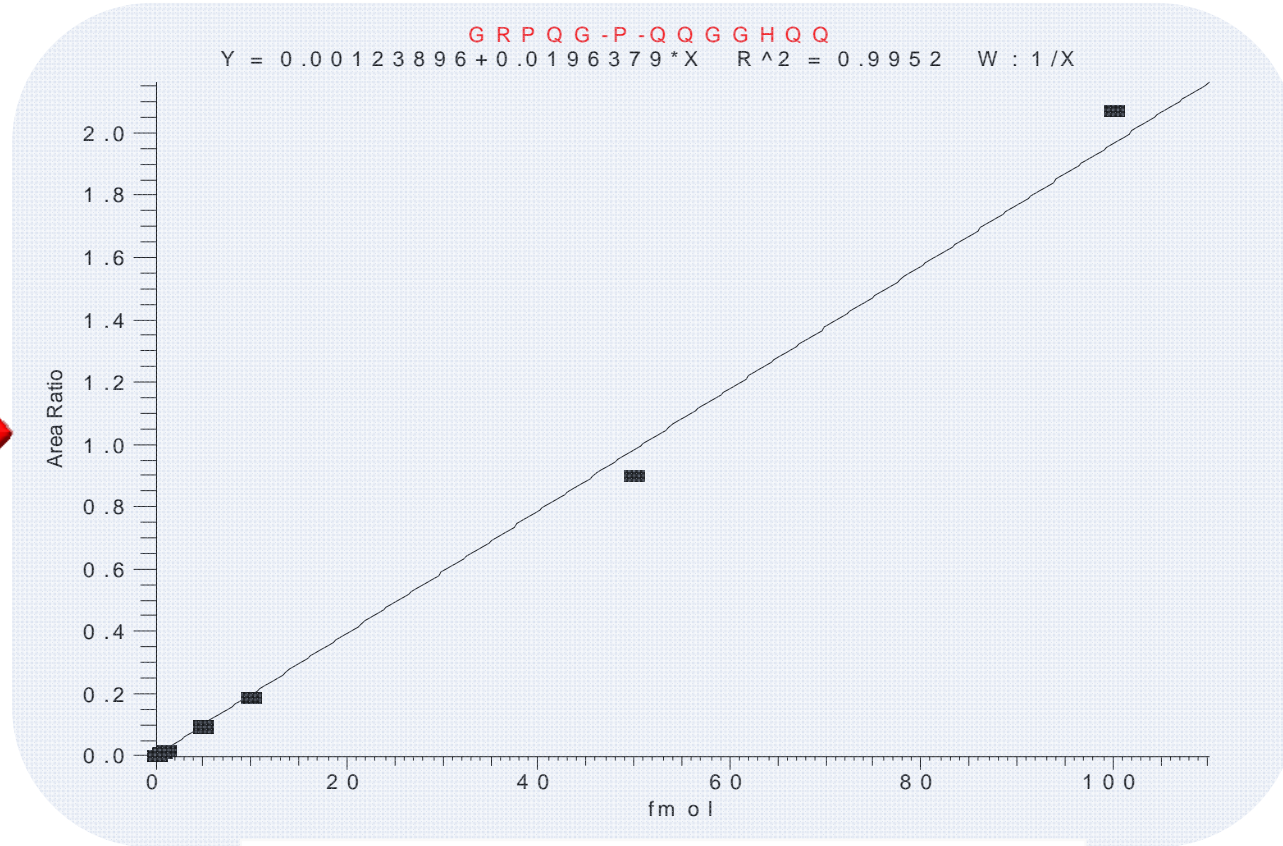
Linear calibration curve (range 0.01–100 fmol) of **Myoglobin** spiked into Ecoli digest matrix.  
*Note: Absolute area counts used (No ISTD).*

# Proof Statement 1: Targeted Peptide Quantitation

	10 attomol	50 attomol	100 attomol	1 fmol	10 fmol	100 fmol
run 1	3188	22459	44645	781101	16223693	170848122
run 2	2427	23856	38705	777105	14635180	137929457
run 3	3874	22333	62007	901912	12157105	134856941
run 4	2537	17128	51554	1071270	12060978	140034146
run 5	1880	14797	40517	954965	11746329	129911605
Average	2781	20115	47486	897271	13364657	142716054
% CV	27.6	19.5	20.0	13.8	14.8	11.3

*Nanospray application; 1uL injected; flow rate 300nL/min*

# Proof Statement 2: Targeted Peptide Quantitation



**4 orders of LDR\* with linear 1/x fit**

Linear calibration curve (range 0.02–100 fmol) of isotopic labeled peptide **GRPQGP\*QQGGHQQ** spiked in the pooled saliva sample.

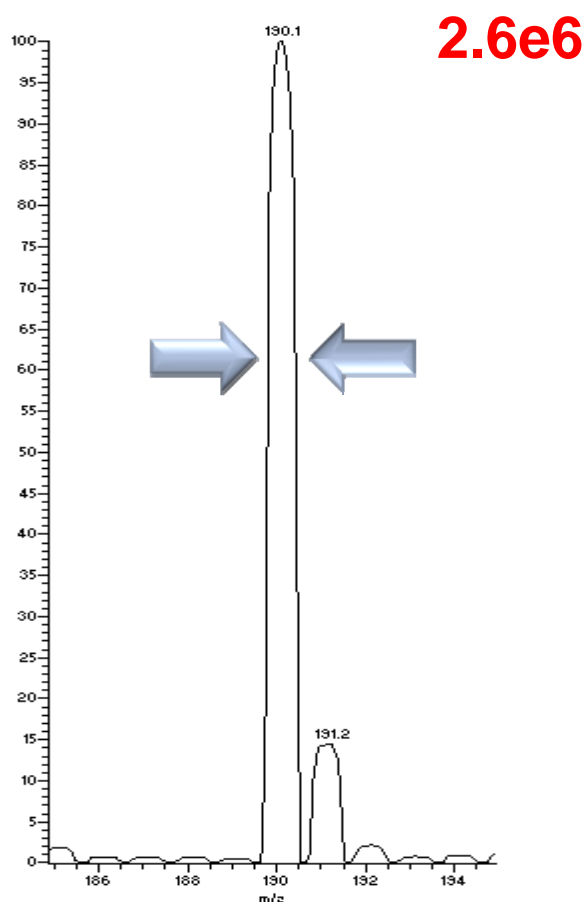
*Nanospray application; 1uL injected; flow rate 300nL/min*

# Targeted Peptide Quantitation: Summary

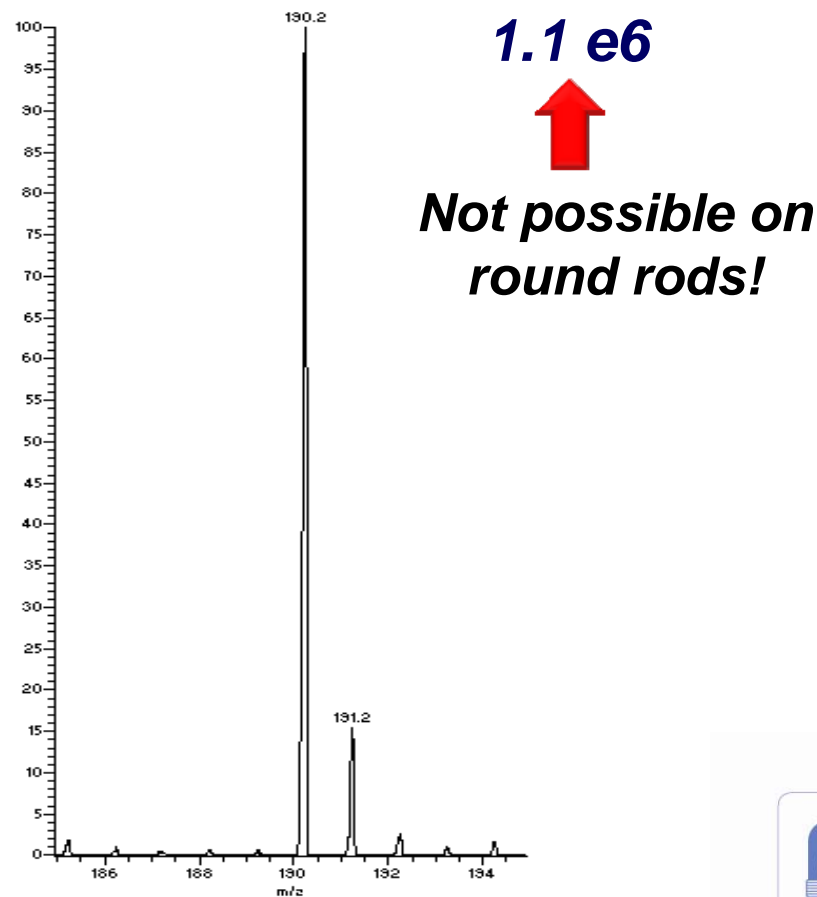
- Highly sensitive peptide quantitation
- **H-SRM advantage**
- > 4 orders of *Linear* Dynamic Range with or without internal standard
- Exceptional low flow rate performance



# H-SRM Refresher (Hyperbolic Rod Advantage)



0.7u FWHM



Not possible on  
round rods!

0.1u FWHM

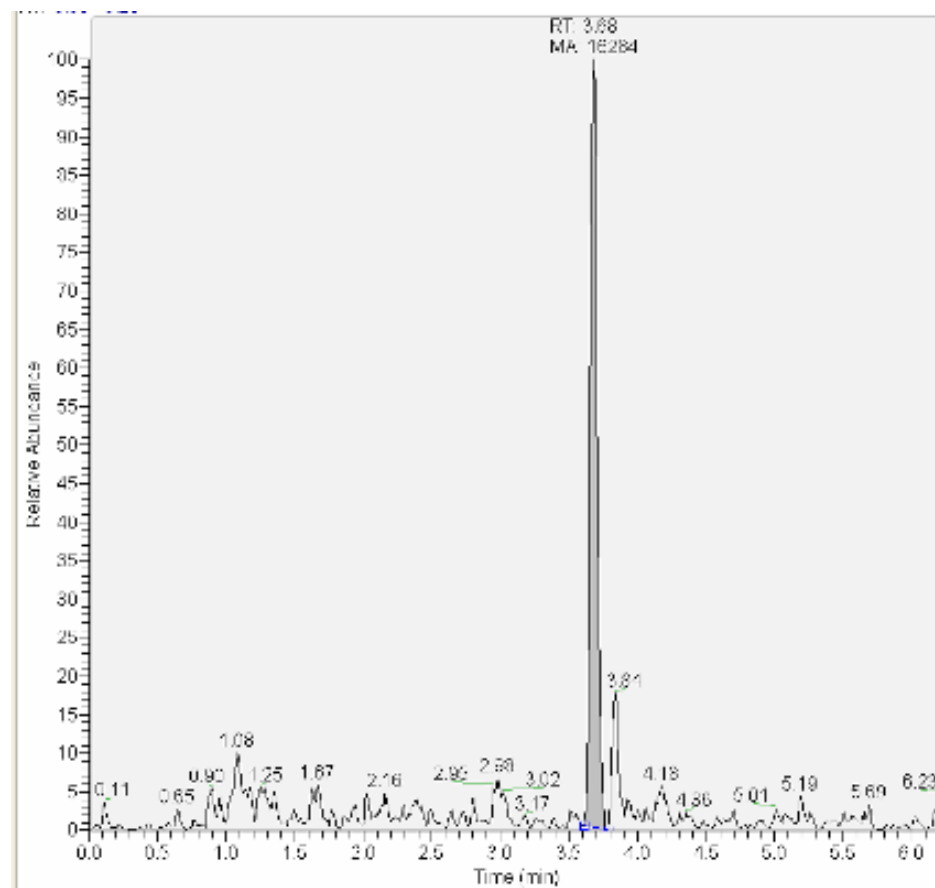
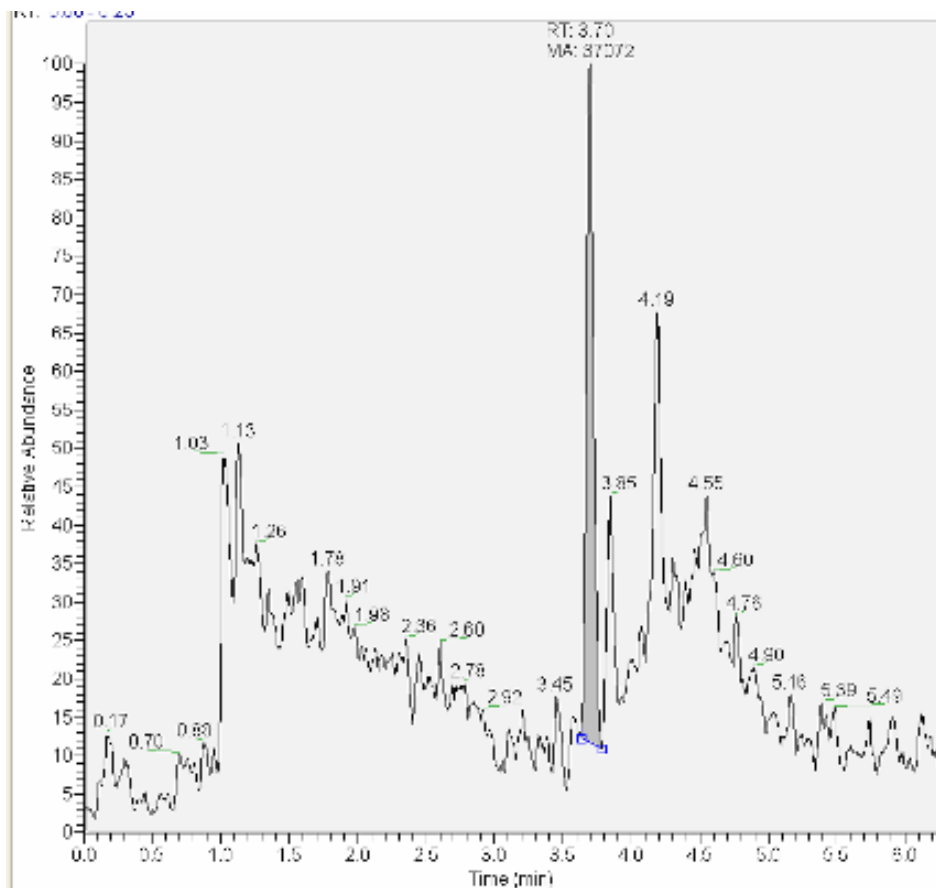
*Quadrupoles matter!*



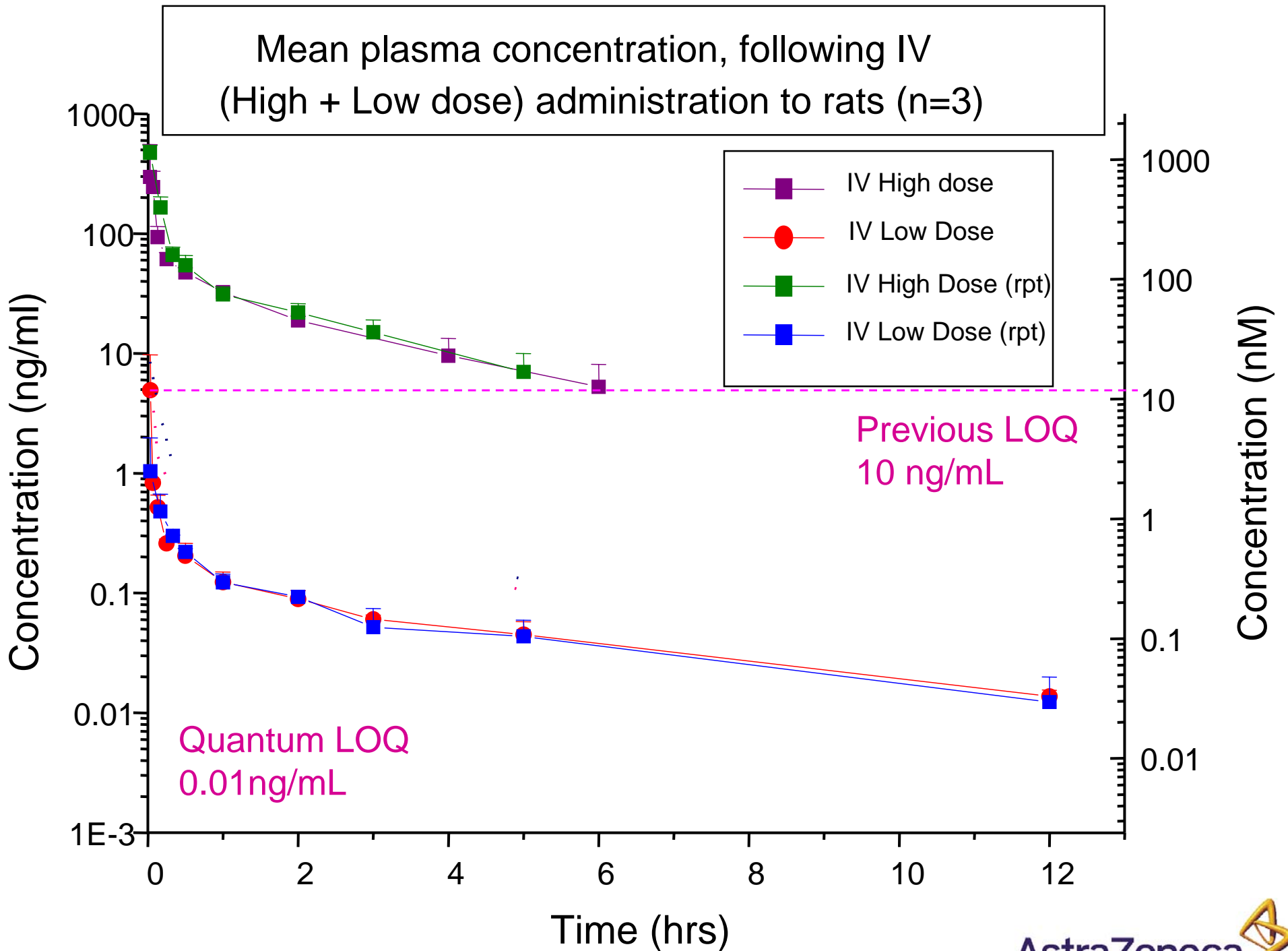
# High resolution Comparison

0.7 fwhm

0.1 fwhm

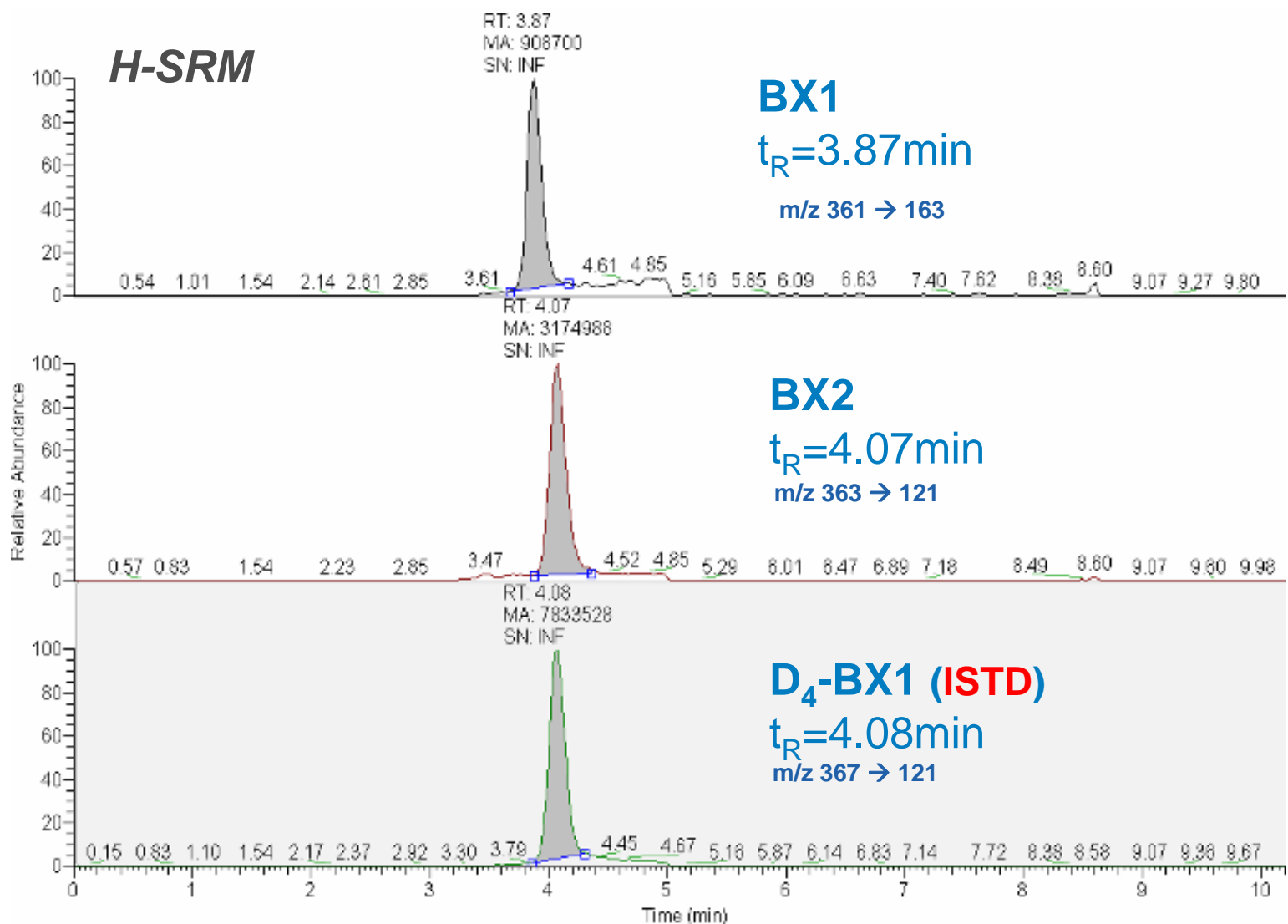


0.5ng/ml hydrolysed Amino Acid standard



# SPE-LC-Quantum MS/MS method application

## Analysis of BX1 & BX2 50 $\mu$ l Human Plasma



- Detection of endogenous biomarkers
- RSD < 10%
- Analytical Method OK

# TSQ Vantage: *Highest Sensitivity, Lowest Noise*

***Robustness***

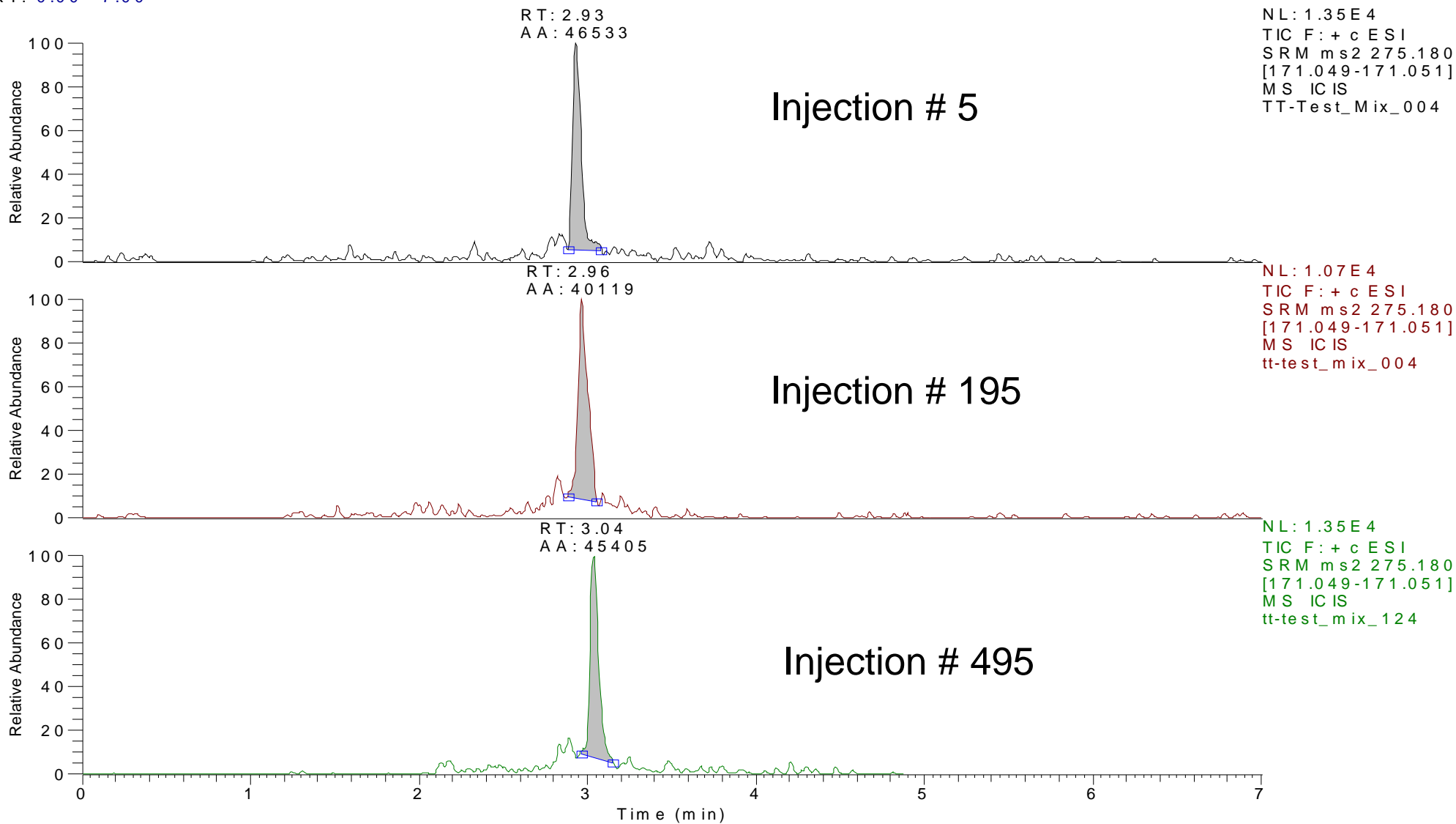


# Dextromethorphan-d3 (Robustness at LLOQ 0.01 ng/ml )

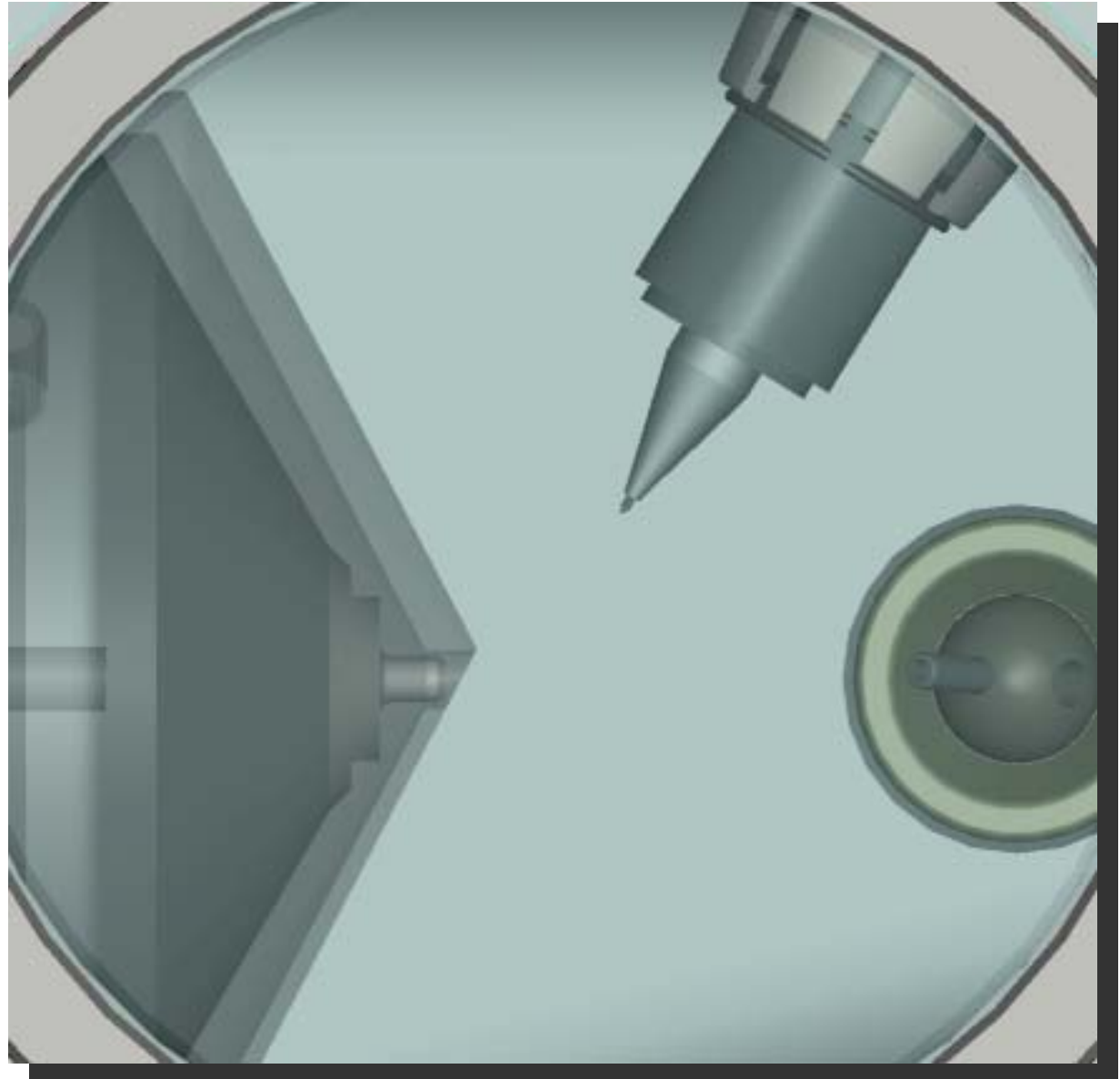
c:\xcalibur\...\rawfiles\tt-test\_mix\_004  
0.01 ng/ml

3/11/2008 1:44:42 PM

RT: 0.00 - 7.00

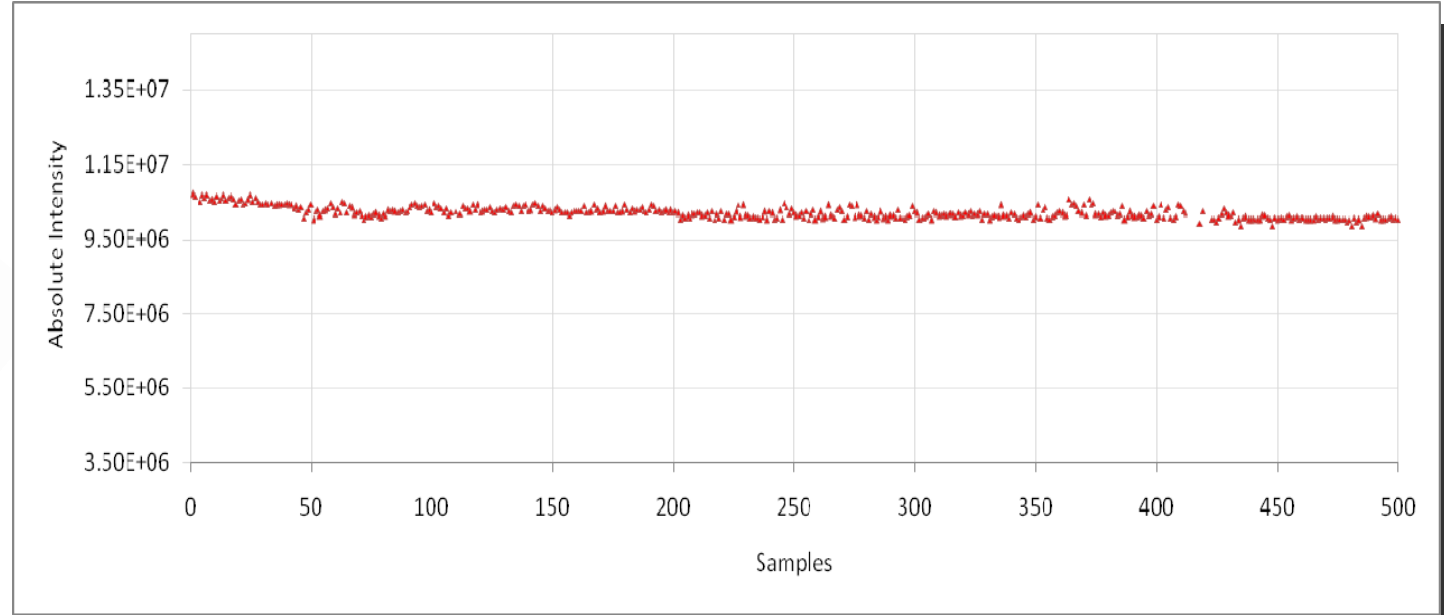


# Ion Source Robustness for Biological Matrix

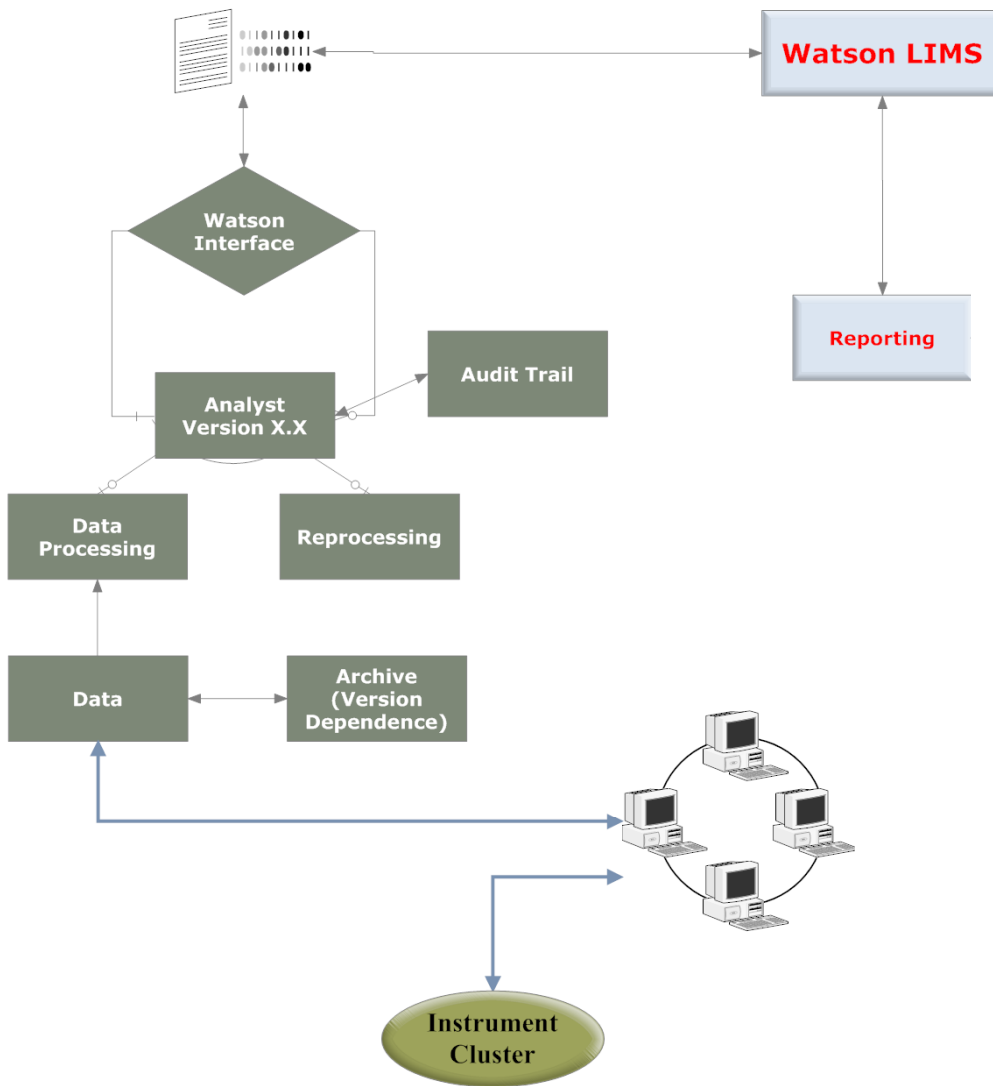


# Feature Summary: Robustness

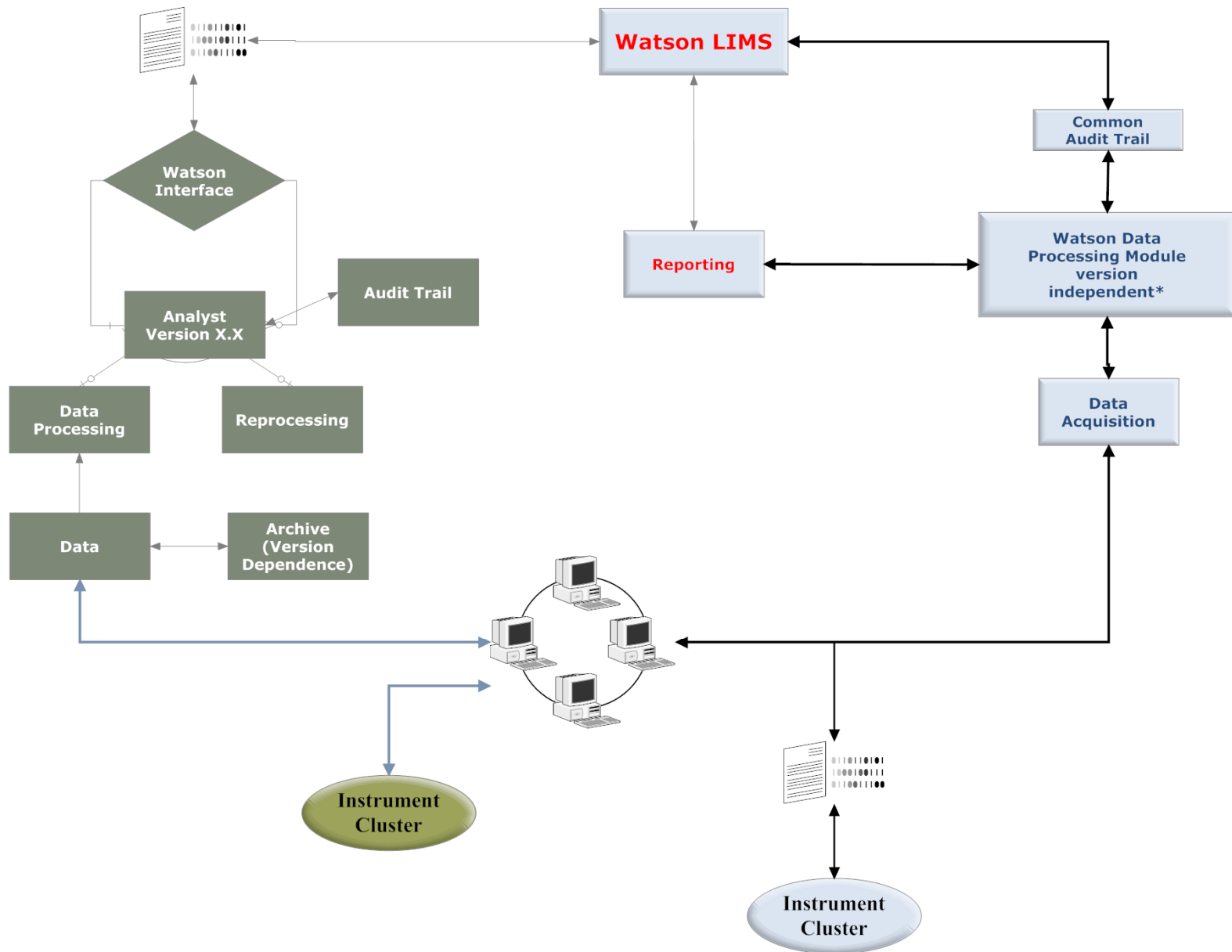
- New Ion Sweep Cap withstands repeated exposure to biological matrix without compromising sensitivity
- Plug & play position on the TSQ Vantage ensures superior ruggedness for extended periods



# Current Workflow (Analyst or LCquan)



# Enhancing the Software Experience (LCquan+Watson)



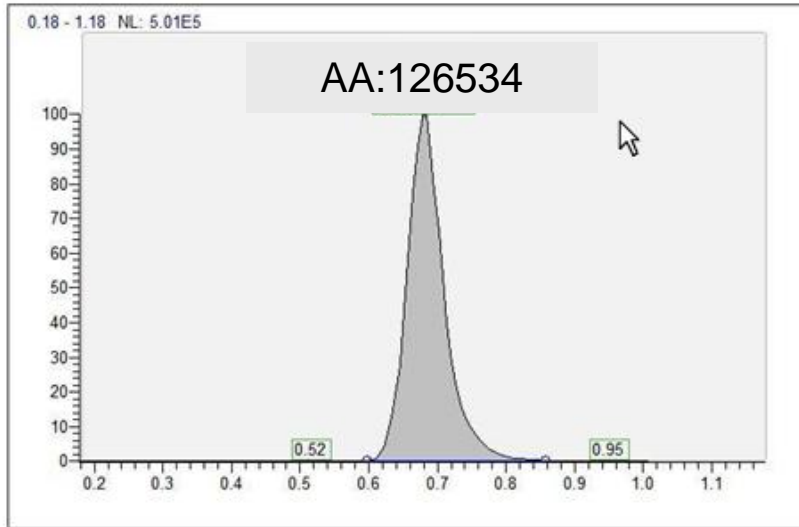
# Peak Integration/Re-Integration Within Watson 7.4

Review run 1 - Study - Pain Relief

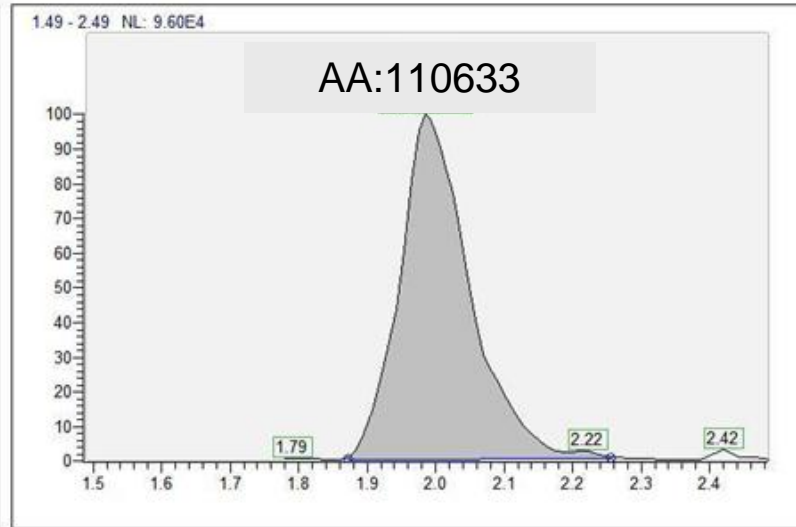
Sample ID	Sample Type	Integration Type	Response	ISTD Response	Response Ratio	Level	Retention Time	Exclude	Delta RT	Integration Code	Relative RT	Start Time	Left Edge	Start Height	End Time
LCQuan Digital v2.5 PV Validation Test STD.1.1	STANDARD	Method	500251	95236	5.253	standardI	0.68	N	-0.038	BB	1.059	0.599	Baseline	406.162	0.857
LCQuan Digital v2.5 PV Validation Test STD.2.1	STANDARD	Method	241748	89004	2.716	standardJ	0.67	N	-0.034	BB	1.053	0.598	Baseline	744.450	0.842
LCQuan Digital v2.5 PV Validation Test STD.2.2	STANDARD	Method	158888	95803	1.658	standardI	0.68	N	-0.035	BB	1.055	0.601	Baseline	-56.788	0.843
LCQuan Digital v2.5 PV Validation Test STD.3.1	STANDARD	Method	56359	95480	0.590	standardI	0.68	N	-0.043	BB	1.066	0.603	Baseline	42.617	0.874
LCQuan Digital v2.5 PV Validation Test STD.3.2	STANDARD	Method	463054	92867	4.986	standardI	0.67	N	-0.031	BB	1.048	0.585	Baseline	44.482	0.926
LCQuan Digital v2.5 PV Validation Test STD.4.1	STANDARD	Method	226610	101357	2.236	standardJ	0.67	N	-0.033	BB	1.052	0.598	Baseline	1158.052	0.830
LCQuan Digital v2.5 PV Validation Test STD.4.2	STANDARD	Method	137272	115100	1.193	standardI	0.68	N	-0.041	BB	1.065	0.588	Baseline	76.006	0.840
LCQuan Digital v2.5 PV Validation Test STD.5.1	STANDARD	Method	1573049	96645	16.277	standardI	0.67	N	-0.029	BB	1.046	0.575	Baseline	276.611	0.904
LCQuan Digital v2.5 PV Validation Test QC.1.1	QC	Method	491417	101155	4.858	QC2	0.69	N	-0.051	BB	1.080	0.601	Baseline	352.735	0.849
LCQuan Digital v2.5 PV Validation Test QC.2.1	QC	Method	221855	93677	2.368	QC1	0.69	N	-0.047	BB	1.073	0.611	Baseline	735.049	0.841
LCQuan Digital v2.5 PV Validation Test QC.3.1	QC	Method	1822514	109928	16.579	QC3	0.69	N	-0.052	BB	1.082	0.586	Baseline	-109.698	0.989
LCQuan Digital v2.5 PV Validation Test CONTROL BLANK	CONTROL	Method	70235	88891	0.790	N/A	0.68	N	-0.039	BB	1.061	0.612	Baseline	142.034	0.823
LCQuan Digital v2.5 PV Validation Test 03446000001 1-001Male A Plasma-1 0h	UNKNOWN	Method	126534	110633	1.144	N/A	0.65	N	-0.011	BB	1.017	0.588	Baseline	1583.541	0.770

All Standards QCs Blanks Unknowns

Analyte: hydrocortisone



Internal Standard: methyltestosterone



Save Selected Sample Integration Save All Integrations Accept Selected Sample Integration Accept All Integrations



Exit

Cancel

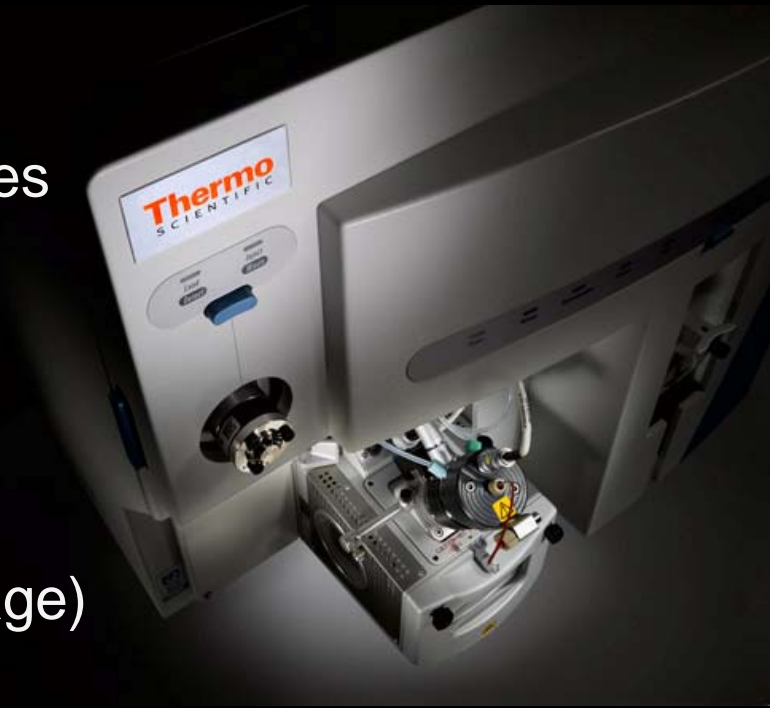
# Positioning Statement

*“We fully intend to support AB/Sciex instruments. No changes will be made to the Analyst user experience in Watson 7.4. **However, the intention is to enhance the LCquan user experience.**”*

*Watson LIMS will continue to fully support a large variety of instruments, and this includes AB/Sciex instruments”*

# TSQ Vantage Summary

- Delivers highest sensitivity, without increasing chemical noise in any matrix
- Excellent linear dynamic range for small molecules and biomolecules
- Stable “easy-to-use” software with full Watson integration
- Method transfer support (API 5000 to TSQ Vantage) through global applications team



# Acknowledgements

- Maurizio Splendore
- Chris Mullen
- Paul Atherton
- Jean-Jacques Dunyach
- Cesar Nepacena
- Bill Siebert
- Michael Belford
- Mark Hardman
- Michael Senko
- Viatcheslav Kovtoun
- Ethan Chan
- Terry Olney
- Alan Schoen
- Scott Stanley (UC Davis)
- Mark Churchill (Aptuit Ltd)
- Rich Lelacheur (Taylor Tech)
- Rich Klein
- Jonathan McNally
- Kevin Cook
- Reiko Kiyonami
- Pixie Kempel
- Joni Miller
- Wendy Weise
- Mark Canales
- Jim Edwards
- Kim Shah

End

**Thank-You  
&  
Any  
Questions**

