

● The Velos Pro: A Versatile, High Performance Ion Trap for Life Science Mass Spectrometry

Users Meeting

Somerset, October 12, 2011

Julie Horner, Ph. D.



Thermo Scientific Velos Pro

The LTQ-series linear ion traps have been established as the premier qualitative instruments on the market at their price point.

The Velos Pro was custom designed for both *Qualitative* and *Quantitative* applications in the laboratory.

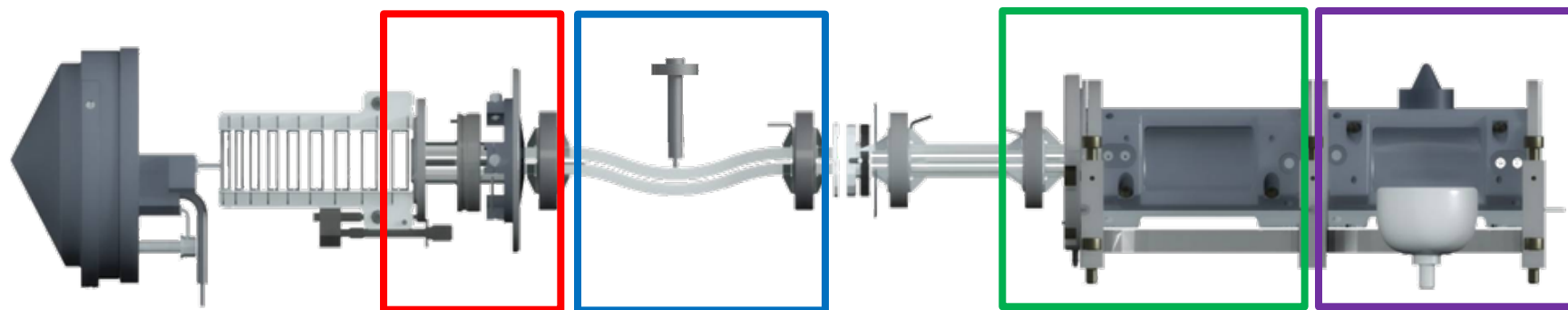
- *CID, PQD, ETD, and now Trap-HCD fragmentation increases flexibility for structural elucidation*
- *Fast, sensitive ion trap for the most demanding applications*
- *Generation II ion optics improve robustness and reduce downtime.*
- *Wide dynamic range, single digit RSD's, great accuracy.*
- *Upgrade to the power of Orbitrap*



Thermo Scientific Velos Pro
Dual-Pressure Linear Ion Trap

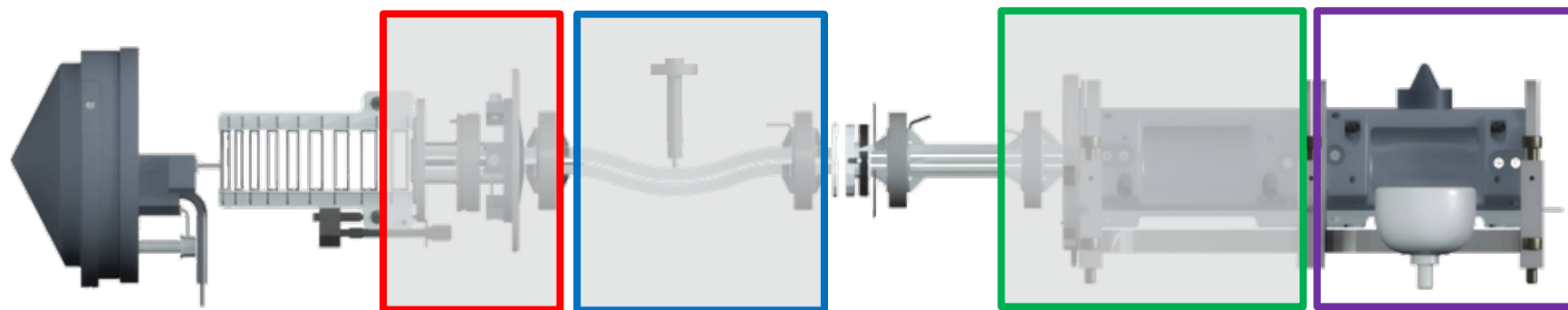
Velos Pro Technology Advancements

	Feature	Customer Benefit
1	UHPLC Cycle Time and High Data Rates	<ul style="list-style-type: none"> • More points across a chromatographic peak gives better quantitative performance • More MS^N during a run • Now scanning at 66kDa/sec
2	Robust and Sensitive Generation II Ion Optics	<ul style="list-style-type: none"> • Improved robustness, reduce down time, all on the most sensitive ion trap. • <i>High sensitivity S-Lens technology</i> • <i>Novel neutral beam blocking technology</i>
3	Novel Detection System for superior Quantitation	<ul style="list-style-type: none"> • Single digit RSDs, up to 6 orders of linear dynamic range on the first ion trap designed for quantitation. • <i>New EM and electrometer detection system matched to handle the ultra-high peak currents from the VELOS trap.</i>
4	Trap-HCD Fragmentation	<ul style="list-style-type: none"> • Increases flexibility for structural elucidation • <i>Trap-HCD fragmentation yields unique product ions for structural determinations.</i>



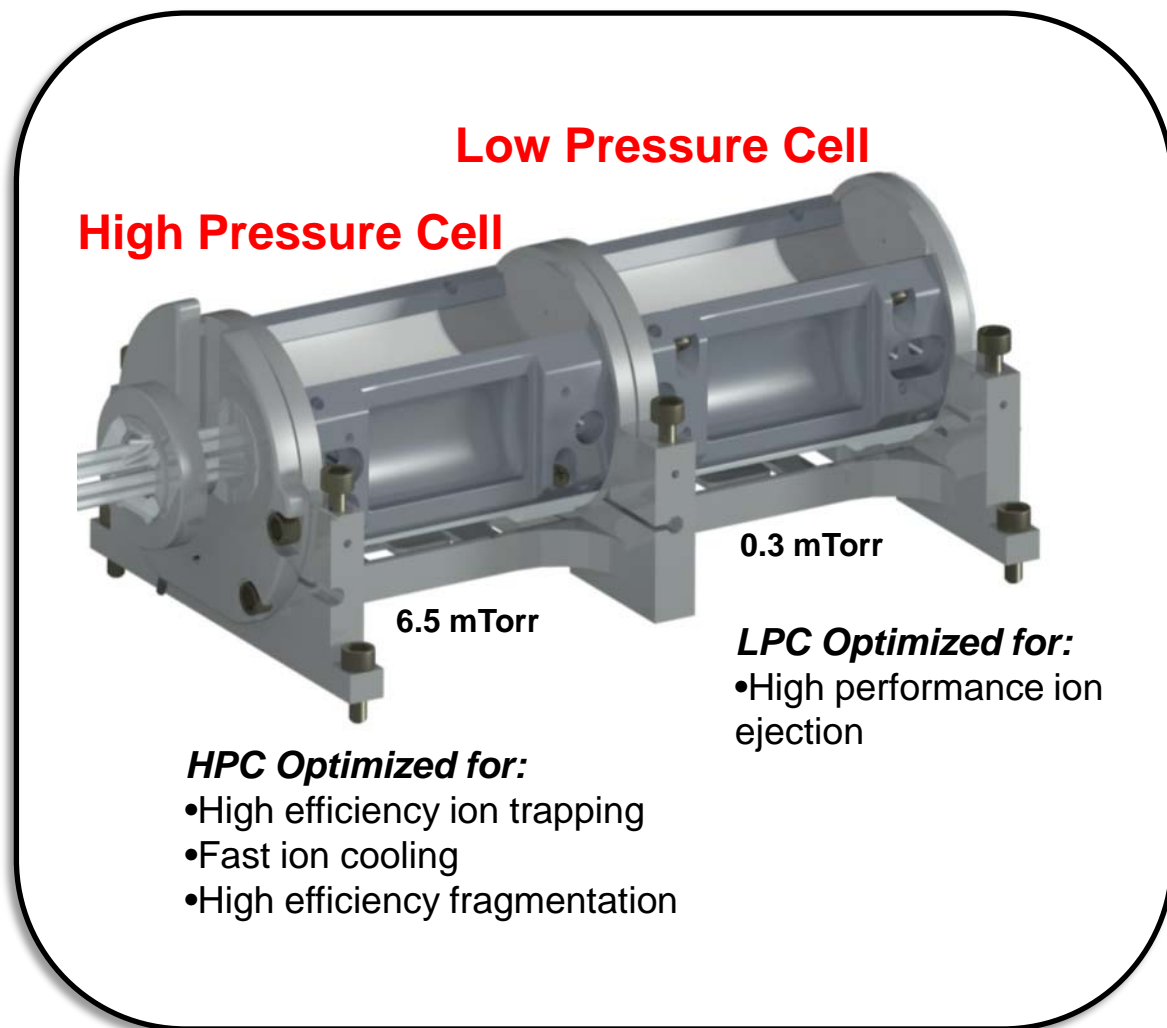
Velos Pro: Rapid Scan Rate

	Feature	Customer Benefit
1	UHPLC Cycle Time and High Data Rates	<ul style="list-style-type: none"> • More points across a chromatographic peak gives better quantitative performance • More MS^N during a run • Now scanning at 66kDa/sec
2	Robust and Sensitive Generation II Ion Optics	<ul style="list-style-type: none"> • Improved robustness, reduce down time, all on the most sensitive ion trap. • <i>High sensitivity S-Lens technology</i> • Novel beam blocking technology
3	Novel Detection System for superior Quantitation	<ul style="list-style-type: none"> • Single digit RSDs, up to 6 orders of linear dynamic range on the first ion trap designed for quantitation. • New EM and electrometer detection system matched to handle the ultra-high peak currents from the VELOS trap.
4	Trap-HCD Fragmentation	<ul style="list-style-type: none"> • Increases flexibility for structural elucidation • Trap-HCD fragmentation yields unique product ions for structural determinations.



Velos Pro: Dual Cell Technology

- *All ion trap mass spectrometers use helium to cool and fragment trapped ions.*
- *A **high pressure** of Helium is required for loading, cooling, and fragmenting ions.*
- *A **low pressure** of Helium is required for ejecting ions.*
- *Historically, all ion trap designs used a **single, compromise**, pressure for both processes.*
- *The Velos Pro uses two cells, each with the optimal pressure for the job at hand!*



Velos Pro: Dual Cell Technology

Since all parts of the scan function operate at the optimized pressure the system is:

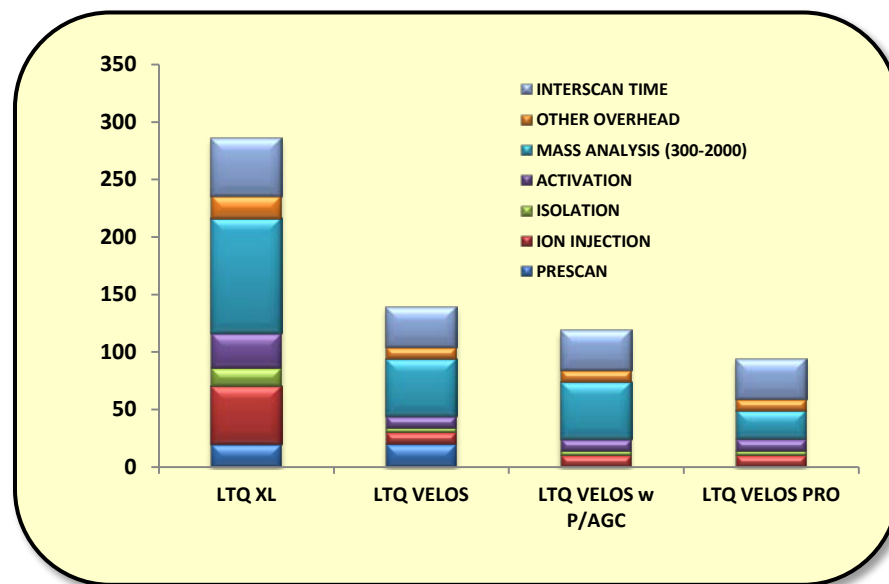
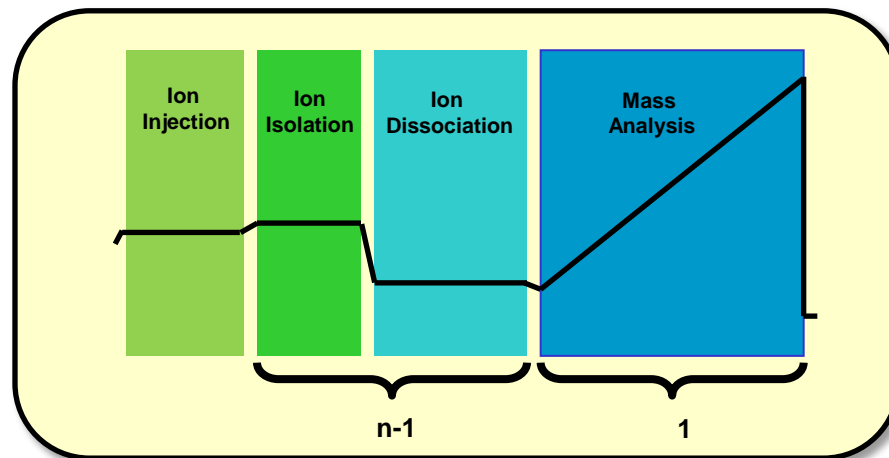
4X faster for ion isolation

3X faster for ion fragmentation

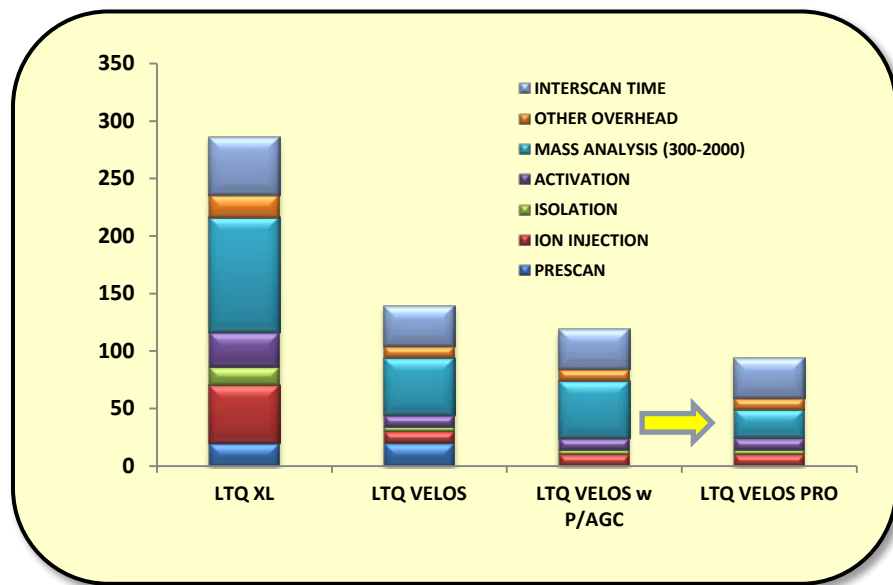
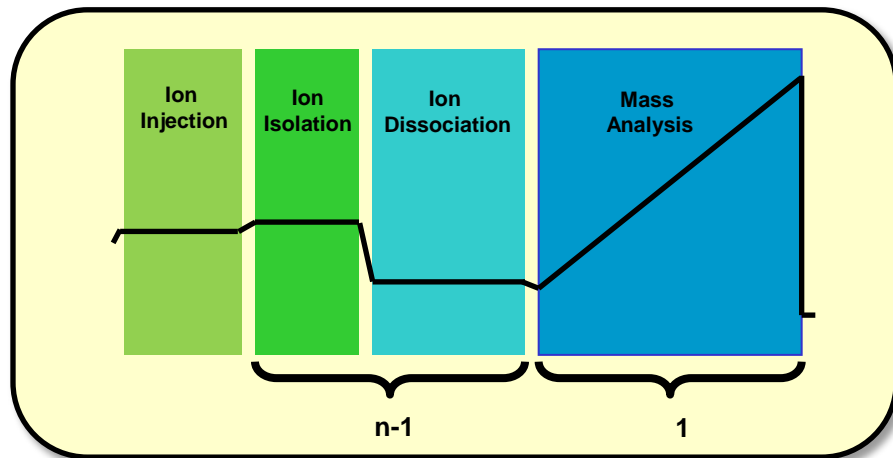
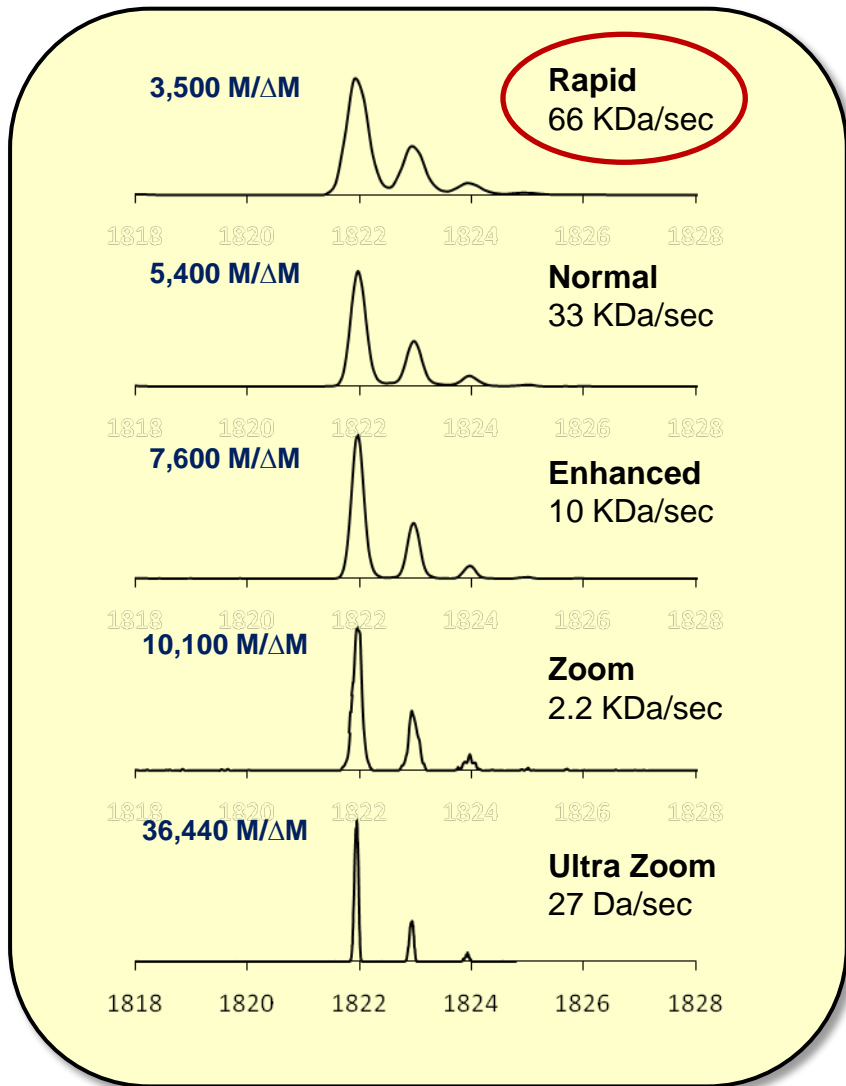
1.3X greater fragmentation efficiency

>90% trapping efficiency

Fastest scanning trap in the world.



New Scan Speed: Rapid Scan



Advantage of Resolution:

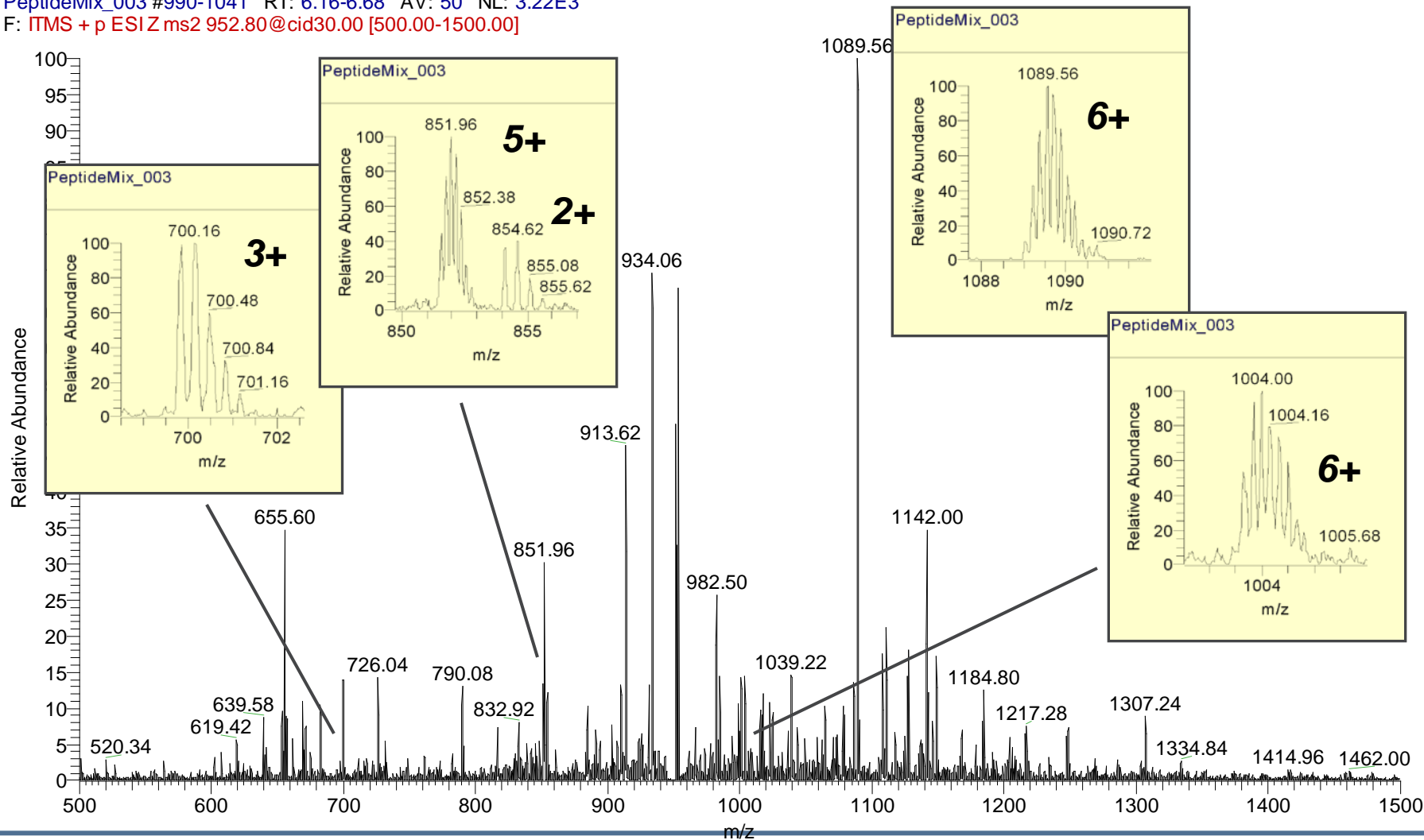
Ubiquitin 9+ Full MS/MS in Zoom Scan Avg 50 scans, 30 s

PeptideMix_003
3 uL/min in 50/50 H2O/ACN .1%FA after cal

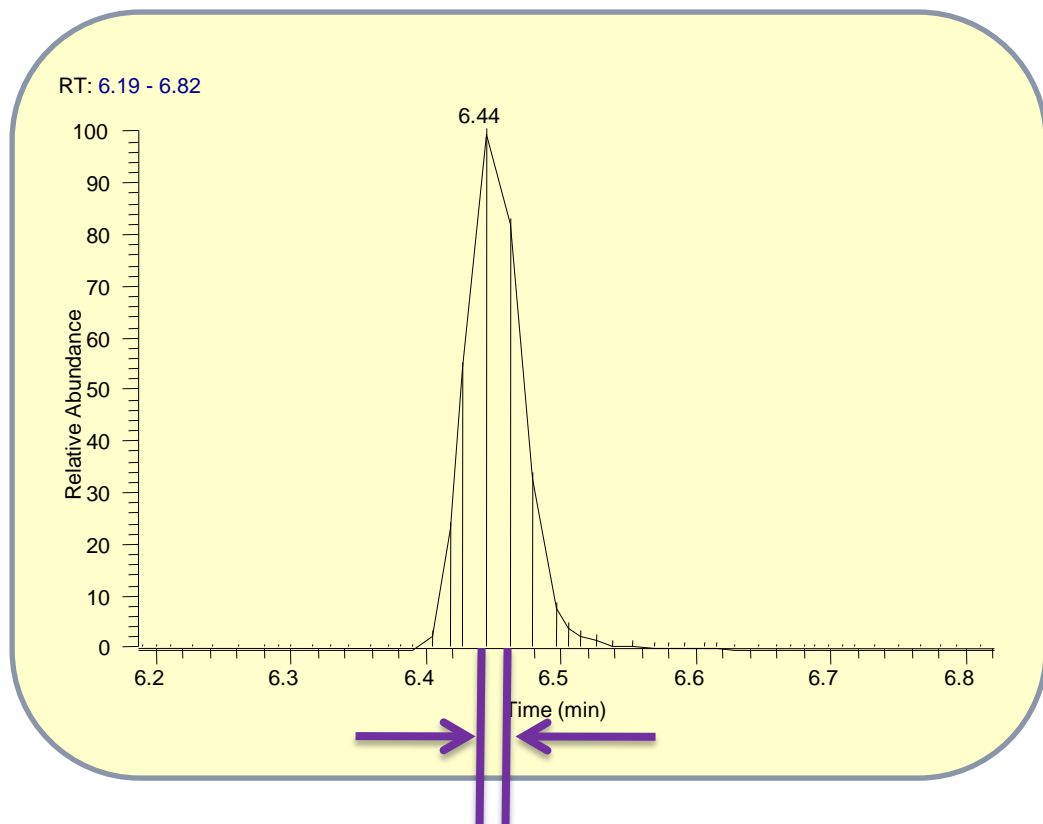
4/1/2009 4:10:08 PM

0.5 ng/uL ea Ang I, Ubiquitin

PeptideMix_003 #990-1041 RT: 6.16-6.68 AV: 50 NL: 3.22E3
F: ITMS + p ESIZ ms2 952.80@cid30.00 [500.00-1500.00]



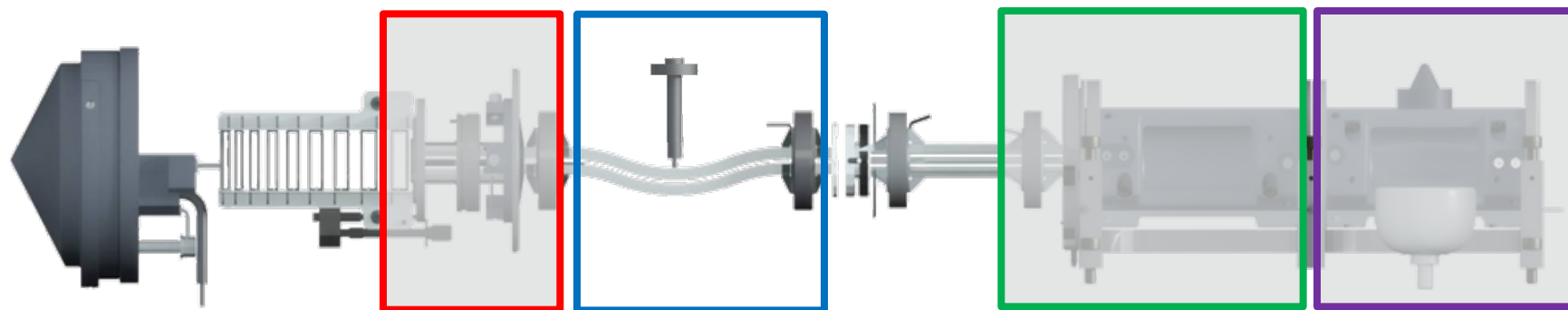
Velos Pro Speed : Full Ion Tree in 1 second!



8 MSⁿ Spectra in 1 second!

Velos Pro: Generation II Optics

	Feature	Customer Benefit
1	UHPLC Cycle Time and High Data Rates	<ul style="list-style-type: none"> • More points across a chromatographic peak gives better quantitative performance • More MS^N during a run • Now scanning at 66kDa/sec
2	Robust and Sensitive Generation II Ion Optics	<ul style="list-style-type: none"> • Improved robustness, reduce down time, all on the most sensitive ion trap. • <i>High sensitivity S-Lens technology</i> • <i>Novel beam blocking technology</i>
3	Novel Detection System for superior Quantitation	<ul style="list-style-type: none"> • Single digit RSDs, up to 6 orders of linear dynamic range on the first ion trap designed for quantitation. • New EM and electrometer detection system matched to handle the ultra-high peak currents from the VELOS trap.
4	Trap-HCD Fragmentation	<ul style="list-style-type: none"> • Increases flexibility for structural elucidation • Trap-HCD fragmentation yields unique product ions for structural determinations.



Velos Pro: High Sensitivity Generation I Ion Optics



S-Lens: Stacked Ring Ion Guide

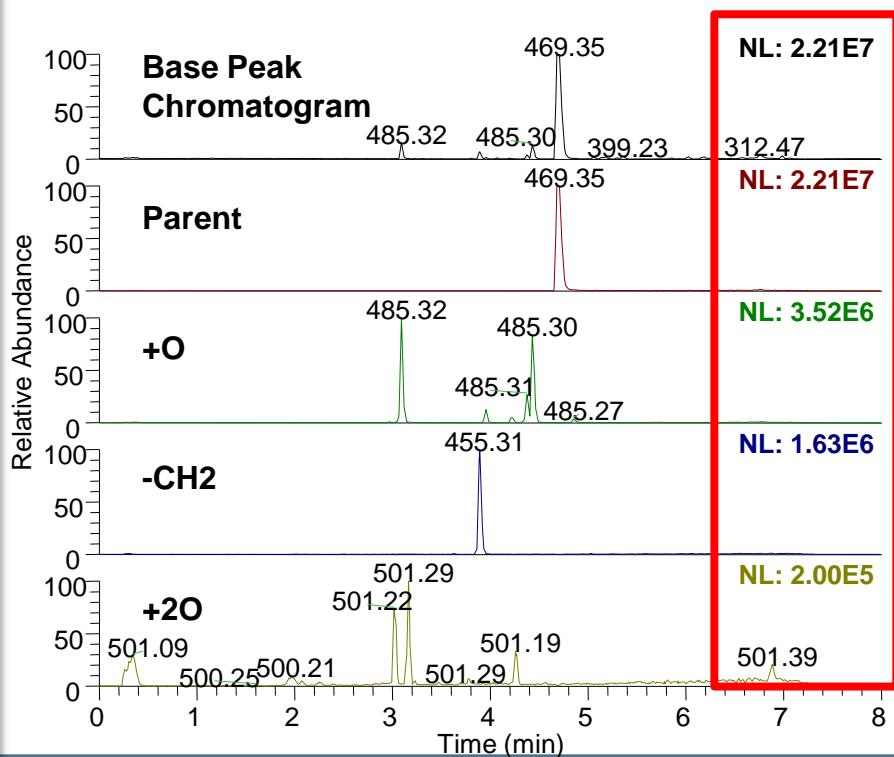
- Dual ID variable spacing Stacked Lenses
 - larger ID lenses capture the entire expansion from the ion transfer tube
 - smaller ID lenses focus the ion beam through the conductance limiting aperture
 - increasing spacing = increasing field penetration to focus ion beam
 - small number of electrodes (18 total in one assembly)
- Relatively Large Exit Lens Aperture versus Skimmer
- **5-10x Transmission Improvement**

Velos Pro: High Sensitivity Generation I Ion Optics

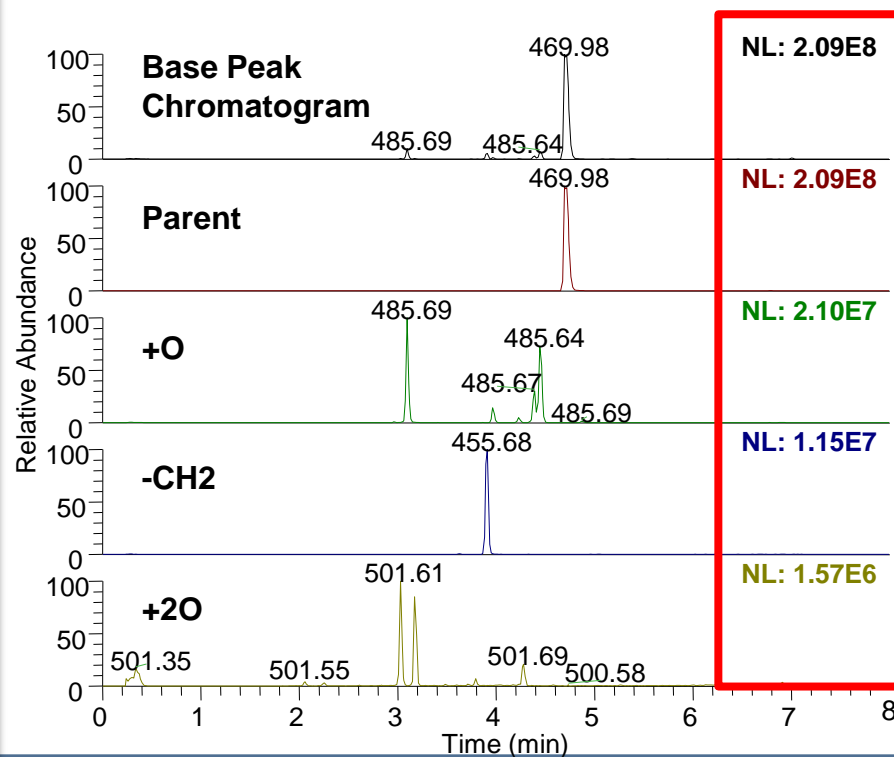
- 10 μm Maropitant
- 60 min Incubation in Dog Liver Microsomes
- 2.1x50mm 1.9 μm Hypersil GOLD
- 8 minute 10%-90% MeOH at 600 $\mu\text{L}/\text{min}$
- Velos S-lens optics vs LTQ Tube Lens optics

10X↑ in signal with S-lens

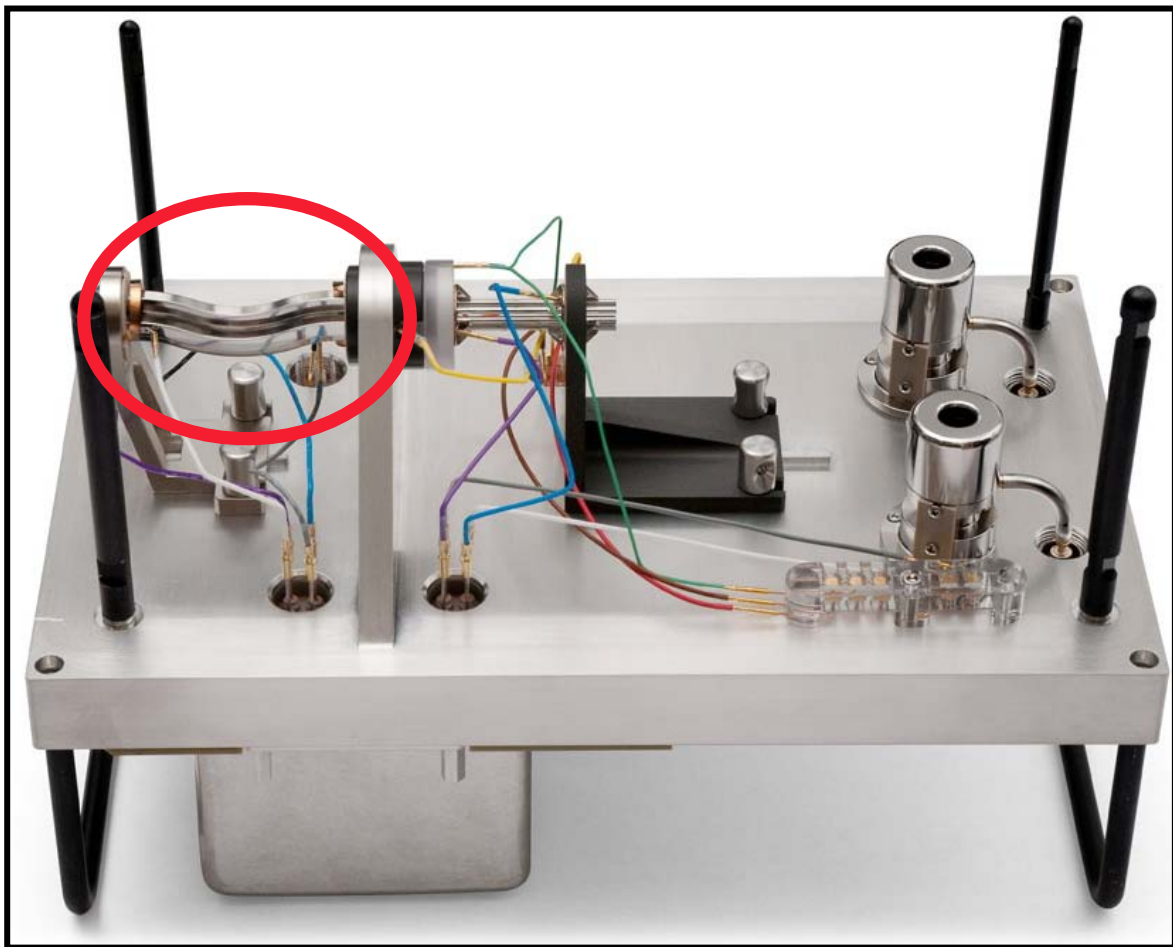
LTQ XL with Tube Lens



Velos Pro with S-Lens

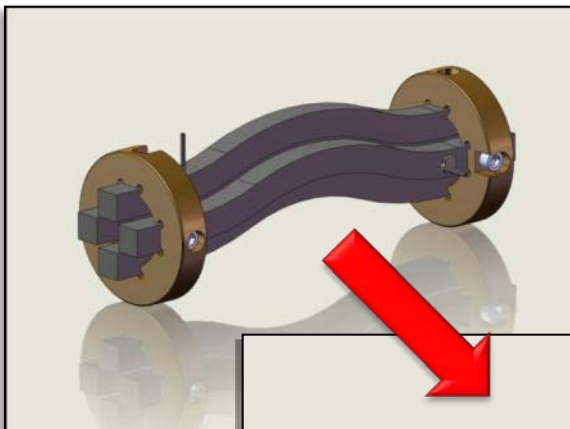


LTQ Velos - Generation I Ion Optics

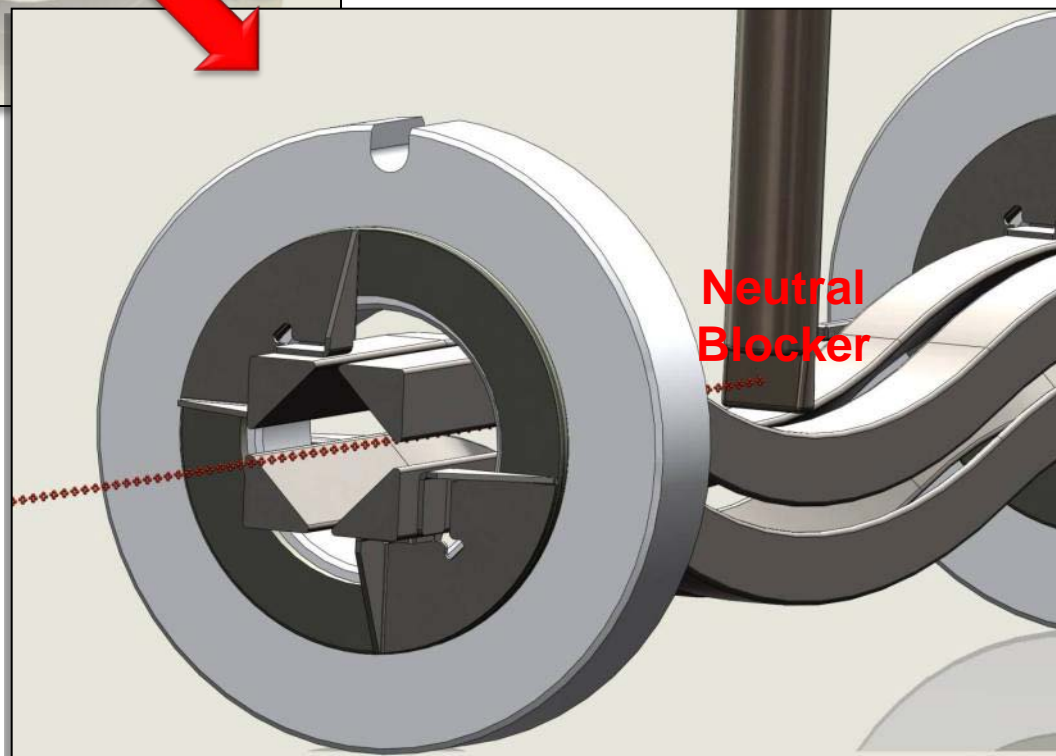


- “S-LENS” in Velos increased Sensitivity by 10
- Bent Q0 Optics Designed to Catch Neutrals
- Can Improve Robustness Further

The “Rotated Quadrupole” with Neutral Beam Blocker



To prevent material from building up on the rods, the Q0 quadrupole has been rotated 45°. This opens up a new line of sight for the neutral beam.

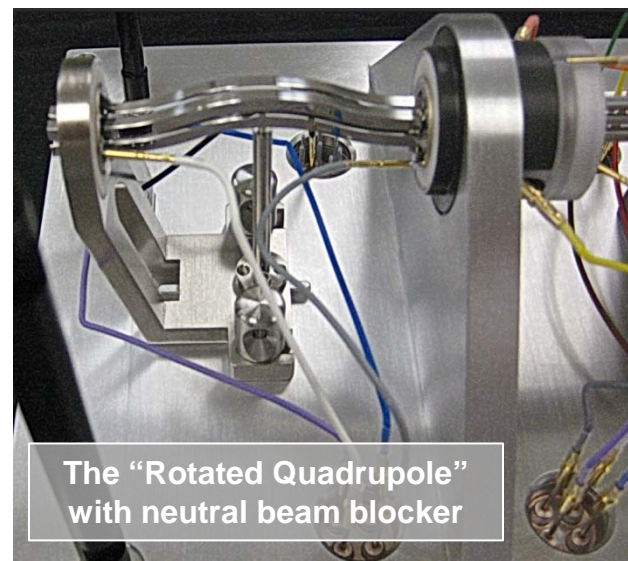
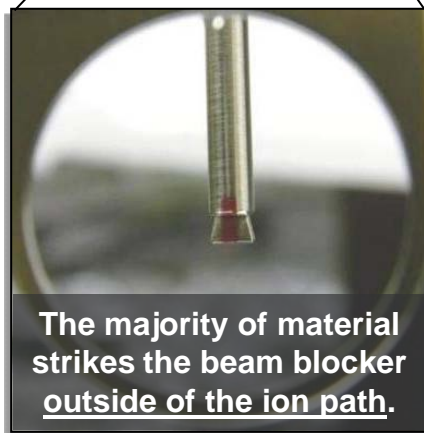
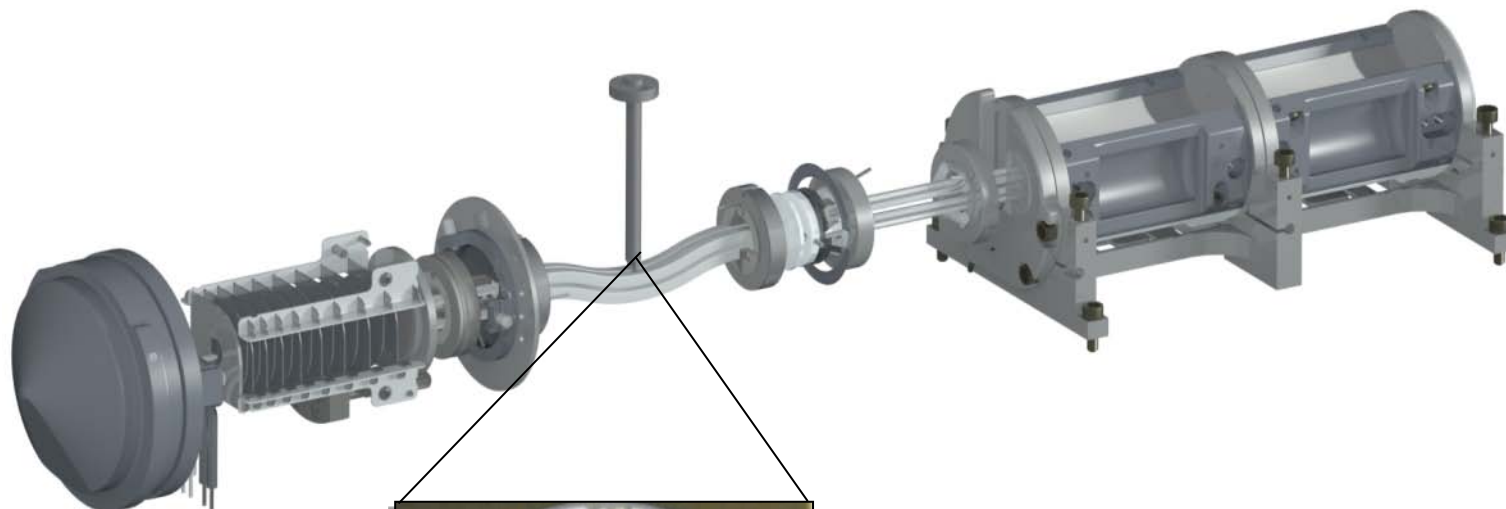


A beam blocker is now able to capture the neutral beam outside the body of the ion optics.

Neutrals can no longer reach the mass analyzer.

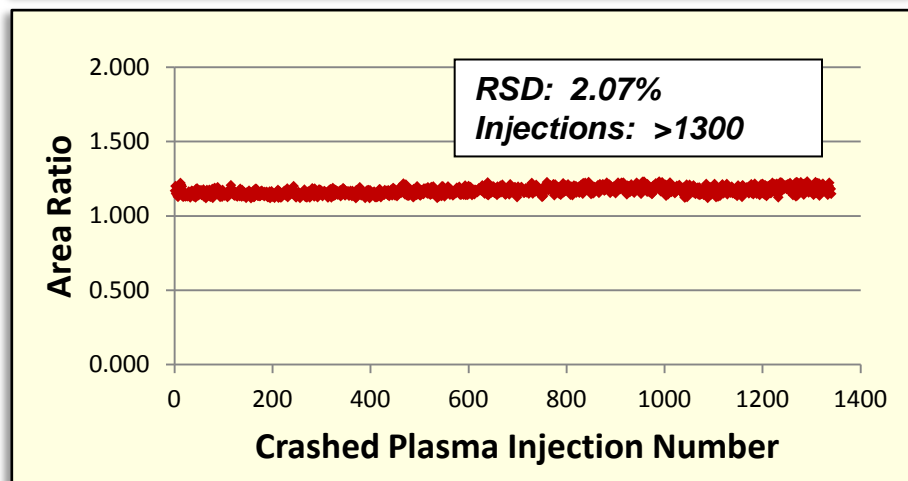
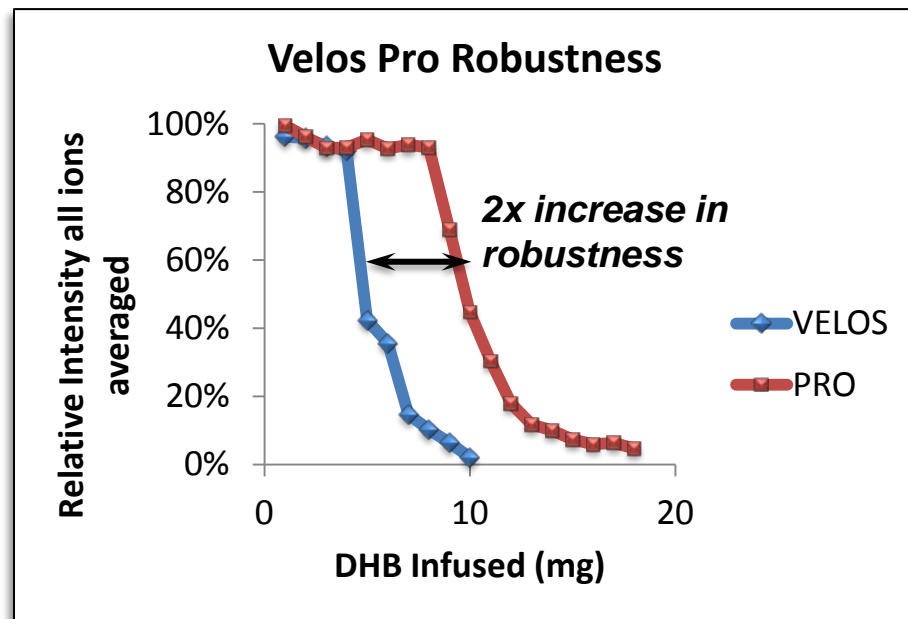
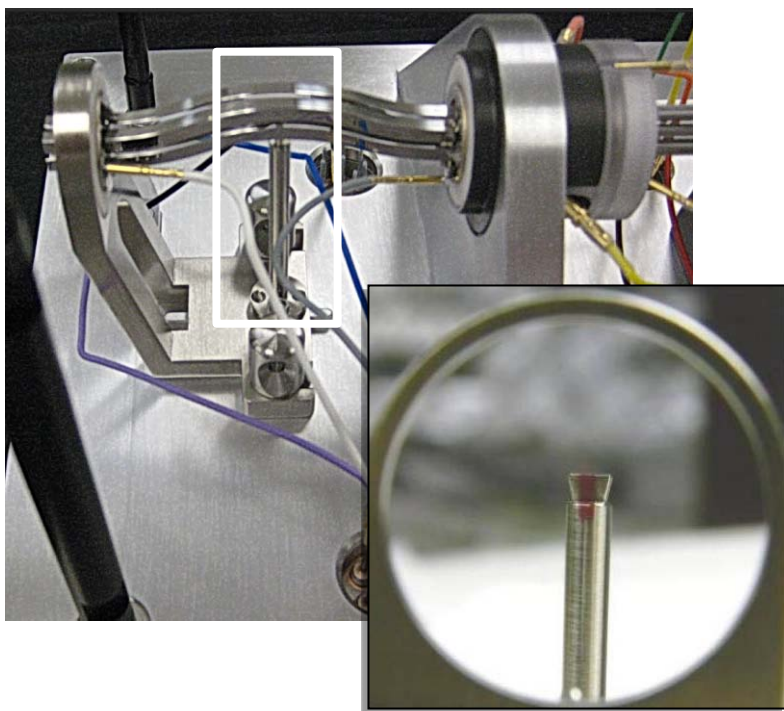
This allows, for the first time, neutrals and ions to be separated in a linear optics design.

Velos Pro Generation II Ion Optics



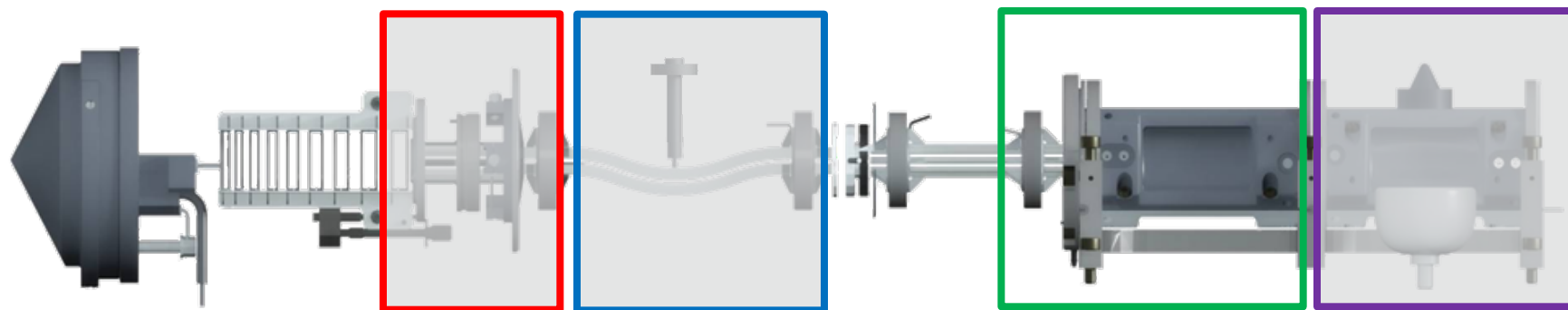
Velos Pro Generation II Optics Robust Signal Intensity

- Using the new rotated Q0, the Velos Pro is twice as robust as the LTQ Velos.
- The Velos Pro has increased robustness in complex matrices; e.g. crashed plasma.



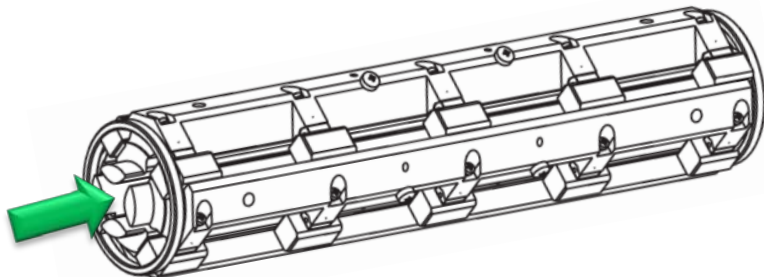
Velos Pro: Ultra Linear Quantitation

	Feature	Customer Benefit
1	UHPLC Cycle Time and High Data Rates	<ul style="list-style-type: none"> • More points across a chromatographic peak gives better quantitative performance • More MS^N during a run • Now scanning at 66kDa/sec
2	Robust and Sensitive Generation II Ion Optics	<ul style="list-style-type: none"> • Improved robustness, reduce down time, all on the most sensitive ion trap. • <i>High sensitivity S-Lens technology</i> • Novel beam blocking technology
3	Novel Detection System for superior Quantitation	<ul style="list-style-type: none"> • Single digit RSDs, up to 6 orders of linear dynamic range on the first ion trap designed for quantitation. • New EM and electrometer detection system matched to handle the ultra-high peak currents from the VELOS trap.
4	Trap-HCD Fragmentation	<ul style="list-style-type: none"> • Increases flexibility for structural elucidation • Trap-HCD fragmentation yields unique product ions for structural determinations.



Triple Quadrupole vs Velos Pro

Transmission Quadrupole



1. In a triple quadrupole, the Q1 rod set acts like a filter to isolate the precursor ion.
2. The precursor ions are then fragmented to produce product ions.
3. The product ions are then filtered in Q3 and detected.

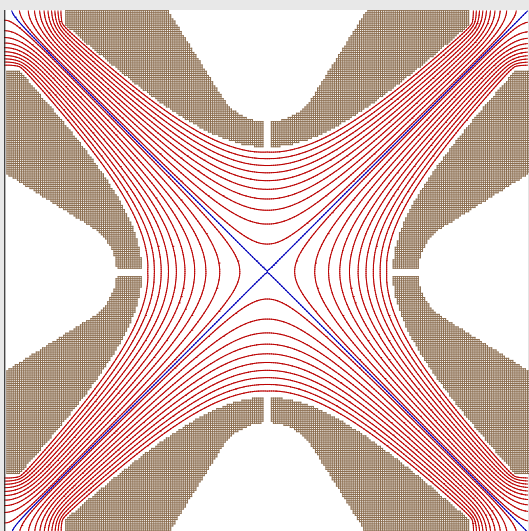
Velos Pro



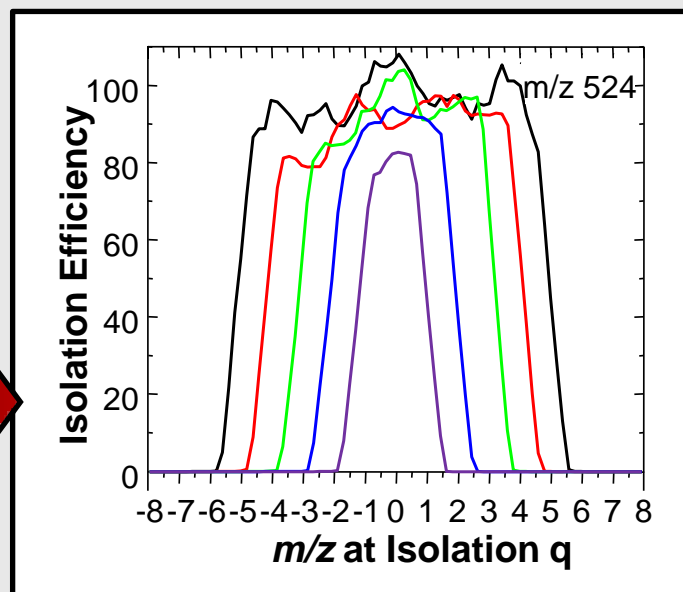
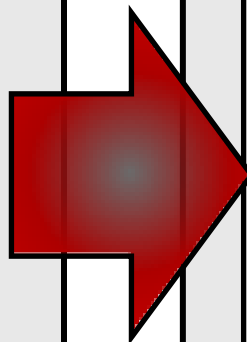
1. In the Velos Pro, the first trap acts like a filter to isolate the precursor ion **while loading into the trap**.
2. The precursor ions are then fragmented to produce product ions.
3. The product ions are then scanned in the second trap and detected.

Injection Waveforms

**With new injection=isolation waveforms,
the HPC acts *just like a good single quadrupole!***



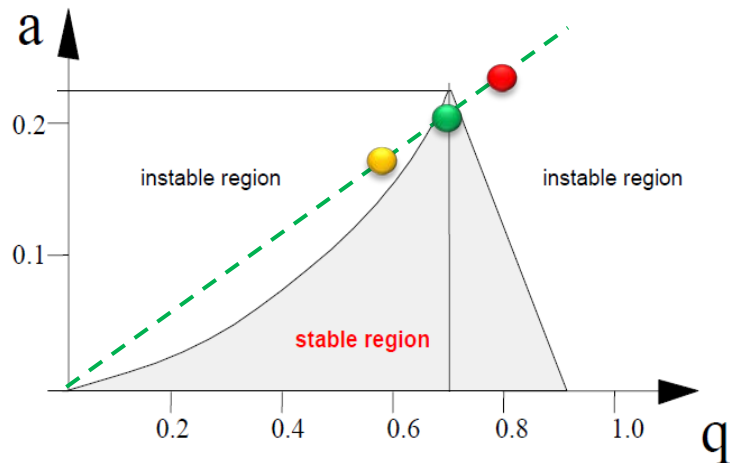
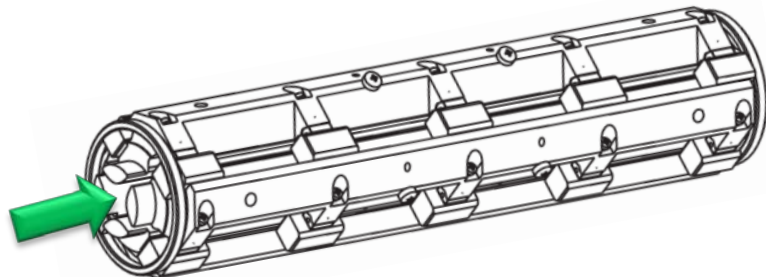
Velos symmetric rod stretch
rf field: $E=0$ on axis



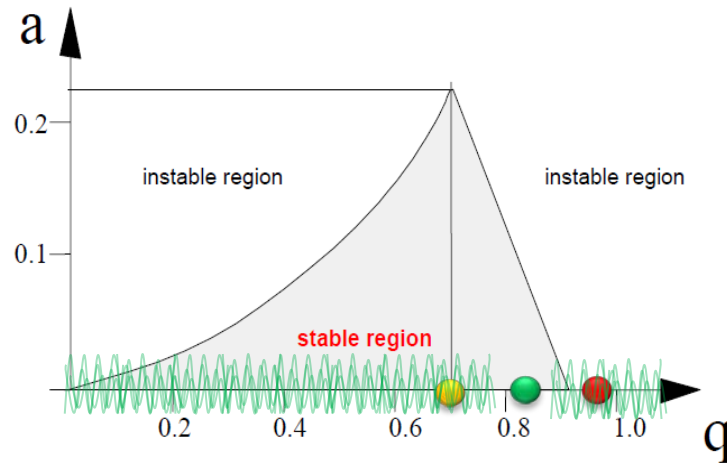
Isolation profiles for Velos
instruments during loading.
Even better isolation after loading.

Isolation before Analysis is Key

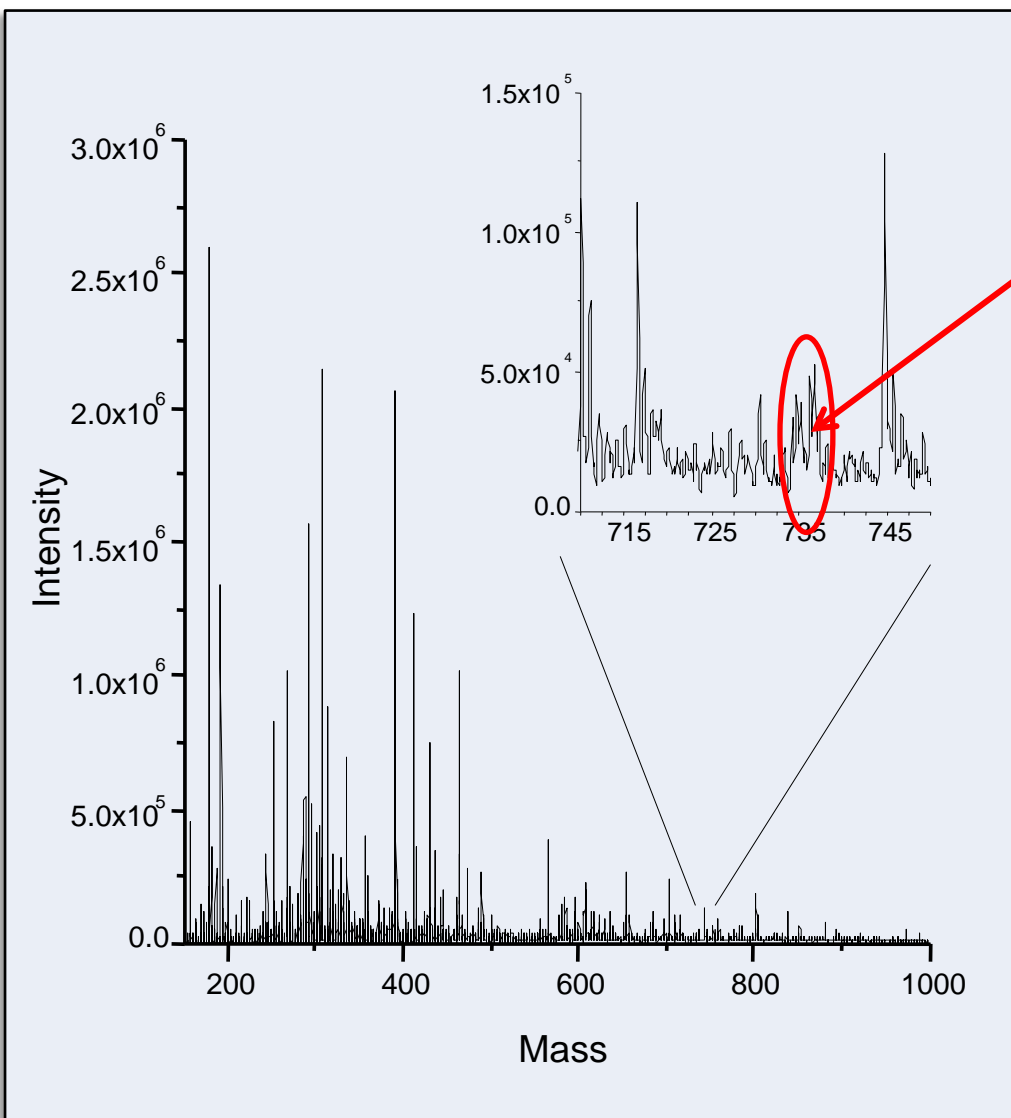
Transmission Quadrupole



Velos Pro

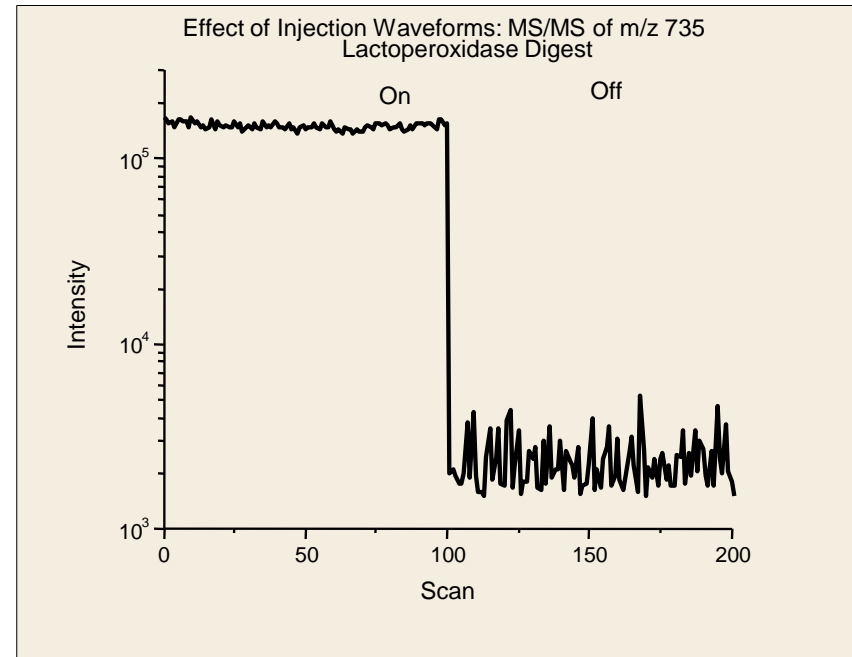
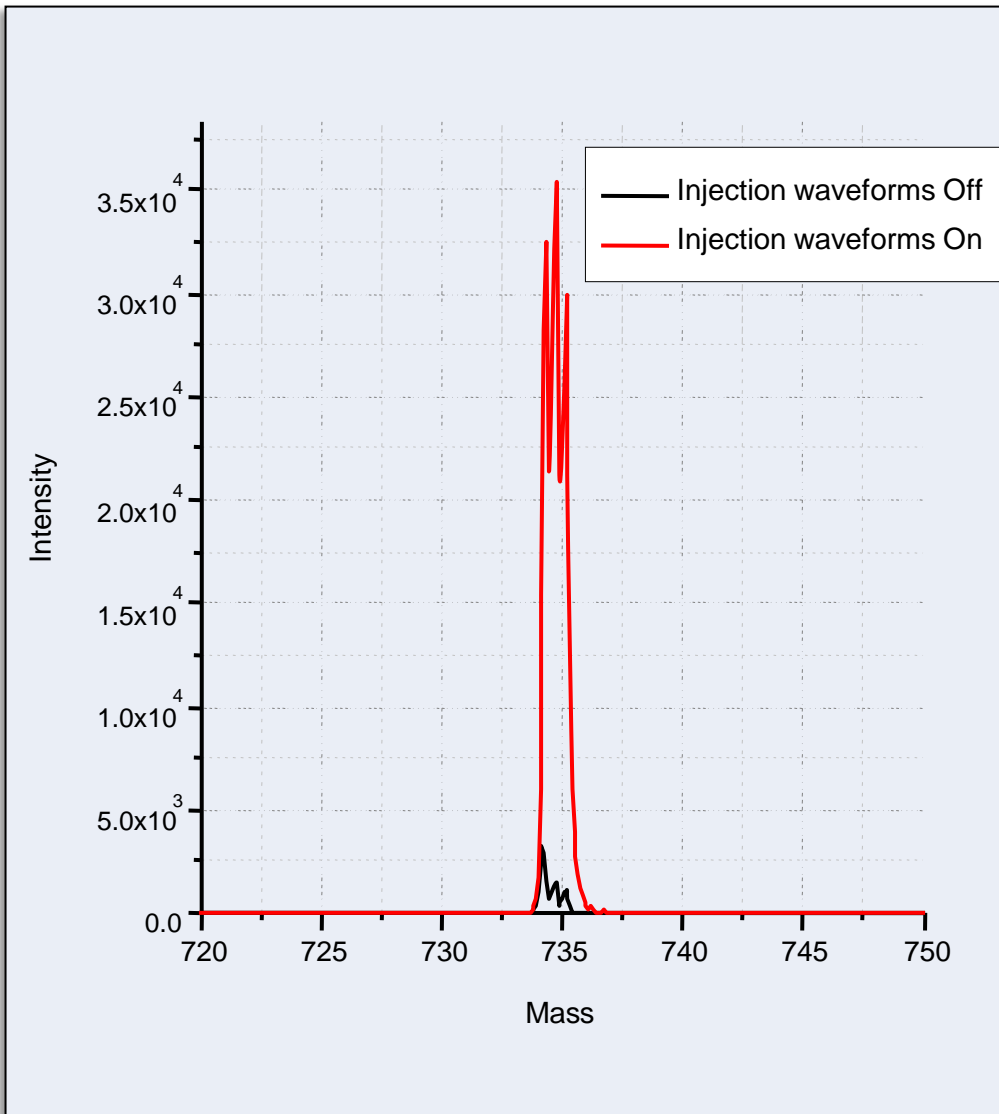


Isolation while loading is key



Select only
this ion while
loading into
the trap

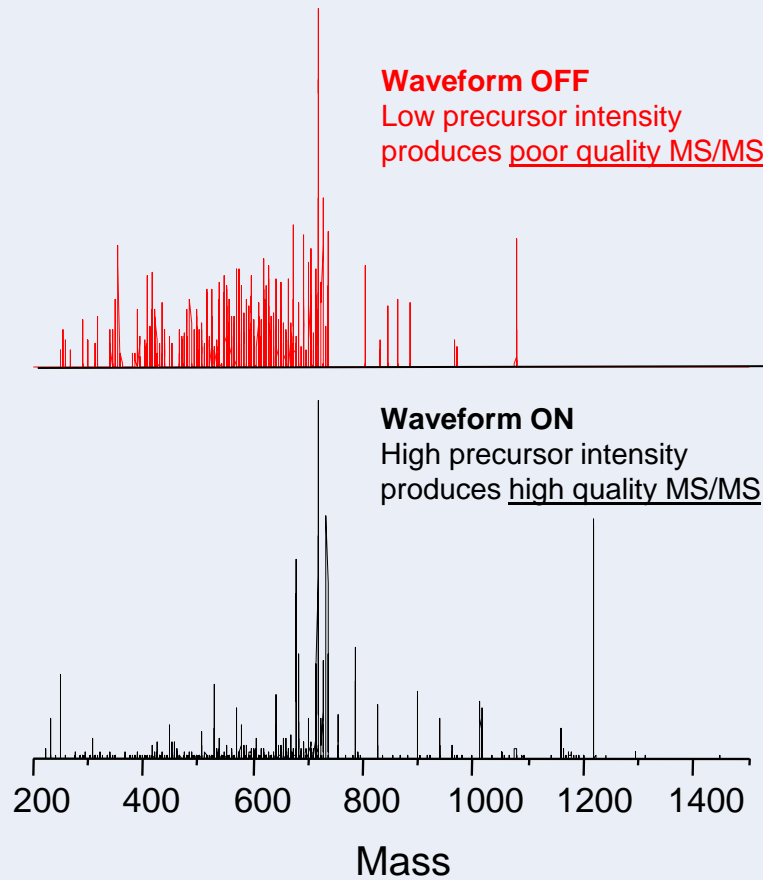
Isolation while loading is key



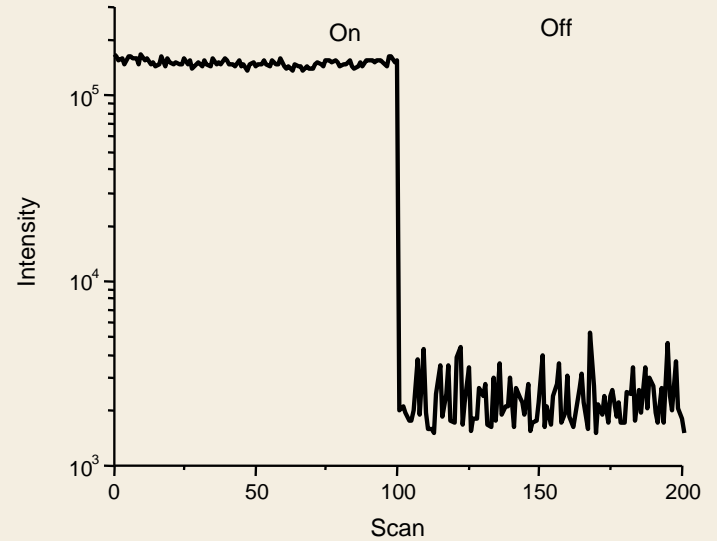
- Injection waveforms allow selective ion accumulation
- Injection waveforms prevent signal variation due to matrix based space charge

Isolation while loading is key

Effect of Injection Waveforms: MS/MS of m/z 735
Lactoperoxidase Digest

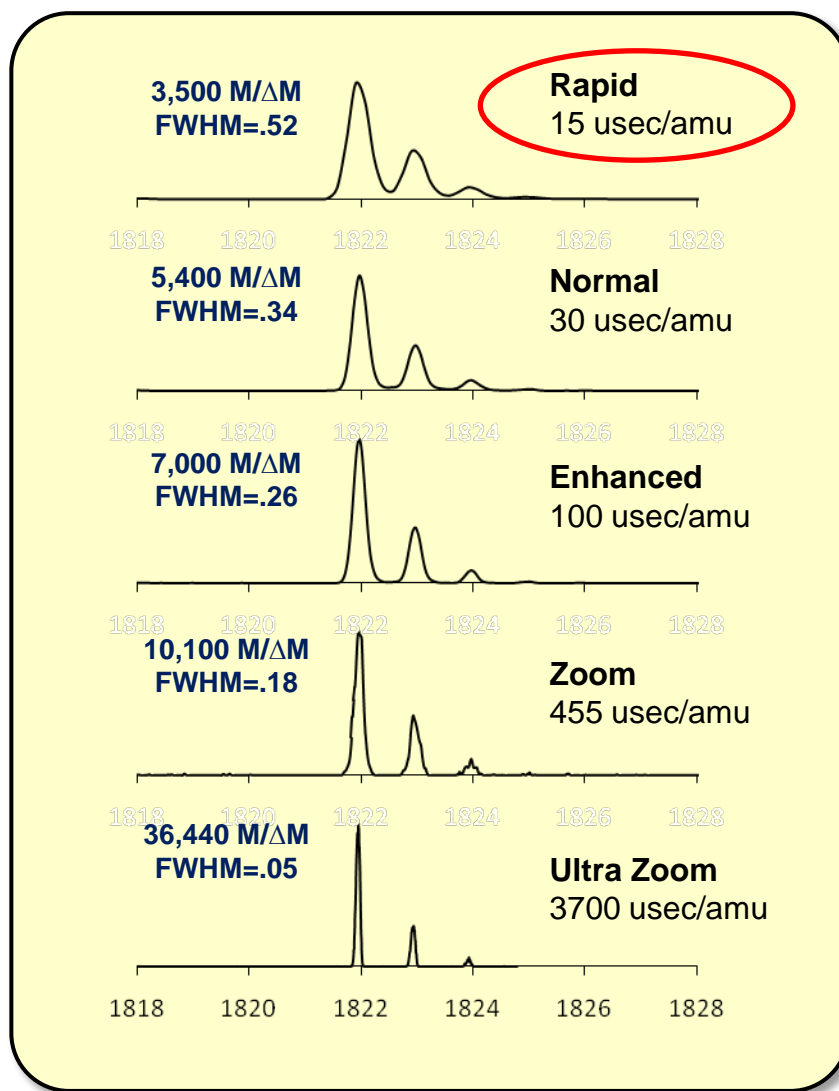


Effect of Injection Waveforms: MS/MS of m/z 735
Lactoperoxidase Digest

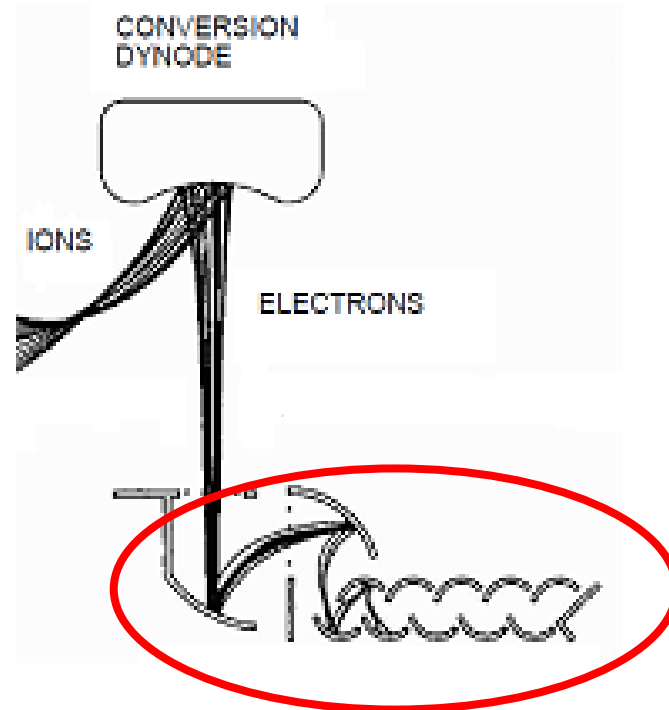
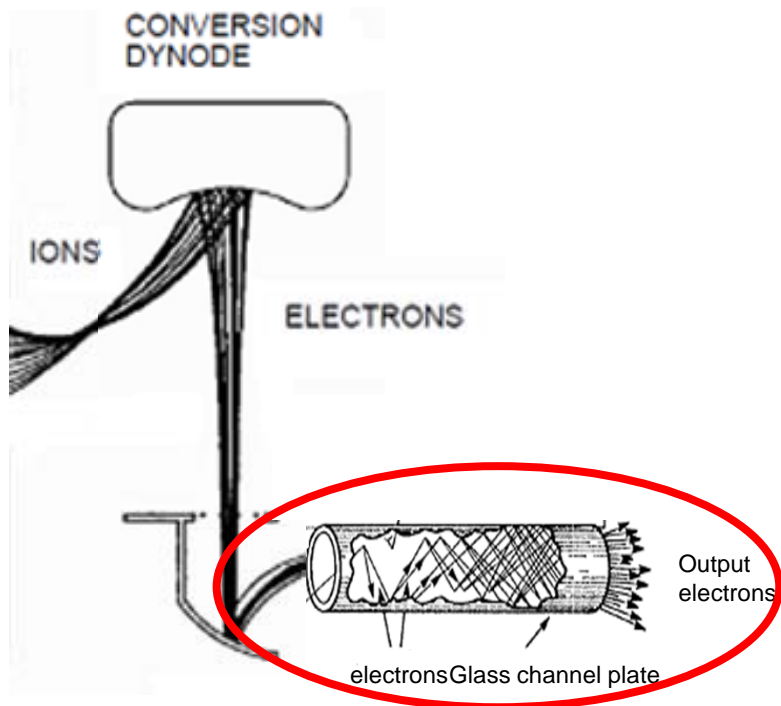


- Injection waveforms allow selective ion accumulation
- Injection waveforms prevent signal variation due to matrix based space charge

Faster Scan Rates – Higher Detected Ion Currents



Continuous Dynode Electron Multiplier (CDEM) *versus* Discrete Dynode Electron Multiplier (DDEM)



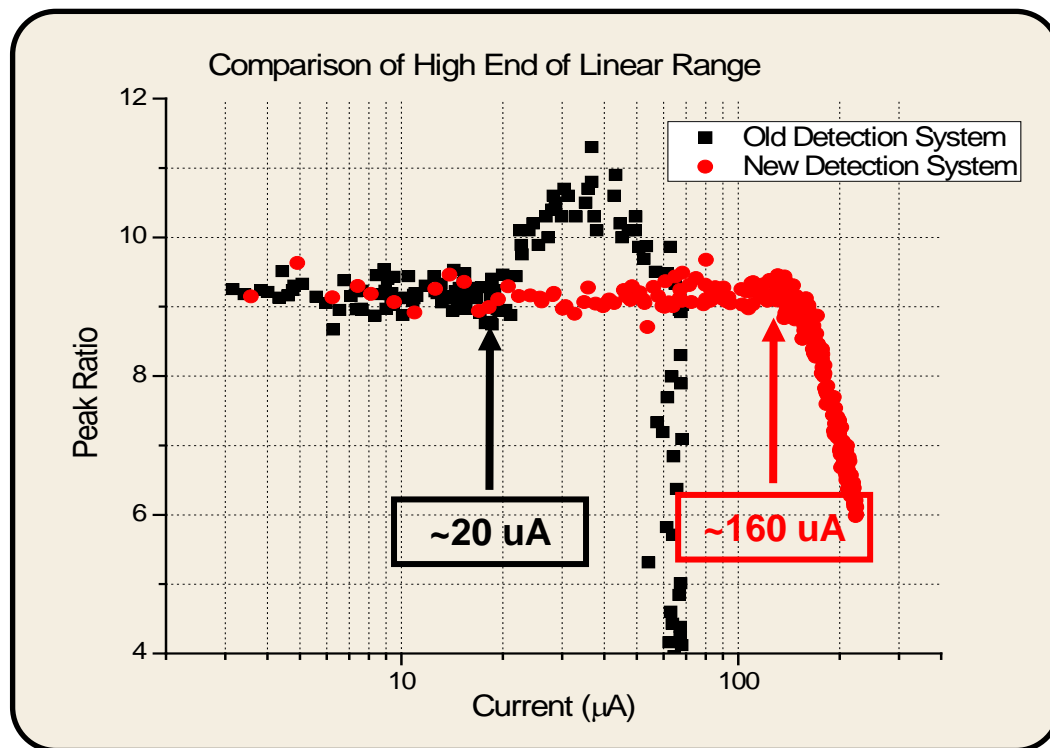
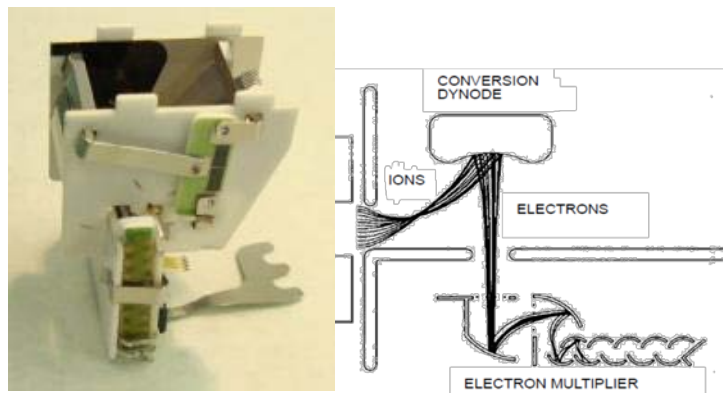
Advantages:

- Smaller
- Cost Effective

Advantages:

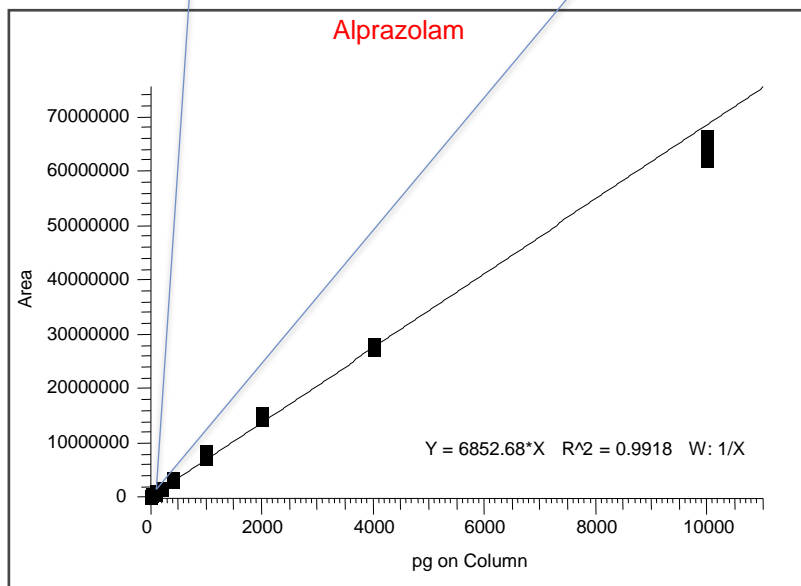
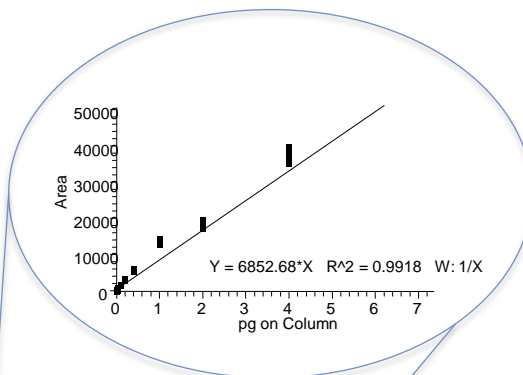
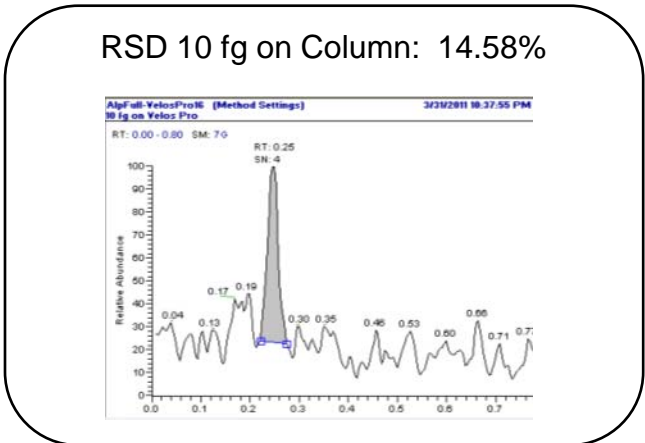
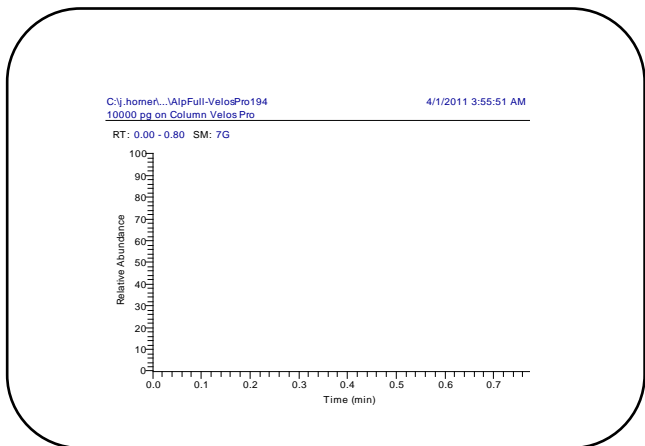
- Larger Surface Area
- Larger linear Dynamic Range
- Longer life times

Velos Pro – Novel Detection System



- Novel 160 μA electrometer board designed to handle high peak currents found in fast scanning micropackets. (now 24bit)
- Custom designed discrete dynode electron multipliers have larger surface area to produce an extended dynamic range with great RSDs
- New multiplier from ETP has slower aging and longer lifetimes than previous design

Velos Pro: Alprazolam 6 Order Quantitation



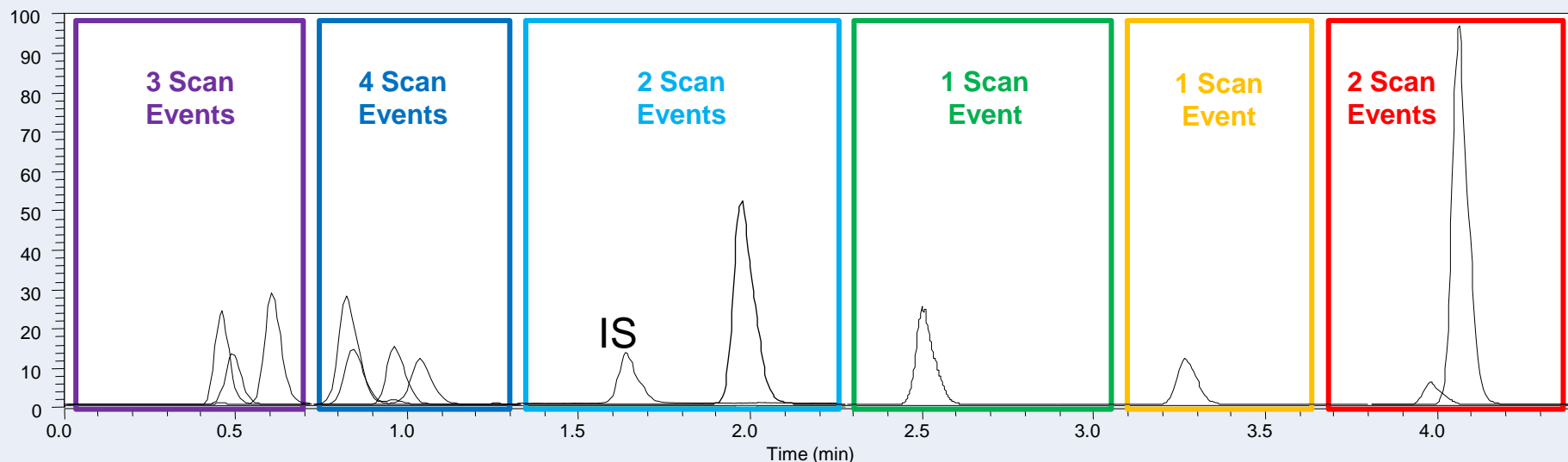
Pg On Column	RSD%
0.01	14.58
0.02	10.76
0.04	6.63
0.1	4.25
0.2	3.21
0.4	3.70
1	3.59
2	3.57
4	3.47
10	2.48
20	1.98
40	4.03
100	4.33
200	2.03
400	3.40
1000	3.85
2000	1.66
4000	1.27
10000	2.16

12 Compounds + 1 IS in plasma (5 min run)

C:\j.homer\...ASMS_VelosPro_Run2_088
200 pg/uL

4/9/2011 12:42:13 PM

RT: 0.00 - 4.40



Norcodeine	286.30 CID	4
Codeine-d3	303.37 CID	5
Hydrocodone	300.37 CID	>5

Methamphetamine	150.24 CID	5
Methoxymethamphetamine	180.26 CID	>5
Lidocaine	235.34 CID	>5
Sulfamethazine	279.09 CID	4

Methylthioamphetamine IS	165.22 CID	4.2%
Bupropion	240.11 CID	>5

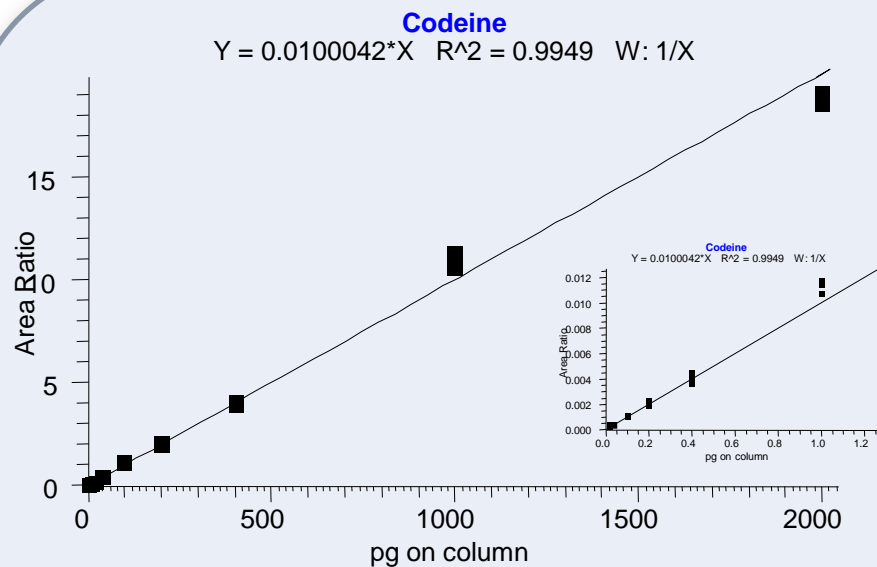
Propranolol	260.16 CID	>5
-------------	------------	----

Alprazolam	309.09 PQD	>5
------------	------------	----

Testosterone	289.43 CID	5
Terfenadine	472.32 CID	6

Codeine 20 fg – 2 ng

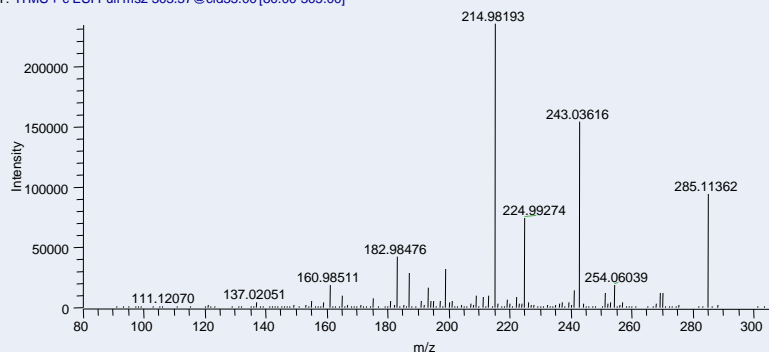
5 orders



C:\j.homer\...\ASMS_VelosPro_Run2_071
20 pg/uL

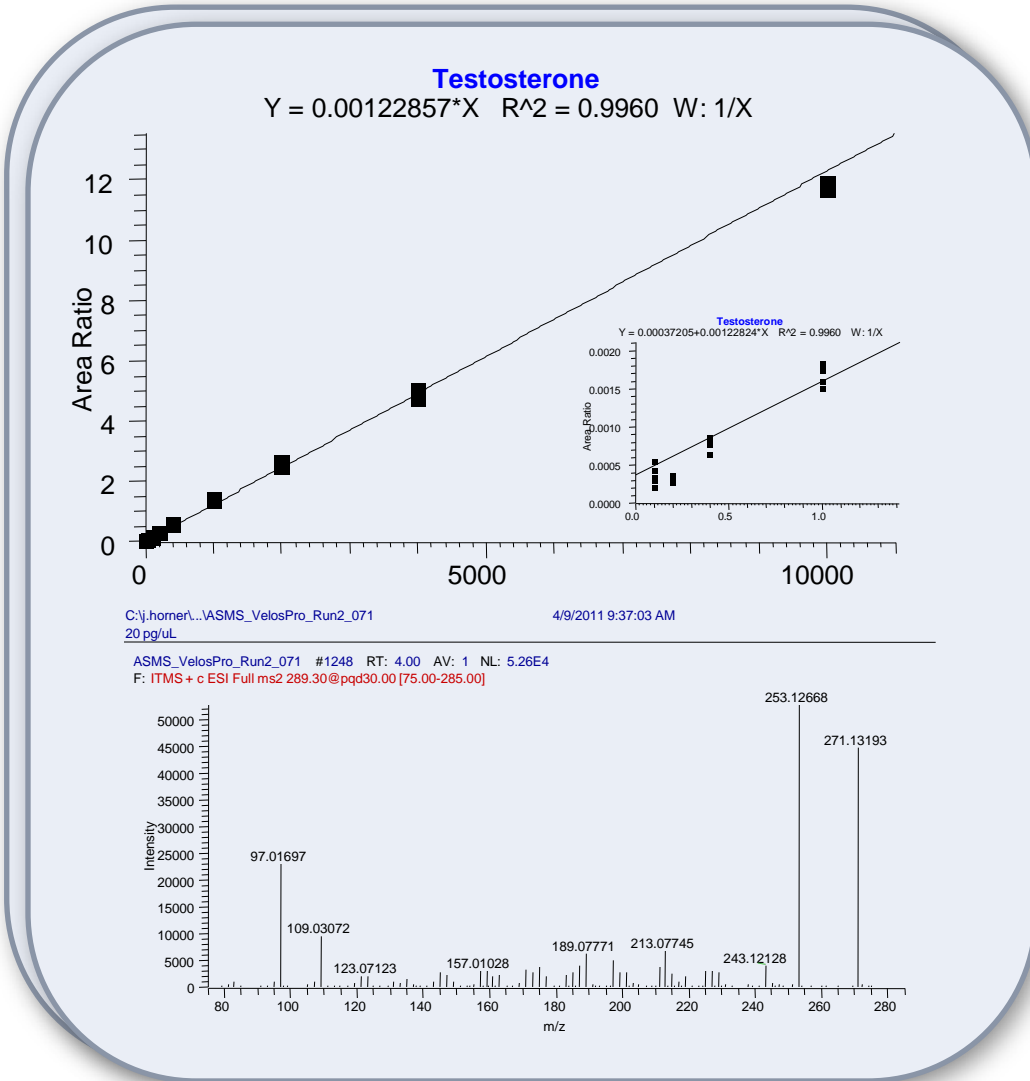
4/9/2011 9:37:03 AM

ASMS_VelosPro_Run2_071 #136 RT: 0.47 AV: 1 NL: 2.33E5
T: ITMS+ c ESI Full ms2 303.37@cid35.00[80.00-305.00]



pg on Column	%RSD
0.020	9.22
0.040	6.50
0.100	5.84
0.200	4.77
0.400	5.17
1.00	3.79
2.00	3.61
4.00	1.96
10.00	1.48
20.00	1.86
40.00	1.14
100.0	1.88
200.0	1.73
400.0	1.69
1000.0	2.65
2000.0	1.09
4000.0	2.27
10000.0	3.13

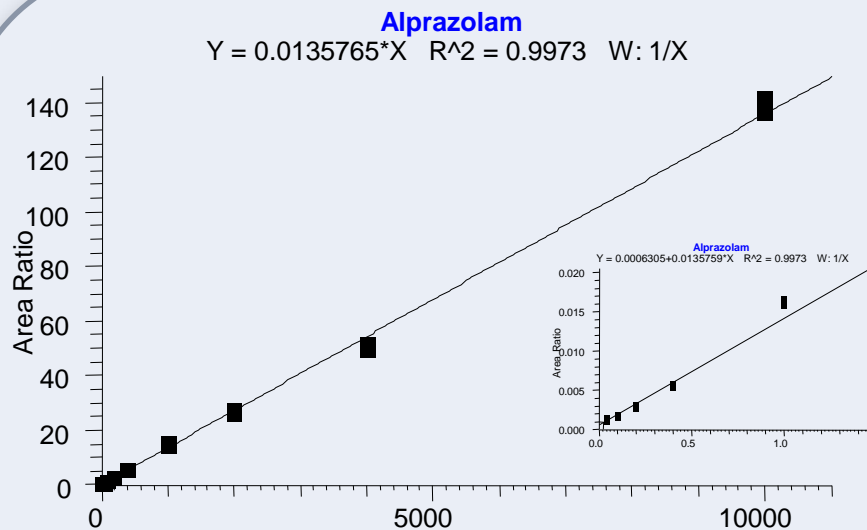
Testosterone 100 fg – 10 ng 5 orders



pg on Column	%RSD
0.100	36.18
0.200	12.50
0.400	5.28
1.00	1.41
2.00	1.16
4.00	2.40
10.00	2.85
20.00	1.99
40.00	2.02
100.0	3.16
200.0	1.96
400.0	2.80
1000.0	2.60
2000.0	2.63
4000.0	2.43
10000.0	1.03
4000.0	2.27
10000.0	3.13

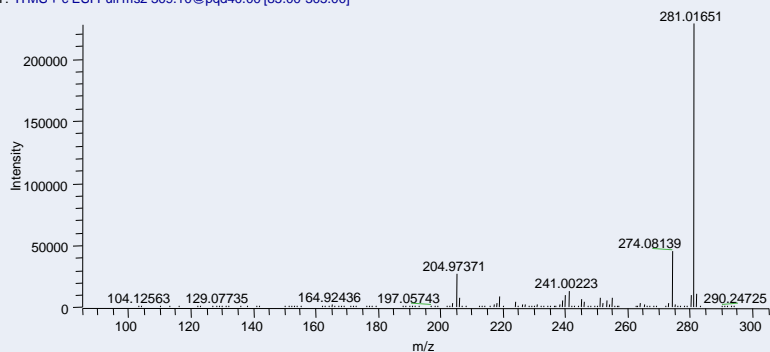
Alprazolam 40 fg – 10 ng

>5 orders



C:\j_homer\... \ASMS_VelosPro_Run2_071 4/9/2011 9:37:03 AM
 20 pg/uL

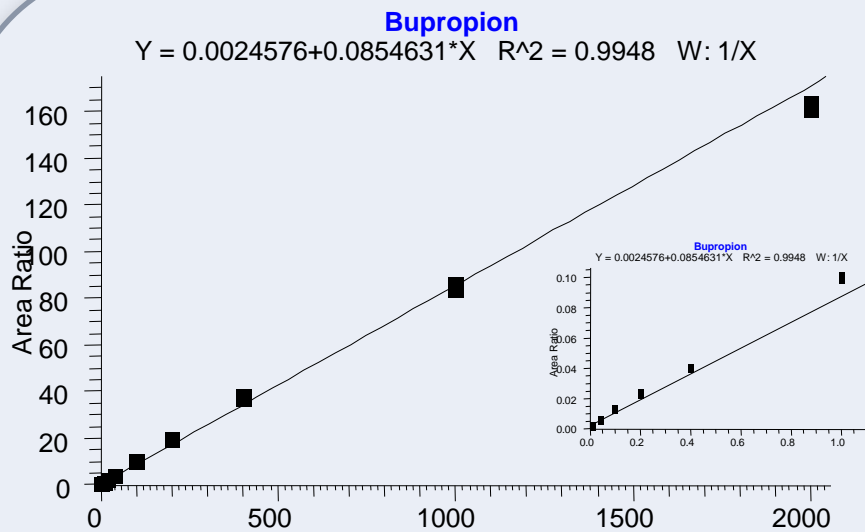
ASMS_VelosPro_Run2_071 #1035 RT: 3.29 AV: 1 NL: 2.27E5
 T: ITMS + c ESI Full ms2 309.10@pqd40.00 [85.00-305.00]



pg on Column	%RSD
0.040	19.86
0.100	11.86
0.200	7.45
0.400	3.97
1.00	2.47
2.00	2.15
4.00	1.48
10.00	1.66
20.00	1.75
40.00	1.28
100.0	1.95
200.0	1.98
400.0	1.78
1000.0	3.67
2000.0	2.59
4000.0	1.79
10000.0	1.67

Bupropion 10 fg – 2 ng

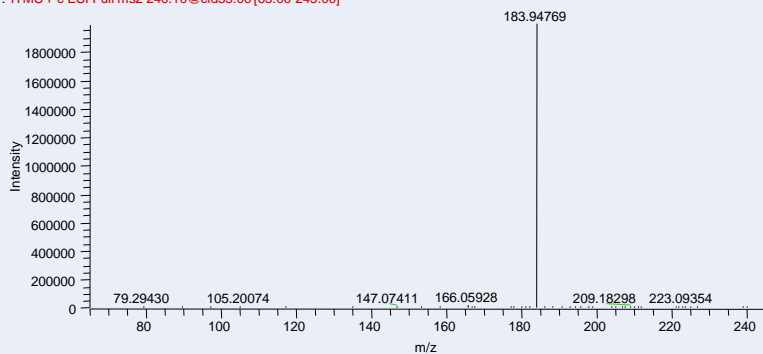
>5 orders



C:\j.homer\...\ASMS_VelosPro_Run2_071
 20 pg/uL

4/9/2011 9:37:03 AM

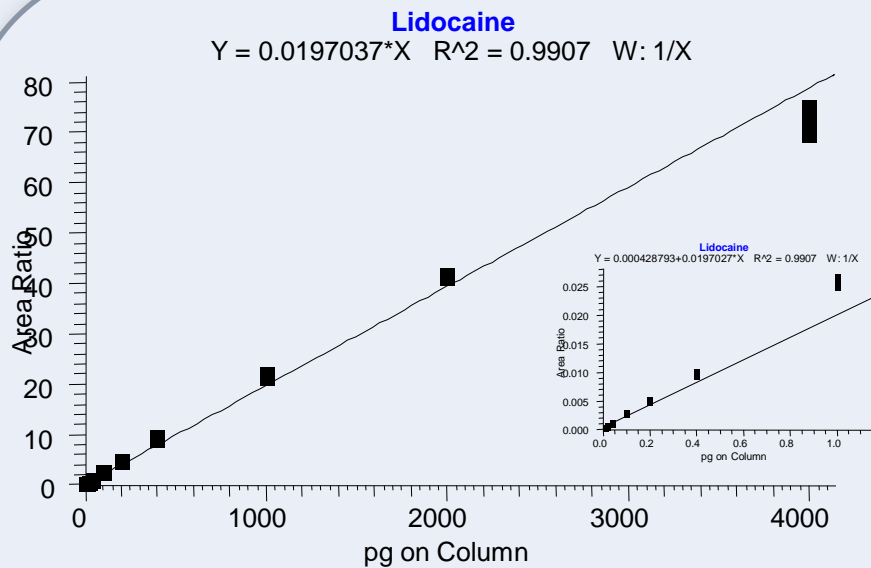
ASMS_VelosPro_Run2_071 #611 RT: 1.99 AV: 1 NL: 1.99E6
 F: ITMS + c ESI Full ms2 240.10@cid35.00[65.00-245.00]



pg on Column	%RSD
0.010	38.92
0.020	6.54
0.040	6.01
0.100	5.60
0.200	4.43
0.400	1.58
1.00	1.44
2.00	1.14
4.00	1.47
10.00	1.88
20.00	1.60
40.00	1.54
100.0	2.51
200.0	1.02
400.0	1.46
1000.0	1.35
2000.0	0.49
4000.0	1.50
10000.0	2.78

Lidocaine 10 fg – 4 ng

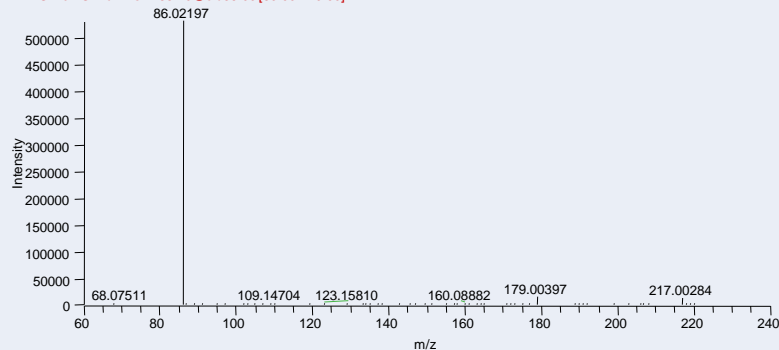
>5 orders



C:\j.homer\...VASMS_VelosPro_Run2_071
 20 pg/uL

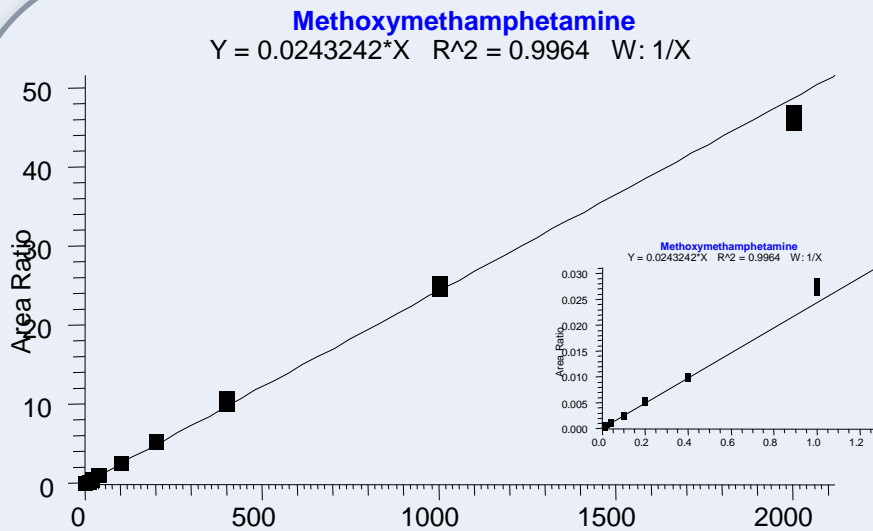
4/9/2011 9:37:03 AM

ASMS_VelosPro_Run2_071 #331 RT: 1.06 AV: 1 NL: 5.30E5
 F: ITMS + c ESI Full ms2 235.20@cid35.00[60.00-240.00]



pg on Column	%RSD
0.010	25.45
0.020	19.69
0.040	9.66
0.100	6.05
0.200	4.31
0.400	3.90
1.00	2.77
2.00	2.12
4.00	2.18
10.00	1.82
20.00	2.63
40.00	2.92
100.0	2.34
200.0	1.04
400.0	1.52
1000.0	1.89
2000.0	0.88
4000.0	2.80
10000.0	1.47

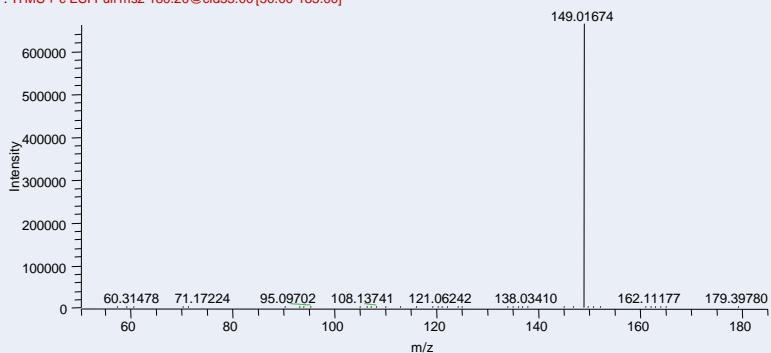
Methoxymethamphetamine 10 fg – 2 ng >5 orders



C:\j.hornef\...ASMS_VelosPro_Run2_071
 20 pg/uL

4/9/2011 9:37:03 AM

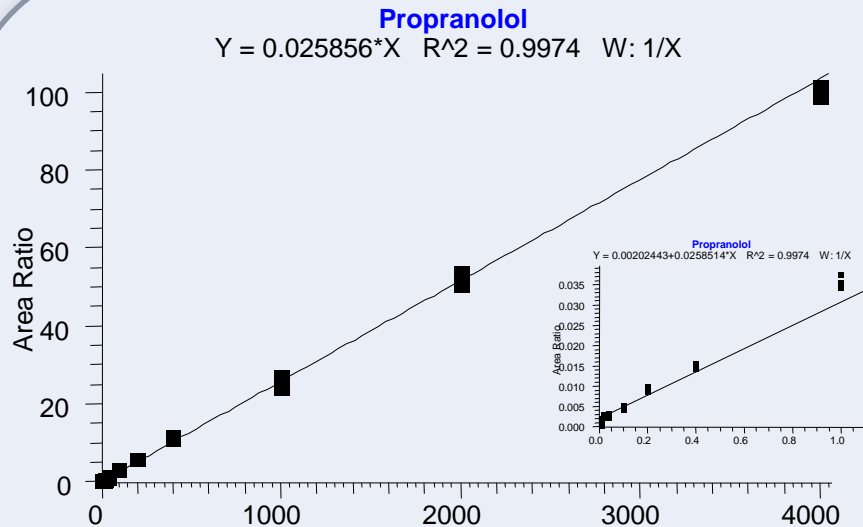
ASMS_VelosPro_Run2_071 #302 RT: 0.98 AV: 1 NL: 6.60E5
 F: ITMS + c ESI Full ms2 180.20@cid35.00 [50.00-185.00]



pg on Column	%RSD
0.010	32.16
0.020	11.42
0.040	8.56
0.100	3.85
0.200	3.60
0.400	2.88
1.00	3.25
2.00	2.77
4.00	2.69
10.00	2.91
20.00	1.49
40.00	1.26
100.0	1.94
200.0	1.40
400.0	2.86
1000.0	1.37
2000.0	1.16
4000.0	1.41
10000.0	2.54

Propranolol 10 fg – 4 ng

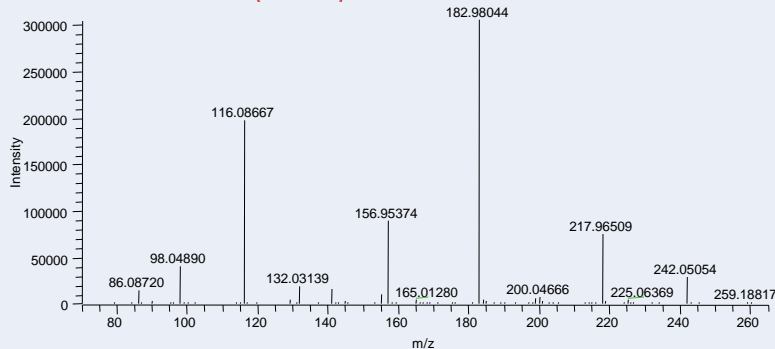
>5 orders



C:\j.homer\...ASMS_VelosPro_Run2_071
 20 pg/uL

4/9/2011 9:37:03 AM

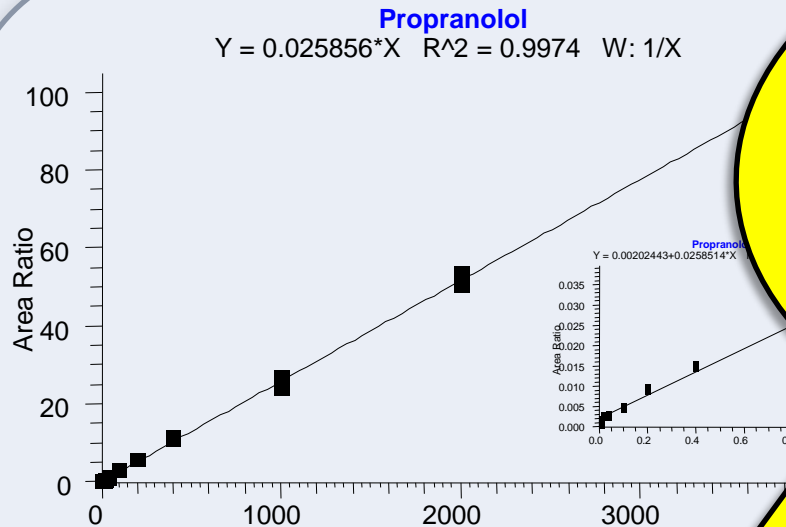
ASMS_VelosPro_Run2_071 #785 RT: 2.52 AV: 1 NL: 3.04E5
 F: ITMS+ c ESI Full ms2 260.20@cid35.00 [70.00-265.00]



pg on Column	%RSD
0.020	14.22
0.040	8.95
0.100	9.65
0.200	4.75
0.400	3.45
1.00	3.43
2.00	2.22
4.00	1.04
10.00	1.31
20.00	1.30
40.00	1.45
100.0	3.11
200.0	1.69
400.0	0.75
1000.0	3.19
2000.0	2.45
4000.0	1.20
10000.0	1.74

Propranolol 10 fg – 4 ng

>5 orders

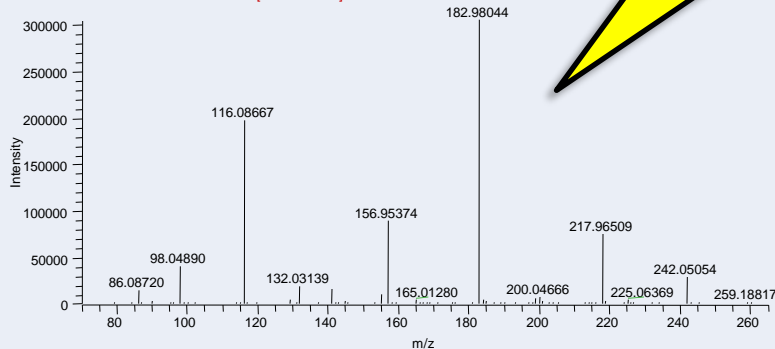


High Quality Full Scan MS/MS at EVERY Data Point

C:\j.homer\...ASMS_VelosPro_Run2_071
20 pg/uL

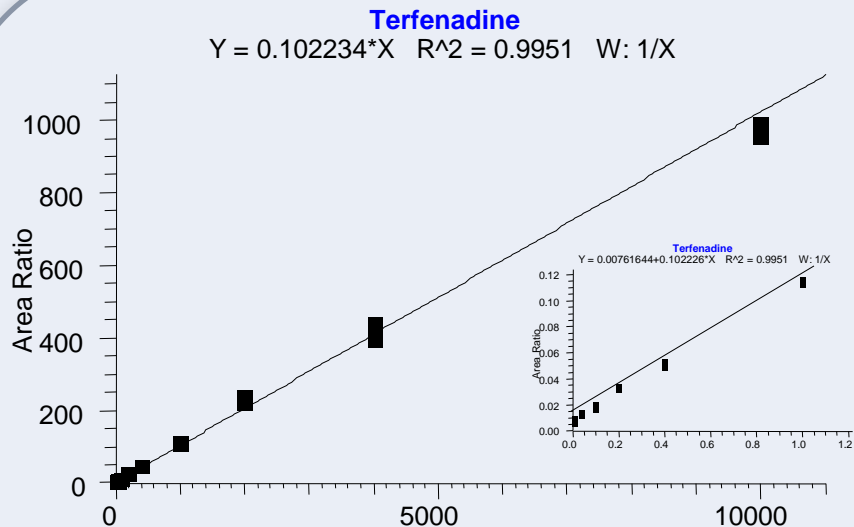
4/9/2011 9:37:03 AM

ASMS_VelosPro_Run2_071 #785 RT: 2.52 AV: 1 NL: 3.04E5
F: ITMS+ c ESI Full ms2 260.20@cid35.00 [70.00-265.00]



Order	Concentration (pg/uL)	Retention Time (min)
1	2.22	1.04
2	10	1.31
4	20	1.30
1	40	1.45
2	10	3.11
4	20	1.69
1	40	0.75
00	100	3.19
00	200	2.45
1	400	1.20
10	1000	1.74
40	2000	
100	4000	
1	10000	

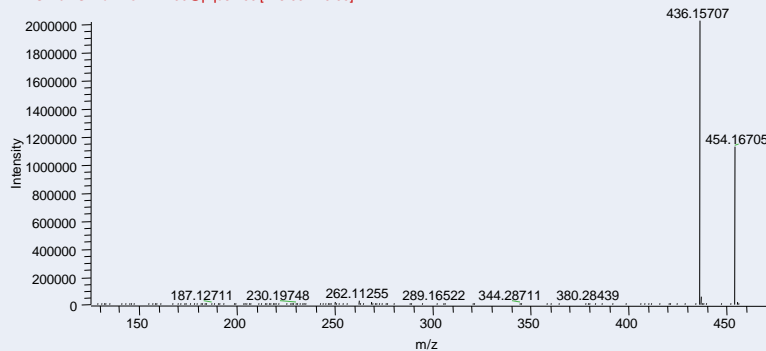
Terfenadine 10 fg - 10 ng 6 orders



C:\j_homer\...ASMS_VelosPro_Run2_071
 20 pg/uL

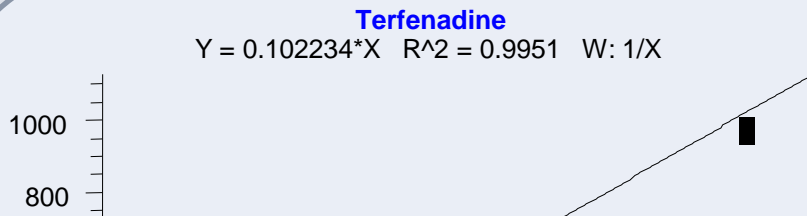
4/9/2011 9:37:03 AM

ASMS_VelosPro_Run2_071 #1281 RT: 4.08 AV: 1 NL: 2.01E6
 F: ITMS+ c ESI Full ms2 472.00@pqd32.00 [125.00-470.00]

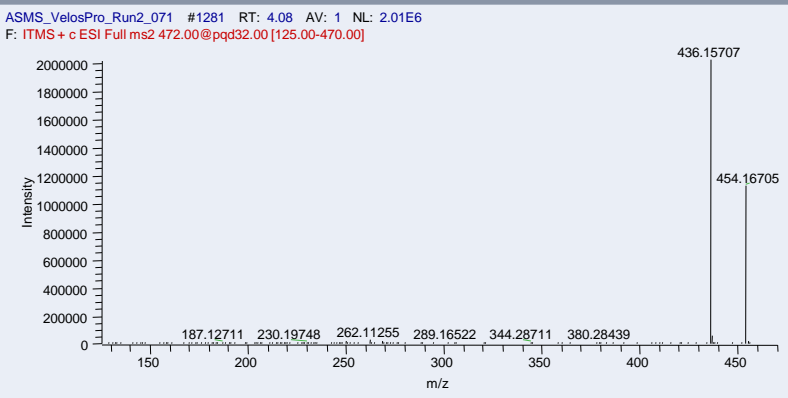


pg on Column	%RSD
0.010	20.75
0.020	8.69
0.040	7.21
0.100	4.36
0.200	2.87
0.400	2.34
1.00	1.22
2.00	2.32
4.00	2.14
10.00	2.15
20.00	1.54
40.00	2.13
100.0	2.28
200.0	3.69
400.0	1.76
1000.0	2.63
2000.0	3.94
4000.0	2.72
10000.0	1.93

Terfenadine 10 fg - 10 ng 6 orders



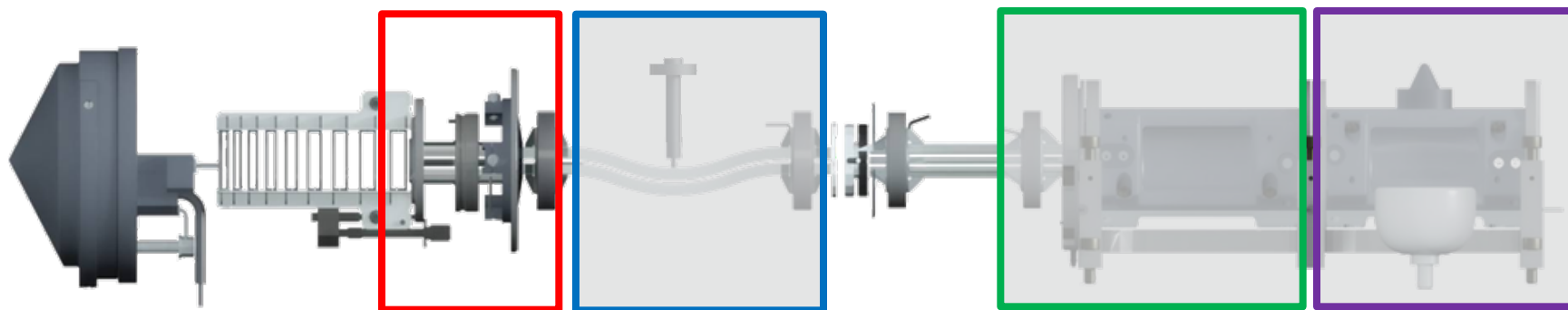
Velos Pro – Designed For Quantitation



pg on Column	%RSD
0.010	20.75
0.020	8.69
0.040	7.21
0.080	4.36
0.160	2.87
0.320	2.34
0.640	1.22
1.280	2.32
2.560	2.14
5.120	2.15
10.240	1.54
20.480	2.13
40.960	2.28
81.920	3.69
163.840	1.76
327.680	2.63
655.360	3.94
1310.720	2.72
2621.440	1.93

Velos Pro: Trap-HCD Fragmentation

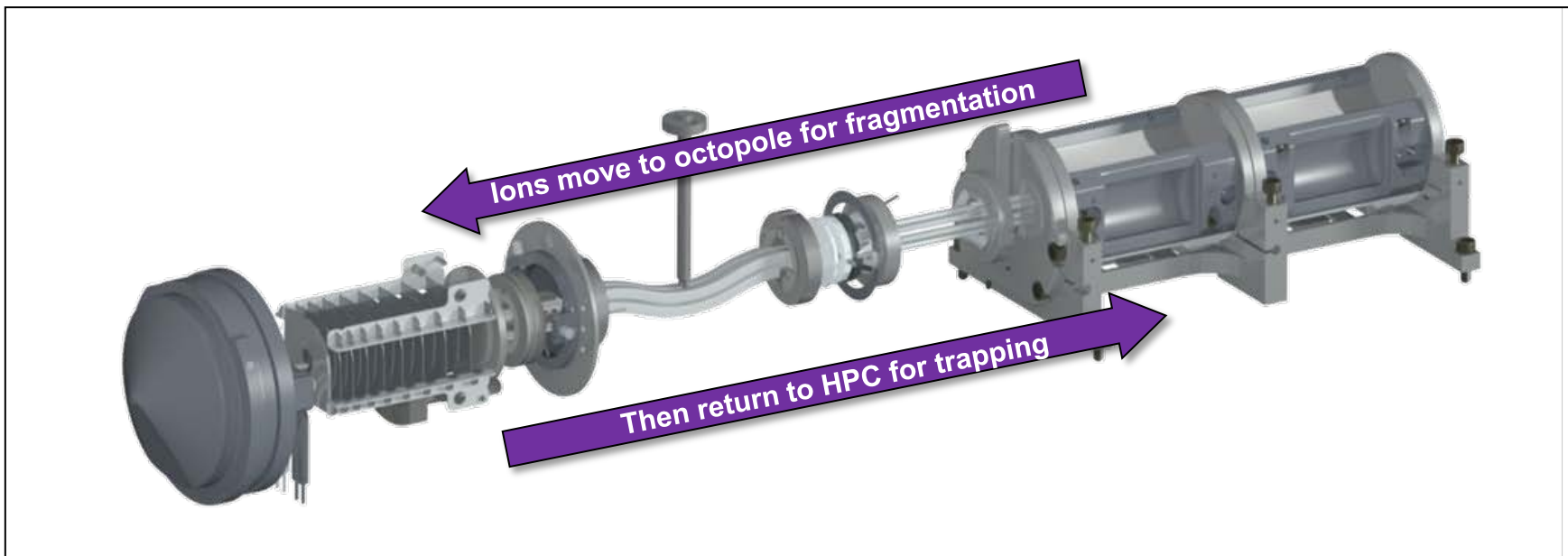
	Feature	Customer Benefit
1	UHPLC Cycle Time and High Data Rates	<ul style="list-style-type: none">• More points across a chromatographic peak gives better quantitative performance• More MS^N during a run• Now scanning at 66kDa/sec
2	Robust and Sensitive Generation II Ion Optics	<ul style="list-style-type: none">• Improved robustness, reduce down time, all on the most sensitive ion trap.• <i>High sensitivity S-Lens technology</i>• Novel beam blocking technology
3	Novel Detection System for superior Quantitation	<ul style="list-style-type: none">• Single digit RSDs, up to 6 orders of linear dynamic range on the first ion trap designed for quantitation.• New EM and electrometer detection system matched to handle the ultra-high peak currents from the VELOS trap.
4	Trap-HCD Fragmentation	<ul style="list-style-type: none">• Increases flexibility for structural elucidation• Trap-HCD fragmentation yields unique product ions for structural determinations.



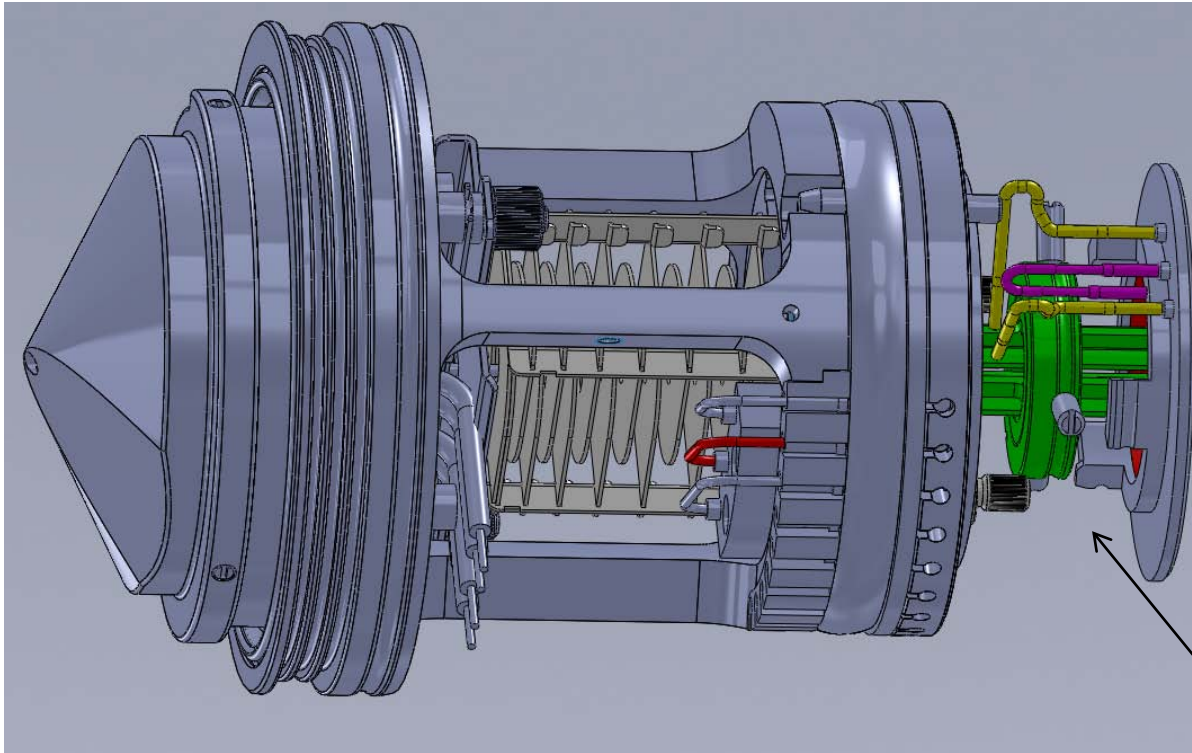
The Collision Cell You Never Knew You Had!

TRAP-HCD

1. Isolate parent ions in the HPC
2. Pass the ions back to the Q00 (100mTorr) at high energy.
3. Send fragments back to the Dual pressure trap for analysis.

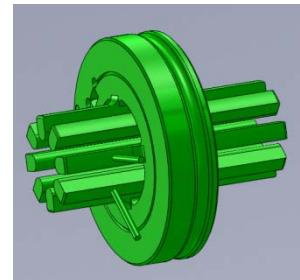


Replace Square Quadrupole with Octopole for Q00



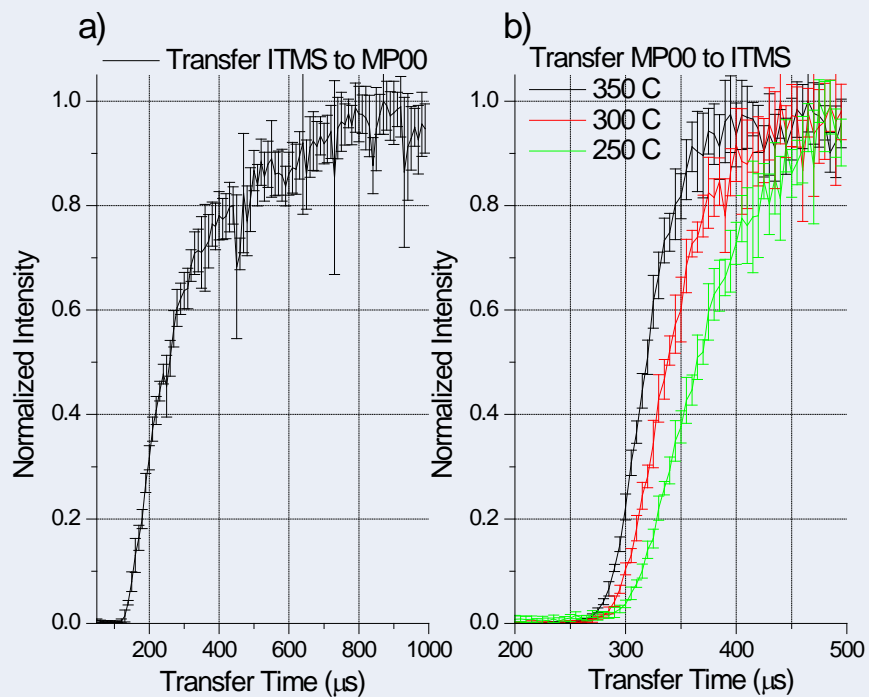
Advantages:

- Higher Ion Capacity
- Lower Mass Discrimination

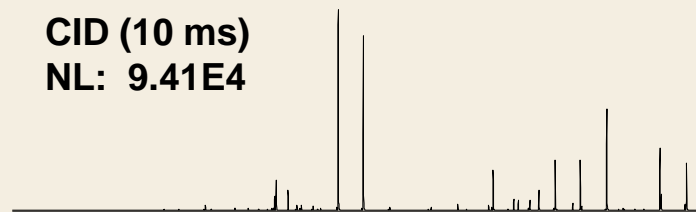


Trap-HCD Technology

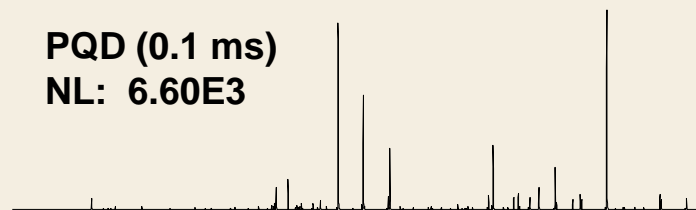
The transfer of ions from the Ion Trap to the Octopole and back is fast and efficient.



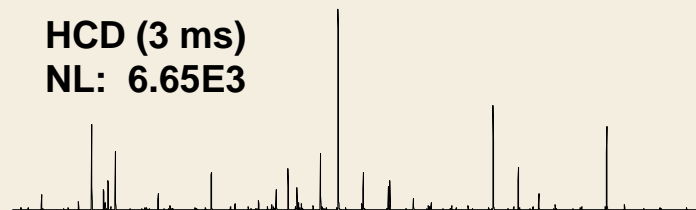
CID (10 ms)
NL: 9.41E4



PQD (0.1 ms)
NL: 6.60E3



HCD (3 ms)
NL: 6.65E3



In General:

- CID is the most sensitive and has a wide range of efficiency.
- PQD is selective and has a very narrow optimum.
- Trap-HCD is typically $\frac{1}{2}$ as efficient as CID.

Trap-HCD vs TSQ fragmentation

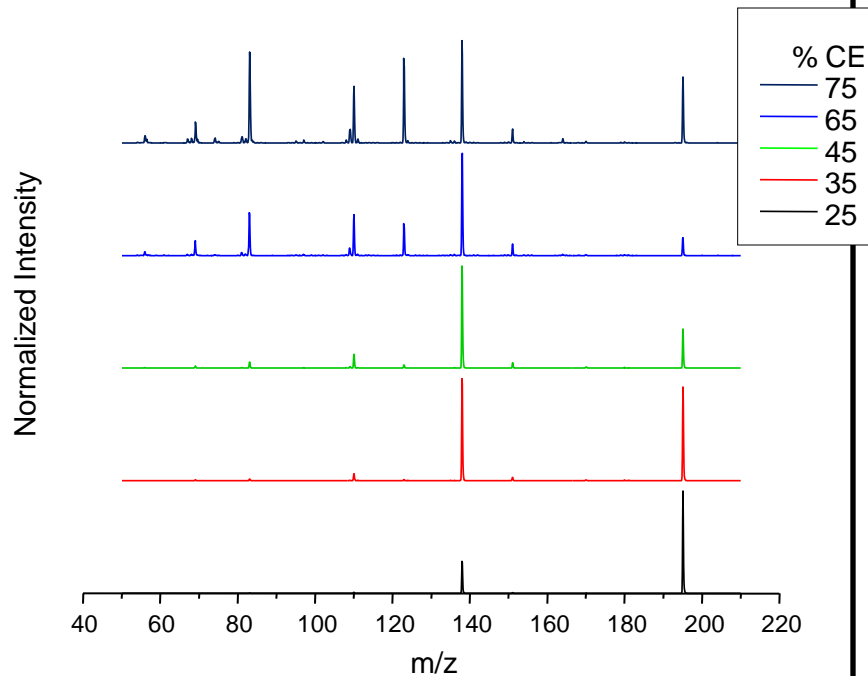


Velos Pro

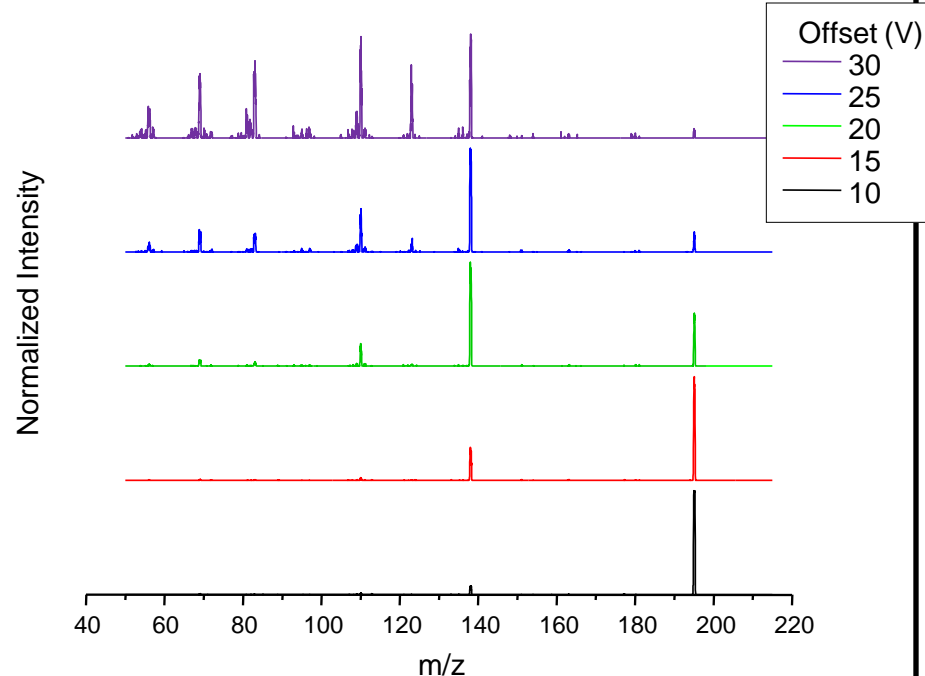


TSQ Vantage

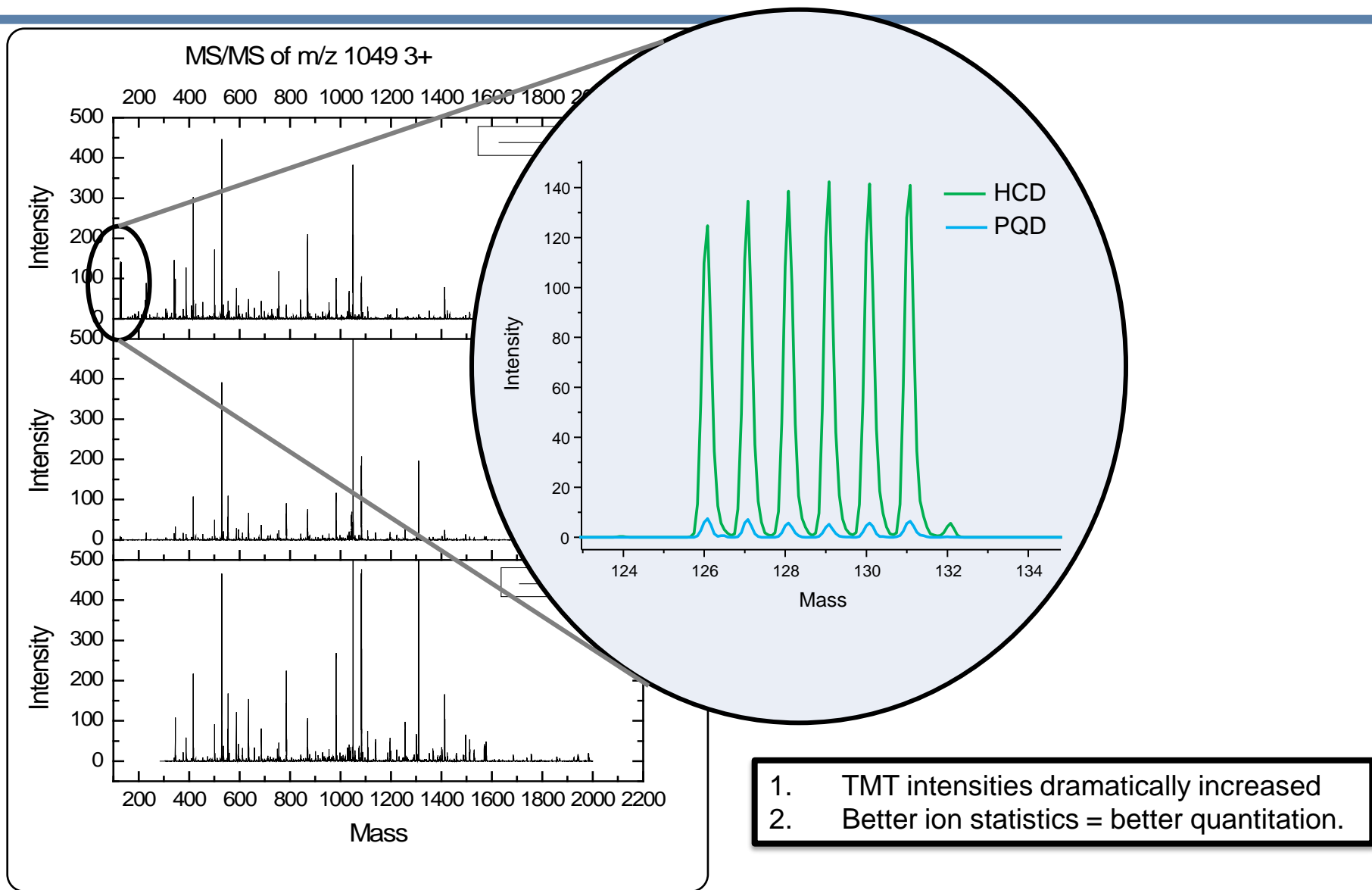
HCD of Caffeine at Various Collision Energies on LTQ Velos Pro



HCD of Caffeine at Various Collision Energies on TSQ Vantage



Protein Quantitation – TMT Labeled Peptides

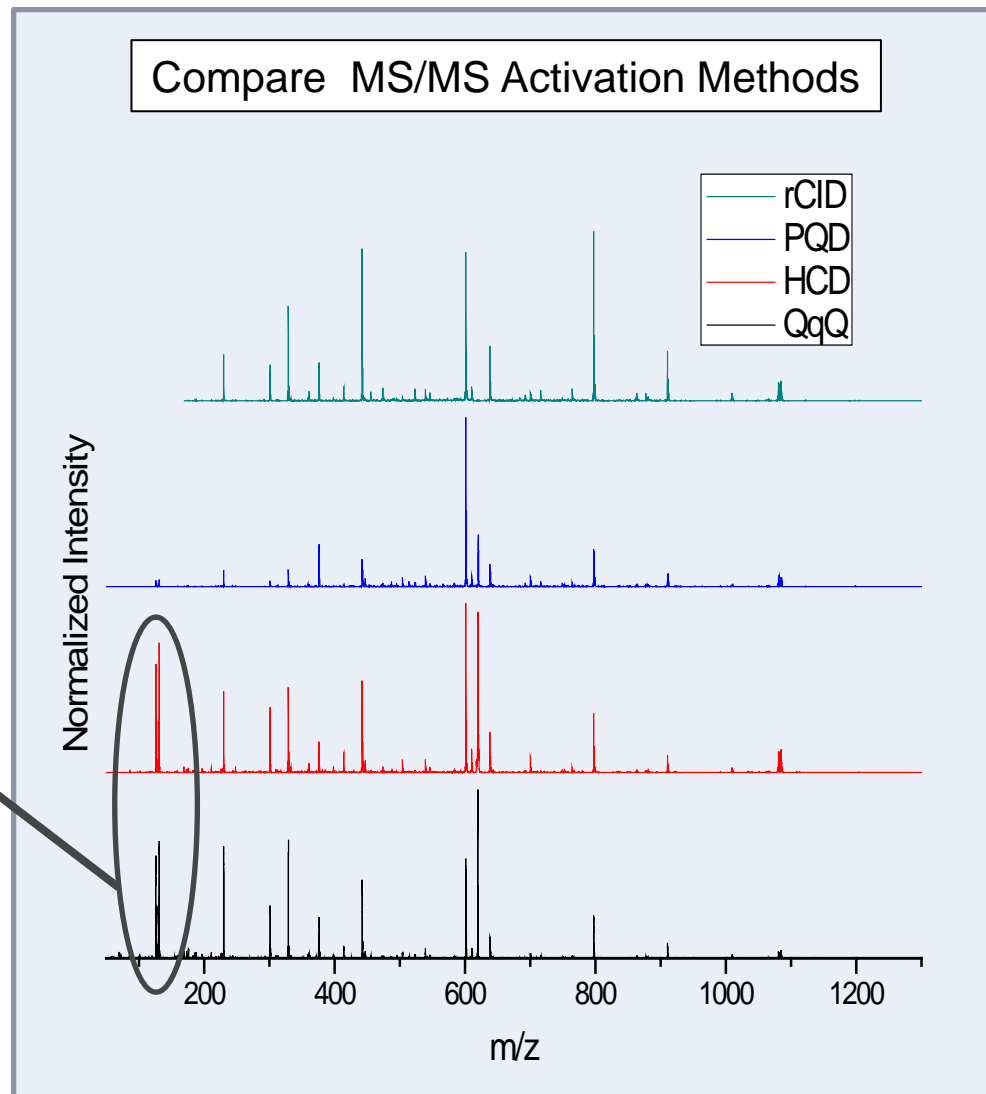
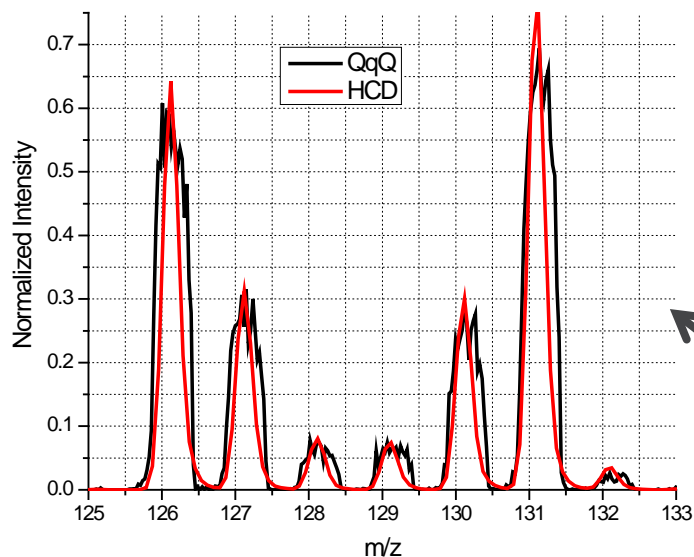


Trap HCD – TMT Labeled Peptide Comparison

m/z 620²⁺

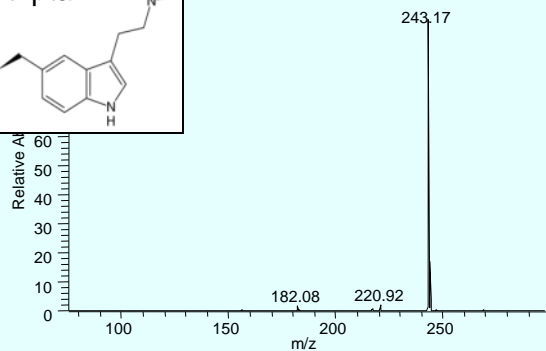
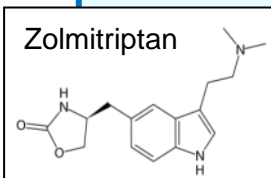
Expected Ratio 10:4:1:1:4:10

Compare Reporter Ion Regions for QqQ and Trap HCD Spectra

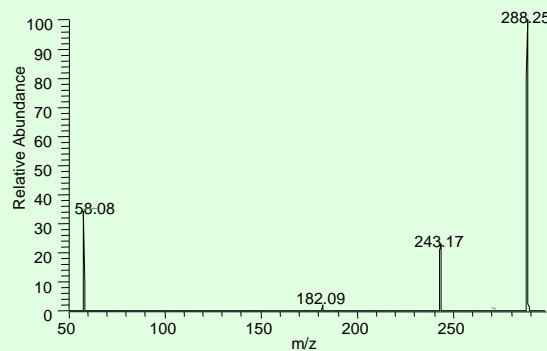


Small Molecule Activation Comparison

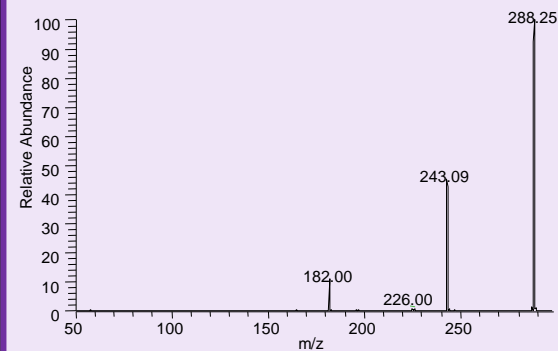
CID



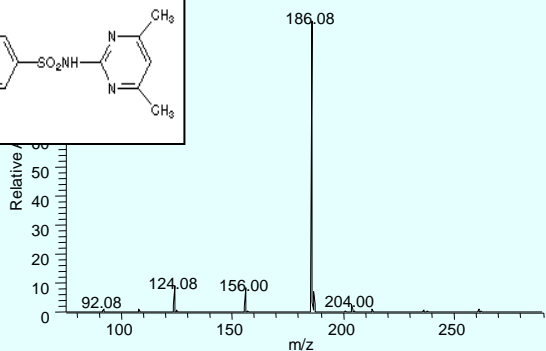
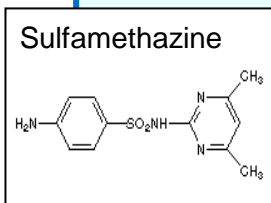
HCD



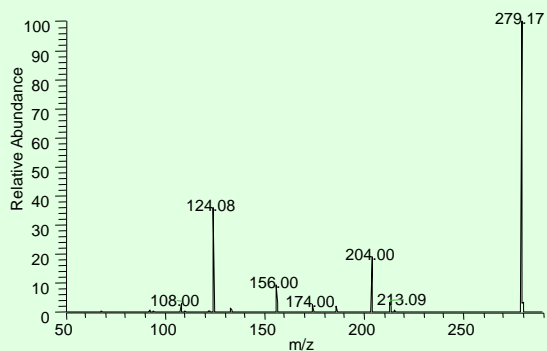
PQD



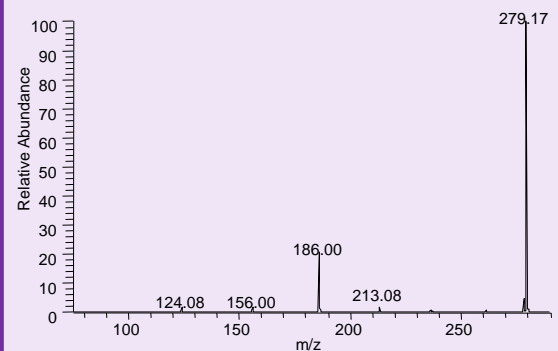
CID




HCD



PQD



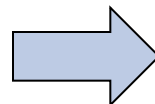


**Velos Pro for Metabolite Structure
Identification and Quantitation**

Trifluoropirazine
Dextromethorphan

New Fragmentation Possibilities

- ❖ Multiple Fragmentation Techniques
- ❖ MSⁿ and Fragment Heritage

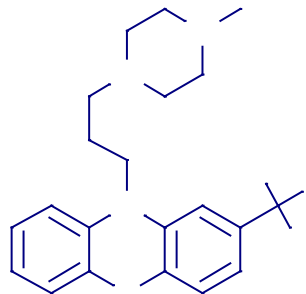


Velos Pro:

Ability to use CID and HCD combinations at multiple different MSⁿ levels.

Sample:

Trifluopirazine metabolites from human liver S9 incubation



LC-MS:

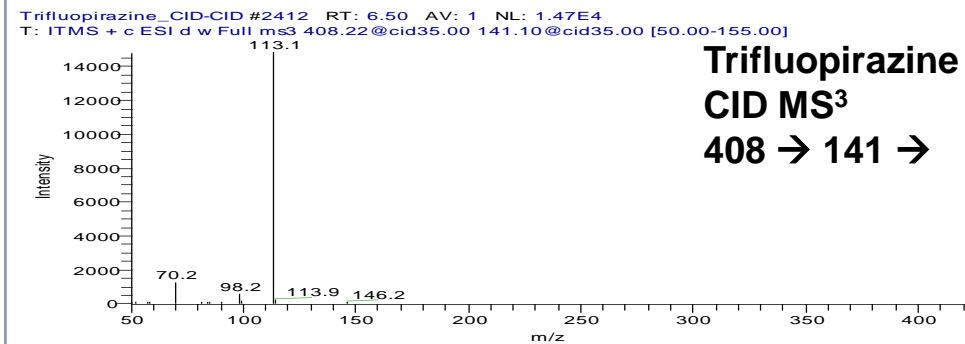
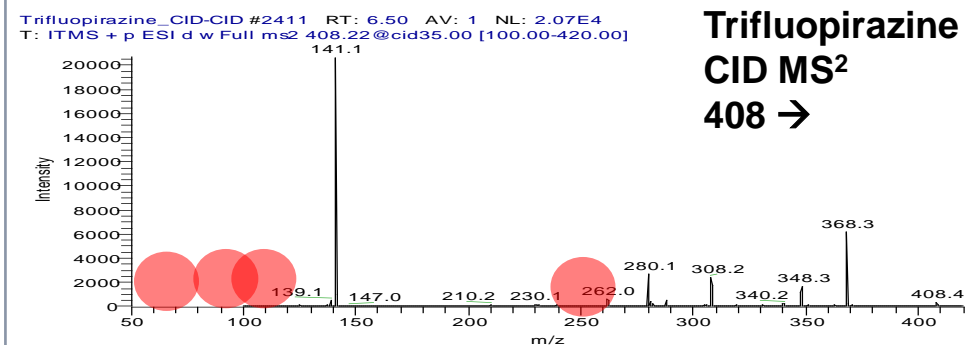
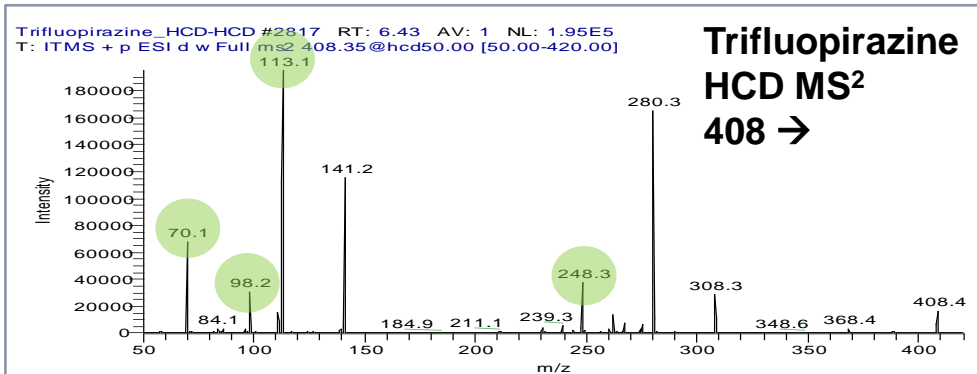
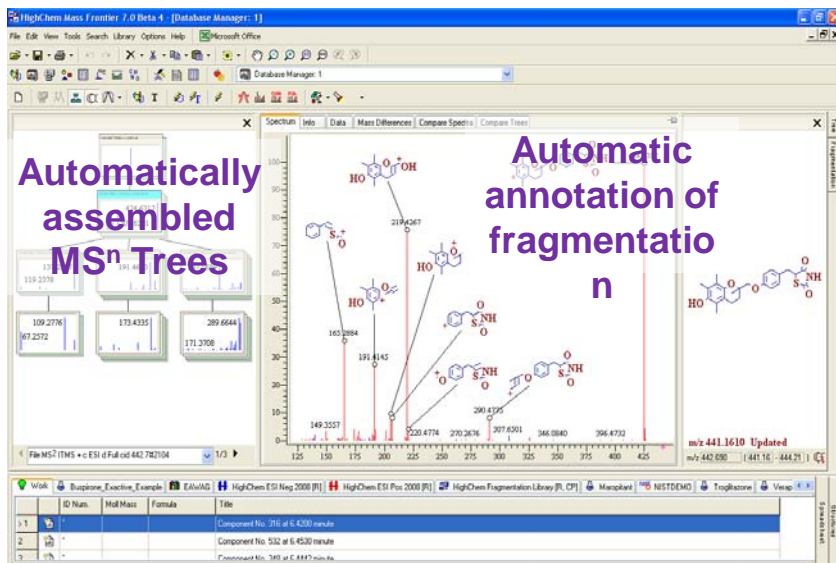
Linear 10 minute gradient of Water:ACN w/ 0.1% Formic.

Hypersil Gold aQ 50X2 1.9 μ column

1. Full scan + CID only MSⁿ
2. Full Scan + HCD MS² + CID MS³
3. Full Scan + HCD MS² + HCD MS³

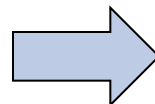
New Fragmentation Possibilities – HCD, CID MSⁿ

- ✓ HCD fragmentation provides a broader mass range of fragments.
- ✓ CID fragmentation provides similar fragment detail with additional MSⁿ steps.
- ✓ Mass Frontier provides automated annotation of fragmentation spectra facilitating structure elucidation



MSⁿ Quantitation – Ultimate Selectivity in Real Matrix

- ❖ MSⁿ Quantitation
- ❖ Ultimate Selectivity

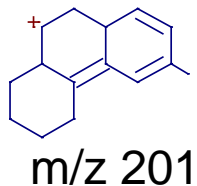
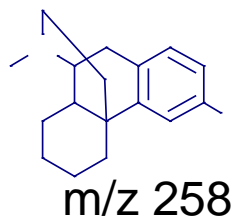


Velos Pro:

Robust MSⁿ fragmentation and multiple fragmentation techniques.

Sample:

Human plasma standards with Dextromethorphan and Metabolites.



LC-MS:

Linear 5 minute gradient of Water:MeOH w/ 0.1% Formic.

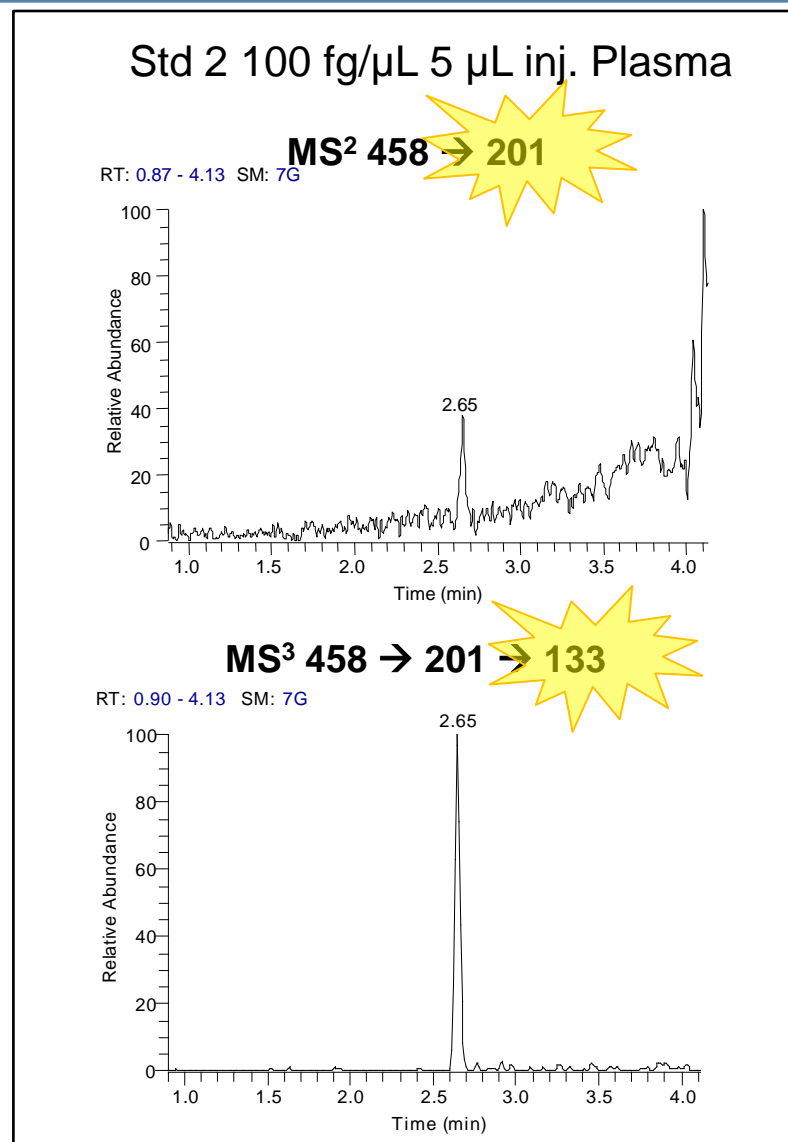
Hypersil Gold aQ 50X2 1.9 μ column

CID (258) \rightarrow CID (210) \rightarrow MS³

MSⁿ Quantitation – Ultimate Selectivity in Real Matrix

- Without using MS³, detection and quantitation was not possible below 0.1 fg/μL.
- With MS³, quantitation was possible down to 0.01 fg/μL and detection possible below that.
- Triplicate Standard curves were prepared and analyzed, the limit of Quantitation and Detection as well as the relative standard deviation (RSD) are shown below.

Std pg/μL	MS ²
1000	8%
100	5%
10	4%
1	11%
0.1	LOQ/LOD, 12%
0.01	X
0.005	X





**Velos Pro in Proteomics Discovery
and Relative Quantitation**

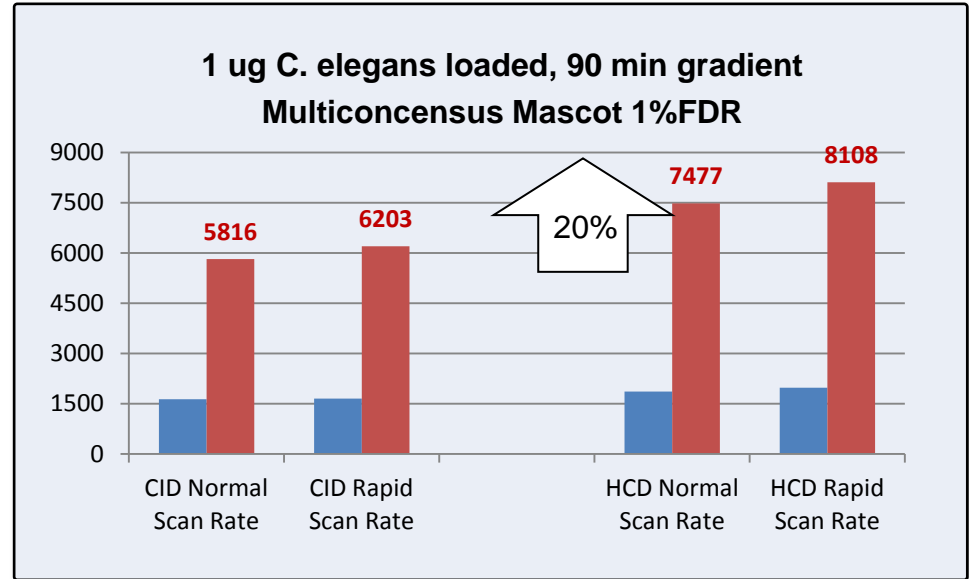
C. elegans

Tandem Mass Tags

Proteomics Discovery Experiments

Velos Pro Benefits for Proteomics Discovery Experiments

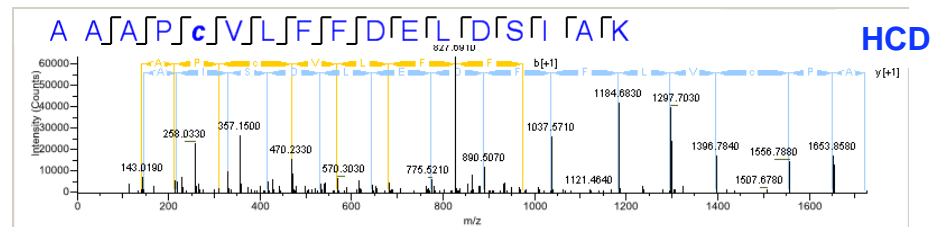
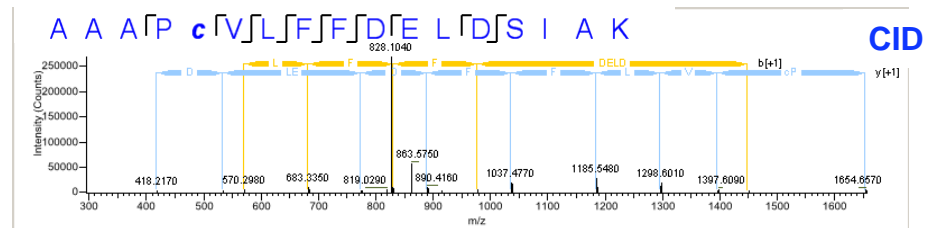
- **Rapid Scanning**
 - Improves total numbers of proteins and peptides identified
- **HCD**
 - Improves total numbers of proteins and peptides identified
 - Low m/z fragment ions can confirm terminal residue identification



Proteome Discoverer 1.3 CID/HCD Venn Diagram C. elegans Peptides



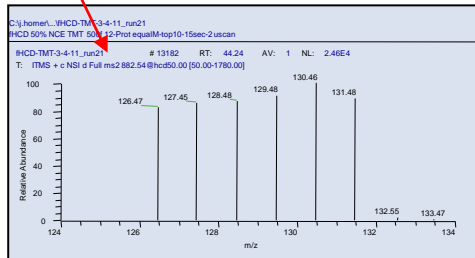
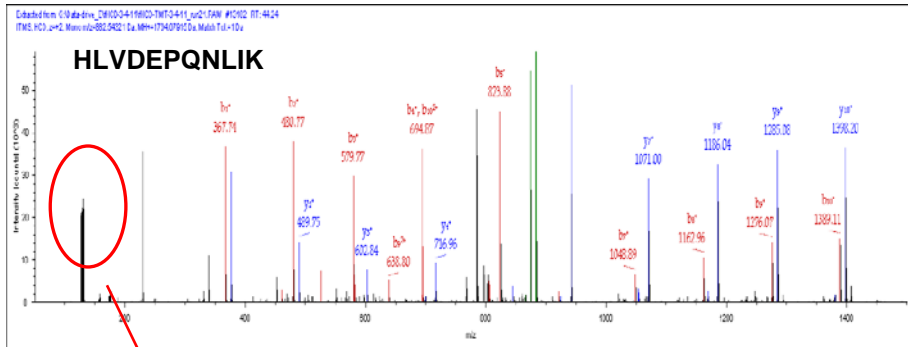
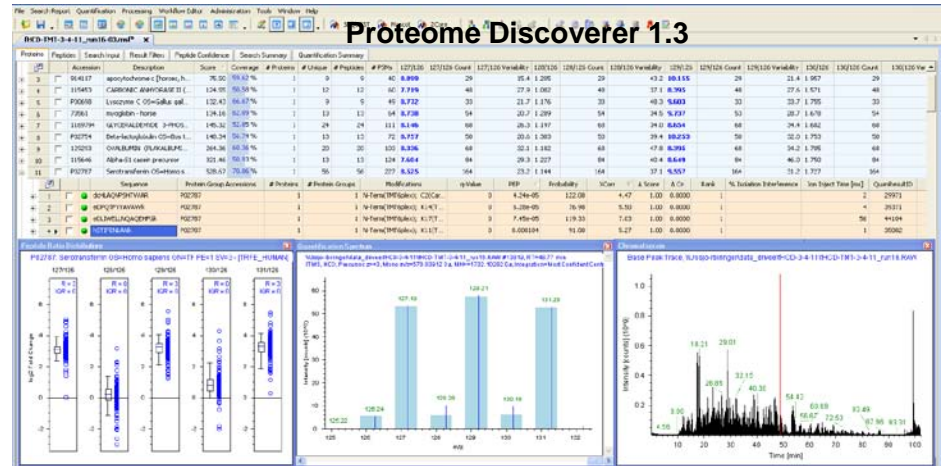
Exclusive	Total	Label	Description
1143	3796	Input File: CID-RR-Top20_Short_002.Thr...	Input File: CID-RR-Top20_Short_002.Thr...
2643	5296	Input File: HCD-RR-Top20_Short_007.Thr...	Input File: HCD-RR-Top20_Short_007.Thr...
2653	2653	Input File: CID-RR-Top20_Short_002.Thr...	Input File: CID-RR-Top20_Short_002.Thr...
Sum	6439		



Proteomics Relative Quantitation with TMT

TMT-HCD Experiments on Velos Pro

- 12 protein mixture digest
- Each peptide labeled with TMT 6-plex at 1:1:1:1:1:1 or 1:10:1:10:1:10
- 500 fmol each or 50:500 fmol loaded on column
- 90 minute gradient 5% → 30% ACN
- Top 10 data dependent Trap-HCD, dynamic exclusion repeat count 1




Results of Trap-HCD-TMT Experiments for 1:1:1:1:1:1 Labeled Peptides

Peptide Spectral Matches	4363
Number of Peptides Quantified	3269
Percent of Identified Peptides Quantifiable	75%
Average Protein Level CV	2.5%

Velos Pro HCD gives:

1. Dramatic increase in TMT intensities
2. Better ion statistics = Better quantitation.
3. Low m/z sequence ions
4. Increased peptide identifications



**Velos Pro for Clinical Screening
and Quantitative Analyses**

Vitamin D2 and D3
Opiates

Vitamin D Standard Curves

Vitamin D2 & D3 Analysis

Sample Preparation

LLE of plasma samples done at collaborator site

Chromatography

Accela Open AS and Accela 1250 uHPLC pump

Gradient and column duplicated from Collaborator Lab

Source

APCI source conditions optimized for best signal to noise

Mass Spectrometry

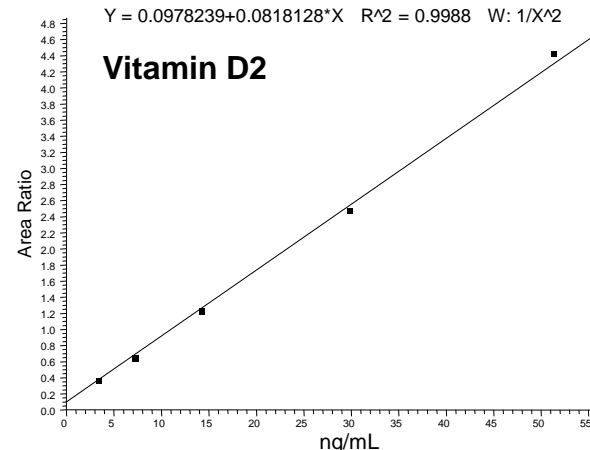
3 Full Scan MS3 scan events

Data Processing

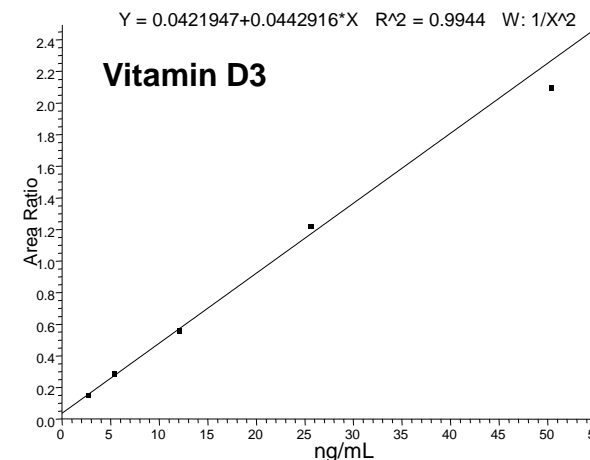
All samples processed using LCQuan 2.6.1

Patient Sample results compared to Collaborator Lab

Vitamin D2	
Conc. (ng/mL)	% Difference
3.3	1.54
7.5	-3.33
14.1	-0.24
29.7	-1.47
51.2	3.50

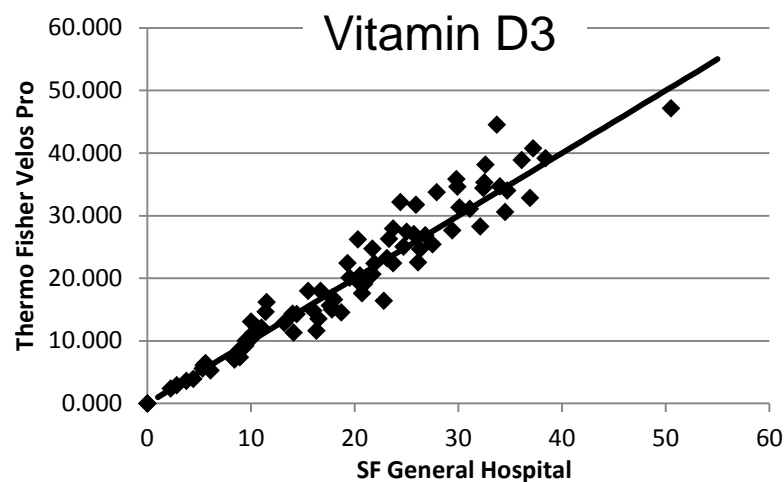
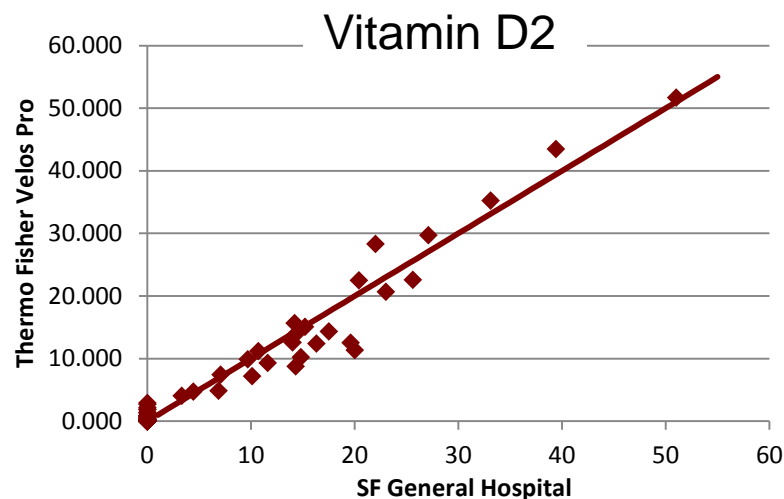
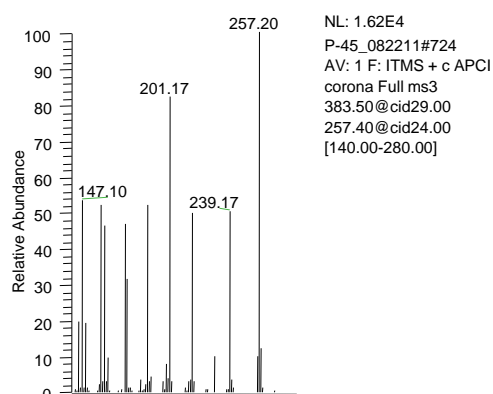
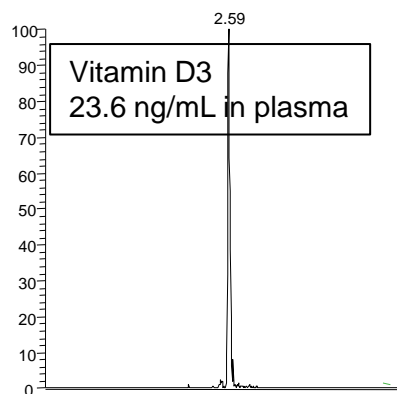
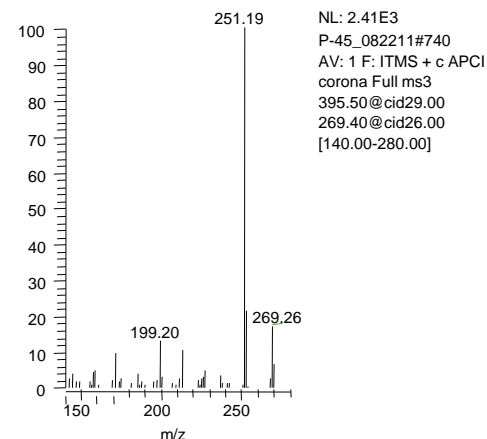
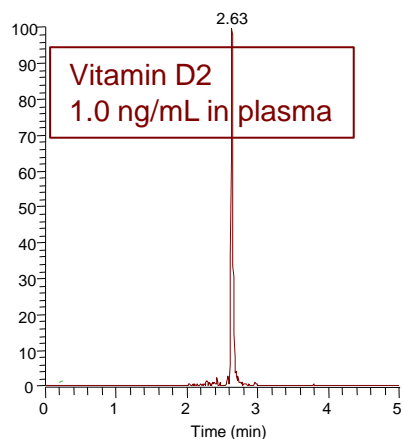


Vitamin D3	
Conc. (ng/mL)	% Difference
2.6	-3.10
5.3	6.49
12.0	-0.93
25.5	4.98
50.3	-7.43



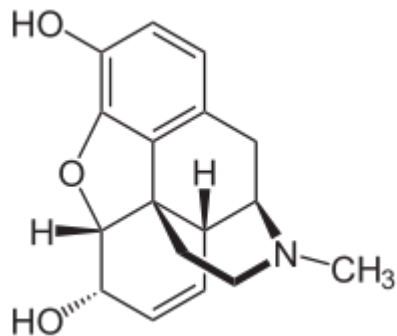
Vitamin D Analysis in Patient Plasma Samples

MS3 Extracted Ion Chromatograms P45

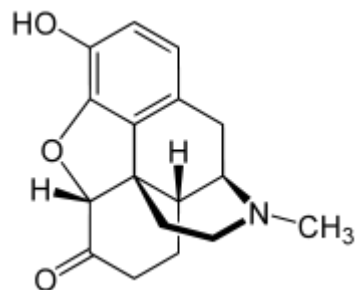


Opiates Analysis

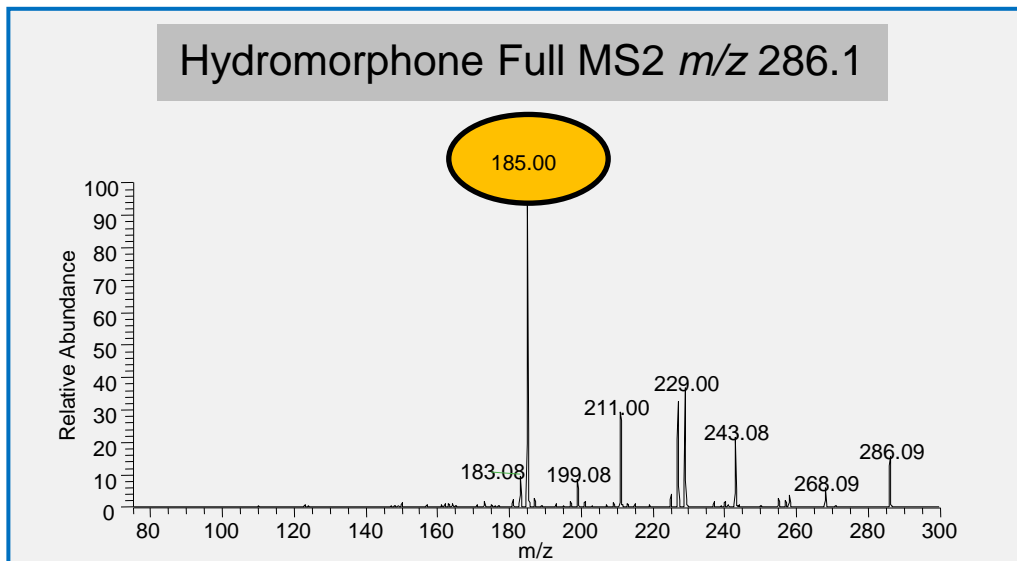
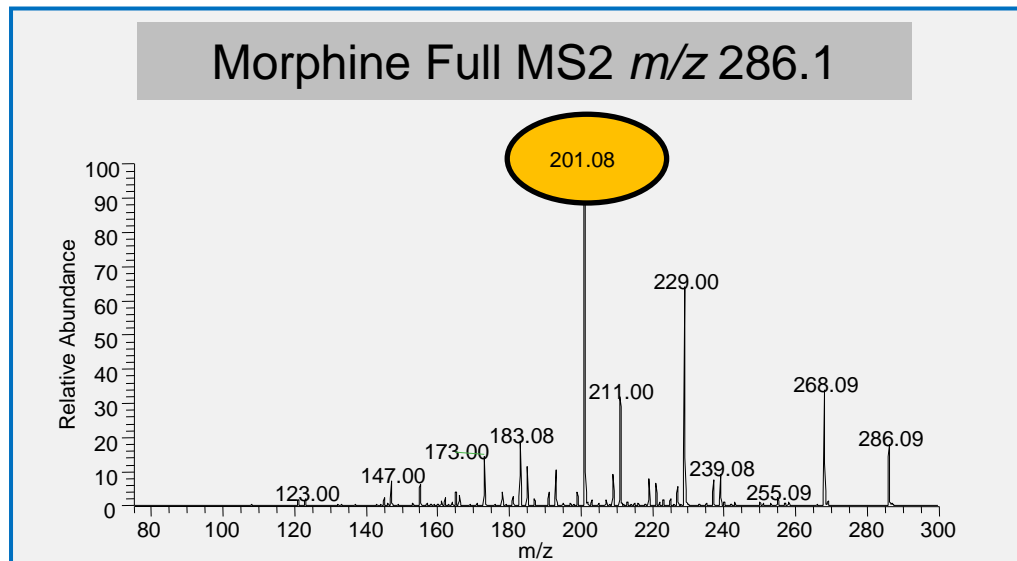
- Sample Preparation
 - 12 Opiates
 - 40X diluted urine
- Chromatography
 - Accela Open AS and Accela 1250 uHPLC pump
 - 5 minute chromatographic method
- Source
 - Heated ESI
- Mass Spectrometry
 - 8 MS2 scan events, 2 MS3 scan events
 - Segmented method
- Data Processing
 - LC Quan 2.6.1



Morphine
C₁₇H₁₉NO₃



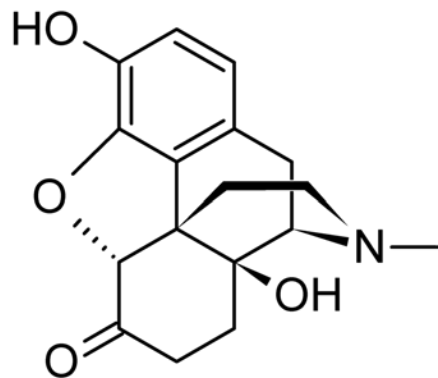
Hydromorphone
C₁₇H₁₉NO₃



Alere Opiates Analysis

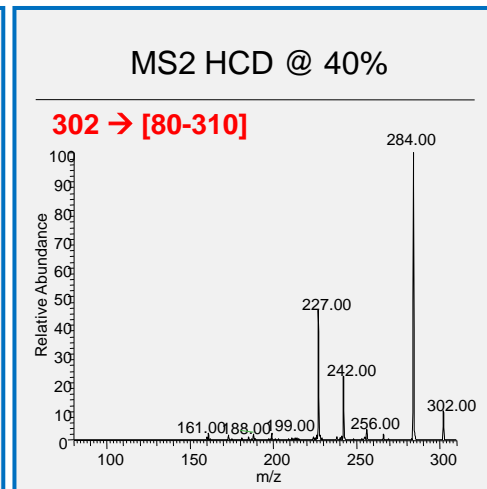
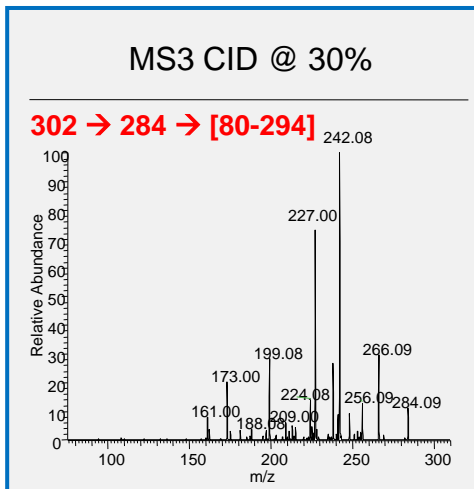
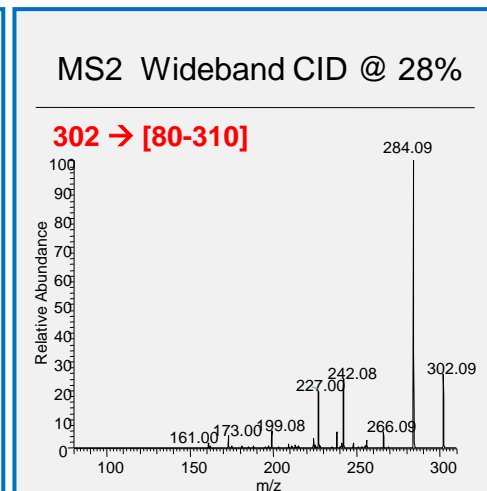
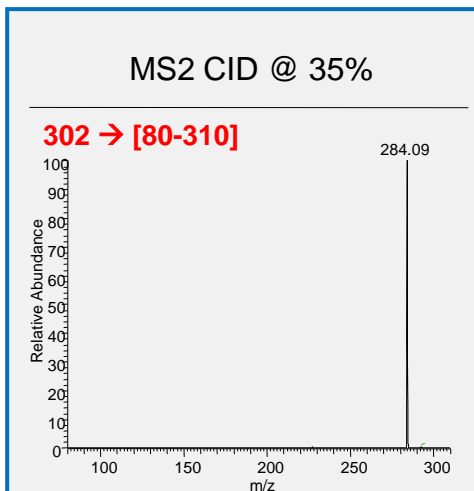
Optimize Fragmentation for Best Sensitivity **and** Selectivity

- Sample Preparation
 - 12 Opiates
 - 40X diluted urine
- Chromatography
 - Accela Open AS and Accela 1250 uHPLC pump
 - 5 minute chromatographic method
- Source
 - Heated ESI
- Mass Spectrometry
 - 8 MS2 scan events, 2 MS3 scan events
 - Segmented method
- Data Processing
 - LC Quan 2.6.1



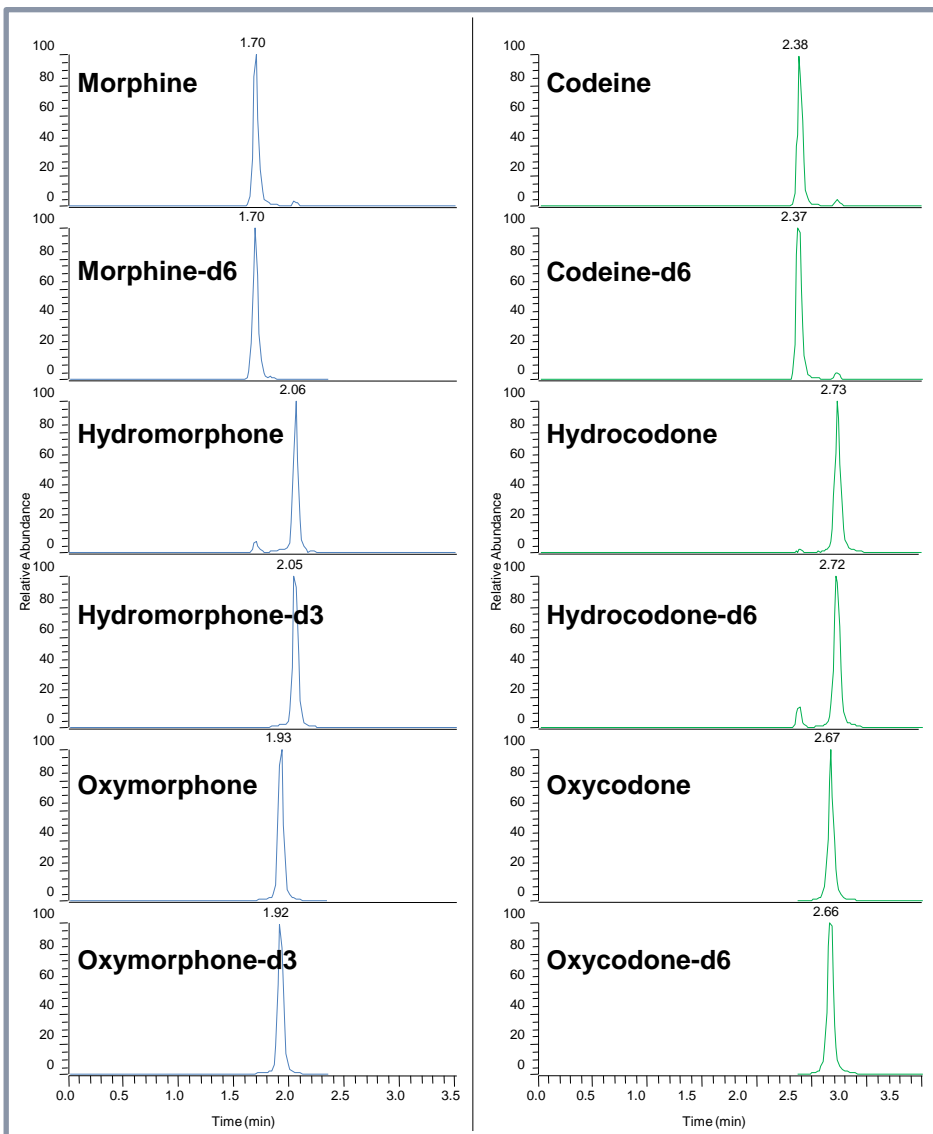
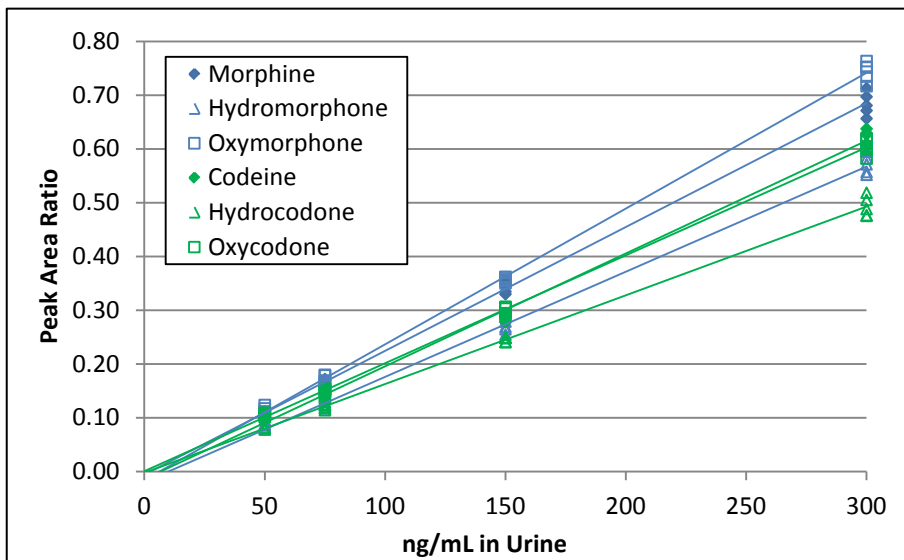
Oxycodone
C₁₇H₁₉N₃O₄

Oxycodone Fragmentation by CID, HCD



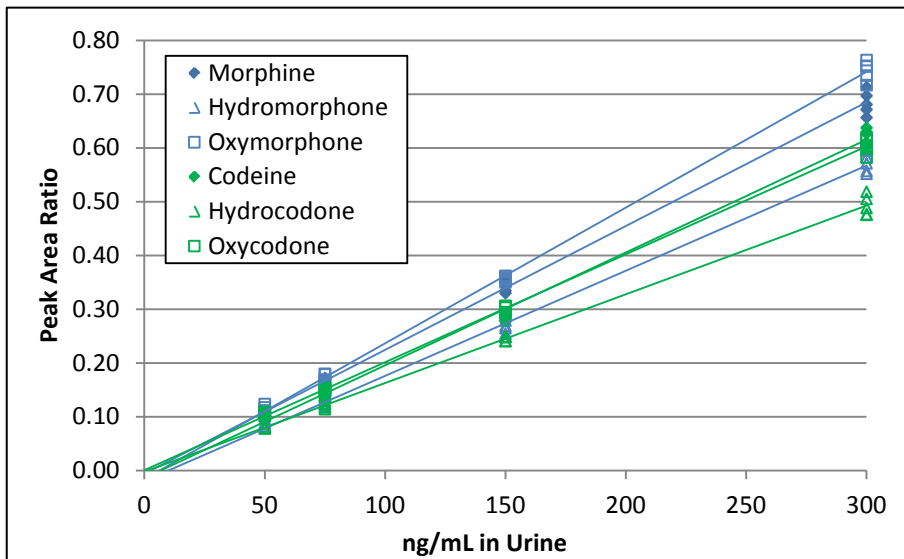
Alere Opiates Analysis

- Velos Pro sensitivity and scan speed are sufficient for Opiates screening and quantitation.
- Flexibility in selection of fragmentation affords highest sensitivity.
- Limits of quantification for all opiates < 10 ng/mL (estimated from S/N for low levels)
- Percent difference between known and calculated levels $\leq 5\%$ for all compounds.



Alere Opiates Analysis

- Velos Pro sensitivity and scan speed are sufficient for Opiates screening and quantitation.
- Flexibility in selection of fragmentation affords highest sensitivity.
- Limits of quantification for all opiates < 10 ng/mL (estimated from S/N for low levels)
- Percent difference between known and calculated levels $\leq 5\%$ for all compounds.



Compound		Cal 1 50 ng/mL	Cal 2 75 ng/mL	Cal 3 150 ng/mL	Cal 4 300 ng/mL
Morphine	Average % Difference	-3.98%	-2.45%	0.91%	0.69%
	%RSD	2.40%	4.9	3.1	3.2
Hydromorphone	Average % Difference	-3.10%	-5.43	-2.5	3.02
	%RSD	4.40%	6.70%	4.30%	3.50%
Oxymorphone	Average % Difference	-3.63	-4.87	-1.48	2.44
	%RSD	5.20%	4.00%	1.40%	2.50%
Codeine	Average % Difference	-0.09	-3.12	-5.05	3.32
	%RSD	4.90%	5.50%	2.60%	2.30%
Hydrocodone	Average % Difference	-1.83%	-1.21%	0.18%	0.46%
	%RSD	1.70%	4.80%	1.80%	3.80%
Oxycodone	Average % Difference	4.90%	-1.86%	-0.63%	0.12%
	%RSD	3.40%	5.10%	2.40%	2.50%

Velos Pro LC-MSⁿ

✓ Accurate

•All background ions are rejected – minimal space charge issues – **maximum accuracy**

✓ Precise

•Fast scanning and novel detection system yield **single digit RSD's** at UHPLC speeds

✓ Sensitive

•HESI-II , S-LENS, and Dual pressure trap give superb **low femtogram sensitivities** with FULL SCAN product ion information

✓ Robust

•Generation II ion optics produce **a robust and stable signal.**

✓ Versatile

•CID, Trap-HCD, PQD, and ETD for **maximum information** from every sample.



Velos Pro LC-MSⁿ