The ThermoFinnigan TSQ Quantum

High Performance Quantitative and Qualitative Applications on a High Resolution Triple Quadrupole Mass Spectrometer

Gary Paul, Witold Winnik, Maurizio Splendore

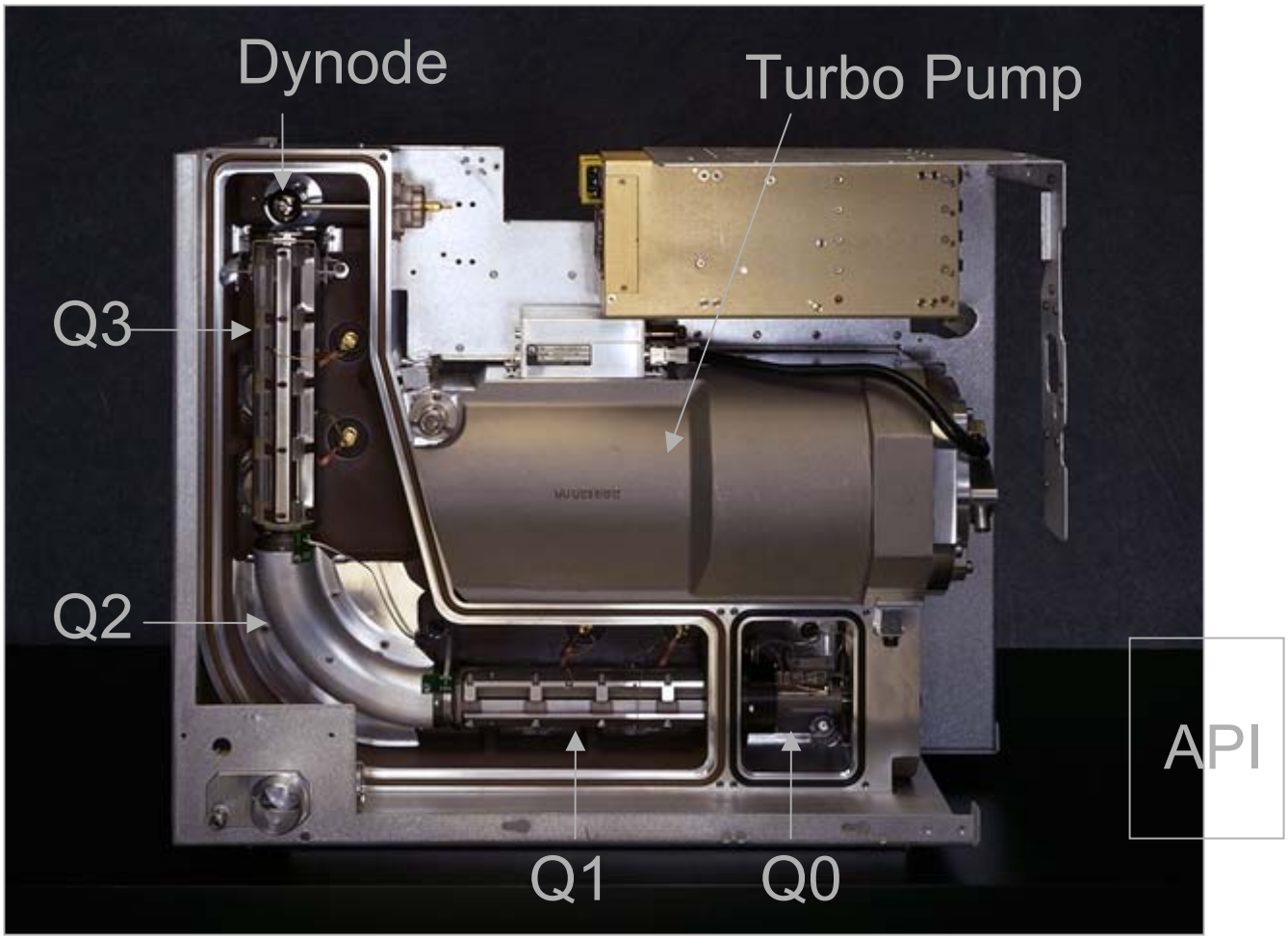
Thermo Finnigan

The TSQ Quantum

The only high resolution triple quadrupole mass spectrometer available capable of accurate mass measurement.

Identification and high performance quantitation of components such as metabolites in one small package!

TSQ Quantum - How So Small?
A Revolutionary 90 Degree Bent Collision Cell

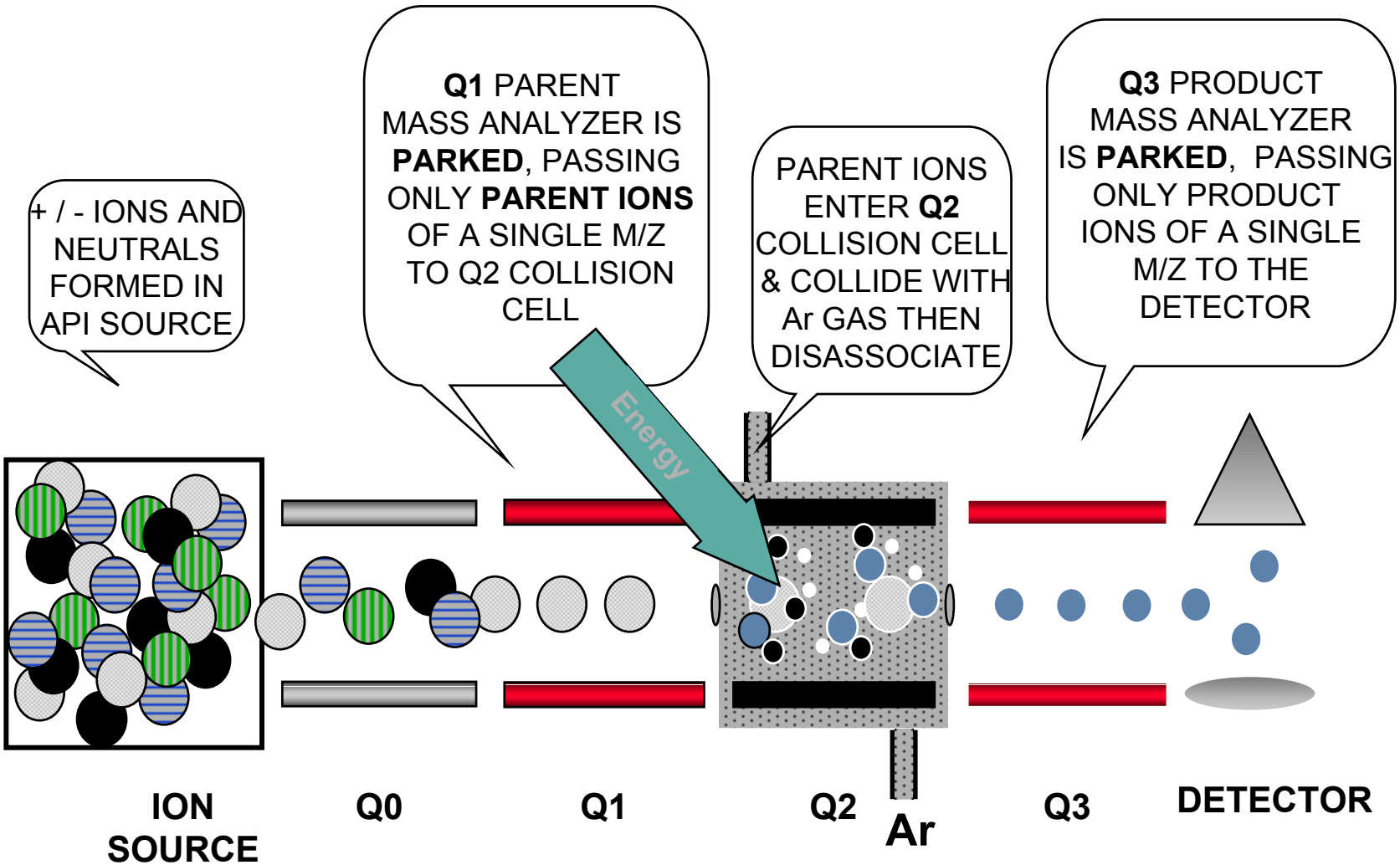


TSQ Quantum - Quantitation

High performance quantitation is the "bread and butter" application of the triple quadrupole mass spectrometer market

TSQ Quantum was primarily designed to achieve maximum **sensitivity**, **accuracy**, **precision** and **linear dynamic range**

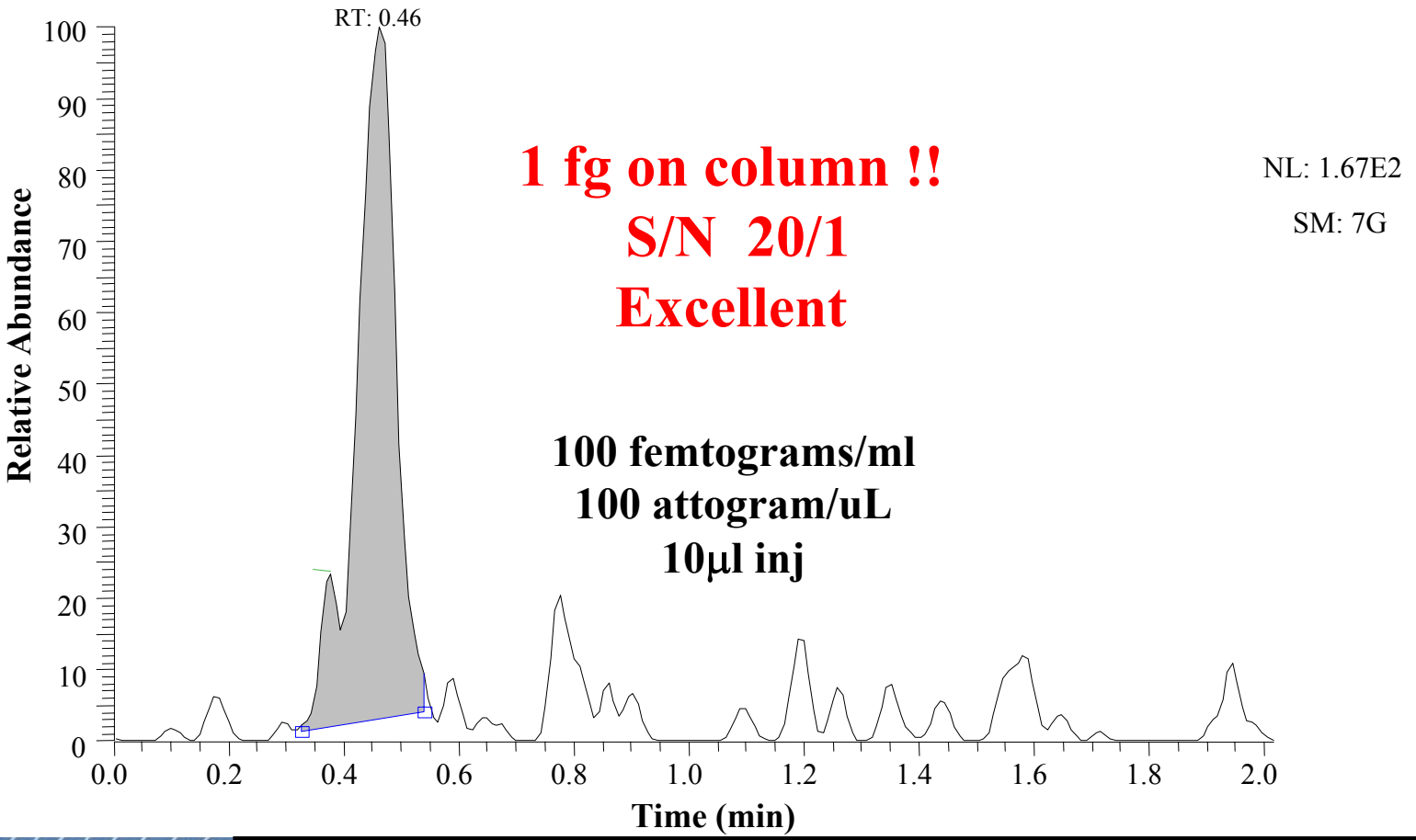
API MS/MS Selective Reaction Monitoring (SRM)



Sensitivity
What Everyone Wants

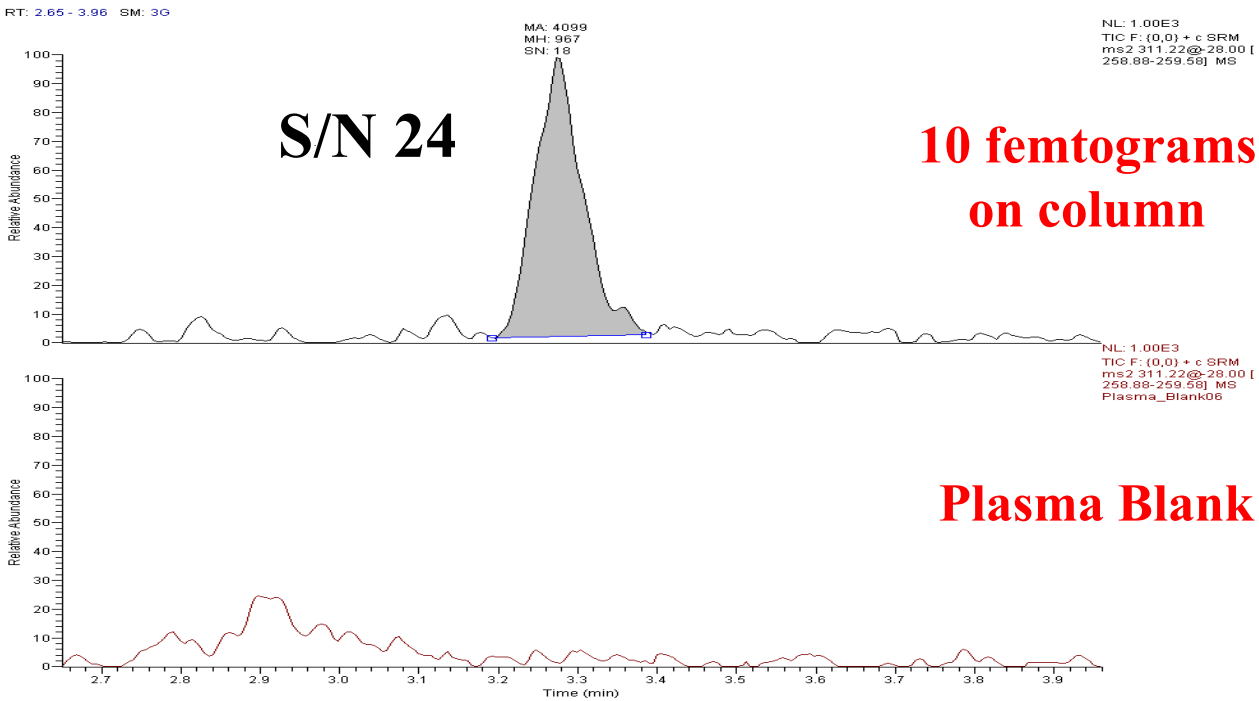
TSQ Quantum, Low Femtogram +ESI/SRM at Unit Resolution (Q1, Q3 - 0.7 Da FWHM)

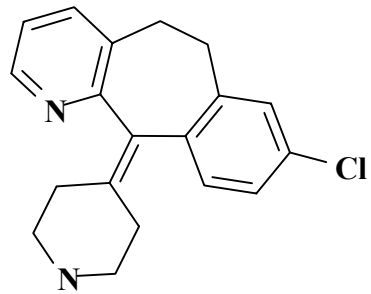
Proprietary Pharmaceutical in Mobile Phase, 3µ Phenyl Hexyl 20 x 2mm, ACN/0.1%TFA/H_2O/NH_4CH_3COO, +ESI, SRM m/z 425 - 218, Collision Energy 28V



TSQ Quantum
Low Femtogram +ESI/SRM at Unit Resolution

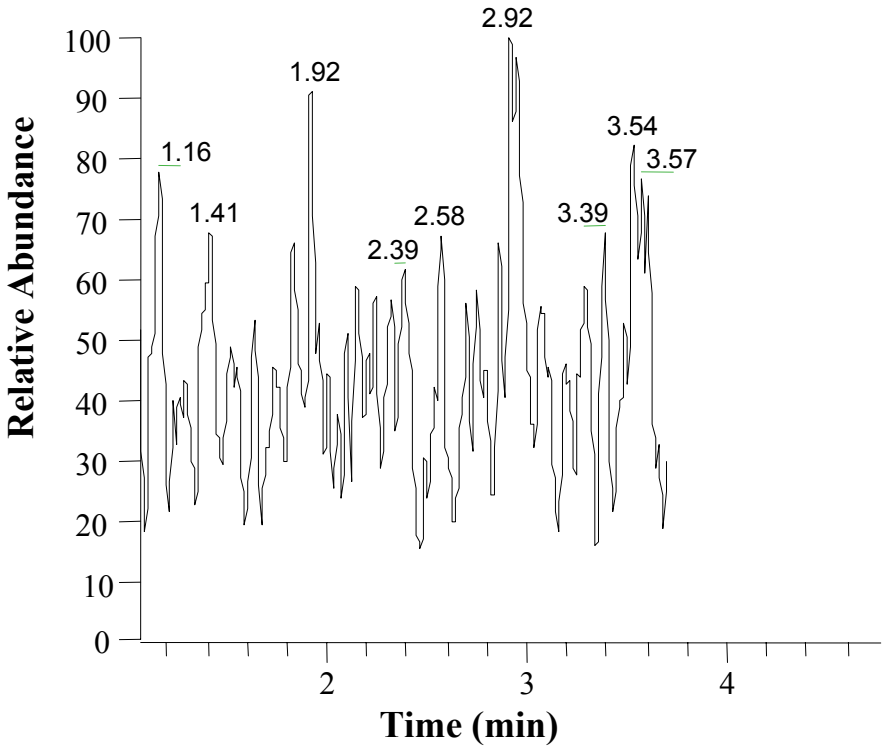
Descarboethoxyloratadine in Human Plasma, H_2O/MeOH/ACN/0.1% formic, 250 µl/min, 100 x 2 mm C8, +ESI, SRM m/z 311 - 259, Collision Energy 28V





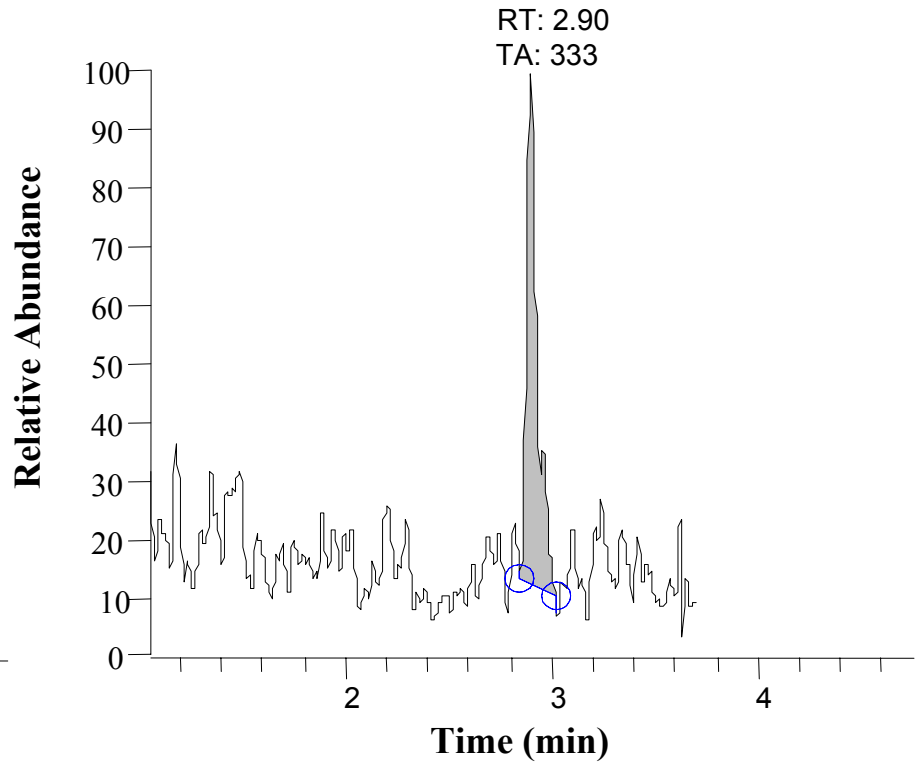
TSQ Quantum, Low Femtogram +ESI/SRM at Unit Resolution

Plasma Blank



10 fg on column

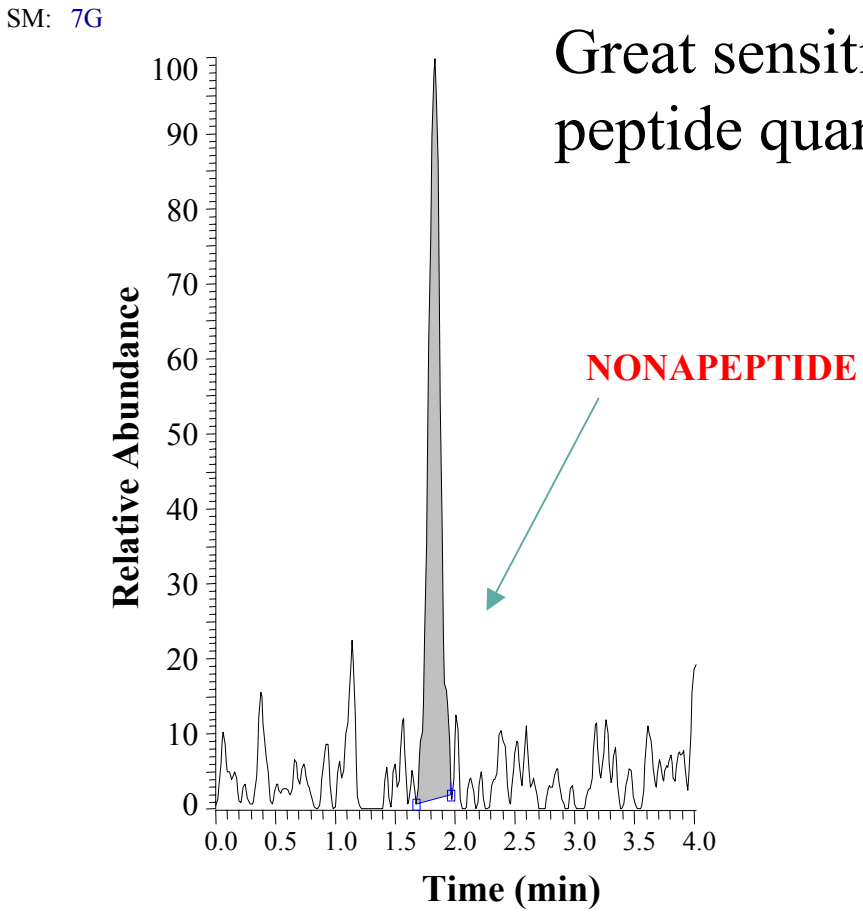
Formoterol



Thanks to Mark Churchill and Mark Harrison for data

TSQ Quantum – Nonapeptide (MW 1209) in Human Plasma 0.8 fM on column, +ESI/SRM in High Resolution

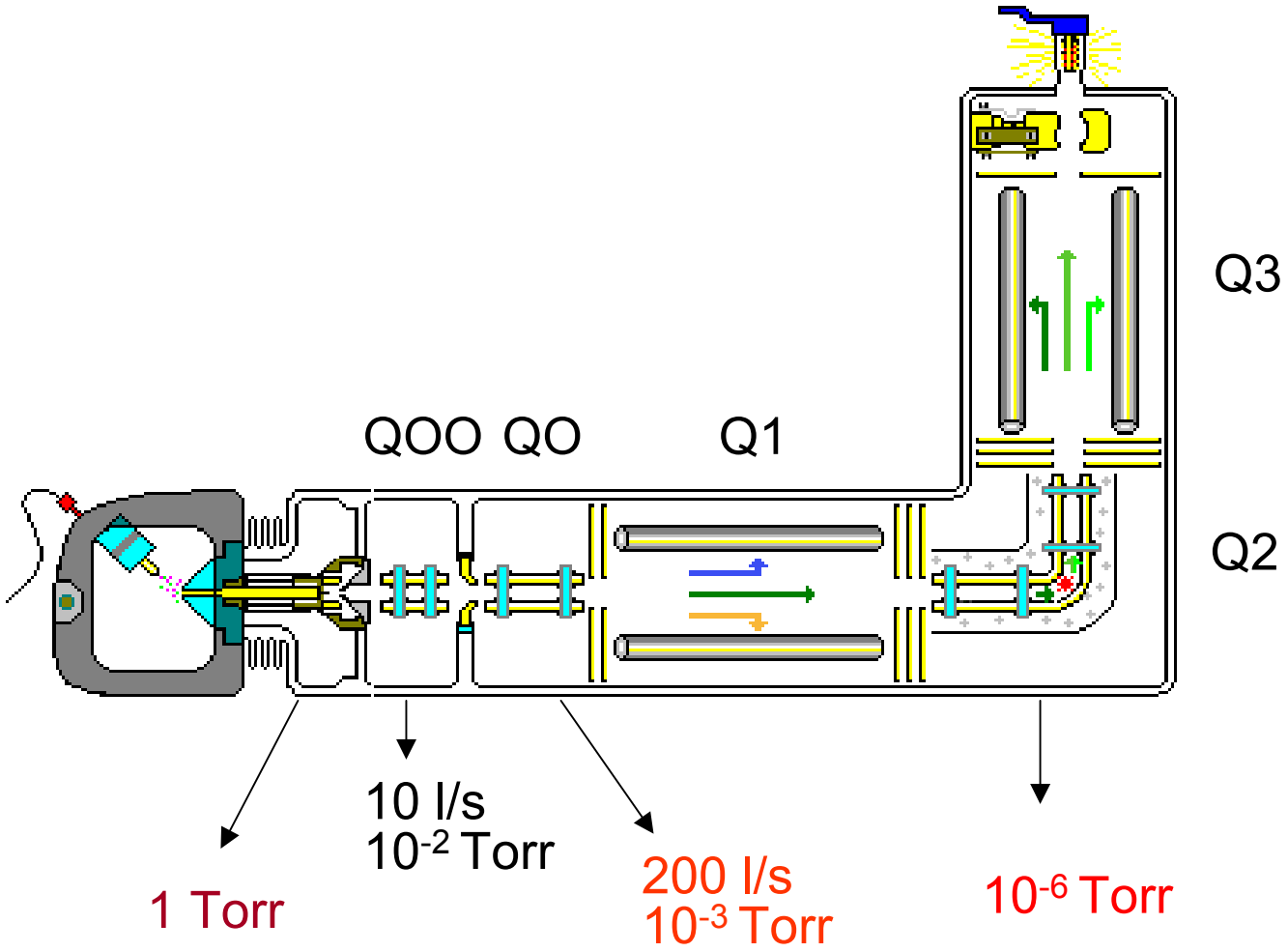
C18 50 mm x 4.6 mm, ACN/0.1%FA/H_2O/0.1%FA, SRM m/z 605 - 249, CE 30V



Great sensitivity for peptide quantitation

Why So Sensitive?

The New TSQ Quantum Analyzer



TSQ QUANTUM - Reasons for Improved S/N

1. New Orthogonal API Source - Less Noise

2. New Square Quadrupole Ion Guides – More Signal

3. New Hyperbolic Quadrupoles - More Signal

4. New 90 Degree Collision Cell –
More Signal, Less Noise

TSQ Quantum
SRM Quantitative Assays at Unit Resolution

ALSO ESSENTIAL FOR HIGH PERFORMANCE QUANTITATION

1. Extended Linear Dynamic Range

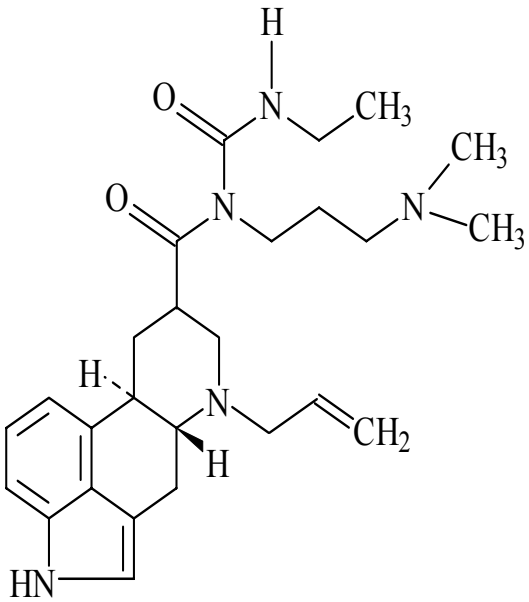
2. Excellent Precision

3. Excellent Accuracy

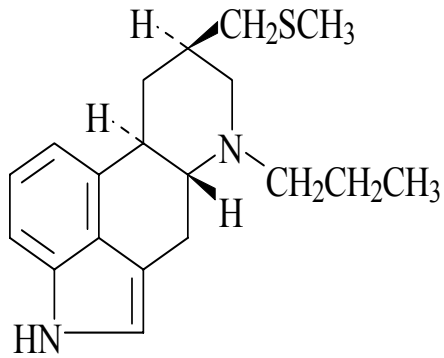
TSQ Quantum
+ESI/SRM Quantitative Assay of Cabergoline

Used in the treatment of Parkinson's disease

Highly potent – typically present in extremely low levels in plasma



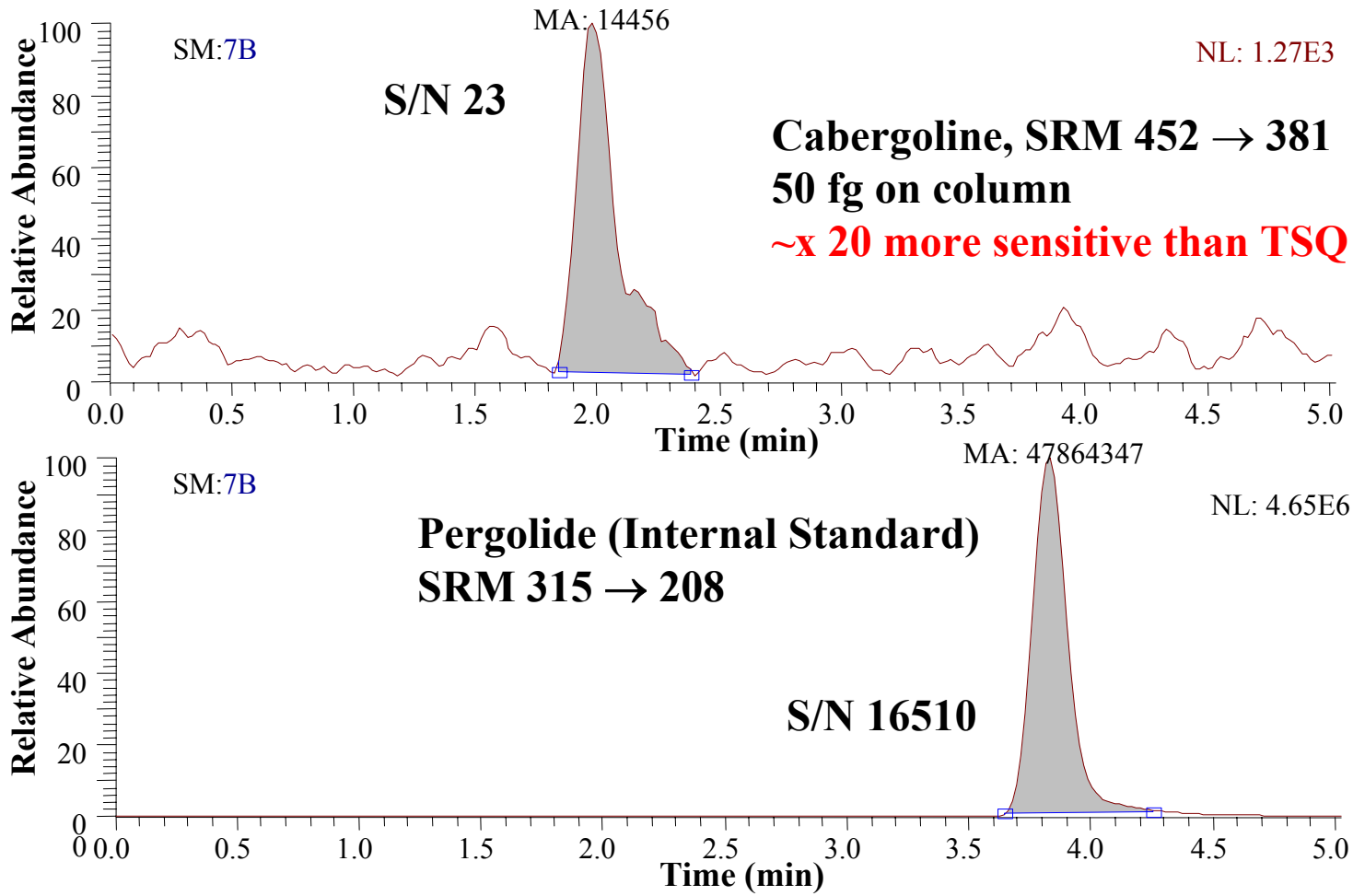
Cabergoline



Pergolide
(Internal Standard)

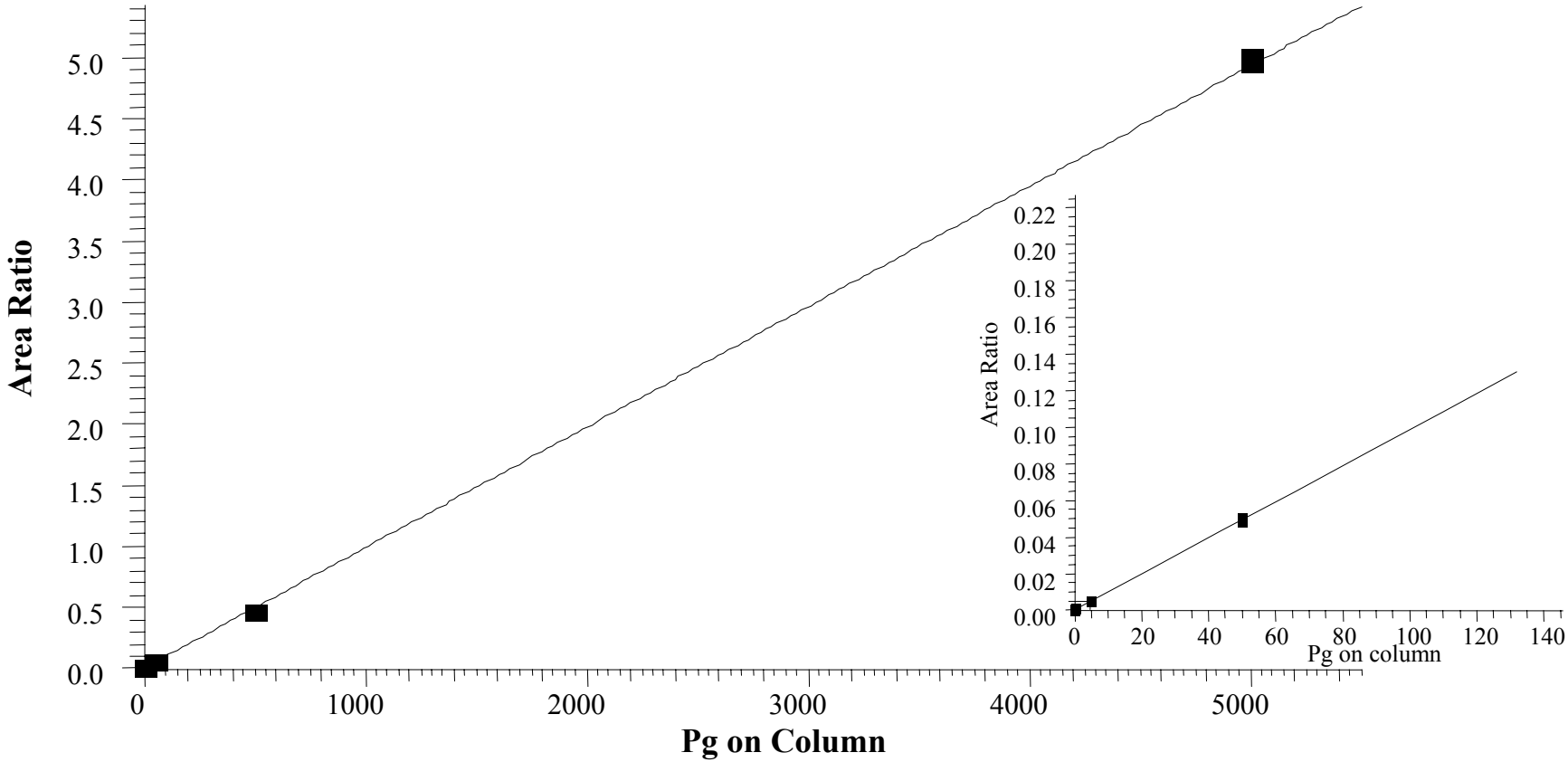
TSQ Quantum
+ESI/SRM Quantitative Assay of Cabergoline in Bovine Plasma

5µ Xterra MS18 150 x 2.1mm, ACN/NH_4CH_3COO (60/40, v/v), CE 19V, Q1 0.7 Da Q3 0.7 Da



TSQ Quantum
+ESI/SRM Quantitative Assay of Cabergoline in Bovine Plasma

150 x 2.1mm 5µ Xterra MS18 LC Column, ACN/NH_4CH_3COO (60/40, v/v), SRM m/z 452 - 381
Collision Energy 19V, Internal standard Pergolide, Q1 0.7 Da Q3 0.7 Da



50 fg - 5 ng on column, 5 orders of Linear Dynamic Range - Excellent

TSQ Quantum
+ESI/SRM Quantitative Assay of Cabergoline in Bovine Plasma

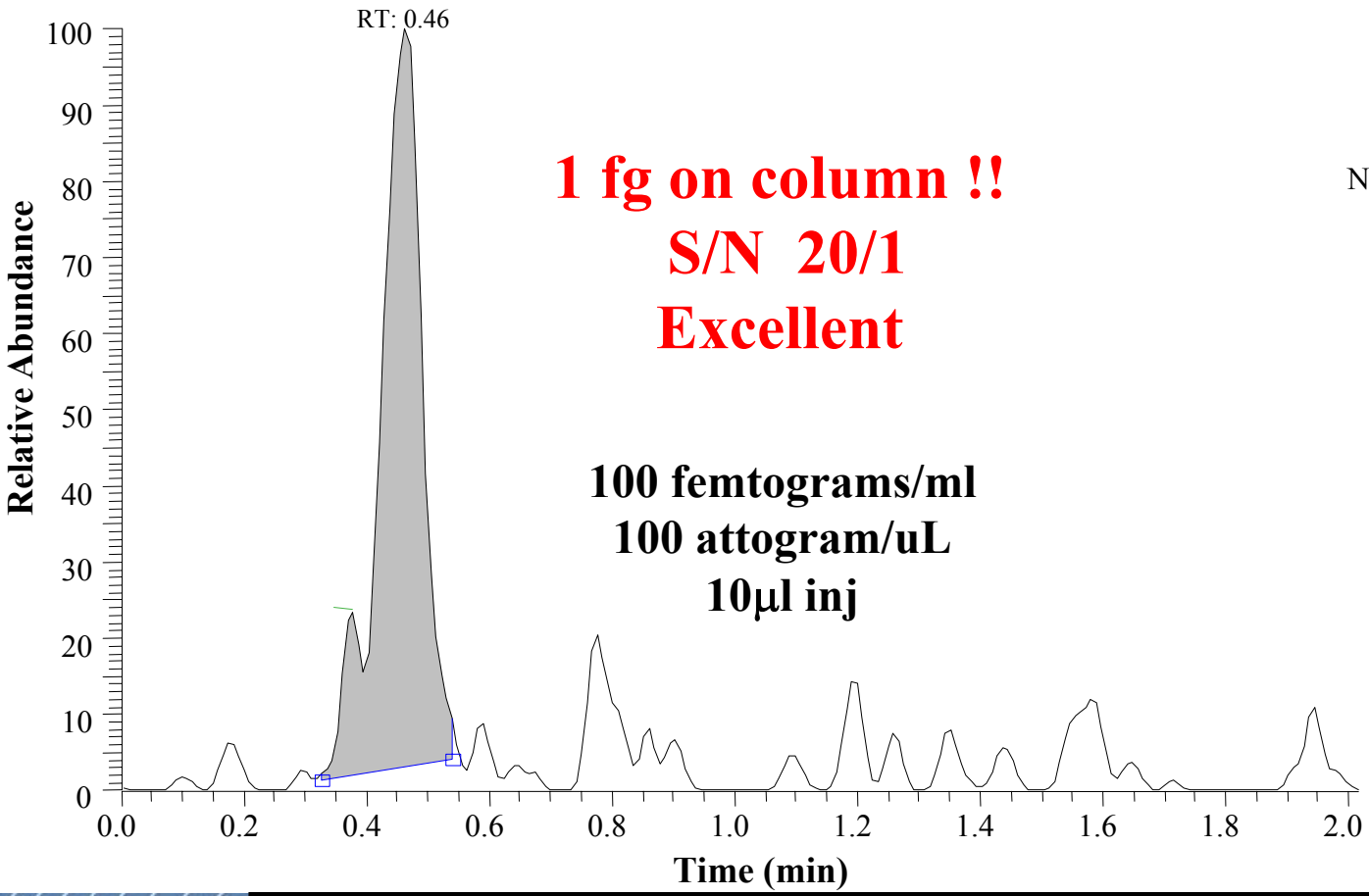
150 x 2.1mm 5µ Xterra MS18 LC Column, ACN/NH_4CH_3COO (60/40, v/v), SRM m/z 452 - 381
Collision Energy 19V, Internal standard Pergolide, Q1 0.7 Da Q3 0.7 Da

Nominal Amount (pg on column)	Mean Amount (pg on column)	Accuracy (% RE)	Precision (% CV)
0.050	0.057	13.6	9.3
0.500	0.508	1.6	8.4
5.000	4.709	-5.8	2.3
50.000	49.861	-0.3	1.0
500.000	462.354	-7.5	1.3
5000.000	5038.062	0.8	0.5

Excellent Accuracy and Precision

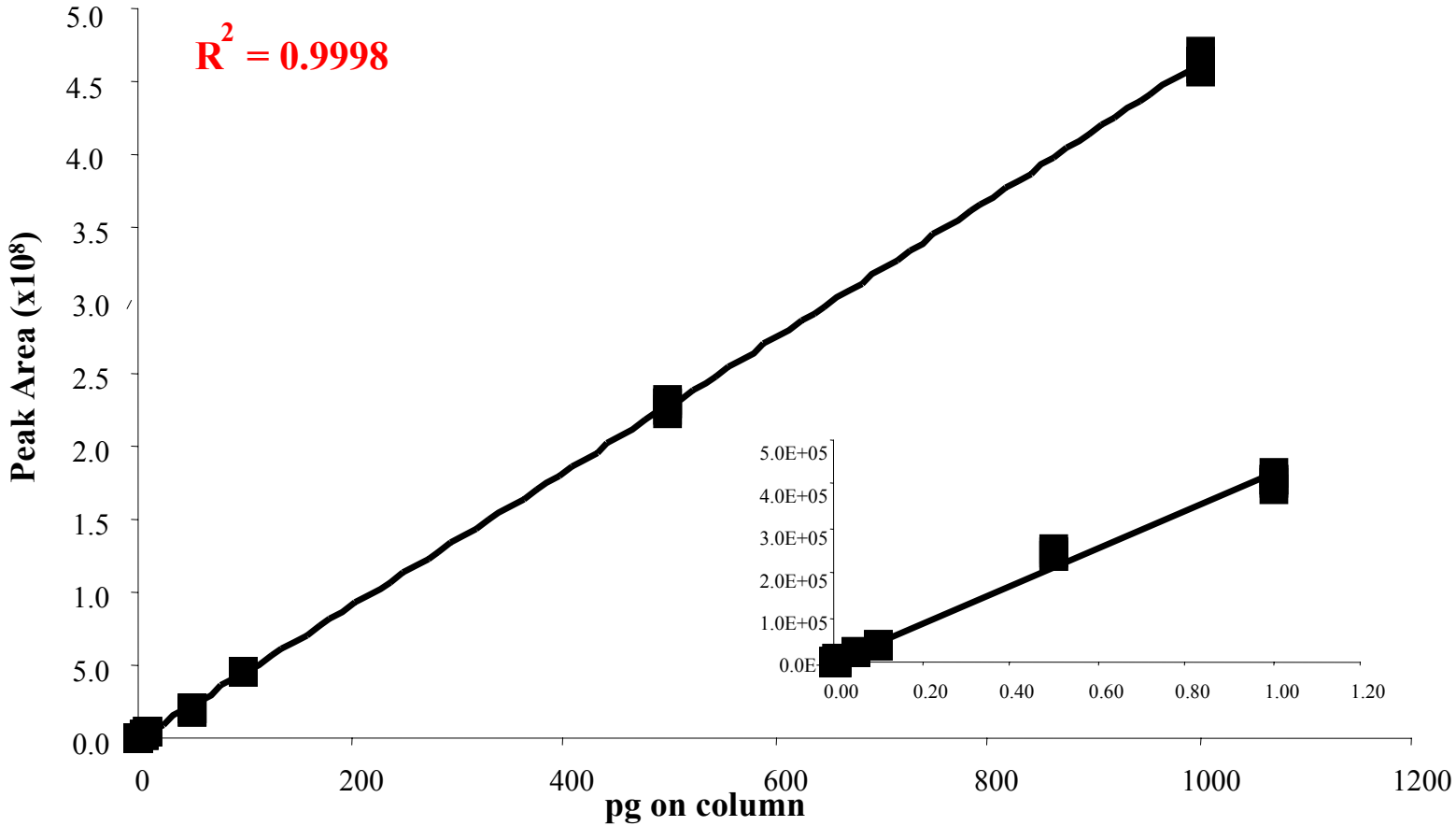
TSQ Quantum, Low Femtogram +ESI/SRM at Unit Resolution

Proprietary Pharmaceutical in Mobile Phase, 3µ Phenyl Hexyl 20 x 2mm, ACN/0.1%TFA/H_2O/NH_4CH_3COO, +ESI, SRM m/z 425 - 218, Collision Energy 28V



TSQ Quantum
+ESI/SRM Quantitative Assay of Drug at Unit Resolution

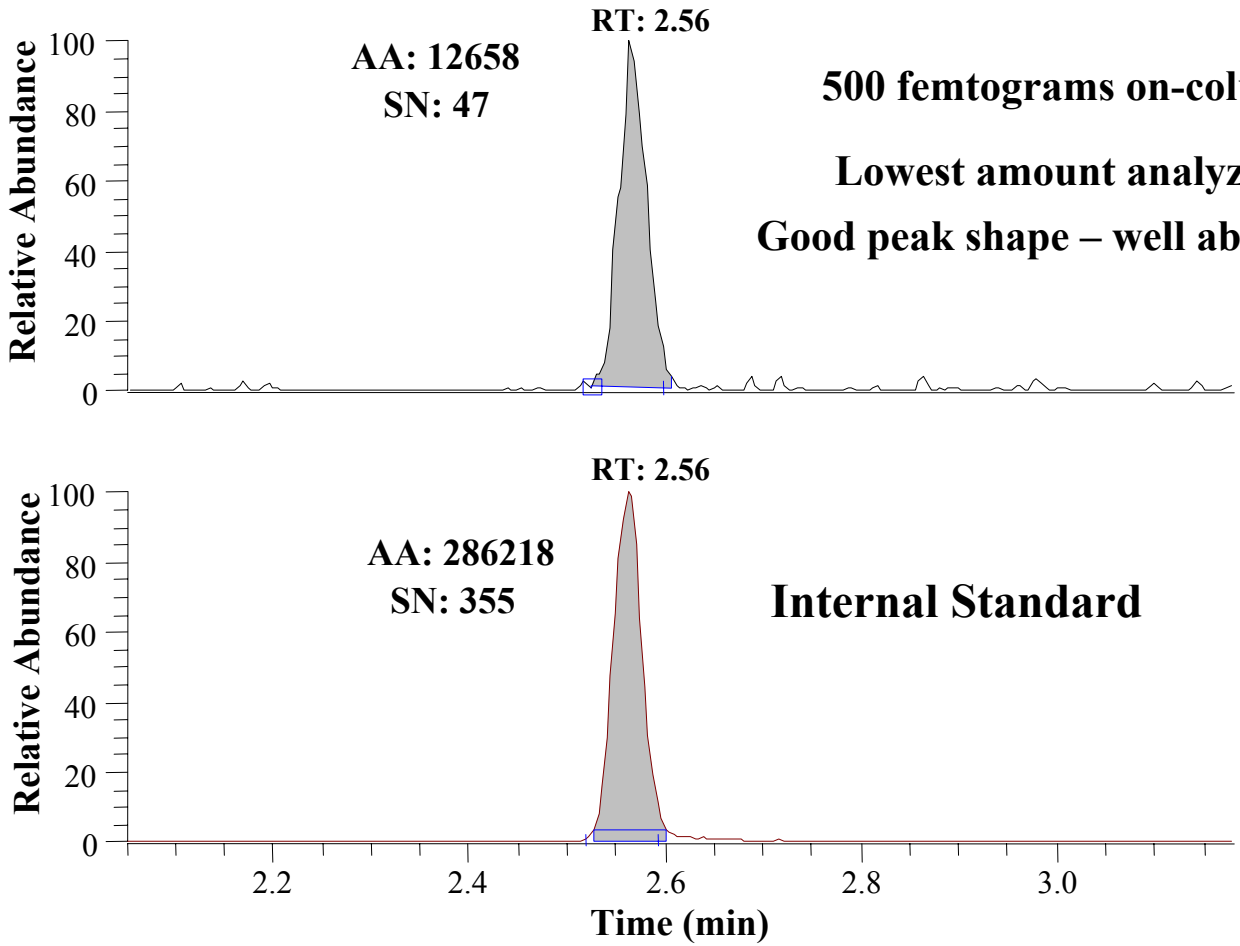
Proprietary Drug in Mobile Phase, 3µ Phenyl Hexyl 20 x 2mm, ACN/0.1%TFA/H_2O/NH_4CH_3COO, SRM m/z 425 - 218, Collision Energy 28V



1 fg - 1 ng on column (6 orders of linear dynamic range!)

High Performance Quantitation of a Drug in Bovine Plasma on the TSQ Quantum

Proprietary Drug, Unit Resolution, +ESI/SRM m/z 644 - 114, Collision Energy 38V



500 femtograms on-column

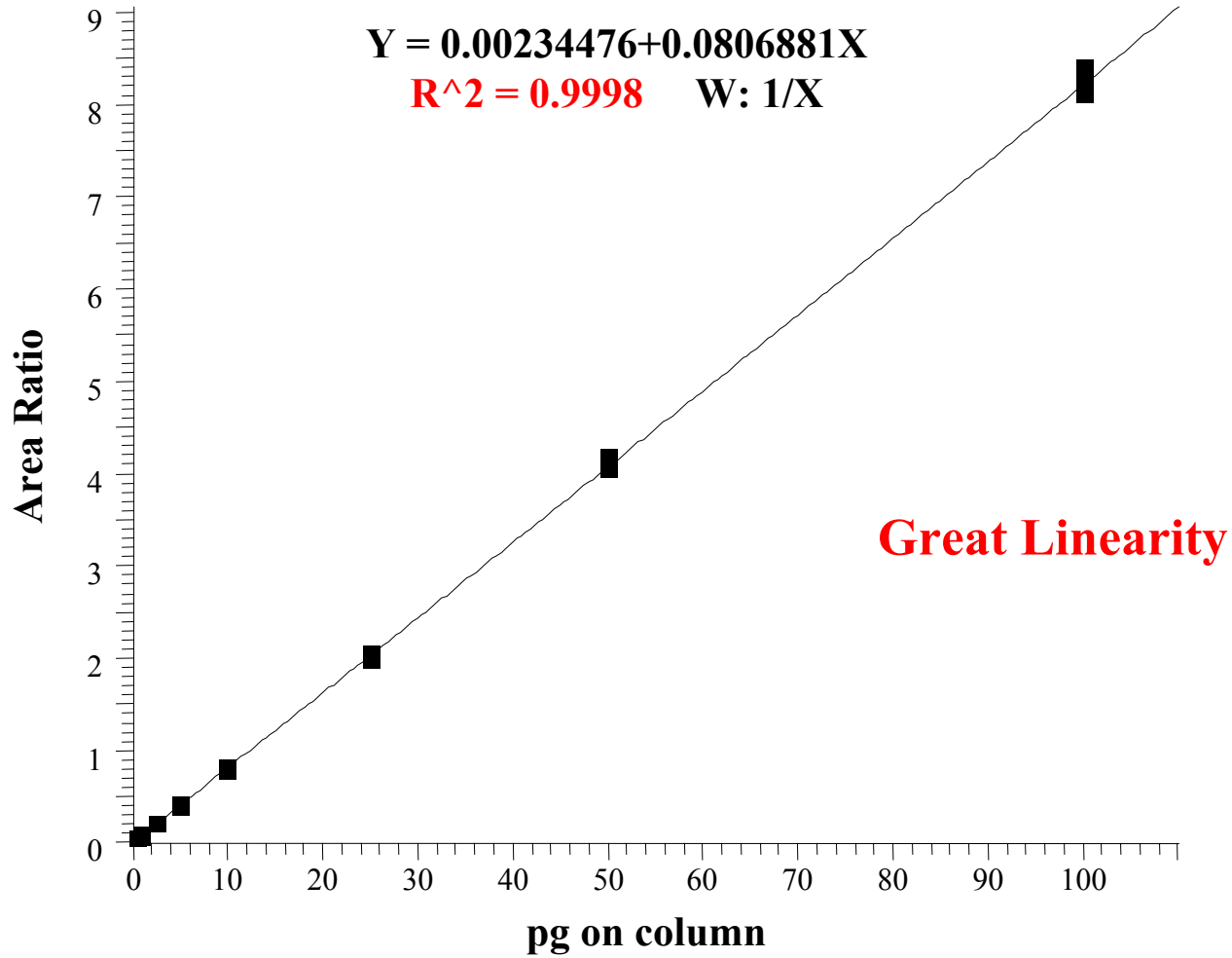
Lowest amount analyzed –

Good peak shape – well above LOQ

Internal Standard

High Performance Quantitation of a Drug in Bovine Plasma on the TSQ Quantum

Proprietary Drug, Unit Resolution, +ESI/SRM m/z 644 - 114, Collision Energy 38V



High Performance Quantitation of a Drug in Bovine Plasma on the TSQ Quantum

Proprietary Drug, Unit Resolution, +ESI/SRM m/z 644 - 114, Collision Energy 38V

CALIBRATION STANDARDS - 8 replicates

Concentration	Area	ISTD Area	Area Ratio	Specified Amount (pg on column)	Calculated Amount (pg on column)	%Diff	%RSD
0.05pg/µL	14112	319466	0.0441	0.500	0.518	4%	3.3%
0.1pg/µL	25875	320259	0.0808	1.000	0.972	3%	3.3%
0.25pg/µL	61483	298452	0.206	2.50	2.52	-1%	2.3%
0.5pg/µL	128639	316553	0.406	5.00	5.00	0%	2.4%
1pg/µL	247948	313325	0.791	10.00	9.76	-2%	1.3%
2.5pg/µL	680210	335203	2.03	25.0	25.0	0%	1.3%
5pg/µL	1363255	332396	4.10	50.0	50.4	1%	1.1%
10pg/µL	2847221	347401	8.20	100.0	99.8	0%	1.2%

Excellent Precision and Accuracy

High Performance Quantitation of a Drug in Bovine Plasma on the TSQ Quantum

Proprietary Drug, Unit Resolution, +ESI/SRM m/z 644 - 114, Collision Energy 38V

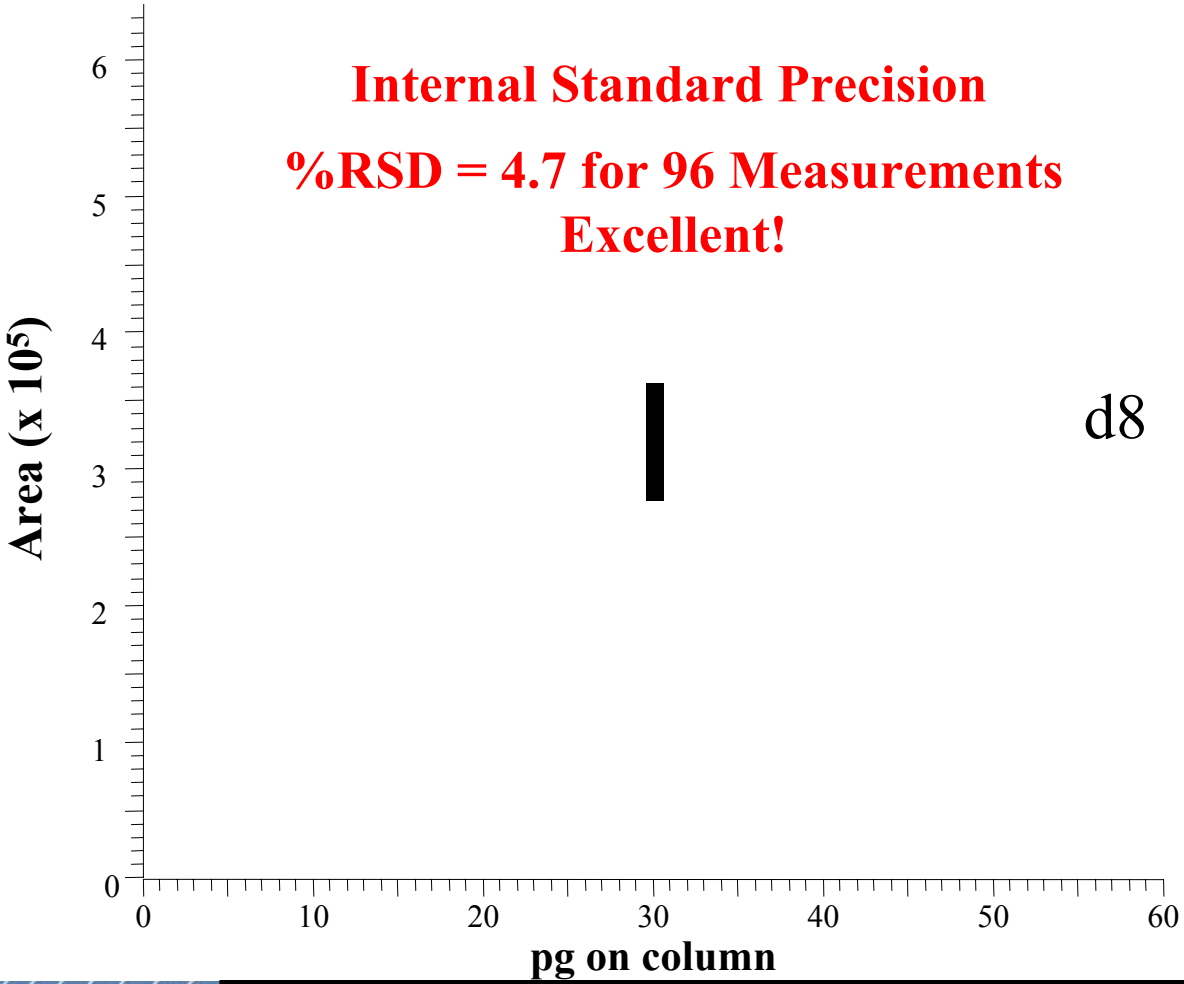
Quality Control Samples - 8 replicates

Concentration	Area	ISTD Area	Area Ratio	Specified Amount (pg on column)	Calculated Amount (pg on column)	%Diff	%RSD
0.05pg/µL	13631	311163	0.04380	0.500	0.514	3%	3.4%
0.1pg/µL	26188	313428	0.08356	1.00	1.01	1%	2.8%
1pg/µL	251810	31593	0.79686	10.0	9.83	-2%	0.7%
10pg/µL	2649398	323837	8.18253	100	99.7	0%	2.2%

Excellent Accuracy and Precision Again

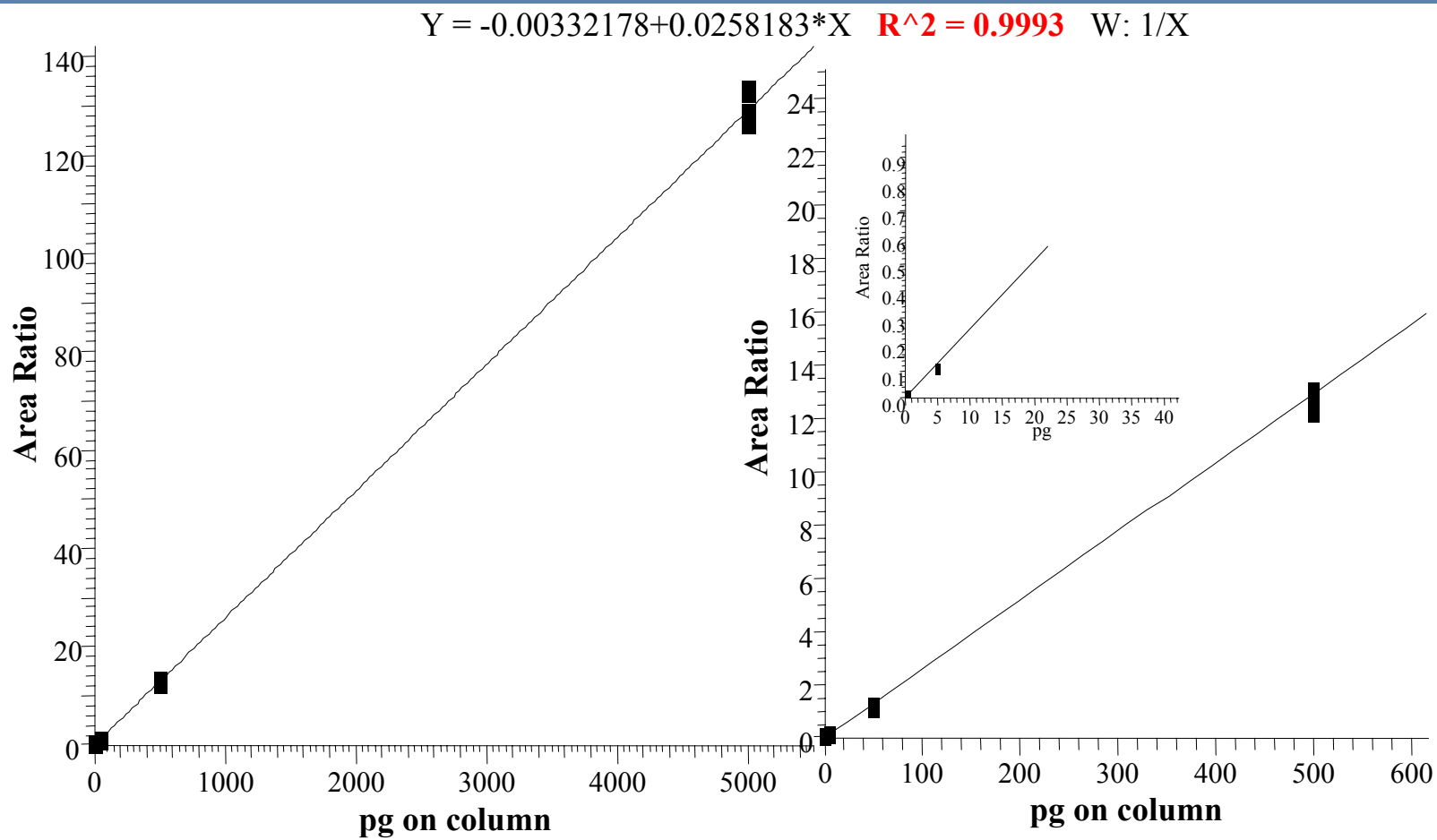
High Performance Quantitation of a Drug in Bovine Plasma on the TSQ Quantum

Proprietary Drug, Unit Resolution, +ESI/SRM m/z 644 - 114, Collision Energy 38V



TSQ Quantum

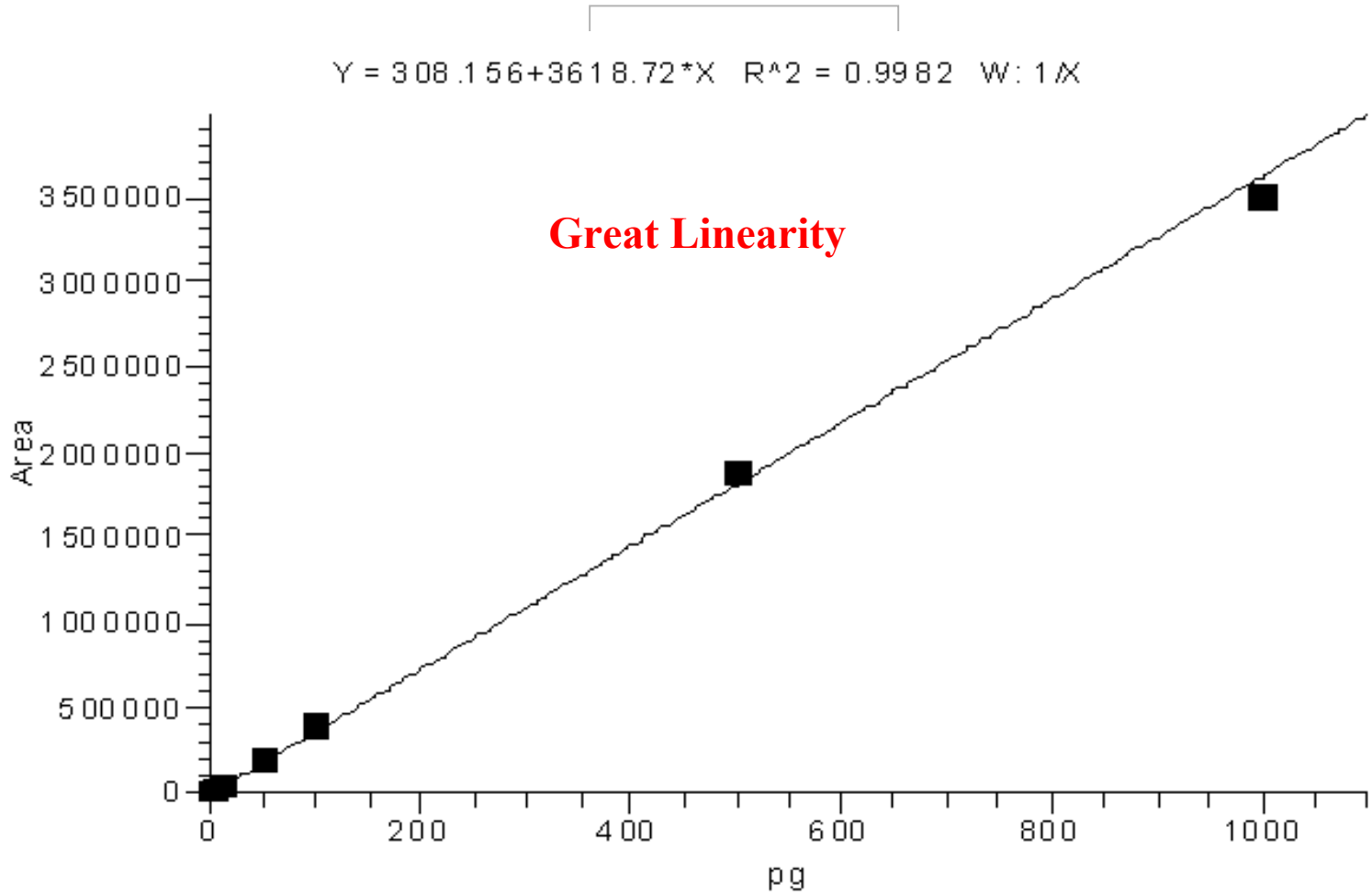
Positive APCI/SRM of a Proprietary Drug in Bovine Plasma



500fg – 5000pg on column (10^4 dynamic range)

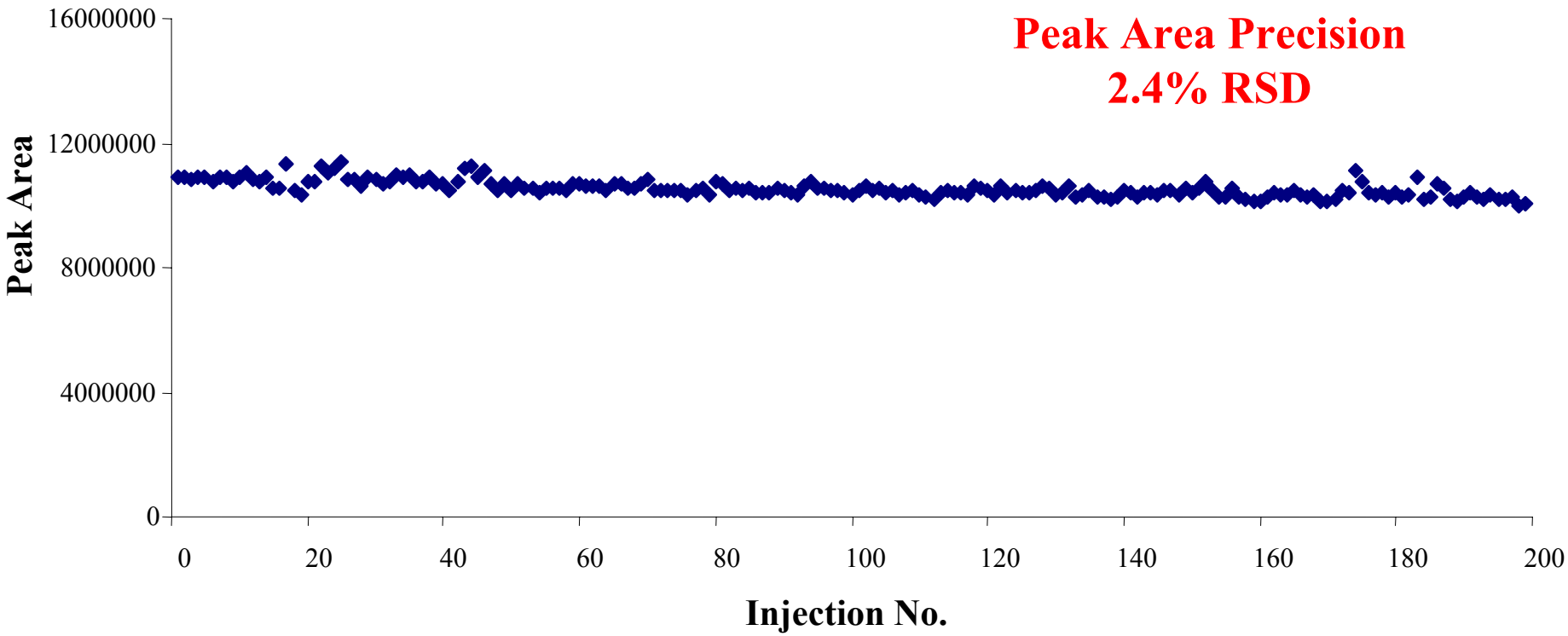
TSQ Quantum
Negative ESI/SRM Quantitative Assay - 10^4 Dynamic Range

Proprietary Drug, Bovine Plasma. Unit Resolution, SRM m/z 440 - 190, Collision Energy 32 V



TSQ Quantum
Ruggedness Over 200 Injections in Plasma

Peak area response of 200 injections of Paroxetine in protein precipitated plasma



Thanks to Mark Churchill and Mark Harrison for data

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TSQ QUANTUM – HIGH RESOLUTION

TSQ Quantum is the Only
Triple Quadrupole Mass Spectrometer
With High Resolution Capability

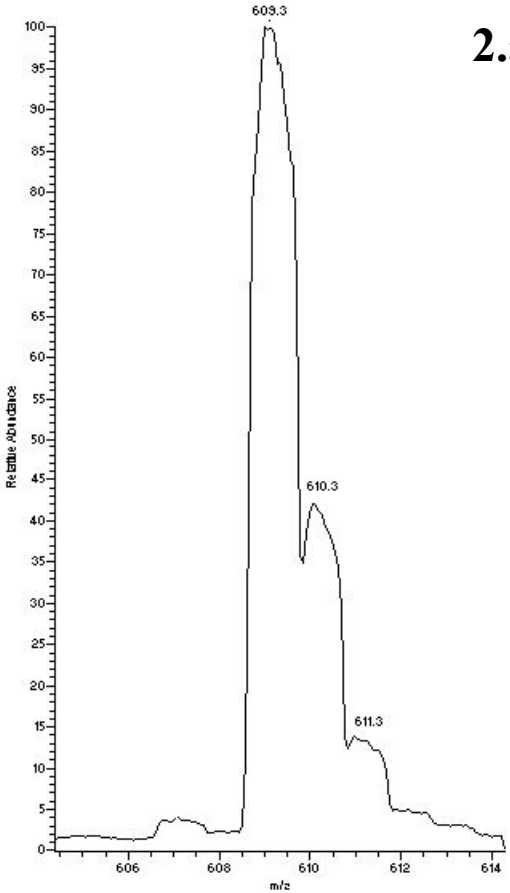
What is High Resolution?

How can High Resolution be Beneficial
In Quantitation?

TSQ Quantum: Increasing Resolution For Reserpine (MW 608) in +ve ESI/MS

1.0 Da FWHM

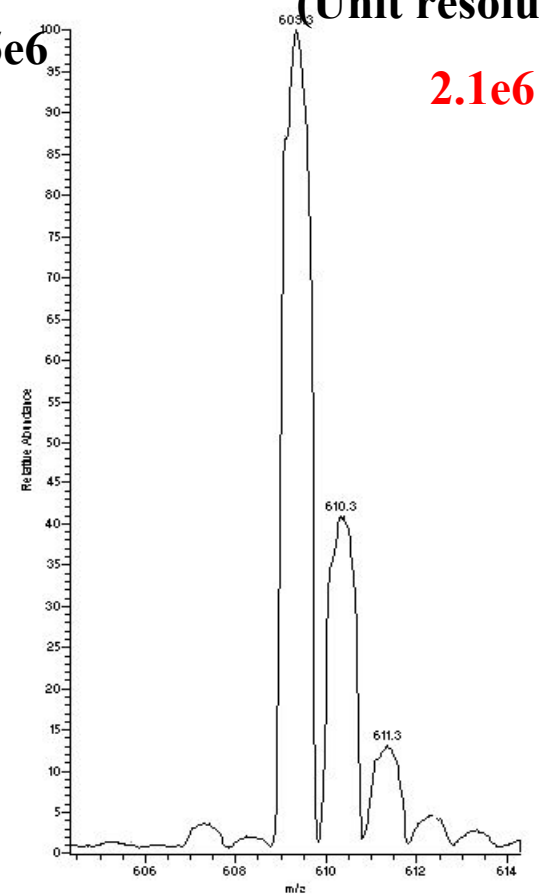
2.5e6



0.7 Da FWHM

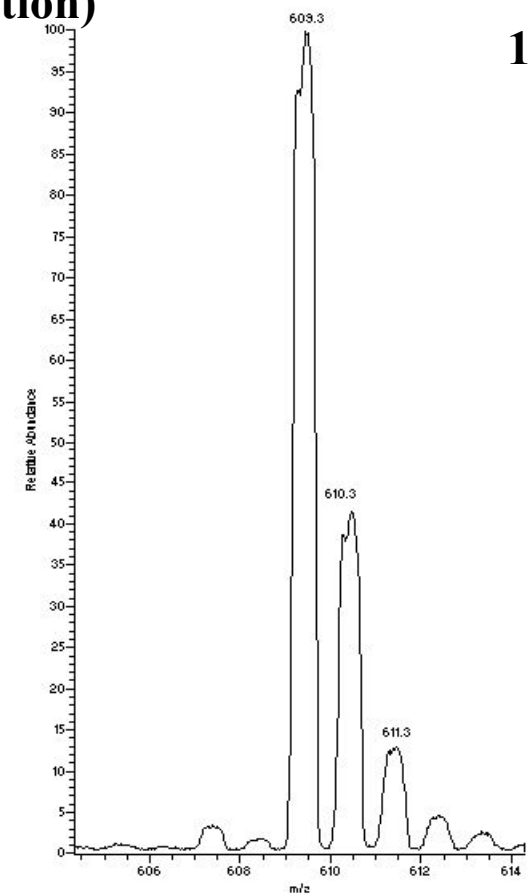
(Unit resolution)

2.1e6



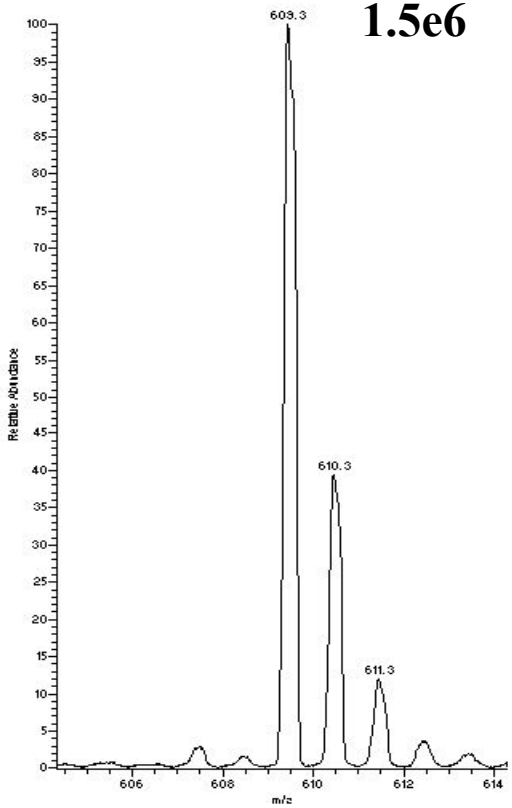
0.5 Da FWHM

1.9e6

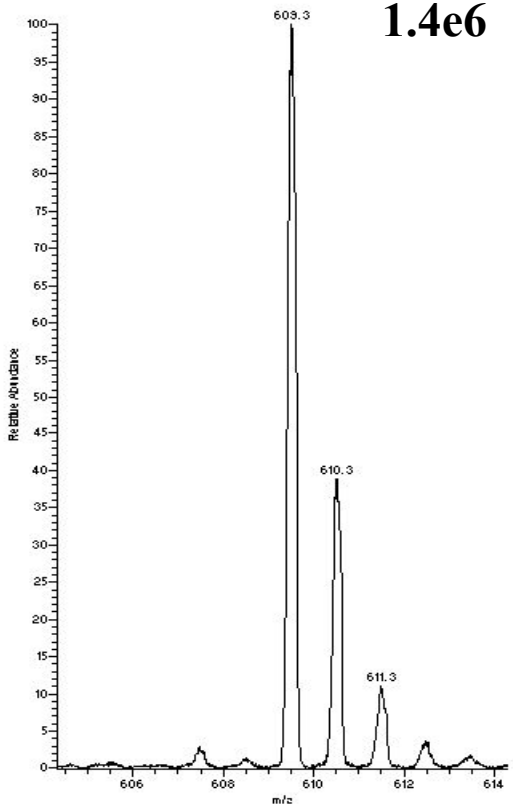


TSQ Quantum: Increasing Resolution For Reserpine (MW 608) in +ve ESI/MS

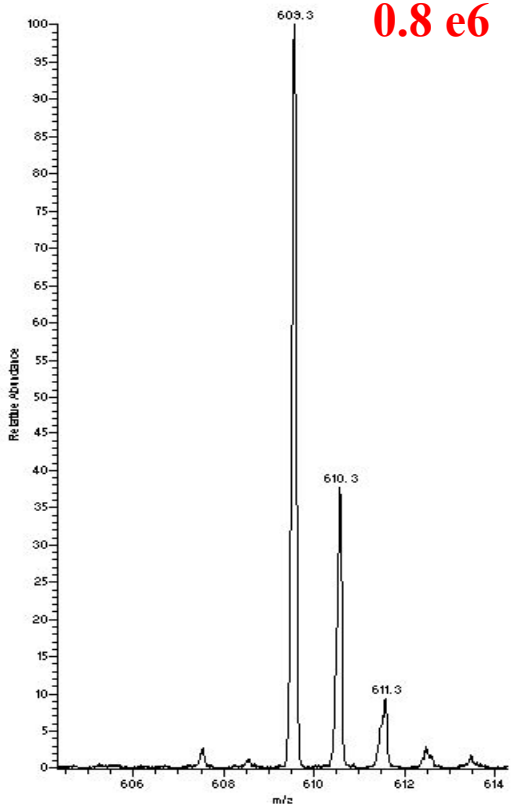
0.3 Da FWHM **1.5e6**



0.2 Da FWHM **1.4e6**



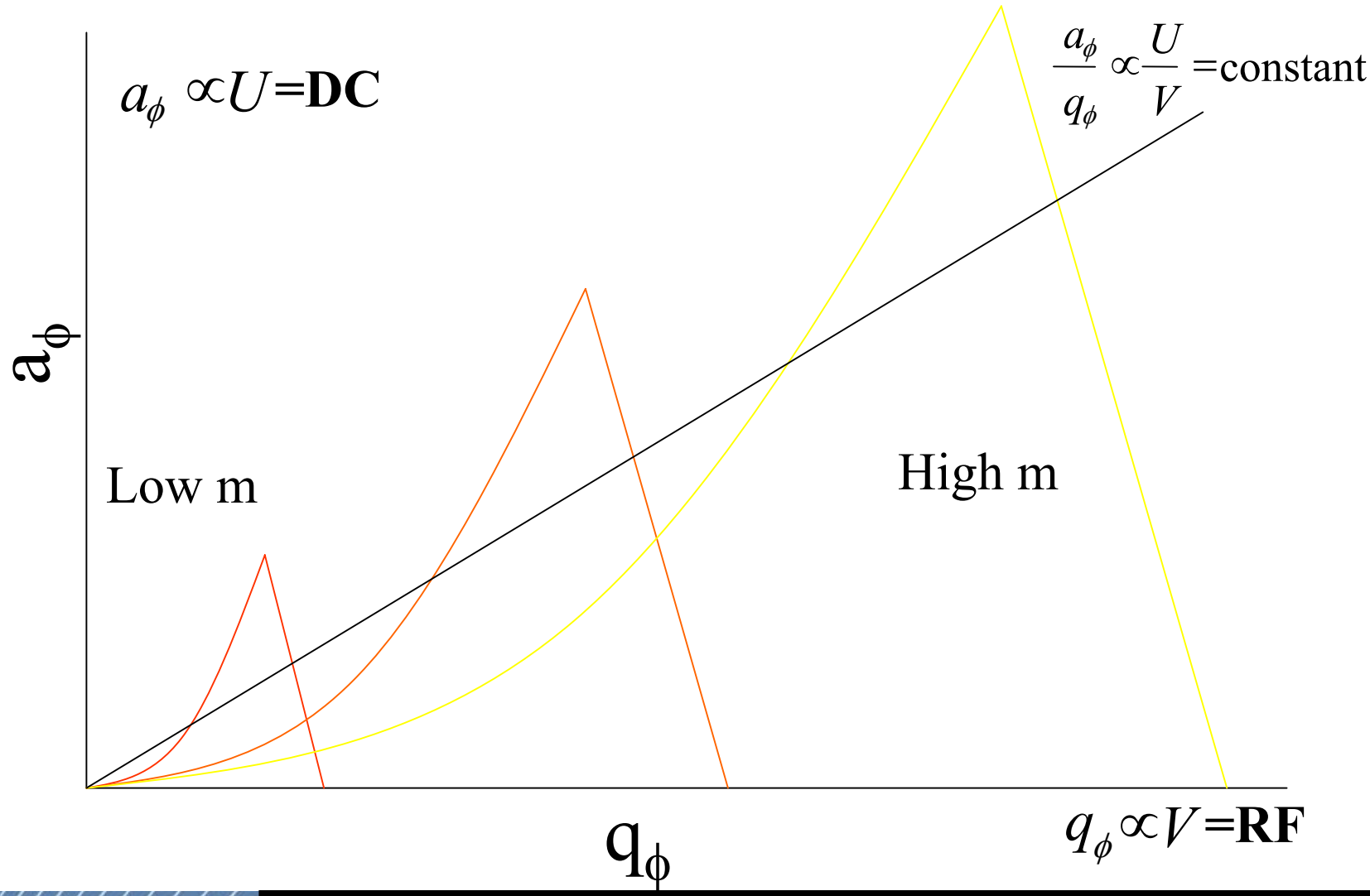
0.1 Da FWHM
(High Resolution) **0.8 e6**



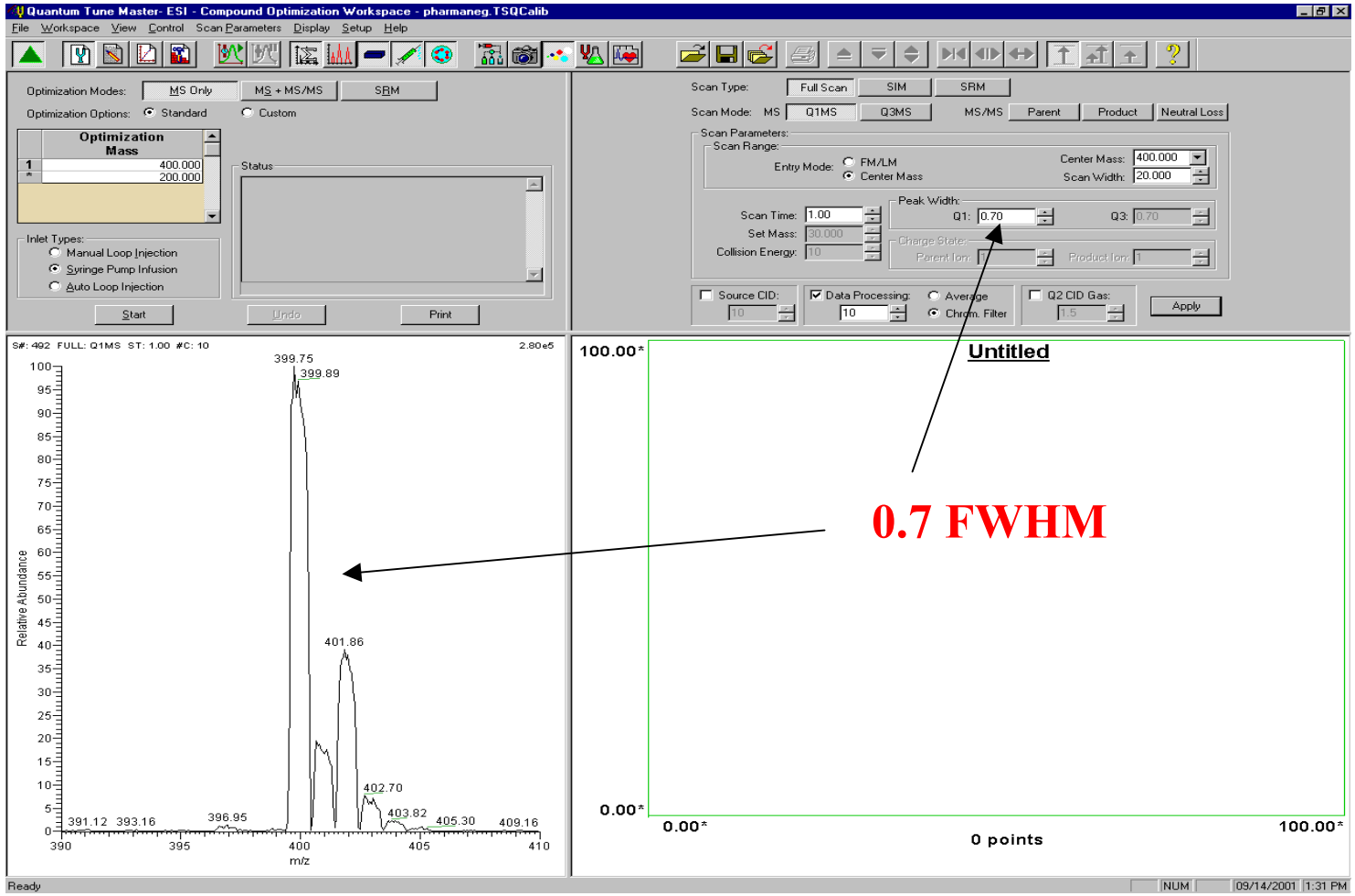
***Signal Intensity at Unit Resolution = 2.1e6**

Loss of intensity of ONLY x2-3 typically seen from Unit to High Resolution

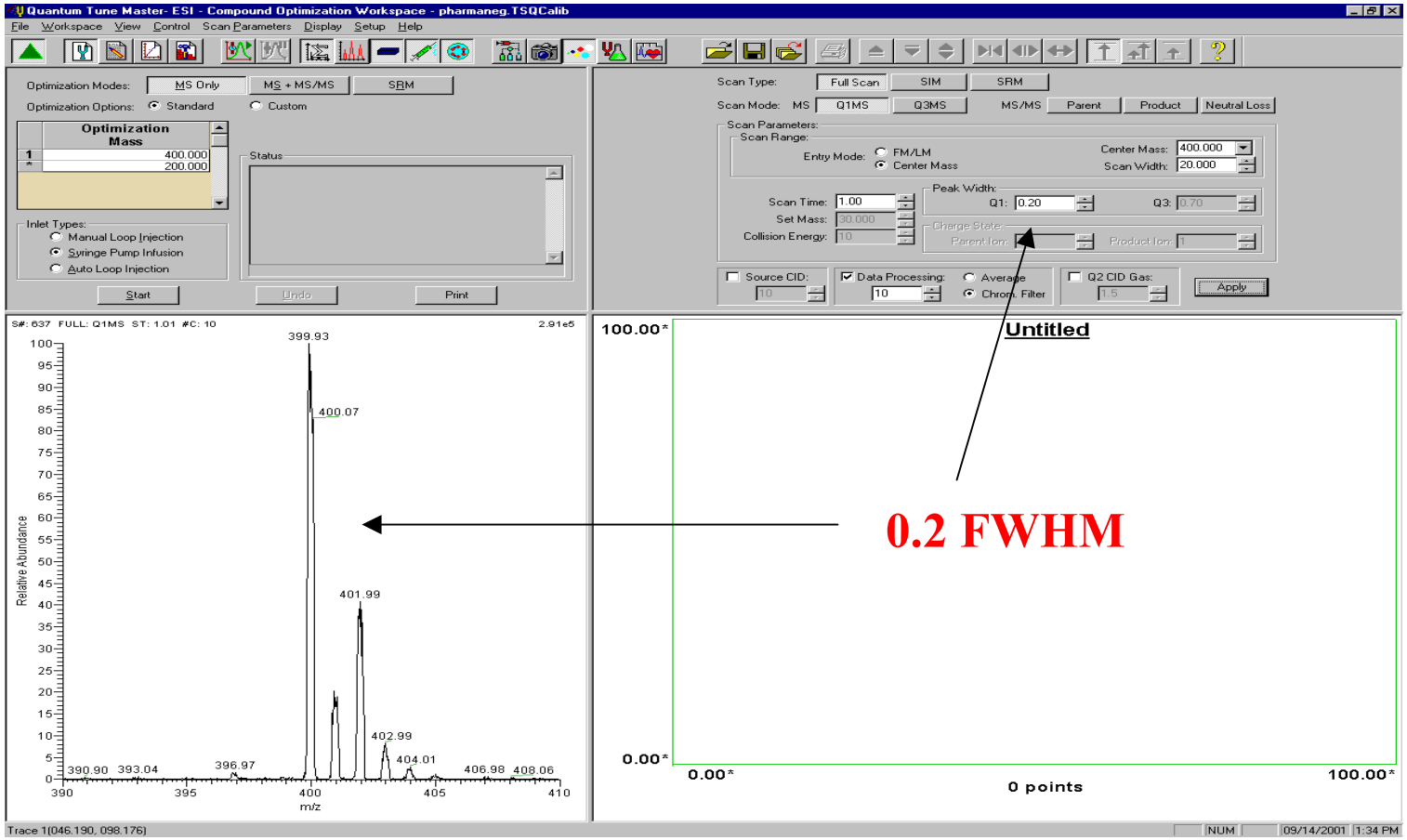
Scanning Ion Stability for Quadrupoles



TSQ Quantum - Instrument Tune Window
Compound Optimization - Resolution Setting



TSQ Quantum - Instrument Tune Window
Compound Optimization - Resolution Setting



High resolution achieved by a simple click of the mouse button

High Resolution on the TSQ Quantum

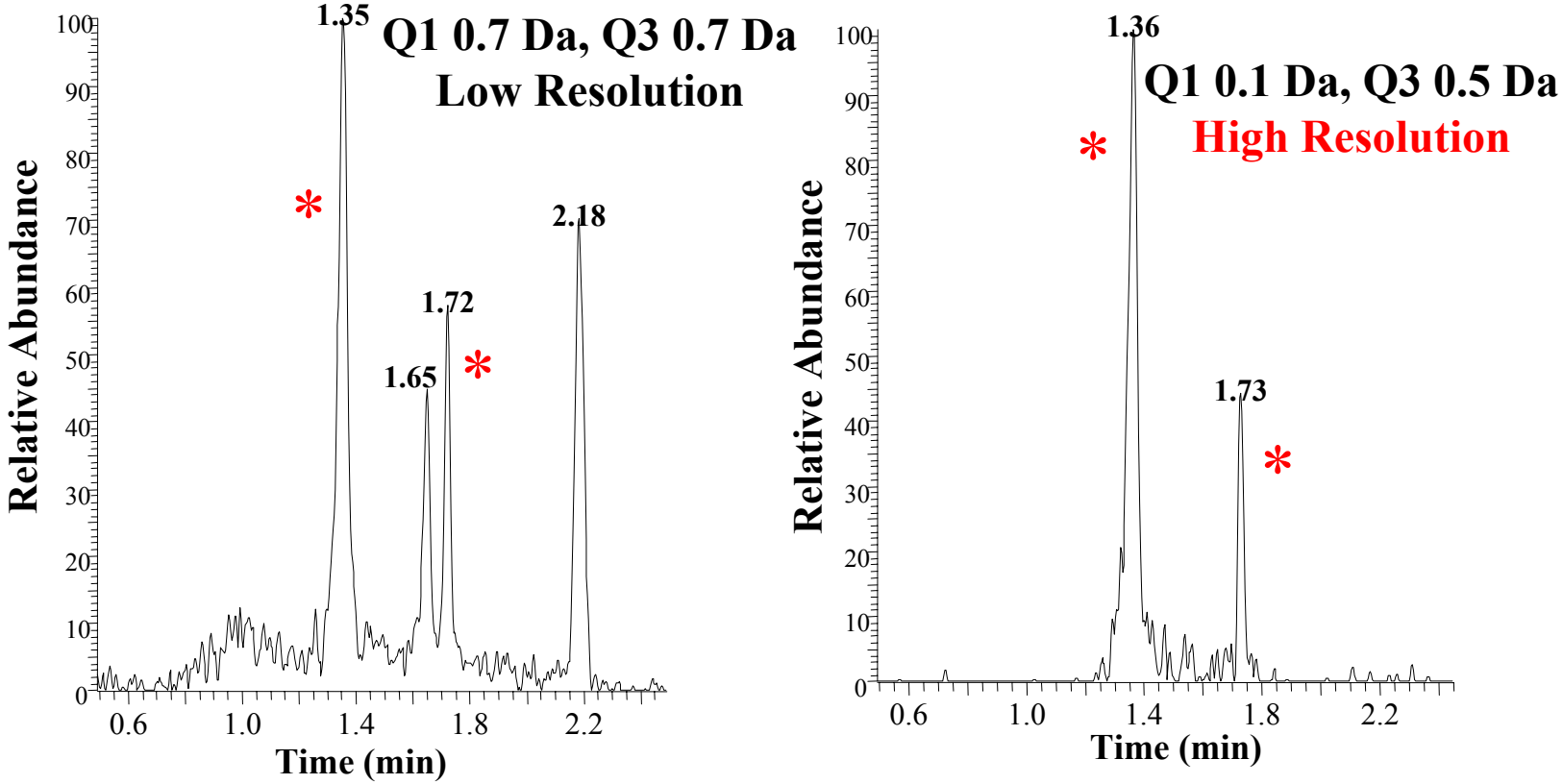
Quadrupole mass filtering fields are defined by:

1. electrode shape
2. field radius
3. frequency
4. voltage
5. length

The TSQ Quantum has true hyperbolic electrodes, large field radius, high frequency and voltage, and a long length (25 cm) and hence has the ability to achieve high mass resolution

TSQ Quantum
SRM Selectivity at High Resolution for Quantitation

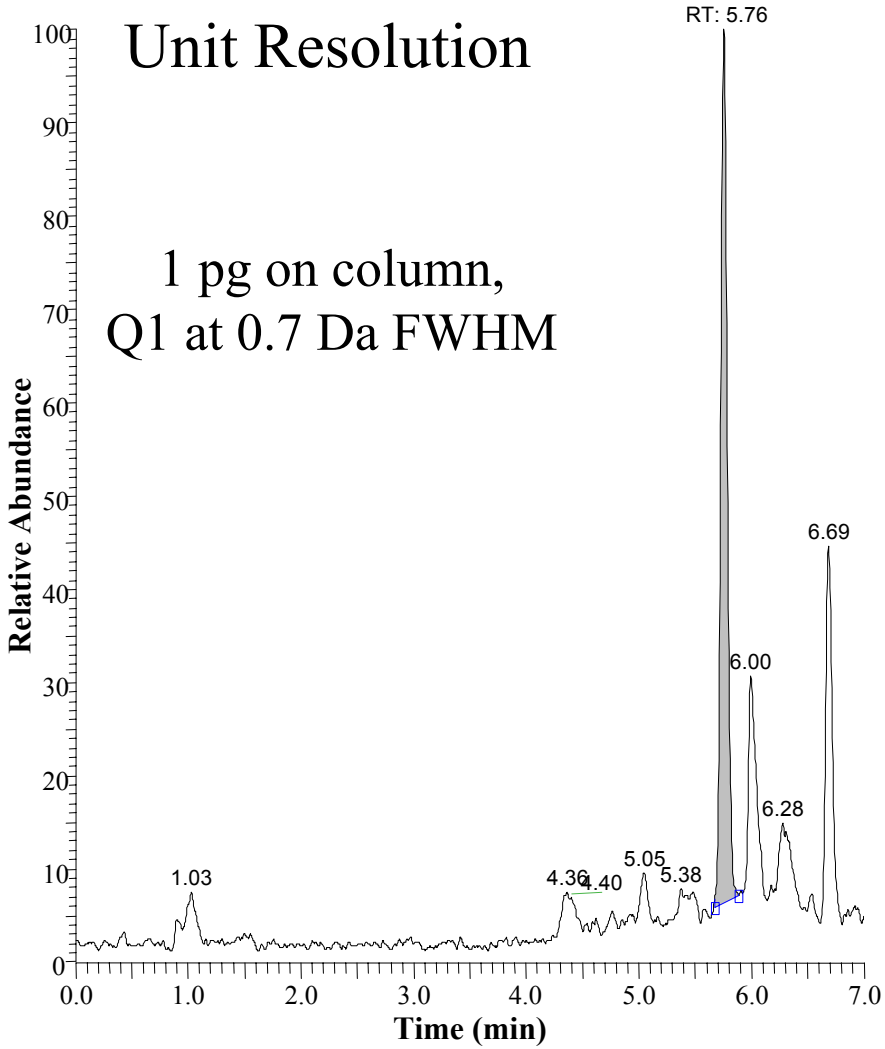
Metabolite Quantitation, Plasma extract, MeOH/H_2O, 500 ul/min, +ESI/SRM 372–328, Fast LC/MS

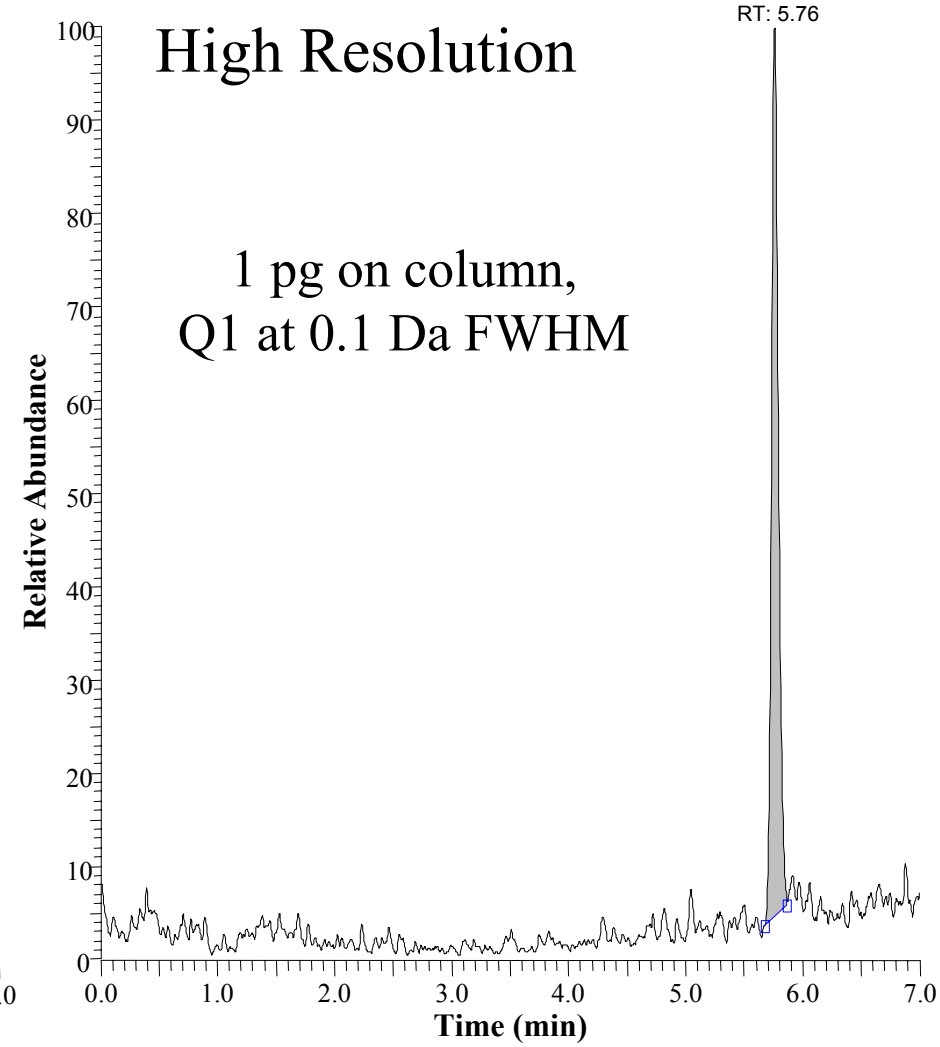


Specificity of high resolution allows removal of two rogue peaks!!

Much less noise in high resolution

TSQ Quantum
LC/ESI/SRM of Clenbuterol – Urine Matrix





Thanks to Mark Churchill and Mark Harrison

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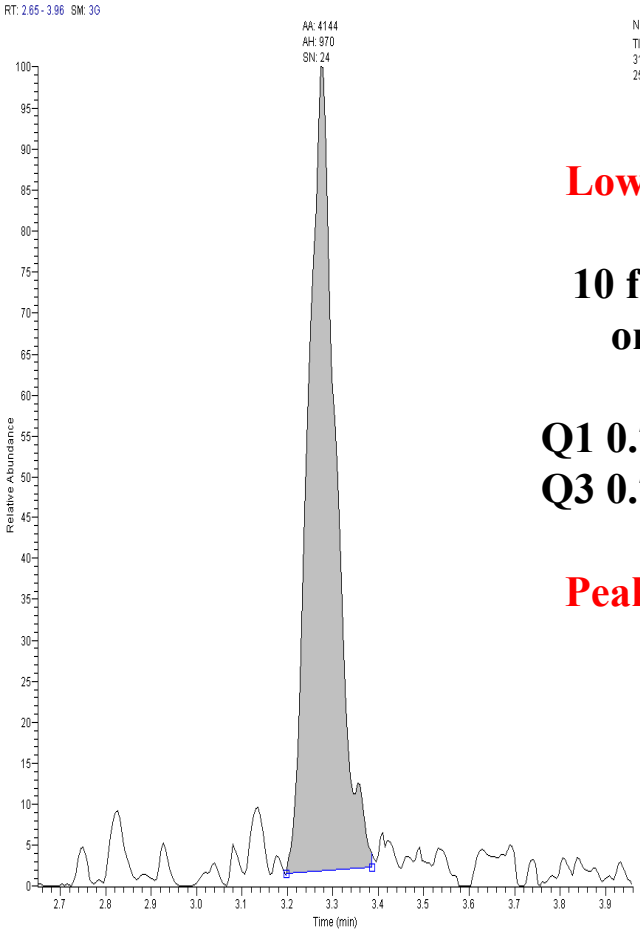
TSQ Quantum - Unit vs. High Resolution +ESI/SRM
Example of Great Sensitivity in HR

Pharmaceutical compound in Plasma, H_2O/MeOH/ACN/0.1% formic,
250 µl/min, 100 x 2 mm C8, +ESI, SRM m/z 311 - 259, Collision Energy 28V

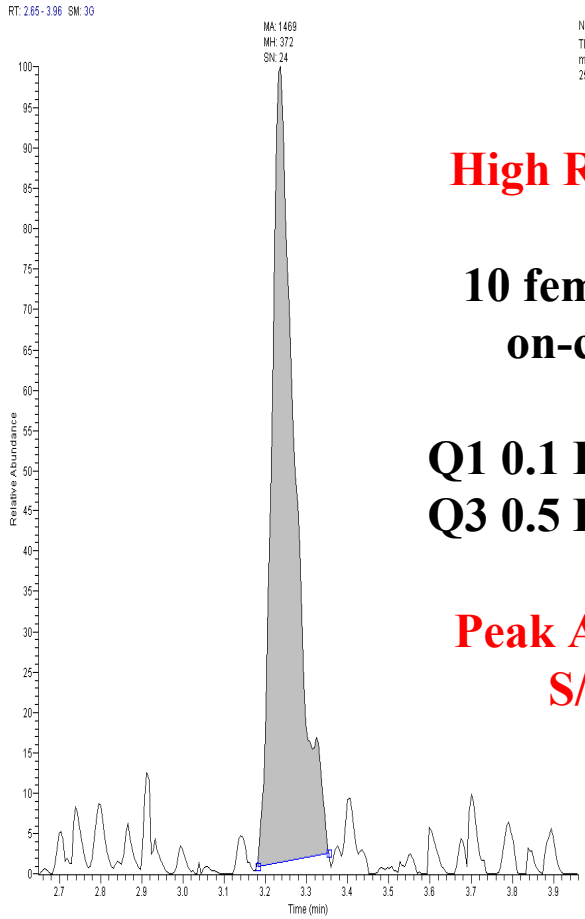


Low Resolution

10 femtograms on-column

Q1 0.7 Da FWHM
Q3 0.7 Da FWHM

Peak Area 4144
S/N 24



High Resolution

10 femtograms on-column

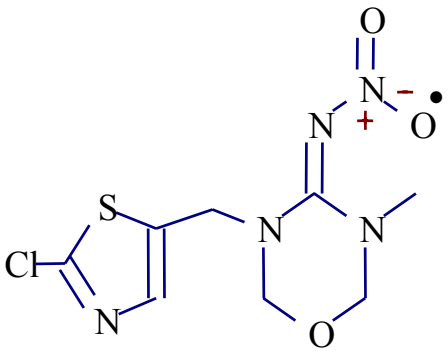
Q1 0.1 Da FWHM
Q3 0.5 Da FWHM

Peak Area 1469
S/N 24

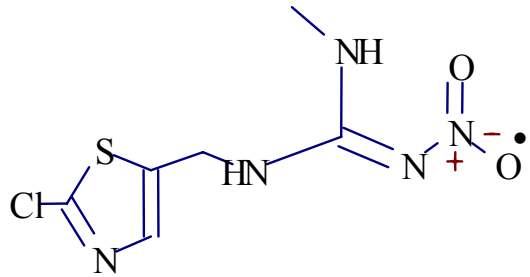
Can use High Resolution Capabilities as low as 10 fg on-column!!

TSQ Quantum
High Resolution Quantitative Assays

+ESI/SRM Quantitation for a Metabolite of the Insecticide, Thiamethoxam, using High Resolution



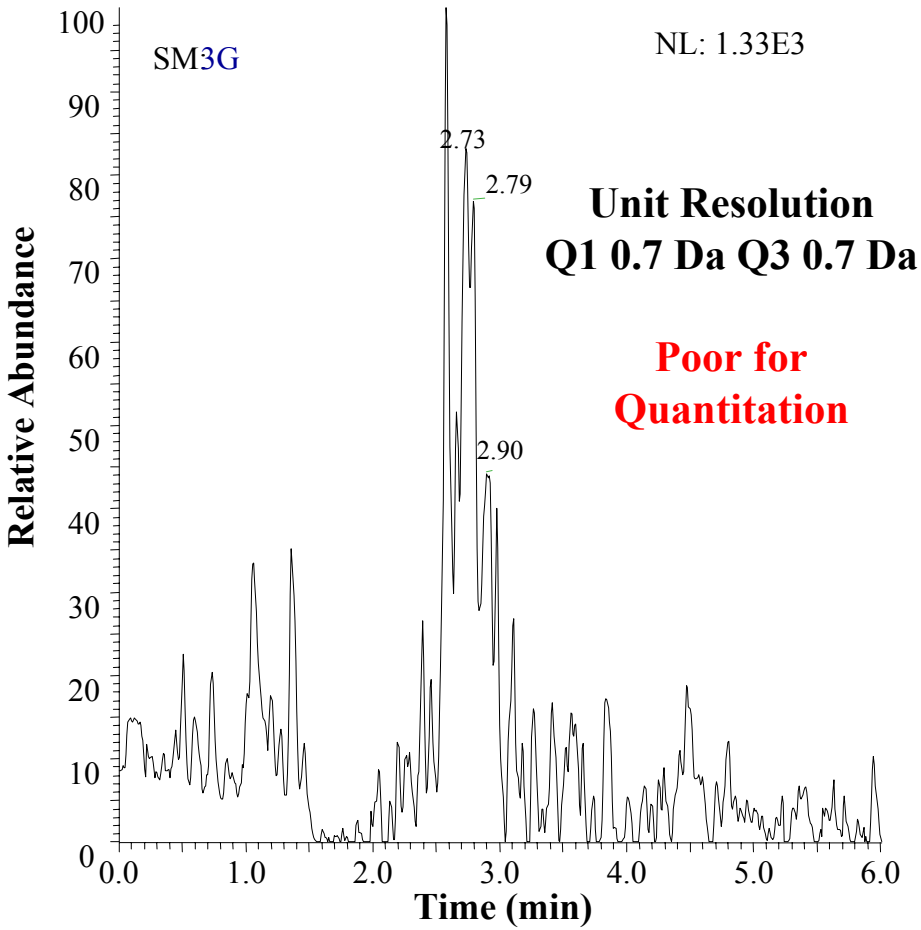
Thiamethoxam
MW 291

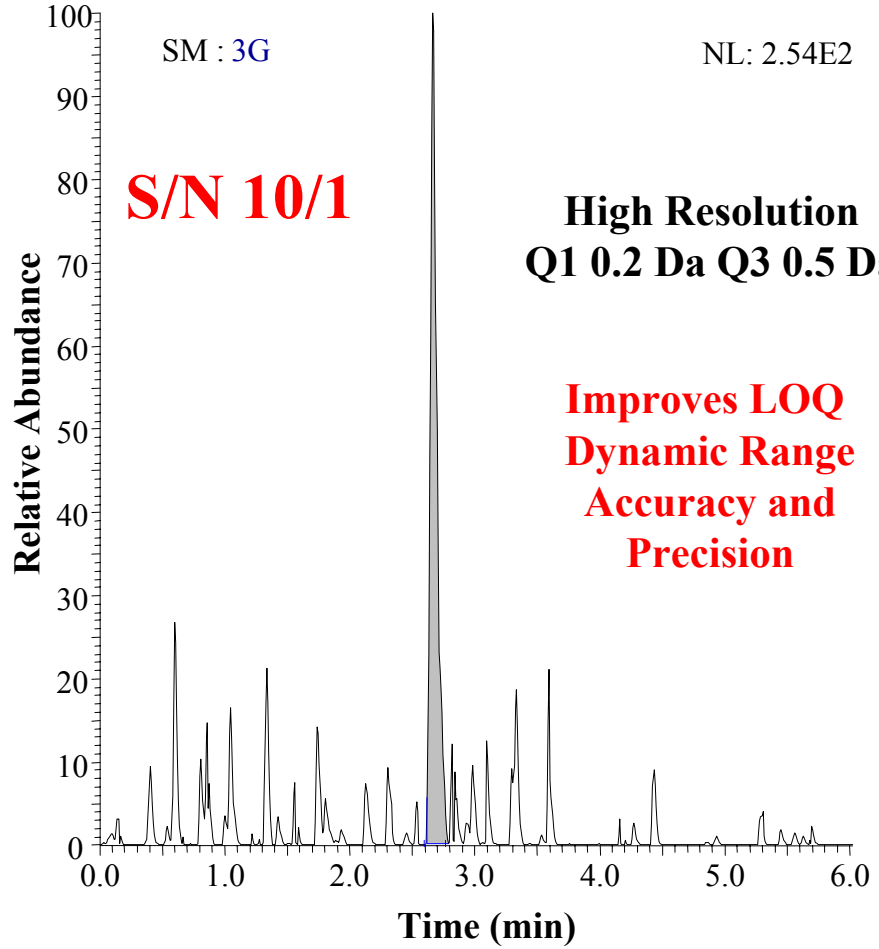


Thiamethoxam Metabolite
MW 249

+LC/ESI/SRM of Thiamethoxam Metabolite in Blueberry Extract TSQ Quantum, Unit versus High Resolution, 100 fg on column

C8 100 x 2.0 mm, ACN/0.1%FA:H_2O/ 0.1%FA, SRM m/z 250-132, Collision Energy 14V

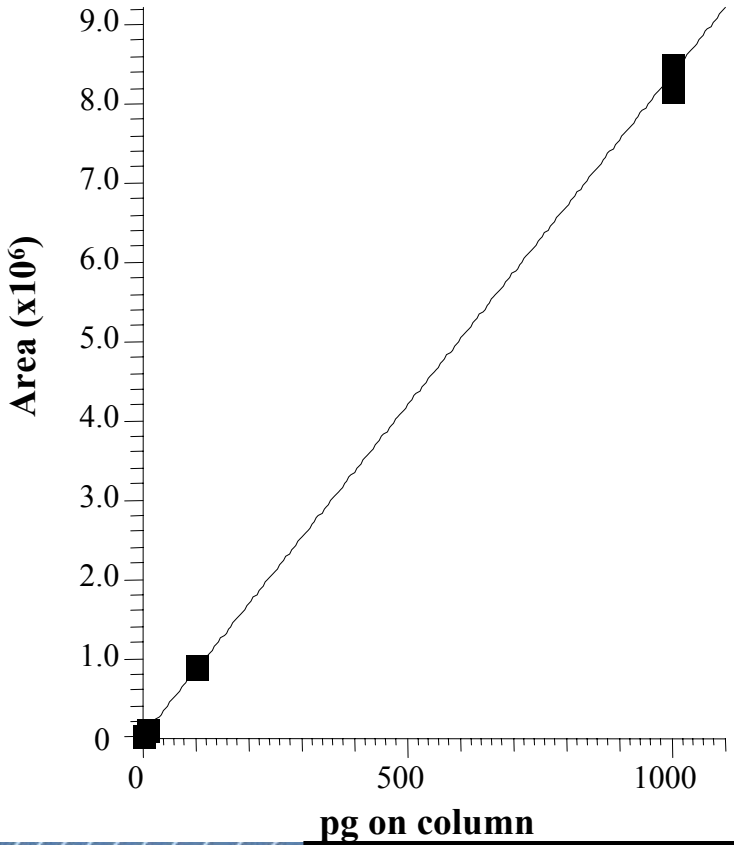


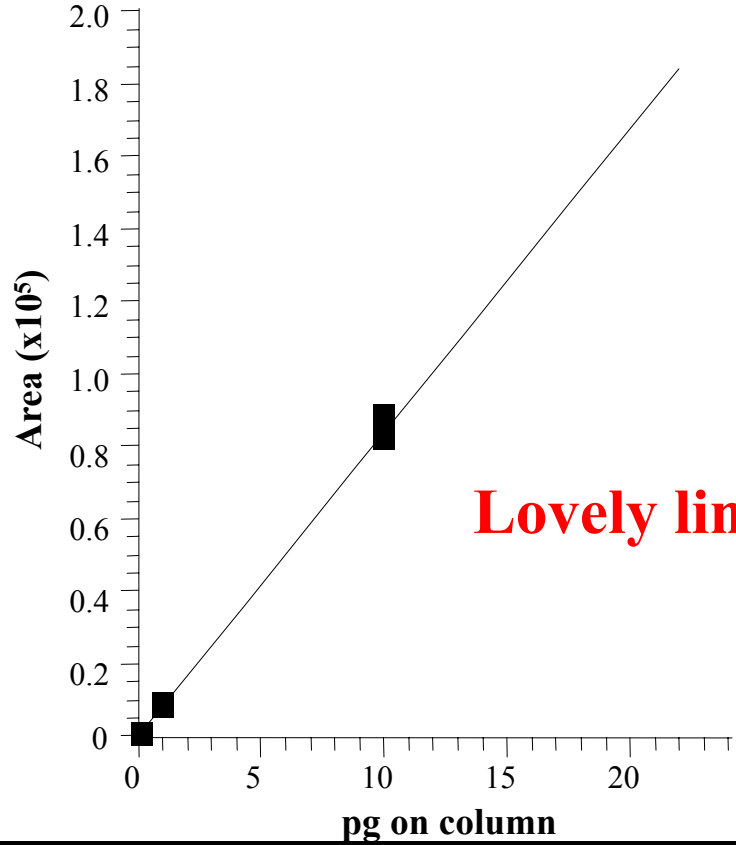


TSQ Quantum: High Resolution +ESI/SRM Quantitation of Thiamethoxam Metabolite in Blueberry Extract

Blueberry Extract, C8 100 x 2.0 mm, Q1 0.2 Da Q3 0.5 Da, ACN/0.1%FA:H_2O/ 0.1%FA, 100 fg – 1 ng on column - 4 orders of magnitude, SRM m/z 250-132, CE 14V

Y = 228.387+8365.93*X **R^2 = 0.9994** W: 1/X





Lovely linearity

TSQ Quantum: High Resolution +ESI/SRM Quantitation of Thiamethoxam Metabolite in Blueberry Extract

Matrix = Blueberry Extract, C8 100 x 2.0 mm, Q1 0.2 Da Q3 0.5 Da, ACN/0.1%FA:H_2O/0.1%FA, SRM m/z 250-132, Collision Energy 14 V

Curve Index	Weighting Index	Origin Index	Equation	
Linear	1/X	Ignore	Y = 228.387+8365.93*X	R^2 = 0.9994

Amount	Area	Specified Amount (pg)	Calculated Amount (pg)	%RSD
100fg on column	964	0.100	0.088	14.3%
1pg on column	9037	1.000	1.053	1.6%
10pg on column	85375	10.000	10.177	3.4%
100pg on column	882912	100.000	105.509	1.6%
1ng on column	8318237	1000.000	994.272	2.0%

Conclusion

Because of the high resolution of the TSQ Quantum, we gain at least an order in magnitude in quantitation limits

Quantitation down to 100 fg of the metabolite on column is possible without interferences of the severe Blueberry matrix. This is 50 times lower than the method proposed by the producer

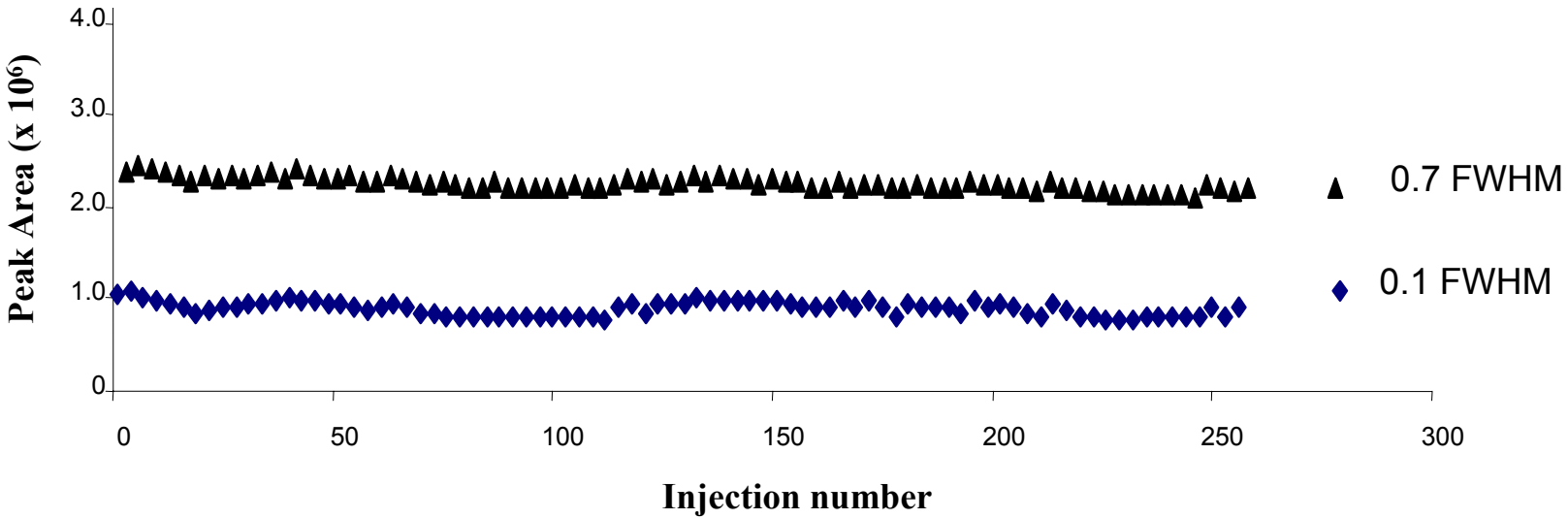
The TSQ Quantum has over 4 orders of magnitude a linear dynamic range in high resolution mode

TSQ Quantum - Ruggedness
Peak Area Stability at Unit (0.7 Da FWHM) and High Resolution (0.1 Da FWHM) with Time

Clenbuterol Peak Area at 0.7 Da and 0.1 Da FWHM

250 injections over 45 hours

ThermoHypersil Keystone C18 100 x 2.1 mm
Methanol / Ammonium acetate 5mM, 0.3 ml/min
10 µl injection of 10 pg/µl



Thanks to Mark Churchill and Mark Harrison for data

TSQ Quantum – Qualitative Applications

Tools For Structural Elucidation

Accurate Mass Measurement
Clean High Quality MS/MS
Different Scanning Functions

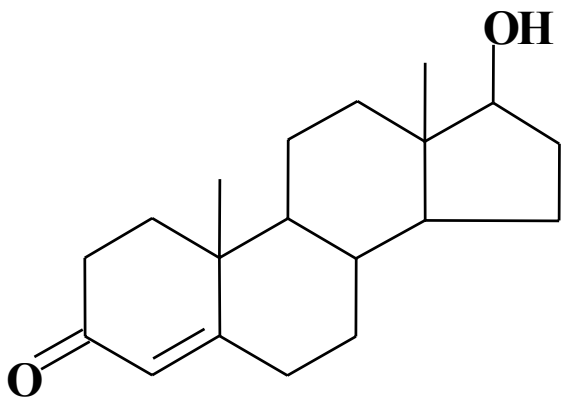
TSQ Quantum – Qualitative Applications

Tools For Structural Elucidation

Accurate Mass Measurement

TSQ Quantum: Accurate Mass Determination of Parent Molecular Ion of Testosterone by LC/ESI/MS

Testosterone, MW 288

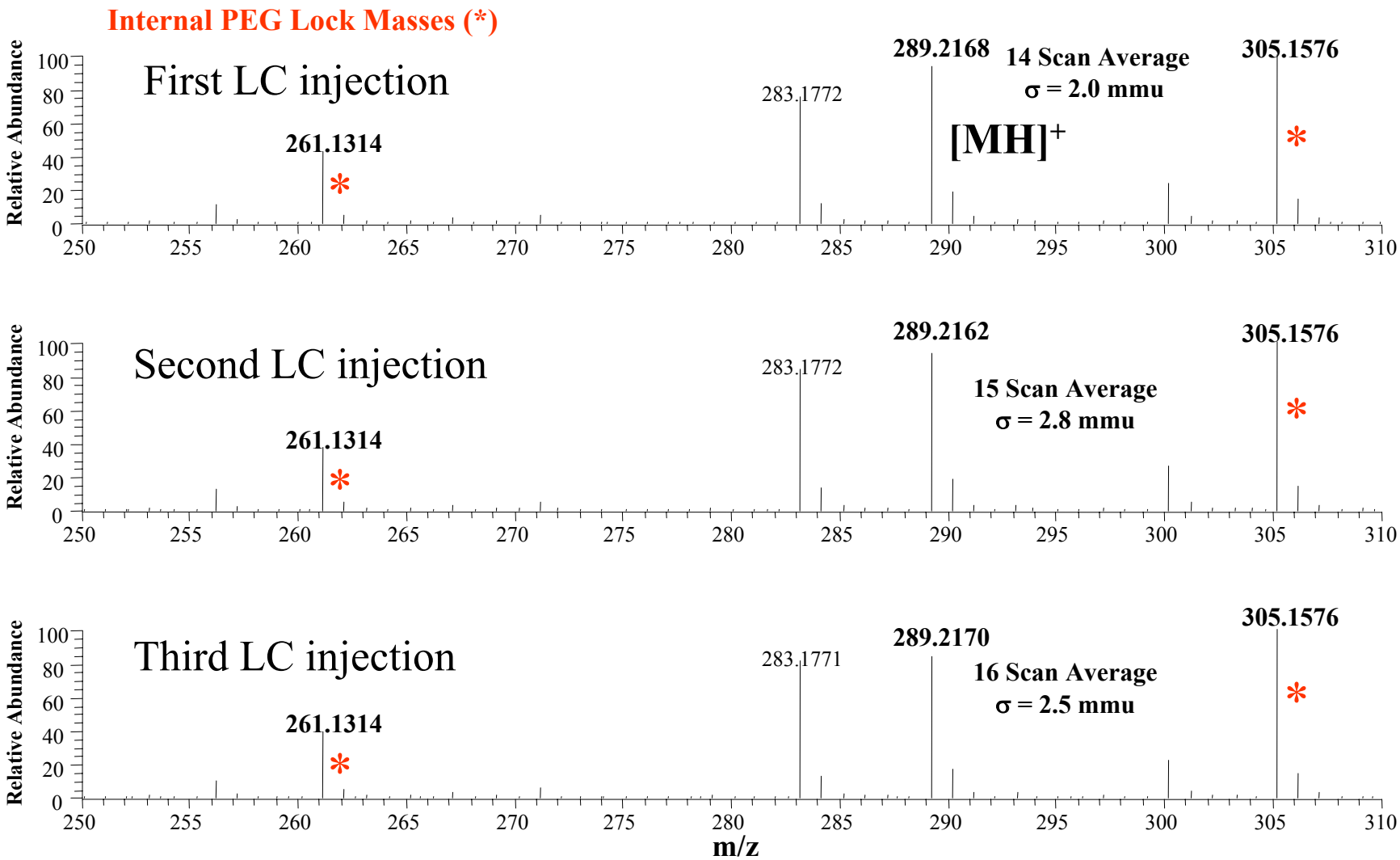


$MH^+ = C_{19}H_{29}O_2$ Exact mass – 289.2168

PEG Internal Lock Masses, Mass Range scanned ~ 60 amu

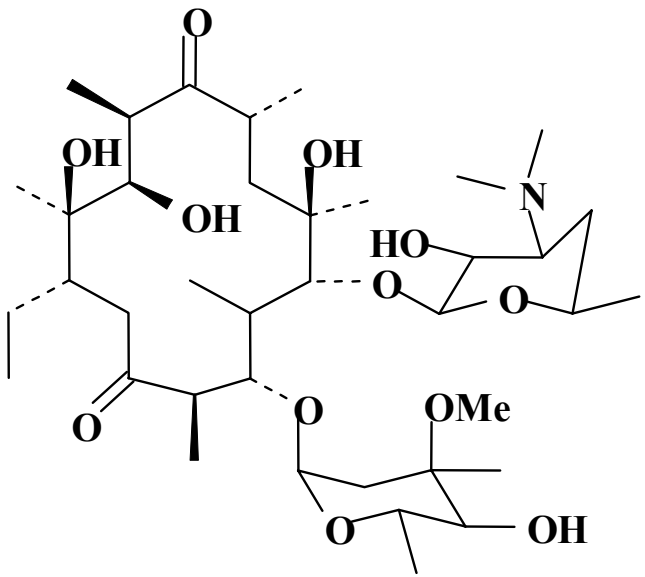
TSQ Quantum, Accurate Mass Determination of MH^+, m/z 289, of Testosterone by LC/ESI/MS (Q3 = 0.1 Da)

Aver. Determined Mass = 289.2166, Proposed Exact Mass = 289.2168, Diff < 0.5 mmu



TSQ Quantum: Accurate Mass Determination of Parent Molecular Ion of Erythromycin by LC/ESI/MS

Erythromycin, MW 733

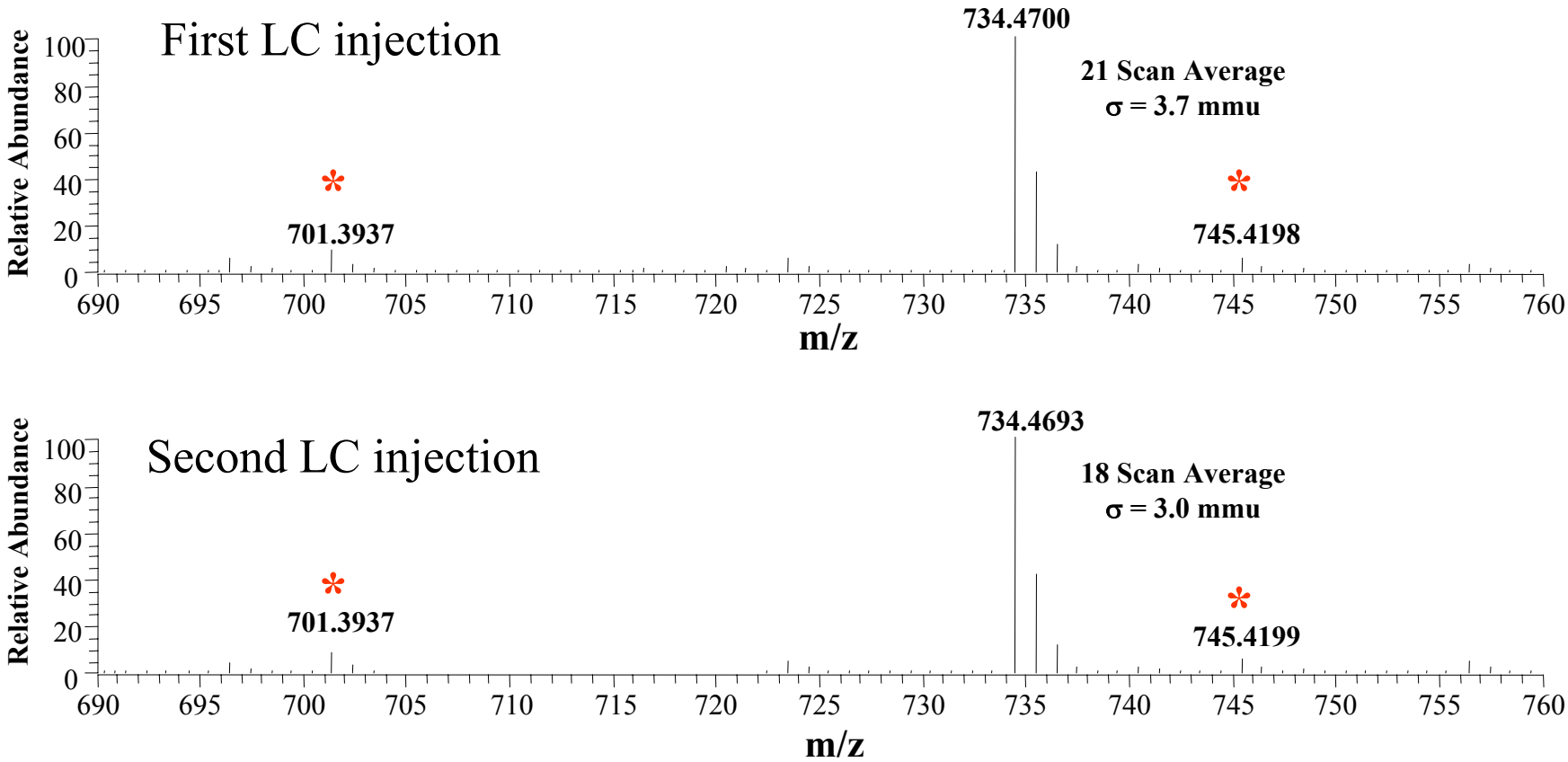


$MH^+ = C_{37}H_{68}NO_{13}$ Exact mass – 734.4688

PEG Internal Lock Masses, Mass Range scanned ~ 60 amu

TSQ Quantum, Accurate Mass Determination of MH^+, m/z 734, of Erythromycin by LC/ESI/MS (Q3 = 0.1 Da)

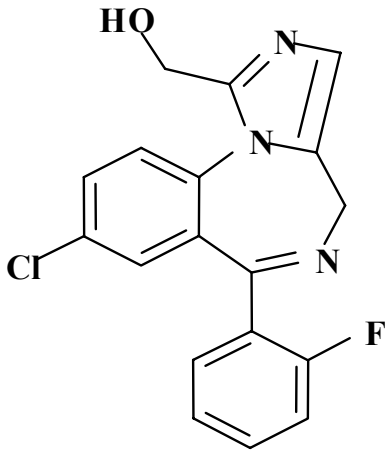
Internal PEG Lock Masses (*)



Aver. Determined Mass = 734.4696, Proposed Exact Mass = 734.4688, Diff < 1mmu

TSQ Quantum – Metabolite Identification through Accurate Mass Measurement

1-Hydroxymidazolam, Metabolite



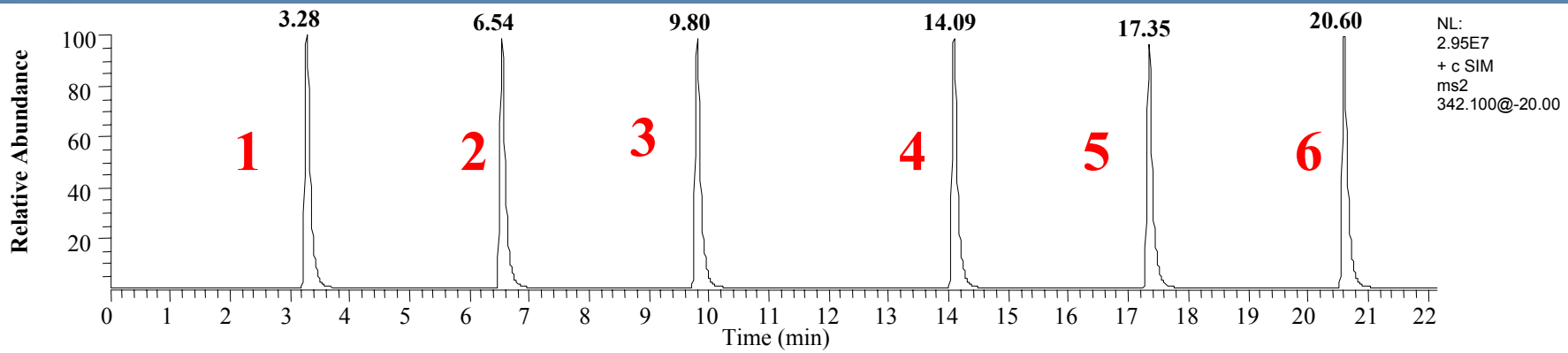
$C_{18}H_{14}N_3OClF$

Exact Mass MH^+ = 342.0809

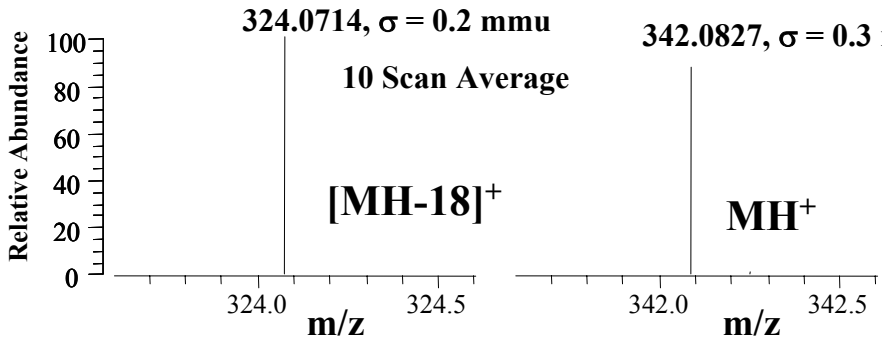
Experiment

Accurate Mass Measurement of MH^+ and Fragment Ion, $[MH-18]^+$ by LC/ESI/MS/MS using External PEG Lock Masses

TSQ Quantum: Accurate Mass of MH^+ and $[MH-18]^+$ by LC/ESI/MS/MS using External PEG Lock Masses (m/z 305, 349) (6 LC injs, SIM, Q3 = 0.1 Da, Collision Gas = 1.2 mtorr, CE = 20 eV)

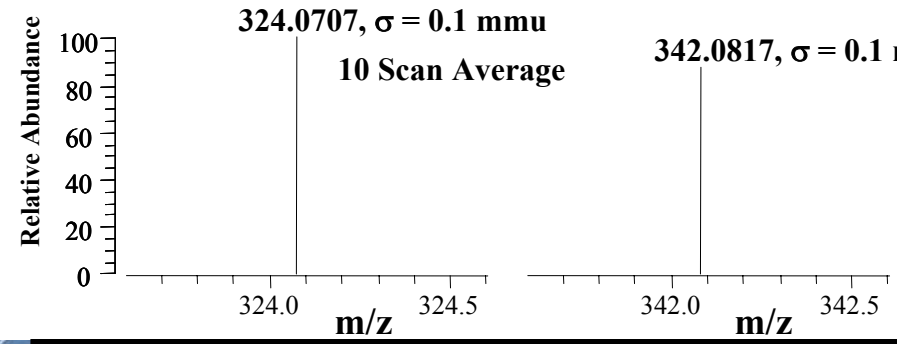


First inj 1



#441-450RT:3.25-3.32AV:10
+ c SIM ms2 342.100@-20.00

Last inj 6



#2783-2792RT:20.57-20.64AV:10
+ c SIM ms2 342.100@-20.00

TSQ Quantum: Accurate Mass of MH^+ and $[MH-18]^+$ by LC/ESI/MS/MS using External PEG Lock Masses (m/z 305, 349) (6 LC injs, SIM, Q3 = 0.1 Da, Collision Gas = 1.2 mtorr, CE = 20 eV)

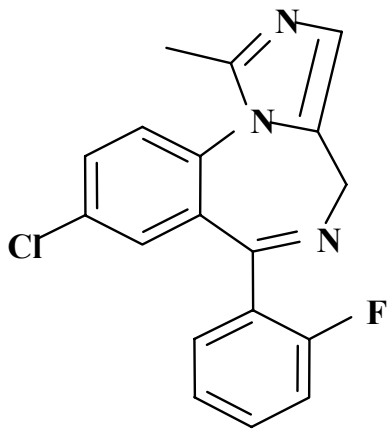
	Time of Elution (min)	Accurate Mass MH^+ (u)	Standard Deviation (mmu)	Accurate Mass $[MH-18]^+$ (u)	Standard Deviation (mmu)
LC Inj 1	3.28	342.0827	0.3	324.0714	0.2
LC Inj 2	6.54	342.0832	0.3	324.0732	0.1
LC Inj 3	9.80	342.0830	0.1	324.0727	0.3
LC Inj 4	14.09	342.0828	0.3	324.0718	0.1
LC Inj 5	17.35	342.0820	0.4	324.0721	0.2
LC Inj 6	20.60	342.0817	0.1	324.0707	0.1
Average		342.0826		324.0720	
Exact Mass	MH^+	342.0809	$[MH-H_2O]^+$	324.0704	

Good agreement between exact masses of known MH^+, proposed $[MH-H_2O]^+$ and the average determined accurate masses (<2 mmu)

TSQ Quantum
Metabolite Identification through Accurate Mass Measurement

Typical situation - the metabolite (R) is unknown and metabolite identification is required through MS

However the active drug (M), in this case Midazolam, is known



Midazolam

$C_{18}H_{13}FN_3Cl$

MW = 325

Since by ESI/MS it is known that the metabolite RH^+ is m/z 342 we know we have a [M+16] metabolite

TSQ Quantum
Metabolite Identification through Accurate Mass Measurement

Accurate Mass determined for the Metabolite, $[M+16]H^+$ on the TSQ Quantum = 342.0826

Using an elemental composition calculator where the elemental lower limits are set by the composition of parent drug, Midazolam, (C = 18, H = 13, F = 1, N = 3, Cl = 1) the ONLY elemental compositions corresponding to the TSQ Quantum-determined accurate mass for $[M+16]H^+$ (50 mmu window) are:

Exact mass of $[M+O]H^+$ = 342.0809, **1.7 mmu different**

Exact $[M+NH_2]H^+$ = 342.1048, **22.1 mmu different**

Exact $[M+CH_4]H^+$ = 342.1173, **34.7 mmu different**

Thus, Accurate Mass Measurement on the TSQ Quantum Provides Clear Identification of $[M+16]H^+$ as the $[M+O]H^+$ Metabolite

TSQ Quantum – Qualitative Applications

Tools For Structural Elucidation

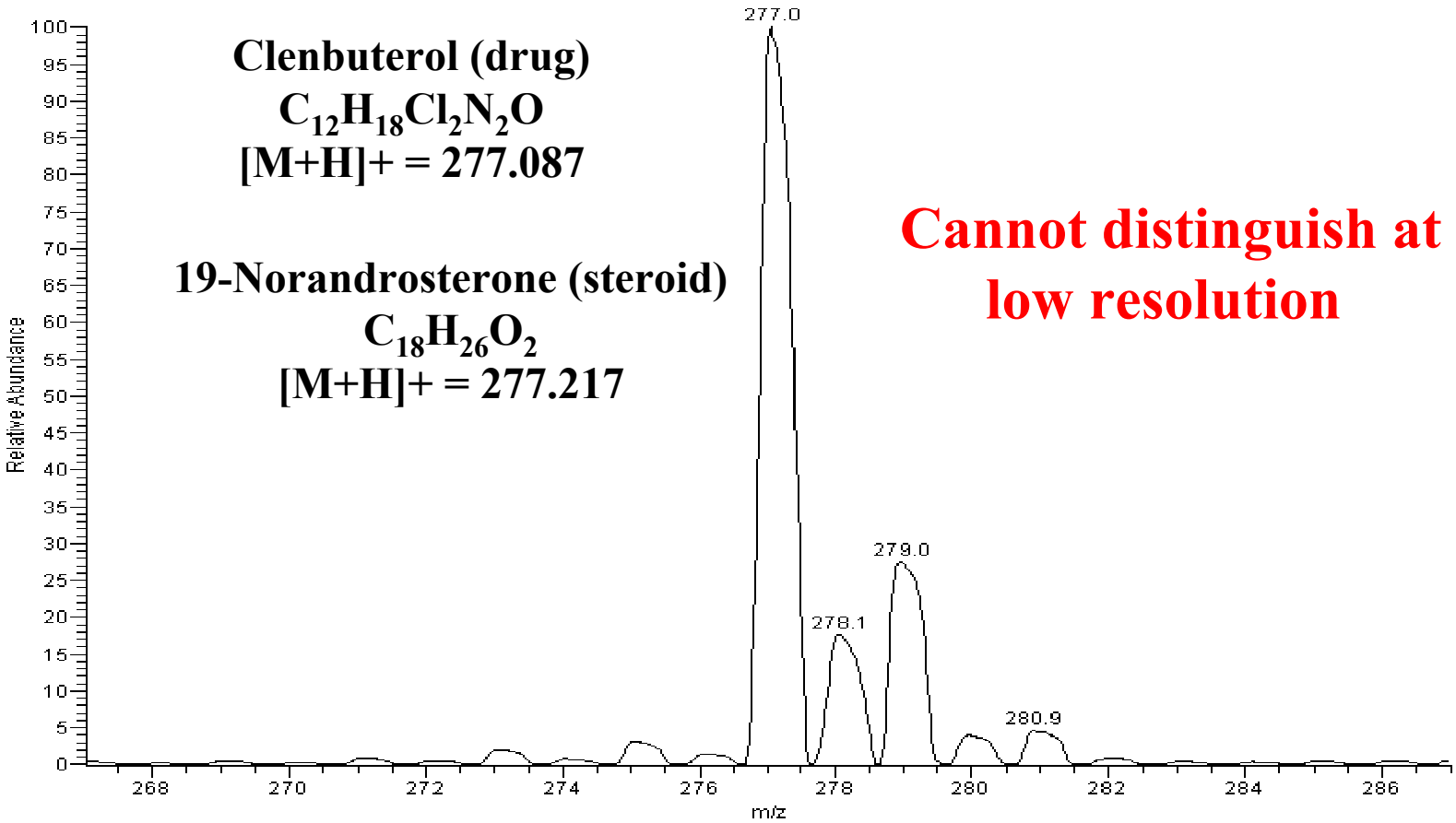
Clean High Quality MS/MS

TSQ Quantum, Separation of Isobaric Compounds By High Resolution for Structural Elucidation

Mixture of Clenbuterol and 19-Norandrosterone, +ESI/MS, Q1 at Unit Resolution

Clenbuterol (drug)
$C_{12}H_{18}Cl_2N_2O$
[M+H]+ = 277.087

19-Norandrosterone (steroid)
$C_{18}H_{26}O_2$
[M+H]+ = 277.217



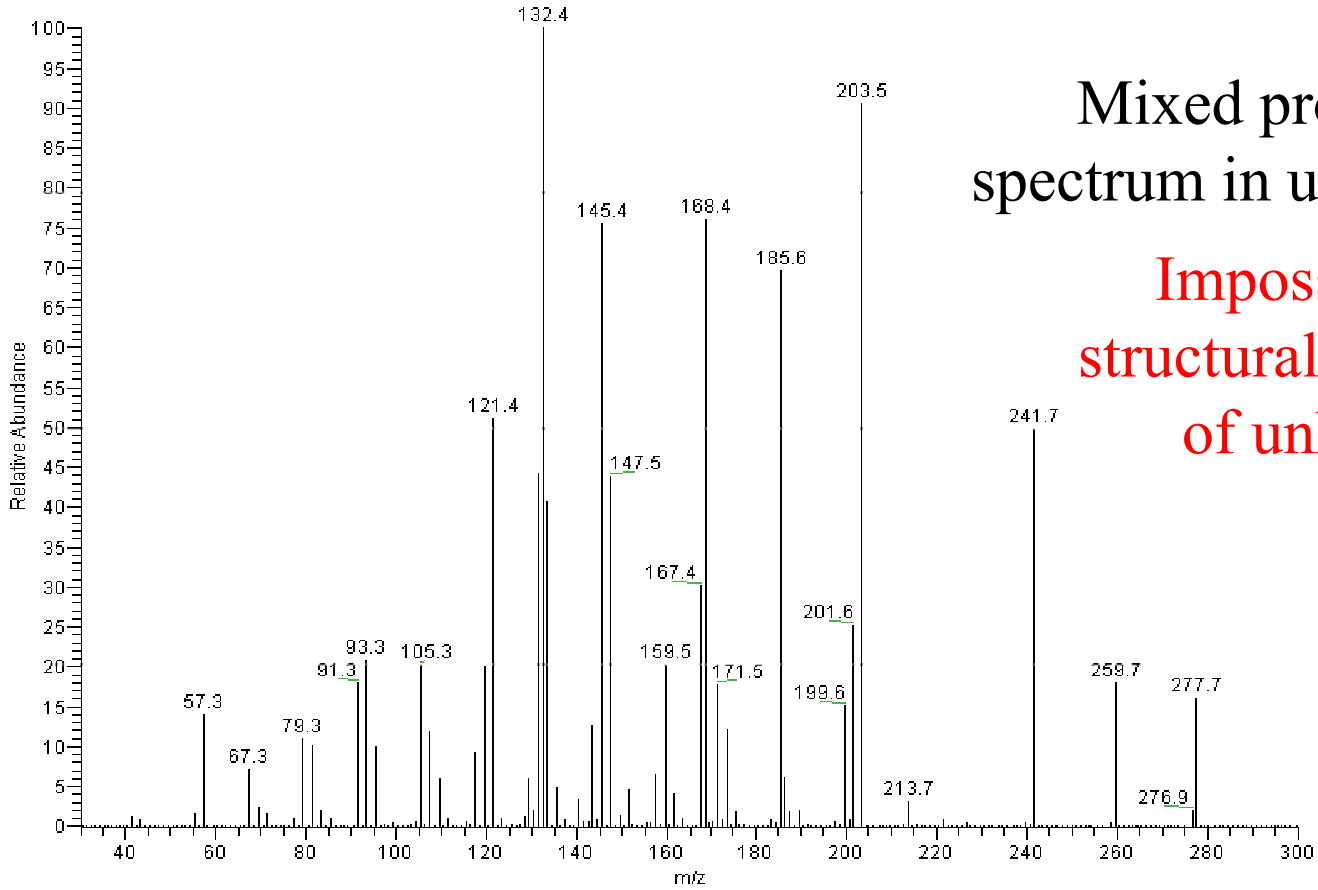
Cannot distinguish at low resolution

Thanks to Mark Churchill and Mark Harrison for data

Thermo Finnigan

TSQ Quantum, Separation of Isobaric Compounds By High Resolution for Structural Elucidation

Mixture of Clenbuterol and 19-Norandrosterone, +ESI/MS/MS, Q1 at Unit Resolution



Mixed product ion spectrum in unit resolution

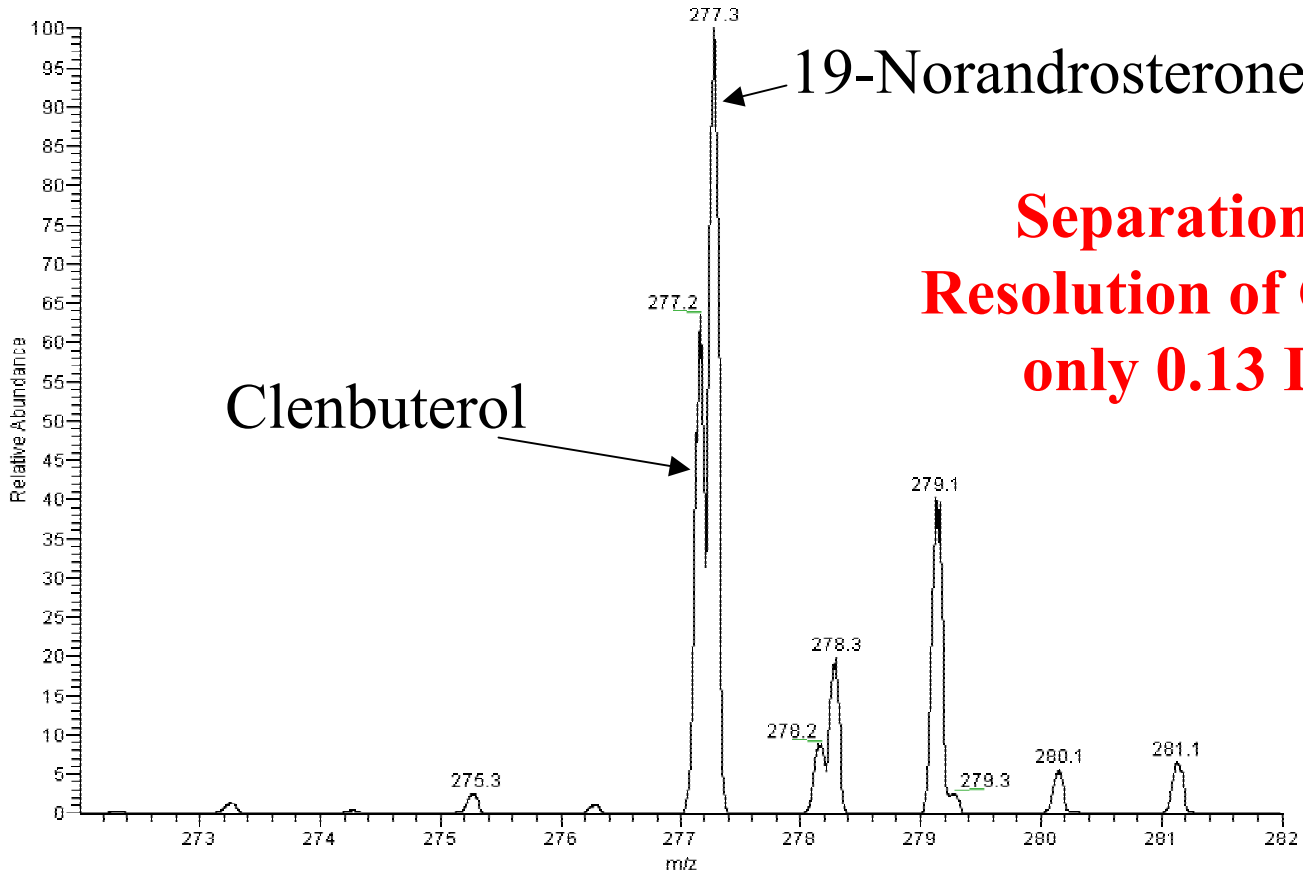
Impossible for structural elucidation of unknowns

Thanks to Mark Churchill and Mark Harrison for data

ThermoFinnigan

TSQ Quantum, Separation of Isobaric Compounds By High Resolution for Structural Elucidation

Mixture of Clenbuterol and 19-Norandrosterone +ESI/MS, Q1 at High Resolution



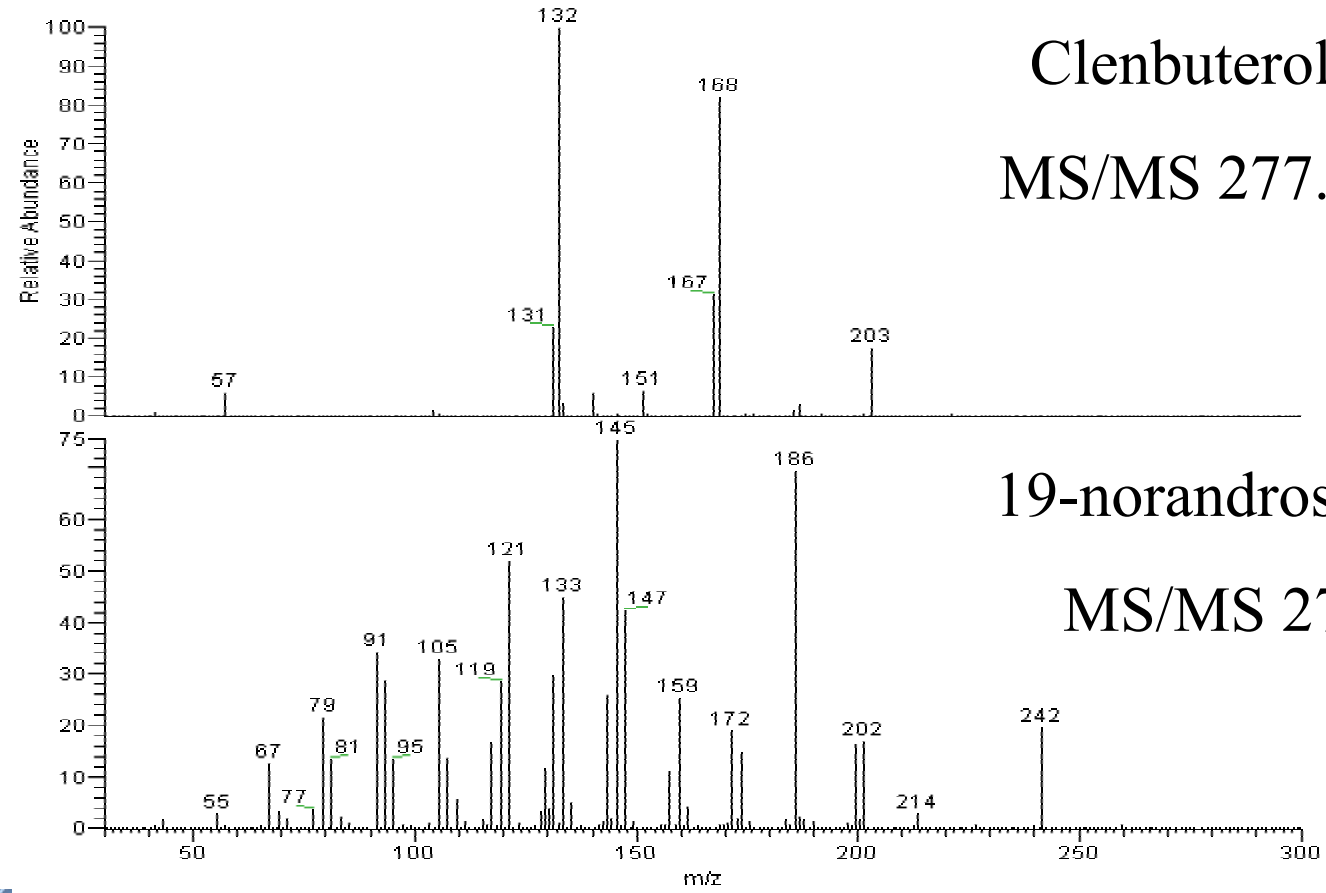
Separation by High Resolution of Components only 0.13 Da Apart

Thanks to Mark Churchill and Mark Harrison for data

ThermoFinnigan

TSQ Quantum, +ESI/MS/MS, Q1 High Resolution Separation and MS/MS Characterization of Isobaric Species

Mixture of Clenbuterol and 19-Norandrosterone +ESI/MS/MS, Q1 at High Resolution



Clenbuterol

MS/MS 277.1

19-norandrosterone

MS/MS 277.2

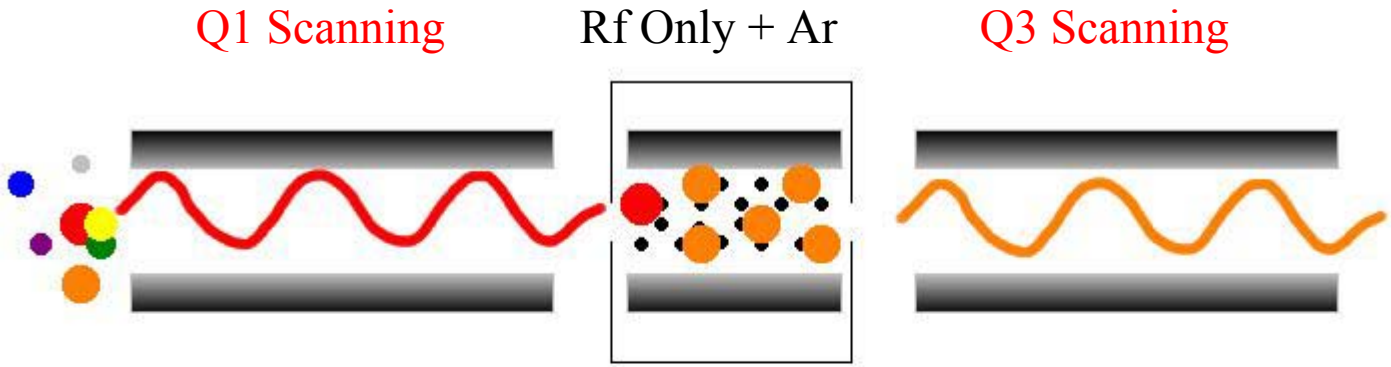
Thanks to Mark Churchill and Mark Harrison for data

TSQ Quantum – Qualitative Applications

Tools For Structural Elucidation

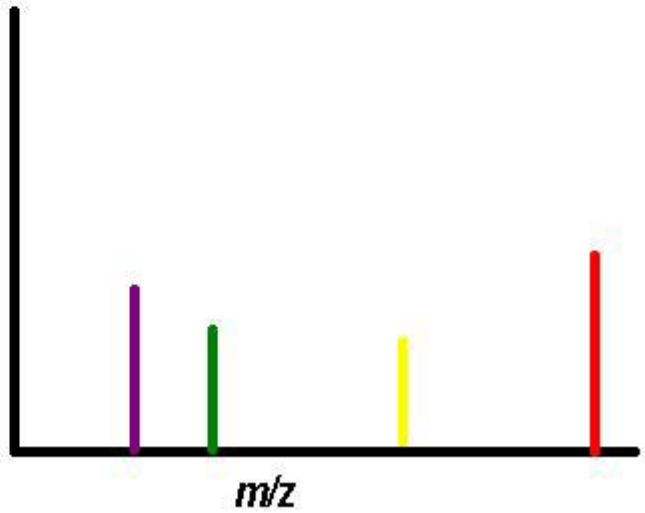
Different Scanning Functions

Constant Neutral Loss Scanning



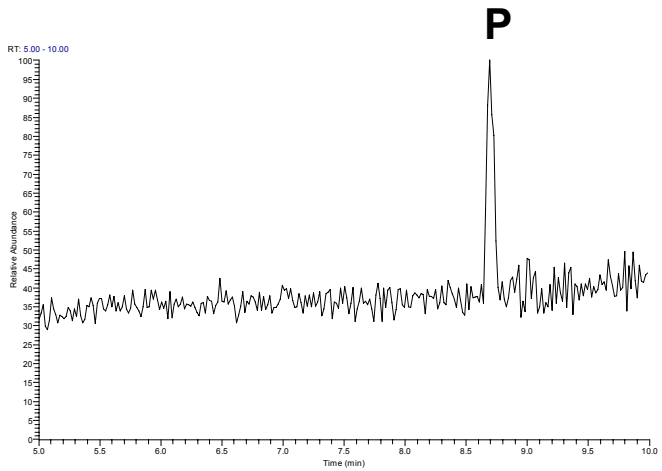
Q3 is offset by the neutral loss under investigation

Only those compounds which give a fragment having that specific loss are detected



TSQ Quantum - Constant Neutral Loss Scanning Increases Specificity and Decreases Chemical Noise

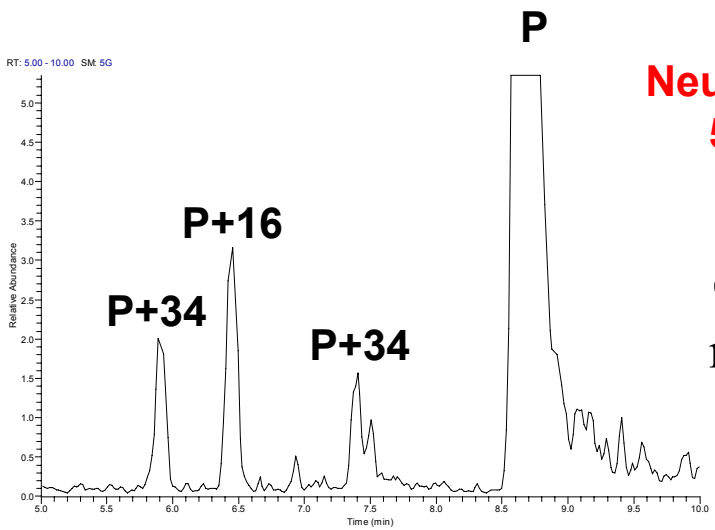
Invitro Microsomal Incubation of Drug P (5 uM)



Full-Scan MS
50 → 450 da

No sign of metabolites

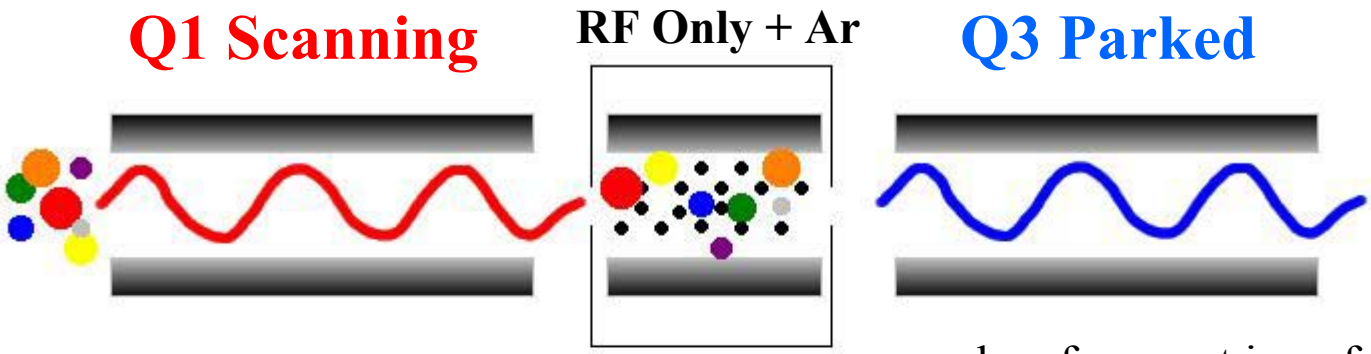
Generate Metabolites with masses 16 and 34 da greater than parent drug



Neutral Loss Scan
50 → 450 da
NL = 116 da

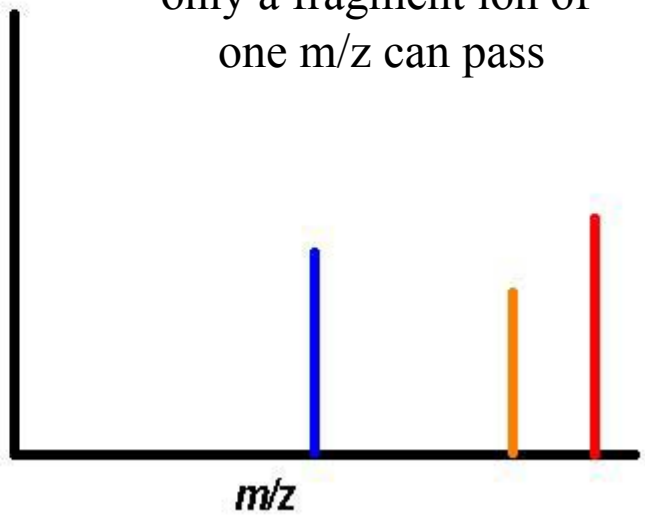
Clearly see metabolites

Precursor Ion Scanning

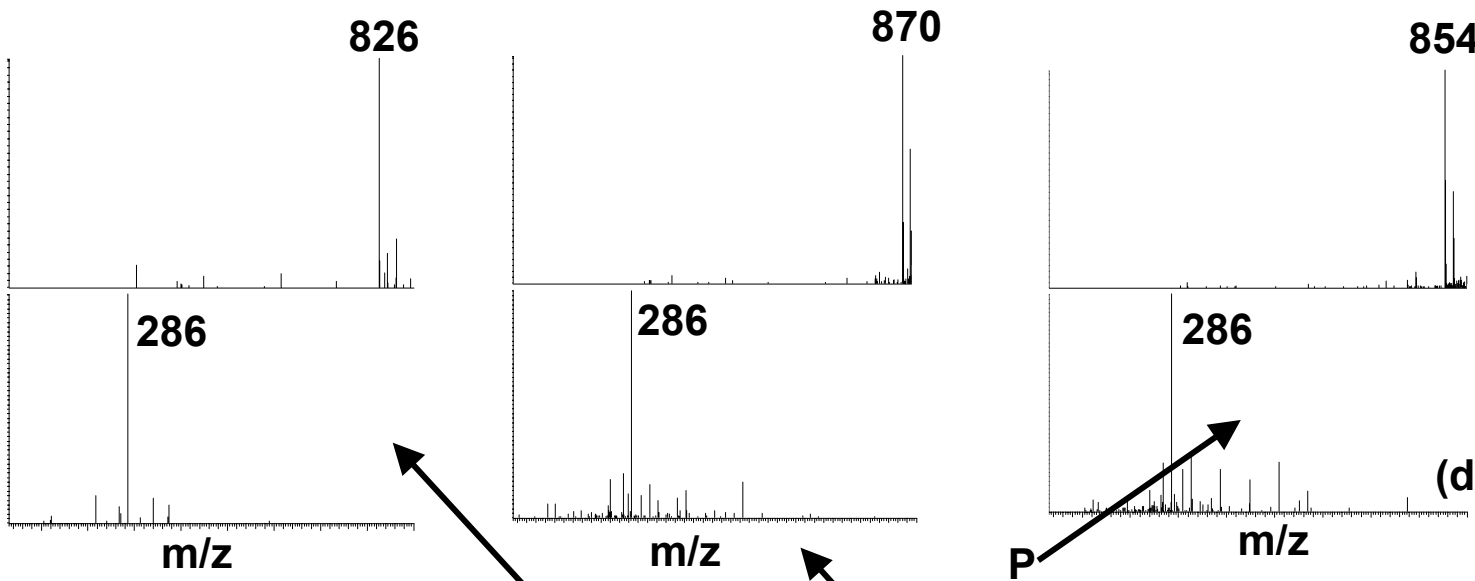


only a fragment ion of one m/z can pass

Only those compounds which give a specified fragment ion are detected



TSQ Quantum, Precursor Ion Analysis of Proprietary Drug and its Metabolites



- P = Drug
- OH-P = Hydroxy Metabolite
- P-28 = Metabolite with a mass 28 da less than parent

Precursor Ion Scan Mass Chromatogram Product Ion = 286 da

Specificity of experiment allows metabolite detection

TSQ Quantum - Summary

TSQ Quantum Project Team

Product Development
Lance Arbini
Paul Atherton
Huy Bui
Clay Campbell
Pascual Cardenas
Richard Currell
Wayne Dewey
Jean-Jacques Dunyach
Phil Fong
Jerry Glass
Nigel Gore
Trevor Hall
Richard Hartford
Rex Heller
Steve Hurwitz
Hugh Leonard
Ray Lewis
Jaime McGregor
Teresa McLaughlin
Iain Mylchreest
Terry Olney
Carlos Rojas
Alan Schoen
Hans Schweingruber
Bill Siebert
Dennis Taylor
Eugene Zhuk

Manufacturing
Alvin Abdon
Ken Bradbury
Rey Brito
Steve Brown
Bob Cook
Rudy Cruz
Armando Delacruz
Lenis Doan
Gary Fontana
Bret Johnson
Joe Leon Guerrero
Alex Manalo
Frank Marcos
Misael Martinez
Ed Matias
Jose-Cruz Medina
Gerry Rojas
Phillip Tran

Marketing
Gary Paul
Mark Churchill
Nelson Cooke
Iain Green
Mark Harrison
Louis Maljers

Product Support
Fred Ayres
Ed Gonzales
Bruce Hillestad

Technical Publications
Antony Harvey
Rob Heather
Toby Kraft
Susan Lee
Franklin Mason

Chemists
Witold Winnik
Mark Kagan
Maurizio Splendore
Liam Moran