Finnigan LTQ FT

Ultra High Performance Mass Spectrometer

Finnigan LTQ FT – Ultra High Performance

- **Ion Trap based Fourier Transform ICR Mass Spectrometer**
- **FTICR (FTMS) at LC timescale**
- **Accurate Mass**
- **High Sensitivity**
- **Ultra-high Resolution**
- **Above All - Simple to Operate**

Finnigan LTQ FT – Ultra High Performance

- Surveyor HPLC
- API, MALDI Sources
- 2-D Linear Ion Trap
- ICR Cell
- Actively Shielded Superconducting Magnet

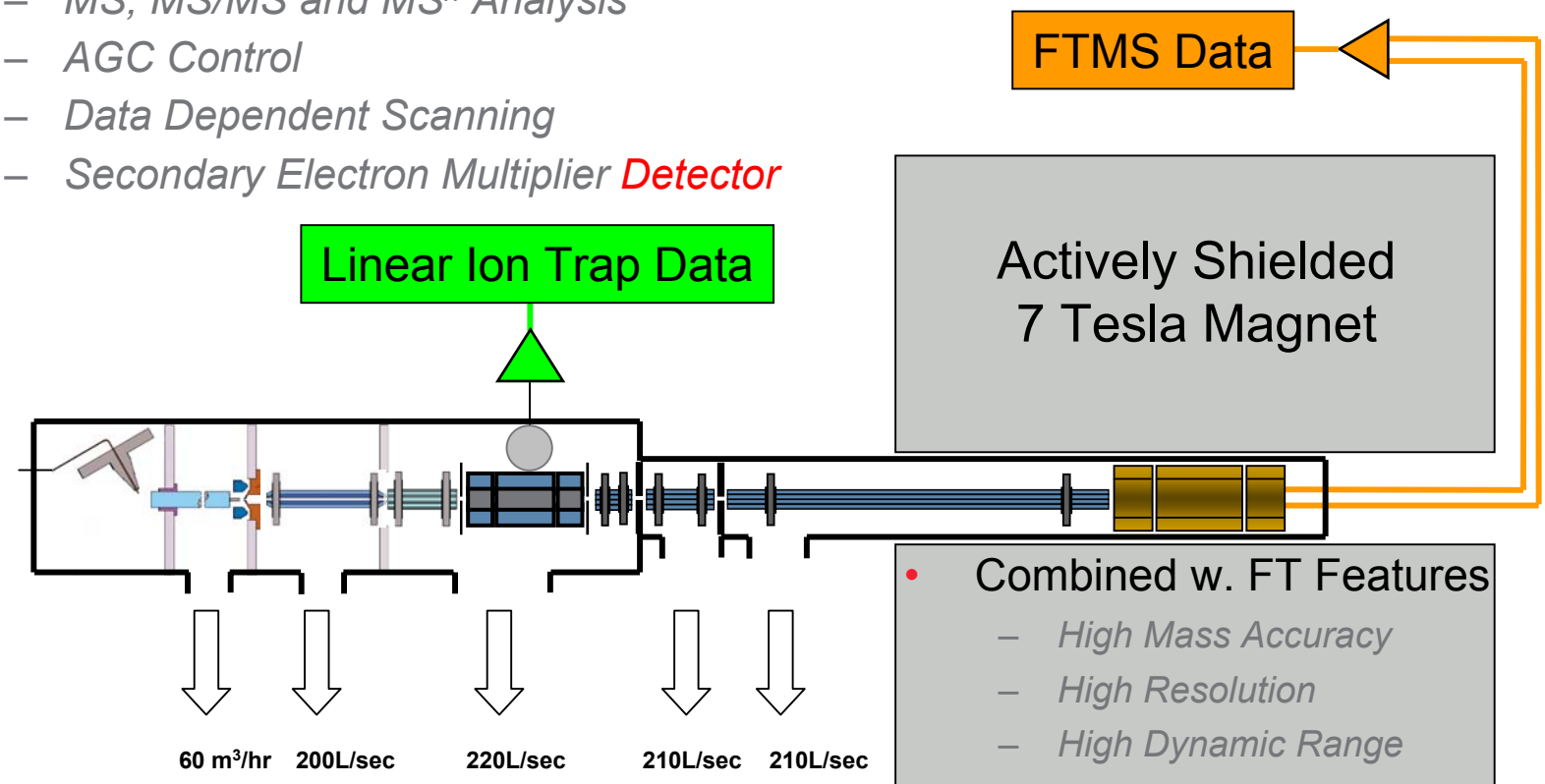
- **For details – have a look at the dummy**

Finnigan LTQ FT – Ultra High Performance

- First Routine FTMS Mass Spectrometer
  - *Fast*
  - *Automated*
  - *Data dependent scanning*
  - *Robust technology*
- Most Advanced Mass Spectrometer Available
  - *Proteomics*
  - *Metabolomics*
  - *Pharmaceutical and Drug Discovery*
  - *Small Molecule Analysis, Structural Elucidation*

Finnigan LTQ FT – Combination of Performance

- All Useful Ion Trap Features
  - *API with APCI, ESI and MALDI Ion Sources*
  - *MS, MS/MS and MS^n Analysis*
  - *AGC Control*
  - *Data Dependent Scanning*
  - *Secondary Electron Multiplier Detector*



Finnigan LTQ FT – Experiment Description

- Generate Ions
  - *API: ESI, APCI, APPI, MALDI Source*
- Store and Prepare Ions in 2-D Linear Trap
  - *MS, MS^n*
  - *AGC to regulate number of ions*
- Transfer Ions to ICR Cell
  - *RF ion guides*
- Excite Ions in ICR Cell
  - *RF frequency sweep or SWIFT excitation*
- Detect Ion Transient Signal
- Fourier Transform Data Processing
  - *Data dependent information passed to 2-D Linear Trap*

Finnigan LTQ FT – Performance Specifications

- Resolution
  - *100 000 resolution at m/z 400 at 1 Hz repetition rate*
  - *>500 000 resolution broadband mode*
- Mass Range
  - *m/z 50-4000 (standard range)*
  - *1-order-magnitude in single scan (e.g. m/z 200-2000)*
- Mass Accuracy
  - *2 ppm RMS, external mass calibration*
  - *<1 ppm RMS, internal mass calibration*
- Dynamic Range
  - *5 000 within mass spectrum*
  - *>500 000 between mass spectra*

Finnigan LTQ FT – Accurate Mass

- The magnetic field is very stable, therefore mass calibration is valid for several days
- Accurate Mass with External Calibration
  - *Must control the number of ions in ICR cell - Ion cyclotron frequency varies with ion density*
  - *Number of ions controlled with AGC on the 2-D Linear Trap*
  - *Mass accuracy of 2 ppm RMS for MS and MS/MS mode*
- Accurate Mass with Internal Calibration
  - *A reference substance of known mass is analyzed with the unknown substance and used as a mass calibrant*
  - *Mass accuracy of better than 1 ppm RMS*

Finnigan LTQ FT – High Resolution

- Resolution Varies with Mass
  - *Resolution is inversely proportional to mass*
- Resolution Depends on Detection Time
  - *Double the detection time to double the resolution*
  - *Detect time of 745 ms for 100,000 resolution at m/z 400*
- Resolution Depends on Magnet Field Strength
  - *Double the magnet field to double the resolution*
  - *Magnet price increases immensely with field strength !*

Finnigan LTQ FT – 100 000 Resolution at 1 Hz

Calibration Mixture
m/z 195 to 1922

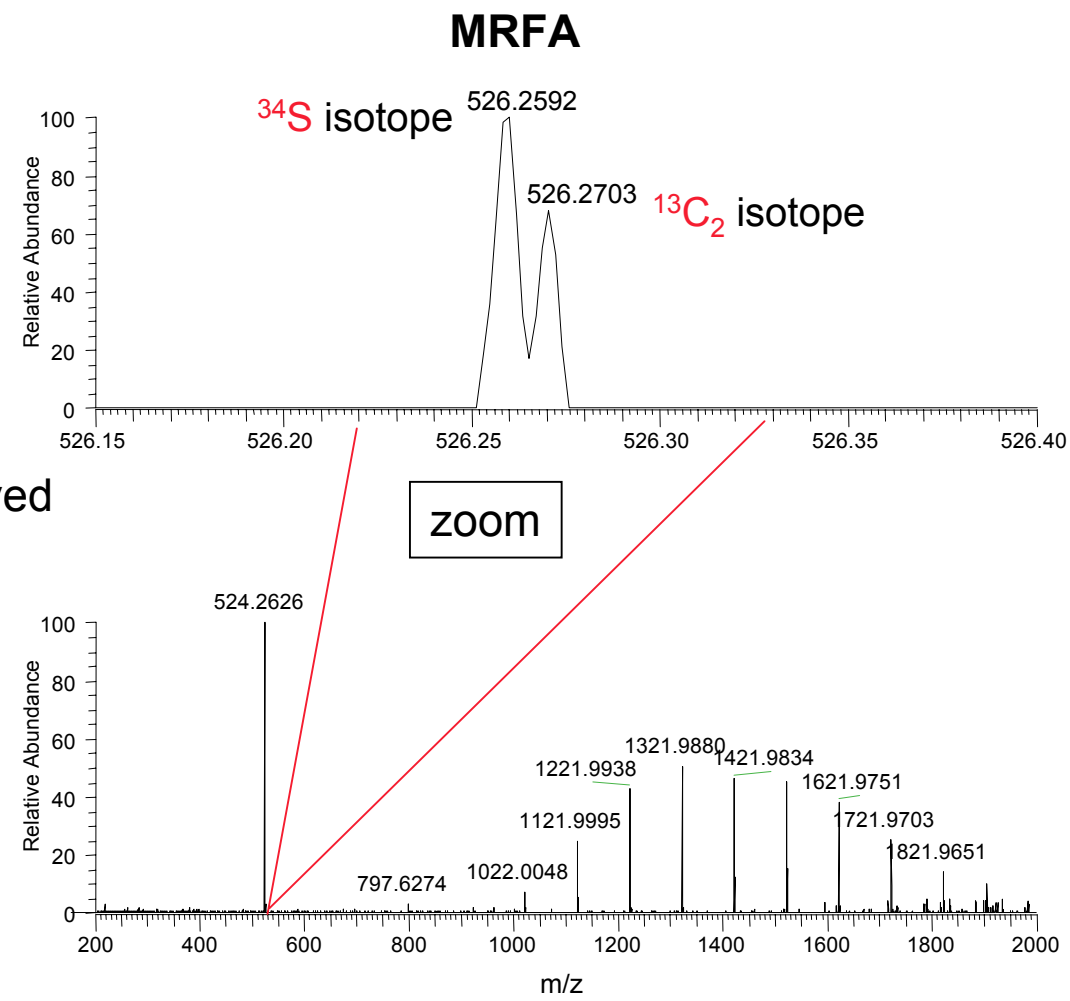
1024 k Word transient
Acquisition rate 1 Hz

MRFA – ^{13}C and ^{34}S Isotopes resolved

Res. Power at m/z 526:
90 000 (FWHM)

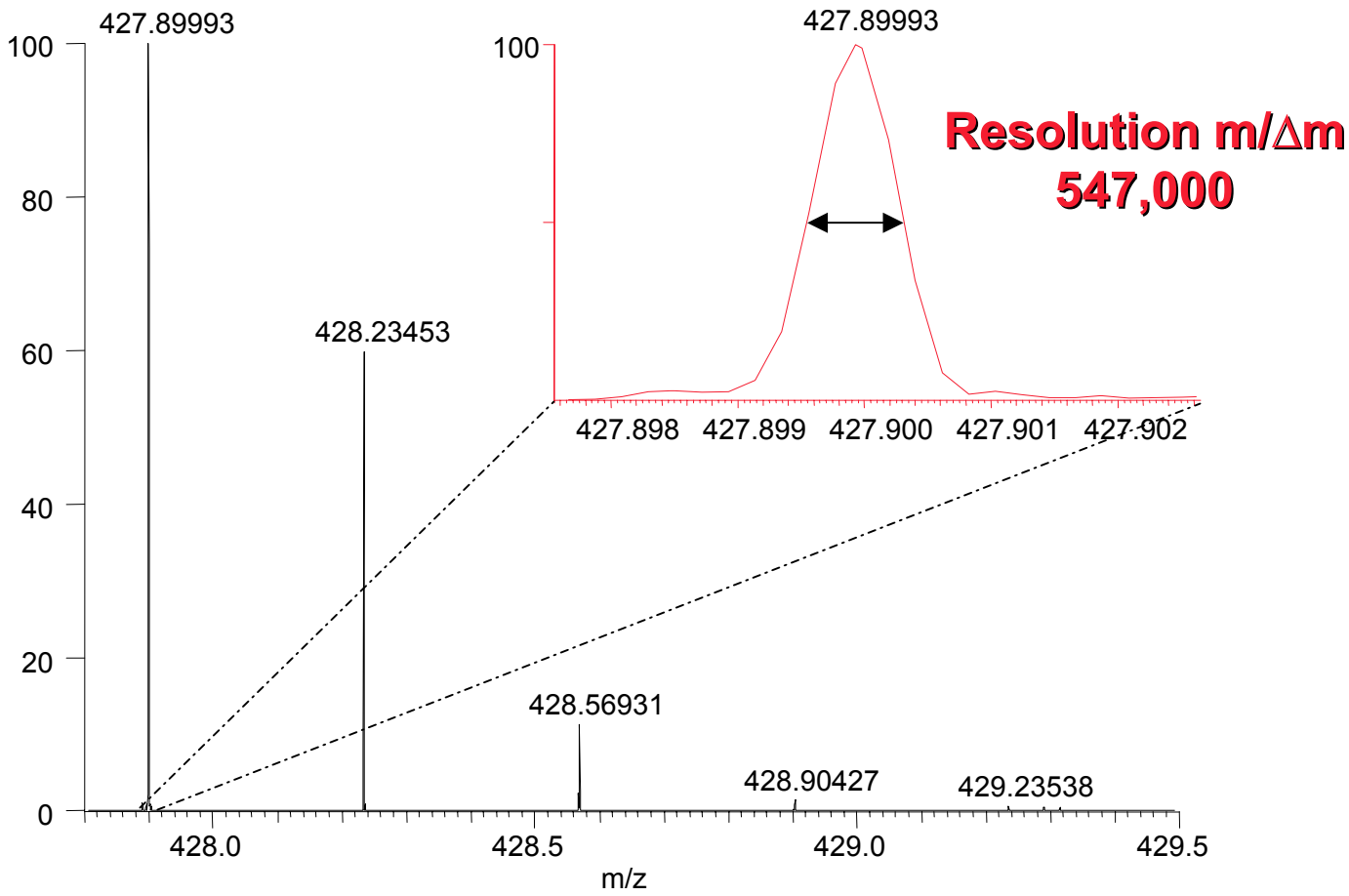
This is equivalent to

Res. Power at at m/z 400:
118,000 (FWHM)



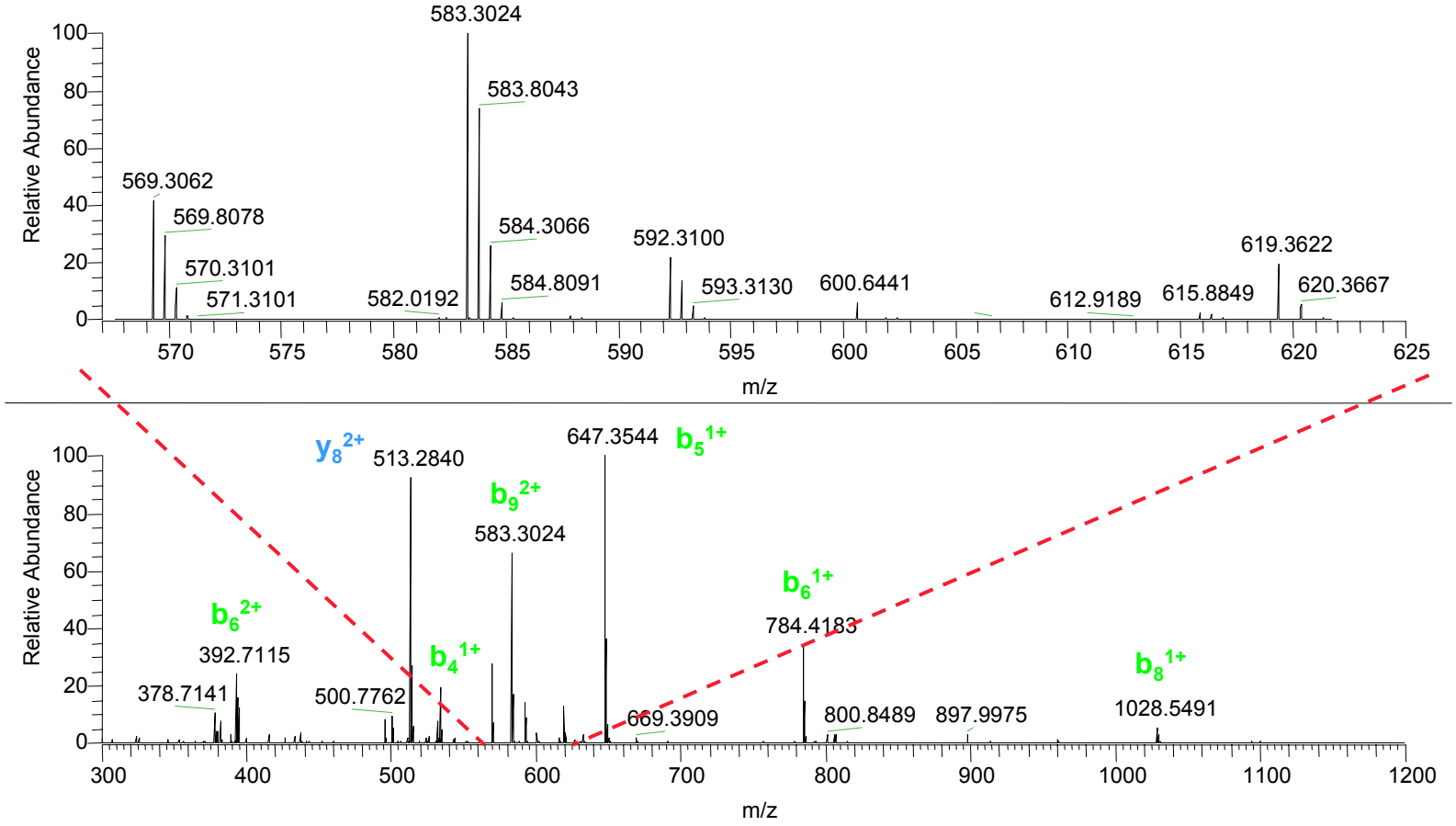
Finnigan LTQ FT – Ultra High Resolution

NRVYVHPFHL (3+) 427.89988 (Angiotensine from Goosefish)



Finnigan LTQ FT – MS/MS Analysis

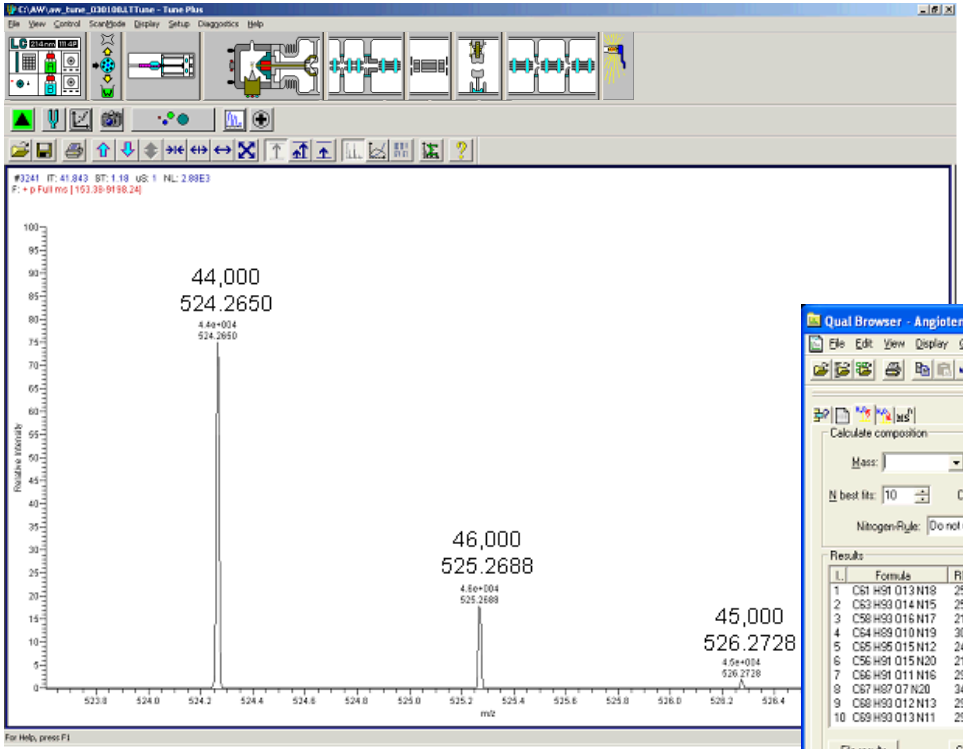
MS/MS Angiotensin I DRVYIHPFHL m/z 432.9 (3+), single Scan



Finnigan LTQ FT – Productive Software

- Known and Accepted Software Platform
  - *LCQ TUNE PLUS enhanced for FTICR analysis*
  - *Data dependent scanning*
  - *Xcalibur enhanced for accurate mass*
  - *Bioworks*
  - *DeNovoX*
  - *Metabolite ID*
  - *Mass Frontier*

Xcalibur™ Software

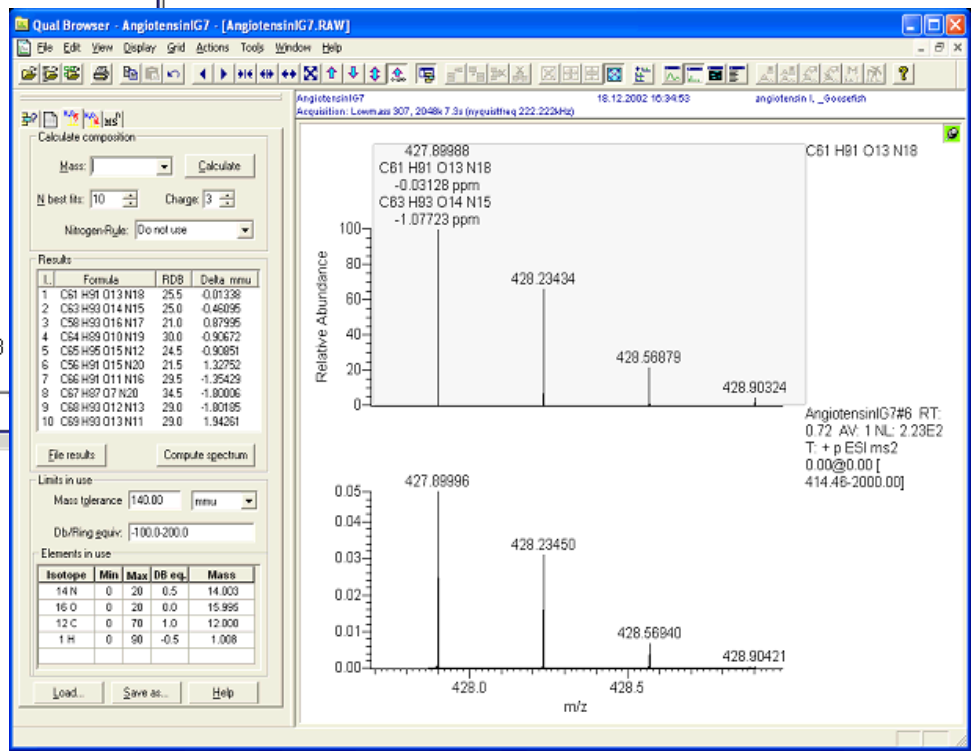


TunePlus

- Simple to use
- Intuitive GUI

Xcalibur 1.4

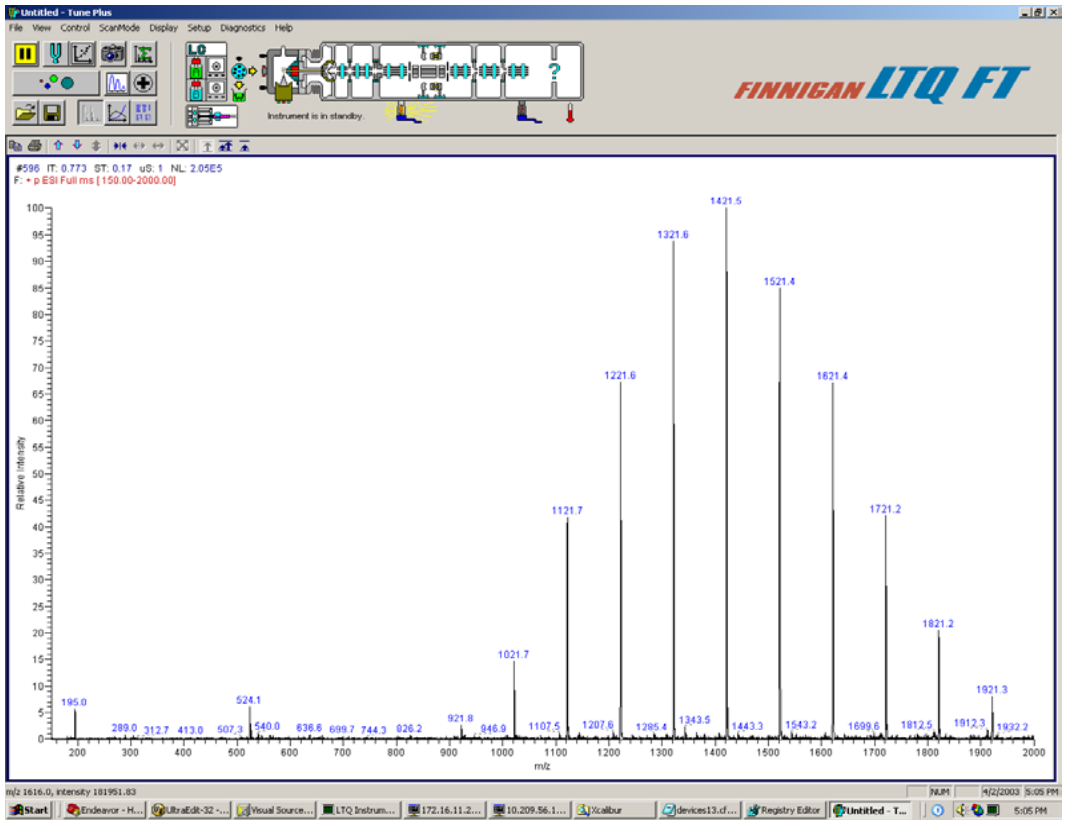
- Extended functions for high resolution and accurate mass



Finnigan LTQ FT – Productive Software

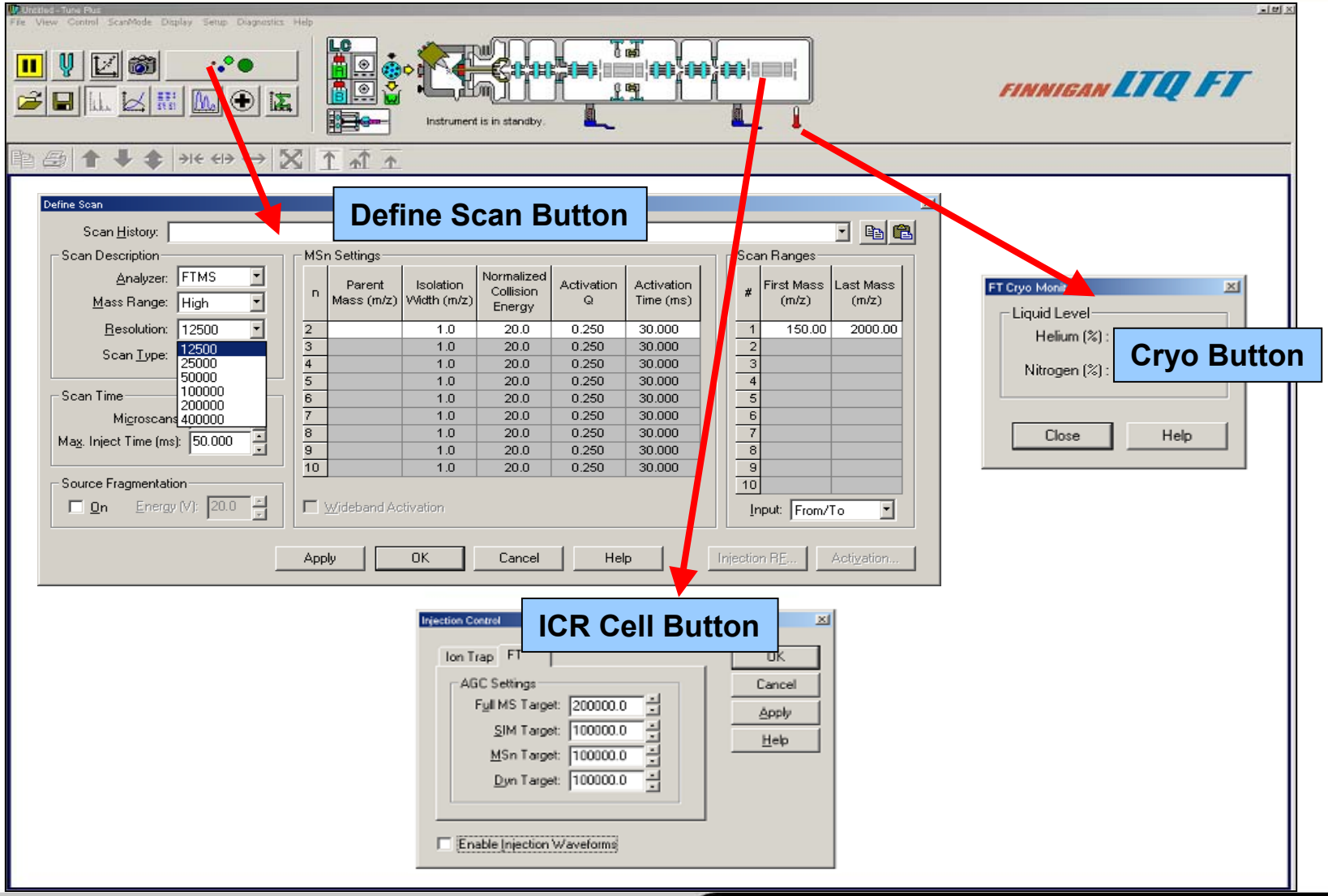
Known Platform

- LCQ TUNE PLUS, enhanced for FTICR analysis
- Method Editor, with data dependent scanning
- Xcalibur
- Bioworks, DeNovoX, Metabolite ID, Mass Frontier
- Accurate Mass Data Evaluation Software

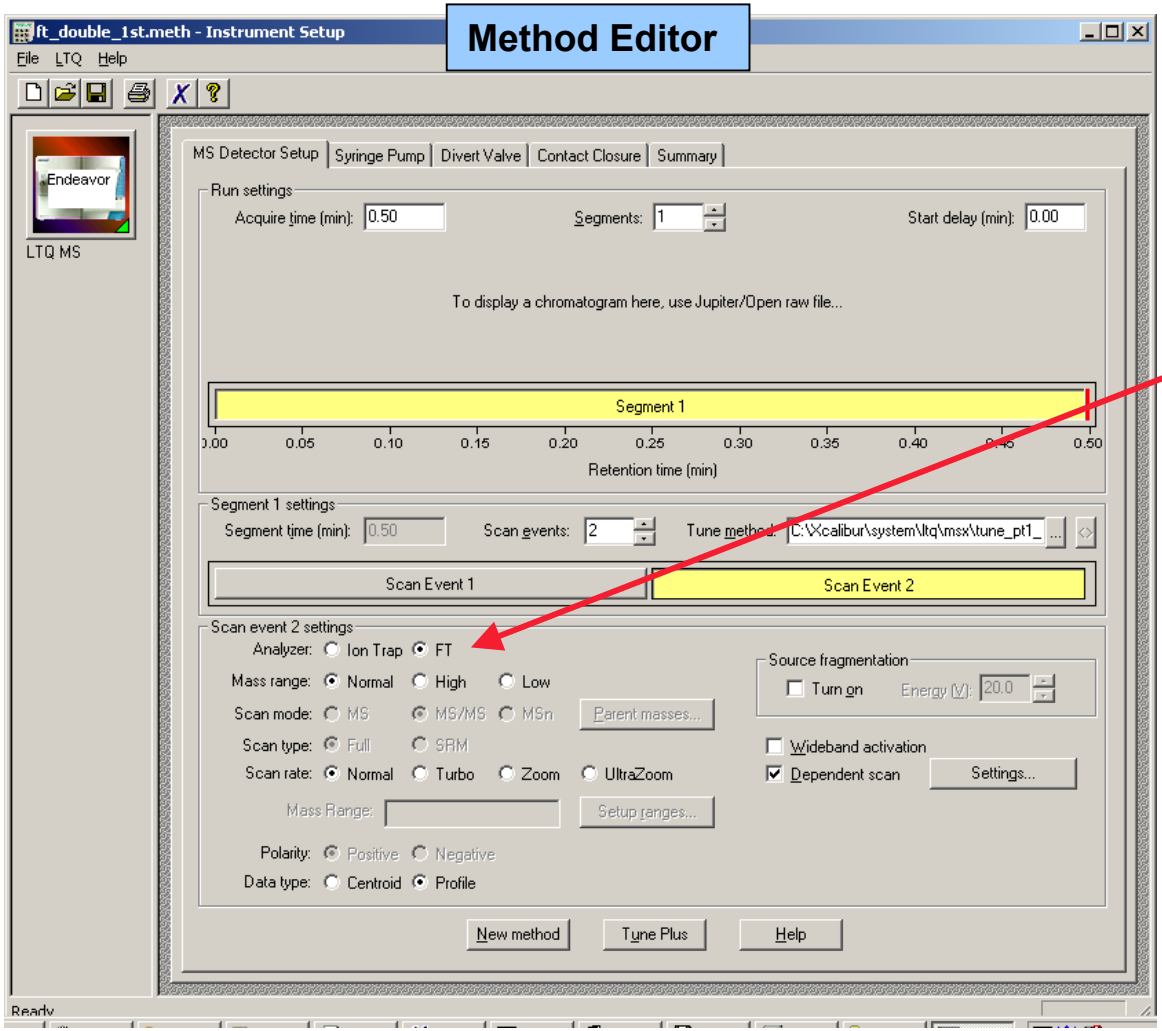


If you can run a Finnigan LCQ you can run an LTQ FT

LTQ FT – Tune Plus Window

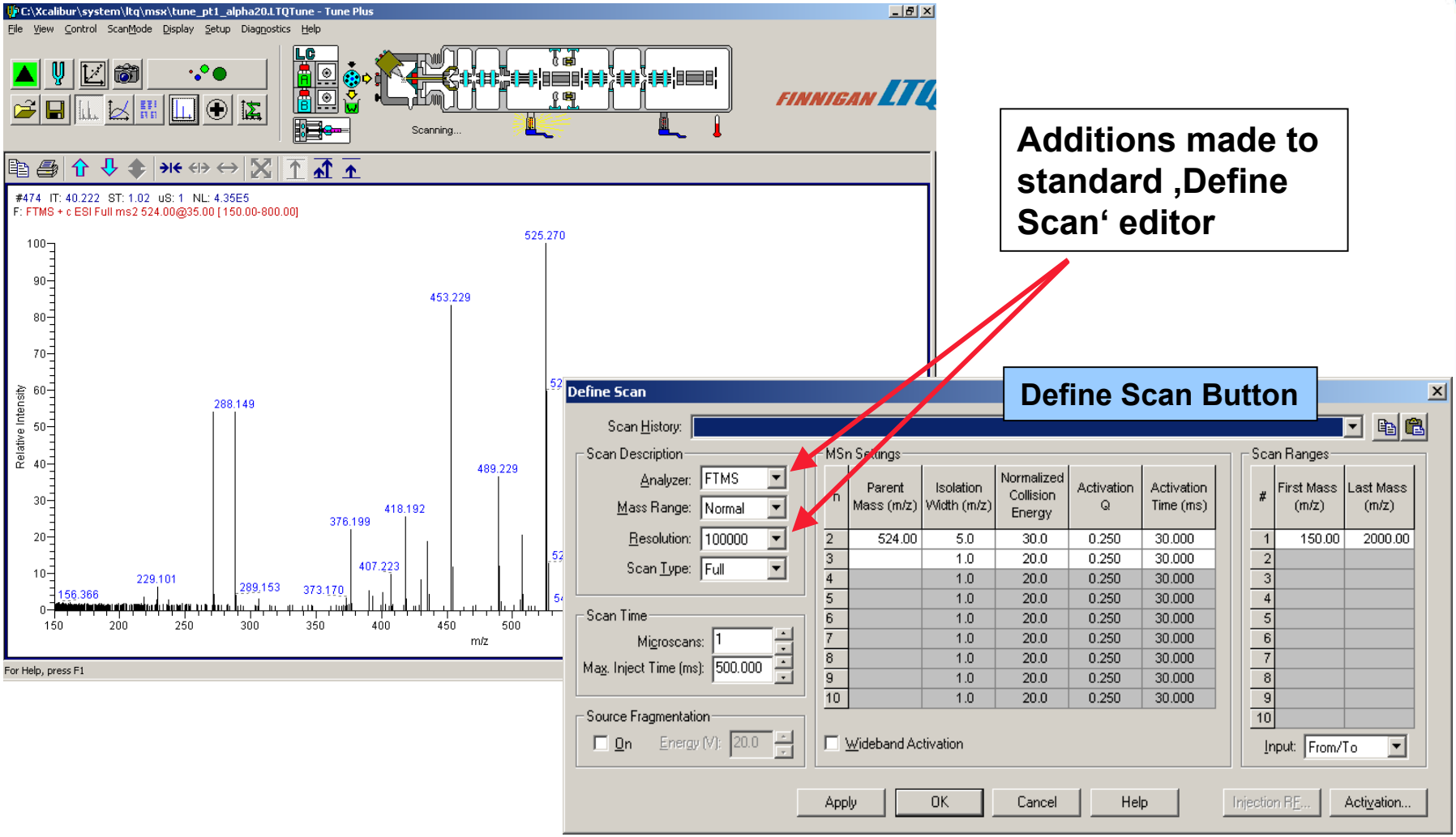


Method Setup for Data Dependant Scanning



Additions made to standard method editor

MS/MS of MRFA – Scan Setup and Tune Plus



Finnigan LTQ FT – Comparison

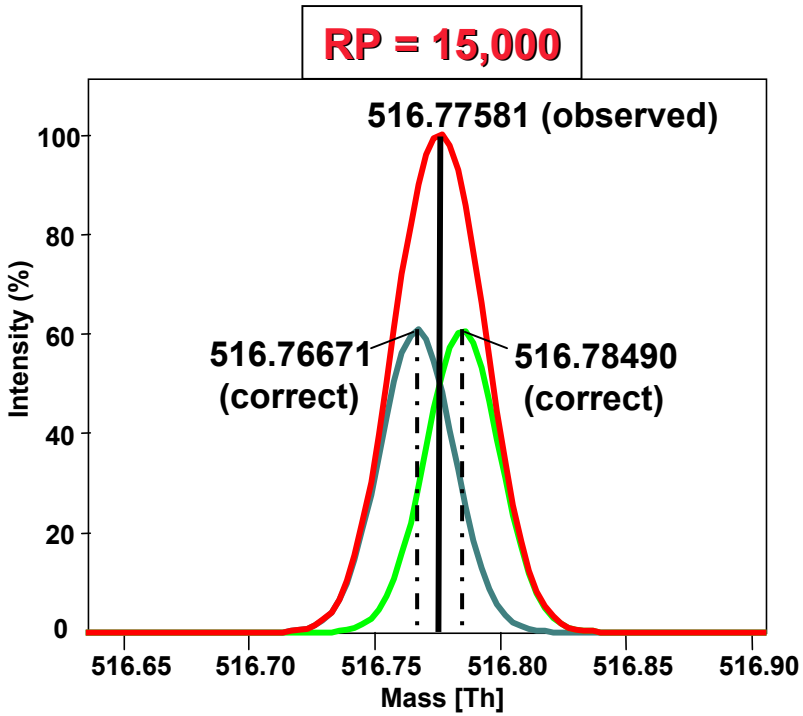
- Unlike Other FTICR Instruments…
  - *no trapping gas is pulsed into the ultra-high vacuum region to trap ions in the ICR cell*
    - don't need to wait several seconds to pump away gas before detecting ICR signal
  - *no collision gas is pulsed into the ICR cell for MS/MS*
    - MS/MS performed in 2-D Linear Trap
    - again, don't need to wait to pump away gas prior to detection
  - *minimized transmission discrimination*
    - short path length between 2-D Linear Trap and ICR cell and octopole ion optics, reduces mass discrimination
  - *ultra High Speed Signal Processing*
    - Fourier Transformation performed in real time

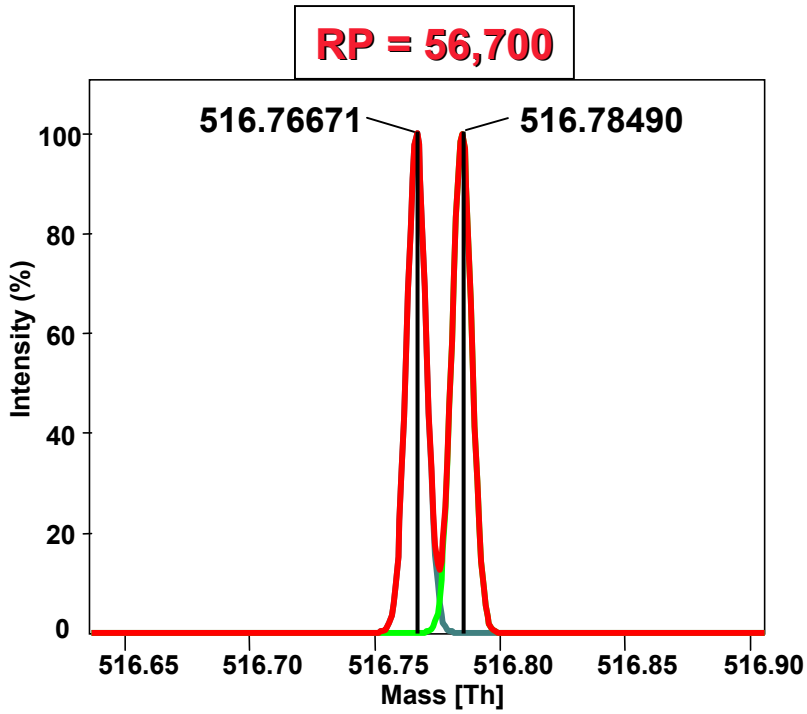
Finnigan LTQ FT – What About ?

- High Resolution Parent Ion Isolation?
  - *possible in ICR cell with SWIFT isolation*
- Collision Activated Dissociation in the ICR Cell?
  - *need pulsed gas inlet. Not planned*
  - *MS/MS performed in 2-D Linear Trap*
- IRMPD?
  - *possible, and in progress*
- ECD?
  - *possible, and in progress*

High Resolution and Accurate Mass

Peptides:	**[Val^5]-Angiotensin II**	**Lys-des-Arg^9-Bradykinin**
Sequence:	**DRVYVHPF**	**KRPPGFSPF**
Formula:	**$C_{49}H_{69}N_{13}O_{12}$**	**$C_{50}H_{73}N_{13}O_{11}$**
Exact mass:	**$[M+2H]^{2+}$ = 516.76671**	**$[M+2H]^{2+}$ = 516.78490**
Δm (mmu):		**18.2 mmu**
Δm (ppm):		**35 ppm**
Required Resolution:		**56,700 (FWHM)**





Mass Error and Elemental Composition

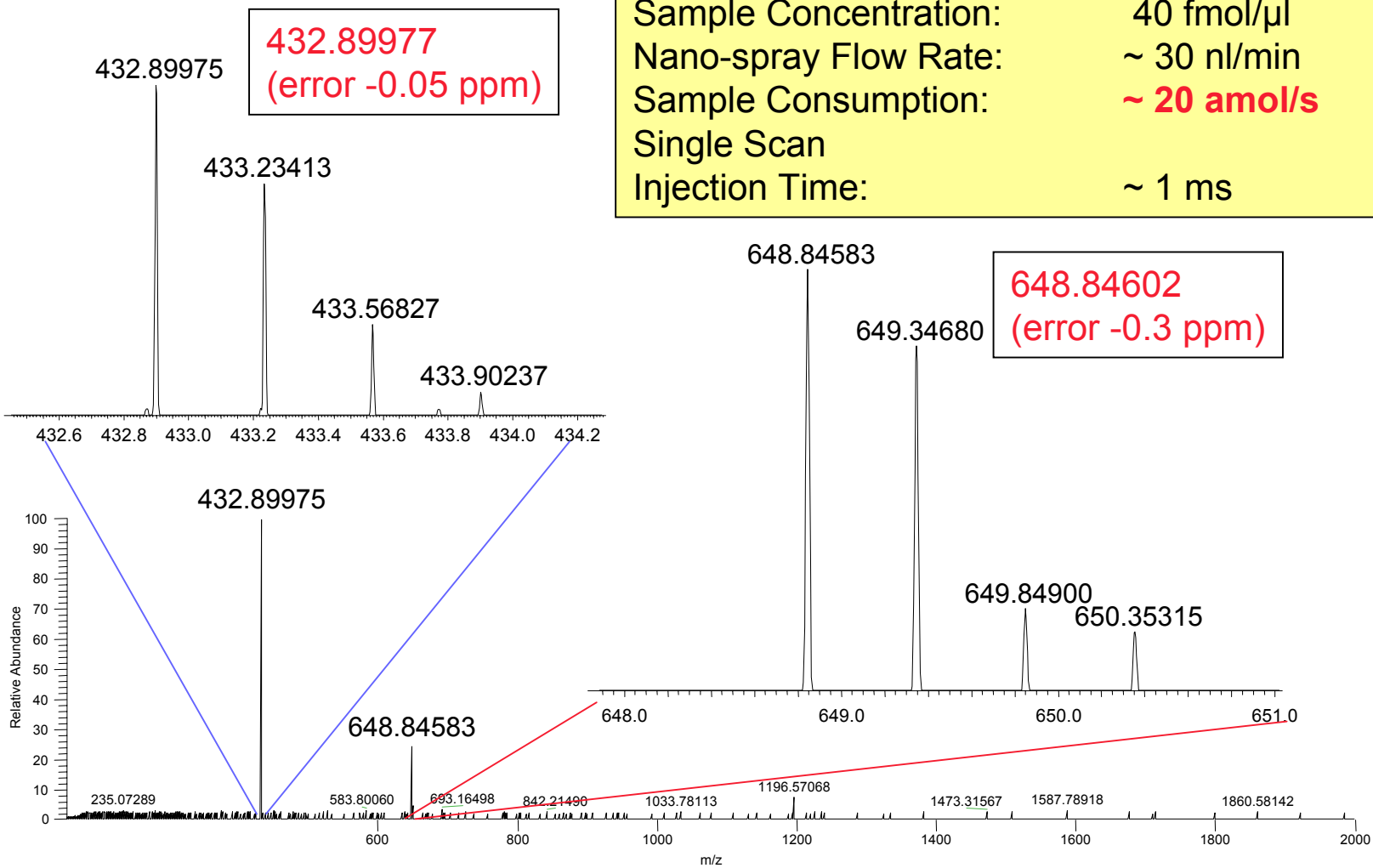
Entering the exact mass of a peptide into a program to calculate the elemental composition will yield different numbers of proposals. These numbers only depend on the maximum allowed mass errors and limits for the elements in use. The below listed limits of elements is for an average peptide composed of common amino acids. The mass error differs with instrument type and is not directly related to the resolution if a single compound is analyzed.

Example Peptide: [Val^5]-Angiotensin II, $[M+2H]^{2+}$ = 516.77671
Limitation for Elements: C 35-70, H 45-100, N 8-16, O 9-16, S 0-2

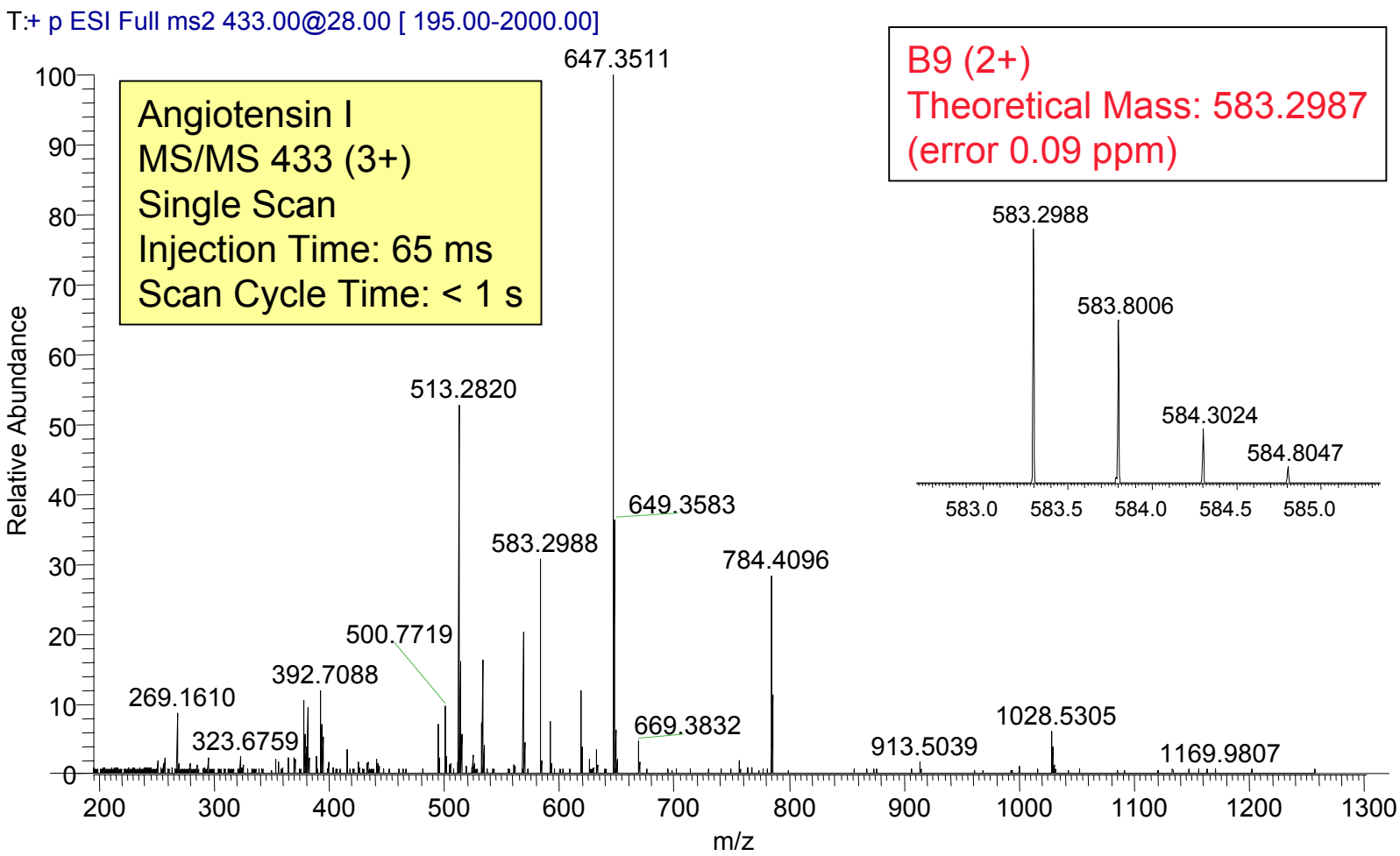
Instrument			QqTOF	LTQ-FT	LTQ-FT
Mass Error		10 ppm	5 ppm	2 ppm	1 ppm
# of Proposals for m/z 516.76671		49	23	10	4

Finnigan LTQ FT – Angiotensin I

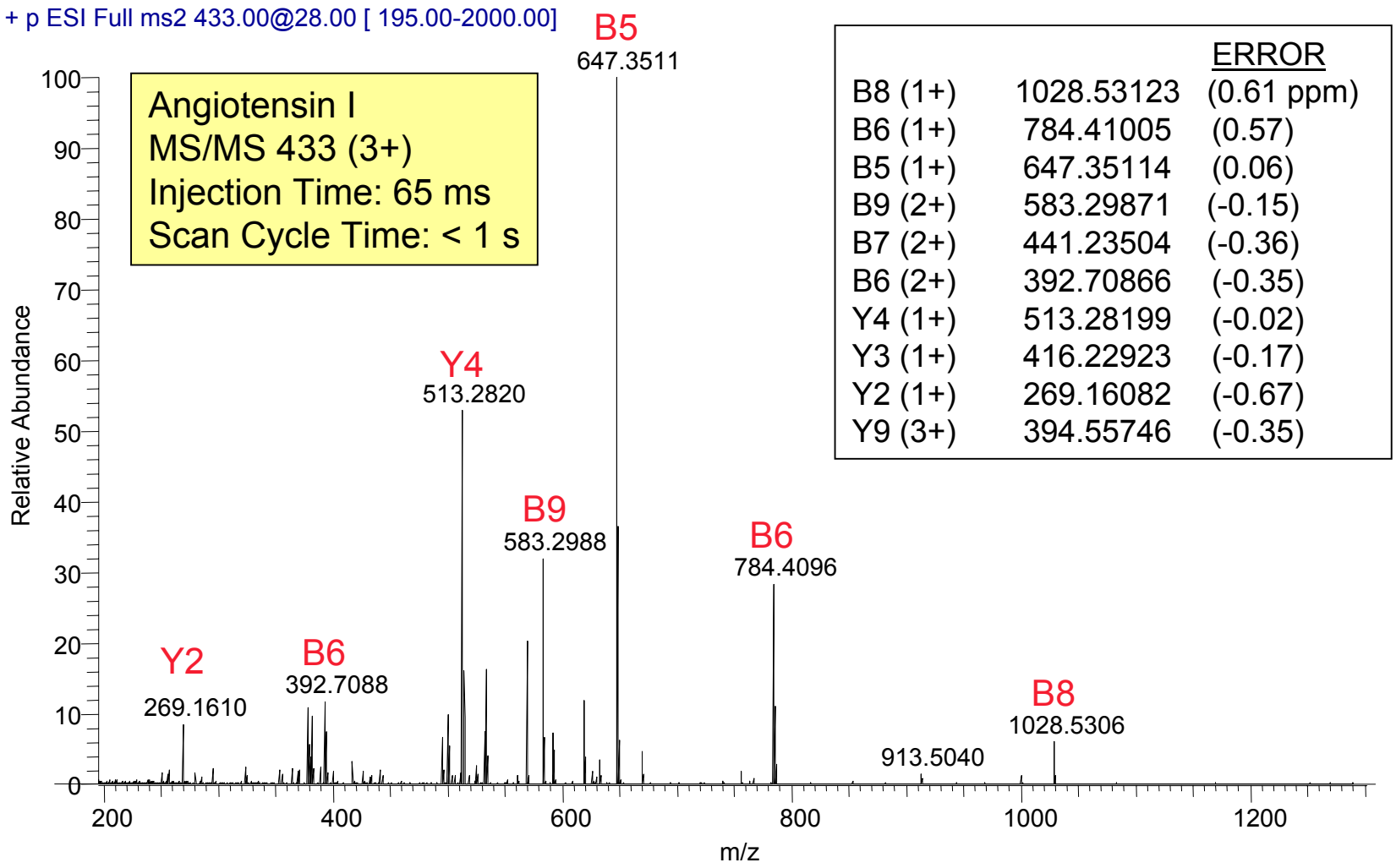
Sample Concentration:	40 fmol/µl
Nano-spray Flow Rate:	~ 30 nl/min
Sample Consumption:	**~ 20 amol/s**
Single Scan	
Injection Time:	~ 1 ms



Finnigan LTQ FT – MS/MS Angiotensin I



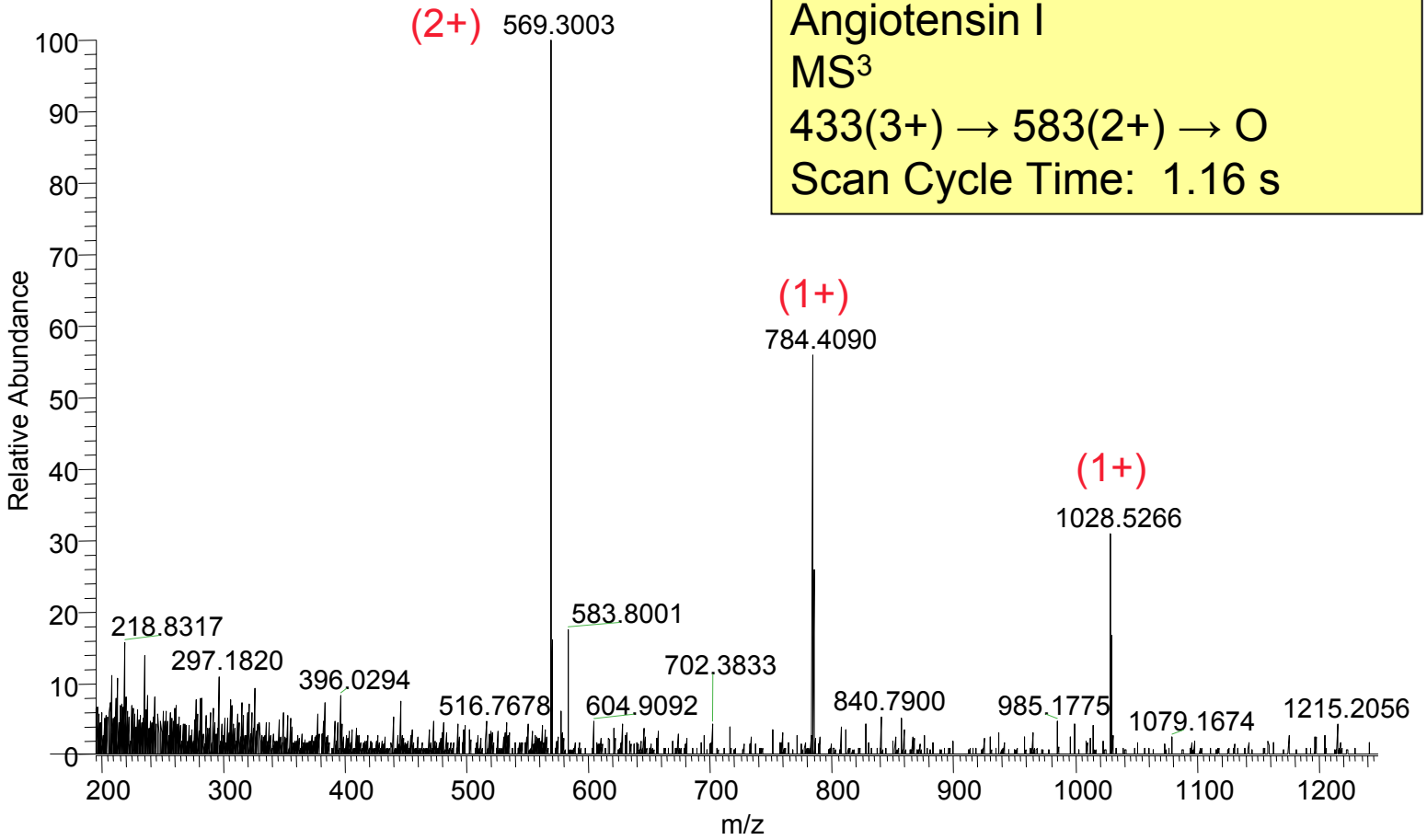
Finnigan LTQ FT – MS/MS Angiotensin I



		ERROR
B8 (1+)	1028.53123	(0.61 ppm)
B6 (1+)	784.41005	(0.57)
B5 (1+)	647.35114	(0.06)
B9 (2+)	583.29871	(-0.15)
B7 (2+)	441.23504	(-0.36)
B6 (2+)	392.70866	(-0.35)
Y4 (1+)	513.28199	(-0.02)
Y3 (1+)	416.22923	(-0.17)
Y2 (1+)	269.16082	(-0.67)
Y9 (3+)	394.55746	(-0.35)

Finnigan LTQ FT – MS^3 Angiotensin I

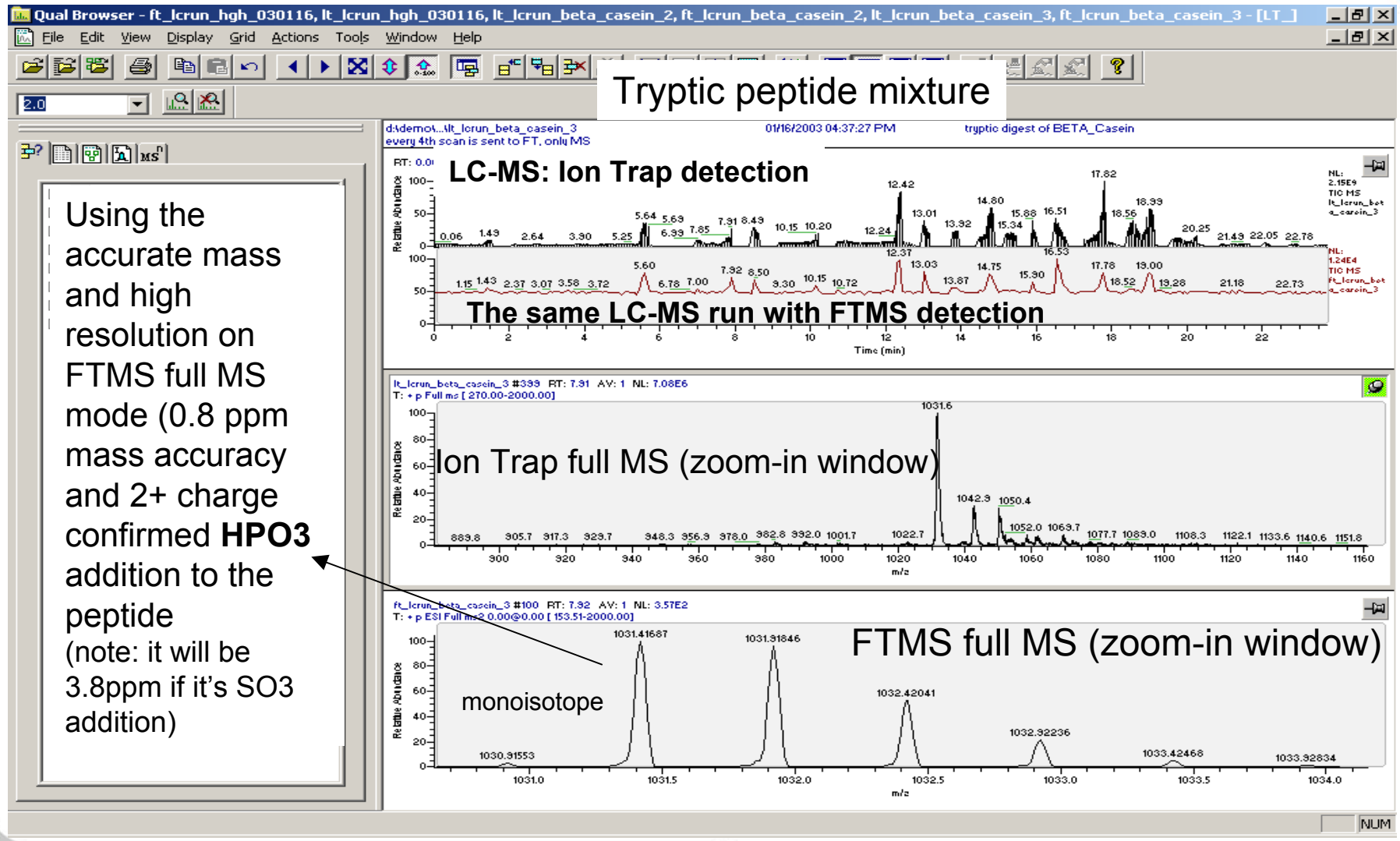
+ p ESI Full ms3 433.00@32.00 583.00@14.00 [195.00-2000.00]



Angiotensin I
MS^3
433(3+) → 583(2+) → O
Scan Cycle Time: 1.16 s

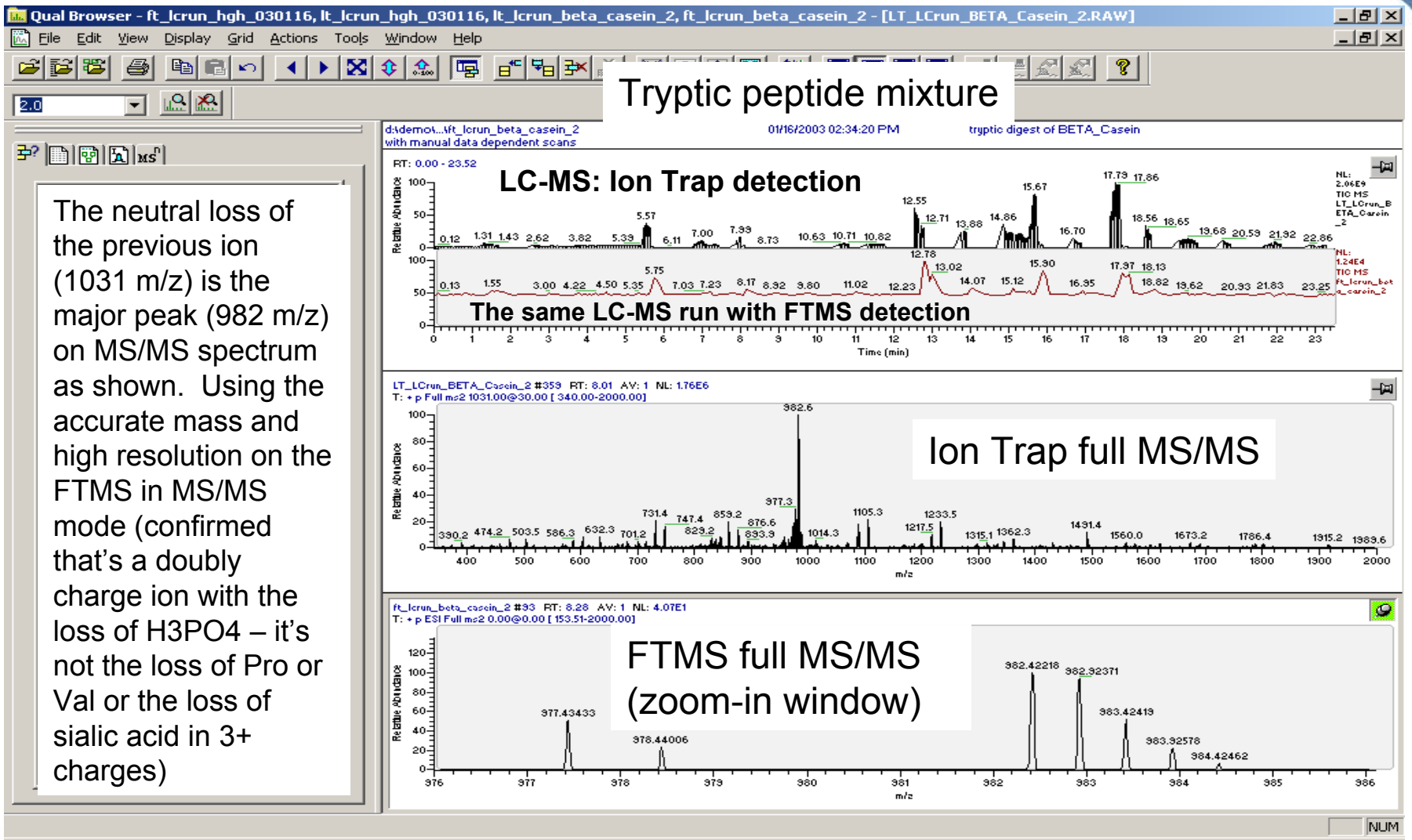
Using the two MS detectors (Linear ion trap and FTMS) for post-translational modification assignment

Using the two MS detectors to pinpoint the PTM – phosphopeptides



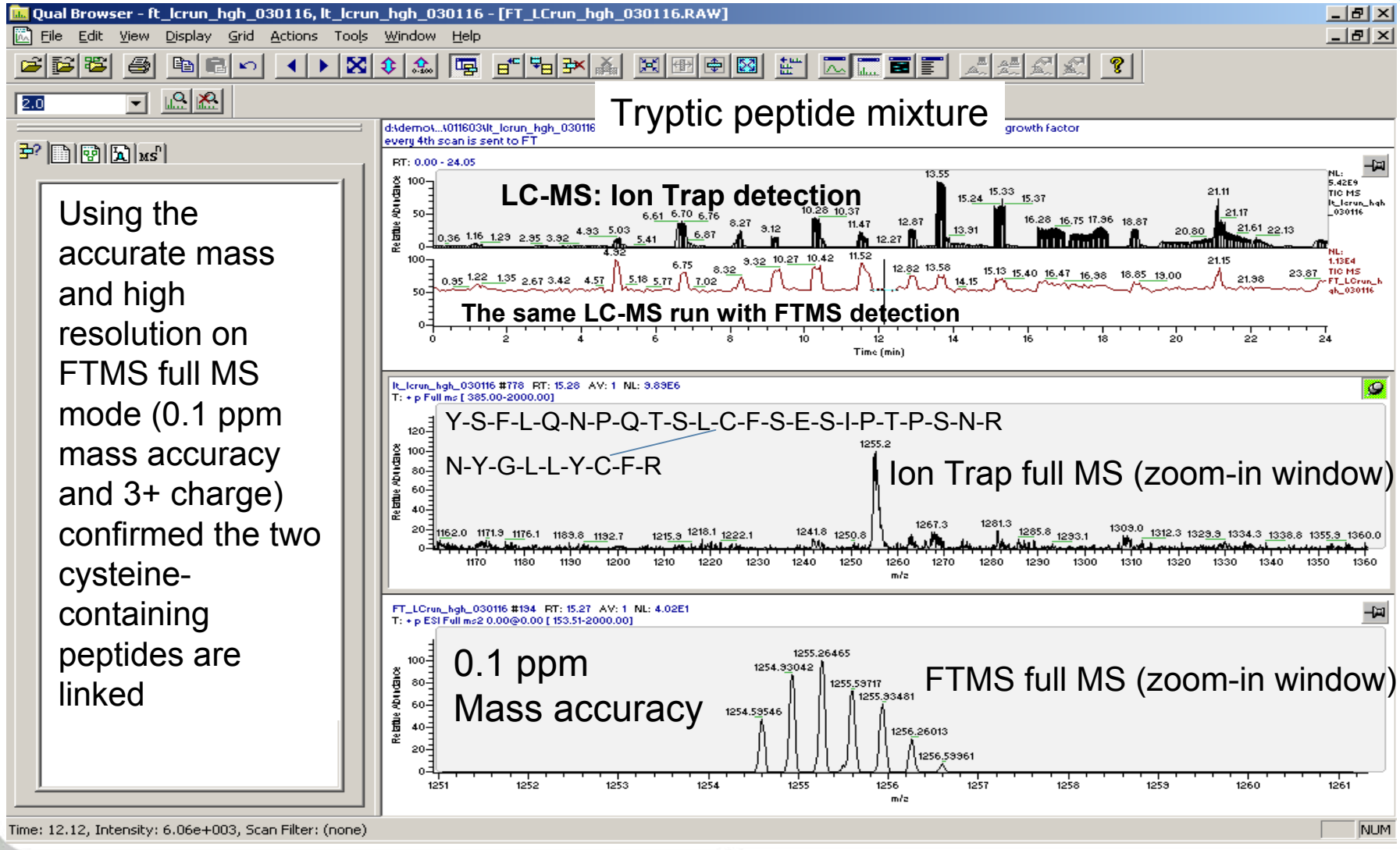
Using the accurate mass and high resolution on FTMS full MS mode (0.8 ppm mass accuracy and 2+ charge confirmed **HPO3** addition to the peptide
(note: it will be 3.8ppm if it's SO3 addition)

Using the two MS detectors to pinpoint the PTM – phosphopeptides



The neutral loss of the previous ion (1031 m/z) is the major peak (982 m/z) on MS/MS spectrum as shown. Using the accurate mass and high resolution on the FTMS in MS/MS mode (confirmed that's a doubly charge ion with the loss of H3PO4 – it's not the loss of Pro or Val or the loss of sialic acid in 3+ charges)

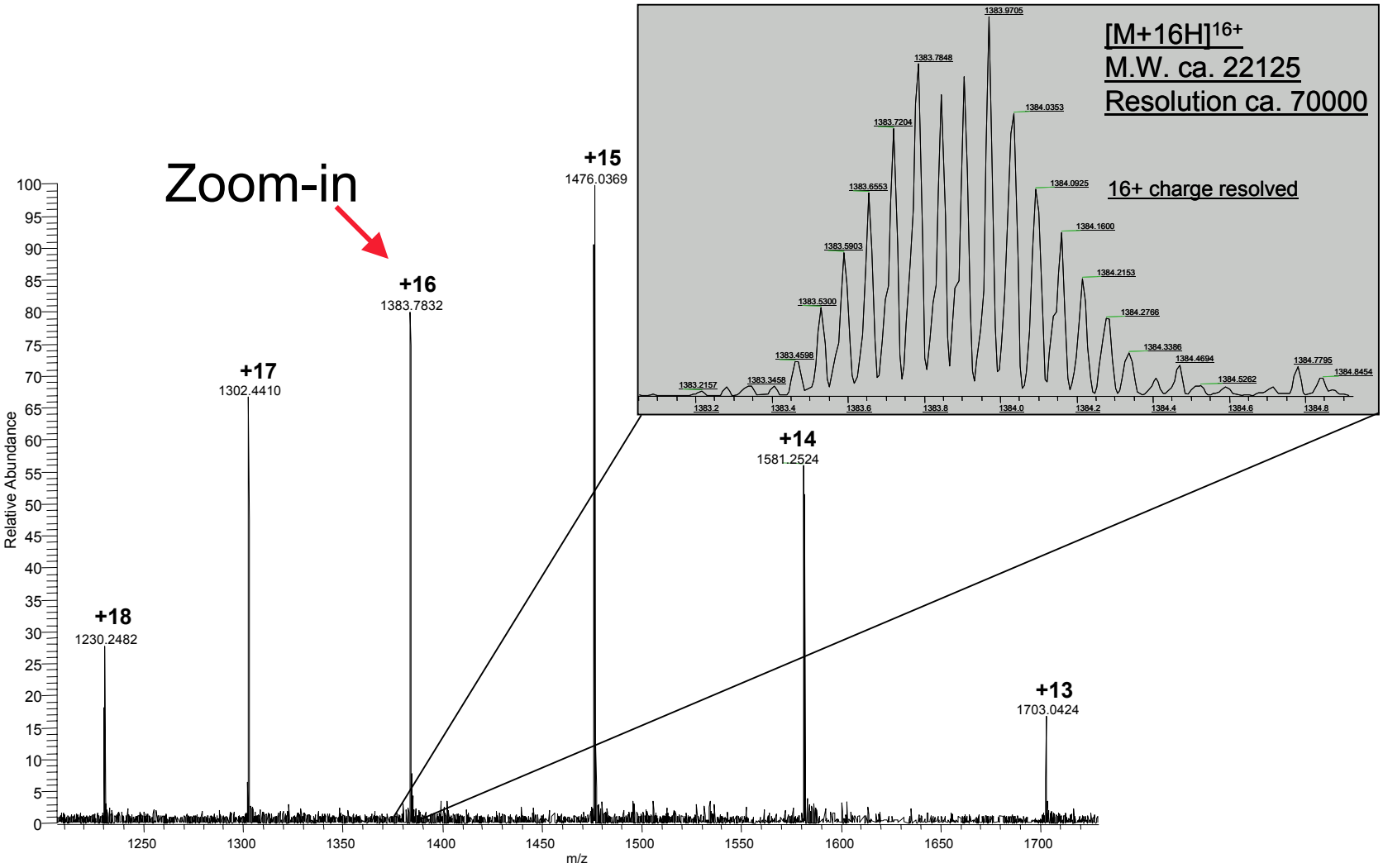
Using the two MS detectors to pinpoint the PTM – disulfide-linked peptides



Using the accurate mass and high resolution on FTMS full MS mode (0.1 ppm mass accuracy and 3+ charge) confirmed the two cysteine-containing peptides are linked

Using the two MS detectors (Linear ion trap and FTMS) for intact protein assignment

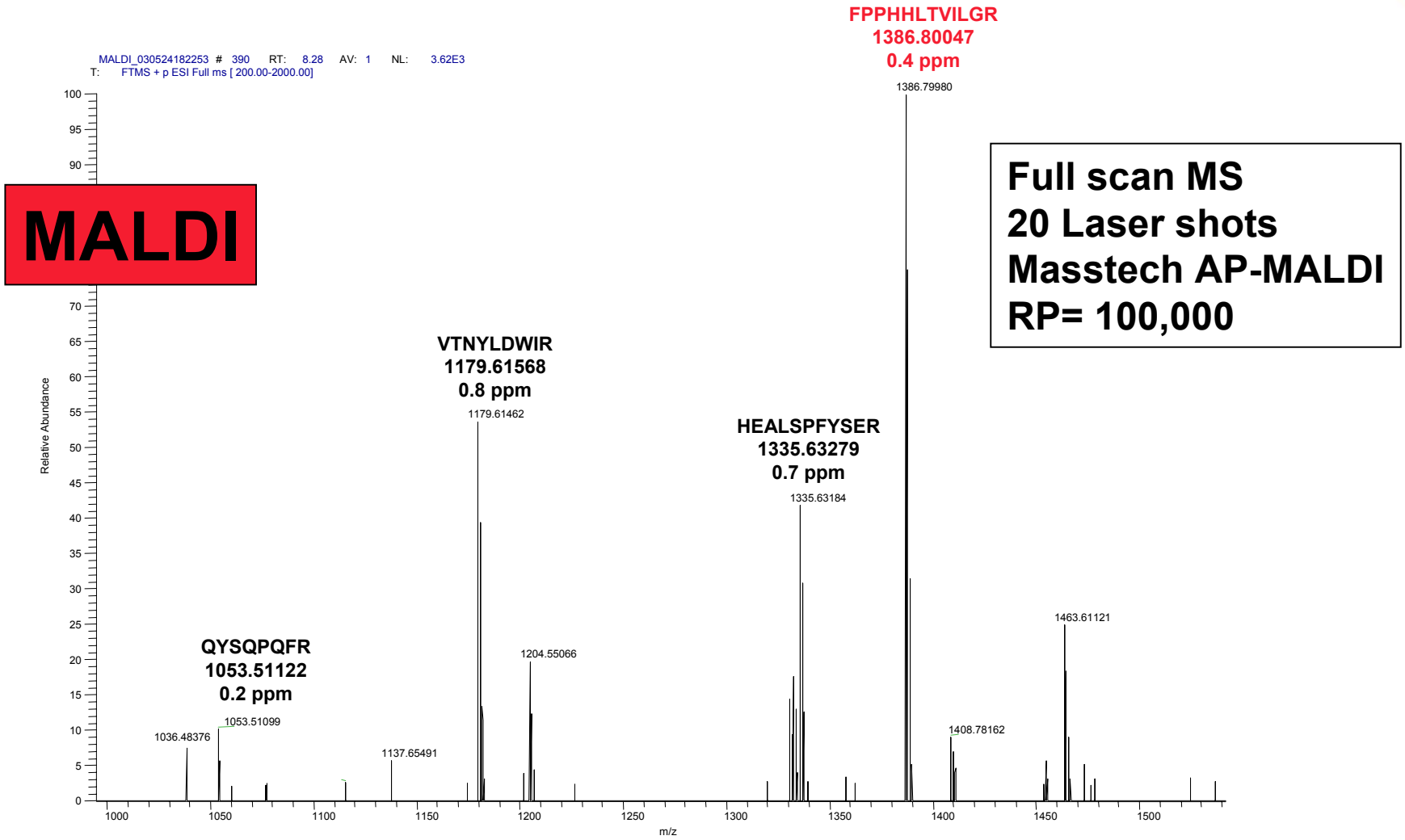
Human Growth Hormon (intact): FTMS Spectrum



Finnigan LTQ FT

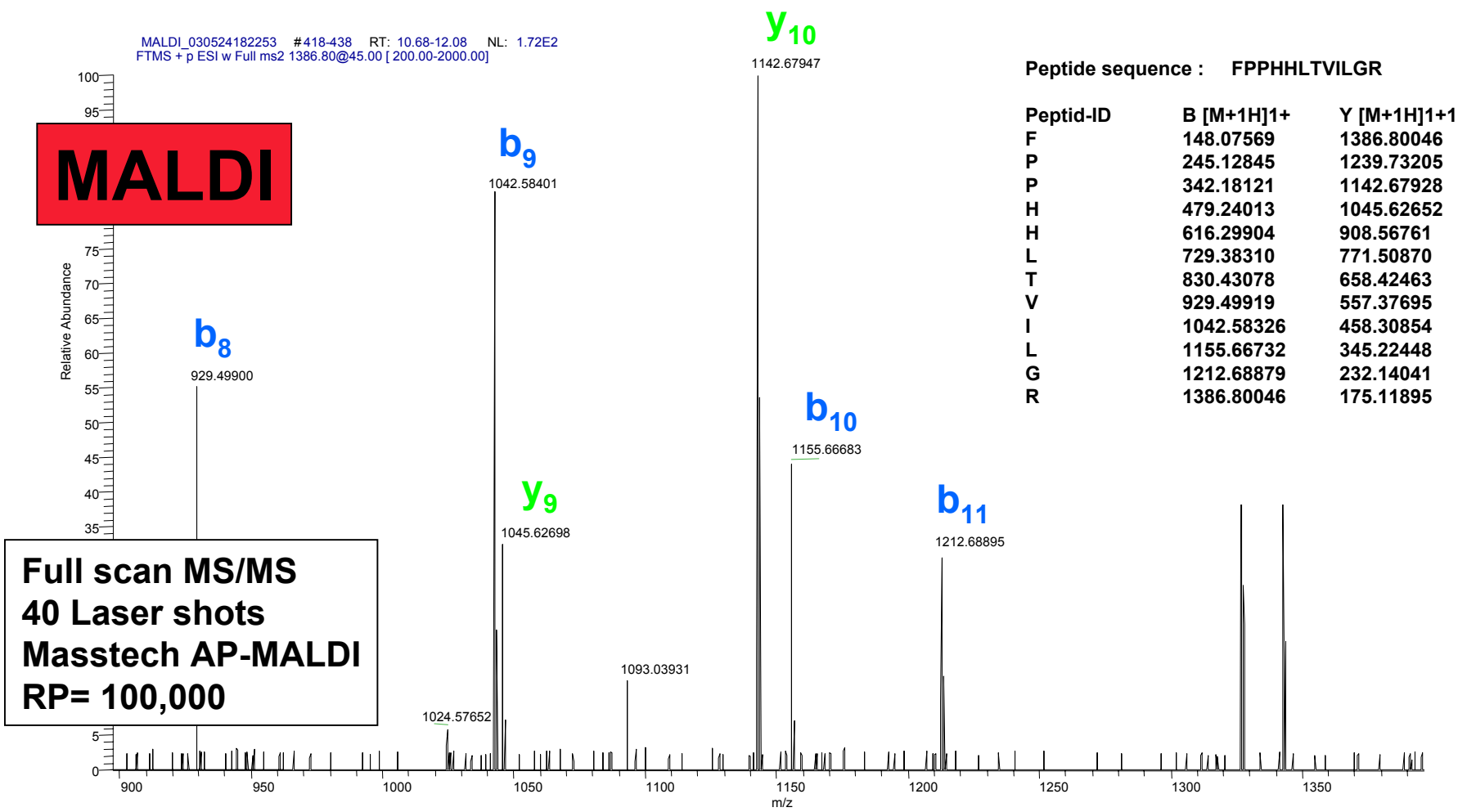
Recent Results

AP-MALDI on the Finnigan LTQ FT



Full scan MS
20 Laser shots
Masstech AP-MALDI
RP= 100,000

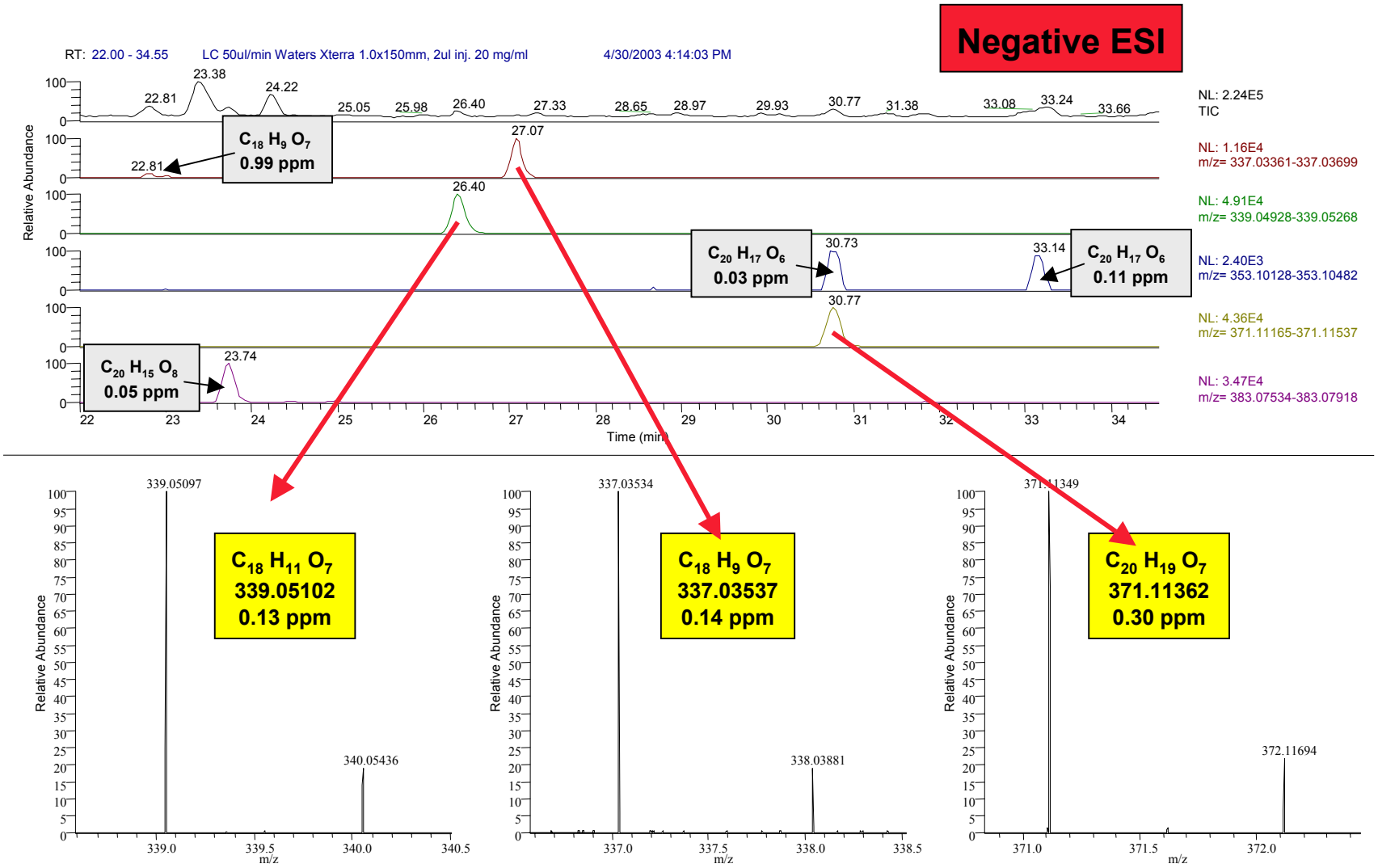
AP-MALDI MS/MS with the Finnigan LTQ FT



Peptide sequence : FPPHHLTVILGR

Peptid-ID	B [M+1H]1+	Y [M+1H]1+1
F	148.07569	1386.80046
P	245.12845	1239.73205
P	342.18121	1142.67928
H	479.24013	1045.62652
H	616.29904	908.56761
L	729.38310	771.50870
T	830.43078	658.42463
V	929.49919	557.37695
I	1042.58326	458.30854
L	1155.66732	345.22448
G	1212.68879	232.14041
R	1386.80046	175.11895

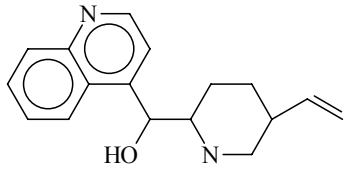
LC-MS Accurate Mass with the LTQ FT



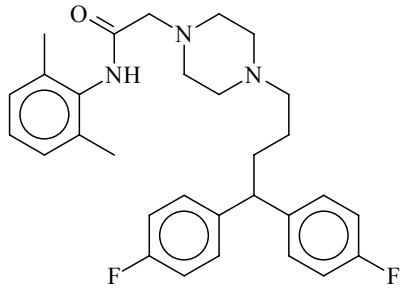
Accurate Mass for Small Molecules

On-line HPLC measurements of 4 different compounds were performed on the LTQ FT at a resolution power of 200,000. AGC was used to control the number of ions.

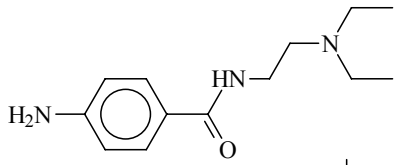
Cinchonidine			
Scan 55 to 155			
Accurate mass:		295.18049	
Aver:	295.1802		-0.91 ppm
Min:	295.1801		-1.24 ppm
Max:	295.1804		-0.21 ppm
Lidoflazine			
Scan 521 to 551			
Accurate mass:		492.28210	
Aver:	492.2816		-1.02 ppm
Min:	492.2813		-1.74 ppm
Max:	492.2820		-0.31 ppm
Procainamide			
Scan 55 to 155			
Accurate mass:		236.17574	
Aver:	236.1756		-0.51ppm
Min:	236.1755		-1.1 ppm
Max:	236.1757		0.0 ppm
Trimipramine			
Scan 480 to 630			
Accurate mass:		295.21688	
Aver:	295.2166		-0.97 ppm
Min:	295.2165		-1.34 ppm
Max:	295.2168		-0.31 ppm



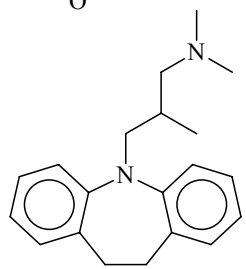
$[M+H] = C_{19} H_{23} N_2 O$
295.18049



$[M+H]^+ = C_{30} H_{36} F_2 N_3 O$
492.28210



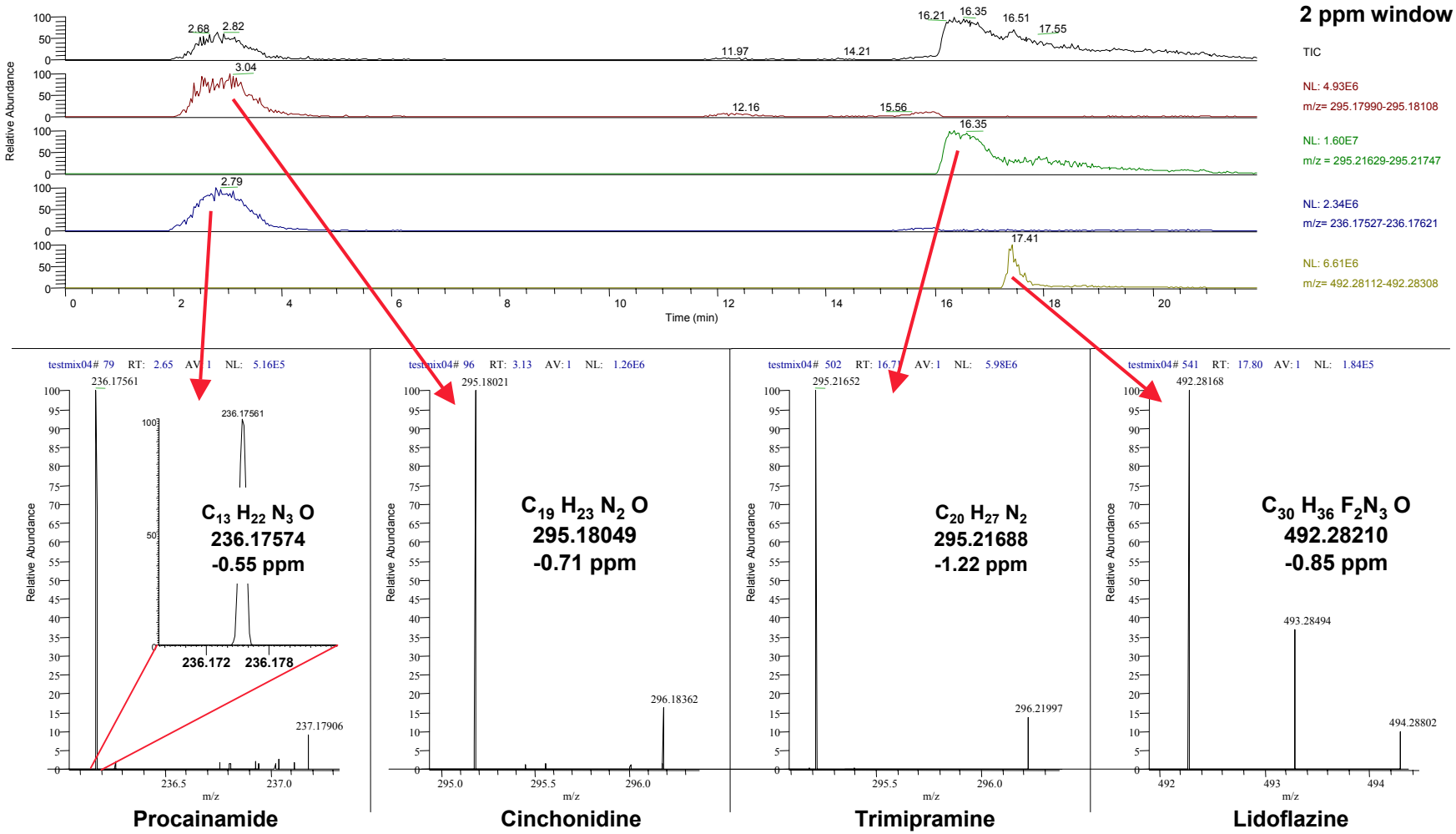
$[M+H]^+ = C_{13} H_{22} N_3 O$
236.17574



$[M+H]^+ = C_{20} H_{27} N_2$
295.21688

Accurate Mass for Small Molecules

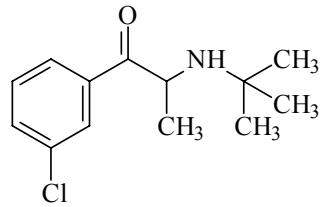
Resolution = 200,000

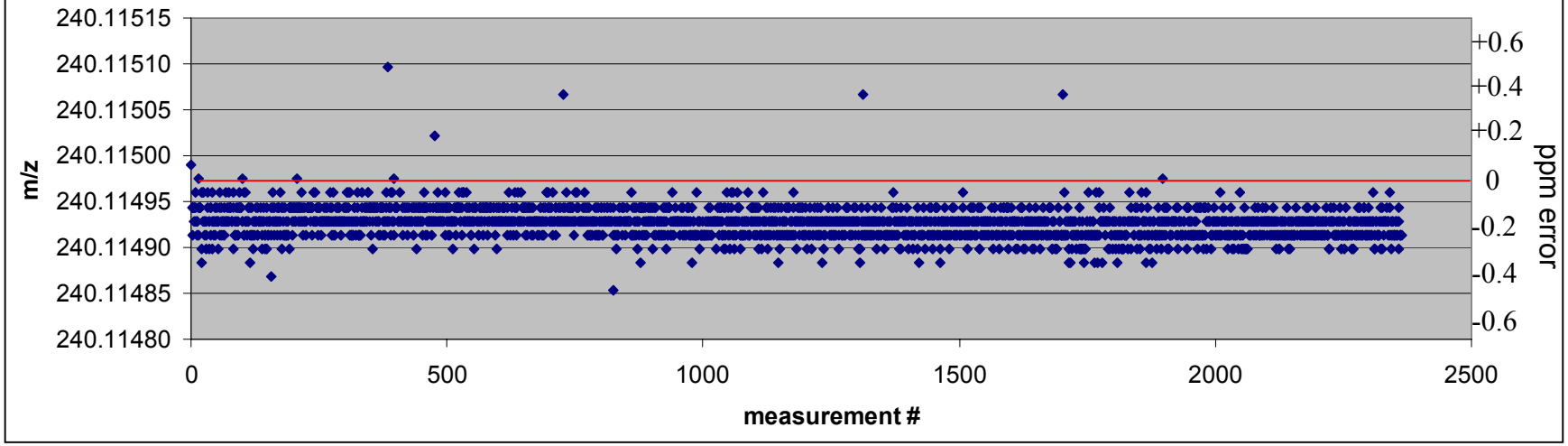


The Advion NanoMate 100 on the Finnigan LTQ FT

Mass Accuracy for Repetitive Measurements

Bupropion (BUP)
[M+H] = 240.11497
C_{13} H_{18} N O Cl

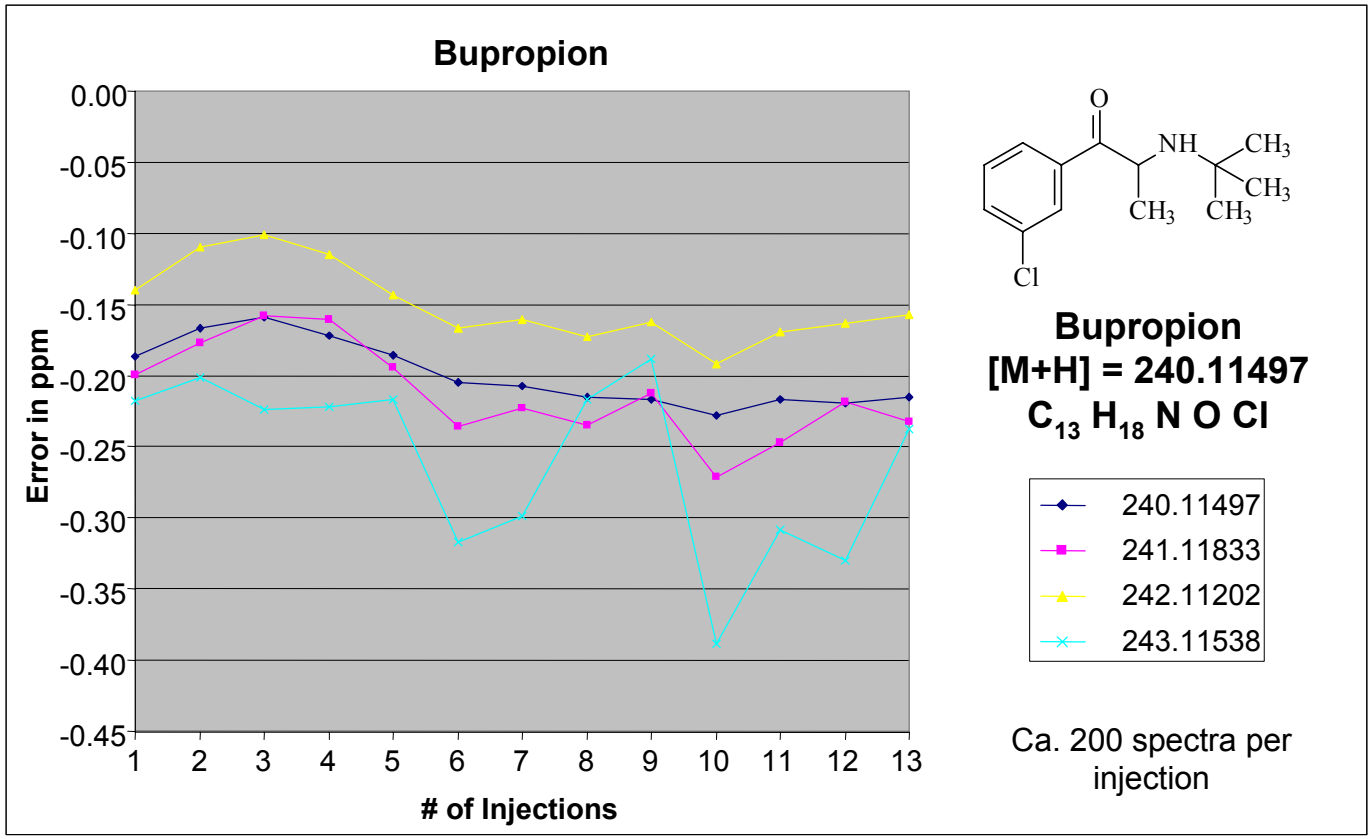




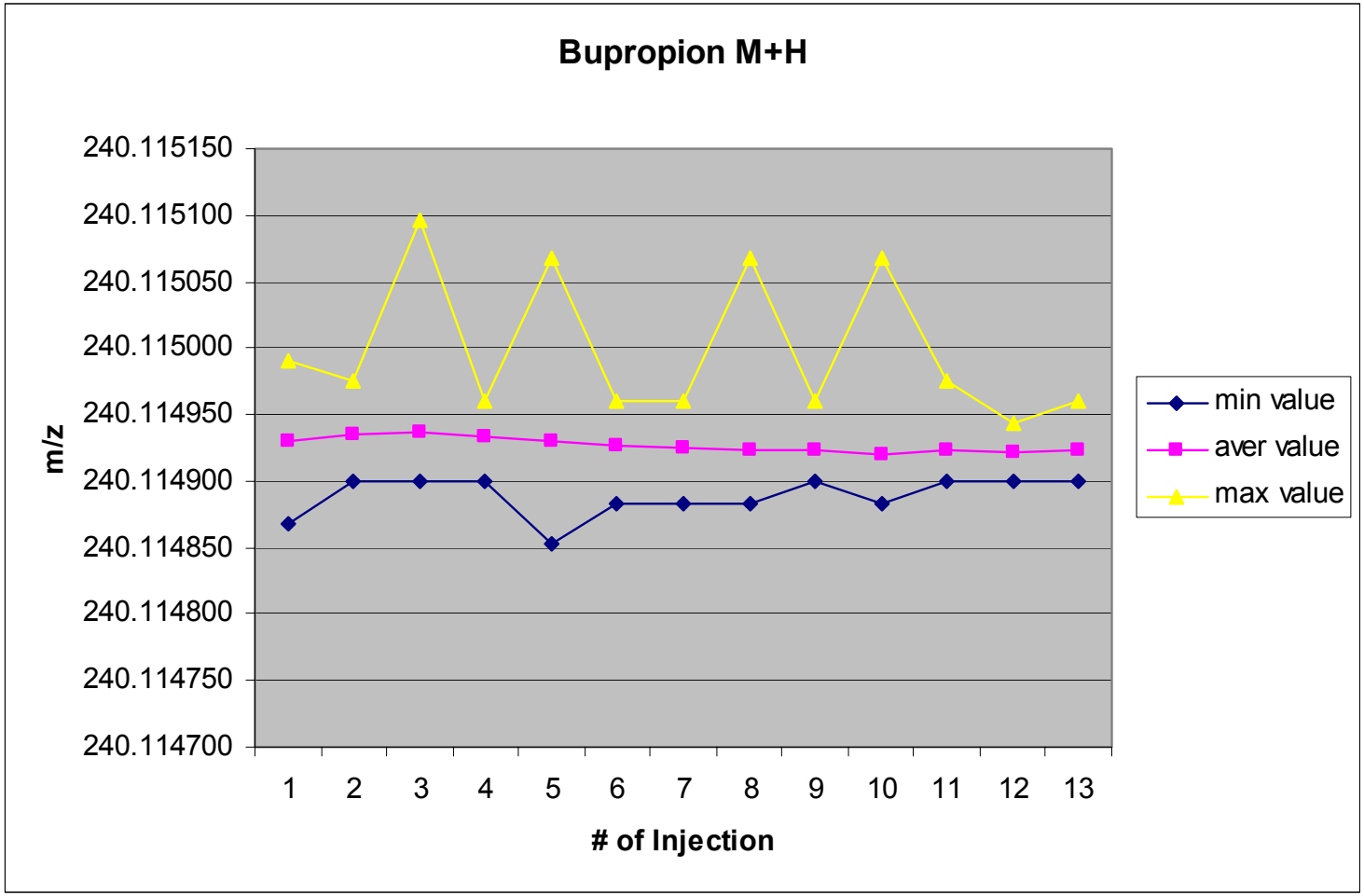
Mass accuracy for repetitive measurements of the MH^+ Peak of Bupropion on the LTQ FT with the NanoMate 100 attached . 13 injection were carried out giving the 2400 data points. Same results are obtained for 3 other small molecules, Threohydrobupropion, Hydroxybupropion (HB), D_6-BUP and D_6-HB. The red line indicates the theoretical mass. The average error is -0.20ppm.

Accurate Mass with the LTQ FT

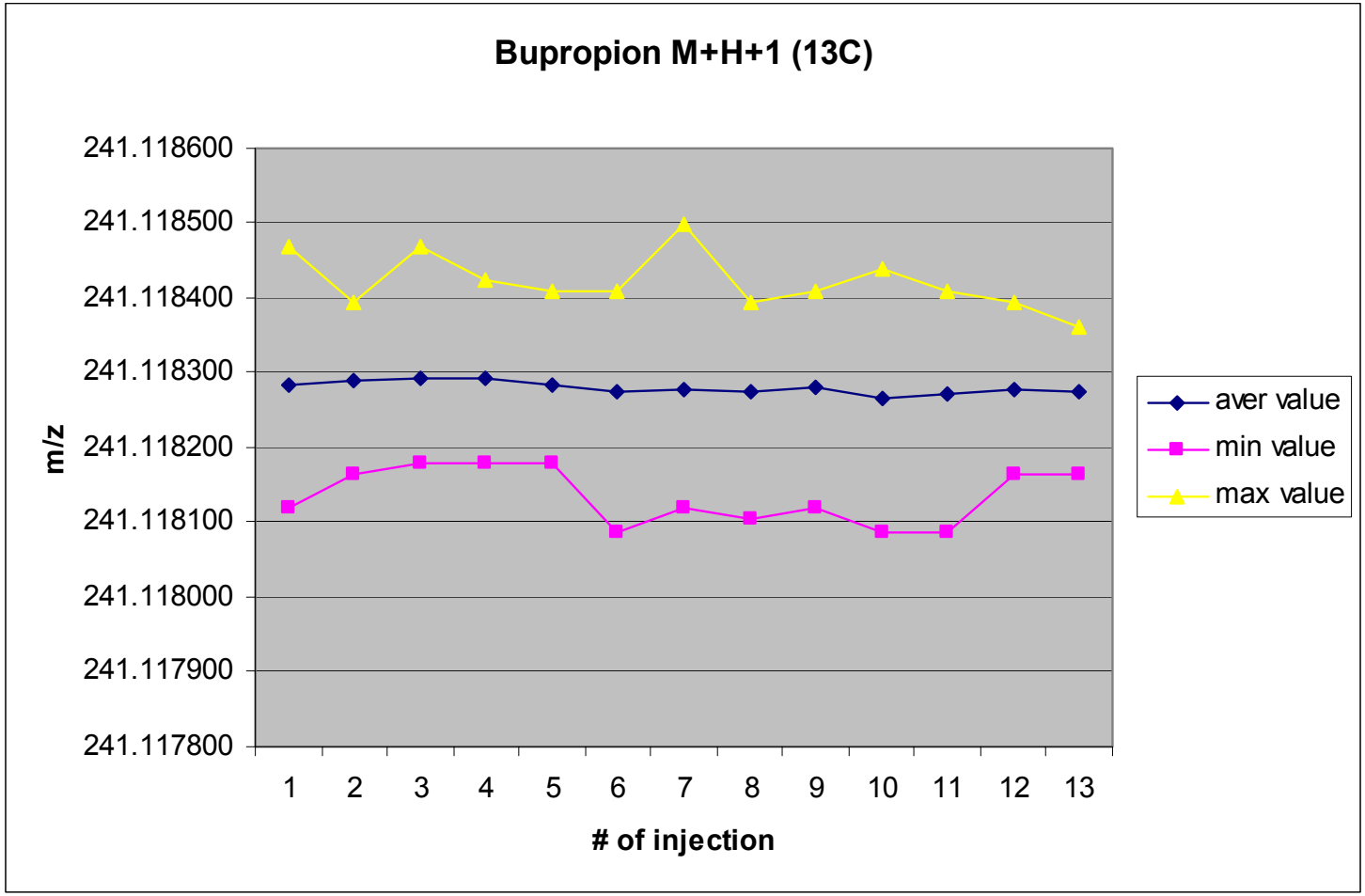
The combination of the Advion Nanomate system with the LTQ FT mass spectrometer was used to make 13 repetitive measurements of Bupropion. Statistics for the molecular ion and its most abundand isotope peaks are shown in the graph. For each injection the Nanomate performed a nanospray at ca. 200 nL/min. The complete set of data was acquired in less than 1 hour.



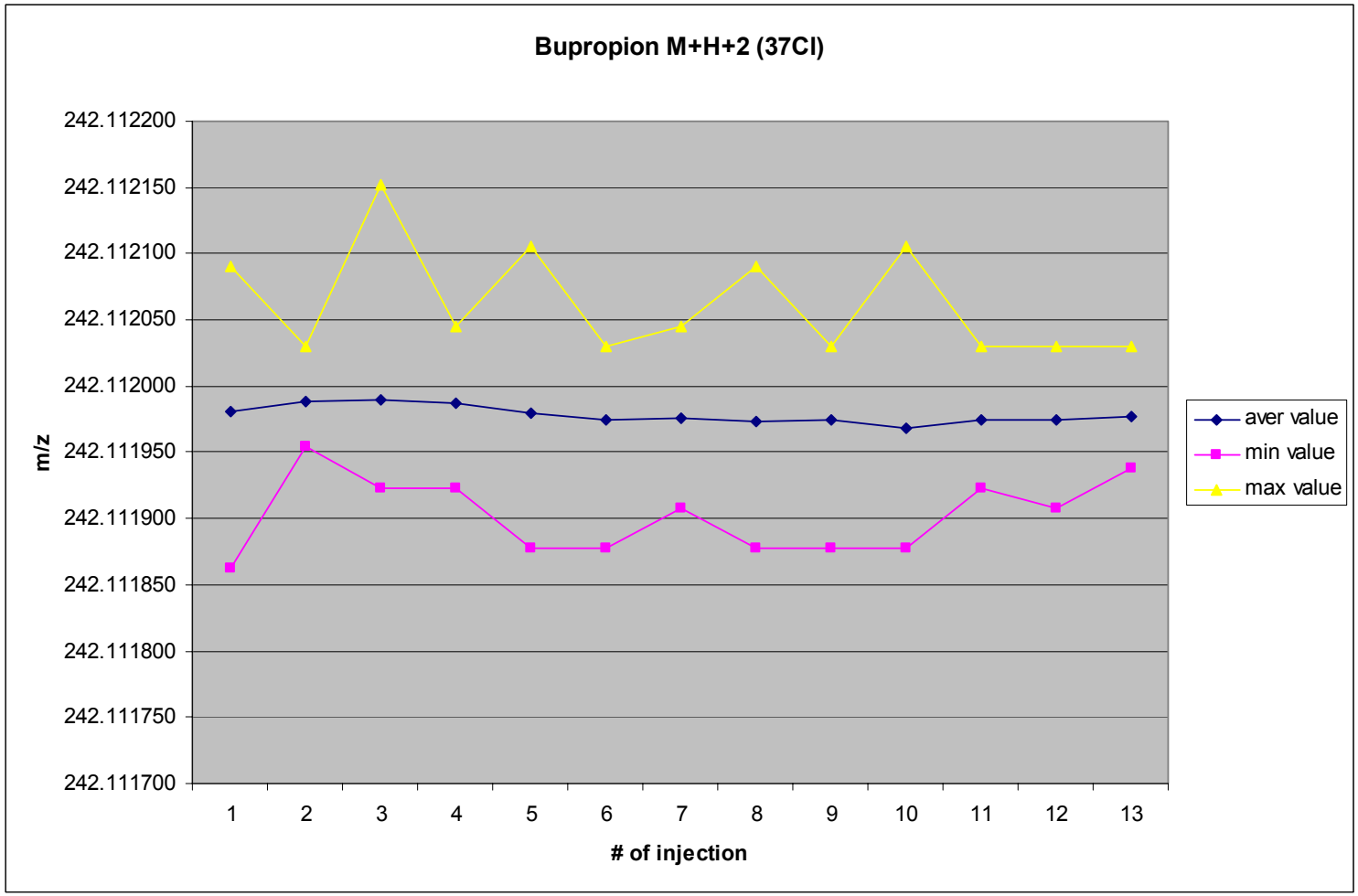
Accurate Mass Statistics for Bupropion M+H



Accurate Mass Statistics for Bupropion M+H+1

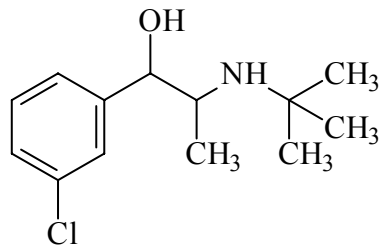


Accurate Mass Statistics for Bupropion M+H+2

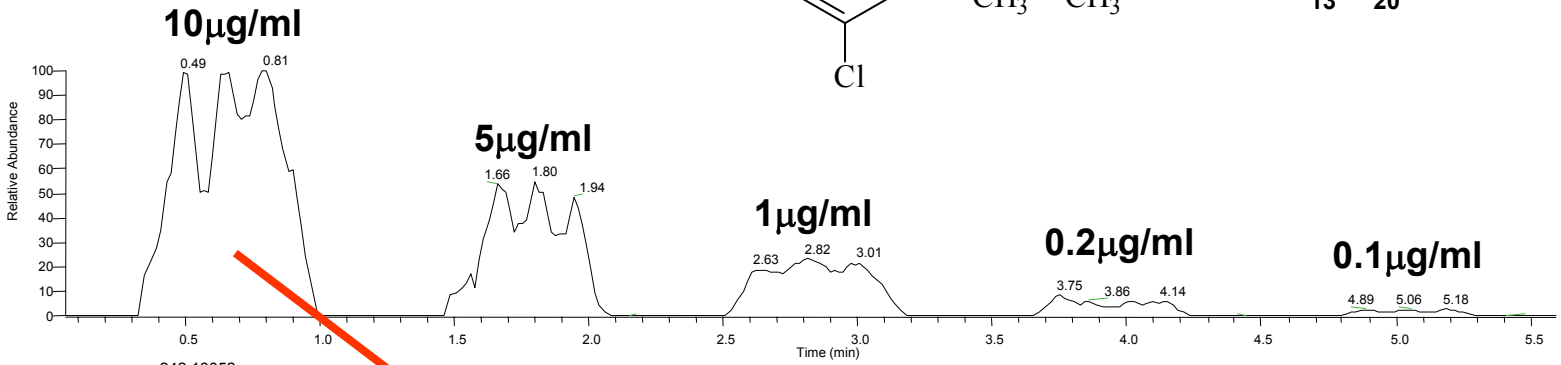


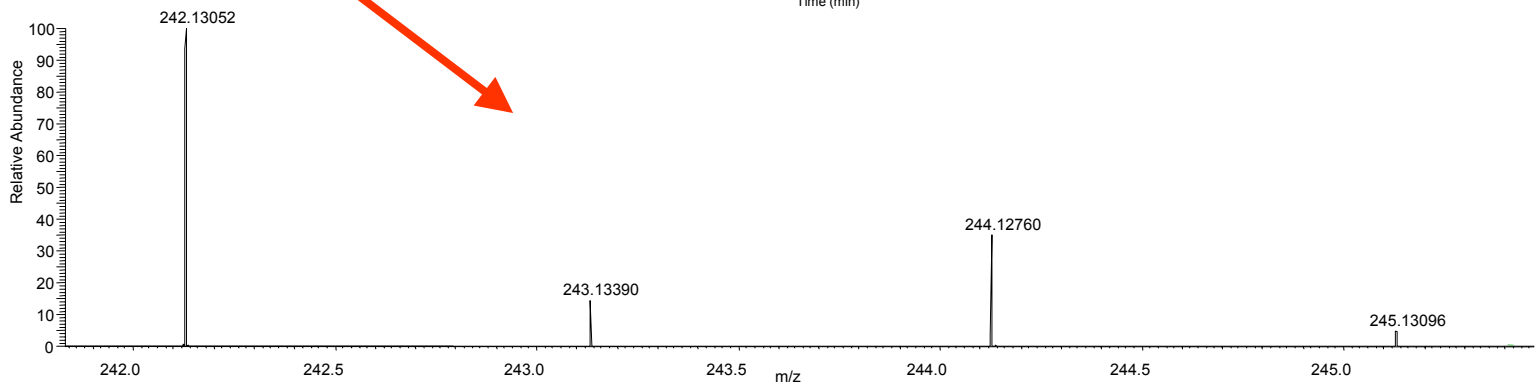
The Advion NanoMate 100 on the Finnigan LTQ FT

Variation of Sample Concentration for the Measurement of Threohydrobupropion



Threohydrobupropion
[M+H] = 242.13062
C_{13} H_{20} N O Cl

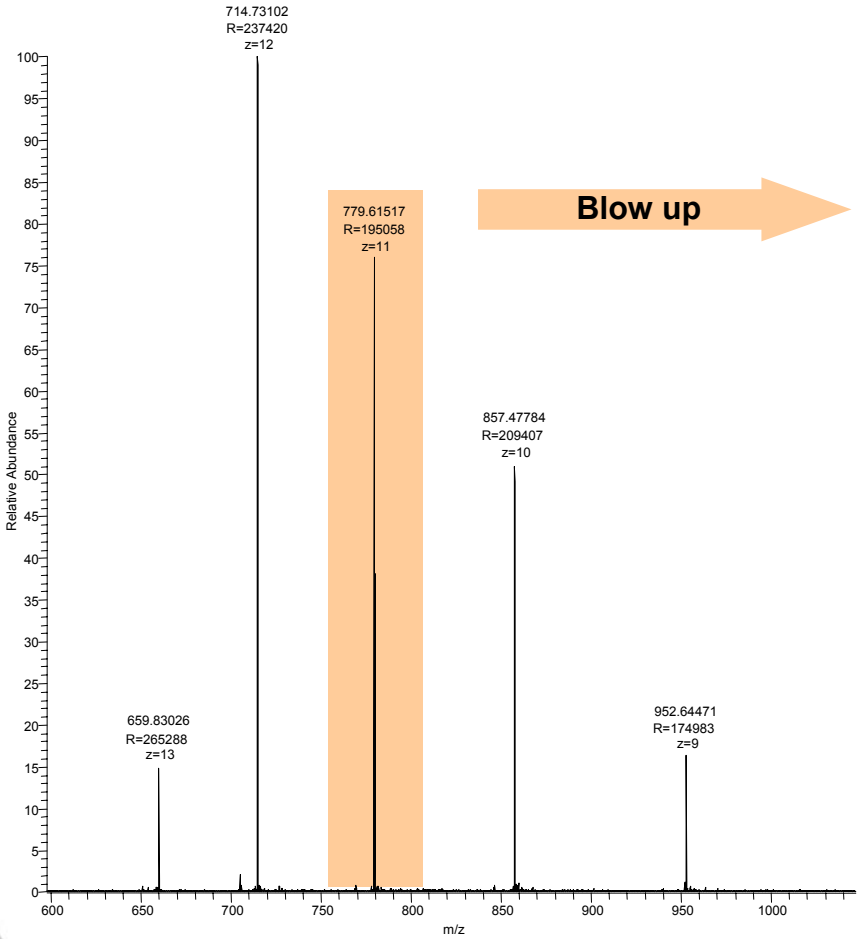




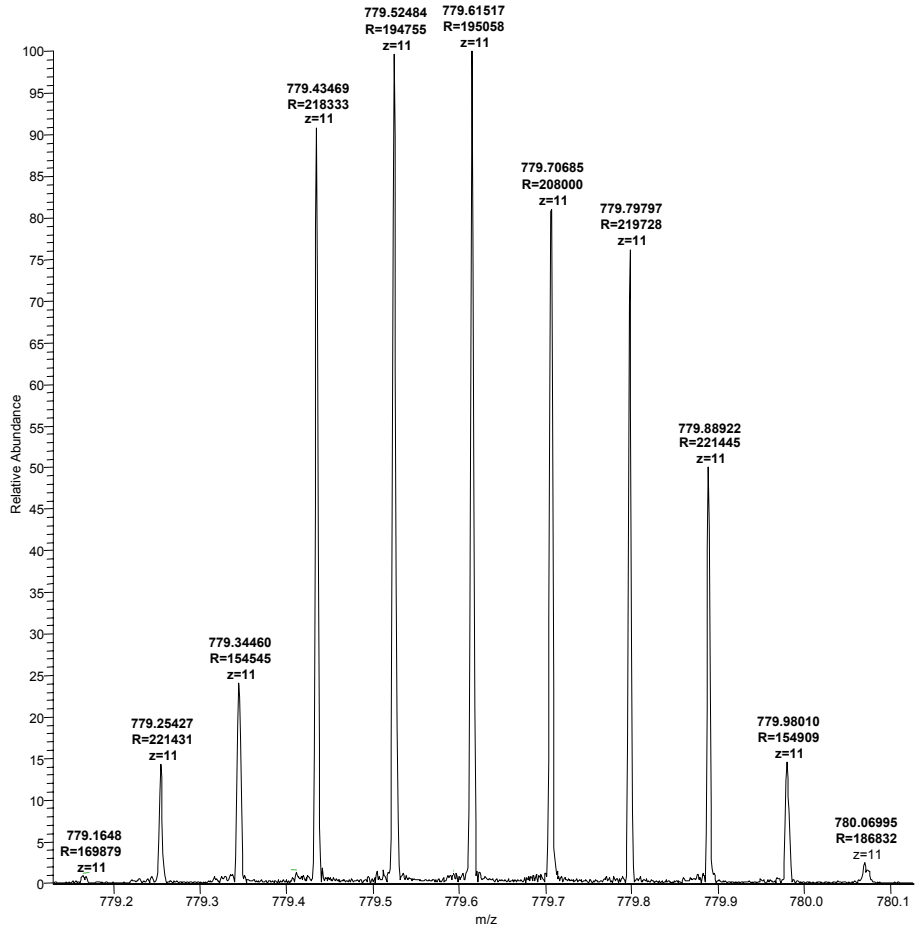
The acquired spectra were analyzed for the accurate masses of the four isotope peaks on the protonated molecule. The mass chromatogram for the MH^+ ion of Threohydrobupropion is shown as well as one exemplary spectrum. Again, the first four isotope peaks on the MH^+ ion were evaluated. As expected, the mass accuracy for all different concentrations are equally good and there is no trend visible with regard to mass accuracy. The errors are randomly distributed across the measurements, all results are better than 0.5 ppm.

Ubiquitin at Very High Resolution

Settings: 1e-6 M, infusion @ 1 ul/min, 5 transients averaged, 4.5 ms inject time / transient



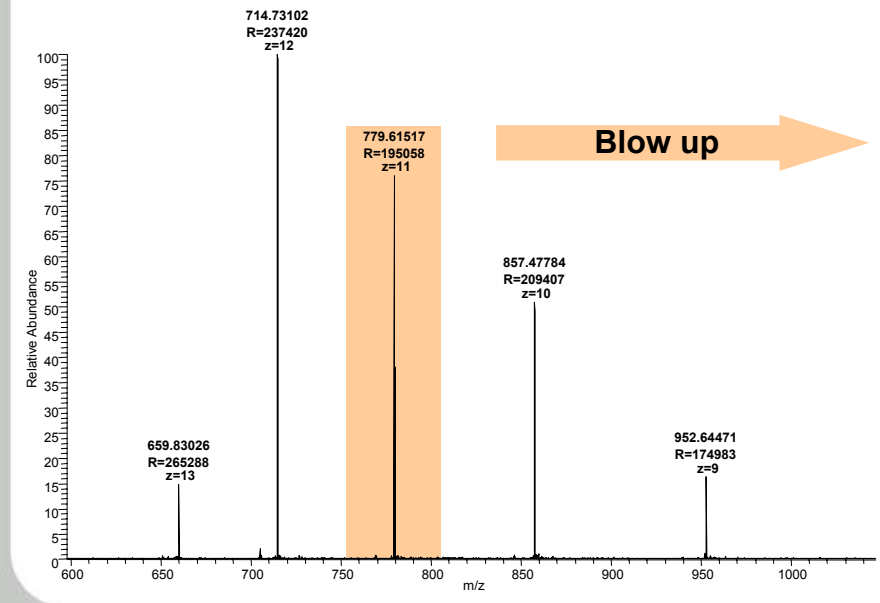
Blow up



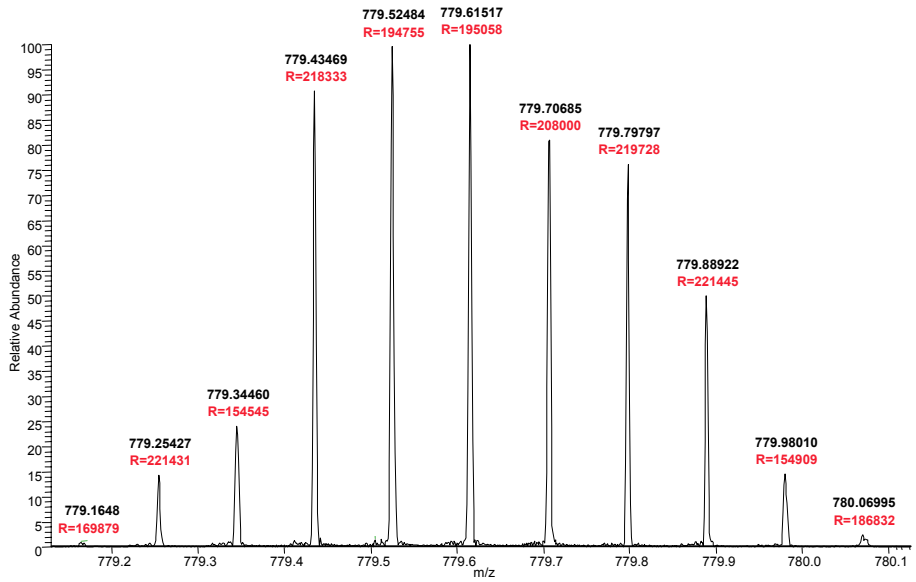
Ubiquitin at Very High Resolution – A Comparison

Settings: 1e-6 M, infusion @ 1 ul/min, 5 transients averaged

`IonSpec Experiment`: (Web page)	Resolution	200,000 @ m/z 779
	Sample consumption	30 fmol
	S/N	115 : 1
	Inject time	360 ms
Finnigan LTQ-FT:	**Resolution**	**200,000 @ m/z 779**
	Sample consumption	**408 amol**
	S/N	**270 : 1**
	Inject time	**24.5 ms**



Blow up



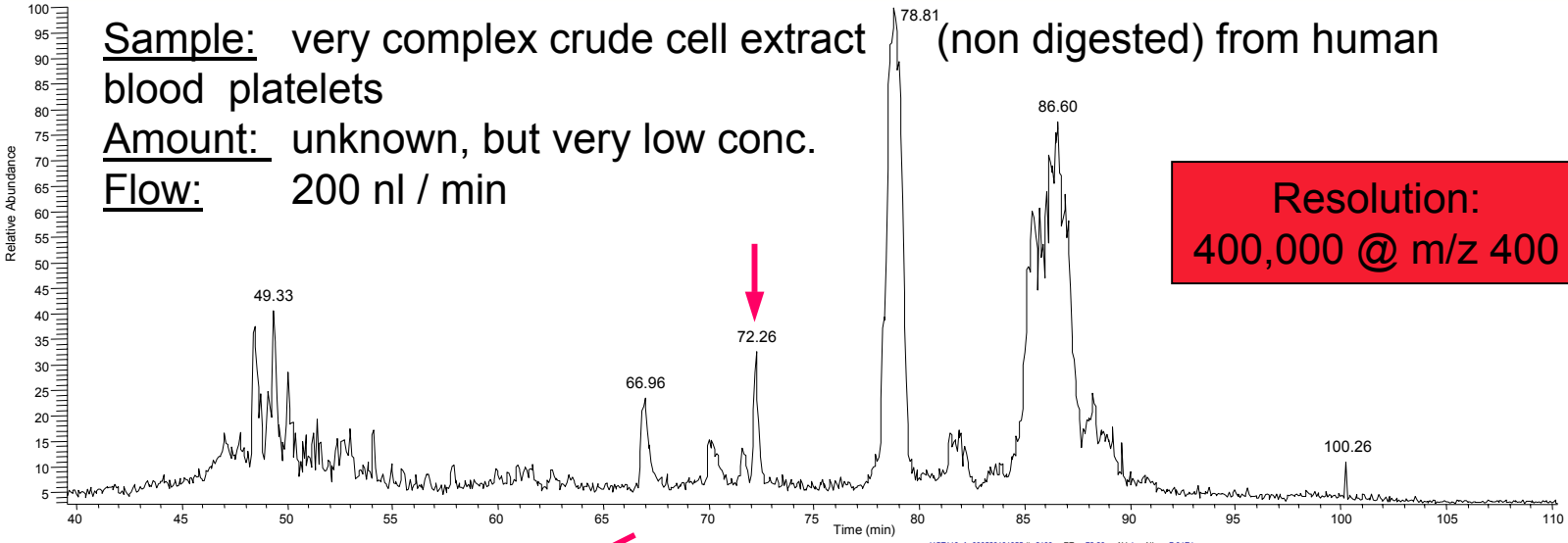
On-line Nano HPLC-MS at Ultra High Resolution

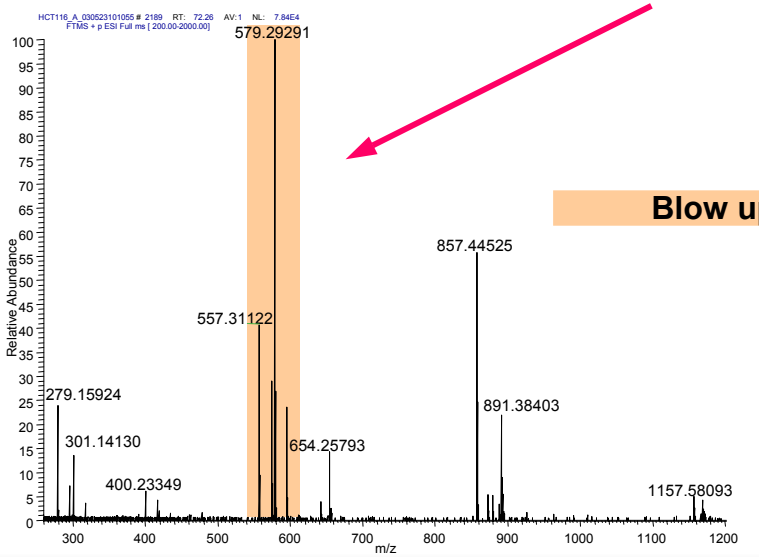
Sample: very complex crude cell extract (non digested) from human blood platelets

Amount: unknown, but very low conc.

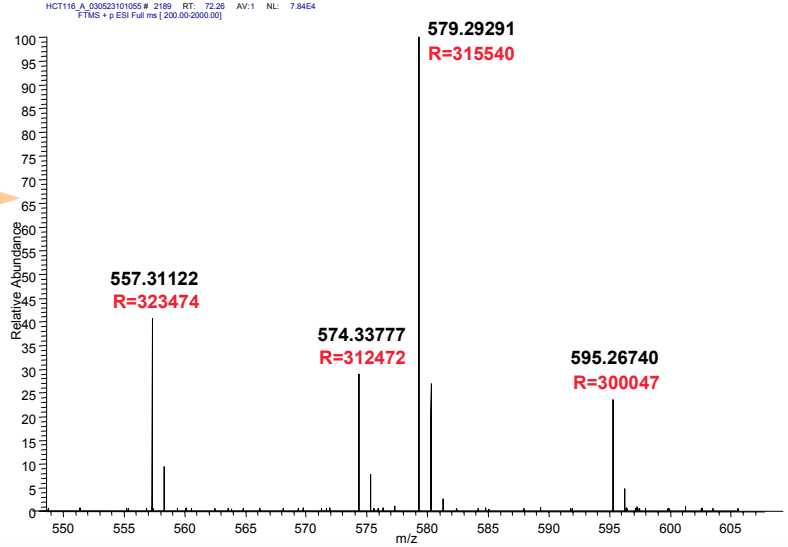
Flow: 200 nl / min

Resolution: 400,000 @ m/z 400





Blow up



Workshop
Finnigan LTQ FT

Thermo Electron‘s Annual Customer Forum
Sunday, June 9, 2003
Montreal, Canada

Agenda

- **Welcome and Introduction**
  Helmut Muenster, Thermo Electron Corp., Bremen, Germany
- **Finnigan LTQ FT – A Technical description of the new Hybrid FTMS**
  Stevan Horning, Thermo Electron Corp., Bremen, Germany
- **High Resolution FTMS for Quantitative Proteomics Measurements**
  Thomas P. Conrads, National Cancer Institute (NCI) Frederick, NC
- **First Results with the new LTQ FT**
  Michael Linscheid, Humbold University, Berlin, Germany

Demo Chemist – Jens Griep-Raming

Jens Griep-Raming
Bremen, Germany
+49-421-5493-219

Poster MPI 182

Fully Automated High-Throughput Accurate Mass Determination using FT-ICR Mass Spectrometry

Jens.Griep-Raming@Thermo.com

Demo Chemist – Wolfgang Metelmann-Strupat

Wolfgang Metelmann-Strupat
Bremen, Germany
+49-421-5493-255

Poster TPX 449

An Integrated LC-MS Platform for Rapid and Precise Identification of Proteins and their Post-Translational Modifications using a Linear Ion Trap coupled with a FT-ICR Mass Spectrometer

Wolfgang.Metelmann-Strupat@Thermo.com

Demo Chemist – Billy Wu

Shiaw–Lin (Billy) Wu
San Jose, USA
+1-408-965-6308

Poster TPE 091

Fourier Transform Ion Cyclotron Resonance (FT-ICR) Mass Spectrometry – A Rapid Method for Metabolite Identification

Billy.Wu@Thermo.com

Additional LTQ FT Posters at ASMS

Poster MPI 189

Timo Hagemeister, Humbold Univ. Berlin, Germany

Fragmentation Pathways of Cisplatin Adducts to Dinucleotides Determined by FT-ICR-MS

Poster MPX 457

Michael Linscheid, Humbold Univ. Berlin, Germany

Structure Elucidation of Structural Proteins from Yersina Phages using MALDI-ToF and ESI-FTMS Data

Finnigan LTQ FT

Installation Requirements

Superconducting Magnet – Requirements

- Transportation
  - *Cold shipping possible*
- Positioning in Laboratory
  - *Active shielding so no influence on lab equipment*
  - *Must not be positioned near elevator, large electric motor or massive steel structural supports*
- Maintenance
  - *Autofill for LN2*
- Safety
  - *Venting*
  - *Oxygen depletion sensors*

Finnigan LTQ FT

Conclusion

Highlights

- High resolution @ 1 Hz repetition rate
- Automation
- High mass accuracy
- On-line LC-MS
- MS, MS/MS and MS^n
- Low maintenance
- Small footprint
- And overall – simple to use

Bremen Development Team

… plus R&D Team in San Jose !!!