Development of High Performance LC-QIT-TOF

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- The Need for Highly Precise Mass Spectrometry
- Objective of LC-QIT-TOF
- Technology Developed for LC-QIT-TOF
- Fundamental Performance Data

The Need for Highly Precise Mass Spectrometry

- Precision mass spectrometry allows inferring the elemental composition of molecules
- The higher the precision, the more the number of constituent candidates can be reduced

Example: (reserpine) The number of elemental combination candidates listed for a mass of $(M+H)^+=609.2822$

Constituent Elements:

Carbon	20 - 40	Hydrogen	30 - 60
Nitrogen	3 - 10	Oxygen	5 -15
Sulfer	0 - 5		

Precision	10 ppm	5 ppm	2 ppm
Number of Combinations	29	16	4

Objective of LC-QIT-TOF

- Ion Trap MS

Allows MS^n

High precursor resolution

- TOF MS

Highly precise mass values

High resolution

QIT-TOF

An instrument that allows high precision MS^n spectrometry

Technologies Developed for LC-QIT-TOF

1685 x 685 x 570 mm, 280 kg

Differences Between
MALDI-QIT-TOF and ESI-QIT-TOF

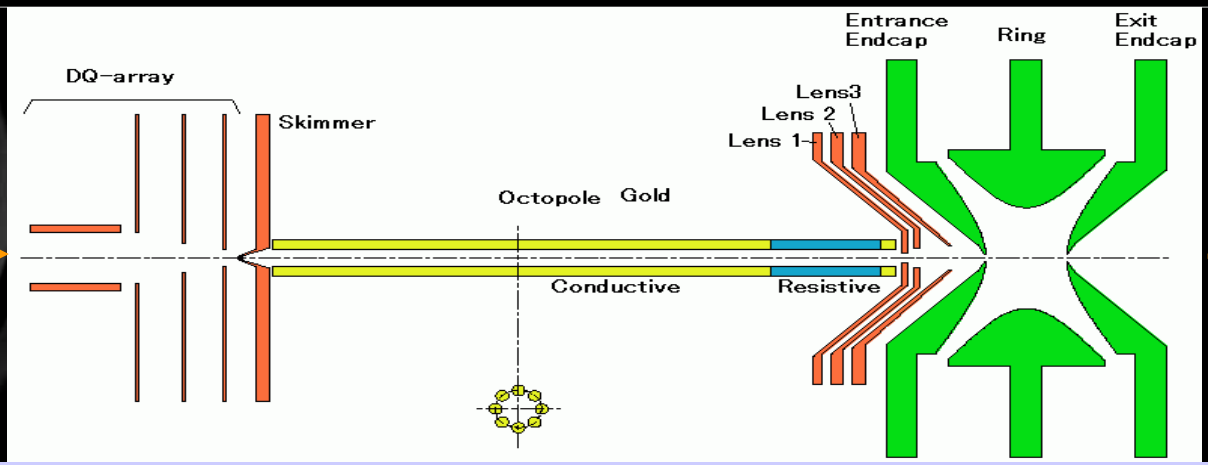
Key newly developed technologies:

1. Compressed ion introduction (introduction to QIT)
   MALDI: Pulsed ionization ESI: Continuous ionization
2. Improvements to Dual Stage Reflectron
   (High sensitivity and high resolution for QIT-TOF)
3. Ionic cooling using argon gas
   (High resolution)
4. Temperature controlled interior of instrument
   (Stability of mass precision)
5. Ballistic Ion Ejection

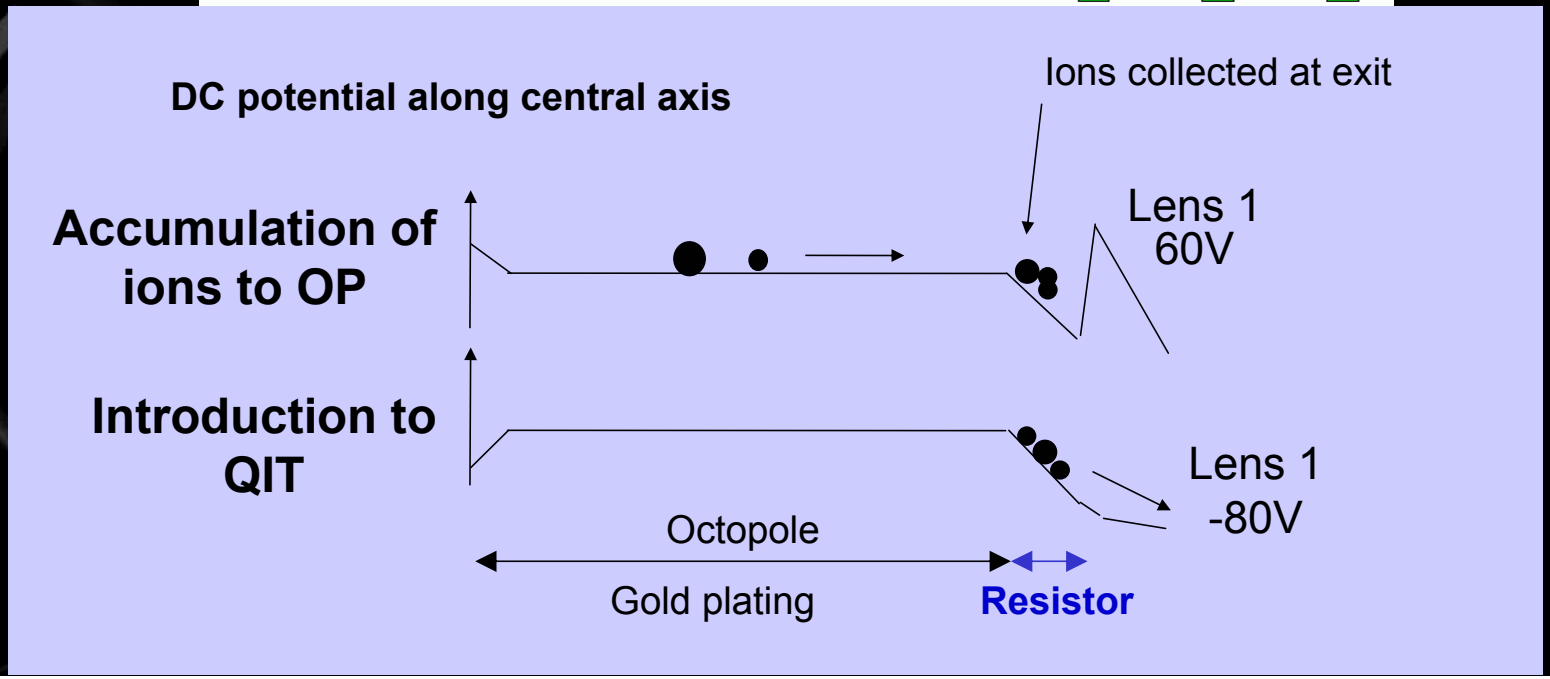
   (Accuracy and stability of mass measurements)

Compressed Ion Introduction

Ion Source



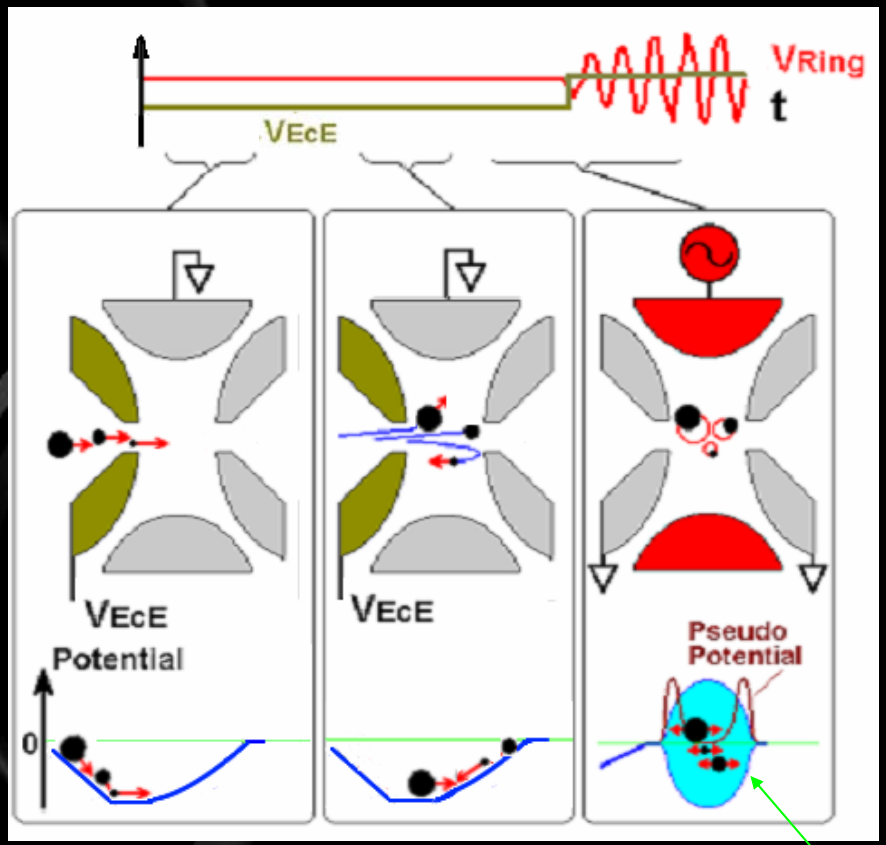
TOF

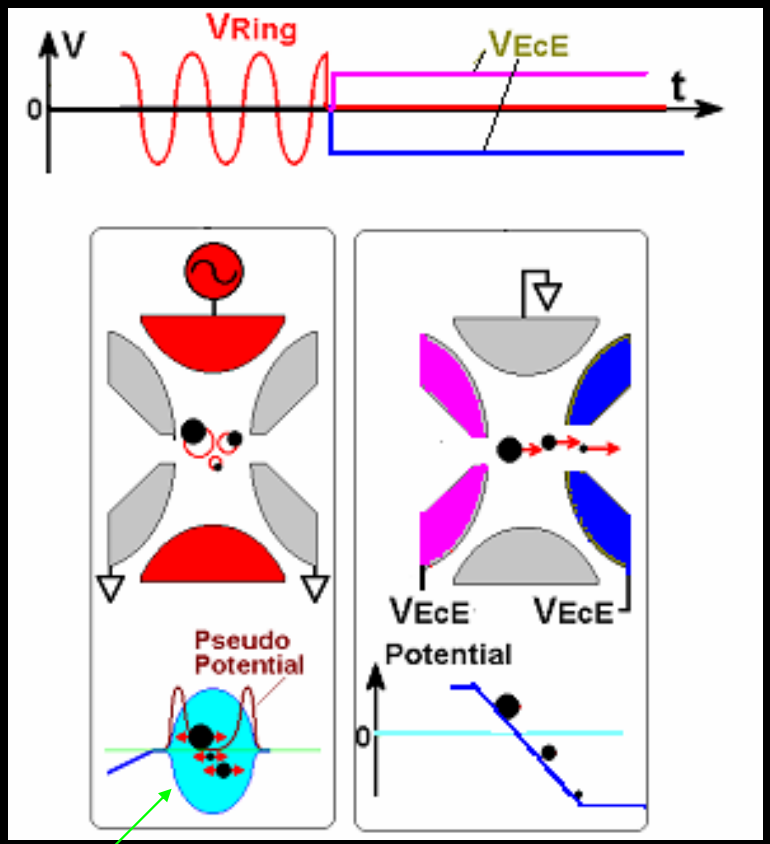


Introducing Ions into the Trap and Discharging Them

Delivery

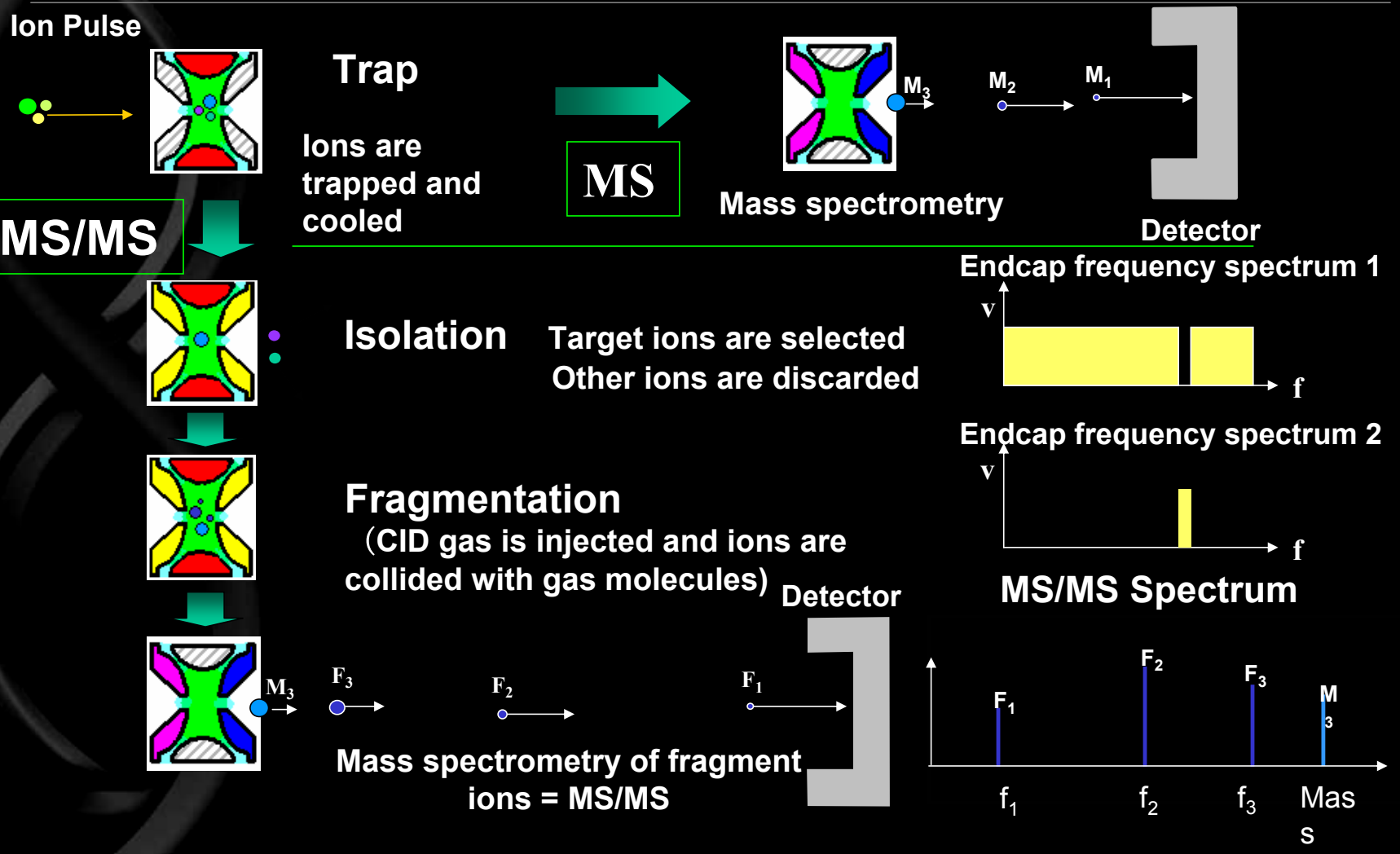
Discharge





Ion trap and localization

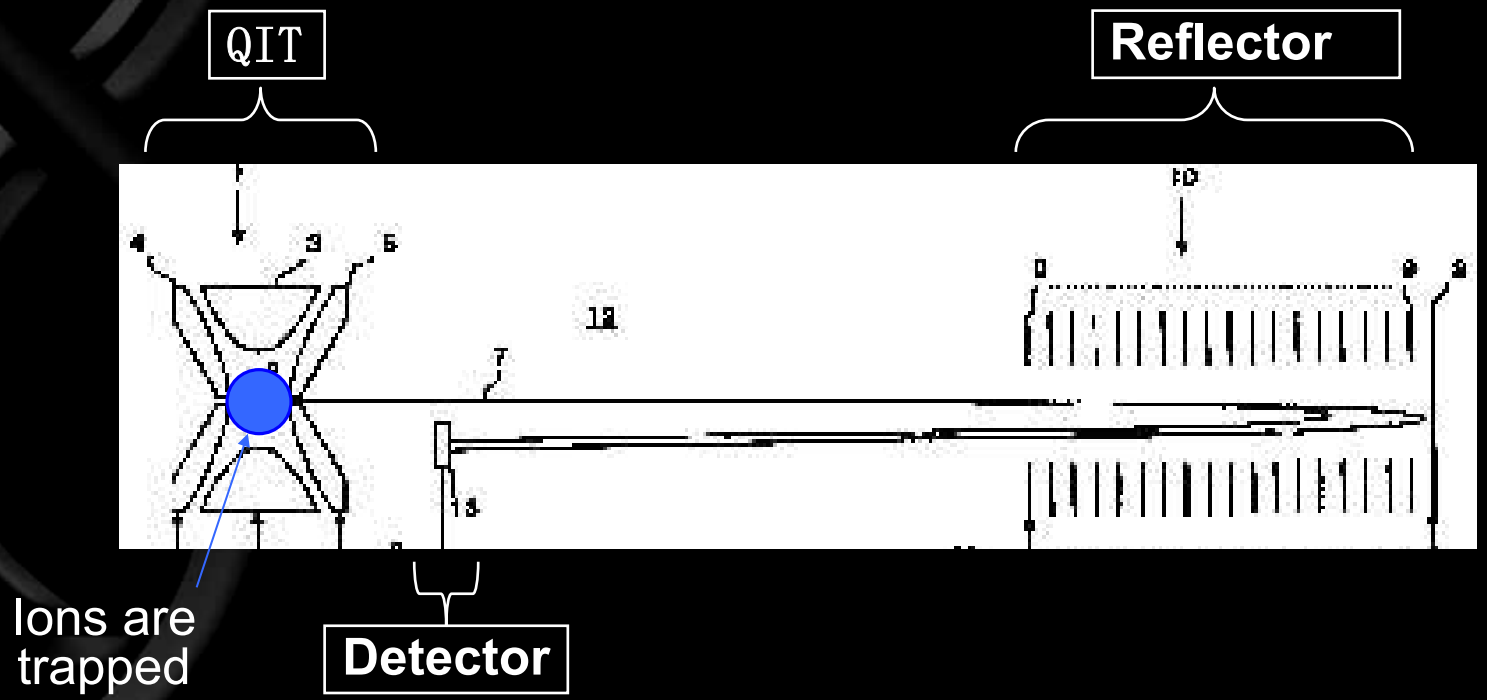
Basic Process of QIT-TOF



Dual Stage Reflectron Improvements

The shape of the electric field was changed for second stage of the dual stage reflectron.

-> Improves the time convergence with respect to ion distribution during acceleration

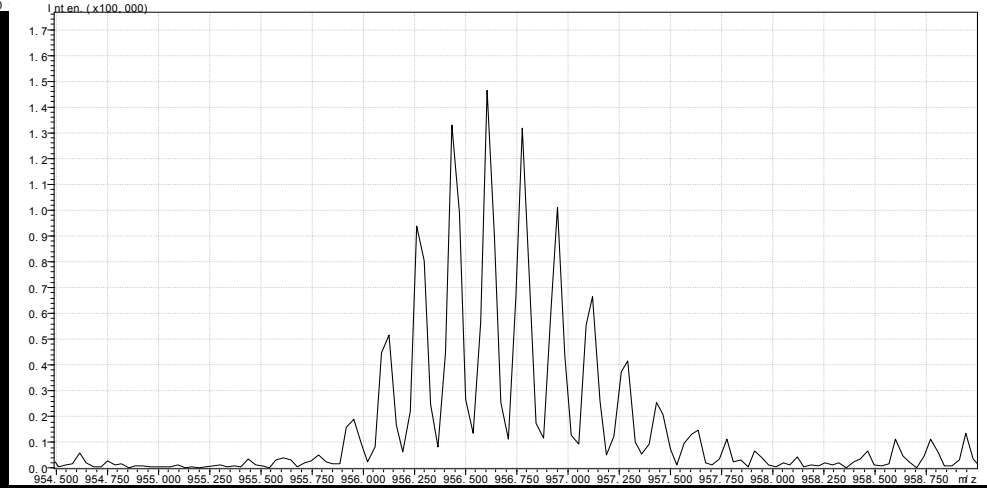


Cooling with Argon Gas

Bovine Insulin m/z 956 $[M+6H]^{6+}$

He: 4.5×10^{-2}Pa

Ar :7.5×10^{-3}Pa



Controlling the Instrument's Internal Temperature to Stabilize Mass Precision

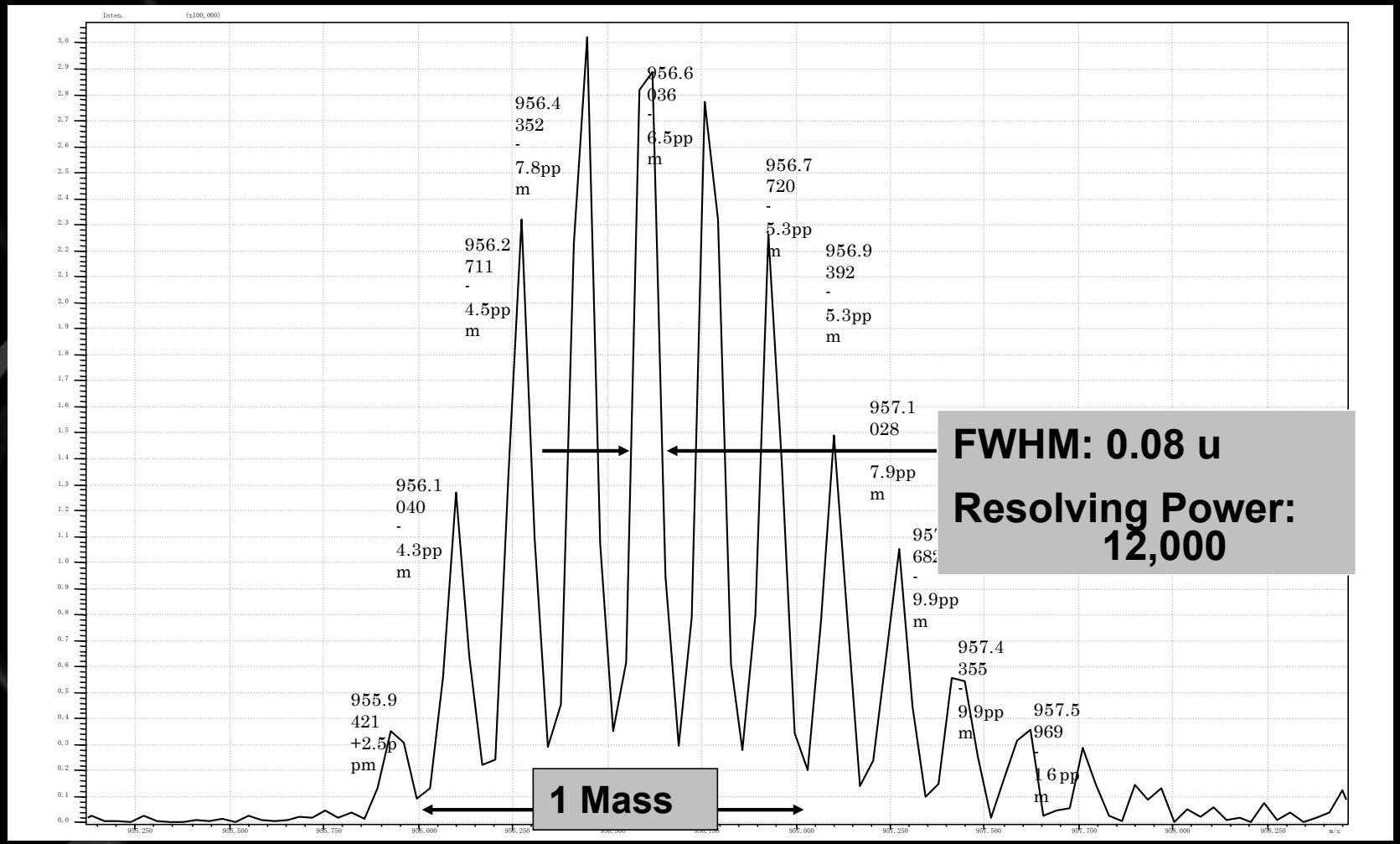
Temperature Control

- Flight Tube (Representative temperature point)
- High Voltage Switch (Ion Trap EC, Ring)
- High Voltage Power Supply (Ion Trap, Flight Tube, Reflectron)
- RF Generator (Ion Trap)

Controls representative temperature point to 40±0.3℃

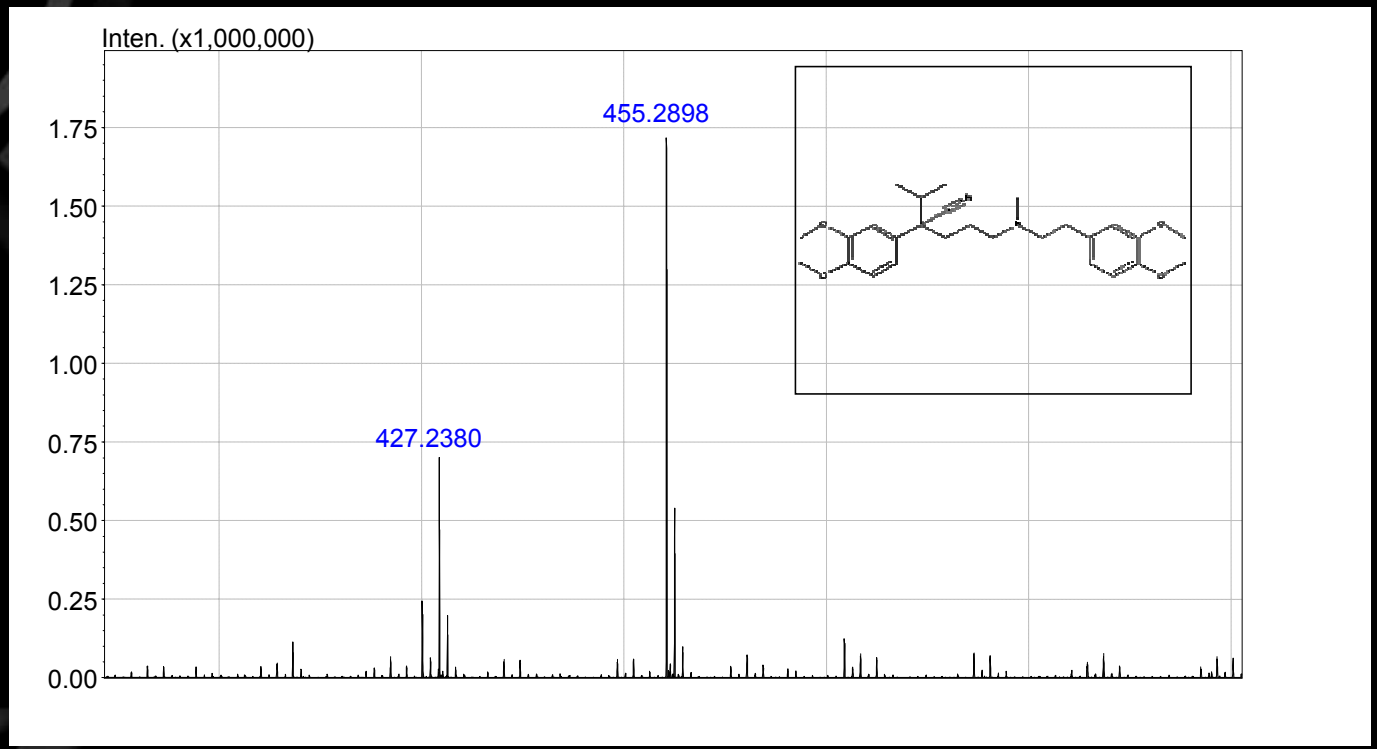
Fundamental Performance Data

Mass Spectrometry of Bovine Insulin 6+(5733m/z)



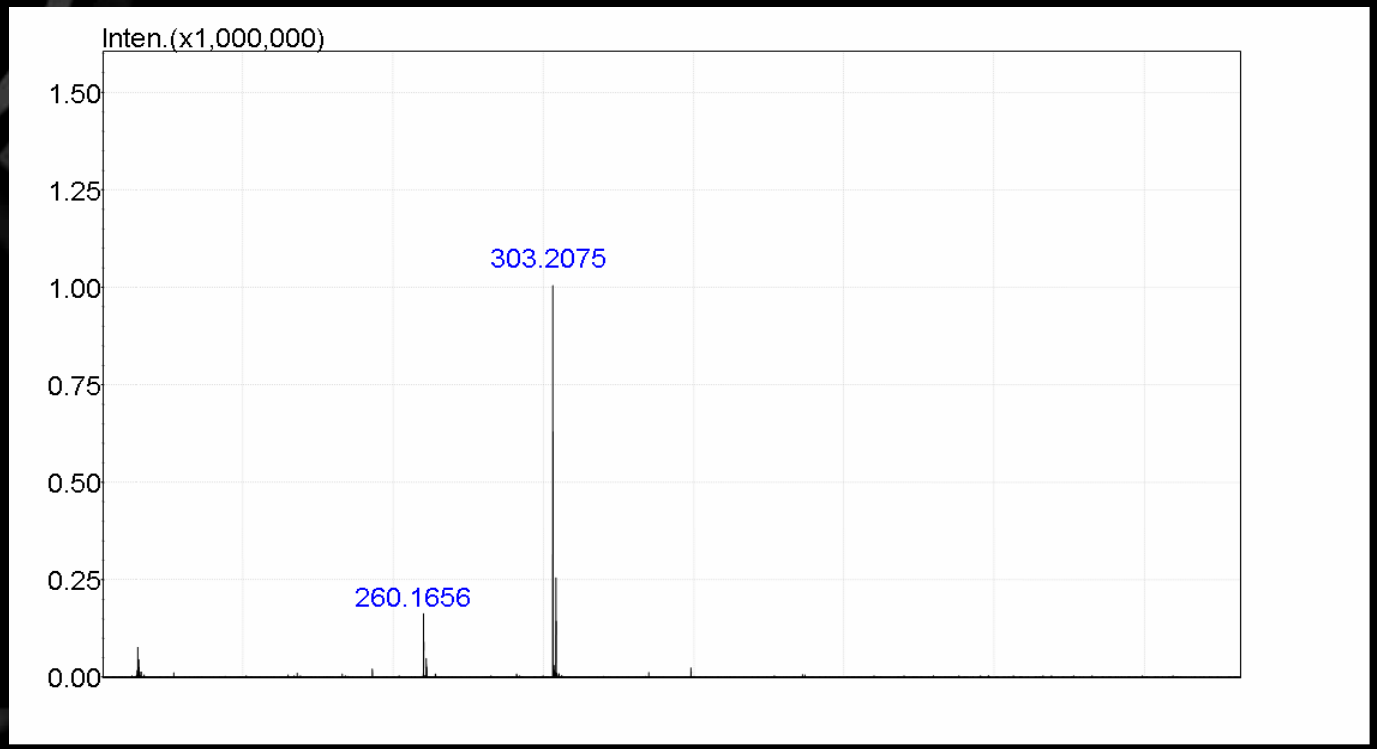
Mass Spectrometry of Verapamil

Sample		Expected	Found	Error
Verapamil	$C_{27}H_{39}N_2O_4$	455.2910	455.2898	- 2.64 ppm



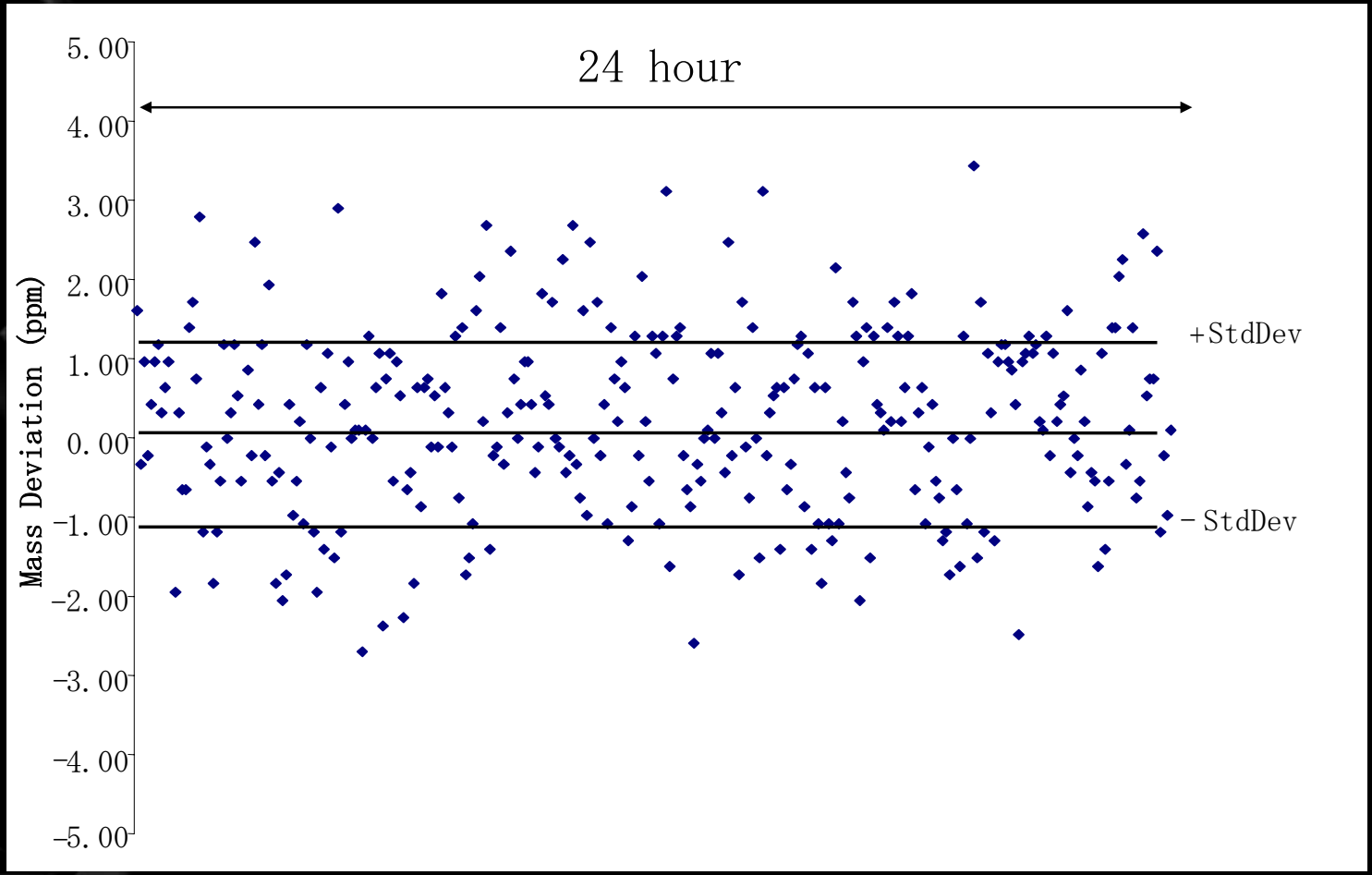
MS/MS Measurement of Verapamil

MS/MS ID	Expected	Found	Error
C18H27N2O2	303.2073	303.2075	+ 0.66 ppm



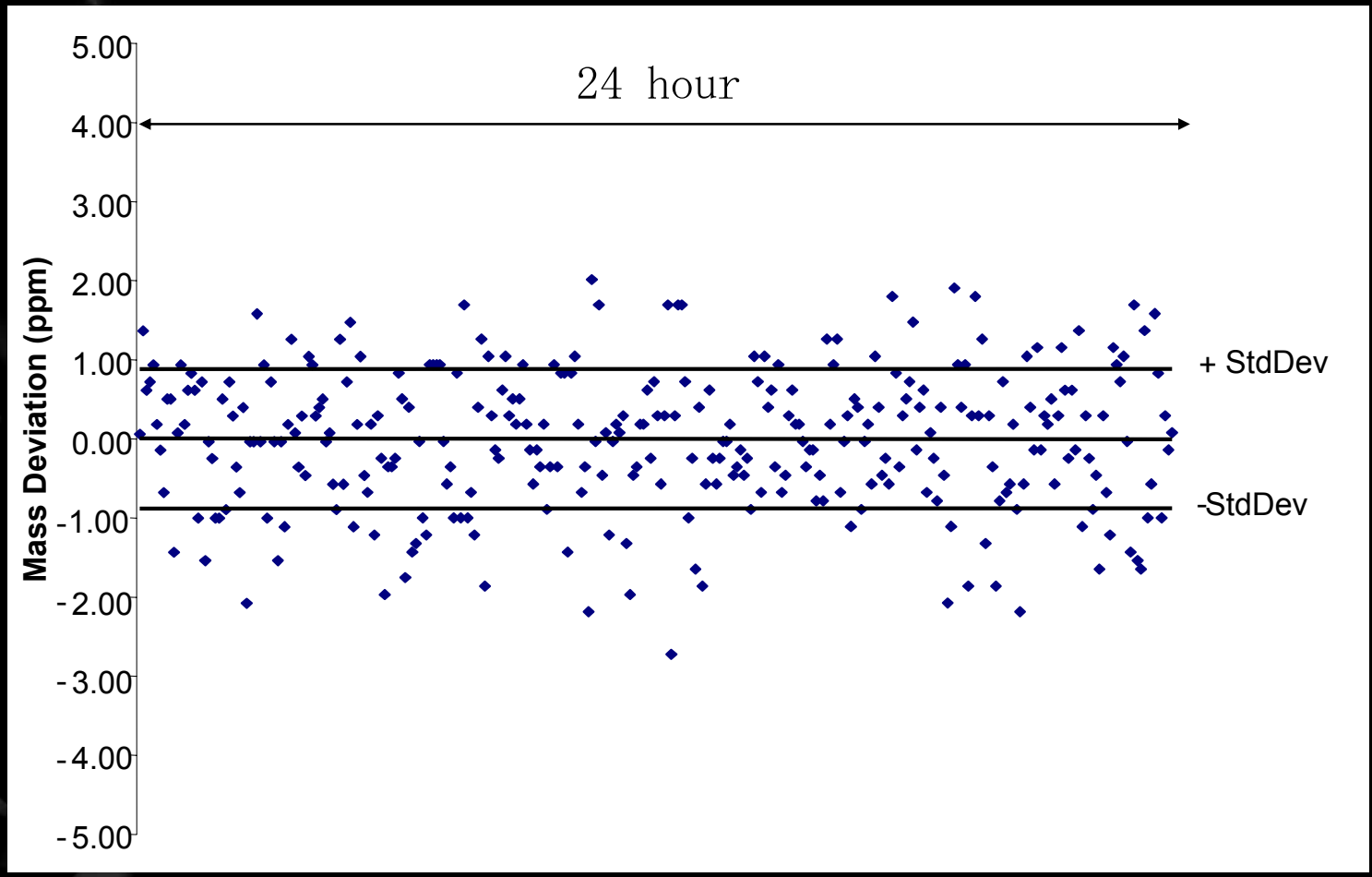
24-Hour Stability of Mass Precision

External Standard Method (m/z928)

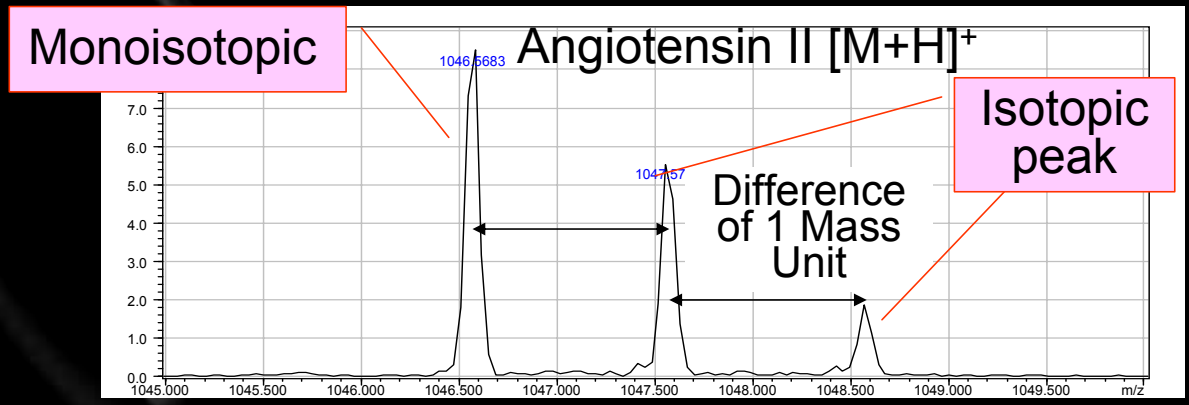


24-Hour Stability of Mass Precision

Internal Standard Method (m/z928)

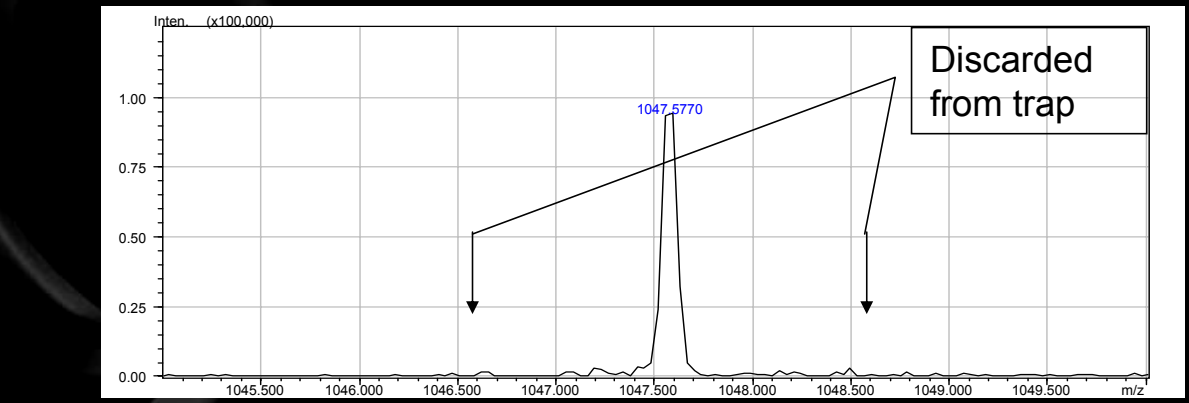


Precursor Selection Using Ion Trap



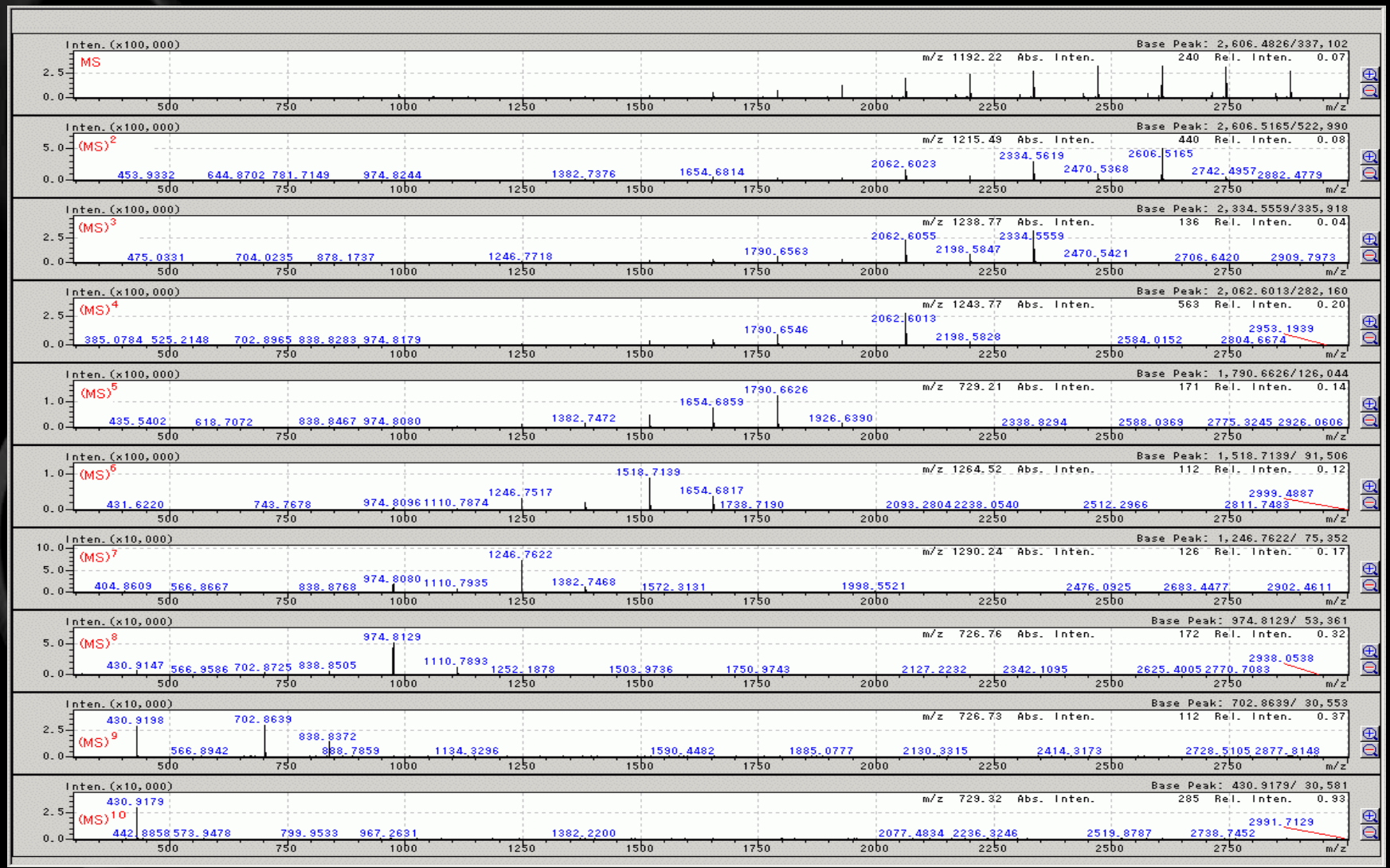
Before selecting precursor ion

Trap selection of m/z 1047.57



After selecting precursor ion

MS^{10} using NaTFA sample



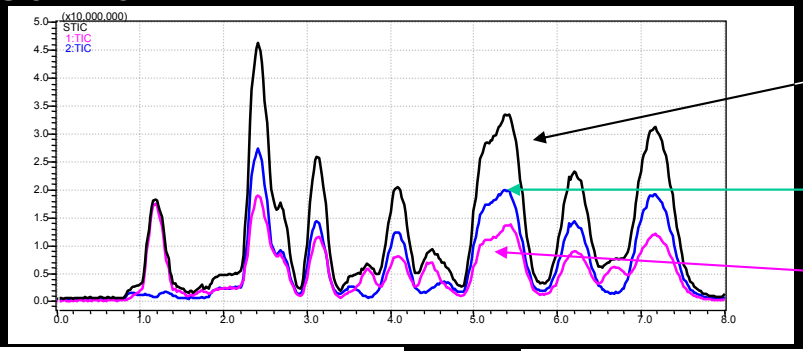
High Speed Pos/Neg Ion Switching (Max 2.5Hz)

Sample: glycosylceramide (C16,18,20,22,23,24:0-d18:1), (C24:1-d18:1) From Riken

Column Imtakt Cadenza CD-C18

Mobile Phase: 5mM acetate methanol solution

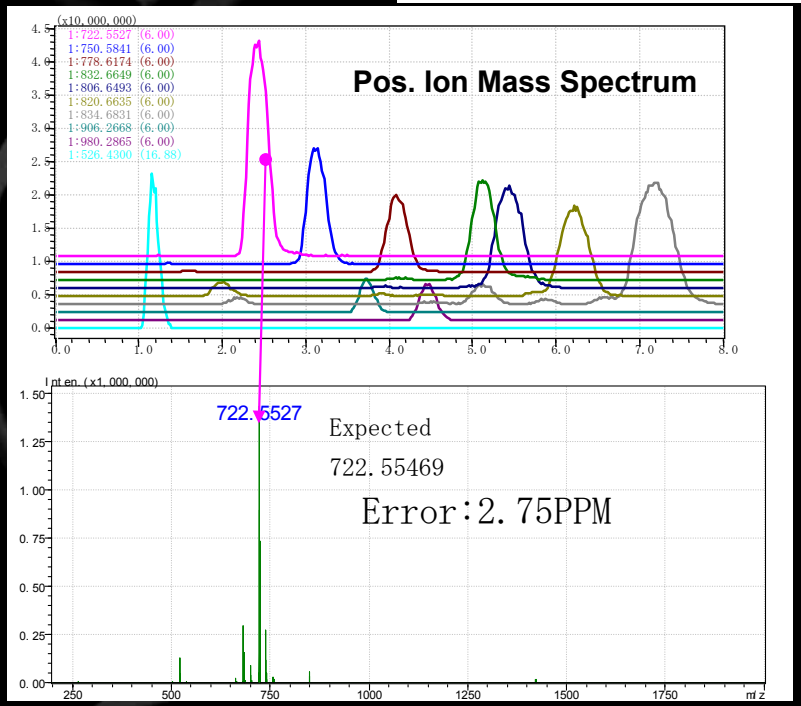
Flow Rate: 0.2ml/min isocratic

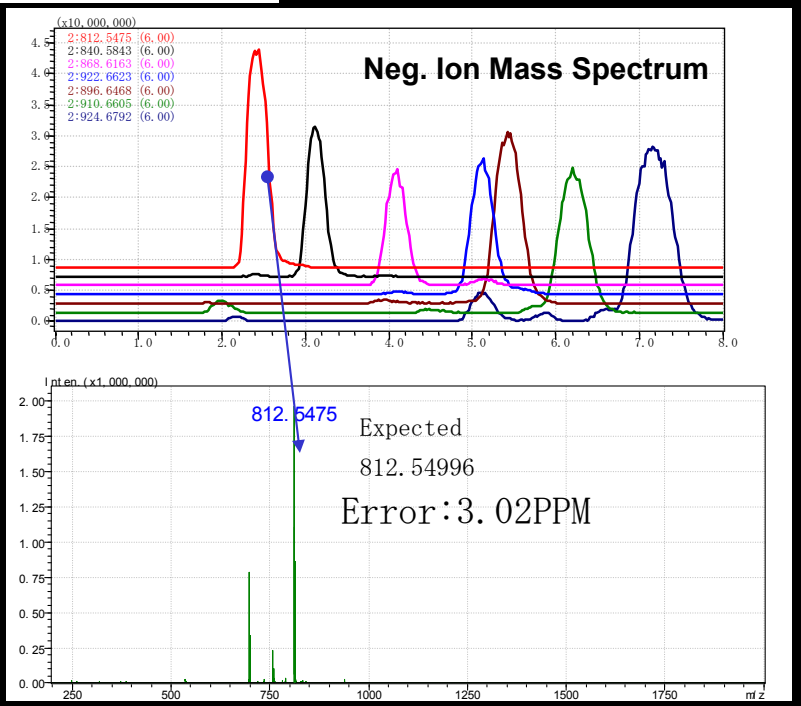


With Pos/Neg Ion TIC

Neg. Ion TIC

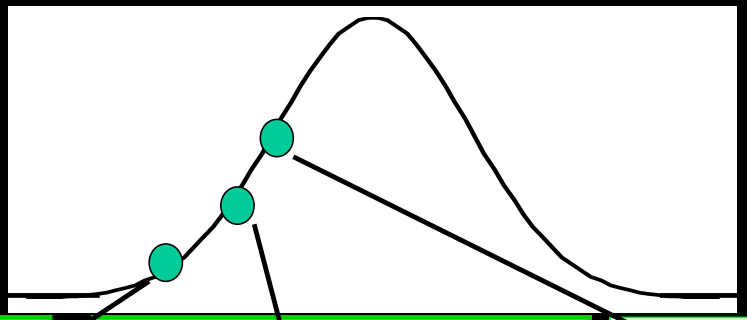
Pos. Ion TIC

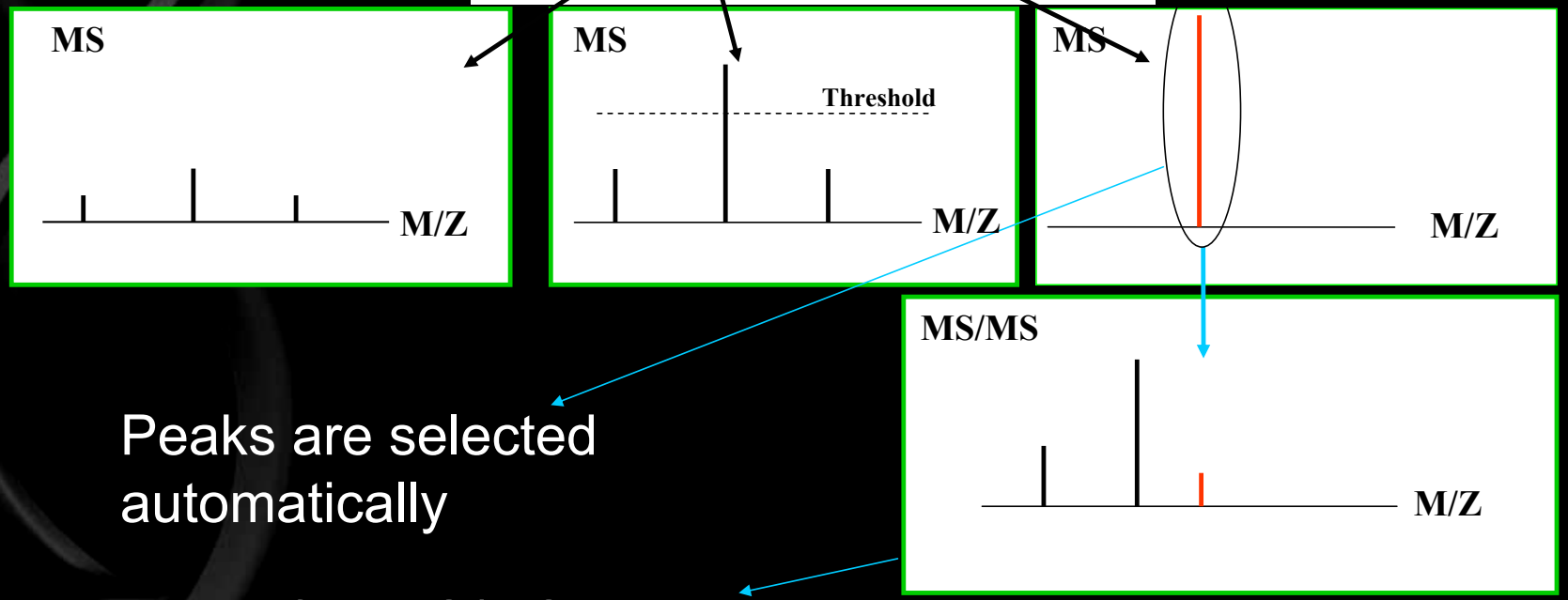




Auto MS/MS

TIC

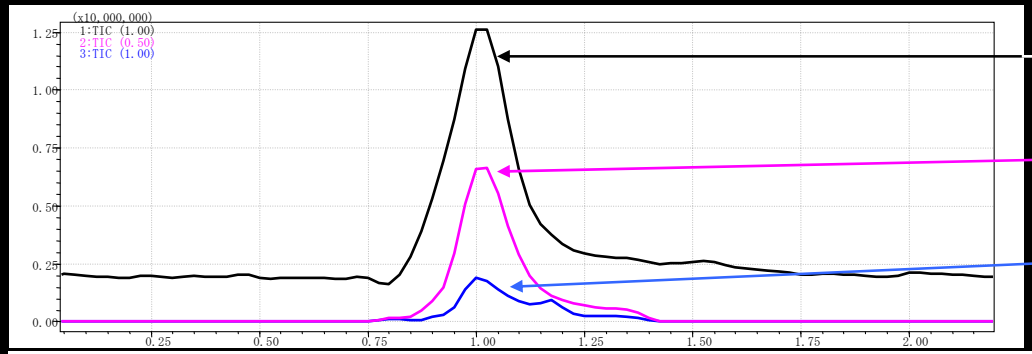




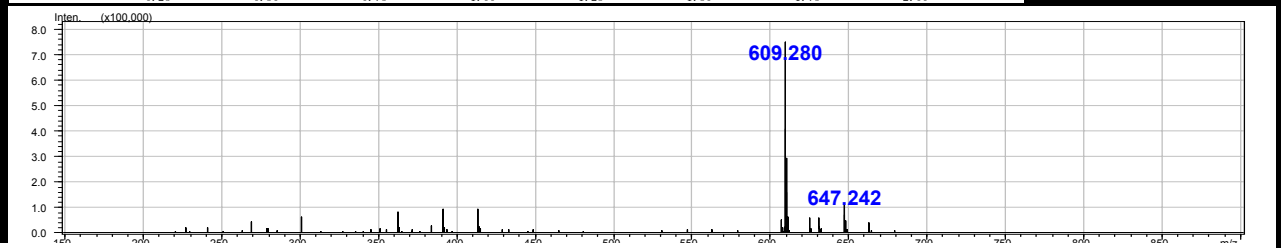
Peaks are selected automatically

Data after MS/MS analysis

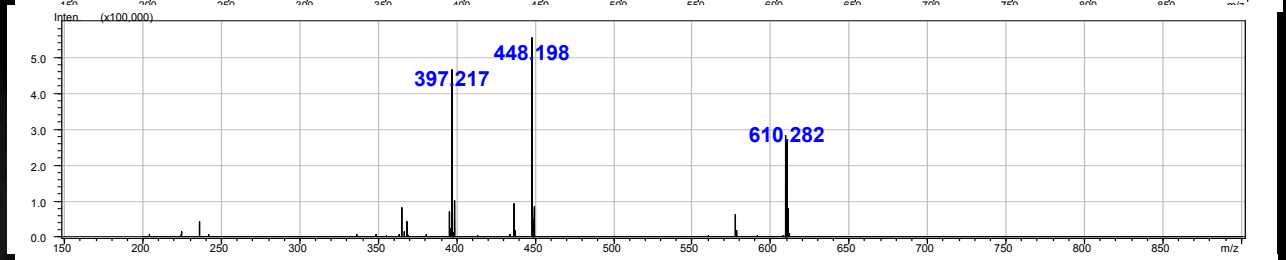
Auto MS/MS



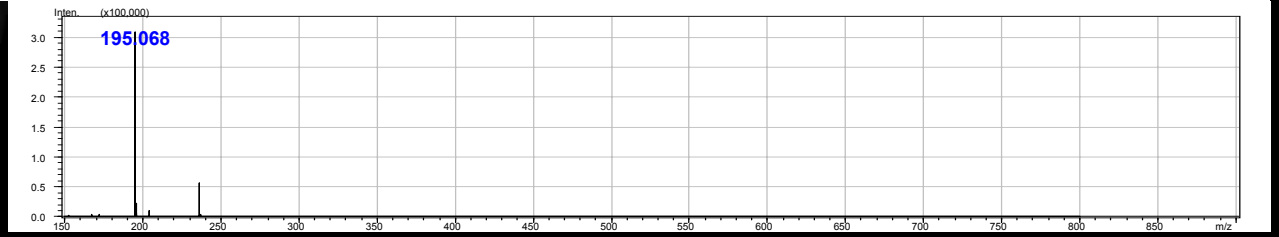
MS1



MS2



MS3



Summary of New Technologies

Compressed Ion Introduction Method
(Continuous ionization -> Pulsed ionization)

Ballistic Ion Ejection

(Transfers ions to TOF all at the same time with similar velocities for higher mass accuracy)

Ion Cooling using Argon Gas

(High resolution analysis of multivalent ions)

Dual Stage Reflectron Improvements
(Hign sensitivity and high resolution for QIT-TOF)

Temperature controlled interor of instrument housing
(Stability of high mass precision)