Liquid Chromatograph Mass Spectrometer

LCMS-9030 Q-TOF

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LCMS-9030
LIQUID CHROMATOGRAPH MASS SPECTROMETER

Understand the rationale behind the Q-TOF LC/MS that stably sustains sub-ppm mass accuracy.

Atsuhiko (Ash) Toyama, Ph.D.

MS Application Specialist,
Analytical & Measuring Instruments Division,
Shimadzu Corporation.

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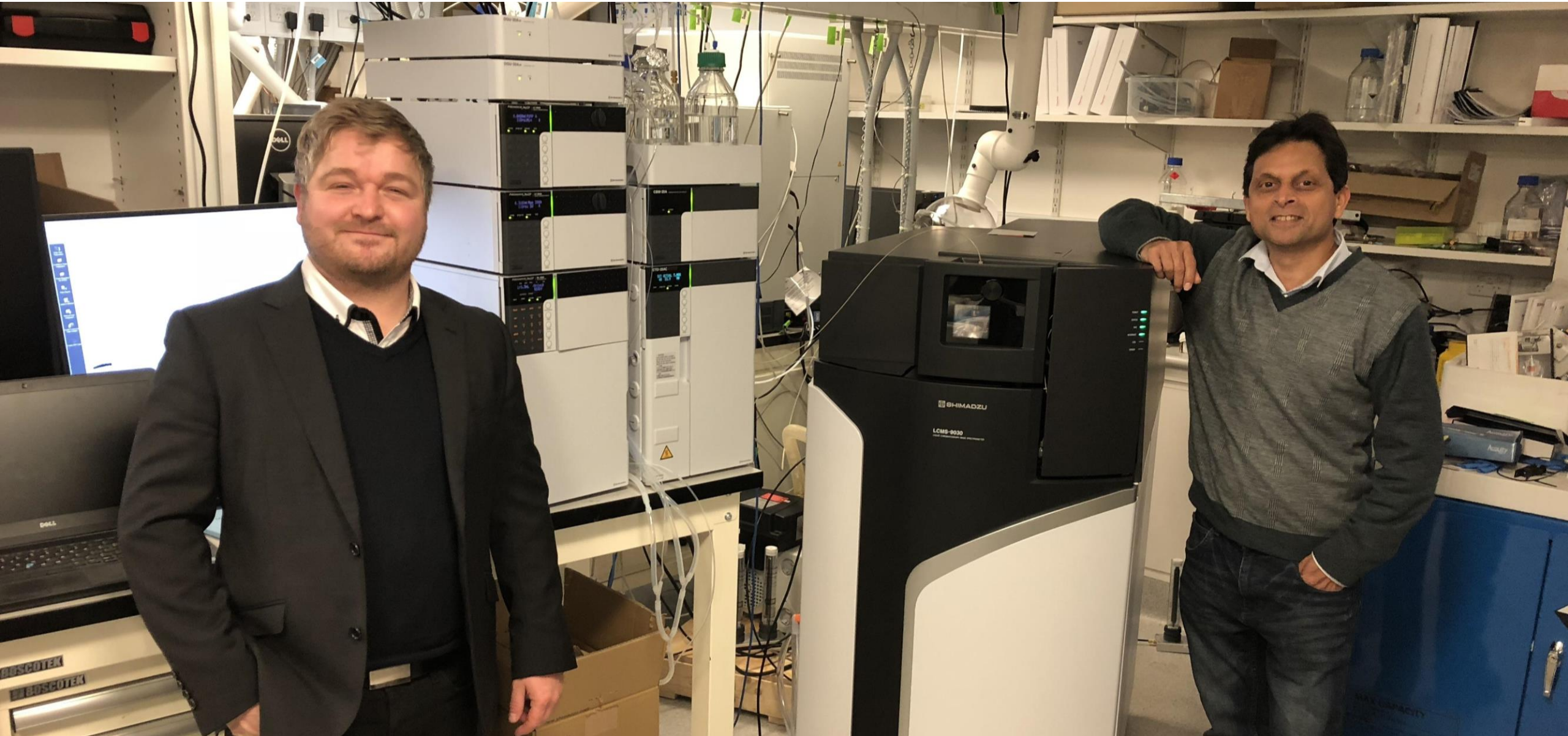
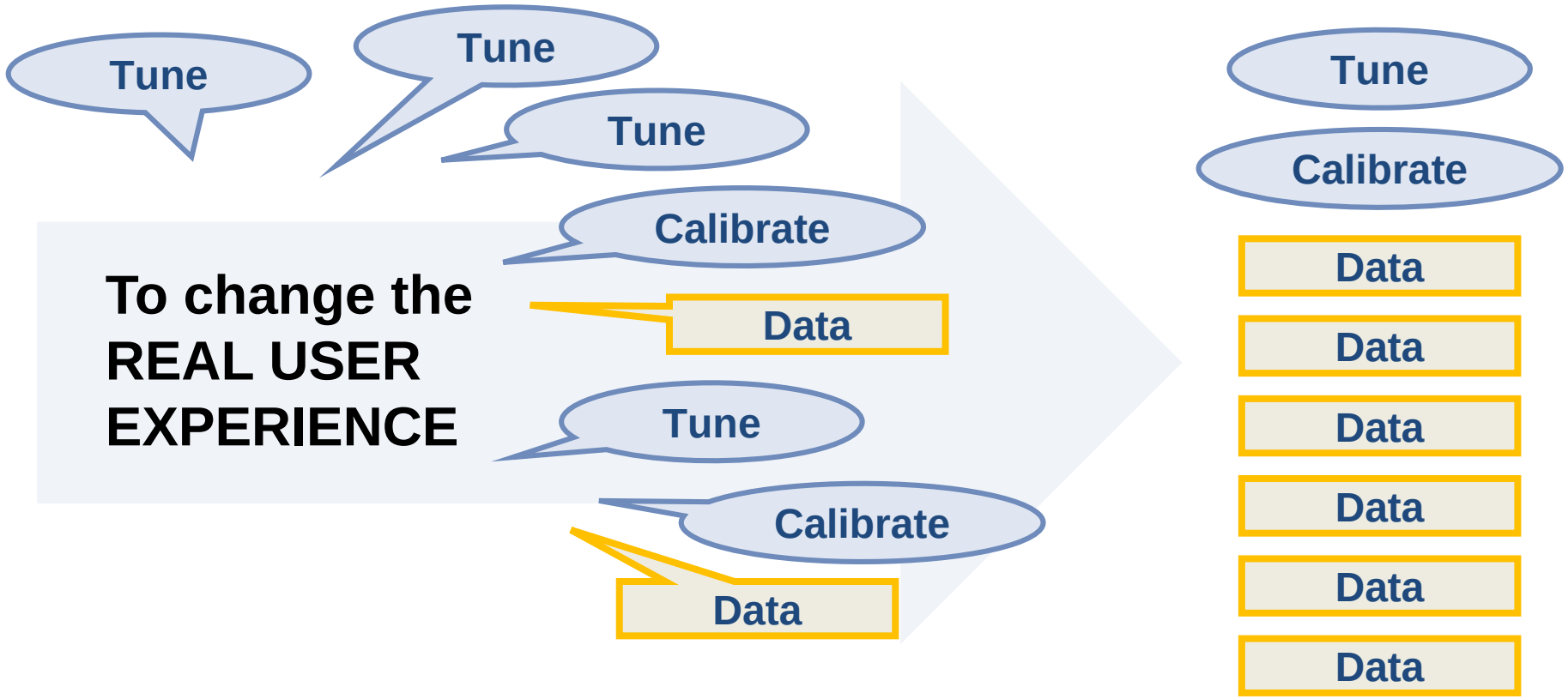
LCMS-9030 is at its Take Off



Engineering Concept

Effortless Performance



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The Q-TOF with MASS APPEAL

MASS
ACCURACY
SENSITIVITY
SPEED

Mass accuracy, particularly the stability of mass accuracy, represents the overall performance of a Q-TOF instrument.

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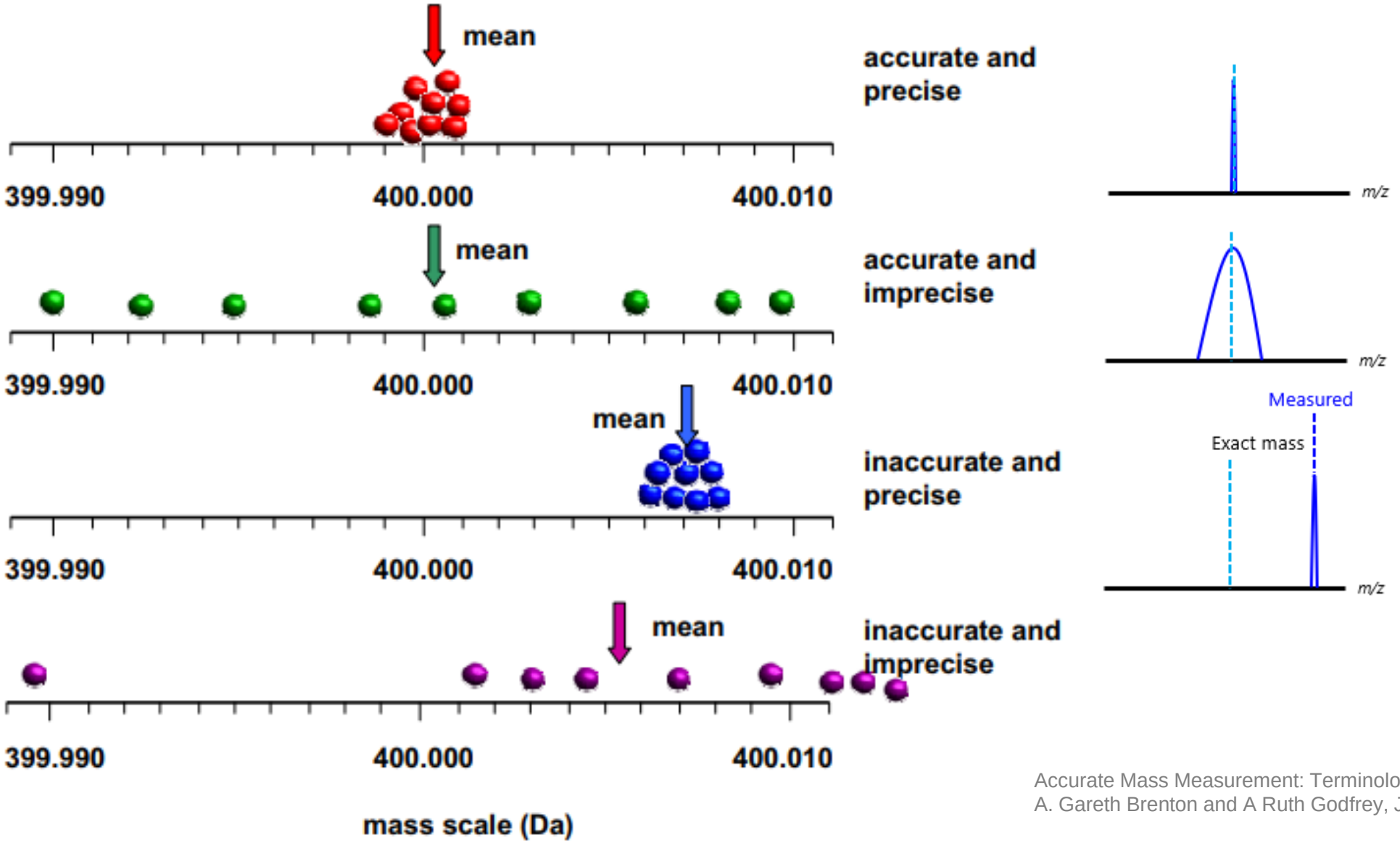
Topics

- Discuss how various parameters affect the accuracy of mass measurement
- Demonstration of mass drift caused by temperature change
- Learn how to assess whether a HRAM MS is practically capable of measuring at sub-ppm mass accuracy

- Shimadzu LCMS-9030 QTOF:

- Mass Resolution 30,000 FWHM at *m/z* 1,972
- Mass Accuracy <1 ppm at *m/z* 622.5662
- Mass Accuracy Stability 1 ppm/24h, 18 to 28 ^{o}C at constant temperature

Accuracy and Precision

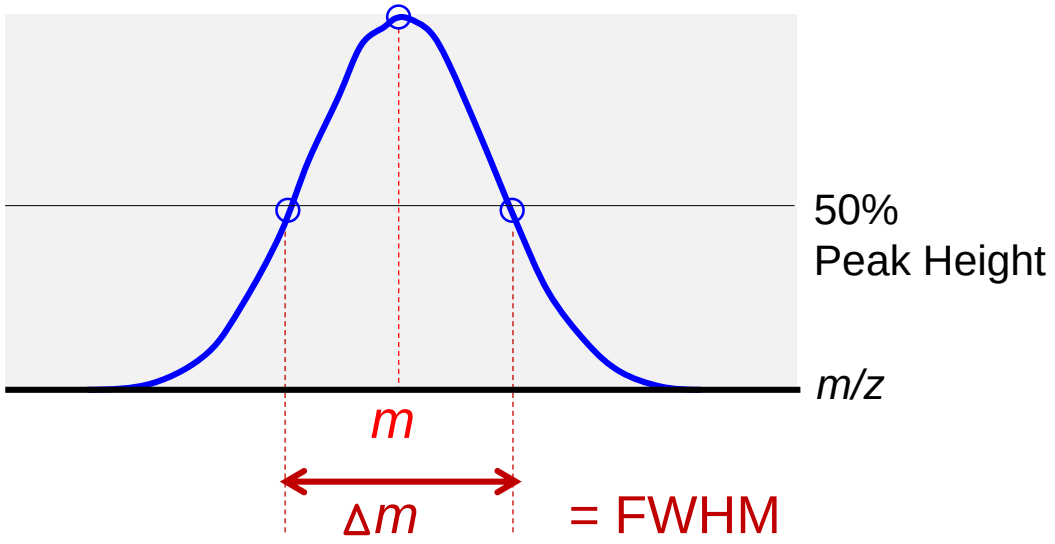


Accurate Mass Measurement: Terminology and Treatment of Data
A. Gareth Brenton and A Ruth Godfrey, JASMS 2010

Defining Mass Resolution

- IUPAC defines mass resolution as $m/\Delta m$, where m is the mass of the ion of interest and Δm is the peak width (peak width definition)
- Peak width at 50% height is typically used and denoted as Full Width Half Maximum (**FWHM**)

Deriving mass resolution ($m/\Delta m$) from a measured mass spectrum



For $m = 300.00$

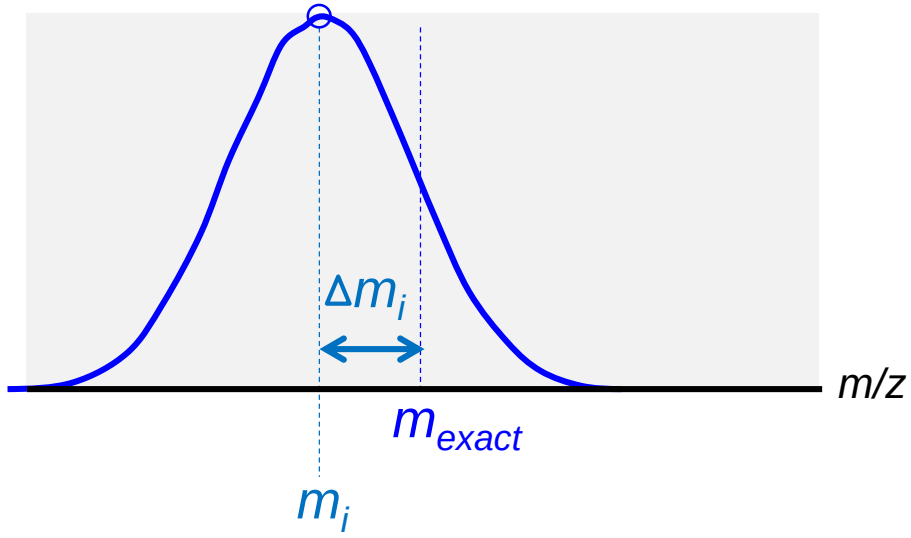
$\Delta m = 0.01$

then $R = 300/0.01 = 30{,}000$

Defining Mass Measurement Accuracy

- Mass Measurement Accuracy (MMA) is **deviation of measured mass (m_i , i for individual) from the exact mass (m_{exact}).**
- It is customary to specify MMA as a relative unit expressed in parts-per-million, ppm

Deriving mass measurement accuracy (Δm_i) from a measured mass spectrum

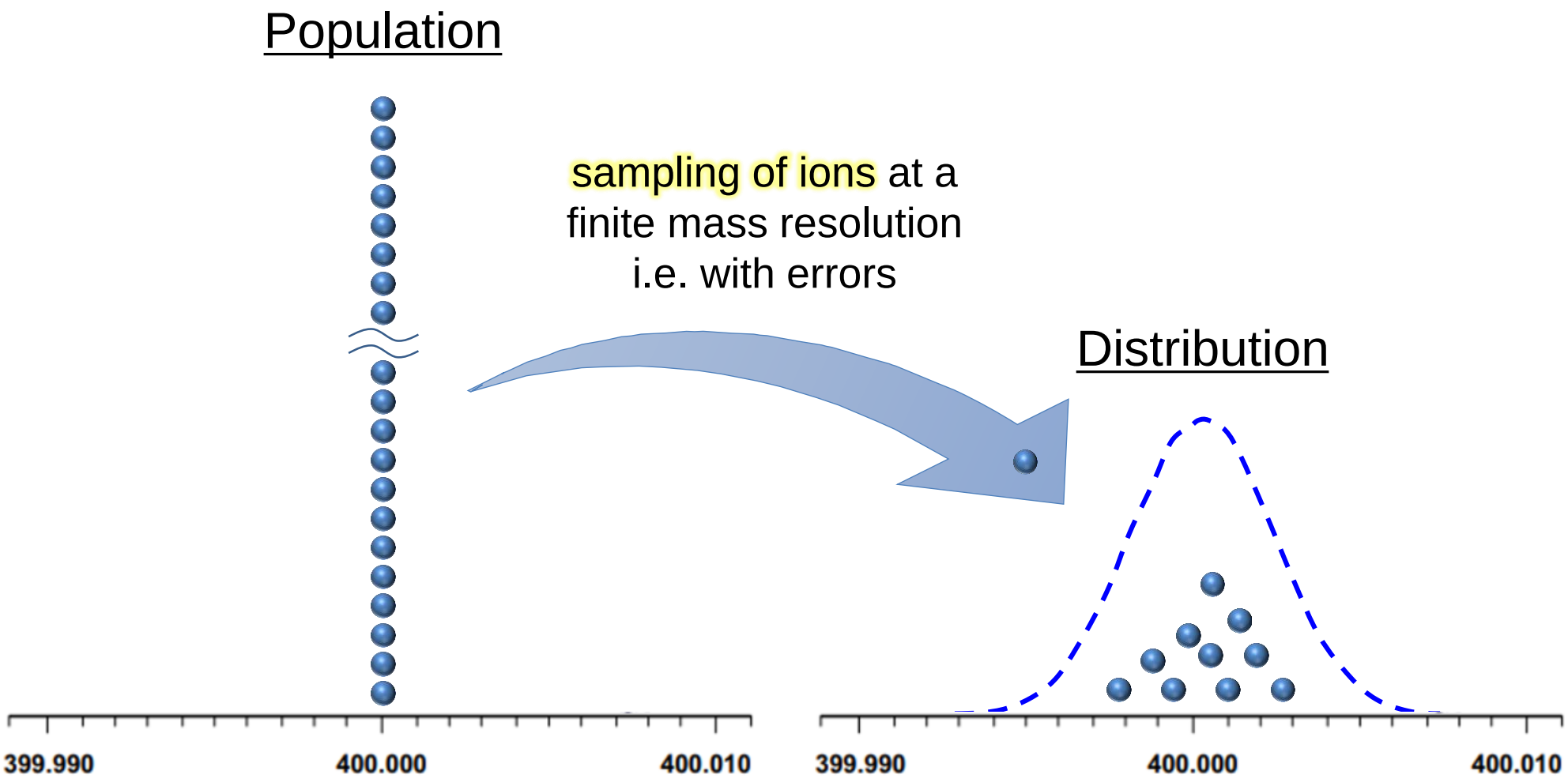


$$\Delta m_i = (m_i - m_{exact}) \quad \text{in Da}$$

$$= (m_i - m_{exact}) \times 10^3 \quad \text{in mDa}$$

$$= \frac{(m_i - m_{exact}) \times 10^6}{m_{exact}} \quad \text{in ppm}$$

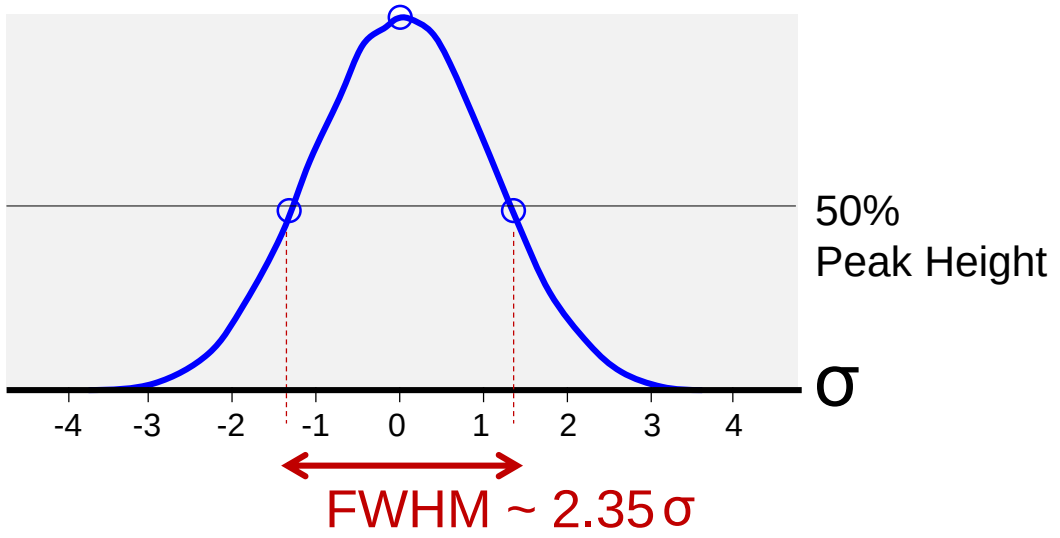
Mass Measurement is Ion Statistics

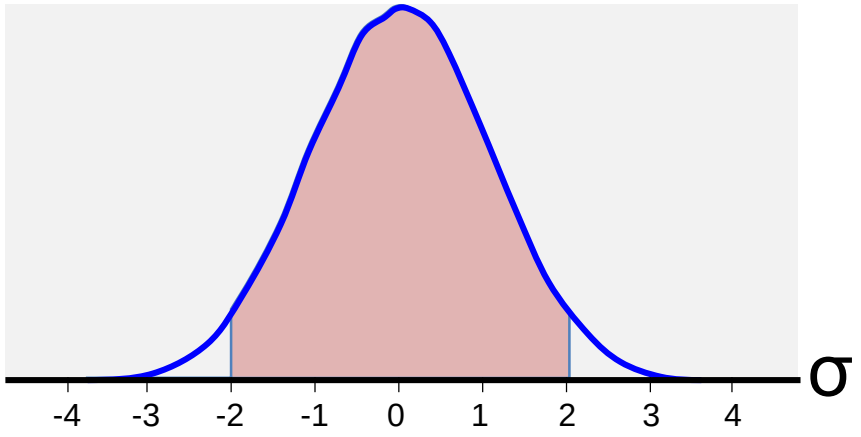


Statistical Interpretation of Mass Measurement

- Assuming Gaussian distribution allows evaluation of **precision of mass measurement** in terms of **standard deviation** (S.D., or sigma, σ)
- Mass resolution (R) and precision (σ) are interchangeable as FWHM ≒ 2.35σ

Interpreting mass peak as distribution of measured masses





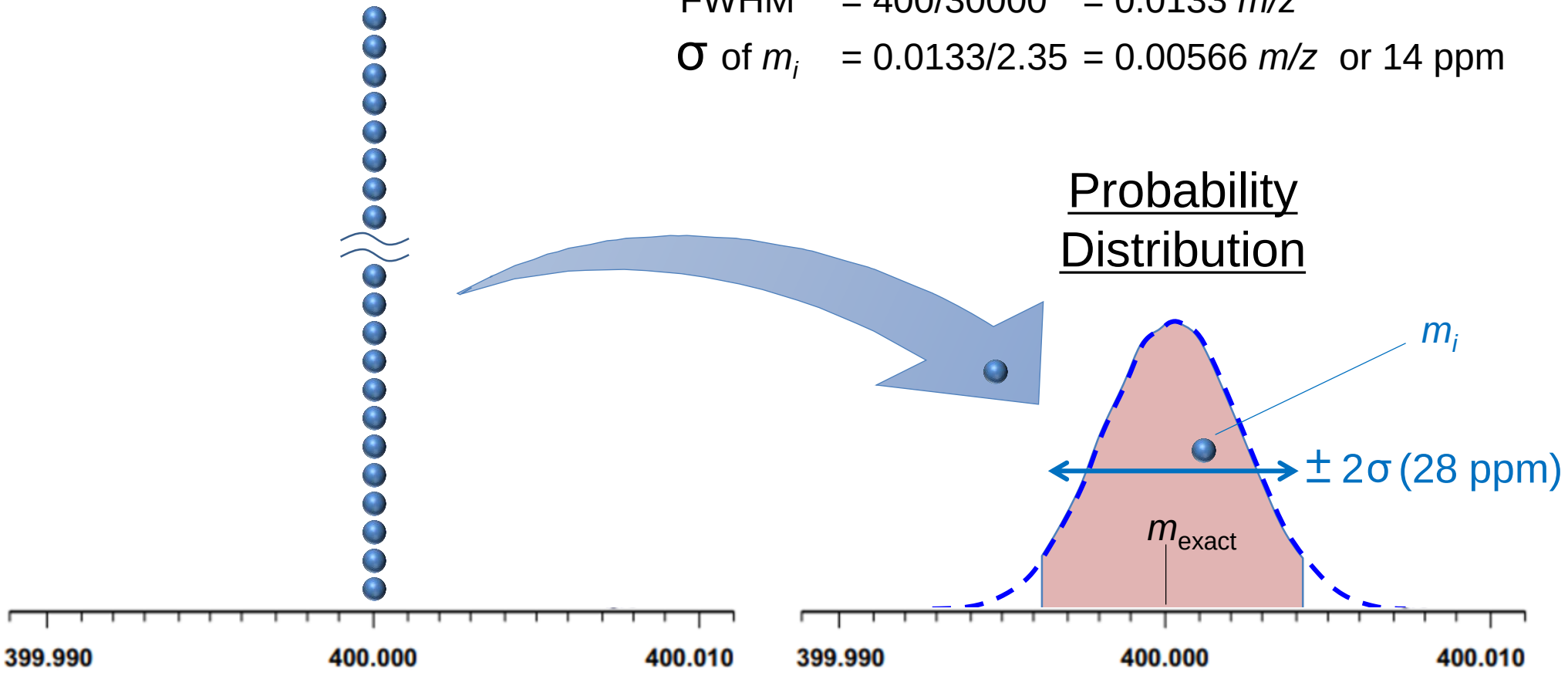
Consider a single sampling event...

Population

For R = 30,000 and m_{exact} = 400.000

FWHM = 400/30000 = 0.0133 *m/z*

σ of m_i = 0.0133/2.35 = 0.00566 *m/z* or 14 ppm

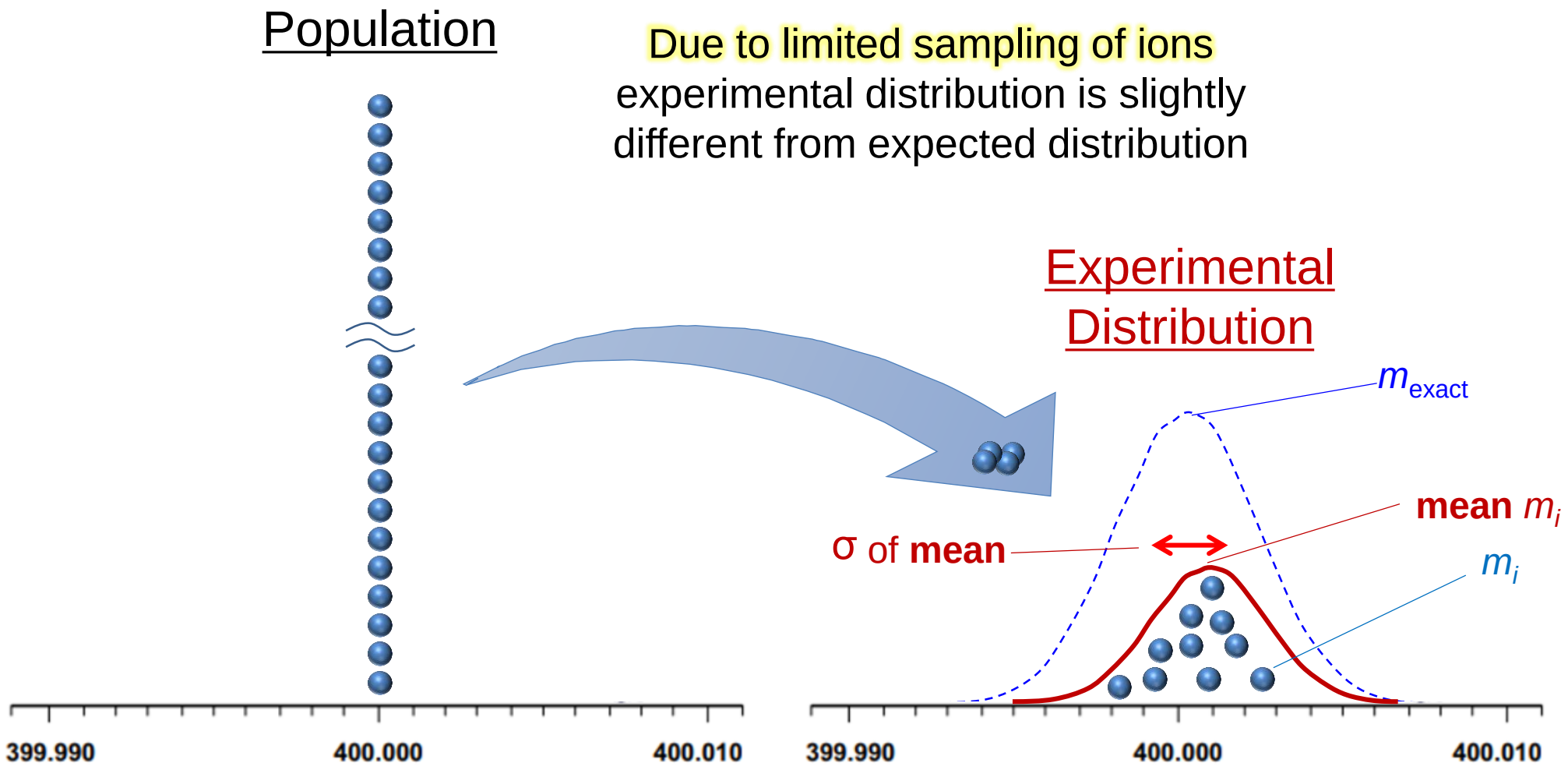


- **Repeatability m_i is directly related to R and dictates MMA**

- Obviously mass resolution is an important factor for achieving good MMA

- **However, mass resolution alone <u>does not</u> guarantee good MMA**

Now consider sampling of multiple ions



- **Experimentally measured mass = mean of all m_i**

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Theoretical Limit of Mass Accuracy

Repeatability of experimentally measured mass:

$$\sigma_{mean} = \frac{\sigma_{m_i}}{\sqrt{N}} \propto \frac{1}{R\sqrt{N}}$$

- N = number of ions

Relationship between ion statistics and theoretical limit of mass measurement accuracy for mass resolution of 30,000

Number of Ions	Theoretical Limit of MMA (95% confidence interval)
1	28 ppm
10	9 ppm
100	2.8 ppm
1,000	0.9 ppm
10,000	0.28 ppm
100,000	0.09 ppm

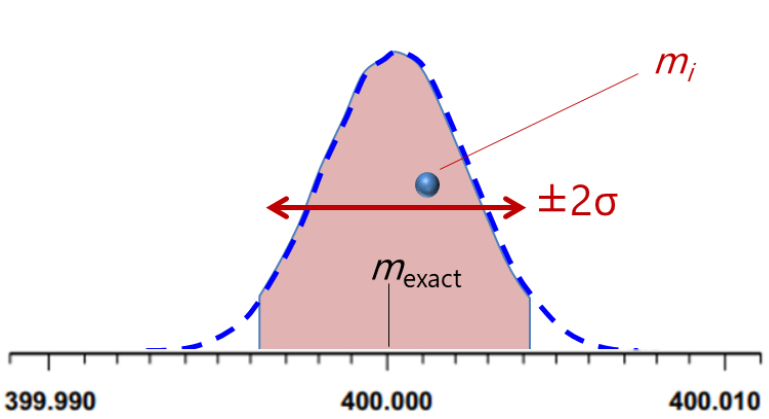
- **With 30,000 resolution, LCMS-9030 should ideally achieve <1 ppm error in this practical range of detection**

Mass Accuracy Triangle

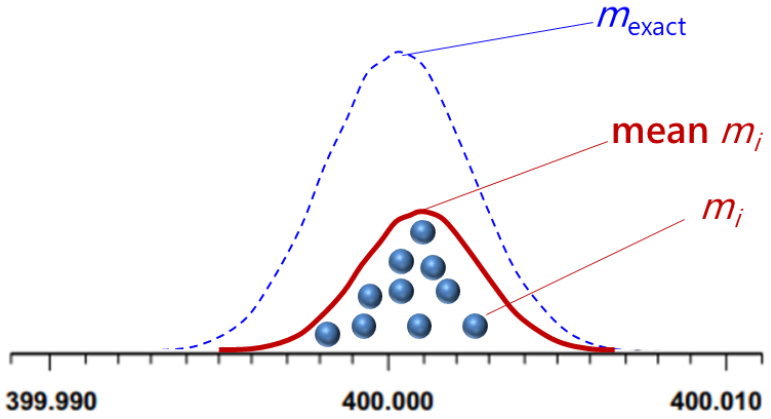
Stability

$$\frac{k}{R\sqrt{N}}$$

Resolution



Sensitivity



What factors affect the stability?

Sources of systematic error (bias):

- Temperature fluctuation
- Dead time effect of detector
  (particularly with time-to-digital converter)
- Selection of calibration standard

Sources of non-systematic error (random):

- Lack of electronic robustness
- Interference of chemical background
- Inadequate mass correction using lock-mass

Temperature: the Biggest Issue for Q-TOF

- Higher temperature gives ↑ bias
- Lower temperature gives ↓ bias to mass measurement
- Typically, a few ºC change can result in <u>10~50 ppm shift</u>

Room temp change
or parameter change

▼

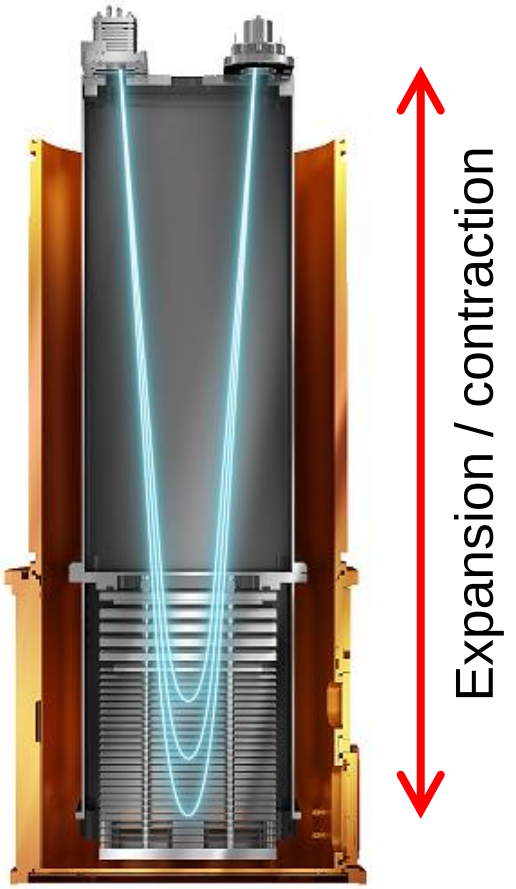
Flight tube temp change

▼

Flight tube size change

▼

TOF change

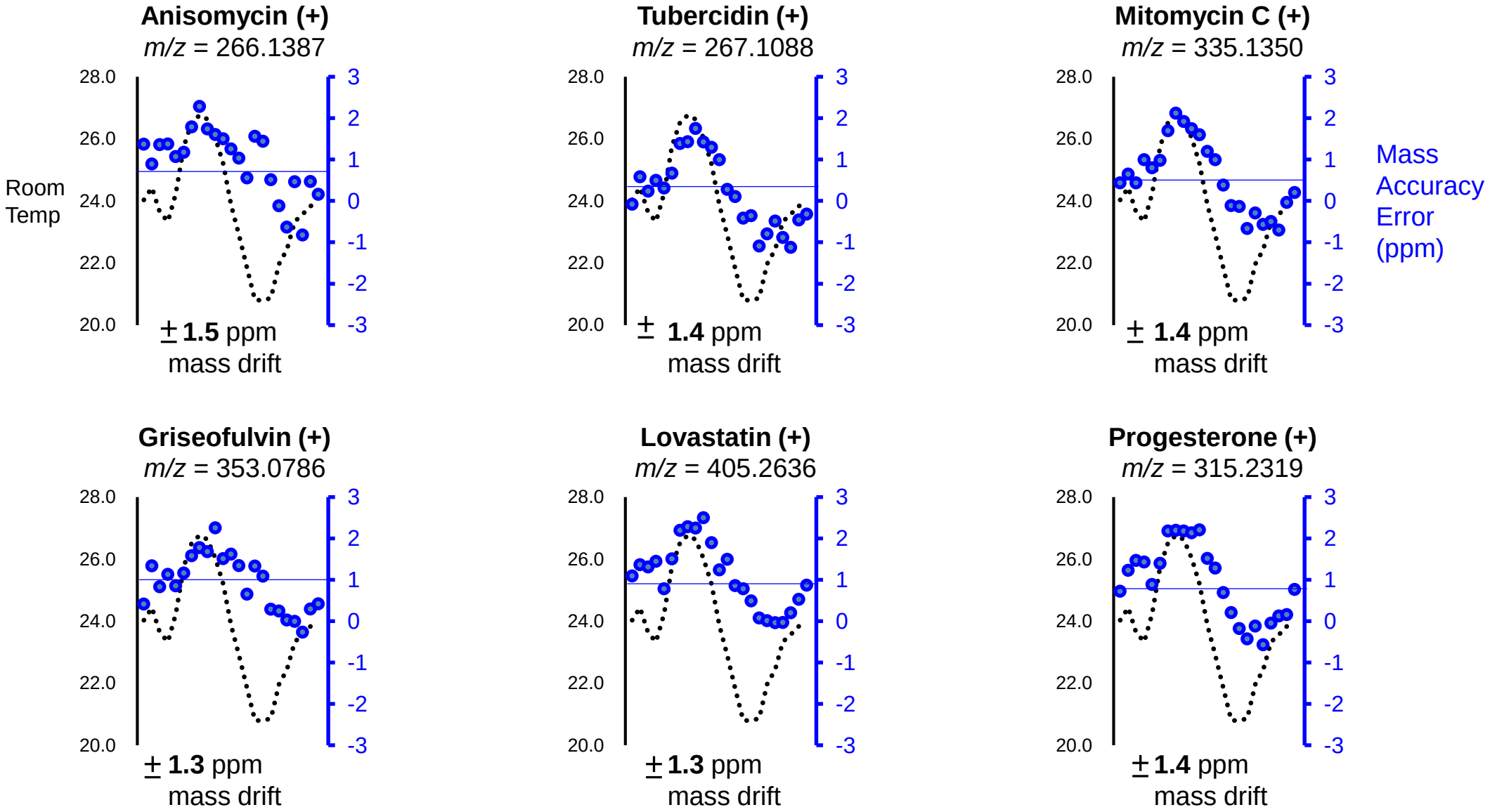


Shimadzu Quality Center at Kyoto HQ

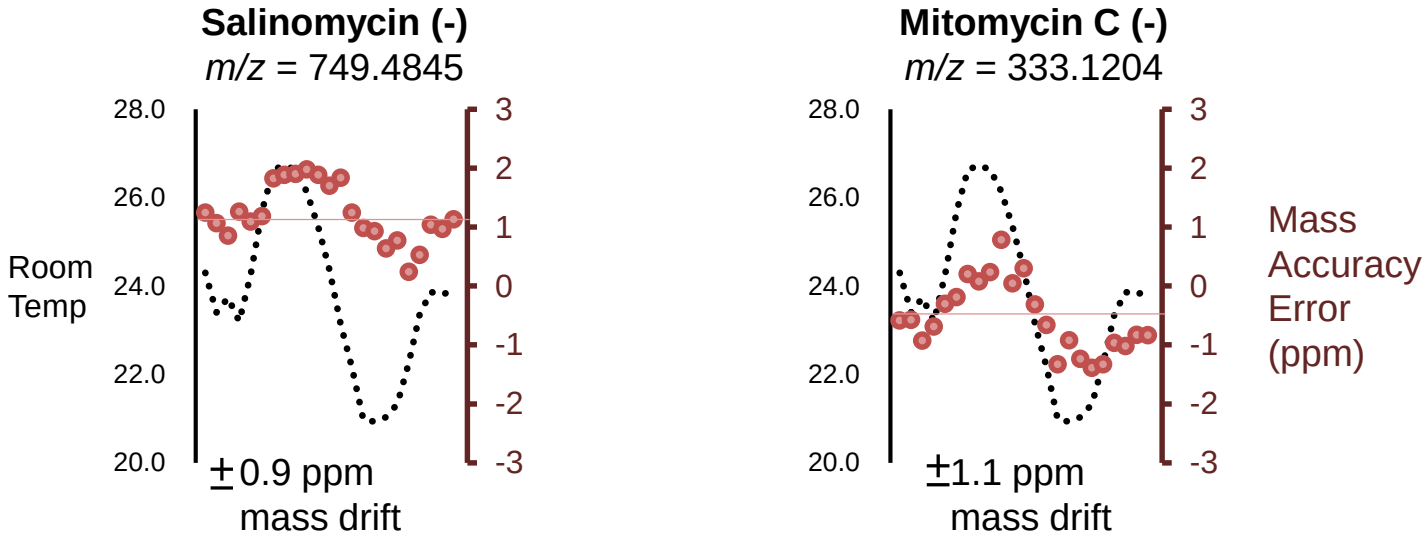
One building block fully dedicated for product quality assessment

- Performance test under harsh environment (temperature/humidity),
- Accelerated stress test,
- EMC assessment,
- Drop impact test

Temp. Stress Test (+3 ~ -3 ºC in 24h, Day 1)



Temp. Stress Test (+3 ~ -3 ºC in 24h, Day 2)



- Results showed < ±1.5 ppm mass drift, ALL at < 3 ppm error
- Only by external calibration before experiment
- No lock-mass = no correction for temperature change
- Clearly demonstrates that the REAL flight tube temp is SUPER STABLE

Sources of Non-Systematic (Random) Error

Sources of systematic error (bias):

- ~~Temperature fluctuation~~
- ~~Dead time effect of detector (particularly with time-to-digital converter)~~
- Selection of calibration standard

Temperature is under control

Analogue-to-digital converter is used

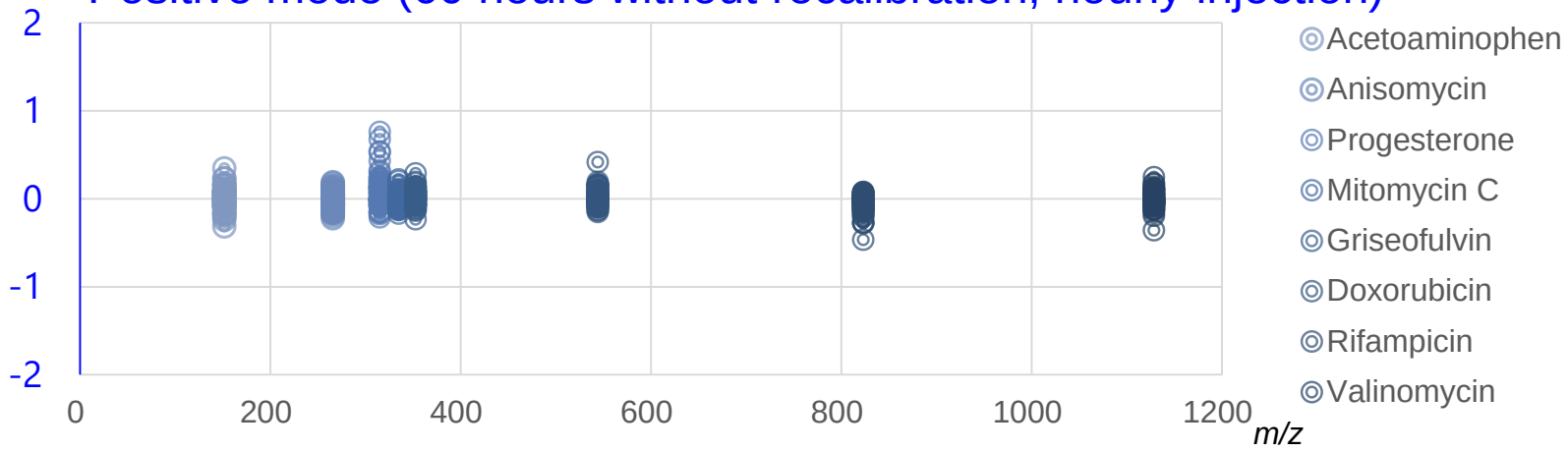
Sources of non-systematic error (random):

- Lack of electronic robustness
- Interference of chemical background
- Inadequate mass correction using lock-mass

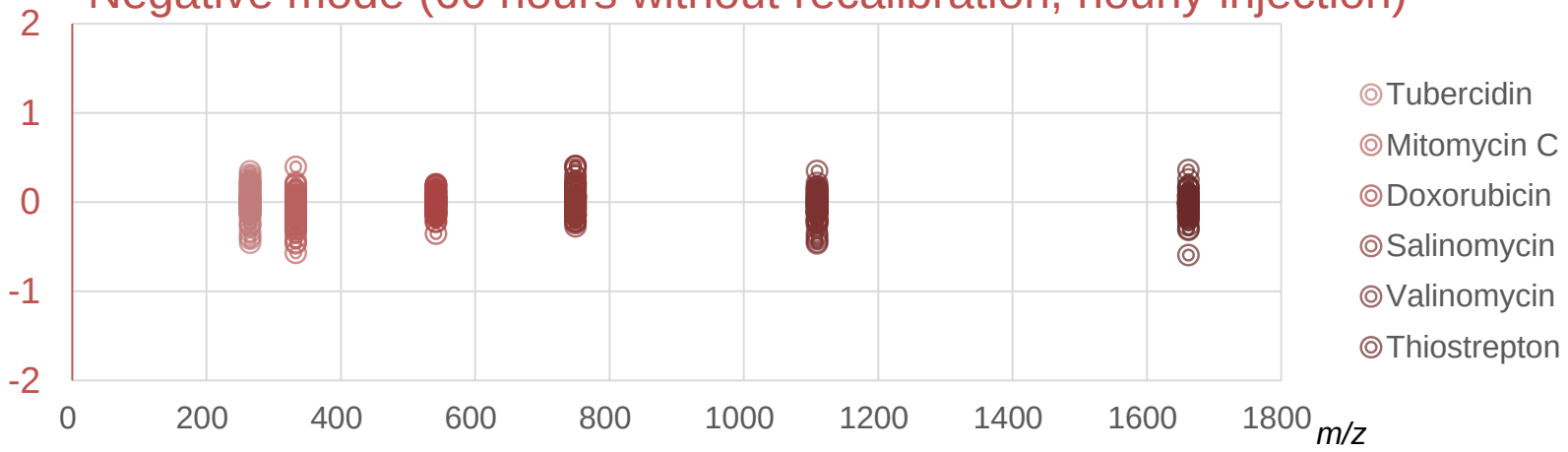
How will the data be without the systematic error?

Mass Accuracy Stability Test (60 hours)

Positive mode (60 hours without recalibration, hourly injection)



Negative mode (60 hours without recalibration, hourly injection)



<1 ppm error maintained for 60 hours only by single external calibration before the experiment

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Mass Accuracy Statistics (60 hours, n=60)

Compound	Exact Mass	Amount (pg)	Mean error (ppm)	S.D. (ppm)
Acetaminophen (+)	152.0706	2000	**0.002**	0.118
Anisomycin (+)	266.1387	100	**-0.022**	0.092
Progesterone (+)	315.2319	200	**0.090**	0.194
Mitomycin C (+)	335.1350	200	**-0.002**	0.079
Griseofulvin (+)	353.0786	100	**0.017**	0.090
Doxorubicin (+)	544.1813	2000	**0.025**	0.099
Rifampicin (+)	823.4124	200	**-0.057**	0.101
Valinomycin (+)	1128.6650	20	**-0.008**	0.097
Tubercidin (-)	265.0942	2000	**-0.002**	0.170
Mitomycin C (-)	333.1204	200	**-0.097**	0.172
Doxorubicin (-)	542.1668	2000	**-0.015**	0.113
Salinomycin (-)	749.4845	20	**0.025**	0.164
Valinomycin (-)	1109.6239	200	**-0.045**	0.160
Thiostrepton (-)	1662.4851	2000	**-0.033**	0.138

- Statistically correct – mean absolute error converges to zero as N increases
- All data under same external calibration i.e. no bias or drift was observed.

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- Without the systematic error (bias), **S.D.** of multiple measurement represents **the degree of system stability against non-systematic error**

NB. Relative Mean Square (RMS) is frequently used to approximate S.D. for small number of repeats.

Stability of a Q-TOF Instrument

Sources of systematic error (bias):

- ~~Temperature fluctuation~~
- ~~Dead time effect of detector (particularly with time-to-digital converter)~~
- ~~Selection of calibration standard~~

Sources of non-systematic error:

- ~~Lack of electronic robustness~~
- Interference of chemical background
- ~~Inadequate mass correction using lock-mass~~

Temperature is under control

Analogue-to-digital converter is used

How will the data be without systematic error?

Extremely robust and stable

No need to use lock-mass correction

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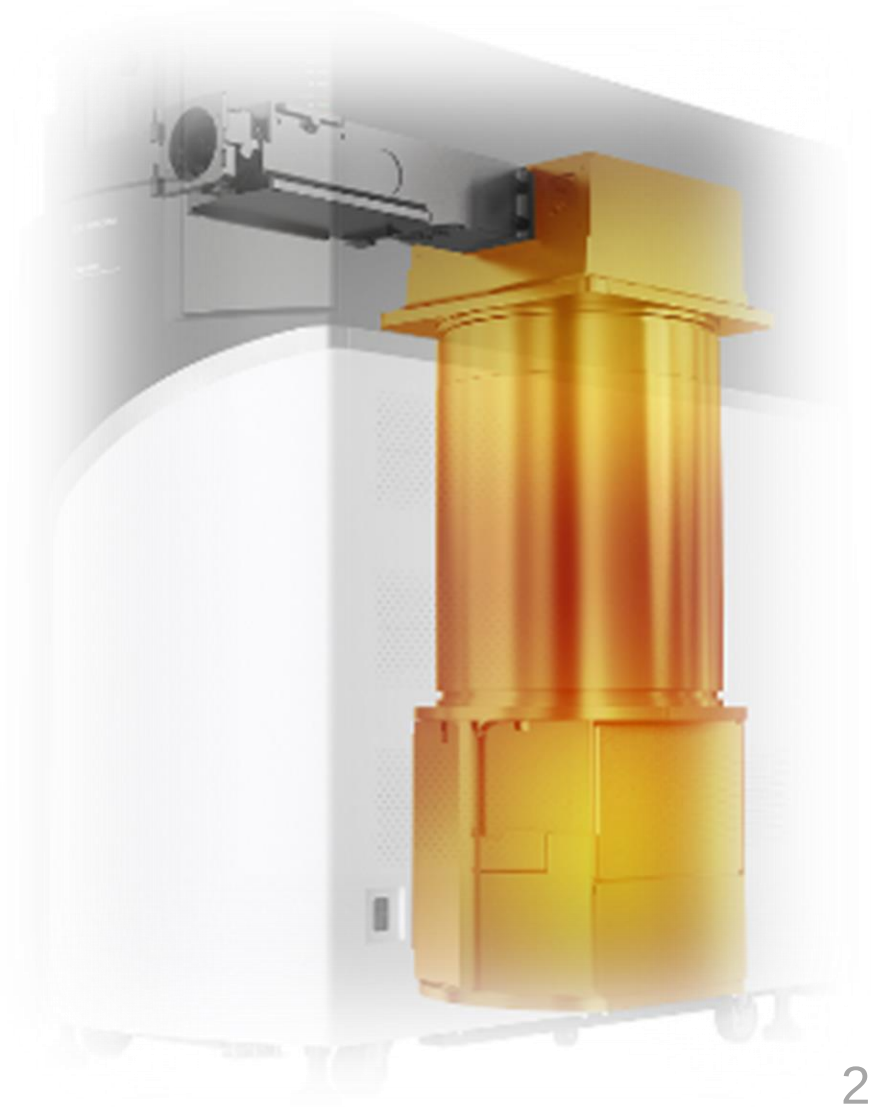
How Temperature was Put under Control

THREE unique technologies were newly developed for the **UF-Flighttube™**

1. **Computational approach to optimization of heater/sensor positions**

2. **Novel feedback/feedforward algorithm**

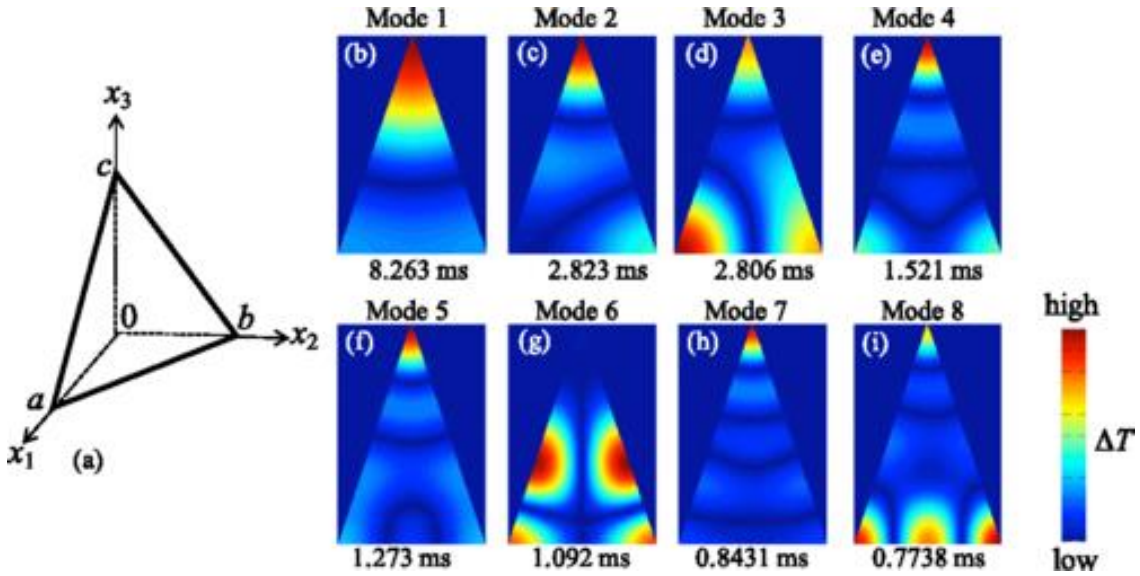
3. **Patented Ni-plating for maximizing radiation**



Optimization by Heat Eigenmode Expansion

H Ogi *et al.*, reported in Physical Review Letters (2016) that they observed the thermal modes of objects for the first time.

https://doi.org/10.1103/PhysRevLett.117.195901



Thermal modes of the aluminum triangular pyramid

Heat does not conduct uniformly but **in waves** in the first instance, similar to vibrational modes.

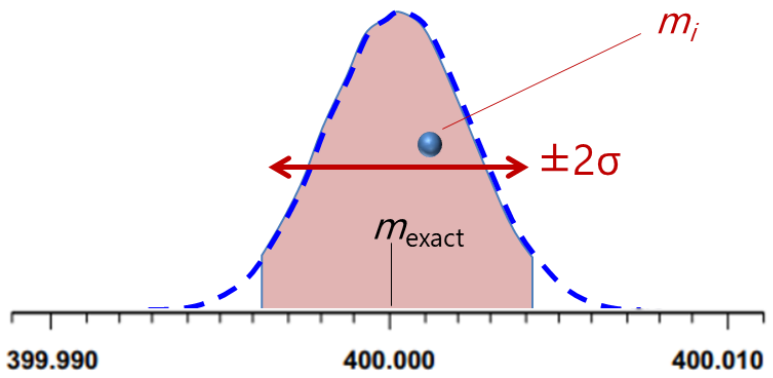
Computationally modeling the thermal modes helps identifying potential hot spots and cold spots and how the **position of heater and sensor** can be optimized to mitigate the inefficiency.

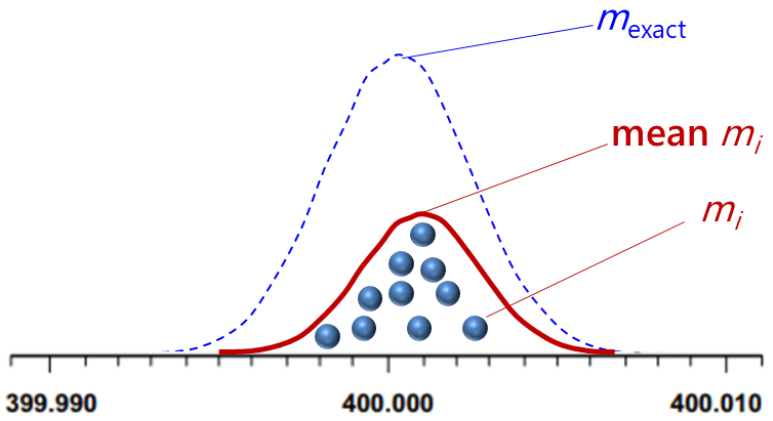
Balancing the Mass Accuracy Triangle

Stability

$$\frac{k}{R\sqrt{N}}$$

Resolution **Sensitivity**



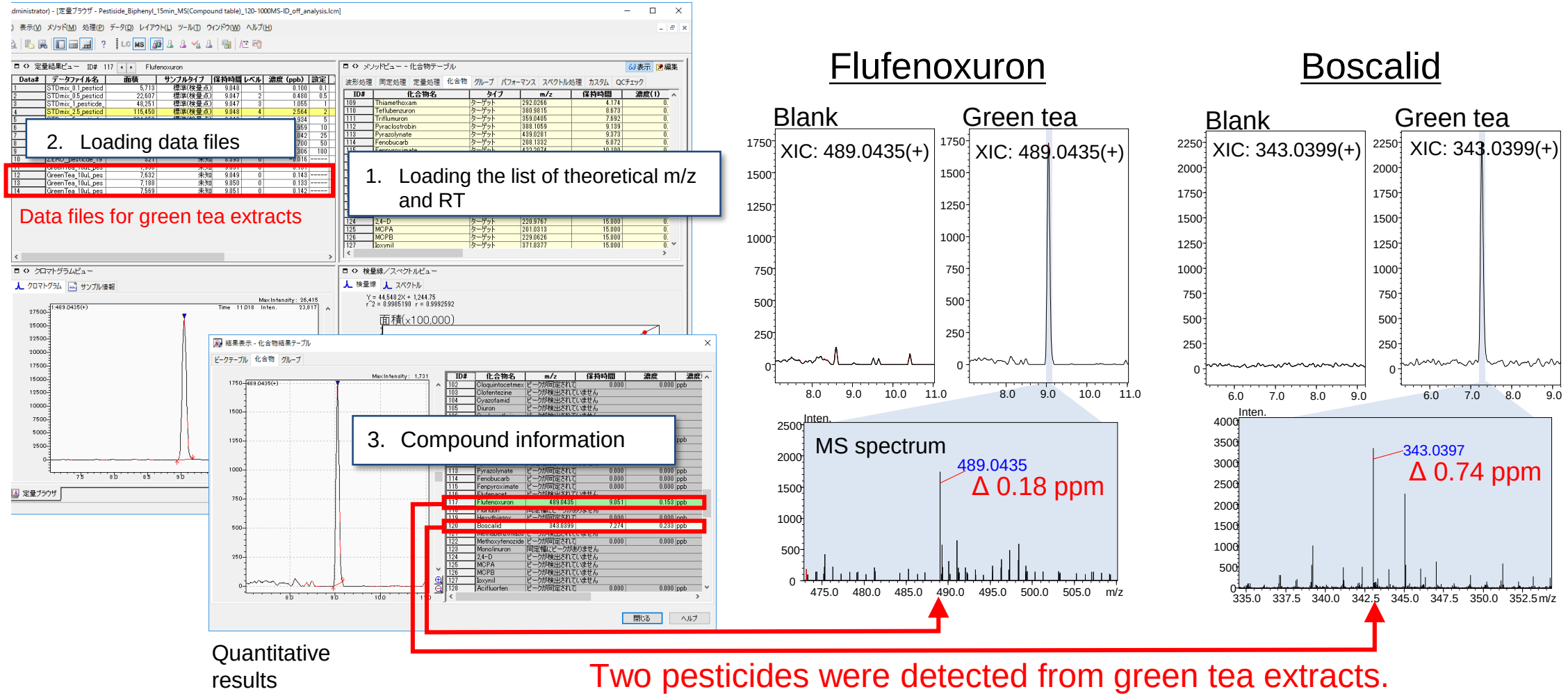


Key Take-aways

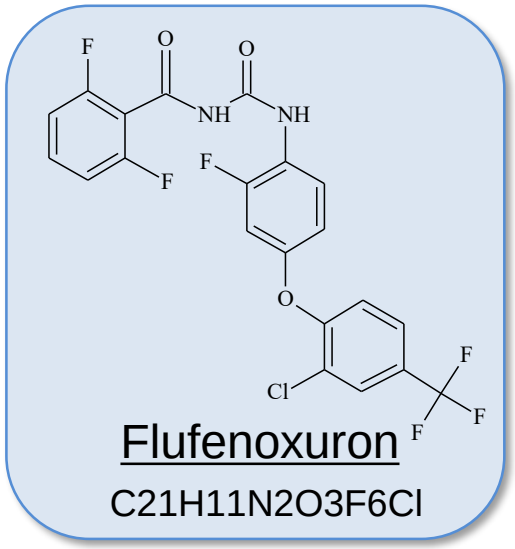
- **Balancing Resolution, Sensitivity and Stability is important**
  - 30,000 resolution was sufficient to deliver <1 ppm MMA
  - High sensitivity and stability can complement the resolution
- **Temperature fluctuation is the most critical issue for a Q-TOF, often necessitating the use of internal calibration or correction.**
  - LCMS-9030 has very sophisticated temperature control system, alleviating the need for internal correction
- **Achieving <1 ppm in one measurement can occur by chance. Benchmark should be S.D. of error through series of analysis.**
  - LCMS-9030 achieved S.D. <0.2 ppm in the 60 h batch without re-calibration showing remarkable stability against non-systematic errors.

Using High Mass Accuracy Data

A database of RT and accurate mass will identify pesticides from non-targeted MS1 data



Elucidation of Elemental Composition



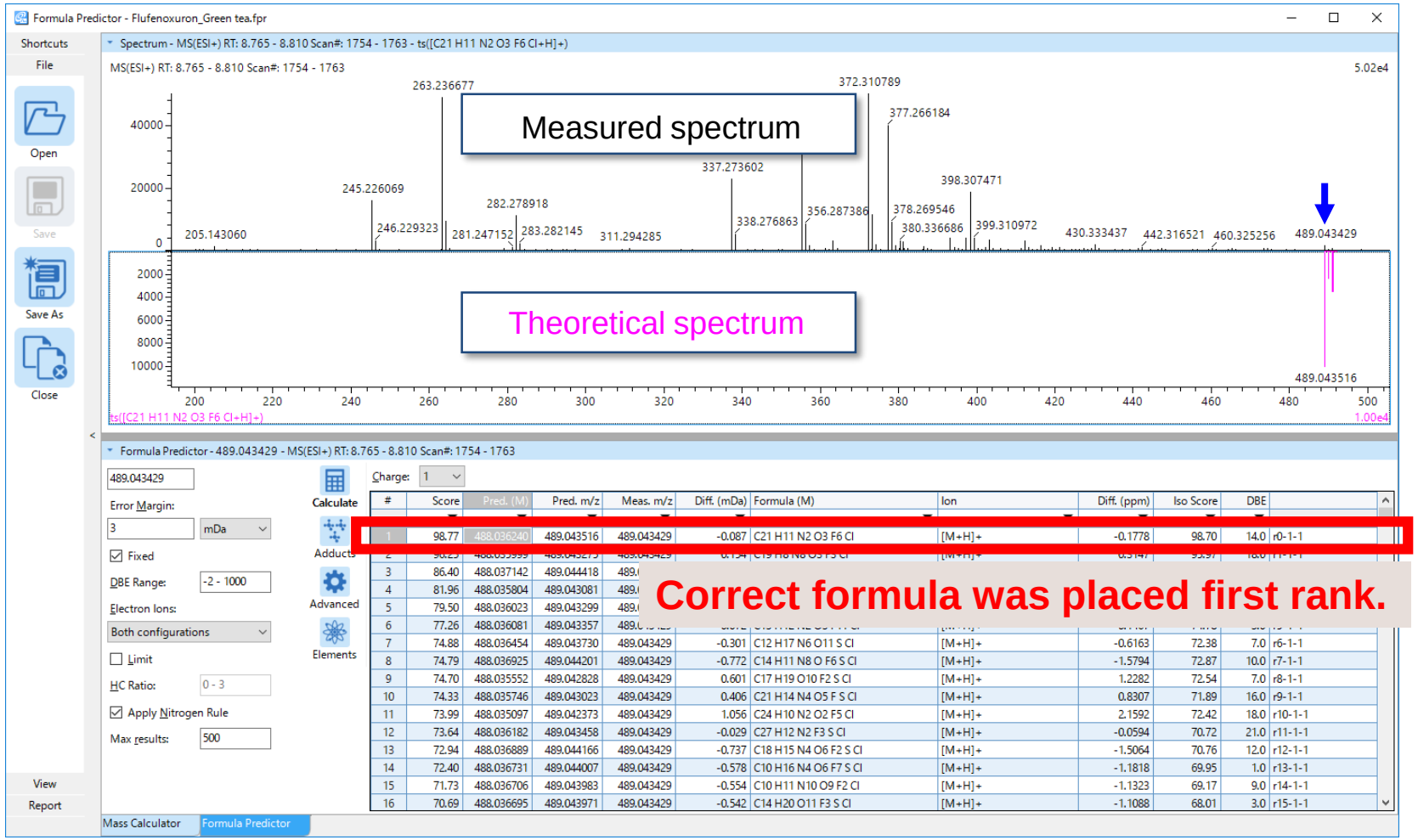
Flufenoxuron

C21H11N2O3F6Cl

Element table

Name	Min	Max
Carbon	0	150
Hydrogen	0	300
Nitrogen	0	12
Oxygen	0	12
Fluorine	0	12
Sulphur	0	12
Chlorine	0	12

Verifying the screening result by Formula Predictor using MS1 data only



Correct formula was placed first rank.

Impact of Mass Accuracy Window for Prediction

- Example for m/z 542.1

- Number of possible formulae using C, H, O, N

4090 Possible +/- 1 amu

2066 Possible +/- 0.5 amu

522 Possible +/- 0.1 amu

58 Possible +/- 20 ppm

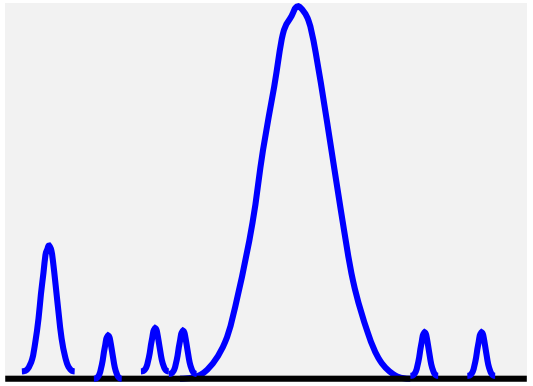
15 Possible +/- 5 ppm

3 Possible +/- 1 ppm

Impact of Mass Accuracy on Quantitation

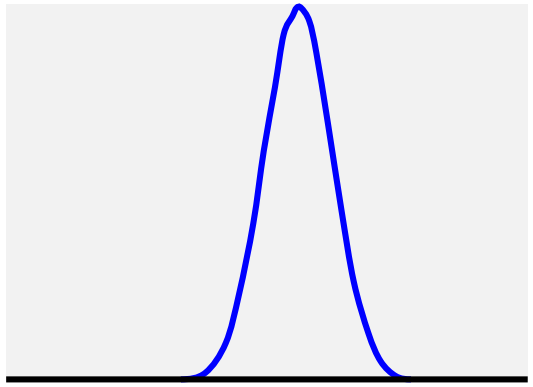
- **Mass accuracy window is also important for generating extracted ion chromatogram (XIC) of high S/N ratio**

XIC window too wide

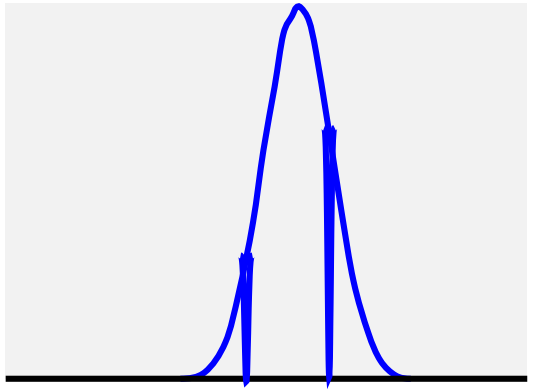


High noise

Good XIC window



XIC window too narrow



Missing data points

Conclusion

- **Benchmark the repeatability under laboratory condition (e.g. 24 hour batch).**
  - Evaluate the mean MMA of multiple analyses as the indicator of systematic error.
  - Use the S.D. as the metric for system stability.
- **S.D. provides the basis to predict the expected range of MMA.**
  - If S.D. was 0.2 ppm,
    - 95% of data is expected to fall within 2 σ = 0.4 ppm
    - 99% of data is expected to fall within 3 σ = 0.6 ppm around the mean MMA
- **Set appropriate mass accuracy window for:**
  - Effective Compound Identification
  - Generating XIC of high S/N ratio

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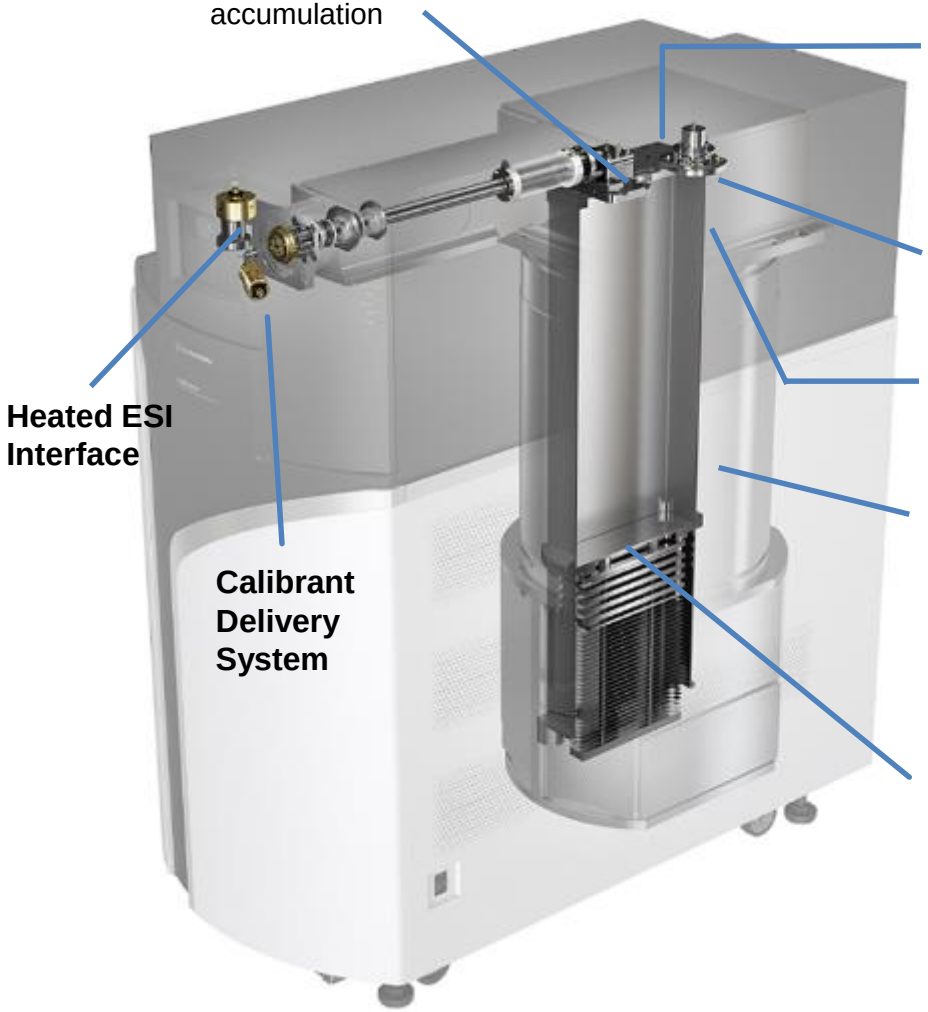
Effortless Performance LCMS-9030

MASS
ACCURACY
SENSITIVITY
SPEED



UF Grating™
(Patent WO 5772967)
Shimadzu's patented puller electrode with outstanding mechanical strength delivers higher sensitivity and resolution.

Funnel MCP
A new detector design for minimal loss

High-speed Digitizer
High-speed data acquisition up to 100 Hz

UF-FlightTube™
Novel simulation algorithm based on latest physics enabled the patented control system for flight tube temperature, needed for stability of mass accuracy.

iRefTOF™
(Patent WO 5629929 and 5924387)
A computationally ideal electrostatic field has become a reality.

Thank you

Contact us

https://www.shimadzu.com/an/contact/index.html