



Chemalys

Massworks

Extend the Limits of Your LC/MS-System

Jan Nordin

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Background

- In early times of Mass Spectrometry Sector Instruments with high resolution >10.000 was used for exakt mass determination.
- Later on Quadrupoles also was used in MS/MS mode for investigation of structures.
- Today most people like to use TOF instrument or preferable OrbiTrap's for as good formula id as possible.
- But do we have other choises?



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Why did we look into this type of software?

- When working with DART & AccuTOF we realized that the resolving power of $R=8000$ was not enough for a correct formula id.
- Even with higher resolution $R=18.000$ we got to many choices
- When dealing with totally unknown samples we realized that we need better tools.
- We looked into several different programs for accurate mass determination and also isotope pattern recognition but found that spectral accuracy was a even better method to use



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- We started a cooperation with

cerno
BIOSCIENCE

- Two programs:
 - **CLIPS** for low resolution instrument like Quadrupoles
 - **sCLIPS** for self calibration on TOF or higher resolution instruments



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CLIPS and Spectral Accuracy (SA)

Calibrated Lineshape Isotope Profile Search

$$SA = \left(1 - \frac{\| \mathbf{r} \|_2}{\| \mathbf{s} \|_2} \right) \times 100$$

\mathbf{s} – mass spectrum as a continuum vector

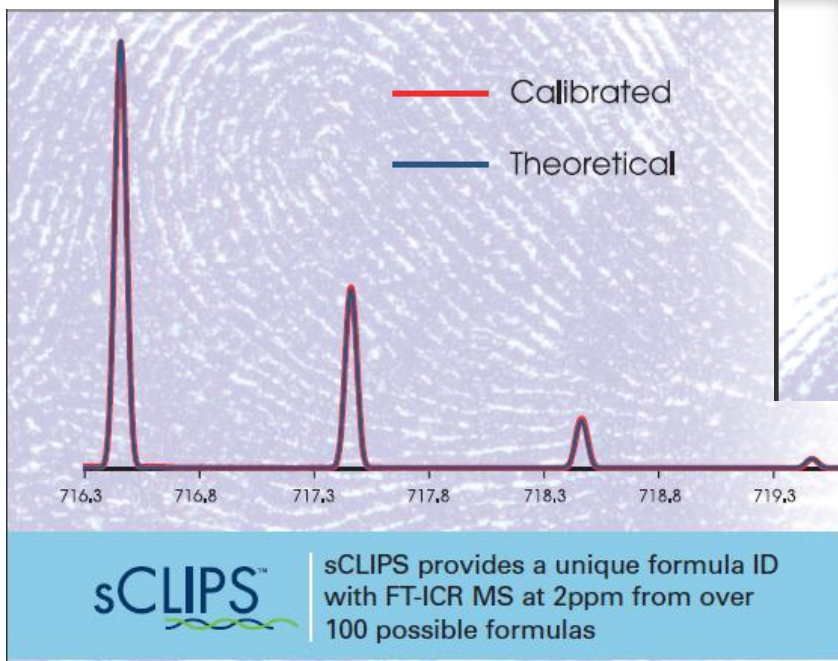
\mathbf{r} – least squares fitting residual, also as a continuum vector



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sCLIPS

self Calibrated Lineshape Isotope Profile Search



Improved Formula ID from High Resolution MS

- ✓ Line-shape calibration for exact isotope modeling without standards
- ✓ Compare calibrated spectra to theoretical with up to 99.9% Spectral Accuracy
- ✓ Relaxes the need for constant accurate mass [re]calibration
- ✓ For use with all TOF, Orbitrap, FT-ICR, high res quadrupole, and magnetic sector instruments



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Content

- Confirmation of a molecular weight
- Find an unknown compound
- Strategy
- Examples from:
 - GC/MS Environmental
 - LC/MS Pharmaceutical
 - TOF



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Confirmation of a molecular weight

- When you know the expected composition of your compound you have the advantage of:
 - Knowing the elements
 - Having the exact mass formula



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A totally unknown compound

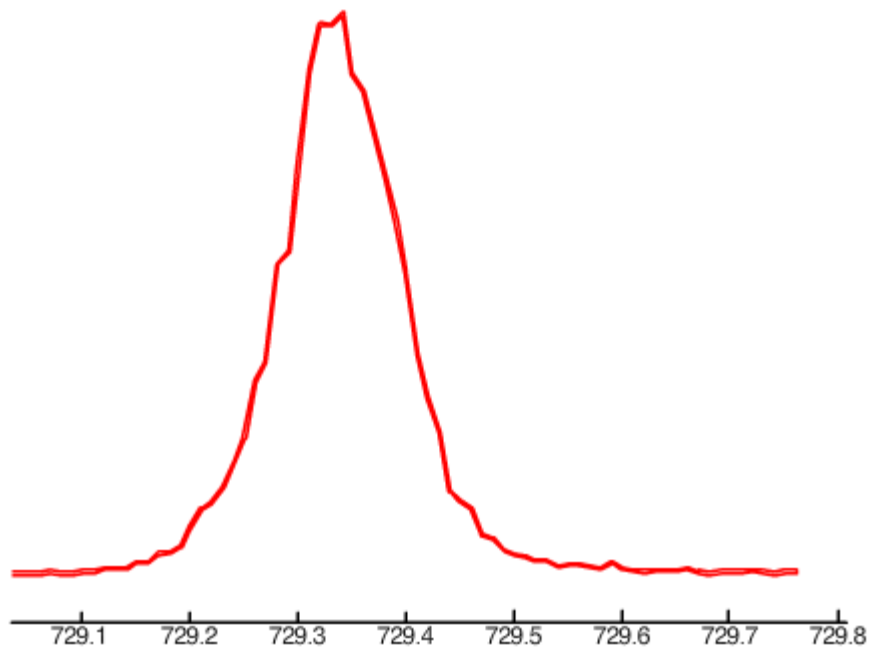
- You don't have a clue of the chemical composition
- The numbers of choices are to many even if you use a TOF-instrument



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MS data is not acquired as centroid

MS data is acquired as a continuum

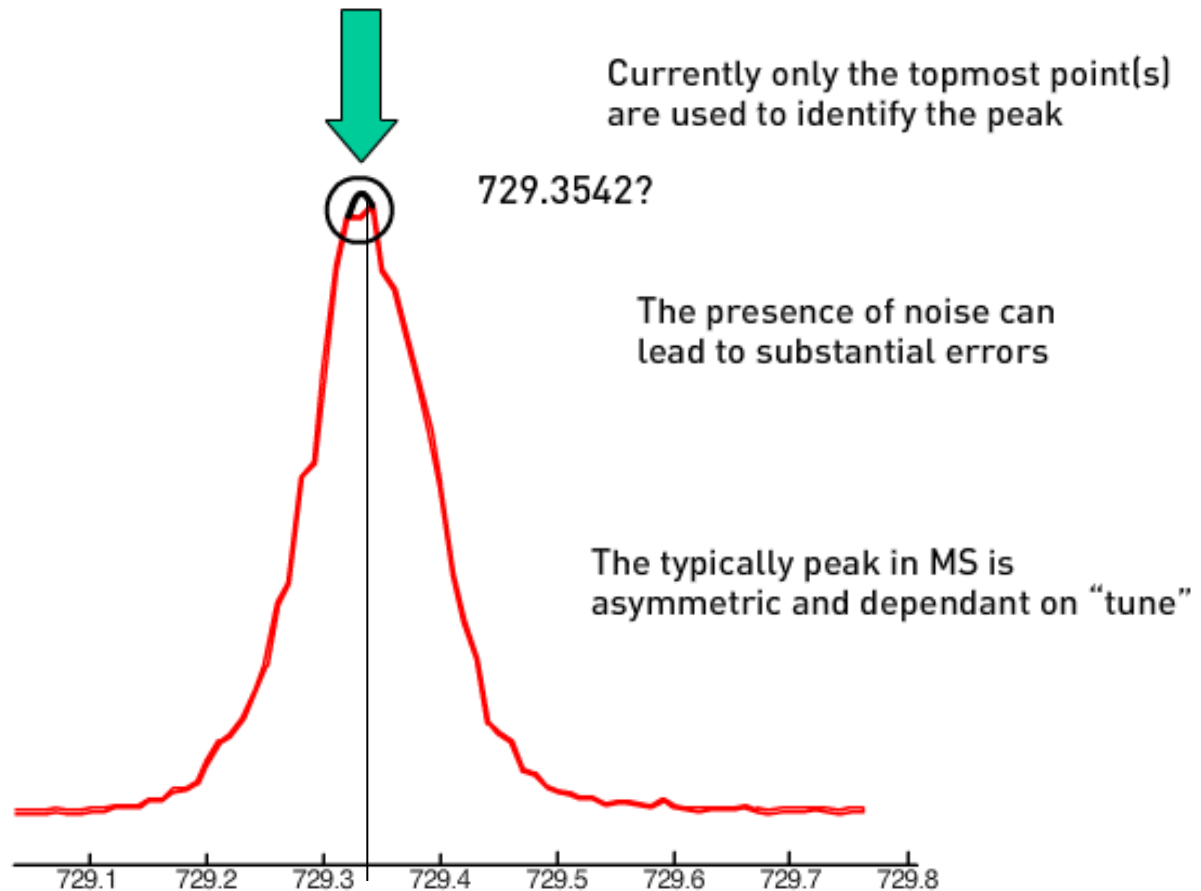




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Only the top of the data is used for information

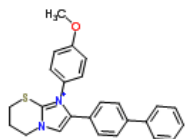
MS data is processed to locate the peaks





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Theoretical composition

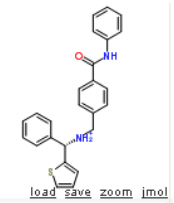
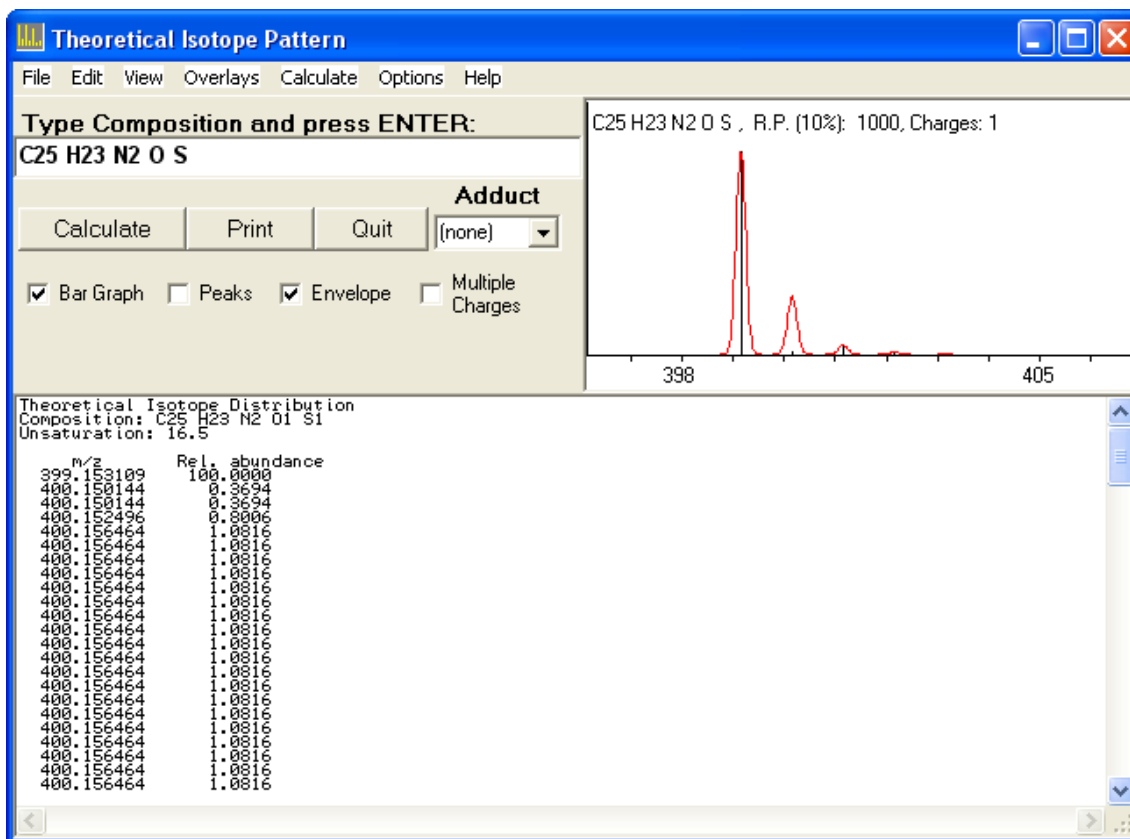


N-[1-(3,4-diphenylthiazol-3-ium-2-yl)methyl]vinyl]-4-methoxy-aniline

1664434

C₂₅H₂₃N₂O₂S 399.5274 399.15256

load save zoom imol

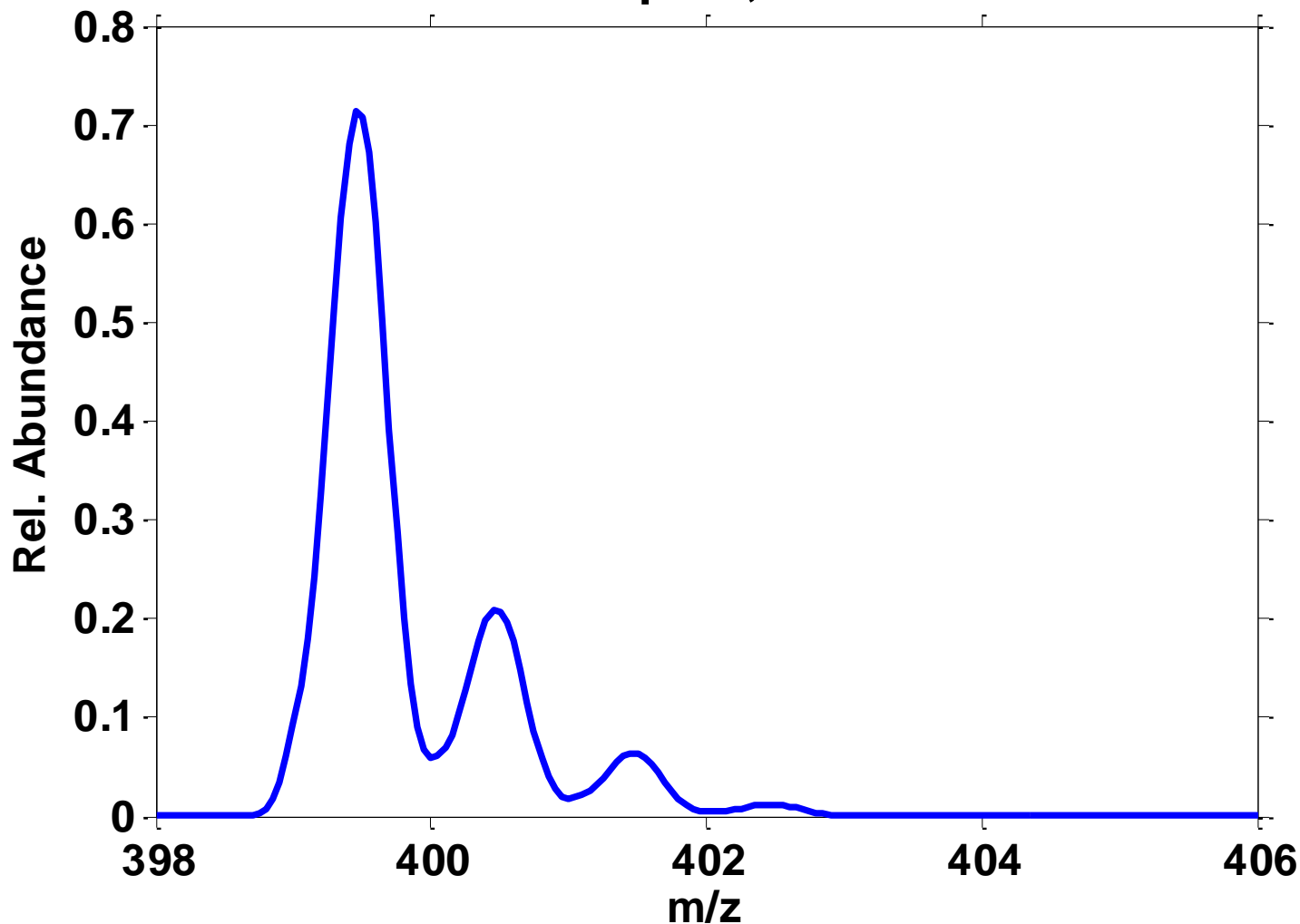




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Actual MS (Profile/Raw): $C_{25}H_{23}N_2OS^+$

Quadrupole, R = 800

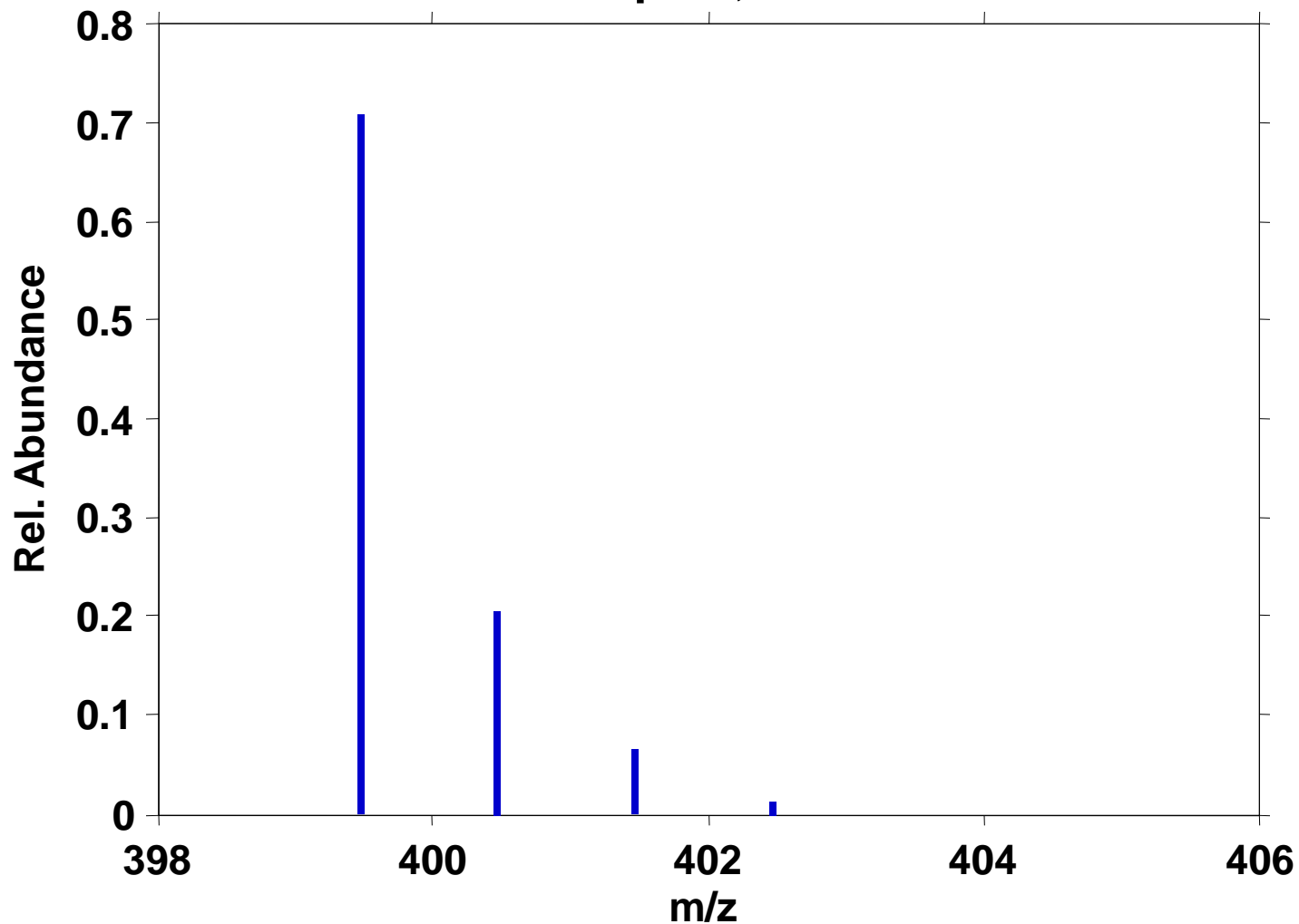




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After MS Centroiding: $C_{25}H_{23}N_2OS^+$

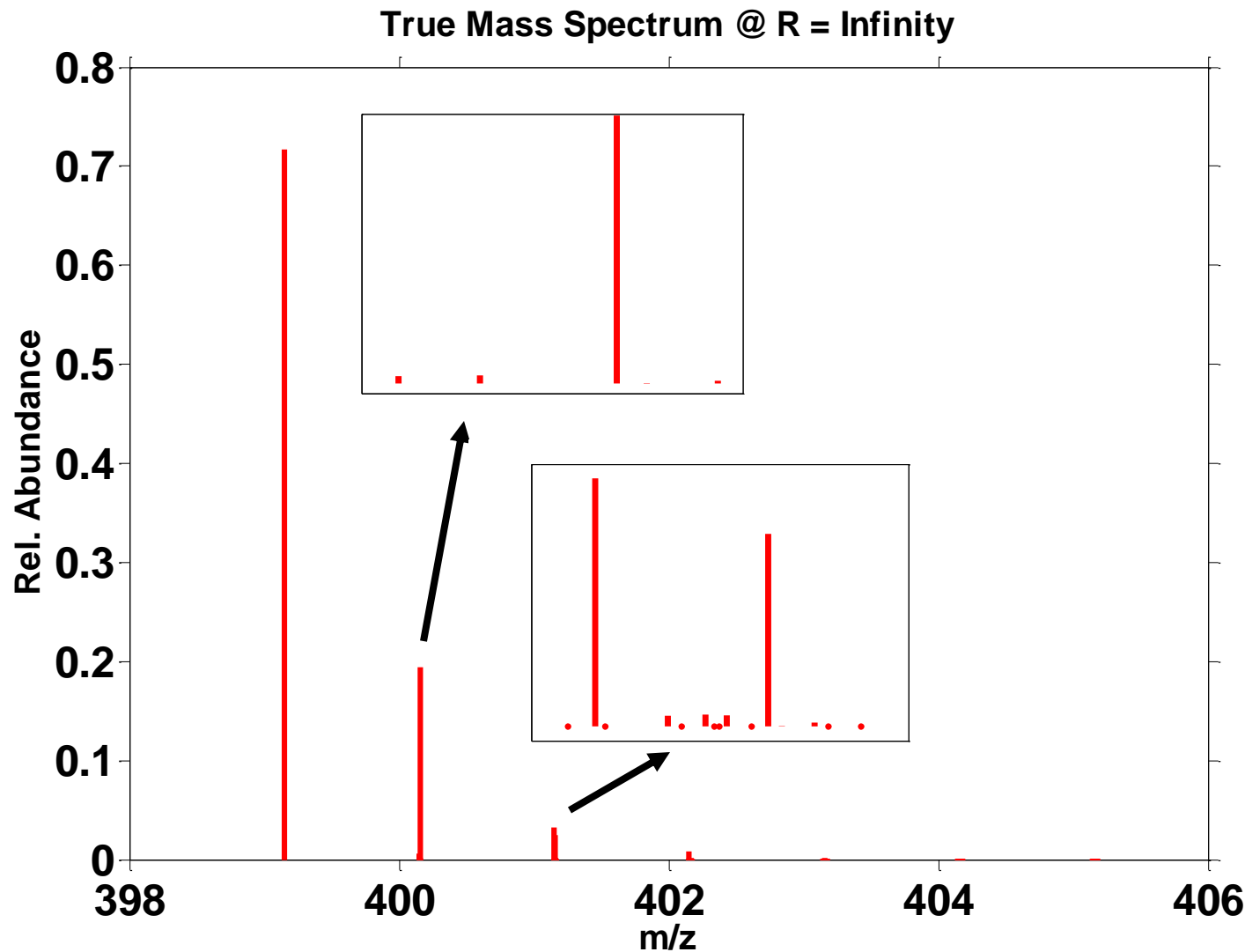
Quadrupole, R = 800





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The True Mass Spectrum: $C_{25}H_{23}N_2OS^+$

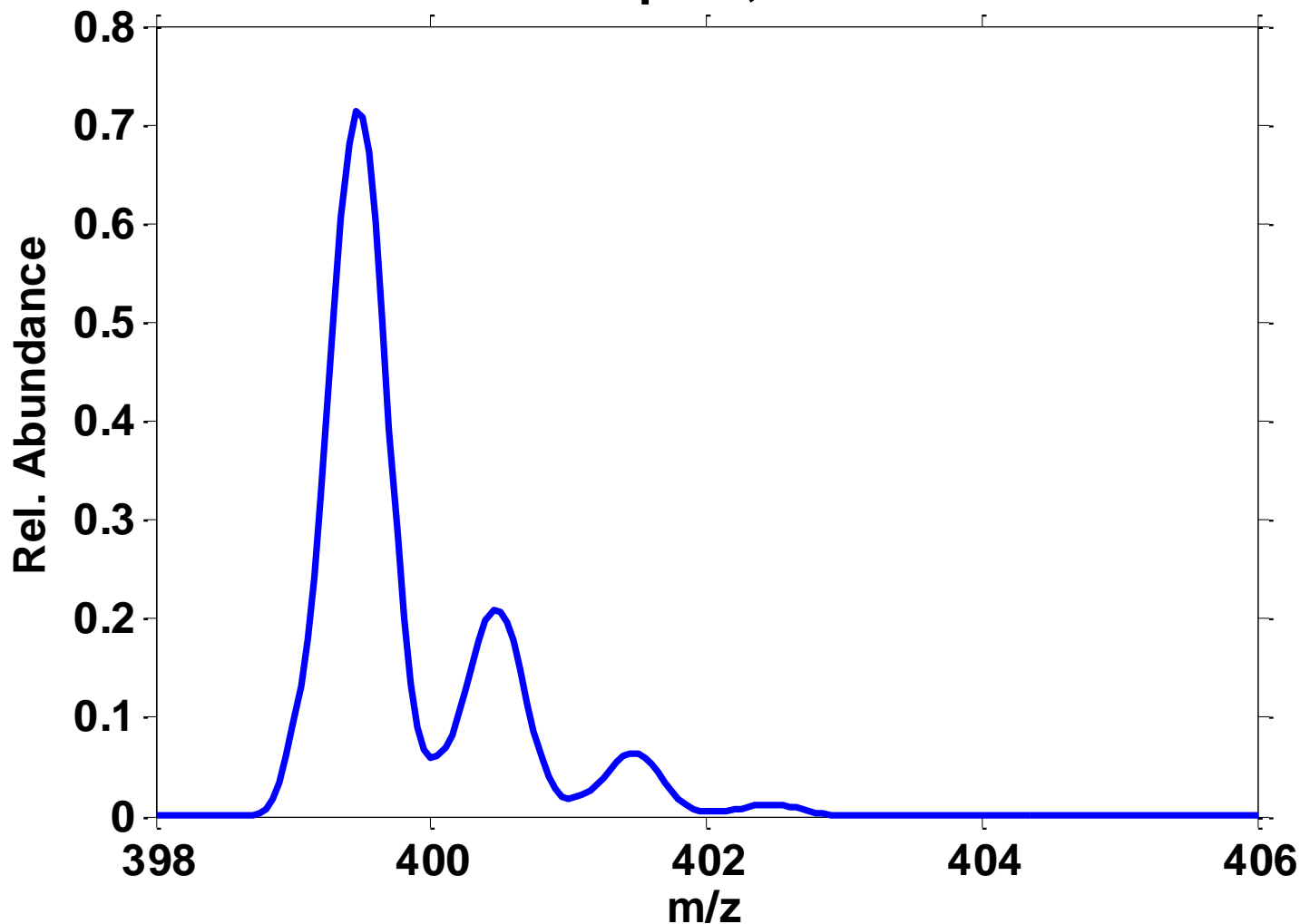




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Actual MS (Profile/Raw): $C_{25}H_{23}N_2OS^+$

Quadrupole, R = 800

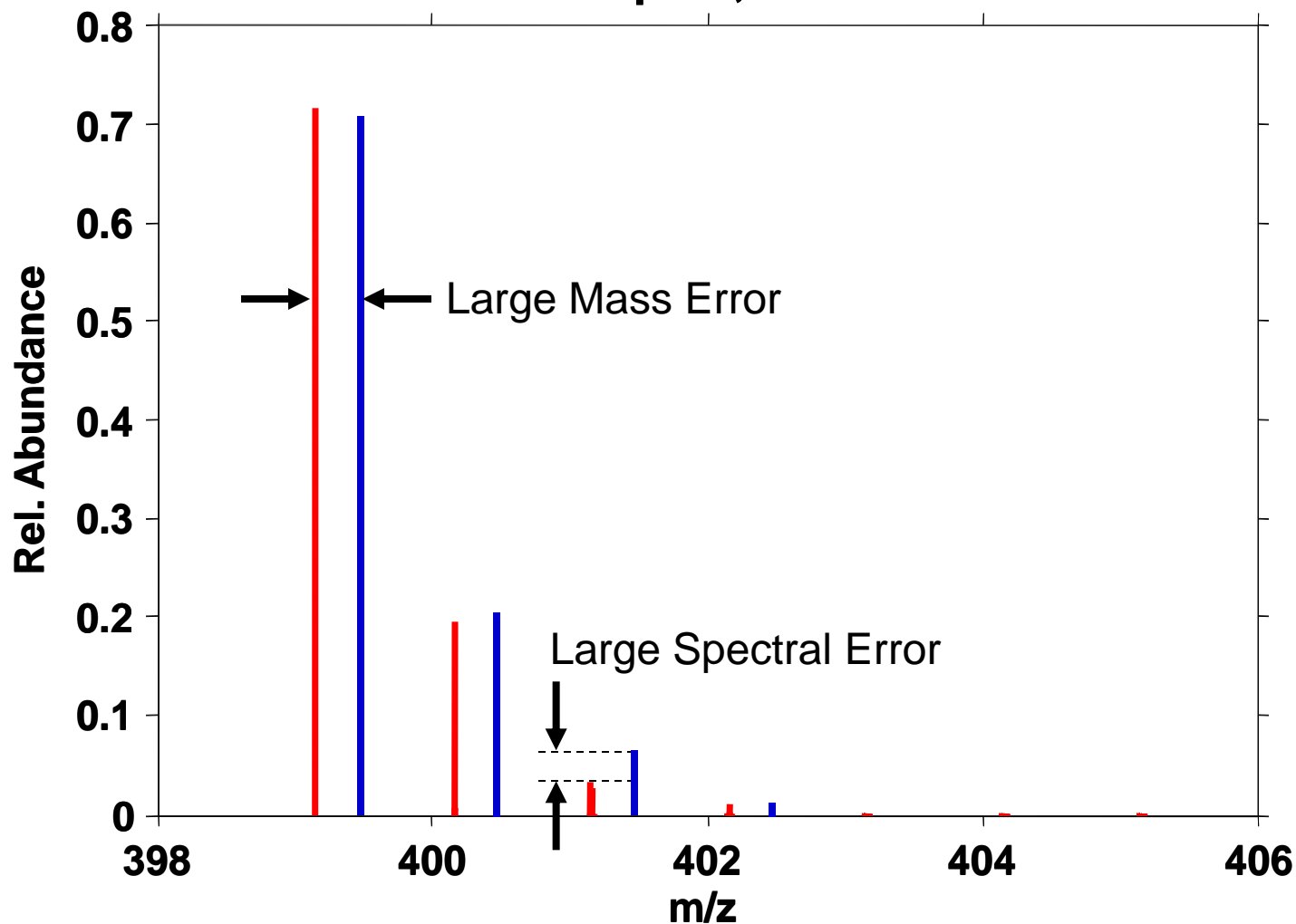




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Centroid vs True MS: $C_{25}H_{23}N_2OS^+$

Quadrupole, $R = 800$

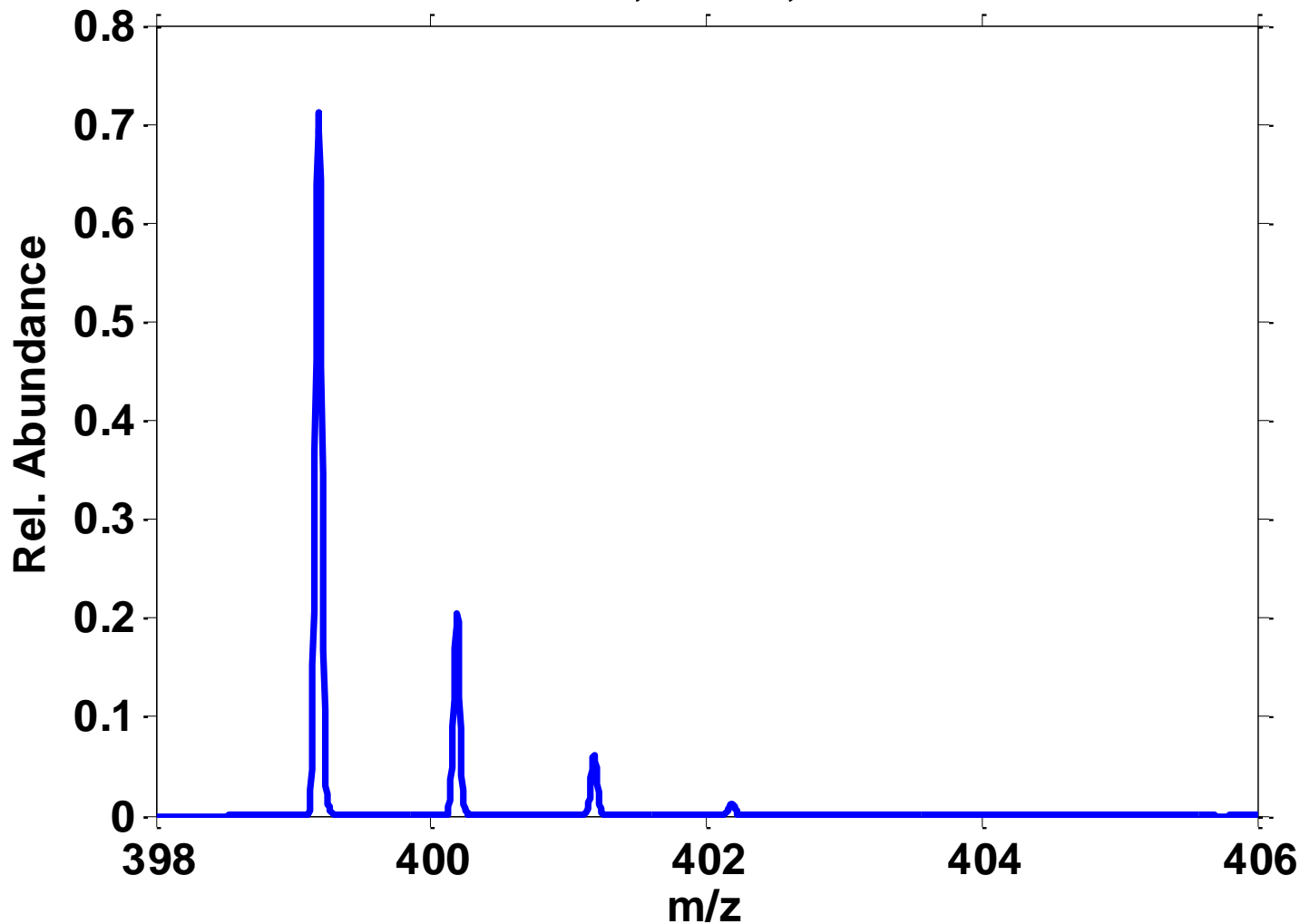




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Mass Spectrum: $C_{25}H_{23}N_2OS^+$

TOF, R = 8,000

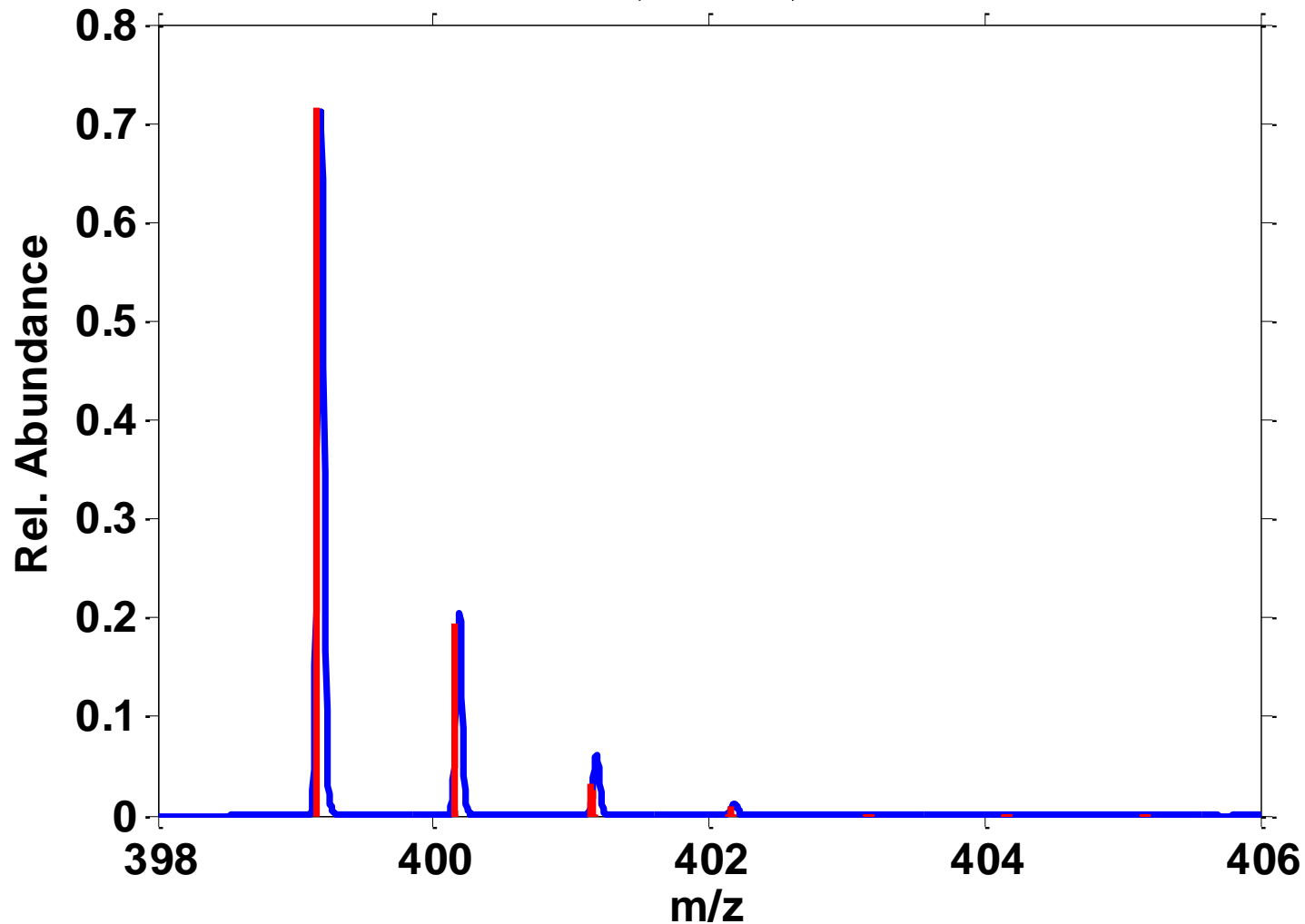




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Mass Spectrum: $C_{25}H_{23}N_2OS^+$

TOF, R = 8,000

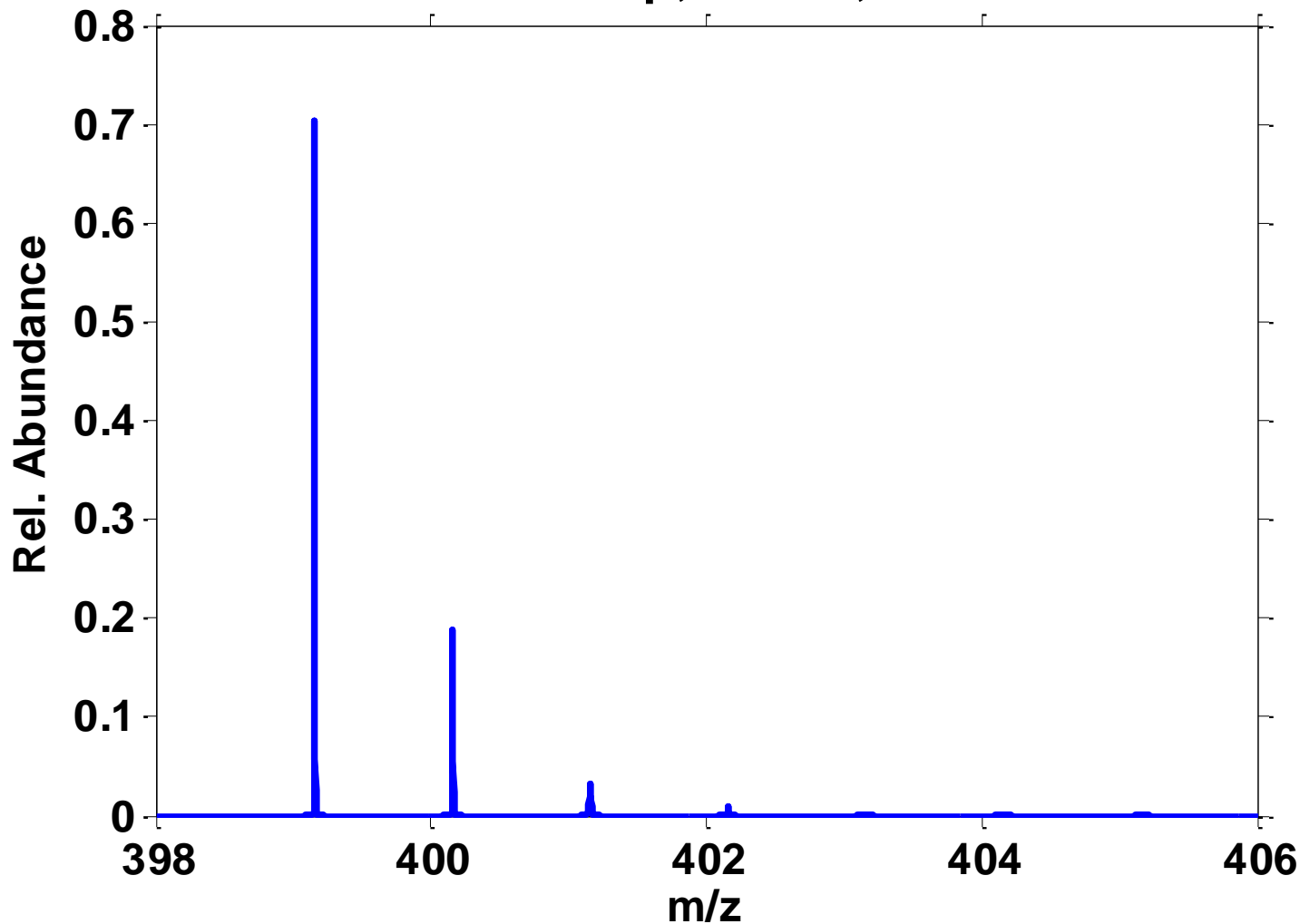




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Mass Spectrum: $C_{25}H_{23}N_2OS^+$

OrbiTrap, R = 80,000

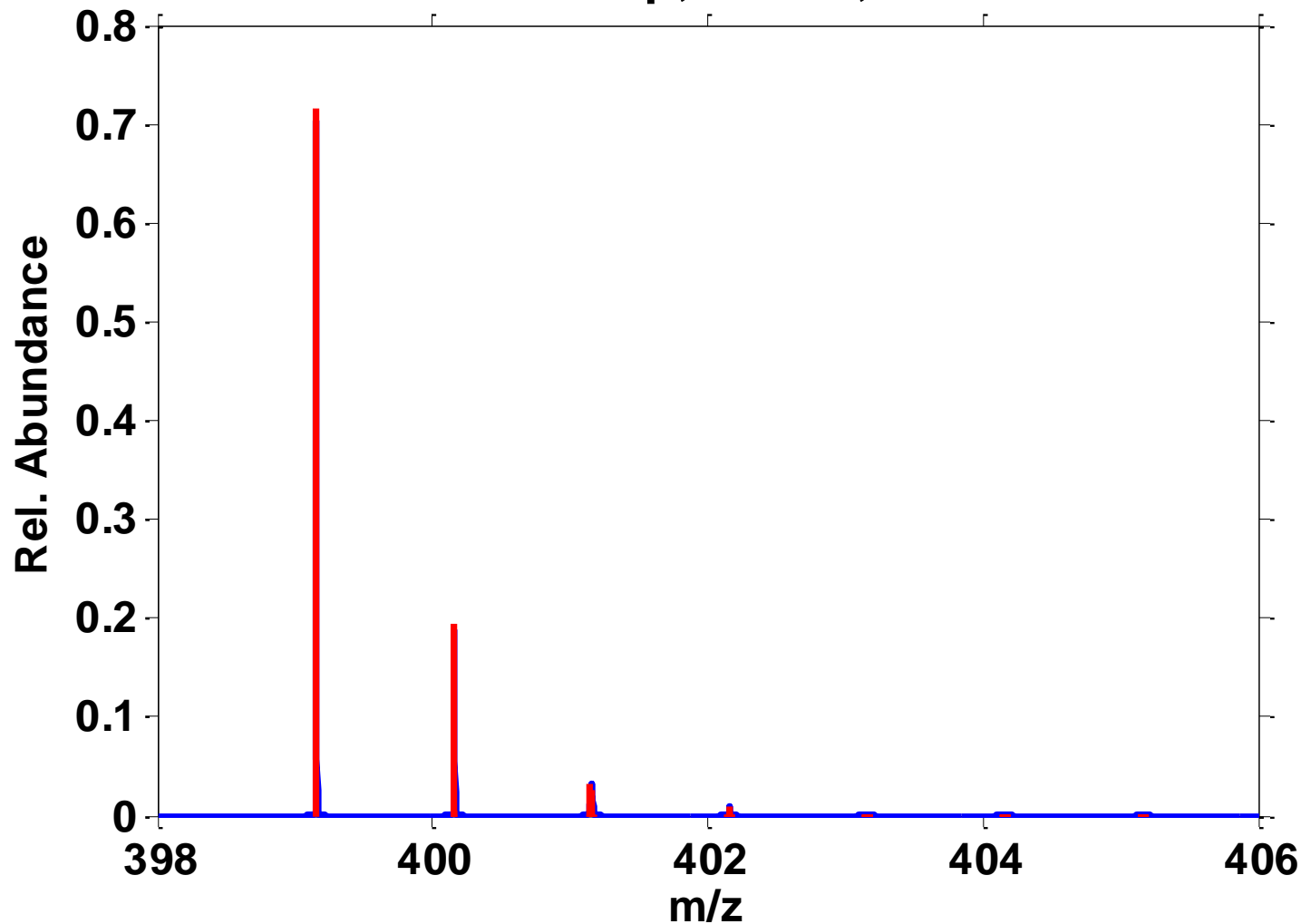




Chemalys

Mass Spectrum: $C_{25}H_{23}N_2OS^+$

OrbiTrap, R = 80,000

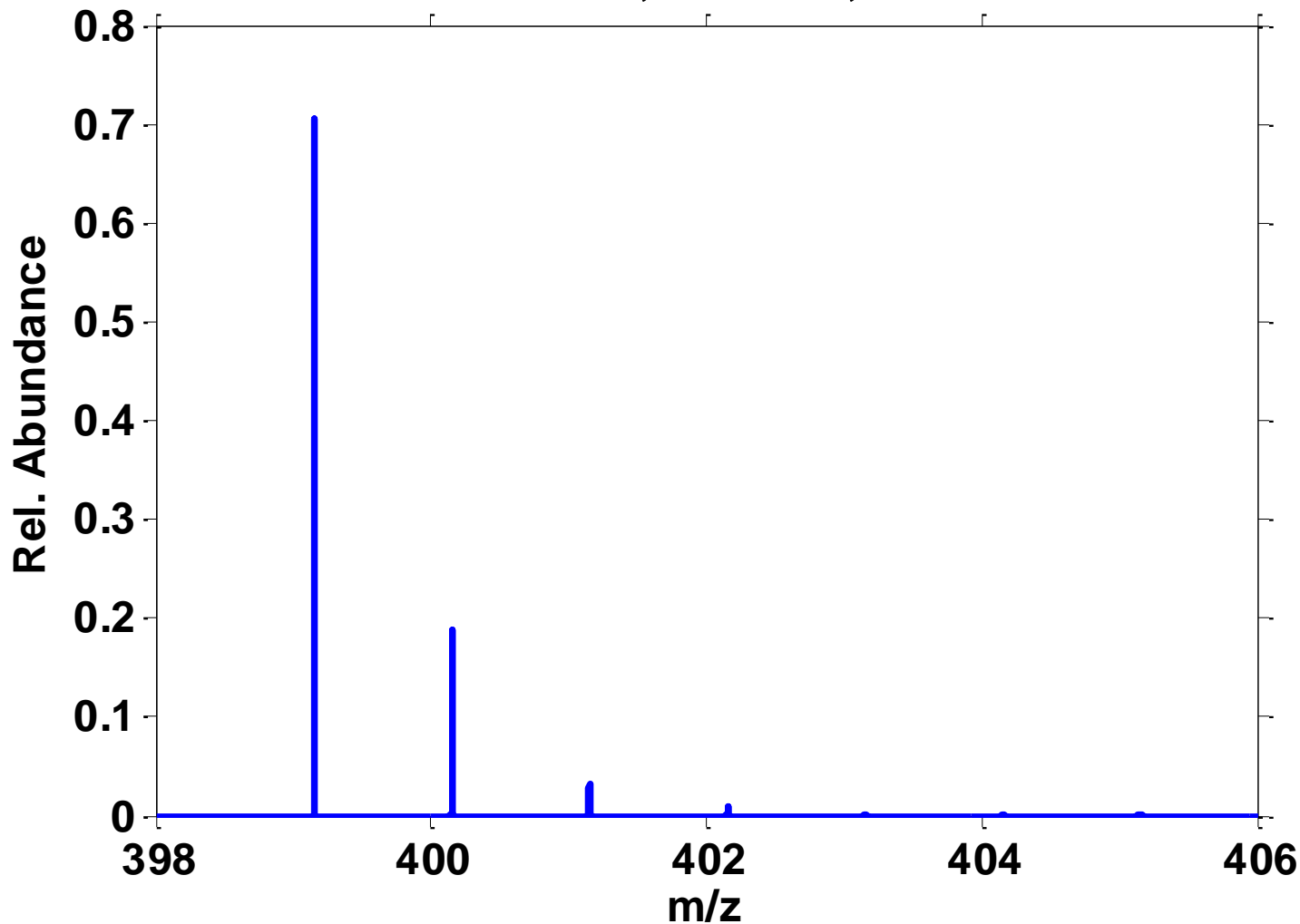




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Mass Spectrum: $C_{25}H_{23}N_2OS^+$

FT ICR, R = 800,000

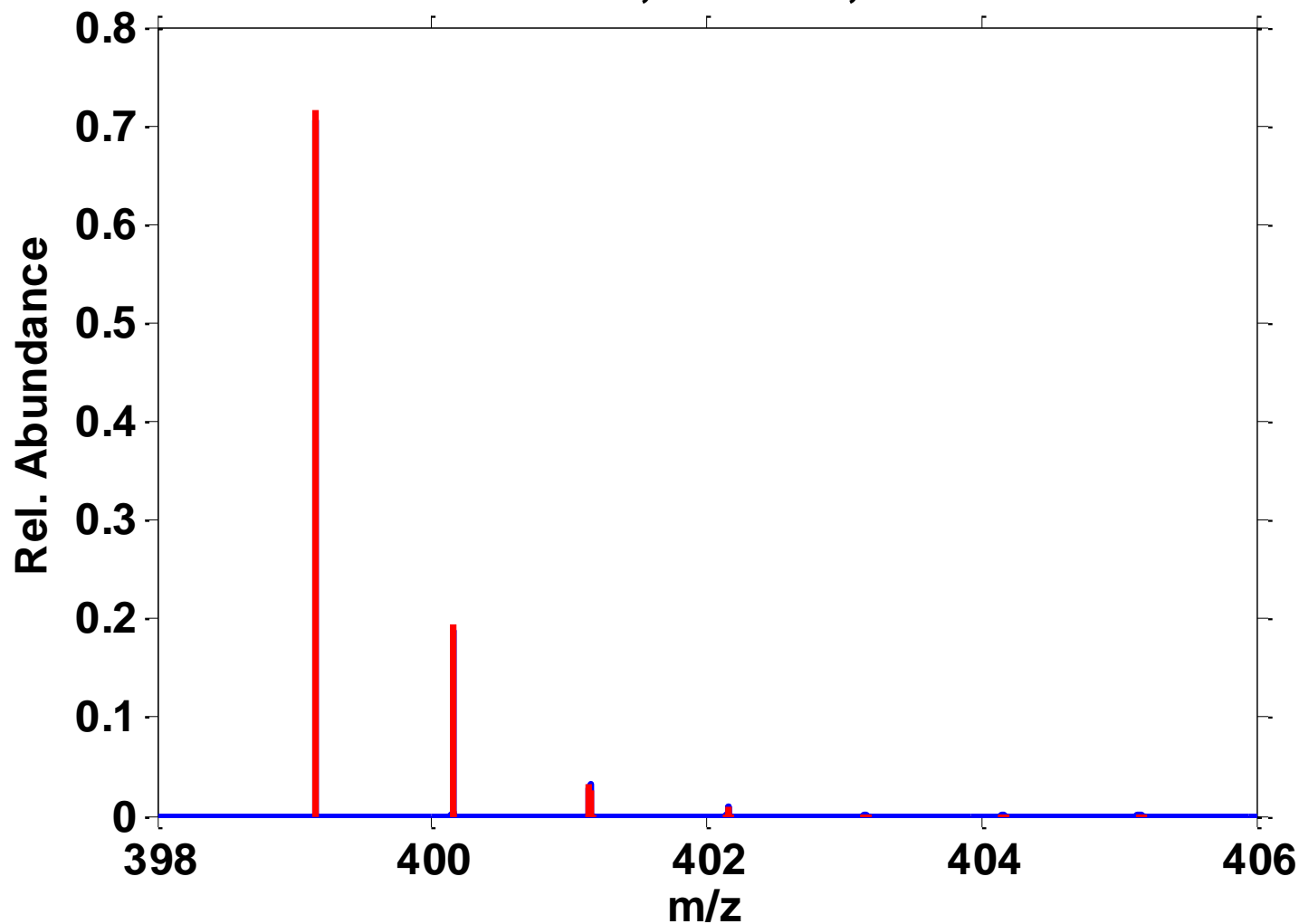




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Mass Spectrum: $C_{25}H_{23}N_2OS^+$

FT ICR, R = 800,000

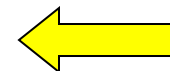




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Search for Possible Formulas

Exact Mass (Da)	399.153109
Charge	1
Mass Tolerance (PPB)	100
Electron State	Even
Double Bond Equivalent Minimum	-3
Double Bond Equivalent Maximum	35



Element	Minimum	Maximum
C	0	33
H	0	396
N	0	28
O	0	24
F	0	21
Na	0	17
S	0	12
P	0	12



Formulas within 100 ppb Error

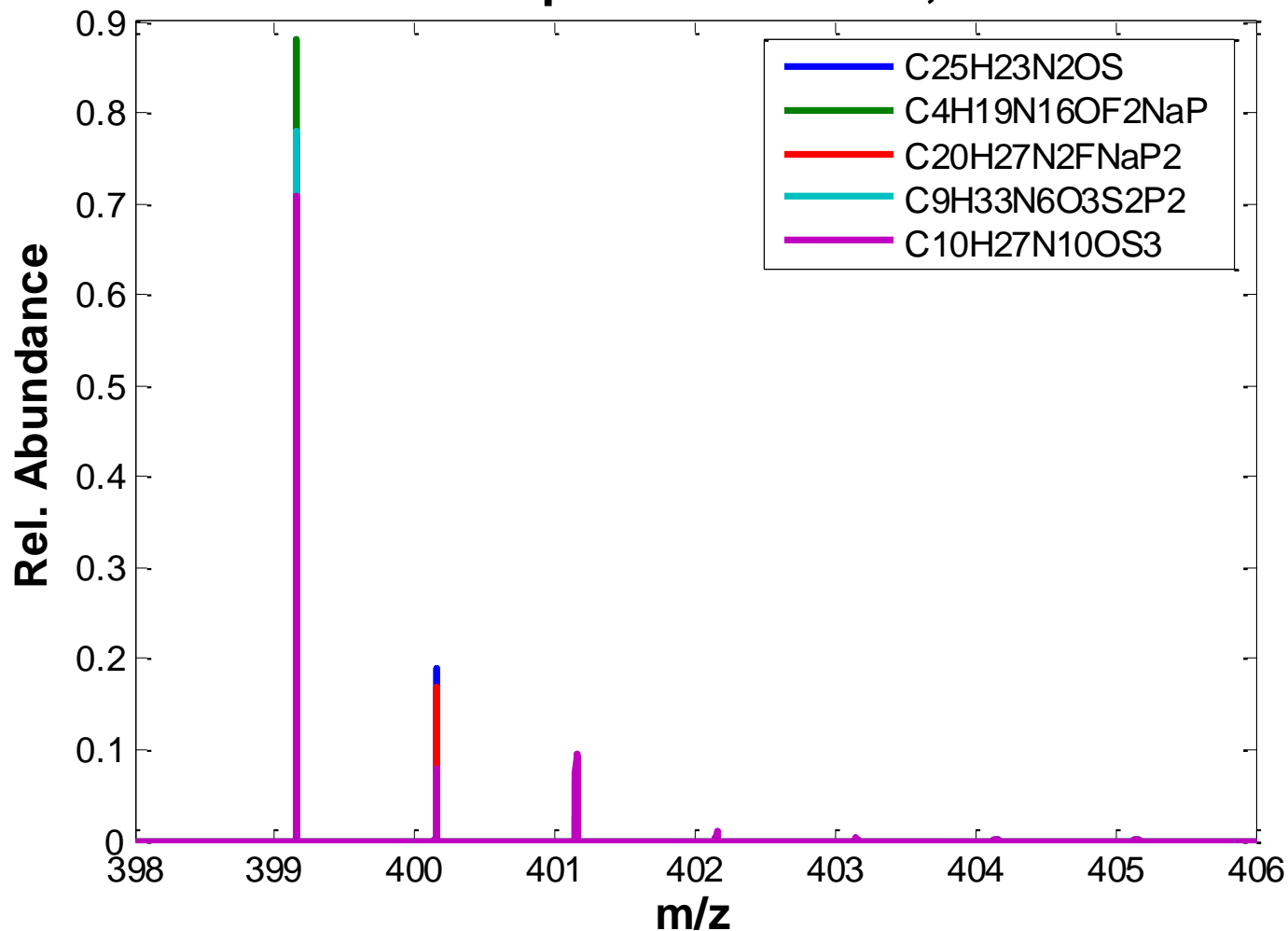
Rank	Formula	Mono Isotope (Da)	Mass Error (mDa)	Mass Error (PPB)	DBE
1	C ₂₅ H ₂₃ N ₂ O ₂ S	399.15311	-0.0001	0	15.5
2	C ₄ H ₁₉ N ₁₆ O ₂ NaP	399.15311	-0.0029	-7	2.5
3	C₁₃H₂₆N₆O₂Na₃S	399.15310	0.0052	13	2.5
4	C ₂₀ H ₂₇ N ₂ FNaP ₂	399.15312	-0.0108	-27	8.5
5	C₁₁H₂₇N₂O₈F₂Na₂	399.15311	0.0224	56	-2.5
6	C ₉ H ₃₃ N ₆ O ₃ S ₂ P ₂	399.15308	0.0306	77	-2.5
7	C ₁₀ H ₂₇ N ₁₀ O ₃ S	399.15314	-0.0336	-84	2.5



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Their Theoretical FT ICR MS?

Mass Spectra @ R=800,000

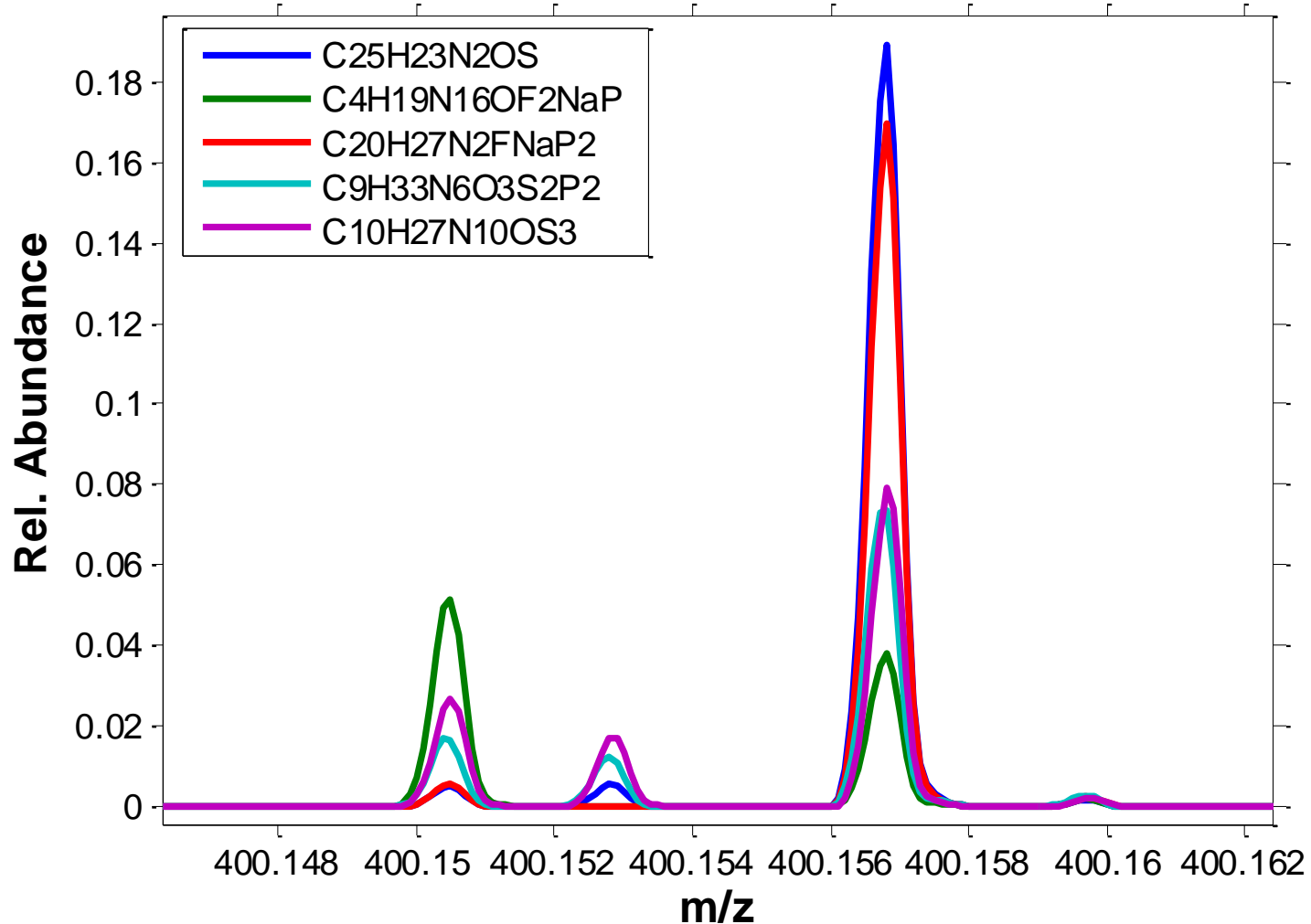




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Opportunities in Isotope Patterns

Mass Spectra @ R=800,000

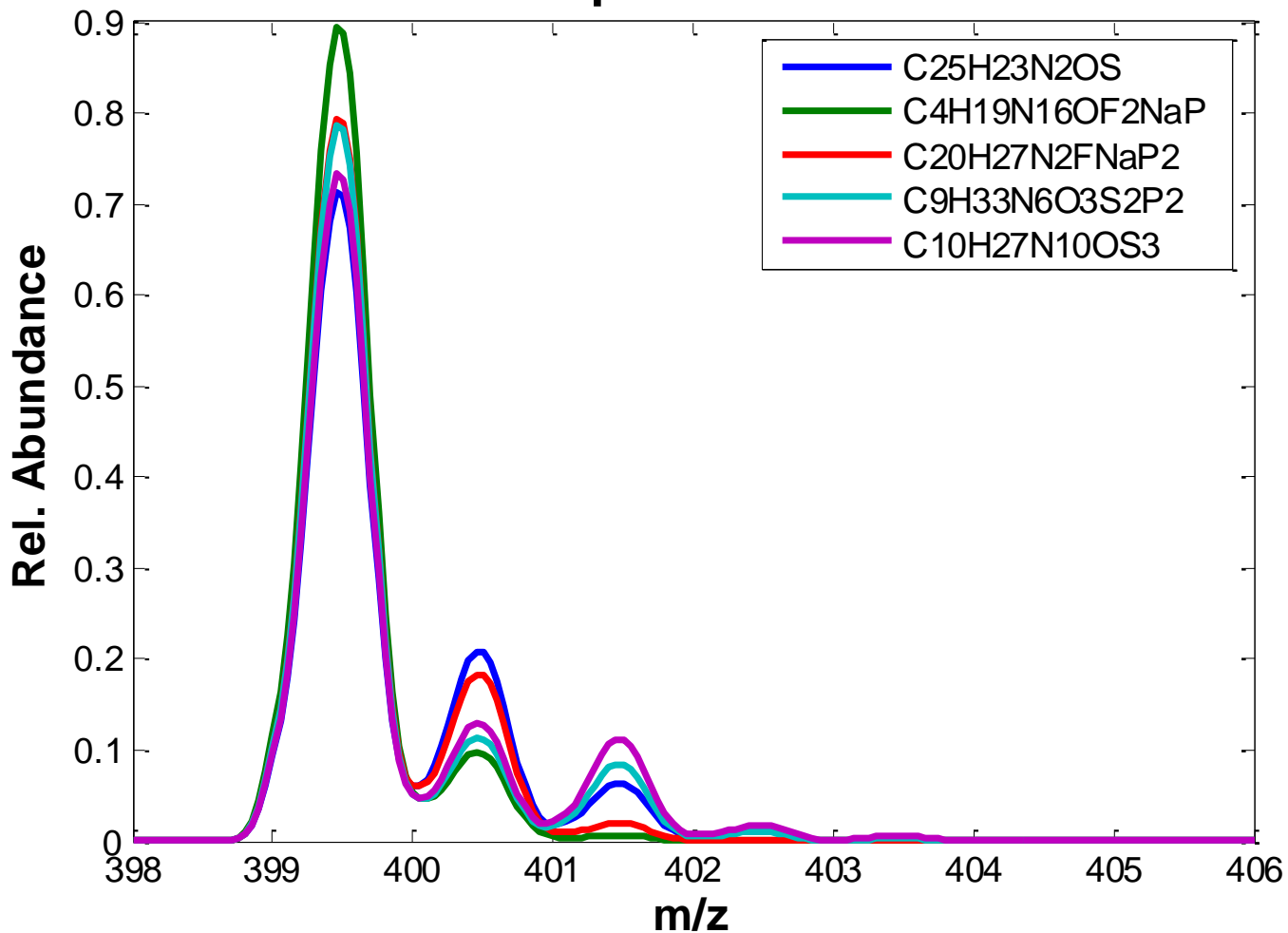




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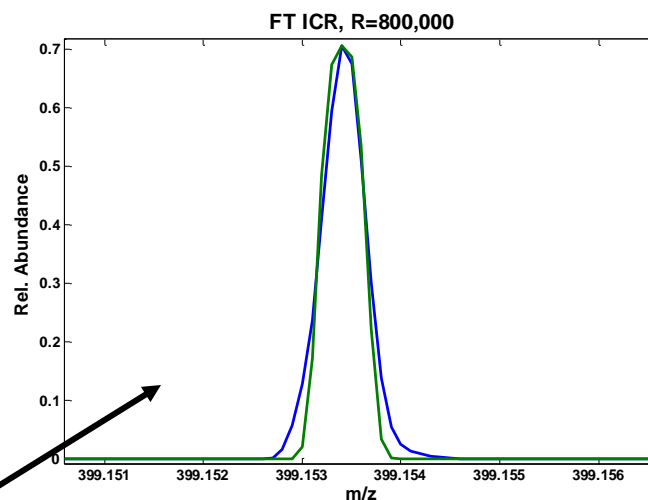
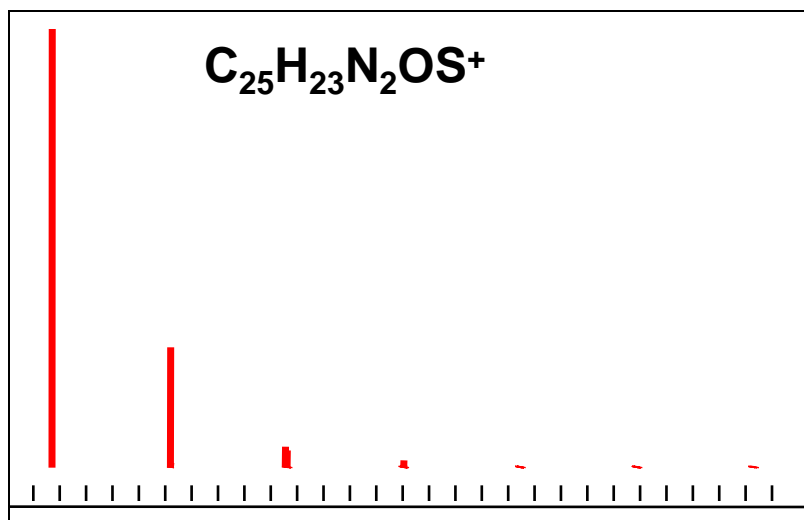
Useful Details Even at Low Res

Mass Spectra @ R=800





Profile Complications

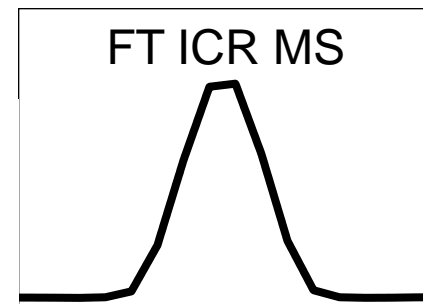
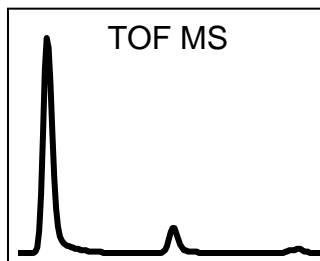
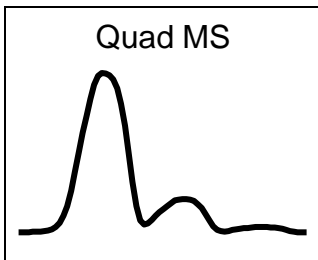


- Instrument specific line shape, not part of the elemental composition, typically not known!
 - Every measurement, without exception, has the imprint of the instrument line shape



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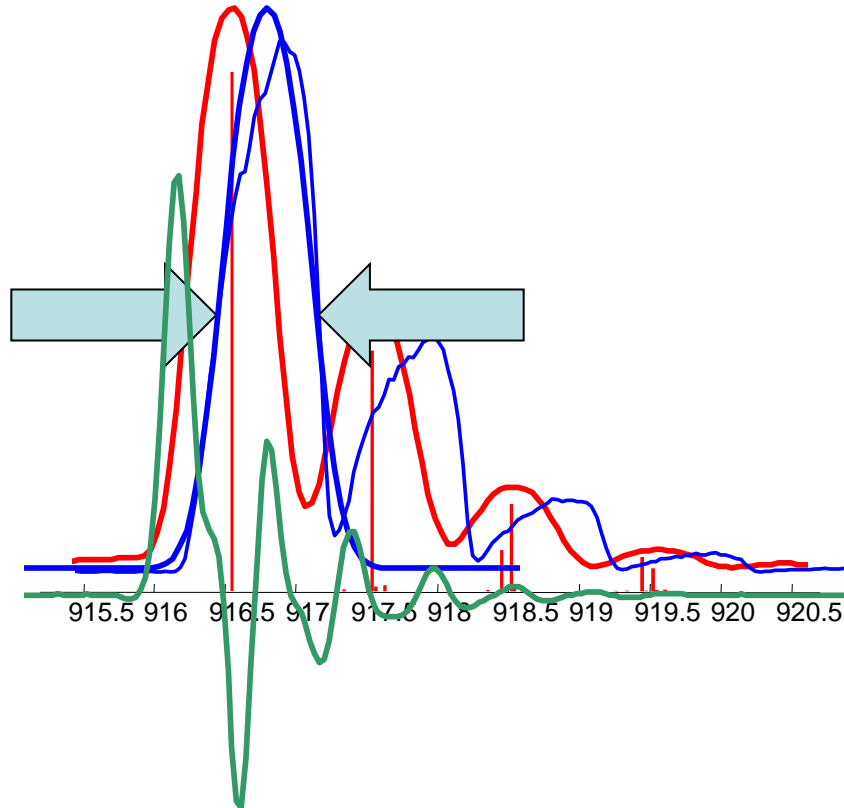
The Need for Standardized Lineshape





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Very Different Kind of MS Calibration

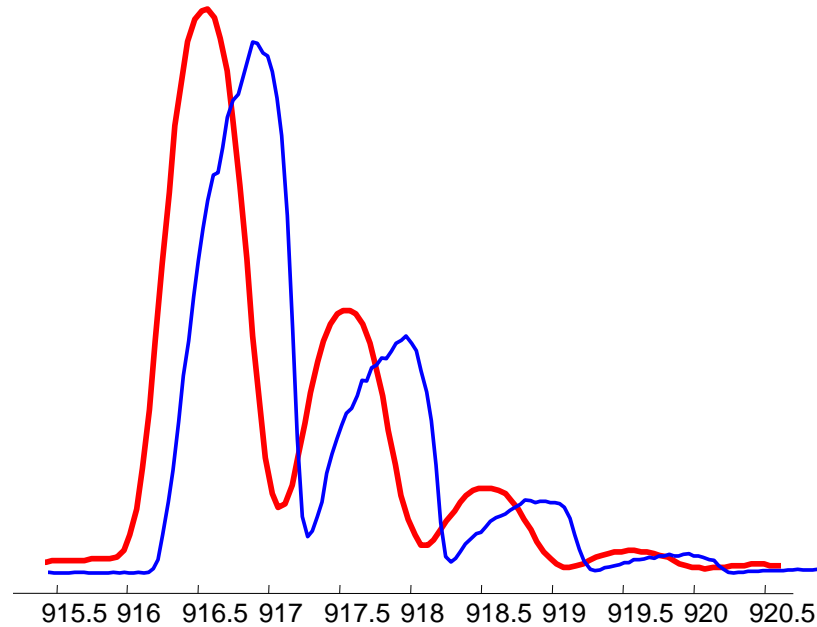


Frame # 4



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Applying the Calibration

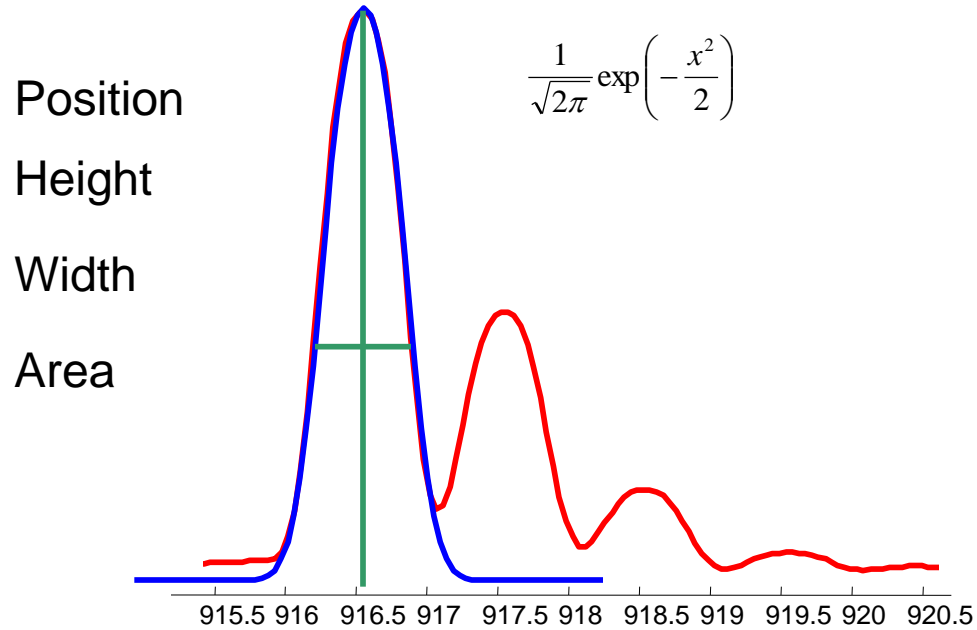


Frame # **4**



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Far Better Centroiding

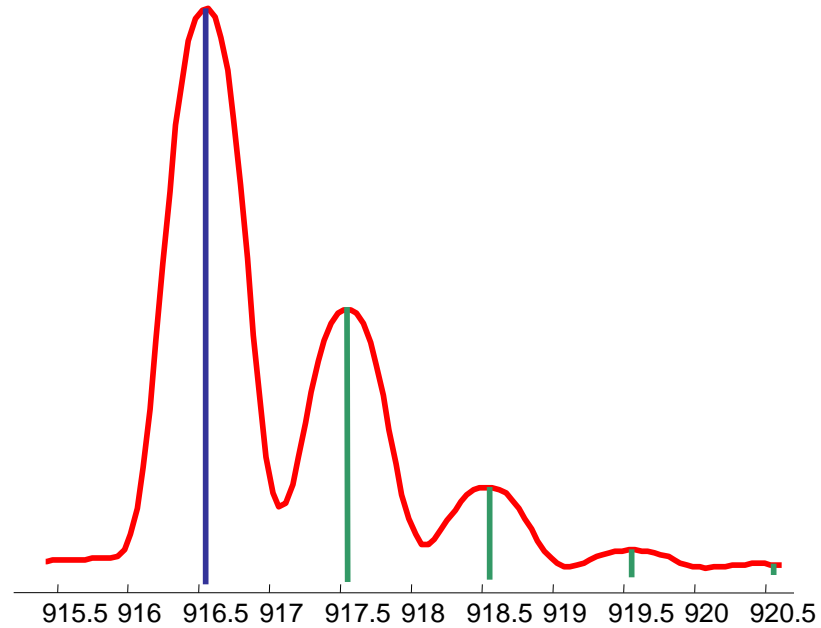


Frame # 4



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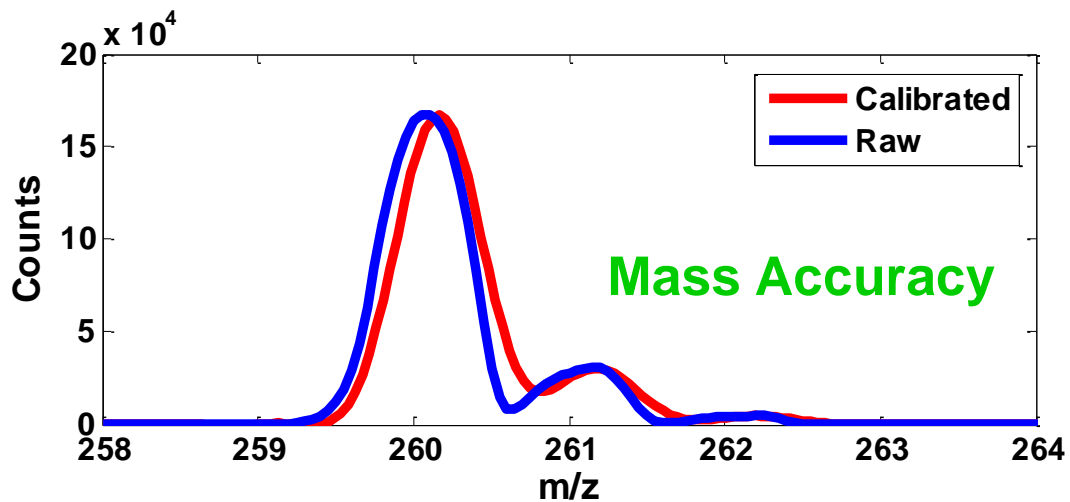
Full vs Reduced Data





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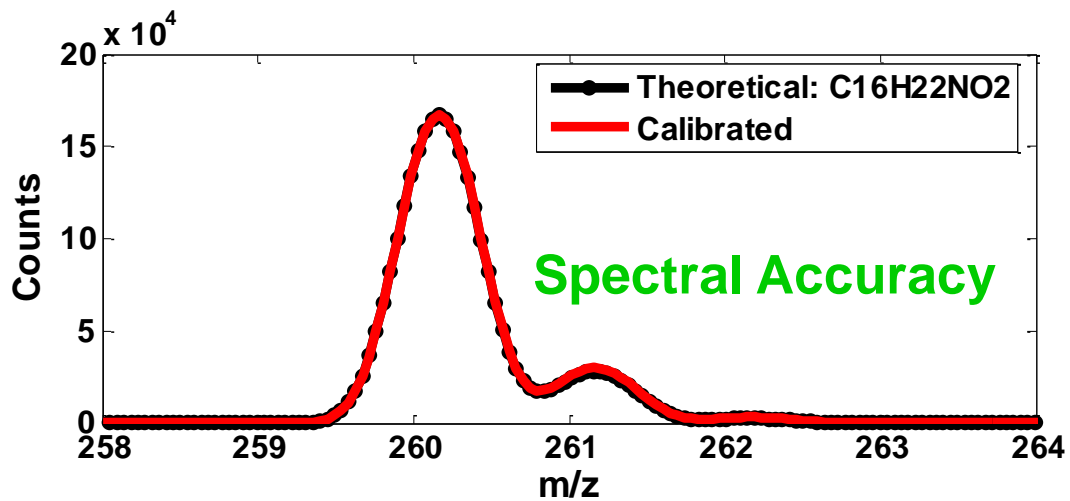
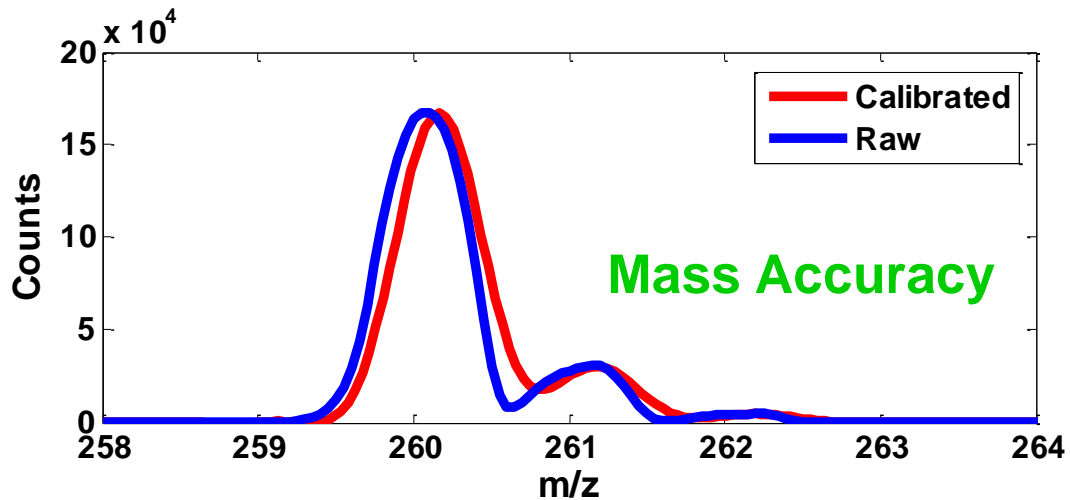
Formula ID w/Single Quad





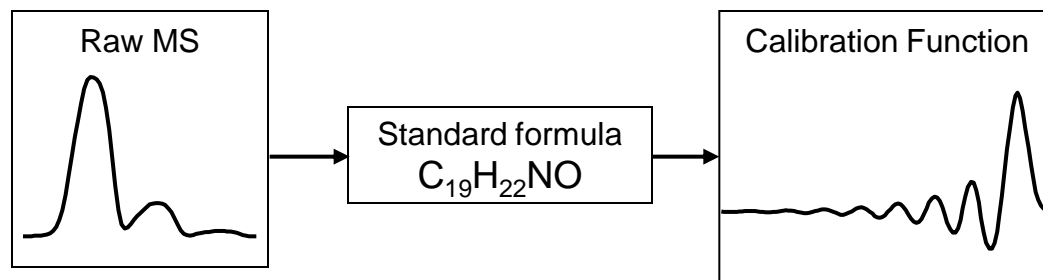
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Formula ID w/Single Quad





Calibration @ Unit Mass Resolution



- Requires external or internal standard(s)
- Requires the formula of the standard(s)
- Standards close in m/z to unknown: +/-50Da
- Standards close in time to unknown: same day



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sCLIPS Process

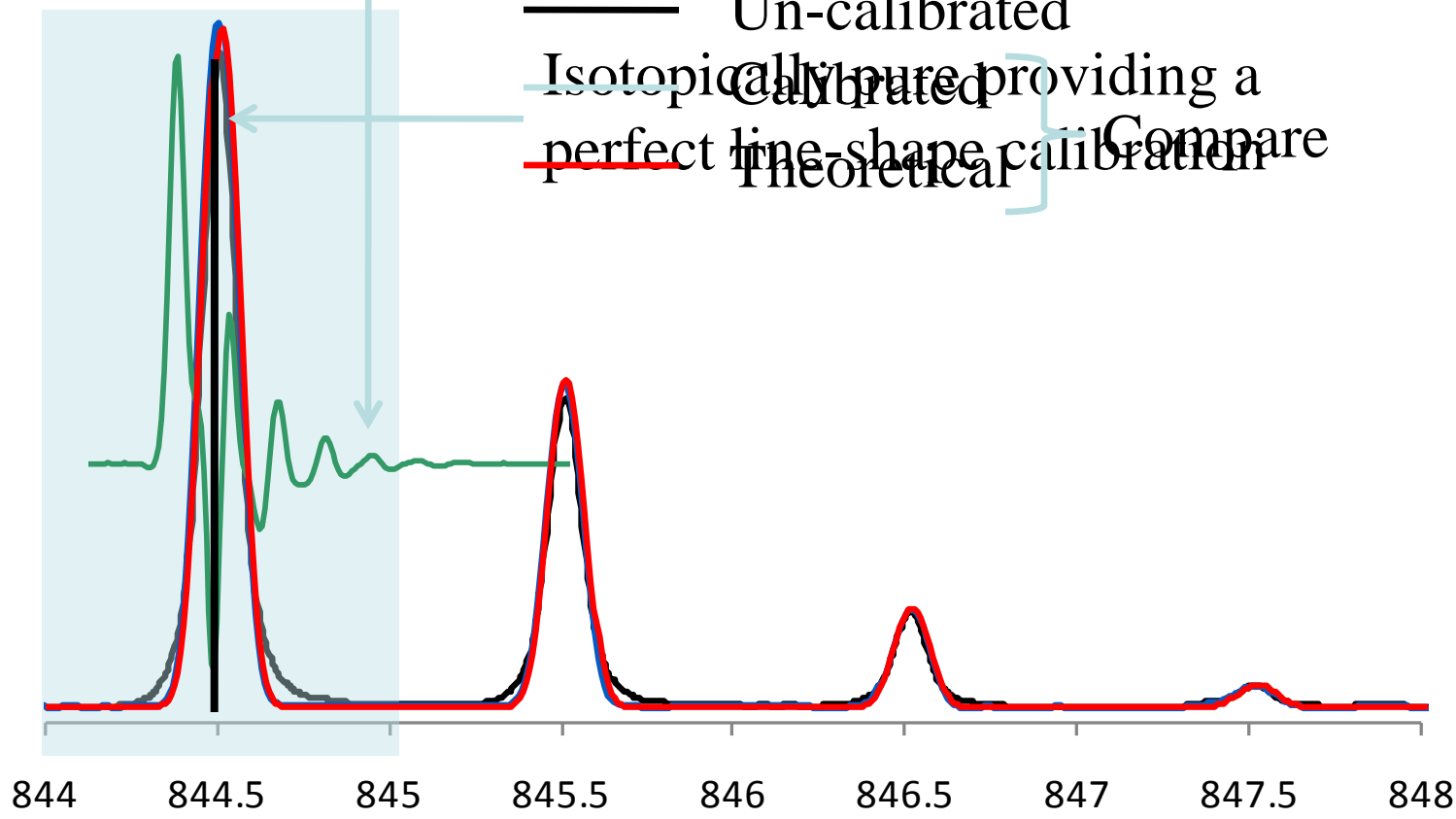
Calculate line-shape calibration

Apply Calibration

— Un-calibrated

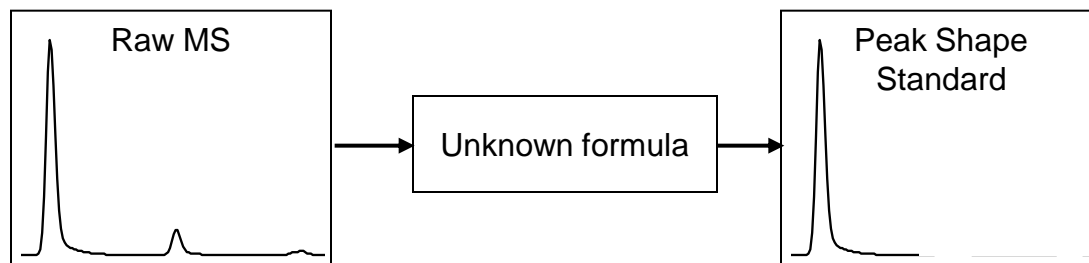
— Isotopically pure providing a perfect line-shape calibration

— Theoretical } Compare





Calibration @ Higher Resolution



- Monoisotopic peak of the unknown itself as “the standards”
- No formula required of “the standard(s)”
- No actual external or internal standard(s)
- “The standards” as close in m/z to unknown as possible
- “The standards” as close in time to unknown as possible
- Peak-shape-only calibration w/o mass adjustment
- sCLIPS: self calibration



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How good does MassWork perform on different instruments

• Agilent GC/MSD	100%
• Agilent LC/MSD	95%
• Waters ZQ	95%
• Waters Quattro Premier	95%
• Perkin Elmer GC/MS	95%
• Thermo Quantum LC/MS	90%
• Thermo DSQ GC/MS	90%
• Jeol AccuTOF (LC or GC)	90%
• Varian Tripple Quad	85%
• Varian Single Quad	85%
• Shimadzu LC/MS Quad	85%
• Thermo OrbiTrap FT MS	80%
• Thermo FT ICR MS	80%
• Bruker FT ICR MS	80%
• Bruker TOF	80%



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Summary

- Mass accuracy is important
- **Spectral accuracy** is even more important
- MS centroiding is BAD
- Line shape calibration is the key to differentiating similar formula candidates in profile mode
- Enabling formula ID on single quad MS (CLIPS): **>100x**
- Enhancing formula ID on high resolution MS (sCLIPS): **>20x**
- Enabling exact mixture analysis, suitable for isotope labeling application



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Thank you for your attention !

More information

www.chemalys.com

Thank's to
LSM lab in Uppsala.