

Finnigan LTQ FT

A Word on High Resolution and Accurate Mass Definitions

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Finnigan LTQ FT System



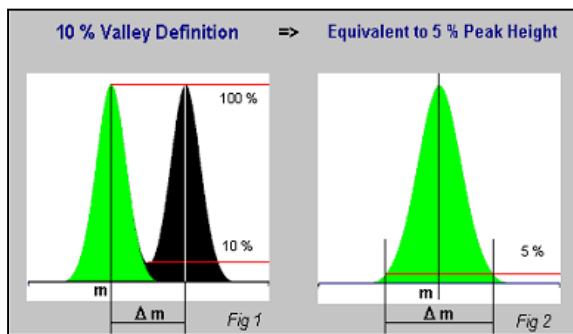
Resolution Definition

Resolution is defined as $m/\Delta m$

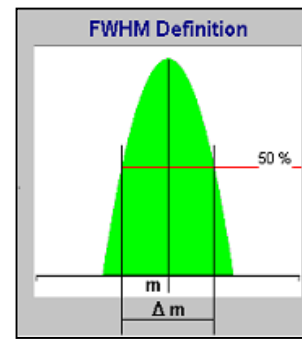
The definition of Δm is different for the different types of analyzers.

- Sector field MS (MAT 95 XP)
- 10 % valley definition
- Corresponds to peak width at 5 % peak height

- All other MS (LTQ FT, Q-TOF, TOF-TOF, LCQ Deca, Quantum)
- FWHM definition
- Corresponds to peak width at 50 % peak height



Resolution
10,000 (10 % valley)
=
20,000 (FWHM)



Mass Errors in Accurate Mass Determination

Error Limit – mmu and ppm

The number of possible elemental compositions at a given mass is limited by the applied error value. Errors are expressed either in terms of parts per million (ppm), millimass units (mmu) or atomic mass units (amu).

$$\text{ppm} = 10^6 * \Delta m / m$$

Where Δm is the difference between theoretical and observed mass and m is the mass.

Errors expressed in ppm are mass dependant and get smaller at higher mass, e.g.:

$$1 \text{ mmu @ } m/z \text{ 1000} = 1 \text{ ppm}$$

but

$$1 \text{ mmu @ } m/z \text{ 100} = 10 \text{ ppm}$$

Explanation of ppm, amu and mmu

Absolute values:

Th – atomic mass units

$$= \text{mmu} * 1000$$

mmu – millimass units

$$= \text{Th}/1000$$

Relative value:

ppm – parts per million

$$= 10^6 * \Delta m/m$$

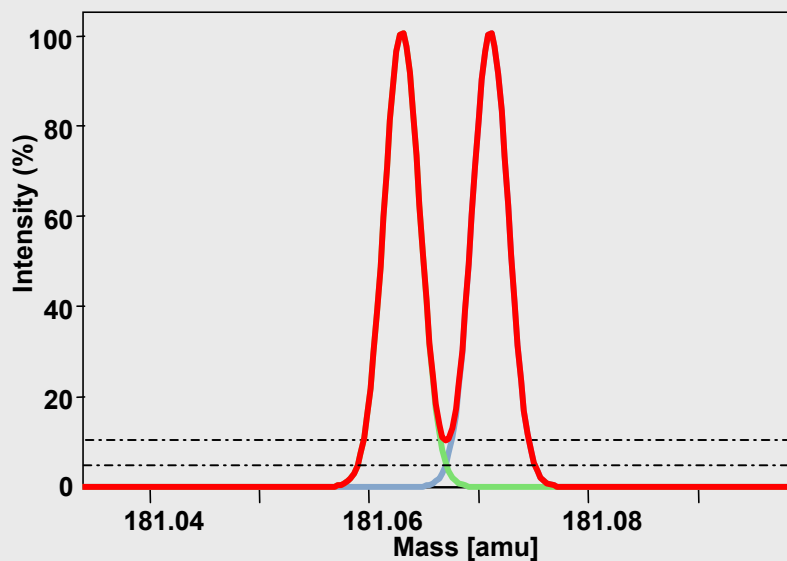
To convert from mmu to ppm:

$$1000 / \text{mass} * \text{mmu} = \text{ppm}$$

Resolution Definition

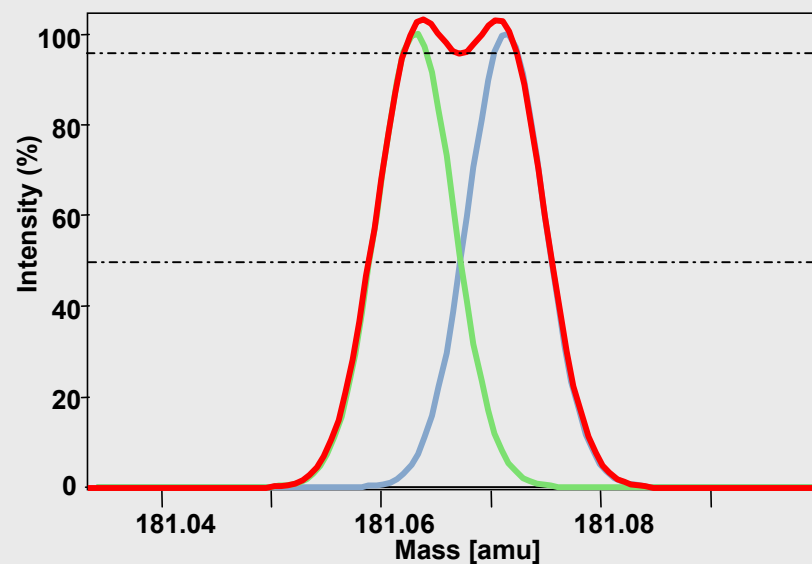
RP = 22,000 (10 % valley)

- Sector field MS (MAT 95 XP)
- 10 % valley definition
- Δm = peak width at 5 % peak height



RP = 22,000 (FWHM)

- All other MS (LTQ FT, TOF-TOF, Q-TOF LCQ Deca, Quantum)
- FWHM definition
- Δm = peak width at 50 % peak height



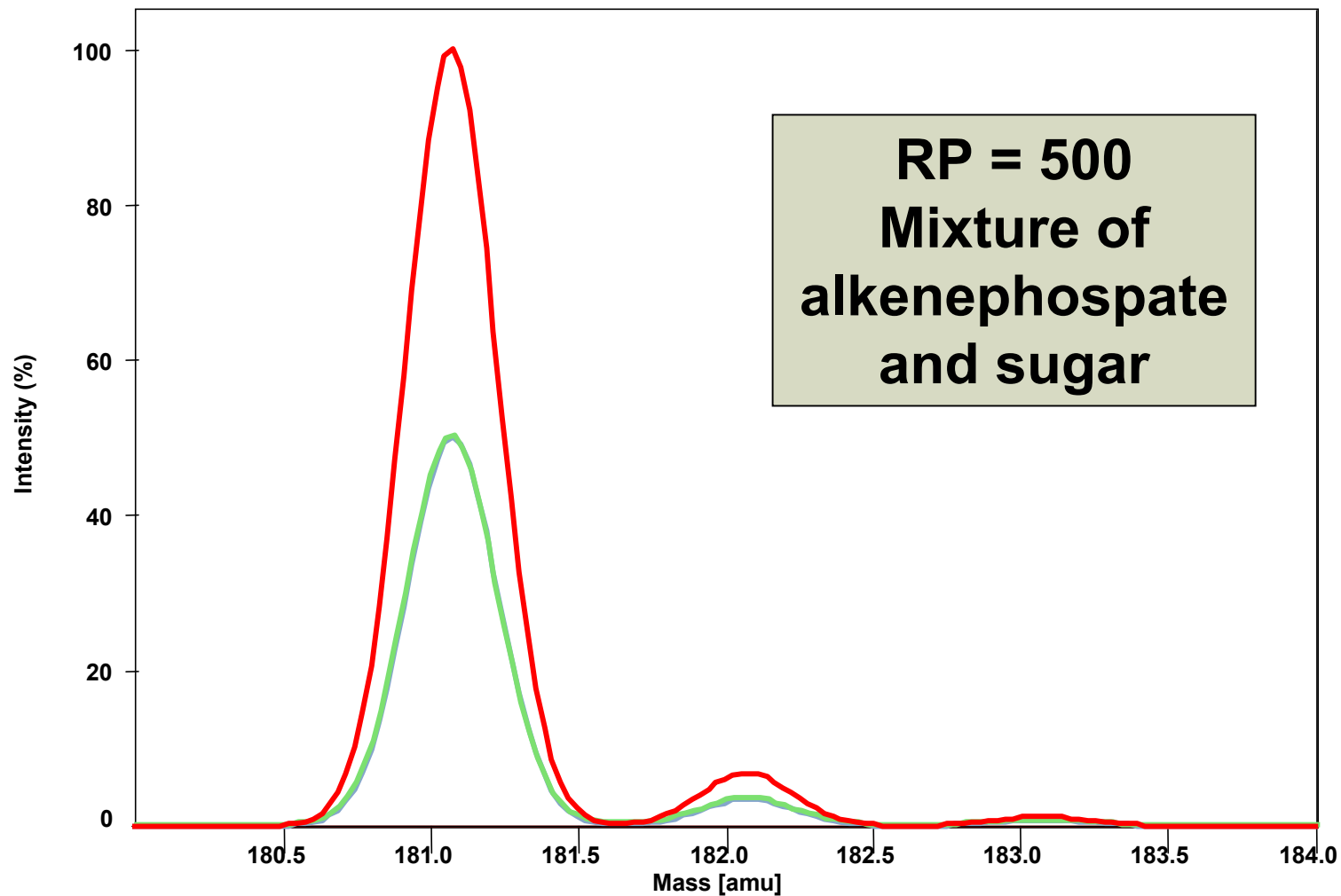
Example for High Resolution

Alkenephosphate
 $[M+H]^+ = C_6 H_{14} O_4 P$
181.06242

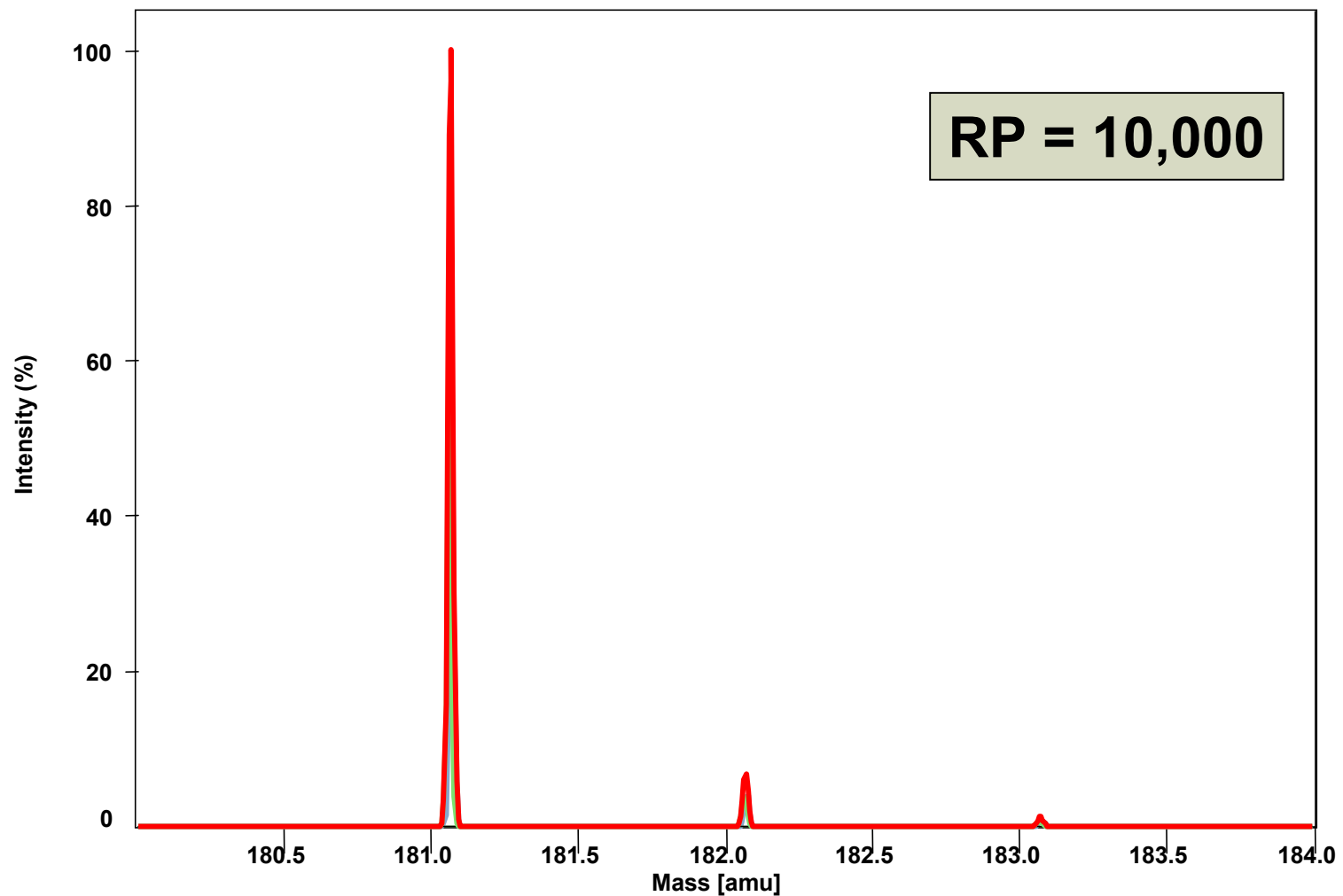
Hexose (Sugar)
 $[M+H]^+ = C_6 H_{13} O_6$
181.07066

$\Delta m = 0.00824 \text{ Th}$
Calculated Resolution at FWHM
 $RP = 181 / 0.00824 = 22,000$
Required resolution for separation:
 $RP = 2 * m / \Delta m$
 $RP = 44,000$

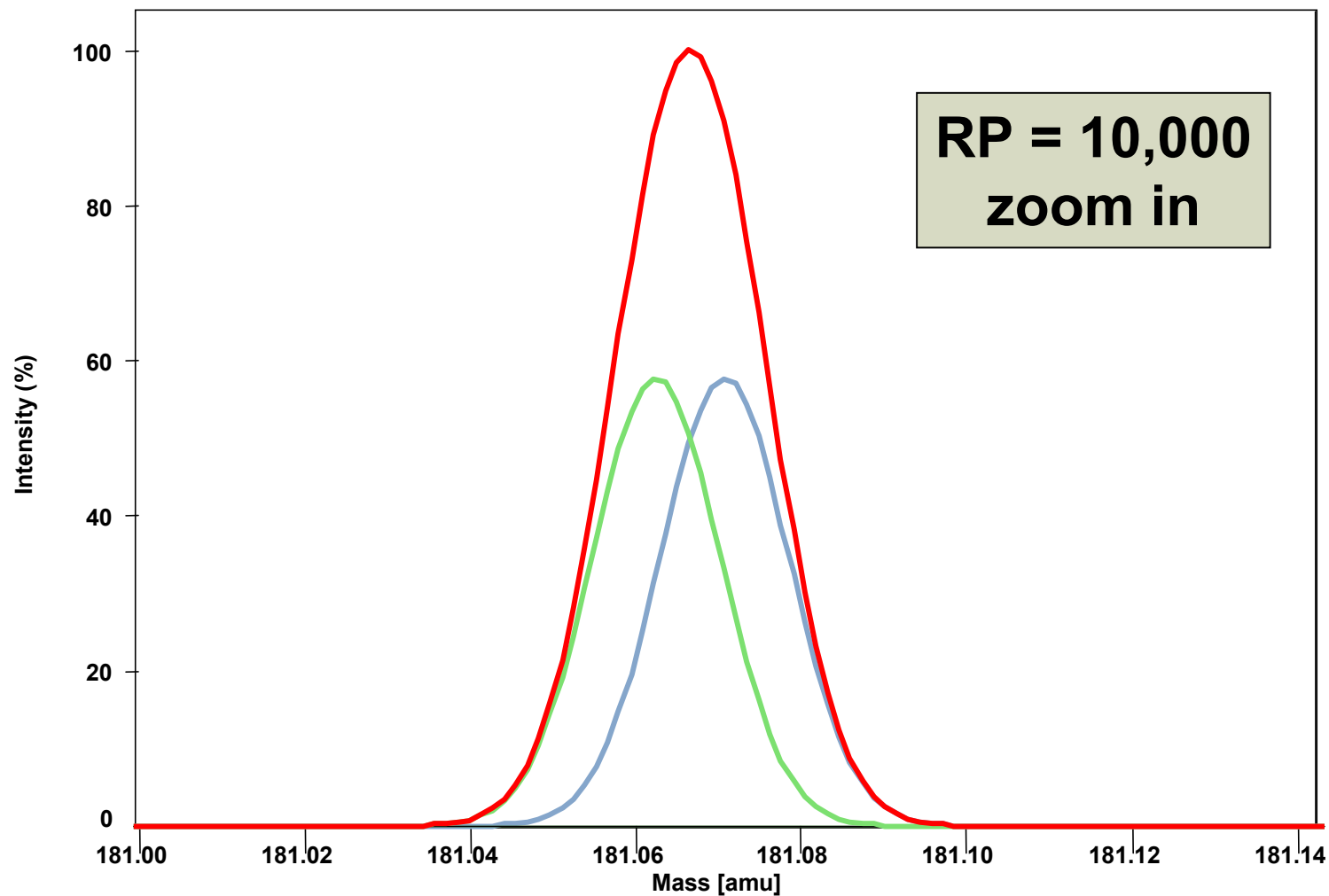
Example for High Resolution



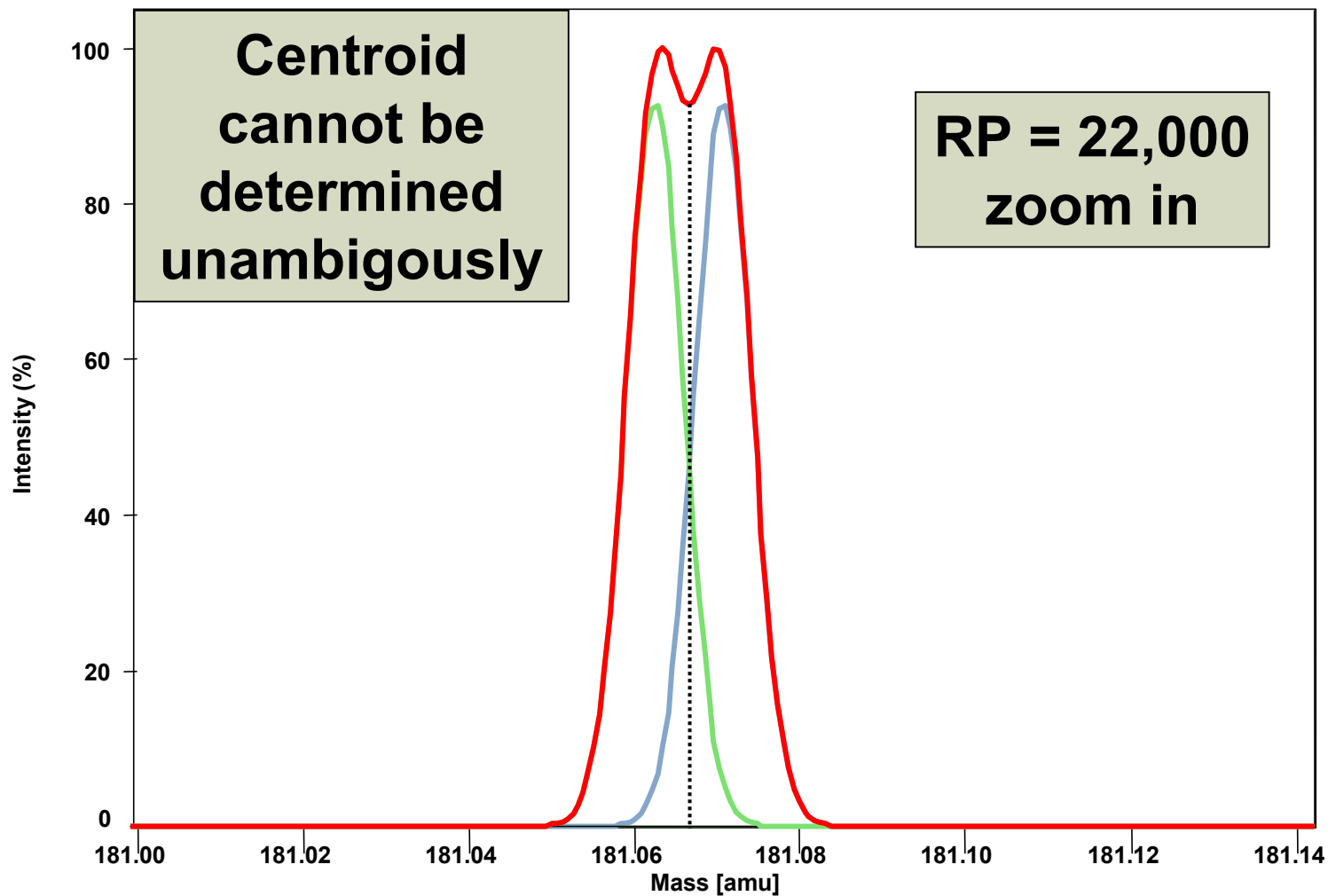
Example for High Resolution



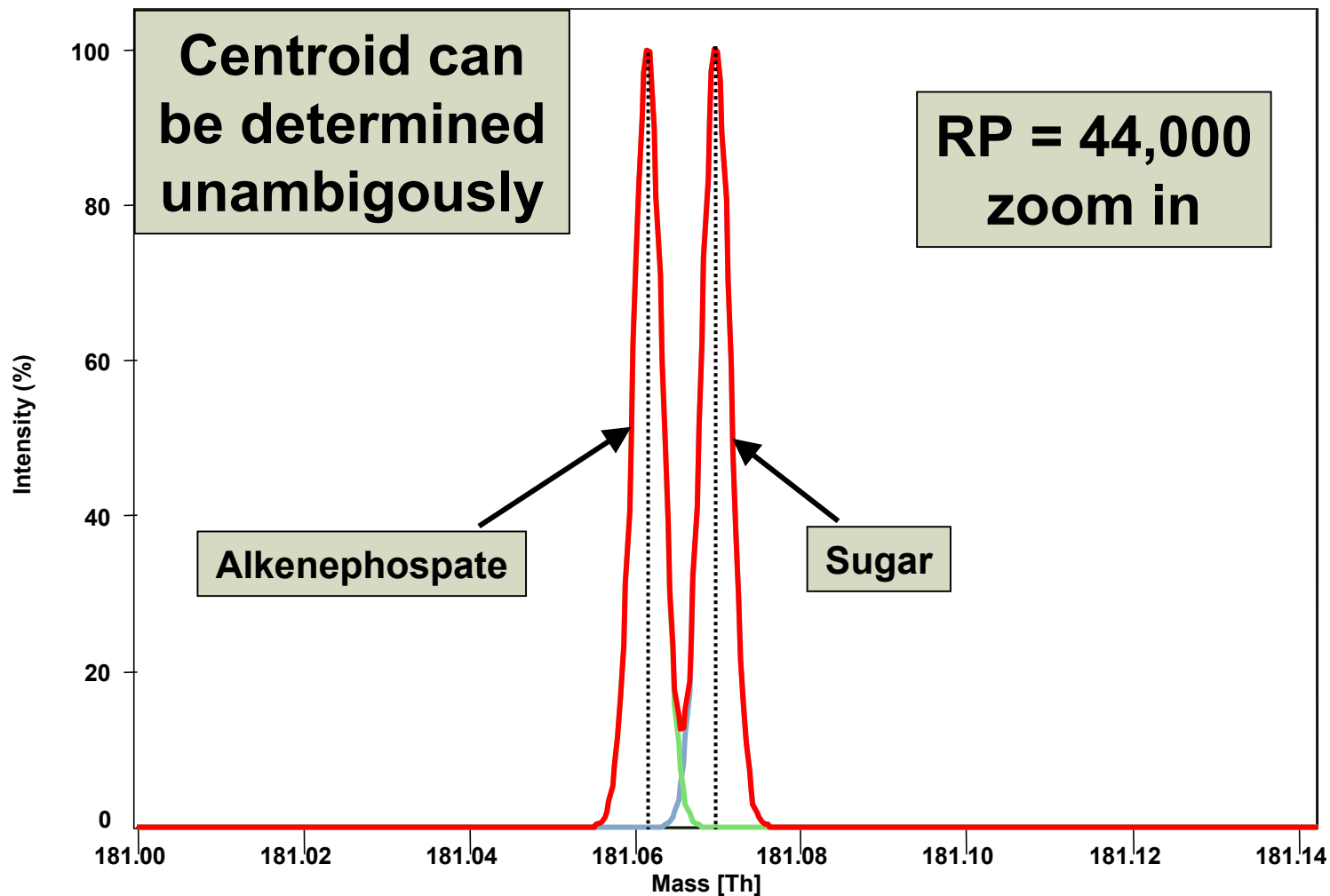
Example for High Resolution



Example for High Resolution



Example for High Resolution



Accurate Mass and Elemental Composition

All chemical compounds are built by combination of different Elements. With the exception of carbon (by definition the exact mass is 12.0000), all other atoms have either a negative or a positive mass defect. That means the exact mass of the atoms are not equal to nominal mass.

Elements:	Carbon,	Hydrogen,	Nitrogen,	Oxygen	Sulfur	etc.
Formula:	^{12}C	^1H	^{14}N	^{16}O	^{32}S	
Exact mass:	12.0000	1.0078	14.0031	15.9949	31.9721	



Amino Acids, the subunit of peptides are built by the different atoms

Accurate Mass and Elemental Composition

All amino acids are built by combination of different elements, mostly C, H, N, O and S. Most amino acids differ by their nominal mass but two of them are isobaric and differ only in their exact mass.

Amino acid:	Lysine	Glutamine
Letter code:	K or Lys	Q or Gln
Formula:	$C_6H_{14}N_2O_2$	$C_5H_{10}N_2O_3$
Exact mass:	146.10498	146.06859

Δm (mmu):	36.4 mmu
Δm (ppm):	250 ppm
Required Resolution:	8,000 (FWHM)



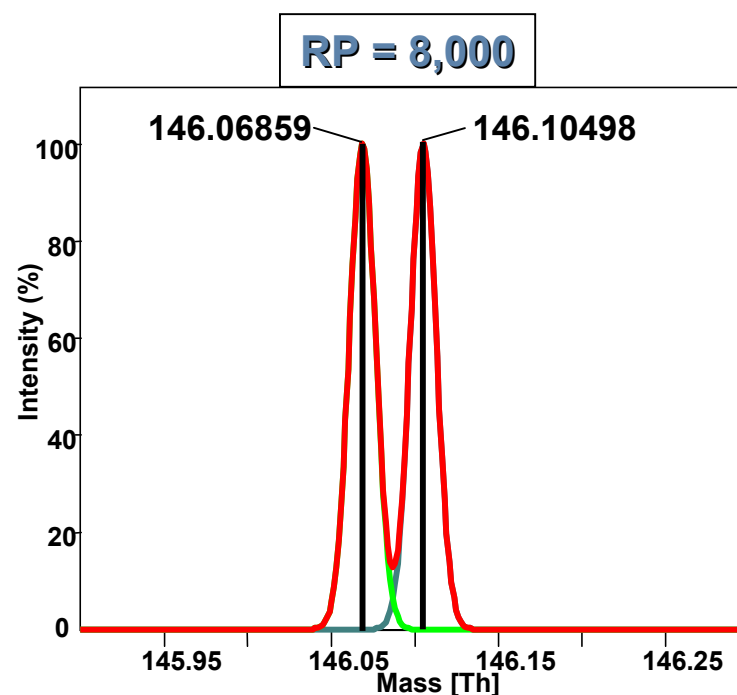
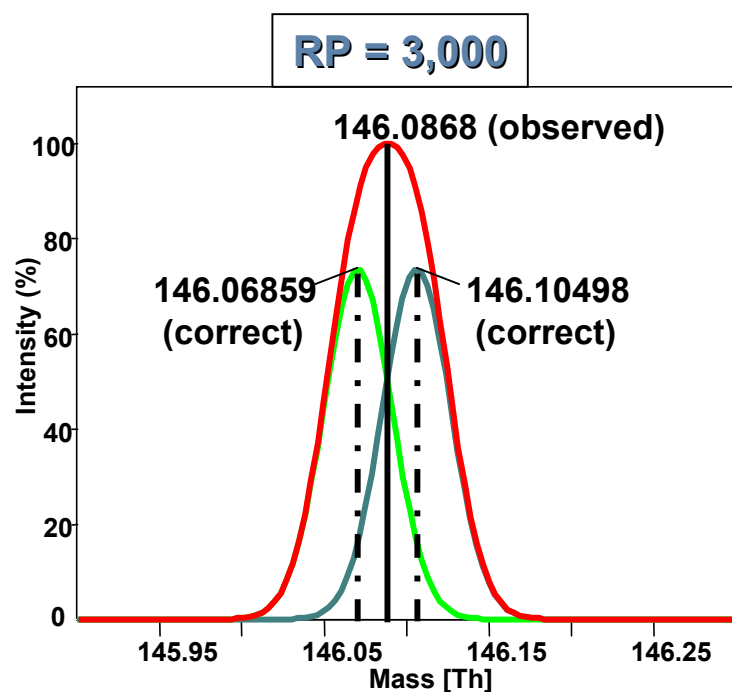
Peptides are built by the different amino acids with MW < 5,000 Da.

High Resolution and Accurate Mass

From the previous examples of the amino acids Lysine and Glutamine, it is obvious that the elemental composition can be determined for both compounds individually with instruments already providing mass accuracies in the range of 5 to 10 ppm.

If we look at mixture of the amino acids the correct mass assignment requires baseline resolved peaks and therefore, in our example resolution power of up to 8,000 is necessary.

At low resolution (RP = 3,000), the error of the accurate mass determination is more than 120 ppm. At higher resolution, the instrumental error is the limitation for the mass determination.



Accurate Mass and Elemental Composition

Peptides are built by combination of different amino acids. All kind of variations of amino acids are possible and build the various peptides, the subunit of proteins.

Peptides: [Val⁵]-Angiotensin II
Sequence: DRVYVHPF
Formula: C₄₉H₆₉N₁₃O₁₂
Exact mass: [M+2H]²⁺ = 516.76671

Lys-des-Arg⁹-Bradykinin
KRPPGFSPF
C₅₀H₇₃N₁₃O₁₁
[M+2H]²⁺ = 516.78490

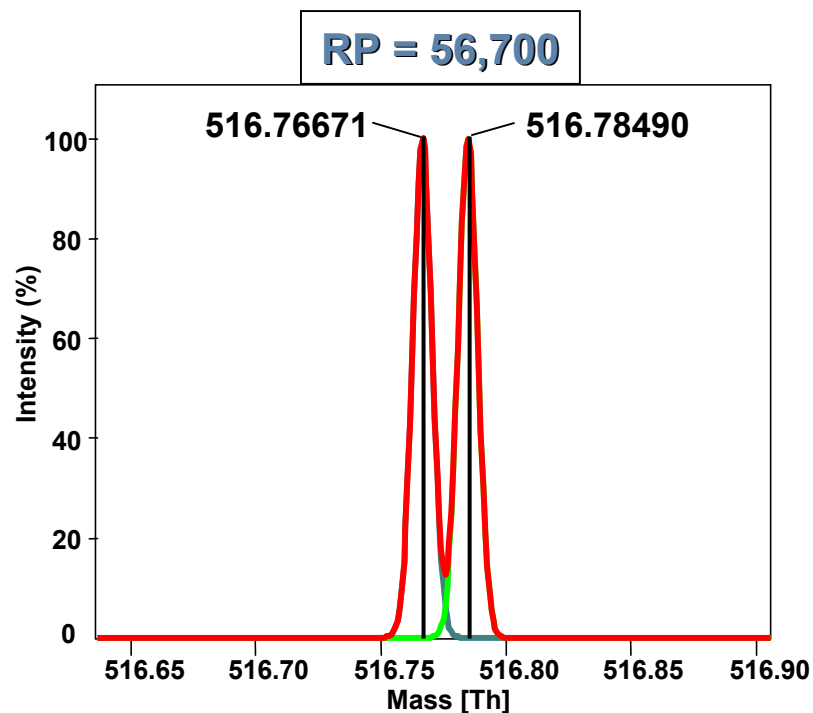
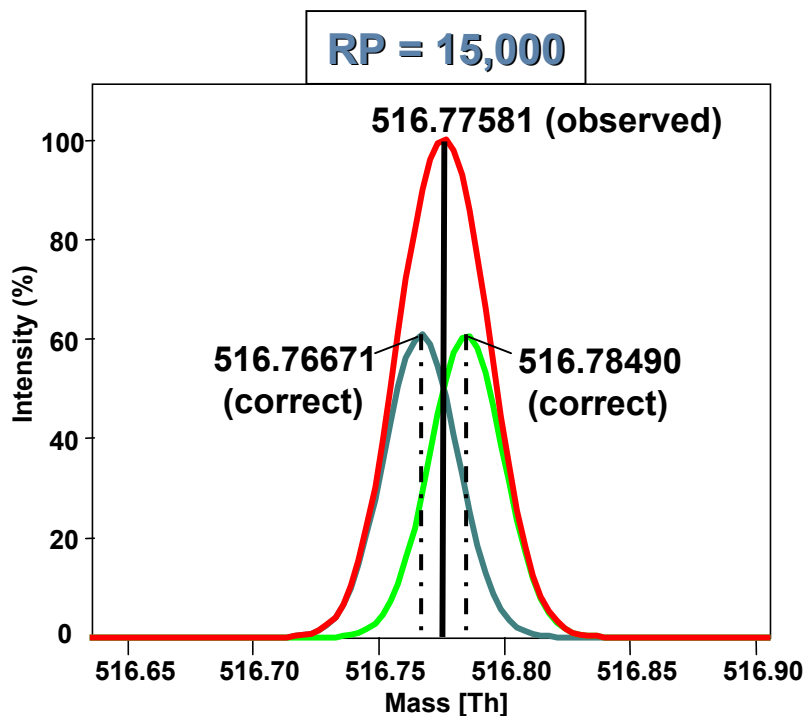
Δm (mmu): 18.2 mmu
 Δm (ppm): 35 ppm
Required Resolution: 56,700 (FWHM)



Proteins are built by the different peptides with MW > 5,000 Da.

High Resolution and Accurate Mass

Another more practical example are the peptides [Val⁵]-Angiotensin II and Lys-des-Arg⁹-Bradykinin. Although the individual elemental compositions can be determined easily with instruments providing moderate mass accuracies (5 to 10 ppm), the appearance of both peptides in a mixture will cause an incorrect mass assignment at QqTOF resolution of $RP = 15,000$. Only applying high resolution to separate these two ions (here a resolution of $RP = 56,700$ is necessary) will yield the correct mass assignment and hence the right elemental composition.



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: [Val⁵]-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

Limits for Elemental Composition Calculation

Isotope	Min.	Max.	DB Eq.	Mass
12 C	35	70	1.0	12.000
1 H	45	100	-0.5	1.008
16 O	9	16	0.0	15.995
14 N	8	16	0.5	14.003
32 S	0	2	0.0	31.972
<new>				

By using statistical occurrences of the amino acids from a protein data base, an average amino acid, *averagine*, was calculated with the following formula:



$$\text{MW}_{\text{av}} = 111.1254 \text{ Da [2].}$$

To calculate the minimum and maximum number of C, H, N, O and S for a given molecular mass of a peptide, the number of *averagine* units is calculated from the molecular mass of the peptide. This number is then multiplied with the average number of each element in the *averagine* formula and a tolerance of +/- 30% is added.

[1] M. W. Senko et al, J. Am. Soc. Mass Spectrom. 1995, 6, 229-233

Results for error limit set to 10 ppm

Mass	Theoretical Mass	Delta [ppm]	Delta [mmu]	RDB	Composition
516.76671	516.76671	0.0	0.0	21.0	C ₄₉ H ₇₁ O ₁₂ N ₁₃
	516.76647	0.5	0.2	15.0	C ₄₉ H ₇₉ O ₁₁ N ₉ S ₂
	516.76638	0.6	0.3	12.0	C ₄₁ H ₇₅ O ₁₄ N ₁₅ S ₁
	516.76705	-0.7	-0.3	11.5	C ₄₃ H ₇₇ O ₁₅ N ₁₂ S ₁
	516.76604	1.3	0.7	16.0	C ₄₈ H ₇₅ O ₁₆ N ₉
	516.76738	-1.3	-0.7	20.5	C ₅₁ H ₇₃ O ₁₃ N ₁₀
	516.76604	1.3	0.7	21.5	C ₄₇ H ₆₉ O ₁₁ N ₁₆
	516.76580	1.8	0.9	15.5	C ₄₇ H ₇₇ O ₁₀ N ₁₂ S ₂
	516.76772	-2.0	-1.0	16.5	C ₄₄ H ₇₃ O ₁₁ N ₁₆ S ₁
	516.76773	-2.0	-1.0	11.0	C ₄₅ H ₇₉ O ₁₆ N ₉ S ₁
	516.76805	-2.6	-1.3	25.5	C ₅₂ H ₆₉ O ₉ N ₁₄
	516.76537	2.6	1.3	16.5	C ₄₆ H ₇₃ O ₁₅ N ₁₂
	516.76807	-2.6	-1.4	7.0	C ₃₈ H ₇₉ O ₁₄ N ₁₅ S ₂
	516.76513	3.0	1.6	10.5	C ₄₆ H ₈₁ O ₁₄ N ₈ S ₂
	516.76513	3.1	1.6	16.0	C ₄₅ H ₇₅ O ₉ N ₁₅ S ₂
	516.76839	-3.3	-1.7	16.0	C ₄₆ H ₇₅ O ₁₂ N ₁₃ S ₁
	516.76479	3.7	1.9	20.0	C ₅₂ H ₇₅ O ₁₁ N ₉ S ₁
	516.76872	-3.9	-2.0	25.0	C ₅₄ H ₇₁ O ₁₀ N ₁₁
	516.76470	3.9	2.0	17.0	C ₄₄ H ₇₁ O ₁₄ N ₁₅
	516.76874	-3.9	-2.0	6.5	C ₄₀ H ₈₁ O ₁₅ N ₁₂ S ₂
	516.76446	4.3	2.2	11.0	C ₄₄ H ₇₉ O ₁₃ N ₁₁ S ₂
	516.76897	-4.4	-2.3	12.5	C ₄₀ H ₇₃ O ₁₆ N ₁₆
	516.76907	-4.6	-2.4	15.5	C ₄₈ H ₇₇ O ₁₃ N ₁₀ S ₁
				
	516.76220	8.7	4.5	19.5	C ₅₃ H ₇₇ O ₉ N ₈ S ₂
	516.76211	8.9	4.6	16.5	C ₄₅ H ₇₃ O ₁₂ N ₁₄ S ₁
	516.77142	-9.1	-4.7	15.5	C ₄₆ H ₇₇ O ₉ N ₁₄ S ₂
	516.76178	9.5	4.9	7.5	C ₃₇ H ₇₇ O ₁₄ N ₁₆ S ₂
	516.76176	9.6	4.9	20.5	C ₅₂ H ₇₃ O ₁₄ N ₈
	516.77166	-9.6	-4.9	16.0	C ₄₇ H ₇₅ O ₁₅ N ₁₁
	516.76176	9.6	4.9	26.0	C ₅₁ H ₆₇ O ₉ N ₁₅

Instrument		
Mass Error		10 ppm
# of Proposals for m/z 516.76671		49

Results for error limit set to 5 ppm

Mass	Theoretical Mass	Delta [ppm]	Delta [mmu]	RDB	Composition
516.76671	516.76671	0.0	0.0	21.0	C ₄₉ H ₇₁ O ₁₂ N ₁₃
	516.76647	0.5	0.2	15.0	C ₄₉ H ₇₉ O ₁₁ N ₉ S ₂
	516.76638	0.6	0.3	12.0	C ₄₁ H ₇₅ O ₁₄ N ₁₅ S ₁
	516.76705	-0.7	-0.3	11.5	C ₄₃ H ₇₇ O ₁₅ N ₁₂ S ₁
	516.76604	1.3	0.7	16.0	C ₄₈ H ₇₅ O ₁₆ N ₉
	516.76738	-1.3	-0.7	20.5	C ₅₁ H ₇₃ O ₁₃ N ₁₀
	516.76604	1.3	0.7	21.5	C ₄₇ H ₆₉ O ₁₁ N ₁₆
	516.76580	1.8	0.9	15.5	C ₄₇ H ₇₇ O ₁₀ N ₁₂ S ₂
	516.76772	-2.0	-1.0	16.5	C ₄₄ H ₇₃ O ₁₁ N ₁₆ S ₁
	516.76773	-2.0	-1.0	11.0	C ₄₅ H ₇₉ O ₁₆ N ₉ S ₁
	516.76805	-2.6	-1.3	25.5	C ₅₂ H ₆₉ O ₉ N ₁₄
	516.76537	2.6	1.3	16.5	C ₄₆ H ₇₃ O ₁₅ N ₁₂
	516.76807	-2.6	-1.4	7.0	C ₃₈ H ₇₉ O ₁₄ N ₁₅ S ₂
	516.76513	3.0	1.6	10.5	C ₄₆ H ₈₁ O ₁₄ N ₈ S ₂
	516.76513	3.1	1.6	16.0	C ₄₅ H ₇₅ O ₉ N ₁₅ S ₂
	516.76839	-3.3	-1.7	16.0	C ₄₆ H ₇₅ O ₁₂ N ₁₃ S ₁
	516.76479	3.7	1.9	20.0	C ₅₂ H ₇₅ O ₁₁ N ₉ S ₁
	516.76872	-3.9	-2.0	25.0	C ₅₄ H ₇₁ O ₁₀ N ₁₁
	516.76470	3.9	2.0	17.0	C ₄₄ H ₇₁ O ₁₄ N ₁₅
	516.76874	-3.9	-2.0	6.5	C ₄₀ H ₈₁ O ₁₅ N ₁₂ S ₂
	516.76446	4.3	2.2	11.0	C ₄₄ H ₇₉ O ₁₃ N ₁₁ S ₂
	516.76897	-4.4	-2.3	12.5	C ₄₀ H ₇₃ O ₁₆ N ₁₆
	516.76907	-4.6	-2.4	15.5	C ₄₈ H ₇₇ O ₁₃ N ₁₀ S ₁

Instrument		QqTOF
Mass Error		5 ppm
# of Proposals for m/z 516.76671		23

Results for error limit set to 1-2 ppm

			Mass	Theoretical Mass	Delta [ppm]	Delta [mmu]	RDB	Composition
			516.76671	516.76671	0.0	0.0	21.0	C ₄₉ H ₇₁ O ₁₂ N ₁₃
				516.76647	0.5	0.2	15.0	C ₄₉ H ₇₉ O ₁₁ N ₉ S ₂
				516.76638	0.6	0.3	12.0	C ₄₁ H ₇₅ O ₁₄ N ₁₅ S ₁
				516.76705	-0.7	-0.3	11.5	C ₄₃ H ₇₇ O ₁₅ N ₁₂ S ₁
				516.76604	1.3	0.7	16.0	C ₄₈ H ₇₅ O ₁₆ N ₉
				516.76738	-1.3	-0.7	20.5	C ₅₁ H ₇₃ O ₁₃ N ₁₀
				516.76604	1.3	0.7	21.5	C ₄₇ H ₆₉ O ₁₁ N ₁₆
				516.76580	1.8	0.9	15.5	C ₄₇ H ₇₇ O ₁₀ N ₁₂ S ₂
				516.76772	-2.0	-1.0	16.5	C ₄₄ H ₇₃ O ₁₁ N ₁₆ S ₁
				516.76773	-2.0	-1.0	11.0	C ₄₅ H ₇₉ O ₁₆ N ₉ S ₁
Instrument		LQ-FT						
Mass Error		2 ppm						
# of Proposals for m/z 516.76671		10						

			Mass	Theoretical Mass	Delta [ppm]	Delta [mmu]	RDB	Composition
			516.76671	516.76671	0.0	0.0	21.0	C ₄₉ H ₇₁ O ₁₂ N ₁₃
				516.76647	0.5	0.2	15.0	C ₄₉ H ₇₉ O ₁₁ N ₉ S ₂
				516.76638	0.6	0.3	12.0	C ₄₁ H ₇₅ O ₁₄ N ₁₅ S ₁
				516.76705	-0.7	-0.3	11.5	C ₄₃ H ₇₇ O ₁₅ N ₁₂ S ₁
Instrument		LQ-FT						
Mass Error		1 ppm						
# of Proposals for m/z 516.76671		4						

What about the mass of the Electron

An electron weights about 0.55 mmu and has a significant contribution to the exact mass calculation especially with increasing charge state. Add or deduct $n * 0.55$ mmu from the calculated mass, where n is the number of charge states.

# of charges	0	+1	+2	+5	+10
Exact mass	518.26764	518.26709	518.26654	518.26494	518.26244

Detection Time & Mass Resolution

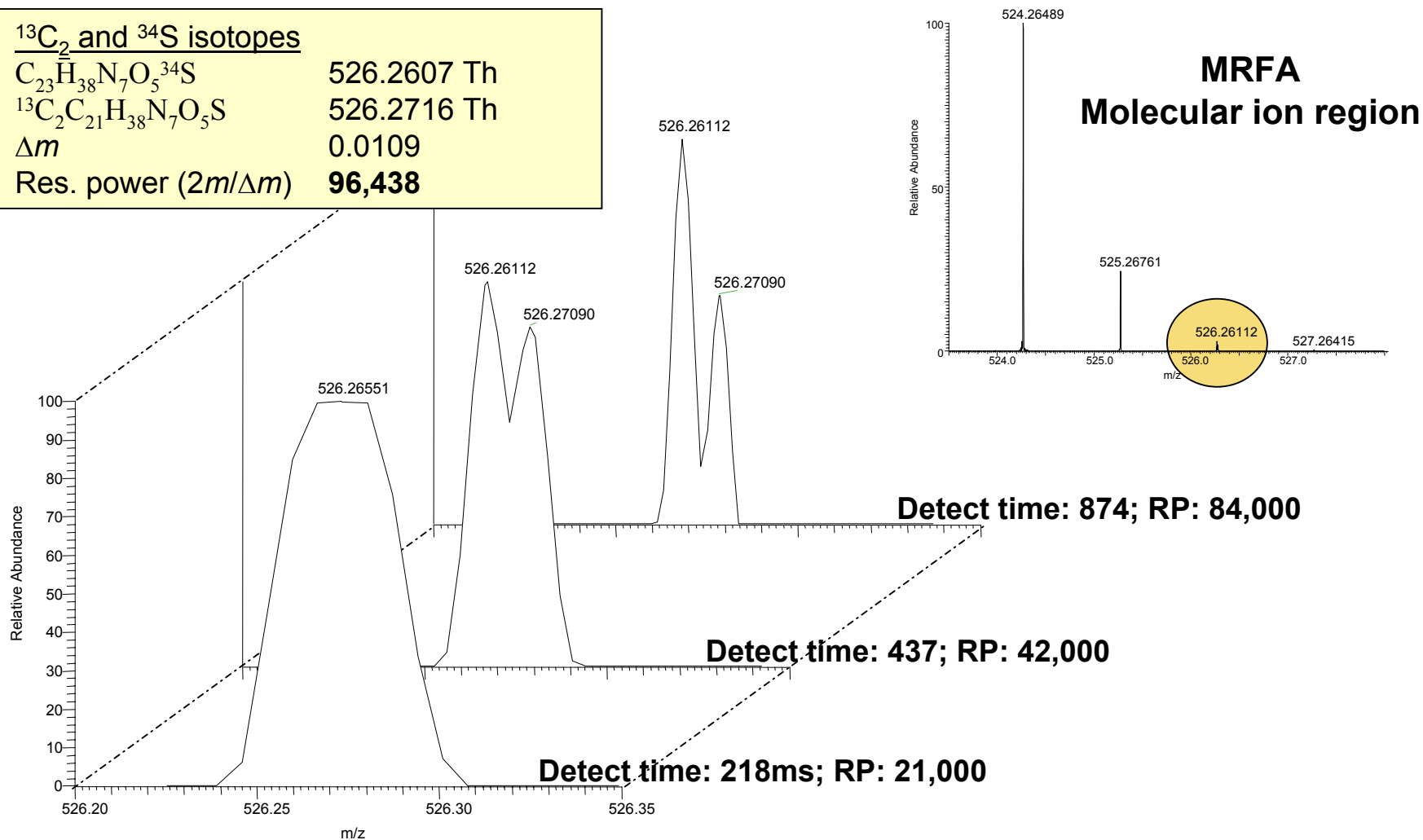
$^{13}\text{C}_2$ and ^{34}S isotopes

$\text{C}_{23}\text{H}_{38}\text{N}_7\text{O}_5^{34}\text{S}$ 526.2607 Th

$^{13}\text{C}_2\text{C}_{21}\text{H}_{38}\text{N}_7\text{O}_5\text{S}$ 526.2716 Th

Δm 0.0109

Res. power ($2m/\Delta m$) **96,438**



Detected Mass Resolution vs. Simulation

MRFA - ^{13}C and ^{34}S isotopes

$\text{C}_{23}\text{H}_{38}\text{N}_7\text{O}_5^{34}\text{S}$ 526.26076 Th

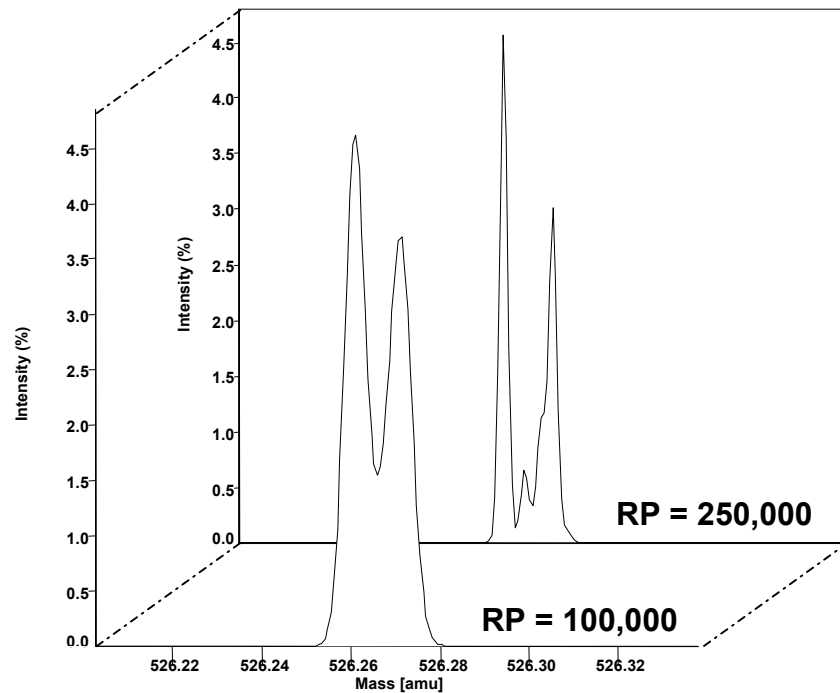
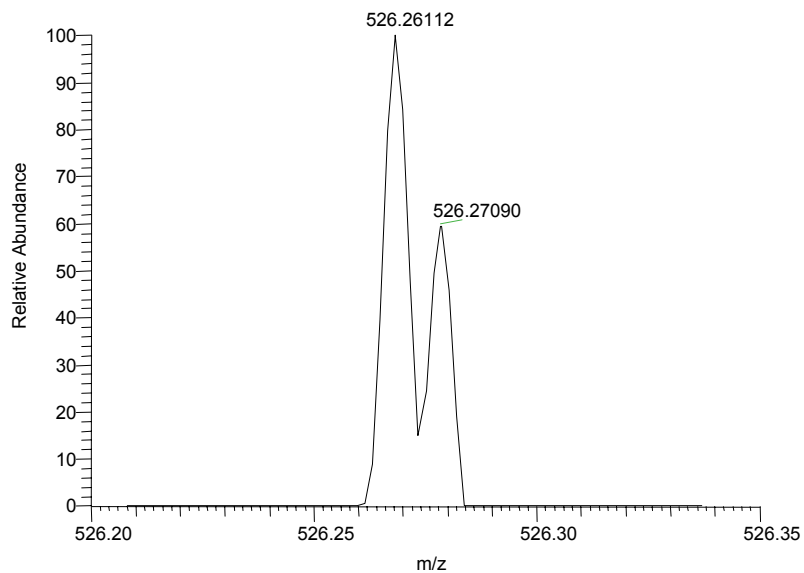
$^{13}\text{C}_2\text{C}_{21}\text{H}_{38}\text{N}_7\text{O}_5\text{S}$ 526.27167 Th

Δm 0.01091

Res. power ($2m/\Delta m$) **96,473**

Detect time: 874ms

Resolution: 84,000

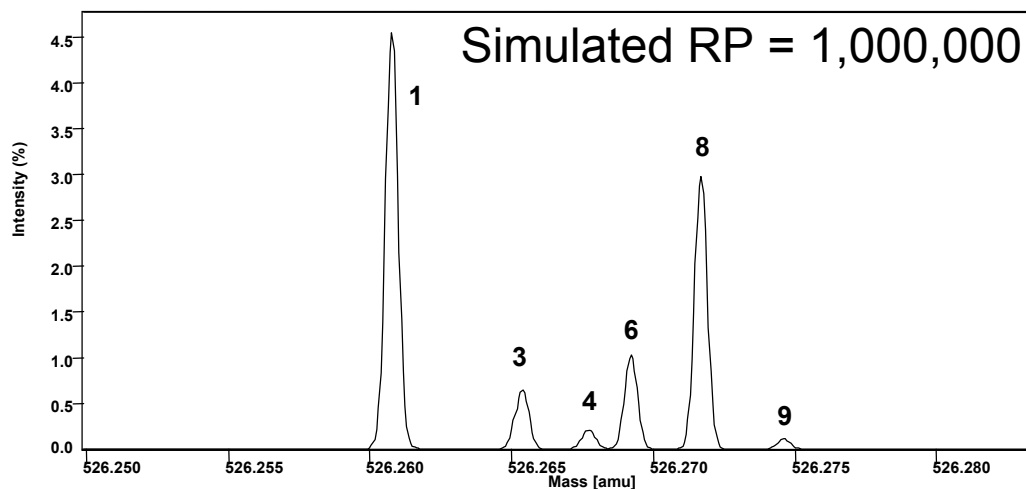


Never Trust Isotope Peaks

Simulation of the [M+H+2]-isotope pattern for MRFA

Formula #1: c23 h38 o5 n7 s

Index	Mass	Intensity	Formula
1	526.26076	4.51912	[12]C23 [1]H38 [16]O5 [14]N7 [34]S1
2	526.26139	0.02070	[12]C23 [1]H38 [16]O5 [14]N6 [15]N1 [33]S1
3	526.26535	0.64318	[12]C22 [13]C1 [1]H38 [16]O5 [14]N6 [15]N1 [32]S1
4	526.26771	0.19916	[12]C22 [13]C1 [1]H38 [16]O5 [14]N7 [33]S1
5	526.26857	0.00152	[12]C23 [1]H38 [16]O4 [17]O1 [14]N7 [33]S1
6	526.26921	1.02750	[12]C23 [1]H38 [16]O4 [18]O1 [14]N7 [32]S1
7	526.27063	0.00350	[12]C23 [1]H37 [2]H1 [16]O5 [14]N7 [33]S1
8	526.27167	2.95959	[12]C21 [13]C2 [1]H38 [16]O5 [14]N7 [32]S1
9	526.27460	0.10872	[12]C22 [13]C1 [1]H37 [2]H1 [16]O5 [14]N7 [32]S1



Finnigan LTQ FT – Ultra High Resolution

NRVYVHPFHL (3+) 427.89988 (Angiotensin from Goosefish)

