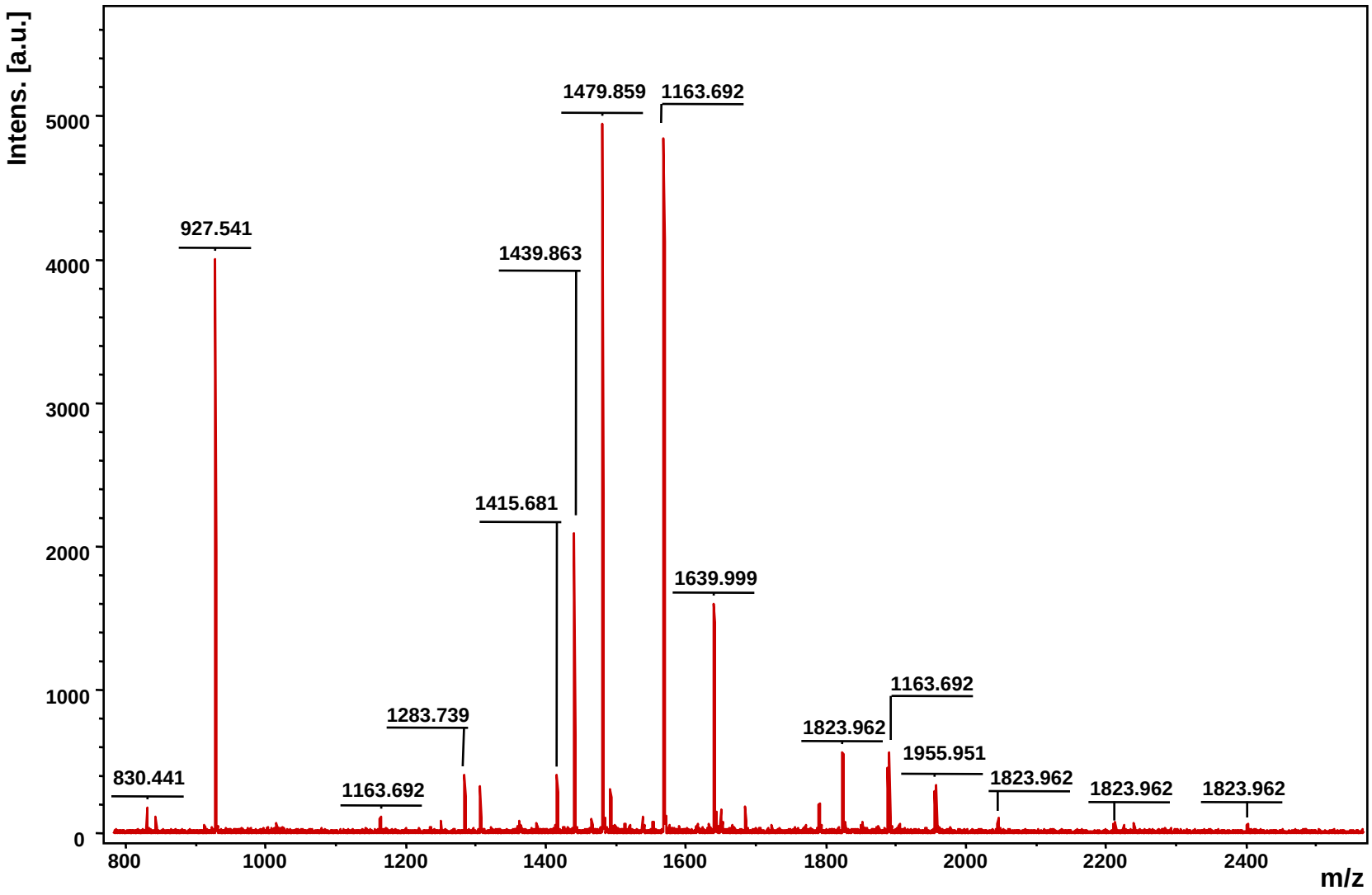
ESPECTROMETRIA DE MASAS

CARACTERISTICAS DE LA ESPECTROMETRIA DE MASAS COMO TECNICA ANALITICA

- **POSIBILITA LA DETERMINACION DE LA RELACION MASA/CARGA (M/Z) DE UN ION EN UNA MEZCLA Y EN CONSECUENCIA SU MASA MOLECULAR, ej. El pm de un péptido o una proteina.**

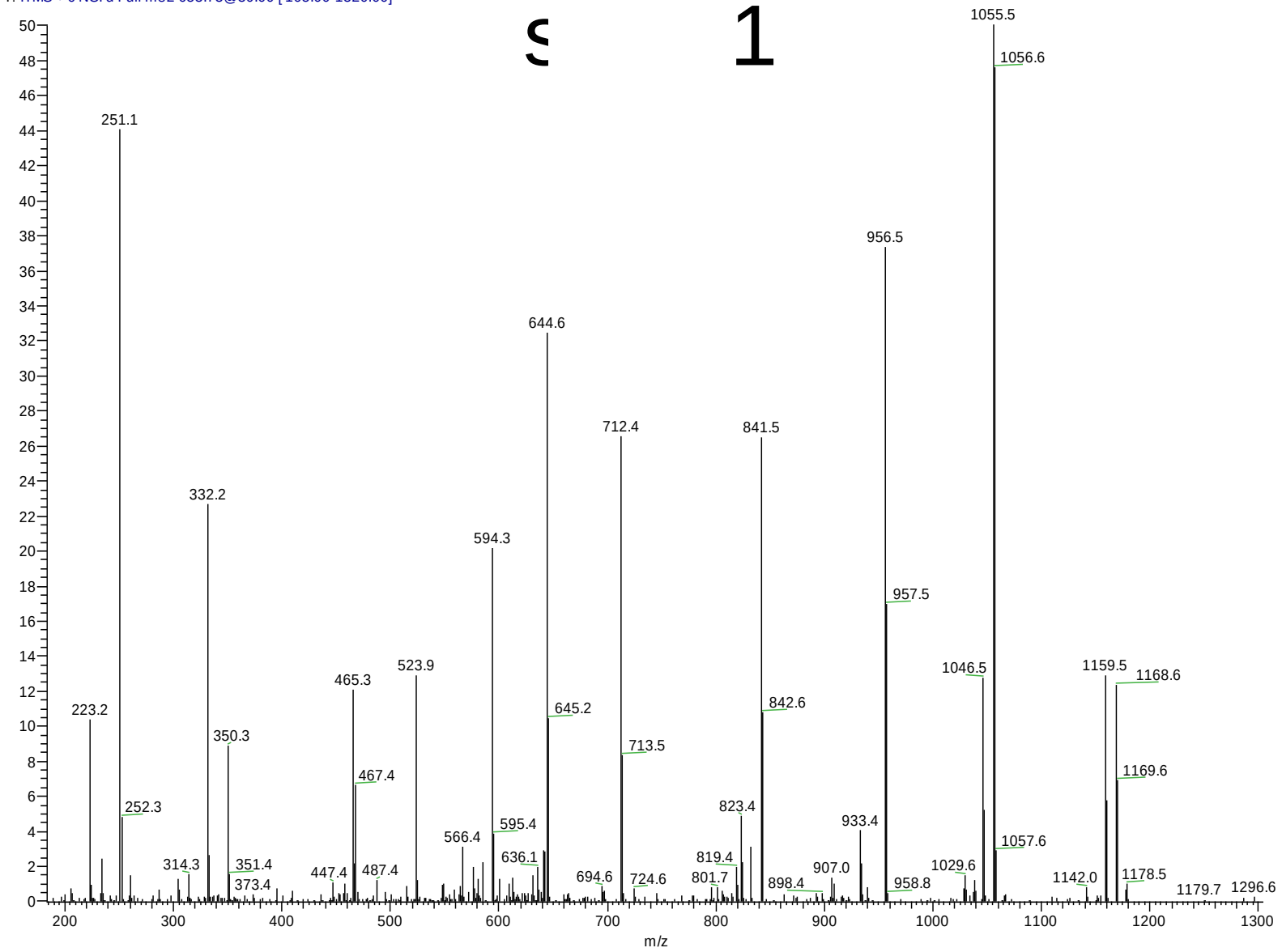
- **OFRECE INFORMACION ESTRUCTURAL DE UN ION SELECCIONANDO ANALIZANDO LAS MASAS DE LOS FRAGMENTOS QUE SE PRODUCEN EN LA COLISION ENTRE ESTE ION Y LAS MOLECULAS DE UN GAS NEUTRO, ej. La secuencia de aminoácidos de un péptido o modificaciones en su estructura primaria.**

Mapa MALDI-TOF de un digerido en gel de BSA



HLVDEPQNLIK

BSA_031203_NEWDIGEST02 #4327-4332 RT: 18.43-18.44 AV: 2 NL: 3.49E3
T: ITMS + c NSI d Full ms2 653.73@30.00 [165.00-1320.00]

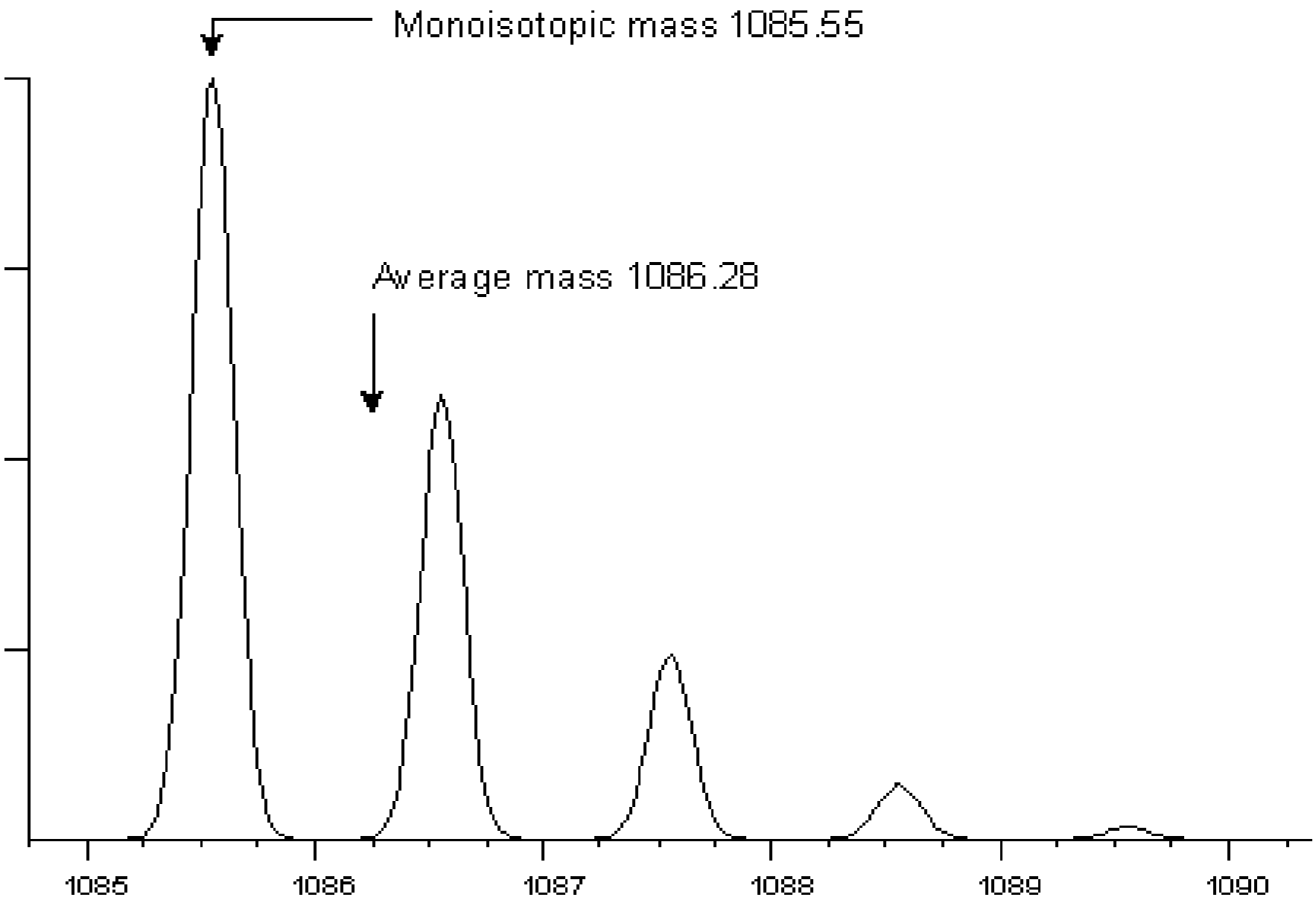


m/z Value

$$m/z = (M + nH)/n \approx (M + n)/n$$

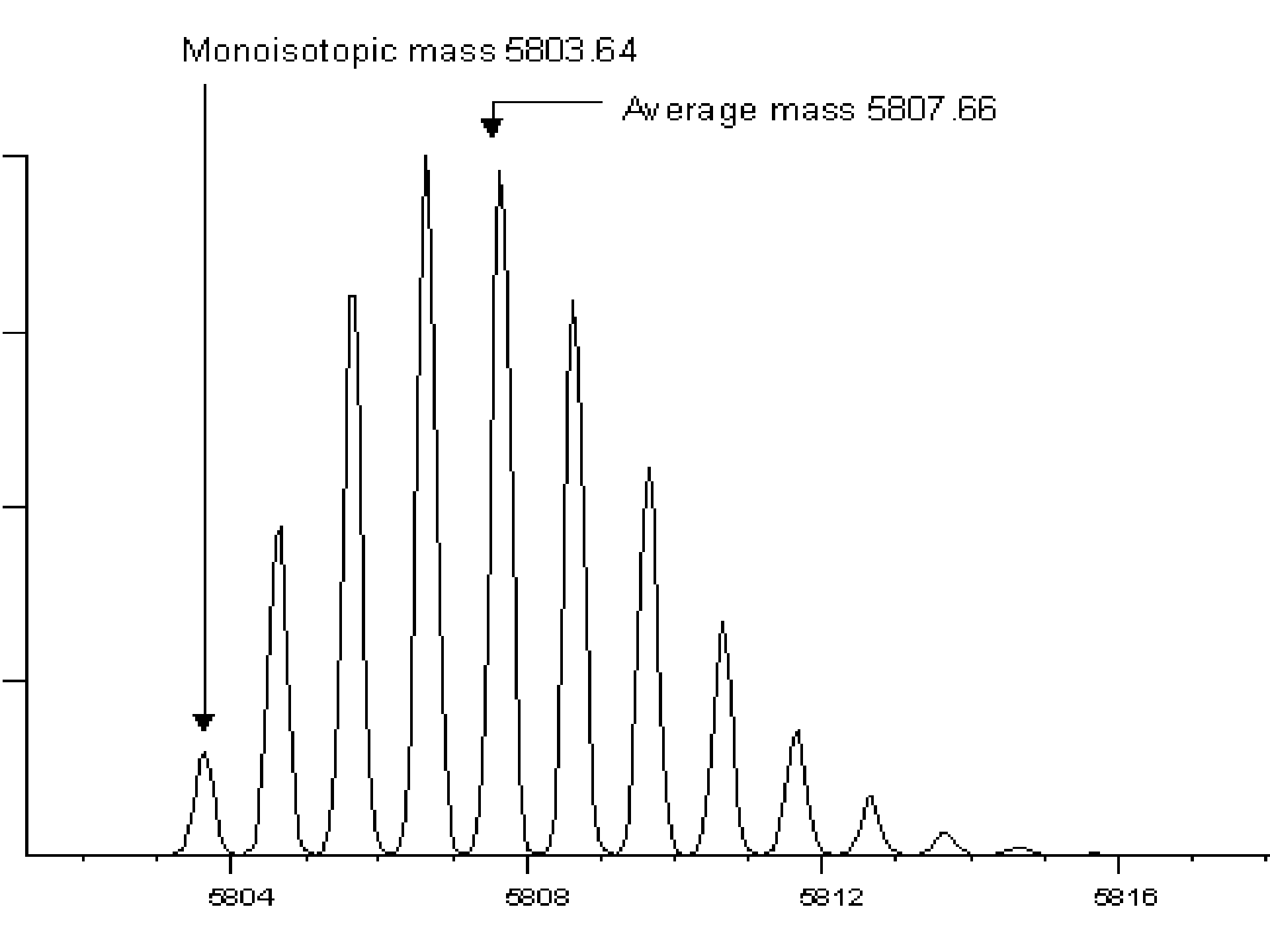
$$M = (m/z)\, n - nH \approx (m/z)\, n - n$$

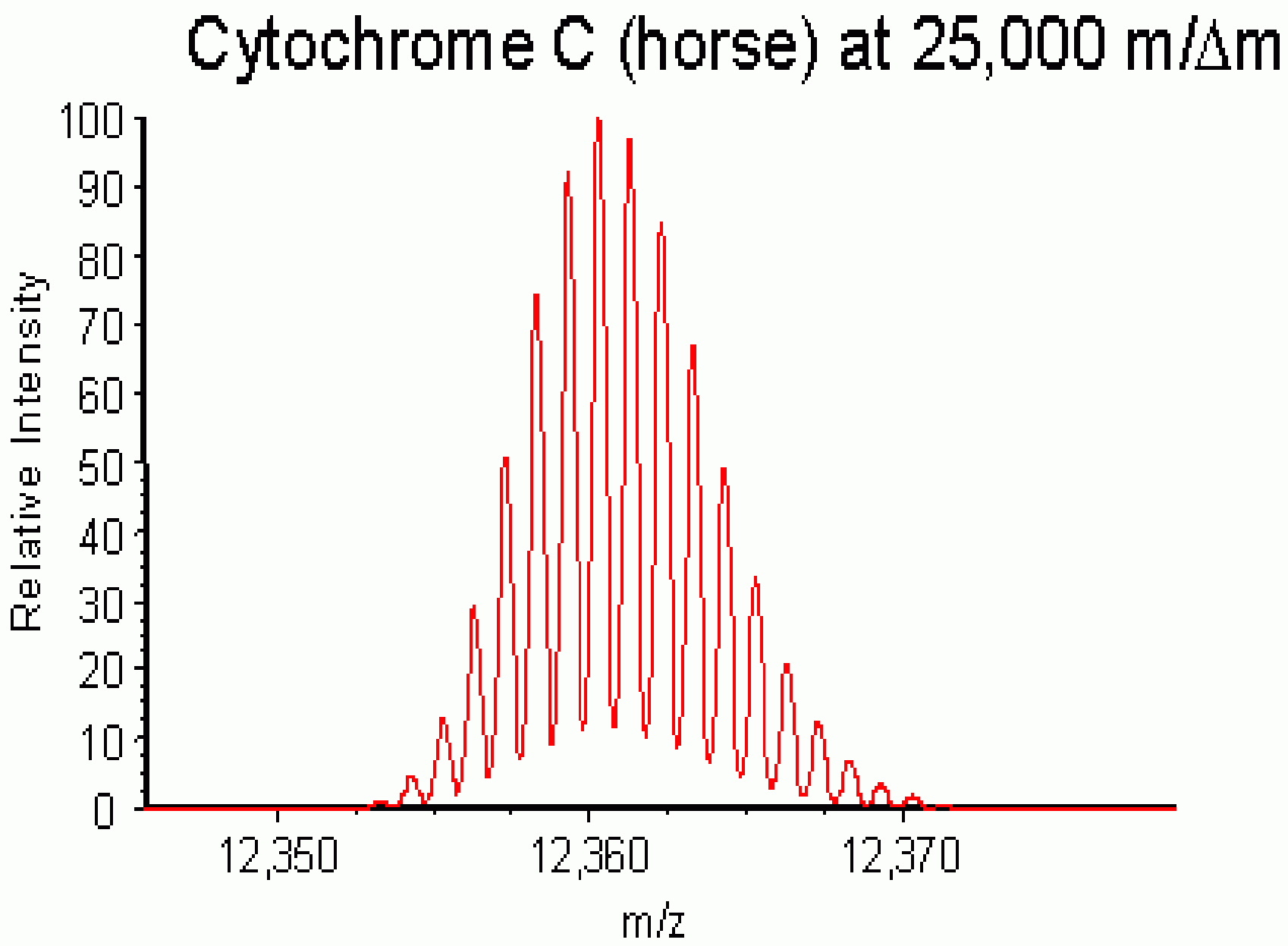
M: mass of the measured molecule
n: number of H^+
H: H^+`mass

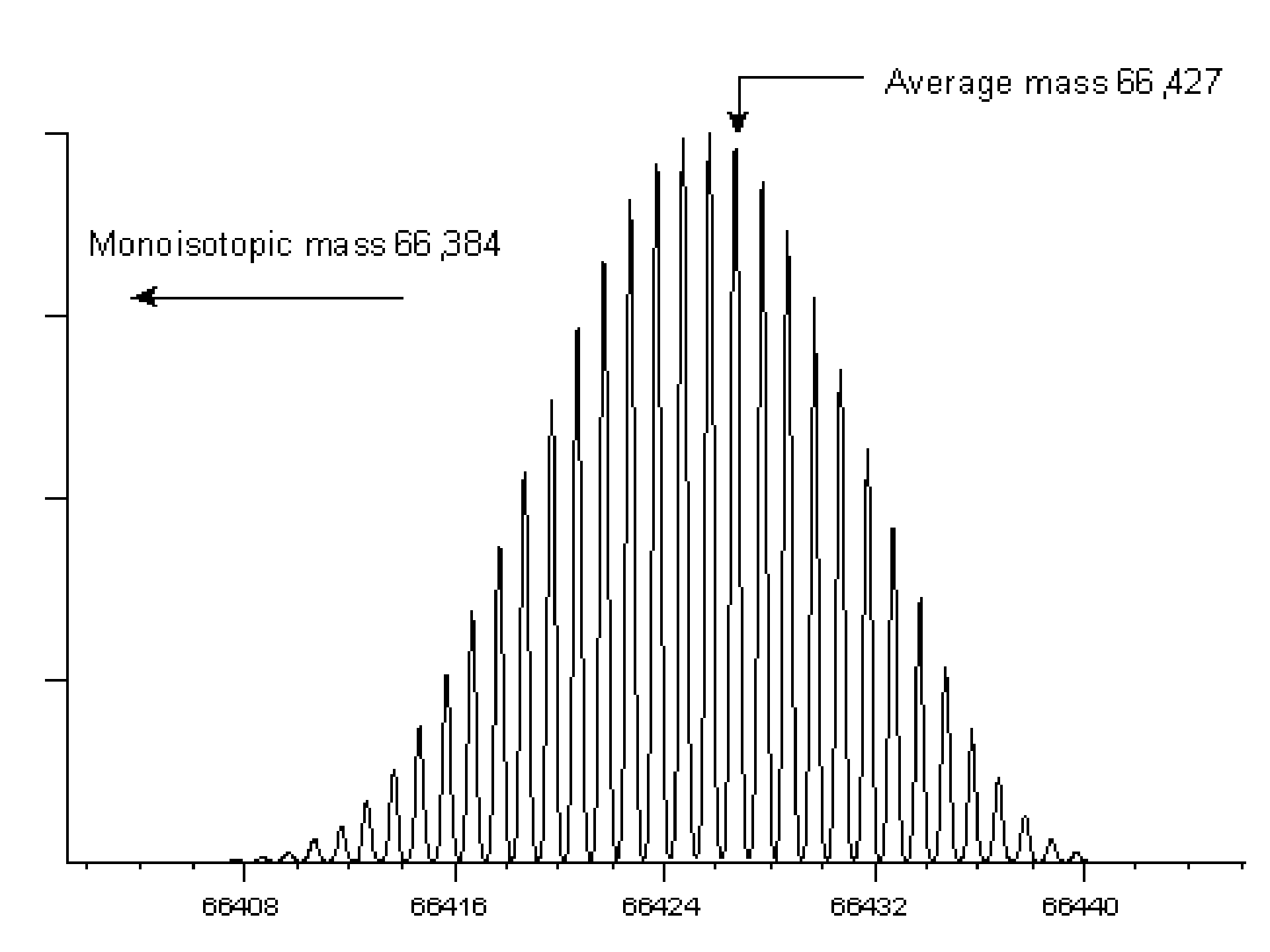

Monoisotopic mass 1085.55
Average mass 1086.28
1085
1086
1087
1088
1089
1090

Isotopic abundances of some common elements

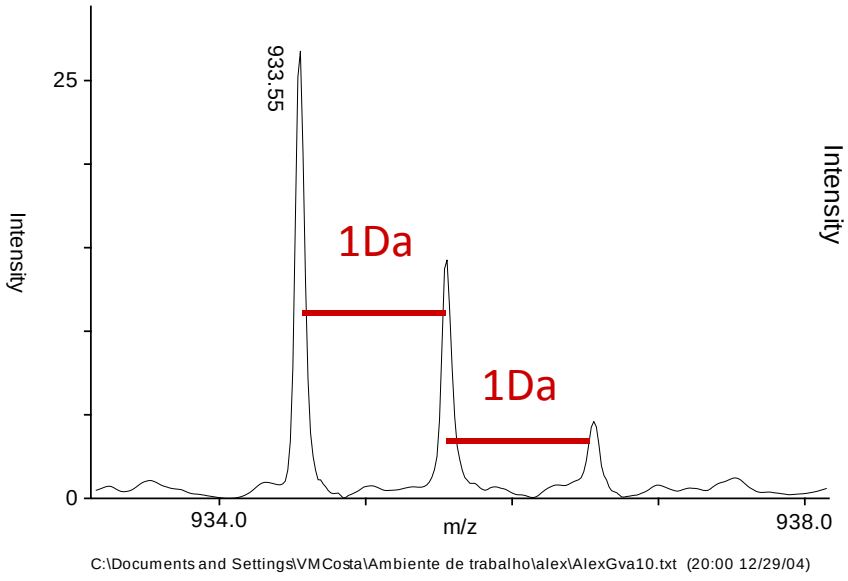
Element	Mass	Natural Abundance
H	1.0078 2.0141	99.985% 0.015
C	12.0000 13.0034	98.89 1.11
N	14.0031 15.0001	99.64 0.36
O	15.9949 16.9991 17.9992	99.76 0.04 0.20
F	18.9984	100
S	31.9721 32.9715 33.9679 35.9671	95.00 0.76 4.22 0.02

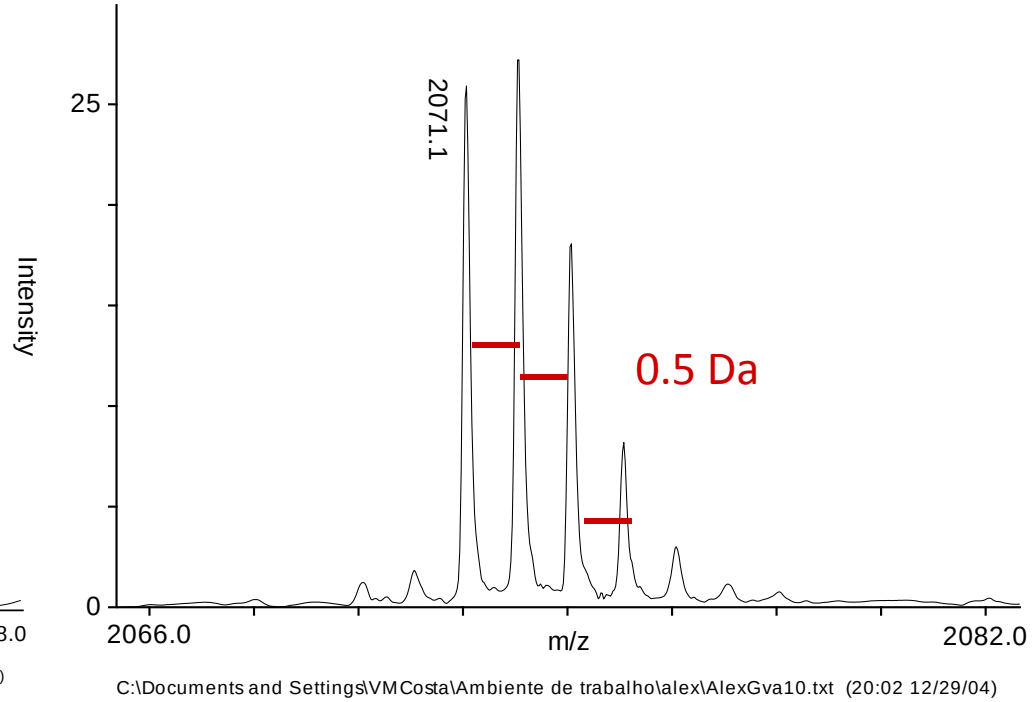

Monoisotopic mass 5803.64
Average mass 5807.66
5804
5808
5812
5816

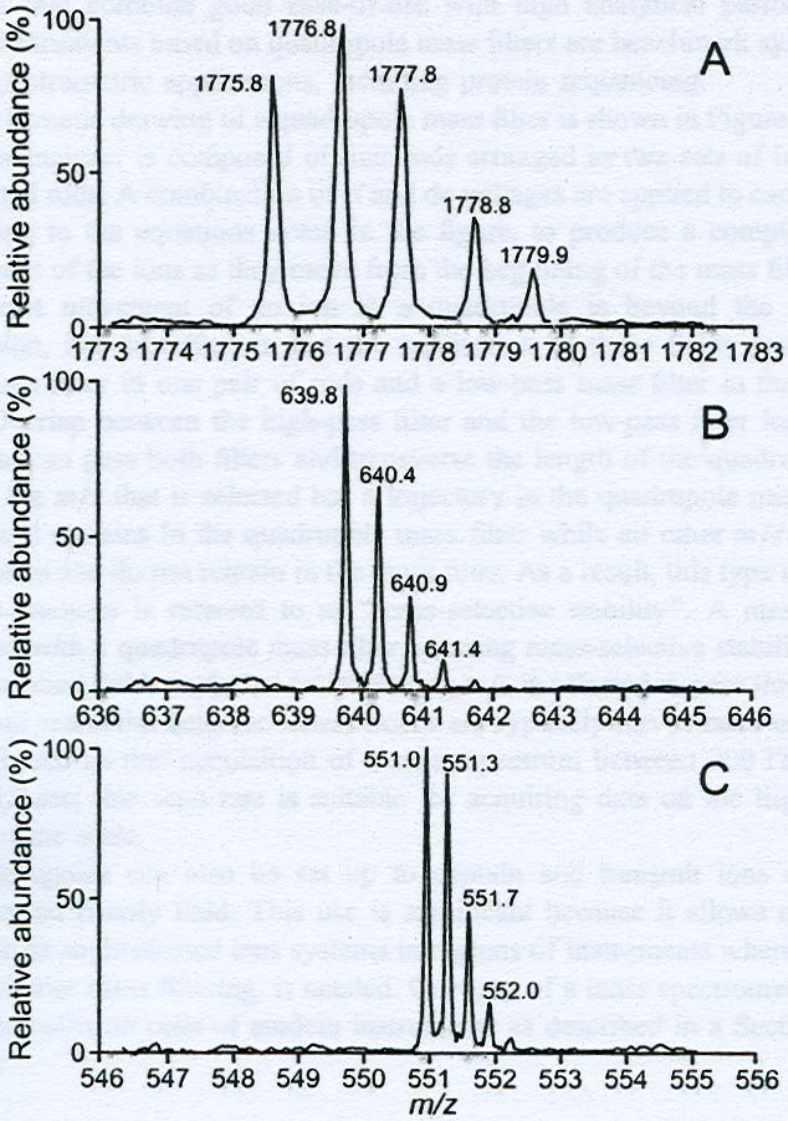

Cytochrome C (horse) at 25,000 m/Δm
Relative Intensity
100
90
80
70
60
50
40
30
20
10
0
12,350
12,360
12,370
m/z


Average mass 66,427
Monoisotopic mass 66,384
66408
66416
66424
66432
66440

Isotopic cluster

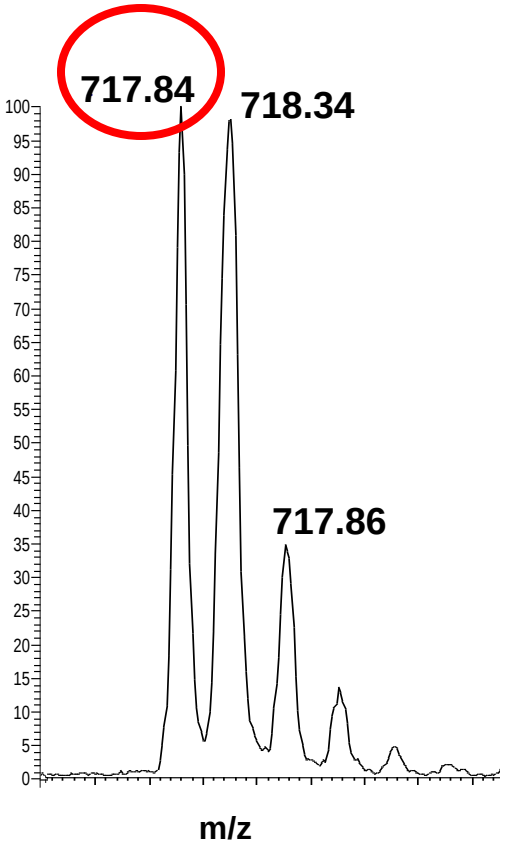






igure 3.7. Resolved isotope clusters of peptide ions at different charge states. Resolution of ıe isotope cluster of a peptide ion allows the charge state of that ion to be determined. (A) The otope cluster for a singly charged peptide ion $(M + H)^+$ is characterized by an m/z difference of .0 Th. (B) The isotope cluster for a doubly charged peptide $(M + 2H)^{+2}$ is characterized by an $ı/z$ difference of 0.5 Th. (C) The isotope cluster of a triply charged peptide $(M + 3H)^{+3}$ is ıaracterized by an m/z difference of 0.33 Th.

m/z Value



Elemental Compositon: C64 H93 N18 O20
Monoisotopic Mass: 1433.6813

H^+

GFGFGQGAGALVHSK

H^+

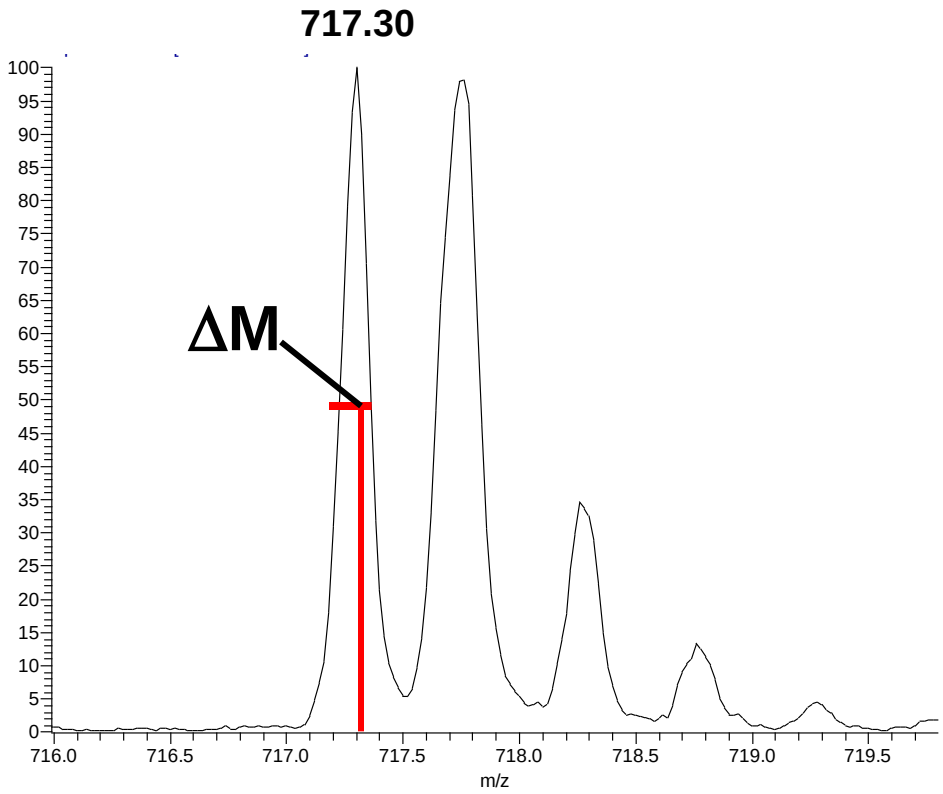
$$m/z = (M + nH)/n \approx (M + n)/n$$

$$m/z = (1433.6813 + 2H)/2$$

$$\approx (1433.6813 + 2)/2$$

$$m/z = 717.8406$$

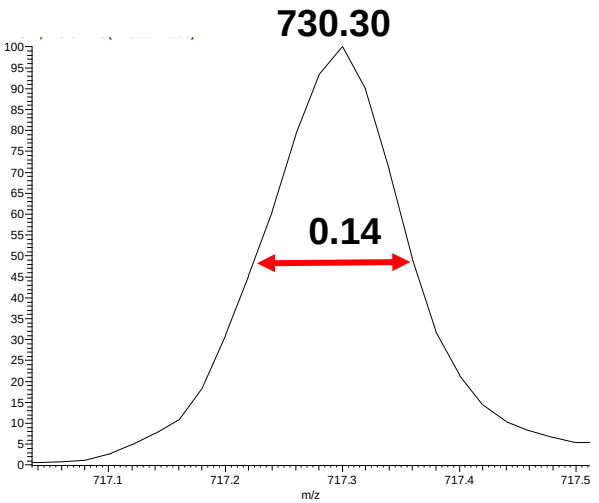
Resolution



Resolution: M/ΔM

Resolution = 730.30/0.14

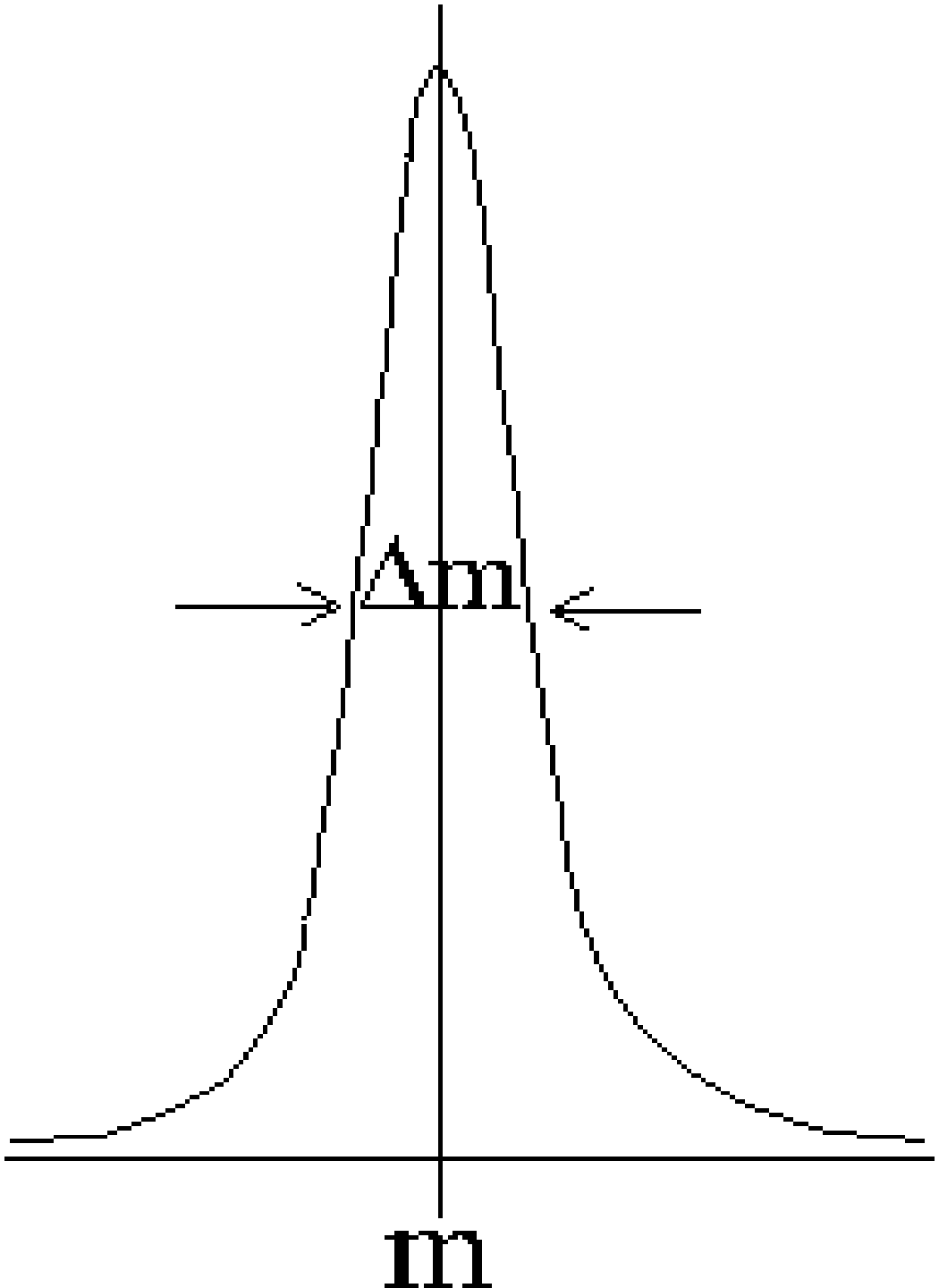
= 5216.42



RESOLUCION:

R= M/ΔM

Cuando se mide la anchura de pico a altura media: FWHM



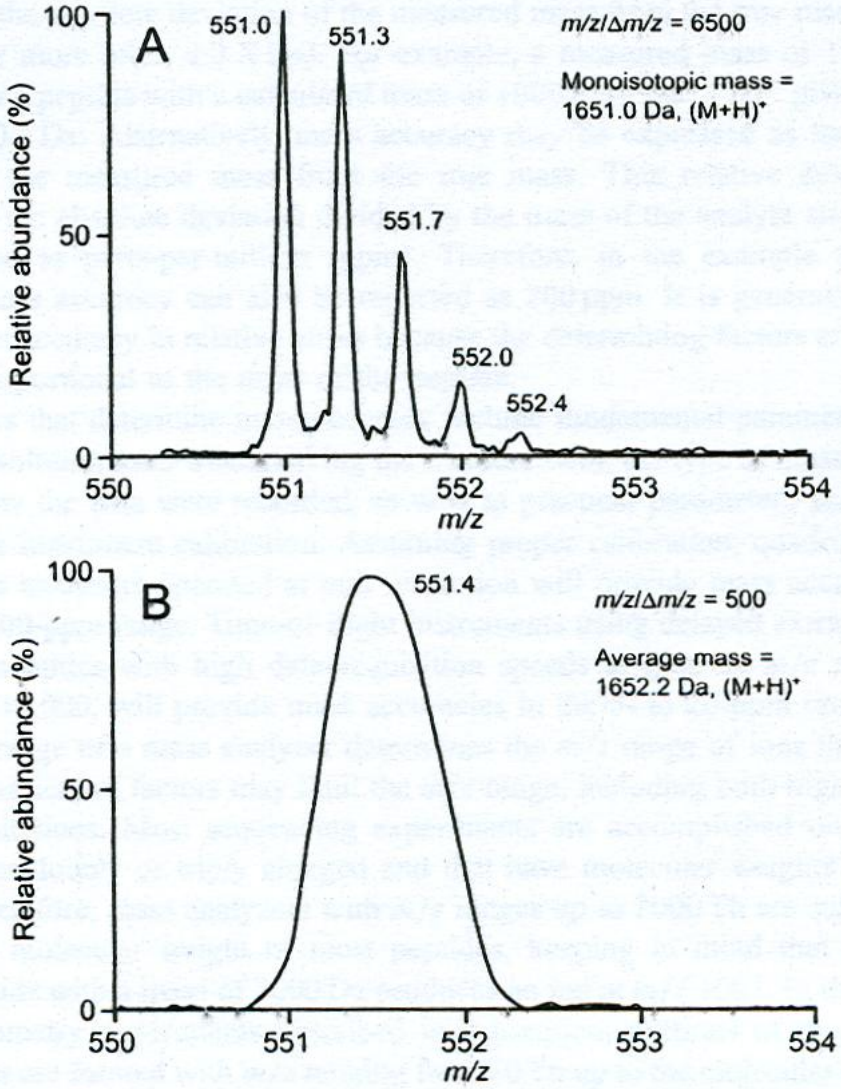
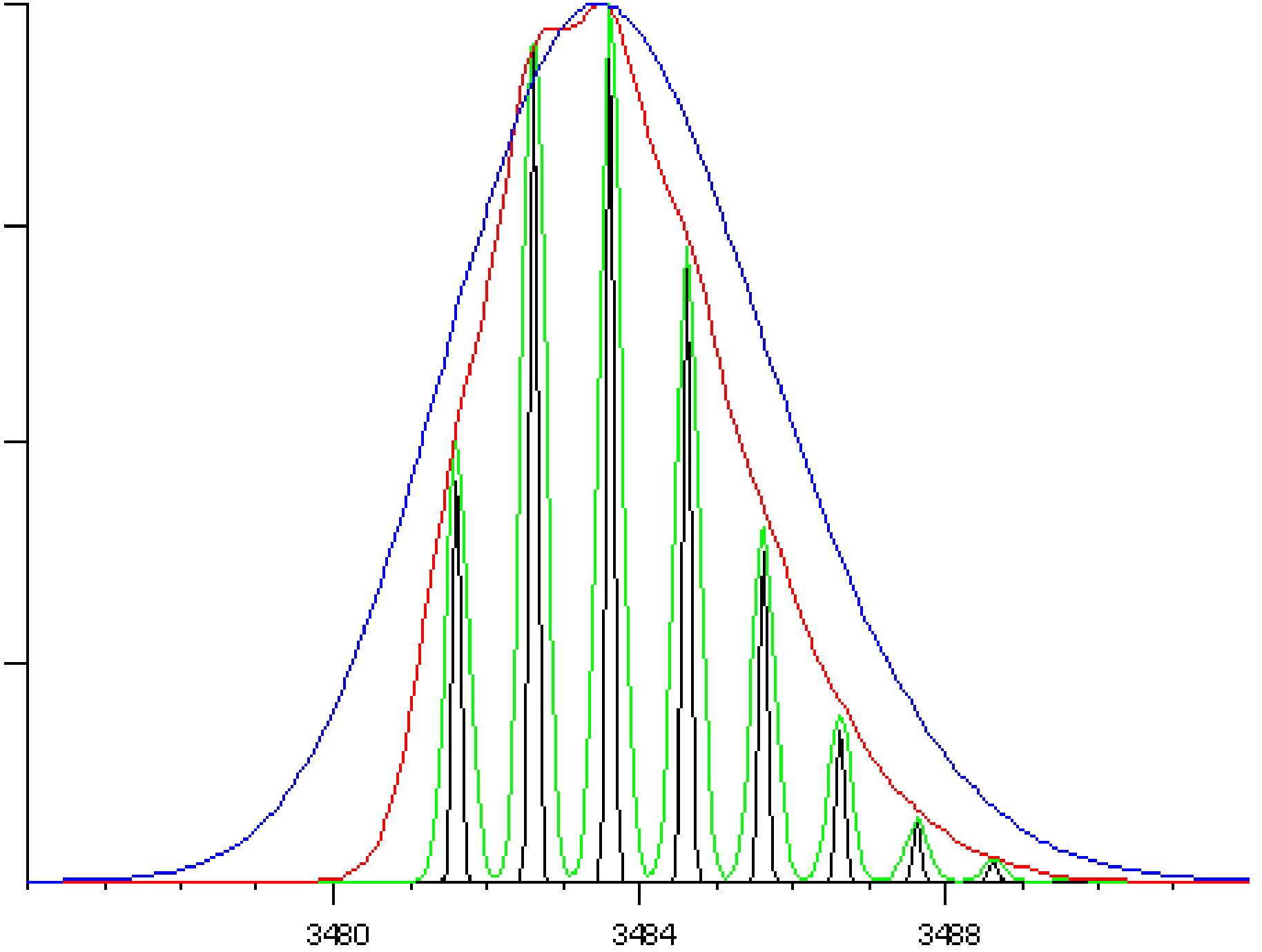
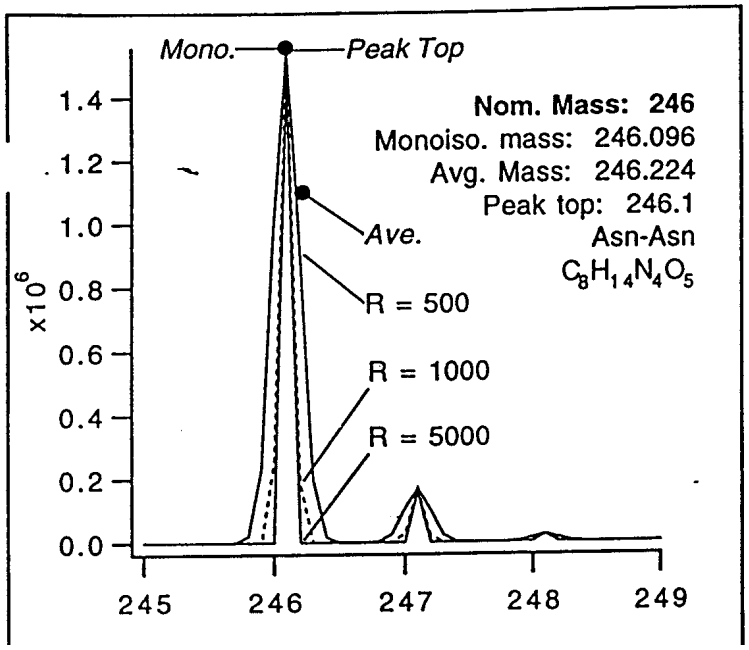
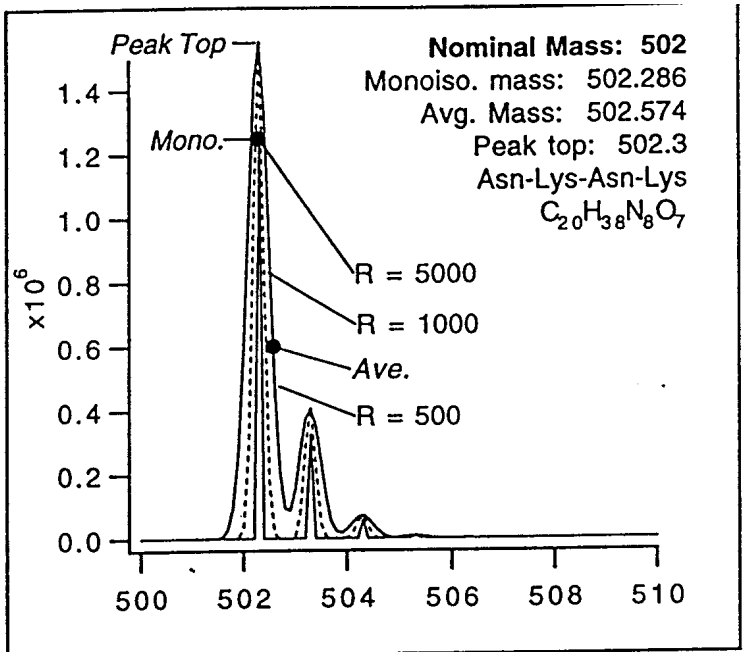
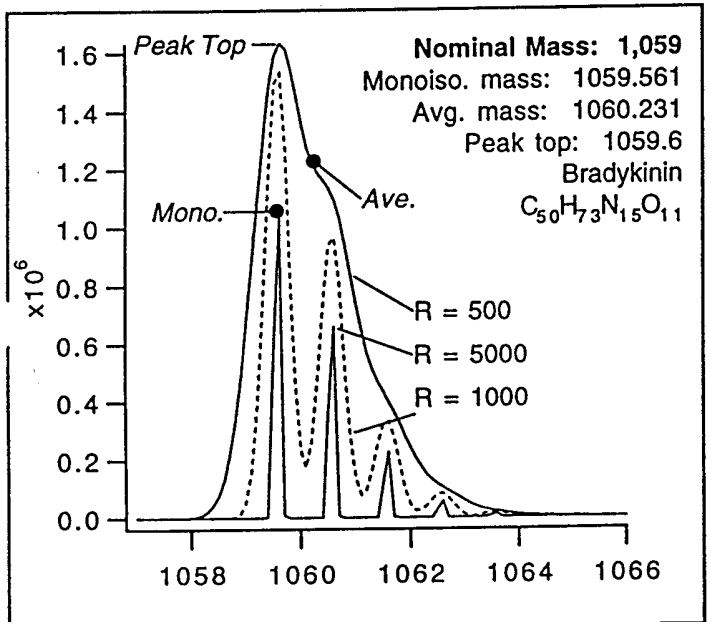


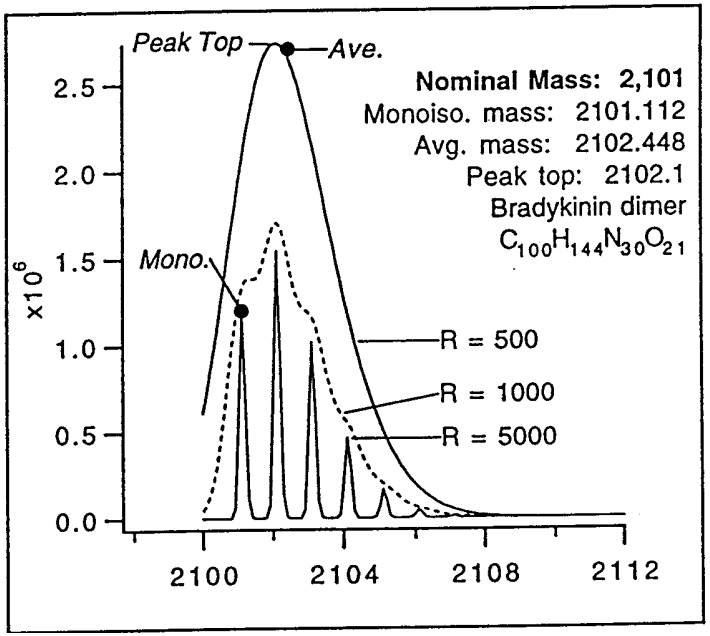
Figure 3.6. Resolution of the isotope cluster of a peptide. (A) Mass analysis of a triply charged peptide ion at a mass resolution of 6500. This resolution is sufficient to resolve the components of the isotope cluster and to measure the monoisotopic mass. (B) Mass analysis of a triply charged peptide ion at a mass resolution of 500. This resolution cannot resolve the isotope cluster of the ion, and the average mass of the peptide ion is determined.

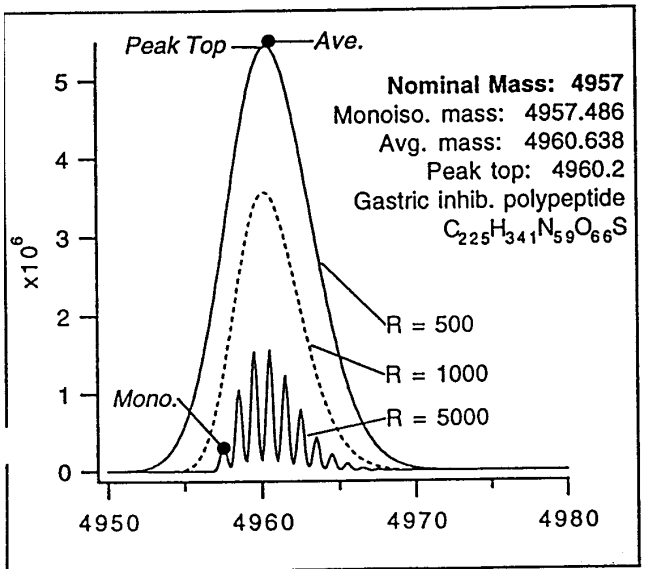

3480
3484
3488

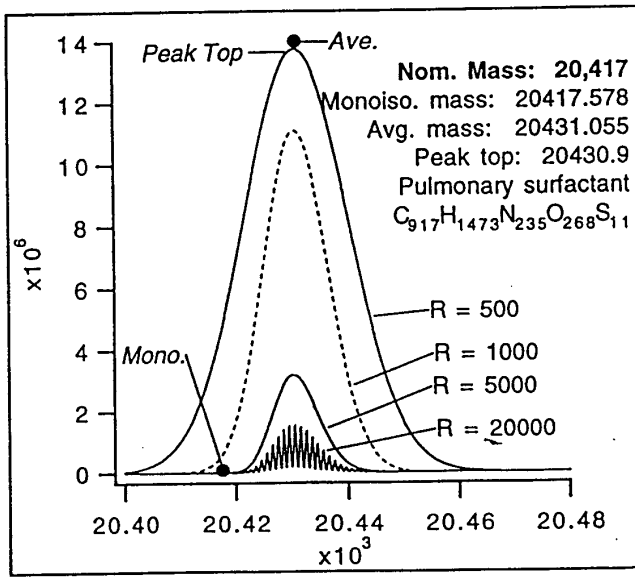

Mono.
Peak Top
Ave.
R = 500
R = 1000
R = 5000
Nom. Mass: 246
Monoiso. mass: 246.096
Avg. Mass: 246.224
Peak top: 246.1
Asn-Asn
$C_8H_{14}N_4O_5$
x10^6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
245
246
247
248
249


Peak Top
Mono.
R = 5000
R = 1000
Ave.
R = 500
Nominal Mass: 502
Monoiso. mass: 502.286
Avg. Mass: 502.574
Peak top: 502.3
Asn-Lys-Asn-Lys
$C_{20}H_{38}N_8O_7$
x10^6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
500
502
504
506
508
510

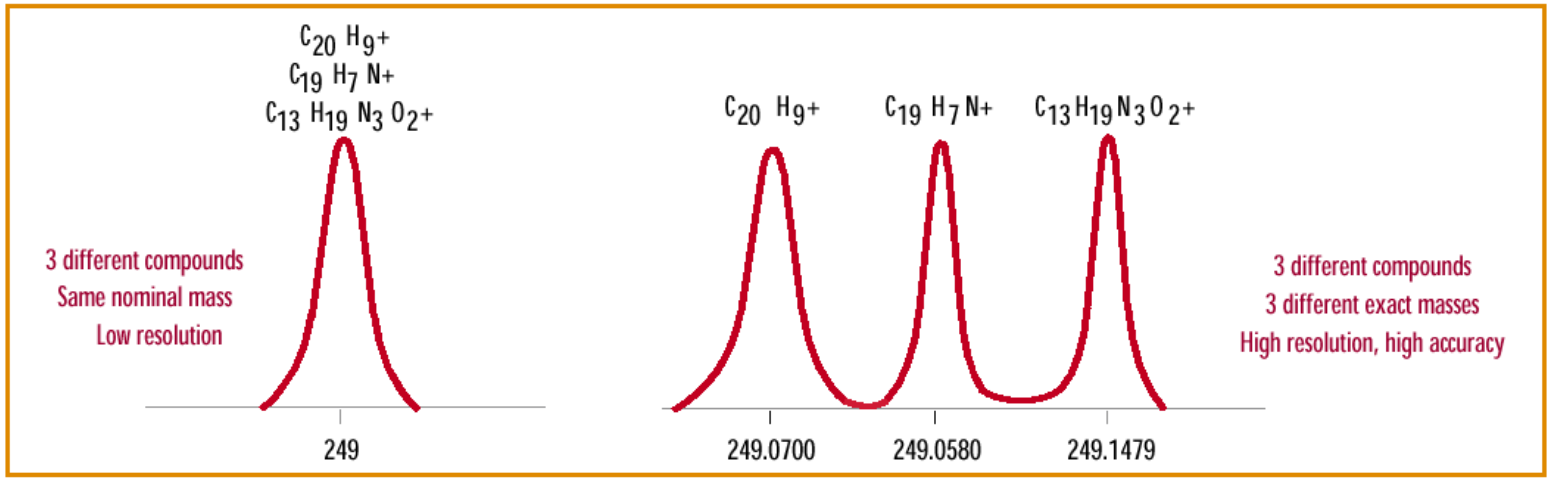

Peak Top
Ave.
Mono.
R = 500
R = 5000
R = 1000
Nominal Mass: 1,059
Monoiso. mass: 1059.561
Avg. mass: 1060.231
Peak top: 1059.6
Bradykinin
$C_{50}H_{73}N_{15}O_{11}$
x10^6
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
1058
1060
1062
1064
1066


Peak Top
Ave.
Mono.
R = 500
R = 1000
R = 5000
Nominal Mass: 2,101
Monoiso. mass: 2101.112
Avg. mass: 2102.448
Peak top: 2102.1
Bradykinin dimer
$C_{100}H_{144}N_{30}O_{21}$
x10^6
2.5
2.0
1.5
1.0
0.5
0.0
2100
2104
2108
2112


Peak Top
Ave.
Nominal Mass: 4957
Monoiso. mass: 4957.486
Avg. mass: 4960.638
Peak top: 4960.2
Gastric inhib. polypeptide
$C_{225}H_{341}N_{59}O_{66}S$
R = 500
R = 1000
R = 5000
Mono.
x10^6
0
1
2
3
4
5
4950
4960
4970
4980


Peak Top
Ave.
Nom. Mass: 20,417
Monoiso. mass: 20417.578
Avg. mass: 20431.055
Peak top: 20430.9
Pulmonary surfactant
$C_{917}H_{1473}N_{235}O_{268}S_{11}$
R = 500
R = 1000
R = 5000
R = 20000
Mono.
x10^6
0
2
4
6
8
10
12
14
20.40
20.42
20.44
20.46
20.48
x10^3

High Mass Accuracy Measurements



Mass Accuracy

- Mass accuracy in *parts per million* (ppm)

$$\frac{(\text{measured mass} - \text{observed mass})}{\text{true mass}} \times 10^6$$

- Measured mass
  - 545.4200
- Known (calculated) mass
  - 545.3234
- Mass accuracy = (545.4200-545.3234)/545 =0.00011724 Da

= 0.00011724 x 10^6 = 117 ppm

A monoisotopic mass can be measured as accurately as the instrument allows, as long as the monoisotopic peak has been correctly identified. If the wrong peak is selected, the mass value will be false by one or two Daltons.