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EPA

ICE* is Nice

Version 1: 9/1/00

Created, Written, Narrated, and Prepared by:

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U.S. Environmental Protection Agency

P.O. Box 93478, Las Vegas, NV 89193

William C. Brumley, Ph. D. & G. Wayne Sovocool, Ph. D.

*Ion Composition Elucidation

Mt. Hood, Oregon

Click

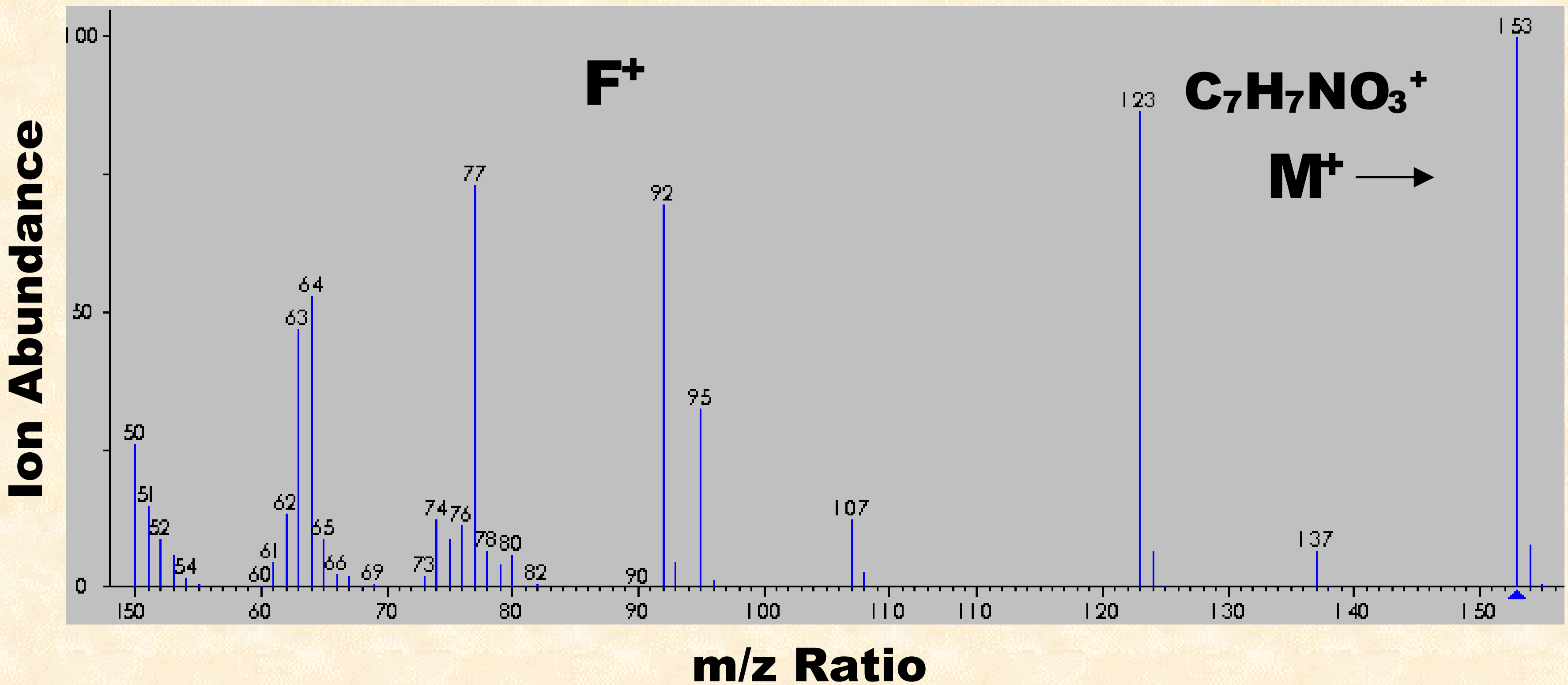
7 C

7 H

1 N

3 O

→ $C_7H_7NO_3^+$



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ICE Instructional Video

Problem Solved by ICE

Scientific Basis, Advantages, Limitations of ICE

Data Acquisition:

Mass Peak Profiling from Selected Ion Recording Data (MPPSIRD)

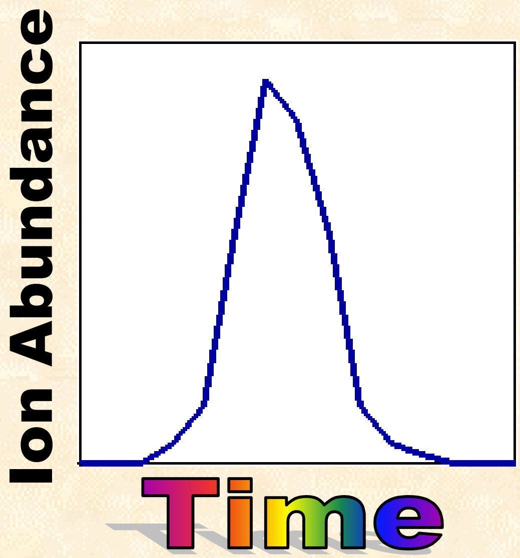
Automated Data Interpretation:

Profile Generation Model (PGM)

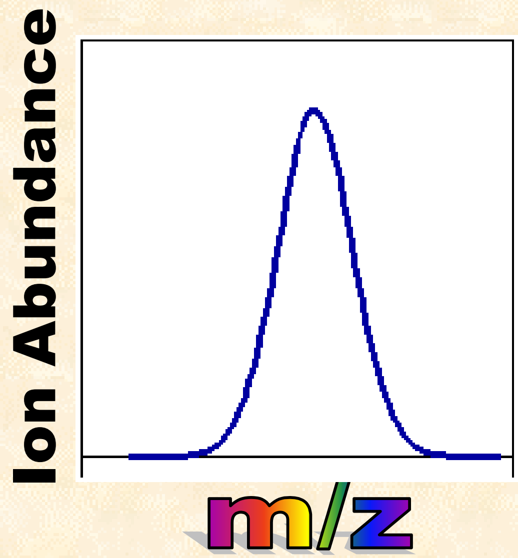
3 Example Applications of ICE

Click

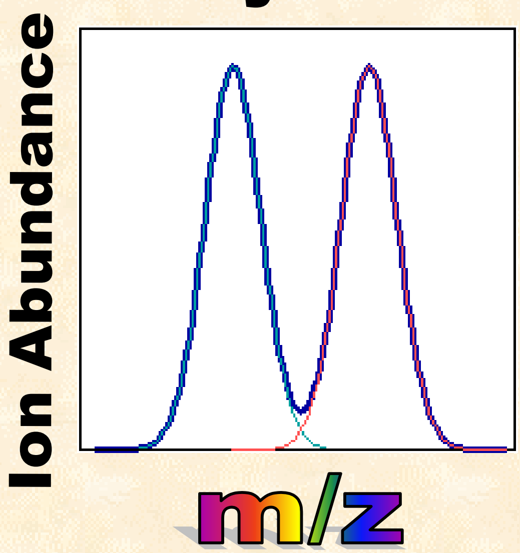
Jargon Review



Total Ion
or
Single Ion
Chromatogram



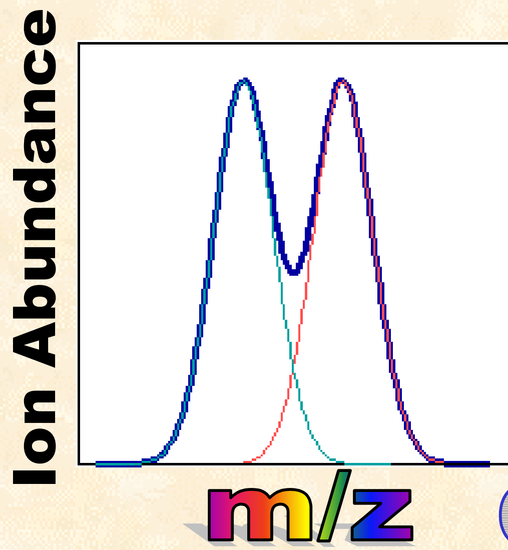
10% valley



– Δ

10,000 (10%)

14,700 (50%)



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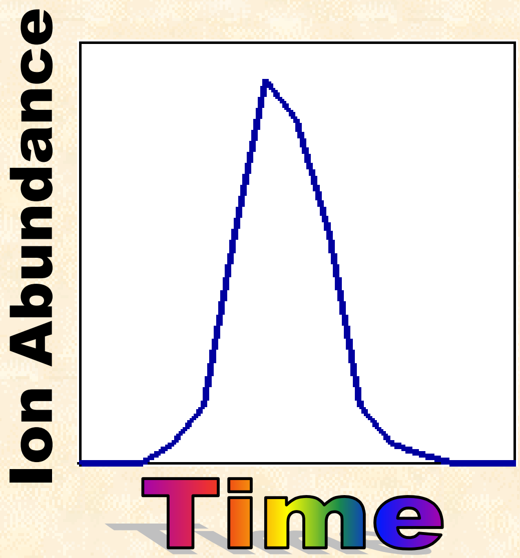
Jargon Review

Chromatographic Peak



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or
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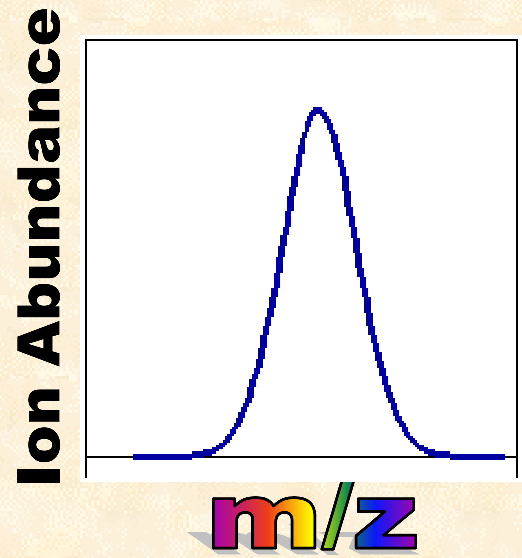
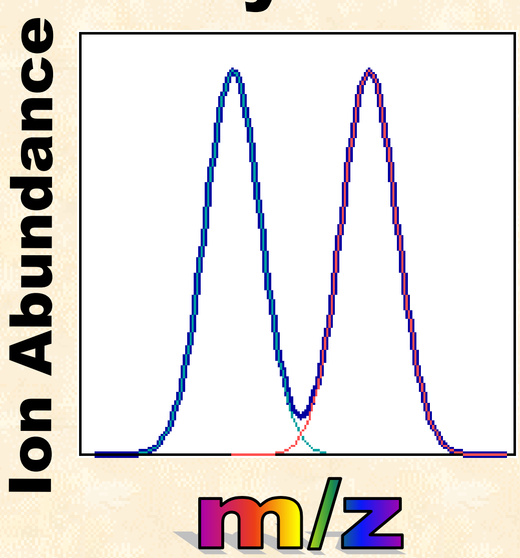
Chromatogram



10% valley



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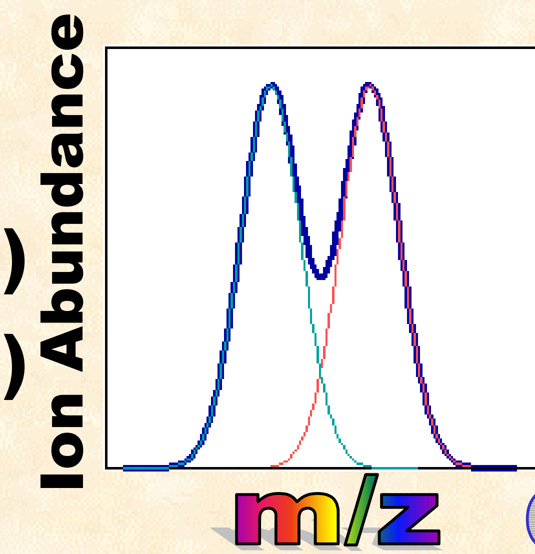

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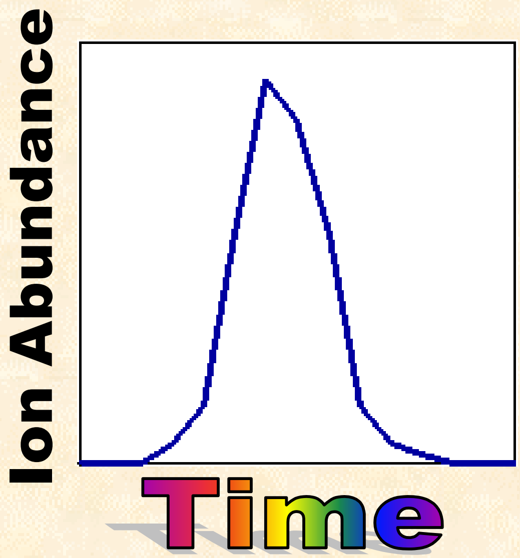
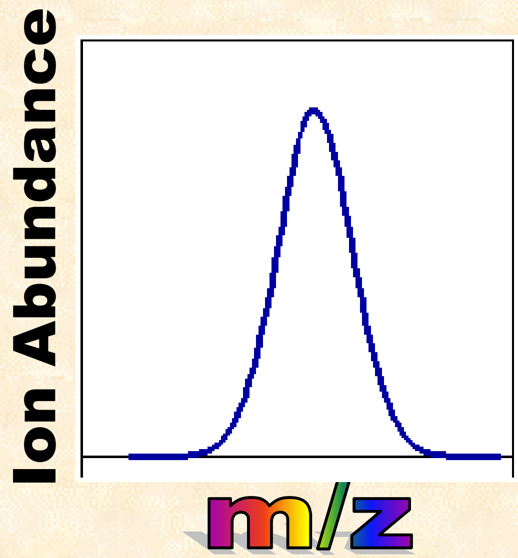
Jargon Review

Chromatographic Peak

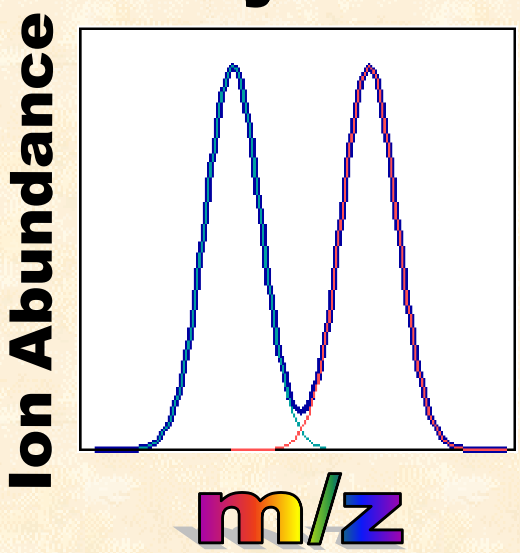


Total Ion
or
Single Ion
Chromatogram

Mass Peak Profile



10% valley



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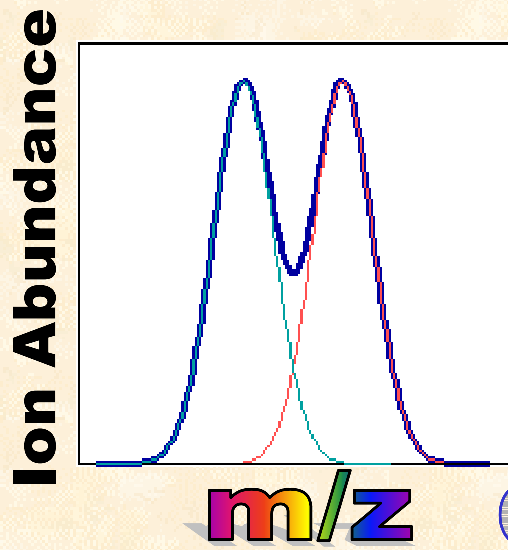

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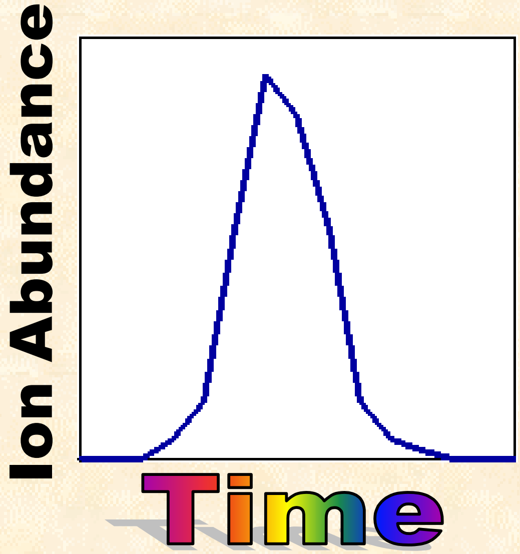
Jargon Review

Chromatographic Peak



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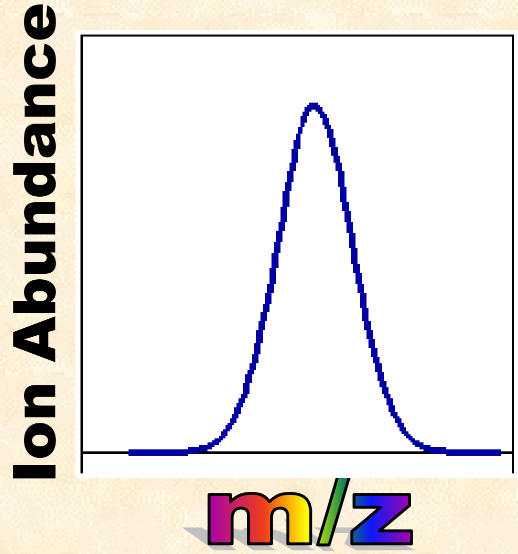
or
Single Ion
Chromatogram

Mass Peak Profile



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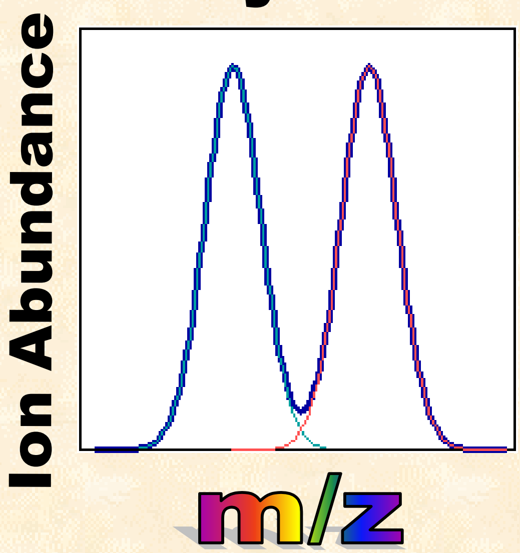
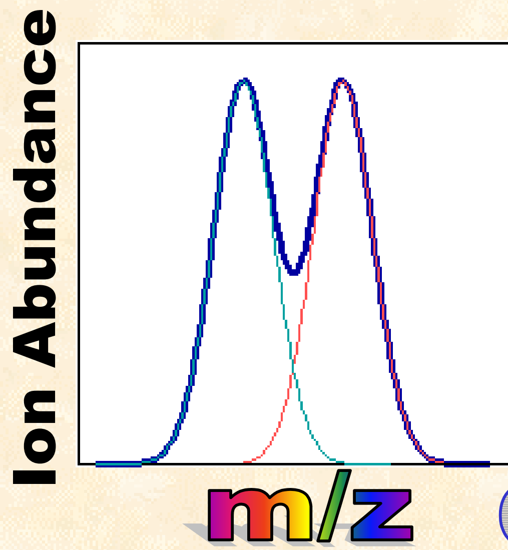


Mass Resolution

— Δ

10,000 (10%)

14,700 (50%)



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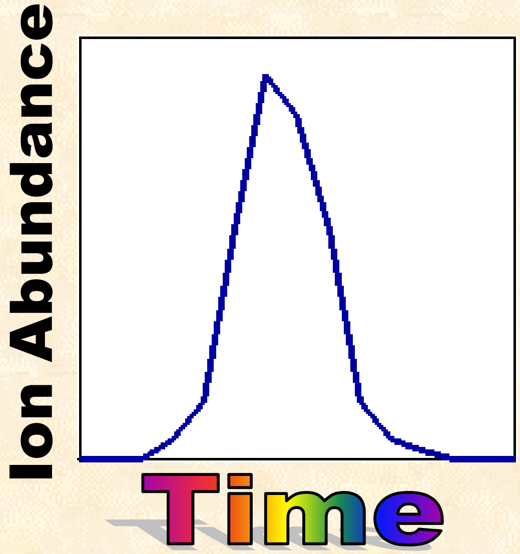
Jargon Review

Chromatographic Peak



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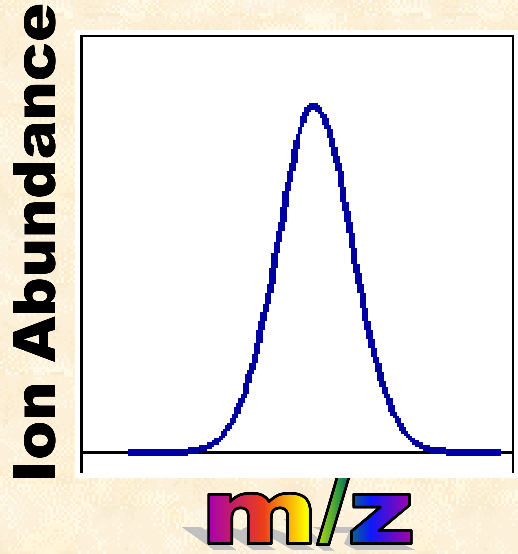
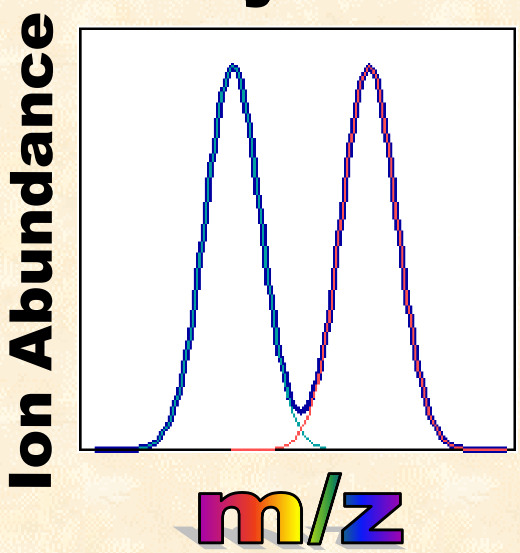


10% valley

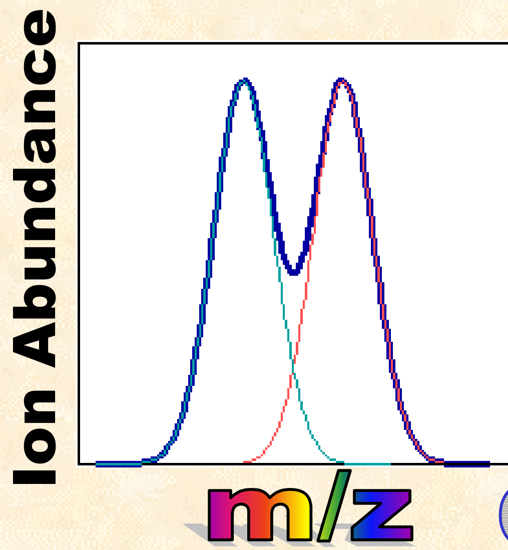


Mass Resolution

$\bar{M}/\Delta M$

10,000 (10%)

14,700 (50%)



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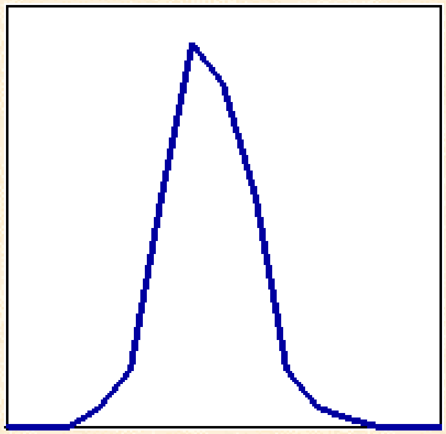
Jargon Review

Chromatographic Peak



Total Ion
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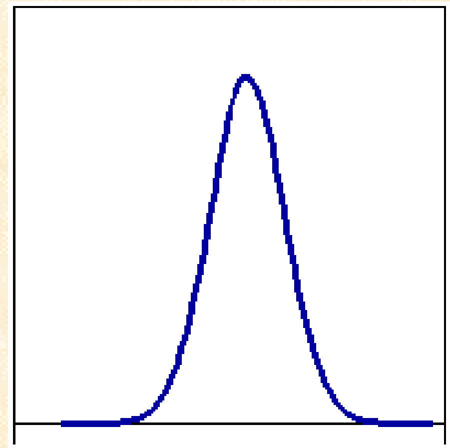
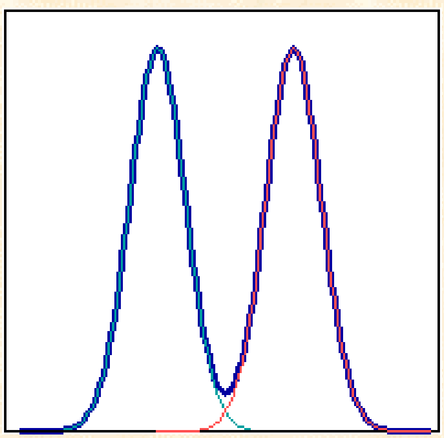
Mass Peak Profile



Mass Resolution

10% valley



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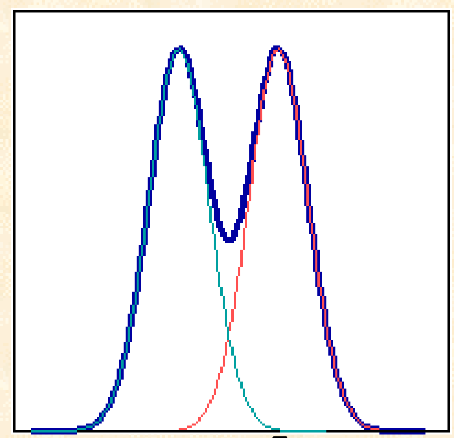
50% valley



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What's the Problem, ICE Man?

Tentative Identification of organic compounds by LRMS:

- Clean mass spectrum
- Several mass peaks
- Mass spectrum in the NIST or Wiley library

Limitations - No Tentative Identification:

- Multiple library matches
- No library matches
- Liquid sample introduction – ESI or APCI
  - Soft ionization – few ions for comparison
  - No libraries of ESI or APCI mass spectra

Majority of Organic Compounds are not Identified by LRMS

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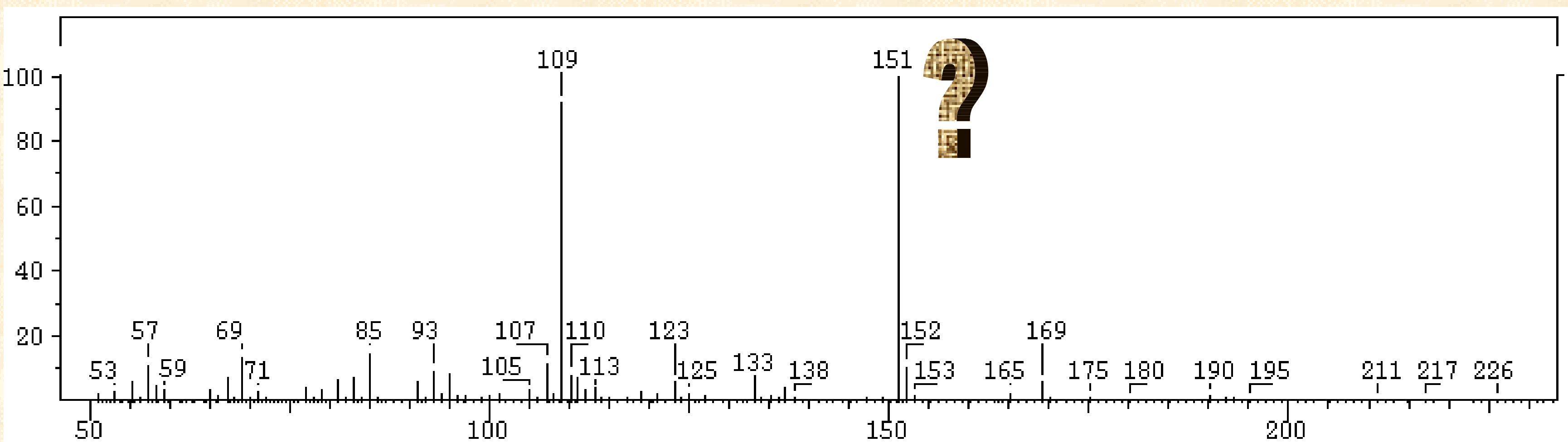
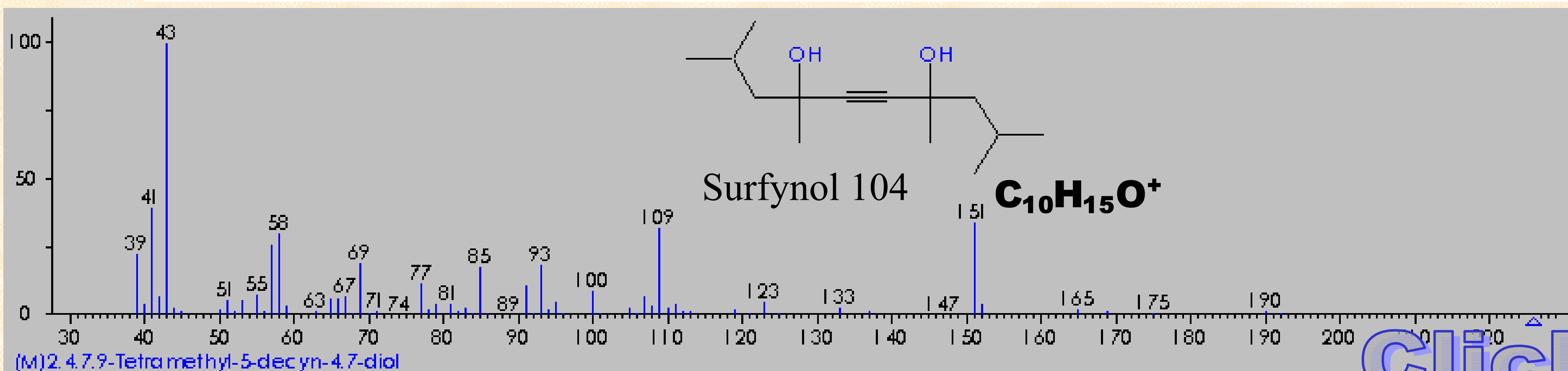
Example:

Multiple Matches

$C_8H_9NO_2^+$

Acetaminophen





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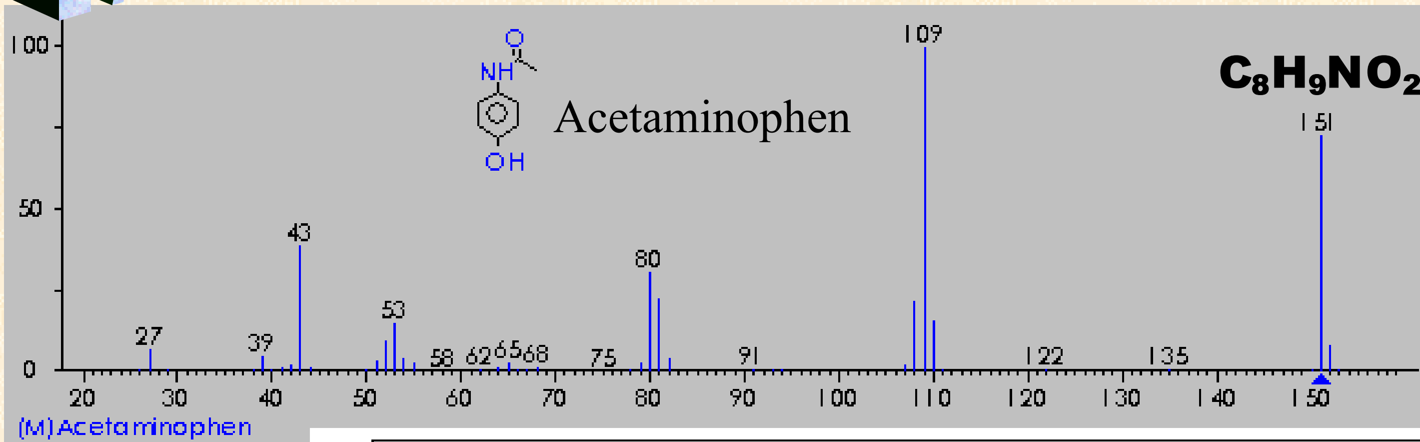
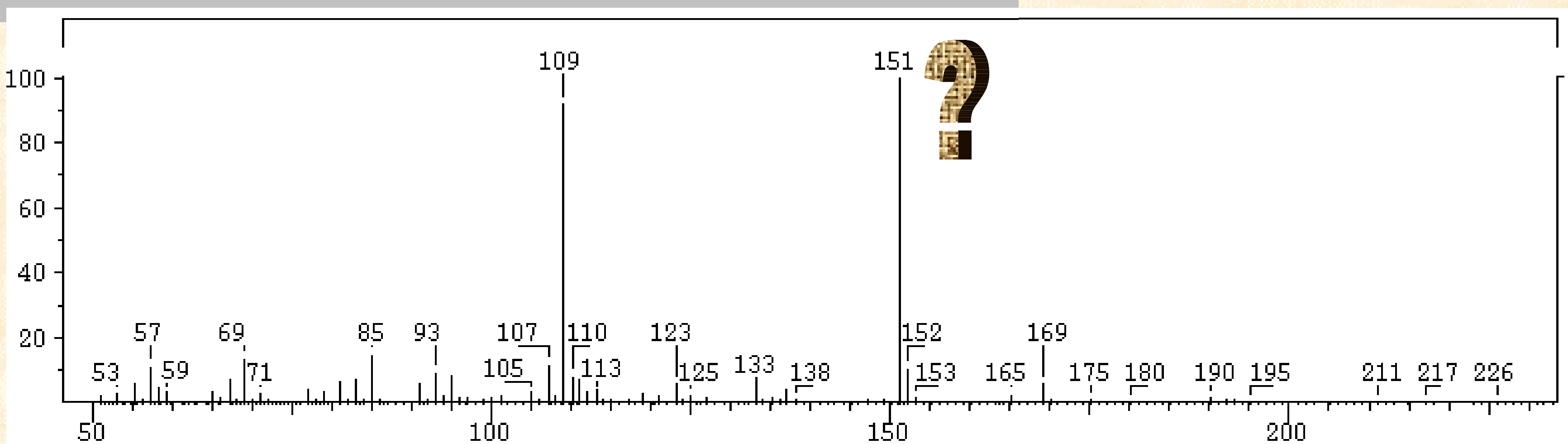
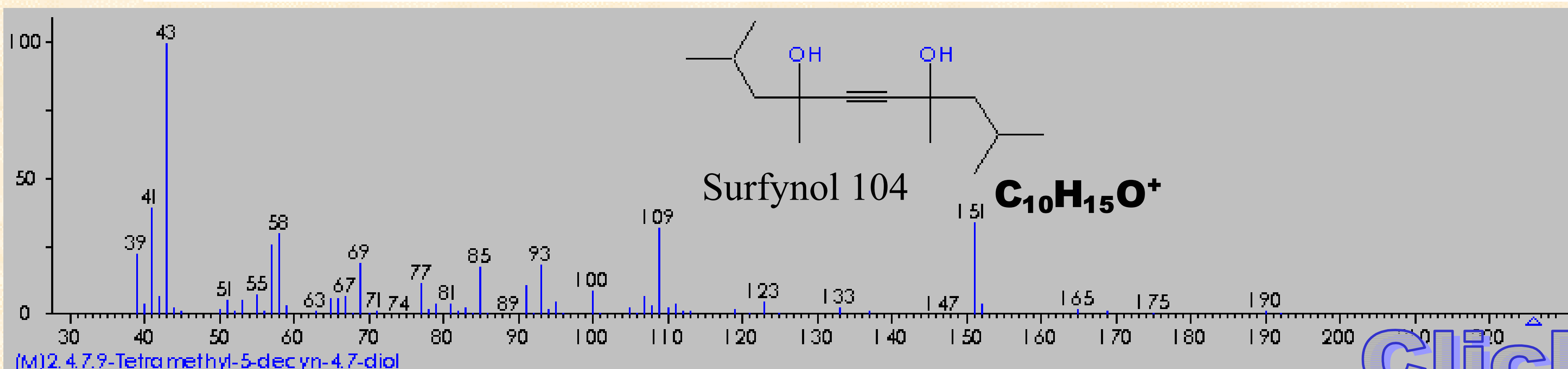
Example

Multiple Matches







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What properties differ between these ions?

High Mass Resolution

$C_8H_9NO_2^+$

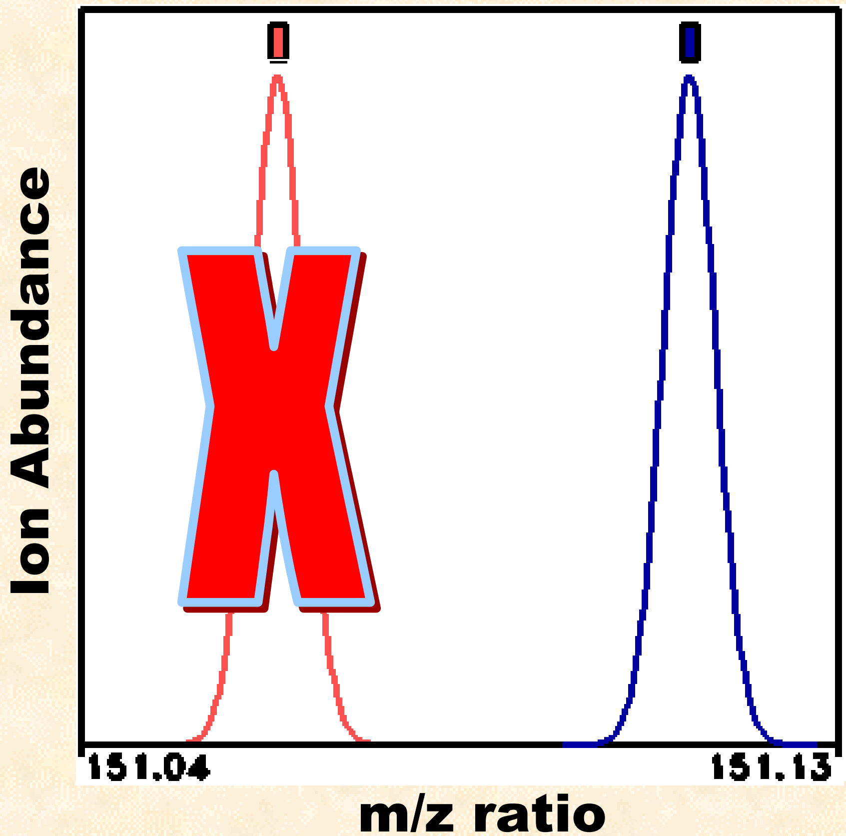
8 x 12.00000
9 x 1.00783
1 x 14.00307
2 x 15.99491

151.06333

$C_{10}H_{15}O^+$

10 x 12.00000
15 x 1.00783
1 x 15.99491

151.11229

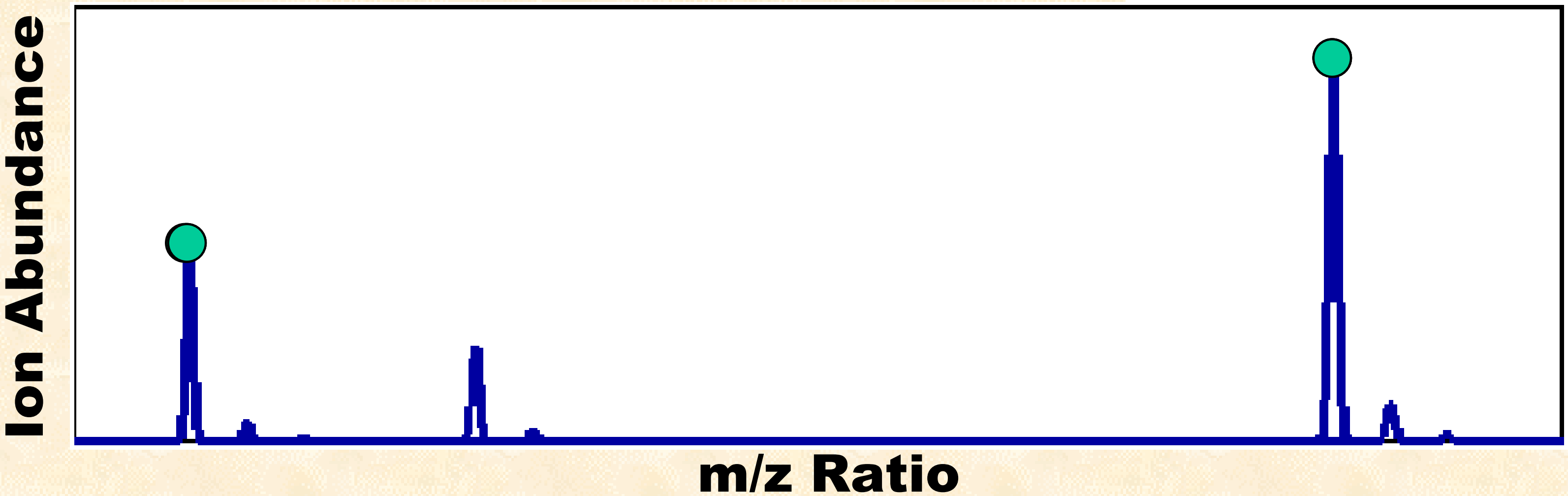


One property is Exact Mass.

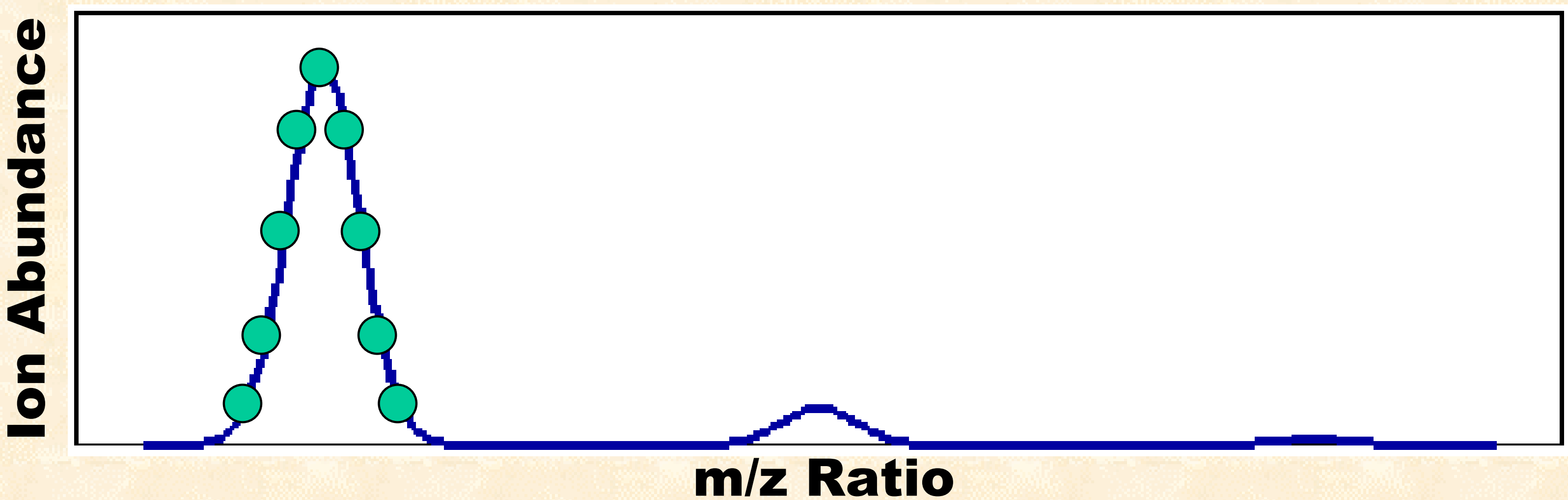
Click

Scan Modes

Selected Ion Recording



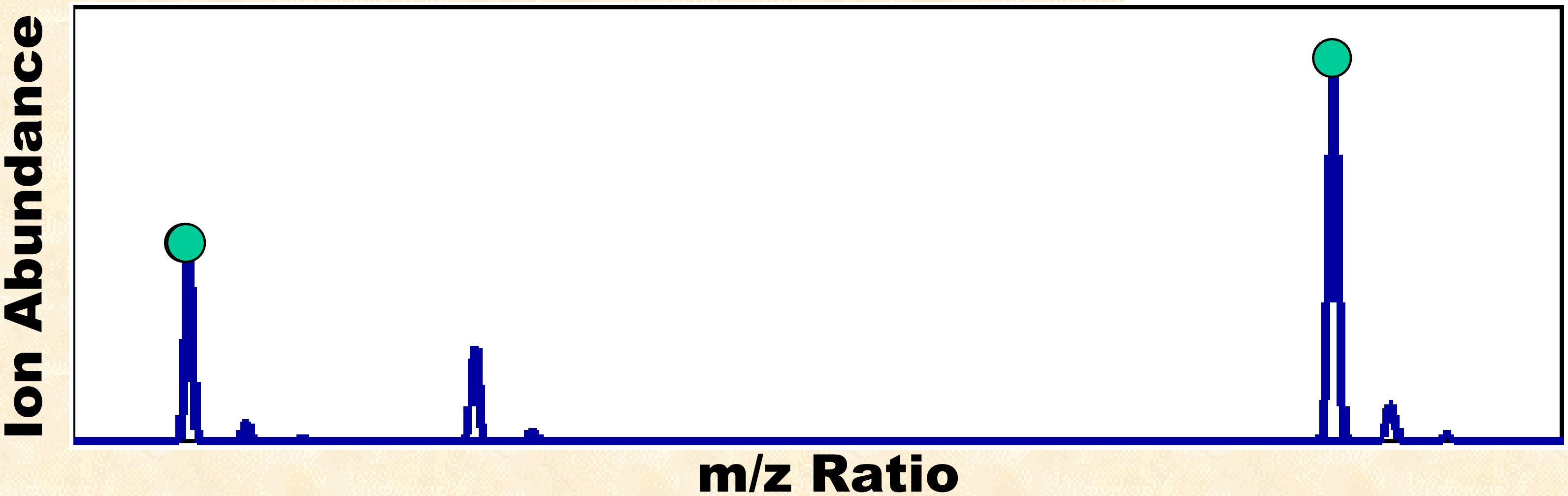
Hybrid Scan



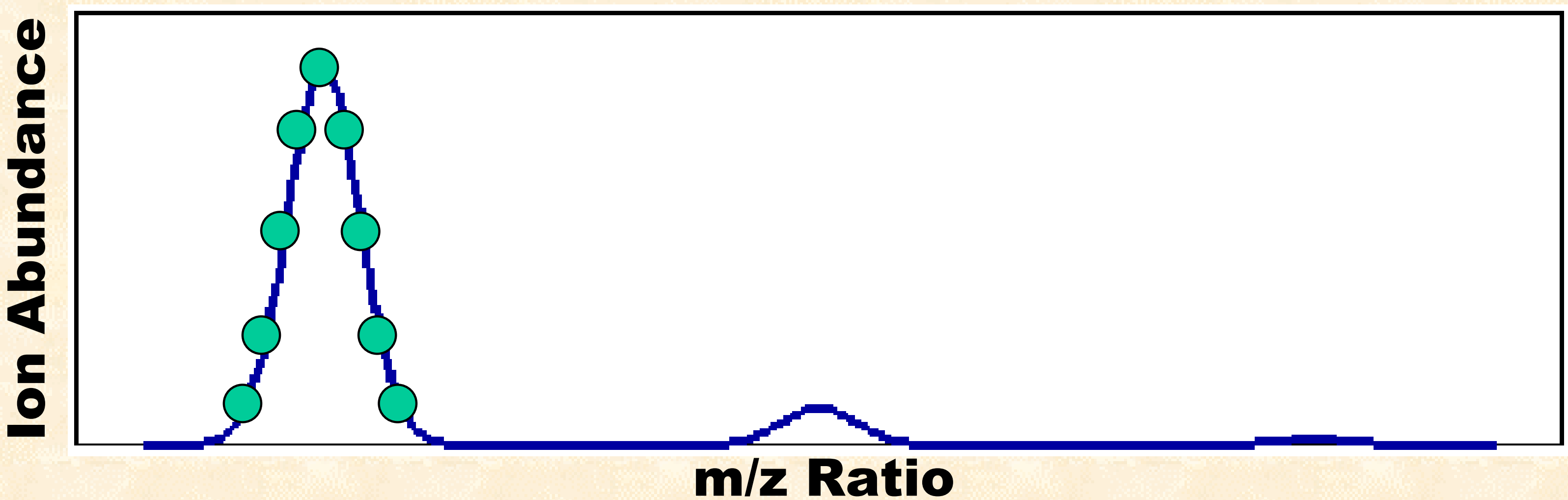
Click

Scan Modes

Selected Ion Recording



Hybrid Scan



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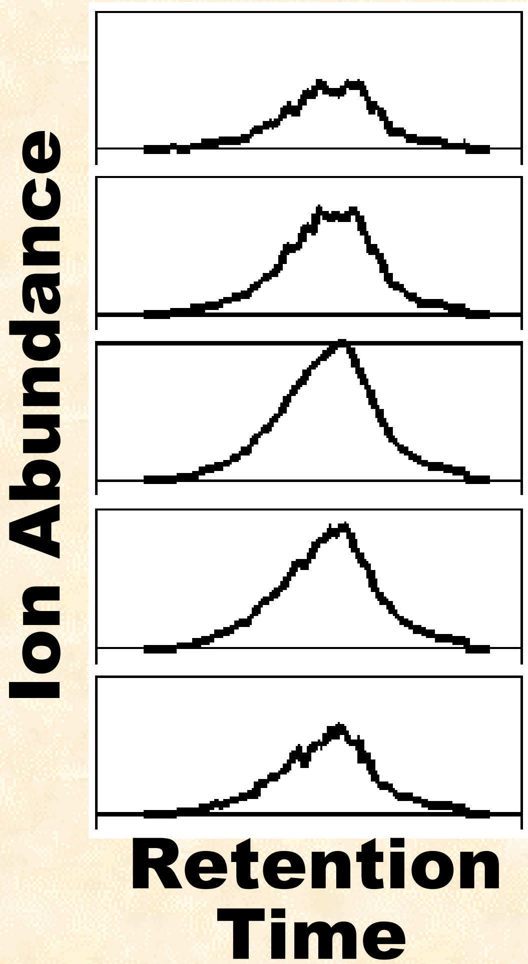
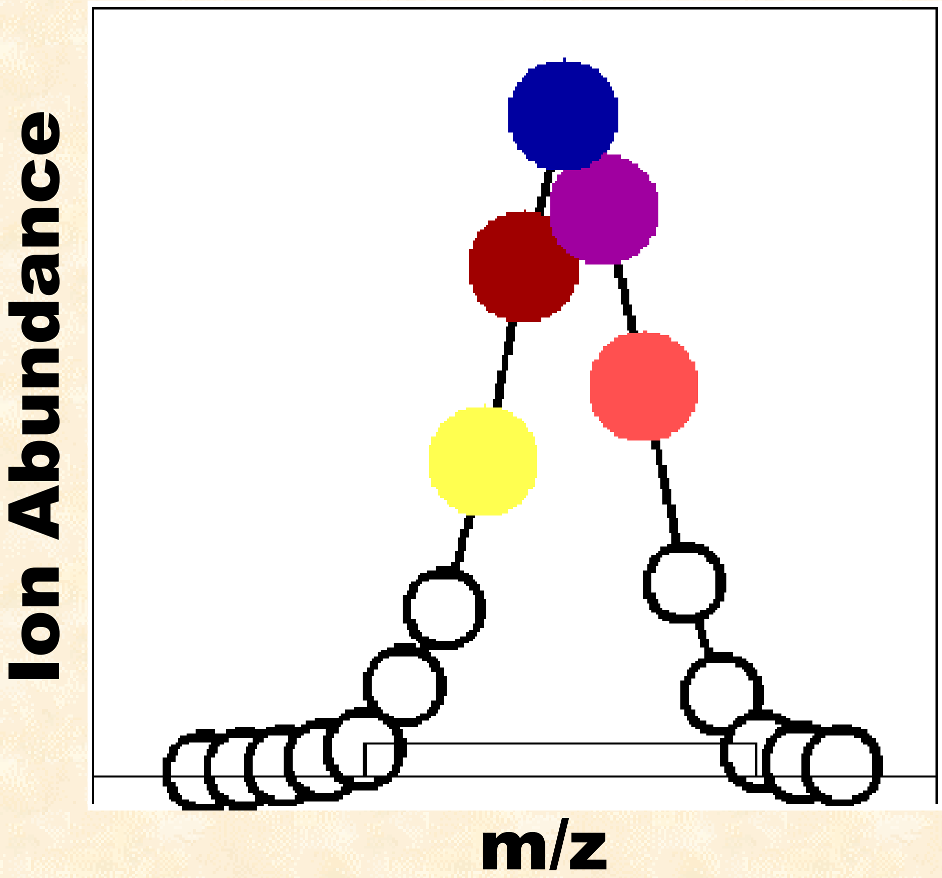
MPPSIRD Definition





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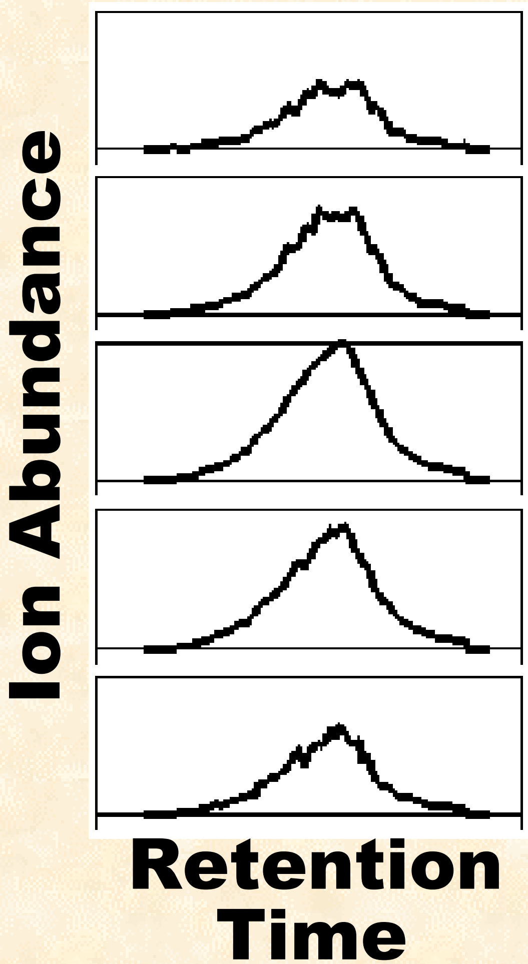
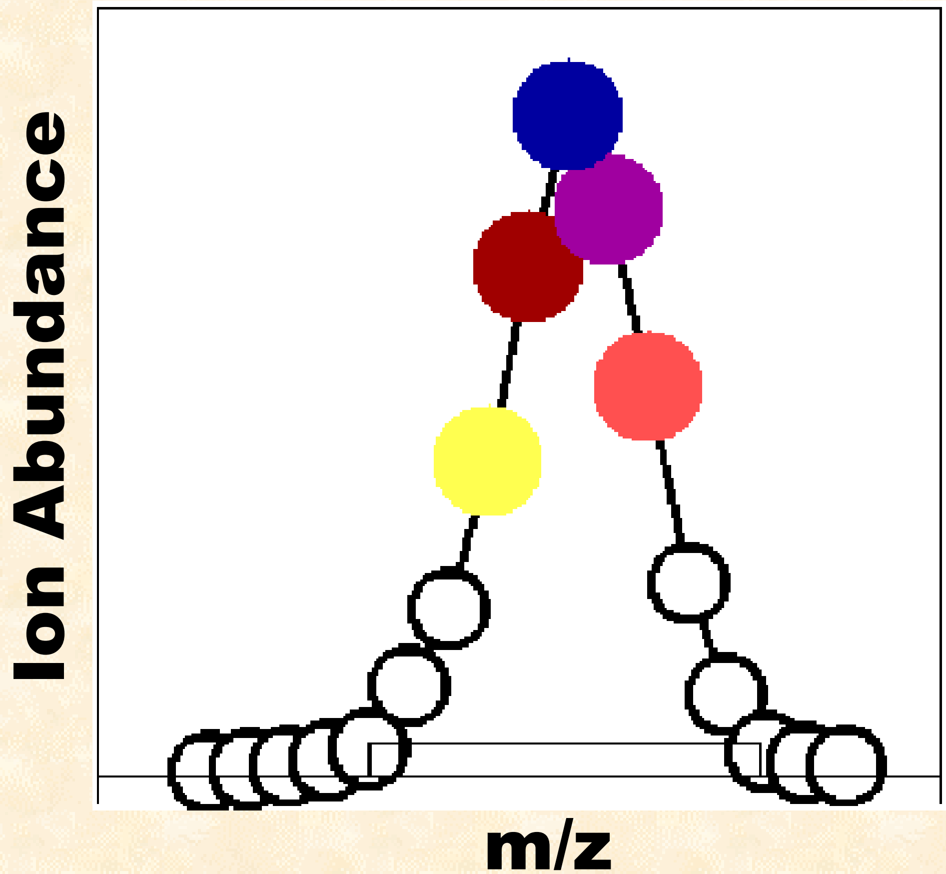
MPPSIRD Definition





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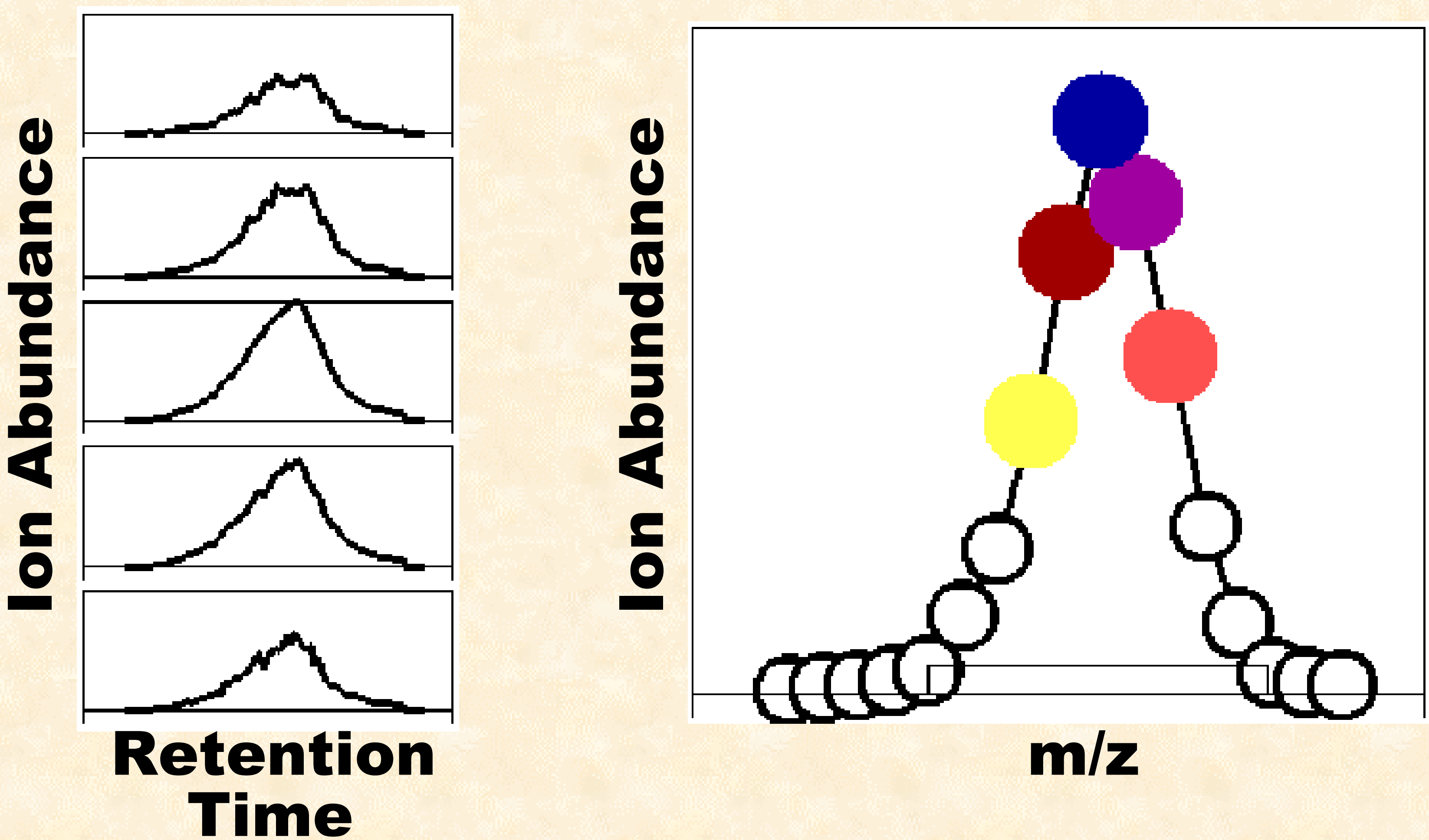
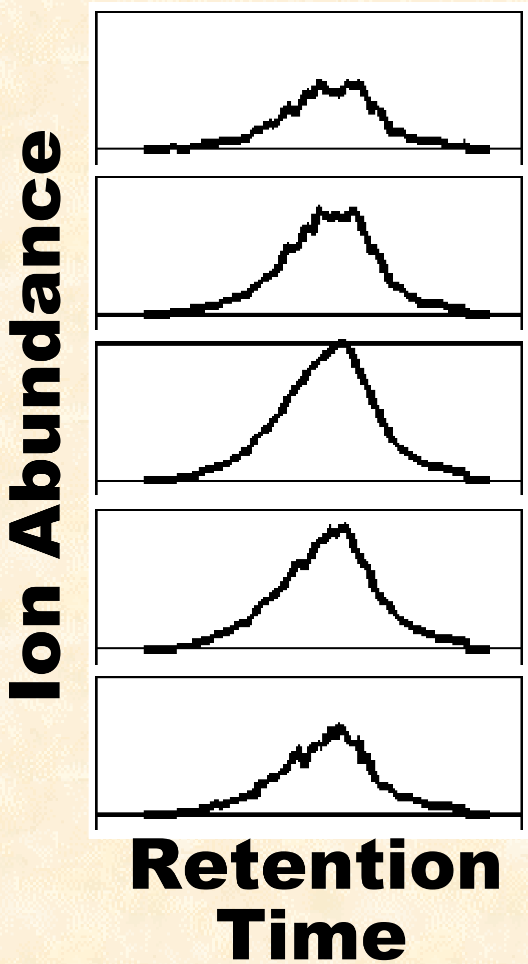
MPPSIRD Definition

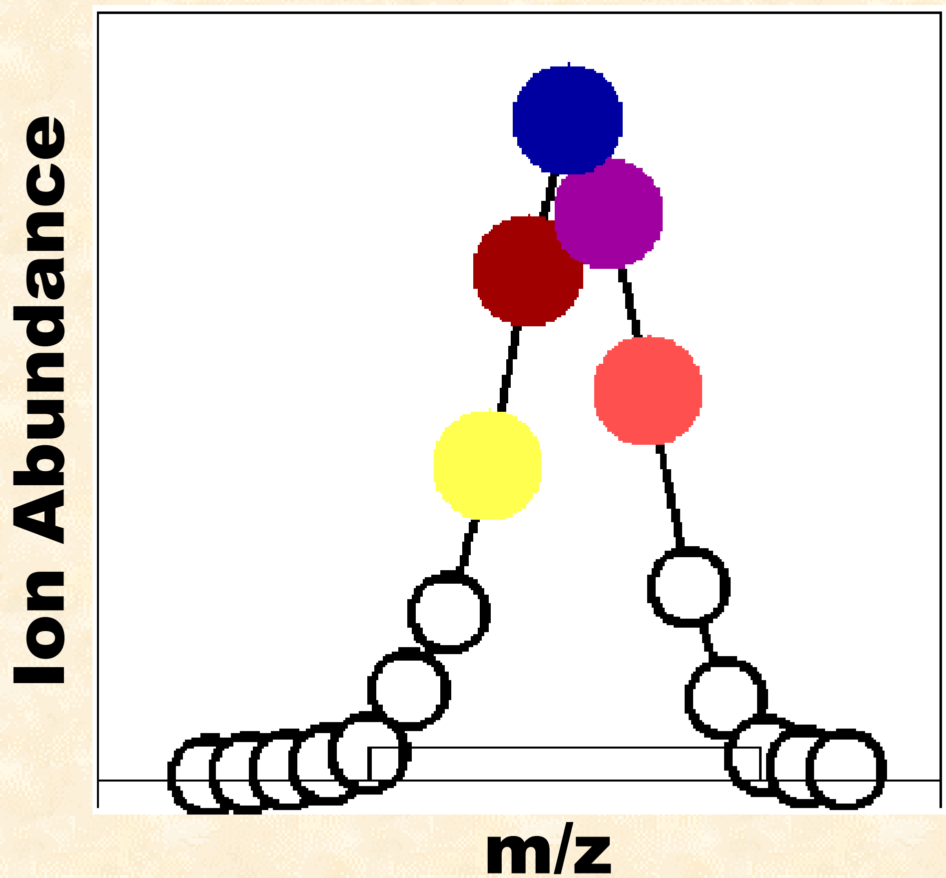


Mass Peak Profiling from Selected Ion Recording Data

Click

MPPSIRD Definition





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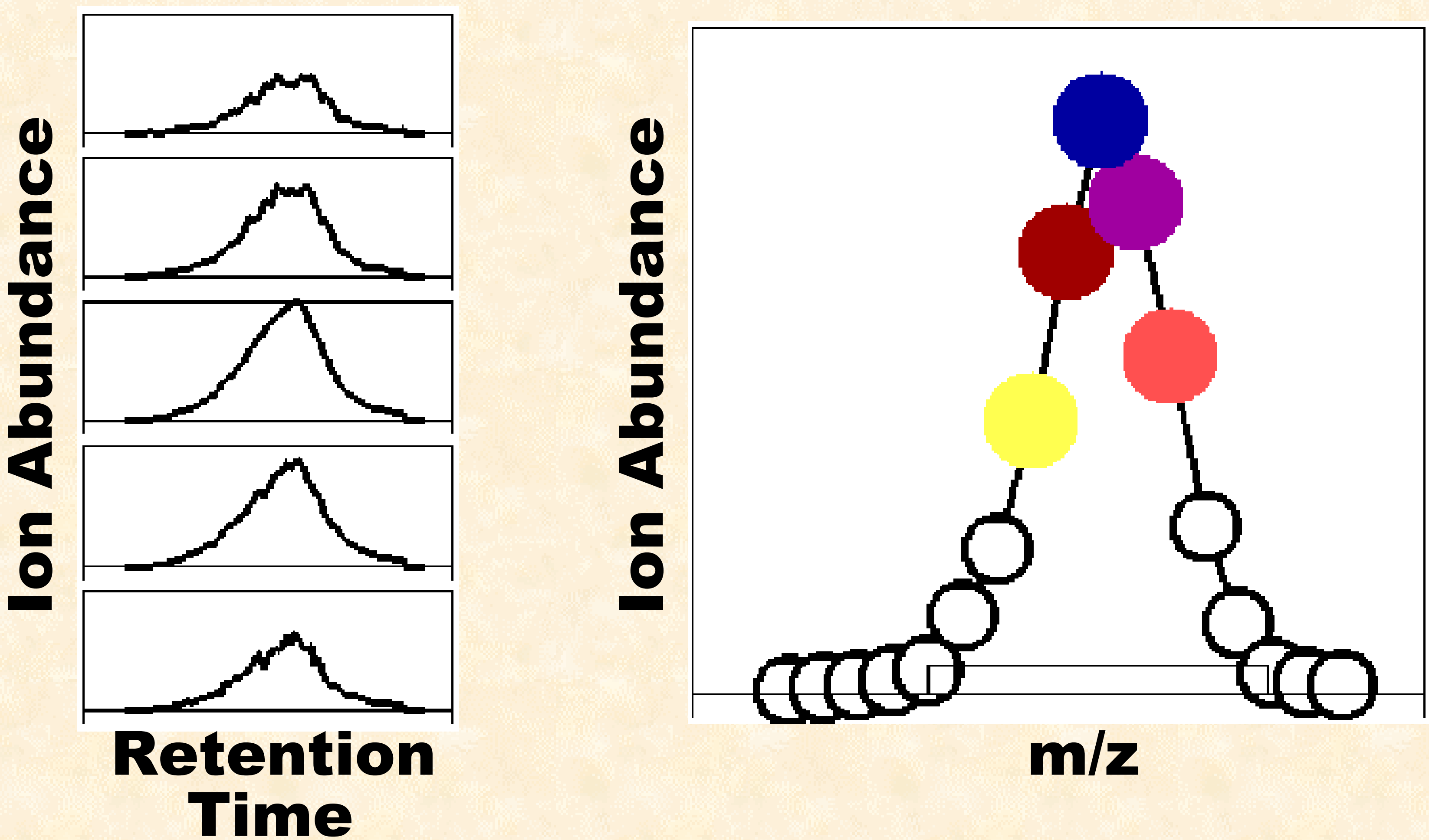

Mass Peak Profiling from Selected Ion Recording Data

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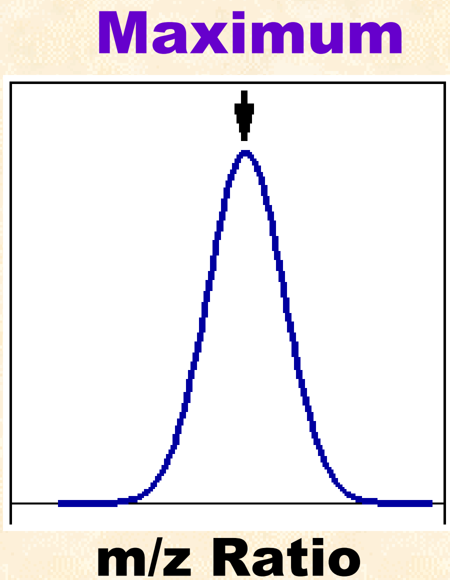
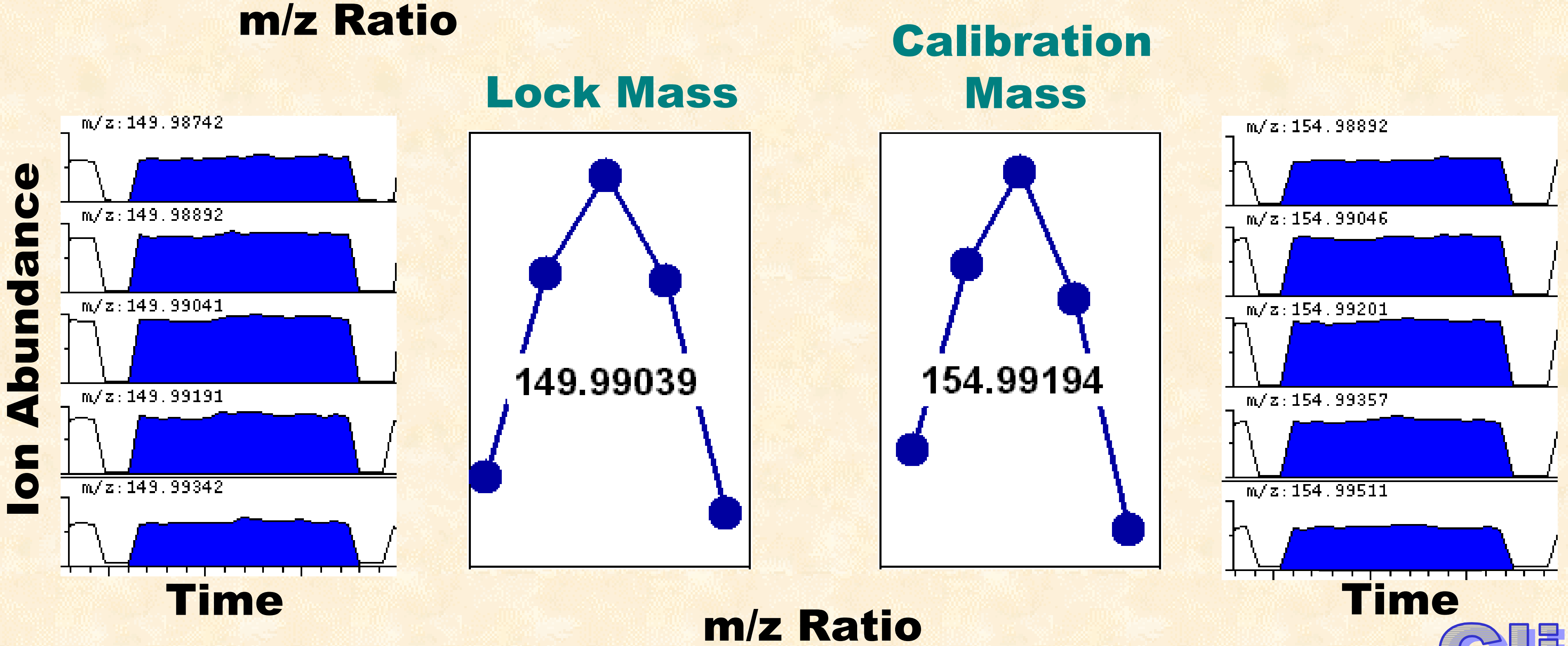
Calibration



Recalibration each cycle



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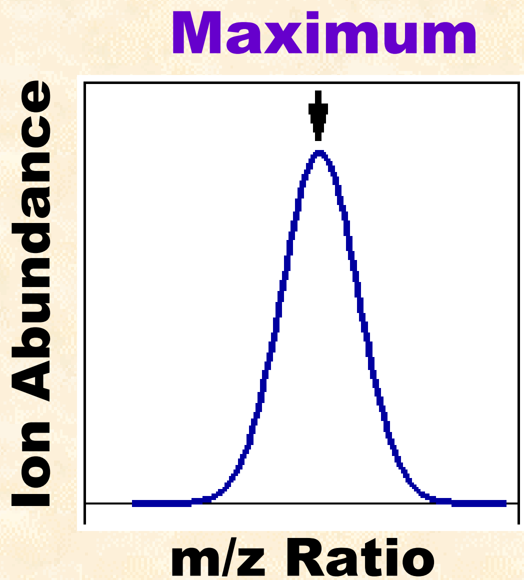
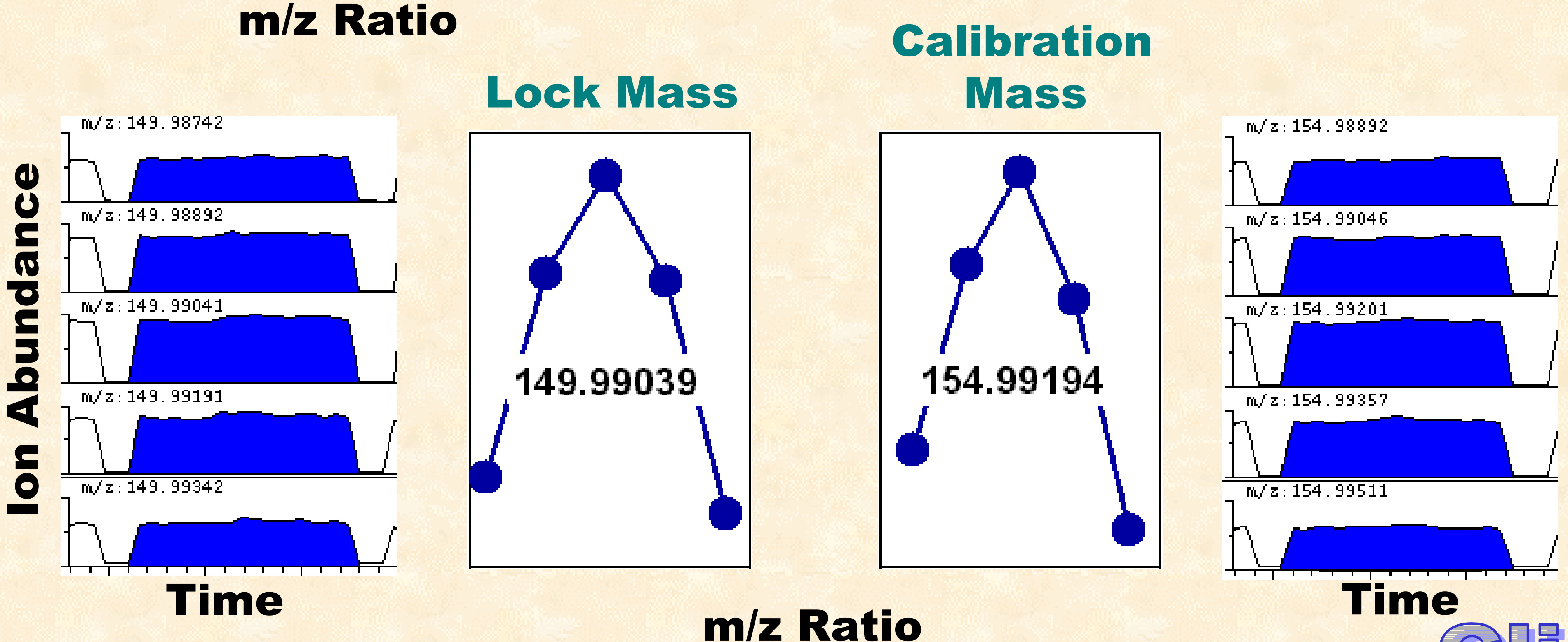
Calibration



Recalibration each cycle

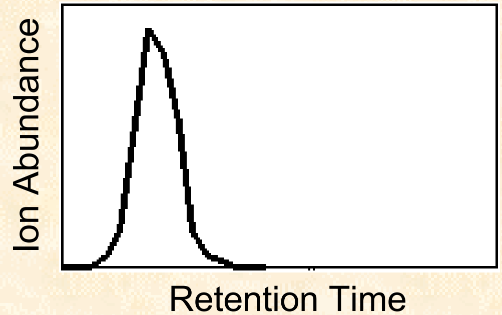


Click

Instrumental Advantages of MPPSIRD

Delineate chromatographic peaks

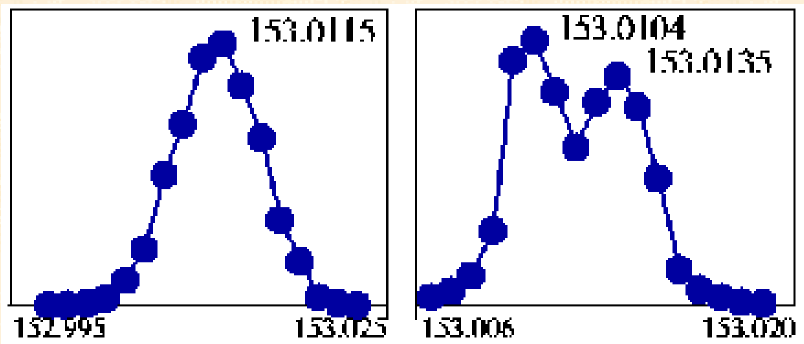
For a single m/z ratio



100-fold greater than full scans

Higher resolution feasible

10,000 Resolution



24,000 Resolution

Lock mass recalibration each cycle

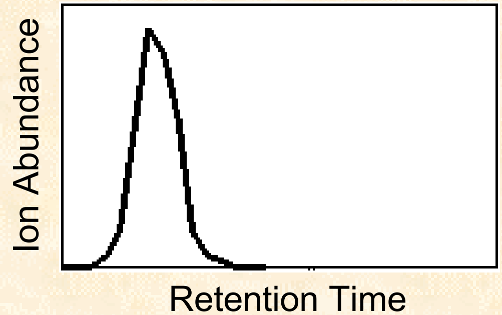
Click

Instrumental Advantages of MPPSIRD

Speed

Delineate chromatographic peaks

For a single m/z ratio



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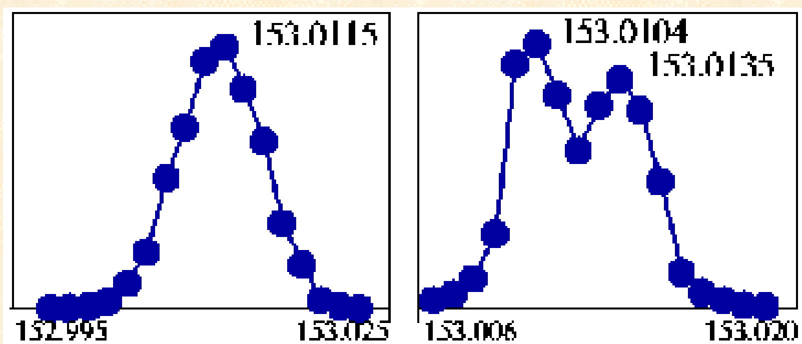

24,000 Resolution

Lock mass recalibration each cycle

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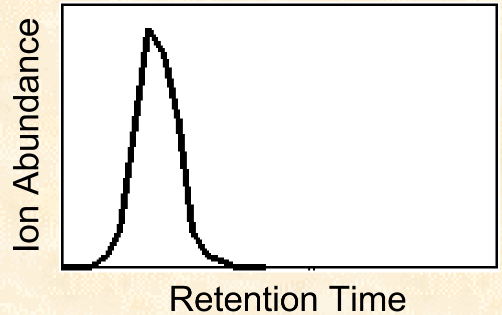
Instrumental Advantages of MPPSIRD

Speed Delineate chromatographic peaks

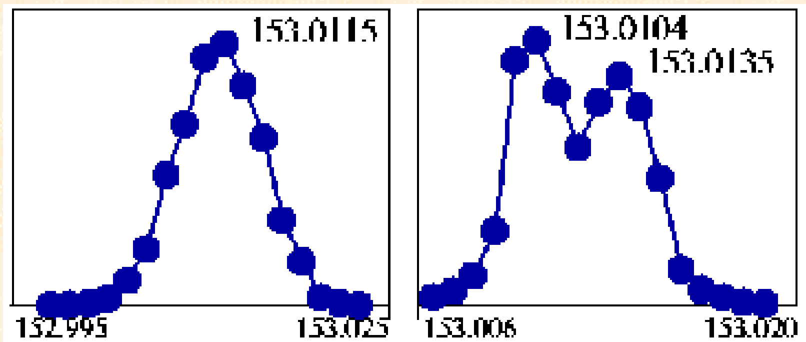
For a single m/z ratio



Sensitivity 100-fold greater than full scans

Higher resolution feasible

10,000 Resolution



24,000 Resolution

Lock mass recalibration each cycle

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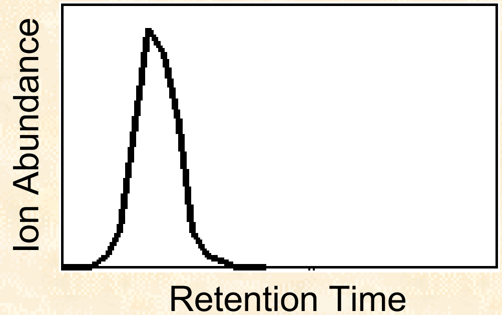
Instrumental Advantages of MPPSIRD

Speed Delineate chromatographic peaks

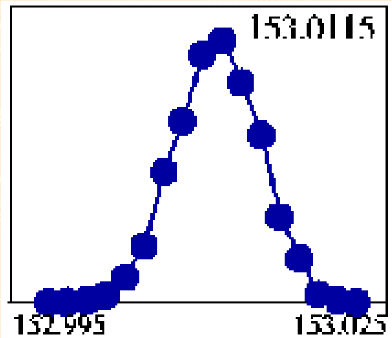
For a single m/z ratio

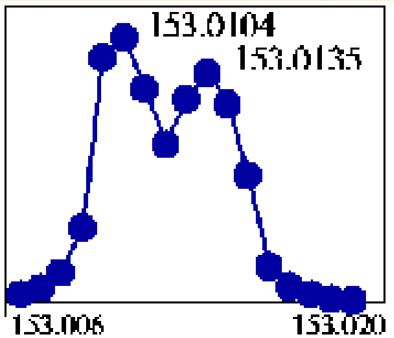


Sensitivity 100-fold greater than full scans

Selectivity Higher resolution feasible

10,000 Resolution





24,000 Resolution

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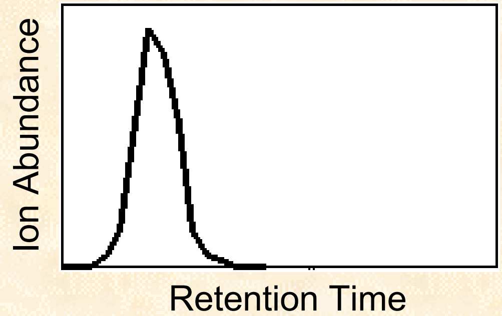
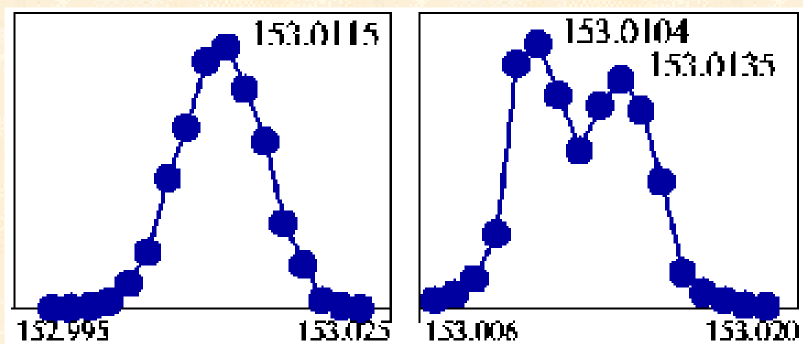
For a single m/z ratio



Sensitivity 100-fold greater than full scans

Selectivity Higher resolution feasible

10,000 Resolution



24,000 Resolution

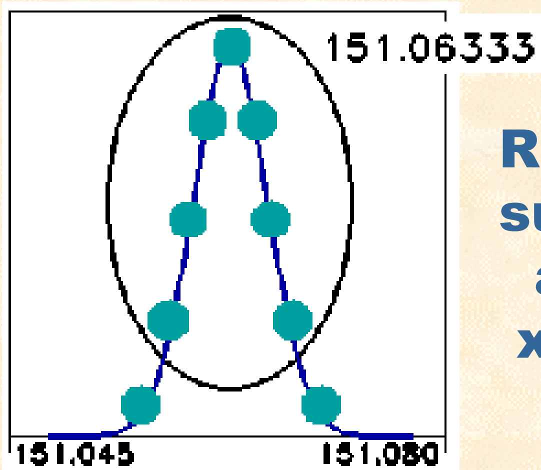
Stability Lock mass recalibration each cycle

Click

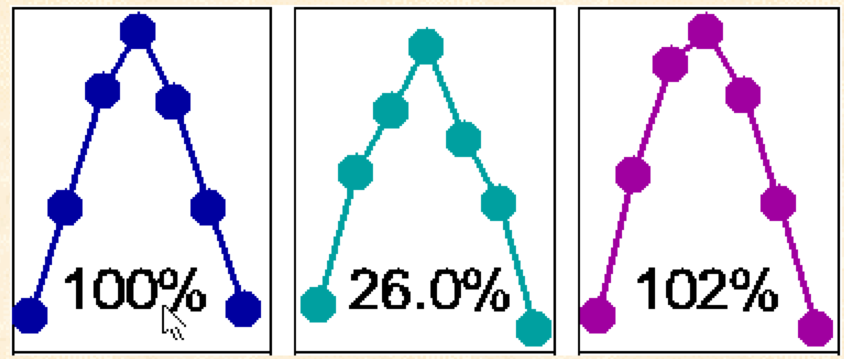
Measurement Advantages of MPPSIRD

Accurate Exact Masses & Relative Abundances

Weighted average of several points



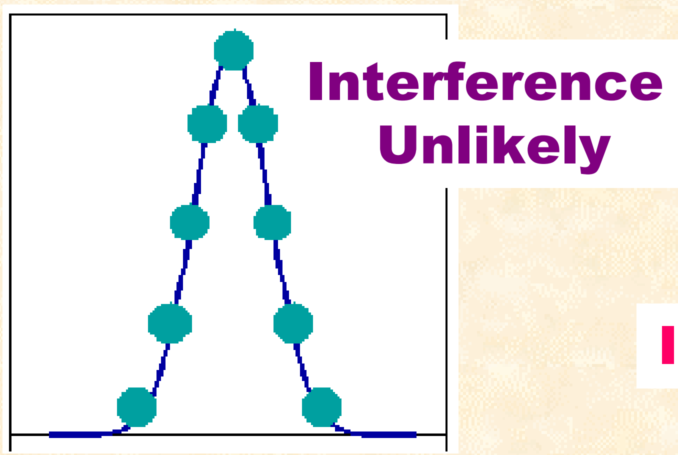
Ratios of summed areas x 100%

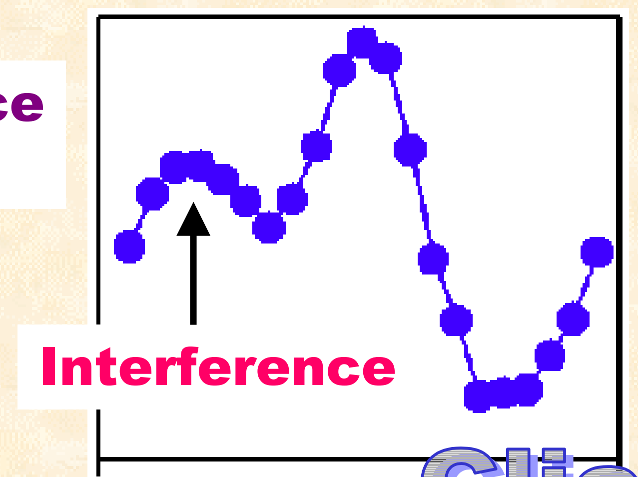


Quality Assurance Advantage of MPPSIRD

Interferences Revealed

Gaussian shape usually indicates no major Interferences





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Limitations of MPPSIRD

Monitor four or fewer Analyte Profiles

- 1 Full Analyte Profile
- 2 Full Analyte Profiles
- 3 Partial Analyte Profiles
- 4 Partial Analyte Profiles

Multiple Experiments required

Double Focusing Mass Spectrometers are expensive

Ancillary DOS capable Computer required

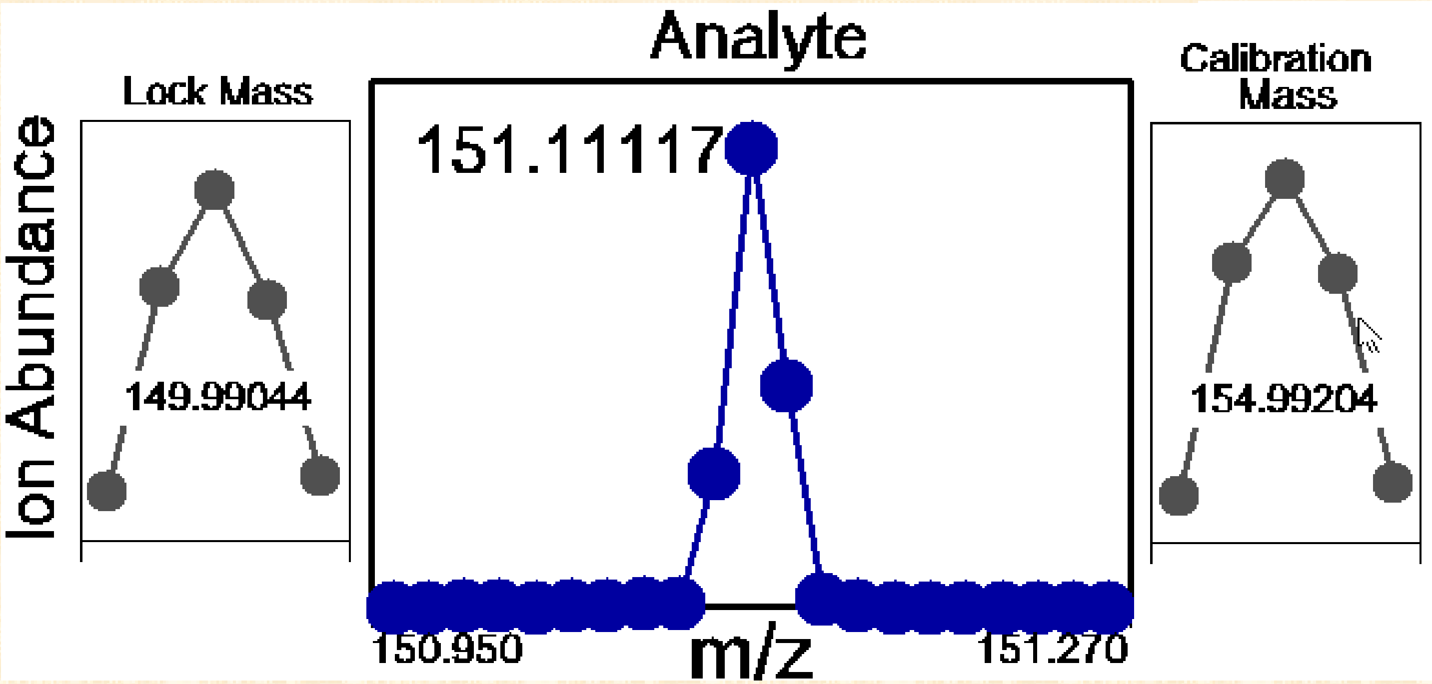
Lotus 2.2, WordPerfect 5.1, QuickBasic 4.5

– Procurement inconvenient

+ Batch files supported, simple code

Click

Survey Data

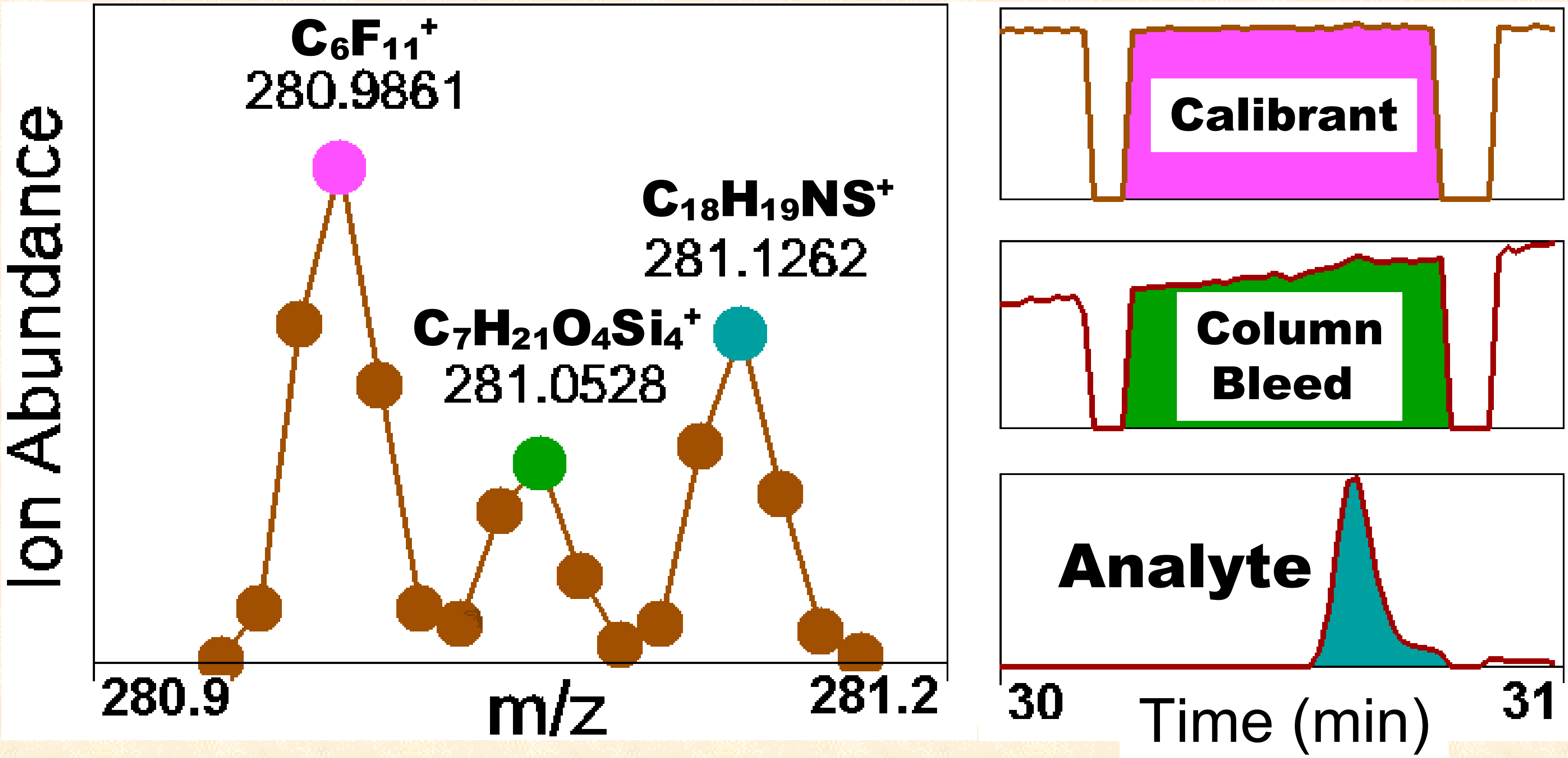


Wide mass range
3,000 mass resolution

Coarse estimate of the exact mass for the most abundant *Analyte* ion

Click

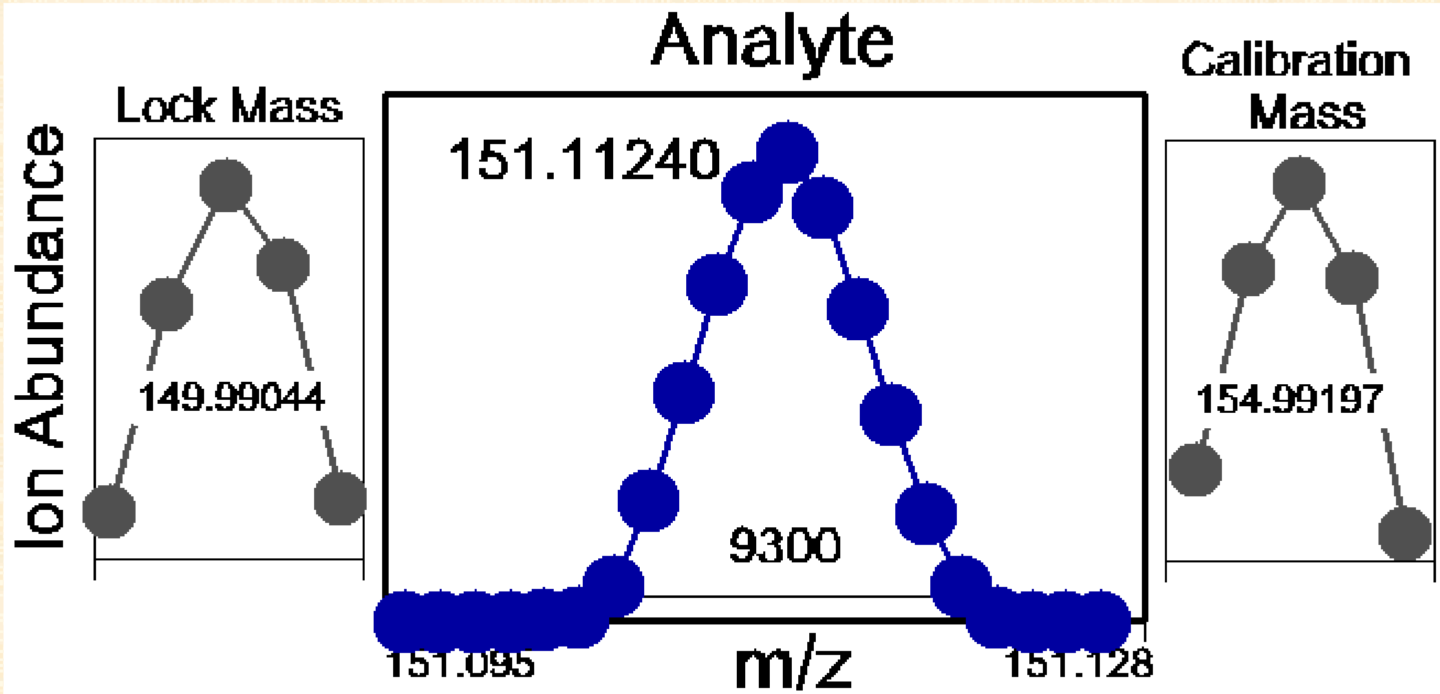
Sources of Ions



Investigate the Analyte ion

Click

Exact Mass of Analyte Ion



Elements Considered: C H N O F P S Si

m/z 151.11240 ± 6 ppm Resolution 10,000

PGM:

#	Err(mmu & ppm)		Composition
1	-0.3	-2.0	C5 H14 N3 O F
2	-0.1	-0.7	C10 H15 O

~~$C_8H_9NO_2$~~ 151.06333 -325 ppm

Click

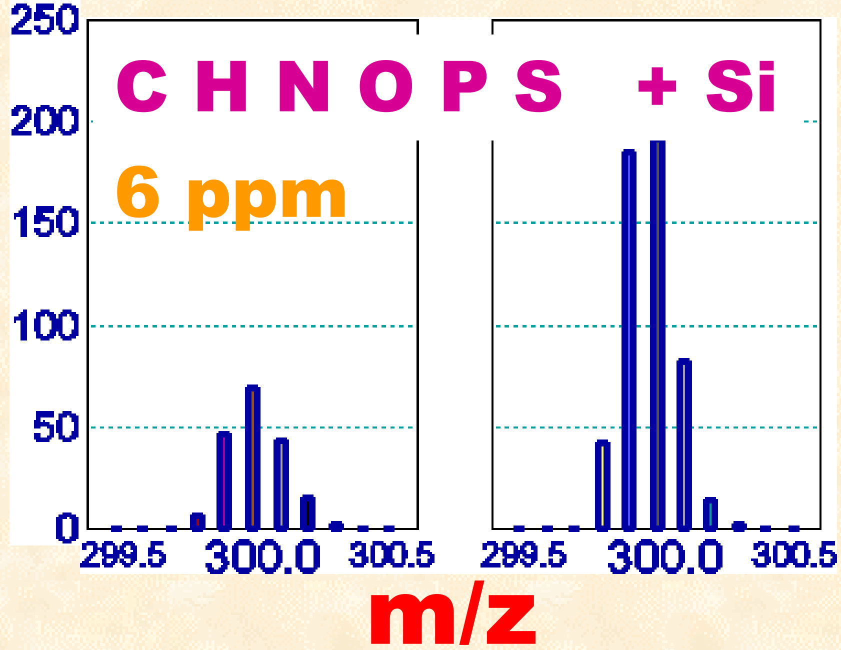
Number of Possible Compositions

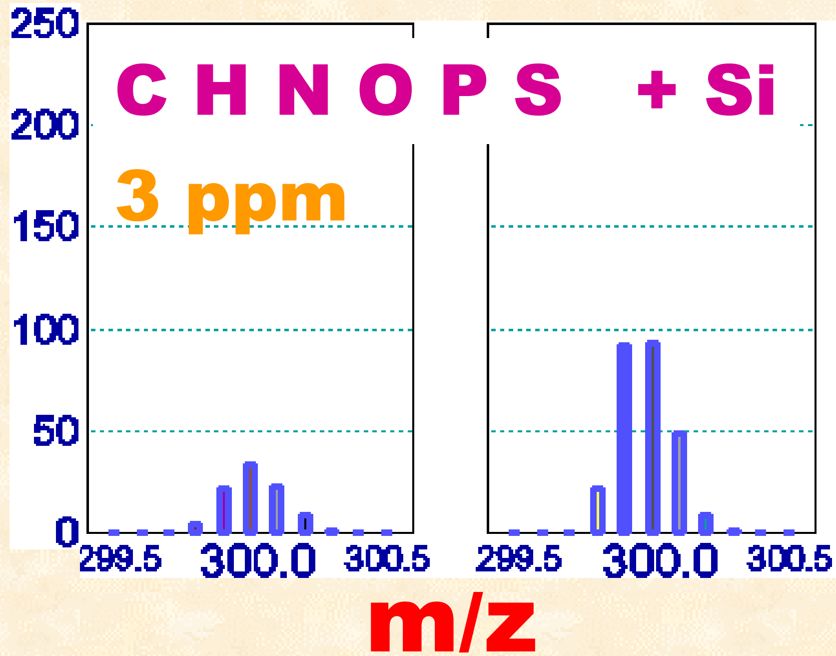
3 Factors

m/z

Error Limit

Number of Elements





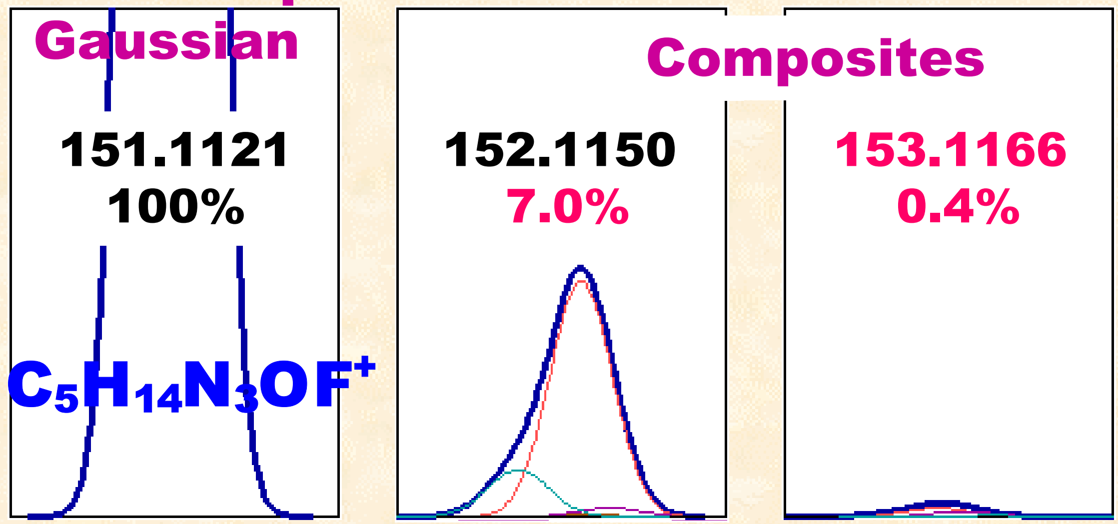
Click

Isotopic Abundances

+1

^{13}C	1.10%
^{29}Si	4.67%

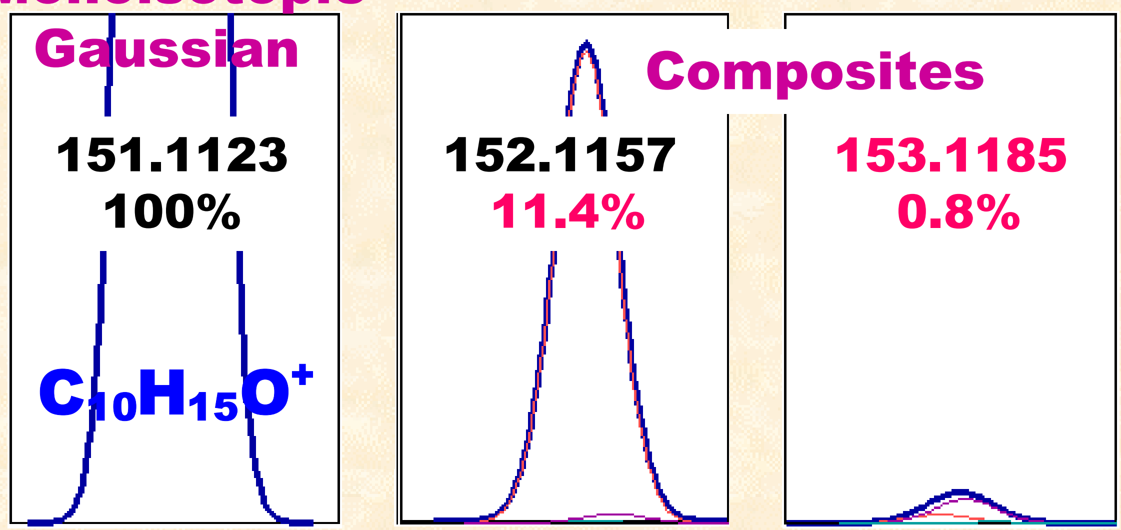
Monoisotopic +1 +2



+2

^{34}S	4.21%
^{30}Si	3.10%
^{37}Cl	24.23%
^{81}Br	49.31%

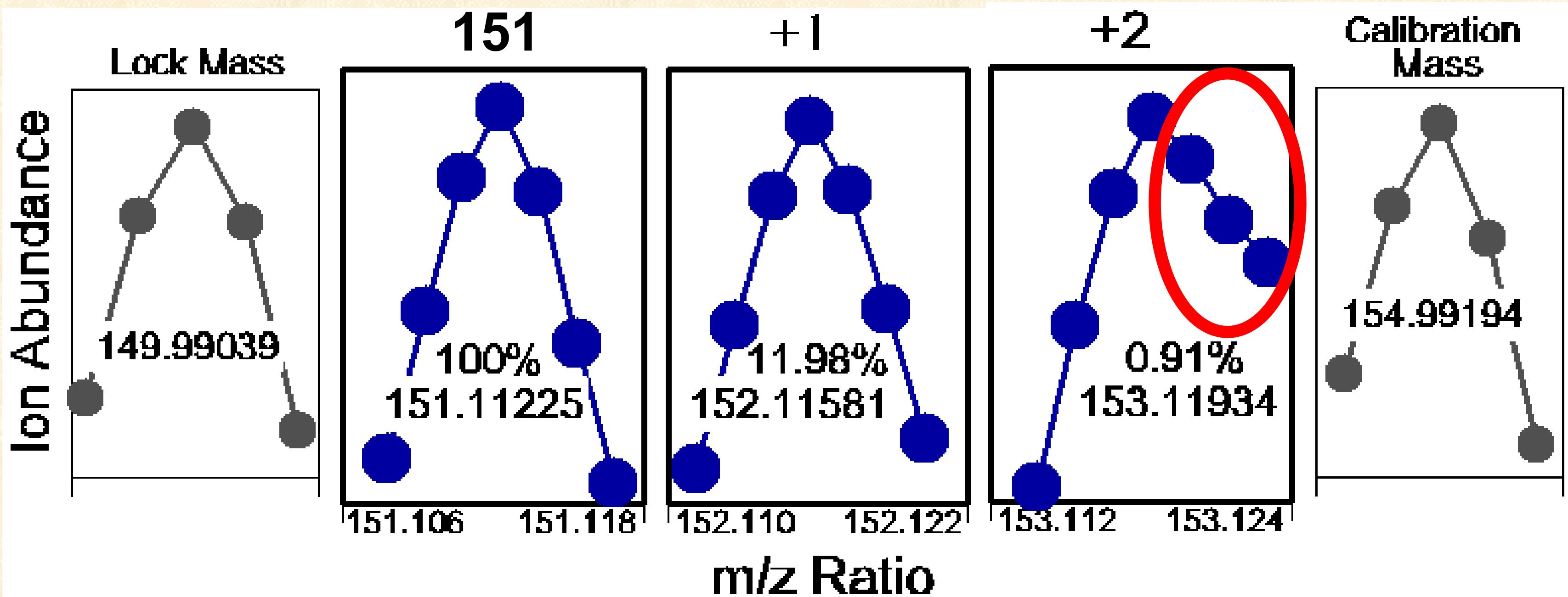
Monoisotopic +1 +2



10,000 Resolution (±6 ppm)

Click

Monitor Partial Profiles with MPPSIRD



PGM:

Elements Considered: C H N O F P S Si

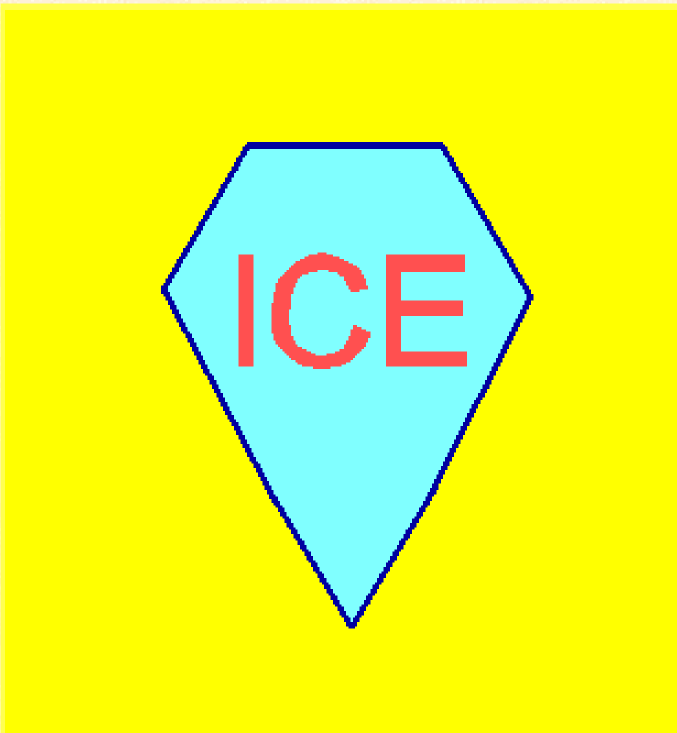
m/z 151.11225 ± 6 ppm Resolution 10,000

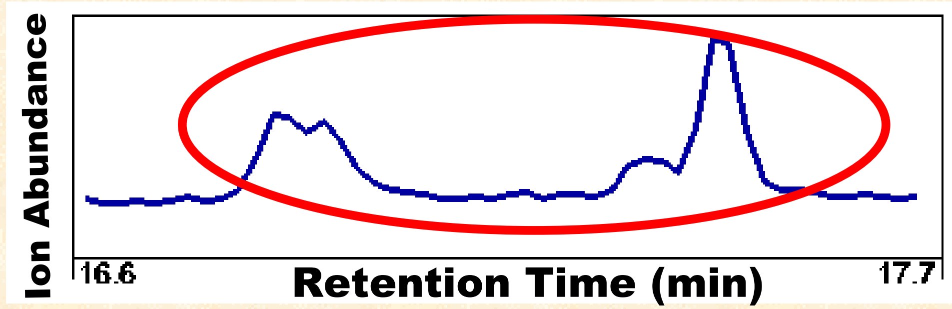
#	RDB	Composition	Mass Defects 151	+1	+2		Relative Abundances %+1(Range %+1)		%+2(Range %+2)	
1	0.0	C5 H14 N3 O F	.11209	.11499	.11686	X	5.72(4.29- 7.23)	X	0.20(0.09-0.33)	X
2	3.5	C10 H15 O	.11229	.11570	.11848		11.32(9.50-13.14)		0.71(0.49-0.92)	
Experimental Values:			*.11225*	*.11581*	*.11934*		*11.98*		*0.91*	

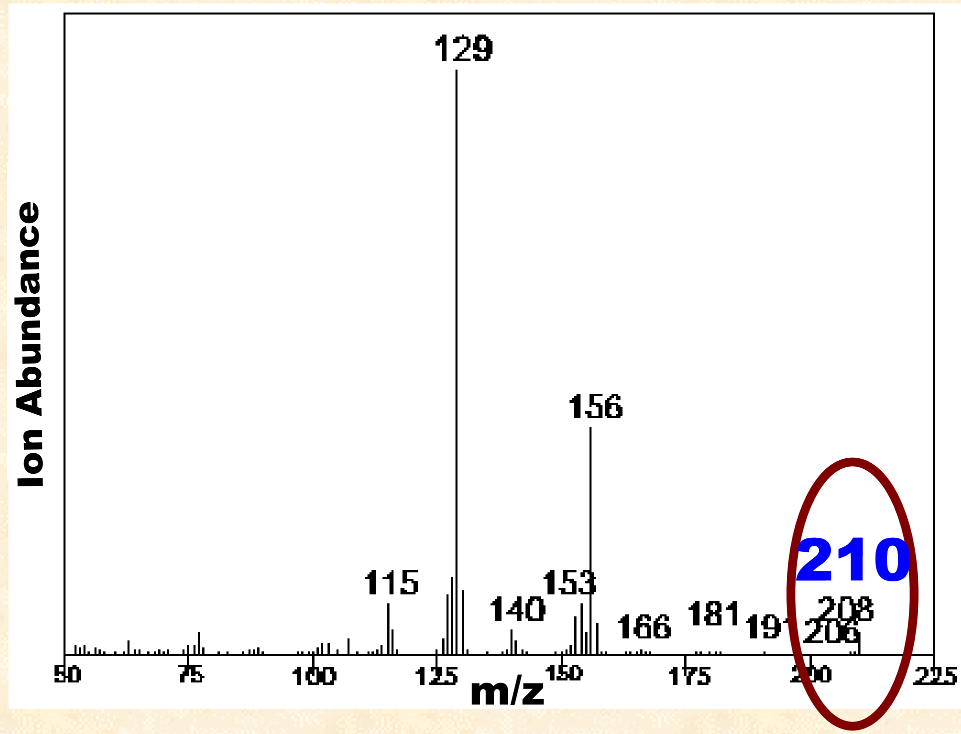
Click

Toms River, NJ - Well Water Extract

This looks like a job for...







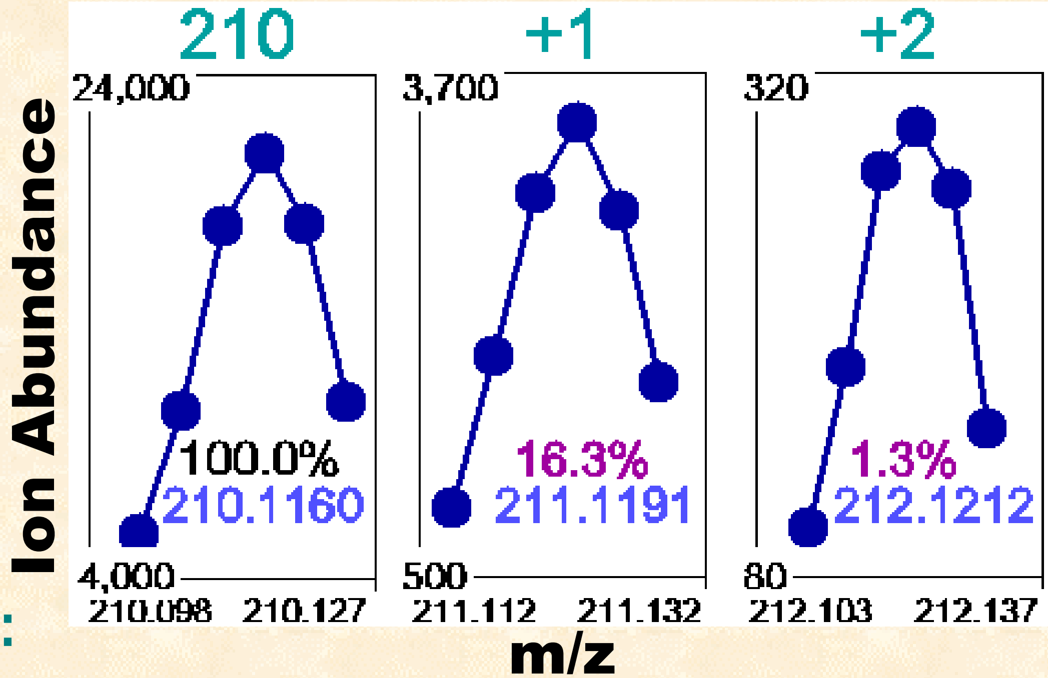
Not identified by conventional low or high resolution mass spectrometry

Click

PGM:

Elements Considered:
C H N O F P S Si

m/z 210.1160 ± 6 ppm
Resolution 10,000



Hypothetical Composition: $C_{14}H_{14}N_2$

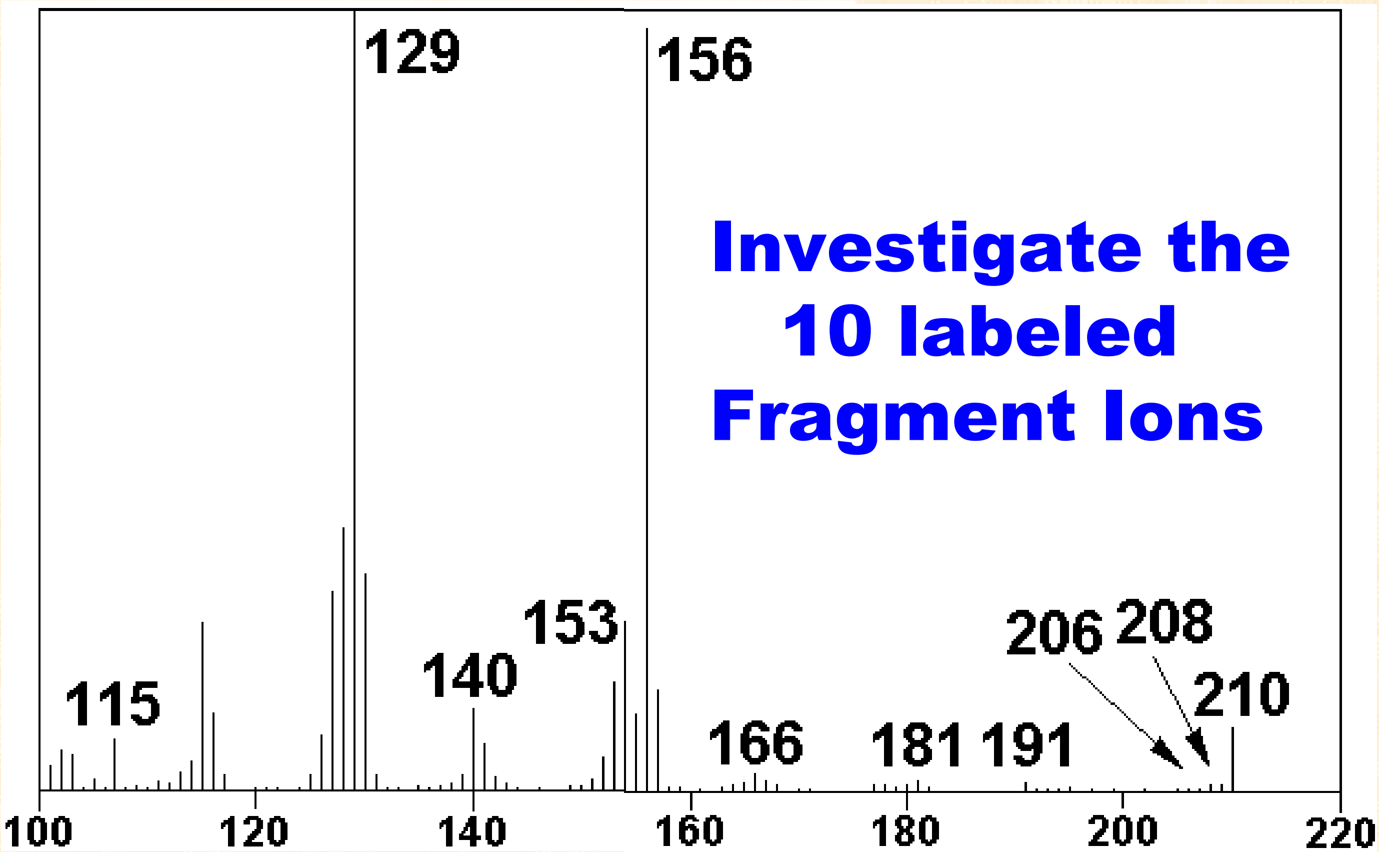
#	RDB Range	Composition	Mass Defects 151	+1	+2	Relative Abundances %+1(Range %+1)	%+2(Range %+2)
1	2.0	~~$C_4H_{12}N_8F_2$~~	.11530	.11638 X	.11697 X	5.08(3.39- 6.93) X	0.11(0.06-0.18) X
2	0.0 1.0	~~$C_5H_{18}N_4O_3Si$~~	.11482	.11598 X	.11286 X	7.95(5.15-10.84) X	0.32(0.03-0.76) X
3	1.0	~~$C_5H_{16}N_6FP$~~	.11581	.11770 X	.11941 X	6.54(4.78- 8.40) X	0.21(0.14-0.28) X
4	0.0	~~$C_6H_{20}N_4P_2$~~	.11632	.11886	.12138	7.90(6.23- 9.66) X	0.28(0.22-0.34) X
5	0.0 1.0	~~$C_6H_{18}N_4O_2S$~~	.11505	.11731 X	.11148 X	7.87(6.16- 9.61) X	0.18(0.00-0.53) X
6	1.5	~~$C_6H_{14}N_5OF_2$~~	.11664	.11894	.12089	8.21(6.32-10.24) X	0.50(0.37-0.64) X
7	-0.5 0.5	~~$C_7H_{20}NO_4Si$~~	.11616	.11805	.11476 X	12.39(9.21-15.67) X	1.01(0.34-2.03)
8	0.5	~~$C_7H_{18}N_3OFP$~~	.11715	.11999	.12217	8.88(7.48-10.34) X	0.55(0.44-0.66) X
9	-0.5 1.5	~~$C_8H_{20}NO_3S$~~	.11639	.11939	.11298 X	10.22(8.58-11.89) X	0.64(0.11-1.47)
10	5.5	~~$C_9H_{13}N_5F$~~	.11550	.11811	.12078	10.77(8.47-13.11) X	0.59(0.48-0.71) X
11	4.5	~~$C_{10}H_{17}N_3P$~~	.11601	.11899	.12210	12.02(10.17-13.90) X	0.65(0.51-0.81) X
12	5.0	~~$C_{11}H_{15}N_2OF$~~	.11684	.11998	.12266 X	12.88(11.06-14.74) X	0.92(0.71-1.12) X
13	9.0	**$C_{14}H_{14}N_2$**	.11570	.11888	.12203	16.22(13.91-18.57)	1.20(0.89-1.52)
Experimental Values:			.1160	.1191	.1212	16.3	1.3

Click

Limiting Possible Compounds

The number of possible compounds was greatly reduced, but further reduction is required.

Determine fragment ion compositions from full profiles with 10,000 resolution – 0-14 C, 0-14 H, and 0-2 N are the elemental limits.



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EPA

Exact Masses Determined for 10 Fragment Ions from the Isomers

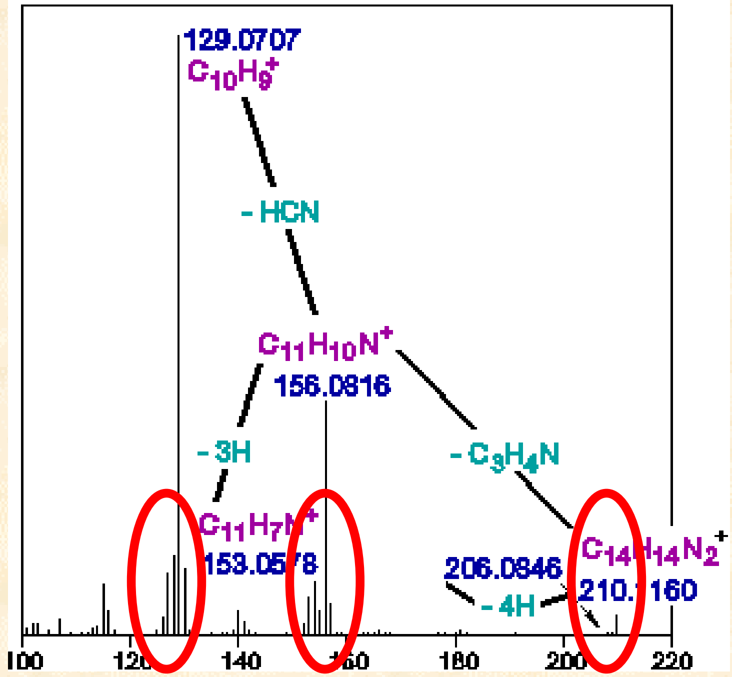
Experimental Mass	0 N	Error (mmu)	1 N	Error (mmu)	2 N	Error (mmu)
208.1001					$C_{14}H_{12}N_2$	+0.1
206.0846					$C_{14}H_{10}N_2$	+0.2
191.0607			$C_{14}H_9N$	- 12.8	$C_{13}H_7N_2$	- 0.2
181.0887	$C_{14}H_{13}$	-12.6	$C_{13}H_{11}N$	- 0.4	$C_{12}H_9N_2$	+12.5
166.0661	$C_{13}H_{10}$	-12.2	$C_{12}H_8N$	+0.4	$C_{11}H_6N_2$	+13.0
156.0816	$C_{12}H_{12}$	-12.3	$C_{11}H_{10}N$	+0.3	$C_{10}H_8N_2$	+12.9
153.0578	$C_{12}H_9$	-12.6	$C_{11}H_7N$	0.0	$C_{10}H_5N_2$	+12.5
140.0501	$C_{11}H_8$	-12.5	$C_{10}H_6N$	+0.1	$C_9H_4N_2$	+12.7
129.0707	$C_{10}H_9$	+0.3	C_9H_7N	+12.9	$C_8H_5N_2$	+25.4
115.0548	C_9H_7	0.0	C_8H_5N	+12.6	$C_7H_3N_2$	+25.2

Exact Masses of Composite Neutral Losses Determined as the Mass Differences Between the Molecular and Fragment Ions

Experimental Mass	0 N	Error (mmu)	1 N	Error (mmu)	2 N	Error (mmu)
2.0159	H_2	+0.2				
4.0314	H_4	+0.1				
19.0553	CH_7	+0.5	NH_5	+13.1		
29.0273	C_2H_5	- 11.8	CH_3N	+0.8	N_2H	+13.3
44.0499	C_3H_8	- 12.7	C_2H_6N	- 0.1	CH_4N_2	+12.5
54.0344	C_4H_6	- 12.6	C_3H_4N	0.0	$C_2H_2N_2$	+12.6
57.0582	C_4H_9	- 12.2	C_3H_7N	+0.4	$C_2H_5N_2$	+12.9
70.0659	C_5H_{10}	- 12.4	C_4H_8N	+0.2	$C_3H_6N_2$	+12.8
81.0453	C_6H_9	- 25.1	C_5H_7N	- 25.1	$C_4H_5N_2$	0.0
95.0612	C_7H_{11}	- 24.9	C_6H_9N	- 24.9	$C_5H_7N_2$	+0.3

Click

A limited number of possible isomers



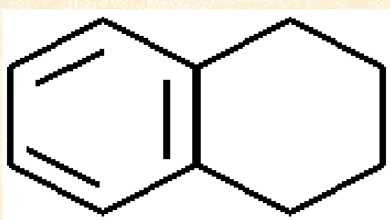
A core portion loses several H atoms

$C_{10}H_9$ is characteristic of Tetralin, which loses multiple H atoms to increase aromaticity

Two N atoms are external to the rings

Two CN groups account for 4 RDB

Possible isomers contain:



Tetralin

+ —CN +

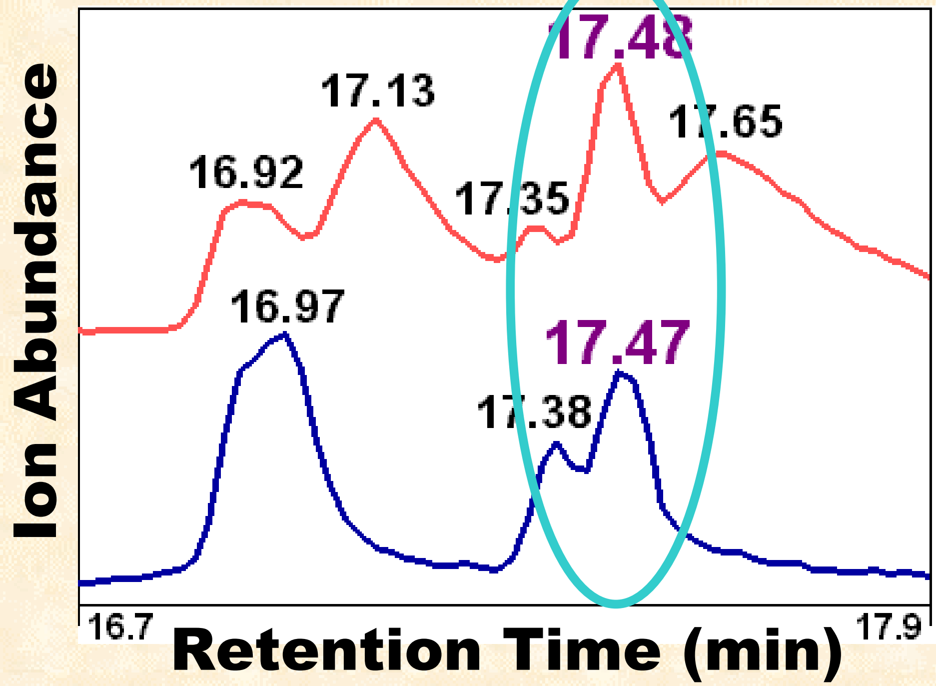
$CH_3\overset{|}{C}HCN$

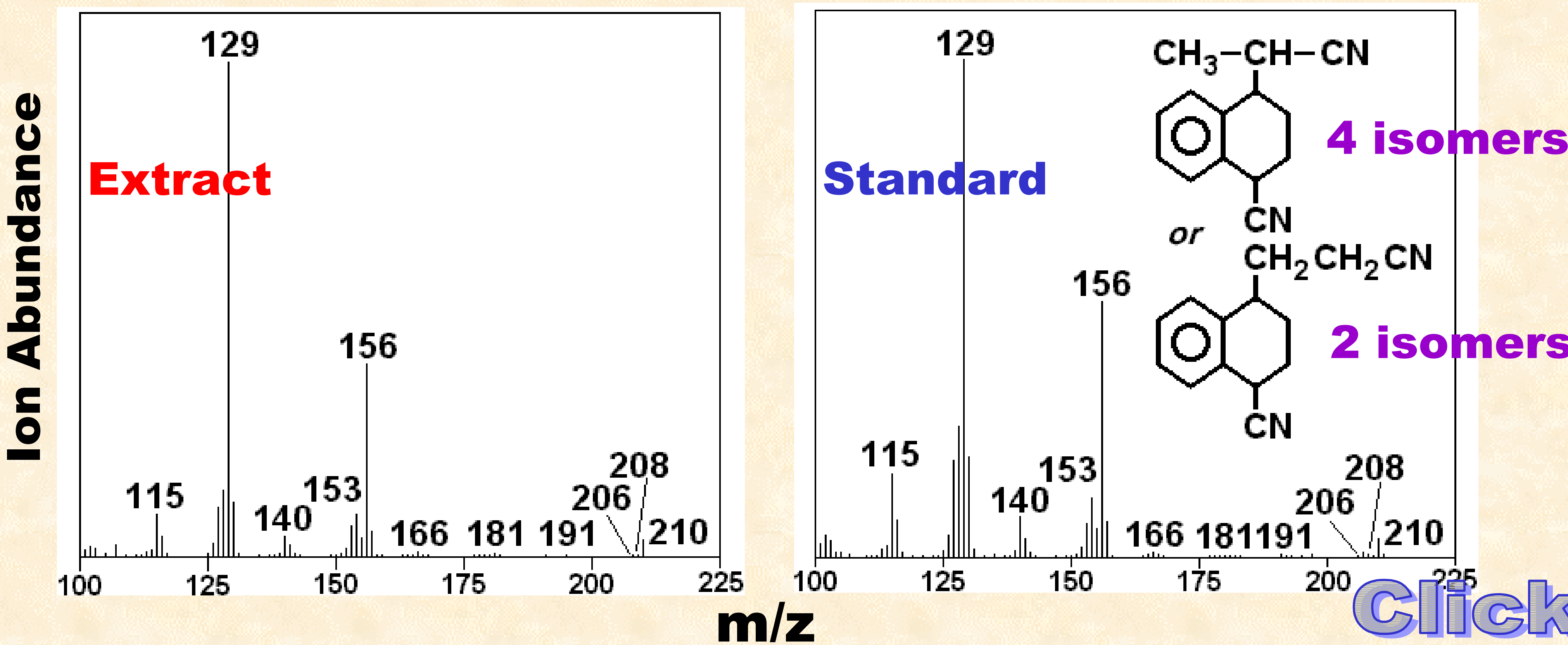
or

– CH_2CH_2CN

Click

Confirmation





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Legal Proceedings

Compound Identification is 1 link in the chain of evidence

ICE provides a Preponderance of Evidence:

- Exacts masses of molecular and fragment ions
- Exact masses, relative abundances, and shapes of +1 and +2 profiles
- Rejection of all but one composition for each ion
- Tables of fragment ion and composite neutral loss compositions
- Logical fragmentation pattern
- Superimposed ion chromatograms obtained with high mass resolution

Opposing Council – Attack a Weaker Link, e.g. the Transport Model

Click

Simplicity

Errors:

Variation in Isotopic Abundances

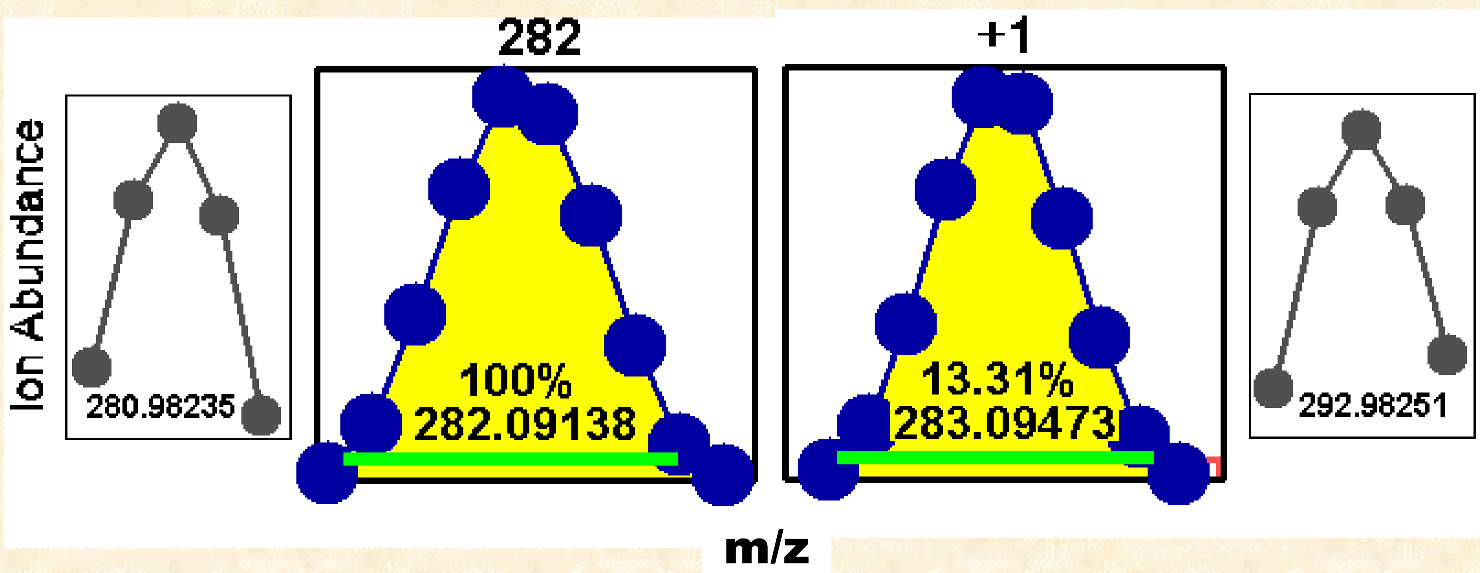
Instrumental Precision

Partial Profile Errors:

Center Mass Offsets for Other Compositions

Up to 1 mass increment experimental offsets

Up to 10% mass resolution error



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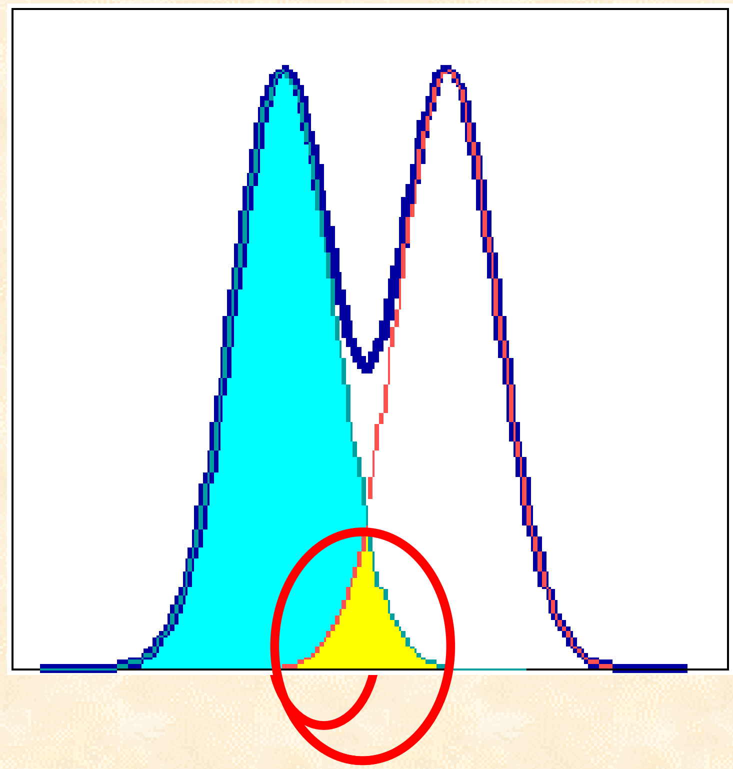
Partial or Full Profiles?

Partial profile advantages:

- Five measurements –
  3 exact masses &
  2 relative abundances
- Less profile overlap
  with interferences

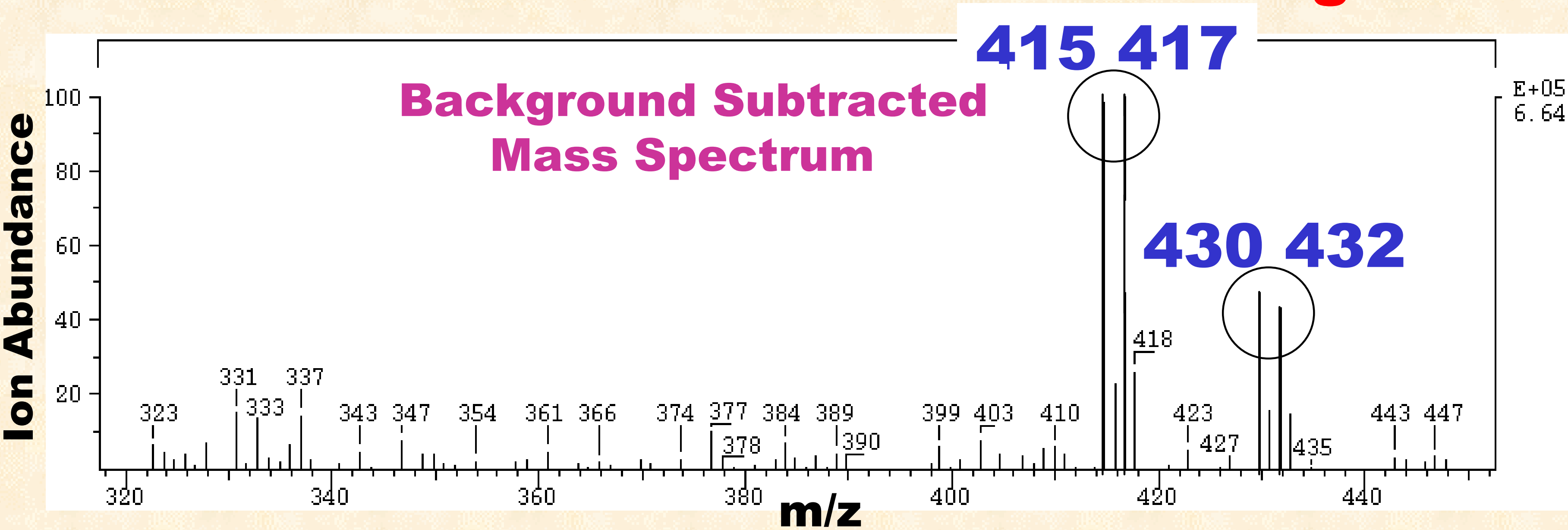


Full profile advantages:

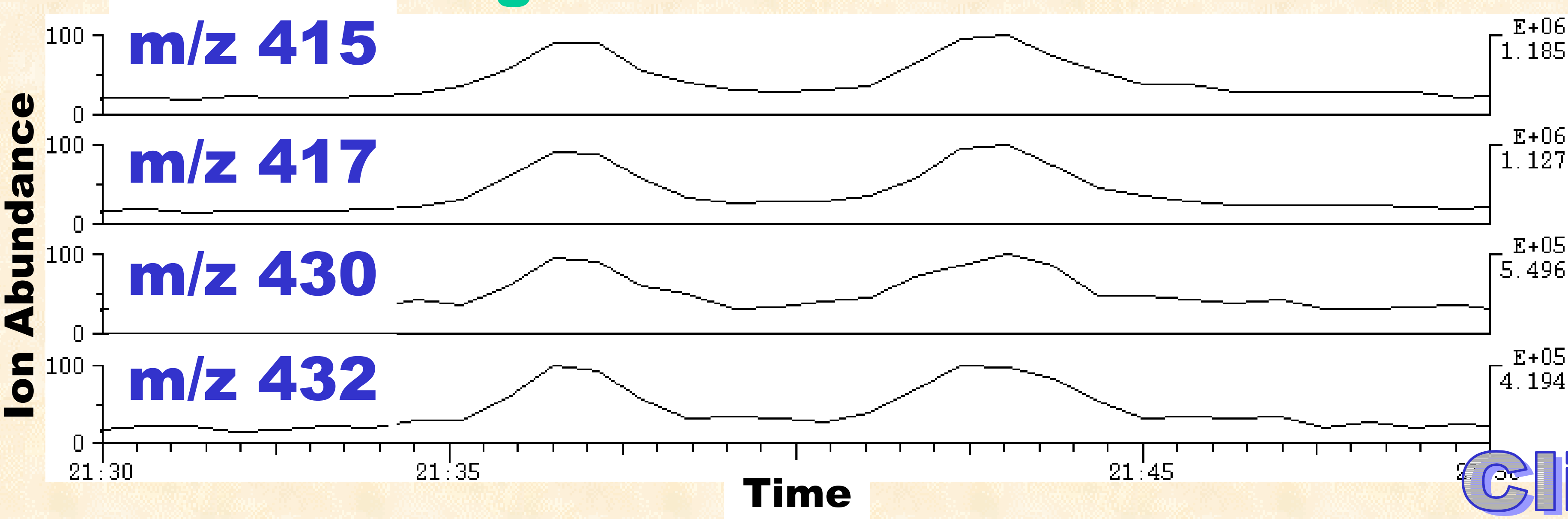
- Fewer errors –
  narrower relative abundance ranges
- Simpler to explain in legal proceedings

Utility of Limited ICE Information

Extract of chlorine-disinfected drinking water



Ion Chromatograms



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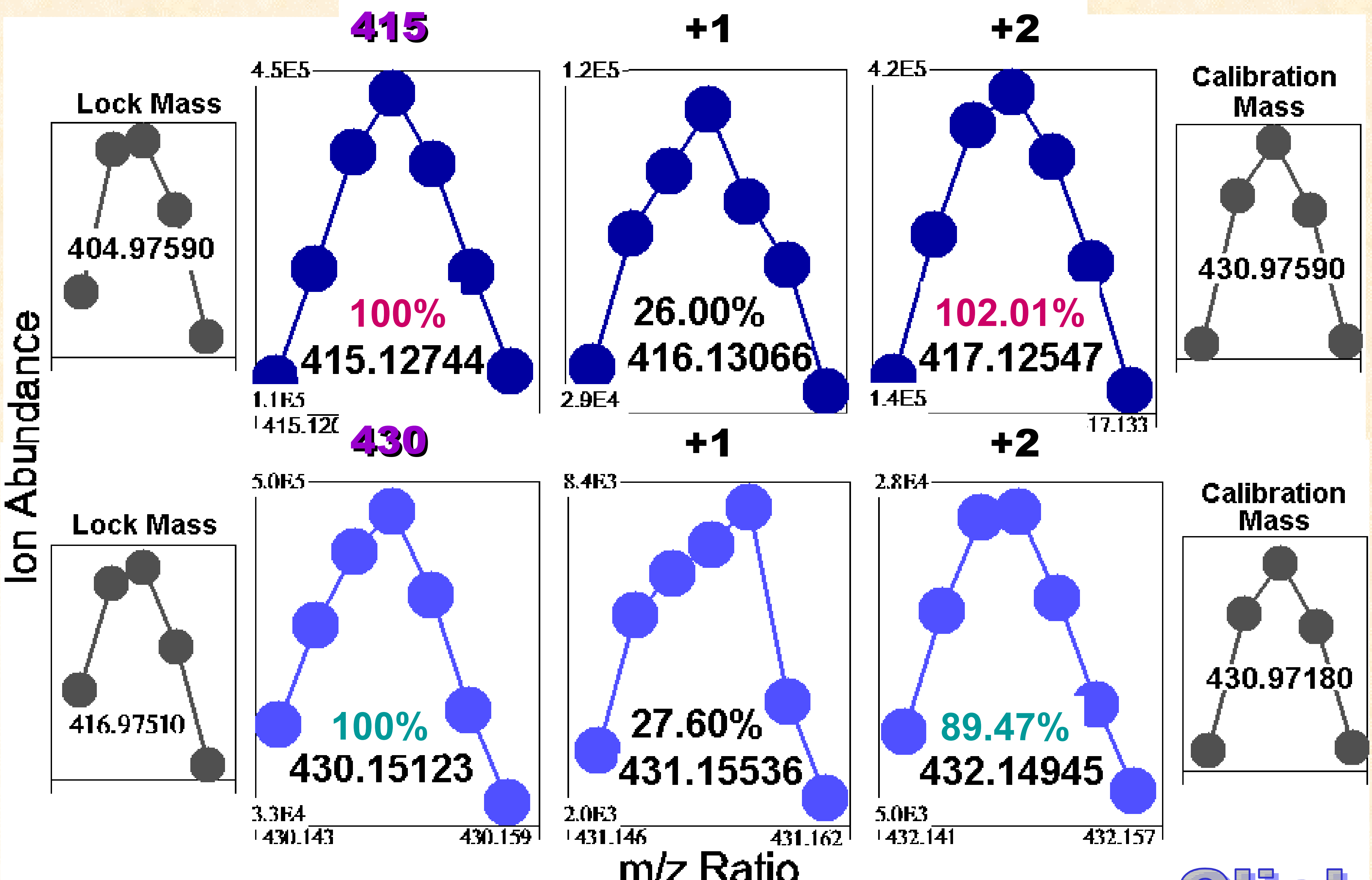
ICE is Nice A.H. Grange ORD, NERL, ESD, ECB

Partial Profiles for m/z 415 Fragment Ion and m/z 430 Molecular Ion



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PGM:

430.15123
- 415.12744
15.02379

15.02348
CH_3

ICE is Nice   A.H. Grange   ORD, NERL, ESD, ECB

m/z = 415.12744 ± 3 ppm   Elements: C H N O F P S Cl Br   Resolution: 20000

#	RDB Range	Composition	415	+1	+2		%+1 (Range +1)		%+2 (Range+2)	
1	4.5	C19 H25 F5 S CL	.12857	.13184	.12561		22.11 (19.03-25.22)	X	37.71 (28.31- 48.09)	X
2	5.5	C19 H22 O F6 CL	.12634	.12974	.12358	X	21.70 (18.25-25.22)	X	32.27 (25.25- 40.01)	X
3	6.5 7.5	C19 H31 O2 P4	.12745	.13087	.13391	X	21.58 (18.12-25.05)	X	1.01 (0.24- 1.98)	X
4	5.0 6.0	C19 H31 N O2 P BR	.12758	.13092	.12567		21.79 (18.26-25.33)	X	98.23 (85.51-111.10)	
5	8.0 9.0	C19 H25 N O3 F2 P2	.12777	.13110	.13393	X	21.79 (18.74-24.87)	X	1.11 (0.29- 2.13)	X
6	8.0 10.0	C19 H23 N O5 F2 S	.12650	.12971	.12559		22.52 (19.32-25.78)	X	5.83 (4.62- 7.36)	X
7	7.5	C19 H27 N2 O F P2 CL	.12712	.13037	.12438		21.98 (18.87-25.15)	X	32.63 (25.51- 40.49)	X
8	6.5 8.5	C19 H28 N2 O2 S2 CL	.12807	.13109	.12504		23.47 (19.74-27.26)		42.11 (31.83- 53.28)	X
9	9.5 11.5	C19 H19 N2 O4 F4	.12810	.13134	.13399	X	21.95 (18.86-25.09)	X	1.23 (0.36- 2.29)	X
10	9.0 10.0	C19 H21 N3 O2 F3 CL	.12744	.13060	.12473		22.14 (18.99-25.37)	X	32.73 (24.87- 41.33)	X
11	8.5	C19 H23 N4 F2 CL2	.12678	.12987	.12403	X	22.37 (19.14-25.71)	X	63.74 (48.92- 79.67)	X
12	12.5	C19 H21 N6 F P S	.12701	.12983	.12496		23.35 (19.78-27.01)		5.52 (4.58- 6.70)	X
13	12.5 13.5	C19 H20 N6 O3 CL	.12854	.13148	.12588		23.03 (19.47-26.71)		33.89 (25.77- 42.90)	X
		☻		☻			☻		☻	
43	12.5	C24 H22 O2 F2 CL	.12764	.13103	.12512		27.01 (22.68-31.34)		33.14 (26.27- 40.93)	X
44	12.0	C24 H24 N F CL2	.12698	.13031	.12413	X	27.24 (23.44-31.07)		64.12 (48.92- 80.59)	X
45	16.0	C24 H22 N3 P S	.12721	.13031	.12683	X	28.32 (24.28-32.47)		5.84 (4.55- 7.51)	X
46	16.5 17.5	C25 H20 N2 O F S	.12804	.13121	.12849	X	29.19 (25.06-33.37)		5.80 (4.53- 7.48)	X
47	15.5 16.5	C26 H24 O P S	.12855	.13185	.12975	X	29.92 (25.76-34.10)		5.76 (4.63- 7.27)	X
48	16.5 17.5	C26 H21 O2 F P	.12632	.12971	.13288	X	29.59 (24.89-34.39)		2.14 (0.59- 4.11)	X
49	16.5	C27 H21 O F CL	.12650	.12988	.12389	X	30.35 (26.16-34.55)	X	32.78 (25.22- 41.25)	X
50	20.5	C28 H19 N2 S	.12689	.13008	.12888	X	32.50 (27.91-37.17)	X	6.34 (4.71- 8.44)	X
16	5.5	C20 H29 O3 F BR	.12841	.13182	.12652		22.51 (18.86-26.12)		97.84 (85.25-110.56)	
39	9.5	C23 H28 O2 BR	.12727	.13067	.12541		26.04 (22.43-29.65)		98.59 (86.48-110.97)	
Experimental Values:			.12744	.13066	.12547		26.00		102.01	

m/z = 430.15123 ± 3 ppm   Elements: C H N O F P S Cl Br   Resolution: 20000

#	RDB Range	Composition	430	+1	+2	%+1 (Range +1)	%+2 (Range+2)
31	5.0	C21 H32 O3 F BR	.15188	.15530	.15001	24.11 (19.75-28.57)	98.91(86.86-111.21)
54	9.0	C24 H31 O2 BR	.15074	.15415	.14891	26.73 (21.79-31.62)	98.66(86.59-111.01)
Experimental Values:			.15123	.15536	.14945	27.60	89.47

Click

ICE Utilizes:

Reject incorrect compositions mechanically:

Sums of Atomic Masses

Sums of Isotopic Abundances

Valences of Elements

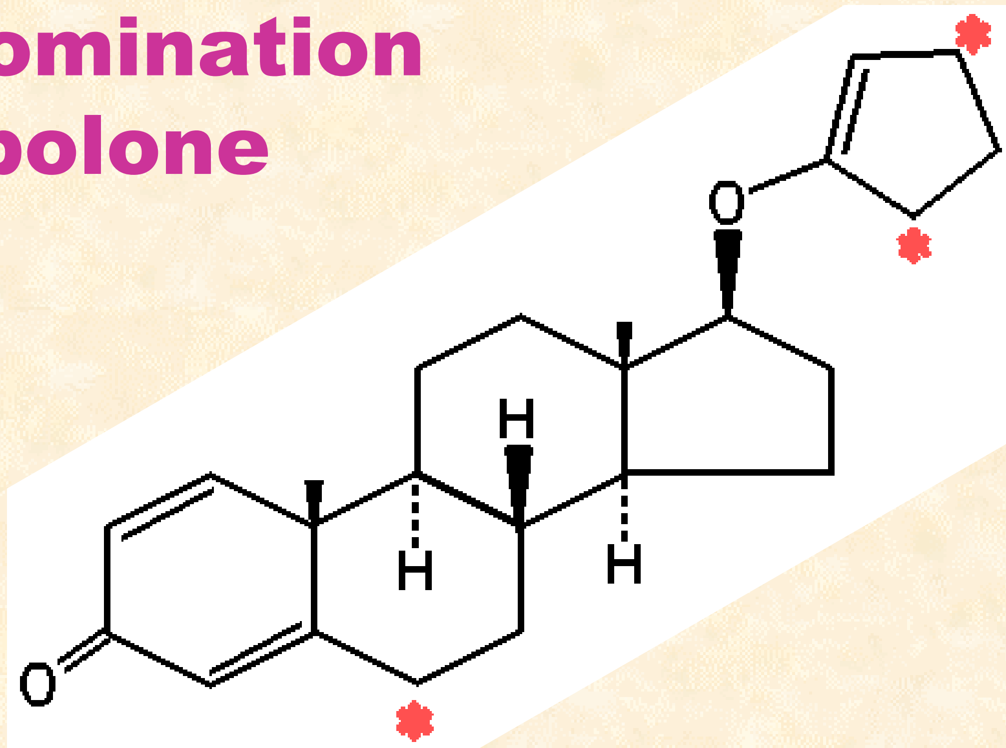
For multiple remaining compositions, invoke chemical and commercial arguments

Commercial argument:

Anabolic steroids could be used to enhance cattle growth in a nearby feedlot.

Click

* Possible Allylic Bromination Sites for Quinbolone



Multiple Isomers

Next Steps:

Purchase Quinbolone

Add to well water

Chlorinate

Extract products

Obtain mass spectra and retention times for comparison

$C_{24}H_{32}O_2Br$

Click

Characterization or Identification

Analysis & Data | **Number of Possible Compositions**

Low Resolution MS

Nominal Mass and
No Library Matches

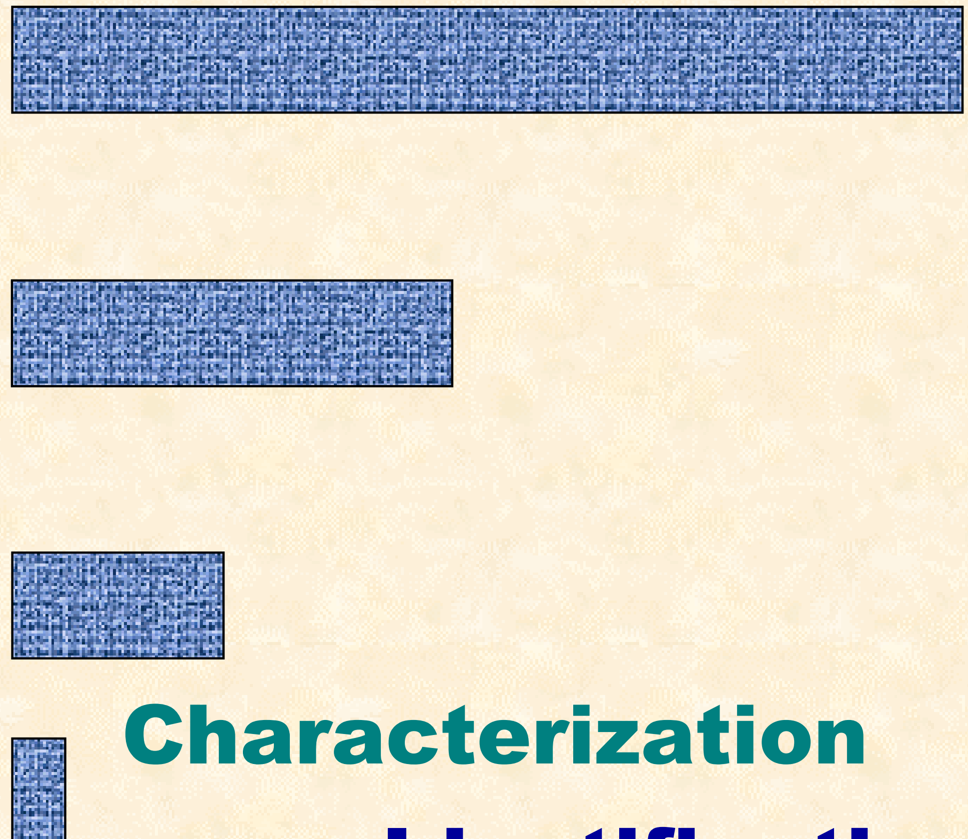
Conventional High Res MS

Exact Mass of
Apparent Molecular Ion

ICE

Ion Composition of
Apparent Molecular Ion

Ion Composition of
Multiple Fragment Ions



Identification

Chemical Literature
Commercial Literature → Single Compound

Click

Selected ICE Publications and Posters

Grange, A.H.; Donnelly, J.R.; Brumley, W.C.; Billets, S.; Sovocool, G.W.
Mass Measurement by an Accurate and Sensitive Selected-Ion-Recording Technique
Anal. Chem., 1994, 66, 4416-4421

Grange, A.H.; Donnelly, J.R.; Brumley, W.C.; Sovocool, G.W.
Determination of Elemental Compositions from Mass Peak Profiles of the Molecular (M), M+1 and M+2 Ions
Anal. Chem., 1996, 553-560

Grange, A.H.; Brumley, W.C.
A Mass Peak Profile Generation Model to Facilitate Determination of Elemental Compositions of Ions Based on Exact Masses and Isotopic Abundances
J. Amer. Soc. for Mass Spectrum., 1997, 8, 170-182

Grange, A.H.; Sovocool, G.W.; Donnelly, J.R.; Genicola, F.A.; Gurka, D.F.
Identification of Pollutants in a Municipal Well Using High Resolution Mass Spectrometry
Rapid Commun. Mass Spectrom., 1998, 12, 1161-1169

6 Posters - http://www.epa.gov/crdlvweb/chemistry/ecb-posters.htm

The End