An Introduction to Mass Spectrometry and Applications to Trace and Ultra-Trace Analysis

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What is Trace? Ultra-Trace?

Trace:
ppt (thousand)
-ppm

					Grams in
	1	0.001	0.000001	0.000000001	1L (1000g)
amu	1ppt	1ppm	1ppb	1ppt	
10	1.00E-01	1.00E-04	1.00E-07	1.00E-10	
100	1.00E-02	1.00E-05	1.00E-08	1.00E-11	
1000	1.00E-03	1.00E-06	1.00E-09	1.00E-12	
10000	1.00E-04	1.00E-07	1.00E-10	1.00E-13	molar

Ultra Trace:
<ppb

Front Ends:
- GC
- LC
- SPME
- Electrophoresis
- Ion Mobility

Ionization Sources:
- Electron Impact (EI)
- Atmospheric Pressure Chemical Ionization (APCI)
- MALDI
- Electrospray/ Nanospray Ionization (ESI)
- Corona Discharge
- Inductively Coupled Plasma (ICP)
- FAB, SIMS

Mass (Ion) Analyzers:
- Linear Quadrupole
- Quadrupole Ion Trap (QIT)
- Time-of-Flight (TOF)
- Ion Cyclotron Resonance (FT-MS)
- Sectors
- OrbiTrap
- Ion Mobility
- Any Combination Hybrids (Triple Quads, Q-TOF, TOF-TOF, IM-TOF, IM-Q)

Detection:
- Faraday Cup
- Electron Multiplier
- Microchannel Plate

Sensitivity vs Selectivity:

$$Sensitivity = \frac{\#\text{-}of-species-\det ected}{total\text{-}\#\text{-}of-species}$$

$$1 - Selectivity = \frac{\#\text{-}of-false-positives-\det ected}{total\text{-}\#\text{-}of-false-positives}$$

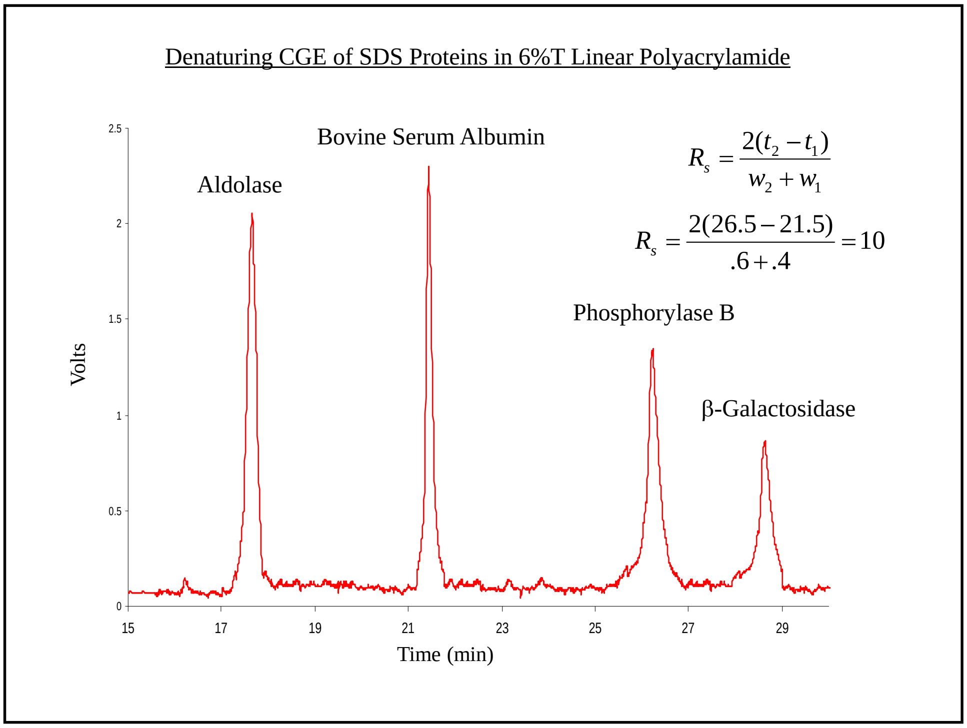
Resolution vs Resolving Power (MS):

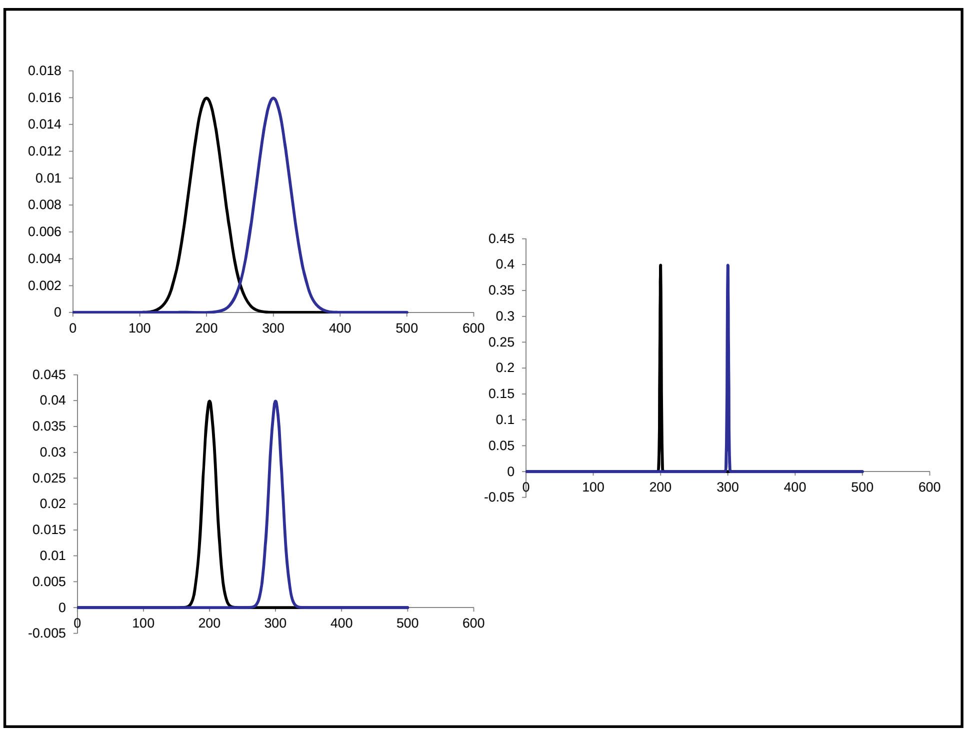
$$\mathrm{Re}\,solution = \frac{m}{\Delta m}$$

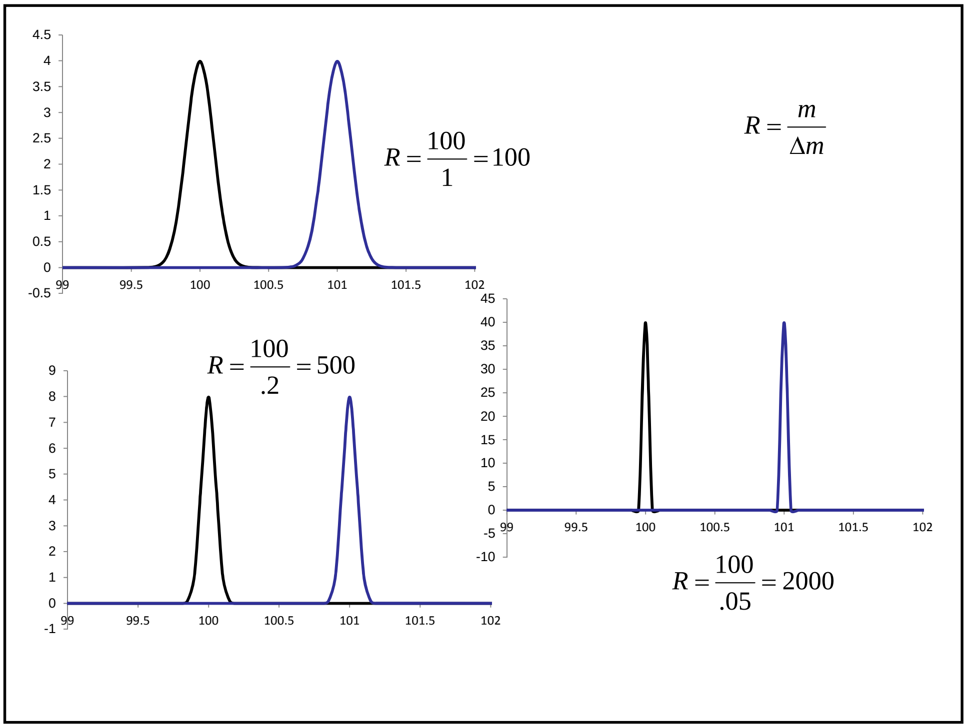
$$\mathrm{Re}\,solvingPower = \Delta m$$

Resolution Chromatography:

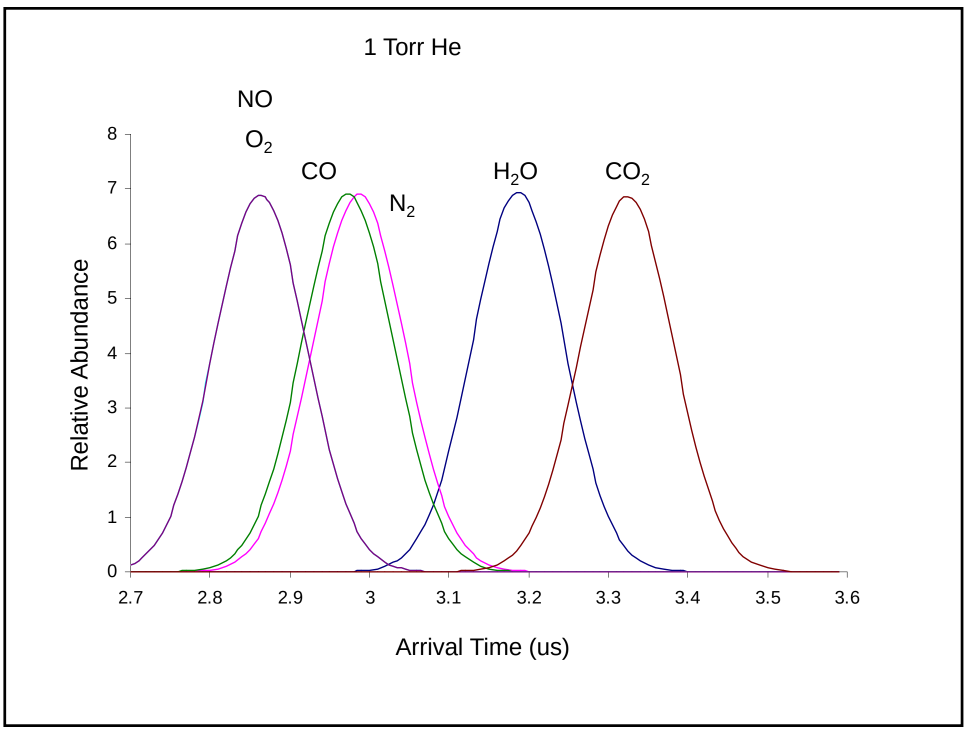
$$R_s = \frac{2(t_2 - t_1)}{w_2 + w_1}$$


Denaturing CGE of SDS Proteins in 6%T Linear Polyacrylamide
Bovine Serum Albumin
Aldolase
Phosphorylase B
β-Galactosidase
$R_s = \frac{2(t_2 - t_1)}{w_2 + w_1}$
$R_s = \frac{2(26.5 - 21.5)}{.6 + .4} = 10$
Volts
Time (min)
0
0.5
1
1.5
2
2.5
15
17
19
21
23
25
27
29

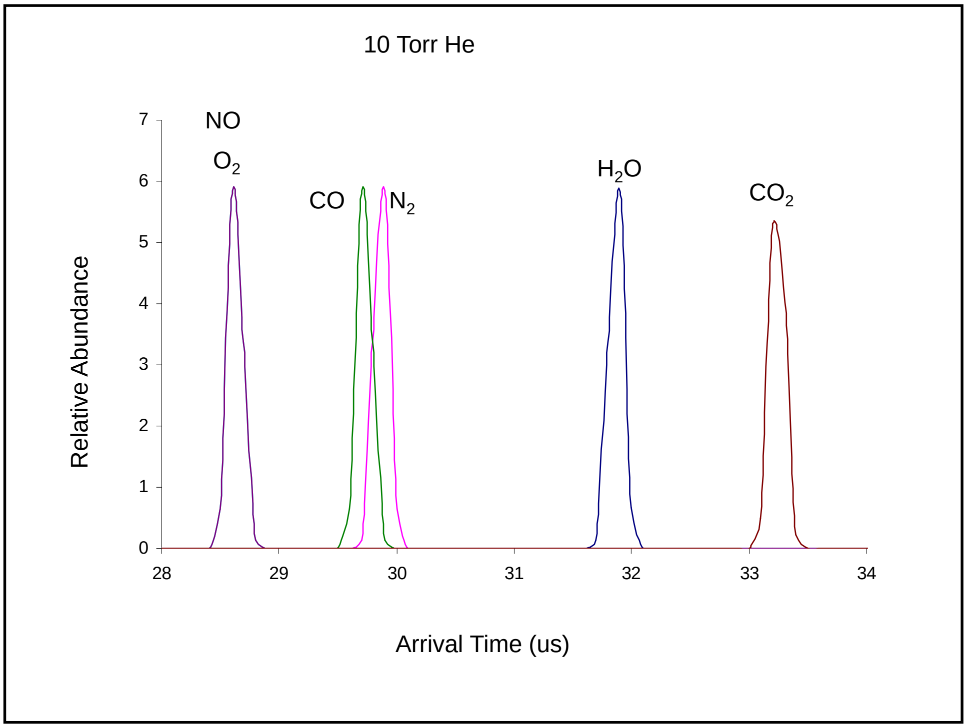

0.018
0.016
0.014
0.012
0.01
0.008
0.006
0.004
0.002
0
100
200
300
400
500
600
0.045
0.04
0.035
0.03
0.025
0.02
0.015
0.005
-0.005
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
-0.05

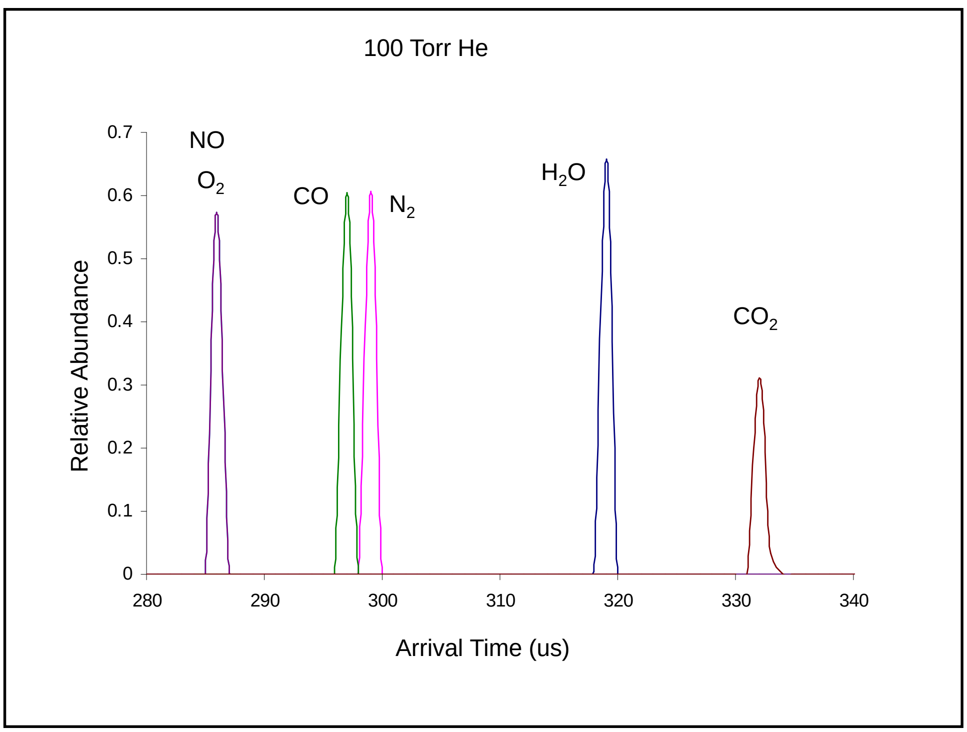


R = m/Δm
R = 100/1 = 100
R = 100/.2 = 500
R = 100/.05 = 2000



1 Torr He
NO
O2
CO
N2
H2O
CO2
Relative Abundance
Arrival Time (us)

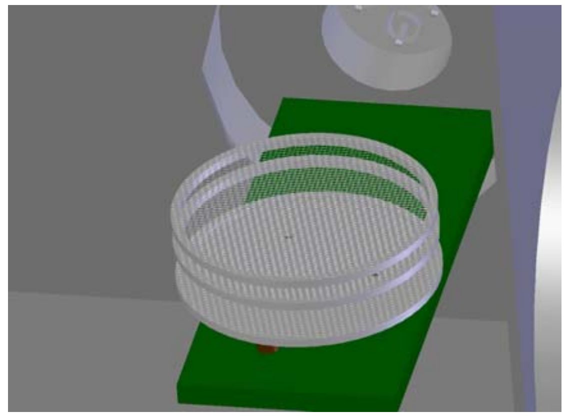

10 Torr He
NO
O2
CO
N2
H2O
CO2
Relative Abundance
0
1
2
3
4
5
6
7
28
29
30
31
32
33
34
Arrival Time (us)


100 Torr He
NO
O2
CO
N2
H2O
CO2
Relative Abundance
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
280
290
300
310
320
330
340
Arrival Time (us)

Why is resolution so important?

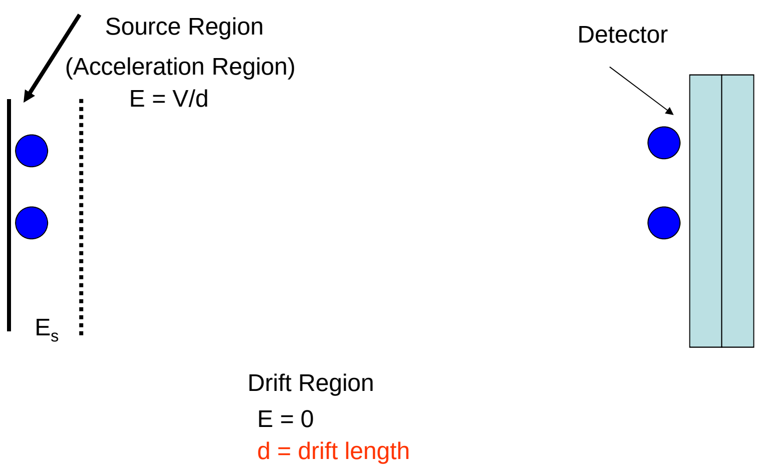
Z	Name	Symbol	Mass of Atom (u)	% Abundance
1	Hydrogen	^{1}H	1.007825	99.9885
	Deuterium	^{2}H	2.014102	0.115
	Tritium	^{3}H	3.016049	*
6	Carbon	^{12}C	12.000000	98.93
		^{13}C	13.003355	1.07
		^{14}C	14.003242	*
7	Nitrogen	^{14}N	14.003074	99.632
		^{15}N	15.000109	0.368
8	Oxygen	^{16}O	15.994915	99.757
		^{17}O	16.999132	0.038
		^{18}O	17.999160	0.205

Mass Analyzers:



Time-of-Flight Mass Spectrometry

Time-of-Flight (TOF)



Time-of-Flight (TOF)

$$eV = \frac{1}{2}mv^2$$ Kinetic Energy given to the Ion in the Source Region

$$v = \left(\frac{2eV}{m}\right)^{\frac{1}{2}}$$ Solving for Velocity

$$v = \frac{d}{t}$$ Solve for Flight Time $$t = \left(\frac{m}{2eV}\right)^{\frac{1}{2}} d$$

So, with a constant acceleration voltage and a known drift length, the drift time is proportional to the square root of the mass to charge ratio (m/e).

Time-of-Flight (TOF)

Mass spectrometrists define resolution as: $$\frac{m}{\Delta m}$$

In TOF we start from the drift time equation:

$$m = \left(\frac{2eV}{d^2}\right)t^2$$ And the derivative is: $$dm = \left(\frac{2eV}{d^2}\right)2tdt$$

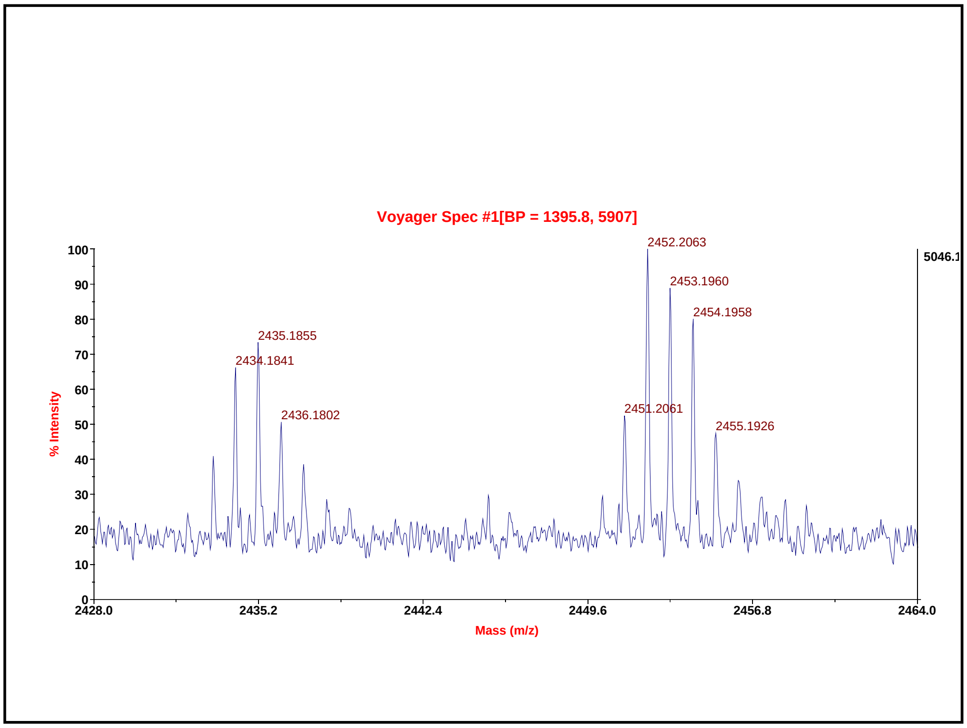
So,

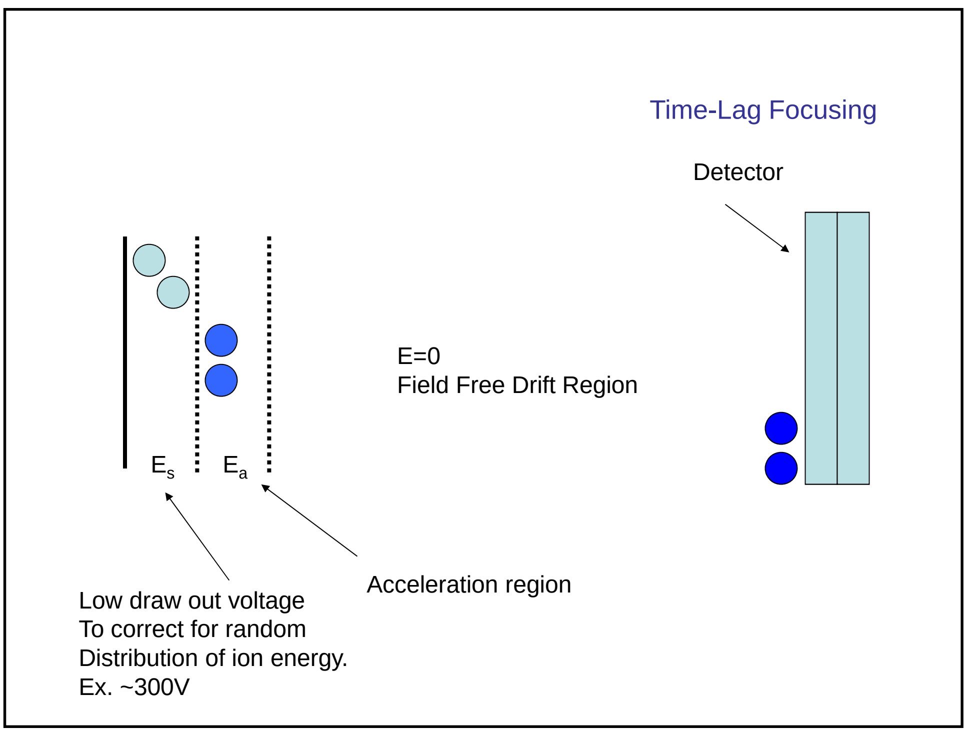
$$\frac{m}{dm} = \frac{t}{2dt}$$

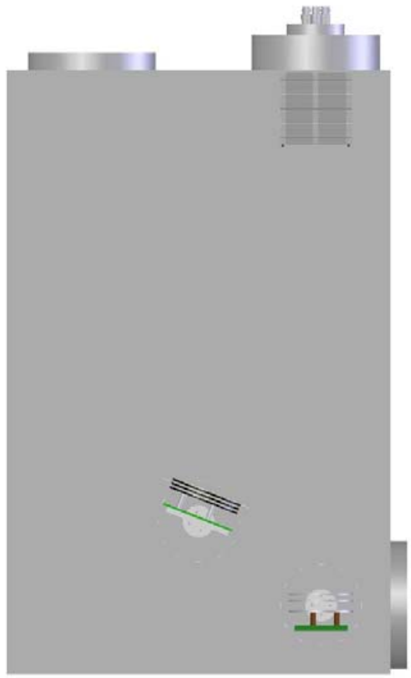
So time-of-flight resolution is defined by:

$$R = \frac{m}{\Delta m} = \frac{t}{2\Delta t}$$ $$R \propto \frac{L}{E_z}$$

Δt is usually defined a peak width at half height.


Voyager Spec #1[BP = 1395.8, 5907]
% Intensity
Mass (m/z)
2452.2063
2453.1960
2454.1958
2435.1855
2434.1841
2436.1802
2451.2061
2455.1926
5046.1
100
90
80
70
60
50
40
30
20
10
0
2428.0
2435.2
2442.4
2449.6
2456.8
2464.0


Time-Lag Focusing
Detector
E=0
Field Free Drift Region
E_s
E_a
Low draw out voltage
To correct for random
Distribution of ion energy.
Ex. ~300V
Acceleration region



Reflectron Time-of-Flight

Quadrupole Mass Spectrometry

The Quadrupole Field

The potential at any point (x,y,z) is defined as:

$$\phi = \frac{\phi_o}{r_o^2}(\lambda x^2 + \sigma y^2 + \gamma z^2)$$

Where,

ϕ_o= the applied field

λ, σ, γ are weighing constants for their coordinate

r_o= constant for the device

The applied field is the combination of a RF and DC (U) field:

$$\phi_o = U - V\cos(\Omega t)$$

V= 0 to Peak
Ω (rad/s) = 2πf(Hz)

Linear Quads

$$\phi = \frac{\phi_o}{r_o^2}(\lambda x^2 + \sigma y^2 + \gamma z^2)$$

We remember the constraints from the Laplace eq.:

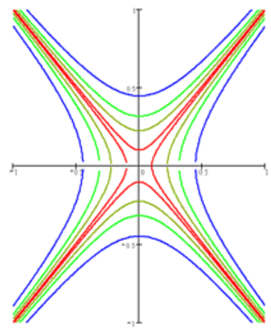
$$\lambda + \sigma + \gamma = 0$$

If we are only interested in quadrupole MS (x,y) then:

$$\gamma = 0$$
$$\lambda = -\sigma$$

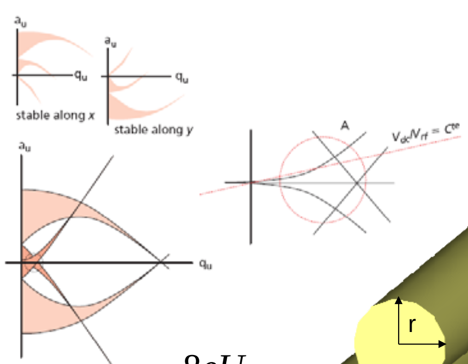
If we set λ=1, then:

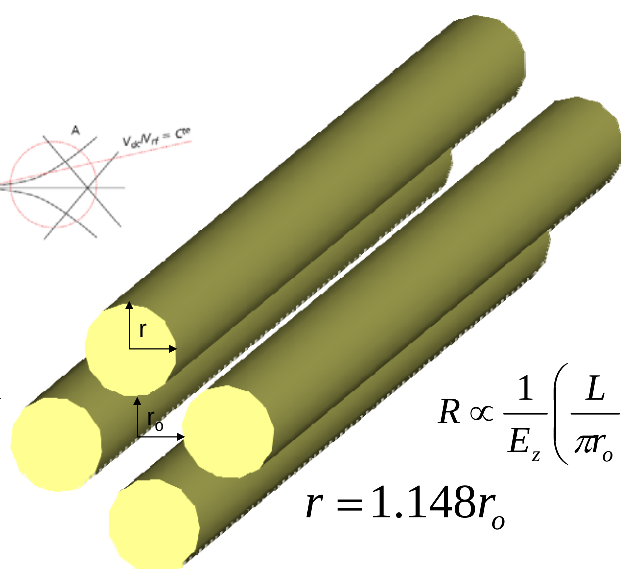
$$\phi = \frac{\phi_o}{r_o^2}(x^2 - y^2)$$



The equipotential field plot

Linear Quads





$$a_x = -a_y = \frac{8eU}{mr_o^2\Omega^2}$$

$$q_x = -q_y = \frac{4eV}{mr_o^2\Omega^2}$$

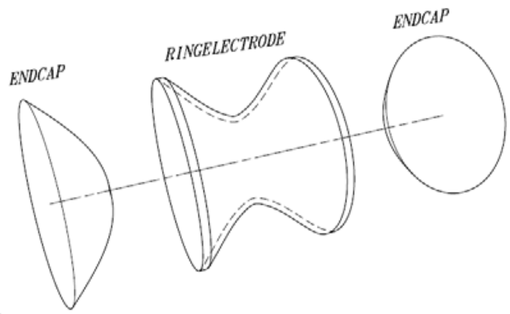
$$R \propto \frac{1}{E_z}\left(\frac{L}{\pi r_o}\right)^2$$

$$r = 1.148 r_o$$

Quad Ion Traps

$$\lambda + \sigma + \gamma = 0$$
$$\lambda = \sigma = 1$$
$$\gamma = -2$$

$$\phi = \frac{\phi_o}{r_o^2}(x^2 + y^2 - 2z^2)$$



Transform into cylindrical coordinates:

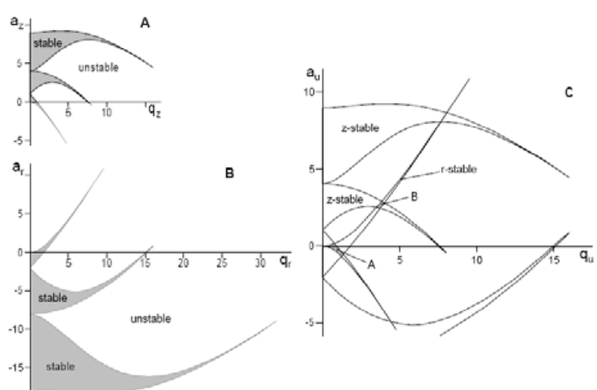
$$\phi = \frac{\phi_o}{r_o^2}(r^2 \cos^2\theta + r^2 \sin^2\theta - 2z^2) \qquad (\cos^2\theta + \sin^2\theta = 1)$$

$$\phi = \frac{\phi_o}{r_o^2}(r^2 - 2z^2)$$

Geometrical constraints:

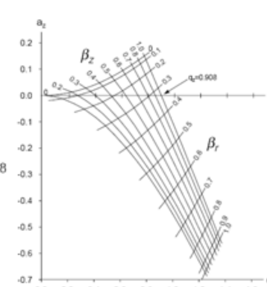
$$r_o^{\ 2} = 2z_o^{\ 2}$$

Quad Ion Traps

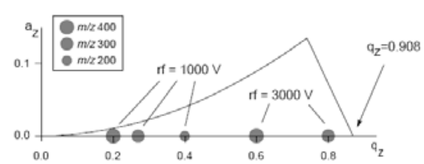


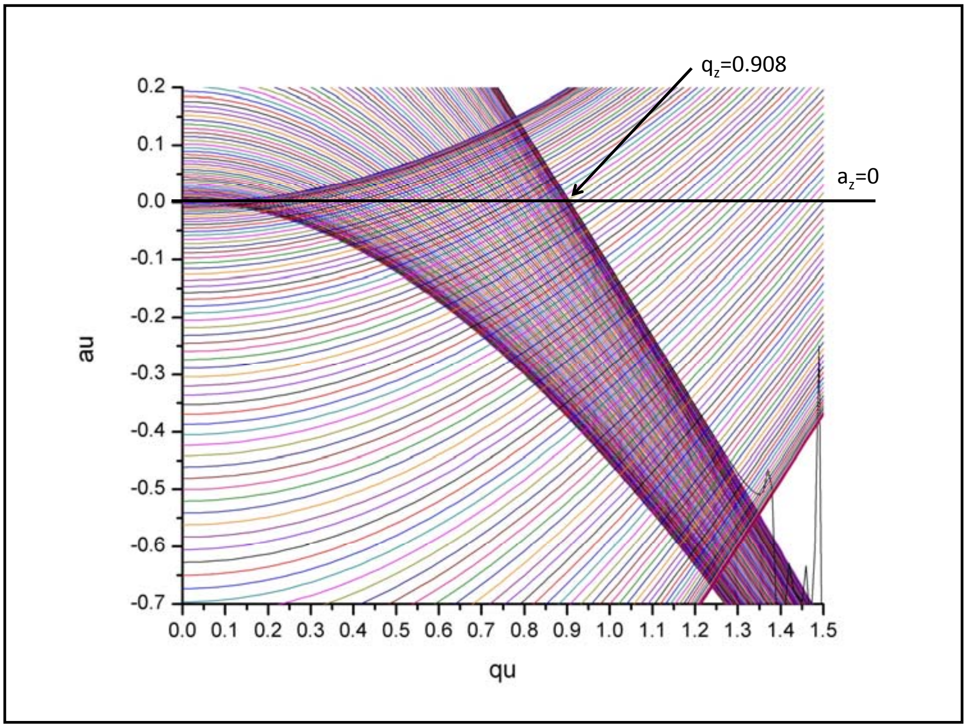
$$a_z = -2a_r = \frac{-16eU}{mr_o^2\Omega^2}$$

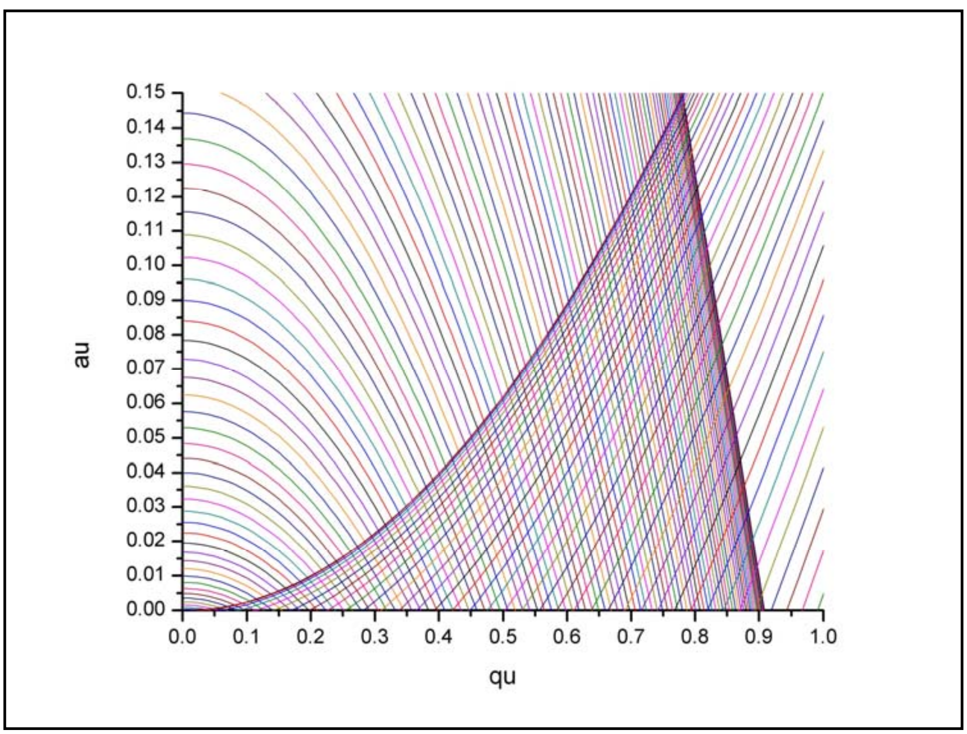
$$q_z = -2q_r = \frac{-8eV}{mr_o^2\Omega^2}$$

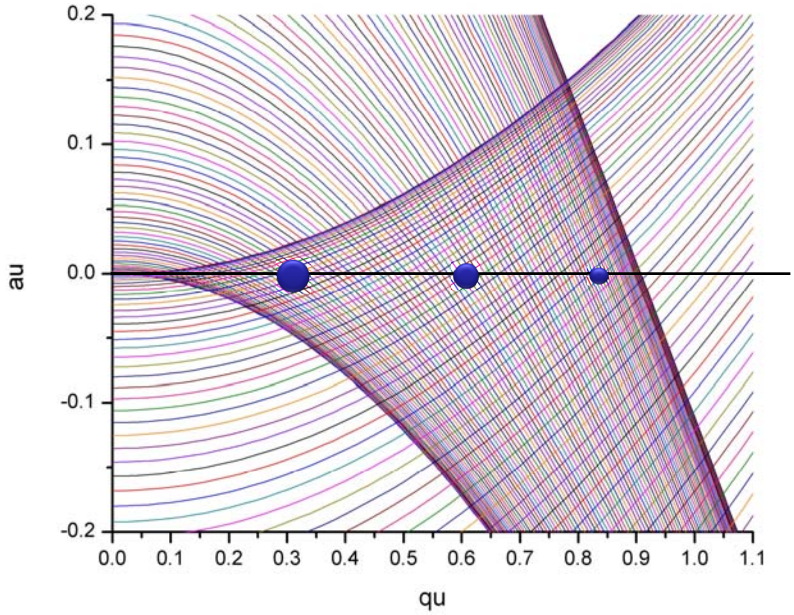


$$R \propto t_{trap} \approx \# RFcycles$$

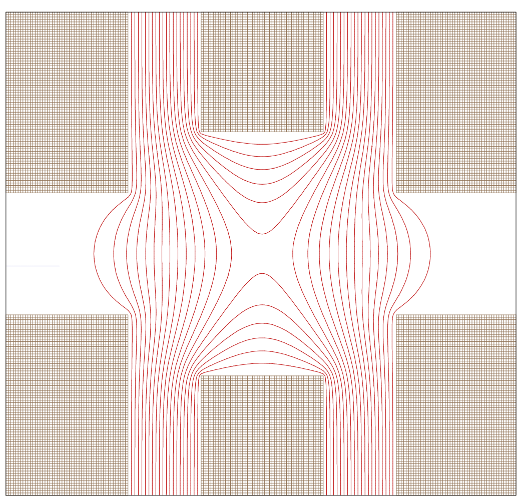



q_z=0.908
a_z=0
au
qu
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
-0.7
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0 1.1 1.2 1.3 1.4 1.5


au
qu
0.15
0.14
0.13
0.12
0.11
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0



au
0.2
0.1
0.0
-0.1
-0.2
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1
qu

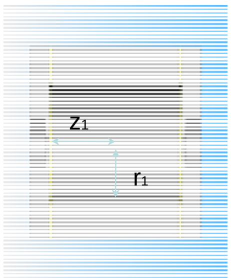


$$a_z = \frac{-16eU}{m(r_0^2 + 2z_0^2)\Omega^2}$$

$$a_z = 0$$

$$q_z = \frac{-8eV}{m(r_0^2 + 2z_0^2)\Omega^2}$$

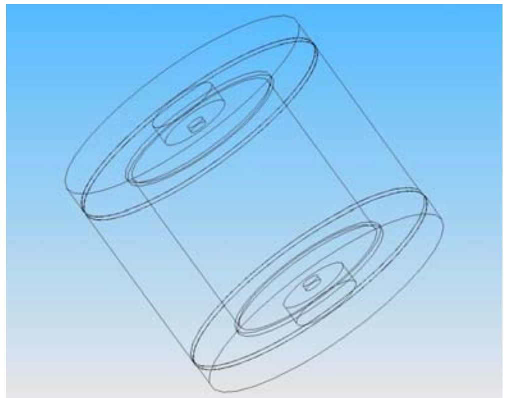
For a cylindrical ion trap, where



$$r_1^2 = 2z_0^2$$

$$a_z = \frac{-8eU}{m(r_1^2)\Omega^2}$$

$$q_z = \frac{-4eV}{m(r_1^2)\Omega^2}$$

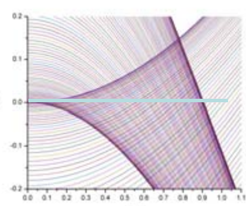


e= 1.60E-19 C

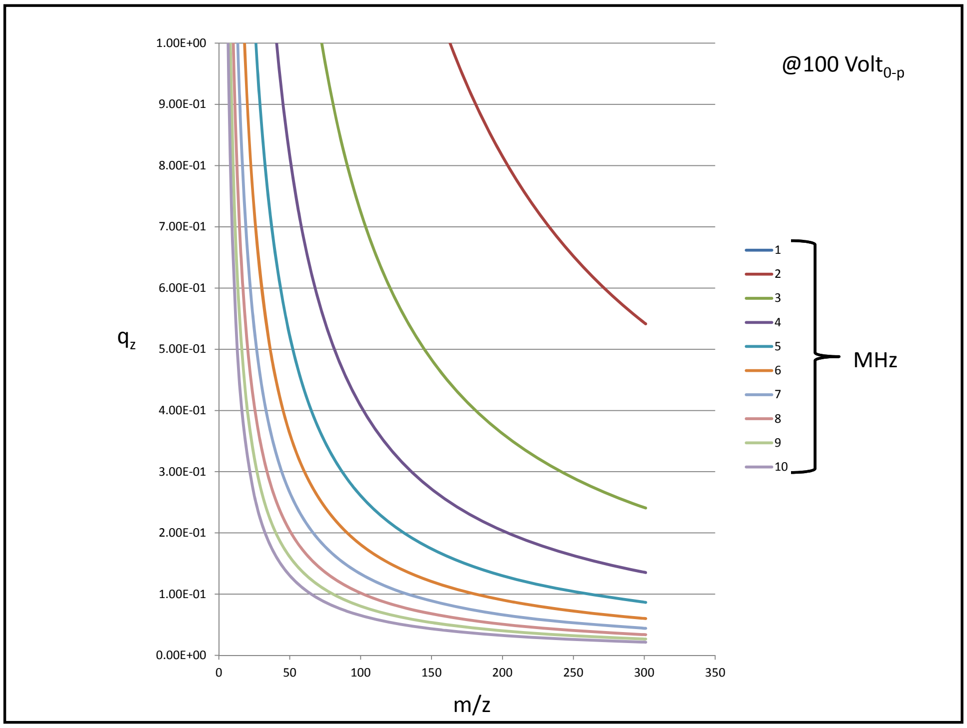
r= 0.001 m

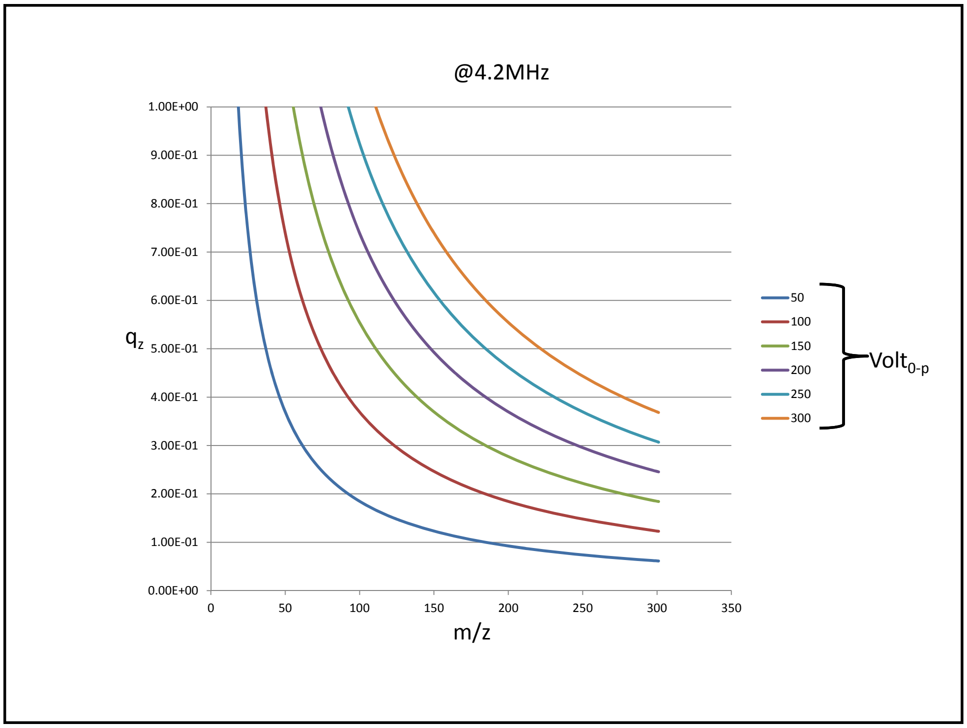
Omega= 6000000 Hz 37699112

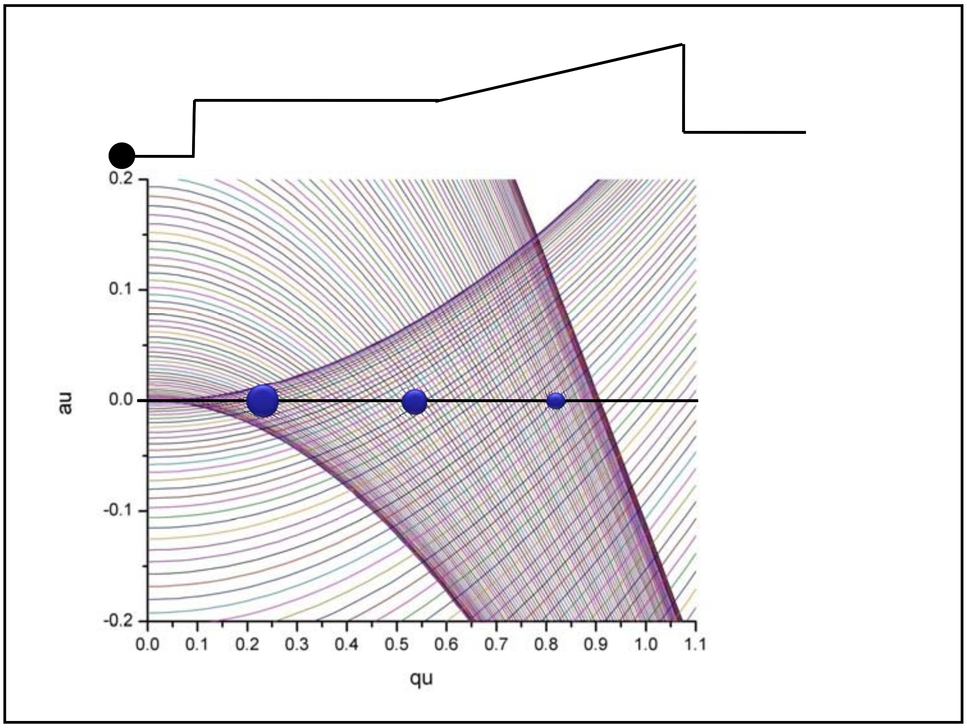
m/z					q					
Vo-p		0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
	10	6.788834807	3.394417	2.262945	1.697209	1.357767	1.131472	0.969834	0.848604	0.754315
	20	13.57766961	6.788835	4.52589	3.394417	2.715534	2.262945	1.939667	1.697209	1.50863
	30	20.36650442	10.18325	6.788835	5.091626	4.073301	3.394417	2.909501	2.545813	2.262945
	40	27.15533923	13.57767	9.05178	6.788835	5.431068	4.52589	3.879334	3.394417	3.01726
	50	33.94417403	16.97209	11.31472	8.486044	6.788835	5.657362	4.849168	4.243022	3.771575
	60	40.73300884	20.3665	13.57767	10.18325	8.146602	6.788835	5.819001	5.091626	4.52589
	70	47.52184365	23.76092	15.84061	11.88046	9.504369	7.920307	6.788835	5.94023	5.280205
	80	54.31067846	27.15534	18.10356	13.57767	10.86214	9.05178	7.758668	6.788835	6.03452
	90	61.09951326	30.54976	20.3665	15.27488	12.2199	10.18325	8.728502	7.637439	6.788835
	100	67.88834807	33.94417	22.62945	16.97209	13.57767	11.31472	9.698335	8.486044	7.54315
	110	74.67718288	37.33859	24.89239	18.6693	14.93544	12.4462	10.66817	9.334648	8.297465
	120	81.46601768	40.73301	27.15534	20.3665	16.2932	13.57767	11.638	10.18325	9.05178
	130	88.25485249	44.12743	29.41828	22.06371	17.65097	14.70914	12.60784	11.03186	9.806095
	140	95.0436873	47.52184	31.68123	23.76092	19.00874	15.84061	13.57767	11.88046	10.56041
	150	101.8325221	50.91626	33.94417	25.45813	20.3665	16.97209	14.5475	12.72907	11.31472
	160	108.6213569	54.31068	36.20712	27.15534	21.72427	18.10356	15.51734	13.57767	12.06904
	170	115.4101917	57.7051	38.47006	28.85255	23.08204	19.23503	16.48717	14.42627	12.82335
	180	122.1990265	61.09951	40.73301	30.54976	24.43981	20.3665	17.457	15.27488	13.57767
	190	128.9878613	64.49393	42.99595	32.24697	25.79757	21.49798	18.42684	16.12348	14.33198
	200	135.7766961	67.88835	45.2589	33.94417	27.15534	22.62945	19.39667	16.97209	15.0863
	210	142.5655309	71.28277	47.52184	35.64138	28.51311	23.76092	20.3665	17.82069	15.84061
	220	149.3543658	74.67718	49.78479	37.33859	29.87087	24.89239	21.33634	18.6693	16.59493
	230	156.1432006	78.0716	52.04773	39.0358	31.22864	26.02387	22.30617	19.5179	17.34924
	240	162.9320354	81.46602	54.31068	40.73301	32.58641	27.15534	23.27601	20.3665	18.10356
	250	169.7208702	84.86044	56.57362	42.43022	33.94417	28.28681	24.24584	21.21511	18.85787
	260	176.509705	88.25485	58.83657	44.12743	35.30194	29.41828	25.21567	22.06371	19.61219
	270	183.2985398	91.64927	61.09951	45.82463	36.65971	30.54976	26.18551	22.91232	20.3665
	280	190.0873746	95.04369	63.36246	47.52184	38.01747	31.68123	27.15534	23.76092	21.12082
	290	196.8762094	98.4381	65.6254	49.21905	39.37524	32.8127	28.12517	24.60953	21.87513
	300	203.6650442	101.8325	67.88835	50.91626	40.73301	33.94417	29.09501	25.45813	22.62945



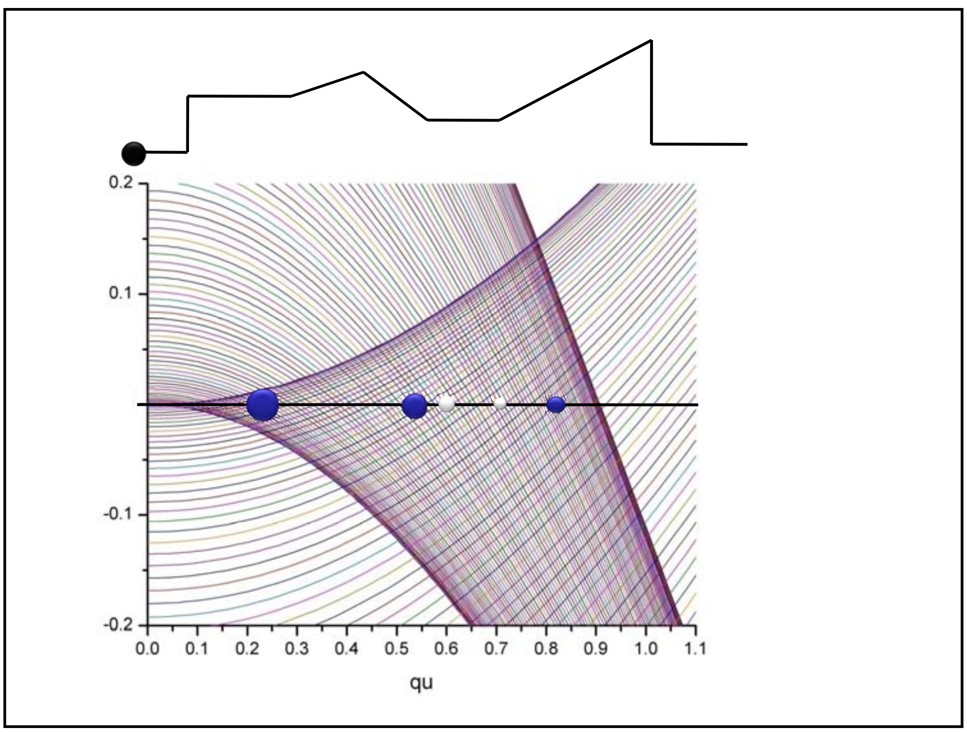
For au=0,
0<qu<0.908


@100 Volt0-p
1.00E+00
9.00E-01
8.00E-01
7.00E-01
6.00E-01
5.00E-01
4.00E-01
3.00E-01
2.00E-01
1.00E-01
0.00E+00
qz
0
50
100
150
200
250
300
350
m/z
1
2
3
4
5
6
7
8
9
10
MHz


@4.2MHz
1.00E+00
9.00E-01
8.00E-01
7.00E-01
6.00E-01
5.00E-01
4.00E-01
3.00E-01
2.00E-01
1.00E-01
0.00E+00
qz
0
50
100
150
200
250
300
350
m/z
50
100
150
200
250
300
Volt0-p

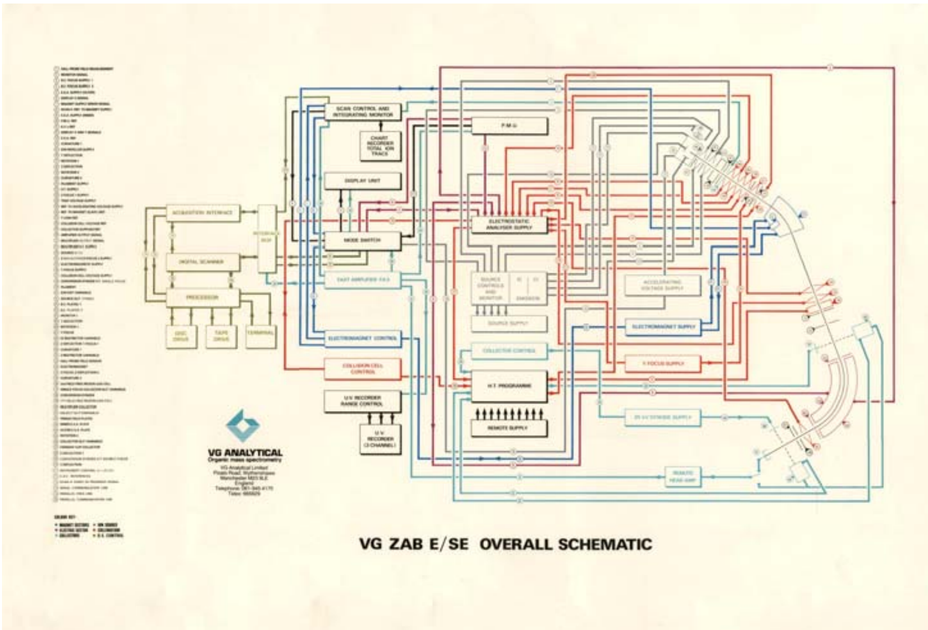


0.2
0.1
0.0
-0.1
-0.2
au
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0 1.1
qu



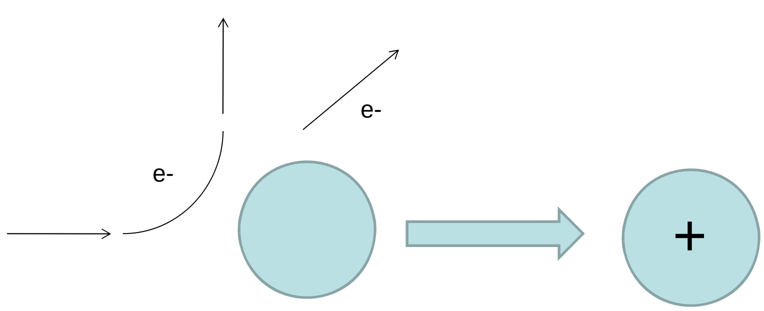
0.2
0.1
-0.1
-0.2
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0 1.1
qu

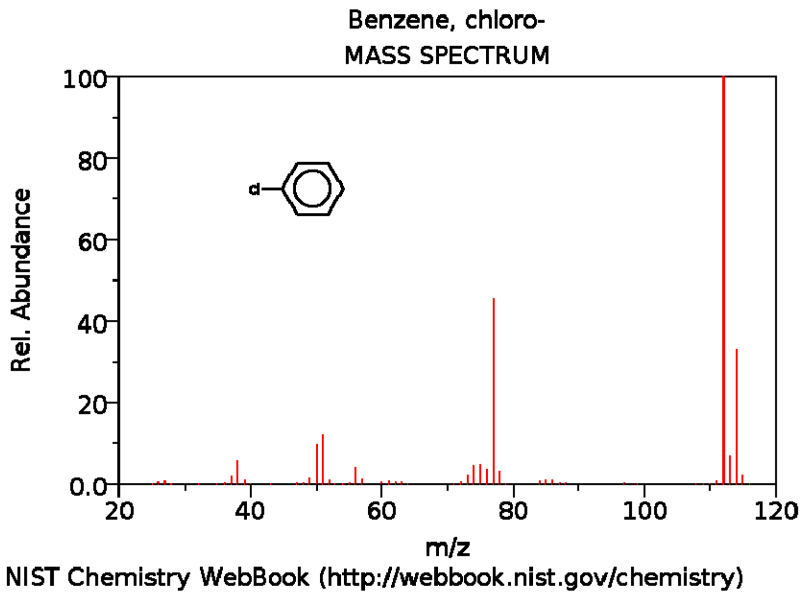
Sector Mass Spectrometry



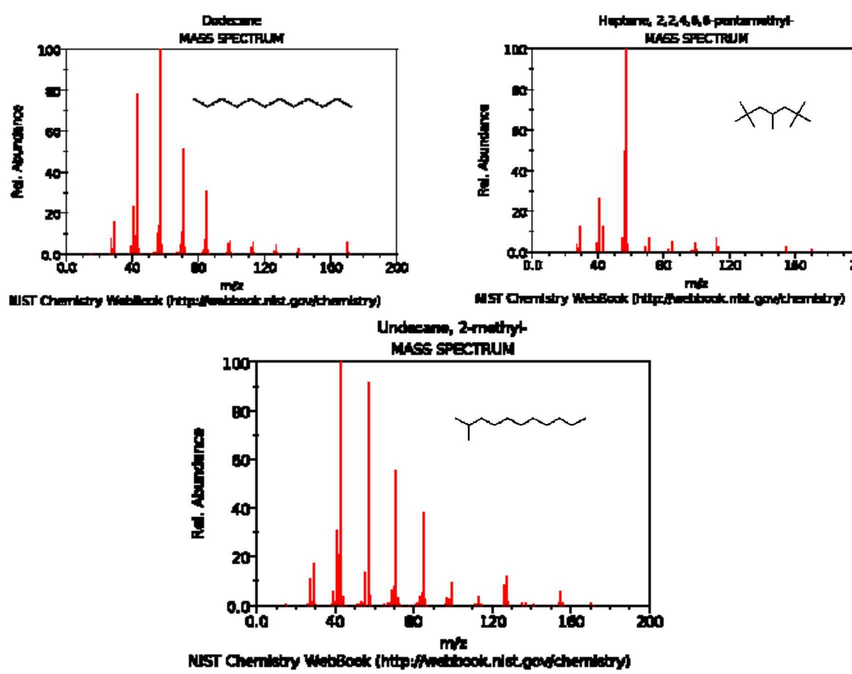
Sources:

Electron Impact:

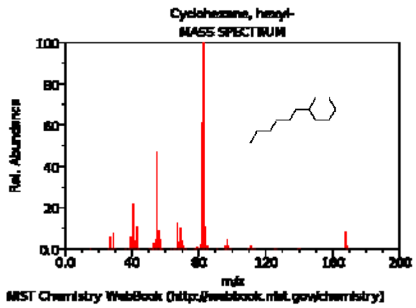


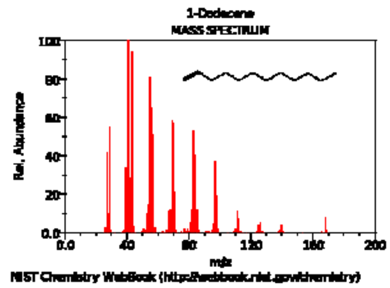


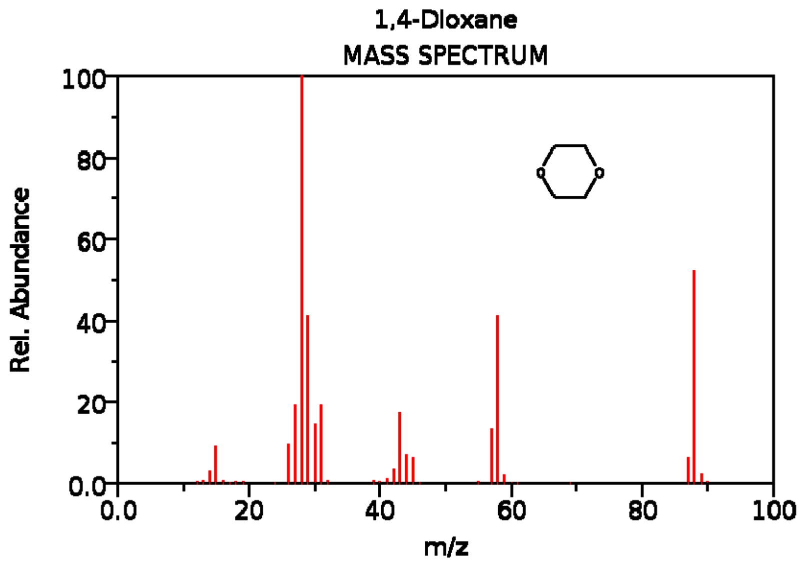
Benzene, chloro-
MASS SPECTRUM
Rel. Abundance
m/z
Cl
NIST Chemistry WebBook (http://webbook.nist.gov/chemistry)

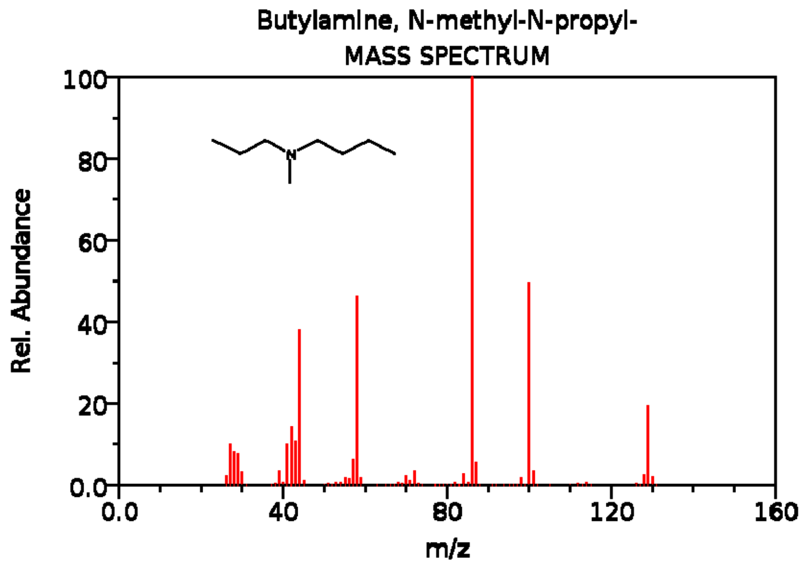


Dodecane
MASS SPECTRUM
Heptane, 2,2,4,6,6-pentamethyl-
MASS SPECTRUM
Undecane, 2-methyl-
MASS SPECTRUM
Rel. Abundance
m/z
NIST Chemistry WebBook (http://webbook.nist.gov/chemistry)


Cyclohexane, hexyl-
MASS SPECTRUM
Rel. Abundance
m/z
NIST Chemistry WebBook (http://webbook.nist.gov/chemistry)


1-Dodecene
MASS SPECTRUM
Rel. Abundance
m/z
NIST Chemistry WebBook (http://webbook.nist.gov/chemistry)


1,4-Dioxane
MASS SPECTRUM
Rel. Abundance
m/z



Electrospray/Nanospray Ionization

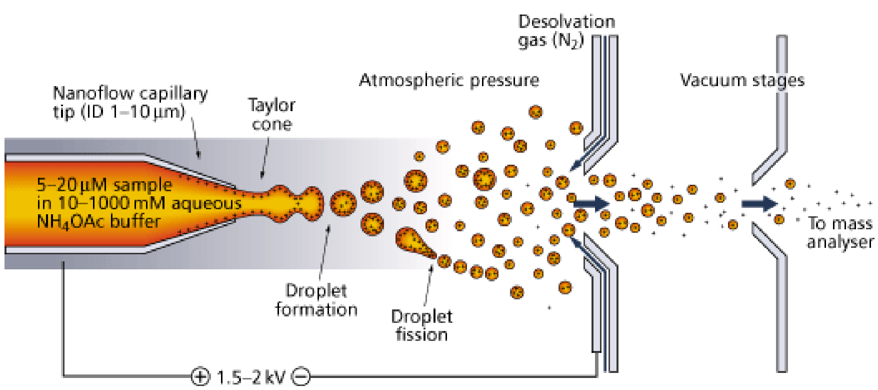


Image from: http://www.uab.edu/botanicals/pdfs/08_Sam090902.pdf

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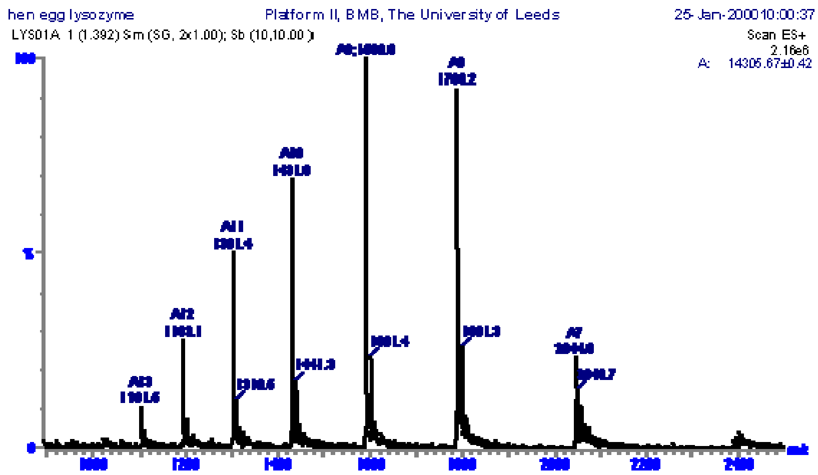
ESI


hen egg lysozyme
Platform II, BMB, The University of Leeds
25-Jan-2000 10:00:37
Scan ES+
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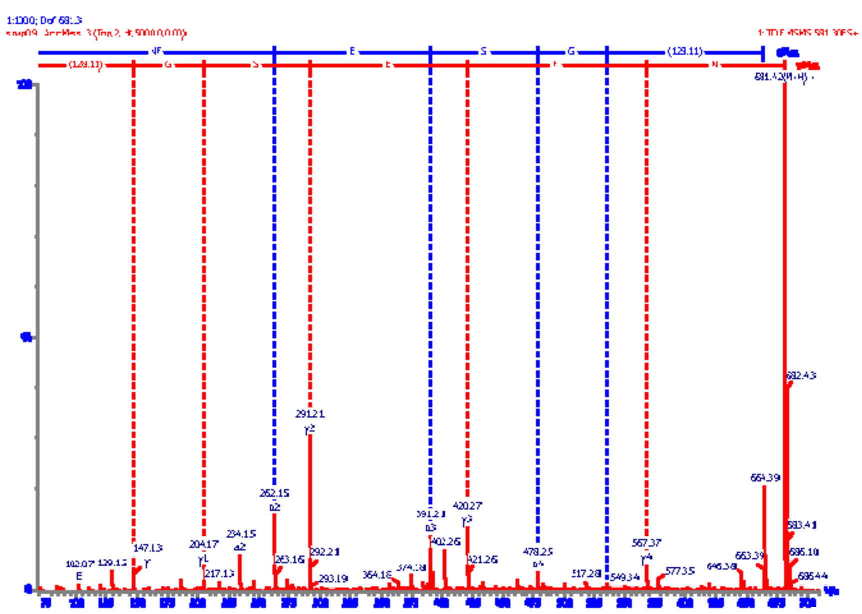
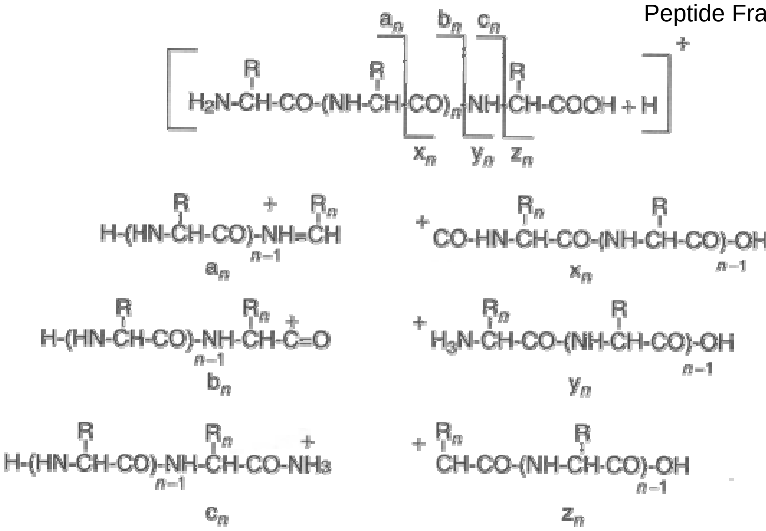


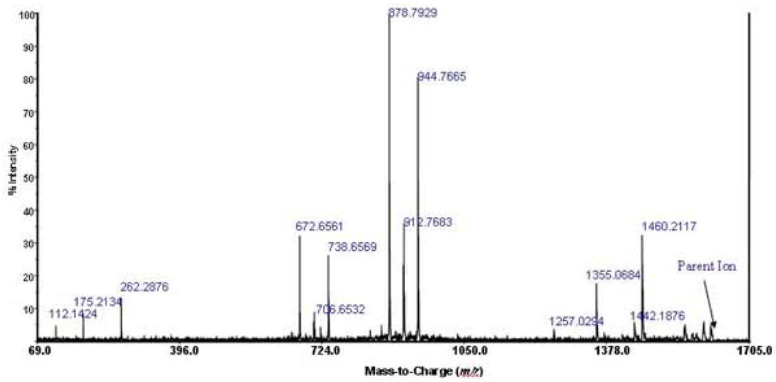
Amino Acid	3 Letter Code	Single Letter Code		Residue Mass	
				Monoisotopic	Average
Glycine	Gly	G		57.02147	57.052
Alanine	Ala	A		71.03712	71.079
Serine	Ser	S		87.03203	87.078
Proline	Pro	P		97.05277	97.117
Valine	Val	V		99.06842	99.133
Threonine	Thr	T		101.04768	101.105
Cysteine	Cys	C		103.00919	103.144
Isoleucine	Ile	I		113.08407	113.160
Leucine	Leu	L		113.08407	113.160
Asparagine	Asn	N		114.04293	114.104
Aspartic Acid	Asp	D		115.02695	115.089
Glutamine	Gln	Q		128.05858	128.131
Lysine	Lys	K		128.09497	128.174
Glutamic Acid	Glu	E		129.04260	129.116
Methionine	Met	M		131.04049	131.198
Histidine	His	H		137.05891	137.142
Phenylalanine	Phe	F		147.06842	147.177
Arginine	Arg	R		156.10112	156.188
Tyrosine	Tyr	Y		163.06333	163.170
Tryptophan	Try	W		186.07932	186.213
Homoserine Lactone				83.03712	83.090
Homoserine				101.04768	101.105
Pyroglutamic acid				111.03203	111.100
Carboxyamidomethyl Cysteine				160.03065	160.197
Carboxymethylcysteine				161.01466	161.181
Pyridylethylcysteine				208.06703	208.284

Peptide Fragmentation

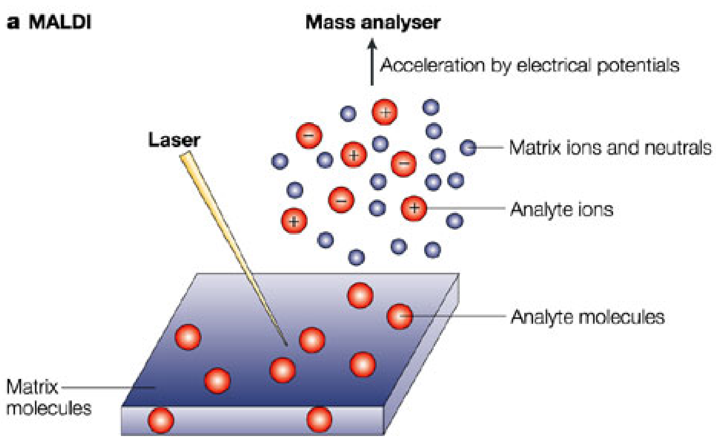


1. Biemann, K., Contributions of mass spectrometry to peptide and protein structure. Biological Mass Spectrometry 1988, 16 (1-12), 99-111.
1. Roepstorff, P.; Fohlman, J., Letter to the editors. Biological Mass Spectrometry 1984, 11 (11), 601.

Sample MS/MS Spectrum acquired on the 4700 TOF-TOF of a Digested Protein from Industry



MALDI Ionization



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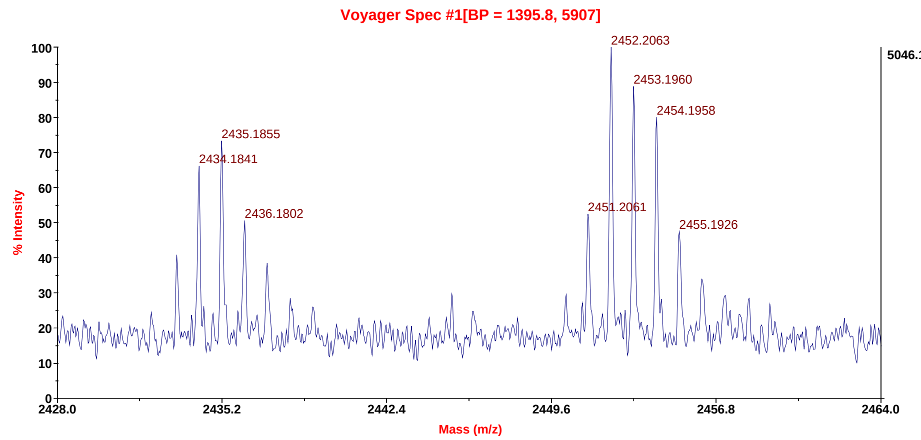
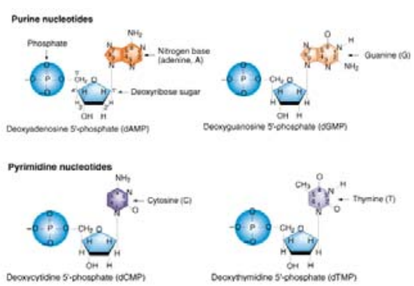
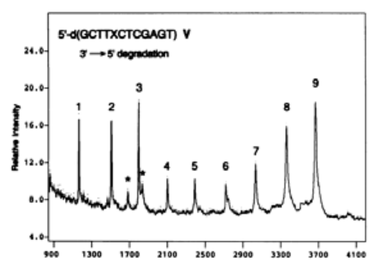
Hanno Steen & Matthias Mann *Nature Reviews Molecular Cell Biology* **2004,** *5*, 699.







Peak	Sequence	Mass calc.	Mass found	Δm
1	5'-d(GCTT)	1163.8	*1163.9*	-0.1
2	5'-d(GCTTX)	1507.1	*1506.9*	0.2
3	5'-d(GCTTXC)	1796.2	*1796.2*	0
4	5'-d(GCTTXCT)	2100.4	*2100.5*	-0.1
5	5'-d(GCTTXCTC)	2389.6	*2389.8*	-0.2
6	5'-d(GCTTXCTCG)	2718.9	*2719.3*	-0.4
7	5'-d(GCTTXCTCGA)	3032.1	*3032.8*	-0.7
8	5'-d(GCTTXCTCGAG)	3361.3	*3362.0*	-0.7
9	5'-d(GCTTXCTCGAGT)	3665.5	*3666.0*	-0.5

Nucleic Acids Research, 1993, Vol. 21, No. 14 3195