The Powers of Using MRM^3 and High Resolution MS with Fast Scan Rates to Conquer Previously Difficult Large Molecule Compounds

Yong-Xi Li, Ph.D.
Executive Director
Medpace Bioanalytical Laboratories
E-Mail: y.li@medpace.com
Phone: (513)226-3185

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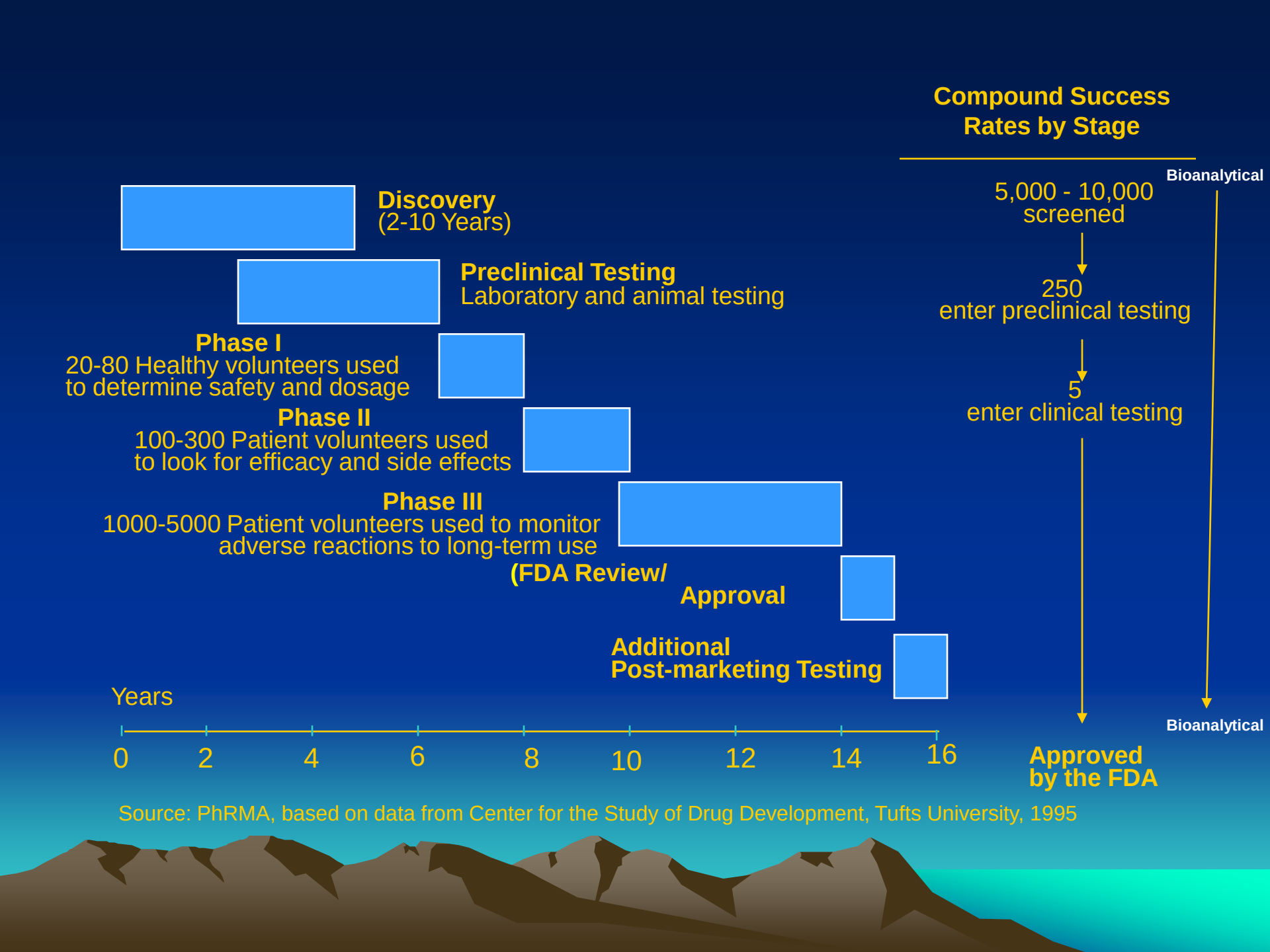


Source: PhRMA, based on data from Center for the Study of Drug Development, Tufts University, 1995

Therefore:

For GLP methods:

Animal Studies: 250 methods → Rat, Dog, Monkey

At least 750 Methods

Clinical Studies: 5 Methods

DRF, BID DRF, Juvenile Rat, 7 day TK, 28 day TK, 13 week TK, Telemetry, Irwin, Micronucleus, Carc. ……..

800,000-1,500,000 Samples need to be analyzed in Bioanalytical Labs

Challenges for Bioanalysis of Large Molecules:

In recent years (30 years), therapeutic large molecules increased significantly, but :

Regulatory: ***GLP, Guidance, White papers…***

Science & Technology:

Sensitivities (for LC-MS/MS), *(Poor Ionization, m/z limitation, Multi-Charge Species, difficult get dominate product ions)*

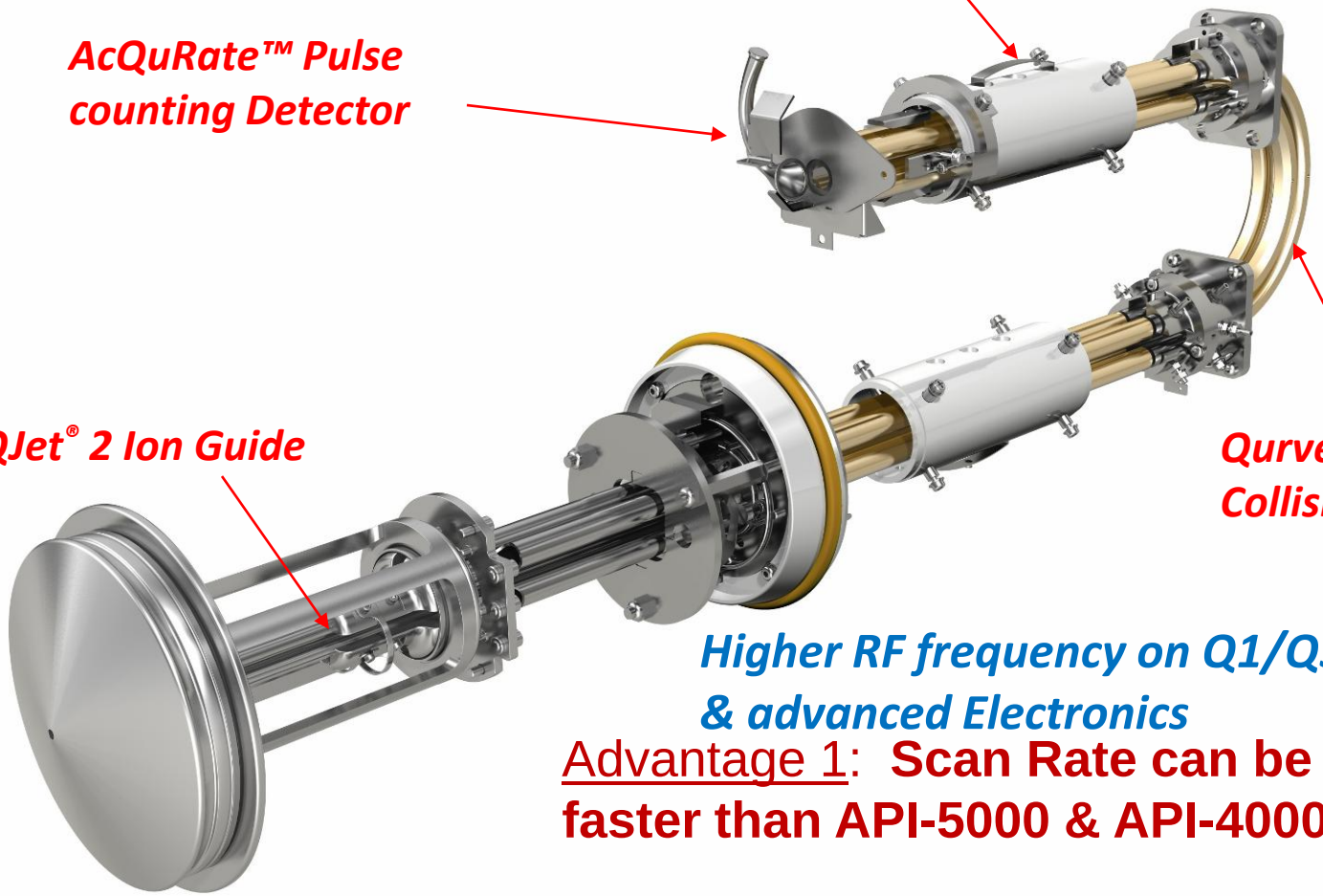
Stabilities, *(blood, plasma, especially pro-drugs);*

Chromatography, *(endogenous, interference, Metabolites)*

Examples: Exenatide (Byetta, MW. 4,187), Glucagon (MW. 3,485) and Salmon Calcitonin (MW. 3,455)

How to solve these challenges easily ? Take advantages of Power of Next Generation……. (API-5500 Q Trap or others)

Ion Rail with a Qurved LINAC® Collision Cell (API-5500 Q Trap)



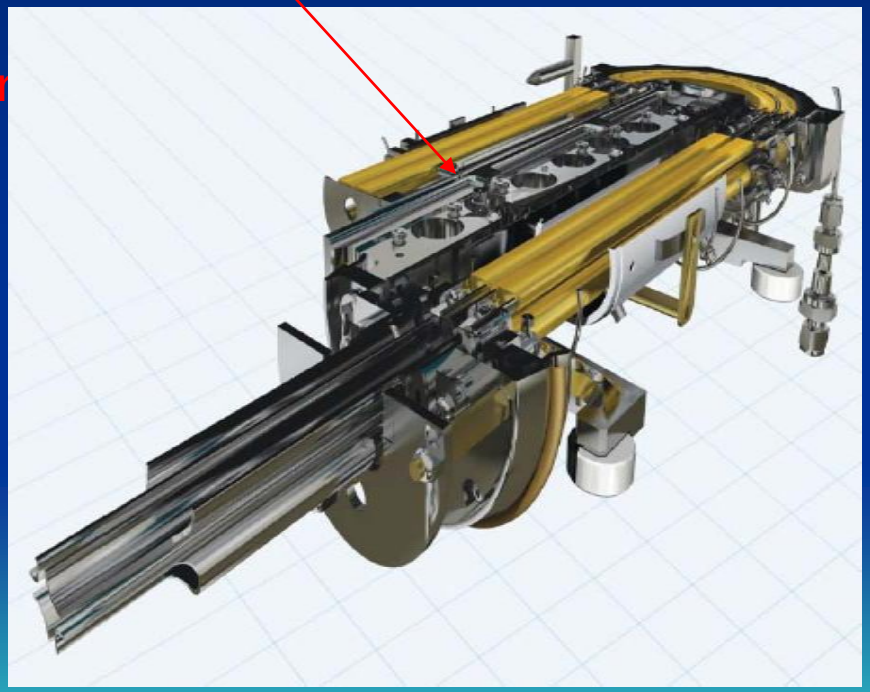
Higher RF frequency on Q1/Q3 rods & advanced Electronics

Advantage 1: **Scan Rate can be much faster than API-5000 & API-4000 !**

Linear Accelerator™ Trap Technology

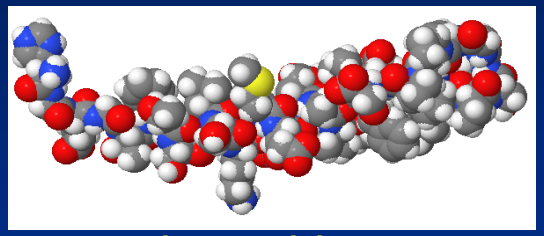
Advantage 2: Speed and Sensitivity in Ion Trap

- **Higher Linear Ion Trap Sensitivities**
  - **Linear Acceleration Ion Trap Technology**
    - **Addition of axial fields *within* the linear ion trap**
    - **Move ions toward the extraction region just before the mass scan**
  - **Fringing Field Modifications**
    - **Addition of properly phased auxiliary RF to the ion extraction region**
  - **More Efficient In-Trap Fragmentation**
- **Faster Scan Speeds and Cycle times**
  - **Scan rates up to 20,000 amu/sec with better than unit resolution.**
    - **Shorter in-trap fragmentation times**

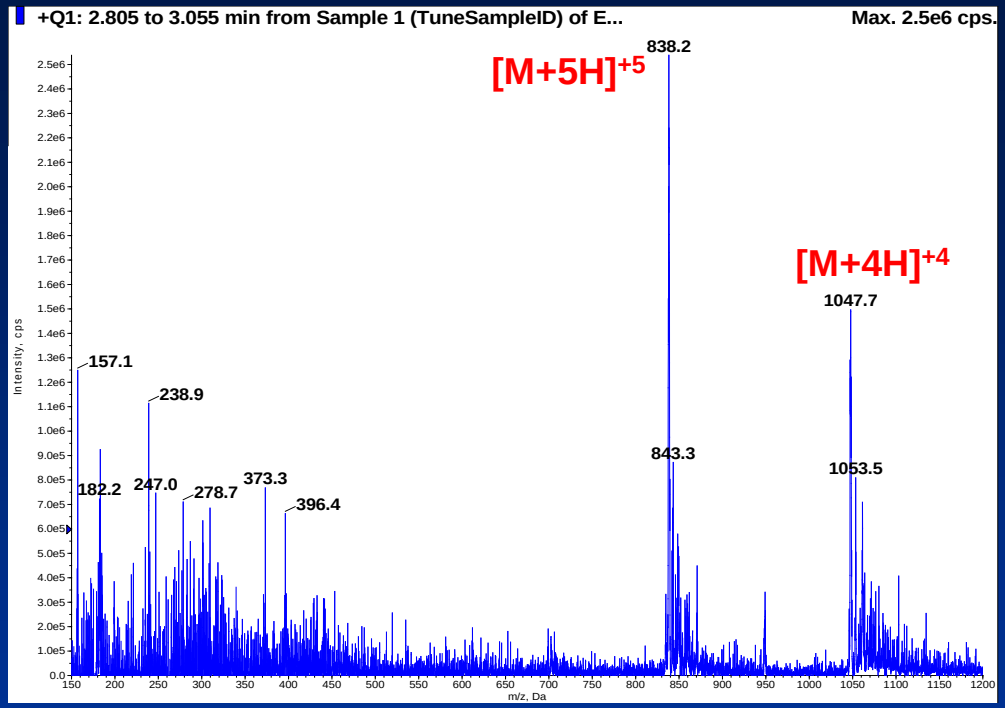


Sensitivity

Exenatide (Byetta)

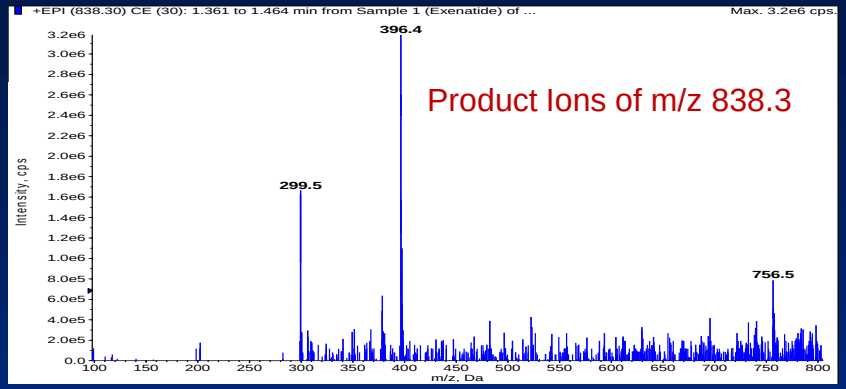


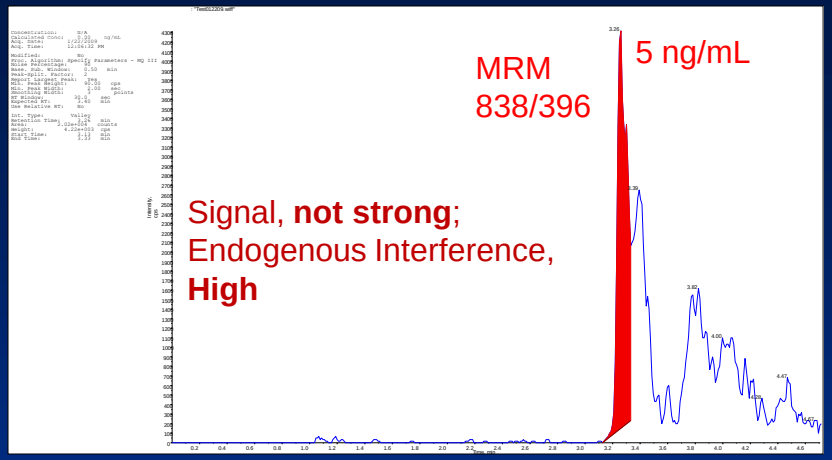
39 amino acids:
MW: 4186.6 Da



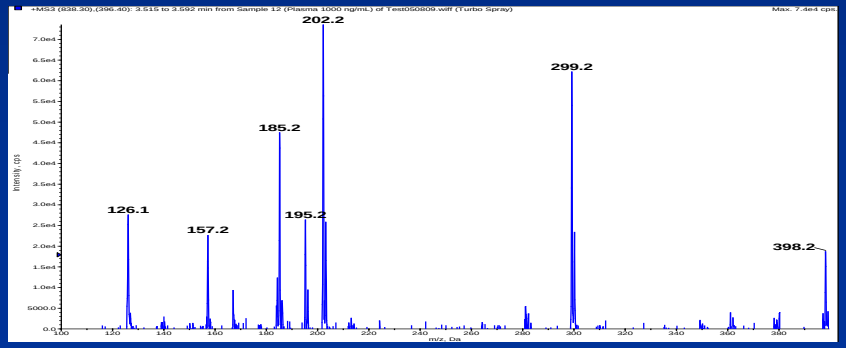
Lose Ions;
Plus: Endogenous Compounds Interferences

Exenatide

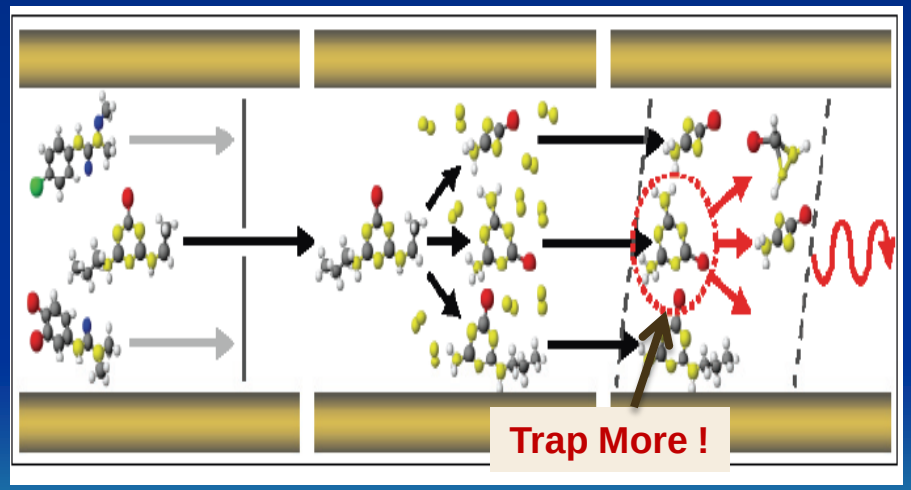


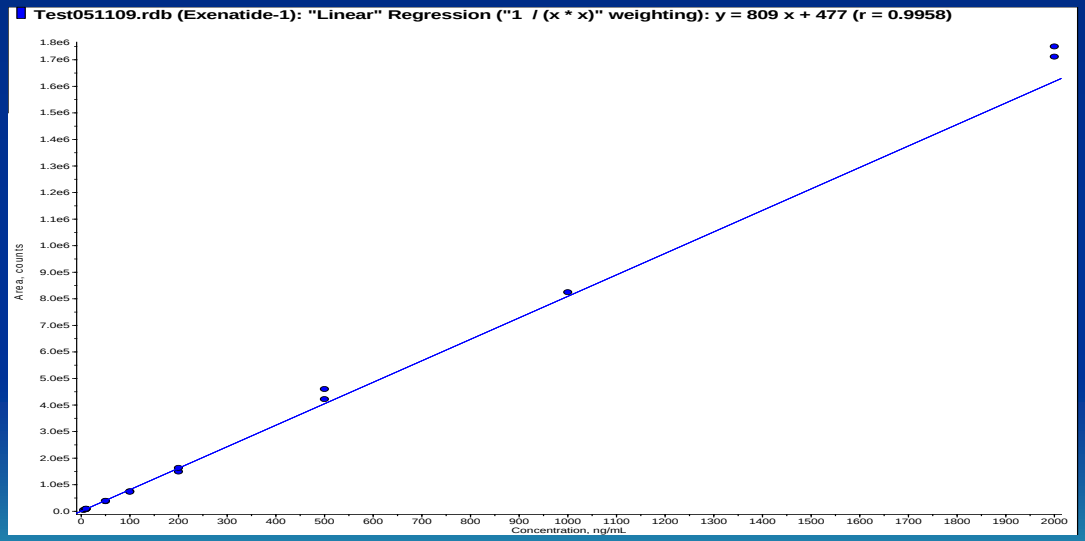


Accumulate Ions in Trap (API-5500 Q Trap) to Enhance the Signal (<u>note:</u> Fast Scan Rate with good fill time !)
MRM^3 to get rid of Interference.



MRM^3





	LLOQ	LQC	MQC	MHQC	HQC
Conc (ng/mL)	5.00	15.0	50.0	800	1800
	5.14	15.9	46.4	779	1650
	4.32	16.9	47.2	767	1549
	5.65	12.0	41.7	821	1521
	4.54	13.5	43.7	729	1641
	3.69	17.1	50.0	658	1745
	4.22	17.4	45.3	751	1672
Mean	4.59	15.5	45.7	751	1630
SD	0.701	2.22	2.85	54.8	82.4
CV	15.3%	14.3%	6.2%	7.3%	5.1%
RE	-8.2%	3.2%	-8.6%	-6.1%	-9.5%

Method in Clinical Sample Analysis: LLOQ is 0.1 ng/mL

Comparison of Sample Analysis Data for Exenatide in K_2EDTA Human Plasma by LC-MS/MS & ELISA Methods

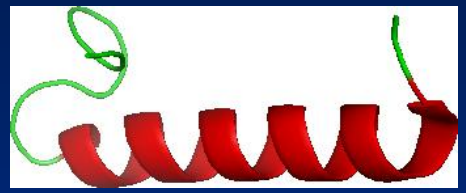
Sample #	Spiked Conc. (ng/mL)	Calculated Conc. By LC-MS/MS (ng/mL)	Accuracy (%)	Calculated Conc. by ELISA (ng/mL)	Accuracy (%)
Sample 1	1	0.960	96	1.42	142
Sample 2	1	0.915	92	1.34	134
Sample 3	1	0.916	92	1.49	149
Sample 4	6	5.43	91	8.80	147
Sample 5	6	6.20	103	9.80	163
Sample 6	6	6.09	101	7.90	132
Sample 7	25	24.1	96	31.5	126
Sample 8	25	26.5	106	30.9	124
Sample 9	25	20.7	83	32.9	131
Sample 10	50	49.2	99	61.5	123
Sample 11	50	50.8	102	54.6	109
Sample 12	50	52.2	104	60.0	120
Sample 13	200	221	111	260	130
Sample 14	200	226	113	255	128
Sample 15	200	224	112	279	140
Sample 16	400	376	94	488	122
Sample 17	400	401	100	464	116
Sample 18	400	403	101	444	111
		Mean	**99.8**		**130.4**

1) In general, Results from ELISA are higher which may be from cross-reactions with Exendin-4 (3-39), GLP-1 or Glucagon…..
2) Exenatide kit from AB BioLabs Inc. (Cat#: SEK 0130-01B).

Power 1:

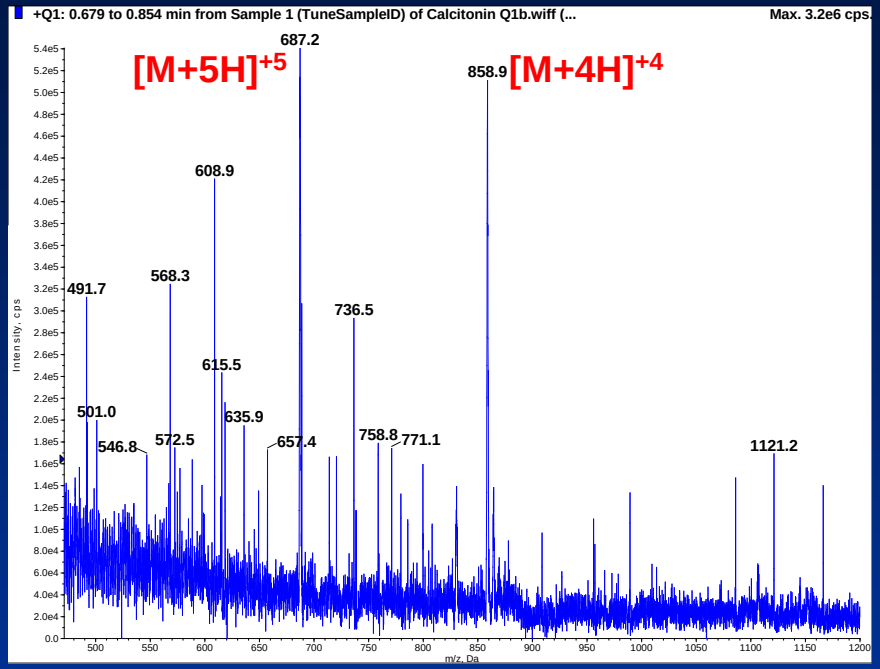
On API-5500 Q Trap: Take advantage of Fast Scan rate with appropriate fill time in Trap to Enhance Ion Intensity (Sensitivity) & Reduce Background to increase S/N ratio as well by MRM^3.

Salmon Calcitonin:

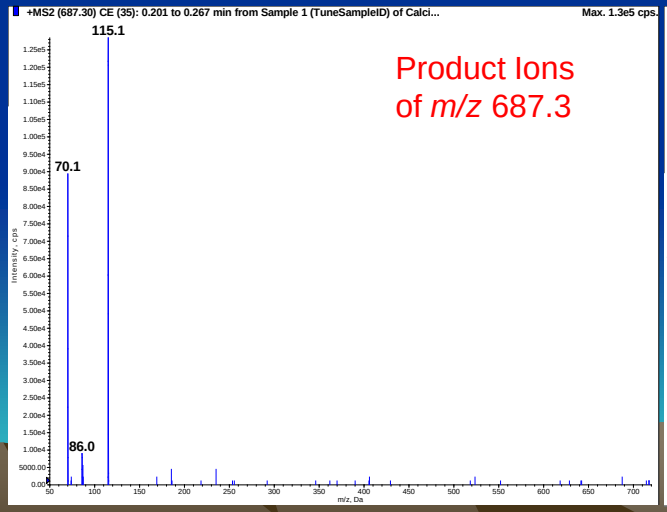


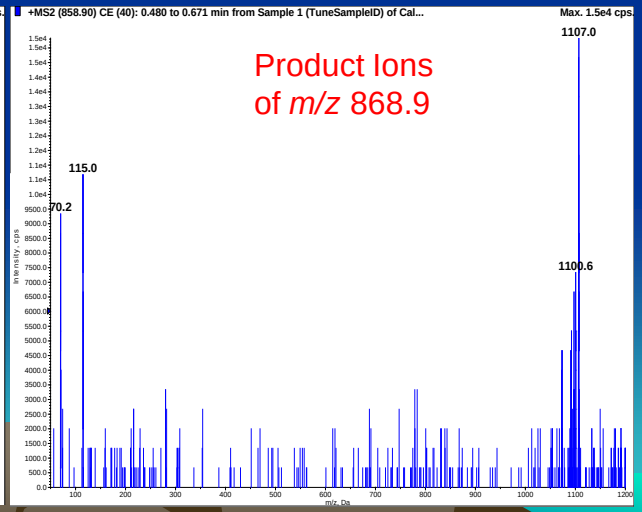
Calcitonin is a 32-amino acid linear polypeptide hormone produced by the C-cells

MW. 3,455 Da



1) Multi-Charge Ions; 2) Week ionization

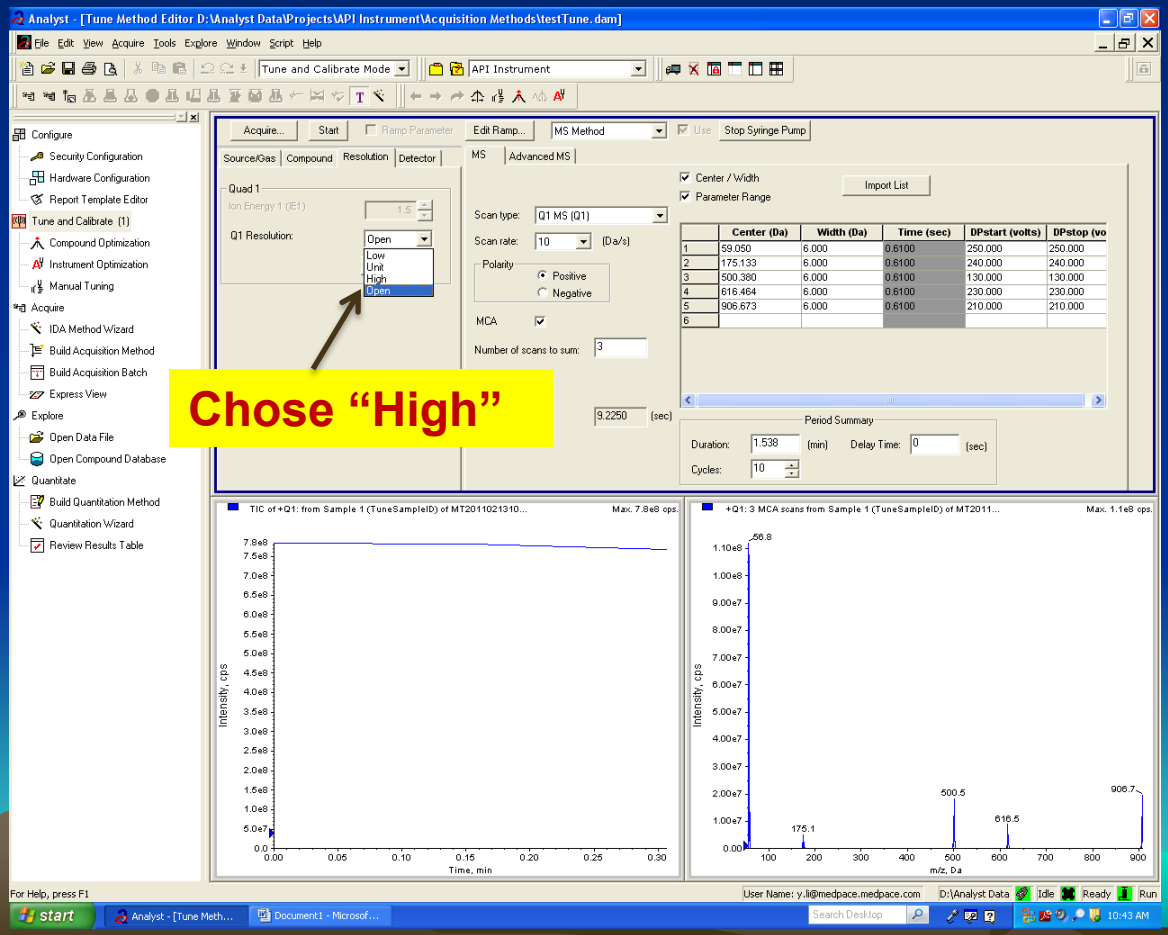


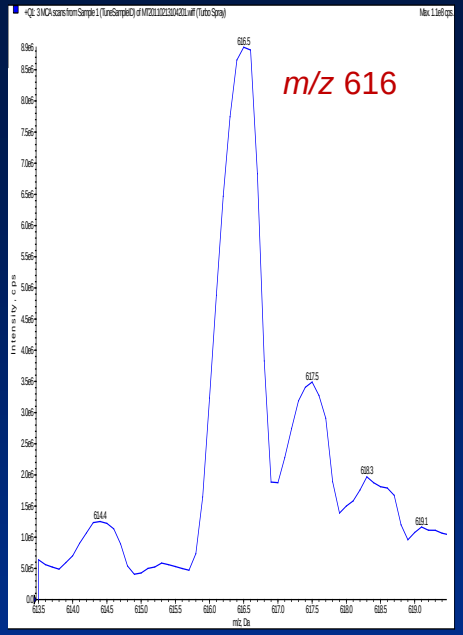


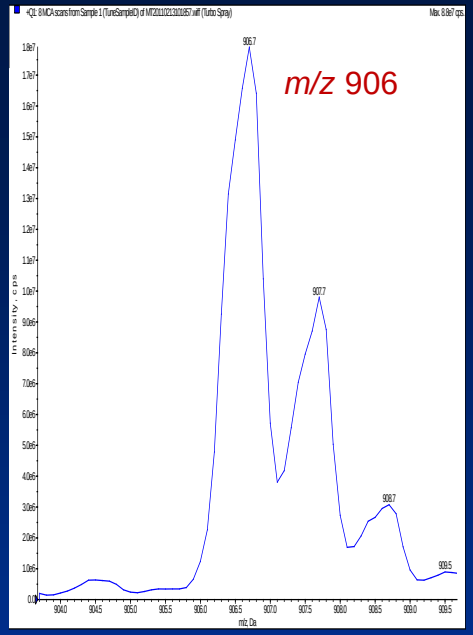
1) Week Product Ions; 2) Small fragment ions (no dominate ions; no grand daughters)

Therefore: in these cases, MRM^3 can not be used; the "POWER 2" can be considered (Take advantage of high resolution with fast scan rate of API-5500 Q Trap)

But, here "High Resolution" is not from TOF instruments for Accurate Mass Determination ! Not MRM^{HR}



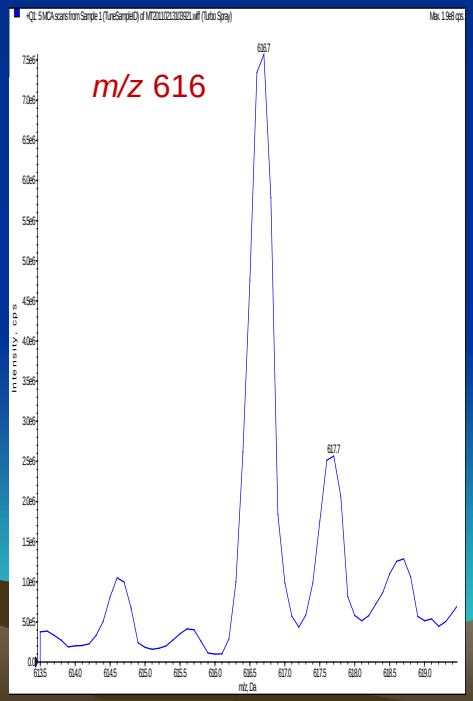


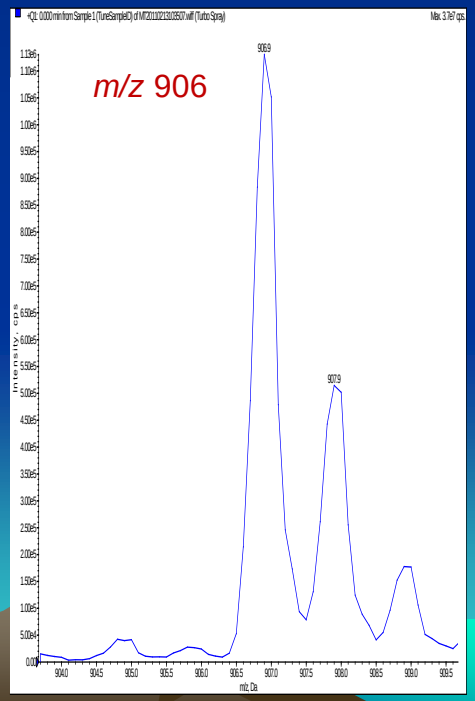


Unit Resolution

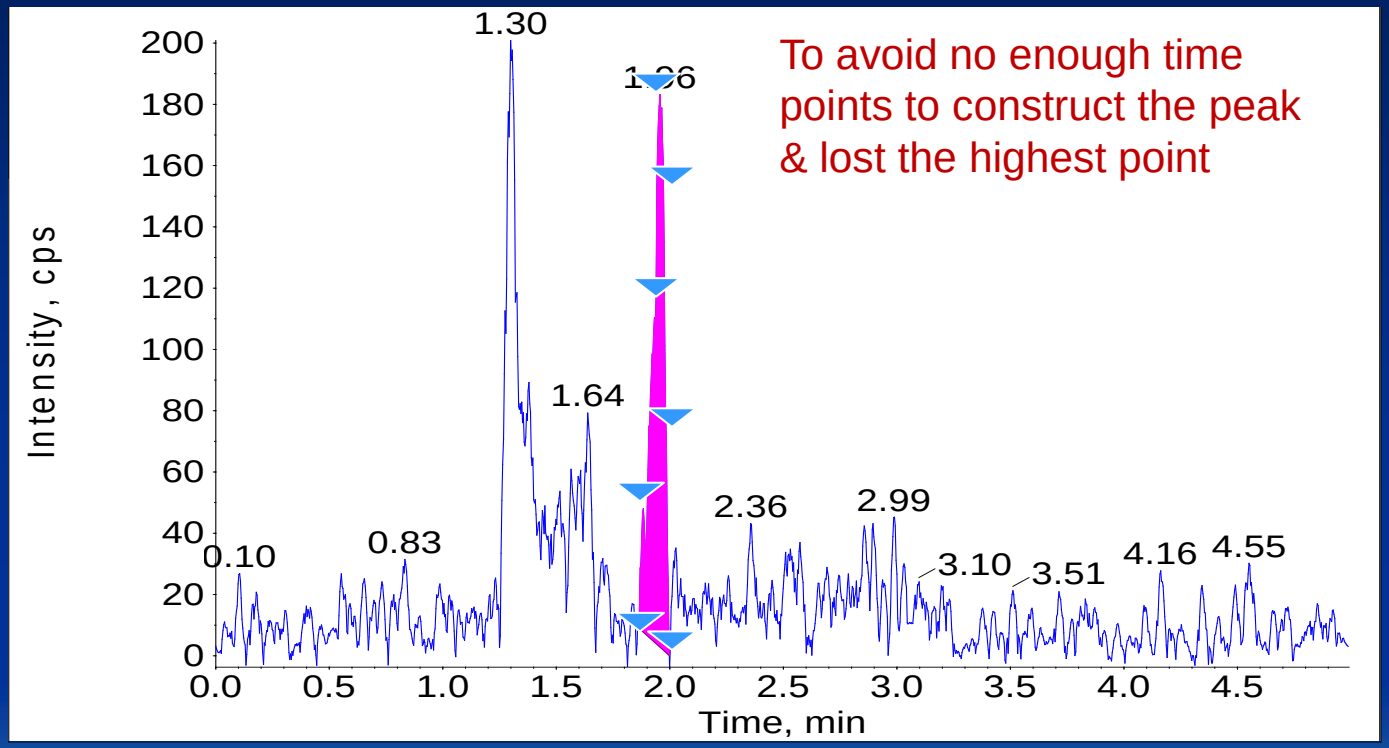
High Resolution

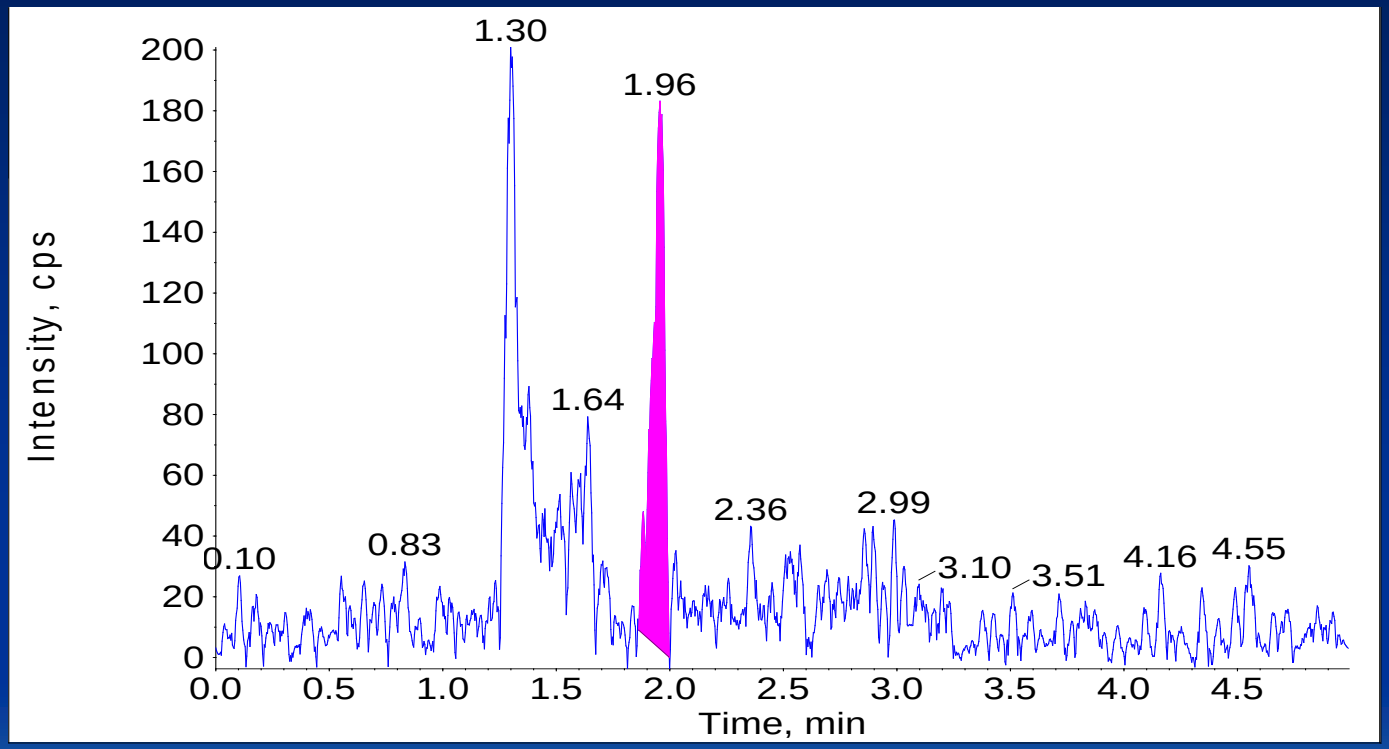
<u>Note:</u> Need tuning high resolution voltages, not default numbers

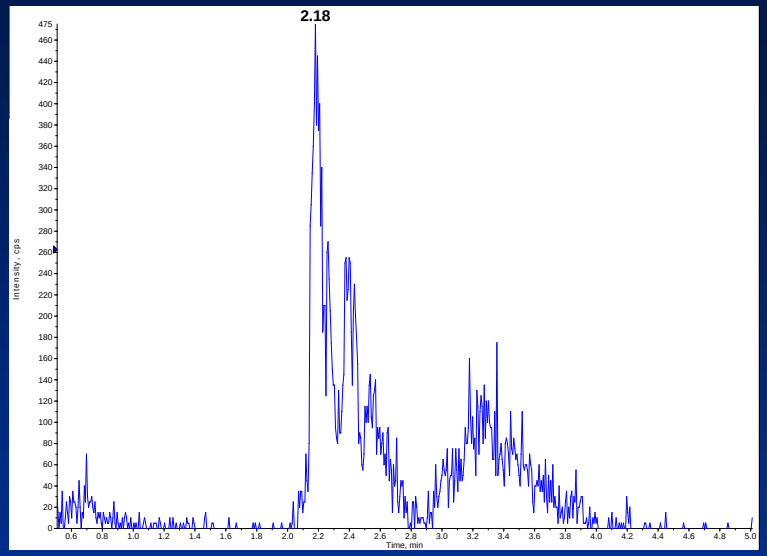




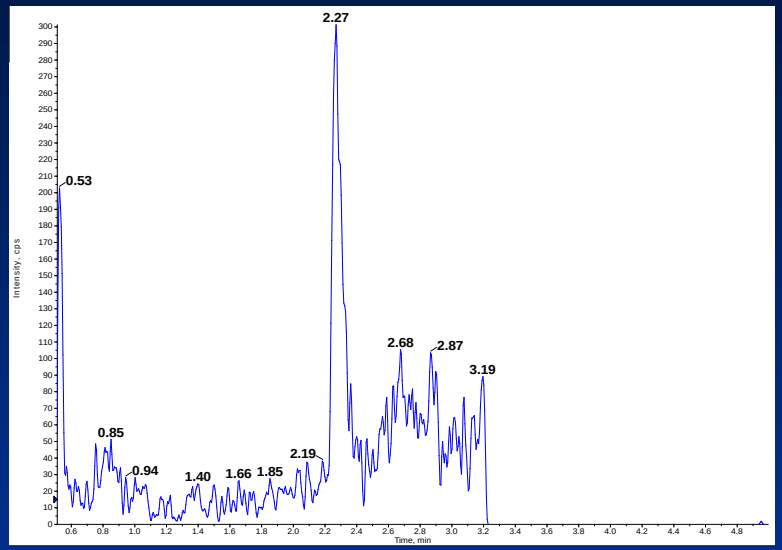
Note: Need carefully chose **"Fast"** scan rate combines with the "high resolution" (or Why do we need "Fast" scan rate with "High" resolution?)



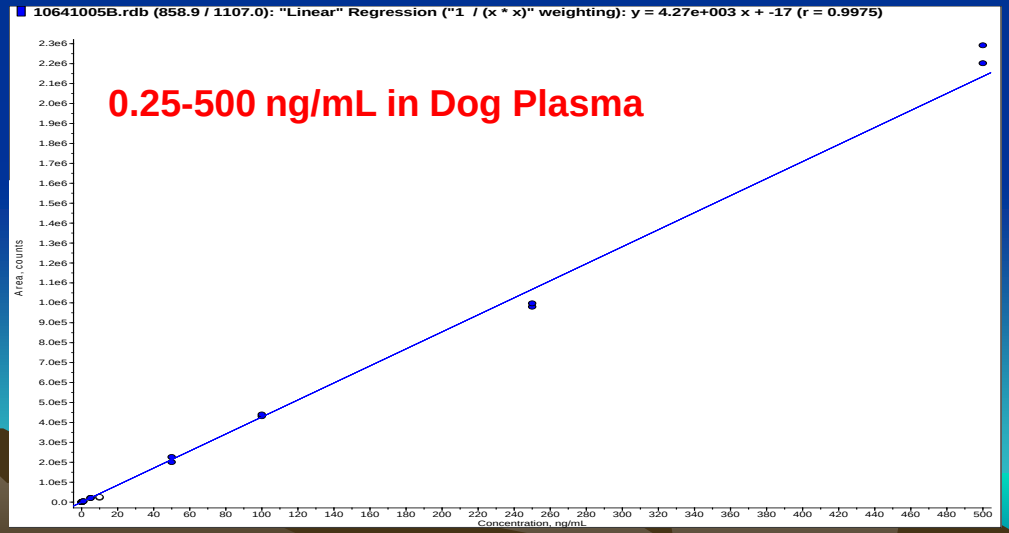

Intensity, cps
200
180
160
140
120
100
80
60
40
20
0
1.30
1.96
1.64
2.36
2.99
0.10
0.83
3.10
3.51
4.16
4.55
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
Time, min



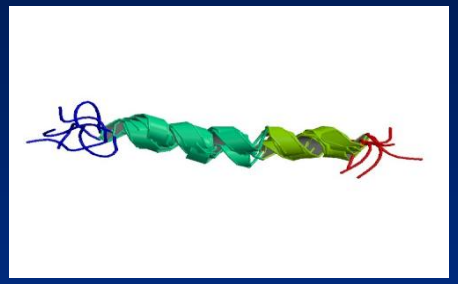
Unit Resolution



High Resolution

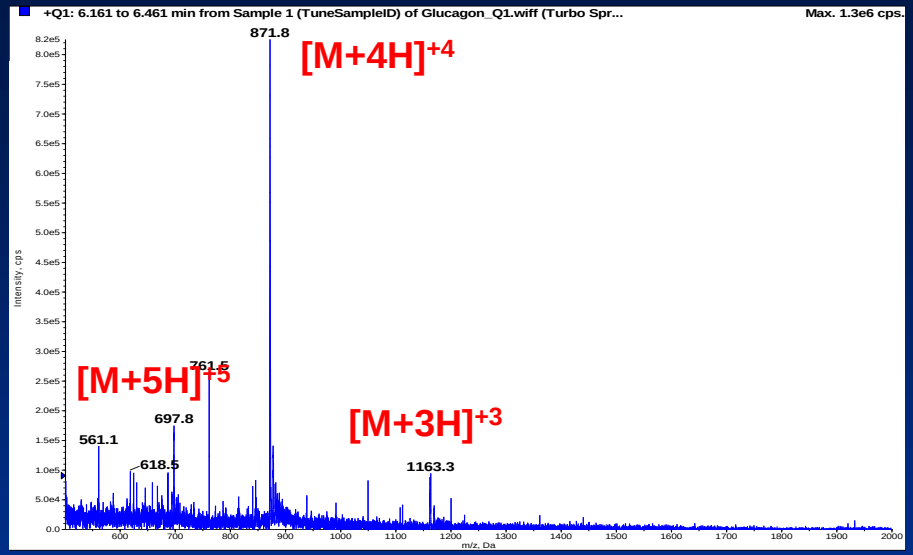


Glucagon (GlucaGen)

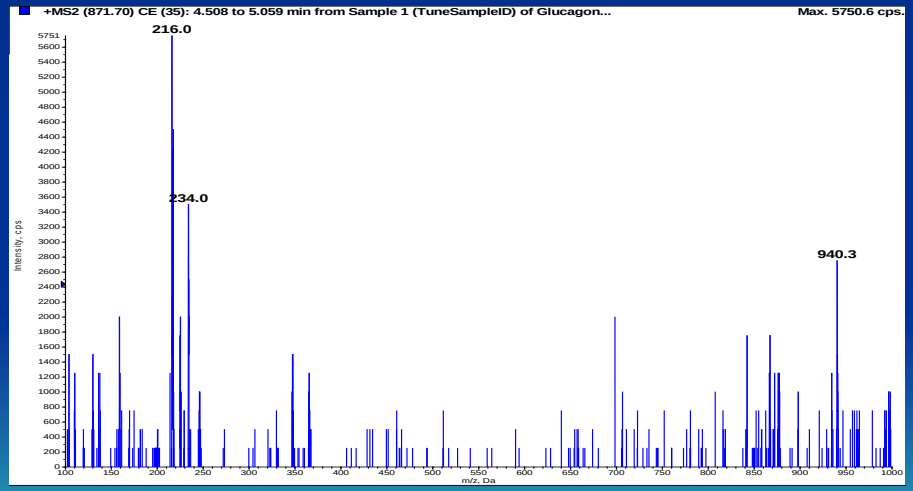


A single-chain polypeptide containing 29 amino acid residues

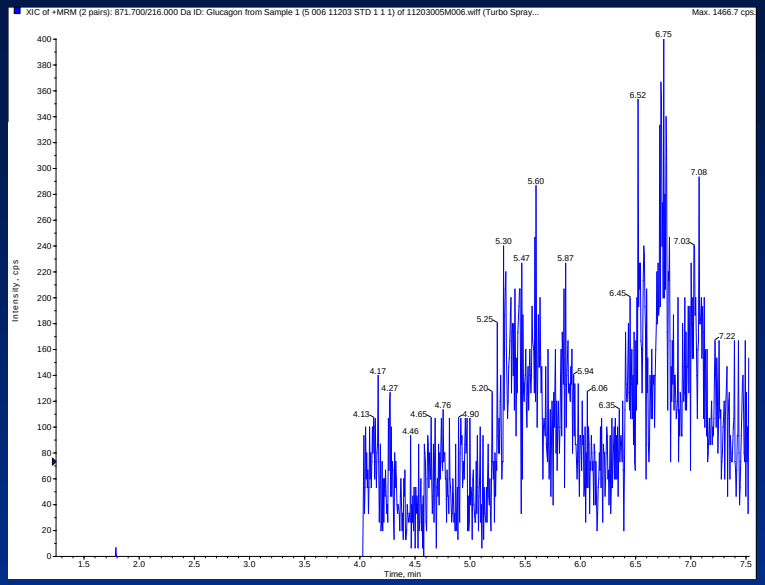
MW. 3483

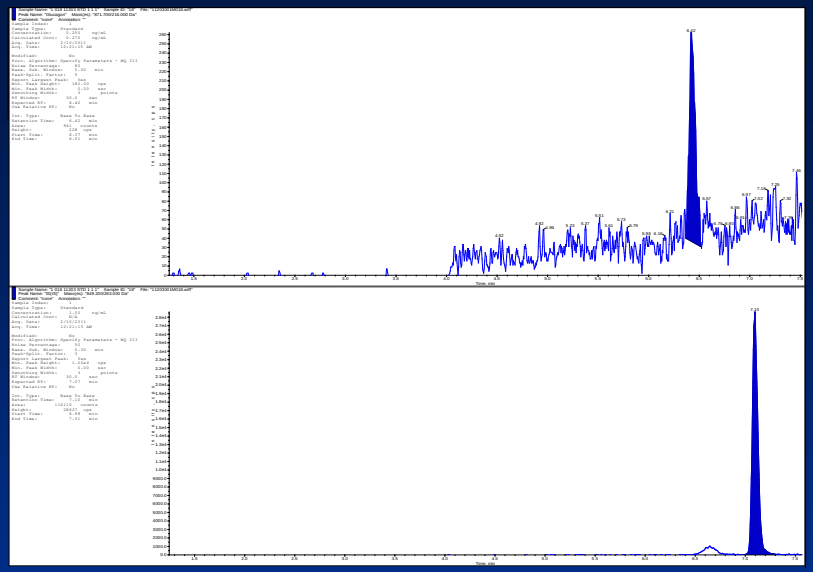


Multi-Charge Ions

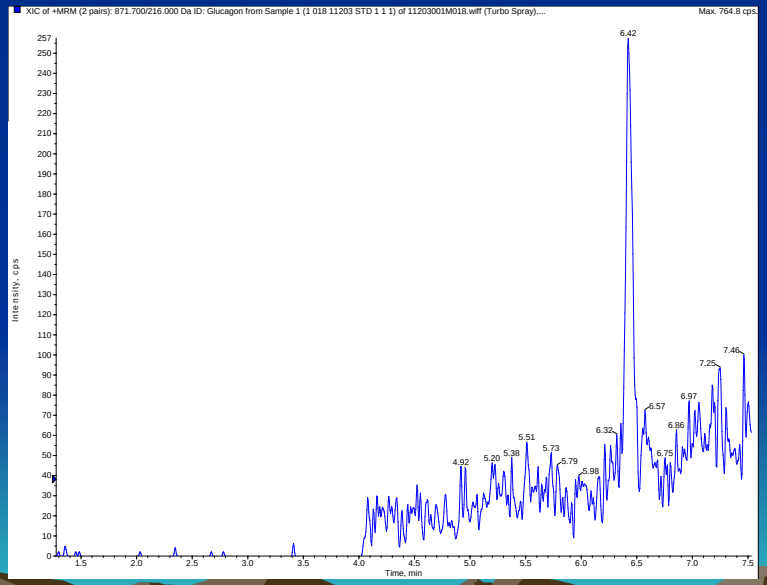


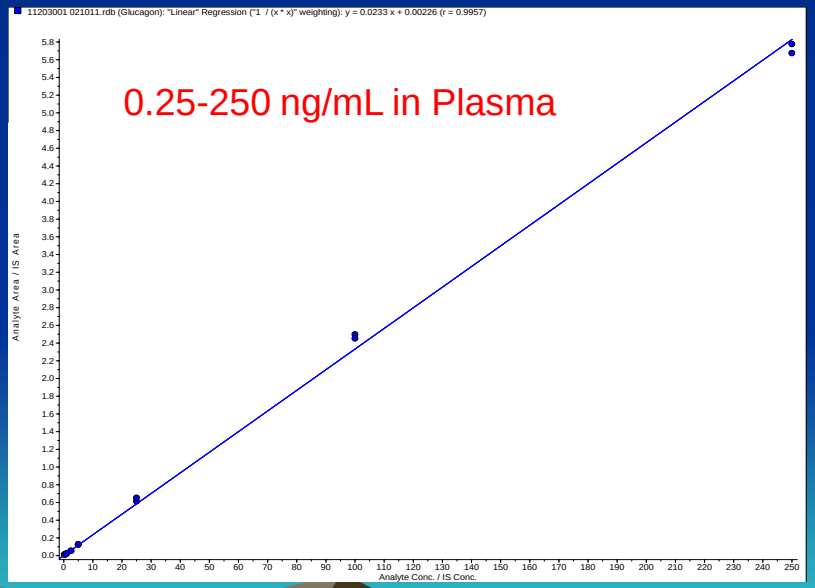
Poor Product Ions, For sure grand daughter even worse !, i.e. MRM^3 does not work.





Unit Resolution





High Resolution with fast scan

Comparison of Sample Analysis Data of Glucagon in K_2EDTA Human Plasma by LC-MS/MS & ELISA

Sample #	Spiked Concentration (ng/mL)	Calculated Conc. By LC-MS/MS (ng/mL)	Accuracy (%)	Calculated Conc. By ELISA (ng/mL)	Accuracy (%)
Sample 1	0.25	0.216	**86**	0.30	**120**
Sample 2	0.25	0.292	**117**	0.29	**116**
Sample 3	0.5	0.428	**86**	0.56	**112**
Sample 4	0.5	0.565	**113**	0.56	**112**
Sample 5	0.75	0.802	**107**	0.86	**115**
Sample 6	0.75	0.759	**101**	0.87	**116**
Sample 7	1	0.829	**83**	1.05	**105**
Sample 8	1	0.96	**96**	1.04	**104**
Sample 9	2.5	2.23	**89**	2.93	**117**
Sample 10	2.5	2.26	**90**	3.01	**120**
Sample 11	5	5.24	**105**	5.00	**100**
Sample 12	5	5.39	**108**	4.92	**98**
Sample 13	25	26.2	**105**	27.2	**109**
Sample 14	25	27.5	**110**	26.7	**107**
		Mean	**99.7**		**111**

1) Two method results acceptable, but ELISA had high end data may be due to cross-reactions with Insulin, Proinsulin, GLP-1, GLP-2 etc.;
2) ELISA kit from Peninsula Labs, LLC (Cat. No. S-1211)

Power 2:

On API-5500 Q Trap: Take advantage of Fast Scan rate with appropriate “High Resolution” in Q1 & Q3 to Reduce Background & Interference to increase S/N ratio by high resolution MRM.

Limitations:

Conclusion:

Take advantages of High Scan Rates & Trap more Ions to overcome the challenges in Sensitivities, Interferences and chromatography area, in some cases, to establish Reliable Bioanalytical Methods to meet regulatory & Science needs.

Thanks & Questions