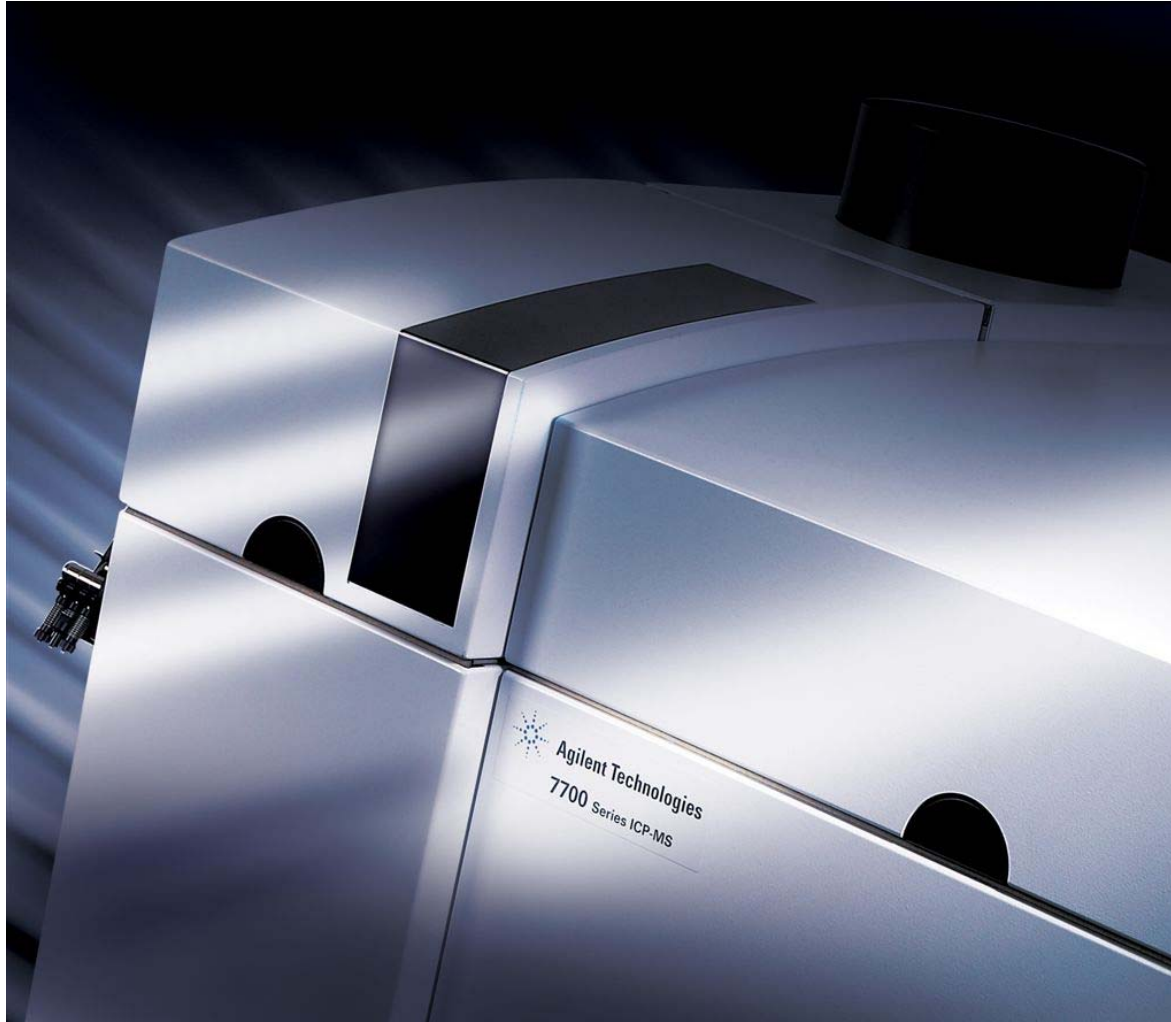


# Analytical Challenges in the Determination of Metals in Nutritional Supplements



***Emmett Soffey  
Agilent Technologies  
AOAC 10/7/2011***

# Why Measure Metals ?

- Two Main Reasons

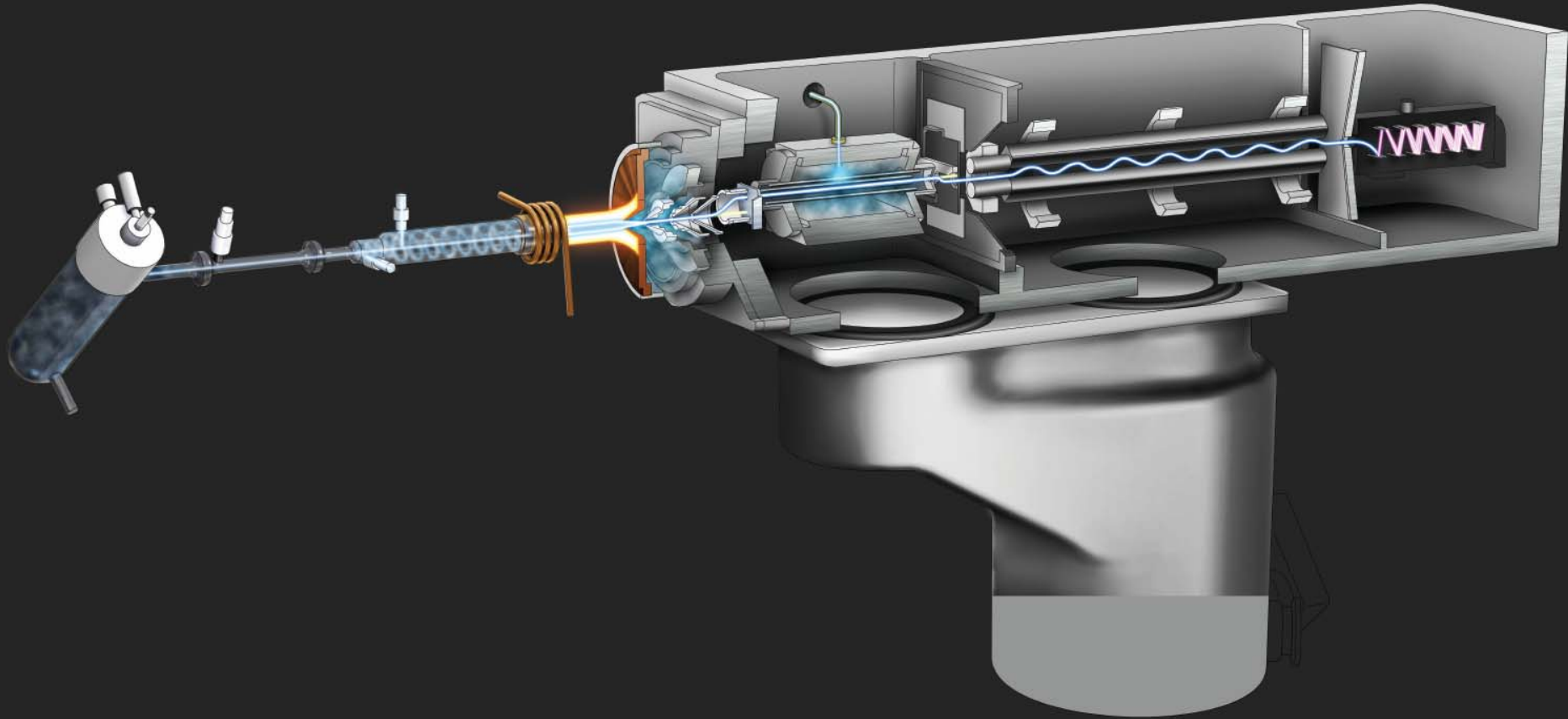
- Regulatory Requirements for Toxic Metals: Prop 65 (As, Cd, Hg, Pb)
- Determination of % Composition or Major Constituents within the Sample.

-Therefore, must be able to measure trace toxic elements at very low levels, with as little dilution as possible, and at the same time be able to measure high level components such as Ca, P, Cr.....

# Analytical Challenges

- Removal of Interferences.
- Measuring Low and High Concentrations Simultaneously (sensitivity and LDR).
- Washout and carry over issues.
- Minimal Dilution for Lowest Detection Limits.
- Controlling Contamination (Prop 65, Pb).
- Speciation

# ICP-MS Overview



# Samples Must be Digested or Solubilized in Acid Prior to Analysis

-Some samples will dissolved readily in dilute acids. However, most require some form of digestion.

## ■ Microwave

Parameter	
<b>Microwave Oven</b>	
<b>Digestion</b>	
Sample weight	0.2g
HNO <sub>3</sub>	1mL
HCl	0.25mL
H <sub>2</sub> O <sub>2</sub>	0.5mL
De-ionized Water	3.5mL
<b>Oven Program</b>	
Pre-digestion (room temperature)	15 minutes
Ramp (to 1200W, 150°C)	15 minutes
Hold (at 1200W, 150°C)	10 minutes
Cool down	15 minutes
<b>Final Dilution</b>	
De-ionized Water	To 50mL
Total dilution factor	250x

## ■ Open Vessel

Hot Block or Open Vessel Digestion			
Sample Wt		0.5 g	
HNO <sub>3</sub>		10 mls	
HCl		5 mls	
H <sub>2</sub> O		~20 mls	
Digest Temp		95 Deg	
Reflux down to 10 mls, add H <sub>2</sub> O, reflux to 10 again.			
Remove and cool. Add peroxide.			
Dilute to volume with H <sub>2</sub> O:		50 mls	
Dilution Factor: 100x			

\*Peroxide is added to remove organics.

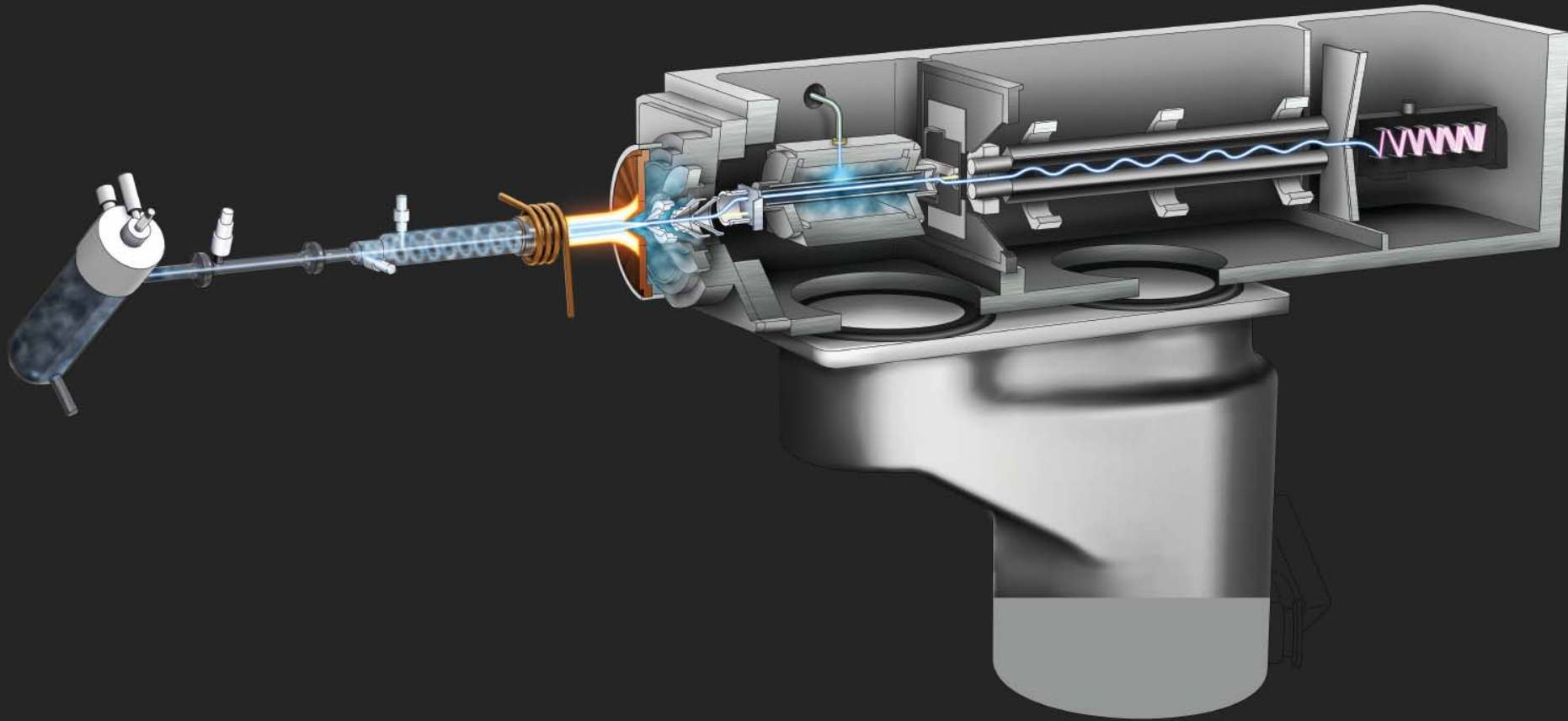
\*\*HCl is added to stabilize Hg.

# Removal Of Interferences

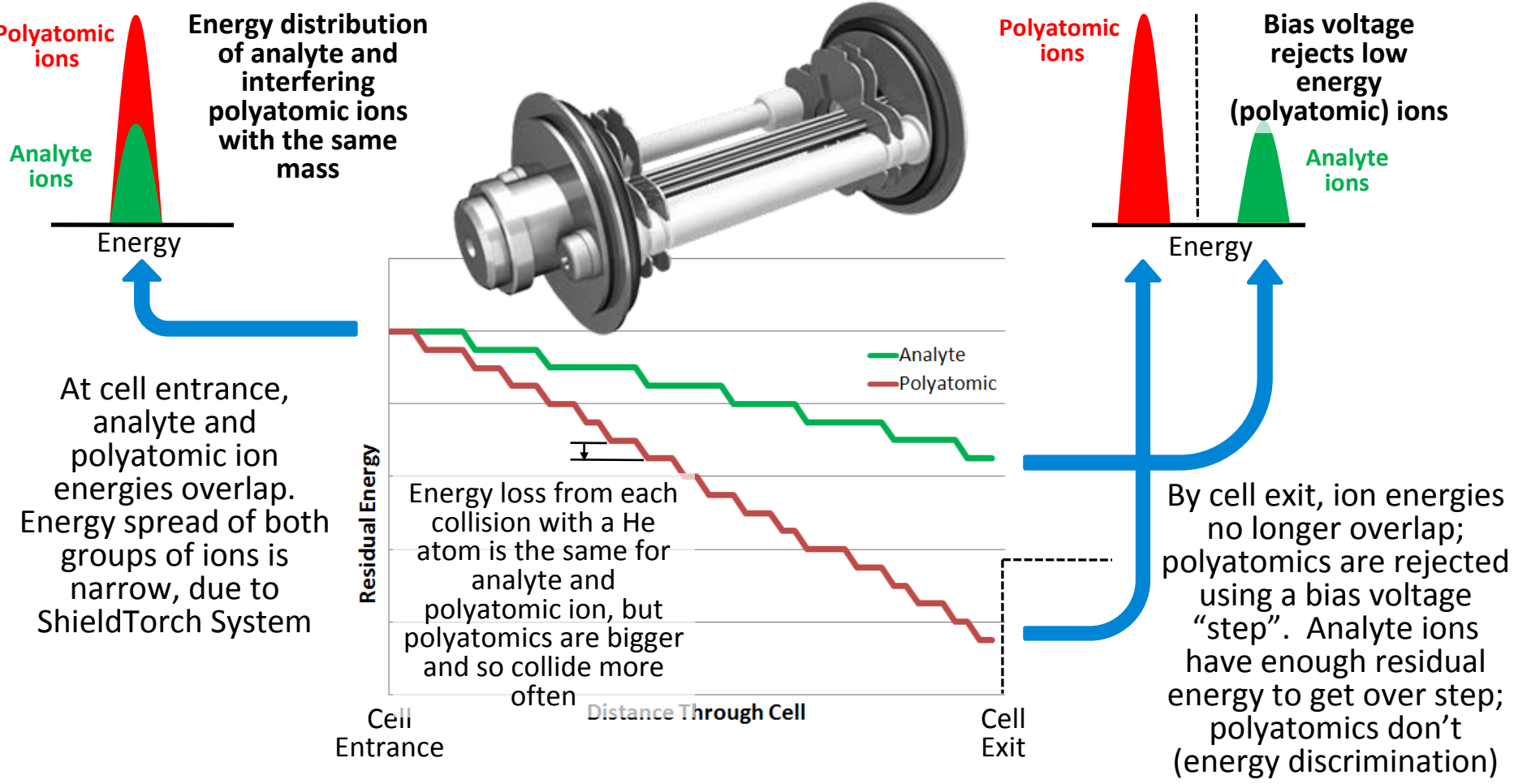
# Troublesome Region of the Periodic Table

H																	He
Li	Be										B	C	N	O	F	Ne	
Na	Mg										Al	Si	P	S	Cl	Ar	
K	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr
Rb	Sr	Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe
Cs	Ba	L	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rn
Fr	Ra	A															
		L	La	Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
		A	Ac	Th	Pa	U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lr

# ICP-MS Overview



# Principle of Helium Collision Mode and Kinetic Energy Discrimination (KED)



# Helium Collision Mode: Energy Discrimination

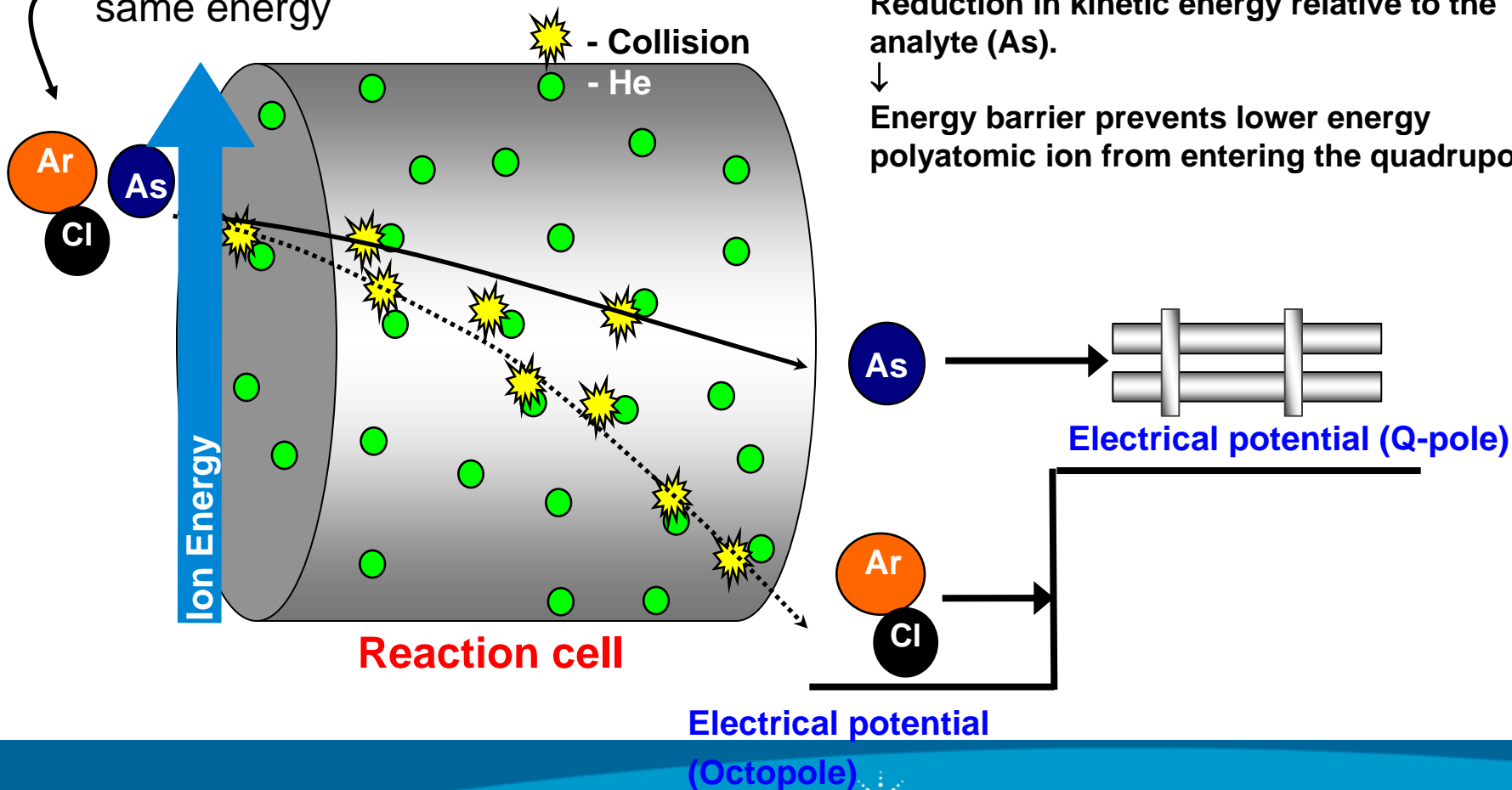
Molecular ion (ArCl) has larger cross section than the analyte at same mass (As).

↓  
More frequent collisions with He.

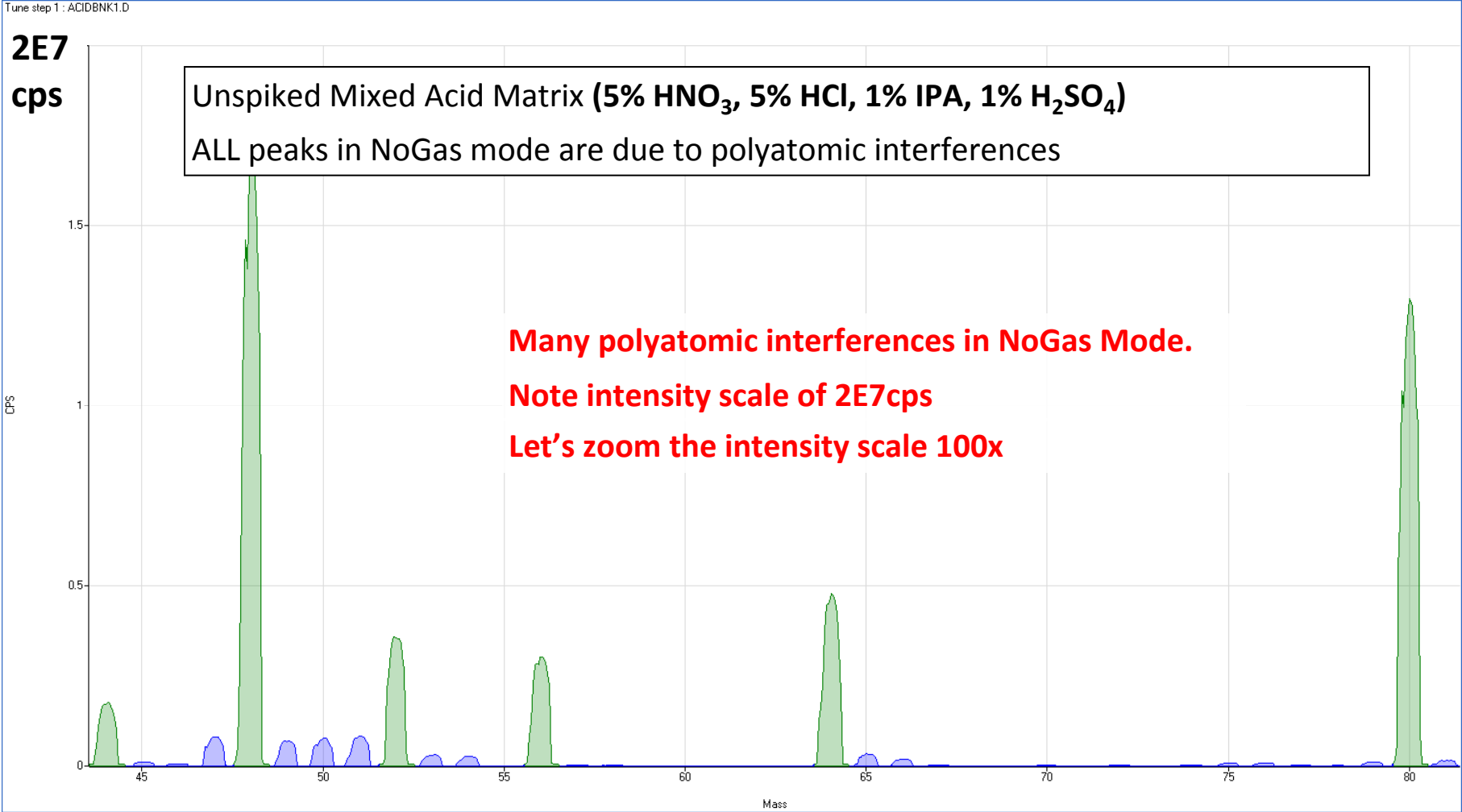
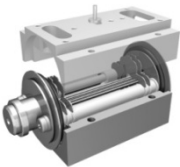
↓  
Reduction in kinetic energy relative to the analyte (As).

↓  
Energy barrier prevents lower energy polyatomic ion from entering the quadrupole

As (monatomic ion) and ArCl (polyatomic ion) – enter cell with same energy

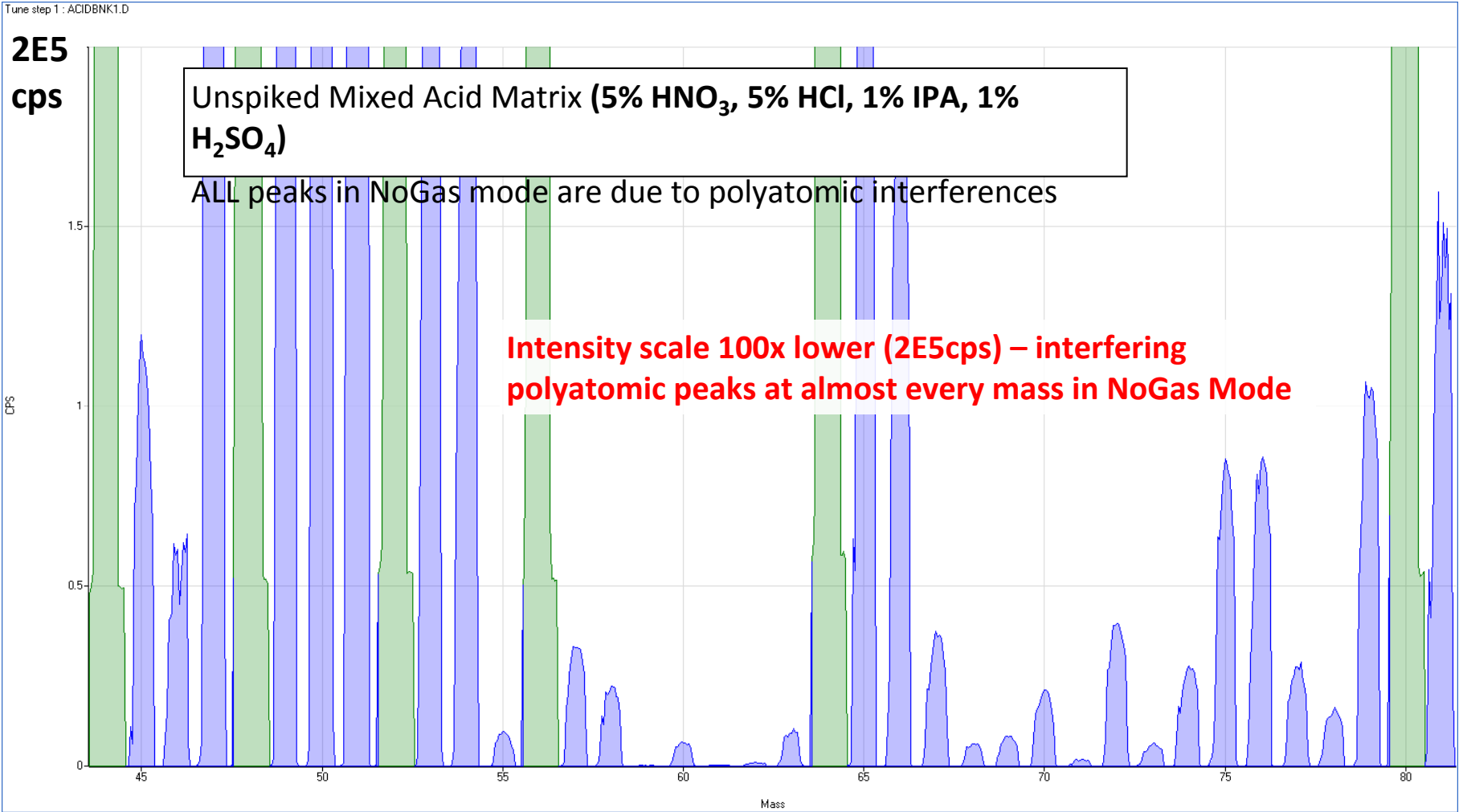
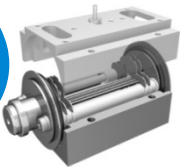


# Mixed Acid Matrix in NoGas Mode



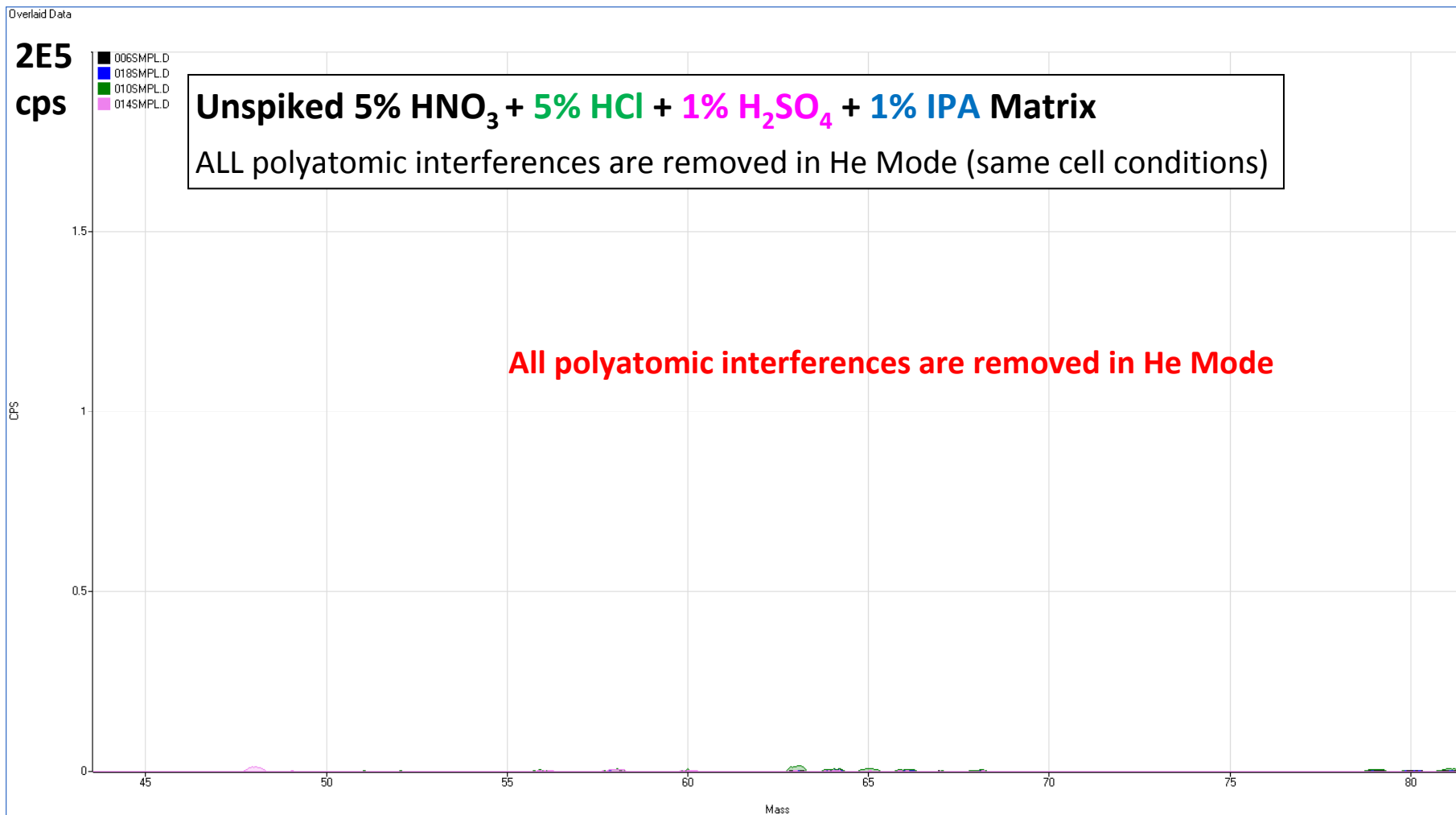
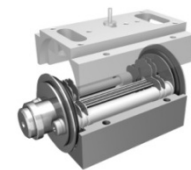
NoGas Mode

# Mixed Acid Matrix in NoGas Mode (100x Zoom)



NoGas Mode

# Single Acid Matrices and IPA in He Mode ( $\text{HNO}_3 + \text{HCl} + \text{H}_2\text{SO}_4 + \text{IPA}$ ) – same scale as NoGas

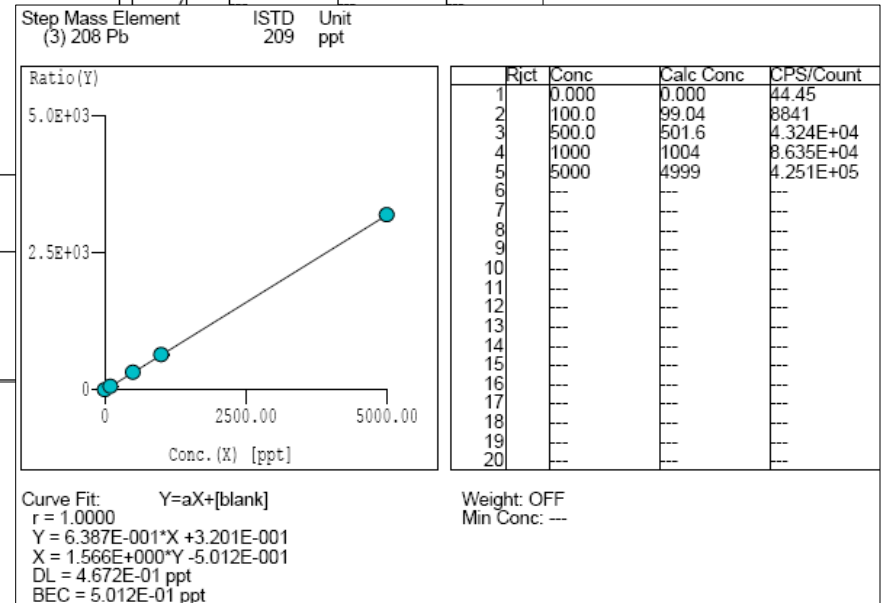
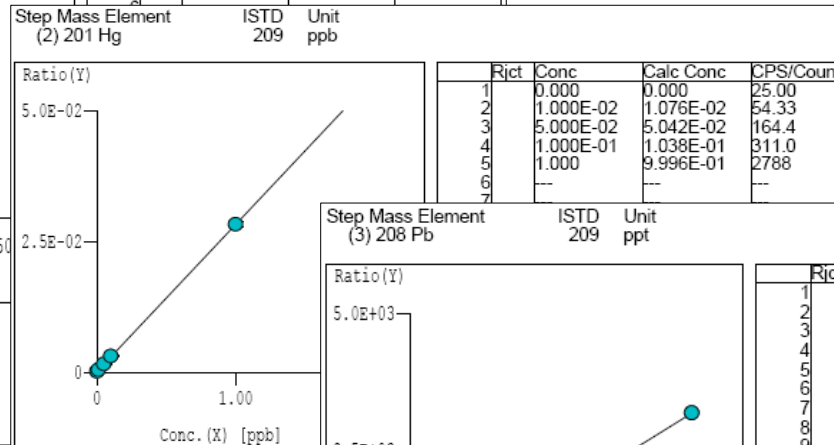
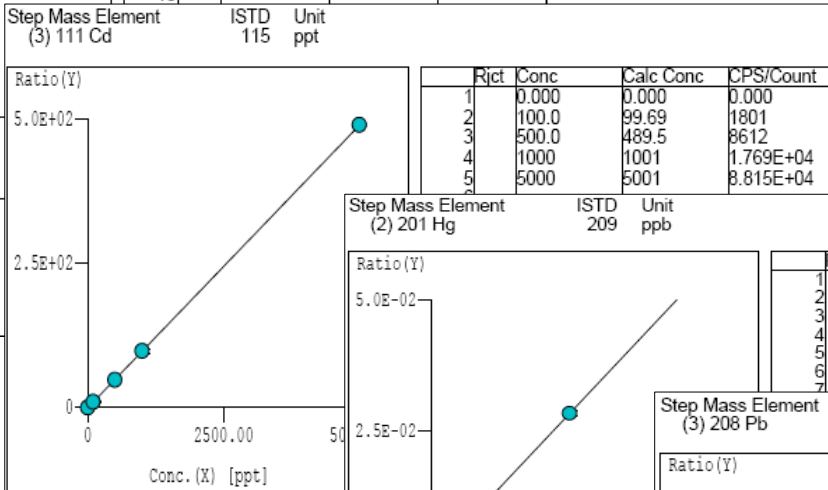
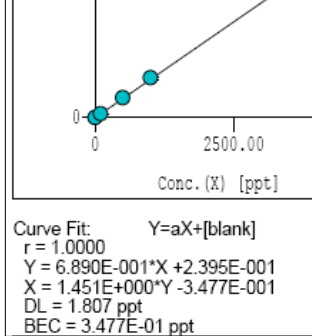
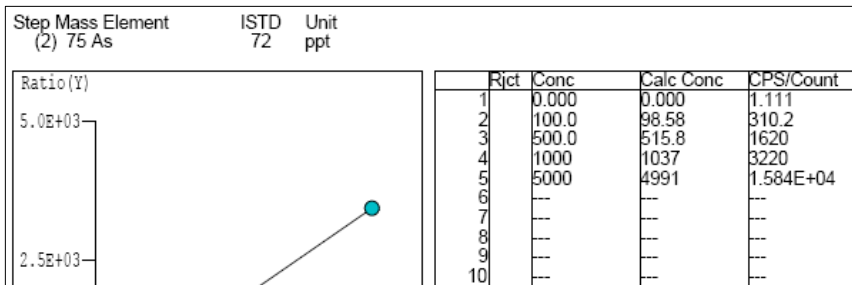


He Mode



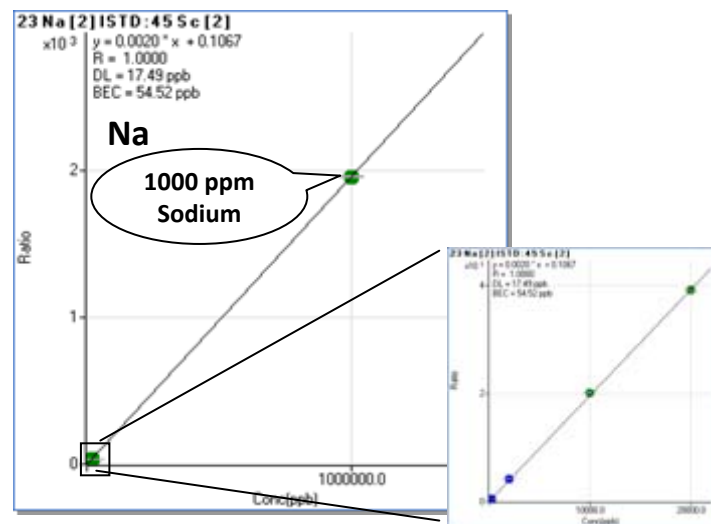
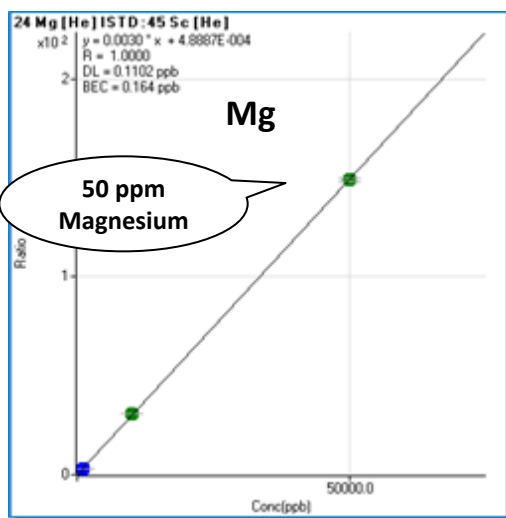
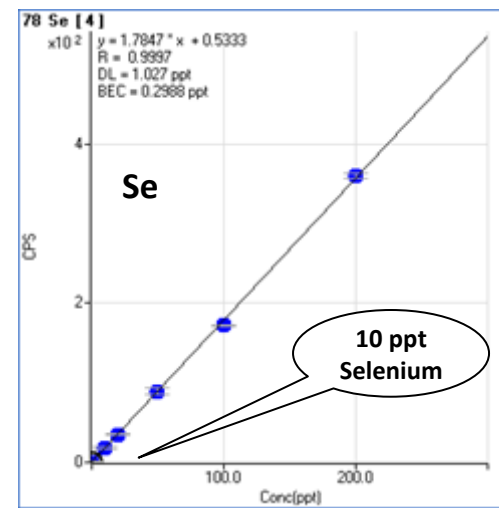
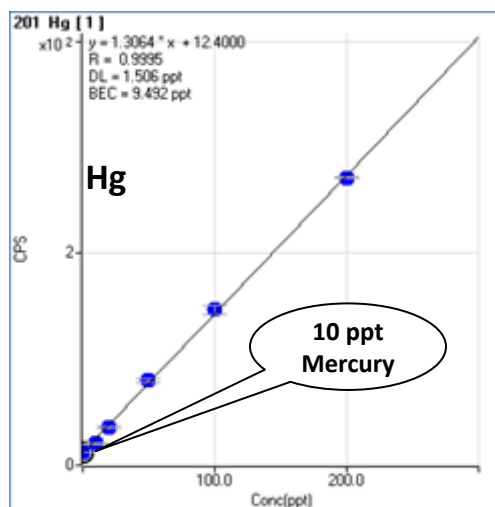
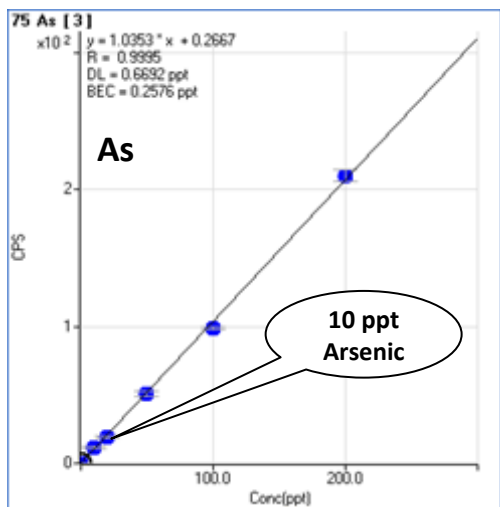
# Measuring Low and High Concentrations Simultaneously

# Prop 65 Elements



# Single Calibration at Low and High Concentrations.

## Minimizing the Need for Dilution and Re-Analysis



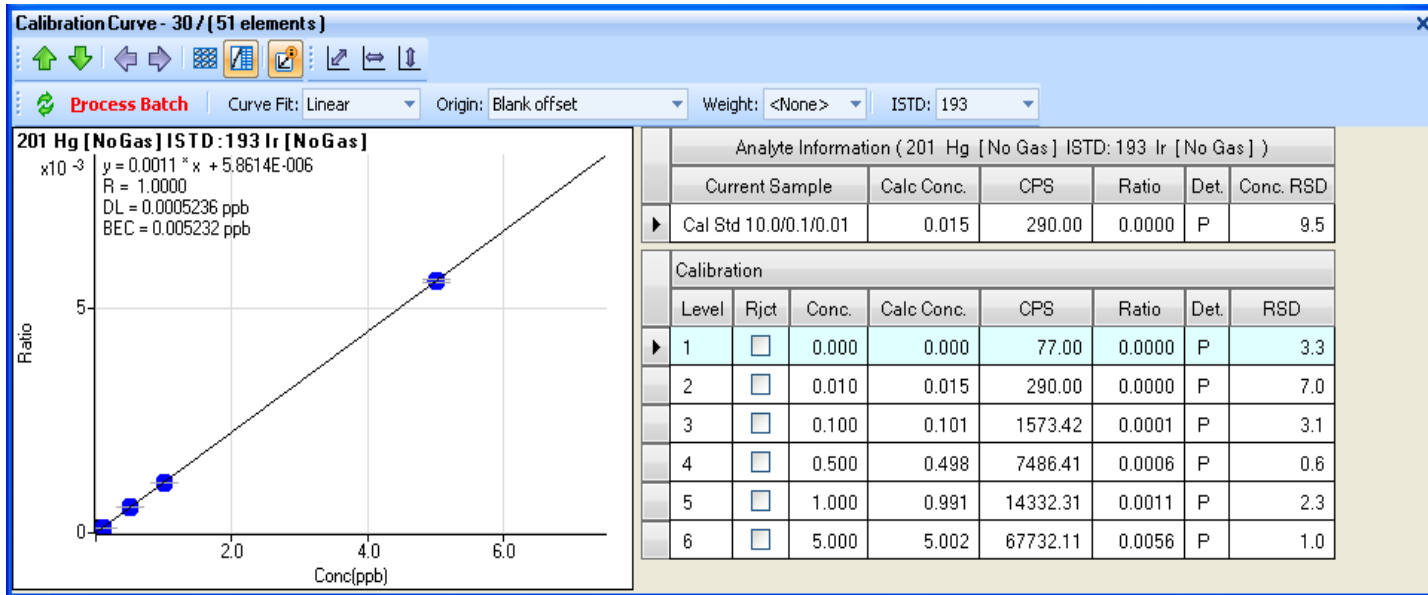
# Preparing a Good Calibration Curve can Save Time.

- Mix the concentration ranges within the calibration standards.
- Calibration should match the range of your samples.
- Do not use multi-element stock solutions with cations and trace elements at the same concentration. Example: Ca and As are at the same concentrations in the stock.
- Cross calibrate the detector for low and high sensitivity modes.

	Blank	Cal #1	Cal #2	Cal #3	Cal #4
Na	0.0	100.0	1,000	10,000	200,000
Mg	0.0	100.0	1,000	10,000	100,000
Ca	0.0	100.0	1,000	10,000	200,000
As	0.0	1.0	10	100	-
Cd	0.0	1.0	10	100	-
Hg	0.0	0.1	1	2	-
Pb	0.0	1.0	10	100	-
	*Units in ppb				

# Washout and Carry Over Issues

# Mercury Washout

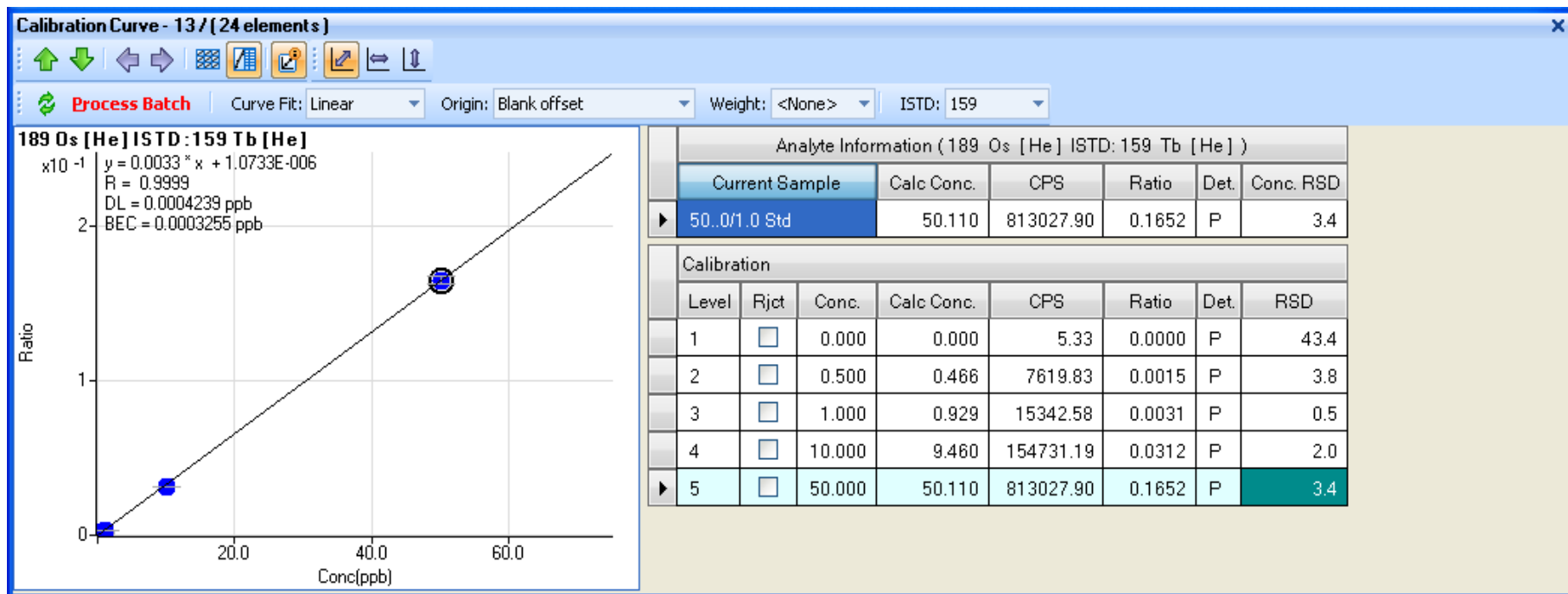


	201 Hg	
	Conc. [ppb]	CPS
Blank	0.000	77.0
0.010	0.015	290.0
0.100	0.101	1573.4
0.500	0.498	7486.4
1.000	0.991	14332.3
5.000	5.002	67732.1
Blank	0.013	271.0

-While addition of HCl (0.5%) stabilizes Hg and minimizes carry over, there will still be approximately 0.2% carryover for this element.

-In order to maintain low detection limits, do not calibrate to unnecessarily high levels.

# Osmium Washout



	189 Os	
	Conc. [ppb]	CPS
Blank	0.000	5.3
0.5	0.466	7619.8
1	0.929	15342.6
10	9.460	154731.2
50	50.110	813027.9
Blank	0.071	1156.1

-As is the case with Hg, ~0.15% carry over. Again, limit the calibration range in order to maintain low detection limits.

-Other elements: B, Ag, Sn, Sb, Tl, Th (Teflon neb, clean lines).

# Minimal Dilution for Lowest Detection Limits

# The Importance of Oxides (CeO/Ce)

-CeO/Ce ratio is a performance criterion that all vendors cite, however the actual relevance of this is often overlooked.

-Ce: m/z 140, CeO m/z: 156 (Formed in the Plasma). Expressed as a ratio of 156/140.

-Two important factors affecting CeO/Ce:

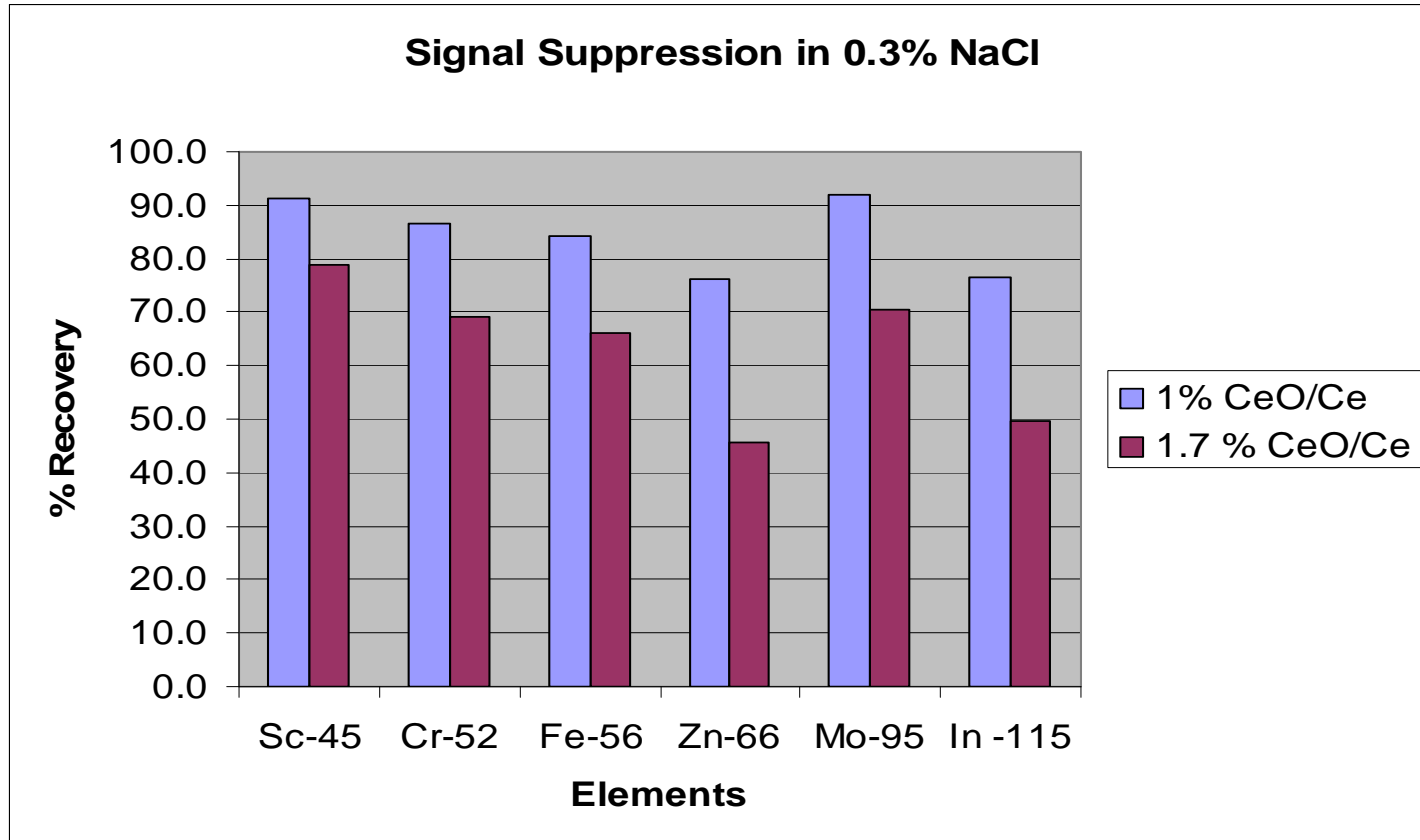
- Vapor loading in plasma (more water, more available Oxygen)
- Plasma temperature (Higher temperature plasma more readily breaks down CeO complex).

■ A hotter plasma will demonstrate lower CeO/Ce ratios.

-Think of CeO/Ce as an inverse plasma thermometer.

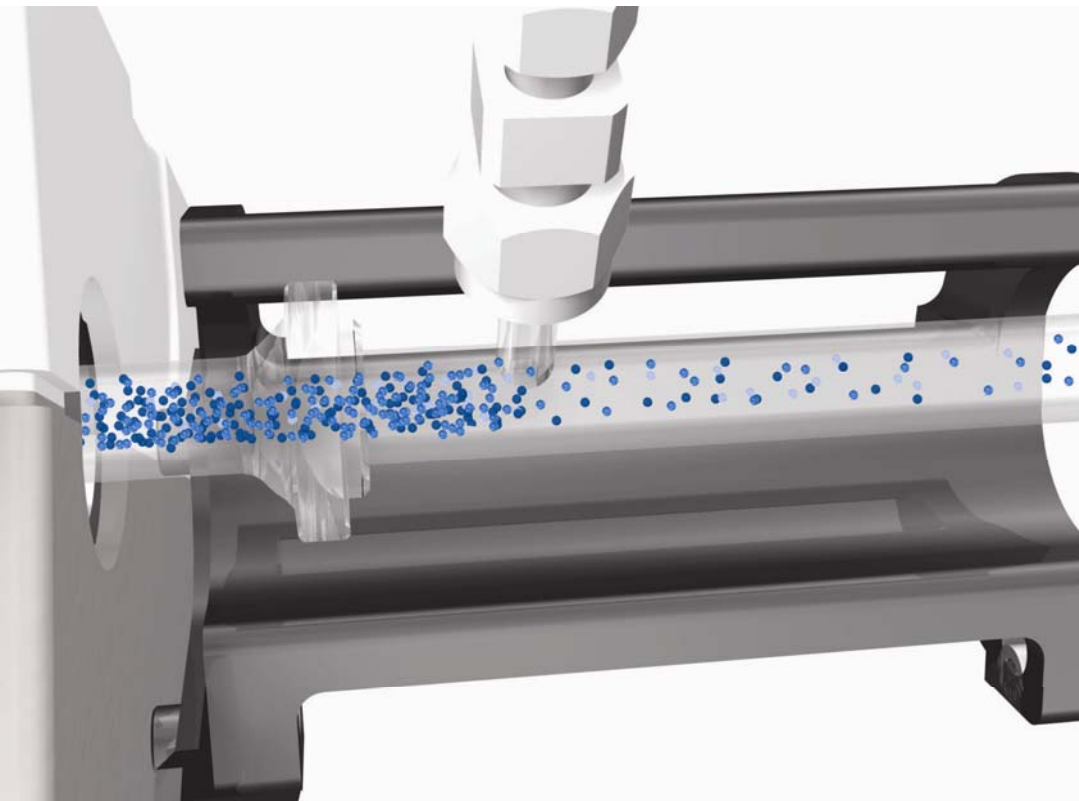
# CeO/Ce Ratio – Effect on Matrix Suppression

When a high sample matrix (e.g. 1/10 seawater) is introduced, the plasma is overloaded and analyte signal drops (suppression). Suppression is worse when the CeO/Ce ratio is higher (plasma temperature is lower)



\*Spike recovery in a high matrix is better with a robust plasma (low CeO/Ce ratio)

# High Matrix Introduction (HMI) – How it Works

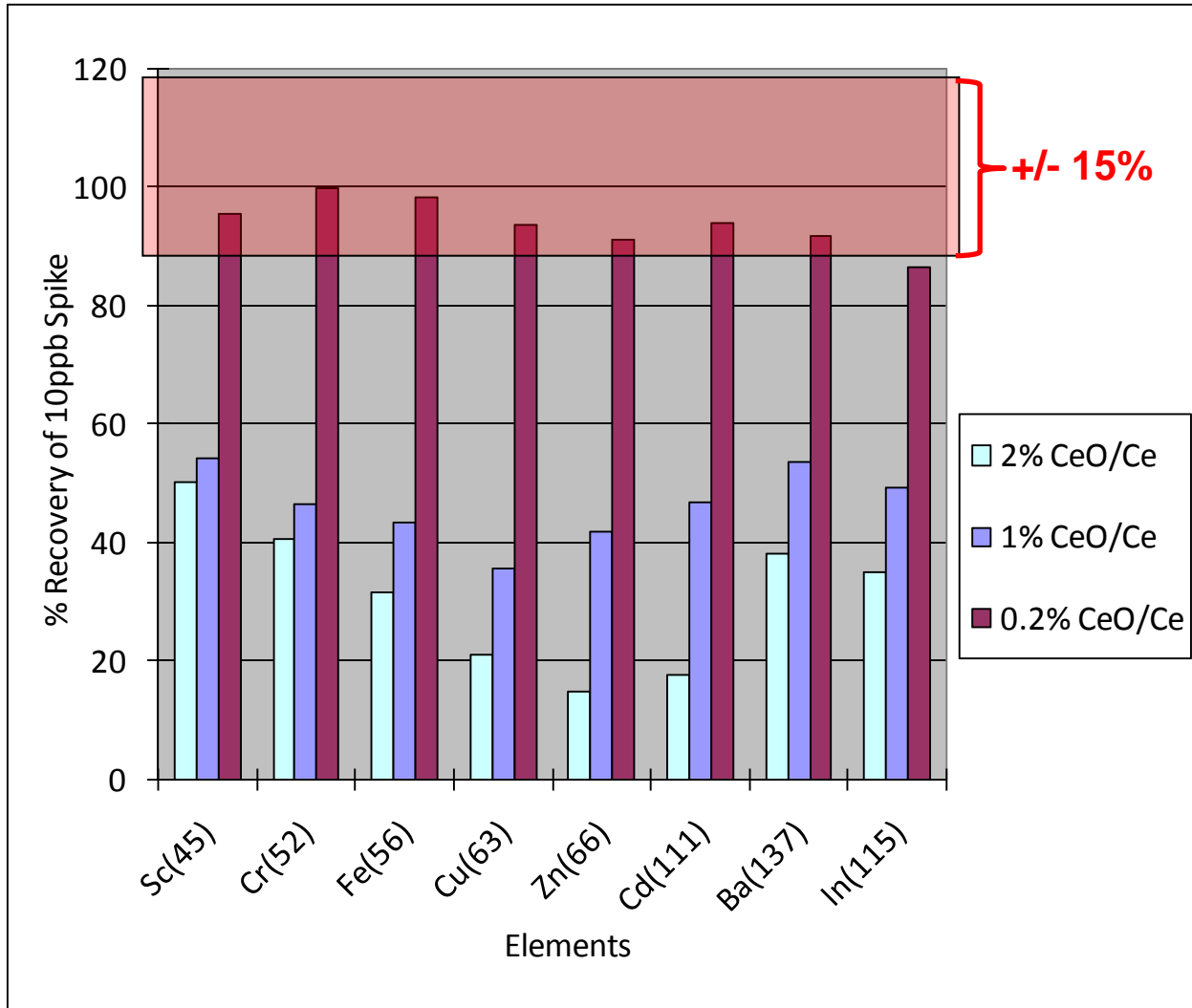


HMI is a sample dilution technique but, uniquely, it dilutes the sample in the gas state, using aerosol dilution.

This removes the main problems of liquid sample dilution:

- Time
- Reagents
- Errors
- Contamination

# Undiluted Sea Water: Example of Matrix Suppression



HMI dilutes aerosol density & water vapor, as well as sample matrix.

Gives much higher plasma temp; much better matrix decomposition. Matrix suppression is almost eliminated.

Plot shows % recovery in undiluted seawater vs aqueous calibrations.

**With HMI, ALL results are within +/- 15% recovery (shaded area)**

**Without HMI, ALL results are below 60% recovery**

# Tuning the ICP-MS for Low Oxides

- Increase Plasma RF – higher plasma temperature.
- Decrease Nebulizer Gas flow – longer sample residence time.
- Increase Sample Depth from Torch to Sampler Cone – longer residence time.
- Decrease Sample Uptake rate – less water vapor.
- Use Aerosol Dilution Hardware if Available.

# Controlling Contamination

# You Don't Need A Clean Room

- Reagents – Use at least ACS grades.
- Maintain a clean environment.
- Air currents in lab.
- Soak/Rinse all plastic ware.
- 18.2 Meg Water.
- Clean Enclosure for Autosampler.
- Soak pipet tips.
- Minimize amount of transfer between vessels.
- Plastic vs. Glass.
- Zn & Pb easy to control.



\* Don't take anything for granted. Review all aspects of your sample and calibration prep techniques.

# Putting it All Together



- Removal of Interferences.
- Measuring Low and High Concentrations Simultaneously (sensitivity and LDR).
- Washout and carry over issues.
- Minimal Dilution for Lowest Detection Limits.
- Controlling Contamination.

# 7700x Nutritional Supplement Sample #1

		Sample Results with Dilutions (ppb)		
Acq. Date-Time		2/4/2010 7:16 PM	2/4/2010 7:20 PM	2/4/2010 7:24 PM
Sample Name				
Dilution		1	5	20
31 P [1]	Conc. [ppb]	717567	732680	734758
44 Ca [1]	Conc. [ppb]	1497859	1454310	1510245
52 Cr [1]	Conc. [ppb]	3.4237	2.1199	<10.000
66 Zn [1]	Conc. [ppb]	6.7672	7.1697	7.8313
75 As [1]	Conc. [ppb]	1.8524	1.8786	1.9115
95 Mo [1]	Conc. [ppb]	6.3477	6.4526	6.4807
111 Cd [1]	Conc. [ppb]	0.2793	0.3099	0.2930
118 Sn [1]	Conc. [ppb]	0.0875	<0.4000	<0.4000
121 Sb [1]	Conc. [ppb]	1.1277	1.1001	1.1117
201 Hg [1]	Conc. [ppb]	<0.0200	<0.4000	<0.4000
208 Pb [1]	Conc. [ppb]	0.7906	0.8485	0.8259

- Major constituents can be determined at any dilution.
- Trace toxics measureable at even the highest dilution.

		Sample Results with Dilutions (ppb)		
Acq. Date-Time		2/4/2010 9:11 PM	2/4/2010 9:15 PM	2/4/2010 9:27 PM
Sample Name				
Dilution		5	20	50
31 P [1]	Conc. [ppb]	<20.000	<400.00	<1000.0
44 Ca [1]	Conc. [ppb]	222.10	<200.00	<500.00
52 Cr [1]	Conc. [ppb]	435740	433897	428843
66 Zn [1]	Conc. [ppb]	18.100	19.217	19.843
75 As [1]	Conc. [ppb]	<0.0200	<0.4000	<1.0000
95 Mo [1]	Conc. [ppb]	1.7025	1.9635	2.2291
111 Cd [1]	Conc. [ppb]	<0.0100	<0.0400	<0.1000
118 Sn [1]	Conc. [ppb]	<0.1000	<0.4000	<1.0000
121 Sb [1]	Conc. [ppb]	<0.0500	<0.2000	<0.5000
201 Hg [1]	Conc. [ppb]	<0.1000	<0.4000	<1.0000
208 Pb [1]	Conc. [ppb]	0.2645	0.2375	<0.5000

# 7700x Nutritional Supplement Sample #2

		2A Sample Results with Dilutions (ppb)		
Acq. Date-Time		12/22/2009 10:35 PM	12/22/2009 10:41 PM	12/22/2009 10:46 PM
Sample Name		2A	2A 10x	2A 50x
Dilution		1	10	50
23 Na [ He ]	Conc. [ ppb ]	15544	16505	16311
24 Mg [ He ]	Conc. [ ppb ]	190345	194858	200006
39 K [ He ]	Conc. [ ppb ]	155052	159369	158686
44 Ca [ He ]	Conc. [ ppb ]	725631	710679	716385
51 V [ He ]	Conc. [ ppb ]	45.300	44.720	45.697
52 Cr [ He ]	Conc. [ ppb ]	99.470	101.11	102.43
55 Mn [ He ]	Conc. [ ppb ]	19155	19543	19247
56 Fe [ He ]	Conc. [ ppb ]	36458	36918	38048
63 Cu [ He ]	Conc. [ ppb ]	6596.0	6870.6	6858.4
66 Zn [ He ]	Conc. [ ppb ]	49028	52879	51803
75 As [ He ]	Conc. [ ppb ]	4.7025	4.6959	4.9547
78 Se [ He ]	Conc. [ ppb ]	88.2032	106.04	111.25
95 Mo [ NoGas ]	Conc. [ ppb ]	93.939	94.283	101.76
111 Cd [ NoGas ]	Conc. [ ppb ]	0.5655	0.5646	0.6465
201 Hg [ NoGas ]	Conc. [ ppb ]	<0.0300	<0.3000	<1.5000
208 Pb [ NoGas ]	Conc. [ ppb ]	1.3960	1.5038	1.7007

-Concentrations across all dilutions for cations the same.

-All elements using main isotope or “on mass” isotope.

-Measurement of Fe at 56.

-Excellent sensitivity across all dilutions (Pb, Cd).

# 7700x Nutritional Supplement Sample #3

		Sample 325-024 Results with Dilutions (ppb)		
		3/25/2010 6:09 PM	3/25/2010 6:15 PM	3/25/2010 6:20 PM
Acq. Date-Time				
Sample Name		325-024	325-024 5x	325-024 20x
Dilution		1	5	20
10 B [NoGas]	Conc. [ppb]	5496.9	5311.4	5577.5
24 Mg [He]	Conc. [ppb]	755012	734548	743697
44 Ca [He]	Conc. [ppb]	1677826	1609765	1548722
51 V [He]	Conc. [ppb]	117.55	115.08	114.19
52 Cr [He]	Conc. [ppb]	617.81	624.81	623.50
55 Mn [He]	Conc. [ppb]	19332	19984	20310
56 Fe [He]	Conc. [ppb]	14500	14971	14693
63 Cu [He]	Conc. [ppb]	3.5950	4.1805	5.5750
66 Zn [He]	Conc. [ppb]	96695	103313	108815
75 As [He]	Conc. [ppb]	2.5474	2.4437	2.3635
78 Se [He]	Conc. [ppb]	193.23	192.27	189.80
95 Mo [He]	Conc. [ppb]	460.90	447.12	444.13
121 Sb [NoGas]	Conc. [ppb]	0.2388	0.3001	0.6368
137 Ba [NoGas]	Conc. [ppb]	12.374	12.450	12.190
201 Hg [NoGas]	Conc. [ppb]	0.0880	0.1323	0.3175
208 Pb [NoGas]	Conc. [ppb]	1.9550	2.0787	2.1223

-Mg ~740 ppm & Ca ~1600 ppm

-Fe ~14.6 ppm at mass 56

-As same values even in presence of ArCl & CaCl

# Speciation with LC-ICP-MS



# USP<231> Replacement

**USP<231>**



**USP<232>**

Elemental Impurities  
Limits

**USP<233>**

Elemental Impurities  
Procedures

At the same time, USP developed a new protocol for dietary supplements



**USP<2232>**

Elemental Contaminants  
in Dietary Supplements

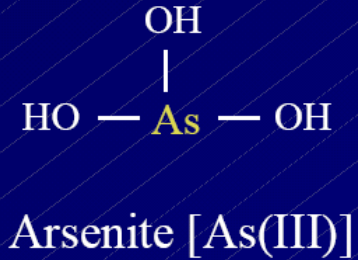
- Limits based on 10g max daily intake
- If total [As] > limit, **Inorganic As speciation is required**
- If total [Hg] > 0.2 µg/g, **CH<sub>3</sub>-Hg speciation is required**

**Table 1. Elemental Limits**

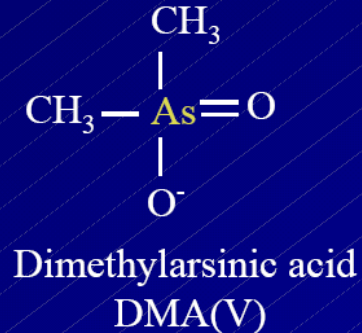
Element	Individual Component Limit <sup>a</sup> (µg/g)	PDE <sup>b</sup> (µg/day)
Arsenic (inorganic) <sup>c</sup>	1.5	15
Cadmium	0.5	5
Lead	1.0	10
Mercury (total)	1.5	15
Methylmercury (as Hg) <sup>d</sup>	0.2	2

# Common Arsenic Species (Oxyanions & Carboxylates)

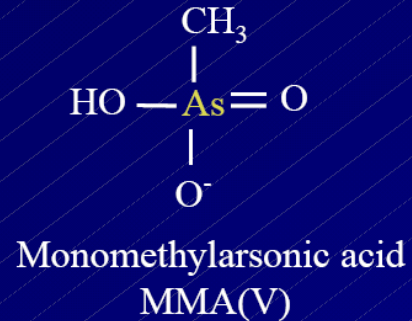
pKa = 9.3



pKa = 6.2



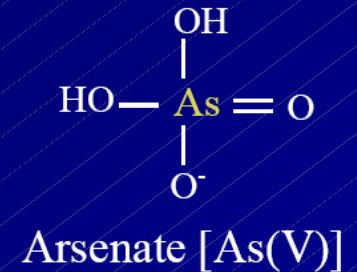
pKa = 3.6



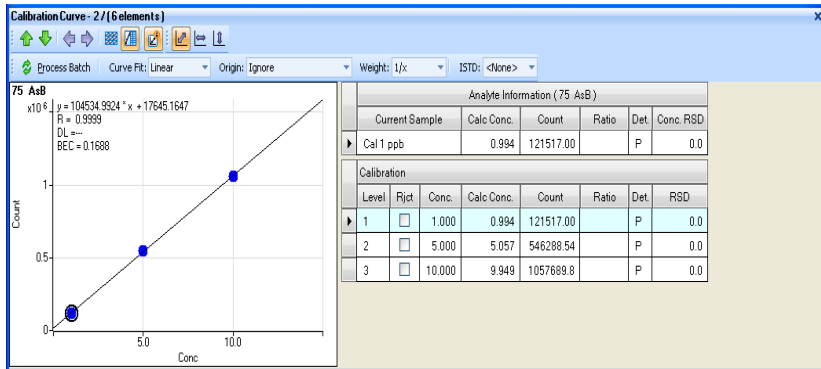
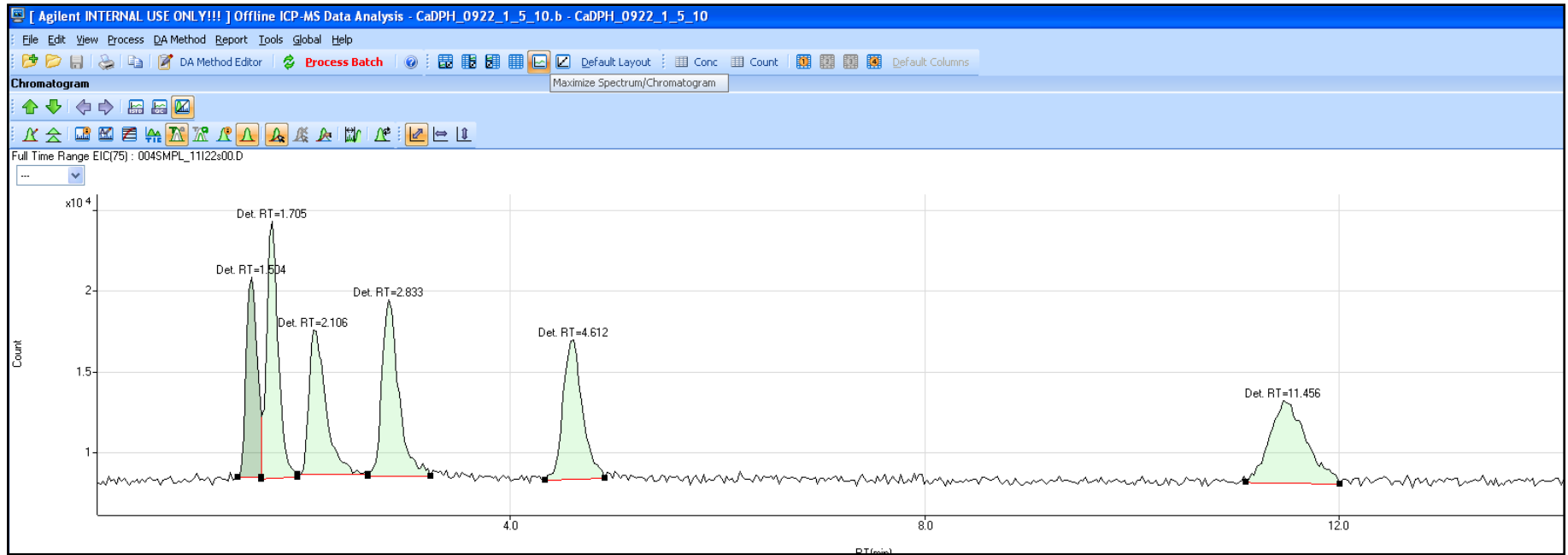
pKa = 4.7



pKa = 2.3



# Mixed Arsenic Species at 1.0 ppb



- Typically inject 10-50 uL of sample.
- Column flow connected directly to nebulizer.
- Anion exchange or C18 paired ion.

# In Summary

- Remove or minimize interferences by using Collision/Reaction technology.
- Prepare your calibration mixes to reflect the element concentration ranges in your sample digests.
- Do not calibrate to needlessly high levels. Especially for those elements that are known to have washout and carry over issues.
- Tune to the lowest oxides possible in order to minimize the need for dilution.
- Be mindful of anything that can lead to contamination of samples or calibration standards. Soak, rinse, clean environment, air currents, covers, etc.

**Thank You**