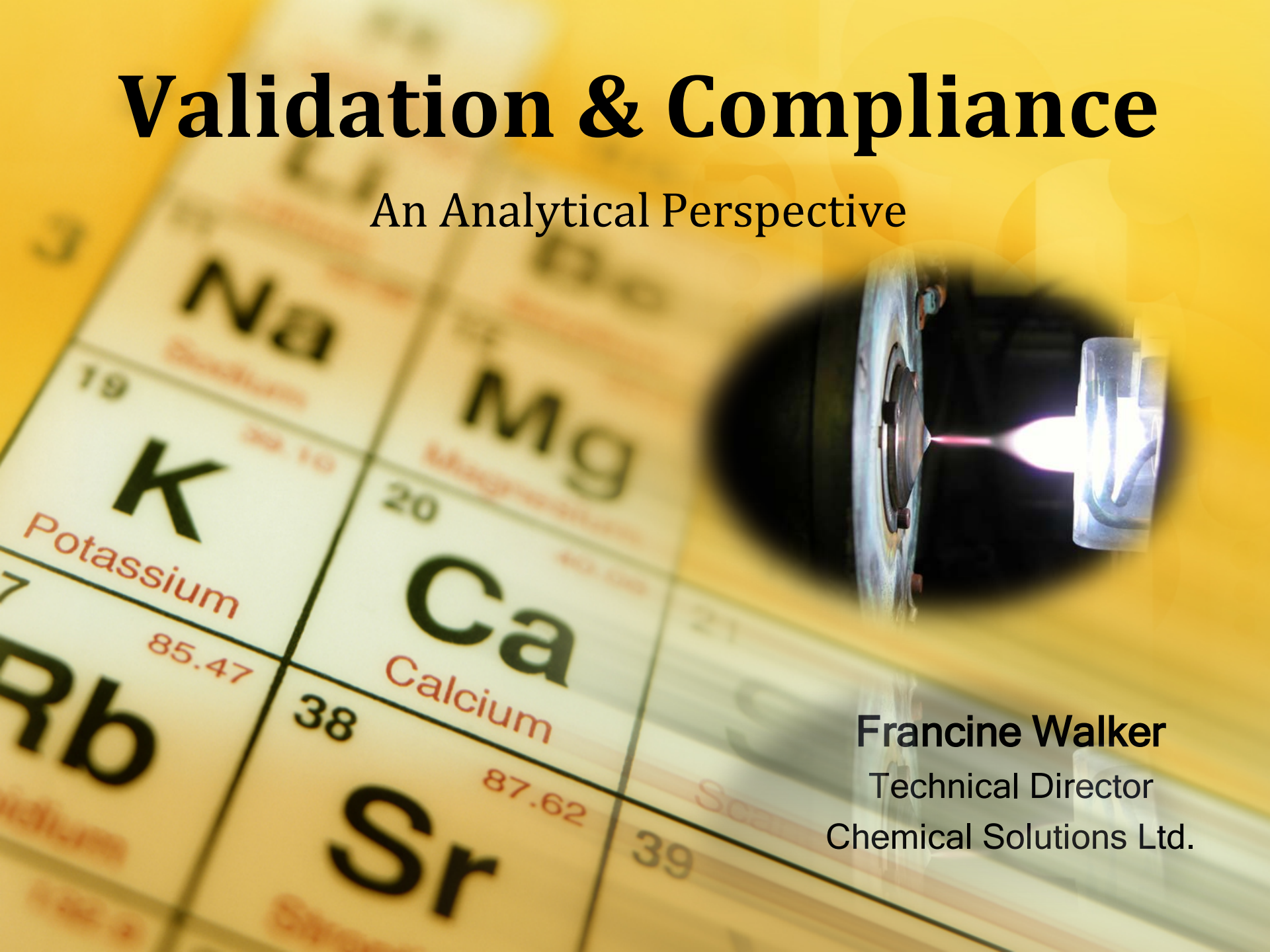
Validation & Compliance

An Analytical Perspective



Francine Walker
Technical Director
Chemical Solutions Ltd.

Validation & Compliance

Context

- **Independent Contract Testing Lab**
- **Dedicated to ICP-MS/OES for 20 years**
- **Historical testing in support of Proposition 65**
  - **Nutritional Supplements**
  - **Pb @ 0.5 ug/day as a Reproductive Toxicant**
  - **As @ 10 ug/day Cd @ 4.1 ug/day (often lower)**
  - **Hg @ 1 – 3 ppm**
- **Sum of 10 elements reactive in USP <231>**
  - **As, Cd, Pb, Hg + Ag, Bi, Cu, Mo, Sb, Sn**
  - **Summation of LOQs to be < 10 ppm**

Validation & Compliance
Discussion Topics

- **Compliance Strategies**
  - **Component vs Finished Product**
  - **Screening and Validation**
  - **Combining Similar Materials**
    - ✓ Flavors, Strengths, Augmentation with Excipients/Actives
- **Specification and Calibration Considerations**
  - **Semantics**
    - ✓ PDE and J = target or specification level in ug/day
    - ✓ MDI MDD = amount (g) per day
    - ✓ PDE/MDD = ug/g
  - **Fractional "J" value targets**
- **Validation Approaches and Questions**
  - **Traditional vs Creative**

Component vs Finished Product

1+1 = 3 ?

Vanadium = V^{51}

$-3.127\ (ClO^{53} - (0.113 * Cr^{52}))$

Berberine Chloride
$C_{20}H_{18}ClNO_4$

+

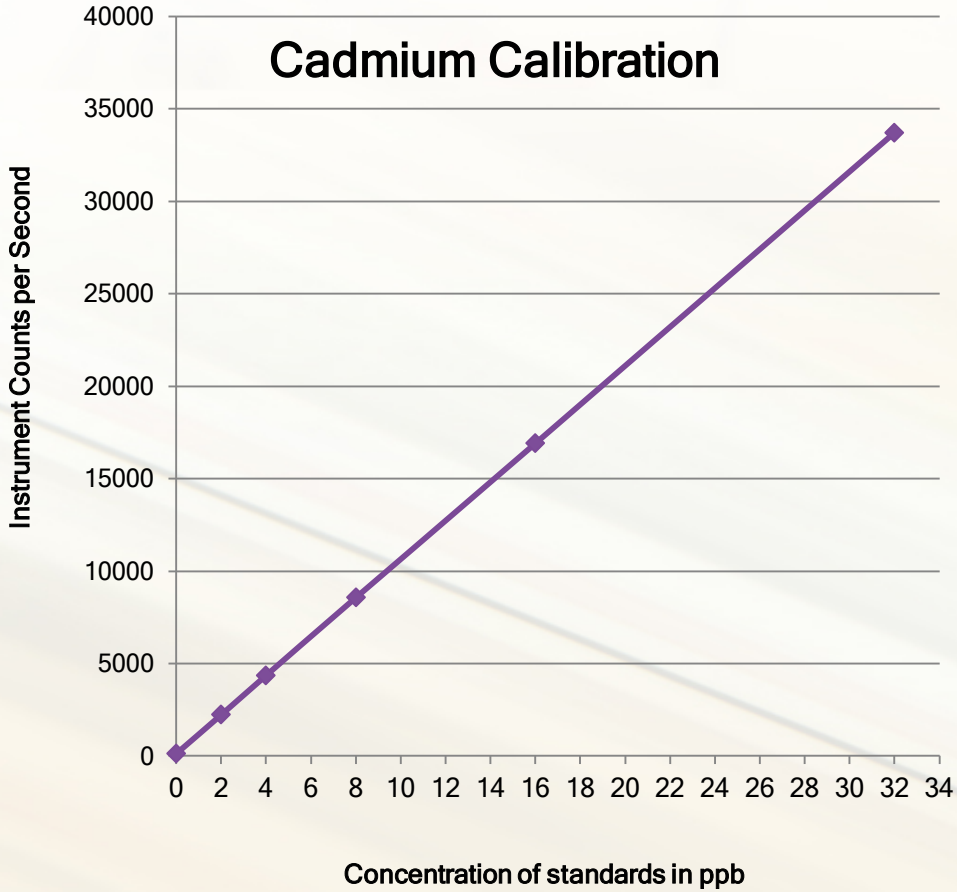
Chromium
Polynicotinate

= or ≠

Interferences appear with combination of raw materials

Validation & Compliance

Compliance Strategies



J Value	Standard	Response (cps)
0	0ppb	123
0.25 J	2ppb	2,220
0.5 J	4ppb	4,350
J	8ppb	8,560
2J	16ppb	16,900
4J	32ppb	33,700

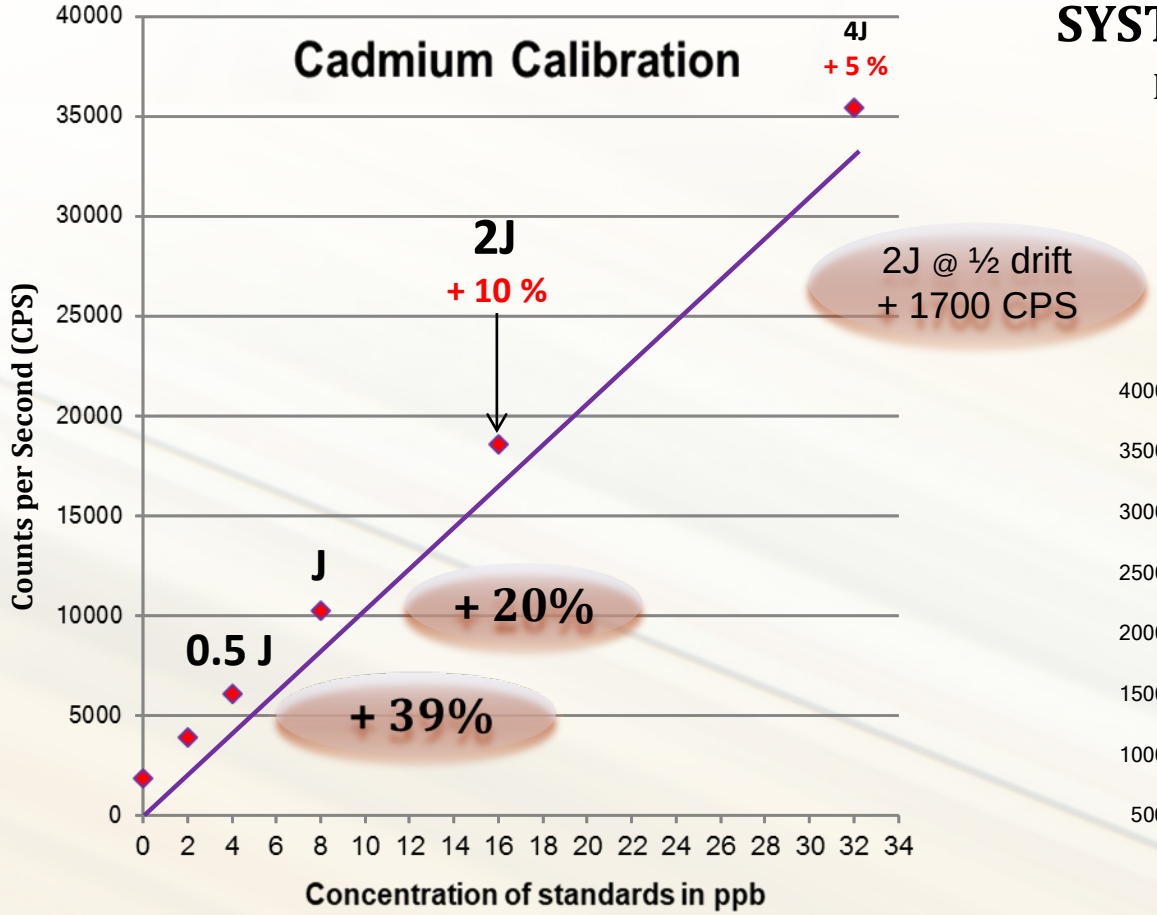
$$y = 1048.8x + 137.8$$

$$R^2 = 1.000$$

1+1 ≠ 2 ?

Summation of Error

Validation & Compliance
Compliance Strategies

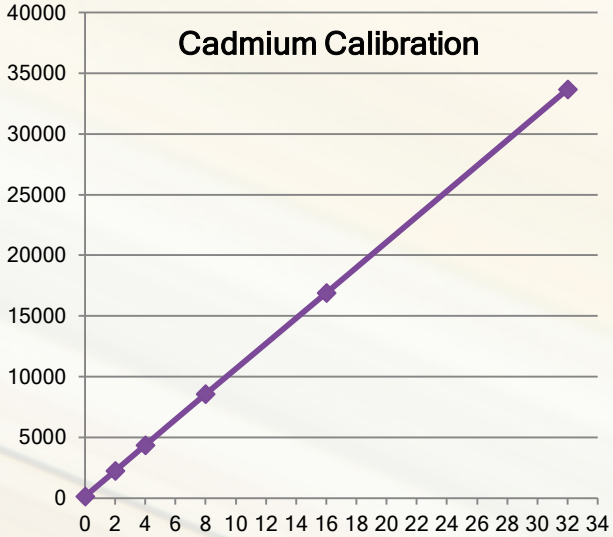


SYSTEM SUITABILITY

Drift NMT 20% for each Target Element

Standardization solution 1 = 2J

Read before and after sample solutions



Validation & Compliance

Compliance Strategies

Screening

- 1 Sample 'Unspiked'
- 1 Predigestion Matrix Spike
  @ 10 - 30% PDE
- 2 Additional Lots of Material

- 26 Elements Common
  24 EI + Fe + Mn
- More Raw Materials Screened than Validated
- Often precedes Validation for finished materials

Parameter	Result	Serving Size	Matrix Spike Recovery	Matrix Spike Level	Specification
Antimony	<45 ug/serving	4.76 g	118%	75.6 ug/g	≤ 1200 ug/serving
Arsenic	<0.57 ug/serving	4.76 g	94%	0.94 ug/g	≤ 15 ug/serving
Cadmium	<0.38 ug/serving	4.76 g	103%	0.32 ug/g	≤ 5 ug/serving
Chromium	<410 ug/serving	4.76 g	98%	693 ug/g	≤ 11000 ug/serving
Cobalt	<1.8 ug/serving	4.76 g	99%	3.15 ug/g	≤ 50 ug/serving
Copper	<110 ug/serving	4.76 g	111%	189 ug/g	≤ 3000 ug/serving
Lead	<0.19 ug/serving	4.76 g	103%	0.32 ug/g	≤ 5 ug/serving
Mercury	<1.1 ug/serving	4.76 g	96%	1.89 ug/g	≤ 30 ug/serving
Molybdenum	<110 ug/serving	4.76 g	116%	189 ug/g	≤ 3000 ug/serving
Nickel	<7.4 ug/serving	4.76 g	97%	12.6 ug/g	≤ 200 ug/serving
Selenium	<5.6 ug/serving	4.76 g	101%	9.45 ug/g	≤ 150 ug/serving
Silver	<5.6 ug/serving	4.76 g	96%	9.45 ug/g	≤ 150 ug/serving
Tin	<220 ug/serving	4.76 g	104%	378 ug/g	≤ 6000 ug/serving
Vanadium*	<3.7 ug/serving	4.76 g	99%	6.30 ug/g	≤ 100 ug/serving

Validation

By means of verification studies, analysts will confirm that the analytical procedures described herein, as well as alternative analytical procedures, are suitable for use on specified material . ***USP <233> Introduction***

Almost exclusively clients are following the **Quantitative Procedures** criteria under **Alternate Procedure Validation.**

Accuracy - spike @ 0.5J, J, 1.5J recoveries 70-150%

Repeatability – 6 preparations of J RSD ≤ 20%

Ruggedness – repeat above differing days, instruments, analysts RSD ≤ 20%

Specificity – references USP <1225>

LOQ, Range, and Linearity – demonstrated by meeting Accuracy

Validation

Accuracy

- ? Target the Fractional Control Threshold as "J"
  - Calibration at 0.3* PDE = J
    - Standards at 0.15* PDE, 0.3* PDE and 0.6 * PDE
  - Spiking Levels for Screening
    - 0.3 * J or 0.09 PDE = control threshold

Specificity

- ? Meets Accuracy Criteria
- ? Digestion blanks ≤ 0.15 PDE/MDD
- ? Recovery of Alternate Isotopes
  - As Available to polyisotopic Elements

LOQ

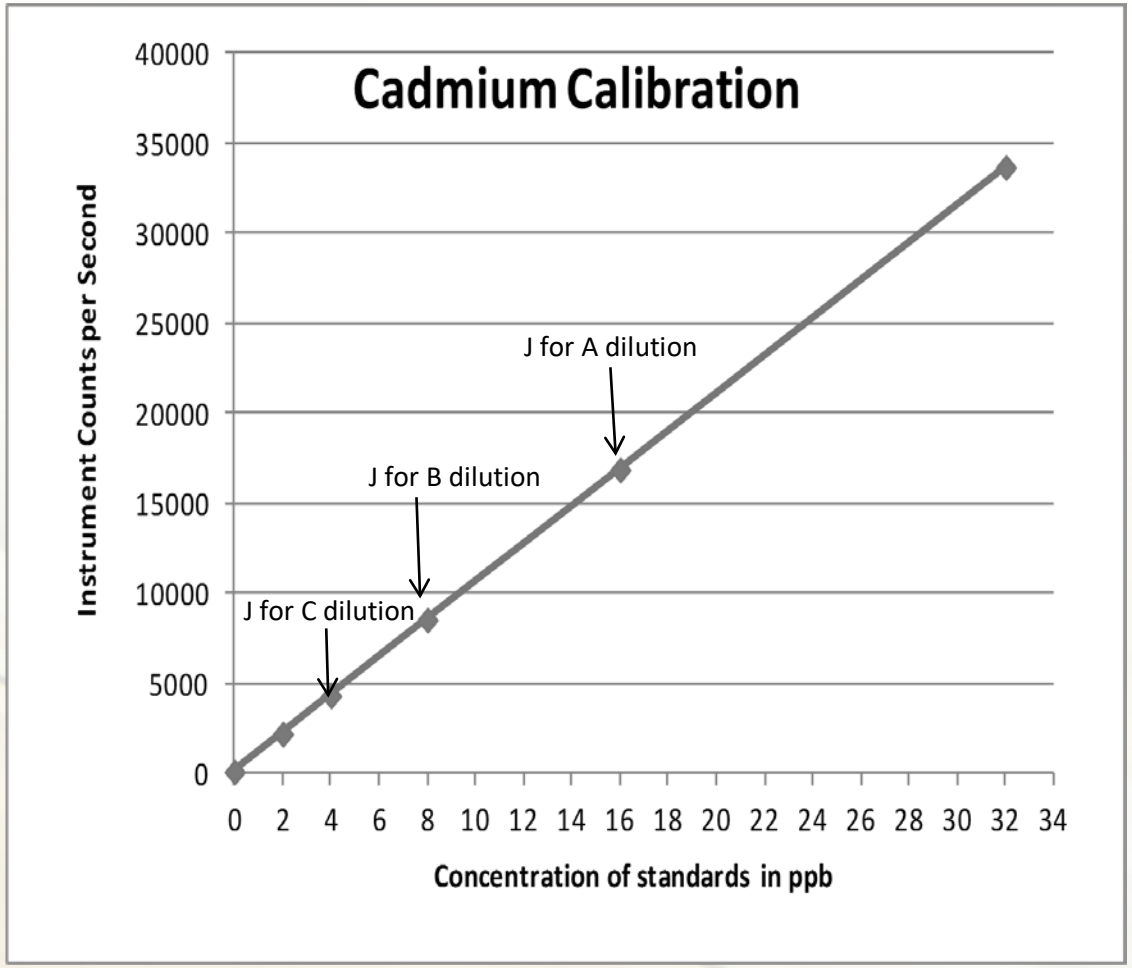
- ? Meets Accuracy Criteria
- ? Lowest Spiking Level = LOQ
- ? Lowest Standard on Calibration Curve * Dilution
  - Should Standards recovery criteria apply
- ? Other

Validation & Compliance

Compliance Strategies

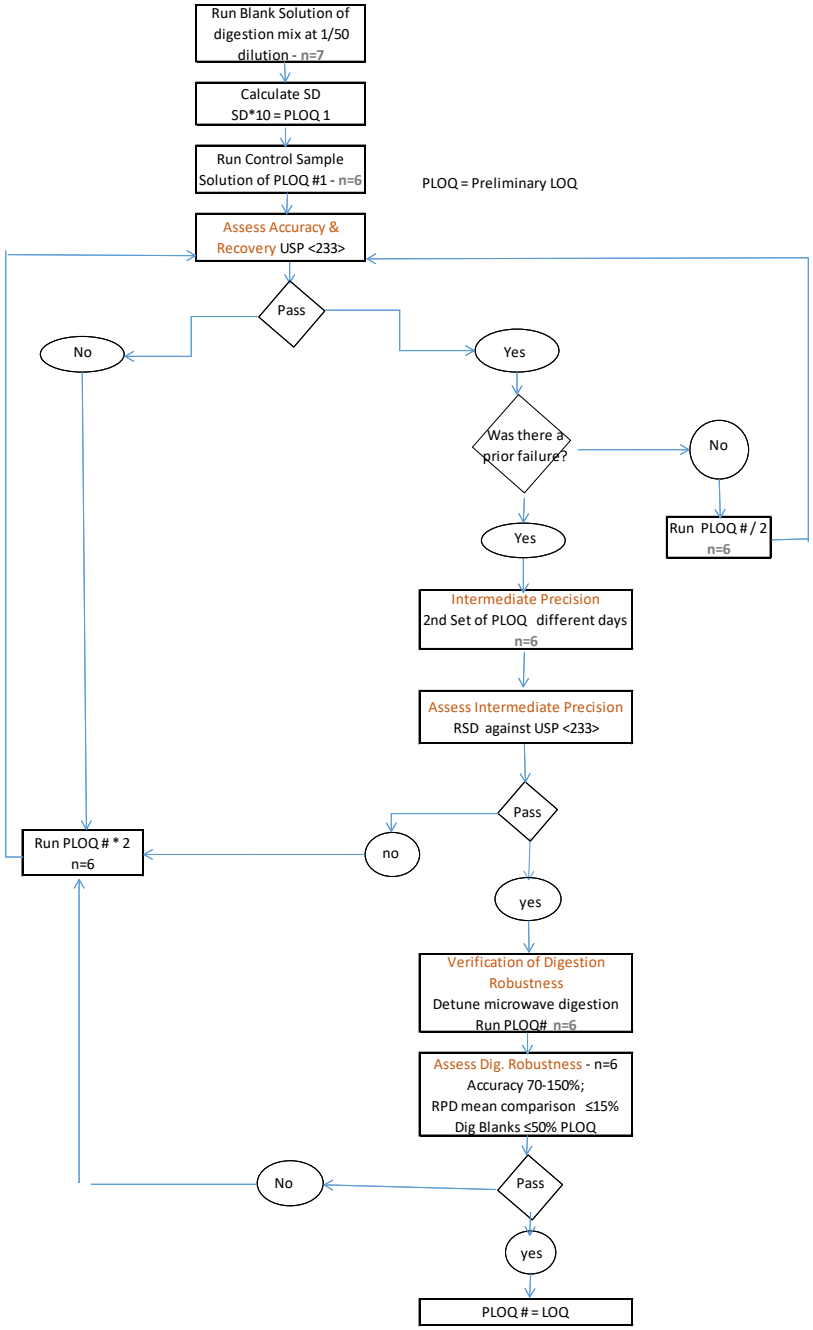
Cadmium Specification (J): 8 ppm or 8000 ppb

Initial Sample:	0.500 gram		
Final Volume:	50 grams (ml)		
Initial Dilution Factor:	50/0.5 = 100 X		
Secondary Dilutions:	A = 1/5	B = 1/10	C = 1/20
Final Dilutions:	A = 500 X	B = 1000 X	C = 2000 X
Corresponding J:	A = 8000/500 = 16 ppb		
	B = 8000/1000 = 8 ppb		
	C = 8000/2000 = 4 ppb		



LOQ

Standard * Dilution



LOQ
in blank digestion fluid

Determine PLOQ
10*SD of Digestion Fluids "Blank"
n = 7 @ 1/50 dilution

Run Control Sample to Confirm
n = 6 @ PLOQ

Assess against USP <233> criteria
Accuracy 70-150% Precision ≤ 20% RSD

Run More Control Samples
Higher or Lower Level n=6

Assess against USP <233> criteria
Intermediate Precision or Repeat Accuracy

Robustness criteria
"Detuning" microwave and instruments

Assess against USP <233> criteria

Validation & Compliance

Compliance Strategies

Validation

USP <233> VALIDATION CRITERIA

QUALITY OBJECTIVE	MEASUREMENT ACTIVITY	SPECIFICATION	RESULTS
Accuracy	1) Prepare and analyze one Control Sample (1) at each of 3 levels for elements of interest (2). 2) 3 Test Samples (3) at each of 3 levels for the elements of interest (2).	1) 80 – 120% Recovery for each sample 2) 80 – 150% Recovery for the mean of the 3 replicates at each concentration	1) Recovery for control sample at each spike concentration: 50% Recovery: 98.5 % 100% Recovery: 98.6 % 200% Recovery: 99.0 % 2) Recovery for the mean of the 3 replicates at each spike concentration: 50% Mean Recovery: 113.8 % 100% Mean Recovery: 113.9 % 200% Mean Recovery: 110.6 %
Precision/ Repeatability	6 Test Samples (3) spiked at the specification level.	1) % Relative Standard Deviation (RSD) NMT 20.0 %	1) RSD of 6 Results: 1.06 %
Intermediate Precision/ Ruggedness	A second set of 6 Test Samples (3) spiked at the specification level prepared by a second analyst and run by a second analyst on a second day.	1) % RSD overall for both sets of precision samples = NMT 25.0%	1) RSD of 12 Results: 6.37 %
Specificity/ False Negative	Spiking for accuracy studies done with mixed elemental standards	1) No interfering signals are detected - see Accuracy	1) Accuracy: PASS
Specificity/ False Positive	3 Aliquots of sample digested and analyzed without added spiking solutions performed in conjunction with Accuracy Requirements.	1) No interfering signals are detected - see Accuracy	1) Accuracy: PASS
Limit of Quantitation (LOQ)	Tagged to Accuracy Specification	1) Meet Accuracy Criteria	1) Accuracy: PASS
Sensitivity	Tagged to Accuracy Specification	1) Meet Accuracy Criteria	1) Accuracy: PASS

Aluminum

Table 1: Specification Spiking Levels

Element of Interest	Specification Limit	½ Specification Limit	2x Specification Limit
Aluminum	340 µg/g	170 µg/g	680 µg/g

Validation & Compliance

Compliance Strategies

Validation

17A1234

1st Analyst

Method Identification				Digestion Blank	Unspike			0.3J Spikes			0.3J Spike			Mean
Mass (g)	Isotope	ISA	Instrument/Mode	0.25000				0.250	0.250	0.250	% Recovery			0.3J
As	75	Ge	NEX350X / KED	0.27	0.27	0.27	0.27	2.89	2.85	2.83	94.3%	92.8%	92.3%	93.1%
Cd	111	In	E9000 / STD	-0.06	-0.04	-0.06	-0.05	0.85	0.91	0.85	96.7%	103.6%	96.6%	98.9%
Co	59	In	E9000 / STD	0.01	0.27	0.27	0.25	9.18	9.00	8.76	96.3%	94.3%	91.7%	94.1%
Pb	208	Ho	E9000 / STD	0.03	0.08	0.10	0.25	0.97	1.13	1.10	89.6%	106.9%	103.3%	99.9%
Hg	201	Ho	E9000 / STD	0.47	0.15	0.07	-0.02	5.66	5.87	5.64	100.6%	104.4%	100.4%	101.8%
Ni	60	Ge	E9000 / STD	1.20	1.20	1.24	4.99	35.82	36.09	34.92	90.0%	90.8%	87.6%	89.5%
V	51	Sc	E9000 / STD	0.52	2.51	2.42	2.11	19.78	20.68	20.26	94.1%	99.0%	96.7%	96.6%

Serving Sizes Matter

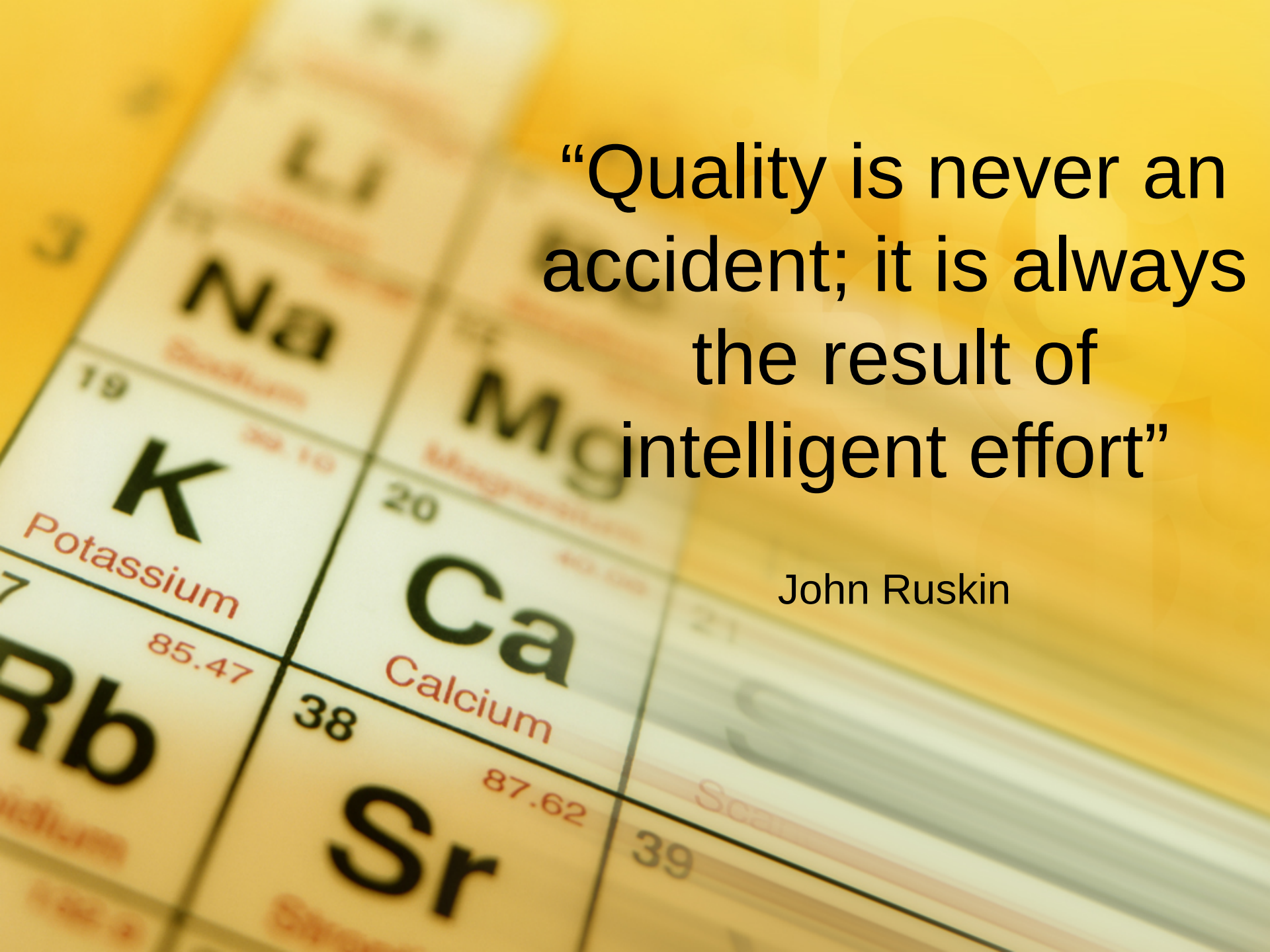
0.10 g = 15 ppm Cadmium spike at PDE

300 g = 0.07 ppm Cadmium spike at PDE

Fractional "J" Matters

300 g = 0.02 ppm Cadmium spike at 0.3 * PDE

300 g = 0.007 ppm Cadmium spike at 0.1 * PDE



“Quality is never an accident; it is always the result of intelligent effort”

John Ruskin