Trace Heavy Metal Analysis in Animal Feed by ICP-MS

Wei Li, Ph.D.
Office of the Texas State Chemist
October 21, 2011

What’s Heavy Metal

- Term used loosely for 60 years under various definitions, none accepted by the International Union of Pure and Applied Chemistry (IUPAC)

- A metal having an atomic weight greater than sodium, a density greater than 5 g/cm^3

- Some notion of toxicity

Heavy Metal Toxicity

Metal	Sources	Description
Arsenic (As)	Chemical processing plants, cigarette smoke, drinking water, fungicides, meats and seafood, metal foundries, ore smelting plants, pesticides, polluted air, specialty glass products, weed killers, wood preservatives, etc.	Extremely poisonous as well as colorless and odorless, arsenic can enter the body through the mouth, lungs and skin. Arsenic toxicity seems to predominantly affect the skin, lungs and gastrointestinal system, and may cause nervous disorders, deteriorated motor coordination, respiratory diseases, and kidney damage as well as cancers of the skin, liver, bladder and lungs.
Cadmium (Cd)	Air pollution, batteries, ceramic glazes/enamels, cigarette smoke (both first and second hand), tap and well water, food (if grown in cadmium-contaminated soil), fungicides, mines, paints, power and smelting plants, seafood, etc.	Exposure to cadmium can occur through inhalation or ingestion in places or situations where cadmium products are used, manufactured, or ingested. Cigarette smoke is the biggest source of cadmium toxicity, which seems to primarily affect the lungs, kidneys, bones, and immune system. It may lead to lung cancer, prostate cancer and heart disease, and also causes yellow teeth and anemia. Cadmium also seems to contribute to autoimmune thyroid disease.
Chromium (Cr)	Stainless steel welding, chromate or chrome pigment production, chrome plating, leather tanning, handing or breathing sawdust from chromium treated wood	Exposure to high level chromium can damage and irritate your nose, lungs, stomach, and intestines. Ingesting very large amounts of chromium can cause stomach upsets and ulcers, convulsions, kidney and liver damage, and even death.
Lead (Pb)	Air pollution, ammunition, auto exhaust, batteries, containers for corrosives, contaminated soil, cosmetics, fertilizers, foods (if grown in lead-contaminated soil), hair dyes, insecticides, lead-based paints, lead-glazed pottery, pesticides, solder, tobacco smoke, water (if transported via lead pipes), etc.	Lead is a naturally-occurring neurotoxin. Although many lead-containing products (such as gasoline and house paints) were banned in the 1970s, contamination still occurs today mostly by drinking lead-contaminated water, breathing lead-polluted air, and living in or near older painted buildings and certain toxic industrial areas. Lead toxicity primarily targets the nervous system, kidneys, bones, heart and blood, and poses greatest risk to infants, young children and pregnant women. It can affect fetal development, delay growth, and may also cause attention deficit disorder, learning disabilities, behavioral defects, and other developmental problems.
Mercury (Hg)	Air pollution, barometers, batteries, cosmetics, dental amalgam fillings, freshwater fish (such as bass and trout), fungicides, insecticides, laxatives, paints, pesticides, saltwater fish (such as tuna and swordfish), shellfish, tap and well water, thermometers, thermostats, vaccines, etc.	Both poisonous and dangerous, mercury is found throughout our environments in many forms and also in many household items. Mercury often permeates the ground we walk on, and is also found in some childhood vaccines today because of its use as a preservative. Mercury as used in dental fillings is the primary source of toxic exposure, and in vapor form accounts for the majority of all exposures (via inhalation). Mercury toxicity can affect the central nervous system, kidneys and liver. Research suggests that this heavy metal may also contribute to autism and multiple sclerosis.
Thallium (Tl)	Infrared and electric eye optical devices, foods (if grown in thallium-contaminated soil), light-sensitive crystals, photocells, rodent and ant poisons (now discontinued), contaminated cocaine (or what is thought to be cocaine), semiconductors, etc.	Thallium is a toxic heavy metal with no known biological function. Human contamination can occur from oral ingestion as well as through the skins and lungs, especially if exposed to thallium-contaminated dust from lead and zinc smelting plants, pyrite burners, and similar processing sites. Thallium toxicity mainly affects the nervous system, and can lead to maladies such as hair loss, nerve degeneration, extremity numbness, and cataracts.

Heavy Metal Toxicity to Animal Health

Animal Specie	Toxic dose of Cadmium	Effect observed
Cattle	Diet Containing 5 to 30 mg of Cd/Kg	Decrease in performance of cattle
	Diet Containing ≥30 mg of Cd/Kg	Disorder of cattle's health
Sheep	Diet Containing >40 mg of Cd/Kg	Animals presenting parakeratosis, reduction on appetite, body weight gain and testicle environment
	Diet Containing 5 to 60 mg of Cd/Kg	Increased Zn concentration in liver and kidney

National Research Council (NRC). Minerals In: National Research Council, editors. Nutrient requirements of dairy cattle. Washington: National Academy Press; **2001**. p.105-61.

Heavy Metal Poisoning

Animal Specie	Cadmium Concentration on Diet (mg/Kg)		Cadmium concentration in tissue (mg/Kg)	
			Liver	Kidney
Cattle and sheep	0.1 to 0.2	Normal	0.02 to 0.05	0.03 to 0.10
	0.5 to 5.0	High	0.1 to 1.5	1.0 to 5.0
	>50	Toxic	50 to160	100 to 250
Pig	0.1 to 0.8	Normal	0.1 to 0.5	0.1 to 0.5
	1.0 to 5.0	High	1.0 to 5.0	2.0 to 5.0
	>80	Toxic	>13	<270

Reis, L.S.; Pardo, P.E.; Camargos, A.S. and Oba E., Mineral element and heavy metal poisoning in animals, Journal of Medicine and Medical Sciences, **2010**, 1(12), 560-79.

Heavy Metal Contamination in Feed

- Mineral nutrient additive

- Fishmeal

  Mercury contamination

- Crops

  Source: Irrigation water, soil

OTSC GOALS

- 2011-2012 FSIS FERN Cooperative Agreement
  - High priority project (Chemistry):

Arsenic, Selenium, Cadmium, Thallium, Lead and if practical Mercury

- FDA Office of Regulatory Affairs and Forensic Chemistry Center, 2012
  - Multi-elements quantitative analysis of trace elements (23 in total) in aqueous solutions by ICP-MS

Heavy Metal Analysis

Quantitative and Qualitative Analysis

- Atomic Absorption Spectrometry(AAS)
  - Flame, Graphite furnace
- Inductively Coupled Plasma(ICP)
  - Optical Emission Spectrometry, Mass Spectrometry
- Other methods
- X-ray Fluorescence Spectrometry
- Instrumental Neutron Activation Analysis
- Prompt Gamma-ray Activation Analysis
- Microwave Plasma-Atomic Emission Spectrometry

Official Methods

Analyte	CEN Published Standard	AOAC Official Method	ISO Method
As	EN15510:2007*	986.15**	ISO 17239:2004
Cd	EN15550:2007*	986.15**	ISO 6561:2005
Cr	EN14082:2003	974.27	NA
Hg	EN13806:2002	977.15, 990.04	ISO 6637:1984
Pb	EN15550:2007*	986.15**	ISO/TS 6733:2006
Se	EN14627:2005	986.15**	NA
Tl	NA	NA	NA

CEN: European Committee for Standardization or Comité Européen de Normalisation.
AOAC: Association of Analytical Communities.
ISO: International Organization for Standardization.
*: in animal feeding stuff; **: in pet food.

Maximum Tolerable Levels

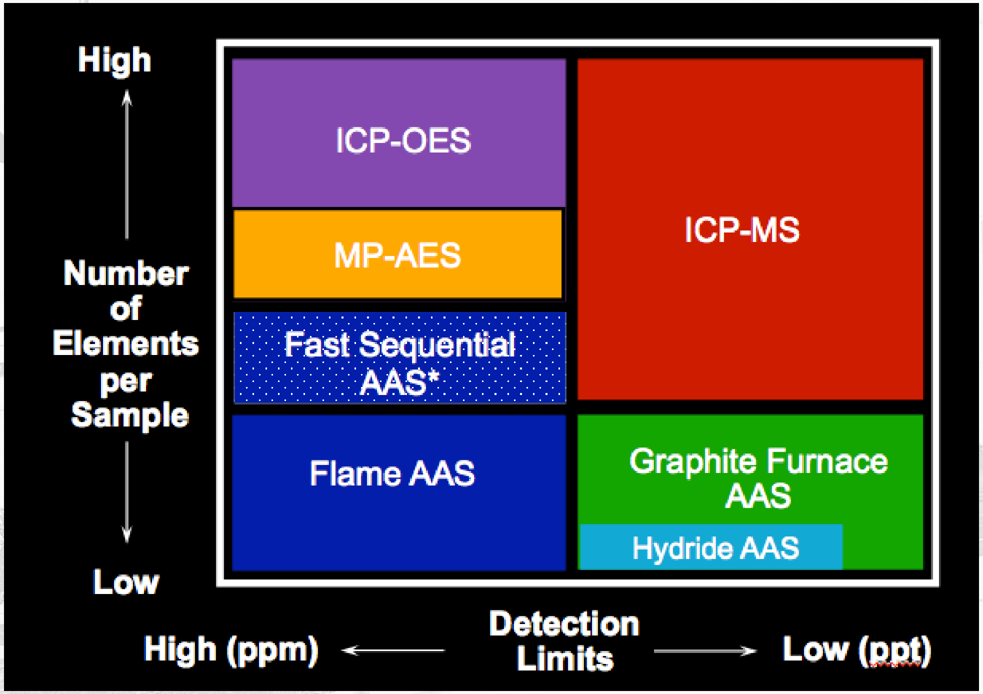
Analyte	Maximum Tolerable Level in Complete Feed (ppm) a	Maximum Level in food supplements (ppm) b	LOQ by ICP-OES in mineral additive and fishmeal (ppm) OTSC
As	30	NA	10
Cd	10	1	1
Cr	100	NA	20
Hg	0.2	0.1	NA
Pb	10	3	10
Se	3	NA	40
Tl	1	NA	NA

a: info was provided by Dr. Lynn Post
b: EU regulation No 1881/2006

Comparison of Detection Limits

Analyte	ICP-MS	ICP-OES	Flame AAS	GF-AAS
As	0.05	5	500	1
Cd	0.01	0.5	5	0.03
Cr	0.1	1	10	0.5
Hg	0.01	1	300	0.6
Pb	0.005	5	20	0.5
Se	0.1	5	1000	1
Tl	0.01	5	40	1.5

Unit: ug/L or ppb in solution

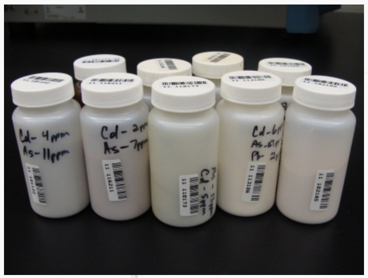


Agilent website-Atomic Spectroscopy

ICP-MS Instrumentation

- ICP-MS in house: NexION 300
- Highlight:
  - Three modes of operation (Standard, Collision and Reaction)
  - High sensitivity
  - High throughput
  - Minimal user maintenance

Sample Preparation

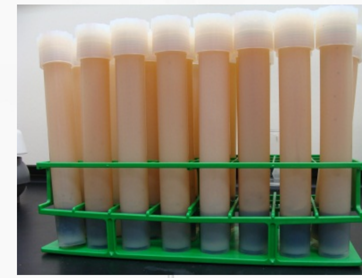


Sample

→

Acid Digestion

HNO_3:HCl 3:1



↓

Microwave Digestion

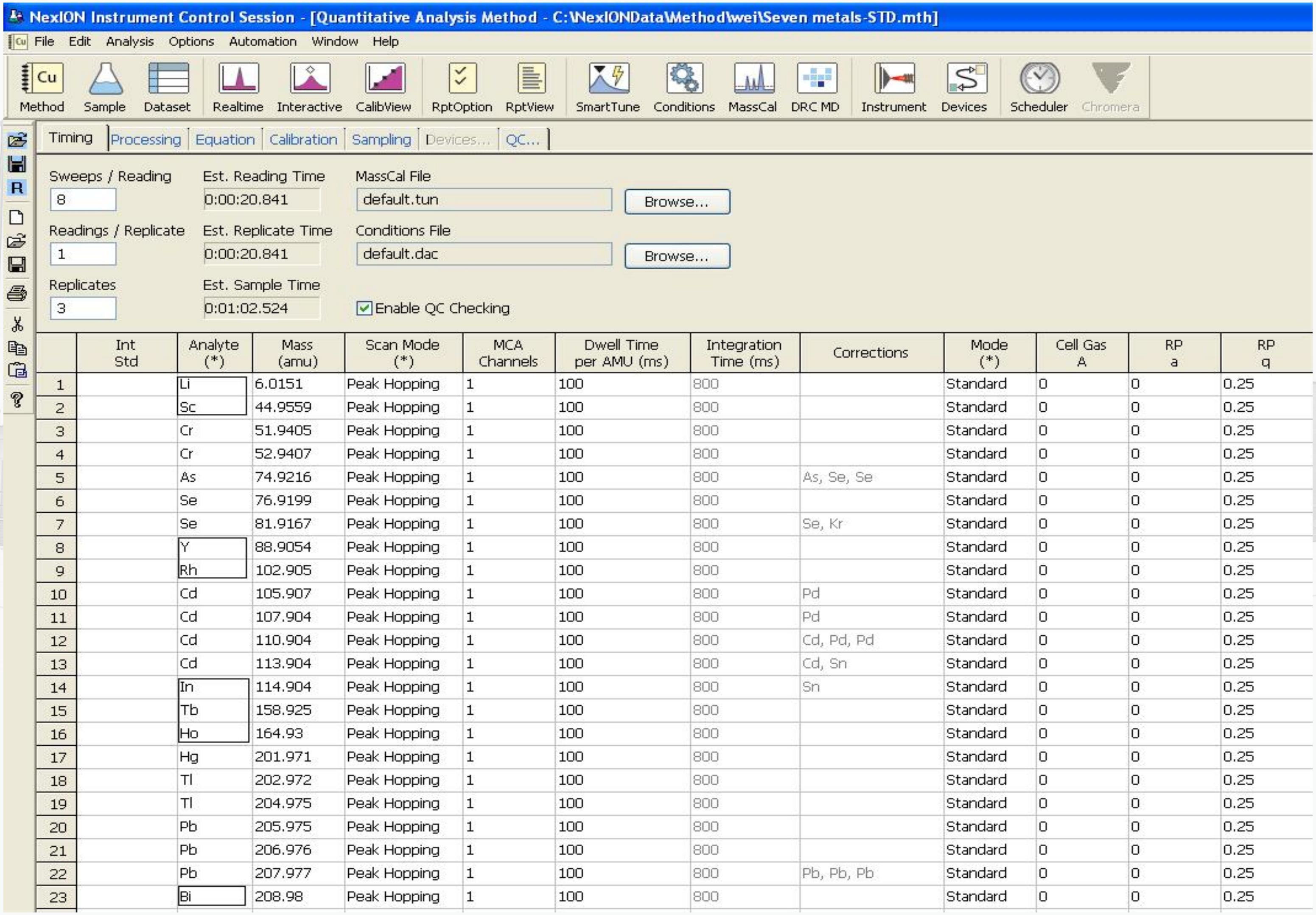
←

Dilution/Filtration

↘

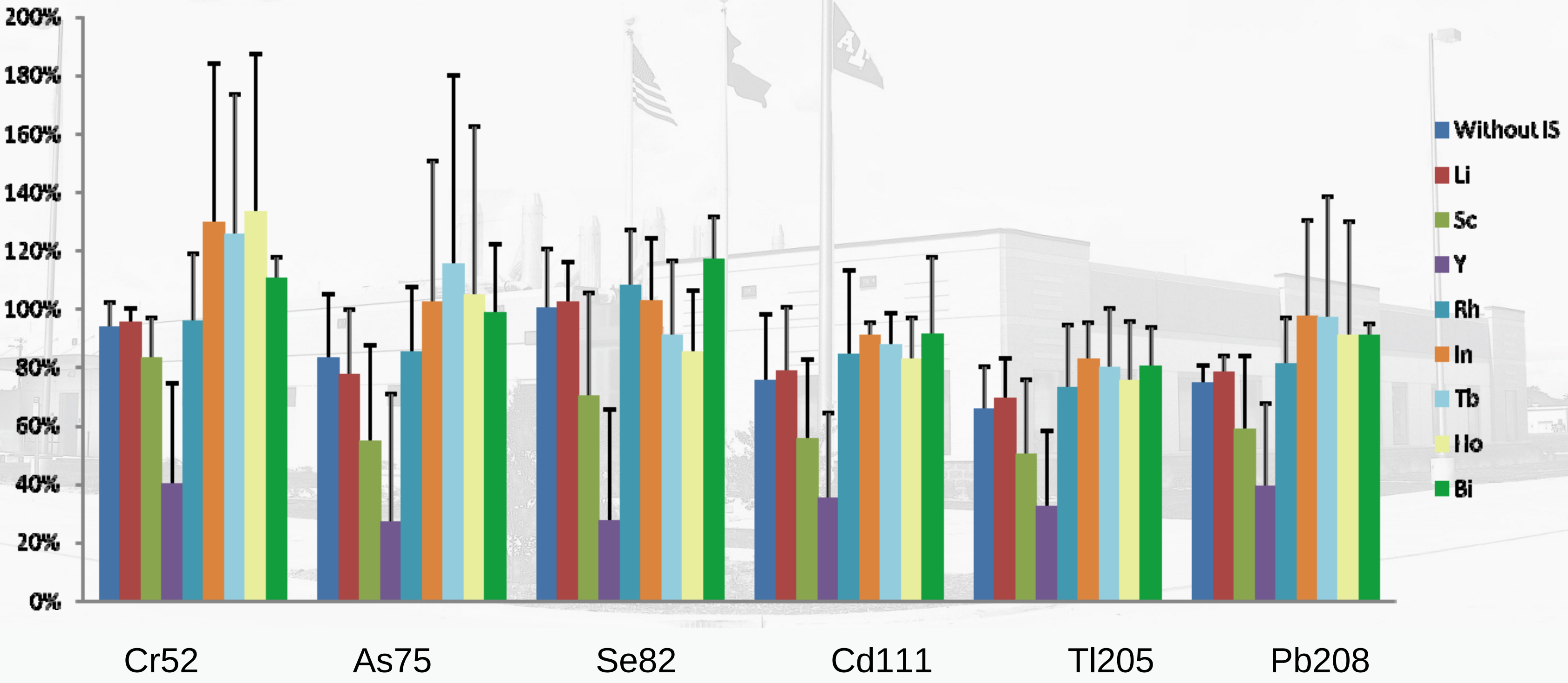
ICP-MS Analysis

Method Development-Standard Mode



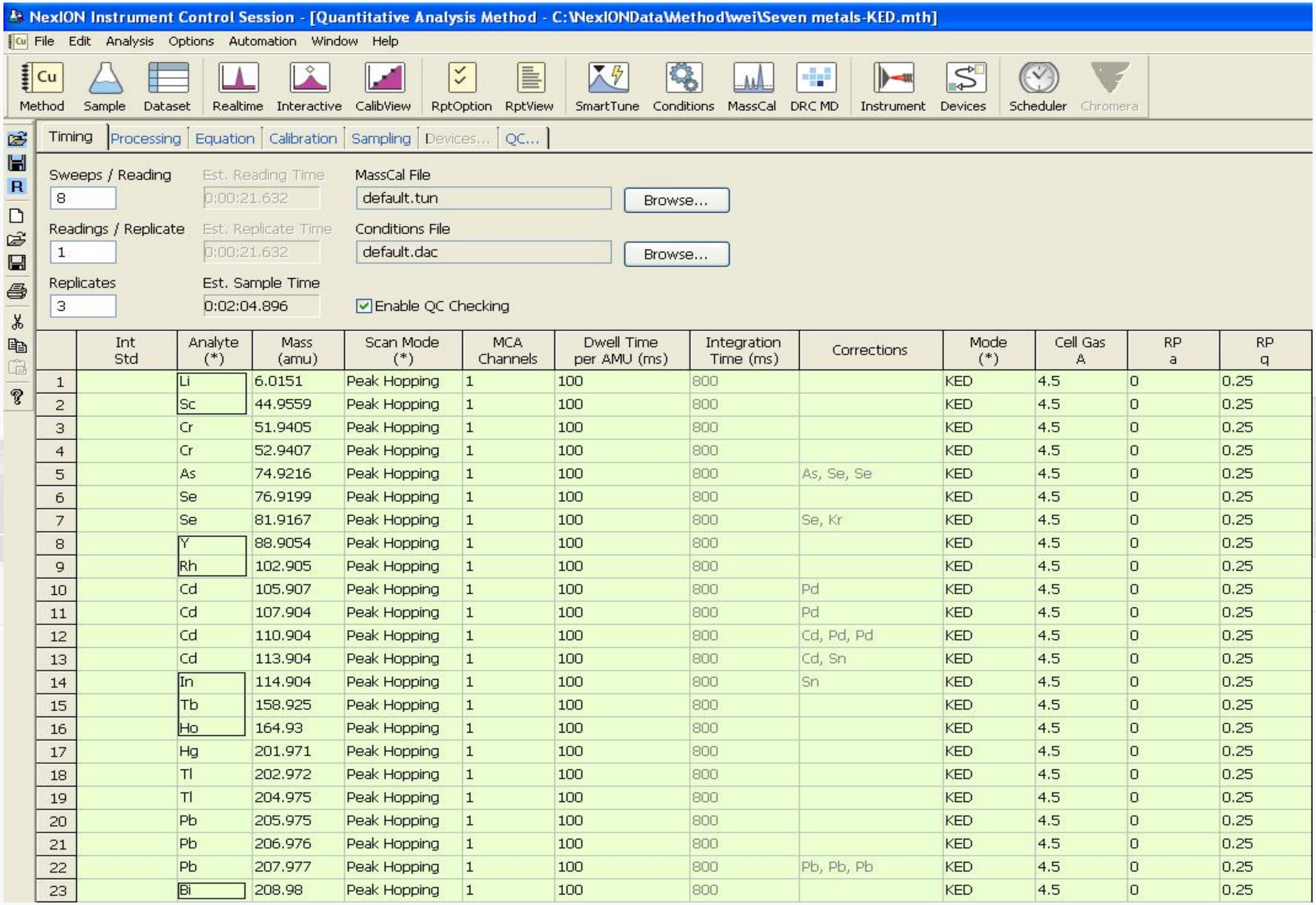
	Int Std	Analyte (*)	Mass (amu)	Scan Mode (*)	MCA Channels	Dwell Time per AMU (ms)	Integration Time (ms)	Corrections	Mode (*)	Cell Gas A	RP a	RP q
1		Li	6.0151	Peak Hopping	1	100	800		Standard	0	0	0.25
2		Sc	44.9559	Peak Hopping	1	100	800		Standard	0	0	0.25
3		Cr	51.9405	Peak Hopping	1	100	800		Standard	0	0	0.25
4		Cr	52.9407	Peak Hopping	1	100	800		Standard	0	0	0.25
5		As	74.9216	Peak Hopping	1	100	800	As, Se, Se	Standard	0	0	0.25
6		Se	76.9199	Peak Hopping	1	100	800		Standard	0	0	0.25
7		Se	81.9167	Peak Hopping	1	100	800	Se, Kr	Standard	0	0	0.25
8		Y	88.9054	Peak Hopping	1	100	800		Standard	0	0	0.25
9		Rh	102.905	Peak Hopping	1	100	800		Standard	0	0	0.25
10		Cd	105.907	Peak Hopping	1	100	800	Pd	Standard	0	0	0.25
11		Cd	107.904	Peak Hopping	1	100	800	Pd	Standard	0	0	0.25
12		Cd	110.904	Peak Hopping	1	100	800	Cd, Pd, Pd	Standard	0	0	0.25
13		Cd	113.904	Peak Hopping	1	100	800	Cd, Sn	Standard	0	0	0.25
14		In	114.904	Peak Hopping	1	100	800	Sn	Standard	0	0	0.25
15		Tb	158.925	Peak Hopping	1	100	800		Standard	0	0	0.25
16		Ho	164.93	Peak Hopping	1	100	800		Standard	0	0	0.25
17		Hg	201.971	Peak Hopping	1	100	800		Standard	0	0	0.25
18		Tl	202.972	Peak Hopping	1	100	800		Standard	0	0	0.25
19		Tl	204.975	Peak Hopping	1	100	800		Standard	0	0	0.25
20		Pb	205.975	Peak Hopping	1	100	800		Standard	0	0	0.25
21		Pb	206.976	Peak Hopping	1	100	800		Standard	0	0	0.25
22		Pb	207.977	Peak Hopping	1	100	800	Pb, Pb, Pb	Standard	0	0	0.25
23		Bi	208.98	Peak Hopping	1	100	800		Standard	0	0	0.25

Selection of Internal Standards



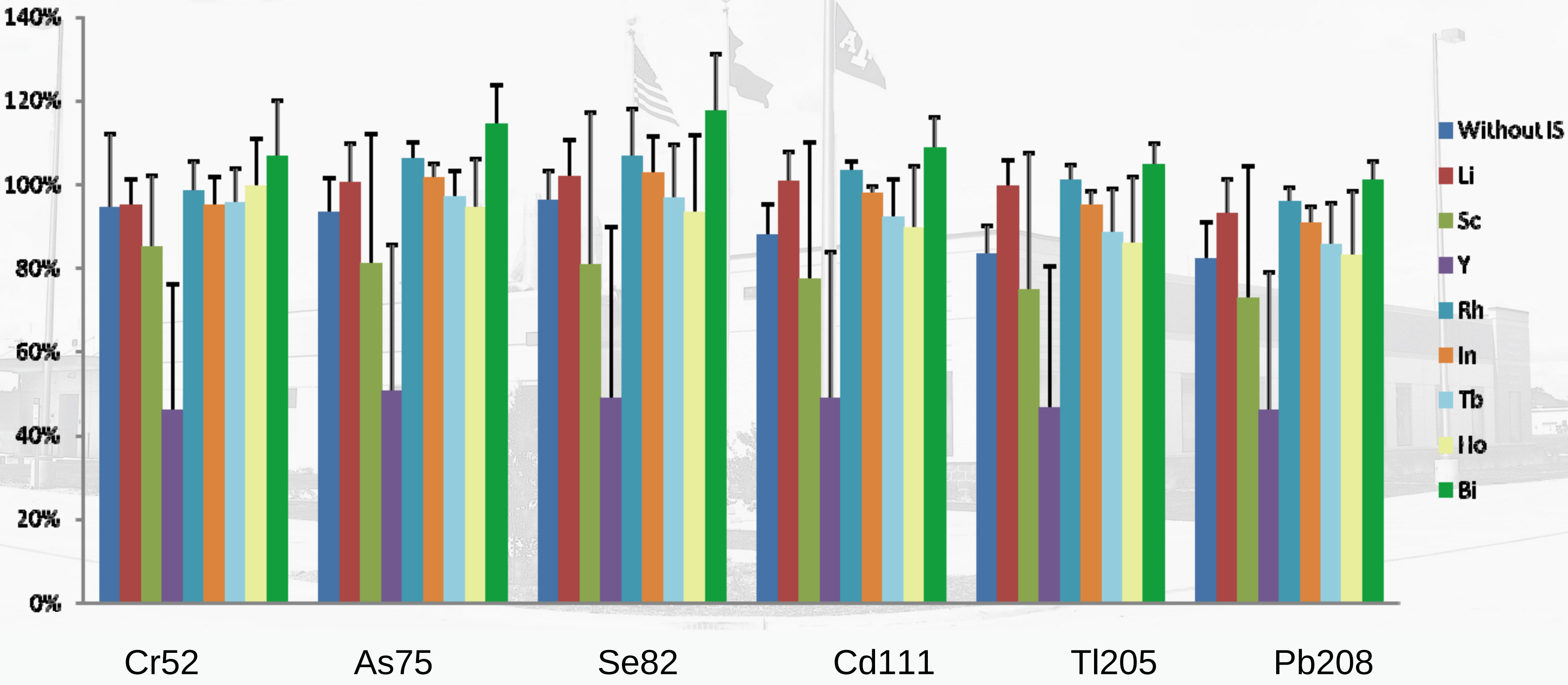
Recovery study of Standard addition (10 ppm) into 12 samples with **Standard Mode**.

Method Development-Collision Mode



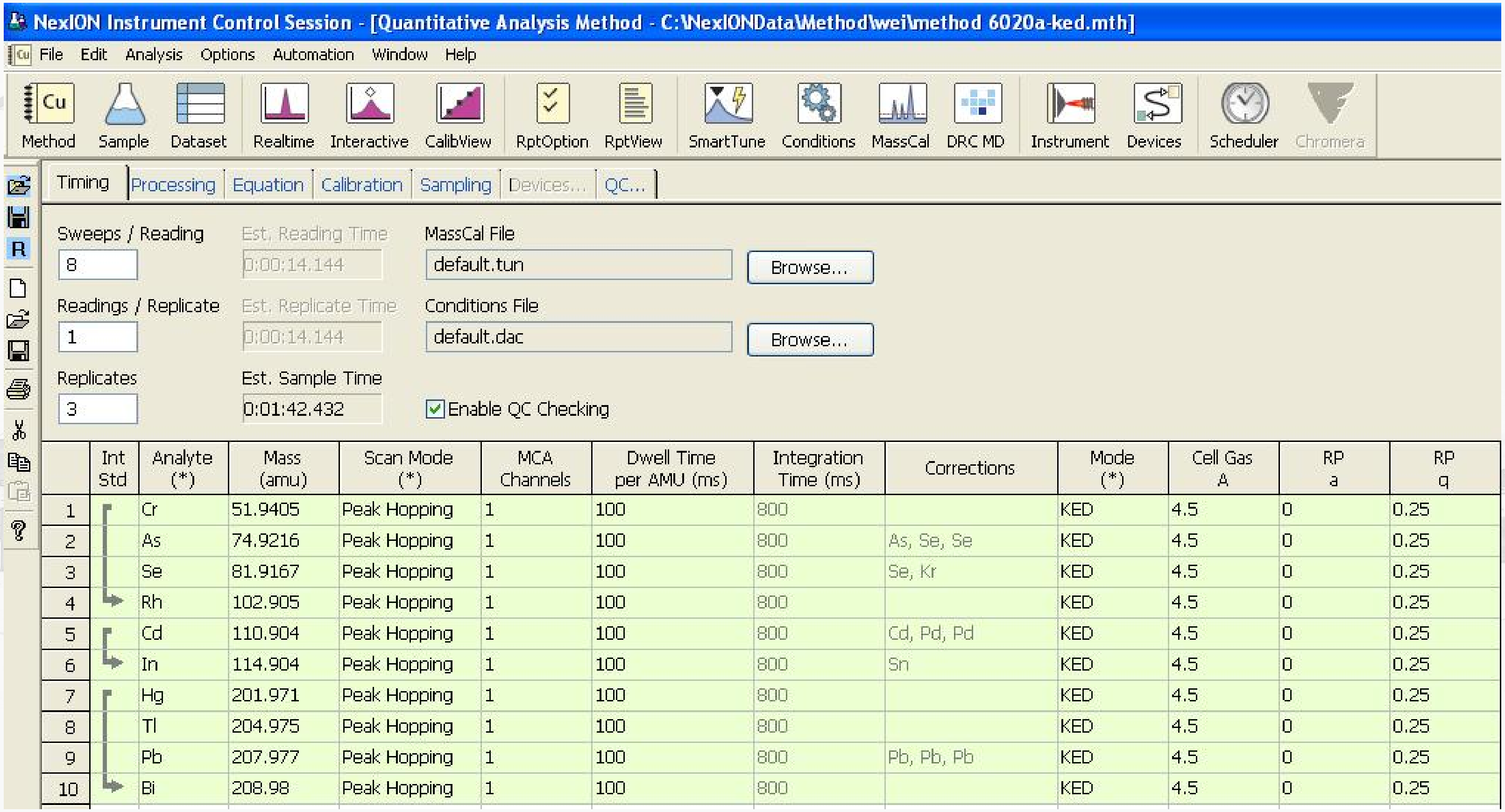
	Int Std	Analyte (*)	Mass (amu)	Scan Mode (*)	MCA Channels	Dwell Time per AMU (ms)	Integration Time (ms)	Corrections	Mode (*)	Cell Gas A	RP a	RP q
1		Li	6.0151	Peak Hopping	1	100	800		KED	4.5	0	0.25
2		Sc	44.9559	Peak Hopping	1	100	800		KED	4.5	0	0.25
3		Cr	51.9405	Peak Hopping	1	100	800		KED	4.5	0	0.25
4		Cr	52.9407	Peak Hopping	1	100	800		KED	4.5	0	0.25
5		As	74.9216	Peak Hopping	1	100	800	As, Se, Se	KED	4.5	0	0.25
6		Se	76.9199	Peak Hopping	1	100	800		KED	4.5	0	0.25
7		Se	81.9167	Peak Hopping	1	100	800	Se, Kr	KED	4.5	0	0.25
8		Y	88.9054	Peak Hopping	1	100	800		KED	4.5	0	0.25
9		Rh	102.905	Peak Hopping	1	100	800		KED	4.5	0	0.25
10		Cd	105.907	Peak Hopping	1	100	800	Pd	KED	4.5	0	0.25
11		Cd	107.904	Peak Hopping	1	100	800	Pd	KED	4.5	0	0.25
12		Cd	110.904	Peak Hopping	1	100	800	Cd, Pd, Pd	KED	4.5	0	0.25
13		Cd	113.904	Peak Hopping	1	100	800	Cd, Sn	KED	4.5	0	0.25
14		In	114.904	Peak Hopping	1	100	800	Sn	KED	4.5	0	0.25
15		Tb	158.925	Peak Hopping	1	100	800		KED	4.5	0	0.25
16		Ho	164.93	Peak Hopping	1	100	800		KED	4.5	0	0.25
17		Hg	201.971	Peak Hopping	1	100	800		KED	4.5	0	0.25
18		Tl	202.972	Peak Hopping	1	100	800		KED	4.5	0	0.25
19		Tl	204.975	Peak Hopping	1	100	800		KED	4.5	0	0.25
20		Pb	205.975	Peak Hopping	1	100	800		KED	4.5	0	0.25
21		Pb	206.976	Peak Hopping	1	100	800		KED	4.5	0	0.25
22		Pb	207.977	Peak Hopping	1	100	800	Pb, Pb, Pb	KED	4.5	0	0.25
23		Bi	208.98	Peak Hopping	1	100	800		KED	4.5	0	0.25

Selection of Internal Standards



Recovery study of Standard addition (10 ppm) into 12 samples with **Collision Mode**.

Method Development



	Int Std	Analyte (*)	Mass (amu)	Scan Mode (*)	MCA Channels	Dwell Time per AMU (ms)	Integration Time (ms)	Corrections	Mode (*)	Cell Gas A	RP a	RP q
1		Cr	51.9405	Peak Hopping	1	100	800		KED	4.5	0	0.25
2		As	74.9216	Peak Hopping	1	100	800	As, Se, Se	KED	4.5	0	0.25
3		Se	81.9167	Peak Hopping	1	100	800	Se, Kr	KED	4.5	0	0.25
4		Rh	102.905	Peak Hopping	1	100	800		KED	4.5	0	0.25
5		Cd	110.904	Peak Hopping	1	100	800	Cd, Pd, Pd	KED	4.5	0	0.25
6		In	114.904	Peak Hopping	1	100	800	Sn	KED	4.5	0	0.25
7		Hg	201.971	Peak Hopping	1	100	800		KED	4.5	0	0.25
8		Tl	204.975	Peak Hopping	1	100	800		KED	4.5	0	0.25
9		Pb	207.977	Peak Hopping	1	100	800	Pb, Pb, Pb	KED	4.5	0	0.25
10		Bi	208.98	Peak Hopping	1	100	800		KED	4.5	0	0.25

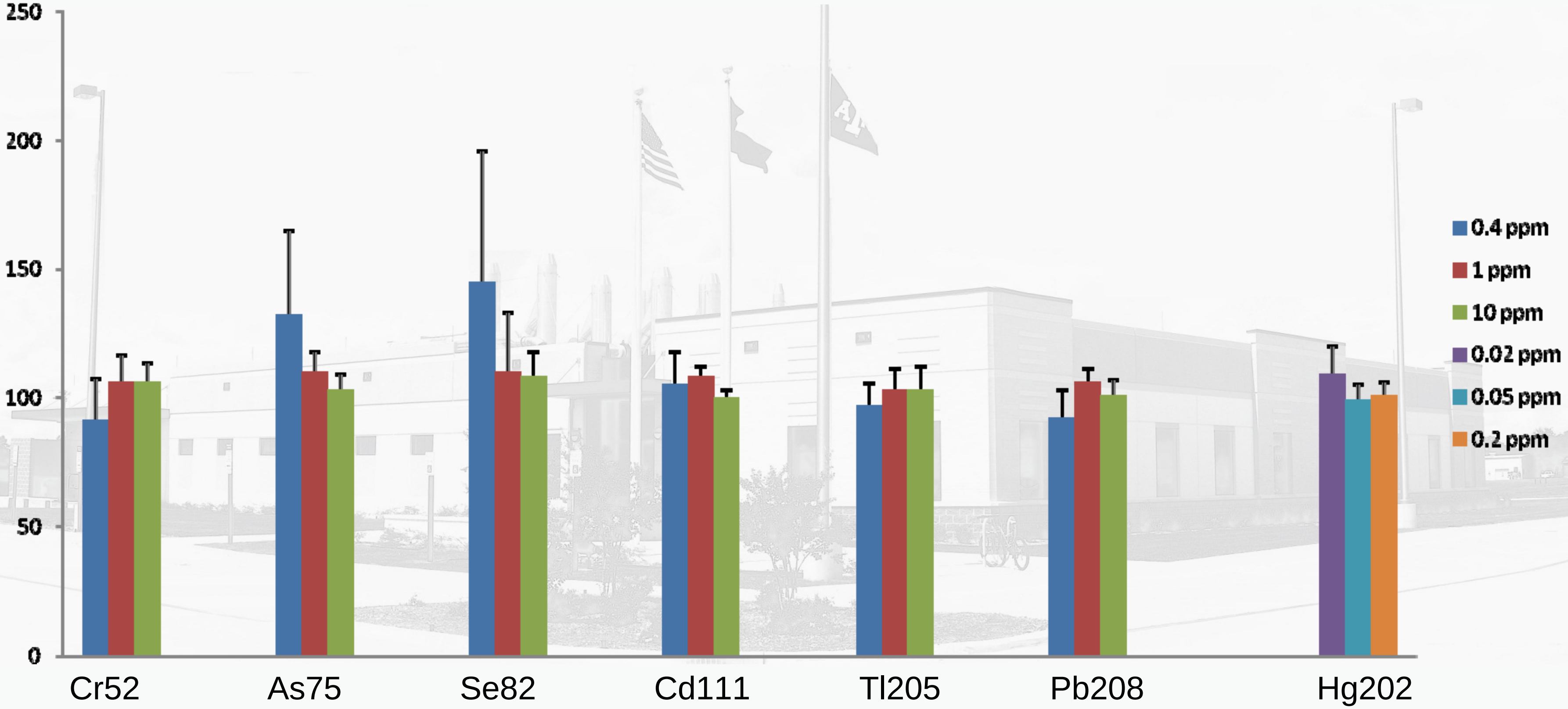
Calibration Curves

Analyte	Mass	Correlation Coefficient	Calibration Range (ppb)
Cr	51.941	0.999929	1~100
As	74.922	0.999974	1~100
Se	81.917	0.999953	5~100
Cd	110.904	0.999990	1~100
Tl	204.975	0.999999	1~100
Pb	207.977	0.999991	1~100
Hg	201.971	0.999911	0.05~1

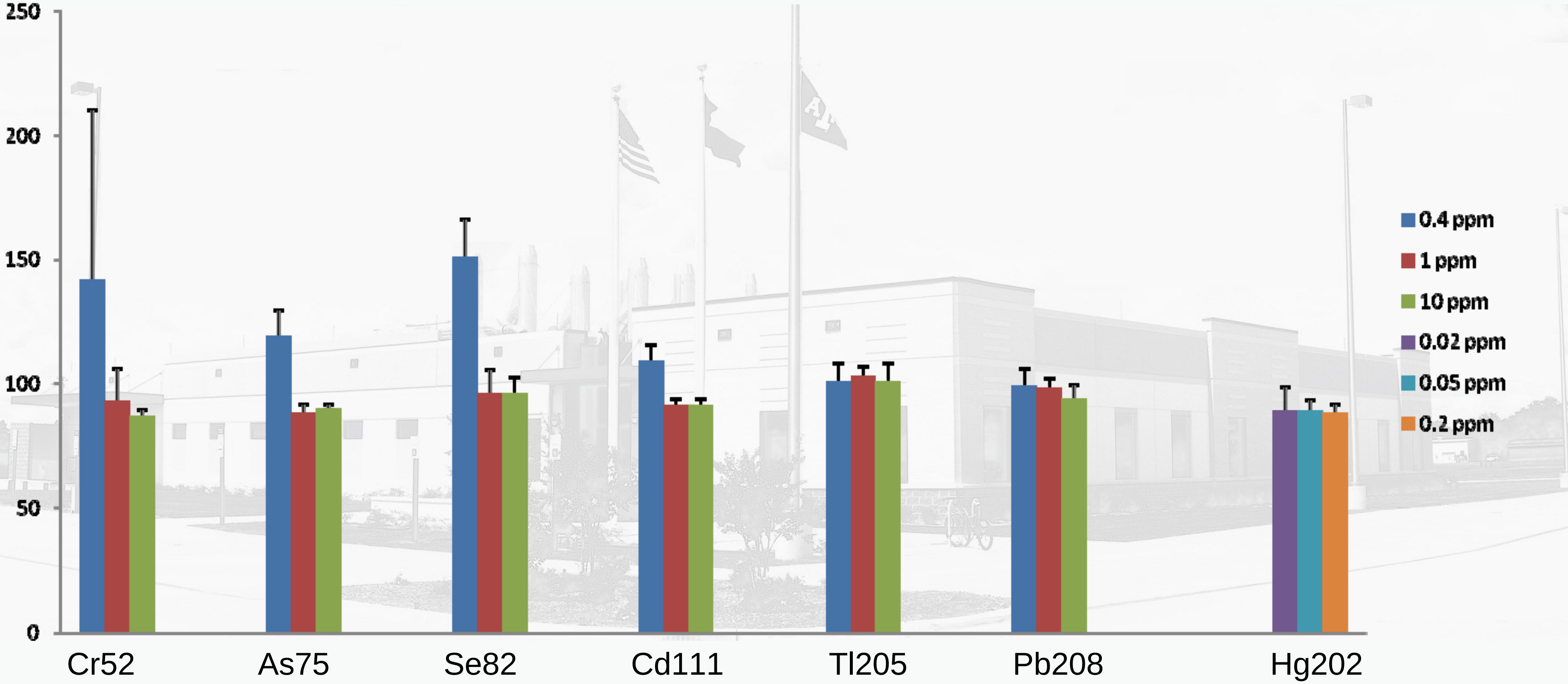
Limit of Quantitation (LOQ)

Analyte	Maximum Tolerable Levels in Complete Feed (ppm)	LOQ by ICP-OES (ppm)	LOQ by ICP-MS in mineral (ppm)	LOQ by ICP-MS in fishmeal (ppm)
Chromium (Cr)	100	20	0.35	1.80
Arsenic (As)	30	10	0.55	0.35
Selenium (Se)	3	40	2.50	2.50
Cadmium (Cd)	10	1	0.35	0.45
Thallium (Tl)	1	NA	0.03	0.12
Lead (Pb)	10	10	0.02	0.20
Mercury (Hg)	0.2	NA	0.02	0.02

Recovery Study in Mineral Matrix



Recovery Study in Fishmeal Matrix



Results of SRM 695b

Analyte	Expected Value ($\mu g\ g^{-1}$)	ICP-MS measurement ($\mu g\ g^{-1}$)	RSD (%) of ICP-MS measurements (N=9)
Cr	244 ± 6	237	11
As	200 ± 5	201	6
Se	2.1 ± 0.1	4.8	25
Cd	16.9 ± 0.2	16.5	3
Tl	N/A	0.6	7
Pb	273 ± 17	269	4
Hg	1.96 ± 0.036	1.73	4

Conclusion

- An ICP-MS method was developed and validated for quantitation of seven metals in feed sample.
- The new ICP-MS method has much lower LOQs than the ICP-OES method.
- The ICP-MS method can be applied for testing mineral and fishmeal matrices.

Acknowledgement

- Dr. Tim Herrman
- Ben Jones
- Dr. Susie Dai
- Dr. Jim Balthrop
- Dr. Lynn Post
- Sara Williams
- James Embry
- Elemental Analysis Group

Office of the Texas State Chemist

OFFICE OF THE TEXAS STATE CHEMIST

445 Agronomy Road • College Station, TX 77840

(979) 845 1121 http://otsc.tamu.edu

Questions