Metallomics, Toxicology and Trace Elemental Analysis – Use of TXRF for Clinical Applications

Bruker AXS
Madison, WI

Welcome

Today's Topics

- TXRF – how does it work?
- Trace elemental analysis in clinical chemistry
- Tissue analysis
- Biological liquid applications
- Interactive Q & A

Speakers

Michael Beauchaine
Business Development Scientist TXRF
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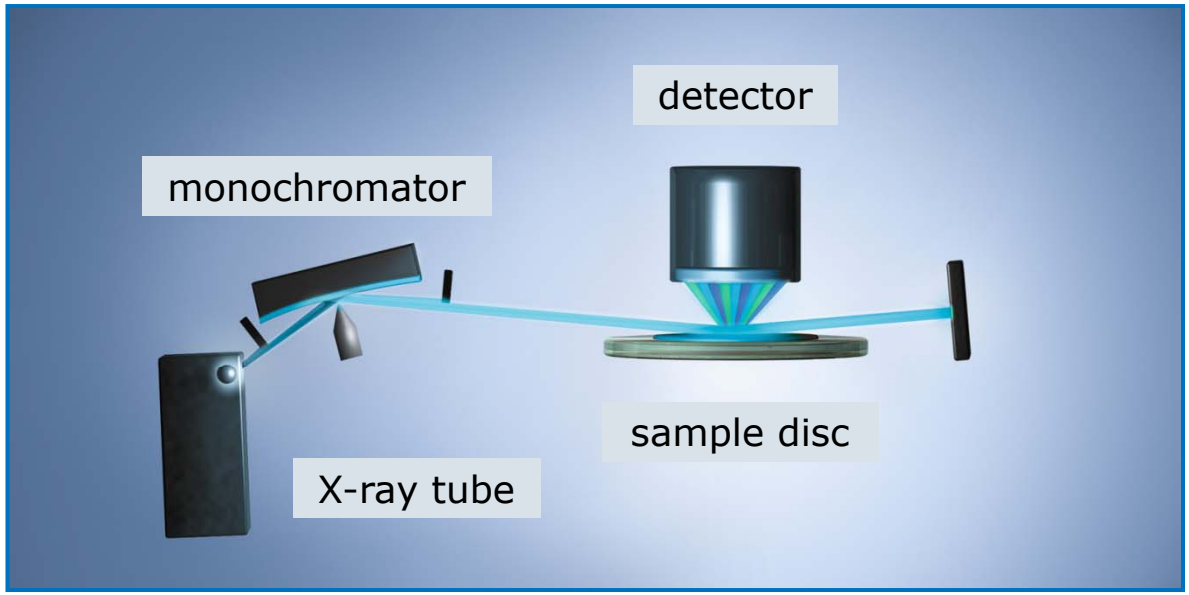
Global Product Manager TXRF
Berlin, Germany

TXRF – How does it work?

Principles of total reflection X-ray fluorescence (TXRF) spectroscopy

Total reflection X-ray fluorescence spectroscopy



Beam angle: 0° / 90°

- Samples must be prepared on a reflective media
- Polished quartz glass or polyacrylic glass disc
- Dried to a thin layer, or as a thin film or microparticle

Principles of total reflection X-ray fluorescence spectroscopy

Samples for TXRF

- Powders: Direct preparation or as suspension
- Liquids: Direct preparation
- Always as a thin film, micro fragment or suspension of a powder
- Necessary sample amount: Low µg respectively µl range

Simple quantification

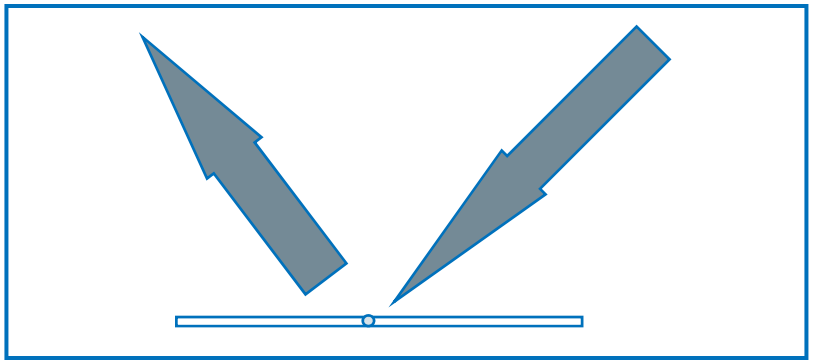
➔ Matrix effects are negligible due to thin layer

➔ Quantification is possible by internal standardization

Principles of total reflection X-ray fluorescence spectroscopy

In TXRF the samples are prepared as thin films or layers

- ➔ Matrix effects are negligible
- ➔ Quantification is possible

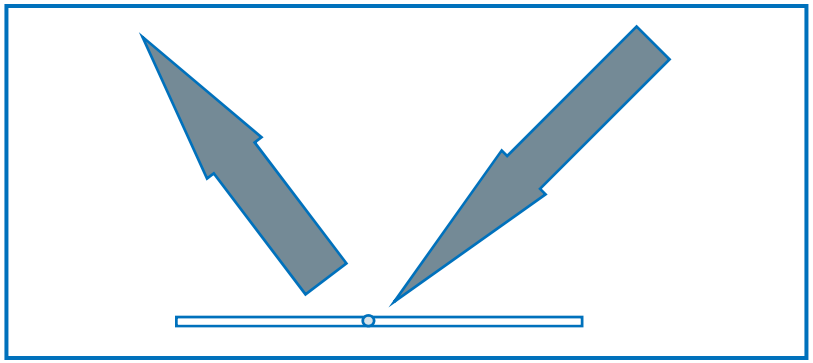


Principles of total reflection X-ray fluorescence spectroscopy

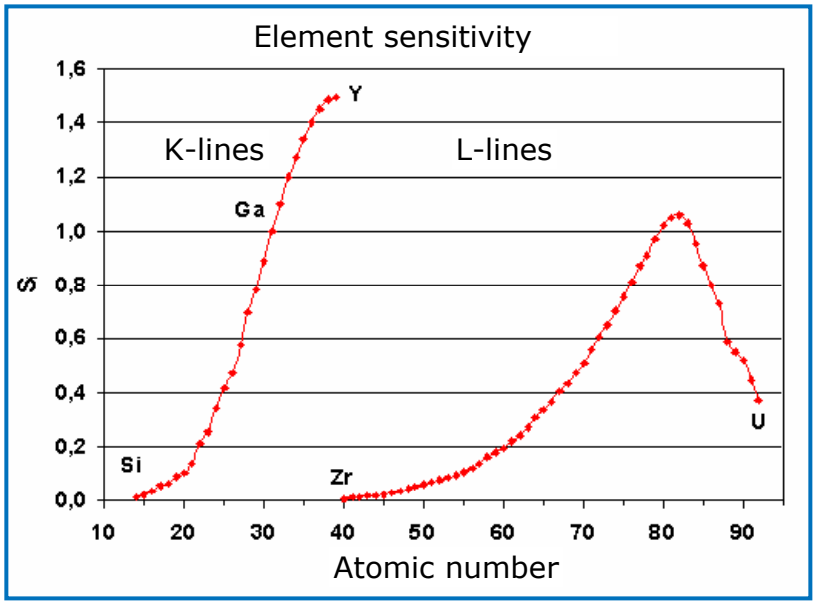
In TXRF the samples are prepared as thin films or layers

➔ Matrix effects are negligible

➔ Quantification is possible



- TXRF detects elements from Na(11) to U(92)
- The element sensitivities depend on the atomic number
- The sensitivity factors are calibrated ex works
- Quantification requires the addition of one standard element



The instrument S2 PICOFOX

Benchtop TXRF spectrometer
S2 PICOFOX

- Metal-ceramic X-ray tube
  - Mo anode
  - Air-cooled
  - Optionally other tubes available
- Multilayer monochromator
- XFlash® silicon drift detector
  - Electro-thermally cooled
  - ≤149 eV @ MnKα 100 kcps
- Automatic version
  - 25 sample cassette

Introduction to Trace Elements in Clinical Chemistry

Metallomics

New frontier within interdisciplinary science investigating trace elements and the role of metals in biological, environmental and clinical systems.

* Metallomics Journal – RSC Publishing

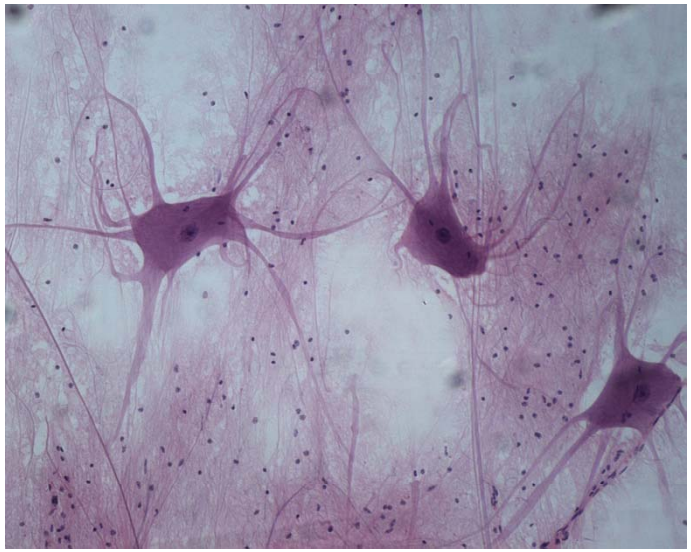
Metallomics

Research topics include

- Regulation of the uptake, accumulation and metabolism of metals and other trace elements in biological systems
- The interaction of metallodrugs, incl. chemotherapy agents
- Elemental distribution and concentrations linked to genomics
- Chemical speciation, dynamics, and kinetics of trace elements in biological systems.
- Physiological and pathological mechanisms related to trace elements in human health and disease
- Metal exchange between biota and the environment
- Biosensing of metals, including diagnostic and therapeutic radioactive metals
- Instrumentation and methods demonstrating solutions in are of metallomics

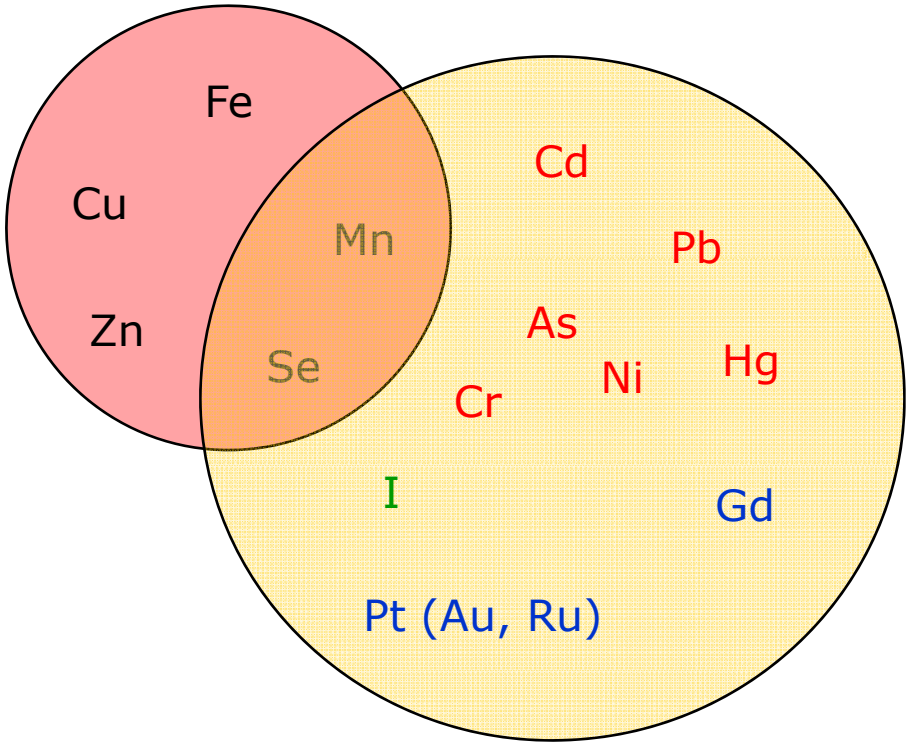
Trace Essential Elements
Impact on Clinical Chemistry

- Metals are increasingly used in dietary supplements for health and prevention of disease
- Essential trace elements or micro-nutrients are those with concentrations below 50 ppm in humans
- Classified as “essential” when a deficiency causes a medical symptom and a nutritional supplementation will avoid or relieve such symptom
- Excessive uptake can lead to intoxication (Se, Cu, Mo, and Cr)



Elemental analysis in medicine

Standard method in clinical chemistry
Atomic absorption spectrometry (AAS)



Toxicology, forensic medicine
Oncology, Endocrinology
Research

Inductively Coupled Plasma Optical Emmission Spectrometry (ICP-OES) / Mass Spectrometry (ICP-MS)

Trace Essential Elements
Biological Function

Element	Good nutrition sources	Metabolic function	RDA*	Deficiency symptoms
Chromium	meat, whole grain, vegetable oil, beer	compound of Glucose Tolerance Factor (sugar metabolism)	35 µg	depression
Cobalt	meat, shellfish, milk, eggs	compound of Cobalamin (Vit B-12)	2 - 3 µg	fatigue, depression
Iron	meat, green vegetables, fish, eggs, whole grain	compound of many enzymes, e.g. P450 monooxygenase	8 mg	iron deficiency anemia
Iodine	seafish, shellfish	compound of thyroid hormones	150 µg	goitre, cretinism
Copper	whole grain, nuts, cocoa, green vegetables, fish, shellfish	compound of many redox enzymes, e.g. cytochrome c oxidase	900 µg	anemia-like symptoms, risk factor for cancer
Manganese	black tea, nuts, whole grain, green vegetables	activator of many enzymes -> anti-oxidant metabolism, bone synthesis, gluconeogenesis	2.3 mg	immune deficiency, blood coagulation disorder
Molybdenum	ubiquitary	compound of the universal molybde-num cofactor	45 µg	risk factor for cancer, immune deficiency
Nickel	nuts, vegetables, cereals	compound of many enzymes, e.g. urease or hydrogenases	not det.	not fully clearified
Selenium	meat, nuts, fish	compound of 30-50 selenoproteins, e.g. glutathione peroxidase	55 µg	risk factor for cancer, immune deficiency
Zinc	animal food, cheese, fish, shell-fish, whole grain, seeds	zinc dependent enzymes are involved in almost all metabolic and cell signal-ing functions, e.g. alcohol dehydroge-nase, carbonic anhydrase	11 mg	dermatitis, risk factor for cancer, immune deficiency

*) Recommended Dietary Allowance, US Department of Agriculture

Trace Essential Elements
Biological Matrices Analyzed with TXRF

Biological matrix	Typical volume	Sample preparation for TXRF
Blood - whole blood [1]	500 µl	1 : 1 dilution with H_2O, addition of internal Ga standard
Blood - serum [1]	500 µl	1 : 10 dilution with H_2O, addition of internal Ga standard
Blood - serum, small volumes	< 10 µl	1 : 2 dilution with H_2O, pipetting on carrier addition of 1 µl Ga standard solution
Urine	ml	direct addition of internal standard, fume off chlorine with HNO_3
Tissue homogenates from mice	15 µl	1 : 1 dilution with Y standard solution or digestion in 65 % HNO_3, 1 h, 70°C
Seminal fluid	µl	direct addition of internal standard
Cerebrospinal fluid	µl	direct addition of internal standard
Mother's milk	ml	direct addition of internal standard
Tear fluid	µl to ml	direct addition of internal standard

1) for details see Lab Report XRF 77, Trace Element Analysis of Blood Samples

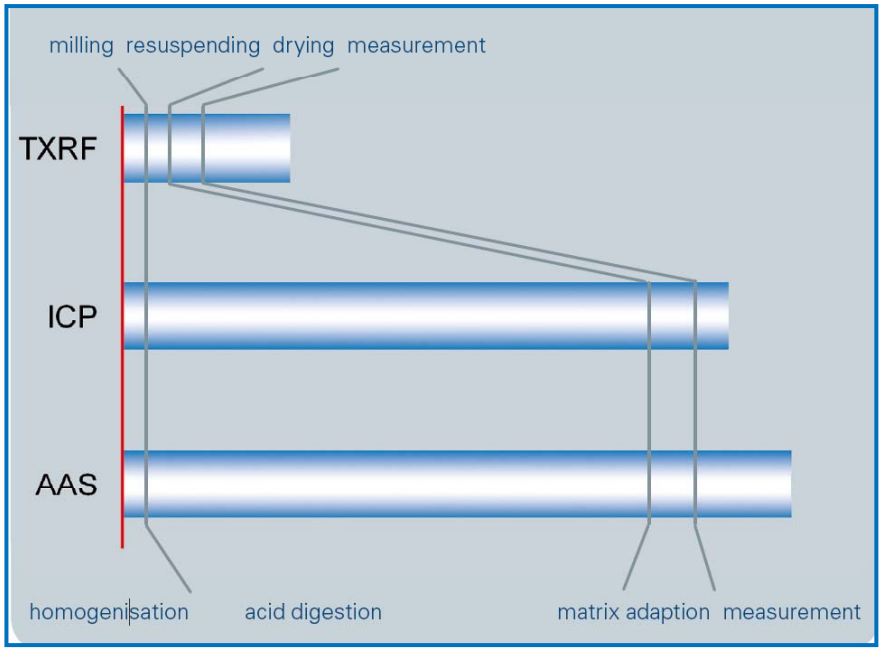
Trace Essential Elements Measurement Techniques

Common techniques for trace element analysis

- ICP-OES (inductively coupled plasma optical emission spectroscopy)
- AAS (atomic absorption spectroscopy)

Both techniques require

- Time consuming sample preparation
- Use and digestion of harmful acids
- Larger amount of sample
- Dilution of sample



Tissue Analysis by TXRF Spectroscopy

Application studies
Rapid screening of fish samples

Fish as nutrient

- Nutrition relevant elements like Cu, Zn and Se in food are regularly monitored

Fish as bioindicator

- Accumulation of heavy metals varies with route of uptake and species of fish
- Use as biomonitors for assessment of bioaccumulation of contaminants within ecosystems

Zebrafish *Danio rerio*

Application studies
Rapid screening of fish samples

Fish as nutrient

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Task

- Analysis of metal content in fish standard DORM-3*

Zebrafish *Danio rerio*

*) Fish protein,
Canadian National Research Council
Preparation by removal of bones and oil, subsequent enzyme hydrolysis; protein hydrolysate was spray dried, sieved (297 µm screen), blended and bottled.

Application studies
Sample preparation for rapid screening

Sample preparation of plants, tissues, grains

Solid materials are ground to fine particle size and resuspended for direct analysis without digestion

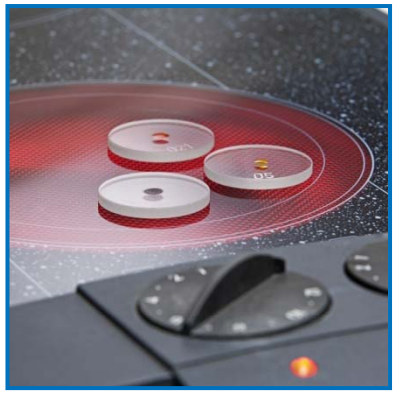


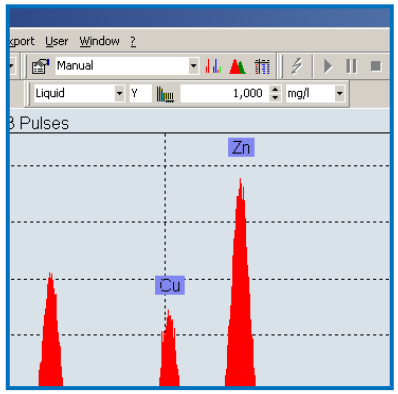

- fill powder in mortar
- grind carefully
- weigh about 20-50 mg
- transfer to tube

Application studies
Sample preparation for rapid screening


- suspend in detergent solution
- add standard
- homogenize
- pipette on carrier

Application studies
Sample preparation for rapid screening



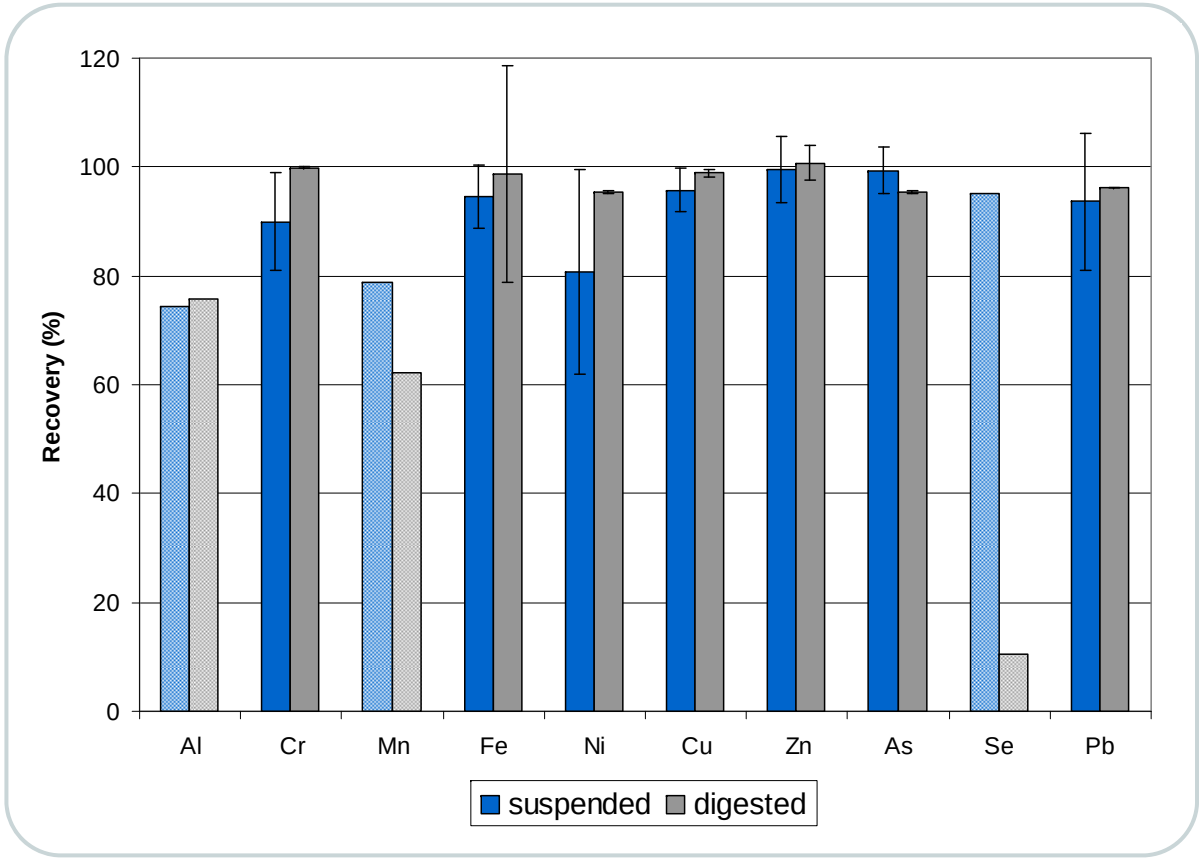


Dry through heat/vacuum → Load the instrument → Start data acquisition

Application studies
Rapid screening of fish samples

Recovery

- Cd, Sn and Hg could not be determined due to line overlaps with Molybdenum unit
- All other element concentrations are in good concordance with reference values
- Only informational values are available for Al, Mn and Se

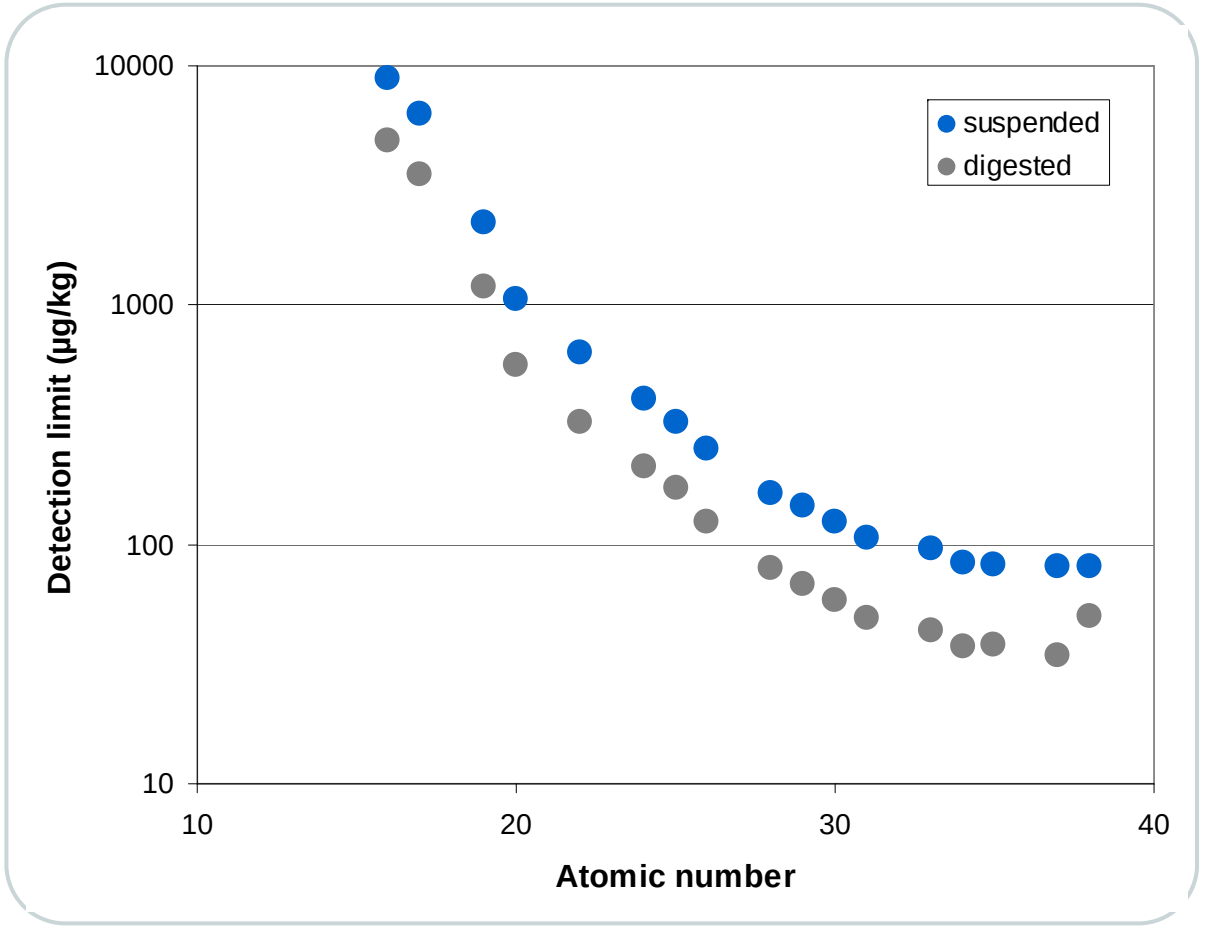


n = 10

Application studies
Rapid screening of fish samples

Detection limits

- Detection limits are improved by a factor of 2 by sample digestion
- Digestion causes partial loss of Cl, Se and Br
- Reproducibility remains unchanged



Application studies
Analysis of Se

The analysis of Se (and other elements) in biological matrices is not a trivial task!

Application studies
Analysis of Se

Sample preparation required for Se analysis in blood samples by ICP-OES

- Digestion with a "cocktail" of 80% H_2SO_4, 12% $HClO_4$ and 8% HNO_3
- Operation of a hydride generator required separates elements like Se from matrix
- Achievable detection limit is 5 µg/l

Recknagel, S. et al. (1993): Determination of Selenium in blood serum by ICP-OES including an on-line wet digestion and Se-hydration formation procedure, Fresenius J. Anal. Chem., 346, 833-836

Application studies
Analysis of Se

Conclusion

- Se analysis with ICP-MS or ICP-OES is laborious and costly
- AAS is not suitable for multi-element analysis

Opportunities

- TXRF enables fully quantitative results for extremely small sample amounts
- Required detection limits accomplished (10 – 200 µg/L)

Application studies
Analysis of Se

Known facts

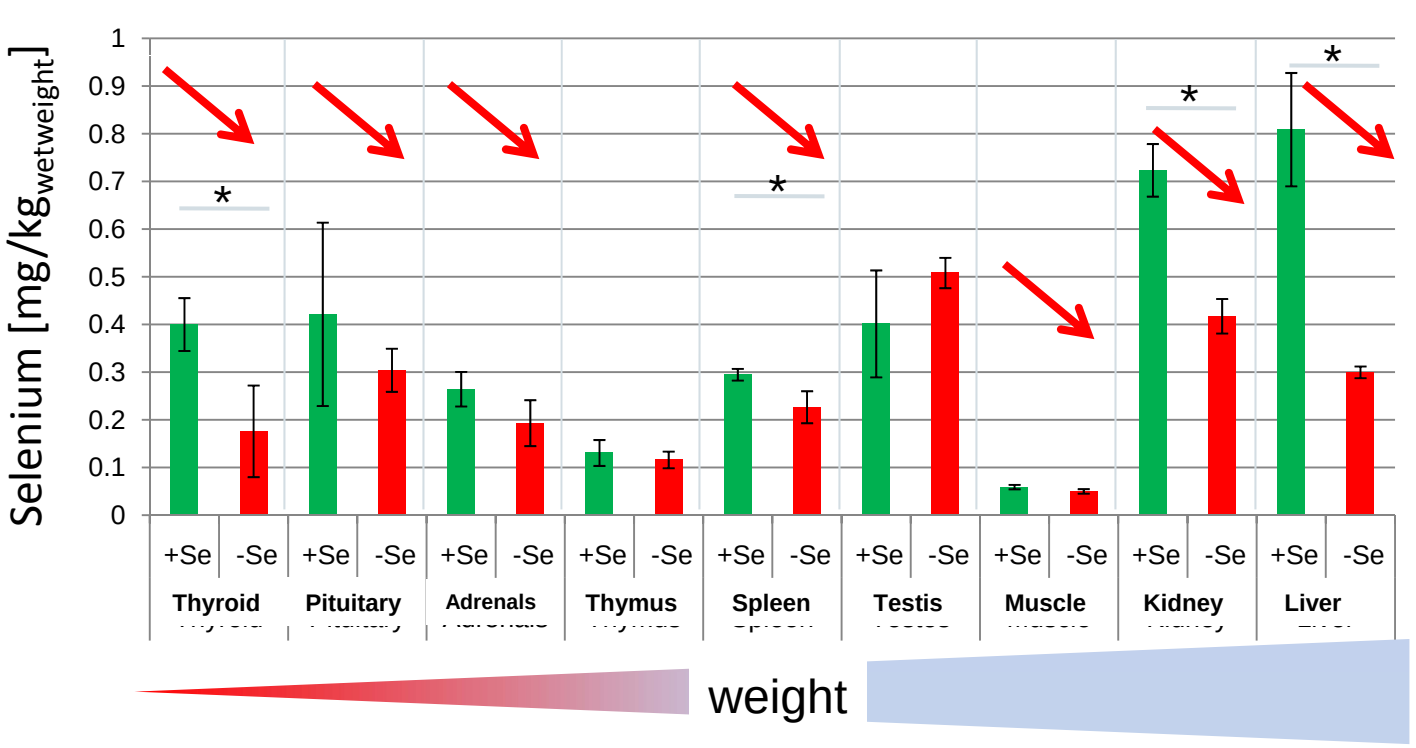
- Se status is related to heavy metal metabolism, accumulation, excretion
- SePP form stable adducts with heavy metals in vitro
- SePP receptors were identified, mainly expressed in kidney, brain and thyroid glands, where heavy metals accumulate at physiological conditions

Hypothesis

- SePP binds heavy metals and triggers biological inert deposition of heavy metals in target tissues

Application studies
Analysis of Se

- Diet with high Se content (3 weeks)
- Diet with low Se content



Effect of dietary Se content

Organ specificity

Suitability of TXRF

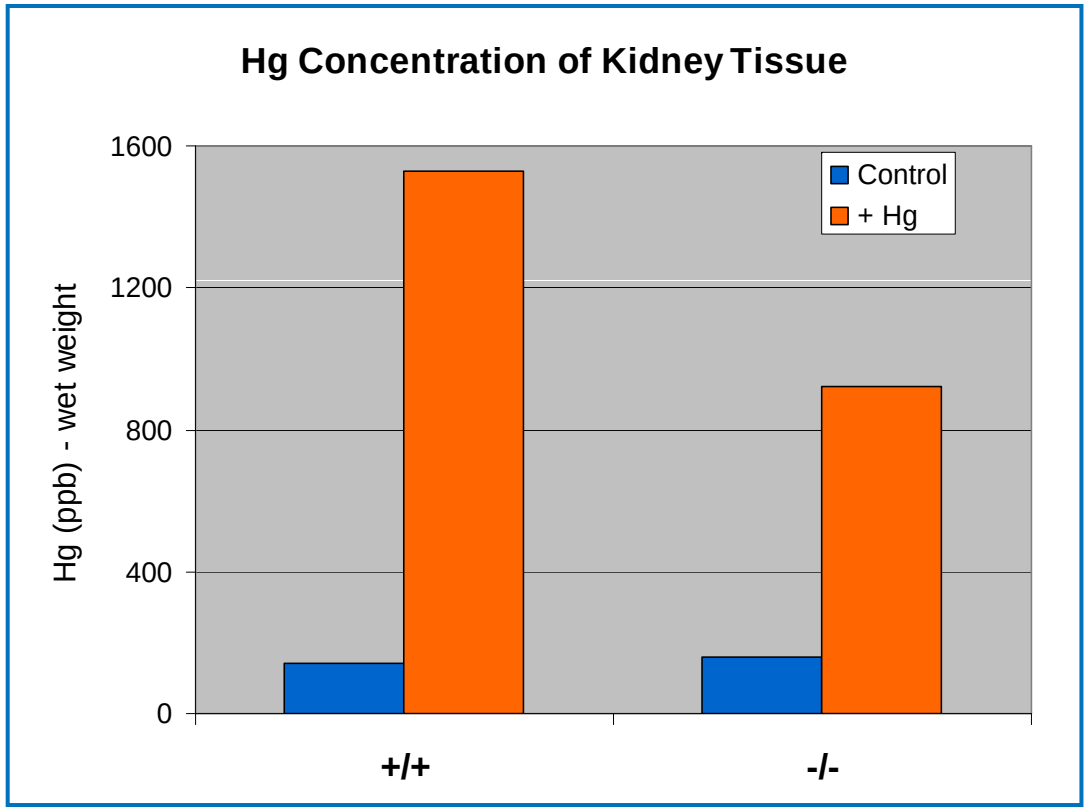
Application studies
Analysis of Se

Setup

- Mice were treated through drinking water for 12 days with 5 mg/L $HgCl_2$
- Genotypes represent the wildtype situation (+/+), as well as the homozygouse knockout (-/-) of the Se-transport protein SePP

First trends

- 2 mice each
- Without SePP, the transport of heavy metal species (i.e. Hg) to classical SePP- target tissues might be reduced



Trace Elemental Analysis in Biological Liquids

Liquid samples
Urine

Introduction

- The trace element content of urine is an indicator for the human health status

Task

- Participation in round robin test for urine samples

Sample preparation and measurement

- Direct application after addition of a Ga standard
- Measurement time 1000 s
- After treatment with HNO_3 directly on the carrier, samples were measured again

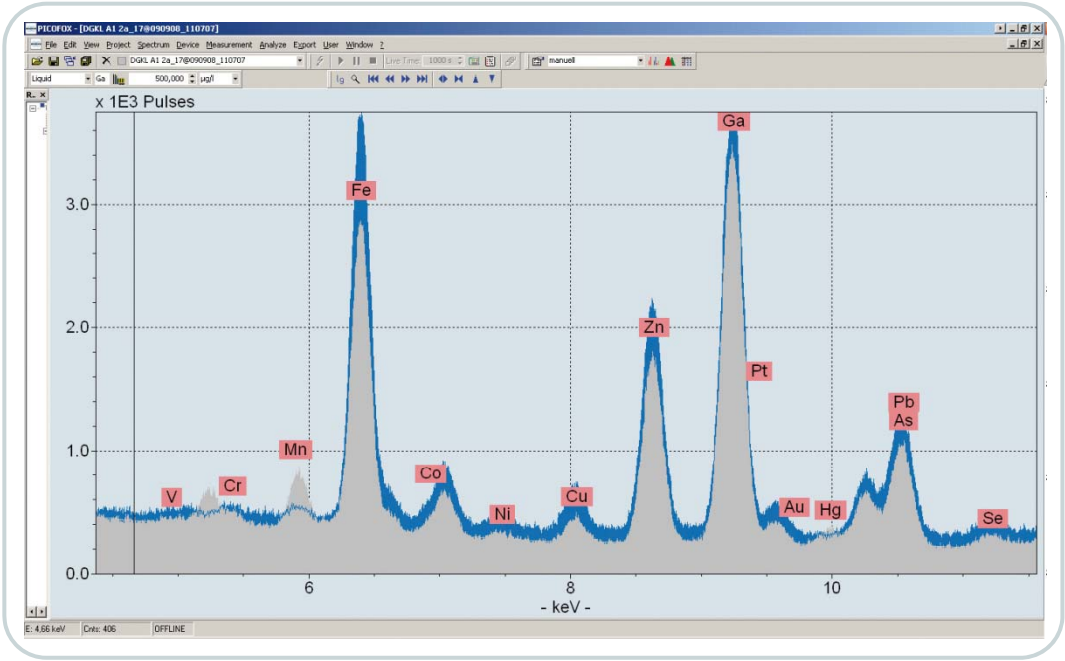
Liquid samples
Urine

Challenges of urine samples testing:

- Detection limits in the low ppb range required
- High amount of chlorine and calcium disturb TXRF measurements with sum peaks of matrix elements

Solution

- Vaporize Cl with HNO_3
- Perform second measurement of same sample



TXRF spectrum of urine sample
grey = before, blue = after HNO_3 treatment

Liquid samples
Urine

Results

- TXRF applications lab received certification for the elements shown below

Conclusion

TXRF allows precise and accurate urine analysis after direct sample preparation

Element	TXRF (µg/l)	Ref. (µg/l)	Recov. (%)	TXRF (µg/l)	Ref. (µg/l)	Recov. (%)
As	154,0	144,0	107	195,0	194,0	101
Cr	20,0	14,9	134	12,0	10,3	117
Fe	760,0	800,0	95	573,0	605,0	95
Ni	14,0	15,5	90	7,8	10,3	76
Se	11,0	13,4	82	91,0	91,0	100
Zn	1160,0	1220,0	95	797,0	838,0	95

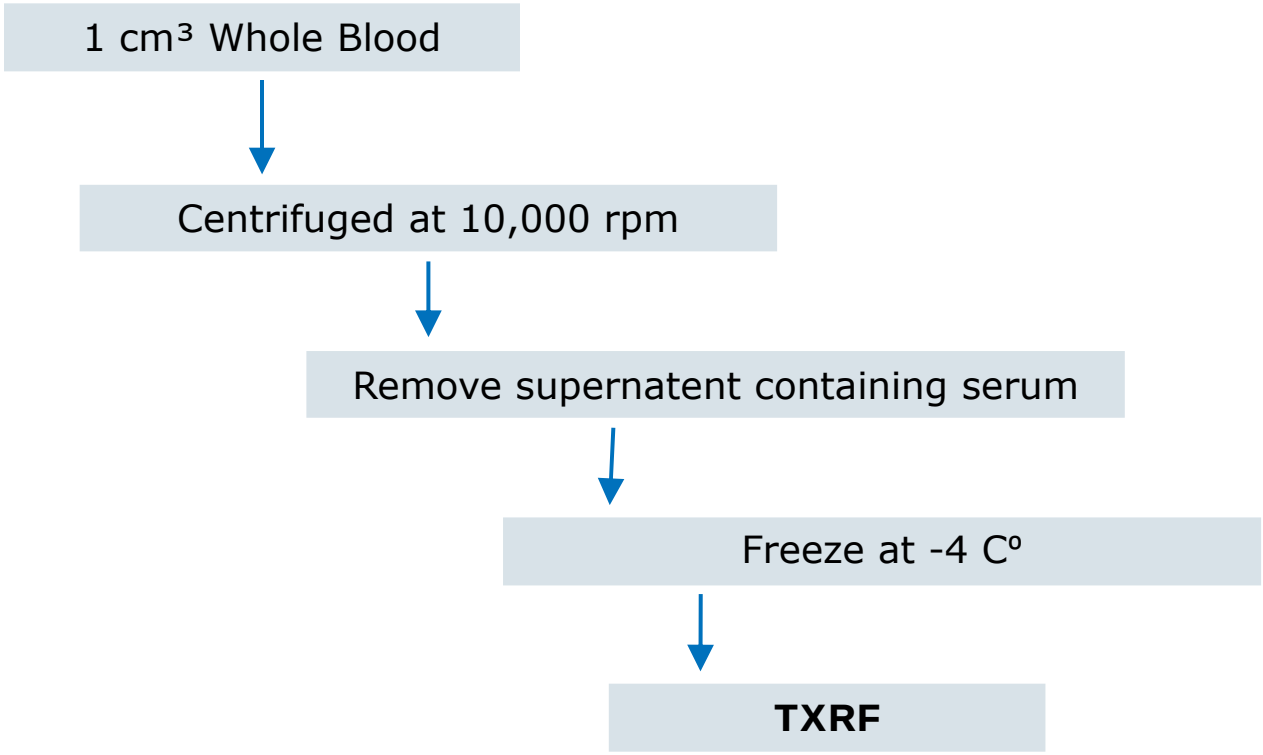
Liquid samples
Platinum in Blood

Application - Platinum analysis of Cancer patients during Chemotherapy treatment

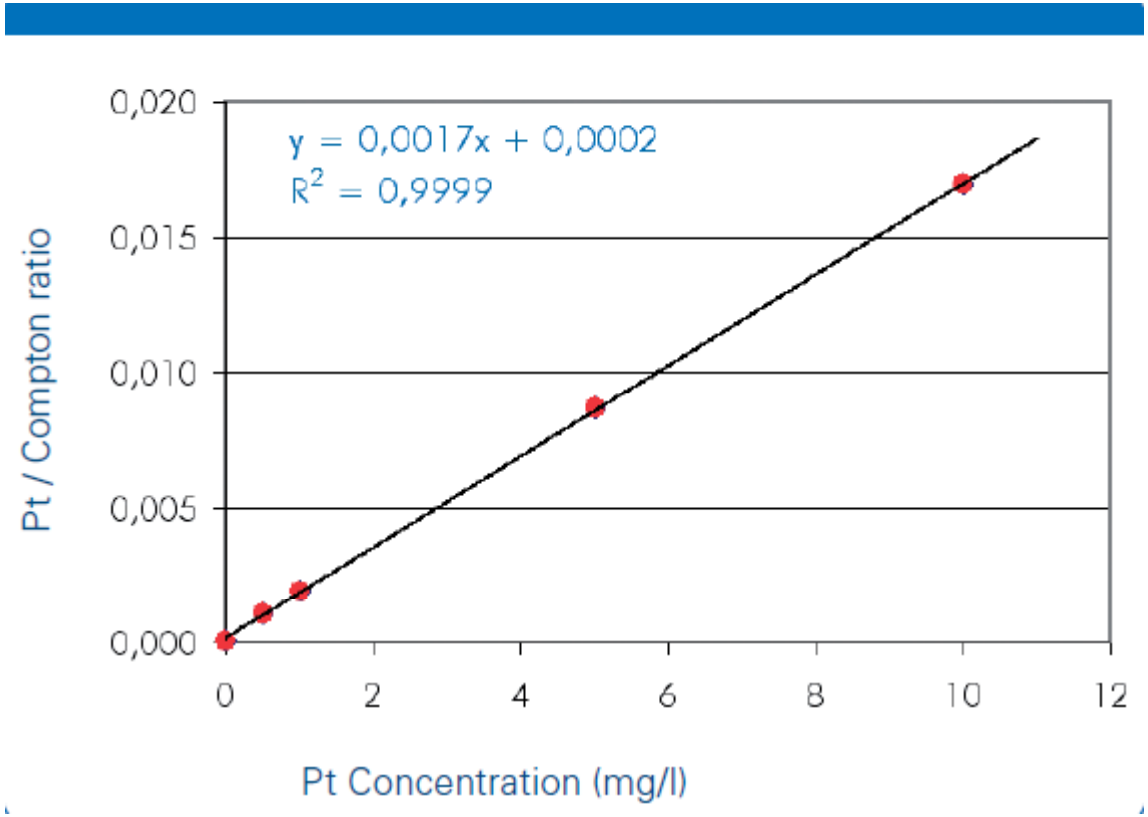
- Platinum is highly toxic – narrow therapeutic window
- Need to monitor individual patients as there is a variability in kidney functions and treatment must be adapted
- Variety of Platinum based cancer drugs and new ones being developed
- Study of Pharmacokinetics: absorption, distribution, and elimination
- Tolerable dosage levels must be established

Liquid Samples
Platinum in Blood

Measurements and sample preparation

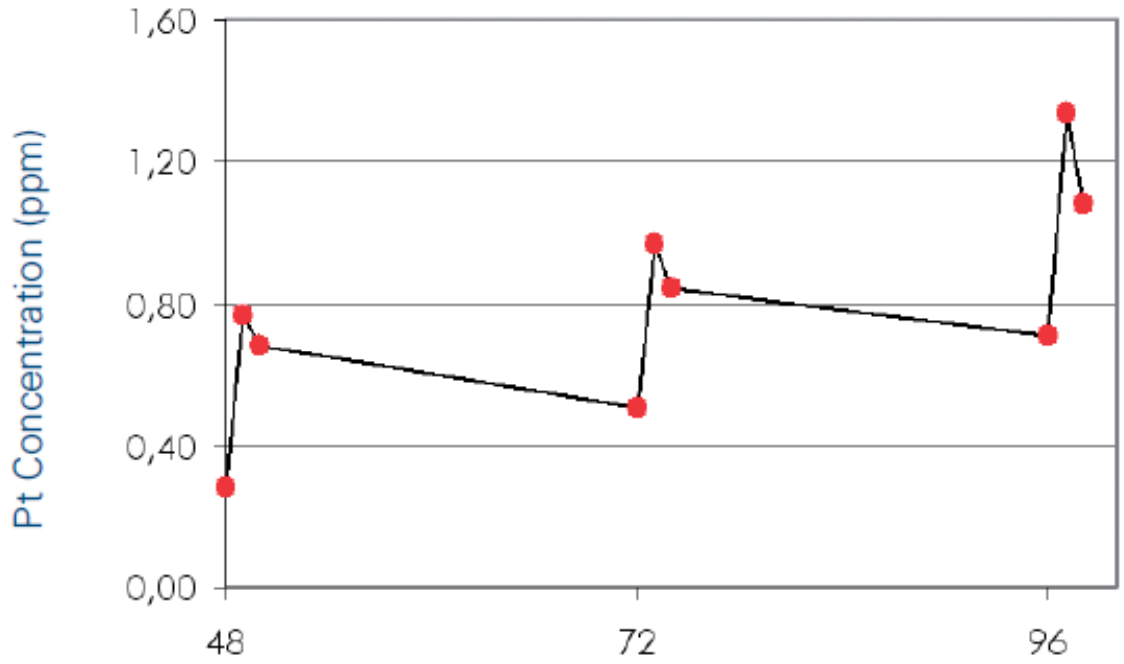


Liquid Samples
Platinum in Blood



- Serum samples spiked with different Pt standards
- Level of detection calculated at 67 µg/l
- Compton Peak at 15 keV was used as Internal Standard

Liquid Samples
Platinum in Blood

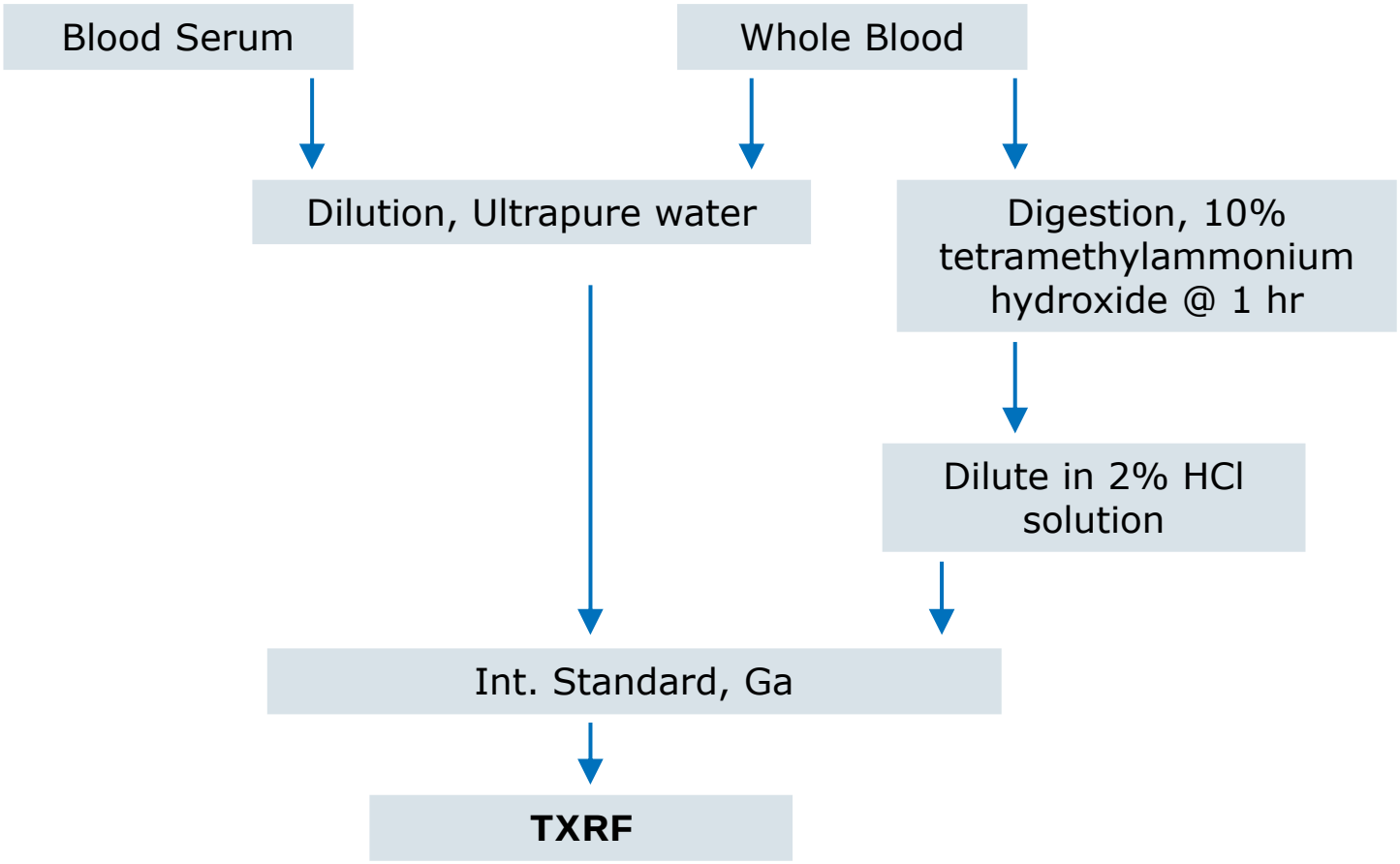


- Pt monitoring over a 4 day period for toxicological control of medication
- Slow decrease of Pt after application indicates kidney function

* Prof. Eduardo D. Greaves, Universidad Simon Bolivar, Caracas, Venezuela

Liquid Samples
Trace Element Analysis in Serum & Blood

Measurements and sample preparation



Liquid Samples
Trace Element Analysis in Serum & Blood

Whole blood standard				
	TXRF	Std. dev.	ICP-MS	Std. dev.
Fe	440000	900	435000	12000
Cu	662	43	623	21
Zn	5010	118	5038	69
Se	123	18	123	10
Pb	399	6.5	396	100
	All values in µg/l			

- Comparison of TXRF to ICP-MS reference values for trace elements in whole blood
- Good concordance of TXRF with reference values for essential elements
- Other elements (P, S, Cl, K, Ca, Br, Rb, Sr) could also be determined during **one** measurement
- Samples analyzed for 600s

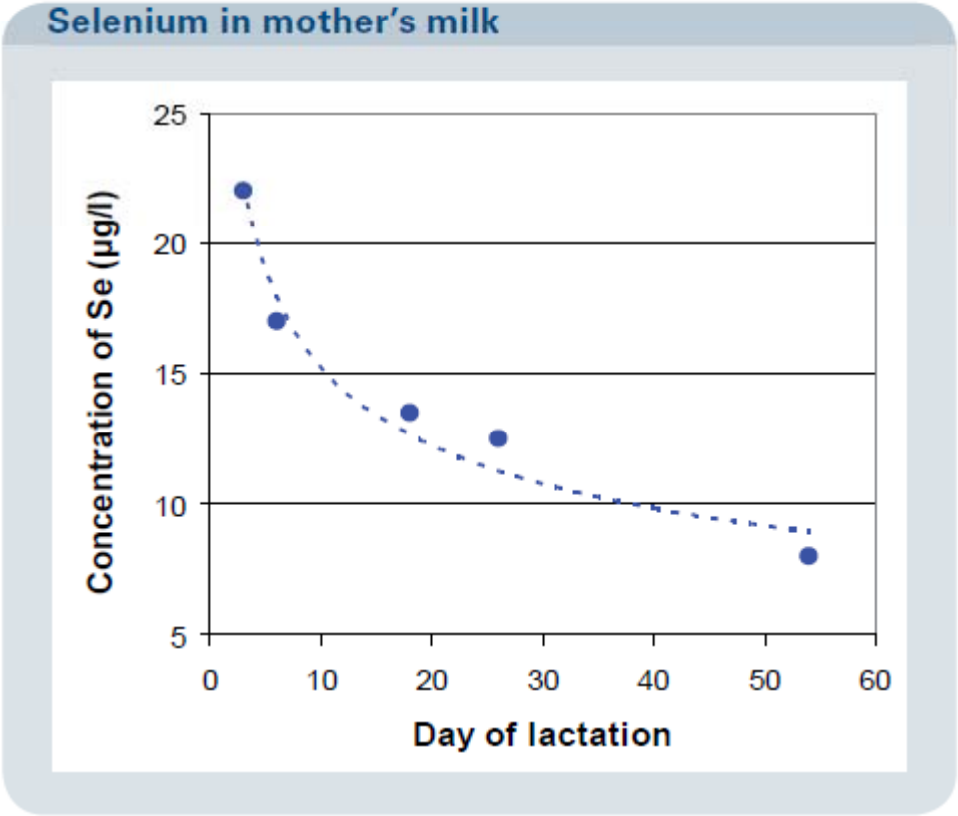
Liquid Samples
Trace Element Analysis in Serum & Blood

	Serum standard			
	TXRF	Std. dev.	AAS	Std. dev.
Fe	2920	87	1964	196
Cu	1690	43	1562	312
Zn	2190	118	2225	334
Se	97	18	102	26
Au	1343	13	1965	393
Pb	11	5.8	n.d.	n.d.
	All values in µg/l			

- Comparison of TXRF and AAS reference values in blood serum
- TXRF has better standard deviations compared to AAS
- **No Digestion** procedure was applied

Liquid Samples
Se in Mother‘s Milk

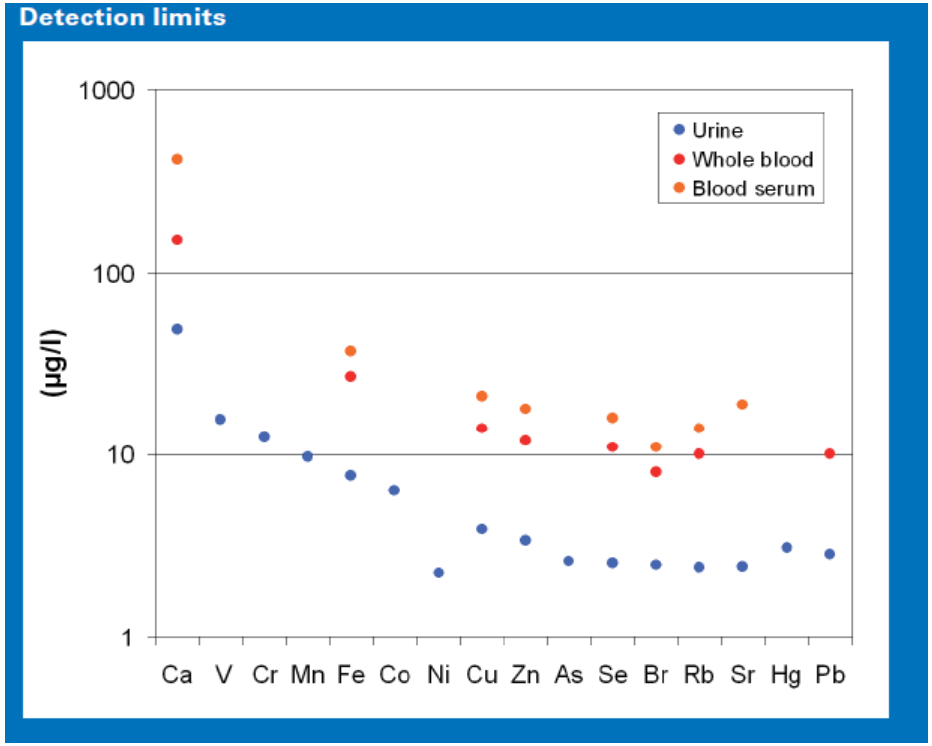
- Se concentration during lactation period was monitored with whole milk
- **No Pretreatment** of milk was done, only addition of Internal Standard
- Detection limits were calculated to **3 µg/l**
- Good concordance to milk powder standards (NIST BCR 150)



Liquid Samples
Conclusion

Accuracies and Sensitivities comparable to AAS or ICP without the need for complex and time-consuming sample preparation and instrument calibration

- Ability to analyze minute samples
- Allows for monitoring of trace metal profiles or metabolic interactions for detection of unsuspected nutritional deficiencies
- Element determinations in distinct fractions of blood (lipids, proteins, etc.) is possible
- Elements Na to U within one measurement
- Detection limits of 1 to 100 ppb for most elements



TXRF vs. AAS/ICP-OES

TXRF

- Small footprint/portable
- Microgram sample size
- %-ppb levels
- Single std. calibration
- Low maintenance
- Non-destructive
- Fast learning curve
- Fast sample prep

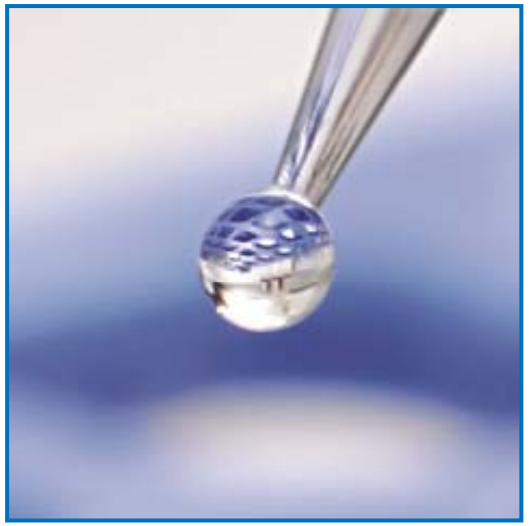
AAS / ICP-OES

- Large, fixed installation
- Milligram sample size
- ppm – sub ppb levels
- Multi-standard calibration
- High maintenance
- Destructive
- Long learning curve
- Laborious sample prep

Q & A

Any Questions?

Please **type** any questions
you may have for our speakers
in the **Q&A panel** and
click **Send**



Thank you for your attention!

Upcoming:

14th International Conference on
Total Reflection X-ray Fluorescence
and Related Methods
06–10 June 2011, Dortmund, Germany

3rd International Symposium on Metallomics
15-18 June, 2011, Muenster, Germany

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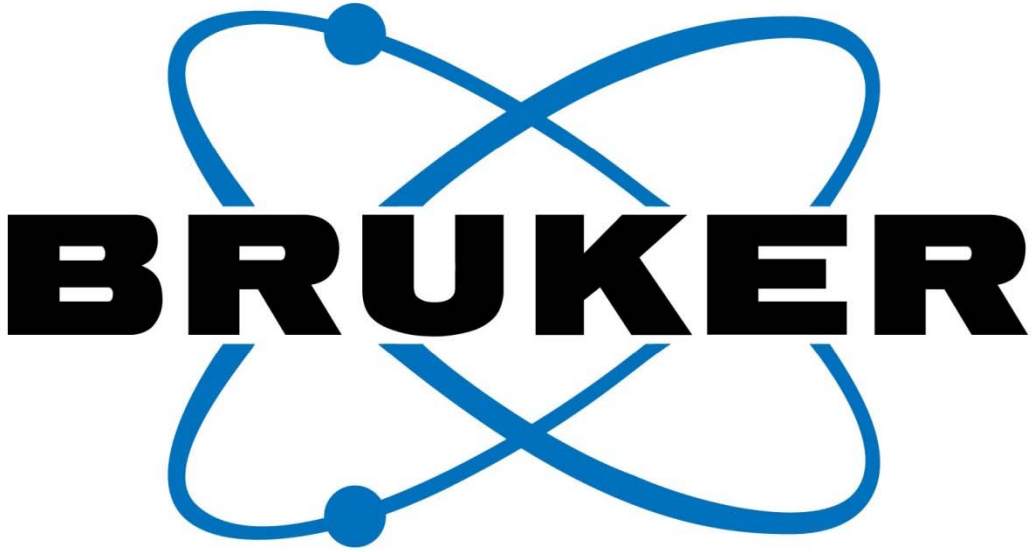

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