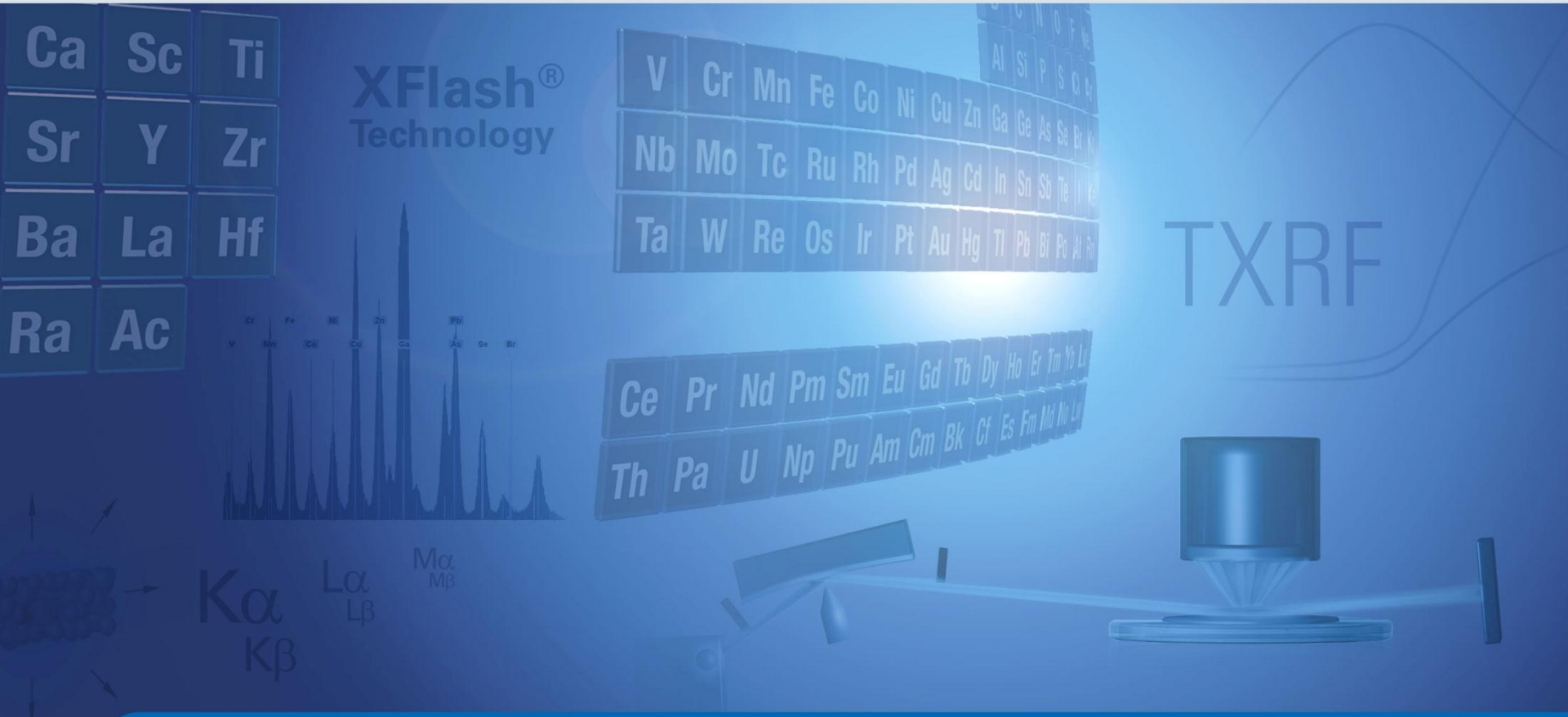
TXRF for Trace Element Analysis in Clinical Chemistry, Metallomics, and Cancer Research

Bruker AXS
Madison, WI, USA



Welcome

Moderator

Karen Roscoe
Digital Marketing Manager
Madison, WI, USA

Speaker

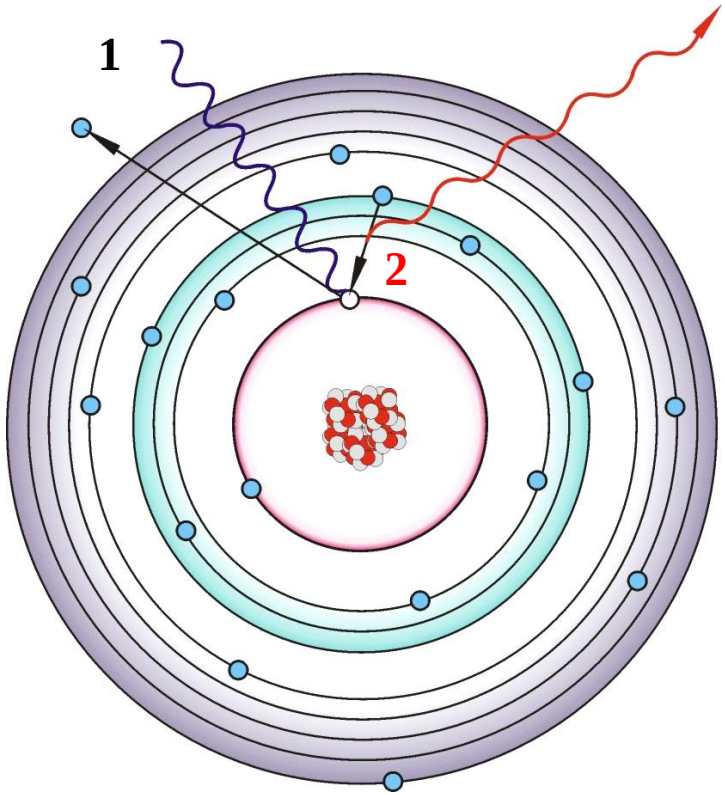
Michael Beauchaine
Business Development Manager, Americas - XRFi
Madison, WI, USA

Today's Topics

- TXRF – how does it work?
- Trace elemental analysis in blood and urine
- Sample preparation
- Comparison with Atomic Spectroscopy methods
- Trace elemental analysis in tissues and hair
- Interactive Q & A

TXRF – How does it work?

Principles X-ray fluorescence (XRF) spectroscopy



1. An X-ray quantum hits an inner shell electron in a (sample) atom. The electron is removed leaving the atom in an excited state
2. The missing inner shell electron is replaced by an electron from an outer shell
3. The energy difference between the inner and outer shell is balanced by the emission of a photon (fluorescence radiation)

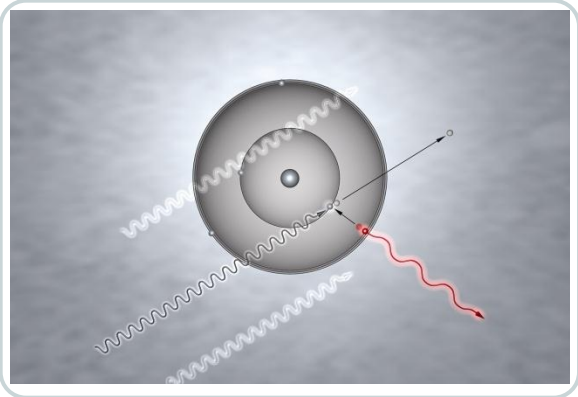
Principles X-ray fluorescence (XRF) spectroscopy

- The energy, and therefore the wavelength, of the X-ray fluorescence radiation is characteristic for the different chemical elements.

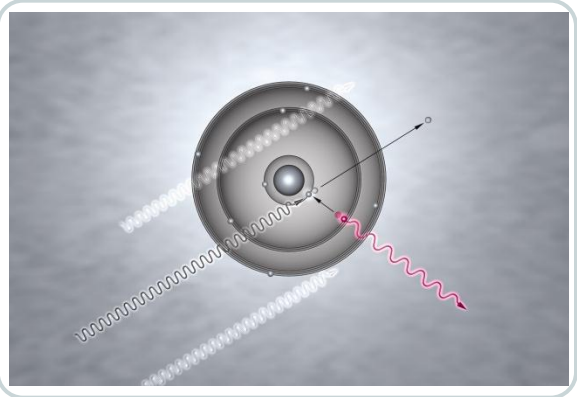
  QUALITATIVE ANALYSIS

- The intensity of the X-ray fluorescence radiation is, in first approximation, proportional to the element concentration.

  QUANTITATIVE ANALYSIS



Low Z

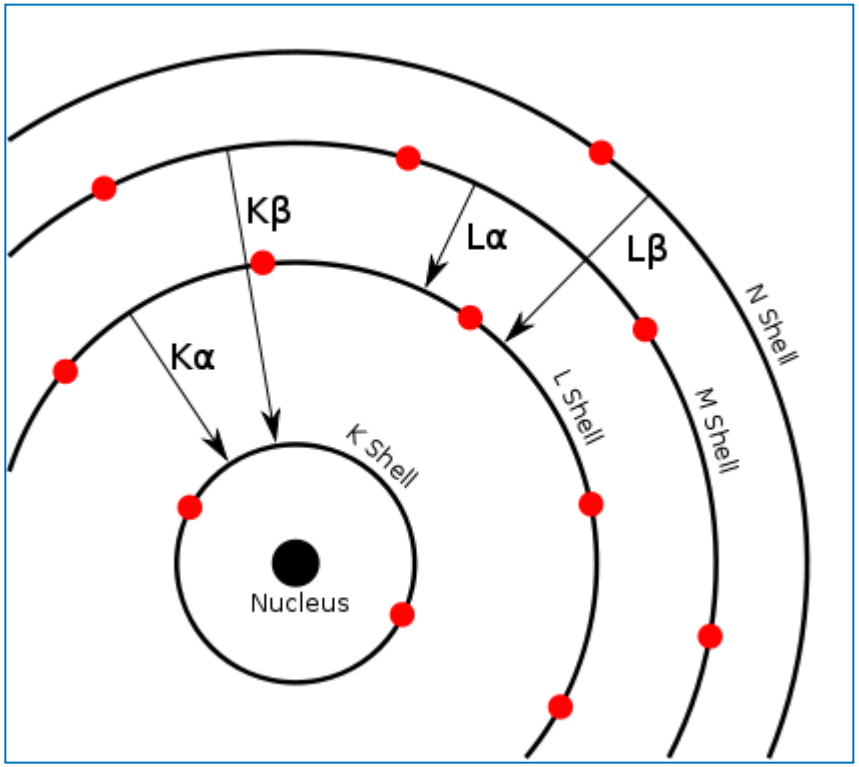


High Z

Principles X-ray fluorescence (XRF) spectroscopy

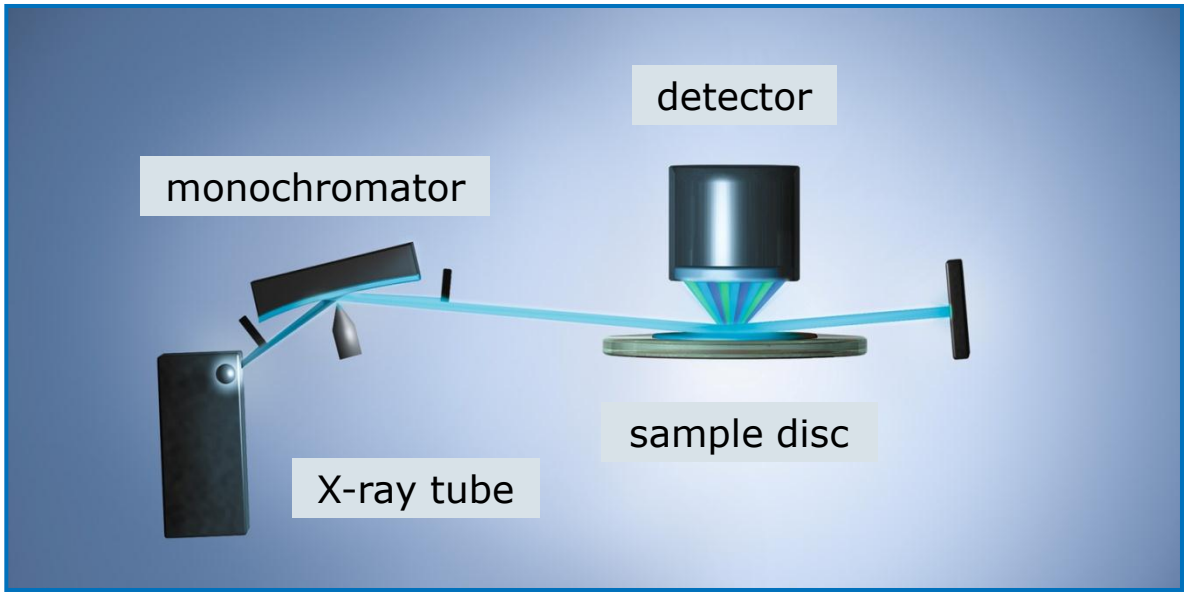
Each element shows a specific line pattern in a spectrum depending on the orbitals involved

- L→K transition = Kα line
- M→K transition = Kβ line
- M→L transition = Lα line
- N→L transition = Lβ line



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

Total reflection X-ray fluorescence spectroscopy



Beam angle: 0.1° / 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

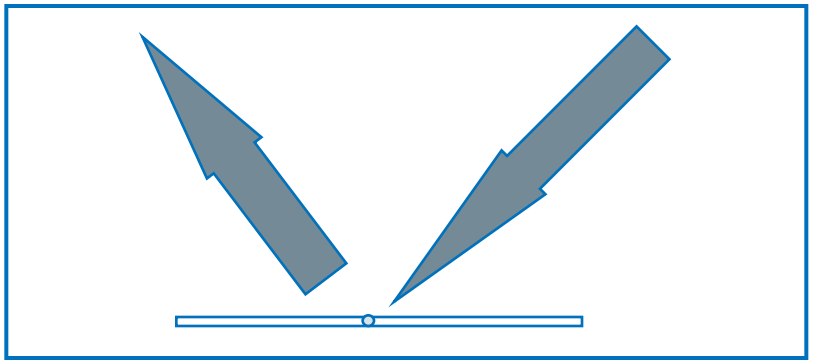
➔ Matrix effects are negligible due to thin layer

➔ 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

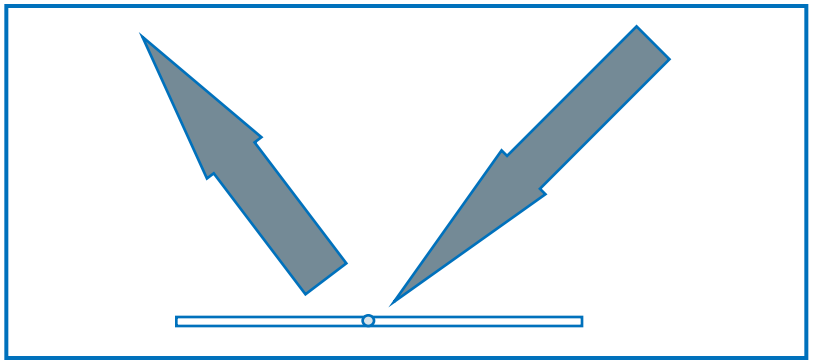
- ➔ Matrix effects are negligible
- ➔ Quantification is possible



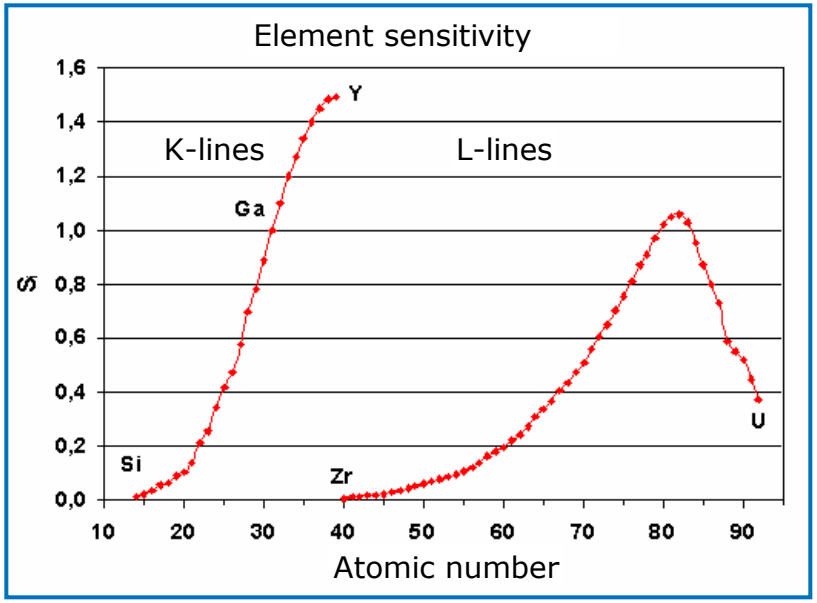
Principles of total reflection X-ray fluorescence spectroscopy

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



Principles of total reflection X-ray Quantification

$$C_i = \frac{C_{IS} \cdot N_i \cdot S_{IS}}{N_{IS} \cdot S_i}$$

C_i: Element concentration

C_{IS}: Internal standard concentration

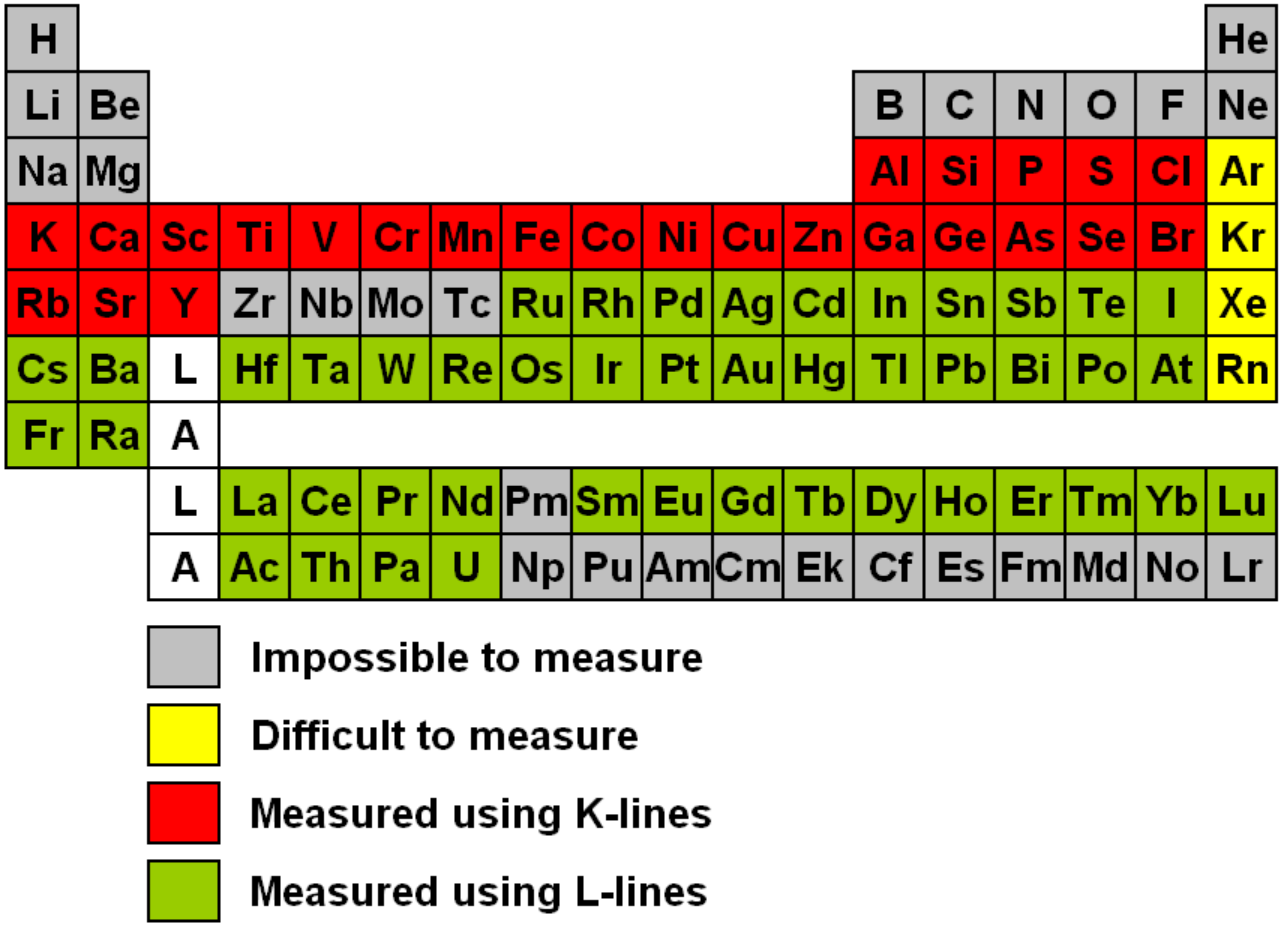
N_i: Element net count rate

N_{IS}: Internal standard net count rate

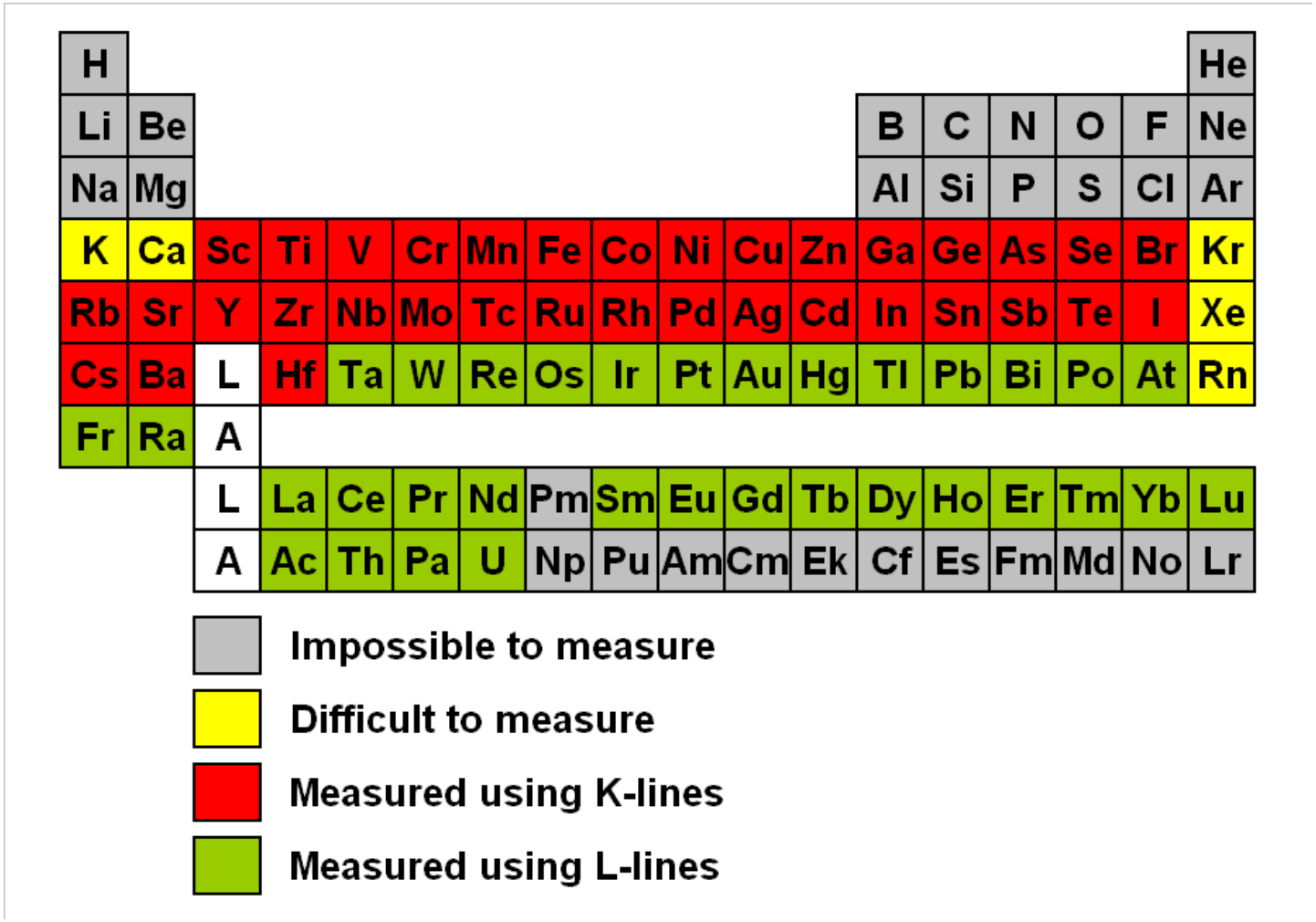
S_i: Element sensitivity factor

S_{IS}: Internal standard sensitivity factor

Elements measured by the Mo PICOFOX

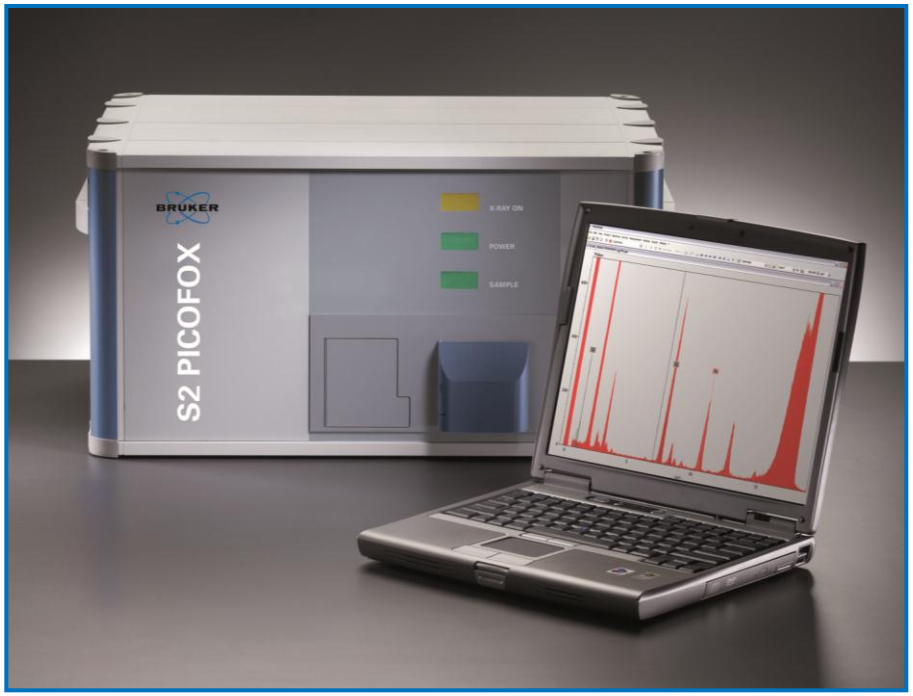


Elements measured by the W PICOFOX



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

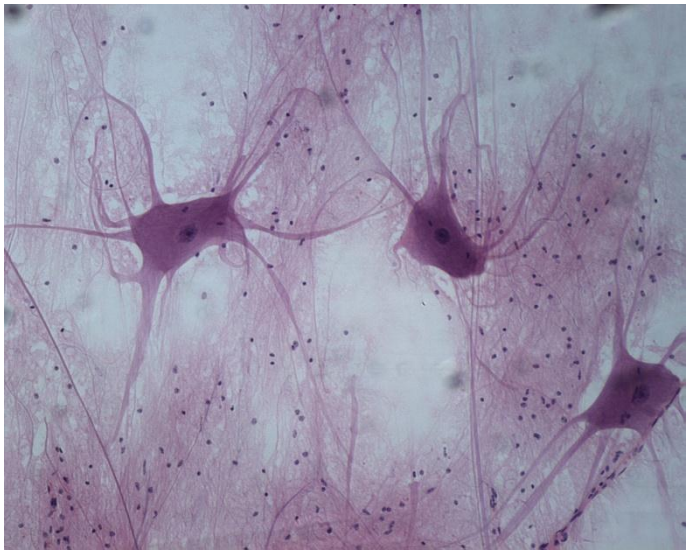
Automatic version

- 25 sample cassette

Introduction to Trace Elements in Clinical Chemistry

Trace Essential Elements
Impact on Clinical Chemistry

- Elements and metals are increasingly used in dietary supplements for health and prevention of disease
- Essential trace elements or micronutrients 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
- In contrast, an excessive uptake can lead to intoxication (Se, Cu, Mo, and Cr)



Metallomics

Metallomics: *new frontier within interdisciplinary science investigating trace elements and the role of metals in biological, environmental and clinical systems.*

Research Topics Include:

- Regulation of the uptake, accumulation and metabolism of metals and other trace elements in biological systems
- The interaction of metallodrugs, including 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

** Metallomics Journal – RSC Publishing*

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

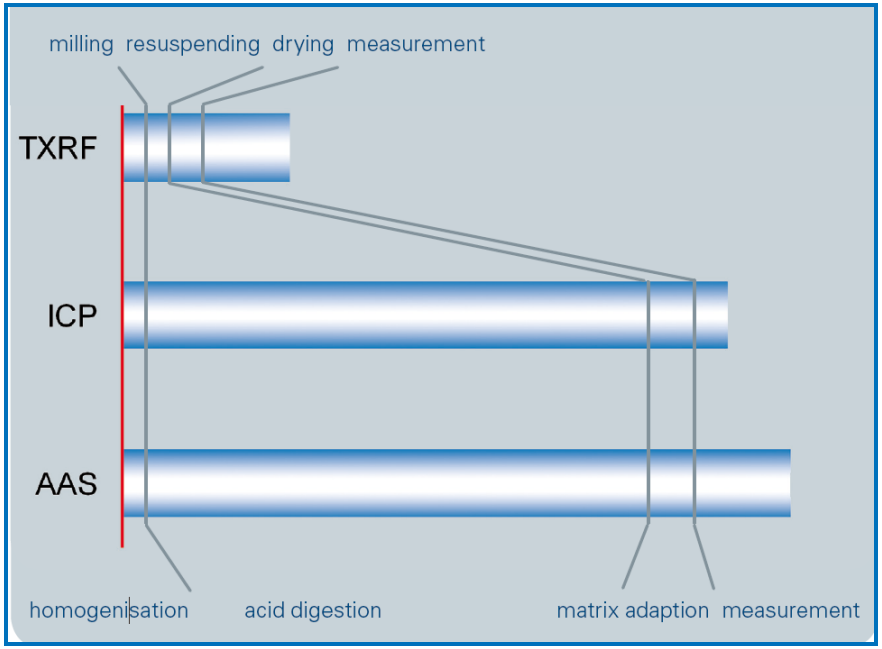
Trace Essential Elements
Biological Matrices Analyzed with TXRF

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 used to measure trace elements include ICP-OES (inductively coupled plasma optical emission spectroscopy) or AAS (atomic absorption spectroscopy)



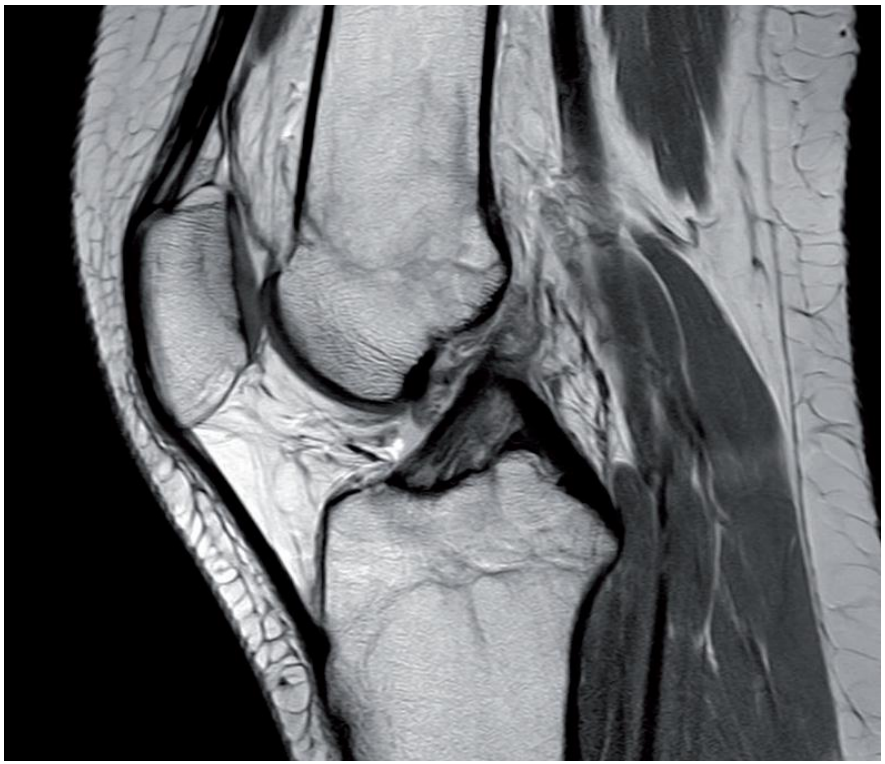
Both techniques require

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

Measurement of Gadolinium Based MRI Contrast Agents

Gd Based Contrast Agents

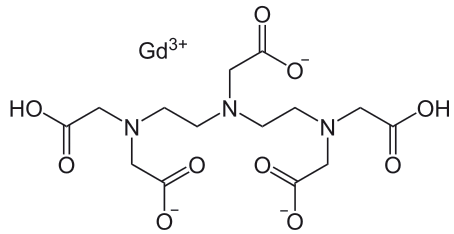
- Paramagnetic Gd^3 ions are imaging enhancement contrast agents used in MRI diagnostics
- Due to its toxicity, Gd^3 is complexed with chelating agents like gadopentetate (Gd-DTPA)
- Nephrogenic Systemic Fibrosis (NSF) prevents the body from releasing Gd contrast agents and causes kidney problems and hardening of the skin and organs
- Patients with kidney problems need to have blood and urine continuously monitored for trace Gd



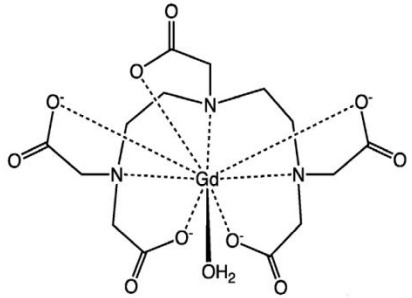
Gd Based Contrast Agents

Sample Preparation

1. Urine samples were collected from 2 patients up to 20 hours after MRI
2. Blood samples were collected from 10 patients immediately after MRI
3. 495 µL of urine or blood was collected and added to 5 µL of a Ga standard solution to obtain a concentration of 10 mg/L of Ga.
4. Samples were homogenized and 10 µL of sample was deposited to a quartz glass carrier.
5. Each sample was prepared in triplicate.
6. Samples were analyzed for 1000s with a S2 PICOFOX
7. For ICP-MS, measurements were digested in a microwave oven and diluted. A standardized Ho was used.
8. For both methods, the Gd concentrations in urine were normalized with creatinine concentrations.



Gd-based contrast agent DTPA 1



Gd-based contrast agent DTPA 1

Gd Based contrast Agents

Measurements and sample preparation

Urine

↓

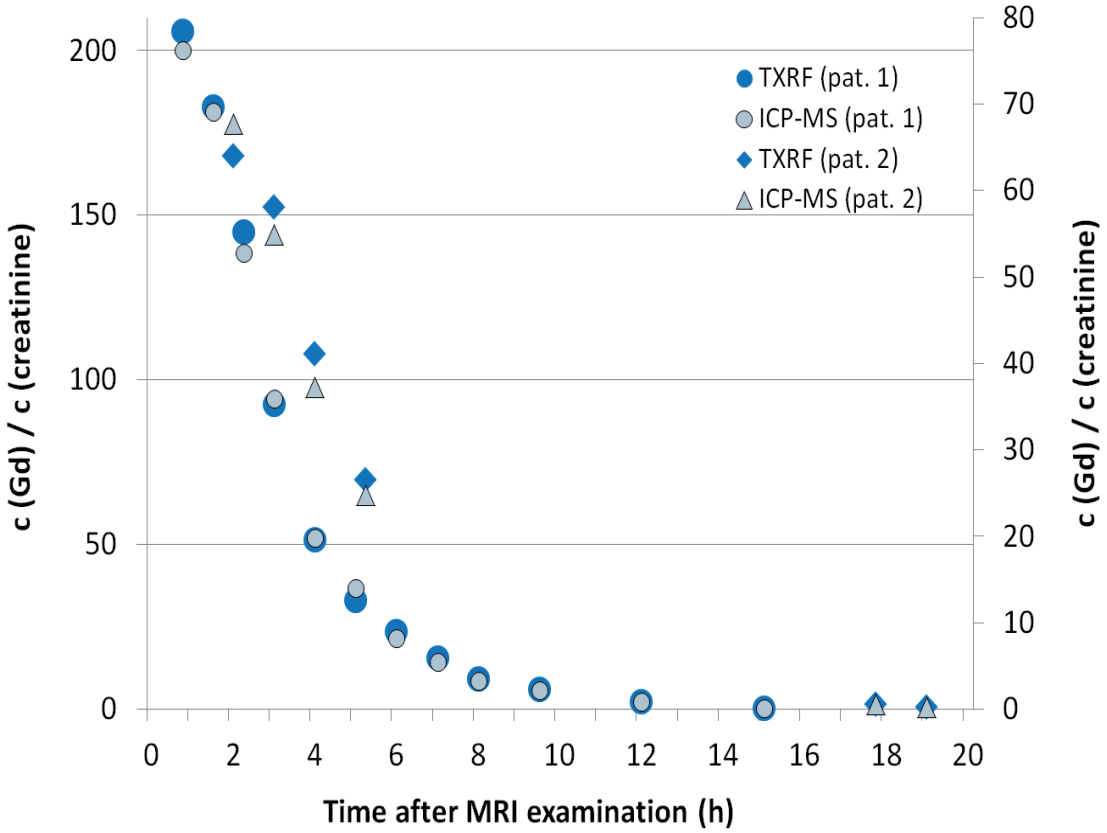
Int. Std., 10 ppm Ga

↓

TXRF

Gd Based Contrast Agents

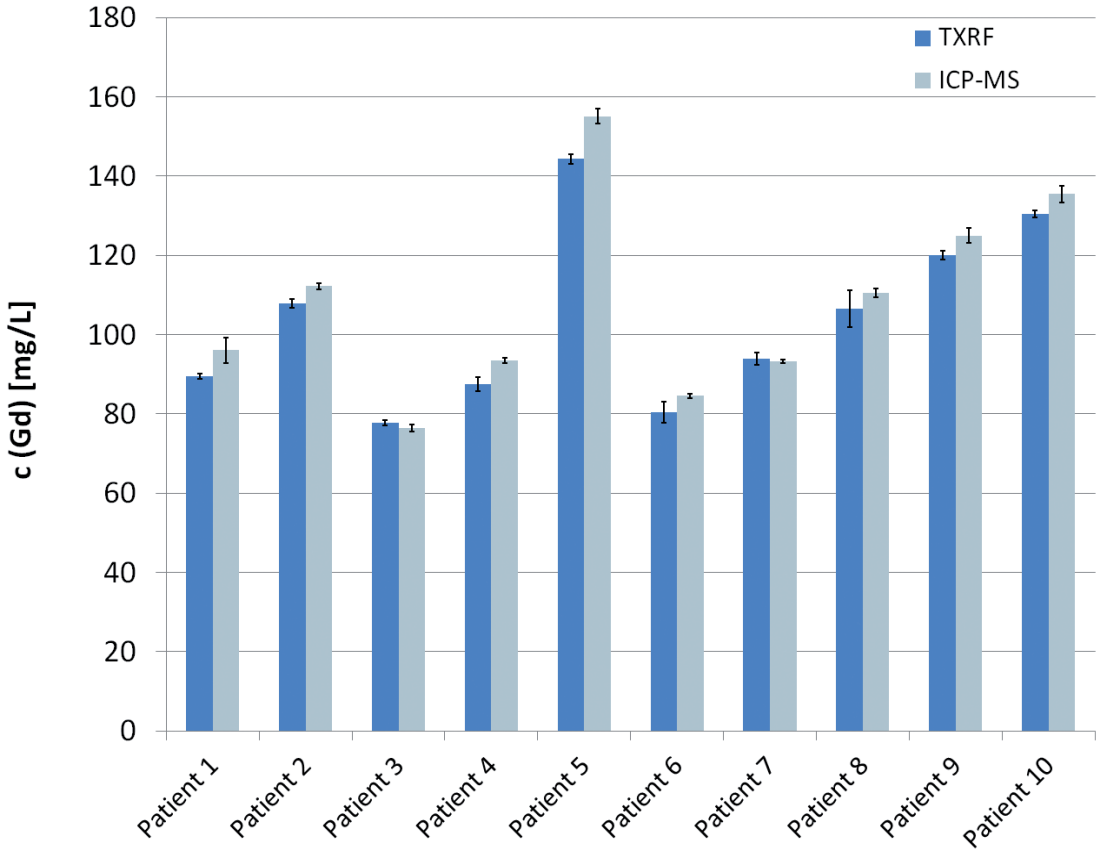
Results - Urine



- Gd concentrations in urine were highly reproducible showing RSD less than 1.6%
- Excellent correlation to ICP-MS results
- Lowest limit of detection for Gd was found to be 100 ppb.

Gd Based Contrast Agents

Results – Blood serum



- Good reproducibility with a RSD of 4.8%
- Blood serum varied depending on the size and weight of the patient
- Good agreement with ICP-MS
- Detection limit for Gd was at 80 ppb
- Easy sample preparation and complete analysis in under 30 minutes

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 at 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

Quantification of Gold and Platinum based drugs in Cell Suspensions

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

Gold and Platinum Based Drugs

Introduction

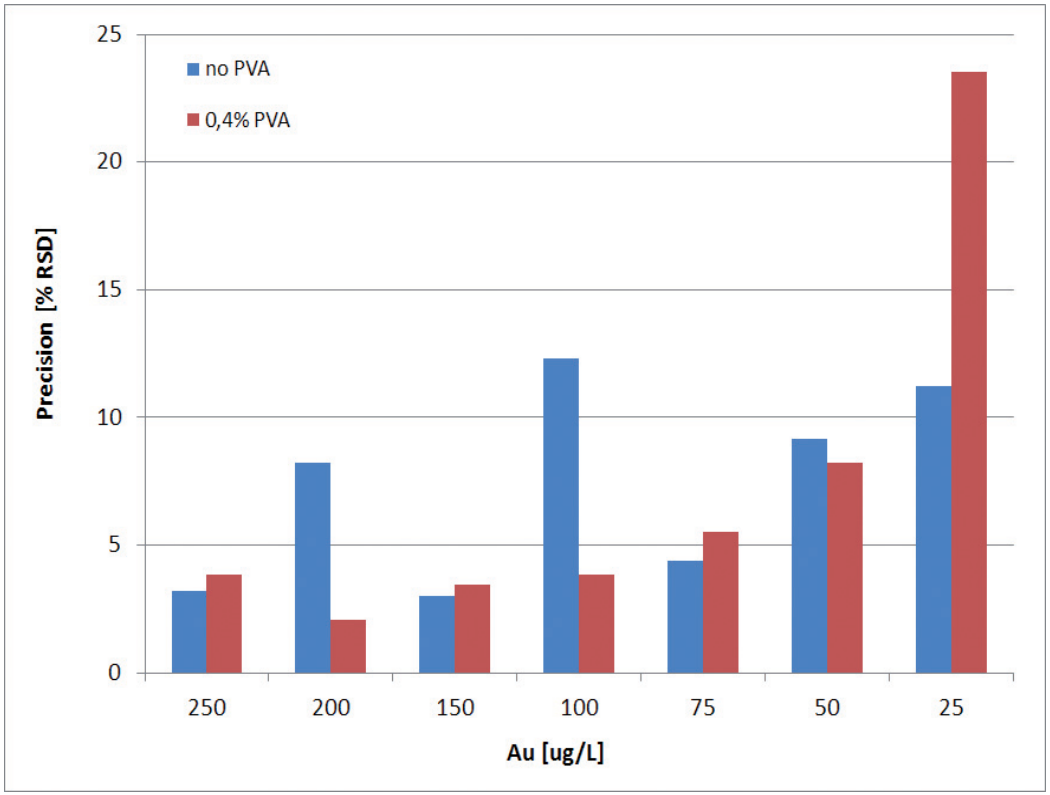
- Cisplatin and Auranofin were chosen as relevant chemotherapeutical metal-based drugs
- Experiments were performed with lysates of HT-29 colon carcinoma cells

Sample Preparation

- Various concentrations of auranofin and cisplatin were prepared with 2 different concentrations of aqueous lysates
- Mn and Y at 1000 µg/L were used internal standards
- 10 µL were pipetted onto a quartz carrier and dried in an oven for approx. 20 minutes
- Samples measured for 250s

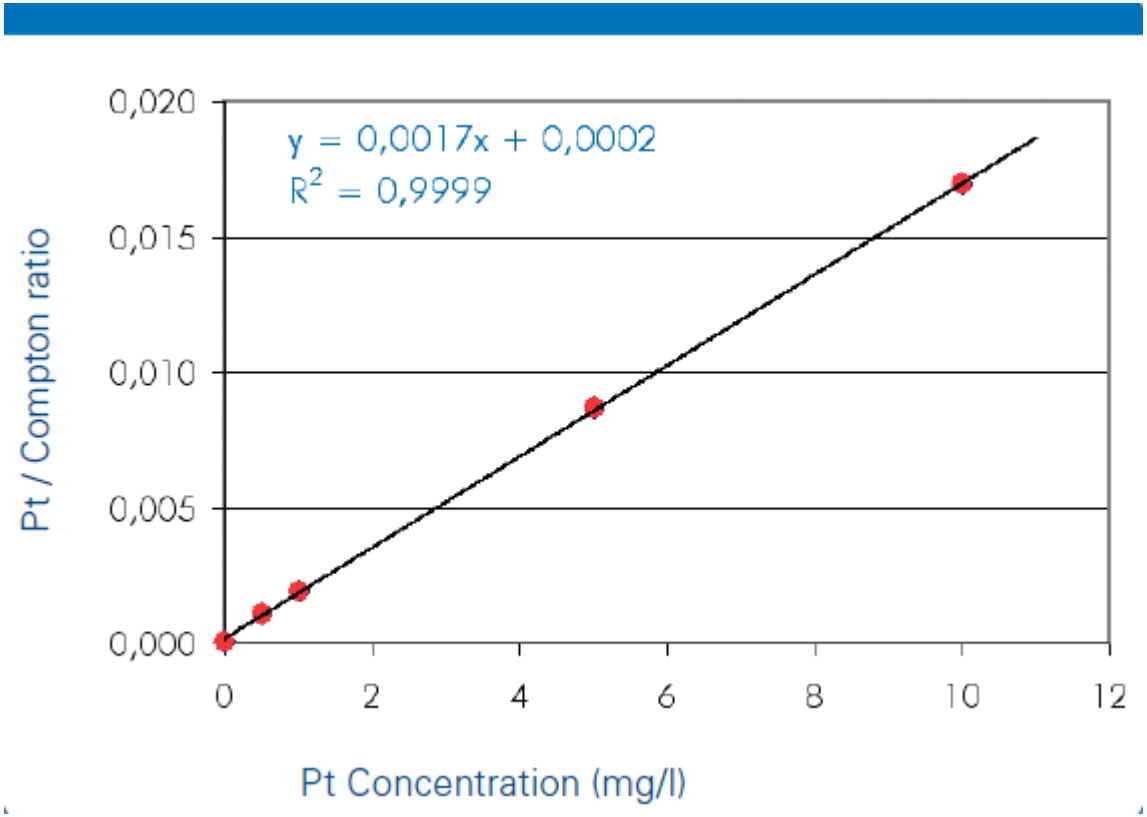
Gold and Platinum Based Drugs

Results



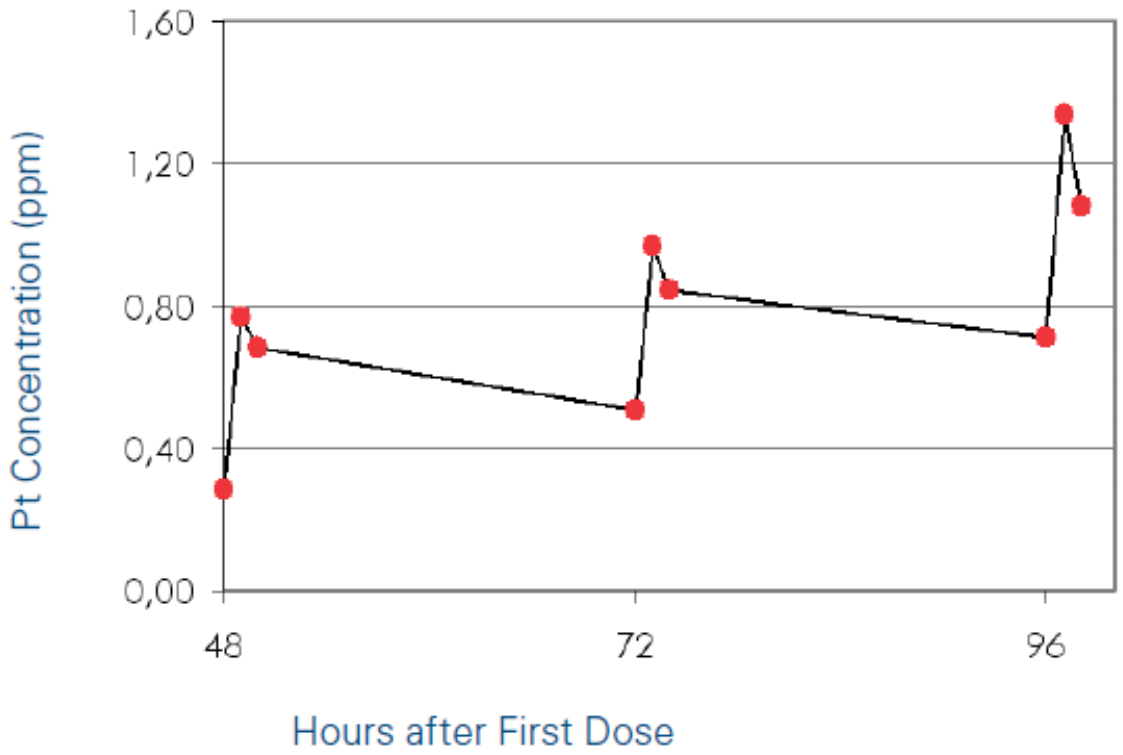
- Recovery rate of Cisplatin were in the range of 80 – 120%
- Recovery of Auranofin was close to 100%
- Precision was in the range of 3 to 12% but when Polyvinyl Alcohol was added, the precision improved to 2 to 8% for 50 ppb and above
- Measurement times were tested at 10 to 100 s. Using a auranofin conc. Of 10 ppm, a RSD better than 5 % was achieved. 10 s measurement times lead to RSD of 10%.

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

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

Tissue Analysis by TXRF Spectroscopy

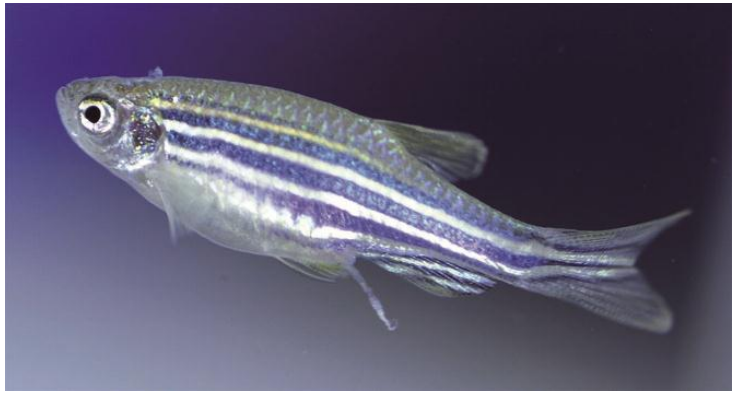
Application studies
Rapid screening of fish samples

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

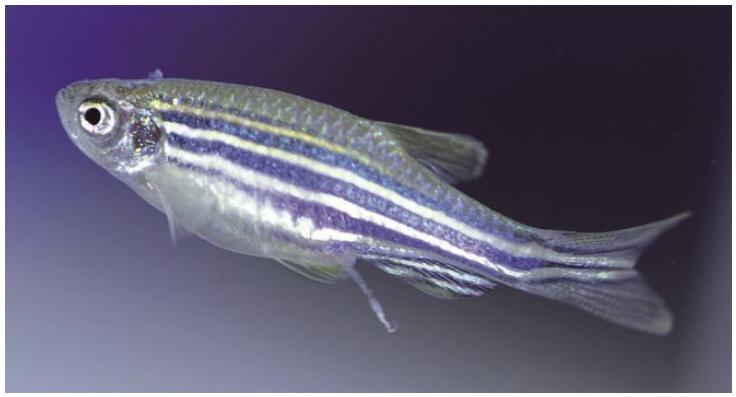
- Nutrition relevant elements like Cu, Zn and Se in food are regularly monitored

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

Task

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



Zebrafish *Danio rerio*

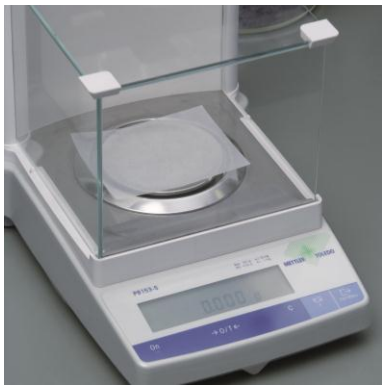
*) Fish protein,
Canadian National Research Council
Preparation by removal of bones and oil, subsequent enzyme hydrolysis; protein hydrolysate was spray dried, sieved (297 µm screen), blended and bottled.

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

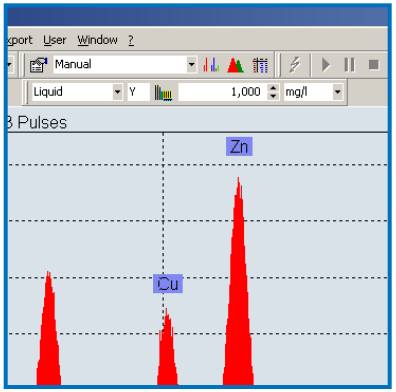
Application studies
Sample preparation for rapid screening

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

Sample preparation
Suspensions and particulate matter

Dry through heat/vacuum

Load the instrument

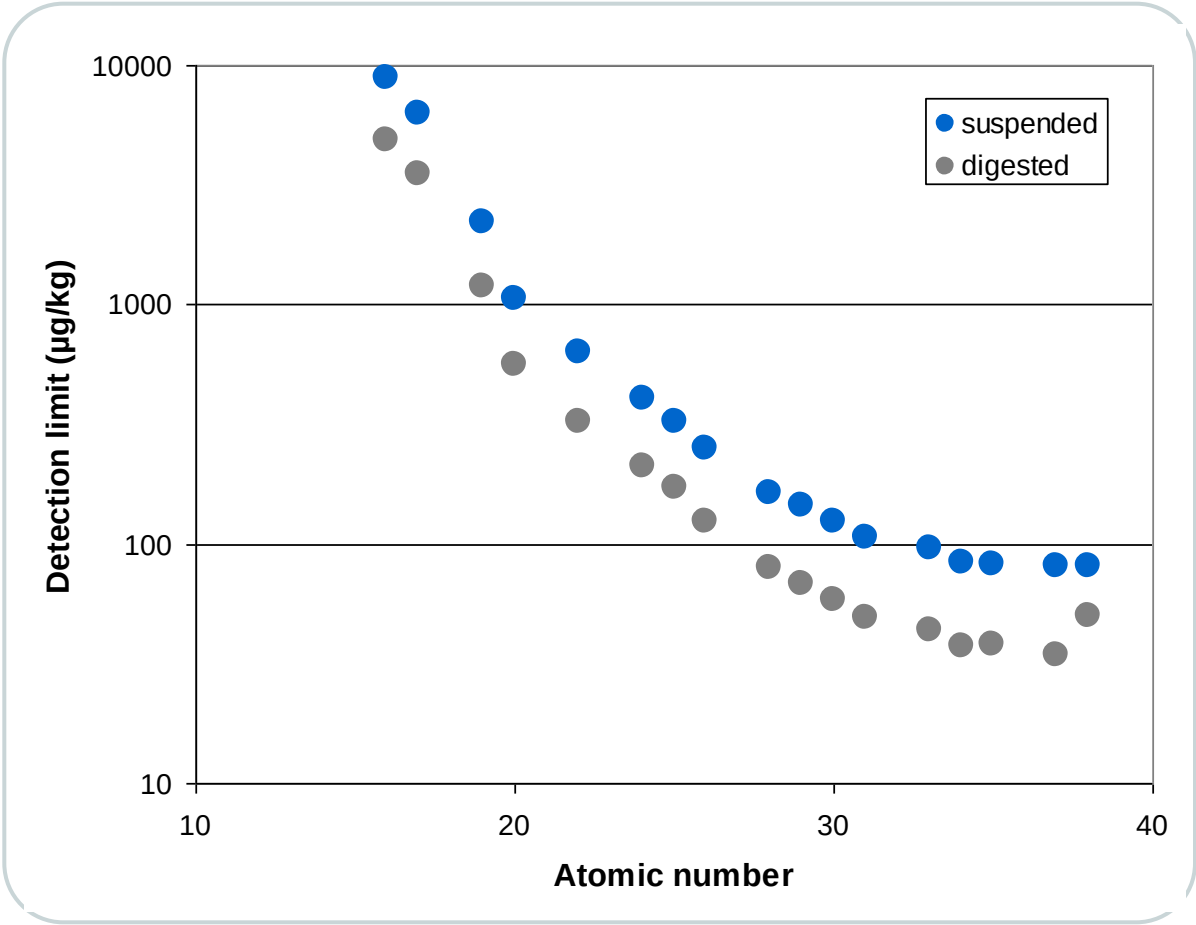


Start data acquisition

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



Trace Elemental Analysis of Hair

Application studies
Trace Elements in Hair

- Biological market for environmental pollution on human health
- Represent mean level in human body during 2-5 month period
- Interest in nutrition relevant elements of Ca, Cu, and Fe
- Accumulation of heavy and toxic metals
- Difficult application because of the inhomogeneity of hair

Application studies
Trace Elements in Hair

Direct Preparation

- About 5 mm of hair was placed on quartz disk
- Placed directly on hot plate with 10 µL nitric acid (65%) added to samples
- Average Zn concentration (200 ppm) was used as internal std.

Sample Digestion

- 80 mg were weighed and placed in Teflon beakers
- 4 mL of nitric acid and 20 µL of Y (1 g/l) standard added
- Beakers heated on hot plate for 20 min.
- 10 µL of digested solution were transferred to quartz disk and dried in a vacuum

Application studies
Trace Elements in Hair

Element	Direct 1	Direct 2	Direct 3	LLD	Digested	LLD
Ca	5163	5246	2169	4.4	1413	2.6
Cu	3381	19106	5473	1.4	6376	0.48
Fe	199	157	62	2.2	40.4	0.66
Mn	5.4	5.4	4.7	2.7	3.9	0.79
Zn	200	200	200	1.8	173	0.32
Pb	28	1.8	26	0.73	5.1	0.21

Sample #1 – Direct preparation vs. Digested

Element	Direct 1	Direct 2	Direct 3	LLD	Digested	LLD
Ca	3985	4786	3408	4.1	1302	0.85
Cu	65	2199	186	0.47	265	0.11
Fe	23	97	60	0.78	29	0.17
Mn	3.4	2.9	3.3	0.98	2.8	0.22
Zn	200	200	200	0.424	156	0.09
Pb	16	1.3	0.41	0.32	2.6	0.07

Sample #2 – Direct preparation vs. Digested

Application studies
Trace Elements in Hair

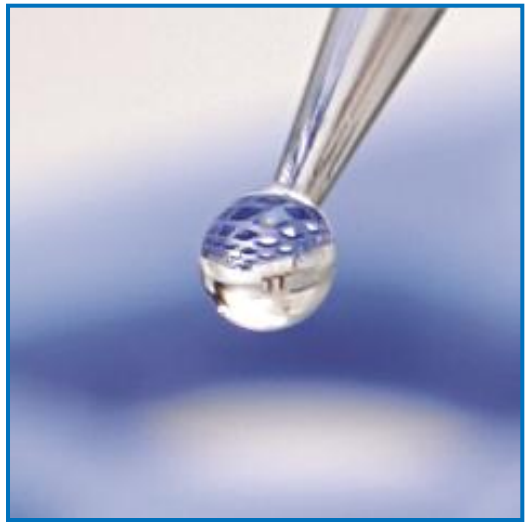
Conclusion

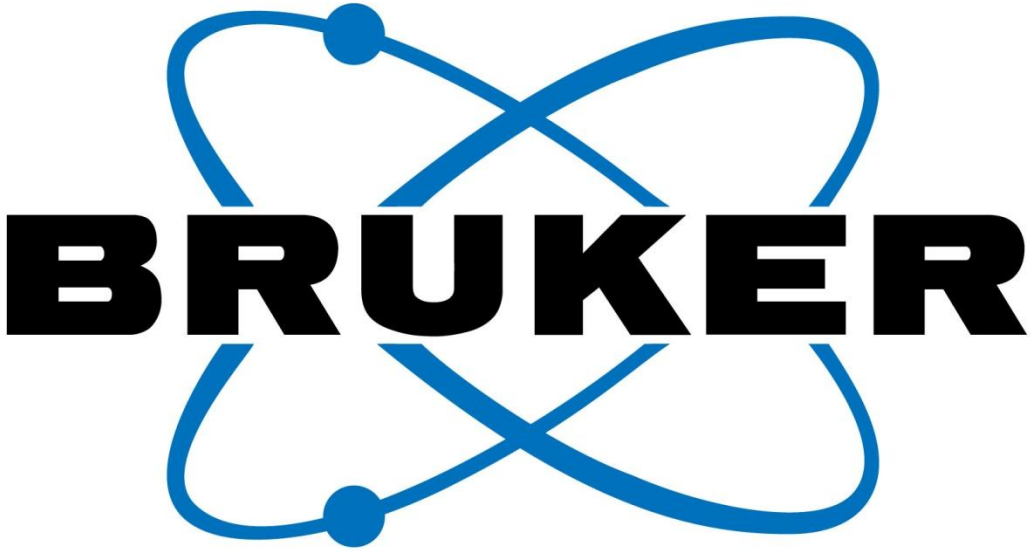
- Hair analysis for trace elements can be measured by TXRF
- Sample inhomogeneity of separate sections suggest a digestion step is required for a better average result
- Ca, Cu, and Fe are detected in each sample
- Other elements found present include: S, K, Ti, Mn, Ga, Se, Br, Sr, Pb, and in parts of samples Al, Mg, P, Cl, Ni, La, and Hg.

Q & A

Any Questions?

Please **type in** the questions you may have for our speakers in the **Questions form** and click **Submit**





www.bruker.com