TXRF analysis of cell culture media and medical microsamples – saving time, money and lives

Bruker Nano Analytics, Berlin
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Welcome

Speakers

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Berlin, Germany

Itinerary

Part I: Cell cultures

- Cell cultures – background
- Method development for element analysis of cell culture media
- TXRF analysis of high performance cell culture media
- Summary and conclusion

Part II: Biological and medical microsamples

- Protein analysis
- Bioassay analysis
- Summary and outlook

Part I: Cell cultures

Background

Cell cultures
Background

Application of cell cultures

- Biopharmaceuticals
  pharmaceutically active proteins and nucleic acids
- Clinical research: tissue cells for therapeutic purposes
- Study of elementary cellular processes without classical animal experiments

Cell cultures
Background

Cell culture media

- Natural environment of cells has to be imitated
- Standardized media, e.g. DMEM,1 g/l or 4.5 g/l glucose
- Media contain amino acids for protein biosynthesis, vitamins and inorganic salts for metabolic processes, carbohydrates for energy production
- Nutrient (P, K, Ca, Zn, Fe) and trace elements (Se, Mn) nutrient sources, co-factors in enzymatic reactions
- Contaminations reduce yield of the biopharmaceuticals
  may cause necrosis of the cells

Cell culture media
Objective

Objective 1

- Development of a TXRF method for cell culture media
  1. Optimize sample layer on disc, standard DMEM media
  2. Determination of LOD / LOQ
  3. Reproducibility

Objective 2

- Measurement of nutrient elements in cell culture media

Objective 3

- Apply method to modern high performance media
  1. QC: identify contaminants
  2. Spike experiments for method detection limits

Cell culture media
Objective

Samples

- Commercially available DMEM, 1 g/l or 4.5 g/l glucose
- Mammalian media from two German suppliers
  Sugars conc. 8 g/l
  - Human Embryonic Kidney (HEK)
  - Chinese Hamster Ovary (CHO)
  - Baby Hamster Kidney (BHK)
  - Invertebrate (IKZ)
  - Feed media (20 g/l glucose)

Cell culture media
TXRF spectrometer

S4 T·STAR - **Unique benefits**

- Three excitation modes — to detect most elements of the PSE
- 60/100 mm² detectors — improved sensitivity for lowest limits of detection
- New analytical capabilities — angle scan for depth profiling and layer analysis
- Sample geometry flexibility — measurement of discs, microscopy slides, wafers etc.
- Motorized beam path — automatic beam adjustment and QC procedures
- Large sample capacity — up to 90 sample discs, multi-user operation
- Most modern software — instrument/measurement status display, statistical functions

Method development for element analysis of cell culture media

Cell culture media
Method development

1. Optimize sample layer on disc

Comparison of different modifier solutions (smoothing agents)

- DMEM media with 50 – 200 ppm P, S, K, Ca
- Addition of 10% modifier
  (1% Triton X-100, Ethanol, PVA (0,3 g/l))
- Dilutions down to 1 : 100
- Internal standard Sc

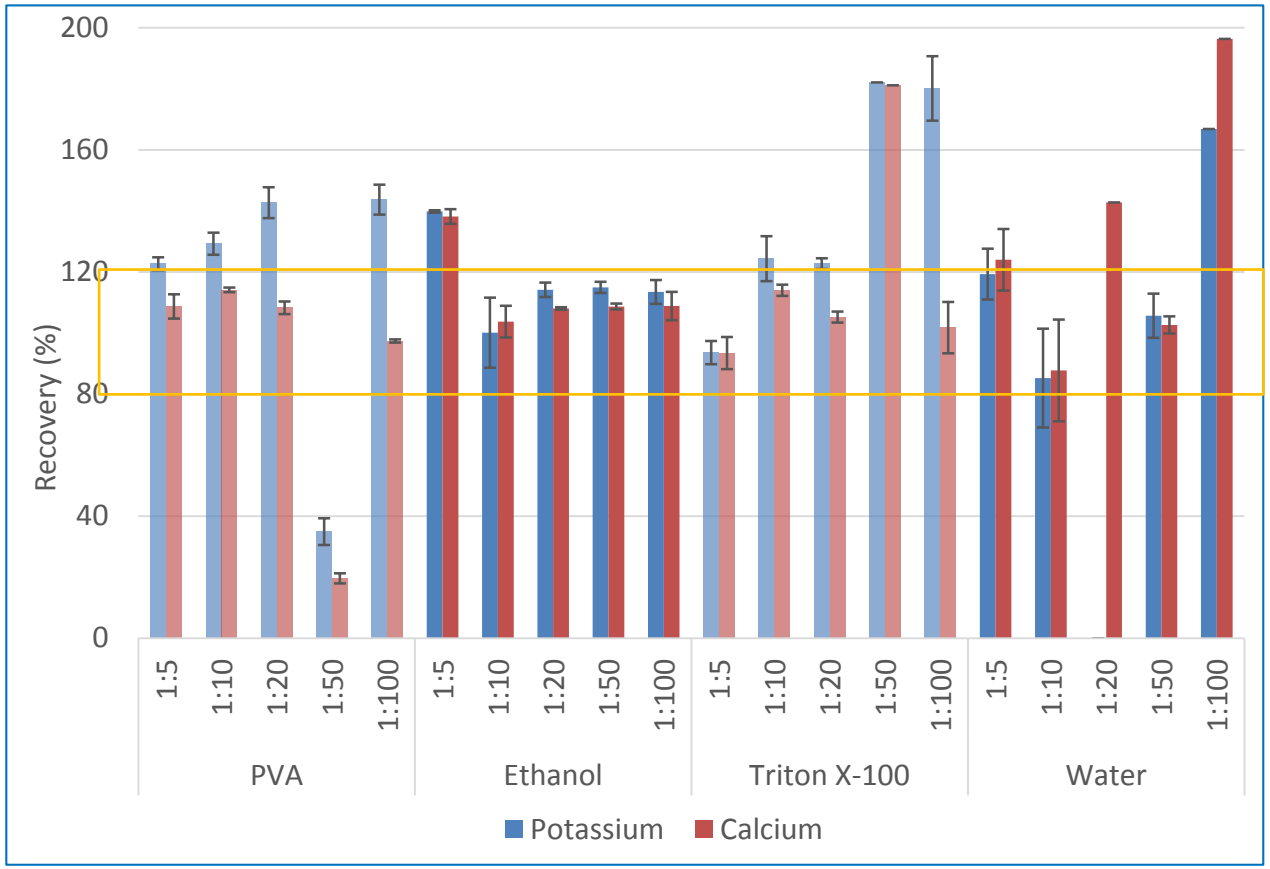
Measurement parameter S4 T-STAR

- Mo excitation, 50 kV, 1000 µA
- W-L excitation, 50 kV, 1000 µA
- Measurement time 1000 s

Cell culture media
Method development

Results

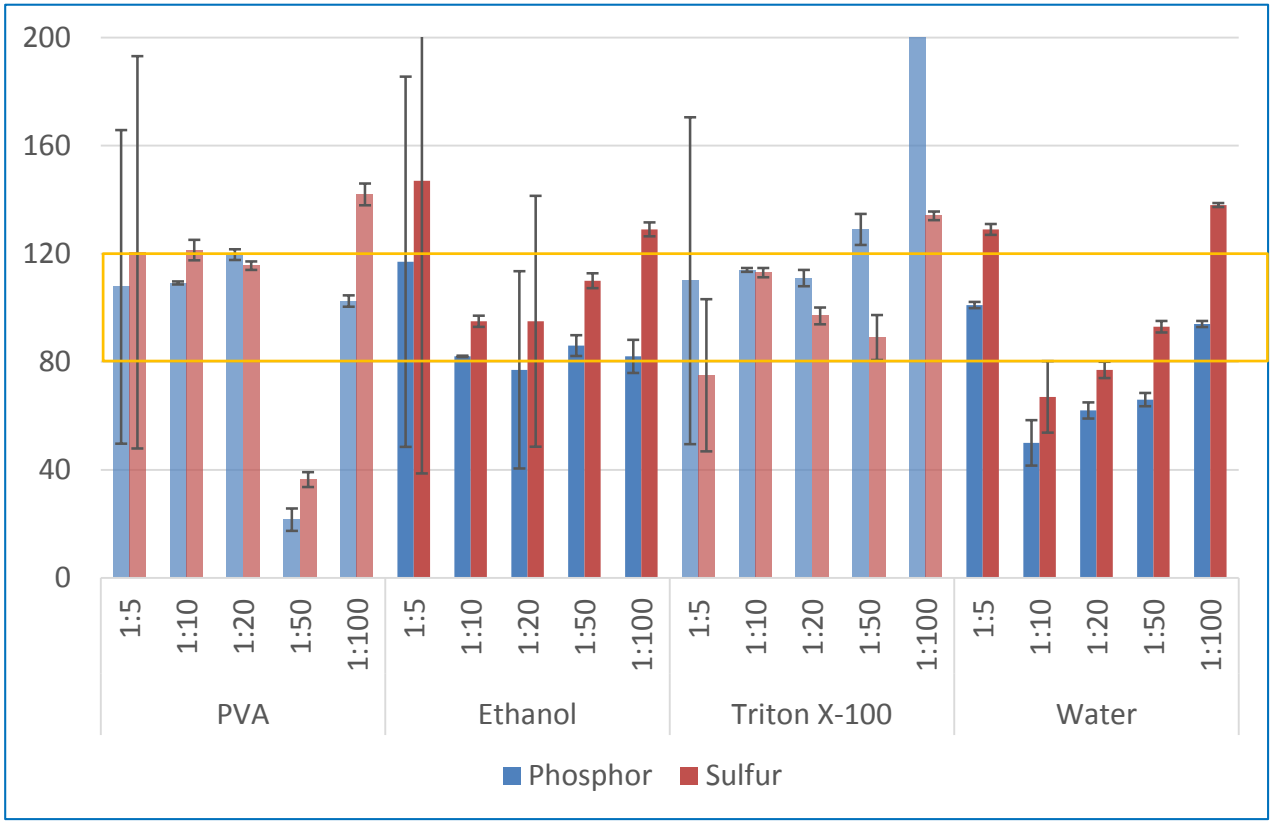
- Ethanol leads to satisfying recovery rates and standard deviations < 5% (< 10% at 1:100)
- Overlap of IS Sc with Ca, following experiments with V
- Mo excitation did not provide good data for P and S



Cell culture media
Method development

Results

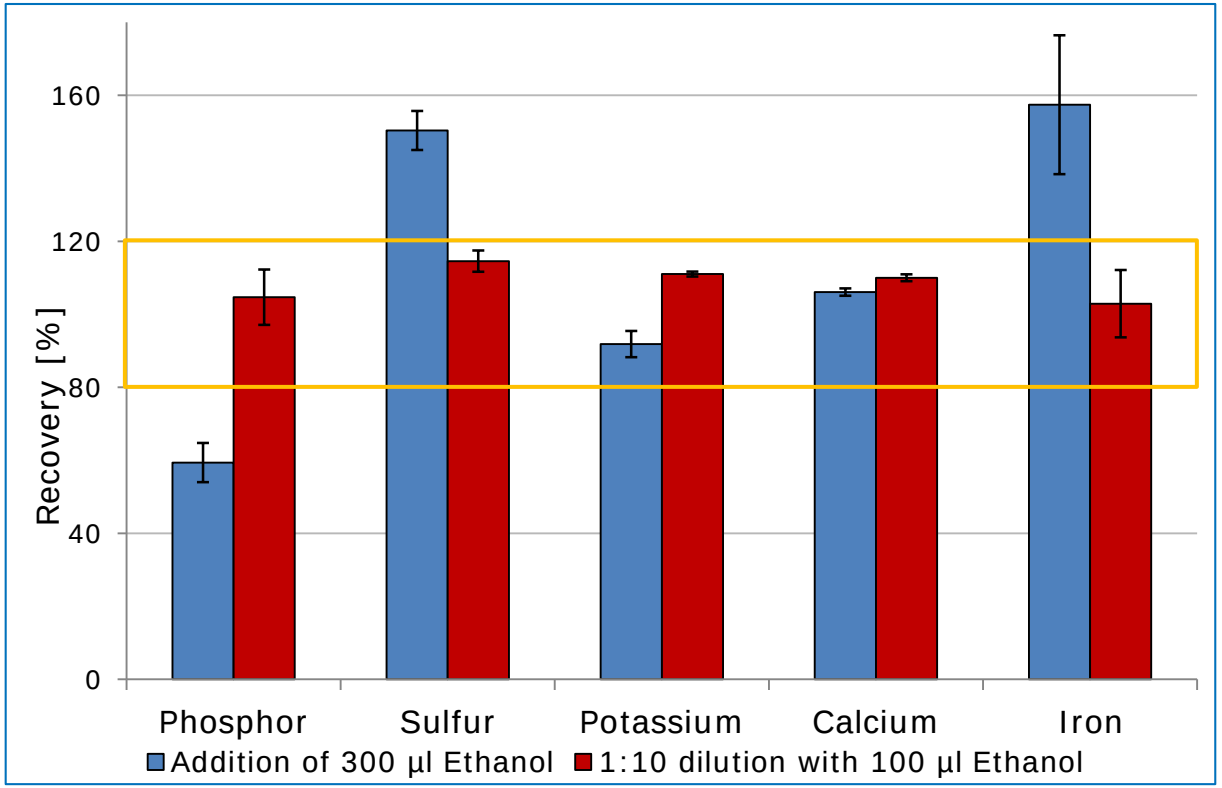
- W-L excitation delivers reproducible results for P and S, if Ethanol was used



Cell culture media
Method development

2. Reproducibility

- Two preparations
  - 700 µl sample + 10 µl IS + 290 µl EtOH
  - 100 µl sample + 100 ml EtOH + 790 µl H_2O + 10 µl IS
- Better than 10%, typically < 3%
- Dilution recommended due to high amount of macro nutrient
- EtOH contained trace impurities (Fe) -> ultrapure or HPLC grade required



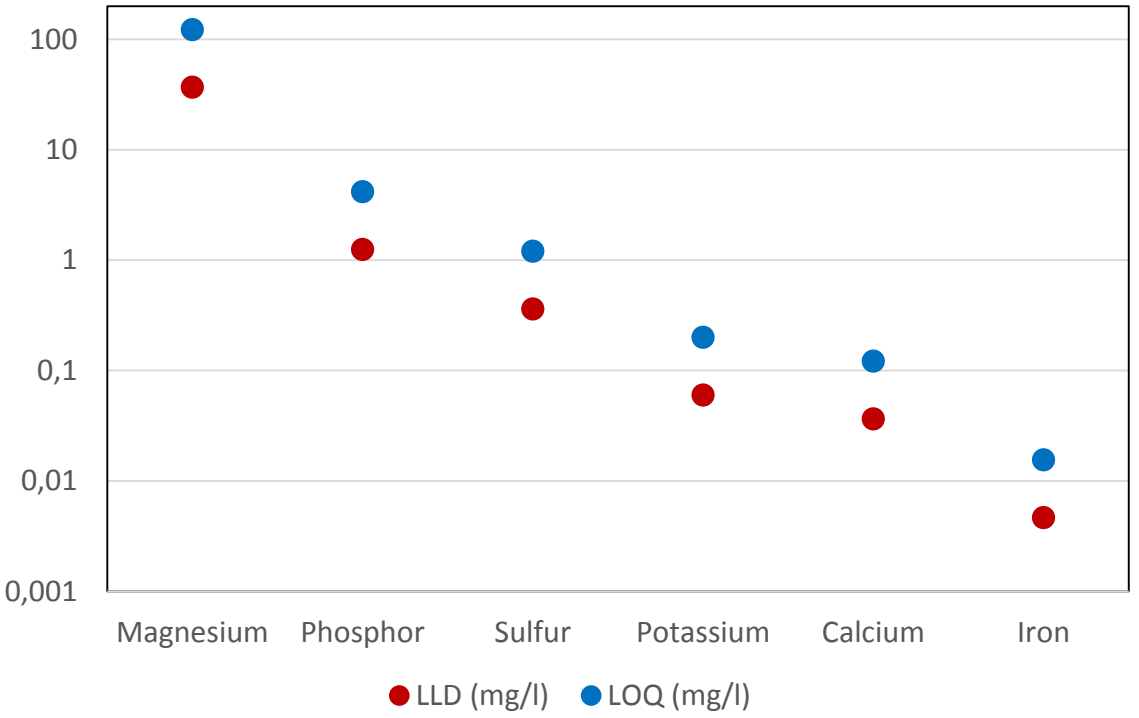
Cell culture media
Method development

3. LOQ

- EtOH treated media

	LOQ (mg/l)
Magnesium*	122,7
Phosphor	4,165
Sulfur	1,205
Potassium	0,200
Calcium	0,122
Iron	0,016

*) W-L excitation



- Sample preparation
  - 700 µl media
  - 290 µl EtOH
  - 10 µl V (IS)

TXRF analysis of high performance cell culture media

Modern high performance media
Quality control

Analysis of mammalian cell culture media

- Method developed for standard media
- 24 media in triplicate
  = 72 discs

Measurement parameter S4 T-STAR

- Mo excitation, 50 kV, 1000 µA
- W-L excitation, 50 kV, 1000 µA
- W-Brems excitation, 50 kV, 1000 µA
- Measurement time 1000 s

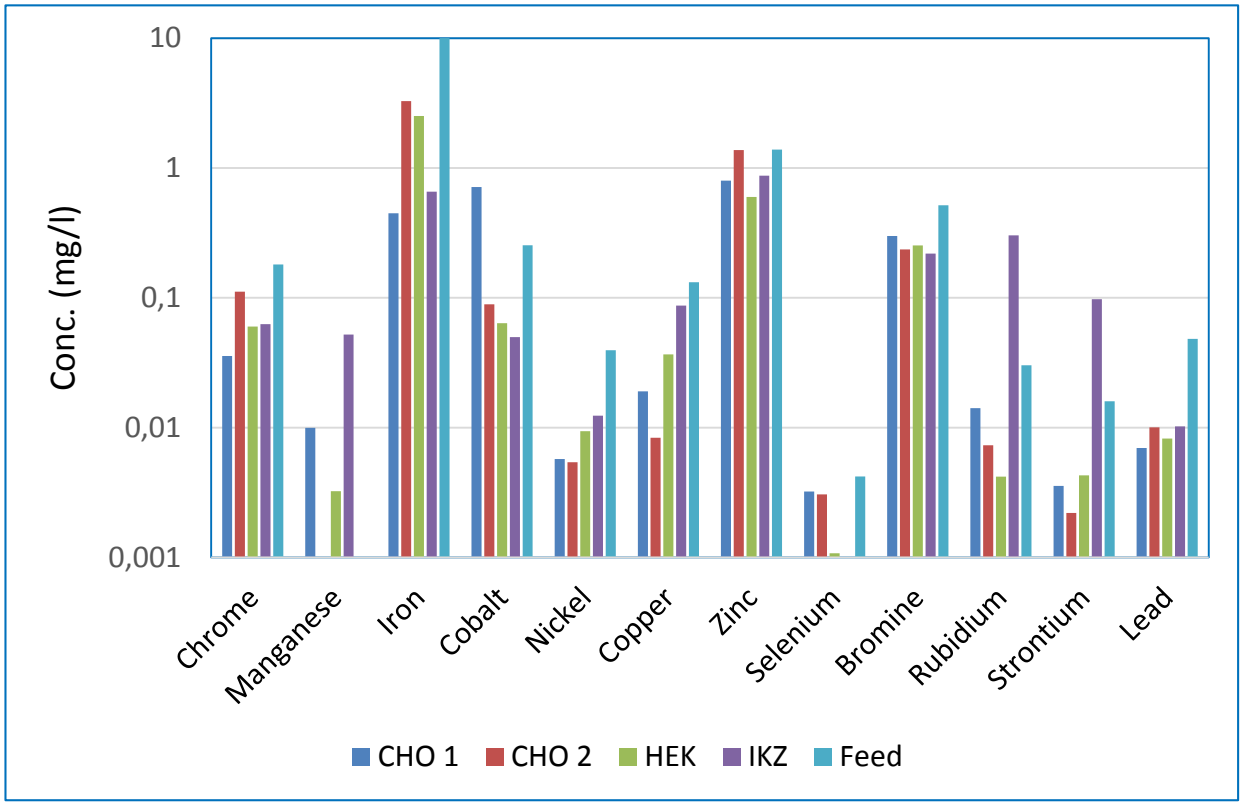
Ethanol	290 µl
IS Vanadium	10 µl
Sample	700 µl

Volume on disc	10 µl
Drying	30° C

Modern high performance media
Quality control

Results mammalian cell culture media

- Micro-nutrient and contamination test of different media batches
- Application of Mo and W-L excitation
- Typical concentration range 1 to 1000 ppb
- Distinct differences between cell lines

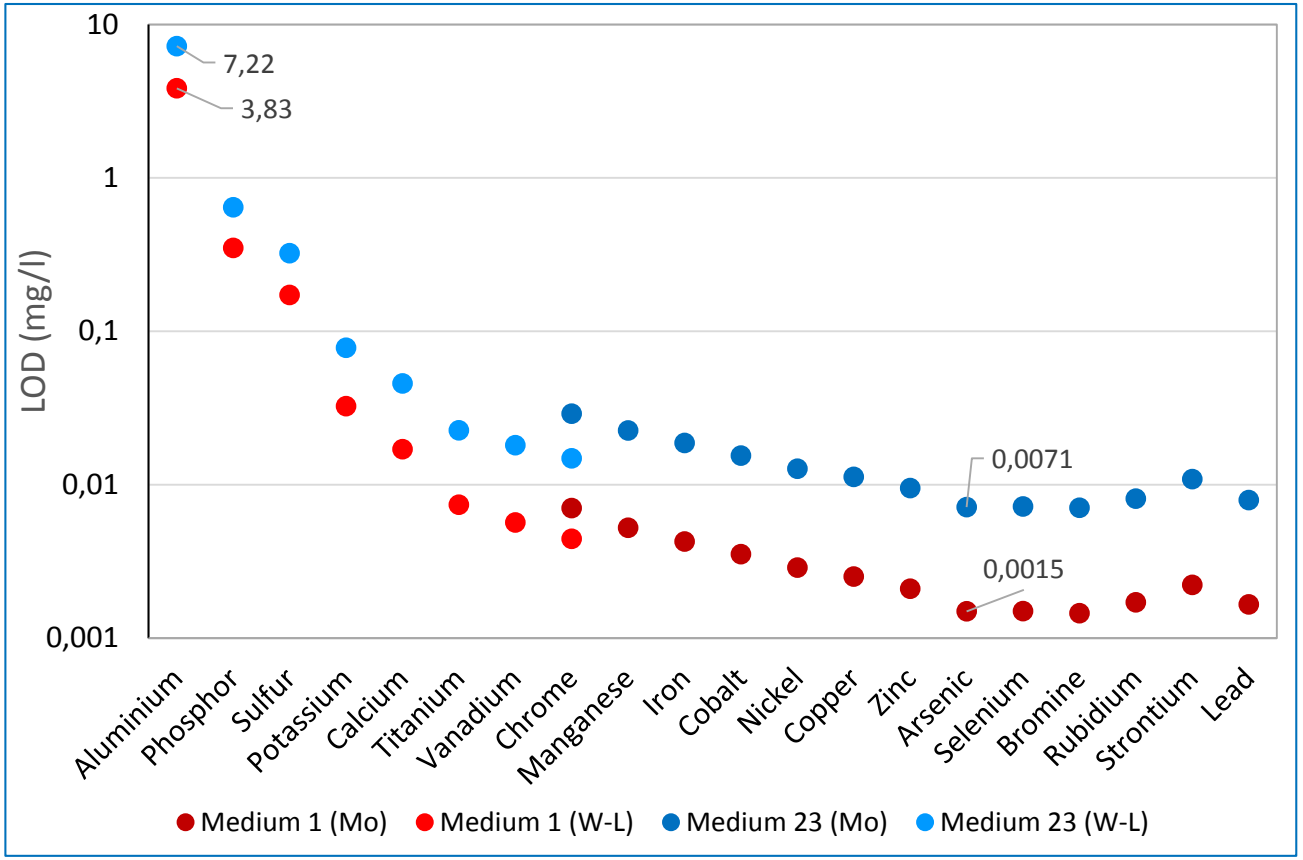


CHO	Chinese Hamster Ovary
HEK	Human Embryonic Kidney
IZK	Invertebrate cell cultures
Feed	Feed media

Modern high performance media
Quality control

Limit of detection

- LOD down to single digit ppb
- W-L significantly improves the detection of light elements (factor 2 - 4)
- Recommended measurement time for routine analysis = 300 s



Modern high performance media
Spike experiments

Preparation of spike media

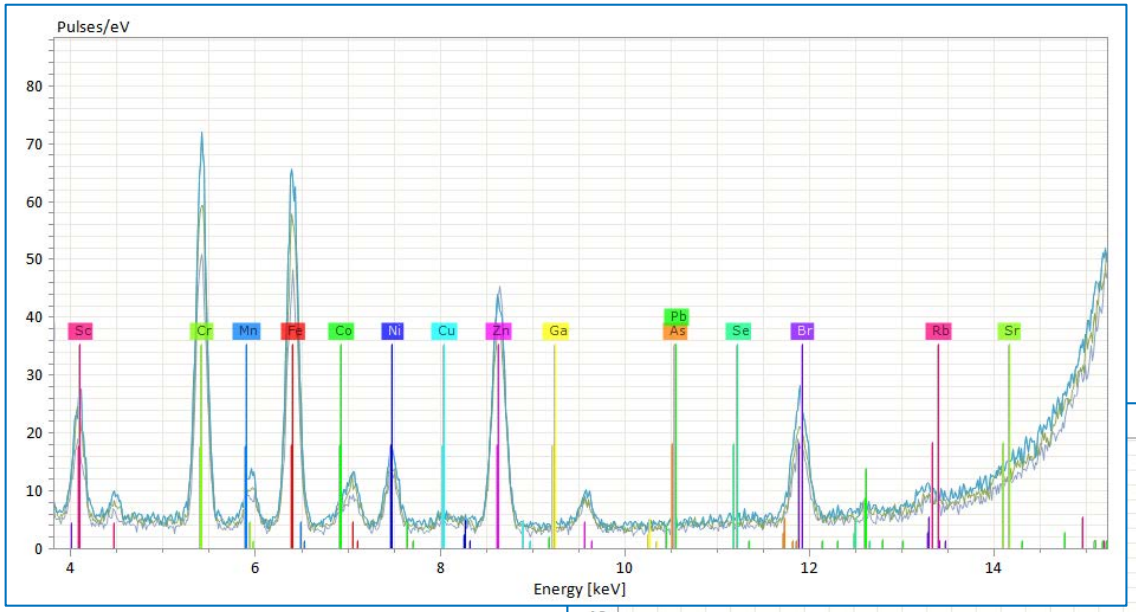
- 1 ml of one high performance medium
- Two spike elements per sample concentrations see table
- Measurement in triplicate
  15 samples = 45 discs

Spiked element concentrations (µg/l)					
Pb	**Mn**	**Se**	**Ni**	**Cr**	**Cd**
5	20	5	10	15	300
10	50	10	20	30	500
20	100	20	50	50	1000
50	200	50	100	100	2000
100	500	100	200	200	4000

Measurement parameter S4 T-STAR

- Mo excitation, 50 kV, 1000 µA
- W-L excitation, 50 kV, 1000 µA
- W-Brems excitation, 50 kV, 1000 µA
- Measurement time 1000 s

Modern high performance media
Spike experiments

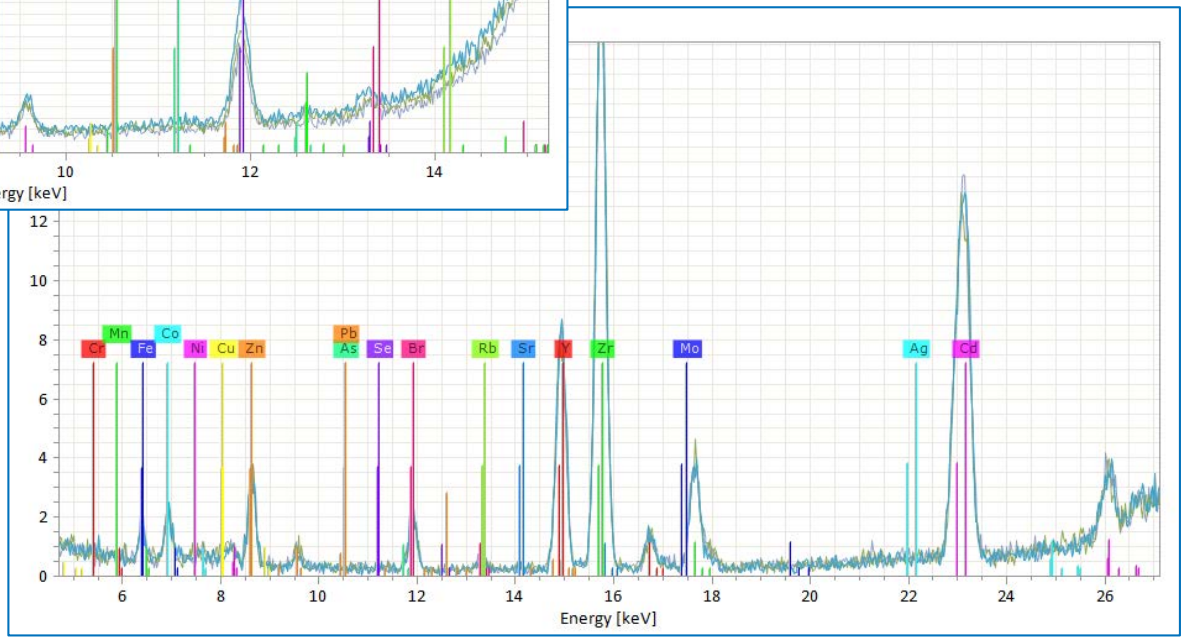


Mo spectra

- Cr spike (200 ppb)

W-Brems spectra

- Cd spike (4 ppm)



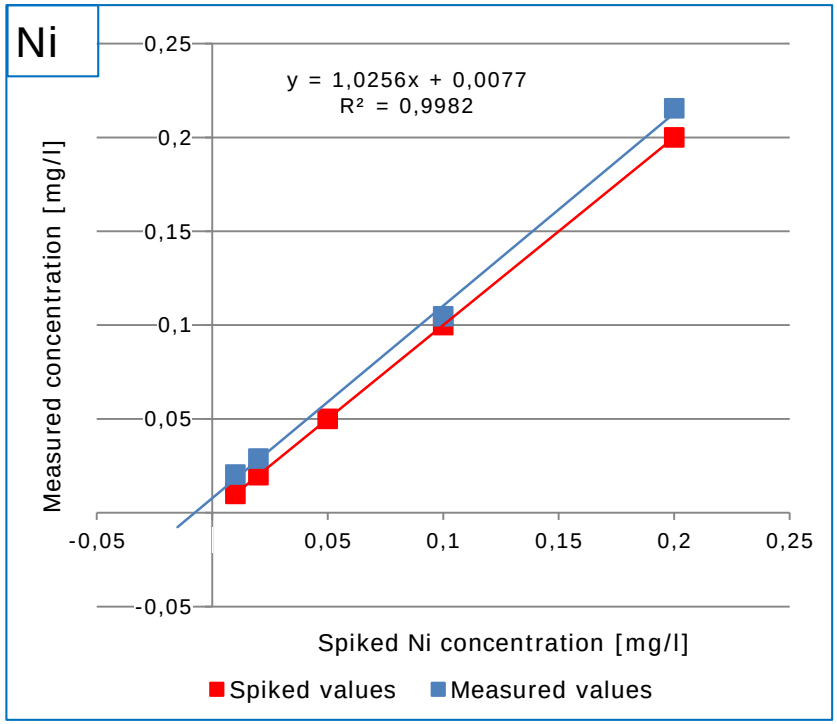
Modern high performance media
Spike experiments

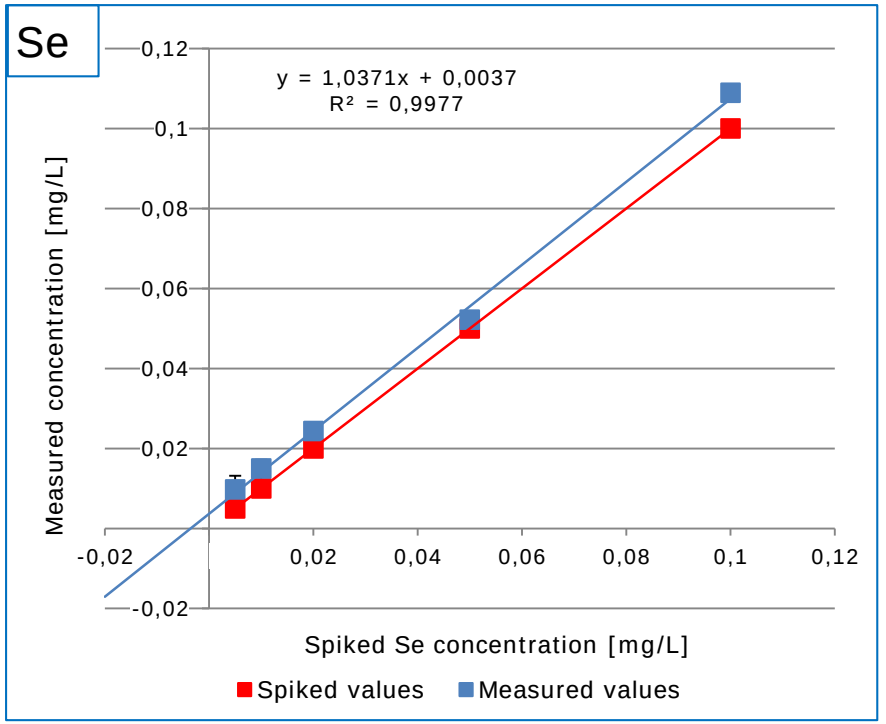
Measurements with Mo excitation

- Very low concentrations of Ni, Se
- Values close to ICP

ICP	2,1 µg/l
TXRF spike	7,5 µg/l
TXRF direct	5,3 µg/l

ICP	5,2 µg/l
TXRF spike	3,6 µg/l
TXRF direct	3,4 µg/l

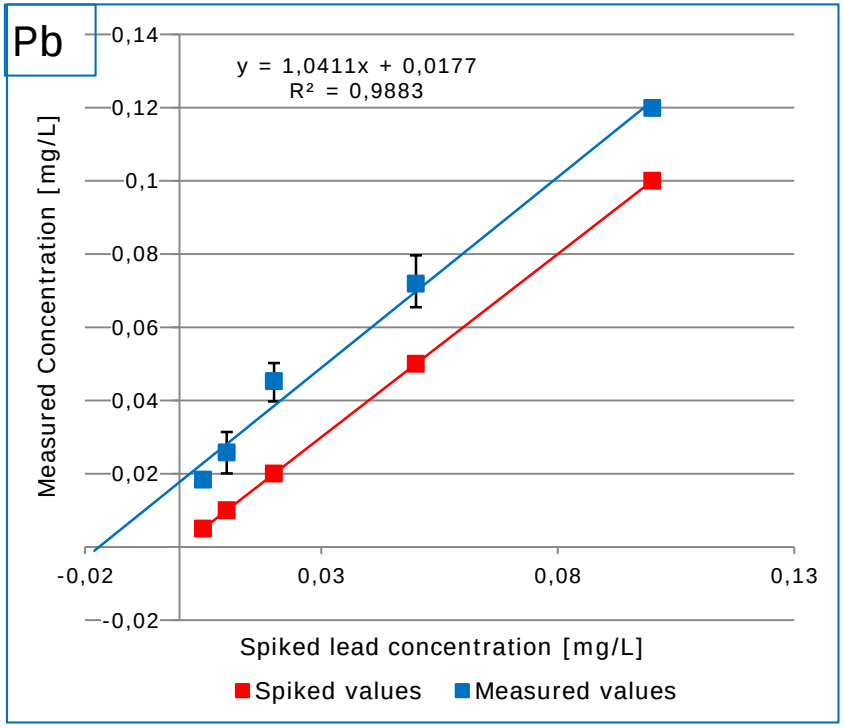




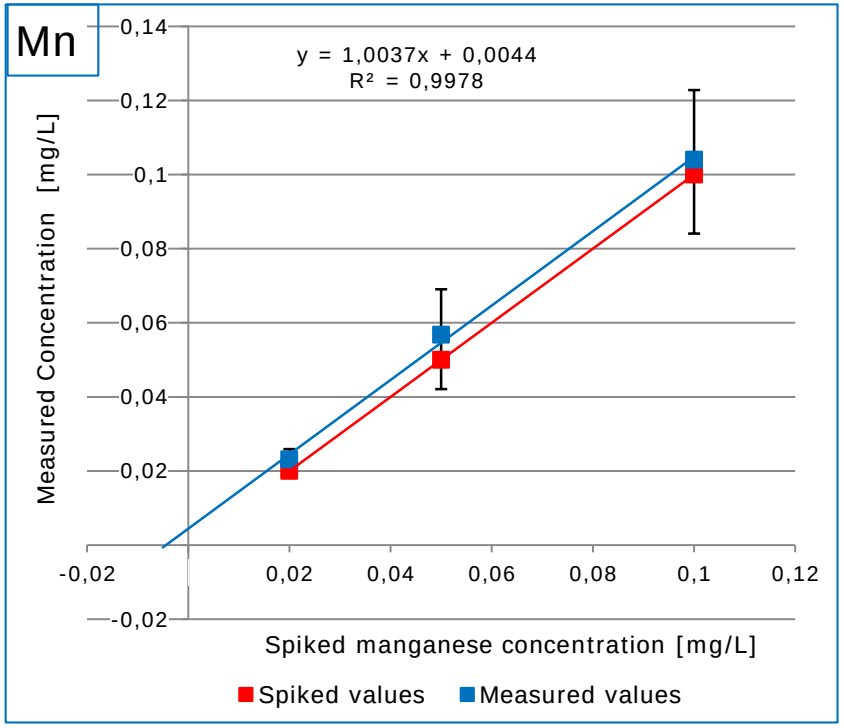
Modern high performance media
Spike experiments

Measurements with Mo excitation

ICP	n.a.
TXRF spike	17 µg/l
TXRF direct	7,6 µg/l



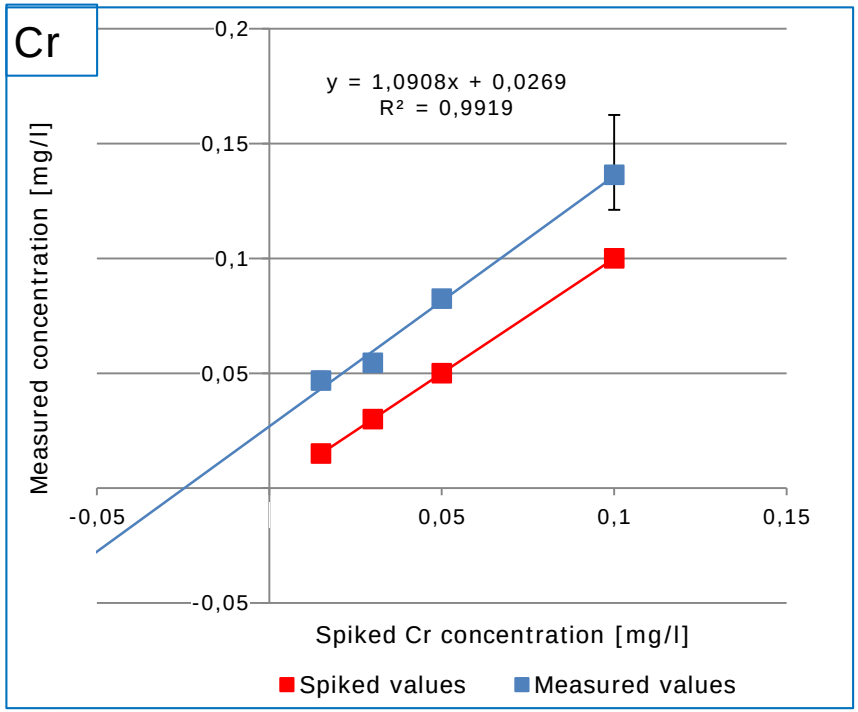
ICP	2,6 µg/l
TXRF spike	4,4 µg/l
TXRF direct	15,8 µg/l



Modern high performance media
Spike experiments

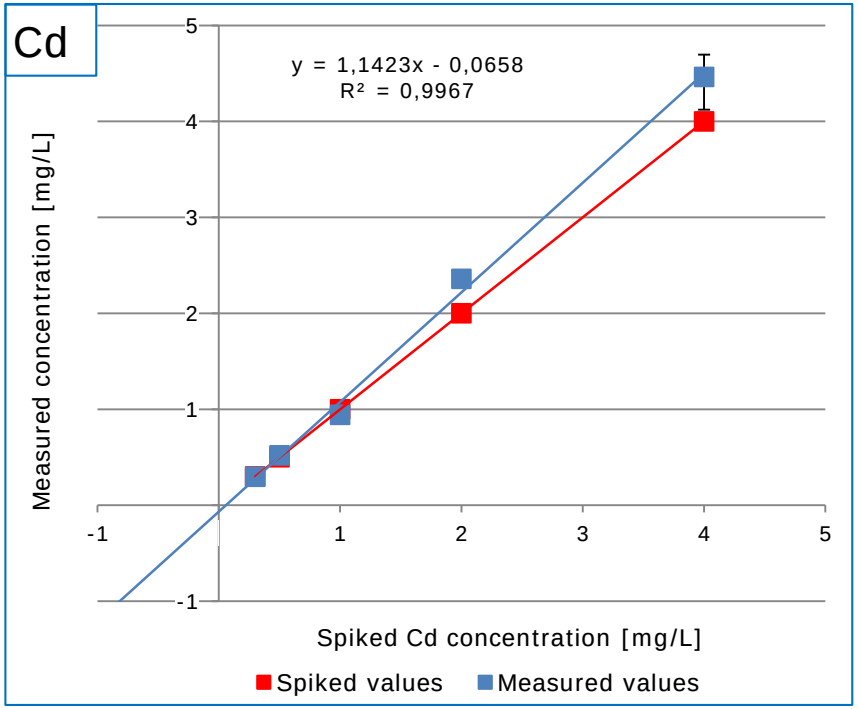
Measurements with W-L and W-Brems excitation

ICP	1,9 µg/l
TXRF spike	24,7 µg/l
TXRF direct	14,7 µg/l



- ICP values for Cr questionable
- Cd quantification not successful

ICP	< 0,2 µg/l
TXRF spike	< 45 µg/l
TXRF direct	< 77,3 µg/l



Summary and conclusion

- A rapid method for TXRF measurements of cell culture media was developed
- EtOH has to be used as smooting agent for optimal layer formation on quartz sample discs
- For contamination control detection limits in the one digit ppb range can be achieved
- The measurement of nutrient elements after a 1:10 dilution provides a reproducibility < 10%

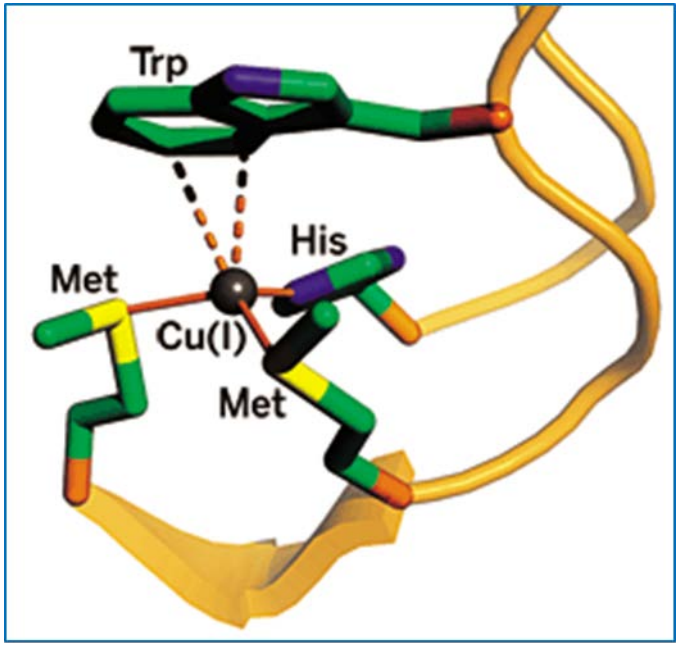
Part II: Biological and medical microsamples

Protein analysis
Introduction

Metal ions and enzymes

- Metal ions are important for the biological function of enzymes
- Various modes of metal-protein interaction: metal-, ligand-, enzyme-bridge complexes
- Metals serve as electron donors or acceptors, Lewis acids or structural regulators

(Riordan JF.: “The role of metals in enzyme activity.”, Ann Clin Lab Sci. 1977 Mar-Apr;7(2):119-29



C. Arnaud: CEN, January 7, 2008
Volume 86, Number 1, p. 8

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Protein analysis
Introduction

Examples of metal ions in enzymes

Role	Metals	Protein
Oxygen transport and storage	Fe, Cu	Haemoglobin
Electron transport	Fe, Cu	Cytochromes
Nitrogen fixation	Fe, Mo, V	Nitrogenase
Oxygen atom transfer	Mo, W	Oxidases, Reductases
Alkyl group transfer	Co	Vitamin B_{12}
Hydrolysis	Zn, Cu, Mn	Hydroxylases, Peptidases
Storage and transport	Fe, Cu, Zn	Ferritin, Metallothioneins

Protein analysis
Introduction

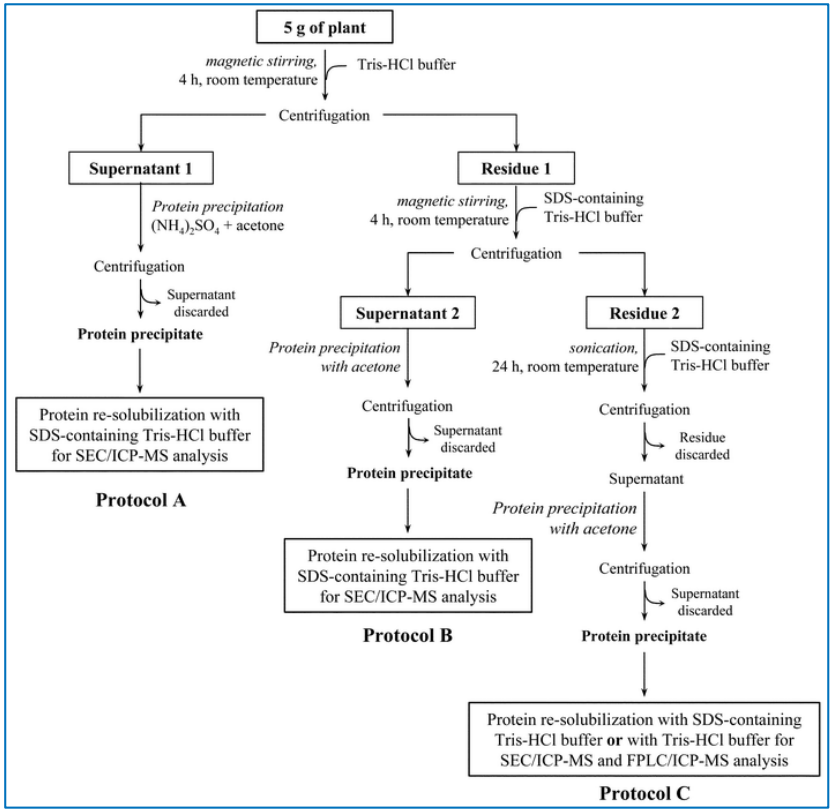
Commonly used analytical techniques

- Atomic Absorption Spectrometry
  - Ca, Co, Cu, Fe, Mg, Mo, Ni, Se, Zn
- Flame/Inductively Coupled Emission Spectrometry
  - for most metals occurring in proteins
- Neutron activation analysis
  - limited availability
  - most reliable technique for a number of elements
  - very sensitive for some elements
  - some critical interferes, e.g. Na
- Inductively Coupled Mass Spectrometry
  - detection of ppb levels of >40 elements in one minute

Protein analysis
Introduction

Analytical issues

- High matrix
  (polypeptides, buffers,salts)
- Sample viscosity, turbidity
- Removal of buffer, salts
  by dialysis or gel filtration
  total destruction of organic matter
- Significant sample amount needed
  microanalysis impossible
- Methods are expensive and
  laborious



Mounicou et al. (2004), Analyst, (2), 116-123

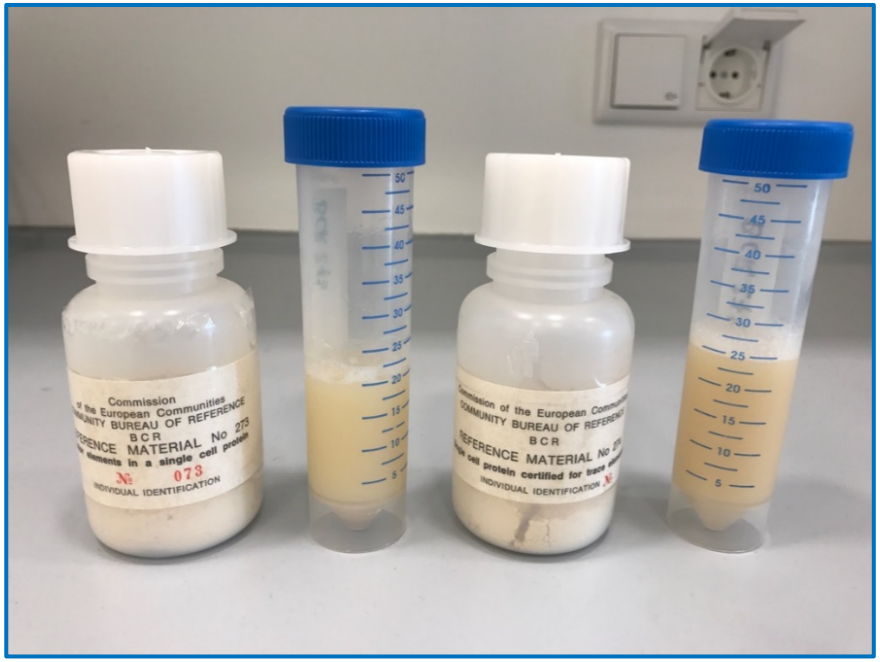
Protein analysis
TXRF spectroscopy

Samples

Certified reference materials

- “BCR 273 – Singe cell protein”
- “BCR 274 – Single cell protein”

500 mg powderous sample were suspended in 25 ml pure water



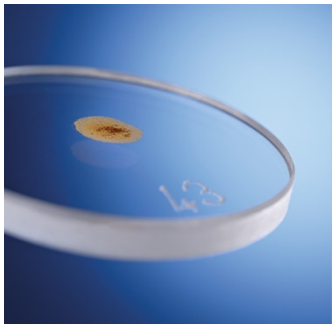
Protein analysis
TXRF spectroscopy

Sample preparation

First approach:
mixing of microsamples in **vials**

- 100 µl sample + internal standard
  + 10 µl Sc (10 mg/l)
  + 10 µl Ga (10 mg/l)

- Preparation of
  - 10 µl
  - 5 µl
  - 3 µl
  - 1 µl
  - 0,5 µl

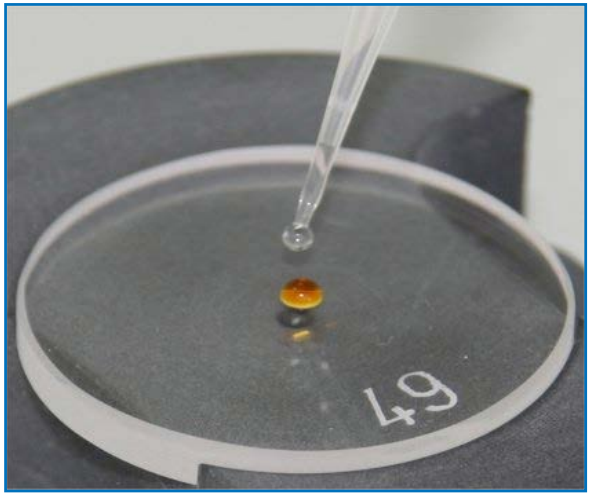


Protein analysis
TXRF spectroscopy

Sample preparation

Second approach:
mixing of microsamples on **discs**

- 5 µl sample + 5 µl Sc/Ga solution (20 mg/l)
- 3 µl sample + 3 µl Sc/Ga solution (20 mg/l)
- 1 µl sample + 1 µl Sc/Ga solution (20 mg/l)



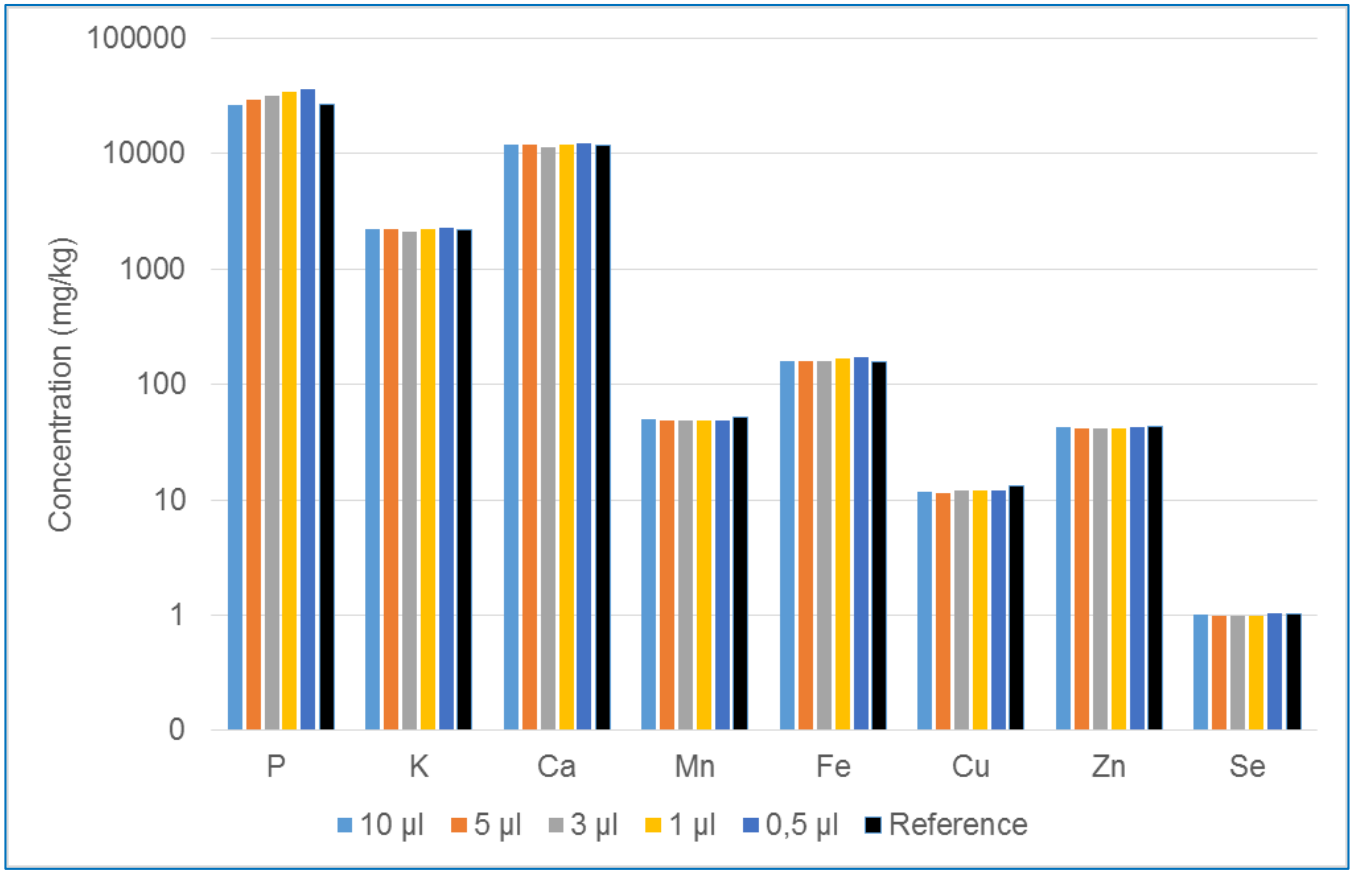
Protein analysis
TXRF spectroscopy

S4 T▪STAR

- Mo tube, 50 kV/1000 µA
- W-tube, 50 kV/1000 µA
- 60 mm^2 XFlash SDD
- 90 position sample changer
- Mo-K excitation, 1000 s
- W-L excitation, 1000 s
- W-Brems, 1000 s

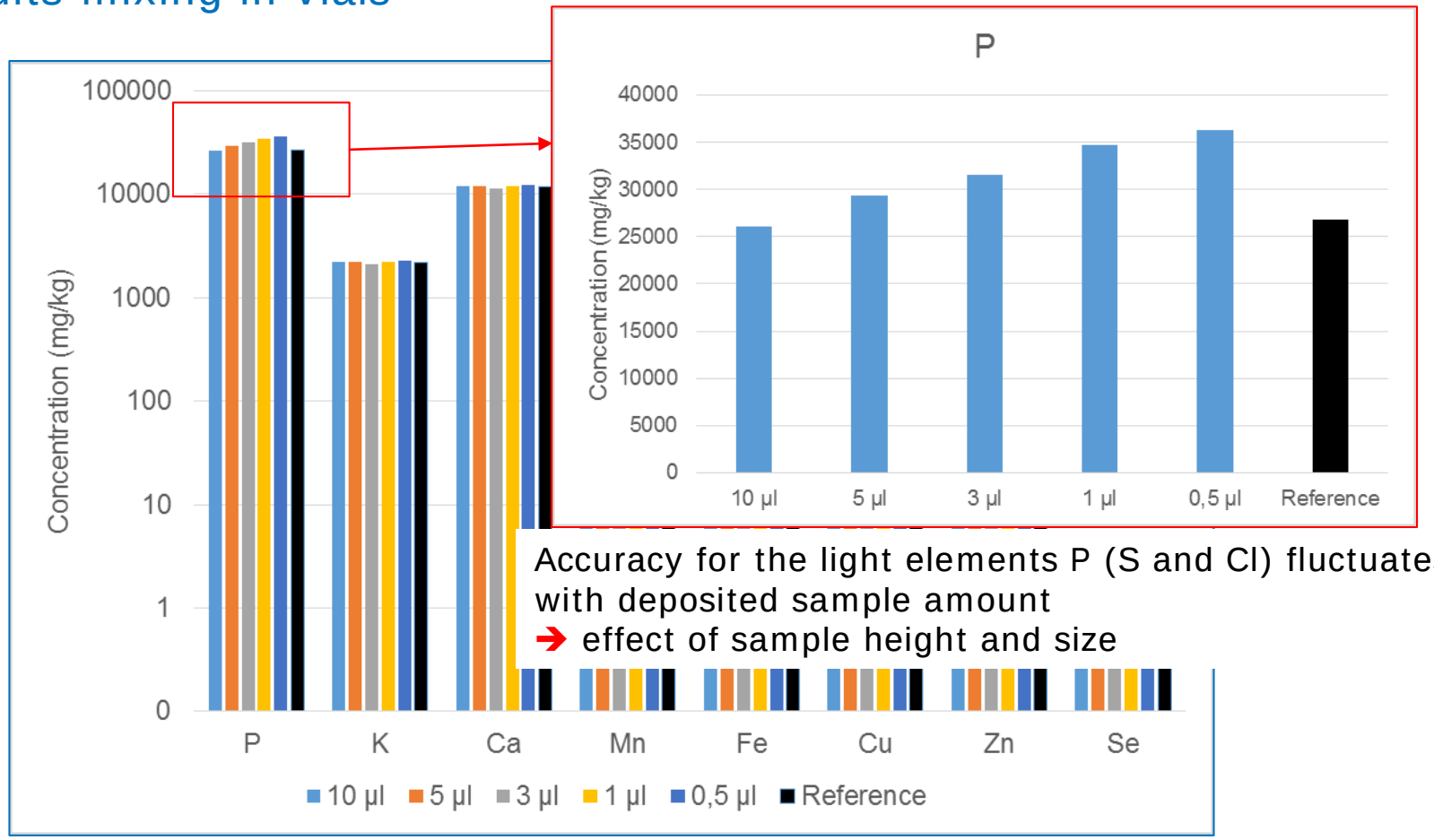
Protein analysis
TXRF spectroscopy

Results mixing in vials



Protein analysis
TXRF spectroscopy

Results mixing in vials



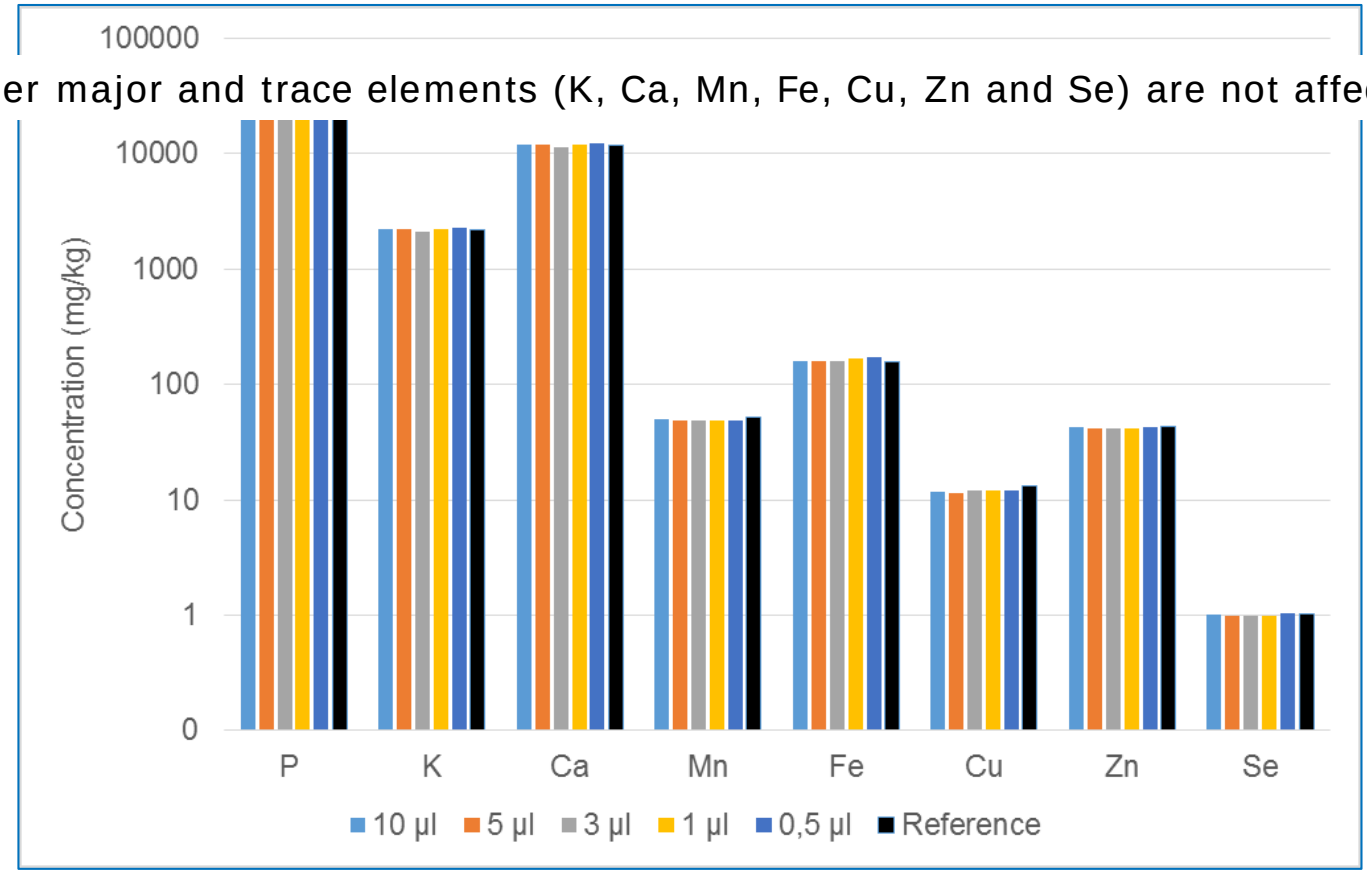
Accuracy for the light elements P (S and Cl) fluctuates with deposited sample amount

➔ effect of sample height and size

Protein analysis
TXRF spectroscopy

Results mixing in vials

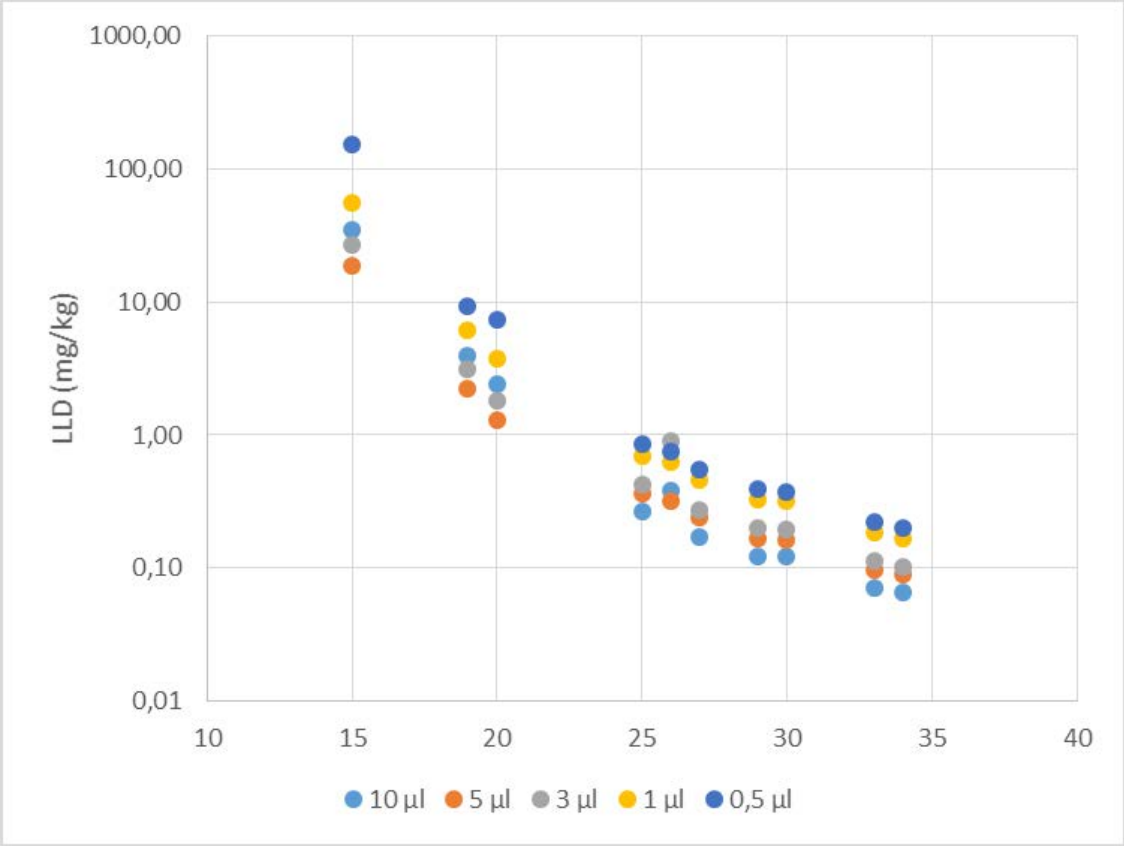
Other major and trace elements (K, Ca, Mn, Fe, Cu, Zn and Se) are not affected



Protein analysis
TXRF spectroscopy

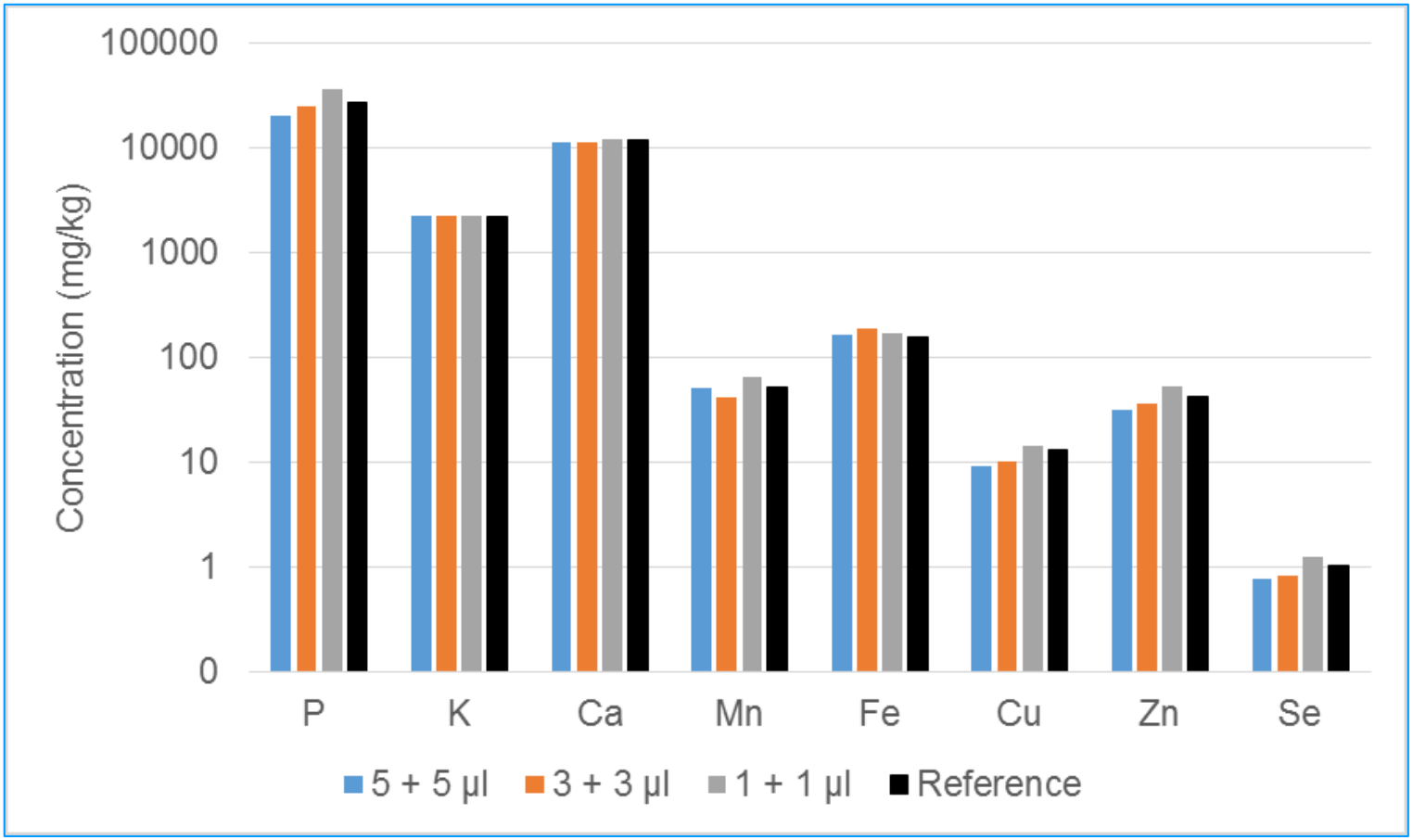
Results mixing in vials

- The detection limits are directly related to the deposited amount



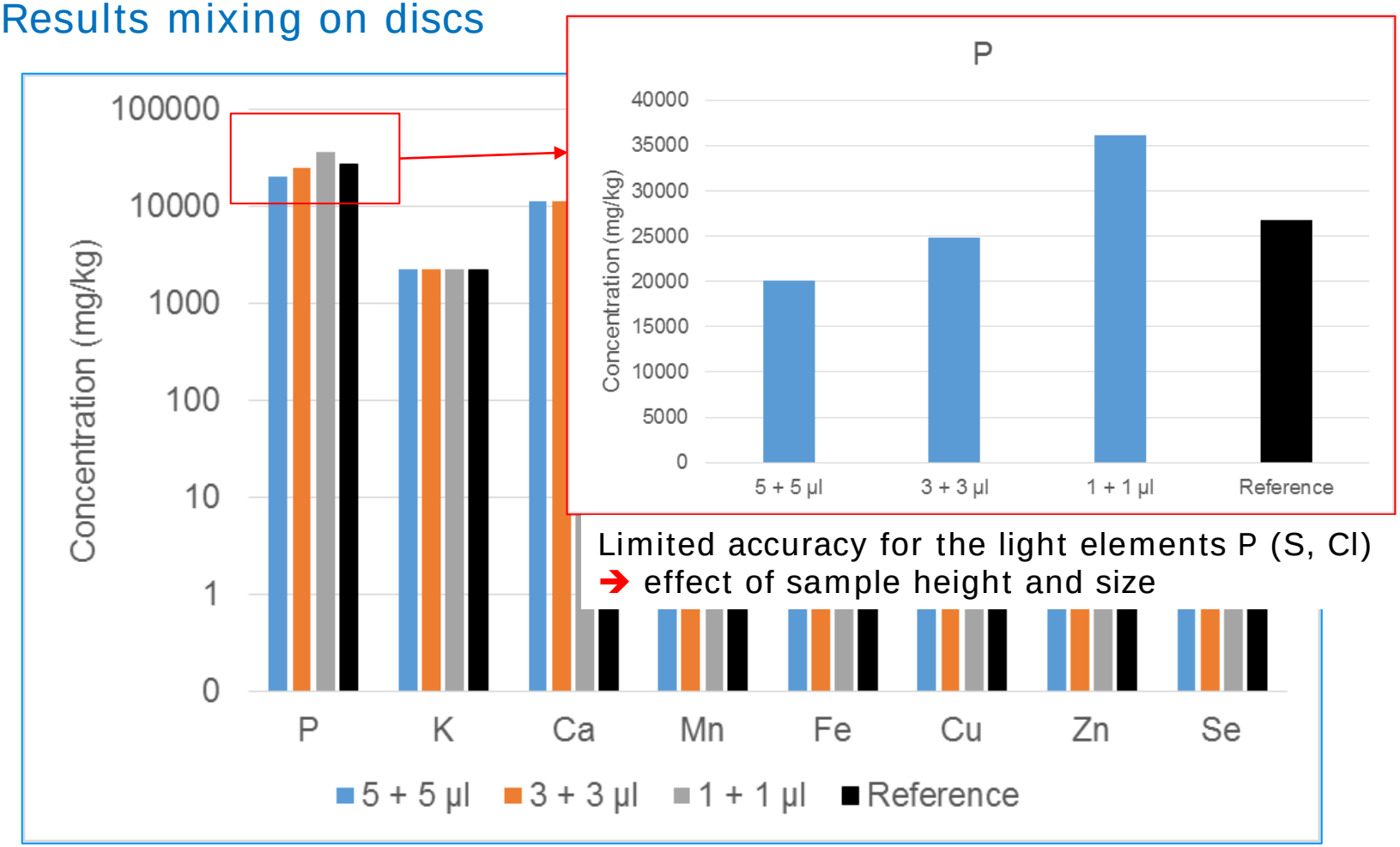
Protein analysis
TXRF spectroscopy

Results mixing on discs



Protein analysis
TXRF spectroscopy

Results mixing on discs

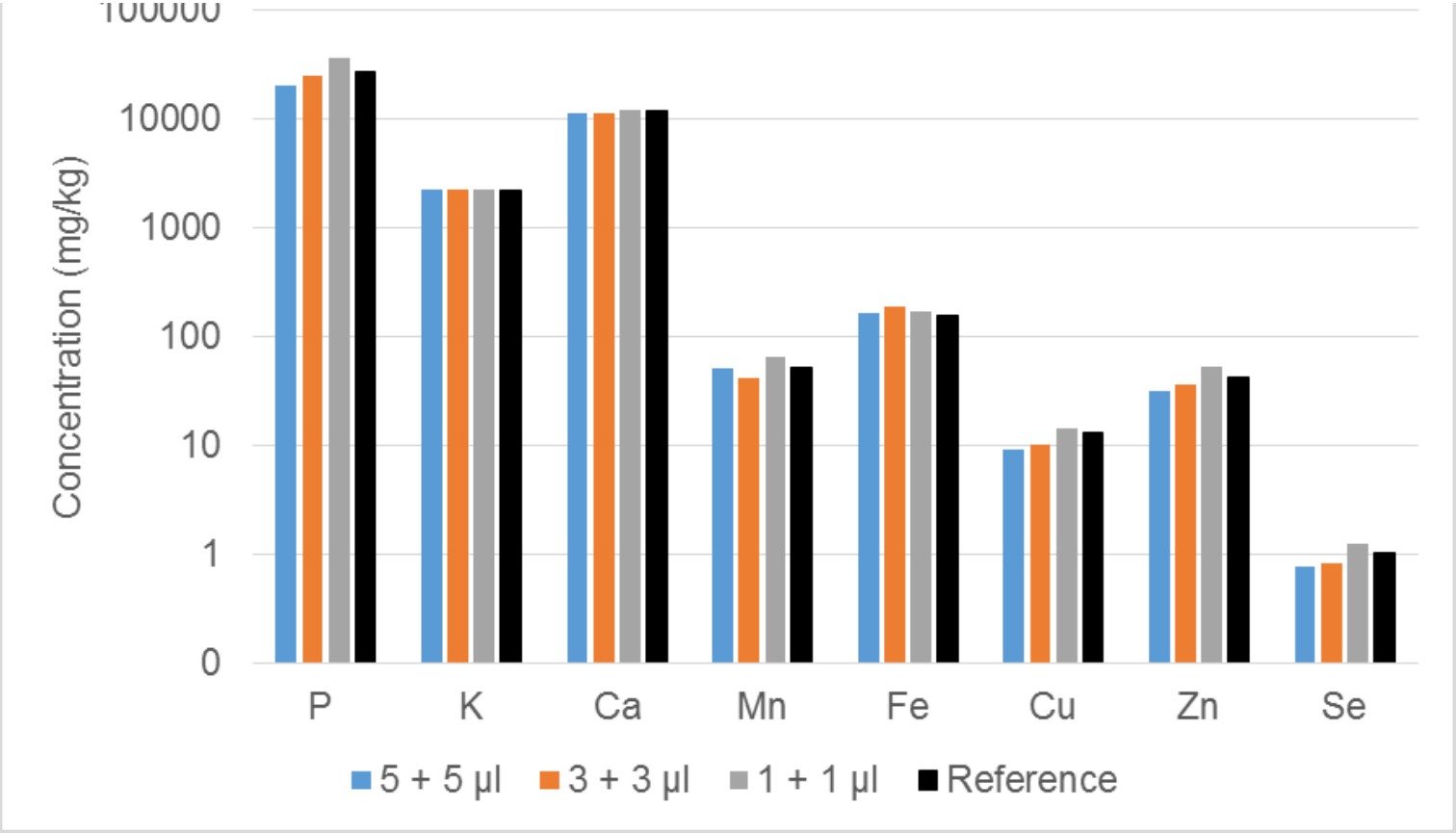


Limited accuracy for the light elements P (S, Cl)
➔ effect of sample height and size

Protein analysis
TXRF spectroscopy

Results mixing on discs

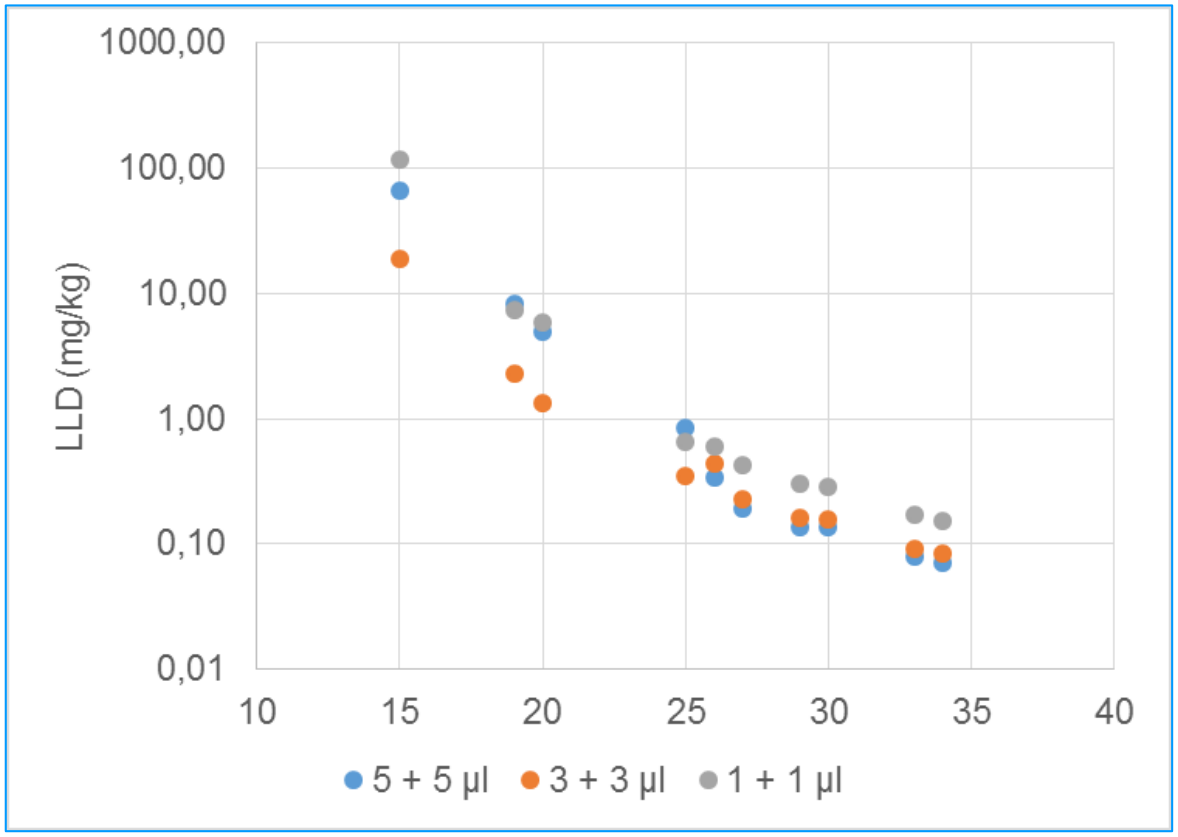
Other major and trace elements (K, Ca, Mn, Fe, Cu, Zn and Se) are not affected



Protein analysis
TXRF spectroscopy

Results mixing on discs

- The detection limits are directly related to the deposited amount



Bioassay analysis
Introduction

Saving lifes…?

Not directly theirs…

Bioassay analysis
Introduction

Saving lifes…?

But theirs…

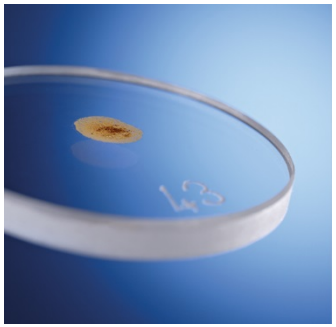
Bioassay analysis
TXRF spectroscopy

Sample preparation

Sampling and preparing non-lethal amounts of blood (urine, saliva …)

20 µl sample
+ 10 µl Ga (4 mg/l)

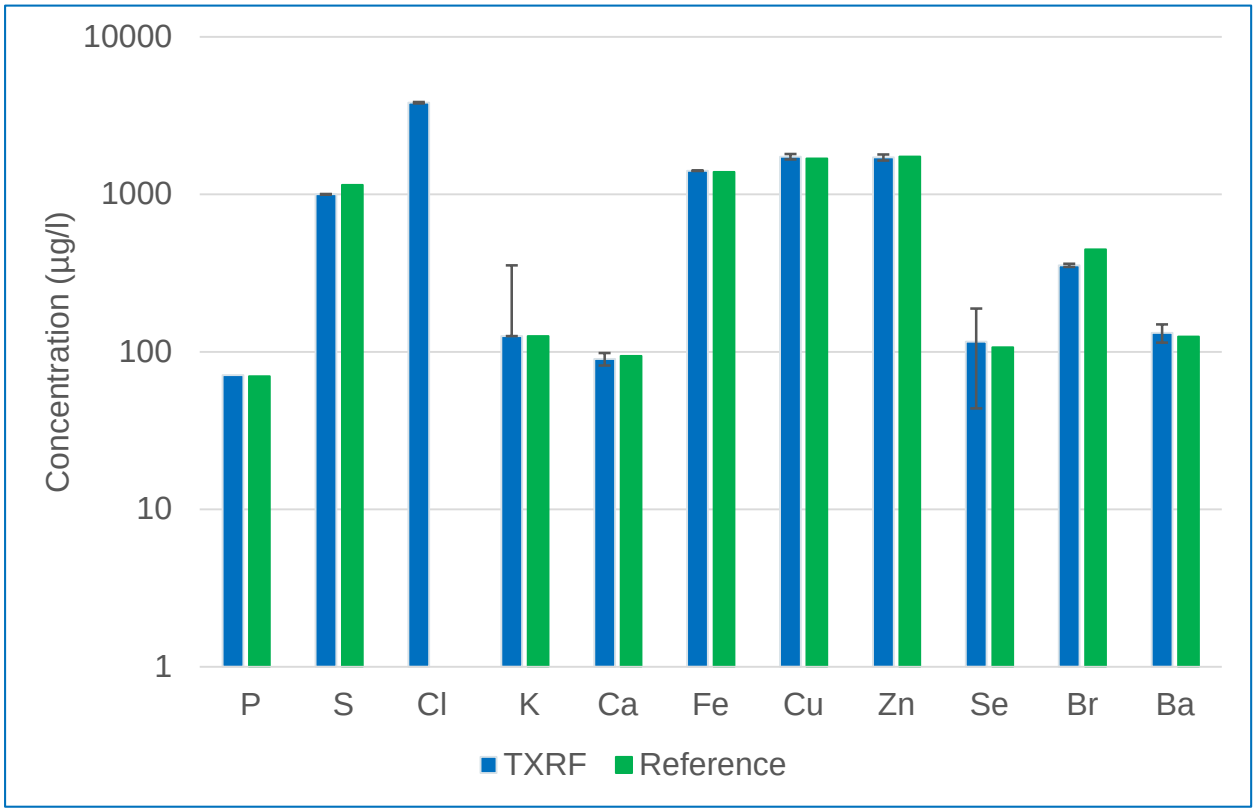
Preparation of
- 10 µl (duplicate)



Bioassay analysis
TXRF spectroscopy

Results

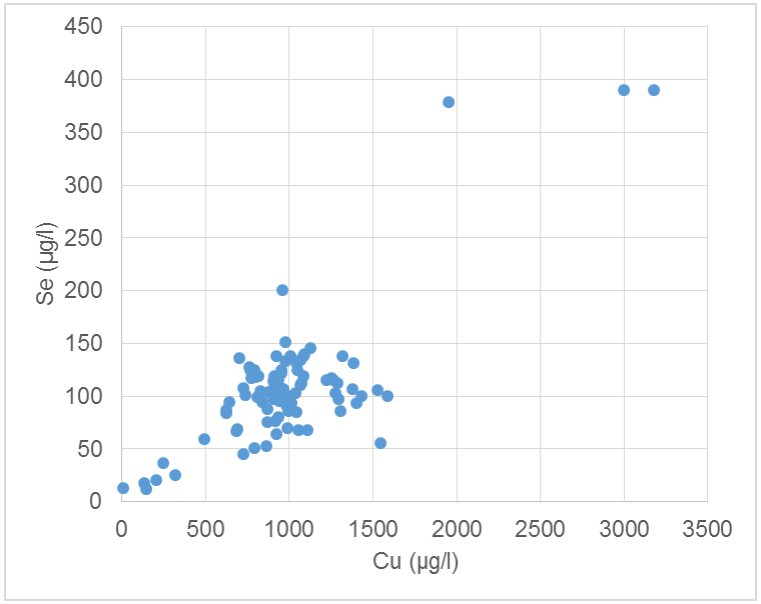
- Seronorm human serum CRM, S2 PICOFOX

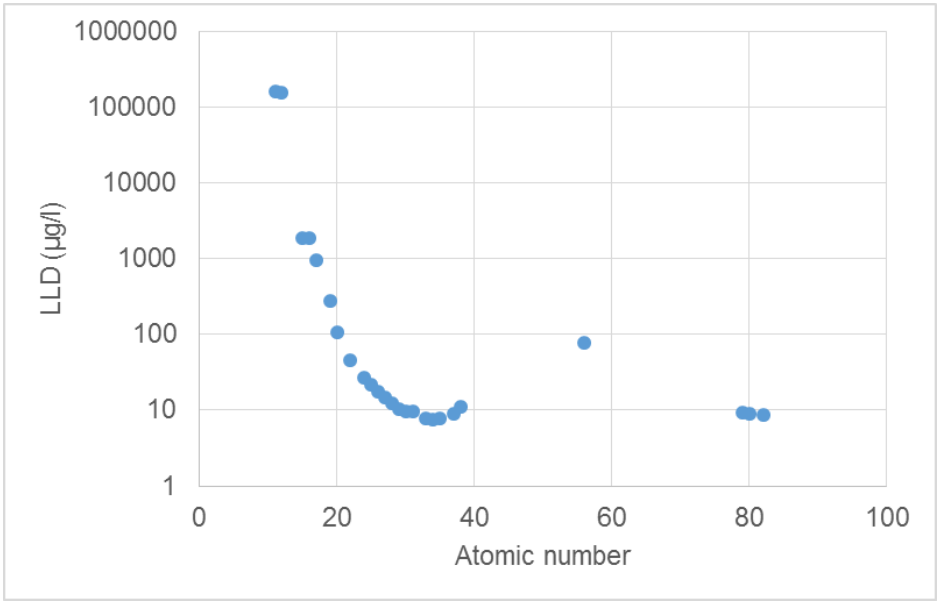


Bioassay analysis
TXRF spectroscopy

Results

- Serum samples from lab mice (publication in preparation)





Summary and Outlook

The analysis of minute protein or serum samples by means of TXRF is possible

- Mixing of internal standard and sample in a vial is recommended
- Determination of detection limits for trace elements requires careful consideration of the sample amount

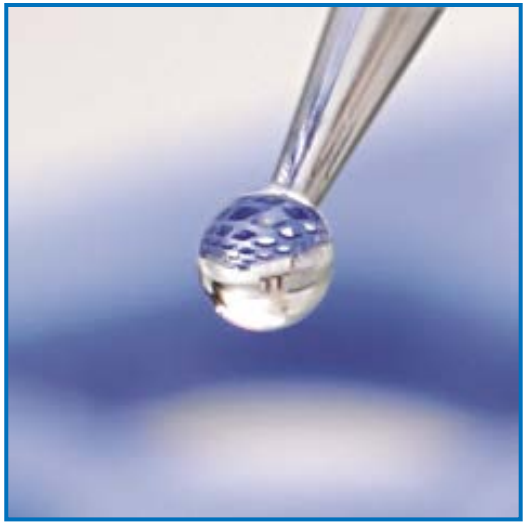
Outlook

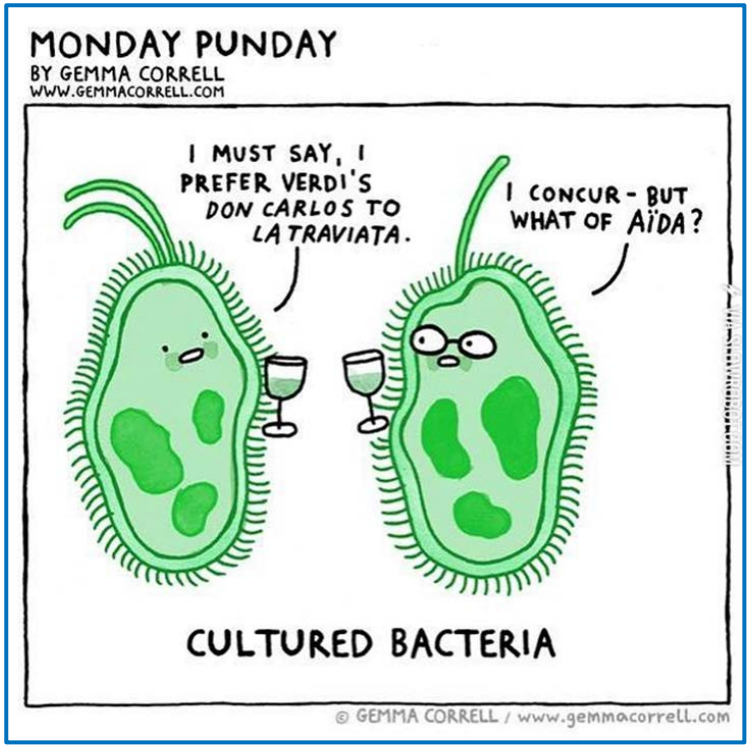
- Intensive analysis of bioassays is ongoing
- Measurements with W-Brems excitation (S4 T▪STAR) to detect elements like Mo, I
- Method development for light elements with optimized calibration factors

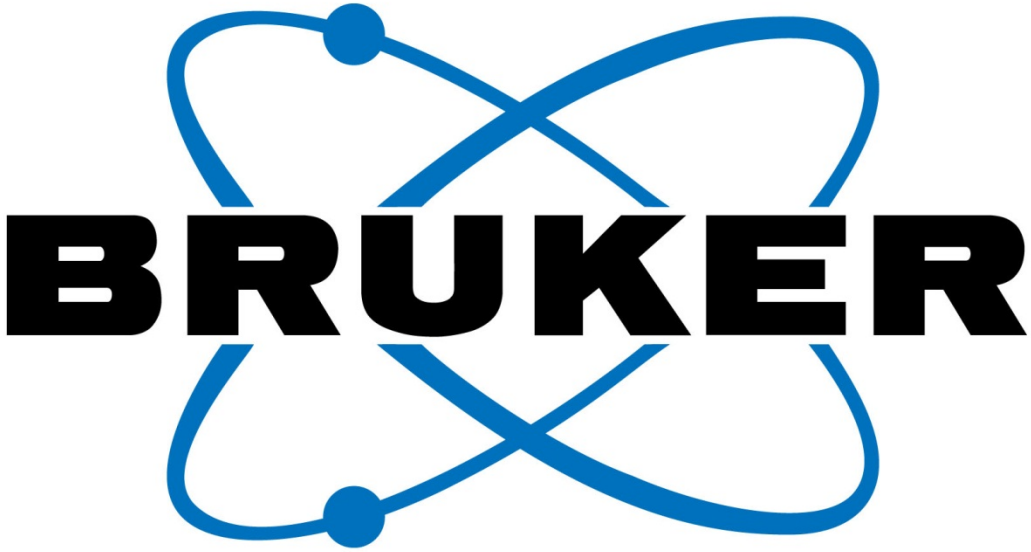
Q & A

Any Questions?

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







Innovation with Integrity

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