Lab Report XRF 434

S2 PICOFOX
Trace Elements in Biological Matrices and their Impact in Clinical Chemistry

Introduction

Trace elements (metals) are increasingly used as dietary supplements in the prevention of widespread diseases and as clinically effective adjuvant therapeutics, and have become a popular research area in basic and applied sciences. These trace elements confer unique reactivities to their respective enzymes.

A sufficient uptake of various vitamins and essential trace elements is of crucial importance to health. Essential trace elements or micronutrients are those with concentrations below 50 ppm (mg/kg) in human bodies or with a daily intake below 50 mg. Elements are classified as "essential", when a deficiency causes a medical symptom and a specific nutritional supplementation will avoid or relieve such a symptom. A list of essential elements and their biological role is shown in table 1.

In contrast, an excessive uptake of essential elements can lead to intoxication. A toxic potential is known particularly for selenium, copper, molybdenum or chromium and a supplementary intake should be medically supervised.

The common usage of these biologically highly active substances is not always accompanied by a diligent monitoring of their concentrations and accumulated amounts within the biological samples. Established analytical methods like atomic absorption spectroscopy (AAS) or inductively coupled plasma optical emmission spectroscopy (ICP-OES) require time-consuming sample preparation steps including digestion with hazardous acids. An even more critical issue is the limited sample amount of biological samples, especially in case of animal experiments or in pediatric medicine.

Table 1: Essential elements and their biological function, which can be detected and quantified by the TXRF spectrometer S2 PICOFOX.

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

Table 2: 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

A sufficient sample amount for an analysis by AAS or ICP requires a dilution, which often leads to element concentrations below the detection limits. Here, the total reflection X-ray fluorescence (TXRF) analysis offers a fast, sensitive and matrix-independent method for the simultaneous quantification of trace elements from minute sample amounts.

Selenium in the focus of medical research

The contribution of the trace element Se in several sophisticated metabolic pathways is in the focus of present medical research [1, 2]. Se is incorporated in enzymes as the 21st proteinogenic amino acid Selenocysteine (SeCys, figure 1). Se-dependent enzymes catalyze the degradation of peroxides or the cleavage of iodine-carbon bonds of thyroid hormones. Both processes are closely connected to further metallo-enzyme activities, which demonstrates the necessitiy of a comprehensive understanding of the enzymatic interaction and the role of the trace elements involved.

In this paper a number of experiments with effect on the trace element content of biological samples are shown:

- Trace element concentrations in gene-modified mice.
- Se concentrations in mother's milk in relation to the eating habits and lactation period.
- Correlation of Se and Zn in human seminal fluid.
- Detection of Se, As and Zn in urine samples.

Instrumentation

All measurements were performed using the benchtop TXRF spectrometer S2 PICOFOX. This instrument is equipped with an air-cooled low power X-ray tube (Mo target), a multilayer monochromator with 80% reflectivity and the liquid nitrogen-free XFlash® Silicon Drift Detector (SDD) with an energy resolution of <150 eV (Mn Kα).

Sample preparation and measurements

Table 2 shows the list of biological matrices and the corresponding sample preparation steps that were analyzed using TXRF in this or recent papers. Unless otherwise stated, the measurement time was 1000 s.

Selenocysteine

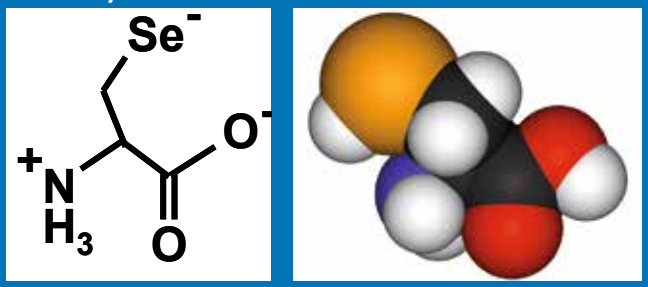


Figure 1: Chemical formula and space fill model of Selenocysteine

Constraints of ICP

Do you know about the constraints of ICP-MS when used for the analysis of blood samples?

- Line interferences of the argon carrier gas with selenium isotopes will prevent Se detection:
  ^{80}Se (49.6% abundance) overlaps with $^{40}Ar_2$
  ^{78}Se (23.8% abundance) overlaps with $^{40}Ar^{38}Ar$.
- Argon adducts are avoided by a matrix adaption and dilution in Butanol, Triton X-100, ammonia, $(NH_4)_2H_2$-EDTA and $(NH_4)_2H$-phosphate.
- However, the detection limit for Se in blood is not better than 2 µg/l for ICP-MS or 0.5 µg/l for ICP-MS with collision cell.

Which sample preparation is required for the analysis of Se 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 hydrid generator that separates elements like Se from the matrix.
- The achievable detection limit is 5 µg/l.

Results

1. Transgenic mice

In this experiment the metal concentrations of wild type mice were compared with a selenium transporter knock-out mutant. Trace metal concentrations of liver and kidney were measured after homogenization of the tissues. Low amounts of the homogenates were directly applied to the sample carrier after internal standardization.

As shown in figure 2 the concentrations of Zn, Se and Pb were increased in the mutant mice, while other elements did not show any significant changes.

Trace elements in transgenic mice

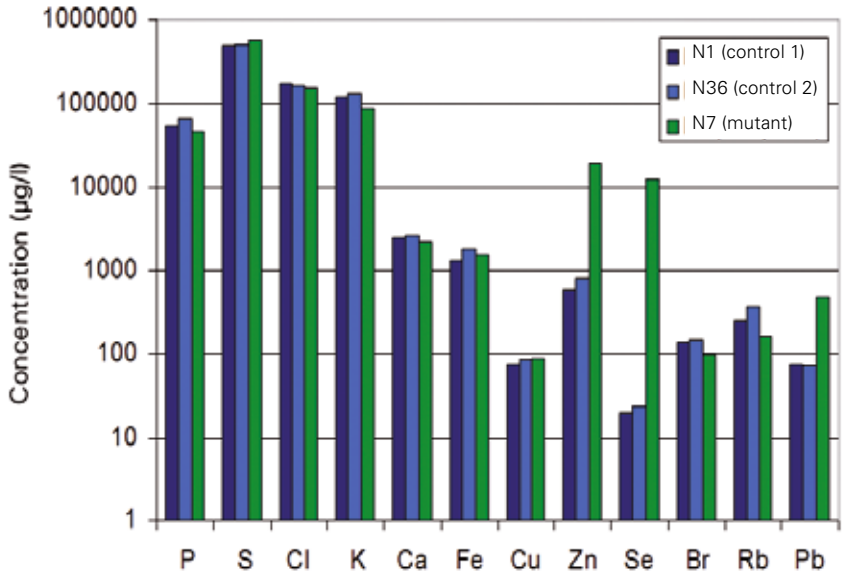
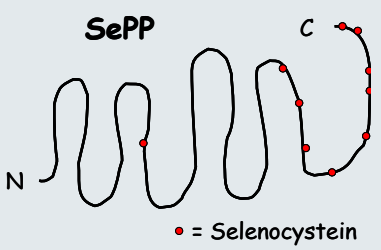


Figure 2: Trace element concentrations of wild type mice compared to a Se transporter knock-out mutant

Se and Selenoproteins

How is selenium transported and stored in human bodies?

- The transport and storage of Se is implemented by a protein called Selenoprotein P (SePP), which is produced in liver hepatocytes.
- It contains up to 10 Se atoms in form of Selenocystein.
- SePP is secreted into the blood circulation to supply brain and peripheral organs with the essential trace element Se.
- While all essential trace elements are incorporated post-translationally into a protein, only Se is incorporated co-translationally into the growing peptide chain of the respective Selenoprotein.
- The individual Se status, which depends on age, sex and nutrition, is considered to be an important health risk factor, e.g. in case of cancer, dementia or cardiovascular diseases.



2. Mother's milk

The adequate maternal micronutrient status is especially critical during pregnancy and lactation [3]. In this study, the Se concentration during the lactation period was monitored. The investigation of the Se status of mother's milk was done with the whole milk, which contains the amount of vitamins and trace elements needed by the baby in a easily resorbable and safe form.

Detection limit and accuracy of the Se measurement in milk was verified with a milk powder standard (NIST BCR 150). Milk powder and whole milk can be analyzed using TXRF without any further pretreatment and immediately after addition of the internal standard. The measured value for Se of 114 ± 9 µg/l was in good concordance with the certified value of 127 µg/l. The detection limit was calculated to 3 µg/l.

Therefore, Se in mother's milk can be detected with TXRF. Figure 3 shows the change in the Se concentration during the lactation period with concentrations starting at 22 µg/l and declining down to 5 µg/l.

Selenium in mother's milk

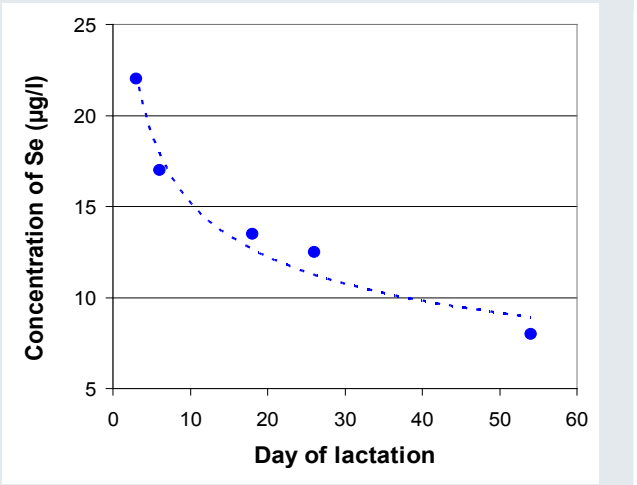


Figure 3: Change in the Se concentration in mother's milk during the lactation period

3. Human seminal fluid

Human fertility is a sensitive issue, and concerns about decreasing sperm quality are regularly discussed among clinicians, environmental toxicologists and endocrinologists [4]. Certain minerals including Zn and Se have been proven essential for optimal spermiogenesis and sperm vitality.

In an attempt to study how far Se correlates to the Zn status, we have analyzed a number of control and subfertile men.

Total Se concentrations were 2 times higher in serum samples compared to seminal fluid samples (108 +/- 18 µg/L versus 49 +/- 21 µg/L, n=18). In serum, Zn and Se were not correlated, while in the seminal fluid, there was a positive correlation between total Zn and total Se concentrations (Fig. 4). Ongoing experiments aim to clarify the chemical and biological form of Se in seminal fluid and whether it represents a meaningful parameter for the characterization of sperm quality in humans.

We conclude that TXRF is a sensitive and reliable analytical technology for trace element analysis even in small amounts of seminal fluid.

Correlation of Se and Zn

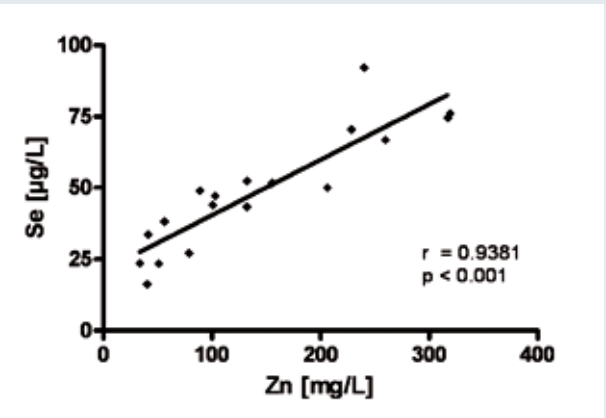


Figure 4: Correlation analysis of Zn and Se in human seminal fluid

4. Urine analysis

Just recently Bruker Nano participated in a round robin test of urine samples conducted by the Reference Institute for Bioanalytics, Bonn, Germany. Urine sample analytics is a challenging task for at least two reasons:

- The accurate detection of metal traces requires detection limits in the low ppb range.
- The high amount of chlorine and calcium disturbs the trace element detection using TXRF with undesirable sum peaks of the matrix elements.

Therefore, the sample preparation was modified to fume off most of the chlorine content. After mixing the urine with the monoelement standard Ga, 10 µl of the urine were pipetted on the carrier and dried. Subsequently, 10 µl of concentrated HNO_3 were added and dried again. Figure 5 shows the disappearance of sum peaks between Cr and Mn. The amount of trace metals like As and Se was not affected by this procedure.

Although the procedures for urine testing with TXRF are not fully optimized, the Bruker Nano application lab received the official certification for accurate measurement values for the elements listed in table 3.

During this first measurement campaign ppb concentrations of the elements Fe, Mn, Pb, Co, Ni, Cr, Cu and Hg could be detected by the S2 PICOFOX. However, the measurement conditions and quantification routines for these elements need to be improved during a forthcoming round robin test.

Table 3: Measurement results of urine samples during a round robin test conducted by the Reference Institute of Bioanalytics, Bonn, Germany

Element	TXRF (µg/l)	Reference (µg/l)
Sample A		
As	116 ± 4	95 ± 29
Se	9.5 ± 1.1	12 ± 3.6
Zn	281 ± 13	253 ± 76
Sample B		
As	228 ± 5	206 ± 62
Se	26.7 ± 1.0	28.5 ± 8.6
Zn	871 ± 40	788 ± 242

TXRF spectrum of a urine sample

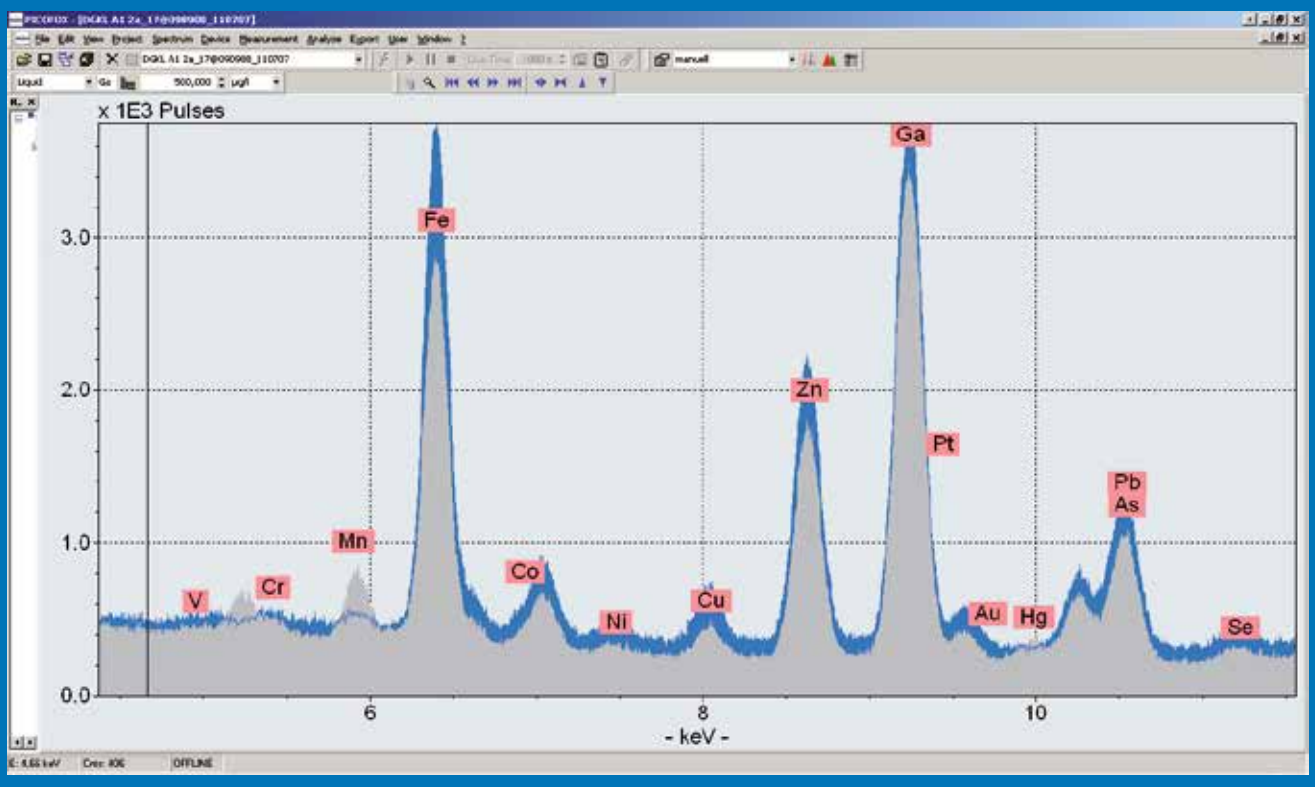


Figure 5: Typical spectrum of a urine sample with (blue spectrum) and without (grey spectrum) HNO_3 treatment

5. Detection limits

The detection limits (3σ) of trace metals in different biological matrices are shown in figure 6. Due to the low matrix content of urine samples detection limits below 10 ppb can be achieved for elements with Z > 24. Mother's milk, seminal plasma and other body fluids can be analyzed with similar sensitivity.

For blood plasma and tissue homogenates, detection limits of 10 to 50 ppb are sufficent for most clinical test procedures. It is important to point out that these detection limits were achieved after an easy dilution step during sample preparation. Further improvements are possible by removal of the organic matrix through microwave digestion or cold plasma ashing [5].

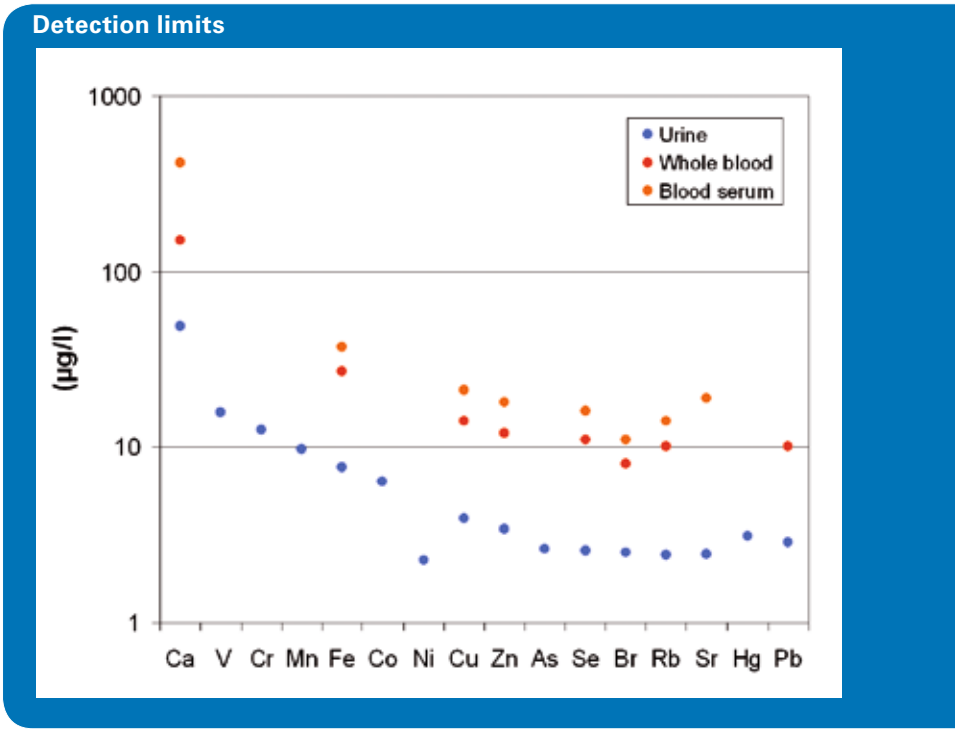


Figure 6: Calculated detection limits for TXRF analysis of biological matrices

Conclusion

The TXRF spectrometer S2 PICOFOX is ideal for trace element determination in any biological matrix during clinical research or routine measurements for several reasons:

- All sample types can be analyzed without any digestion. If not applied directly, simple dilution steps and the addition of an internal standard are sufficient.
- TXRF provides detection limits in a required range of 1 to 100 ppb.
- TXRF is ideally suited for the analysis of extremly low sample amounts.

Literature

[1] Schomburg, L., Köhrle, J., "On the importance of selenium and iodine metabolism for thyroid hormone biosynthesis and human health", Mol. Nutr. Food Res. (2008), 52, 1235-1246.

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[5] Stosnach, H., Mages, M., "Analysis of nutrition-relevant trace elements in human blood and serum by means of total reflection X-ray fluorescence spectroscopy", Spectrochimica Acta Part B 64 (2009), 354-356.

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