



## TXRF

# Trace Element Analysis of Blood Samples

### Application Note # XRF 77

In this report, the suitability of the TXRF spectrometer S2 PICOFOX for blood analysis is evaluated.

In part 1 the analysis of health essential and toxic elements in blood serum and whole blood is described. The determination of Pt in blood samples of cancer patients treated with chemotherapy is the topic of part 2.



**Figure 1**

Whole blood

#### Part 1: Trace element analysis in Serum and whole blood

The determination of trace elements in blood samples is an important analytical task. Essential elements like Fe, Cr, Mn, Ni, Cu, Zn and Se must be controlled because a depletion of these elements leads to serious deficiency diseases. At the same time, small discrete blood sample volumes containing a large proportion of organic molecules in a high salt matrix limit the practicality of such analyses. Typically, sample preparation for AAS or ICP-OES analysis requires time-consuming and hazardous acid digestion.

In terms of environmental and occupational medicine, a more prominent application is the analysis of toxic metals like Pb, As or Hg. In this study, the feasibility and restrictions of blood analysis by means of Total Reflection X-ray Fluorescence (TXRF) spectroscopy without need for time-consuming preparation methods like acid digestion are reported.

## Samples and sample preparation

### Serum samples

The sample preparation for TXRF analysis of blood serum involves simple dilution with ultrapure water. Aliquots of 250 µl serum standard (ClinChek-Control, Level II; Recipe Chemicals + Instruments, Munich, Germany) were diluted with 2500 µl of ultrapure water (Merck). For internal standardisation, 27.5 µl of Ga (Merck, 0.1 g/l) were added. After thorough homogenisation, 10 µl of this solution were transferred to a quartz glass sample carrier and dried in a desiccator for 10 minutes.

### Whole blood samples

Due to the high organic matrix content, whole blood samples require a digestion or at least a dilution step prior to the analysis by TXRF or atomic spectroscopy methods [1]. To evaluate fast preparation without any digestion, 250 µl of whole blood (Serorm, Trace elements in whole blood L2; Sero AS, Billingstadt, Norway) were diluted with 250 ml of ultrapure water (Merck). This treatment led to the disruption of blood cells by osmotic shock and an almost homogenous suspension for direct preparation on a sample carrier.

The analysis of the volatile element Hg demanded a second preparation step: room temperature digestion for the fixation of Hg. 300 µl of whole blood were treated with 300 µl of a 10 % tetramethylammonium hydroxide (TMAH) solution for one hour at room temperature. The mixture was diluted with 600 µl of a 2 % HCl solution and 12 µl of Ga solution (Merck, 0.1 g/l) were added for internal standardisation. An aliquot of 10 µl was transferred to a quartz glass sample carrier and subsequently dried in a desiccator for 10 minutes.

### Measurements

All samples were measured at an X-ray excitation of 50 kV/750 µA for 600 s.

Whole blood standard in µg/l				
	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	366	6.5	396	100

**Table 1**

Comparison of TXRF and ICP-MS reference values for trace elements in whole blood

Serum standard in µg/l				
	TXRF	Std. dev.	ICP-MS	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.

**Table 2**

Comparison of TXRF and AAS reference values for trace elements in blood serum

## Results

The measurement results together with comparison values from ICP-MS and AAS analysis are summarized in Table 1 for the whole blood samples and in Table 2 for blood serum samples.

For all essential elements a good concordance of TXRF with reference values can be observed. It must be highlighted that in addition to the certified element concentrations other elements (P, S, Cl, K, Ca, Br, Rb, Sr) could be determined during one measurement.

For blood serum samples TXRF analysis shows significantly better standard deviations compared to AAS, although no digestion procedure was applied. This example underlines the importance of an easy analytical procedure for blood serum samples.

## Part 2: Analysis of platinum in blood

The continuous monitoring of the Pt concentration in human blood is a crucial task during chemotherapy treatment of cancer patients. Due to the high toxicity only a small therapeutic window for the Pt concentration exists. Additionally, the kidney function of cancer patients is highly variable and thus the treatment must be exactly adapted. Since a number of different anticancer drugs is available, a bioequivalence comparison is of interest, too.

## Samples and sample preparation

The blood samples were gathered during a study under guidance of the Universidad Simón Bolívar, Caracas, Venezuela [2].

1 cm<sup>3</sup> of whole blood sample was centrifuged at 10,000 rpm. The supernatant containing the serum was removed with a pipette and frozen at -4 °C.

## Quantification and measurement results

For measurement with TXRF, the Compton peak at about 15 keV was used as the internal standard. Five different Pt-free serum samples were spiked with different Pt standard solutions (Merck, 1 g/l) and used to create a calibration curve, which is displayed in Figure 2. For this method the detection limit of the S2 PICOFOX was calculated to 67 µg/l by the following formula:

$$DL = 3 \cdot \frac{c}{I_{net}} \cdot \sqrt{2 \cdot I_{bg}}$$

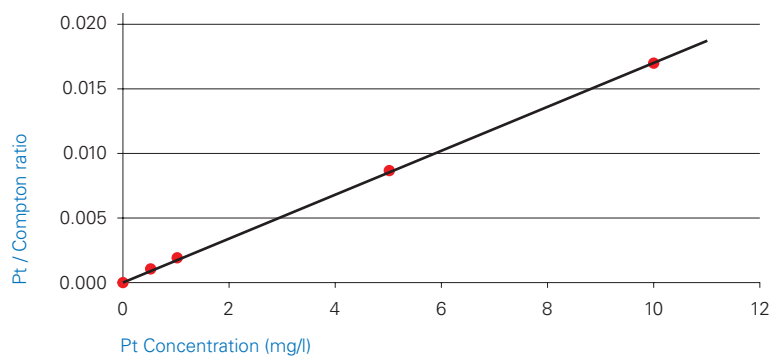
DL: Detection limit (µg/l)

c: Pt concentration (µg/l)

I<sub>net</sub>: Net intensity Pt La line (counts)

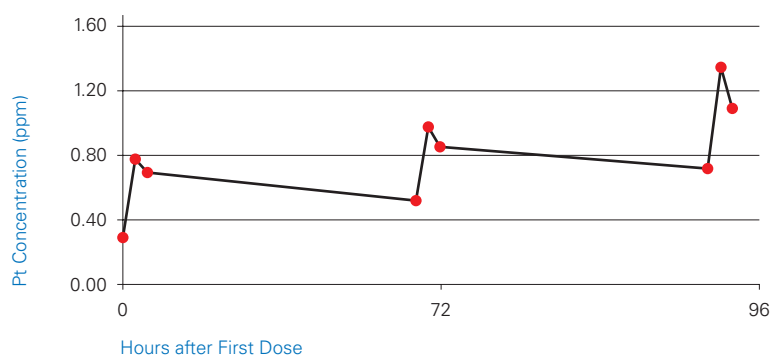
I<sub>bg</sub>: Background intensity Pt La line (counts)

Figure 3 shows the result of Pt monitoring in blood by TXRF. The Pt concentration of a cancer patient, treated with a Pt-containing drug is documented over a period of four days, which allowed the toxicological control of the medication. Furthermore, the slow decrease of the Pt concentration after each application of the drug acts as an indicator of the kidney function.



**Figure 2**

Calibration graph for Pt in blood.



**Figure 3**

Record of Pt concentrations in blood over a period of four days after first dose.

## Conclusion

The results of this study show that TXRF analysis by the S2 PICOFOX is a versatile tool for the analysis of trace elements in blood samples.

Essential as well as toxic elements can be analysed with accuracies and sensitivities comparable to analytical techniques like AAS or ICP.

TXRF is based on a "matrix independent" internal standardisation process leading to a precise and highly accurate quantification without the need for complex and time-consuming sample preparation and instrument calibration.

Besides the detection performance of the S2 PICOFOX, the ability to analyse minute sample amounts offers several advantages:

- It allows monitoring of trace element metabolic interactions or trace metal profiles for the detection of unsuspected nutritional depletions.
- Element determination in distinct fractions of blood, e.g. lipids, proteins etc. is possible.

In addition, TXRF offers a simple and accurate method for the control of chemotherapeutic drugs like platinum in blood samples. The analyses by conventional methods, e.g. ICP-OES or AAS, are prolonged and expensive, preventing routine use in chemotherapy. Again, the effort for sample preparation is significantly reduced in the case of TXRF. Even long-term examinations are possible due to the extremely low amount of cancer patients' blood used.

## Bibliography

- [1]: Prange, A. et al. (1989): Multi-element determination of trace elements in whole blood and serum by TXRF, *Fresenius Z. Anal. Chem.*, 355; 914-918.
- [2]: Greaves, E.D. et al. (2000): Determination of platinum in serum and urine samples from pediatric cancer patients by TXRF, *X-Ray Spectrometry*, 29; 349-353.

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