TXRF

Clinical Application of TXRF spectroscopy

Application Note # XRF 453

Part I – Analysis of gadolinium based MRI contrast agents

For more than 30 years magnetic resonance imaging (MRI) has been applied in medical diagnostics, providing non-invasive tomograms of the body (Figure 1). For image enhancement contrast agents which contain paramagnetic gadolinium (Gd^{3+}) ions are used. Due to its toxicity Gd^{3+} is complexed with chelating agents like in Gadopentetate (Gd-DTPA, Figure 2 a). For about 10 years a new disease called nephrogenic systemic fibrosis (NSF) causing severe kidney problems and hardening of skin and inner organs has been known. Therefore, blood and urine of patients have to be examined continuously when exposed to Gd-based agents.

Although numerous methods for the analysis of Gd-based contrast agents have been described, none of these methods are suitable for clinical routine analysis due to the complexity of sample preparation including digestion and dilution steps. Since TXRF was successfully applied for other clinical applications (see lab reports XRF 77 and 434) it might be suitable for a fast and easy determination of Gd-based contrast agents in blood and urine of patients. This lab report describes the analysis of Gd in clinical samples and compares results derived by ICP-MS.

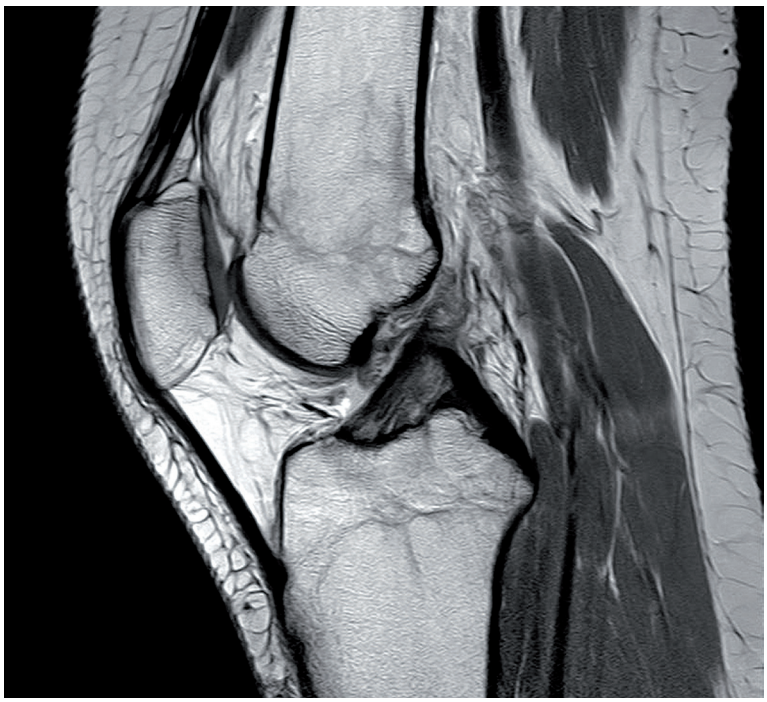

Figure 1
Typical tomogram of a knee after exposure to magnetic resonance imaging (MRI)

Sampling and sample preparation

Urine samples were taken from two patients over a period of up to 20 hours after MRI examination. Blood samples were collected from 10 patients immediately after MRI examination. Since Gd will not be bound to the red blood cells the whole blood was centrifuged to reduce the content of Fe, which shows a strong line overlap with Gd in a XRF spectrum.

5 mL of a Ga standard solution (1 g/L) were added to 495 mL of urine or blood samples, to obtain a Ga concentration of 10 mg/L. The samples were homogenized and 10 µL were deposited as a single drop onto a quartz glass sample carrier. Each sample was prepared in triplicate. While urine samples were dried in vacuum, blood samples were dried in air due to their complex and viscous matrix. TXRF measurements were performed with a S2 PICOFOX with Mo tube (50 kV, 750 µA) for 1000 s. For ICP-MS measurements the blood samples were digested in a microwave oven. Then urine and blood samples were diluted and standardized with Ho. For both methods the Gd concentrations in urine were normalized with the creatinine concentrations.

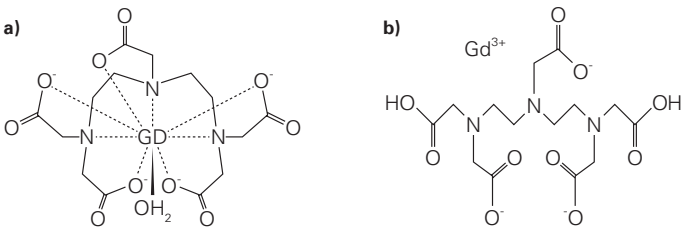

Figure 2
Gd-based contrast agents DTPA (a) and DOTA (b)

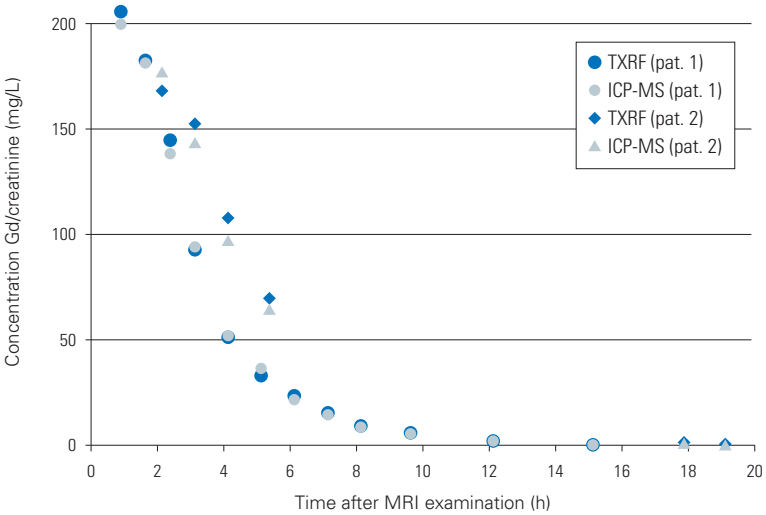

Figure 3
Normalized Gd concentrations in the urine of two patients after MRI examination measured by TXRF and ICP-MS

Results

The Gd concentrations in urine determined by TXRF were highly reproducible showing a relative standard deviation (RSD) of less than 1.6 %. Figure 3 shows the excellent correlation with the ICP-MS data for all times after sampling. The limit of detection (LOD) for Gd in urine was calculated to be 100 µg/L. The linear range of the method extends to more than four orders of magnitude starting at the LOD.

For blood serum samples a RSD of 4.8 % could be achieved demonstrating again the suitability of TXRF for clinical chemistry. Depending on the size and weight of the patient the Gd concentration in blood serum varied signific antly. Nonetheless, the TXRF results were again in close concordance to the values measured by ICP-MS. The detection limit for Gd in blood serum was slightly better than in urine and was calculated at 80 µg/L.

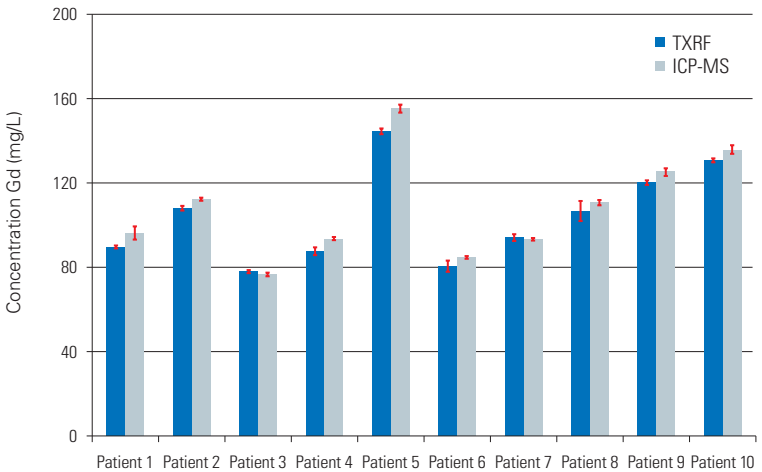

Figure 4
Comparison of Gd concentrations in blood of 10 patients determined by TXRF and ICP-MS

Part II – Quantification of gold and platinum metallodrugs

The objective of this pilot study was the applicability of TXRF for the quantification of low concentrations in the µg/L range of gold and platinum based drugs in biological matrices (cell suspensions). For this purpose cisplatin, a common chemotherapeutic, and auranofin, a gold complex classified as anti-rheumatic agent, were chosen as relevant metal based drugs.

Sample preparation

The experiments were performed with lysates of HT-29 colon carcinoma cells. Before TXRF measurements the cell suspensions were adjusted to the indicated protein concentrations by dilution with distilled water.

Various concentrations of auranofin and cisplatin (62.5–1000 µg/L) were prepared with two different concentrations of aqueous lysates of HT-29 cells (0.5 and 1 mg/mL). Two internal standards (Mn, 1000 µg/L and Y, 1000 µg/L) were added. 10 µl of this sample solution were pipetted on a siliconized quartz glass carrier, dried in an oven for approx. 20 min at 80° C and then measured by the TXRF spectrometer at 50 kV and 600 µA for 250 s.

Results

Recoveries of cisplatin resulted for all concentrations in the range of approximately 90–110 % (Figure 5). For auranofin at concentrations of 500 µg/L and above the recovery was always slightly above 100 %.

Precision was typically in the range of 3–12 % RSD (Figure 6), which demonstrates the influence of the organic sample matrix during deposition on the sample carrier as a thin layer. When adding the film forming lubricant polyvinyl alcohol (PVA) to the suspension, the RSD improved to 2–8 % for gold concentrations at 50 µg/L and above. Due to a higher signal-to-noise ratio the addition of PVA did not work with metal concentrations close to the detection limit.

In a second experiment the precision of TXRF measurements was tested at different measurement times from 10 to 1000 s. At 100 s or longer a precision of better than 5 % RSD was achieved using an auranofin concentration of 10 mg/L. Even a short measurement time of 10 s led to a RSD value of about 10 %.

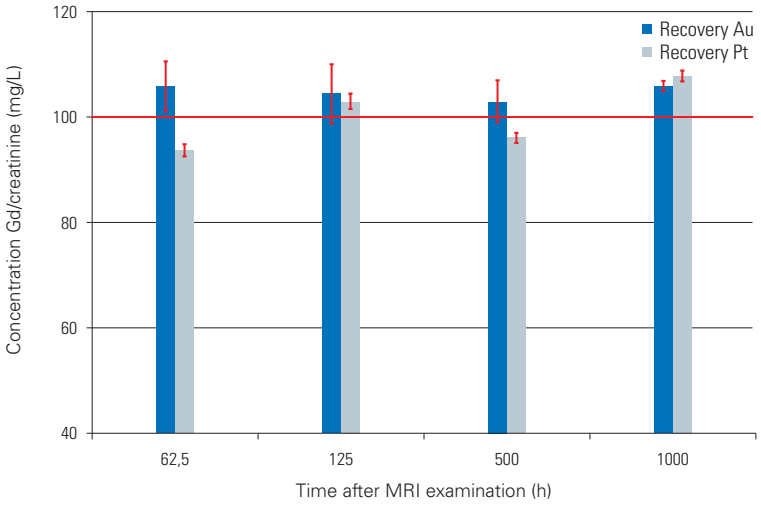

Figure 5
Recovery of cisplatin and auranofin in cell lysate suspensions at a protein concentration of 1 mg/L

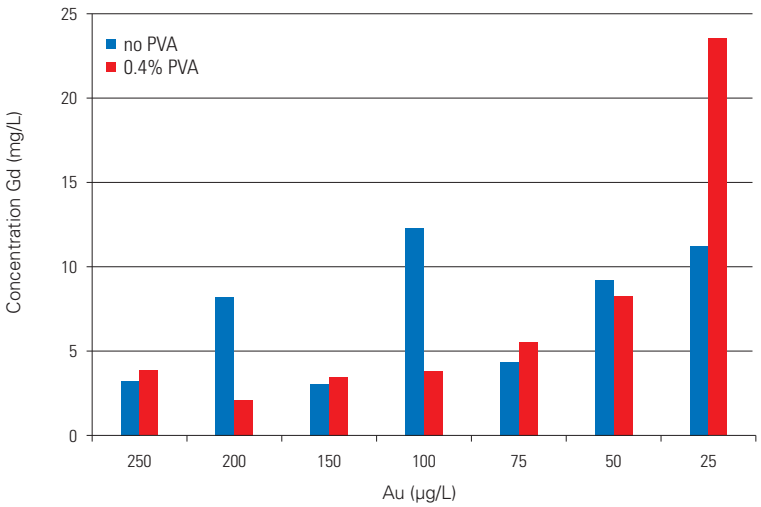

Figure 6
Precision of auranofin measurements in cell lysates

Conclusion

Part I described the analysis of Gd concentration in blood and urine after MRI examination by using TXRF. Analysis results showed an excellent concordance with ICP-MS measurements. The samples of patients could be examined for their Gd excretion in less than half an hour after MRI examination.

Part II showed that gold and platinum from metal based drugs can be quantified by TXRF in the ppb range with acceptable precision in aqueous samples as well as in cell suspensions.

The easy preparation and handling of samples make TXRF very attractive and recommend it as an alternative method for clinical use. TXRF does not require any media or consumables and supports a cost-efficient laboratory practice.

Acknowledgements

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Literature

Telgmann, L. et al., Simple and rapid quantification of gadolinium in urine and blood plasma samples by means of total reflection X-ray fluorescence (TXRF), Metallomics, 2011, 3(10), p. 1035–1040.

Meyer, A. et al., Total reflection X-ray fluorescence spectroscopy as a tool for the quantification of gold and platinum metallodrugs: determination of recovery rates and precision in the ppb concentration range, J. of Pharmaceutical and Biomedical Analysis, V. 70, Nov.2012, p. 713–717.

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