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Thulium spiked gel for internal standardisation in LA-ICP-MS bio-imaging: quantitative elemental distribution of uranium in kidney tissue

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The quantitative analysis of trace metals in different organs or cellular structures is a topic of emerging interest for the assessment of toxicological risk. The kidney is recognized as a major site for uranium accumulation able to induce renal toxicity^{1,2}. Several studies have shown its heterogeneous distribution within the tissue finding areas (S3 segments in the proximal tubule) of high uranium concentration (100-fold above mean renal concentration)³⁻⁵. These studies were carried out employing high-energy synchrotron radiation Xray fluorescence analysis (SR-XRF) whose reduced availability limits its daily use for routine analysis. In this work, mass spectrometry imaging (MSI) using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) has been employed for mapping and quantifying uranium in histological tissue sections of mouse kidney. To the author's knowledge just a single work has been published recently for the semi-quantitative analysis of uranium in mice kidneys due to the lack of an appropriate internal standard⁶. The quantitative monitoring of uranium at tissue level in kidney would facilitate the understanding of its action mechanism in renal toxicity. Therefore, this works presents the development of a correction methodology based on doped gelatine with internal standard as an alternative to current methods⁷. In order to correct matrix effects, lack of tissue homogeneity and instrumental drift, a thulium (Tm) spiked gel was prepared and deposited on the top of glass microscope slides. For quantification purposes, matrix-matched laboratory standards were prepared from a pool of rat kidneys by spiking each level with different concentrations of uranium. The proposed analytical bioimaging approach was successfully applied for quantification of uranium of rat kidney samples.

Bio-distribution and quantification of uranium in kidney tissue. How to do it?

External calibration: matrix-matched standards

The use of matrix-matched standards does not suppress the elemental fractionation but it will happen similarly both in standards and samples.

Internal standardization: spiked gelatine

In addition, the external calibration method needs the use of an internal standard (IS) to compensate matrix effects as well as variations in ablated and transported mass and instrumental drifts during analysis. Ideal IS should behave in a similar manner to the analyte during the ablation process and in the ICP. Additionally, it must be in similar concentration and homogeneously distributed within the samples and standard matrices. General scheme for thulium/thallium 100 ppb doped gelatine preparation





Hypothesis: migration of elements

not able to keep them fixed $(Tm/Tl \neq 1)$.

during drying process as gelatine net is

for an adequate preparation of

gelatine avoiding bubble formation

Contaminated tissue

Drying/setting procedure: 1h room temperature in flat surface (covered) Keep at 4°C until its use

juid ICPMS analysis afte

quid digestion of centra

elatine. Migration? Kinetic

part and borders of the

xperiments in progress

10 areas of 0.5x0.5 mm were ablated, otaining similar response from all (net signal, background corrected)



the central area (up, middle and down – 0.5x0.5 mm area) and Tm/Tl ratio was calculated from net signals at different times after gelatine preparation: 1 day, 2 days and 1.5 months after gelatine preparation.

Different gelatine % were tested, from 2.5%

to 30% m/v. 20% and 30% m/v gelatines

were directly discarded as they are too

viscous to handle comfortably and they dry

into films producing a high amount of

2.5%, 5% and 10% gelatines were ablated in

Optimisation of gelatine %

bubbles.



Sala et al. demonstrated that the gelatines (20 μ L droplet) with the most homogeneous element distribution was prepared from 10% m/v gelatine solution⁸. Therefore, based on bibliographical references and LA-ICPMS homogeneity experiments, 10 % gelatine was chosen for the stated purpose.

Tm/Tl ratio







Instrumentation and experimental conditions



Cryostat cutting at -20°C

16 µm thickness



SM VH



Cryostat cutting (16 µm thickness) Sample ready for analysis at -20°C and deposition in spiked gelatine covered slide

It was taken into account that samples will be analysed by different analytical techniques. Therefore, a « general » sample preparation was done in order to guarantee technical compatibility. However, the authors were aware of the fact that sample preparation might modify the initial metal distribution due to the leaching of metals from the tissue to PFA/sucrose solution⁹. For that purpose, both PFA and sucrose baths (after 24h in contact with kidney samples) were after analysed by ICPMS confirming that no uranium migration happened.

LA-ICP-MS quantitative uranium bio-imaging in kidney samples

A non-contaminated kidney (4 ppb average U by ICPMS, whole organ) and a contaminated kidney (6000) ppb average U by ICPMS, whole organ) were used to obtain these preliminary quantitative images.

Non-contaminated tissue

LA-ICPMS... Calibration curve obtained after the ablation of the

matrix-matched standards

20000





Conclusions

Homogenates are kept at

-20°C in histology moulds

Acknowledgments

In this work the feasibility of an internal standard doped gelatine was assessed for its use in quantitative bio-imaging of U in kidney tissue by laser ablation coupled to ICPMS. Beyond the optimisation of the gelatine itself, a biological sample preparation protocol and matrix-matched standards have been also developed. This new methodology (U spiked matrix-matching standards, Tm spiked gelatine as IS) allowed the visualization of uranium's heterogeneous distribution and its quantification in the analysed kidney tissue. The future goal would be to enhance the image quality by optimizing ablation parameters and the analysis of larger areas for a more accurate reconstruction of the renal distribution of uranium in the whole organ to better understand uranium nephrotoxicity.

Bibliography

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1. Haley DP, Bulger RE, Dobyan DC. The long-term effects of uranyl nitrate on the structure and function of the rat kidney. Virchows Arch B [Cell Pathol]. 1982; 41:181-192. 2. Vicente-Vicente L, Quiros Y, Perez-Barriocanal F et al. Nephrotoxicity of uranium: pathophysiological, diagnostic and therapeutic perspectives. Toxicol Sci. 2010; 118-328-347. 3. Tessier C, Suhard D, Rebiere F et al. Uranium microdistribution in renal cortex of rats after chronic exposure: a study by secondary ion mass spectrometry microscopy. Microsc Microanal. 2012; 18:123-133. 4. Homma-Takeda S, Terada Y, Nakata A et al. Elemental imaging of kidneys of adult rats exposed to uranium acetate. Nucl Instrum Methods Phys Res Sec B. 2009; 267:2167-2170. 5. Homma-Takeda S, Kitahara K, Suzuki K et al. Cellular localization of uranium in the renal proximal tubules during acute renal uranium toxicity. J Appl Toxicol. 2015; 35:1594-1600. 6. Jim V, LaViolette C, Briehl MM, Ingram JC. Spatial distribution of uranium in mice kidneys detected by laser ablation inductively coupled plasma mass spectrometry. J Appl Bioanal. 2017; 3:43-48. 7. Austin C, Fryer F, Lear J et al. Factors affecting internal standard selection for quantitative elemental bio-imaging of soft tissues by LA-ICP-MS. J Anal At Spectrom. 2011; 26:1494:1501. 8. Sala M, Selih VS, Van Elteren JT. Gelatine gels as multi-element calibration standards in LA-ICP-MS bioimaging: fabrication of homogeneous standards and microhomogeneity testing. Analyst, 2017; 142:3356-3359. 9. Bonta M, Török S, Hegedus B et al. A comparison of sample preparation strategies for biological tissues and subsequent trace element analysis using LA-ICP-MS. Anal Bioanal Chem. 2017; 409:1805-1814.

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