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

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IRSN INSTITUT DE RADIOPROTECTION ET DE SÛRETÉ NUCLÉAIRE

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PATERSON — Plateforme de spectrométrie de masse au service de la radioprotection et de la sûreté nucléaire

orano

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 X-ray 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 standard[6]. 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 methods[7]. 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 bio-imaging 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.

General scheme for the preparation of matrix-matched standards

Preparation of uranium standard solution at a know concentration

Preparation of rat kidneys homogenate and divide it into aliquots (blank + standards (minimum 3))

Homogenates are spiked with the U standard solution and homogenized by vortex. After, homogenates are filled in plastic histology moulds and kept at -20°C.

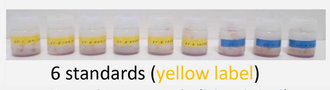
Acid digestion HNO_3:H_2O_2 (2:1) of ≈ 50 mg of frozen homogenates

Homogenate cryo-cutting (-20°C, 16 µm) and place onto the glass substrate

Determination of exact uranium concentration by isotope dilution ICPMS

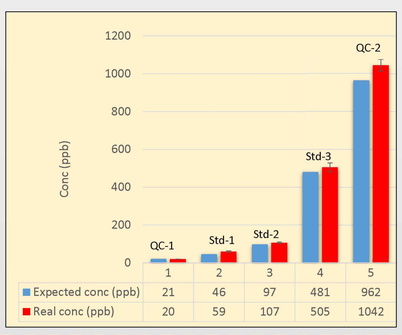
Sections are used as external calibrators for LA-ICP-MS with re-calculated U conc.

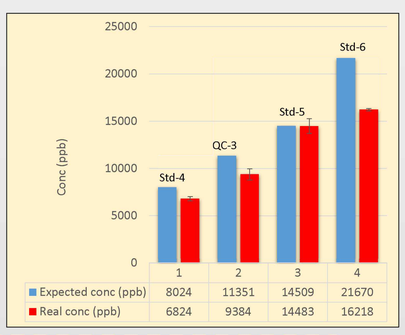
14 rat kidneys ≈ 25g pool

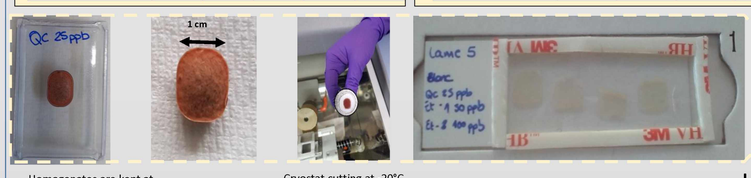


6 standards (yellow label)
3 quality controls (blue label)

Verification of uranium real concentration:
microwave assisted acid digestion + quantification by isotope dilution

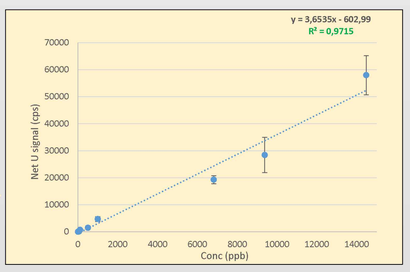




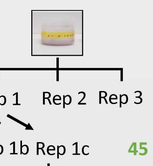


Homogenates are kept at -20°C in histology moulds

Cryostat cutting at -20°C 16 µm thickness



LA-ICPMS... Calibration curve obtained after the ablation of the matrix-matched standards



Rep 1 Rep 2 Rep 3

Rep 1a Rep 1b Rep 1c

45 values per sample

5 replica per ICPMS analysis

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.

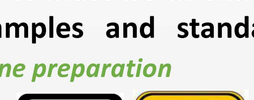
General scheme for thulium/thallium 100 ppb doped gelatine preparation

Attention!!! % of gelatine must be carefully optimised

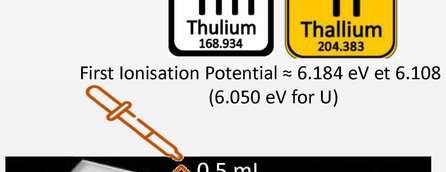
For 100 g of gelatine

Gelatine Type A X g | Selected IS ≈ µL | Milli-Q water 100 - X g

Soft magnetic and hot (80°C) agitation for an adequate preparation of gelatine avoiding bubble formation



First Ionisation Potential ≈ 6.184 eV et 6.108 eV (6.050 eV for U)



Slide coating with IS spiked gelatine

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

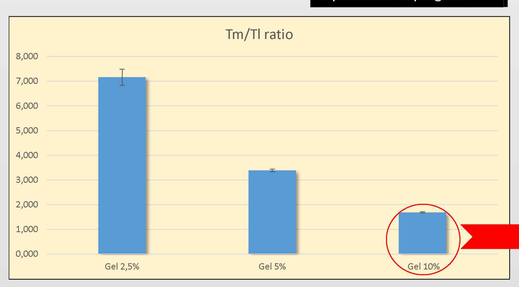
Optimisation of gelatine %

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 bubbles.

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

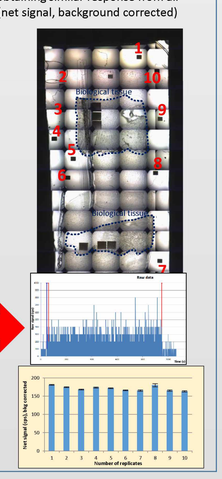
Hypothesis: migration of elements during drying process as gelatine net is not able to keep them fixed (Tm/Tl ≠ 1).

Liquid ICPMS analysis after liquid digestion of central part and borders of the gelatine. Migration? Kinetic experiments in progress



Sala et al. demonstrated that the gelatines (20 µL droplet) with the most homogeneous element distribution was prepared from 10% m/v gelatine solution[8]. Therefore, based on bibliographical references and LA-ICPMS homogeneity experiments, 10 % gelatine was chosen for the stated purpose.

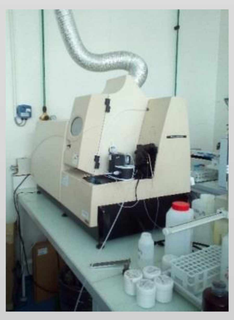
10 areas of 0.5x0.5 mm were ablated, obtaining similar response from all (net signal, background corrected)



Instrumentation and experimental conditions

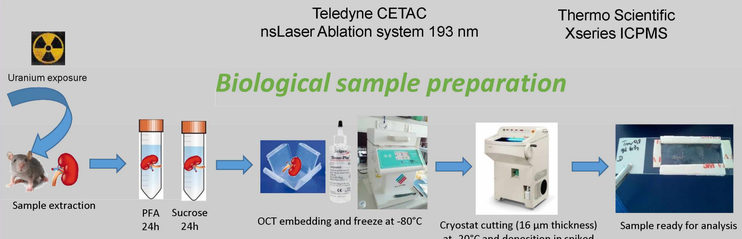
Operating conditions	
Laser ablation system: **Teledyne CETAC 193 nm**	
Wavelength (excimer ArF)	193 nm
Repetition rate	20 Hz
Spot size	35 µm, square
Ablation speed	35 µm/s
Fluence	6.75 J/cm² (need to be optimized)
Carrier Gas (He)	500 mL/min
Mass spectrometer: **Thermo Scientific Xseries**	
RF power	1200
Nebulizer gas (Ar)	0.60 L/min
Auxiliary gas (Ar)	0.8 L/min
Dwell time	10 ms
Signal acquisition mode	Time Resolved Analysis (TRA)
Isotopes	^{13}C, ^{29}Si, ^{169}Tm, ^{235}U, ^{238}U

Teledyne CETAC nsLaser Ablation system 193 nm



Thermo Scientific Xseries ICPMS

Biological sample preparation

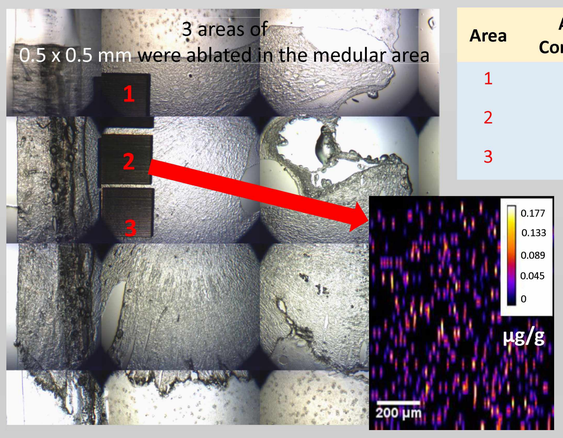


It was taken into account that samples will be analysed by different analytical techniques. Therefore, a « general » sample preparation was done in order to guarantee technical compatibility. However, the authors were aware of the fact that sample preparation might modify the initial metal distribution due to the leaching of metals from the tissue to PFA/sucrose solution[9]. 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

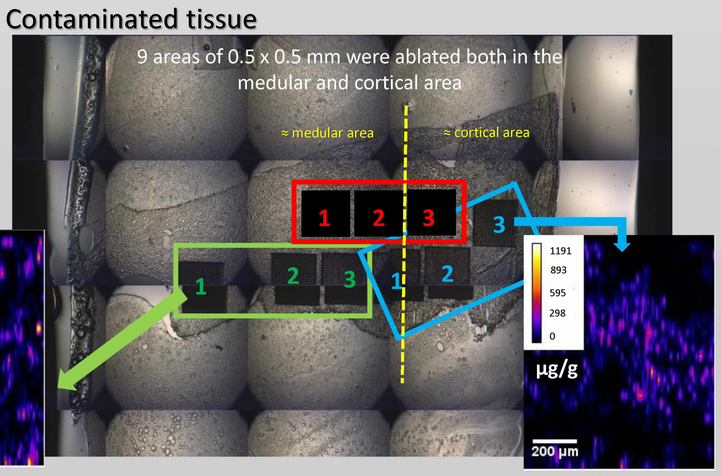
A non-contaminated kidney (4 ppb average U by ICPMS, whole organ) and a contaminated kidney (6000 ppb average U by ICPMS, whole organ) were used to obtain these <u>preliminary</u> quantitative images.

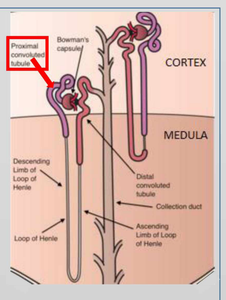
Non-contaminated tissue



Area	Average Conc. U µg/g
1	0.011
2	0.012
3	0.021

Contaminated tissue





Area	Average Conc. U µg/g
1	0.584
2	0.825
3	1.926
1	0.143
2	0.386
3	0.424
1	**18.321**
2	**53.840**
3	**45.382**

These first results on uranium bio-imaging in kidney confirm its heterogeneous distribution and its preferential accumulation in cortical area, in agreement with other studies[5].

Conclusions

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.

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