



HAL
open science

Renal localization and quantification of uranium in rodent exposed to uranyl nitrate by LA-ICP-MS

Nagore Grijalba Marijuan, Alexandre Legrand, Valerie Holler, Celine Bouvier
Capely

► **To cite this version:**

Nagore Grijalba Marijuan, Alexandre Legrand, Valerie Holler, Celine Bouvier Capely. Renal localization and quantification of uranium in rodent exposed to uranyl nitrate by LA-ICP-MS. 15th International Conference on Laser Ablation, COLA, Sep 2019, KAHULUI, HAWAII, United States. 2019. hal-02635607

HAL Id: hal-02635607

<https://hal.science/hal-02635607v1>

Submitted on 27 May 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Copyright

Renal localisation and quantification of uranium in rodent exposed to uranyl nitrate

Nagore GRIJALBA, Alexandre LEGRAND, Valérie HOLLER, Céline BOUVIER-CAPELY

Institut de Radioprotection et de Sûreté Nucléaire, PSE-SANTE/SESANE/LRSI, 31 Av de la Division Leclerc BP 17, 92262 Fontenay-aux-Roses Cedex, France

Context

The overexposure to uranium results in renal toxicity, which is derived from an excessive accumulation of the element in the kidney. This may induce nephrotoxicity characterized by reduced glomerular filtration, exerting its toxic effect by chemical and radiological action (alpha emitter) (1-3). In addition, its tissue distribution is heterogeneous mostly accumulating and producing pathological lesions in the S2 and S3 segments of the proximal tubule located in the cortical zone (100-fold above mean renal concentration) (4-6). Chronic exposure (occupational exposure) to uranium, is linked to its bioaccumulation in kidney and could be associated with renal dysfunction, an increased risk of cancer mortality and kidney failure (7-9).

Rodents were exposed to varying concentration of uranium in their drinking water for a period of 9 months. Total uranium analysis was done by conventional liquid ICP-MS after acid digestion using one kidney from each pair. In parallel, the other kidney was employed for LA-ICP-MS analysis (10). In order to perform quantitative LA-ICPMS bio-imaging analysis, synthetic matrix-matched laboratory standards and a normalisation strategy based on internal standard spiked gelatine were developed. Quantitative images of cryo-sections of rat kidney revealed heterogeneous distribution of uranium within the renal tissue, being the cortical concentration up to 123-fold higher than the medullary concentration.



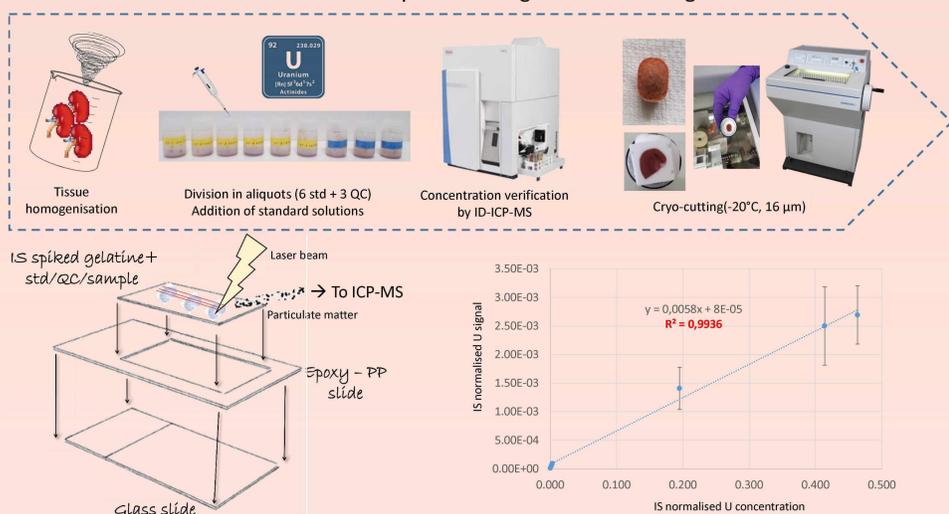
Experimental procedure

External calibration

Objective:

Obtain a concentration – signal linear response to extrapolate the signal obtained from the sample.

Matrix-matched standard = spiked homogenates of analogous tissue



Internal calibration

Objective:

An internal standard (IS) is used for the normalisation of the signal to compensate signal variation and instrumental drift.

Requirements of the IS:

1. IS should behave in a similar manner to the analyte
2. IS must be in similar concentration in samples and standards
3. IS must be homogeneously distributed in samples and standards

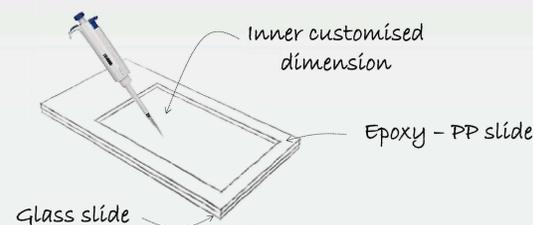
Gelatine as IS substrate:

1. Mimics the texture of biological tissue
2. Ease handling and fast preparation
3. Number of analytes and their concentrations can be adapted to the needs

Slide coating with IS (Tm) spiked gelatine (10% m/v)



First Ionisation Potential = 6.184 eV (6.050 eV for U)



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

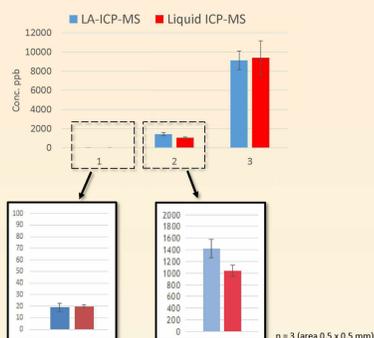
Step 1: Analytical protocol validation by QC samples analysis

Guideline on bioanalytical method validation (EMA et FDA, 2018)

Quantification of Quality Control samples (QC)

Why are they necessary and how must they be analysed?

- A QC is a biological matrix with a known quantity of analyte that is used to monitor the performance of a bioanalytical method and to assess the integrity and validity of the results of study samples
- They should cover the expected study sample concentration range
- Total QC should be at least six in number (low-, mid- and high-QC, in duplicate)
- They must be analysed in triplicate



The calculated concentrations of the QC should be within ± 15-20% of the nominal value

	Expected conc ng g ⁻¹ (Verified by ID-ICPMS)	Obtained conc ng g ⁻¹ (Calculated by LA-ICPMS)
QC 1	20 ± 1	19 ± 3 (15% RSD)
QC 2	1042 ± 92	1420 ± 160 (11% RSD)
QC 3	9384 ± 1792	9128 ± 991 (10% RSD)



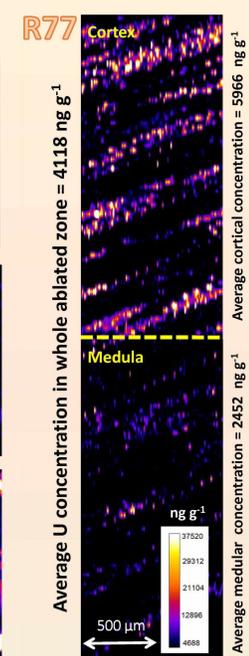
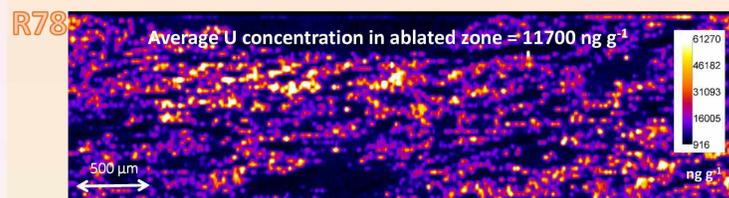
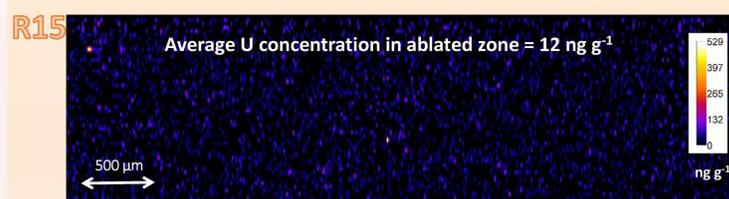
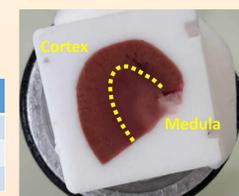
Step 2: Analysis of real samples



Selection of 3 samples

C. Poisson, J. Stefani et al. Free Radical Research, 48(10), 2014

Sample	U contamination?	Ablation zone	Whole organ average conc. (ICP-MS, ng g ⁻¹)
R15	NO	Cortex	4
R77	YES	Cortex/Medula	3256
R78	YES	Cortex	6729



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 ICP-MS. The proposed analytical bio-imaging approach was successfully applied for quantification of uranium of in biological samples (rat kidney). Quantitative images of cryo-sections revealed heterogeneous distribution of uranium within the renal tissue, being the cortical concentration up to 123-fold higher than the medullary concentration which is in great accordance with the studies found in the literature. For the author's knowledge, this is the first quantitative study using an internal standard other than C¹³ to precisely quantify and localise uranium in kidney by LA-ICP-MS.

Bibliography

1. S. Keith, O. Faroon, N. Roney et al. *Toxicological profile for Uranium* (Agency for Toxic Substances and Disease Registry, US, 2013).
2. D.P. Haley, R.E. Bulger, D.C. Dobyen, "The long-term effects of Uranyl Nitrate on the Structure and Function of Rat Kidney", *Virchows Arch [Cell Pathol]*, **41**, 181, 1982.
3. L. Vicente-Vicente, Y. Quiros, F. Perez-Barriocanal et al., "Nephrotoxicity of Uranium: Pathophysiological, Diagnostic and Therapeutic Perspectives", *Toxicological Sciences*, **118** (2), 324, 2010.
4. S. Homma-Takeda, T. Kokubo, Y. Terada et al., "Uranium dynamics and developmental sensitivity in rat kidney", *Journal of Applied Toxicology*, **33**, 685, 2013.
5. T. Konishi, S. Kodaira, Y. Itakura et al., "Imaging uranium distribution on rat kidney sections through detection of alpha tracks using CR-39 plastic nuclear track detector", *Radiation Protection Dosimetry*, **1**, 2018.
6. S. Homma-Takeda, Y. Terada, A. Nakata et al., "Elemental imaging of kidneys of adult rats exposed to uranium acetate", *Nuclear Instruments and Methods in Physics Research B*, **267**, 2167, 2009.
7. L. Stammer, A. Uhl, B. Mayer et al., "Renal effects and carcinogenicity of occupational exposure to uranium: a meta-analysis", *Nephron Extra*, **6** (1), 1, 2016.
8. S.G. Qu, J. Gao et al., "Low-dose ionizing radiation increases the mortality risk of solid cancer in nuclear industry workers: a meta-analysis", *Molecular and Clinical Oncology*, **8** (5), 1590, 2018.
9. A.P. Golden, E.D. Ellis, S.S. Cohen et al., "Updated mortality analysis of the Mallinckrodt uranium processing workers, 1942-2012", *International Journal of Radiation Biology*, **9** (1), 1, 2019.
10. C. Poisson, J. Stefani, L. Manens et al., « Chronic uranium exposure dose-dependently induces glutathione in rats without any nephrotoxicity », *Free Radical Research*, **48**(2), 1218, 2014.

Acknowledgments

The authors acknowledge funding from Orano for the postdoctoral research fellow comprised in the UKCAN project. We also would like to thank IRSN for the access to PATERSON Platform and for its excellent technical and personal assistance. In addition, we thank IRSN PSE-SANTE/SESANE/LRTOX and Dr. Yann Gueguen for the access to the preserved tissue bank. Additionally, thanks to Dr Christophe Pécheyran (PAMAL Platform, UPPA) for ceding us the software for data treatment and image reconstruction (FOCAL).