

Derivatization Strategy to Quantify Doxorubicin in Mouse Tissues using MALDI-Mass Spectrometry Imaging

Charles X.L. Van Assche,¹ Dustin N. Kruger,² Constantijn Franssen,^{2,3} Pieter-Jan Guns,² Ron M.A. Heeren¹ and Berta Cillero-Pastor⁴

¹ Maastricht Multimodal Molecular Imaging Institute (M4i), University of Maastricht, The Netherlands

² Laboratory of Physiopharmacology, Faculty of Medicine and Health Sciences, Faculty of Pharmaceutical, Biomedical and Veterinary Sciences, University of Antwerp, Belgium

³ UZA, Antwerp University Hospital, Edegem, Belgium

⁴ Institute for Technology-Inspired Regenerative Medicine (MERLN), Department of Cell Biology-Inspired Tissue Engineering, Maastricht, Netherlands

Introduction

Doxorubicin is one of the most used chemotherapeutics in cancer treatments, even if its discovery was back in the 1960s. Nowadays it is still highly effective against leukemia, Hodgkin's lymphoma, and several other cancer types such as childhood cancers or breast cancers¹. Nevertheless, the use of Doxorubicin is highly limited to its known cumulative dose depended cardiotoxic effects. In this study, we employed matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI), which has been previously used to directly monitor the distribution of drugs in biological tissues, to evaluate the distribution and potential accumulation of doxorubicin in mouse tissues. Unfortunately, Doxorubicin is not easily ionized by regular electrospray ionization (ESI) or MALDI². To overcome this issue, we optimized an on-tissue derivatization method using Girard's reagent T (Gir T) in mouse-dosed organs being able to study its distribution and quantify the drug abundance.

Method

Doxorubicin-treated organs (Heart, Kidney and Liver) were taken from mice that have been injected with 4 mg/kg concentration of Dox. Tissue sections from two timepoints (3h post- and 24h post-injection of Doxorubicin) have been mounted onto ITO slides. A 6 points Doxo standard curve was manually spotted onto control tissues to perform quantification. 7 layers of Girard reagent T (Gir T in MeOH 0.2% TFA) have been applied using a M3+ sprayer (HTX Technologies). After 150 min incubation time at 40 degrees, 10 layers of CHCA matrix were applied using a TM sprayer. MALDI-MSI images have been acquired with a FT-ICR instrument (Solarix, Bruker), using CASI mode at 50 μm spatial resolution. Quantification has been performed using SCiLS software (Bruker). These experiments have been done in triplicate.

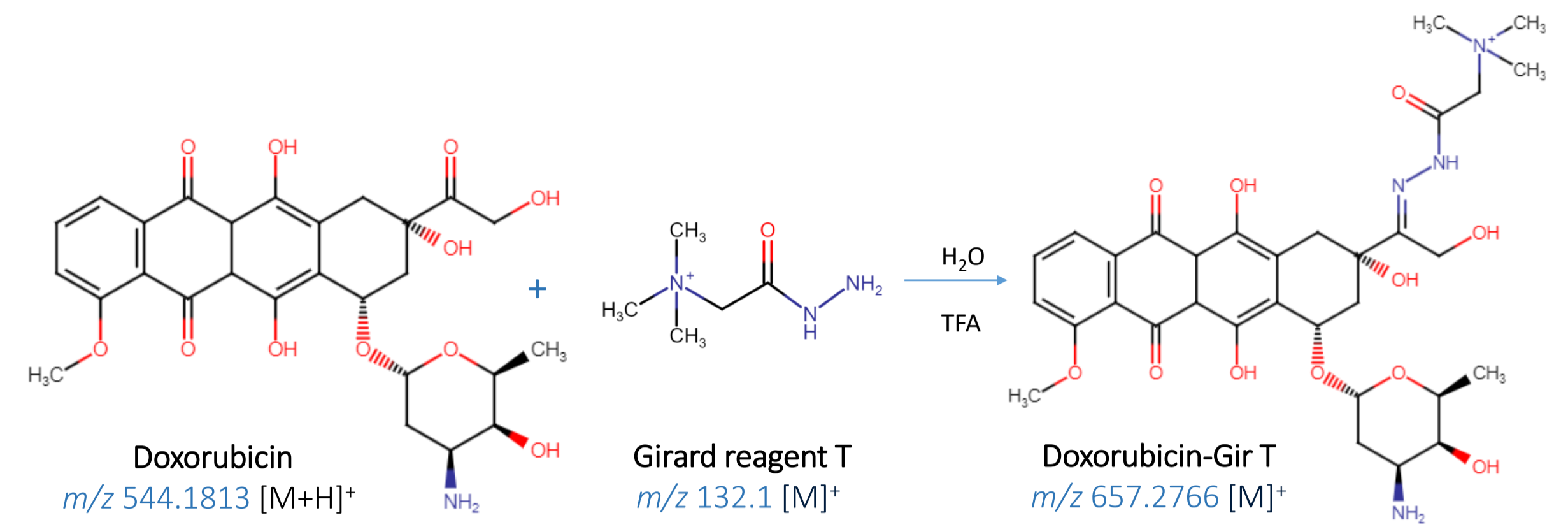


Figure 1. Doxorubicin Derivatization process using Girard reagent T

Results

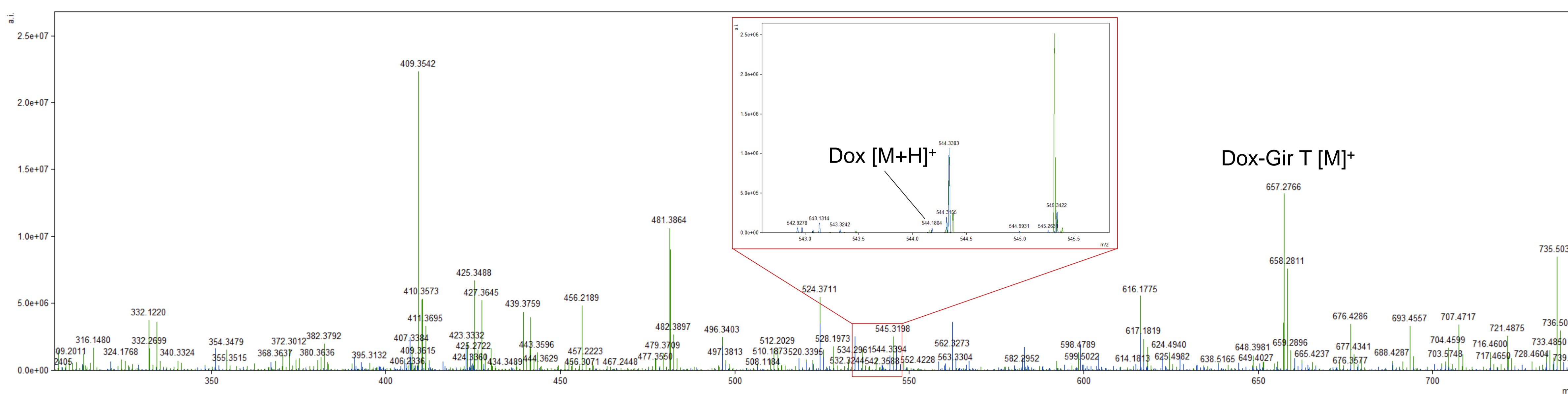


Figure 2. Derivatization of Doxorubicin with Gir T on control tissue. Derivatized Doxorubicin (m/z 657.2766 $[M]^+$) was detected and remains to have a signal 200 times higher than Doxorubicin non-derivatized (m/z (544.1804 $[M+H]^+$)).

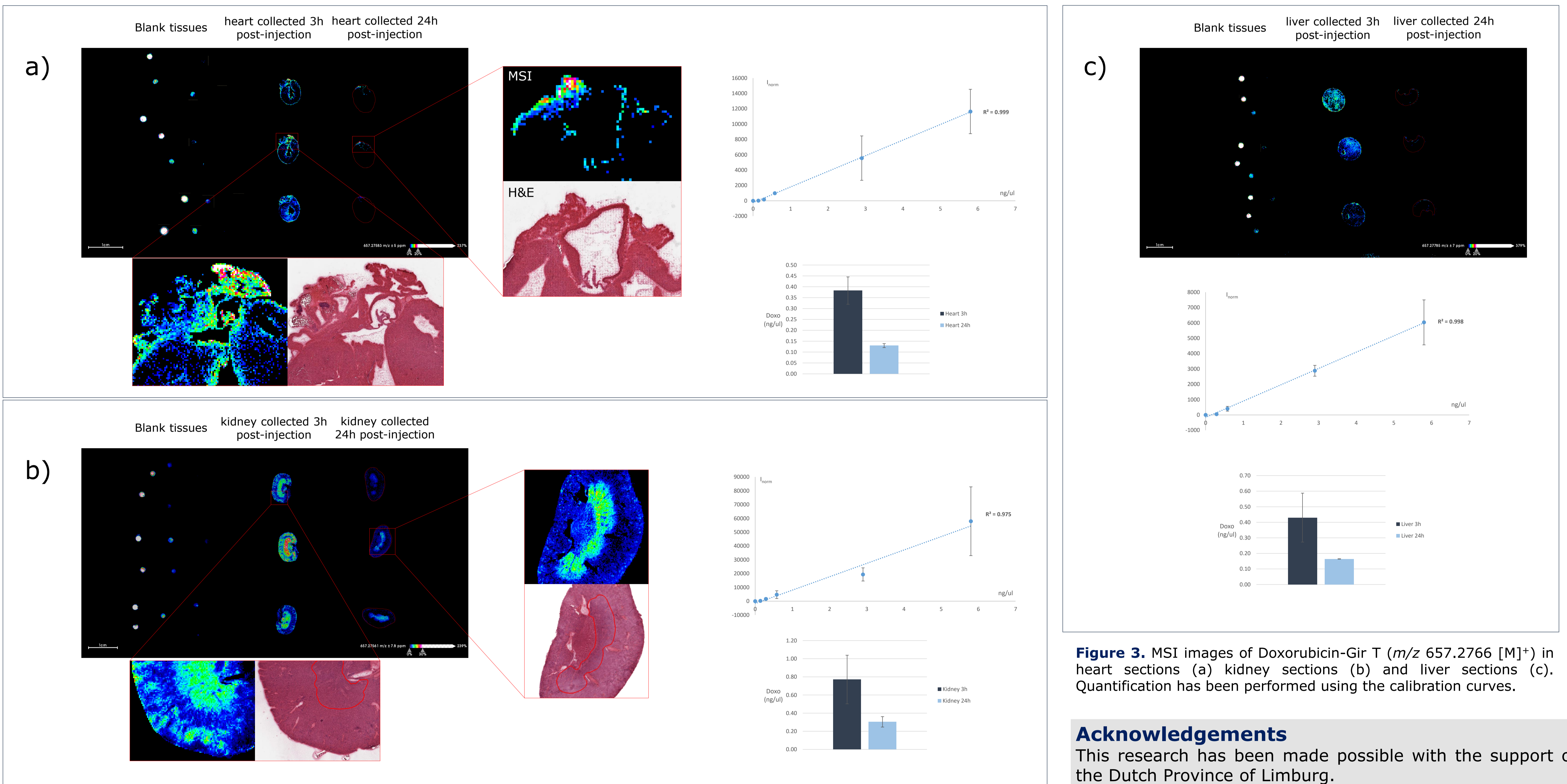


Figure 3. MSI images of Doxorubicin-Gir T (m/z 657.2766 $[M]^+$) in heart sections (a) kidney sections (b) and liver sections (c). Quantification has been performed using the calibration curves.

Conclusions

- In this study, we optimized a derivatization protocol to evaluate the spatial distribution of Doxorubicin in mouse tissues and perform relative quantification of the drug.
- Quantification results shows a higher concentration of Doxorubicin in the kidney (around 0.8 ng/ul for the 3h timepoint). In the heart, the drug was mainly localized in the atrium and the inner part of the left ventricle. As expected, lower concentration was founded in the 24h post-injection organs.
- For absolute quantification, an internal standard (Daunorubicin), analog of Doxorubicin will be sprayed prior the derivatization.

Acknowledgements

This research has been made possible with the support of the Dutch Province of Limburg.

This research has been made possible with the support of the European INSPIRE project (agreement No GA 858070)

References

- Ferreira *et al*, Anthracycline-Induced Cardiotoxicity *Cardiovascular & Hematological Agents in Medicinal Chemistry*, **6**, 278-281 (2008)
- Barré *et al*, Derivatization Strategies for the Detection of TAA in Cartilage by Using MALDI-MSI, *Anal. Chem*, **88**, 12051-12059 (2016)