

# VISUALIZATION AND QUANTIFICATION OF DRUG/METABOLITES BY SENSITIVE AND FAST TARGETED DESI IMAGING ON TQ SYSTEM

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## INTRODUCTION

Mass spectrometry (MS) coupled to liquid chromatography (LC) has been typically used for drug metabolism and pharmacokinetics (DMPK) studies. However, tissues are homogenised and therefore the molecular localisation is lost.

Desorption electrospray ionization (DESI) mass spectrometry imaging (MSI) has proven to successfully map and independently quantify small molecules without radioactive labelling. Including small pharmaceutical molecules, as well as endogenous metabolites directly from tissue without the need of sample preparation after sectioning.

Tandem quadrupole (TQ) MS are renowned for their sensitivity and specificity in targeted applications using Multiple reaction monitoring (MRM) mode of acquisition.

Here, we used a commercial central nervous system (CNS) active drug named pitolisant that treats narcolepsy. It was dosed at pharmacological level and no observed adverse effect level (NOAEL) to show the distribution of the drug and its main metabolite in different organs such as liver and brain using the sensitive and fast DESI TQ system.

## METHODS

### Tissue sample preparation

Pitolisant, a drug enhancing brain activity histaminergic neurons, and its oxidized metabolite were dissolved in MeOH and diluted in 50:50 MeOH:H<sub>2</sub>O from 10 ng/  $\mu$ L down to 0.05/  $\mu$ L ng to establish the level of detection (LOD). 1  $\mu$ L of each dilution series solution was spotted on control porcine liver tissue section.

Mice were dosed at a therapeutic dose of 5 mg/kg and NOAEL toxicologic dose of 75 mg/kg. Livers and brains were collected 2 hours after administration. Organs were immediately frozen in liquid nitrogen and stored at -80°C until sectioning at 18  $\mu$ m using a cryostat.

### Mass spectrometry

MSI experiment were carried out using a Xevo™ TQ Absolute mass spectrometer with a DESI XS source, including the High-Performance sprayer (HPS) for improved sensitivity, spray focus, robustness and ease-of-use.

DESI spray conditions were set at 2  $\mu$ L/min, 95:5 MeOH: H<sub>2</sub>O v/v and the N<sub>2</sub> nebulising gas pressure was set at 10 psi. Capillary voltage was optimised for the most sensitive signal between 0.55 and 0.7 kV in positive ionisation mode. The samples were imaged at 15 and 50  $\mu$ m pixel size.

MRM transitions were obtained from previous UHPLC MS/MS studies. They were transferred onto the DESI-XS source on the Xevo TQ Absolute mass spectrometer and optimised using pitolisant and its main metabolite in rodent (oxidized form) standards spotted onto liver tissue sections, running a confirmatory transition experiment.

MSI experiments were performed at different speeds, with various numbers of MRM transitions, with a minimum dwell time per MRM transition and pixel of 6 ms.

### Data management

DESI imaging datasets were mined using MassLynx™ software as well as processed and visualized using High Definition™ Imaging Software (HDI™) v1.8 (Waters).

A in-house quantitative MicroApp software called MSI Quantify was used to construct calibration curves mapping average signal intensities to known quantities or concentrations. These calibration curves were used to estimate an unknown amount of drug in dosed tissue of interest.

## RESULTS

### A) Dilution series to evaluate LODs of Pitolisant and its oxidized metabolite

The first step of the workflow was to establish the level of detection (LOD) of the drug and its oxidized metabolite. The dilution series ranging from 0.05 to 10 ng was spotted on the control liver and brain tissue sections.

The best MRM transition for the pitolisant was  $m/z$  296.2 > 98.1 and for the oxidized pitolisant was  $m/z$  312.1 > 98.1.

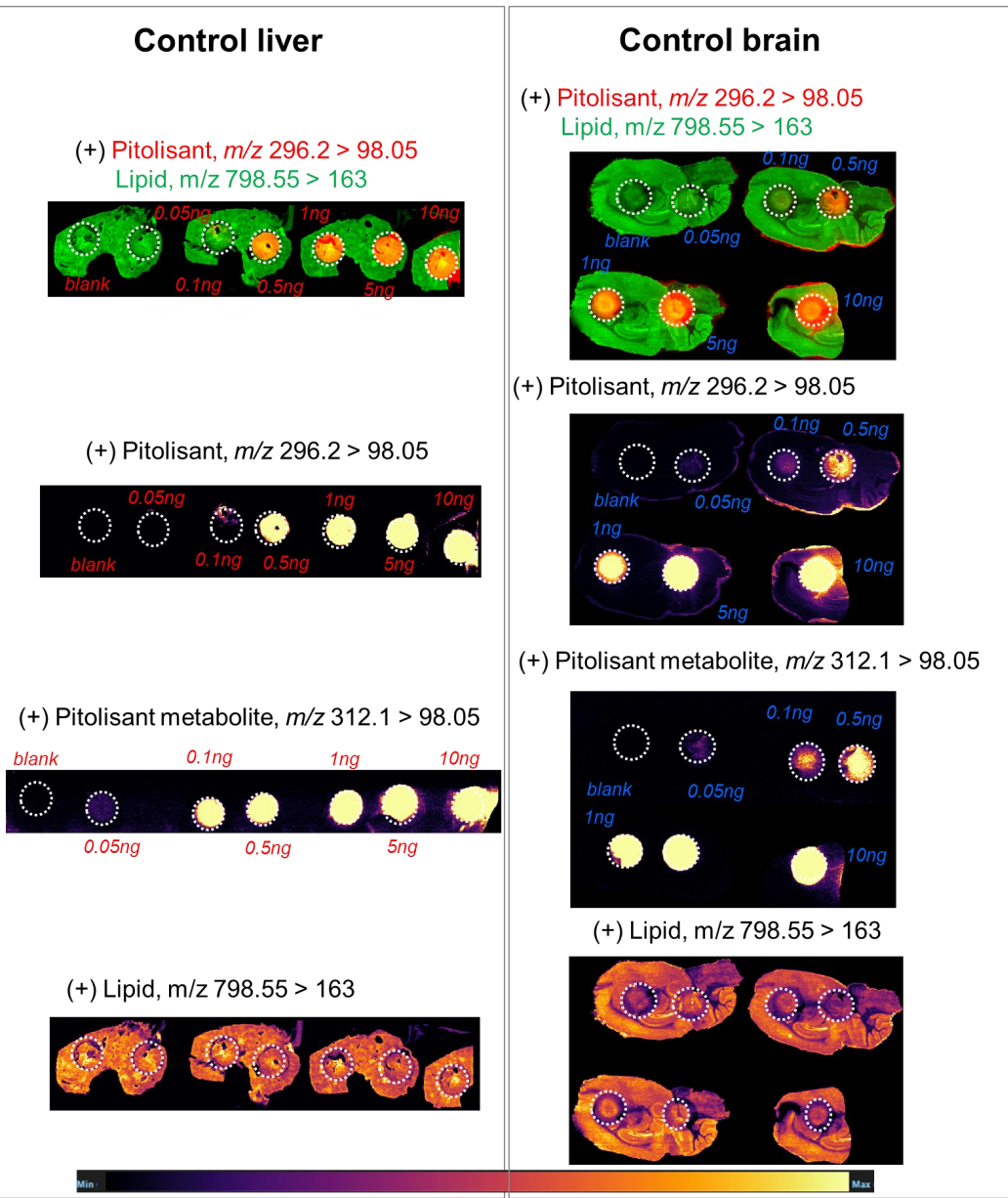


Figure 1. Dilution series of drug pitolisant ( $m/z$  296.2 > 98.05) and its oxidized metabolite (312.1 > 98.05) from 0.05 ng up to 10 ng spotted on control mice liver and brain tissue sections.

Figure 1 displays the ion images of the dilution series of the drug, oxidized pitolisant and potassiated phospholipid PC [34:1]  $m/z$  798.55 . 163 at 50Hz. Pitolisant was detected on liver and brain tissue section at 0.1 ng whereas the oxidized metabolite was detected at 0.05ng spotted on tissues.

### B) Fast detection with high sensitivity experiments

With the Xevo™ TQ Absolute mass spectrometer with a DESI XS source, it was possible to define two MRM transitions ( $m/z$  296.2 > 98.1 and 798.55 > 163) and run the acquisition at 50 Hz acquisition speed and spatial resolution of 50  $\mu$ m x 50  $\mu$ m.

Overall the average acquisition time per tissue sections (control, 5 mg/kg and 75 mg/kg dosage) was 43 min the liver, as they were bigger than the brain tissue sections. Localization of pitolisant and potassiated PC [34:1] in control 5 and 75 mg/kg pitolisant dose liver tissue sections are shown in figure 2.

Furthermore the detection of pitolisant was easily achieved from the pharmacological level dosed liver tissue section with a LOD > 10.

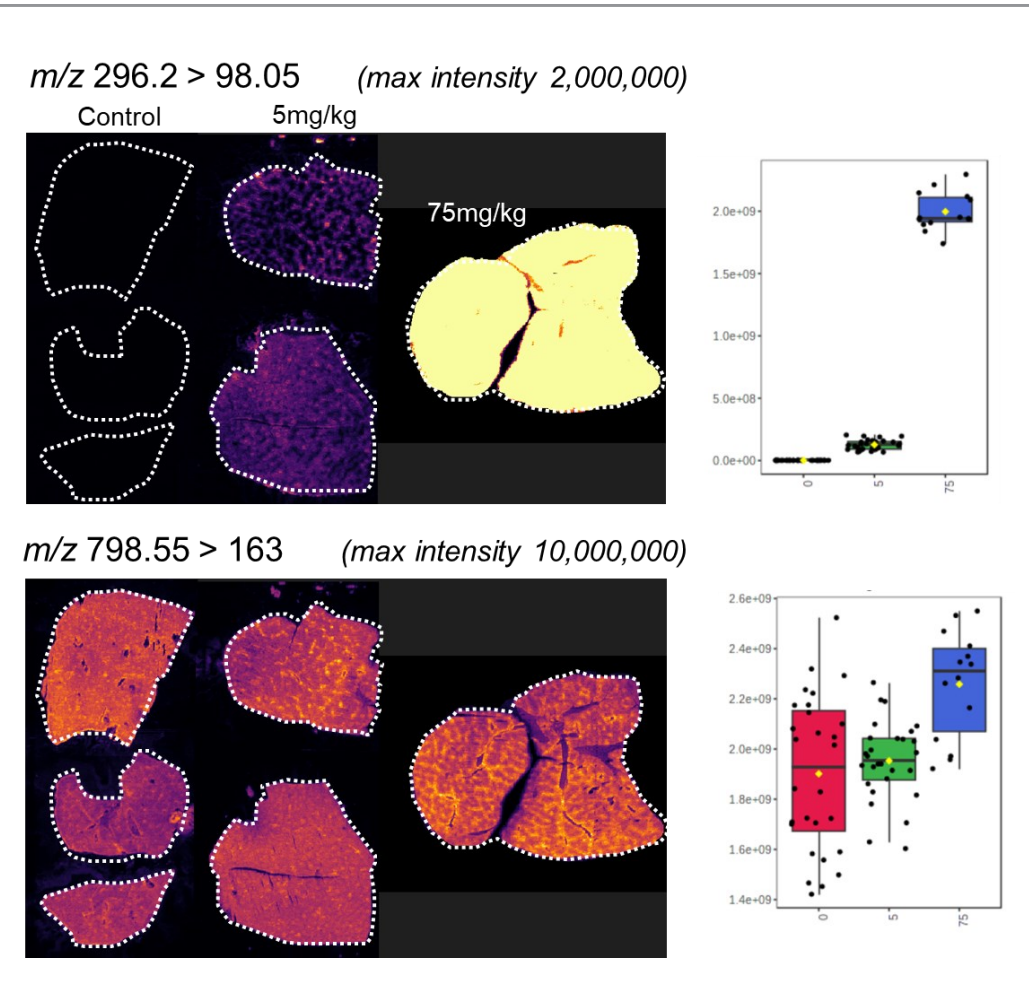


Figure 2. Pitolisant ( $m/z$  296.2 > 98.05) distribution in control, 5 and 75 mg/kg liver dosed tissue sections as well as potassiated PC [34:1] ( $m/z$  798.55 > 163) when MRM DESI targeted experiments were acquired at 50 Hz.

### C) Detection of drug, its oxidized metabolites and biomarkers

Further experiments were performed with ten MRM transitions defined at an acquisition speed of 10 Hz. Two MRM transitions were set for pitolisant  $m/z$  296.2 > 98.05 and 296.2 > 126.15), one for the oxidized metabolite (312.1 > 98.05) and seven MRM transitions for lipids: PC [32:0], K<sup>+</sup>  $m/z$  772.55 > 163; PC [34:1], Na<sup>+</sup>  $m/z$  782.55 > 147; PC [36:2], H<sup>+</sup>  $m/z$  782.55 > 184; PC[34:1], K<sup>+</sup>  $m/z$  798.55 > 163; PC [36:1], Na<sup>+</sup>  $m/z$  810.6 > 147; PC[36:4], K<sup>+</sup>  $m/z$  820.6 > 163; PC[38:4], K<sup>+</sup>  $m/z$  848.6 > 163.

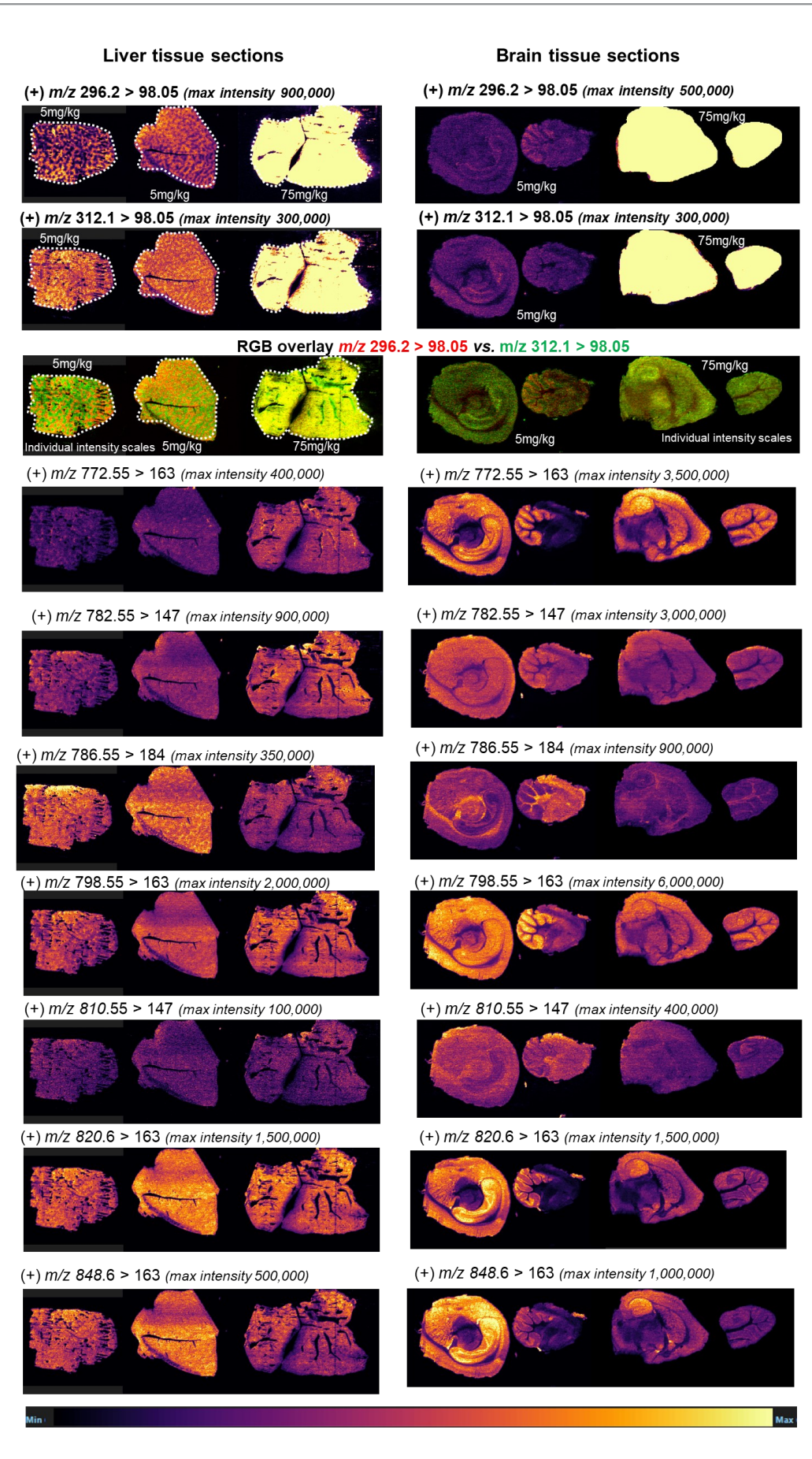


Figure 3. Pitolisant ( $m/z$  296.2 > 98.05) distribution in control, 5 and 75 mg/kg liver dosed tissue sections as well as potassiated PC [34:1] ( $m/z$  798.55 > 163) when MRM DESI targeted experiments were acquired at 50 Hz.

Figure 3 displays the ion images from the 10 MRM transitions for the 5 mg/kg and 75 mg/kg doses mouse liver and brain tissue sections.

Interestingly, it can be seen from the RGB overlay ion images of the pitolisant and oxidized metabolite that they have similar distributions in liver and brain at high dose. This confirms the known high tissue distribution<sup>1</sup>. Whereas, at pharmacological dose, they have different intra tissular distributions, especially in the liver, where pitolisant is more abundant around the lobule and the metabolite is more ubiquitous.

Table 1 shows the summary of the S/N calculated from the raw data acquired from the two dosed liver and brain tissue section.

Overall, the drug and its main metabolite were detected with a good S/N > 10 at pharmacological dose, even in the brain, where both have passed through the blood brain barrier.

|                     | Signal to noise (S/N) |          |         |          |
|---------------------|-----------------------|----------|---------|----------|
|                     | Liver                 |          | Brain   |          |
|                     | 5 mg/kg               | 75 mg/kg | 5 mg/kg | 75 mg/kg |
| Pitolisant          | 39                    | 180      | 10      | 250      |
| Oxidized metabolite | 14                    | 57       | 12      | 171      |

Table 1. Signal to noise (S/N) of pitolisant and its oxidized metabolite analyzed using the XevoTQ Absolute mass spectrometer with a DESI XS source for a 10 Hz MRM experiments from the pharmacological (5 mg/kg) and NOAEL (75 mg/kg) doses in mice liver and brain tissue sections.

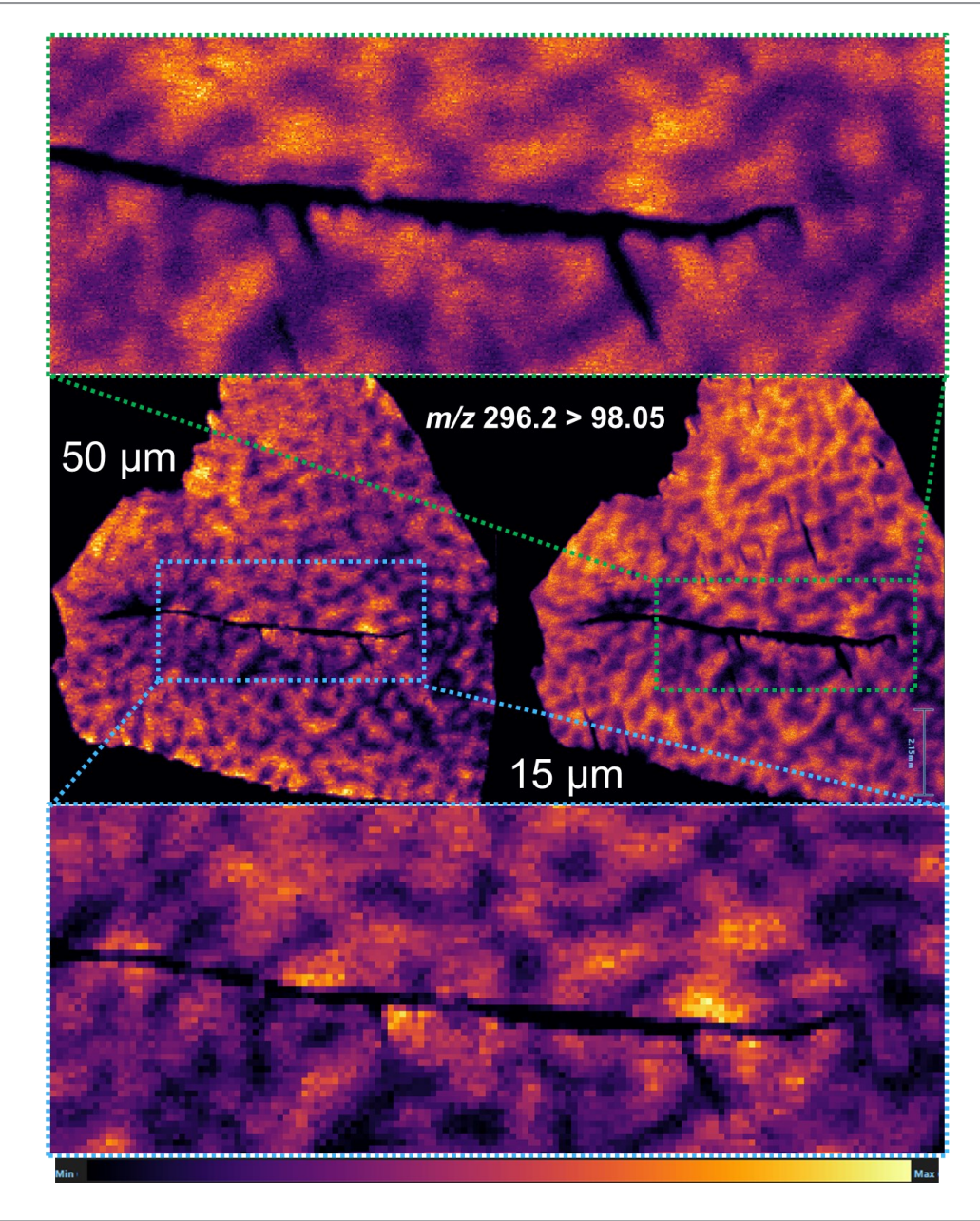


Figure 4. Distribution of pitolisant in the pharmacological dosed liver tissue section, acquired at 15 and 50  $\mu$ m pixel size.

### References

1: X.Ligneau, L.Landais, D.Perrin, J.Piriou, M.Uguen, E.Denis, P.Robert, R.Parmentier, C.Anaclet, J.S.Lin, A.Burban, J.M.Arrang, J.C.Schwartz, Biochemical Pharmacology, Volume 73, Issue 8, 15 April 2007.

### D) Enabling relative quantification

The dilution series with the MRM dataset processed using HDI software was used to generate the calibration curves for pitolisant in MSI Quantify MicroApp.

After uploading the dilution series dataset, a  $m/z$  of interest was selected, ROIs were drawn (figure 5,A), concentration/quantities were specified, calibration curves were generated (figure 5,B) and evaluation of the calibration curve was conducted against the dilution series dataset itself (figure 5,C).

Tissue imaging processed datasets were also uploaded and ROIs were drawn on them. Predicted doses were calculated for each ROI from the control, pharmacological and NOAEL dosed liver tissue section (figure 5,C).

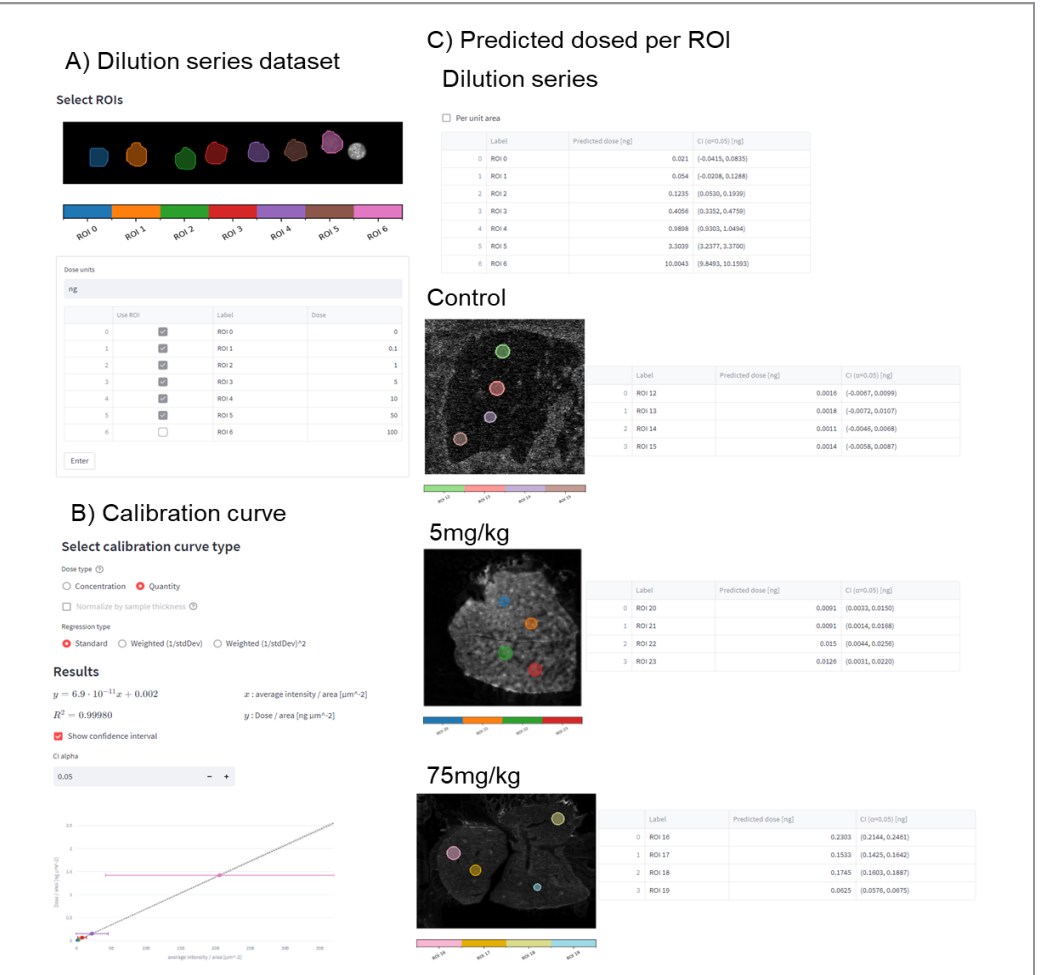


Figure 5. MSI Quantify software workflow to enable MSI quantification from creating the calibration curve from the dilution series dataset up to predicted doses per ROI on the drug tissue datasets.

## CONCLUSION

- Pitolisant and its metabolites were detected sub 0.1 ng when spotted onto tissue section, demonstrating the high sensitivity and specificity of targeted MRM MS Imaging workflow.
- A highly flexibility workflow is evidenced allowing high speed DESI MSI experiments with no compromise in sensitivity or including more MRM transitions to map biomarkers as well as drug.
- Pitolisant and its oxidized metabolite were easily detected at pharmacological dose, illustrating the different distributions in the liver and brain murine tissue sections, information that wouldn't be discovered by traditional DMPK studies.
- MSI quantification workflow is demonstrated with the in-house MicroApp MSI Quantify.