IMPROVING SPATIAL DISTRIBUTION, SENSITIVITY AND SELECTIVITY IN MSI, USING A NOVEL DESI QQQ AND A HR-MULTI REFLECTING TOF SYSTEMS

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INTRODUCTION

Mass spectrometry imaging is a method that determines the tissue distribution of small molecules such as pharmaceutical compounds. Due to tissue complexity and decreasing amounts of analytes sampled with increasing spatial resolution, detection can be challenging, particularly at therapeutic levels.

DESI MSI is known for the mapping of small molecules directly from tissue sections and has been proven to be successful when used on TOF based MS for untargeted analysis. Triple quadrupole MS are renowned for their sensitivity and specificity for targeted application using MRM acquisition.

Here we have evaluated the LOD's for several pharmaceutical compounds (theophylline, ranitidine and atenolol) spotted on control tissue sections and determined their distribution in two time point dosed ileum tissue sections using a discovery untargeted full scan MS mode on a DESI high-resolution MRT MS and a targeted MRM on a DESI TQ-MS.

METHODS

Tissue sample preparation

Theophylline, ranitidine and atenolol were dissolved in MeOH and diluted in 50:50 MeOH:H2O from 100 µM down to 0.1 µM.
1 µL of each dilution series solution was spotted on control porcine liver tissue section.

Sprague-Dawley rats were dosed by intravenous administration of theophylline, ranitidine and atenolol prepared at a concentration of 2.5 mg/ml in sodium phosphate buffer. The animals were euthanized at time points of 4h and 7h, and different parts of the gastrointestinal (GI) tract were collected and flushed with PBS buffer before being stored at -80° C.

The tissue distribution of the compounds was evaluated on fresh frozen 12 µm ileum tissue sections at the 4h and 7h post-dose time points.

Mass spectrometry

Two types of experiments were performed during this study:

1) Full scan MS using a SELECT SERIES™ MRT multi-reflecting Q-ToF with a mass resolution >200,000 FWHM across the lipid mass range and a mass accuracy <500 ppb.
2) MRM using a tandem quadrupole mass spectrometer for high sensitivity and specificity .

The DESI XS source was used on both mass spectrometers with the High-Performance sprayer (HPS) for improved sensitivity, spray focus, robustness and ease-of-use.

	Full scan MS MRT	MRM TQ
Ionisation mode	(+) and (-)	(+) and (-)
Flow rate	2 µl/min	2 µl/min
DESI solvent composition	95% MeOH, 5% water with 100pg/µL Leu-enkephalin	95% MeOH, 5% water with 100pg/uL Leu-enkephalin
Capillary voltage	**1.2 kV (+) / 1.2 kV (-)**	**0.69 kV (+) / 0.75 kV (-)**
Nebulising gas	15 psi	15 psi
Acquisition speed	**2 Hz**	**5 Hz** (dilution series (+)) / **10 Hz**
Pixel size (lateral)	**75µm** (dilution series) / **50 µm** (dosed tissue)	**75 µm** (dilution series) / **15 & 50 µm** (dosed tissue)

Table 1. DESI parameters used for the full scan experiment on the MRT and the MRM experiment on the TQ.

Data management

DESI imaging datasets were mined using MassLynx™ as well as processed and visualized using High Definition™ Imaging Software (HDI™) v1.7 (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

1) Limit of detection (LOD) determination

Limit of detection (LOD) were determined for the three drugs in positive and negative mode from the dilution series spotted on liver tissue sections.

For data acquired in full MS scan mode using the DESI MRT, the mass range was *m/z* 50-2,400.

For data acquired in MRM mode using the DESI TQ, MRM transitions were manually optimized using product ion experiments. Three fragments were chosen per drug compounds to evaluate the best MRM transmission for the Ileum drug dosed MSI experiments (Table 2 and Table 3). Theophylline and ranitidine were detected in both positive and negative ionization mode whereas atenolol was only detected in positive ionization mode.

Compound name	Precursor m/z		Product m/z	Collision energy (V)	Dwell time (msec)
Theophylline	181	>	124	18	4
Theophylline	181	>	96	22	4
Theophylline	181	>	69	21	4
Atenolol	267	>	145	22	4
Atenolol	267	>	190	18	4
Atenolol	267	>	208	18	4
Ranitidine	315	>	130	25	4
Ranitidine	315	>	176	20	4
Ranitidine	315	>	224	15	4

Table 2. DESI TQ MRM transitions for theophylline, atenolol and ranitidine in ***positive ion mode*** *for the dilution series spotted on liver tissue experiment.*

Compound name	Precursor m/z		Product m/z	Collision energy (V)	Dwell time (msec)
Theophylline	179	>	164	20	16
Theophylline	179	>	122	22	16
Theophylline	179	>	135	28	16
Ranitidine	313	>	170	18	16
Ranitidine	313	>	142	18	16
Ranitidine	313	>	91	20	16
Lipid PI (38:4)	885.5	>	283.2	22	16

Table 3. DESI TQ MRM transitions for theophylline and ranitidine in ***negative ion mode*** *for the dilution series spotted on liver tissue experi-ment.*

Data acquired for ranitidine and atenolol in positive mode at 2 Hz on the MRT system resulted in LOD's of 0.5 µM and 1 µM (spotted on a liver tissue section), respectively. In the negative ion mode, theophylline and ranitidine could be detected down to 7.5 µM.

The added value of applying a triple quadrupole for targeted MS imaging was nicely demonstrated by the lower detection limits achieved at a higher speed of acquisition. In the positive ion mode acquired at 10 Hz, ranitidine and atenolol were detected down to 0.1 µM spotted on tissue. Theophylline and ranitidine were detected in negative mode down to 0.5 µM at 5 Hz.

Table 4 reports the LODs for targeted and untargeted in positive and negative ionization mode,

Polarity	Positive		Negative	
Acquisition type	Targeted MRM (5 Hz)	High resolution untargeted full scan MS (2 Hz)	Targeted MRM (10 Hz)	High resolution untargeted full scan MS (2 Hz)
Theophylline	50 µM	n/o	**0.5 µM**	7.5 µM
Atenolol	**0.1 µM**	1.0 µM	n/o	n/o
Ranitidine	**0.1 µM**	0.5 µM	1.0 µM	7.5 µM

Table 3. Summary results for the LODs for theophylline, atenolol and ranitidine spotted on control liver tissue sections, analyzed in positive and negative ionization mode in targeted MRM and untargeted full scan MS mode.

2) Generating calibration curves

HDI processed full scan MS and MRM dataset were used to generate the calibration curves for the three drugs in MSI Quantify MicroApp. Independently of the type of acquisition, the quantitative workflow was the same. Datasets were added, *m/z* of interest were selected, ROIs were drawn, concentration/quantities were specified, calibration curves were generated and evaluation of the calibration curves was conducted against the dilution series datasets themselves.

Examples are displayed in Figure 1 for the drug compound Atenolol acquired in positive ionization full MS scan mode and Figure 2 for theophylline , acquired in negative ionization MRM mode. For both examples the R^2 was better than 0.99 and the predictive dose results matched the amount spotted on tissue within the 95% confidence interval.

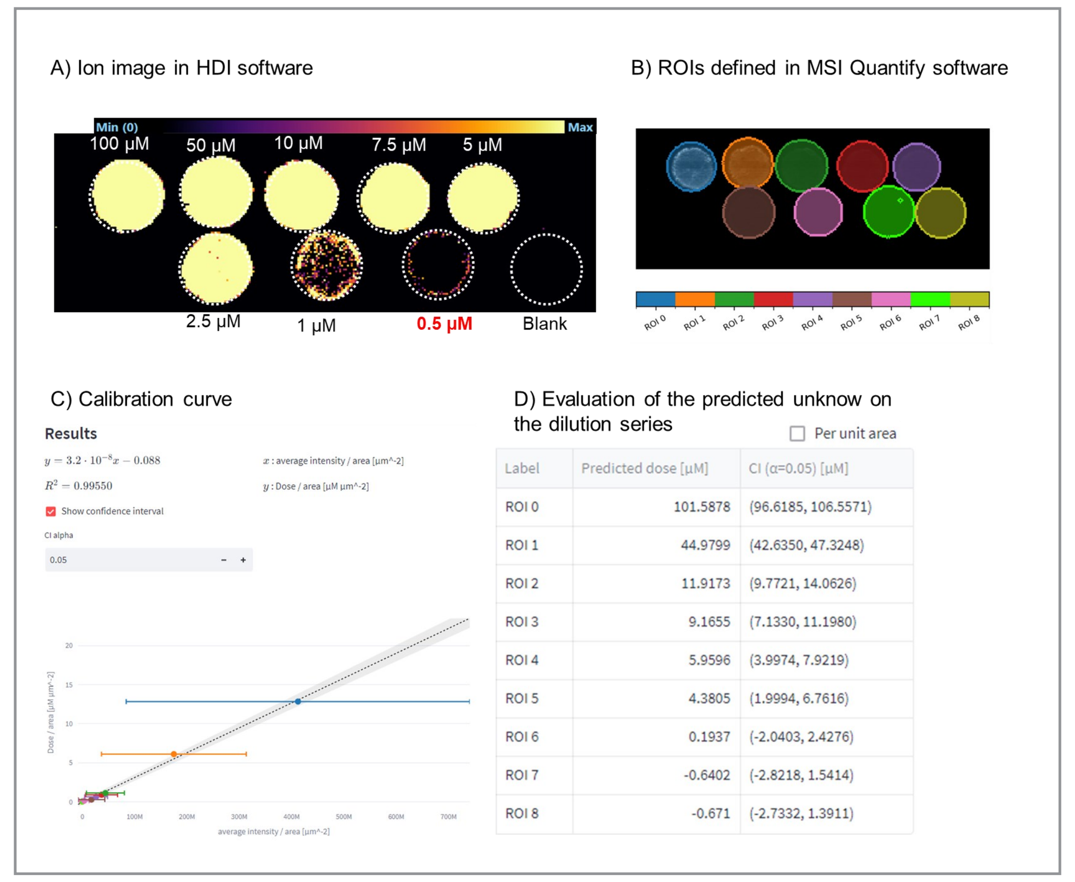

Figure 1. Calibration curve generated in MSI Quantify MicroApp for ***Atenolol*** *acquired in* ***positive ionization full MS scan on the DESI MRT****. A) Atenolol MS ion image displayed in HDI, B) Definition of the ROIs in MSI Quantify, C) Calibration curve and D) Validation of the cali-bration curve with predicted dose calculated on the same dataset.*

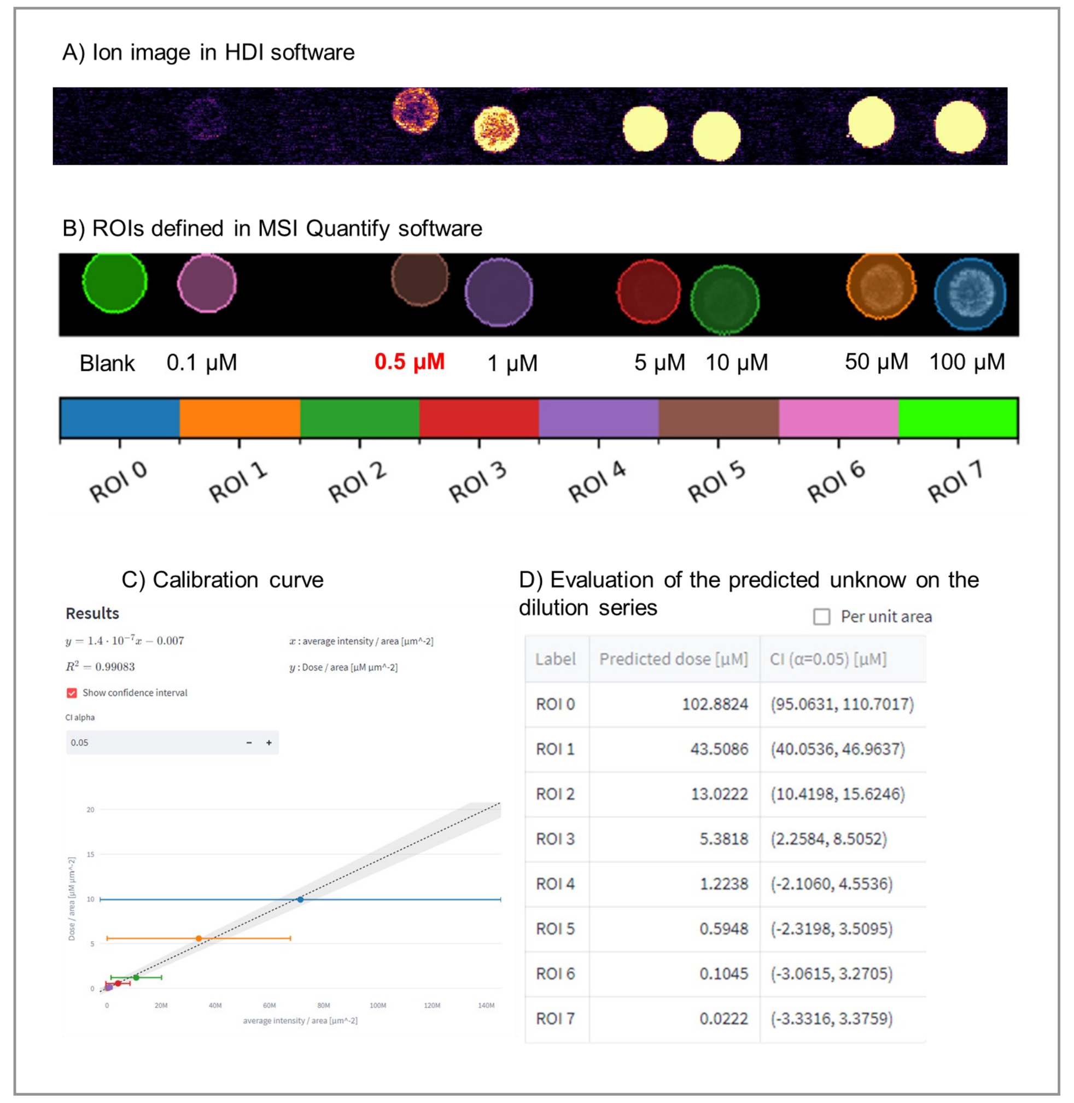

Figure 2. Calibration curve generated in MSI Quantify MicroApp for ***Theophylline*** *acquired in* ***negative ionization MRM on the DESI TQ****. A) Atenolol MS ion image displayed in HDI, B) Definition of the ROIs in MSI Quantify, C) Calibration curve and D) Validation of the calibration curve with predicted dose calculated on the same dataset.*

3) Untargeted DESI MSI of the ileum drug dosed tissue sections

The 4 and 7 hours time point ileum dosed tissue sections were analyzed in a full scan MS in positive and negative mode.

Theophylline was observed in both the 4 hour and 7 hour post dosed tissue sections and distributed in the mucosa, submucosa and tunica muscularis (Figure 3). Atenolol and ranitidine were detected only in the 7 hours post dose tissue section and were mainly localized in the mucosa. In positive mode, interferences were observed outside of the tissue with the same *m/z* as ranitidine. However no interferences were detected in negative mode acquisition.

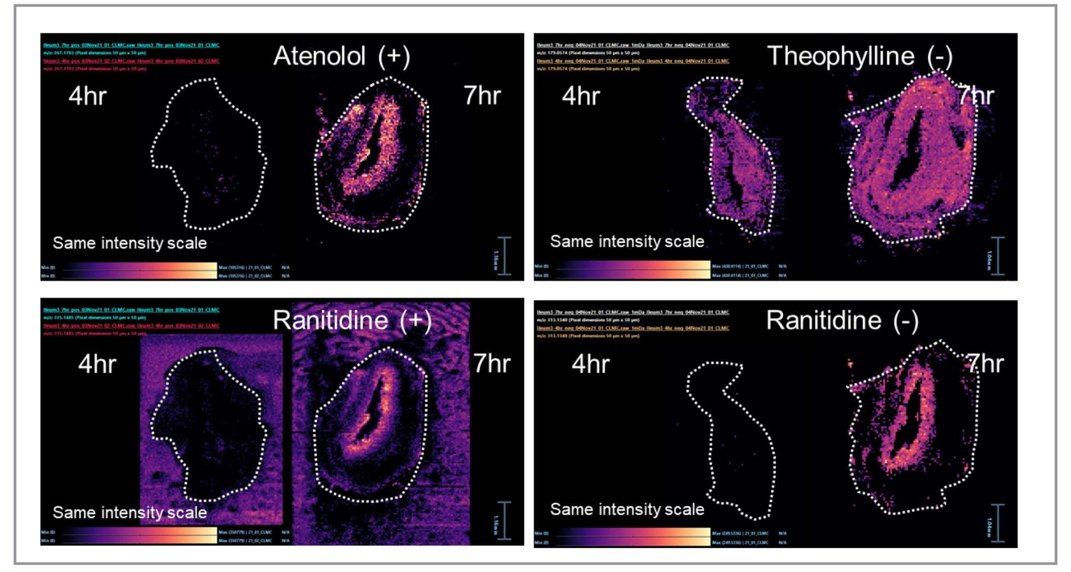

Figure 3. DESI ion images of atenolol (+), theophylline (-) and ranitidine (+/-) in 4 and 7 hours time point ileum tissue sections in ***high resolution untargeted full scan MS****, acquired at 2 Hz with a 50 µm pixel size.*

With the richness of untargeted full scan MS data format, endogenous lipids were imaged and putatively identified with less than 500 ppb mass accuracy (figure 4).

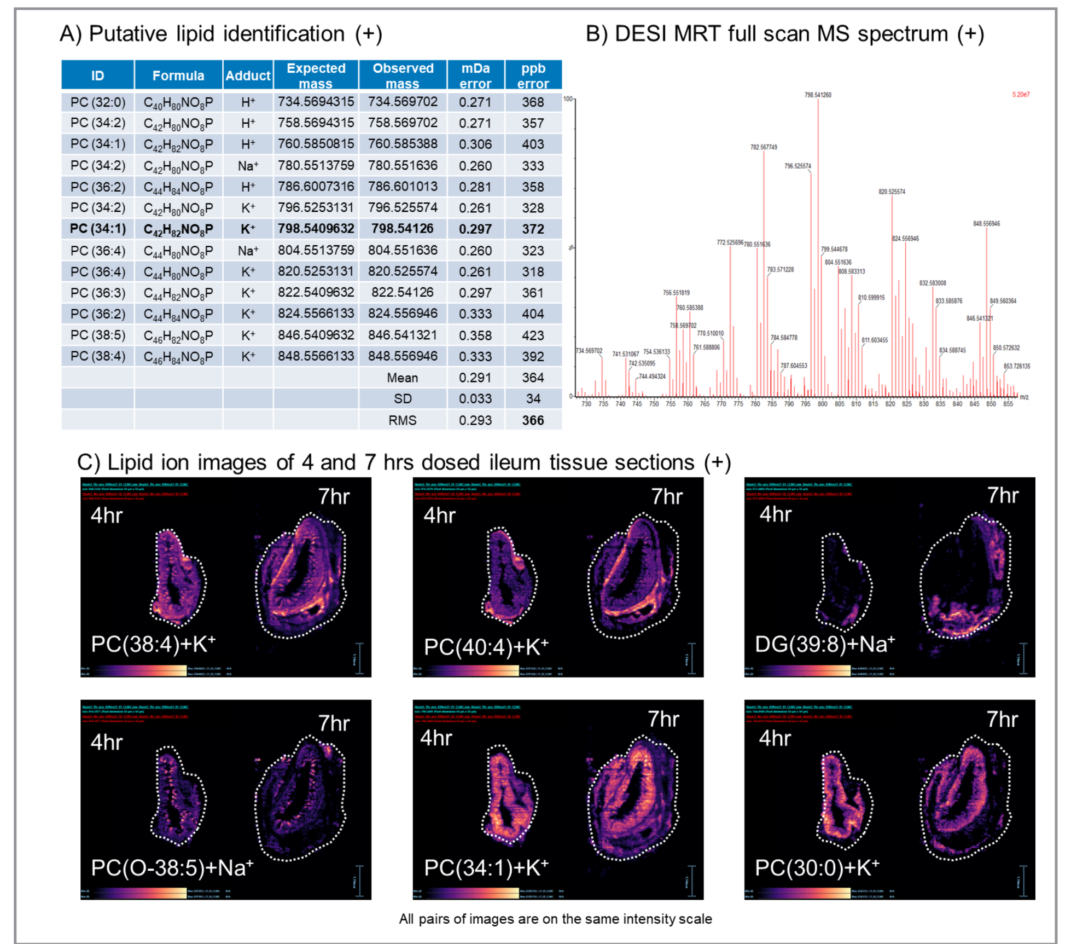

Figure 4. A) Summary table of putative lipid identification in positive mode, B) MS spectrum, C) example of ion images.

4) Targeted DESI MSI of the ileum drug dosed tissue sections

With the enhanced sensitivity of MRM acquisition mode, the three drug compounds were detected directly from the 4 and 7 hours time point ileum dosed tissue sections (Figure 5). Furthermore in positive mode, no interferences were observed outside of the tissue with MRM increased specificity.

Further analyses were conducted on the same tissue sections with a pixel size of 15 µm in positive mode, illustrating the specific localization of ranitidine (Figure 6) in ileum tissue sections.

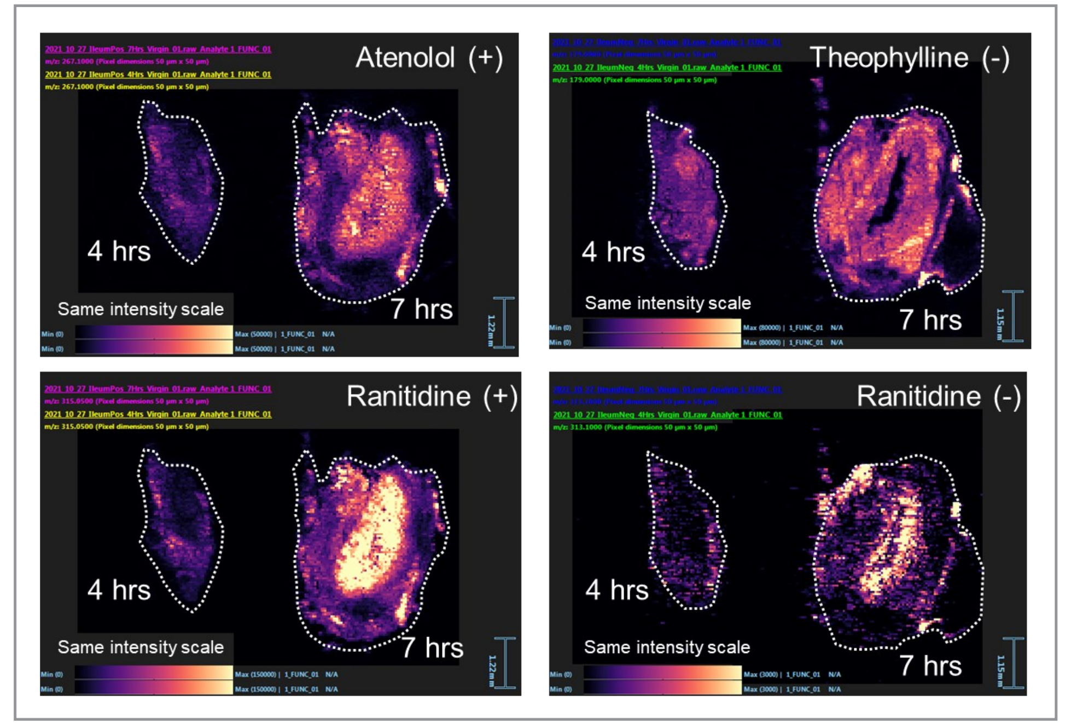

Figure 5. DESI ion images of atenolol (+), theophylline (-) and ranitidine (+/-) in 4 and 7 hours time point ileum tissue sections in ***targeted MRM****, acquired at 10 Hz with a 50 µm pixel size.*

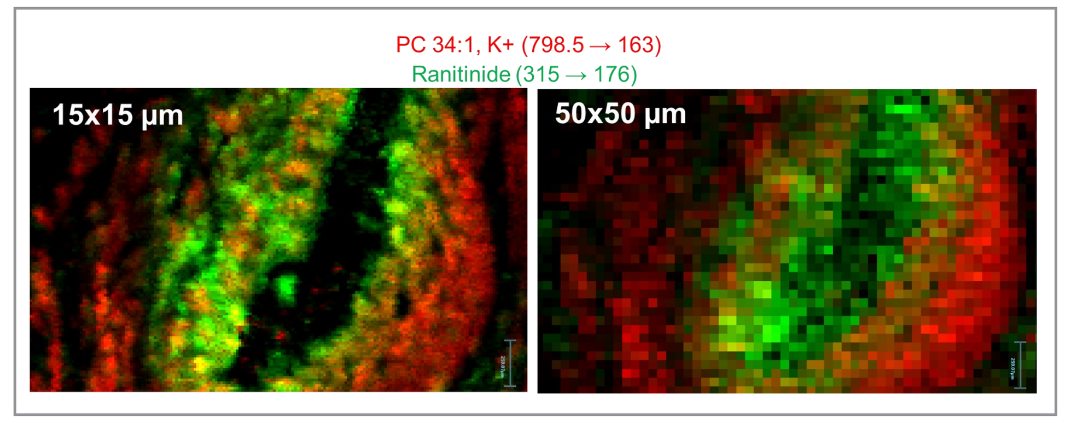

Figure 6. DESI ion images of ranitidine (+ in 7 hours time point ileum tissue section in ***targeted MRM****, acquired at 10 Hz with pixel sizes of* ***15*** *and* ***50 µm****.*

CONCLUSION

- **Demonstrated increased sensitivity and specificity of targeted MRM MS Imaging workflow, allowing lower LODs of drugs spotted on liver tissue section.**
- **Achieved good linear calibration curves in both targeted MRM and untargeted full scan MS modes with R^2>0.99, enabling potential quantitative workflow.**
- **Theophylline, atenolol and ranitidine were all detected in positive or negative ionization mode by DESI MRT only on the 7 hour time point ileum tissue sections, as well as endogenous lipids with sub-500 ppb mass accuracy.**
- **With the targeted MRM workflow, the three drugs were DESI imaged from both 4 and 7 hours time point tissue sections.**

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