TARGETED DESI IMAGING MS OF DRUG DISTRIBUTION AND DRUG-INDUCED LIVER INJURY (DILI) METABOLITES FROM METHAPYRILENE IN THE MALE WISTAR RAT

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INTRODUCTION

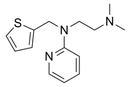
Methapyrilene is a drug known to produce liver damage.[1] Previous discovery and targeted lipidomics LC-MS studies of methapyrilene-dosed male Wistar rats revealed the lipids and biomarkers indicative of the drug's toxicity. Imaging Mass Spectrometry (IMS) adds complementary spatial localization information for the drugs and key metabolites. IMS maps the changes arising from drug metabolism to distinct organ tissue structures.

Desorption Electrospray Ionization (DESI) is an ambient ionization method for tissue imaging offering higher sensitivity for small molecule drugs and metabolites that are more readily ionized with ESI. DESI imaging with MSMS transitions on a tandem quadrupole MS increases specificity because metabolite ID is known, with the much higher sensitivity of a tandem quad.

Targeted DESI Imaging MS of rat liver tissue was used to measure the spatial distribution differences of lipids, drug, and drug metabolite in control (vehicle) vs. dosed rat liver tissue at 50 mg/kg and 150 mg/kg of methapyrilene over a time course of 6 days. The imaging experiments illustrated the changes in localization of the major lipids and metabolites up and down regulated by methapyrilene toxicity.

SAMPLES

Male Wistar rats – liver tissue[2]



$C_{14}H_{19}N_3S$
$[M+H]^+ = m/z$ 262.1839

- Three groups of rats with two rats per group:
  - -Vehicle (control)
  - 50 mg/kg methapyrilene oral dose
  - 150 mg/kg methapyrilene oral dose
  - Methapyrilene given daily for 5 consecutive days
- Livers harvested from rats in each group at 24 h, 72, and 120 h
  - Rinsed in cold NaCl and dried
  - Snap frozen and stored at -80ºC
- Liver tissue: 10 µm thick fresh frozen sections (no embedding) using a cryotome, thaw mounted on standard glass specimen slides for DESI Imaging MS

Reference and calibration standards:

- Leucine-Enkephalin QC internal standard (Waters™ P/N 186006013)
  - 200 pg/µL in DESI solvent
- MS Resolution and Calibrants for TQ-Absolute:
  - Xevo™ TQ-XS Set-Up Solution (Waters P/N 186008719)
- Methapyrilene HCl analytical standard (Sigma Aldrich); 1 mg/mL (aq)

METHODS

Targeted DESI imaging mass spectrometry

Source:

- Waters DESI XS source with High-Performance Sprayer; Heated Transfer Line (HTL)

Mass Spectrometer:

- Waters Xevo TQ-Absolute tandem quadrupole MS (Figure 2)
- Unit mass resolution (0.75 Da) from *m/z* 20 to 2000

DESI conditions:

- 98:2 methanol:water solvent with 0.01%(v/v) formic acid at 2 µL/min
- Nebulizing gas pressure: 0.9 bar N_2 w/ 0.7 kV sprayer voltage
- Cone Voltage: 10 to 50 V; Source Temperature: 120ºC
- Polarity: Positive and Negative

MS mode: Multiple Reaction Monitoring (MRM); 0.2 to 0.5 s/MS scan

Imaging MS Pixel size: 20 and 50 µm

Data management

Imaging MS data were acquired with MassLynx™ 4.2. Experimental parameters were defined, and raw files processed using DESI Method Editor plug-in for High-Definition Imaging (HDI™) software v1.7 (Waters). Imaging data were visualized using HDI.

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DESORPTION ELECTROSPRAY IONIZATION

- High-pressure gas flow from the desolvation gas (N_2) focuses the charged ESI solvent droplets focus into a beam
- The beam washes the surface to desorb analytes on the sample
- Desorbed analytes were then ionized by ESI and carried into a heated inlet capillary that transferred the ions to the MS for analysis
- DESI is minimally destructive and allows multiple imaging analyses from the same sample with adjustable height for thicker samples

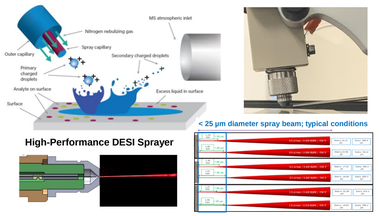

Figure 1. DESI XS high-performance sprayer with nominal 25 µm spray diameter

IMAGING MASS SPECTROMETRY

Tandem quadrupole MS for targeted DESI Imaging MS

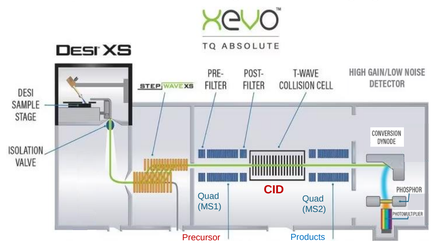

Figure 2. DESI XS TQ-Absolute tandem quadrupole mass spectrometer

Imaging Mass Spectrometry (IMS) basic concept

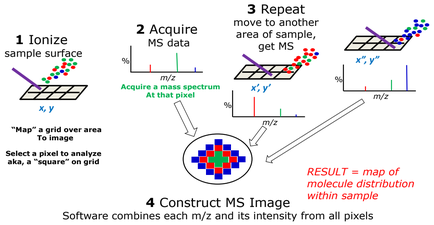

Figure 3. Illustration of how to do Imaging Mass Spectrometry

RESULTS

Methapyrilene Detection Limits – HRMS vs. TQ-Absolute

- Dilution series of methaypyrilene standard spotted at 1 µL on DESI well plate (Figure 4) imaged at 5 MS per s (5 Hz)
- Sum of 3 methapyrilene MRM from TQ-Absolute > 10x lower detection limit vs. MS or MSMS imaging on Qtof HRMS; MSMS less background than MS only detection of m/z 262.14 precursor

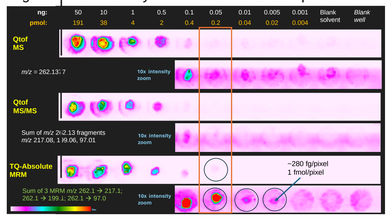

Figure 4. Dilution series of methapyrilene standard analyzed with DESI full scan MS and MSMS with Xevo G3 Qtof vs. targeted MRM detection with TQ-Absolute tandem quadrupole MS

Lipid Imaging MS of vehicle vs. dosed rat liver tissue

- As seen in Figures 5 to 8, the lipid distributions in rat liver tissue have been mapped using MRM acquisition for the transitions shown for the undosed vehicle (control) vs. 50 and 150 mg/kg doses
- These images illustrate changes in the lipid spatial distributions at day 6 as a function of dosage vs. the vehicle (control, no drug); liver morphology.

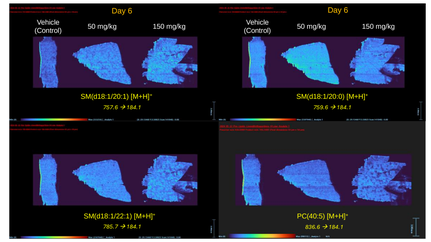

Figure 5. Lipid distribution at 50 µm pixel in vehicle (control); 50/mg/kg, and 150 mg/kg methapyrilene dosed rat liver tissue sections at day 6 of administration (Positive Ion Mode)

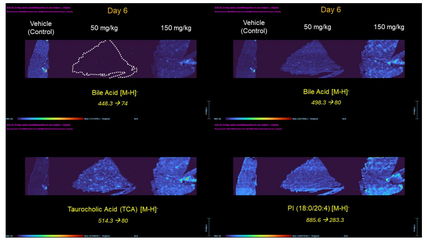

Figure 6. Bile acid distributions at 50 µm pixel in vehicle (control); 50/mg/kg, and 150 mg/kg methapyrilene dosed rat liver tissue sections at day 6 of administration (Negative Ion Mode)

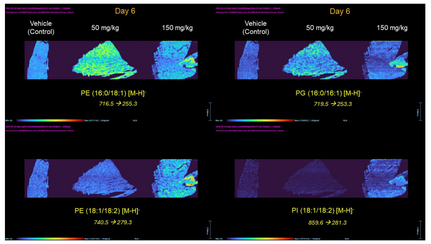

Figure 7. Lipid distributions at 50 µm pixel in vehicle (control); 50/mg/kg, and 150 mg/kg methapyrilene dosed rat liver tissue sections at day 6 of administration (Negative Ion Mode)

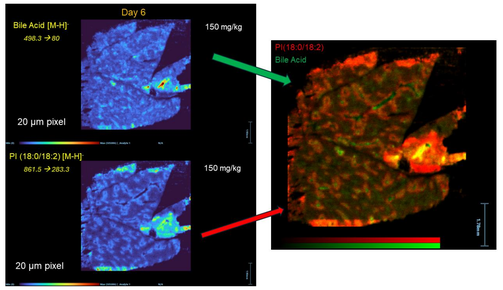

Figure 8. Overlay of 20 µm pixel-size differential distributions of bile acids vs. PI lipids in 150 mg/kg methapyrilene dosed rat liver tissue sections at day 6 of administration (Negative Ion Mode)

DISCUSSION

- Multivariate statistical analyses of the previous LC-MS and LC-MSMS data from rat plasma comparing the vehicle (control), 50 mg/kg, and 150 mg/kg doses showed a select lipids were up regulated in the dosed rats; including sphingomyelins (SM), phosphotidylcholines (PC), and bile acids
- Those lipids were identified using lipid class head group fragment and acyl chain lengths from the MSMS transitions, subsequently used in targeted DESI Imaging MS to link the observed spatial distributions to specifically identified lipids
- Bile acids, phosphotidylinositols (PI), phosphotidylglyerols (PG), and phosphotidylethanolaminds (PE) in negative ion mode show the greatest change vs. methapyrilene dose after day 6; with much smaller changes in the sphingomyelins (SM) in positive ion mode

CONCLUSIONS

- **Targeted DESI imaging MS on a tandem quadrupole (MS) using MRM acquisition provided higher sensitivity than a Qtof high-resolution MS in detecting the drug methapyrilene**
- **Targeted Imaging MS also offered greater selectivity by mapping the spatial distribution of a unique product ion from a known precursor, so that the ID of the drug and lipids were known**
- **Applying targeted DESI imaging MS to methapyrilene drug-induced liver injury (DILI) provided complementary spatially resolved omics to UHPLC-MSMS lipidomics**

References

1. Graichen ME, Neptun DA, Dent JG, Popp JA, and Leonard TB. "Effects of methapyrilene on rat hepatic xenobiotic metabolizing enzymes and liver morphology." Fundam Appl Toxicol 1985; 5:165–174.
2. Dosing animal study conducted, and tissues prepared by EVOTEC (France) SAS; Campus Curie, Toulouse Cedex, France.

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The authors declare no competing financial interest.