DRUG AND METABOLITE LOCALIZATION BY IMAGING MASS SPECTROMETRY UTILIZING MATIX-ASSISTED LASER DESORPTION IONIZATION (MALDI) ON A QUATRUPOLE TIME-OF-FLIGHT MASS SPECTROMETER IN THE ANALYSIS OF DIAZEPAM AND ITS METABOLITE IN RAT BRAIN TISSUE

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ABSTRACT

Matrix-assisted laser desorption ionization (MALDI) is a sensitive solid sampling and soft ionization technique with extensive applications for the analysis of both large and small molecules. Over the years there has been increased interest in the analysis of spatial distribution of small molecules in tissues for drug discovery applications, disease diagnosis or biomarker discovery. Especially, the localization or spatial identification of dosed drug and its metabolites may be critical to understand the mechanism of target-organ toxicity and its relevance to clinical safety. The MALDI-mass spectrometry (MS) signal may be obtained directly from tissue sections[1]. The resulting three-dimensional image may become very useful for the investigation of localization of dosed drug and its metabolites in tissue, which would help to understand the distribution of the drug and metabolites in different tissues. The combination of MALDI/quadrupole time-of flight (QTOF) MS provides excellent sensitivity and selectivity for tissue imaging experiments, which can be very useful for the study of drug candidates and their metabolites distributions earlier on in drug discovery. For this work, after diazepam was intravenously administered to rats at a doses of 10,30 and 100mg/kg, a study using rat brain tissue containing diazepam and its metabolite was analyzed. The tissue was sliced using a Cryostat (Leica CM-3050, Leica Microsystems Inc.) at a tissue thickness of 10 micro-m. The MALDI matrix used for this study was α-Cyano-4-hydroxycinnamic acid (CHCA) which was dissolved in 0.1% trifluoroacetic acid with 50% acetonitrile. The matrix mixture was then deposited on a rat brain tissue slice using a TLC sprayer. All the data was acquired in positive ion mode on a Waters MALDI QTOF Premier Mass Spectrometer. The instrument was operated in MS/MS mode for both the parent drug and the metabolite adding an extra degree of selectivity. The data collected were transferred into BioMap (Novartis) for imaging conversion. The results obtained showed clear localization and semi-quantitative assessment of the parent drug and its metabolite in the rat brain tissue. In conclusion MALDI/QTOF MS proved sensitive, specific, and highly amenable to the image analysis of traditional small molecule drug candidates directly in tissues.

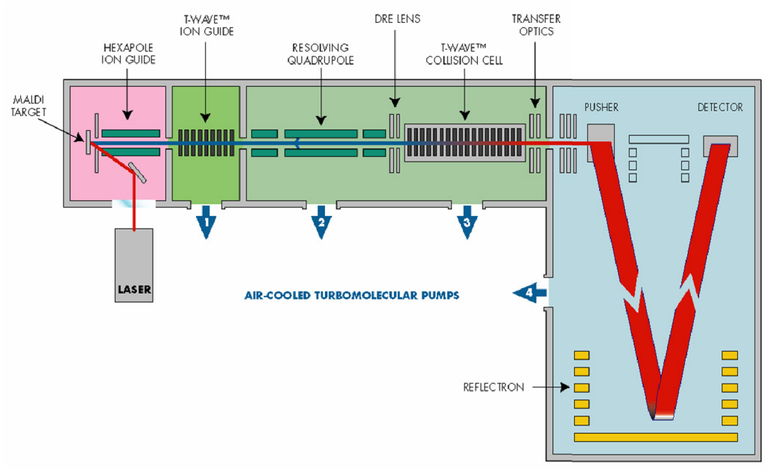

Figure 1. Schematic of Maldi Q-Tof Premier

METHODS

Preparation of Brain Slice:

- Diazepam was intravenously administered to 7-week-old male Sprague-Dawley rats at 3 doses of 10,30 and 100 mg/kg.
- Five minutes after administration, the rats were sacrificed by exsanguinations from the abdominal aorta with a syringe treated with heparin under anesthesia with ether.
- The isolated brain from the control and dosed rats were frozen by dry ice and embedded in the Tissue-Tek O.C.T. compounds (Sakura Finetek Japan, Tokyo). The tissue was sliced using a Cryostat (Leica CM-3050, Leica Microsystems Inc., Bannockburn) at a tissue thickness of 10 micro-m at -18°C.
- The slices were mounted onto microscope plates.

Matrix Conditions:

- The matrix used was α-Cyano-4-hydroxycinnamic acid (CHCA) at 15mg/mL in 50/50 Acetonitrile/Water (0.1% TFA).
- A TLC sprayer was used to deposit the matrix and the tissue slides were sprayed 15 times.

MS Conditions:

- Mass Spectrometer: Maldi Q-Tof Premier™ (Waters, Milford USA)
- Mass range: 50-300 m/z
- Laser type: Fast Nd: YAG Laser
- Laser spot size: 250 µm
- Repetition rate: 200 Hz
- Spatial resolution: 200 µm X 200 µm
- Collision energy: 25 eV
- Gas and Collision gas pressure: Argon with a pressure of 5.30 e^{-3} mBar
- The experiments were carried out in MS/MS mode using EDC (enhanced duty cycle) for enhanced sensitivity (Figure 2)
- The use of EDC allowed the selected daughter ions at m/z 154 (Diazepam) and m/z 140 (Desmethyl Diazepam) to be synchronized with the pusher allowing an increased duty cycle increasing the signal up to x5 more than using standard MS/MS conditions

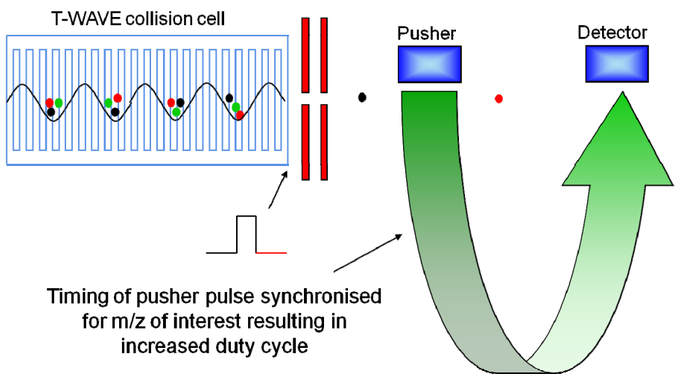

Figure 2. EDC set-up used to obtain enhanced sensitivity in MS/MS mode

RESULTS

- The concentrations of diazepam and desmethyl-diazepam in the rat brain were quantitated by LC-MS/MS, which was to 10% (w/v) homogenate with saline using by a Polytron (Kinematica, Switzerland)

Dose (mg/kg)	Diazepam	Desmethyl Diazepam
10	20.2 ug/g	0.166 ug/g
30	50.5 ug/g	0.595 ug/g
100	266 ug/g	0.080 ug/g

- From this table we can have an estimate of the concentrations of Diazepam and the metabolite in the different dosing experiments
- As it can see from the table above, the concentration of the metabolite, Desmethyl Diazepam on the 10mg/kg and 30mg/kg dose was approximately 1/100 of diazepam
- Below is a typical MS/MS spectra for the Diazepam and the Desmethyl Diazepam metabolite. The ions selected for the tissue image are m/z 154 (Diazepam) and m/z 140 (Desmethyl Diazepam) as shown in Figure 3

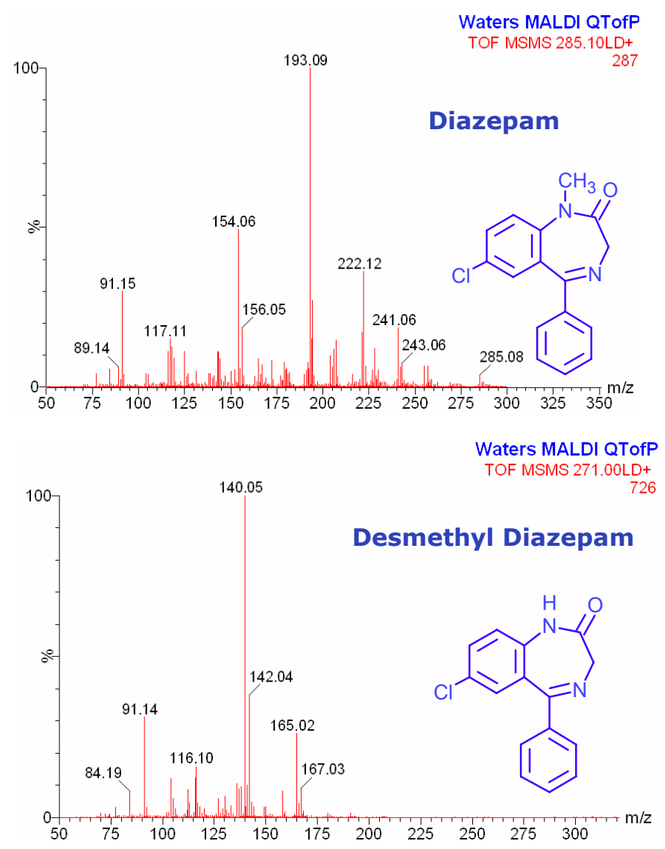

Figure 3. MS/MS spectra for Diazepam and Desmethyl Diazepam

- The 100mg/Kg tissue imaging (Figure 4) below showed an area of increased concentration in the top right corner of the brain with sparse spots of the drug in the tissue
- This last finding was confirmed by the autopsy of the animal as this high dosed lead to the death of the animal and there was cerebral hemorrhage leading to the burst of blood vessels and leakage of the drug in the brain

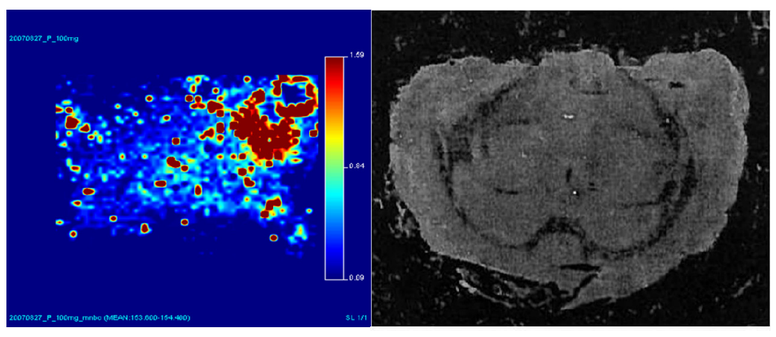

Figure 4. Rat brain tissue image for the 100mg/Kg dose corresponding to Diazepam

- Then, the 30 mg/kg dose was analyzed and it was observed that the drug has now migrated to a different part of the brain, the lower right part with no sign or drug leakage though the vessels as the animal showed no sign of acute toxicity (Figure 5)

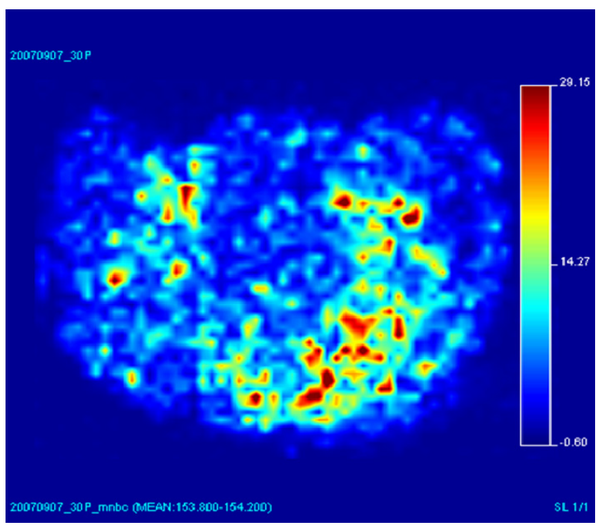

Figure 5. Rat brain tissue image for the 30mg/Kg dose corresponding to Diazepam

- The metabolite for the 30mg/Kg dose was analyzed and the tissue image was generated (Figure 6)
- The localization of the metabolite was not as confined to one region as the drug but more delocalized throughout the entire tissue

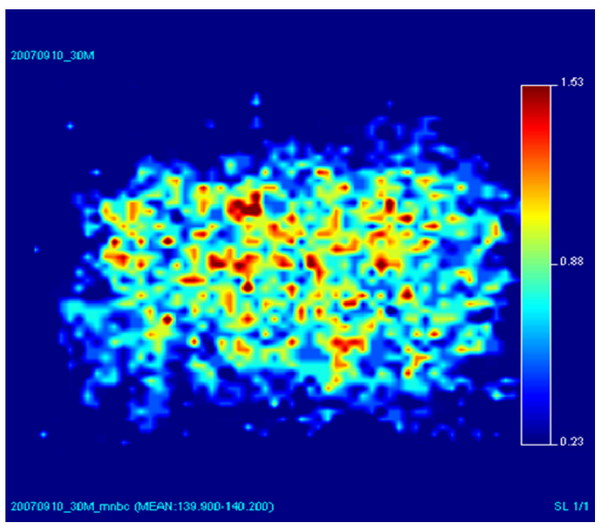

Figure 6. Rat brain tissue image for the 30mg/Kg dose corresponding to the Desmethyl Diazepam metabolite

- The 10mg/Kg dose was analyzed and the localization of the parent drug was in the upper central part of the brain (Figure 7)

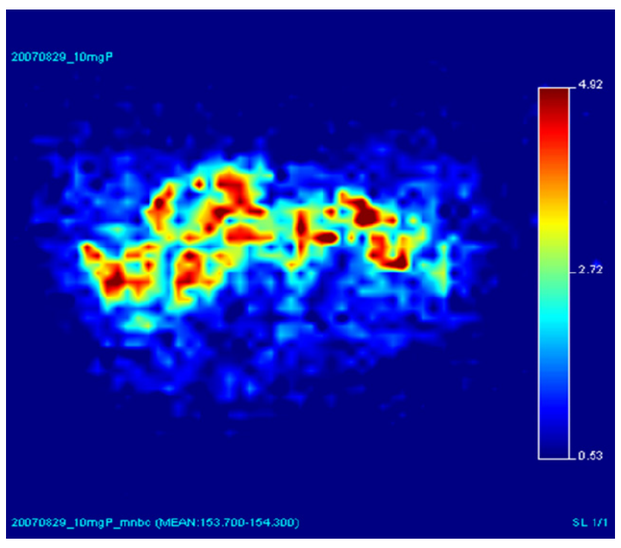

Figure 7. Rat brain tissue image for the 10mg/Kg dose corresponding to Diazepam

- The corresponding 10mg/Kg dose was analyzed for the metabolite and it was also detected as in the case of the 30mg/Kg the metabolite was delocalized throughout the tissue but in this case at a much lower concentration (Figure 8)

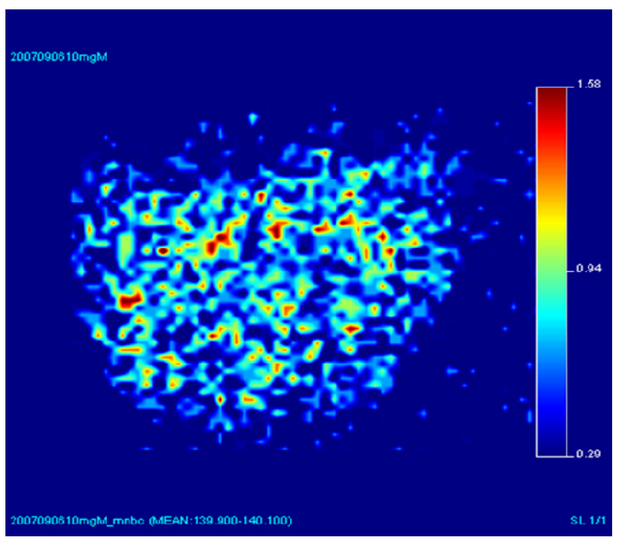

Figure 8. Rat brain tissue image for the 10mg/Kg dose corresponding to the Desmethyl Diazepam metabolite

CONCLUSION

- **Maldi Imaging is a powerful technique to visualize the localization of drug and metabolite in biological tissues**
- **This particular approach using EDC provided enough sensitivity to monitor the drug and metabolite at low levels**

References

1. [1]Stoeckli, M.: Chaurand, P.; Hallahan, D.; Caprioli, R. *Nature Med. 2001, 7(4), 493-6*

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