INCREASED SELECTIVITY FOR THE ION MAPPING OF SYNTHETIC ANTAGONISTS OF DOPAMINE RECEPTORS IN RAT BRAIN SPECIMENS USING MS/MS ON A MALDI Q-TOF MASS SPECTROMETER

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OVERVIEW

- MALDI imaging of small molecules using an orthogonal Tof MS/MS methodology
- High specificity for target compound
  - Mass accuracy
  - MS/MS
- Use of Enhanced Duty Cycle (EDC) improves sensitivity

INTRODUCTION

Imaging the spatial distribution of molecules in tissue using MALDI mass spectrometers is a rapidly developing technique.[1] Figure 1 shows typical MALDI imaging data obtained for endogenous biomolecules. The acquisition of accurate mass data in this type of experiment can be hampered in axial MALDI Tof systems. Even small changes in sample position and laser energy in the source region of this type of mass spectrometer affect mass measurement accuracy and mass spectral resolution. The use of an orthogonal Tof MALDI mass spectrometer circumvents these problems by decoupling the MALDI source from the mass analyser.
Raclopride is retained in tissue as a result of binding to a neurotransmitter receptor. Raclopride is a selective dopamine antagonist with a high affinity for dopamine type 2 (D 2) receptors. After intravenous administration, raclopride localises in the basal ganglia, a region with a high density of dopamine receptors. PET images show stereoselective concentration of raclopride in the region of the putamen relative to the rest of the brain[2].
Data obtained on the spatial distribution of Raclopride and endogenous adenosine monophosphate are shown here.

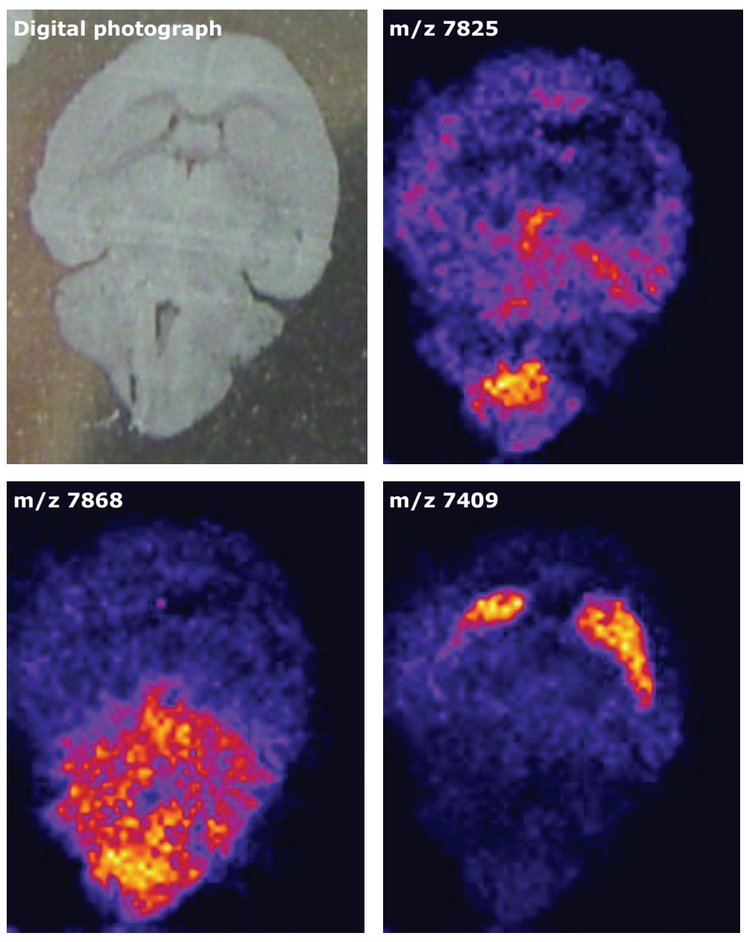

Figure 1: Typical examples of MALDI Imaging results for large molecule analysis. Data acquired on a MALDI micro MX mass spectrometer

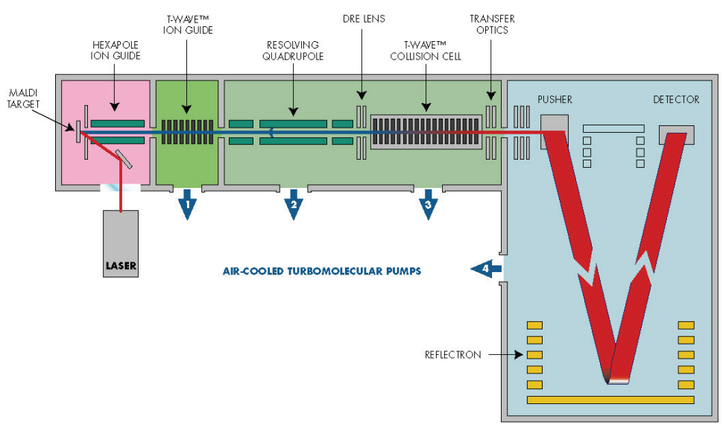

Figure 2: Schematic of the MALDI Q-Tof Premier mass spectrometer used for small molecule imaging work

METHODS

Imaging Sample preparation
Matrix solution was sprayed using a TLC sprayer (Sigma). The matrix used was sinapinic acid (SA) dissolved at a concentration of 20 mg ml^{-1} in 4:1 acetonitrile:aqueous TFA (0.1 % v/v). Matrix was sprayed in layers onto the sample allowing one application to dry before the next application was made. For the results shown here 17 layers were deposited.

MS method
All MS data were acquired on a MALDI Q-Tof Premier (Waters, Milford MA). A schematic of the instrument is shown in Figure 2. The instrument was calibrated over the m/z range 100-900 using a mixture of PEG standards. For increased specificity an MS/MS experiment was used. The MS/MS conditions were optimised for Raclopride, a precursor mass of 347 and collision energy of 25 V were used. A typical MS/MS spectrum obtained from Raclopride standard is shown in Figure 3. In order to increase sensitivity the instrument was operated in enhanced duty cycle mode (EDC) (see Figure 4). This mode enhances the signal intensity across a section of the m/z range (see Figure 5). Ten repeat of MS/MS acquisitions of Raclopride with and without EDC showed an enhancement of a factor of six when EDC was used. For all subsequent experiments EDC (112 Da) was used.
In the fragment ion spectrum of Raclopride, the signal at 112 Da is the most intense ion; and the intensity of the 112 peak was used to produce images of the Raclopride distribution in tissue.

Rastering
Data were acquired in a raster pattern to give a final image consisting of 50 x 50 MS/MS spectra/pixels. Pixel spacing was 200 µm. Hence, a 1 cm x 1 cm area was imaged.

Data processing
Data were processed and imported into BioMap software (Novartis, Basel CH) using a prototype file conversion utility. All data evaluation was carried out using BioMap.

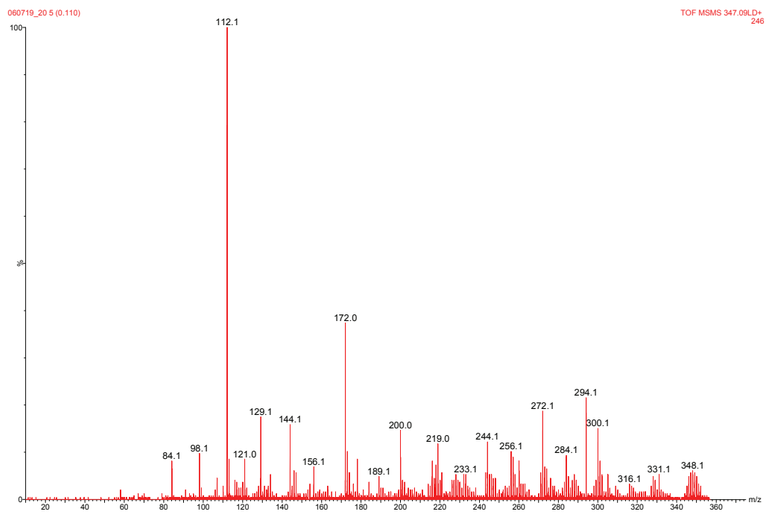

Figure 3: MS/MS spectrum of 20 ng/ml of Raclopride from stainless steel target, in CHCA matrix

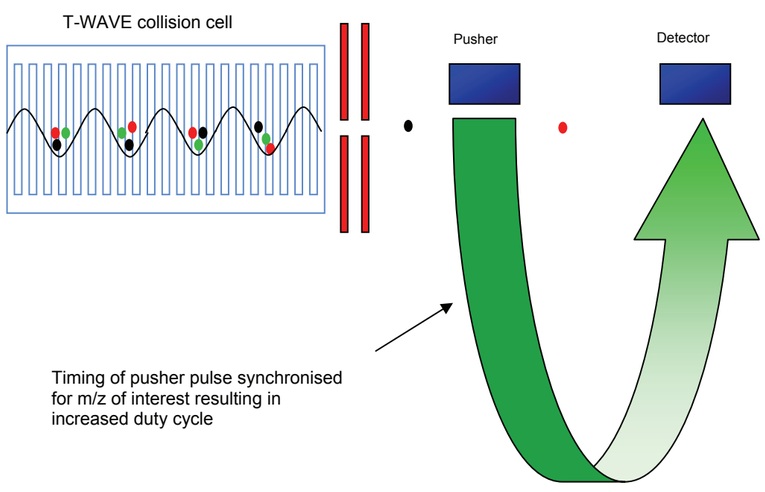

Figure 4: Principle of enhanced duty cycle operation of an orthogonal Tof mass spectrometer

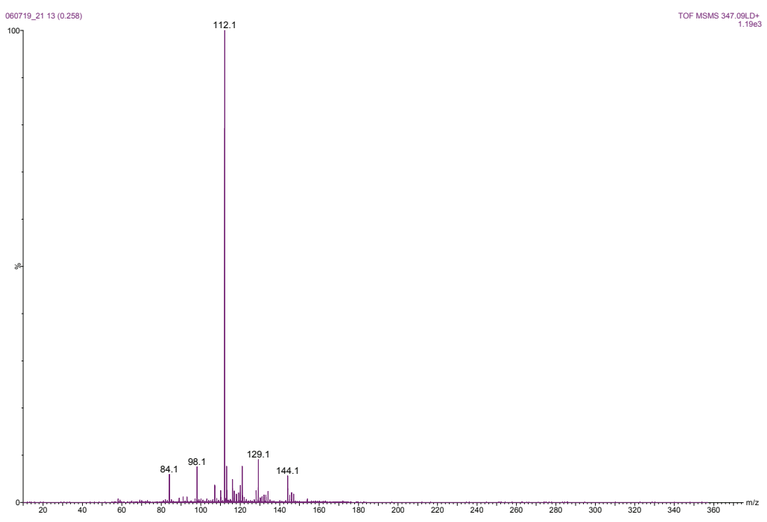

Figure 5: MS/MS spectrum of 20 ng/ml of Raclopride from stainless steel target, in CHCA matrix acquired in EDC mode.

RESULTS

It was not possible to distinguish the 347 Da peak for protonated Raclopride when acquiring from tissue of animals treated with Raclopride at a dose of 2.5 mg/kg (see Figure 6). This was due to interference from other species present in the same mass range.

An MS/MS experiment on the same sample clearly shows fragment ions associated with Raclopride, illustrating the usefulness of monitoring fragment ions when measuring drug molecules in a complex biological matrix (see Figure 7). The 112 Da fragment ion of Raclopride can clearly be seen. It was used to generate an ion intensity map (see Figure 9). An intensity distribution of the drug is shown.

As well as Raclopride, adenosine monophosphate (AMP) a species of similar mass (348 Da) present in the tissue was also fragmented. This leads to a more complex fragment ion spectrum compared to the standard, as can be seen in Figure 7. The fragment peak at 136 Da, corresponding to the base adenine fragment, is typical of AMP. The distribution of this endogenous species was also evaluated using the intensity of this fragment. In Figure 10, it can clearly be seen how AMP is distributed.

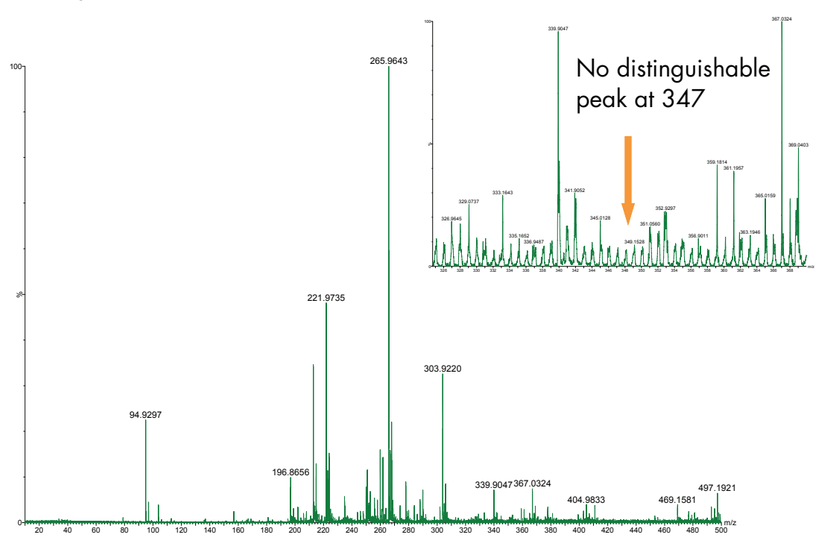

Figure 6: MS spectrum acquired from brain tissue of animals dosed at 2.5 mg/kg with Raclopride. No MS signal was observed at 347, the $(m+H)^+$ of Raclopride.

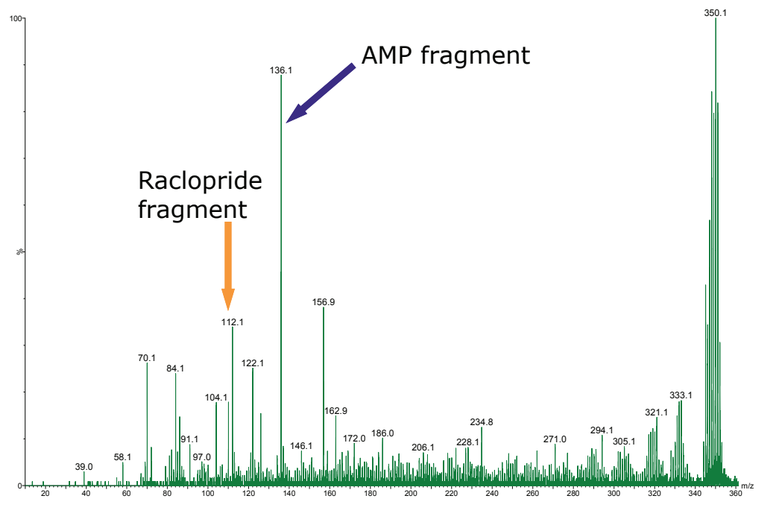

Figure 7: MS/MS spectrum acquired from brain tissue of animals dosed at 2.5 mg/kg with Raclopride. The112 Da fragment ion is indicative of Raclopride and the 136 Da ion is a fragment ion from AMP.

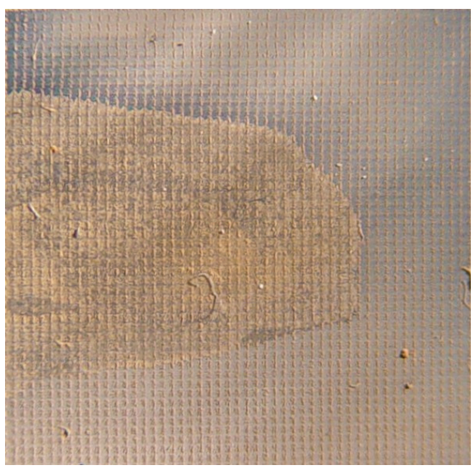

Figure 8: Photograph of rat brain sample taken after MALDI imaging analysis. Laser burn pattern can clearly be seen.

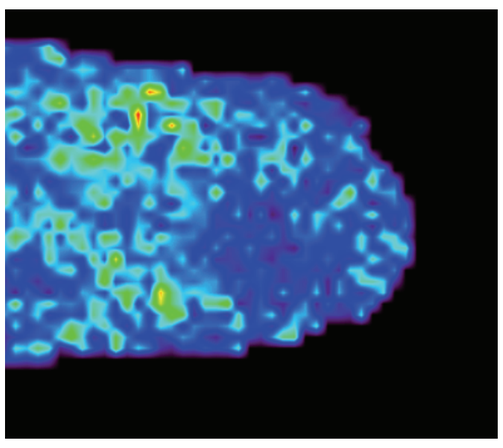

Figure 9: MALDI image of the distribution of the 112 Da fragment of Raclopride

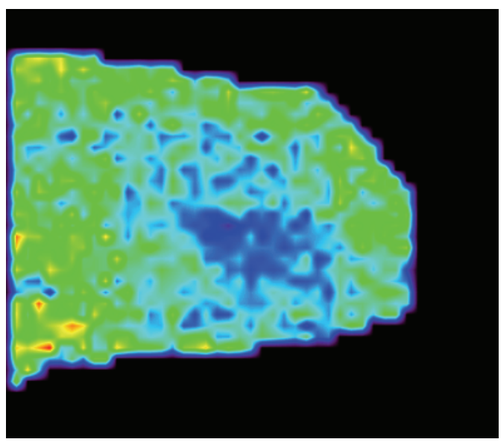

Figure 10: MALDI image of the distribution of the 136 Da fragment of AMP

CONCLUSION

- Using fragment ion information small molecule MALDI imaging in a complex biological matrix was possible.
- Sensitivity was enhanced for specific fragment ions using EDC.
- Data were obtained from tissue from animals that had been dosed at 2.5 mg/kg.

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