



Application Note # MT-87

MALDI Tissue Imaging of Drugs with the ultraflex MALDI TOF/TOF

MALDI-TOF/TOF analysis can be used to detect and image drug molecules and their metabolites in tissue.

In addition to other Mass Spectrometry (MS) methodologies used for determining the localization of small molecules in tissue, MALDI-TOF/TOF can also be used to detect drugs and their metabolites in tissue without the burden of matrix interference.

Introduction

MALDI-TOF MS has previously been used for imaging of large peptides and proteins embedded in tissue sections. This imaging technique is not limited to larger molecules, but can also be used to measure the presence of small molecules directly from tissue. However, for small molecule imaging the method of choice is a selected reaction monitoring approach in MS/MS mode. The benefits of parent ion isolation and subsequent detection of fragment ions by MALDI-TOF/TOF can alleviate matrix interference, and provide enhanced sensitivity due to the specificity of parent ion isolation. Additionally, since the smartbeam™ laser diameter is five to ten times smaller than traditional lasers (1), the ultraflex II™ MALDI-TOF/TOF provides superior high resolution

images of small molecules. It should be noted that the experimental conditions described for this Olanzapine (OLZ) study may not equally apply to other drug molecules. It has been shown (2) that the successful detection of different molecules of interest can vary based upon matrix selection and solvents used to extract analytes from tissue.

The OLZ drug dosing of model animals such as mice, dogs, and monkeys has been previously studied to determine the metabolomic pathways of the drug, by analyzing the total radioactivity in urine, fecal, plasma and blood samples (3). The analytical benefits of a mass spectrometry based approach via direct tissue analysis, provides not only new pharmacological insights about the localization of the dosed drug and its various metabolites in tissue, but can also provide these details within a time course study.

Experimental

Similar experiments for OLZ detection in tissue has been previously published by Khatib-Shahidi et al (4). All OLZ dosed tissues were prepared as described in this previous publication, except for the matrix which has been changed to HCCA due to the increased sensitivity compared to DHB (data not included).

Materials

The MALDI matrix HCCA was purchased from Sigma Chemical Co. (St. Louis, MO). The drug Olanzapine (OLZ) was produced by Lilly Research Laboratories (Eli Lilly and Co., Indianapolis, IN).

Tissue Sectioning

The whole body sample was a 10 week old male Fischer 344 rat orally dosed with 8mg/kg of the OLZ drug. The rat was euthanized after two hours by isoflurane anesthesia followed by exsanguination via cardiac puncture to remove excess blood.

The whole body rat was frozen in hexane/ dry ice and stored at -20 °C, then frozen in an ice block. 20 um thick sagittal tissue sections were collected on acetate film tape (3M, St. Paul, MN) using a cryomacrocut (Leica CM 3600, K100 Microsystems Inc., Bannockburn, IL) at -20 °C. For this study, a frozen section of the abdominal region of the rat was transferred to a MALDI target in preparation for MALDI-TOF/TOF analysis.

Matrix Solution

HCCA matrix was dissolved in 70% Methanol at a concentration of 20mg/mL. The matrix was spray coated onto the tissue using a TLC sprayer (Fisher Scientific TLC Sprayer K4225300019). Repetitive coatings were lightly sprayed onto the tissue to solvate the drug within the tissue,

taking care not to over hydrate the sample which can lead to delocalization of the drug. Upon completion of matrix deposition, the drug was incorporated into the crystallization matrix layer. In addition to performing the tissue imaging experiment on the rat, a serial dilution study of the drug was carried out to determine the limits of detection by MALDI-TOF/TOF analysis for this particular drug.

Instrumentation

The Imaging experiment was performed on an ultraflex™ II MALDI-TOF/TOF MS (Bruker Daltonics, Inc., Billerica, MA) equipped with an all solid state smartbeam™ laser operating at 200Hz. The imaging experiment was run in TOF/TOF mode while isolating the parent mass of the drug which has a molecular weight of 313 amu. Laser Induced dissociation (LID) was used to fragment the drug molecule. The image was acquired and processed using flexImaging™ v. 2.0 (Figure 1). Data was collected with a 400µm pixel density. Four hundred shots were summed at each of the 6,200 pixels, to generate the molecular images.

Results and Discussion

Olanzapine (brand name Zyprexa) is a drug of the thienobenzodiazepine class and is generally used to treat mood disorders such as schizophrenia and acute mania in bipolar patients. Pharmacokinetic studies of OLZ indicate that it is well absorbed after single dose oral administration reaching

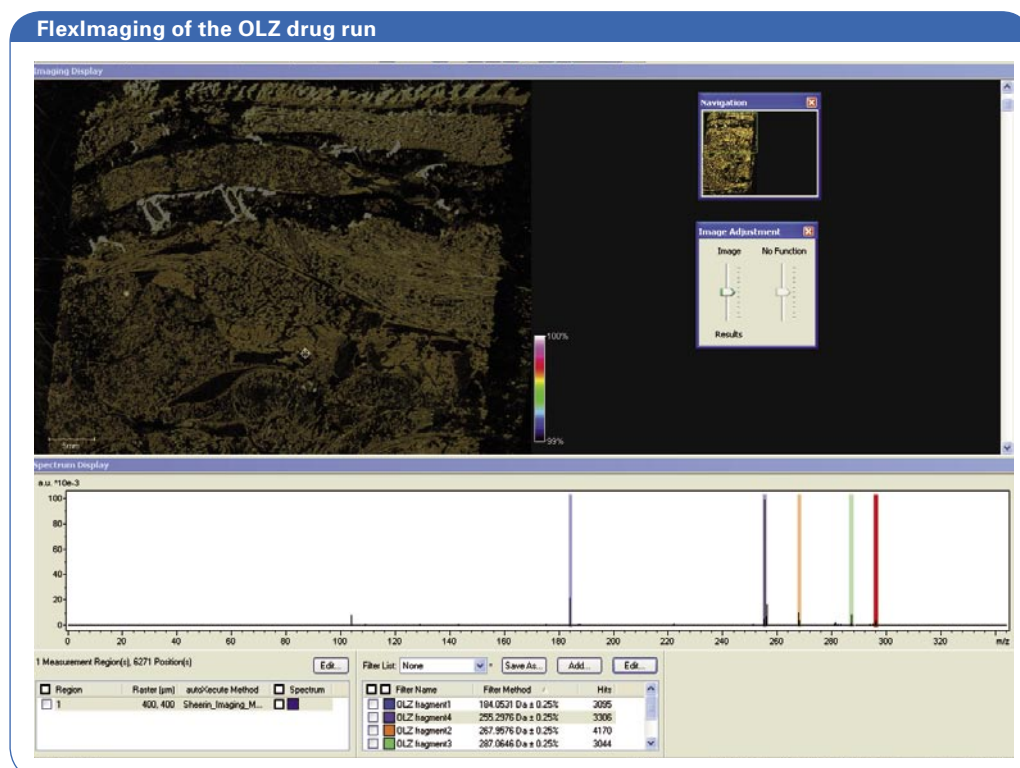


Figure 1: Graphical User Interface of flexImaging™ used to initiate an imaging experiment, as well as providing advanced tools to visualize the resulting molecular ion image.

MALDI TOF-TOF Spectra of OLZ drug from dosed tissue

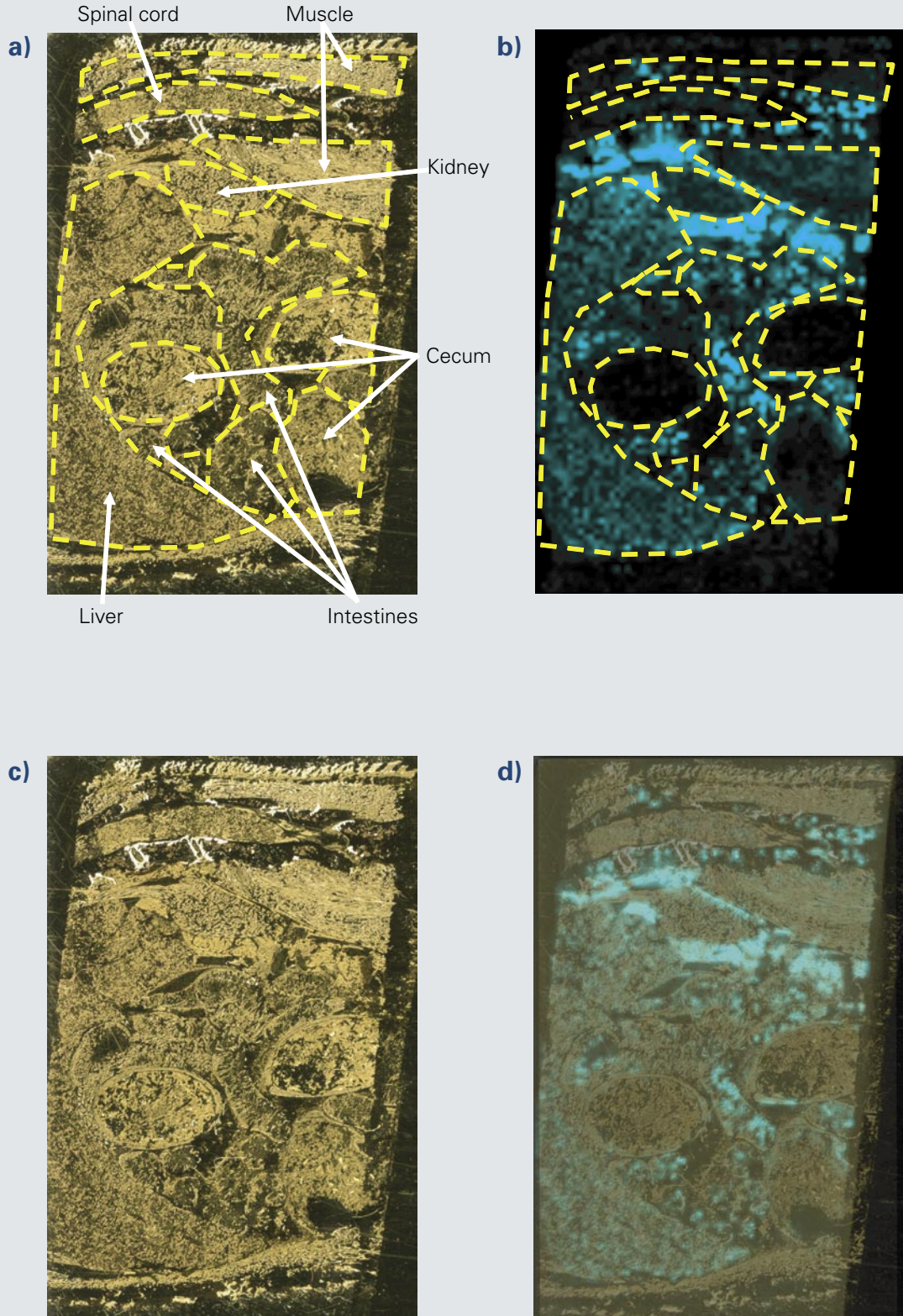


Figure 2: a &c) Optical image of a histological stained 20µm thick section. b) The m/z 256 fragment ion image has been generated indicating the specific location within tissue of the parent OLZ drug by way of Selective Reaction Monitoring. d) The overlay image combines the optical image with the molecular ion image to illustrate the localization of the drug.

peak plasma concentrations, for rats, within 45 minutes. At 2 hour post dose, whole body autoradiography (WBA) data revealed that radioactivity associated with OLZ was readily distributed throughout the tissue of an entire rat. A limitation of WBA is the inability to distinguish labeled drug from the metabolite since the dosed drug cannot be discriminated from its metabolites in the tissue. This indiscrimination can be solved by MALDI TOF-TOF by designing the experiment to detect either the drug molecule fragments, or metabolite fragments. The results of this imaging experiment clearly show the localization of the OLZ drug within the tissue section. The molecular image displays a high concentration of the OLZ in the tissue surrounding the organs, which are void of the drug (Figure 2). Following the isolation of the parent ion 313 m/z, data was collected for the fragment ions. The TOF-TOF mass spectrum including the characteristic 256 m/z fragment ion, is shown (Figure 3).

Conclusion

MALDI-TOF/TOF MS offers numerous analytical advantages for mass analysis which includes high sensitivity, rapid data generation, and the ability to detect a wide range of molecular weights from large proteins to small molecules

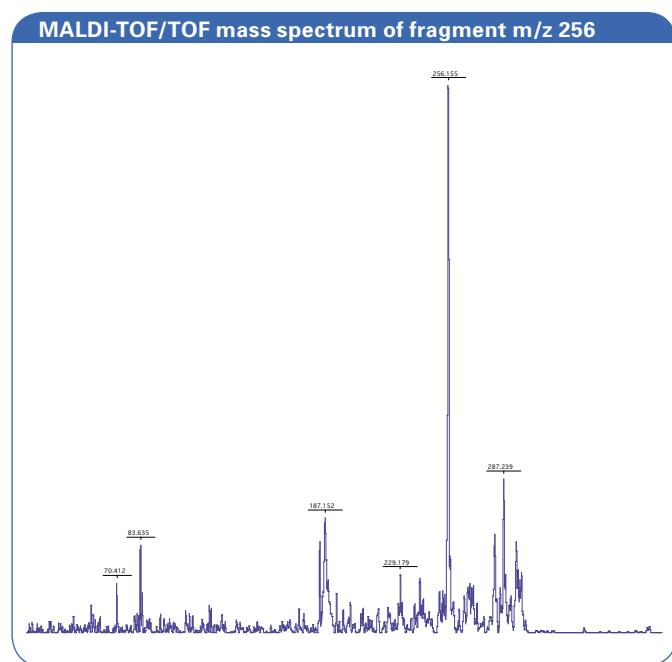


Figure 3: MALDI TOF/TOF Spectra of OLZ drug from dosed tissue.

i.e. drugs and their metabolites. For tissue imaging in MS mode, parent molecules can be measured to determine their presence and location within tissue sections, but in TOF/TOF mode, the gained selectivity and enrichment, resulting from parent ion isolation, allows the detection of small molecules. Unlike WBA, whereby the radioactive labeling is non-specific and cannot discriminate between dosed drugs and their metabolites, these results clearly illustrate the localization of dosed drugs in tissue sections. This is a key benefit which uniquely positions MALDI-TOF/TOF as a viable technique for conducting metabolomic studies by generating unambiguous molecular images of dosed tissue sections.

Keywords:

MALDI tissue imaging
Small molecules imaging
Drugs tissue-localization

Instrumentation & Software

ultraflex II TOF/TOF
flexControl
flexAnalysis
flexImaging

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