MALDI Imaging of Lipids and Drug Metabolites in Brain Tissue

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Overview

Purpose: To evaluate the advantages of high resolving power and mass accuracies of <3 ppm in the MALDI Imaging of **lipids**, **drugs**, and **cerebrosides** from rat brains with different sample preparations.

Methods: Two MALDI matrices were tested. MS MALDI Imaging was performed on a MALDI Orbitrap MS. ImageQuest software was used to visualize the distribution.

Results: The high resolving power and dynamic range of the Orbitrap analyzer allowed differentiation in the distribution of abundant lipid species from cerebrosides and drugs.

Introduction

Advances in MALDI-MS have made the direct analysis of biomolecules and drugs from tissue possible. The profiling and imaging of molecules **less than 1 kDA** for drug and lipid analysis has increased dramatically in recent years, especially for lipids in general and to a lesser extent for drugs. Due to the *in situ* nature of direct tissue analysis, purification and chromatographic separation steps are not possible. Therefore, the mass range below 1 kDA can be extremely complex and difficult to interpret due to interference from MALDI matrix ions or preparation of tissue sections. In this work, a MALDI LTQ Orbitrap XL™ mass spectrometer (MALDI Orbitrap MS) is used for imaging of ions below 1 kDA using different matrix applications, gold nanoparticle and 2,5-dihydroxy benzoic acid (2,5-DHB) for drug and lipid analysis. The high resolving power providing separation of isobaric species and mass accuracies of <3 ppm aids in analyte identification.

Methods

Instrumentation: The ion source in the MALDI Orbitrap MS has a direct beam nitrogen laser (337.7 nm), 60 Hz frequency and average energy of 250 micro joules/pulse. For MS with Orbitrap detection, ions formed in the ion source are transmitted via the C-Trap. The Orbitrap mass spectrometer is capable of up to 100K resolving power (@ *m/z* 400) with typical mass accuracies of <3 ppm. ImageQuest™ (1.0.1) software was used for mapping analyte distributions.

Sample Preparation: All the animal work in this study abides by the Guide for the Care and Use of Laboratory Animals (NIH). Male Sprague-Dawley rats (Harlan Industries, Indianapolis, IN) between 300 and 420 g were euthanized with isoflurane. Brains were quickly removed and flash-frozen in dry ice-chilled isopentane for 15 seconds at -30°C, prior to storage at –80°C. For the cocaine experiments, rats received an injection of cocaine hydrochloride (30 mg/kg free base in normal saline) and were euthanized with an overdose of isoflurane 15-25 min later. Frozen brain tissue was cut into 18 µm-thick sections in a cryostat. The tissue samples were attached to the cryostat sample stage using DI water/ice slush. Brain sections were loaded onto a MALDI sample target. Matrix was prepared by mixing 2,5-DHB (40mg/ml) and 5mM sodium acetate in 70/30 MeOH/H_2O. The 2,5-DHB matrix mix was sprayed on the tissue sections with an airbrush sprayer (Aztek A4809, The Testors Corp., Rockford, Il) or a chemical inkjet printer (Shimadzu, ChIP-1000). The gold nanoparticle deposition was used for the analysis of cerebrosides on tissue. Gold nanoparticles (nanoComposix, San Diego, CA) were spray-deposited on tissue using the airbrush sprayer. Tissue was sprayed several times to ensure complete coating of matrix.

Results:

Drug Analysis: Cocaine (benzoylmethyl ecgonine) - methyl (1*R*,2*R*,3*S*,5*S*)-3-(benzoyloxy)-8-methyl-8-azabicyclo[3.2.1] octane-2-carboxylate is a well-known stimulant of the central nervous system. Cocaine is addictive in nature affecting the mesolimbic reward pathway acting as a reuptake inhibitor to dopamine, norepinephrine and serotonin. The distribution of cocaine at *m/z (monoisotopic)* 304.154 and it's major fragment at *m/z* 182.117 are shown in Figures 1 and 2 respectively.

FIGURE 1. Distribution of cocaine at *m/z* 304.154 on rat brain tissue at 100um spatial resolution

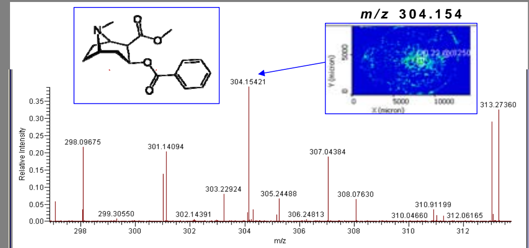
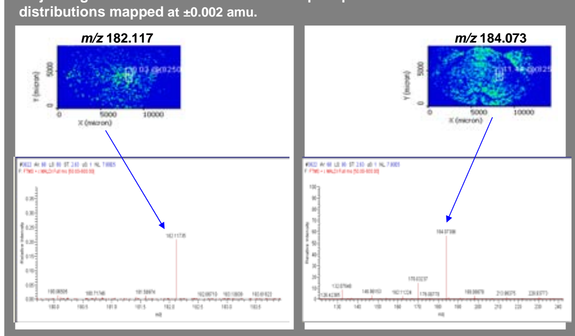


FIGURE 2. Distribution of cocaine's major fragment on rat brain tissue. All distributions mapped at ±0.002 amu.

FIGURE 3. Distribution of phosphocholine on rat brain tissue.



Since cocaine tends to fragment easily, we observed its major fragment at *m/z* 182.117 in the Full MS mode. The presence of both MH+ ion and the fragment ion for cocaine within 1ppm mass accuracy gives credence to the distribution of cocaine on the rat brain tissue.

Figure 3 shows the distribution of phosphocholine at *m/z* 184.073 which is distributed evenly in both the white and gray matter in rat brain, as it is a common fragment of all phospholipids.

The color legend in all images is: red (most intense)>green>yellow>blue=zero intensity.

Lipid Analysis on Rat Brain Tissue: All of the lipid species observed in the positive ion mode in Figure 5 were extracted from a single data file that was collected with *m/z* range 700-900. The two main phosphatidylethanolamine (PE) species that were observed are shown in Figure 4; plasmalogens and diacyl species. Figure 5 shows the images of the phospholipids; in addition to phospholipids, we observed the distribution of a cerebroside that is uncommon for MALDI data analysis resulting from Full MS spectra.

FIGURE 4. Structures of two main PE groups; plasmalogen and diacyl species that were observed in positive ion mode mass spectra from rat brain tissue.

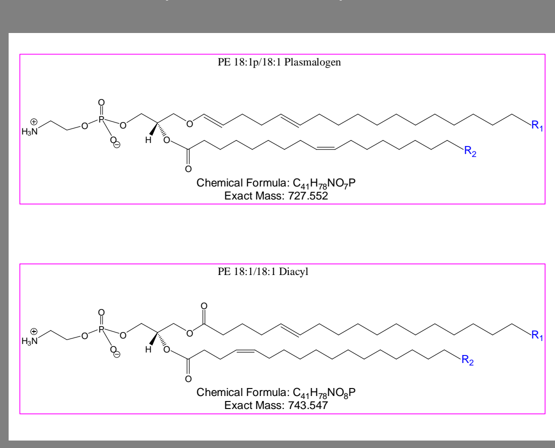
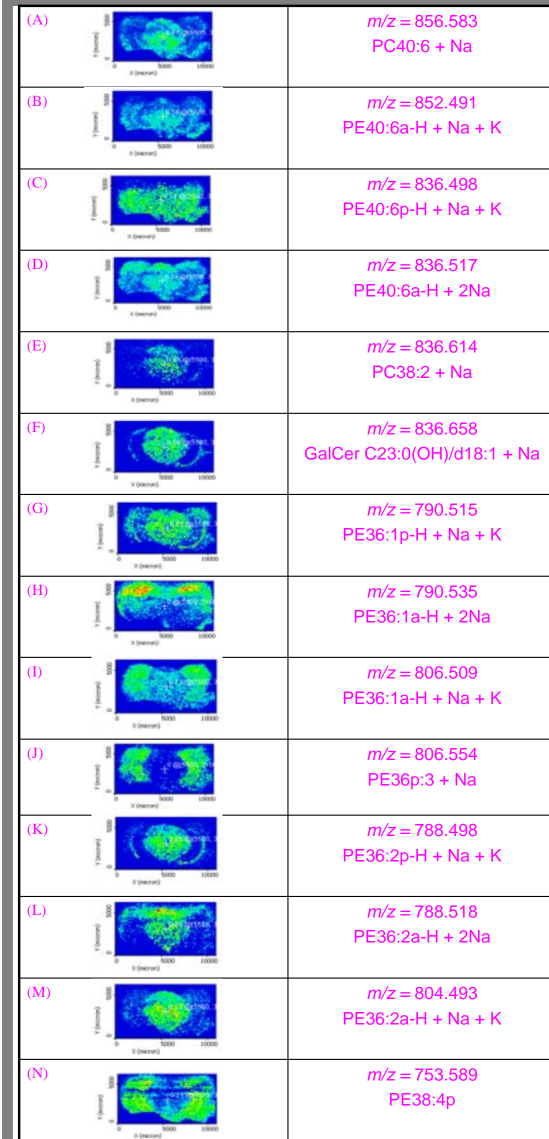


FIGURE 5. Distribution of plasmalogens (PE, p), diacyl (PE, a) species from PE group and cerebroside as shown in Figure 5F. 2,5-DHB matrix, data acquisition at 100um spatial resolution, 2D maps at ± 0.002 amu.



As shown in Figure 5, PE species, which are comprised of plasmalogens and diacyl groups, show plasmalogens preferentially distributed, with greater relative abundance, in white as compared to gray matter. The PE species comprising the diacyl groups show greater relative abundance in gray versus white matter. Some of the more abundant plasmalogens show more homogeneous distribution in white and gray matter as shown in Figures 5C and 5G, whereas the plasmalogens in Figures 5E and 5K show greater relative distribution in the white matter. Figure 5F shows the cerebroside distribution in the white matter including the myelin sheets which has been shown before. Figures 5L, 5M, 5N show diacyl species distribution in the gray matter. Since sodium acetate was used for sample preparation as an additive to DHB, the PE species show the presence of sodium and potassium adducts. Several isobaric species are distinguishable from each other; for example, Figures 5C, 5D and 5E show different lipid distribution for *m/z* 836.498, 836.517, 836.658, all these masses being within a 0.5 amu window. Similarly, Figures 5G and 5H show different lipid profiles even though their *m/z* distribution is within 0.5amu.

Detection of Lipid Species Using Gold Nanoparticles, Cerebroside analysis: The gold nanoparticles matrix application on tissue resulted in better analysis of cerebrosides compared to traditional matrices. Figure 6 shows the distribution of two cerebrosides from rat brain tissue in positive ion mode.

FIGURE 6. Distribution of cerebroside (A) GalCer C24:0(OH)/d18:1+Na at *m/z* 850.676 and (B) GalCer C24:1or 24:0/d18:1ord18:2+Na at *m/z* 832.665

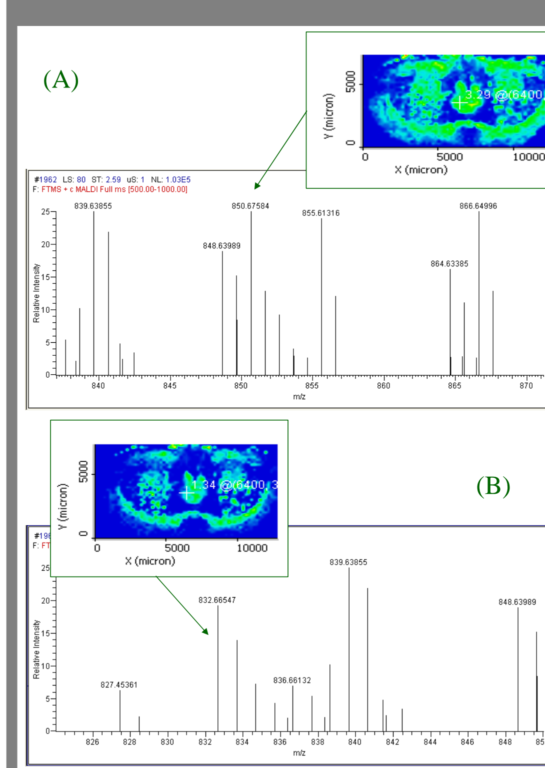
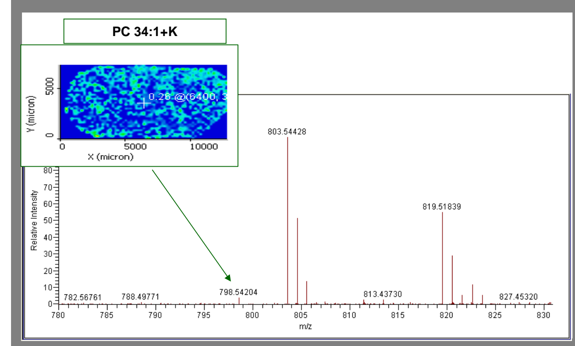


FIGURE 7. Distribution of phosphocholine; PC 34:1 + K at *m/z* 798.542 on rat brain tissue



Previous studies have used implanted gold clusters (1,2) and gold nanoparticles (3) to analyze cerebrosides in tissue; compared to traditional organic acid matrices, such as 2,5-DHB, in which PC species are the dominant signals in this lipid mass range in positive ion mode. The figures above show a non hydroxylated and hydroxylated cerebroside specie in Figures 6A and 6B, respectively, and a PC specie in Figure 7. Both cerebroside species are highly concentrated in white matter regions in the brain, while the PC species, PC 34:1, is distributed throughout the tissue section.

Conclusions

✓ MALDI-MS imaging with the Orbitrap XL instrument was successfully used in MS mode to detect cocaine and it's major metabolite, both occuring below *m/z* 1kDa, a mass region known for its complexity. This MS platform is validated for drug analysis of cocaine and its metabolites.

✓ Extraction of PC, PE and cerebroside species from a single full MS spectrum (positive ion mode, 2,5-DHB matrix) was possible due to the extraordinary resolving power and ultra high mass accuracy of the Orbitrap detector.

✓For cerebroside analysis, gold nanoparticle deposition worked best for the analysis of several different cerebrosides which do not ionize well with traditional MALDI matrices.

✓ All the data collected for this poster showed mass accuracy below 3ppm with external mass calibration at resolving power of 60,000 (m/z 400).

References

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