Chapter 12

Detection of Diagnostic Plant-Derived Psychoactive Biomarkers in Fingerprints by MALDI-SpiralTOF-Mass Spectrometry Imaging

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Abstract

One of the current challenges in forensics is establishment of a connection between an individual and substances to which they have been exposed, and which might have relevance in crime scene investigation. An example of a situation in which this arises is when an individual has handled, and is under the influence of any one of a large number of currently unscheduled plant-based mind-altering substances. In such instances as a medical emergency or a crime scene investigation, one way to establish a connection between an individual and their exposure to such substances is to take advantage of the high information content of their fingerprint. The fingerprint pattern not only establishes the identity of the individual, but also contains rarely exploited chemical information about molecules to which they have been exposed that might have a bearing on a crime. If the fingerprint image is based on the spatial distribution of diagnostic molecular markers indicative of a substance, then an individual's identity can be definitively tied to exposure to the substance. The fingerprint image derived from the spatial distribution of diagnostic molecules can be obtained by mass spectrometry imaging (MSI). Here, we demonstrate how the handling by an individual of a plant-derived psychoactive brew called ayahuasca can be established through determination, by matrix-assisted laser desorption ionization (MALDI) MSI, of ion images featuring biomarkers from the plants from which the brew is made.

Key words Mass spectrometry, MALDI, Ayahuasca, Biomarkers, Fingerprints, Forensics

1 Introduction

The United States is currently in the midst of an opioid drug abuse crisis, the acuteness of which has relegated to the back burner a number of other serious challenges associated with drug abuse. One of these is the steady rise, since 2006, in the recreational use of tryptamine-type hallucinogens [1–4]. Tryptamine derivatives, which are powerful hallucinogens that act primarily as agonists of the 5-HT_{2A} receptor, are present in numerous plant species, although new next-generation synthetic tryptamines are also being observed with increasing frequency. Aside from their potent effects

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on sensory perception, their appeal lies in part in the fact that most of them remain unscheduled, which shields distributors and users from prosecution. Even in cases where the purified compound is scheduled, naturally occurring plant materials in which such compounds occur remain legal, thereby providing users with a convenient workaround to bypass the law. A case in point is the natural occurrence of dimethyltryptamine (DMT) in plants. Although in the United States DMT as a single compound is classified as a Schedule I drug under the Controlled Substances Act, neither plants that contain it nor concoctions made from them are illegal. One such substance is ayahuasca, a beverage which has roots in Amazonian religious and ritual ceremonies, and which is gaining popularity as a recreational drug [5]. The brew is comprised of a combination of two plants—one that contains dimethyltryptamine, and the other, a monoamine oxidase inhibitor (MAOI). When DMT is introduced by the oral route, it succumbs to oxidative deamination by monoamine oxidase to yield the non-psychoactive indole acetic acid. However, DMT inactivation can be circumvented (thus enabling it to effectively act as a hallucinogen) if an MAOI is also introduced. Plant-derived harmala alkaloids such as harmine and harmaline are well-known MAOIs, and plants that contain these compounds, such as *Banisteriopsis caapi* and *Peganum harmala*, are usually paired with a DMT-containing plant such as *Mimosa hostilis* or *Psychotria viridis*, to create the ayahuasca brew [6, 7]. One of the challenges of addressing the abuse of ayahuasca and similar products in a forensics context is the difficulty of establishing a connection between an individual and their exposure to the "legal high" plant-based drug. Information of this type is of vital importance to law enforcement and medical professionals who may need to identify cases of abuse [8, 9]. Currently, there are no standard procedures that address the identification of ayahuasca or its use in humans, although it has been demonstrated that the constituent plants from which the brew is made can be identified by chemometric processing of direct analysis in real-time-mass spectrometry-derived chemical signatures [10].

Mass spectrometry imaging (MSI) is a powerful technique that provides spatial distribution information of detected molecules in the analyzed sample. MSI using a matrix-assisted laser desorption ionization (MALDI) source enables imaging of the distribution of high-mass proteins and peptides, as well as organic small molecules and lipids, as depicted in Fig. 1 [11–13]. Utilizing MALDI-MSI, one can obtain fingerprint ridge pattern ion images that feature not only endogenous compounds such as fatty acids, but also compounds that are indicative of exposure to various other substances and which serve to establish exposure to them. MSI can thus serve as a tool to determine whether psychoactive mixtures such as ayahuasca have been handled, as analysis of the fingerprints can report on the presence of diagnostic biomarkers associated with the plants from which they are comprised. In the case of ayahuasca, these

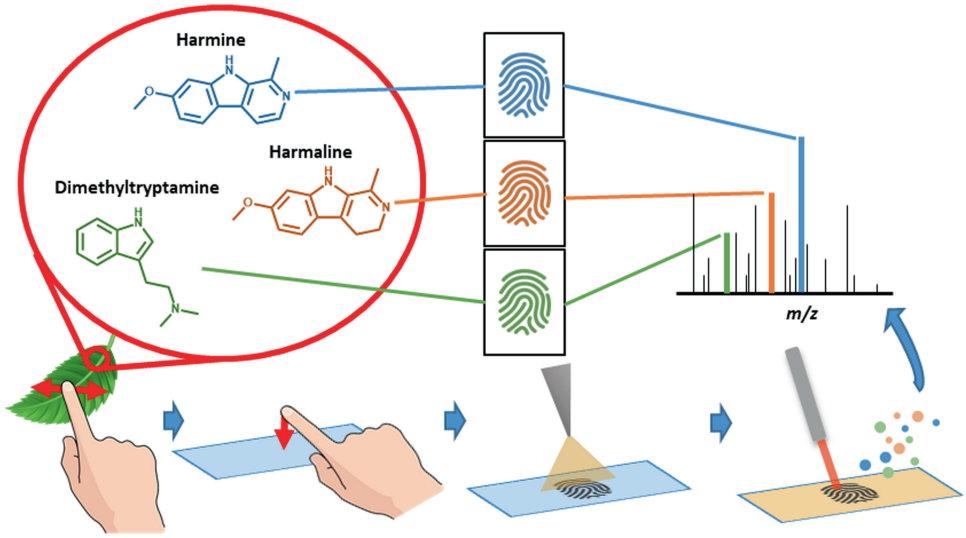

Fig. 1 Overview of MALDI-MSI analysis. A substance containing psychoactive compounds is handled, after which a fingerprint is deposited on an ITO slide. Matrix is applied, and the sample is imaged. From the resulting spectrum, *m/z* values can be chosen to generate ion images corresponding to the psychoactive components present

natural product markers include harmala alkaloids such as harmine and harmaline, as well as DMT. Described here is a method to analyze fingerprints after the handling of ayahuasca in order to demonstrate how to obtain information about exposure to psychoactive plant materials by MALDI-MSI.

2 Materials

2.1 Botanical Samples

1. *Banisteriopsis caapi* shredded vine.
2. *Mimosa hostilis* root bark.
3. *Peganum harmala* seeds.
4. *Psychotria viridis* leaves.

2.2 Solvents and Reagents

1. Matrix solution:
   (a) In a 20 mL via, add 100 mg of α-cyano-4-hydroxycinnamic acid.
   (b) Add 7 mL of acetonitrile.
   (c) Add 3 mL of DI water.
   (d) Add 3 μL of trifluoroacetic acid.
   (e) Cover mixture and sonicate until solid has dissolved fully.
   (f) Make fresh for each sample.

2. Calibrant solution:
   (a) In a 2 mL Eppendorf tube, add 10 mg of polyethylene glycol (PEG) 600.
   (b) Add 1 mg of sodium iodide.
   (c) Add 1 mL of methanol.
   (d) Vortex mixture until solid has dissolved fully.
   (e) Mix 1:1 with matrix solution before spotting.
3. ayahuasca brew:
   (a) Grind 6 g of *P. harmala* seeds or *B. caapi vine* in a coffee grinder or using a mortar and pestle.
   (b) Pull apart 24 g of *M. hostilis* root bark or crumble *P. viridis leaves*.
   (c) Add plant material to a 1 L round-bottom flask.
   (d) Add water until the plant material is covered.
   (e) Add approx. 2 mL of lemon juice.
   (f) Reflux for 6 h.
   (g) Strain liquid to remove solid plant material.
   (h) Boil liquid to reduce volume.

2.3 Instrumentation and Supplies

1. JMS-S3000 SpiralTOF MALDI TOF/TOF mass spectrometer (JEOL USA, Peabody, MA).
2. GREX GCK02 airbrush kit, comprised of an AC1810 compressor with a Tritium TS3 airbrush (GREX, Monterey Park, CA).
3. Indium-tin-oxide (ITO)-coated glass slides, 25 × 75 × 0.7 mm, R_s = 5–15 Ω (Delta Technologies, Loveland, CO).

3 Methods

3.1 Stepwise Procedure

1. Obtain and prepare a small amount of plant material (*see* **Note 1**).
2. Rub fingers against forehead or nose to deposit sebum-rich, "groomed" fingerprints.
3. Rub preparation of plant material between fingertips for approx. 10 s.
4. Press and hold fingertip to the conductive surface of a clean (*see* **Note 2**) ITO glass slide to deposit a fingerprint.
5. Pipette 5–10 μL of calibrant solution on slide adjacent to the fingerprint (*see* **Note 3**).
6. Apply matrix solution as shown in Fig. 2 to the surface of the slide with the airbrush in several even coats (*see* **Note 4**) and allow to dry.
7. Load the slide onto the target plate and into the MALDI-SpiralTOF instrument (*see* **Note 5**).

Fig. 2 Setup for applying matrix with an airbrush. The sprayer is held between 6 and 8 in. from the ITO slide, and many thin layers of matrix are gently applied, taking care not to disturb or damage the fingerprint

Table 1
MALDI operating conditions

Mass range	100–1000 *m/z*
Sampling interval	1 ns
Laser frequency	1 kHz

3.2 Instrument Calibration and Operation

1. Set up the instrument using the conditions listed in Table 1.
2. Calibrate the instrument using the PEG/NaI spot in the 200-600 *m/z* range.
3. Adjust the laser intensity, delay time, and detector voltage parameters to optimize resolution by manually analyzing a small section of the fingerprint until sufficient resolution of peaks of interest is achieved (*see* **Note 6**).
4. Set up 2D auto-acquisition parameters in positive spiral mode (*see* **Note 7**) and perform analysis.

3.3 Data Analysis

1. Load the raw data file in msMicroImager Extract and bin the data for ease of use; keep binning to 1 × 1 spectra to minimize loss of detail.
2. Load the binned data file in msTornado Analysis and generate a peak list at an appropriate cutoff threshold; a lower cutoff threshold will permit generation of images for less abundant *m/z* values.
3. Load the peak list in msMicroImager to view *m/z* values that will be used to generate 2D ion images.

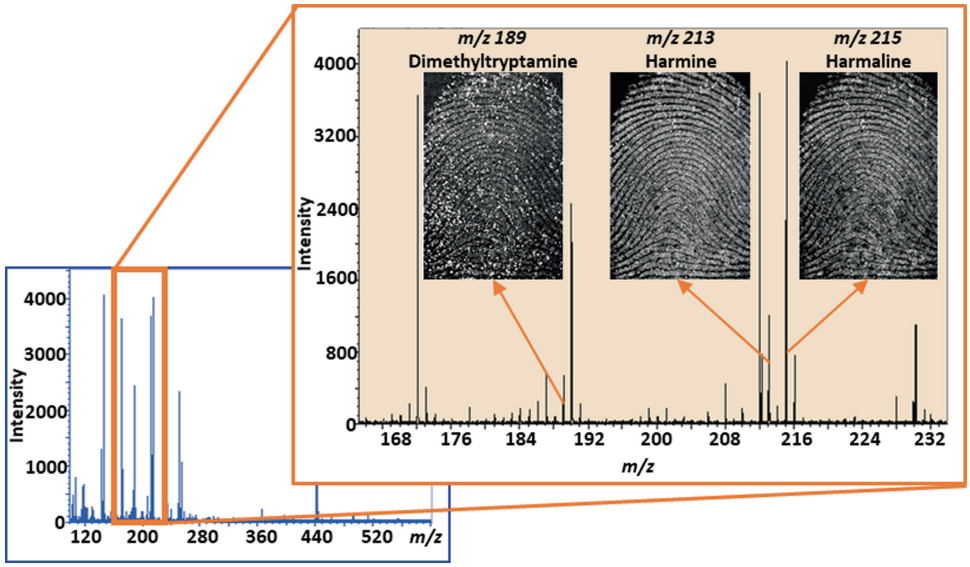

Fig. 3 MALDI spectrum and resulting ion images. Shown are results typical of a fingerprint MALDI-MSI experiment. Analysis of a fingerprint obtained after handling an ayahuasca brew results in a spectrum containing peaks of interest at nominal *m/z* values 189, 213, and 215, corresponding to dimethyltryptamine, harmine, and harmaline, respectively. Using the data analysis software, the spatial distributions of these molecules are mapped, affording the ion images displayed

4. Export mass images of picked peaks (Fig. 3).
5. View and adjust the shading scale using msMicroImager View.

4 Notes

1. Psychoactive plant materials should be prepared in a way consistent with user accounts of preparations for consumption.
2. ITO slides may contain smudges, oil, or fingerprints on them. They should be cleaned with a small amount of isopropyl alcohol prior to use.
3. The calibrant spot should be large enough to be easily located on the slide.
4. Hold the sprayer about 6 in. away from the slide, and lightly apply several coats, allowing the slide to dry between applications. This is to ensure a dense, uniform coating of crystals, which will provide better resolution.
5. It can be helpful to mark the boundary corners of the print with a marker or wax pencil just before inserting the target plate into the instrument to aid in the subsequent selection of the area to be imaged. Taking a picture can also be a useful approach to finding your sample.

6. This will vary slightly between analyses; you will need to adjust these settings each time to ensure that you achieve the best results. Increasing the detector voltage can help with the visualization of low-abundance masses, while adjusting the delay time can increase resolution of peaks of interest. Refer to previous analyses to obtain a starting point for these parameters.
7. To maintain high-detail ion images, keep the integral at or below 0.08 mm.

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