Video Article

Leaf Spray Mass Spectrometry: A Rapid Ambient Ionization Technique to Directly Assess Metabolites from Plant Tissues

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Abstract

Plants produce thousands of small molecules that are diverse in their chemical properties. Mass spectrometry (MS) is a powerful technique for analyzing plant metabolites because it provides molecular weights with high sensitivity and specificity. Leaf spray MS is an ambient ionization technique where plant tissue is used for direct chemical analysis via electrospray, eliminating chromatography from the process. This approach to sampling metabolites allows for a wide range of chemical classes to be detected simultaneously from intact plant tissues, minimizing the amount of sample preparation needed. When used with a high-resolution, accurate mass MS, leaf spray MS facilitates the rapid detection of metabolites of interest. It is also possible to collect tandem mass fragmentation data with this technique to facilitate a compound identification. The combination of accurate mass measurements and fragmentation is beneficial in confirming compound identities. The leaf spray MS technique requires only minor modifications to a nanospray ionization source and is a useful tool to further expand the capabilities of a mass spectrometer. Here, fresh leaf tissue from *Sceletium tortuosum* (Aizoaceae), a traditional medicinal plant from South Africa, is analyzed; numerous mesembrine alkaloids are detected with leaf spray MS.

Video Link

The video component of this article can be found at https://www.jove.com/video/57949/

Introduction

Plants contain a wide range of small molecules with diverse chemical properties. MS is a powerful technique for analyzing plant compounds because it can provide elemental compositions with a high sensitivity and specificity for the detection and identification of metabolites[1]. Most commonly, MS is performed on solvent-extracted samples, which are separated by chromatography prior to the MS analysis[1]. However, the use of liquid chromatography (LC) requires lengthy analysis times and is often associated with an extensive sample preparation[1]. In contrast, direct chemical analysis of intact tissues that circumvents chromatography is a very rapid technique, requiring minimal sample preparation[2]. Thus, in instances where chromatographic steps can be forgone, a direct chemical analysis can be highly advantageous.

Typical LC-MS for natural products and metabolomics research relies on lengthy bulk extractions of dried or frozen plant materials containing multiple tissues and cell types[3]. Alternatively, direct chemical analysis, such as the MS detection of metabolites from plant tissue, can isolate cell types and avoid preparation artifacts[4]. Leaf spray MS, also referred to as tissue-spray[5,6], is a direct ambient ionization MS technique, which requires essentially no sample preparation[5,7]. Leaf spray MS is closely related to paper spray MS, an ambient ionization technique with characteristics of electrospray ionization that allows for the detection of analytes that are deposited onto paper[7]. In spite of the name, leaf spray MS is applicable to various types of plant tissues, not just leaves, and has been demonstrated on fruit, seeds, roots, floral tissues, and tubers, among others[6,8,9,10,11,12]. The technique facilitates the ionization of endogenous phytochemicals directly from the plant materials into the mass spectrometer for detection[8]. Leaf spray MS can also provide information about the spatial distribution of chemicals in different tissue types in plants[13]. When leaf spray MS is compared with solvent extraction and LC-MS, the results suggest leaf spray MS allows for the rapid detection of surface metabolites from unique cell types such as trichomes[13]. **Figure 1** illustrates the leaf spray MS experimental set-up. Direct electrospray ionization occurs after only minor source modifications. A high voltage is applied to the plant tissue via a metal clamp, producing a spray of highly charged droplets forming a Taylor cone that carries the ions to the ion inlet of the MS. Electrospray ionization occurs from the natural liquid of the plant or from the solvent applied to the plant surface. A pointed tip on the tissue facilitates the electrospray and can be naturally occurring or created by cutting.

Leaf spray MS is a fast method for the qualitative and semi-quantitative analysis of intact plant tissues that have found utility for a wide variety of applications. For example, the technique has been used to detect endogenous compounds to distinguish related species, and even to evaluate changes in the same species grown under different conditions. Previous studies have shown this approach by measuring metabolites in beautyberry (*Callicarpa* L.)[12] and American ginseng *(Panax quinquefolium L.)*[6]. In the latter example, ginsenosides, amino acids, and

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oligosaccharides could be detected after wetting raw ginseng tissue. Wild and cultivated American ginseng were differentiated from tuber slices[6]. Ginseng tuber integrity was preserved succeeding leaf spray MS, which allowed for a subsequent morphological and microscopic inspection[6]. Furthermore, exogenous compounds on plant samples can also be detected. A number of pesticides (acetamiprid, diphenylamine, imazalil, linuron, and thiabendazole) have been detected on peel or pulp of fruits and vegetables[9]. While these studies and many others have shown the utility of leaf spray MS for various specific purposes, a detailed protocol has not been previously reported.

Here, the protocol description will not focus on the optimization of the method for a specific tissue or compound. Rather, the detection of mesembrine alkaloids from *Sceletium tortuosum* (L.) N.E.Br. (Aizoaceae) is used as an example to discuss the necessary optimization measures that should be taken when setting up a leaf spray MS experiment for a species, tissue, or compound(s) for the first time. *S. tortuosum* is a succulent endemic to the semi-arid Karroo region of South Africa. A traditional medicine of the San and Khoi Khoi peoples, it was used for appetite and thirst suppression as well as for its psychotropic and analgesic effects[14,15]. Currently, standardized extracts are used for the treatment of neuropsychiatric and neuropsychological disorders[16,17]. The primary compounds of interest include the alkaloid mesembrine and its derivatives, many of which are also found in related *Sceletium* species[15]. Both wild and cultivated populations of *S. tortuosum* have variable concentrations of mesembrine alkaloids, thus presenting a quality control challenge[18]. A method for the rapid detection of mesembrine alkaloids, such as leaf spray MS, may be useful in monitoring *Sceletium* products. Because previously, there had been no detailed visual experimental protocol for the leaf spray MS technique, we will illustrate the method using the example of *S. tortuosum*, and the following is described: the modification of a nanospray source, the selection and preparation of the plant tissues, the acquisition of the data, the interpretation of the results, and the optimization of the MS parameters.

Protocol

1. Modifications to Nanospray Source for Leaf Spray MS

1. Use a modified nanospray source for leaf spray MS. As no fluidic components are necessary for leaf spray MS, modify the source by removing the LC probe from the source.
2. Assemble the leaf spray MS wire that will apply the voltage to the plant tissue with the appropriate pin for plugging into the source. Solder the pin to one end of an insulated wire; solder a clamp to the opposite end of the wire.
   NOTE: The clamp (alligator clip type) may or may not have teeth. For small tissue, a clamp without teeth is preferred. An optional flex arm with a clamp may be added to the nanospray source to assist in positioning the plant tissue. Note that this protocol specifically describes how to perform leaf spray MS on a hybrid quadrupole ion trap mass analyzer MS system (see the **Table of Materials**); however, other MS systems may be altered to perform this technique[6]. Coupling leaf spray MS with a portable mass spectrometer real-time chemical analysis can be performed on-site without the need to transport the plant material to the laboratory[19,20].
3. Position an anti-static floor mat on the floor below the source to reduce the electrical discharge that may occur from the source when using high voltages.

2. Preparation of the MS System for Leaf Spray MS

1. If the system has recently been in use, allow it to cool to the touch and remove any alternative source and the sweep cone. Attach the nanospray leaf spray MS source.
2. Create a tune file with the appropriate ionization parameters set as follows: sheath, auxiliary, and sweep gas to 0; the spray voltage to 2 - 5 kV; the capillary temperature to 150 - 250 °C; and the S-lens RF level to 50. Save the tune file with the desired parameters[8,13]. Optimize the voltage and temperature for the best ionization of the tissue and compound(s) of interest.
   NOTE: Good starting points are 4 kV and 200 °C.
3. Make a method file including the leaf spray MS tune file with: positive and negative full MS; a resolution of 70,000; an AGC target of 1×10^6; a maximum IT of 200 ms; and the desired scan range *m/z*. Alternatively, use only 1 polarity.

3. Preparation of Instrument, Solvents, and Plant Tissue

NOTE: **Always** wear gloves, and do not use plant tissue that has been handled with bare hands. Otherwise, contaminant ions such as polyethylene glycol will dominate the spectra.

1. Bring the plant tissues for the analysis to the same room as the MS system to allow for a rapid sampling.
2. For plant tissue that does not have a naturally pointed tip, use a razor blade on a glass slide to cut a tapered point (**Figure 2**). Determine the amount of tissue needed for the analysis based on the instrument sensitivity, tissue type, and compound(s) of interest (*e.g.*, a young *S. tortuosum* leaf that is ~5 mm in length).
   1. Cut *S. tortuosum* leaves at 10 weeks post-germination into thin strips, each with a tapered end to form a point.
3. Use forceps to gently select the plant tissue at the end that will be clamped. Holding the tissue with forceps, carefully transfer it to the clamp.
   CAUTION: Do not touch the instrument source if the voltage is on.
4. Adjust the flexible arm and the wire with the clamp to position the tissue in line with the MS inlet so that the distance between the plant tissue and the ion inlet of the MS is 5 - 10 mm for the triple quadrupole (*e.g.*, TSQ) and linear trap quadrupole (LTQ) and 10 - 50 mm for the ion trap mass analyzer (*e.g.*, orbitrap)[8].
   1. Plug the opposite end of the wire into the source. If the first attempts produce a low signal intensity, move the plant tissue closer to the ion inlet (refer to the **Discussion** for optimization).
5. Load the method file; name the data file and set the file storage location. Then turn on the MS system by clicking **Play** and click on **Start** to begin acquiring data.

6. Apply a solvent (*e.g.*, methanol) using a pipet with a gel-loading tip to maximize the distance between the hands and the high voltage to protect the user.
   NOTE: The solvent volume required depends on the size, dryness, and texture of the tissue, typically ~2 - 20 µL. *S. tortuosum* leaves do not require any solvent to be added. Carefully apply the solvent and do not touch the instrument source when the voltage is on. Use LC-MS grade solvents and glassware that has been acid washed and is free of detergents. In some tissues, a signal may be observed without the addition of a solvent due to the natural water content of the plant tissue. However, a greater signal intensity and reduced S/N is typically achieved by applying a solvent to the tissue.
7. Acquire data as long as the signal persists or until the adequate spectra have been collected, typically 30 - 60 s. If needed, apply additional solvent to maintain a high signal intensity for a longer amount of time. Stop the data acquisition and pause the MS system.
8. Remove the tissue and wash the clamp with 100% methanol and a lint-free wipe. Clean the MS ion inlet after approximately 1 - 2 h of the acquisition by leaf spray MS according to the vendor's specifications. Also, clean the MS ion inlet between analyses of different tissue types.

4. Data Quality Assessment

1. Open the data file and visually inspect the base peak mass chronogram. Check that the signal intensity is ~1.0×10^7 to 5.0×10^8. If the signal is lower, move the tissue closer to the ion inlet. If higher, the front end of the MS system will become dirty, so move the tissue further from the ion inlet.
2. Based on the presence or absence of the ions of interest in the mass spectra produced, alter the parameters.
   NOTE: The protocol can be paused here.

5. Tandem Mass Fragmentation

1. Decide what ions are of interest for the tandem mass fragmentation (MS/MS); a mass spectra signal that is > 1.0×10^5 is sufficient for the selection of ions for MS/MS.
2. Make a new method file with an inclusion list of *m/z* out to 4 decimal places. **Click on Global lists and Inclusion**. Under **properties of PRM**, select the fragmentation energy [*e.g.*, normalized collision energy (NCE) of 30 - 50 is a good range to start with] and other MS/MS parameters.
   1. To obtain MS/MS data for mesembrine alkaloids, fragment the following ions, 276.1583 *m/z*, 290.1742 *m/z*, and 292.1897 *m/z*, at NCE 35.
      NOTE: The MS/MS data acquisition can be performed immediately after the MS or at a later time. The same tissue can often remain clamped after a full MS and can be reused to acquire MS/MS data. However, if a respraying does not provide a sufficient signal, use a new tissue.
3. Load the MS/MS method file and a named data file. Turn on the MS system, and start acquiring data, adding a solvent if necessary. When adequate spectra have been collected, typically after 30 - 60 s, stop the acquisition.
4. Collect fragmentation at many different energies when assigning fragment ions.
   NOTE: Since leaf spray MS lacks a chromatographic separation, the fragmentation spectra are likely to contain many ions, and fragmenting at different energies will help bring clarity.

6. Putative Identifications by Accurate Mass and Tandem Mass Fragmentation

1. Make putative identifications by referencing accurate mass measurements from publicly available metabolite databases such as Metlin[21], Human Metabolome Database[22], Mass Bank[23], Lipid Maps[24], National Institute of Standards and Technology MS Search[25], ReSpect for Phytochemicals[26], or GNPS[27].
2. As these databases are not exhaustive, perform an additional literature review on the plant species chemically characterized as necessary.
3. Match fragmentation ions from leaf spray MS/MS to the aforementioned databases when MS/MS information is available, or to the literature. Alternatively, use a manual interpretation of MS/MS fragment ions or a fragmentation of an authentic standard performed by the direct injection or LC-MS/MS.

7. Data Analysis

1. Convert the MS raw files to mzXML files with the msConvert tool from Proteowizard[28].
2. Use the XCMS software package implemented in R for peak picking. Use a direct infusion processing method for the leaf spray MS analysis.
   NOTE: The well-annotated scripts used for data processing can be found at https://github.com/HegemanLab/Leaf-Spray-Code.
3. To obtain semi-quantitative measurements, accounting for the experimental variability, normalize the intensity of each metabolite by the total ion current (TIC), as the leaf spray MS signal intensity can vary, in part due to slight variations in the positioning of the leaf in the source and the differences in leaf shape and size.
4. Alternatively, use vendor-provided software for the data analysis or MZmine2 (to be found at http://mzmine.github.io/)[29].

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Representative Results

At 10 weeks post-germination, freshly collected greenhouse-grown *S. tortuosum* leaves were analyzed by leaf spray MS. The experimental workflow for detecting metabolites from *S. tortuosum* leaves using leaf spray MS is illustrated in **Figure 2**. A leaf was selected, cut into a thin strip with a tapered end to form a point, and clamped with the leaf spray MS wire clamp apparatus. The plant tissue was positioned ~30 mm from the ion inlet and in line with the x- and y-axes. Upon the initiation of the leaf spray MS of the *S. tortuosum* leaves, ions were detected without any solvent application. Plants of the *Sceletium* family are succulents with thick leaves with a high water content that enables electrospray ionization by leaf spray MS without the external application of a solvent. However, most typically, plant tissues require a solvent application to detect ions.

A mass chronogram and mass spectra heavily populated with ions were produced after 30 s of acquisition in positive ionization mode (**Figure 3**). The chronogram represents a successful leaf spray MS experiment where a stable signal was maintained for the full duration of the acquisition. In this study, a total of nine mesembrine alkaloids were detected by leaf spray MS and putative identifications were made from the exact mass of the protonated ions of previously characterized compounds (**Table 1**)[30]. It is advantageous to perform leaf spray MS on a high-resolution, accurate-mass (HRAM) mass spectrometer, which makes it possible to resolve slightly differing masses. For instance, the *m/z* at 262.1794 could be putatively identified as dihydrojoubertiamine instead of as mesembrenone-M (demethyl-dihydro-), which would have been at *m/z* 262.1443.

The leaf tissue remained clamped for a tandem mass (MS/MS) fragmentation of a few ions of interest (**Table 1**). **Figure 4** shows three examples of MS/MS fragmentation spectra from leaf spray MS, 276.1583 *m/z*, 290.1742 *m/z*, and 292.1897 *m/z*. Putative identities were verified by mass fragments and confirmed with previously identified fragments[30]. The protonated ion of 276.1583 *m/z* was identified as two different isomers of mesembrine-M (demethyl) and another two isomers of mesembrenone-M due to the presence of diagnostic fragments for each isomer (**Table 1**).

Both mesembrine and mesembrine-M (dihydro-) were detected by leaf spray MS and putatively identified by exact mass as well as by mass fragmentation. The protonated ion of mesembrine at 290.1742 *m/z* is one of the most abundant ions in the spectrum; therefore, it may be a likely candidate as a biomarker for future studies. The two main alkaloids of *S. tortuosum* are mesembrine and mesembrenone, both of which were readily detected by leaf spray MS. Several of the compounds detected by leaf spray MS may be useful in the monitoring of plant materials of *Sceletium* and derived products.

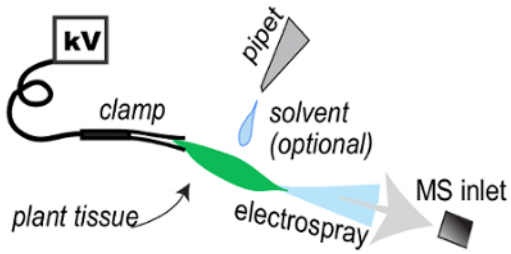

Figure 1: Leaf spray MS diagram of set-up. Leaf spray MS is a metabolite profiling method that allows for the rapid sampling of intact plant tissue. The diagram shows a leaf spray MS with kV high voltage applied with a clamp and the option to apply a solvent to the plant tissue. Leaf spray MS facilitates electrospray ionization directly from the plant tissue into the MS inlet. Please click here to view a larger version of this figure.

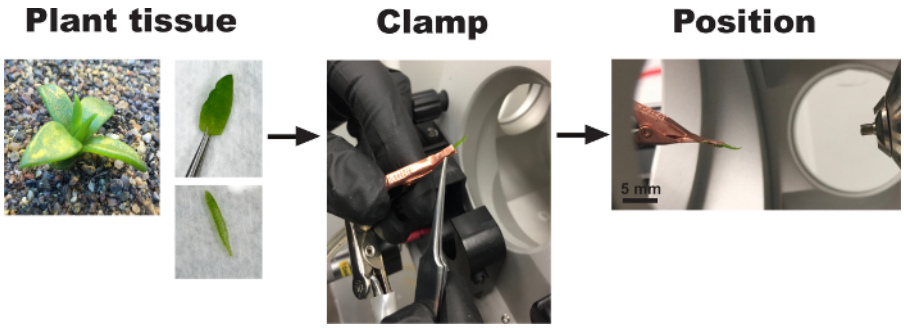

Figure 2: Workflow of leaf spray MS experiment. Selected plant tissue, a *Sceletium tortuosum* leaf, was cut, then transferred with forceps to be clamped, and then positioned in front of the ion inlet prior to the data acquisition. Please click here to view a larger version of this figure.

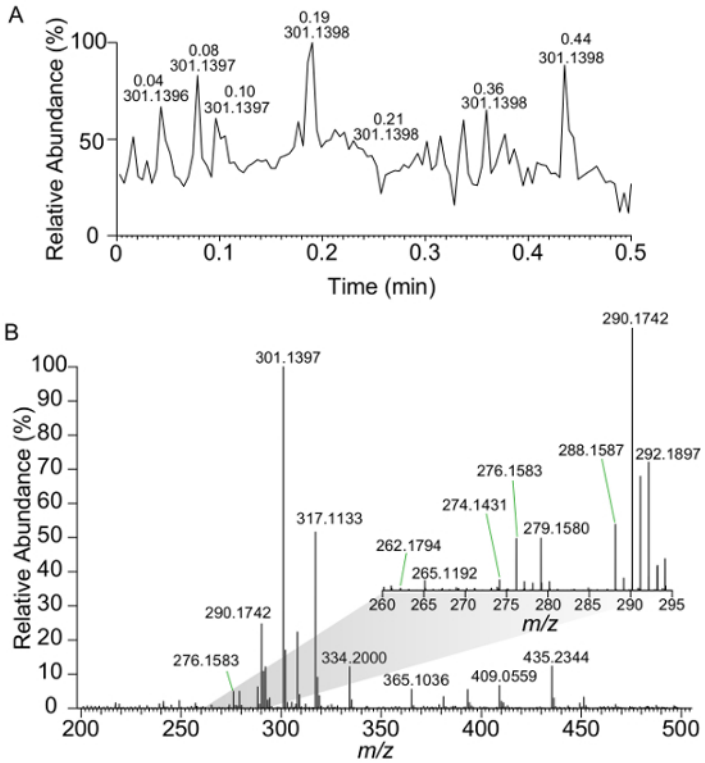

Figure 3: Metabolite profiling of *Sceletium tortuosum* by leaf spray MS with positive ionization. (**A**) This panel shows a leaf spray MS total ion count (TIC) mass chronogram. For each peak, the top number is the time (min) and the bottom is the *m/z*. (**B**) This panel shows the leaf spray MS metabolite profile of a *Sceletium tortuosum* positive mass spectrum. The inset displays 260 - 295 *m/z*. Accurate masses are reported out to 4 decimal places with an error < 6 ppm. Please click here to view a larger version of this figure.

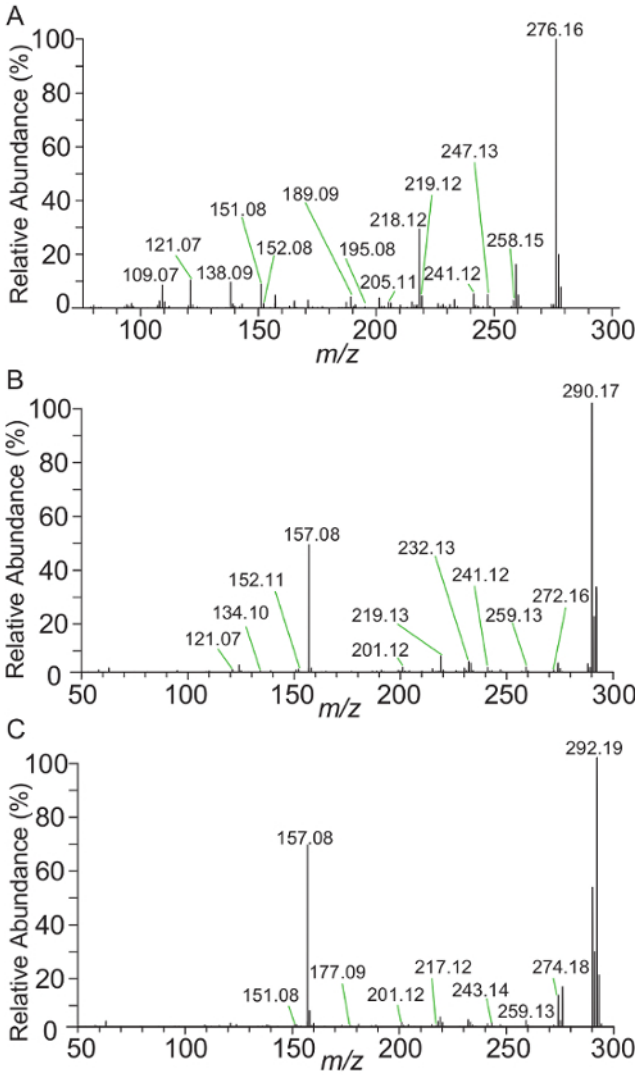

Figure 4: Leaf spray MS positive ionization tandem mass spectra from *Sceletium tortuosum* cut leaf. These panels show tandem mass spectra (MS/MS) collected in positive ionization mode with leaf spray MS. Putative identifications of alkaloids are made from the accurate mass and mass fragmentation for the following: (**A**) mesembrine-M and mesembrenone-M isomers, (**B**) mesembrine, and (**C**) mesembrine-M (dihydro-). Please click here to view a larger version of this figure.

Compound	Molecular Formula	Measured Monoisotopic Mass *m/z* [M+H]	ppm error	Fragments
Dihydrojoubertiamine	$C_{16}H_{23}NO_2$	262.1794	5.0	N/A
Mesembrenone-M (O-demethyl-)	$C_{16}H_{19}NO_3$	274.1431	4.5	N/A
Mesembrine-M (O-demethyl-)	$C_{16}H_{21}NO_3$	276.1583	5.9	121, 152, 195, 201, 218, 219, 258
Mesembrine-M (N-demethyl-)	$C_{16}H_{21}NO_3$	276.1583	5.9	109, 121, 138, 189, 201, 218, 247
Mesembrenone-M (O-demethyl-dihydro-)	$C_{16}H_{21}NO_3$	276.1583	5.9	124, 205, 218, 227, 245
Mesembrenone-M (N-demethyl-dihydro-)	$C_{16}H_{21}NO_3$	276.1583	5.9	120, 151, 210, 229, 241
Mesembrenone	$C_{17}H_{21}NO_3$	288.1587	4.3	124, 151, 191, 199, 226, 230, 257, 270
Mesembrine	$C_{17}H_{23}NO_3$	290.1742	4.9	110, 121, 134, 152, 201, 215, 219, 232, 233, 241, 259, 260, 272
Mesembrine-M (dihydro-)	$C_{17}H_{25}NO_3$	292.1897	5.2	151, 177, 201, 217, 243, 259, 274

Table 1: Putative identifications of *Sceletium tortuosum* mesembrine alkaloids by leaf spray MS. This table reports on positive ionization accurate masses and fragment ions for *Sceletium tortuosum* mesembrine alkaloids.

Discussion

The successful use of this protocol relies on the optimization of various steps for the plant species, tissue type, and target compound(s) of interest. The parameters described in the protocol provide a good starting point. The following experimental decisions need to be made and tested: whether or not to use (1) cut or uncut tissue and (2) solvent or no solvent, (3) what solvent to use and in what volume, (4) what the distance of tissue from the ion inlet should be, and (5) the voltage amplitude. The goal of optimization is to find the conditions that produce a continuous signal that persists for at least 30 s to a few minutes. The conditions should provide an adequate and reproducible signal intensity, which is needed to perform exact mass and MS/MS measurements. High signal intensity is achieved via a successful and reliable spray of ions from the tissue. The spray quality is dependent on the sharpness of the tip of the tissue pointing toward the ion inlet, the distance between the tip of the tissue and ion inlet, and the electrical voltage applied. A successful spray is highly dependent on the sharpness of the point of the tissue tip and, in some cases, the tissue should be cut to form a pointed tip. In particular, slight alterations in the sharpness of the angle of the tapered shape at the tip of the cut tissue have significant effects on the resulting quality of ionization and thus the signal intensity produced[7]. In cases where tissues are already pointed, then no cutting is necessary, as is the case with grass blades or lancet-shaped leaves[5,13].

An ion signal can be suppressed and unstable when plasma discharge occurs from the tissue as a result of being placed too close to the ion inlet or the electrical voltage being too high. A proper positioning of the tissue and a selection of the electrical voltage are required to ensure a stable and consistent spray between samples. The distance of the tissue from the MS ion inlet also impacts the quality and quantity of signal produced. In general, a smaller tissue sample should be placed closer to the inlet, although small-sized tissue may not result in a low signal intensity if the tip is extremely pointed or the compounds are highly concentrated. The intensity and consistency of spectra should be optimized empirically by comparing several placement positions along the z-axis. It is critical for a suitable ionization that the plant tissue is aligned with the MS ion inlet in both the x- and y-axes. However, if the plant tissue is unreasonably close to the ion inlet, this can require a more frequent cleaning of the ion optics and the front end of the MS system.

The volume of solvent applied on top of the tissue can range from 0 - 50µL depending on the water content and the size of the tissue sample. In cases where the tissue is extremely high in water content and is cut, as in the case of the succulent *Sceletium*, no solvent may be added. However, it is more typical to use at least 5 - 10 µL of solvent for at least one application. The addition of a solvent is necessary when using a dried tissue or fresh tissue with a low water content to facilitate the spray. If a small amount of solvent is used on a large piece of plant material, it will likely be absorbed without producing a sufficient spray. Conversely, if too high a volume is used, compounds may be diluted or proper desolvation will not readily and efficiently occur. An alternative option to manually pipetting solvent is to continually apply solvent to the tissue via a syringe pump so that the observed signal decays as a function of time as the compound is depleted from the plant material[10]. Various types of solvents should be tried, and the resulting spectra compared to verify any improvement of the quantity and consistency of the ion(s) for the compound(s) of interest. The addition of a solvent not only produces spray but can also provide a selectivity for the extraction of different compounds. Organic solvents with different polarities (methanol, dichloromethane, hexanes, acetonitrile, chloroform, and acetone) have been compared and result in significantly different ions present in the spectra from a peanut seed[8]. In general, methanol is a good first solvent choice, as it has been shown to work well for many plant tissues and a wide range of phytochemicals, including amino acids, alkaloids, flavonols, carbohydrates, organic acids, fatty acids, and phospholipids[8]. The application of 100% water onto uncut plant tissues does not typically work well but could be improved with the addition of salts[10]. In many cases, in addition to protonated ions of a compound, other abundant adducts are detected such as sodium and potassium adducts. The presence of these salt adducts is even more prevalent when salt is added to the solvent

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and can be advantageous. For example, an increased sensitivity and selectivity of phenolic glycosides from *Populus* species were observed with the addition of sodium and potassium ions to the applied solvent[10].

Two major limitations of the leaf spray MS technique are (1) the low dynamic range and (2) challenges with quantitation. Typically, only the most abundant compounds are ionized and detected by the technique. Decreases in the ionization efficiency due to the ion suppression that occurs dramatically in the absence of chromatographic separation are less of an issue with abundant metabolites. To circumvent this limitation, the scan range can be adjusted to focus on only the *m/z* range of interest. However, low-abundance compounds still may not be detected without the separation and concentration provided by chromatography. Unlike the typical quantitation of compounds from an extract, internal standards cannot be properly mixed into the plant material prior to leaf spray MS. Semi-quantitative measurements and relative concentrations have been obtained by placing a known concentration of a standard solution on the tissue surface and then allowing it to dry prior to a leaf spray MS analysis[8,9,31]. For example, the quantitation standard addition method was used to calculate the ratio of the representative ion for the internal standard to the ion of interest to determine relative quantities[32]. A calibration curve was used to estimate the relative concentration. Using this method, it was possible to compare the ratio of the various glycosides to an internal standard, rebaudioside D, and the relative concentration of specific glycosides could then be calculated within *Ste*via leaves[33]. Alternatively, a more accurate quantitation is possible with an isotopically labeled standard of the compound of interest, though commercial availability may be a challenge. The use of metabolically labeled plant tissue can also improve the quantitation with this method[34].

Given that conventional LC-MS/MS requires an extensive sample preparation and chromatographic separation, other methods for analysis are often desired. Leaf spray MS is a direct chemical analysis technique that may be easily applied and offers simplicity, precision, accuracy and quick metabolite detection, and semi-quantitation. For this reason, we investigated the suitability of leaf spray MS to monitor the chemical content of *S. tortuosum*, which may provide the basis for chemotaxonomic tools to differentiate species of the *Sceletium* genus based on biochemical signatures. Several anatomical properties of this plant make it an ideal test specimen for leaf spray MS. It is a succulent, containing high amounts of water, which is advantageous as the spray can be generated without the application of a solvent. *Sceletium* leaf contains idioblasts (bladder-like cells)[15] which serve as storage reserves where specialized metabolites may accumulate. Leaf spray MS is an *in vivo* analysis technique to characterize plant tissue in a fast manner. The general technique is applicable to many plant species, tissue types, and classes of compounds. Techniques that capture information about plant compounds are of great interest to understand the plant primary and specialized metabolism for human uses of health, nutrition, agriculture, and energy[35].

Disclosures

The authors have nothing to disclose.

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