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Technical Note

Direct analysis in real time high resolution mass spectrometry as a tool for rapid characterization of mind-altering plant materials and revelation of supplement adulteration – The case of Kanna

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ABSTRACT

We demonstrate the utility of direct analysis in real time ionization coupled with high resolution time-of-flight mass spectrometry (DART-HRTOFMS) in revealing the adulteration of commercially available *Sceletium tortuosum*, a mind-altering plant-based drug commonly known as Kanna. Accurate masses consistent with alkaloids previously isolated from *S. tortuosum* plant material enabled identification of the products as Kanna, and in-source collision-induced dissociation (CID) confirmed the presence of one of these alkaloids, hordenine, while simultaneously revealing the presence of an adulterant. The stimulant ephedrine, which has been banned in herbal products and supplements, was confirmed to be present in a sample through the use of in-source CID. High-throughput DART-HRTOFMS was shown to be a powerful tool to not only screen plant-based drugs of abuse for psychotropic alkaloids, but also to reveal the presence of scheduled substances and adulterants.

1. Introduction

For millennia, psychoactive plant-based products have been used to induce or enhance religious experiences, and in therapeutic applications such as pain management. More recently however, many of these psychoactive substances are being marketed as "natural" legal alternatives to scheduled drugs. The widespread availability of these products through the Internet, coupled with the absence of laws governing their use, makes them ideal choices for those wishing to circumvent current drug laws. Furthermore, identification of these mind-altering plant products is often very difficult for forensic laboratories, as the material is commonly dried and ground into a powder. Due to this pre-processing, physical botanical features used for species discrimination are lost and psychoactive plant material cannot be differentiated from innocuous substances. The United Nations Office on Drugs and Crime (UNODC) issued a list of 20 plant-based substances of concern in 2013 as part of a report on the challenges of identifying and regulating new psychoactive substances [1]. Due to the many species and varieties of mind-altering plants and the inability of law enforcement to rapidly screen for these drugs based on physical features, psychotropic plant materials are becoming increasingly popular alternatives to illicit drugs. In fact, these psychotropics now account for nearly 10% of the new psychoactive substances on the global market [2].

Compounding the problem of the unregulated abuse of psychoactive plants is the fact that these substances are usually classified as herbal or dietary supplements. In the United States, this categorization exempts them from mandatory testing by the US Food and Drug Administration (FDA), with the consequence that there is little oversight regarding their ingredient profiles. Thus, although advertised to contain a particular herb or herbal combination, cases of supplements that have been laced with toxic and/or banned substances have arisen, and these incidents are on the rise. Indeed, the ingestion of these products has been associated with poisonings and fatalities and is of growing concern to law enforcement agencies [3,4]. A major bottleneck in addressing this issue is the development of laboratory analysis methods that: (1) enable rapid assessment of the veracity of the claims made on the product label; and (2) facilitate rapid screening for banned adulterants.

Currently, the most common approaches to the identification of plant-based supplements and determination of their chemical content are hyphenated chromatographic-mass spectrometric

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methods such as GC–MS and LC–MS. These well-established techniques can provide definitive information not only on the identity of supplements but also on the presence of adulterants. However, the usual complexity of supplement matrices often requires nuanced method development that is specific for the analyte(s) of interest. While the creation of these protocols is often time and resource intensive, an important additional concern is the sample preparation steps which often include extraction, derivatization, pH adjustments, and in some cases, lengthy chromatographic run times. For example, the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) procedure developed specifically for rapid analysis of adulterants still requires sample preparation time which can be quite lengthy, and additional clean-up steps prior to chromatographic and mass spectrometric analysis [4,5]. Therefore, the methods, even when developed, are often not convenient for use in routine analyses by crime labs. It would be highly advantageous to have analytical protocols that are rapid, widely applicable to a diversity of samples, and which circumvent sample preparation steps such as solubilization, extraction and derivatization. Such methods would in turn, pave the way for the drafting of legislation that addresses the increasing abuse of plant-based psychotropics.

Direct analysis in real time high resolution time-of-flight mass spectrometry (DART-HRTOFMS) is an ambient ionization mass spectrometric technique [6] that yields high-resolution spectra for a wide variety of compounds spanning a range of polarities [7]. Spectra are produced almost instantly, and samples can be tested directly with little or no sample preparation, whether the physical form is a liquid, pill, powder or crushed plant material [7,8]. DART-HRTOFMS has been used successfully for the analysis of psychoactive compounds including synthetic cannabinoids and Kratom (*Mitragyna speciosa*) [9–11], as well as for the analysis of adulterated products including dietary supplements contaminated with anti-diabetic drugs [12], tainted *Berberis aristata* herbal products [13], counterfeit anti-malarial drugs [14], star anise fruits and teas laced with a neurotoxin [15], and milk products containing melamine [16].

Here, we demonstrate the utility of DART-HRTOFMS for the rapid screening of commercially available *Sceletium tortuosum* products, also known as Kanna, an example of a widely available plant supplement that has been identified by the UNODC as a drug of concern [17]. The species identity of the products was confirmed by the DART-HRTOFMS derived characteristic fingerprints that were consistent with the presence of compounds previously detected in the species, including the diagnostic, psychoactive alkaloids mesembrine, mesembrenone, mesembrenol and mesembranol [18–21]. Furthermore, DART-HRTOFMS simultaneously unmasked the presence of ephedrine, an adulterant that would not have been as easily or rapidly detected using more common conventional methods. This work demonstrates how the unique capabilities of DART-HRTOFMS can be harnessed to rapidly screen herbal supplements for the plant species of origin and concurrently reveal the presence of adulterants, even when they are isobars of compounds expected to be present. It also alerts the public as well as law enforcement of the possibility of ephedrine adulteration in Kanna products.

2. Materials and methods

2.1. Kanna products

Kanna 25X extract powder was purchased from World Seed Supply (Mastic Beach, NY, USA). Kanna 5X and 25X powders, as well as Kanna 10X resin and "Smoker's Cut" dried plant material were purchased from Bouncing Bear Botanicals (Lawrence, KS, USA).

2.2. Chemical standards

For structure confirmation studies, authentic standards of hordenine and ephedrine were purchased from Sigma Aldrich (St. Louis, MO, USA) and Cerilliant Corporation (Round Rock, TX, USA), respectively.

2.3. DART-MS mass spectral data collection and analysis

DART mass spectra of plant materials and standards were acquired using a DART-SVP ion source (IonSense, Saugus, MA, USA) coupled to a JEOL AccuTOF high resolution time-of-flight mass spectrometer (JEOL USA, Peabody, MA, USA) in positive-ion mode. The DART ion source parameters were: grid voltage, 250 V; and gas heater temperature, 350 °C. The mass spectrometer settings were: ring lens voltage, 5 V; orifice 1 voltage, 20 V; orifice 2 voltage, 5 V; and peak voltage, 600 V. Mass spectra were acquired over the *m/z* range 60–800 at a spectral acquisition rate of 1 spectrum per sec. The helium flow rate for the DART ion source was 2.0 L s^{-1}. The resolving power of the mass spectrometer was 6000 FWHM.

In-source collision-induced dissociation (CID) was performed on plant material and standards by adjusting the orifice 1 voltage to 90 V to induce fragmentation. The RF ion guide voltage ("Peaks voltage" in the Mass Center software) for CID analyses was set to 400 V, the mass range was set to *m*/z 40–800, and all other ion source and mass spectrometer parameters were as described above.

Kanna powders were tested directly by dipping the closed end of a melting point capillary tube into the powder and presenting the coated surface of the tube to the space between the DART ion source and the mass spectrometer inlet. Hordenine and ephedrine standards were analyzed in the same manner. Kanna dried plant material was sampled by grasping the material with tweezers and suspending it between the ion source and the mass spectrometer inlet. Multiple pieces of the Kanna dried plant material were sampled in each analysis. Kanna resin was sampled in the same manner.

Data calibration, spectral averaging, background subtraction, and peak centroiding were achieved using TSSPro3 software (Shrader Software Solutions, Detroit, MI). Polyethylene glycol (PEG 600) was used as the mass calibration standard. Mass Mountaineer (RBC Software, Portsmouth, NH, available from mass-spec-software.com) was used for mass spectral analysis, spectral elemental composition determination and isotope analysis.

3. Results

3.1. DART-HRTOFMS analysis of Kanna products

As Kanna has been shown to contain characteristic psychoactive mesembrine alkaloids, it was anticipated that several of these might be present in the Kanna products and that their detection could aid in the confirmation of the identity of the plant material using mass spectrometry. Thus, the five Kanna samples available through the internet were analyzed by DART-HRTOFMS. Fig. 1 shows representative soft ionization spectra (i.e. acquired using an orifice 1 voltage of 20 V) of the Kanna samples, with the associated mass measurement data presented in Table 1. The average of five spectra is shown in each case and each of the observed peaks represents a unique protonated compound. The number of peaks above a 1% threshold varied from 30 in the 25X Kanna from World Seed Supply (WSS), to 213 in the Kanna 5X from Bouncing Bear Botanicals (BBB). The mass spectral profiles were most similar for the Kanna 25X from WSS and the Kanna "Smoker's Cut" from BBB. In both cases, the two most prominent peaks appeared at *m/z* 166 and *m/z* 116, with the former being the most abundant peak. Interestingly, although BBB also sells 25X Kanna, a comparison of