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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

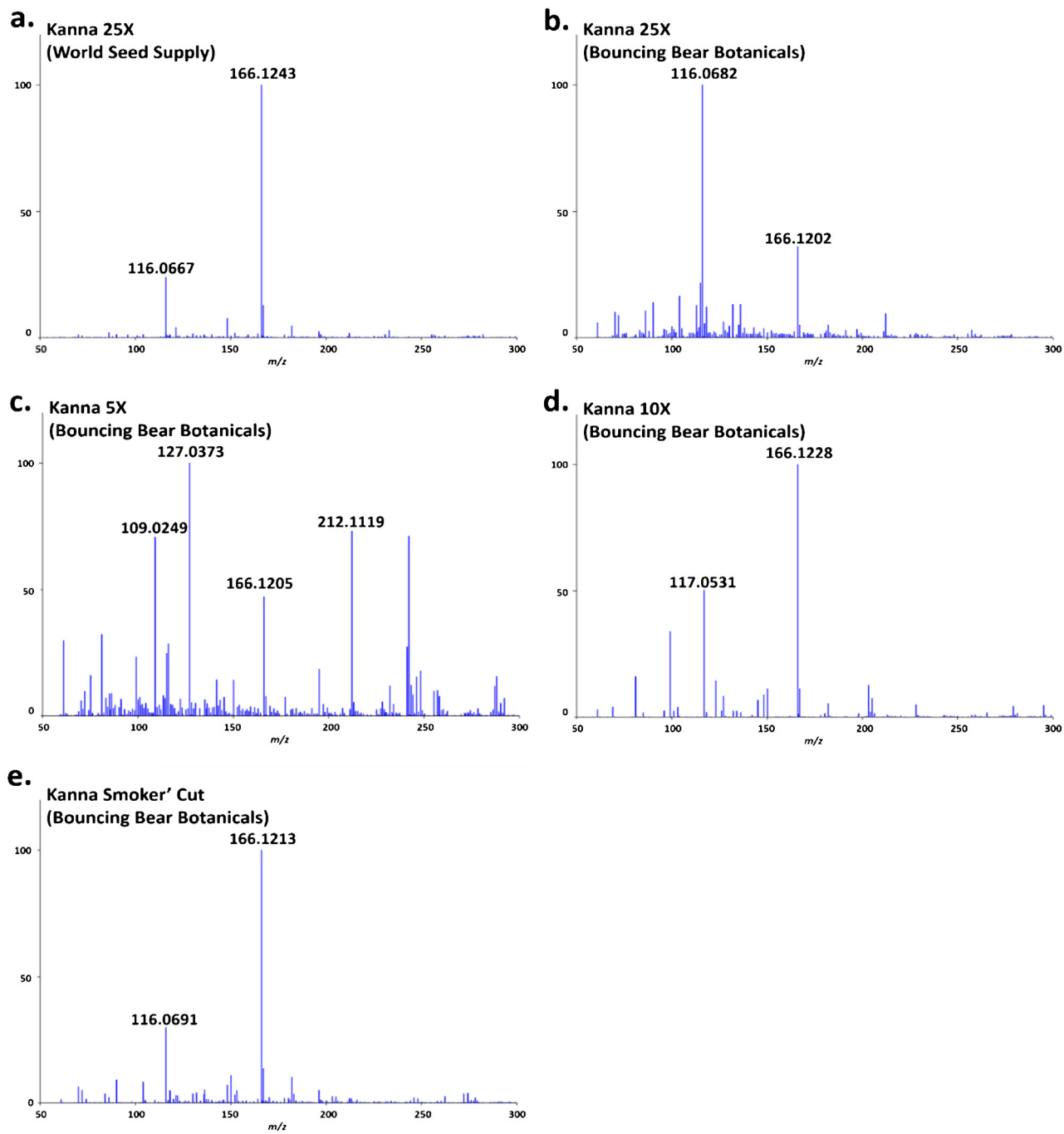

Fig. 1. DART-HRTOFMS soft ionization spectra of *S. tortuosum* (Kanna) products. Panel a: Kanna 25X extract powder from World Seed Supply (WSS); Panel b: Kanna 25X powder from Bouncing Bear Botanicals (BBB); Panel c: Kanna 5X powder from BBB; Panel d: Kanna 10X resin from BBB; Panel e: Kanna Smoker's Cut plant material from BBB. The mass measurement data associated with these spectra are shown in Table 1.

the mass spectral profile of this product to the 25X from WSS showed the two to be quite different. While the major peaks in both cases were at nominal *m/z* 166 and *m/z* 116, the latter was the most intense peak in the BBB product, and the former was the most intense peak in the WSS sample. Comparison of the 5X, 10X and 25X BBB samples showed that the 5X spectrum exhibited the greatest number of components, and the 10X spectrum the least.

Despite the readily apparent distinctions between them, all of the spectra exhibited peaks with masses corresponding to previously isolated alkaloids that are characteristic of *S. tortuosum*. A mass consistent with the formula of protonated hordenine ($[C_{10}H_{15}NO + H]^+$ corresponding to *m/z* 166.1232) was detected in all of the samples. Furthermore, a mass corresponding to protonated mesembranol ($[C_{17}H_{25}NO_3 + H]^+$ at *m/z* 292.1913) was detected in varying relative amounts (0.3–7.0%) in the two 25X samples from both vendors, as well as in the 5X and Smoker's Cut samples from BBB. The peak at *m/z* 290 (corresponding to $[C_{17}H_{23}NO_3 + H]^+$) that was present in the Kanna 5X sample at 5.1% relative abundance, was consistent with the presence of mesembrine. Other possible alkaloids were identified in the Kanna products based on their previous isolation from the species, as well as on elemental composition determination and isotope matching. These included

Table 1
Accurate mass measurements associated with the 20 V DART-HRTOFMS spectra presented in Fig. 1.

	Compound[a]	Formula	Measured	Calculated	Diff.[b]	Rel. Int.[c]
World Seed Supply Kanna 25X	Hordenine	$C_{10}H_{15}NO + H^+$	166.1243	166.1232	−1.1	100.0
	Dihydrojoubertiamine	$C_{16}H_{23}NO_2 + H^+$	262.1835	262.1807	−2.8	0.8
	o-Methyljoubertiamine	$C_{17}H_{23}NO_2 + H^+$	274.1803	274.1807	0.3	0.8
	Mesembranol	$C_{17}H_{25}NO_3 + H^+$	292.1898	292.1913	1.5	0.1
Bouncing Bear Botanicals Kanna 25X	Hordenine	$C_{10}H_{15}NO + H^+$	166.1201	166.1232	3.0	36.0
	Dihydrojoubertiamine	$C_{16}H_{23}NO_2 + H^+$	262.1815	262.1807	−0.8	1.0
	o-Methyldehydrojoubertiamine	$C_{17}H_{21}NO_2 + H^+$	272.1659	272.1651	0.8	0.6
	o-Methyljoubertiamine	$C_{17}H_{23}NO_2 + H^+$	274.1830	274.1807	−2.3	0.8
	Mesembranol	$C_{17}H_{25}NO_3 + H^+$	292.1878	292.1913	3.5	0.4
Bouncing Bear Botanicals Kanna 5X	Hordenine	$C_{10}H_{15}NO + H^+$	166.1205	166.1232	2.7	47.3
	Sceletenone	$C_{15}H_{17}NO_2 + H^+$	244.1345	244.1338	−0.7	8.5
	Dehydrojoubertiamine	$C_{16}H_{19}NO_2 + H^+$	258.1507	258.1494	−1.3	7.8
	Dihydrojoubertiamine	$C_{16}H_{23}NO_2 + H^+$	262.1783	262.1807	2.4	2.0
	o-Methyldehydrojoubertiamine	$C_{17}H_{21}NO_2 + H^+$	272.1611	272.1651	4.0	1.2
	4-*o* -Desmethylmesembrenone	$C_{16}H_{19}NO_3 + H^+$	274.1483	274.1443	−4.0	2.2
	4-*o*-Desmethylmesembrenol	$C_{16}H_{21}NO_3 + H^+$	276.1646	276.1600	−4.7	1.5
	4-*o*-Desmtheylmesembranol	$C_{16}H_{23}NO_3 + H^+$	278.1769	278.1756	−1.3	2.9
	Mesembrenone	$C_{17}H_{21}NO_3 + H^+$	288.1593	288.1600	0.7	15.7
	Mesembrine	$C_{17}H_{23}NO_3 + H^+$	290.1781	290.1756	−2.5	5.1
	Mesembranol	$C_{17}H_{25}NO_3 + H^+$	292.1937	292.1913	−2.4	7.0
Bouncing Bear Botanicals Kanna 10X	Hordenine	$C_{10}H_{15}NO + H^+$	166.1228	166.1232	0.4	100.0
Bouncing Bear Botanicals Kanna Smoker's Cut	Hordenine	$C_{10}H_{15}NO + H^+$	166.1201	166.1232	3.0	36.0
	Sceletenone	$C_{15}H_{17}NO_2 + H^+$	244.1361	244.1338	−2.3	0.8
	Dehydrojoubertiamine	$C_{16}H_{19}NO_2 + H^+$	258.1530	258.1494	−3.6	0.8
	Joubertiamine	$C_{16}H_{21}NO_2 + H^+$	260.1636	260.1651	1.5	0.8
	Dihydrojoubertiamine	$C_{16}H_{23}NO_2 + H^+$	262.1880	262.1807	2.7	2.6
	o-Methyldehydrojoubertiamine	$C_{17}H_{21}NO_2 + H^+$	272.1650	272.1651	0.1	3.7
	o-Methyljoubertiamine	$C_{17}H_{23}NO_2 + H^+$	274.1813	274.1807	−0.6	3.8
	Mesembrane	$C_{17}H_{25}NO_2 + H^+$	276.1958	276.1964	0.6	1.0
	Mesembranol	$C_{17}H_{25}NO_3 + H^+$	292.1922	292.1913	−0.9	0.3

[a] Compound names are tentatively assigned based on the match between elemental compositions determined from accurate mass measurements and compounds that have been previously identified in *Sceletium* species.
[b] Differences are reported in millimass units. Measured masses fell within 5 mmu of the calculated mass.
[c] Relative abundances are reported in percent.

dihydrojoubertiamine ($[C_{16}H_{23}NO_2 + H]^+$ corresponding to *m/z* 262.1807), *o*-methyljoubertiamine ($[C_{17}H_{23}NO_2 + H]^+$ corresponding to *m/z* 274.1807), *o*-methyldehydrojoubertiamine ($[C_{17}H_{21}NO_2 + H]^+$ at *m/z* 272.1651), sceletenone ($[C_{15}H_{17}NO_2 + H]^+$ corresponding to *m/z* 244.1338), dehydrojoubertiamine ($[C_{16}H_{19}NO_2 + H]^+$ corresponding to *m/z* 258.1494), 4-*o*-desmethylmesembrenone ($[C_{16}H_{19}NO_3 + H]^+$ at *m/z* 274.1443), 4-*o*-desmethylmesembrenol ($[C_{16}H_{21}NO_3 + H]^+$ at *m/z* 276.1600), and 4-*o*-desmethylmesembranol ($[C_{16}H_{23}NO_3 + H]^+$ corresponding to *m/z* 278.1756).

3.2. The presence of hordenine was confirmed in the Kanna products

The lack of availability of authentic standards of several mesembrine alkaloids prevented confirmation of the presence of the majority of these compounds. However, the availability of a hordenine standard made it possible to confirm the presence of this compound in the Kanna products through comparison of their positive ion mode in-source collision-induced dissociation (CID) spectra with that of the standard acquired under identical conditions (i.e. orifice 1 potential of 90 V to induce fragmentation). The results, rendered as head-to-tail plots in which the top panel represents the in-source CID spectrum of the Kanna product and the bottom, the in-source CID spectrum of the hordenine standard, are illustrated in Fig. 2a–e. The in-source CID spectrum of hordenine exhibited a protonated parent peak at nominal *m/z* 166, and fragment ion peaks at *m/z* 135, 121, 103, 93, 91, 77 and 58 (bottom panel, Fig. 2a–e). These peaks were present in the in-source CID spectra of all of the Kanna products from both vendors, thus confirming the presence of hordenine in these samples. For example, the BBB Kanna 25X in-source CID spectrum contained not only the protonated parent at *m/z* 166, but also all of the fragment ions found in the hordenine spectrum (Fig. 2a). The same was true for the BBB 5X, 10X and Smokers Cut plant materials (Fig. 2b–d). However, while the hordenine protonated parent and fragment ion peaks were prominent features in the spectra of the products obtained from BBB, the spectrum of the WSS Kanna 25X displayed several additional prominent peaks that were absent from the spectra of the other Kanna samples. These included peaks at nominal *m/z* 148, 133, 105 and 70 (highlighted in orange in Fig. 2e).

3.3. In-source CID confirmed the presence of ephedrine as an adulterant in a Kanna product

Given that the soft ionization DART-HRTOFMS spectrum of the WSS Kanna 25X exhibited a prominent peak at *m/z* 166.1243 (Fig. 1a) that corresponded to a formula consistent with hordenine, the observation under in-source CID conditions of fragments that were so distinctly different from those of the other Kanna samples was unexpected. Moreover, there was no peak in the 20 V spectrum of WSS Kanna 25X (Fig. 1a) that could contribute to the fragment ions at *m/z* 148, 133, 105 and 70. These findings implied that another compound was contributing to the peak at

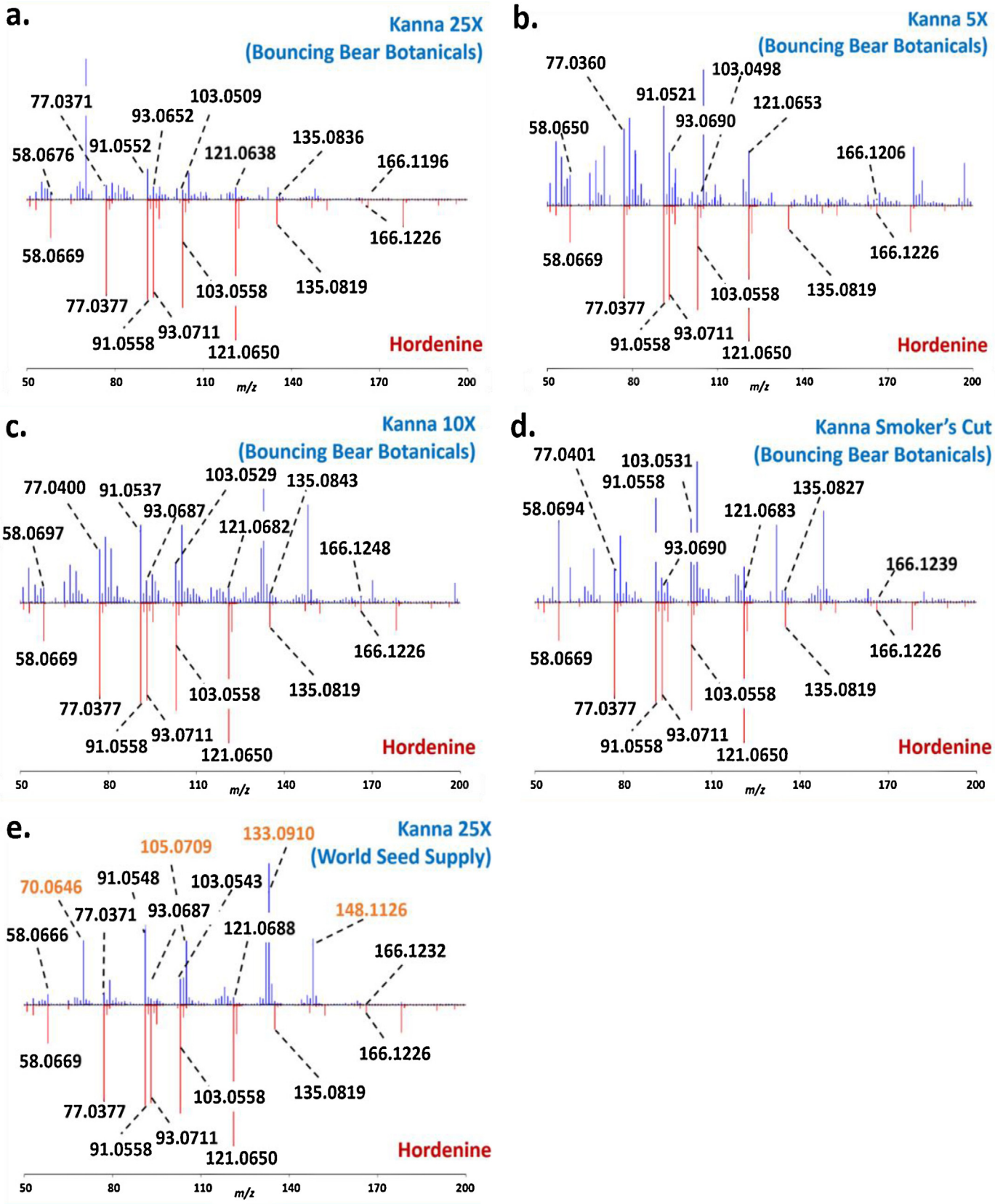

Fig. 2. Comparison of positive ion mode DART-HRTOFMS spectra under in-source CID conditions of *S. tortuosum* Kanna products vs. a hordenine standard, rendered as head-to-tail plots. In each case, the top spectrum is that of the Kanna product and the bottom spectrum that of the hordenine standard. The protonated parent (nominal *m/z* 166) and fragment ions (nominal *m/z* 135, 121, 103, 93, 91, 77, 58) are observed in the hordenine standard and in the Kanna products. Panel a: Comparison of Bouncing Bear Botanicals (BBB) 25X to hordenine; Panel b: Comparison of BBB 5X to hordenine; Panel c: Comparison of BBB 10X to hordenine; Panel d: Comparison of BBB Smoker's Cut to hordenine; Panel e: Comparison of World Seed Supply (WSS) 25X to hordenine.

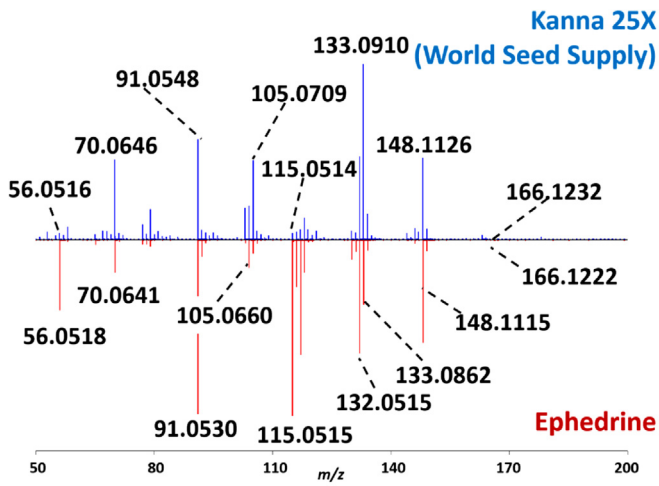

Fig. 3. Comparison of positive ion mode DART-HRTOFMS spectra under in-source CID conditions of the World Seed Supply Kanna 25X product vs. an ephedrine standard, rendered as a head-to-tail plot. The top spectrum is that of the Kanna product and the bottom spectrum, that of the ephedrine standard. The protonated parent (nominal *m/z* 166) and fragment ions (nominal *m/z* 148, 133, 115, 105, 91, 70, 56) are found in both the Kanna product and the ephedrine standard.

nominal *m/z* 166. The investigation of this possibility began with a NIST database search for known molecules with the formula $C_{10}H_{15}NO$, so that their EI spectrum fragmentation patterns could be compared with those observed for hordenine, in order to find a possible match. This inquiry yielded 56 unique compounds, only three of which were known natural products, namely ephedrine, pholedrine, and perillartine. The EI mass spectrum of ephedrine, a constitutional isomer of hordenine, was the only one of the three that exhibited a NIST database EI mass spectrum similar to that of hordenine. Ephedrine was analyzed by DART-HRTOFMS under in-source CID conditions and its spectrum was compared to that of the WSS 25X Kanna powder which was analyzed similarly. Fig. 3 shows a head-to-tail plot featuring both spectra. The protonated parent ion at nominal *m/z* 166 was present in both the Kanna sample (top panel) and the ephedrine standard (bottom panel). Fragment ions at nominal *m/z* 148, 133, 115, 105, 70 and 56 that were not present in the hordenine standard were observed in both the Kanna powder and the ephedrine standard. The observation of these fragment ions in the Kanna powder confirmed that ephedrine was present in the sample. The presence of ephedrine in the WSS Kanna 25X sample was also confirmed using a secondary, traditional analytical method, GC-TOFMS (results not shown).

4. Discussion

The UNODC has identified Kanna as a plant drug of concern due to increases in its use and the potential for abuse. In a forensics context, it would be of interest to be able to identify and distinguish Kanna from other plant material, and the GC–MS, LC–MS, HPLC and NMR methods that have been reported for its analysis have been largely restricted to natural products isolation work rather forensic identification of psychoactives [20–23]. These protocols, although useful, include obligate sample preparation steps, require significant amounts of material, and involve relatively lengthy analysis run times of up to 30 min per sample [21,22]. The intensive sample preparation and long analysis times make these methods impractical for use in routine screenings, particularly in the face of the case work backlog challenges currently confronting many crime labs [24,25].

The Kanna identification process outlined herein is fundamentally different from those delineated in the aforementioned reports, and it provides some unique advantages. As described previously [9,26–29], plant material in both fresh and dried forms exhibits characteristic DART-HRTOFMS chemical fingerprints that are not only consistent, but can be used to definitively identify the material, particularly in those cases where biomarkers characteristic of the genus or species are present. This was found be true for Kanna, where the DART-HRTOFMS spectra for a variety of products from two different vendors all exhibited peaks consistent with the presence of biomarkers known only to be present in the *Sceletium* genus. Unlike earlier procedures [18,20–23], the DART-HRTOFMS method did not require solubilization or other sample preparation steps, since the material could be analyzed in its native form. Furthermore, due to the lack of solubilization, there was no solvent-selection bias introduced in the sample preparation steps, unlike in LC–MS or GC–MS, where hordenine would be preferentially taken up over ephedrine due to the differences in their polarity. In addition to the time savings this engendered, the mass spectral method itself was extremely rapid, with the spectrum acquisition being accomplished in ~3 s. The rapidity of the method lends itself to use as a triage tool that can be applied to tentatively identify Kanna so that it can then be subjected to further more definitive analysis methods such as HPLC, LC–MS or GC–MS.

It was the consistent detection of various alkaloid biomarkers and their characteristic fragmentation profiles in the mass spectra of the various Kanna products that made the apparent adulteration of the 25X WSS Kanna product so obvious. Hordenine is one of the known biomarkers in Kanna and thus it was anticipated that it would be detected at nominal *m/z* 166. Thus, the observation of a peak at that mass in all of the Kanna samples, was consistent with what would be expected for an authentic Kanna product. In that regard, it was confirmed, through in-source CID experiments, that hordenine was indeed present in all of the samples, and in conjunction with other alkaloid biomarkers, the identity of the plant material could be established as Kanna. However, despite the fact that the WSS 25X Kanna and the BBB Kanna Smoker's cut displayed very similar spectra with *m/z* 166 serving as the most abundant peak in both cases, the in-source CID experiments unmasked the presence of an additional compound in the WSS Kanna 25X that was contributing to the *m/z* 166 peak observed in its spectrum. This analysis yielded several fragment ions which were not present in the hordenine in-source CID spectrum (*m/z* 148, 133, 105, 70, and 56). Furthermore, the absence of significant peaks above *m/z* 166 in the WSS Kanna 25X spectrum indicated that the novel fragments were derived from the molecule(s) represented by *m/z* 166. Subsequent comparison of these fragments with those formed from in-source CID of ephedrine, a banned constitutional isomer of hordenine, provided confirmation of ephedrine's presence. Analysis of the derivatized ephedrine standard and Kanna extract by GC-HRTOFMS provided further proof of the presence of the stimulant in the Kanna product. The results presented here demonstrate the use of DART-HRTOFMS not only as a rapid analytical tool for the identification of mind-altering substances, but also for the instantaneous screening for adulterants in complex matrices.

The conclusion that ephedrine was an adulterant, as opposed to a compound biosynthesized in Kanna, was based on the following considerations: (1) several studies of the natural products contained within *Sceletium* genus plants have been reported [19,21,22,30], and in none of them has ephedrine been observed; (2) ephedrine is a biomarker in genus *Ephedra* plants specifically [31–34], and has never been observed in any other genus; (3) ephedrine has been previously documented as an adulterant in herbal supplements [35–37]. The in-source CID DART-HRTOFMS of the natural biomarker hordenine and the adulterant ephedrine

were readily distinguishable (Figs. 2a and 3). Not only was the protonated parent peak retained in both cases (at *m/z* 166), but several unique derivative fragment peaks also appeared (*m/z* of 135, 121, 103, 93, 91, 77 and 58 for hordenine and *m/z* 148, 133, 105, 70, and 56 for ephedrine). Examination of the NIST EI fragmentation database shows that both hordenine and ephedrine exhibit spectra that are very similar, with a base peak at *m/z* 58, and no other discernable fragments or parent peak. By contrast, in-source CID yielded information that was more diagnostic due to the softer ionization conditions, and allowed for rapid detection of an isobaric adulterant. This illustrated the utility of in-source CID DART-HRTOFMS to rapidly provide diagnostic information on the structural identity of small molecule amines without the necessity of solubilization and derivatization steps, pH adjustments, or the utilization of a chromatographic interface.

The identification of ephedrine in herbal supplements is of interest for a host of reasons. Plant-based drugs of abuse, including Kanna, are a concern in and of themselves due to the lack of information on their detrimental consequences, the absence of regulations on their use, and their adverse health effects including poisonings and fatalities [17]. The addition of adulterants to these supplements may impose further dangers and side effects. Ephedrine specifically is a hazard in herbal supplements, as the stimulant effects of ephedrine could be fatal when combined with other stimulants such as caffeine, over-the-counter medicines, or plant-based drugs such as Kanna [38–40]. In a study of 140 ephedrine-related reports submitted to the FDA between 1997 and 1999, nearly half involved cardiac events, with dietary supplements containing ephedrine being implicated in 10 deaths [39]. In addition to cardiac events, other serious side effects of ephedrine exposure include hypertension, kidney stones, tremors, and seizures. As a consequence of the significant health risks to users, ephedrine was banned by the US FDA in 2004 [41].

5. Conclusion

This study demonstrates the utility of the DART-HRTOFMS analysis method in characterizing plant-based drugs and revealing supplement adulteration. Commercially available Kanna products were rapidly analyzed to reveal the presence of alkaloid biomarkers characteristic of the *S. tortuosum* species from which they were derived. The application of in-source CID DART-HRTOFMS was shown to be a viable tool that could be used to rapidly unmask the presence of ephedrine, an adulterant that is an isobar of hordenine, a known Kanna biomarker. The presence of ephedrine might not otherwise have been revealed. The findings illustrate the ingenuity of psychotropic plant vendors in introducing banned substances with characteristics that align with what would be expected in unadulterated products. This approach increases the probability that the adulteration will remain undetected. The findings add to the growing number of examples of how the absence of regulatory controls on the supplement industry provides a convenient conduit for the distribution of banned substances such as ephedrine, through adulteration of unregulated products.

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References

[1] The Challenge of New Psychoactive Substances: List of Plant-based Substances (20 Substances), UNODC, 2013, pp. 101–102.
[2] Global Synthetic Drugs Assessment: Amphetamine-Type Stumulants and New Psychoactive substances., UNODC, 2014.
[3] D.H. Phua, A. Zosel, K. Heard, Dietary supplements and herbal medicine toxicities – when to anticipate them and how to manage them, Int. J. Emerg. Med. 2 (2009) 69–76.
[4] L. Vaclavik, A.J. Krynitsky, J.I. Rader, Mass spectrometric analysis of pharmaceutical adulterants in products labeled as botanical dietary supplements or herbal remedies: a review, Anal. Bioanal. Chem. 406 (2014) 6767–6790.
[5] M. Anastassiades, S.J. Lehotay, D. Stajnbaher, F.J. Schenck, Fast and easy multi-residue method employing acetonitrile extraction/partitioning and dispersive solid-phase extraction for the determination of pesticide residues in produce, J. AOAC Int. 86 (2003) 412–431.
[6] Ambient Ionization Mass Spectrometry, Royal Society of Chemistry, 2015.
[7] R. Cody, J. Laramee, H.D. Durst, Versatile new ion source for the analysis of materials in open air under ambient conditions, Anal. Chem. 77 (2005) 2297–2302.
[8] R. Cody, Observation of molecular ions and analysis of nonpolar compounds with the direct analysis in real time ion source, Anal. Chem. 81 (2009) 1101–1107.
[9] A.D. Lesiak, R.B. Cody, A.J. Dane, R.A. Musah, Rapid detection by direct analysis in real time-mass spectrometry (DART-MS) of psychoactive plant drugs of abuse: the case of *Mitragyna speciosa* aka Kratom, Forensic Sci. Int. 242 (2014) 210–218.
[10] A.D. Lesiak, R.A. Musah, R.B. Cody, M.A. Domin, A.J. Dane, J.R.E. Shepard, Direct analysis in real time mass spectrometry (DART-MS) of bath salt cathinone drug mixtures, Analyst 138 (2013) 3424–3432.
[11] R. Musah, M. Domin, R. Cody, A. Lesiak, A.J. Dane, J. Shepard, Direct analysis in real time mass spectrometry with collision-induced dissociation for structural analysis of synthetic cannabinoids, Rapid Commun. Mass Spectrom. 26 (2012) 2335–2342.
[12] Z. Zhou, J. Zhang, W. Zhang, Y. Bai, H. Liu, Rapid screening for synthetic antidiabetic drug adulteration in herbal dietary supplements using direct analysis in real time mass spectrometry, Analyst 136 (2011) 2613–2618.
[13] V. Bajpai, A. Singh, K.R. Arya, M. Srivastava, B. Kumar, Rapid screening for the adulterants of *Berberis aristata* using direct analysis in real-time mass spectrometry and principal component analysis for discrimination, Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess. 32 (2015) 799–807.
[14] F.M. Fernandez, R.B. Cody, M.D. Green, C.Y. Hampton, R. McGready, S. Sengaloundeth, et al., Characterization of solid counterfeit drug samples by desorption electrospray ionization and direct-analysis-in-real-time coupled to time-of-flight mass spectrometry, Chem. Med. Chem. 1 (2006) 702–705.
[15] Y. Shen, T.A. van Beek, F.W. Claassen, H. Zuilhof, B. Chen, M.W. Nielen, Rapid control of Chinese star anise fruits and teas for neurotoxic anisatin by direct analysis in real time high resolution mass spectrometry, J. Chromatogr. A 1259 (2012) 179–186.
[16] A.J. Dane, R.B. Cody, Selective ionization of melamine in powdered milk by using argon direct analysis in real time (DART) mass spectrometry, Analyst 135 (2010) 696–699.
[17] I. Ujvary, Psychoactive natural products: overview of recent developments, Ann. Ist Super Sanita 50 (2014) 12–27.
[18] E.A. Shikanga, A. Viljoen, S. Combrinck, A. Marston, Isolation of *Sceletium* alkaloids by high-speed countercurrent chromatography, Phytochem. Lett. 4 (2011) 190–193.
[19] N. Gericke, A.M. Viljoen, *Sceletium* – a review update, J. Ethnopharmacol. 119 (2008) 653–663.
[20] S. Patnala, I. Kanfer, Investigations of the phytochemical content of *Sceletium tortuosum* following the preparation of Kougoed by fermentation of plant material, J. Ethnopharmacol. 121 (2009) 86–91.
[21] G.M. Meyer, C.S. Wink, J. Zapp, H.H. Maurer, GC–MS, LC–MS(n), LC-high resolution-MS(n), and NMR studies on the metabolism and toxicological detection of mesembrine and mesembrenone, the main alkaloids of the legal high Kanna isolated from *Sceletium tortuosum*, Anal. Bioanal. Chem. 407 (2015) 761–778.
[22] S. Patnala, I. Kanfer, HPLC analysis of mesembrine-type alkaloids in *Sceletium* plant material used as an African traditional medicine, J. Pharm. Pharmaceut. Sci. 13 (2010) 558–570.
[23] J. Roscher, T.N. Posch, M. Putz, C. Huhn, Forensic analysis of mesembrine alkaloids in *Sceletium tortuosum* by nonaqueous capillary electrophoresis mass spectrometry, Electrophoresis 33 (2012) 1567–1570.
[24] NFLIS 2013 Survey of Crime Laboratory Drug Chemistry Sections, DEA, 2014.
[25] A. Walter, Backlog delays evidence processing at state crime labs. The News and Advance, 2014 6.09.2015.
[26] S. Singh, S. Verma, Study of the distribution profile of piperidine alkaloids in various parts of *Prosopis juliflora* by the application of Direct Analysis in Real Time Mass Spectrometry (DART-MS), Nat. Prod. Bioprospect. 2 (2012) 206–209.
[27] V. Bajpai, R. Pandey, M.P. Negi, N. Kumar, B. Kumar, DART MS based chemical profiling for therapeutic potential of *Piper betle* landraces, Nat. Prod. Commun. 7 (2012) 1627–1629.
[28] C. Lancaster, E. Espinoza, Evaluating agarwood products for 2-(2-phenylethyl)-chromones using direct analysis in real time time-of-flight mass spectrometry, Rapid Commun. Mass Spectrom. 26 (2012) 2649–2656.
[29] R.A. Musah, E.O. Espinoza, R.B. Cody, A.D. Lesiak, E.D. Christensen, H.E. Moore, et al., A high throughput ambient mass spectrometric approach to species identification and classification from chemical fingerprint signatures, Sci. Rep. (2015) 2015.
[30] M.T. Smith, N.R. Crouch, N. Gericke, M. Hirst, Psychoactive constituents of the genus *Sceletium* N.E.Br. and other Mesembryanthemaceae: a review, J. Ethnopharmacol. 50 (1996) 119–130.
[31] J-F. Cui, T-H. Zhou, J-S. Zhang, Z-C. Lou, Analysis of alkaloids in Chinese *Ephedra* species by gas chromatographic methods, Phytochem. Anal. 2 (1991) 116–119.

[32] B.J. Gurley, P. Wang, S.F. Gardner, Ephedrine-type alkaloid content of nutritional supplements containing *Ephedra sinica* (ma-huang) as determined by high performance liquid chromatography, J. Pharmaceut. Sci. 87 (1998) 1547–1553.
[33] M.C. Roman, Determination of ephedrine alkaloids in botanicals and dietary supplements by HPLC-UV: collaborative study, J. AOAC Int. 87 (2004) 1–14.
[34] B.T. Schaneberg, S. Crockett, E. Bedir, I.A. Khan, The role of chemical fingerprinting: application to *Ephedra*, Phytochemistry 62 (2003) 911–918.
[35] D. Lv, Y. Cao, Z. Lou, S. Li, X. Chen, Y. Chai, et al., Rapid on-site detection of ephedrine and its analogues used as adulterants in slimming dietary supplements by TLC-SERS, Anal. Bioanal. Chem. 407 (2015) 1313–1325.
[36] J.J. Ros, M.G. Pelders, P.A. De Smet, A case of positive doping associated with a botanical food supplement, Pharm. World Sci. 21 (1999) 44–46.
[37] Y. Shi, C. Sun, B. Gao, A. Sun, Development of a liquid chromatography tandem mass spectrometry method for simultaneous determination of eight adulterants in slimming functional foods, J. Chromatogr. A 1218 (2011) 7655–7662.
[38] C. Hu, J. Shi, S. Quan, B. Cui, S. Kleessen, Z. Nikoloski, et al., Metabolic variation between japonica and indica rice cultivars as revealed by non-targeted metabolomics, Sci. Rep. (2014) 2014.
[39] C.A. Haller, N.L. Benowitz, Adverse cardiovascular and central nervous system events associated with dietary supplements containing *Ephedra* alkaloids, N. Engl. J. Med. 343 (2000) 1833–1838.
[40] M.J. Cupp, Herbal remedies: adverse effects and drug interactions, Am. Fam. Phys. 59 (1999) 1239–1245.
[41] Final Rule Declaring Dietary Supplements Containing Ephedrine Alkaloids Adulterated Because They Present an Unreasonable Risk. http://www.fda.gov/food/guidanceregulation/ucm072997.htm. 07.1.2015.