Research Article

Rapid identification of synthetic cannabinoids in herbal samples via direct analysis in real time mass spectrometry

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RATIONALE: Dozens of synthetic cannabinoid analogs purposefully meant to circumvent legal restrictions associated with controlled substances continue to be manufactured and promoted as producing 'legal highs'. These designer drugs are difficult to identify in conventional drug screens not only because routine protocols have not been developed for their detection, but also because their association with complex plant matrices during manufacture generally requires labor-intensive extraction and sample preparation for analysis. To address this new and important challenge in forensic chemistry, Direct Analysis in Real Time Mass Spectrometry (DART-MS) is applied to the analysis of these designer drugs.
METHODS: DART-MS was employed to sample synthetic cannabinoids directly on botanical matrices. The ambient ionization method associated with DART-MS permitted the analysis of solid herbal samples directly, without the need for extraction or sample preparation. The high mass resolution time-of-flight analyzer allowed identification of these substances despite their presence within a complex matrix and enabled differentiation of closely related analogs.
RESULTS: DART-MS was performed to rapidly identify the synthetic cannabinoids AM-251 and JWH-015. For each cannabinoid, three hundred micrograms (300 μg) of material was easily detected within an excess of background matrix by mass.
CONCLUSIONS: New variations of herbal blends containing a wide range of base components and laced with synthetic cannabinoids are being produced, making their presence difficult to track by conventional methods. DART-MS permits rapid identification of trace synthetic cannabinoids within complex biological matrices, with excellent sensitivity and specificity compared with standard methods. Copyright © 2012 John Wiley & Sons, Ltd.

Synthetic cannabinoids are psychoactive chemical species that mimic the effects of cannabis (marijuana) when consumed. These compounds were originally designed for therapeutic purposes, to serve as agonists to the same receptors that bind the principal active ingredient of the cannabis plant, tetra-hydrocannabinol (THC).[1] Over 100 synthetic cannabinoid analogs are known. Their core structures vary widely, such that many have no obvious structural similarity to THC. Despite their structural diversity, these substances bind cannabinoid receptors 1 and 2 (CB$_1$ and CB$_2$), in some cases exhibiting receptor binding levels that are several orders of magnitude above that of THC.[1–4] The pharmacological effects associated with cannabinoid receptors and their agonists have been known for several decades, which led to research into developing synthetic cannabinoids as therapeutics.[2,5] Cannabinoid nomenclature is sometimes based on where the chemical was tested. For example, 'HU-210' was developed at the Hebrew University, Israel. Alternately, they have been named to highlight a core structural feature, such as the 'CP-' prefix in the case of CP-47,497, derived from its cyclohexylphenolic core. Two other classifications of synthetic cannabinoid compounds are based on the prefixes JWH- (e.g. JWH-018) and AM- (e.g.

AM-2201), based on the research performed by J.W. Huffman and A. Makriyannis (from Clemson University and Northeastern University in the USA, respectively). All four of these classes of cannabinoids have been identified in herbal products intended for illicit purposes. Synthetic cannabinoids intended for illicit use first appeared in the early to mid 2000s and, within a few years, widespread use was reported.[1,6] Later in the decade, herbal blends containing JWH and HU analogs were added to international controlled drug lists. However, dozens of different herbal samples are widely available, with a large range of active components, present in varying concentrations.[7–9]

To create the herbal products, synthetic cannabinoids are dissolved in a solvent, and the resulting solution is deposited on plant material. The doped plant material is then dried and smoked in a similar fashion to actual cannabis; these products are referred to as synthetic cannabis, but the popularity and extent of use associated with them is such that fashionable brands and related terminology exist. Thus, products such as 'Spice', 'K2', and 'Blaze' have been marketed as 'herbal incense' as a front to their intended purpose. Until recently when synthetic cannabinoids became regulated in the United States, the doped plant material was widely available in convenience stores and petrol stations, and easily found for sale on the internet. In an attempt to misrepresent the understood use and avoid legal scrutiny, the end product is advertised as 'not for human consumption'. None of these cannabinoids have been approved by the United States Food and Drug Administration (FDA) and no oversight exists regarding the manufacture of such substances. Internationally, reports of

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emergency room incidents and poison control data related to herbal formulations containing synthetic cannabinoids increased dramatically from 2008 to 2010.[1,10–12]

Detection and identification of synthetic cannabinoids are complicated by a number of factors. These substances are not part of routine drug screens and metabolites in urine would not show positive for marijuana use.[1,8] Dozens of different cannabinoid agonists have been associated with these illicit products, such that the exact cannabinoid can vary within a single brand, as can the botanical matrix onto which the active chemical has been deposited. The wide range of active ingredients and the variety of botanical matrices on which they are doped substantially complicate the analysis. New 'herbal' products continue to make their way into the supply chain, and novel active analogs have been detected within weeks after related substances have come under a country's regulatory control (such as JWH-073, which is now regulated).[8] Thus, second-, third-, and fourth-generation derivative products have been detected.[1,8] This rapid turn-around and replacement of active components as a means to circumvent legal restrictions demonstrates the seriousness of this problem and the acumen and intelligence of the manufacturers. The producers of these substances have shown the ability to monitor the restrictions in real-time and demonstrate a significant understanding of the underlying chemistry. This level of sophistication is of major concern for authorities and is a strong rationale for the need for rapid, effective measures to identify and detect the active components of these substances.

Limitations on the ability to test for the active components, the rapid emergence of these substances in the counter-culture, and the wide variety of active ingredients identified are serious hindrances to the development of standard analytical techniques for their detection. The different classifications of synthetic cannabinoid compounds are chemically different enough to allow for significant variability in detecting their presence. Manufacturers creating these compounds are knowledgeable enough of the chemistry to introduce subtle structural variations into the molecular framework, limiting the utility of routine methods of analysis for these compounds. None of the synthetic cannabinoids trigger a positive drug test using standard immunological screening procedures, and they are particularly problematic for screening methods that rely on a library search for identification, as these substances are rarely included in standard databases. Very little data associated with advanced methods of analysis have been documented, although a few groups cite liquid chromatography coupled with tandem mass spectrometry (LC/MS/MS) and other advanced techniques as feasible.[1,9,12] Direct Analysis in Real Time Mass Spectrometry (DART-MS) has demonstrated utility for the detection of psychotropic natural products, including THC, opioids and psilocin, largely because of the technique's ability to analyze material directly without labor-intensive or time-consuming extractions.[13,14] DART-MS methods have also shown utility in detecting trace levels of a wide range of controlled substances collected from a variety of surfaces.[15] As a logical extension of these reported works, we present the development of DART-MS methods to analyze and characterize synthetic cannabinoids from complex biological matrices, enabling a more comprehensive, rapid, and sensitive analysis without the need for sample extraction or processing of any kind.

EXPERIMENTAL

Materials

Two cannabinoid agonists were purchased from Sigma-Aldrich (St. Louis, MO, USA). The synthetic cannabinoid JWH-015 (2-methyl-1-propyl-1*H*-indol-3-yl)-1-naphthalenylmethanone) is a chemical in the naphthoylindole family, which acts as a cannabinoid agonist with affinity for CB_2 receptors. The cannabinoid AM-251 (1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-*N*-1-piperidinyl-1*H*-pyrazole-3-carboxamide) is an inverse agonist at the CB_1 cannabinoid receptor.

Preparation of plant samples

The two cannabinoids were dissolved in methanol. The dissolved cannabinoids were applied to dried plant material. For this study, three different plant matrices were used, *Ocimum basilicum*, *Mentha spicata*, and *Coriandrum sativum*, obtained from a local grocery store. Leaves of each plant were lyophilized, weighed, and 300 µL of a 1 mg/mL cannabinoid solution in methanol was pipetted onto 10 mg of dried plant material. Pure cannabinoids dissolved in methanol (~1 mg/mL) were used as standards. DART-MS analysis of cannabinoid samples was conducted by dipping a melting point tube in the solution and holding the liquid droplet briefly between the ion source and the detector.

DART ionization of plant samples

DART-MS experiments were conducted using a JEOL AccuTOF mass spectrometer, interfaced with a DART ion source. Since DART does not require sample preparation, each leaf was simply held with tweezers between the ionization source and the inlet to the mass analyzer. The ion source was operated with helium gas at varied temperature to demonstrate the versatility of the technique. Samples were analyzed 4 to 5 days after doping the plant material with synthetic cannabinoids, to allow for sufficient uptake of the cannabinoid by the plant material and to more closely mimic protocols used in illicit formulations.[16]

DART-MS parameters

An AccuTOF-DART (JEOL USA, Inc., Peabody, MA, USA) time-of-flight (TOF) mass spectrometer was used. The resolving power of the mass spectrometer was 6000 (full width half maximum) measured for protonated reserpine. A polyethylene glycol mass spectrum with average molecular weight 600 was included as a reference standard for exact mass measurements. The atmospheric pressure interface was typically operated at the following potentials: orifice 1 = 20 V, orifice 2 = 3 V, and ring lens = 3 V. In some experiments the voltage was reduced to 12 V for orifice 1 to minimize ion fragmentation. At lower voltages, ion cluster formation was enhanced. The radiofrequency (RF) ion guide voltage was set to 550 V to allow detection of ions greater than *m/z* 55. The DART ion source (IonSense Inc., Saugus, MA, USA) was operated with helium gas at two temperatures: 150°C and 200°C, and a flow rate of 2 L min⁻¹. The glow discharge needle was operated at 3500 V, the intermediate electrode (E1) at 150 V, and the grid electrode at 250 V. TSSPro3 software (Shrader Analytical, Detroit, MI, USA) and Mass Spec Tools programs (ChemSW Inc., Fairfield,

DISCUSSION

The synthetic cannabinoid compounds are solids in their pure form. For ingestion, the solid cannabinoids are incorporated into a matrix to facilitate their uptake. To prepare these substances, the solid is dissolved in a solvent and applied, sprayed, or brushed onto dried plant material. Once the solvent is evaporated and the herbal material re-dried, the doped plant materials can be crushed and smoked in a similar fashion to marijuana. Illicit manufacture has no quality controls or regulations and wide varieties of plant materials, active ingredients, and concentrations have been reported.[1,17] For example, one product lists alfalfa, marshmallow, blue violet, nettle leaf, comfrey leaf, *Gymnema sylvestre*, passion flower leaf, horehound, and neem leaf as ingredients.[11] Herbal matrices are found in a wide range of formulations and, although ingredient listings often cite numerous botanical or herbal components, many of the listed ingredients do not appear to be present.[2] Because the formulation of the botanical matrix is known to vary widely, the synthetic cannabinoids were deposited on three different arbitrary plant matrices. In addition, since these products are unregulated complex mixtures, the determination of actual dosages within a product is complicated by the lack of consistency in ingredient concentrations even between samples of the same brand. It has been estimated that the usual dose, ingested via smoking, would be in the low milligram range,[8] based on the known data from the studies performed during the original development of these substances as therapeutics. Figure 1 shows the structures of the two compounds used in this study, JWH-015 and AM-251, as well as those of THC and JWH-018 for comparison.
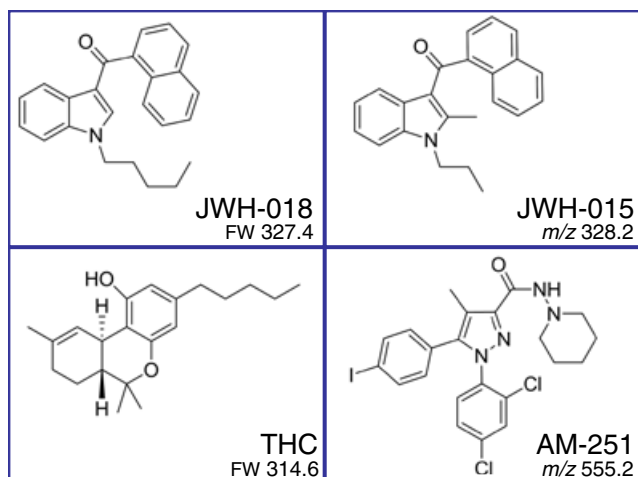
We employed Direct Analysis in Real Time (DART)-MS, which is a significant step forward in mass spectrometry analysis as it requires minimal, if any, sample preparation, and permits the identification of compounds without the use of a database *per se*. As such, while a database search of spectra or library matches would be informative and helpful in the identification of these substances, the high-resolution aspect of the DART-MS instrument can be helpful in the identification of unknowns. The technique allows for the analysis of solid plant material directly at atmospheric pressure by simply presenting the sample between the DART ion source and the mass detector (Fig. 2). Figure 2 illustrates the simplicity of sample analysis, showing a leaf of *Ocimum basilicum* laced with JWH-015, exposed to the DART source. The analysis of samples directly means that no solvents, extractions, or sample preparation are required. The resulting spectrum is produced in seconds. The experimental design results in high-throughput analysis with no carry-over between samples. For example, gas chromatography (GC)/MS analysis of the control and doped leaf samples, including all extractions from the plant material and 30 min GC/MS run times, required approximately 3 days to complete (data not shown). The DART-MS analysis allowed detection of the synthetic cannabinoids, but sampled all the material directly without any processing or extractions, and took less than 2 h. The DART ionization process has similarities to chemical ionization in practice, based on the atmospheric pressure interactions between long-lived electronic excited state atoms and the sample components.[14] The DART ion source uses excited state helium, acting in positive ion mode, which usually resulted in simple mass spectra characterized by the appearance of protonated ions ($[M+H]^+$). The spectra are plots of the molecular ion of each of the detected species versus their relative abundances. DART-MS has the ability to produce high-resolution mass spectra, permitting the immediate determination of the molecular formula of observed species.

A 3×3 sampling configuration was developed consisting of the two agonists deposited on three different botanical matrices, as well as three control samples (Fig. 3). For each
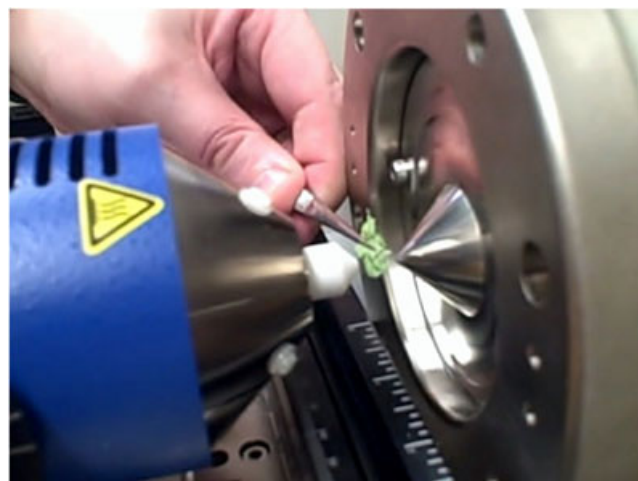

Figure 1. Chemical structures of the two synthetic cannabinoids (JWH-015 and AM-251) used in this study. For comparison, the structures of tetrahydrocannabinol and JWH-018 (currently scheduled by the United States Drug Enforcement Agency) are included.

Figure 2. Direct analysis of synthetic cannabinoid-doped plant material. Since DART-MS can sample solid material directly under ambient conditions, the sample need only be held with tweezers between the ion source (on left, in blue), and the cone of the mass spectrometer inlet (silver cone on right).
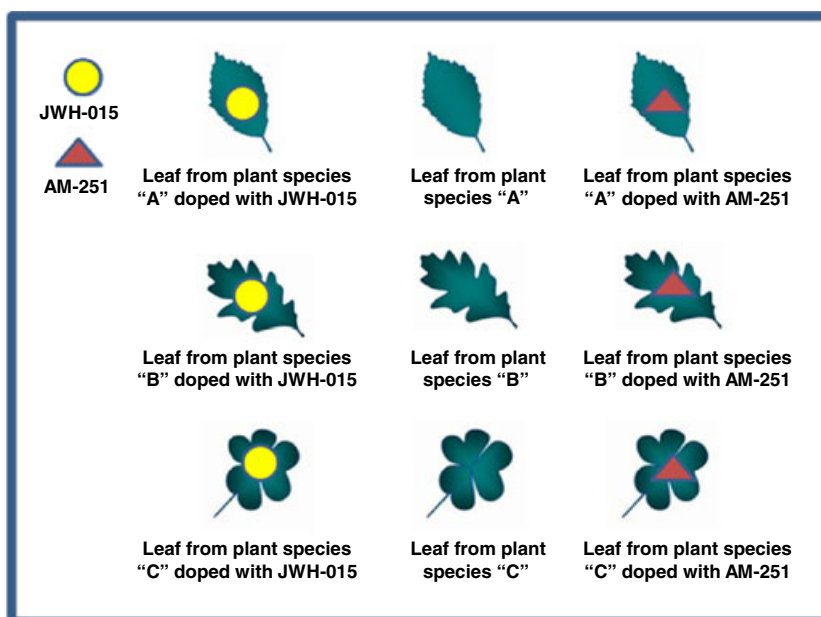
Figure 3. The experimental schematic for the testing of three plant species and two synthetic cannabinoids results in a 3 × 3 sampling strategy, including controls.
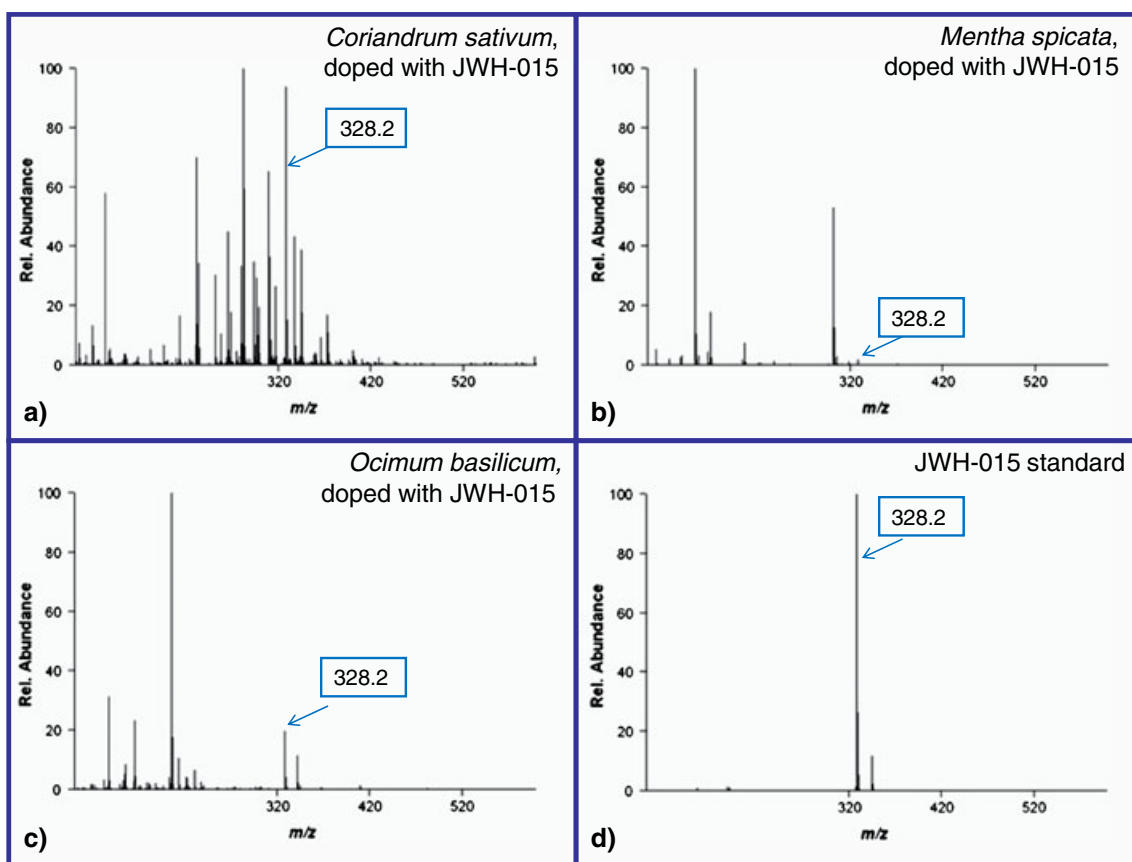

Figure 4. DART-MS analysis of the JWH-015 standard (bottom right), along with the spectra from three doped plant matrices. The [M+H]$^+$ peak is at *m/z* 382.2. All analyses were conducted at a helium temperature of 150°C.

of the spiked samples and controls, two DART analyses were conducted with different ionizing gas (helium) temperatures, to determine the optimum desorption temperature. The two

agonists studied represent the low and high end of the mass ranges of the known synthetic cannabinoids. Higher desorption temperatures were required for the higher mass

species. Spectra exhibiting relatively minimal complexity were observed at the lower temperature, which served to maximize agonist identification. Higher temperatures had the added benefit of yielding more complex spectra that could permit a more detailed identification of the plant matrix based on the natural products detected (data not shown). Initially, analyses of all controls and standards were performed. Supplementary Figs. S1 and S2 (Supporting Information) show the spectra for the three botanical matrices alone at varying desorption temperatures. As expected, the DART spectrum of each of the dried plant materials showed different levels of complexity, with *Coriandrum sativum* showing a rather complex series of peaks. *Mentha spicata* showed the simplest spectrum with the fewest number of peaks. It was relatively straightforward to differentiate the three spectral patterns by eye. In the absence of doping, none of the plant material exhibited peaks for the synthetic cannabinoids. The cannabinoid standards showed molecular ion peaks at m/z 555.1 and 328.2 for AM-251 and JWH-015, respectively (Figs. 5(d) and 4(d)). Figures, 4(a)–4(c) show DART-MS analyses of the plant material doped with JWH-015 as well as the JWH-015 standard at a desorption temperature of 150°C. At this temperature, the plant materials show peaks identical with the controls (Supplementary Figs. S1 and S2), but the JWH-015 (m/z 328.2) is readily visible in all three samples. However, under these same analysis conditions, the AM-251 in doped samples was not detected (data not shown). AM-251 is of considerably higher mass than JWH-015 and required

a higher desorption temperature for detection. Thus, the plant materials doped with AM-251 were reanalyzed with the helium temperature set to 200°C (Fig. 5). Under these conditions, the presence of AM-251 was observed in all three samples based on the peak at m/z 555.1. A possible concern with detecting synthetic cannabinoids is that the complex matrix on which they are doped might obscure detection, particularly if the matrix was comprised of several plants. However, when employing DART-MS with the plant matrices tested herein, no such difficulties were observed. For example, the JWH-015 peak at m/z 328.2 was readily apparent even when all three dried plants were combined (data not shown).

Illicit synthetic cannabinoid formulations continue to change in response to regulation, such that profiling of the known or suspected active ingredients found in these products will continue. Thus, reliable methods that can be used to detect their presence are critical. Given that the manufacturers have demonstrated the ability to rapidly modify the components and formulations that they market, instrumentation and methodologies that can readily identify the presence of prohibited compounds are highly desirable. DART-MS instrumentation and methods are a novel approach to address this problem, providing efficient, sensitive analyses without the requirement of labor-intensive extraction techniques. In total, our data demonstrate the utility of this approach in determining the presence of synthetic cannabinoids in herbal blends. Our experimental observations highlight the utility of DART-MS, but also the complexity of the problem of analyzing Spice products. The
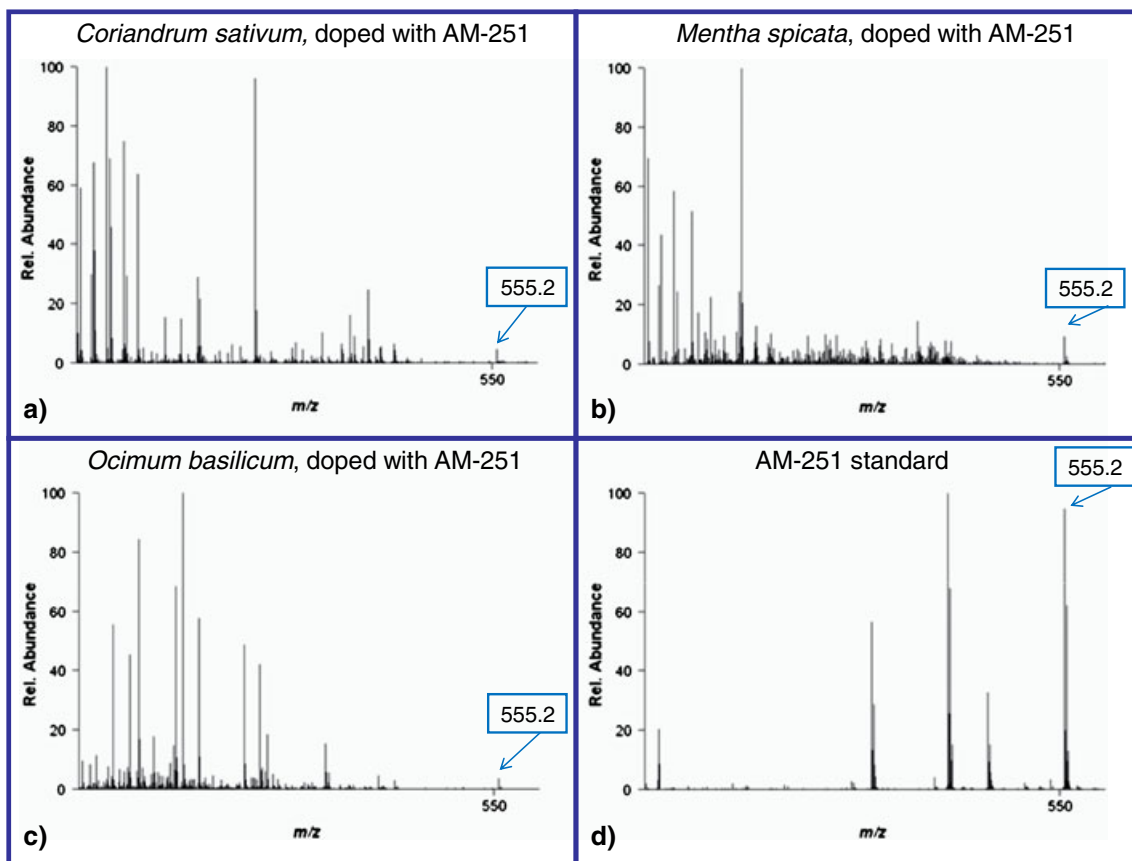

Figure 5. DART-MS analysis of the standard AM-251 (bottom right), along with the spectra from three plant matrices doped with AM-251. The [M]$^+$ peak is at m/z 555.2. All analyses were conducted at a helium temperature of 200°C.

two agonists tested required different desorption temperature conditions for their individual detection, such that method development to target the entire spectrum of compounds is necessary. The three plant matrices used in this study showed relatively complex mass spectral profiles, but this complexity did not prohibit identification of the target compounds via DART-MS. Ultimately this technique can serve as a means to simplify sample analysis by eliminating significant sample preparation and the concomitant loss of material that inevitably accompanies solvent extraction of analytes.

CONCLUSIONS

DART-MS has been shown to be effective for analysis in forensic drug chemistry, demonstrating successful detection of synthetic cannabinoids. While the identification of small molecule drugs is heavily reliant on mass spectral databases containing tens of thousands of known compounds, these libraries are generally devoid of the entire spectrum of synthetic cannabinoids and/or compounds related to the plant matrices in which they are found. However, as shown here, the simple protocols associated with the use of DART-MS can reduce or eliminate many of the problems associated with extraction or other sample preparation steps, and has the potential to *revolutionize* the discipline for forensic small molecule analysis. DART-MS does not require sample extraction or any preparation whatsoever, as it utilizes an ambient ionization source that can analyze solid samples directly. Direct sampling will make for more uniform protocol development and has a substantial time-saving component that will be of value for crime laboratories. DART-MS results are produced instantaneously; thus the rapid screening and the direct sampling ability offered by DART-MS have enormous potential to affect the forensic analysis of illicit substances in terms of cost benefit, time savings, and broad applicability. In particular, analyses associated with plant material, including marijuana, fungi, khat, and other natural products or substances of biological origin, could be streamlined.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article.

Acknowledgements

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