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Rapid Screening and Quantification of Synthetic Cannabinoids in DART-MS and NMR Spectroscopy

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Rapid Screening and Quantification of Synthetic Cannabinoids with DART-MS and NMR Spectroscopy

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The usage of herbal incenses intentionally doped with synthetic cannabinoids has caused an increase in medical incidents and has triggered legislation to ban these products throughout the world. Law enforcement agencies are experiencing sample backlogs due to the variety of the products and the addition of new and still-legal compounds. In our study, proton Nuclear Magnetic Resonance spectroscopy (NMR) was employed to promptly identify the synthetic cannabinoids after their rapid, direct detection on the herbs and in the powders by Direct Analysis in Real Time-Mass Spectrometry (DART-MS). Compared to conventional lengthy pre-NMR sample clean-up and purification, a simple sample preparation protocol was employed on 50 mg of herbal product samples for quick NMR detection. The combined DART-MS and NMR methods can be used to quickly screen synthetic cannabinoids in powder and herbal samples. Subsequently rapid quantification of cannabinoids can be achieved with short proton-NMR scans when an internal standard, maleic acid, is employed.

Introduction

Since 2006, synthetic cannabinoids such as JWH-018 (**Fig. 1** and **Fig. 2**a) have been reportedly mixed with natural herbs and sold as cannabis substitutes all over the world [1]. Smoking these synthetic cannabimimetic compounds in their pure form, and more commonly in herbal blends, has produced adverse effects in users such as anxiety attacks, vomiting and psychotic episodes which resulted in increased emergency room visits. Legislations passed in many countries attempted to ban these compounds with limited effect. Drug users are often inhaling synthetic compounds that are misrepresented with varying concentrations with ever-changing identity. New generations of these so called "Spice" products are constantly being released into the international market and are continuing to cause harm [2]. As a result, it has become urgent for forensic labs to promptly detect, identify, and quantify synthetic cannabinoids in their original powder form and in other consumer products, with minimal sample preparation and clean-up steps.

Many current methods include a combination of chromatographic separation (TLC, LC, and GC) and spectroscopic investigation (FTIR, UV-Vis, NMR and MS). The sample preparation also involves lengthy and expensive steps in order to get pure and clean compounds or mixtures with minimal herbal matrix. We proposed to use DART-MS and NMR to treat with virtually no or little sample preparation while taking advantage of the spectral separation power to rapidly identify and quantify (with proton-NMR) cannabinoids [3].

Direct Analysis in Real Time-Mass Spectrometry (DART-MS) has been previously used to rapidly detect narcotics with essentially no sample preparation and ultra-fast speed analysis under atmospheric conditions [4]. Uchiyama *et al* [5-7] have also utilized DART-MS as one of their confirmatory methods for several purified JWH- compounds extracted and separated from herbal blends. DART ionization occurs by introducing the sample (powder solids and liquids) into the gas stream, sometimes via a coated glass rod [4]. Peaks corresponding to protonated molecules are then detected within seconds by a high resolution Time-of-Flight Mass Spectrometer (TOF-MS). Using exact mass information, isotope peaks and fragmentation data under different cone voltage conditions, a compound of interest can be identified within minutes with minimal interference from the background. More recently, following rapid DART ionization, Musah *et al*. have successfully demonstrated how the fragmentation from the DART mass spectra can indicate the presence of specific structural features in synthetic cannabinoids [8, 9].

DART-MS, however, is not always able to differentiate between two isomers that have identical fragments. Thus it was recommended as a reliable screening tool for forensic

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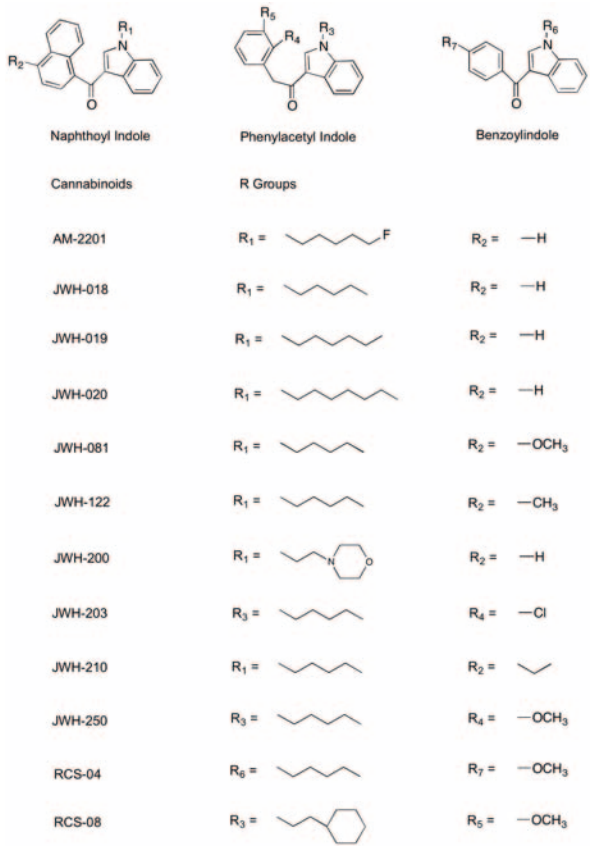
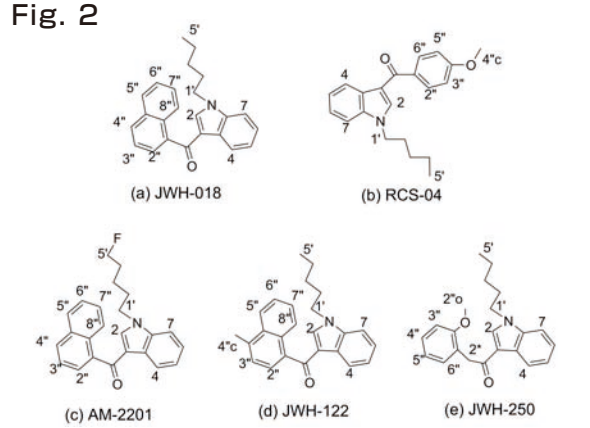
drug analysis [4]. Although time-dependent desorption can occur for compounds with differing volatility, the lack of a chromatographic separation method can in some cases limit the utility of the DART method. Additionally, when more than two synthetic components of varying concentrations are present in the herbal products, it may be difficult to interpret the overlapping fragment-ion mass spectra, thus resulting in the trace components possibly being overlooked. Consequently, additional confirmatory methods such as NMR can enhance the positive identification of positional isomers and all components in a mixture.

Fig. 1



Structures of synthetic indole cannabinoids 154 x 223 mm (300 x 300 DPI)

Fig. 2



Synthetic cannabinoid structures with numbering scheme: (a) JWH-018, (b) RCS-04, (c) AM-2201, (d) JWH-122, (e) JWH-250, 138 x 107 mm (300 x 300 DPI)

NMR has been extensively used to derive the structures of purified synthetic cannabinoids [1, 4, 5, 10, 11]. JWH-series and AM-series compounds (Fig. 1) have distinctive peaks in the proton NMR aromatic regions (6.5-9 ppm) as well as around 4 ppm, with little to no interference from natural components from the herbal base. Because of the high abundance of H-1, only a minimal amount of cannabinoid analyte is necessary to reach very low detection limits with a small amount of herbs (~100 mg or less). To render the dosage effective, usually the concentration of the synthetic cannabinoid ranges from 1-40 mg/g of herb (10). When the synthetic compound is extracted from the surface of the herbs into an NMR solvent, the final concentration range is from 0.1-10 mg/mL, which exceeds the NMR detection limit (~1 µg/mL) by several orders of magnitude.

Conventional structural elucidation by NMR has required cumbersome sample preparation steps to collect enough purified compounds (5 mg or more) and lengthy NMR experiments with H1-NMR, C13-NMR, DEPT, COSY, HMQC and HMBC that can last several days [1, 6, 10]. To ensure clean spectra, the cannabinoid samples had to be extracted from the herbal matrices and separated on TLC plates or chromatographic columns multiple times to get enough pure compounds [1, 5]. Our NMR sample preparation method is designed as a simplified protocol to dramatically reduce the time and sample size needed to positively identify cannabinoids in herbal products. The combination of rapid DART-MS and NMR can provide concrete cannabinoid structural information with no ambiguity, which can be a useful alternative, or complement, to conventional GC-MS and LC-MS methods. With the addition of an internal standard, quantitative proton-NMR can be completed for quantification of cannabinoids.

Experimental

Materials:

The standard cannabinoids were purchased from two sources. Primary standards (see **Table 2**) with good quality control were purchased from Cayman Chemical (Ann Arbor, MI, USA) and all of the other "standards" (stored in round plastic vials like the one in **Fig. 3**a) were purchased online from Mountain Industry (California, USA). The Mountain Industry powders were found to be of low quality with mixtures and/or mislabeled compounds identified within these samples (**Table 1** and Table 2). This company was a major online distributor for other online sellers of "Spice" products. The structures of the standard cannabinoids and the ones detected in our herbal samples are listed in Figs 1 and 2. The sample packages are displayed in Fig. 3 along with a microscopic image of an herb and a plastic vial containing one of the Mountain Industry powders. Figure 3b shows a close-up image of the leafy material in a product called "Moon Spice".

All of the standards were stored in a desiccator at 4°C. Deuterated chloroform ($CDCl_3$) and maleic acid was purchased from Sigma Aldrich (St. Louis, MO, USA). Several pure herbs such as damiana, mullein, and mugwort (from Amazon.com) were used to serve as a background or as blank samples for MS and NMR analyses.

DART-MS methods:

An AccuTOF-DART (JEOL USA, Inc., Peabody, MA, USA) time-of-flight mass spectrometer (TOF-MS) was used for all exact mass measurements (resolving power = 6000, FWHM definition). A mass spectrum of polyethylene glycol (PEG), with an average molecular weight of 600 g/mol, was included in each

Table 1

Label	DART-MS	Proton NMR
"Mountain Industry" Powder Samples		
AM-1221	AM-2201 (with impurity)	AM-2201 (with impurity)
AM-2201	JWH-019	JWH-019
JWH-122	JWH-200	JWH-200
JWH-203	As labeled with impurities	As labeled with impurities
Herbs		
Barely Legal	AM-2201 RCS-04	AM-2201 RCS-04
Melon Code Black	JWH-122 JWH-203	JWH-122 JWH-203
Moon Spice	RCS-04 JWH-018	RCS-04 JWH-018
Sweet Leaf	JWH-210 JWH-122 JWH-250	JWH-210 JWH-122 JWH-250
Apple Jacked	RCS-04 AM-2201	RCS-04 AM-2201
Chillin XXX	RCS-08 AM-2201 JWH-210	RCS-08 AM-2201 JWH-210
Funky Monkey	JWH-122	JWH-122
Ion Lab	RCS-04 AM-2201	RCS-04 AM-2201
Mr. Nice Guy	AM-2201 JWH-122	AM-2201 JWH-122
4 Winds	No Cannabinoid	No Cannabinoid

Identification results for synthetic cannabinoids and herbs.

data set as a reference standard for the exact mass measurements. The AccuTOF atmospheric pressure interface was operated with the potential settings for Orifice 1 = 20 V, Orifice 2 = 5 V, and Ring Lens = 3 V. At these potentials, little to no collision-induced dissociation (CID) occurs and the resulting mass spectra are dominated by protonated molecules ($[M+H]^+$). Fragmentation spectra were obtained via in-source CID with Orifice 1 voltages at 30, 60, 90 and 120 V, respectively. The RF ion guide voltage was set to 600 V to allow the detection of ions greater than *m/z* 60. The DART-SVP ion source (IonSense Inc., Saugus, MA) was operated with a helium gas heater temperature of 300°C and exit grid voltage of 250 V. TSSPro3 software (Shrader Analytical, Detroit, MI) and Mass Spec Tools software (ChemSW Inc., Fairfield, CA) were used for data processing and data interpretation. For standard analysis, the powdered sample was introduced directly into the DART stream on the closed end of a melting point tube. During spice analysis, three random pieces of plant material were selected from a given sample bag. Each sample was then held in the DART gas stream with forceps for 10 seconds. Afterwards, PEG 600 was measured within the same data file for the exact mass calibration. Prior to DART-MS analyses of the herbal blends with cannabinoids, the base herbs were also tested, which yielded no molecular ion peaks comparable to the synthetic cannabinoids. Most of the synthetic compounds possess molecular weights higher than 320 g/mol, and they produce strong, dominating, and distinctive peaks corresponding to protonated molecules.

NMR procedures:

H-1 NMR spectra were obtained on a JEOL JNM-ECS 400 MHz spectrometer (Peabody, MA, USA) with a JEOL 40th 5AT/FG2 5-mm proton/multi-frequency auto-tunable broadband probe and with $CDCl_3$ as the solvent. Chemical shifts were

Table 2

Label	MI "AM-1221"	Cayman AM-2201	MI "AM-2201"	Reference JWH-018 (1,7)	MI "JWH-081"	Cayman JWH-122	MI "JWH-122"	MI "JWH-203"	Cayman JWH-210	MI "JWH-250"	Cayman RCS-04
Actual	AM-2201	A.L.	JWH-19	JWH-018	A.L.	A.L.	JWH-200	A.L.	A.L.	A.L.	A.L.
H-2	7.30-7.41	7.34 S	7.34 S	7.34	7.35 M	7.32-7.38	7.44 M	7.87 S	7.36 M	7.86 S	7.57 S
H-4	8.49 M	8.49 M	8.48 M	8.49	8.46 M	8.48 M	8.52 M	8.39 M	8.48 M	8.40 M	8.36 M
H-5	7.34-7.40	7.34-7.37	7.35 M	7.33-7.39	7.35 M	7.34	7.36 M	7.28 M	7.32-7.40	7.25 M	7.28 M
H-6	7.34-7.40	7.34-7.37	7.33 M	7.33-7.39	7.31-7.41	7.32-7.38	7.33-7.42	7.33 M	7.32-7.40	7.25-7.32	7.31 M
H-7	7.34-7.40	7.34-7.37	7.37 M	7.33-7.39	7.31-7.41	7.32-7.38	7.33-7.42	7.36 M	7.32-7.40	7.25-7.32	7.38 M
H-2"	7.65 M	7.65 D	7.65 dD	7.64	7.65 D	7.55 M	7.65 D	-	7.55 M	-	7.84 D
H-3"	7.51 M	7.50 M	7.50 M	7.51	6.82 D	7.36 M	7.51 M	7.38 M	7.32-7.40	6.87 D	6.98 D
H-4"	7.96 D	7.96 D	7.96 D	7.95	-	-	7.96 D	7.19 M	-	7.21 M	-
H-5"	7.90 D	7.90 D	7.90 D	7.89	8.30 M	8.06 D	7.90 D	7.23 M	8.12 D	6.91 T	6.98 D
H-6"	7.50 M	7.49 M	7.51 M	7.5	7.49 M	7.54 M	7.50 M	7.29 M	7.55 M	7.29 M	7.84 D
H-7"	7.46 M	7.45 M	7.45 M	7.45	7.50 M	7.47 T	7.45 M	-	7.46 T	-	-
H-8"	8.18 D	8.18 D	8.18 D	8.19	8.33 M	8.24 D	8.17 D	-	8.24 D	-	-
H-1'	4.09 T	4.09 T	4.06 T	4.03	4.07 M	4.06 T	4.14 T	4.15 T	4.06 T	4.12 M	4.14 T
H-5'	4.37 dT	4.37 dT	<3	<3	<3	<3	<3	<3	<3	<3	<3
H-4"C1	-	-	-	-	-	<3	-	-	3.17 Q	-	-
H-2"O	-	-	-	-	-	-	-	-	-	3.81 S	-
H-4"O	-	-	-	-	4.05 S	-	-	-	-	-	3.88 S
H-2*	-	-	-	-	-	-	-	4.31 S	-	4.16 S	-

H-1 NMR chemical shift values of the standards used for the confirmation of their presence in herbal extracts.
MI= Mountain Industry. A.L.= as labeled, S=singlet, D=doublet, T=triplet, Q=quadruplet, M=multiplet, dD=doublet of doublets, dT=doublet of triplets

referenced to residual $CHCl_3$ at 7.24 ppm (^1H). The proton sensitivity of the NMR instrument is >=280:1 using 0.1% ethylbenzene in $CDCl_3$ when methyl quartet signal region was evaluated with measured 200 Hz noise width between 3 ppm and 7 ppm. Typically one to five milligrams of the standard powder samples were weighed, dissolved in 1 mL $CDCl_3$, and transferred to NMR sample tubes. Mountain Industry sample concentrations were roughly 5 mg/mL, and Cayman samples 1 mg/mL. The proton spectra were scanned 128 times (18 minutes) in the 0-10 ppm range, unless 512 scans (one hour) was necessary to obtain sufficient signal-to-noise ratio (S/N) for sample amounts less than 1 mg.

For "Spice" plant material sample analysis, ~50 mg of each herbal product was placed into ~1 mL of $CDCl_3$ and vortexed for one minute. The liquid solution was then transferred with a glass pipette to an NMR sample tube for NMR analysis. The proton NMR spectrum of each herbal extract was obtained after 32 scans (4 minutes) with a 4-second relaxation delay and chemical shift ranging from 0-10 ppm. The data were compared with the chemical shifts observed in the spectra of the standards to confirm the presence of the synthetic compounds.

With the powder sample, H-1 NMR was employed to elucidate the structures of synthetic cannabinoids. In most cases when a pure standard was available, matches of all chemical shift values were used to confirm its identity; for herbal samples, the standard chemical shift value ±0.1 ppm range was used to account for peak marking deviation when the DELTA software (JEOL USA) was utilized. The H-1 NMR spectra of the herbal extracts were compared with their standard counterparts, particularly in the aromatic chemical shift region (6.5-9 ppm) and the mid-field region (4-5 ppm) where overlapping signals from both the base herb and the synthetic components were avoided.

Quantitative Herbal Extraction NMR Preparation:

For quantification, between 1 to 2 mg of maleic acid (e.g. 1.5 mg or 1.8 mg) was accurately weighed out and added to ~50 mg of herbal product, also accurately weighed. Approximately 1 ml of d6-acetone was added to extract the cannabinoid and subsequently served as the NMR solvent. The sample was then run utilizing the method previously described for H-1 NMR analysis of the herbal extracts. We have found that the longitudinal relaxation time, T1, for these indole cannabinoids are lower than 4 seconds so 4 seconds relaxation was used to speed up the analytical process without sacrificing the quantitative accuracy.

Results

The DART-MS spectra of JWH-019 and "Moon Spice" herbal sample are presented in **Figs 4**a and 4b, respectively and are typical of the mass spectra observed for DART analyses. Figure 4c shows the comparison between the 90 V fragmentation mass spectrum from the Moon Spice sample and the pure JWH-018 standard. The exact masses for the matching ions within each spectrum were within 5 mmu of each other, thus indicating that they have the same elemental compositions. The other ions depicted in the Moon Spice 90 V spectrum (Fig. 4c) were produced from the fragmentation of the other cannabinoid compound present in the sample, RCS-04. The identification results on all of the other standards and herbal blends along with their NMR confirmations are presented in Table 1. The DART-MS results are generally supported by the NMR results. Sometimes, due to the limitation on NMR sensitivity, the minor ingredients had a poor S/N compared to the major ingredients or in some cases the signals from the minor components dropped below the limit of detection of the instrument. As a result, the ratio of peak areas is only a rough and semi-quantitative measure of each component.

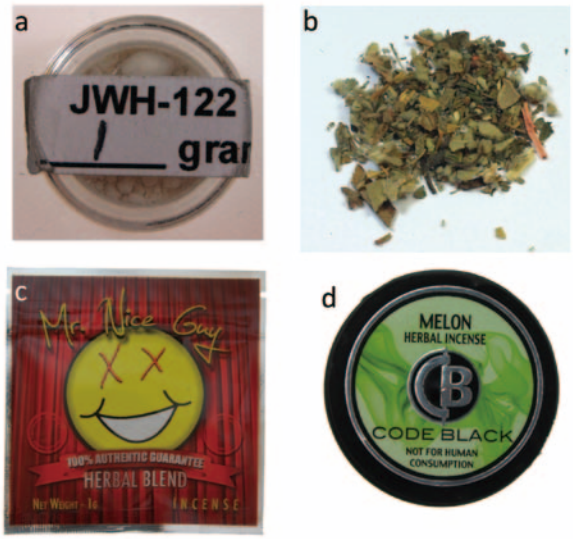
Three of the Mountain Industry powders were mislabeled synthetic cannabinoids and three contained other cannabinoids as contaminants (Table 1). The H-1 NMR chemical shift values of the standards are listed in Table 2, in which the Cayman standards had been correctly labeled and their spectra compared with those from Mountain Industry powders and the herbal extracts (Table 2 and **Table 3**).

Figure 5a is an H-1 NMR spectrum for the $CDCl_3$ extract of 50 mg of cannabinoid-free mugwort leaf. The sharp peak at 7.25 ppm is from protonated chloroform ($CHCl_3$), an impurity in the NMR solvent. As indicated in the spectrum, most of the signals from the leaf are within 1-3 ppm. Besides the residual solvent peak, the $CDCl_3$ extraction method did not produce any strong or noticeable signal from 3-10 ppm. The same phenomena were observed with mullein and damiana leafs, two popular choices for the base herb in incense products as indicated in online discussions among drug users.

Figure 5b is the H-1 NMR spectrum of 1.0 mg RCS-04 cannabinoid standard purchased from Cayman Chemical. As the spectrum indicates, the signals within 3.5-9 ppm do not overlap with blank herbal signals shown in the top panel. The bottom panel is from the $CDCl_3$ extracts of "Moon Spice" herbal incense. The signals for RCS-04 were found at seven locations. The remaining signals from 4-9 ppm are from JWH-018 according to literature values [1, 5] and the correlating chemical shift values are listed in Table 3. JWH-018 and RCS-04 were detected by both DART-MS and NMR (Table 1). Occasionally a proton signal for water (a broad singlet anywhere from 1.2 to 1.8 ppm) is present in the resulting spectra, but has not interfered with our region of interest: 3.5-9 ppm.

As Table 2 indicates, the "AM-1221" compound from Mountain Industry is indeed a mislabeled AM-2201 (Fig. 1).

Fig. 3



Various Spice products: (a) "Mountain Industry" JWH-122 powder, (b) "Moon Spice" leaf, (c) "Mr. Nice Guy" "Spice" package, (d) "Melon: Code Black" "Spice" package. 152 x 139 mm (300 x 300 DPI)

The herbal extract NMR data (Fig. 5 and Table 3) confirmed the results obtained in the DART-MS experiments (Figs 4b and 4c). HPLC-DAD and conventional GC-MS methods were utilized to confirm all positive identifications indicated in Table 1.

To quantify the cannabinoids present in the extract it is important that a well-phased spectrum is obtained. Minor phasing parameter adjustment can be made so the spectrum is in phase. The internal standard, maleic acid (MA), produces a signal at 6.37 ppm due to the two equivalent protons of the methylene group (**Fig. 6**). Well-resolved sample peaks are identified and manual integration is performed (Fig. 6). The MA peak area is normalized and the values obtained are plugged into the equation below to calculate the amount of cannabinoid in milligrams.

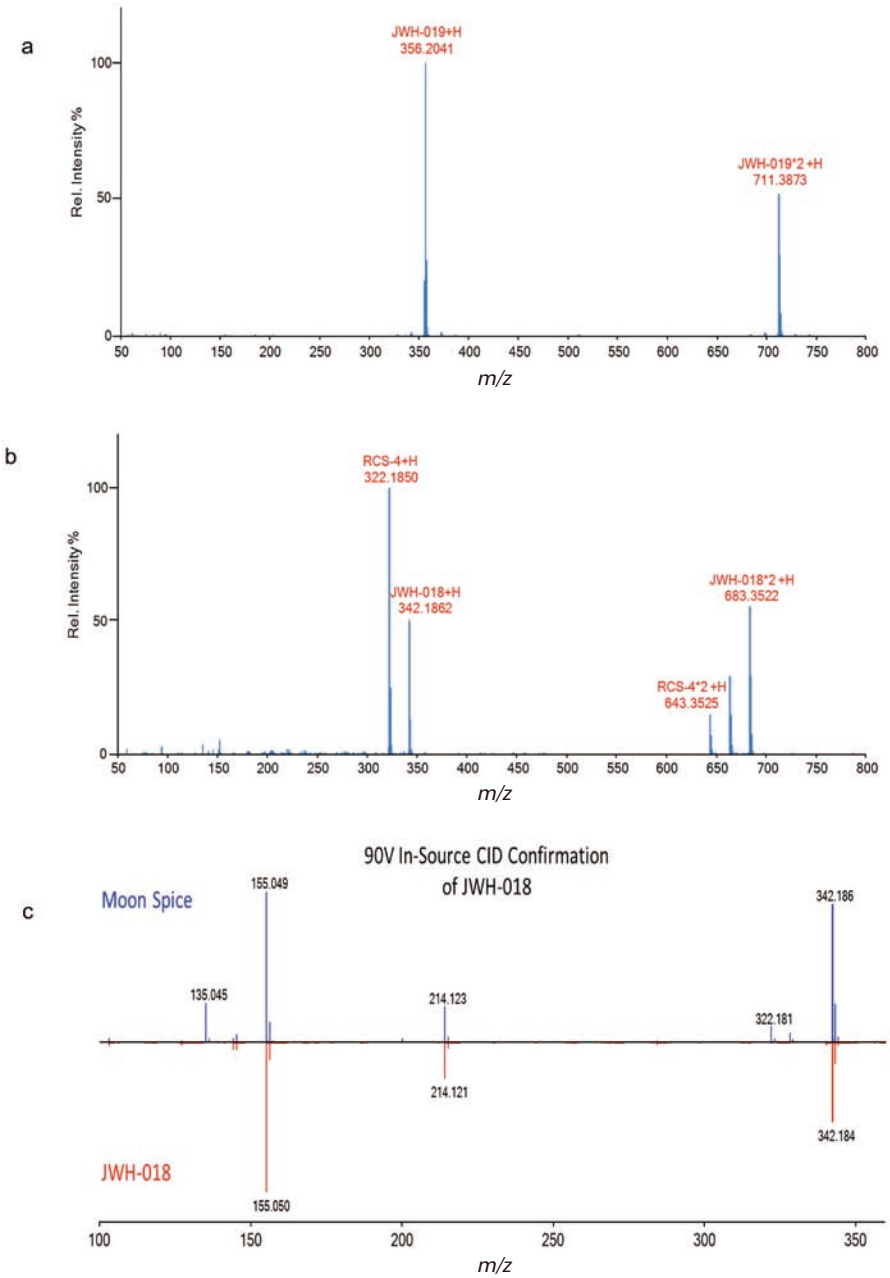
$$mg\ of\ cannabioid = \frac{(mg\ of\ MA)\times(\#\ of\ protons\ in\ MA)\times(Integral\ of\ cannabinoid\ peak)\times(FW\ of\ cannabinoid)}{(Integral\ of\ MA\ peak)\times(FW\ of\ MA)\times(\#\ of\ protons\ represented\ by\ cannabinoid\ peak)}$$

MA = Maleic Acid

FW = Formula weight (in g/mol)

Integral = integrated area under the peak of interest with arbitrary unit.

Fig. 4



DART-MS Spectra of (a) JWH-019 powder standard and (b) one piece of "Moon Spice" leaf, along with (c) the comparison of the 90V-spectra between "Moon Spice" leaf and JWH-018 standard powder. 381 x 508 mm (300 x 300 DPI)

The calculated mass of cannabinoid is divided by the amount of herb (in grams) initially weighed out in order to acquire a concentration in the form of mg of cannabinoid per g of herb in the Spice sample. The entire process of quantifying one sample is completed in less than 10 minutes. The quantitative NMR results are comparable to our chromatographic quantification results, both methods yielding 0.5-122 mg of cannabinoids per gram of herbal product. Because extraction with methanol is less efficient than with acetone, chromatographic quantification results only represent a fraction of the actual amount as indicated in our previous work (Table 2).

The quantitative results of twelve Spice products are displayed in Table 3. Variation in the manual peak integration was found to average about 3% using the same spectrum with five repeated integrations on three different peaks. Some herbal samples (K250, Head Trip, and Extremely Legal) were only quantified using three trials due to low sample availability. The integration results from different proton peaks of the same cannabinoids are very similar. As indicated in Table 3, the relative standard deviation varies (from 7% to 68%) due to the uneven spreading of synthetic components on herbal surface during the manufacturing process. This indicates that there is little to no quality control in the production of these substances, adding to the danger for consumption. Ingestion of even small amounts may result in pronounced effects because of inconsistencies in the dosage, significantly increasing the risk of these drugs. The LOD and LOQ were found to be 0.11 mg/mL and 0.36 mg/mL, respectively, with AM-2201 external standard calibration (0.1-1.5 mg/mL) and accurately-weighed maleic acid internal standard (1-2 mg).

Due to small sample size and uneven coverage of cannabinoids on the herbal samples, the results are only semi-quantitative with short proton scanning (4-second relaxation). Despite that, the methodology accurately represents drug consumption and therefore provides valuable information in this respect. Quantitative scanning takes the same amount of time as a qualitative scanning with $CDCl_3$. The total analytical time for five repeated trials is about one hour.

Summary

The selected blank herbal leaves are popular base-herb choices among makers of synthetic marijuana because they have pleasant aromas, low prices and are readily available. These leaf samples were analyzed through DART-MS as blanks and showed no mass spectral peaks that could be associated with synthetic cannabinoids. For the NMR experiments, the blank leaves were prepared using the same extraction method utilized for the herbal spice samples prior to their NMR analyses. Peaks were not found between 6.5-9 ppm or from 3.5-5 ppm, which is where most synthetic cannabinoids demonstrate strong signals. These results confirmed that the detected signals in the spice samples all originated from the synthetic compounds rather than natural herbal constituents.

The combination of DART-TOF-MS and NMR, used in conjunction with the standards, quickly identified the synthetic cannabinoids in their powder form and as an additive in the herbal products. Total analysis time was under one hour including about five minutes for DART-MS analysis and under 10 minutes for NMR analysis. According to our study, the four-minute 32-NMR scans generated an S/N of 4 to 1 for as little as 50 μg of a cannabinoid sample with successful identification. Our HPLC-Diode Array Detection (DAD) quantification on all the herbs (data not shown) revealed that the concentration

Table 3

Label	Barely Legal		Melon Code Black		Moon Spice		Sweet Leaf		
ID	AM-2201	RCS-04	JWH-122	JWH-203	RCS-04	JWH-018	JWH-210	JWH-122	JWH-250
H-2	7.28-7.41	7.41-7.58	7.32-7.40	7.88 S	7.57 S	7.36 M	7.32-7.40	7.32-7.38	7.86 S
H-4	4.48 M	8.36 M	8.46 M	8.39 M	8.36 M	8.47 M	8.48 M	8.48 M	8.39 M
H-5	7.28-7.41	7.28-7.41	7.32-7.40	7.28 M	7.25-7.38	7.33-7.38	7.32-7.40	7.32-7.38	7.25-7.32
H-6	7.28-7.41	7.28-7.41	7.32-7.40	7.32-7.40	7.25-7.38	7.33-7.38	7.32-7.40	7.32-7.38	7.25-7.32
H-7	7.28-7.41	7.28-7.41	7.32-7.40	7.32-7.40	7.25-7.38	7.33-7.38	7.32-7.40	7.32-7.38	7.25-7.32
H-2"	7.64 M	7.83 M	7.55 M	-	7.84 D	7.64 M	7.55 M	7.55M	-
H-3"	7.41-7.58	6.98 M	7.32-7.40	7.32-7.40	6.98 D	7.52 M	7.32-7.40	7.32-7.38	6.87 D
H-4"	7.96 M	-	-	7.19-7.23	-	7.96 D	-	-	7.21 M
H-5"	7.90 M	6.98 M	8.05 M	7.19-7.23	6.98 D	7.90 D	8.12 D	8.06 D	6.91 T
H-6"	7.41-7.58	7.83 M	7.54 M	7.26-7.30	7.84 D	7.48 M	7.55 M	7.54 M	7.25-7.32
H-7"	7.41-7.58	-	7.47 T	-	-	7.48 M	7.46 T	7.47 T	-
H-8"	8.16 D	-	8.22 M	-	-	8.17 D	8.24 D	8.24 D	-
H-1'	4.09 M	4.15 M	4.06 T	4.15 T	4.15 T	4.05 T	4.06 T	4.06 T	4.12 T
H-4"O	-	3.88 S	-	-	3.88 S	-	-	-	3.81 S
H-5'	4.37 dM	-	-	-	-	-		-	-
H-4"C	-	-	-	-	-	-	3.17 Q	-	-
H-2*	-	-	-	4.31 S	-	-	-	-	4.16 S

Chemical shift values identified in 50 mg herbal extracts. S=singlet, D=doublet, T=triplet, Q=quadruplet, M=multiplet

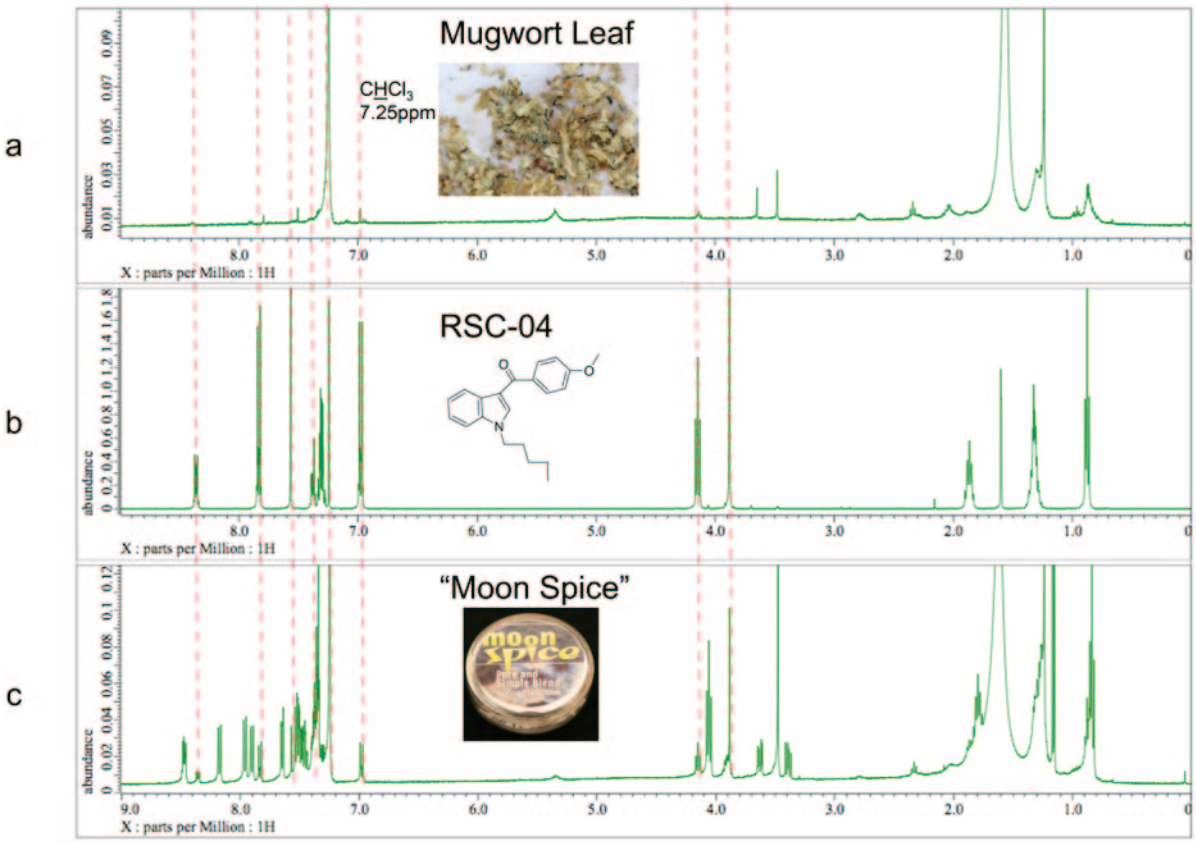
of cannabinoid on herbal base ranges from 1-50 mg/g of herb. 50 µg is usually below the amount we found on 50 mg herbal product. When the sample concentration falls below 0.05mg/mL comparable to DART-MS LOD [4], the NMR scan times have to be increased to four hours or more in order to obtain a spectrum with a S/N higher than 5. The adoption of 50 mg of herbal sample size for NMR investigation implies that at least 50 µg was placed in an NMR tube along with 0.5-1mL $CDCl_3$. The concentration of a cannabinoid was much higher than the detection limit of 1 part per million or 1 µg/g for H1-NMR. Mixtures of two or three cannabinoids were readily identified by using the combined NMR and MS methods (Table 1).

As Table 1 demonstrates, NMR and DART-MS complement each other in the analysis of herbal blends, especially when more than one synthetic cannabinoid is present. If one minor component is missed by one method, the other method usually detects it. The minor ingredient in the NMR spectrum often produces peaks with poor S/Ns so either more scans need to be acquired, which increases experiment time, or an increased sample amount (e.g. 200 mg) is necessary. Additionally, increased sampling with more sample batches is sometimes necessary to get a better representation of the whole package. The herbal sample is not homogenized to demonstrate the variation in concentrations for "hot" and "cold" spots, which could cause great harm for unaware users. Mixtures were detected with DART-MS spectra as signals of various heights, which further confirmed the non-uniformity of the synthetic compound distribution among the herbal bases. Sometimes only one compound was discovered on one piece of leaf while another piece from the same bag at a different location produced peaks responsible for two synthetic compounds in the mass spectrum. These results show that it is important to perform at least three different measurements using different leafs from a particular herbal sample to comprehensively identify all of the components in an herbal mixture. And this also made NMR confirmation very important as the 50-mg sample size usually contains more than a dozen pieces of leafs.

Our recent research efforts have extended towards using 2D NMR techniques for both identification and quantification [12]. The added dimension from the 2D NMR techniques provided additional signals that were easier to differentiate than those acquired by 1D NMR analysis, and valuable correlation signals for screening and comparison.

In summary, the combination of NMR and DART-MS can provide concrete identifications of synthetic cannabinoids rapidly and without ambiguity. The combined method also maximizes the potential of instrumental detection and signal separation power that is inherent in DART-MS and NMR while minimizing cumbersome wet chemistry processing and organic solvent usage. Up to a three-component mixture from herbal Spice sample was detected with the correct isomer identifications (Table 1). The DART-MS+NMR method will hopefully accelerate the drug detection process in the enforcement of current laws and regulations, as well as the detection of future blends sold as "herbal potpourri" or "legal highs".

Fig. 5



Proton-NMR spectra of (a) 50 mg blank herb "Mugwort Leaf" extracted with $CDCl_3$, (b) 5 mg RCS-04 standard powder in 1 mL $CDCl_3$, and (c) 50 mg "Moon Spice" herbal sample extracted with 1 mL $CDCl_3$. 558 x 431 mm (300 x 300 DPI)

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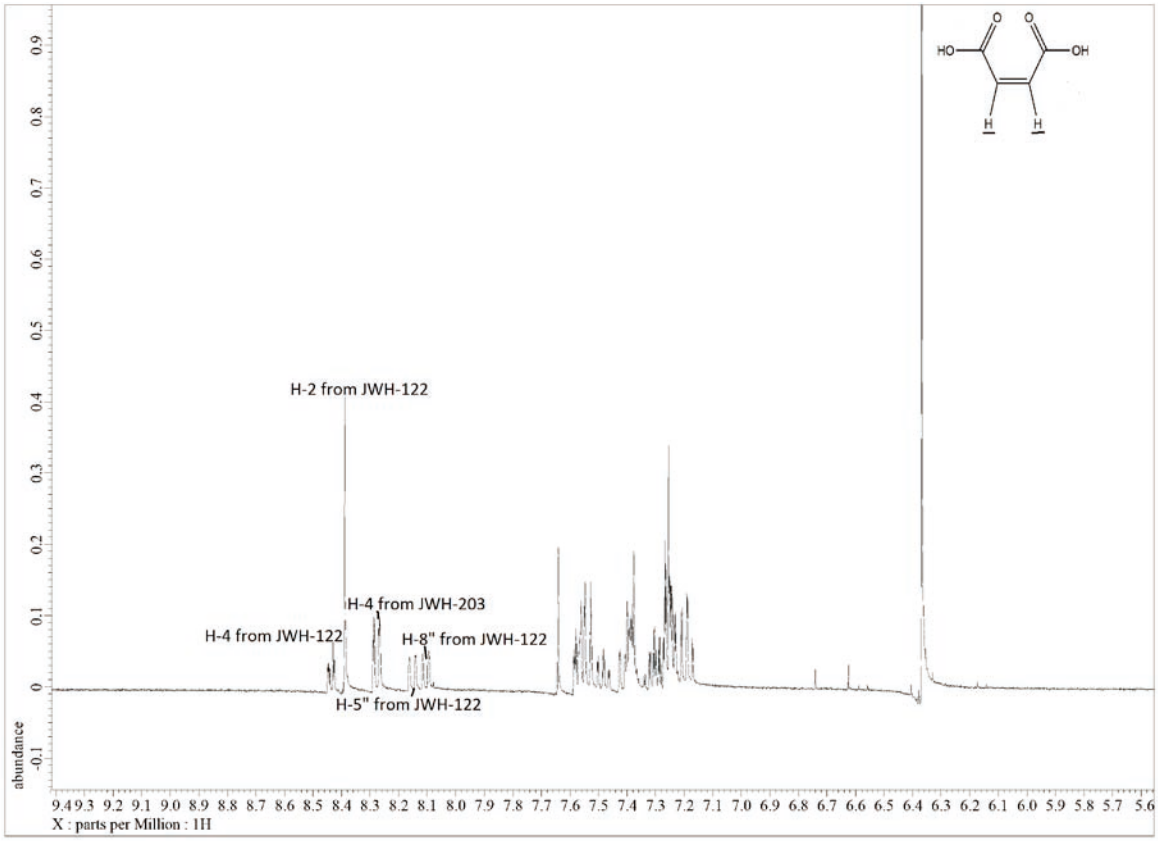
Fig. 6



Quantitative proton NMR spectrum of Melon Code Black with maleic acid internal standard. JWH-122 and JWH-203 were found and quantified.