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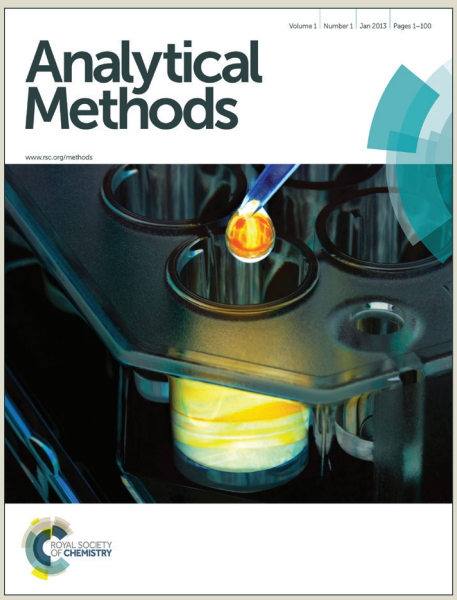

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Rapid screening and quantification of synthetic cannabinoids in herbal products with NMR spectroscopic methods

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Abstract

The abuse of "Spice" designer drugs, herbal incenses containing synthetic cannabinoids, has led to an increase in medical incidents and triggered legislations throughout the world banning these harmful designer substances. As more cannabinoids are added to that list, forensic analytical labs are experiencing sample backlogs due to the variety of the products and the addition of new and still-legal compounds. Here we use NMR spectroscopy exclusively to screen for and quantify synthetic cannabinoids in herbal products. In contrast to other qualitative and quantitative NMR methods that have appeared in the literature, in our methods synthetic cannabinoids were directly extracted with NMR solvent without any conventional lengthy isolation, purification or chromatographic separations. 1H NMR and proton correlation spectroscopy (COSY and TOCSY) were successfully employed to generate molecular fingerprints for synthetic cannabinoids in herbal extracts, taking advantage of the spectroscopic separation power from NMR spectroscopy. The added dimension from the 2D NMR techniques provided additional signals that are easier to differentiate than those acquired by 1D NMR analysis, and valuable correlation signals for screening and comparison. Quantification of cannabinoids by NMR was carried out in d6-acetone solutions with maleic acid as an internal standard, which generated quantitative results that were comparable to our previous HPLC quantification. The overall data suggest that in approximately one hour, NMR spectroscopy can be used exclusively for the non-destructive screening and quantification of synthetic drugs in complex herbal matrices.

Introduction

The use of herbal incense intentionally doped with synthetic cannabinoids has initiated the passage of multiple legislations and temporary regulations in the United States banning these designer drug products.[1-3] Despite that, new generations of "Spice" products such as STS-135 and URB-754 (structures in Figure 1) are continually being released into the international market.[4-5] Rapid identification and quantification of synthetic cannabinoids have become key objectives for forensic labs in recent years. The challenges remain to screen and quantify designer cannabinoids with adequate speed and minimal and economical preparation and clean-up steps.

Many current methods include a combination of chromatographic separation and spectroscopic investigation. The early work done by Auwärter [6], Lindigkeit[7] and Uchiyama [8-10] involved lengthy preparation techniques, including multiple extractions followed by thin layer chromatography (TLC) separation before enough material could be purified and collected for downstream analyses, such as nuclear magnetic resonance (NMR) [6-10] and mass spectrometry (MS)[8-10]. Logan *et al.* describes a method that involved multiple preparatory extractions with both methanol and acid-base reagents prior to TLC, gas chromatography with mass spectrometry (GC-MS), and liquid chromatography LC-MS analyses. [11] GC-MS was used for separation and identification while LC-time-of-flight mass spectrometry (TOF-MS) was employed for molecular and formula confirmation through the comparison of retention times with known standards. Finally, high performance liquid chromatography with diode array detector (HPLC-DAD) was used for quantification of cannabinoids in the herbal samples.[11]

Despite the "gold standard" status of GC-MS in forensic drug analytical labs, sometimes GC cannot sufficiently resolve structurally similar isomers that produce nearly identical mass spectra, making the library matching difficult and unreliable.[12] Retention times of isomers can be extremely close which makes them difficult to differentiate. The elevated temperatures associated with GC separation often degrade or alter the nature of an analyte, as witnessed in the cannabis GC analysis where decarboxylation was usually observed [13]. Another concern is carry-over "ghost" peaks that are often observed on GC chromatograms.[14] As has been pointed out by other investigators [15-16], heat can lead to isomerization and degradation of cannabinoids, (e.g. AM-2201 isomerizes to JWH-018, whereas heat destroys the cyclopropyl ring of UR-144 and XLR-11, structures in Figure 1). In an attempt to compensate for these drawbacks, NMR spectroscopy can be employed. Unlike most GC methods, NMR analysis is

carried out at room temperature so there is no thermal degradation. More significant for evidentiary material, NMR preserves the sample so downstream analyses are possible.

In our previous study, proton NMR was combined with Direct Analysis in Real Time-Mass Spectrometry (DART-MS) for the rapid screening of synthetic cannabinoids in herbal samples. [17] Whereas DART-MS is a relatively new and expensive technique that makes it hard to find in forensic labs, many labs already own an NMR. It is at this juncture that our research has focused on the exclusive use of NMR spectroscopy for the comprehensive screening and quantification of synthetic cannabinoids.

Proton-proton correlation spectroscopy (COSY) NMR technique is used by protein chemists to 'fingerprint' secondary protein structures [18]; therefore it is natural to extend this technique to 'fingerprint' smaller synthetic cannabinoids. In this paper we propose to use 'Dirty' NMR sample preparation method, with which the herbal product is directly immersed in solvent in preparation for NMR analysis and the conventional purification steps are circumvented. This new method is especially advantageous in forensic labs for several reasons: sample preparation and clean-up are comparatively fast and easy, smaller sample sizes are required, extraction efficiency is higher, and one can accurately identify and quantify cannabimimetic compounds in a mixture utilizing NMR alone.

In addition to COSY, the 'Dirty' NMR method can be employed in other NMR experiments, such as Total Correlation Spectroscopy (TOCSY) TOCSY is similar to COSY in that it analyzes proton-proton correlations; however, it has the power to correlate protons beyond two and three bond couplings on the same chain or ring. This provides more structural information and confidence when assigning signals to specific protons.

Experimental

Materials and Methods

Chemicals and Materials

Cannabinoid standards listed in Figure 1 were purchased from Cayman Chemical (Ann Arbor, MI, USA). Solvents (chloroform-D, d6-Acetone, and methanol) and maleic acid were acquired from Sigma-Aldrich (Milwaukee, WI, United States). All solvents and powder standards were used without further purification. Some Cayman standards were shipped in a methanol solution. In those cases, the methanol solvent was evaporated in a vacuum oven heated

at 55°C for 10 minutes to retain dry powder. Herbal products were obtained from various internet vendors, local smoke shops or convenience stores, and some directly from users.

Standard Sample Preparation and NMR Analysis

Standard samples were prepared by adding ~ 1 mL 99.96% chloroform-d ($CDCl_3$) to a cannabinoid standard sample of no less than one milligram, generating 1 mg/mL solution. ^{1}HNMR and 2-D NMR COSY experiments were performed using a JEOL ECS 400 MHz spectrometer with a 5-mm proton/multi-frequency probe. NMR operation and data analysis were carried out with bundled DELTA software. For the single pulse experiments 256 scans (~ 30 minutes) were acquired with a 4 second relaxation delay. Double quantum filtered proton correlation spectroscopy (DQF-COSY) with a 90 degree pulse sequence was obtained with 8 scans and a 1.5 second relaxation delay so that each experiment required approximately 60 minutes. TOCSY experiments on standards were run with the same parameters as COSY experiments. All NMR scanning was carried out at 25 °C.

Extraction from Herbal Sample and NMR Analyses on Extracts

Approximately 50 mg of herbal product was added to 1 mL 99.8% $CDCl_3$, which also served as an extraction solvent. The extraction of the cannabinoids from the leaf surface was encouraged through manual shaking or vortex-assisted mixing for ~1 min. The extracted solution was then transferred to an NMR sample tube. Each sample was subjected to single-pulse, COSY, and TOCSY NMR experiments. For the herbal extracts, 32 scans (4 second relaxation delay, ~ 4 minutes) were acquired for the single-pulse experiments, and a single scan (1.5 relaxation delay, ~ 8 minutes) was run in the COSY experiments. TOCSY experiments on herbs were run in a similar fashion to COSY experiments but with 8 scans (60 minute run time The NMR standard spectra were then used to compare and identify synthetic cannabinoids in the herbs.

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 ^{1}H 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 and Discussion

COSY correlations of synthetic cannabinoids in standards and herbal samples

In this investigation COSY correlations in twelve herbs, Melon Code Black, Cloud Nine, Sweet Leaf, Head Trip, Extremely Legal, Zombie Matter, Moon Spice, Ion Source, Rack City, Nuclear Bomb, and Mr. Nice Guy, were identified using the 'Dirty' NMR method (Table 1). In our previous study, chemical shifts of protons were assigned to these twelve herbs after elucidating the chemical shifts in cannabinoid standards. [17]Proton-proton correlation spectroscopy (COSY) reveals short-range couplings (2 – 3 bonds) between protons and is therefore useful for structurally characterizing compounds. The signals observed in the COSY spectra obtained from each of the herbal extracts were compared to those obtained from COSY spectra of the standard samples. Positive identifications were made when the signals found in herbal extract matched standard values within ±0.01 ppm on both axes. Because herbal samples were minimally processed before being introduced into the NMR sample tubes, the herbal signal could potentially interfere with the cannabinoid signal. In order to investigate potential herbal interferences, three popular base herbs (damiana, mugwort and mullein) were purchased online and processed using the same pre-NMR sample preparation methods. Surprisingly, in the 48 street samples analyzed, virtually no interfering signals were observed in the COSY spectra, probably due to the low concentrations of cannabinoids that minimize any chemical shift change. Despite the fragrances and food dyes that were purportedly added to these products, the NMR spectra obtained of the extracts are relatively free of interfering peaks. In addition to indole cannabinoids, our method can be expanded to study additional compounds with indazole or other core structures by selecting new key signals.

In our previous study, signature peaks were suggested for quick qualification of synthetic cannabinoids in herbal mixtures. For COSY, three signature signals or "spots" are also suggested; however the signal range incorporates a larger span because couplings between neighboring hydrogens may encompass signals with greater variations in their chemical shift values. The first signature signal is produced by the H-4/H-5 coupling interaction and appears between 6.5-8.5ppm. This signal can usually be spotted at the lower left corner of the spectrum. The second signature signal is produced by H-1'/H-2'coupling and appears between 1.5-4.5 ppm. This signal is easily spotted in the upper right corner. In contrast to the spectra acquired from single-pulse experiments where the 1-3 ppm region is crowded with herbal signals, the COSY data suggests that the noise is due to the herbal matrix and does not interfere with the proton-proton correlations of cannabinoid signals in the alkyl region. The third signature COSY signal can be chosen from the range 1.5-4.5ppm or 6.5-8.5ppm, where correlations between neighboring hydrogens located on

aliphatic or aromatic carbons, respectively, appear. Because the correlations usually span over larger chemical shift ranges the visual detection of the signals is easier with the aid of the extra dimension in COSY.

The COSY NMR spectra for Cloud Nine (Figure 2) indicates the presence of these signature spots correlating with the interactions occurring between neighboring hydrogens, H-4 and H-5. In fact, this correlation is evident in the spectra for all cannabinoids found in the herbal sample. Single-pulse NMR analysis confirmed the presence of the cannabinoids JWH -122, JWH-210, and JWH-250 in Sweet Leaf.[17] Quantitative NMR (qNMR) and LC-DAD analysis support that, even at low concentrations (0.2mg cannabinoid/mg herb), peaks corresponding to the three cannabinoids are evident after a 7 minute scan. Therefore, combining 2-D NMR with proton NMR allows for better spectral separation thanks to the added dimension in 2-D NMR.

Proton qualification in the twelve herbal samples was reported in our previous study.[17] Proton NMR alone, however, was not sufficient to consistently differentiate the alkyl substituent off the indole or naphthyl rings from one cannabinoid to the other. This was due to interference by signals produced by the herb that coincide with the signals of the cannabinoids appearing in the aliphatic region of the spectra. Subtle differences between XLR-11 and UR-144 (Figure 1) could be observed in the aliphatic region in the absence of herbal background after lengthy chromatographic separation had been performed. COSY analysis of Cloud Nine, however, made the distinction between the peaks produced by the aliphatic groups and signals from the base herb more obvious so that the identities of the cannabinoid present in the herb could be confirmed. Because of the structural similarities (Figure 1) between XLR-11 and UR-144 (these compounds are identical except for the presence of a fluorine atom at the distal end of the pentyl chain attached to the indole nitrogen of XLR-11); proton-NMR cannot conclusively differentiate between the two cannabinoids when they are present in a mixture.

TOCSY Identification

Total correlation spectroscopy (TOCSY) experiments (Table 2) were run on Melon Code Black (MCB) herbal incense (Figure 4) and JWH-203 standard powder (Figure 1, found in MCB), along with the other 11 herbs. TOCSY spectra incorporate interactions between neighboring protons as well as longer range couplings between protons on a chain or in a ring. Therefore, it is useful to look at a COSY spectrum to discern between neighboring interactions and longer range interactions in the TOCSY spectrum.

In both Melon Code Black and JWH-203, a TOCSY interaction between H4 and H6 on the indole ring was observed, so this could potentially be a signature TOCSY spot. An H4/H6 interaction attributed to JWH-122 is not observed, which could be due to a low concentration of this compound on the herb, or because of other signals overlapping in this region. However, an H5"/H7" interaction from the naphthyl group on JWH-122 is observed. A major advantage of TOCSY is that the overlapping signals often observed in the alkyl regions of "dirty" solution extracted from herbal incenses are more clearly resolved and therefore more easily elucidated. TOCSY provides additional signature spots to those obtained from COSY experiments allowing for more accurate signal elucidation of aliphatic hydrogens that are typically difficult to fingerprint in ^{1}H NMR.

Heteronuclear Single Quantum Coherence HSQC-TOCSY and nuclear Overhauser effect spectroscopy NOESY are NMR techniques that may also be incorporated to further confirm the identity of the compound. For example, 2-D NMR techniques such as HMQC (heteronuclear multiple-quantum correlation spectroscopy) can be implemented to aid in the identification of the more complicated structures, such as the adamantyl group which appears as a substituent in place of a naphthyl and benzyl group in some of the compounds. However, these NMR techniques involve scanning for carbon nuclei and therefore require significantly many more scans than proton 1D or 2D NMR techniques in order to achieve satisfactory signal to noise ratio because the low abundance of 13C in the samples.

Quantitative Proton NMR

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 (Figure 3). Well-resolved sample peaks are identified and manual integration is performed (Figure 3). 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.

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 S2).

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.

It should also be noted that conventional methods of herbal product analysis call for the sample to be ground up with sand paper and stirred to homogenize the distribution of synthetic cannabinoids.[10]The homogenization of Spice samples, however, does not take into account the hot and cold spot variations. This information is critical as an unwary user might smokes a portion of the “cold spot” and feel nothing, then smoke a second batch from the same bag, hit on the hot spot and end up overdosing. The ‘Dirty’ NMR technique circumvents the time-consuming grinding step. In order to evaluate the extraction efficiency with d6-acetone, mock street samples were prepared

using known amount (20 mg) of cannabinoid AM-2201 (in 3 mL methanol) sprayed on one gram of a popular herbal leaf, damiana. The extraction efficiency was found to be 75±6 % (N=4).

Method Validation Study

To validate our NMR results, 11 undergraduate students working in groups of three or four students performed the experimental method outlined above and were able to identify and quantify cannabimimetic compounds, specifically indole cannabinoids, in three herbal products: "Sweet Leaf", "Cloud Nine", and "Melon Code Black", incorporating the use of Proton and COSY NMR. Both the quantitative and qualitative results of the study were representative of our research.

Compared to conventional NMR approaches in the analysis of designer drugs, our 'Dirty' NMR method consumes significantly less time. Our pre-NMR sample preparation takes an average analyst about 10 minutes including weighing, sample extraction and transfer to the NMR tube. The proton NMR procedure takes 4 minutes and COSY takes 7 minutes. Usually the cannabinoid identity can be discovered within 30 minutes from sample in to answer out. Each quantification scan only takes about 20 minutes from weighing to analysis. TOCSY analysis takes less than an hour to complete and can complement the proton and COSY data.

All of the qualitative results from NMR investigations were confirmed with conventional GC-MS methods. The detailed method and results are presented in Table S1 of supplemental information. GC-MS method was successful at separating the multiple cannabinoids presented in the herbal extract, but the library search often generated results of multiple isomers. Some of these isomers have very close retention times that are hard to differentiate. NMR serves as a useful alternative tool for isomer differentiation.

Conclusions

Accurate qualification and quantification of synthetic cannabinoids in herbal samples can be rapidly and exclusively achieved by the NMR spectroscopic techniques described herein. A simplified sample preparation technique that was previously coupled with rapid proton-NMR can be expanded to include COSY, TOCSY, and quantitative NMR methods to quickly detect and quantify synthetic cannabinoid in "Spice" products. The identification and quantification steps can be completed within an hour from sample in to answers out. As a non-destructive method, NMR can clearly identify regio-isomers that are hard to differentiate in conventional GC-MS methods.

Incorporating COSY and TOCSY in conjunction with 1H NMR provides unambiguous structure elucidation so that isomeric cannabinoids, such as JWH-019, JWH-180 and JWH-122 can be differentiated. The simple and rapid quantitative NMR method employs an inexpensive internal standard, maleic acid. Between 0.5-50 mg cannabinoid/g herbal sample were discovered in the "Spice" products. The quantitative results are comparable to results obtained by our previous HPLC-DAD analysis. The NMR-exclusive identification and quantification methods can potentially serve as alternatives or complementary methods to conventional MS-based drug analytical methods for fast screening of designer drugs.

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Table 1 COSY signals of cannabinoids detected in herbal samples (N/D=not/detected)

Label Name	Sweet Leaf			Cloud Nine		Melon Code Black		Ion Source	
Possible ID	JWH-122	JWH-250	JWH-210	XLR-11	UR-144	JWH-122	JWH-203	JWH-203	RCS-08
H-4/H-5	8.49/7.35	8.40/7.26	8.49/7.35	8.39/7.26	8.39/7.26	8.46/7.34	8.38/7.27	8.39/7.29	8.38/7.24
H-5/H-6	N/D	N/D	N/D	7.26/7.33	7.26/7.33	N/D	7.28/7.35	N/D	N/D
H-6/H-7	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
H-2"/H-3"	7.55/7.37	-	7.58/7.38	-	-	7.54/7.36	-	-	-
H-3"/H-4"	-	N/D	-	-	-	-	7.38/7.20	7.38/7.20	6.88/7.24
H-4"/H-5"	-	N/D	-	-	-	-	N/D	N/D	N/D
H-5"/H-6"	8.07/7.55	N/D	8.12/7.54	-	-	8.05/7.53	N/D	N/D	6.91/7.29
H-6"/H-7"	7.54/7.46	-	7.54/7.46	-	-	7.54/7.46	-	-	-
H-7"/H-8"	7.47/8.24	-	7.47/8.24	-	-	7.46/8.22	-	-	-
H-1'/H-2'	4.07/1.80	4.12/1.86	4.07/1.80	4.17/1.94	4.14/1.88	4.05/1.78	4.14/1.87	4.15/1.88	4.14/1.75
H-2'/H-3'	N/D	N/D	1.80/1.26	1.94/1.48	1.88/1.34	1.78/1.24	1.87/1.32	1.88/1.32	1.75/1.75
H-3'/H-4'	N/D	N/D	N/D	1.49/1.72	N/D	N/D	N/D	N/D	1.75/1.75
H-4'/H-5'	1.28/0.85	N/D	1.28/0.85	1.73/4.43	1.34/0.89	1.26/0.83	1.33/0.88	1.32/0.89	1.75/1.25
H-4"Ca/H-4"Cb	-	-	3.19/1.44	-	-	-	-	-	1.25/0.90

Rack City	Extremely Legal		K250	Moon Spice		Zombie Matter	
XLR-11	JWH-122	JWH-210	JWH-250	RCS-04	JWH-18	AM-2201	JWH-210
8.40/7.25	8.47/7.34	8.47/7.34	8.39/7.25	8.35/7.30	8.48/7.35	8.49/7.35	8.49/7.35
7.26/7.32	N/D	7.33/7.40	N/D	N/D	N/D	N/D	N/D
7.32/7.34	N/D	N/D	N/D	N/D	N/D	N/D	N/D
-	7.55/7.35	7.58/7.38	-	7.84/6.98	7.65/7.53	7.65/7.50	7.58/7.38
-	-	-	6.88/7.21	-	7.51/7.96	7.52/7.96	-
-	-	-	7.21/6.91	-	N/D	N/D	-

-	8.07/7.55	8.12/7.54	6.90/7.29	7.84/6.98	7.90/7.50	7.90/7.50	8.12/7.54
-	7.55/7.45	7.55/7.45	-	-	N/D	N/D	N/D
-	7.48/8.23	7.48/8.23	-	-	7.46/8.17	7.45/8.23	7.45/8.23
4.17/1.94	4.06/1.80	4.06/1.80	4.11/1.85	4.15/1.87	4.06/1.80	4.09/1.86	4.07/1.80
1.94/1.50	1.80/1.25	1.80/1.25	1.85/1.31	1.87/1.32	1.80/1.24	1.86/1.39	1.80/1.26
1.49/1.72	N/D	N/D	N/D	N/D	N/D	1.39/1.65	N/D
1.73/4.43	1.27/0.84	1.27/0.84	1.31/0.88	1.33/0.88	1.27/0.85	1.64/4.36	1.28/0.83
-	-	3.18/1.43	-	-	-	-	3.17/1.43

Head Trip		Mr. Nice Guy		Nuclear Bomb
JWH-122	JWH-210	JWH-122	AM-2201	JWH-122
8.47/7.34	8.47/7.34	8.48/7.35	8.48/7.35	8.48/7.33
N/D	N/D	N/D	N/D	N/D
N/D	N/D	N/D	N/D	N/D
7.55/7.35	7.55/7.35	7.55/7.36	7.64/7.51	7.55/7.35
-	-	-	7.52/7.96	-
-	-	-	N/D	-
8.06/7.55	8.12/7.54	8.06/7.54	N/D	8.06/7.55
7.55/7.45	7.55/7.45	N/D	N/D	7.55/7.45
7.45/8.23	7.45/8.23	7.46/8.23	7.46/8.17	7.47/8.24
4.06/1.80	4.06/1.80	4.06/1.80	4.09/1.85	4.06/1.78
1.80/1.24	1.80/1.24	1.79/1.25	1.85/1.39	1.78/1.24
N/D	N/D	N/D	1.40/1.65	N/D
1.28/0.83	1.28/0.83	1.26/0.84	1.64/4.37	1.28/0.83
-	3.18/1.42	-	-	-

Table 2 TOCSY signals of selected herbs (N/D=not detected)

Label	Melon Code Black		Moon Spice		Cloud Nine		Sweet Leaf		
	JWH-203	JWH-122	JWH-018	RCS-04	XLR-11	UR-144	JWH-210	JWH-122	JWH-250
H-2/H-4	N/D	7.35/8.46	7.34/8.48	N/D	N/D	N/D	7.33/8.48	7.33/8.48	N/D
H-4/H-6	N/D	N/D	N/D	N/D	8.40/7.32	8.40/7.32	N/D	N/D	N/D
H-5/H-7	7.30/7.40	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
H-1'/H-3'	4.15/1.32	N/D	N/D	4.15/1.31	4.17/1.50	4.14/1.34	4.07/1.26	N/D	N/D
H-1'/H-4'	N/D	N/D	4.04/1.27	N/D	N/D	4.14/1.32	N/D	N/D	N/D
H-2'/H-4'	N/D	1.80/1.29	1.79/1.26	N/D	N/D	N/D	N/D	N/D	N/D
H-2'/H-5'	1.83/0.82	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
H-3'/H-5'	N/D	N/D	N/D	1.32/0.88	N/D	N/D	N/D	N/D	N/D
H-2"/H-4"	-	-	7.64/7.95	N/D	-	-	-	-	-
H-2"/H-8"	-	N/D	N/D	-	-	-	N/D	N/D	-
H-3"/H-5"	N/D	7.44/8.05	N/D	N/D	-	-	N/D	N/D	N/D
H-3"/H-6"	N/D	7.44/7.55	N/D	N/D	-	-	N/D	N/D	N/D
H-4"/H-8"	-	-	N/D	-	-	-	-	-	-
H-5"/H-7"	-	N/D	7.90/7.46	-	-	-	N/D	N/D	-
H-5"/H-8"	-	8.05/8.23	7.89/8.18	-	-	-	8.12/8.23	N/D	-
H-6"/H-8"	-	N/D	N/D	-	-	-	N/D	N/D	-

Rack City	Ion Source		Extremely Legal XXX		Head Trip	
XLR-11	JWH-203	RCS-08	JWH-122	JWH-210	JWH-122	JWH-210
N/D	7.31/8.38	N/D	7.36/8.46	7.36/8.46	7.34/8.46	7.34/8.46
8.39/7.32	N/D	N/D	N/D	N/D	N/D	N/D
N/D	N/D	N/D	N/D	N/D	N/D	N/D
4.17/1.50	N/D	N/D	4.06/1.24	4.06/1.26	4.06/1.26	4.06/1.26
4.17/1.72	N/D	N/D	4.06/1.28	4.06/1.28	N/D	N/D
1.75/1.73	N/D	N/D	1.80/1.28	1.80/1.28	N/D	N/D

1.95/4.43	N/D	1.75/1.25	1.81/0.83	1.81/0.83	N/D	N/D
1.50/4.43	N/D	1.75/1.25	1.24/0.84	1.26/0.84	1.26/0.84	1.26/0.84
-	-	-	7.56/8.06	-	N/D	N/D
-	-	-	7.57/8.23	7.57/8.23	7.55/8.23	7.55/8.23
-	N/D	6.88/6.92	N/D	N/D	N/D	N/D
-	N/D	6.88/7.29	N/D	N/D	N/D	N/D
-	-	-	-	-	-	-
-	-	-	N/D	N/D	N/D	N/D
-	-	-	8.06/8.23	N/D	N/D	N/D
-	-	-	N/D	N/D	N/D	N/D

Nuclear Bomb	Zombie Matter		Mr Nice Guy		K250
JWH-122	AM-2201	JWH-210	AM-2201	JWH-122	JWH-250
7.35/8.47	7.34/8.47	7.36/8.49	7.34/8.48	7.34/8.48	N/D
N/D	N/D	N/D	N/D	N/D	N/D
N/D	N/D	N/D	N/D	N/D	N/D
4.06/1.25	N/D	4.06/1.26	4.09/1.39	4.07/1.24	4.13/1.30
N/D	N/D	4.06/1.28	4.09/1.64	N/D	4.13/1.30
1.80/1.28	1.80/1.28	1.80/1.28	1.86/1.64	1.79/1.27	1.85/1.31
N/D	N/D	N/D	1.86/4.36	N/D	1.85/0.88
N/D	N/D	1.26/0.84	1.39/4.36	1.24/0.84	1.31/0.88
-	7.65/7.96	-	7.65/7.96	-	-
N/D	N/D	N/D	N/D	7.55/8.06	-
N/D	7.90/7.50	N/D	7.50/7.90	N/D	6.87/6.92
N/D	N/D	7.38/7.54	N/D	N/D	6.88/7.28
-	N/D	-	N/D	-	-
8.06/7.46	N/D	8.12/7.45	7.90/7.45	8.07/7.45	-
8.06/8.23	N/D	8.12/8.24	7.90/8.18	8.06/8.23	-
7.55/8.22	N/D	7.54/8.23	7.47/8.18	7.55/8.23	-

Table 3 Quantitative NMR results with d6-Acetone as NMR solvent (S=singlet, D=doublet, T=triplet, M=multiplet, dD=doublet of doublets, and dM=doublet of multiplets)

Herb Name	Cannabinoid	Peak (ppm)	Assignment	mg of cannabinoid/g herb	SD	RSD
Sweet Leaf	JWH-250	8.27 S	H-2	1.7	1.2	68%
	JWH-250	6.93 D	H-3"	1.9	1.2	65%
	JWH-250	4.12 S	H-2*	1.5	1.0	66%
	JWH-122	8.43 M	H-4	4.7	2.8	59%
	JWH-210	7.64 S	H-2	6.5	3.0	46%
	JWH-210	4.25 T	H-1'	6.5	3.1	47%
	JWH-210	1.39 T	H-4" b	6.5	3.0	46%
Melon Code Black	JWH-203	8.39 S	H-2	31.9	4.0	12%
	JWH-203	8.28 D	H-4	31.2	3.5	11%
	JWH-203	4.37 S	H-2*	31.4	3.8	12%
	JWH-122	8.43 M	H-4	18	2	12%
	JWH-122	8.16 D	H-5"	18	2	13%
	JWH-122	8.11 D	H-8"	18	2	13%
Cloud Nine	UR-144	4.28 M	H-1'	53.5	3.8	7%
	XLR-11	4.46 T	H-5'a	50.6	3.7	7%
Ion Source	JWH-203	4.36 S	H-2*	4.2	1.4	34%
	RCS-08	6.93 D	H-3"	8.1	1.1	14%
Rack City	XLR-11	8.35 M	H-4	118.6	23	19%
Extremely Legal	JWH-122	2.79 S	H-4"a	116.6	20.5	18%
	JWH-210	1.39 T	H-4"b	19.6	2.8	14%
K250	JWH-250	7.51 D	H-7	10.2	0.9	9%
Head Trip	JWH-122	2.79 S	H-4”C	109.2	35.2	30%
	JWH-210	3.17 M	H-4”Ca	13.8	9.7	70%

Nuclear Bomb	JWH-122	8.43 M	H-4	11.8	1.5	13%
Zombie Matter	AM-2201	7.97-8.03 dD	H-4"+H-5"	26.7	4.7	17%
	JWH-210	3.18 M	H-4"a	27.7	4.3	16%
Mr. Nice Guy	AM-2201	8.03 D	H-8"	110.7	15.7	14%
	AM-2201	7.97 D	H-4"	108.2	15.4	14%
	AM-2201	1.64 dM	H-4'	108.6	14.2	13%
	JWH-122	8.17 D	H-8"	32.9	4	12%
	JWH-122	2.73 S	H-4"C	33.2	3.9	12%
	JWH-122	0.80 T	H-5'	33.8	3.6	10%
Moon Spice	RCS-04	8.35 M	H-4	4.6	0.4	8%
	JWH-18	8.48 M	H-4	23.0	7.0	31%

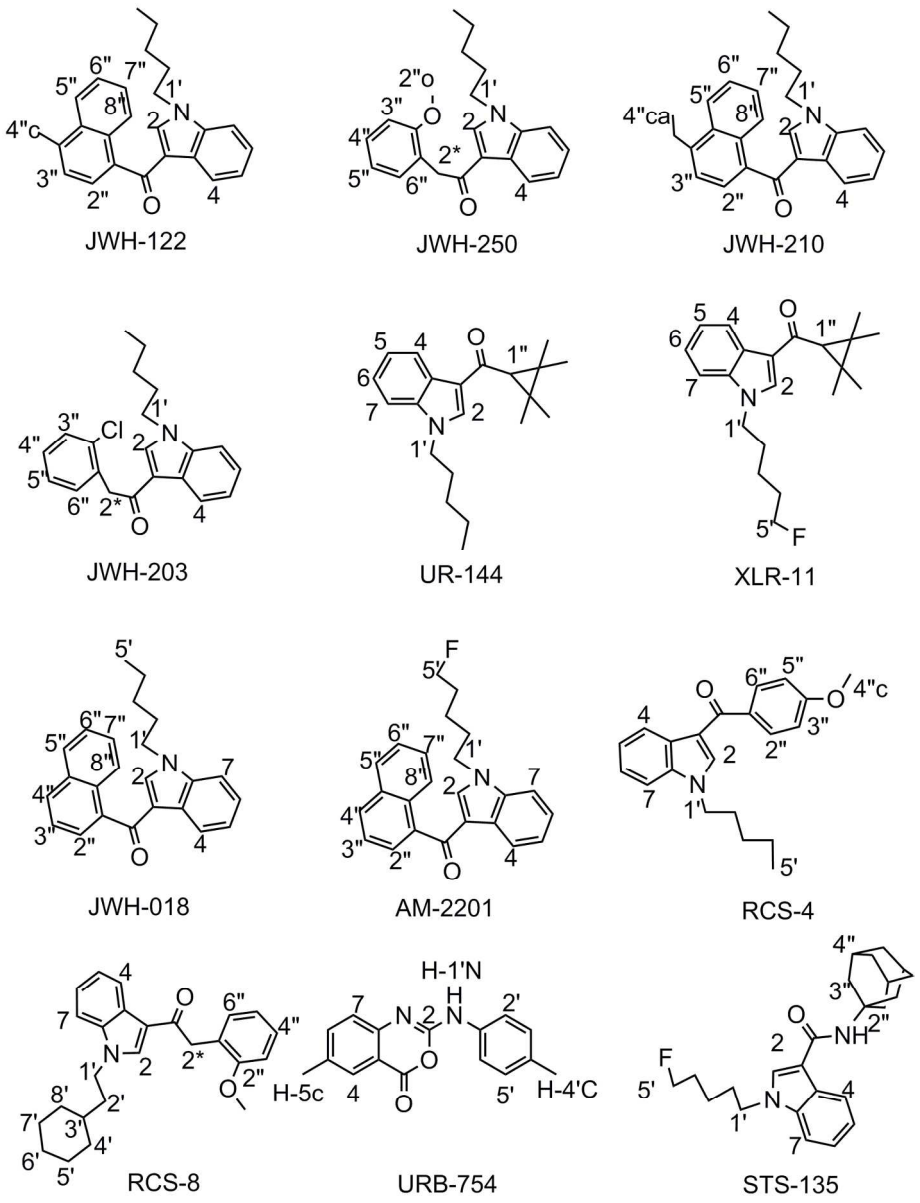


Figure 1 Numbered structures of cannabinoids found in herbal incenses (numbering method adopted from reference 7 with modifications).
159x208mm (300 x 300 DPI)

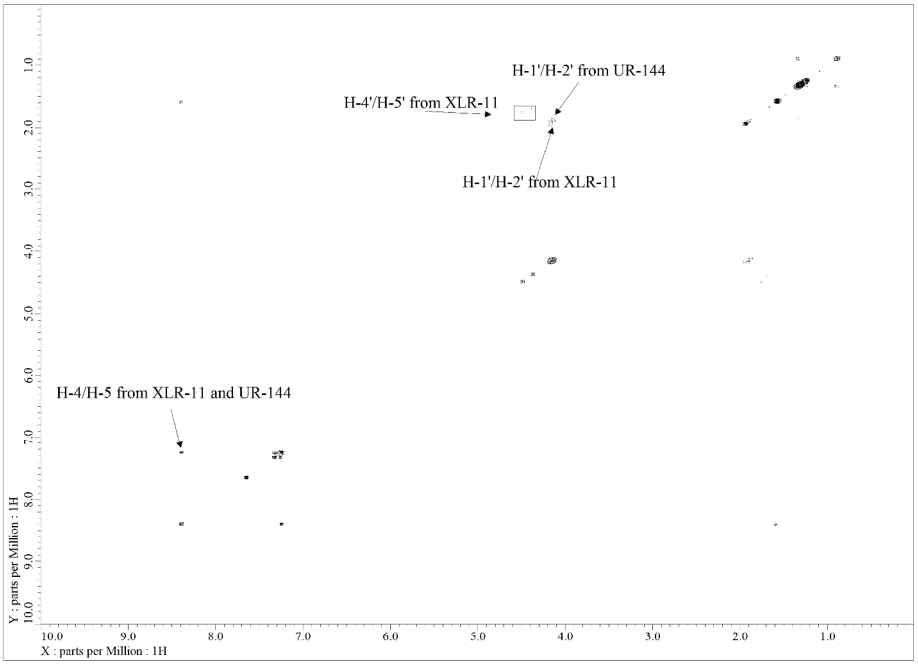


Figure 2 Cloud Nine COSY spectrum with identified signals from XLR-11 and UR-144
279x215mm (300 x 300 DPI)

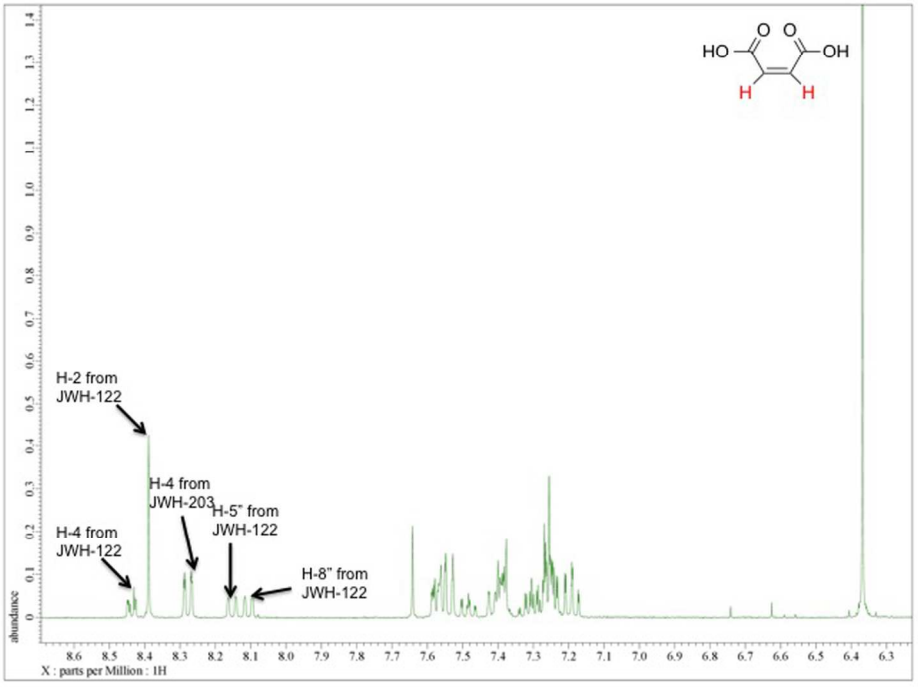


Figure 3 Quantitative proton NMR spectrum of Melon Code Black with maleic acid internal standard. JWH-122 and JWH-203 were found and quantified.
246x190mm (300 x 300 DPI)

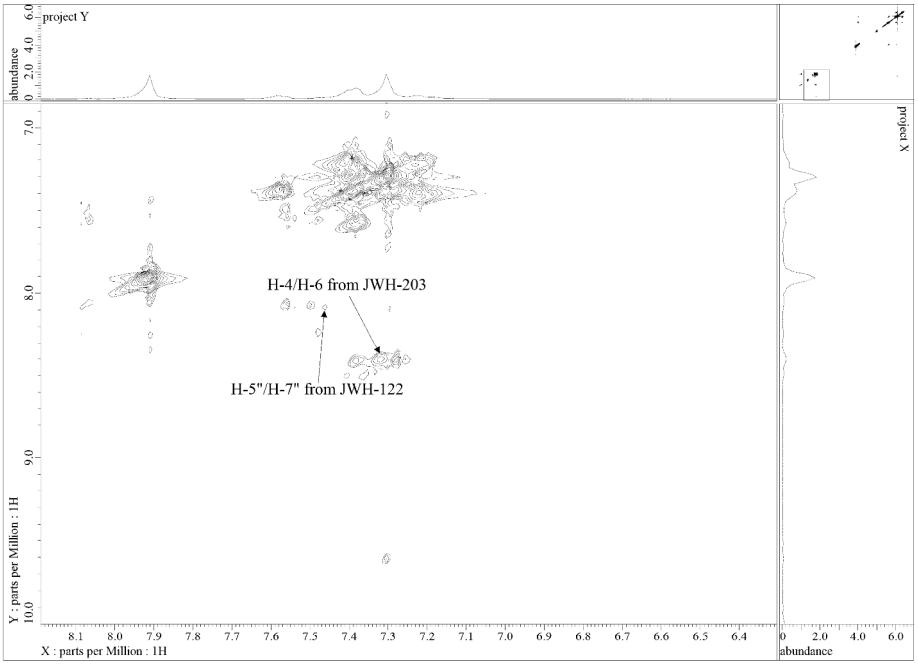
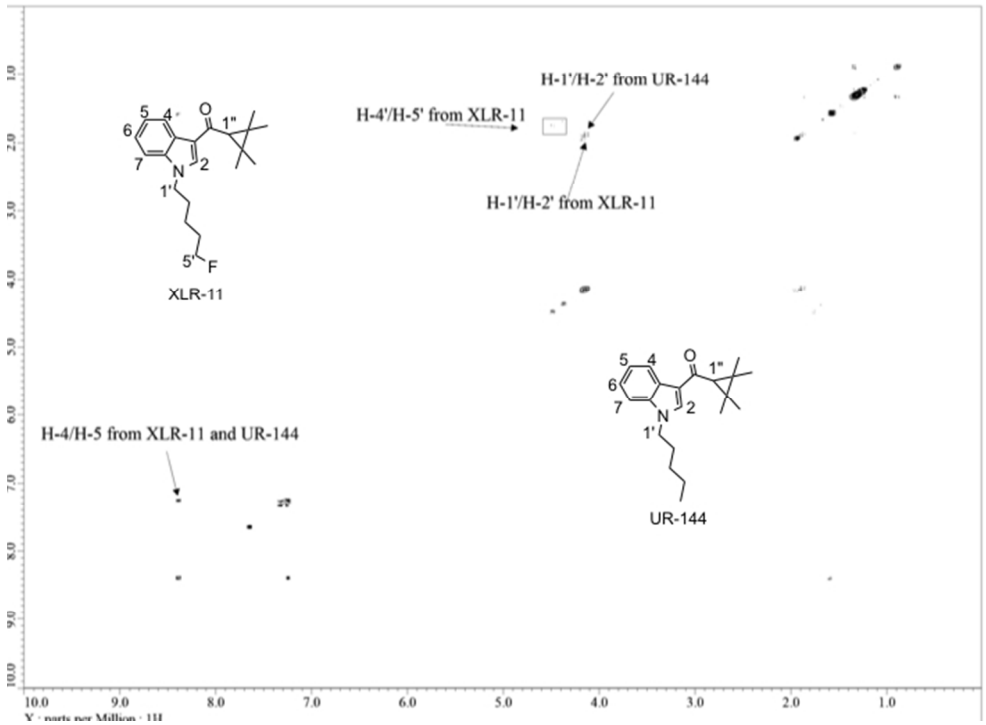


Figure 4 Melon Code Black TOCSY Spectrum
279x215mm (300 x 300 DPI)



1-D and 2-D NMR methods were used exclusively to rapidly screen for and quantify synthetic cannabinoids in herbal products with direct and simple NMR-solvent extraction.
254x190mm (72 x 72 DPI)