Use of SPME-HS-GC–MS for the Analysis of Herbal Products Containing Synthetic Cannabinoids

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The increasing prevalence and use of herbal mixtures containing synthetic cannabinoids presents a growing public health concern and legal challenge for society. In contrast to the plant-derived cannabinoids in medical marijuana and other cannabinoid-based therapeutics, the commonly encountered synthetic cannabinoids in these mendaciously labeled products constitute a structurally diverse set of compounds of relatively unknown pharmacology and toxicology. Indeed, the use of these substances has been associated with an alarming number of hospitalizations and emergency room visits. Moreover, there are already several hundred known cannabinoid agonist compounds that could potentially be used for illicit purposes, posing an additional challenge for public health professionals and law enforcement efforts, which often require the detection and identification of the active ingredients for effective treatment or prosecution. A solid-phase microextraction headspace gas chromatography–mass spectrometry method is shown here to allow for rapid and reliable detection and structural identification of many of the synthetic cannabinoid compounds that are currently or could potentially be used in herbal smoking mixtures. This approach provides accelerated analysis and results that distinguish between structural analogs within several classes of cannabinoid compounds, including positional isomers. The analytical results confirm the continued manufacture and distribution of herbal materials with synthetic cannabinoids and provide insight into the manipulation of these products to avoid legal constraints and prosecution.

Introduction

According to the 2010 World Drug Report issued by the United Nations Office on Drugs and Crime (UNODC), between 155 and 250 million people, or 3.5 to 5.7% of the population aged 15–64, is estimated to have used illicit substances at least once in the previous year. Cannabis users comprise by far the largest number of illicit drug users (129–190 million people), followed by amphetamine-type stimulants, opiates and cocaine. Although these numbers have remained relatively stable over the last decade, the incidence of abuse of “designer drugs,” clandestinely produced drugs that are structurally and pharmacologically very similar to a controlled substance but are not themselves controlled substances, has increased (1). Indeed, the appearance of synthetic cannabinoids marks the latest stage in the development and abuse of controlled substance analogs, the history of which includes the design of fentanyl in the 1980s; the development of the ring-substituted phenethylamines in the late 1980s and the tryptamines in the 1990s; and

the piperazines and cathinone derivatives in the 2000s (2). Smokable herbal mixtures containing non-controlled synthetic cannabinoids have been sold on the Internet and in various retail shops as “Spice” and other misleadingly labeled products since at least 2006. The majority of these products are mixtures of plant/herbal ingredients of varied name and constitution, and little is known about the toxicology of the plant materials purportedly contained in these products (3). Synthetic cannabinoids that have most commonly been reported to be present in herbal Spice and Spice-like samples include JWH-018, JWH-073, JWH-398, JWH-250, HU-210, and CP-47,497 and its homologues (4–8). These and other known and plausible synthetic cannabinoids also have relatively unknown toxicology. Hence, accurate assessment of the potential health risks of these herbal mixtures has become increasingly difficult. Furthermore, although little is known about the prevalence of their use or the extent to which smoking mixtures containing non-controlled synthetic cannabinoids have replaced cannabis in the United States or globally, their prevalence and abuse appears to have significantly increased. In 2009, a survey conducted among 1,463 students aged between 15 and 18 years at schools providing general and vocational training in Frankfurt found that around 6% of respondents reported having used synthetic cannabinoid-containing Spice products at least once (9). In the US, a survey conducted by the Idaho Hospital Association revealed that of the 20 hospitals polled across the state, 11 had knowledge of the drug and more than 80 cases of suspected synthetic cannabinoid (Spice) overdoses occurred between February and August of 2010 (10). According to the United States Drug Enforcement Administration (DEA), Office of Diversion Control’s special report on synthetic cannabinoid and cathinone data within the National Forensic Laboratory Information System, the incidence of synthetic cannabinoids that were submitted to state and local forensic laboratories rose from 13 to 2,977 cases between the same reporting periods in 2009 to 2010 (11).

Several forces may exert some control on the continued design, production or abuse of synthetic designer drugs. Demand reduction is obviously one of the most important factors in controlling abuse, which to the clandestine supplier equates to maintenance of user desire and acceptance of the marketed drug. Another important market controlling factor is law enforcement control of their manufacture and distribution. With respect to law enforcement, the status of control measures and regulation against synthetic cannabinoids also varies across the globe. None of the synthetic cannabinoids are under international control by virtue of the UN drug control conventions, but JWH-018, JWH-073, HU-210 and CP 47,497 (together

with its C6, C8 and C9 homologues) are scheduled drugs in some Member States. The following countries control Spice and/or other synthetic cannabinoids: Austria, Canada, Chile, Denmark, Germany, Estonia, Finland, France, Ireland, Italy, Japan, Latvia, Lithuania, Luxembourg, New Zealand, Poland, Romania, Russia, South Korea, Sweden, Switzerland and the United Kingdom (12). In the United States, Δ^9 -THC and similar forms of synthetic cannabinoids of the "classical cannabinoid" structural motif (HU-210) have been scheduled under federal law for many years. However, in response to the perceived risk associated with these synthetic cannabinoids and herbal products, on November 24, 2010, the United States DEA announced that it intended to use its emergency scheduling authority to temporarily control five synthetic cannabinoids (JWH-018, JWH-073, JWH-200, CP-47,497 and cannabicyclohexanol). This scheduling took effect on March 1, 2011. The DEA clearly considers these, and other synthetic cannabinoids, to be a drug class of concern. Before the action by the DEA, many states had passed acts making it illegal under state law to sell Spice and/or synthetic cannabinoids. Since the DEA edict, other states have also passed or are considering similar legislation (13). Additional bans in the US are being enacted on city and county levels. There is little doubt that the actions of the UN member states, the DEA, and other regulatory agencies will reduce access to these synthetic cannabinoids in herbal formulations, and may dramatically impact their abuse. However, there are hundreds of compounds with cannabinoid receptor affinity and activity, in several chemical classes (14–17), and it is likely that new substances will continue to appear in various formulations for illicit use. This large array of possible compounds presents a constant challenge for the forensic and toxicological identification of new substances that is needed for the prompt assessment of risk and, where necessary, implementation of control measures by both public health and law enforcement agencies.

To analyze commercially available herbal formulations sold as incense, but suspected to contain synthetic cannabinoids, various researchers have developed and applied chromatographic methods and mass spectrometry. Dresen *et al.* and Uchiyama *et al.* have used gas chromatographs coupled to mass spectrometers (GC–MS) in electron ionization mode to identify synthetic cannabinoids in extracts of herbal products, while Hudson *et al.* and Uchiyama *et al.* also performed analysis of herbal extracts with liquid chromatography–mass spectrometry (LC–MS) (5, 7, 8, 18). These methods have allowed for the qualitative identification of numerous synthetic cannabinoids in herbal product matrices. Each of these methods used an extraction step to isolate the analytes of interest followed by concentration before analysis.

In an effort to develop rapid methods to characterize herbal formulations, an automated headspace solid-phase microextraction and gas chromatography–mass spectrometry (HS-SPME-GC–MS) method was developed. The analytical approach allows for the detection of JWH-018, JWH-073 and other synthetic cannabinoid analogs from small quantities of materials (~50 mg) without requiring extraction, concentration or derivatization. Analysis of over 20 products, obtained from retail stores in the Raleigh, Durham and Chapel Hill areas of North Carolina, revealed the presence of at least one synthetic cannabinoid in each product. Several products had two or more analogs, and

some products appeared to have more uncommon or previously unreported active compounds present. The results described here provide evidence to support the SPME-GC–MS approach as an effective means for the rapid analysis, detection and mass spectral confirmation of synthetic cannabinoids in both bulk drug substance and herbal formulations. This method was used to analyze products obtained before and after the DEA's emergency scheduling control of five commonly encountered synthetic cannabinoids. The results show that the legislation has not prevented manufacturers or distributors from dispensing these newly controlled chemicals; however, the use of new cannabinoids in these herbal formulations was also observed. In addition to the analysis of herbal products, over 20 synthetic cannabinoid standards were analyzed to confirm the structural identification of the synthetic cannabinoid compounds detected in the herbal products based on both retention time and mass spectrum. Finally, the gas chromatograms and mass spectra for both the herbal products and the synthetic cannabinoid standards have been submitted to a publically available spectral database (www.forensicdb.org) for future use by the scientific community. The further adoption and continued application of the described method for rapid analysis and identification of synthetic cannabinoid-containing formulations, and the expansion of the spectral information that this method has allowed to be made publically available to date, should prove useful for forensic and special testing laboratories in identifying previously described and novel synthetic cannabinoids in bulk substances and herbal formulations. In turn, these efforts will enable the scientific community to better address the intent of our society's legislation and the effectiveness of the concomitant law enforcement efforts.

Experimental

Materials

Methanol (99.9 + %, capillary GC grade), and 2,6-dichlorotoluene (99 + %) were purchased from Sigma-Aldrich (St. Louis, MO). Ethanol (200 proof, USP grade) was purchased from Pharmco-AAPER (Brookfield, CT). HPLC standard mixture I (item number 13830) containing ($\pm$)-CP 47,497, ($\pm$)-CP 47,497-C8-homolog, ($\pm$)-CP 55,940, HU-308, HU-331, JWH 015, JWH 018, JWH 019, JWH 073, JWH 200, JWH 250 and WIN 55212-2, was purchased from Cayman Chemical (Ann Arbor, MI). AM-694 was obtained from LGC (Teddington, UK). All JWH analogs used in these studies as reference standards were synthesized by Dr. John W. Huffman and provided by Dr. Jenny L. Wiley.

Herbal products were purchased in convenience stores and various commercial venues in the Raleigh, Durham and Chapel Hill area.

Standard and sample preparations

Herbal samples were prepared by accurately weighing approximately 50 mg of each sample into individual 10 mL headspace vials and then spiking each with a 20 μ L aliquot of a 2,6-dichlorotoluene internal standard solution (0.1 mg/mL solution in methanol). The vials were capped with 20-mm magnetic crimp seal caps with PTFE/silicone septa (Restek; Bellefonte, PA) before analysis.

Standard synthetic cannabinoid samples for SPME-HS-GC–MS analysis were prepared by diluting the synthetic cannabinoid standards to a final concentration of 1 mg/mL in ethanol. Sonication was used to aid in the dissolution. A 20- μ L aliquot of each standard solution was then removed and added directly to individual 10-mL headspace vials and dried in a TurboVap LV evaporation system (Caliper LifeSciences; Hopkinton, MA) until dry. The vials were capped as described previously and subsequently analyzed.

HS-SPME-GC–MS

An Agilent 6890 Plus gas chromatograph coupled to a 5973 MSD mass selective detector (Agilent Technologies; Santa Clara, CA) was used for all analysis. A CTC analytics combipal autosampler fitted with a SPME sampling system (Leap Technologies; Carrboro, NC) was used to extract and inject all samples. The SPME system consisted of an SPME fiber holder, a fiber conditioning station and a sample incubation block capable of heating and agitation of the sample. A stableflex 23-gauge 85- μ m carboxen/polydimethylsiloxane (CAR/PDMS) SPME fiber was used for the analysis (Supelco; Bellefonte, PA). The autosampler was controlled by Pal (firmware version 2.5.2) software (Leap Technologies).

Samples were incubated at 200°C with pulse-agitation at 250 rpm (cycles of 5 s on, 2 s off). After one minute of sample equilibration, the SPME fiber was inserted and exposed to the sample headspace for five minutes of incubation. The fiber was then injected into the GC inlet for 15 min to desorb the analytes, and then allowed to condition for 5 min before the following injection.

An Agilent DB-5MS capillary column (30 m $\times$ 0.25 mm $\times$ 0.25 μ m, Agilent Technologies) was used for separation with a helium carrier gas flowing at 1.0 mL/min. A split injection ratio of 1:50 was used at 300°C. The oven temperature program consisted of a one minute hold at 40°C, followed by a 10°C/min ramp to 300°C, a 9 min hold at 300°C, a 15°C/min ramp to 325°C and then a one minute hold. The MSD transfer line heater was set to 300°C. The temperatures of the quadrupole and the ion source were 150 and 230°C, respectively. The duration of each run was 38.67 min. The analytes were detected after electron impact ionization (70 eV) in the SCAN-mode from m/z 50 to 550.

Data analysis

All GC–MS data acquisition and analysis was performed using the enhanced MS Chemstation G1701BA versions B.01.00 software (Agilent Technologies, 1998) and E.02.01.1177 (Agilent Technologies, 2010). Samples were analyzed to obtain retention time and mass spectra for analytes of interest to appropriately identify each analyte. Active compounds found in the samples were identified based on the spectra and retention times of the standards.

Results and Discussion

The SPME-HS-GC–MS analysis of synthetic cannabinoid standards revealed that most of the commonly encountered

cannabinoids in the herbal products were volatilized, absorbed onto the SPME fiber, desorbed in the GC injection port, chromatographically resolved and detected reliably, with very little indication of degradation (Table 1). The direct SPME from the headspace produced by heating as little as 20 μ g of a synthetic cannabinoid standard, including JWH-018, JWH-073, CP-55,940, CP-47,497 (and its octyl analog), HU-308, HU-331, and several other JWH analogs using the SPME method produced intense chromatographic peaks. The high resolution of the capillary chromatography resulted in baseline separation and unique retention times for all of the isobaric synthetic cannabinoid standards analyzed. This is an important consideration in the present work, because most of the synthetic cannabinoids encountered in these herbal products have positional isomers that are relatively indistinguishable from each other based on their mass spectrum alone, but are able to be differentiated based on capillary gas chromatographic retention time. In addition, all of the analytes were baseline separated and well resolved from each other, with the exception of CP-55,940 and JWH-302, which only differed by 0.08 min in retention time (Table 1). Nevertheless, these two compounds are clearly differentiated by their different molecular ions and fragmentation patterns. Hence, suspected synthetic cannabinoid compounds present in the herbal products were identified based on consistencies between both retention time and mass spectrum when compared to those obtained from the analysis of standards, as well as agreement between compound mass spectrum and published structure. This method of analysis, using retention time with mass spectral data, can help to identify both compounds that have been explicitly banned by the DEA along with their structural analogs that are not, which is important given what appears to be the recent migration by herbal product manufacturers away from the use of compounds specifically banned by the DEA to other synthetic analogs that may delay or confound detection.

During the analysis of herbal products, the most intense chromatographic peaks were most often found to correspond to synthetic cannabinoid compounds. Although this has implications regarding the relative abundance and volatility of the synthetic cannabinoid analogs in and between the various herbal samples, it also indicates that the choice of SPME fiber material was appropriate for the type of analyses conducted. SPME has previously been shown to discriminate during adsorption between different molecules based on size and affinity to the fiber material (19). This discrimination is often also the means used to concentrate the analytes relative to the matrix. Thus, these data and other data obtained in our laboratory by LC–MS and other analytical approaches (data not shown) suggest that the synthetic cannabinoid compounds are present in considerable quantity and are readily volatilized or extracted from the herbal material. Previously reported methods of sample preparation such as that of Dresen (5), or the LC–MS method provided by Hudson (18), involve a solvent extraction step entailing a vortexing or sonicating period. Although these extractions are relatively simple, when compared to the automated SPME fiber, they require solvents for extraction and additional efforts to purify and concentrate the sample before delivery to the GC-inlet. In contrast, SPME provides the ability to extract volatile analytes from the headspace vapors produced simply by heating a complex solid sample, thereby

Table 1

Structures and Retention Times of Synthetic Cannabinoid Standards

Compound	Structure	Mass*	Time [†]	Compound	Structure	Mass*	Time [†]
AM-694		435	29.88	JWH-408		371	38.42
JWH-348		435	31.45	JWH-197		371	31.94
JWH-363		435	30.88	JWH-019		355	32.58
JWH-372		435	30.57	JWH-018		341	31.30
JWH-200		384	38.21	JWH-201		335	29.36
Pravadoline (WIN 48098)		378	34.20	JWH-250		335	28.51
CP 55,940		376	28.78	JWH-302		335	28.86
JWH-081		371	36.06	CP 47,497 C8-homolog		332	26.47

JWH-164	371	33.56	JWH-073	327	30.22

JWH-166	371	35.84	JWH-015	327	29.61

JWH-267	371	32.27	CP 47,497	318	25.71

*Nominal mass of compound.

[†]Retention time of compound.

concentrating them and carrying them to the inlet, all in one single sample preparation step. Because there are few sample manipulations, the potential for confounding variables such as solvent artifacts or reactivity, or variations in recovery and detection due to sample solubility, are potentially reduced or eliminated. Additionally, while 2,5-dichlorotoluene was used here as an internal standard to verify suitable method performance, deuterated cannabinoid ligands are commercially available that could prove useful for enabling more quantitative analyses.

In all of the 31 herbal products tested, active synthetic cannabinoids were readily detected (for example, see Figure 1). In this particular herbal product (K2 Summit), the most intense peak, at a retention time of 31.3 min, was identified as JWH-018 based on its mass spectrum (Figure 2) and retention time match to a synthetic JWH-018 standard. Fifteen products were found to contain a single synthetic cannabinoid (Table II). JWH-018, a compound previously reported to be present in numerous herbal products (5, 6, 8, 18), was the analyte most often detected during this analysis; being present in 23 of 31 products sampled. The prevalence of JWH-018 in herbal products is corroborated by previous analyses, including those reported by Hudson *et al.*, as is the occurrence of multiple compounds in certain products (18). Other previously reported synthetic cannabinoids detected in this analysis include JWH-073, JWH-019 and JWH-081. Interestingly, JWH-073 is often found in combination with JWH-018 (Figures 3 and 4), and is seldom present alone in herbal products, suggesting that these analogs are most commonly available for use, or that the combination of these active cannabimimetic analogs affords some advantage to the formulation, such as a pharmacological property of preference to the consumer.

JWH-250 is another synthetic indole cannabinoid that has previously been reported as a constituent in Spice products (5), and was also determined to be present in many of our samples. JWH-250 was found in seven out of 31 herbal products. This compound was the second most abundant psychoactive constituent found in our analyses, which is in contrast to previous reports of the synthetic cannabinoids found in herbal products, where JWH-250 was not as prevalent (17). This may reflect the recent DEA scheduling of JWH-018 and JWH-073, which did not specifically mention JWH-250, leading to a

Figure 1. Total ion chromatogram recorded for the herbal product K2 Summit revealing a peak eluting at 31.3 min that was determined to be due to the presence of JWH-018.

Figure 2. Mass spectrum recorded for the peak eluting at 31.3 min in the total ion chromatogram obtained during the analysis of the herbal product K2 Summit. This constituent was determined to be JWH-018 based on comparison to the retention time and mass spectrum recorded for a synthetic standard. The structure of JWH-018 is provided in the figure for reference.

switch to this cannabinoid compound as an effort to avoid detection and law enforcement. Indeed, the mass spectrum of JWH-250 (Figure 5) is substantially different from JWH-073 and JWH-018 due to the added methylene group between the two ring systems (compare the structures and spectra presented in

Figures 2, 4 and 5). Another indole cannabimimetic compound was detected in an herbal formulation, JWH-019, but failed to follow the trend of producing an intense chromatographic signal. The spectrum was determined to be a match to a synthetic standard with respect to retention time and mass

Table II

Synthetic Cannabinoids Detected in Herbal Products by SPME-HS-GC-MS

Type	Description	JWH-018	JWH-073	JWH-250	JWH-081	JWH-019	Pravadoline	AM-694	AM-2201
K2	Summit (Batch 1)	X							
K2	Summit (Batch 2)	X		X					
Happy Shaman Herbs	Humboldt Gold	X	X						
Happy Shaman Herbs	Pink Tiger	X	X						
Happy Shaman Herbs	Brain Freeze	X							
Meditation	Strawberry (1.5g Package)	X		X	X				
Meditation	Strawberry (3.0g Package)			X	X				
Meditation	Mango			X	X				
Meditation	Blueberry			X	X				
Spice99	Bad 2 the Bone (cinnamon)	X						X	
Mr. Nice Guy	Herbal smoke blend	X				X			
Solowholesale	Cloud 49 (ultra strong, Irish Crème)	X			X				
Unknown	Ultra Cloud 10	X	X						
Unknown	Generic (ultimate) Spice! Double strength	X							
Unknown	Generic (Premium) Spice!	X							
Hot Hawaiian	Exotic Herbal Blend	X							
Tranquility Herbal Incens	Kush	X							
Deviltracks	Newprot (mint)	X			X				
Flawless	Strawberry			X					
Black Magic Smoke	Herbal incense	X							
Purple Flake	N/A	X							
Southern Spice	Herbal incense (mango)	X							
Berry Twist	N/A	X							
Destiny	Exotic Herbal Blend			X					
Southern Spice	Blue Dream (melon)	X							
Southern Spice	Blue Dream (melon)	X							
Happy Shaman Herbs	Humboldt Gold	X	X						
Spice99	Received After DEA Ban								
Spice99	Stinger						X	X	
Spice99	GI Jane						X		
Spice99	GI Joe						X		
Purple Puff	Kush	X							
Yeah Right	Head Funk								X

spectrum, but the peak was barely distinguishable in the gas chromatogram of the herbal product. This suggests that JWH-019 was present at a relatively low concentration in this herbal product or sample aliquot, or that it was less efficiently extracted from this material using the SPME technique. However, the presence of this compound was confirmed with data from a second study using high-resolution LC-MS (data not shown), where the compound appeared to be present in relatively high concentrations. A tentative conclusion, based on our ability to detect JWH-019 easily in a SPME analysis of the synthetic standard, is that heterogeneity in the product manufacture may result in varied concentration across the herbal material, which is consistent with variation noted across packages of the same material (20). Finally, in cases in which more than one type or flavor of herbal product was analyzed from the same brand, it was observed that the active constituents and their relative proportions often remain quite similar. For example, all products tested from the Happy Shaman Herbs

product line except one contained JWH-073 and JWH-018 (see Table 1). Similarly, all products from the Meditation brand contained JWH-081 and JWH-250. Spice99 was also noted as the only brand that we sampled that contained the AM (i.e., AM-694) class of compounds. While varying other aspects of their products, this consistency suggests that brands or manufacturers may attempt to establish a means to recognize or differentiate their products by the mixture of cannabinoids present within them. AM-694 was detected in our analysis of both Spice99 Bad 2 the Bone (cinnamon) and Spice99 Stinger, samples from before and after the DEA legislation took effect, respectively. The identity of this compound eluded identification for some time, as we analyzed JWH compounds with the same nominal mass, such as JWH-363, JWH-348 and JWH-372, and found that their mass spectra did not match the peaks in our herbal product samples. Further analysis of additional synthetic cannabinoids allowed us to arrive at the identification of AM-694 (Figure 6). This identification has been confirmed through analysis of a synthetic standard, and is consistent with the observation that AM-694 has been previously reported in European samples (4).

The herbal products obtained after the DEA ban of five synthetic cannabinoids show both an unexpected continuation in the use of DEA Schedule I controlled substances, along with new active substances, indicating that some manufacturers are modifying their products to attempt to continue to supply illicit user demand in light of new legal constraints and law enforcement activities. For example, Kush, a product from Purple Puff, was shown to contain JWH-018. This may indicate that some producers of herbal smoke products are reluctant to move away from the use of existing supplies, or an available supply of a now illegal compound. However, three other samples analyzed after the ban (Spice99's Stinger, GI Joe and GI Jane) also contained what appear to be new or rarely encountered components in herbal smoking blends, such as

Figure 3. Total ion chromatogram recorded for the herbal product Happy Shaman Herbs Humboldt Gold revealing peaks eluting at 30.2 and 31.3 min that were determined to be due to the presence of JWH-073 and JWH-018, respectively.

Figure 4. Mass spectrum recorded for the peak eluting at 30.2 min in the total ion chromatogram obtained during the analysis of the herbal product Happy Shaman Herbs Humboldt Gold. This constituent was determined to be JWH-073 based on comparison to the retention time and mass spectrum recorded for a synthetic standard. The structure of JWH-073 is provided in the figure for reference.

Figure 5. Mass spectrum recorded for the peak eluting at 28.5 min in the total ion chromatogram obtained during the analysis of the herbal product Destiny Exotic Herbal Blend. This constituent was determined to be JWH-250 based on comparison to the retention time and mass spectrum recorded for a synthetic standard. The structure of JWH-250 is provided in the figure for reference.

Figure 6. Mass spectrum recorded for the peak eluting at 29.9 min in the total ion chromatogram obtained during the analysis of the herbal product Spice99 Stinger. This constituent was determined to be AM-694 based on comparison to the retention time and mass spectrum recorded for a synthetic standard. The structure of AM-694 is provided in the figure for reference.

WIN-48,098 (pravadoline, see Figure 7). Pravadoline was developed originally as an analgesic, but was later found to have cannabinimimetic properties and is considered to be a predecessor of the well known WIN-55,212-2. In addition, the herbal product Head Funk was found to contain what appears to be AM-2201 based on GC–MS (Figure 8) and high resolution LC–quadrupole time-of-flight analysis (data not shown). The putative identification of AM-2201 in this herbal product is also consistent with previous published mass spectral information identifying AM-2201 in samples obtained from the internet (21).

The absence of CP-47,497 and its side chain analogs is notable. Hudson *et al.* were able to detect these compounds more frequently in their herbal product samples than any other synthetic cannabinoid (18). The high frequency with which these compounds have been reported may have contributed to the reasoning used by the DEA when choosing which compounds to place on schedule in March of 2011, because CP-47,497 and its homologs were specifically scheduled. Because all herbal samples found to contain CP-47,497 were purchased before the DEA ban, and subsequently no herbal product has been found to contain this psychoactive

Figure 7. Mass spectrum recorded for the peak eluting at 34.3 min in the total ion chromatogram obtained during the analysis of the herbal product Spice99 Stinger. This constituent was determined to be pravadoline based on comparison to the retention time and mass spectrum recorded for a synthetic standard. The structure of pravadoline is provided in the figure for reference.

Figure 8. Mass spectrum recorded for the peak eluting at 32.4 min in the total ion chromatogram obtained during the analysis of the herbal product Head Funk. This constituent is suspected to be AM-2201 based on the observed mass spectrum and the chemical structure and proposed fragmentation, as provided in the figure.

ingredient, it again suggests that these products are being specifically designed with the legality and policing of synthetic cannabinoids in mind. Indeed, we have also noted that once the US states and federal agencies began to identify and ban or schedule specific substances, the prevalence of the prohibited substances decreased and new analogs began to appear and become more prevalent. Finally, it is important to emphasize that all of our spectral data, including ongoing analytical efforts, are available online at www.forensicdb.org. This database provides a searchable, publicly available cheminformatic library that allows online users to compare and search for spectra to aid in the identification of synthetic cannabinoids

and other compounds of interest in herbal products and other items of interest to the health sciences, drug enforcement and regulatory agencies, and law enforcement efforts.

Conclusions

A novel SPME-HS-GC-MS method for the qualitative analysis of cannabinoid compounds in herbal products was developed and shown to be effective for the detection and identification of a wide variety of synthetic cannabinoids in several structural classes, in both a neat standard form as well as in herbal

product matrices. The analysis of more than 25 herbal products with this method identified several active compounds, some previously reported such as JWH-073, 019, 018 and 081, and newer constituents such as AM-694 and WIN-48,098 (pravadoline). It is clear that although the use of JWH-018 and illegal compounds in herbal products has continued after the DEA ban, manufacturers of these products are pursuing modifications and innovations to the chemical constituents in an attempt to elude detection, identification and law enforcement. The SPME-HS-GC-MS method provides sufficient resolution to discriminate between many of the positional isomers in the various series of synthetic cannabinoids, which could also be employed by manufacturers to evade detection and legal prosecution. The minimization of sample preparation inherent in SPME analysis allows for ease and rapid access to the analysis of the herbal products and synthetic cannabinoids, of particular interest to the forensic and toxicological communities.

The increasing popularity and availability of synthetic cannabinoids and herbal formulations is cause for considerable public concern. For this reason, the implementation of control actions and enforcement by regulatory authorities, and the demand for scientific investigation of these synthetic cannabinoids and herbal products, has intensified. These pharmacological and toxicological studies and regulatory control actions require the development of sophisticated analytical methods that are able to readily sample a variety of complex products, bulk chemical samples or biological matrices, while providing reliable and robust separation and discrimination of their illicit chemical constituents. Finally, the application of these analytical approaches and the interpretation of their results are further supported by the inclusion of the data into publically available analytical databases, such as those described here.

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References

1. United Nations Office on Drugs and Crime. (2010) World Drug Report. <http://www.unodc.org/unodc/en/data-and-analysis/WDR-2010.html> (accessed 5 November 2010).
2. Griffiths, P., Sedefov, R., Gallegos, A.N.A., Lopez, D. (2010) How globalization and market innovation challenge how we think about and respond to drug use: 'Spice' a case study. *Addiction*, **105**, 951–953.
3. European Monitoring Centre for Drugs and Drug Addiction. (2009) Addiction. Understanding the 'Spice' phenomenon. European Monitoring Centre for Drugs and Drug Addiction, Lisbon.
4. Dargan, P.I., Hudson, S., Ramsey, J., Wood, D.M. (2011) The impact of changes in UK classification of the synthetic cannabinoid

receptor agonists in 'Spice.' *International Journal of Drug Policy*, **22**, 274–277.

5. Dresen, S., Ferreiros, N., Putz, M., Westphal, F., Zimmermann, R., Auwarter, V. (2010) Monitoring of herbal mixtures potentially containing synthetic cannabinoids as psychoactive compounds. *Journal of Mass Spectrometry*, **45**, 1186–1194.
6. Emanuel, C.E.J., Ellison, B., Banks, C.E. (2010) Spice up your life: screening the illegal components of 'Spice' herbal products. *Analytical Methods*, **2**, 614–616.
7. Hudson, S., Ramsey, J. (2011) The emergence and analysis of synthetic cannabinoids. *Drug Testing and Analysis*, **3**, 466–478.
8. Uchiyama, N., Kikura-Hanajiri, R., Ogata, J., Goda, Y. (2010) Chemical analysis of synthetic cannabinoids as designer drugs in herbal products. *Forensic Science International*, **198**, 31–38.
9. European Monitoring Centre for Drugs and Drug Addiction. (2010) Drug profile—Synthetic cannabinoids and 'Spice,' European Monitoring Centre for Drugs and Drug Addiction, Lisbon.
10. Hurst, D. (2010) Idaho Reporter.Com—Hospital survey shows more than 80 suspected cases of Spice overdose in past six months. <http://www.idahoreporter.com/2010/hospital-survey-shows-more-than-80-suspected-cases-of-spice-overdose-in-past-six-months/> (accessed 5 November 2010).
11. U.S. Drug Enforcement Administration, Office of Diversion Control. (2011) National Forensic Laboratory Information System: Special Report: Synthetic cannabinoids and synthetic cathinones reported in NFLIS, 2009–2010., U.S. Drug Enforcement Administration, Springfield, VA.
12. Cooper, D.A. Future synthetic drugs of abuse. http://www.erowid.org/library/books_online/future_synthetic/future_synthetic.shtml (accessed 5 November 2010).
13. National Conference of State Legislatures. Synthetic cannabinoids legislation. <http://www.ncsl.org/?TabId=22431> (accessed 5 November 2010).
14. Pertwee, R.G. (2000) Cannabinoid receptor ligands: Clinical and neuropharmacological considerations, relevant to future drug discovery and development. *Expert Opinion on Investigational Drugs*, **9**, 1553–1571.
15. Pertwee, R.G. (2009) Emerging strategies for exploiting cannabinoid receptor agonists as medicines. *British Journal of Pharmacology*, **156**, 397–411.
16. Pertwee, R.G., Howlett, A.C., Abood, M.E., Alexander, S.P.H., Di Marzo, V., Elphick, M.R. *et al.* (2010) International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: Beyond CB1 and CB2. *Pharmacological Reviews*, **62**, 588–631.
17. Thakur, G.A., Tichkule, R., Bajaj, S., Makriyannis, A. (2009) Latest advances in cannabinoid receptor agonists. *Expert Opinion on Therapeutic Patents*, **19**, 1647–1673.
18. Hudson, S., Ramsey, J., King, L., Timbers, S., Maynard, S., Dorgan, P.I. *et al.* (2010) Use of high-resolution accurate mass spectrometry to detect reported and previously unreported cannabinomimetics in "herbal high" products. *Journal of Analytical Toxicology*, **34**, 252–260.
19. Zhang, Z., Pawliszyn, J. (1993) Headspace solid-phase microextraction. *Analytical Chemistry*, **65**, 1843–1852.
20. Hudson, S., Ramsey, J., King, L., Timbers, S., Maynard, S., Dargan, P.I. *et al.* (2010) Use of high-resolution accurate mass spectrometry to detect reported and previously unreported cannabinomimetics in herbal high products. *Journal of Analytical Toxicology*, **34**, 252–260.
21. Nakajima, J.I., Takahashi, M., Nonaka, R., Seto, T., Suzuki, J., Yoshida, M. *et al.* (2011) Identification and quantitation of a benzoylindole (2-methoxyphenyl)(1-pentyl-1H-indol-3-yl)methanone and a naphthoylindole 1-(5-fluoropentyl-1H-indol-3-yl)-(naphthalene-1-yl)methanone (AM-2201) found in illegal products obtained via the Internet and their cannabimimetic effects evaluated by in vitro [35S]GTPγS binding assays. *Forensic Toxicology*, **29**, 132–141.