Analysis of Synthetic Cannabinoids in Botanical Material: A Review of Analytical Methods and Findings

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ABSTRACT: Synthetic cannabinoid analogs have gained a great deal of attention from the forensic community within the last four years. The compounds found to be of most interest to forensic practitioners include those of the following series: JWH, CP, HU, AM, WIN, RCS, and most recently, XLR and UR. Structurally the HU compounds are most similar in structure to Δ^9-tetrahydrocannabinol (THC), the main psychoactive component of marijuana. The novel compounds include cyclohexylphenols, naphthoylindoles, naphthylmethylindoles, naphthylmethylindenes, benzoylindoles, naphthoylpyrroles, phenylacetylindoles, adamantoylindoles, and tetramethylcyclopropylindoles. Many of these compounds are cannabinoid receptor agonists and were originally synthesized for medical research purposes but have recently been appropriated into the illicit drug market. Their psychoactive effects, mimicking those of marijuana, as well as their indeterminate legal status, have made them popular for recreational use. Solutions of the compounds dissolved in organic solvents are sprayed onto botanical material and sold as "herbal incense" products via the Internet, and in smoke shops, convenience stores, and gas stations around the world. Many of the products are labeled "Not for human consumption" in an attempt to circumvent legislation that bans the sale and manufacture of certain compounds and their analogs for human use. The compounds that were first detected following forensic analysis of botanical materials included JWH-018, JWH-073, and CP 47,497 (C7 and C8 homologs). However, in the four years since their appearance the number of compounds has grown, and additional diverse classes of compounds have been detected. Governments worldwide have taken action in an attempt to control those compounds that have become widespread in their regions. This article discusses the history of synthetic cannabinoids and how they have been detected in the illicit drug market. It also discusses the analytical methods and techniques used by forensic scientists to analyze botanical products obtained via the Internet or from law enforcement investigations and arrests.

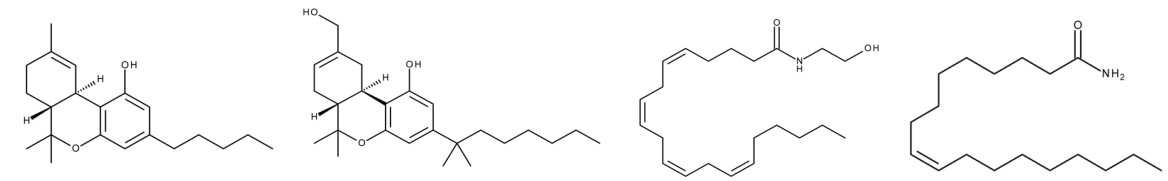
KEY WORDS: Analytical methodology, designer drugs, synthetic cannabinoids, synthetic drug scheduling.

INTRODUCTION

Natural and Endogenous Cannabinoids

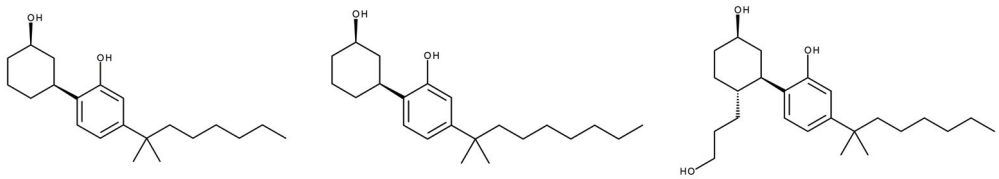
In 1964, the chemical structure of Δ^9-tetrahydrocannabinol (THC) (**Structure 1**), the main psychoactive component of marijuana, was elucidated by researchers Raphael Mechoulam (Daniel Sieff Research Institute) and Yechiel Gaoni (Weizmann Institute of Science) in Rehovoth, Israel [31]. The compound was extracted from hashish provided by Israeli law enforcement and analyzed using several means of molecular identification, most importantly nuclear magnetic resonance (NMR) spectroscopy. This structural determination was a significant breakthrough, as the components of cannabis had been long studied, but until this point no definitive structure or full characterization of the major psychoactive component had been determined. With the characterization of THC, new insights began to develop in the study of what would be known as cannabinoids [7].

During the next 24 years, more findings related to cannabinoid compounds emerged. In 1988, a research group published data describing a G protein-coupled receptor in the brain that bound natural cannabinoids including THC and cannabinol. These studies were performed in conjunction with a research group at Pfizer Inc. The study also included analysis of CP 55,940 (**Structure 2**), which was synthesized by Pfizer and proved to exhibit cannabinoid receptor-binding activity [9]. Pfizer also synthesized CP 47,497 (Structure 2), another compound with significant cannabinoid receptor binding [54]. This work sparked more interest in cannabinoid receptor research and in 1990, an article was released that identified the structure and activity of the CB_1 cannabinoid receptor [30]. Soon after the CB_1 receptor discovery, in 1992 Devane et al. identified



Δ^9-Tetrahydrocannabinol (THC) HU-210 Anandamide Oleamide

Structure 1. Classical Compounds — Structures of THC, HU-210, anandamide, and oleamide.



CP 47,497 (C7) CP 47,497 (C8) CP 55,940

Structure 2. Cyclohexylphenols — Structures of CP 47,497 (C7), CP 47,497 (C8), and CP 55,940.

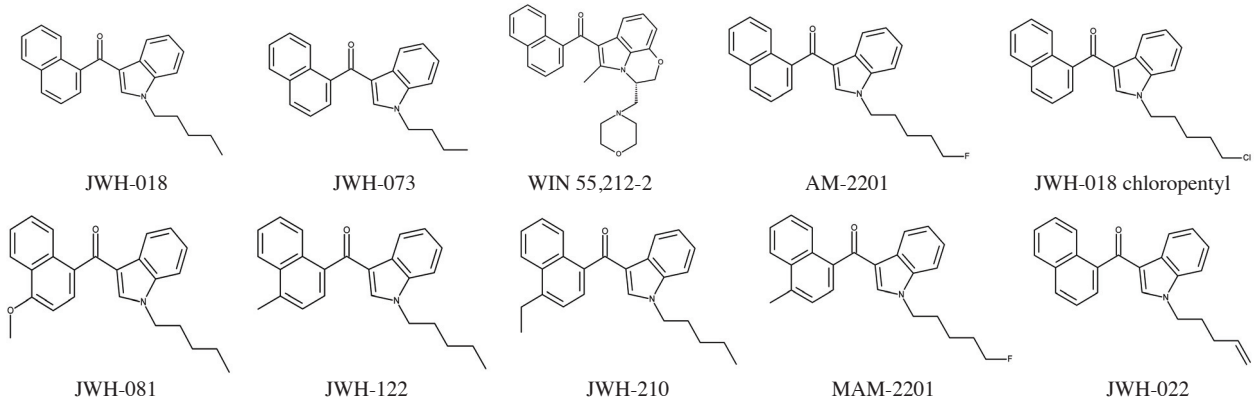
the first endogenous ligand of the cannabinoid receptor, arachidonylethanolamide, also termed anandamide (Structure 1), which was determined during a screen for endogenous cannabinoid receptor ligands [10]. The name is taken from the Sanskrit word *ananda* meaning "bliss" or "delight".

Anandamide is a long-chain fatty acid ethanolamine amide derivative; studies have shown its potential to exhibit analgesic effects and other properties such as the ability to inhibit human breast cancer cell proliferation [3,8,41]. Another endogenous cannabinoid of interest is oleamide (Structure 1). Oleamide is also a long-chain fatty acid amide derivative known to exhibit sleep-inducing properties. The endogenous cannabinoid ligands are known to mimic the effects of THC as they bind to cannabinoid receptors [3,41]. Cannabinoid receptor studies continued and in 1993, the second cannabinoid receptor, CB_2, was discovered by Munro et al. [7,33]. The CB_2 receptor is located throughout the immune system, brain, and gastrointestinal system, and is implicated in immune response.

Synthetic Cannabinoids

Not only has there been an interest in the study of natural and endogenous cannabinoids, but there has also been a significant amount of research performed on synthetic compounds that would bind with the same receptors. In 1999, Pop [41] discussed several synthetic cannabinoids, their chemical structures, and their physiological effects. Some of these compounds include HU-210 (Structure 1) and HU-211, synthesized at Hebrew University in the 1980s, CP 55,940, WIN 55,212-2 (**Structure 3**), and SR 141716A, a selective CB_1 antagonist. Many of these compounds were originally synthesized for medical research by various institutions. Each series is typically named with a two- or three-letter abbreviation for the laboratory discovering them, followed by a three- or four-figure number identifying them within that series. Recently, with widespread illicit synthesis, compound names have more obscure origins, including for example XLR-11 [16], a type of rocket engine from the 1960s, and AKB48 (AP-INACA) [46], a Japanese girl group formed in 2005. The most frequently reported series, and their originators, are JWH (John W. Huffman, Clemson University), CP (Pfizer), HU (Hebrew University), AM (Alexandros Makriyannis, Northeastern University), WIN (Sterling Winthrop), and RCS (Research Chemical Supply).

Dr. John W. Huffman performed research on synthetic cannabinoids at Clemson University (Clemson, SC) in the 1990s through mid-2000s to determine the significance of the structural and geometric features of cannabimimetic compounds as relates to their binding capabilities to the CB_1 and CB_2 cannabinoid receptors [22–25]. A group of compounds that were of interest to Huffman were the alkylaminoindoles, the class containing many of the JWH compounds. Hundreds of these compounds were synthesized in Huffman's research laboratory during those years. These compounds have proven to be some of the strongest cannabinoid receptor agonists, and have come to play a significant role in today's illicit drug market. In an article describing various classes of synthetic drugs, Collins discussed the ease of synthesis of many of the JWH series compounds. A synthetic pathway is provided that details the synthesis of JWH-018 from indole [5].



JWH-018 JWH-073 WIN 55,212-2 AM-2201 JWH-018 chloropentyl

JWH-081 JWH-122 JWH-210 MAM-2201 JWH-022

Structure 3. Naphthoylindoles — Structures of JWH-018, JWH-073, WIN 55,212-2, AM-2201, JWH-018 chloropentyl, JWH-081, JWH-122, JWH-210, MAM-2201, and JWH-022.

Also related to the JWH series of compounds and other synthetic cannabinoids are those synthesized and patented by Alexandros Makriyannis and Hongfeng Deng at Northeastern University, the AM series compounds [29]. Many of the AM series compounds are halogenated analogs of the JWH series. The patent issued on the AM compounds describes their structures' synthetic pathways and intended applications. AM compounds were designed as cannabimimetic indole derivatives having high binding affinities for CB_1 and CB_2 receptors that could be used for the treatment of pain, glaucoma, epilepsy, and nausea associated with chemotherapy [29]. Although many of the synthetic cannabinoids synthesized by researchers were designed to be used for medical research purposes, they have recently been exploited by the illicit drug market. Synthetic cannabinoids are now at the forefront of a worldwide market in "legal high" consciousness-altering research chemicals that are sold directly to consumers without any quality control, manufacturing standards, dosing control, adverse effects assessments, or the general safety assessments that drugs typically undergo before being given to humans.

Abuse Trends of Synthetic Cannabinoids

Synthetic cannabinoids began appearing on the illegal drug market for recreational use around 2004 [2,42]. The various forms in which the drugs were available included powders and capsules, with the most popular being packaged botanical material laced with the synthetic compounds for smoking. The botanical materials were sold as "herbal incense" products, "novelty items", and were labeled "not for human consumption" [42]. Initially the most popular brands of herbal products were sold under the brand names "Spice" and "K2" [42] and these names have persisted as surrogates for this type of product even though the individual product names have continually changed and evolved. At the outset, these products were sold predominantly over the Internet in Germany, Switzerland, and Austria. They soon began to be sold in local smoke shops, gas stations, and convenience stores as well [42]. The second generation of products had names including "Chill Out", "Smoke", "Forest Humus", "Scope Vanilla", "Silent Black", and "Space" [27,39]. The names of the commercial products sold containing these chemicals lack consistency, with many vendors making similarly named products with different chemical composition. In addition, the materials purchased from the same distributor weeks apart may contain different chemicals [11]. The first synthetic cannabinoid identified in a smoking mixture by a laboratory was JWH-018, as a psychoactive component of the product "Spice". This was reported in December 2008 in Frankfurt, Germany, at a laboratory known as "THC Pharma" [1,27]. This discovery highlighted the nature of these products and initiated more extensive testing, leading eventually to a ban in Germany in January 2009 of many synthetic cannabinoids [27].

The botanical material contained in the packaging of the products is very diverse. This includes *Pedicularis densiflora, Nymphacea caerulea, Leonotis leonurus, Leonurus sibiricus, Carnavalia maritime,* and *Zornia latifolia* [50]. To date no clear description of the alleged contribution of the plant substrate to physiological effects has been reported. Some of the products analyzed by laboratories contained additional nonsynthetic bioactive compounds including THC, cannabidiol, and nicotine [11].

Typical manufacturing of these botanical products involves the spraying of an organic solution of the synthetic compounds, often in acetone or alcohols, onto the dried plant material and packaging the treated material for sale [40]. The packaging is generally colorful in appearance, with bright symbols indicating the brand. The plant material itself is sometimes green/brown, but may be colored in a similar manner to the packaging, or suggested by the name of the material (K2 Blueberry, for example, has been seen dyed blue in color), and the material is often perfumed with fruit or "bubblegum" scents. In an article providing a general overview of synthetic cannabinoids, Seely [42] presents several color photographs of both the packaging and the botanical material contained in some synthetic cannabinoid-containing products.

The first peer-reviewed papers that provided details concerning the presence of synthetic cannabinoids in botanical material were published in early 2009 and originated in Germany and Japan. The papers described laboratory work performed within the previous year [2,27,47,48]. During this time period, many forensic laboratories and law enforcement agencies had indicated that the "herbal incense" products were being sold for the purposes of producing a marijuana-like intoxication, giving an indication that the products being sold were more than simply incense. These reports indicated that the products contained synthetic cannabinoid compounds, often characterized as designer drugs, which around that time included predominantly JWH-018, JWH-073 (Structure 3), and CP 47,497 (C7 and C8 homologs) (Structure 2). Over a very short period of time, the number of compounds present in these herbal mixtures has grown significantly and the forms in which the compounds are distributed have also evolved to include some large distributors and sophisticated manufacturing and packaging operations.

Based on a review of published work in the area of synthetic cannabinoids, the compounds of greatest concern from the forensic laboratory point of view are listed in **Table 1**. This table indicates the analytical techniques used for the identification of the compounds and the citations to the papers in which they were reported. The molecular

formula and exact mass of each compound listed in Table 1 is provided in **Table 2**. This review considers the analysis of the synthetic cannabinoid compounds discussed above through mid-2012. It provides details about their significance to the forensic community as well as the various means by which they have been obtained and analyzed from extracts of botanical material and other forms available in the illicit drug market.

I. CHEMICALS OF CONCERN FOR THE ANALYSIS OF BOTANICAL MATERIALS

The synthetic cannabinoid compounds discussed in this review article fall into various categories based on their chemical structure. It should be noted that although the products containing these compounds are currently being used as marijuana substitutes, many of the component structures are not similar to the core structure of THC. The most popular synthetic cannabinoids during the time of this review, as well as in the published literature, are compounds of the JWH series. There are several structural differences among the hundreds of JWH compounds; these compounds can be divided into several classes within this series and each is discussed below. The other classes of compounds that are not related to the JWH series are also described in the following discussion.

Classical

The compounds included in this class are those of the HU series. These are the compounds that have significant structural similarity to traditional cannabinoids such as THC, from which the term "classical" is derived [13]. HU-210 and other HU series compounds have significant binding with the cannabinoid receptors. Anandamide and oleamide, the endogenous cannabinoid ligands, are fatty acid amide derivatives and bind to the CB_1 and CB_2 receptors. These compounds mimic the pharmacological activity of THC and also help regulate various biological processes including pain perception, appetite, immune response, and other effects. The structures of anandamide and oleamide are unrelated to THC, but possess long aliphatic chains which upon folding may mimic portions of the THC structure and contribute to their potency in receptor binding [22,25,41]. Comparison structures of THC, HU-210, anandamide and oleamide are shown in Structure 1.

Cyclohexylphenols

This class of compounds includes the CP (Pfizer) compounds, some of which are CP 47,497 (C7 and C8 homologs), and CP 55,940 (Structure 2); the C8 homolog of CP 47,497 is also termed cannabicyclohexanol. These compounds are structurally distinct from compounds in the JWH, WIN, AM, and RCS series. The CP compounds

Table 1. Compounds discussed in the synthetic cannabinoids analysis papers, the analytical techniques used, and the associated references

Compound	GC-MS	GC-TOF	LC-MS/UPLC-MS/LC-MS-MS	LC-TOF	NMR	TLC	DART-TOF	HPLC	SPME-HS-GC-MS	MALDI-TOF
JWH-007			[21]							
JWH-015	[20,35,45]		[20,35,45]	[35]	[35,45]	[45]	[45]	[45]		
JWH-018	[2,11,20,26, 28,47,49]	[27]	[2,20,21, 26,47,49]	[19,26, 28,43]	[2,26, 27,47]	[2,28, 46]	[47]	[28]	[6]	[18]
JWH-019	[21,28,35]		[20,21,35]	[19,28,35,43]	[35]	[28]		[28]	[6]	[18]
JWH-047			[20]							
JWH-073	[11,20,28, 35,45,55]	[27]	[20,21,35, 45,55]	[19,28,35,43]	[27,35, 45,55]	[28,45, 55]	[45]	[28, 45]	[6]	[18]
JWH-073 methylated analog type 1	[20]		[20]							
JWH-073 methylated analog type 2	[55]		[55]		[55]	[55]				
JWH-081	[20,28,35,45]		[20,35,45]	[19,28,35]	[35,45]	[28,45]	[45]	[28,45]	[6]	[18]
JWH-081 isomer			[20]							
JWH-122	[20,26,34, 35]	[14]	[14,20,21, 26,34,35]	[19,26,34, 35,43]	[14,26, 34,35]	[14]				
JWH-200	[20,28,35]		[20,35]	[28,35]	[35]	[28]		[28]		
JWH-200 piperidinyl variant	[20,45]		[20,45]		[45]	[45]	[45]	[45]		
JWH-203	[4,20]		[4,20]	[4]	[4]					

Table 1. (Continued)

Compound	GC-MS	GC-TOF	LC-MS/UPLC-MS/LC-MS-MS	LC-TOF	NMR	TLC	DART-TOF	HPLC	SPME-HS-GC-MS	MALDI-TOF
JWH-210	[20,35]		[20,35]	[35,43]	[35]					[18]
JWH-210 eythyl derivative	[20,28]		[20]	[28]		[28]		[28]		
JWH-250	[11,20,28, 35,36,45]		[20,35,36, 45]	[19,28,35, 36,43]	[35,36, 45]	[28,45]	[45]	[28,45]	[6]	[18]
JWH-251	[35,45]		[35,45]	[35]	[35,45]	[45]	[45]	[45]		
JWH-253	[20]		[20]							
JWH-387	[20]		[20]							
JWH-398	[20]		[20,21]							
JWH-412	[32]				[32]					
Same exact mass analog of JWH-049/182/213			[21]							
(4-Hydroxymethylphenyl) (1-pentyl-1H-indol-3-yl) methanone	[20]		[20]							
CP 47,497 (C6)			[21]							
CP 47,497	[2,20]		[2,20,21]		[2]	[2]				
CP 47,497 (C8)	[2,11,20,28, 47,48,49]	[27]	[2,20,21, 47–49]	[28]	[2,27, 47,48]	[2,27,28, 47,48]	[47]	[28]		
CP 47,497 (C8) *trans*-diastereomer	[2,49]		[2,49]		[2]	[2]				
CP 47,497 (C8) + C2 variant	[20]		[20]							
CP 47,497 (C9)			[21]							
CP 55,940	[20]		[20]							
AM-679	[26]		[26]	[26]	[26]					
AM-694	[20,28,35,35]		[20,35,35]	[19,28,35,35]	[34,35]	[28]		[28]	[6]	[18]
AM-2201	[28,32,34]		[34]	[19,28,34,43]	[32,34]	[28]		[28]	[6]	
AM-2201 methylated analog	[32]				[32]					
AM-2233				[43]						
AB-001	[26]		[26]	[26]	[26]					
HU-210	[20]		[20]							
HU-308	[20]		[20]							
Nabilone	[20]		[20]							
Pravadoline (WIN 45,098)				[19]					[6]	
WIN 55,212-2	[20]		[20]							
RCS-4	[20,28,34,35]		[21,34,35]	[28,34,35]	[34,35]	[28]		[28]		
RCS-4 methylated analog Type 1	[20]		[20]							
RCS-4 methylated analog Type 2	[20]		[20]							
Positional isomer of RCS-4	[34]		[34]	[34]	[34]					
RCS-8	[28]			[28,43]		[28]		[28]		
CRA-13	[20]		[20]							
Oleamide	[2,49]		[2,49]		[2]	[2]				

Table 2. Compounds discussed in the synthetic cannabinoids analysis papers, their molecular formulae and exact masses (monoisotopic)

Compound	Mol. formula	Exact mass (amu)
JWH-007	$C_{25}H_{25}NO$	355.19361
JWH-015	$C_{23}H_{21}NO$	327.16231
JWH-018	$C_{24}H_{23}NO$	341.17796
JWH-019	$C_{25}H_{25}NO$	355.19361
JWH-047	$C_{25}H_{25}NO$	355.19361
JWH-073	$C_{23}H_{21}NO$	327.16231
JWH-073 methylated analog type 1	$C_{24}H_{23}NO$	341.17796
JWH-073 methylated analog type 2	$C_{24}H_{23}NO$	341.17796
JWH-081	$C_{25}H_{25}NO_2$	371.18853
JWH-081 isomer	$C_{25}H_{25}NO_2$	371.18853
JWH-122	$C_{25}H_{25}NO$	355.19361
JWH-200	$C_{25}H_{24}N_2O_2$	384.18378
JWH-200 piperidinyl variant	$C_{26}H_{26}N_2O$	382.20451
JWH-203	$C_{21}H_{22}ClNO$	331.13899
JWH-210	$C_{26}H_{27}NO$	369.20926
JWH-210 ethyl derivative	$C_{28}H_{31}NO$	397.24056
JWH-250	$C_{22}H_{25}NO_2$	335.18853
JWH-251	$C_{22}H_{25}NO$	319.19361
JWH-253	$C_{23}H_{27}NO_2$	349.20418
JWH-387	$C_{24}H_{22}BrNO$	419.08848
JWH-398	$C_{24}H_{22}ClNO$	375.13899
JWH-412		
Same exact mass analog of JWH-049/182/213	$C_{24}H_{22}FNO$	359.16854
JWH-049/182/213	$C_{27}H_{29}NO$	383.22491
(4-Hydroxymethylphenyl) (1-pentyl-1H-indol-3- yl)methanone	$C_{21}H_{23}NO_2$	321.17288
CP 47,497 (C6)	$C_{20}H_{32}O_2$	304.24023
CP 47,497	$C_{21}H_{34}O_2$	318.25588
CP 47,497 (C8)	$C_{22}H_{36}O_2$	332.27153
CP 47,497 (C8) *trans*-diastereomer	$C_{22}H_{36}O_2$	332.27153
CP 47,497 (C8) + C2 variant	$C_{24}H_{40}O_2$	360.30283
CP 47,497 (C9)	$C_{23}H_{38}O_2$	346.28718
CP 55,940	$C_{24}H_{40}O_3$	376.89775
AM-679	$C_{20}H_{20}INO$	417.05897
AM-694	$C_{20}H_{19}FINO$	435.04954
AM-2201	$C_{24}H_{22}FNO$	359.16854
AM-2201 methylated analog	$C_{25}H_{24}FNO$	373.18419
AM-2233	$C_{22}H_{23}IN_2O$	458.08552
AB-001	$C_{24}H_{31}NO$	349.24056
HU-210	$C_{25}H_{38}O_3$	386.28209
HU-308	$C_{27}H_{42}O_3$	414.31339
Nabilone	$C_{24}H_{36}O_3$	372.26645
Pravadoline (WIN 48,098)	$C_{23}H_{26}N_2O_3$	378.19434
WIN 55,212-2	$C_{27}H_{26}N_2O_3$	426.19434
RCS-4	$C_{21}H_{23}NO_2$	321.17288
RCS-4 methylated analog type 1	$C_{22}H_{25}NO_2$	335.18853
RCS-4 methylated analog type 2	$C_{22}H_{25}NO_2$	335.18853
Positional isomer of RCS-4	$C_{21}H_{23}NO_2$	321.17288
RCS-8	$C_{25}H_{29}NO_2$	375.21983
CRA-13	$C_{26}H_{24}O_2$	368.11763
Oleamide	$C_{18}H_{35}NO$	281.27186

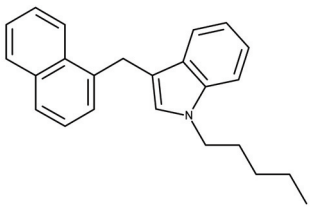
are more similar to the structure of THC with regard to the alkyl chain attached to the central phenol moiety of the compound. This plays a significant role in the interaction of these compounds with the cannabinoid receptors.

Naphthoylindoles

These compounds include the two well-known, prototypical synthetic cannabinoids JWH-018 and JWH-073 (Structure 3). These compounds contain both naphthyl and indole rings joined by a carbonyl group in the center of the compound. These compounds are generally substituted on the nitrogen of the indole ring with varying length aliphatic chains. In the case of JWH-018 there is a pentyl chain attached, and for JWH-073, a butyl substituent. These compounds are also known as alkylaminoindoles. Derivatives of these types of compounds include a well-known medicinal research synthetic cannabinoid WIN 55,212-2 (Structure 3). Halogenated analogs of JWH-018, including AM-2201 and JWH-018 chloropentyl (Structure 3) also belong in this class. Additional analogs of JWH-018 include those that are substituted on the naphthyl ring, such as JWH-081, JWH-122, and JWH-210 (Structure 3). Compounds such as MAM-2201 (Structure 3) are JWH-018 analogs that are both halogenated on the terminal end of the substituted indole moiety and substituted on the naphthyl ring. An analog of JWH-018 that contains a point of unsaturation along the aliphatic chain is JWH-022 (Structure 3). The combination of all these compounds has made the JWH series the largest class of compounds reported on the market to date.

Naphthylmethylindoles

These compounds, such as JWH-175 (**Structure 4**), are structurally similar to the naphthoylindoles. The difference in these compounds is that in place of the two double-ring

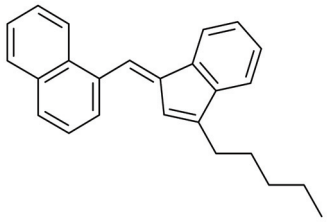


Structure 4. Naphthylmethylindoles — Structure of JWH-175.

structures being connected by a carbonyl functionality, they are attached by a methylene group.

Naphthylmethylindenes

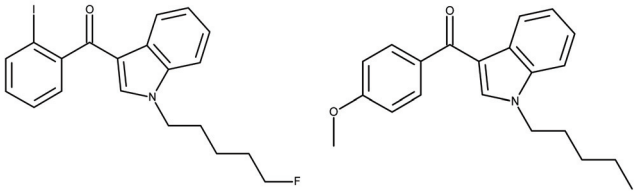
These compounds, including JWH-176 (**Structure 5**) are different from the other compounds discussed in that the indole ring is replaced by an indene ring in the compound. Also, a carbon-to-carbon double bond links the naphthyl ring to the substituted indene ring. Although this compound is structurally different from those previously discussed, it still exhibits cannabimimetic characteristics [25].



Structure 5. Naphthylmethylindenes — Structure of JWH-176.

Benzoylindoles

These compounds include AM-694 and RCS-4 (**Structure 6**) and contain two rings, a phenyl ring and a substituted indole ring. In the case of AM-694, both the phenyl and indole rings are halogenated while RCS-4 is nonhalogenated but possesses a methoxy substituent on its phenyl ring.



AM-694 RCS-4

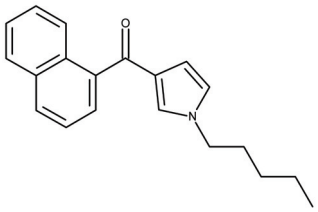
Structure 6. Benzoylindoles — Structures of AM-694 and RCS-4.

Naphthoylpyrroles

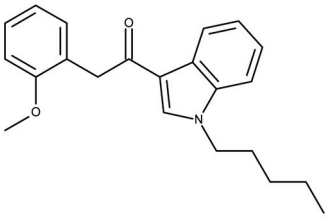
These include compounds such as JWH-030 (**Structure 7**) and contain a naphthyl ring linked to a substituted pyrrole ring by a carbonyl group. These compounds are different from the naphthoylindoles in that the pyrrole ring has replaced the indole ring.

Phenylacetylindoles

These compounds include JWH-250 (**Structure 8**) and are similar to the benzoylindoles but differ only by the presence of a methylene group between the benzene ring and the connecting carbonyl moiety. This is the basis for the term "acetyl" in "phenylacetyl".



Structure 7. Naphthoylpyrroles — Structure of JWH-030.



Structure 8. Phenylacetylindoles — Structure of JWH-250.

Adamantoylindoles

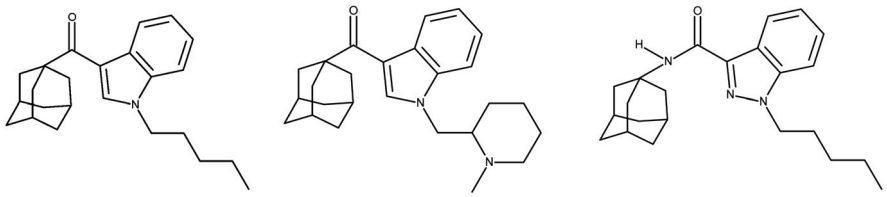
This class of compounds includes AB-001 and AM-1248 (**Structure 9**) and are similar in structure to many of the JWH series compounds, with the main difference being that the naphthyl ring is replaced by an adamantyl ring [12,26]. There are generally substituents attached to the nitrogen of the indole moiety of these compounds. This class of compounds is also referred to as "third generation" synthetic cannabinoids having appeared after the July 2012 changes to the United States Federal Schedule. An additional compound containing an adamantyl ring is AKB48 (Structure 9). It is unique, however, in that it contains an amide functionality adjacent to the adamantyl ring and also contains an indazole ring in place of the indole ring present in many of the other synthetic cannabinoids.

Tetramethylcyclopropylindoles

The latest category to appear on the market in response to changes in the law, these compounds have replaced the aromatic benzyl or naphthyl moiety with a tetramethylcyclopropyl substituent [16]. This bulky aliphatic substituent has similar space-filling properties to the aromatic series. Examples of these compounds are UR-144 and XLR-11 (**Structure 10**), which appeared on the U.S. market in mid-2012. This class of compounds is also considered "third generation".

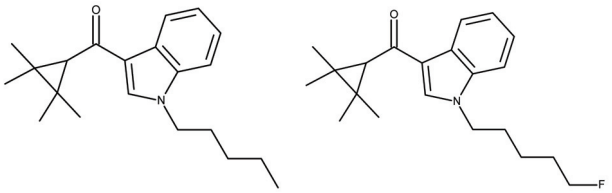
II. SCHEDULING OF SYNTHETIC CANNABINOIDS

In the wake of the large number of adverse events reports related to the use of synthetic cannabinoids, many countries have placed legal bans on the most prevalent compounds. In the United States, the Department of Jus-



AB-001 AM-1248 AKB48

Structure 9. Adamantoylindoles — Structures of AB-001, AM-1248, and AKB48.



UR-144 XLR-11

Structure 10. Tetramethylcyclopropylindoles — Structures of UR-144 and XLR-11.

tice's Drug Enforcement Administration (DEA) initially placed several compounds temporarily into Schedule I of the Controlled Substances Act (CSA), namely JWH-018, JWH-073, JWH-200, and CP 47,497 (C7 and C8 analog). This ban was to expire in February 2012 but was extended through August 2012; in July 2012, however, the United States Congress acted to permanently schedule these and other synthetic cannabinoid drugs and entire classes of their analogs through the Food and Drug Administration Safety and Innovation Act, S.3187 [15]. Under this control measure, individuals can be prosecuted for the manufacture, sale, possession, importation, and/or exportation of these compounds. Additionally, the United States had previously placed HU-210 into the Schedule I list [52]. Compounds listed under Schedule I have no accepted medicinal use, have a high potential for abuse by users, and exhibit a threat to public safety [51]. These control measures fall under legislation that has been established by the CSA as well as the Federal Analog Act. The Federal Analog Act declares that substances that are substantially similar in regards to chemical structure and pharmacological effect, and are also intended for human consumption, may be regarded as analogs of the Schedule I compounds to which they are related [53].

This Federal Analog provision has been challenged by defendants as being vague and overbroad, potentially including many compounds whose scheduling is not warranted based on their unknown pharmacological effect, potentially outlawing compounds with legitimate therapeutic potential and stifling drug development. Within the forensic chemistry community, there is no guidance or consensus around what constitutes an analog. Most chemists may agree that lengthening an alkyl side chain by one methylene unit, or replacing a hydrogen with a halogen, creates an analog, but as changes to structures become more complex (for example, the replacement of the aromatic benzyl/naphthyl ring of benzyl/naphthoylindoles with an aliphatic tetramethylcyclopropyl ring, which arguably creates a new class), the definition of what constitutes an analog of the original drug in the traditional chemical sense may be obscure. Overuse of the term "analog" blurs the lines between distinct chemical classes and undermines the precision of chemical naming. Work is currently being initiated that will attempt to provide better definition in this area, including assessment of the value of more objective methods of evaluation using chemoinformatic indices, such as Jaccard or Tanimoto scoring for evaluating similarity and diversity [38]. This approach is used in the pharmaceutical and drug development fields for designing drugs with potentially similar effects.

Other countries have also taken measures to control the distribution and abuse of synthetic cannabinoids. According to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), Denmark, Germany, Estonia, France, Ireland, Italy, Latvia, Lithuania, Luxembourg, Austria, Poland, Romania, Sweden, and the United Kingdom have also implemented regulations to control synthetic cannabinoids [13]. Luxembourg has placed bans that are similar to the Food and Drug Administration Safety and Innovation Act of the United States, S.3187, placing controls on synthetic substances known to be cannabinoid receptor agonists. It was noted by the EMCDDA that additional member states were also considering the regulation of synthetic cannabinoid substances [13]. Authorities in Australia and New Zealand have also made provisions to control synthetic cannabinoids. In Australia, several compounds have been added to their list of Schedule I drugs and in New Zealand, many compounds have been temporarily controlled through 2013 by the Minister of Health [37,44].

Since the legal status of these compounds changes rapidly and varies by jurisdiction, readers are encouraged to check the statutes and ordinances of their respective localities to determine current drug scheduling. The websites of the United States DEA (www.justice.gov/dea) and the EMCDDA (www.emcdda.europa.eu) provide the current legal status for synthetic cannabinoids in various regions; these are good initial references to utilize.

III. METHODS

The focus for this review was on those articles that discussed the analysis of synthetic cannabinoid compounds found in botanical material and powders identified for recreational use, and the analytical methods used for their identification. We consider reports in the analytical chemistry and toxicology literature of the identity of chemicals present in synthetic cannabinoid products, and the methods used for their detection and identification.

Searches for articles describing the analysis of synthetic cannabinoid compounds were performed through a number of popular search engines, including SciVerse/Science Direct, PubMed, Elsevier, and Springer Link. Search terms that resulted in the most relevant articles included "Synthetic Cannabinoids", "Herbal Incense", "JWH-018", "JWH-073", "HU-210", "Spice and K2", etc. The search was restricted to articles that appeared in the peer-reviewed literature. Twenty-two such papers were obtained and others found were used as supplemental material and supportive references. The data is presented in the following formats. The papers and their analytical approaches are described along with discussion of novel or important conclusions. In addition, Table 1 lists the compounds reported in the articles reviewed, along with the techniques used to characterize them, and the publications in which they were reported. The following section lists the articles in chronological order of publication year.

IV. RESULTS

Lindigkeit et al. (2009) [27] discussed the analysis of some of the earliest synthetic cannabinoids to appear in the German recreational drug market. This includes reports on the analysis of products sold under names such as "Spice Gold", "Chill Out", "Smoke", "Forest Humus", and others. These botanical products were purchased from local smoke shops and Internet suppliers. The workers used a Soxhlet extraction with petroleum ether and methanol, followed by analysis by electron ionization gas chromatography time-of-flight mass spectrometry (EI-GC-TOF-MS) and ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy. Since common mass spectral databases at the time did not include the synthetic cannabinoids, structural identification was accomplished using hydrogen-hydrogen correlation spectroscopy (HH-COSY), nuclear Overhauser effect spectroscopy (NOESY), heteronuclear single quantum correlation (HSQC), and heteronuclear multiple-bond correlation (HMBC) NMR, and by analysis of fragmentation patterns for the principle components of the extracts. This combination of accurate mass, structural information from EI fragmentation, and NMR allowed assignment of the structures of CP 47,497 (C8), JWH-018, and JWH-073. For extraction of CP 47,497 (C8), an additional step using silica gel column chromatography with ethyl acetate and dichloromethane and thin layer chromatography (TLC) was performed after the normal Soxhlet extraction. The authors confirmed the identity of the compounds by synthesizing standards, and characterizing them using the same systems. Finally, based on those standards, they also reported quantitatively the amount of chemical present on the botanical materials, which varied from 2.3 to 22.9 mg/g (0.2–2.3% by weight), showing a 10-fold range overall in concentration. This variability is a significant contributor to adverse effects since the users do not know the potency of the material they are buying.

Auwärter et al. (2009) [2] described the analysis of cannabimimetic compounds extracted from several herbal products, including "Sence", "Skunk", and "Yucatan Fire". Additionally, a self-administration smoking study of the herbal product "Spice Diamond" was reported by the authors. Blood and urine samples were collected for analysis of potential synthetic cannabinoids. For the botanical material, ethanolic extracts were initially screened using conventional EI-GC-MS with traditional chemical library database, multi-target electrospray ionization liquid chromatography tandem mass spectrometry (ESI-LC-MS-MS) using an ion trap mass analyzer, and immunological methods, but did not indicate the presence of any drugs, pharmaceutical or illicit. After observing GC-MS data, several signals with unknown mass spectra were observed. After thorough reanalysis, the unknown compounds were found to be CP 47,497 (C8), along with its *trans*-diastereomer, and JWH-018. After performing a silica gel column separation of TLC extracts of the botanical material, the structures of the CP compounds were elucidated using NMR spectroscopy, LC-MS^3 technology and EI fragmentation patterns. For the CP compounds, GC-MS experiments were performed both with and without derivatization. Derivatized samples were trimethylsilylated or acetylated. Seven herbal mixtures were analyzed and it was determined qualitatively that CP 47,497 (C8) as well as its *trans*-diastereomer, JWH-018, CP 47,497 (C7), and oleamide were present in these mixtures. In the blood samples drawn during the smoking experiment, CP 47,497 (C8), which was isolated using solid phase extraction, was present. However, immunological assays for blood and urine samples all gave negative results.

Uchiyama et al. (2009) [48] gave details about the first identification of CP 47,497 (C8) as an adulterant in an herbal mixture in Japan's illegal drug market. The product, which was purchased via the internet was extracted in methanol, isolated by preparative TLC, and subjected to instrumental analyses using EI-GC-MS, electrospray ionization ultra performance liquid chromatography mass spectrometry (ESI-UPLC-MS), and NMR spectroscopy.

The accurate mass of the compound was determined using an ESI-LC system with the use of an orbit trap mass analyzer.

In a separate article, Uchiyama et al. (2009) [47] provided details on the analysis of an herbal product being sold illegally in Japan around December 2008. This study produced one of the first identifications of two synthetic cannabinoids being used as adulterants in herbal mixtures, namely JWH-018 and CP 47,497 (C8). These compounds were two of the most identified substances used in herbal products for their cannabimimetic effects during this time period. Silica gel TLC and column chromatography-purified methanolic extracts of the herbal mixture were used for identification and structure elucidation of the compounds with the combination of EI-GC-MS, ESI-UPLC-MS, NMR, and direct analysis in real — time–time-of-flight mass spectrometry (DART-TOF). Using these techniques, the structure and fragmentation patterns as well as exact masses were determined for each compound. This was one of the seminal papers which provided details about synthetic cannabinoid identification in herbal products being sold in the illegal drug community.

Dresen et al. (2010) [11] described the analysis of over 140 packages of herbal incense products obtained on dates ranging from June 2008 through September 2009. During this time (January 2009), German legislation implemented a ban to control several synthetic cannabinoids, including CP 47,497 (C8) and JWH-018. The goal of this study was to determine the synthetic cannabinoid components found in these mixtures both before and after the ban was placed into effect. The herbal blends were either purchased via the Internet or submitted for testing by law enforcement officials. Ethanolic extracts of the material were analyzed by EI-GC-MS. Mass spectra obtained were compared to those available in spectral libraries. Many of the products were qualitatively found to contain CP 47,497 (C8), JWH-018, JWH-073, and JWH-250. The authors found that before implementation of the German ban, CP 47,497 (C8) and JWH-018 were the most common synthetic cannabinoids found in the herbal mixtures. After the ban, however, the naphthoylindole JWH-018 had been replaced with its *N*-butyl analog, JWH-073, and a decline in the number of products containing CP 47,497 (C8) and JWH-018 was observed. In addition, a rise in counterfeit products containing no synthetic cannabinoids at all was observed. Some of these mixtures did, however, contain bioactive substances such as Δ^9-tetrahydrocannabinol (THC), cannabidiol, nicotine, and *O*-desmethyltramadol.

Hudson et al. (2010) [21] discussed the determination of the cannabimimetic substance content of 16 different "herbal high" products. One goal of this study was to build a qualitative screening database, which at the conclusion of many analyses contained over 140 cannabimimetic compounds. The authors made use of high-resolution accurate mass spectrometry using ESI-UPLC-MS-MS technology with an orbit trap mass analyzer. This allowed identification of both previously identified and novel compounds to be performed. For analysis of the botanical material, methanolic extracts were made. Upon extraction and analysis, several compounds were found to be contained in the herbal mixtures, including JWH-018, JWH-073, CP 47,497 (C7) (as well as its C6, C8, and C9 homologs), JWH-398, JWH-007/019 (insufficient MS^n data to differentiate), JWH-047/122 (insufficient MS^n data to differentiate), an analog of JWH-081, and an analog with the same exact mass of JWH-049/182/213, although the true identity of the compound was not determined. The limitation of high-resolution mass accuracy is its inability to differentiate between structural isomers, of which there are many within the synthetic cannabinoid classes. Using this method, structural isomers need to be separated chromatographically or identified with the aid of additional analytical methods.

Uchiyama et al. (2010) [49] discussed the analysis of 46 different herbal products obtained via the Internet and suspected to contain synthetic cannabinoid adulterants that were available in the illegal drug market in Japan. Extracts of the material were prepared by powderizing the plant material and mixing with methanol, ultrasonicating, centrifuging, and filtering. The extracts were then analyzed using single quadrupole UPLC-ESI-MS and EI-GC-MS for compound identification. With the use of these systems, emphasizing GC-MS fragmentation patterns, it was found that most of the herbal products contained CP 47,497 (C8), its *trans*-diastereomer, JWH-018, and oleamide, an endocannabinoid. Additional products contained CP 47,497 (C7) and JWH-073. Confirmation of these results was performed by comparing each compound's analytical data such as fragmentation and retention times to purchased standards from commercial vendors. The quantitative values reported for these compounds range from 1.09 to 210.90 mg/g.

Ernst et al. (2011) [14] detailed the analysis of the contents of three packets of an herbal incense blend, "Lava Red", that had become available on the Internet in Germany and gained popularity after the banning of "Spice" brand products. The authors extracted components of the material using a Soxhlet extraction method with petroleum ether and methanol. Afterwards, they purified the extract using silica gel column chromatography with petroleum ether and dichloromethane and screened the eluents by TLC. The extracts from the procedure were analyzed by EI-GC-TOF and ESI-LC-MS-MS using a quadrupole ion trap system, as well as HH-COSY, NOESY, HSQC, and HMBC NMR techniques. Using the accurate mass determination from the TOF mass analyzer, protonated

molecular ion information from ESI, fragmentation patterns from EI, and NMR assignments, the structure of the naphthoylindole JWH-122 contained in the packages was determined. The average concentration of JWH-122 per packet was found to be 82 mg/g.

Hudson and Ramsey (2011) [20] described the analysis of various products containing synthetic cannabinoids including "Spice" herbal blends purchased via the Internet, products seized by customs and border agencies, and synthetic cannabinoid reference materials provided by other laboratories. As a result, the scope of the findings presented in the article is a compilation of work performed by not only their laboratory, but also other affiliated laboratories. The authors' goal was to provide general information about the chemical nature of synthetic cannabinoids and different ways that these compounds can be structurally determined using analytical instrumentation. A methanolic extract of the herbal mixtures was obtained for each product and then analyzed by ESI-LC-MS-MS using an orbitrap mass analyzer, and by EI-GC-MS. The LC-MS^n experiments provided accurate mass measurements for parent molecules as well as fragments, thus facilitating structure determination. With this data and extensive fragmentation patterns provided by EI, the structures of 30 synthetic cannabinoids were determined by the authors. For each compound, the ESI-LC-MS-MS (exact mass) and EI-GC-MS (nominal) mass spectra were provided with an image detailing explanations of fragmentation patterns.

A new cannabinoid agonist analyzed by Hudson and Ramsey, CRA-13 (also known as CB-13), is under investigation and clinical trial for its use in the medical field as an antihyperalgesic drug [17,46]. CRA-13, a product of the Novartis Institute, is structurally similar to the JWH class of synthetic cannabinoids and also exhibits affinity for the CB_1 and CB_2 cannabinoid receptors [17]. This compound has the potential to be abused due to its effect on the cannabinoid receptors and its structural similarities to other synthetic cannabinoids [20]. If this compound were to be released as a legal drug, the possibility for it to be diverted for drug abuse could be high and has the potential to also be found in synthetic cannabinoid-laced herbal mixtures or other forms.

Uchiyama et al. (2011) [45] detailed the analysis of 20 different packages of "Spice-like" herbal material and chemical products in the form of powders containing synthetic cannabinoids. These products were sold commercially via the Internet in Japan during December 2009 and April 2010. The purpose of the authors' work was to determine the presence of any new adulterants in the materials since findings from previous studies to aid in laboratories' compound identification process as well as in building a database detailing pertinent chemical information about various cannabimimetic agents. In this study, some of the compounds were initially isolated using recycling preparative HPLC and TLC methods to produce powders of the suspect cannabimimetic agents. Methanolic extracts of all compounds were used for instrumental analysis including ESI-UPLC-MS, EI-GC-MS, DART-TOF, and ^1H and ^{13}C NMR. With the use of these very precise methods, producing complex compound-specific data, the botanical and powder materials were determined to contain JWH-251, JWH-250, JWH-081, JWH-073, JWH-015, and JWH-200. The concentration of compounds in the mixtures ranged from 3.65 to 340 mg/g, a range in concentration of approximately two orders of magnitude. Purchased reference standards obtained from commercial vendors were analyzed using the same analytical methods used in this study as a means of confirming the identity of the synthetic cannabinoids extracted from the herbal and powdered products.

Bononi et al. (2011) [4] described the analysis of a seized powder identified as a "chemical" imported to Italy from China in November 2010. Methanolic extracts were analyzed using a variety of instrumental methods. ESI-LC-MS-MS, EI-GC-MS, ^1H and ^{13}C NMR, and LC-TOF were used in the structure elucidation of JWH-203 contained within the seized powder sample. The goal of the authors' study was to provide analytical information for the determination and structure elucidation of synthetic cannabinoid JWH-203 by various analytical methods. This information can be used to confirm the presence of this substance being used as an adulterant in various herbal mixtures being sold and distributed illegally in the recreational drug market.

Nakajima et al. (2011) [35] detailed the analysis of herbal and powdered products obtained via the Internet and the synthetic cannabinoids identified. The products analyzed were purchased during September and December 2010. Each packet of material (43 total) was powderized and extracted in methanol as a means of preparation for instrumental analysis. Silica gel column purification was also performed on the extracts. For the analysis and structure elucidation, ESI-LC-MS, ESI-LC-TOF, EI-GC-MS, and ^1H and ^{13}C NMR experiments were performed. After examining data, the products were found to contain: AM-694, RCS-4, JWH-210, JWH-122, JWH-019, JWH-200, JWH-015, JWH-250, JWH-073, JWH-251, and JWH-081 ranging in concentration from 4.0 to 359 mg/package of material. This large range in concentration indicates the inconsistency in the distribution of cannabimimetic substances from package to package.

In a separate article, Nakajima et al. (2011) [34] described the quantitative analysis of herbal and powdered products containing synthetic cannabinoids purchased via the Internet during January and February 2011. The workers used methanolic solutions of each product obtained

for analysis using instrumental methods. The materials analyzed in these experiments were either herbal materials or capsules containing powder. The compounds were first purified using silica gel column chromatography. ESI-UPLC-MS, ESI-LC-TOF, EI-GC-MS, and ^{1}H and ^{13}C NMR were used to identify the following compounds: AM-2201, AM-694, RCS-4, a structural isomer of RCS-4, and JWH-122. The concentrations of these compounds ranged from 11.0 to 185 mg/package of material. Previously isolated and characterized compounds were used as standards in this study to compare the findings and confirm the identity of the newly isolated compounds.

In a third article, Nakajima et al. (2011) [36] discussed the analysis of five products obtained via the Internet containing synthetic cannabinoids and their identification and quantitative analysis. The products, which were in herbal and powdered forms, were purchased during November 2009 and January 2010. The products were found to contain synthetic cannabinoid JWH-250. The structure of this compound was elucidated with the use of ESI-LC-MS, LC-TOF, EI-GC-MS, and ^{1}H and ^{13}C NMR methods. The extraction procedure utilized by the authors involved making a methanolic extract of the herbal and powdered mixtures and isolating using silica gel column chromatography. In the five products obtained, the concentration of JWH-250 ranged from 77.4 to 165 mg/package of material.

Logan et al. (2012) [28] discussed the analysis of 82 "legal high" or "incense" herbal products, liquids, powders, and capsules sold via the Internet in the United States, or submitted for testing as evidence by law enforcement officials or investigating agencies. The products analyzed included the popular "K2" and "Spice" labeled products. These products are of importance in the United States due to recent legislation passed that includes the banning of several synthetic cannabinoids and their analogs due to their harmful effects. The products were analyzed using EI-GC-MS, HPLC, LC-TOF, and TLC. The extraction procedure for aliquots of botanical material taken from the products included first homogenizing samples by grinding them into a fine powder using sandpaper and combining individual fractions. Additional aliquots were taken and dissolved in methanol. An acid/base extraction was also performed for TLC and EI-GC-MS analyses using 10% HCl and concentrated NH_4OH. An internal standard was also used in this procedure. For some GC-MS experiments, samples were derivatized using *N,O*-bis(trimethylsilyl) trifluoroacetamide (BSTFA) producing trimethylsilyl derivatives of the compounds for analysis. Underivatized samples were analyzed by EI-GC-MS as well. LC-TOF experiments allowed for the determination of exact mass as well as the compound's chemical formula. This technique was useful for compounds that did not have readily available certified standards. For the TLC experiments, a number of visualization techniques were used. The compounds detected using all of these methods include JWH-018, JWH-019, JWH-073, JWH-081, JWH-200, JWH-210, JWH-250, CP 47,497 (C8), RCS-4, RCS-8, AM-2201, and AM-694. The concentration of these compounds ranged from 5 to 65 mg/g of material analyzed. Various authentic reference standards were purchased from commercial vendors for confirmation of identified compounds during analysis. These standards were also used to build a database of known cannabimimetic compounds for identification in future studies.

Cox et al. (2012) [6] described the analysis of 32 packets of herbal incense materials obtained from various U.S. locations around Raleigh, Durham, and Chapel Hill, NC. The herbal products were obtained both before and after the U.S. DEA ban on synthetic cannabinoids and were tested to compare the components both before and after the ban. The method used for analysis was solid phase microextraction-headspace gas chromatography-mass spectrometry (SPME-HS-GC-MS). Extraction of the botanical materials included heating a small portion of the solid material along with an internal standard to vaporize the volatile components in preparation for the extraction procedure. Each sample was extracted with a carboxen/polydimethylsiloxane SPME fiber and incubated at 200 °C with pulse-agitation at 250 rpm. After incubation and equilibration, the fiber was injected into the GC and analytes were desorbed. A similar method of preparation and analysis was performed for various synthetic cannabinoid standards that were used to confirm the presence of compounds in the herbal products. Analytes detected using this method of analysis included JWH-018, JWH-073, JWH-250, JWH-081, JWH-019, Pravadoline (WIN 48,098), AM-694, and AM-2201. In addition to the analytes detected, the authors analyzed a multitude of synthetic cannabinoid standards that were not detected in the herbal products. The authors discussed the presence of JWH-250, JWH-019, and AM-694 in several herbal products that were not currently scheduled by the U.S. DEA and also the absence of CP 47,497 and its isomers, which were scheduled. This gives an indication that the clandestine manufacturers of the herbal products continue to find ways to circumvent current laws in place against the use and distribution of synthetic cannabinoids. However, some herbal products obtained after the ban still contained some of the currently scheduled compounds.

Moosmann et al. (2012) [32] detailed the analysis of an herbal mixture, "XoXo", obtained via the Internet and a microcrystalline powder seized by German authorities. The herbal mixture was found to contain a methylated analog of AM-2201 as well as nonmethylated AM-2201, while the microcrystalline powder contained JWH-412,

a positional isomer of AM-2201. GC-MS and ^{1}H and ^{13}C NMR analyses were performed on both samples to facilitate the structural identification of the substances in the materials obtained. For the GC-MS analyses, ethanolic extracts of samples were produced and were either dried down and reconstituted or diluted with ethyl acetate. For the extraction and purification process of the components of the herbal material, a flash chromatography method was developed in which separate ethanolic solutions were filtered and silica powder was added to them. After drying down, the coated silica powder was added to an Rf cartridge; following that, a C_{18} column and Rf apparatus was used to separate the analytes from impurities using aqueous formic acid and methanol mobile phases in a gradient elution. An absorbance wavelength range of 207–360 nm was utilized. The desired components were further processed by the removal of solvent using rotary evaporation and extracting several times with tert-butyl methyl ether (TBME). The final purity of the AM-2201 analog was greater than 95%.

Gottardo et al. (2012) [18] utilized matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) as a screening technique in the analysis of 31 commercial herbal blends. The materials were purchased from local Italian "smart shops". The aim of the experiment was to create a simultaneous, rapid, high-throughput method for the detection of synthetic cannabinoids in herbal blends with minimal sample preparation in which no extraction or chromatographic separation was needed prior to analysis. Sample preparation for the analysis consisted of grinding the botanical material using a mortar and pestle and loading it onto a MALDI plate followed by the addition of a matrix/surfactant mixture of α-cyano-4-hydroxy-cinnamic acid (CHCA): cetyltrimethylammonium bromide (CTAB) (13,250:1). Once the mixture was dried, an additional aliquot of the matrix/surfactant mixture was added and the sample was loaded onto the ion source for analysis. The desorption/ionization laser used in this experiment was a 20-Hz nitrogen laser with a 337-nm emission wavelength. The acceleration voltage used was 20 kV with a 100-ns delay time; mass spectral data obtained was averaged over 50 laser shots. In addition to the molecular ions of interest, the presence of adducts produced was factored into compound identification. Out of the 31 herbal blends analyzed, 21 were positive for synthetic cannabinoids. The following compounds were identified: JWH-018, JWH-073, JWH-081, JWH-250, JWH-210, JWH-019, and AM-694. These results were consistent with prior GC-MS analysis of the herbal blends.

Grabenauer et al. (2012) [19] described the analysis of 32 botanical products obtained both before and after the 2011 U.S. DEA ban of several synthetic cannabinoids. The analysis consisted of a high-resolution UPLC method using a quadrupole time-of-flight (Q-TOF) mass spectrometry system and a mass defect filter in an attempt to discriminate between isomers and analogs of JWH-018 that possess molecular masses whose mass defects are similar in value and can be differentiated with the use of a mass defect filter. The filter used in the analysis was a 50-mDa filter. In the mass spectral analysis both low and high collisional energies were utilized (MS^{E} acquisition) in single reaction monitoring mode (SRM) and the post-data obtained allowed the differentiation of molecules based on the common fragmentation ion/precursor ion differences in mass defect. The compounds identified in the analysis of ethanolic extracts of the herbal products were: JWH-250, JWH-073, JWH-018, JWH-019, JWH-122, JWH-081, AM-694, AM-2201, and Pravadoline (WIN 48,098). The products obtained before the DEA ban was introduced contained all of the previously mentioned compounds except for JWH-122, AM-2201, and Pravadoline. After the ban, the compounds identified in the products were JWH-018, JWH-122, AM-694, AM-2201, and Pravadoline. It was also determined that greater than 80% of all currently published synthetic cannabinoid structures that are related to the core structure of JWH-018 fall within the 50-mDa mass defect filter utilized in this experiment.

Jankovics et al. (2012) [26] provided details on the analysis of four pure bulk powder samples obtained during a seizure by the Hungarian customs office at an international airport. Labels on the powders indicated that they were to contain calcium stearate and malic acid. Dilute methanolic solutions were made for analysis by ESI-LC-MS-MS with a triple quadrupole system, EI-GC-MS, and LC-TOF to aid in elucidating the structures of the compounds contained in the seized powders. With the combination of molecular ion determination, extensive fragmentation patterns, and exact mass, the authors determined that each of the four powders were different substances in their pure form. The first powder contained JWH-018, the second JWH-122, the third AM-679, and the final AB-001, which is the adamantoyl analog of JWH-018. To further characterize the structures of these compounds, ^{1}H and ^{13}C HSQC, HMBC, COSY, and NOESY NMR experiments were also performed. Since the structures of JWH-018 and JWH-122 had been previously determined by other workers, these compounds were used as standards in the study to aid in the structure determination of AM-679 and AB-001.

Westphal et al. (2012) [55] discussed the analysis of an herbal mixture seized by the Bavarian State Criminal Police Office. The authors isolated a crystalline powder from the botanical material and performed an enrichment step using preparative TLC. An ethanolic extract of the

enriched crystalline material was obtained and then analyzed for its contents by various analytical techniques. ESI-LC-MS-MS, EI, and chemical ionization gas chromatography mass spectrometry (CI-GC-MS) using methane as a reagent gas, and distortionless enhancement by polarization transfer (DEPT), COSY, HSQC, and HMBC NMR experiments were used in the structure elucidation. With the use of mass spectral databases and various studies using EI fragmentation findings, as well as CI and ESI confirmation of protonated molecular ion and fragment peaks, the herbal mixture was found to contain JWH-073 and an unknown compound that had similar characteristics to JWH-073. This unknown compound was determined to be an analog of JWH-073 named 1-butyl-3-(1-(4-methyl) naphthoyl)indole. The structures of both compounds were confirmed with the use of ^{1}H and ^{13}C NMR spectroscopy.

Shanks et al. (2012) [43] described the analysis of 98 "legal high" products of various brands for the identifcation of synthetic cannabinoids. The consistency of the products included dried plant material, powders, pills, and capsules. The goal of this study was also to determine the psychoactive components of the products both before and after the US DEA ban. The time periods involved were December 1, 2009, through March 1, 2010, for the preban and March 2, 2010, through April 1, 2012, for postban. The products involved in the time period before the ban were referred to as the "first generation" products and those after the ban were termed "second generation" products. It should also be noted that during the "second generation" time range, legislation was passed in the state of Indiana, effective July 1, 2011, which banned 19 synthetic cannabinoids. The products analyzed were obtained from local convenience stores, smoke shops, or via the Internet. Some products analyzed during the postban time period were obtained from Indiana law enforcement agencies that had seized the products from businesses selling them. The authors extracted the samples with an acetonitrile:methanol mixture (50:50) by sonication in a water bath; the extracts were then diluted 1:50 with acetonitrile:deionized water (20:80) and analyzed by UPLC-TOF-MS. The compounds detected in the analysis were JWH-018, JWH-019, JWH-073, JWH-122, JWH-210, JWH-250, AM-2201, AM-2233, and RCS-8, of which only JWH-018 and JWH-073 were currently federally scheduled by the DEA. Before the ban, JWH-250 was the only compound identified in addition to JWH-018 and JWH-073. After the ban, all of the listed compounds were identified in various packages. The most prevalent compound detected was AM-2201, appearing in 70% of the "second generation" products. This analysis demonstrated that illicit drug manufacturers constantly replace scheduled drugs with nonscheduled compounds in a rapid manner to evade current legislation.

V. DISCUSSION

Analysis in the majority of the articles discussed in this review was performed by GC-MS, LC-MS-MS, or LC-TOF. These techniques offer appropriate sensitivity for the concentrations of synthetic cannabinoids identified in the commercial products tested. Hudson [20] offered a comparison of the relative merits of GC-MS and LC-MS-MS and noted that the extensive fragmentation provided by EI-GC-MS aided in initial identification and provided conditions for subsequent confirmation by LC-MS-MS. High-resolution LC-TOF gives accurate masses for these compounds, enabling calculation of molecular formulae, which is useful information for identifying novel or previously unknown compounds. For many of the JWH series and related compounds that are structural isomers having identical molecular formulae but subtly different structures, EI mass spectrometry was the preferred method, providing distinguishing detail in fragmentation patterns. In their article, Uchiyama et al. [47] discuss various structure elucidation methods used for the determination of the structure of JWH-018. One such method of determination was EI-GC-MS. The EI mass spectrum of JWH-018 along with its chemical structure with labeled fragmentation assignments is provided in **Figure 1**. This is given as an example of one way forensic chemists use mass spectral information to elucidate the structures of novel compounds. The authors of many of the articles discussed in this review provide full explanations concerning the data collected for the structure determination of the compounds of interest and how each different method contributed to the overall identification.

Soft ionization techniques, namely ESI, producing in general only molecular ions, lack the level of discriminating power for closely related compounds that EI-MS has. Additionally, TOF-MS has the disadvantage of being unable to differentiate between isomers since it identifies compounds based on their accurate molecular mass. Some researchers used the less selective technique of HPLC with UV detection as a complementary analytical tool although its value is extremely limited in the identification of unknowns. HPLC was also used as a preparative technique in conjunction with column or thin layer chromatography as a means of purifying samples and preparing extracts to be used in instrumental analysis.

Less commonly, authors used GC-TOF, SPME-HS-GC-MS, DART-TOF, and MALDI-TOF instrumentation, which are more frequently available in research laboratories rather than in forensic laboratories. The DART-TOF interface is an ionization method similar to ESI-MS with the exception that ionization is performed in a totally ambient environment instead of inside the instrument.

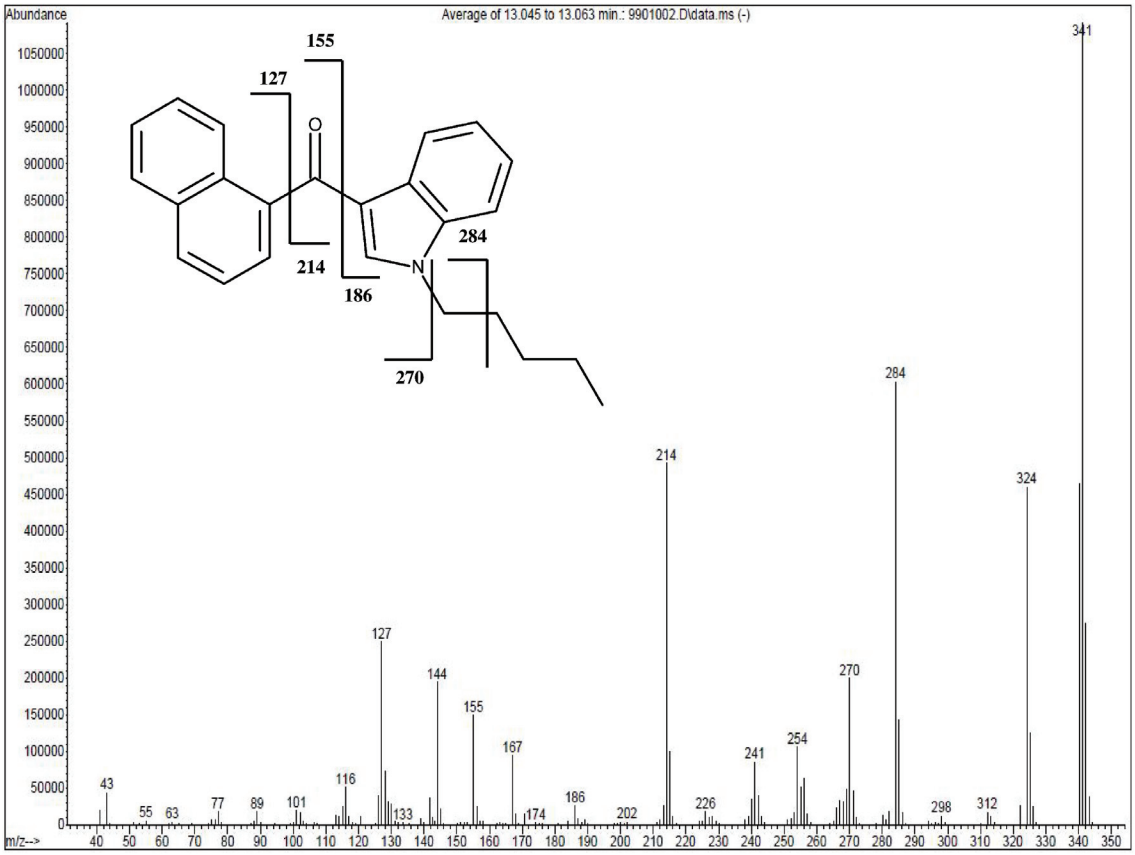


Figure 1. EI mass spectrum of JWH-018 (nominal mass 341 amu) with chemical structure indicating several fragment assignments.

DART-TOF and MALDI-TOF analysis have the advantage of minimal sample preparation, where the need for extraction or chromatographic separation of analytes prior to analysis is not present; this provides the potential for high-throughput analysis, which is attractive in forensic laboratories. MALDI is not as susceptible to ion suppression or enhancement when compared to other types of ionization techniques such as ESI [18]. One drawback for MALDI lies in the analysis of low-molecular-weight molecules where matrix ions can cause interference issues with analyte ions.

Certain compounds, specifically the HU and CP series of compounds that are polar and therefore not well suited to simple GC-MS analysis, required derivatization to allow their analysis by GC-MS. Samples were derivatized with BSTFA to enhance volatility, sensitivity, and chromatographic performance [28]. During the derivatization process, the phenol, cyclohexanol, and other active oxygens of each respective compound (namely HU-210, CP 47,497 and its homologs, and CP 55,940) formed TMS derivatives. These derivatized compounds gave much improved chromatographic performance.

Several authors describe the use of NMR spectroscopy, a technique that is useful for structural elucidation and synthesis confirmation purposes. The use of NMR provides details about the chemical structure of the compounds allowing other structurally similar compounds to be differentiated from one another. Many of the authors used advanced NMR techniques in their analyses that gave further substantiation of structure elucidation, and allowed differentiation of isomers. The technique was also important with the appearance of new synthetic compounds that began to appear whose chemical structures were unknown, and therefore were not included in spectral databases. Although not common in most forensic laboratory facilities, NMR is a critical tool in the verification of the identity of new compounds.

Two authors reported research identifying synthetic cannabinoids that may potentially be used in the future as adulterants in products on the illegal drug market [4,20]. This information is useful since forensic laboratories have the task of determining the substances contained in illicit products on an ongoing basis and need to keep abreast with current trends. For example, there was an emergence of a new generation of compounds reported to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) in 2011 and 2012 that included the adamantoylindoles AB-001, AM-1248 (Structure 9) [46], and the tetramethylcyclopropylindoles such as UR-144 and XLR-11 (Structure 10) [12,16]. More research and investigation still needs to be performed by forensic

practitioners and shared between laboratories and agencies if the forensic community is to remain effective in the analysis of products such as those discussed in this review and those to come.

Some of the factors that work against the rapid identification of novel compounds are the practice in many laboratories of only reporting results for scheduled compounds. This slows development of the awareness of new compounds that might warrant scheduling and lessens some of the urgency by standards suppliers for providing new analytical standards. Additionally, while European laboratories share information and awareness of the appearance of new compounds through groups such as EMCDDA, in the United States there is no equivalent network. The National Forensic Laboratory Information System (NFLIS) (www.nflis.deadiversion.usdoj.gov) attempts to do this but includes only a few laboratories and can only include the compounds they report. NFLIS data are also not available in real time; the reports provided are generally midyear and annual reports that can be outdated by the time they are released. Additional efforts need to be undertaken to rapidly confirm identifications of novel compounds and share that information between laboratories. This includes building analytical databases that contain reference mass spectra of emerging compounds. Some available sources for this information includes Forensicdb (www.forensicdb.org), provided through RTI International and the National Institute of Justice; Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) Mass Spectral Library (www.swgdrug.org/ms.htm); Wiley Mass Spectra of Designer Drugs Library (www.wiley.com/WileyCDA/WileyTitle/productCd-3527333320,miniSiteCd-STMDB2.html); Cayman Spectral Library (CSL), provided through Cayman Chemical (www.caymanchem.com/app/template/SpectralLibrary.vm); Forendex, provided through the Southern Association of Forensic Scientists, with access information to multiple mass spectral libraries and other chemical compound reference information (forendex.southernforensic.org).

CONCLUSION

The growth and continued diversification of the designer drug market is driven by several factors. These include manufacturers' attempts to stay one step ahead of the laws regulating these compounds, demand from the drug-using community for novel highs, and awareness of drug users that the latest chemicals will not be detected in traditional urine drug testing. The forensic science community has responded with the application of both traditional and emerging analytical technologies and methods. This will become increasingly difficult in the future as the array of abused chemicals becomes larger and more complex. Among the major challenges for scientists in this area includes the limited availability of the resources needed to continually update and validate the scope of their testing; coordinated efforts to build databases of relevant analytes; finding appropriate reporting language to characterize novel and often unscheduled compounds in their reports; building and sharing databases to aid in identification, and rapidly communicating findings of novel compounds. It is essential to track prevalence and trends in the illicit drug market in order to know how to apply constrained laboratory resources most effectively, and prioritize which compounds to include in analytical scopes. A growing need exists for certified reference standards of newly detected compounds that are required in order to be able to report the presence of synthetic cannabinoid drugs in forensic case samples. The forensic science community must continue to devote resources to performing research and analysis and reporting the findings through professional groups and publications in this rapidly evolving area.

REFERENCES

1. Ammann J, Drummer O, Gerostamoulos D, Beyer J: Detection of synthetic cannabinoids in biological samples — A review; *TIAFT Bulletin* 41(3):21; 2011.
2. Auwärter V, Dresen S, Weinmann W, Müller M, Pütz M, Ferreirós N: JMS Letter: 'Spice' and other herbal blends: Harmless incense or cannabinoid designer drugs? *J Mass Spectrom* 44:832; 2009.
3. Bisogno T, Katayama K, Melck D, Euda N, De Petrocellis L, Yamamoto S, Di Marzo V: Biosynthesis and degradation of bioactive fatty acid amides in human breast cancer and rat pheochromocytoma cells — Implications for cell proliferation and differentiation; *Eur J Biochem* 254:634; 1998.
4. Bononi M, Belgi P, Tateo F: Analytical data for the identification of the cannabimimetic phenacetylindole JWH-203; *J Anal Toxicol* 35:360; 2011.
5. Collins M: Some new psychoactive substances: Precursor chemicals and synthesis-driven end-products; *Drug Test Anal* 3:404; 2011.
6. Cox A, Daw R, Mason M, Grabenauer M, Pande P, Davis K, Wiley J, Stout P, Thomas B, Huffman JW: Use of SPME-HS-GC-MS for the analysis of herbal products containing synthetic cannabinoids; *J Anal Toxicol* 36:293; 2012.
7. De Fonseca FR, Schneider M: The endogenous cannabinoid system and drug addiction: 20 years after the discovery of the CB_1 receptor; *Addict Biol* 13:143; 2008.
8. De Petrocellis L, Melck D, Palmisano A, Bisogno T, Laezza C, Bifulco M, Di Marzo V: The endogenous cannabinoid anandamide inhibits human breast cancer cell proliferation; *Proc Natl Acad Sci USA* 95:8375; 1998.
9. Devane WA, Dysarz FA, Johnson MR, Melvin LS, Howlett AC: Determination and characterization of a cannabinoid receptor in rat brain; *Molec Pharm* 34:605; 1988.
10. Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, Gibson D, Mandelbaum A, Etinger A, Mechoulam R: Isolation and structure of a brain constituent that binds

to the cannabinoid receptor; *Science* 258:1946; 1992.
11. Dresen S, Ferreirós N, Pütz M, Westphal F, Zimmerman R, Auwärter V: Monitoring of herbal mixtures potentially containing synthetic cannabinoids as psychoactive compounds; *J Mass Spectrom* 45:1186; 2010.
12. EMCDDA: Europol 2011 Annual Report on the implementation of Council Decision 2005/387/JHA; http://www.emcdda.europa.eu/publications/implementation-reports/2011 (accessed June 2012).
13. EMCDDA: Synthetic cannabinoids and 'Spice'; http://www.emcdda.europa.eu/publications/drug-profiles/synthetic-cannabinoids (accessed June 2011).
14. Ernst L, Schiebel H, Theuring C, Lindigkeit R, Beuerle T: Identification and characterization of JWH-122 used as a new ingredient in "Spice-like" herbal incenses; *Forensic Sci Int* 208:e31; 2011.
15. Food and Drug Administration Safety and Innovation Act, S.3187; http://www.gpo.gov/fdsys/pkg/BILLS-112s3187enr/pdf/BILLS-112s3187enr.pdf (accessed August 2012).
16. Frost JM, Dart MJ, Tietje KR, Garrison TR, Grayson GK, Daza AV, El-Kouhen OF, Yao BB, Hsieh GC, Pai M, et al.: Indol-3-ylcycloalkyl ketones: Effects of N1 substituted indole side chain variations on CB2 cannabinoid receptor activity; *J Med Chem* 53:295; 2010.
17. Gardin A, Kucher K, Kiese B, Appel-Dingemanse S: Cannabinoid receptor agonist 13, a novel cannabinoid agonist: First human pharmacokinetics and safety; *Drug Metab Dispos* 37:827; 2009.
18. Gottardo R, Chiarini A, Dal Prà I, Seri C, Rimondo C, Serpelloni G, Armato U, Tagliaro F: Direct screening of herbal blends for new synthetic cannabinoids by MALDI TOF MS; *J Mass Spec* 47:141; 2012.
19. Grabenauer M, Krol W, Wiley J, Thomas B: Analysis of synthetic cannabinoids using high-resolution mass spectrometry and mass defect filtering: Implications for non-targeted screening of designer drugs; *Anal Chem (*doi: 10.1021/ac300509h); 2012.
20. Hudson S, Ramsey J: The emergence and analysis of synthetic cannabinoids; *Drug Test Anal* 3:466; 2011.
21. Hudson S, Ramsey J, King L, Timbers S, Maynard S, Dargan PI, Wood DM: Use of high-resolution accurate mass spectrometry to detect reported and previously unreported cannabinomimetics in "herbal high" products; *J Anal Toxicol* 34:252; 2010.
22. Huffman JW: CB_2 receptor ligands; *Mini-Rev Med Chem* 5:641; 2005.
23. Huffman JW, Dai D: Design, synthesis and pharmacology of cannabimimetic indoles; *Bioorg Med Chem Lett* 4:563; 1994.
24. Huffman JW, Padgett LW: Recent developments in the medicinal chemistry of cannabimimetic indoles, pyrroles and indenes; *Curr Med Chem* 12:1395; 2005.
25. Huffman JW, Zengin G, Wu M, Lu J, Hynd G, Bushell K, Thompson A, Bushell S, Tartal C, Hurst D, et al.: Structure-activity relationships for 1-alkyl-3-(1-naphthoyl)indoles and the cannabinoid CB_1 and CB_2 receptors: Steric and electronic effects of naphthoyl substituents: new highly selective CB_2 receptor agonists; *Bioorg Med Chem* 13:89; 2005.
26. Jankovics P, Váradi A, Tölgyesi L, Lohner S, Németh-Palotás J, Balla J: Detection and identification of the new potential synthetic cannabinoids 1-pentyl-3-(2-iodobenzoyl) indole and 1-pentyl-3-(1-adamantoyl)indole in seized bulk powders in Hungary; *Forensic Sci Int* 214:27; 2012.
27. Lindigkeit R, Boehme A, Eiserloh I, Luebbecke M, Wiggermann M, Ernst L, Beuerle T: Spice: A never ending story; *Forensic Sci Int* 191:58; 2009.
28. Logan BK, Reinhold LE, Diamond FX, Xu A: Identification of synthetic cannabinoids in herbal incense blends in the United States; *J Forensic Sci* 57:1168; 2012.
29. Makriyannis A, Deng H: Cannabimimetic indole derivatives; Patent No.: US 6,900,236 B1; 2005.
30. Matsuda L, Lolait S, Brownstein M, Young A, Bonner T: Structure of a cannabinoid receptor and functional expression of the cloned cDNA; *Nature* 346:561; 1990.
31. Mechoulam R, Gaoni Y: Isolation, structure, and partial synthesis of an active constituent of hashish; *J Am Chem Soc* 86:1646; 1964.
32. Moosmann B, Kneisel S, Girreser U, Brecht V, Westphal F, Auwärter V: Separation and structural characterization of the synthetic cannabinoids JWH-412 and 1-[(5-fluoropentyl)-1H-indol-3-yl]-(4-methylnaphthalen-1-yl)methanone using GC-MS, NMR analysis and a flash chromatography system; *Forensic Sci Int* 220:e17; 2012.
33. Munro S, Thomas K, Abu-Shaar M: Molecular characterization of a peripheral receptor for cannabinoids; *Nature* 365:61; 1993.
34. Nakajima J, Takahashi M, Nonaka R, Seto T, Suzuki J, Yoshida M, Kanai C, Hamano T: Identification and quantitation of a benzoylindole (2-methoxyphenyl) (1-pentyl-1*H*-indol-3-yl)methanone and a naphthoylindole 1-(5-fluorpentyl-1*H*-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 [^{35}S]GTPγS binding assays; *Forensic Toxicol* 29:132; 2011.
35. Nakajima J, Takahashi M, Seto T, Kanai C, Suzuki J, Yoshida M, Hamano T: Identification and quantitation of two benzoylindoles AM-694 and (4-methoxyphenyl) (1-pentyl-1*H*-indol-3-yl)methanone, and three cannabimimetic naphthoylindoles JWH-210, JWH-122, and JWH-019 as adulterants in illegal products obtained via the internet; *Forensic Toxicol* 29:95; 2011.
36. Nakajima J, Takahashi M, Seto T, Suzuki J: Identification and quantitation of cannabimimetic compound JWH-250 as an adulterant in products obtained via the internet; *Forensic Toxicol* 29:51; 2011.
37. New Zealand Temporary Drug Class Notices; http://www.customs.govt.nz/news/resources/listsandguides/Documents/temporary-class-drug-notices.pdf (accessed November 2012).
38. Nikolova N, Jaworska J: Approaches to measure chemical similarity — A review; *QSAR Comb Sci* 22:1006; 2003.
39. Penn HJ, Langman LJ, Unold D, Shields J, Nichols JH: Detection of synthetic cannabinoids in herbal incense products; *Clin Biochem* 44:1163; 2011.
40. Piggee C: Investigating a not-so-natural high: Researchers identify synthetic cannabinoids in herbal incense; *Anal Chem* 81:3205; 2009.
41. Pop E: Cannabinoids, endogenous ligands and synthetic analogs; *Curr Opin Chem Biol* 3:418; 1999.
42. Seely K, Lapoint J, Moran J, Fattore L: Spice drugs are more than harmless herbal blends: A review of the pharmacology and toxicology of synthetic cannabinoids; *Prog*

Neuro-Psychopharmacol Biol Psychiatry; http://dx.doi.org/10.1016/j.pnpbp.2012.04.017 (accessed June 2012).
43. Shanks K, Dahn T, Behonick G, Terrell A: Analysis of first and second generation legal highs for synthetic cannabinoids and synthetic stimulants by ultra-performance liquid chromatography and time of flight mass spectrometry; *J Anal Toxicol* 36:360; 2012.
44. South Australia Controlled Substances (Controlled Drugs, Precursors and Plants) Regulations 2000 under the Controlled Substances Act 1984; Version: 19.7.2012; http://www.legislation.sa.gov.au/lz/c/r/controlled%20substances%20(controlled%20drugs%20precursors%20and%20plants)%20regulations%202000/current/2000.199.un.pdf (accessed November 2012).
45. Uchiyama N, Kawamura M, Kikura-Hanajiri R, Goda Y: Identification and quantitation of two cannabimimetic phenacetylindoles JWH-251 and JWH-250, and four cannabimimetic naphthoylindoles JWH-081, JWH-015, JWH-200, and JWH-073 as designer drugs in illegal products; *Forensic Toxicol* 29:25; 2011.
46. Uchiyama N, Kawamura M, Kikura-Hanajiri R, Goda Y: Identification of two new-type synthetic cannabinoids, *N*-(1-adamantyl)-1-pentyl-1H-indole-3-carboxamide (APICA) and *N*-(1-adamantyl)-1-pentyl-1H-indazole-3-carboxamide (APINACA), and detection of five synthetic cannabinoids, AM-1220, AM-2233, AM-1241, CB-13 (CRA-13), and AM-1248, as designer drugs in illegal products; *Forensic Toxicol* 30:114; 2012.
47. Uchiyama N, Kikura-Hanajiri R, Kawahara N, Goda Y: Identification of a cannabimimetic indole as a designer drug in a herbal product; *Forensic Toxicol* 27:61; 2009.
48. Uchiyama N, Kikura-Hanajiri R, Kawahara N, Haishima Y, Goda Y: Identification of a cannabinoid analog as a new type of designer drug in an herbal product; *Chem Pharm Bull* 57:439; 2009.
49. Uchiyama N, Kikura-Hanajiri R, Ogata J, Goda Y: Chemical analysis of synthetic cannabinoids as designer drugs in herbal products; *Forensic Sci Int* 198:31; 2010.
50. UNODC: Synthetic cannabinoids in herbal products; http://www.unodc.org/documents/scientific/Synthetic_Cannabinoids.pdf (accessed April 2012).
51. U.S. Department of Justice, Drug Enforcement Administration, Office of Diversion Control: Controlled Substance Schedules; http://www.deadiversion.usdoj.gov/schedules/index.html (accessed May 2012).
52. U.S. Department of Justice, Drug Enforcement Administration, Office of Diversion Control: Drugs and Chemicals of Concern HU-210; http://www.deadiversion.usdoj.gov/drugs_concern/spice/spice_hu210.htm (accessed May 2012).
53. U.S. Department of Justice, Drug Enforcement Administration, Office of Diversion Control: Title 21 United States Code (USC) Controlled Substances Act Section 813. Treatment of Controlled Substance Analogues; http://www.deadiversion.usdoj.gov/21cfr/21usc/813.htm (accessed May 2012).
54. Weissman A, Milne G, Melvin L: Cannabimimetic activity from CP-47,497, a derivative of 3-phenylcyclohexanol; *J Pharmacol Exp Ther* 223:516; 1982.
55. Westphal F, Sönnichsen FD, Thiemt S: Identification of 1-butyl-3-(1-(4-methyl)naphthoyl) indole in a herbal mixture; *Forensic Sci Int* 215:8; 2012.

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Dr. Logan is board certified by the American Board of Forensic Toxicologists (ABFT). In recognition of his work and contributions, Dr. Logan has received the American Academy of Forensic Sciences (AAFS) Rolla N. Harger Award and the National Safety Council's Robert F. Borkenstein Award. He currently serves as president-elect of the AAFS.