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A Forensic Analysis of Synthetic Cannabinoids

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Abstract

Synthetic cannabinoids are a drug produced in a laboratory designed to imitate the physiological and psychological effects of a specific controlled substance. They are designed to bind to receptors in the body that recognize THC and CBD metabolites from the cannabis plant. The synthetic cannabinoid was originally created to study these receptors and neurotransmitters in hopes to find a viable alternative to traditional medicine.

Forensic scientists often encounter an issue of detection when dealing with synthetic cannabinoids due to the inconsistencies in the chemical formulas of their derivatives. This leads to difficulty in identifying the drug in biological matrices as it can avoid detection in standard drug tests for cannabinoids, or other related substances. Scientists continue to study how it is metabolized in the body and the effects it causes. A future goal for toxicologists is to develop a standardized test that has the potential to identify them regardless of the inconsistencies in different blends.

Several studies are reviewed which analyze the strongest methods for detection and identification of synthetic cannabinoids under different conditions. Researchers are working towards sharing important information with healthcare professionals to properly treat those who experience severe intoxication. With each scientific advancement toward the identification and regulation of synthetic cannabinoids, research can be applied practically to create laws, standard techniques and treatment plans. They aim to decrease the rates of abuse of synthetic cannabinoids around the world.

Table of Contents

1. Introduction

New drugs are being introduced into society every day. Although most are created with good intentions through the eyes of science, they may not be utilized in the ways expected. Synthetic cannabinoids were created shortly after the discovery of THC in cannabis, for the purpose of research and therapeutic medications. When their work fell into the wrong hands, the public began to produce this designer drug to sell on the market. These drugs are not well regulated by laws because their composition is always changing.

Metabolism of the compounds in the body is a unique process for each strain dependent on what chemicals it contains. With understanding how the synthetic cannabinoids are broken down, they can be connected to the metabolites produced by this process. Knowledge of the correspondence between metabolites and the original substance allows researchers to create valid methods of detection. Numerous studies have been conducted to determine the most reliable and accurate ways to identify and quantify the strain present in biological samples. This information can assist scientists in creating a standardized test for synthetic cannabinoids. A standardized testing method can serve to enforce regulations on this drug and decrease the rates of abuse.

It is known that this drug negatively affects health in various ways. With the reports of intoxication cases, researchers and healthcare professionals can work towards educating the public on the signs and symptoms to watch. Synthetic cannabinoids are also being studied to further understand how they interact with the corresponding endocannabinoid system in the human body to cause these harmful effects. Future goals of the exploration of synthetic cannabinoids includes creation of a common database of information, decreasing hazard for law enforcement when encountering the drug, and developing a standardized method of detection for improved regulation and public safety.

2. History

2.1. Cannabis: The Parent Substance

The natural plant, *Cannabis sativa*, has been utilized for several centuries as both a recreational and medicinal drug. The plant is grown and prepared in various ways. Marijuana is made from dried flower buds, stems and leaves (Sharma et al., 2012). A resin, called hashish, is derived from the plant's flowering buds (Sharma et al., 2012). Each product will vary in potency. These forms are among the most used drugs in the world today. The drug is typically smoked or ingested (NIDA, 2021).

Cannabis sativa contains more than 500 chemicals, where more than 100 of those chemicals are classified as cannabinoids (NIDA, 2021). In the 1960s, the main psychoactive substance of this drug was discovered (Mills et al., 2015). This cannabinoid named delta 9 - tetrahydrocannabinol, most referred to as THC or Δ9-THC, has been most notably used for recreational purposes among adolescents and young adults (Sharma et al., 2012). A survey conducted for children in school in the United Kingdom showed 40% of fifteen- to sixteen-year-olds, and 59% of eighteen-year-olds confirmed using cannabis at least once (Sharma et al., 2012). These rates of abuse are being seen to increase worldwide.

Cannabinoids are the psychoactive chemicals present in the drug that create those "desirable" effects that users want. Some of these effects are a feeling of euphoria, laughter, heightened sensory perception, increased appetite, and change in perception of time (NIDA, 2021). Specifically, the effects of relaxation and pain regulation has attracted researchers to investigate its potential for therapeutic applications and biomedical benefits (Sharma et al., 2012). The clinical conditions in which cannabis has been incorporated into treatment plans are

cancer, epilepsy, asthma, and glaucoma (Sharma et al., 2012). Its purpose is being further investigated as scientists and healthcare professionals gain more knowledge about the drug.

Although not all responses to this drug are positive. These effects produced will vary for each person. Some may even experience fear, paranoia, or anxiety (NIDA, 2021). Many users may endure side effects that are harmful to their health. This can include impaired memory and motor skills, altered judgement, chronic bronchitis, depression, and psychosis (Volkow et al., 2014). Altered brain development, declines in IQ, and addiction are also possible adverse effects, most seen with chronic use by teens and young adults (Volkow et al., 2014).

In the United States, marijuana is now classified as a Schedule I Controlled Substance due to its high potential for abuse, no current accepted medicinal use and lack of accepted safety under medical supervision. A controlled substance is any drug or substance regulated and categorized into schedules by the federal government under the Controlled Substances Act of 1970 (*What Is a Controlled Substance?,* 2019). The strict laws and regulations placed on this drug causes frequent users to search for an alternative to avoid the consequences. This is one of the main factors that led to the development and abuse of synthetic cannabinoids.

2.2. Synthetic Cannabinoids

Commonly referred to as “Spice” or “K2”, synthetic cannabinoids are a chemical derivative of the parent substance, cannabis (Mills et al., 2015). Shortly after the discovery of the psychoactive substance, THC, in cannabis, researchers began to synthesize cannabinoids in order to study the function of this new substance (Mills et al., 2015). Due to the fabrication process of this substance, they are categorized as a designer drug. Designer drugs are defined as a synthetic analog of a substance, that is illegal or prohibited, to circumvent drug laws (Luethi & Liechti,

2020). The intended use for this compound was to study the pharmacological interactions between THC and receptors in the brain (Mills et al., 2015). It was believed further research would help them discover uses of this drug in therapeutic and medicinal means.

By the early 2000s, synthetic cannabinoids were being marketed as “herbal smoking blends", for sale in convenience stores, gas stations, and online shops (Camp, 2011). The drug may be smoked, ingested, or insufflated (Mills et al., 2015). An attractive feature of these new substances was the belief that they are natural, although packages were labeled “not for human consumption” (Evren & Bozkurt, 2013). It is typically sold at a low price and is not detected on a common drug test (Mills et al., 2015). A more recently popular method of use and distribution is selling it in a liquid form to be smoked in e-cigarettes, called “herbal liquid” (Le Boisselier et al., 2017). They were considered a legal alternative to marijuana (Mills et al., 2015). This caused them to gain popularity among high school and college students. A survey of high school students found that one in nine children has admitted to using synthetic cannabinoids (Mills et al., 2015).

The only connection of these synthetics to marijuana is the chemical structure (Mills et al., 2015). They are created from laboratory chemicals mimicking the shape of THC molecules with increased potency (Mills et al., 2015). The synthetic derivative can be one hundred times more potent than the natural substance. This allows them to interact with the same receptors in the body as the natural drug, causing similar desirable effects (Mills et al., 2015). Because of the lack of quality control in their production, the concentrations fluctuate from batch to batch with unknown contaminants (Mills et al., 2015). Individuals cannot truly be aware of what they are taking and at what quantity (Camp, 2011).

The variability of chemicals contained in a synthetic cannabinoid derivative causes unpredictable adverse health effects. Some of the experienced effects reported to hospital emergency departments include tachycardia, lethargy, irritability, agitation, vomiting, hypertension, chest pain, and dizziness (Mills et al., 2015). Studies have also shown that chronic use of synthetic cannabinoids may lead to severe intoxication, psychosis, and even death (Mills et al., 2015). The number of synthetic cannabinoid intoxication is observed to be rapidly increasing as the drug continues to be abused. The American Association of Poison Control has reported a volume of 13,000 calls in 2011 regarding "spice" exposure, which is a drastic increase from 53 calls in 2009 (Evren & Bozkurt, 2013). Education of healthcare professionals, as well as the public, on symptoms of intoxication is important for proper treatment of patients and decreasing rates of abuse.

The deficiency of regulation in the manufacture and sale of synthetic cannabinoids makes it difficult to document all the chemicals the drug may contain. The packages do not list the ingredients (Evren & Bozkurt, 2013). Law enforcement is unable to legally restrict the sale of this drug without knowing the specific synthetic compounds used (Evren & Bozkurt, 2013). Some of the popular derivatives, such as the JWH series, are well-known and banned in many countries (Evren & Bozkurt, 2013). To overcome the ban on any strain, a new analogue is created in its place (Evren & Bozkurt, 2013). Researchers are working to create a standard method to detect synthetic cannabinoids and the chemicals it contains to further assist in legislation against them (Evren & Bozkurt, 2013).

2.3. How Synthetic Cannabinoids Are Produced

As stated above, synthetic cannabinoids are a mixture of chemicals created in a laboratory. The structure of Δ9-THC was the focus behind forming the structure for synthetic cannabinoids (Mills et al., 2015). This is due to the focus of research on this psychoactive component of cannabis. It is a 21-carbon tricyclic structure that contains two chiral centers in trans-configuration (Sharma et al., 2012). The three six member rings, one of which is a benzene ring, was used as a focal point in creating the structure of synthetic cannabinoids. The structure of Δ9-THC is displayed in Figure 1A (Mills et al., 2015). The chemical structure of synthetic cannabinoids, in general, mimics part of this ring structure giving it the ability to interact with our body in a similar way. For example, this specific cannabinoid structure for HU-210, in Figure 1B, contains the same group of three six member rings (Mills et al., 2015).

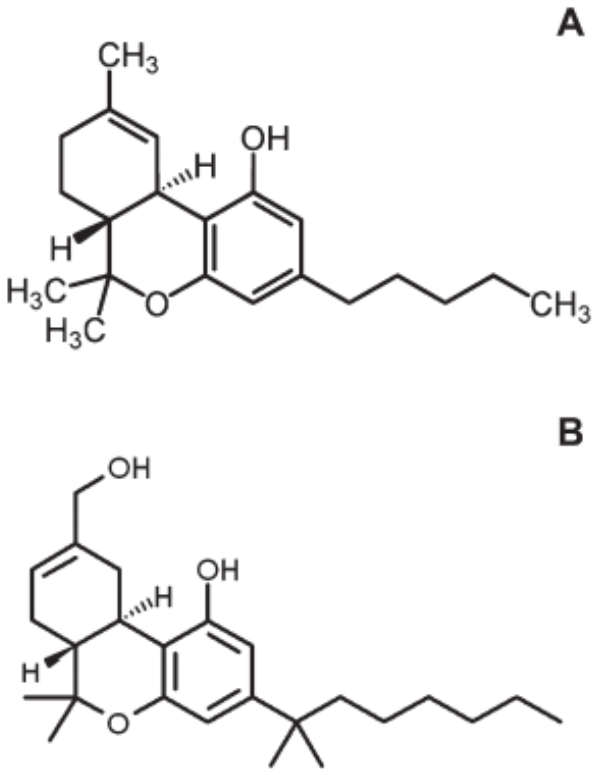
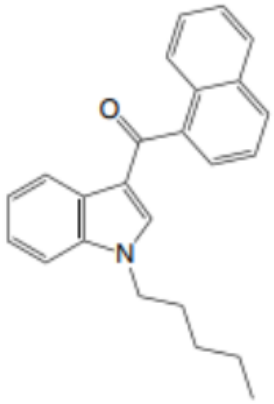


Figure 1. (A) Structure of THC (B) Structure of HU-210.
(Mills, B., Yepes, A., & Nugent, K. (2015). Synthetic Cannabinoids. *The American Journal of the Medical Sciences*, *350*(1), 59–62.)



JWH-018

Figure 2. Structure of JWH-018.
(Wells, D. L., & Ott, C. A. (2011). The "new" marijuana. *Annals of Pharmacotherapy*, *45*(3), 414–417. https://doi.org/10.1345/aph.1P580)

The middle ring contains oxygen in the same place as well as the connections of the benzene ring and the five-carbon tail. The additions to the carbon tail and/or the first ring will differ depending on the synthetic compound made.

As new byproducts are manufactured, the structure became altered, while still being able to function in the same manner. This can be observed when looking at the well-known JWH series of synthetic cannabinoids (Luethi & Liechti, 2020). This compound (Figure 2) contains two, six-member rings and a five-carbon tail similar to the structure of THC. Although, it also has a benzene ring attached to a pentane ring containing nitrogen, which mimic aspects of the structure of the hormone serotonin (Wells & Ott, 2011). The purpose of this was to increase interactions with serotonin receptors in the body, as well as the cannabinoid receptors, to produce more desirable effects (Wells & Ott, 2011).

The ways in which this compound is manufactured depends upon the way it will be used, such as smoked, ingested or insufflated (Mills et al., 2015). The preparation of the drug for each type starts off with a similar process. The compounds created are by, first, dissolving the synthetic chemical into a solvent (Mills et al., 2015). The most common solvents are acetone or ethanol (Mills et al., 2015). The chosen plant material being used is then soaked in the solution until fully saturated (Mills et al., 2015). The material is removed and allowed to dry, causing the solvent to evaporate. The synthetic cannabinoid will be left behind and remain on the plant material (Mills et al., 2015). This material is what will be packaged for sale. Common plant material known to be used in this process includes *Pedicularis densiflora*, marshmallow leaf, damiana leaf, and mullein leaf (Ciolino, 2015). The concentration of cannabinoids left behind on the material will vary based upon the chemicals used, which will also determine the potency of

the strain. A typical package of "spice" sold will contain about three grams of the psychoactive substance (Mills et al., 2015).

As new synthetic compounds are introduced, the manufacturing process can vary. For example, creating synthetic cannabinoid oils for e-cigarette use eliminates the need for plant materials as a carrier. Instead, it may use vegetable glycerin or propylene glycol as the medium (Le Boisselier et al., 2017). Research on the materials can reveal how the drug may be created, which could assist scientists in determining the concentrations of specific variations. This could lead to a better understanding behind the risks and effects of use.

3. Metabolism

3.1. The Endocannabinoid System

The endocannabinoid system is a complex system of receptors, enzymes, and ligands that helps to regulate fundamental processes of the central and peripheral nervous systems, as well as to create responses to endogenous and environmental insults (Lu & Mackie, 2016). The main receptors involved are named CB1 and CB2 receptors. More specifically, they are G-protein-coupled membrane receptors (Battista et al., 2012). The CB1 receptor is the most abundant of this class of membrane receptors identified so far (Marzo et al., 2004). It is expressed at high levels in the central nervous system and several parts of the brain, including the basal ganglia, cortex, cerebellum, and hippocampus (Marzo et al., 2004). They are also observed in the peripheral nervous system, but at a lower concentration (Lu & Mackie, 2016). The mass of their presence is located in the pre-terminal axon segments and axon terminals (Lu & Mackie, 2016).

The strong concentration of CB1 receptors in motor and sensory regions of the brain support the role they play in motivation and cognition (Mechoulam & Parker, 2013). Studies have shown that these receptors are present and active beginning from embryonal stages of human development (Mechoulam & Parker, 2013). This demonstrates the importance of CB1R in neuronal development (Mechoulam & Parker, 2013). Their distribution differs in an adult brain than a neonatal brain. This led researchers to consider their effect on behavioral landmarks associated with different ages (Mechoulam & Parker, 2013). When the CB1R are activated, cyclic adenosine monophosphate (cAMP) accumulation is decreased, inhibiting the function of cAMP dependent protein kinase (Mechoulam & Parker, 2013). It will also stimulate mitogen-activated protein kinase activity. This is the mechanism by which cannabinoids affect cell migration and synaptic plasticity (Mechoulam & Parker, 2013).

The CB2 receptor is abundant in the peripheral nervous system and the immune system tissues and cells (Mechoulam & Parker, 2013). They are also seen in the central nervous system, but at a lower concentration than CB1 (Mechoulam & Parker, 2013). This receptor has been shown to contribute to inflammation and chronic pain management (Marzo et al., 2004). In general, it has been identified as part of a protective system in the body (Mechoulam & Parker, 2013). The lipid endocannabinoid signaling through CB2R allows them to assist in preventing foreign non-protein attacks on the immune system, as well as reducing and repairing inflicted damage (Mechoulam & Parker, 2013). For this reason, CB2R has become an interest for pharmaceutical researchers. They believe formulating drugs that use specific synthetic CB2 antagonists could be used for medicinal purposes, including neuropsychiatric and liver disease (Mechoulam & Parker, 2013).

Lipid mediators in the body, which include amides, esters, and ethers of long chain polyunsaturated fatty acids, are called endogenous cannabinoids that are isolated from brain and peripheral tissues (Mechoulam & Parker, 2013). They could stimulate or inhibit the CB1 and CB2 receptors (Mechoulam & Parker, 2013). These compounds mimic the action of THC in numerous biological processes (Battista et al., 2012). THC is also a lipid compound that is known to bind and interact with these receptors (Mechoulam & Parker, 2013). The most notable types of endogenous cannabinoids produced by the body are 2-arachidonoylglycerol and anandamide (Figure 3) (Lu & Mackie, 2016). These internal cannabinoids can act as modulators of the signaling pathways and activate the involved receptors (Battista et al., 2012).

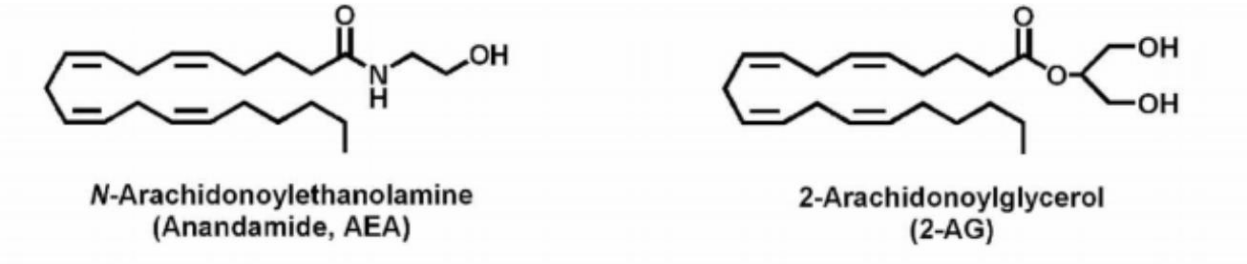


Figure 3. The structure of Anandamide and 2-arachidonoylglycerol.

These interactions of endogenous cannabinoids are reflected in the regulatory effects on brain and behavioral functions (Battista et al., 2012). Anandamide is a high-affinity partial agonist for CB1 receptors and mostly inactive at CB2 receptors (Zou & Kumar, 2018). On the other hand, 2-arachidonoylglycerol acts as an antagonist for both receptors, but it has a low binding affinity (Zou & Kumar, 2018). Both compounds are synthesized when they are needed, and their actions are presynaptic (Mechoulam & Parker, 2013). The primary activity of these endocannabinoids is to act as fast retrograde synaptic messengers (Mechoulam & Parker, 2013). After it crosses the synapse, it is able to activate the cannabinoid presynaptic receptor

(Mechoulam & Parker, 2013). Various neurotransmitter systems that are present there will then be inhibited (Mechoulam & Parker, 2013).

THC is known to interact with the endocannabinoid receptors in the same manner as endogenous cannabinoids (Mechoulam & Parker, 2013). After entering the body, THC takes several hours to be metabolized and synthesized into the molecules that can interact with these receptors (Mechoulam & Parker, 2013). The metabolized THC that activates CB1 receptors causes hindrance of releasing excitatory and inhibitory neurotransmitters in the brain and peripheral nervous system (Mechoulam & Parker, 2013). This activation may also cause a release of dopamine (Mechoulam & Parker, 2013).

Synthetic cannabinoids have a stronger affinity and reaction with these specific receptors than THC. They can release or inhibit the same neurotransmitters; however, it could be at a higher concentration (Zou & Kumar, 2018). The different chemicals that are used in synthetic cannabinoids can interact in various ways. The users experience could differ with each use depending on what neurotransmitters are influenced, although the outcome is not always one that is desired. "As a result of systemic activation of the CB1R, the accompanying side effects always include cardiovascular dysfunction, digestion failure, neurological disorders and potential for addiction" (Zou & Kumar, 2018). The psychoactive effects may be blocked rather than induced by synthetic cannabinoid metabolites (Zou & Kumar, 2018). The prolonged use of synthetic cannabinoids with a high efficacy could also cause chronic drug effects including tolerance, dependency, and withdrawal (Fantegrossi et al., 2014). The inconsistency of chemicals used in each batch will affect the experience for the user depending on how their metabolites engage with CB1R and CB2R.

Although scientists have discovered these interactions with the body, the true purpose of this internal system has yet to be determined. As years progress, numerous studies are conducted to enhance and extend knowledge about it. Some of the internal processes that all categories of cannabinoids can affect include the following: appetite, digestion, liver function, stress, sleep, motor control, cardiovascular function, pain modulation, memory, and mood (Battista et al., 2012). As a result, researchers believe that one of the purposes of this system is to maintain homeostasis in the body (Mechoulam & Parker, 2013). The CB1 receptor is responsible for psychoactive effects in response to THC, as well as affecting cognition, reward systems, and anxiety (Mechoulam & Parker, 2013). The CB2 receptor is responsible for immunosuppression functions including inflammation and related tissue injury (Mechoulam & Parker, 2013). Research is further being explored to determine how the stronger interactions with synthetic cannabinoids could reveal their therapeutic potential (Zou & Kumar, 2018).

3.2. Metabolism of Synthetic Cannabinoids

As previously discussed, synthetic cannabinoids are complex compounds that have a high binding affinity, potency, and efficacy at the CB1 and CB2 receptors (Cannaert et al., 2016). The process by which this drug is metabolized in the body is a major factor that contributes to the stronger interactions. The drug must be metabolized in a specific way to result in the proper chemical structure to fit with the receptors. Metabolites are created as a result of the drug being processed through different biological pathways in the body (*Metabolite - an Overview | Sciencedirect Topics*, n.d.). The resulting compound is specific to the metabolism of the original substance. Understanding the ways this illicit drug is metabolized may allow us to predict the ways it could negatively affect our health, along with identifying proper practices for treatment.

This knowledge will also assist forensic laboratories to predict toxicity and develop effective detection methods.

Most strains of synthetic cannabinoids are extensively metabolized in the body. This becomes problematic when working to identify them in biological matrices (Kong et al., 2018). First, they undergo oxidation by a group of enzymes called cytochromes P450s, or CYPs (Fantegrossi et al., 2014). Next, conjugation occurs to form glucuronic acid, a sugar moiety, by UDP-glucuronosyltransferase enzymes, or UGTs (Fantegrossi et al., 2014). Researchers have explored and documented the metabolic pathways of specific synthetic cannabinoids. For instance, JWH-018, chemically named naphthalen-1-yl(1-pentyl-1H-indol-3-yl)methanone, is a primary and commonly encountered type of synthetic cannabinoid (Kong et al., 2018). This was one of the first strains investigated with an *in vivo* analysis using urine specimens (Fantegrossi et al., 2014). The oxidation of this compound will begin with the primary hepatic P450 isoforms identified as CYP2C9 and CYP1A2, creating N-4 or N-5 hydroxypentyl-JWH-018 (Kong et al., 2018). The CYP2C9 enzyme is abundant in the intestines, therefore, it will metabolize synthetic cannabinoids following oral administration (Fantegrossi et al., 2014). The CYP1A2 enzyme is highly expressed in the lungs, which will be important for the metabolism of synthetic cannabinoids that are smoked (Fantegrossi et al., 2014). This compound will then move on to conjugative metabolism. Major isoforms of UGTs, including UGT1A1 and UGT2B7, will interact with the compound to form glucuronic acid conjugations (Fantegrossi et al., 2014). This new form of the compound is believed to be important for excretion of the metabolites from the body as they are highly expressed in the collected urine samples (Fantegrossi et al., 2014). The

full characterization of the metabolism of JWH-018 can be observed in Figure 4 (Kong et al., 2018).

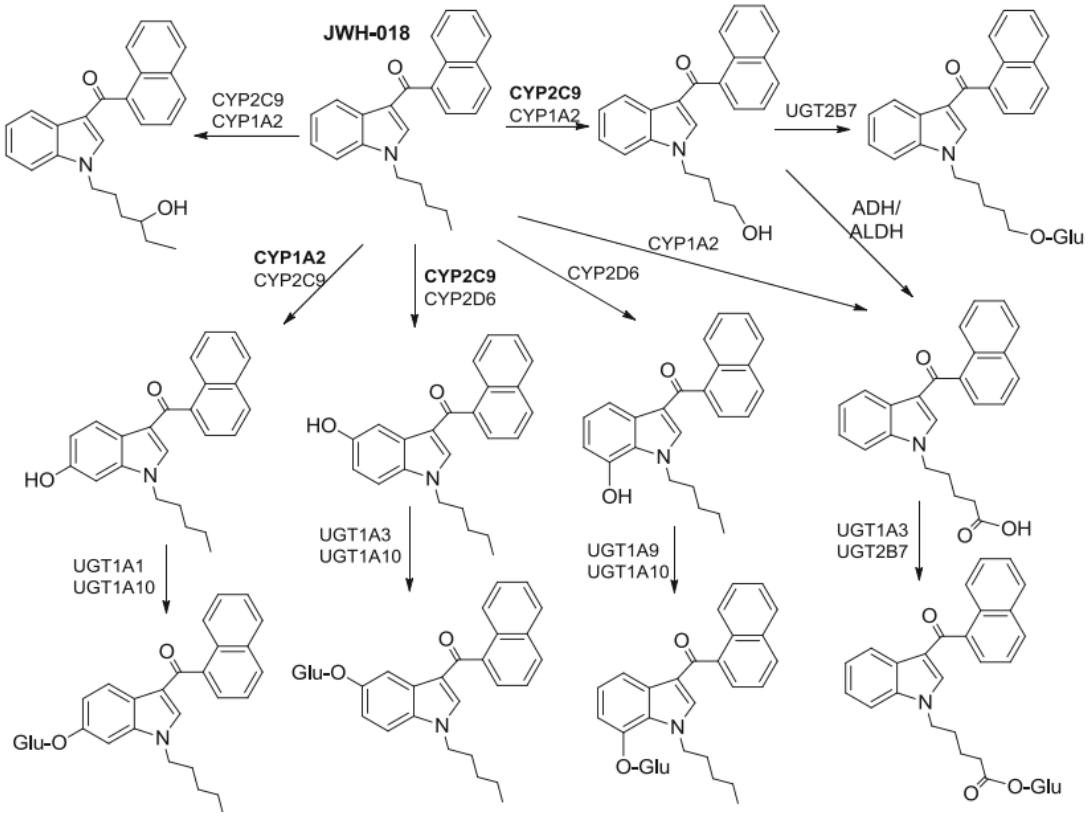


Figure 4. Metabolism characterization of JWH-018. (Kong, T. Y., Kim, J.-H., Kim, D. K., & Lee, H. S. (2018). Synthetic cannabinoids are substrates and inhibitors of multiple drug-metabolizing enzymes. *Archives of Pharmacal Research*, *41*(7), 691–710. https://doi.org/10.1007/s12272-018-1055-x)

Although this is the most frequent pathway, there are several others that synthetic cannabinoids may take through the body that cause different outcomes. Synthetic cannabinoids of the same class will exhibit similar pathways. These pathways may be identified by the drug-metabolizing enzymes that the compound could interact with (Kong et al., 2018). Compounds of differing classes may be broken down by different isoforms of the CYP and UGT enzymes. Below shows the results from a study conducted to identify the pathways of thirteen synthetic

cannabinoids (Figure 5) (Kong et al., 2018). Most classes show interactions between the same enzymes with few variations.

Table 1 Characterization of drug-metabolizing enzymes responsible for the metabolism of synthetic cannabinoids

Chemical class	Synthetic cannabinoids	Drug-metabolizing enzymes	References
Naphthoylindoles	JWH-018	CYPs 1A2, 2C9, 2C19, 2D6, 2E1, 3A4 UGTs 1A1, 1A3, 1A9, 1A10, 2B7 ADH/ALDH	Chimalakonda et al. (2011, 2012, 2013) and Holm et al. (2016)
	JWH-073	UGTs 1A1, 1A3, 1A9, 1A10, 2B7	Chimalakonda et al. (2011)
Halogenated naphthoylindoles	AM-2201	CYPs 1A2, 2C9, 2C19, 2D6, 2E1, 3A4 ADH/ALDH	Chimalakonda et al. (2012, 2013) and Holm et al. (2016)
	MAM-2201	CYPs 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A4	Kong et al. (2017a)
	EAM-2201	CYPs 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 2J2, 3A4/5	Kim et al. (2016)
Indazole carboxamides	AKB-48 (APINACA)	CYPs 3A4, 2D6, C19, 1A2, 2C8, 2C9 ADH/ALDH	Holm et al. (2015, 2016)
	AB-CHMINACA	CYPs 1A2, 2B6, 2C9, 2C19, 2D6, 2E1, 3A4	Erratico et al. (2015)
	AB-FUBINACA	CES1, CES2	Thomsen et al. (2015)
	AB-PINACA	CES1, CES2	Thomsen et al. (2015)
Tetramethylcyclopropyl ketone indoles	UR-144	CYPs 1A2, 2C19, 3A4, 2B6	Nielsen et al. (2016) and Sobolevsky et al. (2012)
	XLR-11 (5F-UR-144)	CYPs 1A2, 2C19, 3A4	Nielsen et al. (2016)
Quinolinyl ester indoles	QUPIC (PB-22)	CES1, CES2	Thomsen et al. (2015)
	5F-QUPIC (5F-PB-22)		

ADH alcohol dehydrogenase, *ALDH* aldehyde dehydrogenase, *CES* carboxylesterase

Figure 5. Metabolism characterization of 13 synthetic cannabinoids. (Kong, T. Y., Kim, J.-H., Kim, D. K., & Lee, H. S. (2018). Synthetic cannabinoids are substrates and inhibitors of multiple drug-metabolizing enzymes. *Archives of Pharmacal Research*, *41*(7), 691–710. https://doi.org/10.1007/s12272-018-1055-x).

It can be concluded that the high affinity retained by the metabolites for the CB1 and CB2 receptors are a result of the method by which they are processed in the body. Our bodies will break down the drugs in our system for them to be excreted. The metabolites that are absorbed into our tissues during the process stay behind and target specific receptors. The way these enzymes change the compound causes them to be structurally fit for later interaction with the receptors. As researchers monitor the intake and changes to these compounds, they can determine how the body may be affected. With the identification of the metabolic profiles of

synthetic cannabinoid classes, new screening methods and toxicity evaluations can be investigated by forensic and clinical laboratories (Kong et al., 2018).

4. Forensic Detection Methods

With the introduction of any new drugs into society, detection and identification methods need to be researched and developed to successfully recognize these compounds. The following four studies work to detect synthetic cannabinoids in various situations. Each provides vital information for further investigation and advancement in drug identification. They have achieved valid and reproducible results in their methods of detection.

4.1. Analysis of Metabolites

Any xenobiotic substance introduced into the human body will be modified into specific metabolites as a byproduct of the metabolism process. These metabolites are the key to detecting a drug in a biological sample. Exploration of the byproduct can provide forensic technicians and healthcare personnel with crucial information leading to the identification of the initial substance consumed. Specifically in the forensic realm, the amount of biological fluid received for an ante mortem analysis is usually limited (Fort et al., 2017). The forensic technicians need to know how to properly handle the sample in order to obtain reliable and accurate results. Maintaining stability of the drug's metabolites within the sample is vital for valid outcomes. Variables, including time, storage, and care, are the main factors that can affect the condition of a sample (Fort et al., 2017).

A study was executed by the Office of the Chief Medical Examiner of Oklahoma and the Forensic Science Institute at the University of Central Oklahoma to research the stability of

different synthetic cannabinoids in biological specimens in several conditions (Fort et al., 2017). More specifically, they used four synthetic cannabinoid compounds in whole blood to establish degradation data. The four compounds used were AB-Pinaca, ABFubinaca, XLR-11, and UR-144 (Fort et al., 2017). With this class of drugs becoming more persistent in society, the researchers can establish imperative material on how casework should be prioritized in a forensic laboratory.

The researchers chose to use liquid chromatography with tandem mass spectrometry (LC-MS/MS) as the technique for analysis (Fort et al., 2017). The technique of LC-MS/MS begins with the separation of the compound being investigated by liquid chromatography. It is an ideal method for separating larger, non-volatile compounds (*Liquid Chromatography Mass Spectrometry (Lc-Ms) Information - Us*, n.d.). The interaction between the molecules with the stationary phase and its affinity for the mobile phase will determine the degree of separation. Following separation, the compounds are eluted off the column and vaporized into a gas phase (*Liquid Chromatography Mass Spectrometry (Lc-Ms) Information - Us*, n.d.). The separated gases will be ionized and introduced into the tandem mass spectrometer. The ions are divided by mass to charge ratio and the information is sent to an electronic output for analysis by the technician (*Liquid Chromatography Mass Spectrometry (Lc-Ms) Information - Us*, n.d.).

The specific instrument used in this experiment consisted of an Agilent 1290 Infinity series liquid chromatograph paired with an Agilent 6420 triple-quadrupole mass spectrometer (Fort et al., 2017). The machine utilized a 10 μL injection (Fort et al., 2017). The flow rate was set for 0.5 mL/min flow of the mobile phase consisting of 0.1% formic acid in water and 0.1% formic acid in acetonitrile (Fort et al., 2017). A temperature of 40°C was regulated in the column compartment (Fort et al., 2017). The run time of the instrument was 8.2 minutes and a 1.4-

minute post time (Fort et al., 2017). The limit of detection (LOD) was determined to be 0.025 ng/mL (Fort et al., 2017). The limit of quantification (LOQ) was measured as 0.1 ng/mL (Fort et al., 2017).

To begin the experiment, a calibration curve needed to be created with standards of each derivative being tested. The researchers chose to create a seven-point calibration curve by using the following concentrations for the four cannabinoids: 0.1, 0.25, 0.5, 1.0, 2.5, 5.0 and 10.0 ng/mL (Fort et al., 2017). Low, 5 ng/mL, and high, 50 ng/mL, quality control samples containing the four compounds were also initially run (Fort et al., 2017). The curve and quality control samples allow for comparison to experimental data and determination of accuracy, linearity, and precision.

Each analyte sample was prepared by spiking a 200 mL sample of whole human blood with 1.0 µg of the synthetic cannabinoid with a concentration of 5 ng/mL (Fort et al., 2017). For each analysis, the sample was aliquoted equally into forty-two 16 × 100 mm^2 borosilicate glass test tubes (Fort et al., 2017). The test tubes were secured with polypropylene screw caps, and 14 for each sample were placed in the appropriate temperature condition (Fort et al., 2017). The study was conducted over a span of twelve weeks with three different temperature variables (Fort et al., 2017). One set of samples for each of the four synthetic cannabinoids were placed at room temperature, 22℃, refrigerated, 4℃, and frozen, -20℃ (Fort et al., 2017). The samples were then tested on the following study days: 0, 3, 7, 14, 21, 28, 35, 42, 56, 70 and 84 (Fort et al., 2017). They were also careful to continually analyze the benchtop stability of the quality control sample over the initial 72 hours of the experiment. This allowed for the elimination of any possible contamination or degradation of the quality control sample. Although, no issues were detected (Fort et al., 2017).

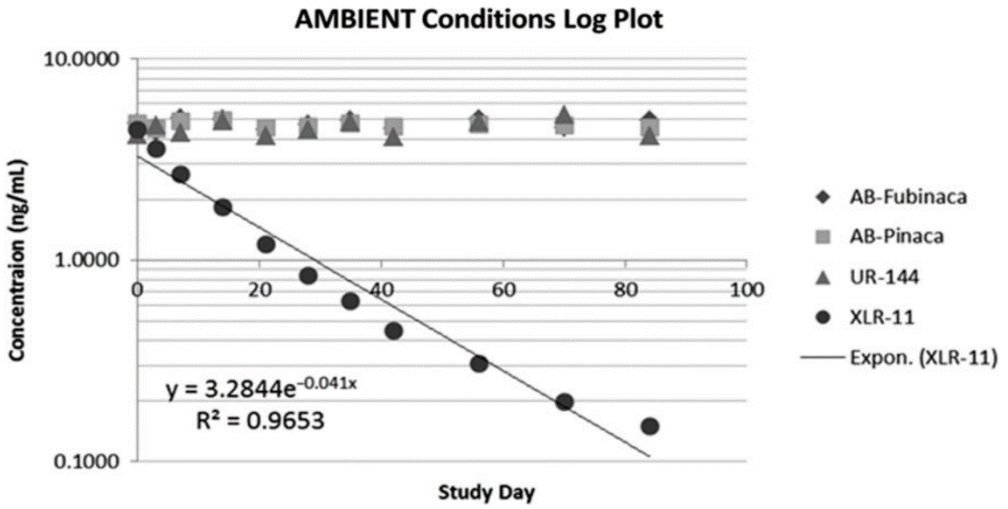


Figure 6. Results for all compounds at 22°C. (Fort, C., Jourdan, T., Jesse Kemp, & Curtis, B. (2017). Stability of synthetic cannabinoids in biological specimens: Analysis through liquid chromatography tandem mass spectrometry. *Journal of Analytical Toxicology*, *41*(5), 360–366. https://doi.org/10.1093/jat/bkx015)

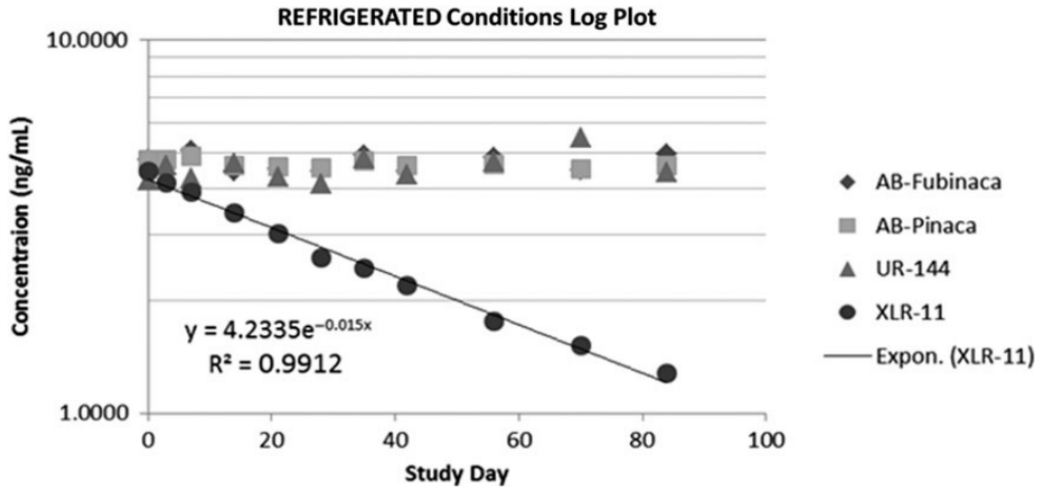


Figure 7. Results for all compounds at 4°C. (Fort, C., Jourdan, T., Jesse Kemp, & Curtis, B. (2017). Stability of synthetic cannabinoids in biological specimens: Analysis through liquid chromatography tandem mass spectrometry. *Journal of Analytical Toxicology*, *41*(5), 360–366. https://doi.org/10.1093/jat/bkx015)

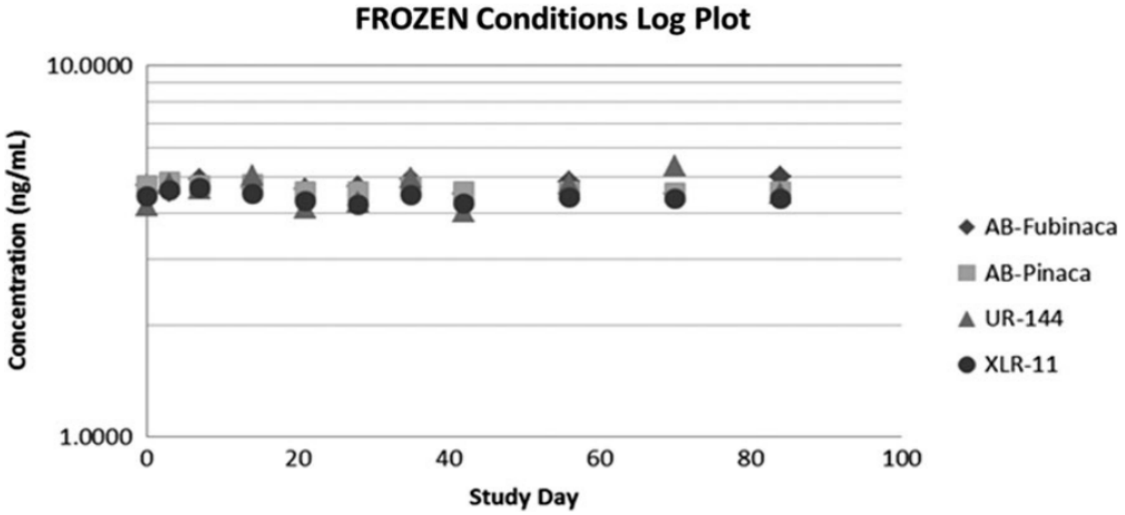


Figure 8. Results for all compounds at –20°C. (Fort, C., Jourdan, T., Jesse Kemp, & Curtis, B. (2017). Stability of synthetic cannabinoids in biological specimens: Analysis through liquid chromatography tandem mass spectrometry. *Journal of Analytical Toxicology*, *41*(5), 360–366. https://doi.org/10.1093/jat/bkx015)

The results of each temperature condition were plotted on a graph for visualization and comparison. Looking at the room temperature data (Figure 6), the stability of AB-Pinaca, ABFubinaca, and UR-144 in the whole blood sample was relatively constant (Fort et al., 2017). The stability of XLR-11 is seen to exponentially decrease over the chosen time period (Fort et al., 2017). Using the equation of the trendline, the researchers were able to anticipate when this compound would reach the LOQ for their experimental parameters (Fort et al., 2017). It was calculated that XLR-11 would reach the LOQ of 0.1 ng/mL at 85.16 days, just outside of the chosen testing period of 84 days (Fort et al., 2017).

In analyzing the results from the refrigerated samples (Figure 7), the stability of AB-Pinaca, ABFubinaca, and UR-144 in the whole blood sample was relatively constant as in the room temperature trial (Fort et al., 2017). Again, we can see that the compound XLR-11 notably degraded in this condition (Fort et al., 2017). The time for XLR-11 was calculated anew. The new time period for it to reach the LOQ when stored at 4°C is 249.71 days (Fort et al., 2017). The stability is significantly higher for this compound when compared to the room temperature results (Fort et al., 2017). Lastly, the findings of the frozen conditions were plotted in the same manner (Figure 8). Here, all four synthetic cannabinoids were observed to maintain lasting stability, with only slight variation over time (Fort et al., 2017).

The percent of degradation loss was calculated for each sample in each temperature condition for weeks three, six, and twelve, based on the initial data from the room temperature samples on day zero. The compared percentages are rather small for all compounds except XLR-11 (Fort et al., 2017). This compound suffered significant degradation in the 22°C and 4°C conditions, which is validated by the information provided in this table (Figure 9) (Fort et al., 2017). It can be concluded that the best storage method for biological samples sent for metabolite

analysis is to be frozen in -20°C (Fort et al., 2017). All four samples avoided degradation in this condition and provided nearly consistent results (Fort et al., 2017).

Table III. Percent loss calculated table for all compounds of interest

% Loss	Week 3 (%)	Week 6 (%)	Week 12 (%)
AB-Fubinaca			
Ambient	6.92	5.55	−3.42
Refrigerated	5.03	6.82	−3.93
Frozen	2.16	4.97	−5.55
AB-Pinaca			
Ambient	4.09	3.18	4.18
Refrigerated	4.19	3.43	3.28
Frozen	3.97	3.71	3.91
UR-144			
Ambient	1.01	2.28	1.63
Refrigerated	−2.34	−3.70	−4.92
Frozen	1.91	4.23	−7.50
XLR-11			
Ambient	73.07	89.95	96.61
Refrigerated	31.76	50.59	71.12
Frozen	2.91	4.11	1.27

Figure 9. Percent loss for all compounds at weeks 3, 6 and 12. (Fort, C., Jourdan, T., Jesse Kemp, & Curtis, B. (2017). Stability of synthetic cannabinoids in biological specimens: Analysis through liquid chromatography tandem mass spectrometry. *Journal of Analytical Toxicology*, *41*(5), 360–366. https://doi.org/10.1093/jat/bkx015)

The chemical structural differences of synthetic cannabinoids cause unpredictable stability conditions for each biological sample received for forensic analysis (Fort et al., 2017). Upon receiving the samples at the lab, it is best to freeze, or at the least, refrigerate the samples to best preserve the concentration of the present metabolites (Fort et al., 2017). This study can be expanded in the future to determine the stability trends of the most used synthetic cannabinoids. This information is imperative for analytical and toxicology laboratories to properly handle and process the biological evidence samples (Fort et al., 2017). Analysis of samples for casework

should be conducted as soon as possible to sustain integrity of the specimen and ensure the results accurately reflect the compound concentration.

4.2. Analysis by GC-MS

Gas Chromatography-Mass Spectrometry (GC-MS) is a common technique used in drug identification. The samples are injected into a gas chromatograph where they are vaporized. It separates the components of the mixture by the times of elution based on boiling point and polarity (*Gas Chromatography Mass Spectrometry (Gc-Ms) Information - Us*, n.d.). The fragments will then pass through an ionization chamber to enter the instrument's mass analyzer. The ions are separated based on mass to charge ratio. All the information is sent through an electronic output where a spectrum for both the retention times and ion fragmentation will be created (*Gas Chromatography Mass Spectrometry (Gc-Ms) Information - Us*, n.d.).

A study conducted in Japan, in 2016, utilized GC-MS with a photoionization (PI) method to analyze sixty-two different synthetic cannabinoids (Akutsu et al., 2017). They aimed to compare the Gas Chromatography-Photoionization-Mass Spectrometry results with those in the database containing Gas Chromatography-Electron Ionization-Mass Spectrometry spectra (Akutsu et al., 2017). They created this study with the intent to show the differences in information provided by both techniques, and how they can further be used to identify the specific synthetic cannabinoid present.

The technique of GC-MS using an electron ionization (EI) method is most used when analyzing synthetic cannabinoids (Akutsu et al., 2017). Electron ionization applies a beam of high energy electrons, of 70 eV, to the gas-phase analyte molecules (Akutsu et al., 2017). The interaction of the electrons and analyte in the low-pressure system will produce single positively

charged ions, which are then directed towards the mass analyzer (Akutsu et al., 2017). Both the Cayman Chemical Compounds Database and SWGDRUG Mass Spectral Library contain spectra of numerous designer drugs using EI spectra at 70eV (Akutsu et al., 2017). GC-EI-MS produces characteristic fragment ions for the identification of compounds. It is known to have a high rate of reproducibility (Akutsu et al., 2017).

A major drawback to this method is it often fails to strongly develop the molecular ion peaks (Akutsu et al., 2017). If the molecular, or pseudo-molecular, ion peak is present on the spectra, it will be very small (Akutsu et al., 2017). This peak is an important factor for individualizing the compound's spectra. Several designer drugs, especially synthetic cannabinoids, are very similar when looking at several strains with a few chemical differences (Akutsu et al., 2017).

The use of photoionization allows for detection of the molecular ion peak. Using photoionization, the radical cation will be formed by ultraviolet light radiation (Akutsu et al., 2017). The radiation will deprive one electron from a target molecule, as opposed to the electron ionization that deprives two electrons at once (Akutsu et al., 2017). Photoionization also uses a low ionization threshold (Akutsu et al., 2017). This contributes to the method being more sensitive. This method allows for a more definitive confirmation of the compound being identified with the display of the molecular ion peak (Akutsu et al., 2017).

For their study, they utilized an Agilent 7890B gas chromatograph connected with an EI/PI combination ion source, then to a JEOL JMS-Q1050 mass spectrometer (Akutsu et al., 2017). The conditions used in the experiment are important for the detection sensitivity and resolution of the analytes being tested. The researchers used a split-less injection of 2µL at 230°C, a DB-5MS fused-silica capillary for the separation column, and a helium carrier gas flow

rate of 1.0 mL/min (Akutsu et al., 2017). The photoionization energy was set to 10.3 eV with the ion source temperature of 150℃ (Akutsu et al., 2017). For comparison, the electron ionization energy was 70 eV with an ion source temperature of 200℃ (Akutsu et al., 2017).

The sixty-two synthetic cannabinoid samples needed to be dissolved in preparation for analysis. Fifty-seven synthetic cannabinoids were dissolved in methanol at a concentration of 100 μg/mL (Akutsu et al., 2017). The remaining five compounds, the carboxylate compounds, were dissolved in acetonitrile at the same concentration of 100 μg/mL (Akutsu et al., 2017). If methanol were used for these five compounds, it may have caused thermal hydrolytic degradation which would affect the results (Akutsu et al., 2017). Each sample was run in the GC-PI-MS and the results were recorded. Twenty of the sixty-two chosen synthetic cannabinoids were also analyzed using the GC-EI-MS for comparative purposes (Akutsu et al., 2017).

All sixty-two of the chosen synthetic cannabinoids were able to be detected using GC-PI-MS (Akutsu et al., 2017). The compounds analyzed were broken down into groups based on their results. The researchers placed thirty-five compounds into “Group 1”. These synthetic cannabinoids only displayed a molecular ion peak on their mass spectra. No fragment ion peaks were observed in the results (Akutsu et al., 2017). The eighteen compounds placed in “Group 2” displayed the molecular ion peak as the base peak with some smaller fragment ion peaks (Akutsu et al., 2017). The remaining nine synthetic cannabinoids were placed into “Group 3”. These compounds produced a fragment ion peak as the base peak with a small molecular ion peak (Akutsu et al., 2017).

Any fragment peaks that appeared in the spectra for Group 2 and Group 3 were also observed in the comparable EI spectra (Akutsu et al., 2017). The image below (Figure 10) shows the results of the compound AMI220, a naphthoylindole, and was classified by the experimenters

in Group 3 (Akutsu et al., 2017). It displays an example of the molecular ion peak being identified in the PI spectra, but completely missing in the EI spectra (Akutsu et al., 2017). Although, the fragment peak at 98 m/z is shown as the base peak in both results (Akutsu et al., 2017).

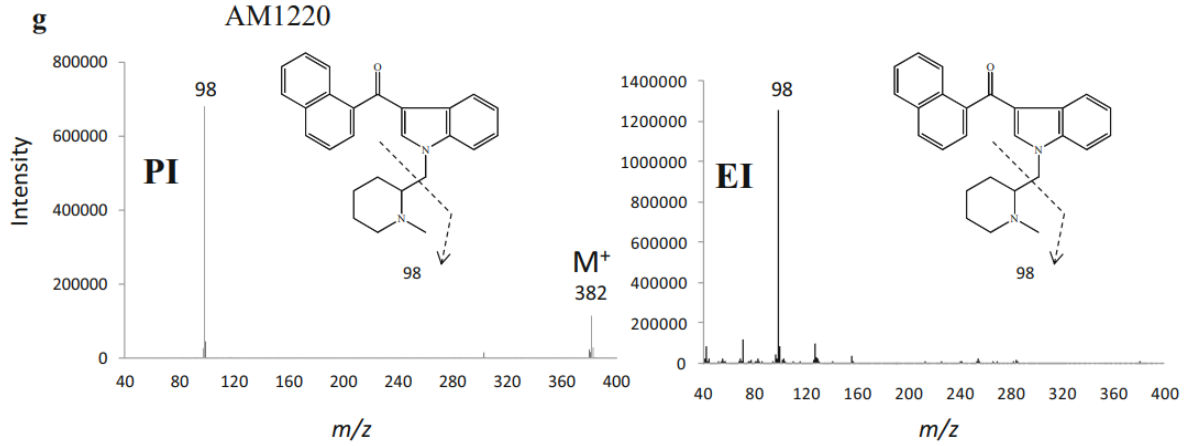


Figure 10. Results of AMI220. (Akutsu, M., Sugie, K., & Saito, K. (2017). Analysis of 62 synthetic cannabinoids by gas chromatography–mass spectrometry with photoionization. *Forensic Toxicology*, *35*(1), 94–103. https://doi.org/10.1007/s11419-016-0342-9)

With the result of this research, conclusions can be made regarding this method of identification. The analysts were able to accurately identify the unique molecular ion peak for all sixty-two synthetic cannabinoids. Although the photoionization spectra showed relatively simple patterns and results, the factor that makes each unique provides vital information for recognition (Akutsu et al., 2017). Therefore, the application of GC-EI/PI-MS analysis and comparison has proved to be a very useful tool to identify the chemicals contained in an unknown sample.

4.3. Analysis by LC-MS/MS

In Australia, there are two established standards used for drug testing in the workplace. These standards are AS/NZS 4308:2008 to test urine samples and AS 4760:2006 to test oral fluid

samples for the presence of drugs of abuse (Williams et al., 2019). Both standards used provide laboratory personnel with the proper practices and procedures that must be followed, as well as the drug classes that should be tested for (Williams et al., 2019).

Choosing to test an oral fluid sample is becoming more favorable over testing urine samples. The oral fluid samples can easily be collected on site under direct observation. This method of testing also has a shorter detection window indicating recent use of any drug identified (Williams et al., 2019). An advantage of using this method over urine sample testing is the resulting compound identified is the parent drug, or the original substance used, rather than the metabolites that result from the drug being processed in the body (Williams et al., 2019).

With the introduction of novel psychoactive substances, some of the current protocols may not be able to uncover their presences in the tests results. Research implemented by the University of New Castle, Faculty of Health and Medicine, in Australia, focuses on the ability to detect and quantify the presence of synthetic cannabinoids specifically in oral fluid using LC-MS/MS analysis (Williams et al., 2019). They aimed to create a protocol that used minimal sample preparation and gave rapid accurate outcomes.

Nineteen distinct strains of synthetic cannabinoids were chosen for the study that the researchers predicted would be popular in their area due to availability of reference materials (Williams et al., 2019). To carry out this experiment, a Shimadzu UHPLC Nexera X2 LC-30AD system to conduct the LC-MS/MS analysis with a total run time of approximately 6 minutes (Williams et al., 2019). A Kinetex Biphenyl column was used to perform the chromatographic separation of the injected compounds at a temperature of 40°C (Williams et al., 2019). The mobile phase used a flow rate of 0.5 mL/min for separation and was prepared using 0.1% formic acid in water and acetonitrile (Williams et al., 2019). The mass spectrometry system worked with

the technique of electrospray positive mode (Williams et al., 2019). The collected data was regulated by Analyst 1.6.3 and refined by the MultiQuant 3.0 system (Williams et al., 2019).

The oral fluid samples being used in the study were collected from the laboratory staff (Williams et al., 2019). A calibration curve was created as an initial step in the experiment. A complete stock solution was made using 10 ng/mL of all the analytes in acetonitrile, taking the analytes from the original 1 mg/mL individual primary standards (Williams et al., 2019). To calculate the higher end of the curve, standards were prepared by adding concentrations of 5, 10, 20, or 50 µL of the analyte stock solution to 1 mL of an oral fluid blank (Williams et al., 2019). These standards were then further diluted 2:1 to generate the lower end of the curve (Williams et al., 2019). The limit of detection and limit of quantitation was determined to have a cutoff value of 5ng/mL (Williams et al., 2019). The analytical samples to be tested were composed of 100 µL of oral fluid, 200 µL of the internal standard acetonitrile mixture, and 300 µL of water (Williams et al., 2019). The sample was centrifuged, and one microliter of the supernatant was injected into the LC-MS/MS for investigation (Williams et al., 2019).

As observed in Figure 11, all nineteen compounds were able to be detected. Each synthetic cannabinoid tested was quantified based on the most notable peak on the spectrum (Williams et al., 2019). Looking at the quantitation, all the analytes were found to have a LOQ of 2.5 ng/mL and a LOD of 1.0 ng/mL (Williams et al., 2019).

A drawback to their method is the maintenance required to keep the database updated. New strains of synthetic cannabinoids are continuously being developed and encountered. These strains would have to be tested as a standard and saved in the database for future reference (Williams et al., 2019). Although this is not a long process to complete, any new derivative will not be discovered until sometime later.

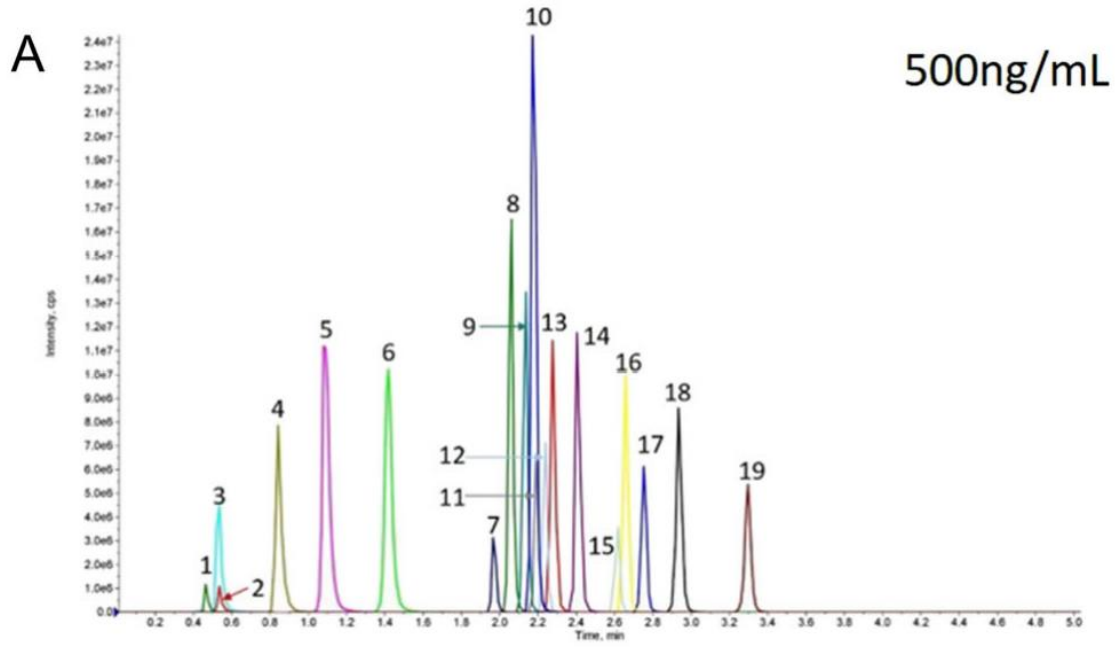


Figure 11. (A) Chromatogram of all analytes 1-AM 2233, 2-JWH-200, 3-AB-005, 4-AB-FUBINACA, 5- AB-PIANCA, 6-AB-CHMINACA, 7-AM 2201, 8-RCS-4, 9-JWH250, 10-STS-135, 11-JWH-73, 12-XLR-11, 13-JWH-250, 14-JWH-18, 15-JWH-122, 16-JWH-19, 17-UR-144, 18-JWH-20, 19-AKB-48. (Williams, M., Martin, J., & Galettis, P. (2019). A validated method for the detection of synthetic cannabinoids in oral fluid. *Journal of Analytical Toxicology*, *43*(1), 10–17. https://doi.org/10.1093/jat/bky043)

Despite synthetic cannabinoids being noted as an increasing drug of abuse, little information on identifying them has been available. Many companies that require drug testing choose oral fluid samples to be collected over urine samples. This is due to easier collection and minimal invasion of privacy (Williams et al., 2019). Their investigation allowed for validation of detection rates for this class of drugs in this specific biological matrix. Companies will now be able to accurately detect and quantify these new compounds that are being used. Minimal preparation of the collected samples is required for analysis with a relatively short run time (Williams et al., 2019).

4.4. Analysis by HPLC-UV

As previously discussed, synthetic cannabinoids are commonly fabricated by lacing a dried plant material with the compound. There is no regulation to how potent the drug may be when purchased. This can lead to accidental over exposure and toxicity of the user (Ciolino, 2015). There are not many legal guidelines in controlling the production and sale of the drug. If any strains are banned, there are numerous others that someone may purchase in its place (Ciolino, 2015). The analysis of the amount of synthetic cannabinoids contained on the plant medium is important for health hazard assessments and regulation of the drug. Quantifying the concentration can provide vital information on what amount will cause harmful intoxication, as well as assist law enforcement in regulating the use and development of this compound.

A study to develop a method for quantification of synthetic cannabinoids on different plant materials was organized and operated by the Forensic Chemistry Center at the U.S. Food and Drug Administration in Ohio (Ciolino, 2015). They worked to develop a method that was broad enough to quantify a large number of available strains without sacrificing the reliability of results. Their method was specifically validated for several classes, including cyclohexyl phenols, naphthoylindoles, benzoylindoles, and phenylacetylindoles (Ciolino, 2015). They selected thirty-four synthetic cannabinoids for analysis using marshmallow leaf, damiana leaf, and mullein leaf (Ciolino, 2015).

High Performance Liquid Chromatography with UV Detection (HPLC-UV) was the technique chosen to achieve their goal. HPLC-UV follows the same principles of liquid chromatography (LC) discussed in section 4.1. It starts with the chromatographic separation of a liquid sample (Zhang & Rock, 2016). After isolating all components of the mixture, an ultraviolet-visible light detector is used to identify the analytes present (Zhang & Rock, 2016).

Each analyte will absorb light at a different wavelength, which is recorded and compared to known standards in a database (Zhang & Rock, 2016). This method has a high sensitivity and does not require the use of standard additions or internal standards (Ciolino, 2015). The equipment used throughout the study was specifically an Agilent 1100, 1200 or 1260 HPLC-DAD system (Ciolino, 2015). The injection volume for all analytes was 10 µL with a flow rate of 1.0 mL/min (Ciolino, 2015). The analytical columns were Phenomenex Luna phenyl hexyl columns, at 5 µm, 4.6 mm ID 9 250 mm length (Ciolino, 2015). The mobile phase used a 70:30 acetonitrile: water mixture as a general buffer due to the neutrality of most synthetic cannabinoids (Ciolino, 2015).

The evidentiary samples were prepared for the study by extracting the synthetic cannabinoids from a 2.5 g portion of plant-based product in 25 mL of acetonitrile (Ciolino, 2015). The material was vortexed, then sonicated for thirty minutes (Ciolino, 2015). A 0.45 µm nylon membrane filter was used to filter a portion of the extract (Ciolino, 2015). The concentration of synthetic cannabinoid contained in the extract was diluted with acetonitrile, if necessary, to achieve a target concentration of 10-100 µg/mL for HPLC analysis (Ciolino, 2015). This process allowed the researchers to achieve a 1.0-10.0 mL sample of the isolated synthetic cannabinoid (Ciolino, 2015).

Spike-and-recovery experiments were conducted prior to the analysis of the evidentiary samples. The spike-and-recovery experiment was performed for thirty-two synthetic cannabinoids to be used in assessing any observed differences between the standard curve and sample matrix (*Spike and Recovery and Linearity of Dilution Assessment - Us*, n.d.). They used the negative control plant materials, marshmallow leaf, damiana leaf, and mullein leaf, and was allowed to soak in a spiked solution for 15 to 30 minutes (Ciolino, 2015). The material was then

placed under a bench top vacuum for the solvent to completely evaporate (Ciolino, 2015). These samples underwent the same extraction procedure as the evidentiary samples to be analyzed by HPLC-UV (Ciolino, 2015).

TABLE 1—*HPLC-UV synthetic cannabinoids parameters and performance summary.*

Structural Class	Compound	Ret Time* (min)	λ Maxes† (nm)	Det λ (nm)	Response as Slope	Linear Range‡ (µg/mL)	LOQ (µg/g)
Cylohexylphenol/dibenzofuran	CP 55,940	4.9	277,219	278	3.9	8.2–821	16
	CP 47,497	7.8	276,218	278	4.9	7.0–700	13
	CP 47,497 C8 homolog	9.3	276,218	278	4.5	9.1–780	15
	HU 210	11.3	279,232	278	1.5	10–1000	44
	HU 211	11.3	279,232	278	1.5	7.9–787	44
Naphthoylindole	AM 1220	3.6	310,244,223	315	22	1.0–854	2.9
	JWH-200	6.3	314,247,218	315	25	1.0–750	2.6
	WIN 55,212 mesylate§	7.9	328,250,220	328	11	0.82–1090	6.3
	JWH-015	10.2	318,250,219	315	26	1.1–525	2.6
	AM 2201	10.3	315,247,218	315	26	1.3–635	2.6
	JWH-073	12.2	315,247,218	315	26	1.0–1000	2.8
	JWH-018	14.8	315,247,218	315	23	1.0–1000	2.9
	JWH-081	15.8	318,235,213	315	25	1.0–520	2.6
	JWH-122	17.2	315,249,222	315	28	1.0–515	2.5
	JWH-019	18.0	315,247,218	315	27	1.0–510	2.6
	JWH-398	21.2	316,249,220	315	24	1.0–500	2.9
	JWH-210	21.4	314,246,222	315	26	1.0–501	2.6
Benzoylindole	WIN 48,098 (pravadoline)	4.7	321,274,214	315	12	1.1–1008	6.9
	RCS-4 C4 homolog	8.0	319,266,214	315	28	1.1–525	2.4
	AM 694	8.8	315,252,214	315	20	1.0–508	3.4
	RCS-4	9.4	319,265,214	315	30	1.1–419	2.2
Phenylacetylindole	JWH-302	9.7	305,248,213	304	25	1.0–1000	2.6
	JWH-250	10.7	303,247,215	304	26	1.1–1070	2.5
	JWH-251	11.9	304,247,214	304	25	1.0–1000	2.6
	JWH-203	12.5	304,247,214	304	26	1.1–1060	2.5
	RCS-8	16.8	304,248,215	304	23	0.11–421	5.7
Tetramethylcyclopropylindole	XLR 11	11.0	304,252,219	304	29	1.0–622	1.6
	UR 144	15.3	304,252,219	304	30	0.84–526	1.5
Miscellaneous	JWH-370	20.0	249,216	255	35	1.2–534	2.0
	AKB48	20.2	303	304	17	43–1000	3.8
	JWH-147	21.7	255,217	255	37	1.0–512	1.8
	JWH-175	29.4	284,223	284	18	1.0–1000	3.8

*Reported retention times for mobile phases comprising 70:30 ACN:water or 70:30 ACN:buffer.
†Peak maxima approximate in some cases due to generally broad spectral bands. See text for discussion.
‡Linearity may exceed tested ranges. All correlation coefficients (r) fell in range 0.9997–1.0000.
§The slope for WIN 55,212 is given as the free base form.

Figure 12. HPLC-UV analysis results of all tested synthetic cannabinoids. (Ciolino, L. A. (2015). Quantitation of synthetic cannabinoids in plant materials using high performance liquid chromatography with uv detection(Validated method). *Journal of Forensic Sciences*, *60*(5), 1171–1181. https://doi.org/10.1111/1556-4029.12795)

The researchers were successfully able to extract and resolve all the compounds being tested (Figure 12). A phenyl hexyl stationary phase proved to be the best choice since all the tested synthetic cannabinoids contained an aromatic ring, and some with aliphatic chains (Ciolino, 2015). The retention time ranged from 3.6 to 29.4 minutes (Ciolino, 2015). Ample

retention times and excellent peak shapes were obtained for various structural classes (Ciolino, 2015). The wavelengths obtained by the UV detector were in the range of 255 to 315 nm (Ciolino, 2015). Synthetic cannabinoids of the same class commonly resulted in the same wavelength. The represented slope values were determined from the calibration curve and allow for quantitative comparison of sensitivity between the compounds (Ciolino, 2015).

This method has been applied to the investigation of synthetic cannabinoids that have been obtained as evidence from 2009 through 2013 (Ciolino, 2015). The results of this analysis are displayed in Figure 13 (Ciolino, 2015). These evidentiary samples were either packaged in small foil packets or glass jars, with a content weight ranging from 400 mg to 30 g (Ciolino, 2015). Although no official botanical analysis was conducted on the plant material, all samples were visually similar to the control material, damiana, mullein, and marshmallow leaf (Ciolino,

TABLE 4—*Quantitation of synthetic cannabinoids in evidentiary samples.*

Seizure Date	Description and Product Name*	Nominal Sample Weights (mg)	Extraction Volume (mL)	Synthetic Cannabinoid(s)	*n*	Amount Found* (mg/g)	%RSD
December 2009	Foil packet "K2 Blonde"	60	5.0	JWH-018	5	34	5.3
				JWH-073	5	28	4.6
October 2010	Foil packet "Mr. Nice Guy"	65	5.0	JWH-018	5	113	9.8
				JWH-073	5	0.24	16
June 2011	Bulk adulterated damiana	2500	25	JWH-018	3	52	0.37
	Bulk adulterated damiana	2500	25	RCS-4	3	48	2.3
January 2012	Foil packet "Cloud 9"	110	10	AM 2201	3	75	3.7
	Foil packet "Zero Gravity"	110	10	AM 2201	3	64	3.5
	Foil packet "Primo Gold"	110	10	AM 2201	3	63	11
February 2012	Bulk powder	50	10	AM 2201	3	99% w/w	1.3
	Bulk powder	60	10	AM 2201	3	99% w/w	0.51
	Bulk powder	65	10	AM 2201	3	98% w/w	0.74
April 2012	Plastic jar "Lawless"	10	1.0	XLR11	2	46	10
	Plastic jar "Lawless"	100	10	XLR11	3	32	4.2
October 2012	Foil packet "Newprot Spice"	100	3.0	JWH-081	3	28	18.5
				JWH-018	3	6.3	18.0
February 2013	Foil packet	175	3.0	XLR11	3	46	4.7
	Foil packet	175	3.0	XLR11	3	82	2.8
October 2013	Foil packet	110	10	5-fluoro PB22	9	49	16
	Foil packet	110	10	5-fluoro PB22	5	66	5.1
	Foil packet	140	10	5-fluoro PB22	5	35	19
	Foil packet	130	10	AB-FUBINACA	9	55	10

*Product contents are plant materials and analysis units are mg/g unless noted in table. Product names are withheld for some exhibits.

Figure 13. Quantification results for evidentiary samples collected. (Ciolino, L. A. (2015). Quantitation of synthetic cannabinoids in plant materials using high performance liquid chromatography with uv detection (Validated method). *Journal of Forensic Sciences*, *60*(5), 1171–1181. https://doi.org/10.1111/1556-4029.12795)

2015). They were tested “as is” following the same procedure as the experimental trials (Ciolino, 2015).

The concentration of synthetic cannabinoids found in each sample was observed to span from 0.24 mg/g to 113 mg/g (Ciolino, 2015). This shows the large variability of the products being marketed. The packages they are sold in do not typically specify the concentration or potency.

The proven success of using HPLC-UV to quantify the concentration of synthetic cannabinoids collected as evidence will be helpful to assist forensic scientists in casework. With the knowledge of the amount of synthetic cannabinoids in a confiscated sample, law and regulations can be created to control them. It will also assist healthcare providers when encountering emergency intoxication cases. A specific concentration of the drug in a person’s system will aid in identifying the best course for treatment relative to an individual case.

5. Case Studies

5.1. Adolescent Studies

The rates of synthetic cannabinoid abuse have greatly been increasing over the last two decades (Besli et al., 2015). These herbal blends laced with psychoactive ingredients are easy to purchase and inexpensive. These factors are contributing to the widespread use of synthetic cannabinoids among adolescents around the world (Besli et al., 2015). In order to analyze and document this trend, a study was conducted at the Istanbul Medeniyet University, Faculty of Medicine, Goztepe Training and Research Hospital, Department of Pediatrics, in Istanbul,

Turkey following sixteen cases presented to the Emergency Department (Besli et al., 2015). They wanted to focus on the social history and clinical features of adolescents admitted for synthetic cannabinoid intoxication (Besli et al., 2015). A goal of their study was to highlight and present the dangers of this drug to public health (Besli et al., 2015).

Researchers worked with the emergency department personnel to identify synthetic cannabinoid intoxication cases that occurred between January and November of 2014 (Besli et al., 2015). All patients of the chosen sixteen cases were under the age of 18, with the mean age of 15.4 ± 1.7 years (Besli et al., 2015). A retrospective chart analysis for each patient was performed to ascertain the following information: demographic data, social history, and physical, psychoactive and metabolic effects of intoxication (Besli et al., 2015).

The researchers found that the route of administration for all patients was smoking the synthetic cannabinoid products (Besli et al., 2015). Although, the intoxication for five patients was induced by co-administration with another drug; the presence of alcohol was found in the results of four patients and the presence of ecstasy was found in the results of one patient (Besli et al., 2015).

When the patients were admitted to the emergency department, they underwent a routine evaluation. This evaluation was a standardized clinical assessment to collect medical history, perform a physical examination and a 12-lead electrocardiography (Besli et al., 2015). Furthermore, it was documented that fifteen of the patients received blood gas analysis and blood chemistry testing (Besli et al., 2015). Also, twelve patients received urinary toxicology screenings (Besli et al., 2015). If any of the studied patients displayed life-threatening conditions, or their altered mental condition worsened, they were transferred from the emergency department to the intensive care unit (Besli et al., 2015).

The psychoactive effects and symptoms experienced by the patients were relatively similar to one another. The common psychoactive reactions reported were anxiety, agitation, hallucinations, and perceptual changes to their senses and body perception (Besli et al., 2015).

Table 3. Physical Effects of Synthetic Cannabinoids

Variable	No. of Patients/Total Sample	%
Eye redness	14/16	87.5
Nausea/vomiting	13/16	81.2
Altered level of consciousness	12/16	75.0
Sweating	11/16	68.7
Mydriasis	9/16	56.2
Slurred speech	9/16	56.2
Hypotension	8/16	50.0
Tachycardia	6/16	37.5
Bradycardia	5/16	31.2
Syncope	5/16	31.2
Hypertension	3/16	18.7
Respiratory failure	3/16	18.7
Muscle tremor/spasms	2/16	12.5
Chest pain	1/16	6.2
Seizure	1/16	6.2

Figure 14. Recorded physical symptoms of 16 patients. (Besli, G. E., Ikiz, M. A., Yildirim, S., & Saltik, S. (2015). Synthetic cannabinoid abuse in adolescents: A case series. *The Journal of Emergency Medicine*, *49*(5), 644–650. https://doi.org/10.1016/j.jemermed.2015.06.053)

An analysis of all the reported physical symptoms with their frequency among the 16 cases is displayed above in Figure 14 (Besli et al., 2015). The most frequently reported physical symptoms included increased perspiration, nausea/vomiting, redness of eyes and altered mental states (Besli et al., 2015).

The results remain consistent with findings in literature of expected and reported symptoms caused by synthetic cannabinoids (Besli et al., 2015). One of the largest associated health risks are the cardiovascular effects, which was observed about half the time (Besli et al., 2015). The blood testing conducted for fifteen of the patients revealed that six of them

experienced elevated creatine kinase levels, and nine experienced mild hyperglycemias (Besli et al., 2015). These increased levels could pose a threat to their health in the near future if not taken care of immediately.

The social history of twelve patients was considered for this study as well (Figure 15). The social data for four patients that were transferred to the intensive care unit could not be obtained (Besli et al., 2015). This data is important to study the factors that contribute to the rising rate of abuse. Almost all the patients stated they purchased the drug illegally from a street dealer (Besli et al., 2015). Two-thirds of the patients repeatedly used synthetic cannabinoids (Besli et al., 2015). This displays how accessible the drug is for adolescents to gain access to. Only two of them had some knowledge regarding the dangers posed by synthetic cannabinoids prior to use (Besli et al., 2015). All twelve patients also revealed poor school performance and attendance records (Besli et al., 2015). This showed the significance of providing future education for the target population on the serious health hazard this drug presents. Education for families and teachers is equally important to identify school or behavior problems that could be associated with abuse of the drug (Besli et al., 2015).

Table 4. Social Work Data of the Patients

Variable	No. of Patients/Total Sample*	%
Site of purchase		
Street dealers	11/12	91.7
Friend	1/12	8.3
Frequency of SCPs use		
Once	4/12	33.3
Multiple times	8/12	66.7
Knowledge about the harmful and addictive effects of SCPs	2/12	16.7
Poor school performance	12/12	100.0
School attendance problem	12/12	100.0
Alcohol habit	8/12	66.7
Cigarette smoking	11/12	91.7
Other drug usage (marijuana, amphetamines)	4/12	25.0

SCP = synthetic cannabinoid product.
* Detailed social work data for 4 patients who transferred to another hospital's intensive care unit could not be obtained.

Figure 15. Social data collected on the patients. ((Besli, G. E., Ikiz, M. A., Yildirim, S., & Saltik, S. (2015). Synthetic cannabinoid abuse in adolescents: A case series. *The Journal of Emergency Medicine*, *49*(5), 644–650. https://doi.org/10.1016/j.jemermed.2015.06.053)

Synthetic cannabinoids are classified as an illegal drug in Turkey. Regardless, they are still easily obtained and abused by adolescents as the target population (Besli et al., 2015). This study could be expanded by reviewing more patient cases over a larger area, or from more hospitals. Investigators in other countries can benefit from this research. They may apply it to a study of their own to determine the rates of abuse in their own area. Providing education for the public about the risks associated with synthetic cannabinoid use may help to stop the rapid spread of products (Besli et al., 2015). It could also assist physicians in recognizing the symptoms of intoxication to provide the proper treatment and avoid future cases (Besli et al., 2015).

5.2. Cases of Acute Intoxication

An increase in emergency cases induced by the use of synthetic cannabinoids is being observed in countries around the world. In Freiburg, Germany, four cases of acute intoxication in which the patients all used a different strain of synthetic cannabinoids were individually reviewed (Hermanns-Clausen et al., 2013). The strain used was recorded, along with social data and the symptoms exhibited by the patient. Their identities are not revealed in the study to maintain confidentiality. With the treatment of the four patients in the emergency department, serum and urine samples were collected for detection and quantification of the drug (Hermanns-Clausen et al., 2013).

Case 1:

A 17-year-old male was admitted to the emergency department not long after using the herbal mixture called 'Jamaican Gold' (Hermanns-Clausen et al., 2013). During a break in the school day, he decided to smoke this drug. The first reactions he experienced were nausea and increased rates of respiration (Hermanns-Clausen et al., 2013). Upon arriving home, his parents noticed he was acting out of the ordinary. They reported mild agitation, trembling and laugh attacks (Hermanns-Clausen et al., 2013). These symptoms quickly escalated into vomiting and panic attacks (Hermanns-Clausen et al., 2013).

The parents called emergency medical services. At the time of their arrival, the patient was conscious but agitated and exhibited myoclonic jerking (Hermanns-Clausen et al., 2013). He was immediately brought into the hospital. A standard examination was conducted and found his vitals to be in the normal range with the exception of sinus tachycardia at 112 beats per minute (Hermanns-Clausen et al., 2013). Further laboratory testing revealed a slight increase in blood glucose concentration and the presence of hypokalemia (Hermanns-Clausen et al., 2013).

Over the next six hours, the patient received intravenous fluids and substitution of potassium (Hermanns-Clausen et al., 2013). He completely recovered after this period for all the experienced symptoms (Hermanns-Clausen et al., 2013).

Case 2:

A 17-year-old male smoked an herbal mixture that was purchased via the internet with the assumption that it contained 'Salvia Divinorum' (Hermanns-Clausen et al., 2013). He originally was using the product with a friend. The friend left shortly after, leaving him to continue using the drug on his own (Hermanns-Clausen et al., 2013). Bystanders that happened to pass by saw the young male every drowsy, leaning against a garden fence, and called emergency medical services (Hermanns-Clausen et al., 2013).

A standard examination was conducted upon his arrival at the emergency department that found his vitals to be in the normal range. The testing did reveal sinus tachycardia at 160 beats per minute and a leukocytosis (Hermanns-Clausen et al., 2013). The patient then underwent a neurological assessment. This revealed a mild somnolence, mydriasis, anisocoria, and retrograde amnesia (Hermanns-Clausen et al., 2013). His symptoms were resolved twelve hours after being admitted (Hermanns-Clausen et al., 2013).

Case 3:

A 19-year-old male smoked a derivative of synthetic cannabinoids called 'Bonzai' (Hermanns-Clausen et al., 2013). He used the drug in the evening, and then repeated use the next morning (Hermanns-Clausen et al., 2013). The patient developed tonic-clonic seizures immediately after the second use (Hermanns-Clausen et al., 2013). He was found in a comatose state after vomiting repeatedly while lying on his back (Hermanns-Clausen et al., 2013).

Due to a lack of sufficient respiration, the patient was intubated and mechanically ventilated immediately after arriving at the emergency department (Hermanns-Clausen et al., 2013). A bronchoscopy was conducted, removing aspirated gastric content from his main bronchi (Hermanns-Clausen et al., 2013). After three hours, the mechanical ventilation was no longer needed (Hermanns-Clausen et al., 2013).

Further testing showed his heart rate and blood pressure were normal. A slight increase of blood glucose concentration was observed (Hermanns-Clausen et al., 2013). Leukocytosis, mild thrombocytosis and increased creatine kinase levels were observed the next day (Hermanns-Clausen et al., 2013). The patient was discharged from the hospital after three days (Hermanns-Clausen et al., 2013).

Case 4:

A 20-year-old male smoked the synthetic cannabinoid variation known as 'Lava Red' while at a party with a few friends (Hermanns-Clausen et al., 2013). Only a few minutes after using the drug, he became pale and vomited heavily (Hermanns-Clausen et al., 2013). Emergency medical services were called to transport him to the hospital. The patient was unable to communicate with the physicians for some time (Hermanns-Clausen et al., 2013).

An examination revealed mild tachycardia at 100 beats per minute, severe drowsiness and mydriasis (Hermanns-Clausen et al., 2013). Further laboratory testing revealed leukocytosis, hypokalemia and slight increase in creatine kinase levels (Hermanns-Clausen et al., 2013). The patient was administered intravenous fluids, benzodiazepines and a substitution of potassium (Hermanns-Clausen et al., 2013). His symptoms subsided after eight hours, and he was discharged the next day (Hermanns-Clausen et al., 2013).

A serum and urine sample were collected from all four patients for further drug analysis. This analysis allowed physicians to detect and quantify the synthetic cannabinoids, using the serum sample, and the metabolites, using the urine sample (Hermanns-Clausen et al., 2013). They used liquid chromatography-electrospray ionization-tandem mass spectrometry to conduct their investigation (Hermanns-Clausen et al., 2013).

The serum was analyzed using a formerly published method that was expanded, allowing for qualitative detection of thirty-nine synthetic cannabinoids (Hermanns-Clausen et al., 2013). The analysis of urine samples was compared to previous samples paired with a serum sample that tested positive for the presence of synthetic cannabinoids (Hermanns-Clausen et al., 2013). The metabolites in the patients' urine were considered identified with presence of two characteristic ion transitions that displayed a chromatographic peak at the correct retention time (Hermanns-Clausen et al., 2013). The patient results for all four cases are displayed in the figures below (Figure 16-19). The patient in case 1 showed a very high concentration of 13 ng/mg of JWH-081 (Hermanns-Clausen et al., 2013). It is assumed the patient had repeated use of the drug in a short amount of time. Physicians concluded this was the main cause of the patient's intoxication (Hermanns-Clausen et al., 2013).

The samples from the patient in case 2 tested positive for UR-144 (Hermanns-Clausen et al., 2013). This is the only case to contain a derivative outside the JWH series. The signals given by the metabolites of UR-144 indicated a very high serum concentration, but it could not be accurately quantified (Hermanns-Clausen et al., 2013).

Compound	Metabolite	Concentration [ng/mg]
JWH-018	N-(3-OH-pentyl)	**0.03**
	N-(4-OH-pentyl)	**0.28**
	N-(5-OH-pentyl)	**0.12**
	N-(5-carboxypentyl)	**0.11**
JWH-073	N-(4-carboxybutyl)	**0.10**
JWH-081	N-(5-OH-pentyl)	**~13***
	OH-indole	**Positive****
	OH-naphthyl	**Positive****

* Concentration ranged above the highest calibrator and was extrapolated

** No reference material available for quantification

Figure 16. Results of Case 1. (Hermanns-Clausen, M., Kneisel, S., Hutter, M., Szabo, B., & Auwärter, V. (2013). Acute intoxication by synthetic cannabinoids - Four case reports: Acute intoxication by synthetic cannabinoids - Four case reports. *Drug Testing and Analysis, 5(9–10), 790–794. https://doi.org/10.1002/dta.1483)*

Compound	Metabolite	Concentration [ng/mg]
JWH-018	N-(5-carboxypentyl)	**0.11**
JWH-122	N-(5-OH-pentyl)	**1.6**
UR-144	OH-indole	**Positive***
	OH-pentyl	**Positive***

* No reference material available for quantification

Figure 17. Results of Case 2. (Hermanns-Clausen, M., Kneisel, S., Hutter, M., Szabo, B., & Auwärter, V. (2013). Acute intoxication by synthetic cannabinoids - Four case reports: Acute intoxication by synthetic cannabinoids - Four case reports. *Drug Testing and Analysis, 5(9–10), 790–794. https://doi.org/10.1002/dta.1483)*

Compound	Metabolite	Concentration [ng/mg]
JWH-018	N-(3-OH-pentyl)	**0.03**
	N-(4-OH-pentyl)	**0.49**
	N-(5-OH-pentyl)	**0.28**
	N-(5-carboxypentyl)	**0.12**
JWH-073	N-(4-carboxybutyl)	**0.10**
JWH-122	N-(4-OH-pentyl)	**~ 11***
	N-(5-OH-pentyl)	**3.5**
	OH-indole	**Positive****
	OH-naphthyl	**Positive****
JWH-210	N-(4-OH-pentyl)	**0.06**

* Concentration ranged above the highest calibrator and was extrapolated

** No reference material available for quantification

Figure 18. Results of Case 3. (Hermanns-Clausen, M., Kneisel, S., Hutter, M., Szabo, B., & Auwärter, V. (2013). Acute intoxication by synthetic cannabinoids - Four case reports: Acute intoxication by synthetic cannabinoids - Four case reports. *Drug Testing and Analysis, 5(9–10), 790–794. https://doi.org/10.1002/dta.1483)*

Compound	Metabolite	Concentration [ng/mg]
JWH-018	N-(4-OH-pentyl)	**0.06**
	N-(5-OH-pentyl)	**0.04**
	N-(5-carboxypentyl)	**0.03**
JWH-122	N-(4-OH-pentyl)	**4.8**
	N-(5-OH-pentyl)	**0.74**
	OH-indole	**Positive***
	OH-naphthyl	**Positive***

* No reference material available for quantification

Figure 19. Results of Case 4. (Hermanns-Clausen, M., Kneisel, S., Hutter, M., Szabo, B., & Auwärter, V. (2013). Acute intoxication by synthetic cannabinoids - Four case reports: Acute intoxication by synthetic cannabinoids - Four case reports. *Drug Testing and Analysis, 5(9–10), 790–794. https://doi.org/10.1002/dta.1483)*

The patient in case 3 showed the highest concentration of 11 ng/mg of JWH-122 in his serum sample (Hermanns-Clausen et al., 2013). Case 3 was the most severe intoxication reviewed. This compound was also seen in cases 2 and 4, but at a significantly lower amount (Hermanns-Clausen et al., 2013). Physicians believed this was the result of using the drug twice

in a 12-hour period (Hermanns-Clausen et al., 2013). The severity of case 4 compared to case 2 as they both exhibited moderate cases of intoxication (Hermanns-Clausen et al., 2013).

In this case review, researchers were able to display a range of life-threatening conditions that could result from the use of synthetic cannabinoid abuse (Hermanns-Clausen et al., 2013). An important feature of their study is how they were able to apply a previously developed detection and quantification method to determine the presence and concentration of the drugs (Hermanns-Clausen et al., 2013). Two of the four patients were confirmed to have a repeated intake of the drug in less than 12 hours (Hermanns-Clausen et al., 2013). This action causes significantly more severe symptoms and takes a longer time to treat (Hermanns-Clausen et al., 2013).

With the knowledge of the consumption pattern and identification of the metabolites, the physicians were able to properly treat all four patients accordingly (Hermanns-Clausen et al., 2013). They were all able to successfully recover in a short period of time. This information could assist further research and education to improve interpretation of clinical and analytical findings (Hermanns-Clausen et al., 2013). Healthcare professionals can benefit from this study to learn more about applying validated analytical methods to determine suitable medications and treatment methods.

5.3. Cause of Death

In the previous cases reviewed, all patients were successfully able to recover from their symptoms of synthetic cannabinoid intoxication. Unfortunately, due to the inconsistency of chemicals contained in the drug, not all cases and reactions have the same outcome. Numerous cases have resulted in the death of the patient. The ages of these fatalities range from 17 to 52

(Langford & Bolton, 2018). While some of the symptoms observed in the patients may have been similar, the overall circumstances of each case varied from the others.

In 2015, the Department of Applied Sciences at Northumbria University in the United Kingdom reviewed a fatality case of a 35-year-old Caucasian man (Langford & Bolton, 2018). The man was found deceased in an alleyway about 30 minutes after using a smoking pipe containing 'Pandora's Box' (Langford & Bolton, 2018). The herbal mixture 'Pandora's Box' contained the synthetic cannabinoid 5F-PB-22 (Langford & Bolton, 2018). Emergency medical services administered a shock advisory defibrillator but were unable to revive him (Langford & Bolton, 2018). No drug paraphernalia was found at the scene (Langford & Bolton, 2018).

The post-mortem examination revealed no internal or external injuries (Langford & Bolton, 2018). Samples of urine, femoral blood and gastric contents were collected for toxicological analysis (Langford & Bolton, 2018). Unquantified amounts of 5F-PB-22 and 5F-AKB-48 synthetic cannabinoids were identified in the samples (Langford & Bolton, 2018). The drug 'Pandora's Box' was only identified to contain the 5F-PB-22 cannabinoid in this case. It can be assumed that the presence of the 5F-AKB-48 cannabinoids was due to an earlier ingestion (Langford & Bolton, 2018). The time or dosage of ingestion was inconclusive.

The analysis found the presence of alcohol at a concentration of 311 mg/100 mL in the femoral blood, and 389 mg/100 mL in the urine sample (Langford & Bolton, 2018). The man was reported to have ingested an unknown quantity of alcohol about 4 hours prior to this event (Langford & Bolton, 2018). The synergistic effects of the two drugs most likely caused severe respiratory depression, and further leading to sudden cardiac arrhythmia (Langford & Bolton, 2018). The cause of death was because of the combination of the synthetic cannabinoids and alcohol (Langford & Bolton, 2018).

The investigators found four other cases of death where they identified a 5F derivative of a synthetic cannabinoid strain in their system (Langford & Bolton, 2018). Only one of these cases reported to have used alcohol in combination with the drug (Langford & Bolton, 2018). This reveals that the use of this strain can cause sudden onset of fatal cardiac arrhythmias (Langford & Bolton, 2018). This effect becomes more likely to occur when used with alcohol (Langford & Bolton, 2018).

The review of these cases shows the increasing danger in using synthetic cannabinoids, especially as more derivatives are being created. This information allows toxicologists and pathologists to recognize and understand qualitative and quantitative issues during investigation (Langford & Bolton, 2018). It may assist them in better identifying how these compounds directly cause these effects, as well as ways to counteract them.

5.4. Law Enforcement Safety Evaluation

Law enforcement may be exposed to the dangers of synthetic cannabinoids more often than they realize. Field agents have grown concerned about the chemicals they encounter during the unannounced investigations of a spice lab (Ramsey et al., 2016). Also, they were worried that the evidence collected and stored may be a threat to their health while in the office (Ramsey et al., 2016).

In 2013, a federal law enforcement agency requested for the Health Hazard Evaluation Program to conduct a study, evaluating any hazards present (Ramsey et al., 2016). This program is run by the U.S. Department of Health and Human Services, the Centers for Disease Control and Prevention, and the National Institute for Occupational Safety and Health (Ramsey et al.,

2016). Investigators in the program accompanied agents during a raid in December 2013 in order to accurately collect samples and data (Ramsey et al., 2016).

Synthetic cannabinoids are manufactured by spraying a chemical mixture onto a dried plant material, then air dried and packaged for sale (Ramsey et al., 2016). Agents believed that the residual chemicals in the atmosphere of the lab could be harmful to their health during a raid. The U.S. Drug Enforcement Administration (DEA) informed researchers that the synthetic cannabinoids being used in this specific lab were most likely AB-PINACA and mitragynine (Ramsey et al., 2016). The agents collected both treated and untreated plant material, packaged products, and equipment used in the lab, properly documenting it in an evidence log (Ramsey et al., 2016). All evidence was properly packaged in plastic or paper bags for further analysis (Ramsey et al., 2016).

The study was conducted by assessment of several aspects. First, the researchers evaluated the potential for exposure to chemical and psychoactive substances that were airborne (Ramsey et al., 2016). Six air samples were obtained in thermal desorption tubes from both the spice lab and the agency's office (Ramsey et al., 2016). Sixteen personal and four area air samples were also collected in charcoal tubes (Ramsey et al., 2016). The charcoal tubes were initially analyzed for several compounds including ethanol, acetone, toluene, cyclohexanone and methyl butyl ketone (Ramsey et al., 2016).

To investigate the potential chemicals the agents were exposed to by contact with their skin, a total of seventeen surface wipe samples were collected from several areas of the spice lab and the office (Ramsey et al., 2016). Cross contamination was avoided in this process by the investigators wearing sterile nitrile gloves and changing them before each wipe (Ramsey et al., 2016). To perform the swab, sterile foam tip applicators, wipes, and cotton swabs were used

(Ramsey et al., 2016). Gas chromatography-mass spectrometry (GC-MS) was the chosen method to perform an analysis on all samples. The parameters of this technique and extraction method for the specimen were set by the DEA (Ramsey et al., 2016).

Biological monitoring of the agents' urine samples was performed to test for the presence of synthetic cannabinoids in their system (Ramsey et al., 2016). A urine sample was collected from each agent for three consecutive days at the times listed below:

> "1. Day 1 (baseline): in the afternoon of the day before entry and search of the spice lab
>
> 2. Day 2 (post-shift): immediately after the work shift during which entry, search, handling, and processing evidence of synthetic cannabinoids and other psychoactive drugs occurred
>
> 3. Day 2 (bedtime): at bedtime the same day of the raid
>
> 4. Day 3 (morning): the next morning before sorting evidence
>
> 5. Day 3 (post-shift): at the end of the shift after sorting evidence"
>
> (Ramsey et al., 2016).

The techniques used to assess the amounts of both AB-PINACA and mitragynine present in the specimen were liquid chromatography-tandem mass spectrometry (LC-MS/MS) and enzyme linked immunosorbent assay (ELISA), both with parameters set forth by the DEA (Ramsey et al., 2016). Using these methods will allow the researchers to identify any metabolites present, which can then be connected to the original substance.

In an analysis of all air samples, researchers found concentrations of acetone (0.02-0.09 ppm), ethanol (0.02-0.2 ppm), toluene (0.002 ppm), and methyl isopropyl ketone (0.002 ppm)

(Ramsey et al., 2016). No concentration of AB-PINACA or mitragynine were detected in the samples. It was suspected that they were present below the detection limit of 0.02-0.1 mg/m³ (Ramsey et al., 2016). Only one of the surface wipe samples showed the presence of AB-PINACA at 250 µg/100 cm² (Ramsey et al., 2016).

The results from the biological monitoring are summarized in Figure 20 (Ramsey et al., 2016). All specimens tested on day one were negative for any metabolites. On days two and three, the agents' specimen showed quantifiable amounts of metabolites present (Ramsey et al., 2016). Most of the agents with these levels present reported not wearing gloves while handling most of the evidence (Ramsey et al., 2016). Two agents reported that they did not handle any

Table 4. Cannabinoid and mitragynine levels in agents' urine samples, by LC-MS/MS (n = 9)

Compound	Number of agents with quantifiable levels (range of concentrations, ng/mL)				
	Day 1: Baseline	Day 2: Post-shift	Day 2: Bedtime	Day 3: Morning	Day 3: Post-shift
AB-PINACA*	0	3 (< LLOQ–0.29)	1 (< LLOQ–0.12)	0	0
AB-PINACA N-(4-hydroxypentyl)*	0	1 (< LLOQ–0.11)	1 (< LLOQ–0.12)	0	0
AB-PINACA N-pentanoic acid*	0	2 (< LLOQ–1.98)	3 (< LLOQ–3.45)	2 (< LLOQ–0.43)	2 (< LLOQ–0.62)
Mitragynine†	0	4 (< LLOQ–3.70)	5 (< LLOQ–12.62)	5 (< LLOQ–0.94)	5 (< LLOQ–5.28)

LLOQ = lower limit of quantification

*The LLOQ for AB-PINACA, AB-PINACA N-(4-hydroxypentyl) metabolite and AB-PINACA N-pentanoic acid metabolite is 0.10 ng/mL.

†The LLOQ for mitragynine is 1.0 ng/mL.

Figure 20. Biological monitoring results. (Ramsey, J. G., Tapp, L., & Burr, G. (2016). *Health hazard evaluation report: Evaluation of law enforcement agents' potential exposures during a raid of a clandestine "spice" lab* (No. 2014-0039–3246; pp. 1–31). U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HHE.)

evidence at the scene or at the office (Ramsey et al., 2016). One agent was present at the scene and wore gloves while handling evidence (Ramsey et al., 2016). Agents most reported symptoms

of eye irritation, throat irritation, dizziness, skin irritation, and headaches as a result of exposure (Ramsey et al., 2016).

The findings from the Health Hazard Evaluation Program indicate the risk of potential exposure to synthetic cannabinoids for agents on the job. These hazard assessments are vital to understanding how to keep law enforcement officers safe while working. Agencies are working to have more assessments performed around the world to maintain a safe working environment and protect the officers that are working to eliminate this drug of abuse.

Although the study was limited to one raid of a spice lab and number of agents that were monitored, it still provided useful information on exposure. The use of personal protective equipment is important for the safety of the agents working in these conditions (Ramsey et al., 2016). This equipment, such as protective gloves and disposable clothing, should be required for those who may encounter these dangerous chemicals (Ramsey et al., 2016). Most agents who wore this equipment avoided exposure or reported very minimal contact (Ramsey et al., 2016).

6. Future Goals

6.1. Legislation in the United States

Since rates of abuse have rapidly been increasing from the early 2000s, nineteen nations, not including the U.S., have bans on numerous spice products (Spaderna et al., 2013). It is evident that there are many challenges around the regulation and laws pertaining to synthetic cannabinoids (Spaderna et al., 2013). This is especially true in the United States of America. Some states and the military have passed laws that prohibit the manufacture and marketing of

any spice products (Spaderna et al., 2013). However, these laws have low success rates to date on stopping the consumption and abuse of this drug (Spaderna et al., 2013). Any existing laws need to be consistently updated to account for new variations of the drug that become available.

Statistics can be used to determine the most prominent variations of synthetic cannabinoids. Knowing this information, law enforcement can ban specific strains that are contained in most products. For example, in 2009, the synthetic cannabinoid JWH-018 was responsible for 86.67% of exposures (Spaderna et al., 2013). This compound was named illegal shortly after this information came to light. By 2010, exposure to JWH-018 had decreased by 63.39% (Spaderna et al., 2013). To avoid the laws put in place, those who manufacture the drug continued to create and sell new derivatives of synthetic cannabinoids.

In 2012, the *Synthetic Drug Abuse Prevention Act of 2012* named 15 common synthetic cannabinoids to the Schedule I category of the Controlled Substances Act (Spaderna et al., 2013). The fifteen compounds were of five distinct chemical structures (Spaderna et al., 2013). Knowing the common structures, legislators may be able to identify more common derivatives and add them to the list.

Consequences for possession, manufacture or sale of these compounds vary for each state, but typically include jail time, fines, and prosecution under federal law. “On July 26, 2012, the US DEA conducted ‘Operation Log Jam,’ seizing 18.4 million packets of synthetic cannabinoids, $36 million in cash, and arresting more than 90 people from 109 US cities” (Spaderna et al., 2013). It was later reported that many of the synthetic cannabinoids seized were not listed in the Controlled Substances Act but were further prosecuted under the Analogues Act (Spaderna et al., 2013). This act includes chemicals that are structurally or functionally similar to ones that are prohibited (Spaderna et al., 2013).

Introduction of these laws have allowed for some progression in regulating the distribution of synthetic cannabinoids. Rates are still on the rise as new derivatives are being introduced on the market. Collection and analysis of synthetic cannabinoids as evidence may provide information on the most common method of sale and variations of the drug available. This could assist states on controlling the abuse of this drug within a specific area. As scientific advancements in research are made, the identification of variations available will further assist legislators to control the substances on the market.

6.2. Availability of a Shared Database

There is high-quality research available on many aspects of synthetic cannabinoids (Williams et al., 2014). Although, this research may lag behind the development and usage patterns of the drug (Williams et al., 2014). One institution may discover new strains being sold, or study toxicological effects, that another may not have access to until years later. With the technology we have access to today, the collection and reporting of all data on synthetic cannabinoids is an easy task.

However, not all poison control centers and researchers are reporting this information (Williams et al., 2014). To avoid this lag of information and create progress in all fields, the sharing of information from all research should be encouraged by creating a national database among law enforcement, forensic scientists, and healthcare professionals.

There are many aspects needed to take a national approach to synthetic cannabinoid research. On the clinical side, all case data should be collected and reported by poison control centers, hospitals and emergency medical services (Williams et al., 2014). This information would allow for knowledge of metabolic profiles and toxicological patterns. They can report on

how each case was handled, any symptoms the patient exhibited, and statistics on recovery rates using specific treatment methods. Healthcare providers that encounter similar cases in the future will be able to determine the best plan for treatment with known clinical outcomes (Williams et al., 2014).

A database can provide important information on methods of rapid screening test and standardized testing panels used to identify exposure and early symptoms (Williams et al., 2014). This can further lead to the successful development of quantitative assays with rapid turnaround times that are available to all related clinical laboratories (Williams et al., 2014). The use of a national database will benefit forensic scientists in their research on synthetic cannabinoids as well.

The diversity of synthetic cannabinoids circulating in society creates several challenges for professionals who encounter the drug in their field of work. "This would include police, pathology laboratories, poisons centers, drug and alcohol centers, and forensic services" (Williams et al., 2014). Often, professionals are unaware of the compounds available until they are encountered in that location, or country. Even then, the information may only be shared among a small group of people. The knowledge of all chemicals being used to manufacture synthetic cannabinoid derivatives will allow for advancements in detection and identification techniques.

Shared research may allow for other countries to get ahead of a strain that may become popular on the market. This can lead to the development of a national mass spectrometry library with a standardized method for identifying compounds in both biological and artificial matrices (Williams et al., 2014). A standardized detection method will further support the creation of laws

and regulations on these compounds before they become too widespread to control (Williams et al., 2014).

A national database may also serve to educate the public on the dangers of this drug (Williams et al., 2014). A program can be created in conjunction with police and medical services to provide this education (Williams et al., 2014). It can inform parents and teachers of information regarding the symptoms and behaviors that result from synthetic cannabinoid abuse. It may also work to educate at-risk groups, including adolescent populations, to deter them from using the drug (Williams et al., 2014). A program could also be developed to implement a service that provides synthetic drug testing in any areas that it may be needed, such as schools, police precincts, and workplaces (Williams et al., 2014).

The establishment of a national database focused on synthetic cannabinoids will benefit many institutions all over the world. Granting access to this vital knowledge will aid several countries to reach goals of standard methods of detection and identification, providing education of this hazardous drug to the public, and administration of proper treatment in intoxication cases. Recognition of the materials available will assist law enforcement, researchers, and healthcare providers in managing the widespread abuse of synthetic cannabinoids. We can further work to decrease the high rates of abuse.

7. Conclusion

Although research is advancing, there is still not enough known about synthetic cannabinoids to control them on the market. The illegal manufacture of this drug in laboratories forms countless variations that could remain unknown until encountered later as evidence, or in

hospitals. Investigation of the interactions between synthetic cannabinoids and specific CB1 and CB2 receptors assists scientists in identifying how the chemicals are manipulated by metabolism. Knowledge of the metabolites formed allows scientists to predict the ways it causes harmful effects to our health.

A review of the diverse studies regarding validated techniques allows for continuous progression towards stronger identification. Combining these findings plays an important role in the groundwork of a standardized testing method that recognizes these compounds in a biological sample, regardless of the inconsistencies in batches. Furthermore, expanding education on the potential dangers of using this substance may deter at-risk groups, resulting in a decline in cases of intoxication. Informing parents and teachers on the symptoms caused by synthetic cannabinoid use could be crucial to identifying and reporting cases of intoxication. In conducting a forensic analysis of synthetic cannabinoids, scientists can work towards establishing a standardized method of detection and identification to regulate and decrease the widespread abuse of this drug.

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