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Analysis of Synthetic Cannabinoids by Direct Analysis in Real Time Quadrupole Time-of-Flight Mass Spectrometry and Gas Chromatography Quadrupole Time-of-Flight Mass Spectrometry

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FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

ANALYSIS OF SYNTHETIC CANNABINOIDS BY DIRECT ANALYSIS IN REAL
TIME QUADRUPOLE TIME-OF-FLIGHT MASS SPECTROMETRY AND GAS
CHROMATOGRAPHY QUADRUPOLE TIME-OF-FLIGHT MASS
SPECTROMETRY

A thesis submitted in partial fulfillment of the
requirements for the degree of

MASTER OF SCIENCE

in

FORENSIC SCIENCE

by

Tyler S. Torbet

2015

To: Dean Michael R. Heithaus
College of Arts and Sciences

This thesis, written by Tyler S. Torbet, and entitled Analysis of Synthetic Cannabinoids by Direct Analysis in Real Time Quadrupole Time-of-Flight Mass Spectrometry and Gas Chromatography Quadrupole Time-of-Flight Mass Spectrometry, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this thesis and recommend that it be approved.

Anthony DeCaprio

Bruce McCord

José Almirall, Major Professor

Date of Defense: June 1, 2015

The thesis of Tyler S. Torbet is approved.

Dean Michael R. Heithaus
College of Arts and Sciences

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University Graduate School

Florida International University, 2015

DEDICATION

I dedicate this work to my family, especially my parents, Bethany and Scott Torbet.

There are no words to express how grateful I am for all of your support and encouragement.

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I would like to thank my major professor and my thesis committee. I thank Dr. José Almirall, my major professor, for his guidance over the last two years. I appreciate the time and opportunities he has given me during my graduate education at Florida International University. I also thank my committee members, Drs. Anthony DeCaprio and Bruce McCord for offering their time and advice.

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ABSTRACT OF THE THESIS

ANALYSIS OF SYNTHETIC CANNABINOIDS BY DIRECT ANALYSIS IN REAL TIME QUADRUPOLE TIME-OF-FLIGHT MASS SPECTROMETRY AND GAS CHROMATOGRAPHY QUADRUPOLE TIME-OF-FLIGHT MASS SPECTROMETRY

by

Tyler S. Torbet

Florida International University, 2015

Miami, Florida

Professor José Almirall, Major Professor

The aim of this study was to investigate the utility of direct analysis in real time quadrupole time-of-flight mass spectrometry and gas chromatography quadrupole time-of-flight mass spectrometry in the analysis of 162 different synthetic cannabinoids. Direct analysis in real time quadrupole time-of-flight mass spectrometry is shown to be a rapid and accurate analytical method for synthetic cannabinoids. Spectra can be generated with less than 1.5 ng of the drug in under a minute and be successfully searched against previously generated ESI-QTOF libraries in most cases (118/130 drugs tested) as well as can also be applied to the identification of synthetic cannabinoids in a mixture. Gas chromatography quadrupole time-of-flight mass spectrometry, while requiring a much longer analysis time, is shown to accurately distinguish all but 19 compounds (140/159). These two instruments have proven to be viable alternatives in synthetic cannabinoid analysis and will greatly benefit forensic laboratories.

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1. INTRODUCTION

Drug abuse is no new problem to society, however the material and method of consumption has changed drastically. The earliest referenced substance of abuse in the United States was alcohol in the mid 1800's. As the United States entered the Civil War era, alcohol consumption decreased because of the first attempts at state prohibition which led to a significant increase in use of tobacco and imported opiates. After the end of the Civil War and the repeal of state prohibition laws, the use of these legal substances steadily increased in addition to the emergence of psychoactive compounds such as cocaine and heroin. In the late 1800s and early 1900's, the United States was instituting alcohol prohibition for the second time as well as creating laws regulating opium and cocaine. These laws had little effect as the consumption of opiates, cocaine, and tobacco increased during this time leading Congress to pass one of the first laws related to substance abuse, the Food and Drug Act of 1890. This act prohibited "the importation of adulterated or unwholesome food, drugs, or liquor," with a focus on opiate and cocaine-containing products. As in the past however, stricter regulation of these compounds had a limited effect on abuse as well as led to the emergence of additional psychoactive substances such as marijuana and LSD in the first half of the 20th century followed by an explosion of abused substances including amphetamines, barbiturates (1960's), ecstasy (1970-80's), and designer drugs in the modern sense (1980-90's) [1-3]. As science has advanced and government has attempted to keep up with legislation, the number of designer drugs has increased dramatically with no signs of slowing down.

1.1. Synthetic Cannabinoids

Synthetic cannabinoids are a class of designer drugs which mimic the psychoactive effects of Δ^9 -tetrahydrocannabinol (THC), the active ingredient in cannabis, on the CB₁ and CB₂ receptors [4]. Synthetic cannabinoids are typically liquid solutions that are sprayed on plant leaves, flowers, or stems, and packaged as herbal incense mixtures with the intention of being smoked, inhaled, or ingested (Figure 1). Although products containing synthetic cannabinoids are typically marked “not for human consumption” in order to avoid government regulation, this does not prevent consumers from using them to achieve the marketed “legal highs” [5-8]. Available in head shops and online, these products are sold under brand names such as “Spice,” “K2,” “Dream,” “Yucatan Fire” among many others [9].

Figure 1. Commercial synthetic cannabinoid herbal incense mixtures [10].

Synthetic cannabinoids, when smoked, are said to have similar results to marijuana, however it has been reported that use can lead to adverse side effects such as anxiety, fatigue, disorientation, panic, dehydration, paranoia, insomnia, disturbed vision, tachycardia, severely exacerbated psychotic episodes and death [4,5]. It has also been shown that the potency levels for these compounds can vary greatly and have been

reported at levels significantly higher than THC, 500-600 times more potent in the case of AM694 and HU-210 [11].

First developed in the 1970's, synthetic cannabinoids were used as investigational drugs to study the effects of cannabinoids on brain functioning, specifically their effects on the CB₁ and CB₂ receptors, and their efficacy in treating pain as well as controlling appetite, blood pressure, and nausea [6-8,12]. In particular, a large group of these synthetic cannabinoids were developed by John W. Huffman, a professor emeritus of chemistry at Clemson University. His research, funded by the National Institute on Drug Abuse, was focused on developing drugs to target the CB₁ and CB₂ endocannabinoid receptors in the body to assist multiple sclerosis, HIV/AIDS, and chemotherapy research. Two decades of research led to the synthesis of hundreds of novel cannabinoids, designated "JWH" followed by a number, several of which were among the first to be detected by law enforcement agencies in herbal incense mixtures [13-15]. In addition to the JWH compounds developed by Huffman's group at Clemson University, several other groups of compounds emerged (Table 1) [13,16].

Table 1. Developers of Synthetic Cannabinoids.

Synthetic Cannabinoid Series	Synthesis by:
JWH	John W. Huffman, Clemson University
HU	Raphael Mechoulam, Hebrew University
CP	Pfizer, Inc.
AM	Alexandros Makriyannis, Northeastern University
RCS	Research Chemical Suppliers
TMCP (incl. UR-144)	Abbot Laboratories
WIN	Sterling Winthrop

The presence of synthetic cannabinoids in herbal incense products was first detected in Europe in 2004. These herbal mixtures were sold in head shops and online, marketed as incense products and air fresheners. After internet posts about the cannabis-

like effects when smoked, popularity of “Spice” and similar mixtures skyrocketed, starting the “Spice” phenomenon. In 2008, analysis of these “Spice” mixtures showed that the main active components were JWH 018 and CP 47,497 (C8 homolog) [17,18]. In November 2008, synthetic cannabinoids were detected for the first time in the United States when the U.S. Customs and Border Protection (CBP) seized a shipment of “Spice” in Dayton, OH [6]. As a result of the perceived status of “legal high,” and the ever increasing number of these compounds, the abuse of synthetic cannabinoids has continued to rise. These compounds have been shown to pose severe health risks because of high potency levels, no set dosage, and lack of research. In 2010, it was reported that 59% of all U.S. Emergency Department visits involved synthetic cannabinoids and from 2010 to 2011, the total number of visits increased from 11,406 to 28,531 [19-21].

As a consequence of the abuse of synthetic cannabinoids and the potential health risks, on March 1st, 2011, the first synthetic cannabinoids (JWH 018, JWH 073, JWH 200, CP 47,497, and CP 47,497 C8 homolog) were temporarily scheduled followed by permanent listing as Schedule I on July 9, 2012. Additional compounds such as AM2201, UR-144, XLR11, AKB48 were also added with the latter three being temporarily scheduled on May 16, 2013. As of 2014, the most recent emerging synthetic cannabinoids were PB-22, 5F-PB-22, AB-FUBINACA, and ADB-PINACA and on February 10, 2014, these four compounds were temporarily scheduled. In addition to scheduling, the U.S. government has also passed several laws including the Federal Analogue Act in 1986 and most recently the Synthetic Drug Abuse Prevention Act of 2012 in order to decrease the abuse of these compounds. However, success has been limited by the ability of drug chemists to develop novel compounds in order to

circumvent this legislation. So far, a solution has not been achieved and the already vast number of synthetic cannabinoids continues to increase [6].

As previously mentioned, synthetic cannabinoids are compounds that target the CB₁ and CB₂ endocannabinoid receptors. The CB₁ receptor is located primarily in the brain and spinal cord and mediates the physiological and psychotropic effects of cannabis. The CB₂ receptor is present in the spleen and immune system cells and is responsible for immune-modulatory effects. The binding at these two receptors determines the pharmacological activity of the drug, allowing for division into three categories: Cannabinomimetics, antagonists, and compounds that do not bind to either of these receptors. Cannabinomimetics are drugs which show similar pharmacological activity to cannabis and are primarily agonists at the CB₁ receptor. Antagonists bind to either the CB₁ or CB₂ receptor, inhibiting binding of other compounds at that location, but do not result in cannabis-like effects. The final category is made of the remaining synthetic cannabinoids, ones which do not bind at either receptor, meaning that the endocannabinoid receptors are not responsible for any pharmacological activity [12, 22-24].

In addition to being distinguished according to the pharmacological effects of the drug, synthetic cannabinoids are also classified according to their chemical structure. This area has been a region of debate as they can be classified into as many as ten different groups, however, the three main classifications are classical cannabinoids, cyclohexylphenols, and aminoalkylindoles. Classical cannabinoids such as HU-210 have similar structures to THC with only minor substituent differences (Figure 2a,b). It is important to note that although synthetic cannabinoids are advertised to have marijuana-

like effects, the classical cannabinoid category is relatively small and the majority of synthetic cannabinoids differ significantly in structure. Cyclohexylphenols and aminoalkylindoles are examples of this where the chemical structure is almost entirely different from that of THC and in the case of cyclohexylphenols, different than any other compounds (Figure 2c,d). In this three group classification system, aminoalkylindoles is the largest and most prevalent group that includes compounds such as indole- and pyrrole- based analogs [12,25].

Figure 2. Chemical structures of THC and 3 main synthetic cannabinoid classifications a) THC b) HU-210 c) CP 47,497 d) JWH 018.

Although effective, this classification system is designed with three broad categories of substances where even within the group, structure varies greatly. In a more detailed classification described by Presley, Varnum and Logan, synthetic cannabinoids are classified into ten groups. The additional groups allow for further division of the large number of JWH series compounds as well as inclusion of newly synthesized, more complex synthetic cannabinoids [25]. Under this classification, the ten categories are:

1. Classical
2. Cyclohexylphenols
3. Naphthoylindoles
4. Naphthylmethylindoles
5. Naphthylmethylindenes

6. Benzoylindoles
7. Naphthoylpyrroles
8. Phenylacetylindoles
9. Adamantoylindoles
10. Tetramethylcyclopropylindole

Classical cannabinoids and cyclohexylphenols have the same definitions here as in the three category classification system with HU-210 and CP 47,497 providing representative examples (Figure 3a,b). The major difference here is the division of the aminoalkylindole category into eight different groups based on specific functional groups present in the chemical structure. Naphthoylindoles and naphthylmethylindoles are similar in that they each contain a naphthyl and indole ring, however the difference is the linking functional group. Naphthoylindoles are linked by a carbonyl group whereas naphthylmethylindoles are connected by a methylene groups. Example compounds of these two groups are JWH 018 and JWH 175 respectively (Figure 3c,d) [25].

Figure 3. Chemical structures of 10 synthetic cannabinoid classifications a) HU-210 b) CP 47,497 c) JWH 018 d) JWH 175 e) JWH 176 f) JWH 030 g) AM694 h) AM1248 i) JWH 250 j) XLR-11

Naphthylmethylenes such as JWH 176 are similar to these two groups, however the indole ring is replaced by an indene ring which is linked to the naphthyl ring by a carbon-carbon double bond (Figure 3e). Naphthoylpyrroles are also similar to the previously mentioned naphthoylindoles with the only difference of a substituted pyrrole ring in place of an indole ring. An example compound of a naphthoylpyrrole is JWH 030 (Figure 3f). The final four categories show much more significant differences than the previous six. Benzoylindoles and adamantoylindoles both contain a substituted indole ring, however exhibit a clear difference of a phenyl ring versus an adamantyl ring. AM694 and AM1248 respectively are example compounds of these two groups (Figure 3g,h). Phenylacetylindoles such as JWH 250 contain a methylene group between the benzene ring and connecting carbonyl (Figure 3i). The compounds categorized into the final category represent some of the most recently detected synthetic cannabinoids. Tetramethylcyclopropylindoles contain a tetramethylcyclopropyl substituent in place of a benzyl or naphthyl group. XLR-11 is a representative compound for this final group (Figure 3j) [25].

As a result of the large number of synthetic cannabinoids, their widespread abuse, and the high potential for synthesis of novel compounds, these compounds have become a major research focus in order to keep up with the new and emerging drugs. Synthetic cannabinoids are also becoming more common in law enforcement seizures and a significant percentage of emergency room visits, meaning that better detection and identification of these compounds is required.

1.2. Direct Analysis in Real Time

The direct analysis in real time (DART) ion source was first developed and published by Robert B. Cody, James A. Laramée, and H. Dupont Durst in 2005. Direct analysis in real time is an ambient ion source, meaning that ionization takes place at atmospheric pressure in open air. Direct analysis in real time is extremely versatile as there are multiple methods that can be used depending on the sample type or the desire for automation. Examples of these methods are direct introduction to the ionization stream by holding the sample by hand or the use of a computer-controlled linear rail that can be combined with various sample holders including tweezers, a tablet holder, a plate holder, a tube holder, TLC plates and sample tips. This allows for a wide variety of samples to be analyzed with little to no sample preparation as analytes can be desorbed and ionized directly off of a surface. Direct analysis in real time is also able to be used for both positive-ion and negative-ion detection, expanding the types of compounds that can be analyzed. Another advantage to DART over other ionization and analysis techniques is that the analyte is ionized immediately after entry into the gas stream and directly passed into the mass spectrometer or ion mobility spectrometer, allowing for rapid analysis time. Direct analysis in real time has been successfully used to sample solid, liquid, and gaseous samples containing chemical residues such as concrete, glass, currency, and clothing [26-28].

Direct analysis in real time ionization works by flowing helium or nitrogen gas through a chamber where an electrical voltage is applied, exciting the gas and producing ions, electrons, and metastable species. These products are heated to a desired

temperature, removing the charged particles, and the gas stream leaves the DART source into the ambient environment which is then contacts the sample (Figure 2) [26-28].

Figure 4. Schematic diagram of the DART ion source [26].

The mechanism of DART ionization not completely understood as depending on the reaction gas and the ionization potential of the analyte, multiple ionization mechanisms are possible. However a proposed method for positive ionization with helium is that helium atoms are excited which then interact with water molecules present in the atmosphere, producing protonated water clusters. These water clusters then interact with the sample through proton transfer resulting in ionization of the analyte (Figure 3). The ionization using protonated water clusters proves to be much softer than electron ionization (EI) allowing for the observance of molecular ions in the mass spectra [26-28].

Figure 5. Proposed scheme of positive mode DART ionization [27].

For negative ionization, it is proposed that electrons produced from collisions with gas molecules undergo electron capture from oxygen present in the atmosphere resulting in O_2^- ions which then ionize the analyte. The ability to use positive and negative DART

ionization allows for analysis of samples of varying composition including pharmaceuticals, synthetic organics, organometallics, explosives, and drugs of abuse among many others [26-28].

As interest in alternative laboratory techniques has grown, DART has become one of the most widely studied because of the rapid analysis time. Direct analysis in real time coupled with a mass spectrometer (MS) and time-of-flight mass spectrometer (TOF) have been previously demonstrated in the analysis of a wide variety of samples, including synthetic cannabinoids. Musah et al. were able to rapidly analyze two synthetic cannabinoids, JWH 015 and AM251, by DART-MS when doped directly onto a leaf species with no additional sample preparation. In addition, they also showed that the biological matrices did not affect the ability to detect the two compounds [29]. Musah et al. were also able to use DART-TOF and collision induced dissociation (CID) to detect and differentiate five synthetic cannabinoids (AM2201, JWH 122, JWH 203, JWH 210, and RCS-4) in commercially available “Spice” products [30]. Lesiak et al. were also able to detect five synthetic cannabinoids (AM2201, JWH 122, JWH 203, JWH 210, and RCS-4) in a series of six “Spice” products by using DART-TOF and CID as a screening technique. Here they were able to detect the compounds individually and in tandem at concentrations of 4-141 mg/g of material [31]. Dunham et al. used DART-TOF to identify JWH 018 in three different herbal incense products with no sample preparation in a total analysis time of under five minutes [32]. Gwak and Almirall were able to show results for 35 psychoactive substances, including a limited number of synthetic cannabinoids, demonstrating the rapid analysis and high resolution capability of quadrupole time-of-flight mass spectrometry, while also showing that DART-QTOF

spectra can be compared with ESI-QTOF generated spectral libraries [33]. Significant research has also been shown outside of the United States by Uchiyama et al. In a series of publications over the last several years, they have used DART-TOF in combination with UPLC-ESI-MS, GC/MS, and NMR to identify newly distributed synthetic cannabinoids including JWH 081, JWH 015, JWH 200, JWH 073, cannabipiperidiethnone, URB 754, APICA, and APINACA. In these studies, DART-TOF was used following UPLC-ESI-MS and GC/MS to obtain accurate mass spectra. Additionally, in each study, additional compounds were also described as newly distributed in Japanese markets [34-37].

1.3. Gas Chromatography

Chromatography is the separation of a mixture on the basis of the interaction between the analytes, mobile phase, stationary phase. The mobile phase is used to transport the sample through the stationary phase which is typically a polymer layered tubing. As the sample passes through the column, the analytes interact with the stationary phase coating the column walls. This interaction results in separation of the analyte and the elution of each component at differing times based on the relative affinity of the analyte with the stationary phase. The elution time is known as the retention time. In forensic laboratories, both gas and liquid chromatography are commonly used to analyze seized drugs. Liquid chromatography (LC) uses a liquid mobile phase whereas gas chromatography (GC) uses an inert gas as the mobile phase [38-39].

Gas chromatography is typically performed using hydrogen, helium, or nitrogen as the mobile phase as these gases are inert, do not interfere with the sensitivity of the instrument, and are inexpensive. However, the choice of gas can affect the analysis.

Nitrogen tends to yield the best separation because of its high molecular mass and low diffusion coefficient, but results in longer run times than when using other gases because lower velocity is required. Hydrogen can also be used, however because of its flammability, additional safety precautions are required. As helium provides the best results and is safe to use, it is the typical mobile phase used in gas chromatography analysis [39]

The stationary phase used in GC analysis must also be carefully selected according to the type of samples that will be analyzed. A stationary phase should be thermally and chemically stable to prevent decomposition and contamination of the sample. There are a variety of commercial stationary phases that can be used, ranging from non-polar to polar (Table 2). As expected, polar stationary phases have function groups that interact with the analyte, meaning that elution depends on these interactions. Inversely non-polar stationary phases lack these functional groups and interact with the analyte through dispersive forces [39].

Table 2. Common Commercial Stationary Phases for GC Analysis [40].

Stationary Phase Composition	Name (Agilent)	Polarity	Max. Temp. Limit (°C)	Use
100% Dimethylpolysiloxane	DB-1, HP-1	Non-polar	325/350	Amines, hydrocarbons, pesticides, PCBs, phenols, flavours, fragrances
5% Phenyl-95% dimethylpolysiloxane	DB-5, HP-5	Non-polar	325/350	Semi-volatiles, alkaloids, drugs, FAMES, halogenated compounds, pesticides, herbicides
35% Phenyl-65% dimethylpolysiloxane	DB-35, HP-35	Mid	300/320	Pesticides, aroclors, pharmaceuticals, drugs of abuse
50% Phenyl-50% dimethylpolysiloxane	DB-17	Mid	280/300	Drugs, glycols, pesticides, steroids
50% Cyanopropyl-50% methylpolysiloxane	DB-23	High	250/260	FAMES
88% Cyanopropyl-12% arylpolysiloxane	HP-88	High	250/260	FAMES
35% Trifluoropropyl-65% dimethylpolysiloxane	DB-200	Mid	300/320	Residual solvents, pesticides, herbicides

50% Trifluoropropyl-50% dimethylpolysiloxane	DB-210	High	240/260	EPA Methods 8140 and 609
50% Cyanopropylphenyl-50% dimethylpolysiloxane	DB-225	Mid/high	220/240	Cis/trans FAMES, alditol acetates, neutral sterols
6% Cyanopropylphenyl-94% dimethylpolysiloxane	DB-1301	Low/mid	280/300	Aroclors, alcohols, pesticides, VOCs
14% Cyanopropylphenyl-86% dimethylpolysiloxane	DB-1701	Low/mid	280/300	Pesticides, herbicides, TMS sugars, aroclors

Gas chromatography instruments are typically automated as it is important that the amount of sample injected onto the column is within the capacity range and detector range. Although sample mixtures may be solids, liquids, or gases, any solid or liquid samples must be easily vaporized. Another key factor is that the sample must be thermally stable so that it does not degrade in the injection port [38,39].

Depending on the analyte mixture, GC analysis can be performed using either split or splitless injection modes. Splitless injection means that the injection volume is deposited onto the column in its entirety whereas when using split injection, only a fraction of the sample is introduced to the column. Split injection is most commonly used as it reduces the possibility of overloading the column while also resulting in improved peak shape. However, split injection also results in lower sensitivity because only a small fraction of the sample is used and the remaining is wasted. For this reason, splitless injection is advantageous for trace analysis [39].

Gas chromatography as well as liquid chromatography are common analytical techniques in forensic science, however because on their own they only provide limited information about the mixture components by determining the retention time, they are typically coupled with a mass spectrometer. The inclusion of a mass spectrometer allows for determination of structural information about the components as well as additional identification capabilities.

1.4. Quadrupole Time-of-Flight Mass Spectrometry

Mass spectrometry is an analytical technique that is commonly coupled with liquid and gas chromatography. It works by ionizing a solid, liquid, or gaseous sample in a vacuum which breaks the compound into charged fragments. These charged fragments are then separated according to their mass-to-charge (m/z) ratio, the ratio of the mass of a particle to the number of electrostatic charge units carried by that particle. The mass-to-charge ratio is useful as it reveals characteristic and structural information about the compound because similar molecules will fragment into predictable patterns.

The first step in mass spectrometry is ionization of the analyte. Ionization is typically done through electron ionization (EI) or chemical ionization (CI). As with gas chromatography, it is important that the analyte is volatile and thermally stable allowing for ionization and preventing degradation. Electron ionization is the most commonly used ionization method with mass spectrometry. It is recognized as a “hard” ionization technique resulting in significant fragmentation, meaning that the molecular ion may not be present in the mass spectra. Electron ionization works by heating a filament which produces electrons. These electrons are accelerated toward an anode and interact with the analyte molecules, causing them to absorb energy and emit an electron. The energy is typically set at 70 eV in commercial instruments, providing more than enough energy to produce a large amount of fragmentation. Chemical ionization first bombards a reagent gas with generated electrons and then these ionized reagent gas molecules collide with the analyte causing fragmentation. Chemical ionization is a “soft” ionization technique as there is limited fragmentation of the parent ion, meaning the molecular ion is commonly present in the mass spectra [41].

Mass spectrometry data are displayed as a mass spectrum representing the masses of the sample fragments versus the frequency of the fragment/intensity. The mass analyzer, typically a quadrupole or ion-trap instrument, separates the ions based on the stability of their trajectory through an oscillating electric field. After passing through the quadrupole or ion trap, the ion reaches the detector. Mass spectrometers are kept under vacuum at low pressures in order to prevent unwanted collisions of gas molecules and analyte ions before injection [41].

A quadrupole time-of-flight (QTOF) mass spectrometer operates by measuring the time required for an ion to travel from the ion source to the detector. As the ions are introduced to the mass spectrometer, they are selected by a mass filter and accelerated, with all ions receiving the same kinetic energy. As the ions pass into the collision cell, they undergo collision induced dissociation. The ions are reaccelerated after passing through the collision cell and focused into an ion beam by the ion modulator before reaching the field-free region where TOF separation takes place. An ion mirror is then used to focus the ions to the detector. (Figure 4) [42,43].

Figure 6. Schematic diagram of quadrupole time-of-flight mass spectrometer [43].

As the ions are all accelerated at the same kinetic energy, the weight of the ions are what influence the TOF separation. The smaller, lower molecular weight ions move faster, reaching the detector first, followed by the heavier, slower moving ions. The molecular ion, or the peak of highest abundance, is the most important ion in the mass spectra because it is an indicator of the molecular weight of the compound. However, as mentioned, depending on the ionization method used and the energy and stability of the compound, the molecular ion may decompose and not appear in the mass spectra [41-43].

Although both function in a similar manner, a quadrupole time-of-flight mass spectrometer has several advantages over its time-of-flight counterpart. The additional quadrupole allows the instrument to be able to focus on a particular ion or ions of interest which means a specific drug or compound in a mixture can be fragmented. The QTOF is also highly sensitive, accurate and capable of resolving the exact mass of not only the parent ion, but also the product and fragment ions of the analyte. The high mass accuracy, resolution (DART-QTOF, $R=20,000$ /GC-QTOF, $R=15,000$), and sensitivity allows for improved identification of analytes not only when compared to TOF instruments, but also gas chromatography coupled with triple quadrupole mass spectrometry (GC-QQQ) which has previously been used for analysis of synthetic cannabinoids [42-43].

With synthetic cannabinoids recently becoming more abused, more research has been designated to this field. As a result numerous articles have been published on the analysis of these compounds with analytical techniques such as GC-MS, LC-MS, LC/ESI-MS, GC-QQQ-MS, and tandem mass spectrometry variations [17,44-50]. Analysis of synthetic cannabinoids using GC-TOF has also been demonstrated. Ernst et

al. were able to identify JWH-122 as an ingredient in “Lava red,” a “Spice-like” herbal mixture by providing accurate masses to the molecular fragment ions [51]. Lindigkeit et al. were able to use GC-TOF to analyze several synthesized alkylaminoindoles as well as three synthetic cannabinoids (CP 47,497-C8, JWH 018, and JWH 073) present in commercially available herbal incense mixtures [52]. In addition, GC-TOF has been described by Vincenti et al. and Fernandez-Peralbo and Luque de Castro as an alternative method in the analysis of hair and urine samples for synthetic cannabinoids [53,54]. Specifically relating to this study, no publications on analysis of synthetic cannabinoids have been found, however it has been used to analyze materials such as pesticides, organics, and volatile organic compounds (VOCs) [55-57].

2. SYNTHETIC CANNABINOIDS BY DART-QTOF

2.1. Materials

Reference standards of 160 synthetic cannabinoids, cannabidiol and (-)-11-nor-9-carboxy- Δ^9 -THC were provided by Cayman Chemicals (Ann Arbor, MI) (Table 3). Methanol (Optima®, LC/MS grade) was purchased from Fisher Scientific (Fair Lawn, NJ). The standard stock solutions of each synthetic cannabinoid in powder form were prepared with methanol at varying concentrations depending on the amount of standards, 1000, 2000, 5000, 10000 $\mu\text{g/mL}$. These stock solutions were diluted to 10 $\mu\text{g/mL}$ in methanol in order to prepare samples for analysis.

Table 3. Synthetic Cannabinoid Standards

Name	Molecular Formula	Molecular Weight (amu)
AM2201 2'-naphthyl isomer	C ₂₄ H ₂₂ FNO	359.1685
AM2201 N-(2-fluoropentyl) isomer	C ₂₄ H ₂₂ FNO	359.1685
AM2201 N-(3-fluoropentyl) isomer	C ₂₄ H ₂₂ FNO	359.1685
AM2201 N-(4-fluoropentyl) isomer	C ₂₄ H ₂₂ FNO	359.1685
AM694 3-iodo isomer	C ₂₀ H ₁₉ FINO	435.0495
AM694 4-iodo isomer	C ₂₀ H ₁₉ FINO	435.0495
JWH 018 2'-naphthyl-N-(1-ethylpropyl)	C ₂₄ H ₂₃ NO	341.1780
JWH 018 2'-naphthyl isomer	C ₂₄ H ₂₃ NO	341.1780
JWH 018 2'-naphthyl-N-(1,1-dimethylpropyl) isomer	C ₂₄ H ₂₃ NO	341.1780
JWH 018 2'-naphthyl-N-(1,2-dimethylpropyl) isomer	C ₂₄ H ₂₃ NO	341.1780
JWH 018 2'-naphthyl-N-(1-methylbutyl) isomer	C ₂₄ H ₂₃ NO	341.1780
JWH 018 2'-naphthyl-N-(2,2-dimethylpropyl) isomer	C ₂₄ H ₂₃ NO	341.1780
JWH 018 2'-naphthyl-N-(2-methylbutyl) isomer	C ₂₄ H ₂₃ NO	341.1780
JWH 018 2'-naphthyl-N-(3-methylbutyl) isomer	C ₂₄ H ₂₃ NO	341.1780
JWH 018 N-(1,1-dimethylpropyl) isomer	C ₂₄ H ₂₃ NO	341.1780
JWH 018 N-(1,2-dimethylpropyl) isomer	C ₂₄ H ₂₃ NO	341.1780
JWH 018 N-(1-ethylpropyl) isomer	C ₂₄ H ₂₃ NO	341.1780
JWH 018 N-(1-methylbutyl) isomer	C ₂₄ H ₂₃ NO	341.1780
JWH 018 N-(2,2-dimethylpropyl) isomer	C ₂₄ H ₂₃ NO	341.1780
JWH 018 N-(2-methylbutyl) isomer	C ₂₄ H ₂₃ NO	341.1780
JWH 018 N-(3-methylbutyl) isomer	C ₂₄ H ₂₃ NO	341.1780
JWH 073 2'-naphthyl isomer	C ₂₃ H ₂₁ NO	327.1623
JWH 073 2'-naphthyl-N-(1,1-dimethylethyl) isomer	C ₂₃ H ₂₁ NO	327.1623
JWH 073 2'-naphthyl-N-(1-methylpropyl) isomer	C ₂₃ H ₂₁ NO	327.1623
JWH 073 2'-naphthyl-N-(2-methylpropyl) isomer	C ₂₃ H ₂₁ NO	327.1623
JWH 073 N-(1,1-dimethylethyl) isomer	C ₂₃ H ₂₁ NO	327.1623
JWH 073 N-(1-methylpropyl) isomer	C ₂₃ H ₂₁ NO	327.1623
JWH 073 N-(2-methylpropyl) isomer	C ₂₃ H ₂₁ NO	327.1623
JWH 081 2-methoxynaphthyl isomer	C ₂₅ H ₂₅ NO ₂	371.1885
JWH 081 3-methoxynaphthyl isomer	C ₂₅ H ₂₅ NO ₂	371.1885
JWH 081 5-methoxynaphthyl isomer	C ₂₅ H ₂₅ NO ₂	371.1885
JWH 081 6-methoxynaphthyl isomer	C ₂₅ H ₂₅ NO ₂	371.1885
JWH 081 7-methoxynaphthyl isomer	C ₂₅ H ₂₅ NO ₂	371.1885
JWH 081 8-methoxynaphthyl isomer	C ₂₅ H ₂₅ NO ₂	371.1885
JWH 122 2-methylnaphthyl isomer	C ₂₅ H ₂₅ NO	355.1936
JWH 122 3-methylnaphthyl isomer	C ₂₅ H ₂₅ NO	355.1936
JWH 122 5-methylnaphthyl isomer	C ₂₅ H ₂₅ NO	355.1936
JWH 122 6-methylnaphthyl isomer	C ₂₅ H ₂₅ NO	355.1936
JWH 122 7-methylnaphthyl isomer	C ₂₅ H ₂₅ NO	355.1936
JWH 122 8-methylnaphthyl isomer	C ₂₅ H ₂₅ NO	355.1936
JWH 200 2'-naphthyl isomer	C ₂₅ H ₂₄ N ₂ O ₂	384.1838
JWH 203 3-chlorophenyl isomer	C ₂₁ H ₂₂ ClNO	339.1390
JWH 203 4-chlorophenyl isomer	C ₂₁ H ₂₂ ClNO	339.1390
JWH 210 2-ethylnaphthyl isomer	C ₂₆ H ₂₇ NO	369.2093
JWH 210 3-ethylnaphthyl isomer	C ₂₆ H ₂₇ NO	369.2093
JWH 210 5-ethylnaphthyl isomer	C ₂₆ H ₂₇ NO	369.2093
JWH 210 6-ethylnaphthyl isomer	C ₂₆ H ₂₇ NO	369.2093
JWH 210 7-ethylnaphthyl isomer	C ₂₆ H ₂₇ NO	369.2093
JWH 210 8-ethylnaphthyl isomer	C ₂₆ H ₂₇ NO	369.2093

JWH 251 3-methylphenyl isomer	C ₂₂ H ₂₅ NO	319.1936
JWH 251 4-methylphenyl isomer	C ₂₂ H ₂₅ NO	319.1936
JWH 398 2-chloronaphthyl isomer	C ₂₄ H ₂₂ ClNO	375.1390
JWH 398 3-chloronaphthyl isomer	C ₂₄ H ₂₂ ClNO	375.1390
JWH 398 5-chloronaphthyl isomer	C ₂₄ H ₂₂ ClNO	375.1390
JWH 398 6-chloronaphthyl isomer	C ₂₄ H ₂₂ ClNO	375.1390
JWH 398 7-chloronaphthyl isomer	C ₂₄ H ₂₂ ClNO	375.1390
JWH 398 8-chloronaphthyl isomer	C ₂₄ H ₂₂ ClNO	375.1390
RCS-4 2-methoxy isomer	C ₂₁ H ₂₃ NO ₂	321.1729
RCS-4 3-methoxy isomer	C ₂₁ H ₂₃ NO ₂	321.1729
RCS-8 3-methoxy isomer	C ₂₅ H ₂₉ NO ₂	375.2198
RCS-8 4-methoxy isomer	C ₂₅ H ₂₉ NO ₂	375.2198
(-)-11-nor-9-carboxy- Δ 9-THC	C ₂₁ H ₂₈ O ₄	344.1988
(-)-CP 47,497	C ₂₁ H ₃₄ O ₂	318.2559
(-)-CP 55,940	C ₂₄ H ₄₀ O ₃	376.2978
(+)-CP 47,497	C ₂₁ H ₃₄ O ₂	318.2559
(+)-CP 55,940	C ₂₄ H ₄₀ O ₃	376.2978
(+)WIN 55212-2	C ₂₇ H ₂₆ N ₂ O ₃	426.1943
($\pm$)3epi CP 47,497-C8-homolog	C ₂₂ H ₃₆ O ₂	332.2715
($\pm$)5-epi CP 55,940	C ₂₄ H ₄₀ O ₃	376.2978
($\pm$)CP 47,497	C ₂₁ H ₃₄ O ₂	318.2559
($\pm$)-CP 47,497-C8-homolog	C ₂₂ H ₃₆ O ₂	332.2715
($\pm$)-CP 55,940	C ₂₄ H ₄₀ O ₃	376.2978
($\pm$)-epi CP 47, 497	C ₂₁ H ₃₄ O ₂	318.2559
($\pm$)WIN 55212 (mesylate)	C ₂₇ H ₂₆ N ₂ O ₃	426.1943
1'-Naphthoyl Indole	C ₁₉ H ₁₃ NO	271.0997
4-Quinolone-3-Carboxamide CB2 Ligand	C ₂₆ H ₃₄ N ₂ O ₃	422.2569
AKB48	C ₂₃ H ₃₁ N ₃ O	365.2467
AM1220	C ₂₆ H ₂₆ N ₂ O	382.2045
AM1235	C ₂₄ H ₂₁ FN ₂ O ₃	404.1536
AM1241	C ₂₂ H ₂₂ IN ₃ O ₃	503.0706
AM1248	C ₂₆ H ₃₄ N ₂ O	390.2671
AM2201	C ₂₄ H ₂₂ FNO	359.1685
AM2232	C ₂₄ H ₂₀ N ₂ O	352.1576
AM2233	C ₂₂ H ₂₃ IN ₂ O	458.0855
AM251	C ₂₂ H ₂₁ Cl ₂ IN ₄ O	554.0137
AM630	C ₂₃ H ₂₅ IN ₂ O ₃	504.0910
AM679	C ₂₀ H ₂₀ INO	417.0590
AM694	C ₂₀ H ₁₉ FINO	435.0495
Cannabidiol	C ₂₁ H ₃₀ O ₂	314.2246
CB-13	C ₂₆ H ₂₄ O ₂	368.1776
CB-25	C ₂₅ H ₄₁ NO ₃	403.3086
CB-52	C ₂₆ H ₄₃ NO ₃	417.3243
CB-86	C ₂₆ H ₄₃ NO ₃	417.3243
CP 47,497-para-quinone analog	C ₂₁ H ₃₂ O ₃	332.2351
HU-210	C ₂₅ H ₃₈ O ₃	386.2821
HU-211	C ₂₅ H ₃₈ O ₃	386.2821
HU-308	C ₂₇ H ₄₂ O ₃	414.3134
HU-331	C ₂₁ H ₂₈ O ₃	328.2038
IMMA	C ₂₃ H ₂₃ ClN ₂ O ₄	426.1346
JP104	C ₂₅ H ₃₀ N ₂ O ₃	406.2256
JWH 007	C ₂₅ H ₂₅ NO	355.3000

JWH 011	C ₂₇ H ₂₉ NO	383.2249
JWH 015	C ₂₃ H ₂₁ NO	327.1623
JWH 016	C ₂₄ H ₂₃ NO	341.1780
JWH 018	C ₂₄ H ₂₃ NO	341.1780
JWH 018 6-methoxyindole analog	C ₂₅ H ₂₅ NO ₂	371.1885
JWH 018 adamantyl analog	C ₂₄ H ₃₁ NO	349.2406
JWH 018 adamantyl carboxamide	C ₂₄ H ₃₂ N ₂ O	364.2515
JWH 018 N-(4,5-epoxypentyl) analog	C ₂₄ H ₂₁ NO ₂	355.1572
JWH 018 N-(5-bromopentyl) analog	C ₂₄ H ₂₂ BrNO	419.0885
JWH 018 N-(5-chloropentyl) analog	C ₂₄ H ₂₂ ClNO	375.1390
JWH 019	C ₂₅ H ₂₅ NO	355.1936
JWH 020	C ₂₆ H ₂₇ NO	369.2093
JWH 022	C ₂₄ H ₂₁ NO	339.1623
JWH 030	C ₂₀ H ₂₁ NO	291.1623
JWH 031	C ₂₁ H ₂₃ NO	305.1780
JWH 072	C ₂₂ H ₁₉ NO	313.1467
JWH 073	C ₂₃ H ₂₁ NO	327.1623
JWH 073 2-methylnaphthyl analog	C ₂₄ H ₂₃ NO	341.1780
JWH 073 4-methylnaphthyl analog	C ₂₄ H ₂₃ NO	341.1780
JWH 081	C ₂₅ H ₂₅ NO ₂	371.1885
JWH 098	C ₂₆ H ₂₇ NO ₂	385.2042
JWH 122	C ₂₅ H ₂₅ NO	355.1936
JWH 122 N-(4-pentenyl) Analog	C ₂₅ H ₂₃ NO	353.1780
JWH 145	C ₂₆ H ₂₅ NO	367.1936
JWH 147	C ₂₇ H ₂₇ NO	381.2093
JWH 175	C ₂₄ H ₂₅ N	327.1987
JWH 180	C ₂₅ H ₂₅ NO	355.1936
JWH 182	C ₂₇ H ₂₉ NO	383.2249
JWH 200	C ₂₅ H ₂₄ N ₂ O ₂	384.1838
JWH 201	C ₂₂ H ₂₅ NO ₂	335.1885
JWH 203	C ₂₁ H ₂₂ ClNO	339.1390
JWH 210	C ₂₆ H ₂₇ NO	369.2093
JWH 249	C ₂₁ H ₂₂ BrNO	383.0885
JWH 250	C ₂₂ H ₂₅ NO ₂	335.1885
JWH 251	C ₂₂ H ₂₅ NO	319.1936
JWH 302	C ₂₂ H ₂₅ NO ₂	335.1885
JWH 307	C ₂₆ H ₂₄ FNO	385.1842
JWH 309	C ₃₀ H ₂₇ NO	417.2093
JWH 368	C ₂₆ H ₂₄ FNO	385.1842
JWH 369	C ₂₆ H ₂₄ ClNO	401.1546
JWH 370	C ₂₇ H ₂₇ NO	381.2093
JWH 398	C ₂₄ H ₂₂ ClNO	375.1390
JWH 424	C ₂₄ H ₂₂ BrNO	419.0885
JZL 184	C ₂₇ H ₂₄ N ₂ O ₉	520.1482
JZL 195	C ₂₄ H ₂₃ N ₃ O ₅	433.1638
MAM2201	C ₂₅ H ₂₄ FNO	373.1842
MDA 19	C ₂₁ H ₂₃ N ₃ O ₂	349.1790
MDA 77	C ₂₁ H ₂₃ N ₃ O ₃	365.1739
Cannabipiperidiethanone	C ₂₄ H ₂₈ N ₂ O ₂	376.2151
RCS-4	C ₂₁ H ₂₃ NO ₂	321.1729
RCS-4-C4 Homolog	C ₂₀ H ₂₁ NO ₂	307.1572
RCS-8	C ₂₅ H ₂₉ NO ₂	375.2198

STS-135	C ₂₅ H ₃₄ FNO	383.2624
UR-144	C ₂₁ H ₂₉ NO	311.2249
URB447	C ₂₅ H ₂₁ ClN ₂ O	400.1342
URB597	C ₂₀ H ₂₂ N ₂ O ₃	338.1630
URB602	C ₁₉ H ₂₁ NO ₂	295.1572
URB754	C ₁₆ H ₁₄ N ₂ O ₂	266.1055
URB937	C ₂₀ H ₂₂ N ₂ O ₄	354.1580
WIN 54461	C ₂₃ H ₂₅ BrN ₂ O ₃	456.1049
XLR11	C ₂₁ H ₂₈ FNO	329.2155

Two compounds that are important to distinguish out of the 162 compounds analyzed are Cannabidiol and (-)-11-nor-9-carboxy- Δ 9-THC. Cannabidiol is not classified as a synthetic cannabinoid as it is a naturally occurring compound present in cannabis. However, cannabidiol is a compound of recent interest as different cannabis strains contain varying concentrations of this compound, resulting in changes in the effects of the cannabis when smoked. For example, when cannabis strains with higher concentrations of the cannabidiol were smoked, it was found that the short-term memory impairments that are often seen with cannabis use did not occur. Studies have attributed these results to the CB₁ antagonist properties of cannabidiol and there is a large amount of research being performed to determine its potential in medicinal applications. (-)-11-nor-9-carboxy- Δ 9-THC is an inactive secondary metabolite of Δ 9-THC. The importance of this compound is that it has a long half-life in the body, meaning it is the main metabolite analyzed for in blood or urine testing for cannabis use. As the cannabidiol and (-)-11-nor-9-carboxy- Δ 9-THC standards had been previously obtained and because of the interest in the compounds, these two compounds were included in the 162 analyzed compounds [58,59].

2.2. Methodology

2.2.1. Instrumental Parameters for DART-QTOF

Analysis was performed using an IonSense DART[®]-SVP (Saugus, MA) coupled to an Agilent 6530 Q-TOF (Agilent Technologies, Santa Clara, CA) using the linear rail setup in 1-D transmission mode (Figure 1). Qualitative Analysis MassHunter Workstation software (Agilent Technologies) was used for data processing.

Figure 7. IonSense DART[®]-SVP coupled to Agilent 6530 Q-TOF.

The instrumental parameters were adapted from Gwak and Almirall, with the only modification of the fragmentor voltage in order to produce higher peak intensities for synthetic cannabinoids. Helium at 350°C with a flow rate of 3 L/min was used as the ionization gas. The linear rail was set at a sample speed of 1.0 mm/sec with a contact closure delay of 5 seconds. Data were collected over a mass range of 50-600 amu at Skimmer and RF voltages of 65 V and 250 V respectively [33].

2.2.2. Optimization of the Fragmentor Voltage

A 1 μ L spike of the chosen 10 μ g/mL synthetic cannabinoid standard was deposited onto a wire mesh strip which was then inserted into the linear rail. Two different compounds were analyzed simultaneously in triplicate by spiking the first

compound in the second, third, and fourth slots and the second compound in the sixth, seventh, and eighth slots. The first and fourth slots were left as blanks (Figure 8). In order to prevent any possible carry over between samples, analysis was performed on the mesh without any additional sample being added in between each sample run as a blank.

Figure 8. Linear rail with wire mesh strip insert. Slots numbered beginning with 1 as the farthest to the right and first to enter the DART gas stream.

Analysis was performed using the previously reported parameters with varying fragmentor voltages of 50, 100, 150, 200, 250, 300, 350, 375 and 400 V and full scan total ion chromatograms were obtained. In order to prevent carryover from previous samples, a blank run of the linear rail with mesh with no sample deposited on any slot was run in between every sample analysis.

Using the MassHunter software, the ion chromatogram were extracted by manually inputting a range that encompassed the molecular ion. No specific range value was used, however it was carefully determined by examining the maximum and minimum values present for the molecular ion within a reasonable range of mass accuracy. Each peak was then integrated and an average peak area and standard deviation for each fragmentor voltage were calculated. A fragmentor voltage versus average peak area plot was then used to determine the optimal voltage.

Six synthetic cannabinoids (AM2201, Cannabidiol, HU-210, JWH 018, JWH 200, and JWH 203) were used as a representative group of compounds for optimization. The

data obtained for JWH 200 and JWH 203 (Figure 9) showed a maximum peak area at a fragmentor voltage of 350 V and 375 V, respectively.

Figure 9. Comparison of peak areas for JWH 200 and JWH 203 at fragmentor voltage between 50 and 400 V.

For both AM2201 and JWH 018, a maximum peak area was determined to be at 350 V, although it is important to note that for AM2201, the peak areas at 300 and 350 V are similar (Figure 10).

Figure 10. Comparison of peak areas for AM2201 and JWH 018 at fragmentor voltage between 50 and 400 V.

For HU-210, the peak area of the extracted ion did not change between 200-375 V (Figure 11), and cannabidiol showed a maximum peak area at 350 V with a similar value at 250 V (12). Because of a large difference between peak area for these two compounds, they were plotted individually in order to clearly view the maximum peak areas.

Figure 11. Comparison of peak areas for HU-210 at fragmentor voltage between 50 and 400 V.

Figure 12. Comparison of peak areas for Cannabidiol at fragmentor voltage between 50 and 400 V.

Interday replicates showed the same results for all compounds except for JWH 018 and HU-210. JWH 018 only differed slightly as 300 V showed the maximum peak area with 350 V a close second. HU-210 showed a bit more variation with a much larger area at 200 V, however because of substantial deviation between intraday replicates, this sample was excluded from consideration. On the basis of these results, all synthetic cannabinoid analysis by DART-QTOF was performed using the parameters listed in Table 4, however it is important to note that because of the large error bars, no definitive conclusion can be reached on the effect of the fragmentor voltage on the intensity of the peaks.

Table 4. Optimized DART-QTOF Parameters

IonSense DART [®] -SVP	Agilent 6530 Q-TOF Mass analyzer	
Sample introduction 1-D Transmission (Positive)	Fragmentor Skimmer RF	350 V
Ionization gas He at 350°C, 3L/min		65 V 250 V
Sample speed 1.0 mm/sec	Mass range 50-600 amu	

2.2.3. Limit of Detection

Using the optimized parameters (Table 4), limits of detection were determined for a representative sample of 14 synthetic cannabinoids. 10 µg/mL standard solutions were first diluted to 1 µg/mL. 5 replicates of each compound were then spiked onto the wire mesh strip in the second through sixth slots of the linear rail. Limit of detections were determined over a range of 0.5 to 3 ng by spiking the respective volume of standard (Table 5), with the addition of a blank analysis run of the mesh with no sample deposited.

Table 5. Limit of detection deposit volumes and amounts

Standard Concentration (µg/mL)	Volume Deposited (µL)	Amount Deposited (ng)
1	0.5	0.5
1	1.0	1.0
1	1.5	1.5
1	2.0	2.0
1	2.5	2.5
1	3.0	3.0

After DART-QTOF analysis, the ion chromatograms were extracted using the same method as previously described for the optimization of the fragmentor voltage and the peak area versus the amount deposited was plotted (Figure 13).

Figure 13. Calibration curve for LOD determination of JWH 018.

The average for the five replicates for each amount deposited was calculated and used to calculate the overall standard error. The standard error in accordance with the slope of the curve was used to calculate the limit of detection using the equation.

$$LOD = 3 \left(\frac{SE}{m} \right)$$

Although DART is currently used only as a qualitative screening technique because of the inconsistency caused by the constantly changing ambient conditions, the limits of detection for the representative sample of 14 synthetic cannabinoids can be seen in Table 6 to show the high sensitivity of this technique.

Table 6. Limit of detections for representative list of synthetic cannabinoids

Compound	LOD (ng)
JWH 018 2'-naphthyl isomer	1.2
JWH 073 2'-naphthyl-N-(1-methylpropyl) isomer	0.77
JWH 081 5-methoxynaphthyl isomer	0.92
(-)-CP 47,497	0.52
(±) WIN 55212 (mesylate)	1.5
AKB48	0.83
HU-210	0.94
JWH 018	0.56
AM2201	0.92
AM694	1.3
RCS-4	0.65
RCS-8	0.47
UR-144	1.2
XLR11	1.5

2.2.4. DART-QTOF Standard Analysis

Analysis of the 162 synthetic cannabinoid standards was performed using the previously described linear rail setup. A 1 μ L spike of each 10 μ g/mL synthetic cannabinoid standard was deposited onto the wire mesh strip (10 ng). As with the fragmentor optimization, two different compounds were analyzed simultaneously in triplicate by spiking the first compound in the second, third, and fourth slots and the second compound in the sixth, seventh, and eighth slots, with the first and fourth slots left as blanks (Figure 8). To prevent sample carryover, a full scan of the linear rail with wire mesh was run in between each set of samples. In addition to a full total ion chromatogram scan, product ion scan mass spectra were also obtained at three collision energies

(CE=10,20, and 40 eV) in the auto MS/MS mode. All product ion scan mass spectra were searched against an existing ESI-QTOF spectral library.

2.2.5. DART-QTOF Mixture Analysis

In order to determine the ability of DART-QTOF to identify synthetic cannabinoids in a mixture, three mixtures were prepared according to the conditions in Tables 7, 8, and 9 so that the concentration of each compound in the mixture was 10 µg/mL. Compounds with different molecular weights were chosen in order to demonstrate the ability to show multiple compounds in a single analysis run.

Table 7. DART-QTOF Mixture 1 Preparation

Analyte	Conc. of Standard µg/mL	Total Mixture Vol. (mL)	Vol. of Standard (µL)	Conc. of Analyte (µg/mL)
AM2201 N-(2-fluoropentyl) isomer	1000	200	2	10
JWH 210	1000	200	2	10

Table 8. DART-QTOF Mixture 2 Preparation

Analyte	Conc. of Standard µg/mL	Total Mixture Vol. (mL)	Vol. of Standard (µL)	Conc. of Analyte (µg/mL)
RCS-4 3-methoxy isomer	10000	200	2	10
CB-25	1000	200	0.2	10

Table 9. DART-QTOF Mixture 3 Preparation

Analyte	Conc. of Standard µg/mL	Total Mixture Vol. (mL)	Vol. of Standard (µL)	Conc. of Analyte (µg/mL)
AM2201 N-(2-fluoropentyl) isomer	1000	200	2	10
AM694 3-iodo isomer	1000	200	2	10
HU-210	1000	200	2	10
JWH 210	1000	200	2	10

Analysis was performed using the same method as described previously where a 1 µL spike of the mixture was deposited on the wire mesh in triplicate. Only one mixture was

analyzed at a time, meaning the sample was placed in the second, third, and forth slots with the first slot left as a blank. A full scan with no sample added was run in between mixtures to prevent any carryover. For each of the mixtures, a full total ion chromatogram scan and product ion mass spectra at the three collision energies (CE=10, 20, and 40) in the auto MS/MS mode were obtained.

2.3. Results/Discussion

2.3.1. DART-QTOF Standard Analysis

One-hundred and thirty-four out of the 162 synthetic cannabinoid standards provided DART-QTOF data. The full results including the most abundant MS/MS peaks for each compound at the three different collision energies are summarized in Table 10. As not all spectra will be discussed because of the large number of compounds analyzed and the fact that all mass spectra were obtained using the same method and parameters, page number references to the spectra for each compound are also provided in the table.

Table 10. Summary of major peaks present in MS/MS spectra obtained by DART-QTOF for synthetic cannabinoids. Molecular ions are shown in bold. Abundance rounded to whole number.

Analyte	[M], [M+H] ⁺ (amu)	Major Peaks in order of Relative Abundance (%)	CE (eV)	Spectra Page #
AM2201 2'-naphthyl isomer	359.1685, 360.1758	360.1751 (100) 361 (27) 155 (8) 155 (100) 360.1769 (93) 361 (32) 232 (26) 127 (100) 155 (97) 144 (28) 232 (17)	10 20 40	83
AM2201 N-(2-fluoropentyl) isomer	359.1685, 360.1758	360.1748 (100) 361 (29) 155 (7) 155 (100) 360.1766 (95) 361 (36) 232 (26) 127 (100) 155 (91) 232 (20) 128 (13)	10 20 40	83
AM2201 N-(3-fluoropentyl) isomer	359.1685, 360.1758	360.1769 (100) 361 (38) 155 (9) 155 (100) 360.1765 (97) 361 (33) 232 (26) 127 (100) 155 (97) 232 (17) 156 (14)	10 20 40	84
AM2201 N-(4-fluoropentyl) isomer	359.1685, 360.1758	360.1750 (100) 361 (29) 155 (7) 360.1765 (100) 155 (99) 361 (29) 232 (20) 127 (100) 155 (98) 144 (18) 156 (16)	10 20 40	85
AM694 3-iodo isomer	435.0495, 436.0568	436.0550 (100) 437 (32) 230 (14) 230 (100) 436.0570 (93) 437 (27) 231 (10) 230 (100) 202 (59) 231 (10)	10 20 40	86
AM694 4-iodo isomer	435.0495,	436.0562 (100) 437 (27) 230 (14)	10	87

	436.0568	230 (100) 436.0563 (83) 437 (24) 231 (9) 230 (100) 202 (35) 231 (8)	20 40	
JWH 018 2'-naphthyl-N-(1-ethylpropyl)	341.1780, 342.1853	342.1845 (100) 343 (31) 155 (10) 155 (100) 342.1852 (52) 343 (21) 214 (10) 127 (100) 155 (88) 144 (63) 128 (14)	10 20 40	87
JWH 018 2'-naphthyl isomer	341.1780, 342.1853	342.1844 (100) 343 (34) 155 (10) 155 (100) 342.1849 (60) 343 (23) 214 (20) 127 (100) 155 (77) 144 (19) 128 (13)	10 20 40	88
JWH 018 2'-naphthyl-N-(1,1-dimethylpropyl) isomer	341.1780, 342.1853	342.1843 (100) 343 (35) 54 (25) 272 (25) 272 (100) 155 (33) 273 (30) 144 (24) 144 (100) 155 (93) 127 (53) 156 (17)	10 20 40	89
JWH 018 2'-naphthyl-N-(1,2-dimethylpropyl) isomer	341.1780, 342.1853	342.1852 (100) 54 (60) 343 (32) 155 (11) 155 (100) 342.1841 (84) 343 (30) 156 (13) 155 (100) 127 (98) 144 (71) 156 (15)	10 20 40	89
JWH 018 2'-naphthyl-N-(1-methylbutyl) isomer	341.1780, 342.1853	342.1844 (100) 54 (66) 343 (37) 191 (17) 155 (100) 342.1843 (69) 343 (24) 156 (13) 127 (100) 155 (73) 144 (52) 128 (14)	10 20 40	90
JWH 018 2'-naphthyl-N-(2,2-dimethylpropyl) isomer	341.1780, 342.1853	342.1843 (100) 343 (34.16) 155 (8) 342.1840 (100) 155 (94) 343 (35) 214 (16) 155 (100) 127 (94) 144 (31) 156 (14)	10 20 40	91
JWH 018 2'-naphthyl-N-(2-methylbutyl) isomer	341.1780, 342.1853	342.1841 (100) 343 (36) 155 (10) 155 (100) 342.1839 (61) 343 (22) 214 (15) 127 (100) 155 (73) 144 (36) 128 (20)	10 20 40	92
JWH 018 2'-naphthyl-N-(3-methylbutyl) isomer	341.1780, 342.1853	342.1852 (100) 343 (33) 54 (16) 155 (12) 155 (100) 342.1840 (63) 343 (21) 156 (19) 127 (100) 155 (82) 144 (17) 128 (12)	10 20 40	92
JWH 018 N-(1,1-dimethylpropyl) isomer	341.1780, 342.1853	342.1848 (100) 272 (26) 108 (13) 202 (11) 272 (100) 155 (38) 273 (24) 144 (22) 155 (100) 144 (85) 127 (74) 156 (13)	10 20 40	93
JWH 018 N-(1,2-dimethylpropyl) isomer	341.1780, 342.1853	342.1847 (100) 343 (32) 155 (7) 342.1846 (100) 155 (99) 343 (33) 272 (17) 155 (100) 127 (99) 144 (52) 155 (19)	10 20 40	93
JWH 018 N-(1-ethylpropyl) isomer	341.1780, 342.1853	342.1841 (100) 343 (42) 54 (36) 55 (11) 155 (100) 342.1848 (87) 343 (32) 156 (16) 155 (100) 127 (89) 144 (48) 128 (14)	10 20 40	94
JWH 018 N-(1-methylbutyl) isomer	341.1780, 342.1853	342.1850 (100) 343 (32) 155 (10) 155 (100) 342.1850 (86) 343 (28) 156 (17) 127 (100) 155 (99) 144 (45) 128 (15)	10 20 40	95
JWH 018 N-(2,2dimethylpropyl) isomer	341.1780, 342.1853	342.1842 (100) 343 (32) 155 (6) 342.1844 (100) 343 (35) 155 (8) 155 (100) 127 (94) 144 (30) 128 (15)	10 20 40	95
JWH 018 N-(2-methylbutyl) isomer	341.1780, 342.1853	342.1844 (100) 343 (36) 155 (8) 155 (100) 342.1840 (77) 343 (33) 214 (19) 127 (100) 155 (82) 144 (20) 156 (10)	10 20 40	96
JWH 018 N-(3-methylbutyl) isomer	341.1780, 342.1853	342.1848 (100) 343 (34) 155 (9) 155 (100) 342.1843 (86) 343 (28) 214 (21) 127 (100) 155 (78) 144 (13) 128 (11)	10 20 40	97
JWH 073 2'-naphthyl isomer	327.1623, 328.1696	328.1690 (100) 329 (34) 155 (14) 328.1687 (100) 329 (34) 155 (13) 127 (100) 155 (55) 144 (20) 128 (12)	10 20 40	97
JWH 073 2'-naphthyl-N-(1,1-dimethylethyl) isomer	327.1623, 328.1696	328.1692 (100) 329 (32) 272 (20) 155 (7) 272 (100) 155 (48) 144 (30) 273 (28) 144 (100) 155 (64) 127 (50) 91 (19)	10 20 40	98
JWH 073 2'-naphthyl-N-(1-methylpropyl) isomer	327.1623, 328.1696	328.1689 (100) 329 (34) 155 (13) 155 (100) 328.1687 (49) 329 (17) 156 (15) 127 (100) 155 (63) 144 (41) 128 (12)	10 20 40	99

JWH 073 2'-naphthyl-N-(2-methylpropyl) isomer	327.1623, 328.1696	328.1700 (100) 91 (43) 149 (41) 329 (33) 155 (100) 328.1687 (48) 91 (38) 149 (22) 127 (100) 155 (57) 144 (24) 128 (13)	10 20 40	99
JWH 073 N-(1,1-dimethylethyl) isomer	327.1623, 328.1696	328.1690 (100) 329 (33) 272 (22) 155 (6) 272 (100) 155 (48) 273 (26) 144 (25) 155 (100) 127 (83) 144 (82) 156 (14)	10 20 40	100
JWH 073 N-(1-methylpropyl) isomer	327.1623, 328.1696	328.1685 (100) 329 (34) 155 (10) 155 (100) 328.1694 (61) 329 (27) 156 (17) 127 (100) 155 (75) 144 (34) 128 (17)	10 20 40	101
JWH 073 N-(2-methylpropyl) isomer*	327.1623, 328.1696	328.1695 (100) 329 (34) 155 (12) 155 (100) 328.1690 (61) 329 (20) 200 (20) 127 (100) 155 (57) 144 (19) 128 (12)	10 20 40	101
JWH 081 2-methoxynaphthyl isomer	371.1885, 372,1958	372.1952 (100) 185 (39) 373 (29) 186 (8) 185 (100) 186 (20) 372.1942 (10) 185 (100) 142 (22) 170 (13) 127 (12)	10 20 40	102
JWH 081 3-methoxynaphthyl isomer	371.1885, 372,1958	372.1952 (100) 373 (34) 185 (7) 372.1948 (100) 185 (85) 214 (43) 373 (36) 129 (100) 185 (92) 144 (65) 215 (39)	10 20 40	103
JWH 081 5-methoxynaphthyl isomer	371.1885, 372,1958	372.1950 (100) 373 (38) 185 (15) 185 (100) 372.1950 (45) 186 (15) 373 (11) 185 (100) 157 (48) 127 (39) 129 (30)	10 20 40	103
JWH 081 6-methoxynaphthyl isomer	371.1885, 372,1958	372.1951 (100) 373 (35) 214 (6) 372.1951 (100) 185 (82) 214 (74) 373 (33) 157 (100) 185 (52) 144 (45) 214 (30)	10 20 40	104
JWH 081 7-methoxynaphthyl isomer	371.1885, 372,1958	372.1951 (100) 185 (89) 373 (32) 186 (13) 185 (100) 186 (15) 170 (100) 185 (71) 171 (12) 127 (10)	10 20 40	105
JWH 081 8-methoxynaphthyl isomer	371.1885, 372,1958	372.1947 (100) 185 (93) 373 (35) 186 (14) 185 (100) 186 (14) 170 (100) 185 (70) 171 (13) 186 (11)	10 20 40	105
JWH 122 2-methylnaphthyl isomer	355.1936, 356.2009	356.1999 (100) 357 (34) 169 (9) 214 (6) 356.2000 (100) 169 (95) 214 (49) 357 (36) 141 (100) 169 (71) 144 (66) 214 (37)	10 20 40	106
JWH 122 3-methylnaphthyl isomer	355.1936, 356.2009	356.1995 (100) 357.2037 (32) 169 (7) 169 (100) 356.2005 (89) 214 (44) 357 (33) 141 (100) 169 (54) 144 (29) 214 (15)	10 20 40	107
JWH 122 5-methylnaphthyl isomer	355.1936, 356.2009	356.2001 (100) 357 (36) 169 (10) 169 (100) 356.2010 (74) 357 (40) 214 (28) 141 (100) 169 (61) 144 (16) 142 (16)	10 20 40	108
JWH 122 6-methylnaphthyl isomer	355.1936, 356.2009	356.2003 (100) 357 (35) 169 (8) 169 (100) 356.2002 (85) 214 (40) 357 (31) 141 (100) 169 (61) 144 (26) 142 (16)	10 20 40	108
JWH 122 7-methylnaphthyl isomer	355.1936, 356.2009	356.2012 (100) 357 (34) 169 (10) 169 (100) 356.1997 (77) 357 (26) 214 (23) 141 (100) 169 (76) 144 (23) 170 (15)	10 20 40	109
JWH 122 8-methylnaphthyl isomer	355.1936, 356.2009	356.2006 (100) 357 (33) 169 (7) 356.2005 (100) 169 (82) 357 (35) 214 (31) 141 (100) 169 (80) 144 (30) 214 (21)	10 20 40	109
JWH 200 2'-naphthyl isomer*	384.1838, 385.1911	385.192 (100) 386 (36) 155 (24) 155 (100) 114 (28) 385.1911 (24) 156 (16) 155 (100) 114 (71) 127 (68) 156 (13)	10 20 40	110
JWH 203 3-chlorophenyl isomer	339.1390, 340.1463	340.1463 (100) 342 (39) 341 (30) 340.1464 (100) 125 (90) 214 (77) 188 (61) 125 (100) 144 (59) 214 (37) 127 (34)	10 20 40	111
JWH 203 4-chlorophenyl isomer	339.1390, 340.1463	340.1463 (100) 342 (33) 341 (26) 340.1466 (100) 125 (97) 188 (39) 342 (35)	10 20	11

		125 (100) 127 (34) 144 (24) 214 (14)	40	
JWH 210 2-ethylnaphthyl isomer	369.2093, 370.2166	370.2156 (100) 371 (34) 370.2156 (100) 183 (58) 214 (43) 371 (35) 144 (100) 183 (83) 155 (82) 214 (67)	10 20 40	112
JWH 210 3-ethylnaphthyl isomer	369.2093, 370.2166	370.2161 (100) 371 (35) 370.2161 (100) 183 (77) 214 (42) 371 (38) 155 (100) 183 (84) 144 (51) 214 (36)	10 20 40	113
JWH 210 5-ethylnaphthyl isomer	369.2093, 370.2166	370.2154 (100) 371 (35) 183 (8) 370.2155 (100) 183 (98) 371 (39) 214 (21) 183 (100) 155 (78) 153 (33) 144 (30)	10 20 40	113
JWH 210 6-ethylnaphthyl isomer	369.2093, 370.2166	370.2161 (100) 371 (35) 183 (8) 370.2157 (100) 183 (78) 214 (35) 371 (34) 155 (100) 183 (77) 144 (25) 214 (21)	10 20 40	114
JWH 210 7-ethylnaphthyl isomer	369.2093, 370.2166	370.2159 (100) 371 (35) 183 (8) 183 (100) 370.2156 (97) 371 (33) 214 (26) 155 (100) 183 (88) 144 (26) 184 (15)	10 20 40	114
JWH 210 8-ethylnaphthyl isomer	369.2093, 370.2166	370.2156 (100) 371 (34) 183 (7) 370.2156 (100) 183 (97) 371 (36) 214 (29) 155 (100) 183 (86) 144 (36) 214 (22)	10 20 40	115
JWH 251 3-methylphenyl isomer	319.1936, 320.2009	320.2010 (100) 321 (34) 105 (8) 105 (100) 214 (92) 320.2008 (72) 188 (23) 105 (100) 144 (92) 214 (47) 145 (11)	10 20 40	116
JWH 251 4-methylphenyl isomer	319.1936, 320.2009	320.2008 (100) 321 (32) 105 (5) 320.2013 (100) 321 (31) 105 (6) 105 (100) 144 (47) 214 (26) 119 (19)	10 20 40	116
JWH 398 2-chloronaphthyl isomer	375.1390, 376.1463	376.1472 (100) 378 (36) 377 (31) 189 (8) 376.1476 (100) 378 (43) 377 (38) 189 (10) 189 (100) 161 (52) 191 (35) 163 (16)	10 20 40	117
JWH 398 3-chloronaphthyl isomer	375.1390, 376.1463	376.1462 (100) 378 (40) 377 (26) 189 (11) 189 (100) 376.1465 (92) 378 (35) 191 (34) 161 (100) 189 (95) 191 (33) 163 (29)	10 20 40	118
JWH 398 5-chloronaphthyl isomer	375.1390, 376.1463	376.1468 (100) 378 (41) 377 (35) 189 (9) 189 (100) 376.1466 (87) 378 (35) 191 (32) 189 (100) 161 (97) 191 (36) 163 (33)	10 20 40	119
JWH 398 6-chloronaphthyl isomer	375.1390, 376.1463	376.1461 (100) 378 (35) 377 (30) 189 (8) 189 (100) 376.1466 (85) 378 (35) 191 (33) 161 (100) 189 (85) 163 (34) 191 (30)	10 20 40	120
JWH 398 7-chloronaphthyl isomer	375.1390, 376.1463	376.1461 (100) 378 (34) 377 (29) 189 (10) 189 (100) 376.1472 (82) 378 (37) 191 (37) 189 (100) 161 (99) 191 (40) 163 (38)	10 20 40	121
JWH 398 8-chloronaphthyl isomer	375.1390, 376.1463	376.1455 (100) 378 (40) 377 (34) 189 (8) 189 (100) 376.142 (94) 378 (55) 191 (40) 189 (100) 161 (66) 191 (34) 163 (21)	10 20 40	121
RCS-4 2-methoxy isomer	321.1729, 322.1802	322.1798 (100) 135 (42) 323 (25) 324 (8) 135 (100) 322.1783 (11) 136 (10) 135 (100) 136 (9)	10 20 40	122
RCS-4 3-methoxy isomer	321.1729, 322.1802	322.1760 (100) 323 (28) 135 (12) 135 (100) 322.1787 (43) 323 (11) 136 (10) 107 (100) 135 (82) 144 (15) 108 (9)	10 20 40	123
RCS-8 3-methoxy isomer	375.2198, 376.2271	376.2218 (100) 377 (31) 121 (7) 376.2241 (100) 121 (99) 254 (44) 377 (37) 121 (100) 254 (59) 144 (31) 255 (13)	10 20 40	124
RCS-8 4-methoxy isomer	375.2198, 376.2271	376.2208 (100) 377 (38) 121 (5) 376.2239 (100) 121 (44) 377 (31) 149 (15) 121 (100) 135 (38) 122 (12) 254 (10)	10 20 40	124

(+)WIN 55212-2*	426.1943, 427.2016	<i>No data obtained</i> 155 (100) 427.2017 (28) 428 (18) 156 (16) 155 (100) 127 (28) 100 (17) 156 (9)	10 20 40	125
1'-Naphthoyl Indole	271.0997, 272.1070	272.1021 (100) 273 (26) 155 (19) 144 (12) 155 (100) 144 (42) 272.1051 (14) 156 (10) 127 (100) 144 (33) 116 (20) 155 (14)	10 20 40	126
4-Quinolone-3-Carboxamide CB2 Ligand	422.2569, 423.2642	423.2606 (100) 424 (29) 135 (9) 135 (100) 423.2592 (97) 424 (37) 272 (26) 135 (100) 136 (13) 107 (11) 93 (10)	10 20 40	126
AKB48	365.2467, 366.2540	366.2545 (100) 135 (78) 367 (35) 136 (13) 135 (00) 136 (12) 366.2517 (5) 135 (100) 93 (31) 79 (21) 136 (12)	10 20 40	127
AM1220	382.2045, 383.2118	<i>No data obtained</i> 112 (100) 98 (78) 383.2111 (58) 286 (39) 98 (100) 112 (48) 155 (26) 127 (16)	10 20 40	128
AM1235	404.1536, 405.1609	405.1601 (100) 406 (33) 223 (12) 297 (10) 405.1600 (100) 277 (41) 155 (36) 406 (34) 155 (100) 127 (75) 189 (44) 207 (44)	10 20 40	128
AM1241	503.0706, 504.0779	504.0769 (100) 505 (29) 98 (20) 98 (100) 504.0779 (58) 505 (17) 112 (11) 98 (100) 112 (10) 99 (6)	10 20 40	129
AM1248	390.2671, 391.2744	391.2739 (100) 392 (36) 393 (6) 391.2739 (100) 392 (36) 135 (32) 112 (10) 135 (100) 112 (41) 98 (34) 136 (14)	10 20 40	129
AM2201	359.1685, 360.1758	360.1761 (100) 361 (35) 155 (8) 360.1765 (100) 155 (99) 361 (30) 232 (22) 127 (100) 155 (93) 144 (19) 156 (12)	10 20 40	130
AM2232	352.1576, 353.1649	353.1643 (100) 354 (32) 155 (22) 155 (100) 353.1623 (35) 225 (17) 156 (14) 127 (100) 155 (75) 144 (17) 225 (13)	10 20 40	131
AM2233	458.0855, 459.0928	459.0870 (100) 445 (53) 460 (27) 429 (24) 98 (100) 112 (76) 459.0885 (76) 341 (29) 98 (100) 112 (29) 230 (24) 99 (8)	10 20 40	131
AM251	554.0137, 555.0210	<i>No data obtained</i> 555.0162 (100) 454 (62) 557 (44) 456 (28) 454 (100) 456 (65) 455 (18) 84 (10)	10 20 40	132
AM630	504.0910, 505.0983	505.0884 (100) 135 (59) 506 (27) 135 (100) 505.0916 (18) 136 (10) 114 (8) 135 (100) 114 (20) 136 (8)	10 20 40	132
AM679	417.0590, 418.0663	418.0648 (100) 419 (28) 230 (8) 230 (100) 418.0653 (82) 419 (25) 291 (15) 230 (100) 202 (50) 231 (8) 234 (7)	10 20 40	133
AM694	435.0495, 436.0568	436.0571 (100) 437 (26) 230 (10) 436.0571 (100) 230 (84) 437 (30) 309 (15) 230 (100) 202 (55) 231 (12) 234 (9)	10 20 40	133
CB-13	368.1776, 369.1849	369.1854 (100) 370 (34) 299 (7) 155 (7) 155 (100) 171 (75) 299 (64) 369.1848 (55) 171 (100) 155 (84) 127 (61) 143 (23)	10 20 40	134
CB-25	403.3086, 404.3159	404.3148 (100) 405 (27) 58 (13) 347 (8) 58 (100) 347 (32) 181 (18) 83 (16) 58 (100) 207 (20) 111 (18) 281 (10)	10 20 40	134
CB-52	417.3243, 418.3316	418.3342 (100) 58 (48) 419 (32) 361 (9) <i>No data obtained</i> <i>No data obtained</i>	10 20 40	135
CB-86	417.3243, 418.3316	<i>No data obtained</i> 58 (100) 418.3348 (31) 292 (18) 419 (18)	10 20	135

		58 (100) 71 (12) 57 (10) 85 (8)	40	
HU-210	386.2821, 387.2894	387.2902 (100) 388 (37) 355 (12) 371 (12) <i>No data obtained</i> <i>No data obtained</i>	10 20 40	136
JWH 011	383.2249, 384.2322	384.2317 (100) 385 (30) 155 (5) 384.2324 (100) 155 (76) 385 (33) 156 (9) 155 (100) 127 (54) 158 (24) 156 (10)	10 20 40	136
JWH 015	327.1623, 328.1696	328.1694 (100) 329 (31) 155 (12) 155 (100) 328.1696 (44) 329 (15) 200 (15) 127 (100) 155 (56) 128 (13) 158 (11)	10 20 40	137
JWH 016	341.1780, 342.1853	342.1846 (100) 343 (34) 54 (19) 155 (10) 155 (100) 342.1851 (58) 343 (18) 214 (14) 127 (100) 155 (69) 128 (16) 158 (7)	10 20 40	138
JWH 018	341.1780, 342.1853	342.1854 (100) 343 (30) 155 (8) 155 (100) 342.1868 (70) 343 (21) 214 (19) 127 (100) 155 (56) 144 (15) 128 (11)	10 20 40	139
JWH 018 6-methoxyindole analog	371.1885, 372.1958	372.1958 (100) 373 (33) 155 (10) 155 (100) 372.1959 (53) 373 (19) 156 (13) 127 (100) 155 (99) 156 (13) 128 (13)	10 20 40	140
JWH 018 adamantyl analog	349.2406, 350.2479	350.2478 (100) 351 (34) 135 (5) 350.2480 (100) 135 (37) 351 (36) 135 (100) 107 (15) 79 (14) 136 (13)	10 20 40	141
JWH 018 adamantyl carboxamide	364.2515, 365.2588	365.2581 (100) 366 (35) 367 (4) 365.2584 (100) 135 (64) 366 (32) 214 (15) 135 (100) 93 (16) 107 (15) 136 (12)	10 20 40	141
JWH 018 N-(4,5- epoxypentyl) analog	355.1572, 356.1645	356.1645 (100) 357 (35) 155 (28) 155 (100) 356.1647 (17) 156 (14) 127 (100) 155 (78) 128 (13) 156 (12)	10 20 40	141
JWH 018 N-(5- chloropentyl) analog	375.1390, 376.1463	376.1470 (100) 378 (35) 377 (28) 155 (7) 155 (100) 376.1467 (98) 378 (38) 377 (21) 155 (100) 127 (92) 144 (14) 156 (12)	10 20 40	142
JWH 019	355.1936, 356.2009	356.2004 (100) 357 (34) 155 (6) 356.2019 (100) 155 (92) 357 (35) 228 (22) 127 (100) 155 (89) 144 (17) 128 (11)	10 20 40	142
JWH 020	369.2093, 370.2166	370.2166 (100) 371 (38) 155 (6) 370.2166 (100) 155 (81) 371 (35) 242 (17) 155 (100) 127 (87) 156 (13) 144 (13)	10 20 40	143
JWH 022	339.1623, 340.1696	340.1680 (100) 341 (35) 155 (7) 155 (100) 340.1694 (59) 212 (26) 341 (22) 127 (100) 155 (58) 128 (10) 144 (8)	10 20 40	144
JWH 030	291.1623, 292.1696	292.1686 (100) 155 (79) 293 (30) 156 (10) 155 (100) 156 (15) 127 (9) 127 (100) 155 (24) 128 (12)	10 20 40	144
JWH 072	313.1467, 314.1540	341.1539 (100) 315 (32) 155 (13) 155 (100) 314.1533 (39) 186 (24) 156 (14) 127 (100) 155 (38) 144 (13) 128 (13)	10 20 40	145
JWH 073	327.1623, 328.1696	328.1694 (100) 329 (26) 155 (9) 155 (100) 328.1687 (61) 200 (25) 329 (23) 127 (100) 155 (57) 144 (16) 128 (13)	10 20 40	146
JWH 073 2-methylnaphthyl analog	341.1780, 342.1853	342.1853 (100) 343 (28) 169 (7) 169 (100) 342.1851 (94) 200 (58) 343 (30) 141 (100) 144 (51) 169 (47) 200 (22)	10 20 40	146
JWH 073 4-methylnaphthyl analog	341.1780, 342.1853	342.1853 (100) 343 (30) 169 (5) 169 (100) 342.1848 (71) 200 (33) 343 (22) 141 (100) 169 (73) 144 (30) 142 (10)	10 20 40	147

JWH 081	371.1885, 372.1958	372.1959 (100) 373 (34) 185 (8) 185 (100) 372.1957 (88) 214 (32) 373 (29) 185 (100) 157 (43) 144 (25) 214 (17)	10 20 40	148
JWH 098	385.2042, 386.2115	386.2110 (100) 387 (39) 185 (10) 387 (6) 185 (100) 386.2109 (70) 228 (28) 387 (25) 185 (100) 157 (37) 158 (22) 186 (14)	10 20 40	148
JWH 122	355.1936, 356.2009	356.2011 (100) 357 (34) 169 (9) 169 (100) 356.2004 (88) 357 (32) 214 (30) 141 (100) 169 (96) 144 (26) 214 (16)	10 20 40	149
JWH 122 N-(4-pentenyl) Analog	353.1780, 354.1853	354.1855 (100) 355 (36) 169 (11) 169 (100) 354.1856 (80) 212 (31) 355 (26) 141 (100) 169 (80) 144 (16) 170 (15)	10 20 40	150
JWH 145	367.1936, 368.2009	368.2016 (100) 155 (55) 369 (39) 156 (6) 155 (100) 156 (17) 127 (5) 155 (100) 127 (97) 156 (16) 128 (13)	10 20 40	150
JWH 147	381.2093, 382.2166	382.2169 (100) 155 (48) 383 (37) 156 (7) 155 (100) 156 (15) 382.2178 (5) 127 (5) 155 (100) 127 (97) 156 (19) 128 (11)	10 20 40	151
JWH 175	327.1987, 328.2060	328.2131 (100) 141 (66) 149 (45) 91 (42) 141 (100) 142 (22) 91 (16) 149 (12) 141 (100) 114 (40) 58 (27) 59 (25)	10 20 40	152
JWH 180	355.1936, 356.2009	356.2017 (100) 357 (35) 197 (8) 197 (100) 356.2015 (94) 186 (42) 357 (34) 197 (100) 141 (99) 186 (54) 144 (47)	10 20 40	152
JWH 182	383.2249, 384.2322	384.2327 (100) 385 (36) 386 (5) 384.2319 (100) 197 (68) 385 (37) 214 (26) 197 (100) 141 (58) 214 (34) 144 (28)	10 20 40	153
JWH 200	384.1838, 385.1911	385.1839 (100) 386 (39) 155 (27) 114 (17) 155 (100) 114 (42) 385.1872 (25) 156 (14) 114 (100) 155 (90) 127 (51) 156 (8)	10 20 40	154
JWH 201	335.1885, 336.1958	336.1918 (100) 337 (33) 121 (9) 121 (100) 336.1939 (66) 149 (39) 135 (31) 121 (100) 135 (33) 144 (13) 122 (110)	10 20 40	154
JWH 203	339.1390, 340.1463	340.1435 (100) 342 (44) 341 (35) 125 (9) 125 (100) 340.1445 (96) 342 (37) 127 (34) 125 (100) 127 (28) 144 (12) 214 (8)	10 20 40	155
JWH 210	369.2093, 370.2166	370.2172 (100) 371 (35) 183 (6) 370.2171 (100) 183 (78) 371 (37) 214 (26) 183 (100) 155 (57) 144 (30) 214 (24)	10 20 40	156
JWH 249	383.0885, 384.0958	386 (100) 384.0946 (88) 385 (25) 387 (14) 386 (100) 384.0938 (90) 168 (82) 170 (64) 168 (100) 170 (94) 214 (28) 144 (26)	10 20 40	157
JWH 250	335.1885, 336.1958	336.1958 (100) 337 (33) 121 (32) 121 (100) 336.1950 (12) 122 (11) 121 (100) 130 (27) 144 (23) 131 (12)	10 20 40	157
JWH 251	319.1936, 320.2009	320.2009 (100) 321 (34) 105 (7) 105 (100) 320.2002 (88) 214 (74) 321 (28) 105 (100) 144 (59) 214 (34) 106 (10)	10 20 40	158
JWH 302	335.1885, 336.1958	336.1957 (100) 337 (32) 121 (15) 188 (80) 121 (100) 214 (33) 336.1957 (26) 158 (20) 121 (100) 144 (93) 214 (55) 145 (12)	10 20 40	159
JWH 307	385.1842, 386.1915	386.1909 (100) 155 (53) 387 (35) 371 (13) 155 (100) 156 (14) 386.1920 (6) 155 (100) 127 (88) 156 (16) 128 (12)	10 20 40	159
JWH 309	417.2093, 418.2166	418.2168 (100) 155 (44) 419 (78) 420 (9) 155 (100) 156 (16) 418.2158 (9) 419 (6)	10 20	160

		155 (100) 127 (77) 156 (22)	40	
JWH 368	385.1842, 386.1915	386.1908 (100) 387 (31) 388 (5) 155 (100) 156 (16) 386.1889 (8) 155 (100) 127 (90) 156 (16) 128 (12)	10 20 40	161
JWH 369	401.1546, 402.1619	402.1620 (100) 155 (54) 404 (43) 403 (35) 155 (100) 156 (13) 402.1615 (7) 155 (100) 127 (80) 156 (13) 128 (9)	10 20 40	161
JWH 370	381.2093, 382.2166	382.2163 (100) 155 (53) 383 (42) 156 (8) 155 (100) 156 (23) 382.2229 (13) 127 (9) 155 (100) 127 (90) 156 (12) 128 (7)	10 20 40	162
JWH 398	375.1390, 376.1463	376.1494 (100) 378 (42) 377 (35) 189 (10) 189 (100) 376.1462 (99) 378 (60) 190 (41) 189 (100) 161 (67) 191 (33) 163 (18)	10 20 40	163
JWH 424	419.0885, 420.0958	422 (100) 420.0960 (82) 421 (28) 423 (16) 422 (100) 420.0960 (81) 234 (51) 232 (50) <i>No data obtained</i>	10 20 40	163
JZL 195	433.1638, 434.1711	434.1738 (100) 435 (47) 183 (35) <i>No data obtained</i> 183 (100) 155 (29) 148 (23) 184 (23)	10 20 40	164
MAM2201	373.1842, 374.1915	374.1922 (100) 375 (40) 376 (7) 169 (6) 374.1912 (100) 169 (74) 375 (34) 232 (24) 169 (100) 141 (64) 144 (20) 232 (19)	10 20 40	164
MDA 19	349.1790, 350.1863	350.1865 (100) 105 (73) 351 (30) 106 (5) 105 (100) 106 (7) 77 (4) 105 (100) 77 (56) 106 (8)	10 20 40	165
MDA 77	365.1739, 366.1812	<i>No data obtained</i> 105 (100) 77 (42) 366.1745 (18) 106 (8) 77 (100) 105 (32) 51 (14) 78 (7)	10 20 40	166
Cannabipiperidiethanone	376.2151, 377.2224	<i>No data obtained</i> 112 (100) 98 (60) 121 (41) 377.2255 (34) 98 (100) 91 (70) 70 (46) 58 (37)	10 20 40	166
RCS-4	321.1729, 322.1802	322.1826 (100) 323 (35) 135 (10) 135 (100) 322.1828 (27) 136 (9) 323 (8) 135 (100) 107 (25) 136 (10)	10 20 40	167
RCS-4-C4 Homolog	307.1572, 308.1645	135 (100) 77 (29) 308.1655 (24) 107 (21) 135 (100) 77 (52) 92 (18) 107 (16) 77 (100) 135 (52) 92 (46) 51 (32)	10 20 40	167
RCS-8	375.2198, 376.2271	376.2286 (100) 377 (44) 121 (19) 121 (100) 376.2299 (99) 377 (15) 122 (9) 121 (100) 144 (28) 122 (6) 254 (6)	10 20 40	168
STS-135*	383.2624, 384.2697	135 (100) 383 (47) 93 (21) 79 (18) 135 (100) 93 (31) 79 (30) 383 (22) 135 (100) 93 (70) 79 (62) 107 (24)	10 20 40	169
UR-144	311.2249, 312.2322	312.2289 (100) 215 (65) 89 (31) 55 (100) 125 (63) 57 (50) 144 (44) 55 (100) 116 (96) 130 (76) 144 (59)	10 20 40	169
URB754	266.1055, 267.1128	160 (100) 104 (67) 77 (42) 106 (28) 77 (100) 160 (98) 104 (73) 106 (27) 77 (100) 104 (30) 51 (16) 78 (15)	10 20 40	170
XLR11	329.2155, 330.2228	330.2180 (100) 332 (30) 125 (4) <i>No data obtained</i> <i>No data obtained</i>	10 20 40	170

Of the 162 cannabinoid standards that were analyzed, 142 of these compounds were present in the existing ESI-generated library. Data were obtained for a total of 134 compounds, including four synthetic cannabinoids which were not present in the existing library (marked with an asterisk in Table 10). Twelve compounds that were present in the comparison library did not provide data, meaning there were a total of 130 compounds where data were obtained and the compound was in the ESI-generated library. The twelve compounds that produced inconsistent data and heavy fragmentation when analyzed by DART-QTOF were (-)-11-nor-9-carboxy- Δ^9 -THC, Cannabidiol, HU-211, HU-308, HU-331, JP104, JWH 018 N-(5-bromopentyl), JWH 031, URB447, UR597, URB602, and WIN 54461. Because these twelve compounds belong to different classifications and vary greatly in structure, it is difficult to determine a specific reason why no data was obtained. It is important to note that compounds related to these twelve did produce data. Further study or modification of the analysis parameters may be required in order to better analyze these compounds. Library searching resulted in library matches at one or more collision energy for 118 out of 130 compounds.

The results of the library search against the ESI-generated library produced two results. More commonly, the search resulted in a match to a single compound as in the case of JWH 145 (Figure 14).

Figure 14. DART-QTOF data for JWH 145. a) Product ion mass spectra for JWH 145 at CE=20 eV using DART ionization b) Product ion mass spectra for JWH 145 at CE=20 using ESI ionization c) ESI-generated library search using MassHunter software.

In this example for JWH 145, the two most abundant peaks are the 368 molecular ion peak and the 155 peak corresponding to the combination of the naphthalene and carbonyl groups. In the identification of this compound, the 155 peak is not extremely helpful as the naphthalene and carbonyl bridge are a common component of many synthetic cannabinoids. However, because JWH 145 is the only compound analyzed with a molecular ion of 368, this provides additional details of the compound which when compared with the fragmentation pattern allow for the compound to be identified.

As a result of the analysis of different isomers, it is also important to note the second observed result where there was not a match to a single compound. In this case, it was also not uncommon for the first library match to not always be the analyzed compound as in the case for JWH 073 2'-naphthyl isomer (Figure 15).

Figure 15. DART-QTOF data for JWH 073 2'-naphthyl isomer. a) Product ion mass spectra for JWH 073 2'-naphthyl at CE=20 eV using DART ionization b) Product ion mass spectra for JWH 073 2'-naphthyl at CE=20 using ESI ionization c) ESI-generated library search using MassHunter software.

In Figure 15, it can be seen that the JWH 073 2'-naphthyl is the third library match behind JWH 073 and JWH 015, two closely related compounds (Figure 16). JWH 073 2'-naphthyl is a positional isomer of JWH 073 and JWH adds only a methyl group to these compounds, but the fragmentation pattern at this collision energy is similar enough for a library match to be noted. In the case of these three compounds, the fragmentation pattern observed only shows a molecular ion peak of 328 and a peak with a m/z of 155. As mentioned, the 155 peak corresponds to the combination of the naphthalene and carbonyl bridge groups, a commonality among these three compounds. In this case,

because the molecular weights of these compounds are the same and there is limited and similar fragmentation of the molecules, discrimination by DART-QTOF is not possible.

Figure 16. Chemical structures of a) JWH 073 b) JWH 015 c) JWH 073 2'-naphthyl isomer.

Out of the 130 synthetic cannabinoid standards that spectral data were obtained for, 77 of these compounds were isomers. In the majority of these cases, analysis was not able to distinguish among isomers which was to be expected since differentiation was determined only by fragmentation. Because these compounds are positional isomers, meaning same molecular weight with only minor substituent changes, this similar fragmentation is not unexpected. However, 27 out of the 77 isomers were able to be differentiated using the high-mass accuracy spectra obtained using DART-QTOF, as was the case with the JWH 018 N-(1,1-dimethylpropyl) and JWH 018 N-(1,2-dimethylpropyl) isomers (Figure 17).

Figure 17. Product ion mass spectra of JWH 018 isomers. N-(1,1-dimethylpropyl) (top) and N-(1,2-dimethylpropyl) (bottom) at CE=20 eV.

As can be seen in Figure 17, the JWH 018 N-(1,1-dimethylpropyl) and JWH 018 N-(1,2-dimethylpropyl) isomers can be clearly distinguished on the basis of the relative intensities of the major peaks (342, 272, and 155) as well as the presence of the 214 peak for the 1,2-dimethylpropyl isomer. The 1,1-isomer shows a significantly higher 272 fragment whereas the 1,2-isomer shows a significantly higher 342 and 155 peak. The fragment with the m/z of 272 corresponds to the molecule after loss of the alkyl chain. The difference in intensity for this peak between the two isomers can be explained by the relative stability of the lost alkyl chain. In the case of the 1,1-isomer, when the bond between the alkyl chain and the nitrogen in the indole ring breaks, the resulting fragment is 2-methylbutane with a charge at the tertiary position. With the 1,2-isomer, the alkyl chain fragment is 3-methylbutane and the charge is at the secondary position. When these two compounds fragment, it is more favorable to have a tertiary charge, meaning that two relatively stable fragments are produced for the 1,1-isomer than compared with only the molecular ion for the 1,2-isomer. Although the peak with the m/z of 155 is common between both isomers, it can be determined that the 1,1-isomer with the loss of

the alkyl chain is the most stable fragment whereas for the 1,2-isomer, the 155 and 368 molecular ion peaks are similar in stability. Because of these differences in stability of the resultant fragments, when searched against the ESI-generated library, the other isomer does not show up as a match.

2.3.2. DART-QTOF Mixture Analysis

Full total ion chromatogram and product ion mass spectra were obtained for each of the three mixtures. The first mixture contained two compounds: AM2201 N-(2-fluoropentyl) and JWH 210. The second mixture contained two compounds: RSC-4 3-methoxy isomer and CB-25. The third mixture contained four compounds: AM2201 N-(2-fluoropentyl) isomer, AM694 3-iodo isomer, HU-210, and JWH 210.

The full total ion chromatogram and product ion mass spectra at two of the three collision energies (CE=10, 20 eV) can be seen in Figure 18. In all three spectra, it is clear that two compounds are present, one with a molecular weight of 360 and the other with a molecular weight of 370, although it can be seen that the mass accuracy is off. A collision energy of 40 eV did not produce any data.

Figure 18. Full total ion chromatogram and product ion mass spectra for DART-QTOF Mixture 1.

On the basis of the analysis of each of the standards individually, at collision energies of 10 and 20 eV, for AM2201 N-(2-fluoropentyl) isomer and JWH 210, it is expected to see the following fragmentation (Table 11).

Table 11. Fragmentation of AM2201 N-(2-fluoropentyl) and JWH 210 at CE=10, 20 eV.

AM2201 N-(2-fluoropentyl) isomer	359.1685,	360.1748 (100) 361 (29) 155 (7)	10
	360.1758	155 (100) 360.1766 (95) 361 (36) 232 (26)	20
JWH 210	369.2093,	370.2172 (100) 371 (35) 183 (6)	10
	370.2166	370.2171 (100) 183 (78) 371 (37) 214 (26)	20

However, the product ion mass spectra by themselves do not show anything other than the molecular ions as a result of their high abundance when compared with the rest of the peaks in the spectra. When looking at the mixture using the MS/MS product ion spectra for each collision energy, these fragmentation patterns are seen which allow for identification (Figure 19 and 20).

Figure 19. Product ion mass spectra obtained in auto MS/MS mode for DART-QTOF Mixture 1 at CE=10 eV. a) AM2201 N-(2-fluoropentyl) isomer b) JWH 210.

Figure 20. Product ion mass spectra obtained in auto MS/MS mode for DART-QTOF Mixture 1 at CE=20 eV. a) AM2201 N-(2-fluoropentyl) isomer b) JWH 210.

By utilizing the product ion mass spectra collected in the auto MS/MS mode for this mixture, the two compounds are separated from each other, showing the expected

fragmentation peaks shown in Table 11. Because the two compounds have different structures corresponding to the respective fragmentation patterns and molecular ion present, these two compounds are easily distinguished. Although the mass accuracy was off, when a library search was run for each of the product ion spectra at a collision energy of 10 eV (Figure 20a and b), the search came up with the correct compound match, confirming that the two compounds are AM2201 N-(2-fluoropentyl) isomer and JWH 210.

The same process was used with the second mixture and the full total ion chromatogram and product ion mass spectra are shown (Figure 21).

Figure 21. Full total ion chromatogram and product ion mass spectra at CE=10 eV for DART-QTOF Mixture 2.

As with the first mixture, both spectra show the presence of two compounds, although it can be seen that the intensity of the 322 peak is much greater than that of the 404. In the full total ion chromatogram, the 404 peak almost looks like it is part of the background,

however, when using a collision energy of 10 eV, it can be clearly seen that it represents a compound. The spectra collected at collision energies of 20 and 40 eV did not provide any data as the increased fragmentation of the compounds did not allow for discrimination. As with the first mixture, these spectra on their own do not produce the fragmentation pattern expected after analysis of the individual standards (Table 12), but inclusion of the auto MS/MS data allows for this (Figure 22).

Table 12. Fragmentation of RCS-4 3-methoxy and CB-25 at CE=10, 20 eV.

RCS-4 3-methoxy isomer	321.1729, 322.1802	322.1798 (100) 135 (42) 323 (25) 324 (8)
CB-25	403.3086, 404.3159	404.3148 (100) 405 (27) 58 (13) 347 (8)

Figure 22. Product ion mass spectra obtained in auto MS/MS mode for DART-QTOF Mixture 2 at CE=10 eV. a) RCS-4 3-methoxy isomer b) CB-25.

Looking at the product ion mass spectra collected at a collision energy of 10 eV, it is much more evident that the 404 peak corresponds to a compound, as the relative intensity is 100%. Although the abundances of the peaks do not match up with what is shown for

the individual standard, the peaks for RCS-4 3-methoxy isomer match up well. The spectra for CB-25 show much more deviation from the fragments but the presence of the 404 and 347 peaks aid in identification and clearly show that a second compound is present in the mixture.

The final mixture prepared was a combination solution of four synthetic cannabinoids. Once it was shown that DART-QTOF was able to differentiate between compounds in a mixture, it was important to show that a more complex mixture, similar to what is present in the analysis of an herbal incense mixture, could be analyzed. The analysis of a mixture of four different compounds was complicated by the fact that only the full total ion chromatogram provided any useable data as fragmentation of the four compounds was not able to be discriminated (Figure 23).

Figure 23. Full total ion chromatogram for DART-QTOF Mixture 3.

No fragment patterns were observed in the chromatogram as no collision energy was used. However, although there is no fragmentation, it is still clearly observed that there are four compounds present in the mixture with the molecular ion peaks of 360, 370, 387, and 436 corresponding to the synthetic cannabinoids AM2201 N-(2-fluoropentyl) isomer, AM694 3-iodo isomer, HU-210, and JWH 210 respectively.

2.4. Conclusions

On the basis of the results shown here, DART-QTOF is shown to be a viable analytical technique for the identification of synthetic cannabinoids. The DART-QTOF spectra can be generated with approximately 1.5 ng or less of spiked standards and analyzed in triplicate in less than 2 minutes. DART-QTOF spectra can also be successfully searched against previously generated ESI-QTOF libraries in most cases as demonstrated by library matches for 118 out of 130 compounds. Direct analysis in real time quadrupole time-of-flight mass spectrometry is able to distinguish between a limited number of isomers, 27 out of 77 without the requirement of any separation technique. Direct analysis in real time quadrupole time-of-flight mass spectrometry has also been shown to be able to differentiate between analytes in a mixture for two to four compounds, mimicking what is often seen in marketed herbal incense products. This is limited by the fact that compounds with similar molecular weight may not be able to be distinguished in mixtures because of the appearance of only the molecular ion when only the full total ion chromatogram is available. Isomers may also limit the identification in mixture as they have the same molecular weight and as shown, result in the appearance of the same molecular ion and similar fragmentation. The addition of a separation technique in addition with the mass spectrometry may provide better identification of synthetic cannabinoids and in particular, the many isomers that are present.

3. SYTHETIC CANNABINOIDS BY GC-QTOF

3.1. Materials

Reference standards of 160 synthetic cannabinoids, cannabidiol, and (-)-11-nor-9-carboxy- Δ^9 -THC were provided by Cayman Chemicals (Ann Arbor, MI) (Table 3). Methanol (Optima®, LC/MS grade) was purchased from Fisher Scientific (Fair Lawn, NJ). The standard stock solutions of each synthetic cannabinoid in powder form were prepared with methanol at varying concentrations depending on the amount of standards, 1000, 2000, 5000, 10000 $\mu\text{g/mL}$. These stock solutions were diluted to 10 $\mu\text{g/mL}$ in methanol in order to prepare samples for analysis.

3.2 Methodology

3.2.1. Agilent Parameters

Qualitative analysis of the 162 cannabinoid standards was performed on an Agilent 7200 GC/Q-TOF (Santa Clara, CA) with a DB5-MS column (30m $\times$ 0.25mm (i.d.), 0.2525 μ film thickness) and Ultra Inert (split) liner; P/N 5191-2295. The retention time was locked with SKF-525A (Proadifen) to provide consistent retention time for analytes over time. Full scan mode GC parameters were as follows, the injection volume of 5 μL , split ratio of 1:10, inlet temperature of 280°C, transfer line temperature of 300°C, helium as a carrier gas at a constant flow rate of 1.2 mL/min. Oven temperature was programmed with an initial temperature of 60°C (1 min) and increased 10°C/min to 325°C, held after the RT of WIN 55-212-2 (RT 33.850). Quadrupole time-of-flight mass spectrometer parameters for the full scan mode were as follows, a threshold value of 0, a gain factor of 1, source temperature at 300°C, solvent delay of approximately 5

minutes, ionization energy of 70 eV, and an acquisition range of 45-570 m/z. Analysis time per each sample was approximately 35.5 minutes.

3.2.2. GC-QTOF Standard Analysis

Using the previously listed parameters provided by Agilent for analysis of the 162 synthetic cannabinoid standards, 5 μL of the each of the 10 $\mu\text{g/mL}$ diluted standards were individually injected into the GC-QTOF. All compounds were analyzed in electron ionization (EI) mode in triplicate and methanol blanks were run after every two sets of analytes to ensure no carryover or contamination of the column. Data analysis was performed using the Agilent MassHunter Workstation software. The peak corresponding to the compound was integrated and then the peak spectrum was extracted, subtracting out the background by averaging the background before and after the peak.

3.2.3. GC-QTOF Mixture Analysis

After analysis of all 162 synthetic cannabinoid standards, three mixtures were prepared from the standard stock solutions according to Tables 13, 14, and 15. Mixture components were chosen because of the presence of a molecular ion in EI mode and retention times that differed to prevent coelution. All analysis and data processing was performed in accordance with the previously described parameters.

Table 13. Preparation for GC-QTOF Mixture 1. 1000 μL total volume with 10 $\mu\text{g/mL}$ concentration for each analyte in the mixture.

Analyte	RT	Conc. of Standard ($\mu\text{g/mL}$)	Vol. of Standard (μL)
Cannabidiol	21.343	1000	10
CP 47,497-para-quinone analog	22.489	1000	10
($\pm$)3epi CP 47,497-C8-homolog	23.536	1000	10
JWH 175	25.002	10000	1
($\pm$)5-epi CP 55,940	25.532	10000	1

JWH 072	26.008	1000	10
JWH 018 adamantyl analog	26.651	1000	10
JWH 210 2-ethylnaphthyl isomer	27.009	1000	10
JWH 122 8-methylnaphthyl isomer	27.315	10000	1
JWH 018 2'-naphthyl-N-(2-methylbutyl) isomer	27.653	10000	1
JWH 018 2'-naphthyl isomer	28.014	10000	1
JWH 020	28.362	1000	10
CB-52	28.899	1000	10
JWH 098	29.247	1000	10
JWH 309	34.238	1000	10

Table 14. Preparation for GC-QTOF Mixture 2. 1000 μ L total volume with 10 μ g/mL concentration for each analyte in the mixture.

Analyte	RT	Conc. of Standard (μ g/mL)	Vol. of Standard (μ L)
($\pm$)-epi CP 47, 497	22.778	1000	10
($\pm$)-CP 47,497-C8-homolog	23.644	1000	10
RCS-4-C4 Homolog	25.197	1000	10
(+)-CP 55,940	25.666	1000	10
JWH 073 N-(1,1-dimethylethyl) isomer	26.020	10000	1
JWH 018 N-(1,1-dimethylpropyl) isomer	26.470	10000	1
JWH 018 2'-naphthyl-N-(1,2-dimethylpropyl)	27.107	10000	1
AM2201 N-(4-fluoropentyl) isomer	27.426	1000	10
JWH 018 2'-naphthyl-N-(3-methylbutyl) isomer	27.727	10000	1
JWH 398 3-chloronaphthyl isomer	28.026	1000	10
JWH 210	28.367	1000	10
AM2201 2'-naphthyl isomer	28.849	1000	10

Table 15. Preparation for GC-QTOF Mixture 3. 1000 μ L total volume with 10 μ g/mL concentration for each analyte in the mixture.

Analyte	RT	Conc. of Standard (μ g/mL)	Vol. of Standard (μ L)
($\pm$)CP 47,497	22.896	1000	10
URB754	23.789	1000	10
RCS-4 3-methoxy isomer	25.368	10000	1
AM679	25.801	1000	10
JWH 015	26.206	1000	10
JWH 018 N-(2-methylbutyl) isomer	26.776	10000	1
AM2201 N-(2-fluoropentyl) isomer	27.114	1000	10
JWH 073 4-methylnaphthyl analog	27.417	1000	10

JWH 180	27.803	1000	10
MDA 77	28.305	1000	10
JWH 182	28.943	1000	10

3.3. Results/Discussion

3.3.1. GC-QTOF Standard Analysis

One-hundred and fifty-nine out of the 162 synthetic cannabinoid standards provided GC-QTOF data. The full results including the most abundant MS peaks for each compound can be seen in Table 16. As not all will be discussed, page number references to the chromatograms and spectra for each compound are also provided in the table.

Table 16. Summary of major peaks present in EI full scan MS spectra obtained by GC-QTOF. Molecular ions are shown in bold. Compounds that do not show molecular ion marked with asterisk.

Analyte	[M] (amu)	Major Peaks in order of Relative Abundance (%)	RT (min)	Spectra Page #
AM2201 2'-naphthyl isomer	359.1685	359.1685 (100), 284 (91.67), 232 (89.89), 127 (62.17)	28.849	171
AM2201 N-(2-fluoropentyl) isomer	359.1685	359.1683 (100), 284 (90.94), 127 (87.41), 232 (83.11)	27.114	171
AM2201 N-(3-fluoropentyl) isomer	359.1685	284 (100), 359.1686 (93.49), 127 (72.45), 342 (70.25)	27.299	171
AM2201 N-(4-fluoropentyl) isomer	359.1685	284 (100), 359.1682 (71.32), 127 (63.85), 232 (46.48)	27.426	172
AM694 3-iodo isomer	435.0495	435.0504 (100), 232 (94.68), 359 (67.56), 144 (37.64)	27.300	172
AM694 4-iodo isomer	435.0495	435.0499 (100), 359 (78.64), 232 (64.40), 230 (41.53)	27.591	172
JWH 018 2'-naphthyl-N-(1-ethylpropyl)	341.1780	312 (100), 155 (56.88), 341.1776 (56.30), 127 (33.42)	26.978	173
JWH 018 2'-naphthyl isomer	341.1780	341.1781 (100), 214 (78.33), 284 (59.62), 127 (46.39)	28.014	173
JWH 018 2'-naphthyl-N-(1,1-dimethylpropyl) isomer	341.1780	271 (100), 144 (94.92), 341.1778 (56.93), 270 (55.10)	27.432	173
JWH 018 2'-naphthyl-N-(1,2-dimethylpropyl) isomer	341.1780	298 (100), 155 (29.59), 341.1775 (28.37), 127 (27.35)	27.107	174
JWH 018 2'-naphthyl-N-(1-methylbutyl) isomer	341.1780	298 (100), 341.1778 (61.27), 155 (31.70), 127 (30.29)	27.204	174
JWH 018 2'-naphthyl-N-(2,2-dimethylpropyl) isomer	341.1780	284 (100), 341.1768 (51.31), 285 (42.45), 127 (30.85)	27.236	174
JWH 018 2'-naphthyl-N-(2-methylbutyl) isomer	341.1780	284 (100), 341.1776 (64.55), 127 (37.64), 285 (26.21)	27.653	175
JWH 018 2'-naphthyl-N-(3-methylbutyl) isomer	341.1780	341.1779 (100), 285 (74.70), 155 (70.62), 127 (59.53)	27.727	175
JWH 018 N-(1,1-dimethylpropyl) isomer	341.1780	270 (100), 254 (59.79), 341.1769 (33.94), 271 (30.01)	26.470	175
JWH 018 N-(1,2-dimethylpropyl) isomer	341.1780	298 (100), 341.1767 (27.49), 155 (26.65), 127 (24.53)	26.197	176
JWH 018 N-(1-ethylpropyl) isomer	341.1780	312 (100), 341.1783 (54.81), 155 (51.87), 127 (29.35)	26.055	176
JWH 018 N-(1-methylbutyl) isomer	341.1780	298 (100), 341.1780 (61.67), 127 (37.90), 144 (27.89)	26.283	176
JWH 018 N-(2,2dimethylpropyl) isomer	341.1780	284 (100), 341.1779 (39.95), 127 (28.10), 285 (23.79)	26.341	177
JWH 018 N-(2-methylbutyl) isomer	341.1780	284 (100), 341.1780 (54.88), 127 (39.70), 285 (20.92)	26.776	177
JWH 018 N-(3-methylbutyl) isomer	341.1780	341.1777 (100), 284 (75.39), 127 (54.55), 324 (50.23)	26.856	177
JWH 073 2'-naphthyl isomer	327.1623	327.1625 (100), 200 (89.49), 284 (63.24), 127 (43.19)	27.450	178
JWH 073 2'-naphthyl-N-(1,1-dimethylethyl) isomer	327.1623	144 (100), 271 (86.26), 327.1618 (64.34), 270 (55.20)	26.980	178
JWH 073 2'-naphthyl-N-(1-methylpropyl) isomer	327.1623	298 (100), 327.1623 (33.09), 155 (33.09), 127 (31.33)	26.801	178
JWH 073 2'-naphthyl-N-(2-methylpropyl) isomer	327.1623	284 (100), 327.1624 (66.54), 127 (36.87), 200 (22.53)	26.995	179
JWH 073 N-(1,1-dimethylethyl) isomer	327.1623	270 (100), 254 (57.43), 327.1623 (46.04), 144 (30.72)	26.020	179
JWH 073 N-(1-methylpropyl) isomer	327.1623	298 (100), 327.1622 (65.79), 127 (36.99), 144 (36.39)	25.894	179
JWH 073 N-(2-methylpropyl) isomer	327.1623	284 (100), 327.1624 (65.11), 127 (44.00), 144 (26.18)	26.140	180
JWH 081 2-methoxynaphthyl isomer	371.1885	371.1889 (100), 354 (79.60), 200 (75.95), 144 (50.19)	27.564	180
JWH 081 3-methoxynaphthyl isomer	371.1885	371.1885 (100), 354 (65.57), 214 (58.71), 314 (57.91)	28.584	180
JWH 081 5-methoxynaphthyl isomer	371.1885	371.1886 (100), 354 (61.02), 214 (47.40), 197 (44.08)	28.921	181

JWH 081 6-methoxynaphthyl isomer	371.1885	371.1883 (100), 354 (73.92), 214 (56.88), 314 (54.51)	28.982	181
JWH 081 7-methoxynaphthyl isomer	371.1885	340 (100), 200 (99.43), 270 (66.79), 144 (42.54)	27.482	181
JWH 081 8-methoxynaphthyl isomer	371.1885	340 (100), 200 (99.53), 270 (70.82), 144 (46.66)	27.483	182
JWH 122 2-methylnaphthyl isomer	355.1936	298 (100), 355.1934 (95.60), 168 (63.46), 338 (46.38)	26.814	182
JWH 122 3-methylnaphthyl isomer	355.1936	355.1927 (100), 338 (68.70), 298 (65.61), 214 (56.80)	27.617	182
JWH 122 5-methylnaphthyl isomer	355.1936	355.1926 (100), 338 (76.10), 298 (68.12), 214 (58.71)	27.962	183
JWH 122 6-methylnaphthyl isomer	355.1936	355.1919 (100), 338 (77.92), 298 (69.70), 214 (56.46)	27.823	183
JWH 122 7-methylnaphthyl isomer	355.1936	355.1930 (100), 181 (91.38), 298 (87.51), 214 (69.85)	27.454	183
JWH 122 8-methylnaphthyl isomer	355.1936	298 (100), 338 (93.46), 355.1927 (72.80), 130 (55.40)	27.315	184
JWH 200 2'-naphthyl isomer	384.1838	100 (100), 127 (7.80), 70 (7.76), 101 (5.29)	31.886	184
JWH 203 3-chlorophenyl isomer*	339.1390	214 (100), 144 (30.67), 215 (13.45), 116 (8.61)	25.564	184
JWH 203 4-chlorophenyl isomer*	339.1390	214 (100), 144 (29.86), 215 (14.08), 116 (8.00)	25.705	185
JWH 210 2-ethylnaphthyl isomer	369.2093	182 (100), 312 (68.27), 369.2080 (60.14), 154 (55.01)	27.009	185
JWH 210 3-ethylnaphthyl isomer	369.2093	369.2077 (100), 352 (77.45), 312 (65.71), 214 (60.58)	28.055	185
JWH 210 5-ethylnaphthyl isomer	369.2093	369.2088 (100), 352 (82.01), 312 (60.93), 214 (59.05)	28.400	185
JWH 210 6-ethylnaphthyl isomer	369.2093	369.2079 (100), 352 (97.41), 312 (68.27), 214 (56.18)	28.437	186
JWH 210 7-ethylnaphthyl isomer	369.2093	369.2083 (100), 195 (85.91), 312 (79.65), 214 (69.30)	27.761	186
JWH 210 8-ethylnaphthyl isomer	369.2093	130 (100), 352 (84.54), 312 (74.69), 369.2080 (74.17)	27.248	186
JWH 251 3-methylphenyl isomer*	319.1936	214 (100), 144 (27.50), 215 (14.14), 116 (8.06)	24.728	187
JWH 251 4-methylphenyl isomer*	319.1936	214 (100), 144 (28.31), 207 (26.42), 215 (14.54)	24.945	187
JWH 398 2-chloronaphthyl isomer	375.1390	214 (100), 375.1386 (91.10), 144 (52.95), 270 (46.88)	27.670	187
JWH 398 3-chloronaphthyl isomer	375.1390	375.1384 (100), 214 (86.37), 318 (84.09), 144 (61.63)	28.026	188
JWH 398 5-chloronaphthyl isomer	375.1390	375.1379 (100), 214 (86.90), 318 (83.14), 144 (60.58)	28.392	188
JWH 398 6-chloronaphthyl isomer	375.1390	375.1383 (100), 214 (84.84), 318 (80.56), 358 (56.97)	28.370	188
JWH 398 7-chloronaphthyl isomer	375.1390	318 (100), 214 (94.66), 375.1386 (90.28), 144 (54.21)	28.205	189
JWH 398 8-chloronaphthyl isomer	375.1390	340 (100), 270 (54.54), 341 (25.37), 144 (9.46)	28.220	189
RCS-4 2-methoxy isomer	321.1729	304 (100), 321.1722 (87.07), 264 (81.27), 135 (46.20)	24.410	189
RCS-4 3-methoxy isomer	321.1729	214 (100), 321.1724 (96.07), 264 (57.65), 144 (42.71)	25.368	190
RCS-8 3-methoxy isomer*	375.2198	254 (100), 207 (33.88), 144 (17.78), 255 (17.48)	28.467	190
RCS-8 4-methoxy isomer*	375.2198	254 (100), 144 (17.35), 255 (17.34), 158 (8.11)	28.881	190
(-)-11-nor-9-carboxy- Δ^9 -THC*	344.1988	100 (100), 142 (68.96), 58 (49.18), 101 (6.32)	7.946	191
(-)-CP 47,497*	318.2559	215 (100), 233 (68.32), 216 (22.81), 81 (16.57)	22.897	191
(-)-CP 55,940	376.2978	273 (100), 147 (97.02), 121 (64.44), 135 (43.79)	25.673	191
(+)-CP 47,497*	318.2559	215 (100), 233 (67.05), 216 (21.91), 81 (16.27)	22.894	192
(+)-CP 55,940	376.2978	147 (100), 273 (99.83), 121 (63.65), 135 (43.36)	25.666	192

(+)WIN 55212-2*	426.1943	100 (100), 70 (6.25), 127 (5.42), 101 (4.98)	33.850	192
(±)3epi CP 47,497-C8-homolog	332.2715	215 (100), 233 (70.23), 216 (18.91), 147 (11.28)	23.536	193
(±)5-epi CP 55,940	376.2978	147 (100), 121 (58.54), 273 (53.22), 135 (36.84)	25.532	193
(±)CP 47,497	318.2559	215 (100), 233 (66.32), 216 (22.79), 81 (16.42)	22.896	193
(±)-CP 47,497-C8-homolog	332.2715	215 (100), 233 (70.22), 216 (23.09), 81 (15.76)	23.644	194
(±)-CP 55,940	376.2978	147 (100), 273 (96.71), 121 (56.53), 187 (37.34)	25.668	194
(±)-epi CP 47, 497	318.2559	215 (100), 233 (70.59), 216 (19.29), 147 (12.16)	22.778	194
(±)WIN 55212 (mesylate)	426.1943	100 (100), 70 (6.38), 127 (5.47), 101 (5.12)	33.848	195
1'-Naphthoyl Indole	271.0997	270 (100), 271.0984 (69.69), 254 (66.72), 144 (60.66)	26.163	195
4-Quinolone-3-Carboxamide CB2 Ligand	422.2569	272 (100), 202 (62.23), 377 (25.74), 365 (21.21)	35.215	195
AKB48	365.2467	215 (100), 145 (59.79), 337 (44.40), 294 (42.68)	26.887	196
AM1220*	382.2045	98 (100), 70 (11.34), 99 (6.21), 127 (4.79)	29.956	196
AM1235	404.1536	404.1523 (100), 387 (93.23), 127 (76.42), 403 (73.78)	32.153	196
AM1241*	503.0706	98 (100), 70 (11.10), 99 (5.92), 96 (2.14)	31.475	197
AM1248*	390.2671	98 (100), 70 (8.40), 99 (6.72), 98 (4.82)	29.439	197
AM2201	359.1685	359.1680 (100), 284 (90.05), 342 (70.03), 232 (67.27)	27.838	197
AM2232	352.1576	352.1571 (100), 225 (76.43), 335 (72.72), 351 (71.94)	29.854	197
AM2233*	458.0855	98 (100), 70 (10.23), 99 (5.91), 230 (2.26)	28.355	198
AM630*	504.0910	100 (100), 135 (10.48), 70 (6.68), 73 (6.64)	32.754	198
AM679	417.0590	417.0585 (100), 214 (75.81), 220 (70.48), 144 (29.31)	25.801	198
AM694	435.0495	435.0500 (100), 232 (88.71), 220 (68.16), 359 (28.98)	26.551	198
Cannabidiol	314.2246	231 (100), 232 (15.68), 174 (7.59), 193 (5.62)	21.343	199
CB-13	368.1776	281 (100), 171 (99.79), 368.1776 (88.54), 297 (85.26)	28.357	199
CB-25	403.3086	124 (100), 267 (35.37), 207 (33.71), 138 (28.71)	28.726	199
CB-52	417.3243	123 (100), 261 (27.58), 346 (23.03), 207 (20.10)	28.899	200
CB-86	417.3243	58 (100), 207 (92.38), 192 (69.32), 152 (59.95)	28.170	200
CP 47,497-para-quinone analog	332.2351	231 (100), 152 (56.73), 163 (53.83), 203 (53.81)	22.489	200
HU-210	386.2821	302 (100), 284 (55.24), 386.2818 (37.10), 269 (32.88)	25.873	201
HU-211	386.2821	302 (100), 284 (54.41), 386.2823 (36.66), 269 (32.40)	25.871	201
HU-308	414.3134	277 (100), 353 (68.36), 318 (64.90), 91 (30.22)	23.988	201
JP104*	406.2256	88 (100), 67 (20.98), 79 (20.51), 76 (16.59)	15.271	202
JWH 007	355.3000	355.1933 (100), 354 (83.88), 340 (81.67), 155 (76.98)	27.330	202
JWH 011	383.2249	270 (100), 155 (77.42), 383.2244 (77.01), 368 (64.85)	27.590	202
JWH 015	327.1623	326 (100), 327.1621 (95.83), 310 (51.80), 127 (40.66)	26.206	203
JWH 016	341.1780	341.1778 (100), 340 (87.34), 127 (57.40), 326 (50.85)	26.783	203

JWH 018	341.1780	341.1780 (100), 284 (80.52), 324 (64.59), 214 (58.55)	27.108	203
JWH 018 6-methoxyindole analog	371.1885	371.1883 (100), 314 (43.18), 354 (33.02), 127 (28.44)	28.667	204
JWH 018 adamantyl analog	349.2406	214 (100), 207 (21.36), 144 (16.21), 215 (14.33)	26.651	204
JWH 018 adamantyl carboxamide	364.2515	214 (100), 307 (51.74), 144 (44.32), 364.2508 (42.02)	28.358	204
JWH 018 N-(4,5-epoxypentyl) analog	355.1572	270 (100), 355.1566 (92.01), 127 (82.5), 284 (56.13)	29.021	205
JWH 018 N-(5-bromopentyl) analog	419.0885	284 (100), 127 (76.22), 402 (61.26), 421 (52.08)	30.581	205
JWH 018 N-(5-chloropentyl) analog	375.1390	284 (100), 375.1391 (89.62), 127 (69.73), 358 (65.91)	29.545	205
JWH 019	355.1936	355.1937 (100), 284 (88.26), 338 (65.20), 354 (53.52)	27.701	206
JWH 020	369.2093	369.2091 (100), 284 (89.32), 352 (68.76), 368 (53.66)	28.362	206
JWH 022	339.1623	339.1616 (100), 155 (88.54), 127 (80.50), 127 (56.14)	27.096	206
JWH 030	291.1623	291.1619 (100), 290 (91.58), 94 (58.39), 164 (55.84)	23.722	207
JWH 031	305.1780	305.1780 (100), 304 (89.97), 155 (67.25), 127 (65.53)	24.468	207
JWH 072	313.1467	313.1467 (100), 186 (77.36), 296 (70.70), 284 (68.57)	26.008	207
JWH 073	327.1623	327.1626 (100), 284 (77.08), 310 (70.36), 200 (68.80)	26.523	208
JWH 073 2-methylnaphthyl analog	341.1780	341.1779 (100), 298 (95.15), 168 (63.13), 324 (45.92)	26.208	208
JWH 073 4-methylnaphthyl analog	341.1780	341.1780 (100), 324 (76.25), 298 (70.45), 200 (58.55)	27.417	208
JWH 081	371.1885	371.1880 (100), 354 (66.35), 314 (63.16), 370 (51.22)	29.026	209
JWH 098	385.2042	370 (100), 385.2031 (72.23), 185 (65.08), 384 (37.81)	29.247	209
JWH 122	355.1936	355.1937 (100), 298 (77.44), 338 (75.95), 354 (52.82)	27.966	209
JWH 122 N-(4-pentenyl) Analog	353.1780	353.1779 (100), 169 (92.91), 298 (48.73), 352 (45.15)	27.952	210
JWH 145	367.1936	367.1936 (100), 155 (87.05), 366 (62.92), 127 (54.85)	27.814	210
JWH 147	381.2093	381.2097 (100), 155 (99.89), 127 (59.45), 380 (57.66)	28.457	210
JWH 175	327.1987	327.1984 (100), 270 (67.83), 200 (44.49), 186 (41.18)	25.002	211
JWH 180	355.1936	355.1929 (100), 338 (87.3), 326 (62.72), 354 (60.15)	27.803	211
JWH 182	383.2249	383.2250 (100), 366 (86.29), 326 (76.68), 382 (55.03)	28.943	211
JWH 200*	384.1838	100 (100), 70 (7.49), 127 (6.30), 101 (5.33)	30.324	211
JWH 201*	335.1885	214 (100), 144 (29.94), 215 (14.58), 116 (7.41)	26.034	212
JWH 203*	339.1390	214 (100), 144 (29.04), 215 (14.67), 116 (8.20)	25.335	212
JWH 210	369.2093	369.2095 (100), 352 (82.43), 312 (76.32), 368 (54.03)	28.367	212
JWH 249*	383.0885	214 (100), 144 (25.28), 215 (14.49), 116 (6.95)	26.010	213
JWH 250*	335.1885	214 (100), 144 (28.05), 215 (14.96), 207 (8.15)	25.410	213
JWH 251*	319.1936	214 (100), 144 (30.65), 215 (14.78), 116 (7.91)	24.725	213
JWH 302*	335.1885	214 (100), 144 (27.76), 215 (14.45), 116 (7.67)	25.675	214
JWH 307	385.1842	385.1836 (100), 155 (78.47), 384 (60.95), 127 (54.56)	27.481	214
JWH 309	417.2093	417.2083 (100), 155 (58.86), 416 (42.18), 127 (41.39)	34.238	214

JWH 368	385.1842	385.1831 (100), 155 (81.91), 384 (58.26), 127 (56.76)	27.514	215
JWH 369	401.1546	155 (100), 401.1534 (86.44), 127 (62.81), 400 (51.16)	28.482	215
JWH 370	381.2093	381.2084 (100), 155 (73.97), 127 (67.66), 380 (67.08)	27.634	215
JWH 398	375.1390	375.1388 (100), 318 (87.53), 214 (72.10), 358 (61.17)	28.289	216
JWH 424*	419.0885	340 (100), 270 (71.51), 341 (25.15), 271 (13.46)	28.870	216
JZL 184*	520.1482	149 (100), 271 (81.52), 121 (12.34), 272 (11.79)	28.938	216
JZL 195*	433.1638	184 (100), 183 (95.23), 143 (84.43), 238 (16.78)	23.156	216
MAM2201	373.1842	373.1840 (100), 298 (83.75), 356 (80.60), 232 (56.77)	28.743	217
MDA 19	349.1790	244 (100), 105 (68.27), 77 (65.00), 118 (59.35)	27.150	217
MDA 77	365.1739	77 (100), 105 (94.19), 176 (86.31), 148 (74.67)	28.305	217
Cannabipiperidiethanone*	376.2151	98 (100), 70 (10.32), 99 (6.24), 207 (5.16)	27.930	218
RCS-4	321.1729	321.1730 (100), 264 (83.16), 135 (78.31), 214 (52.38)	25.806	218
RCS-4-C4 Homolog	307.1572	307.1576 (100), 264 (82.44), 135 (67.15), 200 (60.80)	25.197	218
RCS-8*	375.2198	254 (100), 144 (17.60), 255 (17.31), 158 (7.82)	28.172	219
STS-135*	383.2624	232 (100), 307 (49.14), 144 (39.71), 382 (35.34)	29.224	219
UR-144*	311.2249	214 (100), 144 (29.32), 296 (15.20), 215 (14.25)	22.083	219
URB447	400.1342	275 (100), 105 (96.65), 400.1340 (63.24), 77 (32.19)	28.872	220
URB597*	338.1630	197 (100), 213 (89.80), 169 (45.81), 141 (33.79)	21.437	220
URB602*	295.1572	169 (100), 213 (85.28), 55 (29.35), 195 (28.25)	23.546	220
URB754	266.1055	266 (100), 160 (82.50), 133 (77.95), 104 (49.48)	23.789	221
URB937*	354.1580	149 (100), 167 (16.03), 150 (9.35), 57 (6.48)	24.284	221
WIN 54461*	456.1049	100 (100), 70 (6.66), 135 (5.59), 101 (5.31)	31.117	221
XLR11*	329.2155	232 (100), 144 (33.10), 233 (14.37), 314 (13.23)	22.920	222

As mentioned previously, 159 of the 162 synthetic cannabinoid standards provided GC-QTOF data. The three compounds that did not were AM251, HU-331, and IMMA. These three compounds produced GC chromatograms with several peaks where extensive fragmentation occurred and the data could be determined to correspond to the specific compound. Additional analysis using chemical ionization (CI) mode or multiple reaction monitoring (MRM) will have to be used for these three compounds. All other 159 synthetic cannabinoids produced consistent data with extracted peak spectra that were similar to previous library entries produced using Gas Chromatography-Triple Quadrupole Mass Spectrometry (GC-QQQ).

Out of the total 159 synthetic cannabinoids that data were obtained for, 124 compounds showed a molecular ion in the extracted peak spectra using EI mode, as in the case for JWH 018 (Figure 24). The 35 compounds that did not show a molecular ion will require further study using CI mode.

Figure 24. JWH 018 GC-QTOF analysis. a) GC chromatogram b) Normalized extracted peak spectra at RT 27.108 c) Previous GC-QQQ generated spectra.

In the GC chromatogram for JWH 018 seen in Figure 24a, it clear that the peak with the retention time of 27.108 most likely corresponds to the compound. From the extracted peak spectra for this peak (Figure 24b), it can clearly be seen that the molecular ion of 341.1780 m/z is present, and that there is a characteristic fragment pattern. The QTOF spectrum was then compared with previously generated GC-QQQ data (Figure 24c) with good peak correlation.

Similar data were collected for the other 123 synthetic cannabinoids that showed a molecular ion in the EI mode as seen for AM2201 in Figure 25.

Figure 25. AM2201 GC-QTOF analysis. a) GC chromatogram b) Normalized extracted peak spectra at RT 27.108 c) Previous GC-QQQ generated spectra.

From the GC chromatogram, it appears that the peak corresponding to AM2201 has the retention time of 27.838 minutes. After extraction of the peak spectra, this peak is confirmed as the compound because of the 359.1680 m/z molecular ion is present. As with all of the compounds that showed a molecular ion, comparison with the GC-QQQ generated spectra shows good correlation between the two databases.

However, this was not the case for all the compounds as it was previously mentioned that 35 of the synthetic cannabinoids analyzed did not show a molecular ion, as was the case for XLR11 (Figure 26).

Figure 26. XLR11 GC-QTOF analysis. a) GC chromatogram b) Normalized extracted peak spectra at RT 27.108 c) Previous EI-GC-QQQ generated spectra d) Previous CI-GC-QQQ generated spectra.

Similar to the compounds that showed a molecular ion in EI mode, it was relatively easy to determine which peak in the GC chromatogram likely corresponded to the analyte. However, in this case, when the peak spectra was extracted from the peak with a retention time of 22.920 minutes, the molecular ion for XLR11, 330.2228 m/z was not present. After comparison with the EI-GC-QQQ data for this compound, the fragments matched up, meaning that for full analysis of this compound, CI mode would have to be used (Figure 26b and c). Although, CI ionization was not performed because of timing, a CI spectra for XLR11 that was previously produced using CI-GC-QQQ was included to show that the softer ionization technique allows for significantly less fragmentation of the compound and allows the molecular ion to be present (Figure 26d).

Identifying isomers was difficult using DART-QTOF as the analytical technique only relies on fragmentation through mass spectrometry and does not include any

separation technique. Gas chromatography quadrupole time-of-flight mass spectrometry on the other hand, not only produces data about the fragmentation of the molecule, but also provides a retention time that can be used to further characterize and identify a compound. The GC-QTOF analysis showed that using the retention time and the fragmentation of the compound, all but eight isomer groups, a total of 19 compounds, could be discriminated by GC-QTOF (Table 17).

Table 17. Isomer groups that could not be discriminated by GC-QTOF.

Analyte	M.W. (amu)	RT (min)	Major Peaks
JWH 081	371.1885	28.392	371.1880 (100), 354 (66.35), 314 (63.16), 370 (51.22)
JWH 081 6-methoxynaphthyl		28.370	371.1883 (100), 354 (73.92), 214 (56.88), 314 (54.51)
JWH 081 7-methoxynaphthyl	371.1885	27.482	340 (100), 200 (99.43), 270 (66.79), 144 (42.54)
JWH 081 8-methoxynaphthyl		27.483	340 (100), 200 (99.53), 270 (70.82), 144 (46.66)
JWH 122	355.1936	27.966	355.1937 (100), 298 (77.44), 338 (75.95), 354 (52.82)
JWH 122 5-methylnaphthyl		27.962	355.1926 (100), 338 (76.10), 298 (68.12), 214 (58.71)
JWH 210 5-ethylnaphthyl	369.2093	28.437	369.2088 (100), 352 (82.01), 312 (60.93), 214 (59.05)
JWH 210 6-ethylnaphthyl		28.400	369.2079 (100), 352 (97.41), 312 (68.27), 214 (56.18)
JWH 398 5-chloronaphthyl	375.1390	28.392	375.1379 (100), 214 (86.90), 318 (83.14), 144 (60.58)
JWH 398 6-chloronaphthyl		28.370	375.1383 (100), 214 (84.84), 318 (80.56), 358 (56.97)
(-)-CP 55,940	376.2978	25.673	273 (100), 147 (97.02), 121 (64.44), 135 (43.79)
(±)-CP 55,940		25.668	147 (100), 273 (96.71), 121 (56.53), 187 (37.34)
(+)-CP 55,940		25.666	147 (100), 273 (99.83), 121 (63.65), 135 (43.36)
(+)-WIN 55212-2	386.2821	25.873	100 (100), 70 (6.25), 127 (5.42), 101 (4.98)
(±)-WIN 55212 (mesylate)		25.871	100 (100), 70 (6.38), 127 (5.47), 101 (5.12)
HU-210	426.1943	33.850	302 (100), 284 (55.24), 386.2818 (37.10), 269 (32.88)
HU-211		33.848	302 (100), 284 (54.41), 386.2823 (36.66), 269 (32.40)

Analysis of synthetic cannabinoids using GC-QTOF allowed for additional discrimination over DART-QTOF because of the addition of GC as a separation technique. Out of the 159 compounds that data were obtained for, 140 were able to be distinguished by the RT and/or fragmentation. Examples of two different compounds with molecular ions that can be differentiated have been shown with JWH 018 (Figure 24) and AM2201 (Figure 25). As mentioned previously though, 77 isomers were analyzed in this study and in case of GC-QTOF, 58 of these 77 were discriminated. The

eight groups of compounds that were not able to be differentiated from one another have been shown in Table 17.

A simple example of two isomers that are able to be discriminated by retention time alone are RCS-4 2-methoxy isomer and RCS-4 3-methoxy isomer. The retention times for these two compounds are 24.410 and 25.368 minutes.

Figure 27. RCS-4 2-methoxy and RCS-4 3-methoxy GC-QTOF analysis. a) Overlaid GC chromatogram b) Normalized extracted peak spectra for RCS-4 2-methoxy c) Normalized extracted peak spectra for RCS-4 3-methoxy.

It can be seen from the GC chromatograms for these two compounds, although the molecular weight is the same, 321.1724 m/z, the retention times are clearly different (Figure 27a). This difference in retention time is helpful because when examining the extracted peak spectra for the two compounds, with the exception of the 304 peak for the RCS-4 2-methoxy isomer and the 214 peak for the RCS-4 3-methoxy isomer, the spectra

are very similar. Although no determination of a fragment with an m/z of 304 could be made, the closest being the loss of a methyl group from the alkyl chain, the 214 peak may correspond to the loss of the benzene and methyl ether groups. This could show that the methyl ether group is more stable in the ortho-position on the benzene ring in the 2-methoxy isomer instead of the meta-position in the 3-methoxy isomer.

However in some cases it was not possible to discriminate between the isomers using both the retention time and spectra, as for the eight groups of compounds listed in Table 17. The overlaid GC chromatograms and extracted peak spectra for the JWH 081 7-methoxynaphthyl and JWH 081 8-methoxynaphthyl isomers are seen in Figure 28.

Figure 28. JWH 081 7-methoxynaphthyl and JWH 081 8-methoxynaphthyl analysis. a) Overlaid GC chromatogram b) Normalized extracted peak spectra for JWH 081 7-methoxynaphthyl c) Normalized extracted peak spectra for JWH 081 8-methoxynaphthyl.

In this case, when looking at the overlaid spectra of the two compounds, they cannot be differentiated on the basis of the retention time, 27.482 versus 27.483 minutes (Figure

28a). If these two compounds were in a mixture, by using only the retention time the drugs would not be able to be distinguished from one another because they elute at the same time. Typically deconvolution would be used in this case to separate the two peaks based on the spectra. However, in this example the two compounds have the same extracted peak spectra as the compounds fragment into the same peaks including 371 (molecular ion), 340 (loss of methyl ether group), 270 (loss of methyl ether and alkyl chain) and 144 (indole and carbonyl bridge groups). In addition, the minor, less abundant peaks are also similar, meaning that RCS-4 2-methoxy and RCS-4 3-methoxy isomers cannot be differentiated by GC-QTOF (Figure 28b and c).

3.3.2. GC-QTOF Mixture Analysis

The GC chromatograms were obtained for the three mixtures previously described in Tables 13, 14, and 15 and spectra were extracted for each peak. The first mixture of 15 different compounds can be seen in Figure 29.

Figure 29. GC chromatogram for GC-QTOF Mixture 1.

In the GC chromatogram for the first mixture, there are 11 peaks that are automatically integrated. First important note is that in the mixture preparation, 15 compounds were combined in this mixture (Table 13), meaning that either coelution occurred or several of the compounds did not show up. The spectra for each of these 11

peaks were extracted (Figure 30) to determine which compound corresponded to the peak.

Figure 30. Normalized extracted mass spectra for GC-QTOF Mixture 1. a) RT 21.351 min b) RT 23.507 c) RT 24.995 d) RT 25.970 e) RT 26.607 f) RT 26.938 g) RT 27.201 h) RT 27.542 i) RT 28.299 j) RT 29.163 k) RT 34.052.

Using the peaks present in each of the extracted mass spectra, firstly the molecular ion followed by the fragments, and the retention times recorded for the individual standards (Table 16), the peaks in the GC chromatogram were identified (Table 18).

Table 18. Identification of GC Peaks for GC-QTOF Mixture 1

GC Peak	Compound Identified	Figure 30
21.351	Cannabidiol	a
23.507	($\pm$)3epi CP 47,497-C8 homolog	b
24.995	JWH 175	c
25.970	JWH 072	d
26.607	JWH 018 adamantyl analog	e
26.938	JWH 210 2-ethylnaphthyl isomer	f
27.201	JWH 122 8-methylnaphthyl isomer	g
27.542	JWH 018 2'-naphthyl-N-(2-methylbutyl) isomer	h
28.299	JWH 020	i
29.163	<i>No compound determined</i>	j
34.052	JWH 309	k

Overall, for the first mixture, 10 of the 11 peaks in the GC chromatogram were identified. The extracted spectra for the peak at RT 29.163 (Figure 30j) did not correspond to any of the compounds that were added to the mixture and 5 compounds did not result in a peak; CP 47,498-para-quinone, ($\pm$)5epi CP 55,940, JWH 018 2'-naphthyl, CB-52, and JWH 098. Several other smaller peaks were present in the chromatogram however, the extracted peak spectra only showed the background signal. It is important to note that although the concentration of all 15 compounds was 10 $\mu\text{g/mL}$ and each analyte produced a spectra when the individual standard was analyzed, it is possible that the intensity of these peaks were significantly lower in comparison with the other compounds, meaning they merged in with the background noise.

The same process was performed with the second and third mixtures. Per mixture preparation, the second mixture was made up of 12 compounds (Table 14) and the third mixture was a combination of 11 compounds (Table 15). Mixture 2 produced 10 peaks in the GC chromatogram for the 12 compounds (Figure 31) and a summary of the results can be seen in Table 19.

Figure 31. GC chromatogram for GC-QTOF Mixture 2

Table 19. Identification of GC Peaks for GC-QTOF Mixture 2

GC Peak	Compound Identified	Spectra Page #
22.760	($\pm$)-epi CP 47,497	222
23.615	($\pm$)-CP 37,397-C8 homolog	222
25.183	RCS-4 C4-homolog	222
25.624	($\pm$)-CP 55,940	222
27.019	JWH 018 2'-naphthyl-N-(1,2-dimethylpropyl) isomer	222
27.332	AM2201 N-(4-fluoropentyl) isomer	223
27.616	JWH 018 2'-naphthyl-N-(3-methylbutyl) isomer	223
27.946	JWH 398 3-chloronaphthyl isomer	223
28.310	JWH 210	223
28.710	AM2201 2'-naphthyl	223

Ten of the 12 compounds in mixture 2 were able to be distinguished using the retention time of the standard, the molecular ion, and the fragmentation pattern. The two compounds that did not show a peak in the GC chromatogram were the JWH 073 N-(1,1-dimethylethyl) and JWH 018 N-(1,1-dimethylpropyl) isomers. Again, it is possible that these two compounds exhibited lower peak intensities relative to the other 10 compounds, resulting in no observance of peak in the GC chromatogram.

As stated the final mixture was prepared using 11 compounds and resulted in the observance of 9 GC chromatogram peaks and a large, questionable region between 28 and 28.8 minutes (Figure 32). The summary of the identification results can be seen in Table 20.

Figure 32. GC chromatogram for GC-QTOF Mixture 3

Table 20. Identification of GC Peaks for GC-QTOF Mixture 3

GC Peak	Compound Identified	Spectra Page #
22.877	($\pm$)-CP 47,497	223
23.795	URB 754	223
25.761	AM679	224
26.161	JWH 015	224
26.698	JWH 018 N-(2-methylbutyl) isomer	224
27.022	AM2201 N-(2-fluoropentyl) isomer	224
27.366	JWH 073 4-methylnaphthyl isomer	224
27.756	JWH 180	225
28.877	JWH 182	225

The third and final mixture analyzed resulted in the identification of nine of the 11 compounds added to the mixture. The two compounds that were not present were the RCS-4 3-methoxy isomer and MDA 77. It was originally thought that MDA which had a retention time of 28.305 minutes for the individual standard had merged with the large region between 28 and 28.8 minutes, however examination of the region showed only background signal. It is possible that MDA 77 as well as the RCS-4 3-methoxy isomer had significantly lower peak intensities than the other nine compounds in the mixture and were merged in with the background.

3.4. Conclusions

Gas chromatography quadrupole time-of-flight mass spectrometry proved to be a viable alternative to traditional methods such as GC-MS for the analysis of synthetic cannabinoids. Analysis of the total 162 compounds produced data for 159 analytes,

including 124 that showed a molecular ion in EI mode, and 35 that did not. Although not performed in this study, these 35 compounds that did not show a molecular ion when analyzed in the EI mode should be analyzed using CI mode in order to get a full representation of these synthetic cannabinoids. The discrimination capability of GC-QTOF was also demonstrated. One-hundred and forty out of the 159 compounds were able to be differentiated based on the retention time and peak spectra, with only 8 groups of compounds, and 19 total, unable to be distinguished from one another. When looking only at the 77 isomers analyzed in the study, GC-QTOF was able to discriminate 58 out of 77 isomers. When it came to the analysis of mixtures, GC-QTOF showed a good ability to separate out mixtures, however not all of the compounds were able to be resolved; Mixture 1: 10/15 compounds, Mixture 2: 10/12 compounds, and Mixture 3: 9/11 compounds. In addition to showing the ability to separate and identify different compounds, the results here confirmed that separation of isomers could be achieved as in Mixture 2, the JWH 018 2'-naphthyl-N-(1,2-dimethylpropyl) and JWH 0182'-naphthyl-N-(3-methylbutyl) isomers were resolved in the same mixture of compounds.

4. CONCLUSIONS

Since their first detection in herbal incense products in the U.S. in 2008, the number of detected and abused synthetic cannabinoids has increased dramatically. The use of these drugs cause similar psychoactive effects to THC and have also been seen to have severe health effects on the cardiovascular and nervous system. Although federal scheduling of synthetic cannabinoids under the Controlled Substance Act and Synthetic Drug Abuse Prevention Act of 2012 have significantly reduced the amount of seizure and detection of a number of these compounds, the number of emerging synthetic cannabinoids is even greater. For this reason, the analysis of synthetic cannabinoids is of great interest to forensic science and toxicology.

This study has shown the usefulness of DART-QTOF and GC-QTOF for the analysis of synthetic cannabinoids. DART-QTOF has proved to be a rapid analysis technique as little to no sample preparation is required and the linear rail setup used here allows for a level of automation, meaning the instrument is easier to use. In this study, DART-QTOF parameters were optimized for this specific class of designer drugs with single sample analysis times under two minutes each. Limits of detection were determined for a representative sample of 14 compounds in the range of approximately 1.5 ng and below, showing that the method is very sensitive. Spectral data was obtained for 134 synthetic cannabinoids at three different collision energies and has shown that DART-QTOF spectra can be successfully searched against existing ESI-QTOF generated libraries in most cases. This was demonstrated by library matches for 118 out of 130 compounds that data was obtained for. Direct analysis in real time quadrupole time-of-flight mass spectrometry is also able to distinguish between a limited number of isomers,

27 out of 77, without the requirement of any separation technique. In addition, this study has shown that DART-QTOF is able to discriminate between synthetic cannabinoids in a mixture for two to four compounds, mimicking the common components of herbal incense mixtures. However, there are limitations to DART-QTOF in the analysis of synthetic cannabinoids. Identifying isomers can be difficult because with this analytical technique, the results are based on the molecular weight and fragmentation of each compound. In the case of isomers, the molecular weight is the same, but the fragmentation can also occur in the same way. This means that there are synthetic cannabinoid isomers that are not able to be differentiated using DART-QTOF. Mixture analysis is also limited as the only data obtained may be a full total ion chromatogram, meaning that identification is based only on the molecular ion. Although the addition of a separation technique is beneficial, overall, DART-QTOF has been shown to be a promising analytical technique for the detection and identification of synthetic cannabinoids.

In terms of GC-QTOF, although the analysis time is much longer than that of DART-QTOF, this technique is capable of full automation where a set of samples can be started at the beginning of the day and allowed to run until completion, allowing for other tasks such as data analysis to be completed without losing any time. Analysis of the 162 synthetic cannabinoids in the EI mode resulted in data for all but three of the compounds and extraction of GC chromatogram peaks allowed for determination of molecular fragments in the full scan mode. Using only the EI mode, molecular ions were observed for 124 of the compounds, with no molecular ion presence for only 35. This study showed that the discrimination capability of GC-QTOF is high as 140 out of 159 of

compounds were able to be differentiated based on the retention and molecular fragments, with only a total of 19 compounds spanning eight groups, not being resolved from one another. This is much greater than the 118 compounds that were able to be identified by DART-QTOF. It is important to note that GC-QTOF is able to identify peaks to a specific compound using the fragmentation pattern and retention time whereas not all of the DART-QTOF library matches were to a single compound. Gas chromatography quadrupole time-of-flight mass spectrometry showed an ability to discriminate between isomers as 58 of the 77 isomers were able to be resolved, much higher than the 27 isomers using DART-QTOF. This study also showed that GC-QTOF is able to separate and resolve complex mixtures, including isomers, with high accuracy and consistent spectral data, however, it is important to state that additional research will be beneficial as some compounds were not able to be detected in the mixture. A limitation with GC-QTOF is that because EI ionization is a hard technique, analytes are more likely to fragment, meaning molecular ions may not be present for all analyzed compounds. This means that any analysis of synthetic cannabinoids using GC-QTOF should be performed using both EI and CI ionization in order to fully characterize and identify the compounds present.

Both of these relatively new analytical techniques have shown to be viable options to replace traditional instrumentation, allowing for high resolution, accurate, and in the case of DART-QTOF, rapid analysis results. All of these qualities would greatly benefit forensic laboratories in the analysis of synthetic cannabinoids as well as the ever increasing number of novel designer drugs.

LIST OF REFERENCES

- [1] Brown, Lawrence S. Substance Abuse and America: Historical Perspective on the Federal Response to a Social Phenomenon. *Journal of the National Medical Association* 1981, 73, 6, 497-506.
- [2] Meyer, Jerrold S. 3,4-methylenedioxymethamphetamine (MDMA): current perspectives. *Substance Abuse and Rehabilitation* 2013, 4, 83-99.
- [3] Henderson, GL. Designer drugs: past history and future prospects. *Journal of Forensic Science* 1988, 33, 2, 569-75.
- [4] Vandrey, R.; Dunn, K. E.; Fry, J. A.; Girling, E. R. A survey study to characterize use of Spice products (synthetic cannabinoids). *Drug and Alcohol Dependence* 2012, 120, 238-241.
- [5] Berkovitz, R.; Arieli, M.; Marom, E. Synthetic cannabinoids – the new “legal high” drugs. *Harefuah* 2011, 150, 12, 884-887.
- [6] U.S. Department of Justice, Drug Enforcement Administration. Rules – 2014. *Federal Register* 2014, 79, 7.
- [7] U.S. Department of Justice, Drug Enforcement Administration. Special Report: Synthetic Cannabinoids and Synthetic Cathinones Reported in NFLIS, 2009-2010. 2011.
- [8] Di Marzo, V.; De Petrocellis, L. Plant, Synthetic, and Endogenous Cannabinoids in Medicine. *Annu. Rev. Med.* 2006, 57, 553-574.
- [9] Every-Palmer, S. Synthetic cannabinoid JWH-018 and psychosis: An explorative study. *Drug and Alcohol Dependence* 2011, 117, 152-157.
- [10] Crews, B. O. Synthetic Cannabinoids: The Challenges of Testing for Designer Drugs. *Clinical Laboratory News* 2013, 39, 2.
- [11] Gurney, S. M. R.; Scott, K. S.; Kacinko, S. L.; Presley, B. B.; Logan, B. K. Pharmacology, Toxicology, and Adverse Effects of Synthetic Cannabinoid Drugs. *Forensic Sci Rev* 2014, 26, 53-77.
- [12] United Nations Office on Drugs and Crime (UNODC). Synthetic Cannabinoids in Herbal Products. 2011.
- [13] Wiley, J. L.; Marusich, J. A.; Huffman, J. W.; Balster, R. L.; Thomas B. F. Hijacking of Basic Research: The Case of Synthetic Cannabinoids. *Methods Report (RTI Press)* 2011, 17971.

- [14] Brownstein, J. K2 Giving People Another Dangerous Way to Get High. *ABC News*. 17 March 2010.
- [15] Wang Linda. C&EN Talks With John W. Huffman. *Chemical & Engineering News* 2010, 88, 26, 43.
- [16] Zawilska, J. B.; Wojcieszak, J. Spice/K2 drugs – more than innocent substitutes for marijuana. *International Journal of Neuropsychopharmacology* 2014, 17, 509-525.
- [17] Dresen, S.; Kneisel, S.; Weinmann, W.; Zimmermann, R.; Auwarter, V. Development and validation of a liquid chromatography-tandem mass spectroscopy method for the quantitation of synthetic cannabinoids of the aminoalkylindole type and methanandamide in serum and its application to forensic science. *J. Mass. Spectrom.* 2011, 46, 163-171.
- [18] Dresen, S.; Ferreira, N.; Putz, M.; Westpahl, F.; Zimmermann, R.; Auwarter, V. Monitoring of herbal mixtures potentially containing synthetic cannabinoids as psychoactive compounds. *J. Mass. Spectrom.* 2010, 45, 1186-1194.
- [19] SAMHSA, *Drug-Related Emergency Department Visits Involving Synthetic Cannabinoids* 2012.
- [20] SAMHSA, *Drug Abuse Warning Network, 2011: National Estimates of Drug-Related Emergency Department Visits* 2013.
- [21] University of Maryland Center for Substance Abuse Research. *CESAR FAX* 2013, 22, 10.
- [22] Compton, D.R.; Rice, K. C.; De Costa, B. R.; Razdan, R. K.; Melvin, L. S.; Johnson, M. R.; Martin, B. R. Cannabinoid structure-activity relationship: correlation of receptor binding and in vivo activities. *J Pharmacol Exp Ther* 1993, 265, 1, 218-226.
- [23] Munro, S.; Thomas, K. L.; Abu-Shaar, M. Molecular characterization of a peripheral receptor for cannabinoids. *Nature* 1993, 365, 6441, 61-65.
- [24] Porter, A. C.; Felder, C. C. The endocannabinoid nervous system: unique opportunities for therapeutic intervention. *Pharmacol. Ther.* 2001, 90, 1, 45-60.
- [25] Presley, B. C.; Jansen-Varnum, S. A.; Logan, B. K. Analysis of Synthetic Cannabinoids in Botanical Material: A Review of Analytical Methods and Findings. *Forensic Science Review* 2013, 25, 27-46.
- [26] Cody, R.; Laramée, J. A.; Durst, H. D. Versatile New Ion Source for the Analysis of Materials in Open Air under Ambient Conditions. *Anal. Chem.* 2005, 77, 8, 2297-2302.

- [27] Song, L.; Dykstra, A. B.; Yao, H.; Bartmess, J. E. Ionization Mechanism of Negative Ion-Direct Analysis in Real Time: A Comparative Study with Negative Ion-Atmospheric Pressure Photoionization. *J. Am. Soc. Mass Spec.* 2009, *20*, 1, 42-50.
- [28] Cody, R. B.; Laramée, J. A.; Nilles, J. M.; Durst, H. D. Direct Analysis in Real Time (DART) Mass Spectrometry. *JEOL News* 2005, *4*, 1, 8-12.
- [29] Musah, R. A.; Domin, M. A.; Walling, M. A.; Shepard, J. R. E. Rapid identification of synthetic cannabinoids via direct analysis in real time mass spectrometry. *Rapid Commun. Mass Spectrom.* 2012, *26*, 1109-1114.
- [30] Musah, R. A.; Domin, M. A.; Cody, R. B.; Lesiak, A. D.; Dane, A. J.; Shepard, J. R. E. Direct analysis in real time mass spectrometry with collision-induced dissociation for structural analysis of synthetic cannabinoids. *Rapid Commun. Mass Spectrom.* 2012, *26*, 2335-2342.
- [31] Lesiak, A. D.; Musah, R. A.; Domin, M. A. Shepard, J. R. E. DART-MS as a Preliminary Screening Method for "Herbal Incense": Chemical Analysis of Synthetic Cannabinoids. *Journal of Forensic Science* 2014, *59*, 2, 337-343.
- [32] Dunham, S. J. B.; Hooker, P. D.; Hyde, R. M. Identification, extraction and quantification of the synthetic cannabinoid JWH-018 from commercially available herbal marijuana alternatives. *Forensic Science International* 2012, *223*, 241-244.
- [33] Gwak, S.; Almirall, J. R. Rapid screening of 35 new psychoactive substances by ion mobility spectrometry (IMS) and direct analysis in real time (DART) coupled to quadrupole time-of-flight mass spectrometry (QTOF-MS). *Drug Testing and Analysis* 2015.
- [34] Uchiyama, N.; Kikura-Hanajiri, R.; Ogata, J.; Goda, Y. Chemical analysis of synthetic cannabinoids as designer drugs in herbal products. *Forensic Science International* **2010**, *198*, 31-38.
- [35] Uchiyama, N.; Kikura-Hanajiri, R.; Goda, Y. Identification of a Novel Cannabimimetic Phenylacetylindole, Cannabipiperidietanone, as a Designer Drug in a Herbal Product and Its Affinity for Cannabinoid CB₁ and CB₂ Receptors. *Chem. Pharm. Bull.* 2011, *59*, 9, 1203-1205.
- [36] Uchiyama, N.; Kawamura, M.; Kikura-Hanajiri, R.; Goda, Y. Identification of two new-type synthetic cannabinoids, *N*-(1-adamantyl)-1-pentyl-1*H*-indole-3-carboxamide (APICA) and *N*-(1-adamantyl)-1-pentyl-1*H*-indazole-3-carboxamide (APINACA), and detection of five synthetic cannabinoids, AM-1220, AM-2233, AM-1241, CB-13 (CRA-

- 13), and AM-1248, as designer drugs in illegal products. *Forensic Toxicology* **2012**, 30, 114-125.
- [37] Uchiyama, N.; Kawamura, M.; Kikura-Hanajiri, R.; Goda, Y. URB-754: A new class of designer drug and 12 synthetic cannabinoids detected in illegal products. *Forensic Toxicology* **2013**, 227, 21-32.
- [38] Grob, R. L.; Barry, E. F. *Modern Practice of Gas Chromatography*. 4th ed. New Jersey: Wiley 2004.
- [39] Robards, K.; Haddad, P. R.; Jackson, P. E. Principles and practice of modern chromatographic methods. Elsevier, New York: 2004.
- [40] Agilent Capillary GC Columns, Agilent Technologies.
- [41] Watson, J. T. *Introduction to Mass Spectrometry: Biomedical, environmental and forensic applications*. New York: Raven Press 1976.
- [42] Steen, H.; Küster, B.; Mann, M. Quadrupole time-of-flight versus triple-quadrupole mass spectrometry for the determination of phosphopeptides by precursor ion scanning. *Journal of Mass Spectrometry* **2001**, 36, 782-290.
- [43] Chernushevich, I. V.; Loboda, A. V.; Thomson, B. A. An introduction to quadrupole-time-of-flight mass spectrometry. *Journal of Mass Spectrometry* 2001, 36, 849-865.
- [44] Hudson, S.; Ramsey, J. The emergence and analysis of synthetic cannabinoids. *Drug Testing and Analysis* 2011.
- [45] De Castro, A.; Piñeiro, B.; Lendoiro, E.; Cruz, A.; López-Rivadulla, M. Quantification of selected synthetic cannabinoids and Δ^9 -tetrahydrocannabinol in oral fluid by liquid chromatography-tandem mass spectrometry. *Journal of Chromatography A* 2013, 1295, 99-106.
- [46] Choi, H.; Heo, S.; Choe, S.; Yang, W.; Park, Y.; Kim, E.; Chung, H.; Lee, J. Simultaneous analysis of synthetic cannabinoids in the materials seized during drug trafficking using GC-MS. *Anal. Bioanal. Chem.* 2013, 405, 3937-3944.
- [47] Hutter, M.; Broecker, S.; Kneisel, S.; Auwärter, V. Identification of the major urinary metabolites in man of seven synthetic cannabinoids of the aminoalkylindole type present as adulterants in 'herbal mixtures' using LC-MS/MS techniques. *Journal of Mass Spectrometry* 2012, 47, 54-66.

- [48] Kneisel, S.; Auwärter, V. Analysis of 30 synthetic cannabinoids in serum by liquid chromatography-electrospray ionization tandem mass spectrometry after liquid-liquid extraction. *Journal of Mass Spectrometry* 2012, 47, 825-835.
- [49] Kneisel, S.; Speck, M.; Moosmann, B.; Corneillie, T. M.; Butlin, N. G.; Auwärter, V. LC/ESI-MS/MS method for quantification of 28 synthetic cannabinoids in neat oral fluid and its application to preliminary studies on their detection windows. *Anal Bioanal Chem* 2013, 405, 4691-4706.
- [50] Gwak, S.; Arroyo-Mora, L. E.; Almirall, J. R. Qualitative analysis of seized synthetic cannabinoids and synthetic cathinones by gas chromatography triple quadrupole tandem mass spectrometry. *Drug Testing and Analysis* 2015, 7, 2, 121-130.
- [51] Ernst, L.; Schiebel, H.; Theuring, C.; Lindigkeit, R.; Beuerle, T. Identification and characterization of JWH-122 used as new ingredient in "Spice-like" herbal incenses. *Forensic Science International* 2011, 208, 31-35.
- [52] Lindigkeit, R.; Boehme, A.; Eiserloh, I.; Luebbecke, M.; Wiggermann, M.; Ernst, L.; Beuerle, T. Spice: A never ending story? *Forensic Science International* 2009, 191, 58-63.
- [53] Vincenti, M.; Salomone, A.; Gerace, E.; Pirro, V. Application to Mass Spectrometry to Hair Analysis for Forensic Toxicology. *Mass Spectrometry Reviews* 2013, 32, 312-332
- [54] Fernandez-Peralbo, M. A.; Luque de Castro, M. D. Preparation of urine samples prior to targeted or untargeted metabolomics mass-spectrometry analysis. *Trends in Analytical Chemistry* 2012, 41, 75-85.
- [55] Zhang, F.; Yu, C.; Wang, W.; Fan, R.; Zhang, Z.; Guo, Y. Rapid simultaneous screening and identification of multiple pesticide residues in vegetables. *Analytica Chimica Acta* 2012, 757, 39-47.
- [56] Hernández, F.; Portolés, T.; Pitarch, E.; López, F. Gas chromatography coupled to high-resolution time-of-flight mass spectrometry to analyze trace-level organic compounds in the environment, food safety and toxicology. *Trends in Analytical Chemistry* 2011, 30, 2, 388-400.
- [57] Kang, W.; Zhang, F.; Su, Y.; Guo, Y. Application of gas chromatography-quadrupole-time-of-flight-mass spectrometry for post-target analysis of volatile compounds in *Fructus Amomi*. *European Journal of Mass Spectrometry* 2013, 19, 2, 103-110.

[58] Huestis, M. A.; Mitchell, J. M.; Cone, E. J. Detection times of marijuana metabolites in urine by immunoassay and GC-MS. *Journal of Toxicology* 1995, 19, 6, 443-449.

[59] Morgan, C. J. Impact of cannabidiol on the acute memory and psychotomimetic effects of smoked cannabis: naturalistic study. *British Journal of Psychiatry* 2010, 197, 4, 285-290.

APPENDICES

Appendix 1: Full scan total ion chromatogram and product ion mass spectra obtained at CE=10, 20, 40 eV for synthetic cannabinoids by DART-QTOF.

AM2201 2'-naphthyl isomer

AM2201 N-(2-fluoropentyl) isomer

AM2201 N-(3-fluoropentyl) isomer

AM2201 N-(4-fluoropentyl) isomer

AM694 3-iodo isomer

AM694 4-iodo isomer

JWH 018 2'-naphthyl-N-(1-ethylpropyl)

JWH 018 2'-naphthyl isomer

JWH 018 2'naphthyl-N-(1,1-dimethylpropyl) isomer

JWH 018 2'-naphthyl-N-(1,2-dimethylpropyl) isomer

JWH 018 2'-naphthyl-N-(1-methylbutyl) isomer

JWH 018 2'-naphthyl-N-(2,2-dimethylpropyl) isomer

JWH 018 2'-naphthyl-N-(2-methylbutyl) isomer

JWH 018 2'-naphthyl-N-(3-methylbutyl) isomer

JWH 018 N-(1,1-dimethylpropyl) isomer

JWH 018 N-(1,2-dimethylpropyl) isomer

JWH 018 N-(1-ethylpropyl) isomer

JWH 018 N-(1-methylbutyl) isomer

JWH 018 N-(2,2dimethylpropyl) isomer

JWH 018 N-(2-methylbutyl) isomer

JWH 018 N-(3-methylbutyl) isomer

JWH 073 2'-naphthyl isomer

JWH 073 2'-naphthyl-N-(1,1-dimethylethyl) isomer

JWH 073 2'-naphthyl-N-(1-methylpropyl) isomer

JWH 073 2'-naphthyl-N-(2-methylpropyl) isomer

JWH 073 N-(1,1-dimethylethyl) isomer

JWH 073 N-(1-methylpropyl) isomer

JWH 073 N-(2-methylpropyl) isomer

JWH 081 2-methoxynaphthyl isomer

JWH 081 3-methoxynaphthyl isomer

JWH 081 5-methoxynaphthyl isomer

JWH 081 6-methoxynaphthyl isomer

JWH 081 7-methoxynaphthyl isomer

JWH 081 8-methoxynaphthyl isomer

JWH 122 2-methylnaphthyl isomer

JWH 122 3-methylnaphthyl isomer

JWH 122 5-methylnaphthyl isomer

JWH 122 6-methylnaphthyl isomer

JWH 122 7-methylnaphthyl isomer

JWH 122 8-methylnaphthyl isomer

JWH 200 2'-naphthyl isomer

JWH 203 3-chlorophenyl isomer

JWH 203 4-chlorophenyl isomer

JWH 210 2-ethylnaphthyl isomer

JWH 210 3-ethylnaphthyl isomer

JWH 210 5-ethylnaphthyl isomer

JWH 210 6-ethylnaphthyl isomer

JWH 210 7-ethylnaphthyl isomer

JWH 210 8-ethylnaphthyl isomer

JWH 251 3-methylphenyl isomer

JWH 251 4-methylphenyl isomer

JWH 398 2-chloronaphthyl isomer

JWH 398 3-chloronaphthyl isomer

JWH 398 5-chloronaphthyl isomer

JWH 398 6-chloronaphthyl isomer

JWH 398 7-chloronaphthyl isomer

JWH 398 8-chloronaphthyl isomer

RCS-4 2-methoxy isomer

RCS-4 3-methoxy isomer

RCS-8 3-methoxy isomer

RCS-8 4-methoxy isomer

(+)WIN 55212-2

1'-Naphthoyl Indole

4-Quinolone-3-Carboxamide CB2 Ligand

AKB48

AM1220

AM1235

AM1241

AM1248

AM2201

AM2232

AM2233

AM251

AM630

AM679

AM694

CB-13

CB-25

CB-52

CB-86

HU-210

JWH 011

JWH 015

JWH 016

JWH 018

JWH 018 6-methoxyindole analog

JWH 018 adamantyl analog

JWH 018 adamantyl carboxamide

JWH 018 N-(4,5-epoxypentyl) analog

JWH 018 N-(5-chloropentyl) analog

JWH 019

JWH 020

JWH 022

JWH 030

JWH 072

JWH 073

JWH 073 2-methylnaphthyl analog

JWH 073 4-methylnaphthyl analog

JWH 081

JWH 098

JWH 122

JWH 122 N-(4-pentenyl) Analog

JWH 145

JWH 147

JWH 175

JWH 180

JWH 182

JWH 200

JWH 201

JWH 203

JWH 210

JWH 249

JWH 250

JWH 251

JWH 302

JWH 307

JWH 309

JWH 368

JWH 369

JWH 370

JWH 398

JWH 424

JZL 195

MAM2201

MDA 19

MDA 77

Cannabipiperidiethanone

RCS-4

RCS-4-C4 Homolog

RCS-8

STS-135

UR-144

URB754

XLR11

Appendix 2: GC chromatograms and normalized extracted mass spectra for synthetic cannabinoids obtained by GC-QTOF.

AM2201 2'-naphthyl isomer

AM2201 N-(4-fluoropentyl) isomer

AM694 3-iodo isomer

AM694 4-iodo isomer

JWH 018 2'-naphthyl-N-(1-ethylpropyl)

JWH 018 2'-naphthyl isomer

JWH 018 2'-naphthyl-N-(1,1-dimethylpropyl) isomer

JWH 018 2'-naphthyl-N-(1,2-dimethylpropyl) isomer

JWH 018 2'-naphthyl-N-(1-methylbutyl) isomer

JWH 018 2'-naphthyl-N-(2,2-dimethylpropyl) isomer

JWH 018 2'-naphthyl-N-(2-methylbutyl) isomer

JWH 018 2'-naphthyl-N-(3-methylbutyl) isomer

JWH 018 N-(1,1-dimethylpropyl) isomer

JWH 018 N-(1,2-dimethylpropyl) isomer

JWH 018 N-(1-ethylpropyl) isomer

JWH 018 N-(1-methylbutyl) isomer

JWH 018 N-(2,2dimethylpropyl) isomer

JWH 018 N-(2-methylbutyl) isomer

JWH 018 N-(3-methylbutyl) isomer

JWH 073 2'-naphthyl isomer

JWH 073 2'-naphthyl-N-(1,1-dimethylethyl) isomer

JWH 073 2'-naphthyl-N-(1-methylpropyl) isomer

JWH 073 2'-naphthyl-N-(2-methylpropyl) isomer

JWH 073 N-(1,1-dimethylethyl) isomer

JWH 073 N-(1-methylpropyl) isomer

JWH 073 N-(2-methylpropyl) isomer

JWH 081 2-methoxynaphthyl isomer

JWH 081 3-methoxynaphthyl isomer

JWH 081 5-methoxynaphthyl isomer

JWH 081 6-methoxynaphthyl isomer

JWH 081 7-methoxynaphthyl isomer

JWH 081 8-methoxynaphthyl isomer

JWH 122 2-methylnaphthyl isomer

JWH 122 3-methylnaphthyl isomer

JWH 122 5-methylnaphthyl isomer

JWH 122 6-methylnaphthyl isomer

JWH 122 7-methylnaphthyl isomer

JWH 122 8-methylnaphthyl isomer

JWH 200 2'-naphthyl isomer

JWH 203 3-chlorophenyl isomer

JWH 203 4-chlorophenyl isomer

JWH 210 2-ethylnaphthyl isomer

JWH 210 3-ethylnaphthyl isomer

JWH 210 5-ethylnaphthyl isomer

JWH 210 6-ethylnaphthyl isomer

JWH 210 7-ethylnaphthyl isomer

JWH 210 8-ethylnaphthyl isomer

JWH 251 3-methylphenyl isomer

JWH 251 4-methylphenyl isomer

JWH 398 2-chloronaphthyl isomer

JWH 398 3-chloronaphthyl isomer

JWH 398 5-chloronaphthyl isomer

JWH 398 6-chloronaphthyl isomer

JWH 398 7-chloronaphthyl isomer

JWH 398 8-chloronaphthyl isomer

RCS-4 2-methoxy isomer

RCS-4 3-methoxy isomer

RCS-8 3-methoxy isomer

RCS-8 4-methoxy isomer

(-)-11-nor-9-carboxy-Δ⁹-THC

(-)-CP 47,497

(-)-CP 55,940

(+)-CP 47,497

(+)-CP 55,940

(+)WIN 55212-2

(±)3epi CP 47,497-C8-homolog

(±)5-epi CP 55,940

(±)CP 47,497

(±)-CP 47,497-C8-homolog

(±)-CP 55,940

(±)-**epi** CP 47, 497

(±)WIN 55212 (mesylate)

1'-Naphthoyl Indole

4-Quinolone-3-Carboxamide CB2 Ligand

AKB48

AM1220

AM1235

AM1241

AM1248

AM2201

AM2232

AM2233

AM630

AM679

AM694

Cannabidiol

CB-13

CB-25

CB-52

CB-86

CP 47,497-para-quinone analog

HU-210

HU-211

HU-308

JP104

JWH 007

JWH 011

JWH 015

JWH 018 6-methoxyindole analog

JWH 018 adamantyl analog

JWH 018 adamantyl carboxamide

JWH 018 N-(4,5-epoxypentyl) analog

JWH 018 N-(5-bromopentyl) analog

JWH 018 N-(5-chloropentyl) analog

JWH 019

JWH 020

JWH 022

JWH 030

JWH 031

JWH 072

JWH 073

JWH 073 2-methylnaphthyl analog

JWH 073 4-methylnaphthyl analog

JWH 081

JWH 098

JWH 122

JWH 122 N-(4-pentenyl) Analog

JWH 145

JWH 147

JWH 175

JWH 180

JWH 182

JWH 200

JWH 201

JWH 203

JWH 210

JWH 249

JWH 250

JWH 251

JWH 302

JWH 307

JWH 309

JWH 368

JWH 369

JWH 370

JZL 195

MAM2201

MDA 19

Cannabipiperidiethanone

RCS-4

RCS-8

STS-135

URB597

URB754

URB937

WIN 54461

XLR11

Appendix 3: Normalized extracted mass spectra for GC-QTOF Mixture 2.

Appendix 4: Normalized extracted mass spectra for GC-QTOF Mixture 3.