Boston University

OpenBU **http://open.bu.edu**

Boston University Theses & Dissertations Boston University Theses & Dissertations

2022

Detection of pesticides in cannabis flowers: a comparative study utilizing DART®-MS, LC-MS/MS, and QuEChERS

https://hdl.handle.net/2144/45569

Downloaded from DSpace Repository, DSpace Institution's institutional repository

BOSTON UNIVERSITY

SCHOOL OF MEDICINE

Thesis

DETECTION OF PESTICIDES IN CANNABIS FLOWERS: A COMPARATIVE STUDY UTILIZING DART®-MS, LC-MS/MS, AND QuEChERS

by

ABBY NICOLE HOULISTON

B.A., College of Saint Benedict, 2020

Submitted in partial fulfillment of the

requirements for the degree of

Master of Science

2022

© 2022 by
ABBY NICOLE HOULISTON
All rights reserved

Approved by

First Reader ____

Adam B. Hall, Ph.D., ABC-FD
Assistant Professor of Anatomy & Neurobiology

Second Reader ____

Christopher J. Hudalla, Ph.D.
Founder and CSO of ProVerde Laboratories, Inc.

ACKNOWLEDGMENTS

First off, I would like to thank Dr. Adam Hall for his continued support, encouragement, and advice throughout this entire project. Without him, this project would not have been possible. I would like to also thank Professor Sabra Jones, Dr. Chris Hudalla from ProVerde Laboratories Inc., and Jamie Foss from PerkinElmer Inc. Their insight and aid in the methodology of this project and their assistance with keeping the instruments running was crucial and very much appreciated. Thank you to Patty Jones for assisting in the ordering of all laboratory supplies.

A big thank you to PerkinElmer for providing the QSight 220 LC-MS/MS instrument and to IonSense, Inc. for providing the DART®-MS. I would also like to thank the Center for Advanced Research in Forensic Science (CARFS) for funding this project.

Finally, I want to thank my family and friends who have shown their unwavering support during my graduate studies. I truly wouldn't have been able to accomplish any of this without you.

DETECTION OF PESTICIDES IN CANNABIS FLOWERS: A COMPARATIVE STUDY UTILIZING DART®-MS, LC-MS/MS, AND QuEChERS

ABBY NICOLE HOULISTON

ABSTRACT

Cannabis has been used for thousands of years, though only recently has its use become more accepted and even legalized in some states. Cannabis is a schedule I substance and is considered federally illegal. Each state legislature can regulate the production, sale, and consumption of cannabis along with cultivation practices and the use of plant growth regulators or pesticides. With the lack of restrictions and required testing, there is minimal standardized testing for pesticides with limited development of novel analytical methods. The quick, easy, cheap, effective, rugged, and safe sample preparation method known as QuEChERS has shown to be a promising and effective method for the isolation of pesticides. This is a dispersive SPE method to preconcentrate the pesticides and can be modified depending on the sample matrix. In this study, two different methods for sample clean up within QuEChERS were compared along with a solvent extraction of five separate strains of cannabis flower. Instrumentation included ambient ionization using direct analysis in real time coupled to a single quadrupole mass spectrometer (DART®-MS) and liquid chromatography - tandem mass spectrometry (LC-MS/MS).

Method optimization was performed using a 16-component standard pesticide mixture on both instruments. The DART®-MS instrument required a higher concentration of the standard mixture due to a decrease in sensitivity in comparison to the LC-MS/MS approach. Sample preparation was performed with QuEChERS extractions with one clean-

up method containing ChloroFiltr® and one without. Solvent extractions were also performed in both acetonitrile (ACN) and methanol (MeOH). Because the DART® instrument is coupled to a single quadrupole, the scan will detect all ions present in the sample along with any background ions from the ambient environment. In solvent extraction, the most intense peaks corresponded to THC and CBD followed by THCA and CBDA. When utilizing the QuEChERS procedure, the CBD and THC peaks were still present, but the THCA and CBDA peaks were significantly diminished. There were many peaks at a relatively small intensity that, without an analyte library, couldn't be identified. This suggests DART®-MS could potentially serve as a general screening technique for pesticide residues. LC-MS/MS was also used to analyze the effectiveness of both QuEChERS extraction techniques and the solvent extraction with both an ACN mobile phase and a MeOH mobile phase. The QuEChERS extractions did not show a significant difference between utilizing the ChloroFiltr® or not. The QuEChERS extraction detected significantly more analytes than the solvent extraction, and the MeOH mobile phase showed much higher ion counts compared to the ACN.

TABLE OF CONTENTS

ACKNOWLEDGMENTS iv

ABSTRACT v

TABLE OF CONTENTS vii

LIST OF TABLES x

LIST OF FIGURES xi

LIST OF ABBREVIATIONS xii

1. INTRODUCTION 1

1.1 Origins of Cannabis 1

1.1.1 Cannabis Sativa L. and its uses 1

1.1.2 Legal Status of C. Sativa 2

1.2 Growing Methods 4

1.2.1 Cannabis Growing Environments 4

1.2.2 Environmental determinations for Pesticide Use 5

1.3 Properties of Pesticides 5

1.3.1 Pesticide effects on the human body 5

1.3.2 Legal Status of Pesticide Use 7

1.4 Analytical Techniques for pesticide detection 8

1.4.1 Extraction Techniques 8

1.4.2 Instrumental Analysis 10

2. MATERIALS AND METHODS 13

2.1 DART®-MS 13

2.1.1 Standard Preparation 13
2.1.2 Method Development 15
2.1.3 Analytical Method 16
2.1.4 Cannabis Sample Preparation 19
2.1.5 Solvent Extraction 19
2.1.6 QuEChERS Extraction 20
2.2 LC/MS/MS Analysis 22
2.2.1 Reagent Preparation 22
2.2.2 Standard and Sample Preparation 23
2.2.3 Method Development 23
2.2.4 Analytical Method 23
3. RESULTS AND DISCUSSION 27
3.1 DART®-MS Analysis 27
3.1.1 Pesticide Standard Optimizations 27
3.1.2 Cannabis solvent extraction 35
3.1.3 Cannabis QuEChERS Extraction 37
3.2 LC/MS/MS Analysis 40
3.2.1 LC Column 40
3.2.2 Optimizing LC Gradient 40
3.2.3 Arcata Trainwreck Analysis 43
3.2.4 Ghost Train Haze Analysis 47
3.2.5 Black Triangle Kush Analysis 49

3.2.6 GG #4 Analysis....52
3.2.7 Walker Kush Analysis....55
3.2.8 Comparative Analysis....57
4. Conclusions....59
BIBLIOGRAPHY....61
CURRICULUM VITAE....65

LIST OF TABLES

Table 1. Legal Status of Marijuana by State as of January 2022 3

Table 2. DART® Parameters and Waters™ ACQUITY® QDa Mass Detector Parameters 16

Table 3. MRM Transition Values for SPEX Standard. 25

Table 4. LC Method and MS Source Conditions. 26

Table 5. Pesticide Analytes and Their Respective Ion Peaks Detected in DART®-QDa Analysis 29

Table 6. Direct Comparison of Detected MRM Transitions in Arcata Trainwreck Sample 46

Table 7. Direct Comparison of Detected MRM Transitions in Ghost Train Haze Sample 48

Table 8. Direct Comparison of Detected MRM Transitions in Black Triangle Kush Sample 51

Table 9. Direct Comparison of Detected MRM Transitions in GG #4 Sample 54

Table 10. Direct Comparison of Detected MRM Transitions in Walker Kush Sample 56

LIST OF FIGURES

Figure 1. DART® Ion Source Schematic 10

Figure 2. Structures and molecular weights of all 16 analytes in the SPEX pesticide standard. 14

Figure 3. QuickStrip™ sample card in card holder...17

Figure 4. DART® Ion source interfaced to a Waters™ ACQUITY® QDa Mass Detector 18

Figure 5. Solvent Extraction Procedure Flowchart. 20

Figure 6. QuEChERS Extraction Procedure Flowchart 22

Figure 7. Mass spectra for pesticide standard mixture 28

Figure 8. Mass spectrum of the 1000 ng/mL standard dilution 30

Figure 9. Pesticide Standard (1000 ng/mL) Temperature Optimization 32

Figure 10. Pesticide Standard (1000 ng/mL) Cone Voltage Optimization.......................33

Figure 11. Pesticide Standard (1000 ng/mL) Linear Rail Speed Optimization 35

Figure 12. DART®-MS Mass Spectrum for Solvent Extracts of GTH in MeOH and ACN 36

Figure 13. DART®-MS Mass Spectrum of QuEChERS Extraction on GTH...................39

Figure 14. Chromatograms of Varying Starting Mobile Phase Concentrations 41

Figure 15. Aldicarb Sulfoxide MRMs at Each Starting Mobile Phase Concentration......43

LIST OF ABBREVIATIONS

ACN ..... Acetonitrile

AT ..... Arcata Trainwreck

BC ..... Before Christ

BTK ..... Black Triangle Kush

C. Sativa ..... Cannabis Sativa L.

CBCA ..... Cannabichromenic acid

CBD ..... Cannabidiol

CBDA ..... Cannabidiolic Acid

CCL2 ..... Collision Cell Lens 2

CE ..... Collision Energy

CPS ..... Counts per Second

CSA ..... Controlled Substances Act

DART® ..... Direct Analysis in Real Time

DART®-MS ..... Direct Analysis in Real Time – Mass Spectrometry

dSPE ..... dispersive Solid Phase Extraction

EPA ..... Environmental Protection Agency

EV ..... Entrance Voltage

FIFRA ..... Federal Insecticide, Fungicide, and Rodenticide Act

g ..... Grams

GC-MS ..... Gas Chromatography - Mass Spectrometry

GCB ..... Graphitized Carbon Black

GG .... GG #4

GTH .... Ghost Train Haze

H_2O .... Water

HS-SPME .... Headspace-Solid Phase Microextraction

ID .... Internal Diameter

LC .... Liquid Chromatography

LC-MS .... Liquid Chromatography - Mass Spectrometry

LC-MS/MS .... Liquid Chromatography – Tandem Mass Spectrometry

LLE .... Liquid-Liquid Extraction

$[M+H]^+$ .... Protonated Molecular Ion

m/z .... Mass-to-Charge Ratio

MS .... Mass Spectrometry

MS/MS .... Tandem Mass Spectrometry

MeOH .... Methanol

mg .... Milligram

$MgSO_4$ .... Magnesium Sulfate

mL .... Milliliter

mL/min .... Milliliter per Minute

mm .... Millimeter

mM .... Millimolar

mm/sec .... Millimeter per Second

MRM .... Multiple Reaction Monitoring

NaCl .... Sodium Chloride

NETA .... New England Treatment Access

ng .... Nanogram

ng/mL .... Nanogram per Milliliter

PSA .... Primary Secondary Amine

Q-TOF .... Quadrupole Time of Flight

Q1 .... QuEChERS with ChloroFiltr®

Q2 .... QuEChERS without ChloroFiltr®

QuEChERS .... Quick, Easy, Cheap, Effective, Rugged, Safe

RPM .... Rotation per Minute

sec .... Seconds

SPE .... Solid Phase Extraction

SPME .... Solid Phase Microextraction

SPME-TM .... Solid Phase Microextraction-Transmission Mode

SVP .... Standard Voltage and Pressure

THC Δ9-Tetrahydrocannabinol

THCA .... Tetrahydrocannabinolic Acid

US .... United States

USDA .... United States Department of Agriculture

V .... Volts

WK .... Walker Kush

°C .... Degrees Celsius

μg/mL .... Microgram per Milliliter

μL .... microliter

μm... Micrometer

1. INTRODUCTION

1.1 Origins of Cannabis

1.1.1 Cannabis Sativa L. and its uses

Cannabis Sativa L. (C. Sativa) has been used for thousands of years, originating in central Asia as an herbal medicine in the BC era. In the years since discovery, C. Sativa has contributed significant economic value and medicinal value worldwide. Early cultivators of the plant targeted the economic value of the fibers that it produced, allowing them to make anything from fishnets to textile materials (1). While most known today for the psychoactive drugs in the leafy portion of the plant, the entirety of the plant has its own variety of uses. The fibers of the plant, as mentioned previously, can be used for paper and cloth. The seeds of the plant are used to extract Cannabis Sativa seed oil, which is gaining popularity in cosmetics, skincare, and even as an additive in many foods (2).

Not only does C. Sativa have a historic economic value, but there is also a significant religious value to the plant. The Rastafari religious and political movement, rooted in Jamaica, views marijuana as a “holy herb” (3). Depending on the religion, the oils from the seeds, the flower, and the burning of the whole plant have been utilized in various religions including Hindu, Buddhism, and even referenced in the bible. C. Sativa has been linked to healing powers and connecting with spirits (1)(4).

The psychoactive components of cannabis are in increasingly high demand. The most potent and targeted psychoactive component of cannabis is Δ-9-tetrahydrocannabinol or THC (1). THC is a cannabinoid or a chemically active compound that is found in cannabis. Some cannabinoids have psychoactive properties, these include both THC and

cannabidiol or CBD. The THC and other cannabinoids in a cannabis plant are found in the trichomes, or cystolithic hairs, of the female plant (1). These trichomes appear as little clear buds underneath a microscope. These cannabinoids are the main components that help to classify the difference between hemp and marijuana plants. Marijuana is classified as containing more than 0.3% THC whereas hemp contains less than 0.3% THC. The legal status of both growing processes and consumer access is also dependent on the established level of 0.3% THC content.

1.1.2 Legal Status of C. Sativa

In 1970, the Controlled Substances Act (CSA) was passed as law in the United States. This act divides all federally controlled substances into various classes. The level of class starts at schedule I which has high potential for abuse and no accepted medical use and goes down to schedule 5 substances which have low potentials for abuse and an accepted medical use. Marijuana is considered a hallucinogen and is classified as a schedule I substance (5).

As mentioned earlier, hemp is a different classification of C. Sativa that differs by the threshold of a lower content of THC. Hemp is not covered in the Controlled Substances Act, but rather by the USDA Farm Bill which was passed in December of 2018. The Farm Bill defines hemp as any part of the cannabis plant or its extracts that contain less than 0.3% THC by weight, thus excluding it from the CSA. Because hemp isn't federally regulated, states can provide individual requirements and regulations for the cultivation of

hemp. If states don't provide these regulations, then the United States Department of Agriculture provides general guidelines that must be followed (5).

Each state in the US has its own individual laws for the legality of marijuana use and cultivation. These laws are further separated between medical marijuana and recreational use marijuana. Table 1 shows the legal status of marijuana for each state.

Table 1. Legal Status of Marijuana by State as of January 2022

Fully Legal		Medical and Decriminalized	
Alaska	Montana	Delaware	
Arizona	Nevada	Hawaii	
California	New Jersey	Louisiana	
Colorado	New Mexico	Maryland	
Connecticut	New York	Minnesota	
District of Columbia	Oregon	Missouri	
Illinois	Vermont	New Hampshire	
Maine	Virginia	North Dakota	
Massachusetts	Washington	Ohio	
Michigan		Rhode Island	
Medical	**Medical CBD Oil Only**	**Decriminalized**	**Fully Illegal**
Alabama	Georgia	Mississippi	Idaho
Arkansas	Indiana		Kansas
Florida	Iowa		Nebraska
Oklahoma	Kentucky		North Carolina
Pennsylvania	Tennessee		South Carolina
South Dakota	Texas		Wyoming
Utah	Wisconsin		
West Virginia			

As of January 2022, 19 states have fully legalized marijuana. Ten states have legalized medical marijuana and decriminalized possession of small amounts of marijuana (amounts varying by state), seven states have legalized medical marijuana but not decriminalized it, and Mississippi has decriminalized marijuana but has not legalized medical marijuana. Seven states have legalized only CBD oil use for medical purposes and in six states marijuana is fully illegal for any purpose (6).

1.2 Growing Methods

1.2.1 Cannabis Growing Environments

Cannabis can be grown in a variety of environments depending on what is available, what state laws require, and what type of license to grow is obtained. Grow environments include indoor grows, greenhouse grows, and field crops or outdoor growing. An indoor cannabis grow doesn't have natural ventilation or lighting as seen in greenhouse and outdoor grows, and if humidity isn't controlled correctly fungal pathogens and bacteria can thrive in these growing conditions. Outdoor grows or field crops are susceptible to animal activity and insect pests (7).

There is no given "good environment" to grow cannabis in. States can determine which environment a farmer can grow in, and any environment can grow good or bad cannabis. To the best of the authors knowledge, based on the literature available, there is no test or analysis that can be performed to determine the environment in which the cannabis plant was grown. An indoor and greenhouse grow will be significantly more expensive than an outdoor as they require significantly higher costs in soil, fans, specific

lighting, humidifiers and dehumidifiers and HVAC systems (8). The chosen growing environment for each farmer will further indicate what forms of pest prevention are necessary.

1.2.2 Environmental determinations for Pesticide Use

Pesticides can be broken down into various classes most commonly including fungicides, rodenticides, insecticides, and herbicides. A recent study by Simon Fraser University in Burnaby, BC, Canada looked at the prevalence of molds, fungus and other pathogens in cannabis plants grown in various environments. These pathogens, molds, and fungi all affect the quality of the harvested plant no matter the grow environment along with the plants ability to grow (9). The authors focused specifically on fungi affecting the roots, leaves, and flowers of the cannabis plant. Each growing environment showed different types of fungi. When comparing indoor to greenhouse grows, the indoor grow had a higher content of fungi. Fungi and pathogens also varied by season especially in outdoor grows (9). While not all fungi stunt plant growth, it can be difficult to contain and manage fungi that suppresses plant growth when the fungi strains are changing over time.

1.3 Properties of Pesticides

1.3.1 Pesticide effects on the human body

Pesticides in agricultural settings pose a wide variety of risks. Not only is there a risk to the consumer of the product in whatever form it may be in, but there is also an occupational risk to the grower or farmer. Cannabis, whether it be for recreational or medical use, does not have well established regulations for pesticide use. Those users in

the medical cannabis community can be more susceptible to the adverse effects that pesticides have on the human body. For states that have legalized medical marijuana use, the most common qualifying classes of medical conditions consist of pain conditions and movement disorders (10).

In a study by Pinkhasova et al., a digital database called the Comparative Toxicogenomics Database was utilized to simulate the interactions of common pesticides and cannabinoids with different medical conditions. It was found that pesticide exposure takes similar signaling pathways that relate to seizures (10). There is a pressing need for further research around pesticide interactions with common medical conditions that qualify for the use of medical marijuana.

The way that different pesticides can affect human health depends on a variety of different factors. Pesticide exposure can occur from direct contact with skin or inhalation, typically by the grower, both of these, along with ingestion, can affect the consumers (11). Nicolopoulou-Stamati et al. published a review of the many health effects seen in various classes of pesticides. The most common that were found included endocrine-disrupting activity, reproductive toxicity, and neurotoxicity (12). Another study found that pesticides can cause hormonal imbalances, asthma and increased cancer risk (11). Some health effects are specific to the class of pesticide while others are widespread and not specific to class or type.

While there is a large amount of caution that needs to be taken when determining what pesticides growers may need to use, it is also important to understand how often one person may be handling these chemicals. Safely using these chemicals doesn't mean one

will immediately be at high risk for severe health effects. Often the adverse health risks associated with exposure to pesticides have other cofounding health risks or diseases, which is currently a field in need of further research and understanding (13).

1.3.2 Legal Status of Pesticide Use

Pesticides used in agriculture must be registered with the United States Environmental Protection Agency (EPA). Each pesticide is regulated and controlled for the crop that it can legally be applied to. There are, however, several pesticides that aren't required to be registered with the EPA. FIFRA, or the Federal Insecticide, Fungicide, and Rodenticide Act, is a federal statute that aids in regulating the sale and use of pesticides. FIFRA requires that for a pesticide to be registered, it must show that it "will not cause reasonable adverse effects on the environment" (14). The only pesticides that aren't required to be registered come from a select list known as the 25b minimum risk products. Any pesticide product that contains these specific ingredients isn't required to be registered with the EPA as a pesticide.

Due to hemp being excluded from the CSA, the EPA regulates what pesticides and plant growth regulators can be applied to the plant during the cultivation process. There is currently a list of 59 substances approved for use on hemp, 58 of these pesticides are considered biopesticides and one is a conventional pesticide (15). Unlike hemp, the EPA does not have any regulations on plant growth regulators or pesticides for marijuana because it is still considered federally illegal. Due to the fact that marijuana legality depends on state legislature, the rest of the cultivation process, including the use of

pesticides is also covered by state law. With the legalization of marijuana being relatively new and recent, so is state regulation of pesticide use. Some states are very stringent on pesticide use and some states have minimal to no restrictions on what can be used on the plant and what testing is required on the plant material.

1.4 Analytical Techniques for pesticide detection

1.4.1 Extraction Techniques

One of the more recent and promising extraction techniques for detection of trace amounts of pesticides is by the quick, easy, cheap, effective, rugged, and safe method known as QuEChERS. This extraction method is a dispersive solid phase extraction method (dSPE). It utilizes buffer salts in an aqueous/organic solvent mixture to extract the pesticides from the sample matrix, which is then followed by a clean-up step that limits the amount of signal inhibitors in the sample. This method was first proposed back in 2003 for the extraction of pesticides from fruits and vegetables (16). While there are several published official methods for QuEChERS extractions including the original unbuffered, AOAC 2007.01, and EN 15662 methods, the extraction process is made to be modified due to sample quantity, matrix, and even the targeted analytes (17) (18). QuEChERS as a sample preparation method has been widely used in pesticide analysis in different food and crop samples including cereals (19), red wine (20), medicinal herbs (21), and orange juice (18).

The original unbuffered methods for QuEChERS contain magnesium sulfate ($MgSO_4$) and sodium chloride (NaCl). The buffered methods vary in salt components, but

all still contain $MgSO_4$. In the clean-up step, the basic components of the sorbents are $MgSO_4$ and primary secondary amine (PSA). $MgSO_4$ is used to remove excess water in the sample while PSA is used to remove the sugars and fatty acids from certain matrices. C18 as a sorbent is very useful in higher fat containing products including avocados and milk. Graphitized carbon black (GCB) is used in matrices with high levels of non-polar pigments. This is commonly seen in leafy green plant material including spinach. GCB removes these pigments very well but also risks the loss of planar pesticides. To counteract the removal of planar pesticides, a new sorbent called ChloroFiltr® has been developed. ChloroFiltr® works to do the same as GCB without inhibiting the detection of any planar pesticides (22). Any combination of these sorbents can be chosen in combination for the clean-up step.

Other various extraction techniques have been shown for the recovery of pesticides in various sample matrices. Solid-phase extraction (SPE), liquid-liquid extraction (LLE), and solid-phase microextraction (SPME) have been utilized in pesticide analysis (23). The most difficult aspect of LLE is that it can't be automated. LLE is also very labor intensive and requires extensive solvent handling and produces large amounts of waste. SPE overcomes the lack of automation though still lacks the needs of selectivity and repeatability. SPME has shown to be a very promising technique. SPME shows better sensitivity, reproducibility and is a relatively cheap sample preparation process. In cannabis specifically, headspace-SPME (HS-SPME) has been used to target cannabinoids and terpenes (24). More recently, a new SPME process called transmission mode or SPME-TM has been developed. When dealing with trace amounts of pesticide residues, detection limits and sample preconcentration are very important. Two separate studies have shown

SPME-TM to be a significantly quicker sample preparation method while also decreasing the limit of detection and limit of quantitation with Direct Analysis in Real Time, or DART®, instrumentation (25)(26).

1.4.2 Instrumental Analysis

DART® is an ambient ionization technique that utilizes a flow of gas and heat to ionize samples in open air. Most commonly, the ions produced are protonated ions or $[M+H]^+$ ions. The schematic for this process is shown in figure 1. Because the sampling region is open air, a variety of sample types and matrices can be analyzed directly. A solid sample can be held in front of the ion source while a liquid or gas sample can be exposed to the ion source. Because of this wide variety of sampling, DART® also requires minimal sample preparation techniques. Unlike other instrumental techniques, there is no chromatographic separation with DART®. This can cause problems when multiple analytes have the same $[M+H]^+$ ions.

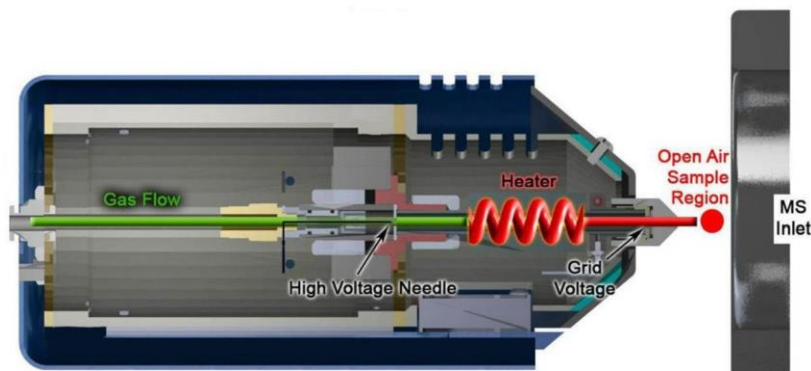


Figure 1. DART® Ion Source Schematic. Gas flow, most commonly helium, enters the ion source and is met with a high voltage needle and heat creating metastable gas atoms. These atoms enter the open-air sampling region where they interact with the air to create electrons. These electrons interact with the sample to create ions which will enter the mass spectrometer. *Photo courtesy of IonSense®*.

Because there is no chromatographic separation, the DART® source is most commonly interfaced to a mass spectrometer to detect and confirm the ions being produced. DART® mass spectrometry (DART®-MS) has gained popularity among many areas of testing including analyzing plant material, pesticide residues on fruits and vegetables, and many other forensic-related fields that require quicker time-sensitive testing (27). Crawford and Musselman have shown successful detection of pesticides in fruits and vegetables by simply swabbing the surfaces and placing the swab directly in the sample region of the DART® source (28). Due to the labor and time of previously mentioned sample preparation methods, along with the growing variety of cannabis containing matrices including gummies and lotions, DART® has been more widely explored as a promising detection method for pesticides (29).

The two biggest limitations of DART®-MS are the reduced sensitivity compared to chromatographic techniques and the manual labor of running samples. Many chromatographic instruments are equipped with an autosampler that allows the analyst to load large quantities of samples to be analyzed at once. With DART® analysis, there are several attachments that allow around a dozen samples to be run. Numerous states also have specific residue limits which requires exact sample quantitation, which is most easily performed with chromatographic techniques (29).

Chambers and Musah have posed the role of DART® as a “triage approach” to analysis in edible matrices and personal care products (30). As a presumptive/detection method, DART® has the quick and efficient capabilities to detect these substances. These are also more complex matrices that often require difficult-to-perform sample preparation

methods for analysis using chromatographic techniques. Though the initial article was targeting cannabinoids, similar approaches can be taken to the analysis of pesticides.

The most utilized instrumentation methods for pesticide detection have been both liquid chromatography and gas chromatography coupled to a mass spectrometer, LC-MS, and GC-MS respectively. The use of a triple quadrupole with tandem mass spectrometry (MS/MS) with these chromatographic instruments allows for the analyte of interest to be detected at very low levels then also quantitated when necessary. LC-MS/MS has been utilized to detect pesticide residues with QuEChERS sample preparation in marijuana and illegally seized cannabis plant material (31),(32),(33) and by SPE in medical marijuana (34). GC-MS is also utilized because not all pesticides will ionize by ionization methods commonly interfaced to liquid chromatography methods (34)(35).

2. MATERIALS AND METHODS

2.1 DART®-MS

2.1.1 Standard Preparation

A 16-component pesticide standard was purchased from SPEX (Metuchen, NJ). This standard includes aldicarb, aldicarb sulfoxide, aldicarb sulfone, imidacloprid, iprodione, imazalil, piperonyl butoxide, chlorantraniliprole, pirimicarb, azoxystrobin, acetamiprid, boscalid, trifloxystrobin, tebufenpyrad, fenoxycarb, and thiacloprid. Figure 2 shows each analyte's structure and molecular weight. Each analyte in the mixture came at a concentration of 100 µg/mL in acetonitrile (ACN). Four dilutions were prepared for DART®-QDa analysis. 1 µL of pesticide standard was added to 999 µL of ACN (Fisher Scientific, Waltham, Massachusetts) to make a 100 ng/mL dilution. 2µL of pesticide standard was added to 990 µL of ACN to make a 200 ng/mL dilution. 5 µL of pesticide standard was added to 995 µL of ACN to make a 500 ng/mL dilution. Finally, 10 µL of pesticide standard was added to 990 µL of ACN to make a 1000 ng/mL dilution. The pesticide standard and each dilution were stored at -20°C for future use.

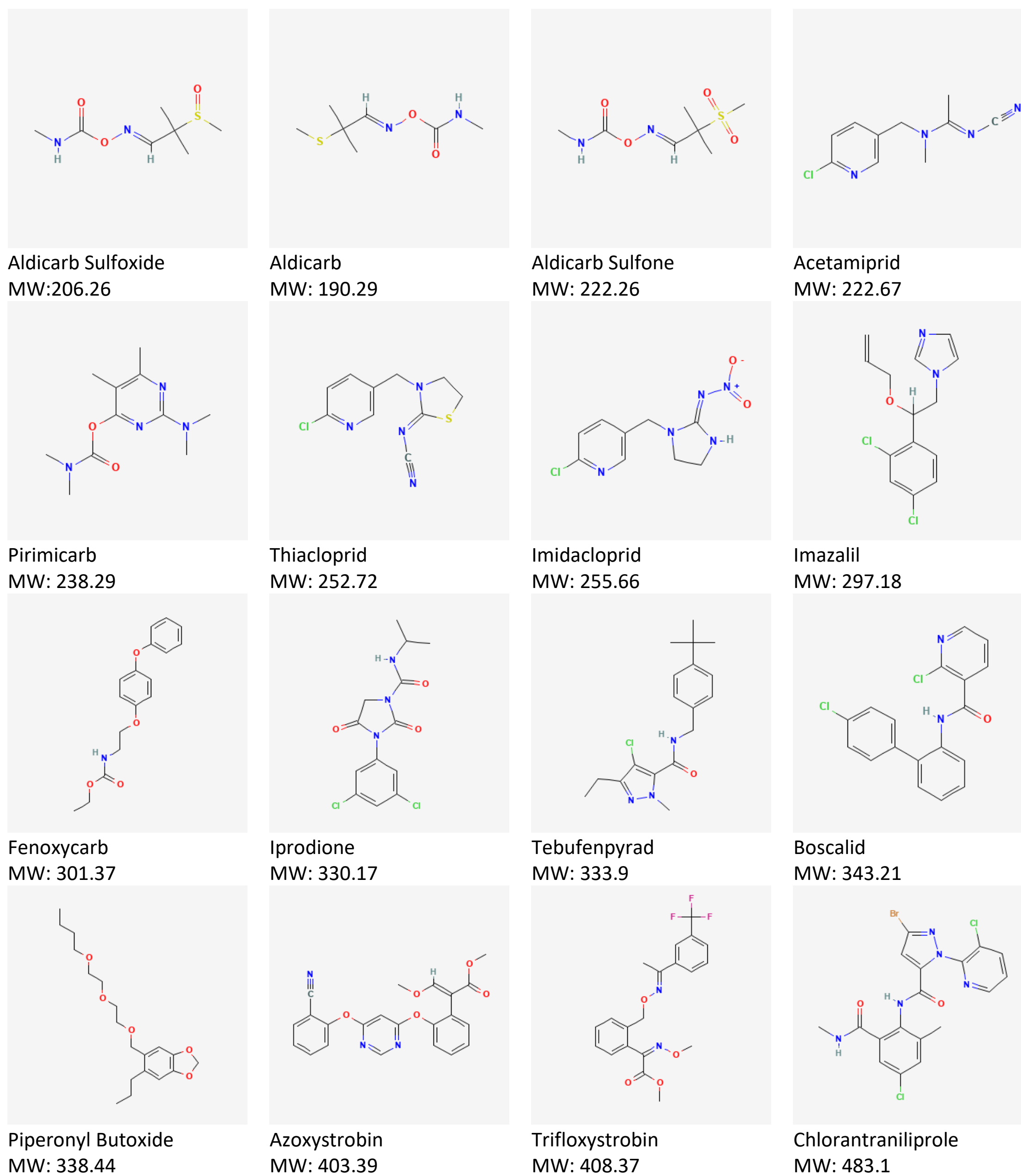


Figure 2. Structures and Molecular Weights of all 16 Analytes in the SPEX Pesticide Standard Mixture

2.1.2 Method Development

The DART®-QDa instrument consists of a DART® standard voltage and pressure ion source (IonSense® Inc., Saugus, Massachusetts) interfaced to a Waters™ ACQUITY® QDa Mass Detector with MassLynx™ software from Waters Corporation (Milford, Massachusetts). Due to lower sensitivity of this instrument, the 1000 ng/mL standard dilution was utilized for method optimization. For each optimization experiment, 5 μL of sample were spotted onto a QuickStrip™ sample card (IonSense® Inc., Saugus, Massachusetts) in triplicate after a card blank (no solvent) and a solvent blank (ACN) where applicable. In total, 5 ng of sample were spotted on each sample card spot.

Experiments were performed to optimize the temperature of the ion source. A temperature profile was run in the DART® software. Temperatures of 200, 250, 300, and 350°C were utilized. If the temperatures are too high, pesticides can degrade before entering the mass analyzer and parent ions may not be detected. If too low, pesticides may not be optimally desorbed off the QuickStrip™ and background ions will be more abundant.

The cone voltage was then changed over a number of different runs. The cone voltage in the mass analyzer helps to attract the ions into the mass analyzer. While lower voltages will attract ions into the mass analyzer, the cone voltage can also be used to induce fragmentation at higher values.

Experiments to optimize the linear rail speed were also conducted. Changing the rail speed affects how long each spot on the QuickStrip™ remains within the open air gap between the ion source and the inlet of the mass analyzer. At slower rail speeds, the sample

has more time to ionize and enter the mass analyzer, but so do background ions. At faster rail speeds the analytes that ionize most readily will enter the analyzer and background ions won't be as abundant. Target analytes that don't ionize as easily may not be detected if the rail speed is too fast.

2.1.3 Analytical Method

The QDa Mass Analyzer from Waters Corporation (Milford, Massachusetts) utilizes MassLynx™ software. An online DART® software allows for control of the DART® ion source. The linear rail speed, as mentioned above, is the speed at which the QuickStrip™ moves in front of the ion source. The heater wait time is how long the instrument allows gas flow once the target temperature has been met. The utilized parameters for both components are listed in table 2.

Table 2. DART® Parameters and Waters™ ACQUITY® QDa Mass Detector Parameters. These are the values used in the final analysis of the cannabis samples.

DART® Parameters	
Linear rail speed	0.4 mm/sec
Run Temperature	250°C
Ion Mode	Positive
Heater Wait Time	30 sec
QDa Parameters	
Mass Range	m/z 150-550
Ionization Mode	ES+
Data collection	Continuum
Cone Voltage	15 V

All Samples were run on QuickStrip™ sample cards with 5 μL of sample on each sample spot. The QuickStrip™ sample card was left in the card holder until all sample had been absorbed into the mesh shown in figure 3. The card was then placed onto the QuickStrip™ sample card holder attached to the linear rail as seen in figure 4. Under the DART® software, the method called "QuickStrip" was used to run the samples.

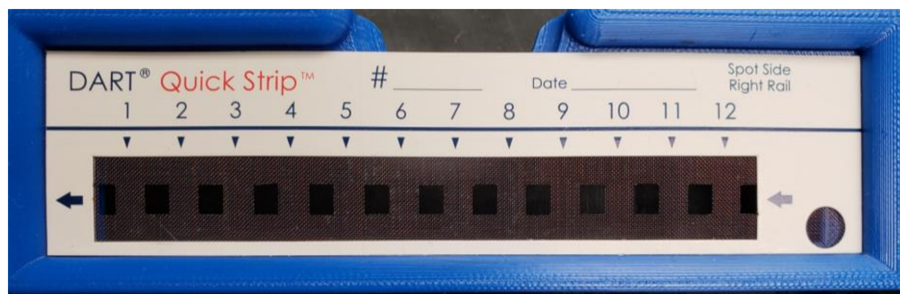


Figure 3. QuickStrip™ sample card in card holder. Each square shown is a blank space between each sample spot and each number points to its corresponding sample spot. Each card holds up to 12 sample spots.

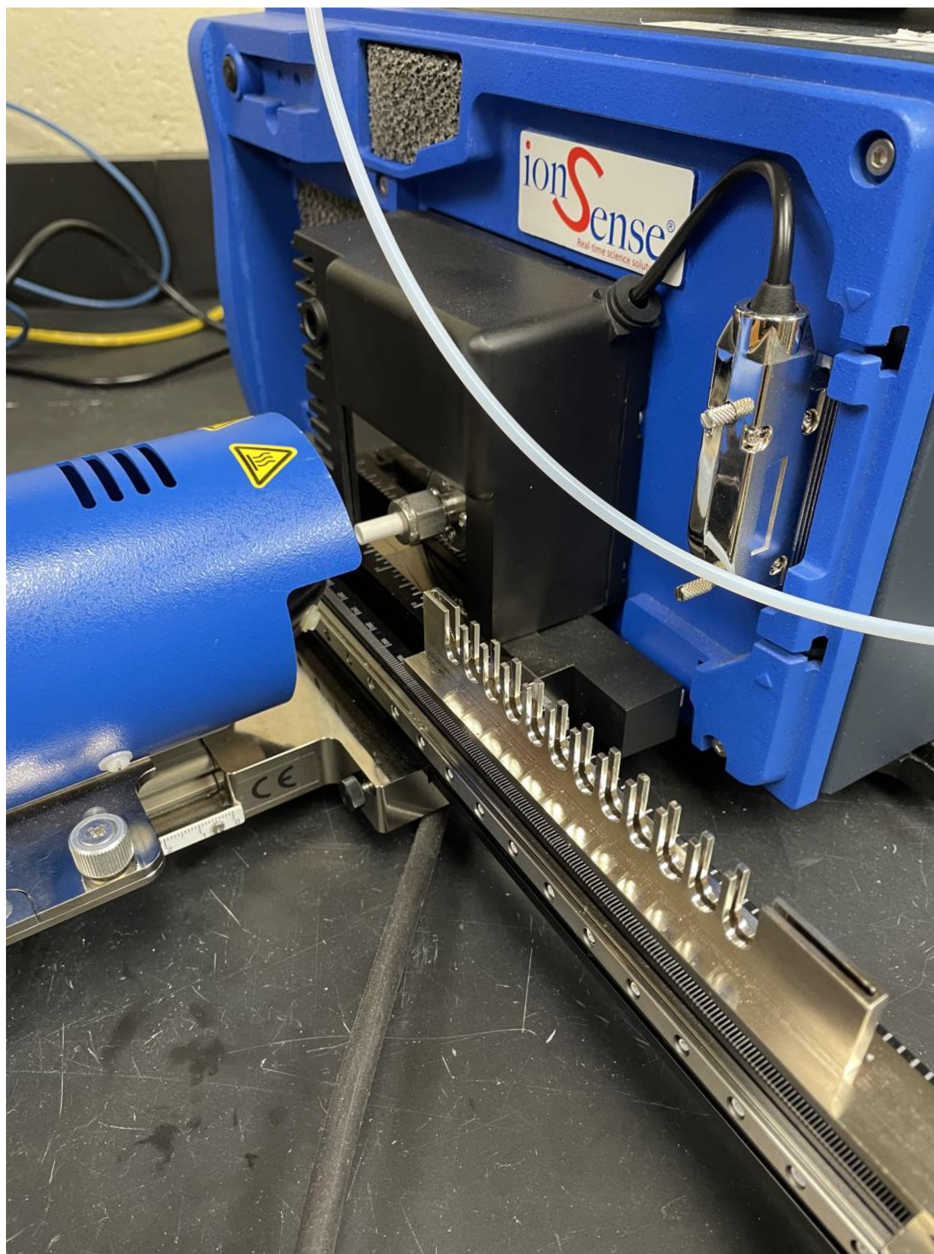


Figure 4. DART® Ion source interfaced to a Waters™ ACQUITY® QDa Mass Detector. The DART® ion source seen on the left is across from the inlet to the mass analyzer on the right. The QuickStrip™ card holder and linear rail are in the middle and lower right. The DART® software allows the rail to move samples between the ion source and mass analyzer at a set speed.

2.1.4 Cannabis Sample Preparation

Five cannabis flower samples were obtained from the New England Treatment Access (NETA) located in Brookline, MA. These samples consist of Ghost Train Haze (GTH), Arcata Trainwreck (AT), GG #4 (GG), Walker Kush (WK), and Black Triangle Kush (BTK). Approximately 1 g of each sample was weighed out in a weigh boat and transferred to a Lonzen® Rechargeable Electric Dry Herb Grinder (Amazon.com). Samples were homogenized then transferred to Qorpak™ clear borosilicate sample vials (Clinton, PA). Samples were stored at room temperature.

2.1.5 Solvent Extraction

The "Ghost Train Haze" cannabis sample was used in sample testing by DART®-MS. Solvent extractions were performed with both ACN and methanol (MeOH). 5 mg of sample was weighed onto a sheet of weigh paper using a Mettler Toledo ME analytical scale (Mettler Toledo, Columbus, Ohio). This sample was then transferred to a 1.5 mL Eppendorf tube. This was repeated for 1 mL of either ACN or MeOH which was added to the tube and vortexed for 10 seconds. The tube was then left to settle for 15 minutes. After settling, 950 µL of solution was transferred to a clean 1.5 mL Eppendorf tube, avoiding transferring any plant material. The new tube was then stored at room temperature for analysis. This process is also outlined in figure 5.

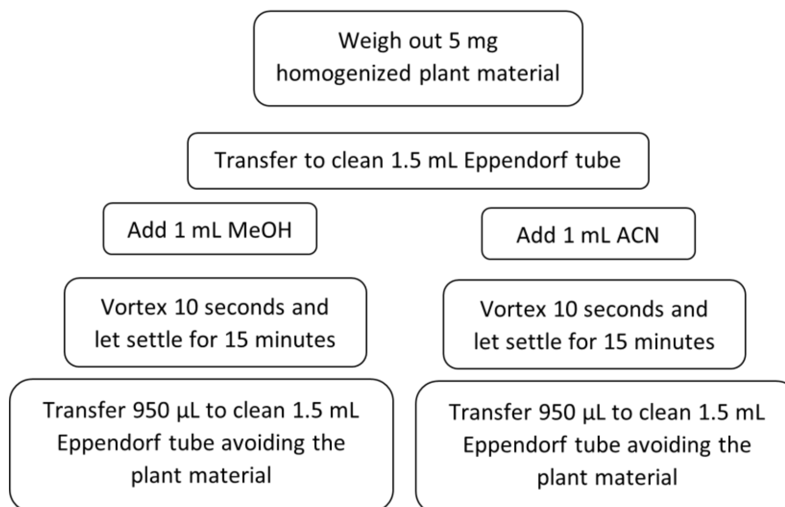


Figure 5. Solvent Extraction Procedure Flowchart

2.1.6 QuEChERS Extraction

QuEChERS extraction methods have been previously utilized for extractions of food and plant materials and can be modified given the nature of the sample. While there are published standard methods, the process is made to be modified as previously discussed in section 1.4.1. Figure 6 outlines the QuEChERS procedure utilized. Due to the limited quantity of sample available, 500 mg of homogenized cannabis sample was utilized in the extraction. 500 mg of homogenized sample was weighed out in a weigh boat and transferred to a clean 50 mL conical tube. 10 mL of water (Thermo Fisher Scientific, Waltham, Massachusetts) was added to the tube and left to hydrate for 10 minutes. 10 mL of ACN was then added to the hydrated sample and vortexed for 1 minute. The contents of this tube were then poured into a 50 mL conical tube that contained the QuEChERS buffer salt mixture (Thermo Fisher Scientific, Waltham, MA). This mixture consists of 4000 mg

anhydrous magnesium sulfate, 1000 mg sodium chloride, 1000 mg sodium citrate tribasic dihydrate, and 500 mg disodium citrate sesquihydrate. This salt mixture was vortexed for 1 minute then centrifuged for 5 minutes at 5000 rpm in a Sorvall Biofuge Primo Centrifuge (Thermo Fisher Scientific, Waltham, Massachusetts). After centrifuging, the salts gathered at the bottom of the tube, and there is an organic layer on top and the aqueous layer on bottom, separated by the plant material. The organic layer is a clear green liquid which is the targeted layer. The bottom aqueous layer is a cloudy yellow layer. 1 mL of the top layer is transferred to a clean-up tube with 150 mg MgSO4 and 50 mg PSA (Thermo Fisher Scientific, Waltham, Massachusetts). Another aliquot of 1 mL of the top layer is transferred to a clean-up tube with 150 mg MgSO4, 50 mg PSA, and 50 mg ChloroFiltr® (Thermo Fisher Scientific, Waltham, MA). Each of these clean up tubes is vortexed for 1 minute and centrifuged for 5 minutes at 4500 rpm in a Sorvall ST 8 Centrifuge (Thermo Fisher Scientific, Waltham, MA). The supernatant was transferred to a clean 1.5 mL Eppendorf tube and stored at 4°C for further analysis.

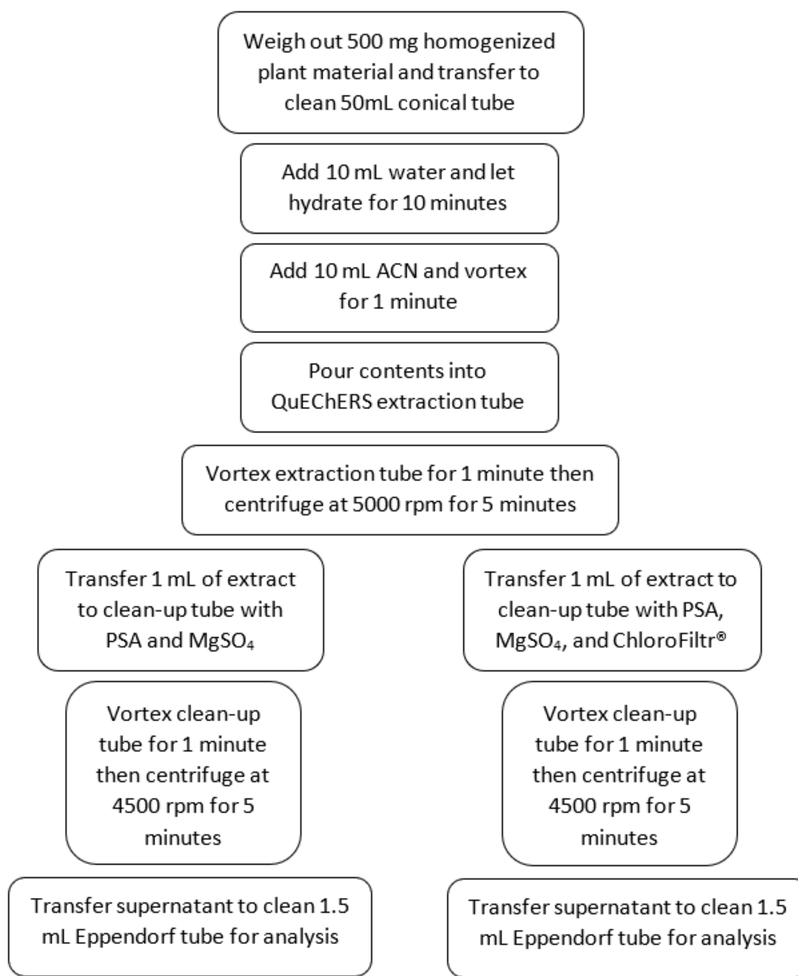


Figure 6. QuEChERS Extraction Procedure Flowchart

2.2 LC/MS/MS Analysis

2.2.1 Reagent Preparation

Reagents used for wash solutions include MeOH, and water (Thermo Fisher Scientific, Waltham, Massachusetts). The strong wash consists of 25:75 ACN:MeOH, weak wash is 50:50 MeOH:H_2O, and the seal wash is 30:70 MeOH:H_2O. The aqueous mobile phase was prepared by weighing out 0.315 g ammonium formate (Thermo Fisher

Scientific, Waltham, MA) in a weigh boat. The ammonium formate along with 1 mL formic acid (Sigma Aldrich, St. Louis, MO) were added to 999 mL of Millipore water (Millipore Sigma, Burlington, MA) to make a 5mM ammonium formate and 0.1% formic acid in water mobile phase. The organic mobile phases were prepared by combining 999 mL of ACN with 1 mL formic acid in one bottle and 999 mL of MeOH with 1 mL formic acid in another bottle.

2.2.2 Standard and Sample Preparation

The same standard that was prepared for DART® analysis was also utilized in LC-MS/MS analysis. Due to the improved sensitivity of the LC-MS/MS instrument, only the 100 ng/mL standard dilution was prepared for analysis. Solvent extraction and QuEChERS extractions as outlined previously were performed on all five cannabis samples for analysis.

2.2.3 Method Development

The mobile phase gradient was based off values from a compendium of pesticides analysis from PerkinElmer (36). Four mobile phase gradients were created with varying starting organic and aqueous concentrations. The starting mobile phase concentration affects the chromatography and how narrow and sharp the peaks are. The mobile phases were also optimized to give the strongest ion counts in the mass spectral data.

2.2.4 Analytical Method

LC-MS/MS analysis was performed on a PerkinElmer QSight 220 with Simplicity 3Q™ software provided by PerkinElmer Inc. (Waltham, MA). The transitions for each of the sixteen analytes in the pesticide standard were obtained from a provided library from

PerkinElmer. The multiple reaction monitoring (MRM) transitions and their masses, collision energies (CE), entrance voltages (EV) and collision cell lenses 2 (CCL2) are shown in table 3. The finalized gradient along with the LC method and MS source conditions are outlined in table 4. For analysis of the cannabis samples, two batches were run, one with an organic mobile phase consisting of ACN and another run with a MeOH mobile phase.

QuEChERS extractions were performed once for each cannabis sample and run by each LC-MS/MS method. All five cannabis samples were extracted by ACN solvent extraction and analyzed by the ACN method. All samples were also extracted by MeOH solvent extraction and analyzed by the MeOH method.

Table 3. MRM Transition Values for SPEX Standard

Name	Q1 Mass	Q2 Mass	CE	EV	CCL2
Aldicarb sulfoxide-1	207.0	89.0	-19	25	-38
Aldicarb sulfoxide-2	207.0	132.0	-13	25	-32
Aldicarb sulfoxide-3	207.0	89.0	-21	25	-40
Aldicarb-1	208.15	116.0	-11	25	-31
Aldicarb-2	208.15	89.0	-25	25	-43
Aldicarb sulfone-1	223.1	148.0	-15	25	-36
Aldicarb sulfone-2	223.1	166.0	-15	25	-36
Aldicarb sulfone-3	223.1	86.1	-23	25	-43
Acetamiprid-1	223.2	126.1	-30	25	-49
Acetamiprid-2	223.2	99.1	-56	25	-73
Pirimicarb-1	239.2	72.1	-38	25	-58
Pirimicarb-2	239.2	182.2	-20	25	-42
Thiacloprid 1-1	253.0	99.0	-66	20	-100
Thiacloprid 1-2	253.0	126.0	-30	20	-80
Thiacloprid-1	253.1	126.1	-26	25	-49
Thiacloprid-2	253.1	99.1	-60	25	-79
Imidacloprid-1	256.2	209.0	-18	25	-42
Imidacloprid-2	256.2	175.2	-26	25	-49
Imazalil-1	297.1	159.2	-31	25	-58
Imazalil-2	297.1	201.0	-25	25	-52
Imazalil-3	297.1	255.1	-25	25	-52
Imazalil-4	297.1	161.2	-31	25	-63
Fenoxycarb-1	302.2	88.0	-34	25	-61
Fenoxycarb-2	302.2	116.0	-15	25	-44
Iprodione-1-1	330.1	245.0	-20	25	-55
Iprodione-3-1	332.1	247.0	-20	25	-55
Tebufenpyrad-1	334.0	117.0	-52	25	-80
Tebufenpyrad-2	334.0	145.0	-34	25	-64
Boscalid-1	343.0	307.0	-25	25	-57
Boscalid-2	343.0	140.0	-28	25	-60
Piperonyl butoxide-1	356.2	177.0	-13	25	-47
Piperonyl butoxide-2	356.2	119.0	-37	25	-69
Azoxystrobin-1	404.1	372.1	-18	25	-57
Azoxystrobin-2	404.1	344.1	-34	25	-71
Trifloxystrobin-1	409.0	186.0	-26	25	-64
Trifloxystrobin-2	409.0	206.0	-20	25	-59
Chlorantraniliprole-1	484.0	452.8	-20	25	-66
Chlorantraniliprole-2	484.0	285.8	-18	25	-65

Table 4. LC Method and MS Source Conditions

<table>
<tr><th colspan="2">LC Method</th></tr>
<tr><td>Column</td><td>PerkinElmer Brownlee SPP C18, 4.6 X 100 mm, 2.7 µm</td></tr>
<tr><td>Mobile Phase</td><td>Solvent A: 5 mM ammonium formate with 0.1% formic acid in water
Solvent B1: 0.1% formic acid in acetonitrile
Solvent B2: 0.1% formic acid in methanol
<table><tr><th>Time (min)</th><th>%A</th><th>%B</th></tr><tr><td>0</td><td>95</td><td>5</td></tr><tr><td>0.5</td><td>90</td><td>10</td></tr><tr><td>1</td><td>90</td><td>10</td></tr><tr><td>15</td><td>5</td><td>95</td></tr><tr><td>17</td><td>5</td><td>95</td></tr><tr><td>17.1</td><td>90</td><td>10</td></tr><tr><td>20</td><td>95</td><td>5</td></tr></table></td></tr>
<tr><td>Flow Rate</td><td>0.600 mL/min</td></tr>
<tr><td>Oven Temperature</td><td>40°C</td></tr>
<tr><td>Auto Sampler Temperature</td><td>15°C</td></tr>
<tr><td>Injection Volume</td><td>3 µL</td></tr>
<tr><th colspan="2">MS Source Conditions</th></tr>
<tr><td>ESI Voltage</td><td>5000 V</td></tr>
<tr><td>Drying Gas</td><td>140</td></tr>
<tr><td>Nebulizer Gas</td><td>350</td></tr>
<tr><td>Source Temperature</td><td>325°C</td></tr>
<tr><td>HSID Temperature</td><td>200°C</td></tr>
<tr><td>Detection Mode</td><td>Time Managed MRM</td></tr>
</table>

3. RESULTS AND DISCUSSION

3.1 DART®-MS Analysis

3.1.1 Pesticide Standard Optimizations

The first card run contained the pesticide standard at all four concentrations prepared (100, 250, 500, and 1000 ng/mL). The mass spectrum for each concentration is shown in figure 7. The 1000 ng/mL dilution is shown on top and subsequently has the largest ion count as shown in the upper right-hand corner of the spectrum. This dilution also shows the smallest amount of background ions as compared to the 100 ng/mL dilution shown on the bottom. Background ions such as m/z 132 and 320 are seen at the largest intensities in the 100 ng/mL standard dilution.

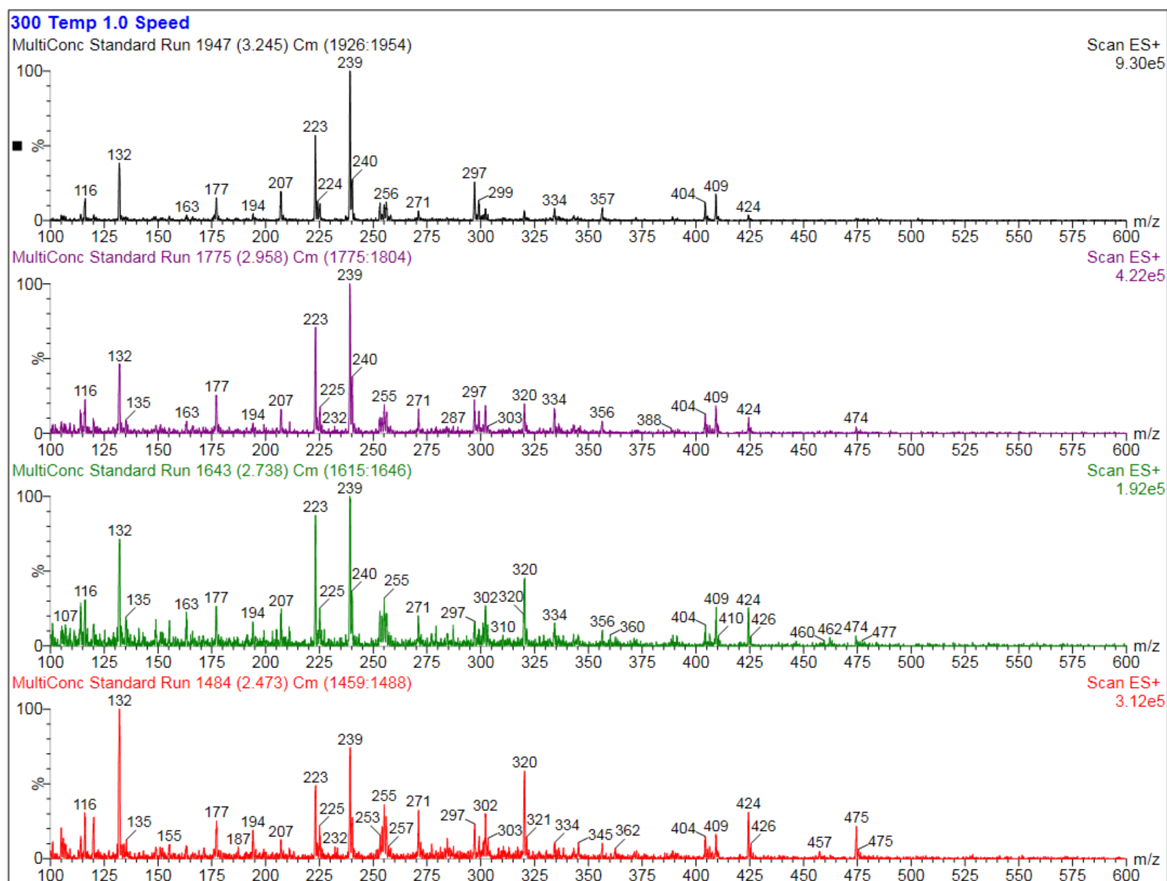


Figure 7. Mass spectra for pesticide standard mixture. Concentration shown at 100 ng/mL (red), 250 ng/mL (green), 500 ng/mL (purple), and 1000 ng/mL (black). The card was run with a source temperature of 250°C and a cone voltage of 15V.

After attempting to optimize the 100 ng/mL standard, these background ions were continually overpowering in relative intensity compared to the target analytes. As a result of the presence of significant background ions, lower concentrations of the standard weren't suitable for detection of all target ions. The 500 ng/mL standard was then utilized for source temperature optimization. The intensity of the background ions was still higher than expected. The 1000 ng/mL standard was then analyzed and showed significantly lower intensity of background ions. Figure 8 shows the mass spectrum of the 1000 ng/mL

standard with each of the target analyte peaks in red boxes or with red arrows. Table 5 shows which analyte corresponds with which peak. Of the sixteen analytes in the standard mixture, fourteen were detected in DART®-QDa analysis and two share the same parent ion. Only aldicarb and iprodione were not detected.

Table 5. Pesticide Analytes and Their Respective Ion Peaks Detected in DART®-QDa Analysis.

Analyte	Ion Peak (m/z)
Aldicarb Sulfoxide	207
Aldicarb Sulfone	223
Acetamiprid	223
Pirimicarb	239
Thiacloprid	253
Imidacloprid	256
Imazalil	297
Fenoxycarb	302
Tebufenpyrad	334
Boscalid	343
Piperonyl Butoxide	356
Azoxystrobin	404
Trifloxystrobin	409
Chlorantraniliprole	484

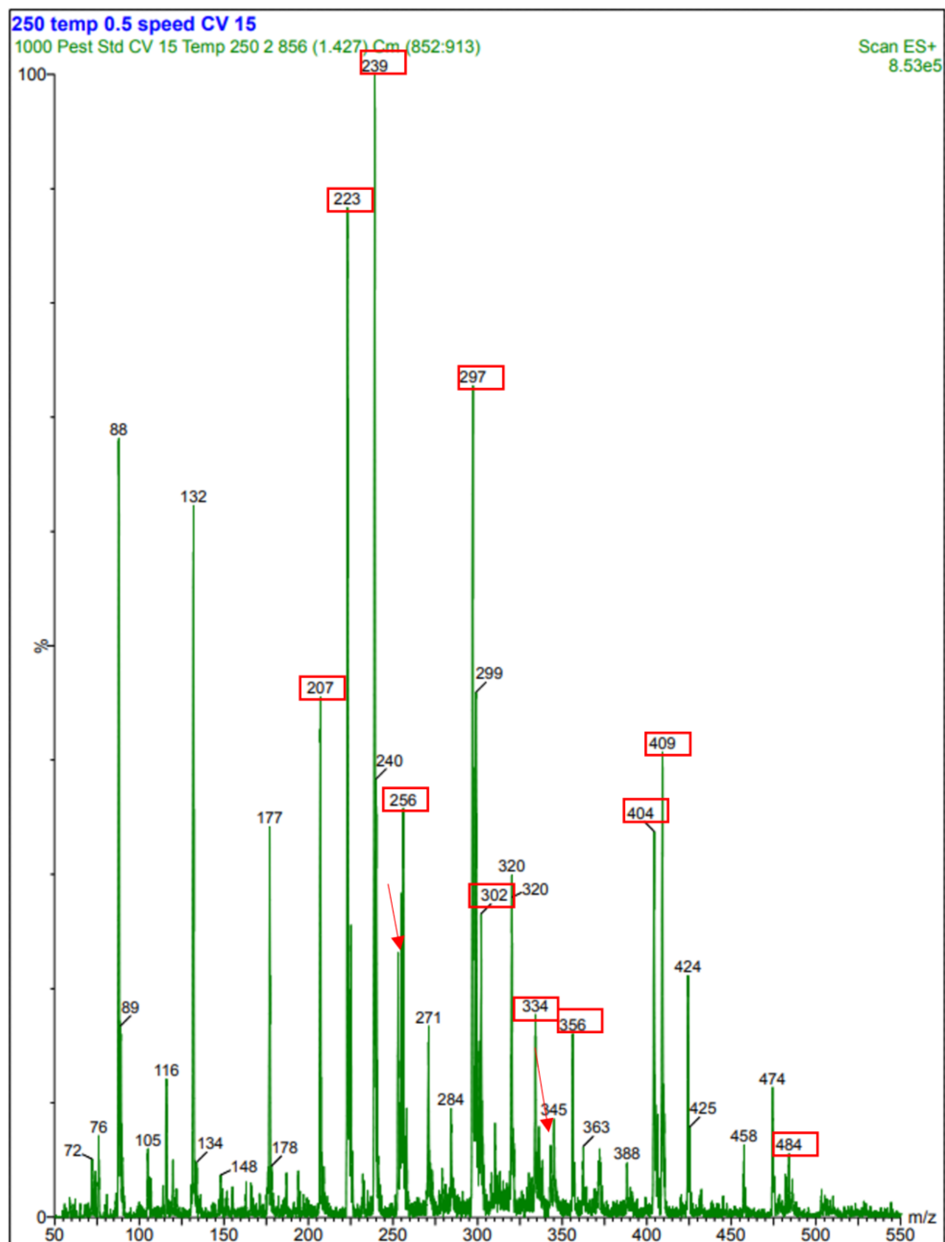


Figure 8. Mass spectrum of the 1000 ng/mL standard dilution. Standard was run at 250 °C source temperature, 0.5 mm/sec rail speed, and 15 V cone voltage. Each red box and arrow represent one of the targeted analytes in the standard mixture. The ions indicated by the two red arrows are m/z species at 253 and 343 respectively

After determining that this dilution would give the best results, a temperature optimization was then performed. Temperatures ranged from 200°C-350°C. The mass

spectrum of each temperature is shown in figure 9. The bottom red spectrum is at 200°C and increases by 50°C increments for each successive stacked spectrum. The m/z 239 ion was consistently the most abundant ion until reaching 350°C. It was determined that this temperature induced significant fragmentation and was excluded when determining optimal values. At 200°C not all targeted analytes were detected and was determined to be too low of a temperature. When comparing 250 and 300°C, the total ion count in the top right corner was considered. The ion count was higher at the 250°C run and was therefore determined to be the most optimal source temperature.

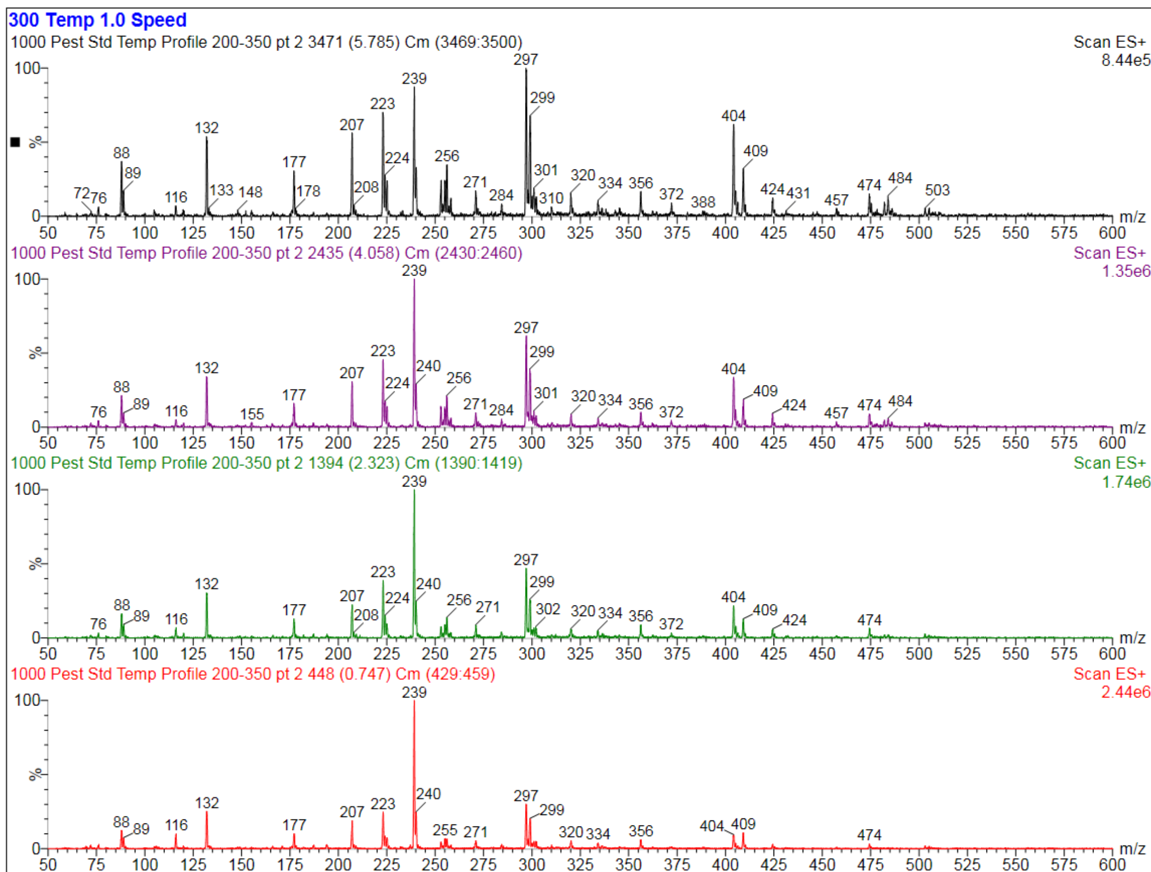


Figure 9. Pesticide Standard (1000 ng/mL) Temperature Optimization. Temperatures ranging from 200°C (bottom) to 350°C (top) in 50°C increments. The temperature profile was run with a 1.0 mm/sec rail speed and 15V cone voltage.

After the temperature optimization was completed, a cone voltage optimization was performed. This included running multiple QuickStrip™ sample cards at the same temperature (250°C) and rail speed (0.5 mm/sec) and various cone voltages from 10V-25V over increments of 5V. The mass spectrum from these runs is shown in figure 10. The spectrum at 10V is shown on the bottom and increases by 5V for each successive stacked spectrum. Although the 10V spectrum also has the largest ion count, a cone voltage that doesn't have a large relative intensity of a single peak was necessary for all target ions to be observed. As seen in the temperature optimization, m/z 239 ion is typically the most

abundant and likely ionizes the easiest. When the cone voltage is at 25V, that peak is significantly decreased in abundance, likely being fragmented by the high voltages. Comparing 15V and 20V it was noted that the background ion m/z 88 was the most abundant ion and therefore 15V was chosen as the optimal cone voltage.

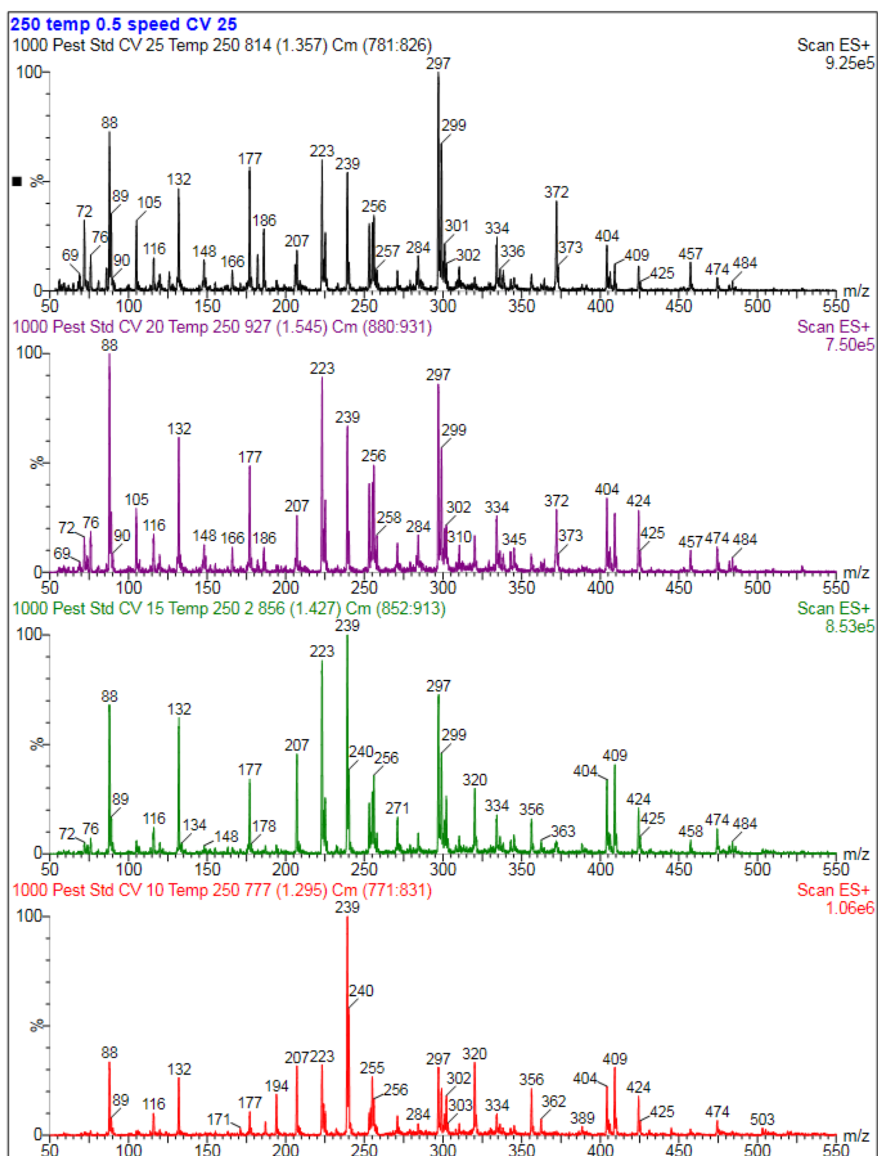


Figure 10. Pesticide Standard (1000 ng/mL) Cone Voltage Optimization. All runs were performed at a temperature of 250°C and linear rail speed of 0.5 mm/sec. Cone voltages were run at 10V (red), 15V (green), 20V (purple) and 25V (black).

The final optimization run with the standard was a linear rail optimization. The temperature profile method defaulted at 1.0 mm/sec and the QuickStrip method defaulted at 0.5 mm/sec. Both of these speeds were chosen along with a slower speed of 0.3 mm/sec. In figure 11, the slowest speed is shown on the bottom with 0.5 mm/sec in the middle and 1.0 mm/sec on top. The fastest speed allows the sample the least amount of time to ionize and enter the mass analyzer, so we see a much larger m/z 239 ion peak relative to all other peaks. This ion, which is contributed by pirimicarb, is consistently the most abundant ion indicating that it ionizes much more readily that other ions in the standard mixture. Due to the relatively low intensities of all other ions, 1.0 mm/sec was determined to be too slow of a rail speed. The targeted ions were all seen in both 0.5 and 0.3 mm/sec rail speeds. While 0.5 mm/sec still had a relatively large difference between m/z 239 peak and other target peaks, 0.3 mm/sec had the most abundant ion as m/z 88 which is a background contributor. Another batch was run at a rail speed of 0.4 mm/sec in an attempt to lower the intensity of the background ions seen in the 0.3 mm/sec run but increase the intensity of target ions as seen in the 0.5 mm/sec run. The run at 0.4 mm/sec achieved that goal and 0.4 mm/sec was further utilized for sample analysis.

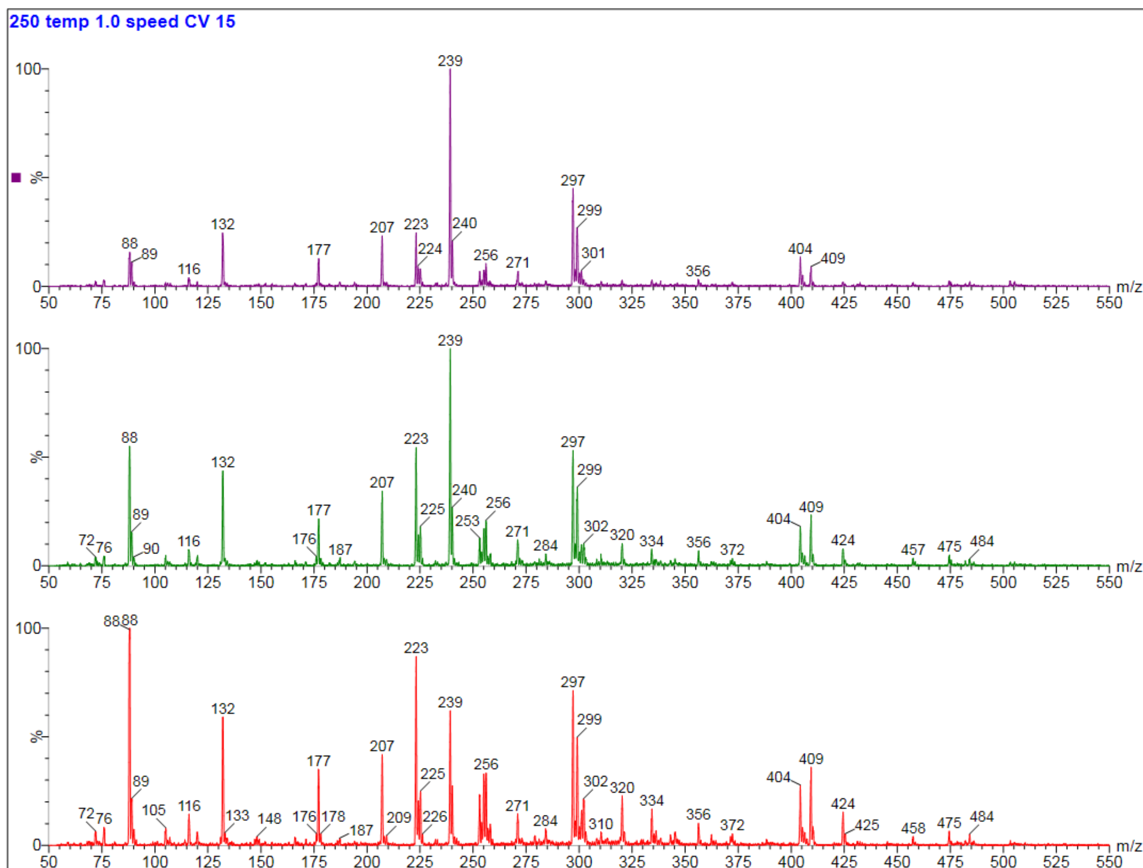


Figure 11. Pesticide Standard (1000 ng/mL) Linear Rail Speed Optimization. Rail speeds at 0.3 (bottom), 0.5 (middle), and 1.0 mm/sec (top), were compared. Run parameters include 250°C source temperature and 15V cone voltage.

3.1.2 Cannabis solvent extraction

After all parameters were optimized, solvent extractions were performed on the GTH sample in both ACN and MeOH. Two different QuickStrips™ were prepared with sample in triplicate and analyzed with the optimized conditions. Figure 12 shows the mass spectrum for GTH in MeOH and in ACN. In figure 12, ACN extraction is shown on bottom and MeOH is shown on top, with both spectra shown at a 4X magnification.

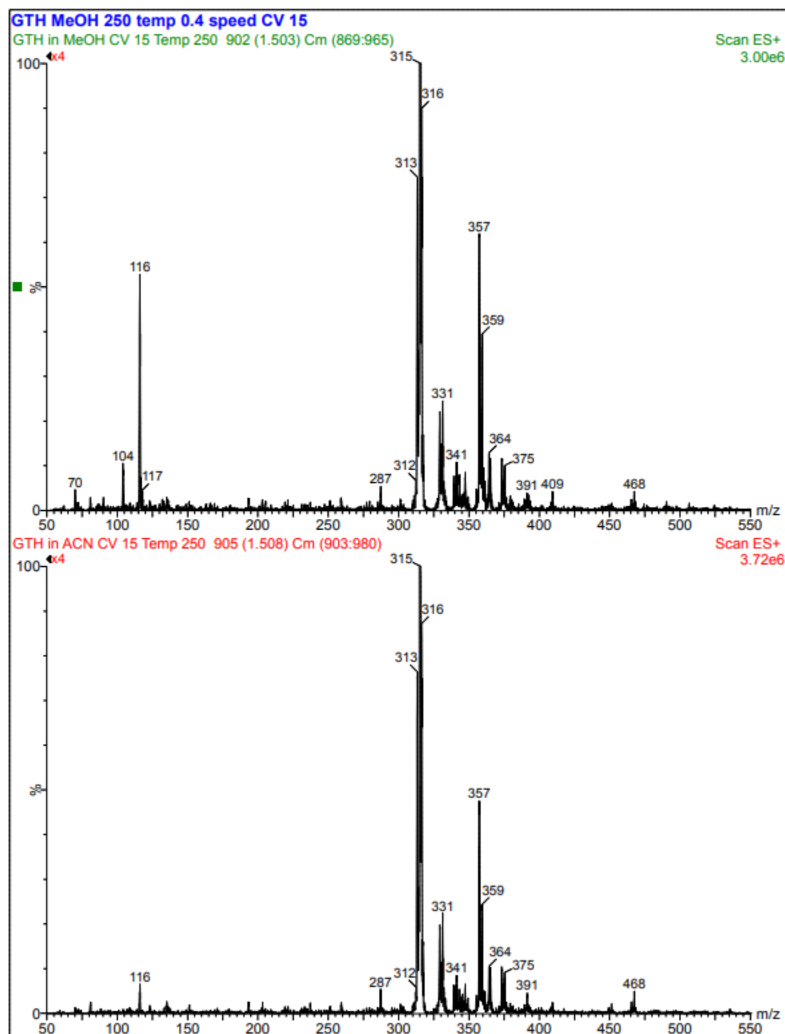


Figure 12. DART®-MS Mass Spectra for Solvent Extracts of GTH in MeOH and ACN. The bottom spectrum shows the solvent extract in ACN, and the top spectrum shows the solvent extract in MeOH. Both spectra are at a 4X magnification. Run parameters included cone voltage of 15V, source temperature of 250°C and a rail speed of 0.4 mm/sec.

When viewing each spectrum without any magnification, the most abundant peaks consisted of cannabinoids at m/z 313, 315, and 316. The m/z 315 ion is the $[M+H]^+$ ion of THC and CBD. The m/z 313 was observed in a previous project analyzing cannabis samples although the contributor is unknown. The m/z 316 peak contribution is unknown as well. The peaks at m/z 357 and 359 were the next most abundant species. M/z 359 is the

$[M+H]^+$ ion for THCA, CBCA and CBDA. Though there isn't a known contributor for the m/z 357 ion, a previous study found that this peak was unique to marijuana samples over hemp samples (37). Due to the nature of the DART®-MS analysis, there was no analyte library that the remainder of the peaks could be identified by.

Comparing the solvent extractions in ACN and MeOH, the most visible difference is the presence of the low mass ions detected between m/z 70-117. Because these peaks don't correspond to any parent ion peaks, the mass range for analysis was condensed to m/z 150-550. The ion count for the MeOH extraction was lower but all other peaks relative to the base peak were more relatively intense. If further analyses were done with cannabis samples, MeOH would be the chosen solvent extraction method.

3.1.3 Cannabis QuEChERS Extraction

QuEChERS as mentioned earlier is an dSPE extraction method to pre-concentrate the pesticides in the cannabis samples. Two different clean-up methods were used, one with PSA and $MgSO_4$, and the second with PSA, $MgSO_4$, and ChloroFiltr®. The goal was to see if the ChloroFiltr® would suppress the recovery and detection of any pesticides. After running both extractions on both clean-up methods, there was no observed difference between the ChloroFiltr® clean-up and clean-up tube without ChloroFiltr®. All DART® QuEChERS data will be shown using the data from the ChloroFiltr® clean-up tube.

The three most abundant peaks when QuEChERS extraction is performed is still the m/z 313, 315, and 316 ion peaks. Interestingly the m/z 357 and 359 ion peaks seen in the solvent extractions aren't visible in the QuEChERS extraction even at a 4X

magnification. Figure 13 shows the mass spectrum of the QuEChERS extraction with no magnification on top and at 4X magnification on bottom. Two of the largest differences between the QuEChERS and solvent extractions is the lack of the m/z 357 and 359 ions but also the lower mass ions at m/z 152 and 166.

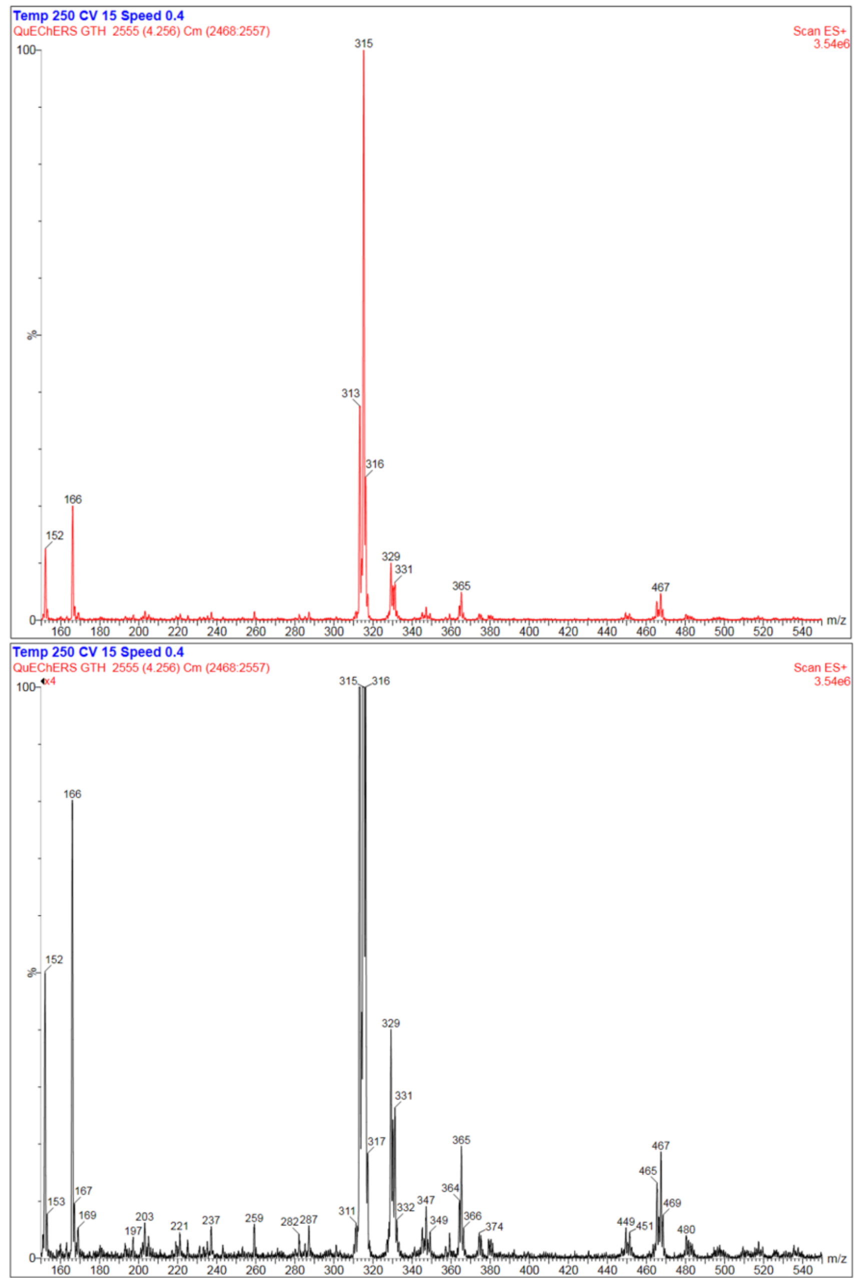


Figure 13. DART®-MS Mass Spectra of QuEChERS Extraction on GTH. The top spectrum is GTH extracted with QuEChERS, and the bottom spectrum is GTH extracted with QuEChERS at a 4X magnification.

3.2 LC/MS/MS Analysis

3.2.1 LC Column

In the initial steps of creating the methods, many parameters were taken from the compendium produced by PerkinElmer on pesticide analysis (36). The initial column utilized was a phenyl-hexyl 2.1X100 mm, 2.7 µm column. The internal diameter (ID) was determined to be too small, and the chromatography showed significant peak fronting. The column that was then utilized for analysis had an increased ID at 4.6X100 mm, 2.7 µm. The increase in internal diameter significantly improved the chromatographic peaks making them sharper and more symmetrical.

3.2.2 Optimizing LC Gradient

In order to optimize the starting mobile phase concentrations, the pesticide standard was run on four different gradients. These gradients started at 80%A, 85%A, 90%A, and 95%A starting conditions. Initial optimizations were performed on the initial phenyl-hexyl column. When the column was switched, the elution times were later than the initial times, but the run time was adjusted to see all targeted analytes. The initial targeted flow rate was 0.3 mL/min. When the new column was used for analysis, it was determined that the flow rate needed to be doubled in order to observe similar retention times. Figure 14 shows the chromatography for each mobile phase concentration.

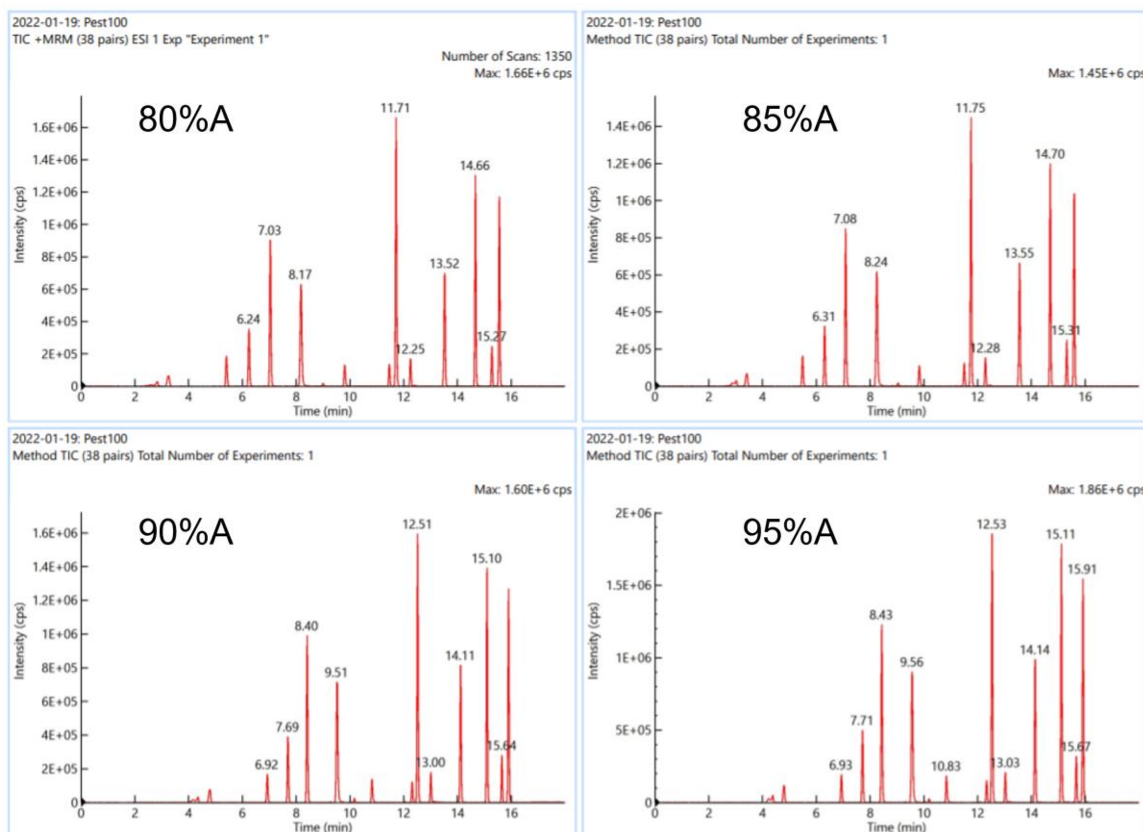


Figure 14. Chromatograms of Varying Starting Mobile Phase Concentrations. Each chromatogram is labeled with the starting percentage of mobile phase A, or the aqueous mobile phase. The standard concentration utilized was 100 ng/mL

Across each different starting mobile phase concentration, there isn't an observed difference in the resolution of the peaks within the chromatography. The only observable difference in each of these chromatograms is the relative ion intensities shown on the y-axis. The chromatogram that represents the starting 95%A concentration shows the most intense ion counts. To further understand the difference between these gradients and concentrations, the MRM data for 3 transitions for the analyte aldicarb sulfoxide were analyzed to observe any differences.

Aldicarb sulfoxide was the chosen analyte because the peak shapes were most affected by altering the mobile phase concentrations. Figure 15 shows each transition for aldicarb sulfoxide at each starting concentration. The peaks have varying amounts of peak fronting at each concentration when zoomed in. The top row is the starting mobile phase concentration of 80%A. The second row is the starting mobile phase concentration of 85%A. The third row is the starting mobile phase concentration of 90%A, and the last row is the starting mobile phase concentration of 95%A. Each transition at 80%A shows peak fronting. Sharper peaks are seen at higher starting %A in the mobile phase. When comparing both 90%A and 95%A, the maximum ion counts for the base peak were taken into consideration. The ion counts at 95%A were the highest, therefore the gradient starting at 95%A mobile phase was chosen as the optimal gradient.

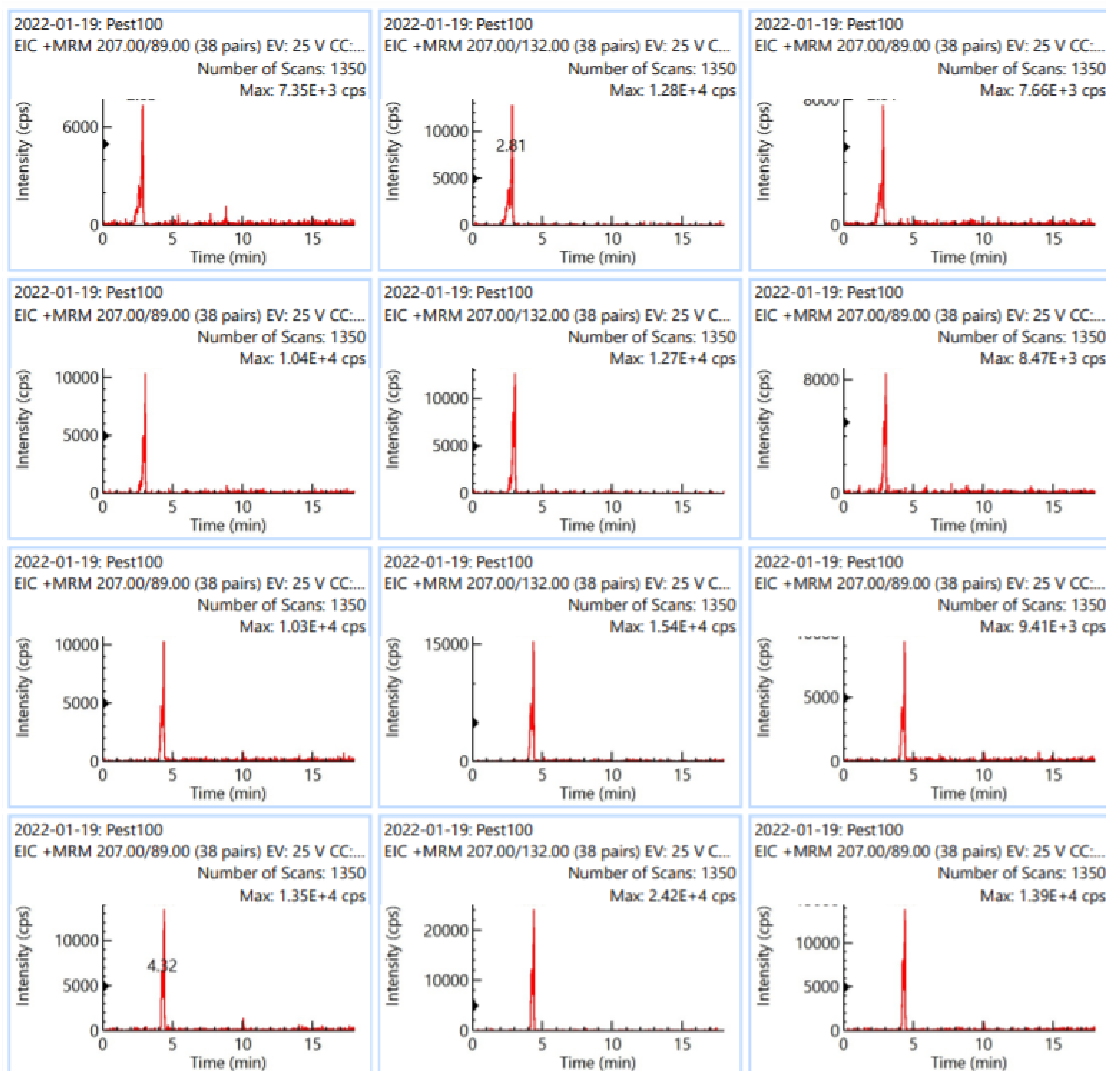


Figure 15. Aldicarb Sulfoxide MRMs at Each Starting Mobile Phase Concentration. The top row shows three aldicarb sulfoxide transitions at the 80% A starting mobile phase concentration. The second row shows the same three transitions at the 85% A starting mobile phase concentration. The third row shows those same three transitions at the 90% A starting mobile phase concentration. The bottom row shows the three transitions of aldicarb sulfoxide at the 95% A starting mobile phase concentration.

3.2.3 Arcata Trainwreck Analysis

All five cannabis flower samples were run on a new MRM method with new MRM transitions but all the same instrument parameters. On one method the QuEChERS extracts

and solvent extract for each flower were run with an ACN mobile phase and on another method the same extracts were run on a MeOH mobile phase. In order for a peak to be positively detected, it was required to be above 1000 ion counts per second (CPS) and have at least a 3:1 signal to noise ratio. This was calculated in the Simplicity 3Q™ software by averaging noise over 5-6 minutes. No quantitation was performed on these samples, positives are reported as detected MRM transitions. The QuEChERS extraction with ChloroFiltr® cleanup will be referred to as Qu1 and the QuEChERS extraction without ChloroFiltr® cleanup will be referred to as Qu2.

All detected transitions for each sample are composed into tables 6-10. This table shows direct comparisons for each detected transition between the extraction technique and the LC mobile phase utilized. Each row is dedicated to a single transition where each column is the specific extraction used. Any blank spots or boxes indicate that the transition in that row wasn't detected by that specific extraction method and mobile phase combination. Numerous transitions were detected by LC-MS/MS utilizing the same extraction methods on both mobile phase conditions. This trend is indicated by rows with bold text in Tables 6-10.

It is important to note that the wash cycles between each sample were not optimized. As denoted in the tables below, there was various amounts of carryover in each sample. Samples that had carryover from the preceding blanks were marked as such but still considered detected in the sample.

In the sample AT, there were many more analyte transitions detected using the MeOH mobile phase than the ACN mobile phase. Mevinphos (Phosdrin)-3 and

Fenoxycarb-1 were the only transitions detected using ACN mobile phase conditions that weren't seen with MeOH. Using the ACN mobile phase, there were four transitions that were only detected when using the Qu1 method and three transitions that were only detected when using the Qu2 method. In total, thirty-seven transitions were detected when using Qu1, thirty-six transitions were detected when using Qu2, and only twenty-four transitions were detected using ACN solvent extraction. Nine transitions were detected when using both QuEChERS methods but not when utilizing the solvent extraction.

Compared to the ACN mobile phase, there are twenty more transitions detected when using MeOH mobile phase conditions across the three extraction methods. Four transitions were detected when using Qu1 and eight were detected when using Qu2. Utilizing the MeOH mobile phase, there were also two transitions, Pyraclostrobin-2 and Etofenprox-1, that were detected only when using solvent extraction. Methiocarb-2 was the only transition in this sample to be detected when using Qu1 and solvent extraction but not Qu2.

Table 6. Direct Comparison of Detected MRM Transitions in Arcata Trainwreck Sample. Transitions detected by both acetonitrile and methanol mobile phases are further separated by the extraction methods. Color key is seen at the bottom and all bolded rows indicate transitions detected in the same extraction methods across both mobile phase methods. *Detected in the blank preceding the sample.

Acetonitrile			Methanol		
QuEChERS With Chlorofiltr	**QuEChERS Without Chlorofiltr**	**Solvent**	**QuEChERS With Chlorofiltr**	**QuEChERS Without Chlorofiltr**	**Solvent**
			Dichlorvos-2	Dichlorvos-2	Dichlorvos-2*
			Dichlorvos-1	Dichlorvos-1	Dichlorvos-1
			Methiocarb-2		Methiocarb-2*
Mevinphos (Phosdrin) - 3	Mevinphos (Phosdrin) - 3				
				Ethoprophos (ethoprofos) - 4	
Ethoprophos (ethoprofos) - 1	**Ethoprophos (ethoprofos) - 1**		**Ethoprophos (ethoprofos) - 1**	**Ethoprophos (ethoprofos) - 1**	
Thiacloprid-2*	**Thiacloprid-2***	**Thiacloprid-2***	**Thiacloprid-2***	**Thiacloprid-2***	**Thiacloprid-2***
Thiacloprid-1			**Thiacloprid-1**		
				Dodemorph-1	
			Methyl parathion-3		
				Myclobutanil-1	
			Myclobutanil-2	Myclobutanil-2	
Bifenazate-2				Bifenazate-2	
Bifenazate-1	**Bifenazate-1**		**Bifenazate-1**	**Bifenazate-1**	
Fenoxycarb-1	Fenoxycarb-1*	Fenoxycarb-1*			
Diazinon-1	**Diazinon-1**	**Diazinon-1**	**Diazinon-1**	**Diazinon-1**	**Diazinon-1**
			Kresoxim-methyl 1-3	Kresoxim-methyl 1-3	Kresoxim-methyl 1-3
			Kresoxim-methyl 1-4	Kresoxim-methyl 1-4	
			Kresoxim-methyl 1-2		
Cinerin-I-1	**Cinerin-I-1**	**Cinerin-I-1**	**Cinerin-I-1**	**Cinerin-I-1**	**Cinerin-I-1***
Cinerin-I-2	**Cinerin-I-2***	**Cinerin-I-2**	**Cinerin-I-2**	**Cinerin-I-2**	**Cinerin-I-2**
Phosmet-2	Phosmet-2		Phosmet-2	Phosmet-2	Phosmet-2
Phosmet-3*	**Phosmet-3***	**Phosmet-3***	**Phosmet-3***	**Phosmet-3***	**Phosmet-3***
Phosmet-1	**Phosmet-1**	**Phosmet-1**	**Phosmet-1**	**Phosmet-1**	**Phosmet-1**
Pyrethrin-I-1	**Pyrethrin-I-1**	**Pyrethrin-I-1***	**Pyrethrin-I-1***	**Pyrethrin-I-1***	**Pyrethrin-I-1***
Pyrethrin-I-2	**Pyrethrin-I-2***	**Pyrethrin-I-2***	**Pyrethrin-I-2***	**Pyrethrin-I-2***	**Pyrethrin-I-2***
Iprodione-1-1	**Iprodione-1-1**		**Iprodione-1-1**	**Iprodione-1-1**	
Malathion-1*	**Malathion-1***	**Malathion-1***	**Malathion-1***	**Malathion-1***	**Malathion-1***
Malathion-2	Malathion-2		Malathion-2	Malathion-2	Malathion-2
Jasmolin-I-1	**Jasmolin-I-1**	**Jasmolin-I-1**	**Jasmolin-I-1***	**Jasmolin-I-1**	**Jasmolin-I-1***
Jasmolin-I-2	**Jasmolin-I-2**	**Jasmolin-I-2***	**Jasmolin-I-2***	**Jasmolin-I-2***	**Jasmolin-I-2***
			Iprodione-3-1	Iprodione-3-1	
Propiconazole-1	**Propiconazole-1***	**Propiconazole-1***	**Propiconazole-1***	**Propiconazole-1***	**Propiconazole-1***
Propiconazole-2	**Propiconazole-2**	**Propiconazole-2***	**Propiconazole-2***	**Propiconazole-2***	**Propiconazole-2***
Thiophanatemethyl-1	**Thiophanatemethyl-1**	**Thiophanatemethyl-1**	**Thiophanatemethyl-1**	**Thiophanatemethyl-1***	**Thiophanatemethyl-1***
Thiophanatemethyl-2	Thiophanatemethyl-2		Thiophanatemethyl-2	Thiophanatemethyl-2	Thiophanatemethyl-2
				Chlorpyrifos-2	
	Tebufenozide-1		Tebufenozide-1	Tebufenozide-1	Tebufenozide-1
				Tebufenozide-2	
Piperonyl Butoxide-2	Piperonyl Butoxide-2		Piperonyl Butoxide-2	Piperonyl Butoxide-2	Piperonyl Butoxide-2*
	Piperonyl Butoxide-1		Piperonyl Butoxide-1	Piperonyl Butoxide-1*	Piperonyl Butoxide-1*
Etoxazole-2	**Etoxazole-2**	**Etoxazole-2**	**Etoxazole-2**	**Etoxazole-2***	**Etoxazole-2***
Etoxazole-1	**Etoxazole-1**	**Etoxazole-1**	**Etoxazole-1**	**Etoxazole-1**	**Etoxazole-1**
Cinerin-II-1	**Cinerin-II-1**	**Cinerin-II-1**	**Cinerin-II-1***	**Cinerin-II-1***	**Cinerin-II-1***
Cinerin-II-2	**Cinerin-II-2**	**Cinerin-II-2**	**Cinerin-II-2**	**Cinerin-II-2**	**Cinerin-II-2**
Coumaphos-1	**Coumaphos-1**		**Coumaphos-1**	**Coumaphos-1**	
				Pyridaben-1	
Pyrethrin-II-1	**Pyrethrin-II-1**	**Pyrethrin-II-1**	**Pyrethrin-II-1**	**Pyrethrin-II-1**	**Pyrethrin-II-1***
Pyrethrin-II-2	**Pyrethrin-II-2***	**Pyrethrin-II-2***	**Pyrethrin-II-2**	**Pyrethrin-II-2***	**Pyrethrin-II-2***
Spirotetramat-2	**Spirotetramat-2**	**Spirotetramat-2**	**Spirotetramat-2**	**Spirotetramat-2**	**Spirotetramat-2**
Jasmolin-II-1	**Jasmolin-II-1**	**Jasmolin-II-1**	**Jasmolin-II-1**	**Jasmolin-II-1**	**Jasmolin-II-1**
Jasmolin-II-2	**Jasmolin-II-2**	**Jasmolin-II-2**	**Jasmolin-II-2**	**Jasmolin-II-2**	**Jasmolin-II-2**
	Pyraclostrobin-1			**Pyraclostrobin-1**	
					Pyraclostrobin--2
Fenpyroximate-2			**Fenpyroximate-2**		
			Bifenthrin-1	Bifenthrin-1	
Bifenthrin-2			Bifenthrin-2	Bifenthrin-2	
					Etofenprox-1
			Cyfluthrin-3-1	Cyfluthrin-3-1	
			Cyfluthrin-1-1	Cyfluthrin-1-1*	Cyfluthrin-1-1*
Both QuEChERS Methods	QuEChERS With Chlorofiltr	QuEChERS Without Chlorofiltr	QuEChERS With Chlorofiltr and Solvent	Solvent Only	

When comparing detected transitions between the two mobile phase conditions, Bifenazate-2 was the only transition detected when using ACN conditions and Qu1 extraction method and in MeOH conditions and Qu2 extraction method. There were twenty-one transitions detected using the MeOH conditions that weren't detected utilizing the ACN conditions and only two transitions detected when using ACN that weren't observed using MeOH. Although there are certain analytes and transitions that are only seen on specific mobile phase conditions and extraction methods, the ability to detect these transitions will be further analyzed in the following samples.

3.2.4 Ghost Train Haze Analysis

In the GTH sample, there was less difference in the number of analytes detected between the two mobile phases. There were forty-eight total transitions detected using the ACN mobile phase run, and fifty-six total transitions detected using the MeOH mobile phase run. The ACN mobile phase run had two transitions detected by Q1 only: Fenpyroximate-2 and Cyfluthrin 3-1. The Fenpyroximate-2 transition was also only seen in Qu1 in the AT sample. There were also four transitions in the ACN mobile phase conditions that were only detected by Qu2. These included Bifentrhin-1, Pyraclostrobin-1, Kresoxim-methyl 1-3, and Kresoxim-methyl 1-4. Malathion-2 was detected when using Qu2 and solvent extraction but not in Qu1. There were fifteen transitions that were detected in both QuEChERS methods, and twenty-six transitions detected in all extraction methods on the ACN mobile phase.

Table 7. Direct Comparison of Detected MRM Transitions in Ghost Train Haze Sample. Transitions detected by both acetonitrile and methanol mobile phases are further separated by the extraction methods. Color key is seen at the bottom and all bolded rows indicate transitions detected in the same extraction methods across both mobile phase methods. *Detected in the blank preceding the sample.

Acetonitrile			Methanol		
QuEChERS With Chlorofiltr	**QuEChERS Without Chlorofiltr**	**Solvent**	**QuEChERS With Chlorofiltr**	**QuEChERS Without Chlorofiltr**	**Solvent**
Dichlorvos-2	Dichlorvos-2		Dichlorvos-2*	Dichlorvos-2*	Dichlorvos-2
Dichlorvos-1	Dichlorvos-1		Dichlorvos-1	Dichlorvos-1	Dichlorvos-1
Mevinphos (phosdrin) - 3	**Mevinphos (phosdrin) - 3**		**Mevinphos (phosdrin) - 3**	**Mevinphos (phosdrin) - 3**	
Ethoprophos (ethoprofos) - 1	**Ethoprophos (ethoprofos) - 1**		**Ethoprophos (ethoprofos) - 1**	**Ethoprophos (ethoprofos) - 1**	
Ethoprophos (ethoprofos) - 4	**Ethoprophos (ethoprofos) - 4**		**Ethoprophos (ethoprofos) - 4**	**Ethoprophos (ethoprofos) - 4**	
Thiacloprid-2*	**Thiacloprid-2***	**Thiacloprid-2***	**Thiacloprid-2***	**Thiacloprid-2***	**Thiacloprid-2***
Methyl parathion-3	**Methyl parathion-3**		**Methyl parathion-3**	**Methyl parathion-3**	
				Metalaxyl-2	
Dodemorph-1	**Dodemorph-1**		**Dodemorph-1**	**Dodemorph-1**	
Myclobutanil-1	**Myclobutanil-1**		**Myclobutanil-1**	**Myclobutanil-1**	
Myclobutanil-2	**Myclobutanil-2**		**Myclobutanil-2**	**Myclobutanil-2**	
Paclobutrazol-1	**Paclobutrazol-1**		**Paclobutrazol-1**	**Paclobutrazol-1**	
			Bifenazate-2	Bifenazate-2	
Fenoxycarb-1*	Fenoxycarb-1*	Fenoxycarb-1*			
				Diazinon-2	
				Tebuconazole-1	
	Kresoxim-methyl 1-3		Kresoxim-methyl 1-3	Kresoxim-methyl 1-3	Kresoxim-methyl 1-3
	Kresoxim-methyl 1-4		Kresoxim-methyl 1-4	Kresoxim-methyl 1-4	Kresoxim-methyl 1-4
				Kresoxim-methyl 1-2	
Cinerin-I-1	**Cinerin-I-1**	**Cinerin-I-1**	**Cinerin-I-1**	**Cinerin-I-1**	**Cinerin-I-1***
Cinerin-I-2	**Cinerin-I-2**	**Cinerin-I-2**	**Cinerin-I-2**	**Cinerin-I-2**	**Cinerin-I-2***
Phosmet-2	Phosmet-2		Phosmet-2	Phosmet-2	Phosmet-2
Phosmet-3*	**Phosmet-3***	**Phosmet-3***	**Phosmet-3***	**Phosmet-3***	**Phosmet-3***
				Phosmet-1	
Pyrethrin-I-1*	**Pyrethrin-I-1***	**Pyrethrin-I-1***	**Pyrethrin-I-1***	**Pyrethrin-I-1***	**Pyrethrin-I-1***
Pyrethrin-I-2*	**Pyrethrin-I-2***	**Pyrethrin-I-2***	**Pyrethrin-I-2***	**Pyrethrin-I-2***	**Pyrethrin-I-2***
Iprodione-1-1*	**Iprodione-1-1**	**Iprodione-1-1**	**Iprodione-1-1***	**Iprodione-1-1***	**Iprodione-1-1***
Malathion-1*	**Malathion-1***	**Malathion-1***	**Malathion-1***	**Malathion-1***	**Malathion-1***
	Malathion-2	Malathion-2	Malathion-2	Malathion-2	Malathion-2
Jasmolin-I-1*	**Jasmolin-I-1**	**Jasmolin-I-1**	**Jasmolin-I-1***	**Jasmolin-I-1***	**Jasmolin-I-1***
Jasmolin-I-2*	**Jasmolin-I-2**	**Jasmolin-I-2***	**Jasmolin-I-2***	**Jasmolin-I-2***	**Jasmolin-I-2***
			Iprodione-3-1		
Propiconazole-1*	**Propiconazole-1***	**Propiconazole-1***	**Propiconazole-1***	**Propiconazole-1***	**Propiconazole-1***
Propiconazole-2*	**Propiconazole-2***	**Propiconazole-2***	**Propiconazole-2***	**Propiconazole-2***	**Propiconazole-2***
			Boscalid-2		
Thiophanatemethyl-1	**Thiophanatemethyl-1**	**Thiophanatemethyl-1***	**Thiophanatemethyl-1**	**Thiophanatemethyl-1***	**Thiophanatemethyl-1***
Thiophanatemethyl-2	Thiophanatemethyl-2		Thiophanatemethyl-2	Thiophanatemethyl-2	Thiophanatemethyl-2
			Chlorpyrifos-1		
Tebufenozide-1	**Tebufenozide-1**		**Tebufenozide-1**	**Tebufenozide-1**	
Piperonyl Butoxide-2	**Piperonyl Butoxide-2**	**Piperonyl Butoxide-2**	**Piperonyl Butoxide-2**	**Piperonyl Butoxide-2***	**Piperonyl Butoxide-2**
Piperonyl Butoxide-1	**Piperonyl Butoxide-1**	**Piperonyl Butoxide-1**	**Piperonyl Butoxide-1**	**Piperonyl Butoxide-1**	**Piperonyl Butoxide-1**
Etoxazole-2	Etoxazole-2	Etoxazole-2		Etoxazole-2	
Etoxazole-1	Etoxazole-1	Etoxazole-1			
Cinerin-II-1	**Cinerin-II-1**	**Cinerin-II-1**	**Cinerin-II-1**	**Cinerin-II-1***	**Cinerin-II-1***
Cinerin-II-2	Cinerin-II-2	Cinerin-II-2		Cinerin-II-2	
Coumaphos-1	Coumaphos-1	Coumaphos-1	Coumaphos-1	Coumaphos-1	
Pyridaben-1	Pyridaben-1				
Pyrethrin-II-1	**Pyrethrin-II-1**	**Pyrethrin-II-1**	**Pyrethrin-II-1**	**Pyrethrin-II-1**	**Pyrethrin-II-1**
Pyrethrin-II-2*	**Pyrethrin-II-2**	**Pyrethrin-II-2***	**Pyrethrin-II-2***	**Pyrethrin-II-2***	**Pyrethrin-II-2***
Spirotetramat-2	**Spirotetramat-2**	**Spirotetramat-2**	**Spirotetramat-2**	**Spirotetramat-2**	**Spirotetramat-2**
Jasmolin-II-1	**Jasmolin-II-1**	**Jasmolin-II-1**	**Jasmolin-II-1**	**Jasmolin-II-1**	**Jasmolin-II-1**
Jasmolin-II-2	**Jasmolin-II-2**	**Jasmolin-II-2**	**Jasmolin-II-2**	**Jasmolin-II-2**	**Jasmolin-II-2**
	Pyraclostrobin-1			**Pyraclostrobin-1**	
Fenpyroximate-2			**Fenpyroximate-2**		
	Bifenthrin-1		Bifenthrin-1		
Bifenthrin-2	Bifenthrin-2		Bifenthrin-2	Bifenthrin-2	Bifenthrin-2
Cyfluthrin-3-1			**Cyfluthrin-3-1**		
			Spinosad D-2		
				Spinosad A-2	
Both QuEChERS Methods	QuEChERS With Chlorofiltr	QuEChERS Without Chlorofiltr	QuEChERS Without Chlorofiltr and Solvent		

The MeOH mobile phase run had seven transitions that were only detected using Qu1. Of these seven, two were the Fenpyroximate-2 and Cyfluthrin 3-1 transitions detected using ACN Qu1 and the other five were not detected in any other method. There were nine transitions that were only detected with Qu2. Six of these transitions were only detected in this method with MeOH conditions, Pyraclostrobin-1 was detected in both mobile phases on this method, and Etoxazole-2 and Cinerin-II-2 were detected among every method on the ACN mobile phase and only Qu2 in MeOH. There were eleven transitions that were detected by both QuEChERS methods and twenty-nine transitions that were detected using all extraction methods and MeOH mobile phase conditions.

When comparing the two mobile phases, thirty-two transitions were detected via the same extraction methods across the two mobile phases. Three transitions (Fenoxycarb-1, Etoxazole-1, and Pyridaben-1) were detected using ACN conditions that weren't observed using MeOH and eleven transitions were detected using MeOH conditions that weren't observed using ACN. Overall, more transitions and analytes were detected using the MeOH mobile phase. Qu1 and Qu2 methods both had analytes that weren't detected in the other, though both are shown to be better extraction methods than the solvent extraction.

3.2.5 Black Triangle Kush Analysis

In the sample BTK, forty-five transitions were detected using the ACN mobile phase conditions and sixty-three analytes were detected using the MeOH mobile phase conditions. The ACN mobile phase had four analytes that were detected when using Qu1

only, including Ethoprophos (ethoprofos)-1, Tebuconazole-2, Pyridaben-1, and Fenpyroximate-2. Five transitions were detected when using Qu2 only, of these transitions, Methyl Parathion-3, Myclobutanil-2 were also seen in both QuEChERS methods on the MeOH mobile phase conditions. Kresoxim-methyl 1-4 and Pyraclostrobin-2 were seen when using all extraction methods in the MeOH mobile phase and Kresoxim-methyl 1-1 was not observed in any extraction method using MeOH mobile phase conditions. Eleven Transitions were observed using both Qu1 and Qu2 extraction methods with ACN mobile phase conditions and twenty-two transitions were observed in all extraction methods using ACN mobile phase conditions.

In the Qu1 extraction of the MeOH mobile phase, nine transitions were detected. Fenpyroximate-2 was also detected in the ACN Qu1 method, but the other eight transitions were exclusively detected in the MeOH Qu1 method. Three transitions were detected in the Qu2 MeOH method. Pyraclostrobin-1 and Methiocarb-2 were only detected by this method, through Pyridaben-1 was detected in both Qu1 ACN and Qu2 MeOH. Cyfluthrin 1-1 was the only analyte to be detected in MeOH conditions by using Qu2 and solvent only. Sixteen transitions were detected in MeOH conditions by both Qu1 and Qu2 methods and thirty-four transitions were detected when using all extraction methods.

When comparing the two mobile phases, twenty-two transitions were detected by the same extraction methods between mobile phases. Two transitions (Fenoxycarb-1 and Kresoxim-methyl 1-1) were detected in ACN conditions that were not detected in MeOH conditions, and twenty transitions were detected using MeOH conditions that weren’t detected by ACN conditions.

Table 8. Direct Comparison of Detected MRM Transitions in Black Triangle Kush Sample. Transitions detected by both acetonitrile and methanol mobile phases are further separated by the extraction methods. Color key is seen at the bottom and all bolded rows indicate transitions detected in the same extraction methods across both mobile phase methods. *Detected in the blank preceding the sample.

Acetonitrile			Methanol		
QuEChERS With Chlorofiltr	**QuEChERS Without Chlorofiltr**	**Solvent**	**QuEChERS With Chlorofiltr**	**QuEChERS Without Chlorofiltr**	**Solvent**
			Carbaryl-2	Carbaryl-2	
			Carbaryl-1		
			Aldicarb-2		
			Propoxur-1		
Dichlorvos-2*	Dichlorvos-2		Dichlorvos-2*	Dichlorvos-2	Dichlorvos-2
Dichlorvos-1	Dichlorvos-1		Dichlorvos-1	Dichlorvos-1	Dichlorvos-1
Mevinphos (phosdrin)-3	Mevinphos (phosdrin)-3		Mevinphos (phosdrin)-3	Mevinphos (phosdrin)-3	
			Mevinphos (phosdrin)-1	Mevinphos (phosdrin)-1	
				Methiocarb-2	
			Pirimicarb-1		
Ethoprophos (ethoprofos)-1			Ethoprophos (ethoprofos)-1	Ethoprophos (ethoprofos)-1	
			Ethoprophos (ethoprofos)-4	Ethoprophos (ethoprofos)-4	
Thiacloprid-2*	**Thiacloprid-2***	**Thiacloprid-2***	**Thiacloprid-2***	**Thiacloprid-2***	**Thiacloprid-2***
			Thiacloprid-1	Thiacloprid-1	
	Methyl Parathion-3		Methyl Parathion-3	Methyl Parathion-3	
			Dodemorph-1		
			Myclobutanil-1	Myclobutanil-1	
	Myclobutanil-2		Myclobutanil-2	Myclobutanil-2	
Paclobutrazol-1	**Paclobutrazol-1**		**Paclobutrazol-1**	**Paclobutrazol-1**	
			Bifenazate-2	Bifenazate-2	
			Bifenazate-1		
Fenoxycarb-1*	Fenoxycarb-1*	Fenoxycarb-1*			
Tebuconazole-2			Tebuconazole-2	Tebuconazole-2	Tebuconazole-2
Kresoxim-methyl 1-3	Kresoxim-methyl 1-3		Kresoxim-methyl 1-3	Kresoxim-methyl 1-3	Kresoxim-methyl 1-3
	Kresoxim-methyl 1-4		Kresoxim-methyl 1-4	Kresoxim-methyl 1-4	Kresoxim-methyl 1-4
	Kresoxim-methyl 1-1				
			Kresoxim-methyl 1-2	Kresoxim-methyl 1-2	
Cinerin-I-1	**Cinerin-I-1**	**Cinerin-I-1**	**Cinerin-I-1**	**Cinerin-I-1***	**Cinerin-I-1**
Cinerin-I-2	**Cinerin-I-2**	**Cinerin-I-2**	**Cinerin-I-2**	**Cinerin-I-2**	**Cinerin-I-2**
Phosmet-2	Phosmet-2		Phosmet-2	Phosmet-2	Phosmet-2
Phosmet-3*	**Phosmet-3***	**Phosmet-3***	**Phosmet-3***	**Phosmet-3***	**Phosmet-3***
			Phosmet-1		
Pyrethrin-I-1*	**Pyrethrin-I-1***	**Pyrethrin-I-1***	**Pyrethrin-I-1***	**Pyrethrin-I-1***	**Pyrethrin-I-1***
Pyrethrin-I-2*	**Pyrethrin-I-2***	**Pyrethrin-I-2***	**Pyrethrin-I-2***	**Pyrethrin-I-2***	**Pyrethrin-I-2***
Iprodione 1-1*	Iprodione 1-1*	Iprodione 1-1*	Iprodione 1-1*	Iprodione 1-1	
Malathion-1*	**Malathion-1***	**Malathion-1***	**Malathion-1***	**Malathion-1***	**Malathion-1***
Malathion-2	Malathion-2		Malathion-2	Malathion-2	Malathion-2
Jasmolin-I-1*	**Jasmolin-I-1**	**Jasmolin-I-1***	**Jasmolin-I-1**	**Jasmolin-I-1***	**Jasmolin-I-1***
Jasmolin-I-2*	**Jasmolin-I-2***	**Jasmolin-I-2**	**Jasmolin-I-2**	**Jasmolin-I-2***	**Jasmolin-I-2***
			Iprodione 3-1	Iprodione 3-1	
Propiconazole-1*	**Propiconazole-1***	**Propiconazole-1***	**Propiconazole-1***	**Propiconazole-1***	**Propiconazole-1***
Propiconazole-2*	**Propiconazole-2***	**Propiconazole-2***	**Propiconazole-2***	**Propiconazole-2***	**Propiconazole-2***
Thiophanatemethyl-1	**Thiophanatemethyl-1**	**Thiophanatemethyl-1**	**Thiophanatemethyl-1***	**Thiophanatemethyl-1***	**Thiophanatemethyl-1***
Thiophanatemethyl-2	Thiophanatemethyl-2		Thiophanatemethyl-2	Thiophanatemethyl-2	Thiophanatemethyl-2
			Chlorpyrifos-1	Chlorpyrifos-1	
			Hexythiazox-2		
Tebufenozide-1	Tebufenozide-1		Tebufenozide-1	Tebufenozide-1	Tebufenozide-1
Piperonyl Butoxide-2	Piperonyl Butoxide-2		Piperonyl Butoxide-2	Piperonyl Butoxide-2*	Piperonyl Butoxide-2
Piperonyl Butoxide-1	Piperonyl Butoxide-1		Piperonyl Butoxide-1*	Piperonyl Butoxide-1	Piperonyl Butoxide-1*
Etoxazole-2	**Etoxazole-2**	**Etoxazole-2**	**Etoxazole-2***	**Etoxazole-2***	**Etoxazole-2***
Etoxazole-1	**Etoxazole-1**	**Etoxazole-1**	**Etoxazole-1**	**Etoxazole-1**	**Etoxazole-1**
Cinerin-II-1	**Cinerin-II-1**	**Cinerin-II-1**	**Cinerin-II-1**	**Cinerin-II-1***	**Cinerin-II-1**
Cinerin-II-2	**Cinerin-II-2**	**Cinerin-II-2**	**Cinerin-II-2**	**Cinerin-II-2**	**Cinerin-II-2**
Coumaphos-1	Coumaphos-1		Coumaphos-1	Coumaphos-1	Coumaphos-1
Pyridaben-1				Pyridaben-1	
Pyrethrin-II-1	**Pyrethrin-II-1***	**Pyrethrin-II-1***	**Pyrethrin-II-1***	**Pyrethrin-II-1***	**Pyrethrin-II-1**
Pyrethrin-II-2*	**Pyrethrin-II-2***	**Pyrethrin-II-2***	**Pyrethrin-II-2***	**Pyrethrin-II-2***	**Pyrethrin-II-2***
Spirotetramat-2	**Spirotetramat-2**	**Spirotetramat-2**	**Spirotetramat-2**	**Spirotetramat-2**	**Spirotetramat-2**
Jasmolin-II-1	**Jasmolin-II-1***	**Jasmolin-II-1**	**Jasmolin-II-1**	**Jasmolin-II-1**	**Jasmolin-II-1**
Jasmolin-II-2	Jasmolin-II-2		Jasmolin-II-2	Jasmolin-II-2	Jasmolin-II-2
	Pyraclostrobin-2		Pyraclostrobin-2	Pyraclostrobin-2*	Pyraclostrobin-2
				Pyraclostrobin-1	
Fenpyroximate-2			**Fenpyroximate-2**		
Cyfluthrin 3-1	**Cyfluthrin 3-1**		**Cyfluthrin 3-1**	**Cyfluthrin 3-1**	
				Cyfluthrin 1-1	Cyfluthrin 1-1
Both QuEChERS Methods	QuEChERS With Chlorofiltr	QuEChERS Without Chlorofiltr	QuEChERS Without Chlorofiltr and Solvent		

3.2.6 GG #4 Analysis

In the sample GG, fifty transitions were detected using the ACN mobile phase, and sixty-eight transitions were detected using the MeOH mobile phase conditions. The ACN mobile phase conditions had four transitions detected using Qu1 only. Thiacloprid-1 was detected using Qu1 in ACN mobile phase conditions and all extractions using MeOH mobile phase conditions. Myclobutanil-2 and Cypermethrin 1-1 were detected using Qu1 in ACN conditions and both Qu1 and Qu2 in MeOH conditions, and Fenpyroximate-2 was detected using Qu1 in both ACN and MeOH conditions. ACN conditions had six transitions detected using Qu2. Of these six, pyraclostrobin-1 was detected only by utilizing Qu2 in both mobile phases while the other five transitions were detected by utilizing both Qu1 and Qu2 in MeOH conditions. Pyraclostrobin-2 was the only transition to be detected using ACN solvent extraction only but by all extraction methods using MeOH conditions. Using ACN conditions, ten transitions were detected using both Qu1 and Qu2 and twenty-nine transitions were detected when using all extraction methods.

Using the MeOH mobile phase, three transitions were detected only when utilizing Qu1 including Fenpyroximate-2, which was also only seen when using Qu1 in ACN conditions, along with Spiromesifen-1 and Propoxur-1 which were only detected when using MeOH conditions and Qu1 extraction. There were nine transitions detected only with Qu2 in MeOH conditions and of those nine, eight were not detected in ACN conditions. Pyraclostrobin-1 was the only transition detected by Qu2 for both mobile phase conditions. There were twenty-two transitions using MeOH conditions that were detected by utilizing

both Qu1 and Qu2 and thirty-four transitions were detected when utilizing all extraction methods.

Table 9. Direct Comparison of Detected MRM Transitions in GG #4 Sample. Transitions detected by both acetonitrile and methanol mobile phases are further separated by the extraction methods. Color key is seen at the bottom and all bolded rows indicate transitions detected in the same extraction methods across both mobile phase methods. *Detected in the blank preceding the sample.

Acetonitrile			Methanol		
QuEChERS With Chlorofiltr	**QuEChERS Without Chlorofiltr**	**Solvent**	**QuEChERS With Chlorofiltr**	**QuEChERS Without Chlorofiltr**	**Solvent**
			Propoxur-1		
Dichlorvos-2	Dichlorvos-2		Dichlorvos-2	Dichlorvos-2	Dichlorvos-2*
Dichlorvos-1	Dichlorvos-1		Dichlorvos-1	Dichlorvos-1	Dichlorvos-1
Mevinphos (phosdrin)-3	**Mevinphos (phosdrin)-3**		**Mevinphos (phosdrin)-3**	**Mevinphos (phosdrin)-3**	
Mevinphos (phosdrin)-1	**Mevinphos (phosdrin)-1**		**Mevinphos (phosdrin)-1**	**Mevinphos (phosdrin)-1**	
				Methiocarb-2	
				Ethoprophos (ethoprofos)-1	
Thiacloprid-2*	**Thiacloprid-2***	**Thiacloprid-2***	**Thiacloprid-2***	**Thiacloprid-2***	**Thiacloprid-2***
Thiacloprid-1			Thiacloprid-1	Thiacloprid-1	Thiacloprid-1
			Methyl Parathion-3	Methyl Parathion-3	
			Spiromesifen-1		
			Myclobutanil-1	Myclobutanil-1	
Myclobutanil-2			Myclobutanil-2	Myclobutanil-2	
	Paclobutrazol-1		Paclobutrazol-1	Paclobutrazol-1	
			Spiroxamine-1	Spiroxamine-1	
Bifenazate-2	**Bifenazate-2**		**Bifenazate-2**	**Bifenazate-2**	
Bifenazate-1	Bifenazate-1		Bifenazate-1	Bifenazate-1	Bifenazate-1
Bifenazate-3	**Bifenazate-3**	**Bifenazate-3**	**Bifenazate-3**	**Bifenazate-3**	**Bifenazate-3**
				Diazinon-1	
Fenoxycarb-1*	Fenoxycarb-1*	Fenoxycarb-1*			
				Tebuconazole-1	
	Kresoxim-methyl 1-3		Kresoxim-methyl 1-3	Kresoxim-methyl 1-3	
			Kresoxim-methyl 1-4	Kresoxim-methyl 1-4	
	Kresoxim-methyl 1-2		Kresoxim-methyl 1-2	Kresoxim-methyl 1-2	
Cinerin-I-1	**Cinerin-I-1**	**Cinerin-I-1***	**Cinerin-I-1**	**Cinerin-I-1**	**Cinerin-I-1***
Cinerin-I-2	**Cinerin-I-2**	**Cinerin-I-2**	**Cinerin-I-2**	**Cinerin-I-2**	**Cinerin-I-2***
Phosmet-2	**Phosmet-2**		**Phosmet-2**	**Phosmet-2**	
Phosmet-3*	**Phosmet-3***	**Phosmet-3***	**Phosmet-3***	**Phosmet-3***	**Phosmet-3***
			Phosmet-1	Phosmet-1	
Pyrethrin-I-1*	**Pyrethrin-I-1***	**Pyrethrin-I-1***	**Pyrethrin-I-1**	**Pyrethrin-I-1***	**Pyrethrin-I-1***
Pyrethrin-I-2*	**Pyrethrin-I-2***	**Pyrethrin-I-2***	**Pyrethrin-I-2***	**Pyrethrin-I-2***	**Pyrethrin-I-2***
Iprodione-1-1	**Iprodione-1-1***	**Iprodione-1-1***	**Iprodione-1-1**	**Iprodione-1-1***	**Iprodione-1-1***
Malathion-1*	**Malathion-1***	**Malathion-1***	**Malathion-1***	**Malathion-1***	**Malathion-1***
Malathion-2	Malathion-2		Malathion-2	Malathion-2	Malathion-2
Jasmolin-I-1*	**Jasmolin-I-1**	**Jasmolin-I-1***	**Jasmolin-I-1**	**Jasmolin-I-1**	**Jasmolin-I-1***
Jasmolin-I-2	**Jasmolin-I-2**	**Jasmolin-I-2***	**Jasmolin-I-2**	**Jasmolin-I-2**	**Jasmolin-I-2***
			Iprodione-3-1	Iprodione-3-1	
Propiconazole-1*	**Propiconazole-1***	**Propiconazole-1***	**Propiconazole-1***	**Propiconazole-1***	**Propiconazole-1***
Propiconazole-2*	**Propiconazole-2**	**Propiconazole-2***	**Propiconazole-2***	**Propiconazole-2***	**Propiconazole-2***
				Boscalid-2	
				Boscalid-1	
Thiophanatemethyl-1	**Thiophanatemethyl-1**	**Thiophanatemethyl-1**	**Thiophanatemethyl-1***	**Thiophanatemethyl-1***	**Thiophanatemethyl-1***
Thiophanatemethyl-2	**Thiophanatemethyl-2**	**Thiophanatemethyl-2**	**Thiophanatemethyl-2**	**Thiophanatemethyl-2**	**Thiophanatemethyl-2**
	Chlorpyrifos-1		Chlorpyrifos-1	Chlorpyrifos-1	
			Chlorpyrifos-2	Chlorpyrifos-2	
Tebufenozide-1	**Tebufenozide-1**	**Tebufenozide-1**	**Tebufenozide-1**	**Tebufenozide-1**	**Tebufenozide-1**
				Tebufenozide-2	
Piperonyl Butoxide-2	**Piperonyl Butoxide-2**	**Piperonyl Butoxide-2**	**Piperonyl Butoxide-2**	**Piperonyl Butoxide-2**	**Piperonyl Butoxide-2***
Piperonyl Butoxide-1	**Piperonyl Butoxide-1**	**Piperonyl Butoxide-1**	**Piperonyl Butoxide-1**	**Piperonyl Butoxide-1***	**Piperonyl Butoxide-1***
Etoxazole-2	**Etoxazole-2**	**Etoxazole-2**	**Etoxazole-2**	**Etoxazole-2***	**Etoxazole-2***
Etoxazole-1	**Etoxazole-1**	**Etoxazole-1**	**Etoxazole-1**	**Etoxazole-1**	**Etoxazole-1**
Cinerin-II-1	**Cinerin-II-1**	**Cinerin-II-1**	**Cinerin-II-1**	**Cinerin-II-1**	**Cinerin-II-1***
Cinerin-II-2	**Cinerin-II-2**	**Cinerin-II-2**	**Cinerin-II-2**	**Cinerin-II-2**	**Cinerin-II-2**
Coumaphos-1	**Coumaphos-1**	**Coumaphos-1**	**Coumaphos-1**	**Coumaphos-1**	**Coumaphos-1**
Pyridaben-1	**Pyridaben-1**		**Pyridaben-1**	**Pyridaben-1**	
Pyrethrin-II-1	**Pyrethrin-II-1**	**Pyrethrin-II-1***	**Pyrethrin-II-1**	**Pyrethrin-II-1***	**Pyrethrin-II-1***
Pyrethrin-II-2	**Pyrethrin-II-2***	**Pyrethrin-II-2***	**Pyrethrin-II-2***	**Pyrethrin-II-2***	**Pyrethrin-II-2***
Spirotetramat-2	**Spirotetramat-2**	**Spirotetramat-2**	**Spirotetramat-2**	**Spirotetramat-2**	**Spirotetramat-2***
Jasmolin-II-1	**Jasmolin-II-1**	**Jasmolin-II-1**	**Jasmolin-II-1**	**Jasmolin-II-1**	**Jasmolin-II-1**
Jasmolin-II-2	**Jasmolin-II-2**	**Jasmolin-II-2**	**Jasmolin-II-2**	**Jasmolin-II-2**	**Jasmolin-II-2**
		Pyraclostrobin-2	Pyraclostrobin-2	Pyraclostrobin-2	Pyraclostrobin-2
	Pyraclostrobin-1			**Pyraclostrobin-1**	
Etofenprox-2	**Etofenprox-2**		**Etofenprox-2**	**Etofenprox-2**	
			Etofenprox-1	Etofenprox-1	
Fenpyroximate-2			**Fenpyroximate-2**		
Cypermethrin-1-1			Cypermethrin-1-1	Cypermethrin-1-1	
				Bifenthrin-1	
	Cyfluthrin-3-1		Cyfluthrin-3-1*	Cyfluthrin-3-1	
			Cyfluthrin-1-1	Cyfluthrin-1-1	
Both QuEChERS Methods	QuEChERS With Chlorofiltr	QuEChERS Without Chlorofiltr	Solvent Only		

When comparing the two mobile phases, there were thirty-six transitions that were detected by LC-MS/MS using the same extraction methods across mobile phases. Fenoxycarb-1 was the only transition detected by the ACN mobile phase that wasn't detected by the MeOH mobile phase. When utilizing the MeOH mobile phase conditions, nineteen transitions were detected that weren't observed in ACN conditions.

3.2.7 Walker Kush Analysis

In the sample WK, forty-six transitions were detected in the ACN mobile phase, and 63 transitions were detected using the MeOH mobile phase. Using the ACN mobile phase, only four transitions were detected using Qu1. Thiacloprid-1 and Myclobutanil-1 were among these four but were detected using both Qu1 and Qu2 in the MeOH mobile phase conditions. Mevinphos (phosdrin)-2 was detected utilizing Qu1 in ACN conditions and not in any extraction method using MeOH mobile phase and Fenpyroximate-2 was detected using only Qu1 in both MeOH and ACN conditions. There were five transitions detected using Qu2 in ACN conditions, Chlorpyrifos-1, Phosmet-2, and Ethoprophos (ethoprofos)-1 were also detected using both Qu1 and Qu2 in MeOH conditions. Malathion-2 was detected utilizing all extraction methods in MeOH conditions, and Pyraclostrobin-1 was detected only when using Qu2 in both mobile phase conditions. Boscalid-2 was detected using ACN conditions only by solvent extraction but was also detected in MeOH conditions only by Qu1. Using ACN, twelve transitions were detected using both Qu1 and Qu2, while twenty-four transitions were detected by utilizing all extraction methods.

Table 10. Direct Comparison of Detected MRM Transitions in Walker Kush Sample. Transitions detected by both acetonitrile and methanol mobile phases are further separated by the extraction methods. Color key is seen at the bottom and all bolded rows indicate transitions detected in the same extraction methods across both mobile phase methods. *Detected in the blank preceding the sample.

Acetonitrile			Methanol		
QuEChERS With Chlorofiltr	**QuEChERS Without Chlorofiltr**	**Solvent**	**QuEChERS With Chlorofiltr**	**QuEChERS Without Chlorofiltr**	**Solvent**
			Carbaryl-2	Carbaryl-2	Carbaryl-2
				Carbaryl-1	
Dichlorvos-2	Dichlorvos-2		Dichlorvos-2	Dichlorvos-2	Dichlorvos-2
Dichlorvos-1	Dichlorvos-1		Dichlorvos-1	Dichlorvos-1	Dichlorvos-1
			Methiocarb-2		
Mevinphos (phosdrin)-3	Mevinphos (phosdrin)-3				
Mevinphos (phosdrin)-2					
				Ethoprophos (ethoprofos)-4	
	Ethoprophos (ethoprofos)-1		Ethoprophos (ethoprofos)-1	Ethoprophos (ethoprofos)-1	
Thiacloprid-2*	**Thiacloprid-2***	**Thiacloprid-2***	**Thiacloprid-2***	**Thiacloprid-2***	**Thiacloprid-2***
Thiacloprid-1			Thiacloprid-1	Thiacloprid-1	
Methyl Parathion-3	**Methyl Parathion-3**		**Methyl Parathion-3**	**Methyl Parathion-3**	
Dodemorph-1	**Dodemorph-1**		**Dodemorph-1**	**Dodemorph-1**	
Myclobutanil-1			Myclobutanil-1	Myclobutanil-1	
				Myclobutanil-2	
Paclobutrazol-1	**Paclobutrazol-1**		**Paclobutrazol-1**	**Paclobutrazol-1**	
			Imazalil-4		
			Bifenazate-1		
Fenoxycarb-1*	Fenoxycarb-1*	Fenoxycarb-1*			
				Tebuconazole-1	
				Kresoxim-methyl 1-3	
			Kresoxim-methyl 1-4	Kresoxim-methyl 1-4	
			Kresoxim-methyl 1-2	Kresoxim-methyl 1-2	
Cinerin-I-1	**Cinerin-I-1**	**Cinerin-I-1**	**Cinerin-I-1**	**Cinerin-I-1**	**Cinerin-I-1***
Cinerin-I-2	Cinerin-I-2		Cinerin-I-2	Cinerin-I-2*	Cinerin-I-2*
	Phosmet-2		Phosmet-2	Phosmet-2	
Phosmet-3*	**Phosmet-3***	**Phosmet-3***	**Phosmet-3***	**Phosmet-3***	**Phosmet-3***
Pyrethrin-I-1*	**Pyrethrin-I-1***	**Pyrethrin-I-1***	**Pyrethrin-I-1***	**Pyrethrin-I-1***	**Pyrethrin-I-1***
Pyrethrin-I-2*	**Pyrethrin-I-2***	**Pyrethrin-I-2***	**Pyrethrin-I-2***	**Pyrethrin-I-2***	**Pyrethrin-I-2***
Iprodione-1-1	**Iprodione-1-1***	**Iprodione-1-1***	**Iprodione-1-1**	**Iprodione-1-1***	**Iprodione-1-1***
Malathion-1*	**Malathion-1***	**Malathion-1***	**Malathion-1***	**Malathion-1***	**Malathion-1***
	Malathion-2		Malathion-2	Malathion-2	Malathion-2
Jasmolin-I-1*	**Jasmolin-I-1**	**Jasmolin-I-1**	**Jasmolin-I-1**	**Jasmolin-I-1**	**Jasmolin-I-1***
Jasmolin-I-2*	**Jasmolin-I-2***	**Jasmolin-I-2**	**Jasmolin-I-2**	**Jasmolin-I-2***	**Jasmolin-I-2**
			Iprodione-3-1	Iprodione-3-1	
Propiconazole-1*	**Propiconazole-1***	**Propiconazole-1***	**Propiconazole-1***	**Propiconazole-1***	**Propiconazole-1***
Propiconazole-2*	**Propiconazole-2***	**Propiconazole-2***	**Propiconazole-2***	**Propiconazole-2***	**Propiconazole-2***
		Boscalid-2	Boscalid-2		
			Boscalid-1		
Thiophanatemethyl-1	**Thiophanatemethyl-1**	**Thiophanatemethyl-1***	**Thiophanatemethyl-1**	**Thiophanatemethyl-1***	**Thiophanatemethyl-1***
Thiophanatemethyl-2	Thiophanatemethyl-2		Thiophanatemethyl-2	Thiophanatemethyl-2	Thiophanatemethyl-2
	Chlorpyrifos-1		Chlorpyrifos-1	Chlorpyrifos-1	
			Chlorpyrifos-2		
Tebufenozide-1	Tebufenozide-1		Tebufenozide-1	Tebufenozide-1	Tebufenozide-1
Piperonyl Butoxide-2	**Piperonyl Butoxide-2**	**Piperonyl Butoxide-2**	**Piperonyl Butoxide-2**	**Piperonyl Butoxide-2**	**Piperonyl Butoxide-2**
Piperonyl Butoxide-1	**Piperonyl Butoxide-1**	**Piperonyl Butoxide-1**	**Piperonyl Butoxide-1**	**Piperonyl Butoxide-1**	**Piperonyl Butoxide-1**
Etoxazole-2	Etoxazole-2	Etoxazole-2	Etoxazole-2*	Etoxazole-2*	
Etoxazole-1	Etoxazole-1	Etoxazole-1	Etoxazole-1	Etoxazole-1	
Cinerin-II-1	**Cinerin-II-1**	**Cinerin-II-1**	**Cinerin-II-1***	**Cinerin-II-1**	**Cinerin-II-1**
Cinerin-II-2	Cinerin-II-2	Cinerin-II-2	Cinerin-II-2	Cinerin-II-2	
Coumaphos-1	Coumaphos-1		Coumaphos-1	Coumaphos-1	Coumaphos-1
Pyridaben-1	Pyridaben-1			Pyridaben-1	Pyridaben-1
Pyrethrin-II-1	**Pyrethrin-II-1**	**Pyrethrin-II-1**	**Pyrethrin-II-1**	**Pyrethrin-II-1**	**Pyrethrin-II-1***
Pyrethrin-II-2*	**Pyrethrin-II-2***	**Pyrethrin-II-2***	**Pyrethrin-II-2**	**Pyrethrin-II-2***	**Pyrethrin-II-2***
Spirotetramat-2	**Spirotetramat-2**	**Spirotetramat-2***	**Spirotetramat-2**	**Spirotetramat-2**	**Spirotetramat-2**
Jasmolin-II-1	**Jasmolin-II-1**	**Jasmolin-II-1**	**Jasmolin-II-1**	**Jasmolin-II-1**	**Jasmolin-II-1**
Jasmolin-II-2	**Jasmolin-II-2**	**Jasmolin-II-2**	**Jasmolin-II-2**	**Jasmolin-II-2**	**Jasmolin-II-2**
			Pyraclostrobin-2	Pyraclostrobin-2	Pyraclostrobin-2
	Pyraclostrobin-1			**Pyraclostrobin-1**	
Etofenprox-2	**Etofenprox-2**		**Etofenprox-2**	**Etofenprox-2**	
Fenpyroximate-2			**Fenpyroximate-2**		
				Cypermethrin-1-1	
				Bifenthrin-1	
				Cyfluthrin-3-1	Cyfluthrin-3-1
					Cyfluthrin-1-1
			Spinetoram(J)-1		
Both QuEChERS Methods	QuEChERS With Chlorofiltr	QuEChERS Without Chlorofiltr	QuEChERS Without Chlorofiltr and Solvent	Solvent Only	

Using the MeOH mobile phase conditions, eight transitions were detected only utilizing Qu1. Of these transitions, six were not detected when using any extraction method with ACN mobile phase conditions. Fenpyroximate-2 was detected using Qu1 in both mobile phase conditions and Boscalid-2 as mentioned above was detected using solvent extraction only with ACN conditions. Eight transitions were detected by using Qu2 with MeOH conditions and all but one, Pyraclostrobin-1, were not detected using any extraction method with ACN mobile phase conditions. Pyridaben-1 and Cyfluthrin-3-1 were detected using Qu2 and solvent but not Qu1 using MeOH conditions while Cyfluthrin-1-1 was detected exclusively when using solvent extraction with MeOH conditions.

When comparing the two mobile phases, only two transitions were detected utilizing ACN conditions that weren't seen in any method when using MeOH conditions. These included Mevinphos (phosdrin)-2 which was only detected using Qu1 and Fenoxycarb which was detected when using every extraction method with ACN conditions. Using the MeOH mobile phase conditions, there were twenty transitions detected that weren't seen in any of the extraction methods with ACN mobile phase conditions.

3.2.8 Comparative Analysis

While some trends differed between samples, it was clear that the QuEChERS methods (Qu1 and Qu2) were more effective extraction methods compared to the solvent extraction for analysis of pesticides in cannabis flower samples. The transitions that were detected with only Qu1 and solvent or Qu2 and solvent were unexpected results. The solvent extraction methods don't have any preconcentration properties that are seen with

QuEChERS methods. The pesticides may have been extracted but at levels too low for detection. When extracting with QuEChERS, the ChloroFiltr® cleanup can help to detect analytes that may have been otherwise suppressed by the chlorophyll of the plant material. The ChloroFiltr® can also itself suppress or inhibit the detection of certain pesticides. It is further unknown which sample preparation methods are most effective when lower levels of pesticides are present. Methiocarb-2 was detected in AT using Qu1 and solvent extraction in MeOH conditions but was also detected in WK using Qu1 only in MeOH conditions, and in BTK by Qu1 only. While it can't be determined which extraction method was best for this analyte, it was only detected in the MeOH mobile phase run. Another confounding transition was that of Etofenprox-1. In AT, this transition was only observed using MeOH solvent extraction but in GG it was observed using MeOH conditions with Qu1 and Qu2, not solvent extraction. When determining what transitions will be targeted in future analyses, it will be important to determine how the analytes and transitions behave over different extraction methods at specific concentrations.

Between the mobile phases, Fenoxycarb-1 was the only transition that was detected in all samples using all extraction methods but only using the ACN mobile phase. Over the five cannabis flower strains analyzed, there was a range of eleven-twenty-one transitions detected using the MeOH mobile phase that were not observed using any ACN mobile phase conditions. In general, the transitions that were detected using both mobile phases had earlier retention times in the MeOH mobile phases. The CPS at each peak were overall greater using MeOH. Given the current presented data, MeOH appears to be the favored mobile phase to run for all extraction methods.

4. CONCLUSIONS

In order to analyze the pesticide standard on DART® with minimal background interference, a concentration ten times what was used for LC-MS/MS was needed for DART®. DART® has been shown to be an effective analysis technique for pesticides when interfaced to other mass spectrometers such as an orbitrap or Q-TOF. Although there was significant contribution of ambient background, there is also a technology called JumpShot® that has shown to significantly decrease the background ions. Given the speed of analysis and minimal need for sample preparation methods, DART® has the potential to be a promising ionization technique for presumptive detection of pesticides in cannabis samples.

During the LC-MS/MS analysis, it was clear that both of the QuEChERS methods were more effective than the solvent extraction methods. Not only were there more transitions detected, but the ion intensities were also greater. When comparing ACN and MeOH as mobile phases, the LC-MS/MS approach utilizing the MeOH mobile phase was able to detect more transitions than the ACN mobile phase. Though fenoxycarb wasn't detected in any MeOH methods, it would be in the analyst's best interest if targeting a different set of analytes to determine if any analytes are mobile phase specific. In the case of this study, MeOH was the more favorable mobile phase.

QuEChERS sample preparation methods were slightly different for each sample. While some showed a large difference between the use of the ChloroFiltr®, some didn't show as much of a difference. There were some transitions that weren't detected by the

same method between samples, but overall, the QuEChERS extraction method without the ChloroFiltr® clean-up yielded a greater number of detected transitions.

In future studies, it will be important to optimize the wash solvents in between each run on the LC-MS/MS. There was significant carry over present in every blank on both mobile phase runs. Further studies are needed to optimize the mobile phase gradient as well. Numerous MRM peaks were detected at the very end of the LC method. As a result of this observation, further chromatographic method development would be required to observe the complete peak for these transitions in the same sample rather than in the blank following the sample. The methods also warrant further development to assess the carryover observed.

BIBLIOGRAPHY

1. Bonini SA, Premoli M, Tambaro S, Kumar A, Maccarinelli G, Memo M, et al. Cannabis sativa: A comprehensive ethnopharmacological review of a medicinal plant with a long history. Journal of Ethnopharmacology. 2018;227:300-315.

2. Piluzza G, Delogu G, Cabras A, Marceddu S, Bullitta S. Differentiation between fiber and drug types of hemp (cannabis sativa L.) from a collection of wild and domesticated accessions. Genetic Resources and Crop Evolution. 2013;60(8):2331-2342.

3. Benard AA. The material roots of rastafarian marijuana symbolism. History and Anthropology. 2007;18(1):89-99.

4. Heilig S. The pot book: A complete guide to cannabis, its role in medicine, politics, science, and culture. Journal of Psychoactive Drugs. 2011;43(1):76-77.

5. Mead A. Legal and regulatory issues governing cannabis and cannabis-derived products in the United States. Frontiers in Plant Science. 2019;10.

6. Map of marijuana legality by state [Internet].; 2022 [updated January; cited January 21, 2022]. Available from: https://disa.com/map-of-marijuana-legality-by-state.

7. Guidance on integrated pest management. Commonwealth of Massachusetts; .

8. Is sun-grown cannabis as good as indoor cannabis? [Internet].; 2020 [updated August 4,; cited January 28, 2022]. Available from: https://news.green-flower.com/outdoor-vs-indoor-cannabis/.

9. Punja ZK, Collyer D, Scott C, Lung S, Holmes J, Sutton D. Pathogens and molds affecting production and quality of cannabis sativa L. Frontiers in Plant Science. 2019;10.

10. Pinkhasova DV, Jameson LE, Conrow KD, Simeone MP, Davis AP, Wiegers TC, et al. Regulatory status of pesticide residues in cannabis: Implications to medical use in neurological diseases. Current Research in Toxicology. 2021;2:140-148.

11. Kim K, Kabir E, Jahan SA. Exposure to pesticides and the associated human health effects. Science of the Total Environment. 2017;575:525-535.

12. Nicolopoulou-Stamati P, Maipas S, Kotampasi C, Stamatis P, Hens L. Chemical pesticides and human health: The urgent need for a new concept in agriculture. Frontiers in Public Health. 2016;4.

13. Falzone L, Marconi A, Loreto C, Franco S, Spandidos DA, Libra M. Occupational exposure to carcinogens: Benzene, pesticides and fibers (review). Molecular medicine reports. 2016;14(5):4467-74.

14. Federal insecticide, fungicide, and rodenticide act (FIFRA) and federal facilities [Internet].; cited January 26, 2022]. Available from: https://www.epa.gov/enforcement/federal-insecticide-fungicide-and-rodenticide-act-fifra-and-federal-facilities#Summary.

15. Pesticide products registered for use on hemp [Internet].; cited January 26, 2022]. Available from: https://www.epa.gov/pesticide-registration/pesticide-products-registered-use-hemp#biopesticide.

16. ANASTASSIADES M, LEHOTAY SJ, STAJNBAHER D, SCHENCK FJ. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and dispersive solid-phase extraction for the determination of pesticide residues in produce. Journal of AOAC International. 2003;86(2):412-31.

17. Waters Corporation. QuEChERS procedure for multi-residue pesticide analysis - DisQuE dispersive sample preparation. 2011 May.

18. Rizzetti TM, Kemmerich M, Martins ML, Prestes OD, Adaime MB, Zanella R. Optimization of a QuEChERS based method by means of central composite design for pesticide multiresidue determination in orange juice by UHPLC–MS/MS. Food Chemistry. 2016;196:25-33.

19. Hakami RA, Aqel A, Ghfar AA, ALOthman ZA, Badjah-Hadj-Ahmed A. Development of QuEChERS extraction method for the determination of pesticide residues in cereals using DART-ToF-MS and GC-MS techniques. correlation and quantification study. Journal of Food Composition and Analysis. 2021;98:103822.

20. Guo T, Fang P, Jiang J, Zhang F, Yong W, Liu J, et al. Rapid screening and quantification of residual pesticides and illegal adulterants in red wine by direct analysis in real time mass spectrometry. Journal of Chromatography A. 2016;1471:27-33.

21. Fu Y, Dou X, Zhang L, Qin J, Yang M, Luo J. A comprehensive analysis of 201 pesticides for different herbal species-ready application using gas chromatography–tandem mass spectrometry coupled with QuEChERs. Journal of Chromatography. B, Analytical technologies in the biomedical and life sciences. 2019 Sep 01,;1125:121730.

22. ChloroFiltr [Internet].; cited February 1, 2022]. Available from: https://www.unitedchem.com/product-category/quechers/dispersive-clean-up/chlorofiltr/.

23. Tadeo JL, Sánchez-Brunete C, Albero B, González L. Analysis of pesticide residues in juice and beverages. Critical reviews in analytical chemistry. 2004 Jul 01,;34(3-4):165-75.

24. Atapattu SN, Johnson KRD. Pesticide analysis in cannabis products. Journal of Chromatography A. 2020 Feb 08,;1612:460656.

25. Gómez-Ríos GA, Pawliszyn J. Solid phase microextraction (SPME)-transmission mode (TM) pushes down detection limits in direct analysis in real time (DART). Chemical communications (Cambridge, England). 2014 Nov 04,;50(85):12937-40.

26. Gómez-Ríos GA, Vasiljevic T, Gionfriddo E, Yu M, Pawliszyn J. Towards on-site analysis of complex matrices by solid-phase microextraction-transmission mode coupled to a portable mass spectrometer via direct analysis in real time. Analyst (London). 2017 Aug 07,;142(16):2928-35.

27. Gross J. Direct analysis in real time—A critical review on DART-MS. Analytical and bioanalytical chemistry. 2013;406.

28. Crawford E, Musselman B. Evaluating a direct swabbing method for screening pesticides on fruit and vegetable surfaces using direct analysis in real time (DART) coupled to an exactive benchtop orbitrap mass spectrometer. Analytical and Bioanalytical Chemistry. 2012 Feb 25,;403(10):2807-12.

29. Nie B, Henion J, Ryona I. The role of mass spectrometry in the cannabis industry. Journal of the American Society for Mass Spectrometry. 2019 Apr 16,;30(5):719-30.

30. Chambers MI, Musah RA. DART-HRMS as a triage approach for the rapid analysis of cannabinoid-infused edible matrices, personal-care products and cannabis sativa hemp plant material. Forensic Chemistry. 2022;27:100382.

31. Daniel D, Lopes FS, do Lago CL. A sensitive multiresidue method for the determination of pesticides in marijuana by liquid chromatography–tandem mass spectrometry. Journal of Chromatography A. 2019 Oct 11,;1603:231-9.

32. Pérez-Parada A, Alonso B, Rodríguez C, Besil N, Cesio V, Diana L, et al. Evaluation of three multiresidue methods for the determination of pesticides in marijuana (cannabis sativa L.) with liquid chromatography-tandem mass spectrometry. Chromatographia. 2016 Jan 30,;79(17-18):1069-83.

33. Schneider S, Bebing R, Dauberschmidt C. Detection of pesticides in seized illegal cannabis plants. Analytical methods. 2014;6(2):515-20.

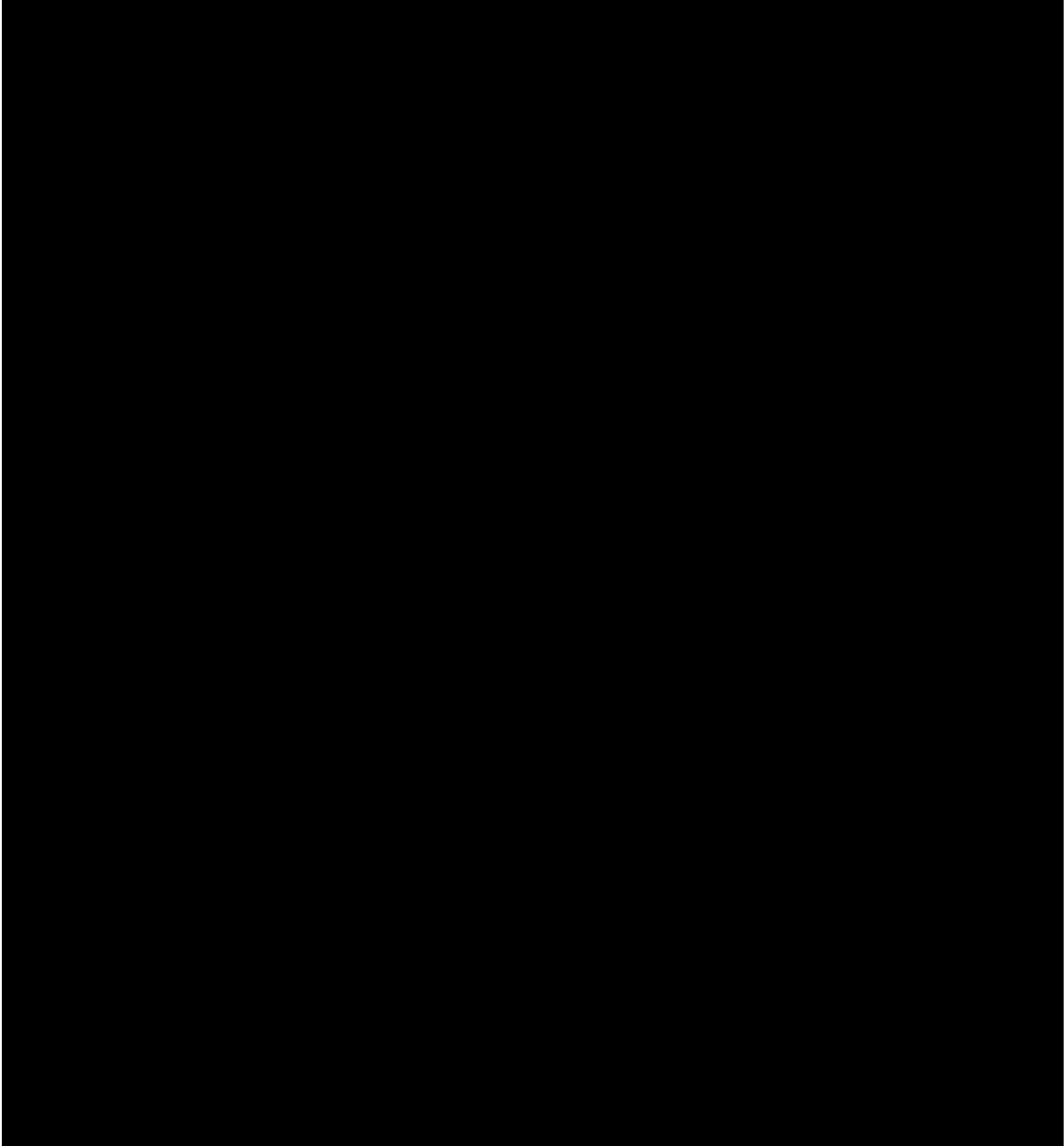
34. Moulins JR, Blais M, Montsion K, Tully J, Mohan W, Gagnon M, et al. Multiresidue method of analysis of pesticides in medical cannabis. Journal of AOAC International. 2018 Nov 01,;101(6):1948-60.

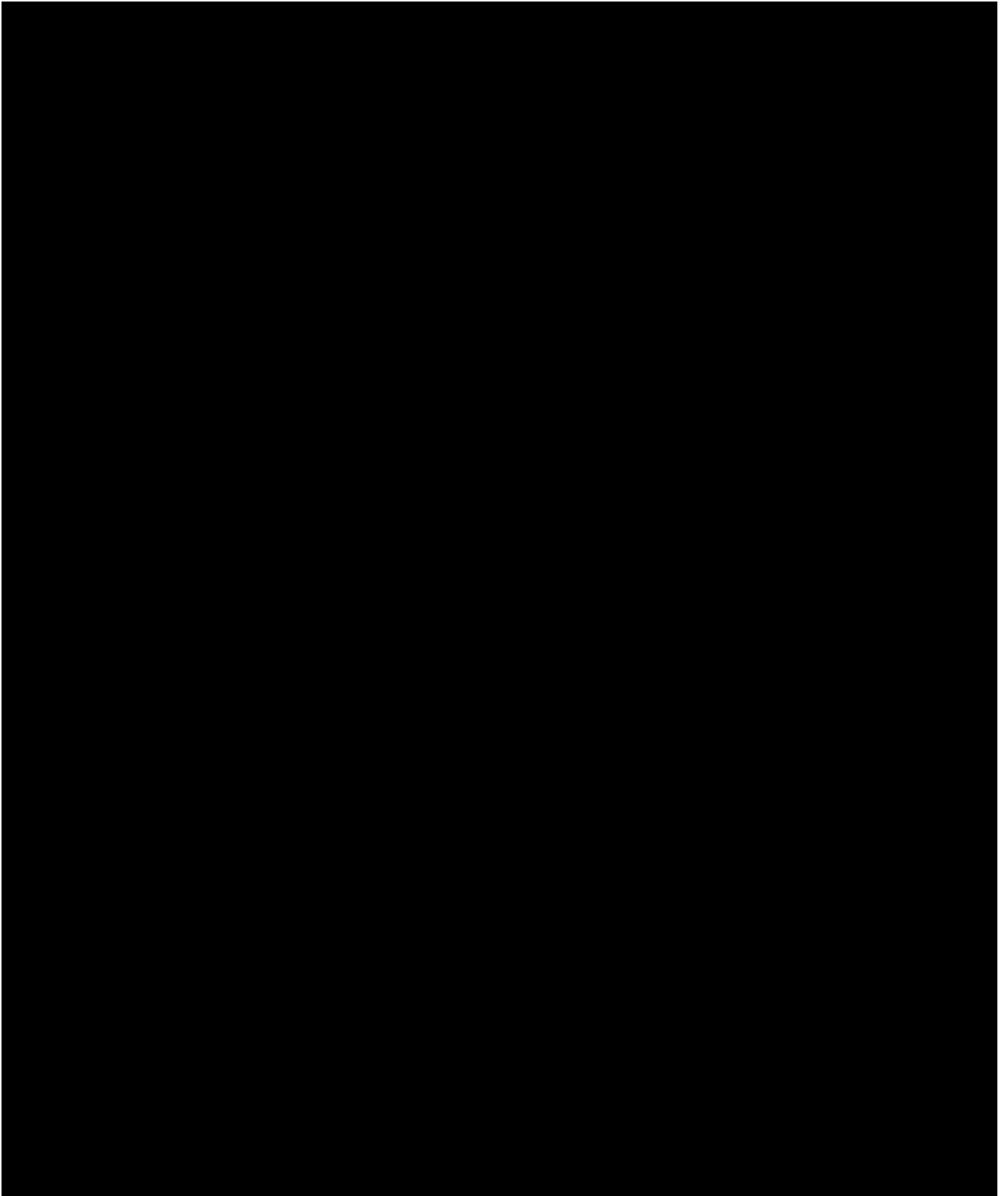
35. Fu Y, Dou X, Zhang L, Qin J, Yang M, Luo J. A comprehensive analysis of 201 pesticides for different herbal species-ready application using gas chromatography–tandem mass spectrometry coupled with QuEChERs. Journal of Chromatography B. 2019;1125:121730.

36. PerkinElmer. Pesticides and organic toxins compendium PESTICIDES AND ORGANIC TOXINS. 2018.

37. Pieslak J. Analytical techniques for the differentiation of hemp and marijuana [dissertation]. Boston University School of Medicine; 2021.

CURRICULUM VITAE





66