Farm to final product; role of chromatography, mass spectrometry in cannabis industry

by

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Abstract

The Cannabis industry is an emerging market that has recently evolved, providing everything from medical treatment for a wide range of maladies to recreational use. In the same way the industry has expanded so quickly and the uses for cannabis are so all-encompassing, so has the demand increased for advanced instrumentations to ensure safety and quality of the cannabis product as it makes its journey from farm to patient. Cannabis-based products (whether they're for medical purposes, industrial hemp, or recreational cannabis) all require various test panels encompassing complex analytical instrumentation to guarantee safety and quality before reaching consumers. The safety of cannabis is of great concern, especially in medical cannabis patients who are immunocompromised. For this reason, contamination from natural or synthetic origin needs to be screened to deem the product's safety.

In the cannabis plant, there are 144 cannabinoids and 120 terpenes, as well as other phytochemicals that are extracted, purified, and processed to cater to specific applications. These techniques include Super Critical Fluid Extraction (SFE), organic solvent extraction, and processing of varying degrees such as infused cannabis product. Each step of processing means introducing potential biological and chemical contaminants such as residual solvents, pesticides, heavy metals, or microbial contaminants. Additionally, storage conditions can exacerbate levels of these contaminants. Lastly, the cannabis plant is an excellent bio-accumulator; this means the potential contamination of pesticides and heavy metals from fertilizer, water sources or pesticide applications are likely.

To ensure the plant's safety, various tests enforced by state or federal regulators are carried out by chemical engineers or chemists as quality control before the release of cannabis-infused products. Some of the processes to ensure cannabis safety include the separation

techniques Liquid Chromatography (LC) and Gas Chromatography (GC), and Ultraviolet (UV) and Mass Spectrometer (MS) to detect levels of contaminants. Most of the mandated tests panels involve LC, GC, UV, and MS analysis. Often, the instruments needed to perform the test panels demand a significant operating cost and are a critical component of a company's operations to ensure compliance with state and regulatory bodies. This forces technical staff to be knowledgeable and involved in designing or operating the instrumentation.

This report contains a detailed historical perspective of Cannabis, tracking its evolution as an industry to its present state. The current status of Cannabis legality and regulation in the U.S is also examined. Also included is an overview of Chromatography and Mass Spectrometry, and how they apply to the Cannabis industry. Additionally, a case study of the medical marijuana (MMJ) program in Missouri and the industrial hemp program in Kansas are explored in greater detail. Lastly, my collaboration with the hemp test lab at Kansas State University in establishing test methods for cannabinoids' characterization is discussed.

Table of Contents

List of Figures vii
List of Tables viii
Acknowledgements ix
Introduction 1
Waters Corporation's Interest 1
Cannabis Chemistry 2
Cannabis Historical Perspective 4
Taxonomy 6
1. Decriminalization 8
2. Medical Marijuana (MMJ) 9
3. Industrial Hemp 10
4. Recreational Cannabis 15
Background 17
The Story of Chromatography- Mass Spectrometer 18
Mass Spectrometer (Quadrupole) 19
Gas Chromatography (GC) 21
Liquid Chromatography 22
Missouri Medical Cannabis Program 26
1. Cultivators 31
2. Manufacturing Facility 31
3. Testing Facility 31
Chemical Contaminant 33
1. Pesticide Residue 34
2. Residual Solvent 35
Biological Contaminant 36
1. Selective Approach 37
2. Total Contaminant testing 37
3. Water activity/Moisture content 38
Metal Testing 38

Kansas Industrial Hemp Program 39
Methodology 43
Cannabinoids Profiling 43
Pesticide Panel Sample Preparation 45
Solvent Residue 47
Biological Contaminants 49
Metal Residue Contamination 50
Results and Discussion 52
Cannabinoids Profile 52
KSU-Collaboration 52
Chemical Contaminant Testing 58
Pesticide Residue 58
Residual Solvent Outcome 61
Biological Contaminant 64
Mycotoxin- LC-MS/MS 64
Microbiological Pathogen 65
Conclusions 68
Future Direction 69
References 72
Appendix A - States Tolerance limit Pesticide Residual test 77
Appendix B - States Solvent Residue Limit 79
Appendix C - Biological Contaminants 81
Appendix D - State Metal Testing 82
Appendix E - Water Activity and Moisture Content 83

List of Figures

Figure 1. Health Benefits of Non-Psychoactive Cannabinoids 2
Figure 2. Major Cannabinoids and their Structure..... 4
Figure 3. Current Cannabis Legalization in America 8
Figure 4. Hemp Licenses issued in U.S from 2016-2019 10
Figure 5. Major Hemp Market segment..... 11
Figure 6. Land area dedicated for Hemp Cultivation from 2016-2019 12
Figure 7. Schematic of MRM Method of MS/MS Experiment 20
Figure 8. Schematic of APGC Source 21
Figure 9. APGC Mode of Ionization..... 22
Figure 10. ALC-100 1st commercial HPLC system By now Waters Corporation..... 24
Figure 11. Van Deemter Plot depicting change in HETP due to linear velocity 25
Figure 12. Sample Preparation Workflow for Cannabinoids Profiling 43
Figure 13. Pesticide Screening Workflow 46
Figure 14. Solvent Residual Work-flow 48
Figure 15. Workflow for Microbial Screening 49
Figure 16. Workflow for Heavy Metal testing..... 50
Figure 17. UPLC -UV Chromatogram of 5 Major Cannabinoids 52
Figure 18. Calibration curve data for major Cannabinoids..... 53
Figure 19. LC-UV Chromatogram of HPLC vs. UPLC 56
Figure 20. TIC for MO Pesticide list 59
Figure 21. Methomyl Calibration curve..... 60
Figure 22. Recovery of Pesticide residue from Flower Matrices 60
Figure 23. Solvent Residue Chromatogram..... 62
Figure 24. Mycotoxin MRM for Aflatoxins and Ochratoxins..... 65
Figure 25. PCR Amplication Curve..... 66

List of Tables

Table 1. Summary of test panels in Cannabis Industries 18
Table 2. Chronological order of particle development 23
Table 3. Qualifying Conditions for Medical Cannabis Program in MO 27
Table 4. Types of Licenses issued MO 29
Table 5. MO State Disease conditions Application for Medical Cannabis 30
Table 6. Sampling size in MO State Medical Cannabis Program 32
Table 7. Labeling Requirement of Major Cannabinoids 33
Table 8. Kansas Approved Seed or Clone plant list for Industrial Hemp 40
Table 9. UPLC-UV Conditions 44
Table 10. Experimental Conditions in Mass Spectrometer 47
Table 11. Experimental Conditions for Solvent Residual Panel 48
Table 12. Experimental conditions for ICP-MS and Microwave digester 51
Table 13. Calibration Curve Parameters 54
Table 14. ROI for UPLC Vs. HPLC 58
Table 15. ROI on LC-MS/MS for Pesticide and Mycotoxin Panel 61
Table 16. ROI on GC- HS-MS/MS for Residual Solvent and Terpene Panel 63
Table 17. qPCR Cq for MO State Microbiological Pathogen 67

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Introduction

Waters Corporation's Interest

The cannabis market is an emerging market that is valued at USD $822 million in 2016 and is projected to grow to USD $1.4 billion in United States by 2021(MARKETSANDMARKETS, 2017). As segments of the cannabis market are getting legalized in midwestern states, there is a huge technical gap within the scientific community and the testing labs to select the correct analytical measurement tools to ensure the safety and quality of cannabis products. Waters Corporation based out of Milford, MA, is a specialty measurement company focused on improving human health and well-being through the application of high value analytical technology and scientific expertise. Waters Corporation sees the emerging cannabis market as an excellent fit. However, the lack of harmonized testing, standardization of regulation, complex matrices, trace detection, and informatics solutions in this market have created a greater challenge to regulators, testing labs, instrumentation vendors and consumers alike.

The focus of this report is to provide an historical overview of the cannabis industry, specifically of the Medical Marijuana program (MMJ) in Missouri commencing in 2021, and the Industrial Hemp program in Kansas commencing in late 2020, and lastly provide scientific expertise and analytical instrumentation solutions that comply with these novel programs. Additionally, I have mentored scientist on industrial hemp programs at Kansas State University (KSU) for the start-up of hemp testing lab by lending my expertise in liquid chromatography, UV and mass spectrometry, to develop the cannabinoids test panel. The example data from these collaborations are reported in this report. My role on this collaboration

was to advise them on their instrumentation need, guide them on method development, and provide training on analytical instrumentation.

Cannabis Chemistry

The cannabis plant is rich in many phytochemicals, including 144 cannabinoids, 120 terpenes, hydrocarbons, glycoproteins, amino acids, flavonoids, enzymes, sugar, and protein vitamins. The surge of the cannabis industry is primarily due to the therapeutic and psychoactive cannabinoids. There are many major cannabinoids with various health effects; however, the discovery of two cannabinoids was a starting point to unravel the complex plant. The first was cannabidiol (CBD), characterized by Rapheael Mechoulam and Y. Shvo in 1963 (Mechoulam & Shvo, 1963). CBD has been found to have therapeutic effect for several maladies. Figure 1 displays various theruaputic effect of major non-psychoactive cannabiniods (Izzo, Borrelli, Capasso, Marzo, & Mechoulam, 2009). Currently, there is a FDA appoved drug “Epidiolex” based on cannabidiol (CBD) to treat epilepsy in human (FDA, 2018).

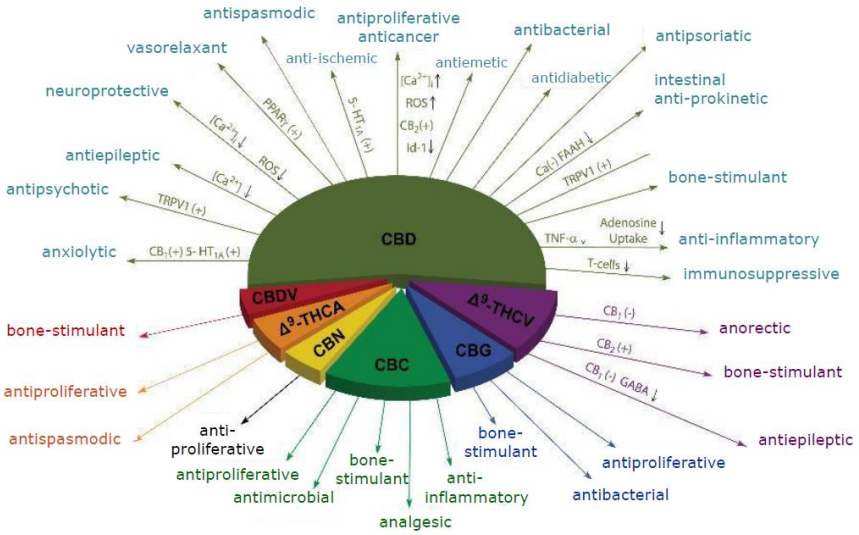

Figure 1. Health Benefits of Non-Psychoactive Cannabinoids

Source: Trends in Pharmacological Science, 2009 Oct; 30(10):515-27

Another breakthrough happened with the discovery and isolation of delta-9-tetrahydrocannabinol (THC) by Y. Gaoni and R. Mechoulam in 1964 (Gaoni & Mechoulam, 1964). The phytochemicals in the cannabis plant, responsible for psychoactive effects, are found in the resin of the plant that is secreted through hair-like structure called trichomes. These trichomes are found in abundance in the female flower, thus cultivators take extreme measures to selectively grow female plant. The FDA has approved the THC-containing products Marinol and Syndros for treatment of nausea associated with cancer chemotherapy and for treatment for anorexia associated with weight loss in AIDS patients (FDA and Cannabis: Research and Drug Approval Process, 2020). The therupetic benifit of CBD and other non-physcoative cannabinoids is the key driver to legalizing industral hemp. Industrial hemp is defined by THC content of less than 0.3% on a dry weight basis. In contrast Medical Marijuana, also known as MMJ in short, does not have any limitation on THC and exploits the medicinal benefit of THC. Cannabis offers numerous phytochemicals with pharmacological properties beyond the two cannabinoids that had been studied to date. New therapeutic understanding of these phytochemicals is at infancy and offer high potential for incorporation into new drugs. Figure 2 indicates the structures of 16 major cannabinoid that have potential as new drugs, though additional research is necessary to validate the health benefit claims.

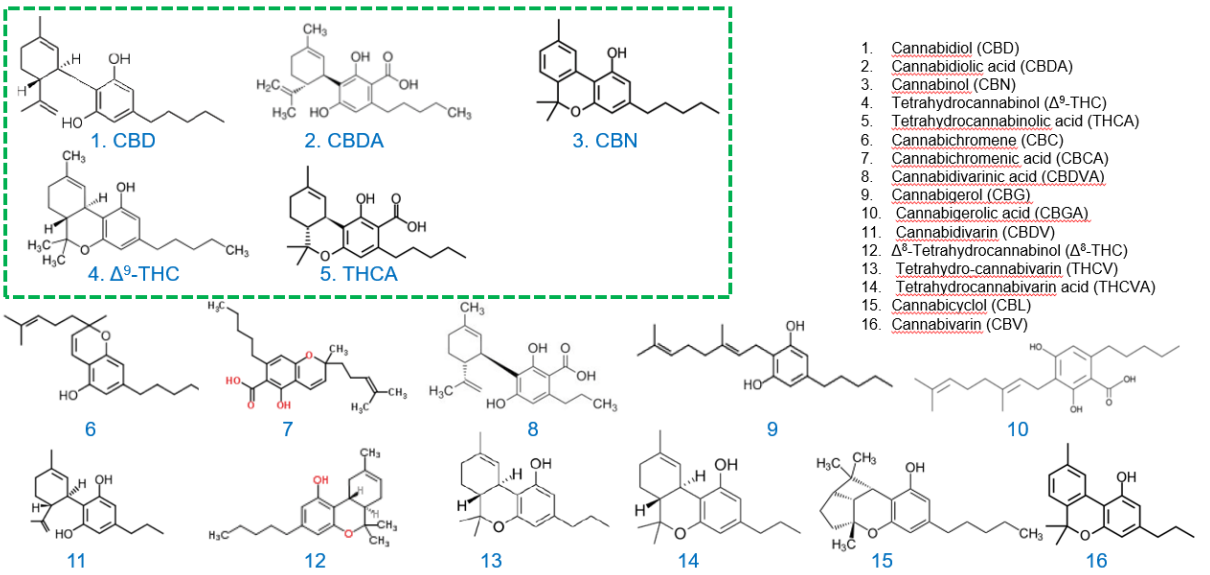

Figure 2. Major Cannabinoids and their Structure

Source: Waters Corporation

Cannabis Historical Perspective

Humanity and the *cannabis* plant share an extensive history, a complicated relationship, and an unsettling present. Typically, plants are cultivated for specific purposes: *Triticum* for wheat, *Gossypium* for cotton, *Papaver* for opium. However, *Cannabis* (genus) belonging in the family Cannabaceae, is one of the most multi-purpose of plants. The early uses were of the medicinal, spiritual and recreational variety, in addition to being used as a rich food source, sturdy textile and for construction materials. Humans have exploited the cannabis plant since the Neolithic era, about 12000 BCE, which was the dawn of the agrarian society (Marijuana, 2019). Cannabis, originating in Central Asia, is considered one of the oldest crops cultivated. Due to its multi-utility, resilience to harsh environments, and shorter seed to harvest cycle, cannabis voyaged the world alongside mankind on trade routes via the silk road and migrations across oceans to reach the New World. Though cannabis' multi-functional reputation earned it worldwide popularity, it also gained infamy.

The earliest resistance to cannabis was reported in the fourth century with the religion Taoism, which viewed intoxication as antisocial. However, this outlook was reversed upon realization of medicinal benefit. (Marijuana, 2019) Similarly, in the seventh century, the religion Islam forbade cannabis use due to its mind-altering ability, citing strict interpretation of Islam's holy text, the Koran.

Beginning in the 20th century, the United States introduced "The Marihuana Tax Act of 1937," which prohibited recreational use and introduced barriers that made cannabis cultivation impossible. (McKenna, 2014) The fear was seeded by labeling cannabis the source of "social evil". The war on cannabis exacerbated scientific advancement in cannabis studies as therapeutics. Only recently has the grassroot approach of legalizing cannabis gained greater momentum, and the medical benefit of this multi-purpose plant is being re-evaluated.

The legality of cannabis has been vacillating throughout human history; however, with the current trend towards decriminalization and the advancement in science and technology associated with cannabis, humanity is in a greater position to rationally explore the benefits and risks associated with cannabis plants. Additionally, isolating the psychoactive component, THC, in the cannabis plant, has opened new possibilities for cannabis use. Scientists can minimize the THC level either by additional processing or by cultivating cannabis of low THC varieties, thus mitigating the traditional concern of abuse and negative psychotropic effect.

Currently in the U.S., cannabis is illegal federally and is classified as a Schedule 1 controlled substance (CSA). Cannabis use is enforced by the U.S Drug Enforcement Agency (DEA); however, prosecution on cannabis-related cases has eased with the present political state. The drug classification is based on 5 distinct schedules. Schedule 1 indicates a drug with few medical uses that is highly addictive. As a Schedule 1 drug, cannabis is currently in the same

schedule as heroin, lysergic acid diethylamide (LSD) 3, 4-methyenedioxymethamphetamine (Ecstasy), methaqualone and peyote (United States Drug Enforcement Agency, 2020). The validity of these drug classifications is heavily debated since the launch of CSA in 1970. The National Commission on Marijuana and Drug Abuse was tasked to investigate the classification of cannabis and suggested declassification of cannabis from Schedule 1. However, politics overruled science, and to date no such change has been made.

The debate on cannabis use continues between advocacy groups and critics. Proponents for Cannabis believe that legalization raises revenue, lowers criminal justice expenditure, improves public health and traffic safety, stimulates the economy, and reduces the crime rate. Critics, on the other hand, argue that legalization exacerbates drug abuse, which will result in a higher rate of legal problems, diminished young people's intellect, higher hospitalization rates, increased crime, and overall negative affect on public health and safety (Zvonarev, Fatuki, & Tregubenko, 2019). Additionally, cannabis users, especially with mental disorder, progress to other illicit drugs (Secades-Villa, Garcia-Rodrigues, Jin, Wang, & Blanco, 2016).

Taxonomy

The term *Cannabis sativa*, as we know it today, was introduced by Carl Von Linnaaeus in 1753 in his book *Species Plantarum*, cataloging all species of the plant. At that time only a single species was known to the west; however, discovery by biologist, Jean-Baptiste Lamarck added *Cannabis indica*, a wild species of cannabis found in India. Four decades later, in 1924 Russian botanist D. E. Janischewsky identified a new species, *Cannabis ruderalis*, native to Central and Eastern Europe and Russia (Janischewsky, 1924). Taxonomical classification of cannabis has been heavily debated the last few centuries. The primary argument is whether cannabis is polytypic with the three separate species mentioned above or monotypic: a single species with

many varieties. There is a lack of consensus among botanical and nomenclature bodies such as the International Code of Nomenclature of Cultivated Plants (ICNCP), regarding taxonomy. The markers used in taxonomical classification includes genetics. The genetic make-up of cannabis plant has been altered over time to cater to the needs of humanity. As one of man's oldest domesticates, the cannabis plant has undergone many natural and anthropogenic selections. Natural selection occurs when plants adapt to survive environmental conditions, resulting in phenotypic and genotypical changes. In contrast, anthropogenic selection is a strategic breeding to produce cultivars of economic value, such as a source of fiber, narcotics, medicine, oils or food (Schultes, Klien, Plowman, & Lockwood, 1974). This propagation over centuries has diluted the native wild species, morphing them into a dynamic hybrid plant. This makes it difficult to fit into the rules of taxonomy. Herein, we refer the Medical Marijuana as MMJ and industrial hemp as just hemp. Also, the term cannabis is broad and will be used to refer to products from cannabis plant.

In the last three decades, many states have adopted various approaches to liberalization of cannabis, which are broadly classified into four categories: decriminalization, MMJ, industrial hemp and recreational cannabis. Decriminalization and MMJ program has traditionally spearheaded easing of cannabis programs in most of U.S. states and has eventually led to adopting recreational cannabis (Pacula & Smart, 2017). Although the categories listed above need varying degrees of analytical instrumentation to comply with regulations, certain categories such as MMJ programs, require very stringent testing and lower detection limits since they are catering to a vulnerable population suffering from life-threatening ailments.

Decriminalization enforced by law enforcement only requires characterizing the legality of cannabis-based products, for example, whether a product is marijuana or hemp. Presently, 33

states have adopted MMJ, 17 have adopted hemp, and 11 have adopted marijuana for recreational use. Figure 3 indicates the current status in 2020 of cannabis legalization in the United States.

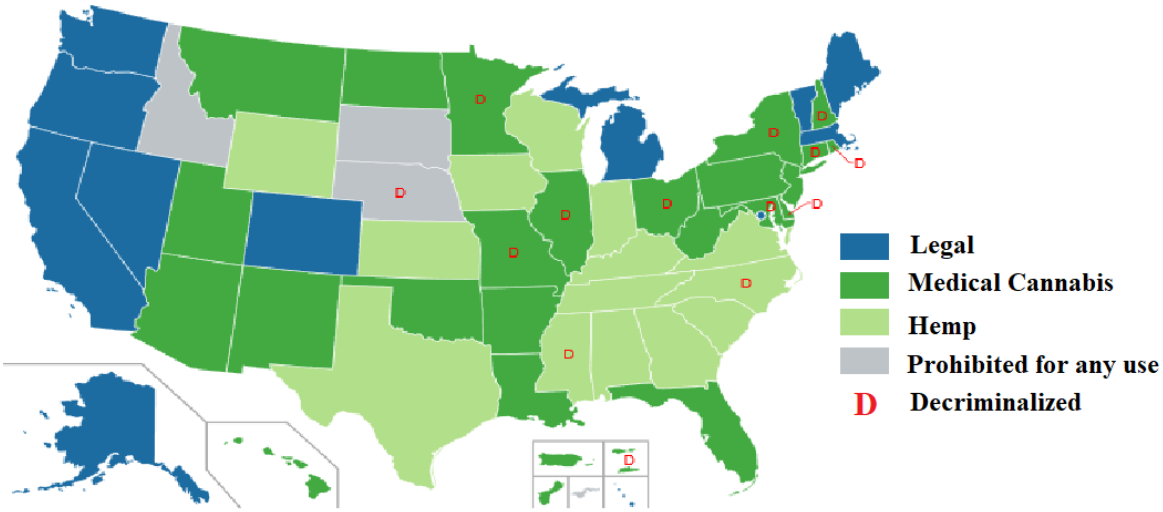

Figure 3. Current Cannabis Legalization in America

Source: Waters Corporation

This report will briefly highlight each of the four categories but will have a deeper dive into MMJ program in Missouri and the Hemp program in Kansas. These categories have the most comprehensive regulations and stringent testing requirements and is addressed in this paper.

1. Decriminalization

In 1970, the 37th U.S. President Richard Nixon launched the "National Commission on Marihuana and Drug Abuse", now known as "The Shafer Commission". It was headed by Governor Raymond P. Shafer to study the impact of cannabis. The commission recommended that non-profit possession of 1 ounce or less of cannabis would not be punishable. Although the Nixon administration did not act upon the Shafer report's conclusion, the study laid the

foundation of decriminalization regulations in the U.S., and 11 states passed decriminalization laws in the early 1970's (Nahas & A, 1974).

The policy that reduces the penalties for cannabis-related offences is classified as decriminalization, however, lack of federal policies has resulted in varied decriminalization approach. Even with the decriminalization mandates, once an individual is charged with cannabis related violation, the stigma of association, results in barriers to access work, student loans and public assistance (Pacula & Smart, 2019). From an analytical testing perspective, decriminalization is hallmarked by the amount of THC level in cannabis. This is mandated by state crime labs, typically using a gas chromatography flame ionization detector (GC-FID) or liquid chromatography (LC) systems with ultra-violet (UV) detection system (LC-UV). The procedure is discussed in detail under cannabinoids profiling.

2. Medical Marijuana (MMJ)

MMJ is more socially acceptable and typically the precursor for any cannabis liberalization agenda. Medical cannabis refers to any strain of cannabis with a THC content to be greater than 0.3% on a dry weight basis. On November 5, 1996, California became the first state to introduce MMJ under Proposition 215, also known as Compassionate Care Act. In the same decade, Alaska, Oregon, Washington and Maine followed, each state modifying regulations to fit their needs and applying the lessons learned from earlier adopters. Presently (July 2020), 33 U.S. States and the District of Columbia have legalized MMJ. In these states, licensed doctors may prescribe cannabis to qualified patients under their supervision. Similarly, to decriminalization states, MMJ rules are varied across the states, constantly evolving from the early adopters. The list of qualified medical conditions for MMJ program in MO is reported in Table 1.

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3. Industrial Hemp

Hemp, also known as Industrial Hemp or CBD, refers to the inebriant version of the cannabis plant that has a lower THC content. Although there are 50,000 hemp products in the market, the most relevant are pharmaceuticals, fiber products such as textiles and plastics, construction materials, and the food source - the edible hemp seed.

Hemp was a critical commodity in U.S history due to its many versatile uses; however, hemp's demand decreased significantly as new alternatives emerged. For example, hemp fiber was replaced by jute and abaca as these alternatives are less expensive, lighter, more buoyant and more resistant to saltwater (USDA, 2019). Recently, hemp demand is on the rise due to the realization of its medical benefits from cannabinoids, the most notable being CBD. CBD is primarily found in the flower and is a major driver of increased hemp cultivation. Additionally, the rest of the plant can be used as a food source, biofuel, construction materials, and bio-remediation corp.

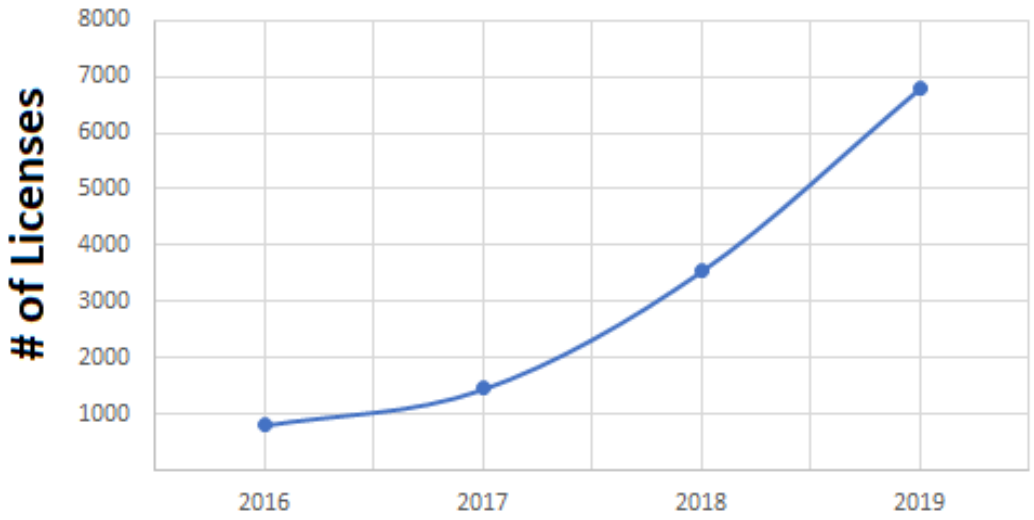


Figure 4. Hemp Licenses issued in U.S from 2016-2019

Source: USDA ARS-Hemp Program (USDA, 2019)

Figure 4 depicts the number of hemp producer licenses issued in the U.S. in the last four years. Although hemp is a multi-functional plant, it is harvested primarily for these three market segments: Inflorescence (flower) for CBD, grain as a food source, and fiber for textiles/materials. Figure 5 indicates the percentages of planted acreage dedicated to these market segments. The planted acreage dedicated for CBD is higher (67%) due to high returns from harvesting the flower. An example of the lucrative returns come from the State of Kentucky. In 2019, the Kentucky Department of Agriculture's estimated revenue per acre yielded a high of $24,000 for CBD, whereas the fiber and grain yielded $1,228 and $453, respectively. The return on investment has driven farmers to consider hemp production for CBD.

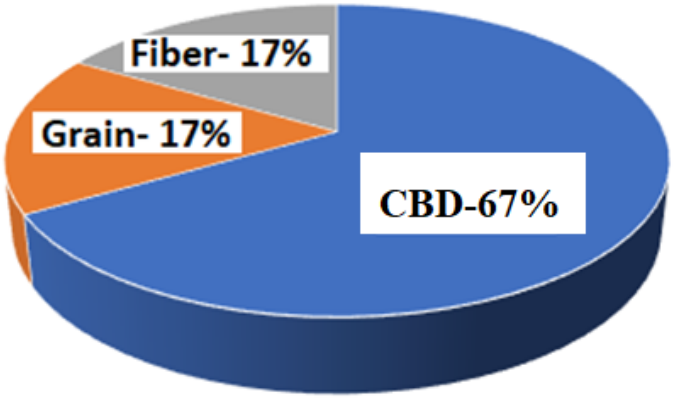


Figure 5. Major Hemp Market segment

Source: USDA ARS-Hemp Program (USDA, 2019)

Figure 6 indicates the planted acreage for hemp in U.S. There was zero hemp acreage in 2013 and a reported 155,688 acres in 2019. The demand for CBD is the key driver in the rise of the U.S. hemp industry.

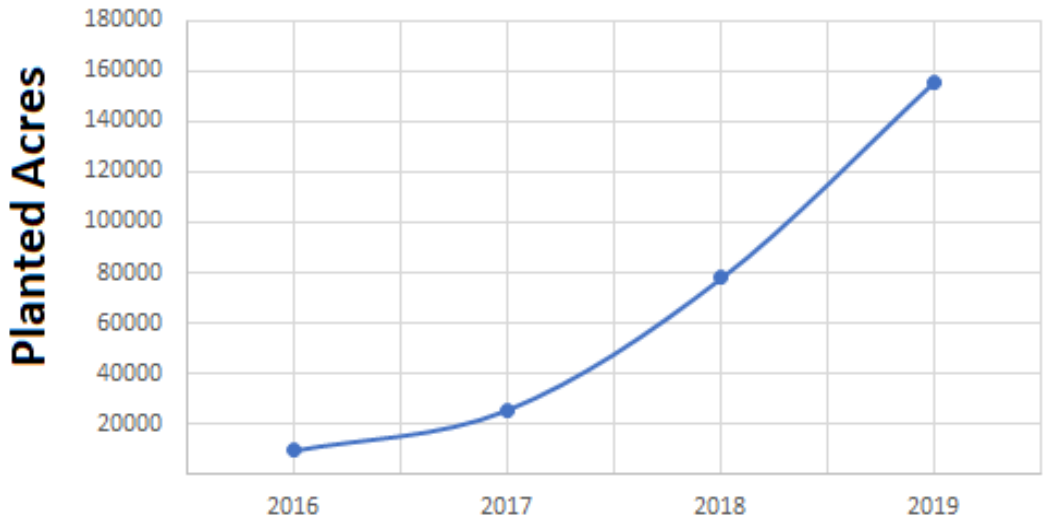


Figure 6. Land area dedicated for Hemp Cultivation from 2016-2019 (USDA, 2019)

President Donald J. Trump signed the 2018 Farm Bill legalizing hemp federally. This bill differentiated hemp from marijuana by removing hemp and hemp seeds from the Drug Enforcement Administration (DEA) Schedule I of controlled substances and defining hemp to contain less than 0.3 % of THC on a dry weight basis. The Farm Bill allows unrestricted interstate transportation and shipment of hemp, which allows manufacturers to market hemp products across state lines and have access to resources in other states, such as sending samples for testing, cultivating and processing hemp (Johnson, 2019).

Another benefit of hemp legalization is the increased accessibility to banking. Prior to the Farm Bill, 46 States had legalized hemp using individual state regulations; however, financial institutions refused to bank with companies addressing the cannabis market as the state rules were murky. Since the federal legalization of industrial hemp, the banking restrictions have been relaxed and entrepreneurs can insure their businesses to mitigate liability. However, banking remains the main challenge in this industry.

The 2018 farm bill authorized the USDA to provide oversight for a consistent regulatory framework in the production of hemp across the U.S. States and Indian tribes. Hemp-legalized states are required to submit plans to the USDA for approval and are required to enforce the state regulations. For states that do not have hemp regulations, the USDA provides guidelines to producers. These guidelines include licensing requirements, maintaining information on the land on which hemp is produced, procedures for testing THC concentration levels for hemp at DEA registered testing labs, and procedures for disposing of non-compliant plants, handling violations, and tracking the product from seed to end product (USDA, 2019).

All producers of hemp require licensing from the state or Indian tribe. Producers in states that do not have a plan will need licensing from the USDA. The application criteria may vary across a state or city depending on the zoning ordinance and the local legislation; however, the USDA requires a complete criminal history for all key participants as the USDA considers those people to ensure compliance. For accountability, hemp producers must report the hemp crop acreage to the Farm Service Agency (FSA). Producers must also report the specific location where the hemp is produced and all greenhouses, buildings, and geolocation boundaries (USDA, 2019).

Farmers typically acquire the certified hemp seed that works for the specific geographical area from state actors or private dealers. Doing so can be challenging, since THC levels vary with geographical location and growing conditions for the same seed. For this reason, the USDA does not have an approved seed list. Cannabis will take 3-4 months from germination to flowering, and the flowering phase can last anywhere from 1-4 months. Cannabis plants can thrive in any harsh condition. Yields can vary depending on the environmental condition or sex of the plant. For example, hot, temperate climates would result in increased resinous cannabis

plants rich in cannabinoids. In cooler environments, cannabis plants have lower levels of cannabinoids but longer stalks for fiber, clothing, paper and construction materials (Prine, 2019).

Once hemp nears the harvest stage, certified sampling agents either from the USDA or state collect the representative sample from a 'lot' and submit it to a testing facility to ensure the THC level is below the legal limit. The lot refers to an area in a field, greenhouse, or indoor space containing the same variety of cannabis. The sampling procedures are designed to ensure the crop is 'hemp', not marijuana. It is recommended that a sample is collected within 15 days prior to the anticipated harvest day for an accurate representation of actual THC levels, as well as to provide the cultivators enough time for submission of a sample to the test labs (USDA, 2019).

As per the USDA guidelines, the sample must be characterized at a DEA registered lab, as the lab might get 'hot samples' (marijuana) - samples with greater than 0.3% THC. The testing lab will need Standard Operating Procedures (SOPs) to ensure disposal of hot samples in accordance with DEA regulations. The total THC concentration on a dry weight basis should be used as the qualifier to determine if the batch is classified as hemp or marijuana.

There are two chromatographic techniques used to calculate a plant's total THC. The first is gas chromatography (GC), which reports the total THC, as the THCA (acidic form) is converted to the THC (neutral form) due to the heat in the injection port. The second technique, liquid chromatography, is most widely accepted approach as it speciates both THCA and THC. The sum of both THC and THCA along with a conversion factor is utilized to report the total THC.

Total THC = (% THCA x 0.877) + % THC ----------Equation 1

Equation 1 is used to calculate total THC. Although THCA is not considered a psychoactive compound, it is a labile compound that converts into THC with heat. Testing labs should report the measurement uncertainty and should meet the AOAC standards (Association of Official Analytical Chemist), an international body that establishes standard methods of analysis to ensure the safety and integrity of foods and other products that impact public health around the world (AOAC international, 2020). An example of state regulated "Industrial Hemp" is covered in this report on the sections "Kansas Industrial Hemp Program." The USDA has approved Kansas' plan submitted by the Kansas Department of Agriculture (KDA), to regulate a commercial Industrial Hemp program.

4. Recreational Cannabis

Recreational cannabis has gained traction in the U.S. As of July 2020, 11 states and the District of Columbia have legalized recreational cannabis. Although other cannabis programs have gained wider popularity, the adoption of recreational cannabis has been slow. The states that legalized recreational cannabis are trailblazing states that have adopted other cannabis programs such as decriminalization, industrial hemp, and Medical Marijuana programs. Colorado and Washington became the first states to legalize recreational cannabis in 2012. Since then, several other states have followed suit. Like all cannabis programs, regulations vary from state to state with some states taking a commercial approach, for example, issuing licenses for cultivators, manufacturers, testing facilities and retailers. Taking a commercial approach enables states to monitor these programs, their impact, and to generate revenue. Other states and cities like the District of Columbia allow individuals 21 and over the ability to grow up to six plants in a primary residence and transfer up to 1 oz of cannabis without any legal ramifications. States

have also taken different approaches about how cannabis is taxed. Some choose sales tax rates on the final product; others tax during cultivation (Pacula & Smart, 2017).

It is vital to mandate testing for cannabis-based products, whether they are for MMJ, hemp, or recreational cannabis use. These regulatory bodies, whether state or federal, ensure quality and safety of the product before it reaches consumers.

Background

A necessary component of the cannabis Industry is the technology required to safely and correctly test samples before they are consumer ready. Cannabis-based products, whether they're medicinal marijuana, industrial hemp or recreational cannabis, all require various test panels encompassing complex analytical instrumentation to ensure proper safety and quality before reaching consumers. These test panels include classification of marijuana or hemp, cannabinoid profile and terpenes as a quality check. Additionally, to address the safety aspect of the product, there are test panels measuring the presence of chemical and biological contaminants. The studies and proposed solutions provided in this report can be extrapolated to other U.S states and countries who have yet to legalize or are on the path to legalization of cannabis.

Table 1 indicates all the regulated test panels in the cannabis Industry, along with the preferred instrumentation (highlighted in bold). However, it's important to note that the U.S regulations vary from state to state. Most of these test panels utilize **LC-UV, LC/MSMS, GC-MS, qPCR, and ICP/MS** and often these instruments demand a significant operating cost and technical expertise.

Table 1. Summary of test panels in Cannabis Industries

Test Type	Safety/Quality	Recommended Methods
Marijuana or Hemp	Quality	**LC/UV,** HPLC, GC, UPC2
Potency	Quality	
Pesticides	Safety	**LC/MSMS and GC/MSMS**
Mycotoxins	Safety	**LC/MSMS**, LC/FLR, Strip test, immunoaffinity
Terpenes	Quality	**GC-MS**, LC-MS
Residual Solvents	Safety	**HS-GC/MS,** HS-GC/FID
Microbial	Safety	Cultures, **qPCR,** ELISA
Heavy Metals	Safety	AA, **ICP/MS**, ICP/OES
Water Activity/Moisture Content	Safety	Gravimetric, Water Activity, pH

The Story of Chromatography- Mass Spectrometer

Chromatography, whether liquid Chromatography(LC) or Gas Chromatography(GC)-Mass Spectrometer(MS) touches every aspect of modern living, whether gleaning out Emerging Contaminants(EC's) in the drinking water by Environmental Protection Agency (EPA), or to monitoring levels of Amino acids and Acylcarnitines to detect up to 45 different hereditary disorders in newborn at Neonatal labs (Regina, 2011). This tool has gained popularity among regulatory agencies, CRO's, Chemical industries, Pharmaceuticals, Biotechnology, Industrial Chemistry, Environmental Testing, Food and Beverages Industries and now the cannabis industries. In this report, only the work of key contributors and accomplishments in the field of LC/GC-MS is detailed and like any important topic, efforts of many scientists and engineers

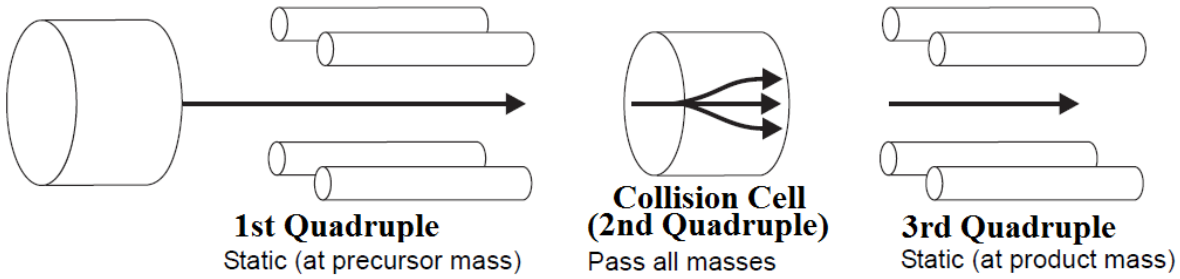
have paved the roads to modern LC/GC-MS technology. The LC, GC and MS journey evolved independently and their marriage into LC-MS or GC-MS gained momentum only after coupling these techniques together. In the cannabis industries, LC, GC and MS are critical instrumentation to ensure safety, quality of cannabis product. Additionally, these tools are essential in understanding cannabis plant.

Mass Spectrometer (Quadrupole)

There are several kinds of mass spectrometers, such as Time of Flight (ToF), Orbitrap, Magnetic sector, Secondary- Ion Mass spectrometry (SIMS); however, none are more important than quadrupole-based mass spectrometer in the cannabis industry. Tandem Quad Mass spectrometry (TQD) is the standard mass spectrometer for targeted analysis for organic molecule such as pesticides contaminant, terpenes, residual solvent, cannabinoids and biological contaminants. Targeted analysis means that only specific chemical species of interest are characterized. The quadrupole-based detector was founded by Professor Wolfgang Paul of University of Bonn in 1953. Today, quadrupole detectors are the most common detectors in mass spectrometer and a good number are sold due to lower cost, smaller footprint, tolerance to extreme conditions and faster scan speed that are optimal for GC and LC inlets. Quadrupole analyzers exploit the direct-current (DC) and radio frequency (RF) applied to quadrupole to filter narrow m/z range pass via a stable trajectory. Ions with unstable trajectory collide with the pole and are removed by high vacuum (Westman-Brinkmalm). Furthermore, in 1970 Jim Morrision along with his students Richard Yost and Christie Enke expanded on quadrupole application by introducing Tandem quadrupole (TQD) (Mass Spectrometry, 2015).

. Once the separation criteria for LC are satisfied, the focus is shifted to developing (Multiple Reaction Monitoring) MRM, a preferred quantitative analysis for the TQD. Since it is targeted

analysis, the monotopic mass is already established, and the MRM involves 1st quadruple is parked to filter only the compound of interest or parent or precursor mass. This allows only the compound of interest to reach the collision cell, filtering out all the other irrelevant ions of the complex matrix. In the collision cell, the parent mass is fragmented by introducing a collision gas, typically Argon, and applying energy in terms of voltage. At specific collision energy the Parent mass fragment and product or daughter ions are generated. Energy applied to fragment is dependent on the stability of the compound. The two or three most abundant product ions are further filtered by the 3rd Quadruple providing further specificity and sensitivity. Figure 7 depict the MRM method of MS/MS experiment. Note each arrow represent a unique mass.



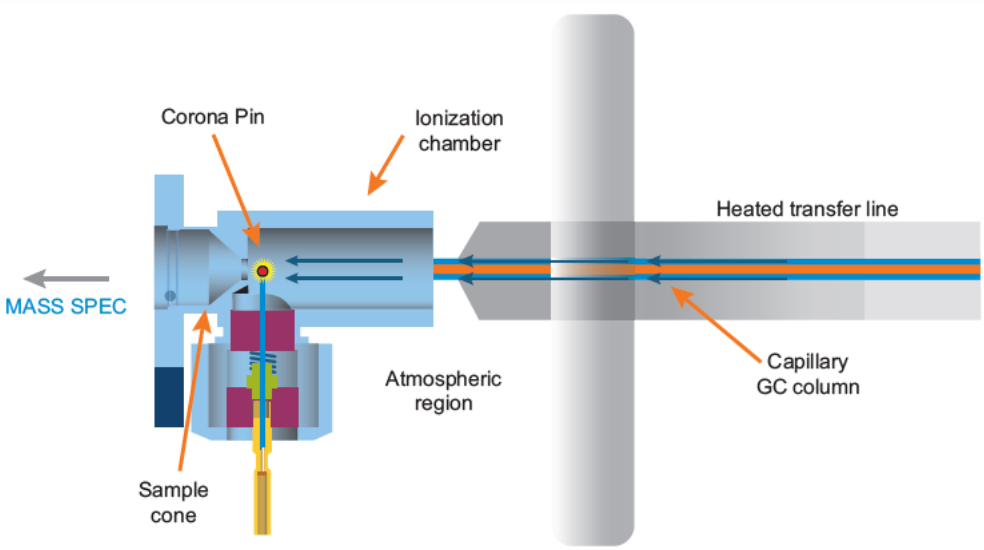
Source: Waters Corporation (Waters Corp, n.d.)

Figure 7. Schematic of MRM Method of MS/MS Experiment

A targeted compound is positively identified by its specific retention time, parent or precursor ion, daughter or product ion, and ion abundance ratios. Typically, identifying with two Multiple Reaction Monitoring (MRM) transitions and their relative ion abundance ratio with matched retention time is enough to identify a compound unequivocally as per the EU identification criteria. The most abundant MRM transition ion is usually assigned as the quantitative ion and used for quantization. The others are used as confirmation ion. Various MRM transition abundance yields a unique ratio at specific collision energy confirming the compound of interest.

Gas Chromatography (GC)

GC is a separation technique to isolate volatile components by exploiting the differential affinity of mobile phase and stationary phase. The sample is vaporized and injected to column head for separation, ideal for very small molecules. In 1967, Robert E. Finnigan coupled GC to a quadrupole mass spectrometer and computerized it as a single system to analyze the constituents of a mixture. In the cannabis industry, a novel GC technique that is gaining momentum is Atmospheric Pressure GC(APGC)-MS. The APGC source is a unique source proprietary to Waters corporation for analyzing GC amenable pesticide, terpenes, and residual solvents.



Source: Waters Corporation Application Notes (Waters, 2014)

Figure 8. Schematic of APGC Source (Waters, 2014)

The source or inlet could be interchanged with an LC source sharing a common mass spectrometer. This reduces the capital cost for cannabis industry. Additionally, APGC-MS is a soft ionization technique, where ionization happens are atmospheric pressure. In the APGC-MS technique, a flow of nitrogen stream under atmospheric condition with a corana pin (voltage discharge) creates a plasma. When separated species exit from the GC column into the plasma,

ionization results via charge transfer or proton transfer (Figure 9). Figure 9 displays the mode of ionization. Charge transfer reaction are favored under dry source conditions, whereas proton transfer is favored in the presence of a protic modifier such as water, methanol or other protic solvents.

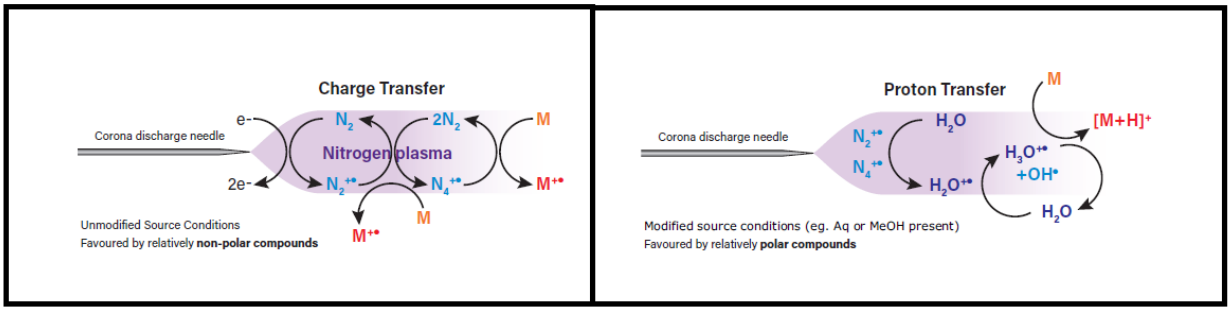


Figure 9. APGC Mode of Ionization

Source: Waters Corporation Application notes (Waters, 2014)

This soft ionization approach results in reporting molecular ions rather than fragment ions in traditional hard ionization technique such as Electron impact (EI). This ultimately, means more selectivity, increased sensitivity and enhanced ease of method development (Waters, 2014).

Liquid Chromatography

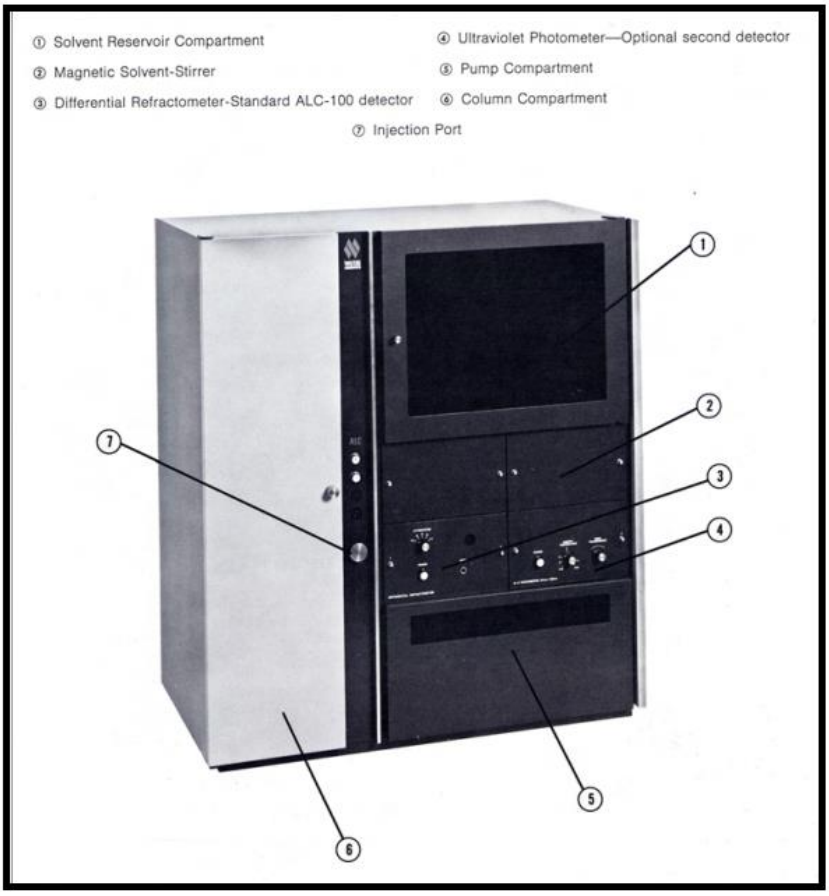
A major transformative phase in the story of Mass spectrometry was in 1980 with the coupling of LC to a mass spectrometer. Liquid chromatography (LC) began with the work of Russian botanist, Mikhail S. Tsvett ,in 1903. He was interested in separating leaf pigment extracted from plants using a solvent in a column packed with particles (Lough & Wainer, 1995). The ability to predict and reproduce, with great precision, competing interactions between analytes in solution (mobile phase) passed over a bed of packed particles (stationary phase) has

led to the widespread use of LC. The LC principles remain the same even after a century, but particle size has reduced from <5µm to sub 2 µm.

In 1940 Archer John Porter Martin and Richard Laurence Millington Synge introduced the concept of two liquid phases to separate compounds with different partitions. They also developed plate theory, a mathematical model for evaluating separations. Their contributions in their area led to a Nobel prize. (Martin, 1941). Another milestone for LC was the introduction of High-Performance Liquid Chromatography (HPLC) during late 1960's that consisted of a pump to propel mobile phase through a column. This allowed the technique to overcome the back pressure resulting from incorporating smaller particle. Reducing the particle size was documented by Martin and Synge to improve resolution. The term HPLC was coined by Professor Csaba Hovath at 1970 Pitcon; however, the commercial HPLC Model ALC -100 (Figure 10) debuted three years earlier by Jim Waters, the founder of Waters Associates, now Waters Corporation (McDonald, n.d.) (Eltre). Table 2 summarize commercial particle developments in chronological order as of the improvement of LC depends on particle size reduction.

Table 2. Chronological order of particle development

Year of development	Most popular Nominal size (μm)	Plates /15 cm
1950	>100	200
1967	50	1000
1972	10	6000
1985	5	12000
1992	3-3.5	22000
2000	2.5	25,000
2003	1.8	32,500



Source: Water Corporation.

Figure 10. ALC-100 1st commercial HPLC system

After three decades of HPLC dominance in the market, the commercial debut of Ultra-Performance Liquid Chromatography (UPLC) systems in 2004 offered improved resolution, sensitivity and shorter analysis time when used in tandem with Mass Spectrometer. In some cases, 10-fold increase in sensitivity, along with 5-fold improvement in speed and superior resolution were documented as compared with traditional HPLC-MS (Churchwell, Twaddle, Meeker, & Doerge, 2005). From HPLC to UPLC, particle sizes (dp) of sub 2 micron has evolved from three decades of research and development. These enhancements include stronger stationary phase particle that could tolerate the backpressure generated as a result of smaller particles, robust

pumping, an auto sampler and fittings to accommodate the high pressure. This led to commercial UPLC systems handling pressure up to 18,000 psi, giving improved separation, resolution, speed and sensitivity. This enhancement can be explained by the Van Deemter curve as shown in Figure 4. This figure plots the Height Equivalent to a Theoretical plate (HETP) as a function of linear velocity (u), where HETP is represented by the Van Deemter equation (equation 1). HETP is a column performance measurement indicator, with lower values indicating higher efficiency, thus greater resolution. In the Van Deemter equation, both A and C terms are proportional to the particle size. The A term (or eddy diffusion) is the efficiency term (N) relating to how well the column is packed (i.e. smaller particle size lowers the A term as A α dp). The B term is the longitudinal diffusion and it decreases with higher flow rate The C term (mass transfer term) is affected by both flow rate and particle size (C α dp^2) (Jerkovich, Mellors, & Jorgenson, 2003) (Evans & Jorgenson, 208).

$$HETP = A + \frac{B}{u} + Cu$$ ----------Equation 2

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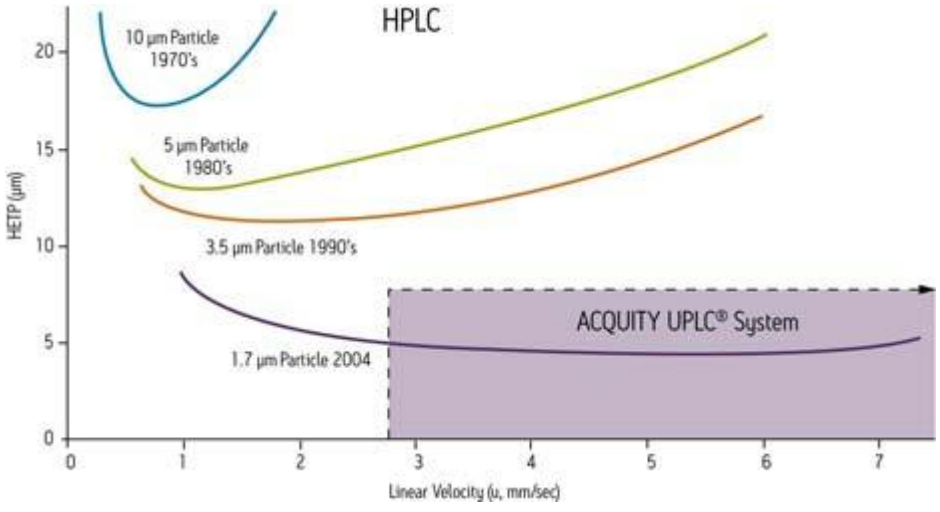


Figure 11. Van Deemter Plot depicting change in HETP due to linear velocity

Figure 11 depicts, Van Deempter curve's of various particle size, 10, 5, 3.5 and 1.7 μm, indicating that with smaller particles, lower HETP is achieved and higher flow rates can be used. These higher flow rate result in faster analysis times. Higher resolution operating can, therefore, be achieved due to improved efficiency leading to narrower peak width. Narrow peak enhances signal to noise ratio, improving the sensitivity (Waters Corp, n.d.).

In this report, a granular detail of Marijuana or hemp profile and Potency test panel is covered, the data generated was carried out by KSU employees, and my mentorship on behalf of Waters corporation.

Missouri Medical Cannabis Program

On November 6, 2018, Missouri voters passed Amendment 2 with 65% voting to legalize Medical Marijuana (MMJ). The legislation later known as Article XIV of the Missouri Constitution gave the Missouri Department of Health and Senior Services (DHSS) the authority to manage the program to ensure safe access to Medical Marijuana. The mission of DHSS is to make Medical Marijuana accessible by allowing physicians (MD and DO) to discuss Medical Marijuana with their patients and recommend cannabis to patients with qualifying medical conditions under the supervision of the physician.

There are many facets of this complex puzzle to ensure the implementation of a successful Medical cannabis program. The program must provide safe and secure access to Medical Marijuana for qualifying Missouri patients through consistent regulation, enforcement and education (Services, 2020). The state of Missouri enforces these standards by issuing licenses for qualified patients, patient cultivators, caregiver applications, cultivators, laboratory testing, dispensary, infused product manufacturing, seed to sale and transportation applications. The key part of the process is the informatic solution in managing these applications, patient

information, caregiver applications, seed to sale tracking, and the vast amount of data needed to comply with a MMJ program. DHSS awarded METRC LLC and sub-contractor Compila a contract to provide an online platform and registry for patient and facility applications. Additionally, DHSS awarded Wise Health Solutions a contract to score the facility licenses applications in accordance with Article X1V (Services M. D., 2020). Qualified resident patients are required to apply online for DHSS issued medical marijuana identification cards once qualified by certified Missouri licensed physicians. Table 3 lists the qualifying medical conditions:

Table 3. Qualifying Conditions for Medical Cannabis Program in MO

Cancer	Epilepsy	Glaucoma	Intractable Migraines
Debilitating psychiatric disorders	PTSD	HIV	Terminal illness
Chronic persistent pain or muscle spasm	Multiple Sclerosis	Seizures	Parkinson's Disease
Tourette's Syndrome	Wasting Syndrome	Hepatitis C	Amyotrophic lateral sclerosis
Inflammatory bowel disease	Crohn's Disease	Huntington's Disease	Autism
Neuropathies	Sickle cell anemia	Alzheimer's disease	Cachexia
A chronic medical condition that is normally treated with prescription medication that could lead to physical or psychological dependence.			

Patients may designate up to two caregivers who are over 21 years of age to purchase or cultivate Medical Marijuana. Any patient or caregiver who chooses to cultivate also needs to apply with the state for authorization and must agree to a state inspection. Additionally, a secured space is needed for cultivation with access to the patient and authorized agent. An approved patient or caregiver may purchase up to 4 oz of dried marijuana and possess up to 8 oz of unprocessed marijuana or its equivalent for 30 days. Patients with a serious medical condition can purchase additional marijuana with approval from two independent doctors (Services M. D., 2020).

For Missouri MMJ, DHSS invited qualified Missourians to apply for licenses in four categories; cultivation, manufacturing, testing facility and dispensary licenses, in accordance with Article XIV. The state awarded 60 cultivation licenses, 86 infused product manufacturing licenses, 192 dispensary licenses and 11 laboratory testing certification licenses. The applicants were selected based on their questionnaire responses to evaluate their expertise as to a specific license. However, there are minimum requirements that must be met, and these are highlighted below:

- Complete ownership structure form
- Prepare written description or visual depiction of ownership structure
- Proof of Missouri residency
- 1000 ft proximity away from an elementary or secondary school, daycare or church, or local government exemption
- Schematics for the facility
- Compliance with local zoning restriction
- Facility ownership

- Common control supporting document

The applicants who meet the minimum requirements are further screened based on the questionnaire that gauges their expertise in the specific facility licensing they are pursuing and are scored blindly to prevent bias. The high-ranking applicants are awarded licenses in their selected category (Services M. D., 2020).

Table 4 shows the revenue collected by regulating licenses for the Medical Cannabis program in Missouri. It is noteworthy to point out that the revenue generated from legalizing Medical Marijuana offers a lucrative income avenue for state governments.

Table 4. Types of Licenses issued MO

License Type	Application Fee	# Applicants	Revenue collected
Cultivation Facility	10,000	640	$6,404,694.02
Dispensary Facility	6,000.00	1312	$7,872,000.00
Infused Product Facility	6,000.00	450	$2,702,000.00
Laboratory Testing Facility	5,000.00	17	$85,000.00
Seed to sale	5,000.00	10	$50,000.00
Transporter	5,000.00	18	$90,000.00
Patient Identification Card	25	23,455	$586,375.00
Cultivation Identification Card	100	783	$783,450.00
Caregiver Identification Card	25	637	$15,925.00
Total			$18,723,704.63

Source: Missouri Department of Health & Senior Services- Annual Report to the Governor FY19

Table 5 below indicates the number of patients who applied in 2019 for an identification card, to receive cannabis treatment for a medical condition. It is important to note that under the

MMJ program in Missouri, the availability of medical cannabis will not be until 2021. So, although this table represents the 22,670 patients who pre-registered in 2019, the actual number of patients will have increased by 2021, creating a larger demand for MMJ based products and services.

Table 5. MO State Disease conditions Application for Medical Cannabis

Disease	# of Patients	% Patient
Psychiatric Conditions	7,379	32.5
Chronic Medical Conditions	6,109	27.0
Physical/psychological Dependence	3,810	16.9
Migraines	876	3.9
Cancer	838	3.7
Epilepsy	324	1.5
Glaucoma	259	1.2
Neuropathies	248	1.1
HIV	203	0.9
Crohn's Disease	170	0.8
Inflammatory Bowel Disease	112	0.5
Hepatitis	95	0.5
Other Conditions*	2,247	9.9
Total	22,670	

Source: Missouri Department of Health & Senior Services- Annual Report to the Governor FY19

*Other conditions: Terminal illness, autism, wasting syndrome, agitation of Alzheimer's, Cachexia, sickle cell anemia, amyotrophic lateral sclerosis, Huntington's disease.

1. Cultivators

The 60 approved cultivation facilities in Missouri are necessary to keep up with the demand of providing a high quality, safe MMJ Program. Licenses are awarded to facilities that grows indoor, outdoors, or in greenhouses. An indoor facility is allowed 30,000 square feet of flowering plants, whereas an outdoor facility is allowed to have 2,800 flowering plants. Cultivators must keep detailed monthly records of all pesticides, herbicides, fertilizers and other agricultural chemicals applied to cannabis plants for at least 5 years. Cultivators are required by Missouri law to use the statewide track and trace platform to monitor the stage of grow, their location, adhere to security measures and comply with HIPPA (Health Insurance Probability and Accountability Act) act of 1996.

2. Manufacturing Facility

There are 86 manufacturing facility licenses awarded within Missouri, to include the manufacturing facility where marijuana-infused products are made. Manufacturing facilities cannot transfer medical marijuana until they have received verification from the testing facilities that they passed all the required testing. Marijuana-infused ingestible products needs must comply with the state's food safety standards (Ashcroft, 2020).

3. Testing Facility

The testing facility plays an integral role in providing quality and safety of the MMJ Program. Since patients who qualify for treatment are often battling life-threatening diseases with compromised immune systems, these patients are much vulnerable to microbial pathogens or chemical residues. Consequently, this makes the screening of marijuana or marijuana-infused

products against contamination paramount to ensure safety and quality. Additionally, packaging and labeling accuracy is of greater relevance.

There are 11 testing facilities that support the MMJ Program. All unprocessed marijuana, concentrates, extract, and marijuana-infused products are tested before being released to the dispensary. These testing facilities must be accredited by ISO 17025 within the first year of their operation, and by law, maintain the certification. ISO 17025 is an independent, non-governmental international standardization body. ISO 17025 caters to general requirements for competency of testing and calibration laboratories.

The participating testing facilities are also required to participate in proficiency testing programs. Proficiency testing is provided by an organization that operates in accordance with the requirement of ISO/IEC 17043 twice every calendar year. Proficiency testing confirms the accuracy, reliability and compliance of the measurement utilized by the testing facility. Additionally, passing proficiency testing further demonstrates the test method technical competency compared to independent measurement (AOAC, 2020). Testing should be conducted at the lot level for unprocessed marijuana and a minimum of 0.5% of a harvest lot is sampled for testing, whereas concentrates, extract and infused products require amounts listed below:

Table 6. Sampling size in MO State Medical Cannabis Program

Concentrate and Extract			**Infused Products**	
Process lot Weight	**Sample Increment (1±0.2g)**		**Unit for Sale**	**Sample increments**
0.0-0.5	4		2-15	2
0.51-1.5	8		16-50	3
1.51-3.00	12		51-150	5
3.01-6.00	16		151-500	8
6.0-10	20		501-3200	13
10+	32		3201-35,00+	20

Once the sample is received from cultivation (processor or manufacturer), the testing facility is required to report test outcomes within 5 business days to the statewide track and trace system. There are multiple test panels required by the testing facility that can be classified into two broad categories: Quality and Safety. Table 1 represents the test panel required by Missouri state regulation, along with its classification and instrument used. In this report, more detail on each testing panel will be dissected.

The cannabinoid panel is one of the largest volume testing panels for cannabis testing labs. These tests are required by regulation, and also required for the labeling claim. Cultivators need this information to ensure they are cultivating a high-quality product. Additionally, manufacturers want verification on their products as they go through various manufacturing processes to know the various concentration levels of cannabinoids. Missouri state legislation requires label claim on all medical marijuana products to list the levels of cannabinoids listed in Table 7.

Table 7. Labeling Requirement of Major Cannabinoids

Cannabinoids	CAS Number
Delta-9 tetrahydrocannabinol (THC)	172-08-3
Tetrahydrocannabinol Acid (THCA)	23978-85-0
Cannabidiol (CBD)	13956-29-1
Cannabidiolic Acid (CBDA)	1244-58-2
Cannabinol (CBN)	521-35-7

Chemical Contaminant

MMJ regulation requires measuring chemical contaminants before the release of the final product. The chemical residue testing can be classified into pesticide screening and solvent

residual screening. Chemical testing requirements can vary from state to state. For example, safety screening for California includes 67 pesticide contaminants compared to 9 for Massachusetts. However, Massachusetts tolerance limit for pesticides contaminants are lower, requiring more sensitive analytical tool. See Appendix A, and B for state specific chemical contaminants acceptable limits.

1. Pesticide Residue

In many ways, pesticides are a necessary evil. They are used to keep pests from destroying plants, thus protecting them, but what is used to protect plants may also be toxic to the environment and the consumer. There are many pesticides available on the market, and their selection varies from plant to plant and according to geographical location. Each state decides the allowance of pesticide use. Some states' regulations are less stringent than others. Appendix A, shows the list of pesticide limits for California, Massachusetts, Missouri, Nevada, Oregon, Colorado, Arkansas and Washington. Massachusetts has the lowest detection limit compared to other states; however, Massachusetts only requires testing for 9 pesticides. On the other hand, California has a comprehensive list of 67 pesticides that need to be monitored. The list is of great significance to the testing facility, since it directly correlates to production cost. The state with more stringent or comprehensive regulations, such as California, needs both methods of GC-MS/MS and LC-MS/MS to be in compliance. Interestingly, even though a state like Massachusetts only has 9 pesticides that are regulated, both LC-MS/MS and GC-MS/MS are needed for compliance due to the low tolerance limits. For example, Bifenthrin and Cyfluthrin are regulated at 10ppb, and these compounds are amenable to GC at that low level, LC-MSMS technique for these specific compounds lack the sensitivity to reach 10 ppb levels. same compounds could be analyzed by LC-MSMS at higher levels.

2. Residual Solvent

Residual solvent is an integral part of the safety panel of tests for the cannabis industry, especially when it's in the processed form of marijuana, such as concentrate, oil, infused food and drinks, products that are inhaled or topically applied. Residual solvents are chemical contaminants that are remnants of solvents which are used in the extraction or purification of cannabinoids and terpenes. According to United States Pharmacopeia (USP) <467>, residual solvents are defined as organic volatile chemicals that are used or produced in the manufacturing of a drug or herbal medicine, or in the preparation of the substance thereof. Since the residual solvent in cannabis does not add value (instead it devalues as its impact is negative), it is removed during the manufacturing/processing process. Nonetheless, trace levels may still exist and different states have varying tolerance levels for those trace levels.

The USP guidelines list 59 common solvents and characterize them into three different classes: Class I, Class II and Class III. The Class I solvents are the most toxic and should be avoided with very low limits. These are solvents known as human carcinogens and have ozone-depleting properties. Class II solvents should be of limited use and are either non-genotoxic animal carcinogens or possibly causative agents of other irreversible toxicity, such as neurotoxicity or teratogenicity. Class III solvents are considered safer alternatives with a low toxicity potential to humans. The 59 solvents in the USP list do not make up a comprehensive list, because different states require additional solvents (Seltenrich, 2019).

Appendix B indicates the residual solvent list of chemicals screening and the limit of individual states with a MMJ Program. Although the list provided is for a handful of states that have legalized MMJ, the Appendix is provided to highlight the variance in the number of solvent residues that are monitored along with the limit requirements. Also noteworthy is that Colorado

only has 6 residual solvents that are screened, however the limit is lower compared to some states like Massachusetts, which has 49 residual solvents. Missouri has a different value for an inhalation product compared to an infused marijuana product. Inhalation products have stricter residual solvents. The table also indicates the list of 59 residual solvents that are listed at USP chapter 467 and their various categories by class (Convention, 2019).

Biological Contaminant

Cannabis plants are prone to biological contaminants such as bacteria, yeast, mold, and other pathogens from the environment. Furthermore, these biological contaminants can inoculate any time between post-harvest and the pre-sale of the cannabis' end product. For example, improper storage or unhygienic processing or handling can be the culprit to introduce these contaminants. The consumption of the final product and its associated contaminants may have adverse health effects, especially to immunocompromised patients under medical marijuana treatment. For this reason, screening for these contaminants is of greater importance.

Since there are no federal regulations currently, consensus among various states regarding which microbes are harmful, along with their acceptable limits, is subjective. The safety screening to ensure cannabis products are safe also varies from state to state. Appendix C depicts biological contaminants screened by select states California, Massachusetts, Missouri, Nevada, Colorado, Oregon, Arkansas and Washington. In the U.S, the lack of agreement on what constitutes "biological contaminants" has resulted in three major tactics to ensure safety. They are the following:

1. Selective approach to regulate pass/fail status
2. Total contaminant testing
3. Water activity/moisture content limits

1. Selective Approach

The selective approach test is for specific toxins or contaminants. For example, there are 180 individual species of mold and the majority are benign; only four species are prevalent in cannabis that cause adverse health conditions. These four species are: *A. fumigatus, A. flavus, A. niger*, and *A. terreus*. There are a couple of common approaches to test these biotoxins. One popular method is a culture-based method. Another common approach is a quantitative polymerase chain reaction (qPCR) based testing, which targets specific DNA of contaminants based on the PCR products. Mycotoxin, which is a secondary metabolite from mold, is also screened. These mycotoxins include ochratoxins and aflatoxins, and are typically screened with LC-MSMS technology. The microbiological tests are selective to specific toxins. California, Colorado and Missouri have adopted this approach to regulate pass/fail criteria for the final products. Appendix C indicates the acceptable tolerance for these mold species.

2. Total Contaminant testing

States like Massachusetts test for total yeast and mold (TYMC), total aerobic microbial count (TAMC). These tests are less discriminative and are considered broad tests as they account for both benign and toxic biological contaminants. The concern with using these broad tests is the potential that they lead to rejection of a safer cannabis product. The unit of measuring contaminants is by counting the number of colony forming units per gram of Cannabis (CFU/g). However, the proponents of these tests argue that this approach is more protective to public health as it has a broader coverage of contaminants. Additionally, the tolerance limit for the total amount of mycotoxins (aflatoxin and ochratoxin) is also mandated (McPartland & McKernan, 2017).

3. Water activity/Moisture content

States such as Oregon and Arkansas regulate biological contaminants by regulating the maximum limit for water activity and moisture content. Lowering water activity provides safer cannabis by preventing microbial growth. The way this works is by measuring partial vapor pressure of water in a headspace that is at equilibrium with sample. The range of water activity(aw) is from 0 (bone dry) to 1.00 for pure water. Values above 0.70 aw would provide favorable growth conditions for mold spores or mycotoxins. Water activity measurement is an intensive property that provides the energy of water, whereas moisture content is an extensive property that accounts for moisture.

A moisture content value of below 15% prevents fungi and bacteria growth. Regulating water activity and moisture content in order to prevent microbial growth and provide chemical and physical stability is a widely accepted practice in both the pharmaceutical and food industries (Carter, 2019). Appendix E indicates the limits of water activity and moisture content.

Metal Testing

Metal contaminants in edible cannabis, inhalants, and topicals pose a health threat as the toxins can bioaccumulate and are known to be carcinogenic. (McPartland & McKernan, 2017) Metal contaminants gain entry to the plant through contaminated soil, tainted fertilizer, and other environmental factors such as volcanic conditions. Once these toxins are absorbed, they could bio-accumulate over a period of time and ultimately reach the flower inflorescence. Additionally, various processing or storage conditions could be a source of contamination (Seltenrich, 2019).

Cannabis plants are considered an excellent bioremediation crop because they can extract toxins from the soil and accumulate them. Common metal contaminants that are mandated by state regulations include cadmium, mercury, lead, arsenic and nickel. Like all other test panels,

metal residue tests also vary from state to state. Appendix D shows the tolerance limit of various metal contaminants in California, Missouri, Massachusetts, Nevada, Colorado, Arkansas and Washington, and the limits reported in USP <232>. It is clear from this table that the majority of these states monitor four metal contaminants in cannabis. Some states, like Missouri, have added total chromium to the safety list.

The majority of the states follow USP <232>. USP chapter on "Elemental Impurities" in drug products for their limits (USP39, 2020), however there are states that deviate from the UPS <232> limits. Additionally, some states have differentiated the route of consumption limits, such as ingestion, inhalation and transdermal applications. It is also important to note that there are some states that do not regulate metals, such as Oregon. USP <233> chapter describes the methods, sample preparation and instruments used for these impurity tests. Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES) or Inductive Coupled Plasma-Mass Spectroscopy can be used to monitor metal residue (USP233, 2020).

Kansas Industrial Hemp Program

The State of Kansas introduced "Alternative Crop Research Act" authorized by Kansas Department of Agriculture (KDA) referred to as Senate Bill 263, which was signed into law in 2018 by Governor Jeff Colyer. The legislation, now known as K.S.A. 2-3901, allows Kansas' farmers to diversify their crops and provide an avenue to a very growing industry (Agriculture K. D., Program Information, 2020). Soon after the beginning of the Kansas industrial hemp program, hemp was legalized nationally with the Agriculture Improvement Act of 2019, also known as "2018 farm bill" that required USDA authority for oversight to implement a consistent regulatory framework in the production of hemp across the U.S. States and Indian tribes. USDA issued an "interim final rule" in 2019 October, which requires states to submit their state hemp

plans to be sanctioned by USDA (USDA, 2019). In April 2020, the USDA approved the commercial industrial hemp plan submitted by the KDA.

The state of Kansas requires four different licenses: Grower Licenses, Distributor Licenses, Processor Licenses and Educational Institution Licenses. Grower Licenses allow farmers to grow and harvest hemp, whereas a Distributors licenses give permission to store, distribute or transport hemp. Processor Licenses are issued for the processing of hemp, and State Educational Institution licenses allow university applicants to cultivate, distribute, process and conduct research on hemp (Agriculture K. D., 2020). Licensees undergo a criminal background check and are fingerprinted. Additionally, licensees are required to provide a legal description and GPS coordinates of the entrance to the lot where the hemp is cultivated. The state of Kansas also requires only authorized seeds or clone plants to be utilized for hemp cultivation. These seeds are characterized to produce THC concentration which is less than 0.3 percent on a dry-weight basis. Table 8 lists the authorized seeds or clone plants.

Table 8. Kansas Approved Seed or Clone plant list for Industrial Hemp

Alyssa	Beniko	Canda	Carmagnola	CC	CFX-1	CFX-2	Cherry Wine
Cobbler #8	Zolotonosha15	Delores	Eletta Campana	Elite	Endurance LF 2/14	Fedora 17	Felina 32
Fibrol	Fibronova	Finola	FranklinLF 2/14	Fedora 17	Felina 32	Fibrol	Fibronova
Finola	Futura 75	Georgina	Grandi	Joey	Katani	KCC13	Martha
Maui's Cherry	Maui's Cherry 2	MS77	MS77-CHG	OT	Otto II:Endurance	Otto II: Stout	Picolo
RR13	Santica 27	Seagul IH 1	Stout LF 2/14	Sweetened LF 2/15	TI	Trump	Tygra
USO 31	Victoria	Wojko	Workhouse	X-59	Zolotonosha15		

Source: Kansas Department of Agriculture- Industrial Hemp Varieties

Farmers have to report their utilized seeds within 15 days of planting. Farmers must record the official name for the hemp variety, the GPS coordinates, the number of acres planted

in the licensed growing area, the number of acres planted in each lot, and the intended use of the hemp plant, plant parts, grain, or seed being cultivated.

It is the responsibility of cultivators to notify the state 30 days prior to the intended harvest date. The state will send a sampling agent to procure the representative sample 15 days prior the harvest day. The procedure for the collection of representative samples is published at the KDA website. In summary, each agricultural lot is tested to confirm its compliance with Kansas Industrial Hemp Program. The test agent collects plant specimens necessary to represent a homogeneous composition of the lot to be sampled. That testing agent is required to verify the GPS coordinates, average height, appearance, approximate density, condition of the plants, homogeneity of the variety, and degree of maturity of the inflorescence.

The requirement of a sample collection includes cutting a minimum of 15 plants for a lot less than 1 acre, and 30 plants for a lot ranging between 1-33 acres. Lots larger than 33acres require additional cuttings of plants as published in the Standard operating procedure(SOP). Plant cuttings must include 20 cm of the vegetative part, which includes meristem shoot material. Cuttings must also include 20 cm of reproductive parts, to include the pistallate flower, peduncle, pedicel and foliar materials.

Cultivators will be billed $225 for the characterization test at the testing lab at Kansas Department of Agriculture (KDA) and additional cost associated with travel time and mileage. If the test samples fail, or any lot of sample that have 0.3% greater THC on dry weight basis, a notice will be send to the cultivator. The cultivators must carry out the disposal in compliance with controlled substance act, 21 U.S.C 801 of the United States Drug enforcement agency (DEA), in the presence of a state actor with 10 days of notice and report the numbers of acres effectively disposed to the department. The cost associated with the disposal are the

responsibility of the licensee and violations are grounds for denial of any future hemp producer licenses. The Kansas Hemp industrial program also restricts cultivation within 50ft of a residential structure, or within 0.25 miles of any public or private K-12 school. Additionally, the licensee shall not interplant any other crop with hemp or any other varieties of hemp within a lot. The lot shall be clearly identified with 36 inches sign, legible from the adjacent public road, intersection of public road with text “Kansas Department of Agriculture Industrial Hemp Program” (Agriculture, 2020).

Methodology

Cannabinoids Profiling

The cannabinoids panel can be easily accomplished by Liquid Chromatography (LC) with UV detection. There are other more selective detectors like Mass Spectrometry, but the cost advantages have resulted in adopting LC-UV. Analytical labs in the cannabis space have developed the cannabinoids panel as a high-volume assay, thus leveraging sub-2um column chemistry in UPLC system, shortening the method while keeping the same resolving power.

In this report, we will address the procedure for cannabinoid profile panel for unprocessed Marijuana such as the cannabis flower. This method can be applied to infused marijuana characterization; however, depending on the matrix, there's potential for added complexity. Complex matrices might require Solid Phase Extraction (SPE) to simplify a complex sample matrix with compound purification. (Arsenault, 2012) Figure 12 illustrates the sample preparation steps involved for cannabinoid profiling of an unprocessed marijuana sample.

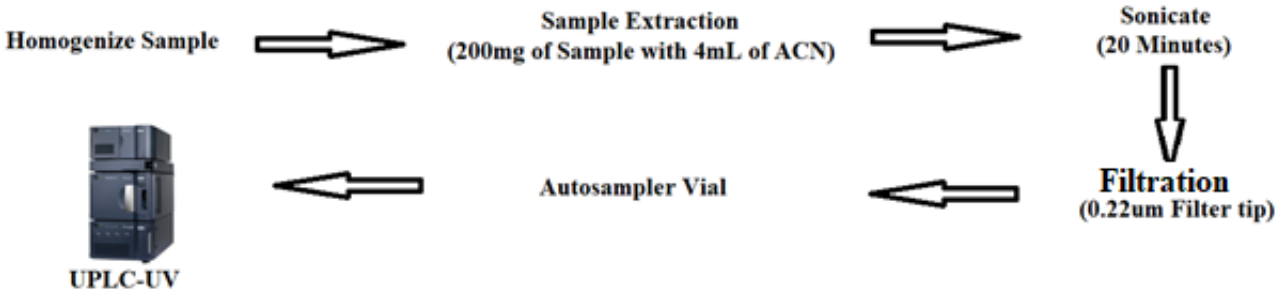


Figure 12. Sample Preparation Workflow for Cannabinoids Profiling

Table 9. UPLC-UV Conditions

Aquity UPLC H-Class-with TUV detector (:LC-UV)

LC Conditions		**Column and UV Conditions**	
Isocratic Flow Rate	0.7 ml/min	Column Temperature	35°C
Mobile Phase A	41% H_2O in 0.1% TFA -	UV Channel	228nm
Mobile Phase B	59% ACN	Column	CORTECS UPLC Sheild RP18, 90A , 1.6 µm, 2.1 x 100 mm

Source: Waters Corporation Application note (Aubin & Layton, 2020).

The sample is compared against the concentration curve based on known concentration levels of cannabinoids by mixing individual cannabinoids or acquiring pre-mixed standards from the accredited ISO vendor (Aubin & Layton, 2020). For Hemp labs that need to distinguish between Hemp and Marijuana, the same method can be utilized to calculate total THC level. The total THC is calculated by the following equation 1.

The THCA (also known as the acidic version) are thermally labile compounds and are easily converted to THC with heat. For this reason, GC-based methods decarboxylate THCA on the injection port, thus GC chromatogram will only have total THC peak, whereas LC technique is temperature neutral and THCA and THC are resolved as separate peak in the chromatogram. The Total THC accounts for the THCA species to convert to the THC form, and 0.877 factor accounts for the loss of mass due to decarboxylation of THCA to THC. Equation 1 is applied to the LC technique to report total THC so as to qualify whether the product is Hemp or Marijuana.

Pesticide Panel Sample Preparation

The pesticide panel tends to be the most complicated of the cannabis panels. The complexity is due to the large number of pesticides that need to be monitored, the detection limit, and the matrix that is used. Additionally, the cannabis plant matrix composition is complex as it contains cannabinoids, terpenes, hydrocarbons, sugar, fatty acids, flavonoids and other substances (Waters Corporation, 2020).

Since the Missouri regulation states that pesticide testing is to be monitored for the final product, the complexity of analysis is dependent on the matrix. Marijuana is often infused into "delivery substrates" such as gummy bears, candies or various other edibles, and often results in preferential ionization of endogenous and exogenous ions rather than the analyte of interest. This is referred to as ion suppression, and it has a negative impact on signal to noise resulting in poor recovery. To mitigate these challenges, QuEChERS, which is a sample preparation technique, is utilized. QuEChERS means it is a Quick, Easy, Cheap, Effective, Rugged and Safe method. This technique allows the screening of pesticides in a wide range of matrices using the same approach via two major steps - extractions and dispersive solid-phase extraction(d-SPE). In other words, the same sample preparation procedure applies for the unprocessed Marijuana plant as it does for the infused Marijuana final product.

The procedure involves using two tubes. The first tube has an extraction content, which improves analyte availability and extract quality. The second tube contains d-SPE sorbent (150mg MgSO4, 50 mg PSA, 50 mg C18 7.5 mg graphitized carbon). This tube's main function is to remove constituents such as fatty acids, sugar, and some ionic liquid. The d-SPE step serves to remove the residual water and further remove matrix interference from the sample, as well as being compatible with GC-MS or LC-MS technology. The QuEChERS sample preparation is the

culmination of 40 years of evolution in multi-residue pesticide analysis. Using the QuEChERS method allows more samples to be screened for a multitude of compounds in a shorter period of time and is an acceptable sample prep for both the European Committee for Standardization (CEN) Method 1566.25 and the AOAC official method 2009.01.

Missouri requires the regulation of 60 pesticides, however the acceptable levels are not as stringent as other states. As a result, all the pesticide screening can be done on one LC-MS/MS platform. This simplifies the pesticide panel for the state of Missouri, as the same sample prep could be applied for all in the list. Furthermore, the panel could be done in one platform which enables higher throughput.

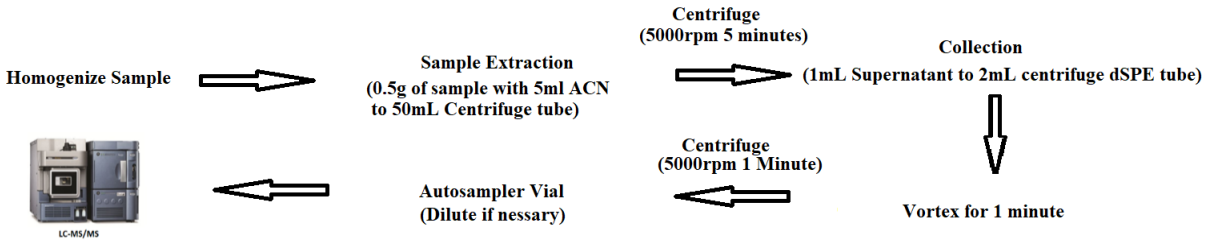


Figure 13. Pesticide Screening Workflow

The UPLC separation is done by XBridge BEH C18 XP, 130A, 2.5um, 2.1mm * 100mm. A 5ul sample volume is injected to a flow rate of 0.5mL/min at 30C.

Table 10 lists the instrumentation condition of LC-MS/MS. The sample area response is compared against the concentration curve based on known concentration levels of pesticide by mixing individual pesticide standards or acquiring pre-mixed standards from the accredited ISO vendor (Tran, et al., 2018).

Table 10. Experimental Conditions in Mass Spectrometer

Xevo TQ-S micro- Aquity UPLC H-Class System (LC-MS/MS)

LC Conditions				Mass Spec Conditions	
Time(min)	%A	%B	Curve	MS Parameters	Values
0.00	98%	2%	-	Ionization Mode	ESI+/ESI-
0.20	98	2	6	Capillary Voltage	2.5kV(+); 2.4 kV(-)
11.50	1	99	6	Cone Voltage	Various V
13.00	1	99	6	Collision Energy	Various eV
13.25	1	99	6	Desolvation Temp	450C
15.00	98	2	1	Source Temp:	150C
Solvent A	5mM NH_4HCO_2 with 0.02% Formic Acid in Water			Desolvation Gas Flow	1000 L/hr.
Solvent B	Methanol			Cone gas	50 L/hr.

Source: Waters Application Notes (Tran, et al., 2018)

Solvent Residue

The MMJ Program in Missouri requires a screening of 19 solvent residues, similar to California's residual solvent requirements (1-Butanol added to MO list). Missouri, however, requires separate limits for the inhaled Marijuana end product (a stringent requirement) compared to the infused marijuana end product (Ashcroft, 2020). Gas chromatography-Mass Spectrometer (GC-MS)/Headspace (HS) is typically utilized for residual solvents testing. The sample is placed on a Headspace vial, heated and set at a specific equilibration time. This will

allow the sample to reach equilibrium and will have constant concentration in the gas phase. An aliquot of the gas sample is injected via the headspace autosampler into the instrumentation so as to screen for residual solvents. Figure 14 illustrates the typical workflow of solvent residue testing, while Table 11 lists the experimental conditions for the Headspace Mass spectrometer.

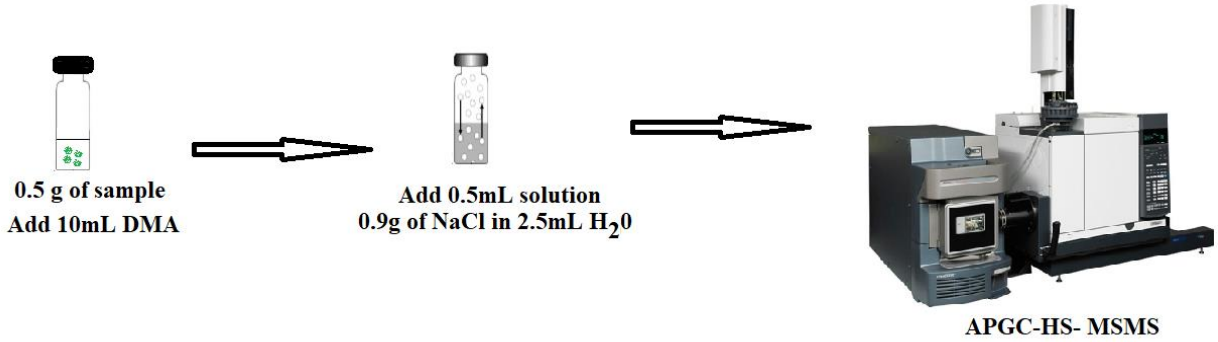


Figure 14. Solvent Residual Work-flow

Source: Restek Corporation (Myers; Herrington, Jason; Steimling, Justin; Gerardi, Ashlee)

Table 11. Experimental Conditions for Solvent Residual Panel

Xevo TQ-S micro- APGC-HS-MSMS(APGC-HS-MS/MS)

HS-GC Conditions		**MS Conditions**	
Sample Volume	500uL	MS Parameters	Values
Incubation Time	120s	Ionization Mode	ESI+/ESI-
Incubation Temperature	80C	Capillary Voltage	2.5kV(+); 2.4 kV(-)
Purge time	90 S	Cone Voltage	Various V
Oven	30C(hold for 3 min) to 85C(hold for 2 min) at 15C/min to 250C by 35C min	Collision Energy	Various eV
Inj. Temp.	280C	Desolvation Temp	450C
Purge flow	3 ml/min	Source Temp:	150C

Carrier Gas	He, Constant flow	Desolvation Gas Flow	1000 L/hr.
Flow Rate	2mL/min	Cone gas	50 L/hr.
Column	Rxi-624SIM MS, 30m x 0.25mm x 1.40 um	Scan Mode	MRM

Source: Restek Corporation (Myers; Herrington, Jason; Steimling, Justin; Gerardi, Ashlee)

Biological Contaminants

The state of Missouri mandates a stricter biological contaminant screening, identical to California requirements. There are 8 targeted pathogens screened to deem the cannabis product to be safe for the MMJ Program. These pathogens include 4 mold species of Aspergillus (*A. fumigatus, A. flavus, A. niger, A. terreu*) - all to be absent in 1g of a sample. Additionally, two bacteria, E. Coli and Salmonella are screened to be under 1g. Figure 15 illustrates a typical workflow for qPCR that is the gold standard for microbiological screening. The qPCR test takes 24-36 hr. time to report the results.

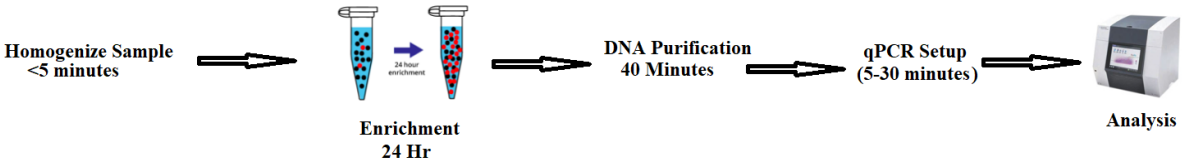


Figure 15. Workflow for Microbial Screening

Mycotoxins are also screened in Missouri to ensure the patient safety in the MMJ. The maximum tolerable limit for Aflatoxin and Ochratoxin are under 20ppb. The LC-MS/MS technique is the gold standard, as it is more selective, less susceptible to Signal-to Noise(S/N), and therefore more sensitive. Additionally, the same sample preparation and instrument procedure as referred to in Figure 13 and Table 10 for pesticide residue screening can be utilized. This is accomplished by adding a multiple reaction monitoring (MRM) channel for Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2 and Ochratoxin into existing methods for pesticide

screening. In other words, identical sample preparation, instrument procedure, and one injection will characterize for both pesticide and mycotoxin test panels (Regina, 2011) (Tran, et al., 2018)

Metal Residue Contamination

The Inductive coupled Plasma-Mass Spectrometry (ICP-MS) is routinely used in the cannabis industry to screen for toxic heavy metal. These heavy metals can bio-accumulate in the cannabis plant, and even at very low concentrations, can have negative consequences. The tolerance limit for metal residue contaminants vary from state to state, as do the limits to how much can be ingested. Most states follow the USP 232 list of metals and their tolerance levels as a guide. Missouri regulation requires a screen for five metals: inorganic Arsenic, Cadmium, total Chromium, Lead, and Mercury - all at sub-ppm levels. The challenge for heavy metal testing (in addition to the sub-ppm level detection) is procuring matrices of the cannabis sample, which are flower, concentrates, edibles, extracts, tinctures, waxes and oils. Sample preparation is a critical step to ensure homogeneity of the sample and to reduce the matrix related interference. Figure 16 depicts the typical work-flow trace level testing for MMJ samples using ICP-MS systems.

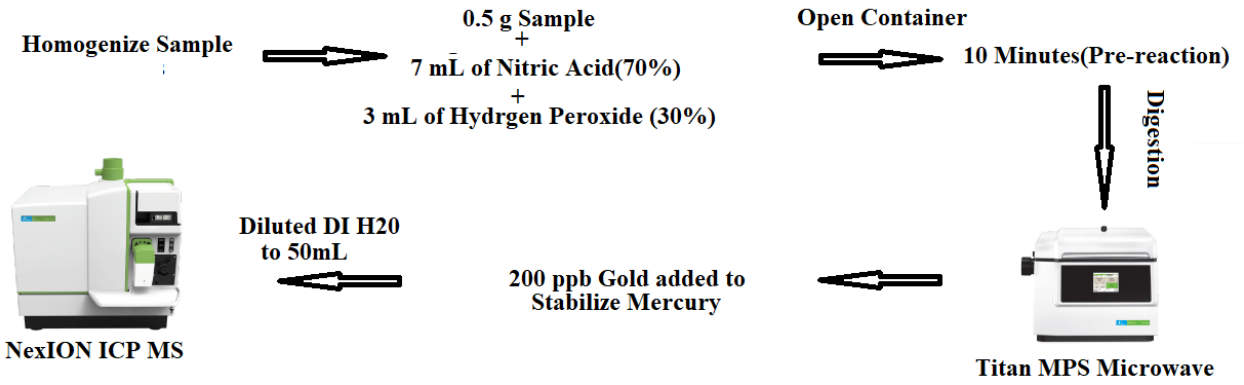


Figure 16. Workflow for Heavy Metal testing

Table 12. Experimental conditions for ICP-MS and Microwave digester

NEXion ICP-MS System

Microwave Conditions					**ICP-MS Conditions**	
Temp(C)	*Pmax*	*Ramp (min*	*Hold*	*Power*	*MS Parameters*	*Values*
160	30	5	5	90	RF Power(W)	1600
200	30	5	20	100	Nebulizer flow(L/Min)	0.88
50	30	1	30	0	Dilution Gas Flow(L/min)	0.11
					Sample Uptake Rate(mL/min)	0.22
					Collision (He) Gas flow(mL/min)	0.20
					Collision (He) Gas Flow (mL/min)	0.4

Source: (Hineman, Purcel, & Astill, 2019)

Results and Discussion

The cannabinoid profile results reported here are part of the method development effort for the hemp testing lab at KSU. The KSU lab in Olathe, Kansas supports the industrial hemp program in Kansas. This method utilized UPLC-UV for cannabinoids profiling and potency testing to confirm the sample are hemp, not Marijuana. Additionally, the example results for chemical and biological contaminants reported here are based on a review of the application notes from various market leading vendor.

Cannabinoids Profile

KSU-Collaboration

Figure 17 displays the UPLC-UV overlay chromatogram of CBD, CBDA, CBN, THC and THCA. The chromatogram indicates acceptable resolution between cannabinoids. These 5 cannabinoids are mandated to be listed for labeling by Missouri MMJ program. The total THC can be calculated using Equation 1 for potency testing to differentiate between hemp and Marijuana samples.

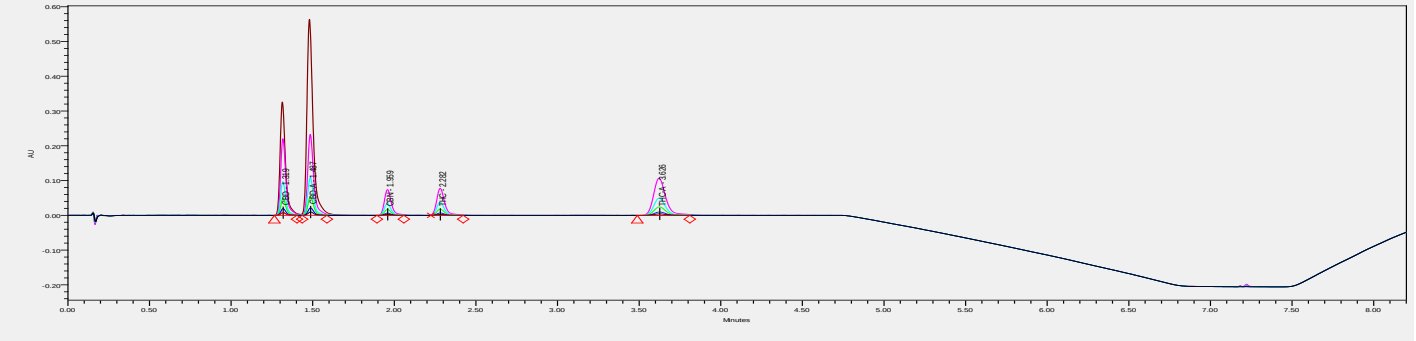


Figure 17. UPLC -UV Chromatogram of 5 Major Cannabinoids

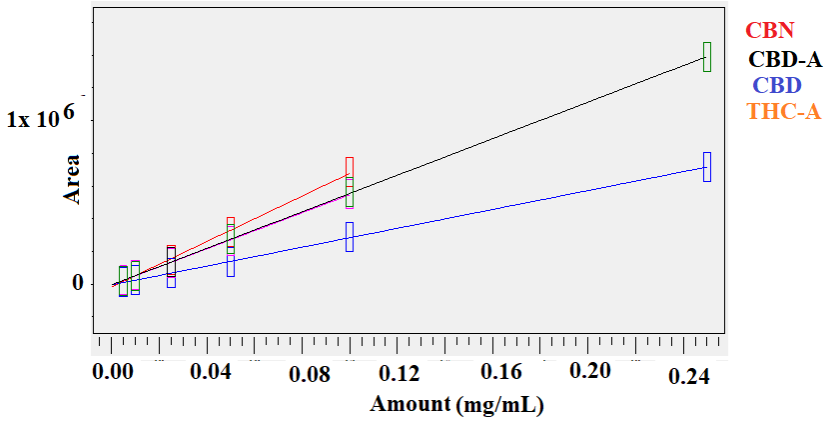


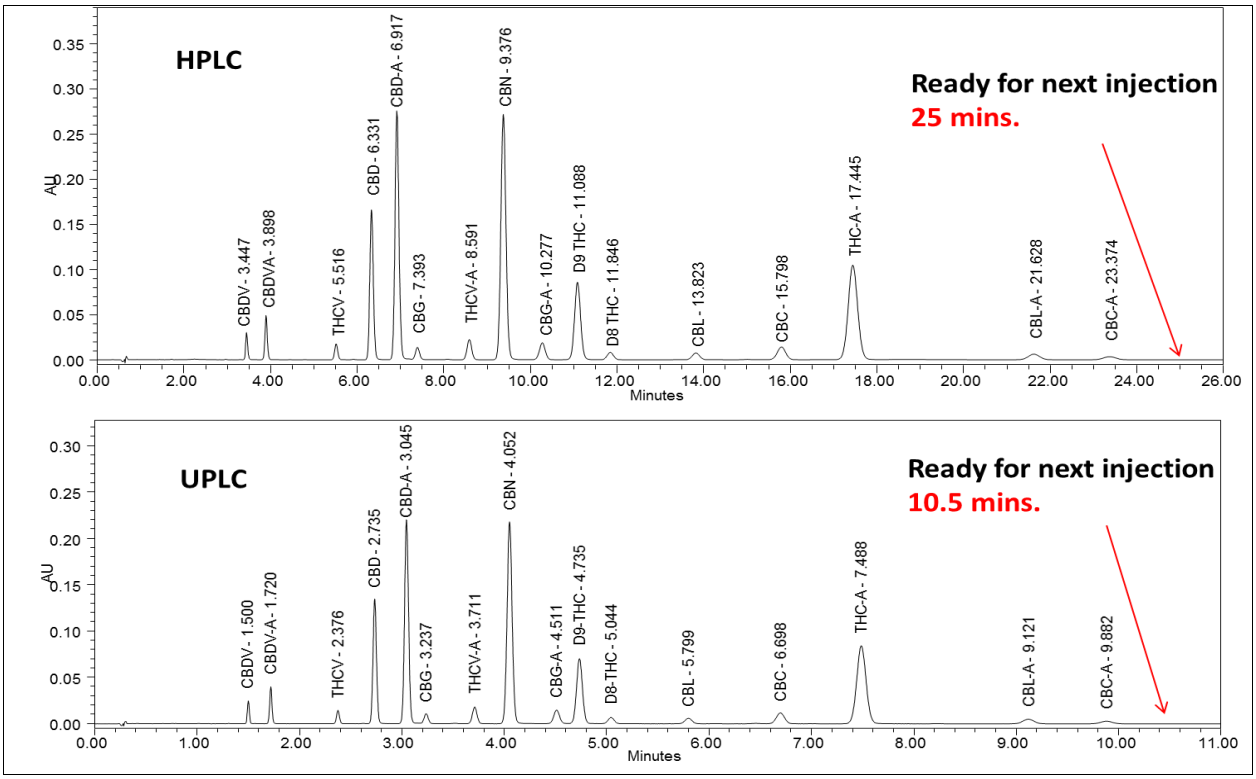
Figure 18. Calibration curve data for major Cannabinoids

Table 13. Calibration Curve Parameters

Project Name	Channel	R^2	Equation	Name	Units	Time
HempProject_9_9_19	PDA Ch1 228nm@4.8nm	0.999026	Y = 5.50e+006 X - 3.77e+003	THC-A	mg/ml	3.750
HempProject_9_9_19	PDA Ch1 228nm@4.8nm	0.999792	Y = 2.88e+006 X - 3.78e+003	CBD	mg/ml	1.350
HempProject_9_9_19	PDA Ch1 228nm@4.8nm	0.999937	Y = 5.58e+006 X - 3.44e+003	CBD-A	mg/ml	1.547
HempProject_9_9_19	PDA Ch1 228nm@4.8nm	0.999075	Y = 6.94e+006 X - 1.66e+004	CBN	mg/ml	2.040
HempProject_9_9_19	PDA Ch1 228nm@4.8nm	0.999075	Y = 6.234e+006 X - 1.01e+004	THC	mg/ml	2.298

Figure 18 displays the calibration curve for major cannabinoids, CBN, CBD-A, CBD, THC-A, the limit of quantitation (LOQ) is at 0.005 mg/mL. Table 13 list the R^2 value greater than 0.999 for the THC-A, CBD, CBD-A, CBN, THC indicating excellent linear fit for the calibration curve. Additionally, the linear equation and retention time of cannabinoids are listed. Cannabinoid concentration of the hemp sample can be determined by corelating area of the unknown sample to the calibration curve of known standards. MMJ regulation in Missouri requires the reporting of five major cannabinoid concentrations for labeling on all cannabis-related products. These cannabinoids are THC, THCA, CBD, CBDA, and CBN.

Figure 19 shows the comparison to HPLC using a 2.7um particle size in column chemistry versus a 1.6um particle size, decreased the run time from 25 minutes to 10.5 minutes when analyzing 16 cannabinoids. This allows the testing lab to run approximately 48 samples in an 8 hour day, with one UPLC system that exploits sub-2 micron column Leveraging sub-2µm particles. Given the significant time savings, UPLC is preferred for high throughput cannabis testing labs.



Source: Waters Application Note (Layton, 2018)

Figure 19. LC-UV Chromatogram of HPLC vs. UPLC

Table 14, shows the return on investment (ROI) for the UPLC method vs. HPLC method. The ROI was based on the method for characterizing 16 cannabinoids, and the cost to run a single cannabinoids panel is $50. Although the UPLC is more expensive than traditional HPLC, the time saved per run would make up for the cost differential. Assuming the lab operates 8 hours, 5 days a week, it would take 6 weeks to achieve a break-even point, as compared to 11 weeks with the HPLC method. The break-even point only accounts for the instrumentation cost and excludes other overhead costs.

Table 14. ROI for UPLC Vs. HPLC

8 hrs./5 days a week	UPLC Method	HPLC Method
# of Cannabinoids Measured	16	
Cannabinoids	CBDV, CBDV-A, THCV, CBD, CBD-A, CBG, THCV-A, CBN, CBG-A, d9-THC, d8-THC, CBL, CBC, THC-A, L-A, CBC-A	
Est. Revenue/Sample	$50	$50
Run Time (minutes)	10	25
Samples/Day	48	19.2
Samples/Week	240	96
Est. Revenue/Week	$12,000	$4,800
Instrument Cost	$68,170	$55,000
Break Even (weeks)	6	11

Chemical Contaminant Testing

Pesticide Residue

Chemical contaminant testing for Missouri's MMJ includes a list of 60 pesticides and 19 solvent residues. These chemicals are listed in Appendix A 1 for pesticides and Appendix B 1 for solvent residue.

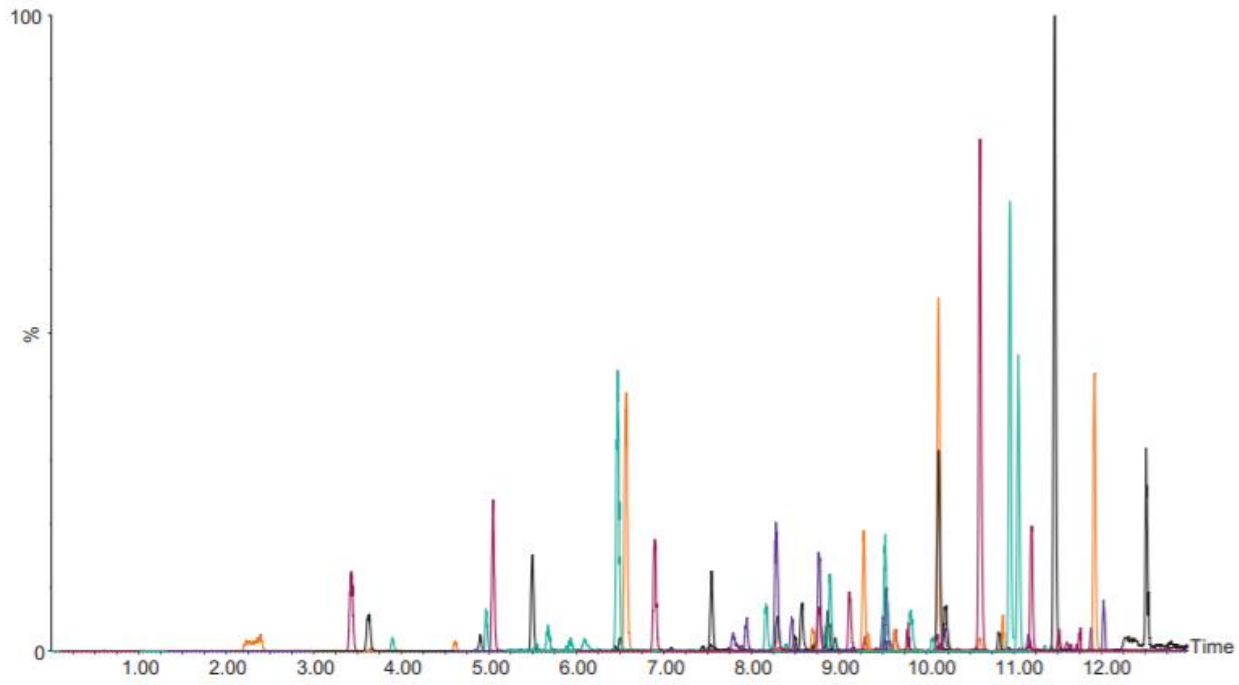


Figure 20. TIC for MO Pesticide list

Source: Waters Application Notes (Tran, et al., 2018)

Figure 20 shows the Total Ion Chromatogram (TIC) of 60 Pesticides analyzed by UPLC-MS/MS. The method time is 13 minutes with the LC-MS/MS system. MRM of individual pesticides can be monitored by selecting corresponding transitions to quantitate against the calibration curve. For example, Figure 21 indicates the quantitating Methomyl sample selected based on its transition 163.1>88 against its calibration curve. The calibration curve for individual pesticides to encompass the linear dynamic range of the instrument is prepared. The calibration curve is compared to the area response of the unknown sample to determine the concentration level of the pesticide.

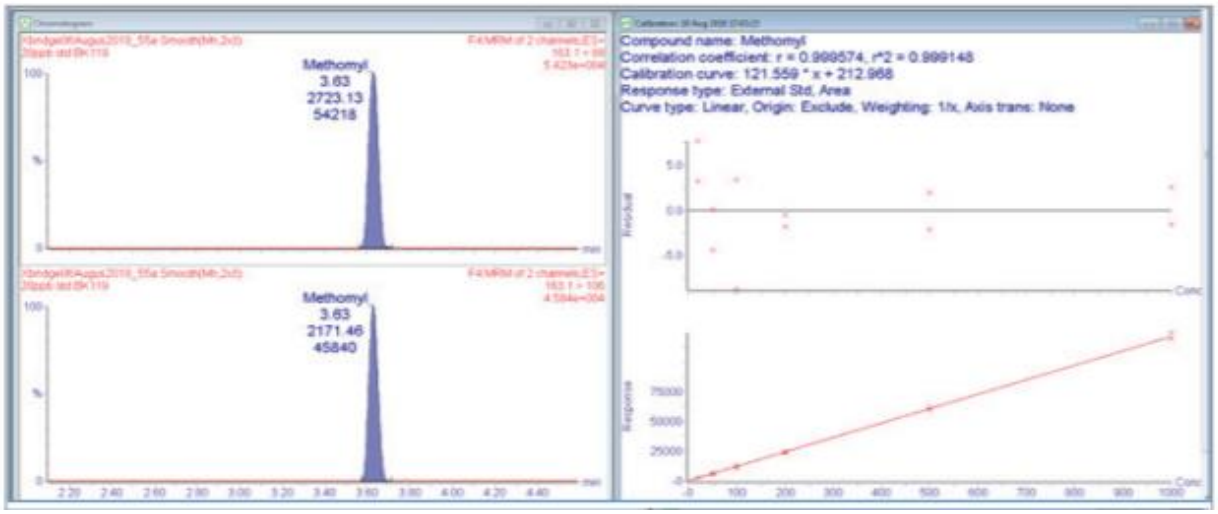


Figure 21. Methomyl Calibration curve

Source-Waters Application Notes (Tran, et al., 2018)

Figure 22 indicates method robustness by recovery data, 200ppb and 1000ppb levels were spiked in the cannabis flower matrix, and comparing the response observed to spiked blank. The recovery range of 80% to 120% matrix suppression was determined. The error bar indicates the standard deviation on each compound.

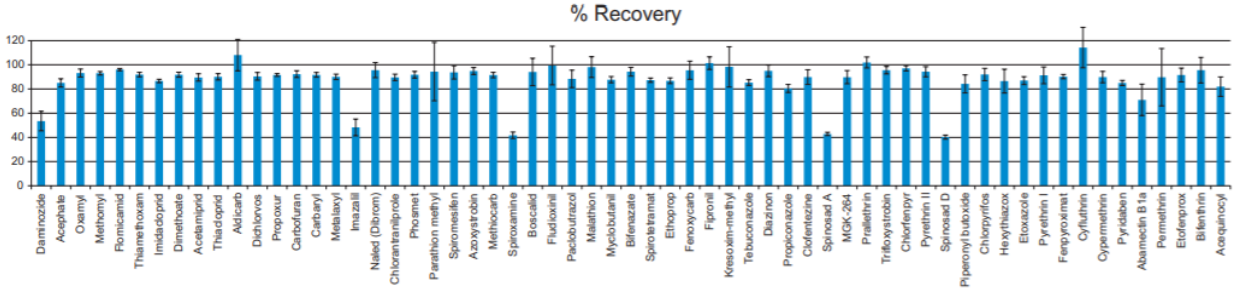


Figure 22. Recovery of Pesticide residue from Flower Matrices

Source: Waters Application Note. (Tran, et al., 2018)

Table 15. ROI on LC-MS/MS for Pesticide and Mycotoxin Panel

8 hrs./5 days a week	LC Pesticides and Mycotoxins UPLC-MS/MS
LC Pesticides	Missouri pesticide list (60 compounds, LOQ>0.2 to 2ppm) - Title 19
Mycotoxins	Aflatoxins (B1, B2, G1, G2) and Ochratoxin A
Est. Revenue/Sample	$150
Run Time (minutes)	15
Samples/Day	32
Samples/Week	160
Est. Revenue/Week	$24,000
Instrument Cost	$228,465
Break Even (weeks)	10

Table 15 shows the return on investment (ROI) for LC-MS/MS on an instrument for a pesticide residual test. Since the same equipment is used to analyze Mycotoxin analysis, this is an added value. Assuming the lab operates 8 hours, 5 days a week, it would take 10 weeks to achieve a break-even point. The break-even point only accounts for the instrumentation cost and excludes other overhead costs. The operation of LC-MS/MS does require a high-level skill set, and additionally testing various matrices add complexity.

Residual Solvent Outcome

The solvent residue test is carried out on a GC-FID or GC-MS system. GC-MS is the preferred technique due to a wide variety of matrices such as concentrates, extracts, edibles,

waxes and oils, having the ability to not only separate with retention time of the compound, but also potentially enabling the mass spectrometer to separate based on molecular weight. This would allow the user to integrate the compound of interest even if other impurities co-elute with same retention time. Figure 23 displays the TIC chromatogram for residual solvents for the cannabis flower.

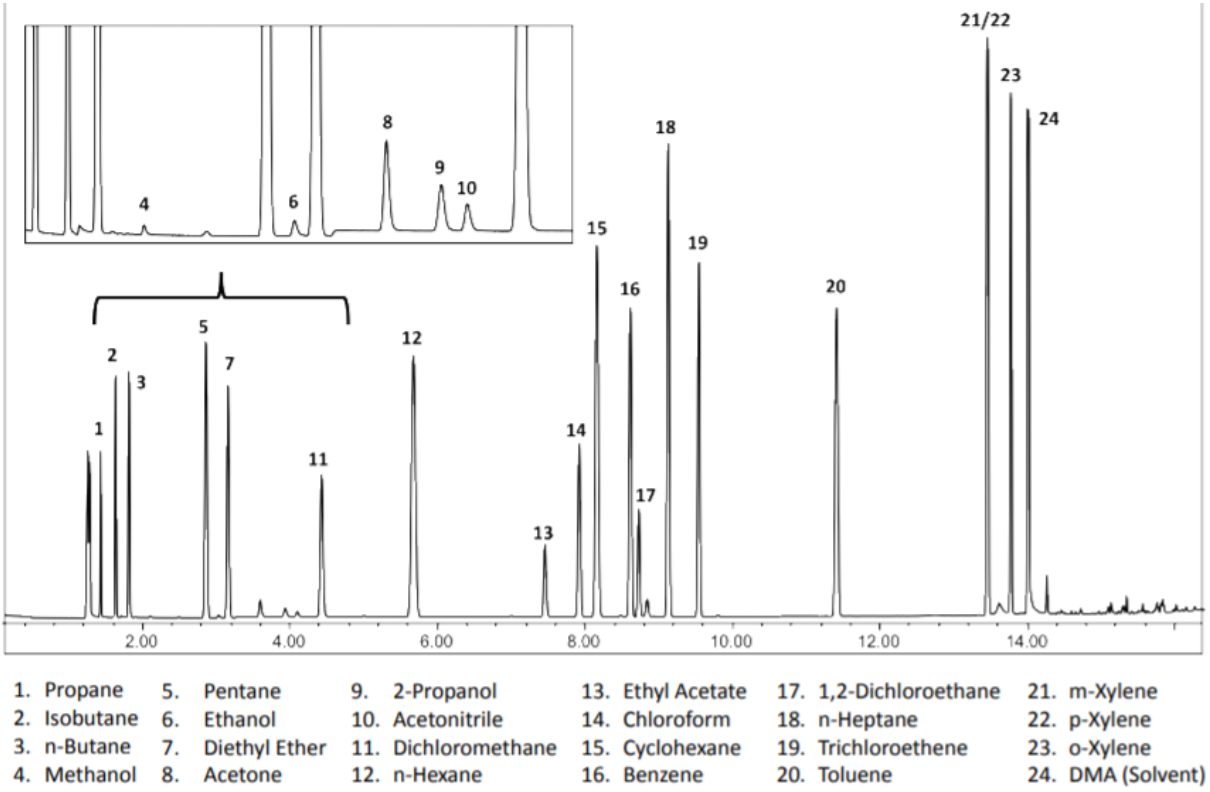


Figure 23. Solvent Residue Chromatogram

Source: Restek Application Note (Myers; Herrington, Jason; Steimling, Justin; Gerardi, Ashlee;)

The calibration curve for each individual residual solvent is created. The calibration curve is compared to the area response of the unknown sample to determine the concentration level of the residual solvent.

Table 16. ROI on GC- HS-MS/MS for Residual Solvent and Terpene Panel

8 hrs./5 days a week	LC Solvent Residue GC-HS-MS/MS
GC Solvent Residue	Missouri Solvent residue list (19 compounds, - Title 19
Terpenes	23 VOCs
Est. Revenue/Sample	$150
Run Time (minutes)	30
Samples/Day	16
Samples/Week	80
Est. Revenue/Week	$12,000
Instrument Cost	$280,000
Break Even (weeks)	23

Table 16 shows the return on investment (ROI) for GC-HS-MS/MS on an instrument for a pesticide residual test. The same equipment is used to analyze Terpene analysis. Although it's not regulated, manufacturers benefit from testing a Terpene panel. Terpenes are aromatic compounds abundant in cannabis and they contribute to the physiological response associated with the cannabis product. Measuring terpenes is an important quality control measure, as it adds to the preference or satisfaction of the cannabis product. Testing a terpene panel is a separate method than what would be used for a solvent residue panel, so it requires additional time with the instrument. Assuming the lab operates 8 hours, 5 days a week, it would take 23 weeks to achieve a breakeven point for running residual solvent panels. The breakeven point only accounts for the instrumentation cost while excluding other overhead costs. The operation of

GC-HS-MS/MS already requires a high-level skill set, and testing various matrices add further complexity.

Biological Contaminant

Mycotoxin- LC-MS/MS

LC-MS/MS technology is the preferred tool to screen mycotoxin. MO state law requires producers to demonstrate that individual Aflatoxins G2, G1, B2 and B1 are under 0.02ppm. Additionally, it also requires Ochratoxin to be under 0.020 ppm. Figure 24 displays MRM chromatogram of cannabis matrix spiked at 0.02µg/g which is the action level set by state of Missouri for mycotoxin testing. The response for individual mycotoxins MRM channel with their transition is reported in Figure 24. For example, MRM channel, 331.1>245.1 indicates, isotopic mass for Aflatoxin G2 as 331.1 Dalton (Da) and the quantitative daughter mass as 245.1Da. The sample response factor (area of the peak) below the spiked 0.02 µg/g area count is considered passed or is withing the tolerance limit for MO state regulation.

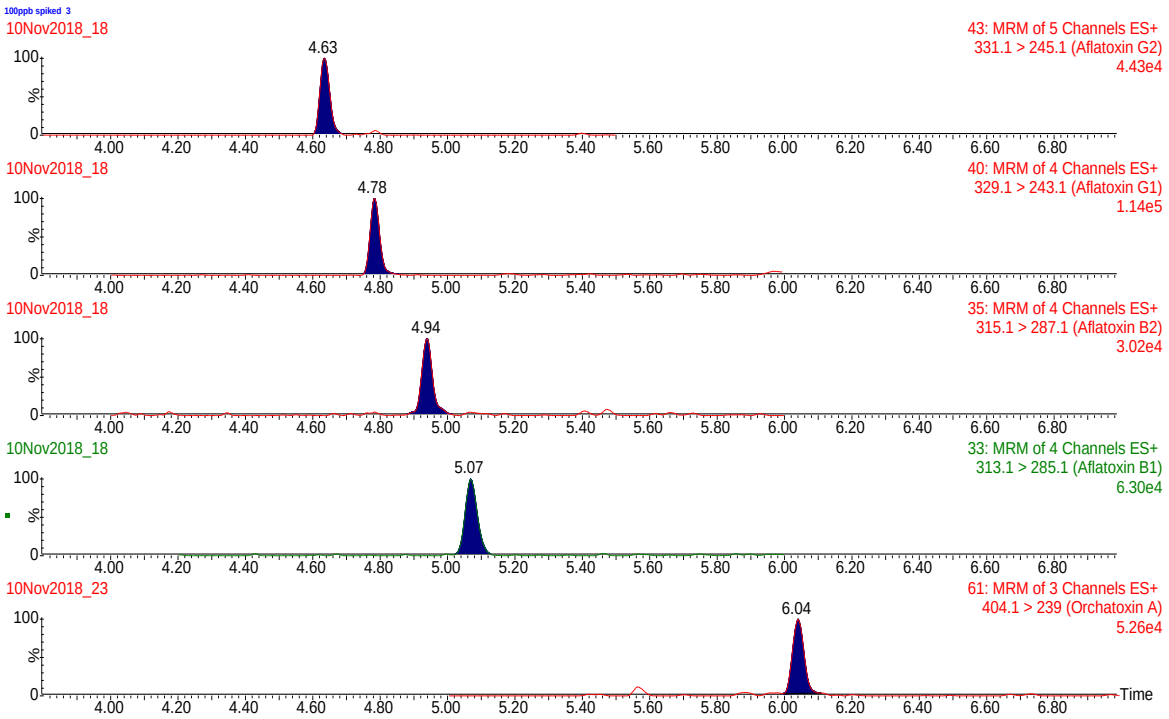


Figure 24. Mycotoxin MRM for Aflatoxins and Ochratoxins

Source: Waters Application Note. (Tran, et al., 2018)

Microbiological Pathogen

Missouri legislation regulates for mold species of Aspergillus, (*A. fumigatus*, *A. flavus*, *A. niger*, *A. terreus)*, *E. Coli* and *E. Salmonella* to all be absent in 1 g of sample. Appendix C lists the microbiological pathogens regulated in various states and the tolerance limit, including MO. PCR can amplify a single copy or copies of targeted microbial DNA by tagging with fluorescence primer. The levels of fluorescence are measured to generate sample application curve shown in Figure 25.

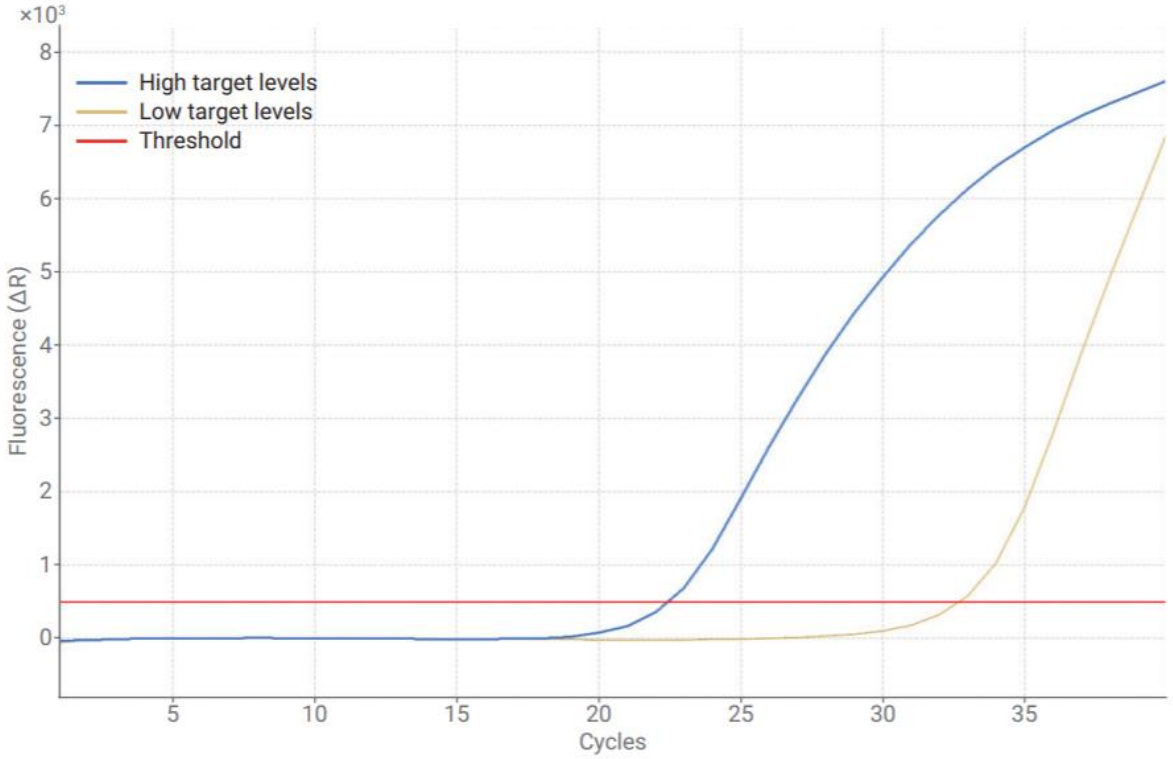


Figure 25. PCR Amplication Curve

Source: Agilent Application notes; (Leppanen & Heather)

The presence of the target DNA molecule is detected with the fluorescence detector, additionally, comparison to a standard or low target level amplication curve can quantitate the microbial levels. The output from the application curve is fraction cycle number(Cq) , a value at which signal curve exceeds the pre-determined value known as threshold(redline in Figure 25). The Cq derived from PCR results is converted to colony forming unit (CFU), a common microbial term reported in the regulations. A mathematical equation provided by the qPCR manufacturer is used to calculate the CFU, the equation is specific for the qPCR system as the machine ramp rate and temperature can alter the equation.

In this report, an example for Cq data converted to CFU for total yeast & mold detection assay using equation 3 provided by medicinal genomics, this equation is derived from plating and qPCR(Agilent Real-Time PCR system) (Genomics, 2020).

$CFU/G = 10^{((42.185\text{-Cq value})/3.6916)}$----------Equation 3

Table 17. qPCR Cq for MO State Microbiological Pathogen

Organism Spiked	qPCR Assay	Cq	CFU
E. Coli	Salmonella/E.coli	22.34	N/A
Salmonella	Salmonella/E.coli	23.9	N/A
Aspergillus flavus	Aspergillus flavus	20.65	6.8^5
Aspergillus fumigatus	Aspergillus fumigatus	20.40	8.0^5
Aspergillus niger	Aspergillus niger	28.45	5.3^3
Aspergillus terreus	Aspergillus terreus	33.03	3.0^2

Source: Recreated from Medicinal Genomics Application notes

This equation above can be used to meet evaluate state tolerance level for total yeast and mold detection. A empirical equation to convert Cq to CFU for each pathogen is needed as a part of method development work to create standard operating procedure (SOPS) from specific instrumentation vendor.

Conclusions

The journey from farm to tangible cannabis product is multifaceted. It involves controlled cultivation and plant gender selection, while ensuring correct variety of cannabis is cultivated following federal guidelines. At the same time, testing is need to ensure the product is of high quality, ensuring intended the presence of the desired cannabinoids and terpenes and the absence of chemical and biological contaminants. It involves manufacturing or processing, extracting targeted cannabinoids and terpenes with platform like Supercritical fluid extraction (SFE), further processing to meet the intended purification levels, and ensuring accurate label claim by testing the final product. Analytical testing to ensure safety and quality of cannabis-based product is paramount to the resurgence of cannabis into the mainstream. Medical Marijuana, industrial hemp and recreational cannabis all require stringent quality measures as they are topically applied, ingested or inhaled whether for medical or recreational purposes. Once industrial hemp was nationally legalized in U.S with the passage of 2018 farm bill, hemp was removed from the definition of Marijuana under the controlled substance act (CSA), thus lifting regulation federally. Demand for hemp is largely driven by Cannabidiol (CBD) and other non-psychoactive cannabinoids for its therapeutic benefits. Currently, there is no uniform federal regulations on these hemp-based products other than potency testing to verify the THC levels. The industrial hemp program instituted by State of Kansas fall into this category. The Kansas Department of Agriculture (KDA) provides testing services to ensure low THC or Hemp based products are cultivated, manufactured and made available to consumers. This places the onus on the manufacturers to ensure safety and quality of the hemp product.

Medical Marijuana is still illegal at the federal level; however, 33 U.S. states have legalized cannabis for medical use. Although state regulation varies from state to state, a requirement of

qualifying health condition is a must. Additionally, medical program adheres to strict quality and safety controls as it caters to sick and immunocompromised patients. To add to the complexity, safety tolerance levels varies from state to state. States like California have more comprehensive test requirements with trace level detection. Furthermore, since marijuana is illegal federally, interstate commerce for marijuana is also prohibited. This means that all of the cultivation, manufacturing, processing and testing is required to be done within the state boundaries. The medical marijuana program in Missouri mentioned in this report only applies to Missourian patients using a product that's cultivated, manufactured, processed and ultimately tested for its safety and quality within the Missouri territorial boundary. Eleven licensed Missouri labs ensure the quality of each lot of cannabis-based product for the potency and cannabinoids profile. Additionally, chemical contaminant and biological contaminant testing is required before the product is released. Liquid Chromatography, Gas Chromatography, Mass Spectrometer, ICP-MS and qPCR are required analytical tool to guarantee quality control and compliance to all regulations.

Future Direction

Cannabis industry whether its MMJ, hemp, decriminalization or recreational is a disorganized market due to the lack of harmonized regulations. Individual states have different approach in regulating cannabis, resulting in confusion in enforcement, barriers in interstate commerce and access to financial institution. A universal regulation in cannabis market are imminent that will standardize the testing requirement to ensure safety and quality of cannabis just like the mature food and drug market. The quality and safety attribute tests currently lack multiplexing as multiple assay, multiple instrumentation is required to enforce regulation. For example, in Missouri MMJ program, 9 separate test panel using multiple different

instrumentation to comply with the regulation. The future of testing a comprehensive quality and safety attributes to minimize the number of test panel with few instrumentation is desired. One such way, is multidimensional approach, such as 2D LC (LC x LC), thus 1st dimension LC can be utilized for reverse phase column for suitable non -polar separation or HILIC column for polar separation. This would minimize number of test panel and promote high throughput.

Currently, there are few cannabis related FDA drug in the market and is expected to grow as we gain greater understanding of the cannabinoids. This greater understanding of cannabinoids in human body requires OMICS based approach, technique characterizing thousands of proteins, metabolite, biomarkers to understand diseases, effect of cannabinoids to target and therapies. These OMICS based approach includes genomics, transcriptomics, proteomics, lipidomic and metabolomics, all within the realm of mass spectrometry. Advancement in MS in terms of imaging, newer source, nano flow all had contributed in unraveling OMICS workflow. One such advancement is in ion mobility technology(IMS) coupled with UPLC-MS. Physiological samples are complex and often certain metabolites or biomarkers are chromatographically unresolved and are isobaric, existing separation and mass spectrometer technology are unable to differentiate these compounds. An alternative approach is to use a technique to separate by shape, charge, mass and size. IMS is a orthogonal separation that involves ion traveling through a drift tube containing a buffer gas, the tube with voltage gradient applied via ring electrodes. With gas in the tube, the larger ions takes longer than the smaller ion as it drift through the tube corresponding to its size. As ion tumbles through the tube, rotationally-averaged- collision cross section (CCS) value of ion is calculated. CCS is its chemical cross-sectional structure value and can be used as a unique identifier for that compound. Additionally, CCS measurements are universal, its unaffected by sample matrix

consistent between instruments. IMS coupled with UPLC-MS system is an advanced investigative tool to study OMICS based approach to cannabis to delineate its therapeutic benefits.

References

Agriculture, K. D. (2020, June 15). *Industrial Hemp Applications*. Retrieved from www.agriculture.ks.gov/divisions-programs/plant-protect-weed-control/industrial-hemp/industrial-hemp-applications

Agriculture, K. D. (2020, April 8). K.S.A. 2-3901-Kansas Plan for Industrial Hemp Production. Manhattan, KS.

Agriculture, K. D. (2020, June 15). *Program Information*. Retrieved from www.Agriculture.ks.gov/divisions-program/plant-protect-week-control/industrial-hemp/program-information

AOAC. (2020, June 20). *AOAC International*. Retrieved from www.aoac.org/news/aoac-cannabis-program-new-working-group-reviews-laboratory-proficincey-testing-needs/

Arsenault, J. (2012). *Beginner's Guide to SPE.* Milford: Waters Corporation.

Ashcroft, J. (2020, June 30). *Missouri Secretary of State: Code of State Regulations.* Retrieved from Division 30-Division of Regulations and Licensure- Chapter 95 Medical Marijuana: www. sos.mo.gov/CMSimages/AdRules/csr/current/19csr/19c30-95.pdf

Aubin, A., & Layton, C. (2020, June 22). UPLC Seperation of the Anlysis of Cannabinoids contents in Cannabis Flower and Extracts. Milford, MA, USA.

Carter, B. (2019). The What, how, and why of Water Activity in Cannabis. *Cannabis Science and Technology, 2*(4). Retrieved from https://www.cannabissciencetech.com/view/what-how-and-why-water-activity-cannabis

Churchwell, M. I., Twaddle, N. C., Meeker, L. R., & Doerge, D. R. (2005). proving LC-MS Sensitivity through increase in chromatographic performance: Comparision of UPLC-ES/MS/MS to HPLC-ES/MS/MS. *Journal of Chromatography B*, 134-143.

Convention, T. U. (2019). *<467> Residual Solvents.*

Eltre, L. (n.d.). The Development of GPC and the First Commercial HPLC Instruments. In L. Eltre, *Chapters in the Evolution of Chromatography* (pp. 391-403). London: Imperial College Press.

Evans, C., & Jorgenson, J. (208). Ultrahigh Pressure Multidimensional Liquid Chromatography. In S. Cohen, & M. Schure, *Multidimensional Liquid Chromatography* (pp. 170-204).

FDA. (2018, June 25). FDA Approves First Drug Comprised of an Active Ingredient Derived from Marijuana to Treat Rare, Severe Forms of Epilepsy. Retrieved July 29, 2020, from https://www.fda.gov/news-events/press-announcements/fda-approves-first-drug-comprised-active-ingredient-derived-marijuana-treat-rare-severe-forms

FDA and Cannabis: Research and Drug Approval Process. (2020, July 22). Retrieved from FDA.gov-Public Health Focus: https://www.fda.gov/news-events/public-health-focus/fda-and-cannabis-research-and-drug-approval-process

Gaoni, Y., & Mechoulam, R. (1964, April 1). Isolation, Structure, and Partial Synthesis of an Active Constituent of Hashish. *Journal of the American Chemical Society*, pp. 1646-1647.

Genomics, M. (2020, July 18). PathogINDICAtor Cq to CFU. Woburn, MA, 01801. Retrieved from https://1280717.app.netsuite.com/core/media/media.nl?id=5398527&c=1280717&h=58d0c07ccf7d5473f8c2&_xt=.pdf

Hineman, A., Purcel, R., & Astill, T. (2019). Digestion, Testing, and Validation of Heavy Metals in Cannabis. San Fransisco, CA, U.S.A. Retrieved from https://www.perkinelmer.com/lab-solutions/resources/docs/app_nexion_icp_ms_titan_mps_heavy_metals_in_cannabis_014714_01.pdf

Izzo, A., Borrelli, F., Capasso, R., Marzo, V., & Mechoulam, R. (2009, October). Non-psychotropic plant cannabiniods: new therapeutic oppertunities from an ancient herb. *Trends in Pharmocological Science*, pp. 515-527.

Janischewsky, D. E. (1924). *A form of hemp in wild areas of southeastern Russia.* In Russia: N. G Cernysevskogo.

Jerkovich, A., Mellors, J., & Jorgenson, J. (2003). The Use of Micrometer-Sized Particles in Ultrahigh Pressure Liquid Chromatography. *LCGC North America* , 600-610.

Johnson, R. (2019). *Defining Hemp-Fact Sheet.* Congressional Research Service.

Layton, C. A. (2018, Febrary). UPLC Seperation for the Anlaysis of Cannabinoids Content in Cannabis Flower and Extracts. Milford, MA, U.S.A. Retrieved from https://www.waters.com/webassets/cms/library/docs/720006509en.pdf

Leppanen, S., & Heather, E. (n.d.). Optimized Cannabis Microbial Testing: Combined Use of Medicinal Genomics Extraction Methods With the AriaMX qPCR Instruemnts. Retrieved July 31, 2020, from https://www.agilent.com/cs/library/applications/application-fod-cannabis_5994-0430en-agilent.pdf

Linnaeus, C. v. (1753). *Species Plantarum.* Stockholm: Laurentius Salvius.

Lough, W., & Wainer, I. (1995). *High Performance Liquid Chromatography.* Glasgow: Blackie Academic & Professional .

Marijuana. (2019, November 15). Hisotory.

MARKETSANDMARKETS. (2017, March). Cannabis Testing Market by Product & Solftware (LC, GC, Spectroscopy(MS, Atomic), Column, Standards, Accessories, LIMS), Service (Potency, Pesticides, Heavy metal, Genetic Testing), End User (Lab, Pharmaceutical, Resarch)-Global Forcast to 2021.

Martin, A. S. (1941). A new form of chromatogram employing two liquid phases: A Theory of Chromatography. 2. Application to the micro-determination of the higher monoamino-acids in protiens. *Biochemical Journal, 35*(12), 1358-1368.

Mass Spectrometry. (2015, October). NewYork, U.S.A. Retrieved March 23, 2017, from http://www.nature.com/milestones/milemassspec/full/milemassspec04.html

McDonald, P. (n.d.). *James Waters and his liquid chromatography people: a personal perspective.* Retrieved from www.waters.com.

McKenna, G. (2014). The Current Status of Medical Merijuana in United States. *Hawai'i Journal of Medicine & Public Health*, 105-108.

McPartland, J., & McKernan, K. (2017). Contaminants of Concerns in Cannabis: Microbes, Heavy Metals and Pesticides. In S. Chandra, H. Lata, & M. ElSohly, *Cannabis Sativa L. Botany and Biotechnology* (pp. 457-474).

Mechoulam, R., & Shvo, Y. (1963). Hashish-I: The Structure of Cannabidiol. *Elsevier*, 2073-2078.

Myers; Herrington, Jason; Steimling, Justin; Gerardi, Ashlee;. (n.d.). A Simple Approach to Analyzing Residual Solvents in Cannabis Concentrates via HS-GC. Bellefonte, PA, USA. Retrieved July 10, 2020, from https://www.restek.com/pdfs/solventsincannabis_csc-poster.pdf

Nahas, G. G., & A, G. (1974, Jan). The first report of the National commmsion on marihuana (1972): signal of misunderstanding or exercise in ambiguity. *Bulletin of New York Academy of Medicine*, 55-75.

Pacula, R. L., & Smart, R. (2017). Medical Marijuana and Marijuana Legalization. *Annual Rev Clin Pyschol*, 397-419.

Pacula, R. L., & Smart, R. (2019). Medical Marijuana and Marjuana Legalization. *Annual Review of Clinical Psychology*, 397-419.

Prine, A. (2019, November 15). History. *Marijuana*. New York, NY: Meredith Corporaiton.

Regina, S. (2011, Jan). LC-MS/MS Progress in Newborn Screening. *Cli*, 21-31.

Release, F. N. (2018, June 25). FDA Approves First Drug Comprised of an Active Ingredient Derived from Marijuana to Treat Rare, Severe Forms of Epilepsy. Retrieved July 29, 2020, from https://www.fda.gov/news-events/press-announcements/fda-approves-first-drug-comprised-active-ingredient-derived-marijuana-treat-rare-severe-forms

Schultes, R. E., Klien, W. M., Plowman, T., & Lockwood, R. E. (1974). *Cannabis: an Example of Taxonomic Neglet.* Boston: Harward University.

Secades-Villa, R., Garcia-Rodrigues, O., Jin, C., Wang, S., & Blanco, C. (2016). Probability and Predictors of hte cannabis gateway effect: A national Study. *International Journal of Drug Policy*, 135-142.

Seltenrich, N. (2019). Cannabis Contaminants: Regulating Solvents, Microbes. and Metals in Legal Weed. *Enviornmental Health Perspectives*.

Services, M. D. (2020, June 16). *About Us*. Retrieved from www.health.mo.gov/safety/medical-marijuana/about-us.php

Services, M. D. (2020, June 18). *Annual Report to the Governor PY19*. Retrieved from Data and Report: www.health.mo.gob/safety/medical-Marijuana/pateint-stats.php

Tran, K., Organtini, K., Twohig, M., Young, M., Cleland, G., Rosnack, K., . . . Hudalla, C. (2018, December). Analysis of Residual Pesticides and Mycotoxins in Cannabis Using UPLC-MS/MS and GC-MS/MS to Meet California Regulatory Requirements. Milford, MA, U.S.A.

Tran, K., Twohig, M., Young, M., Aubin, A., Meruva, N., Fujimoto, G., . . . Hudalla, C. (2018, October). Determination of the Oregon Pesticide List in Cannabis Using a Simple Extraction Prodcure With dSPE Cleanup and UPLC-MS/MS. Milford, MA, U.S.A.

United States Drug Enforcement Agency. (2020, April 29). Retrieved from https://www.dea.gov/drug-scheduling

USDA. (2019, October 31). *The Daily Journal of the United States Government.* Retrieved from Federal Register: https://www.federalregister.gov/documents/2019/10/31/2019-23749/establishment-of-a-domestic-hemp-production-program

USP233. (2020, July 7). *<233> Elemental Impurities-Procedures.* Retrieved from USP: usp.org/sites/default/files.usp/document/our-work/chemical-medicines/key-issues/233_ElementalimpuritiesPorcdures.pdf

USP39. (2020, July 07). *Chemical Tests-232 Elemental Impurities-Limits.* Retrieved from USP: usp.org/sites/default/files/usp/document/our-work/chemical-medicines/key-issues/c232-usp-39.pdf

Waters. (2014). Atmospheric Presure GC(APGC-Expanding Analytical Capabilities in Food and Enviornmental Laboratories. Milford, MA, U.SA. Retrieved July 31, 2020, from https://www.waters.com/waters/library.htm?locale=en_US&lid=134985472

Waters Corp. (n.d.). *Ultra-Performance Liquid Chromatography 1st edition*. Retrieved April 06, 2017, from Waters.com: http://www.waters.com/waters/en_US/UPLC---Ultra-Performance-Liquid-Chromatography-Beginner%27s-Guide/nav.htm?cid=134803622

Waters Corporation. (2020, June 24). QuEChERS Procedure for Multi-Resudie Pesticide Analysis-DisQue Dispersive Sample Prepration. Milord, MA, U.S.A.

Westman-Brinkmalm, A. B. (n.d.). A Mass Spectrometer's Building Blocks. In R. S.-B. Ekman, *Mass Spectrometry Instrumentation, Interpretation, and Applications.* A John Wiley & Sons, Inc.

Zvonarev, V., Fatuki, T., & Tregubenko, P. (2019, Sep 30). The Public Health Concerns of Marijuana Legalization: An Overview of Current Trends. *Cureus*.

Appendix A - States Tolerance limit Pesticide Residual test

			Limits (PPB)									
Pesticides(A-Z)	CAS Number	LC/GC	CA (inhale)	CA (other)	MA	MO	NV	OR	CO	AK	WA	AOAC
abamectin	71751-41-2	LC	100	300		>500	50	500	70	500	500	50
acephate	30560-19-1	LC	100	5000		>400		400		400	400	100
acequinocyl	57960-19-7	LC	100	4000		>2000	4000	2000		2000	2000	100
acetamiprid	135410-20-7	LC	100	5000		>200		200		200	200	100
aldicarb	116-06-3	LC	<100	<100		>400		400		400	400	100
azoxystrobin	131860-33-8	GC	100	40000		>200		200	20	200	200	20
bifenazate	149877-41-8	LC	100	5000	10	>200	15000	200	20	200	200	10
bifenthrin	82657-04-3	GC	3000	500	10	>200	50	200		200	200	10
boscalid	188425-85-6	LC	100	10000		>400		400		400	400	100
captan	133-06-2	GC	700	5000			(50)					50
carbaryl	63-25-2	LC	500	500		>200		200		200	200	200
carbofuran	1563-66-2	LC	<100	<100		>200		200	0	200	200	100
chlorantraniliprole	500008-45-7	LC	10000	40000		>200		200		200	200	200
chlordane (sum of cis-, trans- and oxychlordane)	57-74-9	GC	<100	<100					0			100
chlorfenapyr	122453-73-0	GC	<100	<100		>1000		1000		1000	1000	100
chlomequat chloride		LC				>200					500	0
chlorpyrifos	2921-88-2	GC	<100	<100		>200		200		200	200	100
clofentezine	74115-24-5	LC	100	500		>200		200		200	200	100
coumaphos	56-72-4	GC	<100	<100								100
cyfluthrin (and beta-cyfluthrin) (Baythroid)	68359-37-5	GC	2000	1000	10	>1000	4000	1000		1000	1000	10
cypermethrin	52315-07-8	LC	1000	1000		>1000	50	1000		1000	1000	50
daminozide (Alar)	1596-84-5	LC	<100	<100		>1000	50	1000	0	1000	1000	50
diazinon	333-41-5	GC	100	200		>200		200		200	200	100
dichlorvos (DDVP)	62-73-7	GC	<100	<100		>1000		100		100	100	100
dimethoate	60-51-5	GC	<100	<100		>200		200		200	200	100
dimethomorph	110488-70-5	LC	2000	20000			60000					2000
ethoprophos	13194-48-4	LC	<100	<100		>200		200		200	200	100
etofenprox	80844-07-1	LC	<100	<100		>400		400		400	400	100
etoxazole	153233-91-1	LC	100	1500	10	>200	7000	200	10	200	200	10
fenhexamid	126833-17-8	LC	100	10000			30000					100
fenoxycarb	72490-01-8	LC	<100	<100		>200		200		200	200	100
fenpyroximate	134098-61-6	LC	100	2000		>400		400		400	400	100
fipronil	120068-37-3	GC	<100	<100		>400		400		400	400	100
flonicamid	158062-67-0	LC	100	2000		>1000	7000	1000		1000	1000	100
fludioxonil	131341-86-1	GC	100	30000		>400	20	400		400	400	20
hexythiazox	78587-05-0	LC	100	2000		>1000		1000		1000	1000	100
imazalil	35554-44-0	LC	<100	<100	10	>200		200	40	200	200	10
imidacloprid	138261-41-3	LC	5000	3000	10	>400	50	400	20	400	400	10
kresoxim-methyl	143390-89-0		100	1000		>400		400		400	400	100
malathion (USP sums with Malaoxon)	121-75-5	LC	500	5000		>200		200	50	200	200	50
metalaxyl	57837-19-1	GC	2000	15000		>200		200		200	200	200
methiocarb	2032-65-7	LC	<100	<100		>200		200		200	200	100
methomyl	16752-77-5	LC	1000	100		>400		400		400	400	400
methyl parathion	298-00-0	GC	<100	<100		>200		200	0	200	200	100
Mevinphos I & II (each)		GC	<100	<100					0			100
MGK-264 (N-Octyl bicycloheptene dicarboximide)	113-48-4	GC				>200		200		200	200	200

myclobutanil (systhane)	88671-89-0	LC	100	9000	10	>200	4000	200	40	200	200	10
naled	300-76-5	LC	100	500		>500		500		500	500	100
oxamyl	23135-22-0	LC	500	200		>1000		1000		1000	1000	500
paclobutrazol	76738-62-0	LC	<100	<100		>400	50	400		400	400	50
pentachloronitrobenzene (quintozene)	82-68-8	GC	100	200			200					200
permethrins (sum of cis and trans)	52645-53-1	GC	500	20000		>200		200	40	200	200	40
phosmet	732-11-6	GC	100	200		>200		200		200	200	20 / 200
piperonyl butoxide	51-03-6	LC	3000	8000		>2000	10000	2000		2000	2000	1000
prallethrin	23031-36-9	LC	100	400		>200		200		200	200	100
propiconazole	60207-90-1	LC	100	20000		>400		400		400	400	100
propoxur (baygon)	114-26-1	LC	<100	<100		>200		200		200	200	100
pyrethrins (sum of pyrethrin 1, cinerin 1 and jasmolin 1)	8003-34-7	LC	500	1000		>1000	1000	1000		1000	1000	500
pyridaben	96489-71-3	LC	100	3000		>200		200		200	200	100
spinetoram	187166-40-1	LC	100	3000			1700					100
spinosad	168316-95-8	LC	100	3000		>200	10000	200	60	200	200	60
spiromesifen	283594-90-1	LC	100	12000	10	>200		200	30	200	200	10
spirotetramat	203313-25-1	LC	100	13000		>200	10000	200	20	200	200	20
spiroxamine	118134-30-8	LC	<100	<100		>400		400		400	400	100
tebuconazole	80443-41-0	LC	100	2000		>400		400	10	400	400	10
thiacloprid	111988-49-9	LC	<100	<100		>200		200		200	200	100
thiamethoxam	153719-23-4	LC	5000	4500		>200	20	200		200	200	50
trifloxystrobin	141517-21-7	LC	100	30000	10	>200	11000	200		200	200	10
Total # Pesticides			**67**	**67**	**9**	**60**	**24**	**59**	**18**	**59**	**60**	**103**

Appendix A 1. State Tolerance Limit Pesticide Residual Test

Source: USP; MO, CA, MA, CO, AR, WA, OR State legislation.

Appendix B - States Solvent Residue Limit

		Limits (PPM)										
Solvent Residue	**CAS Number**	**CA**	**MA**	**MO Inhalation**	**MO infused**	**NV**	**OR**	**CO**	**AK**	**WA**	**USP**	**Category**
1-Butanol	71-36-3		5000	>2	>5				5000		5000	Class III
2-Butanol	78-92-2		5000				5000		5000		5000	Class III
2-Butanone	78-93-3								5000			
2-Ethoxyethanol	110-80-5		160				160		160		160	Class II
1,1-Dichloroethene	75-34-3										8	Class I
1,2-Dichloroethane	107-06-2	1	1870						100		1870	Class II
2,2-dimethylbutane	75-83-2								290			
2,3-dimethylbutane	79-29-8								290			
1,2-Dimethoxyethane	110-71-4										100	Class II
1, 4- Dioxane	123-91-1		380				380		380		380	Class II
2-Methoxyethanol	109-86-4		50								50	Class II
1,1,1-Trichloroethane	71-55-6										1500	Class I
1,1,2-Trichloroethylene	79-01-6		80									
3-Methyl-1-Butanol	123-51-3										5000	Class III
2 Methyl-1-Propanol	78-83-1		5000								5000	Class III
1-Pentanol	71-41-0		5000						5000		5000	Class III
2-methylbutane	78-78-4								5000			
2-MethylPentane	107-83-5								290			
3-Methylpentane	96-14-0								290			
3-Methyl-1-Butanol	123-51-3		5000									
1-Propanol	71-23-8		5000						5000		5000	Class III
2-Propanol	67-63-0		5000				5000		5000		5000	Class III
Acetic Acid	64-19-7		5000								5000	Class III
Acetone	67-64-1	5000	5000	>750	>5000		5000		5000	5000	5000	Class III
Acetonitrile	75-05-8	410	410	>60	>410		410		410		410	Class II
Anisole	100-66-3		5000								5000	Class III
Benzene	71-43-2	1		>1	>2		2	<1	2	2	2	Class I
Butane	106-97-8	5000		>800	>5000	<500	5000	800	5000	5000		
Butyl Acetate	123-86-4		5000								5000	Class III
Carbon Tetrachloride	56-23-5										4	Class I
Chlorobenzene	109-90-7		360								360	Class II
Chloroform	67-66-3	1	60	>2	>60						60	Class II
Cumene	98-82-8		70				70		70		5000	Class III
Cyclohexane	110-82-7		3380				3880		3880		3880	Class II
Dichloromethane	75-9-2						600		600			
Dimehtyl Sulfoxide	67-68-5		5000						5000		5000	Class III
Ethanol	64-17-5	5000	5000	>1000	>5000				5000		5000	Class III

Ethylene Glycol	107-21-1						620						
Ethyl Acetate	141-78-6	5000	5000	>400	>5000		5000		5000		5000	Class III
Ethyl glycol	107-21-1		620								620	Class II
Ehtyl Ether	60-29-7	5000	5000	>500	>5000		5000		5000		5000	Class III
Ethyl Formate	109-94-9										5000	Class III
Ethylene Oxide	75-21-9	1		>5	>50		50		50			
Formic Acid	64-18-6										5000	Class III
Formamide	75-12-7										220	Class II
Heptane	142-82-5	5000	5000	>500	>5000	<500	5000	<500	5000	5000	5000	Class III
Hexane	110-54-3	290	290	>50	>290		290	<10	290	290	290	Class II
Isopropyl Alcohol	67-63-0	5000		>500	>5000		5000			5000		
Isobutyl Acetate	110-19-0		5000						5000		5000	Class III
Isopropyl Acetate	108-21-4		5000								5000	Class III
N,N-Dimethyacetamide	127-19-5		1090						1090		1090	Class II
N,N-Dimethyformamide	68-12-2		880						880		880	Class II
N-Methylpyrorolidone	872-50-4		530								530	Class II
Nitromethane	75-52-5		50								50	Class II
Methanol	67-56-1	3000	3000				3000		3000	3000	3000	Class II
Methyl Acetate	79-20-9		5000								5000	Class III
Methylpropane	75-28-5								5000			
Methylbutylketone	591-78-6		50								50	Class II
Methylcyclohexane	108-87-2		1180								1180	Class II
Methyl ethyl Ketone	78-93-3		5000									
Methylene Chloride	75-09-02	1		>125	>600						600	Class II
Methylisobutyl Ketone	108-10-1		5000									
Pentane	109-66-0	5000	5000	>750	>5000		5000		5000	5000	5000	Class III
Propane	74-98-6	5000		>2100	>5000	<500	5000			5000		
Propyl Acetate	109-60-4		5000								5000	Class III
Pyridine	110-86-1		200						200		200	Class II
Sulfolane	126-33-0		160						160		160	Class II
Tetralin	119-64-2		100								100	Class II
tert-Butylmethyl Ether	1634-04-4										5000	Class III
Tetrahydrofuran	109-99-9		720				720		720		720	Class II
Trichloroethylene	79-01-6	1		>25	>80						80	Class II
Toluene	108-88-3	890	80	>150	>890		890	<1	890	890	890	Class II
Total Xylenes	1330-20-7	2170	2170	>150	>2170		2170	<1	2170	2170	2170	Class II
# of Solvent Residue	**65**	**20**	**49**	**19**	**19**	**3**	**24**	**6**	**39**	**11**	**56**	

Appendix B 1. Solvent Residue Limit

Appendix C - Biological Contaminants

Class	Pathogen	CA	MA			MO	NV	CO	OR	AK	WA	
			Unprocessed	Processed	CO[2]						Unprocessed	Processed
Mycotoxins (ppm)	Ochratoxin A	<20	<20	<20	≥20	>20	>20	>20			20	
	Aflatoxins	<20					>20	>20			20	
Bacteria (CFU)	E. Coli	>1	>1			>1	ND	>1	100	100	ND	ND
	Salmonella	>1				>1	ND	>1			ND	ND
	Enterobacteria		10^3	10^3	10^2		$>10^3$	>1			10^4	10^3
	Staphylococcus Aureus							>1				
	Pseudomonas Aeruginosa							>1				
Yeast (CFU)	Canadian Albican							>1				
Mold (CFU)	Aspergillus Species											
	A. Flavus	>1				>1	ND					
	A. Niger	>1				>1	ND					
	A. Terreus	>1				>1	ND					
	A Fumigatus	>1				>1	ND					
CFU	Total Yeast and Mold		10^4	10^4	10^3		$>10^4$	$\geq 10^4$				
CFU	Total Aerobic Microbial Count		10^5	10^5	10^4			$\geq 10^2$				
CFU	Total Coliforms		10^3	10^3	10^2				Yes	Yes		
Total		**8**	**5**	**5**	**6**	**8**	**10**	**10**	**1**	**1**	**5**	**5**

Appendix C 1

ND: Not Detected; CFU: Colony forming unit; PPM : Parts per Million

Source: State of CA, MA, MO, NV, CO, AR, AK, and WA

Appendix D - State Metal Testing

Appendix D 1 Limits (PPM)

	CA			MO		MA		MD/NM/NV	CO			AR	WA	USP	
Metal	**Ing.**	**Inh.**	**T.**	**Ing.**	**Inh.**	**Ing.**	**All**	**All**	**Ing.**	**T.**	**Inh.**	**All**	**All**	**Ing.**	**Inh.**
Inorganic Arsenic	1.5	0.2	3	1.5	0.2	1.5	0.2	2	1.5	3	0.2	200	10	1.5	0.2
Cadmium	0.5	0.2		0.5	0.2	0.5	0.2	0.82	0.5	3	0.2	200	4.1	0.5	0.2
Total Chromium				2	0.6									1100	0.3
Lead	0.5	0.5	10	0.5	0.5	1	0.5	1.2	1	10	0.5	500	6	0.5	0.5
Mercury	3	0.1	1	3	0.1	1.5	0.1	0.4	1.5	1	0.1	100	2	3	0.1
Total	4	4	4	5	5	4	4	4	4	4	4	4	4		

Ing.*=Ingestion; Inh* =Inhalation; T. =Transdermal; All*=All use

Source: CA, MO, MA, NV, CO, AR, WA and USP<232>

Appendix E - Water Activity and Moisture Content

Test	CA (Flower) (Aw	CA (Semi Solid Edible)	WA	MO (Dried)	NV	OR	AR
Water Activity	0.65	0.85	0.65	0.65		0.65	0.65
Moisture Content	5-13%		≥15	5-13%	15	15	16

Appendix E 1