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Optimization of Δ^9 -THC Extraction from Cannabis Edibles for GC-MS Analysis for Forensic Casework Application

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ISBN

9798193266341

Author

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Publication Date

2026-09-08

Peer reviewed|Thesis/dissertation

Optimization of Δ^9 -THC Extraction from Cannabis Edibles for GC-MS Analysis for Forensic
Casework Application

By

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THESIS

Submitted in partial satisfaction of the requirements for the degree of

MASTER OF SCIENCE

in

Forensic Science

in the

OFFICE OF GRADUATE STUDIES

of the

UNIVERSITY OF CALIFORNIA

DAVIS

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2026

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ACKNOWLEDGEMENTS

I would like to give the utmost thanks and recognition to my parents, who have supported my academic journey and career aspirations my entire life. It is only with their encouragement and support that I am able to complete this degree.

I would like to thank the Sacramento District Attorney's Office Laboratory of Forensic Services for welcoming me into their laboratory and providing me with the necessary means and materials to complete this project. I would especially like to thank the criminalists in the Controlled Substances unit, as well as unit supervisor Jonathan Charron, for their mentorship as I conducted my research. I am also grateful to Dr. Ashley Hall and my committee members, Dr. Donald Land, Dr. Benjamin Moeller, and Dr. Cristina Davis, for their feedback and guidance throughout this project.

ABSTRACT

Following the specified legalization of cannabis products in California, the cannabis industry has quickly grown as new products enter the market and offer various ways for consumers to experience the psychoactive effects of cannabinoids. Edible cannabis products, in particular, have become increasingly popular as they are easily accessible, discreet, and produce a longer-lasting psychoactive effect than smoking cannabis. The Sacramento County District Attorney's Office Laboratory of Forensic Services does not currently have a validated method for analyzing cannabis edible evidence. The laboratory hopes to add a method for extracting Δ^9 -THC from edibles to its Controlled Substances Unit's (CSU) technical manual. For this study, pre-existing Δ^9 -THC extraction methods from external sources were compiled and evaluated for their effectiveness, extraction cleanliness, and compatibility with the materials available in the CSU. Five procedures were gathered, including a QuEChERS-based extraction, hexane extraction, ethanol extraction, sonication-assisted extraction, and basic/acidic extraction. After initial testing, the basic/acidic extraction produced the greatest extraction cleanliness while being mostly compatible with the available materials. The original method was modified through experimentation with steps involving the base used, number of washes performed, and amount of starting material used. The optimized method was then used to analyze five unknown edibles, and Δ^9 -THC was successfully detected and identified in all five samples. Overall, this project aimed to develop a robust Δ^9 -THC extraction method for GC-MS analysis that would utilize the instrumentation, chemical reagents, and solvents readily available in the CSU, increase laboratory testing capabilities, and be reliably applied to casework samples.

CHAPTER 1. BACKGROUND

Cannabis and Edibles

Cannabis sativa (commonly known as cannabis) is a plant containing many chemical substances, including hundreds of cannabinoids.¹ The primary psychoactive cannabinoid in cannabis is delta-9-tetrahydrocannabinol (Δ^9 -THC), a Schedule I controlled substance that is constantly relevant in discussions of government regulation and recreational use advocacy.^{2,3} Schedule I drugs are those with a high potential for abuse without having any approved medical applications.⁴ Cannabis can be legally placed into one of two categories: marijuana and hemp.

Marijuana, defined as cannabis containing 0.3% or more Δ^9 -THC by dry weight,⁵ is federally illegal but has been legalized in many states for medical or recreational purposes.³ While marijuana is broadly considered a Schedule I controlled substance, the 2025 Executive Order on Increasing Medical Marijuana and Cannabidiol Research prompted new scheduling and regulation for marijuana products.⁶ In April 2026, the Drug Enforcement Administration “[placed] both FDA-approved products containing marijuana and marijuana products regulated by a state medical marijuana license in Schedule III of the Controlled Substances Act.”⁶ Further administrative action is being pursued to reschedule marijuana from Schedule I to Schedule III entirely.⁶ In California, the specified use of marijuana products has been legal for adults 21 years or older since the passing of Proposition 64: The Adult Use of Marijuana Act in 2016.⁷

Hemp, a non-controlled substance defined as cannabis containing less than 0.3% Δ^9 -THC by dry weight, was federally legalized by the Agricultural Improvement Act of 2018 (commonly known as the 2018 Farm Bill).² However, new legislation regarding the legal definition of hemp will be implemented in November 2026 to address loopholes identified in the 2018 Farm Bill. Hemp will be newly defined as cannabis containing less than 0.3% total tetrahydrocannabinol

(THC) by dry weight, including all cannabinoids, acids, derivatives, and isomers.⁸ There will also be a limit of 0.4mg total THC per product container, along with “any other cannabinoids that have similar effects (or are marketed to have similar effects) on humans or animals as a tetrahydrocannabinol.”⁸ Despite the impending legislation, the specified manufacturing and distribution of products in both categories of cannabis will remain legal in California.

Cannabis edibles have become increasingly popular due to their discreet nature, the longer-lasting effects they offer compared to smoked cannabis,^{9,10} and the continuous development of creative formulations from manufacturers and consumers alike. As laws and regulations for cannabis products continue to change, there is a need for forensic laboratories to implement testing methods for various product matrices. However, edible testing can be difficult, as their ingredients can complicate sample preparation and cannabinoid extraction. If either of these steps is poor or incomplete, subsequent issues can arise during instrumental analysis.¹¹

Edibles come in many forms, from beverages to candies to baked goods, most of which contain complex combinations of ingredients. This project focused on extracting Δ^9 -THC from gummy matrices. While there are many gummy formulations, most present similar analytical issues of being sticky and difficult to break down,¹¹ as well as containing flavoring and fragrance ingredients that are challenging to filter out during extraction. Gummies also contain gelling agents, such as gelatin or agar, that contribute to their texture and can complicate the breakdown of certain matrices, negatively impacting the effectiveness of an extraction.¹¹ Often, heat and water are necessary to initially dissolve a gummy and break down the matrix to access the individual components inside.¹¹

An important consideration for edible analysis is that Δ^9 -THC is nonpolar and largely insoluble in water,¹² a significant component in many edibles. The hydrophobic nature of Δ^9 -

THC prevents it from properly mixing into water-containing matrices, often causing nonuniform distribution within an edible and inconsistency between product batches.¹⁰ Thus, different samples taken from even the same edible can have widely varying amounts of Δ^9 -THC.

Δ^9 -THC Extraction Analytical Methods

Δ^9 -THC extraction methods for edibles often consist of two main steps: sample preparation and solvent extraction. Cannabis edibles typically have solid matrices that need to be broken down before chemical reagents and solvents are introduced. An ideal method for this project would also contain thorough clean-up measures, as extraction cleanliness is important for instrument maintenance. Liquid or gas chromatography are the standard analytical methods for edibles, and running insufficiently clean extracts through chromatography columns can lead to issues such as product build-up and column degradation. Over time, this can result in greater costs for instrument repair and maintenance, which laboratories aim to avoid.

Instrumental analysis for Δ^9 -THC sample extracts from cannabis edibles is often conducted with liquid chromatography (LC) methods such as high-performance LC coupled with a diode array detector (HPLC-DAD) or LC coupled with mass spectrometry (LC-MS).¹³ LC is the preferred technique when analyzing cannabis products, as it allows for direct quantification of Δ^9 -THC separate from Δ^9 -tetrahydrocannabinolic acid (Δ^9 -THCA).¹⁴ Δ^9 -THCA is the non-psychoactive acid precursor to Δ^9 -THC,¹⁴ which decarboxylates to Δ^9 -THC slowly during fermentation or long-term storage.¹⁵ This decarboxylation can also occur rapidly upon extreme heating,¹⁵ as shown in Figure 1.

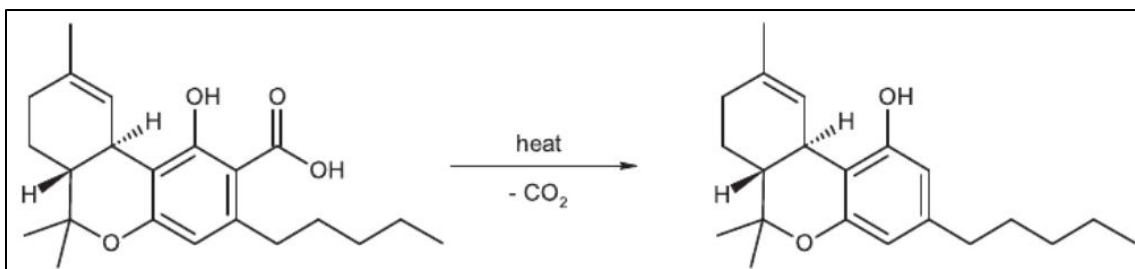


Figure 1. Decarboxylation Reaction of Δ^9 -THCA to Δ^9 -THC.¹⁶

Edible ingestion does not involve fermentation or sufficient heat to cause Δ^9 -THCA decarboxylation to Δ^9 -THC, causing any Δ^9 -THCA in edibles to remain non-psychoactive upon consumption. However, gas chromatography (GC) applies extreme heat to samples, which does cause Δ^9 -THCA to decarboxylate to Δ^9 -THC. Therefore, GC analysis of samples containing both Δ^9 -THCA and Δ^9 -THC makes the two indistinguishable and ultimately causes the Δ^9 -THC content to appear artificially high.¹⁴ As previously noted, new legislation will take effect in November 2026 that will change the definition of hemp to be based on total THC content instead of solely Δ^9 -THC content.⁸ At the time this project was conducted, however, differentiation between Δ^9 -THC and Δ^9 -THCA was still relevant for cannabis product analysis. Under current legislation, testing laboratories that use GC instrumentation can perform a lengthy derivatization to allow separate quantification of Δ^9 -THCA and Δ^9 -THC,¹⁴ but this procedure was unnecessary for the scope of this project.

When analyzing edibles, the Controlled Substances Unit (CSU) at the Sacramento County District Attorney's Office Laboratory of Forensic Services (Sacramento DA Crime Laboratory) aims to detect Δ^9 -THC, not quantify the amount present in a sample. As edibles are legal in the state of California, so long as they are manufactured and sold under legal parameters, the laboratory does not analyze edibles often. However, there are scenarios where cannabis edibles are illegal, such as juvenile use, inmate use, sales, and driving under the influence of

drugs. In these cases, Δ^9 -THC detection is sufficient to determine illegal possession or use of cannabis. As the quantification of Δ^9 -THC in edibles is unnecessary, GC-based instrumentation can be applied for analysis following extraction, without the need for differentiation between Δ^9 -THCA and Δ^9 -THC. Additionally, an important factor in this project is that GC-MS is the most readily available and preferred instrumentation in the CSU. While other instrumentation is available in other units of the laboratory, the CSU desires a method that can be completed entirely within the capabilities of their unit. For these reasons, this project aims to develop an extraction method that is suitable for GC-MS analysis.

Previously, the CSU developed a QuEChERS-based extraction that they hoped to add to their technical manual. However, while the method successfully extracted Δ^9 -THC from edibles, it produced insufficiently clean extracts, required additional material costs (QuEChERS packets and syringe filtration components), and required a high-powered centrifuge. The CSU only has Clay Adams Compact II Centrifuges, which do not have sufficient power or test tube capacity for this extraction, so analysts must go between units in the laboratory to perform this method. Analysts are not automatically granted cross-unit access throughout the laboratory, so movement between units is not ideal. After considering the results and disadvantages, the CSU decided not to implement the QuEChERS-based method into their technical procedures.

Research Objectives and Aims

The objective of this research was to develop a robust Δ^9 -THC extraction method for further GC-MS analysis for the CSU at the Sacramento DA Crime Laboratory. Ideally, this method would utilize the instrumentation, chemical reagents, and solvents readily available in

the unit and be reliably applicable to casework samples, such that CSU analysts can be confident that Δ^9 -THC content in an edible sample will be successfully extracted with this method.

This project consisted of three aims. Aim 1 was to research and analyze Δ^9 -THC extraction methods from external sources, including those from literature or used by cannabis testing laboratories or crime laboratories. All compiled methods were performed in-house with known edible samples from state-licensed dispensaries, and then compared for efficiency and consistency. Aim 2 was to modify and optimize the most efficient and consistent method tested from Aim 1. Aim 3 was to test unknown samples from the Sacramento DA Crime Laboratory and anonymous donors with the optimized extraction method. The findings from this project will provide the Sacramento DA Crime Laboratory CSU with a method for cannabis edible analysis, contribute to improved extraction methods for edible matrices in the forensic science community, and may help inform similar protocols for external laboratories as the need to test edible samples increases.

CHAPTER 2. METHODS AND MATERIALS

Compiling Pre-Existing Methods

The first aim of this project was to research pre-existing methods for Δ^9 -THC extraction from cannabis edibles that allow for analysis by GC-MS. Pre-existing methods offer a foundation to build from, as well as insight into adjustments to methods that have been previously tested or suggested for future experiments.

The first method tested was the previously described QuEChERS-based extraction that was developed in the CSU, which required the following steps (abbreviated):¹⁷

1. Add 1g of finely chopped gummy to 5mL of warm water, and vortex until dissolved
2. Add 1mL of desired solvent (acetone or acetonitrile), 5-7 drops of internal standard, and a QuEChERS packet
3. Vortex for 10-20 seconds, then centrifuge at 4000rpm for 5min
4. Use syringe filtration to extract the organic layer

The QuEChERS-based method calls for either acetone or acetonitrile as the extraction solvent. Acetone was chosen for this method as it is readily available in the CSU, while acetonitrile is not. The internal standard used for this method was methapyrilene, for which the reasoning is described in the *Required Materials* section. For filtration, a 0.2 μ m PTFE syringe filter was used. The researchers who originally developed this method selected these filters, and there was a remaining supply of them available for this project.

The second method was a hexane extraction described in the article “GC–MS as a valuable tool for analysing cannabinoid-containing gummies and identifying the synthetic process used for their production” by Bini et al. (2025), which consists of the following procedure (abbreviated into numerical steps):¹⁸

1. Dissolve approximately 250mg of a gummy in 30.0mL of warm water
2. Extract with three 10.0mL aliquots of hexane
3. Pool the organic extracts together, filter, and analyze

While Bini et al. (2025) used 250mg of gummy sample in their extractions, 1g samples were used for this study to allow for easier comparison of tested methods. The filtration in Step 3 was not described for this method, so filtration was done with 0.2 μ m PTFE syringe filters.

The third method was an ethanol extraction, also from the article by Bini et al. (2025), which called for the following procedure (abbreviated into numerical steps):¹⁸

1. Dissolve approximately 250mg of a gummy in 2.0mL of warm water
2. Add 30.0mL of ethanol in portions while stirring to precipitate sugar/matrix components
3. Filter 1.0mL of the obtained suspension over a 0.45 μ m Nylon syringe filter, then analyze the filtered solution

As with the hexane extraction, Bini et al. (2025) used 250mg of gummy sample in their extractions, but 1g samples were used in this study for easier method comparison. While not explicitly included in the method, centrifugation was performed to better separate the suspension from the precipitated sugars. Due to the volume of ethanol used and the resultant test tube size required, centrifugation could not be done in the CSU and was instead done in a different unit. Additionally, the CSU did not have or desire to purchase the specific 0.45 μ m Nylon syringe filters for this extraction. Thus, filtration here was also done with 0.2 μ m PTFE syringe filters.

The fourth method was a sonication-assisted extraction described in the poster presentation “Cannabis Analysis I: What’s in a Cannabis Gummy Bear?” by Delacruz et al. (2022), which calls for the following steps (abbreviated into numerical steps):¹⁹

1. Dissolve one gummy (approximately 3g) in 9.0mL of water (approximately three times the weight of the gummy) in a sonicating bath for 80min (heated to 65°C)
2. Extract a 1.0mL aliquot of the resulting solution with 2.0mL of hexane (or ethyl acetate), then analyze

Similar to the previous two extraction methods, instead of using 3g of sample (with 9mL of water), 1g samples (with 3mL of water) were used when testing this method for easier comparison. Additionally, while the method calls for heating at 65°C, sonication was done at 60°C, which was the maximum temperature of the sonicator available in the CSU.

The fifth method was a basic/acidic extraction obtained from the Kern Regional Crime Laboratory's technical manual, which involves the following steps (abbreviated):

1. Add hexane to the sample, then vortex and centrifuge
2. Transfer hexane to a new tube, then extract with 0.5N methanolic KOH
3. Wash with at least 3 aliquots of hexane
4. Acidify with 1N HCl, extract with hexane, and dry the extract with sodium sulfate
5. Retain hexane and concentrate through evaporation, then analyze

The basic/acidic extraction did not include a sample preparation step, so Step 1 from the QuEChERS-based extraction was followed for the initial dissolution when testing this method.

In addition to the described methods, a pyrolysis GC-MS (Py/GC-MS) method from the article "Direct analysis of THC containing edibles using Py-GC/MS" by Almadani et al. (2025) was considered. Py/GC-MS is becoming a more common method for edible analysis as it offers many advantages compared to standard GC-MS. While analysis with standard GC-MS requires sample preparation and extraction, Py/GC-MS only requires sample homogenization before

direct injection into the instrument.²⁰ The Sacramento DA Crime Laboratory has a Py/GC-MS instrument, but it is not in the CSU. Additionally, while the lack of required sample preparation allows Py/GC-MS analysis to be faster than standard GC-MS, it also results in unprocessed samples being introduced to the instrument. Directly injecting such samples can cause contamination or build-up in the GC column and produce chromatograms with high background noise and miscellaneous peaks, potentially leading to analytical interference. Thus, while Py/GC-MS analysis was considered, it was ultimately not utilized in this project.

Required Materials

For the five pre-existing extraction methods gathered for this project, the required materials for each original method are listed in Table 1.

Table 1. Required Materials for Collected Extraction Methods.

Extraction Method	Instrumentation and Materials	Chemical Reagents and Solvents
Modified QuEChERS	Hot plate, QuEChERS salt packet, 4000rpm centrifuge, syringe filter	H ₂ O, acetone, methapyrilene internal standard
Hexane	Hot plate, filter	H ₂ O, hexane, methapyrilene external standard
Ethanol	Hot plate, syringe filter, 4000rpm centrifuge	H ₂ O, ethanol, methapyrilene external standard
Sonication-Assisted	Sonicator, centrifuge	H ₂ O, hexane, methapyrilene external standard
Basic/Acidic	Hot plate, centrifuge	H ₂ O, hexane, 0.5N methanolic KOH, 1N HCl, sodium sulfate, methapyrilene external standard

All samples tested during this study were analyzed using an Agilent Technologies 8890 GC System coupled to a 5977C Series GC/MSD with a 7693A Autosampler. The CSU has many analytical methods programmed into its GC-MS instruments, and the methods used during this project were the Drug method and Drug2X method. The Drug and Drug2X methods only differ in split ratio, with the Drug2X method delivering more of the sample into the column than the Drug method. The parameters for both methods are displayed in Table 2.

Table 2. Parameters for GC-MS Methods.2

Method	Initial Temperature (°C)	Final Temperature (°C)	Ramp Rate (°C/min)	Run Time (min)	Injection Volume (µL)	Flow Rate (mL/min)	Split Ratio
Drug	90	300	60	10	0.2	1.0	20:1
Drug2X	90	300	60	10	0.2	1.0	10:1

Throughout this project, all sample extracts were run on the Drug method. The Drug2X method was only used during unknown testing if an unknown sample extract contained too little Δ^9 -THC to be properly detected and identified with the Drug method. The Drug method (20:1 split ratio) vents more of the sample as waste and reduces the chance of overloading the column, while the Drug2X method (10:1 split ratio) sends more of the sample into the column and improves analyte detection of low-concentration samples.

The Drug and Drug2X methods are programmed to look for methapyrilene as the standard and require that methapyrilene act as a point of comparison for calculating relative retention times for detected analytes. If methapyrilene is not detected in a sample, retention times are not reported and relative retention times are not calculated for any analytes, which hinders analysis as both help confirm library matches. With the parameters of the Drug and Drug2X

methods, methapyrilene has a retention time of approximately 5.45 minutes, which is near the middle of the GC-MS run (10 minutes). Very few drugs elute from the column at a similar time, with some exceptions being psilocin and xylazine. Additionally, methapyrilene is not often seen in the evidence samples that the CSU receives, and it has a unique MS fragmentation pattern compared to most drugs analyzed in the laboratory.

Extraction methods often use surrogate chemical standards, which behave similarly to the analyte of interest and are added in a known amount before sample processing.²¹ While a surrogate like deuterated Δ^9 -THC would be preferable for a Δ^9 -THC extraction, the use of methapyrilene in this study allows the CSU's GC-MS methods to work properly. Additionally, by using methapyrilene, this project applies available materials and does not require the CSU to purchase a new standard that would only be used for this method. For methods tested during Aim 1 that did not specify the use of a chemical standard, a solution of methapyrilene in toluene was added as an external standard during the final step before sample analysis.

All initial and optimization testing were completed with known edibles purchased from Mainstage in Davis, CA, a state-licensed dispensary (Table 3). Initial method testing was done exclusively with KLRB samples, and optimization testing was done with all three sample types.

Table 3. Description of Known Edible Samples.

Abbreviation	Edible Sample Details
KLRB	KANHA Live Rosin Belts – Cherry Limeade ○ 25mg Δ^9 -THC per belt
KLFWC	KIVA Lost Farms Chews – Watermelon ○ 10mg Δ^9 -THC per chew
KPLG	KANHA Classic Gummies – Pink Lemonade ○ 10mg Δ^9 -THC per gummy

CHAPTER 3. INITIAL METHOD TESTING

QuEChERS-Based Extraction

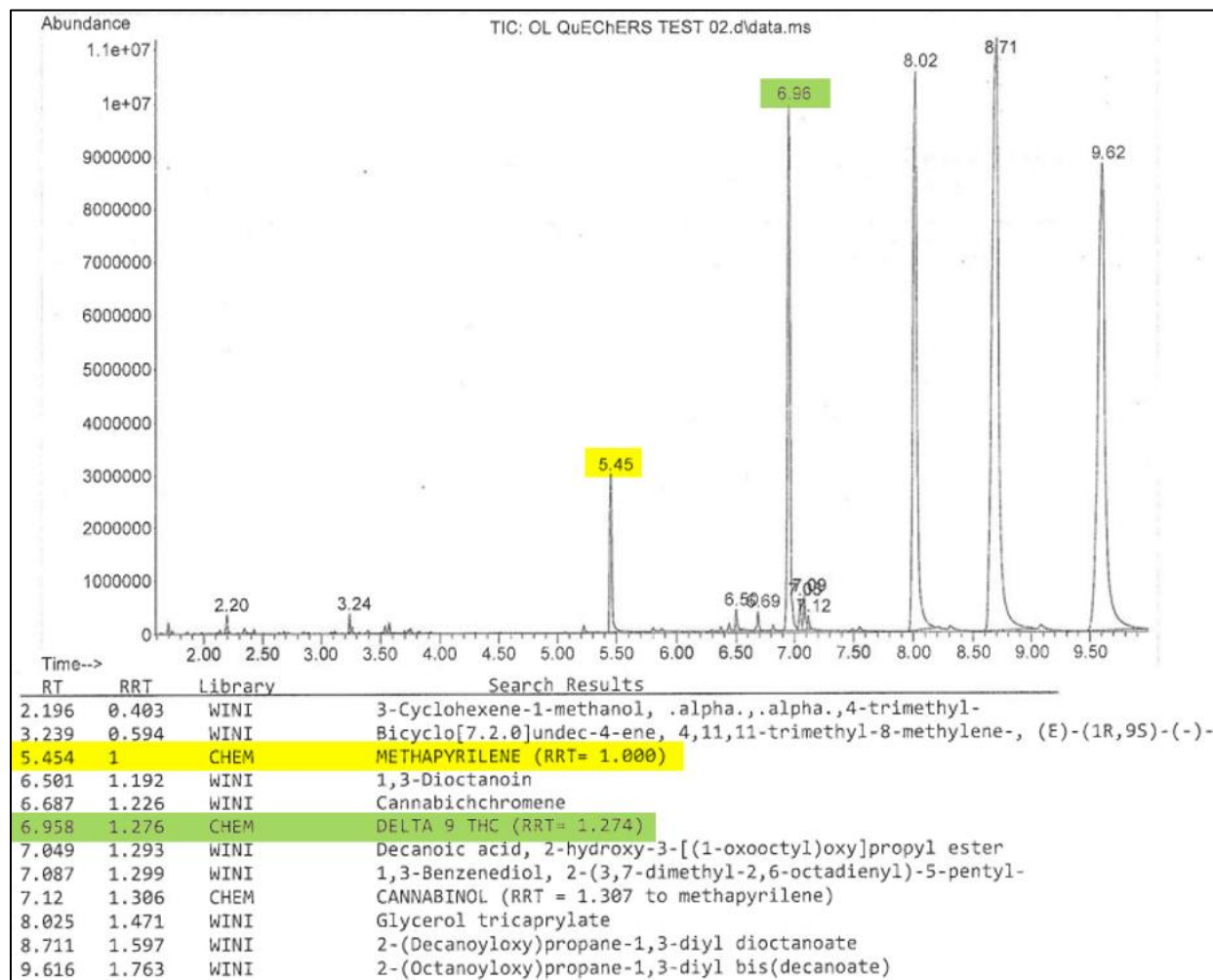


Figure 2. Total Ion Chromatogram of QuEChERS-Based Extraction Sample Extract.

Although the CSU wanted to pivot from the QuEChERS-based extraction, it was tested again in this project for comparison against the other methods. QuEChERS (an acronym that stands for Quick, Easy, Cheap, Effective, Rugged, and Safe) offers many advantages by being relatively quick and user-friendly, but also presents major disadvantages of having insufficient clean-up, requiring additional material costs, and requiring a powerful centrifuge. Another observation about this method is that the syringe filtration could be improved, as it was quite

difficult to filter the organic extract. The syringe material or pore size may not be ideal for this extraction, but other filtration options were not explored for this project.

Hexane Extraction

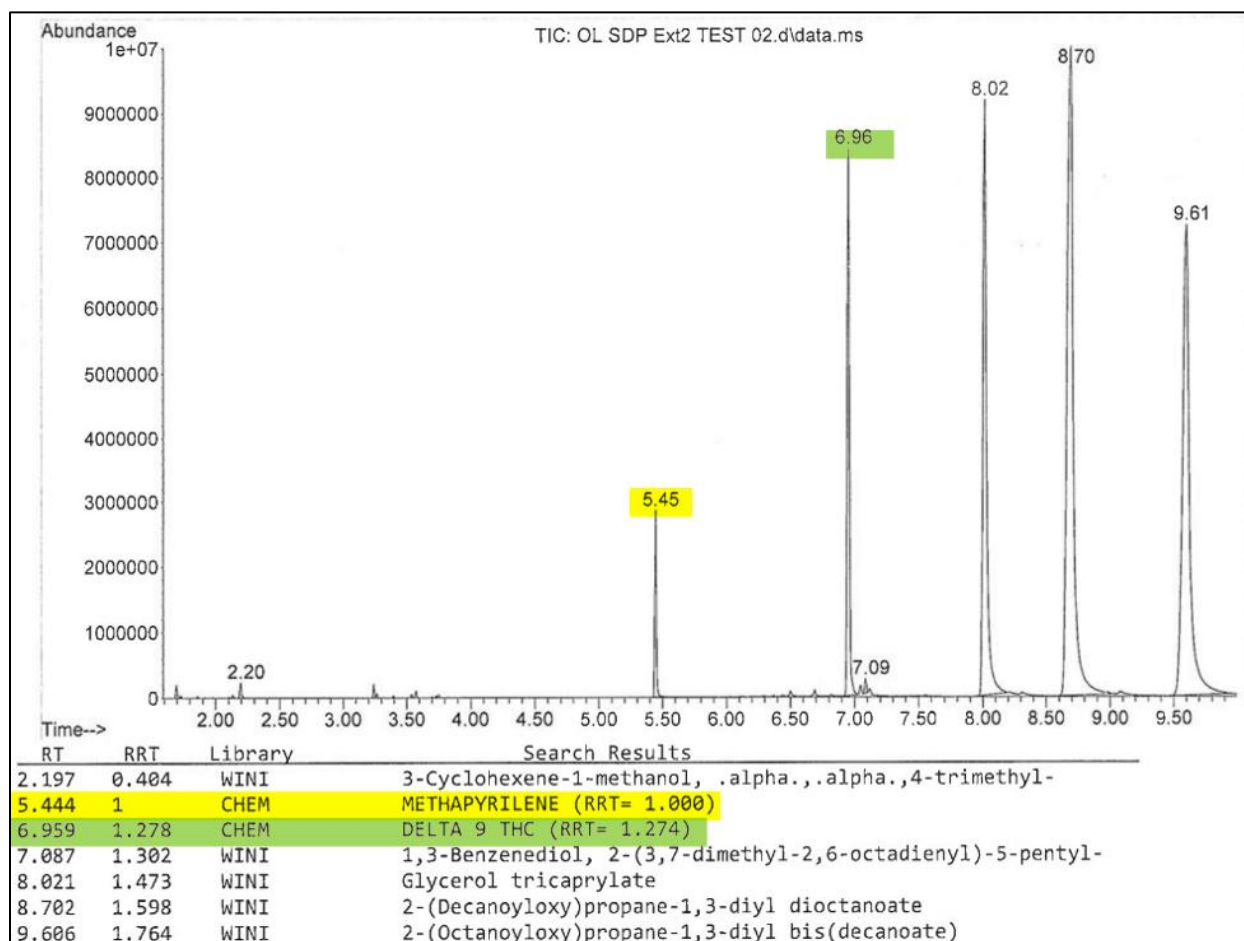


Figure 3. Total Ion Chromatogram of Hexane Extraction Sample Extract.

The hexane extraction (Figure 3) produced similar results to the QuEChERS extraction (Figure 2) and demonstrated comparable advantages and disadvantages. This extraction is quick and requires few steps but also produces insufficiently clean extracts, requires additional material costs, and calls for more solvent than the CSU would like to use for a single extraction.

Ethanol Extraction

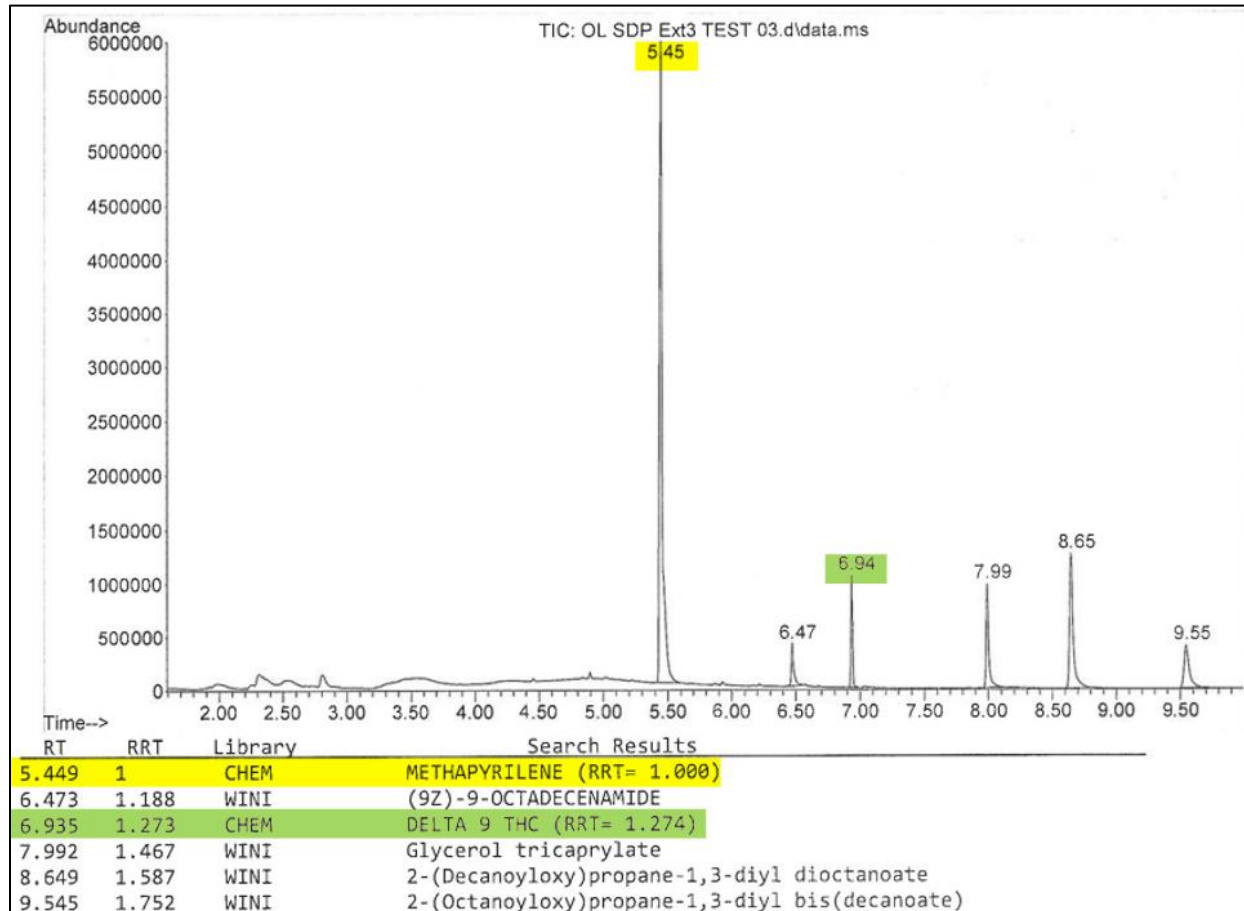


Figure 4. Total Ion Chromatogram of Ethanol Extraction Sample Extract.

The ethanol extraction is another quick method with few steps, but it produced unclear extracts similar to the previous methods. This method shares the same disadvantages as the hexane extraction, requiring additional materials for filtration and using a large volume of solvent. Compared to the QuEChERS (Figure 2) and hexane (Figure 3) extractions, the ethanol extraction produces greater noise along the baseline (Figure 4), suggesting greater presence of co-extracted matrix components. An important note is that the ethanol extraction calls for filtration with 0.45µm Nylon syringe filters, but 0.2µm PTFE syringe filters were used during this project as they were available in the CSU. The change to the filtration method may have

influenced the extraction results, but no additional testing was conducted during this project to determine the potential impact of using different filters.

Sonication-Assisted Extraction

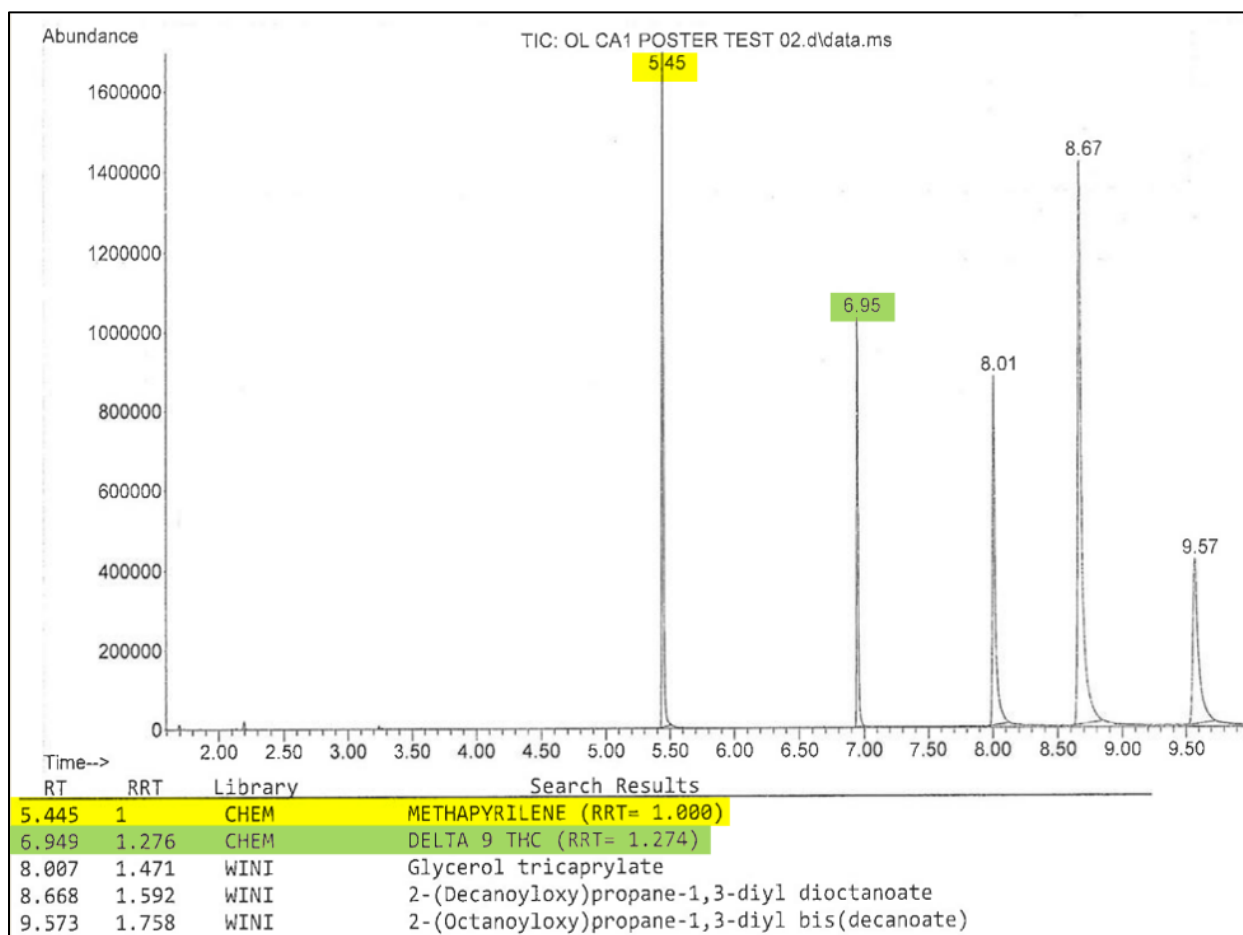


Figure 5. Total Ion Chromatogram of Sonication-Assisted Extraction Sample Extract.

The sonication-assisted extraction (Figure 5) produces very similar results to the hexane extraction (Figure 3), which was anticipated due to both methods using hexane as the solvent. An advantage of the sonication-assisted extraction over the hexane extraction is that it requires much less hexane. While the sonication system is quick and easy to set up, a major disadvantage of this

method is the lengthy sonication period of 80 minutes. However, it is a passive procedure that allows analysts to step away or complete other tasks as they wait.

Basic/Acidic Extraction

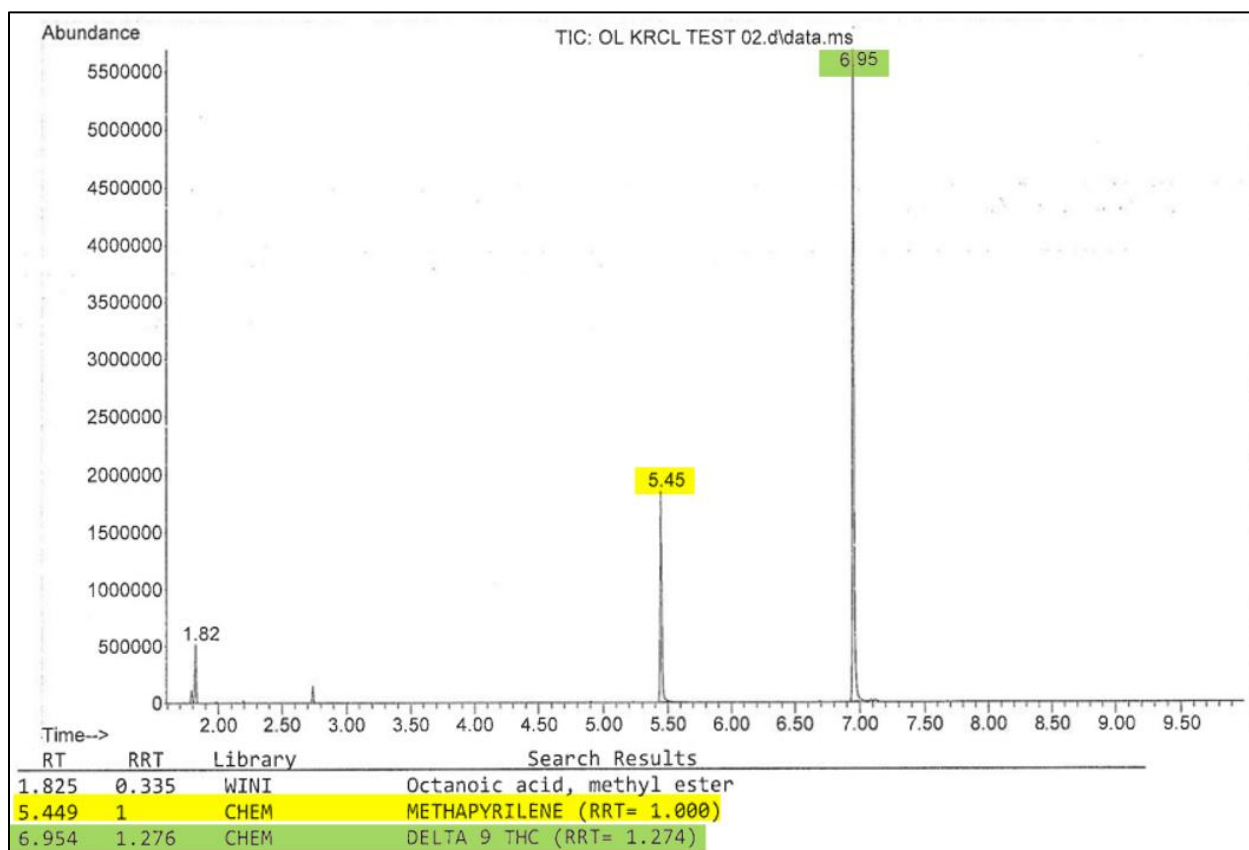


Figure 6. Total Ion Chromatogram of Basic/Acidic Extraction Sample Extract.

The basic/acidic extraction included multiple clean-up steps and produced the cleanest extracts of the tested methods (Figure 6). Compared to the other tested methods, the basic/acidic extraction results in fewer co-extracted compounds, which are at a lower relative abundance compared to Δ^9 -THC. However, it requires many steps, is time-consuming, and calls for methanolic KOH as the base, which the CSU does not have readily available.

Initial Method Testing Conclusions

After testing the compiled methods, observations about the extraction procedures themselves were considered along with the analytical results. The main factors considered when comparing the methods were whether the method required the purchase of additional materials, if the method could be completed entirely in the CSU, and how clean the sample extracts were.

Table 3. Initial Methods Comparison.

Extraction Method	Requires Additional Material Costs	Can be Completed Entirely in CSU	Cleanliness Ranking
QuEChERS	Yes	No	4
Hexane	Yes	Yes	3
Ethanol	Yes	No	5
Sonication-Assisted	No	Yes	2
<u>Basic/Acidic</u>	No	Yes	1

After comparing the methods (Table 3), the basic/acidic method was selected to move forward with for optimization, as it met all goals the CSU had for this method. While the methanolic KOH for the basic/acidic extraction is not readily available in the laboratory, the CSU possesses the materials to make batches as needed.

CHAPTER 4. METHOD MODIFICATIONS

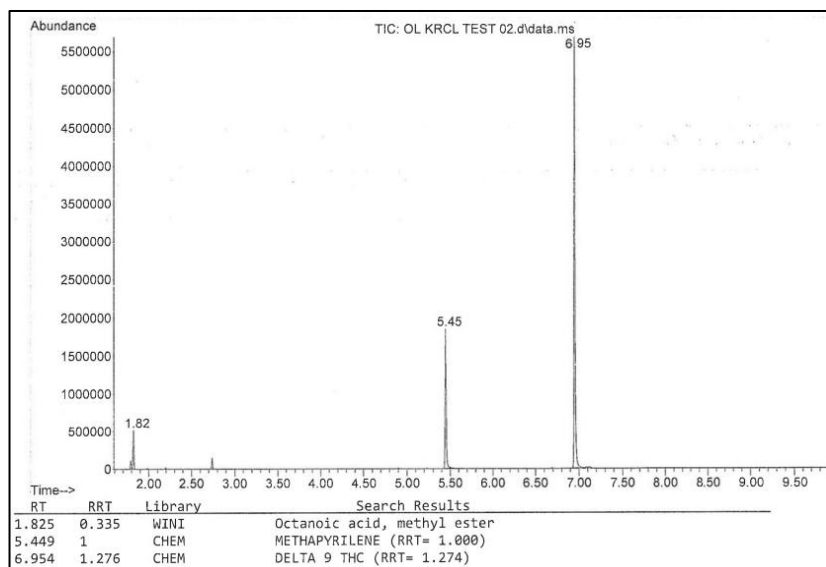
The original basic/acidic method from the Kern Regional Crime Laboratory is as follows, verbatim from the laboratory's manual.²²

“Marijuana products (i.e., edibles);

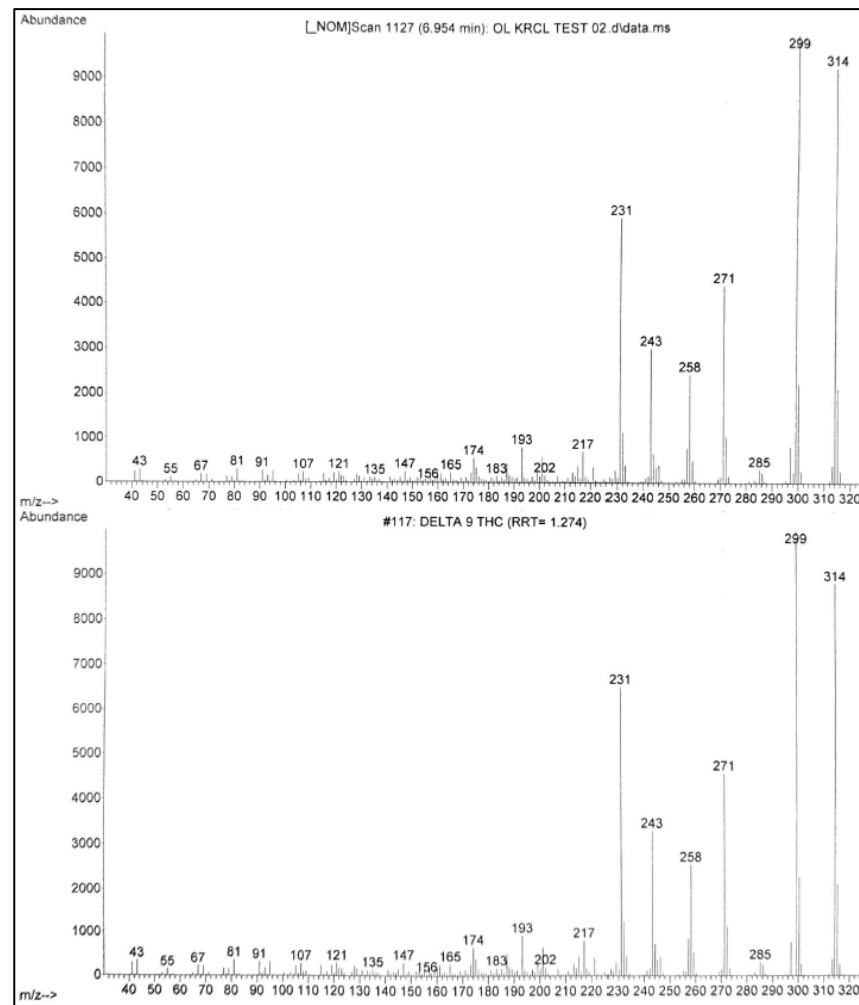
a. Basic/Acidic extraction (petroleum ether can be used instead of hexane);

- Add hexane to suitable quantity of sample;
- Vortex and centrifuge;
- Transfer hexane to new test tube
- Extract with 0.5N KOH (methanolic solution). The bottom layer retains THC if present;
- Discard top hexane layer;
- Wash with at least 3 aliquots of hexane;
- Acidify using 1N HCl;
- Extract with hexane (top layer retains THC if present);
- Dry hexane extract with sodium sulfate;
- Remove and retain hexane;
- Concentrate hexane through evaporation; and
- Resultant concentrate will yield THC if present”

The original method produces a mostly clean total ion chromatogram (TIC) with a Δ^9 -THC peak whose mass spectrum (MS) is consistent with the reference standard, allowing for confident identification of Δ^9 -THC (Figure 7). The GC-MS instruments in the CSU directly compare the MS of an integrated analyte peak to that of the best library match, displaying the sample analyte MS above the reference standard MS (Figure 7b).



(a)



(b)

Figure 7. (a) Total Ion Chromatogram and (b) Mass Spectrum for Original Basic/Acidic Extraction Sample Extract.

Base Modification

The first modification attempted was the substitution of methanolic KOH, as it is not otherwise used or readily available in the CSU. While the CSU has the necessary materials to make methanolic KOH, analysts would ideally use reagents that are already available at their workspaces. Aqueous NaOH is commonly used in the CSU and is readily available at 0.5N, which is the same concentration that the method specifies for the base. Thus, the basic/acidic extraction was tested using 0.5N aqueous NaOH instead of 0.5N methanolic KOH.

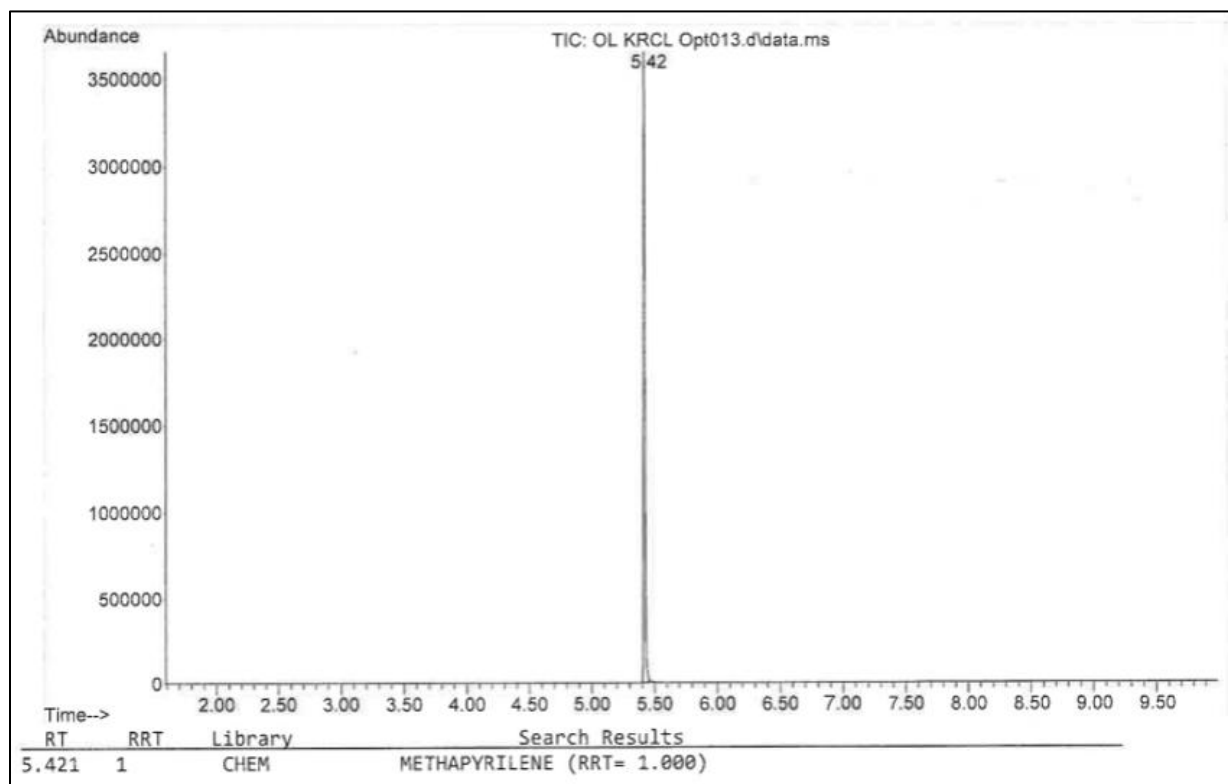


Figure 8. Total Ion Chromatogram of Basic/Acidic Extraction with 0.5N Aqueous NaOH.

The base substitution modification was unsuccessful, as methapyrilene was the only integrated compound in the TIC, and no Δ^9 -THC was detected (Figure 8). The absence of Δ^9 -

THC in the final sample extract is likely due to its poor solubility in aqueous solutions.¹² In the original method, after adding the base, the hexane layer is discarded while the base layer is retained. When using methanolic KOH to basify, Δ^9 -THC partitions into the base layer and is retained. However, when using aqueous NaOH to basify, the nonpolar nature of Δ^9 -THC caused it to preferentially partition into the hexane layer instead of the aqueous base layer. Thus, the Δ^9 -THC was discarded along with the hexane layer, and no Δ^9 -THC was detected at the end of the extraction. As a result, aqueous NaOH was not found to be a viable substitute for methanolic KOH in this method, and the original 0.5N methanolic KOH base was used for all further extractions.

Wash Modification

The next modification attempted was adjusting the number of washes performed. The original method calls for at least three washes with hexane, but there was concern about the potential negative effects of performing multiple washes. While more washing is beneficial for removing more impurities from a sample, it is likely to cause some loss of the target analyte. To determine the effect of the number of washes on the extraction cleanliness and Δ^9 -THC peak area, extractions were performed with three washes (Figure 9a), two washes (Figure 9b), and one wash (Figure 9c).

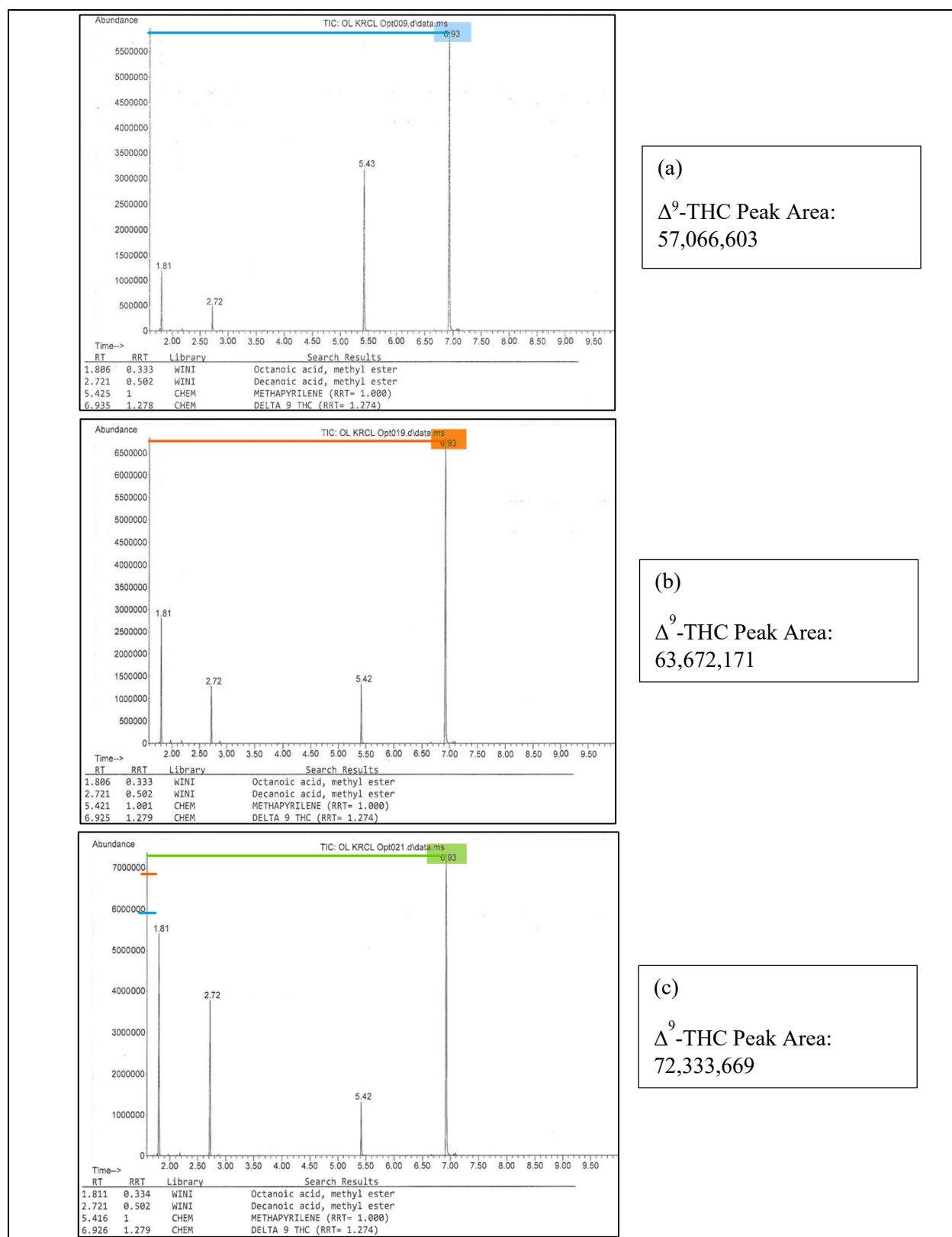
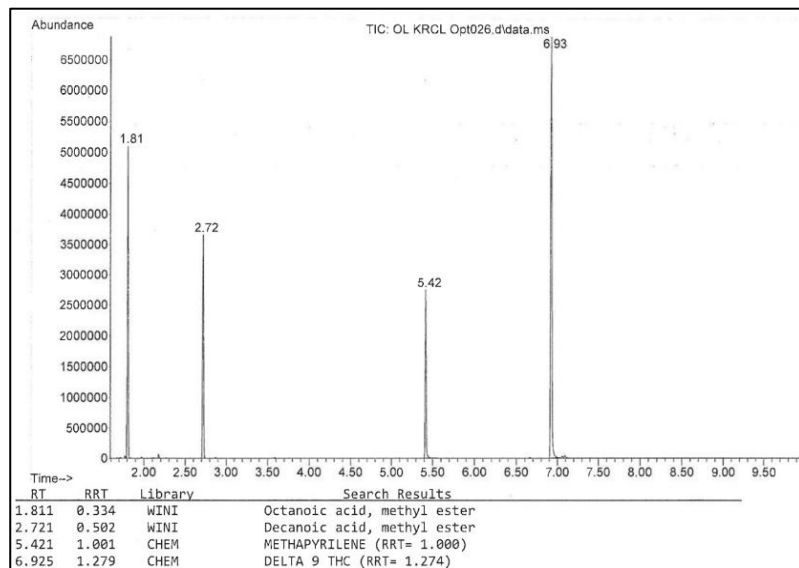


Figure 9. Total Ion Chromatograms and Δ^9 -THC Peak Areas for Basic/Acidic Extractions Performed with (a) Three Washes, (b) Two Washes, and (c) One Wash.

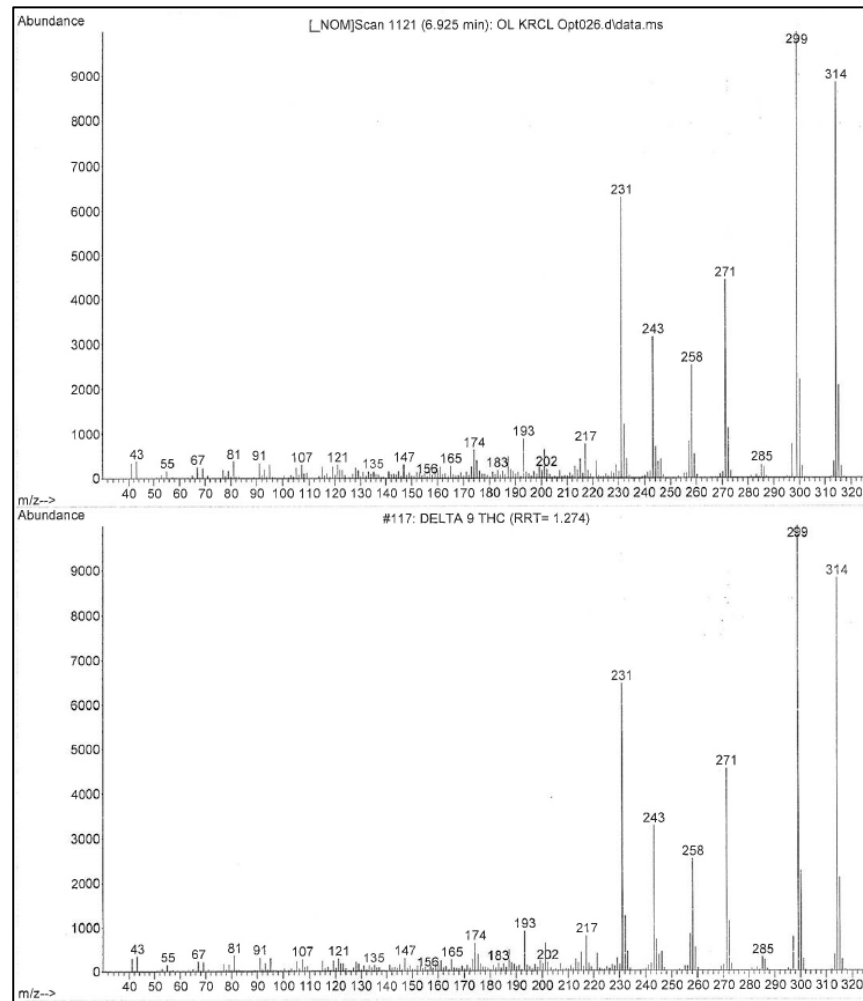
The same contaminants were present in the final extracts of all three samples (Figure 9). Increasing the number of washes reduced the peak areas of the contaminants but did not fully remove any from the final extract. Simultaneously, increasing the number of washes also caused the Δ^9 -THC peak area to decrease. As the number of washes increased, the Δ^9 -THC peak area decreased from 72,333,669 after one wash (Figure 9c) to 63,672,171 after two washes (Figure 9b) to 57,066,603 after three washes (Figure 9a). All TICs in Figure 9 represent extractions of 1g KLRB samples, which have a Δ^9 -THC concentration of 25mg per belt. With such concentrated samples, there was no concern that the additional washes would cause enough Δ^9 -THC loss to prevent detection. However, the final method is intended for casework samples, for which the Δ^9 -THC concentration and sample amount are variable and unpredictable. Some casework samples may be highly concentrated, while others have low or trace concentrations of Δ^9 -THC. It is also possible that a casework edible is too small to take a 1g sample from for analysis. In anticipation of scenarios where the Δ^9 -THC concentration is low or where the sample is very small, it was decided that the final method would only require one wash, which would produce sufficient cleanliness while preserving Δ^9 -THC content.

Starting Material Trials

Finally, the amount of starting material was experimented with to determine what amount would produce optimal results, as well as the minimum sample size threshold for this extraction that would still allow for successful Δ^9 -THC detection and identification. The original method does not specify a starting material amount, and 1g samples were used for initial testing. To test for the optimal and minimal sample amounts, extractions were performed for samples with 1g (Figure 10), 0.5g (Figure 11), 0.25g (Figure 12), and 0.125g (Figure 13) of starting material.

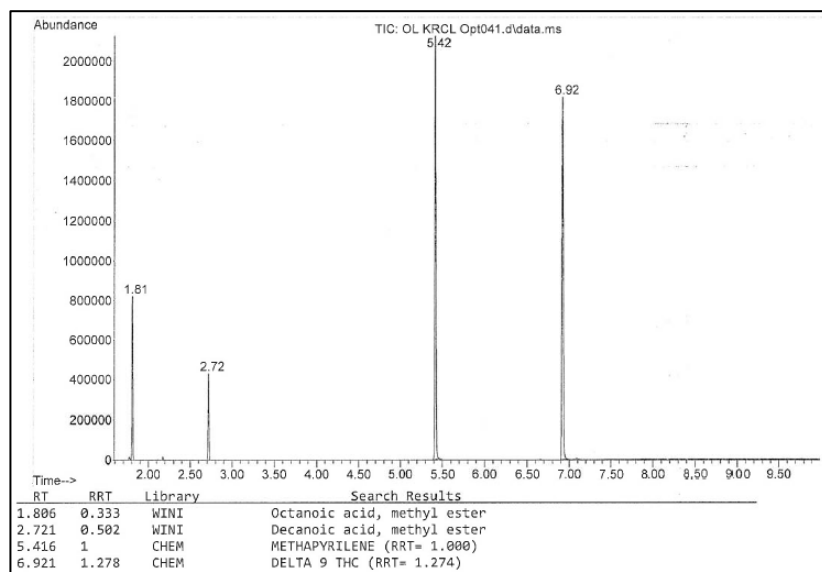


(a)

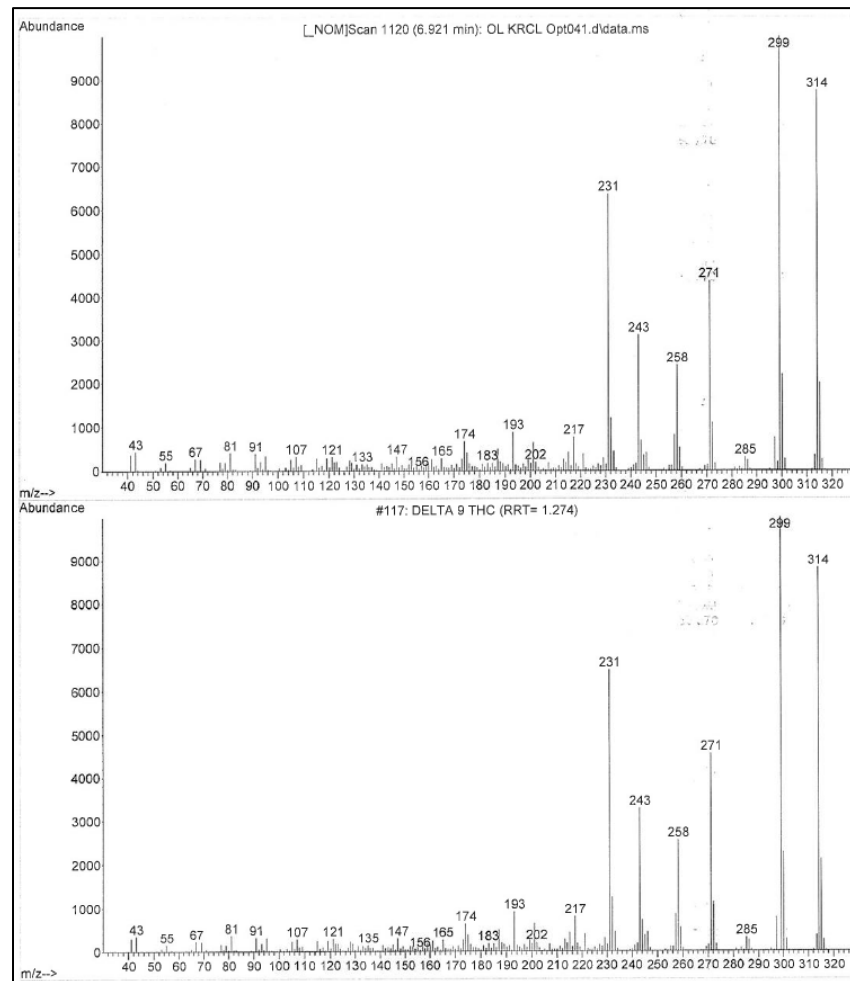


(b)

Figure 10. (a) Total Ion Chromatogram and (b) Mass Spectrum for Modified Basic/Acidic Extraction with 1g Sample.

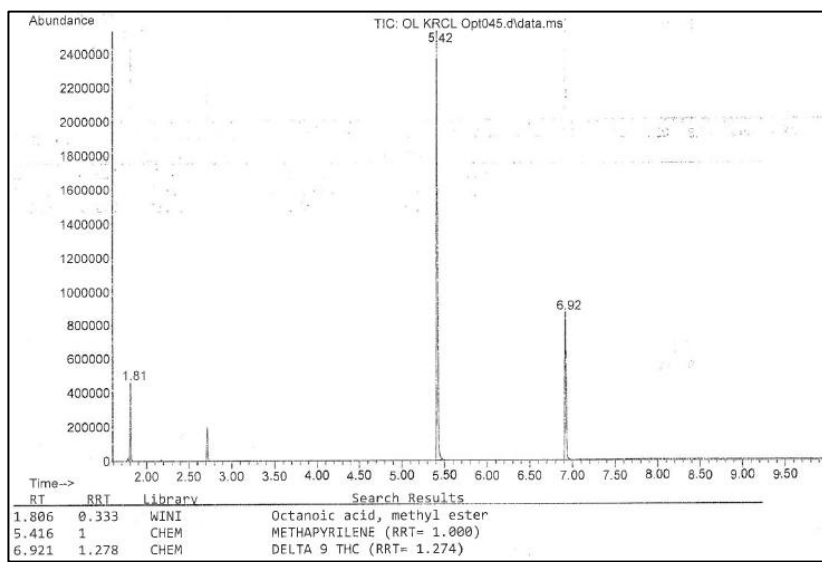


(a)

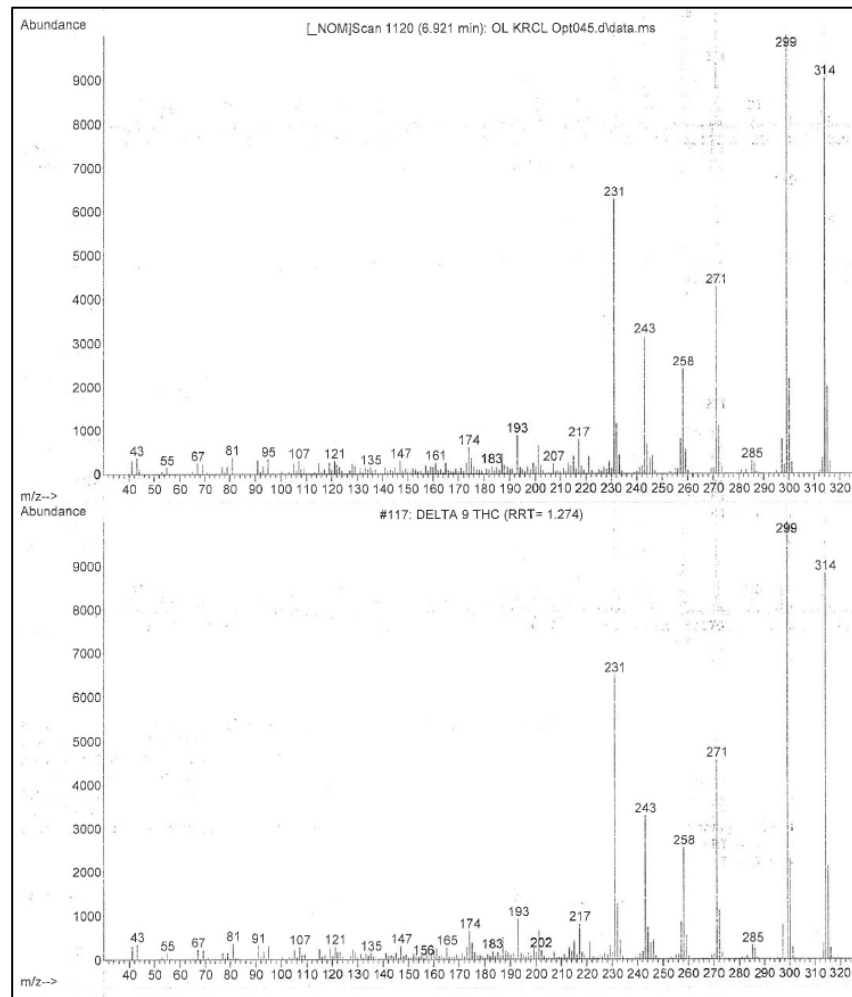


(b)

Figure 11. (a) Total Ion Chromatogram and (b) Mass Spectrum for Modified Basic/Acidic Extraction with 0.5g Sample.

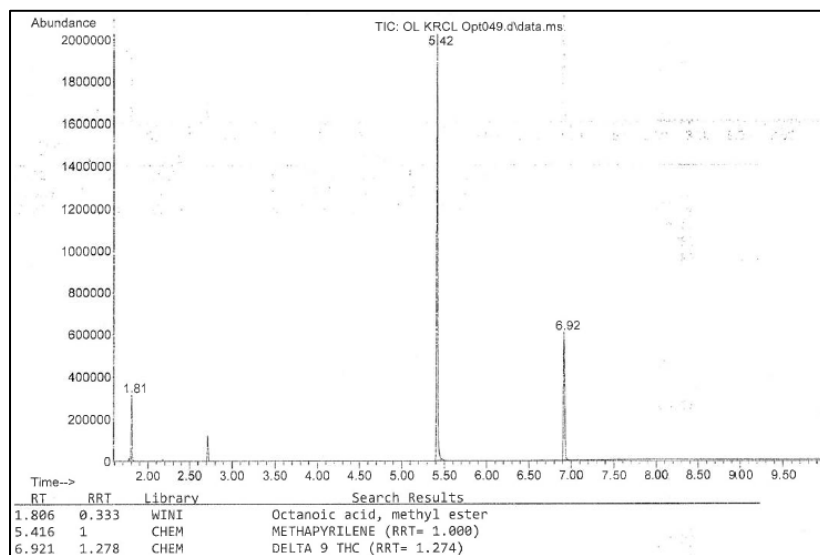


(a)

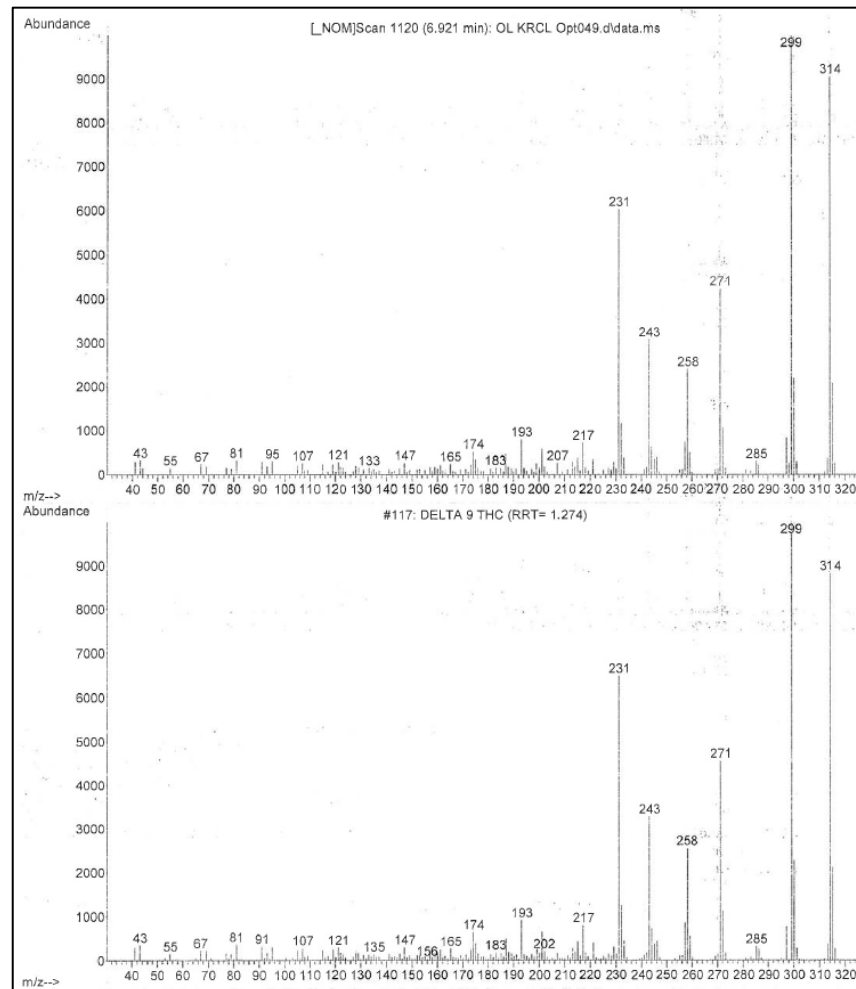


(b)

Figure 12. (a) Total Ion Chromatogram and (b) Mass Spectrum for Modified Basic/Acidic Extraction with 0.25g Sample.



(a)



(b)

Figure 13. (a) Total Ion Chromatogram and (b) Mass Spectrum for Modified Basic/Acidic Extraction with 0.125g Sample.

The TICs for each extraction were similar, with an expected decrease in Δ^9 -THC peak area as the starting amount decreased. The MS were closely analyzed when comparing the different sample sizes, as the goal was to determine the minimum amount needed to allow for confident Δ^9 -THC identification. The 1g sample (Figure 10b) and 0.5g sample (Figure 11b) produced comparable results, with both analyte mass spectra displaying the complete ion fragmentation pattern consistent with the Δ^9 -THC library reference MS. The analyte MS for the 0.25g sample (Figure 12b) and 0.125g sample (Figure 13b) did not display some ions that were present at very low abundances in the Δ^9 -THC reference MS, but the overall fragmentation pattern was sufficiently consistent with the reference MS for confident Δ^9 -THC identification.

Experimenting with different amounts of starting material revealed that Δ^9 -THC can be confidently identified from samples as small as 0.125g. Again, it is important to note that these extractions were done with samples containing relatively high concentrations of Δ^9 -THC, whereas casework samples are unpredictable. For samples with lower Δ^9 -THC concentrations, using 0.125g or 0.25g may result in an analyte MS with some of the low-abundance peaks going undetected when analyzed by the Drug method. In these cases, the extract can be reanalyzed with the Drug2X method, which may improve detection of low-abundance ions and increase confidence in the Δ^9 -THC identification. While Δ^9 -THC identification is possible with samples as small as 0.125g, a sample size between 0.5g – 1g is preferred, as these analyte mass spectra contain fragmentation patterns that are fully consistent with the Δ^9 -THC library reference MS.

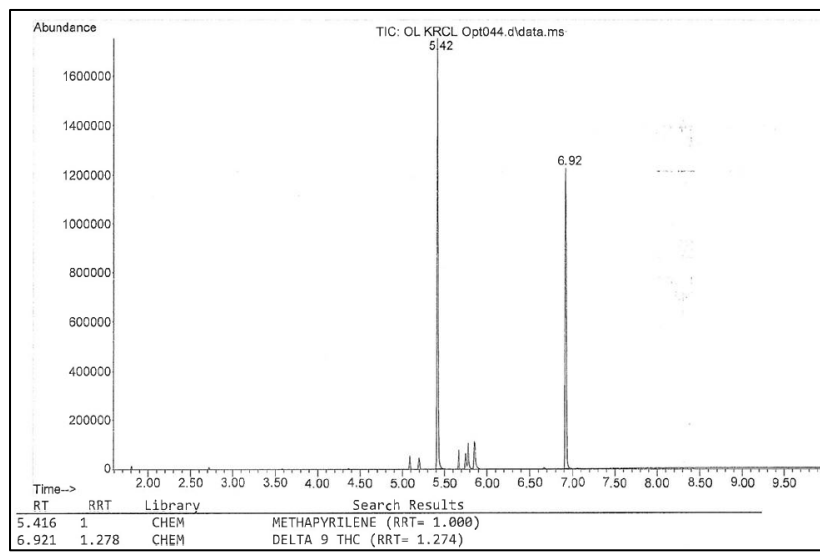
Optimized Extraction Method

Following modification of the original method to better fit the needs and capabilities of the CSU, the optimized extraction method is as follows:

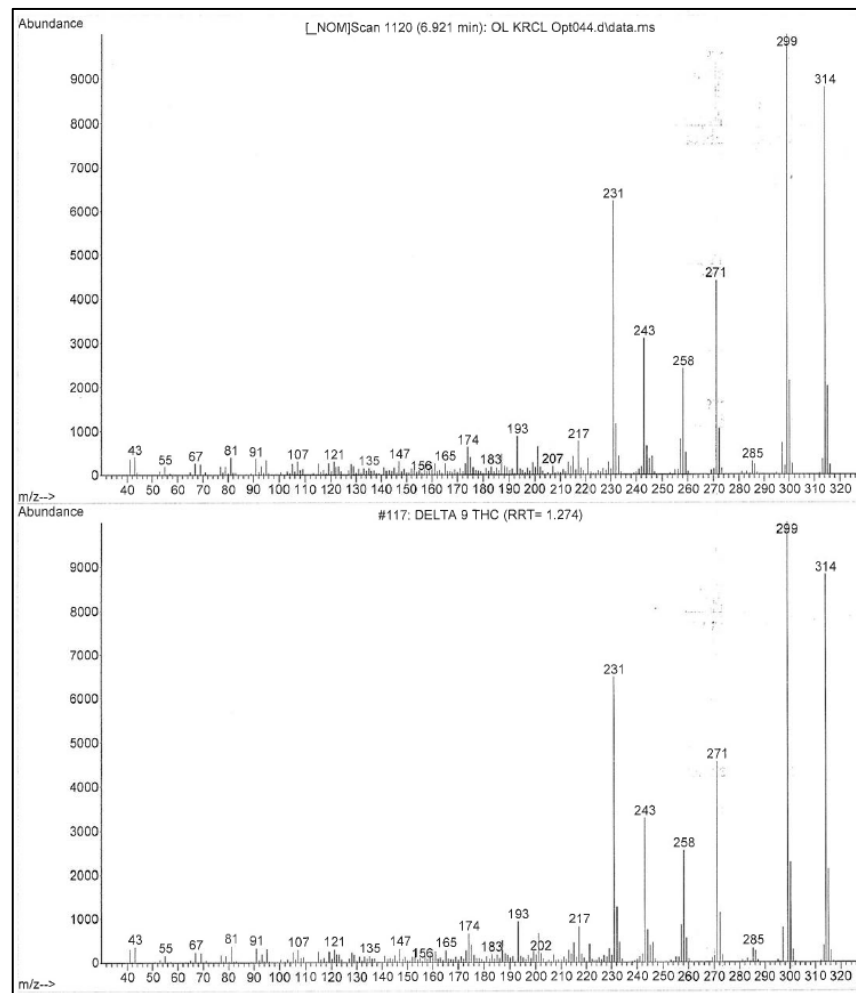
1. Finely chop 0.5 – 1g of edible sample and dissolve in 5mL of hot water (~ 90-95°C)
 - a. Add 2.5mL and vortex for 15sec, then add the remaining 2.5mL and vortex for at least 30sec (some samples may require longer vortexing)
2. Add 2mL hexane to sample
3. Vortex to mix fully, then centrifuge for 2min (some samples may require longer vortexing and/or centrifuging)
4. Transfer hexane layer to new test tube
5. Add 1mL of 0.5N methanolic KOH
6. Vortex and centrifuge briefly (bottom layer retains THC, if present)
7. Discard top hexane layer;
8. Wash with at least one 2mL aliquot of hexane;
9. Acidify with 0.5mL of 1N HCl and vortex briefly
10. Extract with 1mL hexane (top layer retains THC, if present)
11. Transfer hexane layer to new culture tube and add ~1-3 drops of External Standard
 - a. Amount of ES is dependent on amount of material and instrument sensitivity
12. Transfer into autosampler vial and analyze on Drug method

The detailed method provides a general outline of steps to follow for Δ^9 -THC extraction from edibles, but there may be some necessary adjustments to be made when analyzing certain samples. Adjustments may include additional wash steps, concentrating the final extract through evaporation, or using a greater amount of external standard depending on instrument sensitivity.

Before testing unknown samples, the optimized method was used to test a chocolate sample (Figure 14) and a cannabidiol (CBD) gum sample (Figure 15).

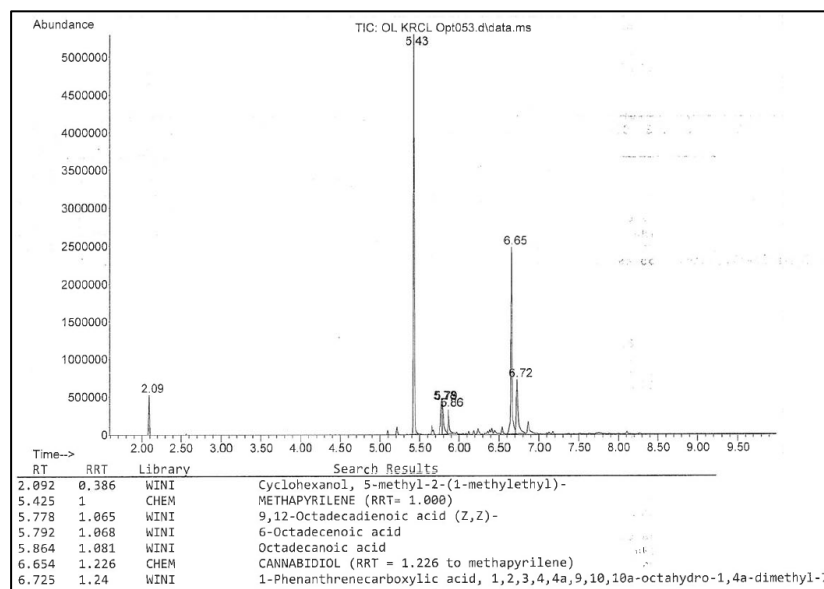


(a)

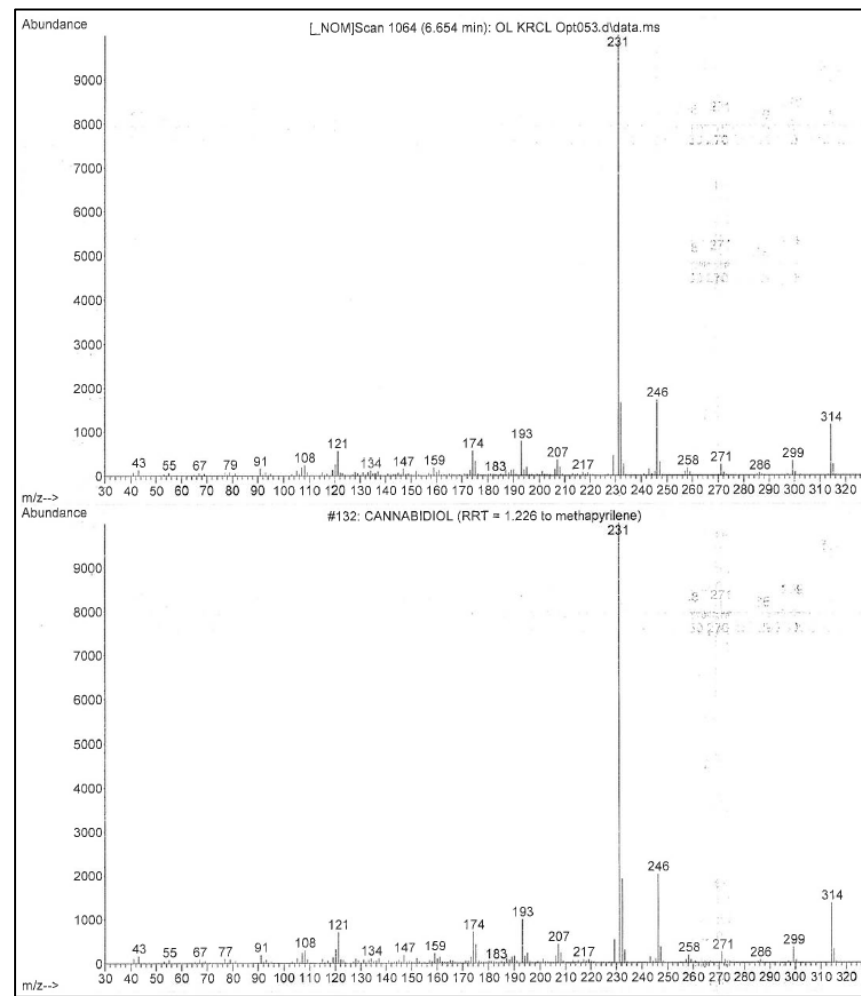


(b)

Figure 14. (a) Total Ion Chromatogram and (b) Mass Spectrum of Chocolate Sample Tested with Optimized Extraction.



(a)



(b)

Figure 15. (a) Total Ion Chromatogram and (b) Mass Spectrum of CBD Gum Sample Tested with Optimized Extraction

The optimized method was used to test a chocolate sample to determine its effectiveness on a non-gummy matrix, as one goal of this project was for the final extraction method to be compatible with multiple edible matrices. A 0.5g sample of HIGHATUS Chron Bons (10mg Δ^9 -THC per chocolate) was extracted using the optimized method, without any adjustments. The resulting extract was relatively clean, with a few miscellaneous peaks that were not integrated by the instrument (Figure 14a), and Δ^9 -THC could be confidently identified from the consistent ion fragmentation pattern between the analyte MS and Δ^9 -THC library reference MS (Figure 14b).

Additionally, the CSU possessed CBD gum from a separate project, which presented another unique matrix. While the gum was marketed to contain only CBD and not Δ^9 -THC, the CSU was still interested in the behavior of gum throughout the extraction due to its increased elasticity compared to gummies. A 0.25g sample of Simply CBD gum was extracted with one adjustment to the optimized method. The gum matrix was specified to be organic and was not able to be fully broken down with hot water, instead getting tacky and adhering to the walls of the test tube. For better breakdown of the gum matrix, 2.5mL of hot water was added to the gum, followed by 2mL of hexane and vortexing, before the remaining 2.5mL of hot water was added. The rest of the extraction procedure was completed without adjustment. The resulting extract was not very clean, as demonstrated by the miscellaneous peaks and noise along the baseline of the TIC (Figure 15a). For future similar samples, it may be beneficial to perform an additional wash. CBD could be confidently identified by the consistency between the analyte MS and CBD library reference MS (Figure 15b). The results from the chocolate and gum sample tests inspired confidence that the optimized method could successfully extract and detect Δ^9 -THC and CBD from various edible matrices.

CHAPTER 5. TESTING UNKNOWN SAMPLES

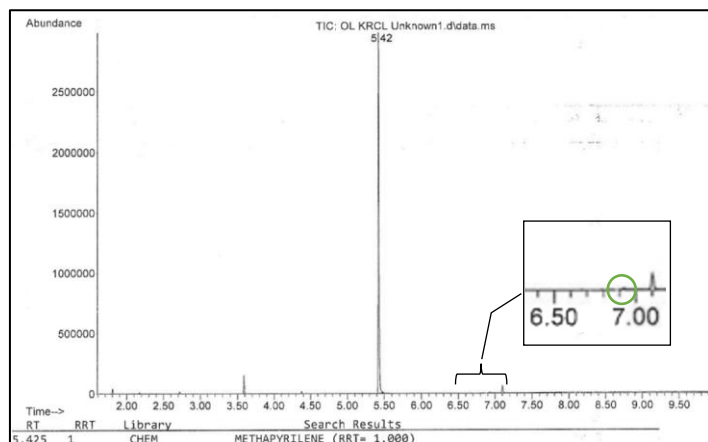
Five unknown edible samples were tested with the optimized method, with two from the Sacramento DA Crime Laboratory (not casework samples) and three from anonymous donors.

Unknown Edible Samples

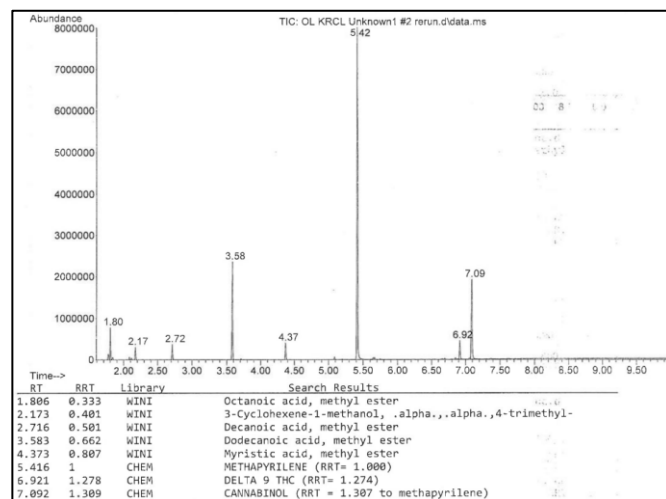


Figure 16. Unknown Edible Samples (a) #1, (b) #2, (c) #3, (d) #4, and (e) #5.

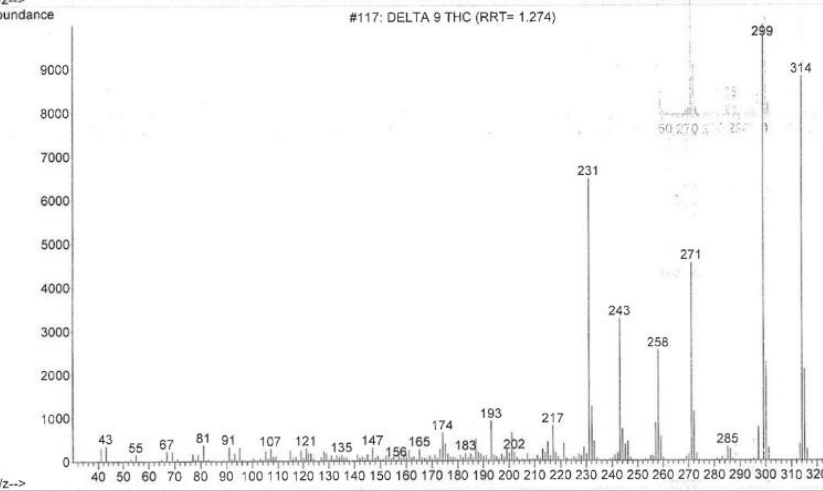
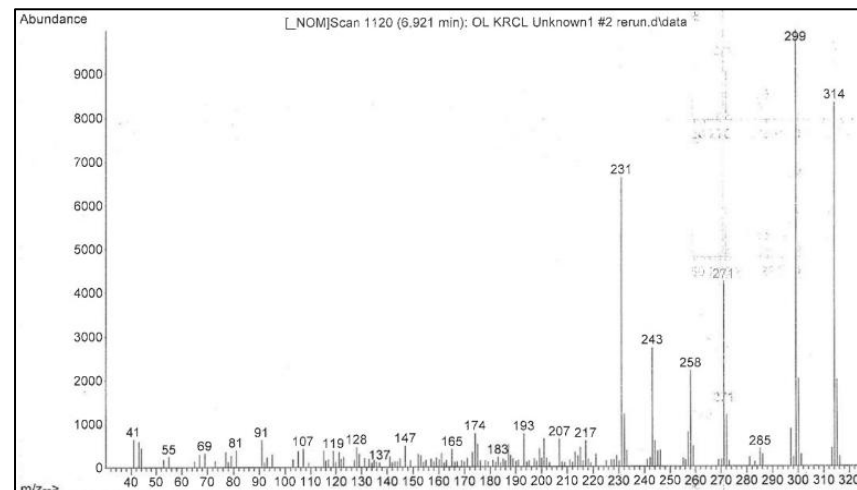
Unknown Sample Results



(a)

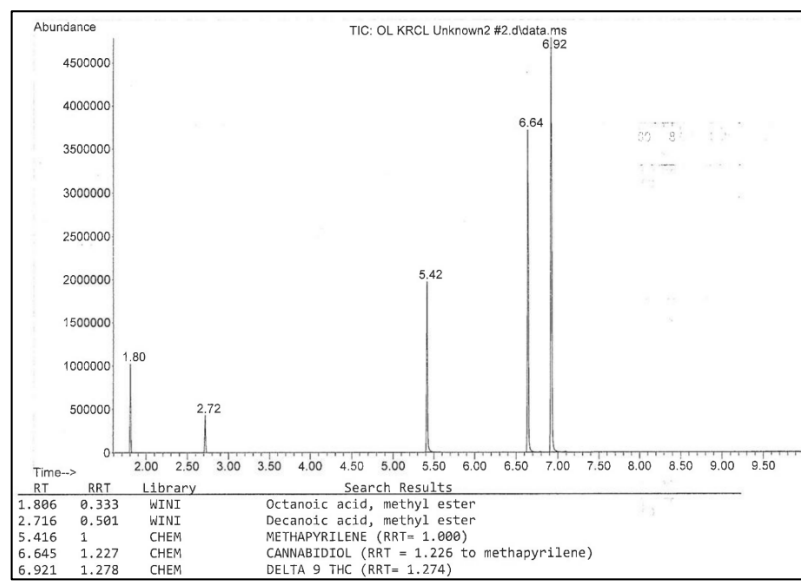


(b)

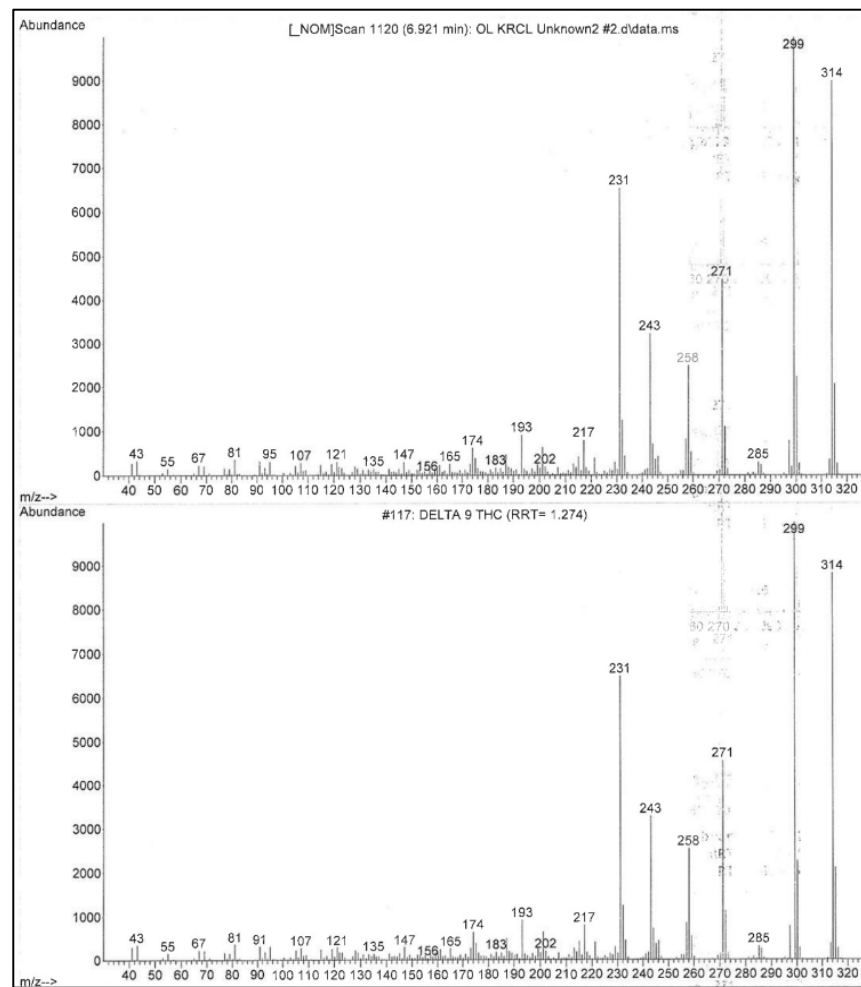


(c)

Figure 17. (a) Total Ion Chromatogram for 0.5g Sample of Unknown #1, (b) Total Ion Chromatogram for 2.23g Sample of Unknown #1 on Drug2X Method, and (c) Mass Spectrum for 2.23g Sample of Unknown #1.

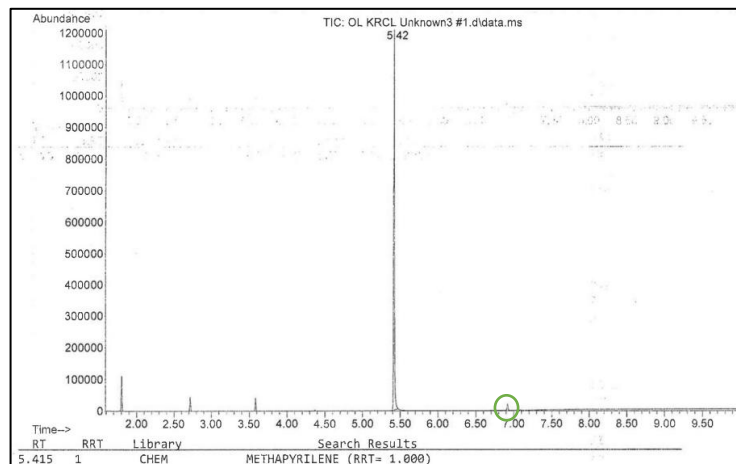


(a)

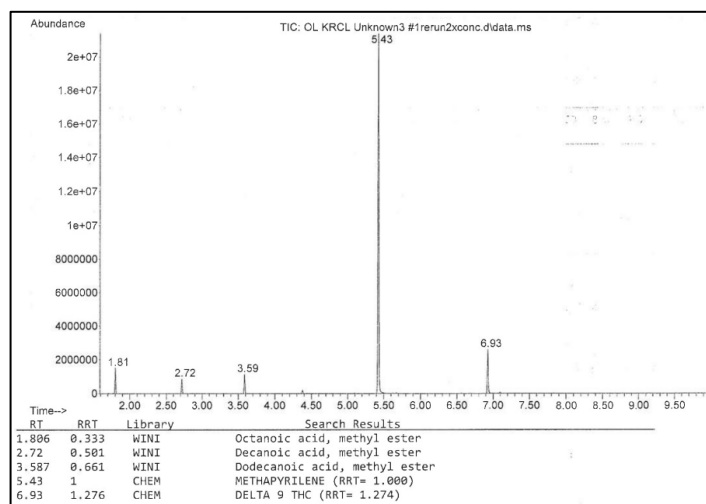


(b)

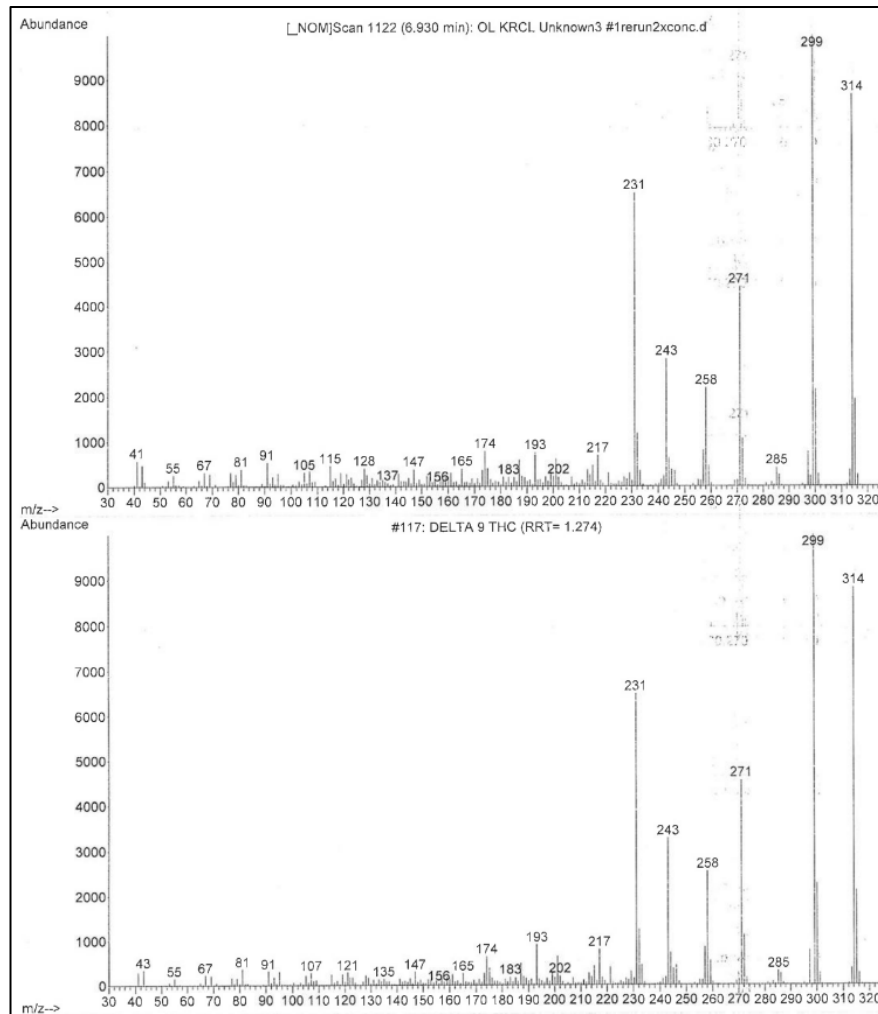
Figure 18. (a) Total Ion Chromatogram and (b) Mass Spectrum for 0.5g Sample of Unknown #2.



(a)

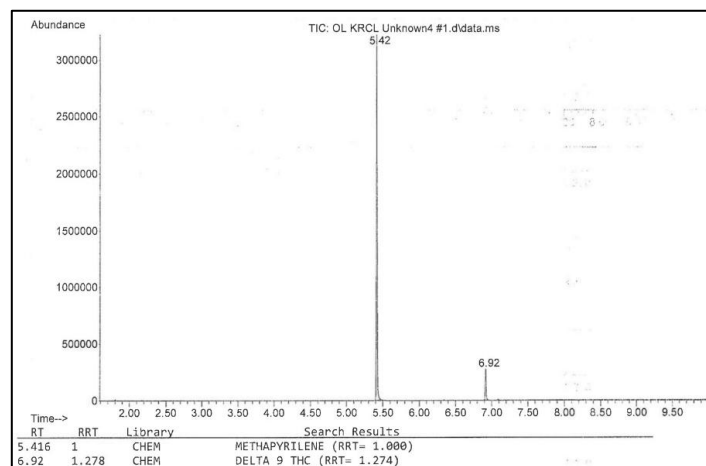


(b)

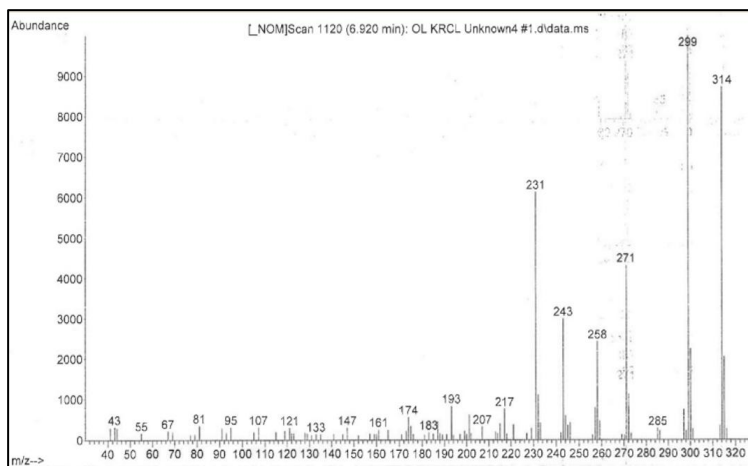


(c)

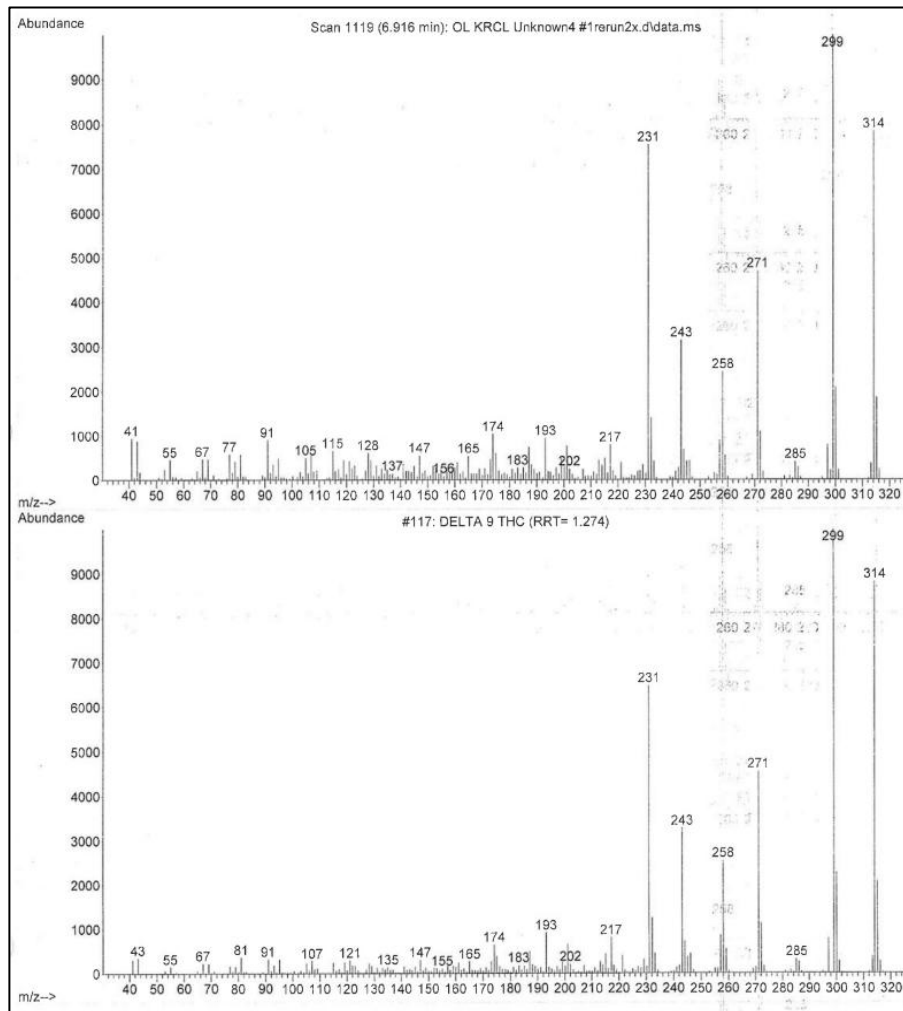
Figure 19. (a) Total Ion Chromatogram for 0.5g Sample of Unknown #3, (b) Total Ion Chromatogram for 0.5g Sample of Unknown #3 on Drug2X Method, and (c) Mass Spectrum for 0.5g Sample of Unknown #3.



(a)

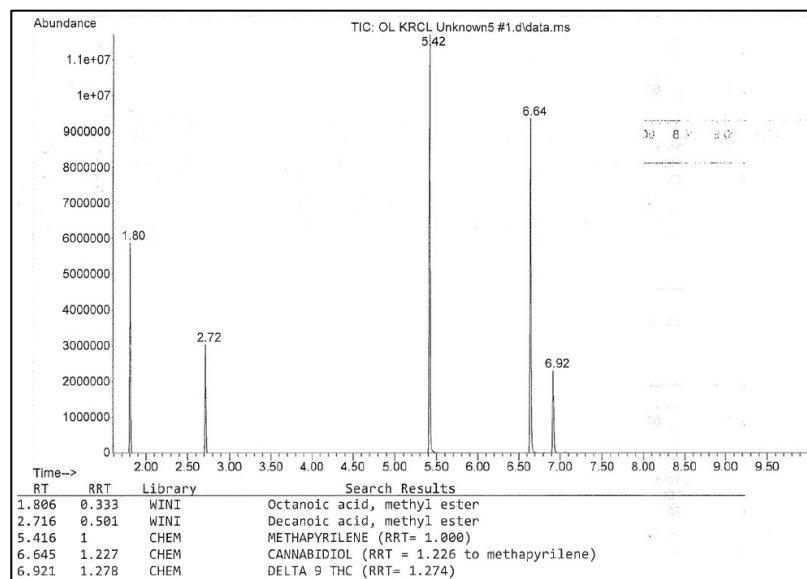


(b)

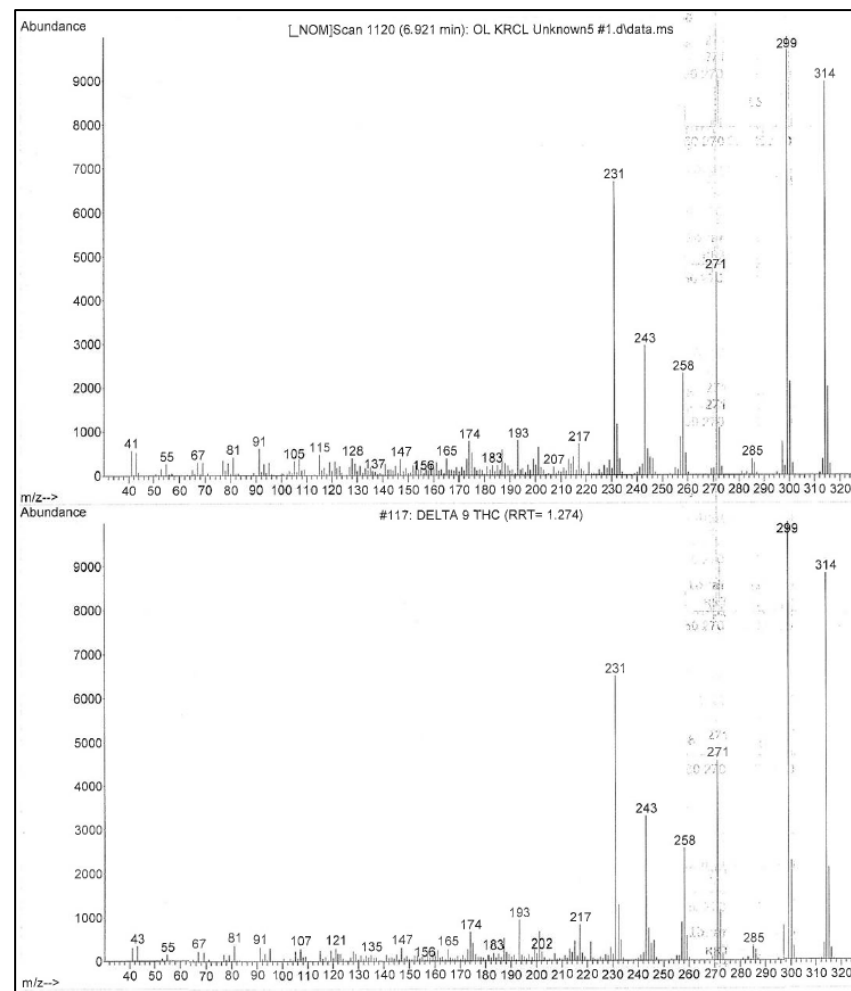


(c)

Figure 20. (a) Total Ion Chromatogram for 0.5g Sample of Unknown #4, (b) Mass Spectrum for 0.5g Sample of Unknown #4 on Drug Method, and (c) Mass Spectrum for 0.5g Sample of Unknown #4 on Drug2X Method



(a)



(b)

Figure 21. (a) Total Ion Chromatogram and (b) Mass Spectrum for 0.5g Sample of Unknown #5.

Unknown Sample #1 was two gummy worms (Figure 16a), and 0.5g from one of the gummy worms was analyzed following the optimized extraction method without adjustment. Zooming into the time point on the TIC where Δ^9 -THC is expected, approximately 6.92 minutes, there is a barely visible peak (Figure 17a). As the retention time of this peak was near the expected retention time for Δ^9 -THC, another extraction was performed using more starting material to determine whether the low intensity of the peak was a result of carryover or an extremely low Δ^9 -THC concentration in the sample. For the second extraction of Unknown Sample #1, approximately half of one gummy worm (2.23g) was used as the starting material, and the final extract was analyzed with the Drug2X method. With these adjustments, the TIC displayed an integrated peak at 6.921 minutes (Figure 17b), which was confidently identifiable as Δ^9 -THC by comparison of the analyte MS to the library reference MS (Figure 17c).

Unknown Sample #2 was two small dense chews (Figure 16b), of which 0.5g from one of the chews was analyzed following the optimized extraction method without adjustment. The TIC displayed an integrated peak at 6.921 minutes (Figure 18a), which was confidently identifiable as Δ^9 -THC by comparison of the analyte MS to the library reference MS (Figure 18b).

Unknown Sample #3 was one small gummy (Figure 16c), of which 0.5g was analyzed following the optimized extraction method without adjustment. The TIC displayed a small, unintegrated peak at 6.916 minutes (Figure 19a) that presented indications of Δ^9 -THC but could not be confidently identified. The same extract was analyzed with the Drug2X method, which resulted in an integrated peak at 6.93 minutes (Figure 19b) that was confidently identifiable as Δ^9 -THC by comparison of the analyte MS to the library reference MS (Figure 19c).

Unknown Sample #4 was one medium-sized dense gummy (Figure 16d), of which 0.5g was analyzed following the optimized extraction method without adjustment. The TIC displayed

an integrated peak at 6.92 minutes (Figure 20a) that presented indications of Δ^9 -THC, but with a significant amount of low-abundance ion fragments that were undetected in the analyte MS (Figure 20b). The same extract was analyzed with the Drug2X method, which resulted in an analyte MS that allowed for confident identification as Δ^9 -THC by comparison of the analyte MS to the library reference MS (Figure 20c).

Unknown Sample #5 was a small gummy (Figure 16e), of which 0.5g was analyzed following the optimized extraction method without adjustment. The TIC displayed an integrated peak at 6.921 minutes (Figure 21a), which was confidently identifiable as Δ^9 -THC by comparison of the analyte MS to the library reference MS (Figure 21b).

CHAPTER 6. CONCLUSIONS AND FUTURE WORK

Research Conclusions

The goal of this project was to develop a method for extracting Δ^9 -THC from cannabis edibles for casework application in the Controlled Substances Unit at the Sacramento County District Attorney's Laboratory of Forensic Services. Five pre-existing methods were compiled and tested in the CSU, then compared for their effectiveness and compatibility with the unit's resources. The basic/acidic extraction obtained from the Kern Regional Crime Laboratory was selected to move forward with for optimization to produce a final method for the CSU.

The original method requires methanolic KOH as the base, which the CSU is equipped to make but does not already use. A substitution attempt was made to instead use aqueous NaOH, a base the CSU routinely uses. However, this resulted in the loss of Δ^9 -THC early in the extraction, as its nonpolar nature caused preferential partitioning into an organic layer that was discarded. As a result, methanolic KOH remained the base for the method, which was less than ideal but acceptable, as the CSU has the means of making the reagent without additional costs. Other aspects of the method that were modified were the number of washes performed and the preferred amount of starting material to use. After testing the effects of three washes, two washes, and one wash, it was decided that one wash is preferred. Additional washes can be performed if needed for complex samples, but one wash produces sufficiently clean samples without risking significant loss of Δ^9 -THC. Regarding the amount of starting material used, it was determined that samples of at least 0.5g are preferable for proper detection and confident identification of Δ^9 -THC. However, in cases where the edible sample received as evidence is very small, it was determined that Δ^9 -THC can be detected and identified from sample sizes as small as 0.125g.

Once the method was modified, it was used to test a chocolate matrix and a CBD gum matrix. Δ^9 -THC was extracted and confidently identified from the chocolate sample, and the extraction was fairly clean. Meanwhile, CBD was extracted and confidently identified from the CBD gum sample, demonstrating the ability of the method to co-extract other cannabinoids with similar chemical properties as Δ^9 -THC. The results from the chocolate and gum sample tests conveyed the extraction's compatibility with multiple edible matrices. However, the extract from the gum was relatively unclean, which is likely due to the sample's organic matrix and tackiness. In future scenarios with similar edible matrices, additional washing may be beneficial.

Future Experimentation

Future experimentation beyond this project includes evaluating the ability of the method to co-extract other controlled substances that may be present in edible samples. Homemade edibles can contain various drug combinations, whether intentionally mixed in or unintentionally present due to using contaminated cannabis. Ideally, this extraction method would allow for the co-extraction and identification of other controlled substances. However, experimentation is needed to determine which drug classes can be successfully extracted when using this extraction method, as well as any drug classes that would be excluded from analysis.

Additionally, there are many edible matrices beyond the gummies, chocolate, and gum samples tested during this project. Future testing can be done to determine the effectiveness and cleanliness of the extraction for other sample matrices, such as more complex chocolates, baked goods like cookies or brownies, and cooking ingredients like cannabis butter. Testing additional matrices can provide insight into ways the method can be adjusted, if necessary, to accommodate a broader sample range and further increase the testing capabilities of the laboratory.

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