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Optimal Extraction Methods of THC from Infused Products

Final Summary Overview

Denver Police Department Crime Laboratory
Forensic Chemistry Unit

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Abstract/Project Overview/Purpose of Project/Results

Delta-9 Tetrahydrocannabinol (Δ9-THC) is the main active chemical in Marijuana that causes intoxicating effects. Colorado has an active market in which Δ9-THC is infused into food and drinks, known as marijuana edibles. The amount of Δ9-THC which should be within a single serving is regulated by the state, but manufacturing processes and quality control within the industry varies. It is unknown how much Δ9-THC is within a given edible, and where within that edible the Δ9-THC is located. Developing and validating a quick, safe, reliable, and inexpensive quantitative method for THC-infused products is an analytical necessity in today's environment.

Quantitation of marijuana edibles is complicated by the various food matrices a laboratory may encounter. This research focused on analyzing various matrices, to include hard candy, a sugar-based drink, and brownies/baked goods (see Fig. 1 in Appendix A). The optimal sample preparation methods, extraction techniques, and instrumental analyses for each matrix were explored. The objective was to obtain at least 80% recovery of Δ9-THC for each matrix using a Gas Chromatograph with Flame Ionization Detector (GC/FID) and/or a Liquid Chromatograph with Mass Spectrometer and Ultraviolet Detectors (LC/MS/UV), and to create a low-cost standard operating procedure to be provided to public crime laboratories (see Appendices B through D).

Difficulties arose during research due to transfer techniques and glassware used, as well as various matrix effects, several of which damaged analytical instrumentation. While numerous matrix clean-up methods and extraction techniques were deemed inadequate or problematic, several methods have proven viable. These include dry ice homogenization, enzymatic digestion of triglycerides, separation of sugars and fats from the organic layer, the use of a Vari-Mixer, heated sonication, centrifugation, and the use of chloroform as the extraction solvent. With optimized methods, the following recoveries were achieved: hard candy ≥ 96%, sugar-based drink ≥ 99%, and brownie ≥ 92%.

Project Subjects & Project Design and Methods

The following matrix clean-up methods and extraction techniques proved to be viable options and were incorporated into the final optimized methods for each food matrix.

Homogeneity

The distribution of THC within an edible may or may not be homogenous. Infused products must therefore be blended/ground to homogenize in order to provide a consistent amount of analyte, thereby ensuring reproducible results. Cold homogenization successfully freezes solid food products – hard candy and brownies – using dry ice (-86^{o} Celsius), and grinds the material at 2500 RPMs into a fine powder using an industrial grade food homogenizer to increase the surface area of the food product (see Fig. 2). Grinding only takes one minute, but the powdered material must be allowed to fully de-gas (to remove carbon dioxide) prior to making any weight measurements.

Standard Spike

There are only three known companies at this time which manufacture Δ9-THC standards, and the quantities in which they are available are limited, as a solid standard is not available. A further complicated is the high cost and unacceptable contamination levels found in some standards. This research was conducted using a purchased 1.0 mg/mL standard in methanol, the concentration of which limited the amount at which samples could be spiked, and standards could be analyzed. Creating an accurate calibration curve is difficult on both the GC and LC when starting at such a low concentration. The LC used in this research had the option to vary the injection volume, so calibration curves could be made by varying the injection volume of a standard at one concentration.

Matrix Cleanup for Hard Candies and Sugar-based Drinks

Very little matrix cleanup is necessary for candies and drinks, as the matrices are relatively simple. Deionized water (DIH_2O) was added to each to dissolve and remove sugars and dyes, separating them from the organic layer to be tested. Without sufficient DIH_2O, sugars formed a sticky layer at the

bottom of the hard candy centrifuge tube, which was confirmed to be corn syrup/starch via Fourier Transform Infrared (FTIR) spectroscopy.

Matrix Cleanup for Brownies

Foods contain a multitude of lipids, carbohydrates, and protein, each of which can cause interference with, and/or inhibit the extraction of, Δ9-THC. Additionally, foods containing high levels of fats can present challenges when trying to separate Δ9-THC from the fats, because Δ9-THC is extremely lipophilic due to its non-polar chain. Various food extraction kits – including those designed to remove lipids – were not viable clean-up techniques for Δ9-THC infused foods, as the kits removed the Δ9-THC along with the lipids. Various filters were also analyzed for effectiveness, including 0.45 µm polytetrafluoroethylene (PTFE) and 0.45 µm GH Polypro (GHP) membrane filters. While the filters did not remove Δ9-THC from the solution, they also did not improve recovery of the analyte, and were therefore deemed unnecessary.

The brownies are an example of a food matrix which contain a significant amount of fat, which can include butter and oils. Lipase digestion using an active enzyme solution (*Thermomyces lanuginosa*) was successful in digesting triglycerides from the matrix, and was more effective than using a basic solution. Glycerol and fatty acids were found following digestion, indicating the enzyme successfully broke down the triglycerides (see Fig. 3). Upon addition of the extraction solvent and DIH2O, many of the fatty acids and sugars segregated into the water layer, thereby significantly improving the organic layer.

Extraction Techniques

The two most successful extraction steps used for each matrix clean-up involved the use of a Vari-Mixer and centrifuge. Several options for mixing the materials during the extraction step were tested, including a tabletop orbital shaker, ambient sonication, a Vari-Mix Aliquot Mixer, and heated sonication. For the hard candy and sugar-based drink, use of the Vari-Mix alone was sufficient for the extraction. For the brownies, the best results were obtained when using the Vari-Mix and heated

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sonication in combination, likely due to the increased surface area with which to mix. Heated sonication following lipase digestion ensured the solid material within the centrifuge tube was thoroughly dissolved and mixed, prior to the addition of the extraction solvent. The Vari-Mix extraction step then ensured adequate mixing of the materials. The use of the Vari-Mix for a sufficient amount of time was critical during the lipase digestion and solvent extractions steps, but less important when dissolving the hard candy matrix in DIH_2O. The optimal brownie digestion time on the Vari-Mixer was 45 minutes (see Fig. 4). Similarly, the optimal extraction time for each matrix following addition of the solvent was 45 minutes (see Fig. 5).

Following extraction, it was necessary to fully separate the various layers using centrifugation. The optimal centrifugation time was ten (10) minutes at 2500 RPMs, which provided higher recoveries than obtained with only five (5) minutes (see Fig. 6).

Solvent Selection

For the hard candy and sugar-based drink matrices, the optimized extractions used chloroform for the organic layer. Chloroform was chosen due to its prevalence within most drug chemistry laboratories and its immiscibility with water, thereby forming two separate layers. With the addition of DIH_2O and chloroform for the extraction, the organic chloroform layer is at the bottom. The aqueous layers of the hard candy and sugar-based drink extracts were not difficult to puncture using a disposable borosilicate glass pipette during sampling. The samples were sufficiently clean of matrix interferences, and exhibited high Δ9-THC recoveries.

Solvent selection for the brownie matrix was complicated by the presence of additional matrix components, such as fatty acids and triglycerides. Much of the initial research focused on identifying a suitable organic solvent for the extraction of Δ9-THC that would not damage the analytical instrumentation. Methanol exhibited poor recovery and chromatography, formed a glue-like

consistency which damaged the GC's injector and cannot be separated into two layers upon the addition of DIH_2O because they are miscible.

Acetonitrile was successful in extracting Δ9-THC from the brownie matrix and was easy to sample because its low density causes it to form the top layer (above the aqueous layer), but extracts exhibited significant compatibility issues with the LC.

Hexane improved sampling because it forms the top layer; however, it requires a different column, mobile phase and parts, and requires switching to normal phase using LC. One goal of this project was to identify similar extraction techniques and instrument parameters across each matrix when possible, for ease of use. Switching the column and mobile phase for the brownie matrix would have added complexity, and was not necessary due to the success of chloroform as the extraction solvent. One issue to note is that hexane is not miscible with the methanol within the standard used to spike the samples during this research. This caused a lack of Δ9-THC recovery (0%) in the hexane extracts, as it remained within the methanol. This is only an issue during method development and validation in which a standard spike is used, and was successfully mitigated by drying down the standard to remove the methanol and reconstituting in hexane. The dry down and reconstitution technique successfully transferred approximately 100% of the Δ9-THC into the hexane, when compared on the GC/FID to the methanol standard.

The optimized method for the brownie extraction uses chloroform for the organic layer. Following lipase digestion, the DIH_2O successfully removes sugars and soluble fatty acids, significantly cleaning up the organic extract. The use of chloroform was successful in extracting Δ9-THC and provided better recoveries and chromatography than other solvents. Sampling the chloroform layer is difficult, however, as its high density causes it to form the layer at the bottom of the centrifuge tube. Several layers – including one which is dense – must be punctured using a disposable borosilicate glass pipette, before reaching the desired layer (see Fig. 7). While such extracts are also difficult to analyze via GC,

they do not cause the significant damage to instrumentation seen with methanol. Analysis is greatly improved when analyzing via LC in reverse phase, but guard columns and an incorporated column wash are critical to protecting the analytical column from damage.

Transfer Techniques

A concern noted during the research process, was the accuracy of the micropipettes used to dispense the standard spike. Upon varying recovery results between two analysts, it was found that the amount pipetted varied between the two analysts, even when using the same techniques. This effect was even more pronounced when pipetting chloroform during the extraction step, due to its high density. They were therefore replaced in the procedures with gastight syringes, graduated cylinders, and Class A glass pipettes, which were found to provide greater accuracy when using solvents such as chloroform and methanol. A gastight syringe was used to transfer the standard spike and the lipase enzyme, graduated cylinders were used to transfer the DIH_2O, and Class A glass pipettes were used to transfer the chloroform extraction solvent. Upon method validation within a laboratory, the accuracy of such glassware would be incorporated into the measurement uncertainty calculations.

Instrumentation

The main difference between using GC or LC for the analysis of Δ9-THC infused edibles, is the concept of decarboxylation. Tetrahydrocannabinolic Acid (THCA) is the most abundant cannabinoid in marijuana. It is the non-intoxicating precursor that becomes Δ9-THC upon being exposed to heat (typically through smoking). This transition from THCA to Δ9-THC is known as decarboxylation (see Fig. 8). Marijuana edibles may be infused with THCA – often in large quantities – in addition to Δ9-THC. A quantitation method must therefore take this into account, particularly when analysis via GC is necessary, as the THCA will convert to Δ9-THC within the instrument's inlet upon being heated. THCA and Δ9-THC are thus indistinguishable when using GC, requiring decarboxylation of the THCA prior to quantitative analysis. This is not a concern, however, when using LC, as the sample is not heated by the

instrument. If not cost-prohibitive, LC is the preferred analytical technique, as THCA and Δ9-THC can be resolved by the instrument and the additional decarboxylation step is not necessary.

There are additional considerations when evaluating whether to use GC or LC for such analyses. While GC is more cost effective, analyses of complicated matrices such as brownies are virtually impossible, as the various matrix components damage the syringe, inlet, and column, even after the clean-up procedures are used. Most of the instrument issues encountered during this research involved injector malfunctions and column damage due to the agglomeration and deposition of fatty acids and triglycerides. Such samples had to be manually injected, provided poor recovery, and caused compounds to elute in later injections causing erratic chromatography.

Similar instrument issues were encountered with the LC when injecting brownie extracts without the use of guard columns or a rinse step incorporated into the run. Column damage was significant and irreparable, as the triglycerides and fatty acids bound to the analytical column's packing. The hard candy and sugar-based drink extracts were not an issue on the LC without guard columns or a rinse step, and therefore an isocratic method was used. For fat-based food matrices, the use of a guard column and an incorporated rinse step is imperative, necessitating a gradient run. For this research, a rinse step was incorporated into the run, as well as a re-equilibration period to return the mobile phase to its starting concentrations prior to the next run. A shutdown method was also necessary as the last sample for each day of use, to rinse the system. Following implementation of such rinse steps and the addition of a guard column, brownie extracts were no longer an issue on the LC, and Δ9-THC retention times were consistent across samples and standards.

While choosing an appropriate column and mobile phase is a challenge, the LC has many benefits over the use of GC. It does not have as many matrix effects, exhibits faster run times, provides higher sensitivity, and includes the option to add a diverter valve, allowing much of the matrix components to be sent to waste rather than entering the MS.

The use of an LC with a MS and/or UV detector was examined. Use of the MS has the added benefit of utilizing Extracted Ion Chromatograms to limit the analytes to those of interest. It also requires a lower level of Δ9-THC concentration than the UV detector, and could be optimized to detect and quantitate lower levels of the analyte within infused products. The MS detector was overloaded at the concentrations used throughout this project, and thus a lower concentration calibration curve would be necessary for the MS recoveries to be accurate.

The UV detector is more affordable, consistently provides accurate data, and is not as prone to being overloaded by a large amount of Δ9-THC. The calibration curves used for this project were optimized for use of the UV detector, and thus these results were more accurate. The quantitative results reported below are those obtained using the UV detector.

Data Analysis & Project Findings

The sample preparation, extraction techniques, and instrumental analyses were optimized for each of the three matrices and significant recoveries were obtained. While each method must be assessed and validated in-house by other laboratories, the following parameters are provided as a starting point, and suggestions for best practices are included.

Recovery Results

Hard Candy

For the optimized hard candy method, the weight of approximately one hard candy was spiked and analyzed, as representative of one serving of a Δ9-THC-infused candy. 20 mL of DIH_2O was added, as less water caused decreased recovery because sugars remain in the organic layer and/or form a bottom layer of starch/corn syrup. 10 mL of chloroform was then added, and the centrifuge tube was placed on the Vari-Mixer to extract for approximately 45 minutes. Less chloroform and extraction time each resulted in lower Δ9-THC recoveries. Prior to sampling, the test tube was centrifuged for ten (10) minutes, as less centrifuge time resulted in lower Δ9-THC recoveries. A sample from the bottom chloroform layer was then removed for analysis. For the full method, see Appendix B.

Qualitative analysis via GC/MS exhibited a Δ9-THC peak with good peak shape and no interfering or co-eluting peaks (see Fig. 9).

With the optimized method, two samples were analyzed via LC/MS/UV. Δ9-THC recoveries for two samples ranged between 96% and 100% (see Fig. 10). With an optimized calibration curve, recovery of Δ9-THC from a hard candy is ≥ 96%.

Sugar-based Drink

The optimized method for a sugar-based drink is similar to that of the hard candy. 12.5 mL of the sugar-based drink was spiked and analyzed. 5 mL of chloroform was added, and the centrifuge tube was Vari-mixed for approximately 45 minutes. Less chloroform and extraction time each resulted in lower Δ9-THC recovery. Prior to sampling, the test tube was centrifuged for ten (10) minutes, as less centrifuge time resulted in lower Δ9-THC recoveries. A sample from the bottom chloroform layer was then removed for analysis. For the full method, see Appendix C.

Qualitative analysis via GC/MS exhibited a Δ9-THC peak with good peak shape and no interfering or co-eluting peaks.

With the optimized method, two samples were analyzed via LC/MS/UV. The sample concentrations were twice the concentration of the optimized calibration curve for the UV detector. This may have contributed to a slight variance in the Δ9-THC recoveries for the two samples, which ranged between 99% and 102% (see Fig. 11). Recovery of Δ9-THC from a sugar-based drink is therefore approximately ≥ 99%.

Brownie

For the optimized brownie method, approximately 5.0g was spiked and analyzed. 25 mL of DIH_2O was added, as less water caused decreased recovery and more water showed no improvement. The water was left in the centrifuge tube throughout the extraction procedure, because removing water removed approximately 0.5% of the analyte with each water wash removed. 600µL of the lipase enzyme was then added, as more lipase did not improve Δ9-THC recovery. Lipase digestion was improved by

placing the tube on the Vari-Mixer for approximately 45 minutes, to increase surface area interaction. Heated sonication (at about 44 to 47^{o}C) then ensured the material was fully dissolved and mixed, with a visual appearance similar to chocolate milk. When heated sonication was conducted prior to the lipase digestion step, recovery of Δ9-THC was lower. Ambient sonication and sonication at 65^{o}C reduced recovery of Δ9-THC. 15 mL of chloroform was then added, and the centrifuge tube was placed on the Vari-Mixer to extract for approximately 45 minutes. More chloroform, less chloroform, and less extraction time each resulted in lower Δ9-THC recoveries. Prior to sampling, the test tube was centrifuged for ten (10) minutes, as less centrifuge time resulted in lower Δ9-THC recoveries. A sample from the bottom chloroform layer was then removed for analysis using a glass pipette, as a syringe provided lower recovery. For the full method, see Appendix D.

With the optimized method, two sample extracts from the same centrifuge tube were analyzed via LC/MS/UV. Δ9-THC recoveries for two samples ranged between 92% and 95% (see Fig. 12 & 13). With an optimized calibration curve, recovery of Δ9-THC from a brownie is ≥ 92%.

Implications for Criminal Justice Policy and Practice in the United States

With an increasing number of states, and potentially the federal government, considering the legalization and regulation of marijuana, a reliable quantitative method for the analysis of Δ9-THC in marijuana edibles is imperative. This research has confirmed the numerous difficulties involved in developing a fast, inexpensive, and accurate quantitative method. Despite difficulties, the project was a success and optimized methods for the quantitation of Δ9-THC within a hard candy, a sugar-based drink, and a brownie (baked goods) were developed. These method parameters can be adopted by laboratories throughout the United States, and validated using those laboratories' particular equipment and supplies. These methods provide a starting point for method validation, and prove that recoveries of over 92% are achievable for each of the marijuana edibles matrices tested.

Appendix A: Figures

Figure 1. Marijuana edibles matrices

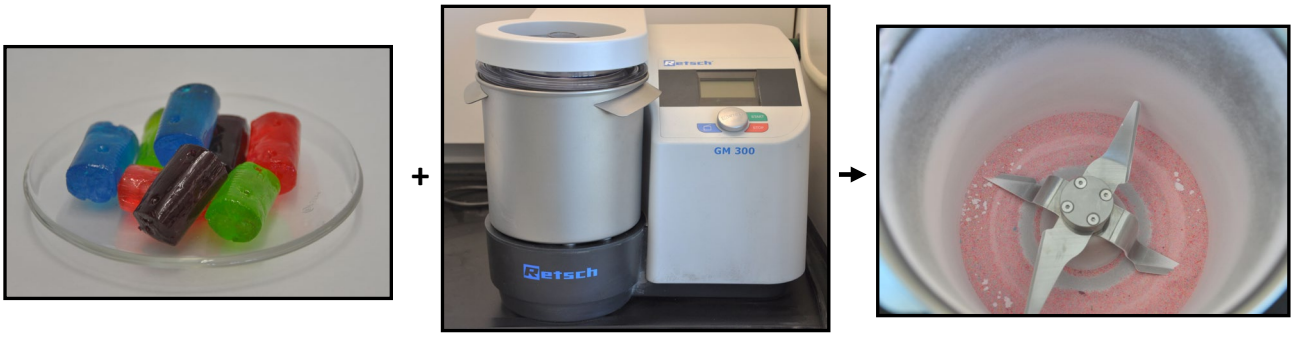

Figure 2. Cold homogenization of hard candy using industrial grade food homogenizer and dry ice

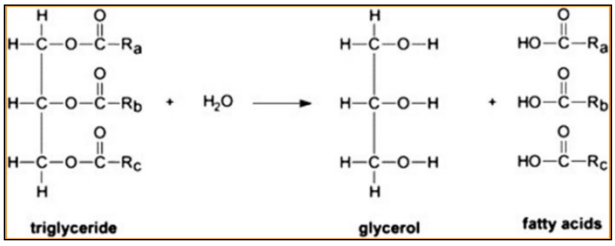

Figure 3. Lipase digestion of triglycerides in brownie matrix

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Figure 4. Vari-Mix lipase digestion step for brownie matrix

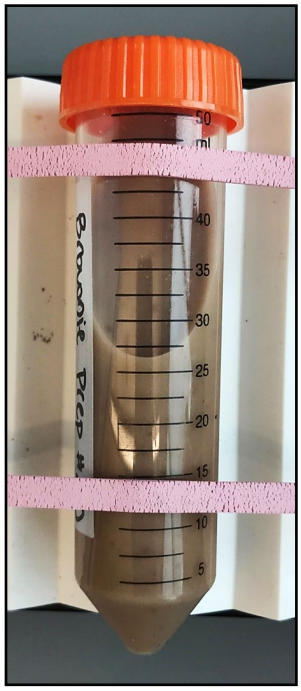

Figure 5. Vari-Mix extraction step for brownie matrix

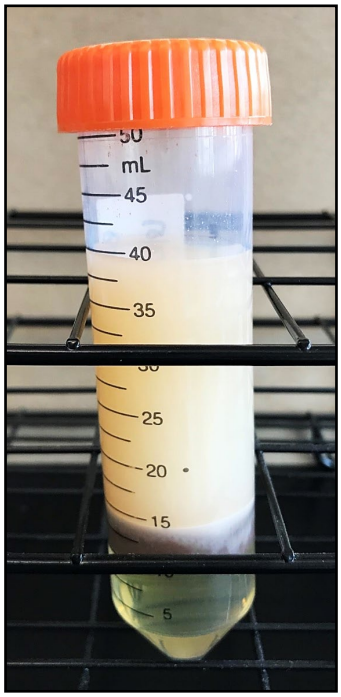

Figure 6. Brownie matrix following centrifugation

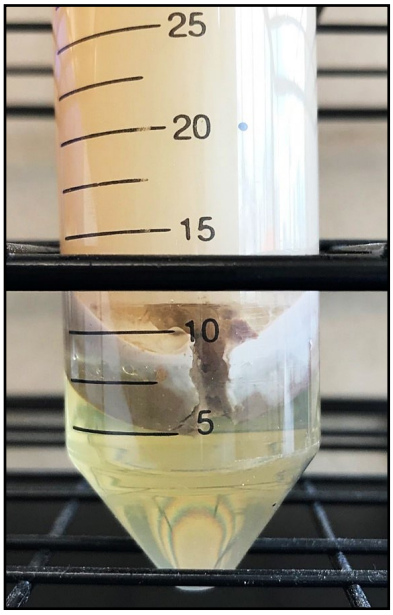

Figure 7. Dense layer that must be punctured when removing chloroform extract from brownie matrix

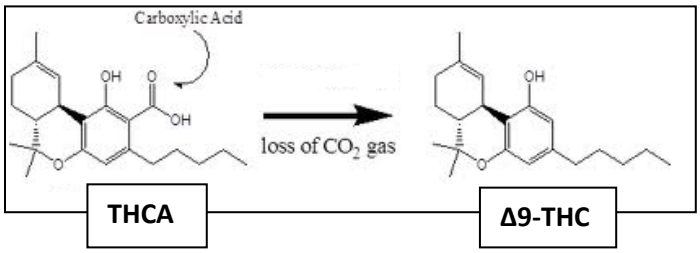

Figure 8. Decarboxylation of THCA to Δ9-THC

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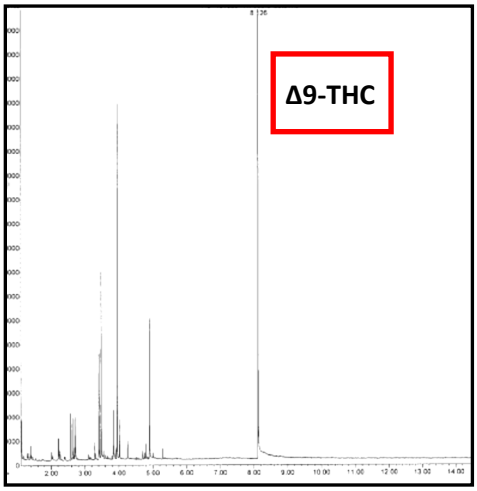

Figure 9. Hard candy chloroform extract on GC/MS

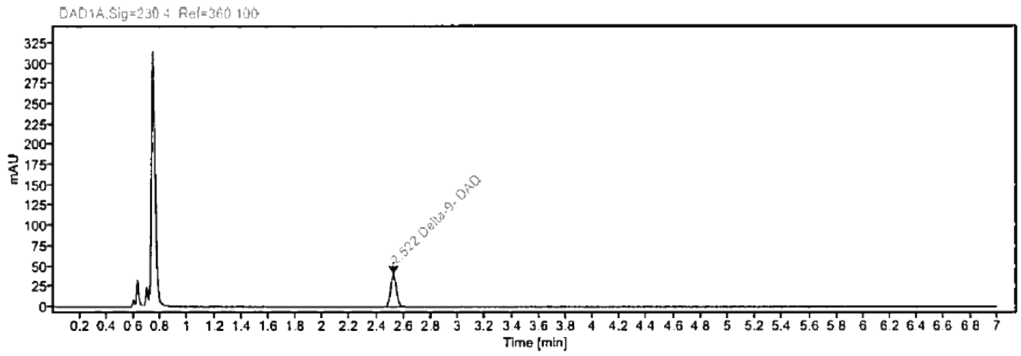

Figure 10. Hard candy chloroform extract on LC/UV

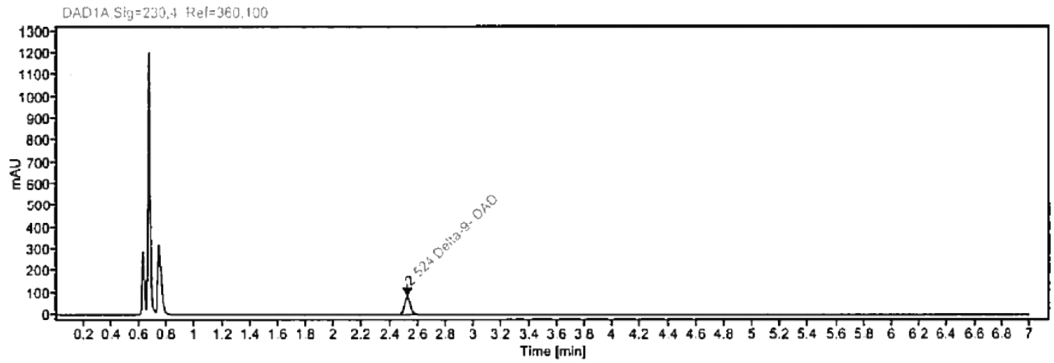

Figure 11. Sugar-based drink chloroform extract on LC/UV

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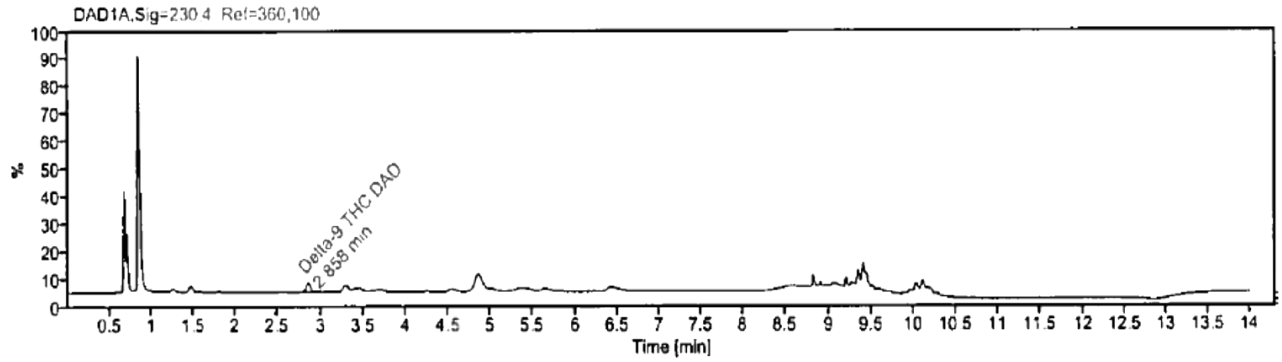

Figure 12. Brownie chloroform extract on LC/UV

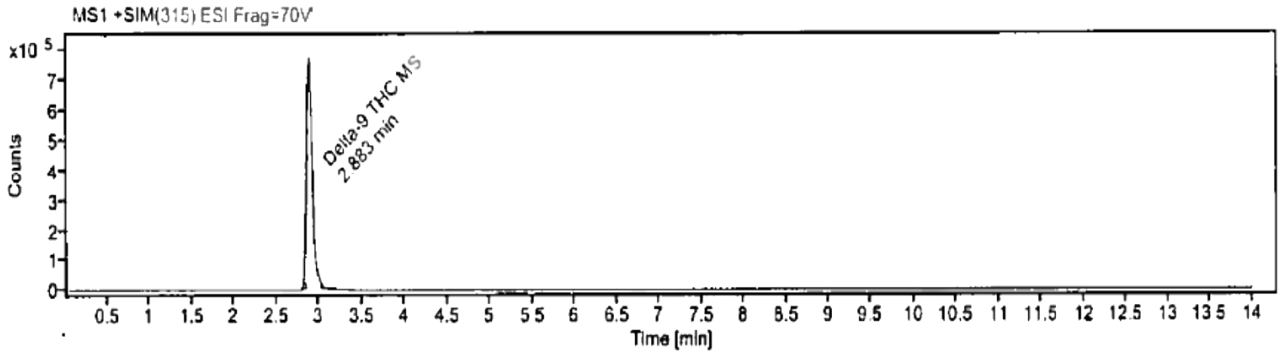

Figure 13. Brownie chloroform extract on LC/MS

Appendix B: Hard Candy
Analytical Procedure and Instrument Parameters

Preparation of Pulverized Hard Candy

1. Place the hard candy/candies inside an industrial grade food homogenizer with an appropriate amount of dry ice. This research used 60g of hard candy to 100g of dry ice.
2. Place the gas lid on the homogenizer, such that generation of gas does not rupture the vessel.
3. Grind the material for 60 seconds at 2500 RPMs. If chunks are still visible, repeat grinding step. The final material should be a fine powder.
4. Allow the resulting pulverized mixture to fully out-gas.

Standard Spike

To aid in the process of method validation, below are the steps taken during this research to spike the hard candy samples.

1. Using a 1.0 mL gastight syringe, transfer 1.0mL of a 1.0 mg/mL Δ9-THC standard into an empty 50mL centrifuge tube as the spike.
2. Dry down the standard spike using an evaporator, until no solvent is visible. (*Note: there may be a slight purple/pink residue visible.*)

Analytical Procedure

1. Place the centrifuge tube on a balance and tare once stable.
2. Weigh and transfer approximately 6.1g of pulverized hard candy into the centrifuge tube.
3. Add 20mL of DIH_2O to the tube using a graduated cylinder.
4. Vortex for about 2-5 minutes until dissolved.
5. Add 10mL of chloroform to the centrifuge tube using a 10mL Class A glass pipette.
6. Vari-mix the mixture for 45 minutes.
7. Centrifuge the tube at 4000 RPMs for 10 minutes.
8. Remove a sample from the chloroform layer (center of the cone at the bottom of the centrifuge tube) using a disposable borosilicate glass pipette and place in an amber autosampler vial, and cap immediately.
    - *Note: Once the pipette tip is within the chloroform layer, expel some air from the bulb, withdraw the sample, then let some of the liquid drip out prior to transferring the sample to the autosampler vial.*

Instrumental Analysis

Instrument: Ultra Performance Liquid Chromatograph (UPLC) with UV and MS and Quaternary Pump

Column: Agilent InfinityLab Poroshell 120 EC-C18 (3.0x100mm; 2.7µm)

- Analyze the chloroform extract(s) via LC/UV or LC/MS using an appropriate calibration curve.
    - With the spike concentration and amount of chloroform used during this research, the expected concentration at 100% recovery would be 0.1 mg/mL of Δ9-THC.
- Run alongside chloroform blanks.

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- Analyze a check standard to check the accuracy of the calibration curve.
- The approximate retention time of Δ9-THC using the parameters below is: 2.52 minutes via UV and 2.55 minutes via MS.

Instrument Parameters

DAD (UV):

Spectrum Range: 190 nm – 400 nm (store all)
Wavelength: 230 nm

Column Oven:

Temperature Control: 50.0°C

Quaternary Pump:

Flow Rate: 0.700 mL/min
Total Run Time: 7.0 min

Solvent Composition:

Isocratic run

A: 10% Deionized water
B: 85% Methanol
C: 5% 1L DIH_2O + 1mL Formic Acid + 2.2 mL 5M Ammonium Formate Solution*

*For 25mL of stock solution, weigh 7.8825g of ammonium formate into a 25mL flask, then fill to volume with 18.2MΩ DIH_2O.

Autosampler:

Injection Volume: 0.50µL

Needle Wash: 4 second flush port 25:25:50 deionized water: isopropanol: methanol

Mass Spectrometer: **(*OPTIONAL*)**

Ion Source: Multimode (MMI)
Scan Type: Selected Ion Monitoring (SIM)/Scan
Delta Electron Multiplier Voltage (EMV) Pos: 200 V
Mass: 315 m/z
Source Parameters:
- Gas Temperature: 300°C
- Gas Flow: 4.9 L/min
- Nebulizer: 15 psi
- Capillary: 3000 V
- Corona Current: 4µA
- Vaporizer: 200°C
- Charging Voltage: 2000 V

Diverter Valve:
- 0 - 2.5 min: sent to waste
- 2.5 - 3.4 min: sent to MS
- 3.4 - 7 min: sent to waste

Appendix C: Sugar-based Drink
Analytical Procedure and Instrument Parameters

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Preparation of Sugar-based Drink

No preparation is necessary for the sugar-based drink prior to the extraction procedure.

Standard Spike

To aid in the process of method validation, below are the steps taken during this research to spike the sugar-based drink samples.

1. Using a 1.0 mL gastight syringe, transfer 1.0mL of a 1.0 mg/mL Δ9-THC standard into an empty 50mL centrifuge tube as the spike.
2. Dry down the standard spike using an evaporator, until no solvent is visible. (*Note: there may be a slight purple/pink residue visible.*)

Analytical Procedure

1. Add 12.5mL of the sugar-based drink to the centrifuge tube using a graduated cylinder.
2. Add 10mL of DIH_2O to the tube using a graduated cylinder.
3. Vortex for 1-2 minutes until mixed.
4. Add 5mL of chloroform to the centrifuge tube using a 5mL Class A glass pipette.
5. Vari-mix the mixture for 45 minutes.
6. Centrifuge the tube at 4000 RPMs for 10 minutes.
7. Remove a sample from the chloroform layer (center of the cone at the bottom of the centrifuge tube) using a disposable borosilicate glass pipette and place in an amber autosampler vial, and cap immediately.
    - *Note: Once the pipette tip is within the chloroform layer, expel some air from the bulb, withdraw the sample, then let some of the liquid drip out prior to transferring the sample to the autosampler vial.*

Instrumental Analysis

Instrument: UPLC with UV and MS and Quaternary Pump

Column: Agilent InfinityLab Poroshell 120 EC-C18 (3.0x100mm; 2.7µm)

- Analyze the chloroform extract(s) via LC/UV or LC/MS using an appropriate calibration curve.
    - With the spike concentration and amount of chloroform used during this research, the expected concentration at 100% recovery would be 0.2 mg/mL of Δ9-THC.
- Run alongside chloroform blanks.
- Analyze a check standard to check the accuracy of the calibration curve.
- The approximate retention time of Δ9-THC using the parameters below is: 2.52 minutes via UV and 2.55 minutes via MS.

Instrument Parameters

DAD (UV):

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Spectrum Range: 190 nm – 400 nm (store all)
Wavelength: 230 nm

Column Oven:
Temperature Control: 50.0°C

Quaternary Pump:
Flow Rate: 0.700 mL/min
Total Run Time: 7.0 min

Solvent Composition:
Isocratic run

A: 10% DIH_2O
B: 85% Methanol
C: 5% 1L DIH_2O + 1mL Formic Acid + 2.2 mL 5M Ammonium Formate Solution*

*For 25mL of stock solution, weigh 7.8825g of ammonium formate into a 25mL flask, then fill to volume with 18.2MΩ DIH_2O.

Autosampler:
Injection Volume: 0.50µL

Needle Wash: 4 second flush port 25:25:50 deionized water: isopropanol: methanol

Mass Spectrometer: **(*OPTIONAL*)**
Ion Source: MMI
Scan Type: SIM/Scan
Delta EMV Pos: 200 V
Mass: 315 m/z
Source Parameters:
Gas Temperature: 300°C
Gas Flow: 4.9 L/min
Nebulizer: 15 psi
Capillary: 3000 V
Corona Current: 4µA
Vaporizer: 200°C
Charging Voltage: 2000 V
Diverter Valve:
0 - 2.5 min: sent to waste
2.5 - 3.4 min: sent to MS
3.4 - 7 min: sent to waste

Appendix D: Brownie
Analytical Procedure and Instrument Parameters

Preparation of Pulverized Brownie

1. Place the brownie(s) inside an industrial grade food homogenizer with an appropriate amount of dry ice. This research used one brownie (178.7g) to 100g of dry ice.
2. Place the appropriate gas lid on the homogenizer, such that generation of gas does not rupture the vessel.
3. Grind the material for 60 seconds at 2500 RPMs. If chunks are still visible, repeat grinding step. The final material should be a fine powder.
4. Allow the resulting pulverized mixture to fully out-gas.

Standard Spike

To aid in the process of method validation, below are the steps taken during this research to spike the brownie samples.

1. Using a 1.0 mL gastight syringe, transfer 0.5mL of a 1.0 mg/mL Δ9-THC standard into an empty 50mL centrifuge tube as the spike.
2. Dry down the standard spike using an evaporator, until no solvent is visible. (*Note: there may be a slight purple/pink residue visible.*)

Analytical Procedure

1. Place the centrifuge tube on a balance and tare once stable.
2. Weigh and transfer approximately 5.0g of pulverized brownie material into the centrifuge tube.
3. Add 25mL of DIH_2O to the tube using a graduated cylinder.
4. Add 600µL of lipase enzyme to the centrifuge tube using a 1.0mL gastight syringe.
5. Vortex the tube for approximately 30 seconds.
6. Vari-mix the mixture for 45 minutes.
7. Sonicate the centrifuge tube using hot tap water – approximately 44 to 47 degrees Celsius – in a sonicator for 10 minutes, with shaking by hand every three minutes. (*Place the tube within a beaker also containing hot tap water, and place in sonicator*.)
8. Place the centrifuge tube on the Vari-mixer for approximately 5 minutes to cool.
9. Add 15mL of chloroform to the centrifuge tube using a 15mL Class A glass pipette.
10. Vortex the tube for approximately 30 seconds.
11. Vari-mix the mixture for 45 minutes.
12. Centrifuge the tube at 4000 RPMs for 10 minutes.
13. Remove a sample from the chloroform layer (center of the cone at the bottom of the centrifuge tube) using a disposable borosilicate glass pipette and place in an amber autosampler vial, and cap immediately.

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- *Note: to remove the chloroform extract, the dense brown layer must be penetrated. Once the pipette tip is within the chloroform layer, expel some air from the bulb, withdraw the sample, then let some of the liquid drip out prior to transferring the sample to the autosampler vial.*

Instrumental Analysis

Instrument: UPLC with UV and MS and Quaternary Pump
Column: Agilent InfinityLab Poroshell 120 EC-C18 (3.0x100mm; 2.7µm)
Guard Column: Agilent InfinityLab Poroshell 120 EC-C18 (4.6x5mm; 2.7µm)

- Analyze the chloroform extract(s) via LC/UV or LC/MS using an appropriate calibration curve.
    - With the spike concentration and amount of chloroform used during this research, the expected concentration at 100% recovery would be 0.033 mg/mL of Δ9-THC.
- Run alongside chloroform blanks.
- Analyze a check standard to check the accuracy of the calibration curve.
- The approximate retention time of Δ9-THC using the parameters below is: 2.86 minutes via UV and 2.89 minutes via MS.

Instrument Parameters

DAD (UV):

Spectrum Range: 190 nm – 400 nm (store all)
Wavelength: 230 nm

Column Oven:

Temperature Control: 50.0°C

Quaternary Pump:

Flow Rate: 0.700 mL/min
Total Run Time: 14 min (includes wash and re-equilibration)

Solvent Composition:

Gradient run

A: DIH_2O
B: Methanol
C: 1L DIH_2O + 1mL Formic Acid + 2.2 mL 5M Ammonium Formate Solution*
D: Isopropanol

*For 25mL of stock solution, weigh 7.8825g of ammonium formate into a 25mL flask, then fill to volume with 18.2MΩ DIH_2O.

Gradient Time Table:

Time (min)	% A	% B	% C	% D
0	10.0	85.0	5.0	0.0
6.50	10.0	85.0	5.0	0.0
6.75	0.0	10.0	0.0	90.0
10.75	0.0	10.0	0.0	90.0
11.00	10.0	85.0	5.0	0.0
14.00	10.0	85.0	5.0	0.0

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Autosampler:

Injection Volume: 0.50µL

Needle Wash: 4 second flush port 25:25:50 deionized water: isopropanol: methanol

Mass Spectrometer: **(*OPTIONAL*)**

Ion Source: MMI
Scan Type: SIM/Scan
Delta EMV Pos: 200 V
Mass: 315 m/z
Source Parameters:
- Gas Temperature: 300°C
- Gas Flow: 4.9 L/min
- Nebulizer: 15 psi
- Capillary: 3000 V
- Corona Current: 4µA
- Vaporizer: 200°C
- Charging Voltage: 2000 V

Diverter Valve:
- 0 - 2.5 min: sent to waste
- 2.5 - 3.4 min: sent to MS
- 3.4 - 14 min: sent to waste