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Accurate THC Determinations in Seized *Cannabis* Samples for Forensic Laboratories

Award DJO-NIJ-20-RO-0009

Final Research Report

Principal Investigator
Walter Brent Wilson
Chemical Sciences Division
National Institute of Standards and Technology
100 Bureau Dr., MS 8392
Gaithersburg, MD 20899
(301) 975-3438
walter.wilson@nist.gov

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U.S Department of Justice
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Washington, DC 20531

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100 Bureau Drive
Gaithersburg, MD 20899

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Summary of Project

Statement of the Problem & Project Overview

Since the 1970s, cannabis (marijuana and hemp) and its psychoactive constituent, Δ^9 – tetrahydrocannabinol (Δ^9-THC), have been classified as Schedule I controlled substances. Seized evidence is tested by forensic laboratories, who verify the identity of the plant through macro- and microscopic evaluation and the presence of Δ^9-THC through presumptive and confirmatory chemical testing. Drug scheduling has directed the testing approaches, as qualitative confirmation of the presence of Δ^9-THC was sufficient to demonstrate possession of a controlled substance. In the late 1990s, however, several states legalized cannabis for medicinal use and in 2012, the first states legalized adult recreational use of cannabis. Currently, marijuana and THC remain on the controlled substances list, although medical marijuana is legal in 37 states and recreational marijuana is legal in 11 states as well as the District of Columbia. The 2018 Farm Bill defined hemp as cannabis containing 0.3% or less of decarboxylated-Δ^9-THC (total THC) and removed hemp from the controlled substances list. With these legal changes, forensic laboratories are now required to quantify the level of Δ^9-THC in seized evidence to determine whether the cannabis is marijuana (an illegal controlled substance) or hemp (a legal commodity).

Prior to 2019, almost no forensic laboratories had experience in or were accredited to perform quantitative analysis on any drugs. An extensive review of the literature and initial workshops with cannabis stakeholders that included numerous forensic laboratories revealed that routine testing approaches for the quantitation of total THC are labor intensive and based on technologies that are currently unavailable in most forensic laboratories. Guidance at the time from the DEA has focused on a non-quantitative screening approach that will not meet the needs of state and local jurisdictions. As a result, state and local forensic laboratories are looking to NIST to

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develop and validate rapid quantitative approaches for distinguishing legal hemp from illegal marijuana in seized cannabis samples.

Historically, most forensic laboratories have utilized a qualitative test scheme for seized cannabis samples, which includes macro- and microscopic identification of plant features, colorimetric testing for presence of THC, and gas chromatography – mass spectrometry (GC-MS) confirmation of the presence of Δ^9-THC. GC and liquid chromatography (LC) are the primary separation techniques used for quantitative determination of individual cannabinoids and total THC in cannabis plant samples. Of these two approaches, GC is generally favored in forensic laboratories because of shorter separation times and no solvent consumption. GC may be coupled to either a flame ionization detector (FID) or mass spectrometer (MS), but MS provides the distinct advantage to permit a positive identification of Δ^9-THC based on its mass spectrum in seized samples. Existing qualitative GC-MS approaches utilized by forensic laboratories are amenable to quantitative evaluations with specific analytical modifications to the method.

First, quantitative sample preparation protocols are necessary to ensure complete extraction of cannabinoids from the seized sample prior to instrumental analysis. Additionally, isotopically labeled internal standards are required to account for variations in the sample preparation, injection volumes, cannabinoid interconversions, and MS responses, to provide greatest accuracy. Lastly, the GC-MS instrument must be double-tasked to obtain a mass spectrum of Δ^9-THC for confirmation by operating in full scan mode ranging from mass-to-charge ratio (*m/z*) 50 to *m/z* 350 and/or in single ion monitoring (SIM) mode for quantitation of *m/z* 299 ion.

Part one of this study focused on the sample preparation and quantitative approaches to the determination of THC in cannabis samples. Method development and validation work is required for quantitative methods to ensure straightforward and accurate assessment of seized evidence

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while maintaining the high throughput environment within a forensic laboratory. First, development of simple and robust extraction methods and cleanup procedures for a range of cannabis plant samples are needed for quantitative measurements. Extraction approaches must be exhaustive for Δ^9-THC as well as its acidic form, tetrahydrocannabinolic acid (THCA), which are the major components in marijuana plant samples. In order to streamline sample analysis, GC-MS approaches should include either: (1) simultaneous collection of both full scan and SIM modes; or (2) a SIM-mode method that provides a simplified mass spectrum for Δ^9-THC with one quantitation ion and four confirmation ion peaks. As THCA is thermally labile and converts to Δ^9-THC by decarboxylation (Figure 1), which may occur by the heating of the sample prior to analysis or in the GC inlet. The conversion rate of THCA into Δ^9-THC in the GC inlet ranges from 50% to 70%, preventing the reliable detection of THCA and limiting the accuracy of total THC determination by traditional GC-MS approaches [1]. The proper use of an isotopically labeled internal standard for THCA in the samples and calibrants may account for the conversion of THCA in the GC inlet (Figure 2), but this approach must be thoroughly studied and validated.

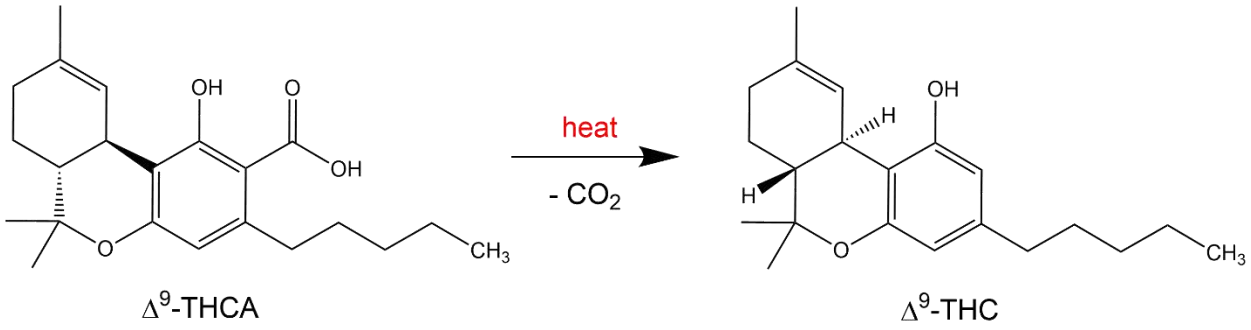

Figure 1. Diagram depicting heat-induced decarboxylation of THCA to Δ^9-THC.

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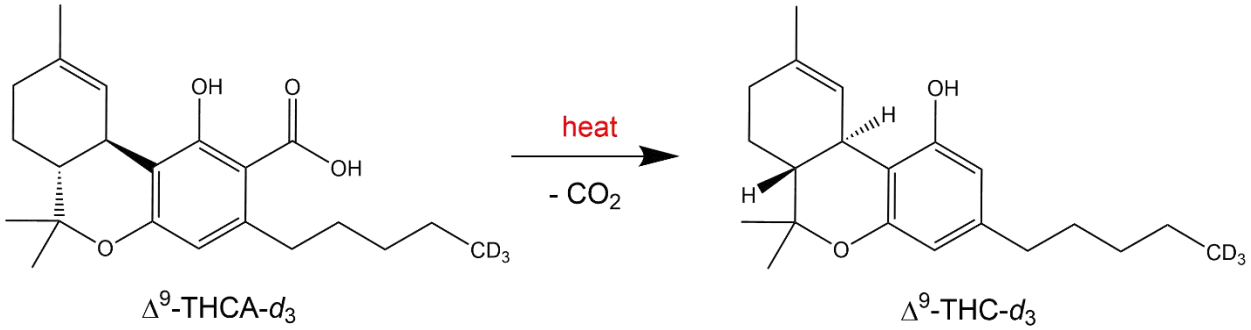

Figure 2. Heat-induced decarboxylation of THCA-d_3 to Δ^9-THC-d_3.

The second part of this study focused on spectroscopic techniques offering rapid, field deployable screening approaches for THC in seized cannabis samples. Infrared (IR) spectroscopy is a commonly used technique in forensic drug analysis [2-6] and is capable of rapid sample analysis with little or no sample preparation requirements when used in conjunction with an attenuated total reflectance (ATR) approach. IR is recognized in seized drug analysis for its high discriminating capability and is designated as a Category A analytical technique for selectivity in SWGDRUG guidance documents [2]. While IR has not been utilized much in the forensic realm for the analysis of cannabis samples, the potential utility for this type of analysis has been demonstrated for the analysis of plant materials and derived extracts.

In this work we conducted method development for the classification of cannabis samples and the quantification of THC using a benchtop research grade FT-IR spectrometer system operating in the near-infrared (NIR) spectral range and utilizing a fiber-optic reflectance probe. Near-infrared (NIR) spectroscopy is a promising approach to quantifying THC and other cannabinoids in cannabis [7]. NIR is widely used in the pharmaceutical, chemical, food, and agricultural industries as a rapid quantitative spectroscopic tool requiring little or no sample preparation for use in field applications, in-line process monitoring, and finished product analysis.

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Four commercially available IR (both NIR and MIR) based cannabis analyzers evaluations are included in this project. These are described as transportable instruments while not necessarily being fit for hand-held operation. These instruments were operated according to the procedures described in their user manuals. Some were capable of measuring intact cannabis flower materials, but all samples were ground for this evaluation to improve homogeneity. In both the MIR and NIR case, data acquisition times are generally short, < 1 min, so it is relatively straightforward to take advantage of multiple samplings to improve accuracy and precision. For the analysis of cannabis plant materials both spectroscopic techniques offer the potential for a rapid, quantitative analysis of total THC content with little sample manipulation requirements. This capability could be valuable in forensic analysis scenarios including field use and to aid in determining whether additional confirmatory sample analysis is necessary.

Major Goals and Objectives

The overall goal of this project is to provide federal, state, and local forensic laboratories with simple, robust, and cost-effective analytical methods for the confident differentiation of hemp from marijuana in seized cannabis samples.

Objective #1: ID-GC-MS Method for Measuring Total THC

This portion of the project was focused on the development of a quantitative ID-GC-MS methods to provide forensic laboratories the ability to confidently distinguish between hemp and marijuana for all seized cannabis samples. For rapid analysis it is imperative that the GC-MS method developed in this study have the ability to operate in the full scan mode collecting *m/z* ion signals for a set range (i.e., *m/z* 50 to *m/z* 350) and/or in selective ion monitoring (SIM) mode for

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a small number of ion signals. The quantitative approach developed in this project needs to be based on the internal standard calibration using isotopically labeled internal standards (Δ^9-THC-d_3 and THCA-d_3) to represent a total THC-d_3 internal standard to help address the issue of THCA decarboxylation in the GC inlet to Δ^9-THC at high temperatures.

Objective #2: Sample Extraction and Cleanup Optimization

Highly efficient and simple extraction methods and sample cleanup procedures were developed to help in the accurate quantitation of total THC. This project will optimize a sample extraction method previously approved by an AOAC expert review panel for the quantitation of cannabinoids in cannabis dried plant using LC-UV [8]. A detailed procedure for the isolation of cannabinoids from cannabis plant samples through sequential ethanol extractions using routine laboratory equipment was developed. While this approach provides thorough extraction and accurate results, the total sample preparation time required (70 to 90 mins) and would not be acceptable in a high throughput laboratory. NIST reduced the sample preparation time to a maximum of 30 min through the investigation of the effect of sample mass, solvent volume, and shaking time on the extraction efficiency.

Objective #3: ID-GC-MS Validation Study

A single laboratory validation study was performed on the ID-GC-MS method following the guidelines outlined in the AOAC Official First Action Method[8]. Calibration and linearity were evaluated based on data obtained for a set of calibration standard solutions using a linear regression function with a particular focus on the fit of the lower region of the calibration curve to facilitate the differentiation of hemp from marijuana. The limits of detection (LOD, S/N = 3) and

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limits of quantitation (LOQ = S/N = 10) for the ID-GC-MS method were determined for total THC. Method accuracy was validated for multiple hemp and marijuana samples with a focus on concentrations around the mass fraction of 0.3% federal limit. Method precision was determined as intermediate precision (RSD_i) using the samples above.

Objective #4: Evaluation of IR Instrumentation

The initial phase of IR method evaluation involved optimization of relevant instrument configuration and acquisition parameters for the benchtop FTIR instrumentation in concert with optimizing sample preparation and replication. Evaluation of sample processing and data acquisition was performed on a small subset of samples, the results of which informed a second large scale data collection effort for quantitative model development based on partial-least squares (PLS) regression and classification model development based on partial-least squares discriminant analysis (PLS-DA). Lastly, four portable IR-based instruments were evaluated for the ability to discriminate between hemp and marijuana samples.

Objective #5: Technology Transfer to Forensic Laboratories

A key component to the success of this project is the implementation of the optimized procedures in federal, state, and local forensic laboratories. To help facilitate this component of the project NIST has prepared standard operating procedures (SOPs), journal publications, conference presentations, and/or in-person training to collaborators at Maryland State Police (MSP and Montgomery Country Police Department (MCPD) crime laboratories.

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Research Questions

Several questions were asked and answered to meet the goals and objective outline above for this project.

1. What are the optimized conditions needed for the development of a GC-MS method to separate Δ^9-THC from the primary cannabinoids detected in seized cannabis samples including Δ^8-THC?
2. Can the use of multiple deuterated internal standards compensate for the decarboxylation of THCA into Δ^9-THC in the GC inlet?
3. What are the optimized conditions for the complete extraction of cannabis samples with total THC concentrations at the 0.3% threshold?
4. Can benchtop and portable IR devices be used to accurately distinguish between hemp and marijuana in seized cannabis samples?

Research Design, Methods, Analytical and Data Analysis Techniques

Research Design

The experiments conducted for this project were designed to provide federal, state, and local forensic laboratories with simple, robust, and cost-effective analytical methods to confidently differentiate hemp from marijuana in seized cannabis samples at the 0.3% threshold value.

Sample Information

For the purposes of cannabis research at NIST, a collaboration with MCPD crime laboratory was established in 2020 to permit the transfer of previously seized and adjudicated cannabis samples to NIST. Four hemp plant reference samples were purchased from the University

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of Kentucky Proficiency Testing (UK-PT) Program. After completion of the PT studies, hemp samples are made available for purchase as reference samples accompanied with a Certificate of Analysis (COA) with assigned mass fraction (%) values and expanded uncertainties (2×SD) summarized in Table 1 for Δ^9-THC, THCA, and total THC. Additional hemp plant samples were purchased from multiple commercial sources. Certified Reference Materials (CRMs) solutions were purchased for an 11-cannabinoid mixture and individual solutions for Δ^9-THC, THCA, Δ^9-THC-d_3, and THCA-d_3 from commercial sources at the highest purity available.

Table 1. Mass fraction (%) values and their expanded uncertainties of Δ^9-THC, THCA, and Total THC.

	Δ^9-THC	THCA	Total THC
HM19SEP-1	0.2480 ± 0.0056	0.03849 ± 0.00346	0.2869 ± 0.0076
HM19SEP-2	0.1097 ± 0.0032	0.0390 ± 0.00320	0.1437 ± 0.0047
HM19NOV-1	0.1609 ± 0.0038	0.1592 ± 0.0050	0.2999 ± 0.0061
HM19NOV-2	0.03506 ± 0.00108	0.0380 ± 0.00199	0.06858 ± 0.00230

Analytical Instrumentation

Sample extractions were performed using ordinary equipment normally found in forensic laboratories including analytical balances, mechanical shakers, and a centrifuge. The LC-PDA measurements were performed on a Shimadzu *Cannabis Analyzer* equipped with a binary pump, degasser, autosampler, column compartment, and a photodiode array detector. Separations were carried out on a NexLeaf CBX for Potency C18 column purchased from Shimadzu with the following characteristics: 15.0 cm length, 4.6 mm diameter, and 2.7 μm average particle diameters. The LC column was protected with the installation of a NexLeaf CBX guard column. The separation conditions were previously optimized by Shimadzu as a "high sensitivity method" and recently evaluated at NIST [9]. The GC-MS measurements were performed using an Agilent HP 6890N gas chromatograph coupled to a 5973 quadrupole mass spectrometer with electron impact ionization. The GC-MS parameters are summarized in Table 2.

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The benchtop, research grade IR instrument utilized in this work was an FTIR system (Bruker Vertex-70) equipped with sources, beam splitters, and detectors for operating in the NIR spectral region using a diffuse reflectance fiber-optic probe for NIR measurements. Classification and quantification models were developed on the data from this instrument using partial-least-squares discriminant analysis (PLS-DA) and partial-least-squares (PLS) regression. Four portable IR detectors were evaluated that included a small, stand-alone, FTIR-ATR unit and three NIR reflectance-based instruments. These devices utilized built-in calibration models and were operated according to the manufacturer's instructions.

Table 2. GC-MS operating parameters.

Inlet/Injection	Split Mode with Wool Liner, 280 °C, 1 µL		
Column	DB-35 ms UI (Agilent Technologies) 15 m × 0.25 mm i.d. × 0.25 µm film thickness		
Carrier Gas Flow	Helium, 1.2 mL/min		
Oven Program	Temp. (°C)	Ramp (C/min)	Hold Time (min.)
	205 °C	Initial	0 min
	230 °C	25 °C/min	0 min
	235 °C	5 °C/min	0 min
	240 °C	2.5 °C/min	2 min
	250 °C	10 °C/min	0 min
	300 °C	40 °C/min	0.75 min
MS Temperature	Transfer Line: 280 °C, Source: 230 °C, and Quadrupoles: 150 °C		
Full Scan Mode	*m/z* 50 to *m/z* 350		
SIM Mode	*m/z* 231, *m/z* 279, *m/z* 299, *m/z* 302, and *m/z* 314		

Expected Applicability of the Research

With the passage of the 2018 Farm Bill, many state and local prosecutors have either stopped prosecuting cases or outsourcing the analytical measurements involving cannabis seizures due to the lack of necessary quantitative analytical methods to confidently distinguish between hemp or marijuana in forensic science. The analytical methods developed in this project consisting of routine instrumentation already utilized in their laboratories for the identification of THC in seized cannabis samples. However, these methods are not limited to only forensic science as there

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is large need for accurate analytical measurements in the discipline of chemistry as the need for research on cannabis is drastically needed. For these reasons, careful attention has been given to making the analytical method and extraction protocols as cost-effective, robust, and straightforward to implement for chemist with a wide range knowledge including undergraduate students, graduate students, and forensic analyst. To support this transition, analytical methods have been technically evaluated by Amber Burns from MSP crime lab. NIST has started to implement and evaluate the analytical methods with personnel at MSP and MCPD crime laboratories. NIST has also prepared a training video to be posted online of the entire sample grinding, extraction, and cleanup prior to analysis via LC-PDA or GC-MS.

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Participants & Other Collaborating Organizations

National Institute of Standards and Technology

Name:	Walter Brent Wilson
Project Role:	Principal Investigator
Contribution to Project:	Dr. Wilson coordinated the entire project.
Name:	Aaron Urbas
Project Role:	IR Specialist
Contribution to Project:	Dr. Urbas conducted NIR and LC-PDA measurements for objective 4.
Name:	Jerome Mulloor
Project Role:	GC-MS Specialist
Contribution to Project:	Jerome conducted all the ID-GC-MS measurements in objective 1 and 3.
Name:	Maryam Abdur-Rahman
Project Role:	Undergraduate Intern
Contribution to Project:	Maryam conducted the sample extractions used for LC-PDA and ID-GC-MS measurements for objective 2, 3, and 4.
Name:	Ewelina Mistek
Project Role:	Post-Doctoral Guest Scientist
Contribution to Project:	Ewelina conducted the data analysis for the NIR and LC-PDA analysis for objective 4.

Project Collaborators

Organization Name:	Maryland State Police
Location of Organization:	Pikesville, Maryland
Point of Contact:	Amber Burns
Organization Name:	Montgomery County Police Department
Location of Organization:	Gaithersburg, Maryland
Point of Contact:	Leah King

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Changes in Approach

The original research plan was to focus on the development of multiple quantitative ID-GC-MS methods to provide forensic laboratories the ability to confidently distinguish between hemp and marijuana for all seized cannabis samples. The first ID-GC-MS method proposed in this project was designed to simultaneously collect full scan and SIM data with quantitative measurements to be performed for Δ^9-THC using the SIM mode at *m/z* 299 and the full scan mode for confirmation purposes through mass spectral matching.

The second ID-GC-MS method proposed in this project was to accommodate GC-MS instrumentation that does not operate in the simultaneous measurement mode. In this method, Δ^9-THC measurements were performed in SIM mode using the *m/z* 299 ion for quantitation and *m/z* 231, *m/z* 279, and *m/z* 314 ions for confirmation purposes using a simplified SIM mass spectrum. The second method approach was investigated to match current forensic laboratory capabilities.

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Outcomes

Activities, Accomplishments, Results, and Findings

Objective #1: ID-GC-MS Method for Measuring Total THC

- Development of a GC-MS method for the separation of Δ^9-THC, Δ^8-THC, and 7 additional commonplace neutral cannabinoids in under 9 min using the method summarized in Table 2. Detailed studies were conducted evaluating the different column stationary phases, temperature programs, column lengths (15 m vs 30 m), and flow rates.
- The GC-MS method was evaluated in both the full scan and SIM modes; however, SIM mode was found to be preferred over full scan mode for quantitation because a selective scan for a handful of ions (*m/z* 231, 271, 299, and 314) as represented in Figure 3 versus a large range (*m/z* 50 – 350) in a given time frame dramatically improved sensitivity.

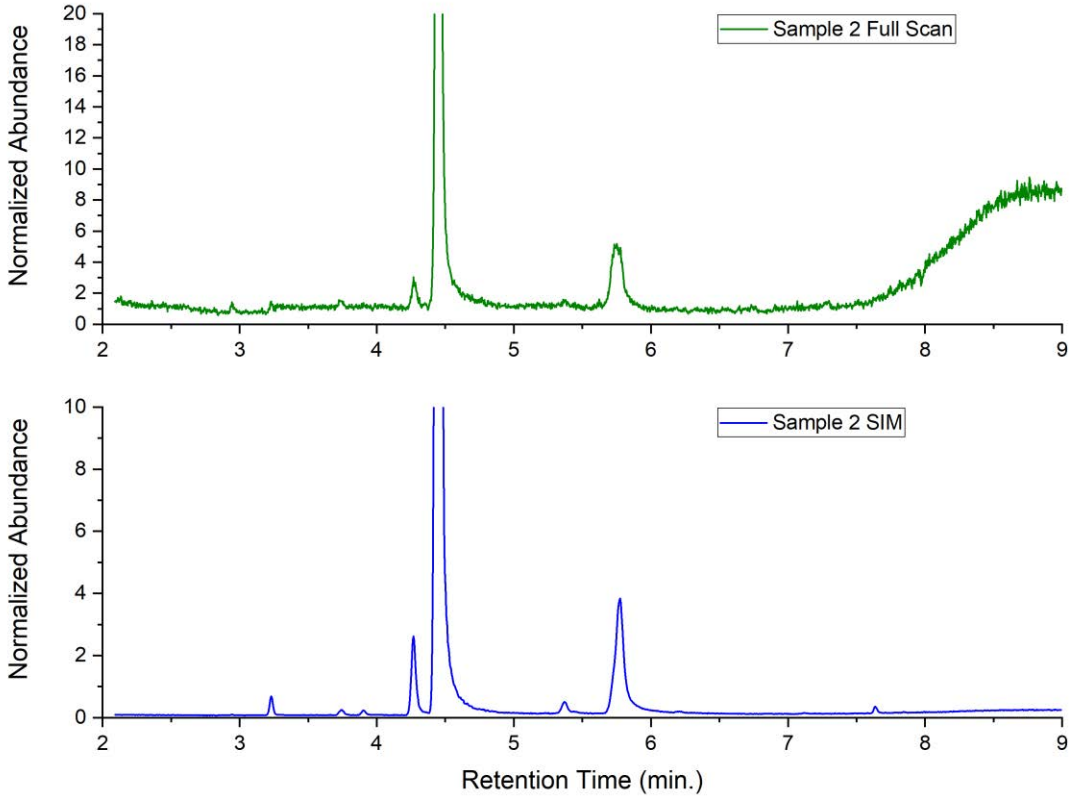

Figure 3. Full scan (top) and SIM (bottom) chromatograms for an example hemp extract.

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- A total THC-d_3 internal standard was evaluated for quantitative purposes using a 1:1 ratio of Δ^9-THC-d_3 and THCA-d_3 to account for the decarboxylation issue of THCA into Δ^9-THC in the GC inlet.
- The GC-MS SIM method developed here enables the forensic analyst to perform quantitative analysis using the *m/z* 299 ion and qualitative analysis using *m/z* 231, *m/z* 279, and *m/z* 314 ions for confirmation purposes using a simplified SIM mass spectrum.
- Sample preparation procedure for the GC-MS method was developed incorporating a 10-fold dilution after sample extraction discussed below.

Objective #2: Sample Extraction and Cleanup Optimization

- Optimization of an extraction method for seized cannabis samples using routine forensic laboratory equipment in less than 15 min was accomplished following the procedure below.
    1. Grind a minimum of 5 g of sample using a small portable high-power grinder in short 10 s intervals to minimize heat generation.
    2. Weigh 0.10 ± 0.01 g of ground samples in a 50 mL centrifuge tube.
    3. Add 20 mL of methanol.
    4. Vortex for 10 s, shake for 5 min, and centrifuge for 1 min at 1500 rpm.
    5. Remove the methanol extract and add a fresh 20 mL of methanol.
    6. Vortex for 10 s, shake for 5 min, and centrifuge for 1 min at 1500 rpm.
    7. Combine the two methanol extracts (40 mL).
    8. Filter the samples using a 0.45 µm PTFE membrane filter.
    9. For GC-MS measurements, sample extracts (100 µL) are spiked with an internal standard working solution of Δ^9-THC-d_3 and THCA-d_3 (≈ 900 µL) prior to analysis.

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10. For LC-PDA measurements, 10-fold and 100-fold sample dilutions are prepared by adding 900 µL and 9900 µL of methanol to the sample extract (100 µL) prior to analysis.

- Approximately 85% of the cannabinoids present in the sample are extracted after 10 s of vortexing.
- A cleaning procedure for the grinding vessel is summarized below to permit their continued use the following day.
    1. Grinding vessel and blade cap were rinsed with hot water for a minimum of 10 s to remove any visible cannabis particles.
    2. Methanol (40 mL) was added.
    3. The blade cap was tightened on the vessel and vigorously shaken by hand for 10 s.
    4. The methanol was removed and fresh methanol (40 mL) was added twice.
    5. Grinding vessel and blade cap were allowed to air dry overnight.

<u>Objective #3: ID-GC-MS Validation Studies</u>

- The analytical figures of merit determined from a single laboratory validation for the developed ID-GC-MS method in objective 1 is summarized below for the analyte Δ^9-THC.
    - Calibration Curve: 1 mg/L to 8 mg/L (0.00013% to 0.001%)
    - Correlation Coefficient (r^2): 0.9950
    - Limits of Quantitation, S/N = 10: 0.25 mg/L (0.000032%)
    - Limits of Detection, S/N = 3: 0.1 mg/L (0.000013%)

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- Measurement accuracy and precision for the determination of total THC mass fraction in eight cannabis samples are summarized Table 3 with (%RSD_r) ranges from ≈ 2.0% to ≈ 7.3%.

Table 3. Mean mass fraction and standard deviation (%) values for total THC.

	LC-PDA	ID-GC-MS
Sample 1	0.291 ± 0.015	0.2574 ± 0.0088
Sample 2	0.1505 ± 0.0040	0.1227 ± 0.0090
Sample 3	0.3228 ± 0.0073	0.279 ± 0.016
Sample 4	0.0609 ± 0.0041	0.0448 ± 0.0027
Sample 5	0.3298 ± 0.0067	0.318 ± 0.011
Sample 6	0.4401 ± 0.0242	0.386 ± 0.026
Sample 7	0.500 ± 0.020	0.478 ± 0.021
Sample 8	0.531 ± 0.015	0.487 ± 0.015

Objective #4: Evaluation of IR Instrumentation

- A total of 75 cannabis samples were analyzed by LC-PDA to develop NIR models and evaluate the commercial IR-based cannabis analyzers
    - These samples included 48 low-THC (< 2%) and 27 high-THC (> 2%) materials. Recognizing that this is an arbitrary threshold, the low-THC materials were high CBD (or CBG) type samples, while the high-THC samples were low CBD and CBG. A single exception to this was observed where a high-THC seized marijuana sample was found to contain a high level of CBD (≈ 9%).
    - Over 50 hemp samples were purchased from multiple commercial sources, including 30 samples analyzed from a single source.
        - 28 of the 30 samples were determined to have a total THC mass fraction value greater than the 0.3% threshold.

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- 26 of the 30 samples were determined to have a Δ^9-THC mass fraction value less than the 0.3% threshold.

- NIR spectroscopic methods were developed using a benchtop research-grade FT-IR system with multivariate statistical data analysis. The full sample set of 75 samples described above bullet point were used in this investigation. Both classification and quantitative models were explored (Table 4).
    - *Partial-Least Squares Discriminant Analysis (PLSDA).* PLSDA models were developed for the differentiation of low-THC (< 2%) and high-THC (> 2%) cannabis samples. Models were constructed and evaluated using both leave-one-sample-out-cross-validation (LOSOCV) and a 60/40 calibration/test (CAL/TEST) splitting. The classification accuracy was found to be 98.5% and 100% accurate for the LOSOCV and CAL/TEST set evaluations, respectively.
    - *Partial-Least Squares (PLS) Regression*. PLS models were developed for the prediction of total THC (%) on an as-received (not dry weight) basis. Models were evaluated using the same LOSOCV and 60/40 CAL/TEST splitting as for the classification models. Summary statistic of the model performance are given in the following table. Prediction errors were on the order of 1% THC.

Table 4. Summary statistics for the NIR reflectance PLS regression models

Metric	LOSOCV	CAL/TEST
RMSEC (THC wt%)	0.582	0.575
RMSECV (THC wt%)	0.846	0.940
RMSEP (THC wt%)	N/A	0.859
Calibration Bias (THC wt%)	0.000	0.000
Cross-Validation Bias (THC wt%)	0.033	0.021
Prediction Bias (THC wt%)	N/A	0.123
r^2 Calibration	0.982	0.982
r^2 Cross-Validation	0.961	0.951
r^2 Prediction	N/A	0.962

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- Four commercial portable IR based cannabis analyzers (both NIR and MIR) were evaluated on a subset of the samples used in this investigation. All instruments reported total THC mass fraction (%) values, which were used to both determine RMSEs for prediction and to classify samples using the low-THC and high-THC designation described based on a 2% total THC threshold as used in the benchtop evaluation. A summary of the results for each system is provided in Tables 5 and 6. The tables were divided into low-THC and high-THC results. Note that for System 2 the LOD for THC was 2% so it was not possible to calculate a RMSEP for the low-THC data set.

Table 5. Summary of results for IR based portable commercial cannabis analyzers for low-THC (< 2%) samples.

	System 1	System 2	System 3	System 4
Samples Measured	39	39	33	33
No Result Reported	3	0	0	0
# < 2 %THC	34	38	29	28
% Correct	87%	97%	88%	85%
RMSEP (THC, %)	1.16	N/A	2.93	1.40

Table 6. Summary of results for IR-based portable commercial cannabis analyzers for high-THC (> 2%) samples.

	System 1	System 2	System 3	System 4
Samples Measured	9	9	11	11
No Result Reported	4	0	0	0
# > 2 % THC	4	8	5	10
% Correct	44 %	89 %	45 %	91 %
RMSEP (THC, %)	2.28	5.73	5.52	2.06

Objective #5: Technology Transfer to Forensic Laboratories

- NIST research chemists have worked directly with state and local laboratories to help in the development and implementation of analytical methods for accurate determination of total THC in seized cannabis samples.

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- Please see the artifacts section below for specific products from this project to help with the technology transfer to forensic laboratories.

Limitations

- The ID-GC-MS method developed here has not been evaluated for cannabis samples with total THC mass fraction values above ≈ 0.6%.
- The internal standards for THCA-d_3 and Δ^9-THC-d_3 are only available for purchase at 100 µg/mL concentrations currently. A higher concentration (1000 µg/mL) would be preferred for the dilution steps.
- All of the ID-GC-MS samples here were extracted and diluted using methanol. Although methanol performed well overall, other solvents, such as acetonitrile or hexane, should also be compared since they are also commonly used for cannabinoid extractions.
- While the developed NIR method proved very effective at differentiating low and high THC plant materials the prediction error for the quantitative models was on the order of 1% THC, precluding the use for accurate differentiation of cannabis plant materials near the 0.3% THC. Lower accuracy for THC content was observed with the commercial portable IR cannabis analyzers. However, some of these systems still performed well for differentiating low and high THC plant materials. Potential interferences that might be encountered in seized cannabis samples were not investigated.

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Artifacts

The following section provides a list of artifacts that were produced or in-production for the dissemination as a result of this award, or related parallel work.

Publications

1. Mulloor, J., Abdur-Rahman, M., Sander, L.C., and Wilson, W.B., A novel approach for accurate measurement of total THC in cannabis plant material by gas chromatography – mass spectrometry using a deuterated total THC internal Standard. *In-preparation*.
2. Urbas, A., Mistek-Morabito, E., Abdur-Rahman, M., Wilson, W.B., and Lednev, I K., Examination of cannabis plant materials by near infrared (NIR) spectroscopy and multivariate data analysis for differentiating low-THC cannabis and high-THC cannabis. *In-preparation*.
3. Mulloor, J., Abdur-Rahman, M., and Wilson, W.B., Development of a gas chromatography – mass spectrometry method for the determination of total THC in seized cannabis samples. *In-preparation*.
4. Wilson, W.B., Urbas, A., and Scott, F., NIST/NIJ Study finds Commercial Hemp Inaccurately Labeled; Legally Marijuana. *In-preparation*.
5. Urbas, A., Abdur-Rahman, M., Romares, A.S., and Wilson, W.B., Determination of Δ^9-THC, THCA, and total THC in commercial hemp samples by liquid chromatography and photodiode array detection.
6. Urbas, A., Abdur-Rahman, M., and Wilson, W.B., Examination of cannabis plant materials by near infrared (NIR) spectroscopy and multivariate data analysis for differentiating low-THC cannabis and high-THC cannabis. *In-preparation*.

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Presentations

1. Mulloor, J., Yarberry, A., and Wilson, W.B., Method development for separation and quantitation of 17 cannabinoids using gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/tandem mass spectrometry (LC-MS/MS). Poster Presentation. American Academy of Forensic Science (AAFS), February 15-19, 2021.
2. Mistek-Morabito, E.M., Lednev, I.K., Wilson, W.B, Urbas, A.A., Multivariate analysis of vibrational spectroscopic data for cannabis plant materials. Oral Presentation. FACSS SciX, September 29, 2021.
3. Mulloor, J., Phillips, M.M., Urbas, A., Yarberry, A., and Wilson, W.B., Evaluation of an isotope dilution gas chromatography – mass spectrometry approach for quantitation of total THC in cannabis plant extracts. Poster Presentation. American Academy of Forensic Science (AAFS), February 21-25, 2022.
4. Urbas, A., Mistek-Morabito, E., Lednev, I., Wilson, W.B., and Phillips, M.M., Analysis of cannabis plant materials by infrared spectroscopy for differentiating hemp and marijuana. Oral Presentation. American Academy of Forensic Science (AAFS), February 21-25, 2022.
5. Wilson, W. B., Accurate THC determinations in seized cannabis samples for forensic laboratories. Oral Presentation. NIJ Symposium, American Academy of Forensic Science (AAFS), March 1-2, 2022.

Training Videos

1. Wilson, W.B., and Sander, L.C., Processing Cannabis Plant Samples. *In-preparation*.

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Disclaimer

Certain commercial products are identified in order to adequately specify the procedure; this does not imply endorsement or recommendation by NIST, nor does it imply that such products are necessarily the best available for the purpose.

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