Forensic Technology
CENTER OF EXCELLENCE
A program of the National Institute of Justice

Published December 2023

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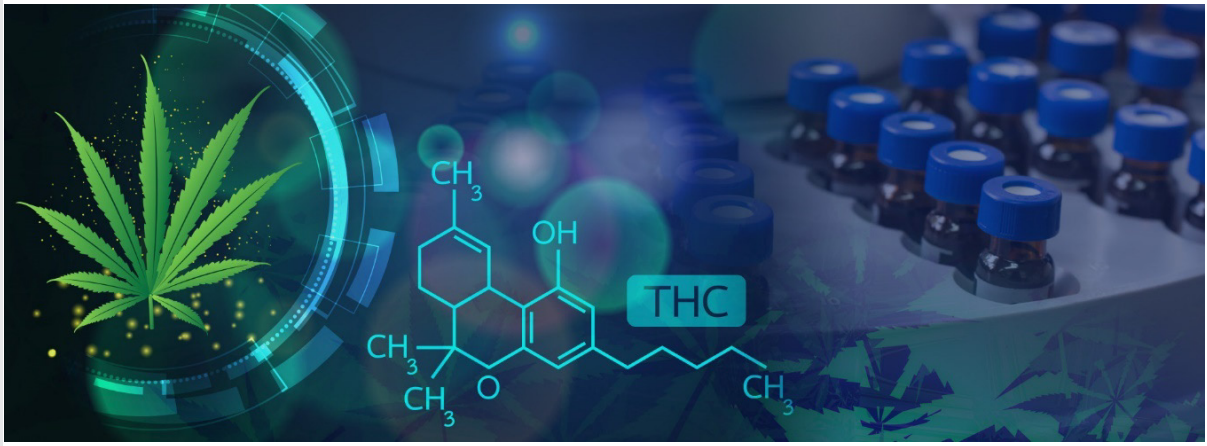
The Forensic Laboratory Needs Technology Working Group (FLN-TWG), formed by the National Institute of Justice (NIJ) in partnership with the Forensic Technology Center of Excellence (FTCOE) at RTI International, created this document in support of NIJ's mission to improve knowledge and understanding of federal, state, local, and tribal forensic science service providers' (FSSPs') technology needs.



TECHNICAL NOTE
Analysis of Marijuana and Marijuana Products

Introduction

In December 2018, the U.S. Congress enacted and subsequently signed the Agriculture Improvement Act of 2018 (Farm Bill) into law. The Farm Bill made two significant changes to drug laws in the United States. First, hemp was defined as "the plant *Cannabis sativa* L. and any part of that plant, including the seeds thereof and all derivatives, extracts, cannabinoids, isomers, acids, salts, and salts of isomers, whether growing or not, with a delta-9 tetrahydrocannabinol concentration of not more than 0.3 percent on a dry weight basis."[1] In the Final Rule, *Establishment of a Domestic Hemp Production Program*,[2] this was further clarified by defining delta-9-tetrahydrocannabionol (Δ^9-THC) as the sum of Δ^9-THC and its associated acid, Δ^9-tetrahydrocannabinolic acid (Δ^9-THCA). Second, the law excluded hemp from the definition of marijuana in the Controlled Substances Act (**21 U.S.C. §801** et seq.). The overarching regulatory authority for hemp resides with the U.S. Department of Agriculture (USDA), and the Farm Bill called for states with hemp production to establish plans and set their own regulatory authorities to meet the requirements. As such, many states immediately began updating their corresponding legislation.

These new legal definitions fundamentally changed the analytical requirements for cannabis testing. Most laboratories had not included methodology in their analytical schemes to evaluate the concentration of total Δ^9-THC.

In fact, a quantitative element or purity determination was never required for the identification of a controlled substance. Therefore, new schemes for evaluating cannabis plant material needed to be developed. Historical colorimetric tests, such as the Duquenois-Levine test, do not have the capability of differentiating between marijuana-type and hemp-type cannabis. Other colorimetric tests, such as 4-aminophenol and Fast Blue BB, have been evaluated for the differentiation of marijuana-type and hemp-type cannabis[3,4] and can be incorporated as a presumptive test in an analytical scheme or used in the field. However, to determine the quantity of Δ^9-THC more accurately, instrumental tests are required.

During this same period, the number of states decriminalizing or legalizing smaller quantities of marijuana, the number of regulated marketplaces selling marijuana and myriad marijuana products, and the number of hemp-derived products containing a variety of cannabinoids greatly increased. This explosion of marijuana- or hemp-derived products created additional analytical difficulties by increasing the number and types of matrices for which the concentration of Δ^9-THC must be measured to determine if it is above allowable limits.

Isomers and derivatives of Δ^9-THC have also increased the complexity of the analysis of marijuana and marijuana products requiring additional method development and validation to ensure proper method selectivity. Some jurisdictions have added additional THC isomers into what is included in the total THC value used to legally define marijuana.[5] In the presence of other common cannabinoids, Δ^9-THC isomers (e.g., Δ^8-THC, Δ^{10}-THC, $\Delta^{6a,10a}$-THC, and exo-THC) can be challenging to separate chromatographically and may or may not be found naturally in marijuana plant material. THC derivatives (e.g., THC-O acetate and THC-P) can also be found in samples submitted to a forensic laboratory. As a result, it is critical that laboratories have access to certified reference materials to ensure that their methodology is fit for purpose.

This technical note provides a summary of the different types of sample preparation and instrumental methodologies that can be leveraged to identify marijuana and marijuana products. In addition, we discuss options for semi-quantitative and quantitative testing. Colorimetric tests may be incorporated into a laboratory's analytical scheme but are not the focus of this technical note. Depending on the type of material or sample matrix, sample preparation may include multiple processes (e.g., sample drying, decarboxylation, homogenization, and derivatization). Typical dissolution extraction methods used in many seized drug analyses may not be the most effective for complex matrices. Solid phase extraction (SPE) and QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) provide useful alternatives. Although not an exhaustive list, the instrumental methods discussed include the following:

- Gas chromatography-flame ionization detector (GC-FID)
- Gas chromatography-mass spectrometry (GC-MS)
- Direct analysis in real time high-resolution mass spectrometry (DART-HRMS)
- Liquid chromatography-ultraviolet spectroscopy (single wavelength or diode array) (LC-UV)
- Liquid chromatography-mass spectrometry (e.g., LC-MS/MS, LC-HRMS).

Sample preparation

Sample preparation varies widely depending on whether a semi-quantitative or quantitative method is necessary to answer the question at hand. For many, but not all, typical suspected marijuana samples submitted to a forensic laboratory, the concentration of total Δ^9-THC far exceeds the legal limit for hemp. Semi-quantitative methods have been demonstrated effective in answering the question, "Does this material have a THC concentration greater than 0.3% on a dry weight basis?".

Semi-quantitative methods

Semi-quantitative methods must reliably determine that the Δ^9-THC concentration in a sample is above or below an established cut-off value and are useful when the actual Δ^9-THC concentration is not required. Many times, an internal standard is used to establish a Δ^9-THC to internal standard ratio of peak height/peak area. This ratio can be compared against a validated cut-off or a control analyzed with the samples.

Sample preparation for semi-quantitative methods can take advantage of the relatively high concentration of Δ^9-THC in most marijuana samples, making it quicker and less

resource-intensive than full quantitative methods. Sample preparation can consist of sample weighing, extraction into a solvent, vortexing for approximately 30 seconds, and filtration (if needed) into an autosampler vial.[6]

Most samples (except for fresh plants) can be analyzed without a drying or decarboxylation step. Decarboxylation of THC is not necessary for methods such as high-performance liquid chromatography (HPLC) that measure Δ^9-THC and Δ^9-THCA independently. For methods based on gas chromatography, approximately 70% of the Δ^9-THCA present is converted to Δ^9-THC in the injection port, which is sufficient for a semi-quantitative method.[7] In some environments, high-concentration cannabidiol (CBD) has been reported to degrade into Δ^9-THC. [8] Laboratories should assess the potential for CBD degradation in their method validation and with method controls.

The National Institute of Standards and Technology (NIST) has prepared several training videos regarding preparation and processing of cannabis samples.[9]

Drying

Dry weight refers to plant material that has been harvested and dried and is therefore ready for use. Water found naturally in fresh plant material increases the overall weight of the material, thus decreasing the percentage of Δ^9-THC by weight.

For quantitative methods, samples can be dried prior to analysis, or the water level within the samples can be measured and accounted for post-analysis in the concentration calculations. The Cannabis Laboratory Quality Assurance Program (CannaQAP) has evaluated several drying and moisture determination methods used by laboratories.[10] Drying methods include desiccator drying, forced air oven drying, or vacuum oven drying. Thermogravimetric analysis was evaluated as the method of moisture determination.

Laboratory-developed drying methods should be validated to ensure that drying conditions do not degrade cannabinoid targets and should designate drying times and temperatures.

Grinding

Unlike other drug samples, such as powders, a cannabis plant sample is known to be non-homogenous with varying concentrations of cannabinoids in different parts of the plant. Quantitative results reflect the concentration in the sample taken; therefore, it may be necessary to homogenize the sample before weighing out an aliquot(s) for analysis.

Mill grinders with disposable grinder cups are convenient and minimize sample contamination from reused equipment. Mills cost $2,000–$5,000, and disposable grinder cups cost approximately $10/each. Coffee grinders can serve as a more affordable alternative; however, they must be cleaned thoroughly between uses because of the potential for cross-contamination.

For more complex matrices, such as food or gummy candy, grinders may need cryogenic capability to make the sample brittle enough for proper grinding.[11] After grinding, these samples must be kept cold or they will return to their previously sticky nature. Mills with liquid nitrogen cooling generally cost between $10,000 and $25,000 depending on their sample capacity.

Decarboxylation

Because the decarboxylation in the injection port is incomplete, samples should be decarboxylated prior to analysis for quantitative methods. If the decarboxylation procedure uses temperatures that are too high or heating for too long, the THC may degrade resulting in inaccurate quantitation results.[12] Decarboxylation can be achieved using an oven or heat block, although a study by the Virginia Department of Forensic Science showed that the heat block proved more efficient for decarboxylation with minimal degradation of THC to cannabinol.[13]

Extraction of marijuana from plant and products

The proliferation of cannabis products, coupled with the need to differentiate marijuana from hemp based on the total Δ^9-THC concentration, has made choosing the proper extraction technique critical for identification. The extraction technique must minimize matrix effects, maximize recovery, reduce ion suppression if followed by LC-

MS/MS, and maximize the use of resources such as analyst time and funds for supplies.

One consideration for any extraction technique is the use of plastic labware. Storing cannabinoid solutions in plastic will dramatically reduce the recovery of the cannabinoids. Cannabinoids in solution tend to adsorb into plastic quickly. This is an issue for both sample preparation and analytical analysis for any extraction technique. Wolf et al. demonstrated that less than 1% of their chosen cannabinoids were left after storage for 16 hours in plastic labware.[14]

To a lesser degree, glass labware can also be problematic, but the use of silanized vials can decrease adsorption and improve the recovery of cannabinoids.[15]

For plant materials and products without added lipids or sugars (e.g., vaping liquids), a simple liquid extraction will often be sufficient to enable extraction and recovery. Care must be taken when selecting an extraction liquid, as methylene chloride has been shown to degrade both Δ^9-THC and CBD.[14] Nonpolar solvents are suitable for the extraction of non-acidic cannabinoids, but for the analysis of total THC (Δ^9-THC + Δ^9-THCA), a more polar solvent is necessary. The United Nations Office on Drugs and Crime (UNODC) recommends a 9:1 methanol:chloroform or 80:20 acetonitrile:methanol (v/v) solution,[16] but 100% methanol has also been found to be effective for plant material.[17] Liquid extraction is relatively fast and inexpensive to carry out and requires no additional specialized equipment.

For other marijuana products, such as gummies, chocolates, baked goods, or beverages, the composition of the matrix will dictate the appropriate extraction technique. For beverages or other liquid preparations, **SPE** may be appropriate. SPE is a technique for separating components dissolved or suspended in a liquid matrix based on their affinity for the solid phase sorbent and solubility in the solvent. Desired analytes are either initially retained on the sorbent material while interferents are washed away or washed in the first elution step. SPE is generally a multi-step process requiring conditioning of the column, loading the sample, washing the column, and eluting the sample. Extraction by SPE takes approximately 3 hours per 48-sample batch, including sample preparation (homogenization and post-extraction solvent evaporation) and costs approximately $5 per sample, accounting for both the columns and solvents needed. SPE generally requires a vacuum manifold, which can allow for multiple samples to be processed together and can be automated at an additional cost. Most manifolds allow for 12–48 samples to be extracted in a batch, are available from a variety of vendors, and cost approximately $775–$2,500. Depending on the manifold capacity, multiple manifold sessions may be necessary for a 48-sample batch. There are many SPE columns available from multiple vendors, some of which are marketed as specific to Δ^9-THC/cannabinoids; however, as is often the case, it may be necessary to optimize the vendor-published methods.

SPE may yield good results for liquids, but it is not capable of separating the large quantities of sugars that may be present in edible marijuana products such as brownies and gummies; it may be able to remove some quantities of oils/fats present. For these products, a modified **QuEChERS** method may be best. This method combines a first phase liquid microextraction with a dispersive solid phase extraction (with the sorbent added directly to the analyte tube creating a slurry) and allows for the advantages of each method while eliminating the need for multiple wash and elution steps that might be necessary on a traditional SPE column. QuEChERS methods cost approximately $3 per sample and take about an hour per 48-sample batch, including equilibration time. Since QuEChERS methods do not require a vacuum manifold, batch sizes are not limited to manifold capacity.

Derivatization

Derivatization may be desired for several reasons. The high temperature of the GC inlet will decarboxylate THCA; therefore, derivatization will be required to detect thermally labile phytocannabinoids. Derivatization can improve chromatography and reproducibility of GC-based quantitation and is typically accomplished with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS). Although derivatization may improve chromatographic peak shape, care should be taken to ensure the method can separate all the derivatized isomers. Holler et al. reported that the use of perfluoroacid anhydrides in combination with perfluoroalcohols can result in isomerization of Δ^9-THC to Δ^8-THC.[18]

Instrumentation

It is important to understand the advantages and disadvantages of different instrumentation as it relates to identification and quantitation of Δ^9-THC and other cannabinoids. GC methods can be developed using a variety of different stationary phases, and it is important to evaluate how well the method separates isomers of Δ^9-THC, such as Δ^8-THC and exo-THC. Typical GC methods use relatively cheap authentic standards and deuterated standards may or may not be used, depending on whether absolute quantitation or threshold values are desired. Ensuring compound separation is critical when using GC-FID because it is a presumptive test that only provides a retention time. If the method is developed for GC-MS, the mass spectrometer may be used in full scan, SIM/scan, or SIM mode, based on the method validation. Full scan and SIM/scan have the added benefit of providing a full mass spectrum to assist in the identification of individual cannabinoids, although the mass spectra of Δ^9-THC isomers may not always be distinguishable. A more polar column, such as a DB-35 stationary phase, can help with separation of these isomers. Like the fentanyls and cathinones, there are new isomers of THC emerging that will have very similar mass spectra to Δ^9-THC.

With dilute and shoot methods, it is important to evaluate the effects of repeated injections on the sensitivity and robustness of the method. The type of inlet liner used can also greatly affect the robustness of the method. Certain liners and higher injection port temperatures can cause conversion of CBD to Δ^9-THC, particularly in samples with a high concentration of CBD.[19] This should be monitored within a quality control program. With marijuana preparations and edibles, the possibility of fouling the injection port is even higher if fats, sugars, oils, and other interfering substances are not removed from the matrix prior to analysis.

LC is capable of identifying cannabinoids and their acids with a fast and simple method. LC has some advantages, especially when dealing with complex matrices of marijuana preparations and edibles, because the methods are more adaptable to handle challenging matrices. LC may also be more appropriate for analyzing thermally labile compounds like THCA without derivatization.

When performing a full quantitative analysis using LC, it is important to have matrix-matched standards, which can be difficult to purchase or make because of the variety of marijuana products on the market. It can also be expensive to purchase certified reference materials of the cannabinoids and deuterated internal standards. LC-MS/MS is appreciably more sensitive than other techniques, thus allowing detection of low levels of THC in a matrix. LC-MS/MS also has a wide dynamic range for quantitation and has been shown to easily differentiate coeluting cannabinoids with different molecular weights. As with GC-based techniques, however, care must be taken to ensure isobaric cannabinoids with similar fragmentation are sufficiently separated by the method. The Δ^9-THC isomers are difficult to separate, and the increased presence of new isomers emerging in the last few years may challenge a method that was created prior to their existence. LC-MS/MS methods can be easily adapted to incorporate new compounds. Instruments like LC-UV can provide presumptive information about the cannabinoids present but will not be as amenable to the incorporation of new compounds in instances where co-elution occurs. Although LC-UV instruments are less expensive than GC-MS or LC-MS/MS instruments, they are also less sensitive and specific, and they require complete chromatographic separation of compounds of interest.

DART-HRMS is a quick screening instrument that can handle high throughput with minimal concerns of carryover. Very little sample preparation is needed prior to determining if a sample has a cannabinoid(s) present.[20,21] However, derivatization may be required to differentiate between Δ^9-THC and CBD because they are isobaric and have similar fragmentation. The complexity of the matrix generally does not affect the ability to detect THC, and therefore this instrument may be useful to screen suspected marijuana preparations and edibles to determine if cannabinoids are present. Given that it is a screening technique, a second instrumental technique would be necessary to confirm or quantitate Δ^9-THC.[22, 23]

Although nuclear magnetic resonance instruments are accurate, reproducible, and insensitive to impurities like chlorophyll and lipids, they are not commonly used in forensic laboratories to quantify Δ^9-THC and related

compounds. The technique lacks the ability to separate the multiple cannabinoids that are similar in structure.

Semi-quantitative (decision point) testing

To screen suspected marijuana samples efficiently, laboratories have developed and validated semi-quantitative screening methods with minimal sample preparation steps that are used within their qualitative analytical scheme for marijuana and hemp testing analysis. If using a GC-based technique, these methods evaluate the total THC concentration by converting Δ^9-THCA to Δ^9-THC via decarboxylation in the injection port. Although as noted previously the conversion is only approximately 70%, it is sufficient for this semi-quantitative determination for many samples that are not near the 0.3% threshold. The resulting total Δ^9-THC instrument response is ratioed against the response of an internal standard (IS). The sample ratio is then compared with the laboratory determined administrative threshold, which is based on the ratio calculated from a 1% THC/IS reference material. Because of this threshold comparison, these methods are also referred to as "yardstick" or "decision point" methods. If the plant material is found to have a THC/IS ratio above the 1% administrative threshold, in addition to other positive tests within the laboratory's analytical scheme, the plant material is reported as marijuana. If the THC/IS ratio is found to be below the 1% administrative threshold, the laboratory may indicate that the plant material is "cannabis" or "inconclusive" and that the sample would require additional quantitative analysis to determine the exact concentration of THC for distinction between no controlled substance (industrial hemp) and marijuana.

Because of the small percentage of samples submitted to forensic science service providers with low total Δ^9-THC concentrations, the semi-quantitative method often provides the required information and the more time-consuming quantitative analysis is unnecessary. LC-based methods are also suitable for semi-quantitative screening methods; however, if the sample is not decarboxylated prior to analysis, the Δ^9-THC and Δ^9-THCA will be assessed separately and combined mathematically using a molecular mass conversion ratio (Δ^9-THC + (0.877*Δ^9-THCA)). Once validated for plant material, semi-quantitative methods can also be validated for use with cannabis extracts, oils, and vape cartridge content samples.

Quantitative testing

If quantitation is required, additional sample preparation is generally needed to ensure that the sample is homogenous, dry, and appropriate for the instrumental technique before analysis. Depending on the method, sample derivatization may also be necessary. Extensive validation is required to ensure that the selectivity, linearity, limits of detection and quantitation, recovery, accuracy, repeatability/ reproducibility, ion suppression/enhancement, and extract stability are fit for purpose. Not all validated methods published to date have assessed the selectivity of the method with regards to the THC isomers and derivatives less likely to be found in natural cannabis plant material (e.g., Δ^{10}-THC, $\Delta^{6a,10a}$-THC, exo-THC, THC-O acetate, and THC-P).

Samples with complex matrices such as edibles (e.g., gummies, brownies, and crisped rice treats) further complicate the analysis. Additional sample preparation is generally needed to maximize analyte recovery and minimize matrix interferences prior to instrumental analysis.

Quantitative testing is made additionally complex by the "by dry weight" language in the legislation. Several questions and interpretations remain about "how dry is dry?" for any material, including plant materials. For example, non-aqueous materials such as oils may have no measurable water but are still liquid. Additionally, the interpretation of Δ^9-THC concentration is complicated in complex matrices, which may contain sufficient absolute quantities of THC to be psychoactive but when ratioed to the entire weight are well below 0.3%. It is unclear in many circumstances if this meets the definition of "hemp" or not.

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Appendix A: Online published standard operating procedures

Arkansas State Crime Lab, Arkansas Forensic Chemistry Quality Manual, Revision 03/05/2021, https://www.dps.arkansas.gov/wp-content/uploads/DRG-DOC-01-Quality-Manual-41.pdf (accessed November 8, 2023)

- 9.10 Semi-quantitative Determination of Δ^9-THC (GC-MS SIM/Scan)
- 9.11 Quantitative Determination of Δ^9-THC (GC-MS SIM)

Drug Enforcement Administration, Summary of Validated Qualitative Methods, Revision 12072022, https://www.dea.gov/documents/2022/2022-12/2022-12-07/summary-validated-qualitative-methods (accessed November 8, 2023)

- THCSCRN – Separation of Controlled and Non-controlled Substances by Gas Chromatography

Drug Enforcement Administration, Summary of Validated Quantitative Methods, Revision 12072022, https://www.dea.gov/documents/2022/2022-12/2022-12-07/summary-validated-quantitative-methods (accessed November 8, 2023)

- DEA 250 – Quantitation of Δ^9-Tetrahydrocannabinol (THC) and Δ^9-Tetrahydrocannabinolic Acid (THCA) by Liquid Chromatography
- Additional information available upon request.

Houston Forensic Science Center, Seized Drugs Standard Operating Procedures, Revision 2021-07-30, https://records.hfscdiscovery.org/Published/Standard%20Operating%20Procedures%202021-07-30.pdf#search=seized%20drugs%20standard%20operating%20procedures (accessed November 8, 2023)

- 8. Gas Chromatography/Mass Spectrometry (GC/MS) Decision-Point Assay for delta-9-Tetrahydrocannabinol (THC) in Plant Substance (SIM/Scan)

Palm Beach County Sheriff's Office, CH Cannabis Methodology, Version 12, https://pbso.qualtraxcloud.com/showdocument.aspx?ID=1952 (accessed November 8, 2023)

- 1% Threshold Testing by Gas Chromatography/Mass Spectrometry (For Cannabis Potentially Containing Tetrahydrocannabinol)

Texas Department of Public Safety, Seized Drug Manual, Revision 8/08/2022, https://txdpslabs.qualtraxcloud.com/ShowDocument.aspx?ID=43051 (accessed November 8, 2023)

- SD-03-06 Instrumental Analysis of Cannabis Sativa L. (Decision Point – SIM/Scan)
- SD-03-07 THC Decision Point Method for Oils and Vape Cartridges

Virginia Department of Forensic Science, Controlled Substances Procedures Manual, Revision 22, https://dfs.virginia.gov/wp-content/uploads/221-D100%20Controlled%20Substances%20Procedures%20Manual-2480-9.pdf (accessed November 8, 2023)

- 6.7 Semi-quantitative Gas Chromatography-Flame Ionization-Mass Spectrometry (GC-FID-MS)
- 6.8 Quantitative Analysis of Total THC in Plant Material using GC/MS (SIM)

Appendix B – Vendor application Notes

The vendor application notes below are provided for reference only and do not indicate any recommendation by the National Institute of Justice, the Forensic Technology Center of Excellence, RTI International, or the members of the Forensic Laboratory Needs – Technical Working Group.

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- Deckers, C., & Roy, J.F. (2022, December 19). Simple and accurate quantification of THC and CBD in Cannabis-infused chocolate edibles using Agilent Captiva EMR—Lipid removal and the Agilent 1260 Infinity II LC System, Agilent Application Note 5994-2873EN. https://www.agilent.com/cs/library/applications/application-thc-cbd-chocolate-captiva-emr-5994-2873en-agilent.pdf

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Appendix C: Webinars

The archived webinars below are provided for reference only and do not indicate any recommendation by the National Institute of Justice, the Forensic Technology Center of Excellence, RTI International, or the members of the Forensic Laboratory Needs – Technical Working Group.

FTCOE marijuana webinars

All Is Not Pot That's Green: An Overview of THC Isomers
Dr. Svante Vikingsson
June 15, 2023 (original viewing date)

Development of Analytical Methods for Measuring Δ9-THC in Cannabis Products
June 27, 2023 (original viewing date)

Cannabinoid Conundrums Webinar Series Expert Panel
August 3, 2023 (original viewing date)

Cayman Chemical webinar

Williams, J. B. (2023). *Isomers, homologs, and analogues of THC - Challenges for Identification and separation of new psychoactive substances*. Presented as part of the 2023 Current Trends in Forensic Toxicology Symposium https://www.caymanchem.com/literature/thc-isomers-homologs-analogues?utm_source=Master+Send+List&utm_campaign=7f5d7c8193-20230622-+FORENSIC%3A+THC+Webinar&utm_medium=email&utm_term=0_56fbb0cb7a-7f5d7c8193-88762813&mc_cid=7f5d7c8193&mc_eid=293dec2237

Additional resources

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The FTCOE, led by RTI International, is supported through a Cooperative Agreement from the National Institute of Justice (15PNIJ-21-GK-02192-MUMU), Office of Justice Programs, U.S. Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this report are those of the author(s) and do not necessarily reflect those of the U.S. Department of Justice. Information provided herein is intended to be objective and is based on data collected during primary and secondary research efforts available at the time this report was written.

Suggested Citation

Jackson, L., McConnell, A., Scott, F. (2023, December). *Analysis of Marijuana and Marijuana Products*. Forensic Technology Center of Excellence. RTI International.