Washington State
Liquor and Cannabis Board

WSLCB Cannabinoid Science Work Group

Report Concerning

Detectable Levels of THC and

Cannabis Product Safety

October 2023

Executive Summary

This is the first report of the Cannabinoid Science Work Group (CSWG). It focuses on providing implementation pathways to determine “detectable” levels of THC as described by E2SSB 5367, now codified as RCW 69.50.101(h)(1)[(8)(a)]. The subgroup critically reviewed, compared, and contrasted materials pertaining to cannabis product testing and production standards from several resources, including but not limited to the Association of Analytical Chemists (AOAC), the American Society for Testing and Materials (ASTM), and the United States Pharmacopeia Food Chemical Codex (USP-FCC). The CSWG asserts that the relationship between the limit of quantification (LOQ) and limit of detection (LOD) is predictable, and relatable 3 to 1. Currently, only LOQ is described in rule (WAC 314-55-102(3)) regarding potency analysis for four cannabinoids (CBD, CBDA, Δ9-THC, and Δ9-THCA). The rule describes an LOQ of 1.0 mg/g or 0.1%. Therefore, the LOD corresponding to this regulatory requirement is 0.03%. This limit can be achieved by laboratories that use methods that have specifications equal to or better than those of the AOAC methods. The group also discussed potential variances in detectable concentrations of THC in various product types and offers ranges of detection based on product type, along with suggestions and considerations for cannabis product safety.

Introduction and Background

In 2012, the legal landscape of cannabis cultivation in Washington shifted when Washington voters approved Initiative 502, allowing for legal production, processing, sale, and use of cannabis. Two years later, the federal Agricultural Act of 2014 (2014 Farm Bill) allowed states to implement pilot programs for the production of industrial hemp. In 2018, the federal Agriculture Improvement Act of 2018 (2018 Farm Bill) went a step further, legalizing hemp more broadly by removing hemp from the federal schedule of controlled substances.

By 2020, several states, including Washington, had established legal hemp production programs. However, by 2021, there was a national oversupply of legal hemp, particularly CBD-rich hemp. While the US Food and Drug Administration (FDA) prohibits CBD in foods and dietary supplements, wellness products like CBD tinctures and gummies gained popularity and became easy to obtain. As this unregulated market for CBD products became increasingly competitive, some hemp product manufacturers began taking advantage of the 0.3% federal limit for THC in hemp products by making food items that contained amounts of THC comparable to or exceeding the limits for THC in adult use cannabis edibles in Washington. Other hemp product manufacturers began chemically converting hemp-derived CBD into other cannabinoids, such as delta-8-THC. Many of these unregulated food products and dietary supplements containing hemp-derived cannabinoids were and continue to be sold in gas stations and convenience stores and can be easily purchased online.

As a result of this evolution in cannabinoid production, additional legislation was considered since the original I-502 system contemplated only products containing delta-9 tetrahydrocannabinol. This limitation narrowed WSLCB's ability to address concerns. For this reason, during the 2022 and 2023 sessions, the Washington state legislature placed emphasis on providing consumers with legal access to products containing cannabinoids that had been tested and met the same standards for quality and safety as products sold in the I-502 system.

During the 2022 session, legislative efforts to address these concerns did not advance including Senate Bill 5981. Introduced in the senate on February 24, 2022, the bill contained a provision that would have established a scientific panel to review available research, data, and regulations of other jurisdictions related to cannabinoids, and make recommendations on potential guidelines for safe methods of manufacturing, extracting, and synthesizing cannabinoids. Instead of waiting for the legislature to act in a subsequent session, the WSLCB established a similar scientific panel when the agency created the Cannabinoid Science Work Group (CSWG) in the fall of 2022.

During the 2023 legislative session, Engrossed Second Substitute Senate Bill 5367 passed and became law on July 23, 2023. The bill expanded the definition of "cannabinoid product" to include "any product intended to be consumed or absorbed inside the body by any means including inhalation, ingestion, or insertion, with "*any detectable amount of THC*" (Emphasis added).

Structure

In early October 2022, WSLCB began to assemble a group of experts through open recruitment to regularly communicate about the rapidly changing issues related to cannabinoids. Recruitment closed on October 24, 2022, and successful applicants were notified in mid-November. The Cannabinoid Science Work Group (CSWG) convened once every other month. Kathy Hoffman, Ph.D., Policy and Rules Manager chaired the CSWG. The first meeting of the CSWG occurred on December 1, 2022. The last meeting is scheduled to occur on December 7, 2023. All CSWG meeting agendas, minutes, and associated materials can be found on the WSLCB outreach and public engagement website.

Since there was not a legislative mandate to frame or guide the work of the CSWG, the stated purpose of the CSWG was to explore and begin to build a foundational understanding of the cannabis plant, in addition to synthetic equivalents of the substances contained in the plant collaboratively and transparently.

The first two CSWG meetings held on December 1, 2022, and February 1, 2023, focused on identifying, narrowing and prioritizing areas of interest that aligned with the Board's interests. Interest areas were subsequently ranked based on the number of members interested in an identified topic, and a vote to confirm rankings was held between meetings by email.

The third meeting held April 6, 2023, focused on the primary area of shared interest, which was diminishing the gap between scientific expression and regulatory/statutory expression, the group began to identify where to begin discussion. Identified topics included:

- What types of processes might be allowable to create safe products?
- Defining "regulation" of cannabis.
- Hemp-derived cannabinoids.
- Education: What type of information can help people make better decisions about what they consume?

The fourth meeting was held on June 6, 2023, during which the group continued the discussion, focusing on:

- Human safety guidance.
- Production/farming guidance.
- Discussion of E2SSB 5367 and "any detectable amount of THC."

During the meeting two subgroups were formed. One group would focus on cannabis product safety guidance. The second group would both explore how to approach the "detectable amount of THC" standard established by E2SSB 5367 and to begin a discussion of a potential framework for future product standards. Multiple subgroup meetings were established. A substantial amount of interaction occurred prior to

subgroup meetings by way of telephone and email exchange, leading to highly productive, efficient subgroup meetings.

The fifth meeting occurred on August 3, 2023, and consisted of subgroup reports. That work is described below.

Detectable Levels of THC and Future Standards

This subgroup met on July 12 and August 22, 2023. Discussion included surveying private Washington State cannabis testing labs to inquire about instrumentation and procedures to measure THC. The results could help the group distinguish theory from what is reliably possible in Washington. The survey may also help the group better understand the limits of current technology and move toward contemplating safety standards. The group also began to create a matrix, or "buckets" by product type to assist in identifying limits of THC detection by product type.

Cannabis Product Safety Guidance

This subgroup met once on July 20 and August 24, 2023, and began to create similar "buckets" by product type (flower, concentrates, edibles). For each product type, the group began to identify product and production standards. The group also began to discuss environmental versus consumption concerns, remediation, food safety, and gaining a better understanding of what "total exposure" means when agencies are performing toxicology work on contaminants.

WSDA Collaboration and Subgroup Merger

On August 21, 2023, Dr. Hoffman learned that WSDA was preparing to distribute a survey to Washington accredited cannabis testing labs to better understand their cannabis testing methods, estimates of measurement uncertainty and variability between labs, and potential shortfalls where labs may not have appropriate methodology if accreditation is selected for any of the three standardized tests being contemplated in WSDA draft rule. Since this aligned with some of the information the CSWG subgroups sought, LCB asked if collaboration was possible to reduce stakeholder survey fatigue and increase efficiencies across agencies. WSDA agreed, and CSWG subgroup members offered feedback on draft survey questions. The agencies met on August 28, 2023, to discuss next steps. As of October 5, it was the groups understanding that the survey had been distributed to labs. This report can be supplemented with the WSDA survey results when they become available.

Additionally, LCB Enforcement & Education staff gathered additional information from RJ Lee to determine cannabinoid LOD/LOQ values to provide additional guidance. A draft of that guidance was shared with the CSWG, and this report can be supplemented with the final version once it becomes available.

Following the August 24 meeting, both subgroups opted to merge since there appeared to be substantial overlap between the topics and approaches. This resulted in a work product draft being produced between September 1 and September 17, 2023, reviewed by workgroup members on September 22 and 29, for discussion at the October 5, 2023, CSWG meeting.

Recommendations

1. Implementation Pathways for "any detectable amount of THC"

The subgroup critically reviewed, compared, and contrasted materials pertaining to cannabis product testing and production standards from several resources, including but not limited to the Association of Analytical Chemists (AOAC), the American Society for Testing and Materials (ASTM), and the United States Pharmacopeia Food Chemical Codex (USP-FCC). Some of the materials reviewed are copyright protected for commercial use. The group did not rely on these materials to formulate recommendations because doing so reduces transparency and creates unnecessary accessibility barriers in public understanding of decision-making processes for those who must comply with and understand current and future regulation. We note below where materials are publicly available.

Further, two terms used in analytical chemistry are referenced below. Limit of quantification (LOQ) refers to the lowest concentration of an analyte that can be measured by a method with acceptable precision and accuracy. Limit of detection (LOD) refers to the lowest quantity of a substance that can be distinguished from the absence of that substance as with a stated confidence level, generally 99%.

<u>Discussion</u>

AOAC SMPRs

- AOAC Standard Method Performance Requirements (SMPRs) provide helpful standards developed by AOAC's Cannabis Analytical Science Program (CASP) expert volunteers.
- AOAC has multiple SMPRs based on matrix, such as flower and oil, and analyte class, such as cannabinoids, pesticides, and others. AOAC uses LOQ for cannabinoids, and LOD for contaminants.
- AOAC SMPRs align with Washington certified cannabis testing labs current practices that rely on high performance liquid chromatography (HPLC) methods and associated testing instruments. Even though WSDA survey results from Washington labs are being finalized, the only additional information that might be useful is instrument manufacturing confirmation that may help to determine instrument and method sensitivity.
- The CSWG worked to assure these recommendations would not result in increased regulatory burden on certified labs. For that reason, one of the major CSWG foci was assuring that labs could comply with potential policy and rule

revisions implementing the legislation requiring additional equipment or instrumentation purchase.

- AOAC SMPRs for quantitation of cannabinoids in dried plant material, cannabis concentrates, and cannabis beverages are publicly available. Specifically, AOAC SMPR® 2018.011 describes Identification and Quantitation of Selected Pesticide Residues in Dried Cannabis Materials, AOAC SMPR® 2018.010 for Screening and Identification Method for Regulated Veterinary Drug Residues in Food, AOAC SMPR® 2022.001 for Quantitation of Cannabinoids in Beverages, and AOAC SMPR® 2017.019 Standard Method Performance Requirements (SMPRs®) for Quantitation of Cannabinoids in Edible Chocolate were reviewed. All of these are publicly available materials.

ASTM

- There are 51 approved ASTM standards for cannabis. Among these is ASTM D875-23, Standard Test Method Determination of Cannabinoid Concentration in Dried Cannabis and Raw Hemp Materials using Liquid Chromatology Tandem Mass Spectrometry (LC-MS/MS).
- The ASTM method is available independent of the ASTM paywall in a peer-reviewed publication. The method in ASTM format must be purchased through ASTM and is not publicly available.
- The ASTM method relies on LC-MS/MS demonstrates greater sensitivity, specificity, accuracy, precision, and stability for analysis of cannabis and hemp samples. However, LC-MS/MS is not widely used for routine analysis of cannabinoids based on the cost of acquiring, maintaining, and operating the instrumentation. This is true in Washington.
- While the application of LC-MS/MS methods may lead to higher data quality and consistency between labs, the most common methods used in Washington labs are HPLC-PDA (high performance liquid chromatography - Photodiode Array) or HLPC-UV (high performance liquid chromatography - ultraviolet).
- E2SSB 5367, now codified in relevant part in RCW 69.50.326 provides that “Nothing in this act shall be construed to require any agency to purchase a liquid chromatography-mass spectrometry instrument.”
- For these reasons, the CSWG does not recommend following the specific ASTM standard for determining cannabinoids by LC-MS/MS at this time, although exploring LC-MS/MS could be a part of future standard discussion.

USP-FCC Hemp Seed Protein Monograph

- This hemp seed protein monograph lists a total THC acceptance criterion of 10 mg/kg – 10 ppm.
- The group viewed this as helpful in justifying LOD but found that it caveats the need to consider LOD versus LOQ, and the intended use or product type being considered. The monograph can be accessed through the USP website through a portal.

USP's Expert Panel Paper on medical cannabis flower (2020)

- While this is limited to flower (inflorescence), this paper provides recommended limits for contaminants (e.g. pesticides, aflatoxins, metals, TYMC, etc.) as well as reporting limits for cannabinoids and terpenes. It also recommends no more than 20% variance against label claims for cannabinoid content.
- This paper was used as a rationale for ASTM D8439 the first Standard Specification for Cannabis Flower products, and is publicly available.

The relationship between LOQ and LOD is predictable, and relatable 3 to 1. Currently, only LOQ is described in rule (WAC 314-55-102(3)) regarding potency analysis for four cannabinoids (CBD, CBDA, Δ9-THC, and Δ9-THCA). The rule describes an LOQ of 1.0 mg/g or 0.1%. Therefore, the LOD corresponding to this regulatory requirement is 0.03%. This limit can be achieved by laboratories that use methods that have specifications equal to or better than those of the AOAC methods 2018.10 and 2018.11.

The group also discussed potential variances in detectable amounts of THC across product type. For that reason, the group suggests offering ranges of detection based on available standards and across product type, rather than a single "detectable amount of THC" based on the following table offered by Dr. Richard Sams, in his draft document entitled "Cannabis Analysis: Determinants of LOD (see also Attachment 1):

Method	Matrix	MDL[1] (mg/mL)	LOD (%, w/w)	LOQ (%, w/w)	MDA[2] Injected (ng)
RP-HPLC-PDA "AOAC 2018.10"	Dried plant material	0.27	0.0067	0.020	0.54
	Oils				
RP-HPLC-PDA "AOAC 2018.11"	Dried plant material	0.40	0.004	0.012	1.2
	Cannabis concentrate	0.40	0.020	0.060	1.2
	Fortified hemp seed oil	0.40	0.002	0.006	1.2
RP-HPLC-PDA	Dried plant material	1.67	0.025	0.05	50
RP-HPLC-PDA	Hashish	1.0	0.04	0.24	2.4

[1] "MDL" is the acronym for "method detection limit" which is the minimum measured concentration of a substance that can be reported with 99% confidence that the measured concentration is distinguishable from method blank results. Also see Method Detection Limit – Frequent Questions.
[2] "MDA injected (ng)" means the minimum amount injected in nanograms that produced a detectable signal in the cited methods.

	Marihuana	1.0	0.04	0.24	2.4
RP-HPLC-PDA	Dried plant material	1.25			6.25
RP-HPLC-PDA	Dried plant material	4.54	0.045	0.136	
RP-HPLC-PDA	Dried plant material	6.15	0.110	0.369	12.3
RP-HPLC-PDA	Dried plant material		0.03	0.06	
RP-HPLC-PDA	Dried plant material	0.76	0.0076	0.023	3.8
RP-HPLC-PDA	Dried plant material	0.33	0.003	0.01	0.67
RP-LC-MS^n	Dried plant material	0.0033	0.000067	0.0002	0.003

The yellow highlighting indicates that the LOQ does not meet SMPR® 2109.003 standard of ≤0.05%

2. Product Safety: Product Specifications and Manufacturing Practices

As part of its critical review of current standards, the group looked at current *product specifications* and *manufacturing practices* by product type (*i.e.,* flower, concentrate, edible). Neither of these phrases are currently defined by rule or statute. However, whether intended or not, E2SSB 5367 resulted in a redefinition of cannabis products.

Several terms, such as “ingredient,” “processing,” “conversion,” “potency” and “synthetic” are not defined in statute or rule. The group also notes that the term “manufacturing” is not defined in statute or rule, although all these terms appear consistently in both.

For example, *intermediate* products, defined in rule as “cannabis flower lots or other material lots that have been *converted* by a cannabis processor to a cannabis mix lot, cannabis concentrate or cannabis-infused product that must be or are intended to be converted further to an end product” (WAC 314-55-010(16) are subject to quality control testing (emphasis added).

Additionally, *end product* is defined in WAC 314-55-101(11) as a cannabis product that requires no further processing prior to retail sale. End products, such as all cannabis, cannabis infused products, cannabis concentrates, cannabis mix packaged, and cannabis mix infused sold from a processor to a retailer must be tested for *potency* (WAC 314-55-102(4)(d), emphasis added). The group offers that the phrase “THC concentration” better represents the concept of potency as it relates to cannabis.

The group recommends defining these terms and phrases, and distinguishing in rule between *production*, which currently appears to be considered as the growth or growing process of the cannabis crop, and *manufacturing*, which appears to be used

interchangeably with the term *processing* in statute. This would not require revising current standards for quality control, or food product safety under LCB's authority, but would require some language alignment throughout chapter 314-55 WAC.

3. Future Discussion and Considerations

Consumption vs. Environmental Concerns

The group's main concern was focused on what cannabis products are being consumed, and that those products contain ingredients deemed to be safe. For example, delta-8-THC and its derivatives are semi-synthetic substances that are often contaminated with side-products that are new chemical entities that have not been subjected to toxicological investigation. As long as the products do not contain any pesticides, the group contemplated whether there was any further need for exploration.

The discussion turned to the environmental impacts of pesticide use in cannabis production. If pesticides are being used, there is environmental impact. Although these products should only be used when needed, the risk of land contamination remains. The group discussed whether cannabis growth should occur on land where other crop growth has occurred.

This issue should be further explored because there is background contamination in many agricultural products and many of these products are consumed in greater amounts than cannabis. An example of salmon was provided: the action level of DDT and DDE for salmon is 0.5 PPM (approximately) which is higher than the action level for DDT and DDE in cannabis in Washington. USDA organic regulations allow residues of *prohibited* pesticides up to 5% of the EPA tolerance "as long as the operator hasn't directly applied prohibited pesticides and has documented efforts to minimize exposure to them." Roughly 4% of randomly tested samples exceeded this limit in a 2010 USDA pilot assessment.[3] Consumers become concerned about this, but theses crops are not tested for these substances.

Cannabis as a Remediator

The group discussed the role of cannabis as a remediation plant that can pull different compounds from soil. While this is beneficial in some cases, it can be detrimental in others. Other plants, like apple trees, aren't known as remediators. The group discussed the unsettled science around whether a compound could get into the fruit of an apple tree or the flower of a cannabis plant because the plants function differently, and whether trials could be done using hemp to determine pesticide and/or heavy metal uptake within the plant.

[3] Pesticide Residue Testing of Organic Produce: 2010-2011 Pilot Study. USDA National Organic Program. United States Department of Agriculture. November 2021, p. 4.

Discussion explored the ability of cannabis plants to pull compounds from soil, and the group agreed that this is where future research would have the most impact. The group discussed where in the plant such compounds are stored, what compound amounts might cannabis pull from supplemented soil, and whether cannabis pulls more compounds than other plants. Once some of these factors have been determined, consumer impact could be assessed. For example, if a compound is being stored at higher concentrations in the plant's root system, there would likely be no consumer impact, but if a compound is stored equally throughout the plant or concentrated in the leaves and flowers, there might be.

Food Safety

Of particular interest was developing a better and careful translation of total exposure. When USDA and other entities are conducting toxicology studies on some of these contaminants, they assess more than just the compound; they assess exposure, and likely daily exposure, which can result in a lack of observed adverse event levels. This is critical, particularly with respect to cannabis, where a heavy user might consume only one or two grams of plant material or a gram of concentrate, contrasted to a person who consumes one or two pounds of salmon each day. There are orders of magnitude difference that come back to the concentration of the contaminant in the product. For this reason, total exposure rather than concentration of the contaminant in the food, is key when considering food safety, as is the method of consumption: ingestion vs. inhalation.

Acknowledgements

The WSLCB wishes to acknowledge the following members of the CSWG subgroups for their significant contribution of time and expertise to this report:

Dr. Richard Sams
Dr. Taylor Carter
Dr. David Gang
Jessica Tonani
Dr. Brad Douglass

ATTACHMENT 1

CANNABIS ANALYSIS

DETERMINANTS OF LOD

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The following representative high performance liquid chromatography (HPLC) methods (see where *C* = analyte concentration determined from the standard curve (micrograms per mL), *V* = total volume of the extraction solvent (mL), *DF* = factor reflecting dilution of the final extract, *W* = sample weigh (grams), and *10,000* = conversion from micrograms per gram to percent (w/w). For example, the analyte concentration in a 0.5-gram sample extracted into 50 mL of solvent, without additional dilution, is 0.05% if the concentration of analyte determined from the standard curve is 5 μg/mL.

It is obvious from this equation that the laboratory's choice of the sample weight taken for analysis, volume of the extracting solvent, and the dilution determine the method's LOD and LOQ.

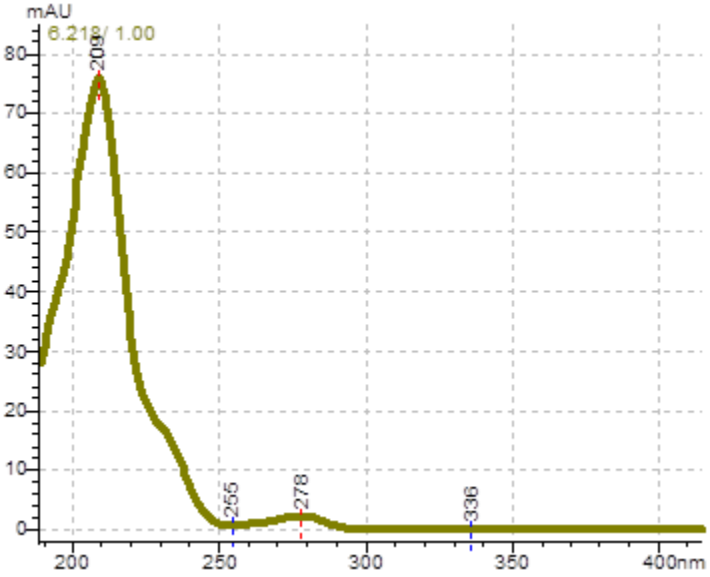
Table 1) have been validated for the determination of cannabinoids including Δ^9-THC in dried plant materials and other sample matrices. The selection includes two methods [1-3] that are designated as AOAC methods because they meet all requirements established by the AORC in the Hemp Standard Method Performance Requirement (SMPR® 2019.003) that specifies, among other requirements, that the Limit of Quantification (LOQ) for all cannabinoids must be ≤0.05%. The tables also include method performance specifications for an HPLC method developed and validated by an independent third-party laboratory that meets the requirements of SMPR® 2019.003. Note that some of the methods [4-8] included in where *C* = analyte concentration determined from the standard curve (micrograms per mL), *V* = total volume of the extraction solvent (mL), *DF* = factor reflecting dilution of the final extract, *W* = sample weigh (grams), and *10,000* = conversion from micrograms per gram to percent (w/w). For example, the analyte concentration in a 0.5-gram sample extracted into 50 mL of solvent, without additional dilution, is 0.05% if the concentration of analyte determined from the standard curve is 5 μg/mL.

It is obvious from this equation that the laboratory's choice of the sample weight taken for analysis, volume of the extracting solvent, and the dilution determine the method's LOD and LOQ.

Table 1 do not meet the requirements for LOQ that are specified in SMPR® 2019.003. Several of these methods were developed for determining THC in cannabis and therefore do not need to meet SMPR® 2019.003 requirements.

Key sample preparation variables are included in *Table 2* because these variables affect the sensitivity of the method but are under the control of the laboratory. Specific method performance data (*viz*., the MDL, LOD, LOQ, and the Minimum Amount Injected into the HPLC instrument) are reported in Table 3 to facilitate comparison of methods.

Figure 1. Absorption spectrum of Δ^9-THC



HPLC instruments equipped with PDA detectors measure the absorption of UV light by substances dissolved in the mobile phase as they exit the column analytical column and pass through the detector. The absorption of light is a physical property of the analyte and differs from one to another although the molar absorptivities of the cannabinoids are similar. The detector has little effect on the intensity of the signal, but the dimensions of the flow cell affect signal strength through Beer's Law and electronic noise (particularly at lower wavelengths) may affect the quality of the signal limiting sensitivity of detection. The intensity of the absorption of UV light depends on the wavelength at which the absorption is measured. For example, the absorption spectrum of Δ^9-THC in *Figure 1* (above) indicates that the absorption maximum is at 209 nm so the greatest response for THC could be obtained by monitoring the column effluent at this wavelength. However, this wavelength isn't routinely used because other substances (*e.g*., terpenes) in the sample extract also absorb at lower wavelengths and create interference. Furthermore, some of the other target cannabinoids have absorption maxima at higher wavelengths. Therefore, higher wavelengths (*e.g*., 220-230 nm) are typically used to determine cannabinoids.

Two of the methods reported in where *C* = analyte concentration determined from the standard curve (micrograms per mL), *V* = total volume of the extraction solvent (mL), *DF* = factor reflecting dilution of the final extract, *W* = sample weigh (grams), and *10,000* = conversion from micrograms per gram to percent (w/w). For example, the analyte concentration in a 0.5-gram sample extracted into 50 mL of

solvent, without additional dilution, is 0.05% if the concentration of analyte determined from the standard curve is 5 μg/mL.

It is obvious from this equation that the laboratory's choice of the sample weight taken for analysis, volume of the extracting solvent, and the dilution determine the method's LOD and LOQ.

Table 1 use wavelengths of 210 nm [4] and 211 nm [5] to determine cannabinoids. However, lower wavelengths are subject to interference from co-eluting substances such as terpenes and other lipophilic plant substances, so the wavelength maximum is not necessarily the best choice for monitoring the cannabinoids. Furthermore, the wavelength of maximum absorption varies from one cannabinoid to another so that the wavelength that is selected for quantification of different cannabinoids is one that provides adequate absorption of target analytes, but which excludes absorption by other components. Those methods with absorption at 220 nm [1; 2; 6][4] meet this requirement reasonably well.

The signal that is used to quantify Δ^9-THC in a cannabis flower is directly proportional to the extent of the absorption of light by the Δ^9-THC molecules at the experimentally-chosen wavelength as they pass through the detector flow cell. The absorption signal decreases as the concentration of Δ^9-THC in the flow cell decreases. As the concentration of the analyte decreases smaller amounts of it reach the detector and, at some concentration of the analyte, the amount of analyte reaching the detector becomes too small to produce a signal that cannot be differentiated from the background noise attributed to the presence of the analyte. For this reason, it may be instructive to determine the minimum amount of analyte required to produce a detectable signal because this figure indicates the response of the instrument to the analyte without the variables of sample preparation. *Table 3* includes a column labelled "MDA injected (ng)" which is the minimum amount injected in ng that produced a detectable signal in the cited methods.

The minimum amount of analyte that produces a detectable signal can be calculated from the Method Detection Limit (MDL) and the volume of the calibrator or sample aliquot analyzed by the HPLC instrument. The MDL can be determined by different approaches but one of the more rigorous procedures is a US EPA procedure[5] which uses the following calculation based on collection of data obtained at concentrations near the detection limit:

$$MDL\ (\mu g/mL) = t_{n-1,1-\alpha)} \times SD$$

where

The intensities of the detector signals from calibrators and test samples are measured and are used to calculate analyte concentrations in the test samples. The concentration of analyte can be determined from the following expression:

$$Analyte\ \left(\%, \frac{w}{w}\right) = \frac{C \times V \times DF}{W \times 10{,}000}$$

where *C* = analyte concentration determined from the standard curve (micrograms per mL), *V* = total volume of the extraction solvent (mL), *DF* = factor reflecting dilution of the final extract, *W* = sample weigh (grams), and *10,000* = conversion from micrograms per gram to percent (w/w). For example, the analyte concentration in a 0.5-gram sample extracted into 50 mL of solvent, without additional dilution, is 0.05% if the concentration of analyte determined from the standard curve is 5 µg/mL.

[4] Independent laboratory

[5] https://www.epa.gov/sites/default/files/2016-12/documents/mdl-procedure_rev2_12-13-2016.pdf

It is obvious from this equation that the laboratory's choice of the sample weight taken for analysis, volume of the extracting solvent, and the dilution determine the method's LOD and LOQ.

Table 1. Representative HPLC-PDA and LC-MS Methods for Determining Various Cannabinoids Including Δ^9-THC in Cannabis

Method	Instrument	Column	Mobile Phase	Matrices	Reference
RP-HPLC-PDA "AOAC 2018.10"	Agilent 1200 HPLC system; Agilent PDA detector at 220 nm (Agilent Technologies, Santa Clara, CA)	Reversed phase C_{18}, 1.7 µm, 100 mm × 3.0 mm i.d. column (Kinetex®, Phenomenex, Torrance, CA)	Acetonitrile/water containing 10 mM ammonium formate, pH 3.6 (gradient)	Plant material Oils	[1; 2]
RP-HPLC-PDA "AOAC 2018.11"	Agilent 1290 Infinity HPLC system; Agilent 1290 PDA Detector at 240 nm (Agilent Technologies, Santa Clara, CA)	Reversed phase C_{18}, 2.0 µm, 150 mm × 2.1 mm i.d. column (Supelco Ascentis Express, Part No. 50814-U)	Acetonitrile/water containing 20 mM ammonium formate, pH 3.2 (gradient)	Plant material Concentrates Oils	[3]
RP-HPLC-PDA "De Backer method"	Agilent HPLC system (Agilent Technologies, Santa Clara, CA); Waters Acquity 2996 PDA detector at 200-400 nm (Waters Corp., Milford, MA)	Reversed phase C_{18}, 5 µm, 250 mm × 2.1 mm i.d. column (Waters XTerra® MS)	Methanol/water containing 50 mM ammonium formate, pH 5.19 (gradient)	Plant material	[8]
RP-HPLC-PDA "Ambach method"	Waters HPLC system; Waters Acquity 2996 PDA at 210 nm (Waters Corp., Milford, MA)	Reversed phase C_8, LiChroCart 125-4, LiChrospher 60, RP-Select B (C_8), 5 µm	Acetonitrile/water containing 25 mM TEAP buffer (isocratic)	Hashhish Marihuana	[4]
RP-HPLC-PDA "Mandrioli method"	Shimadzu HPLC system LC-2030C; PDA at 220 nm (Shimadzu	Reversed phase C_{18}, 2.7 µm, 150 mm × 4.6 mm, (NexLeaf CBX Potency ,	Acetonitrile/water containing 0.085 % phosphoric acid (gradient)	Plant material	[6]

	Scientific Instruments, Columbia, MD)	Shimadzu Scientific Instruments).			
RP-HPLC-PDA "Burnier method"	Waters 1515® HPLC- system; Waters 2998 PDA at 211 nm (Waters Corp., Milford, MA)	Reversed phase $C_{18,}$ 5 µm, 250 mm x 4.6 mm (Nucleodur® C18 Gravity, Machery-Nagel AG, Oensingen, Switzerland)	Acetonitrile/water containing 50 mM phosphoric acid (isocratic)	Plant material	[5]
RP-HPLC-PDA "Birenboim method"	Waters Acquity Arc FTN-R; Waters 2998 PDA at 228 nm (Waters Corp., Milford, MA)	Reversed phase C_{18}, 1.7 µm, 150 mm × 2.1 mm i.d. column (Kinetex® XB-C18 100A, Phenomenex, Torrance, CA, USA).	Acetonitrile/water containing 20 mM ammonium formate, pH 2.9 (isocratic)	Plant material	[7]
RP-HPLC-PDA "Duchateau method)	Waters Acquity Arc FTN-R; Waters 2998 PDA at 228 nm (Waters Corp., Milford, MA)	Reversed phase C_{18}, 1.6 µm, 100 mm × 2.1 mm (CORTECS Shield RP18 90 A, Waters)	Acetonitrile/water containing 0.1% formic acid (isocratic)	Plant material	[9]
RP-HPLC-PDA	Shimadzu HPLC system LC-2030C; PDA at 220 nm (Shimadzu Scientific Instruments, Columbia, MD)	Reversed phase C_{18}, 2.7 µm, 150 mm × 4.6 mm (Raptor ARC-18, Restek).	Acetonitrile, 0.1% formic acid/water containing 5 mM ammonium formate and 0.1% formic acid (isocratic)	Plant material	Independent 3rd party laboratory
RP-HPLC-PDA	Shimadzu Prominence-i LC-2030 C 3D Plus HPLC system; PDA at 230 nm	Reversed phase C_{18}, 2.7 µm, 150 mm × 3 mm (Ascentis® Express C18; MilliporeSigma)	Acetonitrile/water containing 8% (v/v) methanol, 0.035% (v/v) formic acid, 1.8	Plant material	Shimadzu[6]

[6] https://www.ssi.shimadzu.com/sites/ssi.shimadzu.com/files/pim/pim_document_file/ssi/applications/application_note/16984/HPLC-045-Cannabinoid-Cannflavin.pdf

	(Shimadzu Scientific Instruments, Columbia, MD)		mM ammonium formate (gradient)		
RP-LC-MS^n	Agilent 1290 Infinity I UPLC system (Agilent Technologies, Mississauga, ON, Canad); TSQ Quantiva triple quadrupole mass spectrometer (Thermo Scientific, San Jose, CA, USA	Reversed phase C_{18} amide, 3 µm, 100 mm × 2.1 mm (Ace-3, Advanced Chromatography Technologies)	Acetonitrile, 0.1% formic acid/water, 0.1% formic acid (gradient)	Plant material	[10]

Table 2. Sample Preparation Variables for Methods Described in

The following representative high performance liquid chromatography (HPLC) methods (see where *C* = analyte concentration determined from the standard curve (micrograms per mL), *V* = total volume of the extraction solvent (mL), *DF* = factor reflecting dilution of the final extract, *W* = sample weigh (grams), and *10,000* = conversion from micrograms per gram to percent (w/w). For example, the analyte concentration in a 0.5-gram sample extracted into 50 mL of solvent, without additional dilution, is 0.05% if the concentration of analyte determined from the standard curve is 5 μg/mL.

It is obvious from this equation that the laboratory's choice of the sample weight taken for analysis, volume of the extracting solvent, and the dilution determine the method's LOD and LOQ.

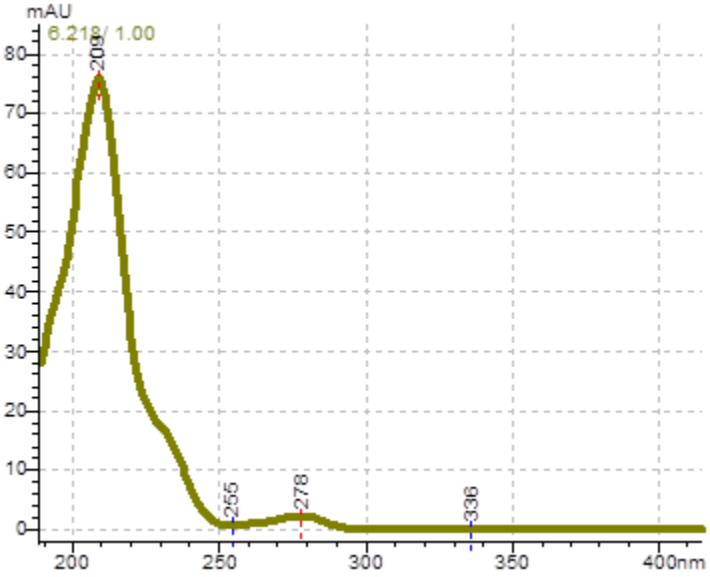
Table 1) have been validated for the determination of cannabinoids including Δ^9-THC in dried plant materials and other sample matrices. The selection includes two methods [1-3] that are designated as AOAC methods because they meet all requirements established by the AORC in the Hemp Standard Method Performance Requirement (SMPR® 2019.003) that specifies, among other requirements, that the Limit of Quantification (LOQ) for all cannabinoids must be ≤0.05%. The tables also include method performance specifications for an HPLC method developed and validated by an independent third-party laboratory that meets the requirements of SMPR® 2019.003. Note that some of the methods [4-8] included in where *C* = analyte concentration determined from the standard curve (micrograms per mL), *V* = total volume of the extraction solvent (mL), *DF* = factor reflecting dilution of the final extract, *W* = sample weigh (grams), and *10,000* = conversion from micrograms per gram to percent (w/w). For example, the analyte concentration in a 0.5-gram sample extracted into 50 mL of solvent, without additional dilution, is 0.05% if the concentration of analyte determined from the standard curve is 5 μg/mL.

It is obvious from this equation that the laboratory's choice of the sample weight taken for analysis, volume of the extracting solvent, and the dilution determine the method's LOD and LOQ.

Table 1 do not meet the requirements for LOQ that are specified in SMPR® 2019.003. Several of these methods were developed for determining THC in cannabis and therefore do not need to meet SMPR® 2019.003 requirements.

Key sample preparation variables are included in *Table 2* because these variables affect the sensitivity of the method but are under the control of the laboratory. Specific method performance data (*viz*., the MDL, LOD, LOQ, and the Minimum Amount Injected into the HPLC instrument) are reported in Table 3 to facilitate comparison of methods.

Figure 1. Absorption spectrum of Δ^9-THC



HPLC instruments equipped with PDA detectors measure the absorption of UV light by substances dissolved in the mobile phase as they exit the column analytical column and pass through the detector. The absorption of light is a physical property of the analyte and differs from one to another although the molar absorptivities of the cannabinoids are similar. The detector has little effect on the intensity of the signal, but the dimensions of the flow cell affect signal strength through Beer's Law and electronic noise (particularly at lower wavelengths) may affect the quality of the signal limiting sensitivity of detection. The intensity of the absorption of UV light depends on the wavelength at which the absorption is measured. For example, the absorption spectrum of Δ^9-THC in *Figure 1* (above) indicates that the absorption maximum is at 209 nm so the greatest response for THC could be obtained by monitoring the column effluent at this wavelength. However, this wavelength isn't routinely used because other substances (*e.g*., terpenes) in the sample extract also absorb at lower wavelengths and create interference. Furthermore, some of the other target cannabinoids have absorption maxima at higher wavelengths. Therefore, higher wavelengths (*e.g*., 220-230 nm) are typically used to determine cannabinoids.

Two of the methods reported in where *C* = analyte concentration determined from the standard curve (micrograms per mL), *V* = total volume of the extraction solvent (mL), *DF* = factor reflecting dilution of the final extract, *W* = sample weigh (grams), and *10,000* = conversion from micrograms per gram to percent (w/w). For example, the analyte concentration in a 0.5-gram sample extracted into 50 mL of

solvent, without additional dilution, is 0.05% if the concentration of analyte determined from the standard curve is 5 μg/mL.

It is obvious from this equation that the laboratory's choice of the sample weight taken for analysis, volume of the extracting solvent, and the dilution determine the method's LOD and LOQ.

Table 1 use wavelengths of 210 nm [4] and 211 nm [5] to determine cannabinoids. However, lower wavelengths are subject to interference from co-eluting substances such as terpenes and other lipophilic plant substances, so the wavelength maximum is not necessarily the best choice for monitoring the cannabinoids. Furthermore, the wavelength of maximum absorption varies from one cannabinoid to another so that the wavelength that is selected for quantification of different cannabinoids is one that provides adequate absorption of target analytes, but which excludes absorption by other components. Those methods with absorption at 220 nm [1; 2; 6] meet this requirement reasonably well.

The signal that is used to quantify Δ^9-THC in a cannabis flower is directly proportional to the extent of the absorption of light by the Δ^9-THC molecules at the experimentally-chosen wavelength as they pass through the detector flow cell. The absorption signal decreases as the concentration of Δ^9-THC in the flow cell decreases. As the concentration of the analyte decreases smaller amounts of it reach the detector and, at some concentration of the analyte, the amount of analyte reaching the detector becomes too small to produce a signal that cannot be differentiated from the background noise attributed to the presence of the analyte. For this reason, it may be instructive to determine the minimum amount of analyte required to produce a detectable signal because this figure indicates the response of the instrument to the analyte without the variables of sample preparation. *Table 3* includes a column labelled "MDA injected (ng)" which is the minimum amount injected in ng that produced a detectable signal in the cited methods.

The minimum amount of analyte that produces a detectable signal can be calculated from the Method Detection Limit (MDL) and the volume of the calibrator or sample aliquot analyzed by the HPLC instrument. The MDL can be determined by different approaches but one of the more rigorous procedures is a US EPA procedure which uses the following calculation based on collection of data obtained at concentrations near the detection limit:

$$MDL\ (\mu g/mL) = t_{n-1,1-\alpha)} \times SD$$

where

The intensities of the detector signals from calibrators and test samples are measured and are used to calculate analyte concentrations in the test samples. The concentration of analyte can be determined from the following expression:

$$Analyte\ \left(\%, \frac{w}{w}\right) = \frac{C \times V \times DF}{W \times 10{,}000}$$

where *C* = analyte concentration determined from the standard curve (micrograms per mL), *V* = total volume of the extraction solvent (mL), *DF* = factor reflecting dilution of the final extract, *W* = sample weigh (grams), and *10,000* = conversion from micrograms per gram to percent (w/w). For example, the analyte concentration in a 0.5-gram sample extracted into 50 mL of solvent, without additional dilution, is 0.05% if the concentration of analyte determined from the standard curve is 5 μg/mL.

It is obvious from this equation that the laboratory's choice of the sample weight taken for analysis, volume of the extracting solvent, and the dilution determine the method's LOD and LOQ.

Table 1

Method	Matrix	Sample Mass (g)	Extraction Volume (mL)	Dilution Factor	Volume Injected (μL)	Reference
RP-HPLC-PDA "AOAC 2018.10"	Dried plant material	0.20	25	2	2	[1; 2]
	Oils	0.05	50	1	5	
RP-HPLC-PDA "AOAC 2018.11"	Dried plant material	0.50	50	1	3	[3]
	Cannabis concentrate	0.05	25	1	3	
	Fortified hemp seed oil	0.05	25	1	3	
RP-HPLC-PDA	Dried plant material	0.20	20	1	30	[8]
RP-HPLC-PDA	Hashish	0.30	20	20	10	[4]
	Marihuana	0.50	10	20	10	
RP-HPLC-PDA	Dried plant material	0.025	25	0.25	5	[6]
RP-HPLC-PDA	Dried plant material	0.10	10	1		[5]
RP-UPLC-PDA	Dried plant material	0.10	4	5	2	[7]
RP-UPLC-PDA	Dried plant material	0.10	10	1	5	[9]
RP-HPLC-PDA	Dried plant material	0.25	25	1	5	Independent 3rd party laboratory
RP-HPLC-PDA	Dried plant material	0-2-0.5	10	2	2	Shimadzu[7]
RP-LC-LC-MS^n	Dried plant material	0.10	20	1	1	[10]

Table 3.Comparisons of Reported LOD and LOQ Values for Determining THC by Different HPLC-PDA and LC-MS Methods

Method	Matrix	MDL (μg/mL)	LOD (%, w/w)	LOQ (%, w/w)	MDA Injected (ng)	Reference
RP-HPLC-PDA	Dried plant material	0.27	0.0067	0.020	0.54	[1; 2]

[7] https://www.ssi.shimadzu.com/sites/ssi.shimadzu.com/files/pim/pim_document_file/ssi/applications/application_note/16984/HPLC-045-Cannabinoid-Cannflavin.pdf

"AOAC 2018.10"	Oils					
RP-HPLC-PDA "AOAC 2018.11"	Dried plant material	0.40	0.004	0.012	1.2	[3]
	Cannabis concentrate	0.40	0.020	0.060	1.2	
	Fortified hemp seed oil	0.40	0.002	0.006	1.2	
RP-HPLC-PDA	Dried plant material	1.67	0.025	0.05	50	[8]
RP-HPLC-PDA	Hashish	1.0	0.04	0.24	2.4	[4]
	Marihuana	1.0	0.04	0.24	2.4	
RP-HPLC-PDA	Dried plant material	1.25			6.25	[6]
RP-HPLC-PDA	Dried plant material	4.54	0.045	0.136		[5]
RP-HPLC-PDA	Dried plant material	6.15	0.110	0.369	12.3	[7]
RP-HPLC-PDA	Dried plant material		0.03	0.06		[9]
RP-HPLC-PDA	Dried plant material	0.76	0.0076	0.023	3.8	Independent 3rd party laboratory
RP-HPLC-PDA	Dried plant material	0.33	0.003	0.01	0.67	Shimadzu[8]
RP-LC-MS^n	Dried plant material	0.0033	0.000067	0.0002	0.003	[10]

The yellow highlighting indicates that the LOQ does not meet SMPR® 2109.003 standard of ≤0.05%.

8 https://www.ssi.shimadzu.com/sites/ssi.shimadzu.com/files/pim/pim_document_file/ssi/applications/application_note/16984/HPLC-045-Cannabinoid-Cannflavin.pdf

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