IN THE UNITED STATES DISTRICT COURT
FOR THE EASTERN DISTRICT OF ARKANSAS
CENTRAL DIVISION

BIO GEN, LLC, DRIPPERS VAPE SHOP, LLC, THE CIGARETTE STORE LLC d/b/a SMOKER FRIENDLY, and SKY MARKETING CORPORATION d/b/a HOMETOWN HERO, **PLAINTIFFS**

v. **Case No. 4:23-CV-718 (BRW)**

GOVERNOR SARAH HUCKABEE SANDERS, in her official capacity; ATTORNEY GENERAL JOHN TIMOTHY GRIFFIN in his official capacity; TODD MURRAY, SONIA FONTICIELLA, DEVON HOLDER, MATT DURRETT, JEFF PHILLIPS, WILL JONES, TERESA HOWELL, BEN HALE, CONNIE MITCHELL, DAN TURNER, JANA BRADFORD, FRANK SPAIN, TIM BLAIR, KYLE HUNTER, DANIEL SHUE, JEFF ROGERS, DAVID ETHREDGE, TOM TATUM, II, DREW SMITH, REBECCA REED MCCOY, MICHELLE C. LAWRENCE, DEBRA BUSCHMAN, TONY ROGERS, NATHAN SMITH, CAROL CREWS, KEVIN HOLMES, CHRIS WALTON, and CHUCK GRAHAM, each in his or her official capacity as a prosecuting attorney for the State of Arkansas; JIM HUDSON, in his official capacity as director of the ARKANSAS DEPARTMENT OF FINANCE AND ADMINISTRATION; GREG SLED, in his official capacity as director of the ARKANSAS TOBACCO CONTROL BOARD; WES WARD, in his official capacity as secretary of the ARKANSAS DEPARTMENT OF AGRICULTURE; and MATTHEW MARSH, in his official capacity as chair of the ARKANSAS STATE PLANT BOARD **DEFENDANTS**

PLAINTIFFS' REPLY[1] IN SUPPORT OF THEIR MOTION FOR A TEMPORARY RESTRAINING ORDER OR ALTERNATIVE MOTION FOR PRELIMINARY INJUNCTION

[1] On August 8, 2023, Defendants filed a document styled as a "Motion to Dismiss and Response in Opposition to Plaintiffs' Motion for Temporary Restraining Order or Alternative Motion for Preliminary Injunction." ECF 38. This Reply focuses exclusively on the substantive issues before the Court. Plaintiffs separately filed an Amended Complaint and Response to Defendants' Motion to Dismiss.

Despite Defendants' allegations contained in their response, Act 629, with or without the apparent changes made by the Arkansas Code Revision Committee, directly conflicts in several different aspects with the 2018 Farm Bill. First, Defendants disingenuously claim that the definition of "hemp" in the Act comports with the federal definition. This is false. The Act clearly alters the definition of "hemp," which the 2018 Farm Bill specifically prohibits states from doing.

Additionally, Defendants allege that the Act's protection of interstate commerce mirrors the language from the 2018 Farm Bill, which expressly prohibits states from interfering with the interstate transportation of hemp and hemp products. However, the Act includes an additional burden to interstate transportation of hemp and hemp products by requiring them to be in "continuous transportation" through Arkansas, a standard that appears nowhere in the 2018 Farm Bill. Moreover, the Act attempts to criminalize certain THCs, chemically converted products, and "psychoactive substances" as Schedule VI "synthetic substances," impermissibly creating a distinction based on method of manufacture in further conflict with the 2018 Farm Bill's definition of hemp, which removed all hemp derived THCs from federal controlled status. Finally, throughout their Response, Defendants erroneously conflate hemp-derived cannabinoids with synthetic cannabinoids based on an invented two prong standard, which, again, directly conflicts with the 2018 Farm Bill that relegalized all extracts and derivatives of hemp.

Defendants further allege that, due to certain substantive revisions made by the Arkansas Code Revision Commission ("Commission") to Sections 16 and 17 of Act 629, the Act does not unconstitutionally interfere with interstate commerce nor is it unconstitutionally vague. However, in making these revisions, the Commission manifestly altered the meaning and substance of the Act by changing which sections of

the Act become effective upon the enjoining of certain sections of the Act. These changes were substantive, and in revising Act 629, the Commission exceeded its powers. As a result, the original wording of Act 629 controls. Therefore, Sections 7 and 10 of Act 629, which attempt, but fail, to cure the Act's impermissible interference with the interstate transportation and shipment of hemp and hemp products, are not currently effective until Sections 2-5 are enjoined as originally contemplated in Section 17.

Moreover, Act 629 is unconstitutionally vague due to those various inconsistencies and for failing to provide notice as to what contemplated conduct is forbidden and what is permitted with regard to production, possession, transportation, and shipment of hemp and hemp products it seeks to ban.

Finally, Act 629's threat of irreparable harm has become a reality for Plaintiffs as Arkansas Tobacco Control officers have directed Plaintiffs' employees to remove delta-9 THC products, regardless of whether those products were naturally extracted or produced as a result of a synthetic chemical process in apparent conflict with Defendants' interpretation of the Act. The Court should grant Plaintiffs' requested temporary restraining order for each of these reasons.

I. THE ACT CONFLICTS WITH THE PLAIN LANGUAGE OF THE 2018 FARM BILL

A. Section 2's definitional change conflicts with the federal definition of hemp.

Section 2 of the Act narrows the definition of "industrial hemp" for purposes of Arkansas's Industrial Hemp Production Act. The Industrial Hemp Production Act establishes a 2018 Farm Bill-authorized plan by which hemp may be legally grown and cultivated in Arkansas. The Industrial Hemp Production Act is, therefore, subject to the

2018 Farm Bill's parameters. *See* 7 U.S.C. § 1639p (requiring a state to submit its 2018 Farm Bill hemp plan to USDA for approval).

Defendants' baseless assertion that the Act's narrowed definition of "industrial hemp" is "coherent" with the 2018 Farm Bill's expanded definition of "hemp" falls woefully short of the two definitions being one in the same—in both a literal and a practical sense. Make no mistake about it: with its definition of "hemp" in the 2018 Farm Bill, Congress deliberately broadened the 2014 Farm Bill's definition of "industrial hemp" to additionally protect "all derivatives, extracts, cannabinoids, isomers"—individual parts of the hemp plant which were not explicitly included in the 2014 Farm Bill's definition. *See United States v. Mallory*, 372 F. Supp. 3d 377, 379, n.1 (S.D.W. Va. 2019) ("Hemp is now defined in [the 2018 Farm Bill] as . . ."). The 2018 Farm Bill did not, however, change the delta-9 THC dry weight standard by which hemp is federally measured. So long as a product contains 0.3% delta-9 THC or less on a dry weight basis, the product constitutes hemp under the 2018 Farm Bill. *See AK Futures LLC v. Boyd St. Distro, LLC*, 35 F.4th 682, 690 (9th Cir. 2022) ("A straightforward reading of [the 2018 Farm Bill] yields a definition of hemp applicable to all products that are sourced from the cannabis plant, contain no more than 0.3 percent delta-9 THC, and can be called a derivative, extract, cannabinoid, or one of the other enumerated terms.").

Arkansas's modified definition of "industrial hemp" in Section 2 of the Act narrows the term to exclude products that the 2018 Farm Bill's definition of "hemp" was expressly intended to protect. As opposed to any product that is sourced from the hemp plant and that contains no more than 0.3% delta-9 THC on a dry weight basis, a product must instead have a delta-9 THC concentration "of no more than three-tenths of one percent (0.3%) of the hemp-derived cannabidiol" to meet the Act's new definition of "industrial

hemp." In addition to being confusing and nearly unintelligible, the Act's new definition conflicts with the definition of "hemp" in the 2018 Farm Bill.[2] Indeed, Defendants' own side-by-side comparison unambiguously depicts the direct conflict between the two definitions.

Act 629	Arkansas Law	Federal Law
Section 2 Definition of Hemp Arkansas Industrial Hemp Production Act	A.C.A. § 2-15-503(5) "Industrial hemp" means the plant Cannabis sativa and any part of the plant, including the seeds of the plant and all derivatives, extracts, cannabinoids, isomers, acids, salts, and salts of isomers, whether growing or not, with a total delta-9 tetrahydrocannabinol concentration of no more than three-tenths of one percent (0.3%) of the hemp-derived cannabidiol on a dry weight basis, unless specifically controlled under the Uniform Controlled Substances Act, § 5-64-101 et seq.	7 U.S.C. 16390(1) The term "hemp" means 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 grow-ing or not, with a delta-9 tetrahydro-cannabinol concentration of not more than 0.3 percent on a dry weight basis.

As one can plainly read, the above highlighted language in the Act's definition of "industrial hemp" is nowhere to be seen in the federal definition of hemp. The Act's definition limits hemp to something with a delta-9 THC concentration that is a percentage concentration of a wholly different cannabinoid, cannabidiol ("CBD")—when the 2018 Farm Bill does not. Defendants nonetheless expect this Court to believe the two

[2] It is telling that Section 7 of the Act preserves the federal definition of hemp. As discussed below, Section 7, which restricts transportation in interstate commerce, incorporates the 2018 Farm Bill's definition of hemp word for word ("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 three-tenths percent (0.3%) on a dry weight basis"). This was perhaps an acknowledgement by the General Assembly that the State cannot narrow its definition of hemp as it chooses. These competing definitions further illustrate the Commission's error in changing the Act's trigger clause provisions.

definitions are the same. As a result of its new definition, the Act recriminalizes certain parts of the hemp plant despite Congress' clear intention to redevelop a domestic supply chain of hemp and hemp products that flow freely in the stream of interstate commerce.

To be clear, Arkansas's definition of hemp is unlike any in the country at the state or federal level. Most states, in fact, match the 2018 Farm Bill's definition verbatim. Arkansas's extremely narrow and unworkable definition puts Arkansas farmers, small businesses, and consumers in jeopardy. First, it turns them into criminals overnight by reclassifying certain hemp-derived cannabinoids (and others that may be chosen in the future) as Controlled Substances. Second, requiring a farmer's hemp to be ". . . no more than three-tenths of one percent (0.3%) of the hemp-derived cannabidiol" creates an insurmountable obstacle to harvest and will eliminate all legal hemp production in Arkansas. This is because the new definition of hemp requires the total delta-9 THC concentration to be limited to—and calculated on account of—the plant's CBD concentration. According to this novel definition of hemp, the hemp plant cannot have a total delta-9 THC concentration that exceeds 0.3% of the CBD. In contrast, the federal definition focuses on the concentration of delta-9 THC, not the CBD concentration. Although Defendants want this Court to believe it to be an "absurd" claim, it is a fundamental reality that this narrower definition of hemp is unworkable for Arkansas farmers, and it is absurd to suggest that the federal and Arkansas definitions of hemp are the same. They are not.

Defendants correctly point out that the 2018 Farm Bill does not "preempt[] or limit[] any law of a State or Indiana tribe that – (i) regulates the production of hemp; and (ii) is more stringent than this subchapter." 7 U.S.C. § 1639p(a)(3). However, in standing by Section 2's narrowed definition of "industrial hemp," Defendants are asking this Court

to ignore the plain meaning of the term "production" in an agricultural sense and interpret this language to allow individual states to criminalize certain parts of the hemp plant on a whim. In fact, Defendants assert that this means "[s]tates are permitted to ban the production of hemp all together" as justification for the Act. ECF No. 38 at 3. This is an unequivocally inaccurate interpretation of the law. While a state may very well choose to not invest in developing a hemp production regulatory program for their farmers, farmers in that state could still apply for a license to grow hemp through USDA's program and that state is still required to permit the interstate transportation of hemp and products. Evidently, Congress anticipated states dragging their feet on relegalizing the domestic production of hemp and codified safeguards to prevent states from doing exactly what the General Assembly did here.

B. Section 7 conflicts with interstate commerce protections.

As Defendants identified in their side-by-side comparison, the 2018 Farm Bill expressly prohibits states from interfering with the interstate transportation of hemp and hemp products. However, yet again, Defendants' own comparison illustrates the conflict between Arkansas law and federal law. While the definition of hemp in Section 7 matches the 2018 definition, an additional burden has been added to interstate transportation of hemp and hemp products.

Act 629	Arkansas Law	Federal Law
Section 7 Uniform Controlled Substances Act – Substances in Schedule VI regarding Transportation and Shipment	A.C.A. § 5-64-215(d) (d) This section does not prohibit the continuous transportation through Arkansas of 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	7 U.S.C. 16390 Statutory Notes and Related Subsidiaries – Interstate Commerce Pub. L. 115–334, title X, §10114, Dec. 20, 2018, 132 Stat. 4914 "(a) RULE OF CONSTRUCTION.—

	delta-9 tetrahydrocannabinol concentration of not more than three-tenths of one percent (0.3%) on a dry weight basis, produced in accordance with 7 U.S.C. § 16390 et seq.	Nothing in this title or an amendment made by this title prohibits the interstate commerce of hemp (as defined in section 297A of the Agricultural Marketing Act of 1946 [7 U.S.C. 16390] (as added by section 10113)) or hemp products. (b) TRANSPORTATION OF HEMP AND HEMP PRODUCTS.— No State or Indian Tribe shall prohibit the transportation or shipment of hemp or hemp products produced in accordance with subtitle G of the Agricultural Marketing Act of 1946 [7 U.S.C. 16390 et seq.] (as added by section 10113) through the State or the territory of the Indian Tribe, as applicable."

As one can plainly read, the above highlighted language is nowhere to be seen in the 2018 Farm Bill. As confirmed by its rules of construction, the 2018 Farm Bill preempts and forbids Arkansas from "prohibit[ing] the transportation or shipment of hemp or hemp products produced in accordance with [the 2018 Farm Bill]"—full stop. *See* 7 U.S.C. § 16390 (Pub. L. 115-334, Title X, § 10114). The Act's attempt to criminalize hemp as a controlled substance when it is not in "continuous transportation" defies Congressional intent as a prohibition against hemp and hemp products in transportation or shipment.

As a practical consequence, Section 7 effectuates a significant burden to the interstate transportation or shipment of hemp and products by requiring them to be in "continuous transportation" through Arkansas. Under Act 629, those who transport and

ship hemp or hemp products may face criminal liability if they fail to demonstrate the products were "in continuous transportation." *See* Ark. Code Ann. § 20-56-412. As a direct result, farmers and other small businesses will be forced to route their products around Arkansas. Consumers will be denied hemp products intended to reach Arkansas. Arkansas, on the other hand—if Section 7 is allowed to take effect—will have been allowed to avoid express preemption by Congress.

Section 7 criminalizes certain hemp products that are not "in continuous transportation," which is not defined in statute, as controlled substances. Would an employee from a state like Tennessee that regulates and taxes hemp products like delta-8 THC be subject to criminal liability when stopping for gas or staying overnight before reaching the final destination outside of Arkansas? By criminalizing such products, the Act attempts to involve law enforcement agencies like the Drug Enforcement Administration ("DEA"). This ignores the fact that the 2018 Farm Bill completely removed any jurisdiction from the DEA over hemp and hemp products. While it is true that USDA and states must consult with the Department of Justice and DEA, the 2018 Farm Bill's plain language limited this interaction to only those situations where a mental state greater than negligence is suspected (like intentionally growing marijuana under a hemp license). In fact, the 2018 Farm Bill explicitly protects farmers who unintentionally grow hemp above 0.3% delta-9 THC from local, ***state***, and federal criminal prosecution. *See* 7 U.S.C. § 1639p(e) (emphasis added).

USDA's Final Rule implementing the 2018 Farm Bill even goes as far as to ***require*** state hemp plans to include remediation options for farmers who produce hemp above 0.3% delta-9 THC. This means that so long as the noncompliant crop is properly

remediated, farmers are still able to get their hemp and hemp products into the stream of commerce despite state and federal marijuana laws. *See* 7 U.S.C. § 990.3(6).

Clearly, Congress did not see a role for the DEA in the redevelopment of a domestic supply chain of hemp and hemp products. And for good reason. The DEA has been attempting to frustrate the development of a domestic hemp production program since its inception in the 2014 Farm Bill. For example, the DEA seized hemp seeds destined for the Kentucky Department Agriculture ("KDA"). KDA was forced to spend time and resources suing the DEA in federal court to gain possession of their legally purchased hemp seeds that the DEA had decided to seize. See *Kentucky Dep't of Ag. v. U.S. Drug Enf. Admin. et al.*, No. 3:14-cv-372-JGH, 2014 WL 2601244 (W.D. Ky. May 14, 2014).

The interstate commerce of hemp and hemp products are unduly burdened as employees would have to route around Arkansas due to the ever-present risk of arrest for transporting the hemp or hemp products through the State. Therefore, Act 629 is unconstitutional under the Commerce Clause of the United States Constitution.

C. Defendants offer no explanation for Section 6's conflicting additions to Arkansas' Uniform Controlled Substances.

Defendants attempt to justify Section 6's addition of certain THCs and "synthetic" or "psychoactive" substances to Arkansas's Uniform Controlled Substances by arguing that "[t]he federal government does not have a Schedule VI under the Controlled Substances Act." ECF No. 38 at 15. Defendants are technically correct—the federal Controlled Substances Act does not have a Schedule VI—but that argument is unhelpful to Defendants' position. Defendants have conveniently omitted the fact that the 2018 Farm Bill removes all hemp derived THCs from federal controlled status. *See* 21 U.S.C. § 812(c)(17).

Act 629	Arkansas Law	Federal Law
Section 6 Uniform Controlled Substances Act – Substances in Schedule VI	A.C.A. § 5-64-215(a) (5) Synthetic substances, derivatives, or their isomers in the chemical structural classes described below in subdivisions (a)(5)(A)-(J) of this section and also specific unclassified substances in subdivision (a)(5)(K) of this section. Compounds of the structures described in this subdivision (a)(5), regardless of numerical designation of atomic positions, are included in this subdivision (a)(5). The synthetic substances, derivatives, or their isomers included in this subdivision (a)(5) are: (A) (i) Tetrahydrocannabinols, including without limitation the following: (a) Delta-1 cis or trans tetrahydrocannabinol, otherwise known as a delta-9 cis or trans tetrahydrocannabinol, and its optical isomers; (b) Delta-6 cis or trans tetrahydrocannabinol, otherwise known as a delta-8 cis or trans tetrahydrocannabinol, and its optical isomers; (c) Delta-3,4 cis or trans tetrahydrocannabinol, otherwise known as a delta-6a,10a cis or trans tetrahydrocannabinol, and its optical isomers; (d) Delta-10 cis or trans tetrahydrocannabinol, and its optical isomers; (e) Delta-8 tetrahydrocannabinol acetate ester; (f) Delta-9 tetrahydrocannabinol acetate ester; (g) Delta-6a,10a tetrahydrocannabinol acetate ester; (h) Delta-10 tetrahydrocannabinol acetate ester; (i) A product derived from industrial hemp that was produced as a result of a synthetic chemical process that converted the industrial hemp or a substance contained in the industrial hemp into delta-8, delta-9, delta-6a,10a, or delta-10 tetrahydrocannabinol including their respective acetate esters; and (j) Any other psychoactive substance derived therein.	The federal government does not have a Schedule VI under the Controlled Substances Act. 21 U.S.C. § 812.

As is clear from the highlighted language above, Section 6 attempts to criminalize THCs "without limitation" in hemp—and other products protected as hemp—in conflict with the 2018 Farm Bill. The 2018 Farm Bill "is unambiguous." *AK Futures LLC*, 35 F.4th

at 692. Its definition of hemp, for purposes of the exclusion of THCs in hemp from federal controlled status, "does not limit its application according to the manner by which [the THCs, as derivatives, extracts, or cannabinoids] are produced." *See id*. "Rather, [the definition] expressly applies to 'all' such downstream products so long as they do not cross the 0.3 percent delta-9 THC threshold." *Id.* (citing 7 U.S.C. § 1639*o*(1)). In other words, there is no "distinction based on manufacturing method." *Id*. It is "the source of the product—not the method of manufacture—[that] is the dispositive factor . . ." *Id*.

Section 6's criminalization of certain THCs, chemically converted products, and "psychoactive substances" as Schedule VI "synthetic substances" creates a distinction based on method of manufacture that the Ninth Circuit rejected in *AK Futures*, Section 6 conflicts with not only the plain language of the 2018 Farm Bill, but the plain language of Arkansas's own Industrial Hemp Production Act as well, which expressly states it does ***not*** regulate hemp processing practices or methodologies and is very clear that if ***any*** of its provisions conflict with federal law relating to hemp, the federal provisions control. *See* Ark. Code Ann. §§ 2-15-502(b) (Department of Agriculture lacks "authority to regulate hemp processing practices or methodologies") and 2-15-506 (federal law controls in the event of conflict with Arkansas law).

D. Defendants erroneously conflate hemp-derived cannabinoids with synthetic cannabinoids.

Despite the plain and unambiguous reading of the 2018 Farm Bill that removed all tetrahydrocannabinols in hemp from the Controlled Substances Act, Defendants assert that popular hemp-derived cannabinoids are the equivalent of "synthetic cannabinoids" based on an invented two prong standard. This invented standard relies on whether or not a compound occurs naturally in the plant and the type of manufacturing process (like

a chemical reaction) used. This invented standard ignores the plain reading of the 2018 Farm Bill that relegalized all extracts and derivatives of hemp.

For the first prong of the invented standard, Defendants state as a matter of fact without evidence that delta-9 THCO[3] and delta-8 THCO do not occur naturally in the plant. The science of testing hemp is still catching up to the market, and scientists do not even know how many cannabinoids are found in the plant. For an example, a recent abstract of a thesis exploring testing methods to properly identify unknown cannabinoids and isomers of cannabinoids discovered a structural isomer of delta-9 THCO in flower material. Mojisola Adisa, *Development of a Validated Method for High Throughput Quantification of up to Twenty Cannabinoids in Cannabis Cigarettes Using Liquid Chromatography Diode Array Detector with Optional Electrospray Ionization Time-of-Flight Mass Spectrometry*, DEP'T OF CHEMISTRY - WESTERN ILLINOIS UNIVERSITY (December 2022), courtesy copy attached as **Exhibit 1**.

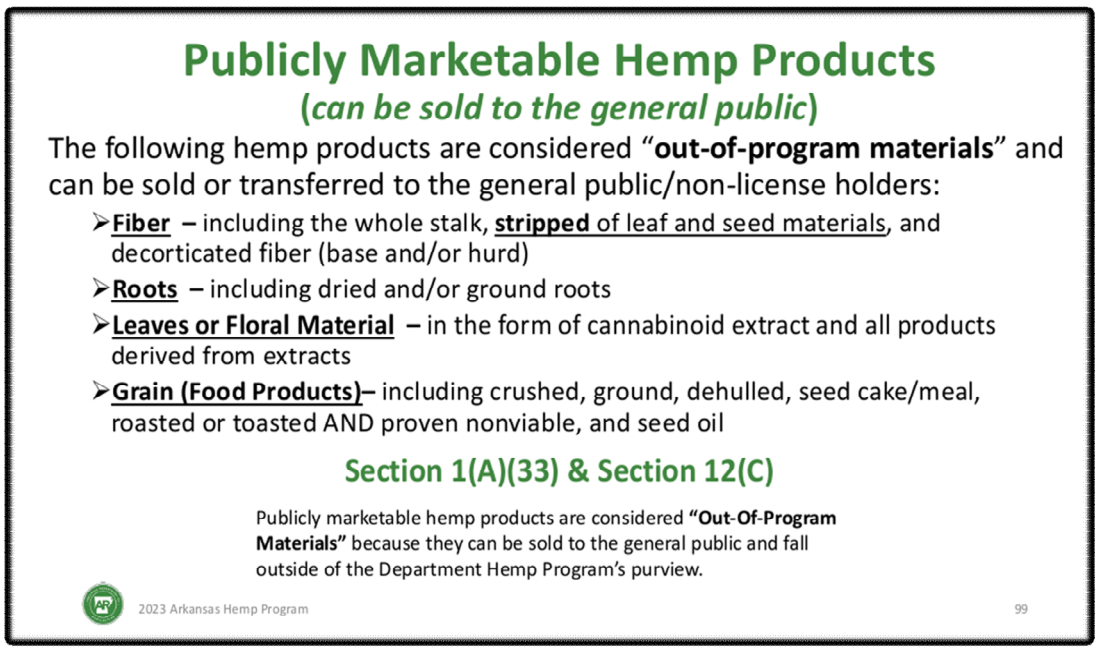
For the second prong of the invented standard, Defendants rely on DEA correspondence (Exhibit B to the Response) that states delta-8 THC ". . . produced from non-cannabis materials is controlled under the CSA as 'tetrahydrocannabinol.'" This private email exchange is dated August 12 and 13, 2021 and is in direct conflict with more publicly available guidance issued a month later to the Alabama Board of Pharmacy indicating delta-8 THC is in fact considered hemp so long as it is 0.3% or less delta-9 THC. *See* ECF No. 1-5. Defendants would have the Court believe that hemp-derived cannabinoids are the same as "synthetic cannabinoids," but these two substances are worlds apart. Plaintiffs agree that true synthetic cannabinoids—man-made products that

[3] To be clear, this case has nothing to do with Delta-8 THCO or any other synthetic product like "spice" or K2.

do not occur naturally—are dangerous and need to be controlled, and in fact they already are. Defendants conflate these two concepts, claiming on one hand that delta-8 THC occurs naturally in the cannabis plant in trace amounts and is 50-75% less potent than delta-9 THC, and simultaneously claiming synthetic cannabinoids are not naturally produced by the cannabis plant and are 100-800 times more potent than the THCs found naturally in the plant. Defendants cannot have it both ways. And despite several subsequent public statements from the DEA that indicate delta-8 THC derived from cannabis is not controlled so long as the delta-9 THC levels do not exceed 0.3%, Defendants instead rely on one sentence in an earlier-dated obscure private email from a DEA staff person to make their case.

To be clear, prior to Act 629, floral material, including cannabinoid extract and all products derived from extracts, were considered "out of program materials" and were allowed to be sold to the general public. In fact, as the below chart from Defendants' Exhibit A demonstrates, the hemp program rules currently in effect specifically permit the sale or transfer of consumable hemp products to the general public, both within and outside the state, without any special license or permit required, deeming them "publicly marketable hemp products."[4] The only compliance metric used for these out of program materials is the delta-9 THC concentration on a dry weight basis—just like the 2018 Farm Bill.

[4] The same information is currently available on the State of Arkansas's hemp program website at https://www.agriculture.arkansas.gov/plant-industries/feed-and-fertilizer-section/hemp-home/restrictions-on-sale-or-transfer/ (last visited Aug. 14, 2023).



Again, the Arkansas Industrial Hemp Act expressly states that if ***any*** of its provisions conflict with federal law relating to hemp, the federal provisions control. *See* Ark. Code Ann. § 2-15-506. Yet, Defendants have adopted the position that compliance with the 2018 Farm Bill now relies on an invented standard that is equal parts incoherent and unverifiable. Finally, this invented standard ignores the fact that the 2018 Farm Bill provided a path to market for all extracts and derivatives of hemp (going so far as to protect them in interstate commerce from individual states), and such an interpretation is in direct conflict with the plain reading of the 2018 Farm Bill.

II. THE CHANGES MADE BY THE ARKANSAS CODE REVISION COMMISSION MANIFESTLY CHANGED THE SUBSTANCE AND MEANING OF ACT 629, AND THEREFORE THE ORIGINAL WORDING OF THE ACT CONTROLS.

Defendants further allege that Act 629 does not unconstitutionally interfere with interstate commerce nor is it unconstitutionally vague due to certain revisions made by the Arkansas Code Revision Commission on June 13, 2023. *See* ECF No. 38 at 3. As originally enacted, Section 17 of Act 629 stated:

> Sections 6-14 of this act shall become effective only upon the certification of the Arkansas Attorney General that the State of Arkansas is currently enjoined from enforcing Sections 2-5 of this act relating to delta-8 tetrahydrocannabinol and delta-10 tetrahydrocannabinol, but no earlier than August 1, 2023.

This provision is a trigger clause, and by altering the numerical section designations, the Commission altered the substance of the Act. Indeed, the Commission presumed that the General Assembly intended Sections 8-15, not 6-14, of the Act to become effective upon enjoining the enforcement of Sections 2-7, not 2-5, of the Act. *See* ECF No. 43-3. Because of this, the Commission altered the sections referenced in this trigger clause along with Section 16, indicating that such references constituted "manifest reference errors." *See id*.

While the Commission, in the process of codifying an Act, is permitted to make certain corrections to spelling, grammar, and clerical errors, "the commission shall not authorize any change in the substance or meaning of any provision of the Arkansas Code or any act of the General Assembly. The bureau [of legislative research] shall not change the substance or meaning of any provision of the Arkansas Code or any act of the General Assembly." Ark. Code Ann. § 1-2-303(d)(1) (emphasis added). Further, except for the clerical-type changes specifically listed in subsection (d)(1), "the wording, punctuation, and format of sections of acts shall appear in the Arkansas Code *exactly as enacted* by the General Assembly." Ark. Code Ann. § 1-2-303(d)(2) (emphasis added); *see also* Norman Singer & Shambie Singer, 2 SUTHERLAND STATUTORY CONSTRUCTION § 36A:1 (7th ed.) (Nov. 2018 update) ("The only truly authentic text of a statute is the exact wording of the act in the form in which it passed both houses of the legislature and was either approved by executive or passed over h[er] veto.").

Arkansas courts have consistently invalidated the Commission's amendments that change the substance and meaning of any provision of the Arkansas Code or any act of

the General Assembly. *See e.g.*, *Harrell v. State,* 2012 Ark. 421, at 4-5, 2012 WL 5462868 ("In reviewing the General Assembly's language and the codified version, it is apparent that the language of the General Assembly was substantially altered by the Arkansas Code Revision Commission."); *Falcon Cable Media LP v. Arkansas Pub. Serv. Comm'n*, 2012 Ark. 463, 11, 425 S.W.3d 704, 710–11 (holding that the Commission's substitution of the word "subchapter" for the word "act" "ha[d] the effect of altering the meaning of the statute, [and] the Code Revision Commission is not authorized to change the substance or meaning of any provision of the Arkansas Code or any act of the General Assembly"); *Porter v. Ark. Dep't of Health & Human Servs.*, 374 Ark. 177, 182, 286 S.W.3d 686, 691 (2008) ("The [Commission] lacked the authority to amend Act 441 in its codification—which became § 9-11-102(b)—in a manner that changed the meaning and substance of Act 441."); *Cox v. City of Caddo Valley,* 305 Ark. 155, 806 S.W.2d 6 (1991) (holding that, although the language of the statute was clear and unambiguous, the language of the act was controlling where the Code Revision Commission omitted a word that changed the meaning of the statute). Where the Commission exceeds its powers in amending an act, the original wording of the act controls. *Porter*, 374 Ark. at 183, 286 S.W.3d at 692.

The Commission lacked the authority to amend Act 629 in a manner that changed the meaning and substance of the Act. While the Commission has the authority to correct reference errors, its revision cannot "manifestly change[] the substance and meaning" of the Act. *Id.*, 286 S.W.3d at 691. Here, the Commission did exactly that by unilaterally amending Section 17 of Act 629 to change the sections of the Act that were to become effective upon the occurrence of a certain triggering event. Such a change goes beyond merely amending reference errors to completely altering the substance of the Act.

This is particularly true in context of Act 629. Act 629 contains a trigger clause, and altering the section references of a trigger clause directly alters its substance. In fact, Plaintiffs have been unable to locate *any* legislation containing a trigger clause that the Commission attempted to amend. The Commission, in revising Act 629 exceeded its powers, and, as a result, the original wording of Act 629 controls. And if not, the Commission's revisions only serve to render Act 629 all the more vague.

A. The interstate commerce provisions from Sections 7 and 10 of Act 629 are not in effect until Section 2-5 of the Act are enjoined.

Given that the language of Act 629 as originally passed by the General Assembly controls, Sections 7 and 10 of Act 629, which attempt, but fail, to cure the Act's impermissible interference with the interstate transportation and shipment of hemp and hemp products, are not currently effective until Sections 2-5 are enjoined as contemplated in Section 17. As a result, no such attempted protection of the transportation of hemp and hemp products is currently in effect, and, thus, Act 629 additionally interferes with the interstate transportation and shipment of hemp and hemp products in direct violation of the express language of the 2018 Farm Bill.

Moreover, even the Code referenced in Defendants' exhibit indicates that the language from Section 7 of the Act is not currently in effect and is only effective if the contingency in Section 17 of Act 629 is met. *See* ECF No. 43-5 at 57; *see also* Ark. Code Ann. § 20-56-412. As discussed above, because the 2018 Farm Bill expressly preempts any prohibition on the interstate transport of hemp and hemp products, Act 629 is unconstitutional under the Supremacy Clause of the United States Constitution.

B. Act 629 remains unconstitutionally vague and constitutes a regulatory taking.

Additionally, given that the language of Act 629 as originally passed by the General Assembly controls, the Act is unconstitutionally vague as those internal inconsistencies remain which prevent a person of average intelligence from knowing whether the possession, transportation, or shipment of hemp or hemp-derived products is subject to criminal punishment. Moreover, besides those internal inconsistencies, Act 629 is unconstitutionally vague for various other reasons as previously outlined in Plaintiffs' Motion for Temporary Restraining Order or Alternative Motion for Preliminary Injunction.

Additionally, as fully explained above, Act 629 does in fact change the definition of hemp from that of the 2018 Farm Bill. But Act 629 does not define "psychoactive substance," and Defendants' attempt to rely on the DEA's interpretations is misplaced. *See* ECF No. 38 at 30. The DEA fact sheet that Defendants cite does not define "psychoactive substance." Instead, it defines "marijuana" as "a mind-altering (psychoactive) drug, produced by the Cannabis sativa plant."[5] A psychoactive substance is commonly defined as "any substance that interacts with the central nervous system," which includes nicotine, alcohol, and caffeine.[6] Hemp products containing 0.3% or less THC and even CBD isolate with no THC could therefore be considered "psychoactive substances," which are banned by Act 629.[7]

---

[5] Marijuana/Cannabis, DOJ/DEA Drug Fact Sheet, https://www.dea.gov/sites/default/files/2020-06/Marijuana-Cannabis-2020_0.pdf (last visited Aug. 13, 2023).

[6] https://www.medicalnewstoday.com/articles/types-of-psychoactive-drugs#risks (last visited Aug. 13, 2023).

In response to Plaintiffs' allegation that Act 629 constitutes a regulatory taking, Defendants direct the Court to review Section 10 which states that a hemp-derived product "shall not be combined with or contain any of the following: . . . Any amount of tetrahydrocannabinol as to create a danger of misuse, overdose, accidental overconsumption, inaccurate dosage, or other risk to the public." However, it is unclear what amount of tetrahydrocannabinol Defendants believe constitutes a "danger of misuse" or a "risk to the public." This metric is also vague. This prohibition could potentially include hemp-derived products that contain 0.3% or less of delta-9 tetrahydrocannabinol as encompassed by the broad definition of hemp under the 2018 Farm Bill. 7 U.S.C. § 16390(1).

Act 629's threat of irreparable harm has become a reality for Plaintiffs. Specifically, one of the retail stores of Plaintiff Drippers Vape Shop was inspected by an Arkansas Tobacco Control officer on August 7, 2023. *See* **Exhibit 2**, *Declaration of Scout Stubbs* (Drippers Vape Shop, LLC). The officer directed the company's employees to remove a hemp product containing 0.3% or less of delta-9 THC, regardless of whether that product was naturally extracted or produced as a result of routine chemical processes. *Id*. This enforcement action shows that the Act not only recriminalizes all hemp products "produced as a result of a synthetic chemical process" but also "[a]ny other psychoactive substance derived [from hemp]," including those hemp products purportedly permitted under the Act, naturally derived hemp products containing 0.3%

[7] Ian Stewart, *It's Time to Set the Record Straight – CBD Is Psychoactive*, https://www.mondaq.com/unitedstates/food-and-drugs-law/809252/its-time-to-set-the-record-straight--cbd-is-psychoactive (last visited Aug. 13, 2023); *see also* https://www.goodrx.com/classes/cannabinoids/does-cbd-get-you-high (last visited Aug. 13, 2023).

or less of delta-9 THC, and even CBD isolate. Therefore, Act 629 constitutes a regulatory taking as it impermissibly deprives Plaintiffs of all, or substantially all, beneficial economic use of their businesses without just compensation.

Inconsistent with a plain reading of Act 629, Defendants state in their Response that "assuming Plaintiffs were already compliant with Arkansas law in their cultivation and sale of help products, there should be no change in their business. If instead their inventory contains synthetic cannabinoids that exceeds the required concentration of delta-9 THC, it is already illegal under both Arkansas and federal law." ECF No. 38 at 31. Plaintiffs cannot take the word of Defendants in their Response as to how to interpret Act 629. The Act appears on its face to recriminalize all hemp products "produced as a result of a synthetic chemical process" – including delta-8, along with "[a]ny other psychoactive substance derived [from hemp]." Demonstrating the public confusion involving Act 629, even Arkansas Tobacco Control officers appear to be inconsistently enforcing the Act. On its face, Act 629 contains numerous internal inconsistencies and fails to provide notice as to what contemplated conduct is forbidden and what is permitted with regard to production, possession, transportation, and shipment of hemp and hemp products it seeks to ban. Therefore, Act 629 is unconstitutional, and the Act should be enjoined.

CONCLUSION

As set forth in Plaintiffs' Motion, Brief, and this Reply, Act 629 is unconstitutional because it is (1) preempted by the 2018 Farm Bill, which solidifies the broad definition of hemp and declares hemp and all derivatives and isomers thereof legal; (2) is preempted by the 2018 Farm Bill by precluding the interstate commerce of hemp; (3) impermissibly restricts the interstate commerce of hemp in violation of the Commerce Clause; (4) its regulatory scheme results in an impermissible regulatory taking, effectively creating a

total ban of hemp containing any amount of tetrahydrocannabinol and thus infringing upon Plaintiffs' businesses; and (5) is void for vagueness due to its failure to provide clarity and fair warning to persons of ordinary intelligence as to its requirements. Moreover, Plaintiffs will be irreparably harmed unless Act 629 is enjoined, and the balance of equities favors entry of an injunction enjoining the Act. Plaintiffs respectfully request that the Court grant Plaintiffs' Motion for a Temporary Restraining Order, or in the alternative, Preliminary Injunction, and for all other just and equitable relief.

Respectfully submitted,

Abtin Mehdizadegan (2013136)
Joseph C. Stepina (2020124)
Allison T. Scott (2020205)
HALL BOOTH SMITH, P.C.
200 River Market Avenue, Suite 500
Little Rock, Arkansas 72201
Telephone: (501) 214-3499
Facsimile: (501) 604-5566
abtinn@hallboothsmith.com
jstepina@hallboothsmith.com
allisonscott@hallboothsmith.com

- and -

Justin Swanson
Paul Vink
Admitted Pro hac vice
BOSE MCKINNEY & EVANS, LLP
111 Monument Circle, Suite 2700
Indianapolis, IN 46204
(317) 684-5404
(317) 223-0404
jswanson@boselaw.com
pvink@boselaw.com

Attorneys for Plaintiffs

DEVELOPMENT OF A VALIDATED METHOD FOR HIGH THROUGHPUT QUANTIFICATION OF UP TO TWENTY CANNABINOIDS IN CANNABIS CIGARETTES USING LIQUID CHROMATOGRAPHY DIODE ARRAY DETECTOR WITH OPTIONAL ELECTROSPRAY IONIZATION TIME-OF-FLIGHT MASS SPECTROMETRY

An Abstract of

A Thesis

Presented to the Faculty of

Department of Chemistry

Western Illinois University

In Partial Fulfillment

Of the Requirement for the Degree

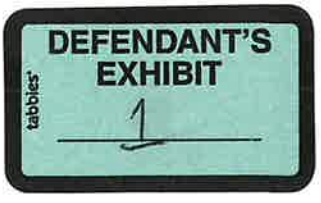
Masters of Science in

Chemistry

By

MOJISOLA ADISA

DECEMBER 2022



Abstract

The modern *Cannabis* market is in a period of dramatic influx and the growing interest in this highly sought out plant could be attributed to the unique class of compounds, i.e., cannabinoids. Over the past few years, the popularity of this plant has resulted in an explosion in cannabis derived products including *Cannabis* cigarettes. As a result, a liquid chromatography diode array detector (LC-DAD) method with optional electrospray ionization time-of-flight mass spectrometry (ESI/TOFMS) has been developed, validated, and applied in the analysis of cannabinoids in six *Cannabis* cigarettes: five samples of marijuana cigarettes and one hemp cigarette sample. The developed method has achieved significant improvements over published methods. It includes the characterization of twenty targeted cannabinoids, quantification of eighteen cannabinoids, baseline resolution among quantified cannabinoids, a lower limit of quantification at 0.02 µg/mL, and a wide linear range (0.02 to 25 µg/mL in 50 µg/mL extracts of the *Cannabis* cigarettes or 0.04 to 50% (w/w) in the *Cannabis* cigarettes). Real time recovery of sample preparation was achieved by spiking abnormal cannabidiol (ACBD) into the samples. The method specificity was confirmed by ESI/TOFMS. Precision and accuracy were assessed using quality control (QC) samples at three concentration levels, i.e., 0.02, 0.5, and 12.5 µg/mL, in triplicates with interday and intraday precision of less than 15% relative standard deviation (RSD) and accuracy of within ±15% relative error, therefore meeting the requirements by the ISO 17025 standards. Additionally, with this method two unknown cannabinoids which includes a structural isomer of cannabinolic acid (CBNA) and a structural isomer of Δ^9-THC acetate were discovered.

Approval Page

This thesis by MOJISOLA ADISA is accepted in its present form by the Department of Chemistry of Western Illinois University as satisfying the thesis requirement for the degree Master of Science.

L. Song 5006

Dr. Liguo Song

Chairperson, Examining Committee

Dr. Jin Jin

Member, Examining Committee

Dr. John Determan

Member, Examining Committee

12/01/2022

Date

DEVELOPMENT OF A VALIDATED METHOD FOR HIGH THROUGHPUT QUANTIFICATION OF UP TO TWENTY CANNABINOIDS IN CANNABIS CIGARETTES USING LIQUID CHROMATOGRAPHY DIODE ARRAY DETECTOR WITH OPTIONAL ELECTROSPRAY IONIZATION TIME-OF-FLIGHT MASS SPECTROMETRY

A Thesis

Presented to the Faculty of

Department of Chemistry

Western Illinois University

In Partial Fulfillment

Of the Requirement for the Degree

Masters of Science in

Chemistry

By

MOJISOLA ADISA

DECEMBER 2022

Acknowledgements

I would like to express my deepest appreciation to my thesis committee members Dr. Liguo Song, Dr. Jin Jin, and Dr. John Determan for their patience and support. I would also like extend my special gratitude to my research mentor, Dr. Liguo Song, for all his practical guidance. I appreciate the open-door to his research group even from my first email and I am very grateful for the learning opportunities he has provided me during my academic career at Western Illinois University.

I would also like to express my profound gratitude to my mother and Uncle for the various forms of support and continuous encouragement throughout my years of study. Furthermore, I would like to thank the rest of my research group colleagues for their collaborative efforts during data collection. I must also thank Kay for her unwavering support and insightful suggestions.

Lastly, I would like to extend my sincerest gratitude to the foundations and institutes that made this work possible. This work was supported by the National Institute of Justice (grant no. 2020-DQ-BX-0021), the National Science Foundation (grant no. CHE-1827209), and the Western Illinois University (university research council grant; graduate student research grants; undergraduate research grants). I would like to thank the National Institute on Drug Abuse (NIDA) Drug Supply Program (DSP) for providing the samples used in this research.

Table of Contents

List of Tables

List of Figures

List of Equations

Introduction

Cannabis plant is considered one of the most controversial plants today; although well-known for its medicinal properties, it is also the most frequently abused drug due to its intoxicating effect. Cannabis is a dioicous annual flowering plant that has been botanically named as *Cannabis sativa* L., with two subspecies called *Cannabis sativa ssp. sativa* and *Cannabis sativa ssp. indica*. [1] *Cannabis Sativa L.* is known to be the only plant that biosynthesizes a group of unique C_{21} terpenophenolics compounds known as cannabinoids,[2] hence the continuous interest in the chemistry of this plant. In addition to cannabinoids which is the main constituent of Cannabis plant, terpenes, fatty acids, and other chemically active compounds have also been identified in the plant.[3-4] So far, 144 cannabinoids have been isolated and identified according to two recent reviews.[5,6]

The biosynthetic pathway for all cannabinoids starts with cannabigerolic acid (CBGA)[7], and cannabigerovarinic acid (CBGVA)[8] (Figure 1). All cannabinoids are synthesized in their acidic form and then decarboxylated by ex vivo stress conditions such as UV light and heat to the neutral forms.[7, 9-10] CBGA undergoes enzymatic catalyzed reactions to produce cannabidiolic acid (CBDA), tetrahydrocannabinol acid (THCA), and cannabichromenic acid (CBCA) using CBDA synthase, THCA synthase, and CBCA synthase enzymes respectively.[7, 9] Thereafter, CBDA, THCA, and CBCA can be degraded upon exposure to heat or UV light to produce their neutral analogs, cannabidiol (CBD), Δ^9-tetrahydrocannabinol (Δ^9-THC) and cannabichromene (CBC), respectively. CBCA may also degrade to cannabicyclolic acid (CBLA) when exposed to UV light. Upon exposure to heat, CBLA produces cannabicyclol (CBL).[11] The oxidative aromatization of Δ^9-THC yields cannabinol, (CBN), and cannabitriol (CBT). [7, 9] CBN may also be produced by the

degradation of its acidic form, cannabinolic acid, CBNA, which exists as a minor cannabinoid in the plant.[10] Additionally, Δ^8-THC can be produced through an acidic isomerization of Δ^9-THC. CBGVA undergoes a similar enzymatically catalyzed reaction to produce cannabidivaric acid (CBDVA), cannabichromevarinic acid (CBCVA), and tetrahydrocannabivarin acid (THCVA).[8] CBDVA, CBCVA, and THCVA degrades upon exposure to heat or light to produce cannabidivarin (CBDV), cannabichromevarin (CBCV), and tetrahydrocannabivarin (THCV), respectively.

The two primary subspecies of *Cannabis Sativa L.* have been classified based on phenotypic characteristics and cannabinoid content.[12] *Cannabis sativa ssp. Sativa which* is also referred to as hemp has a low THC/CBD ratio (<< 1) *while Cannabis sativa ssp. Indica* known as marijuana has high THC/CBD ratio (>> 1).[9, 13-14] Generally, psychotropic effects are exerted by the *Cannabis* plant when Δ^9-THC—the main psychoactive component of marijuana[15]— content approximates 1%.[14] Thus, marijuana generally refers to the cultivated plant used as a psychotropic drug. On the contrary, hemp is characterized by low levels of Δ^9-THC and high levels of CBD, which is non/anti-psychoactive. Before the 1950s, hemp was cultivated and used mainly to produce fibers for industrial applications in the U.S. However, to regulate controlled substances in the U.S., hemp was classified together with marijuana which was later defined to be a "Schedule 1" substance according to the 1970 Federal Controlled Substances Act (CSA). For this reason, the use and cultivation of *Cannabis* for medicinal and recreational purpose was considered illegal.

Notwithstanding, the use of marijuana for medicinal purposes has long been favored in the U.S. Also, there has been a steady increase in the number of states that legalize marijuana for medical use after California passed the law first in 1996, conflicting with the

Federal position on marijuana. As of February 3, 2022, Medical Marijuana Laws have been passed in 37 states, four territories, and the District of Columbia, albeit with considerable state-to-state variation in the specific provisions of the laws.[16] One of the most significant shifts in state law occurred in 2012, where Colorado and Washington State passed laws permitting adult to use marijuana for recreational purpose. As at November 2, 2021, recreational Marijuana Laws have been passed in 18 states, two territories and the District of Columbia.[16] Furthermore, the Farm Bill policy enacted by the Congress in December of 2018, excluded hemp from the statutory definition of *Cannabis* as long as the Δ^9-THC concentration is not more than 0.3% (w/w) on a dry weight basis. This policy also included provisions to facilitate the commercial cultivation, processing, and sale of hemp and hemp-derived products. Thus, hemp was no longer subject to monitoring by the Drug Enforcement Administration (DEA), neither was it regulated as a Schedule I controlled substance under the CSA. Instead, the regulation of hemp cultivation is now under the control of U.S. Department of Agriculture (USDA).[17]

The growing popularity of *Cannabis Sativa L.* and its widespread use for medical and recreational purposes has been accompanied with a significant interest in developing various cannabis derived consumer products including marijuana and hemp cigarettes. Although the two species of *Cannabis sativa L.* have been differentiated, new varieties with different phytochemical properties constantly emerge due to crossbreeding and different climatic conditions around the world. For these reasons, the science community in all parts of the world seek to develop accurate and reliable analytical methods to identify and quantify cannabinoid content in Cannabis plant materials and consumer products. The Association of Official Agricultural Chemists (AOAC) International has recently issued

standard method performance requirements (SMPRs) for quantitative determination of cannabinoids in dried plant materials[18] and plant materials of hemp.[19] These requirements were developed by a group of the AOAC Stakeholder Panel on Strategic Food Analytical Methods. Of all the known cannabinoids, five (5) are required namely: CBD, CBDA, CBN, Δ^9-THC, and Δ^9-THCA. An additional nine (9) cannabinoids are desirable namely: CBG, CBGA, CBC, CBCA, CBDV, CBDVA, Δ^8-THC, THCV, and THCVA. Also, the USDA has clarified that the testing guidelines and methodologies should consider the potential conversion of Δ^9-THCA to Δ^9-THC. Therefore, analysis results must reflect the total available Δ^9-THC derived from the sum of the Δ^9-THC and the Δ^9-THCA content.[17]

The traditional way of quantifying cannabinoids is by the use of gas chromatography (GC)—a method readily employed by authorities and used for federal regulation purposes.[1, 7, 20, 21-23] Usually, the GC is coupled to a flame ionization detector (FID) or mass spectrometry (MS) for detection. However, the direct examination by GC which relies on heating the sample comes with a limitation. Acidic cannabinoids are known to readily decarboxylate into the neutral counterparts under ex vivo conditions[7, 9] such as high temperature.[9, 24-25] Most times the incomplete decarboxylation of acidic cannabinoids takes place at the injection port of the instrument[26] which affects quantification. This problem could be solved by derivatization, but it is a time-consuming process. The most common method includes blocking the -COOH or -OH functional groups with trimethylsilating reagents.[1, 25] In the last few years, liquid chromatography (LC) coupled either ultraviolet (UV)/diode array detection (DAD) or MS detector has emerged as the golden standard for potency testing of cannabinoids. This can be explained by the ability to analyze cannabinoids in their original forms without any form of thermal stress.[7, 22]

However, since LC-MS methods demand expensive instruments that are not commonly available or are inappropriate for routine analysis by *Cannabis* growers, commercial suppliers, and crime labs, LC-UV/DAD methods are mostly favored. Unfortunately, the LC-UV/DAD method comes with a few challenges as well.

The LC-UV/DAD methods published[4, 7, 27-38] so far have only focused on the quantification of thirteen cannabinoids or less except Tran et al and Galettis et al.[4 39] who both recently published a method for the quantification of sixteen and seventeen cannabinoids respectively. Furthermore, some application notes provided by LC companies demonstrates the separation of up to nineteen cannabinoids,[22] but method validation has been rarely performed. This is crucial since the resolution of critical pairs of cannabinoids—CBG/CBD and Δ^9-THC/Δ^8-THC—may not meet the minimum required resolution of 1.5 appropriate for method validation. This has been the case when the LC method involved separation of cannabinoids at high concentration and/or involved many cannabinoids.[4, 30, 37-38, 40] Another problem encountered in published LC-UV/DAD methods is the use of narrow calibration ranges.[4, 7, 9, 21, 27-31, 33-38, 40-41 42 43 44] Since it is expected that plant materials of hemp and marijuana contain varying high concentrations of the major cannabinoids (CBD/CBDA & Δ^9-THC/Δ^9-THCA) and very low concentration of the minor cannabinoids, it is challenging to provide an accurate quantification of both categories of cannabinoids with a single analytical run. Therefore, two different dilutions are often prepared to get all cannabinoids within the linear range limits.

Another problem which complicates method recovery in published methods is the lack true blank cannabinoid *Cannabis* material[45] to meet method validation requirements. Some researchers opt to perform recovery test on surrogate plants matrixes related to

Cannabis. Mudge et al.[33] and De backer et al.[9] both used nettle (*Urtica dioica*) extract due to the fact the genus *Cannabis* was formerly placed in the nettle family (*Urticaceae*)[9]. The representative blank plant matrix used as a cannabinoid reference material were spiked to monitor recovery.[9, 28] Similarly, Zivovinovic et al.[7] used hop strobiles (*Humulus lupulus L.*) —belonging to the same family (*Cannabaceae*) as a surrogate matrix for method validation. Two other methods published by Giese et al.[38] and Wang et al.[31] used exhaustively extracted and completely dried Cannabis plant materials as a blank matrix. The most common assessment of recovery was standard addition.[30, 34, 36] It is important to point out that none of the methods mentioned above track the recovery of sample preparation in real time for each sample. Method validations are complicated by recovery experiments also due to the high cost of cannabinoid standards thereby making spiking impractical and sometimes unaffordable[45] even at small concentrations. Consequently, extracts of cannabinoids-rich *Cannabis* samples were used as an alternative by DeBacker et al[9], Vaclavik et al.[30] and Ciolino et al.[32]

Finally, a few methods have also been reported for analyzing cannabinoids in consumer products such as honey, gummies, beverages, edibles, and cigarettes.[27, 32] In general, specificity of these methods have relied mostly on LC separation, with only a few assessments by MS detection by Citti et al.[35], Brighenti et al.[34], Wang et al.[31], and Vaclavik et al.[30] However, interferences to quantification by untargeted or unknown cannabinoids are yet to be reported in developed methods.

The developed method solved the abovementioned problems. First, a systematic optimization of the LC separation achieved baseline separation of 21 cannabinoids (Figure *2*). These includes the 14 cannabinoids recommended by the AOAC international plus

CBCV, CBNA, CBL, CBLA, CBT, Δ^8-THCA, and an internal standard— abnormal CBD (ACBD). A wide linear calibration range was obtained due to the lowest limit of quantitation ever achieved (LOQ), 0.02 µg/mL. This was attained due to low UV absorption of the mobile phase. Afterward, ACBD—a cannabinoid not naturally present in *Cannabis* plants and commercially available at a reasonable price—was spiked into each sample to track the recovery of sample preparation in real-time. Finally, method specificity was well-assessed by electrospray ionization time-of-flight mass spectrometry (ESI/TOFMS).

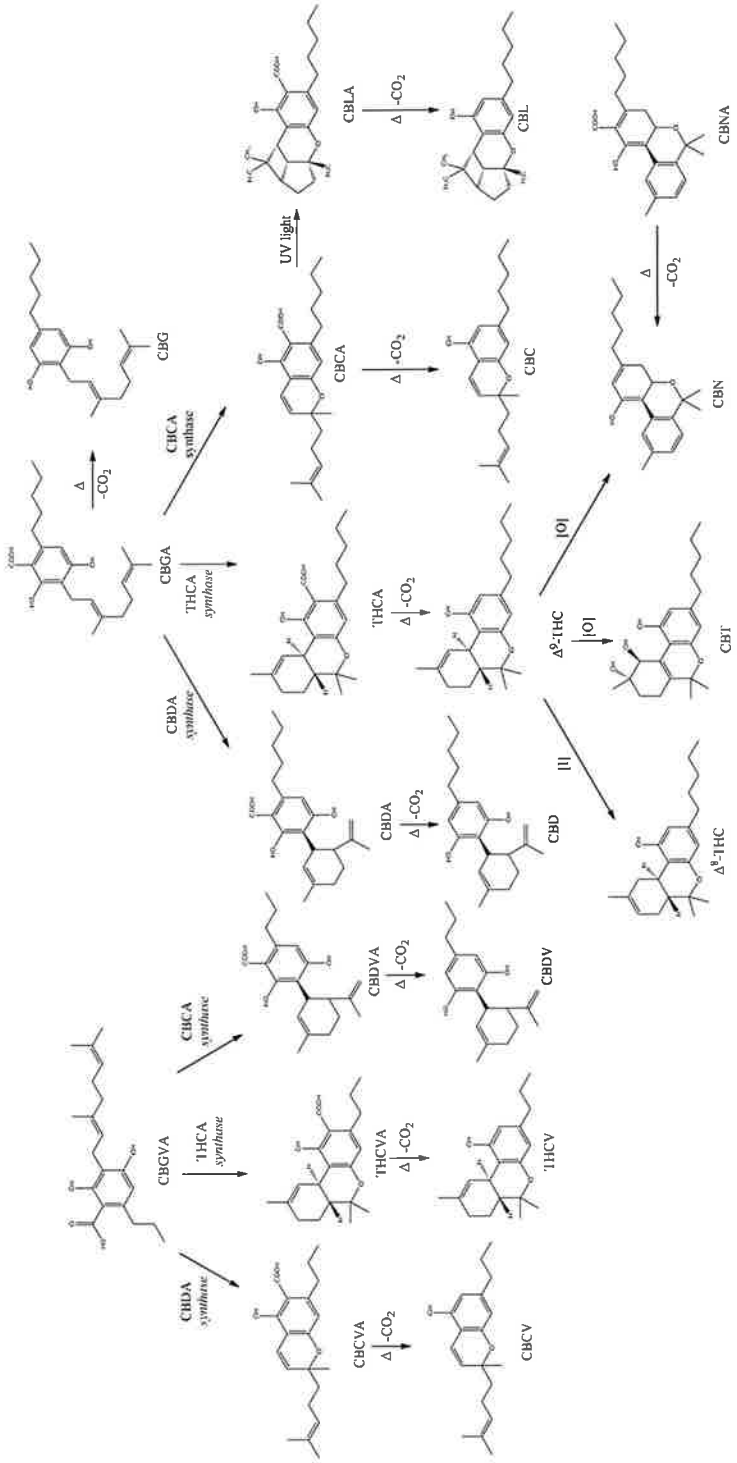
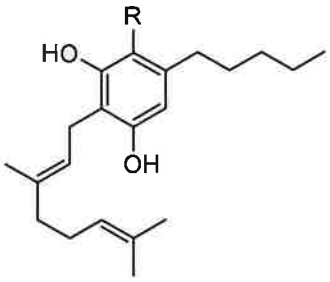
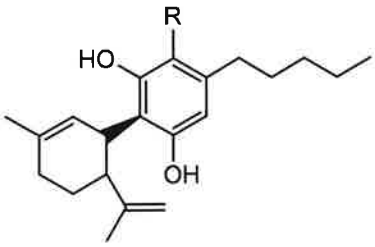


Figure 1. The Biosynthetic Pathway of the 18 Quantified Cannabinoids.

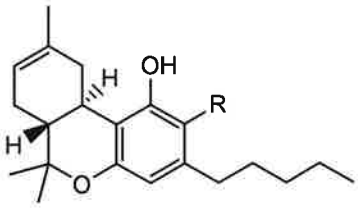


CBG: R=H
CBGA: R=COOH

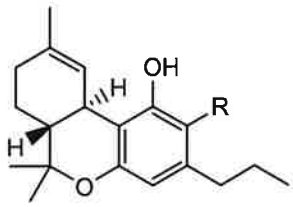


CBD: R=H
CBDA: R=COOH

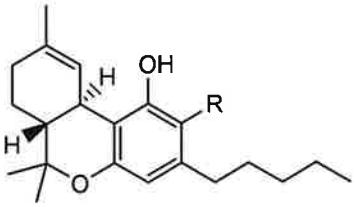
CBDV: R=H
CBDVA: R=COOH



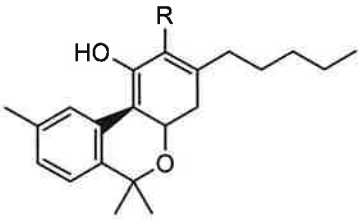
Δ^8-THC: R=H
Δ^8-THCA: R=COOH



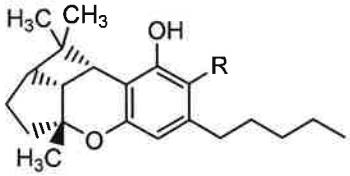
THCV: R=H
THCVA: R=COOH



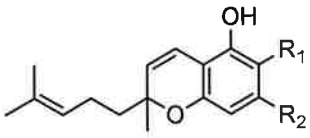
Δ^9-THC: R=H
Δ^9-THCA: R=COOH



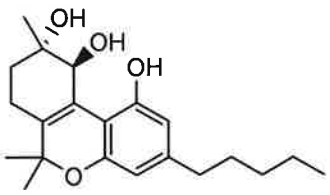
CBN: R=H
CBNA: R=COOH



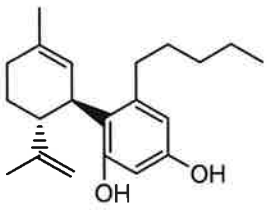
CBLA: R=H
CBLA: R=COOH



CBC: R_1=H; R_2=C_5H_{11}
CBCA: R_1=COOH; R_2=C_5H_{11}
CBCV: R_1=H; R_2=C_3H_7



CBT



ACBD

Figure 2. Chemical structures of 21 cannabinoids separated in the method.

Materials and Methods

Chemicals and Reagents

LC grade Acetonitrile (ACN), methanol (MeOH), water, ammonium formate and MS grade formic acid were purchased from Fisher Scientific (Pittsburgh, PA). All cannabinoid standards were purchased as certified reference materials (CRMs) in Drug Enforcement Administration (DEA) exempt preparations. CBCA, CBDVA, CBNA, Δ^8-THCA, and THCVA were purchased from Cayman Chemical (Ann Arbor, MI) at concentration of 1 mg/mL in acetonitrile. While CBLA was purchased from Sigma-Aldrich (St. Louis, MO) at concentration of 1 mg/mL in acetonitrile. Neutral cannabinoids, including CBCV, CBL, and CBT were purchased from Cayman Chemicals at concentration of 1 mg/mL in methanol. The remaining cannabinoids were purchased as an 11 phytocannabinoids mixture from Cayman Chemical at concentration of 250 µg/mL in acetonitrile. ACBD was also purchased from Cayman Chemical at 250 mg in 10 mL of methanol.

Cannabis Samples

Five marijuana cigarettes samples plus a placebo were provided by the National Institute on Drug Abuse (NIDA) Drug Supply Program (DSP): Research Triangle Institute (RTI), Log No. 12792-1208-77 (MC1), 12792-0109-120 (MC2), 12792-0109-146 (MC3), 10074-0301-97 (MC4), 10604-0203-95 (MC5) and 12944-0509-105 (Placebo, MC0). One hemp cigarette sample, Lucky Leaf (HC1) was purchased from Industrial Hemp Farms (Denver, CO).

Calibration Solution Preparation

A twenty-cannabinoid mixture solution was first prepared in methanol at 50 µg/mL individual concentration. The mixture solution was serially diluted with methanol to obtain nine solutions according to

Table 1 and

Table 2. The concentration of individual cannabinoids in each solution ranged from 25 to 0.04 µg/mL. A previously prepared 1 µg/mL ACBD solution in methanol was then mixed with each solution in a 1:1 ratio (v/v). The final eleven prepared solutions had a concentration of 0.5 µg/mL ACBD and 25, 12.5, 5, 2.5, 1, 0.5, 0.2, 0.1, 0.04, and 0.02 µg/mL individual cannabinoid concentration. Three quality control (QC) samples containing 0.5 µg/mL ACBD with a low concentration (0.02 µg/mL), medium concentration (0.5 µg/mL), and high concentration (12.5 µg/mL) of individual cannabinoids were prepared similarly. Because CBLA was found in CBCA CRMs, calibration solutions of CBLA at 25, 5, 1, 0.2, and 0.04 µg/mL in methanol were similarly prepared to measure the CBLA concentration in a 25 µg/mL CBCA solution in methanol.

Table 1. First Dilution Series

Conc. of cannabinoids	Volume	Methanol
50 µg/mL		
10 µg/mL	100 µL 50 µg/mL cannabinoids	400 µL
2 µg/mL	100 µL 10 µg/mL cannabinoids	400 µL
0.4 µg/mL	100 µL 2 µg/mL cannabinoids	400 µL
0.08 µg/mL	100 µL 0.4 µg/mL cannabinoids	400 µL

Table 2. Second Dilution Series.

Conc. of cannabinoids	Volume	Methanol

25 µg/mL	250 µL 50 µg/mL cannabinoids	250 µL
5 µg/mL	100 µL 25 µg/mL cannabinoids	400 µL
1 µg/mL	100 µL 5 µg/mL cannabinoids	400 µL
0.2 µg/mL	100 µL 1 µg/mL cannabinoids	400 µL
0.04 µg/mL	100 µL 0.2 µg/mL cannabinoids	400 µL

Sample Preparation

The procedure for extracting cannabinoids were adapted from previously published literatures.[28, 33-34, 40] A 75 µg/mL ACBD solution in methanol was first prepared. A waring lab blender (Torrington, CT) was used to homogenize ¼ oz or 7 g of sample for 2 minutes. To further homogenize the sample, 0.5 g cuttings of sample were transferred to a 7 mL tube with two ¼ inch stainless steel balls and then powdered using a SPEX Genolyte 1200 (Methichen, NJ) set to 3000 revolutions per minute (rpm) for 2 minutes. To extract the cannabinoids, 100mg of the powered samples were placed into a 15mL centrifuge tube and suspended in a previously prepared 75 µg/mL ACBD in methanol solution to make a 25 mg/mL mixture. ACBD is therefore spiked into the sample at 0.3% (w/w) level. The centrifuge tube was ultrasonicated for 5 min followed by brief vortex to wash samples on the walls of the tube to the bottom. After four cycles of ultrasonication and vortex, about 2 mL of the supernatant was centrifuged at 13,000 rpm for 10 minutes. This was followed by filtering about 1 mL of the supernatant of the centrifuged sample with a 0.2 µm syringe filter. The filtrate was then diluted serially with methanol according to Table *3* to finally obtain 0.15 µg/mL ACBD and 50 µg/mL sample. The 75 µg/mL ACBD solution was also diluted serially according Table *4* to obtain 0.15 µg/mL ACBD.

Table 3. Preparation of sample solutions in methanol with ACBD.

Sample solution	Volume	Methanol
75 µg/mL ACBD + **25 mg/mL** sample		
15 µg/mL ACBD + **5 mg/mL** sample	200 µL of **75 µg/mL** ACBD + **25 mg/mL** sample	800 µL
1.5 µg/mL ACBD + **500 µg/mL** sample	100 µL of **15 µg/mL** ACBD + **5 mg/mL** sample	900 µL
0.15 µg/mL ACBD + **50 µg/mL** sample	100 µL **1.5 µg/mL** ACBD + **500 µg/mL** sample	900 µL

Table 4. Preparation of ACBD solution in methanol.

Sample solution	Volume	Methanol
75 µg/mL ACBD		
15 µg/mL ACBD	200 µL of **75 µg/mL** ACBD	800 µL
1.5 µg/mL ACBD	100 µL of **15 µg/mL** ACBD	900 µL
0.15 µg/mL ACBD	100 µL of **1.5 µg/mL** ACBD	900 µL

After analysis by LC-DAD, the peak area ratio of 0.15 µg/mL ACBD in the sample solution and 0.15 µg/mL ACBD in methanol was calculated as the recovery of sample preparation at 0.3% (w/w) level using the following equation:

Equation 1:

$$Recovery = \left(\frac{(PA\ of\ ACBD)_{sample\ solution\ in\ methanol\ with\ ACBD}}{(PA\ of\ ACBD)_{ACBD\ solution\ in\ methanol}} \right) \times 100\%$$

The concentration of individual cannabinoids was calculated using a calibration curve generated with the calibration solutions and the calibration range for each individual cannabinoid in the sample was between 0.04 to 50% (w/w).

LC-DAD

An Agilent 1260 Infinity II LC system (Agilent Technologies, Santa Clara, CA) consisting of a solvent degasser, binary pump, temperature controlled autosampler, column oven, and diode-array detector (DAD) was employed. Chromatographic separation was carried out using two Restek Raptor ARC-18 150 × 2.1 mm, 2.7 µm columns (Bellefonte, PA) connected sequentially and protected with a 0.2 µm IDEX (Oak Harbor, WA) ultra-high pressure (UHP) precolumn filter at the entrance. The temperature of the column oven was set to 30°C. The mobile phases consisted of (A) water with 0.5 mM ammonium formate plus 0.02% (v/v) formic acid (pH 3.0) and (B) acetonitrile. Separation was achieved at 75% (v/v) B under isocratic elution. The injection volume was set to 4 µL while the flow rate was 0.4 mL/min. The autosampler was maintained at 8°C. The UV spectra was recorded within 190.0 to 400.0 nm with 2.0 nm steps. A reference wavelength of 360 nm with a 100 nm band length was employed. The UV detection was carried out at 223, 230, 251, 261, 269, and 285 nm wavelength with a 4 nm bandwidth.

ESI-TOFMS

An Agilent 6545 quadrupole time-of-flight (Q-TOF) mass spectrometer, equipped with a Dual Agilent Jet Stream (AJS) ESI source was operated in the positive ion mode. The MS acquisition mass range was set to 100-1000 *m/z* with an acquisition rate of 5 spectra/s. The drying gas temperature was set to 325 °C with a drying gas flow of 10 L/min. Nebulizer pressure was set at 20 psi. The sheath gas temperature and flow were set at 400 °C and 12 L/min respectively. Capillary voltage, nozzle voltage, and fragmentor voltage were at 3000

V, 600 V, and 120 V, respectively. The skimmer was at 45 V and Oct1 RF Voltage was at 750 V. Mass reference ions at 121.0509 *m/z* and 922.0098 *m/z* were used.

Method Validation

The presented method was validated for linearity, precision, accuracy, specificity, and recovery in accordance with ISO 17025 guidelines.

Results and Discussion

Separation Optimization

According to the AOAC SMPRs recommendation for the quantitative determination of cannabinoids in dried plant material[18] and plant materials of hemp[19], the initial focus was on fourteen cannabinoids plus two additional ones—CBLA and ACBD. Focus on CBLA was imperative as it was found in the CBCA certified reference material as an impurity. ACBD which is not naturally occurring in *Cannabis* plants was spiked into both the calibration and sample solutions to normalize retention times and track the recovery of the sample in real-time.

The optimized separation was based on our recent study on the effect of various LC parameters on the retention time of cannabinoids using a Phenomenex (Torrance, CA) Luna Omega Polar C18, 150 × 2.1 mm, 1.6 µm column.[46] After the study, a secondary separation had to be developed due to co-elution of THCVA and CBG. The second optimization developed for the quantification of both cannabinoids in case they were present in a sample.[46] however, One of the shortcomings of this method was the co-elution of CBCA and Δ^9-THCA with unknown neutral cannabinoids that were structural isomers of Δ^9-THC in hemp concentrates. The previously developed method was adequate for quantifying neutral cannabinoids in hemp concentrates— as acidic cannabinoids undergo

decomposition from extensive processing of the products during manufacturing. As a result, this method is inadequate for quantifying cannabinoids in plant materials of marijuana and hemp because of the large number of acidic and neutral cannabinoids present.

The separation optimization strategy involved two steps: first, adequate separation of neutral cannabinoids was achieved under isocratic eluting conditions using an acetonitrile and water eluting system; then, the pH of the mobile phase was adjusted to ensure the acidic cannabinoids did not co-elute with neutral cannabinoids. Separation optimization started by using the optimized conditions with the Luna Omega Polar C18 column with mobile phases being (A) water containing 0.028% (v/v) formic acid and 73% (v/v) (B) acetonitrile. Under this system, two issues were observed. First, the resolution between adjacent neutral cannabinoids was inadequate. This inadequate separation affected two critical pairs: CBG/CBD and Δ^9-THC/Δ^8-THC. Secondly, the pH of the mobile phase was inappropriate, resulting in low resolution of both acidic and neutral cannabinoids.

To resolve these problems theoretically, inadequate resolution between the adjacent neutral cannabinoids could be solved by replacing the LC column with a longer one. The drawback to this solution would be a longer retention time; however, a higher flow rate could be utilized to overcome this. Since the longest available column was 15 cm, two columns were sequentially connected, and the resulting resolution had an improvement of $\sqrt{2}$ times. The resolution between CBG/CBD and Δ^9-THC/Δ^8-THC with one column was 1.1 and 1.2 respectively. The use of two columns yielded a resolution above 1.5 for both pairs required by appropriate method validation.

To increase the pH of the mobile phase, 1 mM ammonium formate was added to the aqueous solvent. To determine the optimal pH of the mobile phase for the retention of acidic cannabinoids, the aqueous solvent was further adjusted by adding 0.0062, 0.0085, 0.010, 0.012, 0.015, 0.018 and 0.021% (v/v) formic acid. The effects of the formic acid content in the aqueous solvent can be seen in *Figure 3*. The retention of the neutral cannabinoids remained relatively constant while acidic cannabinoid retention increased. The change in retention was especially relevant for later eluting acidic cannabinoids—THCVA, Δ^9-THCA, CBCA, and CBLA—with their elution order altered. Interestingly, the elution order of the earlier eluting acidic cannabinoids—CBDVA, CBDA, and CBGA—did not change. From the inquiry, separation of sixteen cannabinoids was achieved at 0.012% and 0.021% (v/v) FA. However, 0.021% FA was further studied as it yielded better robustness for the method.

To further optimize the separation, previously determined mobile phase A consisting of 1 mM ammonium acetate which was adjusted with 0.021% (v/v) formic acid was used while the content of acetonitrile in mobile phase B was varied from 68, 70, 73, 75 to 78% (v/v). The effect of the change in acetonitrile content on the separation of sixteen cannabinoids is seen in Figure *3*. The increase in acetonitrile, mobile phase B, resulted in a decrease in retention time for all cannabinoids. Some changes are also observed in the resolution between the some of the cannabinoids. The resolution of CBD and CBG increased from 0.95, 1.22, 1.57, 1.69 to 1.82 for 68, 70, 73, 75 to 78% (v/v) acetonitrile respectively; however, resolution decreased between Δ^9-THC and Δ^8-THC from 2.24, 2.09, 1.80, 1.57, to 1.33, respectively. The optimal acetonitrile content for this method was determined to be 75% (v/v) acetonitrile where a resolution between CBG/CBD and Δ^9-

THC/Δ^8-THC would be 1.69 and 1.57, respectively. According to method validation parameters, this resolution would be adequate since it also achieved reduced retention times.

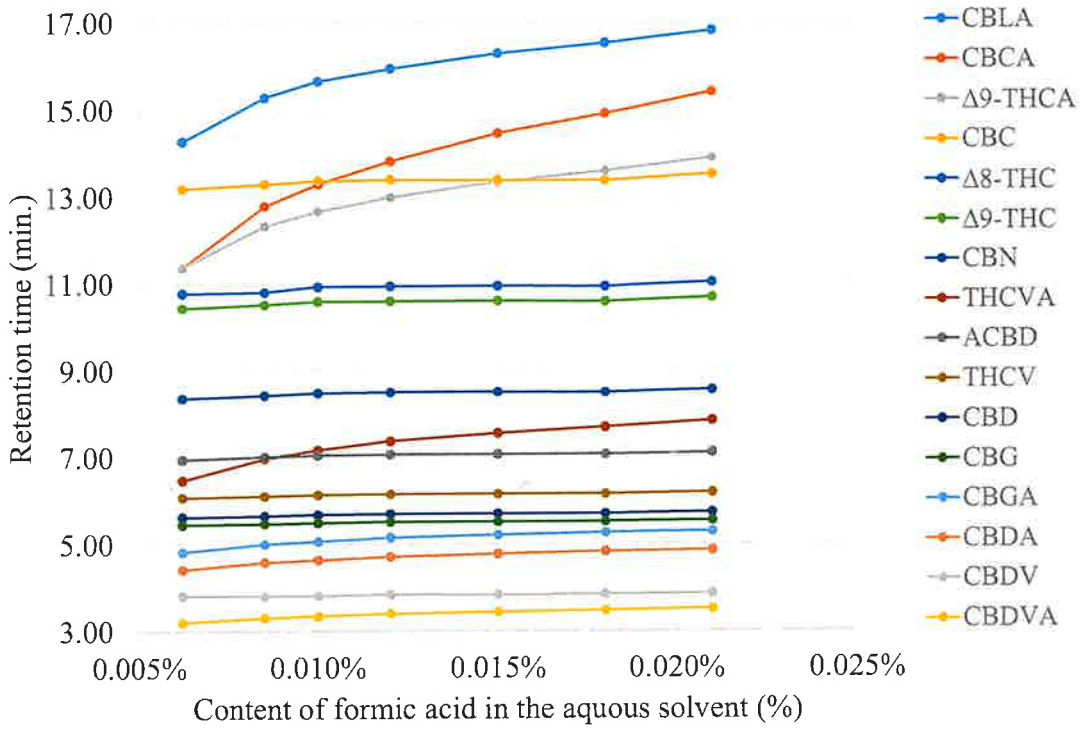


Figure 3. The effects of formic acid content in the mobile phase on sixteen cannabinoids.

The formic acid content was reexamined using 75% (v/v) acetonitrile in organic solvent B and 1 mM ammonium formate in the aqueous solvent (A). This time, formic acid content was varied between 0.022, 0.025 and 0.030% (v/v). The separation achieved with the new formic acid content was similar; however, better resolution was achieved between CBC and Δ^9-THCA: 1.49, 1.51, and 1.84, respectively.

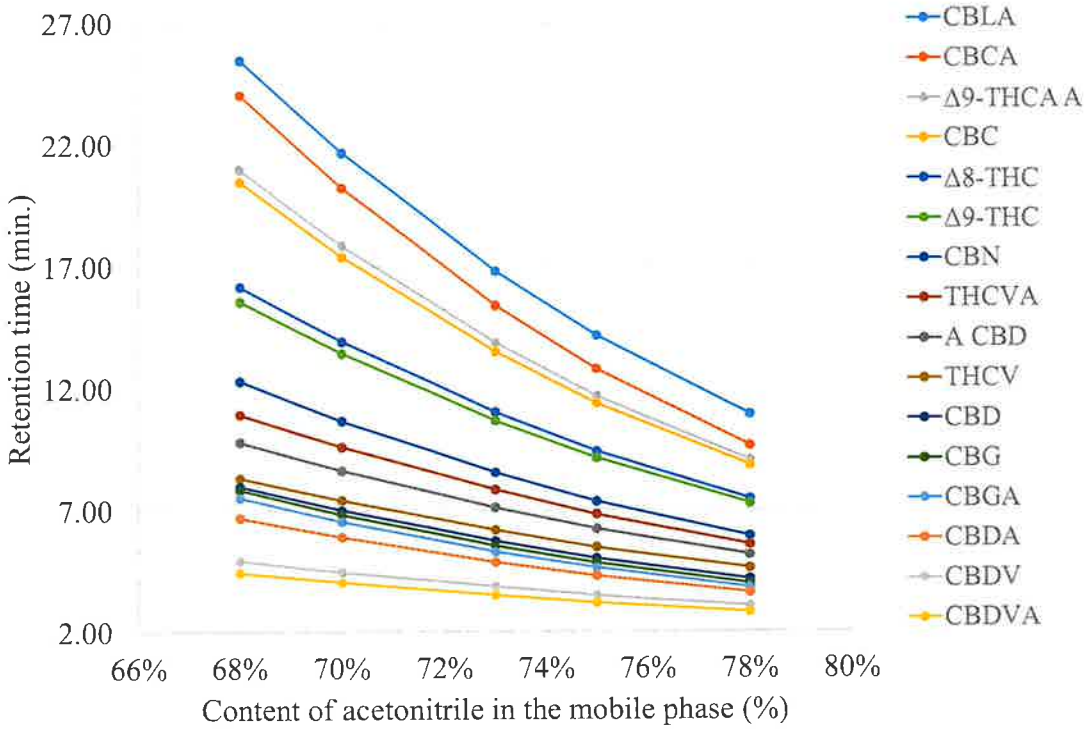


Figure 4. Content of acetonitrile in the mobile phase with the aqueous solvent containing 1 mM ammonium acetate and 0.021% formic acid on retention time of sixteen cannabinoids.

After these series of optimization, the final analysis was carried out using (A) water with 0.5mM ammonium formate plus 0.02% (v/v) formic acid (pH 3.0) and 75% (v/v) (B) acetonitrile at 30˚C. A low concentration of formate in the aqueous solvent was preferred to attain a better limit of quantitation (LOQ) since formate absorbs UV light. Under this condition, separation of twenty cannabinoids, including ACBD, was achieved with satisfactory resolution between several pairs of critical cannabinoid pairs at 1 µg/mL individual concentration. The critical pairs and their resolution include: CBGA/CBG, 2.18; CBG/CBD, 1.55; Δ^9-THC/Δ^8-THC, 1.51; CBC/Δ^9-THCA, 2.50 (Figure *2*). Better resolution could be achieved for CBCV and CBNA with a lower flow rate of 0.3 mL/min; however, it was unnecessary as only eighteen of the twenty targeted cannabinoids,

excluding CBCV and Δ^8-THCA, were present in the samples analyzed. The main difference between the new and old separation is majorly with the longer retention time of acidic cannabinoids, which was necessary to avoid their coelution with unknown neutral cannabinoids that may be present in the samples.

DAD Optimization

For all cannabinoids excluding CBCA and CBNA, a major peak of absorption with its apex at a wavelength below 210 nm was observed. Due to the absorption of the mobile phase and to be consistent with other published methods, all cannabinoids except CBT were quantified at 230 nm. CBT was detected as 223 nm for improved detection. Two cannabinoids, CBCA and CBNA each had a major peak with apexes at wavelengths of 251 and 261 nm respectively. For this reason, these two cannabinoids were quantified at these wavelengths in addition to 230 nm. Additional UV absorption peaks were seen for other acidic cannabinoids with their apexes at wavelengths around 269 and 305 nm. A higher absorption was seen at 269 nm, so the acidic cannabinoids were quantified at 269 nm in addition to the 230 nm. Two neutral cannabinoids, CBN and CBC, showed an additional absorption peak which apexed at approximately 285 nm. CBN and CBC were subsequently analyzed at two wavelengths—230 and 285 nm.

The cannabinoids were quantified at two different wavelengths to increase method specificity. Improved specificity is possible with the discovery of interferences if the results of quantification at two wavelengths are statistically different. An LC-UV chromatogram of eighteen cannabinoids with a concentration of 0.02 µg/mL and ACBD at 0.5 µg/mL at 230, 251, 261, 269, and 285 nm can be seen in Figure 5. The LOQ of the method is reported to be 0.02 µg/mL as it was the lowest point of all the calibration curves; although, it could

be lower for some cannabinoids like CBDVA. Quantification of CBT was the most challenging of the eighteen cannabinoids due to its long retention time and wide peak shape. Therefore, CBT was detected at 223 nm instead of 230 nm, to improve quantification at the LOQ level. Alternatively, a larger sample volume could be injected for quantification purposes since this method employs 4 µL injection volume.

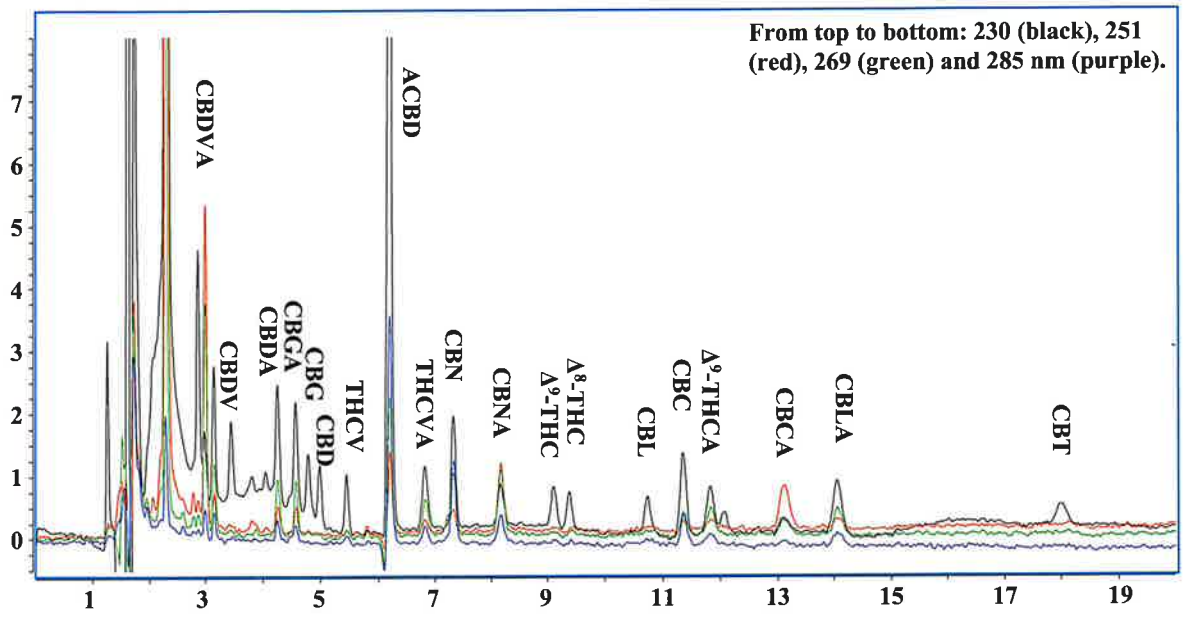


Figure 5.LC-UV chromatogram of eighteen cannabinoids at 0.02 µg/mL and ACBD at 0.5 µg/mL at 230 (black), 251 (red), 261 (purple), 269 (green) and 285 (blue) nm.

Method Validation

In accordance with ISO 17025 guidelines, method validation of eighteen cannabinoids detected in preliminary analysis of the samples was carried out in triplicate over three days using the presented method.

External calibration was chosen over internal calibration as the procedure was simpler and yielded a good performance. The weighted, $1/x^2$, linear calibration curves for all cannabinoids were then constructed by plotting the cannabinoid concentration over a

range of 0.02 to 25 µg/mL against the peak area of the cannabinoid. The obtained R^2 values for all calibration curves for each cannabinoid are shown to be above 0.9948 in Table 5. The LLOQ and the ULOQ of the method are reported to be 0.02 µg/mL and 25 µg/mL, respectively, for all cannabinoids as shown in Table 5.

The precision and accuracy of the method was assessed using three quality control (QC) samples at the lowest concentration (0.02 µg/mL), medium concentration (0.5 µg/mL), and high concentration (12.5 µg/mL). Precision of the QC samples were calculated as intraday and interday relative standard deviations (RSD) as seen in Table 6. Intraday RSD values were calculated by using the results obtained within the same day while interday RSD values were calculated by combining results over the three-day analysis period. The relative standard deviation (RSD) values for the precision of the QC sample at 0.02 µg/mL were < 8.6% for intraday and < 9.4% for interday; QC sample at 0.5 µg/mL RSD values were < 5.4% for intraday and < 5.3% for interday; QC sample at 12.5 µg/mL, the RSD values were < 3.9% for intraday and < 6.1% for interday. The accuracy of the QC samples is shown in Table 7. Accuracy of the method were given as interday and intraday values. For the lowest concentration, 0.02 µg/mL, QC sample accuracy values were between 90.0-110.0% for intraday and between 92.2-106.0% for interday. For medium concentration, 0.5 µg/mL, QC samples accuracy values were between 94.5-112.3% for intraday and between 100.6-107.0% for interday. At the high concentration, 12.5 µg/mL, QC samples accuracy values were between 95.3-108.2% for intraday and between 98.1-105.7% for interday.

Table 5. Calibration curves.

Validation parameters		CBDVA	CBDV	CBDA	CBGA	CBG	CBD	THCV	THCVA	CBN	CBNA	Δ^9-THC	Δ^8-THC	CBL	CBC	Λ^9-THCA	CBCA	CBLA	CBT
R^2	Day 1	0.9983	0.9986	0.9991	0.9993	0.9989	0.9984	0.9992	0.999	0.998	0.9981	0.9975	0.9985	0.998	0.9983	0.9966	0.9988	0.9983	0.998
	Day 2	0.9984	0.9985	0.9987	0.9989	0.9984	0.9977	0.9988	0.9978	0.9989	0.9979	0.9982	0.9969	0.9965	0.9948	0.9987	0.9949	0.9975	0.9979
	Day 3	0.9983	0.9989	0.9987	0.999	0.999	0.9992	0.9989	0.9987	0.9987	0.9991	0.998	0.9972	0.9988	0.9991	0.9989	0.9985	0.9975	0.9975
LLOQ	Day 1	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
	Day 2	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
	Day 3	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
ULOQ	Day 1	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25
	Day 2	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25
	Day 3	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25

Table 6. Precision of the method using QC samples: RSD values were computed using triplicate measurements for both intraday and interday.

Conc	RSD (%)	CBDVA	CBDV	CBDA	CBGA	CBG	CBD	THCV	THCVA	CBN	CBNA	Δ^9-THC	Δ^8-THC	CBL	CBC	Λ^9-THCA	CBCA	CBLA	CBT
0.02	Day 1	1.9	0.1	1.7	1.2	1.6	2.7	1.9	2.9	3.1	2.1	1.6	2.7	4.2	2.5	2.3	2	4.2	6.1
	Day 2	2.5	4.9	0.6	3	4.6	2	3.4	2.8	1.2	3.8	1.5	3.8	1.6	1.6	1.9	3.2	5.3	0.9
	Day 3	8.6	2.5	3.1	3.1	0.7	1.4	3	7	2.9	1.2	5.3	3.5	2.6	1.6	4.9	4.6	2.3	8
	Interday	5.4	2.7	3.2	1.7	8.1	5.6	2.2	3.3	3.9	5.8	4.7	6.9	6	2.3	9.4	5.4	8.7	8.2
0.5	Day 1	0.1	0.2	0.3	0.4	0.3	1	0.6	0.9	0.3	1.1	0.6	0.4	0.6	0.1	1.6	5.4	1.3	2.4
	Day 2	0.5	0.5	0.5	0.7	0.3	0.4	0.4	0.8	0.3	1.3	3	2	0.7	0.3	1.9	2.1	0.7	2.5
	Day 3	1.3	0.2	0.4	1.7	0.1	0.3	0.4	2.1	1.5	0.3	2.1	0.7	0.9	0.5	0.3	0.5	1.8	1.3
	Interday	2.2	1.3	0.5	1.3	5.3	4.4	0.4	0.2	0.5	1	0.8	2.4	1.1	1.7	4.3	4	1.7	1.2
12.5	Day 1	0.1	0	0.1	0.1	0.7	1.8	0.4	0.1	3.3	1.2	0.2	0.1	0.2	0.2	0.1	0.1	0.1	1.2
	Day 2	0.5	0.6	0.5	0.6	1.3	0.4	0.6	0.6	1.9	0.6	3.9	0.5	0.4	0.4	0.5	0.5	0.8	1.6
	Day 3	0.4	0.7	0.4	0.4	0.2	0.4	0.4	0.4	0.4	0.4	0.6	0.5	0.6	0.5	2.2	0.3	1	0.7
	Interday	3.9	0.8	3.6	3.6	4.7	3.3	4.1	3.7	4.2	4.3	3.4	3.3	4.2	2.7	3	2.8	6.1	4.3

Table 7. Accuracy of the method using QC samples: average recovery values were computed using triplicate measurements for both intraday and interday.

Conc	Accuracy (%)	CBDVA	CBDV	CBDA	CBGA	CBG	CBD	THCV	THCVA	CBN	CBNA	Δ^9-THC	Δ^8-THC	CBL	CBC	Δ^9-THCA	CBCA	CBLA	CBT
0.02	Day 1	105.1	102.6	103.3	98.1	97.3	98.6	97.7	100.5	99.6	109.4	96.5	95.9	89.6	108.3	91.1	90.2	90	90.9
	Day 2	98.3	103	99.5	101.1	92.9	96.1	102	96.4	100.9	109.8	105.5	110	88.4	103.6	109.2	95.1	106.1	107.2
	Day 3	94.6	107.7	96.9	100.9	108.6	106.9	101	94.3	107	98.9	104	104.5	98.5	104.5	96.7	100.5	93.9	100.6
	Interday	99.4	104.4	99.9	100.1	99.6	100.5	100.2	97.1	102.5	106	102	103.4	92.2	105.5	99	95.2	96.7	99.5
0.5	Day 1	106.2	104.9	103.3	105.4	102.7	100.2	104.1	105	104.5	103.5	103.4	99	105.6	102.5	112.3	96	107	104.3
	Day 2	102.9	104	102.5	103.1	94.5	108.8	103.3	104.5	104	104.2	101.9	101.9	103.6	105.9	104.7	104	103.9	102.1
	Day 3	101.8	106.7	102.3	103	104.5	102.1	104.1	104.8	104.9	102.2	102.1	103.8	105.8	103.2	104.1	100.5	103.9	104.2
	Interday	103.6	105.2	102.7	103.8	100.6	103.7	103.8	104.8	104.5	103.3	102.5	101.6	105	103.9	107	100.2	104.9	103.5
12.5	Day 1	100.3	97.6	98.5	100.4	96.2	97.5	97.9	98.6	97.7	97.1	97.2	100	97.4	97.4	104.9	102.3	96.5	98.8
	Day 2	102.3	99.3	100.1	98.8	98.9	102.9	98.8	100.5	97	97.1	95.3	97.4	98.4	99.8	99.7	107.5	95.5	98.1
	Day 3	108.2	98.7	105.5	105.8	105.4	103.5	105.4	106	104.5	104.5	101.7	104	105.1	102.8	105.4	107.3	106.4	105.9
	Interday	103.6	98.5	101.4	101.6	100.2	101.3	100.7	101.7	99.8	99.6	98.1	100.5	100.3	100	103.4	105.7	99.5	101

To assess recovery of sample preparation, ACBD, a cannabinoid not naturally present in *Cannabis*, was spiked into all samples at a 0.3% (w/w) level during sample preparation. ACBD is very similar to Δ^9-THC in terms of chromatography, UV absorbance and fragmentation patterns during ESI since it is a structural isomer of Δ^9-THC. Assessment of recovery of each sample was performed for the first time in real-time. Recovery results of ACBD for each sample are as follows: 101.6 (MC0), 96.1 (MC1), 98.0 (MC2), 93.6 (MC3), 95.3 (MC4), 103.3 (MC5) and 99.6 (HC1). The analysis was carried out in triplicate and the RSD values of ACBD recovery are as follows: 1.5 (MC0), 4.6 (MC1), 4.8 (MC2), 1.9 (MC3), 7.0 (MC4), 4.0 (MC5) and 1.4 (HC1). The summary of the recovery can be seen in Table *8*.

Table 8. Average recovery of 0.3% (w/w) ACBD spiked in 7 samples and corresponding RSD values.

Sample	Recovery (%)	RSD (%)
MC0	101.6	1.5
MC1	96.1	4.6
MC2	98.0	4.8
MC3	93.6	1.9
MC4	95.3	7.0
MC5	103.3	4.0
HC1	99.6	1.4

Sample Analysis

The first CBCA CRM (Batch 0601795) purchased on 06/03/2021 was found to contain CBLA. Therefore, another batch of CBCA CRM (Batch 0623807) was gotten on 12/17/2021 and analyzed again. The second batch was also found to contain CBLA as shown in Figure *6*. The first CBCA CRM was analyzed in triplicate and its CBLA content was quantified to be 8.01% with an RSD of 3.4%. The second batch of CBCA CRM contained 8.88% CBLA with an RSD of 4.8%. CBLA content in the CBCA reference

material could be attributed to Cayman Chemical not being able to fully isolate CBLA from CBCA with current experimental protocols. CBCA CRM was purchased from Cayman Chemicals because the previous study[46] indicated that CBCA CRM gotten from Sigma contained CBLA.

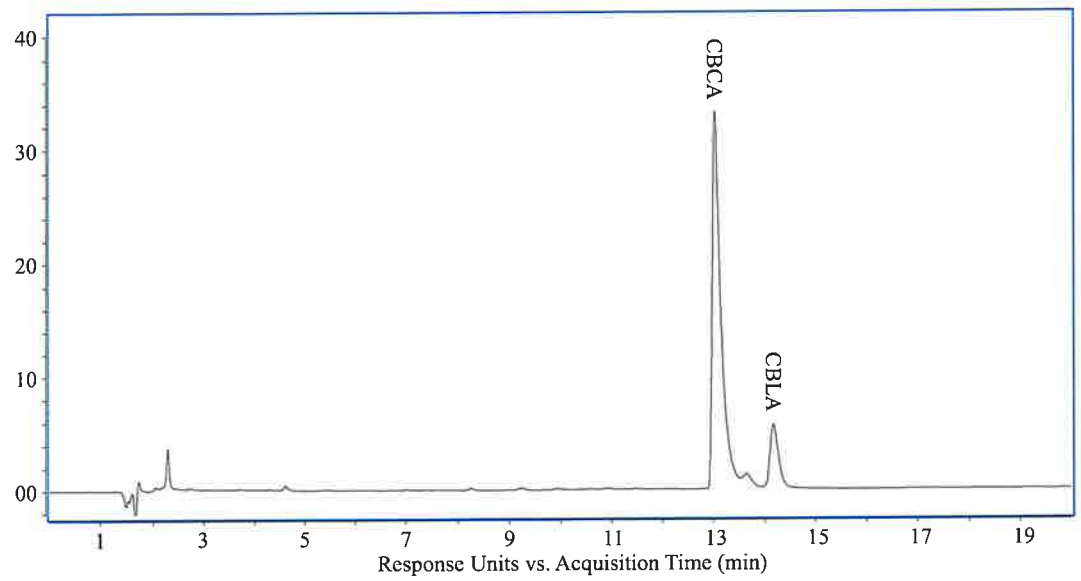


Figure 6. LC-UV chromatogram at 230 nm of CBCA CRM at 25 µg/mL.

The average content (% w/w) of eighteen cannabinoids in seven samples that were analyzed in triplicate are seen in

Table 9 and their corresponding RSD values can be seen in

Table 10. The RSD values ranged from 0.7 to 17.5% with the higher values associated with lower contents. The placebo cigarette sample was not included since its cannabinoid content was below the LOQ of the method. To account for the presence of CBLA in CBCA

CRM, the CBLA content was divided by 108.88%, while the CBCA content was divided by 91.12%. The total cannabinoid content was determined by adding all individual content up and this ranged from 0.04 to 6.12% in the samples. The total THC and CBD content were calculated using the following equations:

Equation 2. Total THC content

$$Total\ THC\ content = \Delta^9 - THC\ content + \Delta^9 - THCA\ content \times \frac{314.5}{358.5}$$

Equation 3. Total CBD Content

$$Total\ CBD\ content = CBD\ content + CBDA\ content \times \frac{314.5}{358.5}$$

Two samples of the marijuana cigarette— MC1 and MC2—contained 0.26 and 0.36% (w/w) Δ^9-THC, respectively, and had a total THC content of 1.73 and 2.88% (w/w), respectively. One of the marijuana cigarettes, (MC4), contained 2.56% (w/w) total THC content. Both MC3 and MC5 contained well above 0.3% (w/w) Δ^9-THC content with a total THC content of 4.61 and 5.88% respectively. The hemp cigarette, Lucky leaf (HC1), had a total THC content below the acceptable amount.

Previous published methods which have assumed good specificity based adequate separation of targeted cannabinoids; however, they could be challenged by the presence of unidentified and unknown compounds or cannabinoids. For this reason, the specificity of the method is examined by simultaneous detection using ESI/TOFMS with DAD detection. Under optimized positive-ion ESI conditions, neutral cannabinoids generated $[M+H]^+$ ions while the acidic cannabinoids generated both $[M+H]^+$ and $[M+H-H_2O]^+$ ions. The fragmentor voltage was optimized at 120 V to produce the maximum intensity of $[M+H]^+$ ions for neutral cannabinoids and acidic cannabinoids. At a lower voltage, $[M+H]^+$

fragment ion was predominant for all acidic cannabinoids except CBGA which had predominantly $[M+H-H_2O]^+$ ions. At a voltage above 120 V, an increase in the abundance of $[M+H-H_2O]^+$ ion was observed and this could be attributed to in-source fragmentation. As such, the fragmentor voltage was set to 120 V to obtain maximum intensity of the $[M+H]^+$ ions. The specificity of the method is summarized in

Table 9 and the obtained LC-UV chromatograms and corresponding LC-TOFMS extracted ion chromatograms for each analyzed sample is shown in Figure 7 through Figure 12. The LC-TOFMS extracted ion chromatograms were obtained using the cannabinoids $[M+H]^+$ ions at ±20 ppm, except for CBGA using its $[M+H-H_2O]^+$ ion. The chromatograms were labeled with coordinating-colored fonts and followed with descriptions.

One unknown cannabinoid were discovered at different UV wavelength absorptions and confirmed using ESI/TOFMS detection (marked in green fonts on

Table 9 and Figure *7* to Figure *12)*. Interferences to CBDVA quantification were discovered in two marijuana cigarette samples (MC3 and MC5) by quantifying at 230 and 269 nm and later confirmed by ESI/TOFMS detection. The interference was due to an unknown cannabinoid—RRT0.50 denoting its relative retention time (RRT) to ACBD at 0.50 min. This interference was found to be a structural isomer of CBNA with a *m/z* 355.1904. This interfering unknown cannabinoid was identified by the developed LC-DAD method since it has a different UV absorption from the targeted cannabinoids.

Lastly, another unknown cannabinoid (RRT2.36, marked in purple in Figure *12*) which did not interfere with the quantification of any targeted cannabinoids was discovered

in hemp cigarette sample during ESI-TOFMS. This unknown cannabinoid RRT2.36 was found to be a structural isomer of Δ^9-THC acetate with *m/z* 357.2440.

Table 9. Content (%, w/w) of cannabinoids in triplicate in *Cannabis* samples (TTHC - Total THC; TCBD - Total CBD).

Sample	CBDVA	CBDV	CBDA	CBGA	CBG	CBD	THCVA	CBN	CBNA	Δ⁹-THC	Δ⁸-THC	CBC	Δ⁹-THCA	CBCA	CBT	Total	TTHC	TCBD
MC1				0.05				0.04	0.06	0.26			1.67	0.22		2.30	1.73	
MC2				0.09				0.07	0.12	0.36		0.05	2.88	0.16		3.68	2.88	
MC3	RRT0.50			0.13			0.04	0.10	0.19	0.45			4.74	0.19		5.84	4.61	
MC4			0.08	0.11			0.06	0.07	0.08	0.46			2.39	0.12		3.38	2.56	0.07
MC5	RRT0.50		0.19	0.37			0.08	0.11	0.19	0.51			6.12	0.16		7.74	5.88	0.17
HC1			1.77	3.96	0.91	2.70				0.14		0.27		0.10	0.11	9.96	0.14	4.26

Table 10. Precision (%) of cannabinoid measurements in cigarette samples (TTHC: Total THC; TCD: Total CBD).

Sample	CBDVA	CBDV	CBDA	CBGA	CBG	CBD	THCV	THCVA	CBN	CBNA	Δ⁹-THC	Δ⁸-THC	CBL	CBC	Δ⁹-THCA	CBCA	CBLA	CBT	Total	TTHC	TCBD
MC1				5.7					2.2	5.9	1.3				2.5	4.1			2.5	2.3	
MC2				8.7					5.5	8.5	10.4				9.5	9.5			9.2	9.6	
MC3				3.7				6.4	1.5	6	5				5.6	2.4			5.4	5.6	
MC4			7.3	12				4.8	5.4	10.4	8.6				16	11.4			13.5	14.5	6.1
MC5			4.2	4.4				5.8	4.2	1.2	3.9				3.5	1.3			3.5	3.5	4.9
HC1			4	6.3	4.8	4					4.5			4		5		9.8	5	3.3	4

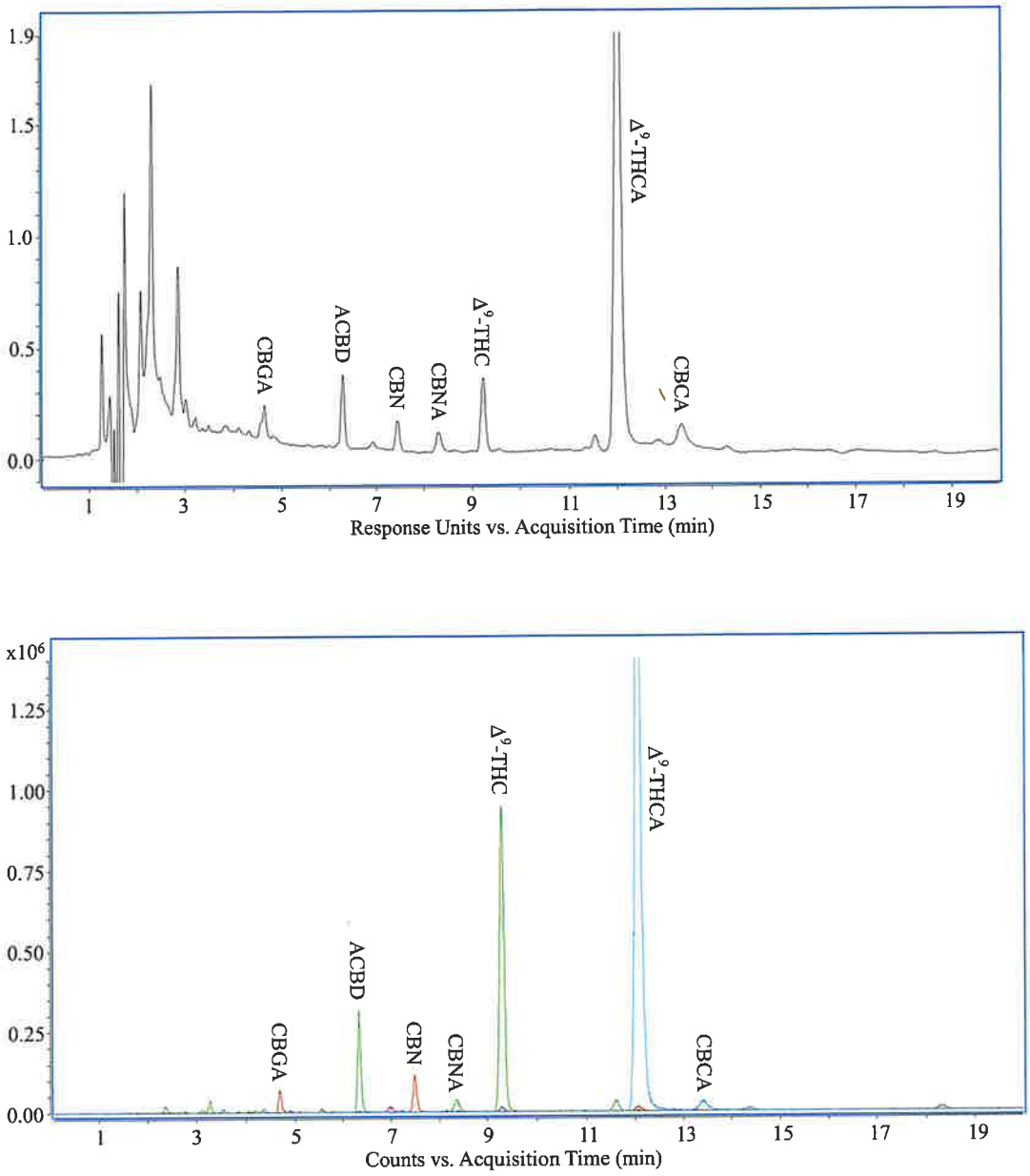


Figure 7. The LC-UV chromatogram (top) at 230 nm and LC-ESI/TOFMS extract ion chromatogram (bottom) using $[M+H]^+$ ion for all cannabinoids except CBGA which used $[M+H-H2O]^+$ ion of sample MC1.

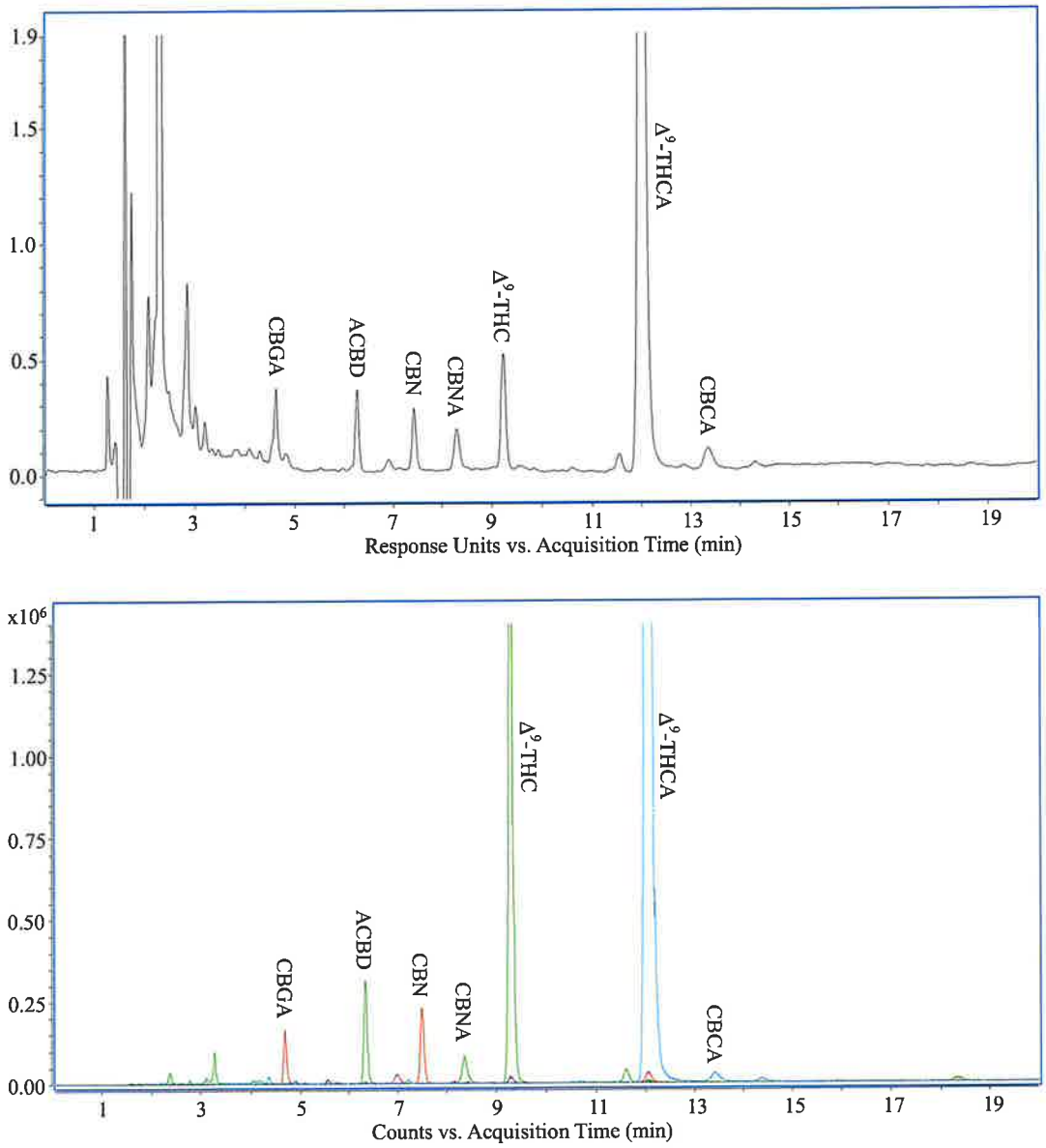


Figure 8.The LC-UV chromatogram (top) at 230 nm and LC-ESI/TOFMS extract ion chromatogram (bottom) using $[M+H]^+$ ion for all cannabinoids except CBGA which used $[M+H-H_2O]^+$ ion of sample MC2.

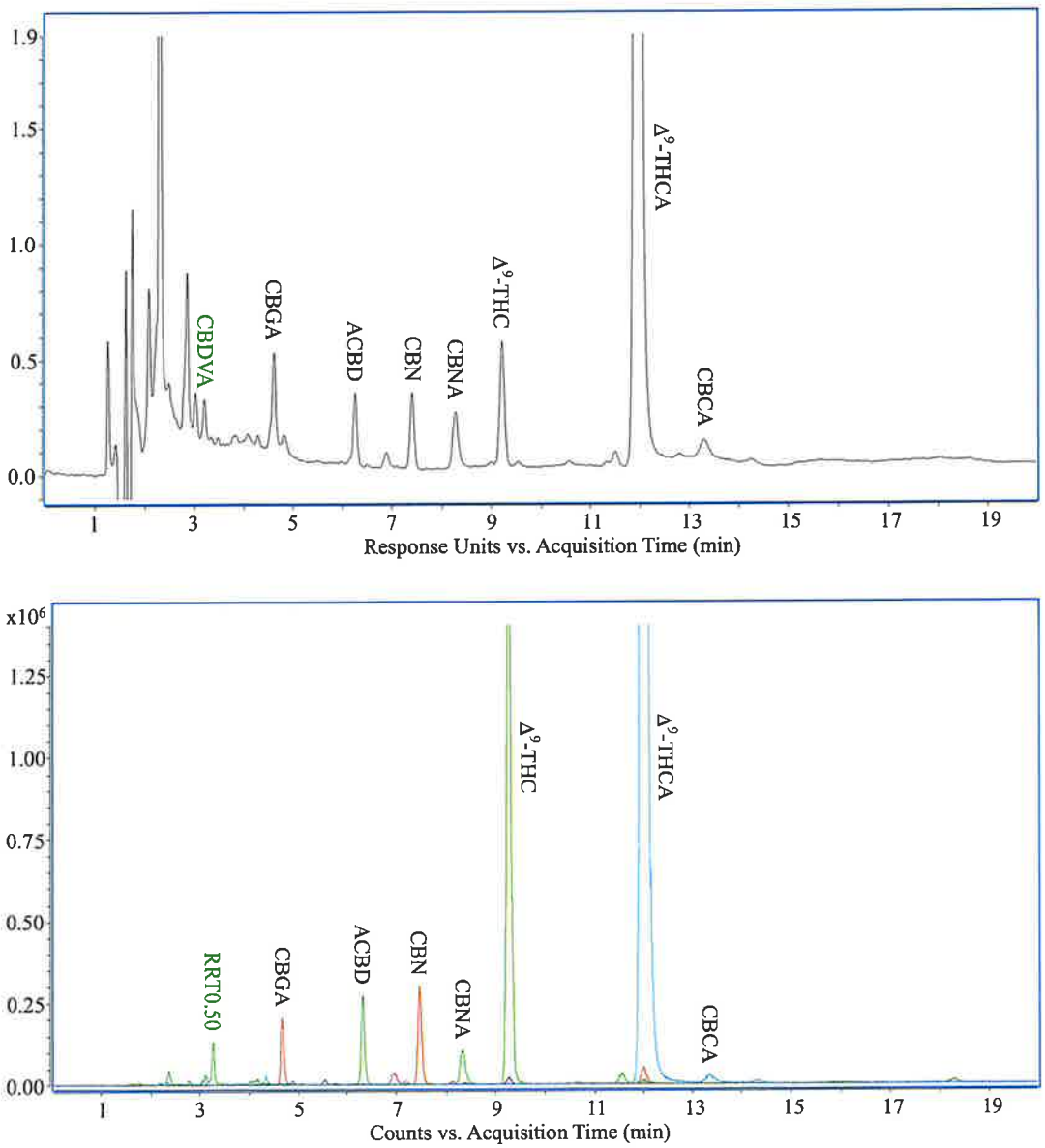


Figure 9. The LC-UV chromatogram (top) at 230 nm and LC-ESI/TOFMS extract ion chromatogram (bottom) using $[M+H]^+$ ion for all cannabinoids except CBGA which used $[M+H-H_2O]^+$ ion of sample MC3.

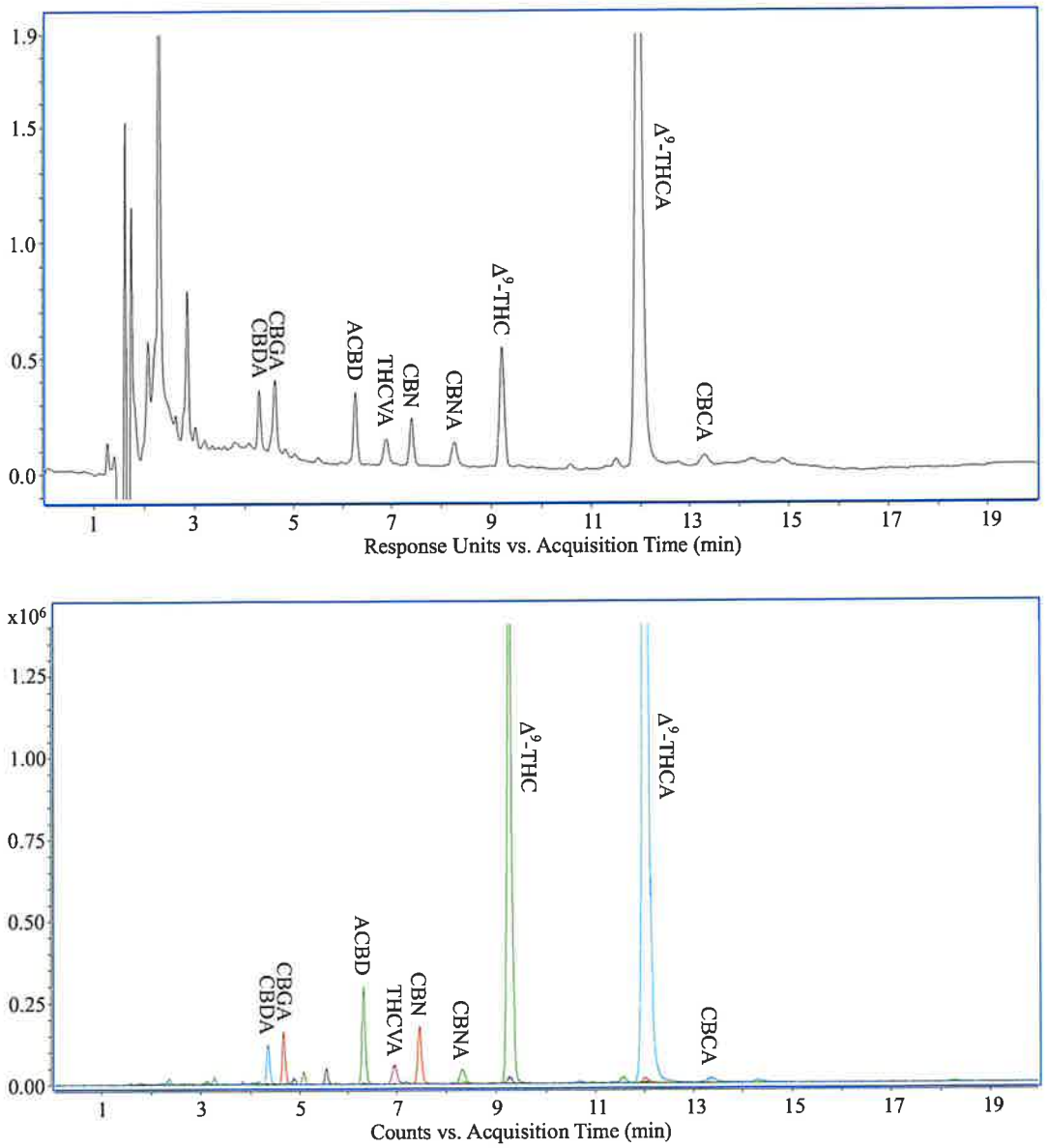


Figure 10.The LC-UV chromatogram (top) at 230 nm and LC-ESI/TOFMS extract ion chromatogram (bottom) using $[M+H]^+$ ion for all cannabinoids except CBGA which used $[M+H-H_2O]^+$ ion of sample MC4

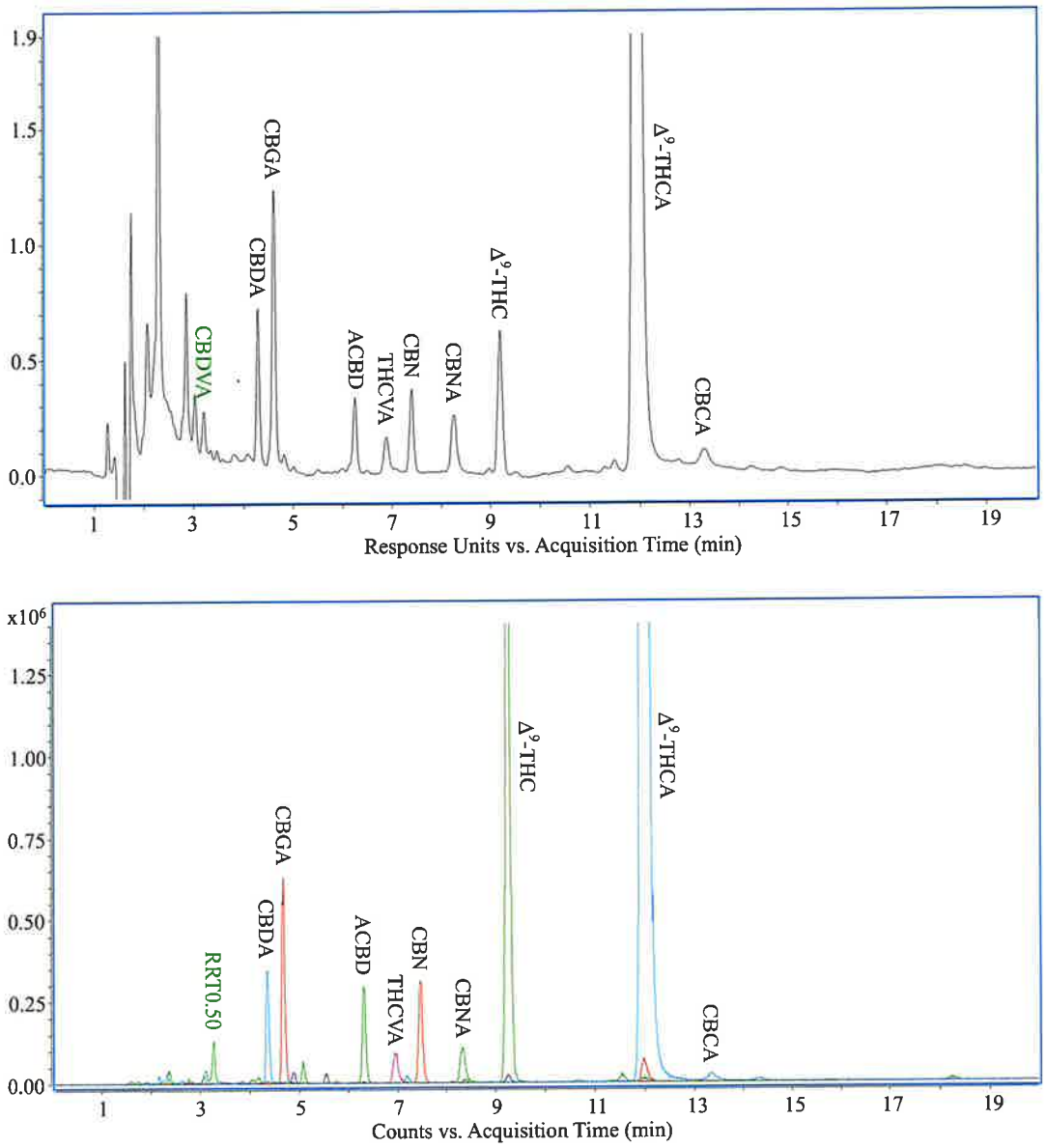


Figure 11. The LC-UV chromatogram (top) at 230 nm and LC-ESI/TOFMS extract ion chromatogram (bottom) using $[M+H]^+$ ion for all cannabinoids except CBGA which used $[M+H-H_2O]^+$ ion of sample MC5

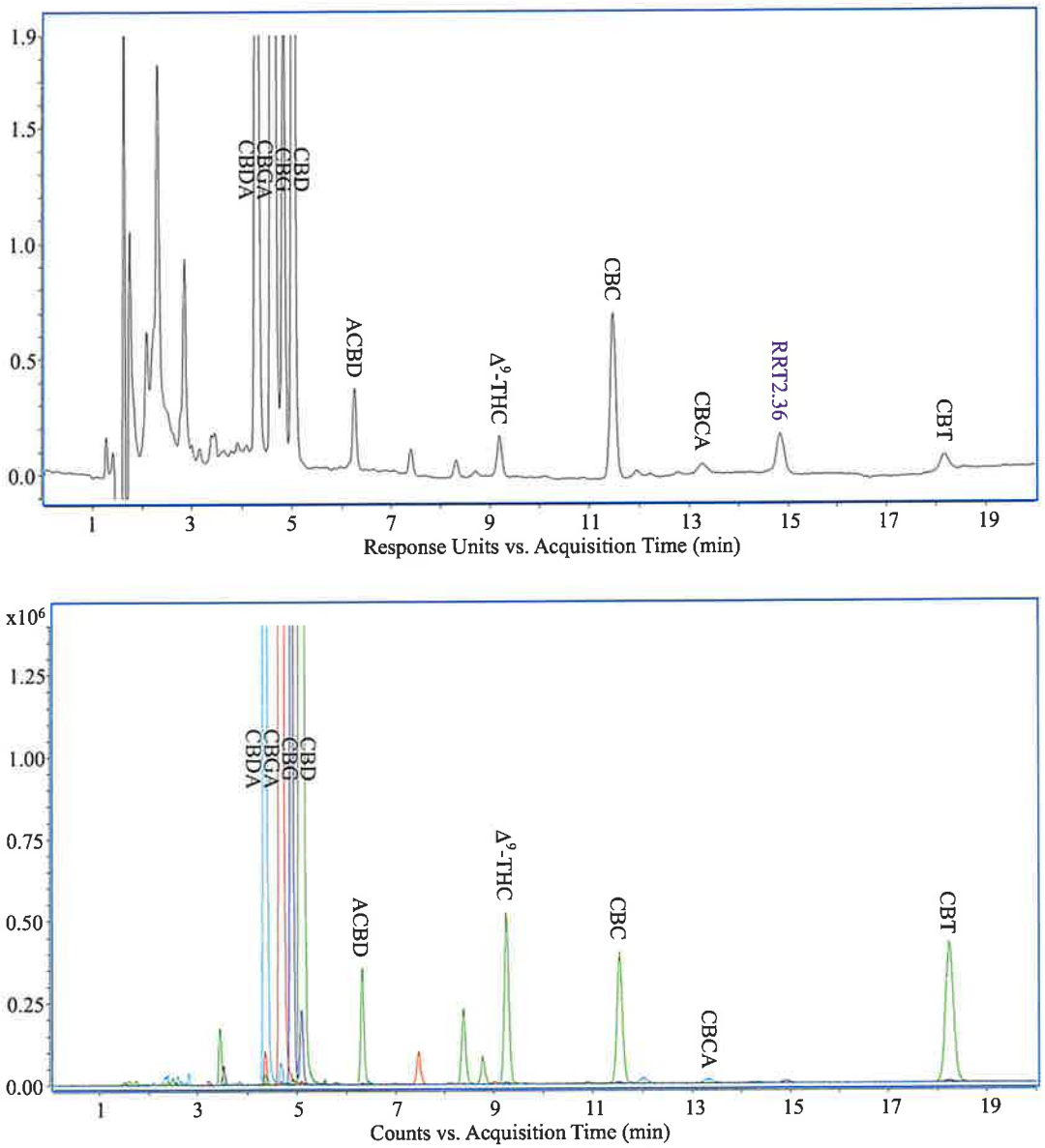


Figure 12. The LC-UV chromatogram (top) at 230 nm and LC-ESI/TOFMS extract ion chromatogram (bottom) using $[M+H]^+$ ion for all cannabinoids except CBGA which used $[M+H-H_2O]^+$ ion of sample HC1

Conclusion

The LC-DAD method described demonstrates significant improvements in comparison to previous published methods. Aside from accounting for more than 13 cannabinoids which is often the maximum number of cannabinoids studied, this method meets the required minimum resolution of 1.5 for appropriate method validation. The developed method is straightforward: samples were analyzed at one concentration, 50 µg/mL in methanol, due to a wide linear calibration range utilized—0.02 to 25 µg/mL in 50 µg/mL extracts of the *Cannabis* cigarettes or 0.04 to 50% (w/w) in the *Cannabis* cigarettes; while published methods have analyzed the same sample at more than one concentration. Recovery of sample preparation was tracked in real-time by spiking ACBD—a non-naturally occurring cannabinoid that is available at a reasonable price— into each sample, while published methods have showed limited result with recovery due to unavailability of cannabinoid-free matrix and the high cost of cannabinoid standards. Overall good specificity was also obtained with only a few minor interferences from compounds in the samples and this was verified by ESI/TOFMS detection. Although possible interference to the quantification of CBDVA was observed close to the LOQ level, this interference could be identified by the method since it utilized two-wavelengths for quantification. On the contrary, another interference which did not affect quantification of any of the target cannabinoids was discovered using ESI-TOFMS.

References

1. Micalizzi, G.; Vento, F.; Fillippo, A.; Donnarumma, D.; Dugo, P.; Mondello, L., *Cannabis Sativa* L.: a comprehensive review on the analytical methodologies for cannabinoids and terpenes characterization. *Journal of Chromatography A* **2021,** *1637* (461864).

2. Hanuš, L. O.; Meyer, S. M.; Muñoz, E.; Taglialatela-Scafati, O.; Appendino, G., Phytocannabinoids: a unified critical inventory. *Nat Prod Rep* **2016,** *33* (12), 1357-1392.

3. ElSohly, M. A.; Slade, D., Chemical constituents of marijuana: The complex mixture of natural cannabinoids. *Life Sci.* **2005,** *78* (5), 539-548.

4. Galettis, P.; Williams, M.; Gordon, R.; Martin, J. H., A Simple Isocratic HPLC Method for the Quantitation of 17 Cannabinoids. *Aust. J. Chem.* **2021,** *74* (6), 453-462.

5. Hanus, L. O.; Meyer, S. M.; Munoz, E.; Taglialatela-Scafati, O.; Appendino, G., Phytocannabinoids: a unified critical inventory. *Nat. Prod. Rep.* **2016,** *33* (12), 1357-1392.

6. ElSohly, M. A. G., Waseem, Constituents of Cannabis Sativa. In *Handbook of Cannabis*, Pertwee, R. E., Ed. Oxford University Press: 2014; pp 3-22.

7. Zivovinovic, S.; Alder, R.; Allenspach, M. D.; Steuer, C., Determination of cannabinoids in Cannabis sativa L. samples for recreational, medical, and forensic purposes by reversed-phase liquid chromatography-ultraviolet detection. *J. Anal. Sci. Technol.* **2018,** *9*, 27.

8. Macherone, A., Chapter One - Comprehensive analytical testing of cannabis and hemp. In *Comprehensive Analytical Chemistry*, Ferrer, I.; Thurman, E. M., Eds. Elsevier: 2020; Vol. 90, pp 3-29.

9. De Backer, B.; Debrus, B.; Lebrun, P.; Theunis, L.; Dubois, N.; Decock, L.; Verstraete, A.; Hubert, P.; Charlier, C., Innovative development and validation of an HPLC/DAD method for the qualitative and quantitative determination of major cannabinoids in cannabis plant material. *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.* **2009,** *877* (32), 4115-4124.

10. Citti, C.; Braghiroli, D.; Vandelli, M. A.; Cannazza, G., Pharmaceutical and biomedical analysis of cannabinoids: A critical review. *J. Pharm. Biomed. Anal.* **2018,** *147*, 565-579.

11. Bonini, S. A.; Premoli, M.; Tambaro, S.; Kumar, A.; Maccarinelli, G.; Memo, M.; Mastinu, A., Cannabis sativa: A comprehensive ethnopharmacological review of a medicinal plant with a long history. *J. Ethnopharmacol.* **2018,** *227*, 300-315.

12. La Tella, R.; Rigano, F.; Guarnaccia, P.; Dugo, P.; Mondello, L., Non-psychoactive cannabinoids identification by linear retention index approach applied to a hand-portable capillary liquid chromatography platform. *Anal Bioanal Chem* **2022,** *414* (21), 6341-6353.

13. E., S. R.; M., K. W.; T., P.; E., L. T., Cannabis: An example of taxonomic neglect. *Bot Mus Leaf Harv Univ.* **1974,** *23*, 337-367.

14. Zuk-Golaszewska, K.; Gołaszewski, J., Cannabis sativa L. – cultivation and quality of raw material. *Journal of Elementology* **2018**.

15. Aizpurua-Olaizola, O.; Omar, J.; Navarro, P.; Olivares, M.; Etxebarria, N.; Usobiaga, A., Identification and quantification of cannabinoids in Cannabis sativa L. plants by high performance liquid chromatography-mass spectrometry. *Anal. Bioanal. Chem.* **2014,** *406* (29), 7549-7560.

16. State Medical Marijuana Laws. https://www.ncsl.org/research/health/state-medical-marijuana-laws.aspx .

17. Johnson, R.; Goodrich, M.; Rodriguez-Cruz, S. Marijuana or Hemp: From Farm Bill to Forensic Analysis. https://forensiccoe.org/webinar/marijuana-or-hemp-from-farm-bill-to-forensic-analysis/ .

18. AOAC SMPR® 2017.002 Standard Method Performance Requirements (SMPRs) for Quantitation of Cannabinoids in Dried Plant Materials. https://www.aoac.org/wp-content/uploads/2020/11/SMPR202017_002.pdf.

19. AOAC SMPR® 2019.003 Standard Method Performance Requirements (SMPRs®) for Quantitation of Cannabinoids in Plant Materials of Hemp (Low THC Varieties Cannabis sp.). https://www.aoac.org/wp-content/uploads/2019/10/SMPR-2019_003.pdf .

20. Buttenbender, S.; Carlos, G.; Steppe, M.; Ortiz, R. S.; Limberger, R. P.; Mendez, A. S. L., Fast and reliable profiling of cannabinoids in seized samples using the method of HPLC-DAD followed by chemometrics. *Forensic Toxicol.* **2022,** *40* (2), 407-413.

21. Citti, C.; Russo, F.; Sgro, S.; Gallo, A.; Zanotto, A.; Forni, F.; Vandelli, M. A.; Lagana, A.; Montone, C. M.; Gigli, G.; Cannazza, G., Pitfalls in the analysis of phytocannabinoids in cannabis inflorescence. *Anal. Bioanal. Chem.* **2020,** *412* (17), 4009-4022.

22. Felletti, S.; De Luca, C.; Buratti, A.; Bozza, D.; Cerrato, A.; Capriotti, A. L.; Lagana, A.; Cavazzini, A.; Catani, M., Potency testing of cannabinoids by liquid and supercritical fluid chromatography: Where we are, what we need. *J. Chromatogr. A* **2021,** *1651*, 462304.

23. Capriotti, A. L.; Cannazza, G.; Catani, M.; Cavaliere, C.; Cavazzini, A.; Cerrato, A.; Citti, C.; Felletti, S.; Montone, C. M.; Piovesana, S.; Laganà, A., Recent applications of mass spectrometry for the characterization of cannabis and hemp phytocannabinoids: From targeted to untargeted analysis. *Journal of Chromatography A* **2021,** *1655*, 462492.

24. Giroud, C., Analysis of cannabinoids in hemp plants. *Chimia* **2002,** *56* (3), 80-83.

25. Cardenia, V.; Gallina Toschi, T.; Scappini, S.; Rubino, R. C.; Rodriguez-Estrada, M. T., Development and validation of a Fast gas chromatography/mass spectrometry method for the determination of cannabinoids in Cannabis sativa L. *J. Food Drug Anal.* **2018,** *26* (4), 1283-1292.

26. Dussy, F. E.; Hamberg, C.; Luginbuhl, M.; Schwerzmann, T.; Briellmann, T. A., Isolation of Delta9-THCA-A from hemp and analytical aspects concerning the determination of Delta9-THC in cannabis products. *Forensic Sci Int* **2005,** *149* (1), 3-10.

27. Dubrow, G. A.; Pawar, R. S.; Srigley, C.; Fong Sam, J.; Talavera, C.; Parker, C. H.; Noonan, G. O., A survey of cannabinoids and toxic elements in hemp-derived products from the United States marketplace. *Journal of Food Composition and Analysis* **2021,** *97*, 103800.

28. Mudge, E. M.; Brown, P. N., Determination of Cannabinoids in Cannabis sativa Dried Flowers and Oils by LC-UV: Single-Laboratory Validation, First Action 2018.10. *J AOAC Int* **2020,** *103* (2), 489-493.

29. Križman, M., A simplified approach for isocratic HPLC analysis of cannabinoids by fine tuning chromatographic selectivity. *European Food Research and Technology* **2020,** *246*.

30. Vaclavik, L.; Benes, F.; Fenclova, M.; Hricko, J.; Krmela, A.; Svobodova, V.; Hajslova, J.; Mastovska, K., Quantitation of cannabinoids in Cannabis dried plant materials, concentrates, and oils using liquid chromatography-diode array detection technique with optional mass spectrometric detection: single-laboratory validation study, first action 2018.11. *J. AOAC Int.* **2019,** *102* (6), 1822-1833.

31. Wang, Y.-H.; Avula, B.; El Sohly, M. A.; Radwan, M. M.; Wang, M.; Wanas, A. S.; Mehmedic, Z.; Khan, I. A., Quantitative Determination of Δ 9 -THC, CBG, CBD, Their Acid Precursors and Five Other Neutral Cannabinoids by UHPLC-UV-MS. *Planta Med.* **2018,** *84* (4), 260-266.

32. Ciolino, L. A.; Ranieri, T. L.; Taylor, A. M., Commercial cannabis consumer products part 2: HPLC-DAD quantitative analysis of cannabis cannabinoids. *Forensic Sci. Int.* **2018,** *289*, 438-447.

33. Mudge, E. M.; Murch, S. J.; Brown, P. N., Leaner and greener analysis of cannabinoids. *Anal. Bioanal. Chem.* **2017,** *409* (12), 3153-3163.

34. Brighenti, V.; Pellati, F.; Steinbach, M.; Maran, D.; Benvenuti, S., Development of a new extraction technique and HPLC method for the analysis of non-psychoactive cannabinoids in fibre-type Cannabis sativa L. (hemp). *J. Pharm. Biomed. Anal.* **2017,** *143*, 228-236.

35. Citti, C.; Ciccarella, G.; Braghiroli, D.; Parenti, C.; Vandelli, M. A.; Cannazza, G., Medicinal cannabis: Principal cannabinoids concentration and their stability evaluated by a high performance liquid chromatography coupled to diode array and quadrupole time of flight mass spectrometry method. *J. Pharm. Biomed. Anal.* **2016,** *128*, 201-209.

36. Peschel, W.; Politi, M., 1H NMR and HPLC/DAD for Cannabis sativa L. chemotype distinction, extract profiling and specification. *Talanta* **2015,** *140*, 150-165.

37. Gul, W.; Gul, S. W.; Radwan, M. M.; Wanas, A. S.; Mehmedic, Z.; Khan, I. I.; Sharaf, M. H. M.; El Sohly, M. A., Determination of 11 cannabinoids in biomass and extracts of different varieties of cannabis using high-performance liquid chromatography. *J. AOAC Int.* **2015,** *98* (6), 1523-1528.

38. Giese, M. W.; Lewis, M. A.; Giese, L.; Smith, K. M., Development and Validation of a Reliable and Robust Method for the Analysis of Cannabinoids and Terpenes in Cannabis. *J AOAC Int* **2015,** *98* (6), 1503-22.

39. Tran, J.; Elkins, A. C.; Spangenberg, G. C.; Rochfort, S. J., High-Throughput Quantitation of Cannabinoids by Liquid Chromatography Triple-Quadrupole Mass Spectrometry. *Molecules* **2022,** *27* (3).

40. Wilson, W. B.; Abdur-Rahman, M., Determination of 11 Cannabinoids in Hemp Plant and Oils by Liquid Chromatography and Photodiode Array Detection. *Chromatographia* **2022,** *85* (2), 115-125.

41. Swift, W.; Wong A Fau - Li, K. M.; Li Km Fau - Arnold, J. C.; Arnold Jc Fau - McGregor, I. S.; McGregor, I. S., Analysis of cannabis seizures in NSW, Australia: cannabis potency and cannabinoid profile. (1932-6203 (Electronic)).

42. Ambach, L.; Penitschka, F.; Broillet, A.; König, S.; Weinmann, W.; Bernhard, W., Simultaneous quantification of delta-9-THC, THC-acid A, CBN and CBD in seized drugs using HPLC-DAD. *Forensic Sci Int* **2014,** *243*, 107-11.

43. Sexton, M.; Shelton, K.; Haley, P.; West, M., Evaluation of Cannabinoid and Terpenoid Content: Cannabis Flower Compared to Supercritical CO2 Concentrate. *Planta Med* **2018,** *84* (4), 234-241.

44. Deville, M.; Dubois, N.; Denooz, R.; Charlier, C., Validation of an UHPLC/DAD method for the determination of cannabinoids in seized materials: Analysis of 213 samples sold in Belgian CBD shops. *Forensic Sci Int* **2020,** *310*, 110234.

45. McRae, G.; Melanson, J. E., Quantitative determination and validation of 17 cannabinoids in cannabis and hemp using liquid chromatography-tandem mass spectrometry. *Anal. Bioanal. Chem.* **2020,** *412* (27), 7381-7393.

46. Song, L.; Carlson, S.; Valenzuela, G.; Chao, M.; Pathipaka, S. B. In *Development of a validated UHPLC-DAD method with optional ESI/TOFMS detection for rapid quantification of delta 9-THC and delta 9-THCA-A among sixteen cannabinoids in hemp concentrates*, NIJ Forensic Science R&D Symposium, Virtual, March 1&2; Virtual, 2022.

DECLARATION OF SCOUT STUBBS

1. My name is Scout Stubbs, and I am over the age of twenty-one, of sound mind, and competent to execute this declaration.

2. I submit this declaration on behalf of Drippers Vape Shop, LLC ("Drippers"), in support of plaintiffs' motion for preliminary injunction.

3. I have personal knowledge of the facts set forth in this Declaration, or know them in my capacity as president and managing member of Drippers, based on records that Drippers keeps in the regular course of its business, and could and would competently testify to them under oath if called as a witness.

4. Drippers is an Arkansas limited liability company with its principal place of business located in Greenbrier, Arkansas.

5. Drippers is a retail store offering, among other things, hemp products to Arkansas consumers and businesses.

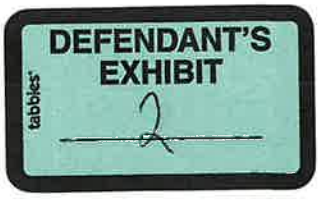
6. On August 8, 2023, Drippers' retail store in Hot Springs, Arkansas was inspected by an Arkansas Tobacco Control officer.

7. That ATC officer directed Drippers' employees to remove from the shelves a certain hemp product, which contained no more than 0.3% delta-9 THC and was not produced as a result of a synthetic chemical process.

8. Nonetheless, Drippers' employees complied with the officer's directive and removed the requested hemp product from the shelves.

9. Drippers no longer retails this hemp product to its customers.

10. As a result of Act 629 and the ATC's enforcement of the Act, Drippers and its employees are in jeopardy of criminal prosecution for possessing, shipping, transporting, packaging, processing, and retailing hemp-derived products in Arkansas.

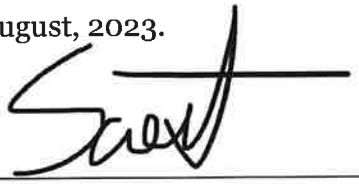


11. Drippers' investment is exposed and threatened as Act 629 has rendered its inventory of hemp-derived products utterly worthless in Arkansas.

12. Drippers will continue to be irreparably harmed until Act 629 is enjoined.

I declare under penalty of perjury, pursuant to 28 U.S.C. § 1746, that the foregoing is true and accurate.

Executed on this 14th day of August, 2023.



Scout Stubbs
President and Managing Member
Drippers Vape Shop, LLC