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RESEARCH

Evaluating Different C_{18} Columns for the LC Separation of Δ^9-THC and Other Related Cannabinoids

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Abstract

The best separation possible of delta-9-tetrahydrocannabinol (Δ^9-THC) in complex samples via liquid chromatography (LC) is particularly challenging due to the potential for interference from other cannabinoids. Over 100 cannabinoids have been detected in *Cannabis sativa* plant samples. The separation of Δ^9-THC in *Cannabis*-derived finished products was believed to be easier than plant extracts to analyze at one time because the acidic cannabinoids are converted to their neutral cannabinoids during material preparation. However, the emergence of synthetic or semi-synthetic cannabinoid products, such as delta-8-tetrahydrocannabinol (Δ^8-THC), has led to more chromatographic interferences due to the presence of synthetic by-products. The original LC separation method implemented in the Chemical Sciences Division (CSD) at the National Institute of Standards and Technology (NIST) was not acceptable when these chromatographic interferences were present. Within this context, the work presented here explores initial separation of an 11 cannabinoid mixture using different monomeric and polymeric octadecylsilane (C_{18}) columns via liquid chromatography. These columns were characterized using the Standard Reference Material 869b as a three-component column selectivity test mixture to determine if an LC C_{18} column is classified as monomeric or polymeric. Monomeric C_{18} columns (NexLeaf C_{18}, ACE 3 C_{18}, and ACE Super C_{18}) provided better separations of the 11 cannabinoids, and baseline separations were obtained in less than 13 min after minor adjustments to the mobile phase program. Using the NexLeaf C_{18} LC-UV method, mixtures of Δ^9-THC and four known chromatographic interferences were analyzed. Cannabinolic acid (CBNA) could not be separated from Δ^9-THC; however, CBNA has drastically different absorbance spectra from Δ^9-THC, and the co-elution of these cannabinoids can easily be recognized using photodiode array detection. When CBNA is present, the sample can be reanalyzed using an alternate NexLeaf C_{18} LC-UV method developed here, which baseline-resolves Δ^9-THC and CBNA in 60 min. These two LC-UV methods will be further evaluated at NIST CSD through quantitative comparisons in future publications, enabling their use in the development of reference materials for *Cannabis* plants and/or *Cannabis*-derived finished products.

Keywords Cannabinoids · Delta-9-tetrahydrocannabinol · Delta-8-tetrahydrocannabinol · Liquid chromatography · C18 columns

Introduction

In 2019, the Chemical Sciences Division (CSD) at the National Institute of Standards and Technology (NIST) established a Cannabis Research Program to provide forensic and cannabis testing laboratories with tools to accurately measure delta-9-tetrahydrocannabinol (Δ^9-THC), its acidic precursor delta-9-tetrahydrocannabinolic acid (Δ^9-THCA), and other important cannabinoids often found in *Cannabis sativa*. The development of this program was initiated in response to the passage of the *Agriculture Improvement Act of 2018* (2018 Farm Bill), which legalized hemp as *Cannabis* plants containing 0.3% or less of Δ^9-THC by weight [1]. Prior to this legislation, forensic laboratories had not been required to perform quantitative measurements to distinguish seized *Cannabis* plant samples as hemp or marijuana. Previous analytical approaches were based on qualitative tests using macro- and microscopic identification [2], a colorimetric test [3–5], and/or gas chromatography-mass

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spectrometry (GC–MS) for Δ^9-THC identification [6–12]. GC–MS is generally preferred by forensic scientists over liquid chromatography with ultraviolet detection (LC-UV) due to its shorter separation times, reduced solvent consumption, and greater simplicity and familiarity for illicit drug analysis [11, 12].

The accurate GC–MS determination of Δ^9-THC in *Cannabis* is dependent on the conversion of analyte precursors during sample introduction in the heated GC inlet. Δ^9-THCA is the predominant form of Δ^9-THC present in *Cannabis* plants. When heated, Δ^9-THCA decarboxylates to yield Δ^9-THC by losing CO_2, and GC–MS analysis yields a single peak representing the total Δ^9-THC. Studies have shown that Δ^9-THCA is not converted with 100% efficiency [7–10]. Due to this limitation, it is widely accepted that accurate total Δ^9-THC measurements necessitate a different approach. Separate determination of Δ^9-THCA and Δ^9-THC can be achieved by derivatization during sample preparation. This prevents the conversion of Δ^9-THCA to Δ^9-THC, allowing the analyst to calculate the total Δ^9-THC using Eq. 1. Recently, Mulloor et al. [7] demonstrated that accurate total Δ^9-THC measurements in *Cannabis* plant samples are possible without derivatization through the use of analyte protectants to passivate the heated injector during sample introduction to yield quantitative conversion of Δ^9-THCA to Δ^9-THC.

$$\%\text{Total}\Delta^9\text{ - THC} = (0.877 \times \%\,\Delta^9 - \text{THCA}) + \%\Delta^9 - \text{THC} \quad (1)$$

To avoid the conversion issue, the *Cannabis* industry and some forensic laboratories have employed liquid chromatography with ultraviolet absorbance detection (LC-UV) to determine Δ^9-THC, Δ^9-THCA, and total Δ^9-THC [13, 14]. NIST CSD has performed extensive research involving LC-UV to support method development [15–18] and sample preparation procedures [15, 16]. These LC-UV studies have utilized the *high-sensitivity method* developed by Shimadzu Instruments, specifically for forensic laboratories, as an all-in-one system. This method targets 11 cannabinoids in *Cannabis* plant and oil samples. Recent studies by NIST CSD have indicated that potential biases in Δ^9-THC measurements may occur for some samples when CBNA or by-products from synthetic Δ^8-THC/Δ^{10}-THC are present due to co-elution issues [18, 19].

The current study investigates the use of different C_{18} columns for the separation of a standard mixture of 11 cannabinoids and known cannabinoid interferences. Columns were selected based on the synthetic processes used in their manufacture and other physical characteristics (particle size, pore size, etc.). C_{18} columns are the most widely used stationary phases for LC separation of cannabinoids, as highlighted in a recent review by Duchateu et al. [20], which summarizes LC methods published between 2018 and 2024. Of the 39 LC methods included in the review, 33 used C_{18} columns of various dimensions and particle sizes. A limited number of studies have been published by column manufacturers that clearly show differences in performance among C_{18} columns for the separation of cannabinoids. In the current study, new LC separation methods were developed for three different C_{18} columns: NexLeaf C_{18}, ACE 3 C_{18}, and ACE Super C_{18}. The three methods provided baseline resolution for the 11 cannabinoids, and the LC-UV method using the NexLeaf C_{18} column was selected for further evaluation to separate four known co-eluting cannabinoids. This LC-UV method provided adequate separation for these cannabinoids in under 15 min, except for CBNA, which completely co-elutes with Δ^9-THC. A more extensive separation method, requiring a 60-min chromatographic run, was developed for the determination of Δ^9-THC in *Cannabis* samples when CBNA is detected using a photodiode array (PDA) detector. Both LC-UV methods will be shown in a future publication to provide accurate quantitative measurements of Δ^9-THC in *Cannabis* samples.

Materials and Methods

Chemicals and Standards

A calibration solution containing 11 cannabinoids in acetonitrile (ACN) was obtained from Shimadzu Instruments, LLC (Columbia, MD). This solution has a nominal concentration of 250 mg/L for each cannabinoid, including cannabidivarin (CBDV), cannabidiol (CBD), cannabidiolic acid (CBDA), cannabigerol (CBG), cannabigerolic acid (CBGA), Δ^9-tetrahydrocannabivarin (Δ^9-THCV), cannabinol (CBN), Δ^9-THC, Δ^8-THC, cannabichromene (CBC), and Δ^9-THCA. Individual calibration solutions at a nominal concentration of 1000 mg/L were obtained from Cayman Chemical for *exo*-THC and (9R)-Δ^7-THC. An individual calibration solution at a nominal concentration of 1000 mg/L of cannabinolic acid (CBNA) was obtained from MillaporeSigma. A single working solution mixture of all cannabinoids was prepared volumetrically to have final mass concentrations of ≈ 50 mg/L. HPLC-grade ACN, water (H_2O), methanol (MeOH), and 85% phosphoric acid (PA) were purchased from Fisher Scientific (St. Louis, MO).

LC-UV

Cannabinoid separations were performed using a Shimadzu *Cannabis Analyzer* with six different C_{18} columns (15.0 cm × 4.6 mm i.d.), summarized in Table 1 as monomeric or polymeric C_{18} columns. The three monomeric

Table 1 Analytical LC columns investigated in the present study

	Column[a] efficiency (N)	Particle size (micron)	Carbon load (%)	Surface area (m^2/g)	Pore size (Å)	$\alpha_{TBN/BaP}$
Monomeric C_{18}						
NexLeaf	31,657	2.7	7	130	90	1.74
ACE 3	24,200	3.0	15.5	300	100	1.57
ACE Super	34,010	2.5	7.0	130	95	1.76
Polymeric C_{18}						
HALO PAH	NA	2.7	9.9	135	90	0.62
Pinnacle II PAH	17,293	4.0	-	180	110	0.74
HYPERSIL Green PAH	NA	3.0	14	170	120	0.86

[a] Column efficiency (N) was provided by the column manufacturers for biphenyl

Table 2 Mobile phase programs investigated in the present study

Time (min)	H_2O (%)	ACN (%)	Time (min)	H_2O (%)	ACN (%)
Method 1: Six C_{18}Columns			Method 2: NexLeaf C_{18}		
0.00	30	70	0.00	28	72
3.00	30	70	10.00	28	72
7.00	15	85	10.01	5	95
7.01	5	95	12.00	5	95
8.00	5	95	12.01	28	72
8.01	30	70	14.00	28	72
10.00	30	70			
Method 3: ACE 3 C_{18}			Method 4: ACE Super C_{18}		
0.00	30	70	0.00	28	72
5.00	30	70	3.00	28	72
5.01	15	85	3.01	15	85
9.00	15	85	7.00	15	85
9.010	5	95	7.01	5	95
10.00	5	95	8.00	5	95
10.01	30	70	8.01	28	72
12.00	30	70	10.00	28	72
Method 5: NexLeaf C_{18}					
0.00	45	55			
50.00	45	55			
50.01	5	95			
55.00	5	95			
55.01	45	55			
60.00	45	55			

C_{18} columns are end-capped, and the three polymeric C_{18} columns are not end-capped. The NexLeaf C_{18} and HALO PAH C_{18} are superficially porous columns, while the ACE 3 C_{18}, ACE Super C_{18}, Pinnacle II PAH, and Hypersil Green PAH are fully porous columns. Separations were carried out at a flow rate of 1.6 mL/min, a column temperature of 40 °C, and five mobile phase programs summarized in Table 2. The injection volume was 5 µL, and a PDA detector collected UV data from 190 to 700 nm; 220 nm was used for all chromatograms shown here.

Results and Discussion

Comparison of LC Columns with Different C_{18} Stationary Phases

Although most LC methods for cannabinoids use C_{18} columns [20], few studies have examined the influence of other physical and chemical column properties on the separation performance of these compounds. Relevant properties may include the synthetic approach used to prepare the stationary phase, particle size (µm), carbon load (%), surface area (m^2/g), and pore size (Å). Six columns are included in the current study, as summarized in Table 1. These columns can be categorized into two groups based on the stationary phase chemistry: monomeric or polymeric. These two stationary phases have been shown to exhibit significant selectivity differences toward other isomeric compound groups, such as polycyclic aromatic hydrocarbons (PAHs) [21–23] and carotenoids [24]. Polymeric syntheses utilize trifunctional silane in the presence of H_2O to form intermediary silane oligomers that react with silica particles. Monomeric syntheses are carried out with monofunctional silanes that directly react with silica to produce a monolayer. Three polymeric C_{18} columns were evaluated here despite being primarily used for the separation of PAHs because of the potential for chromatographic retention differences.

NIST has previously developed a Standard Reference Material (SRM) 869b as a column selectivity test mixture to determine whether an LC C_{18} column is classified as monomeric or polymeric [21–23], as this information is not provided by column manufacturers. The test mixture consists of three PAHs: phenanthro[3,4-c]phenanthrene (PhePhe), benzo[a]pyrene (BaP), and 1,2:3,4:5,6:7,8-tetrabenzonaphthalene (TBN). The elution order of these PAHs is used to calculate the selectivity factor $\alpha_{TBN/BaP}$. C_{18} columns with monomeric phases usually exhibit $\alpha_{TBN/BaP} \geq 1.7$, polymeric C_{18} columns have $\alpha_{TBN/BaP} \leq 1$, and C_{18} columns with $\alpha_{TBN/BaP}$ between 1.0 and 1.7 are considered to have intermediate properties. Table 1 summarizes the $\alpha_{TBN/BaP}$ values for the six columns used in this study, employing a mobile phase of 95/5 (v/v) ACN/H_2O with 0.085% PA at a flow rate

of 1.6 mL/min. The primary importance of these conditions was to mimic Method 1 as much as possible under isocratic conditions. A higher ACN percentage was used to ensure that more non-polar PAH molecules would elute from the C_{18} columns. The HALO PAH, Pinnacle II PAH, and HYPERSIL Green PAH columns had $\alpha_{TBN/BaP} \leq 1$ as expected for PAH columns that normally consist of polymeric C_{18} stationary phases. The NexLeaf C_{18} and ACE Super C_{18} columns had $\alpha_{TBN/BaP} \geq 1.7$, indicating a monomeric stationary phase. The ACE 3 C_{18} column had an $\alpha_{TBN/BaP}$ of 1.57, which is characteristic of a C_{18} column with intermediate properties, but with more similarity to monomeric C_{18} columns than polymeric C_{18} columns. Separations of the 11 cannabinoid mixture are shown in Fig. 1, versus the polymeric C_{18} columns in Fig. 2. For these reasons, the ACE 3 C_{18} column is classified as a monomeric C_{18} column here.

These chromatograms were collected using Method 1 in Table 2, which was originally developed by Shimadzu Instruments using the NexLeaf C_{18} column specifically for the separation of these 11 cannabinoids. These 11 cannabinoids were selected based on their commonality throughout the *Cannabis* industry at the time of the study. NIST has multiple publications that use this method to assign cannabinoid mass fractions (%) to commercial *Cannabis* samples [17], Reference Material (RM) 8210 (Hemp Plant) [25], and test samples for the NIST Cannabis Laboratory Quality Assurance Program (CannaQAP) [13, 14]. Wilson et al. [18, 19] have recently demonstrated in several publications that this method does not provide an adequate separation when CBNA or synthetic by-products of Δ^8-THC or Δ^{10}-THC are present. The two primary differences for the separation of these 11 cannabinoids on the three monomeric C_{18} columns involve CBD/Δ^9-THCV and CBC/Δ^9-THCA. CBD and Δ^9-THCV partially co-elute on the NexLeaf C_{18} column but are baseline resolved on the other two monomeric C_{18} columns. CBC and Δ^9-THCA are baseline resolved on the NexLeaf C_{18} column but co-elute on the other two monomeric C_{18} columns, eluting almost as one peak on the ACE Super C_{18} column.

The separation variations shown in Fig. 1 may also be attributed to other specification differences between the LC columns as summarized in Table 1. All columns in this study had the same dimensions of 150 mm × 4.6 mm. The NexLeaf C_{18} and ACE Super C_{18} columns would be expected to provide similar chromatographic separations because of their similar column efficiency (N ≈ 33,000), carbon load

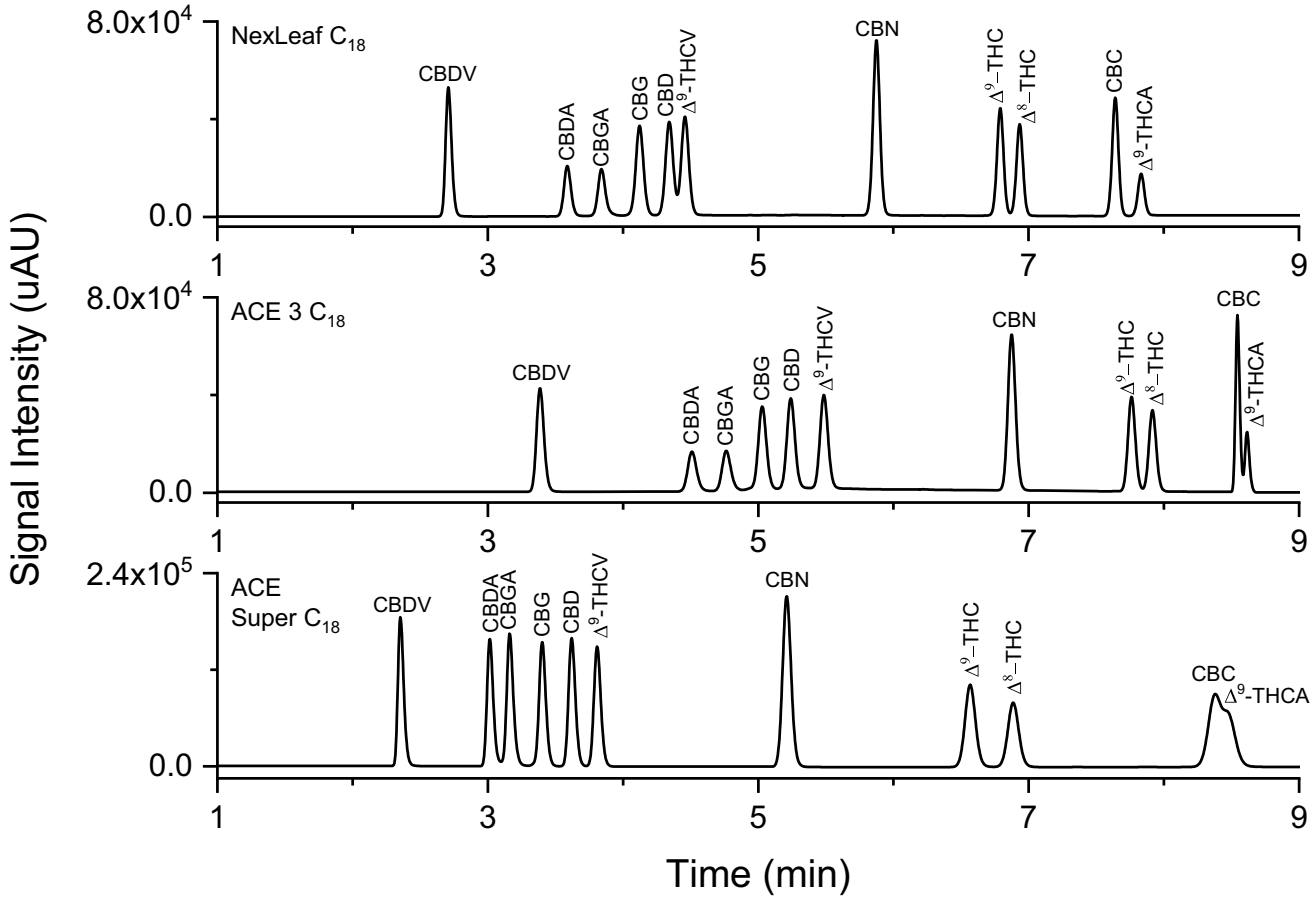

Fig. 1 LC-UV chromatograms collected at 220 nm for 11 cannabinoid mixture on the three monomeric C_{18} columns using Method 1 in Table 2

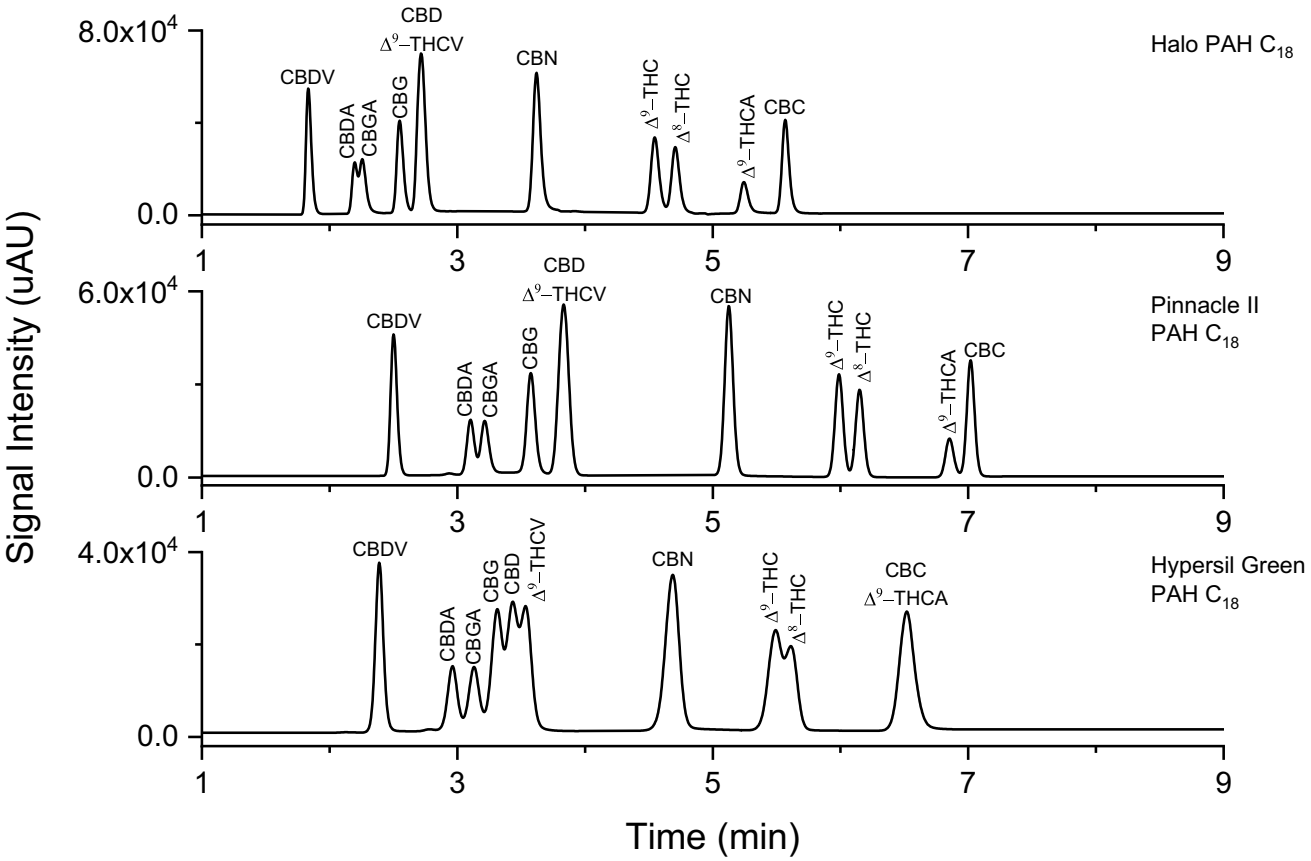

Fig. 2 LC-UV chromatograms collected at 220 nm for 11 cannabinoid mixture on the three polymeric C_{18} columns using Method 1 in Table 2

(7.0%), surface area (130 m^2/g), and pore sizes (90 Å to 95 Å). The separation differences between CBC and Δ^9-THCA may be due to the NexLeaf C_{18} column being superficially porous, whereas the ACE Super C_{18} column is fully porous. The ACE 3 C_{18} column had a slightly higher particle size (3.0 µm) and pore size (100 Å); however, its carbon load (15.5%) and surface area (300 m^2/g) were more than double those of the other two monomeric C_{18} columns. The column efficiency for the NexLeaf C_{18} and ACE Super C_{18} columns was slightly better, with approximately 9000 more theoretical plates than the ACE 3 C_{18} column; however, minimal differences in peak broadening were observed.

Less complete separations of the 11 cannabinoids were achieved with the polymeric C_{18} columns (Fig. 2), and the only selectivity difference occurs with the reversal of the elution order for CBC and Δ^9-THCA. CBDA/CBGA and CBD/Δ^9-THCV partially co-elute on the three polymeric C_{18} columns. Δ^9-THC and Δ^8-THC are baseline resolved on the Halo PAH C_{18} and Pinnacle II PAH C_{18} columns but co-elute on the Hypersil Green PAH C_{18} column. All cannabinoids are less retained on the polymeric C_{18} columns, with the Halo PAH C_{18} column exhibiting the least retention overall. This polymeric C_{18} column probably has the lowest carbon load; however, this value could not be obtained for the Pinnacle II PAH C_{18} column. No additional studies were performed here using polymeric C_{18} columns because monomeric C_{18} columns performed better using Method 1 and are more commonly used for cannabinoid separations [20].

Improving the Separation of 11 Cannabinoids for Three Monomeric C_{18} Columns

The next step in this research was to develop preliminary LC-UV methods for each monomeric C_{18} column for potential use in screening *Cannabis* samples at NIST. These studies focused only on modifying the mobile phase programs, while keeping the organic modifier (0.085% PA), flow rate (1.6 mL/min), and column temperature (40 °C) constant. The use of 0.085% PA was originally established by Shimadzu Instruments during method development, and no attempts were made in the current study to switch to formic acid or an ammonium formate buffer to minimize potential changes for laboratories interested in implementing these methods. The goal for each method was to achieve rapid baseline separation of the 11 cannabinoids within 15 min. The LC-UV chromatograms at 220 nm obtained with the three monomeric C_{18} columns are shown in Fig. 3, using

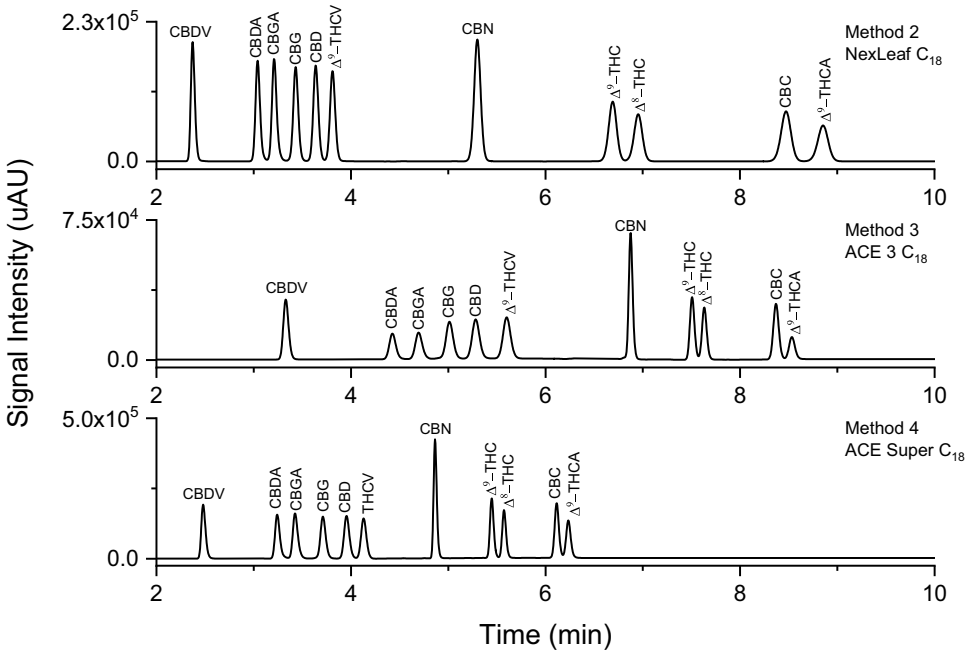

Fig. 3 LC-UV chromatograms collected at 220 nm for 11 cannabinoids using the new methods developed on three monomeric C_{18} columns

the new mobile phase programs summarized in Table 2 for Methods 2, 3, and 4. The initial studies employed an isocratic mobile phase ranging from 60 to 85% ACN to investigate separation differences across columns, as shown in Figs. S1–S3.

Better separations were obtained for the NexLeaf C_{18} column in Fig. S1 using either 70% ACN or 75% ACN. Both isocratic conditions provide baseline separation, except for CBD/Δ^9-THCV (70%) and CBDA/CBGA (75%). The optimal ACN percentage was further studied at 71%, 72%, 73%, and 74%. The separation of the 11 cannabinoids was sensitive to these slight changes, allowing baseline separation with 72% ACN or 73% ACN (Fig. S4). No additional studies were performed to change the mobile phase program because all 11 cannabinoids are baseline resolved, as shown in Fig. 3. However, the mobile phase was adjusted to 95% ACN at 10 min and held for 2 min during the analysis of *Cannabis* samples to ensure the elution of all cannabinoids not included here.

The chromatograms shown in Fig. S2, using the ACE 3 C_{18} column, indicated that better separations were obtained under isocratic conditions at 70% and 75% ACN. The cannabinoid cluster of CBDA, CBGA, CBG, CBD, and Δ^9-THCV was baseline resolved under isocratic conditions at 70% ACN, but CBDA and CBGA co-eluted at 75% ACN. The separation of CBC and Δ^9-THCA improved as the mobile phase composition increased from 70% ACN to 85% ACN; however, the separation of Δ^9-THC and Δ^8-THC was reduced. For this reason, a two-step isocratic mobile phase program was used to achieve baseline separation of the 11 cannabinoids. This mobile phase change was studied by switching between 70% ACN and 85% ACN at the 5 min, 6 min, 7 min, and 8 min time points during the chromatographic run. The best separation was obtained for switching the mobile phase conditions at 5 min, as shown in Fig. S5. The mobile phase is switched to 95% ACN at 9 min and held for 2 min when analyzing *Cannabis* samples, rather than 85%, to ensure baseline separation of Δ^9-THC and Δ^8-THC.

The chromatograms shown in Fig. S3, obtained with the ACE Super C_{18} column, indicate that none of the isocratic conditions could baseline-resolve all 11 cannabinoids. The best separation was obtained at 80% ACN, with only CBDA and CBGA co-eluting, which were better separated with 75% ACN and baseline resolved at 70% ACN. CBC and Δ^9-THCA were baseline resolved at 80% ACN, but co-eluted at 75% ACN and 70% ACN. A two-step isocratic mobile phase program was applied here, similar to the ACE 3 C_{18} column, to obtain the baseline separation shown in Fig. 3. For the separation of CBDA and CBGA, the isocratic mobile phase was studied between 70% ACN and 75% ACN (Fig. S6). Baseline separation was obtained for all cannabinoids except CBC and Δ^9-THCA using 72% ACN or 73% ACN. Δ^9-THC and Δ^8-THC were slightly better separated with 72% ACN, and it was selected for the first isocratic mobile phase for the ACE Super C_{18} column. The best separation for CBC and Δ^9-THCA was obtained in the initial isocratic study at 85% ACN (Figure S3) and was selected for the second isocratic step in this method. The starting time for the second step was investigated by switching between 72% ACN and 85% ACN at the 3 min, 4 min, 5 min, and 6 min time points in the chromatographic run. The best separation was obtained for switching the mobile phase conditions at 3 min, as shown in Fig. S7. The mobile phase is switched to 95% ACN at 7 min

and held for 2 min during analysis of *Cannabis* samples to ensure no sample components remain on the column. The ACE Super C_{18} column provided a separation similar to that of the NexLeaf C_{18} column, as expected given their similar $\alpha_{TBN/BaP}$ values and other column characteristics. The LC-UV method using the NexLeaf C_{18} column was selected here for additional separation studies using four known co-eluting cannabinoids of Δ^9-THC.

Separation of Δ^9-THC and Co-eluting Cannabinoids on the NexLeaf C_{18} Column

Previous NIST CSD studies have shown that Δ^9-THC co-elutes with CBNA and *exo*-THC [14, 18], and elutes closely to (9R)-Δ^7-THC and Δ^8-THC [18]. To the best of the author's knowledge, no method has been published for baseline separation of these five cannabinoids using the NexLeaf C_{18} column. Previous studies [26–29] have reported LC-UV methods capable of baseline separation of Δ^9-THC, Δ^8-THC, *exo*-THC, and CBNA using different C_{18} columns and mobile phase conditions with various organic modifiers (e.g., a combination of ammonium formate and formic acid), which were not used in the current study. For this reason, this study sought to evaluate the potential of the LC-UV using the NexLeaf C_{18} column (Method 2, Table 2) for separating these five cannabinoids.

Figure 4 shows the normalized LC-UV chromatogram at 220 nm for a mixture of Δ^9-THC, Δ^8-THC, *exo*-THC, CBNA, and (9R)-Δ^7-THC, obtained using the NexLeaf C_{18} column (Method 2, Table 2). Δ^9-THC should be resolved enough from *exo*-THC, (9R)-Δ^7-THC, and Δ^8-THC to permit accurate quantitative measurements of Δ^9-THC in *Cannabis* samples. Δ^9-THC completely co-elutes with CBNA,

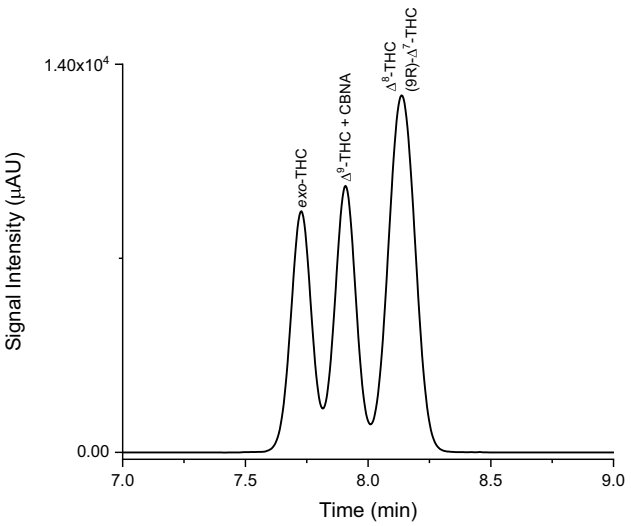

Fig. 4 LC-UV chromatogram collected at 220 nm on the NexLeaf C_{18} column for Δ^9-THC, Δ^8-THC, *exo*-THC, CBNA, and (9R)-Δ^7-THC mixture using Method 2 in Table 2

which can lead to inaccurate measurements of *Cannabis* samples when present. Despite this limitation, Method 2 will be further evaluated at NIST CSD before being implemented to initially analyze all *Cannabis* samples, as CBNA can be identified using the PDA detector, which has a drastically different absorbance spectrum from Δ^9-THC [18]. *Cannabis* samples that are found to contain CBNA would need to be reanalyzed using a different LC-UV method to measure only Δ^9-THC. The reanalysis of *Cannabis* samples could be performed using LC-UV methods with different C_{18} columns and mobile phases [18, 26] or switching the detection method from UV to mass spectrometry (MS or MS/MS) [18]. Previous studies at NIST CSD have demonstrated that these MS-based methods are more selective and sensitive than UV detection [18]; however, several disadvantages could limit their implementation in forensic and *Cannabis* testing laboratories for Δ^9-THC measurements. For example, MS-based methods are more expensive to purchase, entail higher consumables and maintenance costs, and are less straightforward to use.

Laboratories would be more willing to switch to an LC-UV method that could separate all these cannabinoids under similar mobile phase conditions. For this reason, an additional study was conducted to determine the optimal mobile phase conditions for improving the separation of Δ^9-THC and CBNA. Figure 5 shows the LC-UV chromatograms obtained for a mixture of these five cannabinoids separated under different isocratic mobile phase conditions ranging from 50 to 75% ACN. Δ^9-THC and *exo*-THC eluted as a single peak using an isocratic mobile phase containing 50% ACN or 55% ACN, but were baseline-separated from CBNA, Δ^8-THC, and (9R)-Δ^7-THC. However, *exo*-THC would be detected in the initial screening analysis because it is sufficiently separated from Δ^9-THC. As a result, NIST CSD has selected a 55% ACN isocratic mobile phase program, summarized as Method 5 in Table 2, as a second method to be quantitatively evaluated in the future for analyzing *Cannabis* samples when CBNA is present. Method 5 includes a second isocratic step that switches to 95% ACN after 50 min and is held for 5 min to ensure any remaining cannabinoids in the plant sample elute, such as Δ^9-THCA and CBC.

Conclusions

Six commercial LC C_{18} columns were evaluated using a column test mixture (SRM 869b) and a mixture of 11 cannabinoids routinely measured in *Cannabis* samples by most LC-based methods. SRM 869b classified the NexLeaf C_{18} column, ACE Super C_{18} column, and ACE 3 C_{18} column as containing monomeric C_{18} stationary phases. These three

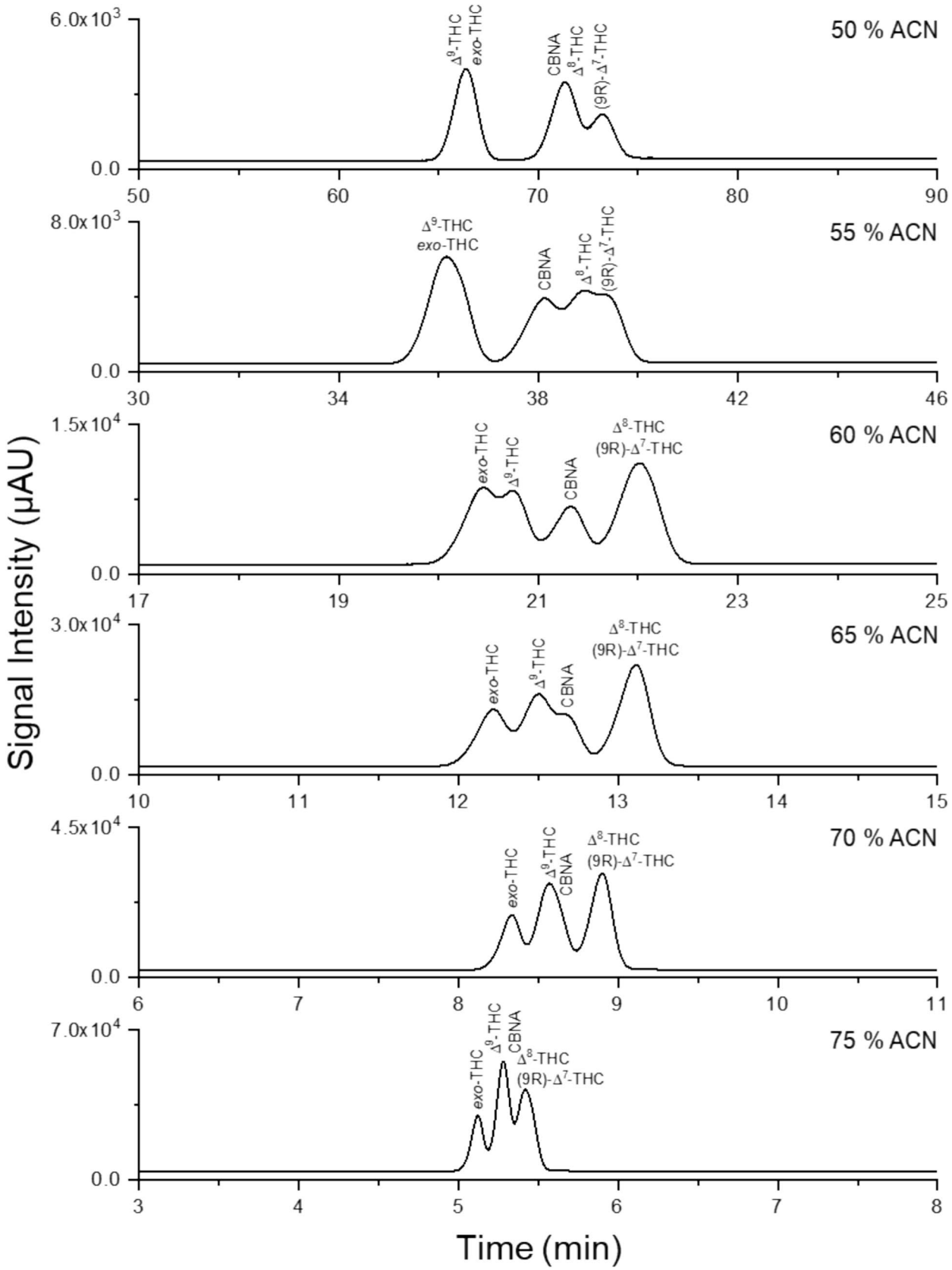

Fig. 5 LC-UV chromatograms collected at 220 nm on the NexLeaf C_{18} column for a five cannabinoid mixture using different isocratic mobile phase conditions

LC columns provided the best separation of the 11 cannabinoids using an LC-UV method previously developed by Shimadzu Instruments. Using these columns, three new mobile phase programs were developed for the baseline separation of the 11 cannabinoids in under 15 min. The organic modifier (0.085% PA), flow rate (1.6 mL/min), and column temperature (40 °C) were kept constant for all methods. An isocratic mobile phase of 72% ACN with the NexLeaf C_{18} column provided the best separation of the 11 cannabinoids, and was further evaluated for the separation of known co-eluting isomers of Δ^9-THC (CBNA and *exo*-THC), as well as closely eluting isomers (9R)-Δ^7-THC and Δ^8-THC. Δ^9-THC partially co-elutes with *exo*-THC but should be resolved enough for quantitative measurements when both are present. Δ^9-THC completely co-eluted with CBNA using this LC-UV method, but CBNA can be easily identified using the PDA detector in any *Cannabis* sample. As a result, another LC-UV method was developed using the same column for the analysis of *Cannabis* plant samples, separating Δ^9-THC and CBNA with an isocratic mobile phase containing 55% ACN. Both LC-UV methods will be further evaluated at NIST CSD in the future to enable accurate quantitative measurements of *Cannabis* plant samples and finished products.

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Data availability Data will be made available on request.

Declarations

Competing interest The authors declare no competing interests.

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