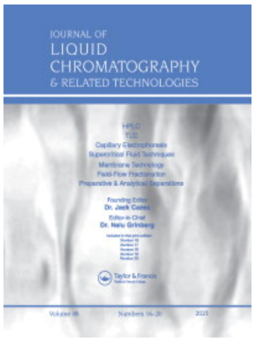

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Simultaneous quantitative analysis of epimeric and Δ^8/Δ^9 positional isomers of synthetic THC analogs and natural cannabinoids in formulated products by UHPLC-DAD with optional ESI/TOFMS confirmation

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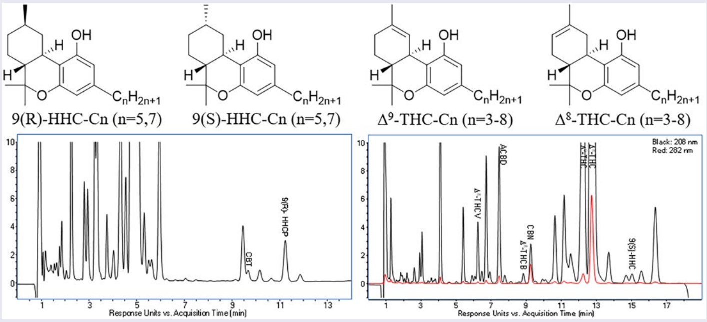
ABSTRACT

The 2018 U.S. Farm Bill legalized hemp but created regulatory gaps that contributed to the emergence of synthetic THC analogs in consumer products. An UHPLC-DAD method was developed and validated in accordance with ISO/IEC 17025 guidelines for the quantitative analysis of twenty-three neutral cannabinoids, including two epimeric pairs and six Δ^8/Δ^9 positional isomer pairs of synthetic THC analogs, six naturally occurring neutral cannabinoids, and one common synthetic THC byproduct. The method was applied to fifteen commercial products comprising four tinctures, six vaping oils, and five gummies. Fourteen cannabinoids were detected above their limits of quantification at concentrations ranging from 0.008–79.6%, with triplicate relative standard deviations of 0.8–18.3%. Analytical results revealed mislabeling in ten of the fifteen samples. Real-time recovery was evaluated using spiked abnormal CBD, a synthetic cannabinoid structurally unrelated to the target analytes, yielding recoveries of 95.0–110.3% with RSDs of 1.0–10.5% across all matrices. High-resolution ESI/TOFMS was used for optional confirmation, verifying method specificity, minimal matrix interference, and the presence of several HHC stereoisomers.

KEYWORDS
Cannabinoids; synthetic THC analogs; cannabis formulated products; UHPLC-DAD; Esi/tofms

GRAPHICAL ABSTRACT



Introduction

Cannabinoids are uniquely synthesized by the *Cannabis sativa* L. plant,[1–4] where they are initially synthesized in acidic forms and then converted to their neutral forms through heat exposure, either in the plant or after harvest. Among the most well-known neutral cannabinoids are Δ^9-tetrahydrocannabinol (Δ^9-THC) and cannabidiol (CBD). Δ^9-THC produces psychotropic effects that are mediated through its binding to CB1 receptors in the brain, which regulate memory, coordination, pleasure, and perception.[5–6] This interaction alters neurotransmitter release, especially dopamine, resulting in euphoria and sensory changes, and it affects brain areas like the hippocampus, cerebellum, and prefrontal cortex, impacting memory, motor function, and decision-making.[5–6] In contrast, CBD is non-psychotropic because it does not directly activate CB1 receptors.[7–8] Instead, it modulates these receptors indirectly and influences other systems, such as serotonin and pain pathways,[7] offering therapeutic benefits like relief from epilepsy,[7–8] anxiety, inflammation, and sleep disorders,[7] without producing a "high."[7–8]

In the USA, Δ^9-THC was classified as a Schedule I controlled substance under the Controlled Substances Act (CSA)

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of 1970 due to its psychotropic effect. Meanwhile, CBD's non-psychotropic property and reported therapeutic potential led to the 2018 Farm Bill, which legalized hemp as an agricultural commodity. According to the bill, hemp is defined as "the plant *Cannabis sativa L.* and any part of that plant, including the seeds thereof and all derivatives, extracts, cannabinoids, isomers, acids, salts, and salts of isomers, whether growing or not, with a delta-9 tetrahydrocannabinol concentration of not more than 0.3 percent on a dry weight basis." The 2018 Farm Bill represented a major turning point in cannabis regulation, opening the door for the legal cultivation of hemp and commercial distribution of hemp-derived products across the United States. However, the broad language of the 2018 Farm Bill inadvertently created a regulatory gray area. As increased hemp production led to a sharp decline in CBD prices, some manufacturers began producing and marketing synthetic THC analogs as a means to reduce surplus hemp-derived materials and recoup prior investments, while also offering products promoted as a "legal high." Synthetic THC analogs are chemical compounds that share a similar structure with Δ^9-THC, enabling them to interact with both CB1 and CB2 cannabinoid receptors. Among these analogs, Δ^8-THC has become the most prominent and widely available in the U.S. market since 2019.[9] Although it is generally acknowledged that Δ^8-THC occurs naturally in hemp, it is present only in trace concentrations, typically far below concentrations capable of producing psychotropic effects. As a result, all Δ^8-THC products available commercially are synthesized from CBD extracted from hemp.[9–11] Under acidic conditions, one of CBD's phenolic hydroxyl groups reacts with its isopropenyl group to form Δ^9-THC (Figure 1). This intermediate can then undergo protonation at the carbon-10 position, generating a tertiary carbocation at carbon-9. This reactive intermediate facilitates isomerization, yielding primarily Δ^8-THC, along with a well-known synthetic THC byproduct, i.e., $\Delta^{9,11}$-THC (also known as exo-THC).

Other synthetic THC analogs currently available in the U.S. market include Δ^9-THC homologs with side alkyl chains ranging from three to eight carbon atoms, i.e., Δ^9- tetrahydrocannabivarin (Δ^9-THCV), Δ^9- tetrahydrocannabutol (Δ^9-THCB), Δ^9- tetrahydrocannabihexol (Δ^9-THCH), Δ^9- tetrahydrocannabiphorol (Δ^9-THCP), and Δ^9-THC-octyl (Δ^9-THC-C8). Studies have shown that the length of the alkyl side chain in these compounds significantly influences their binding affinity to the CB1 receptor.[12–15] At least three carbon atoms are necessary in the side chain to achieve effective receptor binding. As the chain length increases, CB1 receptor affinity also increases, with peak activity typically observed at eight carbons to finally decrease with a higher number of carbon atoms.[12–15]

Although Δ^9-THCV, Δ^9-THCB, Δ^9-THCH, and Δ^9-THCP have been detected in hemp, their concentrations are generally too low to produce psychotropic effects, and their presence depends on the specific hemp strain.[13–16] In contrast, Δ^9-THC-C8 lacks substantial evidence of occurrence in hemp. Commercially available Δ^9-THCV is typically obtained through cyclizing cannabidivarin (CBDV), a CBD analog with a three-carbon alkyl side chain, extracted from selectively bred hemp varieties. On the other hand, Δ^9-THCB, Δ^9-THCH, Δ^9-THCP and Δ^9-THC-C8 are obtained through total organic synthesis. This process generally involves synthesizing a resorcinol derivative bearing the appropriate alkyl side chain (resorcinol-Cn, where n = 4, 6, 7, 8), followed by condensation with (1S,4R)-1-methyl-4-(prop-1-en-2-yl)cyclohex-2-enol to form the intermediate CBD-Cn. CBD-Cn is then cyclized to yield Δ^9-THC-Cn and then to Δ^8-THC-Cn. Δ^9-THC-Cn can be subsequently produced by isomerization of Δ^8-THC-Cn. Usually, the conversion of CBD-Cn to Δ^9-THC-Cn leads to a complex mixture.

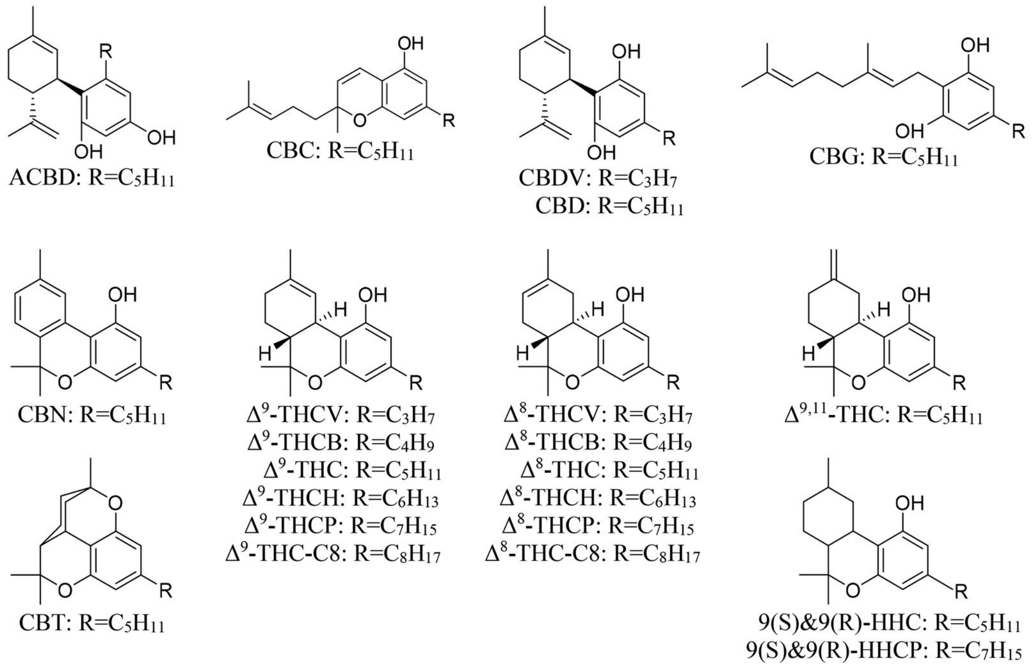

Figure 1. Chemical structure of 23 neutral cannabinoids and ACBD.

Recently, the Cannazza group reported the stereoselective synthesis of (−)-trans-Δ^9-THCB, (−)-trans-Δ^9-THCH, and (−)-trans-Δ^9-THCP in high yields.[13–15] First, resorcinol-Cn was synthesized. This intermediate was then condensed with (1S,4R)-1-methyl-4-(prop-1-en-2-yl) cyclohex-2-enol using para-toluenesulfonic acid (pTSA) as a catalyst for 48 hours to produce the corresponding (−)-trans-Δ^8-THC-Cn. Finally, hydrochlorination of the double bond of the (−)-trans-Δ^8-THC-Cn yielded (−)-trans-HCl-THC-Cn, which was subsequently converted to (−)-trans-Δ^9-THC-Cn through selective elimination at position 2 of the terpene moiety using potassium tert-amylate as base.

Additional synthetic THC analogs available on the U.S. market include 9(R)- and 9(S)-hexahydrocannabinol (HHC), which have been reported globally since 2022.[17–19] However, there is limited evidence supporting the natural occurrence of HHCs in hemp. Commercially, HHCs are typically synthesized through the hydrogenation of Δ^9-THC or Δ^8-THC.[17–18,20–22] Structurally related compounds in this class include their higher homologs, such as 9(R)- and 9(S)-hexahydrocannabiphorol (HHCP).

This study aimed to develop a comprehensive method for quantifying neutral cannabinoids in formulated products infused with synthetic THC analogs. Based on the synthetic pathways, twenty-three cannabinoids were selected for analysis (Figure 1). These included all naturally occurring neutral cannabinoids specified in the AOAC International's Standard Method Performance Requirements (SMPRs) for quantification of cannabinoids in edible chocolate[23] and concentrates,[24] i.e., cannabichromene (CBC), cannabidiol (CBD), cannabidivarin (CBDV), cannabigerol (CBG), and cannabinol (CBN), with the exception of Δ^9-THCV, Δ^9-THC, and Δ^8-THC. These three were instead categorized as synthetic THC analogs, due to their synthetic derivation from CBDV or CBD. Cannabicitran (CBT) was also included, as it frequently exceeded the SMPR's limit of quantification (LOQ).[23–26] These naturally occurring neutral cannabinoids may appear as impurities in the CBDV or CBD used for synthesis. They may also be intentionally added to products to enhance their diversity. The study placed particular emphasis on a broad range of synthetic THC analogs, including Δ^8- and Δ^9-THCV, Δ^8- and Δ^9-THCB, Δ^8- and Δ^9-THC, Δ^8- and Δ^9-THCH, Δ^8- and Δ^9-THCP, Δ^8- and Δ^9-THC-C8, 9(R)- and 9(S)-HHC, as well as 9(R)- and 9(S)-HHCP. A well-known synthetic by-product, i.e., $\Delta^{9,11}$-THC, was also included. Additionally, abnormal CBD (ACBD), a cannabinoid not naturally found in hemp and not structurally related to THC, was used as a procedural and chromatographic control to monitor extraction performance and chromatographic consistency.

Liquid chromatography (LC) and gas chromatography (GC) are both commonly employed for the quantification of neutral cannabinoids.[27–30] Among LC detection methods, diode array detection (DAD) is generally preferred over electrospray ionization tandem mass spectrometry (ESI/MS/MS) due to its greater accessibility for crime laboratories, commercial producers, and agricultural users. However, LC-DAD requires baseline separation of all cannabinoids as well as any unknown compounds present above the LOQ. At present, a method for comprehensive quantification of neutral cannabinoids in samples infused with synthetic THC analogs is still lacking[31–42].[43] To address this gap, the present study sought to develop an LC-DAD method capable of quantifying a wide range of synthetic THC analogs and naturally occurring neutral cannabinoids within formulated products.

Materials and methods

Reagents and standards

LC grade acetonitrile, methanol, water, and MS grade formic acid were purchased from Fisher Scientific (Pittsburgh, PA, USA). All cannabinoid standards were purchased from Cayman Chemical (Ann Arbor, MI, USA). These included the Phytocannabinoid Neutrals Mixture 8, which contained CBC, CBD, CBDV, CBG, CBN, Δ^9-THC, Δ^8-THC, and Δ^9-THCV, each at a concentration of 1 mg/mL in acetonitrile. Twelve standards at 1 mg/mL in acetonitrile included Δ^9-THCB, Δ^8-THCB, Δ^9-THCH, Δ^8-THCH, Δ^9-THCP, Δ^8-THCP, Δ^9-THC-C8, Δ^8-THC-C8, 9(R)-HHC, 9(S)-HHC, 9(R)-HHCP, and 9(S)-HHCP. Three standards at 1 mg/mL in methanol included CBT, $\Delta^{9,11}$-THC, and Δ^8-THCV. Therefore, sixteen cannabinoid standard solutions were used, each at an individual cannabinoid concentration of 1 mg/mL. Additionally, abnormal CBD (ACBD) was acquired at 25 mg/mL in methyl acetate.

Calibration solutions and quality control samples

A mixture solution containing twenty-three neutral cannabinoids was initially prepared in methanol at a maximum individual concentration of 50 µg/mL by combining 50 µL of each of the sixteen 1 mg/mL standard solutions with 200 µL of methanol. This stock solution was then serially diluted in methanol to produce eight additional mixture solutions with individual cannabinoid concentrations of 25, 10, 5, 2, 1, 0.4, 0.2, and 0.08 µg/mL. Separately, an ACBD solution was prepared in methanol at 2 µg/mL. Each cannabinoid mixture was subsequently mixed 1:1 (v/v) with the ACBD solution, yielding nine final calibration solutions containing 1 µg/mL ACBD and individual cannabinoid concentrations of 25, 12.5, 5, 2.5, 1, 0.5, 0.2, 0.1, and 0.04 µg/mL. The original 50 µg/mL cannabinoid mixture (without ACBD) was also used in external calibration to extend the range up to 50 µg/mL. Quality control (QC) samples were prepared similarly, each containing either 50, 1, or 0.04 µg/mL of each cannabinoid.

ACBD was included in the calibration and QC solutions solely to provide a reference for calculating relative retention times to support compound identification and to monitor chromatographic consistency; it was not used as an internal standard for quantitative correction or calibration.

Samples

Two gummy samples, HHC Gummies (G1) and Kyle Kush Gummies – Δ8 + HHC+THCP (G2), were purchased from

3Chi (Carmel, IN, USA). Four tincture samples, HHC-P Tincture – 1000 mg (T1), THC-P Tincture – 1000 mg (T2), THC-H Tincture – 1000 mg (T3), and THC-B Tincture – 1000 mg (T4), along with five vaping oil samples, HHC-P Vape Cartridge – Flavor: Ghost Rider (V1), THC-P Vape Cartridge – Flavor: Fruit Loops (V2), THC-P Live Resin Vape Cartridge – Pura Vida (V3), THC-H Vape Cartridge – Flavor: Death Star (V4), and THC-B Vape Cartridge – Flavor: Cloud Nine (V5), were purchased from Binoid (Northridge, CA, USA). Three gummy samples, Beast Mode Blend Gummies (G3), Knockout Blend Gummies (G4), and Power 9 Blend Gummies (G5), were also purchased from Binoid. One vaping oil sample, iDELTΔ Premium Diamond – THCP Cartridge Full Gram – Flavor: Skywalker (V6), was purchased from iDELT8 (Phoenix, AZ, USA).

Sample identifiers were assigned as follows: G = gummies, T = tinctures, and V = vaping oils. Company names and product labeling are reported to enable comparison of labeling claims with analytically determined cannabinoid composition and to assess differences in mislabeling patterns among commercially available products.

Sample preparation of tinctures

For homogenization, a tincture sample was ultrasonicated for 10 min using a Branson ultrasonic cleaner (Brookfield, CT, USA). To determine the sample's density (mg/µL), 1000 µL of tincture was transferred to a 2 mL centrifuge tube, and its exact mass was recorded. To obtain 100 mg of sample, the required volume was calculated based on the measured density. To prepare 4 mL of a 25 mg/mL mixture, the methanol volume was calculated by subtracting the tincture volume from 2000 µL. This methanol volume and 2000 µL of a 75 µg/mL ACBD solution in methanol were added to a 15 mL centrifuge tube, followed by the required volume of tincture. The exact tincture weight was recorded and its exact concentration in the final mixture was calculated. Since the final mixture contained 37.5 µg/mL ACBD and approximately 25 mg/mL of sample in methanol, an ACBD spiking concentration of approximately 0.15% w/w could be calculated.

The mixture was vortexed for 15 seconds, then sonicated for 5 minutes. This process was repeated three more times. Following extraction, the sample was centrifuged at 13,000 rpm (equivalent to 15.8 g) for 10 minutes using an Eppendorf centrifuge (Hamburg, Germany). The supernatant was filtered through a 0.2 µm PTFE syringe filter (Foxx Life Sciences, Salem, NH, USA). The filtrate was diluted 50-fold with methanol, resulting in a final sample solution containing 0.75 µg/mL ACBD and 500 µg/mL of the sample.

Separately, the 75 µg/mL ACBD stock solution was diluted 100-fold with methanol to prepare a 0.75 µg/mL ACBD reference solution. Both the diluted sample and reference solutions were analyzed using LC-DAD. Recovery at the 0.15% w/w spiking concentration was determined by comparing the ACBD peak area in the spiked sample with that of the reference solution. Individual cannabinoid concentrations were quantified using calibration curves prepared from standards ranging from 0.04 to 50 µg/mL, corresponding to a quantification range of 0.008% to 10% w/w in the original sample.

Sample preparation of vaping oils

To recover vaping oil from a vaping cartridge, its exterior was cleaned with methanol, and both end covers were removed and discarded. About 20 mL of methanol was added to a pre-weighed 50 mL centrifuge tube, and the weight of the methanol was recorded. The cartridge was then added to the tube, and the total weight was measured. The mixture was sonicated for a couple of hours to extract the vaping oil. The resulting solution was transferred as completely as possible to another 50 mL centrifuge tube. The empty cartridge was rinsed with methanol, dried, and weighed to determine the amount of vaping oil recovered for concentration (w/w) calculation.

To obtain 10 mg of vaping oil, the required volume of its methanol solution was calculated using the oil's concentration (w/w) and the density of methanol (0.791 mg/µL). To prepare 4 mL of a 2.5 mg/mL vaping oil solution, the required volume of methanol was determined by subtracting the volume of the vaping oil solution from 2000 µL. This volume of methanol and 2000 µL of 75 µg/mL ACBD in methanol were added to a 15 mL centrifuge tube, followed by the required volume of vaping oil solution. The exact weight of vaping oil solution was recorded and its exact concentration in the final solution was calculated.

The sample was subjected to the same ultrasonication, centrifugation, and filtration procedures as described for the tinctures. The resulting filtrate was diluted 50-fold with methanol, yielding a final solution containing 0.75 µg/mL ACBD and 50 µg/mL of sample. Separately, the 75 µg/mL ACBD stock solution was diluted 100-fold to prepare a 0.75 µg/mL reference solution.

Sample preparation of gummies

For homogenization, five to ten gummies were frozen and then ground into small pieces using a Waring laboratory blender (Torrington, CT, USA). Care must be taken to avoid over-grinding, as excessive processing can cause the gummies to become sticky and difficult to remove. A uniform dispersion of the sample at a concentration of 250 mg/mL in water was prepared by weighing 1 g of the ground sample into a 7 mL SPEX Sample Prep tube (Metuchen, NJ, USA) containing two ¼-inch stainless steel balls. LC grade water was added to the tube, and the mixture was homogenized at 3000 rpm for 5 min using a SPEX Genolyte 1200 homogenizer.

For cannabinoid extraction, 1800 µL of methanol was added to a 15 mL centrifuge tube and sonicated. This was followed by the addition of 2000 µL of a 75 µg/mL ACBD solution in methanol and 200 µL of the prepared water dispersion (250 mg/mL), resulting in a final extraction mixture containing 37.5 µg/mL ACBD and 12.5 mg/mL of the sample in a 95:5 methanol/water solvent. The sample was subjected to the same ultrasonication, centrifugation, and filtration procedures as described for the tinctures. The resulting filtrate was diluted 25-fold with methanol, resulting in a final sample solution containing 1.5 µg/mL ACBD and 500 µg/mL of the sample.

Separately, the 75 µg/mL ACBD stock solution was diluted 50-fold with methanol to prepare a 1.5 µg/mL ACBD reference solution.

LC-DAD

LC-DAD was performed using an Agilent 1260 Infinity II LC system (Agilent Technologies, Santa Clara, CA, USA), equipped with a solvent degasser, binary pump, temperature-controlled autosampler, column oven, and DAD.

Separation was achieved using an Agilent Poroshell 120 EC-C18 column (150 mm × 2.1 mm, 1.9 µm particle size), protected by a 0.2 µm ultra-high pressure (UHP) precolumn filter (IDEX Health & Science, Oak Harbor, WA, USA). The column was maintained at 30 °C in the oven, while the autosampler was kept at 8 °C. The injection volume was 4 µL.

Based on separation optimization studies (Section "Separation optimization"), two complementary UHPLC-DAD separation conditions were employed for quantitative analysis. Quantification of nine neutral cannabinoids with stronger retention on the column was performed using an isocratic mobile phase consisting of 80% (v/v) acetonitrile and 20% (v/v) aqueous 0.02% (v/v) formic acid, at a flow rate of 0.4 mL/min over a 14-min run. Subsequently, fourteen neutral cannabinoids with weaker retention on the column were quantified using a step gradient elution: starting with 68% (v/v) acetonitrile and 32% (v/v) aqueous 0.02% (v/v) formic acid at 0.4 mL/min for 16 min, followed by 98% (v/v) acetonitrile and 2% (v/v) aqueous 0.02% (v/v) formic acid at 0.4 mL/min from 16.01 to 19.00 min. A 7-min post-run re-equilibration at 68% acetonitrile and 32% (v/v) aqueous 0.02% (v/v) formic acid at 0.4 mL/min was applied before the next injection.

UV absorption spectra for the 23 neutral cannabinoids and ACBD were recorded from 190.0 to 400.0 nm with a spectral resolution of 2.0 nm. Most neutral cannabinoids, including CBDV, CBG, CBD, all THC homologs (Δ^9-THCV, Δ^8-THCV, Δ^9-THCB, Δ^8-THCB, Δ^9-THC, Δ^8-THC, Δ^9-THCH, Δ^8-THCH, Δ^9-THCP, Δ^8-THCP, Δ^9-THC-C8, and Δ^8-THC-C8), ACBD, $\Delta^{9,11}$-THC, all HHC homologs [9(S)-HHC, 9(R)-HHC, 9(S)-HHCP, and 9(R)-HHCP], and CBT, exhibited similar UV absorption spectra, each displaying two peaks around 208 and 228 nm. In contrast, CBN showed peaks at approximately 221 and 282 nm, while CBC exhibited peaks near 228 and 282 nm. Based on these observations, UV detection was conducted at multiple wavelengths (208, 221, 228, and 282 nm), each with a 4 nm bandwidth. A reference wavelength of 360 nm (100 nm bandwidth) was used. During method validation, both UV peaks of each cannabinoid were evaluated for quantification. Ultimately, the shorter-wavelength peak was selected for quantification due to its slightly superior signal-to-noise ratio.

Blank solvent injections were performed between sample analyses to assess potential carryover and contamination, and no peaks corresponding to target cannabinoids were observed under the conditions used.

ESI/TOFMS

ESI/TOFMS was conducted using an Agilent 6545 quadrupole time-of-flight (Q-TOF) mass spectrometer equipped with a Dual Agilent Jet Stream (AJS) ESI source operating in positive ion mode. Instrument parameters were optimized for Δ^9-THC using flow injection analysis of a 1 µg/mL solution in methanol delivered at a flow rate of 0.4 mL/min. Under these conditions, all neutral cannabinoids were detected as $[M+H]^+$ ions.

The optimized mass spectrometry conditions included an MS acquisition range of 100 to 1000 *m/z* and a scan rate of 5 spectra per second. The drying gas temperature was set to 325 °C with a flow rate of 10 L/min, while the sheath gas was maintained at 400 °C with a flow rate of 12 L/min. The nebulizer pressure was 20 psi. The capillary voltage was set to 3000 V, and the nozzle voltage was 600 V. Additional parameters included a fragmentor voltage of 120 V, a skimmer voltage of 45 V, and an Oct1 RF voltage (Vpp) of 750 V. Mass calibration was maintained using reference ions at m/z 121.0509 and 922.0098.

Results and discussion

Separation optimization

Chromatographic separation was systematically optimized to achieve baseline resolution of structurally similar neutral cannabinoids, including homologs and positional isomers. Four columns with identical dimensions (150 mm × 2.1 mm, 2.7 µm particle size) but different stationary-phase chemistries were initially evaluated: Poroshell 120 EC-C18, Raptor ARC-18, Cortecs Shield RP-18, and Ascentis Express RP-Amide [41]. Among these, the Poroshell 120 EC-C18 column provided superior resolution of critical $\Delta^{9,11}$-/Δ^9-/Δ^8-THC isomer pairs and was therefore selected for further method development. To further improve separation performance, a column with a smaller particle size (1.9 µm) was selected.

Initial optimization employed isocratic elution with 80% (v/v) acetonitrile and 20% (v/v) aqueous 0.02% (v/v) formic acid at a flow rate of 0.4 mL/min. Under these conditions, nine late-eluting neutral cannabinoids were baseline-resolved ($R \geq 1.5$) within 14 min. Figure 2a shows the UHPLC-UV chromatogram at 208 nm, and Figure 2b presents the corresponding UHPLC-ESI/TOFMS extracted-ion chromatograms (EICs). Resolution between Δ^9- and Δ^8-isomers followed the trend Δ^9-/Δ^8-THC-C8 > Δ^9-/Δ^8-THCP > Δ^9-/Δ^8-THCH > Δ^9-/Δ^8-THC > Δ^9-/Δ^8-THCV. Under these conditions, Δ^8-THCB coeluted with CBN, and 9(R)-HHC coeluted with CBC.

Reducing the acetonitrile content to 75% (v/v) improved resolution between Δ^9- and Δ^8-isomeric pairs (Supplementary Fig. S1), but resulted in longer retention times and the emergence of new coelution behavior, including partial overlap between Δ^8-THCP and CBT. These observations are consistent with previously reported selectivity shifts for CBT under varying acetonitrile contents.[41]

Further reduction of acetonitrile to 68% (v/v) enabled baseline resolution ($R \geq 1.5$) of fourteen early-eluting neutral

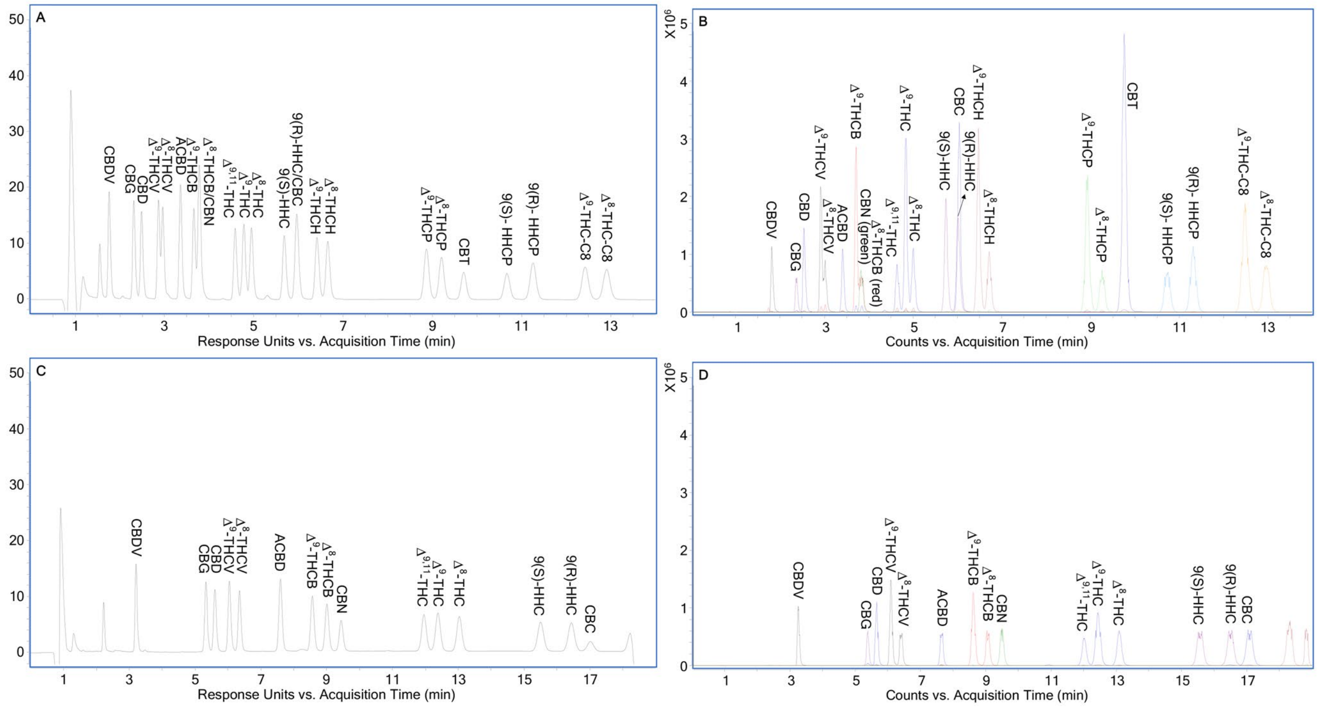

Figure 2. Optimized UHPLC separation of the 23 neutral cannabinoids and ACBD. Each cannabinoid was prepared at an individual concentration of 1 µg/mL, and 4 µL was injected per run. The mobile phase was delivered at a flow rate of 0.4 mL/min. Solvent A consisted of 0.02% (v/v) formic acid in water, and solvent B was acetonitrile. (A and B) The mobile phase contained 80.0% (v/v) B. (C and D) The mobile phase contained 68% (v/v) B from 0 to 16 min, 98% (v/v) B from 16.01 to 19 min, and 68% (v/v) acetonitrile from 19.01 to 26 min. (A and C) UHPLC-UV chromatogram at 208 nm. (B and D) Corresponding UHPLC-ESI/TOFMS EICs for compounds shown in Figures 2a and 2c using $[M+H]^+$ ions with a ± 20 ppm mass tolerance.

cannabinoids, including multiple critical isomeric pairs (Figure 2c and 2d). Under these conditions, however, retention times for the remaining nine late-eluting cannabinoids became excessively long, necessitating a step increase in acetonitrile content to 98% (v/v) to elute these compounds efficiently.

Collectively, these results demonstrated that no single chromatographic condition could provide adequate resolution for all 23 neutral cannabinoids within a practical run time. The target cannabinoids span a wide range of hydrophobicity and include multiple epimeric and positional isomers with very similar chromatographic retention. A single gradient program could not achieve adequate resolution for all compounds without substantially extending run time and introducing UV baseline drift, which adversely affects sensitivity and LOQs at low UV wavelengths.

Accordingly, two complementary UHPLC-DAD separations were selected. The first employed an isocratic elution optimized for nine later-eluting cannabinoids with stronger column retention (Figure 2a and 2b), all of which were baseline-resolved ($R \geq 1.5$) and quantitatively evaluated. Under these conditions, some of the remaining fourteen cannabinoids with weaker retention may exhibit coelution; however, these compounds were not targeted for quantification in this separation. The second separation was optimized specifically for these fourteen weaker-retention cannabinoids using isocratic elution at 68% acetonitrile, under which all fourteen target analytes were baseline-resolved ($R \geq 1.5$), followed by a step to 98% acetonitrile to elute later-retained compounds (Figure 2c and 2d).

Method validation

Method validation was based on the two UHPLC separations shown in Figures 2a and 2C. All synthetic THC analogs-infused products would be first analyzed using a 14-min analysis with 80% (v/v) acetonitrile in the mobile phase (Figure 2a) to quantify nine neutral cannabinoids with stronger column retention: Δ^9-THCH, Δ^8-THCH, Δ^9-THCP, Δ^8-THCP, CBT, 9(S)-HHCP, 9(R)-HHCP, Δ^9-THC-C8, and Δ^8-THC-C8. This was followed by a 26-min step-gradient analysis, starting with 68% (v/v) acetonitrile at 0.4 mL/min for 16 min, increasing to 98% from 16.01 to 19 min, and returning to 68% from 19.01 to 26 min (Figure 2c), to quantify the remaining fourteen neutral cannabinoids with weaker column retention: CBDV, CBG, CBD, Δ^9-THCV, Δ^8-THCV, Δ^9-THCB, Δ^8-THCB, CBN, $\Delta^{9,11}$-THC, Δ^9-THC, Δ^8-THC, 9(S)-HHC, 9(R)-HHC, and CBC.

Method validation was performed three times per day across three separate days. External standard calibration was selected over internal standard calibration due to its simplicity and strong performance. Linear calibration curves were established over the range of 0.04 to 50 µg/mL by plotting the peak area of each cannabinoid against its concentration, using a $1/x^2$ weighting factor. The lowest R^2 value among all calibration curves was 0.9891, as shown in Supplementary Table S1.

To evaluate accuracy and precision, QC samples were analyzed at three concentrations: 50, 1, and 0.04 µg/mL. The results met ISO 17025 standards, with intraday and interday accuracy and precision for low-concentration QCs falling within 80–120% and a relative standard deviation (RSD) below 20%, respectively, and within 85–115% and an RSD below 15%, respectively, for medium and high concentrations (Supplementary Tables S2 and S3).

Figures 3a and 3c show UHPLC-UV chromatograms of the 23 neutral cannabinoids at 0.04 μg/mL and ACBD at 1 μg/mL, recorded at 208, 221, 228, and 282 nm. The corresponding UHPLC-ESI/TOFMS EICs for the same compounds are shown in Figures 3b and 3d. In Figure 3c, significant baseline drift was observed at 208, 221, and 228 nm due to the change of mobile phase from 68% to 98% (v/v) acetonitrile, whereas no such drift occurred at 282 nm. Although gradient elution can accommodate the broad retention range of the analytes, it caused UV baseline drift at shorter wavelengths (208, 221, and 228 nm) where most neutral cannabinoids absorb. Therefore, isocratic elution was preferred for LC-UV quantification of neutral cannabinoids to minimize baseline drift and improve the LOQ. The LOQ for all cannabinoids was set at 0.04 μg/mL, although lower LOQ were achievable for many neutral cannabinoids (Figures 3a and 3c).

Sample preparation recovery was monitored in real time by spiking each sample with ACBD. For the tincture samples spiked with 0.15% w/w ACBD, average recoveries in triplicate measurements were 104.9% (T1), 104.2% (T2), 104.5% (T3), and 103.3% (T4), with RSDs of 3.9%, 1.5%, 2.1%, and 2.2%, respectively. For the vaping oil samples spiked with 1.5% w/w ACBD, average recoveries in triplicate measurements were 99.6% (V1), 109.3% (V2), 102.2% (V3), 110.3% (V4), 102.6% (V5), and 107.1% (V6), with RSDs of 7.5%, 4.8%, 10.2%, 4.4%, 10.5%, and 3.0%, respectively. For the gummy samples spiked with 0.3% w/w ACBD, average recoveries in triplicate measurements were 96.4% (G1), 96.8% (G2), 96.8% (G3), 96.6% (G4), and 95.0% (G5), with RSDs of 6.9%, 1.0%, 2.0%, 3.4%, and 2.3%, respectively.

Sample analysis

A total of four tincture, six vaping oil, and five gummy samples that were labeled as infused with synthetic THC analogs were analyzed in triplicates by the validated UHPLC-DAD method. The contents of cannabinoids are shown in Table 1. Fourteen out of 23 neutral cannabinoids, were detected, with average contents of triplicate measurements ranging from 0.008 to 79.6% w/w. The RSD values of the triplicates ranged from 0.8 to 18.3%, as shown also in Table 1.

All tincture samples were obtained from Binoid and labeled as HHCP (T1), THCP (T2), THCH (T3), and THCB (T4), respectively. The predominant cannabinoid was Δ^8-THC, with an average concentration of 2.45% w/w and 6.1% RSD. 9(R)-HHCP was quantified in all samples at an average concentration of 0.037% w/w and 13.3% RSD. Notably, no THCP, THCH, or THCB was detected above the 0.008% w/w LOQ in any of the respective labeled samples. Overall, despite being marketed under different cannabinoid labels, the four samples shared nearly identical cannabinoid profiles, indicating mislabeling of three out of four products and suggesting a common formulation was used across all samples.

Five of the six vaping oil samples were obtained from Binoid and labeled as HHCP (V1), THCP (V2), THCP Live Resin (V3), THCH (V4), and THCB (V5). The predominant cannabinoid in all five samples was Δ^8-THC, with an average concentration of 73.6% w/w and 5.1% RSD. 9(R)-HHCP

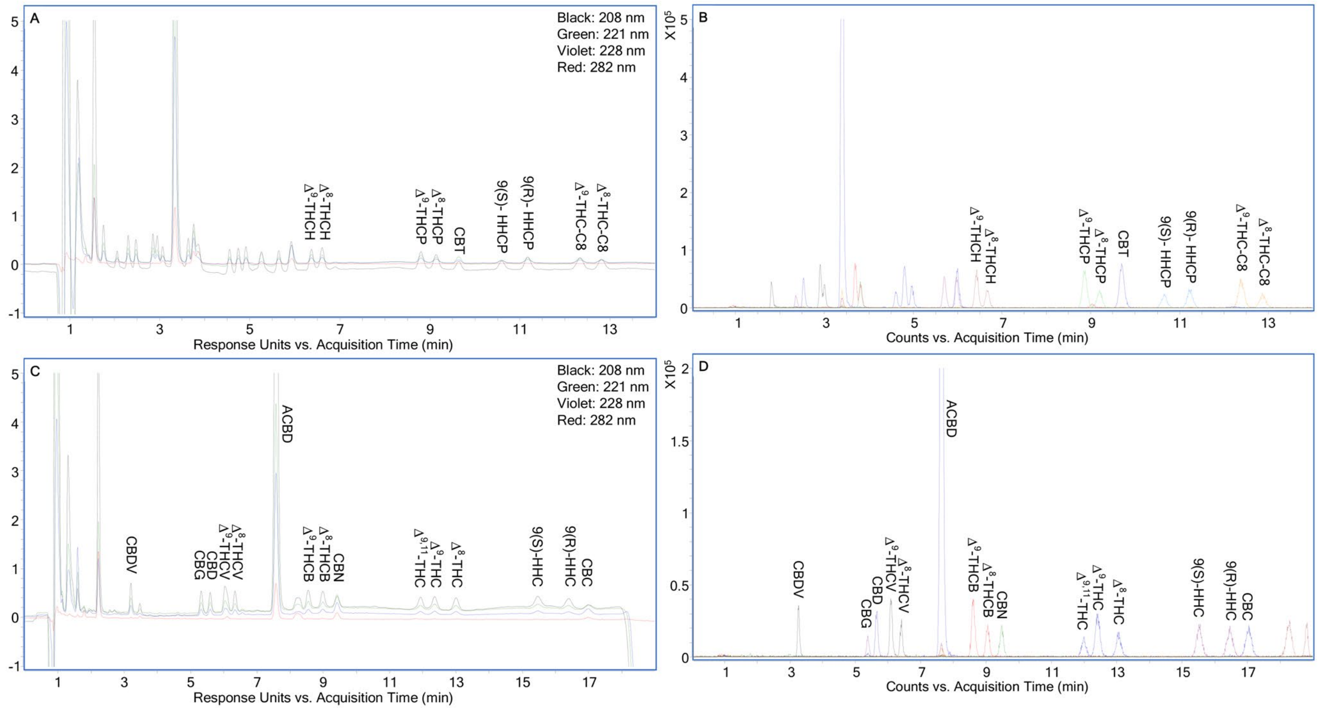

Figure 3. UHPLC separation of the 23 neutral cannabinoids at LOQ level (0.04 μg/mL) and ACBD at 1 μg/mL. (A and C) UHPLC-UV chromatograms using the separation conditions from Figures 2a and 2C, respectively. (B and D) Corresponding UHPLC-ESI/TOFMS EICs for compounds shown in Figures 3a and 3C using $[M+H]^+$ ions with a ± 20 ppm mass tolerance.

Table 1. Average content (%, w/w) and RSD values (%) of neutral cannabinoids in Δ^9-THC analog–formulated products measured in triplicate.

Sample	CBG		CBD		D^8-THCV		D^8-THCB		CBN		$D^{9,11}$-THC		D^9-THC	
T1					0.033	3.9			0.025	3.3	0.025	4.2	0.36	3.2
T2					0.024	2.2			0.024	2.0			0.26	1.4
T3					0.027	3.3			0.024	8.4	0.027	3.0	0.39	3.1
T4					0.056	6.9			0.024	7.3	0.032	7.3	0.40	7.4
V1					0.70	3.1	0.16	9.0	0.76	6.7			7.8	7.0
V2					0.40	11.8	0.17	12.5	0.75	11.3			8.8	11.5
V3					0.45	2.4	0.17	1.6	0.76	1.7			9.9	0.8
V4					1.1	3.4	0.25	1.7	0.56	2.9			6.5	3.0
V5					1.2	10.8	0.19	13.1	1.1	11.9			9.3	11.4
V6			2.3	3.7	0.40	1.4	0.15	13.5	0.43	3.0	0.79	4.0	7.4	3.7
G1														
G2													0.010	2.5
G3	0.078	3.2	0.16	3.1					0.11	2.8			0.060	3.4
G4					0.011	1.7			0.016	1.0			0.12	2.3
G5					0.014	3.8			0.012	7.1			0.087	3.7

Sample	D^8-THC		9(S)-HHC		9(R)-HHC		D^9-THCP		CBT		9(S)-HHCP		9(R)-HHCP	
T1	2.6	3.2											0.034	5.3
T2	2.3	1.2	0.009	14.5									0.033	3.7
T3	2.4	3.2	0.026	8.8					0.010	7.9			0.044	0.9
T4	2.6	7.2	0.008	2.6									0.036	7.2
V1	69.9	6.9	0.24	3.4					0.20	6.5			0.74	6.9
V2	72.2	11.4	0.13	18.3					0.15	5.3	1.5	5.7	0.63	5.0
V3	74.6	1.6	0.11	1.2					0.19	5.0				
V4	79.6	3.1	0.14	2.6					0.15	11.2	0.14	3.2	0.60	5.5
V5	71.7	11.4	0.24	12.8					0.20	13.9	0.16	13.2	0.75	7.0
V6	60.5	3.7	0.16	5.5	0.22	5.7	1.3	8.2	0.66	6.7				
G1			0.13	6.4	0.23	5.9								
G2	0.46	3.0	0.13	3.0	0.30	1.9								
G3	0.46	3.9	0.05	10.7	0.13	4.2	0.014	3.3						
G4	1.1	1.8												
G5	1.2	6.2												

Average content and RSD are shown to the left and right of each cannabinoid column, respectively. Product types are identified as gummies (G), tinctures (T), and vaping oils (V); sample identities are described in Section 2.3.

was quantified in four samples (V1, V2, V4, and V5) at an average concentration of 0.68% w/w and 11.0% RSD. 9(S)-HHCP was also detected in these four samples: its concentration was slightly below the 0.08% w/w LOQ in one sample (V1), slightly above the LOQ in two samples (V4 and V5), and substantially above the LOQ in one sample (V2). Neither 9(R)-HHCP nor 9(S)-HHCP were detected in the THCP Live Resin sample (V3). No quantifiable THCP or THCH was detected in any of the samples labeled as such. Conversely, Δ^8-THCB was quantified in all five samples, averaging 0.19% w/w with a 19.0% RSD. In conclusion, despite the distinct product labels, at least three of the five samples were mislabeled (V2, V3, and V4). Furthermore, three of the five products (V1, V4, and V5) appeared to share a uniform formulation, due to highly similar cannabinoid profiles.

One of the six vaping oil samples was obtained from iDELT8 and labeled as THCP (V6). This sample was characterized by a predominant concentration of Δ^8-THC at 60.5% w/w and a substantial amount of Δ^9-THCP at 1.3% w/w, indicating that it was consistent with its labeling. Additionally, low but quantifiable concentration of Δ^8-THCB was also present, suggesting that the Δ^8-THCB found in the five Binoid vaping oil samples was unlikely an intentionally formulated component.

Two gummy samples were obtained from 3Chi and labeled as HHC (G1) and D8 + HHC+THCP (G2). In the HHC (G1) sample, both 9(S)- and 9(R)-HHC were quantified at concentrations of 0.13% and 0.23% w/w, respectively. In the D8 + HHC+THCP (G2) sample, Δ^8-THC, 9(S)-HHC and 9(R)-HHC were detected at 0.46%, 0.13%, and 0.30% w/w, respectively. The absence of THCP above the 0.008% w/w LOQ indicated mislabeling of the G2 product.

Three gummy samples were obtained from Binoid and labeled as THCB+THCH+HHCP (G3), THCP+THCH+HHCP (G4), and Delta9 + THCJD+THCB (G5), with THCJD commonly referring to THC-C8 in the marketplace. The THCP+THCH+HHCP (G4) and Delta9 + THCJD+THCB (G5) samples appeared to share a uniform formulation based on their nearly identical cannabinoid profiles, characterized by predominant Δ^8-THC at 1.09% and 1.19% w/w, respectively, and notable Δ^9-THC levels at 0.12% and 0.087% w/w. No other synthetic THC analogs, including HHCP, THC-C8, THCP, THCH, or THCB, were detected above the 0.008% w/w LOQ in either sample. In contrast, the THCB+THCH+HHCP (G3) sample contained 0.46% w/w Δ^8-THC and 0.060% w/w Δ^9-THC, along with additional synthetic THC analogs: 0.052% w/w 9(S)-HHC, 0.13% w/w 9(R)-HHC, and 0.014% w/w Δ^9-THCP. However, it lacked quantifiable concentrations of HHCP, THCH, and THCB as listed on the label. Collectively, the data indicate that all three products were mislabeled.

It is worth noting that low but quantifiable concentrations of Δ^8-THCV were detected in all but three gummies (G1, G2, and G3). Similarly, 9(S)-HHC was present at low concentrations in all but one tincture (T1) and two gummies (G4 and G5). Quantifiable concentrations of 9(R)-HHC were found in one vaping oil (V6) and three gummies (G1, G2, and G3).

Supplementary Table S4 summarizes the comparison between product labeling and analytically determined cannabinoid composition for all samples analyzed. Mislabeling was defined as the absence of the labeled THC analog at quantifiable levels, whereas products in which the labeled analog was detected were not classified as mislabeled, regardless of whether it was the predominant constituent. Based on this criterion, ten of the fifteen products were classified as mislabeled: nine of twelve products from Binoid and one of two products from 3Chi, while the single product from IDELT8 was not mislabeled. In addition, several products within the same formulation category exhibited highly similar cannabinoid profiles despite different product labels, suggesting the use of common base formulations. Collectively, these results highlight substantial inconsistencies between product labeling and chemical composition across product types.

Assessment of method specificity using ESI/TOFMS

Method specificity was assessed using ESI/TOFMS following UV detection. However, it is important to recognize that the relative signal intensities observed by ESI/TOFMS and UV can differ substantially. As a result, interference affecting UV-based quantification cannot be reliably evaluated using ESI/TOFMS data alone. For instance, UV detection of CBN at 282 nm demonstrated high selectivity, which may not be apparent from the corresponding mass spectrometric data.

Figure 4 and Supplementary Figs. S2 to S4 show the analysis of the four tincture samples (T1, T2, T3, and T4, respectively), each prepared at 500 µg/mL of product and spiked with 0.75 µg/mL ACBD. Supplementary Figs. S5 to S9 and Figure 5 present the analysis of the six vaping oil samples (V1, V2, V3, V4, V5, and V6, respectively) at 50 µg/mL of product, also spiked with 0.75 µg/mL ACBD. Supplementary Figs. S10 and S11, Figure 6, and Supplementary Figs. S12 and S13 show the analysis of the five gummy samples (G1, G2, G3, G4, and G5, respectively), prepared at 500 µg/mL of product and spiked with 1.5 µg/mL ACBD. In each figure, **subfigures A and C** display UHPLC-UV chromatograms using the separation conditions described in Figures 2a and 2c, respectively. **Subfigures B and D** show the corresponding UHPLC-ESI/TOFMS EICs for the compounds shown in **A and C**, using $[M+H]^+$ ions with a ± 20 ppm mass tolerance.

All samples were characterized by predominant Δ^8-THC content, though the concentrations varied by product type. For gummy samples, Δ^8-THC concentrations reached approximately 0.5–1.0% w/w (corresponding to 5–10% of the validated upper calibration limit of 10% w/w), while tincture samples reached approximately 2.5% w/w (~25% of the upper calibration limit of 10% w/w). In contrast, vaping oil samples reached approximately 50% w/w (~50% of the upper calibration limit of 100% w/w). As a result, the UHPLC-UV chromatograms of the gummy samples showed fewer unknown peaks that did not interfere with cannabinoid quantification. Tincture and vaping oil samples exhibited more unknown peaks; however, these caused minimal interference with the quantification of target cannabinoids. In addition, the UHPLC-UV chromatograms of the nine neutral cannabinoids, which exhibited stronger column retention, showed fewer unknown peaks that did not interfere with the quantification of target synthetic THC analogs, due to their lower concentrations relative to their respective upper calibration limits. Furthermore, most of the unknown peaks observed were identified as structural isomers of

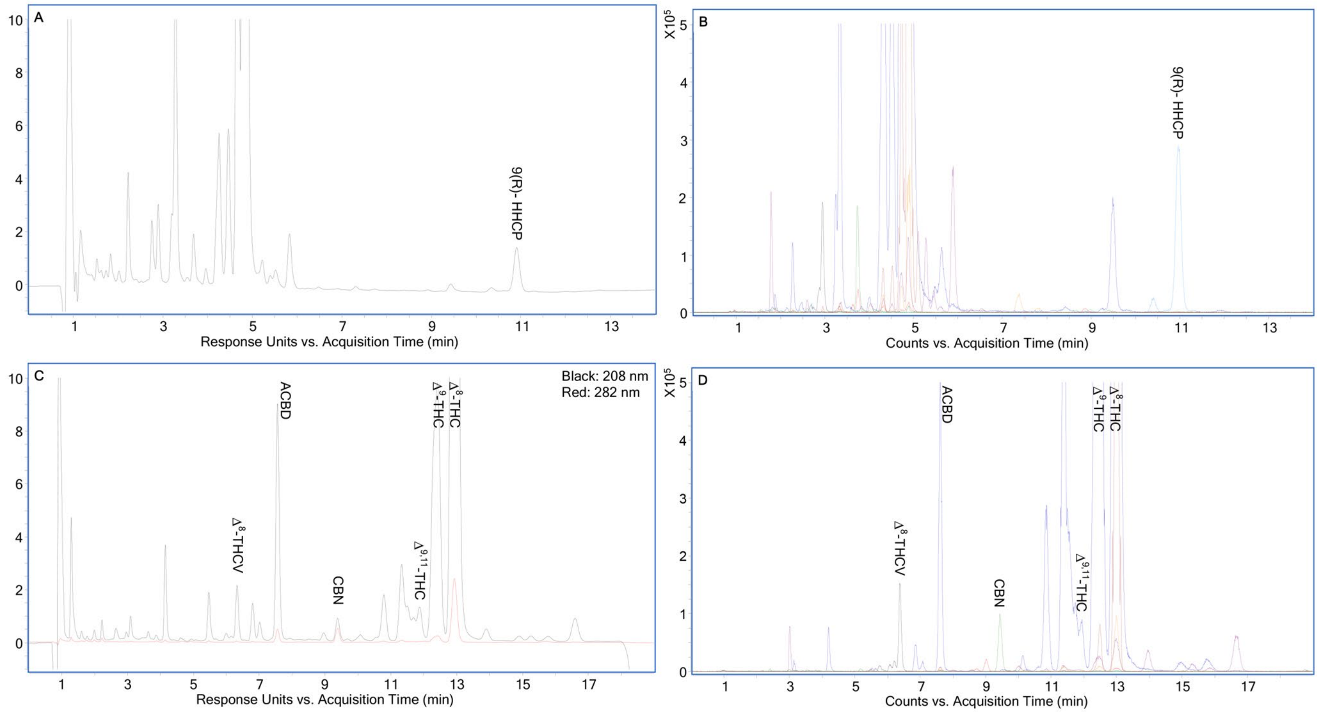

Figure 4. Analysis of 500 µg/mL HHC-P Tincture – 1000 mg (T1) from Binoid spiked with 0.75 µg/mL ACBD. (A and C) UHPLC-UV chromatograms using the separation conditions from Figures 2a and 2C, respectively. (B and D) Corresponding UHPLC-ESI/TOFMS EICs for compounds shown in Figures 4a and 4C using $[M+H]^+$ ions with a ± 20 ppm mass tolerance.

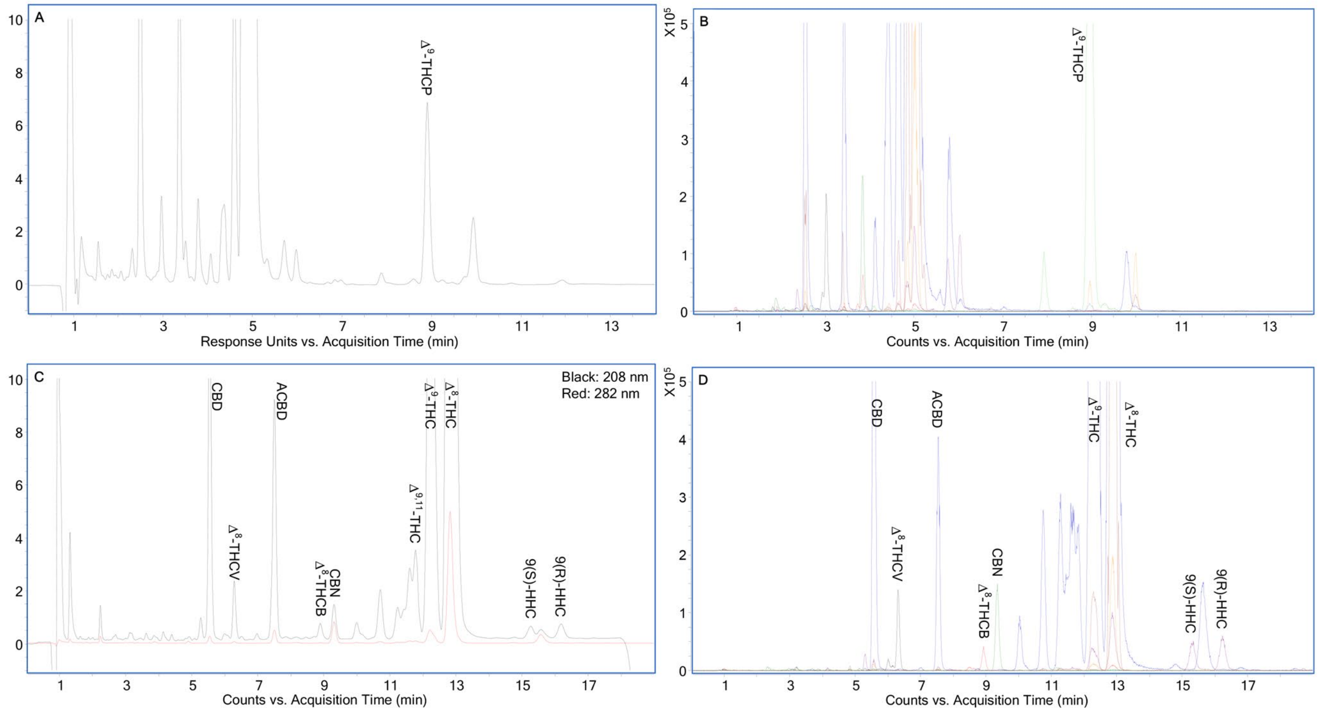

Figure 5. Analysis of 50 µg/mL iDELTΔ Premium Diamond – THCP Cartridge Full Gram – Flavor: Skywalker (V6) from iDELT8 spiked with 0.75 µg/mL ACBD. (A and C) UHPLC-UV chromatograms using the separation conditions from Figures 2a and 2C, respectively. (B and D) Corresponding UHPLC-ESI/TOFMS EICs for compounds shown in Figures 5a and 5C using $[M+H]^+$ ions with a ± 20 ppm mass tolerance.

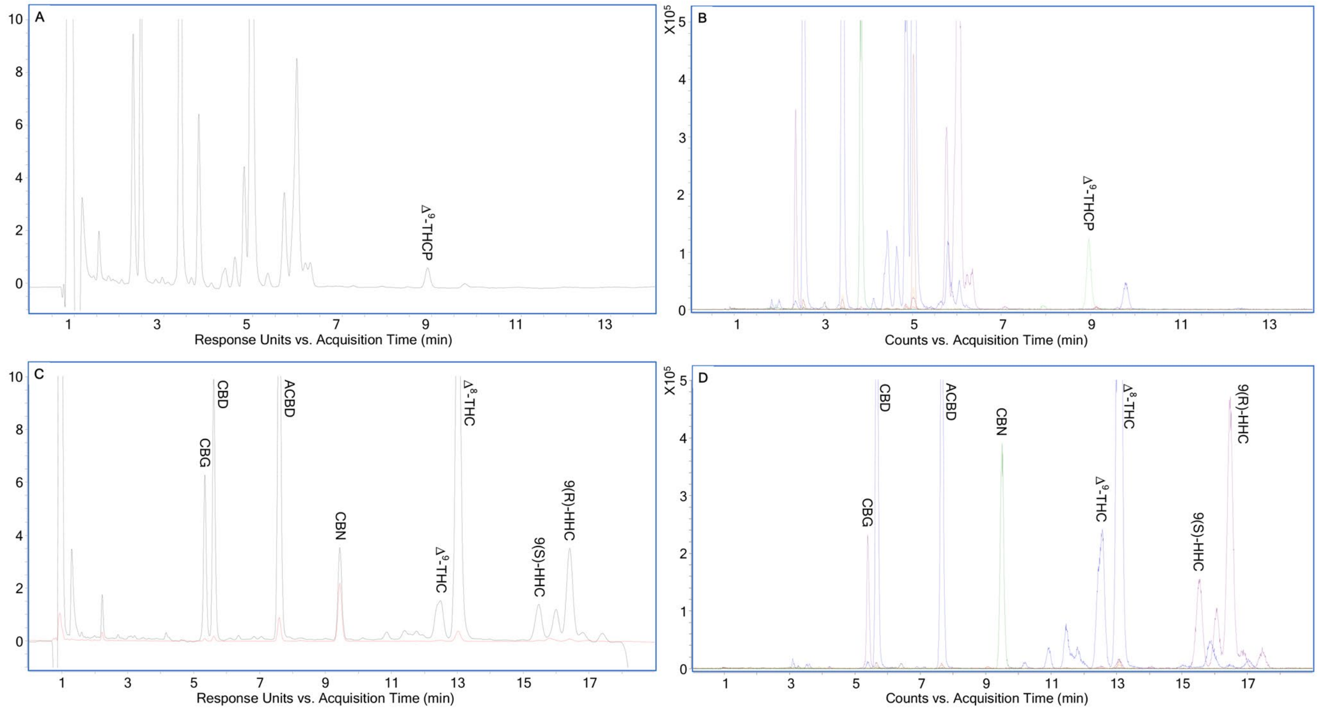

Figure 6. Analysis of 500 µg/mL Beast Mode Blend Gummies (G3) from Binoid spiked with 1.5 µg/mL ACBD. (A and C) UHPLC-UV chromatograms using the separation conditions from Figures 2a and 2C, respectively. (B and D) Corresponding UHPLC-ESI/TOFMS EICs for compounds shown in Figures 6a and 6C using $[M+H]^+$ ions with a ± 20 ppm mass tolerance.

Δ^8-THC, consistent with its predominance in the samples and the lack of specificity in its synthetic production.

Although low but quantifiable concentrations of $\Delta^{9,11}$-THC were detected in all tincture and vaping oil samples, its quantification was affected by peaks identified as structural isomers of Δ^8-THC, which eluted just before $\Delta^{9,11}$-THC and were not adequately resolved. In two samples (T2 and V1), quantification was further complicated by an additional interfering peak, identified as a structural isomer of HHC. While the $\Delta^{9,11}$-THC concentrations reported in Table 1 reflect conditions with relatively less interference, the values may be less accurate due to the need to split overlapping peaks during integration. Fortunately, since $\Delta^{9,11}$-THC is only a synthetic byproduct, precise quantification was not critical to the overall analysis.

Quantifiable concentrations of HHC were detected in all samples except one tincture (T1) and two gummy samples (G4 and G5). The HHC isomer profiles observed in the remaining samples fell into three distinct groups: (1) two 3Chi gummy samples (G1 and G2) and one iDELT8 vaping oil sample (V6); (2) one Binoid gummy sample (G3); and (3) three Binoid tincture samples (T2, T3, and T4) along with five Binoid vaping oil samples (V1 to V5).

The 3Chi gummy samples (G1 and G2) were formulated with HHC, and the expected two epimers, i.e., 9(S)-HHC and 9(R)-HHC, were detected and quantified without interference. In the iDELT8 vaping oil sample (V6), which was not labeled as containing HHC, low but quantifiable concentrations of both 9(S)-HHC and 9(R)-HHC were also detected. While the quantification of 9(S)-HHC in this sample was slightly impacted by a peak identified as a Δ^8-THC structural isomer, which eluted after 9(S)-HHC and was not baseline resolved, 9(R)-HHC was baseline resolved and unaffected.

The Binoid gummy sample (G3) was also not labeled as containing HHC, yet appreciable concentrations of both 9(S)-HHC and 9(R)-HHC were present. Although two additional Δ^8-THC structural isomer-related peaks were observed near the HHC peaks, their impact on quantification was minimal due to low intensity and sufficient resolution. Notably, two additional HHC-related isomers were detected in this sample by ESI/TOFMS, eluting before and after the 9(R)-HHC peak, which was consistent with a Binoid vaping oil sample analyzed in a previous study.[42] Although these were present at concentrations below the LOQ, their detection helped interpret the HHC peaks observed in the Binoid tincture and vaping oil samples.

None of the Binoid tinctures or vaping oils were formulated with HHC, yet low but quantifiable concentrations of three HHC isomers were detected. Among these, only the first peak corresponded to 9(S)-HHC. The second and third peaks matched the two minor isomers observed in the Binoid gummy sample (G3), eluting immediately before and after 9(R)-HHC. HHC has three stereogenic centers, which theoretically give rise to eight stereoisomers, although it has been consistently found as two epimers, i.e., 9(S)-HHC and 9(R)-HHC.[18] Due to the predominant presence of Δ^8-THC in these samples, the quantification of 9(S)-HHC was slightly affected by an interfering Δ^8-THC structural isomer, which eluted before 9(S)-HHC and was not baseline resolved.

Additionally, following the elution of four HHC stereoisomers, a likely fifth stereoisomer was observed with the three gummy samples (G1, G2, and G3).

Low but quantifiable concentrations of CBT were detected in the five vaping oil samples from Binoid (V1, V2, V3, V4, and V5). Its quantification was slightly affected by a peak identified as a structural isomer of Δ^8-THC, which eluted before CBT and was not baseline resolved.

Assessment of method specificity using normalized RRT

Given that synthetic THC analogs often contain byproducts of analogous THC isomers due to the nonspecific nature of their synthetic pathways, in addition that ESI/TOFMS and even ESI/MS/MS cannot distinguish analogous THC isomers,[43] method specificity was further evaluated using chromatographic retention behavior. Specifically, the relative retention time (RRT) of each analyte was calculated with respect to ACBD, which was used as a chromatographic reference compound. For each sample, the average RRT was determined based on triplicate injections. Similarly, the average RRT for each analyte was calculated across calibration standards. A normalized RRT (%) was then obtained by dividing the sample RRT by the calibration RRT and multiplying by 100.

Supplementary Table S5 presents the Normalized RRT values for the quantified neutral cannabinoids in Table 1. Among these, Δ^9-THC showed the largest deviation from 100%, approximately 1%, except for a vaping oil sample from iDELT8 (V6), in contrast to the much smaller deviations observed for Δ^8-THC, approximately 0.1%. In fact, non-Gaussian peak shapes were observed for Δ^9-THC, consistent with the presence of two closely eluting isomers of similar abundance.

Further investigation revealed that the coeluting analogous isomer was $\Delta^{4(8)}$-iso-THC, a minor byproduct formed during the isomerization of CBD to Δ^8-THC. In this process, one of CBD's two phenolic hydroxyl groups can react with the isopropenyl moiety to yield Δ^8-THC, while the second phenolic hydroxyl group may attack the double bond of the cyclohexene ring, forming Δ^8-iso-THC, which rapidly isomerizes to $\Delta^{4(8)}$-iso-THC under acidic conditions (Supplementary Fig. S14).

Under the reversed-phase LC conditions used in this study, it was observed that $\Delta^{4(8)}$-iso-THC coeluted with Δ^9-THC, and Δ^8-iso-THC coeluted with Δ^8-THC. This coelution behavior, though not widely reported, reflects longstanding challenges in separating analogous THC isomers by reversed-phase LC. To date, many analytical studies have focused primarily on differentiating Δ^9- and Δ^8-THC. Several reports have also identified the presence of Δ^8-iso-THC and $\Delta^{4(8)}$-iso-THC in synthetic Δ^8-THC products.[34–36,38] More advanced work has demonstrated the successful separation of Δ^9-, Δ^8-, Δ^8-iso-, and $\Delta^{4(8)}$-iso-THC by GC[32–33] and normal-phase LC,[32] but reversed-phase LC separation of these analogous THC isomers has not yet been reported.

This study emphasized the overall resolution of synthetically targeted THC analogs, encompassing eight distinct pairs (Figure 1). The validated method has been shown to be effective for identifying mislabeling, particularly when a product falsely claims to contain a synthetic THC analog, and for quantifying target THC analogs, with measurements largely unaffected by minor analogous byproducts. Advanced separation of minor isomeric byproducts (i.e., Δ^8-iso- and $\Delta^{4(8)}$-iso-THC) from the target THC analogs (i.e., Δ^9- and Δ^8-THC) via reversed-phase LC for accurate quantification is currently under development and will be reported in a future publication.

Interference from coeluting minor byproducts was observed primarily in products containing high concentrations of Δ^{8}-THC, and similar challenges are expected for other synthetic THC analogs at elevated concentrations. While future analytical methods will likely resolve and quantify only one analog at a time, a comprehensive method for all analogs simultaneously is not feasible. Therefore, the current method functions as a survey analysis, providing an essential overview of product composition, while future methods will serve as detailed analyses for individual analogs. Taken together, this method represents a critical first step in systematically evaluating synthetic THC analogs and establishing a foundation for subsequent, targeted studies.

In Supplementary Table S5, 9(S)-HHC in tincture and vaping oil samples from Binoid exhibited large deviations from 100%, i.e., ≥0.7%, in contrast to the much smaller deviations observed in a vaping oil sample from iDELT8 (V6), two gummy samples from 3Chi (G1 and G2), and a gummy sample from Binoid (G3), i.e., ≤0.1%. While this deviation was not sufficient to confirm misidentification of 9(S)-HHC, it clearly reflected differences in stereoisomers of HHC among the products, warranting further investigation in future studies.

Conclusions

An LC-DAD method was developed and validated for quantifying twenty-three neutral cannabinoids in synthetic THC analogs-formulated products.

Following ISO 17025-compliant validation, the method was applied to analyze four tincture, six vaping oil, and five gummy samples. Analytical results revealed mislabeling in ten of the fifteen samples. Additionally, four tinctures, three of six vaping oils, and two of five gummies showed unexpectedly similar cannabinoid profiles within their respective groups. The extent of mislabeling observed underscores challenges in quality control and regulatory oversight of synthetic THC analog–formulated products, and the validated UHPLC-DAD method provides a practical tool for routine compliance testing and regulatory surveillance using widely available instrumentation.

The method demonstrated high specificity and successfully resolved eight pairs of synthetically targeted THC analogs, including several HHC stereoisomers. Minor analogous byproducts were observed in products with high Δ^{8}-THC content, highlighting the need for further method development to achieve advanced separation for quantification of these minor analogous byproducts. While future methods will allow detailed analysis of individual analogs, a comprehensive approach for all analogs simultaneously is not feasible. Consequently, the current method serves as an essential survey tool, enabling detection of mislabeling and providing a foundation for subsequent, more targeted studies of synthetic THC analogs.

Author contributions

CRediT: **Angelo Bommarito**: Investigation, Resources, Supervision, Validation; **Md Imon Hossain**: Investigation, Methodology; **Emma Joens**: Data curation, Formal analysis, Investigation; **Jeev S. Hora**: Data curation, Investigation, Methodology; **Liguo Song**: Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing; **Matthew McConnell**: Formal analysis, Supervision, Writing – review & editing.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

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