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UPLC–MS/MS Determination of Twenty-Four Cannabinoids in Commercial Cannabis Oils and Gummy Edibles: Potency, Label Accuracy, and Product Quality Assessment

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Abstract

Accurate potency testing supports quality control, regulatory compliance, and informed use of commercial cannabis products. We developed and validated an ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method for simultaneous quantification of 24 cannabinoids in cannabis-derived oils and gummy products. Accuracy was evaluated as percent bias, precision as coefficient of variation (CV%), and extraction recovery as the percentage of fortified analyte recovered from a cannabinoid-free gummy matrix. Analyte-specific calibration ranges extended from 0.05 or 0.10 to 1000 ng/mL; experimentally verified LODs ranged from 0.005 to 0.05 ng/mL and LOQs from 0.05 to 0.10 ng/mL. QC bias at 4, 400, and 800 ng/mL ranged from −19.3% to 10.1%. Most gummy-matrix recoveries were 80–110%; CBDV recovery at 4 micrograms/g was 71.1%. Application to 14 hemp oils and 18 hemp- or cannabis-infused gummies showed substantial variation among the products analyzed and several discrepancies between measured and declared cannabinoid contents. Because the sample set was limited geographically and numerically, and recovery was evaluated in one representative gummy matrix, the market findings should not be generalized to all commercial products. The method provides a practical platform for the tested matrices, but additional matrix-specific validation is needed before broader application.

Keywords: Cannabinoids; cannabis edibles; hemp oil; potency testing; label accuracy; UPLC-MS/MS; minor cannabinoids; full-spectrum profiling

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1. Introduction

The cannabis plant (*Cannabis sativa* L.) has been used for medicinal and industrial purposes for centuries. Although cannabis remains federally controlled in the United States, changes in state laws have expanded medical and adult-use markets. Commercialization and expansion of legal cannabis markets have increased the availability of edibles and oils, while analytical surveys have reported substantial discrepancies between labeled and measured cannabinoid contents [1,2]. Such discrepancies may contribute to unintended exposure, particularly when products contain greater-than-labeled amounts of psychoactive cannabinoids; however, the present analytical study did not evaluate consumer exposure, clinical outcomes, or toxicity. Pediatric ingestion of cannabis edibles has been associated with

central nervous system depression, hospitalization, intensive care admission, and severe or prolonged toxicity [3–5], emphasizing the importance of accurate labeling, child-resistant packaging, secure storage, and appropriate consumer guidance.

AOAC International has established Standard Method Performance Requirements for cannabinoid potency testing in selected complex matrices [6]. The AOAC-related panel includes major cannabinoids used for routine potency assessment. We expanded this panel by adding Δ^8-THCVA, Δ^8-THCV, Δ^8-THCA, CBNA, CBLA, CBL, CBCVA, CBCV, CBGVA, and CBGV (see Figure 1). These additional targets broaden coverage of positional isomers, varin-type compounds, acidic precursors, and oxidation or degradation-related products that may be present in commercial formulations or may complicate identification because of shared MRM transitions. Their inclusion improves analytical characterization of product composition, but detection alone does not establish toxicity, manufacturing history, raw-material origin, or formulation integrity.

The pharmacology of minor cannabinoids remains less established than that of CBD and Δ^9-THC. Contemporary reviews describe potentially distinct receptor, enzyme, and ion-channel activities for CBC, CBG, CBN, CBDV, and THCV, while emphasizing that much of the evidence remains preclinical and that clinical exposure-response relationships are incompletely defined [7,8]. Accordingly, the expanded panel in this study was selected to improve chemical characterization and isomer discrimination, not to infer toxicity or therapeutic activity from detection alone.

Recent literature further supports the need for matrix-specific multianalyte methods. Johnson et al. used LC-MS/MS to examine 56 CBD gummy products and reported that 70% differed from the labeled CBD dose by more than 10%; among 10 products evaluated for within-container consistency, CBD coefficients of variation ranged from 2.1% to 27.1% and Δ^8-THC coefficients of variation ranged from 3.1% to 23.5% [9]. A unified LC-MS/MS study of edible and e-liquid products likewise emphasized that product mislabeling and diverse formulations require methods capable of analyzing multiple cannabinoids across distinct matrices [10]. These findings also show why analytical replication within one package should be distinguished from independent sampling across brands and production batches.

Profiling multi-cannabinoid mixtures in complex matrices presents two major analytical challenges: broad dynamic concentration ranges and severe structural isomerism [11]. First, commercial oils and edibles exhibit extreme concentration disparities between major cannabinoids (CBD and Δ^9-THC, often present at high mg/mL or mg/g levels) and minor or trace cannabinoids (often present at sub-μg or low ng levels). Achieving simultaneous quantification without requiring excessive serial dilutions or risking detector saturation requires an analytical method with high sensitivity coupled with a wide linear dynamic range, usually a tandem mass spectrometry. Second, many of the 24 target cannabinoids are structural or positional isomers that possess identical molecular weights and yield identical multiple reaction monitoring (MRM) mass transitions. For example, the neutral isomer group (CBD, Δ^9-THC, Δ^8-THC, CBC, and CBL) shares the mass transition m/z 315.2→193.1, while the varin isomer group (CBDV, Δ^9-THCV, Δ^8-THCV, and CBCV) shares m/z 287.2→165.2. In addition, the Δ^9/Δ^8cannabinoid pairs and their respective acidic (Δ^9-THCAA vs. Δ^8-THCAA) and varin (Δ^9-THCVA vs. Δ^8-THCVA) analogues differ only in the position of a single carbon-carbon double bond, producing indistinguishable MS/MS fragmentation patterns [12]. Consequently, tandem mass spectrometry alone cannot differentiate these isomeric pairs; baseline chromatographic resolution prior to MS detection is indispensable for accurate identification and quantification.

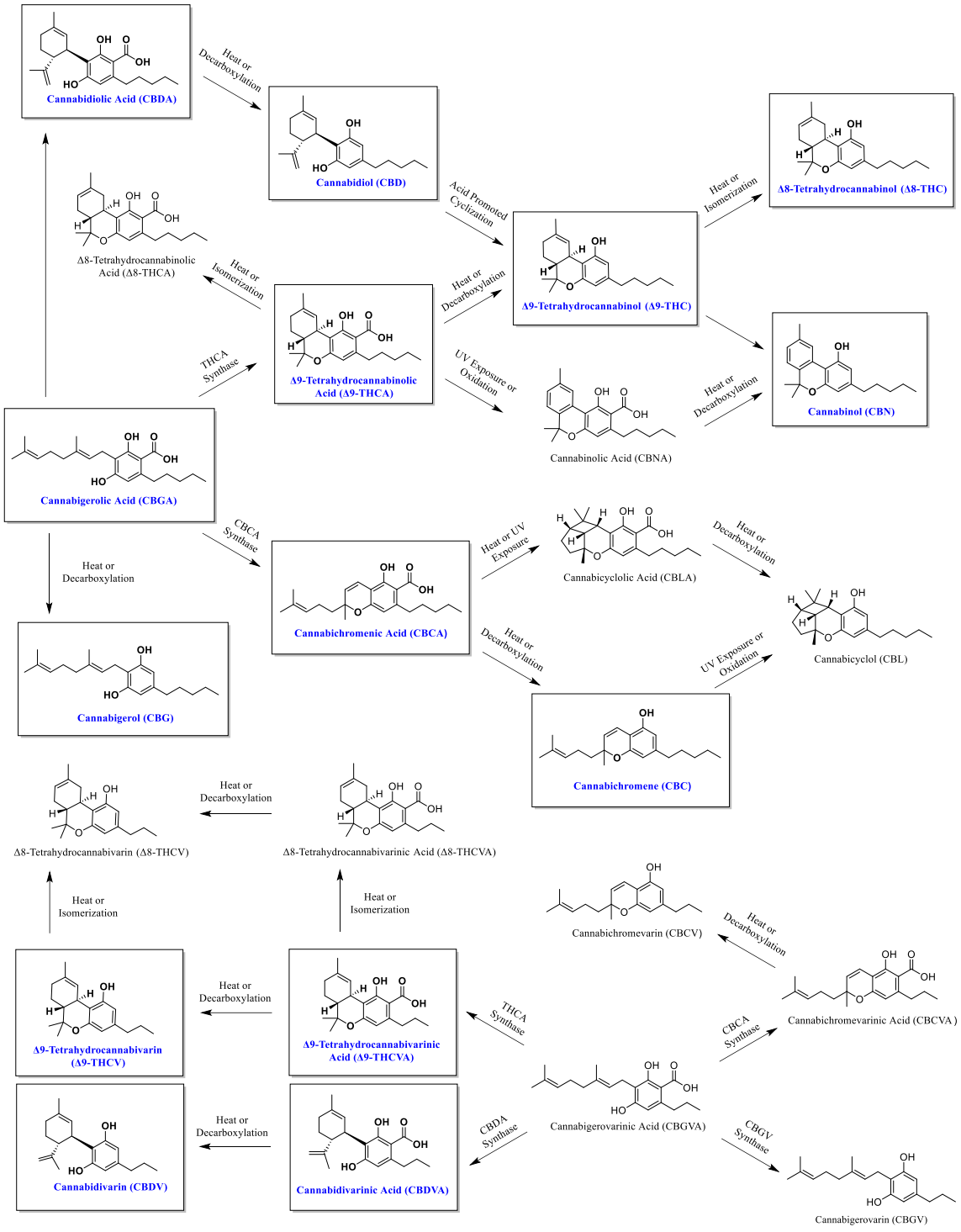


Figure 1. Chemical structures of the 24 target cannabinoids, building upon the 14 AOAC-recommended targets to include expanded minor, varin-type, acidic, and degradation-related analytes.

The primary objective was to develop and validate a selective UPLC-MS/MS method for simultaneous quantification of 24 cannabinoids in hemp oils and gummy matrices. The second objective was to apply the method to 14 commercial hemp oils and 18 hemp- or cannabis-infused gummies to describe cannabinoid profiles and compare measured values with declared label amounts. Conclusions regarding product composition and label agreement are limited to the products analyzed.

2. Methods and Materials

2.1. Chemicals and Reagents

Phytocannabinoid Mixture 10, a certified reference material (CRM), was purchased from Cayman Chemical (Ann Arbor, MI, USA) as an acetonitrile solution containing 250 micrograms/mL of each analyte. The mixture contained CBD, Δ^9-THC, Δ^8-THC, CBC, CBN, CBG, CBDV, CBGA, CBDA, and Δ^9-THCA.

Individual 14 reference standards of cannabidivarinic acid (CBDVA), Δ^9-tetrahydrocannabivarin (Δ^9-THCV), Δ^9-tetrahydrocannabivarinic acid (Δ^9-THCVA), Δ^8-tetrahydrocannabinolic acid (Δ^8-THCA), Δ^8-tetrahydrocannabivarin (Δ^8-THCV), Δ^8-tetrahydrocannabivarinic acid (Δ^8-THCVA), cannabichromenic acid (CBCA), cannabichromevarin (CBCV), cannabichromevarinic acid (CBCVA), cannabinolic acid (CBNA), cannabigerovarin (CBGV), cannabigerovarinic acid (CBGVA), cannabicyclol (CBL), and cannabicyclolic acid (CBLA) were also obtained from Cayman Chemical (Ann Arbor, MI, USA).

QuEChERS extraction salts (ECQUEU750CT-MP) were purchased from United Chemical Technologies (Bristol, PA, USA). LC-MS grade methanol, acetonitrile, and formic acid were obtained from Fisher Scientific (Waltham, MA, USA).

2.2. Cannabinoids Standard Preparation

Individual 14 cannabinoid reference materials were supplied either as 1 mg/mL solutions or as neat material. Neat standards were dissolved in acetonitrile to prepare 1 mg/mL stock solutions. For the 14-analyte mixture, 50 µL of each 1 mg/mL stock was combined and diluted with 300 µL of 50:50 (v/v) acetonitrile/water to a final volume of 1.00 mL, yielding 50 µg/mL of each analyte. Phytocannabinoid mixture 10 was diluted to 50 µg/mL per analyte. Then, 200 µL of phytocannabinoid mixture 10 and 200 µL of the 14-analyte mixture were combined with 600 µL of 50:50 (v/v) acetonitrile/water to produce a 1.00 mL, 24-analyte working solution containing 10 µg /mL of each cannabinoid. Working solutions were stored in amber vials under frozen conditions until use.

2.3. Sample Collection and Preparation

2.3.1. Hemp and Cannabis-Infused Products

Fourteen hemp oil products, seven hemp-derived gummy products, and eleven cannabis-infused gummy products were purchased as distinct commercial products from different brands. Hemp-derived products were obtained from online or local retailers in Kansas, New Jersey, New York, and Nevada; cannabis-infused gummies were obtained from licensed New Jersey dispensaries, from 2022 to 2025. Each product represented one purchased retail unit or batch rather than an independently replicated market sample. Available label information and anonymized product identifiers are provided in the Supporting Information. Samples were stored at −80 degrees C until analysis, and each prepared sample was analyzed in duplicate.

2.3.2. Hemp Oil Preparation

Oil samples were diluted and analyzed directly without extraction. At room temperature, 0.100 mL of oil was mixed with 0.9 mL isopropanol to produce an initial 10-fold dilution, vortexed (Vertex Genie 2, Fisher Scientific, Waltham, MA, USA), and sonicated for 30 min. Aliquots were then serially diluted with acetonitrile to overall dilution factors of 500, 5000, or 50,000. The 50,000-fold dilution was used primarily for CBD and Δ^9-THC, the 500-fold dilution for other minor cannabinoids. The reported product concentration was calculated as the instrument-derived concentration multiplied by the applicable overall dilu-

tion factor. Dilutions were selected to keep responses within the validated analyte-specific calibration range. Samples were injected without filtration.

2.3.3. Gummy Sample Extraction

Two to three gummy units from each product were frozen overnight at −20 degrees C to reduce stickiness, cut into approximately 2 mm pieces, and combined to improve within-product homogenization. A 1.0 g portion was weighed into a 50 mL centrifuge tube, mixed with 5.0 mL deionized water, and shaken (LAB FISH Tube Roller Mixer, Walmart, NJ, USA) for 30 min at room temperature under ambient light to completely dissolve gummy samples. Acetonitrile (10.0 mL) was added, and the mixture was vortexed for 1 min. One packet of QuEChERS extraction salts (ECQUEU750CT-MP) was added, followed by another 1 min of vortex mixing and centrifugation at 4000 rpm for 5 min. The upper acetonitrile layer was transferred directly to autosampler vials, diluted as needed, and analyzed immediately without filtration. Recovery was evaluated in one cannabinoid-free commercial gummy matrix; therefore, matrix-dependent recovery and ionization effects may differ in other formulations.

2.4. Instrumentation Conditions

Sample analysis was performed using a Waters ACQUITY Premier UPLC system coupled to a Xevo TQ Absolute triple quadrupole mass spectrometer (Waters Corporation, Milford, MA, USA).

Separation was achieved on a Waters CORTECS C18 column (3.0 × 150 mm, 2.7 micrometers) at 30 degrees C; the autosampler was maintained at 10 degrees C. Mobile phase A was water with 0.1% formic acid, and mobile phase B was acetonitrile with 0.1% formic acid. At 0.5 mL/min, the gradient was 24% A/76% B from 0.0 to 10.0 min, 1% A/99% B from 10.1 to 12.0 min, and 24% A/76% B from 12.1 to 15.0 min for column re-equilibration. The injection volume was 5 microliters. The effective analytical cycle time was 15 min per injection.

Mass spectrometric detection used a Waters Xevo TQ Absolute (Milford, MA, USA) triple-quadrupole mass spectrometer with a ZSpray electrospray ionization source operated in positive-ion MRM mode. Source settings were: capillary voltage, 3.0 kV; cone voltage, 10 V; desolvation temperature, 450 degrees C; desolvation gas, 1000 L/h; cone gas, 150 L/h; and collision gas, 0.15 mL/min. Nitrogen was used for nebulization and desolvation, and argon was used as collision gas. Analyte-specific precursor/product transitions, cone voltages, collision energies, retention times, and dwell times are reported in Table 1.

Table 1. Optimized retention times, quantifier and qualifier MRM transitions, cone voltages (CVs), dwell time, and collision energies (CEs) for 24 cannabinoids.

Compounds	Retention Time (min)	Mode	Quantifier	CV (V) CE (eV)	Qualifier	CV (V) CE (eV)	Dwell Time (s)
CBD	3.35	Positive	315.2/193.1	20, 25	315.2/123.1	20, 35	0.014
Δ^9-THC	6.49	Positive	315.2/193.1	20, 25	315.2/123.1	20, 35	0.046
Δ^8-THC	6.78	Positive	315.2/193.1	20, 25	315.2/123.1	20, 35	0.046
CBC	8.38	Positive	315.2/193.2	20, 18	315.2/123.2	20, 35	0.046
CBL	7.88	Positive	315.2/235.1	20, 15	315.2/165.2	20, 26	0.046
CBCV	4.63	Positive	287.2/165.2	20, 20	287.2/123.2	20, 35	0.034

Table 1. *Cont.*

Compounds	Retention Time (min)	Mode	Quantifier	CV (V) CE (eV)	Qualifier	CV (V) CE (eV)	Dwell Time (s)
CBDV	2.26	Positive	287.2/165.2	20, 20	287.2/123.2	20, 35	0.014
Δ^9-THCV	3.72	Positive	287.2/165.2	20, 25	287.2/123.2	20, 35	0.071
Δ^8-THCV	3.87	Positive	287.2/165.2	20, 25	287.2/123.2	20, 35	0.071
CBDA	2.83	Positive	359.2/341.2	20, 20	359.2/219.1	20, 30	0.014
Δ^9-THCAA	8.72	Positive	359.1/219.1	20, 35	359.1/341.2	20, 28	0.046
Δ^8-THCAA	9.45	Positive	359.1/219.1	20, 35	359.1/341.2	20, 28	0.046
CBCA	10.36	Positive	359.2/341.2	20, 10	359.2/219.2	20, 25	0.080
CBLA	10.68	Positive	359.2/261.1	20, 25	359.2/81.2	21, 40	0.080
Δ^9-THCVA	4.80	Positive	331.2/191.1	20, 35	331.2/313.1	20, 35	0.034
Δ^8-THCVA	5.16	Positive	331.2/191.1	20, 35	331.2/313.1	20, 35	0.034
CBDVA	2.07	Positive	331.2/191.1	20, 28	331.2/313.1	20, 22	0.014
CBCVA	5.64	Positive	331.2/313.1	20, 13	331.2/191.1	20, 23	0.034
CBN	5.06	Positive	311.2/223.1	20, 23	311.2/208.1	20, 30	0.034
CBNA	6.60	Positive	355.1/235.1	20, 32	355.1/337.1	20, 15	0.046
CBG	3.11	Positive	317.2/193.1	20, 18	317.2/123.1	20, 33	0.014
CBGA	2.92	Positive	361.2/219.1	20, 25	361.2/237.0	20, 10	0.014
CBGV	2.20	Positive	289.2/165.2	20, 23	289.2/123.2	20, 33	0.014
CBGVA	2.18	Positive	333.2/315.1	20, 13	333.2/191.1	20, 25	0.025

2.5. Method Validation

Method validation was conducted in accordance with ANSI/ASB Standard 036, Standard Practices for Method Validation in Forensic Toxicology [13]. Validation parameters included calibration model performance, linearity, limits of detection (LOD), limits of quantitation (LOQ), carryover, bias, precision, and extraction recovery.

2.5.1. Calibration Model, LOD, LOQ, and Carryover

Calibration standards were prepared in triplicate by serial dilution of the 24-cannabinoid working solution with 50:50 (*v*/*v*) acetonitrile/water. The validated range was 0.05–1000 ng/mL for most analytes and 0.10–1000 ng/mL for Δ^9-THCAA, Δ^8-THCAA, and CBCA. A weighted (1/x) linear regression model was used. All reported sample results were obtained from dilutions that fell within the applicable validated range.

LOD and LOQ were established experimentally. The LOD was the lowest tested concentration producing a signal-to-noise ratio greater than 3. The LOQ was the lowest calibration level meeting identification requirements and predefined quantitative performance criteria, including absolute bias within 20% and acceptable replicate precision. Signal-to-noise ratios were calculated from analyte peak response relative to baseline noise in the corresponding retention-time region.

Carryover was evaluated by injecting a solvent blank immediately after the highest calibration standard. Carryover was considered absent when no analyte response above the established LOD was detected in the blank injection.

2.5.2. Bias and Precision

Solvent-based QC samples were prepared independently at 4, 400, and 800 ng/mL by diluting the mixed standard in 50:50 (*v*/*v*) acetonitrile/water. At each level, three

independently prepared QC samples were analyzed in each of three separate analytical runs. Precision was reported as the coefficient of variation (CV, %). Bias acceptance was ±20% at each analyte and QC level.

Bias was calculated as:

$$\text{Bias (\%)} = \frac{\text{Measured Concentration} - \text{Nominal Concentration}}{\text{Nominal Concentration}} \times 100$$

2.5.3. Recovery Study

Extraction recovery of the QuEChERS method was evaluated using Haribo® gummy bears purchased from a local retail store as representative cannabinoid-free matrix. One gram of homogenized gummy sample was fortified with the 24-cannabinoid mixed standard solution at three concentration levels (n = 3): low (0.04 μg g^{-1}), medium (0.4 μg g^{-1}), and high (4 μg g^{-1}). Fortified samples were extracted according to the procedure described in Section 2.3.3.

Recovery was calculated as:

$$\text{Recovery (\%)} = \frac{\text{Measured Concentration}}{\text{Fortified Concentration}} \times 100$$

3. Results and Discussion

3.1. Uhplc-Ms/Ms Method Optimization and Chromatographic Separation

To optimize the multiple reaction monitoring (MRM) transitions, individual cannabinoid standards at 1 μg/mL were injected into the UPLC-MS/MS system. For each compound, the precursor ion was fragmented using a series of collision energies, including 10, 15, 20, 25, 30, and 35 eV, to generate product ions. The most abundant product ion, together with its corresponding collision energy, was selected as the quantifier transition. The second most abundant product ion was selected as the qualifier transition for confirmation.

Positive ionization mode was tested for all neutral cannabinoids. For acidic cannabinoids, both positive and negative ionization modes were evaluated. The results showed that positive ionization mode provided better responses for the acidic cannabinoids. Therefore, positive ionization mode was selected for all 24 cannabinoids in the final method.

The optimized MRM transitions, retention times, cone voltages, and collision energies are listed in Table 1.

A major analytical challenge in cannabinoid profiling is the presence of numerous structural and positional isomers that possess identical molecular weights and highly similar fragmentation patterns. Among the 24 cannabinoids investigated, four prominent isomeric groups were included: (i) CBD, Δ^9-THC, Δ^8-THC, CBC, and CBL; (ii) CBDV, Δ^9-THCV, Δ^8-THCV, and CBCV; (iii) CBDA, Δ^9-THCAA, Δ^8-THCAA, CBCA, and CBLA; and (iv) CBDVA, Δ^9-THCVA, Δ^8-THCVA, and CBCVA. Several compounds within each group shared identical precursor and product ions. For example, CBD, Δ^9-THC, and Δ^8-THC all utilized the same quantifier (m/z 315.2→193.1) and qualifier (m/z 315.2→123.1) transitions, while CBDV, Δ^9-THCV, Δ^8-THCV, and CBCV shared identical transitions of m/z 287.2→165.2 and 287.2→123.2. Consequently, chromatographic resolution rather than MS/MS selectivity was the primary mechanism enabling their reliable identification and quantification.

Chromatographic resolution (Rs) was calculated as $Rs = 2(t_{R2} - t_{R1})/(w_1 + w_2)$, where t_R is retention time and w is baseline peak width. The calculated Rs values were 3.47 for Δ^8/Δ^9-THCAA, 3.29 for Δ^8/Δ^9-THCVA, 2.07 for Δ^8/Δ^9-THC, and 1.74 for Δ^8/Δ^9-THCV. Each exceeded the baseline-separation criterion of $Rs > 1.5$. For compounds sharing MRM transitions, identification required agreement of retention time with the reference standard and acceptable quantifier/qualifier ion behavior in addition to chromatographic separation.

Figure 2 shows the chromatographic separation of 24 cannabinoid standards using the developed UPLC-MS/MS method. Most analytes exhibited sharp, symmetrical peaks with excellent retention-time reproducibility and minimal peak overlap, demonstrating good chromatographic selectivity across the analytical run. The method enabled simultaneous determination of 24 cannabinoids, including neutral cannabinoids, acidic cannabinoids, and their corresponding varin analogues, within a single analysis. The separation of neutral cannabinoids is illustrated in Figure 2A, whereas acidic cannabinoids are shown in Figure 2B.

Compared with previously reported multicannabinoid methods, the present method offers substantially broader analyte coverage while maintaining effective isomer separation in a short run (within 12 min). Song et al. subsequently developed LC-DAD methods for the analysis of up to 16 cannabinoids, respectively, emphasizing the importance of chromatographic resolution for potency testing and achieving baseline separation for numerous analytes [14]. More recently, Owolabi et al. systematically evaluated chromatographic conditions for the separation of 18 cannabinoids and concluded that cannabinoid isomers represent the most significant chromatographic challenge in potency analysis [15]. Notably, Song et al. established one of the broadest panels to date, reporting an LC-DAD method with optional electrospray ionization time-of-flight mass spectrometry for the determination of up to 20 cannabinoids [16]. Although their approach demonstrated the utility of high-resolution chromatography for multi-cannabinoid profiling, optical DAD detection remains susceptible to spectral overlap and attenuated sensitivity when quantifying trace minor cannabinoids in dark or complex matrices such as infused gummies and crude hemp oils.

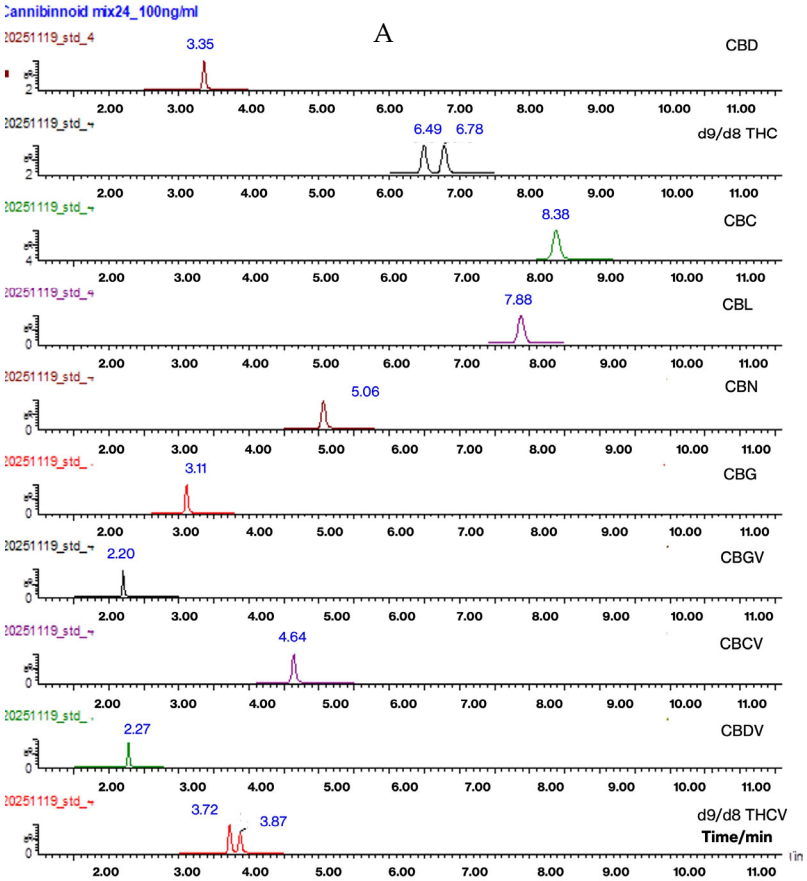


Figure 2. *Cont.*

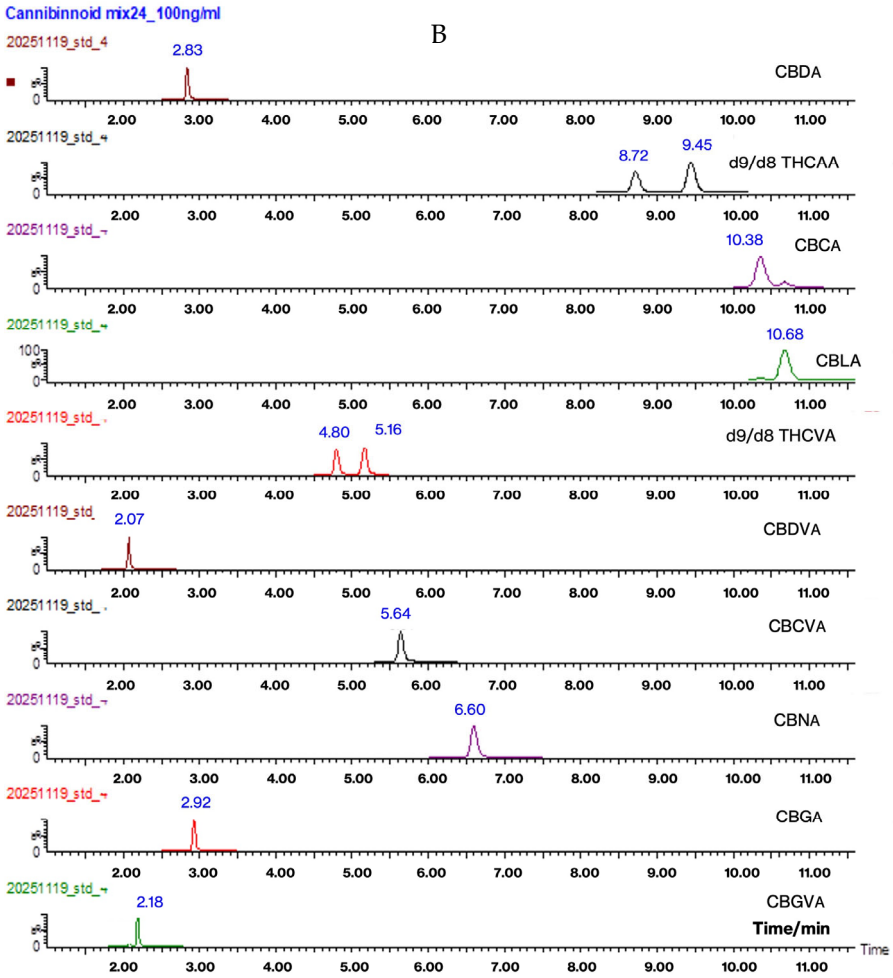


Figure 2. UPLC-MS/MS separation of 24 cannabinoid standards. (**A**) Separation of neutral cannabinoids, including CBD, Δ^9-THC, Δ^8-THC, CBC, CBL, CBDV, Δ^9-THCV, Δ^8-THCV, CBCV, CBN, and CBG. (**B**) Separation of acidic cannabinoids, including CBDA, Δ^9-THCAA, Δ^8-THCAA, CBCA, CBLA, CBDVA, Δ^9-THCVA, Δ^8-THCVA, CBCVA, CBGA, and CBGVA. (Despite the presence of multiple positional and structural isomers sharing identical MRM transitions, all analytes were chromatographically resolved, enabling reliable identification and quantification within a single analytical run).

Cross-method comparisons require more than analyte count. Published methods differ in detector platform, extraction procedure, calibration design, run time, matrix scope, use of internal standards, and criteria for isomer identification. For example, recent LC-MS/MS validation work has demonstrated sensitive simultaneous measurement of eight phytocannabinoids across several CBD product types [17], whereas a unified method for edible and e-liquid products was designed specifically for cross-matrix mislabeling assessment [10].

In comparison, the current UPLC-MS/MS method expands analyte coverage to 24 cannabinoids while simultaneously resolving four sets of structurally related isomers, including neutral, acidic, and varin cannabinoids. The successful separation of Δ^9/Δ^8 cannabinoid pairs and their derivatives is particularly important because these compounds often exhibit identical MRM transitions and may have different regulatory and commercial significance. Therefore, the developed method provides a comprehensive and highly selective platform for cannabinoid potency testing and profiling of commercial cannabis-derived products.

3.2. Method Validation Results

Calibration curves were generated by combining data from triplicate calibration standards for each analyte. A linear regression model was applied with a 1/x weighting factor. The validation results for linearity, sensitivity, accuracy, and precision are summarized in Table 2.

Table 2. Method validation results for 24 cannabinoids, including linearity, LOQ, LOD, and QC accuracy and precision at low, medium, and high concentration levels.

Compounds	R^2	LOQ (ng/mL)	LOD (ng/mL)	Low (4 ng/mL)			Medium (400 ng/mL)			High (800 ng/mL)		
				Mean ± Sd	Bias%	Within Run CV%	Mean ± Sd	Bias%	Within Run CV%	Mean ± Sd	Bias%	Within Run CV%
CBD	0.9971	0.05	0.01	3.9 ± 0.7	−2.5	17.94	376.7 ± 17.9	−5.8	4.76	863.3 ± 92.9	7.9	10.76
Δ^9-THC	0.9956	0.05	0.01	3.9 ± 0.7	−1.7	18.39	370.1 ± 12.2	−7.5	3.28	839.5 ± 87.1	4.9	10.37
Δ^8-THC	0.9977	0.05	0.01	3.9 ± 0.7	−3.3	17.21	379.5 ± 17.1	−5.1	4.51	881.0 ± 96.0	10.1	10.90
CBC	0.9988	0.05	0.01	3.8 ± 0.7	−4.2	18.87	382.0 ± 14.3	−4.5	3.75	836.4 ± 54.8	4.6	6.55
CBL	0.9955	0.05	0.01	3.9 ± 0.7	−1.7	18.39	369.5 ± 7.8	−7.6	2.10	872.6 ± 32.2	9.1	3.69
CBCV	0.9962	0.05	0.01	3.9 ± 0.7	−2.5	17.76	371.5 ± 12.0	−7.1	3.24	858.9 ± 91.0	7.4	10.60
CBDV	0.9960	0.05	0.01	4.0 ± 0.8	−0.8	18.92	373.9 ± 5.3	−6.5	1.42	857.3 ± 89.4	7.2	10.43
Δ^9-THCV	0.9904	0.05	0.005	4.0 ± 0.7	0.8	17.94	347.1 ± 18.9	−13.2	5.45	733.6 ± 70.6	−8.3	9.63
Δ^8-THCV	0.9926	0.05	0.005	3.9 ± 0.7	−1.7	18.39	364.4 ± 19.5	−8.9	5.35	788.7 ± 79.2	−1.4	10.05
CBDA	0.9944	0.05	0.01	3.8 ± 0.6	−4.2	16.57	350.0 ± 16.6	−12.5	4.74	811.0 ± 68.3	1.4	8.42
Δ^9-THCAA	0.9974	0.1	0.05	3.7 ± 0.5	−7.5	14.04	352.0 ± 13.6	−12.0	3.88	857.1 ± 76.3	7.1	8.91
Δ^8-THCAA	0.9966	0.1	0.05	3.7 ± 0.6	−8.3	15.75	357.1 ± 11.7	−10.7	3.27	858.1 ± 76.5	7.3	8.91
CBCA	0.9973	0.1	0.05	3.7 ± 0.6	−8.3	15.75	361.1 ± 14.5	−9.7	4.03	873.4 ± 78.9	9.2	9.03
CBLA	0.9981	0.05	0.01	3.7 ± 0.5	−7.5	14.04	359.5 ± 11.0	−10.1	3.05	868.0 ± 75.4	8.5	8.69
Δ^9-THCVA	0.9920	0.05	0.01	3.7 ± 0.5	−6.7	12.37	339.1 ± 2.7	−15.2	0.80	758.4 ± 49.9	−5.2	6.59
Δ^8-THCVA	0.9925	0.05	0.01	3.7 ± 0.5	−6.7	12.37	340.3 ± 0.8	−14.9	0.22	765.6 ± 49.9	−4.3	6.51
CBDVA	0.9925	0.05	0.01	3.8 ± 0.5	−5.0	13.67	338.3 ± 8.2	−15.4	2.44	768.1 ± 55.4	−4.0	7.21
CBCVA	0.9969	0.05	0.01	3.6 ± 0.5	−9.2	12.71	354.5 ± 6.9	−11.4	1.95	832.9 ± 63.9	4.1	7.67
CBN	0.9968	0.05	0.01	3.9 ± 0.7	−3.3	17.22	373.0 ± 15.6	−6.8	4.18	869.1 ± 94.1	8.6	10.83
CBNA	0.9927	0.05	0.01	3.9 ± 0.6	−3.3	14.93	353.8 ± 7.1	−11.5	2.01	774.8 ± 58.8	−3.1	7.59
CBG	0.9975	0.05	0.005	3.9 ± 0.7	−1.7	18.39	372.8 ± 18.9	−6.8	5.06	851.1 ± 87.6	6.4	10.29
CBGA	0.9941	0.05	0.01	3.9 ± 0.7	−2.5	17.76	349.2 ± 14.2	−12.7	4.06	806.7 ± 75.0	0.8	9.30
CBGV	0.9863	0.05	0.005	4.1 ± 0.7	3.3	17.50	343.9 ± 21.4	−14.0	6.23	719.7 ± 63.9	−10.0	8.88
CBGVA	0.9829	0.05	0.01	4.0 ± 0.6	0.1	15.21	322.9 ± 3.5	−19.3	1.09	708.6 ± 4.6	−11.5	0.66

The developed UPLC-MS/MS method demonstrated good linearity across the evaluated calibration range, with analyte-specific lower limits ranging from 0.05 to 0.1 ng/mL and an upper limit of 1000 ng/mL. The broad calibration range supports the applicability of this method for cannabinoid products with highly variable compositions, including products containing abundant major cannabinoids such as CBD and THC and low concentrations of minor cannabinoids.

Most analytes had R-squared values above 0.99. CBGV (0.9863) and CBGVA (0.9829) were lower, indicating greater calibration variability for these analytes. Their QC biases

remained within the predefined ±20% criterion, but quantitative results for CBGV and CBGVA should be interpreted with additional caution, particularly near the calibration limits. Future work should examine alternative weighting, narrower calibration intervals, and additional replicate calibrators for these analytes.

The method also showed high sensitivity for the 24 cannabinoids. The LOQs ranged from 0.05 to 0.1 ng/mL. Most analytes had an LOQ of 0.05 ng/mL, while Δ^9-THCAA, Δ^8-THCAA, and CBCA had LOQs of 0.1 ng/mL. The LODs ranged from 0.005 to 0.05 ng/mL, with the lowest LODs observed for Δ^9-THCV, Δ^8-THCV, CBG, and CBGV. These results indicate that the method is sufficiently sensitive for trace-level detection and quantification of cannabinoids.

Accuracy and precision were assessed at three QC levels: 4, 400, and 800 ng/mL. Across all analytes, measured concentrations ranged from 3.6 to 4.1, 322.9 to 382.0, and 708.6 to 881.0 ng/mL at the low, medium, and high QC levels, respectively. Bias ranged from −19.3% to 10.1%, meeting the predefined acceptance criterion of ±20%.

Extraction recovery was evaluated in one cannabinoid-free commercial gummy matrix at 0.04, 0.4, and 4 micrograms/g (n = 3 per level) (See Figure 3). Most analyte-level combinations were within 80–110%. CBDV recovery at the high level was 71.1%, below the predefined 80–120% range, indicating possible analyte-specific loss or matrix influence. Consequently, trace and minor-cannabinoid results, particularly CBDV, should be interpreted cautiously. Additional cleanup, matrix-matched calibration, stable-isotope internal standards, and validation across gummies with different sugar, gelatin/pectin, colorant, and lipid compositions should be evaluated in future work.

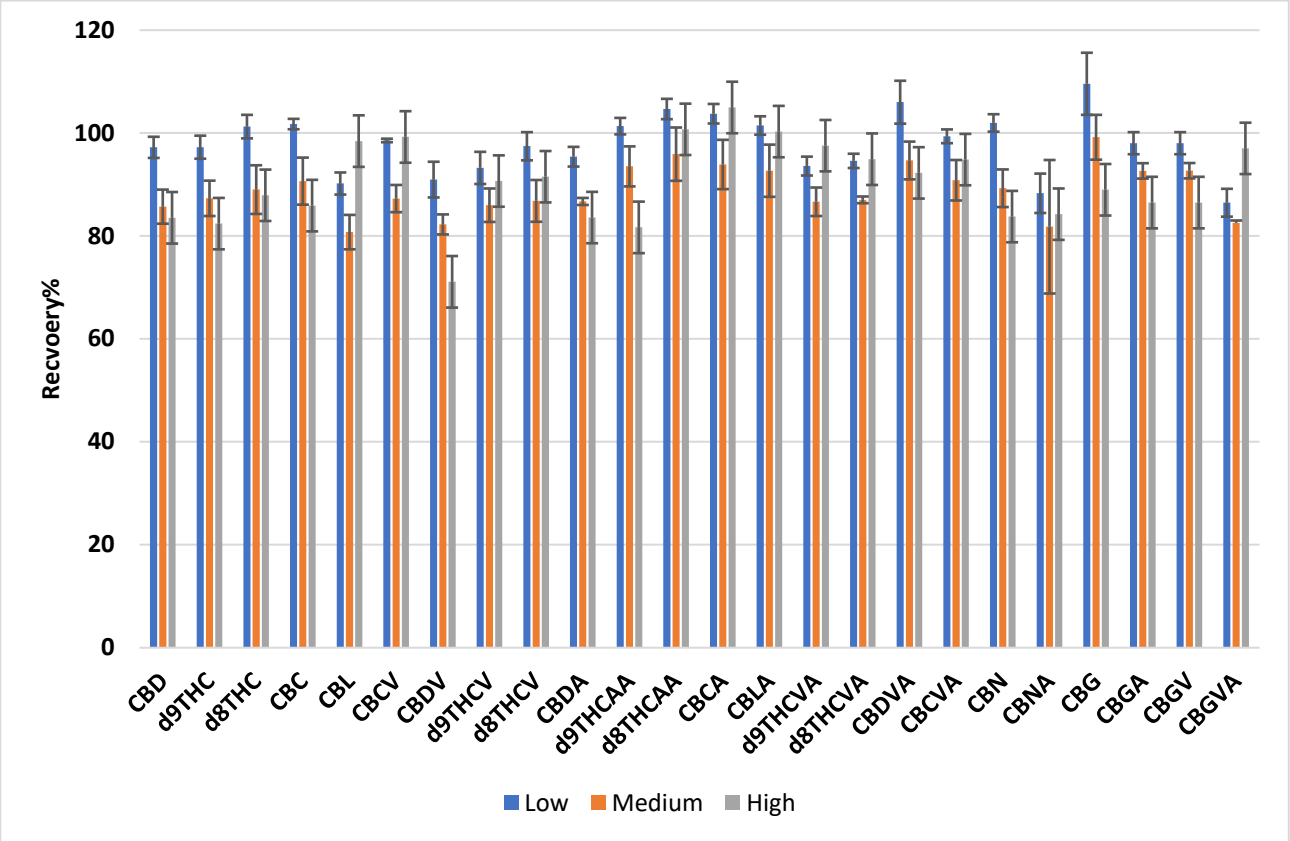


Figure 3. Recovery of 24 cannabinoids in gummy samples at three fortification levels: low, 0.04 µg/g; medium, 0.4 µg/g; and high, 4 µg/g. Recoveries were determined from triplicate analyses, and error bars represent standard deviations.

Absence of a visible interfering peak in a blank gummy addresses chromatographic selectivity but does not quantify electrospray ion suppression or enhancement. A complete matrix-effect assessment would compare post-extraction spiked matrix responses with neat-solution responses, ideally using matrix factors normalized to stable-isotope internal standards. The diversity of gummy formulations, including gelatin- or pectin-based matrices, sugars, acids, colors, flavors, and lipid-containing ingredients, can alter extraction and ionization. Therefore, the recovery results support use in the evaluated control matrix but should not be interpreted as complete validation for every commercial gummy formulation.

3.3. Potency Test of Commercial Hemp Oil Samples

Figure 4 shows the overlay TIC profiles of the solvent blank, gummy control, gummy sample, and oil sample. The solvent blank exhibited minimal background signal, indicating no obvious contamination or carryover during analysis. The gummy control showed limited matrix-related responses, with no major interfering peaks observed in the retention regions of the target cannabinoids. The gummy and oil samples displayed distinct chromatographic peaks corresponding to cannabinoid signals, confirming the applicability of the developed UPLC-MS/MS method to complex commercial matrices.

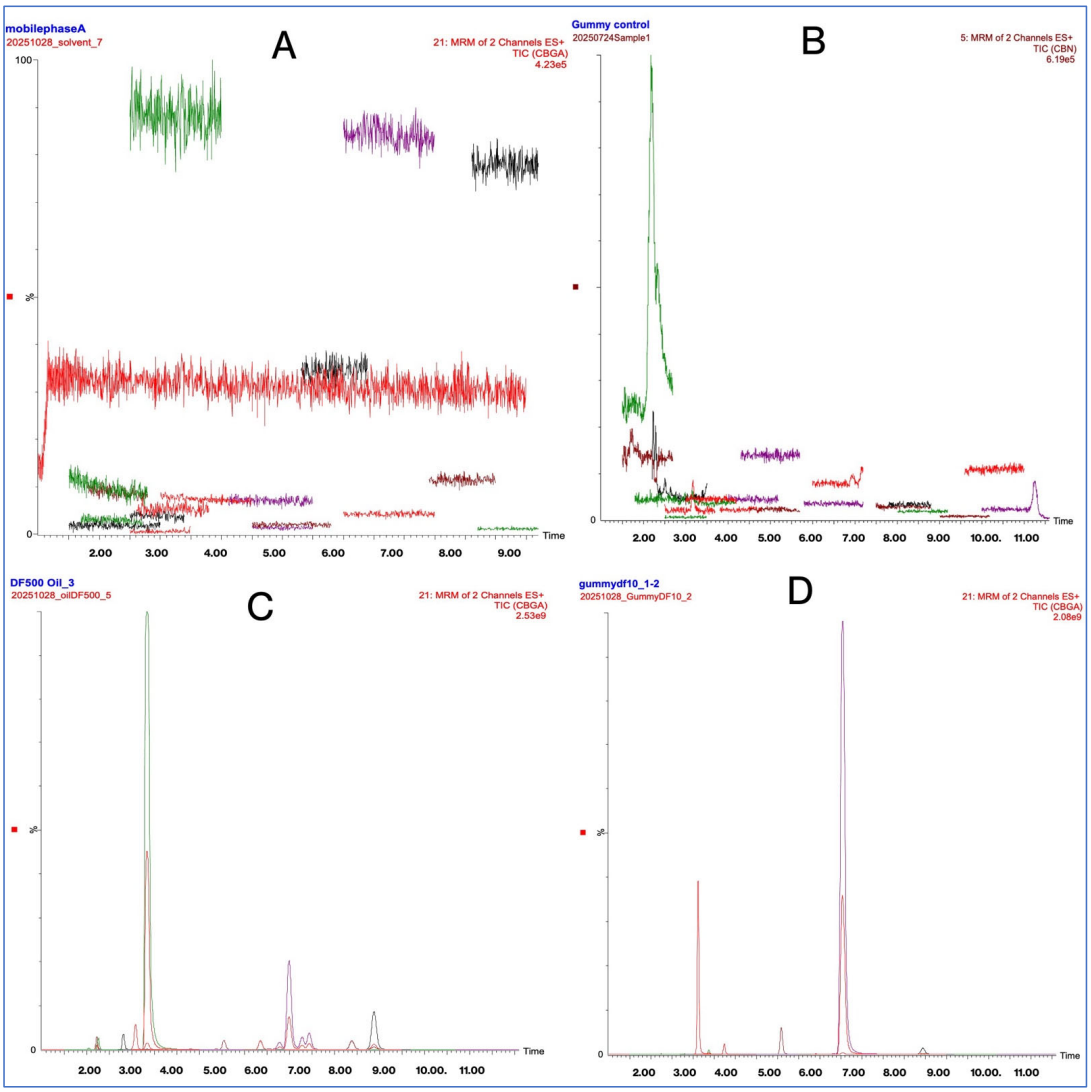


Figure 4. Overlay total ion chromatograms (TICs) of the solvent blank (**A**), gummy control (**B**), gummy sample (**C**), and oil sample (**D**) analyzed by the developed UPLC-MS/MS method.

The developed method was further applied to analyze 14 commercial hemp oil samples, and the quantitative results are summarized in Table S1. The cannabinoid profiles varied substantially among the oil samples, with concentrations spanning from ng/mL to mg/mL levels. CBD was the predominant cannabinoid in most samples, ranging from 0.012 mg/mL in Oil 6 to 45.54 mg/mL in Oil 13, indicating large differences in product potency. Δ^9-THC concentrations also varied widely, from 0.1 to 918.4 µg/mL, with the highest levels observed in Oils 3, 9, and 13. Other neutral cannabinoids, including CBC, CBN, CBG, CBDV, and CBL, were detected at variable concentrations across the samples, whereas acidic and varin-type cannabinoids were generally present at lower µg/mL or ng/mL levels. Several analytes, including Δ^8-THCV, Δ^8-THCAA, Δ^9-THCVA, Δ^8-THCVA, CBCVA, CBNA, and CBGA, were not detected in selected samples, further demonstrating the heterogeneity of commercial hemp oil compositions.

For heatmap visualization (Figure 5), duplicate measurements were averaged and concentrations were expressed as ng/mL for oils and ng/g for gummies. Non-detects and results below the LOQ were assigned zero for visualization only. Data were transformed as $\log10(x + 1)$, then standardized by cannabinoid using row-wise Z-scores. Hierarchical clustering used Euclidean distance and Ward linkage. The heatmaps describe relative compositional patterns and were not used for inferential testing.

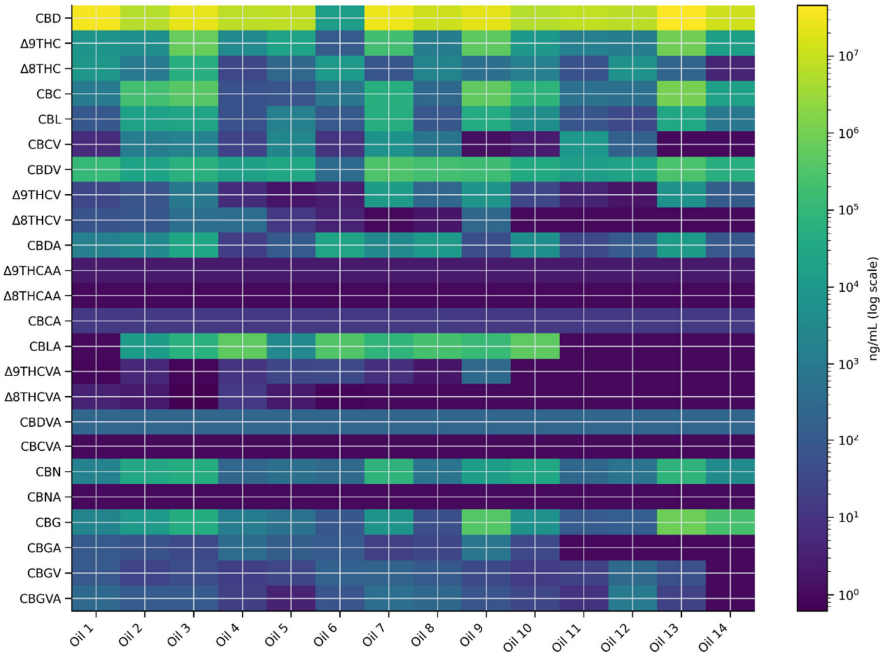


Figure 5. Heatmap visualization of 24 cannabinoids detected in 14 oil samples. Cannabinoid concentrations (ng mL^{-1}) are represented on a log-transformed scale, with color intensity indicating relative abundance. Warmer colors correspond to higher cannabinoid concentrations, whereas cooler colors represent lower concentrations.

Overall, these results demonstrate considerable variability in cannabinoid composition among commercial hemp oil products. The broad concentration range observed for both major and minor cannabinoids highlights the importance of using a sensitive and wide-range analytical method. The developed UPLC-MS/MS method was suitable for comprehensive profiling of hemp oil products, enabling simultaneous quantification of abundant cannabinoids such as CBD and THC as well as trace-level minor cannabinoids.

3.4. Potency Test of Commercial Hemp- and Cannabis-Infused Gummy Samples

The developed UPLC-MS/MS method was applied to 18 commercial hemp- and cannabis-infused gummy samples to evaluate its applicability to edible matrices. The quantitative results showed substantial variation in cannabinoid composition among the gummy products, with concentrations spanning from ng/g to mg/g levels. CBD and Δ^9-THC were the dominant cannabinoids in most samples, but their relative abundance differed markedly across products. CBD concentrations ranged from 2.6 µg/g in Gummy 1 to 9267 µg/g in Gummy 17, whereas Δ^9-THC ranged from 0.37 µg/g in Gummy 13 to 5042 µg/g in Gummy 7. These results indicate large differences in product formulation and cannabinoid potency among commercial gummy samples.

Gummies 1–10 generally showed higher Δ^9-THC concentrations, whereas Gummies 11–18 generally showed higher CBD concentrations. These groupings are descriptive and were interpreted using the product category recorded at purchase. Differences in cannabinoid profiles cannot, by themselves, establish raw-material origin, decarboxylation history, or manufacturing process.

Acidic and varin-type cannabinoids were generally present at lower concentrations and were frequently not detected. These observations describe the analyzed products only. Although processing and storage can alter cannabinoid profiles, the present data do not identify the causes of the observed differences.

To better visualize the broad concentration range and compositional differences among the gummy samples, cannabinoid concentrations were converted to ng/g and displayed using a log-transformed heatmap. As shown in Figure 6, the heatmap clearly separated samples with high Δ^9-THC abundance from those with high CBD abundance. The heatmap also highlighted sample-specific enrichment of minor cannabinoids, including CBC, CBL, CBG, CBDV, and CBCV, while acidic and varin-type cannabinoids appeared at relatively low abundance across most samples. Overall, these results demonstrate the substantial heterogeneity of cannabinoid profiles in commercial gummy products and confirm that the developed UPLC-MS/MS method is suitable for comprehensive quantification of both major and trace cannabinoids in complex edible matrices.

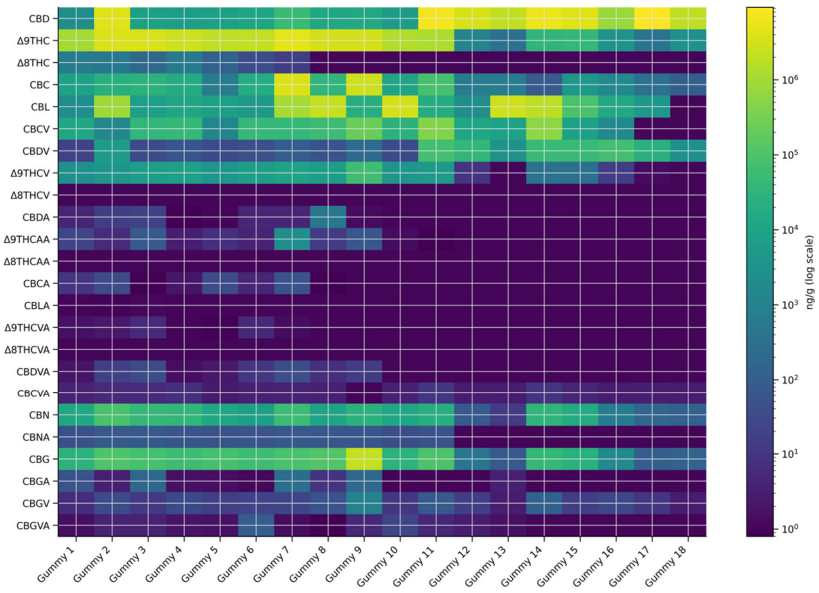


Figure 6. Heatmap visualization of 24 cannabinoids detected in 18 gummy samples. Cannabinoid concentrations (ng g^{-1}) are represented on a log-transformed scale, with color intensity indicating relative abundance. Warmer colors correspond to higher cannabinoid concentrations, whereas cooler colors represent lower concentrations.

3.5. Label Accuracy Assessment

For this study, ±10% reference limits (90–110% of the labeled cannabinoid concentration) were used to evaluate labeling accuracy. Although the U.S. Food and Drug Administration (FDA) has not established cannabinoid-specific regulatory tolerances for potency labeling, the agency emphasizes that analytical measurements used to support product labeling should be generated using scientifically valid and reliable methods [18]. In contrast, the California Department of Cannabis Control explicitly defines a labeling acceptance criterion for cannabinoids, stating that the concentration of THC, total THC, CBD, total CBD, or other claimed cannabinoids is considered accurate when the difference between the laboratory-determined value and the label claim does not exceed ±10% [19]. Therefore, the ±10% tolerance interval adopted in this study represents a regulatory benchmark currently implemented within the U.S. cannabis industry and provides a practical framework for assessing the accuracy of cannabinoid label claims across different product categories.

Figure 7 plots the percent deviation between measured and labeled cannabinoid content (y-axis, roughly −110% to 170%) for seven cannabinoids (CBD, THC, CBN, CBG, CBC, THCV, and THCVA) in commercial gummies (blue points) and oils (orange points). Dashed horizontal lines mark the ±10% descriptive limits around zero. Overall, many products fall outside this band, indicating considerable inconsistency between labeled and measured cannabinoid content, especially for CBD and the minor cannabinoids. CBD has the most data points and the widest spread: gummy values range from about −73% to over +165%, while oil values cluster mostly between about −40% and +55%, with one oil near −100%, indicating that the labeled cannabinoid was essentially undetected. For example, Gummy 3 contained 8.35 µg/g CBD versus 3.12 µg/g declared, Gummy 15 contained 4148.5 versus 1562.5 µg/g, and Gummy 16 contained 796.3 versus 2941.18 µg/g. THC points sit mostly above the upper limit, at roughly +15% to +30%, whereas CBN, CBG, and CBC points are scattered around zero with a few notable outliers, such as about +38% for CBN and about −43% for CBG. THCV and THCVA have only a few points each, several of them far below the lower limit (about −78% and −100%, respectively). All labeled and measured data are provided in the Supplementary Materials.

These findings are consistent with previous studies showing frequent discrepancies between labeled and measured cannabinoid contents in commercial cannabis and hemp-derived products. For example, Geweda et al. evaluated 107 dispensary cannabis flower samples and found that only 30% of samples were within ±20% of labeled Δ^9-THC content, while 70% failed to meet the ±20% acceptance criterion [20]. Similarly, Spindle et al. reported that among 89 hemp-derived topical products with labeled CBD amounts, only 24% were accurately labeled within ±10%, while 18% contained less CBD than advertised and 58% contained more CBD than advertised [21]. Gidal et al. also reported that 74% of 202 commercial CBD products deviated from their CBD potency label claim by at least 10%, further demonstrating widespread labeling inaccuracy among CBD products [22]. The gummy-specific findings of Johnson et al. provide a particularly relevant comparison: 70% of 56 products differed from the labeled CBD dose by more than 10%, 39% contained detectable Δ^9-THC, and individual gummies from the same container sometimes showed appreciable variability [9].

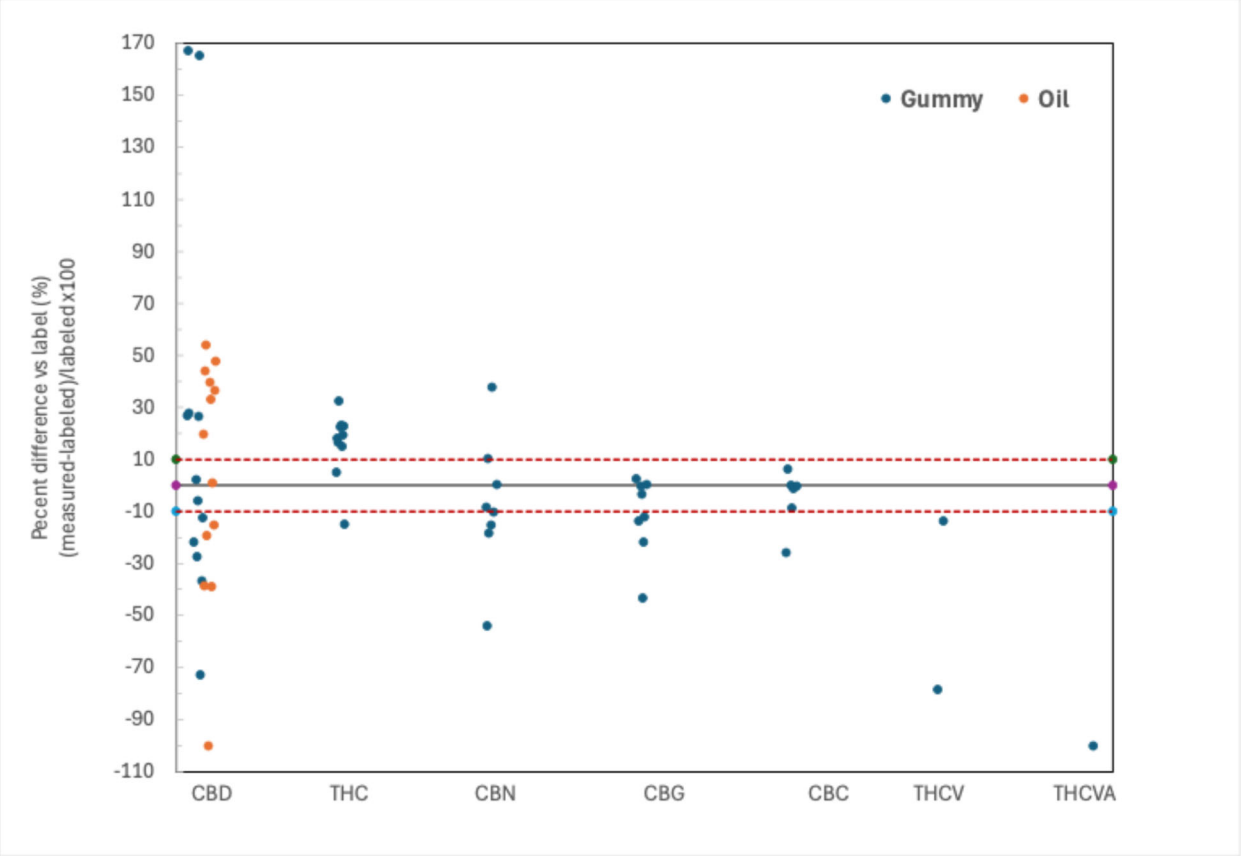


Figure 7. Percentage deviation between measured and declared cannabinoid amounts in commercial oil (n = 14) and gummy (n = 18) products. Percent deviation = 100 × (measured − labeled)/labeled and was calculated only for cannabinoids with a nonzero numerical label claim that could be converted to the same product-basis unit. Blue points represent gummies and orange points represent oils. Dashed lines indicate +10% and −10% descriptive deviation limits; they are not asserted as a universal regulatory acceptance criterion.

Other studies have reported similar labeling issues across different product categories. Melchert et al. found that among 27 products explicitly listing CBD content, only 33% matched the labeled value, whereas 26% exceeded the declared CBD content and 41% contained lower amounts [23]. Dowd et al. analyzed hemp-derived haircare, cosmetic, and edible products and reported that only 12.7% of 71 products with labeled CBD amounts were accurately labeled within ±10%, while 49% were underlabeled and 38% were over-labeled [24]. In addition, studies of broader cannabis product labeling have shown that inaccurate or incomplete labeling extends beyond potency claims. For example, Becker et al. found that unlicensed cannabis products in New York provided significantly less essential label information and fewer safety features than licensed products [25]. Limbacher et al. also reported that labeled THC values in Colorado flower and edible products were generally higher than independently measured values, highlighting ongoing concerns regarding THC label fidelity even in licensed markets [26]. Most recently, a comprehensive matrix-specific review evaluating literature from 2017 to 2025 further underscored these systemic discrepancies, finding that only 31.3% of analyzed CBD products met the standard ±10% label-accuracy threshold and emphasizing that widespread methodological heterogeneity across literature continues to limit direct comparisons among label-accuracy studies [27].

Compared with earlier studies, this work measured a broader 24-cannabinoid panel. Label agreement, however, was evaluated only for cannabinoids explicitly declared with quantitative values.

Overall, the potency-testing results demonstrate that commercial gummy and oil products can differ substantially from their labeled cannabinoid contents, especially for CBD and several minor cannabinoids. While previous studies have clearly documented mislabeling of CBD- and THC-containing products, the present study provides a more comprehensive full-spectrum assessment by simultaneously measuring 24 cannabinoids in both edible and oil matrices. These findings highlight the need for routine broad-spectrum cannabinoid testing to support accurate product labeling, quality control, regulatory compliance, and informed consumer use.

4. Conclusions

A UPLC-MS/MS method was developed and validated for simultaneous determination of 24 cannabinoids in oil and gummy products. The method provided analyte-specific calibration ranges, experimentally established LODs and LOQs, QC bias within ±20%, and generally acceptable recovery in the single gummy matrix evaluated. Chromatographic resolution was essential for isomer pairs sharing MRM transitions. Application to 14 hemp oils and 18 hemp- or cannabis-infused gummies demonstrated substantial variability among the analyzed products and identified several differences between measured and declared cannabinoid amounts. These market observations are descriptive and do not establish toxicity, consumer harm, raw-material origin, or manufacturing history. Future studies should include broader and batch-replicated sampling, matrix-matched or isotope-dilution quantification, formal ion-suppression studies across diverse formulations, and independent verification of label claims.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/foods15234308/s1.

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