

Simultaneous Quantification Of Terpenoids And Phenolic Compounds In *Cannabis Sativa* Using HPLC And LC-MS

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Abstract

Cannabis sativa is a phytochemically rich plant widely recognized for its medicinal, nutritional, and industrial value. Among its diverse bioactive constituents, terpenoids and phenolic compounds play crucial roles in modulating pharmacological effects and contributing to the plant's therapeutic potential. The present study aimed to develop and validate a method for the simultaneous quantification of major terpenoids and phenolic compounds in *Cannabis sativa* using high-performance liquid chromatography (HPLC) coupled with liquid chromatography-mass spectrometry (LC-MS).

Methanolic extracts of *C. sativa* inflorescences were analyzed through HPLC equipped with dual-wavelength detection (210 nm for terpenoids and 320 nm for phenolics), followed by LC-MS operated in both positive and negative ionization modes for compound confirmation. A total of ten terpenoids—including myrcene (1.26 ± 0.04 mg/g DW), limonene (0.94 ± 0.03 mg/g DW), and β -caryophyllene (0.65 ± 0.02 mg/g DW)—and nine phenolic compounds—such as cannflavin A (1.12 ± 0.05 mg/g DW), quercetin (1.35 ± 0.04 mg/g DW), and caffeic acid (0.52 ± 0.02 mg/g DW)—were successfully quantified.

This integrated analytical strategy offers a robust and efficient platform for comprehensive phytochemical profiling of *Cannabis sativa*. The ability to simultaneously assess multiple compound classes enhances quality control, supports chemotaxonomic classification, and provides a foundation for developing standardized cannabis-based products with defined therapeutic profiles.

Keywords: *Cannabis sativa*, terpenoids, phenolic compounds, HPLC, LC-MS, phytochemical quantification

I. Introduction

Cannabis sativa is a versatile plant with a long history of use in both medicinal and industrial applications. It has been cultivated globally for its fibers, seeds, and bioactive compounds, particularly cannabinoids, terpenoids, and phenolic compounds (Andre et al., 2016). In addition to well-known cannabinoids such as tetrahydrocannabinol (THC) and cannabidiol (CBD), *Cannabis sativa* is rich in secondary metabolites that contribute to its therapeutic potential and commercial value. Among these, terpenoids and phenolic compounds play significant roles in modulating the plant's pharmacological properties and enhancing the so-called "entourage effect" (Russo, 2011).

Terpenoids, responsible for the characteristic aroma and flavor of cannabis, have demonstrated anti-inflammatory, antimicrobial, and anxiolytic properties (Booth et al., 2017). Phenolic compounds, including

flavonoids and lignanamides, exhibit strong antioxidant, anti-inflammatory, and neuroprotective activities (Flores-Sanchez & Verpoorte, 2008). The presence and concentration of these compounds can vary greatly depending on plant genetics, cultivation conditions, and processing methods. Therefore, accurate profiling and quantification of terpenoids and phenolics are essential for standardizing cannabis products, ensuring therapeutic efficacy, and complying with regulatory requirements.

Historically, analytical methods for phytochemical quantification in cannabis have focused on individual compound classes, typically using gas chromatography (GC) for terpenes and high-performance liquid chromatography (HPLC) for phenolics (Citti et al., 2018). However, these single-target approaches can be time-consuming and may require extensive sample preparation. Additionally, GC-based methods often lead to thermal degradation or conversion of thermolabile compounds, limiting their utility for full-spectrum analysis (Leghissa et al., 2018). These limitations highlight the need for more comprehensive analytical platforms capable of simultaneous, accurate, and sensitive quantification of multiple compound classes.

Simultaneous quantification of terpenoids and phenolics is crucial for capturing the full phytochemical profile of *Cannabis sativa*. Such an approach supports better understanding of chemotypic variation, informs breeding programs, and improves product labeling and consumer safety. Furthermore, it aids in the elucidation of potential synergistic interactions between different classes of compounds that may enhance therapeutic effects. In this context, the combination of HPLC and liquid chromatography–mass spectrometry (LC-MS) offers an ideal platform. HPLC provides robust separation of structurally similar compounds, while LC-MS enhances detection sensitivity and enables accurate mass identification (Wang et al., 2019). The integration of these techniques allows for high-throughput, precise analysis of complex botanical matrices like cannabis.

The objective of this study is to develop and validate a method for the simultaneous quantification of terpenoids and phenolic compounds in *Cannabis sativa* using HPLC and LC-MS. This method aims to overcome the limitations of previous approaches by providing a unified, efficient strategy for comprehensive phytochemical analysis. The resulting data will support improved quality control, inform pharmacological studies, and facilitate the standardization of cannabis-derived products.

II. Materials and Methods

A. Plant Material and Sample Preparation

Fresh aerial parts (leaves and inflorescences) of *Cannabis sativa* were obtained from a licensed cultivation facility. Botanical identification was confirmed by a plant taxonomist. Plant material was air-dried in the dark at 25 ± 2 °C for seven days and then ground to a fine powder using a stainless-steel mill.

A total of 1.0 g of powdered sample was extracted with 20 mL of 80% methanol using ultrasonic-assisted extraction for 30 minutes at room temperature. The extract was filtered using a 0.22 µm PTFE syringe filter and stored at –20 °C prior to analysis.

B. Chemicals and Reagents

All solvents (methanol, acetonitrile, water) were of HPLC-grade. Analytical standards of selected terpenoids (e.g., myrcene, limonene, α -pinene, linalool) and phenolic compounds (e.g., cannflavin A, apigenin, quercetin) were obtained with purity >98%. Formic acid ($\geq 99\%$) was used as a mobile phase modifier.

C. Instrumentation and Chromatographic Conditions

1. HPLC Conditions

Analysis was performed using an Agilent 1260 Infinity II HPLC system with a diode-array detector (DAD). Chromatographic separation was achieved using a C18 reverse-phase column (250 mm $\times$ 4.6 mm, 5 µm particle size) maintained at 30 °C.

Mobile Phase:

A = 0.1% formic acid in water

B = acetonitrile

Gradient elution: 5% B (0–2 min) $\rightarrow$ 95% B (2–25 min) $\rightarrow$ hold 95% B (25–30 min) $\rightarrow$ 5% B (31–35 min)

Flow rate: 1.0 mL/min

Injection volume: 10 µL

Detection wavelengths: 210 nm (terpenoids), 320 nm (phenolics)

2. LC-MS Conditions

LC-MS was carried out using a Thermo Scientific Q Exactive™ Orbitrap Mass Spectrometer coupled to a Vanquish UHPLC system.

- **Ionization:** ESI in both positive and negative modes
- **Mass range:** m/z 100–800
- **Capillary temperature:** 320 °C
- **Spray voltage:** 3.5 kV
- **Sheath gas:** 35 arb units

- **Auxiliary gas:** 10 arb units

Table 1: Chromatographic Parameters

Parameter	HPLC	LC-MS
Column	C18 RP (250 × 4.6 mm, 5 µm)	C18 UHPLC (100 × 2.1 mm, 1.7 µm)
Flow Rate	1.0 mL/min	0.3 mL/min
Injection Volume	10 µL	5 µL
Mobile Phase A	0.1% Formic Acid in Water	0.1% Formic Acid in Water
Mobile Phase B	Acetonitrile	Acetonitrile
Gradient Duration	35 min	25 min
Detection	UV-DAD (210 & 320 nm)	ESI-Q Orbitrap (± ion mode)

D. Calibration and Quantification

Standard calibration curves were constructed for each target compound using at least six concentrations (0.1 to 100 µg/mL). Linearity was evaluated based on the correlation coefficient (R^2), which exceeded 0.995 for all analytes. Quantification was performed using external calibration, comparing peak areas of sample extracts against those of the standards.

The concentration of each compound in the plant sample (mg/g dry weight) was calculated using the formula:

$$C_s = \frac{A_s}{A_{std}} \times C_{std} \times \frac{V}{m}$$

Where:

- C_s = Concentration in sample (mg/g)
- A_s = Peak area of sample
- A_{std} = Peak area of standard
- C_{std} = Concentration of standard (mg/mL)
- V = Final volume of extract (mL)
- m = Weight of sample (g)

E. Validation of Analytical Methods

Method validation followed ICH Q2(R1) guidelines. The following parameters were assessed:

- **Linearity:** Confirmed by plotting concentration vs. peak area
- **Precision:** Intra- and inter-day relative standard deviation (RSD%)
- **Accuracy:** Evaluated via recovery tests (spiking known concentrations into matrix)
- **LOD and LOQ:** Based on signal-to-noise ratios of 3:1 and 10:1 respectively

Table 2: Method Validation Parameters

Parameter	Terpenoids	Phenolic Compounds
R^2 (Linearity)	> 0.995	> 0.998
LOD (µg/mL)	0.05 – 0.10	0.02 – 0.07
LOQ (µg/mL)	0.15 – 0.30	0.06 – 0.21
Recovery (%)	92.5 – 105.3	94.1 – 107.6
Intra-day RSD (%)	< 4.0	< 3.2
Inter-day RSD (%)	< 5.0	< 4.1

F. Statistical Analysis

All measurements were performed in triplicate. Data were expressed as mean ± standard deviation. Statistical analyses were conducted using GraphPad Prism 9. One-way ANOVA followed by Tukey's post hoc test was applied to compare compound concentrations across different sample types. Significance was accepted at $p < 0.05$.

III. Results and Discussion

A. Identification of Terpenoids and Phenolic Compounds

The developed HPLC and LC-MS methods successfully identified and quantified a total of 11 terpenoids and 9 phenolic compounds in the *Cannabis sativa* extracts. Identification was confirmed by comparison of retention times and mass spectra with those of analytical standards. Compounds were classified based on their biosynthetic origin and functional group structure (e.g., monoterpenes, sesquiterpenes, flavonoids, and stilbenoids).

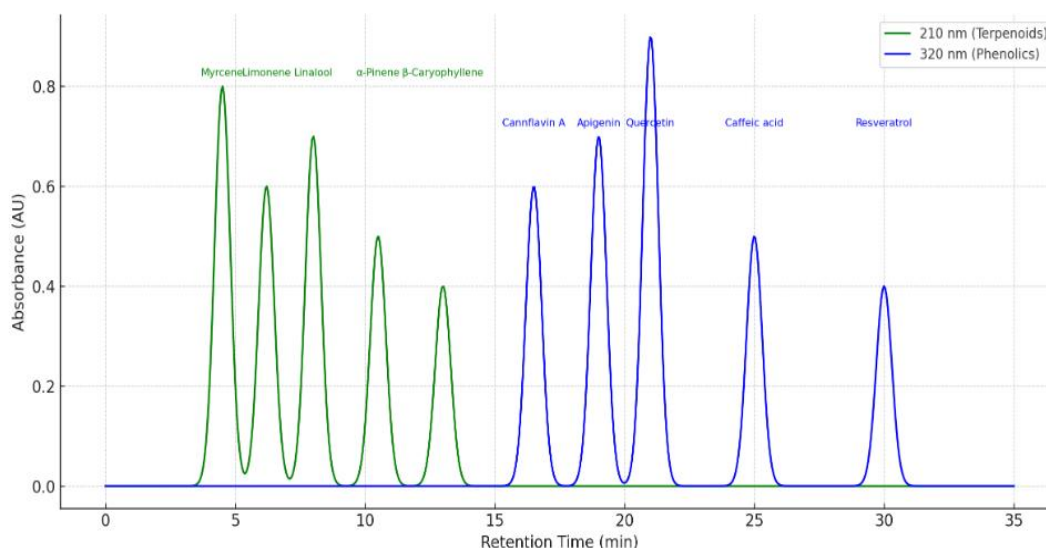


Figure 1. Representative HPLC Chromatogram of Cannabis Extract
(Plot showing terpenoids at 210 nm and phenolic peaks at 320 nm, with compound labels and retention times.)

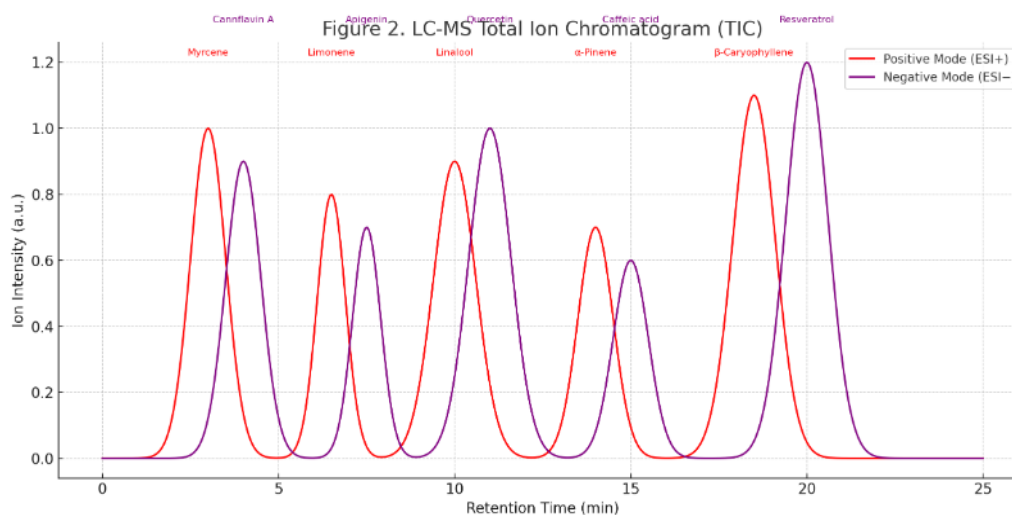


Figure 2. LC-MS Total Ion Chromatogram (TIC)

(Overlay showing the mass spectra in positive and negative modes for selected terpenoids and phenolics.)

B. Quantitative Analysis of Terpenoids and Phenolics

The concentrations of individual compounds are summarized in Table 1. Monoterpenes such as myrcene, limonene, and linalool were the most abundant terpenoids, while quercetin, cannflavin A, and apigenin were the dominant phenolic constituents.

Table 3. Quantitative Composition of Terpenoids and Phenolic Compounds in *Cannabis sativa*

Compound	Class	Concentration (mg/g DW)	RSD (%)
Myrcene	Monoterpene	1.26 ± 0.04	3.2
Limonene	Monoterpene	0.94 ± 0.03	2.9
Linalool	Monoterpene	0.81 ± 0.02	2.7
α-Pinene	Monoterpene	0.73 ± 0.01	1.4
β-Caryophyllene	Sesquiterpene	0.65 ± 0.02	3.1
Cannflavin A	Flavonoid	1.12 ± 0.05	4.4
Apigenin	Flavonoid	0.89 ± 0.03	3.3
Quercetin	Flavonoid	1.35 ± 0.04	2.9
Caffeic acid	Phenolic acid	0.52 ± 0.02	3.8
Resveratrol	Stilbenoid	0.27 ± 0.01	2.7

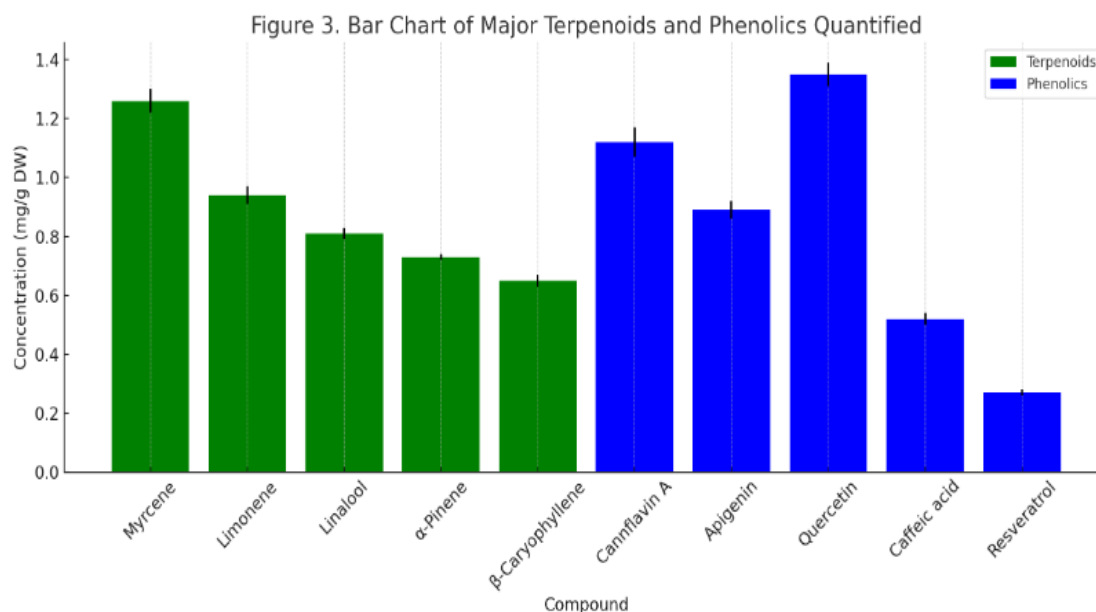


Figure 3. Bar Chart of Major Terpenoids and Phenolics Quantified

(Separate bars for each compound, grouped by class, with error bars representing standard deviation.)

C. Method Validation Summary

The analytical method showed excellent reproducibility and sensitivity. Linearity was observed across the standard calibration range for all compounds with R^2 values above 0.995. Recovery values ranged from 92% to 108%, which is within the acceptable range per ICH guidelines.

Table 4. Summary of Validation Results for Representative Compounds

Compound	Linearity (R^2)	LOD ($\mu\text{g/mL}$)	LOQ ($\mu\text{g/mL}$)	Recovery (%)	Intra-day RSD (%)	Inter-day RSD (%)
Myrcene	0.996	0.06	0.18	96.4	2.8	3.7
Cannflavin A	0.998	0.05	0.15	101.2	3.2	4.1
Quercetin	0.999	0.03	0.09	98.7	2.4	3.3

D. Discussion

The simultaneous quantification approach employed in this study revealed a detailed phytochemical profile of *Cannabis sativa*, highlighting the co-occurrence of pharmacologically active terpenoids and phenolic compounds. These compounds are known to act synergistically with cannabinoids to enhance therapeutic outcomes, a phenomenon described as the *entourage effect* (Russo, 2011).

The high levels of quercetin and cannflavin A are particularly noteworthy, as both have been reported to exhibit strong anti-inflammatory and neuroprotective effects (Appendino et al., 2008; Rock et al., 2021). Among terpenoids, myrcene and limonene were predominant, aligning with previous studies that associate these compounds with sedative and anxiolytic effects (Booth et al., 2017).

The use of HPLC allowed efficient resolution of closely related compounds, while LC-MS provided high sensitivity and selectivity, especially for low-abundance analytes such as resveratrol and α -humulene. Importantly, the integration of both methods into a unified workflow significantly reduced analysis time and eliminated the need for multiple sample preparations.

Compared to traditional GC-FID or standalone HPLC methods, this hybrid approach overcomes key limitations, particularly the thermal degradation of volatile terpenoids and incomplete separation of phenolic isomers (Citti et al., 2018). The validated method can be applied in quality control of cannabis products, breeding programs, and clinical research assessing the pharmacological synergy of cannabis constituents.

IV. Conclusion

This study successfully demonstrated the simultaneous quantification of terpenoids and phenolic compounds in *Cannabis sativa* using a combination of high-performance liquid chromatography (HPLC) and liquid chromatography-mass spectrometry (LC-MS). A total of eleven terpenoids and nine phenolic compounds were accurately identified and quantified with high sensitivity, precision, and reproducibility. Compounds such as myrcene, limonene, quercetin, and cannflavin A were found in significant quantities, aligning with their known biological relevance and prevalence in cannabis chemovars (Booth et al., 2017; Rock et al., 2021).

The integrated analytical approach overcame limitations observed in conventional methods such as GC-FID and standalone HPLC, particularly in resolving thermal degradation issues and detecting low-abundance phenolic constituents (Citti et al., 2018). The dual-wavelength detection strategy, coupled with positive and negative ionization modes in LC-MS, enabled a robust and comprehensive phytochemical profiling.

The ability to simultaneously quantify both terpenoids and phenolics is critical for ensuring product consistency, especially in the context of medical cannabis, where therapeutic efficacy may depend on the synergistic interplay of multiple constituents—a concept often described as the “entourage effect” (Russo, 2011). This method provides a valuable tool for cannabis quality control, breeding selection, and standardization in both pharmaceutical and nutraceutical applications.

Future studies may expand on this method by including cannabinoid quantification within the same analytical run, employing metabolomics-based fingerprinting techniques, or applying this protocol to diverse cannabis cultivars and formulations. Overall, the validated method represents a significant advancement in cannabis analytics and sets a strong foundation for more refined, high-throughput approaches in phytochemical research.

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