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Analytical approaches to cannabinoids detection in hemp: method development

Abstract

The renewed interest in hemp cultivation has been driven by its environmental benefits and the reintroduction of industrial hemp under controlled Δ9-tetrahydrocannabinol (Δ9-THC) levels. Compared to other natural fibers, hemp requires fewer pesticides and less water, while contributing to carbon dioxide (CO_2) sequestration and soil remediation. Despite its benefits, hemp cultivation is controversial due to the association with the production of illegal substances. Therefore, it is essential to develop accurate methods for differentiating between industrial hemp and marijuana. This article is based on existing methodologies for the detection of Δ9-THC, tetrahydrocannabinolic acid (THCa), cannabidiol (CBD), cannabidiolic acid (CBDa) and cannabinol (CBN) in hemp, aiming to develop an improved method using liquid chromatography-tandem mass spectrometry (LC-MS/MS), specifically tailored for the textile industry. Cannabinoids were quantified using calibration curves based on certified reference standards, following solid–liquid extraction of textile samples. Among the five target cannabinoids only CBDa, CBD and CBN were detected in low concentrations. The validated method proved to be robust, accurate, and suitable for routine analysis of cannabinoids in plant-derived textile materials.

Keywords: cannabinoids, Δ9-THC, hemp, LC-MS/MS, textile industry, validation

Volume 11 Issue 5 - 2025

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Received: September 17, 2025 | **Published:** October 03, 2025

Introduction

Hemp is characterized by its rapid growth and high cellulose content, ranging from 60% to 70%, which provides high-quality fibers suitable for diverse applications.[1,2] Since 5000–4000 BC, various parts of the plant have been used for spinning, weaving, papermaking, nutrition in both human and animal diets, as well as for medicinal and health-related purposes.[3–5] However, due to its psychoactive and recreational properties, caused by the high content of Δ9-THC, its cultivation was prohibited in the 20th century.[6] In recent years, there has been renewed interest in hemp, following the reintroduction of legal industrial hemp cultivation under strict Δ9-THC regulations.[7,8] Compared to other natural fibers, such as cotton, hemp requires fewer pesticides and less water resources. Furthermore, hemp offers notable environmental benefits, including its high capacity for atmospheric CO_2 absorption during the growing season and its phytoremediation potential, which contributes to soil decontamination and improvement, particularly through the removal of heavy metals.[9,10]

An additional advantage is the complete utilization of the plant, as all its parts can be used across various industries: the seeds are employed in oil production; the stalks serve as raw material for textile fibers; and the leaves and roots are utilized in the pharmaceutical and cosmetic sectors.[11,12] These features make hemp a highly valuable crop. However, its cultivation can be controversial, due to its close resemblance to the narcotic variety, commonly known as marijuana, as both originate from the same plant species, *Cannabis sativa* L. The key distinction between them lies in their cannabinoid profiles, which is crucial due to the frequent uncertainty regarding the plant material's origin.

Around 144 cannabinoids have been identified in the cannabis plant, with Δ9-tetrahydrocannabinol and cannabidiol being the most prominent and widely studied compounds.[13,14] The main differentiation between industrial hemp and narcotic/medical is regarding the Δ9-THC content. Industrial hemp must contain less than 0.3%, while non-industrial cannabis can contain between 0.5% and 5% Δ9-THC.[15,16] Regarding CBD, industrial hemp can have higher concentrations of this compound as it has no psychoactive properties.

Several analytical techniques have been described in the literature for the identification and quantification of cannabinoids present in the plant and in hemp products, including Gas Chromatography coupled with Mass Detector (GC-MS), High Performance Liquid Chromatography coupled with Ultraviolet Detector (HPLC-UV) or Diode Array Detector (HPLC-DAD), Liquid Chromatography-Tandem Mass Spectrometry, Nuclear Magnetic Resonance (^{1}H-NMR) and Fourier Transform Infrared Spectroscopy (FTIR).[13,17–19] Among these, gas chromatography is one of the most widely employed methods for determination of cannabinoids in hemp matrices.[20–22] However, injector and oven conditions may induce decarboxylation of acidic cannabinoids, namely THCa and CBDa, which, when exposed to high temperatures, are converted into Δ9-THC and CBD, respectively. To prevent this, a derivatization step is required before analysis, enabling the separate determination of THCa, CBDa, Δ9-THC and CBD.[13,17,19]

Liquid chromatography, on the other hand, allows the direct determination of these cannabinoids, as the analysis process does not involve high temperatures.[10] HPLC can be coupled with various detection, such as the ultraviolet detectors (UV) and the diode array detectors (DAD). Among these, DAD provides more comprehensive information as it covers both visible and ultraviolet spectrum. The aforementioned cannabinoids - acidic (THCa and CBDa) and neutral (Δ9-THC and CBD) - exhibit distinct spectra: acidic cannabinoids show absorption peaks around 270 nm and 310 nm, while neutral cannabinoids display absorption peaks around 220 nm.[13]

Although this technique provides results with good specificity and sensitivity for high concentrations of cannabinoids, its performance is limited for trace-level analysis. To overcome this limitation, LC-MS/MS can be employed, as it allows the detection of cannabinoids with low quantification limits.[18]

An alternative technique to gas and liquid chromatography is the nuclear magnetic resonance, which has the advantage of not requiring reference standards for compound identification.[13] Nevertheless, this technique is not sensitive to certain impurities, such as chlorophyll, naturally present in plant material and the equipment is expensive and complex to operate. FTIR has also been used for the identification of cannabinoids structure, as it is a fast, non-destructive technique that requires no sample preparation.

In this study, we describe the development, optimization and validation of a LC-MS/MS method for identifying and quantifying major cannabinoids in hemp. The method was designed to be straightforward and suitable for routine analysis for the textile industry. The study provides comprehensive information on extraction conditions, potential interferences and instrumental analysis. Method validation was performed following standard analytical guidelines, including the evaluation of selectivity, quantification limit, linearity, repeatability, reproducibility, accuracy and extract stability.

Materials and methods

Reagents and chemicals

Analytical reference standards of Δ9-THC (100 µg/mL in methanol solution; CAS 1972-08-3, purity 98.32%), CBD (CAS 13956-29-1, purity 98.68%), CBDa (CAS 1244-58-2, purity 91.7%) and CBN (CAS 521-35-7, purity 96.73%) were purchased from LGC Standards. THCa (CAS 23978-85-0, purity 97.92%) was purchased from Lipomed. CBN-D3 (100 µg/mL in methanol solution; CAS 1435934-54-5, purity 97.76%) was purchased from Supelco.

Grade II water was obtained using a MILI-RX20. LC grade methanol and acetonitrile were purchased from Fisher Scientific, formic acid (≥ 98%) was purchased from Merck and ammonium formate (≥ 98%) was acquired from Thermo scientific.

Sampling and sample preparation

Samples were obtained from licensed producers located in the southern of Portugal. Four varieties of *Cannabis sativa* L. (Futura 75, Santhica 27, Muka 76 and Fibror 79) were analysed. Plant material was collected from various locations within the plantation, without a predefined sampling scheme, to ensure representative coverage of the population.

The samples were air-dried at room temperature and subsequently ground using a cutting mill equipped with 1 mm mesh blade (Retsch, model SM2000). The milled materials were transferred into polypropylene containers and stored protected from light until extraction.

In addition to the *C. sativa* L. samples, twenty-three textile materials containing varying percentages of hemp fibers were collected from different producers and included in the survey. For the analysis of textiles, a representative test specimen was collected. The samples ware homogenized, cut into 5 mm pieces, and stored in polypropylene containers until further processing.

Analytical conditions

Cannabinoid analysis was carried out using an Agilent 1290 Infinity II system coupled to an Agilent 6475 Series triple quadrupole LC-MS. The chromatographic separation was performed on ACQUITY UPLC® BEH C18 column (100 x 2.1 mm internal diameter, 1.7 µm particle size) preceded by a ACQUITY UPLC® BEH C18 guard pre-column (5 x 2.1 mm i.d., 1.7 µm particle size). The mobile phases consisted of: (a) 5 mM aqueous ammonium formate solution buffered with 0.1% formic acid and (b) acetonitrile buffered with 0.1% formic acid. The cannabinoid method used a gradient starting at 57% (b), increasing to 70% (b) at 5 minutes, to 75% (b) at 11 minutes, to 80% (b) at 13 minutes and reverting back to 57% (b) at 14 minutes and equilibrating until 18 minutes. The flow rate was 0.25 mL/min. Column oven temperature was set to 40 °C. The autosampler was maintained at 10 °C and the volume of injection was 5 µL.

Data acquisition was performed using an Agilent 6475 Series triple quadrupole mass spectrometer, set in dynamic multiple reaction monitoring (dMRM) mode. Analyte detection was carried out using both positive (ESI^+) and negative (ESI^-) electrospray ionization modes. The following optimised instrumental parameters were applied: capillary voltage 3500 V; gas temperature 280 °C; gas flow 8 L/min; sheath gas temperature 300 °C; sheath gas flow 12 L/min. The ion transitions and MS voltage parameters are listed in Table 1. Data acquisition and processing were performed using Agilent MassHunter software. Example chromatogram is included in Figure S1 (Electronic Supplementary Material, ESM).

Table 1 LC-MS/MS acquisitions parameters for the cannabinoids

Analyte	Precursor ion	Ionisation state	Product ions	Fragmentor (V)	CAV (V)	CE (V)	Retention time (minutes)
CBD	**315**	[M + H]+	259 **193** 135 123	110	4	30	6.87
CBDa-1	**357**	[M + H]-	339 **245** 227	110 85 110	4	30 40 40	6.23
CBDa-2 [a)]	359	[M + H]+	261 219	110	4	40	
Δ9-THC	**315**	[M + H]+	259 **193** 135	110	4	30	10.4

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Table 1 Continued...

			245	110	4	40	
THCa-1	**357**	[M + H]-	213	85		35	
			191	110		40	12.62
THCa-2 [a)]	359	[M + H]+	261	110	4	40	
			219				
			293			30	
CBN	**311**	[M + H]+	**223**	92	4	22	8.99
			195			30	
CBN-D3	**314**	[M + H]+	241	100	4	18	8.92
			223			22	

Bold values indicate quantification ion parameters. CAV (acceleration voltage) and CE (collision energy) are optimized potentials.[a)] Acid cannabinoids were found to readily lose a molecule of H_2O in source, producing both molecular ion $[M + H]^+$ and $[M\text{-}H_2O + H]^+$.[18]

Method development

Quantification method: Stocks of CBD, CBDa, THCa and CBN were prepared gravimetrically on a micro-analytical balance (SARTORIUS CUBIS 1, model MSA36S-000-DH) and diluted in methanol. Working solutions containing all five target cannabinoids were prepared by proper dilution of stock solutions, with methanol to the final concentration of 5000 ng/mL. These solutions were further diluted with methanol to prepare calibration standards in the concentration range of 1-100 ng/mL (1, 3, 5, 7, 10, 25, 50, 70, 100 ng/mL), as well as quality control (QC) samples. Calibration standards were used to construct calibration curve. Quality control concentrations were determined based on the regressions equations to assess the accuracy and precision.

Deuterated CBN (CBN-D3) was selected as the internal standard (IS), with a stock solution concentration of 10000 ng/mL in methanol. Given the high concentration of cannabinoids in matrix samples, direct spiking of the IS into the matrix prior to extraction was not feasible. Consequently, all samples, standards and QC were transferred (980 µl) into LC vials, and the internal standard (20 µl, 500 ng/mL in methanol) was added prior to injection onto the LC-MS/MS system.

All stock and working solutions were stored at – 18 °C into amber glass vials and calibration standards and QC were freshly prepared on a daily basis.

Any samples with a concentration above working range for cannabinoids were further diluted in methanol into the working range. Quantification of cannabinoids in samples was based on calibration curve, with subsequent calculations accounting for the sample mass, extraction solvent volume, and dilutions, with final results reported in mg/kg.

Sample extraction: During the initial method development phase, the isolation efficiency of cannabinoids from the sample matrix was evaluated with respect to extraction efficiency and extraction duration. Two extraction solvents, methanol and acetonitrile, were compared using one sample material. Aliquots of 0.5 g of sample were extracted in duplicate with 10 mL of extraction solvent. The mixtures were placed on a mechanical shaker at 300 revolutions per minute (rpm) for 30 minutes. Then, the extracts were centrifuged for 5 minutes at 2500 rpm and filtered with a 0.20 µm filter to a LC vial and injected into an LC-MS/MS.

The sample was extracted in duplicate and analysed using the fully optimized procedure.

Method validation

During the method development stage, the chromatographic separation of the target cannabinoids and the internal standard, as well as the extraction parameters and decarboxylation conditions, were systematically evaluated and optimized. The method was then validated for selectivity, limit of quantification (LOQ), linearity of the calibration curve, precision (repeatability and reproducibility), accuracy (recovery) and extract stability.

Selectivity, linearity and limit of quantification: Selectivity was considered appropriate when the following criteria were fulfilled: a signal is visible in at least two product ion for each cannabinoid; the ratio of the peak area from the different transition reactions recorded for each cannabinoid in the sample is within ± 30% compared to the ratio of the calibration solutions; and the retention time of the analyte corresponds to the average retention time of the calibration solutions within a tolerance of ± 0.1 minutes.[23]

Linearity was verified for each cannabinoid over the range of 1 to 100 ng/mL, based on three independent solutions (n = 3) for each calibration point. Reference concentration ratios were plotted on the X-axis and the corresponding instrument response ratios for the analyte and IS were plotted on the Y-axis. All lines of best fit were calculated with linear regression and 1/x weighting following an acceptance criteria for the coefficient of determination of $R^2 \geq 0.995$.

Limit of quantification of the detector response were evaluated by a calibration curve. The LOQ is the lowest concentration at which the analyte can be quantified with a certain level of precision and accuracy. Usually, corresponds to the lowest concentration of the calibration range.[23]

Precision: Precision, including repeatability (r), was evaluated at the lowest concentration (1 ng/mL) and at two different levels in the middle of the working range (5 and 50 ng/mL) for each cannabinoid. Repeatability (intraday) was established using ten independently (n = 10) prepared solution injected once over the course of a day. Repeatability (interday) of the method was evaluated by over three days using ten repeat injections of a single control prepared fresh each day (n = 30), performed by the same operator and equipment. Reproducibility (R) was assessed by comparing the results obtained from the same samples using the identical procedure but conducted on different instrumentation within the laboratory. The method was initially developed on an Agilent 1290 Infinity II system coupled to an Agilent 6475 triple quadrupole LC-MS. The method was also tested on an Agilent 1290 Infinity II system coupled to an Agilent 6470 triple quadrupole LC-MS. Further information on chromatography and columns is included in Tables S1 to S3 (see ESM).

Acceptance criteria for repeatability were set as follows: ≤ 20% for LOQ level and ≤ 15% for the levels in the middle of the working range. For reproducibility, the criteria was set at ≤ 20%.

Accuracy: The accuracy of the method is defined as the agreement between the nominal concentration of the analyte in the sample and the value estimated by the analytical process.[23] Accuracy was evaluated using textiles fortified with different concentrations of all cannabinoids. Each sample was extracted in triplicate: one as such, the second spiked at the LOQ level (1 ng/mL) and the third containing a spiked amount of 70 ng/mL of each cannabinoid. Recoveries for each cannabinoid ranged within the tolerance range of 70 – 120% for all spiking levels.

Extract stability: Extract stability was evaluated using four *C. sativa* L. plant extracts in methanol. Duplicate samples were extracted and analysed (Time 0) and the filtered extracts were stored refrigerated at 4 °C in the dark for 5 weeks. Refrigerated extracts were re-analysed against a calibration curve prepared on the day. The results obtained at time zero were compared with those after five weeks, and the stability was expressed as the percentage difference between these measurements. Extracts were determined to be stable if the percentage between Time 0 and stability results was ≤ 10%.

Results and discussion

Extraction conditions

Solid-liquid extraction is the most widely used isolation technique for cannabinoids from plant matrix.[24] A wide variety of solvents have been employed, including methanol and ethanol, alone or in combination with other solvents such as chloroform.[25] In this study, extraction efficiency was evaluated using methanol and acetonitrile as extraction solvents.

Figure 1 and Figure 2 show the concentration of cannabinoids obtained from two *C. sativa* matrices (leaves and stems), extracted with the two solvents. Through this analysis, it was observed that methanol extraction provided greater efficiency for CBD and CBDa in the Fibror 79 leaf samples. In contrast, the concentrations of the remaining cannabinoids were comparable between the two solvents evaluated. For the Muka 76 stem samples, acetonitrile demonstrated superior extraction performance for CBN and CBDa. Regarding the other cannabinoids, concentrations were either similar across both solvents or showed a slight increase when methanol was used. Consequently, methanol was selected as the extraction solvent for the final optimized method.

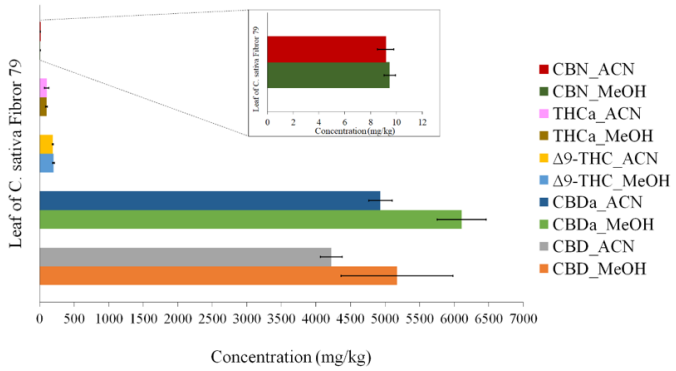

Figure 1 Comparison of extraction solvents for *C. sativa* Fibror 79 leaf. Data represent mean ± SD (n = 2).

Extraction time was also evaluated using the same matrices and methanol as solvent (Figure 3 and Figure 4). Through this analysis, it was determined that an extraction time of 30 minutes was generally optimal for all cannabinoids in high-concentration matrices, such as the Fibror 79 leaf samples. In contrast, lower concentration matrices, like the Muka 76 stem samples, exhibited greater variability in the results. Accordingly, an extraction time of 30 minutes under mechanical agitation at 300 rpm was established as the standardized condition for the extraction.

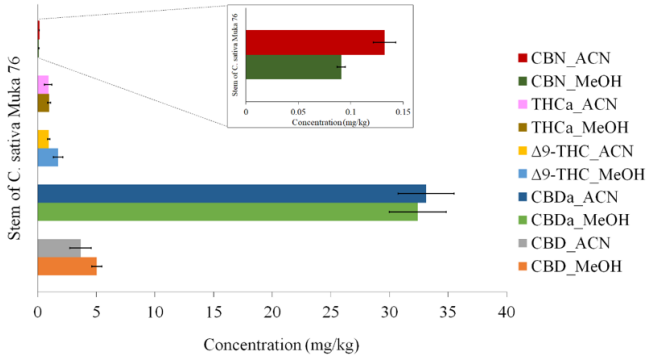

Figure 2 Comparison of extraction solvents for *C. sativa* Muka 76 stem. Data represent mean ± SD (n = 2).

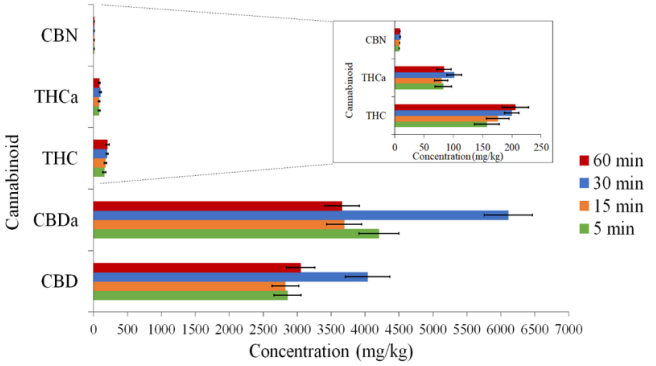

Figure 3 Influence of extraction time on *C. sativa* Fibror 79 leaf. Data represent mean ± SD (n = 2).

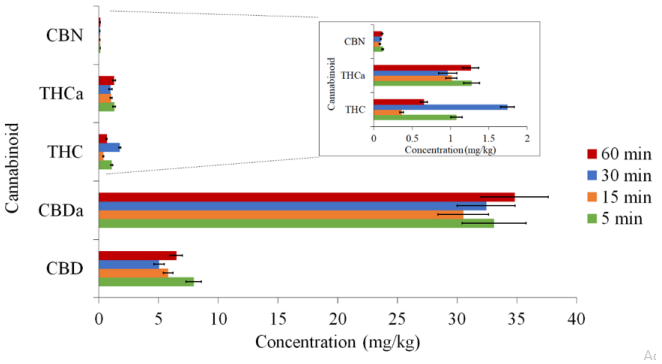

Figure 4 Influence of extraction time on *C. sativa* Muka 76 stem. Data represent mean ± SD (n = 2).

Method validation

Limit of quantification and linearity: Linearity of the detector response was demonstrated across the 1 to 100 ng/mL range (equivalent to in-sample concentrations of 0.02 to 2 mg/kg) for all five cannabinoids. Calibration curves were based on 9 concentration levels prepared in methanol and a good linearity ($R^2 \geq 0.995$) was obtained for all cannabinoids, as shown in Figs. S2 to S6 (see ESM). The LOQ of each analyte was defined as the lowest fortification level considered during validation, which is 0.02 mg/kg for the five cannabinoids studied. This value (summarized in Table S4 of ESM) was adequate for proper compliance testing with regulations on Δ9-THC levels.

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Precision: Repeatability intra and interday were measured to evaluate the performances of the method. These parameters were assessed at the lowest concentration and the middle of the working range. The intraday (CV(r)) and interday (CV(ir)) coefficients of variation for the five cannabinoids were below 8.8% at the LOQ level and below 14.2% at the two different levels in the middle of the working range (Table 2), thus meeting the recommended acceptance criteria of 15% and 20%, respectively.

Table 2 Repeatability at the low and mid concentrations

Cannabinoid	Concentration (ng/mL)	Repeatability			
		Intraday n = 10		Interday n = 30	
		r	CV(r) (%)	r	CV(ir) (%)
CBD	1	0.16	6.9	0.29	12.3
	5	0.5	4.4	1.4	11.6
	50	5.1	3.8	11.5	8.8
CBDa	1	0.15	5.2	0.27	9.5
	5	0.62	3.7	2.4	14
	50	10.1	7.7	18.5	13.5
Δ9-THC	1	0.19	8.8	0.22	9.4
	5	0.37	2.8	1.2	9.7
	50	3.3	2.2	13	9.3
THCa	1	0.17	7.6	0.28	11.8
	5	1.1	7.6	1.9	12.5
	50	5.9	4.6	13.3	10
CBN	1	0.14	7.2	0.34	14.2
	5	0.46	4	0.98	8.1
	50	3.9	3.1	5.8	4.6

Reproducibility was assessed by the agreement between the results obtained for the same sample (leaf Futura 75, seed Santhica 27 and stem Fibror 79) performed on different laboratory equipment. The CV(R) obtained in the reproducibility studies are shown in Table 3. The majority of CV(R) values for the five cannabinoids studied in the three samples were below 20%. Only two values exceeded the acceptance criteria: 22.5% for CBDa and 41.9% for Δ9-THC in the seed Santhica 27.

Table 3 Reproducibility results for *C. sativa* L

Plant *C. sativa* L.	CBD		CBDa		Δ9-THC		THCa		CBN	
	R	CV(R) (%)	R	CV(R) (%)	R	CV(R) (%)	R	CV(R) (%)	R	CV(R) (%)
Leaf Futura 75	193	1.5	362	1.4	102	14.9	27.7	7	7.4	12.5
Seed Santhica 27	11.9	11.5	41.4	22.5	3.5	41.9	1.2	14.6	0.07	13.7
Stem Fibror 79	0.28	1	11.8	6.8	0.28	19.5	0.11	2	0.04	8.8

Accuracy: Accuracy was established across the specified range of the analytical procedure. The analysis was performed using textile samples, with both spiked and un-spiked samples extracted under identical conditions. Recoveries for each cannabinoid ranged between 70 and 120% for all spiking levels.

Extract stability: The stability of the extracted samples was evaluated after five weeks of storage in a dark and refrigerated at 4 °C. Fresh controls were prepared on the day that extracts were re-analysed. Measurements of sample extracts were performed in duplicate and after storage at 4 °C for five weeks produced results within 29% of variation. These results demonstrate that the sample extracts will not be stable when stored for long periods of time. Nevertheless, extracts are not expected to be stored for such long periods during routine examinations.

Application to hemp samples: The validated method was subsequently applied to twenty-three hemp textile samples from different producers. The quantity of each cannabinoid was calculated by interpolation on the respective calibration curve. All sample results were reported in mg/kg (summarized in Table S5 of ESM). As expected, cannabinoids were not detected in most of the hemp textile samples. The highest amount of any cannabinoid found was 0.51 mg/kg of CBD. CBDa and CBN were detected in the same hemp textile at 0.19 mg/kg and 0.061 mg/kg, respectively. CBDa and CBD were detected in two other hemp textile samples at 0.028 mg/kg and 0.092 mg/kg, respectively. These results were expected, as most commercially available products contain controlled cannabinoids at trace levels, and many are labelled as cannabinoid-free.

The method developed in this study was also applied to four different *C. sativa* L. samples. Duplicate extractions of cannabis samples were analyzed on a single day.

All *C. sativa* L. samples contain quantifiable levels of cannabinoids. As demonstrated in Table 4, among the plant material analyzed in this study, CBDa was present at the highest concentrations, followed by CBD. High concentrations of Δ9-THC and CBD were found in

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two *C. sativa* L. (Futura 75 and Fibror 79). These increased levels may be attributed to the high concentrations of CBDa and THCa in these samples, as well as their storage at room temperature, which can promote the decarboxylation of cannabinoid acids to their corresponding neutral forms over time.[26] CBN was detected at low concentrations, consistent with its absence in freshly harvested and properly dried plant material. A high content of CBN generally indicates the product deterioration, as CBN is the oxidized form of Δ9-THC.[26] Furthermore, variations in CBD and THC concentrations can be influenced by multiple factors, including plant age, soil composition, geographic origin, climatic conditions (e.g., temperature and rainfall), harvest timing, and post-harvest storage conditions.[27,28]

Table 4 Cannabinoid concentrations (mg/kg) in *C. sativa* L. samples

Plant *C. sativa* L.		CBD	CBDa	Δ9-THC	THCa	CBN
Futura 75	Seed[1]	2 162 ± 455	6 149 ± 330	119 ± 18	119.3 ± 8.4	10.6 ± 1.3
	Stem[1]	3.8 ± 1.3	26.3 ± 7.3	0.137 ± 0.032	0.874 ± 0.072	0.099 ± 0.019
	Leaf[1]	4 466 ± 32	9 553 ± 1 005	199 ± 15	153.2 ± 9.8	18.5 ± 2.7
Santhica 27	Seed[1]	32.92 ± 0.45	51.5 ± 2.6	1.73 ± 0.47	3.25 ± 0.49	0.1669 ± 0.0045
	Stem[1]	0.1850 ± 0.0022	1.85 ± 0.44	n.d.[2]	0.0251 ± 0.0033	n.d.[2]
	Leaf[1]	1 287 ± 177	1 461 ± 313	45.2 ± 3.2	22.7 ± 3.2	6.752 ± 0.037
Muka 76	Stem[1]	11.2 ± 8.2	32.4 ± 3.4	5.6 ± 5.3	0.96 ± 0.17	0.0910 ± 0.0055
	Leaf[1]	12.4 ± 2.1	60.7 ± 2.3	5.06 ± 0.70	1.75 ± 0.29	0.191 ± 0.051
Fibror 79	Stem[1]	9.7 ± 1.5	58.5 ± 5.5	0.355 ± 0.084	1.24 ± 0.11	0.1720 ± 0.0021
	Leaf[1]	4 473 ± 2 134	6 001 ± 1 248	200 ± 17	102 ± 17	9.49 ± 0.62

[1]Mean ± SD (n = 2)

[2]n.d. - not detected

Overall, the results confirmed that the analyzed samples correspond to industrial hemp, as the Δ9-THC content remained below the legally established threshold.

Conclusion

The renewed interest in hemp cultivation stems from its sustainable characteristics and the recent regulatory frameworks that permit industrial hemp production under strictly limited concentrations of Δ9-tetrahydrocannabinol, a psychotropic substance. This regulatory framework has increased the demand for reliable analytical methods capable of distinguishing between industrial hemp and its narcotic counterpart, by accurately quantifying cannabinoids in *C. sativa* L. specimens.

While analytical techniques such as GC-MS and HPLC-DAD have been widely used, they often present limitations in sensitivity, specificity, or thermal stability of analyte. Liquid chromatography-tandem mass spectrometry offers significantly high sensitivity and specificity, making it particularly well-suited for detecting cannabinoids at low concentrations. However, its use in routine cannabinoid testing remains limited in the textile and industrial hemp sectors.

In this study, we developed and validated an LC-MS/MS method designed to effectively differentiate industrial hemp from medicinal cannabis. The proposed method enables the quantification of five cannabinoids within a range of 0.02 to 2 mg/kg and demonstrated satisfactory performance in terms of selectivity, stability, precision, and accuracy.

The method was successfully applied to both *C. sativa* L. plant material and hemp-derived textiles, utilizing methanol-based extraction. The results confirmed that the Δ9-THC content in the analyzed samples remained below the legally defined thresholds, thereby demonstrating the methods suitability for regulatory compliance, quality control, and routine analysis within the hemp industry.

Acknowledgments

None.

Funding

The authors acknowledge the financial support from the integrated project be@t—Textile Bioeconomy (TC-C12-i01, Sustainable Bioeconomy No. 02/C12-i01.01/2022), promoted by the Portuguese Recovery and Resilience Plan (RRP), Next Generation EU, for the period 2021–2026.

Conflicts of interest

The authors declare no conflicts of interest or competing interests.

References

1. Pierroz G. Need for speed: a breakthrough speed breeding protocol for hemp. *Plant J*. 2023;113(3):435–436.
2. Chaowana P, Hnoocham W, Chaiprapat S, et al. Utilization of hemp stalk as a potential resource for bioenergy. *Mater Sci Energy Technol*. 2024;7:19–28.
3. Kenan Y, Yağmur H, Arican E, et al. Use of cannabis as a dietary supplement. 2022.
4. Karche T, Singh MR. The application of hemp *Cannabis sativa* L. for a green economy: A review. *Turk J Botany*. 2019;43(7):710–723.
5. Schroeder M. The history of European hemp cultivation. 2019.
6. Campiglia E, Gobbi L, Marucci A, et al. Hemp seed production: Environmental impacts of *Cannabis sativa* L. Agronomic practices by life cycle assessment (LCA) and carbon footprint methodologies. *Sustainability (Basel)*. 2020;12(16):6570.
7. Naeem MY, Corbo F, Crupi P, et al. Hemp: an alternative source for various industries and an emerging tool for functional food and pharmaceutical sectors. *Processes*. 2023;11(3):718.
8. Freire HSA, da Costa MM, Rocha S, et al. Potencial de uso de cânhamo industrial (*Cannabis sativa* l.), para a produção de celulose fibra longa.

Boletim Técnico Sif. 2021;1:1–9.

9. Suardi A, Bravo I, Beni C, et al. Carbon footprint of hemp and sunflower oil in southern Italy: A case study. *Ecol Indic*. 2024;160.
10. Novaković M, Popović DM, Mladenović N, et al. Development of comfortable and eco–friendly cellulose based textiles with improved sustainability. *J Clean Prod*. 2020;267.
11. Crini G, Lichtfouse E, Chanet G, et al. Applications of hemp in textiles, paper industry, insulation and building materials, horticulture, animal nutrition, food and beverages, nutraceuticals, cosmetics and hygiene, medicine, agrochemistry, energy production and environment: a review. *Environ Chem Lett*. 2020;18(5):1451–1476.
12. Lazarjani MP, Torres S, Hooker T, et al. Methods for quantification of cannabinoids: A narrative review. *J Cannabis Res*. 2020;2(1):35.
13. Valiaparambil Sebastian JS, Dong X, Trostle C, et al. Hemp agronomy: current advances, questions, challenges, and opportunities. *Agronomy (Basel)*. 2023;13(2):475.
14. Helmi M, Aldawood A, AlOtaibi M, et al. Oral health status among recreational cannabis (marijuana and hashish) users in the USA: A NHANES–based cross–sectional study. *Saudi Dental J*. 2024;36(3):596–602.
15. Cheng YC, Miller JT, McClain K, et al. Interlaboratory validation and one–year retrospective review of hemp and marijuana decision–point assays. *Talanta Open*. 2024;9.
16. Fernández N, Carreras LJ, Larcher RA, et al. Quantification of cannabinoids in cannabis oil using GC/MS: method development, validation, and application to commercially available preparations in Argentina. *Planta Med Int Open*. 2020;07(03):e81–e87.
17. McRae G, Melanson JE. Quantitative determination and validation of 17 cannabinoids in cannabis and hemp using liquid chromatography–tandem mass spectrometry. *Anal Bioanal Chem*. 2020;412(30):7381–7393.
18. Analakkattillam S, Langsi VK, Hanrahan JP, et al. Analytical method validation for assay determination of cannabidiol and tetrahydrocannabinol in hemp oil infused products by RP–HPLC. *Sci Rep*. 2022;12(1):12453.
19. Zekič J, Križman M. Development of gas–chromatographic method for simultaneous determination of cannabinoids and terpenes in hemp. *Molecules*. 2020;25(24):5872.
20. Ahmed AQ, Noshad D, Li PCH. Quantification of cannabinoids in cultivars of *Cannabis sp.* by gas chromatography–mass spectrometry. *Chromatographia*. 2021;84(5–6):711–717.
21. Lachenmeier DW, Kroener L, Musshoff F, et al. Determination of cannabinoids in hemp food products by use of headspace solid–phase microextraction and gas chromatography–mass spectrometry. *Anal Bioanal Chem*. 2004;378(1):183–189.
22. Guidance document on analytical quality control and method validation procedures for pesticide residues and analysis in food and feed. SANTE/11312/2021. 2021.
23. Citti C, Braghiroli D, Vandelli MA, et al. Pharmaceutical and biomedical analysis of cannabinoids: A critical review. *J Pharm Biomed Anal*. 2018;147:565–579.
24. Cheng YC, Kerrigan S. Differentiation of hemp from marijuana using a qualitative decision–point assay. *Forensic Chem*. 2024;37.
25. United Nations. Recommended methods for the identification and analysis of cannabis and cannabis products: manual for use by national drug testing laboratories. 2009.
26. Berthold EC, Yang R, Sharma A, et al. Regulatory sampling of industrial hemp plant samples (*Cannabis sativa* L.) using UPLC–MS/MS method for detection and quantification of twelve cannabinoids. *J Cannabis Res*. 2020;2(1).
27. Koo YM, Ahsan SM, Bin Kwon D, et al. Quantitative analysis of cannabidiol and Δ9–tetrahydrocannabinol contents in different tissues of four cannabis cultivars using gas chromatography–mass spectrometry. *Hortic Sci Technol*. 2023;41(3):339–348.
28. Crini GE, Crini NM. Traditional and new applications of hemp. *Sustainable Agriculture Reviews 42*. 2020.