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RESEARCH ARTICLE

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Discrepancies between validated GC-FID and UHPLC-DAD methods for the analysis of Δ-9-THC and CBD in dried hemp flowers

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Abstract

Herbal products for smoking containing cannabidiol (CBD) are available as "low-tetrahydrocannabinol cannabis products" in most EU countries. In Belgium, Δ9-tetrahydrocannabinol (THC) content of these products must be less than 0.2% w/w, which is also the limit for agricultural hemp. For agricultural hemp, the official and only valid method for European regulators is gas-chromatography coupled to flame ionization detector (GC-FID). There is no such method, for smoking products. Many of these herbal for smoking products are analyzed as part of their quality control and have certificate of analysis. During surveillance by official labs, discrepancies were seen between the official results and the certificate of analysis. In this study, a GC-FID method based on the European method and an ultra-high-performance liquid chromatography coupled to diode array detection (UHPLC-DAD) method were validated and applied for samples analysis in order to investigate these discrepancies. The GC-FID method shows better results for the validation parameters; notably, it has β-expectation tolerance limits within 10% with a β value of 95% while the validated UHPLC-DAD method has β-expectation tolerance limits within 15% with a β value of 90%. Furthermore, the other parameters evaluated are generally better with the GC-FID method. The statistic *t* test shows that the difference between both methods was significantly different for total-THC, but not significantly different for the total-CBD. The authors state that, as for agricultural hemp, the GC-FID method is to be preferred for the analysis of THC and CBD in products for smoking.

KEYWORDS
agricultural hemp, comparison, GC-FID, herbal product for smoking, UHPLC-DAD

1 | INTRODUCTION

Cannabis sativa L. is one of the oldest herbaceous plants coming from Asia, cultivated for multiple agricultural and industrial applications, for dietary use and as a traditional medicine.[1,2] Phytocannabinoids represent the terpenophenolic compounds predominantly produced in cannabis, which accumulate in its glandular trichomes located in the inflorescence.[2] In general, Δ9-tetrahydrocannabinol (THC) and its precursor tetrahydrocannabinolic acid (THCA) are phytocanabinoids present in high concentration in drug-type cannabis, also known as marijuana, and in trace amounts in fiber-type, also known as industrial

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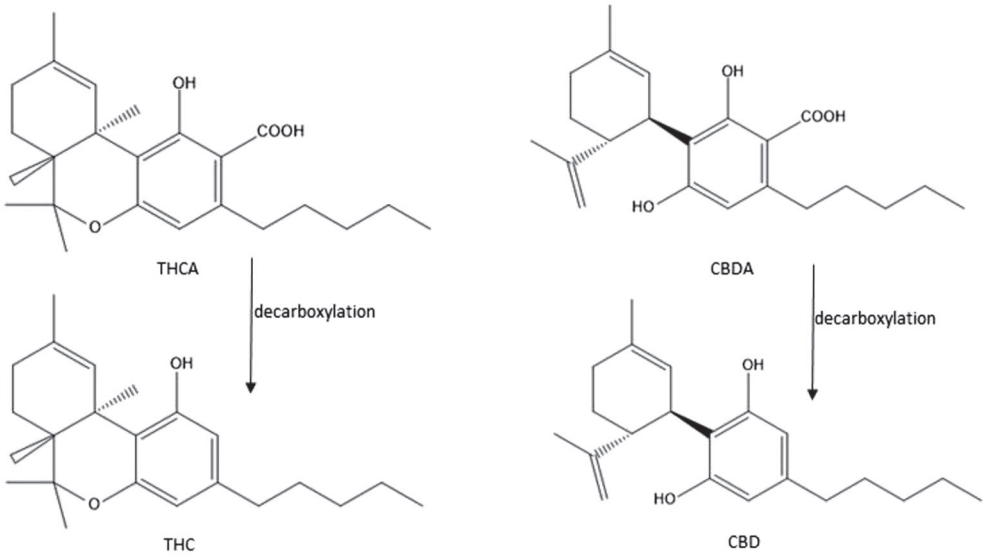

FIGURE 1 Decarboxylated THC and decarboxylated CBD and their carboxylic acid precursor

hemp.[3,4] For the latter cannabidiol (CBD) and its precursor cannabidiolic acid (CBDA) without psychoactive activity are the principal phytocannabinoids.[5] Almost no cannabinoid in decarboxylated form can be found in fresh plant material. Indeed, the carboxyl group is unstable, and therefore, decarboxylation in the decarboxylated form is easily triggered under influence of heat or light[6] (Figure 1).

The cultivation of cannabis was prohibited in many countries due to the presence of the psychoactive cannabinoid, THC.[7] Consequently, this plant is currently considered the most widely used illegal drug in Europe.[8] Further research permitted the development of new varieties leading to hemp cultivation for industrial use with a low concentration of THC.[4,7] The European Union (EU) allowed the marketing of 81 hemp varieties for hemp cultivation and limits the THC content to maximum 0.2% w/w.[9,10] Additionally, low-THC cannabis smoking products are available in most EU countries raising a number of concerns about CBD and THC levels.[11] In Belgium, as for agricultural hemp, the THC content must be less than 0.2% w/w; otherwise, these products are considered as narcotics and prohibited for sale.[12] The concerns about the levels of CBD regard the compliance of label claims, that is, whether or not the producers are misleading the consumers, concerning the labelled levels.

In the scientific literature, several analytical methods are reported, which are used for the determination of the cannabinoids of interest (CBD and THC) in these kinds of products. In the review of Citti et al., the chromatography-based techniques proved to be the methods of choice. Generally, liquid chromatography coupled to diode array detection (LC-DAD) or mass spectrometry and gas chromatography coupled to mass spectrometry (GC–MS) or flame ionization (GC-FID) were the most often used. The major difference between the two mentioned separation techniques is the high temperature employed in GC. That means that the acidic forms (THCA and CBDA), which are thermolabile, are decarboxylated during transit through the system, and so GC is only able to quantify the total-THC and the total-CBD. However, the decarboxylation rate depends on the temperature and the geometry of the injector port.[13,14] The generation of THC would be maximal at 225°C,[15] while decarboxylation of CBDA is already complete at about 100°C.

The European Union method, which is based on GC-FID, is the official method used by the competent authorities to determine the total-THC content in agricultural hemp of *Cannabis sativa* L.[16] Since information of validation was lacking, Sgro et al. evaluated the measurement uncertainty and the compliance assessment of hemp samples analyzed with the European Union method and compared data obtained with GC-FID and GC–MS.[17]

There is a need for quality and safety control of industrial/agricultural hemp and herbal products for smoking. For agricultural hemp, the European method has to be used; though for smoking products, this is not the case. A lot of the latter products come to the market with certificate of analysis, where analysis was performed with liquid chromatography methods, though during surveillance by official labs using the European GC-FID method, discrepancies were seen between the official results and the certificate of analysis, especially concerning the dosage of THC. Béres et al. claimed correctly that the preference for LC or GC methods has been discussed in only a few papers. These authors compared common methods used to analyze cannabinoids by ultra-high performance LC–MS/MS (UHPLC–MS/MS), GC–MS, and GC-FID. They evaluated and compared the selectivity, the linearity, the limit of detection (LOD), the limit of quantification (LOQ), the precision, and the accuracy for the three methods. They concluded that GC–MS was the method of choice due to its accuracy and robustness regarding the determination of cannabinoids in herbal products.[18]

In this study, the validation parameters of a GC-FID method based on the European Union method, implemented, validated, and accredited under ISO 17025 in our laboratory and a validated UHPLC-UV method were compared. Furthermore, 28 samples from agricultural hemp and products for smoking containing CBD were analyzed with the both two methods. The significance of the differences between the means of total-CBD and total-THC in samples was checked by applying a *t* test (alpha = 0.05).

2 | MATERIALS AND METHODS

2.1 | Herbal sample material

2.1.1 | Blank matrix material

Agricultural hemp materials with negligible amounts of the cannabinoids of interest were used as blank matrix for the validation. They were dried within 48 h after delivery in an oven at 68°C for 24 h according to the European standard method[19] to prevent THC oxidation. The dried material was stripped of stems and seeds to retain only the aerial part.

2.1.2 | Sample collection

Twenty-eight cannabis samples (aerial parts) were analyzed at the same time with both validated methods.

Two agricultural hemp samples were obtained from Belgian farmers and were dried within 48 h after delivery. The plants were collected in an oven tray and placed in an oven at 68°C for 24 h. Twenty-six products for smoking containing CBD were seized in shops by the Federal Agency for Medicines and Health Products (FAMHP) and were already dried by the manufacturers.

All samples were passed through a 1 mm mesh sieve. Powders were stored in a dark and dry place.

2.2 | Reagents and chemicals

Methanol absolute (HPLC grade), formic acid, ethanol absolute, dichloromethane, and acetonitrile (HPLC-S Gradient grade) were purchased from Biosolve BV (Valkenswaard, The Netherlands). Ultrapure water was obtained using a milliQ-Gradient A 10 system (Millipore, Billerica, USA). Methyl-arachidate used as internal standard was purchased from Sigma-Aldrich (purity ≥ 99%, Saint-Louis, USA).

2.3 | Standards

The standard CBD was purchased from Fagron (purity 99.1%, Nazareth, Belgium). Standard ethanol solution of THC (purity 100.9%) and CBN (purity 99.952%, powder) were purchased from Lipomed AG (Arlesheim, Switzerland). The standard acetonitrile solutions CBDA (purity 98.9%) and THCA (purity 99.2%) were purchased from Dr. Ehrenstorfer-LGC Group (Wesel, Germany).

2.4 | Solutions preparation

All solutions were prepared in brown glass volumetric flasks and stored in polypropylene tubes at −20°C.

2.4.1 | Preparation of standard stock solutions

For UHPLC-DAD analysis, four stock solutions were prepared in methanol absolute: 0.25 mg/ml of CBD, 0.25 mg/ml of CBD-A, 0.1 mg/ml of Δ9-THC, and 0.1 mg/ml of Δ9-THCA.

For GC-FID analysis, stock solutions of 2 mg/ml CBN and 2 mg/ml CBD were prepared in ethanol absolute. A calibration stock solution of 1 mg/ml and a working stock solution of 5 mg/ml of IS (methyl-arachidate) were prepared in ethanol absolute.

2.4.2 | Preparation of calibration solutions

For UHPLC-DAD analysis, the calibration standard solutions were prepared in methanol absolute from the corresponding standard stock solutions. For CBD, calibration solutions of 6.25, 12.5, 25, 50, 62.5, 75, 125, and 150 μg/ml were prepared. For CBDA, calibration solutions of 10, 40, 100, and 120 μg/ml and, for THC and THCA, calibration solutions of 5, 10, 25, 50, 75, and 100 μg/ml were prepared.

For GC-FID analysis, five calibration solutions containing 2, 40, 80, 200, and 400 μg/ml of CBN and CBD were prepared in ethanol absolute from the stock solutions of CBN and CBD. In these calibration solutions, 100 μg/ml of IS was added from the stock solution of IS.

The different concentrations were chosen in order to cover the three concentration levels validated for each method (Section 3.1.4).

2.5 | Sample preparation

Two distinct extraction procedures were used, making use of different extraction solvents. On the one hand, methanol is used for sample preparation for the UHPLC-DAD method. On the other hand, dichloromethane is used for GC-FID method. This was part of the validation file, submitted for accreditation under ISO17025 by the Belgian National accreditation body.

2.5.1 | Sample preparation for UHPLC-DAD method

In the optimized extraction procedure, a volume of 10.0 ml methanol absolute was added to 100 mg of homogeneous herbal material in a 15 ml polypropylene centrifuge tube. These were protected from light and shaken for 90 min horizontally with the oscillator (Edmund Bühler Swip SM25) set at 200 oscillations per minute. The shaken time was determined to obtain a maximum of CBD extraction. Following the extraction process, the tubes were centrifuged (Heraeus Multifuge 3S-R) for 5 min at 3000*g*-force, and the supernatants were filtered through a 0.22 μm PTFE-filter.

2.5.2 | Sample preparation for GC-FID method

An extraction procedure adapted from the European Union official method[16] for the determination of THC was optimized. A quantity of 200 mg of herbal flowers for smoking or 1 g of agricultural hemp flowers was weighted in a brown glass volumetric flask of 50 ml. To this flask, 1 ml of 5 mg/ml internal standard solution was added, and the sample was brought to volume with dichloromethane. The extraction was performed by sonication during 30 min. The supernatant was removed after waiting 5 min and filtered through a 0.22 μm PTFE-filter.

2.6 | Instrumentation and instrumental conditions

2.6.1 | GC-FID

GC-FID analysis was carried out using an Agilent technologies 5973 system with a FID detector. The instrument was equipped with a capillary column (Agilent, DB-5 ms, 30 m length, 0.25 mm internal diameter, module LTM) impregnated with an 0.25 μm phenyl-methylsiloxane film. The injector temperature was 225°C with an injection volume of 2 μl in split mode (1/20). The carrier gas (He) flow rate was set at 1.5 ml/min. The temperature gradient started at 270°C, which was held for 1 min. Then, the temperature was linearly increased at a rate of 10°C/min until 320°C. The FID detector temperature was set at 250°C. The device was controlled by MSD ChemStation software (E02.02.1431). Each sample was prepared independently in duplicate, and each was injected twice.

2.6.2 | UHPLC-DAD

UHPLC-DAD analysis was carried out using an Acquity UPLC system combined with an Acquity UPLC 2998 PDA detector from Waters (Milford, MA, USA). The output signal was monitored and processed using the Waters Empower® 3 Chromatographic Data Software.

The instrument was equipped with a CORTECS Shield RP18 90 A, 1.6 μm, 2.1 mm × 100 mm column and operated in isocratic mode with a flow rate of 0.7 ml/min and a mobile phase composed of 48% (v/v) 0.1% formic acid in water and 52% (v/v) acetonitrile. The injection volume was 5 μl, and the column temperature was set at 35°C. Cannabinoids were detected at 228 nm. Each sample was prepared independently in duplicate, and each was injected twice.

2.7 | Comparison of the methods

The UHPLC-DAD and the GC-FID method were validated separately applying the total error approach, based on accuracy profiles and the β-expectation tolerance limits. The validation parameters for both methods were compared in order to evaluate their relative performance.

Next to the comparison of the validation parameters, both methods were used to analyze 28 real samples, and statistical analysis was applied to check if the total-THC and the total-CBD content of these real samples obtained with the respective methods were significantly different or not. For that, the means were compared with a *t* test ($\alpha = 0.05$) for paired samples.[20]

2.8 | Relative response factor (RRF) determination

For GC-FID, the validation of total-THC using CBN standard was done using a RRF value of 1.018 as described by Poortman-Van der Meer et al.[21] CBD and CBN are not subjected to many restrictions and available in high purity. The replacement of the reference calibration standard was part of the validation of the method and the dossier submitted for accreditation for agricultural hemp.

3 | RESULTS

3.1 | UHPLC-DAD and GC-FID validation

Both methods were validated using the total error approach (accuracy profiles) taking into account all parameters of the ISO-17025 norm. Accuracy profiles were applied to determine the accuracy, the trueness, the precision, the linearity of the method, and the measurement uncertainties of the methods.

3.1.1 | Selectivity

For both methods, analysis of blank solvent and blank matrix showed no peaks that could interfere with the determination of the cannabinoids of interest. Figure 2a represents an example chromatogram obtained with UHPLC-DAD and shows the presence and the separation of CBD, CBDA, THC, and THCA. A GC-FID chromatogram obtained with GC-FID is shown in Figure 2b with clear separation of the internal standard, total-CBD, total-THC, and CBN.

3.1.2 | LOD and LOQ

The LOD was established as the minimum concentration of analyte that the method can detect in matrix with a signal-to-noise ratio of 3. The LOD was 0.01% (w/w) for both total-THC and total-CBD for the GC-FID method, while it was 0.02% (w/w) for CBDA and CBD, 0.01% (w/w) for THCA, and 0.03% (w/w) for THC for the UHPLC-DAD method.

The LOQ was established as the lowest amount of analyte that the method can detect in the matrix with a signal-to-noise ratio of 10. The LOQ was 0.05% (w/w) for both total-THC and CBD for the GC-FID method and 0.05% (w/w) for CBD, 0.2% (w/w) for CBDA, 0.03%

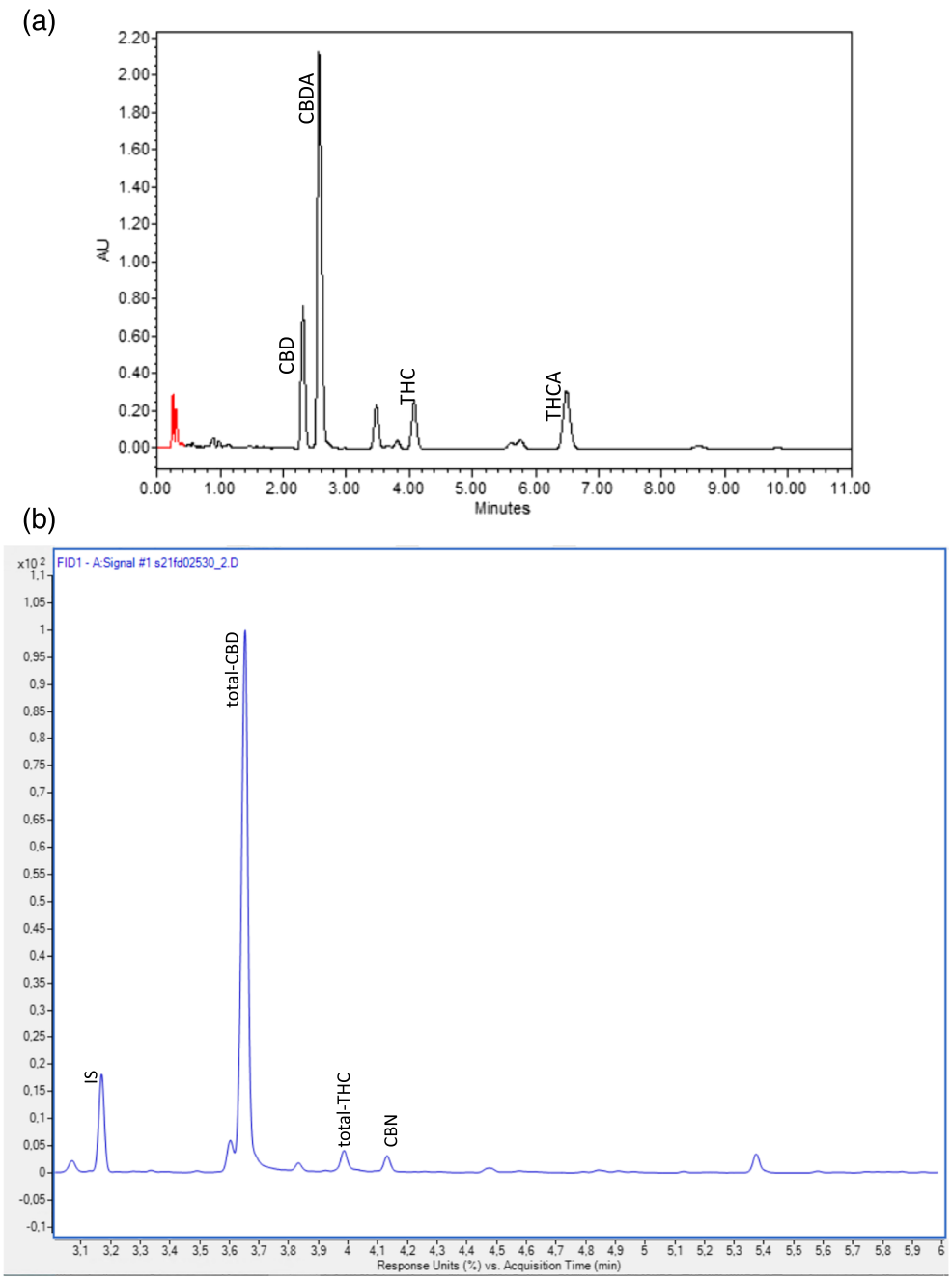

FIGURE 2 Chromatograms of UHPLC-DAD (a) and GC-FID (b) methods showing cannabinoids of interest [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 R^2 and QC values from standard calibration curves obtained with UHPLC-DAD and GC-FID methods

Standard	R^2	QC (%)
UHPLC-DAD		
CBD	0.9995	1.95
CBDA	0.9979	1.64
THC	0.9994	2.24
THCA	0.9990	3.03
GC-FID		
Total-CBD	0.9934	2.91
Total-THC	0.9934	1.76

(w/w) for THCA, and 0.06% (w/w) for THC with the UHPLC-DAD method.

3.1.3 | Linearity of the calibration curves

For UHPLC-DAD and GC-FID, the linearity of the standard calibration curves was validated. Student's t test ($\alpha = 0.05$) was used to test for significance of a quadratic model and revealed that the quadratic coefficient was not significantly different from 0. The quality coefficient (QC)[22] and coefficients of determination (R^2) values are shown in Table 1. The maximal QC value was 3%, and all R^2 values were higher than 0.99 for UHPLC-DAD and GC-FID.

TABLE 2a Trueness, precision, accuracy, and uncertainty of the method for total THC and total CBD for GC-FID method

Compound	Trueness Relative bias (%)			Precision						Accuracy β-expectation limits (%) (β 0.95)			Uncertainty Relative expanded uncertainty (%)		
				Repeatability (RSDr %)			Intermediate precision (RSD %)								
Concentration level (%)	0.2	1	6	0.2	1	6	0.2	1	6	0.2	1	6	0.2	1	6
Total-THC	−1.03	−5.37	−2.73	0.30	0.31	0.59	2.91	0.98	1.83	[−9.84; 7.78]	[−8.22; 2.52]	[−8.02; 2.55]	6.37	2.14	3.99
Concentration level (%)	1	4	10	1	4	10	1	4	10	1	4	10	1	4	10
Total-CBD	−3.98	−5.31	−3.74	0.46	0.63	0.30	1.02	0.95	1.23	[−8.10; 0.14]	[−8.33; −2.30]	[−9.55; 2.07]	2.31	2.11	2.82

TABLE 2b Trueness, precision, accuracy, uncertainty of THCA, neutral THC, CBDA, and neutral CBDD for UHPLC-DAD method

Compound	Trueness Relative bias (%)			Precision						Accuracy β-expectation limits (%) (β 0.9)			Uncertainty Relative expanded uncertainty (%)		
				Repeatability (RSDr %)			Intermediate precision (intermediate RSD %)								
Concentration level (%)	0.1	0.2	0.3	0.1	0.2	0.3	0.1	0.2	0.3	0.1	0.2	0.3	0.1	0.2	0.3
THCA	2.93	2.27	0.12	1.23	1.23	2.03	1.23	1.37	2.03	[0.41; 5.45]	[−0.64; 5.19]	[−3.94; 4.17]	2.66	3.00	4.28
Neutral THC	−0.57	2.21	−0.10	2.69	1.50	1.67	3.63	2.01	2.02	[−9.04; 7.91]	[−2.60; 7.03]	[−6.75; 6.55]	7.95	4.51	4.56
Concentration level (%)	0.2	1	2	0.2	1	2	0.2	1	2	0.2	1	2	0.2	1	2
CBDA	−0.24	0.40	0.63	3.81	1.87	1.15	3.81	2.86	4.08	[−9.00; 8.51]	[−7.19; 7.98]	[−13.13; 14.39]	8.21	6.45	9.43
Concentration level (%)	0.5	2	5	0.5	2	5	0.5	2	5	0.5	2	5	0.5	2	5
Neutral CBD	−1.02	3.28	2.2	2.45	0.76	1.17	4.61	3.40	2.72	[−11.70; 9.66]	[−5.91; 12.48]	[−5.03; 9.43]	10.02	7.82	6.15

TABLE 3 Concentration of total-CBD (a) and total-THC (b) obtained for the two validated methods in 28 cannabis-based products (expressed as mean ± standard deviation)

(a)

				GC-FID		UHPLC-DAD	
Sample code	Sample type	Sample label	Total-CBD (declared) (%w/w)	Total-CBD (%w/w)	Recovery with total-CBD declared (%w/w)	Total-CBD (%w/w)	Recovery with total-CBD declared (%w/w)
1	Smoking product	Caramel candy	3.50	3.40 ± 0.09	97.14	4.0 ± 0.2	114.3
2	Smoking product	OG haze	2.80	2.03 ± 0.08	72.05	2.43 ± 0.07	86.79
3	Smoking product	Lemon haze	/	2.9 ± 0.2	/	3.49 ± 0.02	/
4	Smoking product	Caramel candy	3.5	5.7 ± 0.2	162.9	6.2 ± 0.3	177.1
5	Smoking product	OG haze	2.80	2.10 ± 0.04	75	2.54 ± 0.06	90.71
6	Smoking product	Cheese	3.80	2.77 ± 0.04	72.89	3.6 ± 0.3	94.7
7	Smoking product	Lemon haze	/	5.6 ± 0.2	/	6.6 ± 0.3	/
8	Smoking product	OG haze	2.8	2.90 ± 0.04	103.57	4.4 ± 0.3	157.1
9	Smoking product	OG haze	/	2.51 ± 0.08	/	3.7 ± 0.4	/
10	Smoking product	OG haze	/	2.1 ± 0.1	/	2.8 ± 0.2	/
11	Smoking product	Lemon haze	/	4.4 ± 0.1	/	5.0 ± 0.1	/
12	Smoking product	Cheese	/	2.74 ± 0.04	/	3.45 ± 0.03	/
13	Smoking product	Cannatonic	3.5	3.4 ± 0.2	97.1	4.0 ± 0.6	114.3
14	Smoking product	Ghost train haze	18.0	10.2 ± 0.3	56.67	5.9 ± 0.2	33
15	Smoking product	Ghost train haze	18.0	10.0 ± 0.2	55.6	5.65 ± 0.03	31.4
16	Smoking product	Caramel candy	3.50	3.91 ± 0.04	111.71	2.50 ± 0.09	71.43
17	Smoking product	Candy kush	/	2.7 ± 0.1	/	3.60 ± 0.03	/
18	Smoking product	Candy kush	/	2.45 ± 0.08	/	3.3 ± 0.2	/
19	Smoking product	Lemon haze	/	3.3 ± 0.5	/	4.35 ± 0.02	/
20	Smoking product	Candy kush	3.7	2.8 ± 0.1	75.7	3.84 ± 0.07	103.78
21	Smoking product	Ghost train haze	18	10.34 ± 0.08	57.44	5.5 ± 0.2	30.6
22	Smoking product	/	/	13.8 ± 0.3	/	7 ± 3	/
23	Pot pourri	/	/	2.8 ± 0.1	/	3.7 ± 0.1	/
24	Agricultural hemp	/	/	0.36 ± 0.06	/	0.58 ± 0.05	/

(Continues)

TABLE 3 (Continued)

(a)

Sample code	Sample type	Sample label	Total-CBD (declared) (%w/w)	GC-FID		UHPLC-DAD	
				Total-CBD (%w/w)	Recovery with total-CBD declared (%w/w)	Total-CBD (%w/w)	Recovery with total-CBD declared (%w/w)
25	Agricultural hemp	/	/	1.31 ± 0.08	/	2.0 ± 0.1	/
26	Smoking product	/	/	3.50 ± 0.05	/	3.16 ± 0.01	/
27	Smoking product	/	/	2.35 ± 0.09	/	4.05 ± 0.01	/
28	Smoking product	/	/	4.0 ± 0.1	/	3.55 ± 0.01	/

(b)

Sample code	Sample type	Sample label	Total-THC declared (%w/w)	GC-FID		UHPLC-DAD	
				Total-THC (%w/w)	Recovery with total-THC declared (%w/w)	Neutral-THC[a] (%w/w)	Recovery with total-THC declared (%w/w)
1	Smoking product	Caramel candy	0.18	0.18 ± 0.03	100	0.102 ± 0.003	57
2	Smoking product	OG haze	0.11	<LOQ		<LOQ	
3	Smoking product	Lemon haze	/	<LOQ		0.0576 ± 0.0008	/
4	Smoking product	Caramel candy	0.18	0.184 ± 0.008	102	0.17 ± 0.02	94
5	Smoking product	OG haze	0.11	0.051 ± 0.003	46	0.052 ± 0.001	47
6	Smoking product	Cheese	0.17	0.193 ± 0.007	114	0.040 ± 0.002	24
7	Smoking product	Lemon haze	/	0.096 ± 0.003	/	0.117 ± 0.006	/
8	Smoking product	OG haze	0.11	0.23 ± 0.03	209	0.063 ± 0.007	57
9	Smoking product	OG haze	/	0.064 ± 0.004	/	0.063 ± 0.007	/
10	Smoking product	OG haze	/	<LOQ	/	0.030 ± 0.002	/
11	Smoking product	Lemon haze	/	0.32 ± 0.02	/	0.095 ± 0.003	/
12	Smoking product	Cheese	/	0.196 ± 0.009	/	0.056 ± 0.002	/
13	Smoking product	Cannatonic	0.13	0.081 ± 0.004	62	0.063 ± 0.003	78
14	Smoking product	Ghost train haze	0.2	0.27 ± 0.02	135	0.069 ± 0.008	35
15	Smoking product	Ghost train haze	0.2	0.181 ± 0.008	91	0.082 ± 0.002	45
16	Smoking product	Caramel candy	0.18	0.146 ± 0.003	81	0.055 ± 0.002	31
17	Smoking product	Candy kush	/	<LOQ		0.07 ± 0.01	/

TABLE 3 (Continued)

(b)

Sample code	Sample type	Sample label	Total-THC declared (%w/w)	GC-FID		UHPLC-DAD	
				Total-THC (% w/w)	Recovery with total-THC declared (%w/w)	Neutral-THC[a] (%w/w)	Recovery with total-THC declared (%w/w)
18	Smoking product	Candy kush	/	<LOQ		0.050 ± 0.004	/
19	Smoking product	Lemon haze	/	<LOQ		0.068 ± 0.003	/
20	Smoking product	Candy kush	0.16	<LOQ		0.061 ± 0.001	38
21	Smoking product	Ghost train haze	0.2	0.233 ± 0.002	117	0.090 ± 0.002	45
22	Smoking product	/	/	0.136 ± 0.001	/	0.163 ± 0.006	/
23	Pot pourri		<0.2	<LOQ		0.06 ± 0.005	<0.2
24	Agricultural hemp	/	/	<LOQ		<LOQ	/
25	Agricultural hemp	/	/	<LOQ		0.05 ± 0.03	/
26	Smoking product	/	/	0.084 ± 0.002	/	<LOQ	/
27	Smoking product	/	/	0.063 ± 0.002	/	<LOQ	/
28	Smoking product	/	/	0.110 ± 0.002	/	0.063 ± 0.003	/

[a]For UHPLC-DAD, THCA is <LOD, and total THC is represented by neutral THC.

3.1.4 | Accuracy profiles

Accuracy profiles are a visual representation of the method performance based on the β-expectation tolerance interval and the concept of total error,[23] which integrates trueness, precision, and accuracy in one statistic. Three levels of concentration were used to build accuracy profiles for GC-FID: 0.2–1–6% (w/w) for THC and 1–4–10% (w/w) for CBD. Three levels of concentration were also used to build the UPLC-DAD accuracy profiles: 0.5–2–5% (w/w) for CBD, 0.2–1–2% (w/w) for CBDA, and 0.1–0.2–0.3%(w/w) for THC and THCA. The selected levels were based on the levels encountered during routine analysis at the laboratory as well as values found in literature.[24]

Accuracy, trueness, precision, and measurement uncertainty[25] were determined for both methods. In this section, the discussion is about the legal value of the psychoactive substance THC (0.2% w/w) and values around 5% w/w of CBD. All results are available in Tables 2a and 2b.

Linearity of the results were acceptable with all R^2 values above 0.99.

Precision

Precision is expressed as a relative standard deviation (RSD) of the test results.[26]

In general, it can be observed that precision decreases with the concentration level of analyte. Also, precision is lower for UHPLC-DAD, due to the fact that total-THC and total-CBD are determined by the quantification of THC and THC-A and CBD and CBDA, respectively, while in GC-FID, total-THC and total-CBD are determined in a more direct way.

It might be concluded that these methods were comparable, and these values are considered as acceptable in terms of precision.

Trueness

The trueness[27] is the degree of closeness between the average of measurements and the known true value. It is expressed as relative bias (%).

It can be concluded that values are considered as acceptable since the relative bias is limited to [−3.98; −1.03] for GC-FID method and [−1.02; 3.28]. The results show that the GC-FID method tends to underestimate the CBD and total-THC quantity since the β-expectation limits show a download trend while the UHPLC-DAD tends to overestimate these molecules because of an upward trend.

Accuracy

The β-expectation tolerance limits were set at 95% and 90% and the acceptance limits at 10 and 15% for GC-FID and UHPLC-DAD,

Cannabinoid	Total-CBD		Total-Δ9-THC	
Method	GC-FID	LC-UV	GC-FID	LC-UV
Mean	4.152264787	3.967633065	0.1564	0.07781
Variance	9.809722623	2.16841635	0.00597	0.00155
Observations	28		18	
t Stat (absolute value)	0.461823663		4.35878	
P (*T* ≤ *t*) two-tail	0.647907343		0.00043	
t Critical two-tail	2.051830516		2.10982	

TABLE 4 Paired *t* test for total-CBD and total-Δ9-THC for the two validated methods

respectively. Considering the matrix, these settings were considered as acceptable. The β-expectation tolerance interval did not exceed the acceptance limits fixed for each method.

Measurement uncertainty

The relative expanded uncertainty was below 10.02 for neutral CBD in UHPLC-DAD method and below 6.37 for total-THC in GC-FID method.

The natural variability of samples and the intrinsic uncertainty of the measurement are closely related,[28] and therefore, the obtained values for both methods could be accepted.

3.2 | Samples analysis

The main goal of this work was to do an intra-laboratory comparison analysis of the same samples at the same moment with the two validated methods to quantify total-CBD and total-THC.

Samples (see Section 2.1.2.) were analyzed by GC-FID and UHPLC-DAD, and the averages of total-THC and total-CBD were calculated. The concentrations of the decarboxylated form and the acidic forms are added up by using 0.877 corresponding to the ratio between the molecular mass of the decarboxylated form and the acidic form. The total content of THC was calculated in multiplying the acidic form by 0.877 following by the addition of the decarboxylated form. The results are shown in Table 3a,b. Concentrations of CBDA and neutral CBD are shown in Table S1. Indeed, THCA was always lower than LOQ, and the total-THC was therefore represented by THC.

Afterwards, statistical tests were applied to evaluate the significance of the difference between the mean total-THC and total-CBD obtained by the two validated methods for the same samples. Since there are 10 samples with a total-THC content lower than the LOQ, only 18 samples were taken in account for the comparison of total-THC averages between the two methods. Results of the statistical tests are shown in Table 4.

The paired two sample for means *t* test showed that the *p*-two-tail value is equal to 0.0004 for total-THC, which is lower than the level of significance α (=0.05) and 0.65 for total-CBD, which are higher than the level of significance α (=0.05). The *t* Stat value is between the (−) *t*-critical two-tail and the (+) *t*-critical two-tail values for total-CBD but is not included in the range for total-THC. Therefore, the null hypothesis is not rejected for total-CBD but is rejected for total-THC. The observed difference between the sample means is convincing enough to say that averages of total-CBD concentration between the two validated methods do not differ significantly but differ significantly for total-THC. It shows that the results obtained with the two methods were close to each other but there are some random variances between some samples.

4 | CONCLUSION AND DISCUSSION

Based on the above, it can be stated that both methods showed comparable validation parameters and therefore can both be considered fit-for-purpose for the analysis for total-THC and total-CBD content. Though it has to be pointed out that for the accuracy profiles the β-expectation tolerance was set at 90% with acceptance limits of 15% for UHPLC-DAD, while for GC-FID these parameters were fixed at 95% and 10%, respectively, pointing at a better performance of the GC-FID method for these analysis.

Also from the validation results, it can be seen that the used UHPLC-DAD method tends to overestimate the total-CBD and total-THC content, while the GC-FID has more a tendency of underestimation. These differences are not significant, but can cause problems in this context, especially with the THC content. Generally, the THC content is low, and also the legal limit, to which the products have to be checked, is very low. This means that for products having a THC content close to the limit, discrepancies can occur between both methods, since for the one, the method complies with the legislation, while for the other, it is not. This problem can partly be resolved by taking into account the expanded measurement uncertainty in the decision of compliance. On the other hand, the choice of a standard method for this type of analysis, leading to the same way of working between batch release laboratories and control laboratories, would be a better alternative.

This is also reflected by the results, obtained during real sample analysis. When exploring the results obtained for THC, it can be seen that for several samples, there is a clear difference, and statistical tests for the series of results indicate that these differences are significant. These differences can have different origin: (1) in LC, THC and THC-A

are quantified separately, which mean larger errors and lower quantities to be determined in LC than in GC, since in GC, the total-THC is determined directly. (2) The analysis concerns a herbal matrix, and although it is grinded and sieved, inhomogeneity can affect the extraction process of cannabinoids,[16] especially when the content in cannabinoids is low, like THC(A) in this case.[29] (3) THC(A) amounts are very low, which means that higher measurement errors can occur. The closer you get to the LOQ the more influence small errors will have. THCA is in very low quantity in samples and the UHPLC-DAD method shows a signal lower than the LOQ and could not take it into account in the total-THC calculation. The total-THC is therefore represented by the neutral THC and underestimates the real concentration in THC. The influence of the small amounts to be quantified is also shown by the results of CBD. Here results are much closer to each other for the majority of the samples, although also here some samples show clearly different results.

The label shows generally the "strain" name (e.g., Candy kush) referring to genetic lines of cannabis. Plants are living organisms and have a biological variation and materials taken from the same species give not necessarily the same chemical content. Independently of genetic or species, environmental and growing conditions involve an impact of the end product.[30] Growing of uniform plant material is one major factor to solve it, using cultivation indoor and in greenhouses to control environmental conditions.[31] In this way, the cultivation process offers a better standardization of cannabinoids.[32] However, there is not a control system to check if these parameters were implemented for these kinds of samples.

To conclude, it can be stated that despite the validation of both methods with spiked samples and the statistical evaluation of the results obtained with both methods, differences between the quantification of cannabinoids with GC and LC cannot be neglected. The major causes of this are probably the fact that neutral and acidic forms are determined separately in LC and the fact that in the sample set, more variability in herbal matrices is present than in the spiked samples for validation.

Based on the above, the authors are in favor of the GC-FID method based on the European method, obliged for the analysis of industrial hemp, to be applied to analyze smoking products, derived from *Cannabis Sativa* L. If both producers/distributors as control laboratories and agencies use the same method for analysis, less discrepancies, less discussion, and less products withdrawal from the market will occur. This is both in favor of producers/distributors as of controlling and inspection services.

A supplementary advantage of the GC-FID method is that it can be used with cannabinol as calibration standard, allowing a serious saving of resources for both parties in this context.

DATA AVAILABILITY STATEMENT

Research data are not shared.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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