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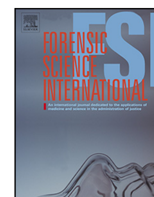

Validation of an UHPLC/DAD method for the determination of cannabinoids in seized materials: Analysis of 213 samples sold in Belgian CBD shops

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ABSTRACT

Purpose: Nowadays, (−)-cannabidiol (CBD) is gaining popularity for the treatment of various problems and can be found easily in many stores in Belgium. However, such product must comply with the law: if the total tetrahydrocannabinol (THC) content [(−)-Δ9-tetrahydrocannabinol + (−)-Δ9-tetrahydrocannabinolic acid A (THC-A)] is higher than 0.2%, it is considered as narcotic by the Belgian legislation. In this context, we have developed a method to quantify major cannabinoids (THC, THC-A, CBD, cannabidiolic acid, cannabigerolic acid, cannabigerol and cannabinol) in plant material.
Methods: After drying, a liquid-liquid extraction was performed on plant materials, followed by dilutions. Extracts were analyzed by ultra-high-performance liquid chromatography combined with a photodiode array detector. Mobile phases consisted of methanol and 0.1% formic acid in water applied in a 16-minute gradient mode. After validating the method, it was applied to 213 samples seized by the police in CBD shops.
Results: The method fulfilled the criteria in terms of specificity, calibration curve, precision, trueness and dosing range. Total THC content ranged from 0.14 to 1.17% (median 0.38%) with 110 samples exceeding the Belgian legal threshold of 0.2%. The amounts measured in the samples varied greatly, some were 6 times below the amount labelled on the packaging, others showed a concentration 4 times higher than stated on the package. Same strain also showed concentration differences from shop to shop.
Conclusion: Our method was successfully validated and applied to samples seized in CBD shops. Half of the products exceeded the Belgian legal threshold. THC and CBD concentrations discrepancies showed that products sold in CBD shops are not pharmaceutical grade.
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1. Introduction

(−)-Cannabidiol (CBD) is one of the 90 phytocannabinoids found in cannabis plants. CBD, as well as (−)-Δ9-tetrahydrocannabinol (THC) is derived from cannabigerolic acid (CBG-A) [1]. In the plant, CBD and THC are synthesized in carboxylated forms, called cannabidiolic acid (CBD-A) and (−)-Δ9-tetrahydrocannabinolic acid A (THC-A), respectively. These three acidic forms (see Fig. 1 from [2,3]) can easily transform into the corresponding neutral forms if they are put under the influence of heat or light, for example: smoking or storage [4]. Finally, there are two other cannabinoids found in aged cannabis: cannabinol (CBN), produced by oxidation of THC and (−)-Δ8-tetrahydrocannabinol produced by isomerization of Δ9-THC [5].

Cannabis is the most commonly tried out and the most widely used illicit drug in Europe [6]. THC is the component which is responsible for the psychoactive effects, see [7]. In Belgium, there is only one registered medicine containing cannabinoids. The so-called *Sativex*® which contains almost the same amount of THC and CBD (2.7 and 2.5 mg/100 μL, respectively). This medicine is indicated in the treatment of spasticity observed in multiple sclerosis [8]. CBD was extensively studied for the treatment of various forms of epilepsy. In June 2018, *Epidiolex*® (oral solution of CBD 10 mg/100 μL) was approved by the Food and Drug Administration for the treatment of seizures associated with Dravet and Lennox-Gastaut syndromes [9]. In September 2019, it was also approved by the European Medicines Agency for the same indications [10].

Although less studied than epilepsy, many other health issues can potentially be treated with CBD. CBD is considered as relatively safe (only benign adverse reactions are reported) and shows neuroprotective, analgesic, anxiolytic, antioxidant, anti-inflammatory and

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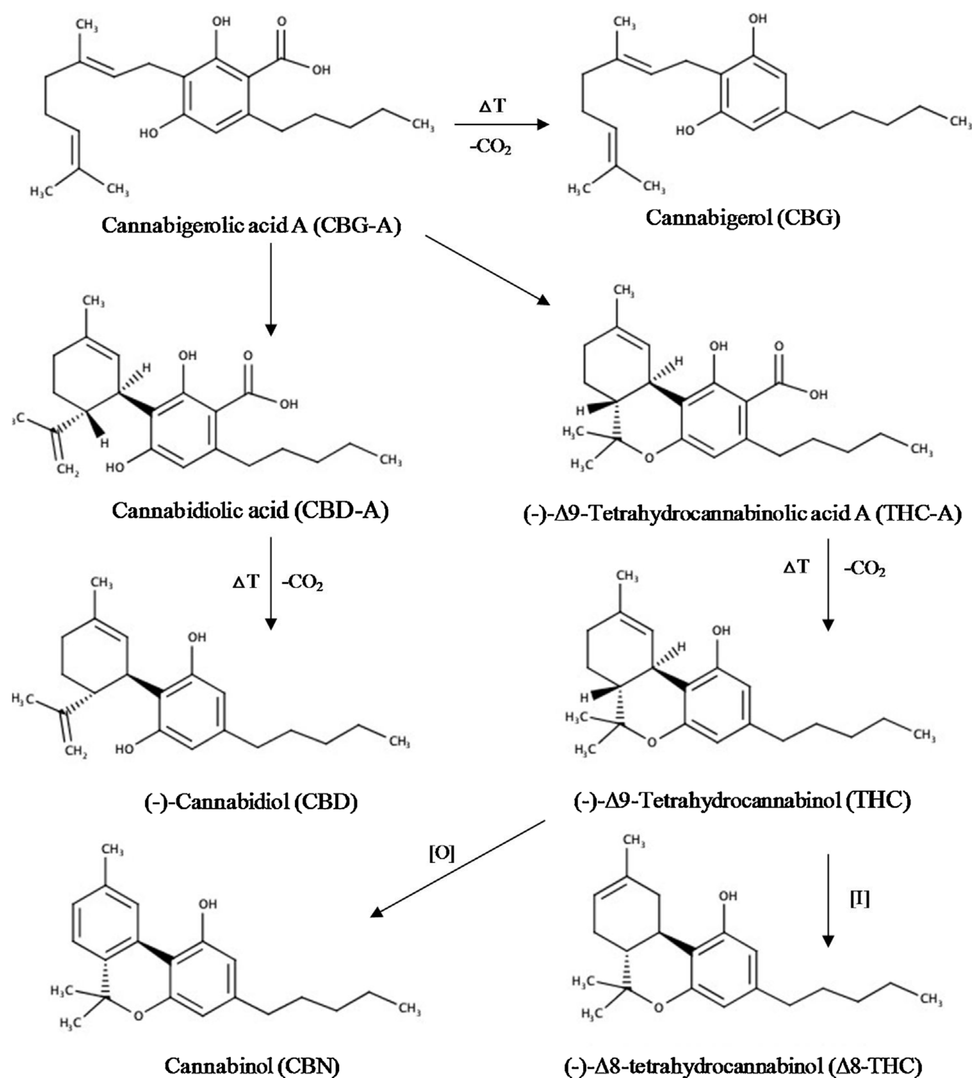

Fig. 1. Cannabinoids structures.
ΔT = heating; [I] = isomerization; [O] = oxidation.

anti-tumor properties, as reviewed in [11–14]. Cannabidiol has gained popularity amongst patients suffering from pain, anxiety and depression. These are the main reasons mentioned to explain cannabidiol consumption [15]. Given its effects on various psychiatric conditions, it would be inaccurate to tell that CBD is not psychoactive.

Since the end of 2018, the increase in popularity has contributed to the proliferation of stores selling low THC cannabis products in Belgium. Most of the products consist of cannabis flowers or, to a lesser extent cannabis resin. Loads of other products are also available, such as lotions, oils, cream, capsules, etc. These products are supposed to be cannabidiol rich, while their THC concentration should be low. Indeed, if their total THC content (THC + THC-A) is higher than 0.2%, they are considered as narcotics by the Belgian legislation. It is also the case in other European Union countries, even if some countries consider that unlicensed products must not contain any THC at all [16]. Currently, flowers and resins, meant to be smoked, are considered as “other tobacco” by the Belgian law.

In order to check if the shops are abiding the law, products are regularly seized by the police and the THC level is measured. Numerous analytical methods are available for the determination of cannabinoids in THC products. These use gas [17–20] or liquid [5,21–23] chromatography (GC or LC) combined with various detectors, such as flame ionization detector (FID), mass spectrometry (MS) or diode array detector (DAD).

In our lab, the high-performance liquid chromatography - diode array detector (HPLC-DAD) method developed by De Backer et al. [2] has been used for years to quantify THC and THC-A in cannabis plant material. However, due to the aging of the HPLC apparatus and the long runtime, a new method was developed using ultra-high-performance liquid chromatography - DAD (UHPLC-DAD).

This study aims at validating this analytical method for quantifying the main neutral and acidic cannabinoids present in plant material. This new method has been applied to samples seized by the police in CBD stores.

2. Materials and methods

2.1. Chemicals and reagents

Analytical standards of THC, CBD-A, CBG, CBG-A were purchased from Cerilliant (Texas, USA) whereas standards of THC-A, CBD and CBN were purchased from LoGiCal (LGC Standards, Teddington, United Kingdom). For quality control samples, THC, CBD and CBN were purchased from LoGiCal (LGC Standards, Teddington, United Kingdom), CBD-A, CBG and THC-A were

purchased from THC-Pham GmbH (Frankfurt, Germany), CBG-A was purchased from Echo Pharmaceuticals BV (Weesp, Holland). Prazepam, used as internal standard, was purchased from Certa (Braine-l'Alleud, Belgium). All solvents were LC–MS or HPLC grade and supplied by J.T. Baker (Phillipsburg, USA). For mobile phase preparation, formic acid was purchased from Biosolve (Dieuze, France).

2.2. Preparation of standards

The internal standard solution was prepared by dissolving 10 mg of prazepam powder in 10 mL methanol. The working internal standard solution was a ten folds dilution of this solution prepared in methanol in order to obtain a final concentration of 100 mg/L.

For the calibration curve, a working solution with a concentration of 50 mg/L of THC-A and THC and 12.5 mg/L of CBD-A, CBG-A, CBD, CBG, CBN was prepared by mixing the compounds in methanol. A ten folds dilution was done in methanol in order to obtain a diluted working solution with a concentration of 5 mg/L of THC-A and THC and 1.25 mg/L of CBD-A, CBG-A, CBD, CBG, CBN. The calibration curves used for quantification were prepared by mixing the appropriate volume of this solution in methanol to obtain a range from 0.10–20% for THC and THC-A and 0.125–5% for other cannabinoids.

2.3. Sample preparation

Samples were dried for 24 h at 40 °C in a ventilated oven. Dried samples were then grinded and 5 mL of a mix of methanol/chloroform (90/10: v/v) were added to 40 mg of the powder before a 30-minute-agitation shaking. Extracts were filtered on cellulose filter paper and 100 μL of the filtrates were diluted in 3100 μL methanol. Finally, 25 μL of the internal standard solution was added to 100 μL of this diluted sample before evaporation under a gentle nitrogen stream. Dried extract was redissolved in 100 μL of a mixture of methanol/water (50/50: v/v) before injection on UHPLC-DAD.

2.4. UHPLC-DAD analysis

The apparatus was an Acquity UPLC system from Waters (Milford, MA, USA) combined with a photodiode array detector (PDA detector). Injection of 10 μL of sample was done on an Acquity UPLC HSS C18 SB 1.8 μm column (2.1 × 100 mm) from Waters. A gradient was applied at 45 °C, with mobile phase A consisting of methanol, while mobile phase B consisted of water with 0.1% formic acid (50/50). A constant flow of 0.35 mL/min was applied using the following gradient: the initial condition of 65% of mobile phase A was held during 1 min. Then the gradient linearly increased to 78% of A in 8 min, then 95% of A in 1 min, held for 2 min. Finally, the gradient returned to initial condition and was maintained for 3.5 min prior to the next injection, for a total runtime of 16 min. Finally, data were collected and processed using the Empower software v2 (Waters). For the quantification, chromatograms were processed at a wavelength of 211 nm for neutral forms and 220 nm for acidic forms.

2.5. Method validation

The method was validated by the *'total error method'* approach using the e-noval® validation software v4.1 (Pharmalex, Mont-Saint-Guibert, Belgium) according to the ISO 17025 guidelines.

2.5.1. Specificity

Specificity was assessed by recording the full spectra of each compound as well as retention times, followed by a comparison with the library.

2.5.2. Calibration curve

Duplicates of eight calibrators were prepared by spiking methanol with an appropriate volume of working solution, in order to obtain final concentrations of 0.10, 0.25, 0.50, 1.00, 2.00, 5.00, 10.0 and 20.0% for THC and THC-A. For the other cannabinoids, the two first levels were not required and final concentrations were 0.125, 0.25, 0.50, 1.25, 2.50 and 5.00%.

2.5.3. Precision and trueness (bias)

Quality control (QC) samples were prepared in the same way, with independent solutions. All eight concentration levels for THC and THC-A (0.10, 0.15, 0.20, 0.50, 5.00, 15.0 and 20.0%) and six concentration levels for other cannabinoids (0.0375, 0.05, 0.125, 1.25, 3.75, 5.0) were analyzed in triplicate for three days. Bias was calculated as percent deviation of mean calculated value from nominal value. The intra- and interassay precisions (repeatability and intermediate precision) were determined as the relative standard deviation (R.S.D) within the analyses carried out in triplicate the same day and over three different days, respectively. The method was considered acceptable if the values were below 15%, or 20% around the LLOQ.

2.5.4. Limits of detection and quantification

Lower and upper limits of quantification were calculated by enoval® as the smallest and highest concentration beyond which the β-expectation limits exceed the acceptance limits (30% for each compound, and 40% around the LLOQ for CBG, with a risk level of 17.5%). Limits of detection are obtained by dividing LLOQ by three.

2.5.5. Dilution and concentration integrity

A 100 μL solution containing 20% CBD and CBD-A was prepared in methanol. Dilution integrity was assessed by preparing a four folds dilution of this sample with methanol before the evaporation step.

On the other hand, 200 μL of the working solution (simulating a 0.025% THC and THC-A content) was submitted to the whole extraction process in order to evaluate the concentration integrity. The concentration step was done by adding 700 μL methanol instead of 3100 μL and evaporating 100 μL of this concentrated sample. Both analyses were done in triplicates. After multiplication or division of the result obtained by the dilution or concentration factor, respectively, accuracy and precision should be below 15%.

2.5.6. Carry-over

Carry over was evaluated by injecting blank samples following the highest level of calibration standard, this operation was repeated four times. Peaks observed in blank samples should be below 20% of the peak area at the LLOQ.

2.5.7. Drying duration

In order to evaluate the appropriate drying duration, fresh cannabis samples were put in a ventilated oven upon reception, and hygrometry was recorded for two days.

Another experiment was performed on five different samples previously grinded. These samples were analyzed in duplicate at the beginning, after 24, 48 and 72 h of drying in the ventilated oven.

2.5.8. Stability during the evaporation process

Five independent samples were used to evaluate the ideal evaporation duration. For each sample, four sampling tubes were submitted to evaporation: two were removed after 3 min (as they were already dried), one was taken out after 15 min and the last one remained under evaporation for a full hour.

2.5.9. Extraction yield

In order to assess if 5 mL of solvent was a sufficient extraction volume to extract all the cannabinoids out of the samples, 5

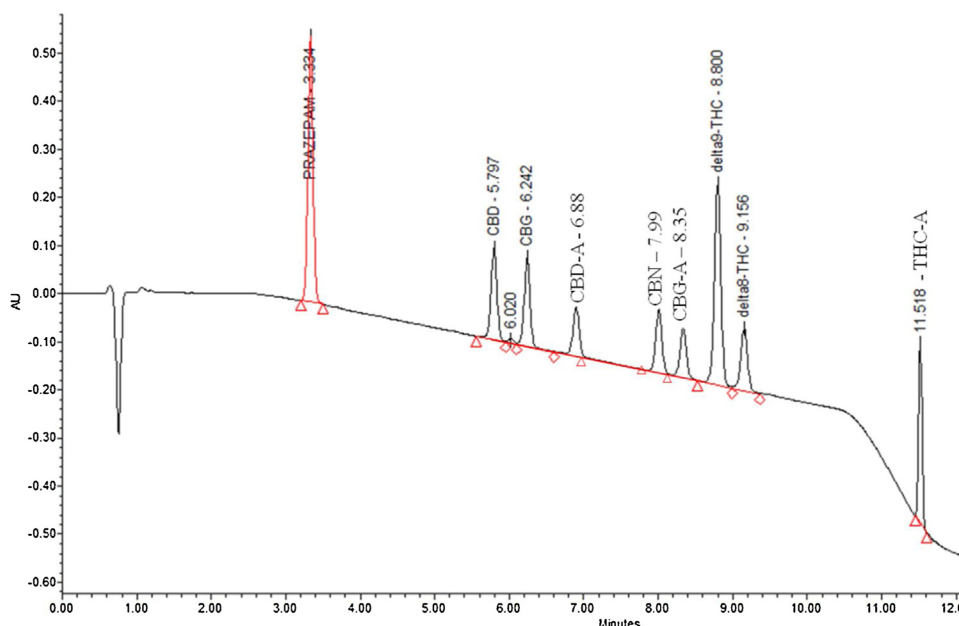

Fig. 2. Chromatogram of a QC sample containing 10% THC, 10% THC-A and 3% of other cannabinoids.

grinded samples were submitted to the classical procedure, followed by another additional extraction with 2 mL of solvent mix. Cannabinoids concentration in the second extract should be below 5% of the concentration measured in the first extract.

2.6. Analysis of real samples

The method was applied to the analysis of 213 samples (192 flowers and 21 resins) seized by the police in 32 shops of the French speaking part of Belgium. Samples were mostly seized in the east part of Wallonia (20 shops in the Province of Liège including the cities of Liège, Waremme, Huy, Stavelot, Verviers, Beyne-Heusay, Ans and Seraing), but also in the south part (6 shops in Arlon, Marche-en-Famenne and Bastogne), in the west part (5 shops in Charleroi, Courcelles, Binche and Ransart) and finally one shop in the center, close to the capital (Rixensart).

Each batch (corresponding to a maximum of 60 samples) was analyzed with a calibration curve and three QC samples (corresponding to three concentrations) injected in triplicate: once after the calibration curve, once in the middle and finally once at the end of the batch. Target concentrations of the QC samples were 0.20, 1.00 and 10.0% for THC and THC-A, whereas only the highest levels were analyzed for other cannabinoids, corresponding to target amounts of 0.30 and 3.00%. Internal quality control samples are used to validate the batch and to check the robustness of the method. When a sample showed a THC (acid or neutral form) amount between 0.10% and 0.20%, combined with the other form (neutral or acid form, respectively) detected below the LOQ, the sum was potentially above the legal limit (total THC content over 0.20%). The analysis was thus repeated from the beginning on a sample four times more concentrated, in order to confirm the first result.

3. Results and discussion

3.1. Method validation

The method we present here shows a considerably shorter runtime, from 36 min on HPLC-DAD to 16 min in UHPLC-DAD. A typical chromatogram is shown in Fig. 2 illustrating the resolution of each cannabinoid.

3.1.1. Specificity

Specificity was ensured by matching all detected peaks (spectrum and retention time) with the library. A 10% range around this retention time was allowed for the compound to be considered as a cannabinoid. Retention time of each cannabinoid is indicated in Table 1. The spectra are presented in Fig. 3. THC, CBD, CBG and CBG-A cannot be distinguished by their UV spectra, increasing the need of a chromatographic technique able to separate each compound, which is done using the described chromatographic conditions.

3.1.2. Calibration curve, precision, trueness

Acceptable precision and trueness results (<15% or <20% around the LLOQ) combined with correlation coefficient greater than 0.99 were obtained with a linear model applying a 1/x weighting factor for THC, a quadratic model applying a 1/x weighting factor for acidic forms (THC-A, CBD-A, CBG-A) and a quadratic regression for CBD, CBG and CBN, as indicated in the table below (Table 2).

Some precision and trueness results did not met the objectives for CBG, CBG-A and CBN. However, it was considered as acceptable as these compounds were not cannabinoids of major interest.

3.1.3. Limits of detection and quantification

Limits of detection and limits of quantification are indicated in Table 3.

3.1.4. Dilution and concentration integrity

Dilution integrity for CBD and CBD-A and concentration integrity for THC and THC-A were acceptable, considering the

Table 1
Retention times of each cannabinoid in the method.

	THC	THC-A	CBD	CBD-A	CBG	CBG-A	CBN
Retention time	8.74	11.5	5.72	6.85	6.20	8.31	7.95

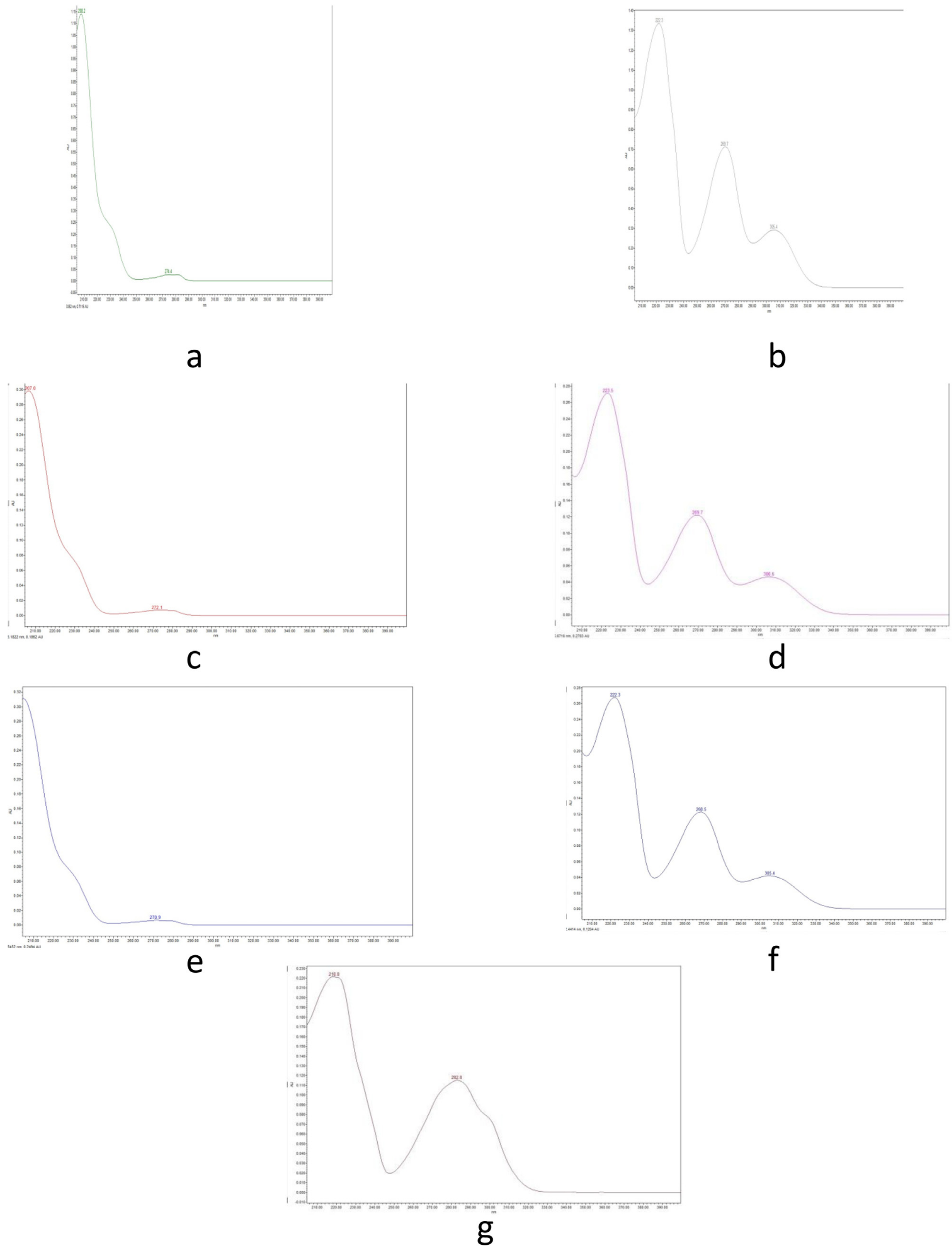

Fig. 3. UV spectra of each cannabinoid. 3a. THC; 3b THC-A; 3c CBD; 3d CBD-A; 3e CBG; 3f CBG-A; 3 g CBN.

Table 2
Precision and trueness results.

Compound	Target concentration (μg/L)	Trueness (relative bias %)	Repetability (RSD %)	Intermediate precision (RSD %)
THC	0.10	7.90	5.00	6.48
	0.15	8.71	6.29	6.32
	0.20	16.0	0.71	5.11
	0.50	5.54	6.19	6.19
	5.00	−1.42	7.57	7.57
	15.0	0.87	5.06	5.06
	20.0	−0.11	3.41	4.64
THC-A	0.10	8.05	1.03	5.79
	0.15	−0.69	3.85	10.6
	0.20	2.46	5.87	12.2
	0.50	−7.30	5.34	8.21
	5.00	−3.79	1.57	7.37
	15.0	−3.59	10.8	10.8
	20.0	−11.7	2.61	5.81
CBD	0.0375	15.0	12.3	12.3
	0.05	11.2	11.1	16.0
	0.125	7.14	7.83	10.7
	1.25	1.57	4.89	4.89
	3.75	2.26	2.29	3.45
	5.00	−0.61	1.87	3.51
CBD-A	0.0375	0.91	5.76	10.3
	0.05	−2.35	3.27	17.6
	0.125	−3.71	7.90	7.90
	1.25	3.88	4.19	7.98
	3.75	−2.26	9.71	12.4
	5.00	−13.3	2.53	5.95
CBG	0.0375	41.7	12.4	52.3
	0.05	19.1	21.1	43.7
	0.125	5.82	9.23	10.6
	1.25	15.0	2.59	6.52
	3.75	18.1	3.32	4.02
	5.00	12.9	1.75	2.26
CBG-A	0.0375	0.29	8.95	27.3
	0.05	1.26	3.66	18.1
	0.125	−3.70	6.88	6.88
	1.25	−0.19	2.51	5.01
	3.75	−1.08	10.9	11.7
	5.00	−10.9	2.89	4.02
CBN	0.0375	−4.65	9.66	26.1
	0.05	10.4	2.92	9.30
	0.125	8.29	9.18	9.61
	1.25	4.48	7.71	7.71
	3.75	6.19	4.64	4.64
	5.00	3.81	3.76	4.08

Table 3
Limits of detection and quantification.

	THC	THC-A	CBD	CBD-A	CBG	CBG-A	CBN
LLOQ	0.100	0.100	0.113	0.087	0.113	0.081	0.063
ULOQ	20.0	20.0	5.00	5.00	5.00	5.00	5.00
LOD	0.03	0.03	0.03	0.03	0.03	0.03	0.02

Table 4
Dilution and concentration integrity results.

	Dilution integrity		Concentration integrity	
	CBD	CBD-A	THC	THC-A
Bias (%)	8.20	11.8	2.00	1.67
RSD (%)	0.50	2.02	3.53	10.6

bias and relative standard deviation indicated in the table below (Table 4).

3.1.5. Carry-over

No carry-over was observed as no peak was detected in blank samples injected after the most concentrated calibration standard.

3.1.6. Drying duration

A significant increase of the humidity level recorded in the oven was observed after the introduction of the fresh plant (from 18% to more than 40% relative humidity), followed by a decrease and stabilization at the basal value after 24 h, meaning that 1 day is enough to dry the samples. The second experiment gave expected results considering the instability of the acidic forms. For all samples, an increase of the neutral forms (CBD, THC, CBG) was observed over time, combined with a decrease of the corresponding acidic forms (CBD-A, THC-A, CBG-A), leading to a stable amount if the sum of both was considered. Indeed, a difference less than 20% between the various sampling times was observed when

considering the total THC content, the total CBD content or the total CBG content.

3.1.7. Stability during the evaporation process

All the extracts were evaporated after a few minutes only (3 min maximum). A longer evaporation time did not lead to a significant variation of the cannabinoid concentration, even after one-hour evaporation.

3.1.8. Extraction yields

THC-A was the only cannabinoid detected in the second extract. THC-A levels in the second extracts were very low (min: 0.08, max: 0.11%) for the five samples, meaning that 5 mL of extraction solvent is a sufficient volume.

3.2. Analysis of real samples

The method was applied to 213 samples coming from 32 CBD shops, including 192 flowers and only 21 resins, as indicated in Supplementary Table S1. Between 1 and 18 samples were analyzed per store. Results are summarized in Fig. 4 below.

Two of the samples can be considered as outliers as their total THC content was really high (10.4 and 11.0%), while CBD and CBD-A were not detected, even if they were seized in a CBD shop, along with four other samples containing CBD. These four samples were all exceeding the legal threshold (total THC content: min: 0.22 – max: 0.61%). These two samples were found in a big plastic bag containing 75 g of flowers, not in small commercial packages of 1 g as usually seen for CBD samples. Also, unexpectedly, two samples seized in another CBD shop contained no cannabinoid at all. So, out of the 213 samples supposed to be CBD rich and THC poor, we decided to leave out 4 samples: two which did not contain any cannabinoid at all and two which contained a really high THC content. If we subtract these four from the 213, we end up with 209 real CBD products.

Thirty-five samples had to be analyzed less diluted due to a THC (or THC-A) content included between 0.10 and 0.20% combined with the other form (THC-A or THC, respectively) detected below the LOQ, leading to a sum potentially higher than 0.20%. Fourteen out of these 35 showed a total THC content higher than 0.2%.

Looking at the legal considerations of these samples, almost half of them (51.6%, 110 samples including one resin) were exceeding the legal limit, that is portraying a sum of THC and THC-A higher than 0.2%. THC and THC-A were detected in all samples except 11 and 16 samples, respectively, in which they were not detected at all. Only nine samples contained both THC and THC-A concentrations below the LOD. If we consider all the samples with a quantifiable amount of THC and THC-A, excluding the two outliers (88 samples, 41.3%), the total THC content ranged from 0.14 and 1.17% (median: 0.38%, mean: 0.40%). After excluding the two samples with an extremely high THC content, and the two samples which did not contain any cannabinoid, the total CBD content for the 209 remaining samples ranged from 0.19 and 22.6% (median: 7.13%, mean: 7.82%).

Cannabinol was rarely found; a CBN amount higher than the LOQ was found in three samples only (0.11%, 0.11%, 0.13%), whereas 17 samples contained a CBN concentration between the LOD and the LLOQ. CBG-A was quantified in 117 samples (min: 0.08%; max: 3.26%; median: 0.22%) whereas CBG was quantified in 67 samples (min: 0.10%; max: 3.26%; median: 0.16%). Finally, nothing is shown about delta-8-THC as it was never detected.

To date, only one other research group has published the results of similar studies. In their first study, Hädener et colleagues [24] analyzed 110 CBD rich flowers and found a total THC content ranging from 0.11 to 0.69% (median 0.45%) which is slightly higher than in our study. Also, they found a CBD content ranging from 3.22 to 18.2% with a median value of 12.4% which is clearly higher than our samples (median 7.13%). In their second study, on 205 CBD rich flowers, Hädener et al. [25] found a CBD content similar to ours (median 8.5%) whereas the THC content was ranging from 0 to 1.7 (median 0.3%). However, we should highlight that the Swiss legislation – applied to their samples – is much more permissive than the Belgian one. Indeed, to be considered as legal, Switzerland allows a total THC content in cannabis hemp up to 1%, which is five times higher than what is allowed in Belgium. If the legislation was the same in Belgium, only three of our 213 samples would be illegal, including the two outliers and one sample with a total THC content of 1.17%.

Out of our 213 samples, 80 had a CBD content mentioned on the packaging. The others had no indication mentioned at all. The concentration stated was ranging from 2.8 to 25%. If we consider the total CBD content, the results measured ranged from 6 times less to 4 times more CBD content, than indicated on the packaging. Seventeen samples (21%) contained a total CBD content higher than labeled, whereas 49 samples contained less CBD content than labeled. Fourteen samples (18%) contained a CBD amount ranging between 90 and 110% of the labeled concentration. This discrepancy of labeling was already described by Bonn-Miller et al. concerning other CBD products (oil, vaporization liquid, etc) [26].

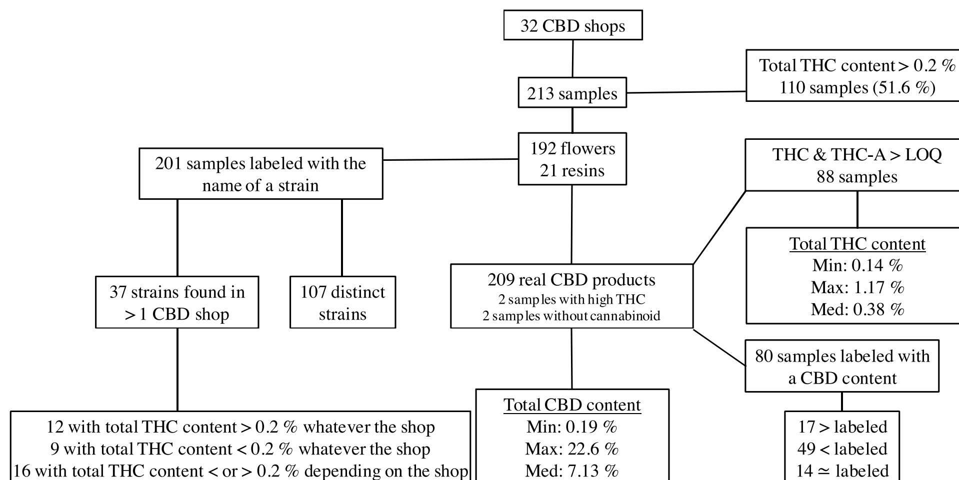

Fig. 4. Main results of the present study.
CBD: (−)-cannabidiol; THC-A: (−)-Δ9-tetrahydrocannabinolic acid A; THC: (−)-Δ9-tetrahydrocannabinol.

The name of usual cannabis strains [27,28] was indicated on 201 samples, corresponding to 107 distinct strains including for example: AC/DC, Amnesia Haze, Blue Dream, Cannatonic, Cheese, Orange Bud, Sour Widow, Strawberry, White Widow, etc.). Out of them, 37 were found in more than one CBD shop, but cannabinoids quantification did not lead to the same conclusion every time. Indeed, 12 strains contained a total THC content higher than 0.2% whatever the CBD shop, nine strains were always considered as legal products based on this threshold, but for 16 strains, the total THC content was sometimes higher, sometimes lower than 0.2% depending on the CBD shop. For the majority of strains, total CBD amount was also highly inconsistent within a strain and varied greatly (up to 9 times more) depending on the CBD store (e.g. Buddha cheese showed a total CBD content of 0.60% in one shop and 9.82% in another shop). These differences can have a huge impact on the consumer who thinks that he is smoking the same product, where actually the CBD concentration is significantly different. Moreover, law abiding citizens can unwillingly be found in possession of narcotics products.

4. Conclusion

We have developed and validated an efficient method allowing the quantification of major cannabinoids in cannabis plant material. The method was successfully applied to 213 samples (mainly flowers but also resins) seized in CBD shops in Belgium. Our study showed that half of the products exceeded the Belgian legal threshold of 0.2% of THC content. Large discrepancies between labeled and measured amounts of CBD were also observed, as well as concentration differences for a same strain sold in various CBD shops.

CRediT authorship contribution statement

Marine Deville: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - original draft. **Nathalie Dubois:** Conceptualization, Methodology, Validation, Writing - original draft. **Raphaël Denooz:** Conceptualization, Methodology, Validation, Writing - original draft. **Corinne Charlier:** Conceptualization, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no conflict of interest

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at https://doi.org/10.1016/j.forsciint.2020.110234.

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