Journal of Pharmaceutical and Biomedical Analysis 205 (2021) 114344

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

CBD oils on the Belgian market: A validated MRM GC-MS/MS method for routine quality control using QuEChERS sample clean up

Duchateau Céline[a,b], Canfyn Michaël[b], Desmedt Bart[b], Kauffmann Jean-Michel[a], Stévigny Caroline[a], De Braekeleer Kris[a,1], Deconinck Eric[a,b,*,1]

[a] Université Libre de Bruxelles (ULB) - Faculty of Pharmacy, RD3, Pharmacognosy, Bioanalysis and Drug Discovery Unit, Boulevard du Triomphe, Campus Plaine, CP 205/6, B-1050 Brussels, Belgium
[b] Sciensano, Scientific Direction Physical and Chemical Health Risks, Medicines and Health Products, Rue Juliette Wytsmanstraat, 14, 1050 Brussels, Belgium

ARTICLE INFO

Article history:
Received 3 June 2021
Received in revised form 20 August 2021
Accepted 25 August 2021
Available online 29 August 2021

Keywords:
Sample clean up
MRM GC-MS/MS
CBD oils
Cannabidiol

ABSTRACT

Quality control of CBD oils on the Belgium market showed that the CBD content not always corresponds to the label claim. There is a pressing need to develop new analytical methods specifically developed to the assay of such oily samples. Analytical issues are, however, encountered for routine analyses due to the matrix complexity, high cost of cannabinoid standards and low Δ9-THC concentrations. An oily matrix could cause technical damages to analytical instruments and reduce the lifetime of the chromatographic columns. This paper proposes a procedure combining a sample cleanup by QuEChERS, removing the oily matrix, followed by a validated MRM GC-MS/MS method for the routine analysis of CBD oil samples. Eighteen CBD samples were selected on the Belgium market for analysis. This method allows the quantification of CBD, the legality check for the Δ9-THC content by a CBN standard and the screening of seven other cannabinoids namely CBN, CBDV, CBT, CBC, Δ8-THC, THCV and CBG. The method was validated at three concentration levels (0.5–1–2% (w/v)) for CBD and (0.05–0.1–0.2% (w/v)) for CBN. The detection limits for CBT, CBD, CBC, Δ8-THC, CBN and for the other cannabinoids of interest, were 10 and 14 ng/mL respectively. The accuracy profile values for CBD and CBN showed that the β-expectation tolerance intervals did not exceed the acceptance limits of ± 20%, meaning that 90% of future measurements will be included within this error range.

1. Introduction

The growing number of cannabis-based products like hemp seeds and more specifically the cannabidiol (CBD) oils and other cannabinoid oils, calls for the establishment of a legal framework specifically for the determination of the permissible residual content of Δ9-tetrahydrocannabinol (Δ9-THC) and the assay of the labeled amount of cannabinoid. For these applications, most European Union countries authorize a maximum concentration of 0.2 (w/w) or 0.3% (w/w) of Δ9-THC in the dried cannabis plant [1]. Since CBD is not controlled in the European Union, there is a lack of specific regulations and analytical controls and no guarantee of the composition and quality of the products [2].

Many analytical methods are described in the literature to quantify the CBD content in oil. The most common cannabinoid quantification techniques for this matrix include gas chromatography (GC) coupled to mass spectrometry (MS) [3,4] and liquid chromatography (LC) coupled to an UV or MS detector [5,6]. One of the important differences between LC and GC is the high column temperature required in GC, which produces a decarboxylation of the acidic form of CBD, cannabidiolic acid (CBDA) [7]. Since the total quantity of CBD is often mentioned on the packaging, GC is the first choice technique. Moreover, it allows the use of only one reference standard, namely CBD. All the published papers, however, do not take into account the negative effects of the oily matrix on the chromatographic columns and the instrument and therefore are not suited for daily routine use for quality control and surveillance of a high number of samples. In addition to the reduced lifetime of the chromatographic column and the increased cleaning frequency necessary, other analytical problems are encountered due to the complexity of the matrix, the high cost of cannabinoid standards and the low concentration levels of Δ9-THC. In the literature often a

* Corresponding author at: Université Libre de Bruxelles (ULB) - Faculty of Pharmacy, RD3, Pharmacognosy, Bioanalysis and Drug Discovery Unit, Boulevard du Triomphe, Campus Plaine, CP 205/6, B-1050 Brussels, Belgium.
E-mail address: Eric.Deconinck@sciensano.be (D. Eric).
[1] Equally contributing project leaders

https://doi.org/10.1016/j.jpba.2021.114344
0731-7085/

direct injection method is used with different split ratios after dilution of the sample in a suitable solvent, though this implies injection of an oily matrix with all negative effects mentioned above. In this paper a QuEChERS (Quick, Easy, Cheap, Efficient, Rugged, Safe) procedure based on the Enhanced Matrix Removal (EMR)-lipid was used for sample clean-up. This technique permits to remove enough fat for the analysis of a great number of samples in one run. High-boiling materials can cause adsorption of analyte in the injection port or in front of a GC column [8]. Furthermore, the small number of sample preparation steps reduces the time and effort and decreases the possible errors by the analyst.

In this work, a MRM GC-MS/MS method was developed and validated to determine the quantity of CBD and Δ9-THC in oily samples. For the latter, cannabinol (CBN) reference standards were used. The method was also evaluated for screening of seven other cannabinoids namely CBN, cannabichromene (CBC), cannabicitran (CBT), delta-8-tetrahydrocannabinol (Δ8-THC), tetrahydrocannabivarin (THCV), cannabidivarin (CBDV) and cannabigerol (CBG). These cannabinoids were selected based on their biosynthesis pathway [9,10] and their medical and pharmaceutical interest [11,12].

2. Materials and methods

2.1. Materials

2.1.1. Instrumentation

GC-MS/MS analyses were performed on an Agilent 7890B gas chromatograph coupled to an Agilent 7000C triple quadrupole detector (Agilent Technologies, Santa Clara, CA, USA). Data acquisition and analysis were performed using MassHunter Workstation 10.0 (Agilent Technologies, Santa Clara, CA, USA).

2.1.2. Reagents, chemicals and extraction material

The extraction solvents n-hexane and ethanol absolute (dehydrated) AR were purchased from Biosolve BV (purity ≥ 99.0%, Valkenswaard,The Netherlands). Ultrapure water was obtained using a milliQ-Gradient A 10 system (Millipore, Billerica, USA). Methyl-arachidate, a fatty acid methyl ester used as internal standard, was purchased from Sigma-Aldrich (purity 99.4%, Saint-Louis, USA). Blank olive oils used during method development and validation were purchased from a local retail shop.

2.1.3. Extraction Kit

The extraction kit was composed of Bond Elut EMR-Lipid dispersive solid phase extraction (D-SPE) and Bond Elut EMR-Lipid Polish Pouch anhydrous $MgSO_4$ sticks (3.5 g) (Agilent Technologies, Santa Clara, CA, USA). After activation of the D-SPE with water, lipids can be extracted selectively from fat samples by the preweighed sorbent. A final cleaning step removes the excess of water, which was necessary to activate the D-SPE. The kit was purchased from Agilent (Diegem, Belgium).

2.1.4. Standards

The standard cannabidiol (100.0% purity) was purchased from Fagron (Nazareth, Belgium). The standard solutions of tetrahydrocannabivarin in methanol (0.9986 mg/mL ± 0.8% (total uncertainty) purity), cannabigerol (97.9% ± 0.2% purity), cannabinol (99.427% ± 0.004% purity), (-)-trans-delta-9-tetrahydrocannabinol (100.9 mg/mL ±0.9%(total uncertainty purity)), (-)-delta-8-tetrahydrocannabinol in ethanol (9 mg/mL ± 2% (total uncertainty purity) and the standard of cannabidivarin (99.45% ±0.02% purity) were purchased from Lipomed AG (Arlesheim, Switzerland). The standard solution of cannabicitran in methanol (99.3% ± 0.6% purity) and (±)-cannabichromene in acetonitrile (100.0% purity) were purchased from LGC standards (Molsheim Cedex, France).

2.1.5. CBD oils samples

Eighteen CBD oils were taken by inspectors affiliated with the Belgian Federal Public Service "Animal, Plant and Food Directorate-General" (DG4) and the Belgian Federal Agency for Medicinal and Health Products (FAMHP).

2.2. Solutions preparation

All solutions were prepared and stored in brown glass volumetric flasks and stored at − 20 °C up to 2 weeks [13].

2.2.1. Preparation of CBD and CBN standard solutions

A stock solution of 2 mg/mL in ethanol was prepared for CBD and stored in a dark flask at − 20 °C.

Five CBD standard solutions were prepared in n-hexane from the stock solution. Solutions of 0, 30, 60, 90 and 120 µg/mL in n-hexane were made. These standard solutions were further used to create a standard addition procedure to create a standard addition calibration curve.

The CBN standard solutions were prepared in the same way with a concentration of stock solution of 100 µg/mL. Solutions of 0, 1.25, 2.5, 3.75, and 5 µg/mL were prepared.

2.2.2. Selection and preparation of the diluent solution

The diluent solution consisted of n-hexane containing 0.4 mg/mL of internal standard (methyl-arachidate). Different solvents were tested as diluent solution: acetonitrile, n-hexane, methanol, isopropanol and mixtures of varying proportions of solvents as acetonitrile/methanol, n-hexane/isopropanol. The selection of n-hexane is in agreement with literature, since n-hexane is generally chosen as the non-polar extraction solvent for vegetable oils for its lipid dissolving properties [14].

2.2.3. Preparation of spiked matrix validation samples

A stock solution of 10 mg/mL CBD in ethanol was prepared from which three levels of CBD concentration were prepared for validation: 0.5% (w/v), 1% (w/v) and 2% (w/v). For preparing these validation samples, 500 µL of a blank olive oil samples were spiked with an aliquot of the stock solution of 250 µL, 500 µL and 1 mL respectively.

In analogy, a stock solution of 1 mg/mL CBN in ethanol was prepared to create the three levels of CBN concentration for validation: 0.05% (w/v), 0.1% (w/v) and 0.2% (w/v). For preparing these validation samples, 500 µL of blank olive oil samples were spiked with an aliquot of the stock solution of 250 µL, 500 µL and 1 mL, respectively.

2.3. Development of the extraction procedure and cleanup

A sample of 500 µL oil was diluted to 10.0 mL with the diluent solution in a brown glass volumetric flask and placed in an ultrasonic bath for 5 min. Different times for the sonication step were tested (5 min, 10 min and 20 min). A volume of 3.0 mL of the previous solution was diluted with the diluent solution to 10.0 mL in a brown glass volumetric flask followed by sonication for 5 min

Two sorbents were tested for sample preparation: Bond Elut EMR-Lipid and the Captiva EMR-Lipid [15]. The latter was rejected because no analyte of interest was detected after the extraction procedure and the cleaning step. Even by using a higher amount of extraction solvent to elute the analytes, they remained on the column.

The sorbent contained in the Bond Elut EMR-Lipid was transferred in a polypropylene tube of 50 mL and activated for optimum performance with 5 mL purified water and homogenized by vortexing for 30 s

The first step of the QuEChERS approach was performed by transferring the sample solution to the polypropylene tube

Table 1
Relative retention time and optimized MRM conditions and transitions for cannabinoids and IS.

N°	Compounds	Relative retention time	Quantifier		Qualifier				Dwell time (min)
			Transition	CE (eV)	Transition	Precursor ion	Product ion	CE (eV)	
1	CBDV	0.914	202.8 -> 174.1	20	217.8 -> 203.0	217.8	203.0	20	24.1
2	CBT	0.992	231.0 -> 174.0	30	314.0 -> 231.0	314.0	231.0	30	24.1
3	IS	1	238.0 -> 101.0	10	326.0 -> 185.0	326.0	185.0	10	24.1
4	THCV	1.029	203.0 -> 174.0	15	286.0 -> 271.0	286.0	271.0	9	24.1
6	CBD	1.120	231.0 -> 174.0	18	231.0 -> 145.0	231.0	145.0	30	24.1
5	CBC	1.143	231.0 -> 174.0	21	231.0 -> 174.0	231.0	173.0	33	24.1
7	D8-THC	1.204	231.0 -> 174.0	21	314.0 -> 231.0	314.0	231.0	18	24.1
8	D9-THC	1.247	231.0 -> 174.0	24	231.0 -> 145.0	231.0	145.0	33	24.1
9	CBG	1.302	193.0 -> 123.0	12	231.0 -> 174.0	231.0	174.0	15	24.1
10	CBN	1.304	295.0 -> 238.0	21	295.0 -> 223.0	295.0	223.0	33	24.1

containing the activated sorbent. The tube was vortexed for 3 min followed by centrifugation for 5 min at 5000 rpm. The optimal stirring device was selected comparing horizontal, oscillating and vibrational (vortex) shakers for different times (3 min, 5 min and 10 min). The second step was made by transferring the supernatant to another 50 mL polypropylene tube together with 1.75 g of anhydrous Polish Poush. The latter contains anhydrous magnesium sulfate which eliminates water.The tube was vortexed for 3 min and centrifugated for 5 min. Half the stick was sufficient because the extraction solvent used in this procedure was highly hydrophobic.

After the extraction procedure and clean-up step, 500 µL of supernatant was transferred in five brown glass vials of 2 mL and 500 µL of each of the calibration standards was added. Vials were vortexed for 5 s. The procedure to create a standard addition calibration is described in Section 2.2.1.

2.4. GC-MS/MS conditions

GC-MS/MS analyses were performed on a fused silica capillary column (VF-5 ms, 30 m ×250 µm inner diameter × 0.25 µm film thickness). The oven temperature was programmed at 200 °C for 2 min, increased to 280 °C at 6 °C/min and held for 2 min at 280 °C. Total run time was 17.3 min. Injection was performed in split mode (10:1) with an inlet temperature of 250 °C and a transfer line temperature of 280 °C. Helium gas, with a flow rate of 1.5 mL/min was used as carrier gas.

The mass spectrometer operated in electron impact mode at 70 eV and the temperature of the ion source was 230 °C.

To analyze the cannabinoids, targeted in this study using GC-MS/MS in MRM mode, it was necessary to optimize quantification and qualification precursor to product ion transitions.

The increased selectivity with the use of MRM transitions enhanced the differentiation among partially co-eluting cannabinoids, such as CBD/CBC and CBG/CBN.

The relative retention times, transitions for quantification and qualification, collision energy and dwell time are shown in Table 1. The first ion mentioned for each compound was used for quantification and the second ion as a qualifier ion.

3. Results and discussion

3.1. Method validation

The validation of the method was performed according to the guidelines of the Food and Drug Administration [16] using accuracy profiles. All parameters were established using CBD and CBN spiked in olive oil.

3.1.1. Selectivity

To test the selectivity of the method, the oil matrix was injected to proof the absence of interference. No peaks were seen in the chromatogram. The target analytes were clearly, though not completely, separated. Chromatograms for the blank matrix and the target analytes in MRM mode are shown in Fig. 1.

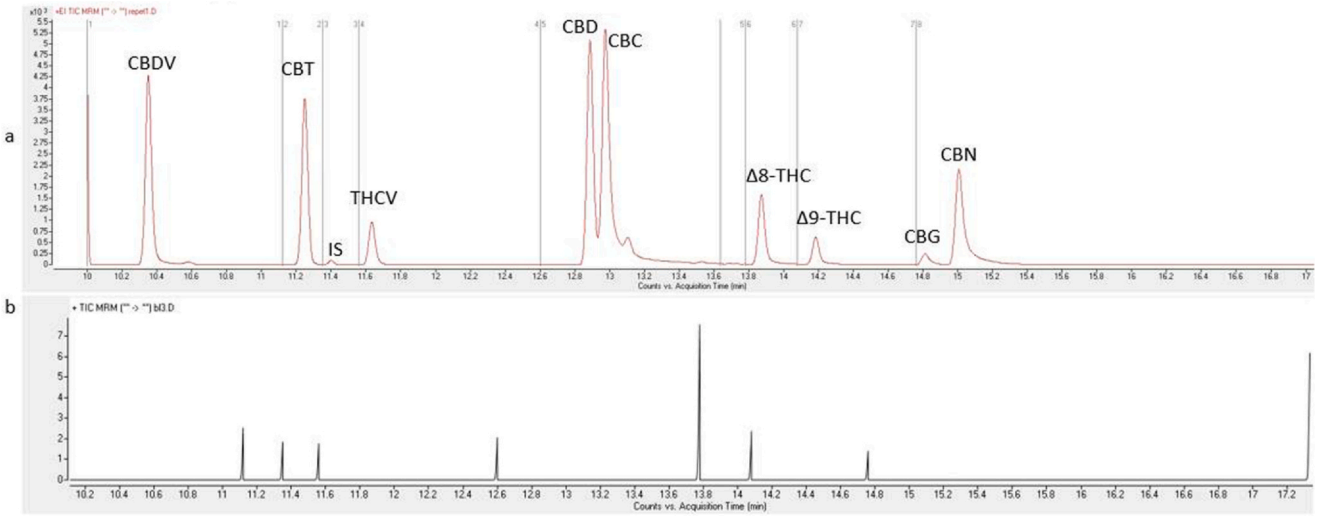

Fig. 1. Total ion chromatogram recorded in MRM mode (a) containing (1) CBDV, (2) CBT, (3) THCV, (4) CBD, (5) CBC, (6) Δ8-THC, (7) Δ9-THC, (8) CBG and (9) CBN and (b) of the blanc matrix.

Table 2
Trueness, precision, accuracy, uncertainty of the method for CBD and CBN.

Compound	Trueness			Precision						Accuracy			Uncertainty		
	Relative bias (%)			Repeatability (RSD %)			Intermediate precision (RSD%)			β expectation limits (%)			Relative expanded uncertainty (%)		
Concentration level (mg/mL)	0.5	1	2	0.5	1	2	0.5	1	2	0.5	1	2	0.5	1	2
CBD	3.89	1.63	8.13	2.11	3.68	2.72	5.89	6.19	4.55	[− 10.29;18.07]	[− 11.50; 14.77]	[− 2.14;18.40]	13.30	13.52	10.57
CBN	-15.44	-16.06	-15.81	2.16	1.26	2.05	2.16	1.60	2.05	[− 19.64;- 11.24]	[− 19.57;- 12.55]	[− 19.79;- 11.83]	3.94	2.98	3.73

3.1.2. Limit of detection (LOD)

The LOD was established as the minimum concentration of analyte that the method can detect with a signal-to-noise ratio of 3. The LOD values were 10 ng/mL for CBT, CBD, CBC, Δ8-THC and CBN and were 14 ng/mL for CBG, THCV and CBDV.

3.1.3. Linearity of the standard addition curves

The linearity of the standard calibration curves was evaluated through the coefficient of determination (R^2) and the quality coefficient (QC) [17]. The R^2 values were above 0.999 and the QC value was maximum 0.24 for CBD curves. The R^2 values were above 0.999 and the QC value was maximum 2.7 for CBN curves.

3.1.4. Accuracy profile

Accuracy profiles are based on the β-expectation tolerance interval and the concept of total error. Equations and theoretical aspect are described in [16,18].

The accuracy estimates the systematic error and the random error in order to determine the differences between observed and true values and is used in various application fields [19–21]. Three concentrations levels (0.5–1–2% (w/v)) for CBD and (0.05 – 0.1 – 0.2% (w/v)) CBN were used to build the accuracy profiles. Concentrations of the validation samples were calculated with the standard addition curves and used to calculate the trueness, the precision and the accuracy. The results are given in Table 2 for the CBD and CBN validation.

Trueness is defined as the closeness of agreement between the mean of a number of test results and the true value. It is an estimate of the systematic error of the method and is calculated as relative bias [22]. In this study, the trueness is acceptable, the highest value was 8.1% for the highest CBD concentration and 16.1% for the middle level concentration of CBN.

Precision is defined by FDA as the closeness of agreement between measurements obtained from multiple samplings of the same homogenous sample under the prescribed conditions. The repeatability (intra-day precision) was estimated by the RSD values of the triplicate injections at each concentration level. The intermediate precision (inter-day precision) was evaluated by the RSD values calculated on the results obtained on four different days. The RSD for CBD was below 3.7% for repeatability and below 6.2% for intermediate precision. The RSD for CBN was below 2.05% for repeatability and below 2.2 for intermediate precision. This was considered acceptable.

The accuracy profile for CBD is shown in Fig. 2. The β-expectation tolerance interval was set at 90% and the acceptance limits at 20%. These settings were acceptable considering the complexity of the matrix. The accuracy profiles showed that the β-expectation tolerance interval did not exceed the acceptance limits of ± 20%.

The measurement uncertainty is a parameter describing the dispersion of the values that could reasonably be ascribed to the measurement. The expanded uncertainty is determined with a 95% confidence level, representing an interval in which the unknown true value can be situated. The maximum value obtained was 13.5% for the middle concentration level for CBD and 3.9% for the low concentration level for CBN, which was considered acceptable.

3.2. Δ9-THC response factor

Δ9-THC reference standard is expensive and often only available in solution. In this study, the response factor of Δ9-THC was compared with the response factor of CBN since this crystalline compound was not subjected to many restrictions and was available in high purity. Additionally, the use of CBN as reference standard to determine the THC concentration was already investigated using other detectors as for example FID [23]. CBN has the advantage that it is normally less present as CBD, which can be present in very high

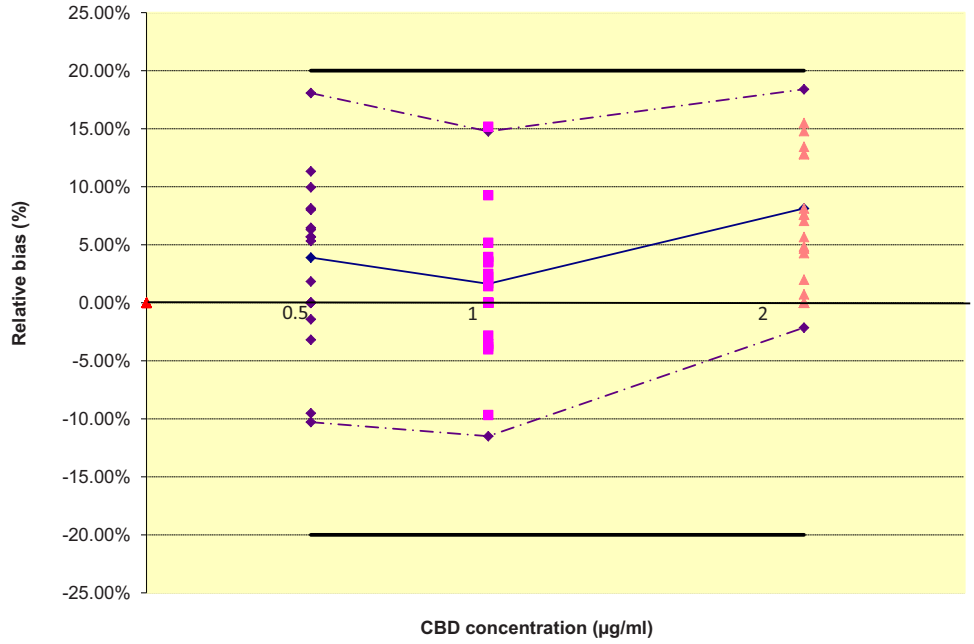

Fig. 2. Accuracy profile with three concentration levels (0.5–1–2% w/v) for CBD with β expectation of 90%. Relative bias (——), β-expectation tolerance limits (——), acceptance limits (__), relative back-calculated concentrations per spiking levels (, ,).

concentration in the sample. Different approaches exist for the calculation of the response factor. The slope method (Eq. (1)) was chosen in this study [24].

$$RF = Slope_{CBN}/Slope_{\Delta 9-THC} \quad (1)$$

To determine the response factor, the method was validated for CBN according to the same procedure as described above for CBD at the levels 0.05% (w/w), 0.1% (w/w) and 0.2% (w/w) to show that CBN can be used as reference standard. Cosmetics do not contain more than 0.05% of Δ9-THC in The Netherlands [25]. The higher level of concentration was chosen because of the only and currently hemp flowers legislation in most European countries. The Δ9-THC slope was (0.03 ± 0.01) and the CBN slope was (0.08 ±0.01). The obtained response factor was equal to 2.75. This response factor was specific to our laboratory and instruments because the RF determined with this detector and this mode depends on several factors such as the gain. It should be determined for each instrument and can not be transferred from one instrument to another.

The determination of Δ9-THC was performed using a CBN standard addition corrected with the RF on olive oils spiked with Δ9-THC at the concentrations 0.05%, 0.1% and 0.2% (w/w) described previously and the recoveries were respectively (105 ± 4) %, (107 ± 4) % and (101 ± 4) %. Since these results were within the error limits of +/- 10% they were considered acceptable and they proved that CBN can be used for Δ9-THC quantification with MS.

3.3. Oil sample analysis

It was found that 45% of the analysed products complied with the acceptance limits for the content of CBD. The summary of the analysis is available in Table 3 and Fig. 3.

Table 3
Analysis of 18 CBD-containing oils products: label strength, strength obtained by MS. Agreement between label strength and label measured, manufacturer, batch, oil matrix, country of manufacturing, supplementary comments on the packaging.

Samples	Label amount (µg/mL)	Lab results (µg/mL)	Assay with respect to label claim (%)	Oil	Country/City
CBD-1-2021	17,000	16,124	94.9	MCT oil	/
CBD-2-2021	29,000	27,173	93.7	Hemp seed oil and hemp extract	The Netherlands
CBD-3-2021	40,000	25,090	62.7	Hemp seed oil and hemp extract	The Netherlands
CBD-4-2021	50,000	59,264	118.5	/	/
CBD-5-2021	50,000	53,040	106.1	/	/
CBD-6-2021	50,000	83,784	167.6	Hemp seed CBD oil	Switzerland
CBD-7-2021	30,000	35,563	118.5	Hemp seed oil and hemp extract	The Netherlands
CBD-8-2021	50,000	65,339	130.7	/	The Netherlands
CBD-9-2021	40,000	44,731	111.8	Hemp seed CBD oil	Switzerland
CBD-15-2021	100,000	47,728	47.7	Hemp seed CBD oil	Switzerland
CBD-16-2021	200,000	38,078	19.0	/	/
CBD-17-2021	100,000	28,539	28.5	/	/
CBD-18-2021	100,000	27,570	27.6	/	/
CBD-19-2021	100,000	31,378	31.4	Cucurbita pepo, seed oil, Cannabis sativa leaves extract	The Netherlands
CBD-20-2021	50,000	83,777	55.8	/	Barcelona
CBD-21-2021	100,000	45,460	45.5	Hemp seed oils and hemp extract	The Netherlands
CBD-22-2021	50,000	216,343	86.5	hemp seed oil	/
CBD-23-2021	50,000	242,569	86.5	hemp seed oil	/

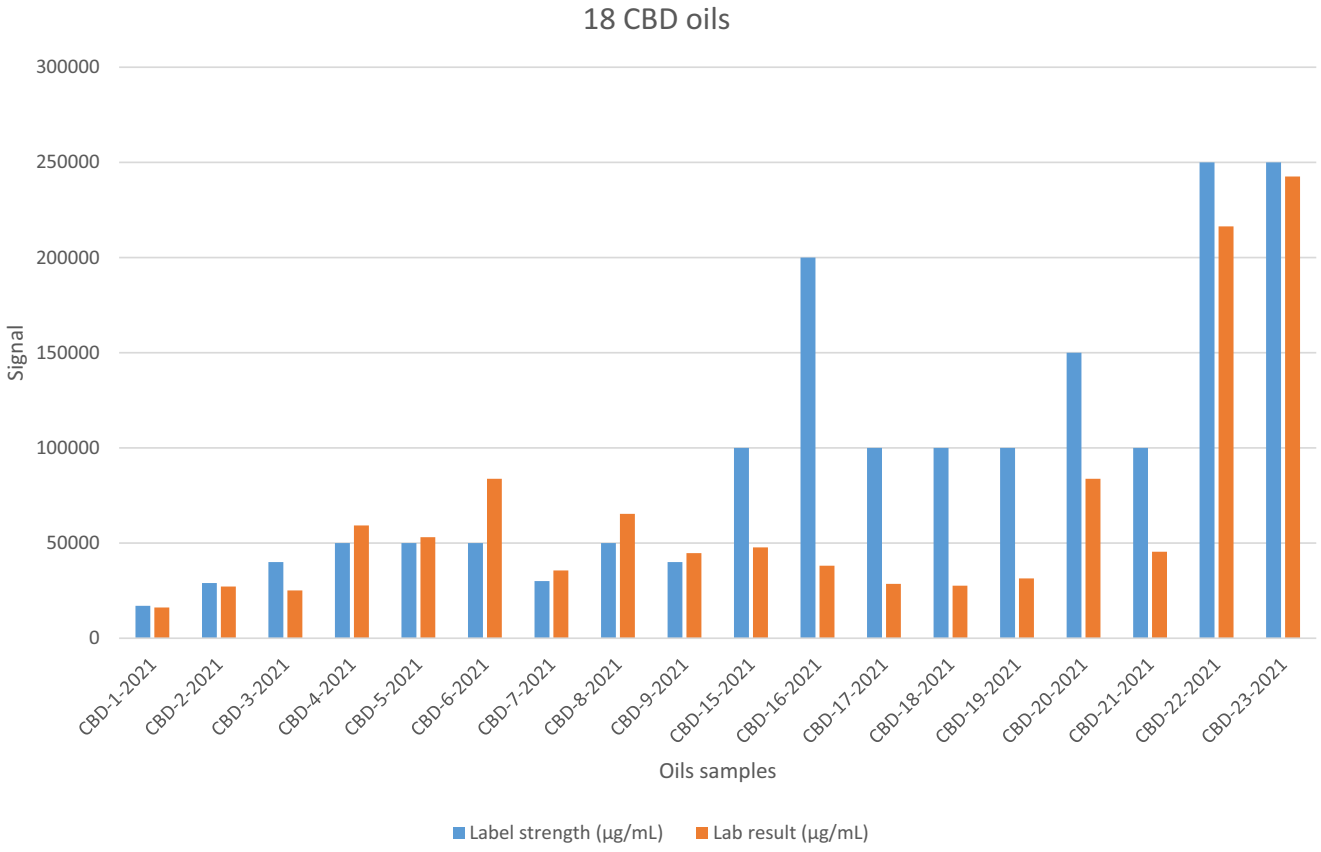

Fig. 3. Comparison of CBD determined by MRM GC-MS/MS validated method and CBD label strength for real samples analysis.

CBDV, CBD and CBC were detected in all oil samples. CBT, Δ8-THC and Δ9-THC were not detected and thus all samples complied with the European legislation. THCV was detected in four samples, CBG in three samples and CBN in ten samples. CBC was present in all samples.

4. Conclusion

A procedure combining sample cleanup by a QuEChERS technique and a validated method by GC-MS/MS was developed. It allowed the quantification of CBD, to check the legality of Δ9-THC and to detect the presence of seven other cannabinoids of interest. To the best of our knowledge this is the first time a validated method of cannabis oily samples is described that includes the protection of the GC instrument, allowing routine analysis of a high number of samples without or with less risk of damaging columns and/or the chromatographic system.

The developed method permits to quantify CBD thanks to a method validated for concentrations from 0.5% to 2%. The obtained accuracy profiles showed that the β-expectation tolerance intervals did not exceed the acceptance limits of ± 20%, meaning that 90% of future measurements will be included within this error range.

After validation, the method was used to analyse eighteen cannabinoid-oil samples. The results showed that 45% of the analysed products complied with the acceptance limits for the content of CBD. All complied to the legislation on the Δ9-THC content. Other cannabinoids were detected as well.

A system specific relative response factor of 2.75 was determined to use a CBN standard instead of Δ9-THC in order to check the legality if its concentration if higher than the LOD.

Funding sources

This research did not receive any specific grant from funding agencies in the public, commercial, or non-profit sectors.

CRediT authorship contribution statement

Duchateau Céline: Methodology, Software, Investigation, Formal analysis, Data curation, Writing – original draft – review and editing, **Canfyn Michaël:** Software, Data curation, **Desmedt Bart:** Formal analysis, review and editing, **Kauffmann Jean-Michel:** Formal analysis, review and editing, **Stévigny Caroline:** review and editing, **De Braekeleer Kris:** Methodology, Validation, Investigation, Supervision, Writing, review and editing **Deconinck Eric:** Conceptualization, Methodology, Validation, Investigation, Writing, review and editing, Visualization, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors are grateful to Agilent Technologies for providing graciously various SPE specimens and to Belgian Federal Agency for Medicines and Health Products (FAMHP) for providing us CBD oils. The authors want also to acknowledge Cedric De Leersnijder for performing some analysis.

References

[1] European Monitoring Centre for Drugs and Drug Adiction (EMCDDA), Cannabis legislation in Europe: an overview, 2017. doi:10.2810/566650.

[2] R. Pavlovic, G. Nenna, L. Calvi, S. Panseri, G. Borgonovo, L. Giupponi, G. Cannazza, A. Giorgi, Quality traits of "Cannabidiol Oils": cannabinoids content, terpene fingerprint and oxidation stability of european commercially available preparations, Molecules 23 (5) (2018) 1230, https://doi.org/10.3390/molecules23051230

[3] V. Cardenia, T. Gallina Toschi, S. Scappini, R.C. Rubino, M.T. Rodriguez-Estrada, Development and validation of a Fast gas chromatography/mass spectrometry method for the determination of cannabinoids in Cannabis sativa L, J. Food Drug Anal. 26 (2018) 1283–1292, https://doi.org/10.1016/j.jfda.2018.06.001

[4] N. Fernández, L.J. Carreras, R.A. Larcher, A.S. Ridolfi, P.N. Quiroga, Quantification of cannabinoids in cannabis oil using GC/MS: method development, validation, and application to commercially available preparations in Argentina, Planta Med. Int. Open 07 (2020) e81–e87, https://doi.org/10.1055/a-1155-6613

[5] A. Nemeškalová, K. Hájková, L. Mikulů, D. Sýkora, Combination of UV and MS/MS detection for the LC analysis of cannabidiol-rich products, Talanta 219 (2020) 121250, https://doi.org/10.1016/j.talanta.2020.121250

[6] C. Citti, P. Linciano, S. Panseri, F. Vezzalini, F. Forni, M.A. Vandelli, G. Cannazza, G. Roda, D. Stewart, Cannabinoid profiling of hemp seed oil by liquid chromatography coupled to high-resolution mass spectrometry, Front. Plant Sci. 10 (2019) 120, https://doi.org/10.3389/fpls.2019.00120

[7] B. De Backer, B. Debrus, P. Lebrun, L. Theunis, N. Dubois, L. Decock, A. Verstraete, P. Hubert, C. Charlier, Innovative development and validation of an HPLC/DAD method for the qualitative and quantitative determination of major cannabinoids in cannabis plant material, J. Chromatogr. B 877 (32) (2009) 4115–4124, https://doi.org/10.1016/j.jchromb.2009.11.004

[8] F. Falaki, Sample preparation techniques for gas chromatography, Gas Chromatogr. - Deriv. Sample Prep. Appl. IntechOpen, 2019, https://doi.org/10.5772/intechopen.84259

[9] H.J. Barrales-Cureno, L.G. Lopez-Valdez, C. Reyes, V.M. Cetina-Alcala, I. Vasquez-Garcia, O.F. Diaz-Lira, B.E. Herrera-Cabrera, Chemical characteristics, therapeutic uses, and legal aspects of the cannabinoids of Cannabis sativa: a review, Braz. Arh. Biol. Technol. 63 (2020), https://doi.org/10.1590/1678-4324-2020190222

[10] F. Degenhardt, F. Stehle, O. Kayser, The biosynthesis of cannabinoids, Handb. Cannabis Relat. Pathol. Elsevier, 2017, pp. 13–23, https://doi.org/10.1016/B978-0-12-800756-3.00002-8

[11] C. Citti, D. Braghiroli, M.A. Vandelli, G. Cannazza, Pharmaceutical and biomedical analysis of cannabinoids: a critical review, J. Pharm. Biomed. Anal. 147 (2018) 565–579, https://doi.org/10.1016/j.jpba.2017.06.003

[12] D.C. Hao, X.-J. Gu, P.G. Xiao, Phytochemical and biological research of Cannabis pharmaceutical resources, Med. Plants (2015) 431–464, https://doi.org/10.1016/B978-0-08-100085-4.00011-6

[13] N. Sannikova, The Effect of Storage Conditions on Cannabinoid Stability, Ascens. Sci. Inc., 2020, pp. 1–7 ⟨https://ascensionsciences.com/wp-content/uploads/2020/06/2020-06-11-Technical-Articles-Ascension-Sciences-Effect-of-Storage-Conditions-on-Cannabinoid-Stability-.pdf⟩.

[14] A.G. Sicaire, M. Vian, F. Fine, F. Joffre, P. Carré, S. Tostain, F. Chemat, Alternative bio-based solvents for extraction of fat and oils: aolubility prediction, global yield, extraction kinetics, chemical composition and cost of manufacturing, Int. J. Mol. Sci. 16 (4) (2015) 8430–8453, https://doi.org/10.3390/ijms16048430

[15] Q.H.H. Le, N.N.C. Tran, T.C.T. Nguyen, H.G. Nguyen, K.T. Lac, T.A. Pham, P.N.S. Chu, Comparison and applicability of Agilent EMR-Lipid and Captiva EMR-Lipid Sorbents in QuEChERS method for food analysis, Vietnam J. Sci. Technol. Eng. 62 (3) (2020) 19–25, https://doi.org/10.31276/vjste.62(3).19-25

[16] U.S. Food, Drug Administration, Bioanalytical Method Validation Guidance for Industry. Food Drug Adm., 2018, pp. 1–41 ⟨https://www.fda.gov/files/drugs/published/Bioanalytical-Method-Validation-Guidance-for-Industry.pdf⟩.

[17] S.M. Moosavi, S. Ghassabian, Linearity of calibration curves for analytical methods: a review of criteria for assessment of method reliability, Calibration Valid. Anal. Methods - A Sampl. Curr. Approaches, InTech, 2018, https://doi.org/10.5772/intechopen.72932

[18] M. Feinberg, Validation of analytical methods based on accuracy profiles, J. Chromatogr. A 1158 (1–2) (2007) 174–183, https://doi.org/10.1016/j.chroma.2007.02.021

[19] B. Desmedt, M. Canfyn, M. Pype, S. Baudewyns, V. Hanot, P. Courselle, J.O. De Beer, V. Rogiers, K. De Paepe, E. Deconinck, HS-GC-MS method for the analysis of fragrance allergens in complex cosmetic matrices, Talanta 131 (2015) 444–451, https://doi.org/10.1016/j.talanta.2014.08.006

[20] Y. Tie, C. Vanhee, E. Deconinck, E. Adams, Development and validation of chromatographic methods for screening and subsequent quantification of suspected illegal antimicrobial drugs encountered on the Belgian market, Talanta 194 (2019) 876–887, https://doi.org/10.1016/j.talanta.2018.10.078

[21] S. Barhdadi, M. Canfyn, P. Courselle, V. Rogiers, T. Vanhaecke, E. Deconinck, Development and validation of a HS/GC–MS method for the simultaneous analysis of diacetyl and acetylpropionyl in electronic cigarette refills, J. Pharm. Biomed. Anal. 142 (2017) 218–224, https://doi.org/10.1016/j.jpba.2017.04.050

[22] ISO 5725–2:2019: Accuracy (trueness and precision) of measurement methods and results, ⟨https://www.iso.org/standard/69419.html⟩, 2019, Accessed 02 February 2021).

[23] A.J. Poortman-Van Der Meer, H. Huizer, A contribution to the improvement of accuracy in the quantitation of THC, Forensic Sci. Int. 101 (1999) 1–8, https://doi.org/10.1016/S0379-0738(99)00004-3

[24] D. Kushwah, H.B. Patel, P.K. Sinha, P.K. Jana, Practical approach for the determination of response factors of impurities in drugs by HPLC, E-J. Chem. 8 (2011) 1504–1511, https://doi.org/10.1155/2011/462364

[25] Cannabinoid Association Netherlands, CBD in Cosmetica, ⟨https://www.cannabinoidenadviesbureau.nl/en/cbd-in-cosmetica/⟩ 2020, accessed 21 March 2021.