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The analysis of cannabinoids in e-cigarette liquids using LC-HRAM-MS and LC-UV

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ABSTRACT

The use of cannabidiol or CBD products has skyrocketed in the last five years due to the alleged therapeutic benefits, a low potential for abuse and lack of the typical psychoactive effects associated with the use of cannabis products containing high levels of Δ9-tetrahydrocannabinol (Δ9-THC). In Belgium, CBD-containing e-liquids with a total THC content lower than 0.2% (w/w) are currently legal. In order to verify the compliance of the different CBD-containing e-cigarette liquids that are available to the Belgian population, a method was developed for screening of 17 cannabinoids and to quantify the major cannabinoids such as CBD, CBDA, Δ9-THC and Δ9-THCA. The latter was fully validated using the 'total error' approach, applying accuracy profiles and conforming to ISO17025. None of the analysed samples exceeded the legal limit for the total amount of Δ9-THC present. However, of the 20 CBD-liquids investigated in this study, only 30% of the samples contained an amount of CBD that was within 10% deviation of the label claim. Moreover, the CBD e-liquids labelled "full/broad spectrum" consisted of several minor alkaloids in comparison to the "classic" CBD e-liquids where the acidic forms of the cannabinoids were not present. Currently, no legislation is available for the regulation of CBD e-liquids, however these results indicate that quality controls are pertinent especially concerning the discrepancy in CBD label accuracy.

1. Introduction

The e-cigarette was invented almost 20 years ago and has since established itself as an alternative for smoking products. In addition to the classical inhalation of nicotine vapours, e-cigarettes have also been used for vaping recreational drugs and cannabinoids, including cannabidiol (CBD) [1]. CBD is a phytocannabinoid derived from *Cannabis sativa L.* and is considered to possess non psychoactive properties when compared to delta-9-tetrahydrocannabinol (Δ9-THC), the well-known principal psychoactive substance of this plant. The content of CBD and Δ9-THC can vary among the different cultivars or chemotypes. Hemp or chemotype III typically has lower concentrations of total THC (Δ9-THC and the different THC isoforms) and may have higher concentrations of cannabidiol (CBD) whereas chemotype I and II have higher concentrations of the psychoactive substances [2].

The last decade saw the use of CBD products gaining tremendous popularity. This can be illustrated by the fact that it is present in different consumers products including cosmetics, edibles, oils and tincture [3]. This wider use of CBD products can very likely be attributed to several health claims, although some can be considered as poorly substantiated [4].

In Belgium, as in many European countries, consumer products containing CBD including e-liquids are considered to be legal provided that their total Δ9-THC (Δ9-THC and Δ9-THCA) content does not exceed 0.2 mass percentage. This limit has been implemented based on the previously established thresholds for the regulation of agricultural hemp production in EU [5]. However, in 2023 the tolerable amount of THC that is allowed for agricultural hemp production will be increased to 0.3% for hemp products under the Common Agricultural Policy (CAP) [6]. For smoking and vaping products in particular, the Tobacco Product Directive 2014/40/EU (TPD) covers the main requirements. Smoking products with CBD are regarded as herbal products in the TPD. These include products based on plants, fruits or flowers, which can be consumed via combustion without tobacco [7]. The current definition of herbal products for smoking does not capture CBD containing oils and e-liquids, used in e-cigarettes.

In the context of the TPD, there is lack of clarity of the regulation of cannabis extracts or synthetic CBD in e-liquids. CBD e-liquids fall out of

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scope of the TPD because they are not a nicotine containing product. If the TPD were applied to non-nicotine containing e-liquids, these products may be regarded as "… other additives that create the impression that a tobacco product has a health benefit or presents reduced health risks;…" which, in that case would prohibit CBD e-liquids as a whole market within the EU. Although this could be easily circumvented by not making any health claims [8]. However up till now, no additional regulation other than the amended threshold value for the total Δ9-THC content has been put in place for CBD e-liquids.

In this study we set out to verify if the different CBD-containing e-cigarette liquids, available in the Belgian market are compliant for their total Δ9-THC content. Additionally, we also aimed to verify the CBD label accuracy for e-liquids as several findings have demonstrated the possible concerns on the label accuracy of CBD products [9–14]. In order to do so, it is pivotal to develop analytical methods that are fit for the purpose. Currently, gas chromatography coupled to flame ionization detectors (GC-FID), is the only legally accepted methodology to determine the total Δ9-THC content of dry herbal material originating from agricultural hemp production in the EU [15]. Consequently GC-FID and GC-coupled to mass spectroscopy have often been used in the past to determine the total Δ9-THC content in other matrices such as oils. These GC methodologies enable a total Δ9-THC content determination, due to the heat induced decarboxylation of the acidic version of Δ9-THC (Δ9-THCA). However, the applicability of GC-based methodologies to assess those products with low total Δ9-THC content has come under scrutiny as it has been revealed that CBD can undergo thermal decomposition resulting in its conversion to Δ9-THC [16]. Moreover, a recent study has also demonstrated that depending on the applied GC-methodology, a presumed cannabielsoin (CBE)-isomer, generated during the heating of the acidic form of CBA (CBDA) in e-liquid matrix could partially overlap with the peak of Δ9-THC, resulting in a overestimation of the amount of total Δ9-THC [17].

Therefore, in order to circumvent these possible heat-induced artefacts, liquid chromatography (LC)-coupled to a high resolution accurate mass spectrometer (HRAM-MS) was used to screen for 17 different cannabinoids. Following which, the cannabinoids of interest (Δ9-THC, Δ9-THCA, CBD and CBDA) were quantified by means of LC coupled to a diode array detector. The method was validated for the e-liquid matrix and the screening and subsequent quantifications were performed on the 20 samples obtained from the Belgian market during the period of 2017–2021.

2. Materials & methods

2.1. Reagents

The solvents used for the LC-MS analysis (acetonitrile, water and formic acid) were MS-grade while the solvents used for LC-UV analysis were HPLC grade. All were purchased from Biosolve (Valkenswaard, the Netherlands). The water used for LC-UV analysis was obtained using a milliQ-Gradient system (Millipore, Billerica, USA).

The components used to generate the matrix solution, propylene glycol and glycerol, were purchased from Merck (Darmstadt, Germany). For the assessment of selectivity, the following terpenes were also purchased at Merck (Darmstadt, Germany); (+)-cedrol, (-)-isopulegol, camphor, eucalyptol, geranyl acetate, hexahydrothymol, trans-caryophyllene, (-)-borneol, (+)-pulegone, (R)-(+)-limonene and L-(-)-fenchone. Linalool, gamma-terpinene and geraniol were bought from Thermo Fisher Scientific (Massachusetts, USA). The analytical standards of the terpenes have a purity of at least 97%.

2.2. Standards and stock solutions

The cannabinoid standards cannabidiol (CBD), Δ9-tetrahydrocannabinol (Δ9-THC) and cannabinol (CBN) were purchased from Lipomed GmbH (Germany). Cannabidiolic acid (CBDA), Δ9-tetrahydrocannabinolic acid A (Δ9-THCA), Δ8-tetrahydrocannabinol (Δ8-THC), cannabinol (CBN), cannabigerol (CBG), cannabichromene (CBC), Cannabicyclol (CBL), cannabidivarin (CBDV), tetrahydrocannabivarin (THCV), cannabicitran (CBT), tetrahydrocannabivarinic acid (THCVA), cannabinolic acid (CBNA), cannabigerolic acid (CBGA) and cannabidivarinic acid (CBDVA) were obtained from Cayman Chemicals Inc. (Michigan, USA).

Cannabielsoin (CBE) was obtained from Toronto Research Chemicals Inc. (Toronto, USA). All analytical standards of 17 cannabinoids had a purity ranging from 94.75% to 101.8%.

The standard stock solutions of 1 mg/ml of either CBD and Δ9-THC were prepared in ethanol prior to a dilution to 5 μg/ml in ACN/water (50:50). These stock solutions were stored at -20 °C and kept for 6 months.

All other commercially purchased reference standards of the different cannabinoids arrived already solubilised in either ACN or methanol at a concentration of 1 mg/ml. These stock solutions were also stored at -20 °C prior to the generation of dilutions in ACN/water mixture.

2.3. E-liquid sample set

A total of 20 e-liquids, collected between 2017 and 2021, were analysed. Twelve samples were obtained from the inspections conducted by the federal government authorities of different vaping shops in Belgium or from interception of online ordered post packages. The remaining 9 e-liquids were purchased from online vape shops. All samples were stored at room temperature (15–25 °C) and protected from light.

2.4. Preparation of standards and control solutions

2.4.1. Preparations for the screening methodology

The matrix components propylene glycol/glycerol were used as negative controls (0.5 g propylene glycol and 0.5 g glycerol in 50 ml ACN/water (50:50) and to mimic any putative matrix effect. During routine screening, a negative and positive control solution was injected prior to each series. The negative control consists of the matrix solution and the positive control, serving as a peak identification and sensitivity test, consisted of a mixture of the 17 cannabinoids in a concentration of 5 μg/ml in ACN/water (50:50). Moreover, in order to minimize carry-over a blank, ACN/water (50:50), was also injected in-between samples.

2.4.2. Preparations for the quantification methodology

A calibration line of at least 5 concentrations, required for quantification purposes, in the range from 2.5 to 50 μg/ml was generated for CBD, CBDA, Δ9-THC and Δ9-THCA by appropriate dilution of the stock solution with ACN/water (50:50). The calibration solutions were kept at -15 °C for no longer than 72 h.

2.5. Sample preparation

The preparation of the samples for screening was carried out using a simple dilute-and-shoot approach wherein the samples were diluted to 1:50 with ACN/water (50:50). Quantification was performed by selecting the appropriate dilution based on the CBD concentration. For the quantification of CBDA and THCA a 1:10 dilution with ACN/water (50:50) was used.

2.6. Chromatographic conditions

2.6.1. Screening methodology

The screening of the cannabinoïds in e-liquids were conducted on a Thermo Scientific™ Vanquish™ ultra-high performance liquid chromatography (UHPLC) system equipped with a Q-Exactive focus mass spectrometer. The applied chromatographic separation methodology

Table 1
Overview of the target analytes, their retention time, exact masses, their fragment ion and their relative intensities.

Cannabinoid	RT (min)	Relative RT (relative to CBD peak)	M+H	M-H	Fragments
CBDV	2.21	0.54	**287.2006**	285.1860	165.0906 (100); 123.0439 (33); 107.0856 (21)
CBE	2.28	0.56	**331.2260**	329.2127	109.1014 (100); 201.0905 (23); 205.1221 (46);
CBDVA	2.27	0.56	331.1904	**329.1760**	217.1223 (100); 151.0765 (37); 243.1029 (36)
THCV	3.56	0.87	**287.2006**	285.1860	165.0909 (100); 123.0440 (33); 107.0857 (20)
THCVA	4.07	1.00	331.1896	**329.1760**	285.1859 (100); 217.1233 (34); 163.0766 (24)
CBD	4.08	1.00	**315.2319**	313.2173	193.1220 (100); 123.0441 (51); 107.0857 (29)
CBDA	4.60	1.13	359.2217	**357.2071**	245.1547 (100); 179.1079 (47); 311.2021 (40)
CBG	4.85	1.19	**317.2475**	315.2330	193.1224 (100); 123.0441 (38); 194.1258 (13)
CBN	6.14	1.50	**311.2006**	309.1860	223.1106 (100); 195.1168 (39); 208.0878 (31)
CBGA	6.97	1.71	361.2373	**359.2228**	309.1862 (100); 310.1895 (23); 279.1394 (13)
Δ9-THC	7.23	1.77	**315.2319**	313.2173	193.1222 (100); 123.0440 (49); 135.1167 (28)
Δ8-THC	7.72	1.89	**315.2319**	313.2173	193.1222 (100); 123.0440 (47); 135.1167 (30)
CBL	8.99	2.20	**315.2319**	313.2173	235.1685 (100); 165.0910 (52); 123.0440 (38)
CBC	9.26	2.27	**315.2319**	313.2173	174.0675 (100); 231.1370 (62); 232.1402 (10)
CBT	9.90	2.43	**315.2319**	313.2173	193.1222 (100); 135.1160 (42); 259.1676 (32);
CBNA	10.11	2.48	355.1890	**353.1760**	309.1862 (100); 310.1895 (23); 279.1394 (13);
THCA	11.74	2.88	359.2217	**357.2071**	313.2174 (100); 357.2074 (25); 314.2211 (23)

was based on the application note of Waters for the determination of cannabinoids in Cannabis flowers [18] but was subjected to minor adaptations to be compatible with our MS system. The chromatographic separation was performed at 35 °C on a CORTECS UPLC Shield RP18, 90 Å, 1.6 μm, 2.1 × 100 mm (Waters, Milford, MA, USA) with a mobile phase consisting of 0.1% formic acid in water (A) and acetonitrile (B) in an isocratic elution (41:59) with a constant flow rate of 0,5 ml/min. The total run time of the method was 13 min and the injection volume was 2 μL. For the detection of target analytes and possibly other components, high resolution tandem mass spectrometry (HRMS/MS) using a Q-Exactive focus mass spectrometer (Thermo Scientific) was operated under full scan MS acquisition mode followed by All Ion Fragmentation mode (AIF). The Q-Exactive focus mass spectrometer was operated in alternating positive and negative heated electron spray ionisation (HESI) mode with subsequent alternating full MS scans of the precursor ions and all ion fragmentation scan (AIF) in which the precursor ions were fragmented by higher energy collisional dissociation (HCD). The MS scan was performed with 70,000 resolution (at *m/z* 200) and the MS/MS scan was performed with 17,500 resolution (at 200 *m/z*). The automatic setting was used for the maximum injection time. The *m/z* range for the full MS scans was set for *m/z* 150–400, and the *m/z* range for AIF scans was 80–400. The target value for the full MS scans was 10^6 ions and the target value for the AIF scans was 3×10^6 ions. The HCD collision energy was set at 30% during the entire run. The heated electrospray ionization (HESI) conditions were as follows: spray voltage: 3.5 kV (positive mode) and 2.5 KV (negative mode); sheath gas flow rate: 50 arb; auxiliary gas flow rate: 13 arb; sweep gas flow rate: 3 arb., heated capillary temperature: 325 °C; S-lens RF level: 50 V. Nitrogen was used for spray stabilization and as the collision gas in the C-trap.

All data were collected in profile mode and were acquired and processed by using the Thermo Xcalibur 4.0 software (Thermo Fisher Scientific, Bremen, Germany). A compound was considered present if the difference in retention was less than or equal to 0.5 min (compared with the retention time of the reference standard of this compound), *m/z* of the precursor ion is equal to the one obtained with the reference standard (error tolerance: 0.01 Da) and the MS2 spectrum corresponds to the MS spectrum of the reference standard (fragment ions and their relative intensities are given in Table 1). The acceptable relative error on the relative intensities of the fragment ions were the following: for relative intensities between 10 and 40: 30%; between 40 and 60: 25% and above 60 an relative error of 10% was deemed accepted. Because of the AIF mode, it might occur that identical fragment ions unrelated to one of the target peaks may be more abundant. Therefore, we accepted that 1 of the 3 selected fragment ions could exceed its relative error, if it is not higher than 100%.

2.6.2. Quantification of CBD, CBDA, Δ9-THC, Δ9-THCA (UPLC-UV)

The LC methodology that was utilised for the screening methodology was also applied for the quantification of CBD, CBDA, Δ9-THC, Δ9-THCA with UHPLC-DAD. The analyses were conducted on an Acquity UPLC™ system (Waters, Milford, USA) equipped with a photodiode array detector. The wavelength used for the quantification was 269 nm.

2.7. Method validation

2.7.1. Validation of the screening methodology

It is mandatory for a screening methodology to correctly identify and distinguish the cannabinoids of interest from each other and from matrix components. This has to be demonstrated for a certain concentration level, the screening detection limit (SDL) for which the respective cannabinoids can be correctly identified in 95% of the samples [19]. The SDL of the different cannabinoids was experimentally determined by serial dilutions in diluted matrix and corresponded to the lowest concentration for which the signal to ratio reached a value equal to or exceeding 3.3. The LC-HR-MS screening method was validated for the intended purpose according to the validation guidelines [19,20]. [5], [6]. The validation samples (n = 10) were prepared by spiking commercial (flavoured) e-liquids with 50 ng/ml of all targeted cannabinoids. Evidently these commercial flavoured e-liquids were devoid of any cannabinoids. In total 20 samples (spiked and non-spiked) were analysed.

2.7.2. Validation of the quantification methodology

For the validation of the selectivity, a 'terpenes'-spiked matrix was prepared containing a selection of 14 terpenes typically found in *Cannabis sativa* [21]. These include (+)-cedrol, (-)-isopulegol, camphor, eucalyptol, geranyl acetate, hexahydrothymol, linalool, trans-caryophyllene, (-)-borneol, (+)-pulegone, (R)-(+)-limonene, gamma-terpinene, geraniol, L-(-)-fenchone. These 14 terpenes were added at a final concentration of 1% (w/w) to the matrix solution. For the assessment of the selectivity of the UHPLC-DAD method, co-elution of these terpenes at the same retention time as the target components (CBD, Δ9-THC, CBDA, Δ9-THCA) was investigated.

Further validation of the UHPLC-DAD quantification methodology method was performed using the total error approach and applying accuracy profiles, compliant to the ISO 17025 guideline [22]. Through this approach, it is possible to calculate trueness, accuracy and precision and estimate the total error and uncertainty of the developed method. For the method validation, the β-expectation tolerance intervals were calculated at 95%. Currently, there is no agreement on the acceptance limits to be used for e-liquids. Considering the wide concentration range, an acceptance limit of ± 10% was therefore regarded as acceptable.

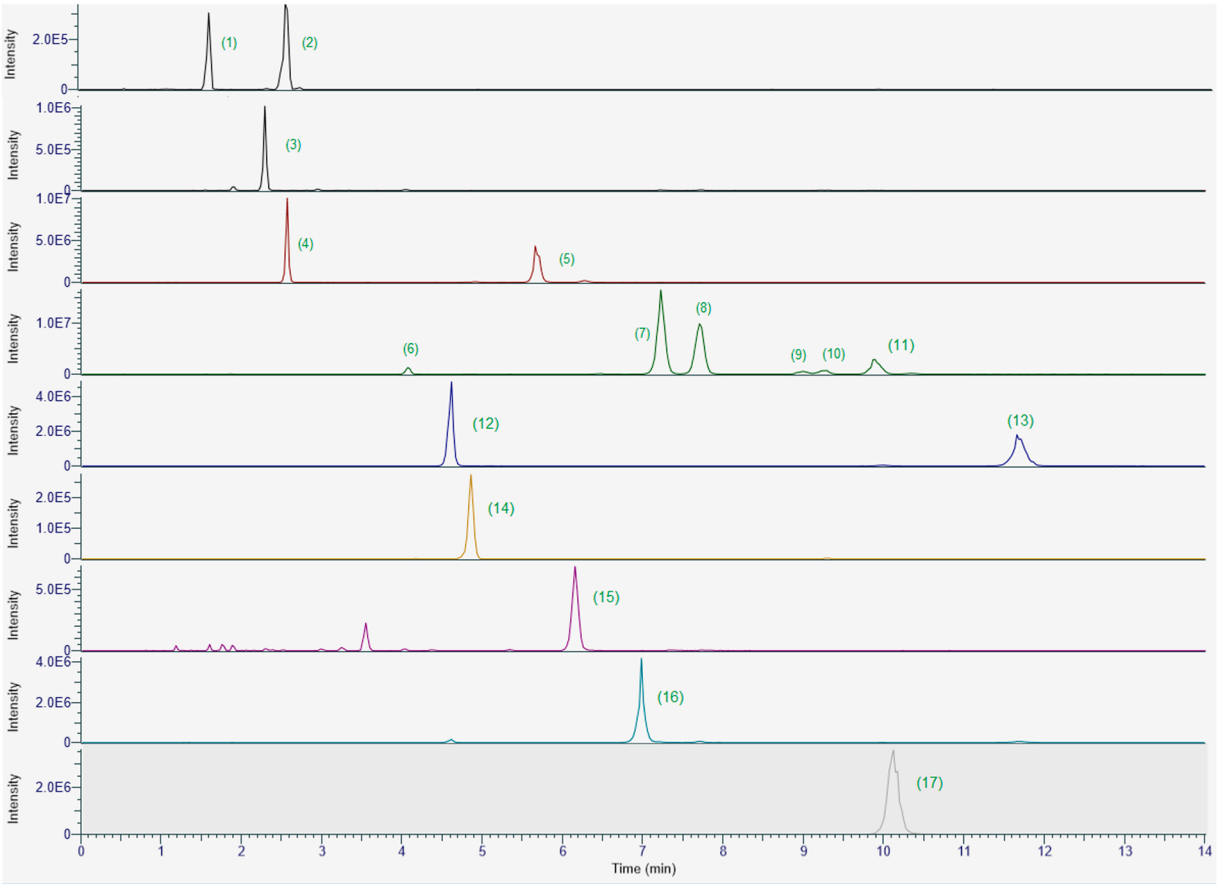

Fig. 1. Extracted chromatograms of the cannabinoids standards by HRAM-LC-MS at a concentration of 5 μg/ml. The *m/z* at which they are extracted corresponds to their *m/z* in Table 1. (1) CBDV (2) THCV (3) CBE (4) CBDVA (5) THCVA (6) CBD (7) Δ9-THC (8) Δ8-THC (9) CBL (10) CBC (11) CBT (12) CBDA (13) THCA (14) CBG (15) CBN (16) CBGA (17) CBNA.

3. Results

3.1. Development and validation of the screening methodology

The applied chromatographic separation methodology was based on a previously developed methodology for the analysis of cannabinoids in *Cannabis* flowers [18] with some minor adaptations to be compatible with downstream MS-detection (see material and methods). It stands to reason that the sample preparation or the extraction of cannabinoids from plant material is different than from e-liquids. Initially we set out to perform a dilute and shoot approach with a dilution in ethanol as it remains the solvent of choice for the extraction of most of the cannabinoids [23] and it is miscible with the e-liquid matrix. However, at least in our case, the use of 100% organic solvent resulted in a broad peak shape, which could potentially interfere with the correct identification of some closely eluting peaks with similar *m/z*. Therefore, we noticed that it was pivotal to prepare the injected samples within a diluent that closely resembled the mobile phase and thus chose to utilise a 50% ACN solution as the diluent for the dilute and shoot methodology. Moreover, as it is known that cannabinoids are thermolabile and light sensitive [24, 25], care was also taken to inject the samples immediately after preparation and leave them for maximum 24 h at a sample temperature of 5 °C. Since we also noticed that small retention time differences occurred between injection series, we deemed it is necessary to also inject a solution for peak identification and as sensitivity test prior to each injection series (as described in 2.4.1.). Nevertheless, after all optimizations we were able to chromatographically separate the majority of the cannabinoids (see Table 1 and Figure 1). This separation might be of utter importance since phytocannabinoids may have identical nominal masses. Indeed, CBD, Δ9-THC, Δ8-THC, CBC, CBT and CBL all have similar masses i.e. [M+H] 315,2319 *m/z* and are separated sufficiently in the chromatogram. The peak pair Δ9-THC/Δ8-THC and CBT/CBC were the most critical pairs to separate. However, their relative retention times remained stable over time.

In addition to the desired chromatographic separation, an accurate mass determination is also essential to distinguish between similar nominal masses. The peaks of CBE and CBDVA have similar nominal masses, but the mass accuracy of the mass spectrometer is high enough to distinguish between both molecules (*m/z* CBE = 331,226; *m/z* CBDVA = 331,190 *m/z*). Moreover, for the above mentioned CBT and CBC molecules with similar nominal mass and comparable retention times, the difference in fragmentation was an additional identification parameter to distinguish both the cannabinoids (see Table 1).

The LC-HRMS screening method was subsequently also validated for the intended purpose. All validation samples, spiked and non-spiked, were correctly identified and the obtained screening detection limit (SDL) was set at 50 ng/ml for all 17 cannabinoids. The SDL is regarded as the lowest concentration at which it has been demonstrated that a given analyte can be detected in at least 95% of the samples. Herein, the SDL was set so as to result in the occurrence of zero false positives and zero false negatives.

3.2. Development and validation of the quantification methodology (CBD, CBDA, Δ9-THC and Δ9-THCA)

In contrast to the LC-HRAM-MS methodology, which has a high specificity, the identification and subsequent quantification by means of UHPLC-UV is solely based on the determination of the retention times

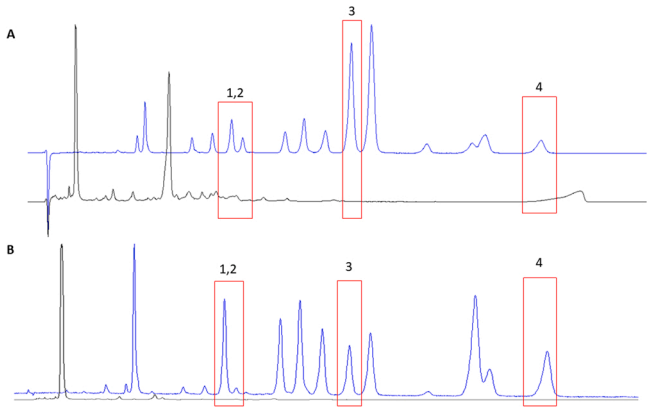

Fig. 2. Comparison of chromatograms of cannabinoids target components (blue) and terpene mix (black) at a wavelength of 228 nm (A) and 269 nm (B). The interference of co-eluting peaks from the terpene mix is negligible at a detection wavelength of 269 nm. (1 = CBD, 2 = CBDA, 3 = Δ9-THC, 4 = Δ9-THCA).

and the matching of the UV-spectra. This method makes it highly susceptible to co-eluting peaks. In CBD containing e-liquids there might be an interference of the matrix components, which may include terpenes. Therefore, in order to assess the selectivity, the co-elution of a selected set of cannabis terpenes with the main cannabinoids was investigated. For the quantification method by UPLC-DAD, co-elution of CBD, Δ9-THC, CBDA and Δ9-THCA would be problematic. The initial setting of the UHPLC methodology utilised the wavelength 228 nm to quantify cannabinoids. However, as can be seen on the chromatogram in Fig. 2 A, there is a small overlap between the chromatogram of the terpenes and CBD and CBDA region. Interestingly, the UV spectra of the target components differ from the terpenes. At the wavelength of 269 nm, a decrease in the UV-absorbance of the selected terpenes is observed and it presented no absorbance in the region of the CBD and CBDA peaks. The target cannabinoids, however still showed significant absorbance for quantification of the peak (see Fig. 2. B). However, co-elution with the other cannabinoids still occurred, demonstrating the utility of the LC-HRAM-MS methodology for initial identification purposes of cannabinoids. To fully disclose the interference of other components with CBD, Δ9-THC, CBDA and Δ9-THCA, all cannabis terpenes along with the added flavourings should be assessed.

Next, the selective and specific methodology was successfully validated using the 'total error' approach and illustrated by accuracy profiles. The obtained accuracy profiles show (Fig. 3) that the β-expectation tolerance intervals did not exceed the acceptance limits of ± 10%, meaning that 95% of future measurements will be included in the [− 10%, 10%] bias limits. All validation parameters are also reported in Table 2. The maximum relative bias was 5.25% for THCA and the highest RSD% of the intermediate precision was 3.4% for CBDA. The limit of quantification is determined as the lowest concentration level that is validated. The linearity of the calibration curve was confirmed using R^2 values (<0.999) and the Mandel fitting test. The Mandel fitting test was not significant for the target components indicating that there was no significant difference between a linear and quadratic calibration model, in which case the linear model was preferred. The linearity of the results, demonstrated as the relationship between the measured concentration and the theoretical concentration, was linear with R^2-values above 0.9999 for all components. Matrix effects were visually inspected through chromatogram overlays of the target components with and without the presence of e-liquid matrix in order to check whether the use of internal standards was necessary. There was no indication of a matrix effect as the chromatogram overlay did not show any response enhancement or suppression of target components. The recovery was also well within the accuracy limits. Additionally the matrix effect was evaluated by comparing the slope of the standard calibration line with the slope of the addition curve by means of a statistical test (t-test). A matrix effect can said to be present when the standard calibration is not parallel with the standard addition line. The slopes of both curves were compared by means of t-test. For all the target components, it can be concluded that there was no significant difference between the slopes ($p > 0.05$). Thus, no internal standard was needed for quantification with UHPLC-DAD.

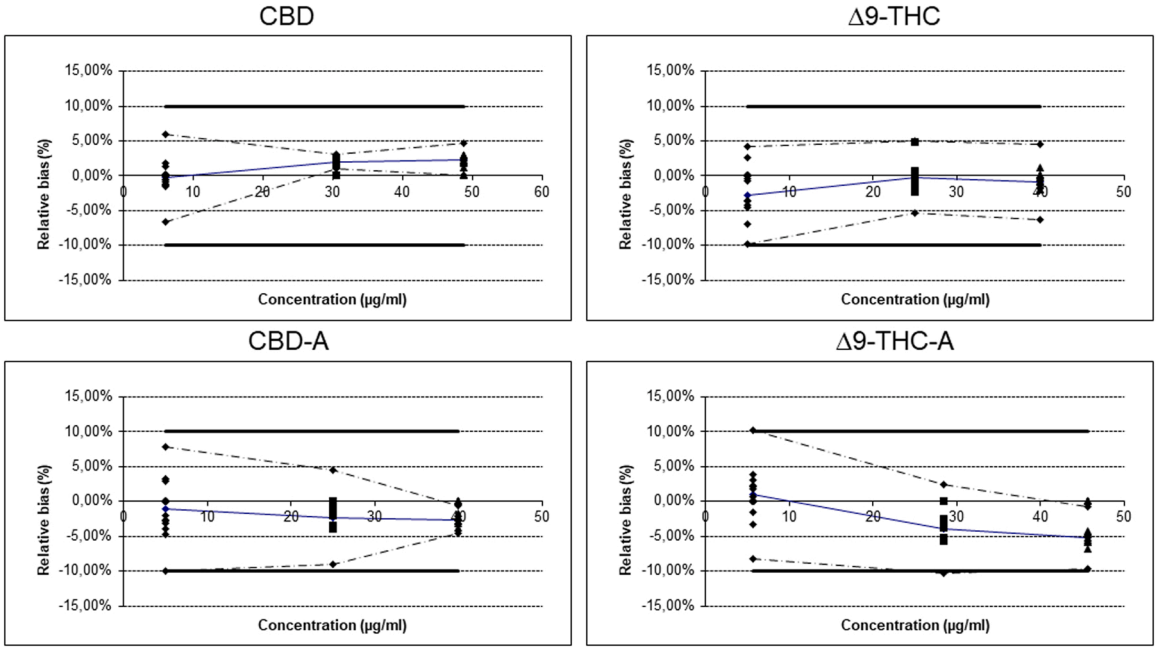

Fig. 3. The obtained accuracy profiles of CBD, CBD-A, Δ9-THC and Δ9-THCA, demonstrating that the β-expectation tolerance intervals did not exceed the acceptance limits of ± 10%. Legend: Relative bias (—), upper and lower β-expectation tolerance limits (— •), upper and lower acceptance limits set at 10% (—), relative back-calculated concentrations per spiking level (◆▲■).

Table 2
Validation parameters of the UPLC-DAD quantitative method for each target component.

	Concentration level (μg/g)	CBD	Δ9-THC	CBDA	THCA
Accuracy (total overall bias %)	5	-0.27%	-2.80%	-1.09%	1.02%
	25	2.05%	-0.18%	-2.27%	-3.97%
	40	2.33%	-0.83%	-2.60%	-5.25%
Repeatability (RSD)	5	0.62	2.903	2.95	1.366
	25	0.387	2.061	0.45	0.403
	40	0.438	0.522	0.452	0.417
Intermediate precision (RSD)	5	1.29	2.903	3.391	2.533
	25	0.387	2.061	1.418	1.345
	40	0.641	1.115	0.651	0.965
β-expectation tolerance limit	5	[-6.54%; 5.99%]	[-9.83%; 4.23%]	[-9.95%; 7.77%]	[-8.16%; 10.19%]
	25	[1.06%; 3.03%]	[-5.31%; 4.95%]	[-9.09%; 4.56%]	[-10.35%; 2.40%]
	40	[1.15%; 4.64%]	[-6.22%; 4.57%]	[-4.55%; -0.65%]	[-9.72%; -0.78%]
LOD/ LOQ (μg/g)		1/5	1/5	1/5	1/5
Linearity (R^2)		1	1	0.9998	0.9998

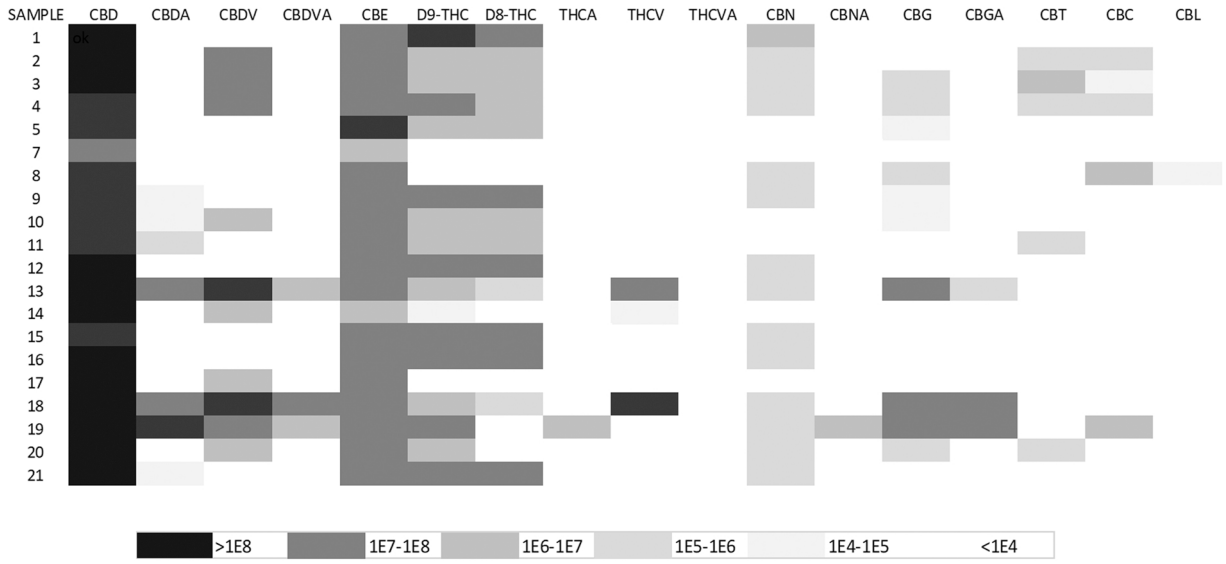

Fig. 4. Overview of the relative amount of each minor cannabinoid normalized to the CBD concentration found in the e-liquids for each sample. For each sample, the intensity of the grayscale represents the relative amount of each minor cannabinoid normalized to the CBD concentration found in the e-liquids.

Table 3
Results of CBD and THC content of the analysed CBD e-liquids.

Sample number	acquired in	Claimed CBD conc (mg/ml)	Actual CBD conc (mg/g)	%CBD	%THC	CBDA (mg/g)	THCA (mg/g)
1	2019	5	2.8	56.7	0.030	-	-
2	2019	10	10.0	100.2	0.004	-	-
3	2019	20	19.0	95.0	0.005	-	-
4	2019	100	45.9	45.9	0.022	-	-
5	2017	5	1.3	25.1	<LOQ	-	-
6	2017	5	0.03	0.5	<LOQ	-	-
7	2018	12	0.8	7.0	<LOQ	-	-
8	2018	30	28.4	94.7	0.030	-	-
9	2018	50	40.5	81.1	0.003	-	-
10	2020	100	102.2	102.2	0.022	-	-
11	2018	5	3.5	69.6	0.004	-	-
12	2021	20	13.5	67.3	0.004	0.023	-
13	2021	10	7.8	77.5	<LOQ	-	-
14	2021	10	3.8	37.7	0.004	-	-
15	2021	10	7.6	76.3	0.015	-	-
16	2021	10	6.9	69.1	0.002	-	-
17 *	2021	10	9.7	97.1	0.002	0.065	-
18 *	2021	10	8.5	85.4	0.006	0.115	0.001
19 *	2021	10	4.9	48.8	0.001	-	-
20 *	2021	10	5.4	54.4	0.001	-	-

- not detected by LC-UV
* labelled as full spectrum CBD e-liquids

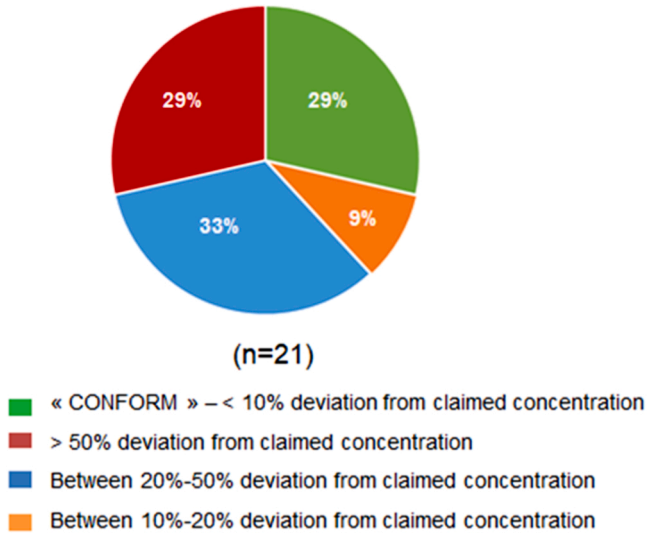

Fig. 5. Results of the quantitative analysis of 21 commercial CBD e-liquids for label accuracy expressed as % deviation of the claimed concentration.

3.3. Sample analysis

3.3.1. Screening for cannabinoids with LC-HRAM-MS

All of the 20 analysed CBD e-liquids contained indeed CBD but also several other cannabinoids. In Fig. 4, an overview of the relative amount of each cannabinoid normalized to the amount of CBD present in the e-liquids for each sample is represented. The minor cannabinoids present in the CBD e-liquids were variable for each sample. CBE was the most abundant phyto-cannabinoid in the e-liquids followed by CBN, CBDV and CBG. While CBL was only detected in one e-liquid. CBD e-liquids labelled as broad spectrum and full spectrum indeed contained various cannabinoids including its acidic forms in relatively higher concentrations. Other conventional CBD e-liquids can also contain atypical minor cannabinoids such as CBT, CBC and CBL. The presence of these minor cannabinoids might be due to degradation of CBD (see below) or because it is particular to the hemp from which the CBD is derived, owing to the different chemotypes and chemovars of *Cannabis sativa* [26].

CBE, which was found in all CBD e-liquids hasn't been mentioned often in the analysis of minor cannabinoids of *Cannabis sativa* and hemp derived product, although it would be typical for Lebanese hashish [27]. It was mainly reported as a decomposition and/or degradation product of CBD due to thermal oxidative conditions and pyrolysis [16,28]. However, this seems unlikely as during the sample preparation no heating nor excessive oxidation took place. Also, to further investigate the relevance of this minor cannabinoid in CBD e-liquids, quantification was needed. Interestingly, there is little known about the bioactivity of this minor cannabinoid.

The psychotropic effects of THC-derivatives are commonly known. They are included in the UN listing of psychotropic substances. CBD, CBDA and CBG are known for the lack of psychotropic effects. For the other cannabinoids there is no certainty about psychotropic effects because of conflicting reports on the activity at the cannabinoid receptors (CBN) or because of the lack of information (CBT, CBL, CBC, etc.) [29,30]. Thus, the clinical relevance of these minor cannabinoids remains unknown.

3.4. Quantification of CBD, THC, CBDA and THCA in commercial samples

The total CBD (CBD and CBDA) and Δ9-THC (Δ9-THC and Δ9-THCA) content were also quantified in the commercial CBD samples. The results demonstrated that only 30% of the samples had a deviation of less than 10% of the claimed CBD concentration (see Table 3). Moreover, more than 60% of the samples contained a CBD concentration which was at least 20% lower than the claimed CBD concentration and almost half of these e-liquids contained half or even less of the claimed amount of CBD (Fig. 5). This large deviation in CBD content could be attributed to the aging of the sample. Indeed, the older samples have higher deviations than the more recent samples. However, the more recent samples from 2021 contained lower amounts of CBD than what was claimed. Only 2 out of these 9 samples were within 20% of the label accuracy. Additionally to the total CBD content, the total THC content was also determined (see Table 3). The total THC levels were found to be below the legal limit of 0,2% (w/w).

Recently, several studies were conducted to investigate the label claim of CBD e-liquids [10,11,25,31,32]. From these studies it can be concluded that the labelling accuracy of commercial CBD e-liquids is indeed poor, considering that in best case scenario 50% of the analysed samples had a CBD content that was within 10% of the label claim [8]. A closer look at the discrepancies revealed that under-dosed CBD e-liquids were more frequent than over-dosed CBD e-liquids [8]. This might be explained by degradation of CBD in the e-liquid matrix (propylene glycol, glycerol). In their study, Mazzetti et al. showed that there were indications that storage at higher temperature (37 °C) showed significantly degradation as well as the exposure to light [25]. Therefore, stability studies considering different storage conditions might be required to determine the shelf life of these type of products.

4. Conclusion

In the last decade, the market has been flooded with CBD products, including CBD e-liquids for e-cigarettes. In this study, a method was optimised to screen for 17 different cannabinoids in CBD e-liquids and quantify accurately the major cannabinoids CBD, CBDA, Δ9-THC and Δ9-THCA, as only CBD products with THC content lower than 0,2% (w/w), are legal in Belgium. The developed methods were used to investigate 20 CBD e-liquid samples. Our results indicated that the THC levels were below the legal limit of 0,2% for all investigated samples. However, for only 30% of the samples the CBD content was within a 10% deviation of the label claim. This could be due to degradation of CBD. Therefore, stability studies considering different storage conditions might be required to determine the shelf life of these type of products before putting them on the market. Moreover, from a public health perspective it is also important to investigate the CBD and THC content in the aerosol emissions. In the study of Czégény et al., it is suggested that a fraction of CBD is transformed to other cannabinoids such as CBN, CBC Δ9-THC and Δ8-THC in certain conditions (high temperature, acidic environment). Therefore, increasing the risk of exposure to substances with potential psychoactive activity. Additionally, the method should be extended with the screening of other emerging phytocannabinoids such as Δ10-THC and HHC and a quantification method for the other cannabinoids for an accurate analysis of the cannabinoid composition. Currently, the regulation of CBD e-liquids is situated in the grey zone as these products are not explicitly defined by the Tobacco Product Directive nor any national legislation, at least in Belgium. Yet, these results indicate that quality controls are pertinent and thus need to be specified more clearly, especially concerning the stability of the CBD in e-liquids.

CRediT authorship contribution statement

Sophia Barhdadi: Conceptualization, Methodology, Investigation, Writing – original draft, Visualization **Patricia Courselle:** Supervision **Eric Deconinck:** Writing – review & editing, Funding acquisition **Celine Vanhee:** Methodology, Validation, Writing – review & editing.

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.

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