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CBD-Containing Liquids for e-Cigarettes: Formation of Psychotropic and Secondary Cannabinoids and Amount of CBD Surviving the Smoking Procedure

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Abstract: Recently, as the interest in cannabidiol (CBD) has grown due to its therapeutic potential, e-cigarette liquids containing CBD have proliferated on the market. Typically, e-liquids contain variable concentrations of CBD (from 2 mg·mL^{-1} to 20 mg·mL^{-1}) in propylene glycol or 70:30 propylene glycol:glycerol mixture and are eventually flavored with food-grade flavors. In this work, carried out by a GC-MS analysis of the condensed smoke produced by a real e-cig, we have demonstrated the actual amount of CBD that can survive the smoking process, and we found that negligible amounts of THCs are formed during the smoking process (i.e., the amount formed was <0.005 mg for each mg of vaped CBD); considering that the threshold dose for Δ^9-THC is around 2.5 mg (smoked or ingested *per os*), it is reasonable to conclude that accidental THC intoxication is unlikely, which is a very important issue from a forensic point of view, as in some court cases the use of e-cig liquids containing CBD has been argued as being the source of THC intoxication. Furthermore, all the other cannabinoids considered in this study and potentially derived from CBD thermal degradation have concentrations below the instrumental LOD.

Keywords: e-cig liquids; cannabidiol; THCs; quantification; GC-MS analysis

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1. Introduction

In recent years the e-cigarette market has been growing, as these devices are seen as a valid and less harmful alternative to traditional cigarettes [1,2].

These devices consist of an atomizer, an electric battery, and a cartridge (also called "tank"). The atomizer, a metallic coil that acts as an electronically heated resistor [3], is a heating element that vaporizes the liquid solution—called "e-liquid"—contained in the cartridge. Instead of smoke, the user inhales vapor, which is why the use of an e-cigarette (e-cig) is often referred to as "vaping".

Typically, e-liquids contain glycerol and/or propylene glycol as solvents and eventually nicotine as the physiologically active ingredient (API); due to the versatility of this device, the nature of the dissolved API can easily be changed, passing from nicotine to other compounds [4]. Recently, a growing interest for cannabidiol (CBD, structure 1 in Figure 1) has arisen, due to its peculiar pharmacological properties [5]: as this last compound has been considered a panacea [5], apart from its established therapeutic potential, e-cig liquids containing CBD have invaded the market [6].

From a pharmaco-toxicological point of view, CBD has well-established uses, such as the 2018 United States FDA approval of CBD for the treatment of two rare forms of epilepsy, Lennox-Gastaut syndrome, and Dravet syndrome [7]. In addition, anxiolytic and antipsychotic properties are well established, as demonstrated by its ability to attenuate psychotomimetic and anxiogenic effects induced by high doses of Δ^9-THC in humans [7].

Tests to evaluate its anti-inflammatory and neuromodulator effects, which would make this compound useful for the treatment of psychiatric or inflammatory conditions, have shown some promising but mixed results, as reviewed in the literature [8].

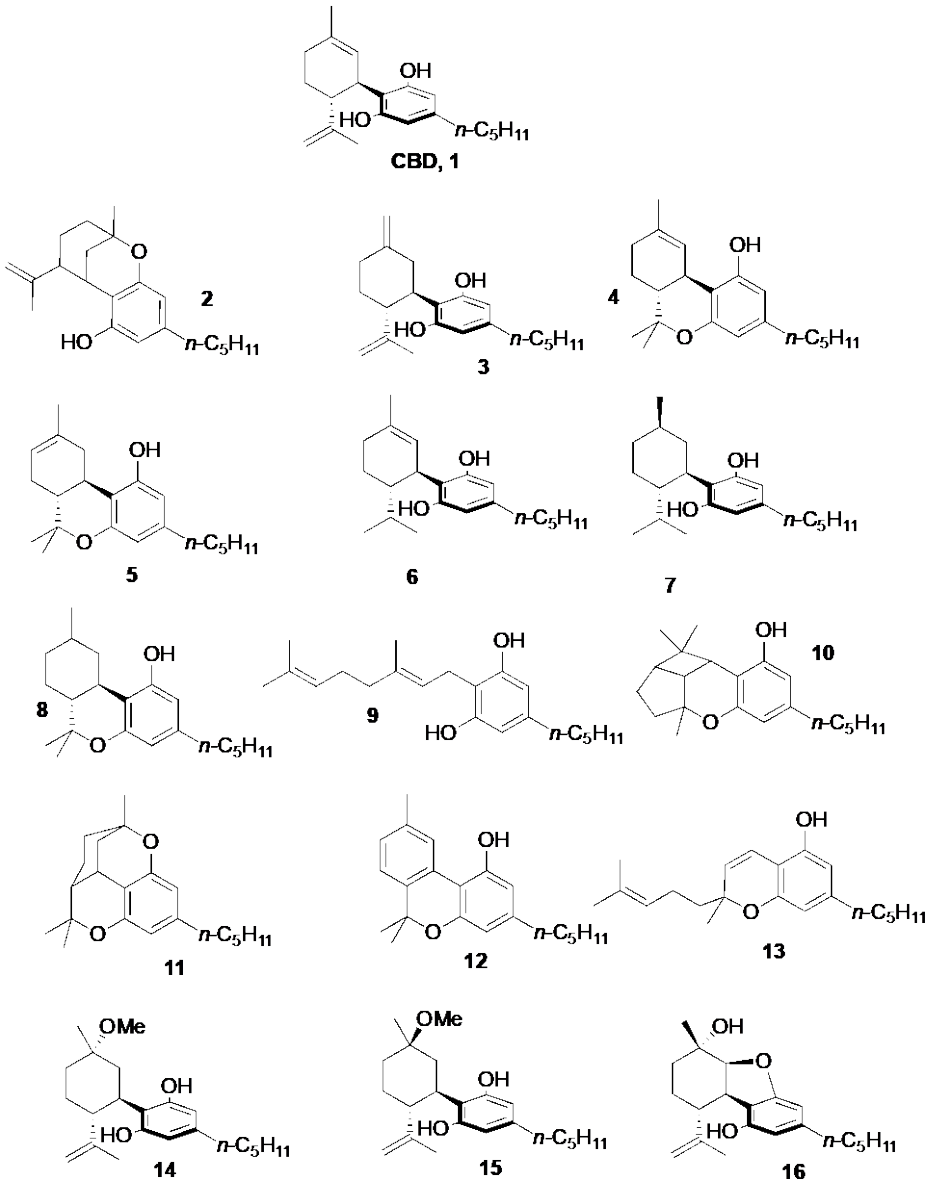

Figure 1. Structure of the cannabinoid derivatives 2–16 considered as possible degradation products in the present work. In Abbreviation section the complete list of the names of these structures is reported.

To the best of our knowledge, CBD has a very good safety profile [8], although the results of toxicological and pharmacological studies are strongly affected by the low bioavailability of CBD, that depend on its route of administration and the vehicle used for the administration, making the comparison of the literature data difficult and making the therapeutic outcomes variable and unpredictable [8]. From data available, it is clear that it lacks euphoric (psychotropic) effects, but nevertheless, its pharmacokinetic characteristics and strong enzyme-inhibiting properties [9] pose the potential for adverse effects and drug–drug interactions [7]. It has been shown that the individual response to CBD is highly variable, requiring ad hoc dosing and a case-by-case approach, with close monitoring of liver enzymes to prevent potential hepatotoxicity; nevertheless, the CBD-related increase

in liver enzymes is not accompanied by hepatic impairment, and its clinical significance is not well understood [9].

Parallelly, the low evidence with respect to the safety of CBD after chronic use poses some questions about its long-term health effects after chronic use, as the absence of evidence does not mean the evidence of absence.

In the commercially available e-liquids, CBD is dissolved in neat propylene glycol or in 70:30 propylene glycol:glycerol mixture. Glycerol alone is not used due to the limited solubility of the API in this medium. Commonly, 2 $mg \cdot mL^{-1}$, 10 $mg \cdot mL^{-1}$, or 20 $mg \cdot mL^{-1}$ CBD solutions are available, eventually aromatized with food-grade aromas [10,11].

In some countries, the ready-to-use e-liquid containing CBD is sold freely while in other ones, in compliance with the laws in force, in a two-container kit containing separately CBD crystals and propylene glycol [12], which are mixed before use [13].

The use of glycerol and propylene glycol as solvents for the e-cig is not as harmless as one might think, as highly reactive species can be formed during smoking, mainly carbonyl compounds such as aldehydes (e.g., formaldehyde, acrolein) and ketones (acetone, hydroxyacetone) [14]. However, the amount of these compounds formed is not considered a health risk by regulatory authorities, so glycerol and propylene glycol are freely sold in both the US and European countries as e-solvents [4,14]. Propylene glycol and glycerol make up most of the whole smoke produced, and their exposure is relatively high compared to other aerosol components. However, propylene glycol has a very low toxicity even at relatively high doses. Mild sensory and respiratory irritation effects may result in concentrations higher than 871 $mg \cdot m^{-3}$, which corresponds to ~17.5 $g \cdot day^{-1}$ exposure for a 70 kg adult assuming 20 m^3 air intake per day, far above the amount assumed by using an e-cig (i.e., few grams) [8]. Weak irritation effects are found only when exposed to high concentration of glycerol vapor, i.e., >> 200 mg $glycerol \cdot m^{-3}$ and, and as a common intermediate of human metabolism, it is not considered toxic once adsorbed [8].

According to commercial claims, the use of CBD-containing e-liquids leads the consumer to believe that e-cigs can be considered a valid method for systemic delivery of CBD, but it is not yet clear how much API can survive the vaping process and whether THCs are formed in real life scenarios. Some doubts arise because, for example, it is known that in the case of nicotine-containing e-liquid, erratic results are reported regarding the amount of alkaloid delivered [15]. In the case of CBD-containing e-liquids, it has been assumed that the active ingredient is transferred to the smoke, but no quantitative data are available, while the formation of other cannabinoids in real cases is completely neglected [16].

In this paper, using GC-MS for the analyses of the smoke produced by an e-cig and condensed in cold chloroform, we have quantified the amount of CBD that can be released and shown that other cannabinoids—in particular, the psychotropic THCs, see Figure 1—are formed in negligible amounts. THCs could, in principle, be formed by CBD cyclization, and there is much concern about the possibility that CBD vaping could lead to psychotropic effects for this reason [17]. The issue is a hot topic from a forensic point of view, as in some countries, CBD is freely sold, while THC is strictly regulated; in other countries this last compound is also deregulated, but some activities are prohibited under its influence (e.g., driving) [18]. Recently, in forensic toxicological laboratories are emerging cases of people positive to Δ^9-THC declaring that the origin of intoxication was the legal use of e-cig-vaped CBD, rather than the use of psychotropic cannabis strains, raising legal concerns that this article helps to unravel [19,20]. In addition, following the reported claims and based on the literature data obtained in a simulated medium, it has been advocated that CBD-containing e-liquids should be withdrawn from sale as a possible source of THCs, and a lively debate on this topic is now active in both clinical and forensic contests [19,21]. Regarding this aspect and using a real e-cig device, we have shown that results obtained with simulated devices are undoubtedly important but do not necessarily reflect the real case scenario, as negligible amounts of THCs are formed under our conditions.

2. Materials and Methods

2.1. GC-MS Conditions and Identification of Degradation Products

Details regarding the chromatographic method and its validation are reported in previous works [22–28] and in the Supporting Information S2.

Spectral data and GC-MS characterization of the compounds (including the fragmentation patterns, the retention times in the described conditions, and the way to evaluate the retention index) are reported in previous works [17,22].

GC-MS analyses have been performed with a Thermo Scientific DSQII single quadrupole GC/MS system (TraceDSQII mass spectrometer, Trace GC Ultra gas chromatograph, TriPlus autosampler—ThermoFisher Scientific, Waltham, MA, USA).

Gas chromatographic runs were performed on a Rxi-5Sil MS capillary column (30 m length × 0.25 mm ID × 0.25 µm film thickness, Restek, Milan, Italy) with helium (>99.99%) as carrier gas at a constant flow rate of 1.0 mL·min^{-1}. An injection volume of 1 µL was employed. The injector temperature was set at 290 °C operating in split mode (split ratio 1:10), with a split flow of 10 mL·min^{-1}. The oven temperature was programmed from 130 °C (isothermal for 2 min) to 300 °C (isothermal for 5 min) at a rate of 5 °C·min^{-1}. Data acquisition started 5 min after injection. Mass transfer line temperature was set at 310 °C. All mass spectra were acquired with an electron ionization system (EI) with ionization energy of 70 eV and source temperature of 250 °C, with spectral acquisition in Full Scan mode, positive polarity, over a mass range of 50 amu·s^{-1} to 950 amu·s^{-1} with a scan rate of 735 amu·s^{-1}.

Assignment of chemical structures to chromatographic peaks was based on the comparison of their mass spectra fragmentation patterns with pure standards; when possible, further confirmation was performed based on the databases for GC/MS NIST Mass Spectral Library (Nist 08) and Wiley Registry of Mass Spectral Data (8th Edition), license number: 35040 (2004 edition and updates), SWGDRUG Mass Spectral Library v3.7 (2020, freely downloadable at https://www.swgdrug.org/ms.htm, accessed 1 April 2023), and Cayman Spectral Library (2019, freely downloadable at https://www.caymanchem.com/forensics/publications/csl upon registration, accessed 1 April 2023) using Xcalibur MS (Version 2.0.7 SP1.48, ThermoFisher, XCALI-64194 (2008)) and AMDIS software (version 11-25-2019, freely available at https://chemdata.nist.gov/dokuwiki/doku.php?id=chemdata:amdis, accessed 1 April 2023).

An orthogonal identification was performed comparing the retention indexes with those published (NIST). A series of n-alkanes (C8-C40, Aldrich (St. Louis, MO, USA), 1000 mg·L^{-1} standard for GC) was used to determine the retention indices.

Peaks representing <2% TIC were not considered unless proper standards were available for their identification.

The standards used for the quantification were synthetized as reported elsewhere [22]; their structure is reported in Figure 1. Cannabielsoin was prepared according to literature [23].

For the quantitative analysis, an aliquot of the samples was spiked with olivetol (200 mg·L^{-1}) as the internal standard (retention time under our conditions: 13.44 min), and the same solutions not spiked with olivetol were also analyzed.

2.2. Set of the Electronic Cigarette

The e-liquids were prepared in the laboratory by dissolving appropriate amounts of pharma-grade CBD (title: >99.5%) in the appropriate solvent and analyzed with GC-MS to confirm the concentration of CBD.

According to the literature [14,29,30], depending on the e-liquid fill level, coil (atomizer) resistance, and voltage settings, the coil temperatures of e-cigs range from 110 °C to 1000 °C. The typical wet coil temperature is 200 °C to400 °C, while extremely high temperatures (around 1000 °C) have only been measured for dry coils [14,29,30].

The electronic cigarette used was a JustFog Q16 PRO with the following specifications: capacity (clearomizer)—1.9 mL; coil cylinder—OCC bottom coil resistance 1.6 ohm; material—Pyrex glass, anodized-Al, chrome-coated brass; dimensions—ø 16 × 60 mm/22 g;

capacity (battery)—900 mAh; voltage—3.5, 3.8, 4.1, 4.4 V (4 levels); charging—micro 5-pin USB, DC 5 V; material—anodized-Al, PC; dimensions—16.5 × 25.2 × 70.4 mm/38.0 g. We considered two different powers applied to the atomizer: "medium" (3.8 V) and "high" (4.4 V). The battery was completely charged before each smoking experiment to reduce the variability of the coil temperature due to the voltage variations. A new atomizer was used for each experiment/replica.

The experiments were performed by connecting the horizontally positioned electronic cigarette to a Drechsel bottle (volume 250 mL, equipped with a G1 glass frit) with a Tygon tube (5 cm) and linking the other outlet of the Drechsel to a vacuum flask (vacuum lung) through a vacuum pump (VWR, VCP 80). A photograph of the apparatus is reported in Supporting Information S3 (Figure S1). During the experiment, the faucet on top of the vacuum flask was left open. The vacuum applied corresponds to approximately 25 mBar below the atmospheric pressure. The Drechsel was filled with 50 mL chloroform and put in a dry ice bath (T = −78 °C) during the experiments. The reservoir of the e-cig was filled with 1.5 mL of e-cig liquid, and aspiration was set to consume around 1 mL in around 100 min sessions. Around 200 puffs were performed by applying a slightly modified protocol [14] set by CORESTA (Cooperation Centre for Scientific Research Relative to Tobacco) for e-cig aerosol sample production (CORESTA, 2015). The puff duration was 5 s, and the puff frequency was 2 puffs·min^{-1} (one puff started every 30 s). The inter-puff interval (distance between the end of one puff and the start of the next) was thus 25 s. With this protocol, no detectable increase in the temperature of the reservoir during the experiment was observed. The amount of CBD containing e-cig liquid consumption was calculated by weighting the reservoir before (full) and at the end of each experiment.

There was no significant loss of chloroform by evaporation from the Drechsel during the smoking session. After the experiment, the Tygon and the Drechsel tubes were washed with 25 mL chloroform, which was added to the chloroform inside the Drechsel. The final volume was kept to 100 mL with $CHCl_3$; 1 mL of this solution was spiked with olivetol and analyzed by GC-MS. The remaining solution was evaporated to dryness with Rotavapor, the residue re-dissolved in 5 mL chloroform to preconcentrate the samples 20 times and analyzed again to verify the presence of minor cannabinoids with a better sensitivity.

At the end of the smoking session, the atomizer was shaken with 5 mL of ethanol, and the solution obtained was analyzed to evaluate the composition of the e-liquid remaining in the reservoir that was in contact with the atomizer when the e-cig was lit. From the difference in weight between a new atomizer and one after the vaping session, we found that approximately 0.2 g of e-liquid impregnates it, thus making it possible to calculate the concentration of CBD in the e-liquid in contact with it.

Once the samples had been collected in a specified operating condition, the e-cig reservoir was drained of e-liquid, carefully washed with ethanol, and dried on air. At this point, the reservoir was rinsed with ~1–2 mL of the next refill liquid, which was discarded, and then tanks were refilled for testing with the same e-liquid. This process was repeated for each test condition to ensure that traces of degradation products and solvents were completely removed from an experiment to another [14].

The experiments were performed with e-cig liquids containing:

- −20 mg·mL^{-1}, 10 mg·mL^{-1}, or 2 mg·mL^{-1} of CBD in propylene glycol;
- 20 mg·mL^{-1}, 10 mg·mL^{-1}, or 2 mg·mL^{-1} of CBD in 70:30 propylene glycol:glycerol mixture;

Each result is the mean value of 3 or 4 independent measures (see Table 1 and Section 3 for details).

A blank obtained by vaping neat propylene glycol was performed (see Figure S2) to ascertain the absence of spurious peaks in the chromatographic region of interest, confirming the literature results [14].

Glycerol (ACS grade, >99% for molecular biology) and propylene glycol (>99.5%) were purchased from Merck–Aldrich. All other reagents were analytical grade or higher. The 70:30 (*v*:*v*) mixture of this solvent was prepared at ambient temperature (20 °C).

Table 1. Results obtained using 20 mg·mL^{-1}, 10 mg·mL^{-1}, and 2 mg·mL^{-1} solutions of CBD. The table shows the composition of e-liquid, the atomizer power, the amount of CBD smoked, the amount of CBD surviving the smoking procedure, and the concentration of CBD in the e-liquid in contact with the atomizer.

Composition of e-Liquid	Atomizer Power [a]	Number of Replicates (between Parentheses the Amount of CBD Vaped, mg [b])	Transfer Efficiency to the Aerosol (between Parentheses the Amount of CBD Surviving the Vaping Procedure, mg [c])	Residual Concentration of CBD in the e-Liquid in Contact with the Atomizer, mg·mL^{-1}
20 mg·mL^{-1} CBD In propylene glycol	medium	1 (17.5)	33% (5.8)	7.48
		2 (12.3)	48% (5.9)	9.75
		3 (10.6)	59% (6.2)	5.68
		4 (13.4)	59% (7.9)	11.83
	mean		50 ± 12%	9 ± 3
	high	1 (17.5)	54% (9.5)	13.43
		2 (15.7)	66% (10.4)	7.37
		3(15.2)	58% (8.8)	12.1
	mean		59 ± 6%	10 ± 4
20 mg·mL^{-1} CBD in propylene glycol:glycerol 70:30	medium	1 (14.2)	46% (6.5)	4.85
		2 (16.7)	31% (5.2)	2.34
		3 (18.8)	37% (6.9)	3.15
		4 (20.7)	43% (8.9)	1.91
	mean		39 ± 7%	3 ± 1
	high	1 (21.9)	37% (8.1)	1.98
		2 (22.5)	44% (9.9)	2.1
		3 (22.0)	41% (9.0)	2.32
	mean		41 ± 4%	2.1 ± 0.2
10 mg·mL^{-1} CBD in propylene glycol	medium	1 (10.2)	60% (6.1)	9.43
		2 (13.2)	55% (7.3)	9.3
		3 (9.5)	62% (5.9)	8.86
	mean		59 ± 4%	9.2 ± 0.3
	high	1 (12.8)	70% (9.0)	5.36
		2 (12.1)	73% (8.8)	7.25
		3 (12.0)	77% (9.2)	9.29
	mean		73 ± 4%	7 ± 2
10 mg·mL^{-1} CBD in propylene glycol: glycerol 70:30	medium	1 (11.6)	35% (4.1)	3.2
		2 (15.5)	36% (5.6)	3.16
		3 (11.9)	44% (5.2)	3.68
	mean		38 ± 5%	3.4 ± 0.3
	high	1 (10.0)	69% (6.9)	0.62
		2 (10.3)	56% (5.8)	0.83
		3 (10.5)	62% (6.5)	0.85
	mean		62 ± 6%	0.8 ± 0.1
2 mg·mL^{-1} CBD in propylene glycol	medium	1 (3.4)	69% (2.3)	0.89
		2 (3.2)	58% (1.9)	0.63
		3 (3.6)	72% (2.6)	0.87
	mean		66 ± 7%	0.8 ± 0.1
	high	1 (3.1)	70% (2.2)	0.44
		2 (3.0)	88% (2.6)	1.11
		3 (3.0)	82% (2.5)	0.77
	mean		80 ± 9%	0.8 ± 0.3
2 mg·mL^{-1} CBD in propylene glycol: glycerol 70:30	medium	1(3.5)	64% (2.2)	0.66
		2 (3.3)	48% (1.6)	0.54
		3 (2.8)	47% (1.3)	0.34
	mean		52 ± 8%	0.5 ± 0.2
	high	1 (2.1)	75% (1.6)	0.32
		2 (3.6)	62% (2.2)	0.22
		3 (3.3)	70% (2.3)	0.31
	mean		69 ± 7%	0.28 ± 0.06

[a] medium: 3.8 V applied; high: 4.4 V applied. Coil resistance: 1.6 ohm. [b] calculated from the residual volume in the tank. [c] evaluated as amount of CBD coming out of the e-cig that can be condensed in cold chloroform, considering that 100% corresponds to the amount of CBD vaped and calculated from the residual volume in the tank (column 2).

3. Results and Discussion

With this research, we wanted to evaluate the maximum amount of CBD that a person can assume using approximately 1.5 mL of e-liquid—a reasonable amount consumed during a day by a regular smoker—containing varying amounts of CBD (from 2 mg·mL^{-1} to 20 mg·mL^{-1}) in propylene glycol or in 70:30 propylene glycol:glycerol mixture, also taking into account the formation of secondary cannabinoids as degradation products. With our experimental setting, all cannabinoids and degradation products formed during vaping were collected by condensing in chloroform (see Material and methods for details), because the smoke could not come out from the tip of the e-cig, being aspirated by the vacuum applied to the device. Moreover, no smoke formation was detected inside the Drechsel bottle during the experiment, suggesting that the condensation process is efficient. For this reason, the amount of CBD found in the collecting liquid (chloroform) corresponds to the neat amount of CBD surviving the smoking procedure (transfer efficiency to the aerosol). Notably, in the lungs of a smoker, only around 15% to30% of the smoke produced is settled, both with classical cigarettes and e-cigs [18,31], although in this last case, an even less value, around 11%, was postulated [32–36].

According to the results shown in Table 1, we found different recovery ranges that depend on the initial CBD concentration and on the e-liquid, and that are generally higher when the atomizer is settled at high power (see Figure 2 and Table 1; the CBD recovery ranges found are expressed as mean ± standard deviation).

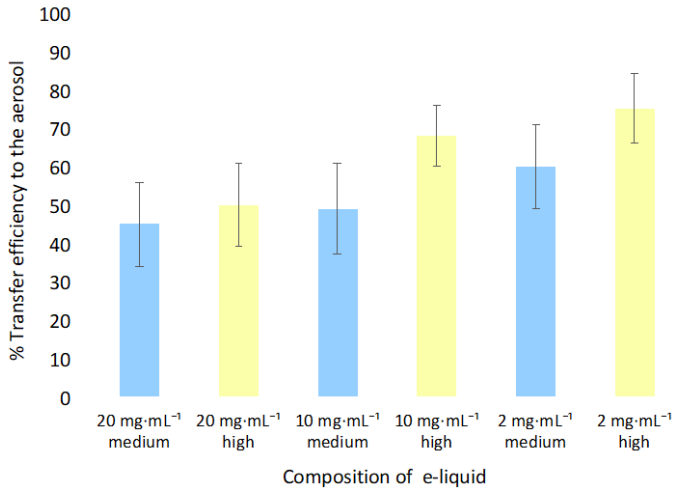

Figure 2. The bars indicate the average percentage of transfer efficiency to the aerosol obtained from 20 mg·mL^{-1}, 10 mg·mL^{-1}, or 2 mg·mL^{-1} solutions of CBD at medium and high atomizer power.

As a general trend, we found that higher voltage applied to the atomizer (i.e., higher temperatures) correlated with higher CBD recovery in the condensed smoke (see Figure 2). Although this may seem counterintuitive, as thermal degradation of CBD happens during the experiments, we believe that higher temperatures are more efficient in vaporizing the solution, thus allowing a shorter residence time of the CBD in contact with the heated coil to produce the desired amounts of smoke. In addition, the rapid evaporation of the liquid helps limit the temperature reached by the coil. This results in less degradation of the API compared to what is observed with an e-cig operating at medium power.

The variability of the results obtained under the same conditions (Table 1) is due to the difficulty in standardizing the manual smoking process, which involves repeatedly pressing the button that activates the coil of the e-cig for the chosen length of time (i.e., the 5 s cycles described in Materials and Methods). Furthermore, it should be taken into account that the e-cig is a technical commercial device that is inherently poorly reproducible, since the state of charge of the battery can drastically affect the temperature of the coil, and the power circuit is not as precise as it would be in a scientific instrument.

The transfer efficiency is higher with pure propylene glycol (see Figure 3) than with the 70:30 propylene glycol:glycerol mixture: it has already been shown that the nature of the e-liquid strongly influences the vaping process and the temperature reached by the coil, despite the same voltage being applied to it [14,29,30]. In fact, the boiling point and evaporation temperature of the mixture are higher than that of pure propylene glycol, and, consequently, a higher temperature is reached in the coil during the smoking process, resulting in greater degradation of the CBD contained. Referring to the literature data, the boiling point of propylene glycol is 188.2 °C at 1 atm, while glycerol boils at 290 °C at 1 atm, and their 70:30 mixture boils at around 205 °C (1 atm) [14].

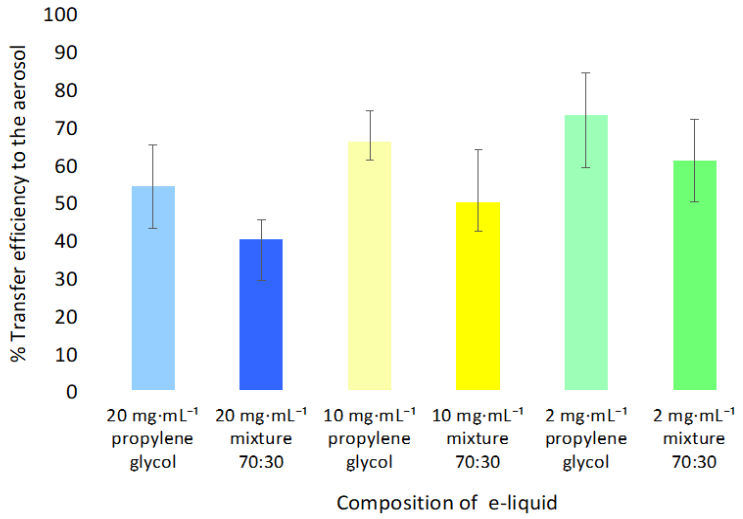

Figure 3. The bars indicate the average percentage of transfer efficiency to the aerosol obtained from 20 (blue), 10 (yellow), or 2 (green) mg·mL^{-1} solutions of CBD in neat propylene glycol and in 70:30 propylene glycol:glycerol mixture.

Moreover, during the e-cig operation, a steam-distillation effect leads to the vaporization of CBD, and this is more evident when higher amounts of vapor are produced, such as in the case of the 70:30 propylene glycol:glycerol mixture.

The actual amount of CBD that can survive the smoking procedure has been evaluated under optimized experimental conditions through a design of experiment (DoE), with a 2^3 factorial design that included CBD concentration (×1), atomizer power (×2), and solvent composition (×3). Two levels were evaluated for each variable: amount of CBD (2 mg·mL^{-1} and 20 mg·mL^{-1}); solvent (PG and PG + glycerin); and atomizer power (medium and high). The elaboration (R-based software CAT, Chemometric Agile Tool, freely available on the site of the Italian Group of Chemometrics, [37]) provided the plot of the coefficients (bi) of the model for the average multiclass transfer efficiency (see Figure 4). Confirming the above reported data, it turned out that the CBD concentration, the nature of the solvent, and the atomizer power were statistically significant with $p < 0.001$ (***), and each variable is independent from each other (see Figure 4). Therefore, it resulted to be advantageous to use high atomizer power and propylene glycol as the solvent with the lowest CBD concentration (i.e., 2 mg·mL^{-1}); the maximum percentage of transfer efficiency to the aerosol predicted by the model (80%) is in good agreement with the one obtained experimentally (80%, $p = 0.05$, $n = 3$).

Looking at Table 1, it can be seen that when around 15.7 mg CBD was smoked, 10.4 mg (66 %) could be found unchanged in the smoke (Table 1, 20 mg·mL^{-1} CBD in propylene glycol, atomizer power: high). Considering that bioavailability of the inhaled smoke ranges from 11 to 30%, the amount of CBD delivered to the body is around 1 to 3 mg. At the same time, no THCs—Δ^8-iso-THC, Δ^9-THC, Δ^8-THC—were found (i.e., the amount formed was <0.005 mg for each mg of CBD smoked; for the corresponding GC-MS chromatogram see Figures 5–8 and in Supporting Information S4). Bearing in mind that the threshold

dose for Δ^9-THC—the most effective psychotropic isomer—is around 2.5 mg [38], it can be concluded that negligible amounts of THCs are assumed by the smoker, excluding intoxication by using an e-cig in the conditions described. The same can be said for the other cannabinoids considered, in all cases, the amounts formed were <0.005 mg for each mg of CBD smoked, contrary to what was expected [21].

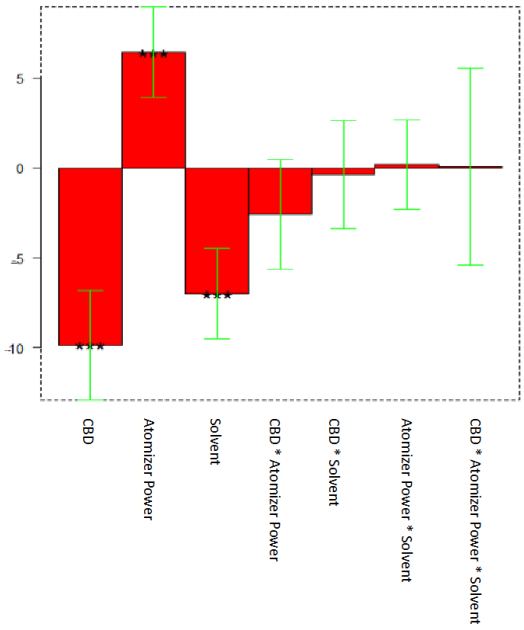

Figure 4. Plots of the coefficients of the model showing the significance of the variables investigated by the chemometric study. Stars indicate the significance of the coefficients, (*** $p < 0.001$), while error bars indicate the confidence intervals ($p = 0.05$).

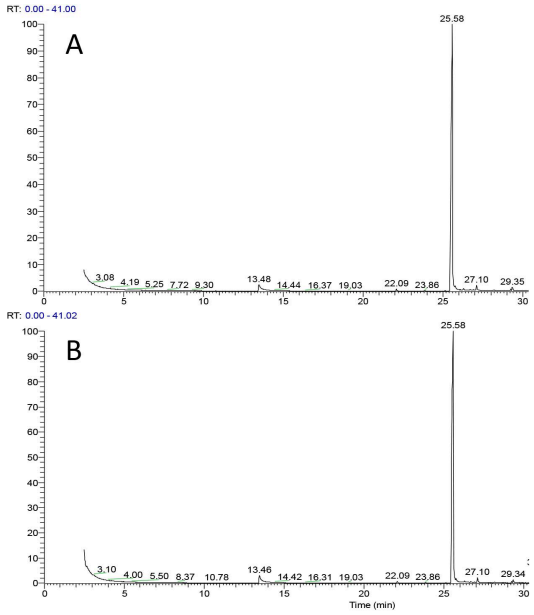

Figure 5. GC-MS chromatograms of the smoke condensed in 50 mL cold chloroform, produced by the e-cigs using 20 mg·mL^{-1} of CBD solution in 70:30 propylene glycol:glycerol mixture, with the atomizer settled at medium power (**A**) and with the atomizer settled at high power (**B**). The main products detected were olivetol, the internal standard, (t_R 13.44 min), CBD (t_R 25.58 min), and Δ^9-THC (t_R 27.10 min).

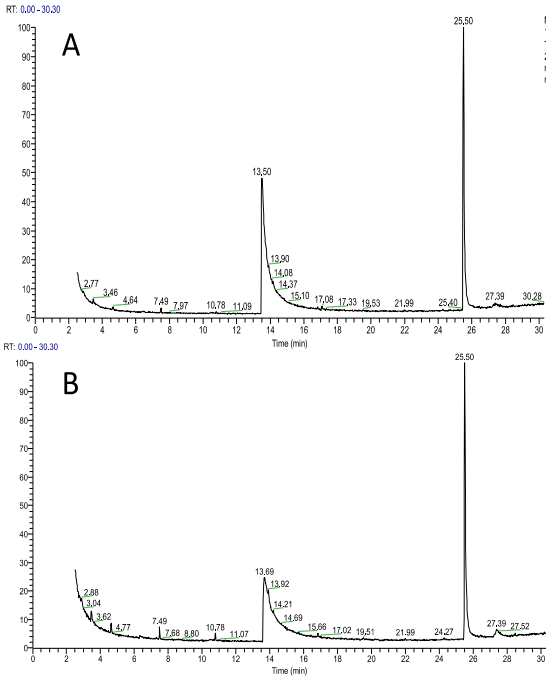

Figure 6. GC-MS chromatograms of the liquid in contact with the atomizer setting at medium power after smoking 20 mg·mL^{-1} of CBD solution in 70:30 propylene glycol:glycerol mixture, (**A**), and with the atomizer settled at high power, (**B**).The main products detected were: olivetol, the internal standard, (t_R 13.44 min), CBD (t_R 25.50 min), and Δ^9-THC (t_R 27.39 min).

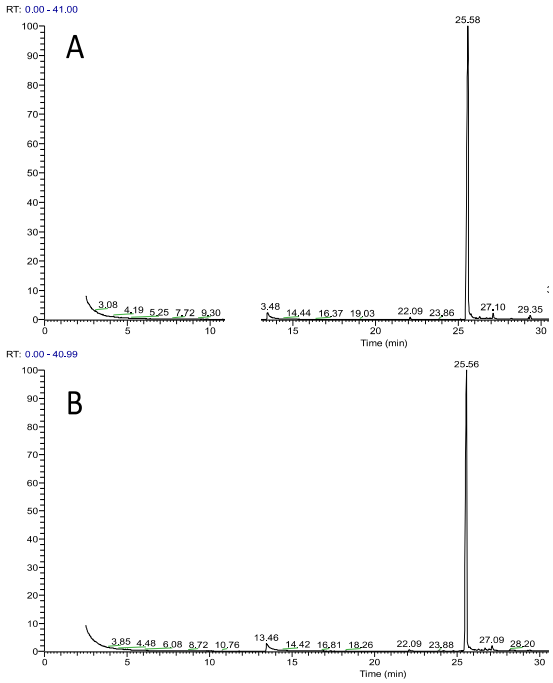

Figure 7. GC-MS chromatograms of the smoke condensed in 50 mL cold chloroform, produced by the e-cigs using 20 mg·mL^{-1} of CBD solution in neat propylene glycol, with the atomizer settled at medium power (**A**) and with the atomizer settled at high power (**B**). The main products detected were olivetol, the internal standard, (t_R 13.44 min), CBD (t_R 25.58 min), and Δ^9-THC (t_R 27.10 min).

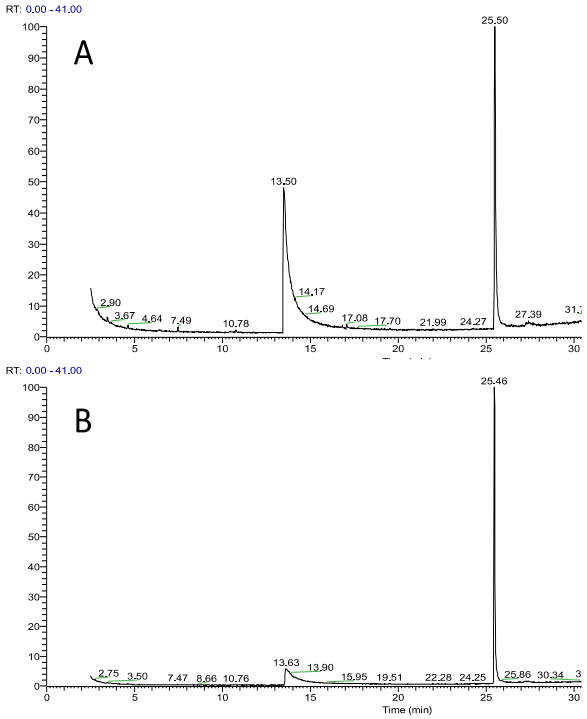

Figure 8. GC-MS chromatograms of the liquid in contact with the atomizer setting at medium power after vaping 20 mg·mL^{-1} of CBD solution in neat propylene glycol (**A**) and with the atomizer setting at high power (**B**). The main products detected were olivetol, the internal standard, (t_R 13.44 min), CBD (t_R 25.50 min), and Δ^9-THC (t_R 27.30 min).

Moreover, we found that the concentration of CBD in the liquid in contact with the atomizer resulted generally lower than in the tank (Table 1, last column). This is due to a partial thermal degradation of the CBD in contact with the warmed coil, and this confirms that CBD is not simply vaporized but also partially degraded during the vaping process. In the liquid in contact with the atomizer, negligible amounts of other cannabinoids (among which THCs) are found. Corresponding chromatograms are reported as Figures 5–8 and in Supporting Information S4 (Figures S2–S10).

4. Conclusions

In this paper, we found that when smoking an e-cig, the CBD transfer efficiency to the aerosol varies according to the solvent used to prepare the e-liquid. In addition, for all concentrations considered, the amount of CBD that can be vaped is greater when pure propylene glycol is used (50–79% vs. 39–69%) and when the atomizer is operating at high power. Commercially available e-liquids are generally a mixture of solvents [29,30,39]; therefore, in most cases, the consumer will take less CBD through an electronic cigarette than expected with the theoretical maximum amount derived from the data obtained with neat propylene glycol (see Table 1). The transfer efficiency to the aerosol also varies with the initial concentration of CBD in the e-liquid used. Higher recoveries are observed by decreasing the initial CBD concentration, passing from (39 ± 7) % for the 70:30 mixture to (50 ± 12)% for pure propylene glycol (n = 4, CBD 20 mg·mL^{-1}, power setting: medium) and from (52 ± 8)% for the 70:30 mixture to (66 ± 7)% for pure propylene glycol (n = 4, CBD 2 mg·mL^{-1}, power setting: medium). However, it should be noted that a higher initial CBD concentration results in a higher amount (in mg) of CBD surviving the vape, even if the percentage of recovery is lower.

Under our experimental conditions, the maximum amount of CBD transferred to the aerosol, from e-liquid containing 20 mg·mL^{-1} CBD in pure propylene glycol and with the atomizer settled at high power, is equal to 10.4 mg for a single smoking session. With

bioavailability ranging from around 30% down to 11% of the smoke produced [33,34], the maximum amount of bioavailable CBD is between 1 mg and 2.5 mg. Considering that a significant amount of smoke escapes the lungs—beyond the transfer efficiency of CBD to the aerosol—we conclude that the e-cig can only be considered as a valid method for the systemic delivery of CBD in strict conditions, such as the one described in the literature [40] and, according to which, the plasma concentration of CBD after e-cig use results in a best case- scenario of about 30% bioavailability of API [31,41,42]. In fact, consuming about 1.5 mL of an e-liquid—which is a reasonable daily dose—delivers an amount of bioavailable API in the lower therapeutic useful range, considering that typically 20 mg·day^{-1} to 1000 mg·day^{-1} *per os* are prescribed [41,42], with a bioavailability of 13 to 19% for this route of administration [31]. These two administration routes—smoked and oral—are comparable only in those subjects where the erratic oral bioavailability of CBD is a concern and only under strictly controlled conditions (i.e., plasma CBD concentration analysis, composition of the e-cig liquid, use of pure propylene glycol) [31,41,42]. In addition, the high variability of the quantity of CBD vaped despite the conditions being set (i.e., the poor repeatability of the smoking process, as can be seen in Table 1), greatly influences the amount of CBD delivered from time to time and, consequently, its bioavailability.

In our experiments, no formation of THC-like compounds (Δ^8-iso-THC, Δ^8-THC, Δ^9-THC: each <0.005 mg for each mg of vaped CBD) is observed: i.e., Δ^9-THC, although it has been found in most analyses, is always <1% compared with the CBD amount smoked. Considering that the threshold of Δ^9-THC, the most active compound of the series, is 2.5 mg, it can be concluded that the smoker takes a negligible dose of THC-like psychotropic cannabinoids, so e-cig vaped CBD-containing liquids cannot be considered a source of intoxication from these compounds. We observe that the literature data that refer to simulated conditions in which conversion of CBD to THCs takes place are clearly useful for understanding the chemistry of CBD, but sometimes they do not accurately reflect the real case scenario; our results are in line with what was found in a strictly controlled clinical setting [42], valorizing from a chemical point of view the data available in the literature.

Although the data presented only refer to one e-cig device (except for those obtained in our laboratory for a limited number of tests carried out with the Exceed Grip Pro device—data not shown), they all operate with the same principles and no significant differences are expected. We chose the JustFog Q16PRO e-cig for these experiments because it is widely used and has a complete description of the operating conditions.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/forensicsci3020019/s1, Table S1: GC-MS characteristic of the considered compounds; Table S2: Recovery test; Figure S1: A photograph of the apparatus used to capture the e-cig smoke. The numbers shown represent the following: (1) Electronic cigarette; (2) Tygon rubber tube (length 12.5 cm); (3) Ice bath; (4) 250 mL; Drechsel bottle; (5) Vacuum lung (500 mL tailed flask); (6) Vacuum pump (VWR, VCP 80); Figure S2: GC-MS chromatogram of the condensed smoke produced by the e-cigs using neat propylene glycol *without* CBD. The peaks at t_R 32.06, 32.17, 35.23, and 35.36 min were above the retention windows of cannabinoids and could not be identified; Figure S3: GC-MS chromatogram of 20 mg·mL^{-1} CBD standard solution in 70:30 propylene glycol: glycerol mixture. The compounds that can be detected are olivetol, the internal standard, (t_R 13.44 min), CBD (t_R 25.58 min), and Δ^9-THC (t_R 27.10); Figure S4: GC-MS chromatogram of 20 mg·mL^{-1} CBD standard solution in neat propylene glycol. The main products detected were olivetol, the internal standard, (t_R 13.44 min), CBD (t_R 25.54 min), and Δ^9-THC (t_R 27.12 min); Figure S5: GC-MS chromatogram of 2 mg·mL^{-1} CBD standard solution in 70:30 propylene glycol:glycerol. The main products detected were olivetol, the internal standard, (t_R 13.44 min), CBD (t_R 25.46 min), and Δ^9-THC (t_R 27.30 min); Figure S6: GC-MS chromatograms of the smoke condensed in cold chloroform (50 mL) produced by the e-cigs using 2 mg·mL^{-1} of CBD solution in 70:30 propylene glycol:glycerol, with the atomizer settled at medium power (A) and with the atomizer settled at high power (B). The main products detected were olivetol, the internal standard, (t_R 13.44 min), CBD (t_R 25.48 min), and Δ^9-THC (t_R 27.16 min); Figure S7: GC-MS chromatograms of the liquid in contact with the atomizer setting at medium power after smoking 2 mg·mL^{-1} of CBD solution in 70:30 propylene glycol:glycerol mixture

(A) and with the atomizer setting at high power (B). The main products detected were olivetol, the internal standard, (t_R 13.44 min), CBD (t_R 25.56 min), and Δ^9-THC (t_R 27.31 min); Figure S8: GC-MS chromatogram of 2 mg·mL^{-1} CBD standard solution in neat propylene glycol. The main products detected were olivetol, the internal standard, (t_R 13.44 min), CBD (t_R 25.46 min), and Δ^9-THC (t_R 27.10 min.); Figure S9: GC-MS chromatograms of the smoke condensed in cold chloroform (50 mL) produced by the e-cigs using 2 mg·mL^{-1} of CBD solution in neat propylene glycol, with the atomizer settled at medium power (A) and with the atomizer settled at high power (B). The main products detected were olivetol, the internal standard, (t_R 13.44 min), CBD (t_R 25.50 min), and Δ^9-THC (t_R 27.14 min); Figure S10: (GC-MS chromatograms of the liquid in contact with the atomizer setting at medium power after smoking 2 mg·mL^{-1} of CBD solution in neat propylene glycol (A) and with the atomizer setting at high power (B). The main products detected were olivetol, the internal standard, (t_R 13.44 min), CBD (t_R 25.56 min), and Δ^9-THC (t_R 27.12 min).

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Abbreviations

List of abbreviation and compounds names

CBD	Cannabidiol (1)
Δ^8-iso-THC	Δ-8-iso-tetrahydrocannabinol (2)
Δ^7-CBD	Δ-7- cannabidiol (3)
Δ^9-THC	Δ-9-tetrahydrocannabinol (4)
Δ^8-THC	Δ-8- tetrahydrocannabinol (5)
DHD	8,9-dihydrocannabidiol (6)
THD	Tetrahydrocannabidiol (7)
HHC	Hexahydrocannabinol (8)
CBG	Cannabigerol (9)
CBL	Cannabicyclol (10)
CBT	Cannabicitran (11)
CBN	Cannabinol (12)
CBC	Cannabichromene (13)
α-MeO-CBD	α-methoxy-dihydrocannabidiol (14)
β-MeO-CBD	β-methoxy-dihydrocannabidiol (15)
CBE	Cannabielsoin (16)

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