CBD-containing liquids for e-cigarettes: formation of psychotropic and secondary cannabinoids and amount of CBD surviving the smoking procedure.

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Supplementary material.

S1. List of abbreviation and compounds names.

S2. Validation of the chromatographic method.

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S1. List of abbreviation and compounds names.

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

S2. Validation of the chromatographic method.

The GC-MS method used was already described and optimized for the analysis of natural cannabinoids (see ref 17 and 22 in the main text), and is substantially unvaried with respect to standard GC-MS methods for this type of analysis [S1-S3].

The calibration curves for CBD were performed in two different ranges: 2 $\mathrm{mg{\cdot}mL^{-1}}$ to 100 $\mathrm{mg{\cdot}mL^{-1}}$ and 100 $\mathrm{mg{\cdot}mL^{-1}}$ to 2000 $\mathrm{mg{\cdot}mL^{-1}}$, using ethyl acetate as solvent. For the other compounds, only one calibration curve in the 2 $\mathrm{mg{\cdot}mL^{-1}}$ to 100 $\mathrm{mg{\cdot}mL^{-1}}$ range was performed. For other details (e.g., the use of the internal standard) see the main text.

S2.1. Determination of LOD and LOQ

LOD and LOQ were calculated from the calibration curve according to According to ICH Topic Q 2 (R1) Validation of Analytical Procedures: Text and Methodology [S6].

$$\mathrm{LOD} = 3.3\frac{Sy/x}{m} \qquad \mathrm{LOQ} = 3\mathrm{LOD}$$

Where *Sy/x* is the residual standard deviation from the linear regression and *m* is the slope of the calibration curve.

For all the cannabinoids considered, LOD was 0.5 $\mathrm{mg{\cdot}mL^{-1}}$, and LOQ is 1.5 $\mathrm{mg{\cdot}mL^{-1}}$, except for compound 16 (LOD 1 $\mathrm{mg{\cdot}mL^{-1}}$ and LOQ 3 $\mathrm{mg{\cdot}mL^{-1}}$). Compounds 13, 14 and 15 were not available as pure compounds, so that their LOQ were estimated basing on the similar compounds considered (16 for 13 and 4 for 14 and 15).

The results obtained are consistent with the methods already described [S1-S3]. The calibration curves showed a correlation (R2) greater than 0.99.

S 2.2. Spectral characteristics and retention indexes of the considered cannabinoids

For the compounds considered, quantification was performed using the most abundant fragment ion (quantification ion), as shown in the table below, generating an extracted ion chromatogram for a given cannabinoid.

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 refs 17 and 22 of the main text.

Table S1. GC-MS characteristic of the considered compounds

Compound	**Quantification Ion, m/z**	**Molecular weight, m/z**	**Retention index**
1 CBD	231	314	2416
Δ^8-*iso*-THC	231	314	2431
Δ^7- CBD	231	314	2477
Δ^9-THC	231	314	2514
Δ^8- THC	231	314	2491
6 DHD	231	316	2444
7 THD	193	318	2468
8 HHC	273	316	2449 (cis), 2461 (trans)
9 CBG	193	316	2569
10 CBL	231	314	2368
11 CBT	231	314	2276
12 CBN	295	310	2578
13 CBC	231	314	2492
14α-MeO-CBD	299	346	2593
15β-MeO-CBD	231	346	2643
16 CBE	205	330	2491

The criteria for identification of the target cannabinoids [S1, S2] were retention time and mass spectral correspondence to pure compounds. Retention time correspondence was calculated as the % relative difference between the observed retention times (min) for the sample and standard as follows:

% relative difference = 100 % * absolute value (sample retention time - standard retention time)/standard retention time

with an allowable variation of < 3 %. Actual retention time correspondences for the cannabinoids analyzed over a year period ranged from 0.3 % to 1.5 %.

In addition, retention times and mass spectra for the target cannabinoids from the spiked products were required to be consistent with the corresponding standard reference materials and that the ion ratio qualifier : quantifier was required to be within ± 20% of the average calibrator ion ratios.

Average retention time correspondences for the target cannabinoids from the spiking study were also in the range 0.1 % to 1.6 %.

It was possible to verify retention times and mass spectra for the cannabinoids from the GC-MS total ion chromatograms [S2].

S 2.3. Recovery test – precision of the method

Solutions in ethyl acetate of some of the compounds considered (CBD, CBN, Δ^9-THC), with concentrations of 5 $\mathrm{mg{\cdot}mL^{-1}}$ and 50 $\mathrm{mg{\cdot}mL^{-1}}$, were used as calibrators and analyzed to evaluate the intraday precision (that resulted in a standard deviation of around 7 % of the title concentration of the compound, using a number of replicates, *n = 3*), and the interday precision, that resulted in a standard deviation of about 11 % of the title concentration of the compound, *n = 3*).

Percentage recovery was evaluated for CBD and Δ^9-THC by smoking 1.5 mL of neat propylene glycol (as described in the article) (samples 1,2,3 in Table S2) or 1.9 mL of 20 $\mathrm{mg{\cdot}mL^{-1}}$ CBD in propylene glycol: glycerol 70:30 (sample 4 in Table S2), condensing the smoke in chloroform and making up to 100 mL with the same solvent. Different amounts of CBD and Δ^9-THC were added to this last solution (see Table S2 for the results).

CBD was quantified in the chloroform solution, while in the case of Δ^9-THC the solution was evaporated to dryness with Rotavapor, the residue re-dissolved in 5 mL chloroform to preconcentrate the samples and analyzed (as described in the main text). Solutions were analyzed in triplicate (apart from case 4, which was analyzed once), and recovery were in accordance with literature published methods [S1,S2,S3,S6,S7]

Table S2. Recovery test

Sample	Spiking	Recovery
1	+ 100 mg·L^{-1} CBD +1 mg·L^{-1} Δ^9-THC	92-110% 85-115%
2	+ 50 mg·L^{-1} CBD +0.1 mg·L^{-1} Δ^9-THC	97-108% 82-118%
3	+ 10 mg·L^{-1} CBD +0.1 mg·L^{-1} Δ^9-THC	96-110% 81-119%
4** CBD content of unspiked solution: 69 mg·L^{-1} Δ^9-THC content of unspiked solution <LOQ	+ 10 mg·L^{-1} CBD +1 mg·L^{-1} Δ^9-THC	92% 110%

** Sample 4 consisted of replicate 3 (medium power) in Table 1 of the main text, obtained by smoking 1.9 mL of 20 mg·mL^{-1} CBD in propylene glycol: glycerol 70:30 and collected in 100 mL chloroform. The solution was divided in 2 equal aliquots: the first one, was analyzed for CBD and Δ^9-THC content, while the second was spiked with CBD and Δ^9-THC and analyzed. Δ^9-THC content was analyzed on the pre-concentrated solution made up to 5 mL, as described in the main text.

S3. Materials and Methods: Set of the electronic cigarette.

Figure S1. A photograph of the apparatus used to capture the e-cig smoke. The numbers shown represent: 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).

S4. Results and discussion: identification of degradation products.

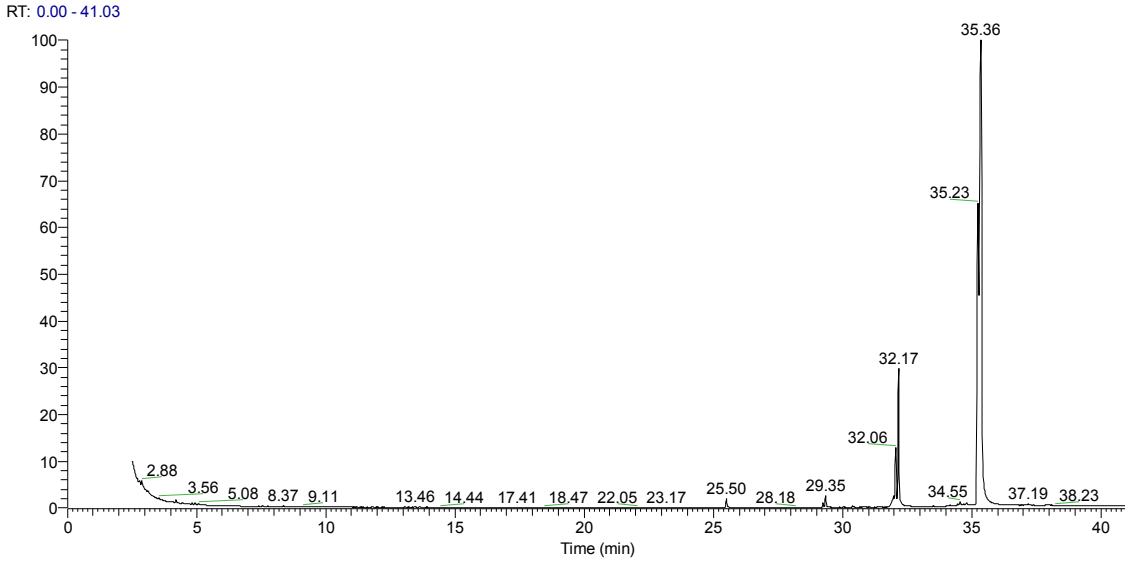


Figure S2. GC-MS chromatogram of the condensed smoke produced by the e-cigs using neat propylene glycol *without* CBD. The peaks at t_R 32.06 min, 32.17 min, 35.23 min and 35.36 min were above the retention windows of cannabinoids and could not be identified.

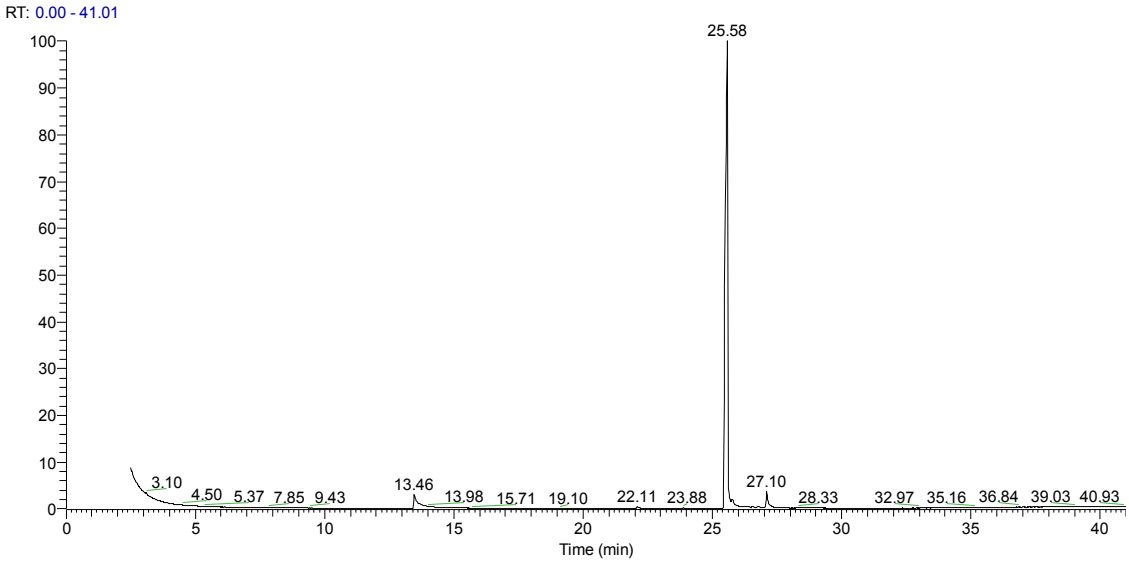


Figure S3. GC-MS chromatogram of 20 mg·mL^{-1} CBD standard solution in 70:30 propylene glycol: glycerol mixture. The compounds detected are olivetol, the internal standard, (t_R 13.44 min), CBD (t_R 25.58 min), Δ^9-THC (t_R 27.10).

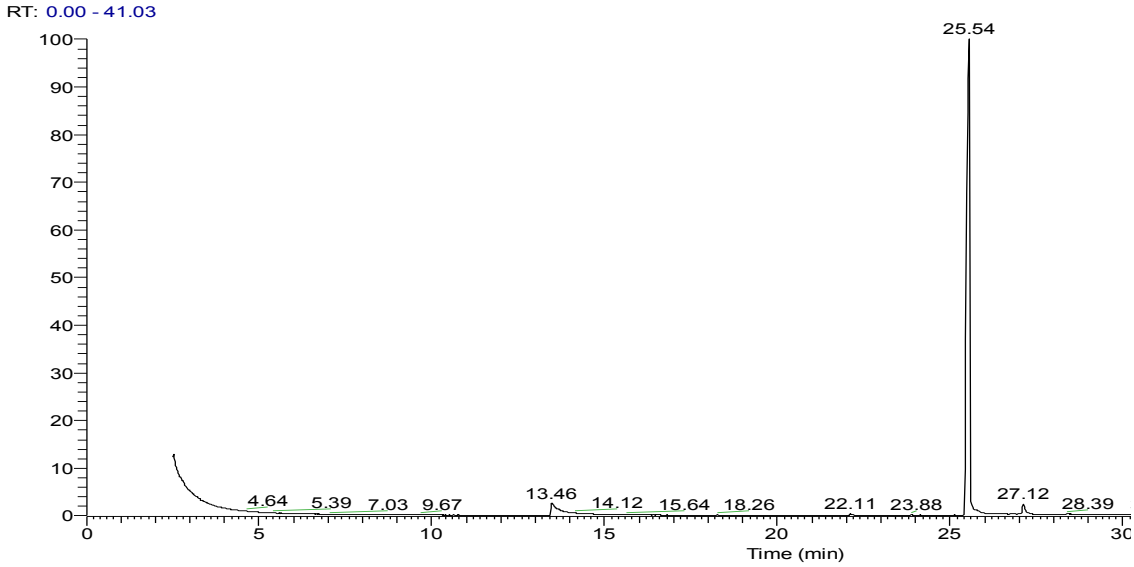


Figure S4. GC-MS chromatogram of 20 mg·mL^{-1} CBD standard solution in neat propylene glycol. The main products detected were olivetol, the internal standard, (t_R 13.44 min), CBD (t_R 25.54 min), Δ^9-THC (t_R 27.12 min).

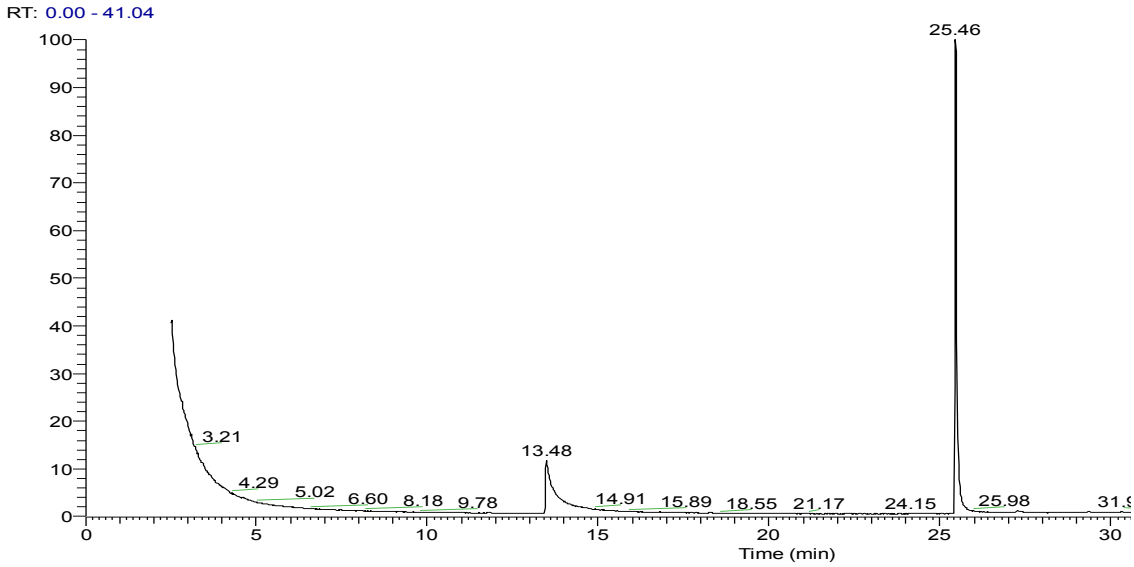


Figure S5. GC-MS chromatogram of 2 mg·mL^{-1} CBD standard solution in 70:30 propylene glycol:glycerol mixture. The main products detected were olivetol, the internal standard, (t_R 13.44 min), CBD (t_R 25.46 min), Δ^9-THC (t_R 27.30 min).

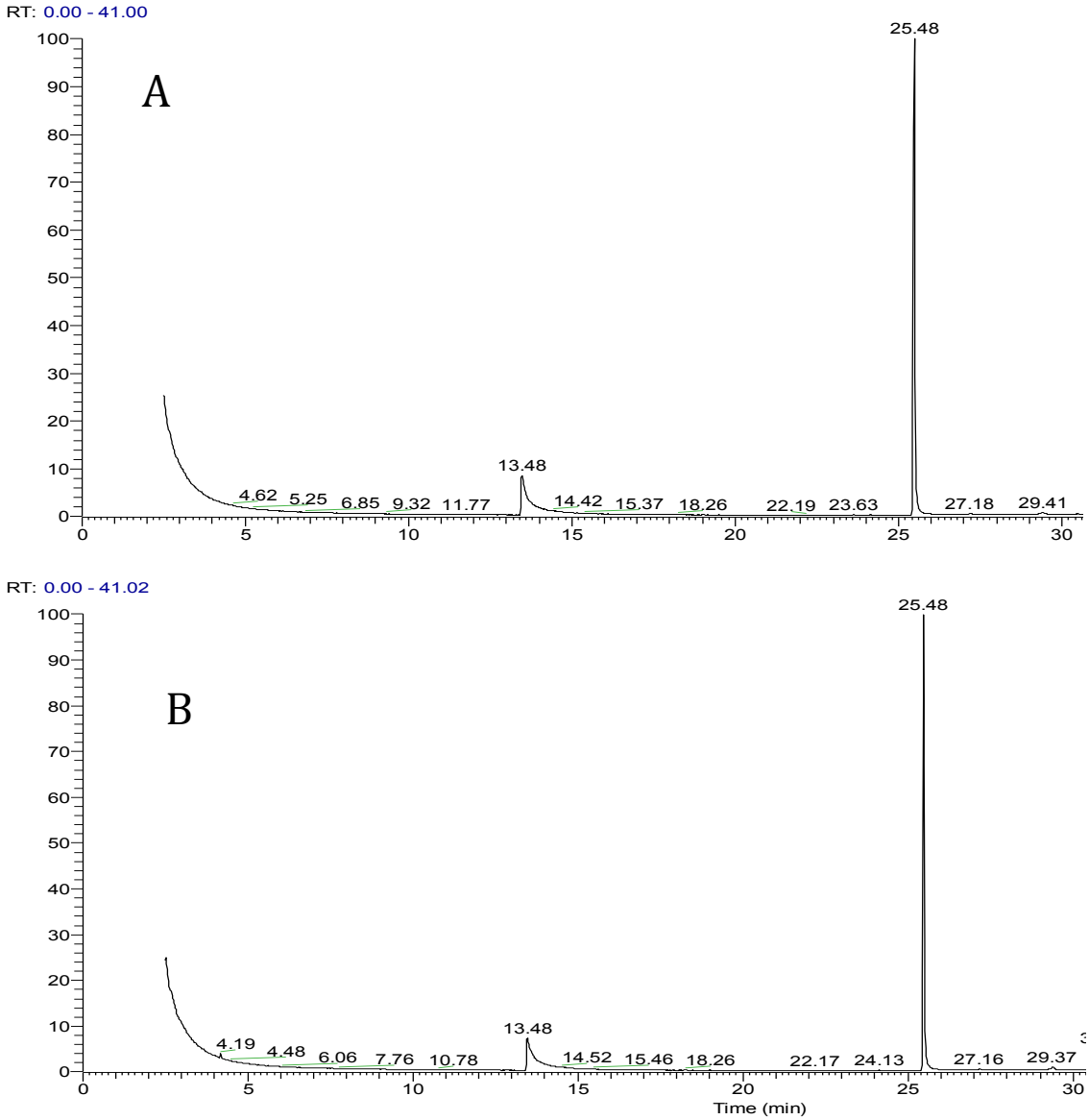


Figure S6. GC-MS chromatograms of the smoke condensed in cold chloroform (50 mL) produced by the e-cigs using 2 mg·mL^{-1}of CBD solution in 70:30 propylene glycol:glycerol mixture, with the atomizer settled at medium power (A) and with the atomizer settled at high power (B). The main products detected were olivetol, the internal standard, (t_R 13.44 min), CBD (t_R 25.48 min), Δ^9-THC (t_R 27.16min).

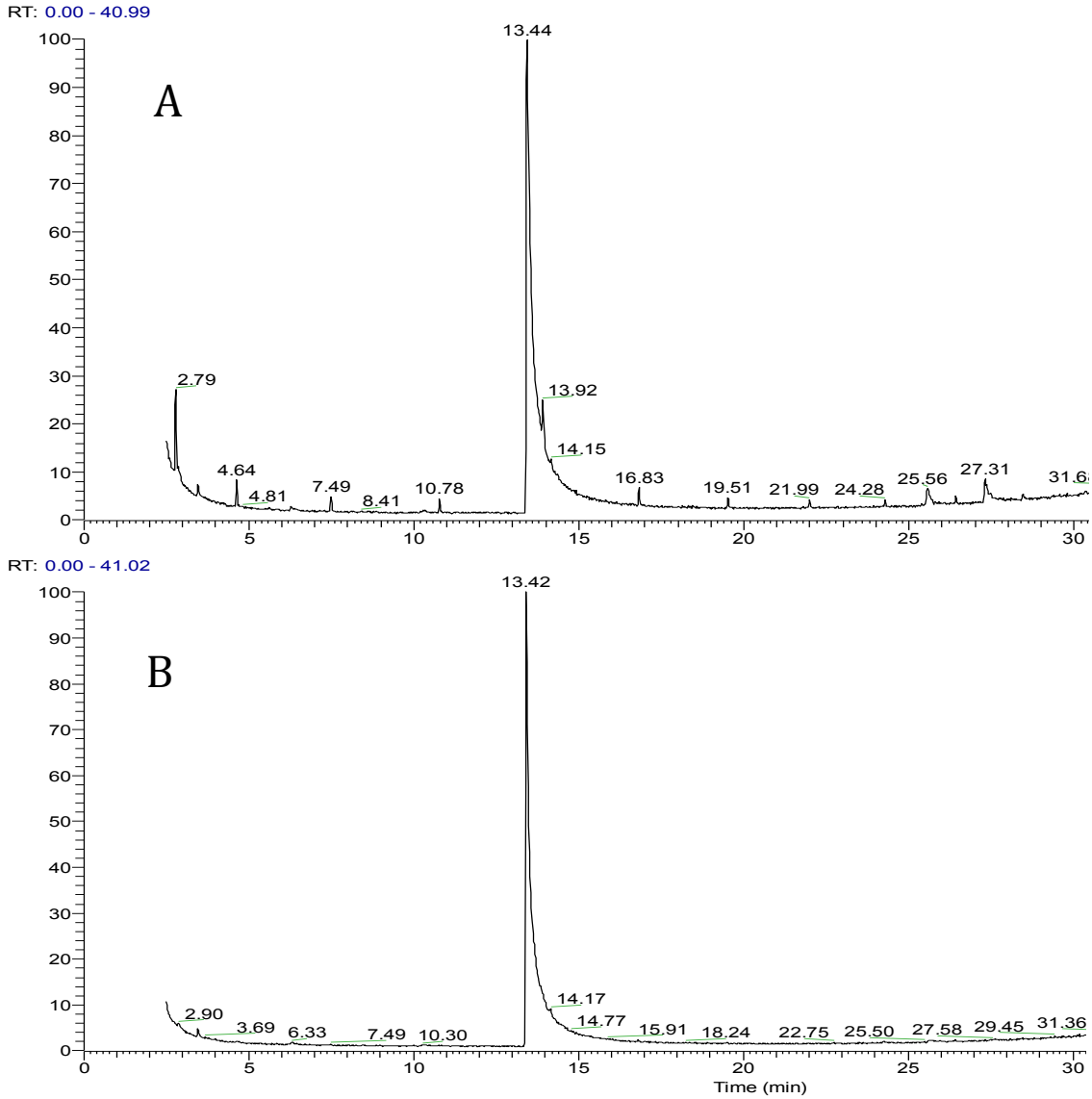


Figure S7. GC-MS chromatograms of the liquid in contact with the atomizer setting at medium power after smoking 2 mg·mL^{-1} of CBD solution in 70:30 propylene glycol:glycerol mixture (A) and with the atomizer setting at high power (B). The main products detected were olivetol, the internal standard, (t_R 13.44 min), CBD (t_R 25.56 min), Δ^9-THC (t_R 27.31 min).

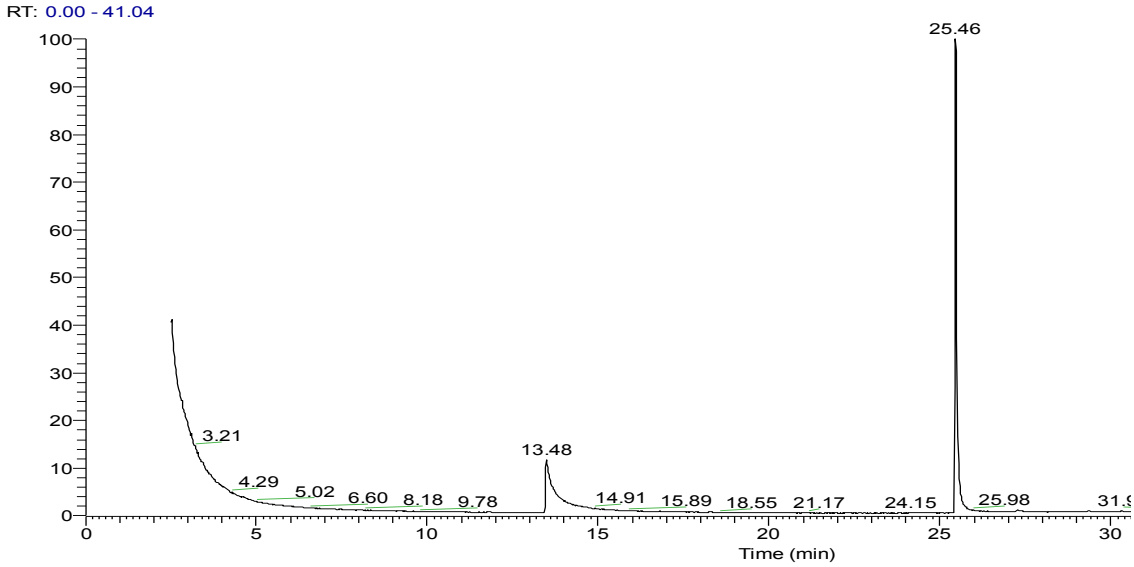
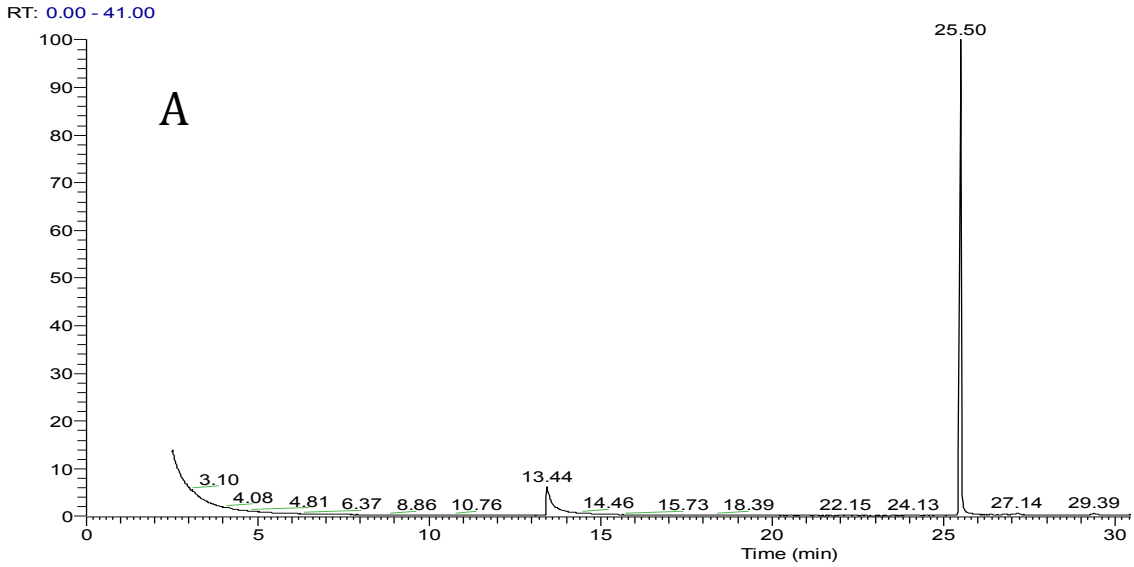


Figure S8. GC-MS chromatogram of 2 mg·mL^{-1} CBD standard solution in neat propylene glycol. The main products detected were olivetol, the internal standard, (t_R 13.44 min), CBD (t_R 25.46 min), Δ^9-THC (t_R 27.10 min.)



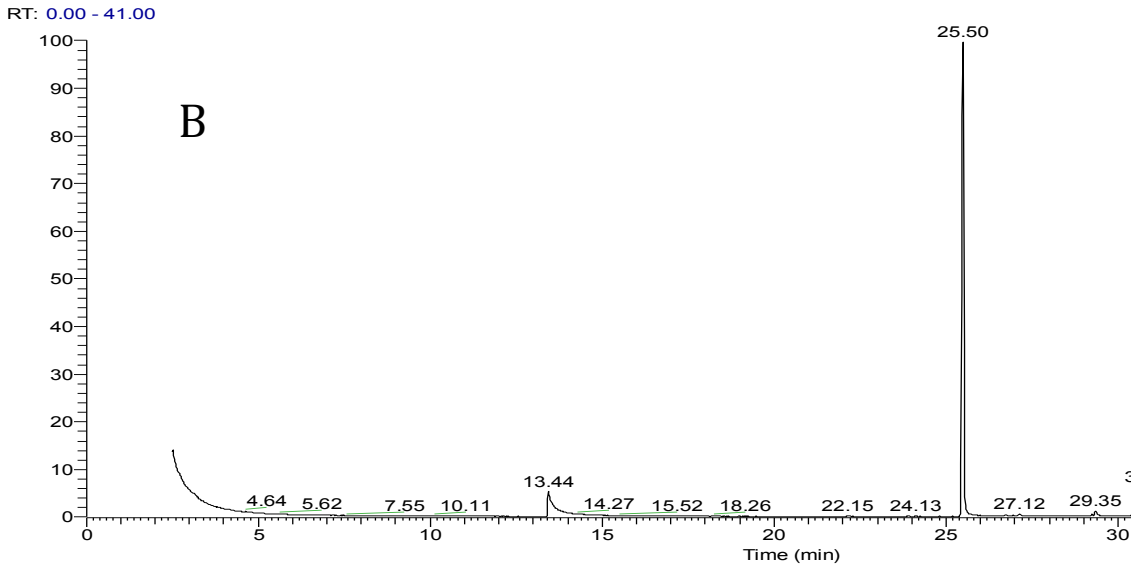


Figure S9. GC-MS chromatograms of the smoke condensed in cold chloroform (50 mL) produced by the e-cigs using 2 mg·mL^{-1} of CBD solution in neat propylene glycol, with the atomizer settled at medium power (A) and with the atomizer settled at high power (B).
The main products detected were olivetol,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

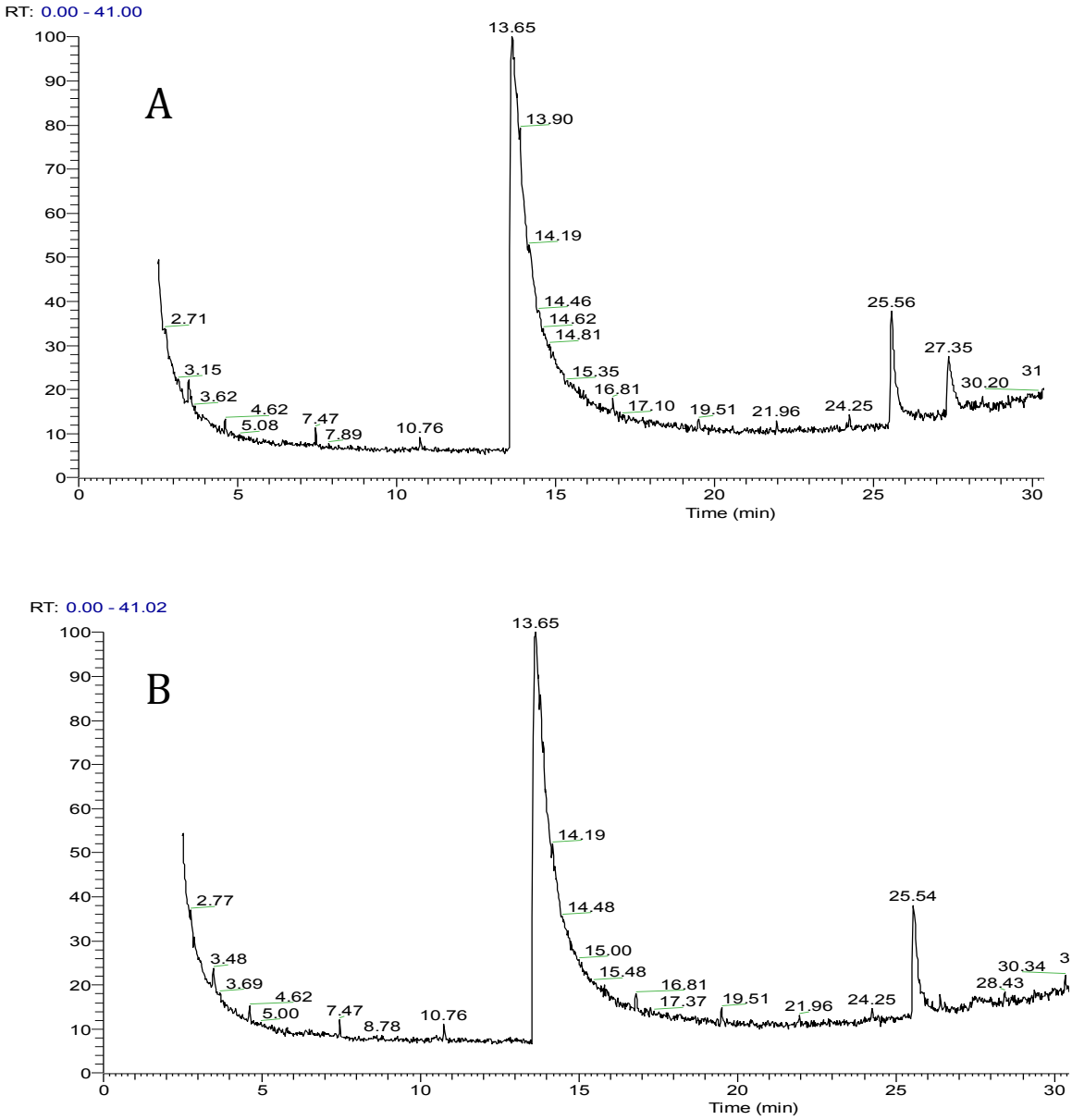


Figure S10. GC-MS chromatograms of the liquid in contact with the atomizer setting at medium power after smoking 2 mg·mL^{-1} of CBD solution in neat propylene glycol (A) and with the atomizer setting at high power (B). The main products detected were olivetol, the internal standard, (t_R 13.44 min), CBD (t_R 25.56 min), Δ^9-THC (t_R 27.12 min).

S5. References for the SI paragraph.

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