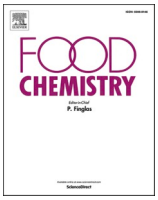
Food Chemistry 440 (2024) 138187

Contents lists available at ScienceDirect

Food Chemistry

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



Comprehensive cannabinoid profiling of acid-treated CBD samples and Δ^8-THC-infused edibles

Si Huang [a,b], Teris A. van Beek [b], Frank W. Claassen [b], Hans-Gerd Janssen [b,c], Ming Ma [a], Bo Chen [a,*], Han Zuilhof [a,b,*], G.IJ. Salentijn [b,d,*]

[a] Key Laboratory of Phytochemical R&D of Hunan Province and Key Laboratory of Chemical Biology & Traditional Chinese Medicine Research of Ministry of Education, Hunan Normal University, No.36, Lushan Road, 410081 Changsha, China
[b] Laboratory of Organic Chemistry, Wageningen University, Stippeneng 4, 6708 WE Wageningen, The Netherlands
[c] Unilever Foods Innovation Centre – Hive, Bronland 14, 6708 WH Wageningen, The Netherlands
[d] Wageningen Food Safety Research (WFSR), Wageningen University & Research, P.O. Box 230, 6700 AE Wageningen, The Netherlands

ARTICLE INFO

Keywords:
Δ^8-THC
CBD conversion
Cannabinoids isomers
Chromatographic analysis
Δ^8-THC gummies

ABSTRACT

Δ^8-Tetrahydrocannabinol (Δ^8-THC) is increasingly popular as a controversial substitute for Δ^9-tetrahydrocannabinol (Δ^9-THC) in cannabinoid-infused edibles. Δ^8-THC is prepared from cannabidiol (CBD) by treatment with acids. Side products including Δ^9-THC and other isomers that might end up in Δ^8-THC edibles are less studied. In this paper, three orthogonal methods, namely reversed-phase (RP)-UHPLC-DAD/HRMS, normal-phase/argentation (silica-Ag(I))-HPLC-DAD/MS, and GC-FID/MS were developed for analysis of cannabinoid isomers, namely Δ^8-THC, Δ^9-THC, CBD, Δ^8-iso-THC, $\Delta^{(4)8}$-iso-THC, and hydrated THC isomers. Eight acid-treated CBD mixtures contained various amounts of Δ^8-THC (0–89%, w/w%), high levels of Δ^9-THC (up to 49%), Δ^8-isoTHC (up to 55%), $\Delta^{(4)8}$-iso-THC (up to 17%), and three hydrated THC isomers. Commercial Δ^8-THC gummies were also analyzed, and issues like overclaimed Δ^8-THC, excessive Δ^9-THC, undeclared Δ^8-iso-THC, and $\Delta^{(4)8}$-iso-THC were found. These findings highlight the urgency of improving regulations towards converting CBD to Δ^8-THC for use as food ingredients.

1. Introduction

Cannabinoid-infused edibles containing e.g. cannabidiol (CBD) and recently Δ^8-tetrahydrocannabinol (Δ^8-THC), but lacking Δ^9-tetrahydrocannabinol (Δ^9-THC), are becoming increasingly popular for therapeutic or recreational purposes (Leas, Nobles, Shi, & Hendrickson, 2022; Peng & Shahidi, 2021). The U.S. Agriculture Improvement Act of 2018 (2018 Farm Bill) legalized hemp and hemp-derived products containing no more than 0.3 % of Δ^9-THC on a dry weight basis (Congress, U.S., 2018). However, the 2018 Farm Bill does not regulate Δ^8-THC, which possesses similar therapeutic and mild psychoactive effects as Δ^9-THC (Casajuana Kögel, López-Pelayo, Balcells-Olivero, Colom, & Gual, 2018). In 1995, Δ^8-THC underwent its initial assessment as an antiemetic remedy in a trial involving eight children undergoing chemotherapy (Abrahamov, Abrahamov, & Mechoulam, 1995). Subsequently, Δ^8-THC was found to produce a dose-dependent response leading to euphoria, blurred vision, mental confusion, and lethargy with around 75 % of the potency of Δ^9-THC (La Maida, Di Giorgi, Pichini, Busardò, & Huestis, 2022). It is therefore not surprising that Δ^8-THC-infused edibles are pursued as a legal substitution of Δ^9-THC by consumers (LoParco, Rossheim, Walters, Zhou, Olsson, & Sussman, 2023).

Due to the negligible occurrence of natural Δ^8-THC in cannabis (Gülck & Møller, 2020), Δ^8-THC for consumption is typically produced by acid-catalyzed intramolecular cyclization of CBD (Duffy et al., 2022). Δ^8-THC-infused products are often marked as “hemp-derived” or “legal high” products in different formulations, e.g., vape oils, gummies, and chocolates, with attractive packaging and are easy to acquire in stores or online. Some “home-kitchen” protocols for making Δ^8-THC edibles from CBD are also easily available on websites. However, there is no regulation or monitoring for producing Δ^8-THC from CBD, and such acid-induced intramolecular cyclization is normally versatile with not only Δ^8-THC but also Δ^9-THC and other cannabinoids formed. Subsequent

* Corresponding authors at: Key Laboratory of Phytochemical R&D of Hunan Province and Key Laboratory of Chemical Biology & Traditional Chinese Medicine Research of Ministry of Education, Hunan Normal University, No.36, Lushan Road, 410081 Changsha, China (B. Chen); Laboratory of Organic Chemistry, Wageningen University, Stippeneng 4, 6708 WE Wageningen, The Netherlands (H. Zuilhof, G.IJ. Salentijn).
E-mail addresses: dr-chenpo@vip.sina.com (B. Chen), Han.Zuilhof@wur.nl (H. Zuilhof), Gert.Salentijn@wur.nl (G.IJ. Salentijn).

https://doi.org/10.1016/j.foodchem.2023.138187
Received 23 April 2023; Received in revised form 7 December 2023; Accepted 7 December 2023
Available online 14 December 2023

infusing into a food matrix would result in concomitant concerns about the safety and legal status of Δ^8-THC edibles. We summarized 53 analysis reports of commercial Δ^8-THC products reported by the US Cannabis Council and the New Leafreport Research in 2021 (Council, U. S. C., 2021; Leafreport, 2022), and there were several issues concerning Δ^8-THC products including (i) unknown ingredients, (ii) no content label or incorrect Δ^8-THC content, and (iii) illegal levels of Δ^9-THC (>0.3 %, Supporting Information (**SI**), **Figure S1**). While the latter is already worrisome, the presence of unknown compounds from the Δ^8-THC producing process, without toxicity data, is potentially of even greater concern. Moreover, increasing hospitalizations induced by consuming Δ^8-THC edibles and other products further underlines the need to analyze the compositions after converting CBD to Δ^8-THC (LoParco et al., 2023).

Cannabinoids in edibles that should at least be analyzed are Δ^9-THC, tetrahydrocannabinolic acid (THCA), CBD, cannabidiolic acid (CBDA), and cannabinol (CBN) (AOAC, 2017; AOAC, 2022). Recent research expanded the analytical scope with Δ^8-THC (Christinat, Savoy, & Mottier, 2020; Pisciottano, Guadagnuolo, Soprano, Esposito, & Gallo, 2021; Song, Meyer, Adejumo, Jovanovich, LeBlanc, & Provis, 2023). As mentioned earlier, most Δ^8-THC found in the market is synthesized and thus unavoidably accompanied by other THC isomers (Golombek, Müller, Barthlott, Sproll, & Lachenmeier, 2020; Helander, Johansson, Andersson, & Villén, 2022). Advanced analytical methods to analyze Δ^8-THC, Δ^9-THC, and CBD in the presence of synthetic isomers have recently been published. These include a 2D high pressure liquid chromatography (HPLC) system (C6 Phenyl column as the first dimension and C18 as the second dimension) for analysis of Δ^8-THC, Δ^9-THC, CBD, $\Delta^{6a,10a}$-THC, and Δ^{10}-THC in e-cigarettes (Chan-Hosokawa, Nguyen, Lattanzio, & Adams, 2022), and poroshell C18 column with complex multi-step gradient conditions for analysis of Δ^8-THC, Δ^9-THC, $\Delta^{6a,10a}$-THC, Δ^{10}-THC, and $\Delta^{9,11}$-THC (Reber, Karschner, Seither, Knittel, Dozier, & Walterscheid, 2022). All these methods rely on reversed-phase separations and multiple reaction monitoring (MRM) mass spectrometry (MS), with the same MS/MS transitions for different isomers. As a consequence, multiple challenges remain to be addressed. First, due to their near-identical retention times, baseline separation between Δ^8-THC and Δ^9-THC is only achieved by resorting to highly efficient HPLC systems such as ultra-high-pressure liquid chromatography (UHPLC), or to the use of core–shell particles. Even then, isomers, e.g., $\Delta^{9,11}$-THC that are coeluting with Δ^9-THC, interfere with their analysis (Reber, Karschner, Seither, Knittel, Dozier, & Walterscheid, 2022). Second, the lack of unique MRM transitions makes the identification of isomers problematic. Thus, given their near-identical retention times and lack of MS-based identification, a small variation in retention time can easily lead to misassignments. Kiselak et al. (Kiselak, Koerber, & Verbeck, 2020) coupled reversed phase (RP)-HPLC-MS with ion mobility spectrometry (IMS) to investigate formed cannabinoids when converting CBD to Δ^9-THC (or Δ^8-THC) at home. Even though they identified multiple unnatural cannabinoids, the isomers Δ^9-THC and Δ^8-THC were just partially separated and had the same MS fragments and collisional cross-section, hampering identification by IMS. Apart from that, two compounds eluting closely to Δ^8-THC and Δ^9-THC with identical MS fragments and collisional cross-sections as Δ^8-THC and Δ^9-THC could not be unambiguously identified. Ciolino et al. (Ciolino, Ranieri, Brueggemeyer, Taylor, & Mohrhaus, 2021) identified Δ^8-THC, Δ^9-THC, $\Delta^{6a,10a}$-THC, Δ^{10}-THC and $\Delta^{9,11}$-THC in commercial vaping liquids by GC–MS. However, all these THC isomers had to be silylated prior to separation, and no absolute quantification of Δ^8-THC and Δ^9-THC was achieved. Thus, despite the attention given to synthetic THC isomers when analyzing Δ^8-THC-related products, more efforts are needed in developing analytical methods and understanding product compositions, especially regarding the synthetic THC isomers, for both forensic purposes and health considerations.

In the current paper, we therefore set out to improve the analysis of cannabinoid isomers formed in the process of converting CBD to Δ^8-THC as well as their occurrence in cannabinoid-infused edibles (Δ^8-THC gummies). We thus aimed to first evaluate eight common synthetic protocols using acid catalysis to convert CBD to Δ^8-THC (including methods suggested for 'use at home'). Different chromatographic methods, that are based on different principles were expected to jointly achieve improved separation and profiling of the cannabinoids from those mixtures: a) an RP-UHPLC-diode array detector (DAD)/high resolution mass spectrometer (HRMS) method; b) a GC-flame ionization detector (FID)/MS method; c) a silica-Ag(I) HPLC-DAD/MS method. For the silica-Ag(I) HPLC-DAD/MS method, we hypothesized that Ag(I) affinity combined with the normal-phase separation mechanism could lead to improved separation of cannabinoids. Apart from that, Ag(I) adduct formation and unique MS/MS transitions were expected due to selective affinities of Ag(I) towards olefins with different numbers and positions of C=C double bonds (Huang et al., 2021; Kaneti, de Smet, Boom, Zuilhof, & Sudhölter, 2002; van Beek & Subrtova, 1995). If so, characteristic fragments in the presence of Ag(I) could be used for more reliable identification of isomeric cannabinoids.

2. Materials and methods

2.1. Chemicals and reagents

Formic Acid (HPLC grade) and silver nitrate (Analytical grade) were purchased from Fisher Scientific (Loughborough, Leicestershire, UK). Acetonitrile (ACN, HPLC grade) and methanol (MeOH, HPLC grade) were obtained from VWR Chemicals (Gliwice, Poland). *n*-hexane (hexane, HPLC grade) was obtained from Honeywell Riedel-de Haën (Seelze, Germany). Ethanol (EtOH, HPLC-grade) was purchased from Biosolve Chimie SARL (57260 Dieuze, France). Methyl *tert*-butyl ether (MTBE) was bought from Biosolve BV (Valkenswaard, the Netherlands). Deionized water was obtained from a Milli-Q^{TM} direct ultrapure water system ((18.2 MΩ·cm, Milli-Q Integral 3 system, Millipore, USA). Δ^9-THC standard was purified from cannabis flowers. Crystalline CBD was purchased from CBDolie.nl. Δ^8-THC and Δ^8-isoTHC standards were isolated from acid-treated CBD (Citti et al., 2019). The group of Prof. Passarella (Marzullo et al., 2020) kindly provided Δ^8-iso-THC (for comparison with our isolated standard Δ^8-iso-THC) and $\Delta^{(4)8}$-iso-THC. According to NMR and peak integrations at DAD 215 nm (**SI**, **Figure S2-S3**), the purity of these five standards was > 98 %. Normal gummies without cannabinoids were purchased from a local supermarket in Wageningen, the Netherlands, and labeled as N#1, N#2, and N#3. Δ^8-THC gummies were bought online and named as C#1 and C#2.

2.2. RP-UHPLC-DAD/HRMS

A 1290 Infinity UHPLC system (Agilent Technologies, Santa Clara, United States) equipped with a Zorbax Eclipse Plus C18 column (2.1 × 100 mm, 1.8 μm; Agilent Technologies) was used with DAD and coupled to a Q-Exactive quadrupole orbitrap high-resolution mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) via an Electrospray Ionization (ESI) interface. The mobile phase consisted of 5 mM formic acid in water (mobile phase A) and ACN (mobile phase B). The injection volume was 8 μL. Isocratic elution was applied with 58 % B at a flow rate of 0.50 mL·min^{-1}, unless otherwise specified. The resolution (R_s) of two peaks was calculated as follows:

$$R_s = \frac{t_{R2} - t_{R1}}{0.85 \times (W_{1,h/2} + W_{2,h/2})}$$

t_{R1}, t_{R2}: retention time of two adjacent peaks; $W_{1,\ h/2}$, $W_{2,\ h/2}$: peak width at half-height of two adjacent peaks.

Positive ionization mode MS was used with a mass resolution of 70,000 FWHM, a maximum injection time of 200 ms, a sheath gas flow rate of 36 (arbitrary units), and an auxilary gas flow rate of 18 (arbitrary units). The capillary temperature was 320 °C and the S-lens RF level was 50 (arbitrary units). All full-scan measurements were performed with a

scan range of *m/z* 100.0——1500.0. A normalized collision energy (NCE) of 40 V was applied for MS/MS fragmentation. Thermo Scientific Xcalibur 2.2 software was used for data acquisition and processing. The intensity of ions with *m/z* values within ± 5 ppm of the theoretical *m/z* was shown in the extracted ion chromatogram (EIC).

2.3. GC-FID/MS

An Agilent 5975C VL MSD GC–MS system (Agilent, Amstelveen, the Netherlands) equipped with a HP-5MS capillary column (5 % phenylmethylpolysiloxane, 30 m × 0.25 mm i.d. and 0.25 μm film thickness, Agilent J&W GC column, Amstelveen, the Netherlands) was used for GC-FID/MS analysis with a split ratio of 1:1 to the FID and MS detector. The injection temperature was 250 ℃ and the carrier gas was He at a linear velocity of 29 cm·s^{-1}. For the fast analysis of Δ^8-THC, Δ^9-THC and CBD, the initial column temperature was 200 ℃, which was ramped with 10 ℃·min^{-1} to 275 ℃ and kept at this temperature for 5 min (total analysis time is 12.5 min). For the analysis of the five isomers and samples, a two-step gradient temperature program was used with an initial column temperature of 200 ℃, ramped with 1 ℃·min^{-1} to 222.5 ℃. Then, the column temperature was ramped to 250 ℃ with 5 ℃·min^{-1} and kept at this temperature for 5 min (total analysis time of 33 min). 1 μL of sample was injected with a split ratio of 1:10. The mass spectrometer was operated in the 70 eV electron ionization (EI) mode, scanning from *m/z* 35 to 500 at 4 spectra·s^{-1}. Actual measurements started 3.0 min after injection to protect the filament of the mass spectrometer.

2.4. Silica-Ag(I) HPLC-DAD/MS

A 1220 Infinity II liquid chromatography system (Agilent Technologies) equipped with a Nucleosil Ag(I) phase column (100 × 4.6 mm, 3 μm; custom packed at Agilent, Middelburg, the Netherlands) was used with DAD, or with a Linear Ion Trap mass spectrometer (ESI-LXQ, Thermo Fisher Scientific) via a custom-built ESI interface. Isocratic elution was applied with 1 % ACN in hexane (v/v%) as mobile phase at a flow rate of 1.00 mL·min^{-1}. 5 μL of sample was injected. Since hexane cannot be easily used for stable ESI (Zhang, Wang, Zhu, Cai, & Guo, 2014), post-column mixing with a solution of $AgNO_3$ (2.5×10^{-5} M) in EtOH was used to assist ESI ionization and form silver adducts (see **SI**, **Protocol S1**, **Figure S4**). Ionization was performed in positive mode with capillary voltage 49 V, tube lens 85 V, capillary temperature 350 °C, and sheath gas flow rate of 15 (arbitrary units). All full-scan measurements were performed with a scan range of *m/z* 100.0–2000.0. For all MS/MS fragmentation measurements, collision-induced dissociation (CID) energies were determined as the energy at which the target product ions had the highest abundance while the precursor ion or ions had not yet disappeared completely. The isolation width was set to include all desired precursor ions. For accurate mass measurements of Ag(I) adducts and relevant fragments, a previously published Ag(I)-paper spray ionization method was used, in combination with a Q-Exactive mass spectrometer (Huang et al., 2021).

2.5. Calibration curve construction and evaluation of LOD and LOQ

Stock solutions of Δ^8-THC, Δ^9-THC, and CBD were prepared in MeOH at 1.00 mg·mL^{-1}. Mixed standard solutions of 333, 167, 100, 10.0, 5.00 μg·mL^{-1} and 0.500 μg·mL^{-1} were prepared in triplicate by mixing stock solutions and serially diluting them with MeOH, and were analyzed by RP-UHPLC-DAD and GC-FID. For silica-Ag(I) HPLC-DAD analysis, considering the incompatibility of the sample solvent with the mobile phase, 100 μL of the mixed standard solution was first blow-dried with N_2 and re-dissolved in 100 μL of mobile phase in a 1.8 mL HPLC vial with a micro-insert (200 μL, Fisher Scientific, Warsaw, Poland). Characteristic peak areas were plotted against the concentration to construct calibration curves for external standard quantification. For the determination of the limit of detection (LOD) and limit of quantification (LOQ), the peak height was used. Blanks (MeOH for RP-UHPLC-DAD and GC-FID; MeOH blow-dried and redissolved in 1 % ACN in hexane (v/v%) for silica-Ag(I) HPLC-DAD) were injected and standard deviations (SD) of the signal at the respective retention times of the analytes were determined. The LODs and LOQs were calculated as follows: LOD = 3 × SD of blank/slope of the calibration curve; LOQ = 10 × SD of blank/slope of the calibration curve (Shrivastava & Gupta, 2011; Villela, Van der Klift, Mattheussens, Derksen, Zuilhof, & Van Beek, 2011). Samples with concentrations close to the calculated LODs were tested for confirmation.

2.6. Preparation of mixtures containing Δ^8-THC by treatment of CBD with acid

Eight reported methods (see **SI**, **Figure S5** and **Table S1**) for producing "Δ^8-THC" samples from CBD were carried out with minor modifications (Kiselak et al., 2020; Marzullo et al., 2020). In summary, different catalytic conditions were employed: p-toluenesulfonic acid with varying acid quantities and solvent types (Entries 1–4), boron trifluoride diethyl etherate (BF_3·OEt_2) catalysis in acetonitrile (Entry 5), sulfuric acid in ethanol (Entry 6), acetic acid in ethanol (Entry 7), and hydrochloric acid in ethanol (Entry 8). After workup (quenched with base solutions, washed with saturated aqueous $NaHCO_3$ and NaCl solutions, dried with Na_2SO_4 and filtered through filter paper. All eight reaction solutions were filtered through 0.2 μm PTFE membrane syringe filters (⌀ 13 mm, Pall Corporation, Port Washington, NY, USA), and the solvent was evaporated under N_2 flow, followed by freeze-drying (FreeZone 2.5 L, Labconco, Kansas City, MO, USA) at −84 ℃ during more than 24 h. The reaction products obtained by each method were dissolved in MeOH at 100 μg·mL^{-1} unless otherwise stated for RP-UHPLC-DAD/MS and GC-FID/MS analysis. For silica-Ag(I) HPLC-DAD/MS analysis, 100 μL of the methanolic solution was first blow-dried with N_2 and re-dissolved in 100 μL of 1 % ACN in hexane (v/v%).

2.7. Gummy extraction

1.00 ± 0.06 g of normal gummy or Δ^8-THC gummy was weighed in a conical stopper flask and extracted with 200.00 mL of an MTBE/water 1/1 (v/v) solution by ice bath ultrasonication (Bandelin Sonorex, Rangendingen, Germany) for up to 40 min to fully dissolve the gummies. After waiting 10 min for phase separation, the MTBE layer was filtered over 0.2 μm PTFE membrane syringe filters and then used for subsequent analysis.

2.8. Extraction recovery

Four aliquots (1.00 mL each) of 100 μg·mL^{-1} CBD methanolic solution were blow-dried. After that, one aliquot was redissolved in MTBE and subjected to GC-FID/MS analysis. The remaining three aliquots were reconstituted with 1.00 mL of MTBE and 1.00 mL of water, followed by hand-shaking of 10 min. After a waiting period of 10 min for phase separation, 60 μL of the MTBE layer was taken for GC-FID/MS analysis.

$$\text{Extraction recovery}(\%) = \left(A_{\text{after extraction}} / A_{\text{without extraction}}\right) \times 100.$$

A is the GC-FID peak area of a specific cannabinoid.

Four aliquots (100 μL each) of methanolic solution consisting of 100 μg·mL^{-1} of Δ^8-THC, Δ^9-THC, Δ^8-iso-THC, $\Delta^{(4)8}$-iso-THC, and CBD (five-cannabinoid solution) went through the above procedure (volumes of 100 μL of MTBE and 100 μL of water) to evaluate the extraction recovery of each cannabinoid.

2.9. Matrix effects, accuracy, and precision

Matrix effects. Hundred μL of 100 μg·mL^{-1} five-cannabinoid methanolic solution was blow-dried and redissolved in 100 μL of

normal gummy extract for GC-FID/MS and silica-Ag(I) HPLC-DAD analysis. The characteristic peak area of each cannabinoid in spiked normal gummy extract (sample type II) was compared with that in solvent (sample type I).

$$\text{Matrix effect}(\%) = ((A_{II}/A_I) - 1) \times 100.$$

A is the peak area of GC-FID or silica-Ag(I) HPLC-DAD (215 nm) of a specific cannabinoid.

Accuracy and precision. Accuracy and precision of the method were evaluated at three concentration levels (weight percentage w/w%: low (0.02 %), medium (0.1 %) and high (0.2 %)) in normal gummy N#2 extract (n = 3 per concentration). 100 μL of 20, 100, 200 μg·mL^{-1} five-cannabinoid methanolic solution was spiked in 1.00 mL of normal gummy extract N#2 (0.01 times of the total gummy weight) and blow-dried. Subsequently, 100 μL of MeOH was added for GC-FID/MS analysis and 100 μL of 1 % ACN in hexane (v/v%) was added for silica-Ag(I) HPLC-DAD analysis. The concentration of each cannabinoid was calculated by the established calibration curves.

The weight percentage was expressed as:

$$\textit{Weight percentage}\ (\%) = \frac{C \times V}{W} \times 100$$

C: calculated concentration in extract (μg·mL^{-1}); V: volume of extraction (mL); W: total gummy weight (μg).

Precision was calculated as the relative standard deviation (RSD%) (n = 3). Accuracy was calculated as the relative deviation (%) of the calculated mean value of weight percentage from the respective reference value.

2.8. Cannabinoid gummy analysis

Cannabinoid gummy C#1 and C#2 were extracted as described above (n = 3). 1.00 mL of the MTBE layer was filtered and then subjected to the analysis by GC-FID/MS and silica-Ag(I) HPLC-DAD methods for quantification of Δ^8-THC. For the quantification of other cannabinoids, the filtrate of the MTBE layer was concentrated 20 times by blow-drying and reconstitution before analysis. The percentage was expressed as aforementioned with a concentrating factor taken into account if used.

3. Results and discussion

3.1. RP-UHPLC-DAD/HRMS for separation and identification of Δ^8-THC, Δ^9-THC, and CBD standards

The ever-popular Δ^9-THC and CBD and increasingly popular Δ^8-THC are isomers, but have a different pharmacology, toxicology, and legal status. Therefore, discrimination and characterization of these three cannabinoids are of great importance. Methods applying RP-HPLC coupled to ultraviolet (UV) detection or DAD have been most frequently used, as reviewed recently by La Maida et al. (La Maida, Di Giorgi, Pichini, Busardò, & Huestis, 2022). Generally, long separation times (>20 min) with high flow rates (>1 mL/min) have been used to achieve partial or baseline separation of Δ^8-THC and Δ^9-THC, since UV absorption spectra show no differences for the three isomers. Very recently, a reversed-phase 2D-LC system (Chan-Hosokawa et al., 2022) and a RP superficially porous column under a multi-step gradient elution (Reber et al., 2022) achieved separation of Δ^8-THC and Δ^9-THC in 10 min with R_s 2.5 and 1.4, respectively. Subsequently, ESI-MS/MS (MRM mode) with identical transitions was used for quantification.

In the present study, Δ^8-THC and Δ^9-THC were, after optimization, also resolved (R_s = 1.8) by reversed-phase UHPLC (Fig. 1**A**, and **SI**, **Table S2 and Figure S6**). When coupled to DAD and HRMS detectors, as expected, there was no selectivity in the UV absorption (**SI, Figure S7**), nor in the high-resolution mass spectra in positive ionization mode (**Fig. 1B**), as described in other references (Chan-Hosokawa et al., 2022; Lin, Amaratunga, Reed, Huang, Lemberg, & Lemberg, 2022; Reber et al., 2022). The attribution of compounds can thus only be made by comparison of chromatographic retention times (RTs) with the standards. However, if the RT shifts slightly across samples due to e.g., column degradation, sample overloading, variations of mobile phase, column temperature, pressure, incorrect analysis might ensue (Wang et al., 2019). Moreover, any co-eluting matrix compound or isomer would be detrimental to the performance, due to many shared fragments between those. Therefore, improved chromatographic separation and more

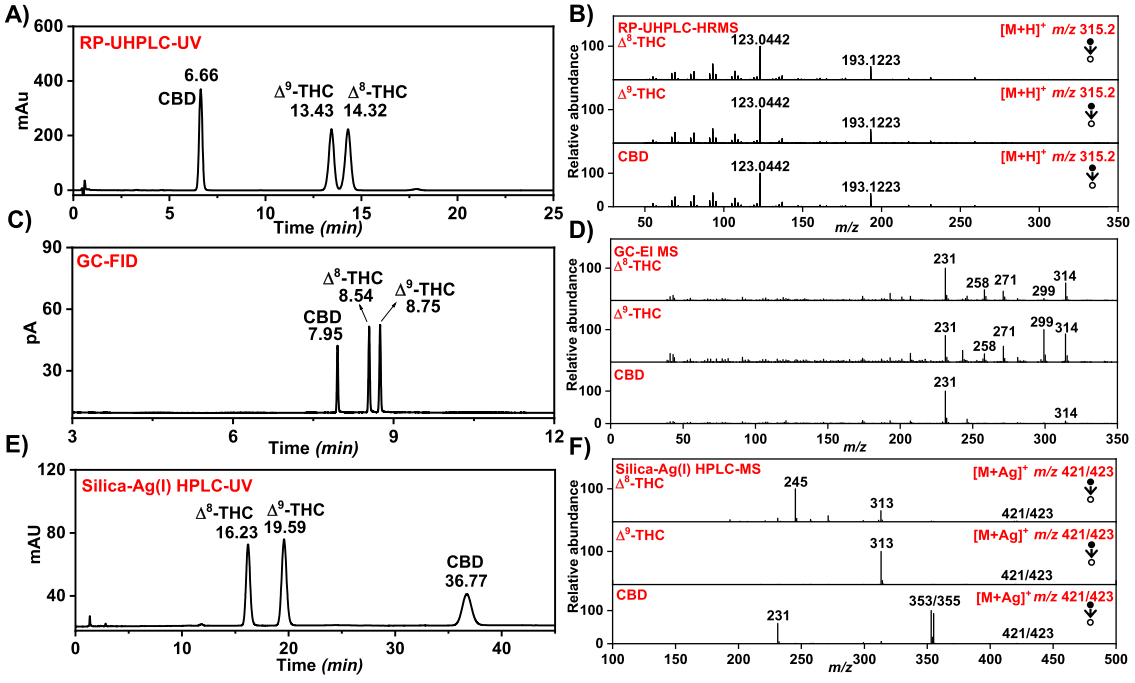


Fig. 1. Δ^8-THC, Δ^9-THC, and CBD standards analyzed by (A) RP-UHPLC-DAD, (B) RP-UHPLC-HRMS/MS of $[M + H]^+$, (C) GC-FID, (D) GC-EI-MS, (E) silica-Ag(I) HPLC-DAD and (F) silica-Ag(I) HPLC-MS/MS of $[M + Ag]^+$.

specific MS information are needed.

3.2. GC-FID/MS for separation and identification of Δ^8-THC, Δ^9-THC, and CBD standards

As second standard method, GC-FID/MS was applied for the analysis of the three neutral cannabinoids. Thermal decomposition should not pose a problem as these synthetic products typically do not contain acidic cannabinoids. Even if the starting CBD would contain acidic cannabinoids, acids were used in treating CBD and this would lead to decarboxylation (X. Zhang, Geng, & Wang, 2011). As shown in Fig. 1**C and 1D**, Δ^8-THC, Δ^9-THC, and CBD were well resolved by the GC method (R_s of Δ^8-THC and Δ^9-THC is 3.1) in 9 min, and have distinct EI spectra. Combined with the merit of a short analysis time, GC-FID/MS is promising in the analysis of Δ^8-THC, Δ^9-THC, and CBD. However, a very recent study (Tsujikawa et al., 2022) observed the thermal decomposition of CBD to Δ^9-THC in the injector of the GC–MS system if the splitless mode was used, due to the longer residence time in the injector compared to split mode. Therefore, some caution is required and split injection with the injector temperature at 200 ℃ should be applied to avoid such decomposition.

3.3. Silica-Ag (I) HPLC-DAD/MS for separation and identification of Δ^8-THC, Δ^9-THC, and CBD standards

In previous work, we separated Δ^9-THC from CBD by Ag(I)-loaded cation exchange HPLC-MS with MeOH as mobile phase and observed unique fragmentation of silver adducts (Huang et al., 2021). This was based on the different affinity of Ag(I) towards a single C=C bond versus that of two C=C bonds in 1,5-dienes. Therefore, we further explored whether Ag(I) affinity could enable improved separation and characterization of Δ^8-THC, Δ^9-THC, and CBD. Not unexpectedly, both Δ^8-THC and Δ^9-THC eluted close to the dead time with Δ^8-THC eluting 0.18 min earlier than Δ^9-THC on the Ag(I)-loaded cation exchanger (**SI**, **Figure S8**), due to their much weaker interactions with Ag(I) compared to CBD. As demonstrated by previous quantum chemical results (Huang et al., 2021), CBD is bound to Ag(I) about 12 kcal·mol^{-1} stabler than Δ^9-THC, and further quantum-chemical computation (**SI**, **Figure S9**) showed that Δ^9-THC is bound to Ag(I) 5 kcal·mol^{-1} more strongly than Δ^8-THC. Due to the lack of separation on the Ag(I)-loaded cation exchange column, an alternative approach was chosen, in which an Ag(I)-coated silica gel column with 1 % ACN in hexane (v/v%) as mobile phase was used for the separation of the three analytes. As shown in Fig. 1**E**, excellent separation of Δ^8-THC and Δ^9-THC was obtained ($R_s = 4.5$), due to the combined contributions from normal-phase adsorption, Ag(I) complexation effects and long separation times (Adlof, 1997; Huang et al., 2022). However, the applied mobile phase (1 % ACN in hexane (v/v%)) cannot be used to produce a stable ESI spray, making it difficult to obtain MS information after the Ag(I)-coated silica chromatographic separation.

To overcome this, we employed the setup as shown in **Figure S4** (**SI**), by which both DAD and mass spectra could be collected after separation. This was achieved by combining the effluent of the HPLC column in a mixer with $AgNO_3$ in EtOH (2.5×10^{-5} M) to produce a stable spray to introduce Ag(I)-cannabinoid adducts to the MS. These adducts have unique fragmentation patterns, even for THC isomers (Fig. 1**F**). Quasi-molecular species at *m/z* 421 and 423 were found for Δ^8-THC, Δ^9-THC and CBD with characteristic silver isotopes, ^{107}Ag (52 %) and ^{109}Ag (48 %). Different fragmentation patterns for Δ^8-THC, Δ^9-THC and CBD were found by utilizing *m/z* 422 as the precursor ion with an isolation width of 4 (thus containing both *m/z* 421 and 423). For CBD, main fragments were observed at *m/z* 353/355. For Δ^9-THC, there was only one product ion at *m/z* 313. However, for Δ^8-THC, apart from a minor fragment at *m/z* 313, the most prominent fragment was found at *m/z* 245.

3.4. Resolving THC isomers by RP-UHPLC-DAD/HRMS, GC-FID/MS, and silica-Ag(I) HPLC-DAD/MS

When treating CBD with acids for producing Δ^8-THC, the formation of various other THC isomers is hardly avoidable as reviewed by Golombek et al. (Golombek et al., 2020). The existence of multiple isomers may ruin the analysis of Δ^8-THC, Δ^9-THC and CBD when less selective methods are used (Lachenmeier et al., 2022). Relatively few papers are devoted to chemical analysis of cannabinoids (only around 2 % of all publications on cannabis) (Gertsch, 2018). Recently published methods for the analysis of THC isomers mainly focus on resolving Δ^8-THC, Δ^9-THC and CBD from $\Delta^{6a,10a}$-THC, Δ^{10}-THC, and $\Delta^{9,11}$-THC (Chan-Hosokawa et al., 2022; Ciolino et al., 2021; Kiselak et al., 2020; Reber et al., 2022). Still, there are also other THC isomers, namely Δ^8-iso-THC and $\Delta^{(4)8}$-iso-THC, which are also found in acid-treated CBD mixtures (Gaoni & Mechoulam, 1968; Marzullo et al., 2020), and even in commercial products (Meehan-Atrash & Rahman, 2021). However, apart from NMR (Marzullo et al., 2020; Meehan-Atrash et al., 2021) there are hardly any other methods available to analyze these isomers in the presence of Δ^8-THC, Δ^9-THC and CBD. Marzullo et al. (Marzullo et al., 2020) applied RP-HPLC in an attempt to resolve Δ^8-iso-THC and $\Delta^{(4)8}$-iso-THC from Δ^8-THC and Δ^9-THC, but Δ^8-THC coeluted with Δ^8-iso-THC, and Δ^9-THC coeluted with $\Delta^{(4)8}$-iso-THC. Even Δ^8-THC and Δ^9-THC were only partially separated. Therefore, in the end, NMR was used together with the RP-HPLC method for the distinction and identification of these isomers in mixtures. However, larger amounts of samples are needed for NMR.

Here, we further evaluated the separation ability of the three chromatography-based methods with Δ^8-iso-THC and $\Delta^{(4)8}$-iso-THC being present in the standard mixture. As shown in Fig. 2**A**, with RP-UHPLC, Δ^8-iso-THC had the same retention time as Δ^8-THC, and $\Delta^{(4)8}$-iso-THC overlapped with Δ^8-THC and Δ^9-THC. Furthermore, these two isomers have the same UV spectra (**SI**, **Figure S7**) and fragmentation patterns for protonated precursor ions (**SI**, **Figure S10**) as Δ^8-THC, Δ^9-THC and CBD. Therefore, when Δ^8-iso-THC and $\Delta^{(4)8}$-iso-THC are present, it is not possible to identify Δ^8-THC and Δ^9-THC by RP-UHPLC-DAD/HRMS (Fig. 2**A and 2B**).

With GC, using the aforementioned 12.5 min temperature program, Δ^8-THC, Δ^9-THC and CBD can be resolved, but $\Delta^{(4)8}$-iso-THC overlapped with CBD (SI, **Figure S11**). In this case, a two-step gradient and a longer (33 min) temperature program was applied for the separation of the five isomers (Fig. 2**C**), and all of them produced distinct EI spectra (Fig. 2**D**). Ciolino et al. (Ciolino et al., 2021) also applied a two-step gradient temperature program with an analysis time of 39.9 min, and resolved various THC isomers that differ in the position of the C=C bond and/or stereochemistry (6aR,10aR-Δ^8-THC, 6aR,10aR-*exo*-THC, 6aR,10aR-Δ^9-THC, $\Delta^{6a,10a}$-THC, 6aR,9S-Δ^{10}-THC and 6aR,9R-Δ^{10}-THC), despite the partial overlap of $\Delta^{6a,10a}$-THC and Δ^{10}-THC. Even though their targets did not include acidic cannabinoids, they still carried out silylation before GC analysis, which took an additional 30 min.

When applying silica-Ag(I) HPLC-DAD/MS, both Δ^8-iso-THC and $\Delta^{(4)8}$-iso-THC were separated from the three major cannabinoids (Fig. 2**E**). Apart from that, each isomer gave a different fragmentation pattern in the presence of Ag(I), with a unique fragment at *m/z* 419 for $\Delta^{(4)8}$-iso-THC, and a characteristic favored fragment at *m/z* 299 for Δ^8-isoTHC when fragmenting precursor ions at *m/z* 422 with an isolation width of 4 (thus including both *m/z* 421 and *m/z* 423) (Fig. 2**F**). We further investigated the fragmentation of precursor ions at *m/z* 421 (isolation width of 1) and *m/z* 423 (isolation width of 1) separately (**SI**, **Figure S12**). It was shown that, for Δ^8-iso-THC, this gave the characteristic fragment at *m/z* 299 in both cases. For $\Delta^{(4)8}$-iso-THC, the precursor ion at *m/z* 423 yielded the fragment at *m/z* 421, while the precursor ion at *m/z* 421 yielded the fragment at *m/z* 419, which seemed to follow the fragmentation pathway $[M + Ag - 2H]^+$.

In our previous work, the MS fragmentation pathways of CBD and Δ^9-THC with Ag(I) were proposed (Huang et al., 2021). Here, we further

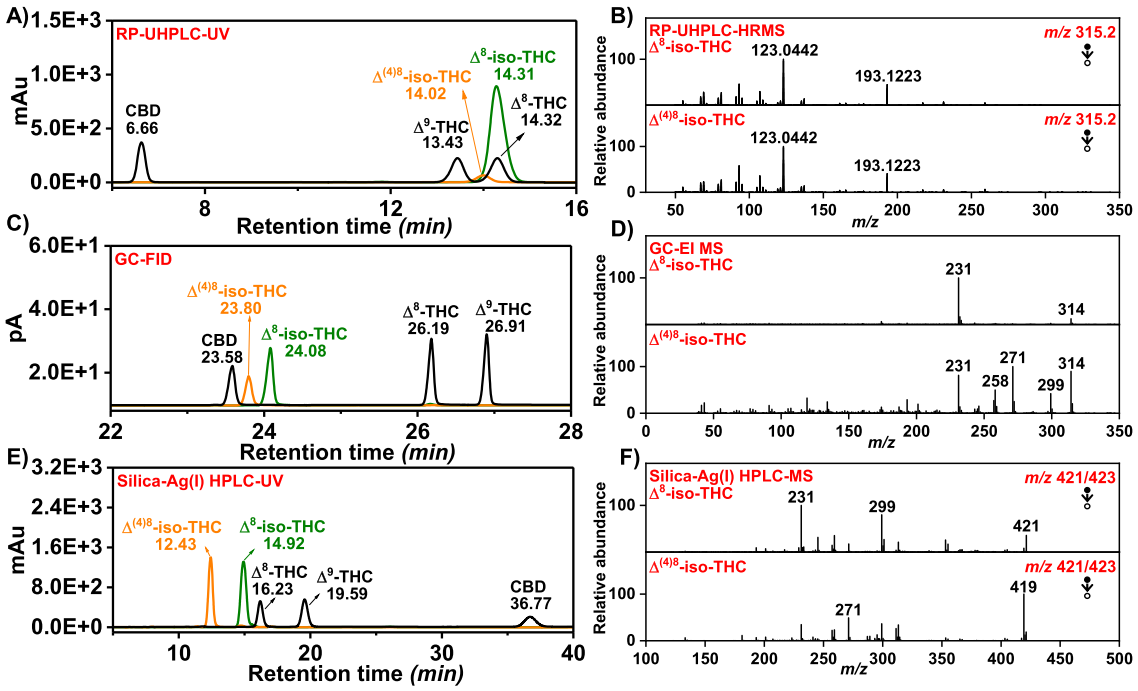


Fig. 2. Δ^8-iso-THC, $\Delta^{(4)8}$-iso-THC, Δ^8-THC, Δ^9-THC, and CBD were analyzed by (A) RP-UHPLC-DAD, (B) RP-UHPLC-HRMS/MS of $[M + H]^+$, (C) GC-FID, (D) GC-EI-MS, (E) silica-Ag(I) HPLC-DAD and (F) silica-Ag(I) HPLC-MS/MS of $[M + Ag]^+$.

propose a mechanism for the MS fragmentation of Δ^8-THC in the presence of Ag(I). The accurate mass of Δ^8-THC Ag(I) adducts and their fragments were determined by Ag(I) paper spray-HRMS and plausible structures and their formation are presented in Fig. 3. The $[\Delta^8\text{-THC} + Ag]^+$ adduct loses AgH during the MS^2 stage to form a tertiary carbocation (path ①). This carbocation undergoes methyl migration and rearrangement to form a new carbocation, stabilized by the lone pair on the oxygen. Afterward, a stable structure at *m/z* 245 was formed by the *retro*-Diels–Alder rearrangement (Tureček & Hanuš, 1984). On the other hand, similar to Δ^9-THC (Huang et al., 2021), $[\Delta^8\text{-THC} + Ag]^+$ adducts could lose AgH during the MS^2 stage to form the fragment at *m/z* 313 stabilized by the aromatic ring with the phenolic group (path ②). However, since the C=C double bond of Δ^8-THC is between carbon atoms 8 and 9 instead of 9 and 10, the fragment is less stable than that of Δ^9-THC. Additionally, the steric hindrance from the aromatic ring makes the H^- loss from carbon atom 10 as AgH less favored compared with path ①. Combined, for Δ^8-THC, the signal at *m/z* 313 is less abundant than the signal at *m/z* 245. These distinctive MS^2 signals could

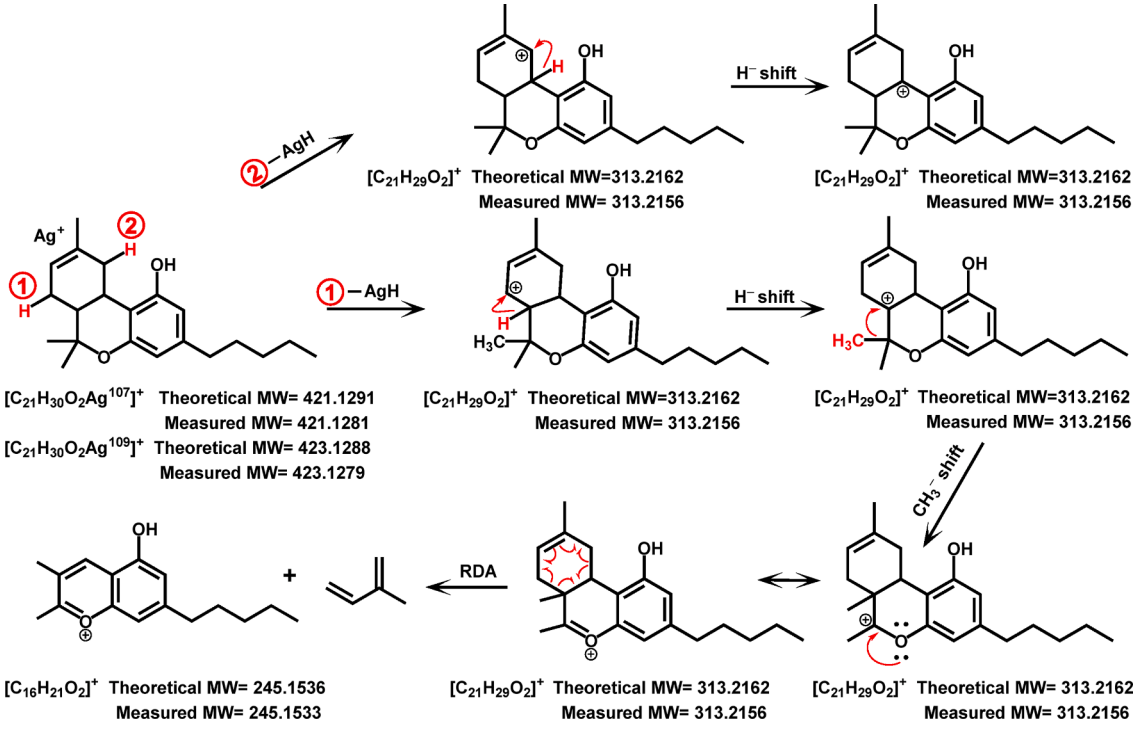


Fig. 3. Proposed mechanism for MS^2 fragmentation of Δ^8-THC in presence of Ag(I).

thus be used for further confirmation of Δ^8-THC, Δ^9-THC, and CBD, in addition to assignments according to HPLC retention times of standards.

3.5. Investigation of acid-treated CBD mixtures by RP-UHPLC-DAD/HRMS, GC-FID/MS, and silica-Ag(I) HPLC-DAD/MS

The increasing popularity of Δ^8-THC products and the insufficient analysis of their composition threaten consumer health and induce potential legal issues. By following several common acid catalysis-based protocols to produce Δ^8-THC from CBD, a better understanding is expected of the possible byproducts or impurities accompanying Δ^8-THC. Briefly, different acids, solvents, and conditions were used to treat CBD. Among those protocols, concerningly, some materials and procedures can be easily accessed and followed, and thus are described online for home-making of Δ^8-THC. These acid-treated CBD mixtures were then analyzed by the developed methods in this study to investigate the composition. Even though the optimized RP-UHPLC-DAD/MS method properly resolved Δ^8-THC, Δ^9-THC, and CBD, it still cannot provide accurate identification of all compounds in the samples, as these (by the other two methods) were shown to contain multiple THC isomers like Δ^8-isoTHC and $\Delta^{(4)8}$-iso-THC. Therefore, GC-FID/MS and silica-Ag(I) HPLC-DAD/MS were used to investigate these acid-treated CBD mixtures.

First, the occurrence of Δ^8-THC, Δ^9-THC, CBD, Δ^8-isoTHC and $\Delta^{(4)8}$-iso-THC was checked by comparing the respective retention times and mass spectra (**SI,** Figure S13) with those of the standards. As shown in Fig. 4, the product profiles vary substantially from protocol to protocol. In mixtures #1 and #2, CBD was not fully consumed and the major product is Δ^9-THC followed by Δ^8-THC, and then a little Δ^8-iso-THC. Increasing the amount of acid (molar ratio of acid to CBD from 0.1 to 2) increased the conversion of CBD. In mixtures #3 and #4, the main product is Δ^8-THC accompanied by trace amounts of Δ^9-THC. $\Delta^{(4)8}$-iso-THC was also detected in these two mixtures. When applying hexane as solvent (#4), $\Delta^{(4)8}$-iso-THC signal was more pronounced, and additionally, Δ^8-iso-THC was found. Compared with #1 and #2 (toluene as solvent), there was a trend of solvent selectivity, with toluene yielding more Δ^9-THC and DCM or hexane more Δ^8-THC. When using $BF_3{\cdot}OEt_2$ as a catalyst and performing the reaction at −10 °C in ACN, Δ^8-THC and Δ^9-THC concentrations decreased, and instead, Δ^8-iso-THC and $\Delta^{(4)8}$-iso-THC dominated. In #6, #7 and #8, easily-accessible materials, namely ethanol (solvent), and battery acid (37 % sulfuric acid), vinegar (5.4 % acetic acid), or muriatic acid (30 % hydrochloric acid) were used, respectively, and the reactions were conducted at 70 °C, which can be performed with ease in a home kitchen. In #6, four THC isomers were formed, predominately Δ^8-iso-THC, and only a bit of CBD remained unreacted. A similar profile was found for #8, except that no CBD was found and less Δ^8-THC was detected. Contrary to #6, in #7, no THC isomers were formed and mainly unreacted CBD was detected.

Except for the peaks identified by comparison with the five reference cannabinoids, there are multiple additional peaks in each. Three of them (initially named X1, X2, and X3) were isolated and RP-UHPLC-DAD/HRMS, GC-FID/MS, and silica-Ag(I) HPLC-DAD/MS (**SI,** Figure S14) analysis were performed. RP-UHPLC could resolve them and X3 had the same RT as CBD. The three isolated compounds also had the same UV spectrum (**SI,** Figure S7), identical MS signal at *m/z* 315.2319 ($[M + H]^+$, $C_{21}H_{31}O_2$) (**SI,** Figure S10), and MS^2 fragments (**SI,** Figure S15) as Δ^8-THC, Δ^9-THC and CBD of *m/z* 315.2319. Apart from that, they all showed an extra signal in full scan MS at *m/z* 333.2408 ($C_{21}H_{33}O_3$) (**SI,** Figure S10), which during fragmentation produced $[M - 18]^+$, likely resulting from the loss of H_2O, at *m/z* 315.2319 ($C_{21}H_{31}O_2$) (**SI,** Figure S16). Separation by the GC method showed molecular ion at *m/z* 332 (**SI,** Figure S17) for all three, confirming a molecular weight of 332 Da. Furthermore, the three compounds shared EI fragments at *m/z* 314 and 231 with Δ^8-THC, Δ^9-THC and CBD, implying a similar chemical structure. On the silica-Ag(I) HPLC column, the three compounds showed similar retention time (7.57 min for X1, 7.89 min for X2, and 7.48 min for X3) and clear Ag(I) adduct signals at *m/z* 439/441 ($[M + Ag]^+$) and *m/z* 771/773 ($[2\ M + Ag]^+$) (**SI,** Figure S18), again

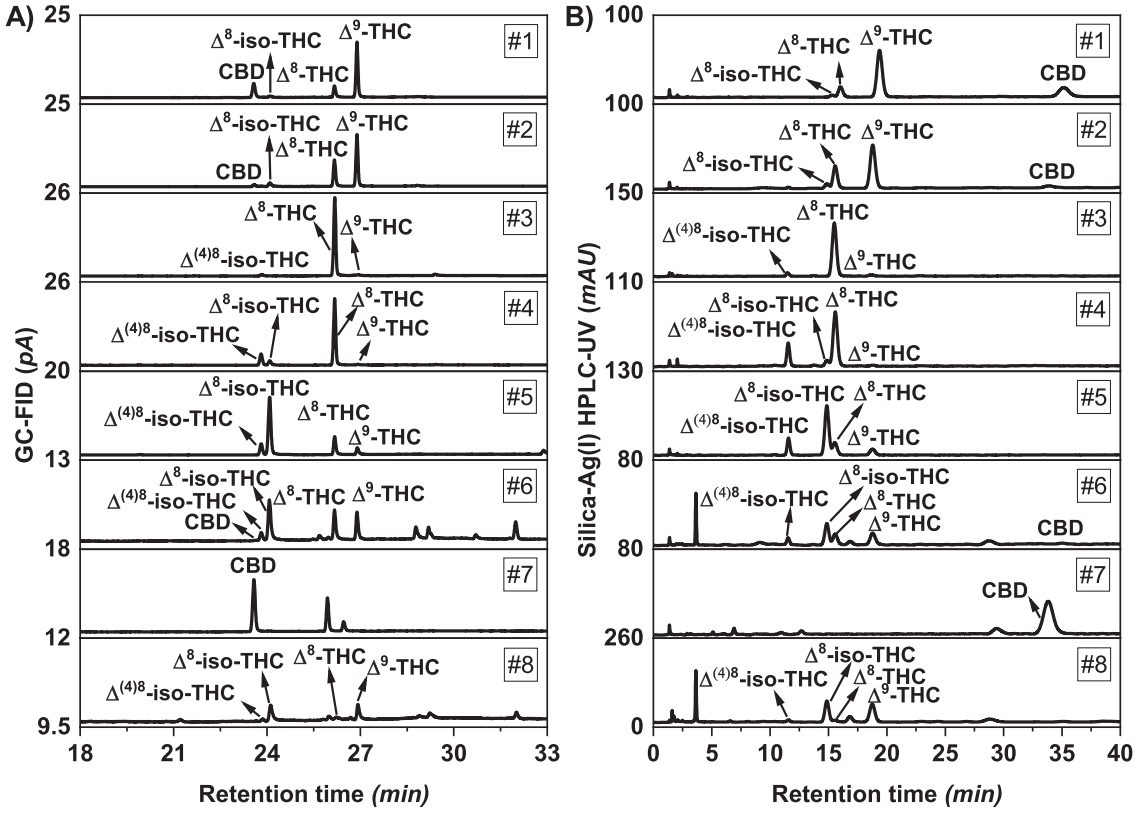


Fig. 4. Detection of Δ^8-THC, Δ^9-THC, CBD, Δ^8-iso-THC, and $\Delta^{(4)8}$-iso-THC in acid-treated CBD mixtures by (A) GC-FID and (B) silica-Ag(I) HPLC-DAD (215 nm).

confirming a molecular weight of 332 Da for the three compounds. All combined, the three compounds were expected to be hydrated forms of Δ^8-THC, Δ^9-THC or CBD. Finally, by performing 700 MHz NMR analysis (**SI, Figure S19**) and comparison with recently identified cannabinoids (Cheng, Xie, Chen, Wang, & Zhou, 2013; Radwan, Wanas, Gul, Ibrahim, & ElSohly, 2023), the compounds were assigned as follows: X1 = 9β-hydroxyhexahydrocannabinol; X2 = 9α-hydroxyhexahydrocannabinol; X3 = 8-hydroxy-isotetrahydrocannabinol. These compounds were observed in mixtures #3, 4, 6, and 8 by the GC-FID method, and in mixtures #1, 2, 3, 4, 5, 6, and 8 by the more sensitive RP-UHPLC-DAD method (**SI, Figure S20**). These findings suggest that these compounds, of which there is currently no toxicological knowledge, occur in many of these CBD reaction mixtures, which warrants further investigation.

In addition to these five THC isomers and three hydrated THC isomers, several minor peaks (especially in the last three samples) can be observed, reflecting the complexity of the product mixtures obtained by treating CBD with acids. There is little information regarding the toxicity and pharmacokinetics of Δ^8-THC, and nothing about Δ^8-iso-THC, $\Delta^{(4)8}$-iso-THC. Since these eight samples were converted from CBD by both lab-based protocols and kitchen-based protocols, they reflect, to some extent, the uncertainties about the composition of existing Δ^8-THC products in the market, and point to potential risks for Δ^8-THC consumers.

3.6. Quantification of Δ^8-THC, Δ^9-THC, CBD, Δ^8-iso-THC, and $\Delta^{(4)8}$-iso-THC by RP-UHPLC-DAD, GC-FID, and silica-Ag(I) HPLC-DAD

As mentioned earlier, there are many issues with commercially available Δ^8-THC products such as incorrectly labeled Δ^8-THC content, and high levels of Δ^9-THC. As such, it is important to understand how much of the different cannabinoids are present in acid-treated CBD mixtures for both investigative and legislative purposes. Therefore, further quantitative analysis of three major cannabinoids (Δ^8-THC, Δ^9-THC, and CBD) in the acid-treated CBD mixtures was conducted.

External calibration curves based on RP-UHPLC-DAD, silica-Ag(I) HPLC-DAD, and GC-FID were constructed (**SI, Figure S21**). LODs were calculated and solutions with cannabinoid concentrations close to the calculated LODs were analyzed (**SI**, **Figure S22**) to confirm the calculated LOD. The GC-FID method and the silica-Ag(I) HPLC-DAD method have the same linear range (5.00–333 μg·mL^{-1}) **(SI, Table S3)**. GC-FID has an LOD of 0.30 μg·mL^{-1} for both Δ^8-THC and Δ^9-THC, and 1.3 μg·mL^{-1} for CBD and the silica-Ag(I) HPLC-DAD method has LODs of 0.50 μg·mL^{-1} for Δ^8-THC, 0.40 μg·mL^{-1} for Δ^9-THC and 0.90 μg·mL^{-1} for CBD. As a comparison, the RP-UHPLC-DAD method could achieve a wider linear range of 0.500–333 μg·mL^{-1} with the lowest LOD: 0.020 μg·mL^{-1} for all the three major cannabinoids. Even though the developed RP-UHPLC-DAD method cannot be used for analyzing the samples prepared in this study, it is a more sensitive method for quantifying Δ^8-THC, Δ^9-THC, and CBD, when there are no interferences of other THC isomers.

Next, all eight acid-treated CBD mixtures were analyzed by the GC-FID method and the silica-Ag(I) HPLC-DAD method. The results are summarized in Table 1. The plot of the quantitative HPLC results against the GC results of the three cannabinoids show good correspondence between the two methods ($R^2 > 0.99$) (**SI, Figure S23**). Still, there is a large discrepancy for the Δ^8-THC percentage in sample #6 between the two methods (8.1 % versus 6.5 %) due to the insufficient separation of the Δ^8-iso-THC and Δ^8-THC peaks by the silica-Ag(I) HPLC-DAD method, resulting in an overestimation of Δ^8-THC. Also, for the quantification of CBD, a deviation (6.9 % versus 8.6 %) is observed in sample #2, which is probably caused by the combined effects of a low CBD concentration in the sample and the relatively high detection limit for CBD by the GC-FID method (1.3 μg·mL^{-1}), resulting an underestimation of CBD. Δ^9-THC shows excellent correspondence between the various methods in all eight mixtures. This is especially important due to the strict legal limit for Δ^9-THC. From the perspective of cannabinoid concentrations in the samples, Δ^8-THC percentages vary from 0 to 89 %. Despite Δ^8-THC being advertised as a "legal high" product, there is little knowledge about its toxicity and pharmacokinetics. Thus, consumers are inhaling or ingesting various amounts of Δ^8-THC without knowing the consequences. On the other hand, with the exception of sample #7, all other samples contain considerable amounts of Δ^9-THC (1.1–49 %, w/w %), which are obviously all above the legal limit of 0.3 % (Leafreport, 2022). With respect to CBD, half of the mixtures contain residual CBD, with levels ranging from 3.5 % to 53 %. In the other mixtures, the complete reaction of CBD under acid and heat is obvious, even when using the mild reactions condition of procedure #8. It would be of interest to investigate to which extent such conversion might occur during the consumption of CBD from e-liquid, which is also heated during vaping (Czégény et al., 2021).

Table 1
Percentage (w/w%, after solvent evaporation) of Δ^8-THC, Δ^9-THC and CBD in acid-treated CBD mixtures analyzed by silica-Ag(I) HPLC-DAD (215 nm) and GC-FID.

Sample	Δ^8-THC (w/w%)		Δ^9-THC (w/w%)		CBD (w/w%)	
	GC-FID	Ag-LC-DAD	GC-FID	Ag-LC-DAD	GC-FID	Ag-LC-DAD
#1	11 (2.6 %)	9.4 (2.1 %)	49 (0.3 %)	49 (2.6 %)	26 (2.0 %)	28 (2.4 %)
#2	26 (4.0 %)	25 (3.9 %)	48 (0.1 %)	49 (3.0 %)	6.9 (0.4 %)	8.6 (5.2 %)
#3	78 (2.1 %)	89 (4.0 %)	1.6* (1.1 %)	1.5* (11 %)	ND	ND
#4	70 (1.8 %)	70 (4.6 %)	1.1* (5.6 %)	1.1** (7.0 %)	ND	ND
#5	14 (3.7 %)	14 (6.7 %)	6.3 (5.2 %)	5.9 (5.7 %)	ND	ND
#6	8.1 (0.5 %)	6.5 (14.4 %)	8.9 (1.2 %)	8.2 (6.3 %)	4.2 (0.2 %)	3.5 (6.9 %)
#7	ND	ND	ND	ND	48 (0.7 %)	53 (12 %)
#8	1.4 (1.1 %)	1.6* (4.6 %)	5.0 (2.5 %)	4.8* (3.6 %)	ND	ND

ND, non-detectable, i.e., <LOD. Values in brackets represent relative standard deviations (n = 3).
*Calculated based on 10 times concentrated sample; **calculated based on 50 times concentrated sample.

Apart from the three major cannabinoids, the existence of Δ^8-iso-THC and $\Delta^{(4)8}$-iso-THC in some acid-treated CBD mixtures was significant and thus they were semi-quantified by silica-Ag(I) HPLC-DAD and GC-FID methods using response factors (**SI**, **Protocol S2**, **Table S4**) (Cuadros-Rodríguez, Bagur-González, Sánchez-Viñas, González-Casado, & Gómez-Sáez, 2007). The majority of these acid-treated CBD mixtures contain Δ^8-iso-THC (six out of eight, with percentages of 1.9–55 %) and $\Delta^{(4)8}$-iso-THC (five out of eight, with percentages of 0.32–17 %). If such acid-treated CBD mixtures or infused samples are analyzed by commonly used reversed-phase chromatography-based methods, overestimation of Δ^8-THC content would occur due to the coelution of Δ^8-iso-THC and Δ^8-THC and the partial overlap of $\Delta^{(4)8}$-iso-THC and Δ^8-THC (Marzullo et al., 2020). Such lack of separation in the most commonly used chromatographic approach could potentially explain issues with incorrectly labeled Δ^8-THC content encountered in Δ^8-THC products.

3.7. Analysis of cannabinoid-infused gummies and evaluation of extraction recovery, matrix effects, accuracy, and precision

Previous experiments were focused on analyzing acid-treated CBD mixtures to understand which cannabinoids might be present after the chemistry, prior to infusion into edibles. While complex due to presence of isomers, as alluded to, the analysis is not complicated in terms of the matrix, as a fairly clean solution is obtained. To further explore the application potential of the developed methods, the composition of cannabinoid-infused edibles was assessed. An extraction and analysis protocol was developed for infused gummies, and extraction recovery, matrix effects, accuracy, and precision were evaluated.

Extraction recovery. Neutral cannabinoids are relatively non-polar and are well-soluble in e.g., ethanol, hexane, and MTBE (López-Olmos, García-Valverde, Hidalgo, Ferrerio-Vera, & Sánchez de Medina, 2022). Major components of gummies (gelatin, starch, sugars, flavors, and colorants), are well-soluble in water (Burey, Bhandari, Rutgers, Halley, & Torley, 2009). Therefore, a liquid–liquid extraction (LLE) with MTBE and water (1/1, v/v) was applied considering the higher polarity and lower toxicity of MTBE compared with hexane, as well as lower polarity than ethanol to exclude co-extracting of flavors and colorants (López-Olmos, García-Valverde, Hidalgo, Ferrerio-Vera, & Sánchez de Medina, 2022). Three normal gummies and two Δ^8-THC gummies were dissolved during ice-bath sonication for 20–40 min. After phase-separation, a colorless, clear, and transparent MTBE layer was obtained (**SI**, **Figure S24**). To evaluate the partitioning of cannabinoids between MTBE and water phases, CBD was first used as a representative cannabinoid and showed an extraction recovery of 101 ± 0.3 % with a total volume of extraction solvent of 2.00 mL, as determined by GC-FID. Afterwards, the five cannabinoid mixtures were also tested in the same way with a smaller volume of extraction solvent (200 μL), and the extraction recovery for different cannabinoid varied from 100 ± 17 % to 101 ± 16 % (**SI**, **Table S5**). Despite the large relative standard deviation (RSD 15–17 %), which can be attributed to the small extraction volume as it was not observed during the CBD extraction with 2 mL, all tested cannabinoids show approximately 100 % extraction recovery.

Matrix effects. Next, matrix effects of the gummies on the two analytical methods were evaluated. Considering the unclear compositional information of commercial Δ^8-THC gummies, as well as the diversity of gummies in terms of composition, texture, and solubility (**SI**, **Table S6**), three varieties of normal gummies without cannabinoids were tested. For the GC-FID/MS method, the extractions of three normal gummies showed minor peaks, which were all distinct from peaks of the tested cannabinoids (**SI**, **Figure S25**). Matrix effects for the five cannabinoids varied from −2.6 % to 14 % (**SI**, **Table S7**). For the silica-Ag(I) HPLC-DAD method, extractions of all three normal gummies exhibited a clean background (**SI**, **Figure S26**), possibly due to the reconstitution step by a different solvent (mobile phase, 1 % ACN in hexane (v/v%)) with different polarity from the extraction solvent MTBE, which provided further selectivity. At the same time, the additional blow-drying and reconstitution potentially introduced experimental errors, especially due to the small sample volume (100 μL). Therefore, even though theoretically the silica-Ag(I) HPLC-DAD method should have limited matrix effects with the three tested normal gummies, deviations of −9.6 % to 11 % were observed for different cannabinoids (**SI**, **Table S7**), although this can likely be improved when larger sample volumes are used. In summary, the two developed methods showed acceptable matrix effects (Scientific Working Group for Forensic Toxicology (SWGTOX) standard practices for method validation in forensic toxicology, 2013) with signal suppression < 10 % and signal enhancement < 14 % for five cannabinoids in three tested normal gummies.

Accuracy and precision. Furthermore, accuracy and precision were evaluated by spiking standards into normal gummy #2, which was most similar in texture and solubility to the tested commercial Δ^8-THC gummies. Five-point calibration curves of Δ^8-THC, Δ^9-THC, CBD, $\Delta^{(4)8}$-iso-THC, and Δ^8-iso-THC were established (**SI**, **Figure S27**) and accuracy and precision at three different spiking weight percentages 0.02 %, 0.1 %, and 0.2 % (low, medium, and high) were assessed. As shown in **Table S8** (**SI**), both methods showed acceptable accuracy (bias from −12 % to 19 %) (Scientific Working Group for Forensic Toxicology (SWGTOX) standard practices for method validation in forensic toxicology, 2013) and good precision (RSD 0.26–6.1 %) for all investigated cannabinoids at different spiking weight percentages. The silica-Ag(I) HPLC-DAD method exhibited better accuracy than the GC-FID/MS method in general (bias −12–8.6 % *vs* 3.0–19 %).

Analysis of Δ^8-THC gummies. Finally, two Δ^8-THC gummies were analyzed by the developed methods (**SI**, **Figure S28**) and weight percentages of five isomeric cannabinoids were compared with the declared information in the certificate of analysis (Table 2). The results obtained by the two developed methods nicely corresponded. For sample C#1, Δ^8-THC (0.63 % by silica-Ag(I) HPLC-DAD and 0.68 % by GC-FID in this study *vs* 0.65 % as declared), Δ^9-THC (0.015 % by silica-Ag(I) HPLC-DAD and 0.014 % by GC-FID in this study *vs* < 0.050 % as declared) and CBD (not detected in all cases) results matched well with the declared contents. However, for sample #2, a lower content of Δ^8-THC (2.8 % by silica-Ag(I) HPLC-DAD and 2.9 % by GC-FID in this study *vs* 3.1 % as declared) and higher content of Δ^9-THC (0.099 % by silica-Ag(I) HPLC-DAD and 0.086 % by GC-FID in this study *vs* < 0.074 % as declared) were detected by the developed methods in this study, whereas the declared CBD content matches with our results (not detected in this study *vs* < 0.0010 % as declared). Moreover, Δ^8-iso-THC and $\Delta^{(4)8}$-iso-THC, about which no information is provided in the certificate of analysis, were found in both tested samples. C#1 only contained a small amount of $\Delta^{(4)8}$-iso-THC (0.031 % by silica-Ag(I) HPLC-DAD and 0.033 % by GC-FID) and no Δ^8-iso-THC but C#2 contained higher amounts of $\Delta^{(4)8}$-iso-THC (0.13 % by silica-Ag(I) HPLC-DAD and 0.12 % by GC-FID) and Δ^8-iso-THC(0.0072 % by silica-Ag(I) HPLC-DAD and 0.0069 % by GC-FID). On the one hand, the presence of $\Delta^{(4)8}$-iso-THC and Δ^8-iso-THC could partly explain the overestimation of Δ^8-THC in the certificate of analysis (reversed-phase HPLC-UV method was used). Crucially, such analysis highlights the necessity of methods to distinguish between THC isomers, such as demonstrated in this work. Moreover, it demonstrates the importance of providing qualitative and quantitative information to consumers, especially due to the fact that intoxication cases induced by consuming Δ^8-THC gummies have already been reported (Akpunonu et al., 2021).

4. Conclusions

Three methods based on RP-UHPLC-DAD/HRMS, silica-Ag(I) HPLC-DAD/MS, and GC-FID/MS have been developed for the analysis of Δ^8-THC related products with special emphasis on THC isomers. RP-UHPLC-DAD/MS provides sufficient separation of Δ^8-THC, Δ^9-THC, and CBD and is the most sensitive method. However, this method cannot resolve Δ^8-THC from the interferences $\Delta^{(4)8}$-iso-THC and Δ^8-iso-THC in "Δ^8-THC" samples and gives identical MS/HRMS spectra for all five isomers. GC-FID/MS separated the five isomers with a two-step gradient temperature program in 33 min, and all isomers showed different EI spectra that thus allows identification. With an analysis time of 40 min, silica-Ag(I) HPLC-DAD/MS also achieved sufficient separation of the five isomers. Importantly, unique Ag(I) adduct fragmentation mass spectra for Δ^8-THC, Δ^9-THC, CBD, Δ^8-iso-THC, and $\Delta^{(4)8}$-iso-THC were obtained, which cannot be achieved by HRMS with protonated adducts. The GC-FID/MS method is faster and more robust in the analysis of cannabinoid mixtures, such as "Δ^8-THC" products, while silica-Ag(I) HPLC-DAD/MS provides an orthogonal method and distinct ESI spectra towards THC isomers. By analyzing eight of such "Δ^8-THC" samples from lab-based and kitchen-based syntheses starting from CBD, potential issues are revealed with regard to Δ^8-THC edibles and other products in the market, including various amounts of Δ^8-THC (0–89 %), illegal levels of Δ^9-THC of up to nearly 50 %, and the presence of other known THC isomers and hydrated THC isomers. Subsequently,

Table 2
Percentage (w/w%) of Δ^8-THC, Δ^9-THC, Δ^8-isoTHC, $\Delta^{(4)8}$-iso-THC in Δ^8-THC gummies analyzed by silica-Ag(I) HPLC-DAD (215 nm) and GC-FID and compared with claimed information.

Sample	Δ^8-THC (w/w%)			Δ^9-THC (w/w%)			CBD (w/w%)			Δ^8-isoTHC (w/w%)			$\Delta^{(4)8}$-isoTHC (w/w%)		
	GC-FID	Ag-LC-DAD	Declared*	GC-FID	Ag-LC-DAD	Declared*	GC-FID	Ag-LC-DAD	Declared*	GC-FID	Ag-LC-DAD	Declared*	GC-FID	Ag-LC-DAD	Declared*
C#1	0.68 (1.8 %)	0.63 (2.6 %)	0.65	0.014 (4.4 %)	0.015 (4.7 %)	<0.050	ND	ND	ND	ND	ND	No information	0.033 (2.3 %)	0.031 (2.6 %)	No information
C#2	2.9 (1.3 %)	2.8 (1.4 %)	3.1	0.086 (3.4 %)	0.099 (3.1 %)	0.074	ND	ND	<0.0010	0.0069 (2.6 %)	0.0072 (3.7 %)	No information	0.12 (1.9 %)	0.13 (6.8 %)	No information

*Information provided by the certificate of analysis. ND, non-detectable, i.e., <LOD.

commercially available normal gummies were analyzed to evaluate matrix effects, accuracy, and precision of the developed methods in the situation of complex food analysis. Our silica-Ag(I) HPLC-DAD/MS method shows limited matrix effects and a higher accuracy for all five cannabinoid isomers compared to the GC-FID/MS method. Finally, two commercial Δ^8-THC gummies were analyzed, and both Δ^8-iso-THC and $\Delta^{(4)8}$-iso-THC were observed. The existence of such, typically unspecified, THC isomers tends to result in overestimated Δ^8-THC content, as evidenced by the certificate of analysis in which a reversed-phase HPLC-UV method (with limited resolution towards Δ^8-THC, Δ^8-iso-THC and $\Delta^{(4)8}$-iso-THC) was used. On the other hand, underestimation of Δ^9-THC in the certificate of analysis was found for one of the two gummies. In short, the developed silica-Ag(I) HPLC-DAD/MS method and GC-FID/MS method showed good applicability in the analysis of cannabinoid products. From a food science perspective, attention should be paid to cannabinoid isomers when analyzing cannabinoid products, considering their common existence, varied health effects and legal status. Moreover, analytical methods that can distinguish and quantify multiple isomers can provide consumers and regulation agencies with more accurate and complete information. From the analysis of real food products in this work, it is clear that such analytical methods are needed to provide a more complete overview of their cannabinoid profile. Moreover, such methods could lay the foundation for future investigations towards the toxicity of (un)known compounds, as well as for the identification of unknown compounds in cannabinoid products.

CRediT authorship contribution statement

Si Huang: Writing – original draft, Validation, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Teris A. van Beek:** Writing – review & editing, Validation, Supervision, Methodology, Conceptualization. **Frank W. Claassen:** Methodology. **Hans-Gerd Janssen:** Writing – review & editing, Methodology. **Ming Ma:** Methodology, Funding acquisition. **Bo Chen:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Han Zuilhof:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **G.IJ. Salentijn:** .

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.

Data availability

Data will be made available on request.

Acknowledgements

The authors thank prof. Daniele Passarella group from the Dipartimento di Chimica, Università degli Studi di Milano, Milano, Italy for kindly providing Δ^8-iso-THC and $\Delta^{(4)8}$-iso-THC, dr. Muthusamy Subramaniam for helpful discussions, and Hans Beijleveld for technical support. We acknowledge support from the National Natural Science Foundation of China (22276050, 22276049), the China Scholarship Council 2020 International Cooperation Training Program for Innovative Talents, and the Hunan Province 100 experts project.

Appendix A. Supplementary data

Δ^8-THC and Δ^9-THC percentages in commercial products; ^{1}H-NMR, reversed-phase UHPLC-DAD/MS analysis of Δ^8-THC, Δ^9-THC, CBD, Δ^8-iso-THC and $\Delta^{(4)8}$-iso-THC; strong cation exchange Ag(I) HPLC-DAD analysis of Δ^8-THC, Δ^9-THC and CBD and quantum-chemical computations of Δ^8-THC and Δ^9-THC with Ag(I); setup of silica-Ag(I) HPLC-

DAD/MS; synthesis of acid-treated CBD mixtures and analysis of these samples by RP-UHPLC-DAD/MS, GC-FID/MS, and silica-Ag(I) HPLC-DAD/MS; analysis of 9β-hydroxyhexahydrocannabinol, 9α-hydroxyhexahydrocannabinol, and 8-hydroxy-iso-tetrahydrocannabinol by RP-UHPLC-DAD/MS, GC-FID/MS, silica-Ag(I) HPLC-DAD/MS, and NMR; calibration curves of Δ^8-THC, Δ^9-THC, and CBD; content of Δ^8-THC, Δ^9-THC, and CBD in acid-treated CBD mixtures analyzed by silica-Ag(I) HPLC-DAD and GC-FID; analysis of gummies and evaluation of extraction recovery, matrix effects, accuracy, and precision of silica-Ag(I) HPLC-DAD and GC-FID. Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2023.138187.

References

Adlof, R. O. (1997). Normal-phase separation effects with lipids on a silver ion high-performance liquid chromatography column. *Journal of Chromatography A, 764*(2), 337–340.

AOAC. (2017). AOAC SMPR® 2017.019 Standard Method Performance Requirements (SMPRs®) for Quantitation of Cannabinoids in Edible Chocolate. Retrieved from: https://www.aoac.org/wp-content/uploads/2020/11/SMPR202017_019.pdf Accessed April 21, 2023.

Abrahamov, A., Abrahamov, A., & Mechoulam, R. (1995). An efficient new cannabinoid antiemetic in pediatric oncology. *Life Sciences, 56*(23), 2097–2102.

Akpunonu, P., Baum, R. A., Reckers, A., Davidson, B., Ellison, R., Riley, M., … Gerona, R. (2021). Sedation and acute encephalopathy in a pediatric patient following ingestion of delta-8-tetrahydrocannabinol gummies. *The American Journal of Case Reports, 22*, e933481–e933488.

Burey, P., Bhandari, B., Rutgers, R., Halley, P., & Torley, P. (2009). Confectionery gels: A review on formulation, rheological and structural aspects. *International Journal of Food Properties, 12*(1), 176–210.

Casajuana Kögel, C., López-Pelayo, H., Balcells-Olivero, M. M., Colom, J., & Gual, A. (2018). Psychoactive constituents of cannabis and their clinical implications: A systematic review. *Adicciones, 30*(2), 140–151.

Chan-Hosokawa, A., Nguyen, L., Lattanzio, N., & Adams, W. R. (2022). Emergence of Delta-8 Tetrahydrocannabinol in DUID Investigation Casework: Method Development, Validation and Application. *Journal of Analytical Toxicology, 46*(1), 1–9.

Christinat, N., Savoy, M.-C., & Mottier, P. (2020). Development, validation and application of a LC-MS/MS method for quantification of 15 cannabinoids in food. *Food Chemistry, 318*, Article 126469.

Ciolino, L. A., Ranieri, T. L., Brueggemeyer, J. L., Taylor, A. M., & Mohrhaus, A. S. (2021). EVALI vaping liquids part 1: GC-MS cannabinoids profiles and identification of unnatural THC isomers. *Frontiers in Chemistry, 9*, Article 746479.

Czégény, Z., Nagy, G., Babinszki, B., Bajtel, Á., Sebestyén, Z., Kiss, T., … Csupor, D. (2021). CBD, a precursor of THC in e-cigarettes. *Scientific Reports, 11*(1), 8951.

AOAC. (2022). AOAC SMPR 2022.001; Quantitation of cannabinoids in beverages. Retrieved from: https://www.aoac.org/wp-content/uploads/2022/11/SMPR-2022_001.pdf Accessed December 20, 2023.

Congress, U. S. (2018). Agriculture Improvement Act of 2018. Retrieved from: https://www.congress.gov/115/plaws/publ334/PLAW-115publ334.pdf Accessed April 21, 2023.

Council, U. S. C. (2021). The unregulated distribution and sale of consumer products marketed as delta-8 THC. Retrieved from: https://irp.cdn-website.com/6531d7ca/files/uploaded/USCC%20Delta-8%20Kit.pdf Accessed April 21, 2023.

Citti, C., Linciano, P., Forni, F., Vandelli, M. A., Gigli, G., Laganà, A., & Cannazza, G. (2019). Analysis of impurities of cannabidiol from hemp. Isolation, characterization and synthesis of cannabidibutol, the novel cannabidiol butyl analog. *Journal of Pharmaceutical and Biomedical Analysis, 175*, Article 112752.

Cheng, L.-J., Xie, J.-H., Chen, Y., Wang, L.-X., & Zhou, Q.-L. (2013). Enantioselective Total Synthesis of (−)-Δ8-THC and (−)-Δ9-THC via Catalytic Asymmetric Hydrogenation and SNAr Cyclization. *Organic Letters, 15*(4), 764–767.

Cuadros-Rodríguez, L., Bagur-González, M. G., Sánchez-Viñas, M., González-Casado, A., & Gómez-Sáez, A. M. (2007). Principles of analytical calibration/quantification for the separation sciences. *Journal of Chromatography A, 1158*(1), 33–46.

Duffy, B. C., Li, L., Lu, S., Dittmar, M. A., Delaney-Baldwin, E., Durocher, L. A., & Spink, D. C. (2022). Chemotyping of Δ8-THC-Containing e-Liquids Analyzed during the 2019–2020 New York State EVALI Investigation. *Journal of Analytical Toxicology, 46*(7), 743–749.

Gaoni, Y., & Mechoulam, R. (1968). The ISO-Tetrahydrocannabinols. *Israel Journal of Chemistry, 6*(5), 679–690.

Gertsch, J. (2018). Analytical and Pharmacological Challenges in Cannabis Research. *Planta Med, 84*(04), 213.

Golombek, P., Müller, M., Barthlott, I., Sproll, C., & Lachenmeier, D. W. (2020). Conversion of Cannabidiol (CBD) into Psychotropic Cannabinoids Including Tetrahydrocannabinol (THC): A Controversy in the Scientific Literature. *Toxics, 8*(2), 41.

Gülck, T., & Møller, B. L. (2020). Phytocannabinoids: Origins and biosynthesis. *Trends in plant science, 25*(10), 985–1004.

Gleb, O. (2022). New leafreport research reveals more than 50% of delta-8 THC hemp-derived products tested had illegal levels of delta-9 THC. Retrieved from: https://www.leafreport.com/education/delta-8-thc-products-market-report-11339 Accessed April 21, 2023.

Helander, A., Johansson, M., Andersson, A., & Villén, T. (2022). Analytical and medico-legal problems linked to the presence of delta-8-tetrahydrocannabinol (delta-8-THC): Results from urine drug testing in Sweden. *Drug testing and analysis, 14*(2), 371–376.

Huang, S., Claassen, F. W., van Beek, T. A., Chen, B., Zeng, J., Zuilhof, H., & Salentijn, G. I. (2021). Rapid distinction and semiquantitative analysis of THC and CBD by silver-impregnated paper spray mass spectrometry. *Analytical chemistry, 93*(8), 3794–3802.

Huang, S., Qiu, R., Fang, Z., Min, K., Van Beek, T. A., Ma, M., Chen, B., Zuilhof, H., & Salentijn, G. I. (2022). Semiquantitative Screening of THC Analogues by Silica Gel TLC with an Ag (I) Retention Zone and Chromogenic Smartphone Detection. *Analytical Chemistry, 94*(40), 13710–13718.

Kaneti, J., de Smet, L. C., Boom, R., Zuilhof, H., & Sudhölter, E. J. (2002). Computational probes into the basis of silver ion chromatography. II. Silver (I)− olefin complexes. *The Journal of Physical Chemistry A*, 106(46), 11197-11204.

Kiselak, T. D., Koerber, R., & Verbeck, G. F. (2020). Synthetic route sourcing of illicit at home cannabidiol (CBD) isomerization to psychoactive cannabinoids using ion mobility-coupled-LC–MS/MS. *Forensic science international, 308*, Article 110173.

La Maida, N., Di Giorgi, A., Pichini, S., Busardò, F. P., & Huestis, M. A. (2022). Recent challenges and trends in forensic analysis: Δ^9-THC isomers pharmacology, toxicology and analysis. *Journal of Pharmaceutical and Biomedical Analysis, 220*, Article 114987.

Lachenmeier, D. W., Habel, S., Fischer, B., Herbi, F., Zerbe, Y., Bock, V., … Sproll, C. (2022). Are adverse effects of cannabidiol (CBD) products caused by tetrahydrocannabinol (THC) contamination? *F1000Research*, 8, 1394.

Leas, E. C., Nobles, A. L., Shi, Y., & Hendrickson, E. (2022). Public interest in Δ8-Tetrahydrocannabinol (delta-8-THC) increased in US states that restricted Δ9-Tetrahydrocannabinol (delta-9-THC) use. *International Journal of Drug Policy, 101*, Article 103557.

Lin, L., Amaratunga, P., Reed, J., Huang, P., Lemberg, B. L., & Lemberg, D. (2022). Quantitation of Δ^8-THC, Δ^9-THC, cannabidiol and 10 other cannabinoids and metabolites in oral fluid by HPLC–MS-MS. *Journal of Analytical Toxicology, 46*(1), 76–88.

LoParco, C. R., Rossheim, M. E., Walters, S. T., Zhou, Z., Olsson, S., & Sussman, S. Y. (2023). Delta-8 tetrahydrocannabinol: A scoping review and commentary. *Addiction*, 1–18.

López-Olmos, C., García-Valverde, M. T., Hidalgo, J., Ferrerio-Vera, C., & Sánchez de Medina, V. (2022). Comprehensive comparison of industrial cannabinoid extraction techniques: Evaluation of the most relevant patents and studies at pilot scale. *Frontiers in Natural Products, 1*, 1043147.

Marzullo, P., Foschi, F., Coppini, D. A., Fanchini, F., Magnani, L., Rusconi, S., … Passarella, D. (2020). Cannabidiol as the Substrate in Acid-Catalyzed Intramolecular Cyclization. *Journal of Natural Products, 83*(10), 2894–2901.

Meehan-Atrash, J., & Rahman, I. (2021). Novel Δ^8-tetrahydrocannabinol vaporizers contain unlabeled adulterants, unintended byproducts of chemical synthesis, and heavy metals. *Chemical research in toxicology, 35*(1), 73–76.

Peng, H., & Shahidi, F. (2021). Cannabis and Cannabis Edibles: A Review. *Journal of Agricultural and Food Chemistry, 69*(6), 1751–1774.

Pisciottano, I. D. M., Guadagnuolo, G., Soprano, V., Esposito, M., & Gallo, P. (2021). A survey of Δ^9-THC and relevant cannabinoids in products from the Italian market: A study by LC–MS/MS of food, beverages and feed. *Food Chemistry, 346*, Article 128898.

Reber, J. D., Karschner, E. L., Seither, J. Z., Knittel, J. L., Dozier, K. V., & Walterscheid, J. P. (2022). An Enhanced LC–MS-MS Technique for Distinguishing Δ^8- and Δ^9-Tetrahydrocannabinol Isomers in Blood and Urine Specimens. *Journal of Analytical Toxicology, 46*(4), 343–349.

Radwan, M. M., Wanas, A. S., Gul, W., Ibrahim, E. A., & ElSohly, M. A. (2023). Isolation and Characterization of Impurities in Commercially Marketed Δ8-THC Products. *Journal of Natural Products, 86*(4), 822–829.

Shrivastava, A., & Gupta, V. B. (2011). Methods for the determination of limit of detection and limit of quantitation of the analytical methods. *Chronicles of Young Scientists, 2*(1), 21–25.

Song, L., Meyer, G., Adejumo, E., Jovanovich, E., LeBlanc, L., & Provis, J. (2023). Potency testing of up to sixteen cannabinoids in hemp-infused edibles using liquid chromatography diode array detector with optional confirmation of identity by electrospray ionization time-of-flight mass spectrometry. *Food Chemistry, 417*, Article 135819.

Scientific Working Group for Forensic Toxicology (SWGTOX) standard practices for method validation in forensic toxicology. (2013). *Journal of Analytical Toxicology*, 37(7), 452-474.

Tsujikawa, K., Okada, Y., Segawa, H., Yamamuro, T., Kuwayama, K., Kanamori, T., & Iwata, Y. T. (2022). Thermal decomposition of CBD to Δ^9-THC during GC-MS analysis: A potential cause of Δ^9-THC misidentification. *Forensic science international, 337*, Article 111366.

Tureček, F., & Hanuš, V. (1984). Retro-Diels-Alder reaction in mass spectrometry. *Mass Spectrometry Reviews, 3*(1), 85–152.

van Beek, T. A., & Subrtova, D. (1995). Factors involved in the high pressure liquid chromatographic separation of alkenes by means of argentation chromatography on ion exchangers: Overview of theory and new practical developments. *Phytochemical Analysis, 6*(1), 1–19.

Villela, A., Van der Klift, E. J., Mattheussens, E. S., Derksen, G. C., Zuilhof, H., & Van Beek, T. A. (2011). Fast chromatographic separation for the quantitation of the main flavone dyes in Reseda luteola (weld). *Journal of Chromatography A, 1218*(47), 8544–8550.

Wang, Y., Ma, L., Zhang, M., Chen, M., Li, P., He, C., … Wan, J.-B. (2019). A simple method for peak alignment using relative retention time related to an inherent peak

in liquid chromatography-mass spectrometry-based metabolomics. *Journal of chromatographic science, 57*(1), 9–16.

Zhang, J.-T., Wang, H.-Y., Zhu, W., Cai, T.-T., & Guo, Y.-L. (2014). Solvent-assisted electrospray ionization for direct analysis of various compounds (complex) from low/nonpolar solvents and eluents. *Analytical chemistry, 86*(18), 8937–8942.

Zhang, X., Geng, Z., & Wang, Y. (2011). Density functional theory study of the mechanism of the acid-catalyzed decarboxylation of pyrrole-2-carboxylic acid and mesitoic acid. *Science China Chemistry, 54*(5), 762–768.