

Development and Validation of a GC-FID Method for the Quantitation of Δ^8 -Tetrahydrocannabinol and Impurities Found in Synthetic Δ^8 -Tetrahydrocannabinol and Vaping Products

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ABSTRACT

Concerns about health hazards associated with the consumption of *trans*-delta-8-tetrahydrocannabinol products were highlighted in public health advisories from the U.S. Food and Drug Administration and U.S. Centers for Disease Control

and Prevention. Simple and rapid quantitative methods to determine *trans*-delta-8-tetrahydrocannabinol impurities are vital to analyze such products. In this study, a gas chromatography-flame ionization detection method was developed and validated for the determination of delta-8-tetrahydrocannabinol and some of its impurities (recently published) found in synthesized *trans*-delta-8-tetrahydrocannabinol raw material and included olivetol, cannabicitran, Δ^8 -*cis*-*iso*-tetrahydrocannabinol, Δ^4 -*iso*-tetrahydrocannabinol, *iso*-tetrahydrocannabinol, Δ^4 -*iso*-tetrahydrocannabinol, Δ^8 -*iso*-tetrahydrocannabinol, 4,8-*epoxy*-*iso*-tetrahydrocannabinol, *trans*- Δ^9 -tetrahydrocannabinol, 8-hydroxy-*iso*-THC, 9 α -hydroxyhexahydrocannabinol, and 9 β -hydroxyhexahydrocannabinol. Validation of the method was assessed according to the International Council for Harmonization guidelines and confirmed linearity with $R^2 \geq 0.99$ for all the target analytes. The limit of detection and limit of quantitation were 1.5 and 5 $\mu\text{g/mL}$, respectively, except for olivetol, which had a limit of detection of 3 $\mu\text{g/mL}$ and a limit of quantitation of 10 $\mu\text{g/mL}$. Method precision was calculated as % relative standard deviation and the values were less than 8.4 and 9.9% for the intraday precision and inter-day precision, respectively. The accuracy ranged from 85 to 118%. The method was then applied to the analysis of 21 commercially marketed vaping products claiming to contain delta-8-tetrahydrocannabinol. The products analyzed by this method have various levels of these impurities, with all products far exceeding the 0.3% of *trans*- Δ^9 -tetrahydrocannabinol limit for hemp under the Agriculture Improvement Act of 2018. The developed gas chromatography-flame ionization detection method can be an important tool for monitoring delta-8-tetrahydrocannabinol impurities in commercial products.

Introduction

The Δ^8 -THC found in minor quantities in the cannabis plant is among more than 125 compounds known as cannabinoids [1]. The term “cannabinoids” refers to terpenophenolic compounds with C-21 and C-22 skeletons that are exclusively found in *Cannabis sativa* L., family Cannabaceae [2, 3]. They are mainly biosynthesized in the glandular trichomes of the female cannabis plant [4, 5]. The structures of natural cannabinoids differ in the C-5 side

chain with the presence and/or absence of carboxylic and hydroxyl groups [4]. The additional cyclization or substitution of additional groups may produce different cannabinoid isomers. As with Δ^9 -THC, a cannabinoid that is primarily responsible for the psychoactive effects experienced after marijuana use, Δ^8 -THC is also naturally occurring (albeit at a much lower concentration) in the plant [6]. Understanding of the molecular targets, bioactivity, and analytical methods to characterize minor cannabinoids, such

ABBREVIATIONS

4,8-epoxy-iso-THC	4,8-epoxy-iso-tetrahydrocannabinol
8-OH-iso-THC	8-hydroxy-iso-tetrahydrocannabinol
9α-OH-HHC	9 α -hydroxyhexahydrocannabinol
9β-OH-HHC	9 β -hydroxyhexahydrocannabinol
AIA	Agriculture Improvement Act
CBD	cannabidiol
CBT	cannabicitran
CDC	U. S. Centers for Disease Control and Prevention
CSA	Controlled Substances Act
DEA	U. S. Drug Enforcement Administration
FDA	U. S. Food and Drug Administration
GC-FID	gas chromatography-flame ionization detection
HHC	hexahydrocannabinol
ICH	International Council for Harmonization of Technical Requirements
IND	investigational new drug
IS	internal standard
iso-THCBF	iso-tetrahydrocannabifuran
LOD	limit of detection
LOQ	limit of quantitation
NMT	not more than
QC	quality control
RSD	relative standard deviation
S/N	signal-to-noise
USP	United States Pharmacopeia
$\Delta^{4,8}$-iso-THC	$\Delta^{4,8}$ -iso-tetrahydrocannabinol
Δ^4-iso-THC	Δ^4 -iso-tetrahydrocannabinol
Δ^8-cis-iso-THC	Δ^8 -cis-iso-tetrahydrocannabinol
Δ^8-iso-THC	Δ^8 -iso-tetrahydrocannabinol
Δ^8-THC	<i>trans</i> - Δ^8 -tetrahydrocannabinol
Δ^9-THC	<i>trans</i> - Δ^9 -tetrahydrocannabinol

as Δ^8 -THC, and impurities from their synthesis is a developing area of science and presents a research gap [7].

The AIA (commonly known as the 2018 Farm Bill) defined the term “hemp” as “the plant *C. sativa*” and any part of that plant, including the seeds thereof and all derivatives, extracts, cannabinoids, isomers, acids, salts, and salts of isomers, whether growing or not, with a Δ^9 -tetrahydrocannabinol concentration of not more than 0.3% on a dry weight basis [8]. The AIA also amended the CSA to exclude hemp from the definition of “marijuana” and removed it from Schedule I status, thereby providing for regulated cultivation of hemp as an agricultural commodity. Following the descheduling of hemp, several U.S. states provided legal pathways for hemp-derived products to enter the market in their jurisdictions, and several hemp-based products are now being marketed as dietary or food ingredients [9]. While the AIA limits the Δ^9 -THC content of hemp to be NMT 0.3% on a dry weight basis, the content of other cannabinoids that are a part of the *C. sativa* L. plant, or their isomers, such as Δ^8 -THC, are not expressly limited

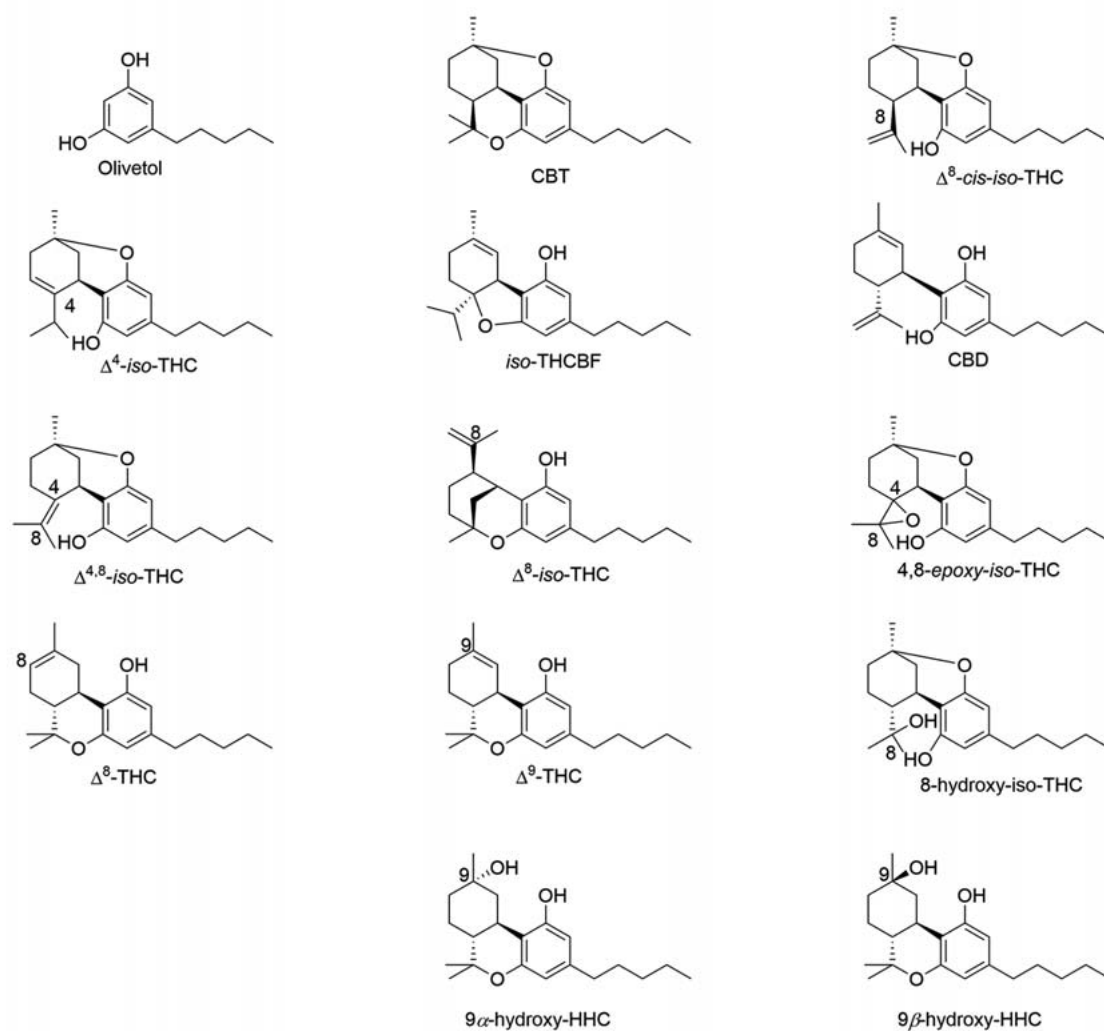
under the definition of hemp. The limit on Δ^9 -THC included in the AIA definition was established to minimize risks to public health and public safety from this psychoactive cannabinoid. However, since Δ^8 -THC is also a psychoactive substance, some groups are attempting to utilize a perceived loophole to bring intoxicating products to the market containing high levels of Δ^8 -THC. To address this, some U.S. state regulatory bodies have created limits and/or definitions for THC that include the Δ^8 isomer or otherwise have found means to limit the amount of Δ^8 -THC in commercial products.

Δ^8 -THC is estimated to be approximately 50 to 75% as psychoactive as Δ^9 -THC, but the plant naturally produces it only in very low levels [10]. Δ^9 -THC is being investigated for the treatment of a variety of medical conditions such as multiple sclerosis, glaucoma, and for the mitigation of chemotherapy side effects [11–13]. Synthetic Δ^9 -THC is approved by the FDA for use in the drug named dronabinol.

Recently, Δ^8 -THC products have flooded the United States market where many products grow in number and are in high demand [14, 15]. Δ^8 -THC in these products is claimed to be derived from hemp extracts and is incorporated into a variety of products such as vape cartridges, gummies, tinctures, and e-cigarettes. Many of these products are sold online, at gas stations or tobacco shops [16], and are labeled with unauthorized and false or misleading drug claims, such as claims that the products are intended for use as a cure for cancer, multiple sclerosis, chronic pain, nausea, and anxiety [17].

Limits for impurities are critical quality attributes because they have the potential to affect the safety of a product. As noted in the FDA guidance about quality considerations for cannabis and cannabis-derived compounds [18], a naturally occurring compound isolated from a botanical source would be expected to have a different impurity profile from the corresponding synthetically produced cannabis-related compound. Products labeled as and containing Δ^8 -THC have a high probability of being synthetically derived, because it is not generally thought to be economically feasible to extract naturally occurring Δ^8 -THC given the low concentrations present in cannabis and hemp [19]. Depending on the reaction conditions and purification processes, synthetic Δ^8 -THC may be associated with unknown impurities, different degradants, and synthetic cannabinoid analogs that are not naturally produced in cannabis/hemp plant material and for which there may be little or no safety or toxicity data [20–23]. A common way that Δ^8 -THC is being obtained is through synthetic or semi-synthetic conversion from hemp-derived CBD. This process normally involves the use of strong acids and catalysts, which tend to be harsh reaction conditions conducive to the formation of other reaction by-products and impurities [24]. Because the methods used to convert CBD to Δ^8 -THC are not specifically addressed in hemp laws, the legality of synthetic Δ^8 -THC stays unclear. This raises safety and product quality concerns for consumers given the unknown and untested nature of Δ^8 -THC, other synthetic analogs, and any other impurities present [24–26].

U.S. health officials are warning about the potential dangers of Δ^8 -THC following hospitalizations tied to the substance. In September 2021, both CDC and FDA alerted consumers to public health concerns from a recent rise in the availability of products



► **Fig. 1** Chemical structures of the identified compounds.

containing Δ^8 -THC, as well as several reports of adverse effects from the products [27]. The DEA, in August 2020, warned that it is in violation of federal law to use any process that produces Δ^9 -THC as a by-product, at any point [15].

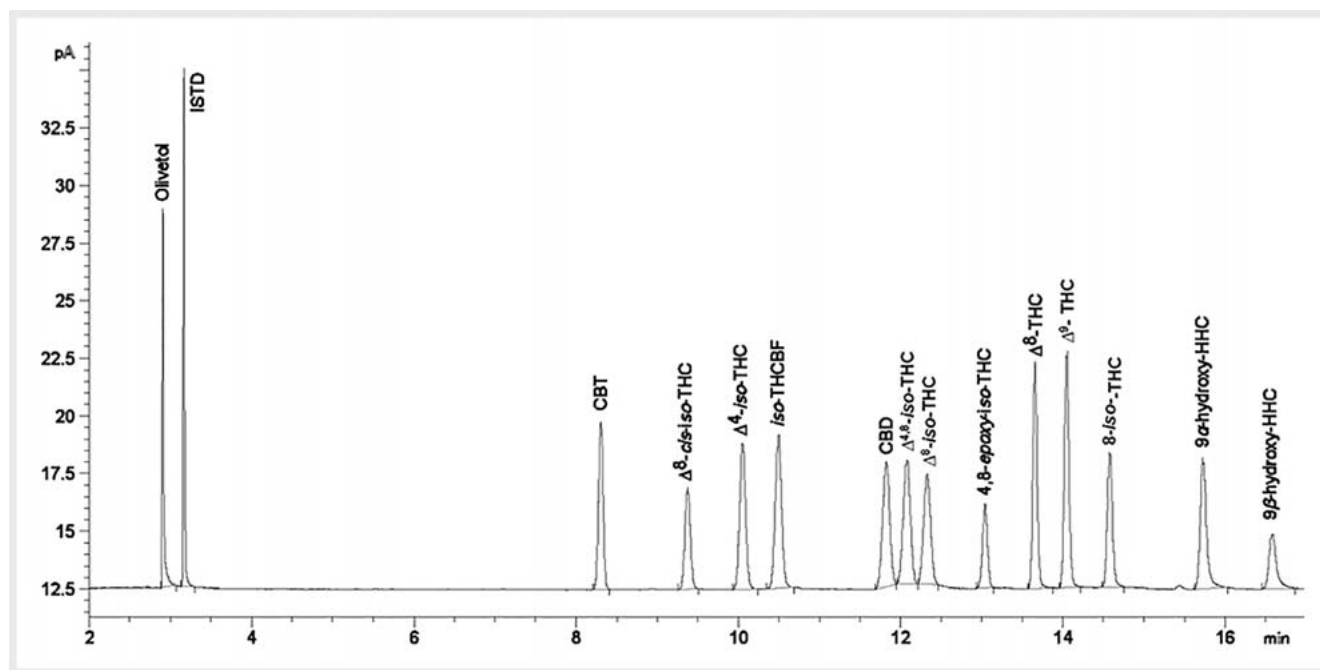
Recently, the FDA issued warning letters for marketed products containing Δ^8 -THC, as these Δ^8 -THC-containing products have not been evaluated or approved. In spite of the safety issues raised by the FDA and the warning letters issued by the agency on the sale of these products, these products are still available online and in stores, putting public health at risk. In the last 2 years, the FDA reported many adverse effects, such as anxiety, vomiting, dizziness, loss of consciousness, tremors, and hallucination, associated with the consumption of Δ^8 -THC-containing products [28].

Several U.S. states are beginning to raise concern about synthetic impurities in Δ^8 -THC products that have not been studied to determine whether they are safe or toxic to humans [29]. In Colorado, USA, tetrahydrocannabinol isomers are not allowed in food, dietary supplements, or cosmetics if they are produced synthetically [29].

In a recent publication, some of the authors reported on the isolation and characterization of chemical impurities in commercial Δ^8 -THC-containing products [30]. In this article, we used these isolated impurities as reference standards for the method development and validation and for the qualitative and quantitative analysis of the marketed Δ^8 -THC products. The isolation process for these impurities will be scaled up to allow for ample materials to be used in toxicological and pharmacological evaluation of these compounds; it is planned that this work will be reported in future studies.

In November 2021, the USP Cannabis Expert Panel provided perspectives [19] to highlight the need for and the value of scientifically valid analytical methods as tools for regulators and manufacturers for testing these products, and to help ensure high-quality materials are used in preclinical and clinical studies, resulting in the increased reproducibility and consistent data from these studies.

This paper reports the analysis of multiple Δ^8 -THC impurities in the complex matrix of synthetic Δ^8 -THC and vaping products, and



► **Fig. 2** GC-FID representative chromatogram of the 14 cannabinoids at 100 µg/mL and the IS at 100 µg/mL.

the development of a GC-FID method for quantitative determination of these impurities. The proposed method was validated according to ICH guidelines [31], and applied to the analysis of 21 commercial Δ^8 -THC vaping products.

Results and Discussion

A GC-FID analytical method was developed and validated for the determination of the concentration of different impurities in a variety of Δ^8 -THC commercial samples. The method was optimized, with minimum sample preparation. The chemical structures of the compound analyzed are shown in ► **Fig. 1**.

The GC-FID method was optimized for reliable determination of Δ^8 -THC and its related impurities that coexist with Δ^8 -THC in commercial samples. After examination of the performance of several GC columns, a DB1-MS column was found to give the best separation under the temperature program adopted in the method. A GC-FID chromatogram for all the target analytes is shown in ► **Fig. 2**.

A simple linear relationship was obtained between the concentration and the area ratio of each compound to the IS. The R^2 obtained was higher than 0.999 for all the target compounds. The regression equations, retention times, and relative retention times for all the target analytes are shown in ► **Table 1**. The calibration curves of the 14 analytes are represented in ► **Figs. 3** and **4**.

Limit of detection and limit of quantitation

LOD was calculated based on the S/N ratio = 3.3 and it was found to be 1.5 µg/mL for all of the analytes except for olivetol, which was 2.5 µg/mL. The LOQ was calculated based on the S/N ra-

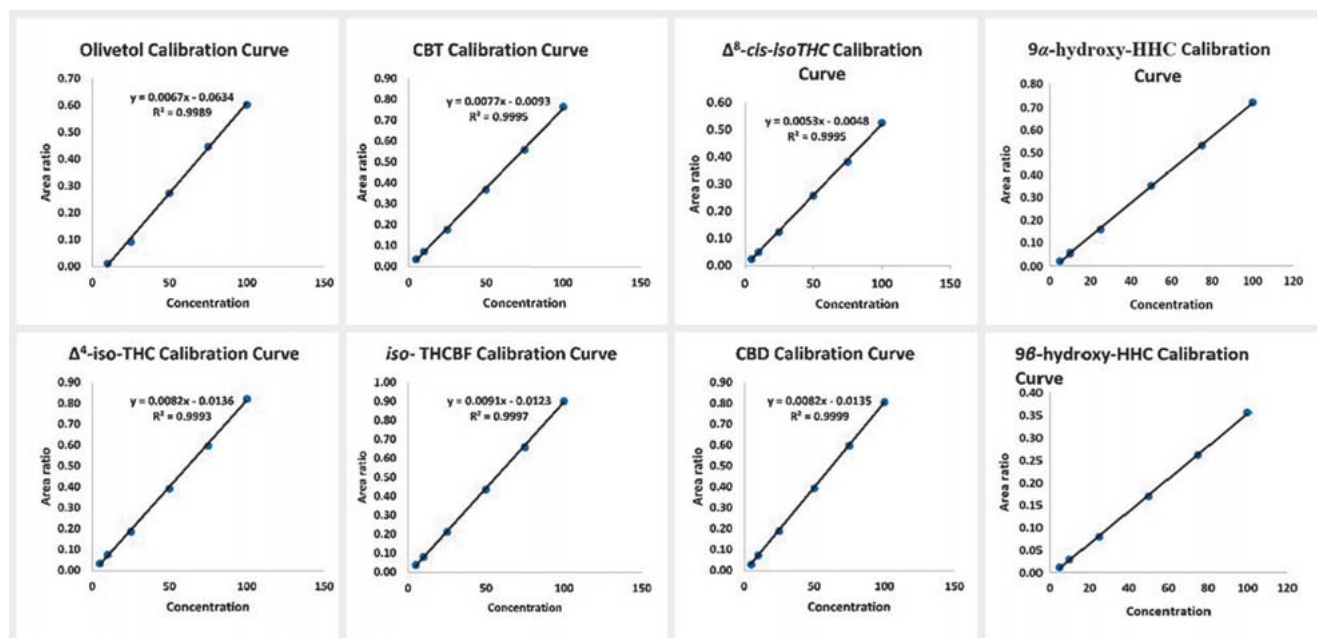
tio = 10, and it was found to be 5 µg/mL for all compounds except olivetol, which had an LOQ of 10 µg/mL (► **Table 1**).

Precision was evaluated at three different concentration levels for intraday and inter-day. For each concentration level of those analytes, %RSD was calculated. As shown in ► **Tables 2** and **3**, the precision of the method is satisfactory, where the %RSD values were not higher than 8.4 and 9.9% for the intraday and the inter-day precision, respectively.

The accuracy of the developed method was assessed at three concentration levels and was found satisfactory for the 14 analytes. The % recovery ranged from 88–118% and 89–112%, for intra- and inter-day accuracy, respectively. Therefore, the method is considered accurate and future results will fall inside the acceptance limits (80–120%). The different accuracy profiles are presented in ► **Tables 2** and **3**.

Twenty-one commercial products were analyzed by the GC-FID method for the determination of Δ^8 -THC and its synthetic impurities. The results show that the actual content of Δ^8 -THC in these products varied from 50 to 335% of the labeled amounts, potentially endangering users with poor quality or super potent products. For example, a manufacturer claimed 84.10% Δ^8 -THC for the sample # EA 316, but analysis showed it contained about 50% less than the labeled amount. This sample contained Δ^9 -THC at 5.29%, far beyond the statutory limit of NMT 0.3% by dry weight for Δ^9 -THC in hemp-derived products. Another sample, #EA 323 contained 69% Δ^8 -THC, which represents 335% of the labeled content of 20.58%. This sample contained Δ^9 -THC at 1.52%, which is also beyond the statutory limit for Δ^9 -THC in hemp-derived products.

Another major observation from the analysis of the 21 samples is that the impurity profiles varied widely. The different impurity



► Fig. 3 Calibration curves of olivetol, CBT, Δ^8 -*cis-iso*-THC, Δ^4 -*iso*-THC, *iso*-THCBF, CBD, 9 α -OH-HHC, and 9 β -OH-HHC.

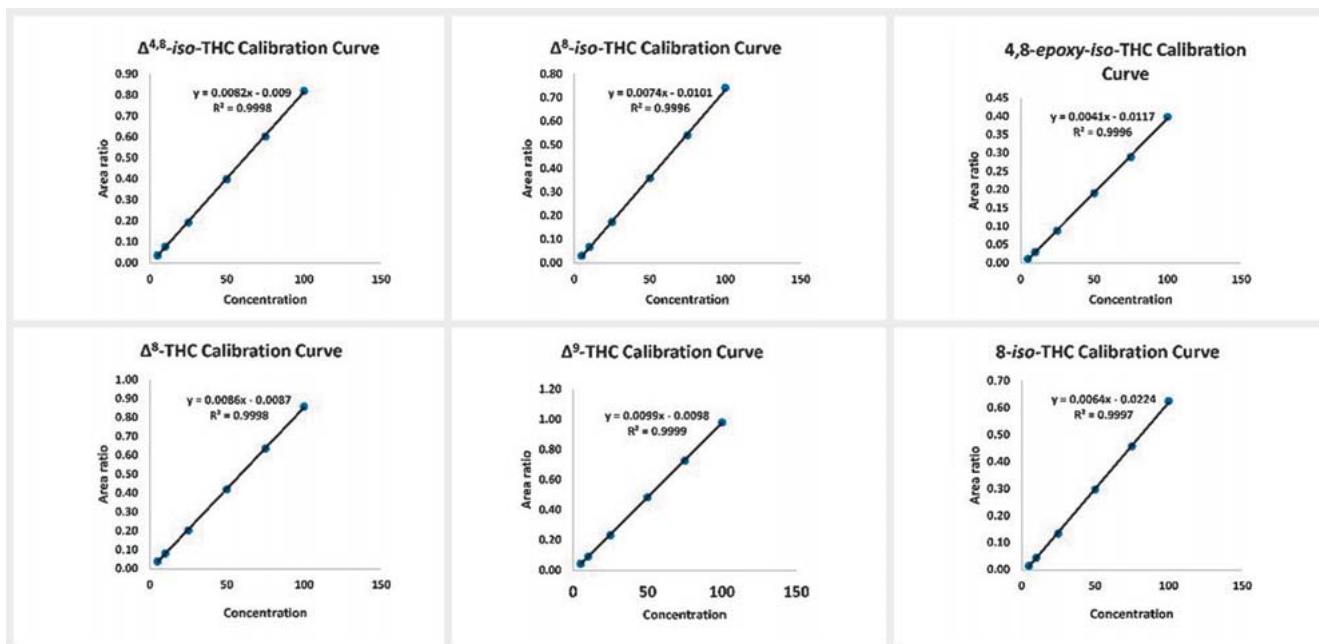
► Table 1 Regression Equation parameters, retention time, LOD, and LOQ of the target analytes.

Compound	Regression equation	R ²	tR	RRT	LOD (µg/mL)	LOQ (µg/mL)
Olivetol	$y = 0.0067x - 0.0634$	0.998	2.90	0.94	2.5	10
CBT	$y = 0.0076x - 0.0091$	0.999	8.30	2.61	1.5	5
Δ^8 - <i>cis-iso</i> -THC	$y = 0.0053x - 0.0048$	0.999	9.38	2.95	1.5	5
Δ^4 - <i>iso</i> -THC	$y = 0.0082x - 0.0136$	0.999	10.01	3.16	1.5	5
<i>iso</i> -THCBF	$y = 0.0091x - 0.0123$	0.999	10.50	3.30	1.5	5
CBD	$y = 0.0082x - 0.0135$	0.999	11.82	3.72	1.5	5
$\Delta^4,8$ - <i>iso</i> -THC	$y = 0.0082x - 0.009$	0.999	12.08	3.80	1.5	5
Δ^8 - <i>iso</i> -THC	$y = 0.0074x - 0.0101$	0.999	12.33	3.88	1.5	5
4,8- <i>epoxy-iso</i> -THC	$y = 0.0041x - 0.0117$	0.999	13.04	4.13	1.5	5
Δ^8 -THC	$y = 0.0086x - 0.0087$	0.999	13.66	4.34	1.5	5
Δ^9 -THC	$y = 0.0099x - 0.0098$	0.999	13.06	4.46	1.5	5
8-OH- <i>iso</i> -THC	$y = 0.0064x - 0.0224$	0.999	14.58	4.63	1.5	5
9 α -OH-HHC	$y = 0.0074x - 0.0208$	0.999	15.72	4.99	1.5	5
9 β -OH-HHC	$y = 0.0036x - 0.0089$	0.999	16.59	5.26	1.5	5

R²= Regression Coefficient; tR= retention time; RRT= relative retention time

profiles in Δ^8 -THC products could likely have resulted from different synthetic processes and possibly a result of the poor quality of CBD used as the starting material. The pharmacology and toxicology of these impurities are unknown and potential harmful effects could result from the use of these products containing these impurities through inhalation of these vape products. While the

impurity profiles varied amongst 21 commercial samples, the results in ► Table 4 show that $\Delta^4,8$ -*iso*-THC is consistently present in every sample, although at varying levels of 1–5%. Future studies should examine if $\Delta^4,8$ -*iso*-THC could be used as a marker for synthesized Δ^8 -THC, since it is not observed at these levels in cannabis plants.



► **Fig. 4** Calibration curves of $\Delta^4,8$ -iso-THC, Δ^8 -iso-THC, 4,8-epoxy-iso-THC, Δ^8 -THC, Δ^9 -THC, and 8-OH-iso-THC.

A chromatogram for a commercial sample of Δ^8 -THC is shown in ► **Fig. 5** for the sample # EA 324, identifying peaks marked that are not found in nature, as well as Δ^9 -THC (► **Fig. 5**). The results of the analysis of the 21 samples are presented in ► **Table 4**. The measured impurity content varied from about 7 to 33%.

► **Table 5** shows the actual levels of both Δ^8 -THC and Δ^9 -THC compared to the values reported by the manufacturers. This shows the wide discrepancy between actual values and claimed values. All tested Δ^8 -THC products in this investigation showed Δ^9 -THC at a level greater than 0.3%, which alone makes them a Schedule 1 controlled substance. While the manufacturers claimed that 19 of the 21 products contained less than 0.3% Δ^9 -THC, our analysis showed that the actual Δ^9 -THC content ranged from 0.68 to 5.78% in these products (see ► **Table 5**).

Emerging concerns from novel substances

The concerns related to synthetic cannabinoids are not limited to Δ^8 -THC. Several synthetic modifications of cannabinoids such as Δ^{10} -THC and Δ^8 -THC-O-acetate (a synthetic derivative of Δ^8 -THC not known to exist in cannabis), HHC, and tetrahydrocannabinol (Δ^8 -THCP) are being introduced into the market with no safety or toxicity data, or data on metabolic fate to support their use, in both ingestible and inhalable forms, and marketed as hemp derivatives.

The emerging use of minor cannabinoids and the cannabinoid analogs should be subjected to systematic preclinical and clinical investigations to characterize and identify any potential toxicities. These studies would be of great importance helping first responders and emergency department clinicians to be prepared to recognize toxicity and treat it. The safety of any new cannabinoid to be introduced to the market for human consumption should be addressed in phase I clinical trials through appropriate IND appli-

cation. The FDA's guidance on cannabis quality considerations for clinical research [18], and the FDA guidance on botanical drug development, [32] provide best practice guidelines for systematic evaluation of cannabis and cannabis-derived compounds. Suitable analytical methods, such as the ones provided in this publication, help ensure that high quality materials are used in such studies, resulting in the increased reproducibility and applicability of pre-clinical and clinical data.

While our study isolated 13 impurities in synthesized Δ^8 -THC, we recognize that a different synthetic route using precursors other than CBD, or different reaction conditions, can result in a different impurity profile. Similarly, the different forms of delivery, such as vaping, gummies, or brownies, could result in different impurity profiles and degradation products with unknown safety under conditions of exposure by inhalation or oral ingestion. Quantitative analysis of these risks may highlight the public health concerns from these synthesized Δ^8 -THC products.

Material and Methods

Isolation and identification of *trans*- Δ^8 -tetrahydrocannabinol impurities

The process for synthesis of Δ^8 -THC from CBD, isolation, and characterization of its impurities has been described in a previous publication [30].

In addition to Δ^8 -THC, 13 compounds were isolated through normal-phase silica gel columns followed by an amino column and reversed-phase chromatography. These impurities were identified, and their structures confirmed using NMR, HRMS, and GC-MS [30]. ► **Fig. 1** shows the chemical structures of the identified compounds.

► Table 2 Intra-day validation results (precision and accuracy).										
Compound	Concentration	Mean	SD	%RSD	Accuracy	Mean	SD	%RSD	Accuracy	
Olivetol		Batch1				Batch2				Batch3
	10 µg/mL	8.92	0.75	8.4%	89%	11.60	0.24	2.0%	116%	11.57
	25 µg/mL	22.22	0.53	2.4%	89%	22.73	0.82	3.6%	91%	22.04
	50 µg/mL	44.17	1.68	3.8%	88%	45.64	2.17	4.8%	91%	49.05
		Batch4				Batch5				Batch6
	10 µg/mL	11.52	0.22	1.9%	115%	11.54	0.19	1.6%	115%	11.83
	25 µg/mL	22.09	1.51	6.8%	88%	21.33	0.61	2.9%	85%	22.70
	50 µg/mL	47.46	1.89	4.0%	95%	49.27	1.56	3.2%	99%	46.26
		Batch1				Batch2				Batch3
CBT	10 µg/mL	10.97	0.43	3.9%	110%	10.72	0.18	1.7%	107%	10.10
	25 µg/mL	24.49	0.35	1.4%	98%	23.91	0.25	1.1%	96%	23.80
	50 µg/mL	48.40	0.94	1.9%	97%	47.90	0.92	1.9%	96%	48.01
		Batch4				Batch5				Batch6
	10 µg/mL	10.36	0.29	2.8%	104%	10.24	0.23	2.2%	102%	10.21
	25 µg/mL	23.99	0.36	1.5%	96%	24.42	0.41	1.7%	98%	23.94
	50 µg/mL	48.48	0.84	1.7%	97%	49.00	0.72	1.5%	98%	48.08
		Batch1				Batch2				Batch3
Δ ⁸ -cis-iso-THC	10 µg/mL	10.02	0.53	5.3%	100%	10.50	0.32	3.0%	105%	10.24
	25 µg/mL	25.02	0.41	1.6%	100%	23.88	0.52	2.2%	96%	23.92
	50 µg/mL	50.78	0.97	1.9%	102%	48.13	1.63	3.4%	96%	48.21
		Batch4				Batch5				Batch6
	10 µg/mL	10.15	0.63	6.2%	101%	10.22	0.35	3.4%	102%	10.24
	25 µg/mL	24.34	0.26	1.1%	97%	24.23	0.83	3.4%	97%	23.95
	50 µg/mL	49.47	1.14	2.3%	99%	48.54	1.06	2.2%	97%	48.43

continued next page

► Table 2 Continued

Compound	Concentration	Mean	SD	%RSD	Accuracy	Mean	SD	%RSD	Accuracy	Mean	SD	%RSD	Accuracy
Δ^4 -Iso-THC		Batch1				Batch2				Batch3			
	10 µg/mL	10.95	0.48	4.3%	110%	10.53	0.16	1.5%	105%	10.66	0.20	1.8%	107%
	25 µg/mL	24.51	0.45	1.8%	98%	23.74	0.36	1.5%	95%	25.09	0.41	1.6%	100%
	50 µg/mL	48.54	0.82	1.7%	97%	47.95	1.00	2.1%	96%	48.30	0.87	1.8%	97%
		Batch4				Batch5				Batch6			
	10 µg/mL	9.87	0.64	6.5%	99%	10.63	0.42	4.0%	106%	10.29	0.19	1.9%	103%
	25 µg/mL	24.30	0.36	1.5%	97%	24.81	0.35	1.4%	99%	23.68	0.54	2.3%	95%
	50 µg/mL	49.28	0.81	1.6%	99%	49.51	0.47	1.0%	99%	47.91	1.06	2.2%	96%
		Batch1				Batch2				Batch3			
Iso-THCBF	10 µg/mL	10.85	0.47	4.3%	108%	10.63	0.23	2.2%	106%	10.20	0.28	2.7%	102%
	25 µg/mL	24.56	0.46	1.9%	98%	23.86	0.43	1.8%	95%	24.06	0.51	2.1%	96%
	50 µg/mL	48.42	1.18	2.4%	97%	48.35	1.36	2.8%	97%	47.98	0.78	1.6%	96%
		Batch4				Batch5				Batch6			
	10 µg/mL	9.95	0.61	6.1%	100%	10.53	0.34	3.2%	105%	9.95	0.27	2.8%	99%
	25 µg/mL	24.33	0.47	1.9%	97%	24.76	0.59	2.4%	99%	23.59	0.40	1.7%	94%
	50 µg/mL	49.57	1.13	2.3%	99%	48.79	0.98	2.0%	98%	47.58	1.29	2.7%	95%
		Batch1				Batch2				Batch3			
	10 µg/mL	11.09	0.46	4.1%	111%	11.02	0.31	2.8%	110%	10.04	0.31	3.0%	100%
CBD	25 µg/mL	24.33	0.41	1.7%	97%	24.13	0.34	1.4%	97%	24.67	0.54	2.2%	99%
	50 µg/mL	48.19	0.69	1.4%	96%	47.39	1.65	3.5%	95%	49.31	0.45	0.9%	99%
		Batch4				Batch5				Batch6			
	10 µg/mL	9.59	0.54	5.7%	96%	10.11	0.41	4.1%	101%	9.53	0.36	3.8%	95%
	25 µg/mL	23.55	0.58	2.4%	94%	24.71	0.73	3.0%	99%	23.38	0.75	3.2%	94%
	50 µg/mL	49.09	0.57	1.2%	98%	49.80	0.82	1.6%	100%	48.23	1.04	2.1%	96%

continued next page

► Table 2 Continued													
Compound	Concentration	Mean	SD	%RSD	Accuracy	Mean	SD	%RSD	Accuracy	Mean	SD	%RSD	Accuracy
Δ^4 - <i>iso</i> -THC		Batch1				Batch2				Batch3			
	10 µg/mL	10.99	0.47	4.3%	110%	10.72	0.06	0.6%	107%	9.95	0.47	4.7%	99%
	25 µg/mL	24.24	0.40	1.6%	97%	24.06	0.31	1.3%	96%	23.78	0.58	2.4%	95%
	50 µg/mL	48.35	0.65	1.3%	97%	48.09	1.53	3.2%	96%	48.27	0.69	1.4%	97%
		Batch4				Batch5				Batch6			
	10 µg/mL	9.57	0.54	5.6%	96%	10.68	0.28	2.6%	107%	10.28	0.19	1.9%	103%
	25 µg/mL	23.86	0.41	1.7%	95%	24.33	0.60	2.5%	97%	24.12	0.29	1.2%	96%
	50 µg/mL	48.95	1.01	2.1%	98%	49.32	0.66	1.3%	99%	48.37	1.26	2.6%	97%
		Batch1				Batch2				Batch3			
	10 µg/mL	10.93	0.53	4.8%	109%	10.27	0.26	2.5%	103%	10.15	0.39	3.8%	101%
Δ^8 - <i>iso</i> -THC	25 µg/mL	24.37	0.49	2.0%	97%	23.59	0.67	2.8%	94%	24.14	0.63	2.6%	97%
	50 µg/mL	48.60	0.53	1.1%	97%	47.15	1.02	2.2%	94%	48.39	0.76	1.6%	97%
		Batch4				Batch5				Batch6			
	10 µg/mL	9.83	0.35	3.5%	98%	10.49	0.33	3.2%	105%	10.20	0.41	4.0%	102%
	25 µg/mL	23.85	0.50	2.1%	95%	24.31	0.68	2.8%	97%	23.91	0.64	2.7%	96%
	50 µg/mL	49.61	0.57	1.2%	99%	48.95	0.79	1.6%	98%	47.88	1.33	2.8%	96%
		Batch1				Batch2				Batch3			
	10 µg/mL	11.08	0.73	6.6%	111%	9.62	0.52	5.4%	96%	10.01	0.28	2.8%	100%
	25 µg/mL	24.63	0.66	2.7%	99%	22.52	0.49	2.2%	90%	24.47	1.08	4.4%	98%
	50 µg/mL	49.20	1.62	3.3%	98%	47.25	1.54	3.3%	95%	48.74	0.68	1.4%	97%
4,8-epoxy- <i>iso</i> -THC		Batch4				Batch5				Batch6			
	10 µg/mL	9.83	0.70	7.2%	98%	10.91	0.45	4.1%	109%	10.22	0.37	3.7%	102%
	25 µg/mL	23.26	1.50	6.4%	93%	25.13	0.75	3.0%	101%	24.45	0.70	2.9%	98%
	50 µg/mL	48.63	1.28	2.6%	97%	49.77	1.00	2.0%	100%	48.39	1.63	3.4%	97%

continued next page

► Table 2 Continued

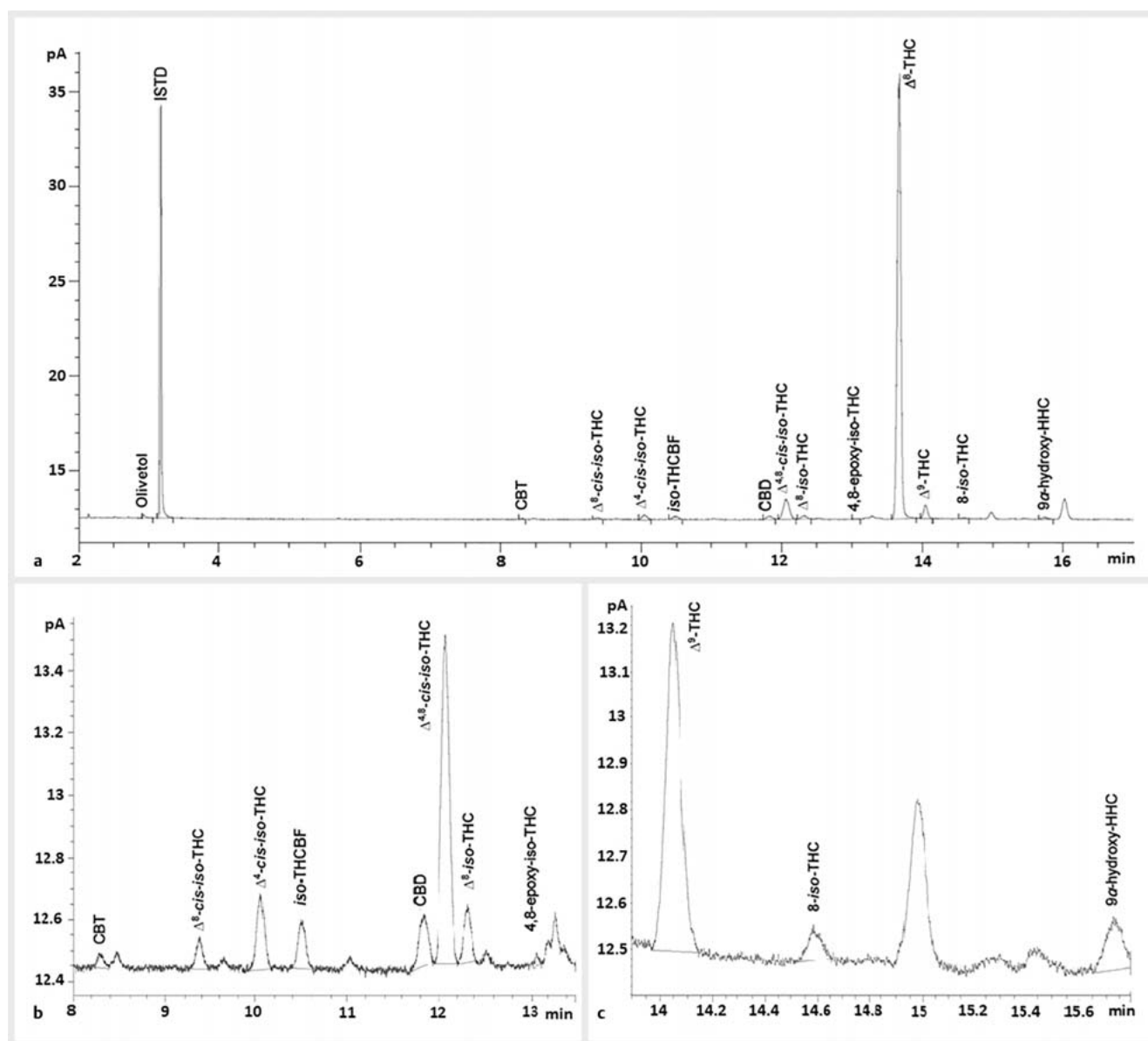
Compound	Concentration	Mean	SD	%RSD	Accuracy	Mean	SD	%RSD	Accuracy	Mean	SD	%RSD	Accuracy
Δ^8 -THC		Batch1				Batch2				Batch3			
	10 µg/mL	11.06	0.45	4.0%	111%	10.78	0.25	2.3%	108%	9.87	0.36	3.6%	99%
	25 µg/mL	24.90	0.44	1.8%	100%	24.44	0.21	0.8%	98%	24.05	0.54	2.2%	96%
	50 µg/mL	49.40	0.75	1.5%	99%	48.63	1.03	2.1%	97%	48.52	0.66	1.4%	97%
		Batch4				Batch5				Batch6			
	10 µg/mL	9.82	0.65	6.6%	98%	10.31	0.14	1.3%	103%	9.93	0.24	2.4%	99%
	25 µg/mL	23.69	0.52	2.2%	95%	24.67	0.75	3.0%	99%	23.92	0.23	1.0%	96%
	50 µg/mL	49.23	1.16	2.3%	98%	49.87	0.72	1.5%	100%	47.94	1.26	2.6%	96%
		Batch1				Batch2				Batch3			
Δ^9 -THC	10 µg/mL	10.94	0.48	4.4%	109%	10.75	0.15	1.4%	108%	9.99	0.32	3.2%	100%
	25 µg/mL	24.76	0.35	1.4%	99%	24.16	0.33	1.4%	97%	24.04	0.40	1.7%	96%
	50 µg/mL	49.03	0.75	1.5%	98%	48.62	0.96	2.0%	97%	48.80	0.54	1.1%	98%
		Batch4				Batch5				Batch6			
	10 µg/mL	9.77	0.50	5.1%	98%	10.31	0.17	1.6%	103%	9.77	0.11	1.1%	98%
	25 µg/mL	23.76	0.73	3.1%	95%	25.02	0.42	1.7%	100%	23.91	0.30	1.2%	96%
	50 µg/mL	49.37	0.74	1.5%	99%	50.51	0.72	1.4%	101%	48.21	1.33	2.8%	96%
		Batch1				Batch2				Batch3			
	10 µg/mL	10.81	0.46	4.2%	108%	9.54	0.48	5.0%	95%	10.48	0.40	3.8%	105%
8-OH-iso-THC	25 µg/mL	24.25	0.46	1.9%	97%	23.08	0.66	2.8%	92%	23.93	0.78	3.3%	96%
	50 µg/mL	48.44	0.93	1.9%	97%	47.59	1.34	2.8%	95%	48.39	1.14	2.3%	97%
		Batch4				Batch5				Batch6			
	10 µg/mL	10.05	0.81	8.1%	101%	9.40	0.18	1.9%	94%	9.48	0.24	2.6%	95%
	25 µg/mL	22.99	1.18	5.1%	92%	23.78	0.48	2.0%	95%	22.83	0.31	1.4%	91%
	50 µg/mL	49.09	0.78	1.6%	98%	49.96	2.72	5.4%	100%	46.52	1.20	2.6%	93%

continued next page

► Table 2 Continued													
Compound	Concentration	Mean	SD	%RSD	Accuracy	Mean	SD	%RSD	Accuracy	Mean	SD	%RSD	Accuracy
9 α -hydroxy-HHC		Batch1				Batch2				Batch3			
	10 μ g/mL	10.89	0.38	3.5%	109%	10.27	0.36	3.5%	103%	10.28	0.31	3.0%	103%
	25 μ g/mL	24.62	0.68	2.8%	98%	23.54	0.27	1.1%	94%	23.71	0.62	2.6%	95%
	50 μ g/mL	48.06	1.17	2.4%	96%	47.24	1.27	2.7%	94%	47.52	1.33	2.8%	95%
		Batch4				Batch5				Batch6			
	10 μ g/mL	9.57	0.79	8.2%	96%	9.48	0.20	2.1%	95%	9.97	0.33	3.3%	100%
9 β -hydroxy-HHC	25 μ g/mL	23.07	1.33	5.8%	92%	23.75	0.53	2.2%	95%	23.31	0.62	2.6%	93%
	50 μ g/mL	48.64	0.89	1.8%	97%	48.36	0.28	0.6%	97%	47.12	1.65	3.5%	94%
		Batch1				Batch2				Batch3			
	10 μ g/mL	10.65	0.76	7.1%	106%	9.41	0.42	4.5%	94%	10.52	0.32	3.1%	105%
	25 μ g/mL	23.91	0.81	3.4%	96%	22.50	1.44	6.4%	90%	23.63	0.91	3.8%	95%
	50 μ g/mL	45.08	1.92	4.3%	90%	45.73	1.78	3.9%	91%	47.94	2.43	5.1%	96%
		Batch4				Batch5				Batch6			
	10 μ g/mL	9.43	0.62	6.6%	94%	9.75	0.71	7.3%	98%	9.06	0.40	4.4%	91%
	25 μ g/mL	22.96	1.23	5.4%	92%	23.42	1.08	4.6%	94%	22.85	0.80	3.5%	91%
	50 μ g/mL	48.47	1.18	2.4%	97%	48.37	1.78	3.7%	97%	45.99	1.32	2.9%	92%

► **Table 3** Inter-day validation results (precision and accuracy).

Compound	Concentration	Mean	SD	%RSD	Accuracy
Olivetol	10 µg/mL	11.16	1.10	9.9%	112%
	25 µg/mL	22.18	0.52	2.3%	89%
	50 µg/mL	46.98	2.00	4.3%	94%
CBT	10 µg/mL	10.43	0.34	3.2%	104%
	25 µg/mL	24.09	0.29	1.2%	96%
	50 µg/mL	48.31	0.40	0.8%	97%
Δ^8 - <i>cis</i> -iso-THC	10 µg/mL	10.23	0.16	1.5%	102%
	25 µg/mL	24.22	0.43	1.8%	97%
	50 µg/mL	48.93	1.03	2.1%	98%
Δ^4 -iso-THC	10 µg/mL	10.49	0.37	3.5%	105%
	25 µg/mL	24.36	0.57	2.3%	97%
	50 µg/mL	48.58	0.68	1.4%	97%
iso-THCBF	10 µg/mL	10.35	0.38	3.6%	104%
	25 µg/mL	24.19	0.44	1.8%	97%
	50 µg/mL	48.45	0.69	1.4%	97%
CBD	10 µg/mL	10.23	0.68	6.6%	102%
	25 µg/mL	24.13	0.56	2.3%	97%
	50 µg/mL	48.67	0.89	1.8%	97%
$\Delta^{4,8}$ -iso-THC	10 µg/mL	10.36	0.53	5.2%	104%
	25 µg/mL	24.06	0.21	0.9%	96%
	50 µg/mL	48.56	0.47	1.0%	97%
Δ^8 -iso-THC	10 µg/mL	10.31	0.37	3.6%	103%
	25 µg/mL	24.03	0.30	1.2%	96%
	50 µg/mL	48.43	0.85	1.8%	97%
4,8- <i>epoxy</i> -iso-THC	10 µg/mL	10.28	0.59	5.8%	103%
	25 µg/mL	24.08	0.98	4.1%	96%
	50 µg/mL	48.66	0.85	1.7%	97%
Δ^8 -THC	10 µg/mL	10.29	0.52	5.1%	103%
	25 µg/mL	24.28	0.47	1.9%	97%
	50 µg/mL	48.93	0.70	1.4%	98%
Δ^9 -THC	10 µg/mL	10.26	0.50	4.9%	103%
	25 µg/mL	24.28	0.50	2.1%	97%
	50 µg/mL	49.09	0.80	1.6%	98%
8-OH-iso-THC	10 µg/mL	9.96	0.59	5.9%	100%
	25 µg/mL	23.48	0.58	2.5%	94%
	50 µg/mL	48.33	1.19	2.5%	97%
9 α -hydroxy-HHC	10 µg/mL	10.08	0.52	5.2%	101%
	25 µg/mL	23.67	0.53	2.3%	95%
	50 µg/mL	47.82	0.62	1.3%	96%
9 β -hydroxy-HHC	10 µg/mL	9.80	0.65	6.6%	98%
	25 µg/mL	23.21	0.53	2.3%	93%
	50 µg/mL	46.93	1.50	3.2%	94%



► Fig. 5 GC-FID chromatogram of a representative sample prepared at 5 mg/mL (sample # EA 324).

Standards and reagents

Olivetol, CBT, Δ^8 -*cis*-iso-THC, Δ^4 -iso-THC, *iso*-THCBF, CBD, $\Delta^{4,8}$ -iso-THC, Δ^8 -iso-THC, 4,8-*epoxy*-iso-THC, Δ^8 -THC, Δ^9 -THC, 8-hydroxy-*iso*-THC, 8-OH-*iso*-THC, 9 α -OH-HHC, and 9 β -OH-HHC were previously isolated and identified by some of the authors according to a published protocol from a commercial product and an in-house prepared Δ^8 -THC mixture using silica gel column chromatography, high-pressure reversed-phase column chromatography using a C18 column, and high-pressure normal-phase column chromatography using an amino column to produce these compounds in addition to Δ^8 -THC in small quantities. The purified compounds were identified, and the structures were confirmed by ^1H and ^{13}C NMR, which were prepared at 1 mg/mL in methanol. The purity of all the reference standards was confirmed using GC-FID and

GC-MS (purity > 98%). The structures of these compounds are shown in ► Fig. 1. Phenanthrene was purchased from Sigma-Aldrich and was used as the IS. HPLC grade methanol was obtained from Fisher Scientific.

Standard solutions preparation

The standard stock solutions of the purified compounds were prepared in methanol at a concentration of 1 mg/mL of each. Subsequently, a mixture of standard solutions was prepared by pipetting 100 μL of each compound. The mixture was vortexed, evaporated under a gentle stream of nitrogen, then the residue was dissolved in 1 mL methanol to reach a final concentration of 100 μg /mL. Serial dilutions were made to prepare the different points in the calibration curve.

► **Table 4** Analysis results of Delta-8-THC Vapes Using GC-FID (%w/w).

Sample Name	Olivetol	CBT	Δ^8 -cis-iso-THC	Δ^4 -iso-THC	iso-THCBF	CBD	$\Delta^{11,8}$ -iso-THC	Δ^8 -iso-THC	4,8-epoxy-iso-THC	Δ^8 -THC	Δ^9 -THC	8-iso-THC	9 α -hydroxy-HHC	9 β -hydroxy-HHC
EA 316	–	0.26	1.76	3.74	2.41	0.74	5.58	0.35	0.90	42.23	5.29	–	–	–
EA 317	0.65	0.22	0.27	0.69	0.59	–	2.11	–	–	74.54	0.68	–	0.36	–
EA 318	0.62	0.13	0.40	0.38	0.54	–	1.87	1.32	–	60.71	1.90	–	–	–
EA 319	–	–	–	0.46	0.42	–	2.21	1.65	–	53.92	1.08	–	0.95	–
EA 320	0.61	–	0.16	0.33	0.42	–	1.90	1.40	–	69.34	1.08	–	0.88	–
EA 321	–	–	–	1.49	0.44	–	3.63	–	–	61.33	1.01	0.80	0.81	–
EA 322	–	0.13	0.17	0.90	0.69	–	2.29	0.22	–	64.96	1.61	–	–	–
EA 323	0.77	–	0.14	1.11	0.78	–	2.56	–	–	69.01	1.52	–	–	–
EA 324	1.14	0.55	0.38	0.94	0.59	0.73	4.24	0.84	0.68	53.80	1.64	0.51	0.68	–
EA 325	–	0.24	0.50	1.54	1.12	–	3.69	–	–	74.09	1.76	–	–	–
EA 326	–	0.20	0.34	0.51	0.64	–	2.50	0.60	0.59	75.17	0.81	–	0.47	–
327	–	–	0.09	0.25	1.01	–	1.14	3.20	1.77	52.21	5.78	–	–	–
EA 328	–	0.28	0.52	1.17	0.50	0.12	5.96	0.74	–	56.01	1.21	1.50	1.81	–
EA 329	–	0.20	0.30	0.81	0.84	–	2.59	–	–	59.76	0.86	–	–	–
EA 330	–	0.24	0.36	1.79	1.55	–	4.77	–	–	67.76	2.38	–	–	–
EA 331	–	0.21	0.28	0.99	0.88	–	3.32	0.29	–	66.37	1.36	0.76	1.45	1.22
EA 332	–	0.19	0.37	0.97	0.83	–	3.00	0.37	–	52.77	1.13	1.10	1.74	1.10
EA 333	–	0.16	0.27	0.72	0.66	–	2.81	0.29	–	66.13	1.88	–	0.37	–
EA 336	8.96	0.26	1.15	2.57	2.87	0.53	5.05	0.93	–	46.44	2.72	–	–	–
EA 337	1.19	0.20	1.12	2.00	1.21	–	4.36	0.31	–	65.34	2.47	–	–	–
EA 338	–	–	0.19	0.43	0.64	–	2.04	1.95	–	69.75	3.43	–	0.52	–

► **Table 5** Concentration of Δ^8 -THC and Δ^9 -THC in the 21 products analyzed vs. manufacturers reported values.

Sample Name	Δ^8 -THC	Manufacturer (%)	Δ^9 -THC	Manufacturer (%)
EA 316	42.23	84.10	5.29	0.00
EA 317	74.54	92.94	0.68	<0.08
EA 318	60.71	82.58	1.90	0.00
EA 319	53.92	NG	1.08	NG
EA 320	69.34	93.41	1.08	<0.04
EA 321	61.33	92.12	1.01	0.23
EA 322	64.96	83.20	1.61	0.02
EA 323	69.01	20.58	1.52	0.41
EA 324	53.80	94.37	1.64	1.56
EA 325	74.09	90.45	1.76	0.03
EA 326	75.17	89.50	0.81	0.00
EA 327	52.21	92.40	5.78	0.00
EA 328	56.01	76.99	1.21	0.03
EA 329	59.76	76.90	0.86	<0.0033
EA 330	67.76	80.59	2.38	0.29
EA 331	66.37	88.97	1.36	<0.05
EA 332	52.77	NG	1.13	NG
EA 333	66.13	82.10	1.88	0.10
EA 336	46.44	NG	2.72	NG
EA 337	65.34	NG	2.47	NG
EA 338	69.75	NG	3.43	NG

NG = Not Given by Manufacturer

Internal standard preparation

The phenanthrene IS (1 mg/mL) was prepared in methanol; the concentration was kept as 100 μ g/mL in each calibration point.

Calibration curves and control samples

A six-point calibration curve was prepared from the stock standard solution mixture in the range of 5–100 μ g/mL for all the analytes except olivetol, which was prepared from 10–100 μ g/mL from the stock standard solution (100 μ g/mL solution mixture). A volume of 10 μ L of IS was added to each calibration curve sample. Calibration curves were obtained in six replicates and constructed by plotting the concentration versus average peak area ratio (peak area of analyte/peak area of IS). QC samples were independently prepared at three different concentrations (low: 10 μ g/mL, medium: 25 μ g/mL, and high: 50 μ g/mL) for each analyte and were similarly prepared and analyzed on 6 consecutive days (one batch every day). All standard stock solutions and QC samples were stored at -20°C until the time of analysis.

trans- Δ^8 -Tetrahydrocannabinol commercial products

Twenty-one Δ^8 -THC vape samples were analyzed. These samples were submitted by multiple companies as hemp-derived products for laboratory analysis to determine their Δ^9 -THC content. A limitation of this study is that other Δ^8 -THC products, such as gummies, were not tested in this study due to restrictions associated with DEA licensing of the labs.

Sample preparation of the *trans*- Δ^8 -tetrahydrocannabinol commercial products

From each Δ^8 -THC vape product, 50 mg was weighed in a 2-dram vial, dissolved in 5 mL of methanol, vortexed for 10 sec, sonicated for 5 min, then transferred to a 10-mL volumetric flask. The volume was adjusted to the mark with methanol to get a final concentration of 5 mg/mL. Each sample was analyzed using 10 μ L (dilute) and 25 μ L (straight) and to each vial 10 μ L of the IS (1 mg/mL) were added and the volume adjusted to 100 μ L. The injection volume was 2 μ L.

Instrumentation and column

GC-FID analysis was performed on an Agilent 6890 Network GC System (Agilent Technologies) fitted with a 7683B series injector. The column used was DB-1MS (15 m \times 0.25 mm and 0.25 μ m film thickness). The carrier gas was helium at a flow rate of 1.4 mL/min and for the detector make up gas. The inlet temperature was set at 270°C with a split ratio of 50:1. The temperature program started at 150°C and was held for 1 min, then ramped to 200°C with a rate of $40^{\circ}\text{C}/\text{min}$ (held for 10 min). Next, the oven temperature was ramped to 220°C at a rate of $15^{\circ}\text{C}/\text{min}$ (held for 4 min). The total run time was 17.58 min. Detector temperature was set at 270°C and the hydrogen, air, and make up gas (helium) flow rates were 30, 300, and 30 mL/min, respectively. Data were acquired and analyzed by Agilent ChemStation software (rev. B.04.02).

Method validation

The GC-FID method validation included linearity, selectivity, LOD, LOQ, trueness, and precision, and was performed according to the ICH Tripartite Guideline for Validation of Analytical Procedures [31]. Trueness was measured by the standard addition method. The intraday and inter-day precisions were assessed using a series of measurements. Six-point standard calibration curves were used to evaluate linearity. Calibration graphs were constructed by plotting the peak area ratio (y) of each analyte to that of the IS versus the analyte concentration (x) by injecting each concentration in triplicate. Linear regression with a $1/x$ weighting factor described the regression relationship. Linearity was considered satisfactory if the correlation coefficient (R^2) of the calibration was higher than 0.99.

LOD and LOQ were determined as 3.3 S/N and 10 S/N, respectively, where S = signal of the response of each cannabinoid and N = noise of the baseline.

To verify method precision, the %RSD of each batch (intraday precision) was calculated for 6 consecutive days ($n=6$) and between batches (inter-day precision) ($n=36$). Trueness was calculated as % recovery and precision (stated as %RSD). The intraday and inter-day precisions were required to be less than 10%.

Accuracy was calculated and reported as the % recovery between the QC series of measurements and the targeted concentrations at the three selected levels. Accuracy was back calculated from the calibration curve, which was run with each validation batch. It was established and expressed in terms of % recovery.

Contributors' Statement

Conception and design of the study: M.A. ElSohly, W. Gul, N. Sarma, K. Nam-Cheol; data collection: W. Gul, I. Shahzadi, M.A. ElSohly; statistical analysis: W. Gul, I. Shahzadi, M.A. ElSohly; analysis and interpretation of the data: M.A. ElSohly, W. Gul, N. Sarma, K. Nam-Cheol, I. Shahzadi; data visualization: M.A. ElSohly, W. Gul, N. Sarma, K. Nam-Cheol, I. Shahzadi; drafting the manuscript: M.A. ElSohly, W. Gul, N. Sarma, K. Nam-Cheol, I. Shahzadi; critical revision of the manuscript: M.A. ElSohly, W. Gul, N. Sarma, K. Nam-Cheol, I. Shahzadi.

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Conflict of Interest

W. G., I. S., and M. A. E. declare that they have no conflict of interest. N. S. and N. C. K. are employees of USPC, which is a non-for-profit organization that sells documentary and physical reference standards to sustain its activities.

Notes

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