Emergence of Hexahydrocannabinol as a psychoactive drug of abuse in e-cigarette liquids

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Article

Keywords:

Posted Date: June 26th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3047132/v1

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Abstract

Electronic cigarettes (e-cigarettes) have tremendously grown into societies. Due to the absence of regulation and standardization in their production, electronic cigarette liquids (e-liquids) present a complex matrix and a debate on their use and efficiency within the public health community. E-liquids are introduced into a delivery device that might include drugs of abuse such as cannabinoids. Despite the health problems associated, novel cannabis vaporizer ingredients continue to arise, such as Δ^8^-Tetrahydrocannabinol (Δ^8-THC), Dronabinol (aka Δ^9-THC) and Hexahydrocannabinol (HHC).

The aim of the present study was to investigate drugs of abuse in e-cigarettes seized by Dubai Police. Four e-liquids were analyzed through Gas Chromatography-Electron Ionization-Mass Spectrometry (GC-EI-MS). Our results showed that all seized e-cigarettes contained HHC and Dronabinol whereas 75% of samples contain Δ^8-THC. Our study revealed a rapid and sensitive GC-MS approach to detect newly trending cannabinoids in a short period of time (30 min/sample) in e-liquids. These findings could be helpful in further investigations considering the rapid evolution of e-cigarettes as mean for delivery of drugs of abuse and the critical need to make lawmakers aware of the legal loopholes used by manufacturers where “legal” drugs of abuse can be delivered to your door without consequences for the sellers.

1. Introduction

Electronic cigarettes, generally recognized as electronic nicotine delivery systems (ENDS) or e-cigarettes, are the most consumed tobacco product among youth. In 2022, 2.55 million U.S. middle and high school students used e-cigarettes, comprising 3.3% (380,000) of middle school and 14.1% (2.14 million) of high school students [1].

E-cigarettes are battery-functioned inhalers that afford nicotine to the consumer eliminating the dangerous combustion reactions of old tobacco cigarettes. Different companies produce dissimilar designs of e-cigarettes with diverse references such as cigalikes, vape pens, Hookah Pens, tank systems and some common components among all [2, 3].

The main components of e-cigarettes, which are common through various manufacturer’s devices, were summarized in Fig. 1. In e-cigarettes, the cartridge containing the electronic cigarette liquid or e-liquid is a common component. In the earliest manufactured e-cigarettes, devices were sold with pre-filled cartridges. Currently, in newer e-cigarettes, cartridges are reusable and refillable allowing consumers to choose the e-liquid according to their individual taste. Generally, nicotine e-liquids contain carrier humectants such as propylene glycol (PG) and vegetable glycerin (VG) in different ratios. In addition, several flavoring chemicals, solvents, preservatives, thickeners and diluents are detected in nicotine e-liquids [4]. Commercial cannabis-based e-liquids contain medium chain triglycerides (MCTs), Polyethylene glycol (PEG) to dissolve cannabinoids and vitamin E acetate (VEA)[5]. However, the precise chemical make-up varies largely depending on the manufacturer.
The second mutual element between e-cigarettes is the atomizing device enclosing the heating component, which vaporizes e-liquid to form a vapor inhaled by the consumer. User can drip e-liquid straight in the atomizer or attach a filled cartridge with e-liquid to produce this vapor. Another constituent found in nearly all devices is the single-use or rechargeable battery [6].

In recent years, e-cigarettes have been emerged as mean for delivery of drugs of abuse since users are able to utilize them unsuspiciously in public in addition to the fast desirable effects through oral usage rather than intravenous injections [7]. Moreover, several internet forums discussing the misuse of e-cigarettes were also trending [8].

Researchers have investigated commercial cannabis-based e-liquids and found that these comprise several drugs of abuse such as methamphetamine, cocaine, fentanyl, heroin, nicotine, cannabidiol (CBD), delta-9-tetrahydrocannabinol (Δ⁹-THC) and delta-8-tetrahydrocannabinol (Δ⁸-THC) [9].

Figure 2 illustrated the chemical structure of some cannabinoids including Δ⁹-THC, Δ⁸-THC and HHC.

From chemical point of view, both Δ⁹-THC and Δ⁸-THC are tetrahydrocannabinol molecules sharing an identical structure with a difference in the position of one double bond. Indeed, Δ⁸-THC has a double bond on the eighth carbon atom in its molecular structure whereas Δ⁹-THC has a double bond on the ninth carbon atom (Fig. 2a, 2b). Dronabinol is a synthetic form of Δ⁹-THC. On the other hand, HHC is a hexahydrocannabinol with no double bond (except in its aromatic cycle) in its chemical structure, but two hydrogenated molecules instead (Fig. 2c).

These dissimilarities in the chemical structure of Δ⁸-THC, Δ⁹-THC and HHC are very significant chemically and legally since different isomers might have unlike pharmacodynamics, pharmacokinetics and legal status [10, 11].

Indeed, Δ⁹-THC, one of over 120 phytocannabinoids produced in Cannabis sativa L. plant, is recognized as the major psychoactive natural compound [12]. Δ⁹-THC is a non-selective partial agonist of Cannabinoid receptor type 1 (CB1) and Cannabinoid receptor type 2 (CB2) receptors. It causes numerous physiological effects comprising analgesia, motor neuron inhibition, and central nervous system (CNS) sedation, when bound to CB1 [13]. Δ⁹-THC is extremely potent with an inhibitor constant $K_i < 50$ nM for CB1 and CB2 in human [14]. Vaping high doses of Δ⁹-THC caused auditory and visual hallucinations [15] and lead to serious hypodopaminergic-anhedonia (depression) and cognitive decline [16]. Moreover, vaping high doses of Δ⁹-THC may cause higher concentrations of Δ⁹-THC metabolites including 11-hydroxytetrahydrocannabinol (11-OH-Δ⁹-THC) and 11-nor-9-carboxytetrahydrocannabinol (Δ⁹-THC-COOH), in blood and oral fluid compared to traditional combustion smoking of the same dose [15]. Dronabinol is the synthetic form of Δ⁹-THC.

Additionally, Δ⁸-THC, isomer of Δ⁹-THC, is a minor phytocannabinoids also produced in Cannabis sativa L. plants. High amounts of Δ⁸-THC detected in items (food, e-cigarettes, etc..) are usually manufactured
in companies from hemp-derived cannabidiol (CBD) through chemical synthesis processes which may produce harmful by-products or contaminants [17]. Δ⁸-THC exhibits psychoactive and intoxicating effects comparatively similar to Δ⁹-THC. The ratio for the relative potency of Δ⁸-THC to Δ⁹-THC is 2:3, as described by Hollister and Gillespie, stating that Δ⁹-THC is significantly more potent than Δ⁸-THC. On another note, the psychoactivity of Δ⁹-THC is 1x whereas it is 0.5x for Δ⁸-THC [18]. Due to this difference in potency and the lower rate of adverse effects associated with Δ⁸-THC, consumers of cannabis seek out Δ⁸-THC products over Δ⁹-THC [18, 19].

In addition to Δ⁹-THC and its isomer Δ⁸-THC, HHC is a naturally occurring compound detected in trace quantities in cannabis plants or phytocannabinoids but can likewise be produced synthetically [20]. HHC was first termed in 1940 by Adams et al[21] in United States through research intended to reveal the chemical structure of psychoactive components of marijuana and hashish. HHC exists in two epimeric forms called 9α-HHC corresponding to (9S)-HHC epimer and 9β-HHC corresponding to (9R)-HHC epimer (Fig. 3)[22].

In hemp derived products, trace amounts of (9R)-HHC and (9S)-HHC were found at approximately 42.0% and 22.6% of the total cannabinoids respectively [23].

Currently marketed HHC is semi-synthetic and is typically a mixture of the (9R)-HHC and (9S)-HHC epimers. Semi synthetic HHC can be obtained from hemp-derived tetrahydrocannabinol isomers by catalytic hydrogenation of Δ⁹-THC [24] or through total synthesis [22, 25, 26]. Research is still developing regarding the synthesis processes, health effects, and potency of HHC. HHC effects have been investigated in cells and animals but not in humans [22].

More recently, many European countries detected e-cigarettes with low-THC cannabis also recognized as “CBD weed” adulterated with a synthetic cannabinoid receptor agonist (SCRA) methyl (S)-3,3-dimethyl-2-(1-(pent-4-en-1-yl)-1H-indazole-3-carboxamido)butanoate, or MDMB-4en-PINACA using gas chromatography coupled with mass spectrometry (GC-MS) [27]. This emerging NPS is considered a full and potent agonist on CB1-receptor triggering psychological and behavioral effects as well as negative effects such as vomiting, paranoia, panic attacks and seizures [28]. Before this case study was carried out, cannabis’ adulteration was not usually perceived, mainly in countries with a reasonably tolerant cannabis juridictive framework [27].

In another study, a third-generation synthetic cannabinoid (SC) 5F-ADB was detected in e-cigarettes [29]. In this case, a 16-year-old man presented intoxication signs minutes succeeding the use of a friend’s e-cigarette. 5F-ADB was identified in e-liquid and in an early collected serum sample (0.50 µg/L).

In the present study, we investigated the presence of different cannabinoids (Δ⁸-THC, Dronabinol, HHC) in four cases of e-cigarette samples seized by Dubai Police using GC-MS. Our results showed that all samples contained different natural cannabinoids including Δ⁸-THC, Dronabinol and HHC, which could
be identified through mass spectral library showing current trends in e-cigarettes containing drugs of abuse in the region.

2. Materials and Methods

2.1. Material

Dubai Police seized four e-cigarettes suspected to contain drugs of abuse. These e-cigarettes comprised cartridges, tanks, and pods that enclosed vaping liquids in variable volumes from residue to 1 mL. HPLC grade methanol was purchased from Merck. Reference standards including Dronabinol, HHC and $\Delta^8$-THC were purchased as 1.0 mg/mL solutions in methanol from Cerilliant (Round Rock, TX, USA).

2.2. Sample Preparation

When appropriate vaping liquid was available with 1–2 mg (1–2 µl for low viscosity samples), samples were prepared following the “Dilute and shoot” procedure where 1–2 mg were dissolved in 1 mL of methanol. Samples were sonicated for 2 min to ensure full dissolution. Following, all samples were centrifuged at 4,000 rpm at room temperature and supernatants were transferred into GC-MS vials for analysis.

2.3. GC-MS Analysis

GC (7890B series, Agilent Technologies, USA) paired with MS (5977A, Agilent) and fitted including an analytical column DB-5 (30 m x 250 µm x0.25 µm) was used for the detection of drugs of abuse in e-liquids injected in split mode. Helium was applied as carrier gas with a flow rate of 1mg/ml. Initial oven’s temperature was first set at 70°C for 3 min then the temperature was increased by 15°C per 1 min. Injector’s temperature was 280ºC and split mode was applied.

MS analysis consisted of single quadrupole at 150ºC with electron ionization (EI) source at 230°C, fixed electron energy of 70 eV and full scan acquisition mode between 40 and 700 m/z. Samples’ identification and mass spectra matching were achieved through SWGDRUG Mass Spectral Library (version 2017/2018), Cayman Spectral Library (version 2018/2019) and Wiley Library (version W9N11/W10N14/W11N17).

3. Results

Four E-cigarettes seized by Dubai Police were available for qualitative analysis of e-liquids conducted using GC-MS. Quantitative analysis was not required since laboratory requests were focused on the determination of the presence of drugs of abuse rather than the drugs’ purity.

The mass spectrum MS of three samples, out of four seized, showed the presence of $\Delta^8$-THC and Dronabinol (Fig. 4). As mentioned earlier, all active drugs could be identified based on certified MS libraries. $\Delta^8$-THC was eluted at 17.5 min with a base peak m/z 231.1 and a second base peak ion m/z
The second active drug detected was Dronabinol, eluted at 17.2 min with a base peak 299.1 and a second base peak 231.1 (Fig. 4b).

Out of four seized samples, one sample examined through GC-MS indicated the presence of Dronabinol (without Δ⁸-THC as observed in Fig. 4) with a base peak at m/z 299.1 (Fig. 5).

In addition to mentioned detected drugs, in all e-liquids analyzed, the mass spectrum of the suspected HHC component in the sample (Fig. 6) matched the HHC library mass spectrum. Indeed, the mass spectrum of a standard 9(S) or 9(R)-HHC exhibited a base peak at m/z 193 and a second base peak at 273 (Fig. 6.b). A similar fragmentation pattern for HHC was observed in all analysed samples as shown in Fig. 6.a. It must be noted that variation in the mass spectra fragmentation ratios may occur in HHC case samples due to the matrix effect or unknown concentration of HHC in the sample. Highly concentrated samples also cause changes in the fragmentation ratios and in such cases, the sample must be diluted and re-injected.

Furthermore, we identified two epimeric forms of HHC, (9R)-HHC and (9S)-HHC epimers in e-liquid in seized samples (Fig. 7). HHC detected in e-liquid contained two epimeric forms eluted respectively at 16.555 and 16.622 min (Fig. 7.a) which could be identified in comparison to mass spectra of standard (9R)-HHC (Fig. 7.b) and (9S)-HHC epimers (Fig. 7.c).

4. Discussion and conclusion

E-cigarettes are considered a latent device for drug abuse with the proficiency of fascinating youth to illicit drugs since illicit e-liquids cannot be perceived morphologically.

Vaping illicit drugs can have several health effects on consumers [30]. For example, in United States, several federal health agencies reported e-cigarettes or vaping product use-associated lung injury (EVALI) with 2807 cases resulting in hospitalization or even death by February 2020 [31]. 82% of patients stated the consumption of THC-comprising products and 57% declared the use of nicotine-containing products [31].

In order to investigate the presence of illicit drugs in e-liquids, generally, several analytical approaches are known in the literature [32]. These includes chromatographic and spectroscopic approaches which can be applied depending on the study context and investigations’ objectives [32]. One of these approaches is the direct analysis in real-time mass spectrometry (DART-MS), an atmospheric ionization MS method allowing the direct analysis of samples with high selectivity and sensitivity, applied for various target analytes in e-liquids of e-cigarettes such as nicotine, CBD, Δ⁹-THC, MCTs, PEG, and VEA [33]. Although DART-MS method could screen vaping liquids for substances of concern in less than 2 min per sample, this technique cannot differentiate between isomers including Δ⁹-THC, CBD, and other isomers due to similarities of MS-MS spectra of cannabinoid isomers whereas GC–MS can distinguish between them [33].
Previously, the forensic chemistry section at General Department of Forensic Science and Criminology in Dubai Police in United Arab Emirates investigated the presence of controlled substances in e-cigarettes seized in Dubai between 2016 and 2020 [34]. From 188 samples, 159 samples (84% of samples) contained drugs scheduled in UAE tablets of Federal Law N0 14 of 1995 on Countermeasure Against Narcotic Drugs and Psychotropic Substances [35]. Qualitative analysis with GC-MS in combination with DART Q-TOF MS-MS with prior QuEChERS extraction technique was conducted. 98% of positive samples contained THC whereas remaining samples contained other illicit drugs such as Fluram, Mephedrone, Ethyl 6-(2-aminopropyl) benzofuran, or N,N-di allyl-5-methoxy tryptamine or 5-MeODALT and Ethylphenidate [34].

In the present study, we investigated the presence of psychoactive cannabinoids in e-liquids of e-cigarettes seized by Dubai Police. Four e-cigarette cases were examined through GC-EI-MS, a common approach for analysis of volatile samples including cannabinoids. EI mode used ionized gaseous molecules by collision with an electron of 70eV energy, generating an excited molecular ion, which dissociates into structurally related fragment ions. Generally, the chosen approach efficiently separates complex mixtures by GC and allows subsequent component identification by MS. All drugs were screened using certified MS libraries.

For each detected drug, the molecular ion peak, \(M^{+}\) was identified in comparison to a standard. Consequently, all peaks at lower m/z value correspond to fragment ions. The most intensive peak or base peak was noted as well as the second base peak ion. A small isotope related peak accompanied all peaks.

Our results showed that all four samples contained HHC and Dronabinol whereas three out of four samples had \(\Delta^8\)-THC. \(\Delta^9\)-THC, significantly more potent than \(\Delta^8\)-THC, was not detected in any e-liquids analysed in this study.

As mentioned earlier, little information about HHC is available. Our case study could identify HHC in e-cigarettes using a routine forensic GC-MS methodology with no modifications. Furthermore, we could identify two epimers of HHC using GC-MS. Proton NMR spectroscopy can also be conducted in order to discriminate between HHC epimers. Indeed, HHC epimers differ only in the spatial position of C-11 methyl moiety. Indeed, in 9α-HHC or (9S)-HHC, methyl group is axial, whereas in 9β epimer or (9R)-HHC, methyl group is equatorial [22]. This dissimilarity might considerably influence receptor binding and HHC metabolism, eventually causing differences in psycho and pharmacological properties of these two epimers.

Currently, human pharmacology, including pharmacokinetics and metabolism of HHC have not been explored yet whereas pharmacological properties of HHC were investigated in vitro and in vivo. In vitro studies showed that 9β epimer or (9R)-HHC was the one wielding the cannabimimetic activity, while 9α-HHC or (9S)-HHC epimer had less psychotropic effect [22]. In vivo, research conducted since 1940 in animals specified that the pharmacology of HHC is analogous to that of \(\Delta^9\)-THC. Consequently, in
human, the behavioral effects of 9β epimer or (9R)-HHC might be qualitatively and quantitatively analogous to those of Δ⁹-THC [22]. This research topic should be further elaborated. Furthermore, researchers must investigate the potency of a cannabis product with HHC that might also be influenced by the abundance of one epimer in regard to the other.

Additionally, the manufacturers of such psychoactive cannabinoids have used legal loopholes to provide HHC to users in addition to other similar cannabinoids that are psychoactive yet not included in any scheduled drug list. A way around such loopholes is to have a general statement included in drug laws, which is the case in the UAE drug schedule legislation, or use more inclusive terminology where psychoactive cannabinoids or “derived psychoactive cannabis products” are specifically mentioned as suggested by Rossheim et al [36]. Interestingly, HHC which was discovered several decades ago and just recently used as a drug of abuse [37]. Therefore, it is crucial for lawmakers to have swift responses to such instances where novel or “rediscovered” drugs of abuse make their way to the legal market.

Finally, the approach we have applied for analysis of e-cigarettes liquids can be further expanded in order to consider supplementary chemicals of interest. This can include pesticides and heavy metals to provide insights about the dangers of using such products and may be used profiling e-liquids [38]. Future work can also focus on determining the potency of HHC to aid lawmakers in adding HHC to the drug schedule in a timely fashion. Advanced research is required in order to determine the long-term effects of e-cigarettes vaping in combination with several cannabinoids of different potencies.

**Declarations**

**Data availability**

*The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.*

**Acknowledgments** Authors express sincere gratitude to Chief of Dubai Police Lt. General Abdulla Khalifa Al Marri, Assistant Commandant for Criminal Investigation Affairs Major General Khalil Ibrahim Al Mansouri, and the Director of the General Department of Forensic Science and Criminology Major General Ahmed Thani Bin Ghalita, for their support by facilitating this research. The authors also thankfully acknowledge the support of faculty at the Department of Chemistry, Forensic Chemistry Track at Khalifa University of Sciences and Technology, Abu Dhabi, United Arab Emirates.

**Author Contributions** M.H has prepared the original and draft version of the manuscript. B.A and F.B.T have contributed to the conception and design of the work. M.J.A has conducted the interpretation of data and reviewed the manuscript. All authors have materially participated in sections and subdivisions preparation. All authors have read and approved the submitted version of the manuscript.

**Competing Interests** The authors have no relevant financial or non-financial interests to disclose.
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about-delta-8-tetrahydrocannabinol-delta-8-thc.


**Figures**

**Figure 1**

Components of an e-cigarette [6].

The cartridge containing electronic cigarette liquid (e-liquid) is a common component. The atomizing device encloses the heating component, which vaporizes e-liquid to form a vapor inhaled by the consumer. E-cigarettes comprise also battery components and indicator light. *Open access article distributed under the terms of the Creative Commons CC BY license. Copyright © 2011, Polosa et al; licensee BioMed Central Ltd.*
**Figure 2**

Chemical structures of different cannabinoids.

a. $\Delta^9$-THC; b. $\Delta^8$-THC; c. HHC. All chemical structures were created using Mestrelab Research Software Bruker.

**Figure 3**

Molecular structure of 9α-HHC corresponding to (9S)-HHC epimer and 9β-HHC corresponding to the (9R)-HHC epimer [22].
HHC epimers differ only in the spatial position of C-11 methyl moiety. In 9α-HHC or (9S)-HHC, methyl group is axial, whereas in 9β epimer or (9R)-HHC, methyl group is equatorial. Adapted with permission from Reference [22]. Copyright 2023, John Wiley and Sons*. Copyright Clearance Center License Number 5562881046616.
Representation of mass spectrum of analyzed e-liquids (n=3).

(a) Δ⁸-THC eluted at 17.5 min with a base peak m/z 231.1 and a second base peak ion m/z 314.2, (b) Dronabinol eluted at 17.2 min with a base peak 299.1 and a second base peak 231.1. GC (7890B series, Agilent Technologies, USA) paired with MS (5977A, Agilent) and fitted including an analytical column DB-5 (30 m x 250 µm x0.25 µm). Network Mass Selective Detector (scan range m/z 40 to m/z 700). Active drugs were identified based on certified MS libraries.

Figure 5

Representation of mass spectrum of analyzed sample (n=1) and detection of Dronabinol.

Dronabinol with a base peak at m/z 299.1. GC (7890B series, Agilent Technologies, USA) paired with MS (5977A, Agilent) and fitted including an analytical column DB-5 (30 m x 250 µm x0.25 µm). Network Mass Selective Detector (scan range m/z 40 to m/z 700). Active drugs were identified based on certified MS libraries.
Figure 6

Mass spectrum of HHC detected in all samples (n=4) in comparison to HHC library match.

(a) HHC with a base peak at m/z 193 and a second base peak at 273, (b) Standard HHC mass spectrum. GC (7890B series, Agilent Technologies, USA) paired with MS (5977A, Agilent) and fitted including an analytical column DB-5 (30 m x 250 µm x0.25 µm). Network Mass Selective Detector (scan range m/z 40 to m/z 700). Active drugs were identified based on certified MS libraries.
Figure 7

Identification of (9R)-HHC and (9S)-HHC epimers in e-liquid in seized samples


GC (7890B series, Agilent Technologies, USA) paired with MS (5977A, Agilent) and fitted including an analytical column DB-5 (30 m x 250 µm x0.25 µm). Network Mass Selective Detector (scan range m/z 40 to m/z 700).