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RESEARCH ARTICLE

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External hair contamination from cannabis and "light cannabis" delivered by smoking and vaping: An in vitro study

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Abstract

External contamination of hair by cannabis smoking requires a careful evaluation in forensic toxicology. Medical and recreational cannabis are increasingly consumed by e-cigarettes, which give rise to side-stream vapor. Moreover, products containing low Δ9-tetrahydrocannabinol (Δ9-THC) and rich in cannabidiol (CBD) started spreading legally. The goal of the present study was to assess whether hair analysis could allow to distinguish the type of delivered product, with low or high Δ9-THC, and the delivering mode, by smoking or vaping. Contamination of blank hair was mimicked by in vitro exposure to low- (0.4%) and high-Δ9-THC (9.7%) products delivered by smoking and vaping within a small confined system. Cannabis vaping extracts were prepared to deliver identical target Δ9-THC doses. Eighty samples were analyzed by ultrahigh-performance liquid chromatography mass spectrometry and quantified for Δ9-THC and CBD. After contamination by cannabis smoking, THC levels were in line with past in vitro and in vivo studies. Samples exposed to cannabis (169.30 ng/mg) showed significantly higher Δ9-THC than hair exposed to "light cannabis" (35.54 ng/mg), and the opposite was seen for the CBD/Δ9-THC ratio. Hair contaminated by vaping or smoking did not show a statistically different Δ9-THC content.

Under our in vitro conditions, hair analysis might allow to discriminate whether external contamination is determined by products containing low or high Δ9-THC, but not the delivering mode. More research is needed in real-life conditions, to see whether the same also applies to the interpretation of forensic casework.

KEYWORDS
drug contamination, hair analysis, marijuana, UHPLC–MS/MS, vaping

1 | INTRODUCTION

Hair analysis is one of the fundamentals of forensic toxicology, allowing to retrospectively assess the exposure to xenobiotics in a wide range of settings. Drugs get incorporated into hair through the bloodstream, via apocrine and sebaceous glands, but also through other external sources such as dust, contaminated hands, or smoke, especially for those drugs that are usually smoked or snorted like cocaine and cannabis.[1,2] For this reason, in hair analyses interpretation, it must be taken into account the differentiation between drug use and external contamination.[3,4]

Smoking is the primary method for recreational consumption of cannabis products. The application of heat causes the decarboxylation of delta-9-tetrahydrocannabinoic acid to its active form Δ9-tetrahydrocannabinol (Δ9-THC), which can be deposited on hair. The external contamination of hair by cannabis smoking or handling has been extensively studied by means of in vitro[2,5,6] and in vivo studies, showing that side-stream smoke can lead to Δ9-THC concentrations as typically measured in excessive cannabis users.[7–9] Therefore, a cut-off for Δ9-THC (0.05 ng/mg) has been established to prove drug use.[4]

"Vaping" is an alternative popular method for medical and recreational cannabis consumption and the term encompasses distinct practices, including the use of cannabis flowers vaporizers and of "cannabis electronic cigarettes" (CECs).[10] Electronic cigarettes were first introduced on the tobacco market and have proliferated for recreational delivery of cannabis extracts,[11–13] particularly among young users.[14] CECs are largely customizable but are mostly composed of a battery, a heating unit characterized by an atomizer, and a fluid reservoir. When the user inhales, a filament in the atomizer, activated by button or by an airflow sensor, generates an aerosol from the mixture of "e-liquids," usually containing flavors and carrier substances (e.g., propylene glycol) with or without nicotine.[11] Among "cannabis vaping" instruments, CECs are usually preferred due to portability, disposability, easy concealment, and easy use.[10] The "heat not burn" or "vaping" is perceived and sold as a safer way to avoid exposure to particulate matter,[11,12] although several reports of lung injury have been reported.[14,15] Nowadays, instructions on how to extract cannabinoids from plants, blending them with solvents such as propylene glycol and glycerol to prepare "homemade" Δ9-THC containing e-liquids, are readily available on several websites. Because vaping leads to the formation of exhaled aerosol, the release of organic compounds from the e-liquid into the environment might determine a Δ9-THC or cannabidiol (CBD) deposition on hair, as seen by smoking.

In addition to marijuana, low-Δ9-THC cannabis products, also referred to as "light cannabis," are being offered for sale in the majority of European countries.[16–18] In Italy, in the absence of explicit laws banning the commercialization of flowering tops with a Δ9-THC content between 0.2% and 0.6%, these products gained popularity because they contain CBD.[19,20] CBD and "light cannabis" products, although often sold under the label "not for human consumption," are popular and commonly consumed by smoking, drinking, or vaping as tobacco or cannabis replacement.[21] Recently, CBD, Δ9-THC, and their metabolites were studied on hair samples from "light cannabis" and cannabis users, and a high CBD/Δ9-THC ratio was proposed as a marker of the type of consumed product.[22]

In the present study, we aimed at identifying whether smoking low-Δ9-THC products could determine an in vitro contamination of hair similarly to high-Δ9-THC cannabis and whether the CBD/Δ9-THC ratio might help recognizing the case. Furthermore, we aimed at assessing whether cannabis vaping could lead to quantifiable levels of cannabinoids on hair comparable with smoking.

2 | MATERIALS AND METHODS

2.1 | Study design and trials

In this in vitro preliminary study, hair samples collected from 10 volunteers were contaminated with high- and low-Δ9-THC products, delivered in approximately equivalent Δ9-THC doses, by smoking and vaping.

Volunteers' hair samples were submitted to ultrahigh-performance liquid chromatography tandem mass spectrometry (UHPLC–MS/MS) analysis, preliminary to the experiment, to verify the absence of drugs of abuse (Δ9-THC, morphine, codeine, methadone, cocaine, amphetamine, methamphetamine, N-methyl-3,4- methylendioaxymethamphetamine (MDMA), 3,4-methylendioxyamphetamine (MDA), and 3,4-methylenedioxy-N-ethylamphetamine (MDEA)).

Smoke contamination was mimicked by exposing blank hair to high amount of cannabis in a small confined system, designed to exceed a realistic scenario, as previously reported.[6] Fifty milligrams of hair sample were tied at one end with strings and suspended by pins to a plastic net. The sample was put inside a 3 L one-necked round-bottom flask fitted with a one-hole stopper, making sure that the loose ends of the hair strands were not in contact one with another, nor with the walls of the flask. The hole of the stopper was fitted with a 30 cm-long tubing, connecting the flask to a pump (Brushless DC pump Model QR30B, 2.3 W, qmax 220L/h). The pump was connected at the other end to a 5 cm-long tube, the extremity of which was connected to the cigarette or e-cigarette (Figure 1a).

By activating the pump and connecting either the cigarette or the mouthpiece of the e-cigarette to the tube, the stream of smoke fell directly in the flask, around the hair locks suspended in it (Figure 1b).

For *smoking trials*, the cigarette was lit by hand and the pump was activated for 2 min, to simulate smoking time, at which point the cigarette was extinguished.

For *vaping trials*, the button to heat the filament in the atomizer was pressed with a puff length of 2 s and a puff frequency of 2 s (simulating a 2 s inhalation followed by a 2 s pause), in order to produce the side-stream vapor. Total e-cigarette puff time was 2 min, during which the pump was activated.

In both smoking and vaping trials, once extinguished the cigarette or terminated the e-cigarette puff volume, the pump was deactivated, and the one-hole stopper was sealed with parafilm. After that, the flask was gently swirled around to obtain a pretty uniform exposure of the hair locks to the smoke for further 13 min (total 15 min of exposure) (Figure 1c); then, hair samples were removed from the flask and the latter was evacuated.

Once removed from the flask, hair samples were stored in individual paper envelopes until analyses performed 1–2 days later.

Trials were repeated for a total of six different sessions, each of which permitted three or four hair strands to be externally contaminated. Between one session and the following one, the flask was accurately washed multiple times with hexane and water and then was left to air until complete dryness. In total, 20 samples were exposed to smoke of high-Δ9-THC containing products (10 delivered

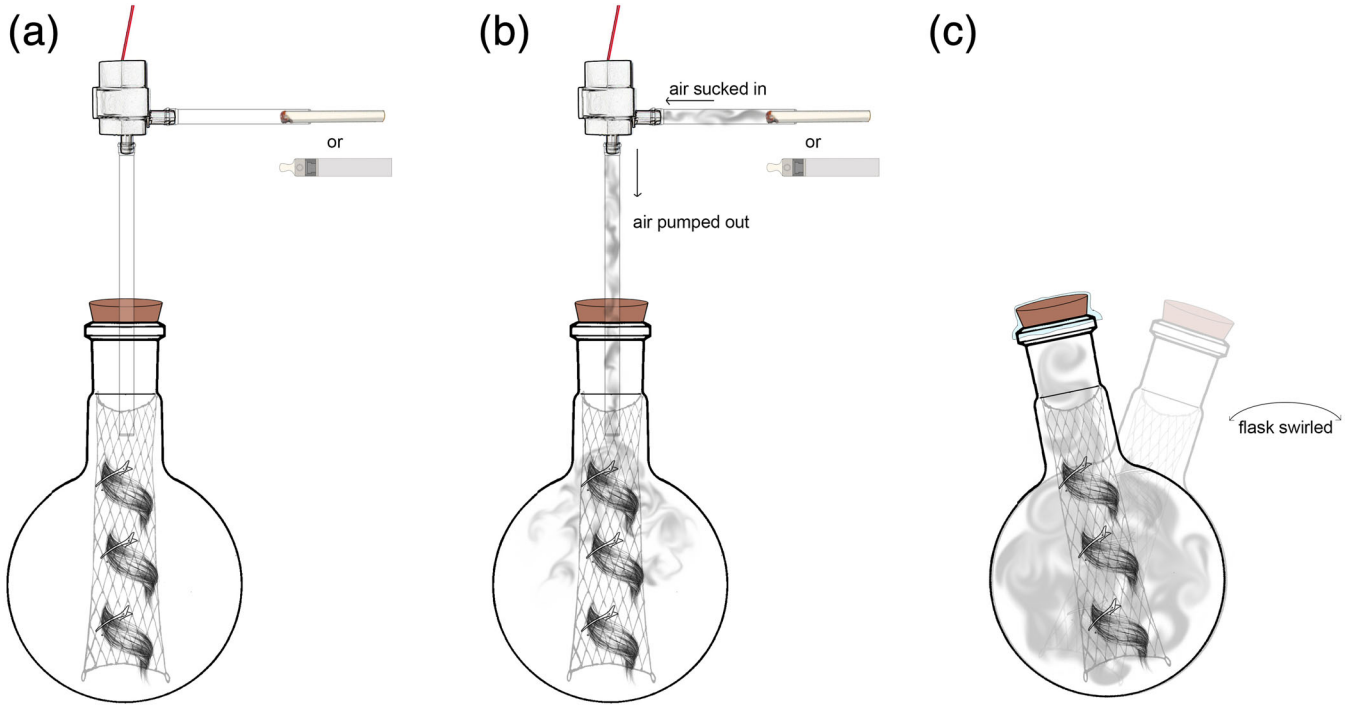

FIGURE 1 (a) The hair strands are suspended through a pin to a plastic net and the flask is connected to the pump by a tube. The pump is connected to the cigarette through a tube. (b) The pump is activated for 2 min, allowing the stream of smoke to fall in the flask. (c) The pump is deactivated and removed, the one-hole stopper is sealed with parafilm, and the flask is gently swirled around for 13 min.

by smoking and 10 by vaping) and 20 were exposed to low-Δ9-THC products (10 by smoking and 10 by vaping).

Because the experimental setup only took place in vitro, no approval by the local ethical committee was necessary.

2.2 | Chemicals and reagents

Acetonitrile, methanol, and formic acid were purchased by Merck Millipore (Darmstadt, Germany). Hexane, ammonium formate, and dichloromethane were obtained from Sigma Aldrich (Steinheim, Germany). M3® reagent and mix of internal standards (Δ9-THC-D_3 and CBD-D_3) were purchased from Comedical (Trento, Italy). All reagents and solvents were of LC/MS grade.

Water was produced through the Elga-Veolia instrument (Lane End, High Wycombe, United Kingdom). Mobile phases were composed of 5 mM ammonium formate and 0.1% formic acid in water (mobile phase A) and methanol (mobile phase B).

The e-cigarette was a Geekvape (M100 mod), equipped with a 13 W, 1.6 Ω tank.

2.3 | Cannabis cigarettes

The high-Δ9-THC products consisted in a seized batch of marijuana, which had been analyzed at the forensic laboratory of the University of Bologna for the determination of the Δ9-THC content according to the Italian legislation. The Judicial Authority authorized the use for research purposes. Cannabis cigarettes were prepared with 500 mg of tobacco and 500 mg of cannabis (flowering tops) with a percentage of 9.7% Δ9-THC and 1.39% CBD.

The low-Δ9-THC products were purchased from a local store of low Δ9-THC cannabis products. Low-Δ9-THC cigarettes were similarly prepared with 500 mg tobacco and 500 mg of flowering tops, with a content of 0.4% Δ9-THC and 31% CBD, purchased.

The percentage of Δ9-THC was tested by gas chromatography mass spectrometry (GC–MS), through an internally validated method, showing linearity (R^2 > 0.999) in the range 0.01–1 mg/mL, lower limit of quantification at the lowest calibration point, limit of detection at 0.0033 mg/mL, and acceptable accuracy (bias% < 15%) and precision (coefficient of variation% < 10%). The GC–MS system consisted of a Shimadzu GC-2010 equipped with a model AOC-20i auto-sampler system (Shimadzu) interfaced with a QP 2010 MS (Shimadzu). Analyte separation was achieved in 15 min on a Restek Rxi-5 MS column, 30 m length × 0.25 mm internal diameter × 0.25 μm film thickness. The oven temperature was programmed at 120°C for 1 min, increased to 270°C, and maintained for 8 min. Samples were analyzed in split 1:40 injection mode. Helium (purity 99.9999%) was the carrier gas at a column flow rate of 1.03 mL/min. The injection port, ion source, and interface temperatures were 290°C, 230°C, and 270°C, respectively.

2.4 | Cannabis vaping extracts

Cannabis vaping extracts were prepared from the high- and low-Δ9-THC cannabis products, in order to deliver approximately identical target Δ9-THC doses to that of smoking cigarettes. The doses were calculated after preliminary analyses, which showed that 2 min of vaping, under our experimental condition, would allow the consumption of approximately 1 mL of e-liquid, including a waste of 0.3 mL in the filter.

For the extraction procedure, 7 g of 9.7% Δ9-THC flowering tops were minced with scissors and spread evenly onto a 10 × 5 cm aluminum container, which was then placed inside a preheated stove at 110°C for about 30 min. The decarboxylated material was put inside a 50 mL beaker and covered with 19 mL of food alcohol; the beaker was then covered with several layers of parafilm and placed in a dark and cool place for 24 h, during which the beaker was stirred gently a couple of times. The mixture was then filtered through layers of sterile cotton gauze, and then, the alcohol was evaporated by bathing in water bath on a heating plate at a temperature around 100°C, until reaching a volume of 1.2 mL of residual liquid. After cooling, the liquid was mixed with propylene glycol (1:2) placed again in water bath for full homogenization and then left to cooling, obtaining a 12.2% Δ9-THC containing working solution.

The same procedure was repeated with 2.1 g of low-Δ9-THC products, to obtain a 2% Δ9-THC containing additional working solution.

Both working solutions were diluted in glycolic solution in order to obtain the final vaping extracts, each consisting in a volume of 1 mL containing target Δ9-THC doses of 4.8% (high-Δ9-THC product) and of 0.2% (low-Δ9-THC product), as analyzed by GC–MS.

CBD content in these vaping extracts was not controlled but was determined by GC–MS as 0.15% in the high-Δ9-THC content vaping fluid and 0.005% in the low-Δ9-THC one.

2.5 | Hair samples preparation

Hair samples with variable color (blonde, brown, or black) and structure (curly or straight) were collected from the vertex of 10 noncannabis users, cutting in close proximity to the skin.

After the experimental trials, hair samples were divided in two aliquots of which one submitted to a washing step and one left unwashed, for a total of 80 samples. The washing step, internally validated for forensic purposes and routinely applied in our laboratory, consisted of 1 mL dichloromethane ×2, manually agitated for 5 min, with no dryness in between. After the drying step, all hair samples (washed and unwashed) were cut phase into 2–3 mm lengths and divided in two aliquots of 25 mg each.

Then, samples were added with 5 μL of internal standards and 0.5 mL M3® and incubated in a Thermoblock at 100°C for 1 h.

At the end of the incubation, samples were cooled, centrifuged for 5 min at 3000 rpm, and transferred into Eppendorf and centrifuged for another 10 min at 14,000 rpm. After centrifugation, the supernatants were transferred in vials and injected into the UHPLC–MS/MS system.

2.6 | UHPLC–MS/MS analysis

A fully validated UHPLC–MS/MS method for the quantification of cannabinoids in hair was applied to the experimental samples after the exposure to either cannabis or light cannabis by means of smoking and vaping. UHPLC–MS/MS analysis was performed with a Waters Acquity UHPLC® (Milford, MA), coupled to a quadrupole mass detector Waters Xevo TQD, equipped with an electrospray ion source operating in positive mode. Chromatographic conditions were as follows: mobile phase B at a starting concentration of 13%, kept constant for 0.5 min, linearly increased to 50% in 9.5 min; then, for 2.5 min, mobile phase B concentration increased to 95%; returning to the starting conditions in the last 2.5 min, with a total run time of 15 min. Flow rate was 0.4 mL/min; column temperature was set to 50°C. Injection volume was 2 μL. Acquisitions were carried out in multiple reaction monitoring mode. In Tables S1 and S2, details of the multiple reaction monitoring method and validation parameters for the analytes of interest are shown.

2.7 | Statistical analysis

In order to check whether data were normally distributed, the sktest (including observation of skewness and kurtosis) was performed with Stata (StataCorpl LLC, Lakeway Drive, Texas, USA). According to the test, if $p < 0.05$, data are normally distributed.

Descriptive statistics was provided for all data. Statistical analyses were performed by paired parametrical or nonparametrical *t*-test, depending on the distribution shown by the sktest. The following comparisons of Δ9-THC and CBD levels took place, considering washed samples only:

- between samples exposed to high and low Δ9-THC. Furthermore, CBD/Δ9-THC ratios were calculated and compared; and
- between samples exposed by washing and by smoking, to evaluate the role of the delivering method, keeping fixed the variable of the cannabis product used for the exposure.

Δ9-THC concentrations in washed and unwashed samples were compared in Supporting Information S1.

Statistical analyses, considered significant with *p*-values < 0.05, and figures were realized by Prism (GraphPad Software, LLC, version 9.0.0).

3 | RESULTS

In total, 40 hair strands were in vitro contaminated, each analyzed with and without a washing step, for a total of 80 samples, and

TABLE 1 Quantification of tetrahydrocannabinol (THC) and cannabidiol (CBD) in washed and unwashed samples exposed to high-Δ9-THC and low-Δ9-THC products by vaping and smoking.

		Δ9-THC (ng/mg) Mean (±*SD*)		CBD (ng/mg) Mean (±*SD*)	
	N	Unwashed	Washed	Unwashed	Washed
Smoking high Δ9-THC	10	286.23 (122.36)	181.50 (100.59)	23.44 (24.21)	6.42 (8.34)
Smoking low Δ9-THC	10	138.14 (61.01)	36.05 (48.85)	>ULOQ	166.43 (142.48)[a]
All smoking	*20*	*212.19 (120.55)*	*108.77 (107.09)*	>ULOQ	*73.09 (119.20)*[a]
Vaping high Δ9-THC	10	241.67 (68.21)	157.11 (33.32)	30.47 (29.51)	20.91 (21.91)
Vaping low Δ9-THC	10	45.68 (39.46)	35.03 (33.47)	34.56 (49.54)	27.05 (37.17)
All vaping	*20*	*143.67 (114.93)*	*96.07 (71.01)*	*32.52 (39.23)*	*23.98 (29.49)*
All samples	*40*	*177.93 (120.72)*	*102.42 (89.39)*	>ULOQ	*46.65 (85.60)*[a]

Note: Results are given as mean and standard deviation (*SD*) between brackets. *N* = number of samples. "›ULOQ" was assigned when seven or more samples were higher than the upper limit of quantification (ULOQ).
[a]Two samples were higher than the ULOQ.

detailed results are shown in Table 1. A comparison between unwashed and washed hair is provided in Supporting Information S1, and all further analyses involved washed samples. Hair samples analyzed before the exposure showed negative results proving the absence of contamination prior to the study. All the experimentally contaminated samples showed quantifiable concentrations of Δ9-THC.

Δ9-THC and CBD appeared normally distributed (sktest $p = 0.0001$).

3.1 | Cannabis versus cannabis light

As shown by *t*-test, the Δ9-THC content was significantly higher ($p = 0.0001$) in hair samples exposed to high Δ9-THC compared with those exposed to low-Δ9-THC containing product, with a mean of 169.30 ng/mg (standard deviation = 19.54) and 35.54 ng/mg (standard deviation = 10.75), respectively. The presence of a significant difference was also shown when considering separately the subsamples of hair exposed to smoking ($p = 0.0064$) and exposed to vaping ($p = 0.0001$).

To compare the level of CBD and the CBD/Δ9-THC ratio, in the two samples above the calibration range, CBD was approximated to 300 ng/mg (i.e., the highest calibration point). CBD was significantly higher ($p = 0.0166$) in hair samples exposed to low Δ9-THC compared with those exposed to high-Δ9-THC containing products. This statistically significant difference was confirmed when analyzing samples exposed by smoking ($p = 0.0074$) but not when considering samples exposed to vaping ($p = 0.7734$).

Additionally, the CBD/Δ9-THC ratio was calculated. Median Δ9-THC/CBD ratio was 0.030 (IQ = 0.010–0.10) for hair contaminated with high-Δ9-THC product and 1.61 (IQ = 0.57–15.13) for low-Δ9-THC containing product, with a statistically significant difference ($p = 0.0204$).

Comparison of samples exposed to high- and low-Δ9-THC containing products for Δ9-THC and for Δ9-THC/CBD ratio is shown in Figure 2.

3.2 | Smoking versus vaping

Δ9-THC level did not show a statistically significant difference ($p = 0.5337$) between hair contaminated by smoking and those contaminated by vaping, when considering together hairs exposed to high- and low-Δ9-THC products.

The absence of a statistically significant difference was also confirmed when separately considering samples exposed to high-Δ9-THC content ($p = 0.5650$) and to low-Δ9-THC content ($p = 0.9003$). Results are shown in Figure 3.

No comparison was performed in the CBD content, because this variable was not experimentally controlled.

4 | DISCUSSION

Despite the recent surge in the commercialization of cannabis vaping instruments, including CECs, and of products labeled as "light cannabis," external hair contamination produced by vaping or by exposure to low Δ9-THC has not been yet investigated, to the best of the authors' knowledge. In this experiment, we simulated an external hair contamination, exposing blank hair strands first to the smoke of 500 mg cannabis and "light cannabis" products, mixed with 500 mg of tobacco.[19]

Unwashed and washed samples contaminated by smoking high-Δ9-THC product were used to assess the consistency with past studies. In our study, the average Δ9-THC content (286.23 ng/mg) in unwashed hair was slightly higher compared with other in vitro settings[2,6] but this seems consistent with the use of a very high-

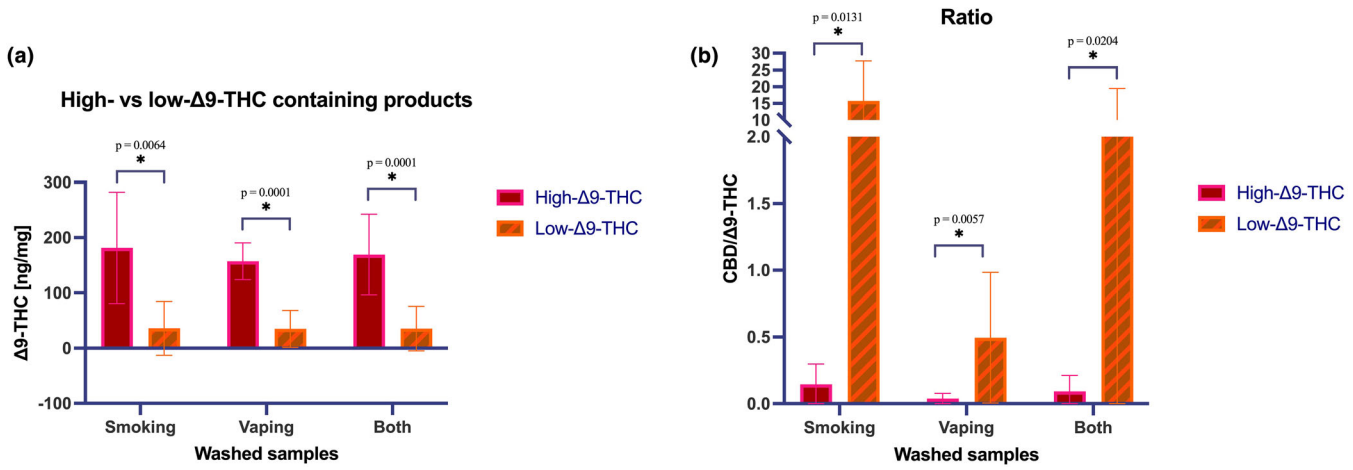

FIGURE 2 Comparisons between washed hair samples exposed to high- and low-Δ9-tetrahydrocannabinol (Δ9-THC) containing products, with particular reference, (a) to the Δ9-THC content and (b) to the cannabidiol (CBD)/Δ9-THC ratio.

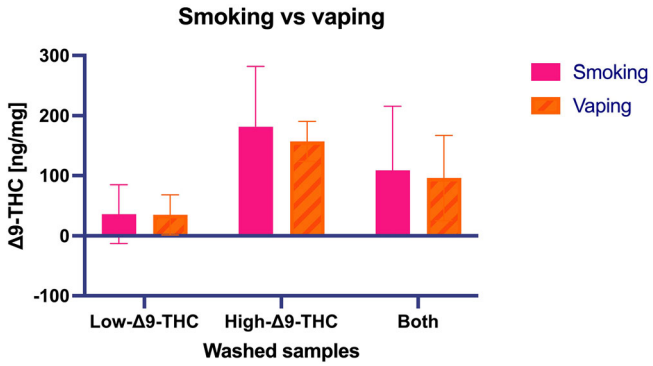

FIGURE 3 Δ9-Tetrahydrocannabinol (Δ9-THC) content of hair samples, showing a comparison between exposures by smoking and vaping for low Δ9-THC, high Δ9-THC, and both samples.

Δ9-THC-containing cannabis batch (9.7% vs. 2% of past experiments in a similar limited chamber of exposure[6]). In vitro levels usually exceed by 10 to hundreds of times those obtained in vivo, where the highest Δ9-THC concentration reported was around 1.7 ng/mg.[7] A similar value (approximately 1.8 ng/mg Δ9-THC) was shown in our study on washed hair by correcting for a factor of 100; this result is consistent with the expected values and confirms the reliability of the herein obtained data.

4.1 | Cannabis versus cannabis light

Considering the growing diffusion of material containing high CBD and low Δ9-THC, a few studies have investigated the impact of "light cannabis" on hair analysis and only in an active consumption setting. Subjects consuming "light cannabis" showed either negative or low amounts of Δ9-THC in hair. Indeed, the results of the studies so far performed demonstrated that hair analysis allows to discriminate between use of THC- and CBD-prevalent products, because the latter would not lead to Δ9-THC levels above the cut-off for drug use.[22–24]

In the present study, we wanted to test whether external contamination by "light cannabis" would also lead to unquantifiable Δ9-THC amounts on hair. As expected, significantly lower Δ9-THC levels were found in hair samples exposed to "light cannabis" compared with those contaminated with high Δ9-THC. Correcting for a factor of 100 to roughly approximate in vivo levels, our results would confirm that hairs contaminated by light cannabis would fall below the Δ9-THC Society of Hair Testing cut-off. However, three samples (two exposed by smoking and one by vaping) would be above it, so that further research on the topic is needed to understand whether "light cannabis" contamination could lead to Δ9-THC levels falsely pointing toward a drug use.

In these cases, the use of the CBD/Δ9-THC ratio, suggested by Casati et al., as a marker to discriminate illegal and "light cannabis" use,[22] might become useful. Indeed, in our study, the hair CBD/Δ9-THC ratio showed a statistically significant difference when considering samples exposed to low and high Δ9-THC, confirming the usefulness of this ratio even in the differential diagnosis within the external hair contamination context.

4.2 | Smoking versus vaping

Since its introduction on the market, cannabis vaping has grown in the market so much to be declared an "epidemic" by the Food and Drug Administration.[25] Because e-cigarettes give rise to side-stream vapor,[26–28] we wanted to ascertain whether it could also give rise to external hair contamination with quantifiable levels of Δ9-THC.

Under our experimental condition, vaping gave rise to hair Δ9-THC levels similar to smoking. This seems to confirm that the decarboxylation of tetrahydrocannabinoic acid into the active Δ9-THC occurs with any process involving the heating of cannabis material, including vaporizer systems (e.g., the Volcano one).[29] Previous studies have suggested that vaporizing the plant flowers might

allow even a more efficient Δ9-THC extraction and delivery compared with smoking, possibly due to the avoidance of combustion and burning of the plant material.[30,31] This was particularly observed when comparing cigarettes with Volcano systems at 270°C.[30]

Nevertheless, several factors should be considered. Contrarily to e-liquids, cigarettes also contained tobacco, which might lead to an improved burning efficiency, higher average temperatures, and improved volatilization of the cannabinoids.[29] Although the influence of tobacco cannot be ascertained, Δ9-THC levels did not differ between hair exposed to smoke and vapor and even higher vapor concentrations could be hypothesized when taking into account this possible bias.

Conventional cigarette continuously emits particles from the combustion process itself, while the e-cigarette aerosol is usually released only during exhalation.[28] In our vaping trial, the model of used e-cigarette directly produced vapor to contaminate hair, while the air exhaled by a possible e-cigarette user would likely contain lower amounts of active cannabinoids. However, e-cigarettes producing vapor with no inhalation or in between inhalations are available on the market. Also, persistence in air of the exhaled vapor is very short (approximately 11 s) compared with that of tobacco smoke (even 20 min).[32] Future studies should thus explore, in a real case scenario, the external hair contamination under exposure to exhaled air produced by CECs.

A possible explanation of our results likely resides in the air saturation obtained in the small experimental chamber, with possible overload also of the incorporation capacity of the hair.

In the field of forensic toxicology, should these results be confirmed, our study would imply that the delivery method, whether smoking or vaping, could not be ascertained by means of a hair analysis and that e-cigarettes do not differ from traditional ones in the determination of external hair contamination.

4.3 | Limitations

The major limitation of the present study resides in the use of an in vitro design, which is not directly transferable to authentic forensic samples, even if the in vitro study provided preliminary information to be transferred in real-life casework. This kind of cannabis exposure does not take into account a wide number of parameters and possibilities, for example, different patterns of smoking and puffing, distribution of the cannabis over the hair shaft via sebum, impact of hair treatments/hygiene, or Δ9-THC accumulation over multiple exposures, as for regular cannabis consumers.[7]

The in vitro setting also included that the hair strands exposition was realized in a small-volume chamber, resulting in higher Δ9-THC concentrations with respect to an in vivo exposure. However, similar to previous studies,[6] this setup was chosen in order to verify the effects of vaping and "light cannabis" at high and easily detectable Δ9-THC levels. A further limitation is represented by the washing step, which was not optimized for the high levels obtained by in vitro experiments.

In vaping trials, a uniform puffing procedure was applied (simulating a 2 s inhalation followed by a 2 s breath hold) to deliver theoretically the same amount of THC as by smoking. We are aware that this might be inconsistent with naturalistic puffing profiles and that individual differences in the puffing procedure (length and interval between puffs), the type and age of e-cigarette in use, might account for variation in dose delivery.[28] Moreover, because users tend to customize the cannabis administration to suit their specific preferences and needs,[30] future studies should be devoted to investigate various settings.

Lastly, it is well known that levels of Δ9-THC are usually several orders of magnitude higher than those of the inactive metabolite 11-nor-9-carboxy-delta9-tetrahydrocannabinol (THC–COOH), the presence of which strongly supports an active consumption of cannabis (Society of Hair Testing).[3] This metabolite was not analyzed in our study, because the distinction between external contamination and use was not the scope of the present work.

5 | CONCLUSIONS

The study demonstrated that, under our experimental conditions, on the basis of the CBD/Δ9-THC ratio, it might be possible to distinguish whether external hair contamination occurred with products containing low or high Δ9-THC.

Side-stream vapor produced by CECs can lead to Δ9-THC and CBD contamination of hair samples and hair analysis does not allow to discriminate the cannabis delivering mode, between smoking and vaping.

Taking into considerations the limits connected to the herein reported in vitro design, more research is needed in order to assess the effect of vaping and of "light cannabis" products on external hair contamination and to evaluate the usefulness of hair analysis for the confirmation of anamnestic information, especially in a realistic scenario.

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CONFLICT OF INTEREST STATEMENT

Nothing to declare.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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