IRIS AperTO

UNIVERSITÀ DEGLI STUDI DI TORINO

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

Determination of cannabinoids in urine, oral fluid and hair samples after repeated intake of CBD-rich cannabis by smoking

This is a pre print version of the following article:

Original Citation:

Availability:

This version is available http://hdl.handle.net/2318/1794741 since 2025-01-17T12:23:57Z

Published version:

DOI:10.1016/j.forsciint.2020.110561

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

09 March 2025

biomedicines

MDPI

1 *Article*

2 Determination of cannabinoids in urine, oral fluid 3 and hair samples after repeated intake of CBD-rich 4 cannabis by smoking

5 **Enrico Gerace [1], Snezhana Petrova Bakanova [2], Daniele Di Corcia [1], Alberto Salomone [1,2,*] and 6 Marco Vincenti [1,2]**

7

8 [1] Centro Regionale Antidoping e di Tossicologia“A. Bertinaria”, Regione Gonzole 10/1, 10043 Orbassano, 9 Turin, Italy; enrico.gerace@antidoping.piemonte.it (E.G.); daniele.dicorcia@antidoping.piemonte.it (D.D.)

10 [2] Dipartimento di Chimica, Università degli Studi di Torino, via P. Giuria 7, 10125 Turin, Italy; 11 snezhana.bakanova@edu.unito.it (S.P.B.); alberto.salomone@unito.it (A.S.); marco.vincenti@unito.it (M.V.)

12 * Correspondence: alberto.salomone@unito.it; Tel+39-348-9330145 (A.S.)

13

14 Received: date; Accepted: date; Published: date

15 **Abstract:** Cannabidiol prevalent (CBD-rich) cannabis derivatives are increasingly popular and 16 widely available on the market as replacement of THC, tobacco substitutes or therapeutics for 17 various health conditions. In this paper, we evaluate the impact of a repeated CBD-rich cannabis 18 intake on levels of cannabinoids in biological samples. Urine, oral fluid and hair (pubic and head) 19 samples were obtained from a naive user during a 26-day smoking period of one 250-mg CBD-rich 20 cannabis joint/day containing 6.0% cannabidiol (CBD; 15 mg) and 0.2% 21 delta-9-tetrahydrocannabinol (THC; 0.5 mg). In total, 35 urine, 8 oral fluid and 4 hair sample were 22 collected. Cannabinoids concentrations were quantified by a UHPLC/MS^n technique. The results 23 suggested that the repeated exposure to CBD-rich cannabis (containing small amounts of THC) can 24 generate positive results in biological samples. Urinary concentrations of 25 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THC-COOH) were quantitatively detected after 8 26 days from the smoking start and exceeded the 15 ng/mL cut-off limit on day-15 even in the urine 27 sample collected 12 hours after the last intake. In the oral fluid collected on day-26, no cannabinoids 28 were found before the cannabis intake, thus excluding accumulation, while THC was detectable up 29 to 3 h after the cannabis intake, at concentrations progressively decreasing from about 18 to 6 30 ng/mL. Hair samples collected one week after the end of the study turned out negative for THC 31 and THC-COOH, suggesting that this matrix is suitable to discriminate the chronic consumption of 32 CBD-rich cannabis from THC-prevalent products. The obtained findings are relevant for the 33 interpretations of cannabinoids levels in biological fluids, also in light of the legal implications of a 34 positive result.

35 **Keywords:** cannabis; CBD; THC; urine; hair; oral fluid; drug testing, workplace.

36

---

37 1. Introduction

38 In the past two decades, a growing interest has been observed in the therapeutic potential of 39 cannabis and single cannabinoids, mainly cannabidiol (CBD) and delta-9-tetrahydrocannabinol 40 (THC). More recently, CBD has gained prevalent attention for its potential therapeutic use as 41 anti-inflammatory, anticonvulsive, anxiolytic, analgesic, neuroprotective, anticancer, and 42 antioxidant agent [1–3]. In this context, a cannabis derived CBD product (Epidiolex®) was approved 43 by the US Food and Drug Administration (FDA) for treatment of pediatric seizure disorders [4]. 44 Likewise, the availability of non-medical CBD-rich products largely increased in recent years [5].

45 Cannabis products rich in CBD and low in THC can be legally sold and purchased in several
46 countries [5,6]. In the European Union, the free cultivation of cannabis varieties containing up to
47 0.2% (0.6% in some specific cases, e.g. Italy) is allowed [7]. In Switzerland, hemp plants containing
48 less than 1 % THC and 3-20% CBD are being sold for smoking as a tobacco replacement, and
49 combined with other ingredients in daily products or with no need of specific declared use [8]. In the
50 US and Canada, the commercialization of cannabis plants with THC concentration ≤0.3% is also
51 allowed [9,10].

52 In this context, the commercialization of CBD-rich cannabis products with low THC content
53 and variable CBD concentration, is proliferating. Moreover, cannabis farmers have been working to
54 create new cannabis varieties rich in CBD. Varieties expressing up to 25% total CBD and less than 1%
55 total THC (typically 0.3–0.7%) within the floral tissue have recently been farmed [11].

56 CBD-rich products can either be sold as dried plant material to be inhaled using conventional
57 methods (e.g., joints, bowls and vaporizers) or as concentrated CBD extracts intended for oral (e.g.,
58 oils, tinctures), pulmonary (e.g., vaporizers or vape pens) or topical (e.g., cream) consumption
59 [12–14].

60 Considering the diffusion of CBD-rich material, several studies were recently addressed to
61 evaluate the consequences of the single or repeated intake of these products on drug testing in
62 biological fluids [8,11,12,15,16]. In particular, it is relevant to know whether or not the consumption
63 of low THC/high CBD products can result in positive testing at cannabinoids screening within, for
64 example, workplace, roadside, or driving relicensing procedures. In this paper, we investigated the
65 impact of repeated CBD-rich cannabis intake on the cannabinoids levels in urine, oral fluid, and hair
66 samples, after a prolonged smoking period of 26 days.

67 **2. Experimental**

68 *2.1 Chemicals and Materials*

69 Δ9-tetrahydrocannabinol (THC), cannabidiol (CBD), cannabidiolic acid (CBDA),
70 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THC-COOH) and deuterated cannabinoids used as
71 internal standard (THC-d3, CBD-d3 and THC-COOH-d3) were purchased from Merck KGaA
72 (Milan, Italy). Methanol, acetonitrile, ethylacetate, dichloromethane, n-exane and glacial acetic acid
73 were provided by Merck KGaA (Milan, Italy) while formic acid (LC-MS grade) was obtained by
74 Fisher Scientific (Geel, Belgium). Sodium hydroxide was purchased from Carlo Erba Reagents
75 (Cornaredo, Milan, Italy). Ultra-pure water was obtained using a Milli-Q® UF-Plus apparatus
76 (Millipore, Bedford, MA, USA). For the determination of THC-COOH in urine, the working solution
77 was prepared by dilution in methanol of THC-COOH at a final concentration of 1 μg/mL. The
78 internal standard working solution was prepared in methanol at THC-COOH-d3 final concentration
79 of 10 μg/mL. For the determination of cannabinoids in oral fluid, the working solution containing
80 THC and CBD at a concentration of 1 μg/mL was prepared in methanol. An equivalent internal
81 standard mixture containing THC-d3 and CBD-d3 at a concentration of 1 μg/mL was prepared in
82 methanol. For the determination of cannabinoids in hair samples, the same working solutions used
83 for oral fluid were used. In addition, two working solutions of THC-COOH and THC-COOH-d3
84 were prepared by dilution in methanol at final concentration of 0.1 μg/mL. All stock and working
85 solutions were stored at -20°C until used.
86
87 *2.2 Study design and biological samples*
88
89 A healthy male subject (45 years, 72 kg, 169 cm, BMI 25.2; regular tobacco cigarette smoker),
90 who declared no consumption of cannabinoid-containing products prior to the study, gave written
91 informed consent and accepted to donate urine, oral fluid and hair samples prior, during and after a
92 daily smoking of a joint containing 250 mg of CBD-rich cannabis for 26 consecutive days. The
93 cannabinoid content of the CBD-rich cannabis (6.0% CBD and 0.2% THC) was preliminarily verified
94 by means of HPLC-UV and GC-MS methods routinely employed in our laboratory. Consequently,

the daily exposure to CBD and THC via inhalation was estimated to be 15 and 0.5 mg, respectively. Observation of side effects was excluded from the study because of the small number of tested subjects. The study was conducted in accordance with ethical standards established in the Helsinki Declaration.

Urine samples were collected every day, 12 hours after smoking, except days 1, 15, and 26 when urine samples were collected both before smoking (U-t_0) and in the time interval 1-10 h after smoking. No urine sampling was carried out on day 16. The oral fluid samples were collected in a test tube on day 26 before smoking (OF-t_0), and 0.25, 0.5, 1, 2, 3, 5 and 6 hours after smoking. Prior of collection, the participant washed his mouth twice with water.

Head and pubic hair samples were collected one day before the beginning of the study and one week after the end of the study [17]. Urine and oral fluid samples were stored at -20°C until the analysis, while hair samples were stored in dark and at room temperature before the analysis.

All collected samples were screened for the presence of cannabinoids (CBD, THC and THC-COOH) by means of validated UHPLC-MS^n methods. The main validation parameters are described in the supplementary material (Table S1). In order to adjust the quantitative confirmation results based on urine dilution, urinary creatinine was measured by the Jaffe method using an Abbott Architect instrument (Abbott Laboratories, IL, USA). Therefore, every cannabinoid level was normalized with respect to 100 mg/dL of excreted creatinine.

2.3 Sample preparation

2.3.1 Urine

Urine samples (500 μL) were spiked with 2.5 μL of internal standard solution and 20 μL of sodium hydroxide solution (10 N). The mixture was incubated at 55°C for 15 minutes. Then the samples were cooled at room temperature and added with 10 μL of glacial acetic acid for neutralization (pH 6.5-7.5). After vortexing, 50 μL of mixture was transferred into a new tube and diluted with 450 μL of a solvent mixture methanol/acetonitrile (80:20 v/v). Finally, the tube was centrifuged at 14,000 rpm for 5 minutes and a 4 μL aliquot of the solution was injected into the UHPLC-MS^n system.

2.3.2 Oral fluid

Oral fluid samples (250 μL) were spiked with 10 μL of internal standard solution, then 500 μL of a solvent mixture methanol/acetonitrile (80:20 v/v) was added. After vortexing for 10 seconds, the samples were centrifuged at 14,000 rpm for 5 minutes. 50 μL of surnatant was transferred into a new tube and a 2μL-aliquot of the solution was injected into the UHPLC-MS^n system.

2.3.3 Hair

The present sample preparation introduced minor modifications with respect to the standard operating procedure [18]. Hair sample (50 mg) was washed twice with dichloromethane (2 mL, vortex mixing for 3 min). After complete removal of the washing solvent, the hair aliquot was dried at room temperature under a gentle nitrogen flow and then cut into 1-2 mm segments. The sample was then fortified with 5 μL of internal standard before the addition of 2 mL NaOH 10N and subsequent incubation at 75 °C for 60 min. After cooling at room temperature, 1 mL of glacial acetic acid was added to adjust the pH value down to 3.5-4.0. Afterwards, the sample was extracted with 10 mL of n-hexane/ethylacetate 90:10 (v/v). The organic phase was separated, dried under a nitrogen flow at 45°C and reconstituted with 50 μL of acetonitrile/methanol 50.50 (v/v). Finally, an aliquot of 5 μL methanol solution was directly injected into the UHPLC-MS^n system.

2.4 UHPLC-MS^n methods

Cannabinoids concentrations in urine and hair samples were determined using an Agilent Infinity 1290 UHPLC system (Agilent Technologies, Milan, Italy) coupled to a 4500 QTRAP hybrid triple quadrupole/linear ion trap mass spectrometer with a Turbo V ion source (SCIEX, Darmstadt, Germany). Analyst software version 1.6.1 (SCIEX, Darmstadt, Germany) was used for data acquisition and analysis. A UHPLC Ace Excel Super C18 column 75 mm × 2.1 mm i.d., 2 µm (Agilent Technologies, Scotland), protected by a C18 guard column, was used for the separation of the target analytes. The column oven was maintained at 45°C. The elution solvents were water/formic acid 5mM (solvent A) and acetonitrile (solvent B). After an initial isocratic condition at 50% A for 0.3 min, the mobile phase composition was varied by a linear gradient (A:B; v/v) from 50% to 100% B in 3.0 min; then isocratic elution at 100% B was maintained for 0.5 min. The flow rate was 0.6 mL/min and the total run time was 5 min, including re-equilibration at the initial conditions before each injection. For urine analysis, mass spectrometric data were detected in negative electrospray ionization (ESI) mode and MS/MS (MS^2) selected reaction monitoring (SRM) acquisition, with an ion spray voltage of -4500 V and an ion source temperature of 600 °C. Nitrogen was employed as the collision gas (5×10^{-3} Pa). For hair analysis, mass spectrometric data were acquired following two different experiments. THC and CBD were detected in positive ESI mode and MS^2 acquisition (ion spray voltage: 4000 V; ion source temperature: 550°C) while THC-COOH was detected in negative ESI mode and MS^3 acquisition (ion spray voltage: -4500 V; ion source temperature: 650°C). THC and CBD in oral fluid samples were determined using a Shimadzu LC-20A Series system (Shimadzu, Duisburg, Germany) interfaced to an API 5500 triple quadrupole mass spectrometer (SCIEX, Darmstadt, Germany) with an electrospray Turbo Ion source operating in the positive ion mode (ion spray voltage: 4000 V; ion source temperature: 550°C). The chromatographic parameters (column, mobile phases and gradient) were the same used for the analysis of cannabinoids in urine and hair samples. The SRM transitions for analytes and internal standard detection are shown in Table 1.

Table 1. SRM transitions for the target compounds and the internal standards

Matrix	Compound	Ionization	SRM transitions (m/z)
Urine	THCCOOH	$[M-H]^-$	343.1 → 299.0 343.1 → 245.0 343.1 → 191.1
	THCCOOH-d3	$[M-H]^-$	346.1 → 302.0
Oral fluid **Hair**	CBD	$[M+H]^+$	313.2 → 244.8 313.2 → 106.6 313.2 → 311.0
	THC	$[M+H]^+$	315.2 → 193.2 315.2 → 123.1 315.2 → 259.2
	CBD-d3	$[M+H]^+$	316.1 → 248.0
	THC-d3	$[M+H]^+$	318.0 → 196.2
Hair	THCCOOH	$[M-H]^-$	343.2 → 299.0 (MS^2) 245.0 (MS^3)
	THCCOOH-d3	$[M-H]^-$	346.2 → 299.0 (MS^2) 245.0 (MS^3)

176 ## 3. Results

177 ### 3.1. Urine

178 A total of 35 urine samples was collected during the study period. THC-COOH was detected in
179 27 samples (77% of the total) at concentrations in the interval <LOQ-23 ng/mL (LOQ: 6 ng/mL). In
180 detail, 20 samples contained THC-COOH in trace (LOD<THC-COOH<LOQ), while in the remaining
181 7 samples the THC-COOH concentration was between 10 and 23 ng/mL. THC-COOH was never
182 detected in the urine samples collected before smoking (U-t_0). The results of all urine samples taken
183 during the 26-day smoking period are shown in Table 2. All urine concentrations are reported as
184 creatinine normalized concentrations.
185
186 **Table 2.** THC-COOH concentration (creatinine normalized) in urine samples at different collection
187 times during the 26-day study period.
188

Day	Collection time[1] (hh:mm)	Creatinine (mg/dL)	THCCOOH (ng/mL)
1	U-t_0	116	<LOD
	02:10	95	<LOQ
	04:30	138	<LOQ
2	13:30	100	<LOQ
3	12:00	126	<LOQ
4	16:00	109	<LOQ
5	16:00	92	<LOQ
6	12:00	169	<LOQ
7	12:00	207	<LOQ
8	12:30	61	13
9	12:30	90	<LOQ
10	12:30	58	12
11	12:00	171	<LOQ
12	12:30	87	<LOQ
13	12:00	120	<LOQ
14	12:00	84	10
15	12:00	113	15
	01:15	64	23
	02:55	85	19
	05:10	114	10
17	12:30	157	<LOQ
18	12:00	150	<LOQ
19	11:30	135	<LOD
20	13:30	124	<LOQ
21	12:20	163	<LOD
22	12:20	102	<LOQ
23	12:50	120	<LOQ
24	12:00	127	<LOD
25	10:30	114	<LOD
26	12:00	127	<LOD
	02:10	99	<LOQ
	03:45	153	<LOQ
	06:40	141	<LOQ
	08:10	179	<LOD
	10:10	166	<LOD

[1] Time elapsed between the last intake and the urine collection.
LOQ = 6 ng/mL

A maximum THC-COOH concentration of 23 ng/mL was found on day 15, particularly 1h15min after the daily intake of CBD-rich cannabis. Moreover, on the same day, the urine samples collected 2h55min and 5h10min after the joint consumption turned out positive at THC-COOH concentration of 19 and 10 ng/mL, respectively. Among the samples collected about 12 hours after consumption, positive samples were observed on days 8, 10, 14 and 15 with THC-COOH concentrations between 10 and 15 ng/mL.

3.2. Oral fluid

Figure 1 shows the concentrations of THC and CBD in oral fluid on day 26, before and after the daily smoking of the CBD-rich cannabis joint.

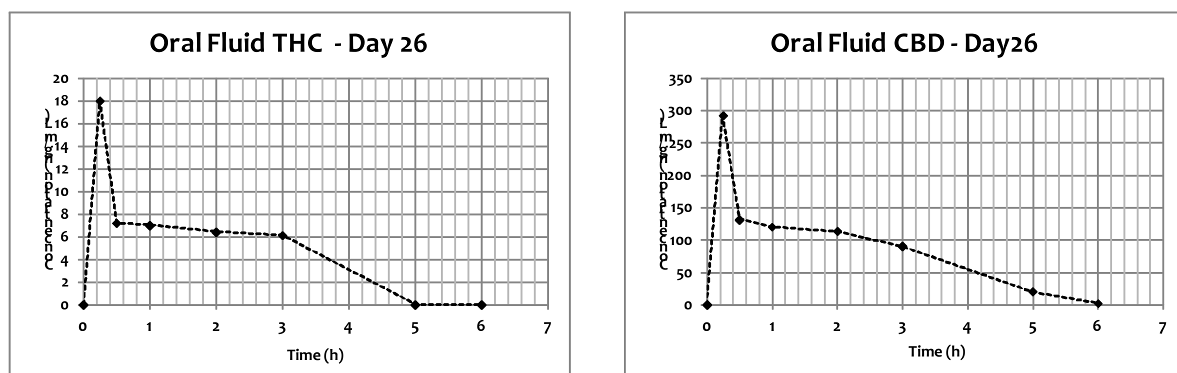

Figure 1. Time course of THC and CBD concentrations in oral fluid on day 26 before (OF-t_0) and after the daily smoking of the CBD-rich cannabis joint.

After a 25-day period of regular intake of CBD-rich cannabis joints, THC and CBD concentration in oral fluid before smoking (day 26) resulted negative. The first collection 0.25 h after the start of CBD-rich cannabis smoking revealed the highest THC and CBD concentrations at 18 and 292 ng/mL, respectively. These concentrations rapidly decreased after 15 min at the levels of 7.2 and 131 ng/mL for THC and CBD, respectively. THC was detectable in oral fluid at concentration of 6.1 ng/mL up to 3 h after the cannabis intake. The sample collected 5 h after the cannabis intake resulted negative for the presence of THC. Similarly, CBD concentration declined after a 5 h period to 20 ng/mL, while 6 h after the cannabis intake the measured CBD concentration was 1.7 ng/mL.

3.2. Hair

Head and pubic hair tested before the study turned out negative for the presence of CBD, THC and THC-COOH. Head and pubic hair collected one week after the end of the study revealed the presence of CBD at concentrations of 312 and 178 pg/mg, respectively. The same samples resulted negative for THC and THC-COOH.

4. Discussion

To the best of our knowledge, this is the first study simulating a repeated intake of CBD-rich cannabis over a prolonged period of time (26 days). Meier and co-workers published a study simulating a frequent use of cannabis (content: 34 mg CBD, 1.6 mg THC) following the intake of 2 joints per day by inhalation, for 10 consecutive days [8]. A maximum THC-COOH concentration of 42.5 ng/mL was observed in the urine sample collected on the day-8. In our study, the amount of daily consumed THC was substantially lower (0.5 mg) and corresponded to the consumption of a single legal joint per day. Nevertheless, the presence of THC-COOH in urine was ascertained at 13

224 ng/mL concentration since day-8. Moreover, on day-15 the urine sample collected before the daily
225 intake of cannabis turned out positive to THC-COOH at 15 ng/mL concentration, that is the
226 recognized international cut-off used to prove recent cannabis consumption in several prohibited
227 circumstances (e.g. workplace, driver license release/renewal, roadside controls, drug addiction,
228 firearm license, etc.) [19]. This finding is coherent with the known THC accumulation in the body fat
229 during the smoking period. On the other hand, after the initial 15 days of the observation period, the
230 THC-COOH urine concentrations showed a decrease, remaining below the LOQ in all samples.
231 Again, the observed decrease is in accordance with the influence of CBD on the excretion profile of
232 certain drugs (including THC), as already reported in the literature [20,21]. However, other factors
233 such as physical exercise, nutrition and other biological parameters may play a role in the individual
234 metabolism.

235 The THC and CBD concentrations in the oral fluid sample collected after a 25-day period of
236 regular intake of CBD-rich cannabis joints but shortly before the last consumption tested negative,
237 possibly excluding the occurrence of a cannabinoids accumulation in this matrix during the smoking
238 period. Conversely, the presence of THC in oral fluid was ascertained up to 3 h after the cannabis
239 intake at concentrations ranging from 6 to 18 ng/mL, while the measured THC/CBD ratio was below
240 0.1 in all samples. These findings are in accordance with previous studies published by Pacifici and
241 co-workers, who analyzed oral fluid after single and repeated intake of "light cannabis" (content: 58
242 mg CBD, 2.0 mg THC) [15,16]. In the single intake simulation study, THC was detected in oral fluid
243 in the concentration range 2.5-21.5 ng/mL 30 min after smoking, and then at a minimum value of 1.0
244 ng/mL after 3 h. As noted in our study, CBD concentrations were usually one order of magnitude
245 higher than those of THC. Considering the repeated intake simulation study [16] (four "light
246 cannabis" cigarette smoked in a 4h period for a total intake of 6.4 mg THC and 232 mg CBD), the
247 mean concentration of THC passed from 17.6 ng/mL (30 min after the last cannabis intake) down to
248 0.9 ng/mL (4 h after the last cannabis intake). Moreover, the THC/CBD ratio resulted below 2 in all
249 samples, suggesting a use of CBD-rich cannabis instead of THC-prevalent products [16].
250 Nevertheless, all these findings revealed that positive THC testing in oral fluid may occur for a 3-h
251 period following a smoking intake of cannabis products containing less than 0.2% of THC. This is
252 particularly relevant in the context of roadside and workplace drug testing, in which legal limits are
253 provided [22–24]. Finally, regarding the keratin matrix, hair samples (head and pubic) turned out
254 negative for the presence of THC and THC-COOH, while CBD was detected at high concentration in
255 them. In this case, the hair matrix proved able to discriminate the chronic consumption of CBD-rich
256 cannabis from the THC-prevalent products.

257 **5. Conclusions**

258 In conclusion, this study based on a single subject suggests that the repeated exposure to CBD-rich
259 cannabis (containing small amounts of THC) can generate positive results when biological samples
260 are tested. In particular, positive urine results for THC-COOH, using the confirmatory cut-off of 15
261 ng/mL, were obtained after a 15-days period of consumption. On the other hand, it was
262 demonstrated that there is no accumulation of cannabinoids in oral fluid after the repeated intake
263 experimented in this study. However, THC concentrations above the confirmatory cut-off of 2
264 ng/mL were observed obtained in the few hours after the CBD-rich cannabis products intake.
265 Finally, the experimental results indicate that no accumulation of THC and THC-COOH in the hair
266 matrix is likely to occur, thus suggesting the potential use of hair analysis to discriminate between
267 the repeated consumption of CBD-rich cannabis and THC-prevalent products. The main limitation
268 of this study is the small number of samples tested. Further studies, increasing the population
269 involved, should be carried out to corroborate these preliminary findings, which are of
270 ever-growing importance with the progressively increased availability of CBD-rich cannabis.
271 Considering the legal implications of a positive result, robust criteria for the interpretations of
272 cannabinoids levels in biological fluids are urgently needed.

273 **Supplementary Materials:** The following are available online at www.mdpi.com/xxx/s1, Table S1: main
274 validation parameters of the UHPLC-MS^n methods used in this study.

275 **Author Contributions:** Conceptualization, E.G. and A.S.; methodology, D.D., E.G. and A.S.; validation, D.D.
276 and S.P.B.; formal analysis, D.D. and S.P.B.; investigation, D.D. and E.G.; data curation, D.D. and E.G.;
277 writing—original draft preparation, E.G., A.S. and M.V.; writing—review and editing, E.G., A.S. and M.V.;
278 supervision, A.S and M.V.. All authors have read and agreed to the published version of the manuscript.

279 **Funding:** This research received no external funding.

280 **Acknowledgments:** In this section you can acknowledge any support given which is not covered by the author
281 contribution or funding sections. This may include administrative and technical support, or donations in kind
282 (e.g., materials used for experiments).

283 **Conflicts of Interest:** The authors declare no conflict of interest.
284

285

286

287 ## References

288 1. Mechoulam, R.; Parker, L.A.; Gallily, R. Cannabidiol: An Overview of Some Pharmacological Aspects. *The*
289 *Journal of Clinical Pharmacology* **2002**, *42*, 11S-19S, doi:10.1002/j.1552-4604.2002.tb05998.x.
290 2. Pisanti, S.; Malfitano, A.M.; Ciaglia, E.; Lamberti, A.; Ranieri, R.; Cuomo, G.; Abate, M.; Faggiana, G.; Proto,
291 M.C.; Fiore, D.; et al. Cannabidiol: State of the art and new challenges for therapeutic applications.
292 *Pharmacology & Therapeutics* **2017**, *175*, 133–150, doi:10.1016/j.pharmthera.2017.02.041.
293 3. Atalay, S.; Jarocka-Karpowicz, I.; Skrzydlewska, E. Antioxidative and Anti-Inflammatory Properties of
294 Cannabidiol. *Antioxidants* **2019**, *9*, 21, doi:10.3390/antiox9010021.
295 4. FDA Approves First Drug Comprised of an Active Ingredient Derived from Marijuana to Treat Rare,
296 Severe Forms of Epilepsy Available online:
297 https://www.fda.gov/news-events/press-announcements/fda-approves-first-drug-comprised-active-ingre
298 dient-derived-marijuana-treat-rare-severe-forms (accessed on Jun 9, 2020).
299 5. Manthey, J. Cannabis use in Europe: Current trends and public health concerns. *International Journal of*
300 *Drug Policy* **2019**, *68*, 93–96, doi:10.1016/j.drugpo.2019.03.006.
301 6. Hazekamp, A. The Trouble with CBD Oil. *MCA* **2018**, *1*, 65–72, doi:10.1159/000489287.
302 7. Hughes, B. *Cannabis legislation in Europe: an overview*; Publications Office of the European Union:
303 Luxembourg, 2017; ISBN 978-92-9497-107-4.
304 8. Meier, U.; Dussy, F.; Scheurer, E.; Mercer-Chalmers-Bender, K.; Hangartner, S. Cannabinoid
305 concentrations in blood and urine after smoking cannabidiol joints. *Forensic Science International* **2018**, *291*,
306 62–67, doi:10.1016/j.forsciint.2018.08.009.
307 9. Bridges, M., Hanson, K. Regulating Hemp and Cannabis-Based Products Available online:
308 https://www.ncsl.org/research/agriculture-and-rural-development/regulating-hemp-and-cannabis-based-
309 products.aspx (accessed on Jun 10, 2020).
310 10. Industrial Hemp Regulations Available online: https://laws-lois.justice.gc.ca/PDF/SOR-2018-145.pdf
311 (accessed on Jun 10, 2020).
312 11. Hädener, M.; Gelmi, T.J.; Martin-Fabritius, M.; Weinmann, W.; Pfäffli, M. Cannabinoid concentrations in
313 confiscated cannabis samples and in whole blood and urine after smoking CBD-rich cannabis as a "tobacco
314 substitute." *Int J Legal Med* **2019**, *133*, 821–832, doi:10.1007/s00414-018-01994-y.
315 12. Spindle, T.R.; Cone, E.J.; Kuntz, D.; Mitchell, J.M.; Bigelow, G.E.; Flegel, R.; Vandrey, R. Urinary
316 Pharmacokinetic Profile of Cannabinoids Following Administration of Vaporized and Oral Cannabidiol

317 and Vaporized CBD-Dominant Cannabis. *Journal of Analytical Toxicology* **2020**, *44*, 109–125,
318 doi:10.1093/jat/bkz080.
319 13. Bonn-Miller, M.O.; Loflin, M.J.E.; Thomas, B.F.; Marcu, J.P.; Hyke, T.; Vandrey, R. Labeling Accuracy of
320 Cannabidiol Extracts Sold Online. *JAMA* **2017**, *318*, 1708, doi:10.1001/jama.2017.11909.
321 14. Singer, L.; Tokish, H.; Park, F.; Campisi, C.; Milanaik, R.L. The cannabidiol conundrum: potential benefits
322 and risks of cannabidiol products for children. *Current Opinion in Pediatrics* **2020**, *32*, 198–205,
323 doi:10.1097/MOP.0000000000000861.
324 15. Pacifici, R.; Pichini, S.; Pellegrini, M.; Tittarelli, R.; Pantano, F.; Mannocchi, G.; Rotolo, M.C.; Busardò, F.P.
325 Determination of cannabinoids in oral fluid and urine of "light cannabis" consumers: a pilot study. *Clinical*
326 *Chemistry and Laboratory Medicine (CCLM)* **2018**, *57*, 238–243, doi:10.1515/cclm-2018-0566.
327 16. Pacifici, R.; Pichini, S.; Pellegrini, M.; Rotolo, M.C.; Giorgetti, R.; Tagliabracci, A.; Busardò, F.P.; Huestis,
328 M.A. THC and CBD concentrations in blood, oral fluid and urine following a single and repeated
329 administration of "light cannabis." *Clinical Chemistry and Laboratory Medicine (CCLM)* **2020**, *58*, 682–689,
330 doi:10.1515/cclm-2019-0119.
331 17. Salomone, A.; Tsanaclis, L.; Agius, R.; Kintz, P.; Baumgartner, M.R. European guidelines for workplace
332 drug and alcohol testing in hair. *Drug Testing and Analysis* **2016**, *8*, 996–1004, doi:10.1002/dta.1999.
333 18. Salomone, A.; Gerace, E.; D'Urso, F.; Di Corcia, D.; Vincenti, M. Simultaneous analysis of several synthetic
334 cannabinoids, THC, CBD and CBN, in hair by ultra-high performance liquid chromatography tandem
335 mass spectrometry. Method validation and application to real samples. *Journal of mass spectrometry : JMS*
336 **2012**, *47*, 604–610.
337 19. Penders, J.; Verstraete, A. Laboratory guidelines and standards in clinical and forensic toxicology. *Accred*
338 *Qual Assur* **2006**, *11*, 284–290, doi:10.1007/s00769-006-0131-y.
339 20. Lucas, C.J.; Galettis, P.; Schneider, J. The pharmacokinetics and the pharmacodynamics of cannabinoids. *Br*
340 *J Clin Pharmacol* **2018**, *84*, 2477–2482, doi:10.1111/bcp.13710.
341 21. van de Donk, T.; Niesters, M.; Kowal, M.A.; Olofsen, E.; Dahan, A.; van Velzen, M. An experimental
342 randomized study on the analgesic effects of pharmaceutical-grade cannabis in chronic pain patients with
343 fibromyalgia: *PAIN* **2019**, *160*, 860–869, doi:10.1097/j.pain.0000000000001464.
344 22. Bosker, W.M.; Huestis, M.A. Oral Fluid Testing for Drugs of Abuse. *Clinical Chemistry* **2009**, *55*, 1910–1931,
345 doi:10.1373/clinchem.2008.108670.
346 23. Van der Linden, T.; Legrand, S.-A.; Silverans, P.; Verstraete, A.G. DUID: Oral Fluid and Blood
347 Confirmation Compared in Belgium. *Journal of Analytical Toxicology* **2012**, *36*, 418–421,
348 doi:10.1093/jat/bks038.
349 24. Brcak, M.; Beck, O.; Bosch, T.; Carmichael, D.; Fucci, N.; George, C.; Piper, M.; Salomone, A.; Schielen, W.;
350 Steinmeyer, S.; et al. European guidelines for workplace drug testing in oral fluid. *Drug Testing and Analysis*
351 **2018**, *10*, 402–415, doi:10.1002/dta.2229.
352

© 2020 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).

353