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Cannabinoids in oral fluid following passive exposure to marijuana smoke

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ABSTRACT

The concentration of tetrahydrocannabinol (THC) and its main metabolite 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH) as well as cannabinol (CBN), and cannabidiol (CBD) were measured in oral fluid following realistic exposure to marijuana in a Dutch coffee-shop. Ten healthy subjects, who were not marijuana smokers, volunteered to spend 3 h in two different coffee shops in Groningen, The Netherlands. Subjects gave two oral fluid specimens at each time point: before entering the store, after 20 min, 40 min, 1 h, 2 h, and 3 h of exposure. The specimens were collected outside the shop. Volunteers left the shop completely after 3 h and also provided specimens approximately 12–22 h after beginning the exposure. The oral fluid specimens were subjected to immunoassay screening; confirmation for THC, cannabinol and cannabidiol using GC/MS; and THC-COOH using two-dimensional GC–GC/MS. THC was detectable in all oral fluid specimens taken 3 h after exposure to smoke from recreationally used marijuana. In 50% of the volunteers, the concentration at the 3 h time-point exceeded 4 ng/mL of THC, which is the current recommended cut-off concentration for immunoassay screening; the concentration of THC in 70% of the oral fluid specimens exceeded 2 ng/mL, currently proposed as the confirmatory cut-off concentration. THC-COOH was not detected in any specimens from passively exposed individuals. Therefore it is recommended that in order to avoid false positive oral fluid results assigned to marijuana use, by analyzing for only THC, the metabolite THC-COOH should also be monitored.

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1. Introduction

Tetrahydrocannabinol (THC) is the active ingredient in marijuana and is generally administered orally or by smoking, resulting in euphoria and hallucinations. The utility of oral fluid as a matrix for testing drugs of abuse has been reported, with applications in many areas including roadside and forensic testing. THC is the predominant analyte detected in oral fluid following marijuana ingestion, however there are issues regarding the stability of the drug, since THC adheres to polystyrene surfaces. There are also concerns about the variability in collection devices and the potential for passive exposure to cannabis causing a false positive test result. It has been reported that native THC can be detected in various biological specimens including urine and blood following passive exposure to cannabis smoke [1,2]. While there is less literature available for oral fluid testing there are two reports addressing this issue. One paper concluded that the risk of positive oral fluid tests from passive cannabis smoke inhalation is limited to a period of approximately 30 min following exposure [3] and a follow-up report showed that oral fluid specimens collected in the presence of cannabis smoke appear to have been contaminated, so falsely raising the measured concentration of THC in oral fluid. When specimens were collected outside the contaminated area, the risk of a positive test for THC was virtually eliminated [4].

Since oral fluid is being considered in the USA as a potential matrix for workplace testing [5], the possibility of passive contamination should be considered further and under realistic conditions. If low concentrations of THC are detectable following passive exposure, then there exists the possibility that an individual may test positively even though he/she did not actively partake in smoking marijuana. The presence of a metabolite in oral fluid would greatly diminish the chances of passive exposure causing false positive results.

This study addressed these concerns by using a Quantisal™ collection device, so that the amount of neat oral fluid collected was known, making quantitative results meaningful. Secondly, the identification of THC-COOH in the oral fluid sample effectively ruled out a passive exposure defense. Normally in drug testing assays, THC is the target analyte for confirmatory testing of oral

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fluid samples, since the concentration of its metabolite, THC-COOH is very low in oral fluid, and therefore requires separate testing methodology. Research groups have attempted to analyze for THC-COOH in oral fluid, but the sensitivity required has made detection difficult using either single quadrupole gas chromatography–mass spectrometric (GC/MS) or liquid chromatography with tandem mass spectral detection (LC–MS/MS) systems. Recently, we developed and reported the detection of THC-COOH in oral fluid using two-dimensional (2d) gas chromatography, cryogenic focusing and negative ion chemical ionization detection (GC–GC/MS) [6], a methodology also used and modified by other researchers [7]. Day et al. used gas chromatography with tandem mass spectrometry (GC–MS/MS) to report similar concentrations of THC-COOH in oral fluid [8]. Later, we also reported that while THC is not bound to glucuronides in saliva, the metabolite THC-COOH is glucuronidated and higher concentrations can be measured following base hydrolysis of the oral fluid specimens [9].

With the collection protocol and analytical procedures in place, the aim of this study was to investigate whether the main marijuana metabolite THC-COOH, is detectable in oral fluid following realistic passive exposure to cannabis. The specimens were analyzed for THC, cannabinol, cannabidiol and THC-COOH using immunoassay as well as GC/MS for THC, cannabinol and cannabidiol; and for THC-COOH by two-dimensional GC–GC/MS.

2. Experimental

Volunteers. The study protocol was authorized by Immunalysis Institutional Review Board in March 2011. Ten healthy Caucasian individuals, five males with an average age of 22.8 y; 84 kg (185 lb); height 1.9 m (6 ft 2 in.); BMI 23.3; and five females with an average age of 23.8 y; weight 62.4 kg (137 lb); height 1.71 m (5 ft 6 in.); BMI 21.2 were exposed to marijuana smoke for 3 h, at two different coffee shop locations in Groningen, The Netherlands (Table 1). Dutch coffee shops are areas where smoking marijuana and hash is permitted under strict conditions. At location #1 the dimensions of the smoking area were 5 m length × 7 m wide ×3.5 m high (16 ft × 23 ft × 11 ft) and the number of active smokers ranged from 4 to 16 (mean 8; median 7) during the exposure time. Location #2 measured 2 m × 7 m × 3 m (6.5 ft × 23 ft × 10 ft) and the number of active marijuana smokers present ranged from 0 to 6 smokers (mean 2.5; median 2).

Two oral fluid specimens per volunteer were collected sequentially using the Quantisal™ oral fluid collection device prior to entering the shop; then two specimens were collected sequentially at the following time points: 20 min, 40 min, 60 min, 120 min, and 180 min during passive exposure to marijuana. Samples were collected outside the coffee shop, on the street. Volunteers left the shops after 3 h of exposure time. A final collection was carried out between 12 and 22 h (average 14.6 h) after leaving the coffee shop. In addition, one oral fluid collection pads at location #1 and two pads at location #2 were opened and allowed to remain on the table of the shop throughout the exposure time-frame.

Table 1
Physical characteristics of subjects.

Subject	Age	Gender	Weight (kg)	Height (m)	BMI (kg/m^2)
S1	23	M	77	1.9	21.33
S2	22	M	88	1.92	23.87
S3	22	F	75	1.82	22.64
S4	23	F	57	1.67	20.44
S5	24	F	65	1.74	21.47
S6	23	M	83	1.86	23.99
S7	23	M	90	1.95	23.67
S8	23	M	84	1.87	24.02
S9	25	F	55	1.58	22.03
S10	25	F	60	1.75	19.59

3. Materials and methods

3.1. Reagents and consumables

Enzyme linked immunosorbent assay (ELISA) kit for the analysis of cannabinoids in saliva/oral fluids (Catalog #224) was obtained from Immunalysis Corporation (Pomona, CA).

3.2. Specimen collection

Quantisal™ oral fluid collection devices (Immunalysis Corporation) were used for sample collection. The device indicates when 1 mL (±10%) of neat oral fluid has been obtained. The pad is then placed into a transportation buffer, designed for maximum recovery of the main drug classes from the collection pad and drug stability in the buffer during transportation. Specimens were not exposed to fluorescent light for extended periods of time, nor were the serum separators allowed to remain in the collection tubes upon laboratory receipt. The oral fluid specimens were shipped from Groningen, The Netherlands, chilled, to the testing facility in Pomona, CA.

3.3. Drug recovery

The percentage recovery of THC-COOH at a concentration of 10 pg/mL from the Quantisal™ oral fluid collection device (80%) has been previously reported [6]; the recoveries of cannabinol (78.2), cannabidiol (71.9%) and THC (89.2%) at a concentration of 4 ng/mL have also been documented [10].

3.4. Sample preparation and analysis

3.4.1. Immunoassay

All specimens were analyzed using enzyme linked immunosorbent assays (ELISA). For THC in saliva/oral fluid a cut-off concentration of 4 ng/mL was used. Δ^9-THC was used as the calibrator (100% cross-reactivity). The kit showed cross-reactivity of 66% towards Δ^8-THC; 4% for cannabinol, 50% for cannabidiol and THC-COOH. A low positive control (2 ng/mL), a cut-off calibrator (4 ng/mL) and a high positive control (8 ng/mL) were analyzed with the specimens. Briefly, an aliquot of the calibrator, control or specimen (25 μL) was added to each well of the microplate along with pre-incubation buffer (25 μL) and the plate was allowed to remain in the dark at room temperature for 30 min. The enzyme conjugate (50 μL) was added to each well and the plate was incubated for 1 h at room temperature. The plate was then washed six times with deionized water, then 3,3″,5,5″-tetra-methylbenzidine with hydrogen peroxide in buffer (TMB) was added as the chromogenic substrate (100 μL). The plate was allowed to remain at room temperature in the dark for 30 min, and then 1 N HCl (100 μL) was added to stop the reaction. The absorbance of each well of the plate was read at a dual wavelength of 450 nm and 650 nm.

3.5. Confirmation

Regardless of the result using immunoassay, all specimens were analyzed for THC, cannabinol and cannabidiol according to a previously published fully validated procedure [10]. In the method the limit of quantitation (LOQ) for THC and CBN was 0.5 ng/mL; CBD 1 ng/mL. In addition, all specimens were analyzed for THC-COOH according to a previously published fully validated procedure, which included base hydrolysis of the oral fluid specimen, and a LOQ of 2 pg/mL [6].

4. Results and discussion

The results from the immunoassay (ELISA) are presented in Tables 2a and 2b. Specimens showing absorbance (B) divided by the negative absorbance (Bo) × 100% of less than that of the cut-off concentration were considered to be positive. The immunoassay results correlated exactly with the confirmation data, in that all presumptive positive specimens identified (>4 ng/mL) confirmed for the presence of THC using GC/MS. Even though some of the confirmatory data were less than 4 ng/mL, the immunoassay cross-

Table 2a
ELISA absorbance values based on THC as calibration standard.

Calibrator concentration	Absorbance (B)	B/Bo (%)
Negative	2.62	100
2 ng/mL	1.36	51.9
4 ng/mL (cut-off)	**0.97**	**37.0**
8 ng/mL	0.67	25.5

Absorbance and B/Bo of the cut-off calibrator are shown in bold.

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Table 2b
Results of oral fluid ELISA analysis: specimens with absorbance values lower than 37% were considered positive (bold) relative to a 4 ng/mL THC cut-off concentration; specimens with an absorbance value greater than 37% were considered negative relative to a 4 ng/mL THC cut-off concentration.

Exposure time (min)	Coffee shop									
	Location #1					Location #2				
	S1(M)	S2(M)	S3(F)	S4(F)	S5(F)	S6(M)	S7(M)	S8(M)	S9(F)	S10(F)
0	83.4	80.9	91.4	90.1	93.5	93.6	97.8	88.6	97.3	98.6
20	60.2	57.7	46.6	49.1	52.0	64.7	75.0	77.7	81.1	72.2
40	44.6	43.3	45.5	50.5	47.1	65.3	73.8	78.7	74.9	77.9
60	42.2	37.1	42.3	45.5	59.9	55.0	71.3	69.8	80.6	78.4
120	**27.2**	**21.5**	**21.8**	**33.2**	48.5	75.0	75.0	76.2	67.3	79.1
180	**31.5**	**26.7**	**23.2**	45.7	43.1	**28.7**	41.8	**13.2**	**18.5**	53.8
Exposure ended after 3 h (180 min)										
12–22 h	54.3	62.7	73.0	92.3	85.9	74.9	83.4	88.2	89.3	62.6

reacts with other cannabinoids which may be present causing some degree of inhibition in the screening assay.

4.1. THC and CBN

Location #1. The larger of the coffee shops was #1, which also had more active smokers present during the experiment (mean 8; median 7). The data for the concentration of THC detected in oral fluid are shown in Table 3 and Fig. 1. All volunteers were negative for cannabinoids before entering the shop. In this location, THC was detected in all the oral fluid samples from all five subjects over the 20 min to 3 h period; and in three of the five it was present at a concentrations >4 ng/mL after 2 h of exposure; in two of those subjects the concentrations remained above 4 ng/mL after 3 h. Cannabinol was detected at the 2 and 3 h time-points in three of the subjects (Table 4).

Location #2. Again, all volunteers were negative for cannabinoids before entering the shop. The smaller shop had less frequent traffic in terms of active marijuana smokers (mean 2.5; median 2). Therefore, it was not surprising that THC was not detected for the first few time-points in any volunteers. However, interestingly, at the 3 h time-point, relatively high concentrations of THC were detected in three subjects (5, 12 and 17 ng/mL). Cannabinol was also detected at the 3 h time-points in those three subjects (Table 4). It is possible that the proximity of these individuals to active smokers may have become intense at that time-point.

4.2. CBD

Cannabidiol (CBD) was not detected in any specimens. The Dutch "Nederwiet" or "Nederweed" (dried cannabis flowers) contain low amounts of CBD, since the illegal growers of marijuana attempt to achieve higher concentrations of THC in the plant at the expense of CBD; therefore the fact that CBD was not detected in passive smokers was not unexpected. The pads which were left exposed in the store had no THC-COOH present; but were positive for THC at 290, 212 and 216 ng/mL; CBD and CBN at much lower concentrations: CBD: 16, 28 and 38 ng/mL; CBN 48, 40 and 42 ng/mL indicating that cannabinoids are absorbed easily into the surrounding atmosphere.

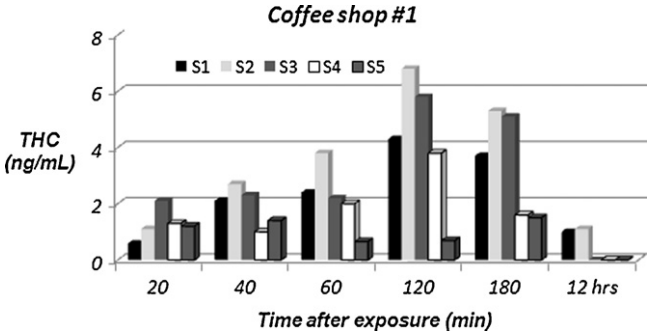

Fig. 1. THC detected in oral fluid after passive exposure to marijuana smoke.

4.3. THC-COOH

The metabolite THC-COOH was not detected in the open pads or in any of the specimens using a limit of quantitation (LOQ) of 2 pg/mL. Other studies have characterized the disposition of THC-COOH in oral fluid following the ingestion of Marinol®. THC was present in only 20.8% of specimens, while THC-COOH was the most prevalent analyte being identified in 98.2% of samples using a limit of quantitation of 7.5 pg/mL. Those authors noted that the identification of THC-COOH in oral fluid minimized the possibility

Table 3
Concentration of THC (ng/mL) in oral fluid detected in subjects realistically exposed to marijuana smoke for 3 h.

Time (min)	Coffee shop									
	Location #1					Location #2				
	S1(M)	S2(M)	S3(F)	S4(F)	S5(F)	S6(M)	S7(M)	S8(M)	S9(F)	S10(F)
0	0	0	0	0	0	0	0	0	0	0
20	**0.5**	**1.1**	**2.1**	**1.3**	**1.2**	0	0	0	0	0
40	**2.1**	**2.7**	**2.3**	**1**	**1.4**	0	0	0	0	0
60	**2.4**	**3.8**	**2.2**	**2**	**0.6**	**0.9**	0	0	0	0
120	**4.3**	**6.8**	**5.8**	**3.8**	**0.6**	0	0	0	**0.7**	0
180	**3.7**	**5.3**	**5.1**	**1.6**	**1.5**	**5.1**	**2.3**	**17**	**12**	**1.3**
12–22 h	**1.0**	**1.1**	0	0	0	0	0	0	0	0

S, subject; M, male; F, female; THC concentrations shown in bold.
Location #1: 5 m length × 7 m wide × 3.5 m high (16 ft × 23 ft × 11 ft); active smokers 4–16 (mean 8). Location #2: 2 m × 7 m × 3 m (6.5 ft × 23 ft × 10 ft); active smokers 0–6 (mean 2.5).

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Table 4
Concentration of cannabinol (ng/mL) in oral fluid detected in subjects realistically exposed to marijuana smoke for 3 h.

Time (min)	Coffee shop									
	Location #1					Location #2				
	S1(M)	S2(M)	S3(F)	S4(F)	S5(F)	S6(M)	S7(M)	S8(M)	S9(F)	S10(F)
0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0	0	0
60	0	0	0	0	0	0	0	0	0	0
120	0	**1.7**	**1.2**	0	**0.8**	0	0	0	0	0
180	0	**0.5**	**1.6**	0	**0.5**	**1.1**	0	**2.0**	**1.6**	0
12–22 h	0	0	0	0	0	0	0	0	0	0

S, subject; M, male; F, female; CBN concentrations shown in bold.
Location #1: 5 m length × 7 m wide × 3.5 m high (16 ft × 23 ft × 11 ft); active smokers 4–16 (mean 8). Location #2: 2 m × 7 m × 3 m (6.5 ft × 23 ft × 10 ft); active smokers 0–6 (mean 2.5).

of passive inhalation and that THC-COOH may be a better analyte for the detection of cannabis use [11]. Our data presented here affirms that conclusion under realistic exposure conditions.

5. Summary

In accordance with other passive exposure studies, it has been demonstrated that THC was absorbed by volunteers under realistic conditions when exposed to marijuana smoke. The concentrations detected in some volunteers after only 2 h of exposure were above proposed cut-offs for both screening and confirmatory assays. However, the metabolite THC-COOH was not detected in any of the specimens using a highly sensitive two dimensional GC–GC/MS technique. Therefore it is recommended that in order to avoid false positive oral fluid results assigned to marijuana by analyzing for only THC, the metabolite THC-COOH is also monitored.

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