Comparison of Cannabinoid Concentrations in Plasma, Oral Fluid and Urine in Occasional Cannabis Smokers After Smoking Cannabis Cigarette

A.Marsot[1], C. Audebert[1], L. Attolini[1], B. Lacarelle[2], J. Micallef[1], O. Blin[1]

[1.] Service de Pharmacologie Clinique et Pharmacovigilance, AP-HM, Pharmacologie intégrée et interface clinique et industrielle, Institut des Neurosciences Timone – AMU-CNRS 7289, Aix Marseille Université, 13385 Marseille.
[2.] Service de Pharmacocinétique Toxicocinétique, AP-HM, Marseille, France.

Received, September 15, 2016; Revised, October 24, 2016; Accepted, October 24, 2016; Published, October 25, 2016.

ABSTRACT - Purpose. A randomized cross-over, double blind placebo controlled study of smoked cannabis was carried out on occasional cannabis smokers. The objective of this research was to describe the pharmacokinetic parameters of THC and its metabolites in plasma, oral fluid and urine, from samples obtained simultaneously to provide estimations of THC and metabolites concentrations after smoking a cannabis cigarette. **Methods**. Blood, oral fluid and urine samples were collected until up to 72 h after smoking the cannabis cigarette (4% of delta-9-tetrathydrocannabinol (THC)). THC, 11-OH-THC and THC-COOH were analyzed by gas-chromatography-mass spectrometry. Pharmacokinetic parameters were estimated from these data**. Results**. Eighteen male healthy adults participated in the study. In total, 560 plasma, 288 oral fluid and 448 urine samples were quantified for cannabinoids. Plasma, oral fluid and urine pharmacokinetic parameters were calculated. A wide range of median THC C_{max} (1.6-160.0 µg/L and 55.4-123120.0 µg/L in plasma and oral fluid, respectively), 11-OH-THC C_{max} (0-11.1 µg/L in plasma) and THC-COOH C_{max} (1.0-56.3 µg/L in plasma) was observed. When expressed as a percentage of the total available THC dose, and corrected for molar equivalents, mean percentage of total THC dose excreted was 1.9 +/-2.5 % with range of 0.2-7.5%. This high inter-individual variability was also observed on other calculated pharmacokinetic parameters. **Conclusion**. Prediction of plasma THC concentration from THC oral fluid concentration or from THC-COOH urinary concentrations is not feasible due to the large variations observed. The results from this study support the assumption that a positive oral fluid THC result or a positive urine fluid result are indicative of a recent cannabis exposure.

INTRODUCTION

Cannabis is the most widely used drug that is still illegal in many part of the world. In addition, delta-9-tetrahydrocannabinol (THC) is frequently detected in the blood or saliva of impaired drivers suspicious of erratic driving or involved in road accidents [1,2]. Although urine and plasma are commonly utilized for cannabinoid testing, the acceptance of oral fluid as an alternative testing device matrix has increased in the past two decades [3]. Oral fluid is an attractive drug-testing tool because the procedure for obtaining the desired specimens is easier, safer and less invasive as compared to urine and plasma.

The knowledge of the pharmacological properties od cannabis in saliva, as an alternative body fluid, is of great importance when this method it should serve as a biological specimen for roadside testing. This is a critical step to allow the assessment of cannabinoid concentration after cannabinoid exposure determination and eventually evaluating driving impairment. THC is the primary psychoactive constituent of cannabis and also one of the main analytes detected in both, oral fluid and plasma [4]. The short-term plasma pharmacokinetics of THC has been relatively well characterized. The inhalation method (by smoking a cannabis cigarette) yields a rapidly rising plasma concentrations with a high peak within a few minutes. Systemic inhaled bioavailability is between 10% in light users, and 23% in heavy users [5]. THC is mainly metabolized in the liver, by cytochrome P450 enzymes such as CYP2C9, CYP2C19 and CYP3A [6,7] which, in turn, is rapidly oxidized to an active metabolite, 11-hydroxy-THC (11-OH-THC) and further to THC-COOH [8].

Corresponding Author : Amélie MARSOT **;** Service de Pharmacologie Clinique **;** Unité de Pharmacométrie **;** Bâtiment F **;** 264 rue Saint Pierre **;** 13005 Marseille, France **;** E-mail : amelie.marsot@ap-hm.fr

The primary metabolite 11-OH-THC is at least as potent as THC, has a similar pharmacokinetic profile, and probably contributes significantly to the effects observed after THC administration whereas THC-COOH is an inactive metabolite [4,9].

The reported few clinical studies, were mainly short termed, with limited information on the metabolites [10-12]. Information on the simultaneous concentration and time-course of THC and its metabolites in oral fluid, plasma and urine are, therefore, needed. Such data would aid the interpretation of test results and enhance the value of impairment assessments involving oral fluid and urine testing. The objective of this research was to describe the pharmacokinetic parameters of THC and its metabolites in plasma, oral fluid and urine, from samples obtained simultaneously to provide estimations of THC and metabolites concentrations after smoking cannabis cigarette.

METHODS

Participants

Male volunteers tobacco smokers (3-8 cigarettes per day), cannabis occasional users (a minimum of one joint per month and a maximum of one joint per week), aged 20 to 45 years, were recruited from the local community. The main inclusion criteria were weight in +/-10% ideal weight, negative urine cannabis test, negative alcohol breath test, a coffee or tea consumption of less than 5 cups per day, without psychiatric troubles (psychiatric interview with scale of Eysenck, scale of anxiety of Cattel, scale of search of sensation of Zuckerman and Barrage tests) and clinically significant abnormality on physical examations and standard biological screening tests. Participants were excluded if they were participants in an official sports competition, or psychoactive medication dependence in the past or at date. Participants were also required to have a seizure-free history, no reported severe head trauma, dementia, or other conditions associated to significant cognitive impairment; no reported heart attack or major cardiac events.

The study was conducted in the Clinical Investigation Center of Marseille (Assistance Publique des Hôpitaux de Marseille) in collaboration with the French Directorate of Security and Road Traffic. The study was approved by the local Ethics Committee (Marseille 2) and the French Drug Agency. The research was conducted in accordance with the 1964 Helsinki Declaration and Good Clinical Practices. Participants gave their written informed consent prior to participation.

Study design

After their full informed consent and screening procedures, eligible subjects were included in a randomized cross over, double blind placebo controlled study. The study comprises an inclusion visit and two sessions separated by a four-week washout. Therefore, all subjects will receive the two products tested (tobacco with and without THC) at a 4-week interval throughout the study. Subjects were asked to abstain from cannabis for 28 days prior to the session. They were also asked to avoid any over-the-counter medication without the investigator's approval. Subjects were asked to abstain from caffeine and alcohol for 12 h prior to and after each experimental session. One subject reported daily tobacco cigarette and THC use was also asked to abstain from smoking for 12 h prior to the session. Participants were hospitalized in the clinical unit of Clinical Investigation Center of Marseille the night before the trial and toxicological blood and urine tests were performed by Laboratory of pharmacokinetics of Marseille (Assistance Publique des Hôpitaux de Marseille). They were allowed to leave the centre in the evening after medical examination. Baseline measurements for biological samples were performed before the start of smoking. The cannabis cigarette contained 20 mg of THC (500 mg cannabis with 4% THC) added to tobacco (DRUM®). Subjects were instructed to smoke the cannabis cigarette under medical supervision, according a standardized computerized procedure described by Leirer et al. [13] to minimize inter subject variability: to inhale the smoke as deeply as possible, hold each inhalation for approximately 4 s and then exhale. This sequence was repeated until the cigarette was smoked as completely as possible within maximum 30 minutes. Participants provided blood, saliva and urine samples up to 72 h after smoking initiation.

Biological fluids collection

At the beginning of the session, prior to smoking procedure, a catheter was inserted into a forearm vein of the subject. Blood was drawn through the catheter into a cooled vacutainer tube containing dipotassium ethylenediaminetetraacetic acid (EDTA). Oral fluid was collected with Salivette Sarstedt system (Nümbrecht Germany). Blood and oral fluid was sampled at baseline and 1, 5, 10, 15, 20, 30 minutes, 1, 2, 4, 6, 8, 12, 24, 48 and 72 h after the onset of smoking; and six urine samples were collected at 1, 2, 4, 8, 12 and 24 h.

Analyses

Plasma, oral and urinary specimens from all subjects were analyzed by gas chromatography with tandem mass spectrometry detection for THC, 11-OH-THC and THC-COOH in the laboratory of pharmacokinetics of Marseille. The mass selective detector was operated in electron ionization-selected ion monitoring (SIM) mode. All fluids used a THC and metabolites assay with a limit of quantification (LOQ) of 1.0 ng/ml.

Pharmacokinetics

We performed noncompartmental analysis with Microsoft Excel 2013. The maximum concentration (C_{max}), time to maximum concentration (T_{max}) and time of the last observed concentration (T_{last}) were obtained from the kinetics. Excretion rate of THC-COOH in urine was calculated. To determine the percentage of total dose excreted as THCCOOH, the molar equivalent dose of THC to THCCOOH was calculated. The adjusted total dose was divided by the cumulative amount of THCCOOH excreted by each individual. The areas under the curve (AUC_{0-t}) from 0 to 72h for plasma and oral samples were estimated using the trapezoidal rule. The elimination half-lives ($T_{1/2}$) were calculated by log-linear regression of the concentration-time curves. Clearance (CL) and volume of distribution (V_d) were calculated from the previously calculated parameters. For statistical purposes, concentrations less than the limit of quantification (LOQ) were set to 0. Oral fluid/plasma and metabolite ratios were calculated when quantifiable (positive) data were available. We compared plasma and oral fluid concentrations, urinary excretion and pharmacokinetic parameters for THC, 11-OH-THC and THC-COOH.

RESULTS

Eighteen male healthy adults (age 20-28 years) participated in the study (Table1). Data were available for 14 subjects for THC plasma observations, for all subjects for THC oral fluid observations and for 7 subjects for THC urine observations (Table 2). Concerning 11-OH-THC, data were available for only 7 subjects in plasma and urine fluids, and THC-COOH observations were available for 14 subjects for plasma and urine fluids. The missing data are due to sampling or analytical problems.

In total, 560 plasma, 288 oral fluid and 448 urine samples were quantified for cannabinoids. Plasma and oral fluid pharmacokinetic parameters are presented in table 2. A high range of THC C_{max} (1.6-160.0 µg/L and 55.4-123120.0µg/L in plasma and oral fluid, respectively), 11-OH-THC C_{max} (0-11.1 µg in plasma) and THC-COOH C_{max} (1.0-56.3 µg/L in plasma) was observed (Table 2). When expressed as a percentage of the total available THC dose, and corrected for molar equivalents, mean percentage of total THC dose excreted was 1.9 +/-2.5 % with range of 0.2-7.5%. This wide interindividual variability was also observed on other calculated pharmacokinetic parameters (Table 2). Table 3 shows a comparison of $T_{1/2}$ urinary excretion rate of THC-COOH and $T_{1/2}$ of plasma THC.

Figure 1 describes individual concentrations of THC, 11-OH-THC and THC-COOH in plasma versus time. The analysis of the concentration-time curve of THC in plasma shows a marked decrease in the thirty minutes after smoking which is equivalent to the distribution phase and plasma THC reached its highest concentration first, followed by 11-OH-THC and by THC-COOH (Figure 1). Figure 2 describes individual THC concentrations in oral fluid versus time. Figure 3a represents individual urinary excretion rate of THC-COOH versus mid time collection and figure 3b represents individual urinary excretion rate of THC-COOH versus AUC of THC.

Table 4 shows oral fluid/plasma THC ratios. Median (range) oral fluid/plasma THC ratio was 59.34 (10.33-82.58).Table 5 shows metabolites ratios in plasma. Median (range) 11-OH-THC/THC in plasma was 0.06 (0.02-0.08). These metabolite ratios did not vary by time. Median (range) THC-COOH/THC in plasma was 1.41 (0.10-8.24). These metabolite ratios showed a substantial inter-individual variability.

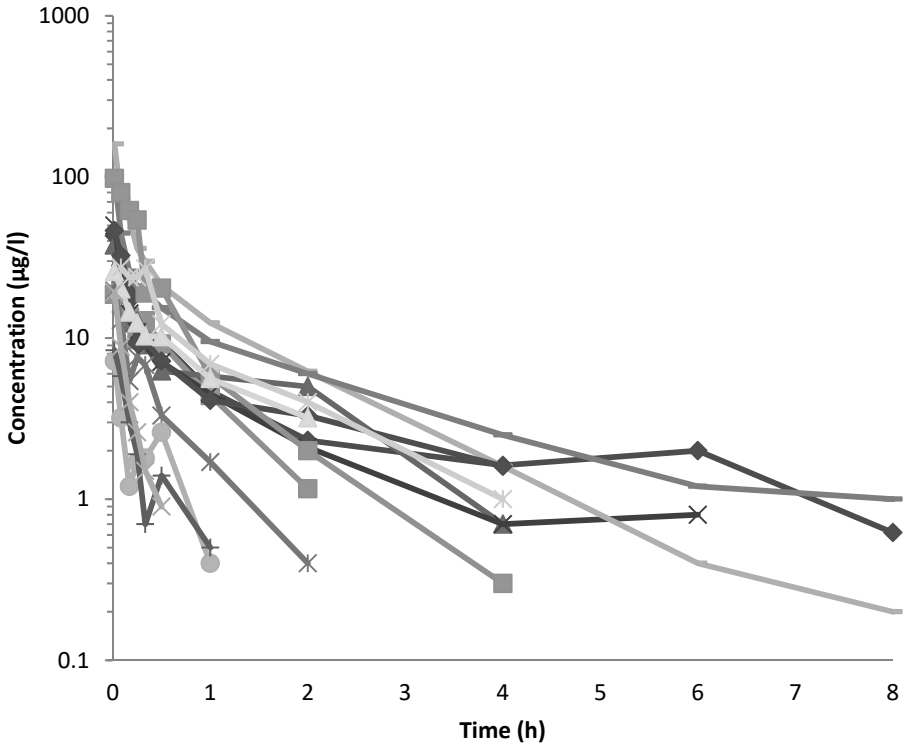
DISCUSSION

The combination of oral fluid (THC), plasma (THC, 11-OH-THC and THC-COOH) and urinary (THC, 11-OH-THC and THC-COOH) concentrations provided an opportunity to compare excretion rates in the three biological fluids. We calculated the pharmacokinetic parameters of THC and metabolites in plasma, oral fluid and urine after administration of mean doses of THC through inhalation for 72 h after onset of smoking. The main interest of the study is to provide values of THC and its metabolites as pharmacokinetic parameters after cannabis cigarettes containing cannabis with a mean concentration of THC mixed with tobacco.

The actual quantity of cannabis smoked was equal to 0.5 g with 4% of THC. The total quantity of THC used during smoking session was approximately 20 mg., As a comparison, Mariani et al [14] reported that an amount of 0.66 g is used in making joints in the USA (generally uncut with

tobacco) while typical European joints contain 0.33-0.4 g of plant material and 20-50 mg of THC [10].

A



B

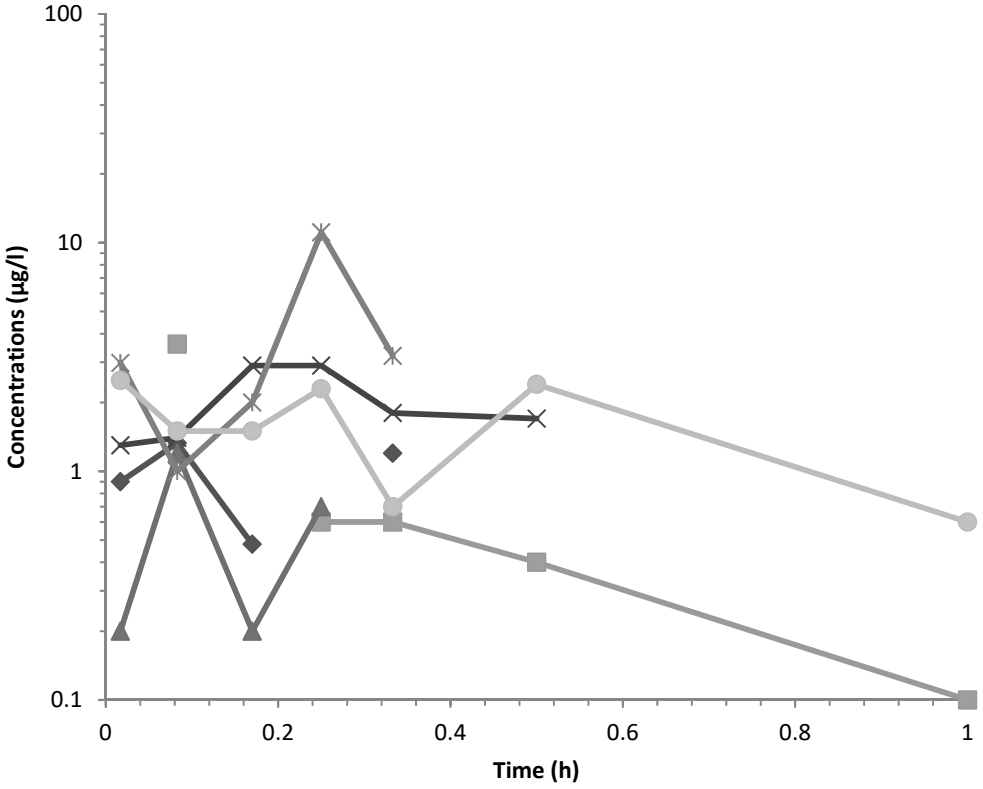


Figure 1. Continued…

C

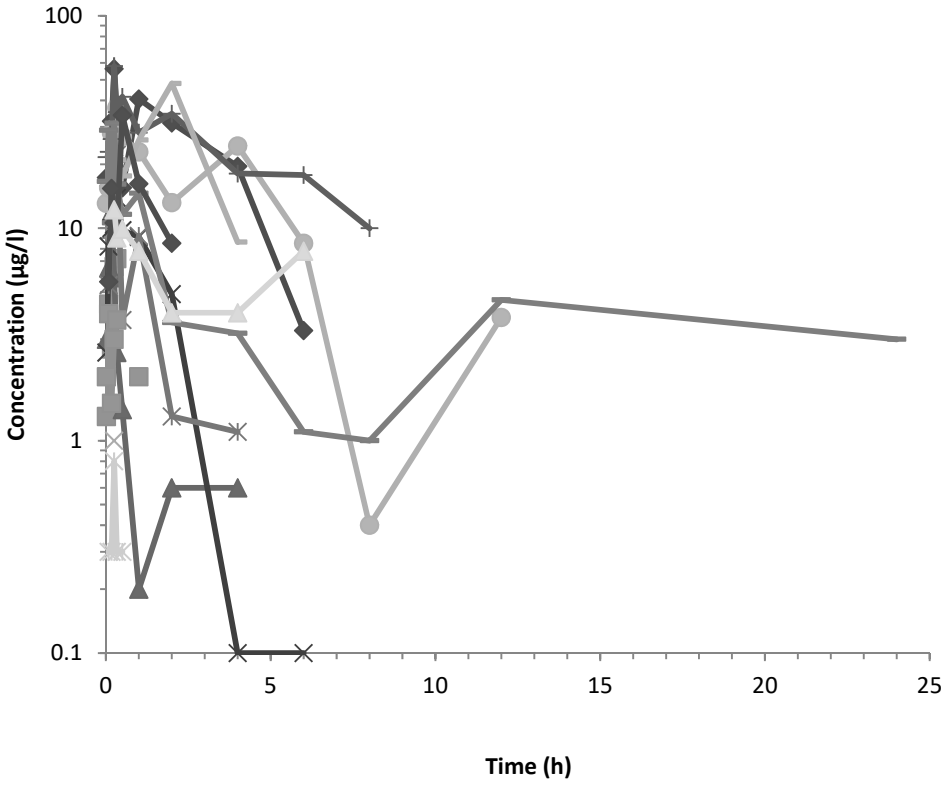


Figure 1. Individual concentrations versus time in plasma for THC(A), 11-OH-THC(B) and THC-COOH(C)

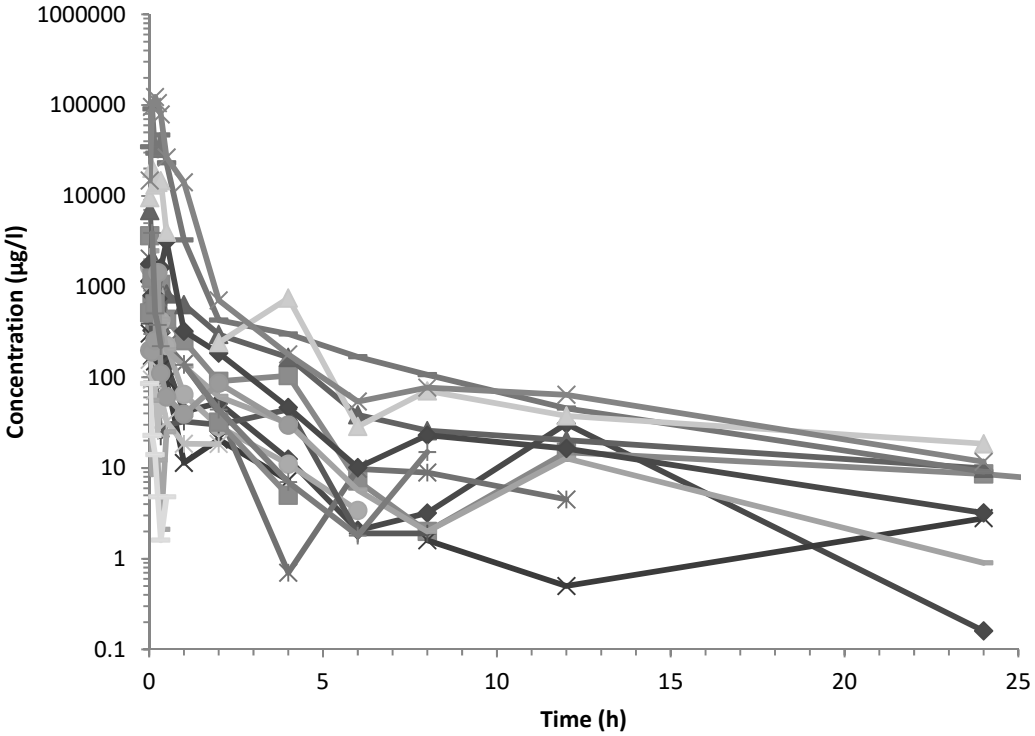
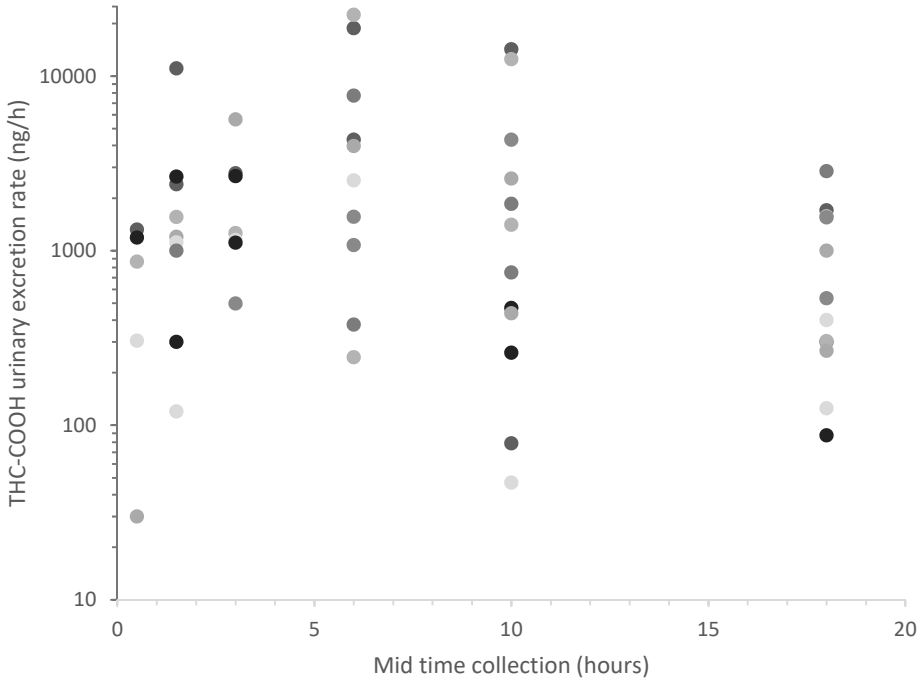


Figure 2. THC individual concentrations versus time in oral fluid.

A



B

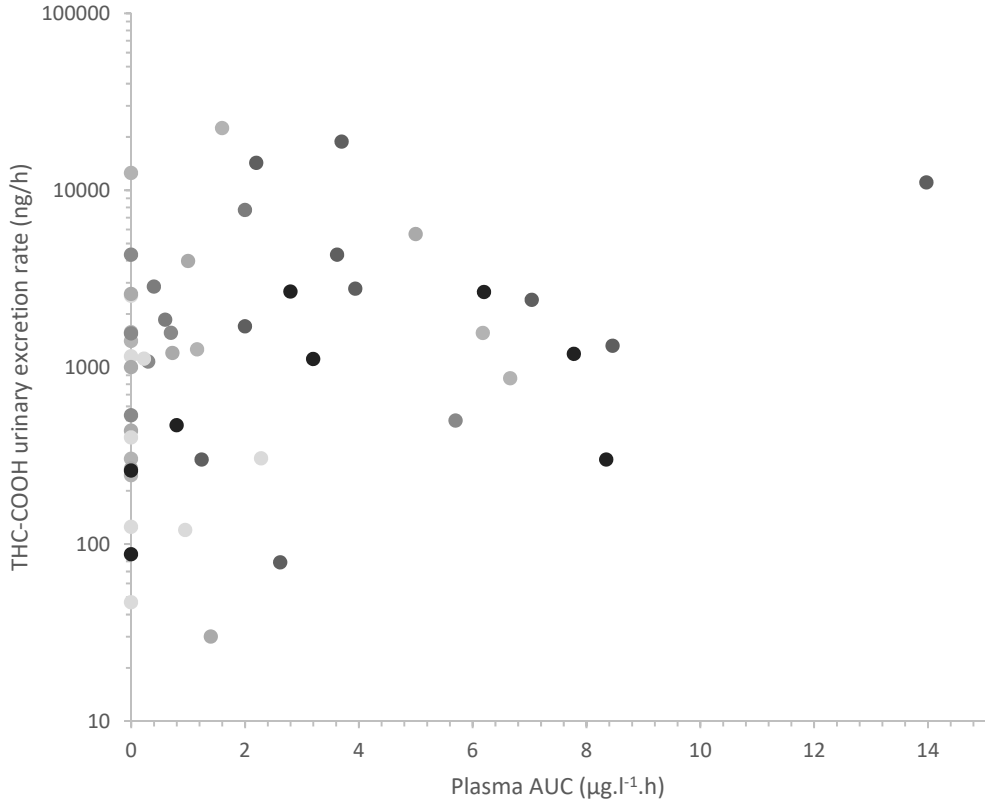


Figure 3. Individual urinary excretion rate of THC-COOH versus mid time collection (A) and versus AUC of THC (bB).

Table 1. Subjects characteristics

Variable	Mean	Range
Number of subjects	18	-
Male/female	18/0	-
Age (years)	22.63	20.00-28.00
Body weight (kg)	70.48	62.50-91.00
Height (cm)	179.81	173.00-187.00
Creatinine serum (µM)	90.06	76.00-99.00
ASAT (IU/L)	20	7.00-36.00
ALAT (IU/L)	22.06	6.00-45.00
Glycemia	4.81	3.50-5.40
Azotemia	5.45	3.20-9.60
THC dose (mg)	20	-
Duration of cigarette (min)	22.88	15.00-30.00

Table 2. Plasma and oral fluid pharmacokinetic parameters following smoking a single cannabis cigarette.

	Plasma				**Oral fluid**			
THC	Median	SD	CV%	Range	Median	SD	CV%	Range
$n_{subject}$	14				18			
C_{max} (µg/L)	25.8	42.9	108	1.6-160	1828	34326	236	55.4-123120
T_{max} (h)	0.02	0.02	76.6	0.017-0.083	0.02	0.12	154	0.017-0.5
T_{last} (h)	2.00	3.20	113	0.017-12.0	18.0	12.7	80.2	0.5-48.0
AUC_{0-t} (µg.l^{-1}.h)	20.3	16.3	73.6	2.12-53.7	622	15047	244	8.62-58496
CL (L/h)	217.5	179	61.0	146-798	6.41	30.8	173	0.08-116
$T_{1/2}$ (h)	1.92	2.31	88.5	0.20-6.84	1.32	5.65	159	0.02-18.9
V_d (L/)	686	695	80.8	113-2020	5.91	71.13	221	0.20-25
11-OH-THC								
$n_{subject}$	7							
C_{max} (µg/L)	2.70	3.69	98.7	0-11.1				
T_{max} (h)	0.08	0.10	82.6	0-0.25				
T_{last} (h)	0.33	0.36	79.0	0-1.0			-	
AUC_{0-t} (µg.l^{-1}.h)	1.02	0.73	72.2	0.17-1.87				
CL (L/h)	7607	9461	83.9	4013-28760				
$T_{1/2}$ (h)	0.15	0.88	156	0.06-2.31				
V_d (L/)	2448	10813	138	394-27547				
THC COOH								
$n_{subject}$	14							
C_{max} (µg/L)	18.30	16.7	78.0	1-56.3				
T_{max} (h)	0.25	0.22	67.0	0.083-1.0				
T_{last} (h)	4.00	6.23	111	0.25-24.0			-	
AUC_{0-t} (µg.l^{-1}.h)	40.9	71.2	88.8	2.72-203				
CL (L/h)	1229	317	136	35.4-972				
$T_{1/2}$ (h)	0.60	1.89	118	0.18-5.96				
V_d (L/)	111	2480	256	22.6-8366				

Table 3. Comparison of $T_{1/2}$ urinary excretion rate of THC-COOH and $T_{1/2}$ of plasma THC

ID	$T_{1/2}$ THC Plasma (h)	$T_{1/2}$ THC-COOH urinary excretion (h)
1	6.44	0.59
2	0.44	0.74
3	1.11	0.61
6	5.34	0.35
8	1.41	0.88
10	0.44	0.50
11	2.43	0.49
12	3.03	0.48
14	1.11	0.48
15	3.75	0.58
17	0.30	0.39
18	0.20	0.60
20	1.08	0.55
21	0.87	0.22
Mean	2.00	0.53
SD	1.97	0.16
CV (%)	98.5	30.3
Range	0.20-6.44	0.22-0.88

Table 4. Oral fluid/plasma THC ratios

Time (h)	Median	SD	CV (%)	Range
0.017	82.6	159	115	8.09-572
0.083	61.7	1261	255	10.7-4737
0.17	63.4	2244	316	17.7-8491
0.25	47.3	2324	300	9.85-8476
0.333	61.5	2099	247	8.15-7666
0.5	67.7	732	208	0-2592
1	57.6	736	237	8.87-2513
2	59.3	62.0	95.8	9.70-220
4	13.2	861	234	1.00-2486
6	10.3	206	187	1.03-420
8	23.0	300	160	5.14-534

Table 5. Metabolite ratios in plasma

		Ratio 11-OH-THC/THC plasma		
Time (h)	**Median**	**SD**	**CV (%)**	**Range**
0.017	0.02	0.01	62.9	0-0.03
0.083	0.03	0.05	102.4	0.02-0.16
0.17	0.03	0.03	65.0	0.01-0.08
0.25	0.06	0.21	138	0.04-0.53
0.333	0.07	0.05	58.4	0.04-0.17
0.5	0.08	0.03	31.5	0.06-0.12
1	0.06	0.06	101	0.02-0.10

		Ratio THC-COOH/THC plasma		
Time (h)	**Median**	**SD**	**CV (%)**	**Range**
0.017	0.10	0.15	82.0	0.05-0.44
0.083	0.36	0.14	36.1	0.21-0.61
0.17	0.97	0.64	68.9	0.17-2.15
0.25	0.90	1.51	94.3	0.46-5.49
0.333	1.41	0.98	64.3	0.29-3.26
0.5	1.33	0.99	64.0	0.22-3.33
1	2.71	2.63	77.8	0.03-8.65
2	2.49	5.13	109	0.12-14.6
4	6.31	5.39	77.6	0.14-15.3
6	8.24	10.26	108	0.13-21.3
8	6.00	5.66	94.3	2.00-10.0

Concerning kinetic profiles and pharmacokinetic parameters, it is known that the bioavailability of THC after cannabis cigarette smoking is variable and influenced by an individual technique and experience [15]. Indeed, the bioavailability of THC and metabolites can be influenced by many factors: how deep the smoke is inhaled in the lungs, the number of puffs and puff volume, the strength of inhalation, the size of smoked particles and the distribution between gas phase, and the particle phase and the residence time in the mouth [16]. The high range of median THC C_{max} (1.6-160.0 µg/L and 55.4-123120.0µg/L in plasma, and oral fluid, respectively), 11-OH-THC C_{max} (0-11.1 µg/L in plasma) and THC-COOH C_{max} (1.0-56.3 µg/L in plasma) indicate that this was also observed in the present study in spite of a standardized smoking procedure. Furthermore, concerning oral fluid samples, extraction efficiency could have been influenced by the type of saliva collector. Indeed, broad variations in the THC concentrations measured in oral fluid were observed between studies. Milman et al. reported a median oral fluid THC C_{max} of 2629 µg/L at 0.25 h after smoking [17]. Huestis and Cone indicated an oral fluid THC C_{max} of 5800 µg/L 0.2 h after inhalation [12]. Several parameters could explain these large variations of THC C_{max} in oral fluid. First, the elevation of THC in the first and second collected oral fluid specimens was obviously caused by THC contamination of oral fluid during the smoking process [18]. Contamination of the oral cavity during and immediately after smoked administration also has been reported for cocaine [19,20] and heroine [19]. Secondly, the devices used for collecting oral fluid differ and may influence the THC levels recovered from the saliva. Thirdly, the bioavailability of THC after cannabis smoking is variable and influenced by individual techniques of inhalation and previous history of use, as already mentioned. Overall, THC concentrations were higher in oral fluid than in plasma. Accordingly, time of the last observed THC concentration was much higher than in plasma with a median of 18.0 h. This confirms previous reports that THC is be longer detectable in oral fluid than in plasma [21,22].

As expected and as presented in previous studies [23,-26], in plasma, THC reached its highest concentration first, followed by 11-OH-THC and by THC-COOH (Figure 1). Median THC T_{max} was 0.017 h, while median 11-OH-THC T_{max} was slightly delayed to 0.083 h and median THC-COOH T_{max} was even more delayed to 0.25 h. These results were similar from those reported by Kauert et al [23] and by Toennes et al [24]. As demonstrated by Huestis et al. [25], the THC-COOH plasma concentrations peaked later, and showed a long-lasting plateau followed by a slow decrease. The urinary peak times of THC and metabolites were in agreement with those found in a different study, involving smoking cannabis cigarettes which contained 3.58% THC [26].

A wide inter-individual variability was also observed concerning the metabolism. Concerning metabolites of THC, 11-OH-THC remains detectable 0.333 h (until 1 h) and 8 h (until 12 h) after administration of THC in plasma and urine, respectively. THC-COOH remains detectable 4 h (until 24 h) and 24 h after administration of THC, respectively. THC is mainly metabolized in the in the liver by microsomal hydroxylation and oxidation catalysed by enzymes of cytochrome P450 complex (CYP 2C subfamily mainly in humans). Lowe et al. [27] studied chronic, heavy cannabis users and found THC and 11-OH-THC to be excreted in urine for up to 24 days. These findings support the hypothesis of Hunt and Jones [28] that the rate-limiting step in the terminal elimination of THC is its slow excretion from tissue stores that may be extended following chronic cannabis use.

In plasma, THC 95-99% is bound to proteins, mostly lipoproteins and a small fraction to albumin [29]. Given that the high protein binding limits the initial bioavailability, early volume of distribution is low for a lipophilic substance, of the order of 2.5 to 3.0L/kg [28]. At steady state, the volume of distribution is around 700L i.e. 10L/kg for a 70kg subject [28,30,31]. These data are in line with the volume of distribution found 686.0 L i.e. 9.74 L/kg in our study. For Wall et al. [30] the average plasma clearance of THC is 197 ± 50mL/min for women and 248 ± 62mL/min for men. Hunt and Jones [28] calculated higher clearances reaching about 600mL/min in naive subjects and 1000mL/min in regular consumers. The latter value corresponds substantially to the hepatic blood flow which is therefore a limiting factor of THC metabolism. These high clearances explain the importance of hepatic first pass and the highest concentration of 11-OH-THC as THC after oral administration contrary to what is observed during inhalation. Indeed, during the inhalation of cannabis, it was shown that the polycyclic hydrocarbons from tobacco smoke induce the action of CYP 1A2. The metabolism of THC also involving the cytochrome P-450, repeated exposure to cannabis can then cause more rapid loss of THC by the enzyme explaining thus our much higher values of clearance [32]. Following a single oral dose of THC, urinary search THC-COOH is generally three to five days [33]. With an examination of urine immunoassay screening with

sensitive threshold 20 µg/L, the first negative urine result is found on average 8.5 days (three to 18 days) for casual users [34]. Urinary elimination is not constant, positive results can succeed negative thus increasing negativity periods indicated above. For Huestis and Cone [35], the half-life of urinary elimination of THC-COOH is about 30 h when the measurement period is seven days. The main urinary metabolite is eliminated such as THC-COOH-glucuronide [36]. THC-COOH free is present in urine in trace amounts [37,38,39]. Mean percentage of total THC dose excreted as THC-COOH metabolite was 1.9 +/- 2.5%. These data are in close agreement with those reported by Huestis et al. [35] who observed similar THC-COOH excretion percentages, 0.54 +/- 0.1 and 0.53 +/- 0.1%, of total dose following smoking of low and high-dose marijuana cigarettes. Manno et al. [26] showed in eight occasional consumers a peak urinary excretion of 21.5µg/L, 77.3µg/L, 179 µg/L for THC, 11-OH-THC and THC-COOH at two, three and four h after smoking a cigarette containing 27mg of THC, respectively. These results are consistent with our values for a dose of 20mg despite slightly lower T_{max}. In addition, the comparison of the half-life values of urinary excretion rate of THC-COOH and plasma THC confirms the observed interindividual variability.

Oral fluid/plasma, the THC ratio over the studied period had a median ratio of 59.34 with a range of 10.33 to 82.58. Similar ratios were reported by Kauert GF et al. while Huestis MA et al. and Lee D et al. showed lower ratios (range) 1.18 (0.5-2.2) and 6.1 (0.2-348.5) respectively [11,12,40]. This study and previous studies showed a large inter-individual variability (Table 3). It appears that the variability in THC oral fluid concentrations precludes exact estimation of plasma THC concentrations from oral fluid test results. Metabolite ratios were examined to evaluate THC and metabolite disposition after smoking cannabis cigarette. 11-OH-THC/THC ratios are low after smoked cannabis; THC enters the blood-stream directly from the alveoli, yielding approximately 5%–10% 11-OH-THC [25]. Median 11-OH-THC/THC ratios increased after all active doses as THC was metabolized to 11-OH-THC (Table 4). Therefore, THC and 11-OH-THC were not regarded as suitable biomarkers for recent cannabis consumption. No consistent THCCOOH/THC ratio pattern was evident. As reported previously [26], THC-COOH showed the highest intra- and inter-ubject variability and was still detectable after 4 days as the result of a 3.58% cannabis cigarette smoked [41]. Thus, the main THC metabolite is not suitable as a urine marker for recent use. Instead, THC-COOH in urine is only suggestive of cannabis consumption at some time in the past [26].

There were several limitations of the study, including the small sample size, potential underreporting of cannabis smoking and an insufficient analytical method. Indeed, THC analysis methods were deemed insufficient to conclude that samples could be analyzed with reasonable accuracy, at least to 1.0 µg/L. For example, in plasma for THC, samples taken at 10h after the dose are around 1.0 ng/ml and, in the study of Heuberger et al. samples taken at 48 h after the dose are around 0.1 µg/L [42]. Numerous samples are below 1.0 µg/L in these three fluids and for these three cannabinoids; therefore, a large number of samples were no detectable.

Another limitation was absence of information on pH of urinary and oral fluid. Indeed, the importance of pH has been shown previously on the pharmacokinetics of methadone for example [43,44]. Earlier studies have indicated that an increase in methadone excretion has been observed on lowering urinary pH [43]. Indeed, urinary pH was found to affect the renal excretion of methadone (clearance) but also its volume of distribution [43]. Urinary pH modulates renal excretion of a number of drugs by the mechanism of nonionic diffusion as described by Milne et al. [45]. The absorption and excretion of THC should also be affected by the pH of saliva and urine, which could explain the observed variability.

CONCLUSION

Kinetic profiles and pharmacokinetic parameters of THC and its metabolites in plasma, oral fluid and urine were described to provide estimations of THC and metabolites concentrations after smoking cannabis cigarette. Direct prediction of plasma THC concentration from oral fluid concentration is not available regardless of large observed concentrations in this biological fluid. On the other hand, urine THCCOOH concentrations could estimate plasma THC concentrations. However, THCCOOH is a metabolite whose development presented also a wide variability of concentrations and moreover is an inactive metabolite, will not reflect performance impairment. Results from this study support the interpretation that positive oral fluid THC results or positive urine fluid results are indicative of a recent cannabis exposure. These data also provide valuable information on how to connect plasma, oral fluid and urinary cannabinoid concentrations after smoking cannabis cigarette.

Conflict of interest statement, Marsot Amélie, Audebert Christine, Attolini Laurence, Lacarelle

Bruno, Micallef Joelle and Blin Olivier have no conflict of interest to declare.

REFERENCES

1. United Nations Office on Drugs and Crime. World drug report 2012. United Nations Publications Sales 2012; n° E.12.XI.I Vienna, Autria
2. Augsburger M, Donzé N, Ménétrey A, Brossard C, Sporkert F, Giroud C, Mangin P. Concentration of drugs in blood of suspected impaired drivers. Forensic Sci Int. 2005; 153(1):11-5.
3. Bosker WM, Huestis MA. Oral fluid testing for drugs of abuse. (2009) Clin Chem. 2009; 55(11):1910-31. doi, 10.1373/clinchem.2008.108670. Epub 2009 Sep 10.
4. Grotenhermen F. Pharmacokinetics and pharmacodynamics of cannabinoids. Clin Pharmacokinet. 2003; 42(4):327-60.
5. Lindgren JE, Ohlsson A, Agurell S, Hollister L, Gillespie H. Clinical effects and plasma levels of delta 9-tetrahydrocannabinol (delta 9-THC) in heavy and light users of cannabis. Psychopharmacology (Berl). 1981; 74(3):208-12.
6. Watanabe K, Matsunaga T, Yamamoto I, Funae Y, Yoshimura H. Involvement of CYP2C in the metabolism of cannabinoids by human hepatic microsomes from an old woman. Biol Pharm Bull. 1995; 18(8):1138-41.
7. Watanabe K, Yamaori S, Funahashi T, Kimura T, Yamamoto I. Cytochrome P450 enzymes involved in the metabolism of tetrahydrocannabinols and cannabinol by human hepatic microsomes. Life Sci. 2007;80(15):1415-9. Epub 2007 Jan 17.
8. Lemberger L, Crabtree RE, Rowe HM. 11-hydroxy- 9 -tetrahydrocannabinol, pharmacology, disposition, and metabolism of a major metabolite of marihuana in man. Science. 1972;177(4043):62-4.
9. Perez-Reyes M, Timmons MC, Lipton MA, Davis KH, Wall ME. Intravenous injection in man of 9 -tetrahydrocannabinol and 11-OH- 9 -tetrahydrocannabinol.
Science. 1972;177(4049):633-5.
10. Fabritius M, Chtioui H, Battistella G, Annoni JM, Dao K, Favrat B, Fornari E, Lauer E, Maeder P, Giroud C. Comparison of cannabinoid concentrations in oral fluid and whole blood between occasional and regular cannabis smokers prior to and after smoking a cannabis joint. Anal Bioanal Chem. 2013; 405:9791-9803
11. Lee D, Vandrey R, Milman G, Bergamashi M, Mendu DR, Murray JA, Barnes AJ, Huestis MA. Oral fluid/plasma cannabinoid ratios following controlled oral THC and smoked cannabis administration. Anal Bioanal Chem 2013;405:7269-7279
12. Huestis MA, Cone EJ.Relationship of Delta 9-tetrahydrocannabinol concentrations in oral fluid and plasma after controlled administration of smoked cannabis. J Anal Toxicol. 2004;28(6):394-9.
13. Leirer VO, Yesavage JA, Morrow DG. Marijuana carry-over effects on aircraft pilot performance. Aviat Space Environ Med. 1991;62(3):221-7.
14. Mariani JJ, Brooks D, Haney M, Levin FR. Quantification and comparison of marijuana smoking practices: blunts, joints, and pipes. Drug Alcohol Depend. 2011 Jan 15;113(2-3):249-51. doi: 10.1016/j.drugalcdep.2010.08.008. Epub 2010 Sep 21.
15. Lindgren JE, Ohlsson A, Agurell S, Hollister L, Gillespie H. Clinical effects and plasma levels of delta 9-tetrahydrocannabinol (delta 9-THC) in heavy and light users of cannabis. Psychopharmacology (Berl). 1981;74(3):208-12.
16. Perez-Reyes M. Marijuana smoking: factors that influence the bioavailability of tetrahydrocannabinol. NIDA Res Monogr. 1990;99:42-62.
17. Milman G, Schwope DM, Schwilke EW, Darwin WD, Kelly DL, Goodwin RS, Gorelick DA, Huestis MA. Oral fluid and plasma cannabinoid ratios after around-the-clock controlled oral Δ(9)-tetrahydrocannabinol administration. Clin Chem. 2011 Nov;57(11):1597-606. doi: 10.1373/clinchem.2011.169490. Epub 2011 Aug 29.
18. Swortwood MJ, Newmeyer MN, Abulseoud OA, Scheidweiler KB, Huestis MA. Cannabinoid disposition in oral fluid after controlled smoked, vaporized,
and oral cannabis administration. Drug Test Anal. 2016 Sep 19. doi: 10.1002/dta.2092.
19. Jenkins AJ, Oyler JM, Cone EJ. Comparison of heroin and cocaine concentrations in saliva with concentrations in blood and plasma. J Anal Toxicol. 1995;19(6):359-74.
20. Cone EJ, Oyler J, Darwin WD.Cocaine disposition in saliva following intravenous, intranasal, and smoked administration. J Anal Toxicol 1997;21(6):465-75.
21. Drummer OH. Review, Pharmacokinetics of illicit drugs in oral fluid. Forensic Sci Int. 2005;,150(2-3):133-42. Epub 2005 Apr 18. Review.
22. Verstraete AG. Detection times of drugs of abuse in blood, urine, and oral fluid. Ther Drug Monit. 2004;26(2):200-5. Review.
23. Kauert GF, Ramaekers JG, Schneider E, Moeller MR, Toennes SW. Pharmacokinetic properties of delta9-tetrahydrocannabinol in serum and oral fluid. J Anal Toxicol. 2007;31(5):288-93.
24. Toennes SW, Ramaekers JG, Theunissen EL, Moeller MR, Kauert GF. Pharmacokinetic properties of delta9-tetrahydrocannabinol in oral fluid of occasional and chronic users. J Anal Toxicol. 2010 May;34(4):216-21.
25. Huestis MA, Henningfield JE, Cone EJ. Blood cannabinoids. I. Absorption of THC and formation of 11-OH-THC and THCCOOH during and after smoking marijuana. J Anal Toxicol. 1992 Sep-Oct;16(5):276-82.

26. Manno JE, Manno BR, Kemp PM, Alford DD, Abukhalaf IK, McWilliams ME, Hagaman FN, Fitzgerald MJ. Temporal indication of marijuana use can be estimated from plasma and urine concentrations of delta9-tetrahydrocannabinol, 11-hydroxy-delta9-tetrahydrocannabinol, and 11-nor-delta9-tetrahydrocannabinol-9-carboxylic acid. J Anal Toxicol 2001; 25:538–549
27. Lowe RH, Abraham TT, Darwin WD, Herning R, Cadet JL, Huestis MA. Extended urinary Delta9-tetrahydrocannabinol excretion in chronic cannabis users precludes use as a biomarker of new drug exposure.Drug Alcohol Depend 2009;105:24–32
28. Hunt CA, Jones RT. Tolerance and disposition of tetrahydrocannabinol in man. J Pharmacol Exp Ther 1980; 215:35–44
29. Wahlqvist M., Nilsson I.M., Sandberg F., Agurell S. Binding of delta-1-tetrahydrocannabinol to human plasma proteins *Biochem Pharmacol* 1970 ; 19 : 2579-2584
30. Wall M.E., Sadler B.M., Brine D., Taylor H., Perez-Reyes M. Metabolism, disposition, and kinetics of delta-9-tetrahydrocannabinol in men and women *Clin Pharmacol Ther* 1983 ; 34 : 352-363
31. Lemberger L., Tamarkin N.R., Axelrod J., Kopin I.J. Delta-9-tetrahydrocannabinol: metabolism and disposition in long-term marihuana smokers *Science* 1971 ; 173 : 72-74
32. Valjent E., Mitchell J.M., Besson M.J., Caboche J., Maldonado R. Behavioural and biochemical evidence for interactions between delta 9-tetrahydrocannabinol and nicotine *Br J Pharmacol* 2002 ; 135 : 564-578
33. Schwartz R.H., Hayden G.F., Riddile M. Laboratory detection of marijuana use: experience with a photometric immunoassay to measure urinary cannabinoids *Am J Dis Child* 1985 ; 139 : 1093-1096
34. Ellis G.M., Mann M.A., Judson B.A., Schramm N.T., Tashchian A. Excretion patterns of cannabinoid metabolites after last use in a group of chronic users *Clin Pharmacol Ther* 1985 ; 38 : 572-578.
35. Huestis M.A., Cone E.J. Urinary excretion half-life of 11-nor-9-carboxy-Δ9-tetrahydrocannabinol in humans *Ther Drug Monit* 1998 ; 20 : 570-576.
36. Williams P.L., Moffat A.C. Identification in human urine of delta 9-tetrahydrocannabinol-11-oic acid glucuronide: a tetrahydrocannabinol metabolite *J Pharm Pharmacol* 1980 ; 32 : 445-448
37. Law B., Mason P.A., Moffat A.C., Gleadle R.I., King L.J. Forensic aspects of the metabolism and excretion of cannabinoids following oral ingestion of cannabis resin *J Pharm Pharmacol* 1984 ; 36 : 289-294,
38. Kelly P., Jones R.T. Metabolism of tetrahydrocannabinol in frequent and infrequent marijuana users *J Anal Toxicol* 1992 ; 16 : 228-235,
39. Alburges M.E., Peat M.A. Profiles of delta 9-tetrahydrocannabinol metabolites in urine of marijuana users: preliminary observations by high performance liquid chromatography-radioimmunoassay *J Forensic Sci* 1986 ; 31 : 695-705
40. Kauert GF, Ramaekers JG, Schneider E, Moeller MR, Toennes SW. Pharmacokinetic properties of delta9-tetrahydrocannabinol in serum and oral fluid.J Anal Toxicol. 2007 Jun;31(5):288-93.
41. Huestis MA, Mitchell JM, Cone EJ. Urinary excretion profiles of 11-nor-9-carboxy-delta 9-tetrahydrocannabinol in humans after single smoked doses of marijuana. J Anal Toxicol 1996; 20:441–452
42. Heuberger JA, Guan Z, Oyetayo OO, Klumpers L, Morrison PD, Beumer TI, van Gerven JM, Cohen AF, Freijer J. Population pharmacokinetic model of THC integrates oral, intravenous, and pulmonary dosing and characterizes short- and long-term pharmacokinetics. Clin Pharmacokinet. 2015; 54: 209-219
43. Nilsson MI, Widerlöv E, Meresaar U, Anggard E. Effect of urinary pH on the disposition of methadone in man. Eur J Clin Pharmacol. 1982;22(4):337-42.
44. Bellward GD, Warren PM, Howald W, Axelson JE, Abbott FS Methadone maintenance: effect of urinary pH on renal clearance in chronic high and low doses..Clin Pharmacol Ther. 1977 Jul;22(1):92-9.
45. Milne MD, Scribner BH, Crawford MA. Non-ionic diffusion and the excretion of weak acids and bases. Am J Med. 1958 May;24(5):709-29.