D9-Tetrahydrocannabinol and Cannabidiol Time Courses in the Sera of "Light Cannabis" Smokers: Discriminating Light Cannabis Use from Illegal and Medical Cannabis Use

Δ9-Tetrahydrocannabinol and Cannabidiol Time Courses in the Sera of "Light

Cannabis" Smokers: Discriminating Light Cannabis Use from Illegal and Medical

Cannabis Use

Simona Pichini, PhD[a], Giulio Mannocchi, BSc[b], Paolo Berretta, PhD [a], Simona Zaami,

MD[c], Filippo Pirani, MD [d], Roberta Pacifici, PhD[a], Francesco Paolo Busardò, MD[d,*]

[a]National Centre on Addiction and Doping, Istituto Superiore di Sanità, Viale Regina

Elena 299, 00161, Rome, Italy.

[b]University of Camerino, Piazza Cavour 19/f – 62032, Camerino, Italy.

[c]Unit of Forensic Toxicology (UoFT), Department of Anatomical, Histological, Forensic

and Orthopedic Sciences, Sapienza University of Rome, Viale Regina Elena 366, 00161,

Rome, Italy.

[d]Department of Excellence-Biomedical Sciences and Public Health, Via Conca 71, 60126,

Ancona, Italy

***Correspondence**: Prof. Francesco Paolo Busardò MD, MSc, DipFMS, PhD, Associate

Professor of Forensic Science and Toxicology**,** Department of Excellence Biomedical

Sciences and Public Health, University "Politecnica delle Marche" of Ancona. E-mail:

fra.busardo@libero.it; Fax:+39 071 5964723; Tel.:+39 071 5964717.

Acknowledgment

Conflicts of Interest and Sources of Funding

Abstract

Background: Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD) time courses in serum, and physiological and behavioral effects associated with smoking 1 or 4 "light cannabis" cigarettes were studied. Biomarkers to differentiate light cannabis vs. illegal and medical cannabis use were also investigated**.**

Methods: Sera were obtained at different times from 6 healthy light cannabis consumers and 6 individuals who smoked 1 and 4 cigarettes, within 4 h via a liquid-liquid method and analyzed by liquid chromatography-tandem mass spectrometry.

Results: In serum, minimal THC concentration was observed after a single cigarette smoke, while repeated smoking increased it by one order of magnitude. CBD concentrations were higher, but did not increase linearly, probably because it does not preferentially volatilize compared to THC. The highest THC and CBD concentrations were observed 0.5 h after the start of the smoking of 1 cigarette. Serum THC ranged from 2.7 to

5.9 ng/mL, while serum CBD varied from 5.7 to 48.2 ng/mL. Similarly, the highest THC and CBD concentrations were observed 0.5 h after the smoking of 4 cigarettes. Specifically, the ranges were THC: 11.0-21.8 ng/mL, and CBD: 19.4-35.3 ng/mL. In both cases and the mean THC/CBD concentration ratio ranged from 0.2 to 0.9. There were no significant changes in blood pressure, heart rate, and body temperature, but participants who smoked 4 cigarettes experienced severe drowsiness.

Conclusions: THC and CBD time courses in the sera of light cannabis smokers were similar to those previously observed in oral fluid and blood. Serum THC/CBD concentration ratio not higher than the mean value of 0.9 might be a useful biomarker to identify use of light cannabis *vs.* that of illegal THC cannabis (where THC/CBD concentration ratios are generally greater than 10) or *vs.* that of medical cannabis (where ratios are greater than 1). Consumers should be advised of possible drowsiness after he repeated smoking of light cannabis cigarettes.

Keywords: light cannabis; THC; CBD; serum; time course

1. Introduction

Although cannabis remains the most widely cultivated, trafficked, seized, and abused illicit drug,[1] the possession of small quantities has been decriminalized in many US states and European Countries (such as Holland, Spain, France, Germany) due to spreading medical cannabis prescription.[2,3] Additionally, in the absence of laws banning the commercialization of cannabis with : Δ9-tetrahydrocannabinol (THC; <0.2%) and variable cannabidiol (CBD) contents (e.g. 2 to 40% CBD content), manufacturers are commercializing a product called "light cannabis".[4,5]

This product is sold as an "environmental perfume" and "not for human use", "herbal incense" and cannot be ingested, following label indications. However, web fora explains how to smoke light cannabis, its effects, and contraindications.[4,6] Indeed, this legal product is gaining popularity because it contains CBD, which is not psychoactive, but said to reduce anxiety, promote sleep via a relaxing sedative effect, and is reportedly used for medical purposes since it can be obtained without medical prescription.[7] The increasing medical cannabis prescription for different pathologies, including less severe ones such as insomnia or anxiety, makes people keener to test these products, freely available both in specialized shops and tobacconists.[6]

The use of light cannabis raises two main questions: 1) can smoking this herbal product result in a THC positive screening test in biological fluids; and 2) how does one distinguish between light cannabis use and that of illegal and medical cannabis when analyzing the biological fluids of consumers of the different products.

Recently, a consumption assessment demonstrated that the consumption of 1 g of light cannabis cigarette containing 0.16% THC and 5.8% CBD did not result in a positive urine screening; hence, confirmation would not be required. Conversely, they might result in positive oral fluid testing with some on-site kits, with THC cut-off lower than 25 ng/ml, at least in the first hour after smoking, requiring subsequent confirmation analysis.[5] A successive study on smokers of 4 light cannabis cigarettes (0.16% THC and 5.8% CBD) showed that oral fluid was a valuable alternative to blood in monitoring light cannabis consumption. Additionally, oral fluid and blood THC/CBD concentration ratios never exceeded 2, possibly providing a useful biomarker to identify light cannabis *vs.* illegal use, where THC/CBD ratios are generally greater than 10.[8]

To complete this first observation in light cannabis smokers, THC and CBD concentration profiles in serum and associated physiological and behavioral effects were

studied after the smoking of 1 or 4 light cannabis cigarettes. Biomarkers to identify light cannabis vs. illegal and medical cannabis use were also investigated.

2. Materials and Methods

2.1. CHEMICALS AND MATERIALS

THC, CBD, and deuterated THC-d_3 (used as internal standard, IS) were supplied by LGC Standards (Milan, Italy). VTA-M3 reagent (acid aqueous buffer) was provided by Comedical s.a.s (**Mattarello**, Trento, Italy). All reagents of analytical grade were purchased from Carlo Erba (Milan, Italy).

2.2. BIOLOGICAL SAMPLE COLLECTION AND PREPARATION

Serum samples were obtained from healthy light cannabis smokers, invited verbally to participate in a light cannabis clinical trial. Eligibility criteria included prior light cannabis intake on at least 5 occasions, successful accomplishment of a general physical examination, and routine laboratory analysis and electrocardiogram. All subjects gave written informed consent, and the study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Ethics Committee (IRCCS-INRCA Ancona). Subjects participated as outpatients in a 10-h experimental session in the Ancona Institute of Patient Care and Scientific Research (IRCCS-INRCA) room equipped for clinical trials with an open balcony where they could smoke *ad libitum*. Before the trial started, urine drug screening (Quantilab DRI, ILabTaurus, Instrumentation Laboratory, Milano, Italy) was performed for all volunteers, and had to be negative for opiates, cocaine, cannabinoids, and amphetamines.

Six volunteers (3 males and 3 females, 37.0 ± 16.1 years) smoked 1 cigarette containing 1.6 mg THC and 58 mg CBD. The second group of 6 volunteers (3 males and 3

females, 39.3 ± 12.3 years) smoked 4 consecutive cigarettes (6.4 mg THC and 232 mg CBD) in 4 h (a cigarette per hour). Blood pressure, heart rate, and body temperature were measured and participants were free to report any sensation felt during the experimental session for up to 24 h.

Serum (2 mL) from whole blood centrifugation was collected in glass tubes 0.5, 1, 2, 3, **4 and 5 h** after smoking 1 or 4 subsequent light cannabis cigarettes, and stored at -20°C until analysis.

Serum samples were thawed at room temperature and pretreated according to the following procedure: 50 µl of sample was added to THC-d$_3$, 50 µl of M3® reagent (Comedical, Trento, Italy), and 100 µl of acetone/acetonitrile (8:2, v/v). After centrifugation, 1 µl of supernatant was injected into the chromatographic system.

2.3. UHPLC–MS/MS ANALYSIS OF SERUM SAMPLES

Chromatographic analysis was carried out using a UPLC instrument Waters Acquity I Class coupled with a Waters XEVO TQ-S Micro (Waters, Milano, Italy). The reversed phase column used for analyte separation was a Waters BEH 1.7 µM 2.1 x 50 mm set at the temperature of 50°C.

Run time was 10 min with a gradient mobile phase of ammonium formate, 0.005 mol/L at pH 3 (mobile phase A) and methanol (mobile phase B) at a 0.4 ml/min flow rate. Initial conditions were A:B 60:40 v/v. Solvent A was held at 60% for 0.5 min, decreased to 0% up to 7.00 min, and then returned to 60% and held for 2 min.

Mass spectrometric analysis was performed in the positive ion multiple reaction monitoring (MRM) mode. The applied ESI conditions were: 2.5 kV capillary voltage, 650 °C desolvation temperature, 150 °C source temperature, 1200 L/h desolvation gas flow rate, 0.18 mL/min collision gas flow rate, and 35 kV cone energy voltage. Selected MRM transitions were: THC: m/z 315.21>193.10 and 315.21>123; and CBD: m/z 315.15>193.15

and 315.15>123; and THC-d$_3$: 318.21>196.10. Transitions in bold were used for quantifications.

The method developed following international criteria,[9] was tested in a validation protocol, following the most recent standard practices.[10] Limit of quantification (LOQ) for THC and CBD was 0.2 ng/mL, with an upper limit of linearity of 100 ng/mL. Intra- and inter-assay imprecision and accuracy were always less than 15%, and analytical recoveries were always higher than 89%.

3. Results

3.1. THC AND CBD TIME COURSES AND THC/CBD CONCENTRATION RATIOS IN SERUM AFTER THE SMOKING OF 1 AND 4 SUBSEQUENT LIGHT CANNABIS CIGARETTES

THC and CBD time courses after the smoking of a single light cannabis cigarette and 4 subsequent cigarettes are reported in Figures 1 and 2.

Table 1 shows the mean, standard deviation (SD), median, and range of maximum and minimum concentration reached by the two cannabinoids in serum samples after the smoking of a single cigarette or 4 subsequent cigarettes in 4 h.

In both cases, THC and CBD presented highest concentrations at 0.5 h after smoking, and lowest concentrations 4 h after smoking. At 5 h, both compounds could not be detected in serum samples.

After the smoking of a single cigarette, THC/CBD concentration ratios ranged between a minimum value of 0.21 at 1 h after smoking to a maximum mean value of 0.44 at 3 h after smoking, while after the smoking of 4 subsequent cigarettes, THC/CBD concentration ratios ranged between a minimum mean of 0.49 at 4 h after smoking to a maximum mean value of 0.86 at 1 h after smoking (Table 1).

3.2. PHYSIOLOGICAL AND BEHAVIORAL EFFECTS OF THE SMOKING OF 1 AND 4 SUBSEQUENT CIGARETTES OF LIGHT CANNABIS

No significant changes were observed during the study session in body temperature, heart rate, and blood after the smoking of 1 and 4 cigarettes of light cannabis. Nevertheless, it has to be reported that the 6 participants who smoked 4 cigarettes experienced drowsiness when returning home at the end of the session.

4. Discussion

The data from this study show that THC and CBD time courses in serum after the smoking of 1 or 4 light cannabis cigarettes matched those previously reported in blood and oral fluid.[8] Unfortunately, our first serum sample was collected 0.5 h after THC and CBD Cmax, which occur just prior to the last puff on a cannabis cigarette. For this reason, we reported our findings at 0.5 h as the highest concentration rather than Cmax. Nevertheless, in spite of this protocol limitation, our aim was to show the time course of THC and CBD in serum following the smoking of light cannabis.

Regardless of smoking a single cigarette or 4 subsequent cigarettes (mimicking repeated product consumption), the two principal constituents, THC and CBD, disappeared from serum within 4 h.

As already reported, in the case of THC, this rapid disappearance from the blood stream is due to the high lipophilicity of the compound and its extremely low content in the consumed product, which is approximately 15 (single cigarette) to 5 (4 cigarettes) times lower than that formerly reported in kinetics studies or in the commonly abused product.[11-15]

Taking into account the THC content in light cannabis, data from this study demonstrate that no psychotropic effect was observed or reported, even in the smoking of more than 1 cigarette.

For this reason, when questioned, light cannabis users declared that they consume the product for the relaxing and calming effects, which are mainly due to the presence of varying CBD quantities, which can be as high as 40%.[6]

The CBD content of the product used in this study was 30 times more than that of THC, but this was not reflected in the CBD concentrations measured in serum, which were only 7 times higher than those of THC after the smoking of a single cigarette and slightly higher than that after smoking of 4 cigarettes.

Two possible reasons can justify this evidence: firstly, CBD does not preferentially volatilize compared to THC.[16] Secondly, this is the first study in which light cannabis was smoked in a controlled clinical trial by participants with limited experience in using this product, but eventually applying compound self-titration.

Indeed, as seen in Figures 1 and 2, a great intersubject variability can be observed in THC and CBD serum concentrations, which could be a function of experience when smoking this product.

Nevertheless, an interesting finding of this study is that mean serum THC/CBD concentration ratios fluctuated between 0.2 (single cigarette smoking) and 0.9 (4 subsequent cigarette smoking). The fact that the mean THC/CBD concentration ratios never exceeded the unit and subjects' time point maximum value was 1.5, might be useful in discriminating light cannabis *vs.* illegal or medical THC cannabis use. Indeed, since illegal cannabis contains high psychotropic THC compared with non-psychotropic CBD, ratios are generally greater than 10.[13] In medical cannabis, in a product with high THC content (about 14-20%) or CBD less than 0.1% (e.g., Bedrocan), THC/CBD concentration

ratios are one order of magnitude higher than 10, and when considering a product with similar THC and CBD content (6.5-7% *vs*. 8-10%), the ratios are always higher than 2.

Although demonstrated in a limited number of subjects, this observation can be useful in several medico-legal and forensic purposes such as driving under the influence of cannabis, road killing under the influence of cannabis, workplace cannabis testing, firearms license release, and adoption and custody of a minor. Although the selection matrix to be examined is blood in some of these situations, serum is the most available body fluid to be stored, transported, and examined in laboratory context.

Finally, as already reported,[8] it is apparent that smoking either a single light cannabis cigarette or 4 subsequent cannabis cigarettes did not affect blood pressure, heart rate, and body temperature; however, severe drowsiness was reported in subjects who smoked 4 subsequent cigarettes. This presents another limitation of the study as the latest time-point considered was 4 h, while subjects that consumed 4 cigarettes experienced drowsiness upon returning home.

4. Conclusions

THC and CBD excretion profiles in serum and the time course of THC/CBD concentration ratios after the smoking of 1 or 4 light cannabis cigarettes confirmed those previously measured in blood and oral fluid. It was important to confirm this in serum, since serum is the most available and manageable matrix in forensic toxicological contexts. The data presented in this study seem to indicate that subjective and physiological effects of light cannabis are related to the presence of non-negligible amounts of the non-psychotropic cannabinoid CBD. This fact should be taken into consideration since this product is gaining importance as a myorelaxant, and because of its alleged sleep inducer and calming effects.[7] Foreseeing a future widespread use of the product, regulations

regarding driving and light cannabis use are needed to avoid a significant increase in road accidents due to drowsiness observed after repeated product smoking.

When drivers are stopped at the roadside or involved in accidents,[16] serum THC/CBD concentration ratio should be used as a biomarker for light cannabis use instead of illegal or medical cannabis use, which in any case can be recognized by the possession of a medical prescription.

References

1. World Drug report 2018: Analysis of drug markets. https://www.unodc.org/wdr2018/prelaunch/WDR18_Booklet_3_DRUG_MARKETS.pdf. Accessed May 3, 2019.

2. Whiting PF, Wolff RF, Deshpande S, et al. Cannabinoids for Medical Use: A Systematic Review and Meta-analysis. *JAMA*. 2015;313(24):2456-2473.

3. Zaami S, Di Luca A, Di Luca NM, et al. Medical use of cannabis: Italian and European legislation. *Eur Rev Med Pharmacol Sci.* 2018;22(4):1161-1167.

4. Cannabis Light guidelines: what's the difference between CBD and THC? https://cbweed.com/en/cannabis-light-guidelines-difference-between-cbd-thc/. Published March 2019. Accessed May 3, 2019.

5. Pacifici R, Pichini S, Pellegrini M, et al. Determination of cannabinoids in oral fluid and urine of "light cannabis" consumers: a pilot study. *Clin Chem Lab Med.* 2018;57(2):238-243.

6. What is Cannabis Light: effects, properties, and benefits. https://cannabio.it/en/blog/what-cannabis-light-is-effects-properties-and-benefits-b12.html. Published January 2019. Accessed May 4, 2019.

7.   Huestis MA, Solimini R, Pichini S, et al. Cannabidiol Adverse Effects and Toxicity. Curr Neuropharmacol. 2019; Jun 3. doi: 10.2174/1570159X17666190603171901. [Epub ahead of print].

8.   **Pacifici R, Pichini S, Pellegrini M, et al. THC and CBD concentrations in blood, oral fluid and urine following a single and repeated administration of "light cannabis".** *Clin Chem Lab Med. 2019 Apr 8.https://www.degruyter.com/view/j/cclm.ahead-of-print/cclm-2019-0119/cclm-2019-0119.xml* **Published April 2019. Accessed May 3, 2019.**

9.   Peters FT, Wissenbach DK, Busardò FP, et al. Method Development in Forensic Toxicology. *Curr Pharm Des.* 2017;23(36):5455-5467.

10.   Wille SMR, Coucke W, De Baere T, et al. Update of Standard Practices for New Method Validation in Forensic Toxicology. *Curr Pharm Des.* 2017:23(36):5442-5454.

11.   Newmeyer MN, Desrosiers NA, Lee D, et al. Cannabinoid disposition in OF after controlled cannabis smoking in frequent and occasional smokers. *Drug Test Anal.* 2014;6(10):1002-1010.

12.   Toennes SW, Ramaekers JG, Theunissen EL, et al. Pharmacokinetic properties of delta 9-tetrahydrocannabinol in OF of occasional and chronic users. *J Anal Toxicol.* 2010;34(4):216-221.

13.   Fabritius M, Chtioui H, Battistella G, et al. Comparison of cannabinoid concentrations in of and whole blood between occasional and regular cannabis smokers prior to and after smoking a cannabis joint. *Anal Bioanal Chem.* 2013;405(30):9791-9803.

14.   Swortwood MJ, Newmeyer MN, Andersson M, et al. Cannabinoid disposition in OF after controlled smoked, vaporized, and oral cannabis administration. *Drug Test Anal.* 2017;9(6):905-915.

15. Huestis MA. Human cannabinoid pharmacokinetics. *Chem Biodivers.* 2007;4(8):1770-1804.

16. Solowij N, Broyd SJ, van Hell HH., et al. A protocol for the delivery of cannabidiol (CBD) and combined CBD and Δ9-tetrahydrocannabinol (THC) by vaporisation. *BMC Pharmacol Toxicol.* 2014;15(1):58.

17 Busardò FP, Pellegrini M, Klein J, et al. Neurocognitive correlates in driving under the influence of cannabis. *CNS Neurol Disord Drug Targets***. 2017;16(5):534-540.**

Figure Legends:

Figure 1. Time course of serum concentrations and concentration ratio of THC and CBD after the smoking of 1 light cannabis cigarette.

Figure 2. Time course of serum concentrations and concentration ratio of THC and CBD after the smoking of 4 subsequent light cannabis cigarettes in 4 h

Table 1. Serum concentrations of Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD) in study participants.

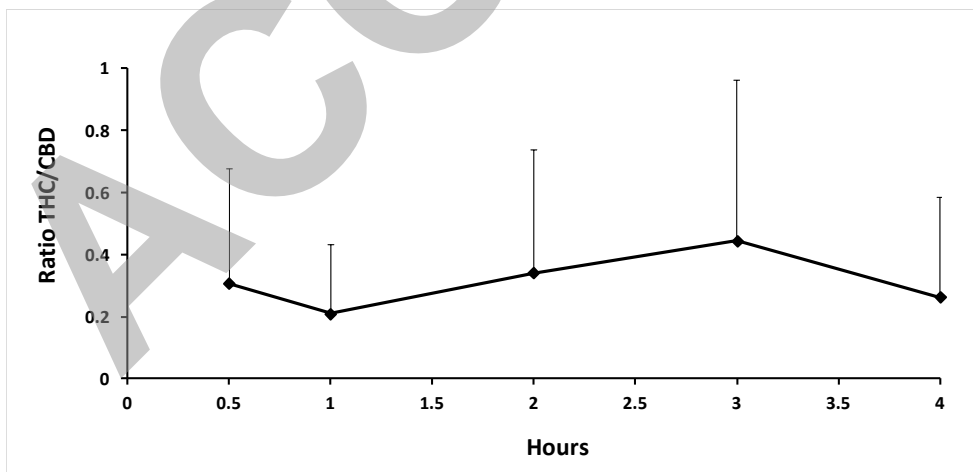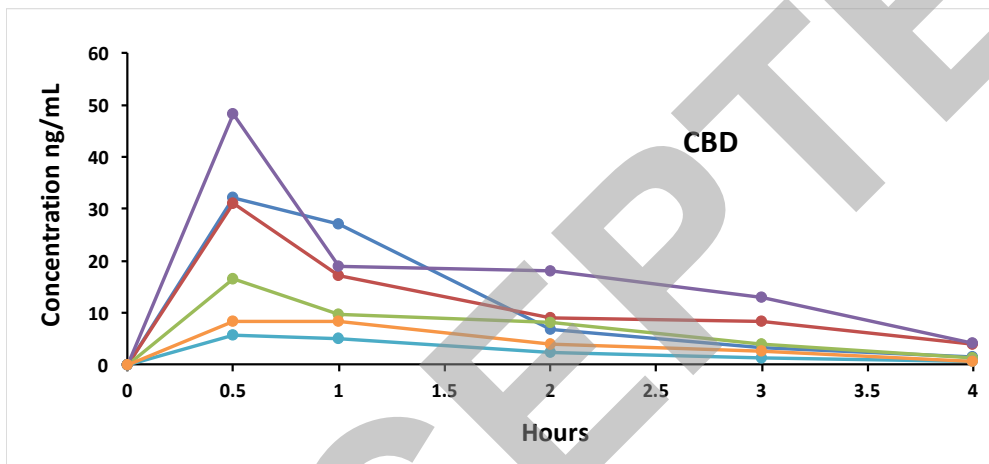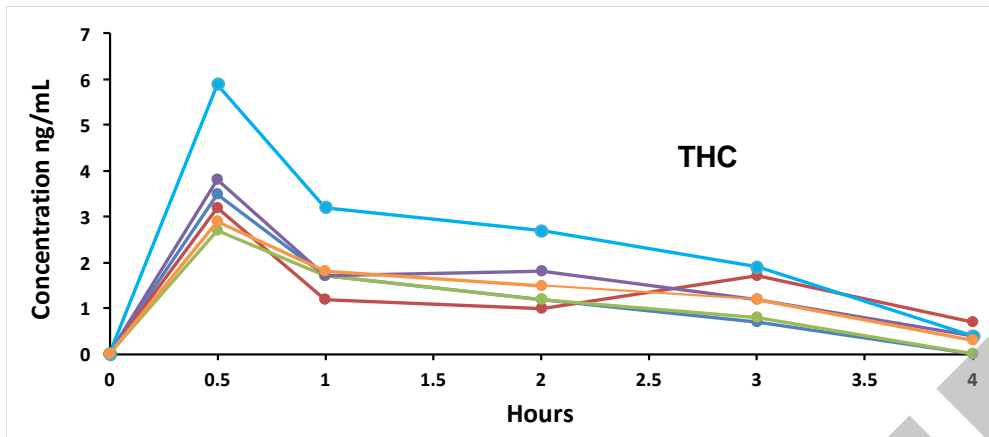|	Serum concentration (ng/mL)			
1 cigarette of light cannabis smoking				
THC	**Mean**	**SD**	**Median**	**Range**
Concentration at 0.5 h	3.7	1.2	3.4	2.7-5.9
Concentration at 4 h	0.3	0.3	0.4	0.0-0.7
CBD				
Concentration at 0.5 h	23.7	16.3	23.8	5.7-48.2
Concentration at 4 h	2.0	1.6	1.4	0.5-4.1
THC/CBD Concentration ratio				
minimum value	0.21	0.22	0.13	0.06-0.64
maximum value	0.44	0.51	0.21	0.09-1.40
4 cigarettes of light cannabis smoking				
THC				
Concentration at 0.5 h	21.8	6.1	23.2	11.0-28.1
Concentration at 4 h	1.0	0.5	0.8	0.5-1.5
CBD				
Concentration at 0.5 h	26.4	5.7	26.6	19.4-35.3
Concentration at 4 h	2.6	1.2	2.8	0.9-4.2
THC/CBD Concentration ratio				
minimum value	0.49	0.40	0.28	0.2-1.0
maximum value	0.86	0.26	0.84	0.6-1.2

SD: standard deviation

Figure. 1. Time course of serum concentrations and concentration ratio of Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD) after the smoking of 1 light cannabis cigarette.
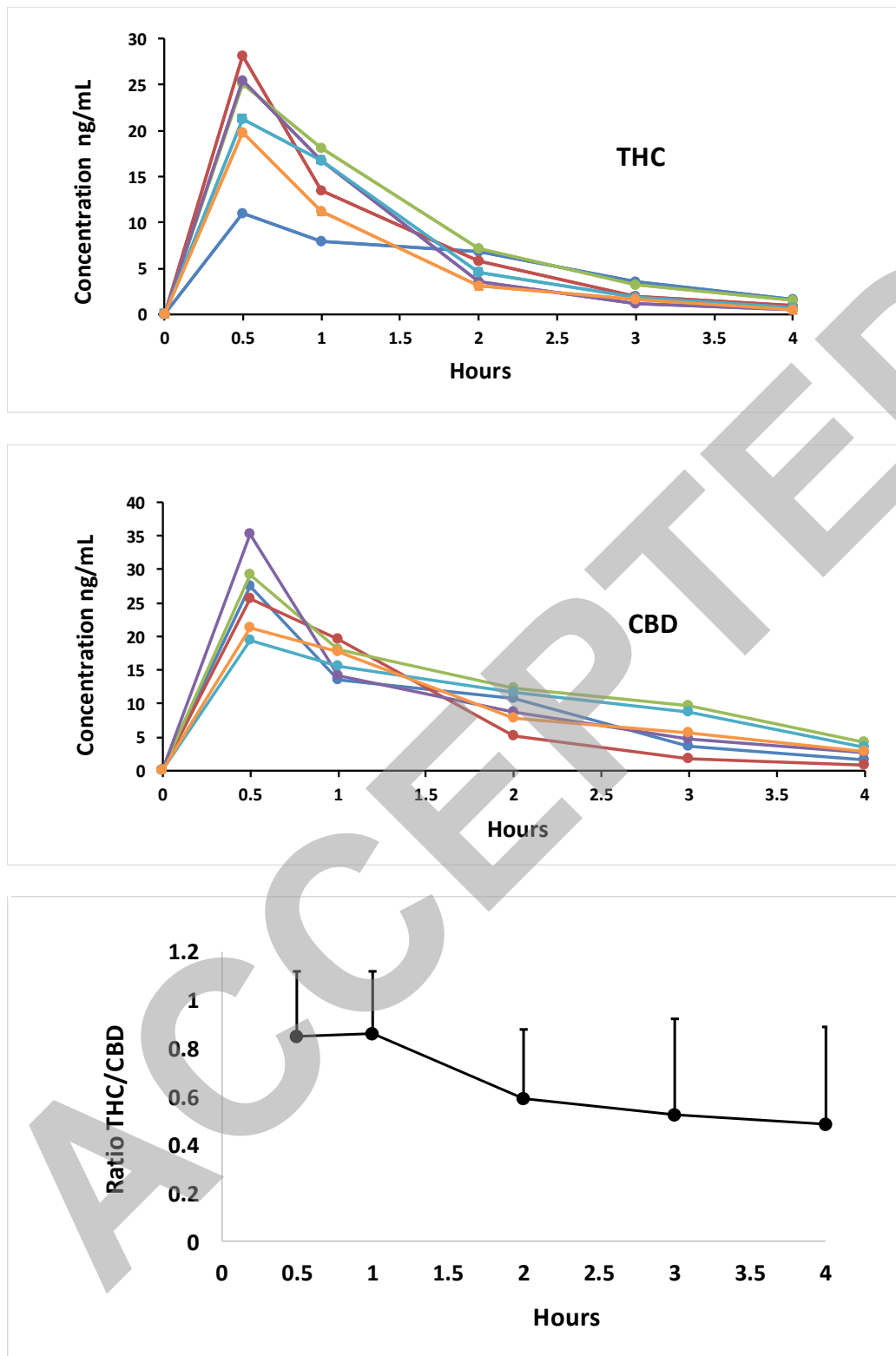
Figure. 2. Time course of serum concentrations and concentration ratio of Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD) after the smoking of 4 subsequent light cannabis cigarettes in 4 h