Clinical Biochemistry 49 (2016) 1072–1077

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

# $\Delta^9$-Tetrahydrocannabinol concentrations in exhaled breath and physiological effects following cannabis intake – A pilot study using illicit cannabis

Line Coucke [a,1], Enrico Massarini [b,d], Zachery Ostijn [c], Olof Beck [d], Alain G. Verstraete [a,c,*]

[a] Department of Laboratory Medicine, Ghent University Hospital, Ghent, Belgium
[b] Department of Public Health, Section of Legal Medicine, University of Rome "Tor Vergata", 00133 Rome, Italy
[c] Ghent University, Department of Clinical Chemistry, Microbiology and Immunology, Ghent, Belgium
[d] Department of Laboratory Medicine, Clinical Pharmacology Unit, Karolinska University Hospital, Stockholm, Sweden

ARTICLE INFO

*Article history:*
Received 27 February 2016
Received in revised form 29 May 2016
Accepted 4 June 2016
Available online 8 June 2016

*Keywords:*
Cannabis
Breath drug testing
Marijuana smoking
THC

ABSTRACT

**Objectives:** $\Delta^9$-Tetrahydrocannabinol (THC) can be measured in exhaled breath by using an aerosol particle collection device. The sampling procedure is simple, non-invasive and takes only 2–3 min. In the present study we measured the amount of THC in exhaled breath of cannabis users at specific time intervals up to 3 h after smoking one cannabis cigarette.

**Design and methods:** The breath concentration-effect relationship was studied by measuring the pulse rate and the pupil diameter to assess physiological changes. THC and the main metabolite 11-nor-9-carboxy-$\Delta^9$-tetrahydrocannabinol were analyzed in exhaled breath by a liquid chromatography-tandem mass spectrometry method. Thirteen subjects (9 males and 4 females, aged 23–24 years) participated. Five of those were using cannabis more frequently than monthly.

**Results:** THC was detected in most subjects already at baseline, concentrations increased following smoking and remained detectable for over 3 h (mean THC concentration in breath at 3 h: 1479 pg/sample). Pulse rate ($p = 0.015$) and pupil diameter ($p = 0.044$) were significantly altered up to 30 min after smoking. The detection window of cannabis in breath after smoking one cannabis cigarette in occasional and chronic smokers was at least 3 h. Only THC was detected, and not the metabolite. The THC concentration in exhaled breath was related to the physiological changes that occur over time.

**Conclusions:** Exhaled breath can be used to detect recent cannabis exposure.



## 1. Introduction

Cannabis is the most used illicit drug in the world: between 128 and 230 million people have used cannabis in 2013 [1]. Around 5.7% of all Europeans aged 15 to 64 reports to have used cannabis at least once during the last year. Cannabis is especially popular in young adults (15–34 years). It is estimated that almost 1% of European adults are daily or almost daily cannabis users [2]. The principal active agent, $\Delta^9$-tetrahydrocannabinol (THC), is a psychodysleptic substance that causes relaxation and euphoria [3]. Cannabis smoking results in tachycardia within a few minutes. The effect of cannabis on pupil diameter is controversial. In several studies, a reduced pupil diameter was measured after cannabis intake [4], but others have found dilated pupils [5]. The associated detrimental effects on concentration, alertness and perceptual function can endanger the user and his environment. This underscores the need for an accurate measurement of THC in samples to detect recent intake, especially in circumstances where impairment needs to be avoided, such as driving a vehicle and in the workplace [6].

Analysis of cannabis in exhaled breath offers an attractive alternative to more invasive blood testing. Breath has regained interest as a matrix for drugs of abuse testing in recent years following the demonstration that a wide spectrum of abused drugs can be detected in breath following recovery from acute intoxication [7]. New sample collection systems have been developed and sensitive bioanalytical techniques allow accurate determination of THC [8]. Cannabis is usually consumed by smoking, whereby THC is inhaled and deposited in the airway lining fluid. Exhaled aerosol particles are composed of airway lining fluid components, including many nonvolatile endogenous substances [9–11]. The possibility of quantitative analysis of cannabis in

*Abbreviations:* LOD, limit of detection; LOQ, limit of quantification; LLOQ, lower limit of quantification; THC, $\Delta^9$-tetrahydrocannabinol; THC-$D_3$, $\Delta^9$-tetrahydrocannabinol-$D_3$; THCCOOH, 11-nor-9-carboxy-$\Delta^9$-tetrahydrocannabinol.

* Corresponding author at: Ghent University, Department of Clinical Chemistry, Microbiology and Immunology, Ghent University Hospital, Department of Laboratory Medicine, De Pintelaan 185 2P8, 9000 Ghent, Belgium.
*E-mail address:* Alain.Verstraete@ugent.be (A.G. Verstraete).
[1] Current address: Clinical Laboratory, O.L.V. van Lourdes Hospital, Waregem, Belgium.

http://dx.doi.org/10.1016/j.clinbiochem.2016.06.003
0009-9120/

exhaled breath collected with a simple collection device has been demonstrated [8,12].

In this study, the breath concentration-effect relationship was investigated in cannabis smokers. Participants smoked cannabis, and heart rate and pupil diameter were measured. To our knowledge, this is the first study that evaluates serial measurements of THC concentration in exhaled breath shortly after cannabis smoking in relation to effects on cardiovascular and physiological parameters.

## 2. Methods

### 2.1. Participants

Participants were recruited by one of the co-authors among students in Ghent, Belgium. A questionnaire was used to register self-reported previous drug use and frequency. The study was approved by the institutional ethics committee. All subjects signed an informed consent form.

### 2.2. Cannabis administration

Participants smoked self-supplied cannabis cigarettes. The quantity of cannabis used per subject varied between 0.1 g and 0.4 g. Participants arrived at the testing site, were informed about the study procedure, signed the informed consent forms and gave the baseline breath sample. The participants were supervised by the researchers for about 15–30 min prior to smoking. Participants prepared their joint and smoked it outside for 5–10 min. Directly after smoking, the participant was called inside for breath sampling and physiological tests. The participants remained inside for the tests at the other time points.

### 2.3. Exhaled breath sampling

Sampling was performed with a SensAbues DrugTrap® device (SensAbues AB, Huddinge, Sweden). The subjects were asked to breathe via a mouthpiece to fill a plastic bag for 2 to 3 min (±30 L of exhaled

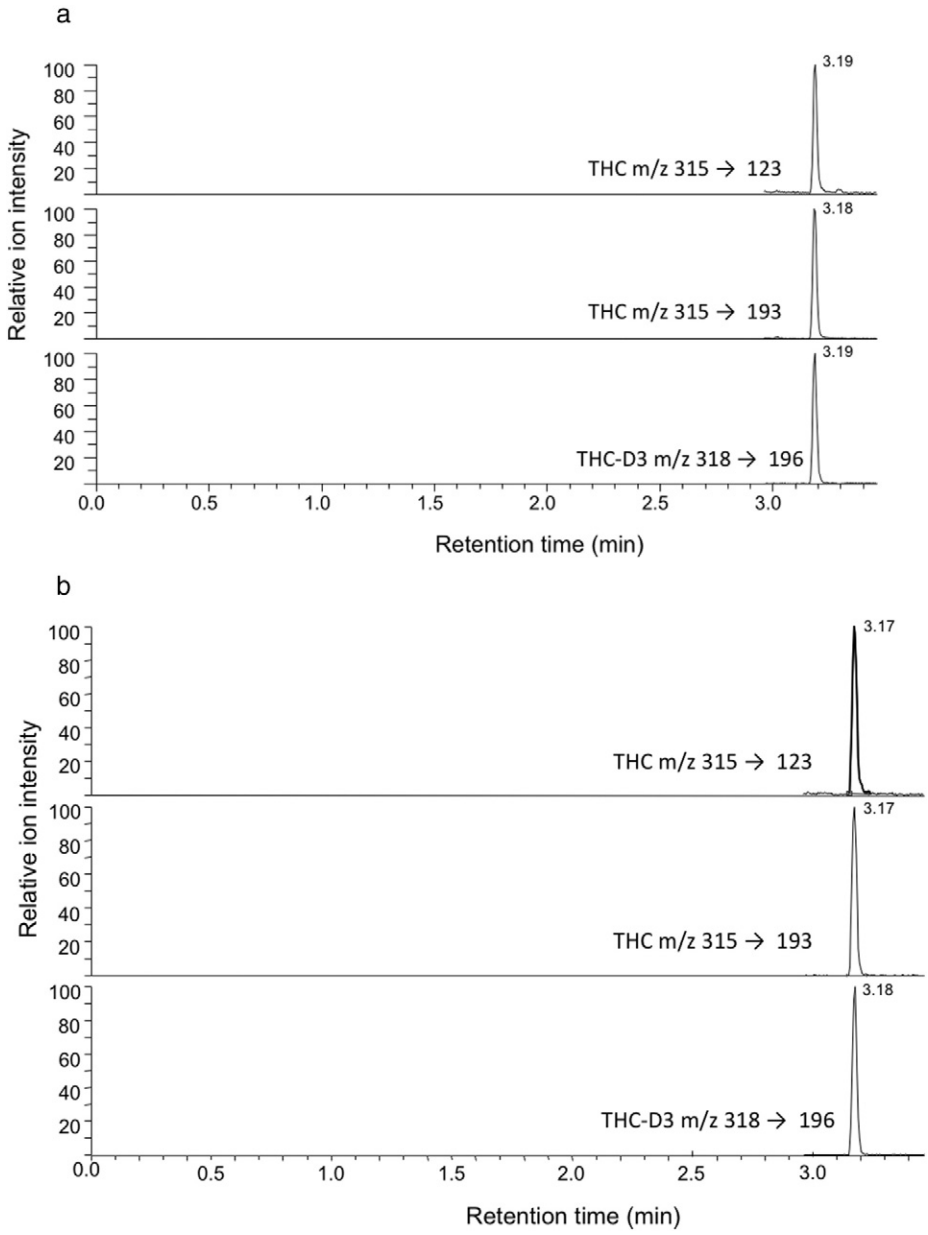


**Fig. 1.** Chromatograms from the LC-MS/MS analysis of THC in a) a breath sample collected from subject M at 120 min quantified to 4132 pg/filter; b) a prepared standard of 3000 pg/filter. Two product ions were monitored for THC. Identification was based on a correct retention time relative to internal standard ($\Delta^9$-tetrahydrocannabinol-$D_3$, THC-$D_3$) and a correct peak area ratio (±20%) between the two ions.