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Cannabis and driving: the use of LC–MS to detect Δ^9-tetrahydrocannabinol (Δ^9-THC) in oral fluid samples

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Abstract

Cannabis is the most widely used illicit drug in the world. There is strong evidence from performance studies that Δ^9-THC has significant effects on the cognitive and psychomotor tasks associated with driving. New, easy and sensitive methods to detect impaired drivers are needed. Therefore, it is necessary the use of alternative biological samples which may be accurate, precise and with trustfully interpretation results. This article presents an LC–MS methodology for detecting Δ^9-tetrahydrocannabinol (Δ^9-THC) in oral fluid samples. The mean recovery was 79%, coefficients of variations were between 2.9% and 6.9% and the limits of detection (LOD) and quantitation (LOQ) were 1.0 ng/ml and 2.0 ng/ml, respectively. The method is sensitive, accurate and reproducible and may be utilized in ongoing controlled cannabinoid administration studies and in roadside studies and thus, important for the fields of forensic toxicology.

Keywords: Δ^9-THC; Oral fluid; LC–MS

1. Introduction

Cannabis is the most widely used illicit drug in the world [1]. The incidence of driving while affected by cannabis is rising in parallel with increased cannabis use in the community [2] and it is clear from prevalence studies that cannabis is frequently used before and during driving [3]. There is strong evidence from performance studies that Δ^9-tetrahydrocannabinol has significant effects on the cognitive and psychomotor tasks associated with driving [3]. New, easy and sensitive methods to detect impaired drivers are needed. Therefore, it is necessary the use of alternative biological samples which, presenting more advantages than the traditional ones, may be accurate, precise and with trustfully interpretation results: the ease and non-invasiveness of sample collection, reduced hazards in specimen handling and testing makes oral fluid a useful alternative matrix [4] for detection of recent cannabis use.

Only recently, LC–MS techniques have been introduced in order to achieve better detection limits than GC–MS, becoming a more sensitive methodology [5]. Thus, we present a method using high-performance liquid chromatography with electrospray ionization mass spectrometry (LC–MS) to determine Δ^9-THC in oral fluid samples.

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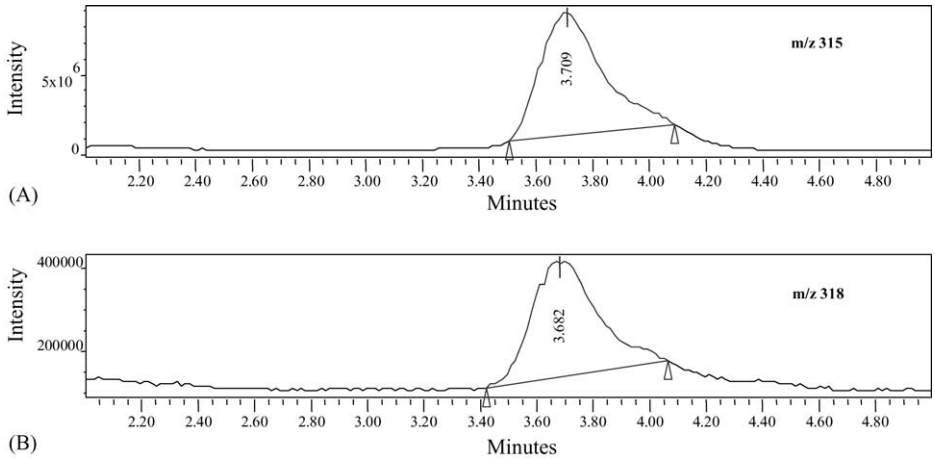

Fig. 1. Δ^9-THC chromatogram at 100 ng/mL (A) and Δ^9-THC-d_3 at 25 ng/mL (B).

2. Experimental

2.1. Sample preparation

To each tube, 500 μL of oral fluid was added after addition of 25 ng of Δ^9-THC-d_3 as the internal standard (IS) and Δ^9-THC at seven different concentrations. Samples were extracted using a solid phase extraction procedure with Bond Elut LRC-Certify columns (10 cc; 300 mg). The dried extracts were reconstituted with 100 μL of mobile phase used in the LC–MS system.

2.2. Apparatus

The LC–MS analyses were conducted on a 2695 Alliance System liquid chromatograph from Waters. The analytical column was an XTerra™ MS C_{18} (2.1 mm × 50 mm, 3.5 μm) from Waters and the mobile phase was acetonitrile and ammonia 0.05% (70:30), with a 0.3 mL/min flow rate, in the isocratic elution mode. The detector was a Waters ZQ 2000 single quadrupole mass spectrometer. Single ion recording (SIR) was performed in positive mode for the ions *m/z* 315 and *m/z* 318 for the protonated molecules [THC + H^+] and [d_3-THC + H^+], respectively.

2.3. Validation of the method

The validation of the method was carried out by establishing recovery values, linearity, intra- and inter-assay accuracy and precision, limits of detection (LOD) and quantitation (LOQ). Calibration curves were prepared daily by spiking blank oral fluid samples with corresponding analytical working solutions to obtain calibration concentrations of 2, 5, 10, 25, 50, 75 and 100 ng/ml of Δ^9-THC. Inter- and intra assay accuracy and precision data for Δ^9-THC were determined with the low, medium and high QC samples. The recovery or extraction efficiency (%) was determined at low, medium and high concentrations.

3. Results and discussion

No interferences were detected in 10 blank oral fluid samples of different origin. Fig. 1 shows two examples of the chromatograms obtained with the LC–MS analysis of an oral

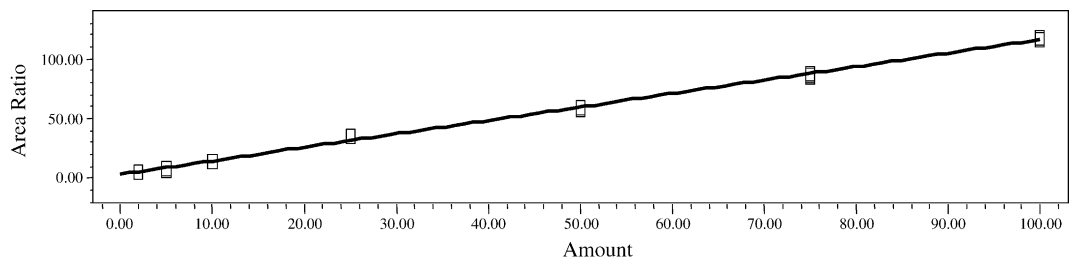

Fig. 2. Calibration curve for Δ^9-THC between 2 ng/mL and 100 ng/mL, in oral fluid.

Table 1
Analytical method validation results

	Parameters				
	LOD (ng/mL)	LOQ (ng/mL)	Accuracy (n = 10) (%) intra/inter[a]	Precision (n = 10) (%) intra/inter[a]	Recovery (%)
Results	1	2	6.9/5.8	2.9/3.8	79

[a] Intra-assay and Inter-assay results.

fluid sample spiked with Δ^9-THC at the concentration of 100 ng/mL and with 25 ng/mL of Δ^9-THC-d_3. Calibration curves for Δ^9-THC were performed in methanol solutions and in oral fluid samples, achieving linearity between 2 ng/ml and 100 ng/ml (Fig. 2).

The confidence parameters of the validated method for the determination of Δ^9-THC are shown in Table 1. In this table, the extraction efficiency of the method is presented as percent recovery. The method provided a good recovery of 79% for this cannabinoid across the linear dynamic range.

This report describes a sensitive and specific LC–MS procedure for the quantification of Δ^9-THC in oral fluid samples. The method has suitable linearity, accuracy and precision with high analyte recoveries and thus, may be a useful analytical procedure for the fields of forensic toxicology, including roadside studies and cannabinoid pharmacology.

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