FORENSICS AND TOXICOLOGY ANALYSIS

DETERMINATION OF CANNABINOIDS, THC AND THC-COOH, IN ORAL FLUID USING AN AGILENT 6490 TRIPLE QUADRUPOLE LC/MS

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Solution Note

Forensics

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Abstract

A simple and rapid method was developed for the quantitation of cannabinoids, namely Tetrahydrocannabinol (THC) and its metabolite Carboxy-tetrahydrocannabinol (THC-COOH), in oral fluid (OF) using an Agilent 6490 Triple Quadrupole LC/MS system. The method was validated according to forensic guidelines and presented excellent data in terms of selectivity, sensitivity and linearity. Also an evaluation of the matrix effect is reported. The results confirmed the suitability of the present method in forensic routine analysis of cannabinoids in oral fluid.

Introduction

Oral fluid (OF) is a new biological matrix for clinical and forensic drug testing, offering easy and non-invasive sample collection mainly accomplished with commercial disposable devices. Due to a stronger correlation than urine with blood concentrations, screening based on OF is greatly gaining value in DUID (Driving under the influence of drugs) programs worldwide [1]. Furthermore cannabinoids are usually the most prevalent analytes in illicit drug testing and for this reason application of OF testing requires sufficient reliable data to support sensitive and specific cannabinoid detection for forensic purposes [2].

Cannabinoids analysis has been historically performed using GC-MS technology, either single quadrupole or triple quadrupole systems. However in the last decade the role of LC-MS/MS in modern forensic toxicology laboratories has gained more relevance because of less extensive sample preparation, reliable results and versatility.

This application note describes a simple fully validated LC-MS/MS method for the determination of Tetrahydrocannabinol (THC) and its metabolite Carboxy-tetrahydrocannabinol (THC-COOH) in OF. Sample preparation is fast and is based on the "dilute and shoot" approach. Results are presented in terms of selectivity, matrix effect, linearity and reproducibility.

Experimental Setup

Materials

Oral fluid samples were from healthy volunteers gathered from the people in the laboratory who denied any drug consumption. Samples were collected by spitting. Water was of milli-Q grade from Sartorius arium® pro VF | UF, Goettingen, Germany. Acetonitrile was of LC-MS grade from SigmaAldrich, St. Louis, MO, USA.

Instrumentation

LC	1290 Infinity
MS	6490A

Chromatographic Conditions

Injection volume	5.0 µL	
Column	Agilent ZORBAX Eclipse Plus C18 RRHT, 2.1 mm x 100 mm x 1.8 µm	
Column thermostat	35 °C	
Needle wash	45 sec	
Mobile phase A	5 mM ammonium formate + 0.1 % formic acid in water	
Mobile phase B	Acetonitrile + 0.1 % formic acid	
Flow rate	0.200 mL/min	
Gradient	Initial	30 %B
	5.0 minutes	95 %B
	8.0 minutes	95 %B
	8.1 minutes	30 %B
Stop time	8.0 minutes	
Post run	1.5 minutes	

MS Parameters

Ionisation	ESI Jet Stream
Polarity	(+)
Gas temperature	120°C
Gas flow	11 L/min
Nebuliser pressure	30 psi
Sheath gas temperature	400°C
Sheath gas flow	12 L/min
Capillary voltage	3000 V
Nozzle voltage	2000 V
Ion funnel voltages	150/100 V
MRM transitions	
THC	315.2→193.2 @20
	315.2→123.3 @30
THC-COOH	345.2→299.2 @18
	345.2→327.2 @18
THC-COOH-D3 (IS)	348.2→330.2 @18

Sample treatment

Oral fluid samples were collected in plastic tubes by spitting. They were then centrifuged at 3000rpm for 10 minutes. The "dilute and shoot" approach consisted of sample dilution in pure water and direct injection onto the LC-MS system. In order to evaluate matrix effect different dilution factors were considered: 1/5, 1/8, 1/10 in water and 1/5 in methanol. Best results were obtained through a 1/8 dilution in water.

Software

MassHunter acquisition, qualitative, quantitative

Results and Discussion

Validation of the method

The validation was carried out according to the international forensic guidelines [3]. The method was validated for selectivity, linearity and sensitivity, precision and accuracy. All calculations were performed using the MassHunter software.

Matrix effect (ME)

In order to quantitatively evaluate ME, the Matuszewski method [4] was adopted. Three dilution factors in water (1/5, 1/8 and 1/10) and one dilution factor in methanol (1/5) were evaluated through standard spiking at three different levels, 0.1, 1.0 and 10 ng/mL, for THC and THC-COOH. Average ME, expressed as coefficient of variation (CV) in %, are reported in Table 1.

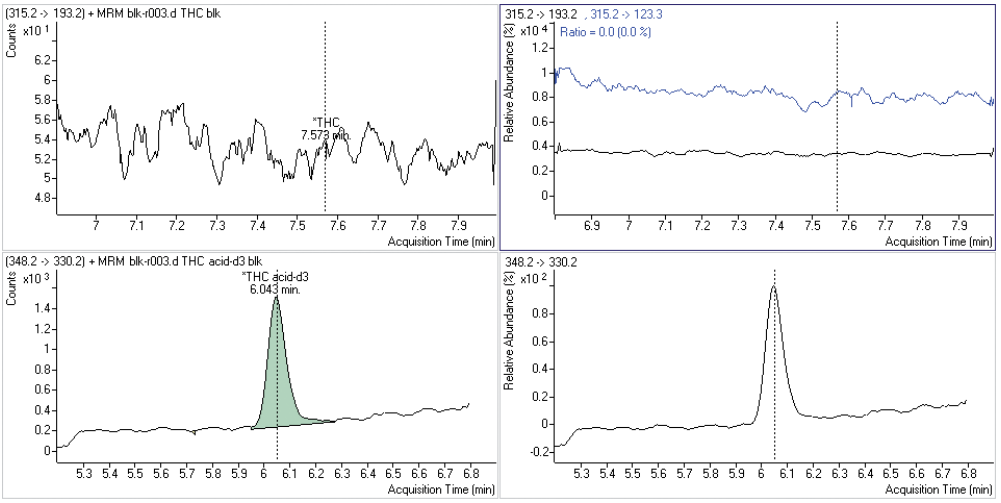
Compound	CV, % Dil 1/5 water	CV, % Dil 1/8 water	CV, % Dil 1/10 water	CV, % Dil 1/5 MeOH
THC	15	10	17	35
THC-COOH	4	12	12	15

Table 1. Average CV (%) for THC and THC-COOH in different matrix dilutions.

In order to fulfil the CV < 15% requirement for both analytes, the dilution factor of eight in water was selected for method validation.

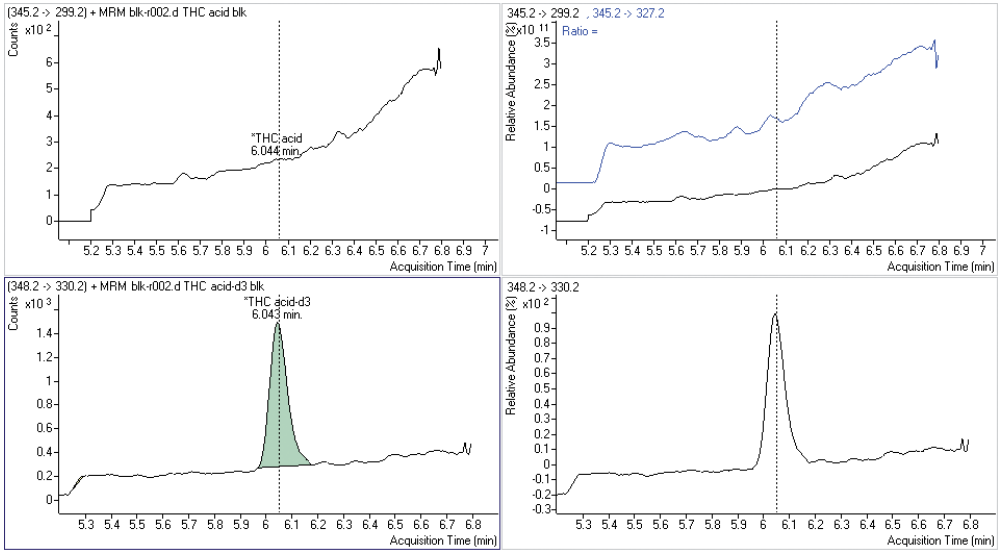
Selectivity

Five different samples from volunteers were tested with and without internal standard (IS) in order to determine any endogenous interferent. All tested samples showed no interferent peak at the retention time of the analyte and IS (figures 1A and 1B).



A

Figure 1 A. OF blank sample spiked with IS (THC-COOH/D3). No interfering peaks were found at the RT of THC.

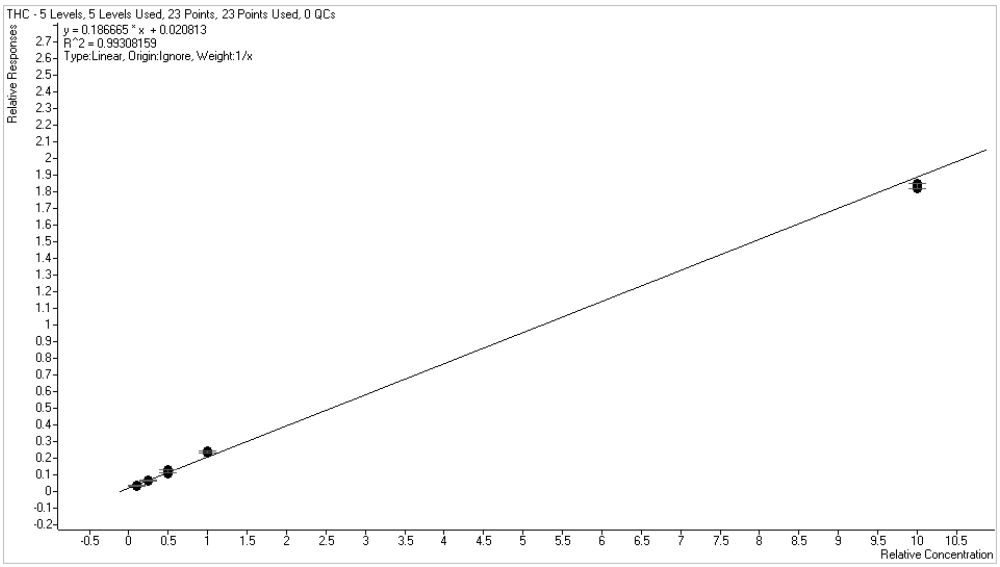


B

Figure 1 B. OF blank sample spiked with IS (THC-COOH/D3). No interfering peaks were found at the RT of THC-COOH.

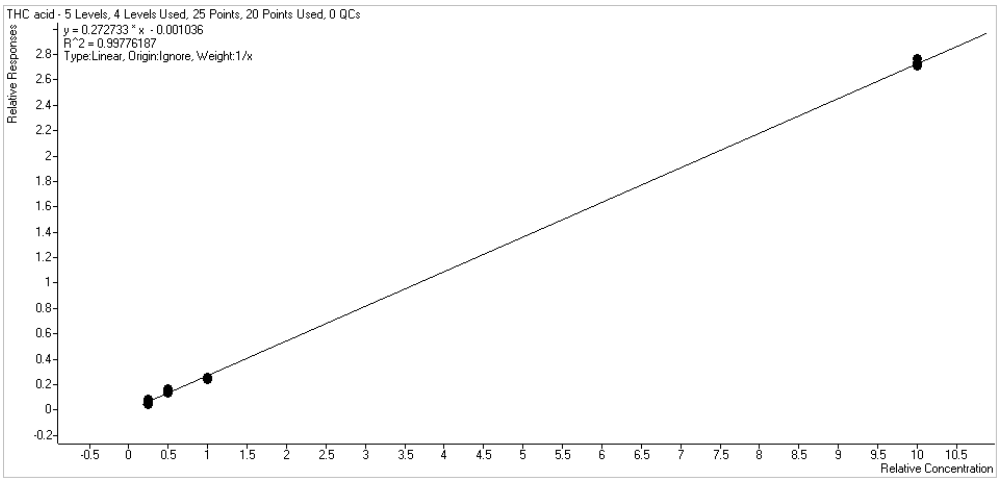
Linearity and sensitivity

The calibration was evaluated by analysing five replicates of spiked OF at 0.1, 0.25, 0.5, 1.0, 10.0 ng/mL for THC and 0.25, 0.5, 1.0, 10.0 ng/mL for THC-COOH. The peak area response relative to the IS was plotted against concentration for both analytes. The calibration curves, determined by the least squares regression method, were linear over the range, with equations y = 0.186665x + 0.020813, R square 0.9930 and y = 0.272733x – 0.001036, R square 0.9977 for THC and THC-COOH respectively [figures 2A and 2B].



A

Figure 2 A. THC spiked OF calibration curve. Calibration range 0.1-10 ng/mL.



B

Figure 2 B. THC-COOH spiked OF calibration curve. Calibration range 0.25-10 ng/mL.

The lower limit of quantifications (LLOQ), defined as the lowest concentration with an intraday and inter-day accuracy RSD < 20%, was 0.1 ng/mL in OF (0.0625 pg on column) for THC (figure 3) and 0.25 ng/mL in OF (0.156 pg on column) for THC-COOH (figure 4). The limit of detection (LOD) defined as signal to noise ratio equal to 3 was 0.01 ng/mL in OF for THC (0.00625 pg on column) and 0.1 ng/mL (0.0625 pg on column) in OF for THC-COOH. Signal to noise ratios were calculated with the RMS algorithm in MassHunter quantitative software.

Precision and accuracy

Precision and accuracy of the method were assessed by analysing spiked OF samples at concentrations of 0.1 (LOQ for THC), 0.25 (LOQ for THC-COOH), 1.0 and 10.0 ng/mL. Five replicates each were analysed on three non-consecutive days. The intra- and inter-day precision and accuracy values fitted the requirements of the forensic guidelines with RSDs always below 20% at the LOQ level and < 15% for higher concentrations. The results of the inter-day testing are summarised in Table 2.

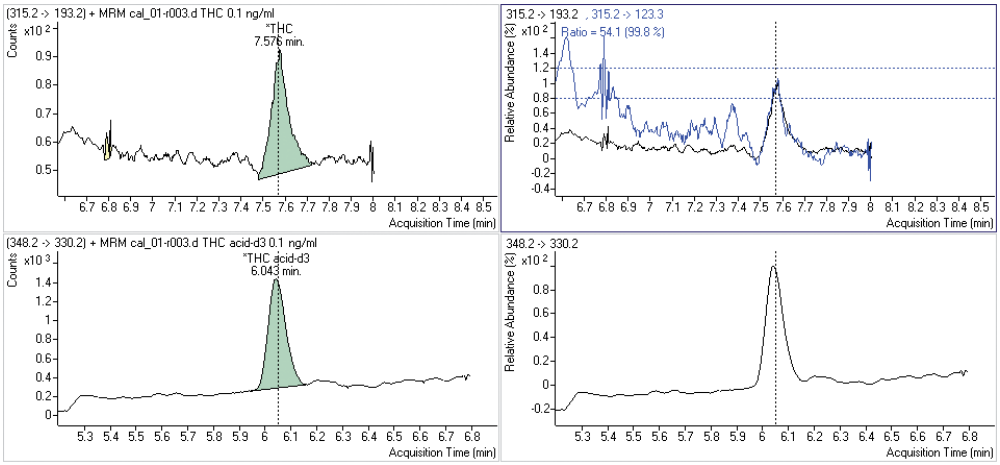


Figure 3. THC at 0.1 ng/mL (LLOQ) in spiked OF sample with IS.

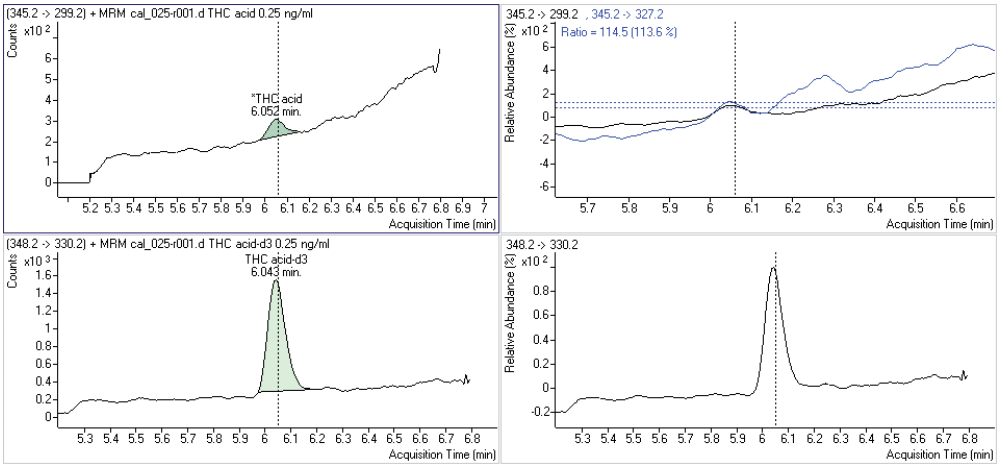


Figure 4. THC-COOH at 0. 25 ng/mL (LLOQ) in spiked OF sample with IS.

Parameter	THC 1.1 ng/mL	THC-COOH 0.25 ng/mL	THC 1.0 ng/mL	THC-COOH 1.0 ng/mL	THC 10.0ng/mL	THC-COOH 10.0 ng/mL
Mean (ng/mL)	0.088	0.24	1.06	0.97	9.8	10
SD	0.017	0.03	0.12	0.07	0.3	0.11
RSD, %	19	12	11	7	3	1.1
Bias, %	-11	-3	+6	-3	-2	+0.12

Table 2. Summary of inter-day results for THC and THC-COOH in spiked OF samples.

Conclusion

The increasing need for testing for drugs of abuse imposes high demands in terms of sensitivity and accuracy of toxicological procedures. An ultra-high performance liquid chromatography/ tandem mass spectrometry method was developed, mainly proposed for screening but also for simultaneous confirmation of cannabinoids in OF samples in a single run. In fact, this developed method demonstrated that it fulfilled the requirements of selectivity, sensitivity, accuracy and precision presented in the international forensic guidelines. The advantage of this LC-MS technique over traditional GC-MS cannabinoids analysis is that it limits sample preparation and exploits the "dilute and shoot" approach, offering an alternative to time-consuming and labour-intensive sample preparation procedures.

References

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