Application Note

Forensics

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Determination of Δ^9-Tetrahydrocannabinol in Seized Marijuana by QuEChERS and LC/MS/MS

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Abstract

This Application Note describes an analytical method for the determination of Δ^9-tetrahydrocannabinol (Δ^9-THC) in marijuana samples using QuEChERS sample preparation followed by liquid chromatography/tandem mass spectrometry (LC/MS/MS). The proposed method is fast, simple, and presents linear calibration curves in the range of 10 to 1,000 µg/kg (R^2 >0.999), with relative standard deviation (RSD) for replicate injections lower than 3.8 %. The limit of detection (LOD) and limit of quantification (LOQ), based on the signal-to-noise ratio (S/N), were 1.3 µg/kg and 4.5 µg/kg, respectively. Precision and accuracy were verified through recovery of spiked samples at three distinct levels of concentration, in five replicates. Recovery values ranged from 90 to 106 % with RSD lower than 6 %. The method was successfully applied to the detection of Δ^9-THC in marijuana samples seized by police in Rio de Janeiro, Brazil. The Δ^9-THC content in the samples varied between 1.53 and 4.82 %, with an average of 2.3 %, (% w/w).

Introduction

Cannabis, the source of marijuana and other preparations, is one of the oldest cultivated plants, especially in the tropical regions of South America such as Peru and Brazil[1]. Some components of the cannabis plant have psychoactive properties, such as Δ^9-tetrahydrocannabinol (Δ^9-THC). This is present in high concentrations in the plant, and is the main compound responsible for the effects of marijuana. Δ^9-THC is rapidly absorbed by inhalation or ingestion. Due to its lipophilic properties, it tends to accumulate in fatty tissue, remaining in the body for as long as 30 days[1,2]. Figure 1 shows the molecular structure of Δ^9-THC.

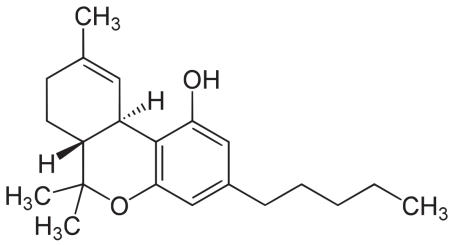

Figure 1. Molecular structure of Δ^9-THC.

Despite the global trend toward marijuana legalization, the legality of cannabis for medicinal or recreational use varies from country to country. In many countries, the use, sale, and possession of all forms of cannabis is illegal[1]. As a result, many forensic and testing labs are looking for a simple, economic, fast, and robust method to determine the Δ^9-THC concentration in marijuana and cannabis-infused foods. This Application Note describes an LC/MS/MS method for the determination of Δ^9-THC in marijuana samples seized by police in Rio de Janeiro, Brazil.

Experimental

LC conditions

Parameter	Value
Instrument	Agilent 1290 Infinity II Series UHPLC, with the following configuration: Agilent 1290 Infinity II high speed pump (G7120A) Agilent 1290 Infinity II multisampler (G7167B) Agilent 1290 Infinity II multicolumn thermostat (G7116B)
Column	Agilent ZORBAX Rapid Resolution High Definition Eclipse Plus C18 2.1 mm × 100 mm, 1.8 µm (p/n 959758-902)
Column temperature	40 °C
Injection volume	1 µL
Mobile phase	A) Water acidified with 0.1 % formic acid B) Acetonitrile
Gradient	Time (min) %A %B 0.0 50 50 0.2 50 50 2.5 10 90 6.0 10 90 6.1 50 50 7.0 50 50
Flow rate	0.300 mL/min

MS conditions

Parameter	Value
Instrument	Agilent 6470 triple quadrupole LC/MS (G6470AA)
Ion mode	Agilent Jet Stream Electrospray, positive ionization
Capillary voltage	4,000 V
Sheath gas heater	300 °C
Sheath gas flow	11 L/min
Drying gas flow (N_2)	10 L/min
Drying gas temperature	300 °C
Nebulizer pressure	30 psi

The MS was operated in positive multiple reaction monitoring (MRM) mode using three specific transitions for Δ^9-THC. The most intense transition was used for quantification, and the others were used as qualifying ions. Table 1 lists the retention time (RT), monitored ions, and other MS/MS acquisition parameters used for the identification and quantification of Δ^9-THC in marijuana.

Table 1. Retention time (RT) and MS/MS acquisition parameters used for the identification and quantification of Δ^9-THC in marijuana.

Compound	RT (min)	Q1[a] (*m/z*)	Q3[b] (*m/z*)	CE[c] (V)	FE[d] (V)
Δ^9-THC	4.52	315.2	259.2* 193.1 123.0	20 20 40	150

[a] Precursor ion (Q1) ,[b] Fragment ion (Q3), [c] Collision energy, [d] Fragmentor energy

Sample preparation

Marijuana samples were provided by the Service of Forensic Chemistry of the Carlos Éboli Institute of Criminalistics, Rio de Janeiro Department of Technical and Scientific Police, Brazil. We had access to five different samples. Extraction of Δ^9-THC from marijuana samples was performed using the QuEChERS method. A 1.0-g aliquot of sample was placed into a 50-mL polypropylene tube. Afterwards, 10.0 mL of acetonitrile was added to each tube, as well as an Agilent Original QuEChERS extraction packet (p/n 5982-7550) containing 4 g of magnesium sulfate ($MgSO_4$) and 1.0 g of sodium chloride (NaCl). Sample tubes were capped tightly, hand-shaken vigorously for one minute, and centrifuged at 5,000 rpm for five minutes. The cleanup step was performed using the QuEChERS Dispersive SPE Kit for Fruits and Vegetables, EN method (p/n 5982 5021CH). A 1-mL aliquot of the supernatant was placed into a 2-mL PP tube containing 25 mg of PSA sorbent and 150 mg of $MgSO_4$. This was vortexed for one minute, then centrifuged for five minutes at 5,000 rpm. A 1-mL aliquot of the supernatant was diluted with mobile phase 1:1 (v/v, 50 % organic). This was filtered through a 0.2 µm PVDF and PP membrane (Agilent Captiva filter cartridges, p/n A5300002), and analyzed.

Results and discussion

Figure 2 shows an example of the response for Δ^9-THC, using the Agilent MassHunter Qualitative software (B.08.00).

The linearity of the analytical curve was studied at nine different concentration levels ranging from 10 to 1,000 µg/kg. Each concentration level was analyzed in triplicate. The determination coefficient (R^2) calculated by linear regression was greater than 0.999. The LOD and LOQ were determined to produce a signal 3 and 10 times the baseline noise, relative to each of the corresponding concentrations, in a region close to the retention time of Δ^9-THC. The proposed method enabled us to detect Δ^9-THC at a 1.4 µg/kg level. Table 2 shows the regression equations and other related parameters of the developed method, and Figure 3 shows the calibration curve for Δ^9-THC, using the MassHunter software (B.08.00).

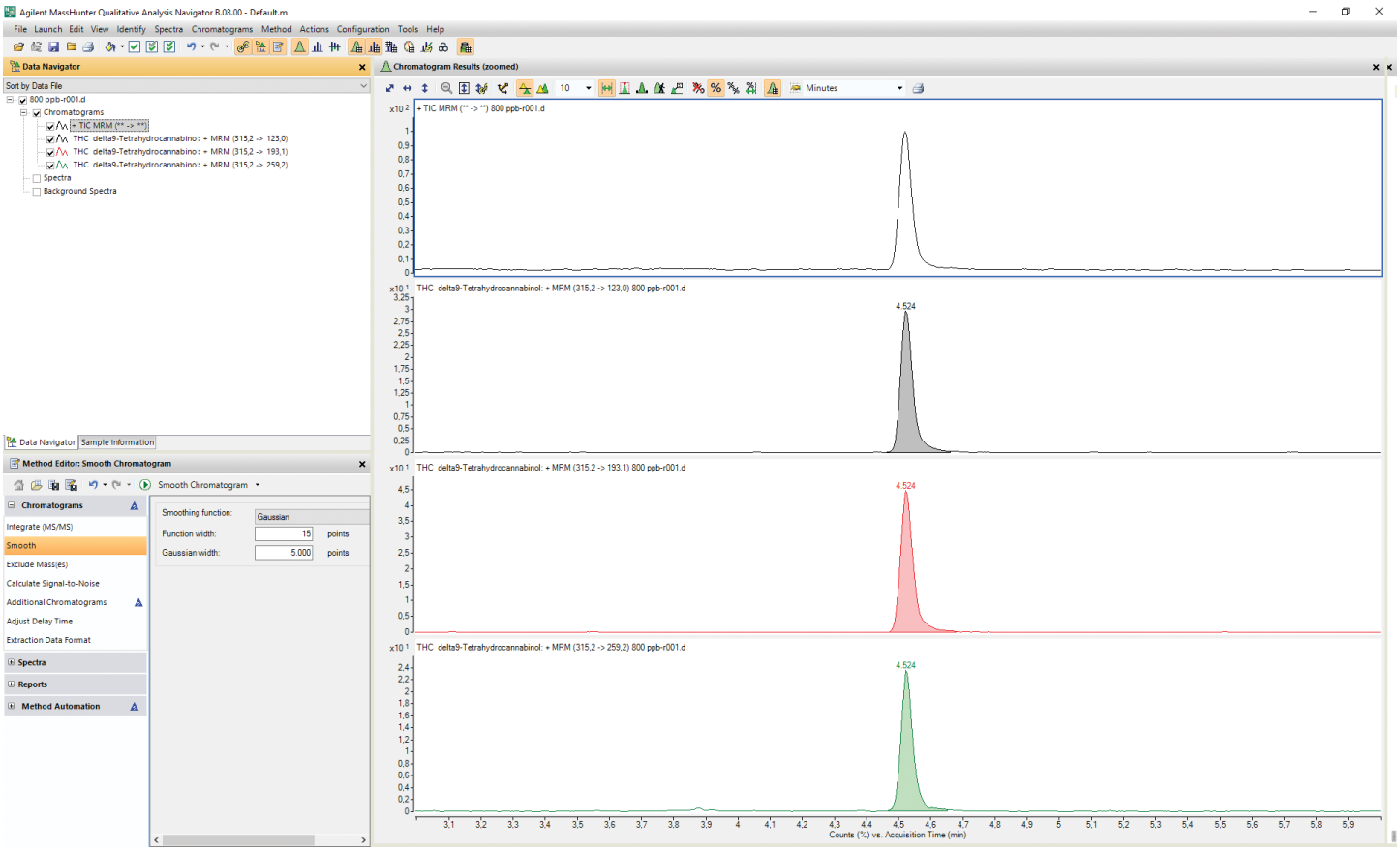

Figure 2. Example of response for Δ^9-THC, using the MassHunter Qualitative software (B.08.00).

Table 2. Analytical characteristics of the proposed LC/MS/MS method for Δ⁹-THC detection in marijuana.

Compound	y = a + bx	R^2	LOD (µg/kg)	LOQ (µg/kg)
Δ⁹-THC	y = 26.216 x – 238.57	0.999	1.3	4.5

a = Intercept; b = Slope; R^2 = Determination coefficient; LOD = Limit of detection; LOQ = Limit of quantitation

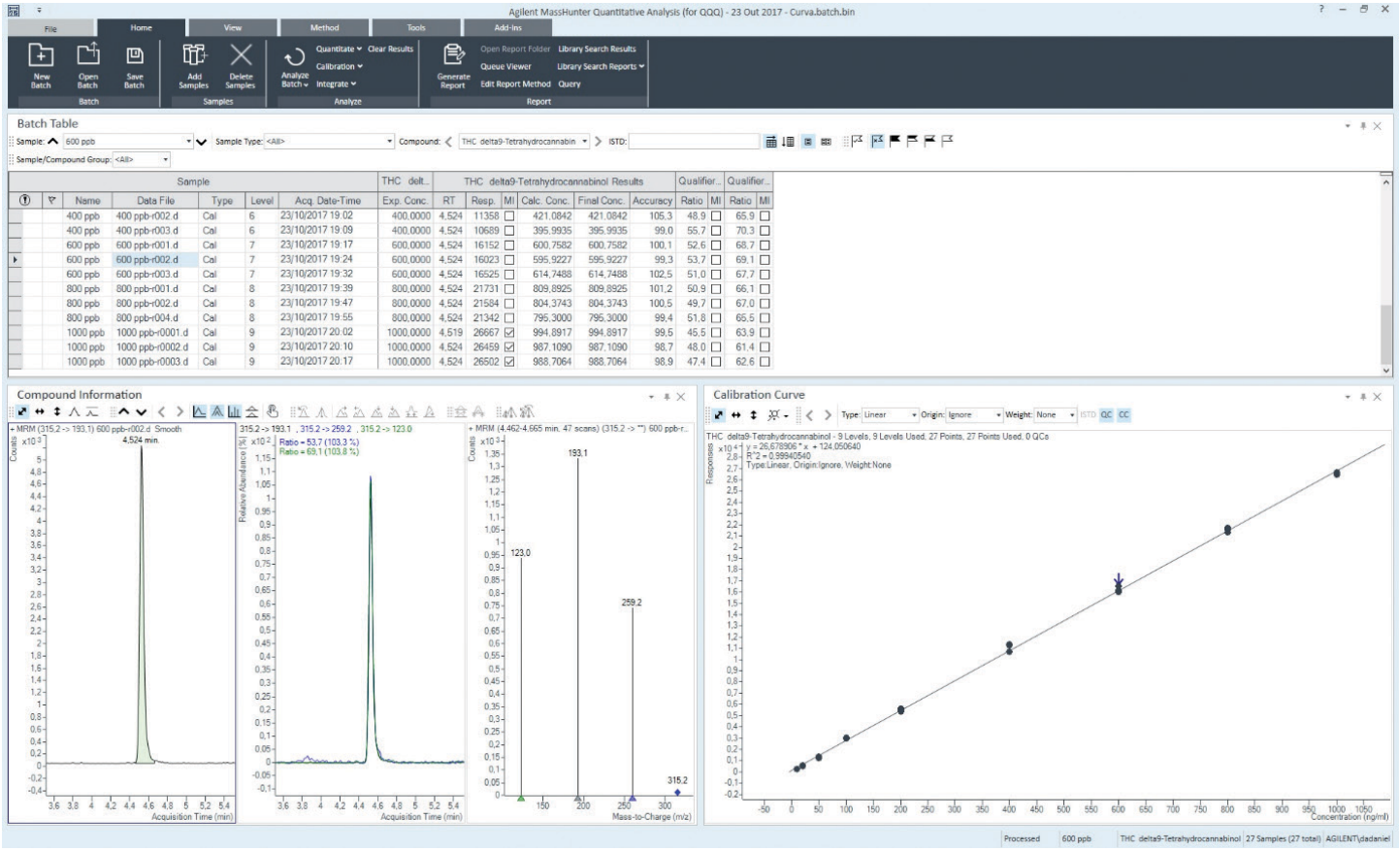

Figure 3. Calibration curve for Δ⁹-THC, using the MassHunter Quantitative software (B.08.00).

Table 3. Levels of Δ⁹-THC (% w/w) obtained by LC/MS/MS in marijuana samples (n = 3).

Sample	Δ⁹-THC (%)[a]	RSD (%)
A	1.84 ± 0.02	1.2
B	4.82 ± 0.04	0.8
C	1.53 ± 0.02	1.3
D	3.62 ± 0.02	0.6
E	1.97 ± 0.01	0.5

[a] The level is expressed as a percentage of the Δ⁹-THC over the total mass of the sample.

Precision and accuracy were verified through recovery tests carried out by spiking samples before the QuEChERS extraction step treatment with a known amount of Δ^9-THC. This resulted in three different concentration levels (50, 100, and 500 µg/kg), in five replicate measurements. The recovery values ranged between 90 to 106 %, with RSD lower than 3 %. The Δ^9-THC levels in marijuana samples were determined by a standard addition calibration curve. Table 3 shows the results and standard deviations expressed in percentages. Although there are no studies focused on the potency of the marijuana consumed in Latin America countries, the results obtained are consistent with those observed by other researchers[1].

Conclusion

The LC/MS/MS method described here proved to be suitable for the measurement of Δ^9-THC levels in marijuana. The proposed method presented a linear response to Δ^9-THC in the concentration range from 10 to 1,000 µg/kg, with excellent precision data for replicate injections and a LOD lower than 1.4 µg/kg. The QuEChERS sample preparation was simple and efficient, with recoveries from 90 to 106 % from marijuana matrix. The method is simple and fast, requiring less than seven minutes per sample, and shows excellent potential for use in forensic laboratories.

Agilent products and solutions are intended to be used for cannabis quality control and safety testing in laboratories where such use is permitted under state/country law.

References

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