FORENSICS ANALYSIS

THC-COOH DETERMINATION IN HAIR WITH LC/MS/MS: A CHALLENGING REQUEST

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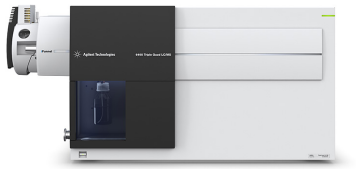

ABSTRACT:

Traditionally, to achieve concentrations in the order of pg/mg, as recommended by the Society of Hair Testing (SoHT), 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THC-COOH) determination in hair is carried out with GC/MS/MS after sample derivatization. Here, a very sensitive method for the quantitation of THC-COOH down to 0.2 pg/mg in hair matrix using an Agilent 6495 Triple Quadrupole LC/MS system is presented.

INTRODUCTION:

Testing hair for drugs of abuse was introduced over 50 years ago and since then a large body of literature has been produced. One advantage of hair testing is its larger detection window compared to other biological fluids and in most cases drugs are better preserved in hair. Applications of hair testing include criminal investigations, i.e. verifying drug use history and identifying drug facilitated sexual assault, proving drug use in child custody cases, monitoring abstinence of parolees, drug treatment participants or employees, and documenting in utero exposure. Guidelines for drug testing in hair have been proposed by the Society of Hair Testing (SoHT)[1] and, according to them, THC-COOH is considered an incontestable proof of tetrahydrocannabinol (THC) uptake.

However, if implemented, the guidelines establish a confirmation cutoff of 0.2 pg THC-COOH /mg hair, thus requiring the use of very sensitive, specific instrumentation such as tandem mass spectrometry. This solution note describes a simple, very sensitive LC/MS/MS method for the determination of THC-COOH in hair matrix.

EXPERIMENTAL:

Materials

Blank hair samples were from healthy laboratory volunteers who denied any drug consumption. Positive samples were collected from volunteers who admittedly declared regular cannabis consumption. Water was of ultrapure grade from Sartorius arium ® pro VF|UF, Goettingen, Germany. All solvents were of LC/MS grade from Sigma Aldrich, St. Louis, MO, USA. Salts were of reagent grade and were also from Sigma Aldrich, St. Louis, MO, USA. SPE cartridges were Bond Elut Certify, 130 mg, 6 mL (p/n 12256146) from Agilent Technologies.

Instrumentation	
LC	1290 Infinity II
MS	6495 Triple Quadrupole LC/MS

Chromatographic Conditions	
Injection volume	5.0 µL
Column	Agilent Zorbax Eclipse Plus C18 Rapid Resolution HD
Column thermostat	50°C
Needle wash	10 sec flushport
Mobile phase A	ammonium fluoride
Mobile phase B	acetonitrile/methanol/isopropanol
Flow rate	0.150 mL/min

MSD Parameters	
Ionization	ESI Jet Stream
Polarity	(-)
MRM transitions	
THC-COOH	343.2 ⇢ 299.0 @ 21
	343.2 ⇢ 245.1 @ 33
	343.2 ⇢ 191.0 @ 37
THC-COOH-D3	346.2 ⇢ 194.0 @ 37

Sample treatment

Hair samples were decontaminated with dichloromethane and methanol for 10 minutes each and dried at room temperature. They were then cut into lengths of 1-2 mm with scissors. 20 mg of each sample was weighed into a clean tube. Blank samples were spiked at 0.1, 0.2, 0.5, 1, 5 and 10 pg/mg of THC-COOH. An internal standard (IS), THC-COOH-D3, was added to all samples at concentration of 1.25 pg/mg. Hydrolysis was carried out in 1 mL of 1M NaOH for 30 minutes at 70°C. After samples cooled down, they were centrifuged at 4000 rpm for 3 minutes and then extracted by SPE. 5 µL of the reconstituted extract was injected into the LC/MS/MS system.

Software

MassHunter acquisition, qualitative, quantitative

RESULTS AND DISCUSSION:

Under the described conditions, THC-COOH and THC-COOH-D3 (IS) eluted in 11 minutes. Three MRM transitions were monitored for THC-COOH (1 quantifier and 2 qualifiers) and one for THC-COOH-D3. Figure 1 shows the MRM transitions of THC-COOH and THC-COOH-D3 in a blank and a spiked hair sample (at a concentration of 0.2 pg/mg). As many analysts can confirm, trace analysis of THC-COOH in hair suffers from possible matrix interferences on some or all of the MRM transitions. In our experiments, the transition m/z 343.2 ⇢ 299.0 may result less significant, though still present, at very low concentrations i.e. 0.2 pg/mg, yet it is confirmed significant at higher levels (Figure 2). Figure 2 shows the MRM transitions at 1 pg/mg of THC-COOH in hair. At the 0.1 pg/mg THC-COOH concentration level, a signal for the quantifier is produced but criteria for the qualifiers are not met. For this reason, linearity was tested in the 0.2-10 pg/mg concentration range, with 5 repetitions at each level (R2 = 0.9952) (Figure 3). Intraday repeatability and accuracy bias were evaluated on spiked hair samples at concentrations of 0.2 and 1.0 pg/mg on 8 runs. Results were satisfactory and are reported in Table 1.

Hair samples from cannabis users were then analyzed using this method resulting positive at THC-COOH, with concentrations of 1.5 and 0.98 pg/mg, respectively (Figure 4).

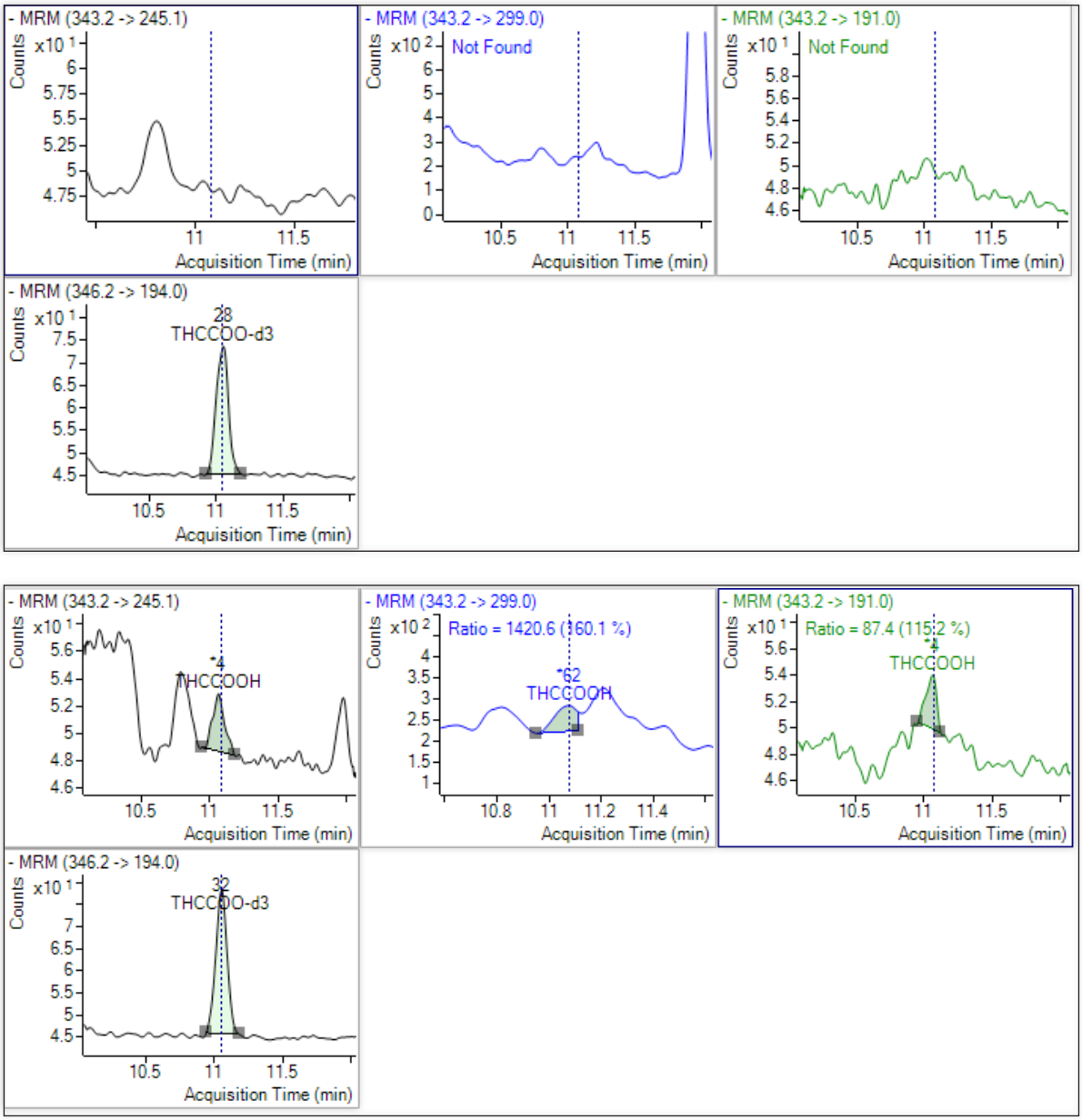

Figure 1. Negative hair sample (above) and spiked hair sample at 0.2 pg/mg (below). Both were spiked with IS.

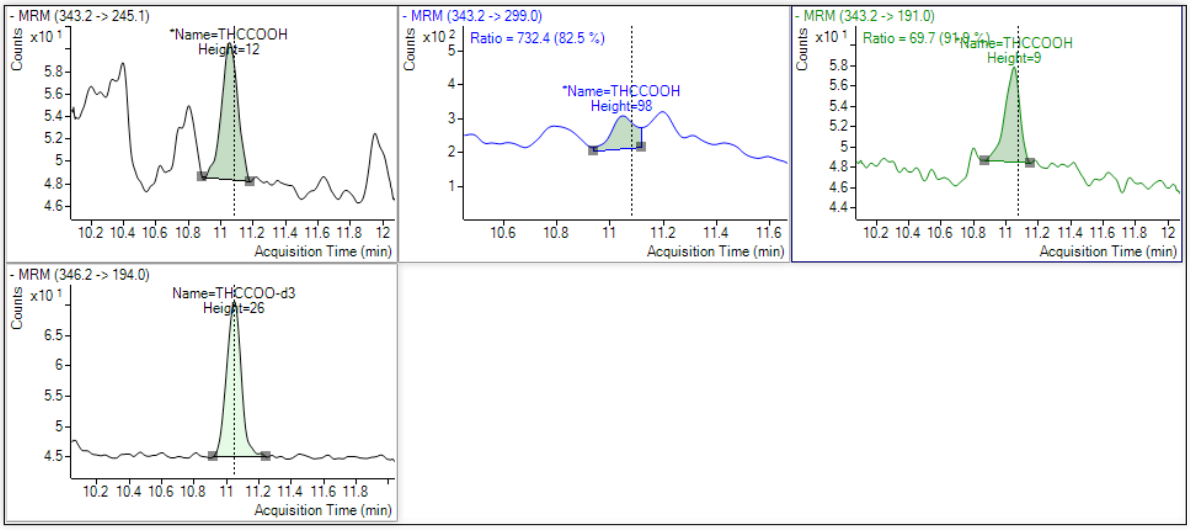

Figure 2. Spiked hair sample at the concentration of 1 pg/mg.

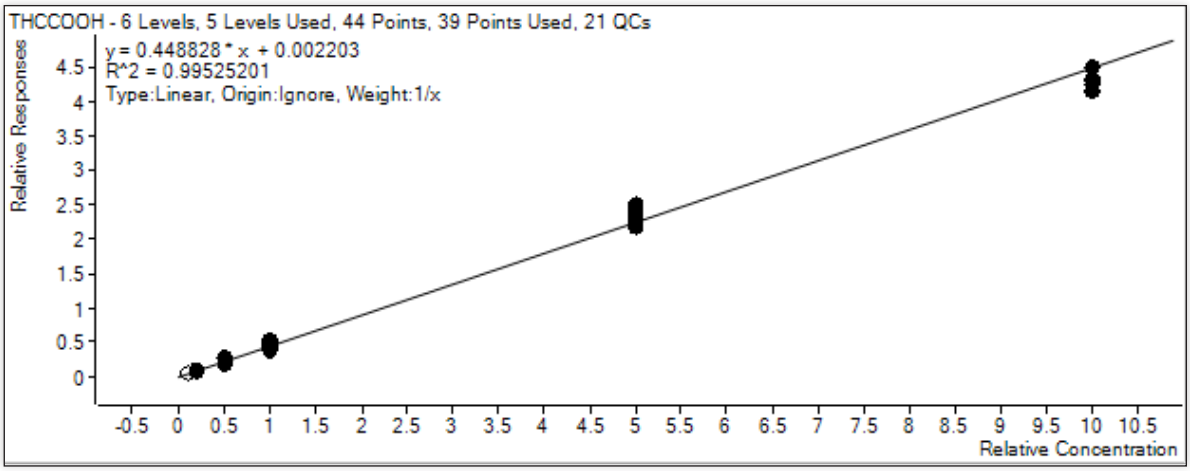

Figure 3. Linearity was tested in the range 0.2-10 pg/mg in spiked hair matrix, with 5 repetitions at each level.

Concentration level (pg/mg)	Intraday precision	Bias 8 runs (%)
0.2	8	1.5
1.0	9	-2.3

Table 1. Intraday precision and bias results on 8 runs at concentrations of 0.2 and 1 pg/mg.

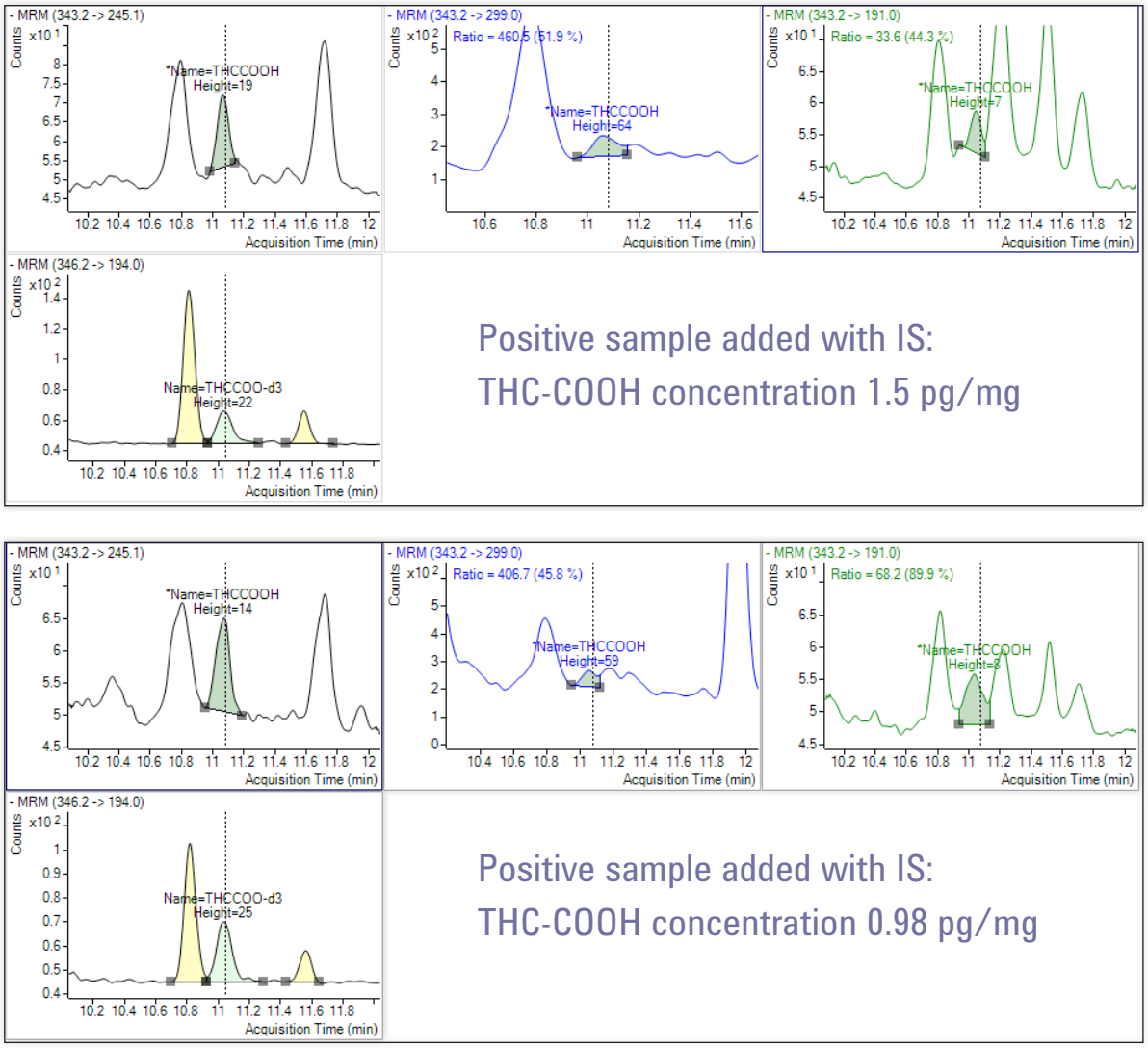

Figure 4. MRM transitions and results for these hair samples.

CONCLUSIONS

The continuing emergence of drugs of abuse imposes high demands in terms of sensitivity and accuracy of toxicological procedures. A very sensitive ultra-high performance liquid chromatography/tandem mass spectrometry (LC/MS/MS) method was developed for the determination of THC-COOH in hair matrix down to levels recommended by the Society of Hair Testing.

REFERENCES

1. G.A.A. Cooper, R. Kronstrand, P. Kintz, Society of Hair Testing guidelines for drug testing in hair, Forensic Science International 218 (2012) 20–24.

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