ANALYSIS OF CARBOXY-THC USING UPLC-MS/MS

Jane Cooper,[1] *Godo Bosch,*[2] *and Simone Donzelli*[3]

[1]Waters Corporation, UK, [2]Waters Corporation, France, [3]Waters Corporation, Italy.

ABSTRACT

This poster describes a robust UPLC-MS/MS method for the analysis of 11-nor-9 -carboxy-Δ9- tetrahydrocannabinol (cTHC) in hair, to satisfy the confirmation cut-off values as recommended by the Society of Hair Testing (SoHT).[1]

INTRODUCTION

The use of hair as a biological matrix for forensic testing has increased in popularity over the last decade. Drug substances can be incorporated into the hair by various mechanisms including, as shown in Figure 1.

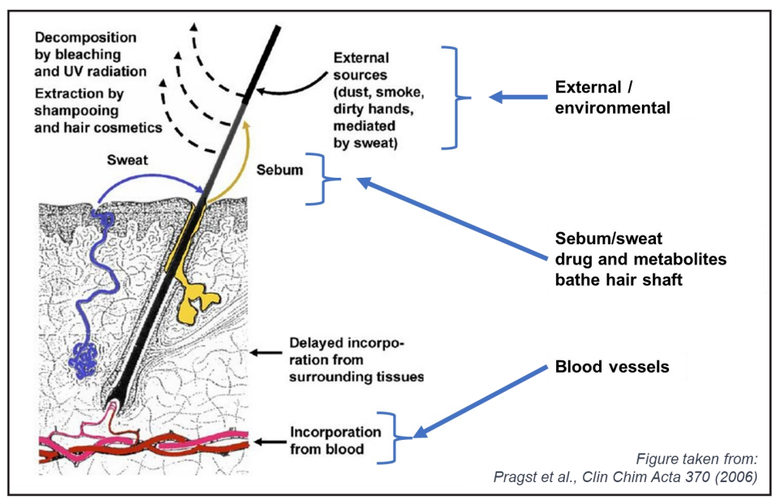

Figure 1. *Mechanisms for incorporation of drugs into hair.*

The use of hair as a specimen offers several benefits, hair collection is simple, and does not require medically trained staff to collect the sample. Collection of the sample does not require privacy, meaning that collection can be supervised, thus reducing the potential for sample adulteration. Further, once collected, hair can be easily stored.

Hair provides an extended window of detection for drug substances, drugs can be detected months and even years after use. As hair grows at ~1 cm per month, a typical hair sample of 20-25 mg hair, and collected from the posterior vertex of the head according to the SoHT recommendations, provides an accumulated specimen which can provide an insight into drug usage in recent months.

The most widely used drug substance in the world is cannabis, and long-term use can lead to dependency. Cannabinoids produced from cannabis are one of the most detected classes of drugs, therefore their analysis is of key importance in forensic testing. Delta-9-tetrahydrocannabinol (THC) is the major psychoactive cannabinoid present in the plant Cannabis sativa and produces several metabolites including cTHC. Positive identification of THC in hair can also be attributed to passive environmental cannabis smoke exposure, therefore SoHT requires that the positive identification of THC in hair samples must be confirmed by measuring the metabolite cTHC. However, the analysis of cTHC is very challenging, it is typically found at low pg/mg concentrations, with limited sample availability, making the requirement for high sensitivity analytical techniques essential.

EXPERIMENTAL

CHROMATOGRAPHIC CONDITIONS

System:	UPLC-I-Class (FTN)
Needle or Loop Size:	30µL needle or 15µL needle with 50 µL extension loop
Mobile Phase A:	0.4mM ammonium fluoride containing 0.0025% ammonium hydroxide
Mobile Phase B:	Methanol
Flow Rate:	0.35 mL/min
Column:	ACQUITY Premier BEH C18 Column with VanGuard FIT, 1.7 µm, 2.1 x 150 mm (p/n = 186002353)
Column Temperature:	55°C
Sample Temperature:	10°C
Injection volume:	15 µL
UPLC gradient:	See Table 1.

Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
Initial	70	30
0.5	70	30
4.0	20	80
4.2	2	98
5.2	2	98
5.25	70	30
7.5	70	30

Table 1. *UPLC gradient conditions.*

MS CONDITIONS

Acquisition:	ESI negative
Capillary voltage:	2.5 kV
Desolvation temperature:	600 ºC
Desolvation flow:	1000 L/Hr
Cone:	150 L/Hr
Source temperature:	150 ºC
MRM conditions:	See Table 2

Analytes and Int. stds	Precursor Ion (*m/z*)	Product Ion (*m/z*)	Cone Voltage (V)	Collision Energy (eV)
cTHC	434.1	191.0 (quantifier)	30	31
		245.1 (qualifier)	30	33
cTHC-D3	346.1	248.1 (Int. std)	37	23

Table 2. *MRM conditions for analytes and internal standard.*

SAMPLE ANALYSIS

Hair samples were sourced from volunteers and the M3 Reagent was supplied by Comedical, Trento, Italy. http://www.comedical.biz/ . Certified reference material for cTHC and the deuterated analogue cTHC-d3 was from Merck (Dorest, UK).

Pre-decontaminated hair samples (25 mg) were weighed into a centrifuge tube with a sealed cap and spiked with cTHC at concentrations ranging from 0.2 to 10 pg/mg, deuterated internal standard (cTHC-d3) was added along with M3 Reagent. The samples were incubated for 60 min at 100 °C and once cooled the entire sample was loaded onto an OASIS PRiME HLB 30 mg Cartridge (p/n 186008055). The sample was washed with an acetonitrile solution followed by hexane. The cTHC was eluted with acetonitrile/ methanol (9:1 v/v) and following evaporation of the solvent, the samples were reconstituted with 100µL 50% methanol containing 0.25% ammonia solution (5mL methanol, 4.9mL de-ionised water, 100µL 25% ammonia solution), vortexed and transferred to Waters Total Recovery Vials. The workflow for the determination of cTHC in hair is shown in Figure 2.

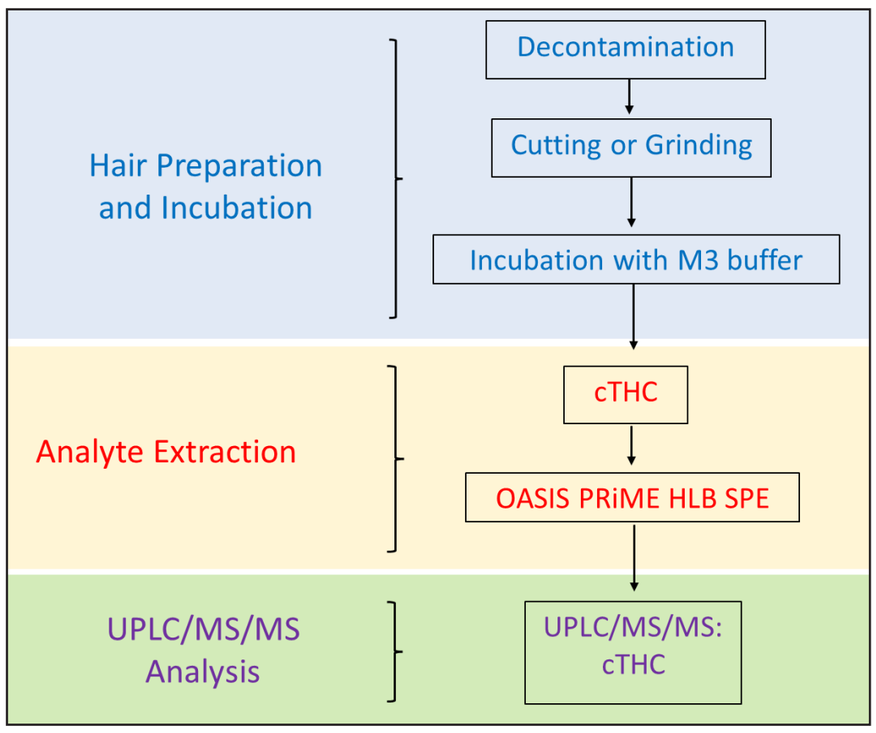

Figure 2. *Workflow for the Determination of cTHC in Hair.*

RESULTS AND DISCUSSION

The MRM chromatograms for a 1 pg/mg spiked hair sample are shown in Figure 2A. The MRM chromatograms comparing blank hair extracts with hair samples spiked at 0.2 pg/mg cTHC, are shown in Figure 3B.

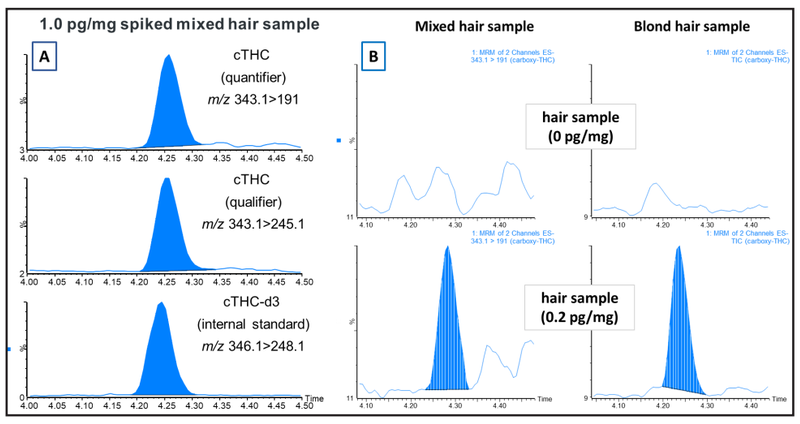

Figure 3. A) *Chromatograms showing the quantifier (top), qualifier (middle) and internal standard (bottom) MRM transitions for a 1.0 pg/mg spiked mixed hair sample.* ***B)*** *Chromatograms showing quantifier MRM transitions for a control (0 pg/mg) hair samples (upper*

The signal to noise values calculated for the quantifier and qualifier MRM transition from 0.2 pg/mg cTHC spiked hair samples are shown in Figure 4.

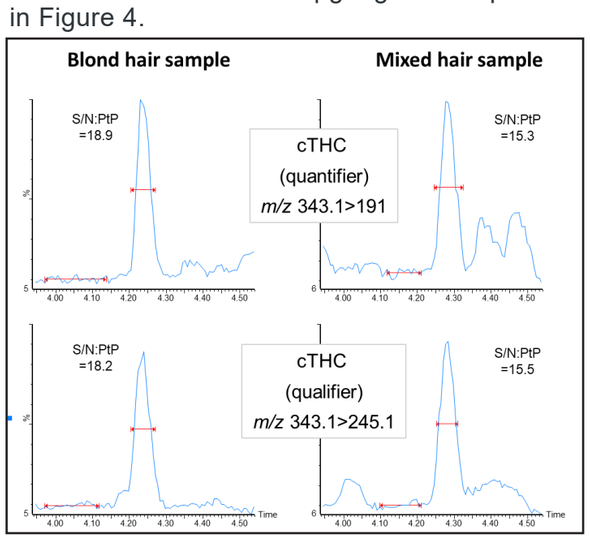

Figure 4. *Chromatogram showing signal to noise calculations for quantifier (upper trace) and qualifier (lower trace) MRM transitions for 0.2 pg/mg spiked hair sample, considering different hair samples (mixed and blond).*

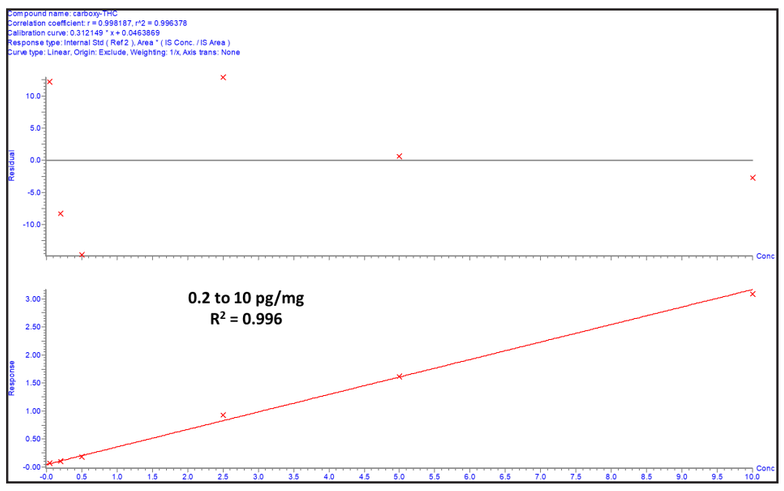

Figure 5*. Linearity of cTHC over the range 0.2 to 10 pg/mg in spiked hair.*

The linearity of the assay was investigated over the range 0.2 to 10 pg/mg, the calibration curve along with the residuals plot are shown in Figure 5.

The robustness of the assay was investigated considering the analysis of mixed hair samples (n=5), each sample extract was injected in triplicate, as shown in Figure 6.

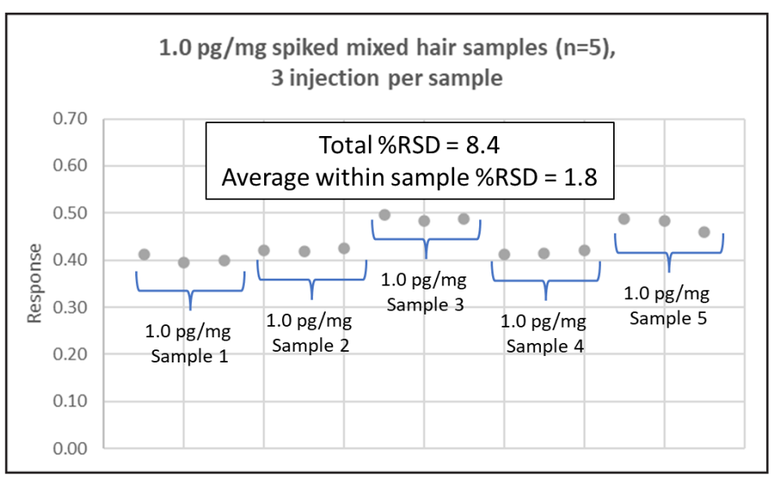

Figure 6*. Robustness data for cTHC in spiked hair* samples (n=5).

To assess the potential carryover owing to transfer/ contamination from a previous sample, injection of a high-level spiked hair standard at 5 pg/mg was carried out followed by a blank (injection solvent) injection. No detectable carry over was achieved as shown in Figure 6.

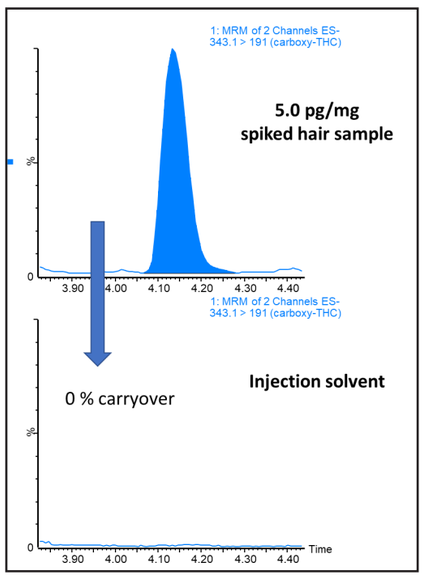

Figure 6. cTHC carryover was assessed by considering the injection of a high-level spiked hair standard at 5 pg/mg followed by a blank (injection solvent) injection.

CONCLUSION

Conclusions:

- **The requirement for quick, accurate, reliable, and robust methods to quantify compounds for forensic toxicology testing in various biological metrices is critical for confident detection and reporting.**
- **Simple, supervised, and non-invasive sample collection for the detection of relevant compounds typically tested in such schemes, can be achieved using hair as the biological matrices.**
- **The ACQUITY UPLC I class / Xevo TQ-Absolute System has demonstrated the required sensitivity, to sub pg/mg levels, permitting the detection of cTHC at the cut- offs as recommended by SoHT (0.2 pg/mg).**

Reference

1. G.A.A. Cooper, R.Kronstrand, P. KIntz. Society of Hair Testing guidelines for drug testing in hair. Forensic Science International 281 (2012) 20-24.

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