ANALYSIS OF CARBOXY-THC USING UPLC-MS/MS

Jane Cooper
Waters Corporation, UK

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ABSTRACT

This poster describes a robust UPLC-MS/MS method for the analysis of 11-nor-9 -carboxy-Δ9-tetrahydrocannabinol(carboxy-THC) in hair, to satisfy the confirmation cut-off values as recommended by the Society of Hair Testing (SoHT).[1,2]

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, as shown in Figure 1.

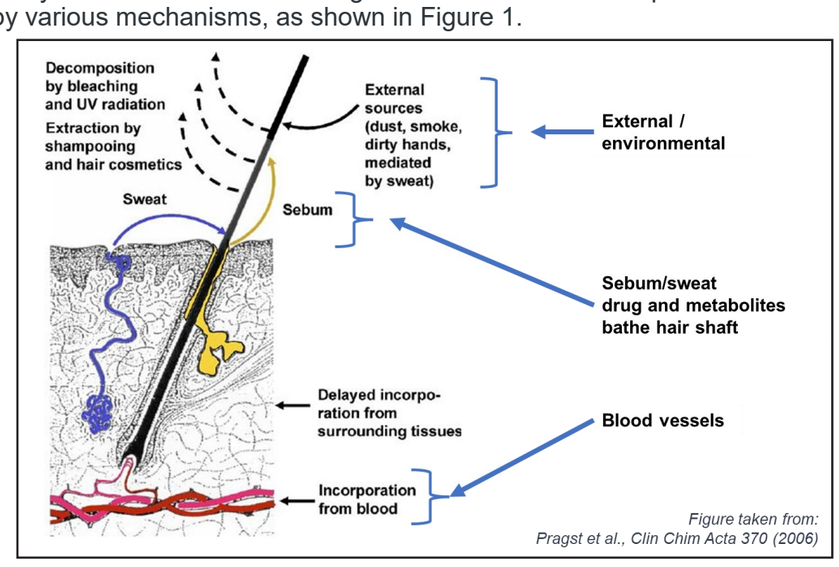

Figure 1. *Mechanisms for incorporation of drugs into hair.*

- 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.
- Drugs in hair can be detected months and even years after use, as hair grows at ~1 cm per month, a typical hair sample 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.
- 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 carboxy-THC.
- 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 carboxy-THC. However, the analysis of carboxy-THC 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) PLUS
Mobile Phase A:	0.4 mM 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

System:	Xevo TQ-Absolute
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

Analyte and Internal standard	Precursor Ion (*m/z*)	Product Ion (*m/z*)	Cone Voltage (V)	Collision Energy (eV)
carboxy-THC	343.1	191.0 (quantifier)	30	31
		245.1 (qualifier)	30	33
carboxy-THC-D3	346.1	248.1 (Int. std)	37	23

Table 2. *MRM conditions for carboxy-THC and the internal standard, carboxy-THC-D3.*

SAMPLE ANALYSIS

Hair samples were sourced from volunteers and either analyzed as a single sample or blended. The M3 Reagent was supplied by Comedical, Trento, Italy (http://www.comedical.biz/). Certified reference material for carboxy-THC and the deuterated analogue carboxy-THC-D3 was from Merck (Dorset, UK).

Decontaminated, scissor minced hair samples (25 mg) were weighed into a centrifuge tube with a sealed cap and for calibrators spiked with carboxy-THC (0.2 to 10 pg/mg), deuterated internal standard (carboxy-THC-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 carboxy-THC 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 (5 mL methanol, 4.9 mL de-ionised water, 100 µL 25% ammonia solution), vortexed and transferred to Waters Total Recovery Vials. The workflow used for the determination of carboxy-THC in hair is shown in Figure 2.

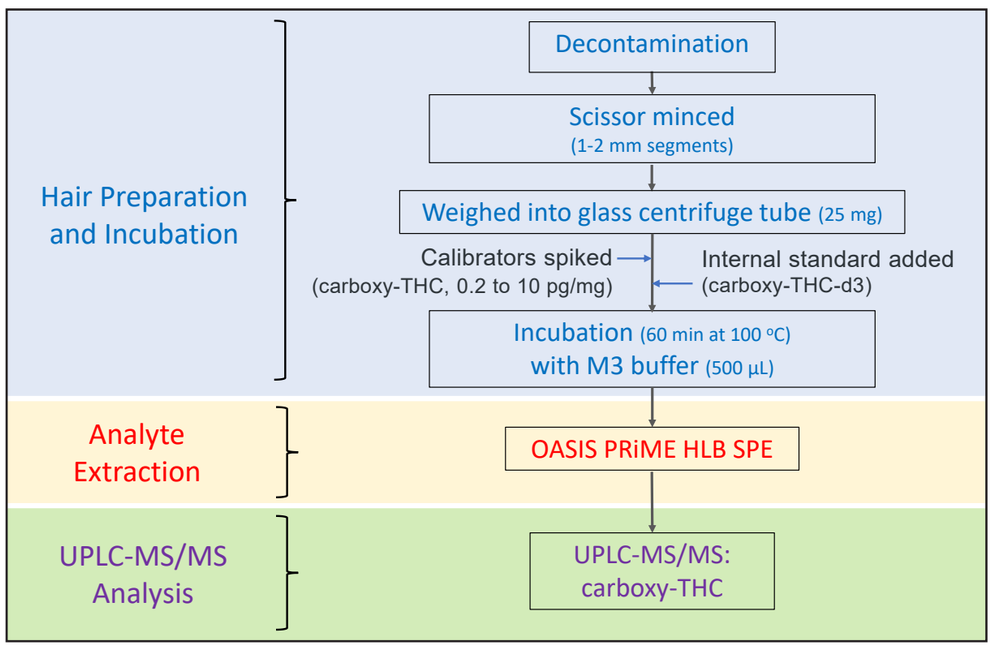

Figure 2. *Workflow for the determination of carboxy-THC in hair.*

RESULTS AND DISCUSSION

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

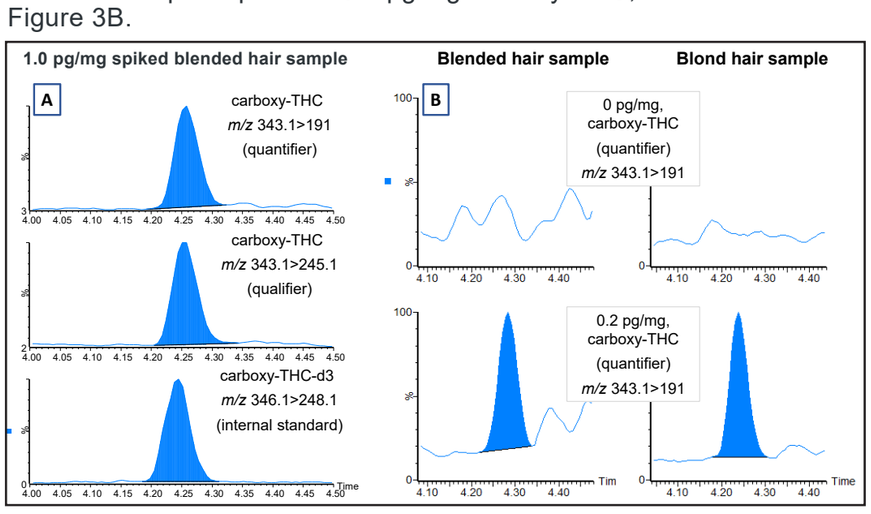

Figure 3. ***A****) Integrated chromatograms showing the quantifier (top), qualifier (middle) and internal standard (bottom) MRM transitions for a 1.0 pg/mg spiked blended hair sample.* ***B****) Integrated chromatograms showing quantifier MRM transitions for a control (0 pg/mg) hair samples (upper traces) and carboxy-THC spiked (0.2 pg/mg) samples (lower traces).*

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

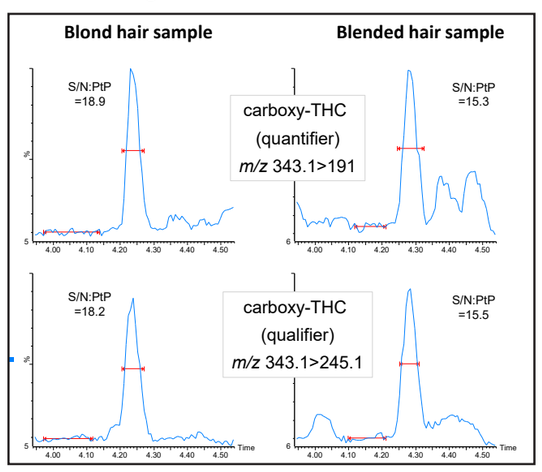

Figure 4. *Chromatograms on raw unsmoothed data, showing signal to noise calculations for quantifier (upper trace) and qualifier (lower trace) MRM transitions for 0.2 pg/mg spiked hair samples, considering different hair samples (blended and blond).*

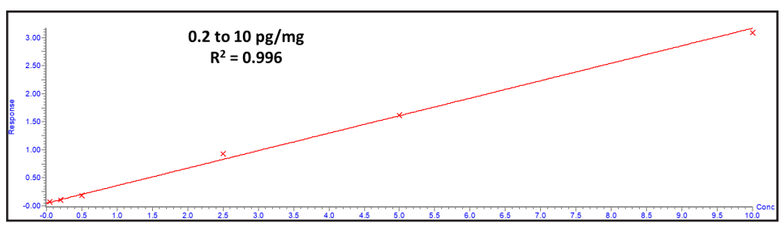

Figure 5*. Linearity of carboxy-THC over the range 0.2 to 10 pg/mg in spiked hair.*

The **linearity** of the assay was investigated over the range 0.2 to 10 pg/mg over 3 separate batches. All coefficient of determinations (R^2) were greater than 0.99, with all determined concentrations within 15 % with the expected values, with exception of the lowest calibrator (within 20 %), an example calibration curve is 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.

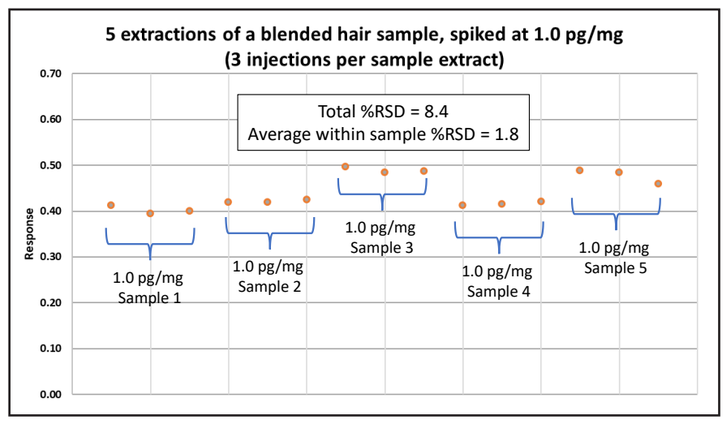

Figure 6*. Robustness data for carboxy-THC in spiked hair* samples (n=5).

As hair is a complex matrix, an isotopically labelled internal standard (carboxy-THC-D3) was added during the sample preparation to compensate for **recovery** and **matrix effects**. The matrix effect can be measured as a matrix factor (MF), by comparing the peak area response a carboxy-THC solvent standard (0.05 ng/mL) to the equivalent matrix standard (0.2 pg/mg, n=5), without using the internal standard the MF= 64 %, and when using internal standard MF= 100 %.

These results indicate that the method demonstrates 36 % ion suppression, but this is compensated extremely well by the addition of the internal standard.

To assess the potential **carryover** owing to transfer/contamination from a previous sample, an injection of a spiked hair calibrator 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.

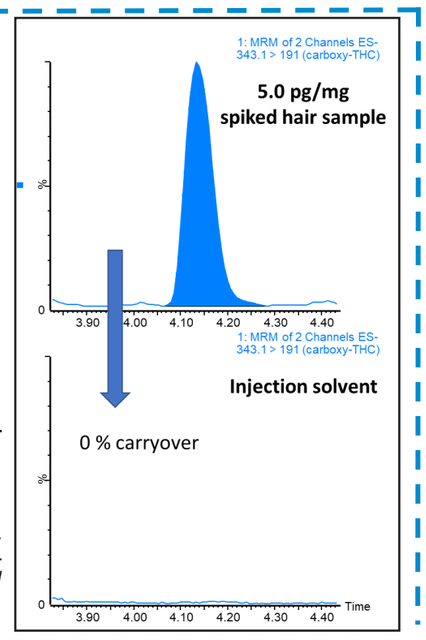

Figure 6*. carboxy-THC carryover was assessed by considering the injection of a high-level spiked hair standard at 5 pg/mg followed by a blank (injection solvent) injection.*

CONCLUSIONS

- **The requirement for quick, accurate, reliable, and robust methods to quantify compounds for forensic toxicology testing in various biological matrices 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 PLUS / Xevo TQ-Absolute System has demonstrated the required sensitivity for carboxy-THC in hair, to sub pg/mg levels (0.2 pg/mg) meeting the cut-off recommended by SoHT.**

References

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.
2. Statements of the Society of Hair Testing concerning the examination of drugs in human hair [cited 14th Aug 2023]. Available from: https://www.soht.org/statements

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