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Applikationsbericht

Analysis of Carboxy-THC in Hair using UPLC-MS/MS for Forensic Toxicology

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Abstract

This application brief 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]

Benefits

- Matrix provides long drug detection window and collection is non-invasive
- Simple and effective SPE protocol for extract clean-up
- Robust method using UPLC-MS/MS for the sensitive determination of carboxy-THC in hair

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:

- passive diffusion from the blood supply at the follicle into the growing hair matrix
- diffusion into the hair shaft from sweat or sebum
- external contamination such as smoke or contaminated hands

The use of hair as a specimen offers several benefits. In contrast to other matrices such as blood, hair collection is simple, and does not require medically trained staff to collect the sample. Sample collection is not considered intrusive, meaning that collection can be supervised, thus reducing the potential for sample adulteration. Further, once collected, hair can be easily transported and stored at room temperature prior to analysis.

However, the key benefit of hair is that it provides an extended window of detection for drug substances. In contrast to some of the traditional matrices such as blood and urine, where drugs may only be detected within hours/days of use, drugs can be detected in hair months and even years after use. As hair is understood to grow at a rate of approximately 1 cm per month, a typical hair sample is 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 over recent months.

The most widely used drug substance in the world is cannabis, thus cannabinoids are one of the most detected drug class and their analysis of cannabinoids is of key importance in forensic drug testing. Although delta-9-tetrahydrocannabinol (THC) is the major psychoactive component of cannabis sativa and can be detected within the hair of users, a positive identification of THC in hair can also be attributed to passive smoke exposure and alone does not categorically prove cannabis use of an individual. Thus, the SoHT recommends that a positive identification of THC in hair should 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, often with limited sample availability, making the requirement for high sensitivity analytical techniques essential.

Experimental

Hair samples were sourced from volunteers and analyzed as single samples or blended. The M3 reagent was supplied from Comedical (Trento, Italy. http://www.comedical.biz/ <http://www.comedical.biz/>). Certified

reference material for carboxy-THC and the deuterated analogue carboxy-THC-D3 were from Merck (Dorset, UK).

Following successive decontamination with dichloromethane, methanol, and diethyl ether, hair samples were scissor minced into 1 to 2 mm segments. The hair samples were weighed (25 mg) into glass centrifuge tubes with a sealed cap, and for matrix calibrators were spiked with carboxy-THC at concentrations ranging from 0.2 to 10 pg/mg. Deuterated internal standard (carboxy-THC-D3) was added along with 0.5 mL 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 <https://www.waters.com/nextgen/global/shop/sample-preparation--filtration/186008055-oasis-prime-hlb-1-cc-vac-cartridge-30-mg-sorbent-per-cartridge-1.html>). The samples were washed with an acetonitrile/water (1:1 v/v) followed by hexane. The carboxy-THC was eluted with acetonitrile/methanol (9:1 v/v) and following evaporation of the solvent, samples were reconstituted with 100 µL 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 (p/n: 186000385c < https://www.waters.com/nextgen/global/shop/vials-containers--collection-plates/186000385c-lcgc-certified-clear-glass-12-x-32-mm-screw-neck-vial-total-reco.html>). The workflow used for the determination of carboxy-THC in hair is shown in Figure 1.

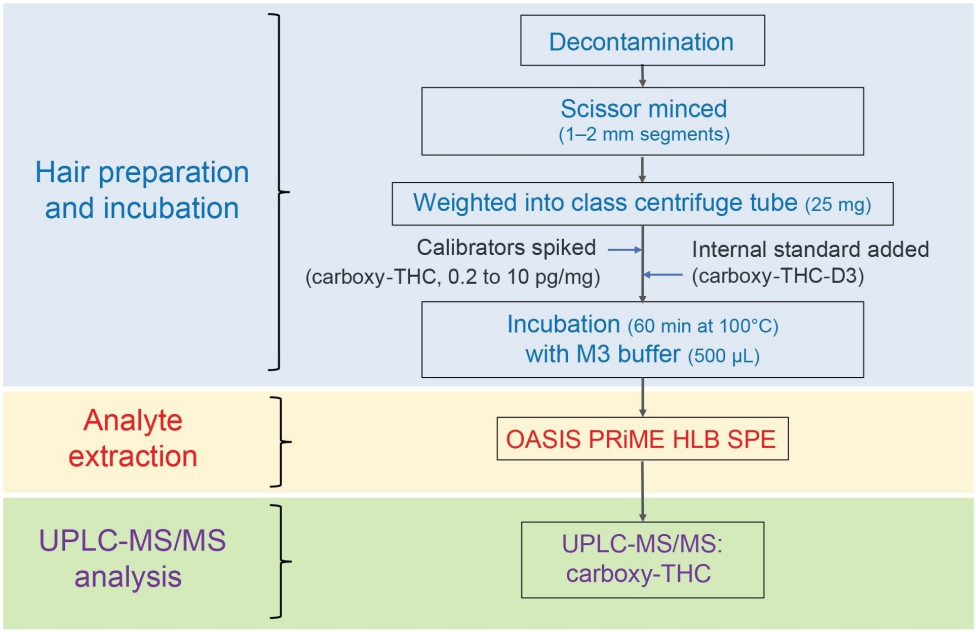

Figure 1. Workflow used for the determination of carboxy-THC in hair.

Chromatographic separation of carboxy-THC was achieved using a gradient of ammonium fluoride (pH 9.5) and methanol on an ACQUITY™ Premier BEH™ C_{18} Column with VanGuard™ FIT, 1.7 µm, 2.1 x 150 mm. Two MRM transitions for carboxy-THC were monitored using the Xevo™ TQ Absolute Mass Spectrometer *i.e.*, *m/z* 343.1 > 191.0 (quantifier), and *m/z* 343.23 > 245.1 (qualifier). The internal standard (carboxy-THC-D3) was monitored using the transition *m/z* 346.1 > 248.1.

Results and Discussion

Chromatograms for the quantifier, qualifier, and internal standard MRM transitions for a hair sample spiked with carboxy-THC at 1 pg/mg are shown in Figure 2A.

The smoothed and integrated quantifier MRM chromatograms comparing control (blank) hair extracts with hair samples spiked at the cut-off concentration recommend by SoHT for carboxy-THC (0.2 pg/mg) are shown in Figure 2B, for a blended (from multiple donors) and a blond hair sample (from a single donor).

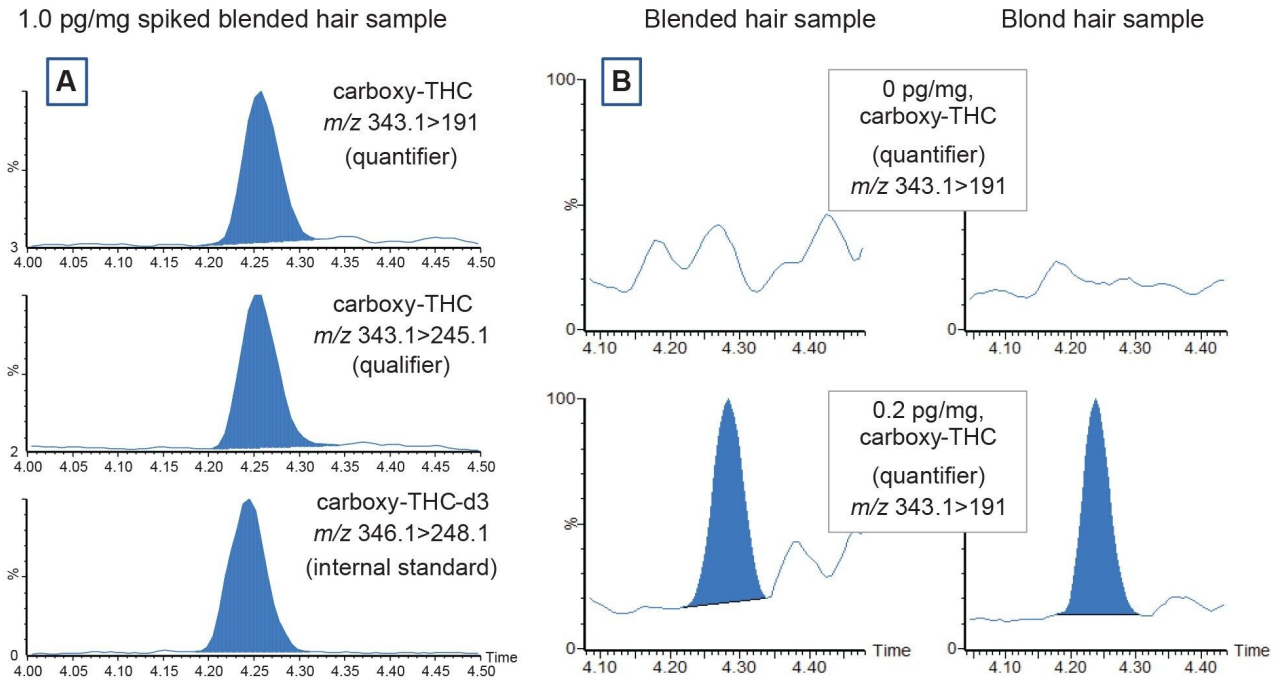

Figure 2. A) Integrated MRM chromatograms for the quantifier (upper), qualifier (middle), and internal standard (lower) for a 1.0 pg/mg spiked blended hair sample. B) MRM chromatograms showing quantifier MRM transitions for a control (0 pg/mg) hair samples (upper traces) and carboxy-THC spiked (0.2 pg/mg) samples (lower traces) for a blended and a blond hair sample.

The signal to noise (peak to peak; PtP) values calculated for the quantifier MRM transition from 0.2 pg/mg carboxy-THC spiked hair samples are shown in Figure 3, for mixed and blond hair samples.

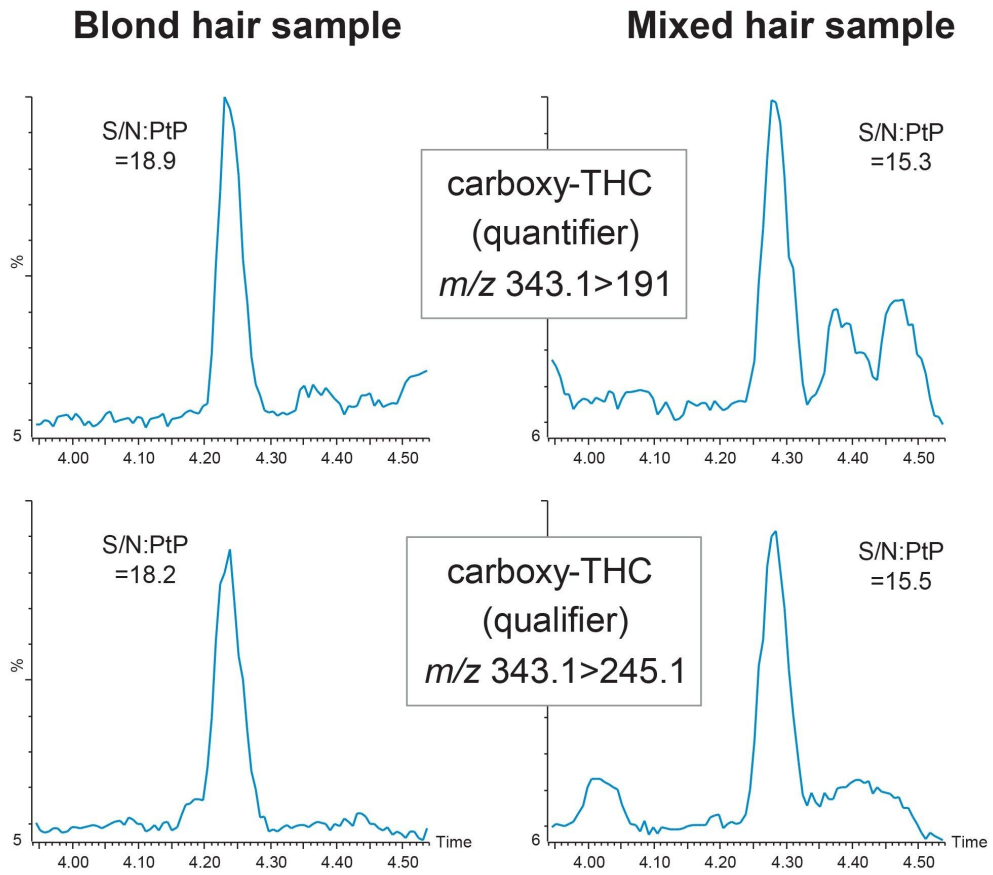

Figure 3. Chromatograms on raw unsmoothed data, showing signal to noise calculations for quantifier (upper trace) and qualifier (lower trace) MRM transitions for 0.2 pg/mg spiked hair samples (blond and blended).

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%).

The robustness of the assay was investigated considering the analysis 5 extractions of a blended hair sample. Within hair sample analysis demonstrated an average precision performance of 1.8% RSD (% relative standard deviation) and across hair samples a total precision of 8.4% RSD was observed, as shown in Figure 4.

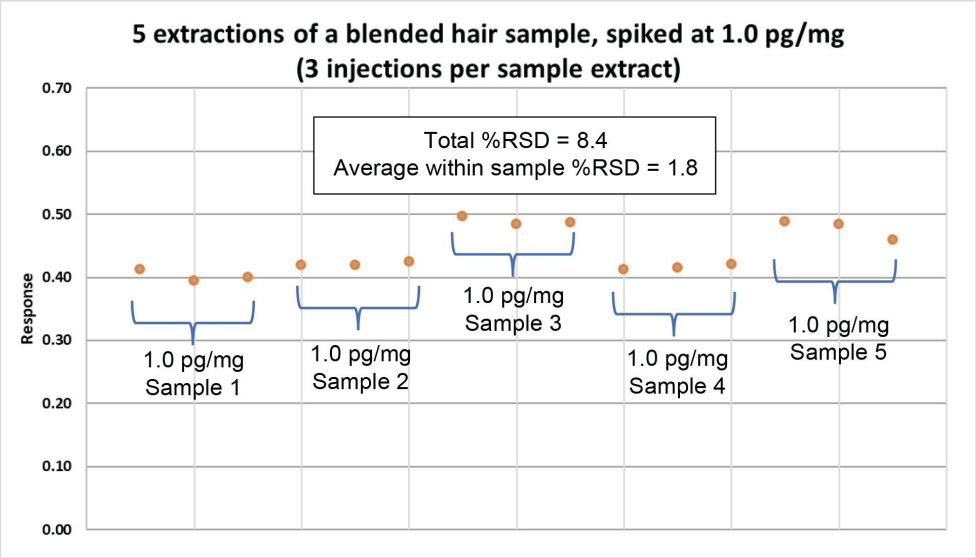

Figure 4. Robustness data for carboxy-THC in a spiked blended hair sample.

To assess the potential carryover attributed 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 carryover was achieved as shown in Figure 5.

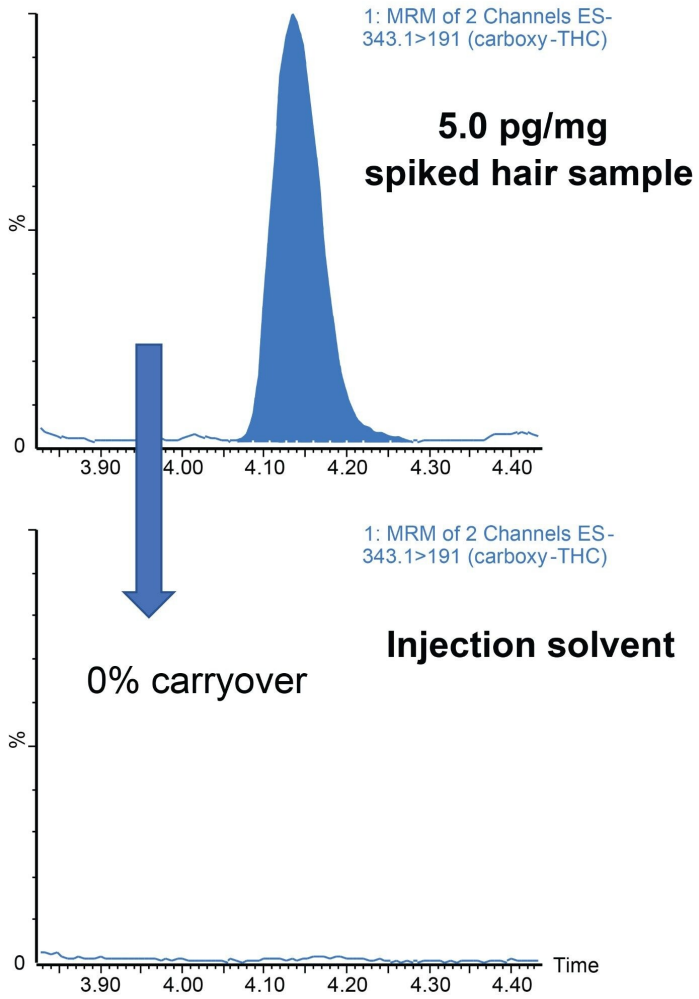

Figure 5. Carboxy-THC carryover was assessed by injection of a spiked hair standard at 5 pg/mg followed by a blank (injection solvent) injection.

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 demonstrate 36% ion suppression, but this is compensated extremely well by the addition of the internal standard.

Conclusion

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 <https://www.soht.org/statements>

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