CANNABINOID ANALYSIS IN NAIL AND HAIR SAMPLES WITH MASS SPECTROMETRY

DISCOVER THE QUANTIFICATION OF CANNABINOIDS IN HAIR & NAIL SAMPLES WITH PRECELLYS

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CONTEXT

In the last decades, considerable efforts have been extended to develop more effective methods for drug analysis in forensic science. Hair testing is a convenient and non-invasive technique for the detection of many controlled drugs and drugs of abuse, with several advantages compared to blood and urine such as a longer window of detection and an easy collection. In cases where hair cannot be collected, nail testing can be a suitable alternative, as it also allows for long-term drug analysis. Due to their slower growth, nails can allow for the detection of lower doses, and the absence of melanin can reduce the inter-individual variability. While many analytical methods exist for the detection of drugs in hair samples, including cannabinoids, there are still only a few methods to test for the presence of drugs in nail samples, and an even smaller number of methods for the detection of cannabinoids in nail samples. In this study, two methods for the quantification of cannabinoids in hair and nail samples based on liquid chromatography-tandem mass spectrometry (LC-MS/MS) were developed and validated. The methods were developed for the determination of cannabidiol (CBD), cannabinol (CBN) and Δ-Tetrahydrocannabinol (THC), and THC's main metabolites (11-nor-9-carboxy-THC (THCCOOH), 11-hydroxy-THC (OHTHC), and 8-β-11-dihydroxyTHC (diOHTHC)), and were used to analyze matched hair and nail specimens from 23 cannabis users. The sample preparation protocol consisted of a decontamination step followed by a pulverization step with the Precellys 24 (Bertin Technologies, Montigny-le-Bretonneux, France) and an alkaline hydrolysis step.

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PROTOCOL

- Sample collection

Authentic specimens were collected from 23 chronic cannabis users between May 2016 and February 2019. Matched fingernail, toenail, and hair samples from each individual were collected within the same week. Nails were obtained by cutting the overhang (nail clippings). Fingernails and toenails were stored separately in individual plastic bags at room temperature. Hair was collected by cutting a hair-lock as close as possible to the scalp in the posterior vertex of the head and stored at room temperature in paper envelopes.

- Sample preparation

1) Decontamination: Hair segments and nail clippings were decontaminated with 3 and 5 consecutive 2 mL dichloromethane washes, respectively, vortex mixing for 2 min each. The last wash solvent was dried under nitrogen after the addition of 25 µL IStd mixture, reconstituted in 75 µL mobile phase, and 20 µL were injected in the LC-MS/MS.
2) Pulverization: Decontaminated samples were pulverized with the **Precellys 24** (Bertin Technologies, Montigny-le-Bretonneux, France), with the **MK28 2mL lysing kit** (2.8mm stainless steel beads in 2mL tubes, ref: P000910-LYSK0-A, Bertin Technologies, Montigny-les-Bretonneux, France) and the following protocol: 2 cycles of 3x60 s at 6500 rpm.
3) Incubation: Fifty mg of hair powder and 30 mg of nail powder were incubated with 1 mL NaOH 1N for 15 min at 95ºC after the addition of 25 µL IStd mixture.

- Sample extraction

Samples were submitted to solid phase extraction (SPE) using Oasis MAX cartridges previously conditioned with 2 mL MeOH and 2 mL water as follows:
1) After loading the sample, four washing steps with 2 mL formic acid 5% in water, 2 mL water, 2 mL NH4OH 5% in water and 2 mL MeOH:water (60:40, v/v) were applied, and cartridges were subsequently dried for 10 min.
2) Elution was performed by addition of 2x1.5 mL MeOH:formic acid (98:2, v/v). Eluates were evaporated with nitrogen in a TurboVap LV evaporator (Zymark, Hopkinton, MA, USA) at 35ºC.
3) a. Nail samples: nail samples were reconstituted in 75 µL formic acid 0.1% in water:acetonitrile (60:40, v/v).
b. Hair samples: hair samples were reconstituted in 200 µL MeOH and 2 mL NaOH 2N, then extracted again using Oasis MCX cartridges previously conditioned with 2 mL MeOH and 2 mL water, with the protocol detailed in [1].
4) Reconstituted samples were transferred to glass inserts and centrifuged using Eppendorf tubes at 14500 rpm for 10 min with a MinispinTM Plus (Eppendorf Ibérica, San Sebastián de los Reyes, Madrid, Spain). Inserts were transferred to vials, and 20 µL injected onto the LC-MS/MS.

- LC-MS/MS analysis:

The HPLC system was an Alliance 2795 Separation Module with an Alliance series column heater/cooler coupled to a Quattro Micro™ API triple quadrupole (Waters Corp.). Chromatographic separation was performed using a Kinetex C18 (2.1 mm x 50 mm, 2.6 µm) analytical column (Phenomenex, Torrance, CA, USA), at 25°C.
Formic acid 0.1% (A) and acetonitrile (B) were used as mobile phase at 0.3 mL/min, applying the following gradient: 40% B until 0.2 min, increased to 100% over 5.8 min, and held for 0.5 min before returning to initial conditions at 6.8 min. Total chromatographic run was 10 min.
The MS was operated in electrospray in positive mode (ESI+) with the following parameters: capillary voltage, 3 kV; source block temperature, 150 °C; desolvation gas (nitrogen) temperature, 400 °C; desolvation and cone gas (nitrogen) flow rate, 800 and 80 L/h, respectively. Argon was employed to promote analyte fragmentation in the collision cell. Data acquisition was controlled with Masslynx 4.0 software and processed with Quanlynx software (Waters Corp.).
Results can be found in **Figure 1** and **Table 1**.

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RESULTS

Compound	Matrix	*n*	Concentration range (pg/mg)	Median [IQR] (pg/mg)
CBD	Fingernails	17	120–12,945	1,982 [2,801]
	Toenails	4	137–639	221 [397]
	Proximal hair	8	118–1,150	335 [548]
	Distal hair	6	339–959	890 [607]
CBN	Fingernails	19	26–2,712	460 [881]
	Toenails	12	20–191	57 [77]
	Proximal hair	9	57–1,315	95 [114]
	Distal hair	7	56–1,131	175 [160]
THC	Fingernails	20	60–24,569	5,284 [8,587]
	Toenails	17	20–6,150	183 [585]
	Proximal hair	12	68–1,237	249 [344]
	Distal hair	7	213–1,624	549 [491]

Table 1: Positive samples for each analyte and matrix with the respective concentration range, median and interquartile range [IQR]. CBD: cannabidiol, CBN: cannabinol and THC: Δ-Tetrahydrocannabinol. From [1].

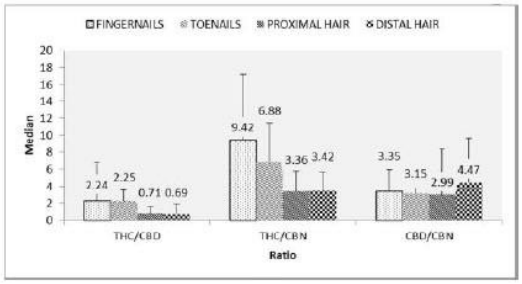

Figure 1. Median analyte THC/CBD, THC/CBN and CBD/CBN ratios in fingernails, toenails and proximal and distal hair segment. CBD: cannabidiol, CBN: cannabinol and THC: Δ-Tetrahydrocannabinol. From [1].

CONCLUSION

Only CBD, CBN, and THC were detected in the hair and nail samples, with much higher concentrations in fingernails than in toenails and hair. No THC metabolites were detected. Fingernails had higher concentrations than hair, and hair CBN and THC concentrations correlated better with toenail than with fingernail concentrations, which are more susceptible to external contamination. These results show that nails can provide a useful alternative to hair for the detection of long-term cannabis consumption. The Precellys homogenizers can efficiently grind hair and nail samples into powder. The resulting powder is suitable for mass spectrometry workflows for drug analysis, including cannabinoid analysis.

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[1] Cobo-Golpe, María, et al. "Determination and distribution of cannabinoids in nail and hair samples." Journal of analytical toxicology 45.9 (2021): 969-975.