Determination and distribution of cannabinoids in nail and hair samples

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Abstract

Hair has been used for decades in toxicology as a biological matrix for long-term detection of substances. Nails are another keratinized matrix that is being studied as an alternative when hair cannot be obtained. Although cannabis is the most prevalent illicit drug in the world, cannabinoid distribution in nails compared to hair has been scarcely studied. In this work, we described two methods for the determination of cannabidiol (CBD), cannabinol (CBN) and Δ^9-Tetrahydrocannabinol (THC), and THC's main metabolites (11-nor-9-carboxy-THC (THCCOOH), 11-hydroxy-THC (OHTHC) and 8-β-11-dihydroxyTHC (diOHTHC)) in nail and hair samples. After an alkaline hydrolysis, samples were submitted to solid-phase extraction and analysed by LC-MS/MS. The methods were fully validated, with good linearity (r^2>0.99) in the range of 20-100 to 20000 pg/mg. No endogenous or exogenous interferences were found. Accuracy was from 99.5 to 109.8% and imprecision was <6.9%. Ion suppression (up to -74.4%) was observed for all the analytes, except for diOHTHC at low concentrations in hair (46.1%). Extraction efficiency ranged from 21.5% to 84.5%. The methods were applied to matched nail and hair specimens from 23 cannabis users to study the incorporation and distribution of the cannabinoids into these matrices. Only CBD, CBN and THC were detected in the samples, with much higher concentrations in fingernails than in toenails and hair. Correlations between analyte concentrations in the different matrices and with reported drug consumption were studied. A preliminary cut-off for THC in toenails was calculated using the cut-off proposed by the Society of Hair Testing (SoHT) in hair for the identification of chronic cannabis use.

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Keywords

Cannabis, hair, nails, LC-MS/MS

Introduction

In the last decades hair has been profusely used in forensic analysis as an alternative biological matrix to detect chronic drug consumption because of its several advantages over blood and urine (1). Namely, collection is easy and non-invasive, but also substances are stable, it has a longer window of detection and allows segmental analysis (2). However, there are some special situations where hair cannot be collected such as short hairstyles, alopecia, chemotherapy and burn or drowning victims.

Nail, another keratinized matrix, also allows long-term drug detection and has a simple and non-invasive collection (3). Due to the absence of melanin and a continuous and slower growth, nail allows for a constant incorporation of substances and the detection of lower doses (4).

There are many analytical methods for the determination of different drugs in hair samples (2,5,6), including cannabinoids (7-10). However, to date only few analytical methods for the determination of drugs of abuse in nail samples were published (11-17), and even fewer for cannabinoids (16-21). Finally, only one study (21) compares hair and nail samples from the same individuals.

The aims of our work were to develop and validate two liquid chromatography – tandem mass spectrometry (LC-MS/MS) methods for the quantification of cannabinoids in both nail and hair samples and to compare the incorporation of these analytes into hair, fingernails and toenails from cannabis users in order to study the viability of nail samples as an alternative to hair.

Materials and methods

Chemicals and reagents

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Δ^9-Tetrahydrocannabinol (THC), cannabidiol (CBD), cannabinol (CBN), 11-nor-9-carboxy-THC (THCCOOH) and 11-hydroxy-THC (OHTHC) standards at 1 mg/mL, and THC-d_3, CBD-d_3, CBN-d_3, THCCOOH-d_3, OHTHC-d_3 and 8-β-11-dihydroxyTHC-d_6 (diOHTHC-d_6) at 0.1 mg/mL were purchased from Cerilliant™ (Round Rock, TX, USA). DiOHTHC at 0.1 mg/mL in methanol was obtained from ElSohly Laboratories (Oxford, MS, USA). Water was purified with a Milli-Q water system (Millipore, Le-Mont-sur-Lausanne, Switzerland). Reagent grade formic acid 98-100%, glacial acetic acid 99.8%, HPLC grade 2-propanol and dichloromethane, and LC-MS grade methanol (MeOH) were from Sharlau (Sentmenat, Spain). LC-MS grade acetonitrile was from Panreac (Castellar del Vallès, Spain). Ammonium hydroxide (NH_4OH) 32% was from VWR Prolabo Chemicals (Fontenay-Sous-Bois, France). Sodium hydroxide (NaOH) was from Merck (Darmstadt, Germany).

Oasis MAX cartridges and Oasis MCX cartridges (3 cc, 60 mg) were purchased from Waters Corp. (Mildford, MA, USA).

Nail and hair samples

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), and 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. Data about demographic characteristics [sex, age and body mass index (BMI)], consumption habits and cosmetic treatments were collected. This study was approved by the Galician Clinical Research Ethics Committee (Xunta de Galicia, Spain).

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Blank hair samples for the preparation of calibration curves and quality controls (QC), voluntarily donated by the laboratory staff, were black or brown, except for the determination of matrix effect and external interferences, where different hair colours were tested. Blank nail samples were likewise obtained from the laboratory staff.

Preparation of calibration and quality control (QC) solutions

Working solutions at 10, 1, 0.5, 0.1 and 0.04 µg/mL were prepared by serial dilutions of the appropriate standard solutions with MeOH. For the QC samples, different working solutions were prepared for THC and CBN (at 10, 1 and 0.02 µg/mL) and for CBD, OHTHC, diOHTHC and THCCOOH (at 10, 1 and 0.2 µg/mL), both in MeOH. The internal standard (IStd) solution containing all deuterated analytes at 1 µg/mL was prepared in MeOH.

In nail, calibrators at 10, 20, 100, 200, 500, 1000, 2000, 10000 and 20000 pg/mg were prepared in 30 mg of blank nail powder. QC samples at low (30 or 300 pg/mg), medium (1500 pg/mg) and high (15000 pg/mg) concentrations were also prepared in 30 mg of blank nail powder.

In hair, calibrators at 40, 100, 200, 500, 1000, 2000, 10000 and 20000 pg/mg were prepared in 50 mg of blank hair powder. QC samples at low (120 or 300 pg/mg), medium (1500 pg/mg) and high (15000 pg/mg) concentrations were also prepared in 50 mg of blank hair powder.

Hair and nail decontamination and incubation

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.

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Decontaminated samples were pulverized with a ball mill (Precellys, Montigny le Bretonneux, France) by two cycles of 3x60 s at 6500 rpm. 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.

Hair and nail extraction

After cooling, samples were submitted to solid phase extraction (SPE) using Oasis MAX cartridges previously conditioned with 2 mL MeOH and 2 mL water. After loading the sample, four washing steps with 2 mL formic acid 5% in water, 2 mL water, 2 mL NH_4OH 5% in water and 2 mL MeOH:water (60:40, v/v) were applied, and cartridges were subsequently dried for 10 min. 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, and nail samples were reconstituted in 75 µL formic acid 0.1% in water:acetonitrile (60:40, v/v).

Hair samples were reconstituted in 200 µL MeOH and 2 mL NaOH 2N, and extracted again using Oasis MCX cartridges previously conditioned with 2 mL MeOH and 2 mL water. After loading the sample, three washing steps with 2 mL NaOH 0.1N in water, 2 mL water and 2 mL MeOH:water:formic acid (39:59:2 v/v/v) were applied, and cartridges were dried for 10 minutes. Elution was performed by addition of 2 mL isopropanol:dichloromethane (75:25, v/v). Eluates were evaporated and reconstituted in 75 µL formic acid 0.1% in water:acetonitrile (60:40, v/v).

Reconstituted samples were transferred to glass inserts and centrifuged using Eppendorf tubes at 14500 rpm for 10 min with a Minispin™ 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

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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.).

Method validation in nail and hair

The following parameters were evaluated during method validation: selectivity, linearity, limit of detection (LOD), limit of quantification (LOQ), imprecision, accuracy, matrix effect, extraction and process efficiency, and autosampler stability according to the Standard practices for method validation of the Scientific Working Group for Forensic Toxicology (SWGTOX) (Supplementary Table S1).

Data analysis

Statistical analysis was performed using SPSS software (version 24.0, SPSS Inc., Chicago, IL, USA). Normality of data was tested with the Shapiro-Wilk test. Mean±standard deviation (SD) was used for continuous variables that fit normal distribution (age and BMI) and median [interquartile range (IQR)] for the rest of the

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variables. Categorical variables are presented as frequencies and percentages. Correlations between analytes and matrices were assessed using Spearman correlations. Using the regression coefficients β0 and β1, the existing cut-off concentration for THC in hair was translated to a preliminary cut-off concentration in toenail samples. A p-value <0.05 was considered statistically significant.

Results

Two analytical methods for the simultaneous quantification of THC, CBN, CBD, OHTHC, THCCOOH and diOHTHC in nails and hair were developed and validated.

The most abundant MRM transition was used for quantification, and a second transition was monitored for qualification purposes to fulfil the European Union identification criteria (22). CBD and THC, isomers that share the same MRM transitions, were chromatographically separated (Table 1). Chromatographic elution of all analytes was achieved in 6.5 minutes.

Method validation

Selectivity of the method was proved as no quantifiable peaks were detected in the 10 different blank nail and hair samples, or in the blank samples fortified with other common drugs of abuse and medicines at the specific retention time of each analyte.

Linearity was verified by least square regression using 1/x (diOHTHC, OHTHC, THCCOOH and CBD) or $1/x^2$ (CBN and THC) weighting factor. Linear dynamic range was from the LOQ to 20000 pg/mg for all the analytes, except for diOHTHC (LOQ to 2000 pg/mg). LOQ was 20 pg/mg for CBN and THC, and 100 pg/mg for the rest of the analytes in nails, and 40 pg/mg for CBN and THC and 100 pg/mg for the rest in hair. LOD in nails was 10 pg/mg for THC, 20 pg/mg for CBN, 50 pg/mg for THCCOOH and 100 pg/mg for the rest; in hair LOD was 40 pg/mg for all analytes except for diOHTHC

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(100 pg/mg). No evidence of carryover was detected since the blank samples (n=3) injected after the highest calibration point calculated a concentration less than the LOD. Accuracy was satisfied for all the analytes, with calculated concentrations within 99.5-109.1% of the nominal concentration in nails, and within 101.0-109.8% in hair. Intra-assay, inter-assay and total imprecision were <6.4%, <4.7% and <6.4%, respectively, in nails; and <5.4%, <5.6% and <6.9% in hair, fulfilling the required criterion in all cases (Supplementary Table S2).

Matrix effect and extraction and process efficiency results are shown in Table 2. Due to sample complexity, significant ion suppression (up to -54.1%) was observed for all the analytes in nails. In hair, matrix effect varied from a -74.4% ion suppression to an ion enhancement of 46.1%. IStds presented similar matrix effects to their respective analogues, compensating these variations. Extraction efficiency ranged from 21.5% to 84.5% in nails and from 12.9% to 75.9% in hair. Process efficiency ranged from 16.5% to 49.1% in nails, and from 5.6 to 90% in hair.

All analytes were stable after 72 h in the autosampler, with a %loss <14.7% and <9.0% in nails and hair, respectively.

Application to real samples

Both analytical methods were applied to nail and hair specimens obtained from 23 chronic cannabis users: 12 participants provided fingernails, toenails and hair, 6 only finger and toenails, 4 fingernails and hair and 1 toenails and hair. The proximal segment of hair was analysed in the 23 cases and the distal segment (the next 2 cm) in 10. All participants were Caucasian, with black or dark brown hair. Men represented 65% of participants, aged 18-67 (mean±SD=34.9±15.9), and with BMI 18.1-28.7 (mean±SD=22.8±3.2).

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All the participants were active cannabis users, with the same cannabis consumption frequency maintained for years, of 0.05-15 joints/day (mean±SD=3.4±3.5).

Analyte distribution in hair and nails

CBD, CBN and THC were the only compounds detected in hair and nails. Table 3 shows the number of positive samples, concentration ranges, median and IQRs for each analyte and matrix. For all the analytes, the highest median concentrations were found in fingernails (8-28.9 times higher than in toenails, and 4.9-21.2 times higher than in hair), followed by hair. THC was the predominant analyte detected in all matrices, followed by CBN.

Median THC/CBD and THC/CBN ratios were higher in nails than in hair, and similar in both hair segments. Median CBD/CBN ratios were similar in all matrices (Figure 1).

THC was detected in 3 out of 4 cases of cosmetic-treated (nail polish and nail polish-remover) nails (Supplementary Table S3), as only one case tested negative in treated nails while positive at low concentrations in the non-treated. On the other hand, antifungal-treated toenails had much lower concentrations than those found in the paired fingernails (not treated), as CBN and THC were 52 and 228 times lower, respectively (Supplementary Table S3). This huge difference in concentrations was not seen in the non-treated cases, for which median CBN and THC concentrations were 7 and 4 times lower, respectively, in toenails than in fingernails.

Correlations between matrices

The correlation between reported consumption habits and analyte concentrations was studied for all the matrices, but was statistically significant only for hair. This good correlation was observed for all the analytes and in both hair segments (Spearman ρ, 0.588-0.862).

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On the other hand, all the studied correlations between the concentrations of the different cannabinoids in the same matrix were significant (ρ, 0.479-0.962).

Finally, correlations between concentrations in the 3 matrices were also assessed. Significant correlations were observed between CBD concentrations in fingernails vs. proximal hair (ρ=0.715), and for CBN and THC concentrations in toenails vs. proximal and distal hair (ρ=0.594 and ρ=0.884, respectively for CBN; ρ=0.843 and ρ=0.830, respectively, for THC). Lastly, correlations between both hair segments were also significant for all analytes (ρ_{CBD} =0.974, ρ_{CBN} =0.884, ρ_{THC} =0.975).

Preliminary THC cut-off in toenails

A THC cut-off of 50 pg/mg was proposed by the Society of Hair Testing to enable identification of chronic drug use (23). As hair concentrations correlated better with toenail than with fingernail concentrations, a preliminary cut-off for THC in toenails was calculated. By using the regression coefficients β0 and β1 of the toenails vs. proximal-hair concentrations for THC, a preliminary THC cut-off concentration of 16.5 pg/mg in toenail samples was determined.

Discussion

In the present work we described two LC-MS/MS methods for the determination of THC, CBD, CBN, THCCOOH, OHTHC and diOHTHC in nails and hair, with LOQ for CBN and THC of 20 and 40 pg/mg in nails and hair, respectively, and 100 pg/mg for the remaining analytes in both matrices. To date, there are only six publications analysing cannabinoids in nails (16-21), with higher (100 to 200 pg/mg (17-20)), similar (20 pg/mg, (16)) or lower (0.02 pg/mg, (21)) LOQs.

For the decontamination of authentic samples we tested different washes with water and dichloromethane, using up to five cycles. Decontamination of hair and nails was finally achieved after three and five cycles of 2 mL dichloromethane, respectively, vortexing

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for 2 min. This is a more thorough washing method than those used by Busardò et al. (17), Jones et al. (21) and Mannocchi et al. (16), which consisted of one (17,21) or two (16) washes with dichloromethane. Nevertheless, it did not include multiple washing solvents, like in the methods developed by Kim et al. (20) (one wash with water and three washes with MeOH) or Lemos et al. (19) and Takaichi (18) (one wash with 0.1% dodecyl sulfate, three washes with water and three washes with MeOH). Different incubation solvents, times and temperatures were also optimized, obtaining the best results with NaOH 1M, 15 minutes at 95ºC. This solvent was already used by other authors (18-21), although we reduced the incubation time from 30-60 minutes to 15 minutes, which proved to be enough for the extraction of the analytes. Different clean-up procedures were also considered, including a liquid-liquid extraction with ethyl acetate and n-hexane:ethyl acetate 9:1 (v/v), and different SPE cartridges. The best results were achieved using mixed mode anion exchange-reversed phase cartridges. For hair samples, a second SPE extraction was necessary, obtaining the best results using mixed mode cation exchange-reversed phase cartridges. Among the previous published methods, only Jones et al. (21) and Busardò et al. (17) utilized an SPE, both at acidic pH. Kim et al. (20) and Lemos et al. (19) chose liquid-liquid extractions; the first combining two extractions in basic and acid pH (20), while Lemos evaluated basic pH (used for most of the samples) and acid pH (only for some samples), to investigate the effect of pH on the extraction (19). Takaichi compared Lemos' extraction after incubation at basic pH with an extraction using MeOH after cryogenic grinding of the samples, skipping the incubation step (18). Mannochi et al. directly analysed the incubation solvent without performing an extraction of the sample (16).

We only detected THC, CBD and/or CBN in fingernail, toenail and hair specimens, no THC metabolites. Lemos et al. (19), who analysed 14 fingernail samples from cannabis

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consumers for THC and THCCOOH after extraction at basic pH, also found THC at concentrations between 130 and 6970 pg/mg, but not THCCOOH. After acidic extraction, they found THCCOOH in fingernail samples in two out of three cases, at levels of 9820 and 29670 pg/mg. This points to THCCOOH needing an acidic pH to be successfully extracted after incubation in basic conditions, and may explain why we did not detect THCCOOH in any sample. Kim et al. (20) analysed 9 fingernail samples from drug users, and although they combined basic and acid extractions, they only detected THCCOOH in one sample at their LOQ. Takaichi (18) did not detect THCCOOH when extracting the samples after alkaline hydrolysis, whereas extracting the samples with MeOH after cryogenic grinding showed the presence of THCCOOH and higher concentrations of CBD and 11-OHTHC. THC concentrations, on the other hand, were higher in samples extracted after alkaline hydrolysis.

Regarding cannabinoids incorporation into the different matrices, we found the highest concentrations in fingernails, with a median concentration 5-20 times higher in fingernails than in hair for THC, CBD and CBN. Nevertheless, we cannot rule out the incorporation of the cannabinoids into the fingernails through external contamination due to drug manipulation. Jones et al. (21), comparing THCCOOH concentrations in matched hair and fingernail samples (n=60), also detected a mean concentration 5 times higher in fingernails (1.81 pg/mg) than in hair (0.36 pg/mg). In their case the difference cannot be explained by external contamination, since THCCOOH is a metabolite of THC not present in the cannabis plant. Conversely, Mannocchi et al. (16) detected higher THC concentrations in hair (400-2300 pg/mg) than in fingernails (17-69 pg/mg), but using non-matched samples, therefore no comparison can be made between both matrices. Finally, Busardò et al. (17) found THC only in two out of twelve fingernail samples (at concentrations of 100 and 230 pg/mg), but no paired hair samples from the

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same individuals were analysed. The present study is the first one to assess the effect of the cosmetic treatment on cannabinoids incorporation into the nails. Nail polish and nail polish-remover did not produce a clear effect, as only in one out four cases THC was not detected in fingernails cosmetically treated, while it was detected in matched toenails (non-treated) at low concentrations. On the other hand, nail antifungal treatment, or even the onychomycosis itself, may affect cannabinoid incorporation, considering the results of our only case, with concentrations of THC and CBN much higher in fingernails (not treated) than in toenails (treated), with concentration ratios between both matrices of 52 (THC) and 228 (CBN). These ratios were much higher than those observed in the cases where the treatment was not applied (ratios from 4 to 7).

Finally, dose intake only correlated with hair concentrations, both in the proximal and distal segments. On the other hand, THC, CBD and CBN concentrations correlated between them within each matrix, but not between different matrices. The best correlation was observed between THC hair and toenail concentrations (ρ=0.843 in proximal hair and ρ=0.830 in distal hair), probably because incorporation through external contamination has a smaller contribution in toenails than fingernails. For this reason, we estimated a possible cut-off concentration of 16.5 pg/mg for THC in toenails based on the SoHT cut-off concentrations of 50 pg/mg in hair for this analyte. Moreover, Jones et al. (21) found significant correlations between THCCOOH concentrations in fingernails and hair (Pearson coefficient= 0.97), much better than those found for the analytes detected in the present work, where external contamination cannot be excluded. Lastly, the good correlation between THC, CBD and CBN concentrations in the proximal and distal hair segments (ρ=0.884 to 0.981) supports the chronic consumption habits reported by the participants.

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The main limitation of this study was the high LOQs for THC's metabolites, which hindered the detection of THCCOOH; in addition, the small number of treated nail samples did not allow a statistic assessment of the effect of external treatments on analyte concentrations. The number of real samples used to determine correlations was low; therefore, the presented correlations may not be totally reliable. Nonetheless, this study is the first to compare cannabinoids (CBD, CBN and THC) incorporation into fingernail, toenail and hair samples, and to report the possible effect of antifungal treatment on concentrations in nails. Moreover, a preliminary cut-off for THC in toenails was proposed.

Conclusion

Two LC-MS/MS methods for the determination of 6 cannabinoids in nails and hair were developed and validated, and applied to matched nail and hair samples from 23 chronic cannabis users. 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. In summary, nails are a useful alternative to hair for detection of long-term cannabis consumption. A preliminary cut-off for THC in toenail samples was proposed, although future studies are needed to confirm its usefulness.

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Table 1. Selected MRM transitions, cone voltage (CV), collision energy (CE), retention time (Rt), and internal standards (IStd) used for each analyte. Underlined transitions were used for quantification.

Analyte	MRM transition	CV (V)	CE (eV)	Rt (min)	IStd
diOHTHC	347.3->329.2	25	10	2.6	diOHTHC-d$_6$
	347.3->311.2	25	12		
diOHTHC-d$_6$	353.3->335.1	25	12	2.5	
OHTHC	331.3->313.2	30	14	4.31	OHTHC-d$_3$
	331.3->193.3	30	24		
OHTHC-d$_3$	334.3->316.1	30	16	4.305	
THCCOOH	345.3->327.2	35	16	4.415	THCCOOH-d$_3$
	345.3->299.2	35	20		
THCCOOH-d$_3$	348.3->330.1	35	16	4.44	
CBD	315.3->193.3	35	22	5.28	CBD-d$_3$
	315.3->123.0	35	36		
CBD-d$_3$	318.0->196.2	35	37	5.25	
CBN	311.3->223.2	35	20	5.74	CBN-d$_3$
	311.3->293.2	35	16		
CBN-d$_3$	314.0->223.1	35	21	5.735	
THC	315.3->193.3	35	22	6.025	THC-d$_3$
	315.3->123.0	35	36		

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THC-d_3	318.0->196.2	35	37	5.975

Table 2. Matrix effect, extraction and process efficiency at low (30 pg/mg for CBN and THC in nails, 120 pg/mg for CBN and THC in hair or 300 pg/mg for the rest of the analytes in nails and hair) and high (15000 pg/mg for all analytes) QC concentrations in nail and hair samples.

Analyte	Concentration	Nails			Hair		
		Extraction efficiency (n=10)	Matrix effect % [%CV] (n=10)	Process efficiency (n=10)	Extraction efficiency (n=10)	Matrix effect % [%CV] (n=10)	Process efficiency (n=10)
diOHTHC	LOW	63.6	-31.2 [13.4]	43.7	61.6	46.1 [34.5]	90.0
diOHTHC-d_6	LOW	63.2	-43.8 [13.3]	35.5	115.1	14.4 [29.6]	131.7
OHTHC	LOW	67.1	-45.7 [14.8]	36.4	73.8	-31.2 [36.0]	50.7
	HIGH	61.9	-54.1 [13.5]	28.4	60.2	-31.7 [23.5]	41.1
OHTHC-d_3	LOW	68.9	-48.0 [14.6]	35.8	132.2	-54.3 [38.7]	60.4
	HIGH	66.0	-48.2 [13.6]	34.2	52.3	-41.5 [29.7]	30.6
THCCOOH	LOW	69.8	-44.8 [15.6]	38.6	75.9	-61.6 [45.3]	29.1
	HIGH	72.5	-52.3 [13.9]	34.6	64.3	-45.3 [30.1]	35.2
THCCOOH-d_3	LOW	71.4	-39.7 [14.3]	43.0	154.8	-76.9 [50.8]	35.8
	HIGH	76.7	-42.7 [12.0]	44.0	62.6	-48.8 [32.7]	32.0
CBD	LOW	21.5	-23.4 [19.1]	16.5	18.5	-69.8 [43.2]	5.6
	HIGH	24.8	-29.4 [18.2]	17.5	12.9	-52.6 [30.3]	6.1
CBD-d_3	LOW	22.0	-24.5 [15.7]	16.6	18.9	-71.6 [44.3]	5.4
	HIGH	27.7	-21.3 [17.5]	21.8	13.5	-54.2 [31.8]	6.2
CBN	LOW	59.5	-45.8 [17.5]	32.2	72.0	-74.4 [53.9]	18.4

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	HIGH	84.5	-47.0 [22.9]	44.8	53.2	-59.6 [34.1]	21.5
CBN-d_3	LOW	72.1	-50.6 [22.2]	35.6	77.4	-78.0 [54.5]	17.0
	HIGH	80.4	-42.4 [21.2]	46.3	52.9	-60.1 [36.1]	21.1
THC	LOW	63.3	-38.1 [21.1]	39.2	63.8	-66.2 [52.8]	21.6
	HIGH	77.7	-36.8 [24.3]	49.1	44.9	-47.1 [29.7]	23.7
THC-d_3	LOW	69.3	-41.6 [23.6]	40.5	66.1	-71.0 [52.1]	19.2
	HIGH	70.7	-29.9 [23.1]	49.6	43.9	-48.8 [32.5]	22.5

Table 3. Positive samples for each analyte and matrix with the respective concentration range, median and interquartile range [IQR].

Compound	Matrix	n	Conc. range (pg/mg)	Median [IQR] (pg/mg)
CBD	Fingernails	17	120 - 12945	1982 [2801]
	Toenails	4	137 - 639	221 [397]
	Proximal hair	8	118 - 1150	335 [548]
	Distal hair	6	339 - 959	890 [607]
CBN	Fingernails	19	26 - 2712	460 [881]
	Toenails	12	20 - 191	57 [77]
	Proximal hair	9	57 - 1315	95 [114]
	Distal hair	7	56 - 1131	175 [160]
THC	Fingernails	20	60 - 24569	5284 [8587]
	Toenails	17	20 - 6150	183 [585]
	Proximal hair	12	68 - 1237	249 [344]
	Distal hair	7	213 - 1624	549 [491]

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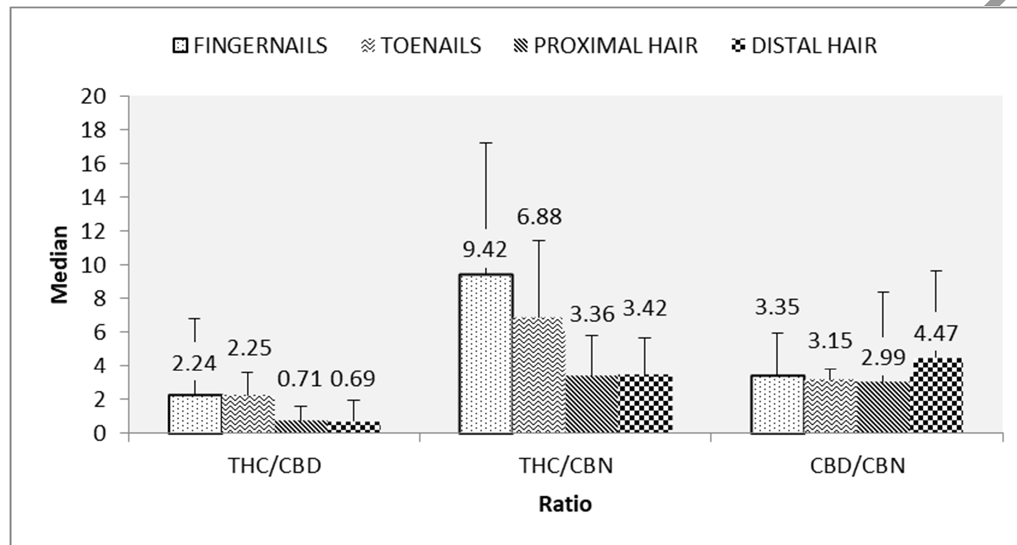
Figure 1. Median analyte THC/CBD, THC/CBN and CBD/CBN ratios in fingernails, toenails and proximal and distal hair segment.



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