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Simultaneous analysis of Δ^9-tetrahydrocannabinol and 11-nor-9-carboxy-tetrahydrocannabinol in hair without different sample preparation and derivatization by gas chromatography–tandem mass spectrometry

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ABSTRACT

The present study describes a gas chromatography/tandem mass spectrometry-negative ion chemical ionization assay (GC/MS/MS-NCI) for simultaneous analysis of Δ^9-tetrahydrocannabinol (THC) and 11-nor-9-carboxy-tetrahydrocannabinol (THCCOOH) in hair. Each hair sample, of approximately 20 mg, was weighed and the sample was dissolved in 1 ml of 1 M sodium hydroxide (30 min at 85 °C) in the presence of THC-d_3 and THCCOOH-d_3. For the analysis of THC, hair samples were extracted with n-hexane:ethyl acetate (9:1) two times; acetic acid and sodium acetate buffer were added for the analysis of THCCOOH, and hair samples were re-extracted with n-hexane:ethyl acetate (9:1) two times. The extracts were then derivatized with pentafluoropropionic anhydride (PFPA) and pentafluoropropanol (PFPOH). This method allowed the analysis of THC and THCCOOH using the GC/MS/MS-NCI assay. This method was also fully validated and applied to hair specimens ($n = 54$) collected from known cannabis users whose urine test results were positive. The concentrations of THC and THCCOOH in hair ranged from 7.52 to 60.41 ng/mg and from 0.10 to 11.68 pg/mg, respectively.

In this paper, we simultaneously measured THC and THCCOOH in human hair using GC/MS/MS-NCI without requiring different sample preparation and derivatization procedures. The analytical sensitivity for THCCOOH in hair was good, while that for THC in hair needs to be improved in further study.

1. Introduction

Cannabis is the most widely abused drug around the world and the second most commonly abused drug in South Korea following methamphetamine (MA). Urine drug testing is usually the most commonly used method, but may not detect the drugs of abuse [1]. Hair drug testing is the most effective and alternative test for long-term drug use. Low levels of cannabinoids in hair require forensic laboratories to employ sensitive and reliable methods to assist police investigations. Δ^9-tetrahydrocannabinol (THC) is the most active constituent in cannabis, but because THC (parent drug) in hair might come from external environmental exposure, the detection of 11-nor-9-carboxy-tetrahydrocannabinol (THCCOOH, metabolite of THC) in hair is important [2]. The analytical tools for determination of THC in hair include gas chromatography combined with mass spectrometry (GC/MS) by electron impact ionization (EI) [1,3–7] and gas chromatography/tandem mass spectrometry-negative ion chemical ionization (GC/MS/MS-NCI) [8]. GC/MS-EI is one of the most widely used techniques in cannabis analysis in both urine and hair samples [2]. THCCOOH in hair is mainly analyzed by GC/MS-NCI [1,4,9–11] and GC/MS/MS-NCI [8,12–15]. Some previously described analytical procedures are targeted for separate analysis of THC and THCCOOH in hair, using different derivatizing reagents despite the same extraction [4,15,16] and using different ionization modes (EI mode or CI mode) [1,4] or different CI modes [positive chemical ionization (PCI) or NCI] [15]. Some researchers have presented validation of hair analysis using GC/MS-EI or GC/MS-NCI, but the limit of detection (LOD) for THCCOOH in hair was high, data on the application of the method to real hair samples were not shown, or the concentrations of THCCOOH in hair were very high [1,4,9]. Therefore, GC/MS/MS-NCI technique seems to be a good solution to increase the sensitivity for THCCOOH in hair.

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The purpose of this paper is to evaluate the sensitivity of GC/MS/MS-NCI for simultaneous analysis of THC and THCCOOH in hair without different sample preparation and derivatization, and to validate the method and apply it routinely to real hair samples. Because of low-level of THCCOOH in hair, the results could be changed according to the extraction procedure and the condition of the instrument. Therefore, several parameters that could affect the sensitivity of instrument were also investigated.

2. Method

2.1. Chemicals and reagents

THC, THCCOOH, THC-d_3 and THCCOOH-d_3 were obtained from Cerilliant (TX, USA). The derivatizing reagents pentafluoropropionic anhydride (PFPA) and pentafluoropropanol (PFPOH) and the silanizing reagents dichlorodimethylsilane and trimethylchlorosilane were purchased from Sigma–Aldrich (MO, USA). Another silanizing reagent surfasil siliconizing fluid was obtained from Pierce (IL, USA). Methanol, sodium hydroxide, sodium acetate trihydrate, toluene, acetic acid, glacial acetic acid, ethyl acetate and n-hexane were of analytical reagent grade.

2.2. Standards and solutions

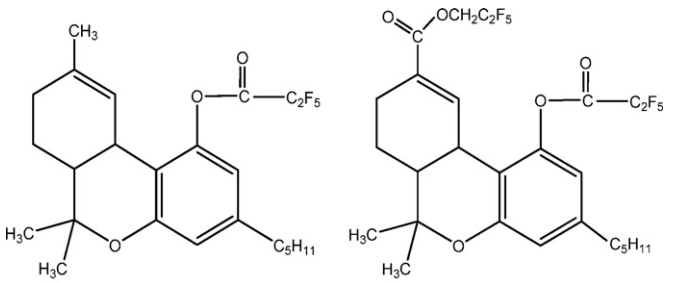
Standards. THC (1 mg/ml) was diluted in methanol to give a working solution of 10 μg/ml. THCCOOH (100 μg/ml) was diluted in methanol to give working solutions of 10 ng/ml, 1 ng/ml and 100 pg/ml.

Internal standards. THC-d_3 (100 μg/ml) was diluted in methanol to give a working solution of 10 μg/ml. THCCOOH-d_3 (100 μg/ml) was diluted in methanol to give a working solution of 1 ng/ml. These standards and internal standards were stored at −20 °C.

Samples for calibration were prepared by spiking hair (20 mg) at six concentrations ranging from 7.5 to 100 ng/mg for THC and from 0.5 to 12.5 pg/mg for THCCOOH.

2.3. Sample collection and extraction protocol

Hair samples were collected at the posterior vertex from cannabis users whose urine test results were positive. The data mostly related to the full hair length, but in case of long hair, the proximal 0–12 cm segment was used. Only glassware (screw-cap tubes, autosampler vial inserts and pipettes) were used during sample preparation. Each hair sample (about 20 mg) was washed two times with 1 ml of methanol in order to remove external contamination. Sample extraction was performed using slightly modified methods described in some papers [4,10,11,17]. Hair samples were digested with 1 ml of 1 M NaOH at 85 °C for 30 min in the presence of internal standards of THC-d_3 (25 ng/mg) and THCCOOH-d_3 (2.5 pg/mg). THC and THCCOOH were extracted from hair by a simple liquid–liquid extraction (LLE). After cooling, hair samples were extracted with 2 ml of n-hexane:ethyl acetate (9:1) for 10 min by quick vertical shaking (approximately 2000 rpm/min) for the analysis of THC. After centrifugation at 2000 rpm for 2 min, the organic extract was transferred into a screw-cap tube. The extraction procedure was performed twice. For the analysis of THCCOOH, after adding 1 ml of 0.1 M sodium acetate buffer (pH 4.5) and 200 μl acetic acid, hair samples were re-extracted with 2 ml of n-hexane:ethyl acetate (9:1) and the organic extract was transferred into the screw cap tube. Hair samples were extracted with 2 ml n-hexane:ethyl acetate (9:1) one more time and the organic extract was transferred into the screw-cap tube and then evaporated to dryness at 45 °C under a gentle stream of nitrogen. The mixture was derivatized with 50 μl PFPA and 25 μl PFPOH for 30 min at 70 °C. The solution was evaporated, and the residue was reconstituted in 40 μl of ethyl acetate and transferred to an autosampler vial. One μl was injected into the GC/MS/MS-NCI system.



Δ^9-THC-PFP
$C_{27}H_{28}F_{10}O_5$
MW:622

THCCOOH-2PFP
$C_{24}H_{29}F_5O_3$
MW:460

Fig. 1. The structures of PFP derivatized THC and THCCOOH.

2.4. Instrumentation

The extracts were analyzed using an Agilent Technologies 6890 N gas chromatograph and 7683 autosampler (CA, USA) coupled to a Waters Quattro Micro GC tandem mass spectrometer (Manchester, UK) operating in the NCI mode using methane (99.9%) as the reagent gas at a pressure between 7 and 8 psi. The MS/MS experiments were based on collision-induced dissociation (CID) occurring in the collision cell with an argon collision gas at a pressure of 3.5 mTorr for multiple reaction monitoring (MRM). The electron multiplier was operated at 650 V. The instrument was optimized daily according to the tuning parameters. The analytical column for GC was a HP-5 MS (30 m × 250 μm × 0.25 μm) (CA, USA). High-purity helium (99.9999%) was used as the carrier gas with a constant flow of 1 ml/min; splitless injection was performed at a purge time of 2 min and a purge flow of 40 ml/min. The oven temperature was programmed from 100 °C (1-min hold) to 275 °C (3-min hold) at 35 °C/min to 300 °C (15-min hold) at 25 °C/min and finally to 200 °C (2-min hold) at 10 °C/min in the MRM mode. The equilibration time was 1 min. The transfer line was set at 250 °C and the source temperature was 150 °C. The PFP derivatized ions monitored for the precursor and product ions of THC, THCCOOH, THC-d_3 (internal standard) and THCCOOH-d3 (internal standard), along with their respective retention times are shown in Table 1 and the structures of PFP derivatized THC and THCCOOH are shown in Fig. 1 [1,10]. The values in boldface represent quantification ions in Table 1. The cut-off value for THCCOOH in hair was 0.05 pg/mg by GC/MS/MS-NCI confirmation [18].

2.5. The effects of some parameters on extraction efficiency and sensitivity

2.5.1. Procedures to silanize glassware

Three silanizing reagents for glassware were used. Silanization was performed using the slightly modified methods described in paper [19]. Five ml of each silanizing reagent trimethylchlorosilane, dichlorodimethylsilane and surfasil siliconizing fluid were added to 95 ml toluene to make 5% silane in toluene solutions. Each 5% silane solution was poured into the glassware (screw-cap tube and autosampler vial insert) and incubated for 3 min. After discarding the solution, the glassware were washed with ethanol 6 times and dried for 30 min at 60 °C in the oven. THC (500 ng) and THCCOOH (500 pg) in methanol solution without matrix and THC (500 ng) and THCCOOH (5 pg) with hair matrix (20 mg) were analyzed in untreated and silane-treated glassware, and the concentrations of THC and THCCOOH were compared.

Table 1
The PFP derivatized ions monitored for the precursor and product ions and retention times (RT) used for the qualification and quantitation of THC and THCCOOH from hair using the multiple reaction monitoring (MRM).

Compound name	Precursor ions (*m*/*z*)	Product ions (*m*/*z*)	Dwell time (s)	Collision (V)	RT (min)
THC	459.29	**339.09**	0.03	15	6.96
		415.07			
THCCOOH	602.12	**474.00**	0.03	11	7.73
		512.99			
THC-d_3	462.22	**341.99**	0.03	13	6.95
		418.55			
THCCOOH-d_3	605.11	**477.03**	0.03	13	7.72
		516.02			

Ions in bold face were used for quantitation.

2.5.2. Extraction efficiency according to solvent composition (hexane:ethyl acetate) and the number of extractions

The solvent composition is important to simultaneously extract THC and THCCOOH from hair. The solvent composition (hexane: ethyl acetate) was as follows: 1:9, 3:7, 5:5, 7:3, 3:1, 9:1, and the extraction efficiencies with 25 ng/mg of THC and 250 pg/mg of THCCOOH were compared. We also investigated the extraction efficiency following extraction one time and two times for low and medium concentrations (20, 75 ng/mg) of THC and low, medium and high concentrations (0.75, 4, 10 pg/mg) of THCCOOH.

2.5.3. Derivatization time and temperature and derivatization rate

Derivatization efficiency is dependent on incubation temperature and the derivatization time; therefore, incubation temperature and derivatization time should be considered. During the derivatization process, the conditions varied at 70 °C for 30 min, 70 °C for 1 h and 95 °C for 30 min to investigate the derivatization efficiency.

Derivatization rate was determined by direct evaporation and derivatization of methanolic solution at four concentrations of THC (300, 400, 800, 1500 ng) and at four concentrations of THCCOOH (5, 15, 80, 200 pg), and by evaporation and derivatization of THC and THCCOOH standards added to solution obtained after blank hair sample (20 mg) was hydrolyzed and extracted, in which 300 ng of THC and 5 pg of THCCOOH standards for reference peak area and 200 ng of THC-d_3 and 50 pg of THCCOOH-d_3 internal standards were used.

2.6. Method validation

For cannabis hair analysis, the method was validated by establishing specificity, selectivity, limit of detection (LOD), limit of quantification (LOQ), linearity, intra- and inter-assay accuracy and precision, percentage recovery and stability. To evaluate specificity and selectivity, five drug-free hair samples (no analyte or internal standard added) were analyzed to check for peaks that might interfere with the detection of analytes or internal standards. To other drug-free hair samples, common drugs of abuse were added at concentrations of 2.5 pg/mg. Amphetamine (AP), methamphetamine (MA), methylenedioxyamphetamine (MDA), methylenedioxymethamphetamine (MDMA), methylenedioxyethylamphetamine (MDEA), pseudoephedrine, norephedrine, norketamine, ketamine, methcathinone, dextromethorphan, dextrorphan, amfepramone, fenfluramine, phentermine, phendimetrazine, phenmetrazine, cannabinol (CBN), hydroxy-tetrahydrocannabinol (THC–OH) and cannabidiol (CBD) were all added to drug-free hair samples, extracted and analyzed. To investigate whether the concentration of THCCOOH (0.75 pg/mg) in hair was affected by THC, THC–OH, CBN or CBD, which have similar structures, THC, THC–OH, CBN and CBD (25 pg/mg) were added. The LOD was determined from extracted samples spiked with decreasing concentrations of the compounds, and defined as the concentration at which the response of qualifying ions was equivalent to three times the background noise. Limit of quantification (LOQ) also was evaluated in triplicate and defined as the concentration that met LOD criteria for which the signal-to-noise ratio was at least 10:1 and the measured concentration was within 20% of target in three replicates. Six sets of calibrators ranging in concentrations from 7.5 to 100 ng/mg for THC and from 0.05 and 12.5 pg/mg for THCCOOH were prepared using 20 mg of blank hair. Spiked drug-free hair samples (20 mg) containing 20 and 75 ng/mg of THC and 0.75, 4 and 10 pg/mg of THCCOOH were prepared to assess the intra-assay accuracy and precision. The inter-assay accuracy and precision were also examined in series on five consecutive days. Percentage recovery was determined at 20 and 75 ng/mg of THC and 0.75, 4 and 10 pg/mg of THCCOOH and peak areas of THC and THCCOOH were compared with those of methanolic strandards and derivatization. Stability of analytes after derivatization was also examined. GC autosampler vials containing three derivatized samples of THC (200, 400 and 800 ng/mg) and three derivatized samples of THCCOOH (0.05, 0.25 and 0.5 pg/mg) were stored at −20 °C for 5 weeks. Concentrations of analytes in stored vials were compared to THC and THCCOOH concentrations of freshly prepared quality control samples.

3. Results

3.1. The effects of some parameters on extraction efficiency and sensitivity

Three silanizing reagents for glassware in cannabis hair analysis were used to investigate extraction efficiency. Inner surfaces of the glassware were inactivated with three 5% (v/v) silanizing reagent in toluene. The concentrations of THC and THCCOOH were compared after using untreated and silane-treated glassware. For THC (500 ng) and THCCOOH (500 pg) in methanol without matrix, the concentrations of THC and THCCOOH using glassware treated with trimethylchlorosilane and dichlorodimethylsilane increased but the difference was not statistically significant ($p > 0.05$). The concentrations of THC (500 ng) in hair matrix (20 mg) analyzed in all silane-treated glassware increased, but the difference was not statistically significant ($p > 0.05$). In contrast, the concentrations of THCCOOH (5 pg) in hair matrix (20 mg) analyzed in silane-treated glassware decreased (Fig. 2).

There was no statistically significant difference according to solvent composition (hexane: ethyl acetate) ($p > 0.05$), but the solvent composition that yielded the best extraction efficiency for both THC and THCCOOH in hair was 9:1. For THC, low and medium concentrations of THC in hair after extraction one time were 99 and 98% of those after extraction two times, respectively. For THCCOOH, low, medium and high concentrations of THCCOOH in hair after extraction one time were 79, 86, and 90% of those after extraction two times, respectively. During the derivatization process, there was no statistically significant difference according to incubation tem-

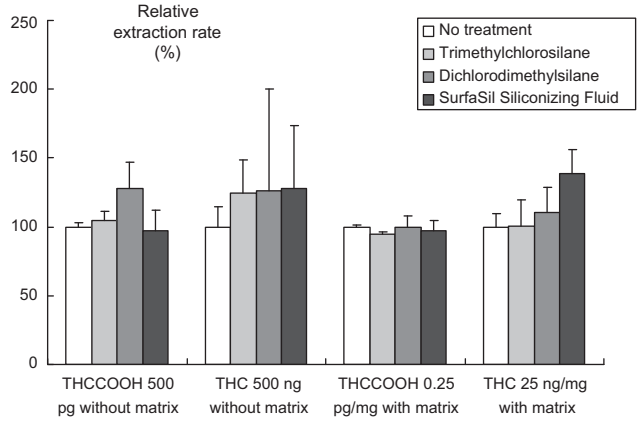


Fig. 2. Comparisons of concentrations of THCCOOH and THC without/with hair matrix after using silane-treated screw-cap tube and autosampler vial insert with 3 types of silylation agents (trimethylchlorosilane, dichlorodimethylsilane and surfasil siliconizing fluid) *Extraction rate: no treatment = 100%.

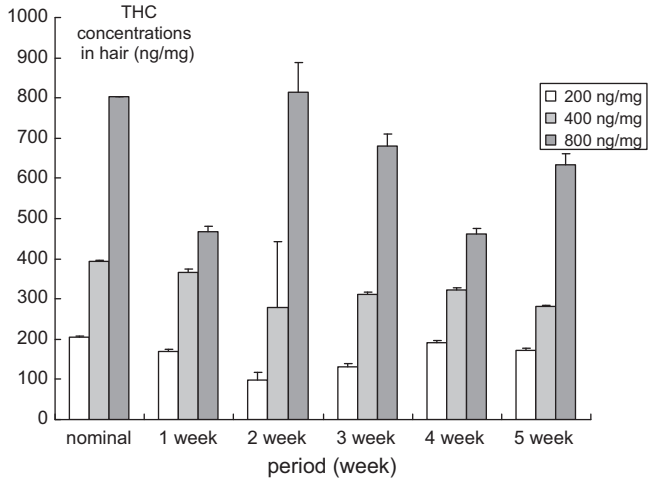


Fig. 4. Stability of derivatized THC in capped GC autosampler vials for 1–5 weeks after storing at −20 °C.

perature and derivatization time; therefore, derivatization process at 70 °C for 30 min was selected for efficiency. Derivatization rate is presented in Fig. 3. Derivatization rates of THC in methanolic solution were low, but those in the hair-matrix solution were good except for high THC. Derivatization rates of THCCOOH in methanolic solution and in the hair-matrix solution were good. We found that derivatization rate decreased with concentrations of THC and THCCOOH.

When we analyzed THCCOOH in hair using GC/MS/MS-NCI, we made some efforts to improve sensitivity. However, when more steps were added, the reproducibility was lower, peak tailing appeared or no peak was identified from the sample. For example, filtering extract with sodium sulfate before derivatization to remove water, filtering the derivatized and reconstituted extract with PVDF or PTFE syringe filter, or using solvents (ethylacetate or toluene) other than PFPOH in derivatization process caused poor reproducibility and peak tailing (data not shown). And no peak was identified from the sample without sodium acetate buffer during sample extraction.

3.2. Validation of method

Table 2 shows the results of the method validation for THC and THCCOOH in hair. For specificity and selectivity, all 5 hair samples were free of co-eluting peaks at the retention times of THC, THCCOOH, THC-d_3 and THCCOOH-d_3. None of the 20 commonly encountered drugs, which were added at high concentrations, produced any interference when added to hair samples. THC, THC–OH, CBD and CBN, which have similar structures, did not interfere with accurate THCCOOH quantitation. The limit of detection of THC and THCCOOH using 20 mg of hair was 2.5 ng/mg and 0.025 pg/mg, respectively. The limit of quantification of THC and THCCOOH using 20 mg of hair was 7.5 ng/mg and 0.05 pg/mg, respectively. The calibration curves for THC and THCCOOH were linear in the concentration range of 7.5–100 ng/mg and 0.05–12.5 pg/mg and R^2 was 0.9925 and 0.9985, respectively. The inter-assay accuracy and precision were determined using all calibration points analyzed during the study. Accuracy was expressed as % bias of the estimated concentrations. Precision was expressed as % CV. The intra- and inter-assay accuracy and precision for THC and THCCOOH in hair were all less than 13.94% at different concentrations. The percentage recovery of THC and THCCOOH in hair was more than 90% and 100% at different concentrations, respectively. Short and long-term stability for THC (200 and 400 ng/mg) in processed samples was within the acceptable ±20% range of the target concentrations except for after 2 weeks, but stability for THC (800 ng/mg) in processed samples fluctuated each week, and the measured value of THC after 1 week was below 500 ng/mg, although the initial value was 800 ng/mg (drop by over 40%) (Fig. 4). THCCOOH (0.05 and 0.25 pg/mg) in processed samples was within the acceptable ±20% range of the target concentration, but the measured value of THCCOOH (0.5 pg/mg) after 3 weeks was below 0.3 pg/mg, although the initial value was 0.5 pg/mg (drop by over 40%) (Fig. 5). The stability in high concentrations of THC (800 ng/mg) and THCCOOH (0.5 pg/mg) unexpectedly fluctuated compared with that in low concentrations and the stability was not acceptable.

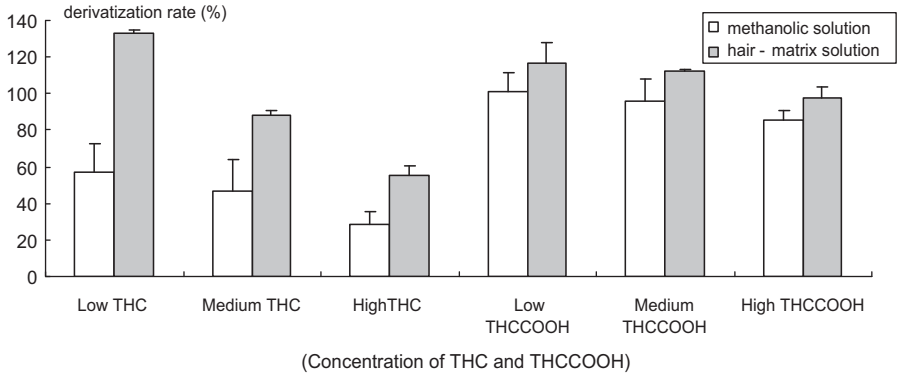


Fig. 3. Derivatization rate of THC and THCCOOH in methanolic solution and in the hair-matrix solution. Low THC; 400 ng, medium THC; 800 ng, high THC; 1500 ng, low THCCOOH; 15 pg, medium THCCOOH; 80 pg, high THCCOOH; 200 pg.

Table 2
Validation data of THC and THCCOOH in hair.

Parameters	Concentration targeted for THC (ng/mg)	THC in hair	Concentration targeted for THCCOOH (pg/mg)	THCCOOH in hair
LOD[a]		2.5 (ng/mg)		0.025 (pg/mg)
LOQ[b]		7.5 (ng/mg)		0.05 (pg/mg)
Accuracy (%)[c]				
Intra-day (n = 3)	20	−11.14%	0.75	13.94%
	75	1.59%	4	2.03%
			10	−5.41%
Inter-day (n = 15)	20	−2.22%	0.75	4.22%
	75	6.39%	4	5.72%
			10	−0.76%
Precision (%)[d]				
Intra-day (n = 3)	20	2.83%	0.75	7.26%
	75	10.03%	4	3.67%
			10	2.73%
Inter-day (n = 15)	20	8.55%	0.75	12.27%
	75	7.96%	4	3.81%
			10	7.29%
Percentage recovery (%) (n = 3)	20	97%	0.75	100%
	75	90%	4	115%
			10	110%

[a] Limit of detection.
[b] Limit of quantitation.
[c] Calculated as [(mean calculated concentration − nominal concentration)/nominal concentration] × 100 (% bias).
[d] The coefficient of variance (% CV): SD/mean × 100%.

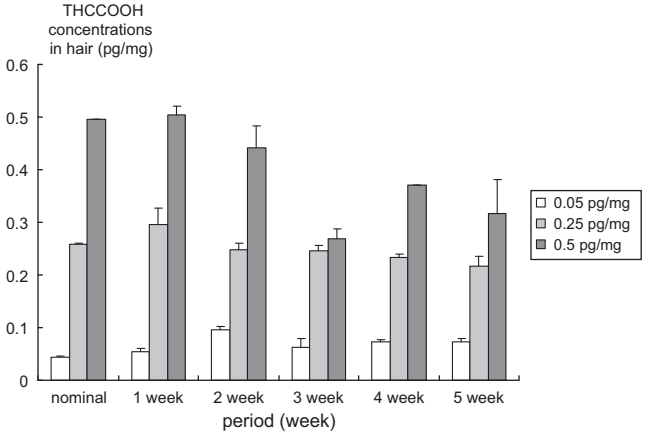


Fig. 5. Stability of derivatized THCCOOH in capped GC autosampler vials for 1–5 weeks after storing at −20 °C.

3.3. Application of validated method to real hair samples (n = 54)

Figs. 6 and 7 show the chromatograms for a blank hair sample, THC and THCCOOH in a spiked control and a positive THCCOOH and THC hair sample. This method was fully validated and applied to hair specimens (n = 54) collected from known cannabis users whose urine test results were positive. Table 3 shows the concentrations of THC and THCCOOH in real positive hair samples. Of these 54 hair samples, 18 (33%) were positive for both THC and THCCOOH and 36 (67%) were positive for THCCOOH but not for THC.

To examine the reliability in the quantification of THC, the cross validation study of the proposed method and the previous method was performed. A comparison of the present method to those reported previously [20], in which 50 μl of N,O-bis(trimethylsilyl trifluoroacetamide) plus trimethylchlorosilane (BSTFA + 1% TMCS) and 20 μl of acetonitrile were used for derivatization and performed using GC/MS-EI, shows that our method represents slightly higher concentrations of THC in 3 cases although statistical limitations in relation to sample size (n = 3) and insufficient comparison were found. The concentrations of THC in hair ranged from 2.43 to 3.29 ng/mg (average 2.87 ng/mg) in GC/MS-EI and ranged from 2.27 to 14.84 ng/mg (average 7.74 ng/mg) in our GC/MS/MS-CI.

Table 3
The concentrations of THC and THCCOOH in real positive hair samples.

Analyte	Positive hair samples (n)	Concentration range	Average concentration
THC	18	7.52–60.41 (ng/mg)	22.79 ng/mg
THCCOOH	36	0.10–11.68 (pg/mg)	2.27 pg/mg

4. Discussion

When analyzing THC and THCCOOH in hair simultaneously, using different extraction methods [15], additional clean-up processes [9,16], different derivatization reagents [15,16], different instruments [13], different ionization modes (EI, CI) [1,4] or PCI and NCI mode under the same CI mode [15] in one experiment is time-consuming, costly and requires longer stabilization times for the instruments and frequent changes of tuning parameters. Therefore, we designed combined extraction, derivatization, and NCI mode for GC/MS/MS to analyze THC and THCCOOH in hair simultaneously and efficiently.

To mask silanol groups and decrease the hydrophilicity of the surface, various silanes has been frequently used to coat the glass surface [21,22]. Silanized glassware is generally used in forensic laboratories to increase sensitivity, but there has been no comparison study of extraction efficiency for THC and THCCOOH in hair using untreated and silane-treated glassware. Therefore, in this study, three silanizing reagents for glassware in cannabis hair analysis were used to investigate extraction efficiency. For THCCOOH in hair matrix, silane treatment appeared to be an inhibitory factor in extraction; therefore all validation and applications of the method to hair samples were performed using untreated glassware because we especially focused on increasing the sensitivity for THCCOOH. Extraction efficiency increased with extractions two times for THC and THCCOOH in hair.

There was no interference because of high specific and selective GC/MS/MS-NCI method. All 5 negative hair samples and 20 common drugs of abuse were free of co-eluting peaks at the retention times for THC, THCCOOH, THC-d_3 and THCCOOH-d_3. Compounds with similar structures did not interfere with accurate THCCOOH quantitation. Some studies on the short- and long-term stability

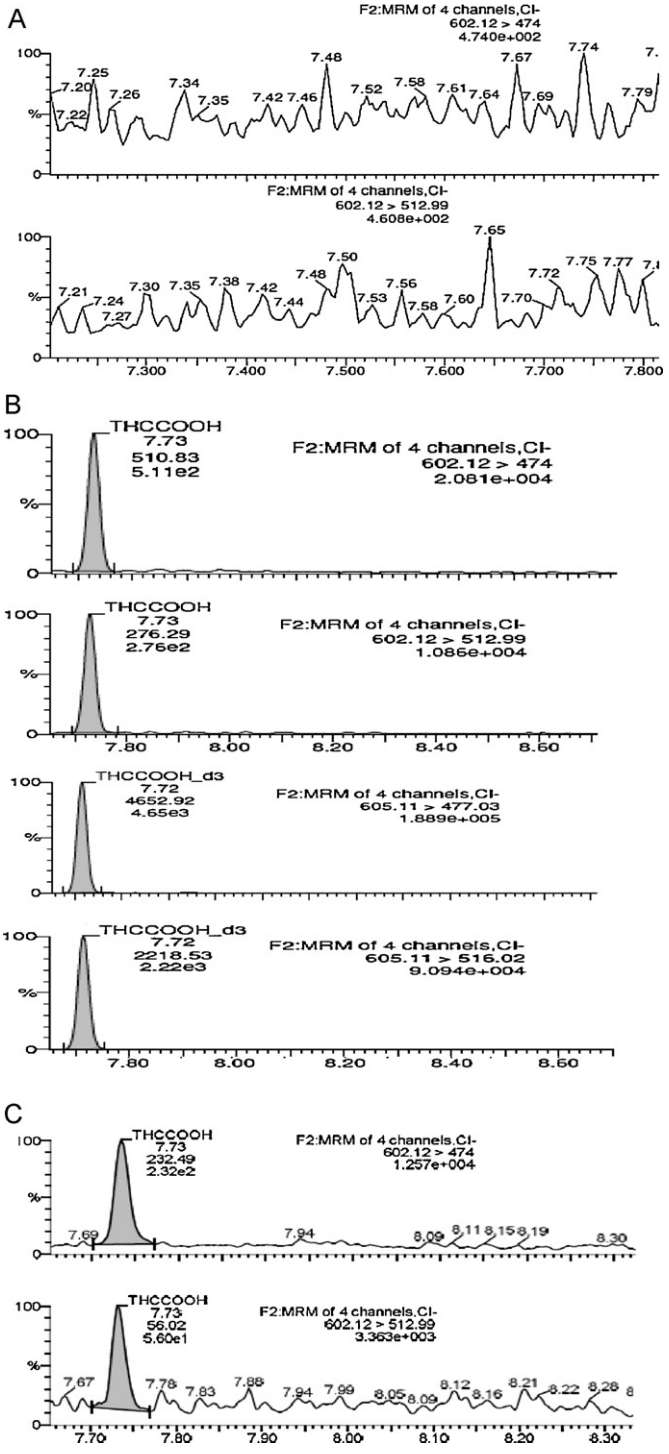


Fig. 6. Chromatograms for THCCOOH in hair. (A) Chromatograms for a blank hair sample. (B) MRM chromatograms m/z 602.12→474 and m/z 602.12→512.99 for THCCOOH in a spiked control at 0.25 pg/mg hair (peak to the first and second top); MRM chromatogram m/z 605.11→477.03 and m/z 605.11→516.02 for THCCOOH-d_3 used as an internal standard at 2.5 pg/mg hair (peak to the third and last bottom). (C) MRM chromatograms for a positive THCCOOH hair sample (measured concentration: THCCOOH = 0.41 pg/mg).

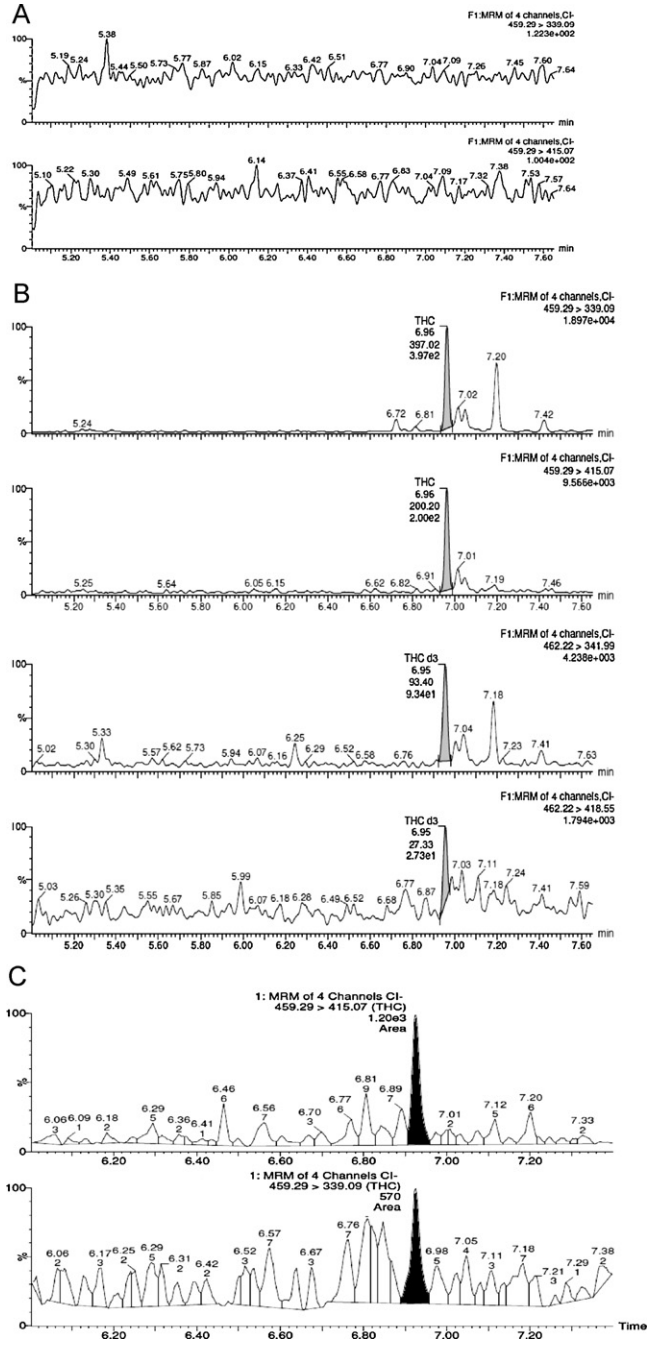


Fig. 7. Chromatograms for THC in hair. (A) Chromatograms for a blank hair sample. (B) MRM chromatograms m/z 459.29→339.09 and m/z 459.29→415.07 for THC in a spiked control at 100 ng/mg hair (peak to the first and second top); MRM chromatogram m/z 462.22→341.99 and m/z 462.22→418.55 for THC-d_3 used as an internal standard at 25 ng/mg hair (peak to the third and last bottom). (C) MRM chromatograms for a positive THC hair sample (measured concentration: THC = 25.53 ng/mg).

of THC [23–27] and THCCOOH [23,25,27] have been published in blood, serum and plasma. However, the stability of cannabinoids in hair samples has rarely been addressed [22]. In this study, stability of analytes after derivatization was also examined. THC (200 and 400 ng/mg) and THCCOOH were (0.05 and 0.25 pg/mg) relatively stable and did not significantly decrease over a 5-week period although higher concentration of THC and THCCOOH dropped by 40%. Skopp et al. [22] performed the study to elucidate the stability of cannabinoids in hair exposed to sunlight and they found that THC decreased to 77% of its initial concentration 10 min after the experiment started. Similary, the reason why so large fluctuation in our study occurred is that the stability of THC and THCCOOH itself was likely to be low and affected by external environment.

In this study, THC concentrations were similar to those reported by Tsanaclis et al. [28] but higher than those reported in other papers [3,4,6,15,29,30]. THCCOOH concentrations were similar to those reported in some papers [9,15], but much lower than

those reported in other papers [1,4,6,16,28,29] because of different limits of detection and quantitation in each instrument. As previously reported, THCCOOH concentrations were maximally 3.87 ng/mg [4,6]. Some authors represented the LOD, confirmation cut-off and the concentration range of THCCOOH in hair as LOD of 5.00 pg/mg and average concentration of 0.028 ng/mg [1], LOD of 0.01 ng/mg and concentration range of 0.05 to 0.39 ng/mg [6], confirmation cut-off of 0.001 ng/mg and concentration range of 0.001–0.589 ng/mg [28], and LOD of 0.1 ng/mg and concentration range of 0.07–0.83 ng/mg [29]. The sensitivity of the proposed method in this study could increase the sensitivity for THCCOOH compared to those of known methods.

The confirmations of THC and THCCOOH in hair generally agree [8], but a significant correlation or dose-concentration relationship between the concentrations of THC and THCCOOH in hair is not always observed [9,28]. We generally did not expect to find many cases where THCCOOH was detected but THC was not because THC is the parent drug of THCCOOH. However, in this study, we found many cases (67%) in which THCCOOH was detected but THC was not. Some researchers reported similar cases in which THC was not detected, although THCCOOH was present [1,8,9]. Quantification of THCCOOH using GC/MS/MS-NCI after PFPA and PFPOH derivatization was found to be more sensitive than that of THC. The lower recoveries observed for polar compounds were widely compensated for by the use of NCI after derivatization with fluorinated agents [17].

When using GC/MS/MS-NCI mode in this study, a common disadvantage of chemical ionization is frequent contamination of ion source and column. We improved the frequent contamination by using an oven temperature method (cool-down method) that lowered the temperature slowly and a 1-min equilibrium time on GC. Moreover, the assay running time in mass spectrometry was fixed to 8 min to reduce the contamination of the ion source.

The aim of this study was to reduce the burden of routine work in cannabis hair analysis. Therefore, our analysis was focused on saving time with efficient procedures. We firstly designed the procedures focusing on the sensitivity for THCCOOH and then that for THC and combined sample preparation, derivatization and GC/MS/MS analysis. This study is the sum of several difficult steps including hair analysis for an acidic drug and the use of tandem mass spectrometry and NCI mode. Despite these difficulties, high sensitivity for THCCOOH in hair was possible through GC/MS/MS-NCI. The ability to acquire THC and THCCOOH in hair without changing the source and by using the same derivatization reagent and reagent gas results in increased productivity. However, the sensitivity for THC was poor; therefore high sensitivity for THC is a prerequisite for further study.

There were limitations on the sensitivity for THC in this study. First, the performances of the method may not fit the requested modern performances because LOD and LOQ for THC were high. The SoHT recommended cut-off for THC is 0.1 ng/mg. We more focused on the sensitivity for THCCOOH because of discrimination between direct and indirect inhalation. Second, the measured concentrations of THC in real hair samples were high. The results seem strange because in 36 cases only the THC-COOH was detected. In hair analysis, generally the mother drug is the predominant compound and there still remains a problem with the sensitivity of the method for the THC. Last, the cross validation study for THC (comparison of measured values by the proposed method and previously reported method using same samples) need to be carried out for evaluation of the reliability of the proposed method using sufficient hair samples for further study. In spite of the limitations on the THC hair analysis, our study allows the simultaneous determination of THC and THCCOOH in hair with combined methods, in which the derivatization and gas chromatographic analysis were the same.

5. Conclusions

This paper describes a GC/MS/MS-NCI procedure for quantitative analysis of THC and THCCOOH in human hair. We used a traditional LLE method that allows collective elution of THC and THCCOOH, and the method was fully validated. Derivatized THC and THCCOOH in hair were relatively stable and did not significantly decrease over a 5-week period although higher concentration of THC and THCCOOH dropped by 40%. Although the sensitivity for THC in hair was low, good validation results and high sensitivity for THCCOOH in hair could allow more effective documentation of cannabis use than the detection of the parent drug (THC), which can come from environmental exposure. To the best of our knowledge, this is the first combination of LLE with GC/MS/MS-NCI for determination of THC and THCCOOH in hair without different sample preparation and derivatization, although it is essential to improve the THC signal in GC/MS/MS-NCI for future studies.

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