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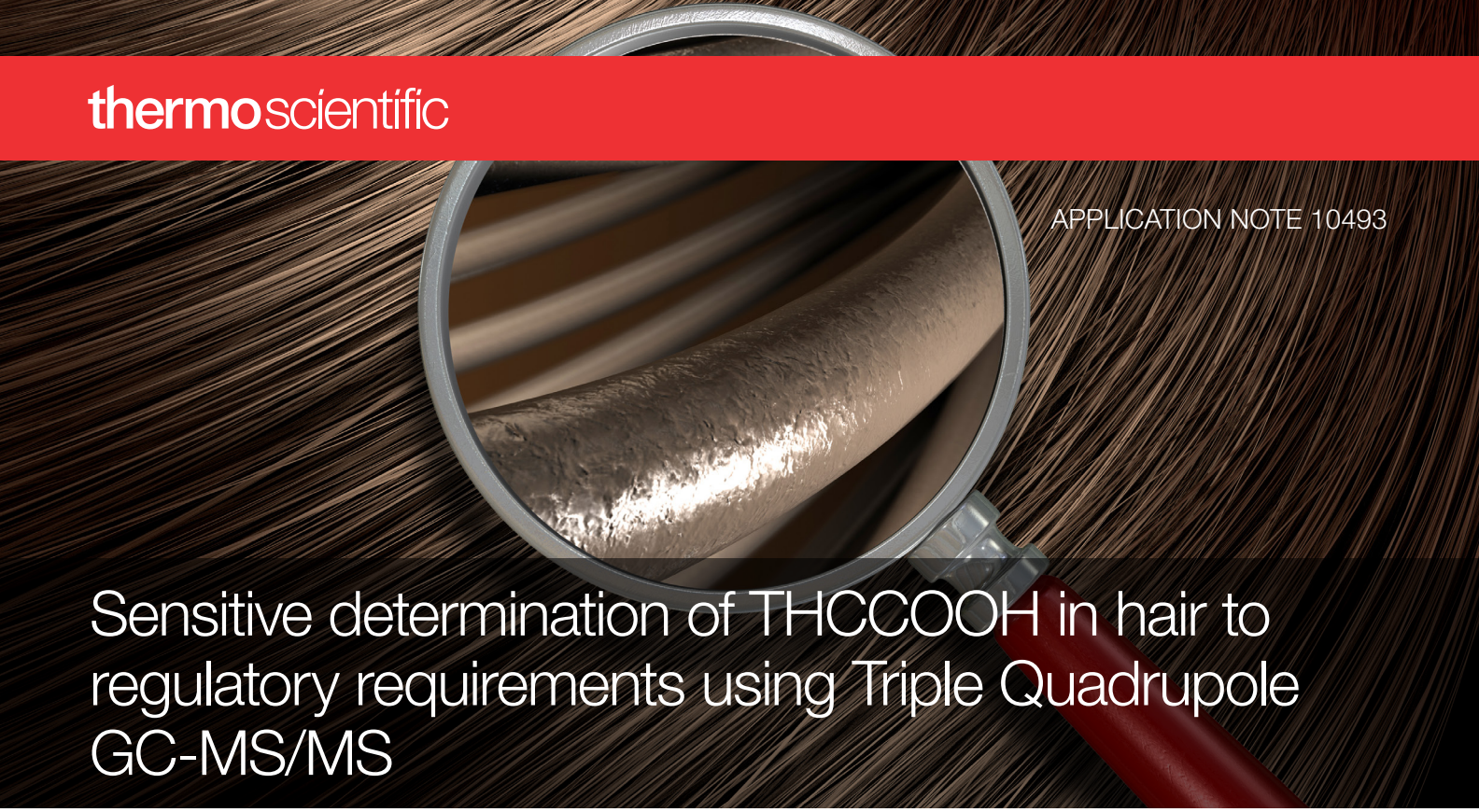
APPLICATION NOTE 10493



Sensitive determination of THCCOOH in hair to regulatory requirements using Triple Quadrupole GC-MS/MS

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Keywords

THCCOOH, hair, GC-MS/MS, chemical ionization, cannabis, TSQ 8000 Evo*

Goal

To optimize the analytical setup for the determination of 11-nor-D9-tetrahydrocannabinol-9-carboxylic acid (THCCOOH) in hair using GC-MS triple-quadrupole technology to obtain maximum sensitivity.

*Equivalent or better performance with the Thermo Scientific™ TSQ™ 9000 GC-MS/MS system

Introduction

Analysis of THCCOOH in hair is becoming one of the most common topics in hair analysis. This relevance lies in the necessity to obtain evidence of deliberate drug consumption and dispel any doubt of passive contamination of the hair through the air. When cannabis is consumed, THC is primarily adsorbed into the hair during exposure to side stream smoke.[1] This can lead to the occurrence of low level THC results through the non-intentional passive exposure of individuals to cannabis smoke. Detection of metabolites makes it possible to distinguish deliberate ingestion (effective drug consumption) from contamination. The main metabolites, which are formed via phase 1 and phase 2 metabolism, are 11-hydroxytetrahydrocannabinol (THC-OH) and THCCOOH. The possibility of external contamination events has induced SAMHSA to recommend guidelines that set the cut-off concentrations for cannabinoids in hair at 1 pg/mg for screening and 0.05 pg/mg of THCCOOH for confirmation testing[2–5] (Table 1).

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Table 1. Cut-off limit of THCCOOH in hair.

Agency Organization	Cut-off Level (pg/mg)
Society of Hair Testing (SOHT)	0.20
Geslleschaft fur Forensiche und Toxikologische Chemie (GFTCh)	0.05
Substance Abuse and Mental Health Services Administration (SAMHSA)	0.05

Delta 9 Tetrahydrocannabinol (THC) is the most commonly abused illicit drug and THCCOOH is the most abundant metabolite, but its concentration in hair is very low[6,7] (Figure 1).

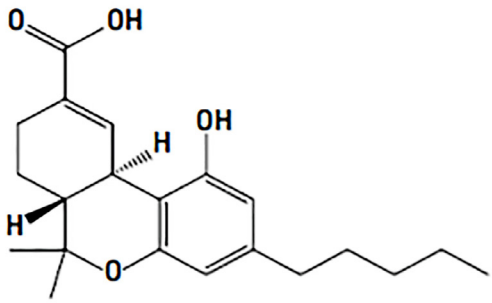

Figure 1. Structure of THCCOOH.

This analysis can be extremely challenging due to the low concentrations of THCCOOH typically found in hair, and due to the small sample of hair that is typically available for analysis. In addition, matrix interferences can limit the low detection levels required for this assay. Immunological techniques are widely used for the detection of all types of abuse drugs in biological fluids by virtue of their simplicity and expeditiousness, but they are unsuitable for determining drugs in hair owing to the high proportion of false positives and negatives.[8–10]

Other instrumental techniques have been studied during these last years like liquid chromatography coupled with mass spectrometer. But, in most of the cases, the very low detection limits are not reached. A sensitive and selective method was developed using a technology (GC-MS/MS) capable of increasing the signal to noise ratio and to allow the quantitation of THCCOOH in hair at the picogram level.

Experimental conditions

Sample preparation

All working solutions of drugs used in the present study were hand-prepared from certified standard solutions acquired from Cerilliant® (LGC Standard). Organic solvents were acquired from Thermo Fisher Scientific. The derivatizing agents used include: pentafluoropropionic anhydride (PFPA) (P/N TS65193) and hexafluoro-isopropanol (HFIP) (Sigma-Aldrich S.r.l.). Hair samples of 50 mg were washed with 2–3 mL of dichloromethane and cut into 10–20 mm pieces. The samples were spiked with standard (THCCOOH), deuterated internal standard (20 pg THCCOOH-d3), and then hydrolyzed at 75 °C in 1 mL of 5 M NaOH for 45 minutes. Four points of calibration (0.05, 0.2, 0.5, 1 pg/mg) were used and the internal standard was added to obtain a final concentration of 0.2 pg/mg. The basic solution was acidified up to pH 4 with concentrated acetic acid. After a strong agitation by vortex mixing, THCCOOH was extracted by a liquid acidic extraction with 4 mL ν-hexane:ethyl acetate (9:1). Dried extracts were derivatized with 50 µL PFPA and 25 µL HFIP at 70 °C and then reconstituted in 50 µL hexane; 2 µL was analyzed by GC-MS/MS in NCI mode for the determination of THCCOOH.

Instrument and method setup

To provide a comprehensive view of method development and validation, the details for sample preparation, acquisition and analysis are described in detail below. It is an essential requirement that any analytical method is fully validated using hair matrix. The analysis was performed using Thermo Scientific™ TriPlus™ RSH Liquid Autosampler mounted on a Thermo Scientific™ TSQ™ 8000 Evo* triple quadrupole GC-MS/MS system. Data collection and processing was performed using Thermo Scientific™ TraceFinder™ software. The system was equipped with one Instant Connect Split/Splitless Injector for the TRACE™ 1300 GC Series system using a single taper splitless liner with quartz wool (P/N 453A1925). A Thermo Scientific™ TraceGOLD™ TG-5MS column was used with the following dimensions: 20 m × 0.18 mm × 0.18 µm (P/N 26098-5780).

*Equivalent or better performance with the TSQ 9000 GC-MS/MS system

Table 2. Transitions and collision energies used for THCOOH GC-MS/MS analysis.

	Rt (min)	Ion Polarity	Mass (*m/z*)	Product Mass (*m/z*)	Collision Energy (eV)
THCCOOH-d3	6.52	Negative	623	386.2	10
THCCOOH-d3	6.52	Negative	623	534.3	12
THCCOOH	6.53	Negative	620	383.2	10
THCCOOH	6.53	Negative	620	531.3	12

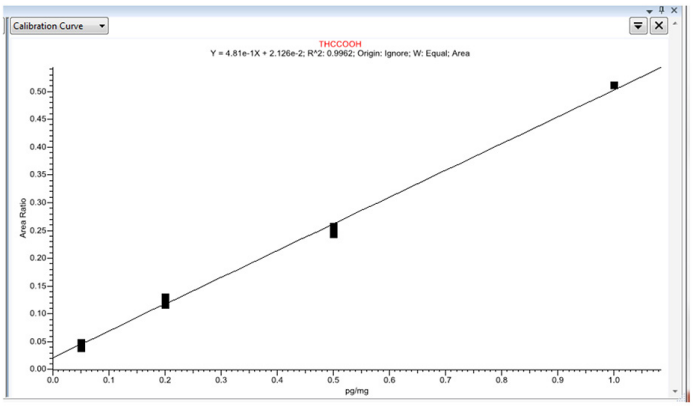

Figure 2. Calibration curve for THCCOOH. Each standard is injected five times.

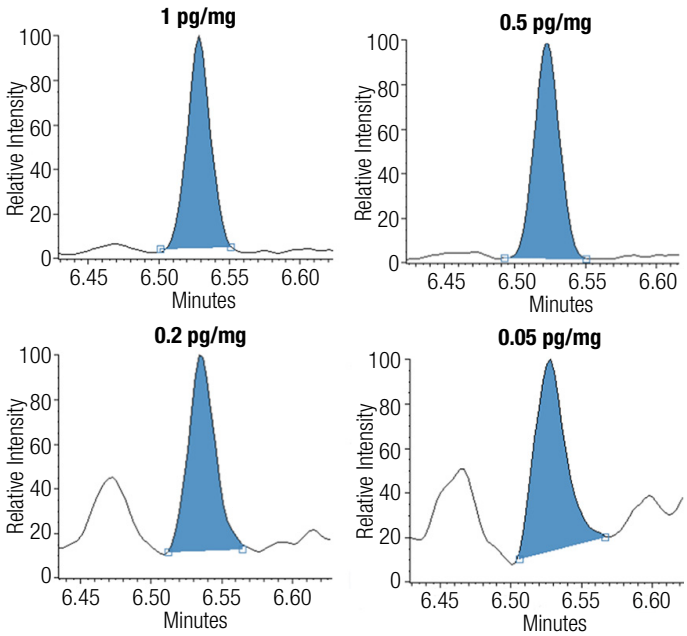

Figure 3. Matrix spike at different concentrations of standard.

Carrier gas flow was set to a constant flow of 1.0 mL/min of helium. The initial temperature on the TRACE 1310 GC system was set to 150 °C. Upon injection of the sample (1 minute), the oven temperature was immediately ramped at 20 °C/min to a final temperature of 310 °C with a final hold of 4 minutes, for a total run time of 13 minutes. The transfer line temperature was set at 250 °C. The split/splitless injector temperature was set to 280 °C. A 2 µL injection volume was programmed on the TriPlus RSH Autosampler, and a splitless injection was used with 1 minute of splitless time. The TSQ 8000 Evo* GC-MS/MS system source temperature was set to 200 °C, the mass spectrometer was tuned using AutoTune™ NCI parameters. A flow of 2 mL/min of methane was used as ionization gas. The transition and the collision energies optimized are reported in Table 2.

Results and discussion

All the standards used were prepared by spiking THCCOOH and THCCOOH-d3 into drug-free human hair prepared following the sample preparation method described. Each calibration standard was analyzed five times. The TSQ 8000 Evo* GC-MS/MS system provided excellent quantitative results, the calibration curve using internal standards showed good linearity with r^2>0.996 over 0.05–1 pg/mg (Figure 2 and Figure 3).

Matrix interferences were investigated for THCCOOH by comparing standard 0.05 pg/mg and sample drug-free labeled matrix blank (Figure 4). No interferences were detected for any transition.

Figure 5 shows the quantitation, the confirming ion of THCCOOH at 0.05 pg/mg, and the ion overlay between quan and confirm ion.

To obtain the LOD, five injections of the spiked standard 0.05 pg/mg were analyzed. The LOD was automatically calculated by the TraceFinder software using the student's *t*-test method at 99% of confidence. The LOD obtained was 0.022 pg/mg (Table 3).

*Equivalent or better performance with the TSQ 9000 GC-MS/MS system

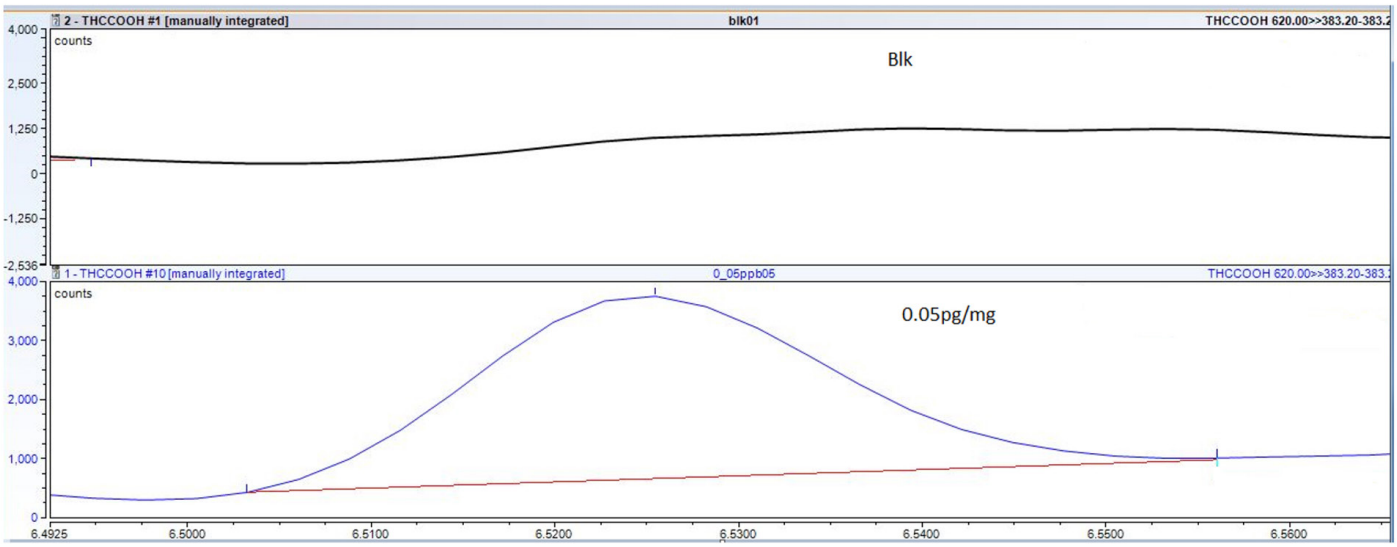

Figure 4. Comparison between the lowest matrix standard and the matrix blank.

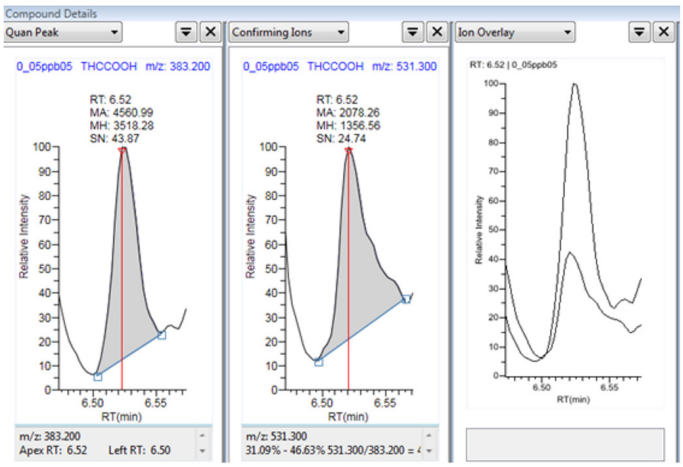

Figure 5. Quan, confirm, and ion overlay at 0.05 pg/mg.

Table 3. Reproducibility of 5 injections of spiked standard 0.05 pg/mg and LOD.

	Area	Resp Factor/ Ratio	Calculated Concentration (pg/mg)
0_05ppb05	4905	0.042	0.043
0_05ppb04	5066	0.043	0.044
0_05ppb03	5419	0.047	0.053
0_05ppb02	4716	0.044	0.046
0_05ppb01	5173	0.048	0.056
average	5056	0.045	0.049
Std dev	267	0.0026	0.0057
RSD%	5.3	5.8	11.9

Compound	Avg Conc	Std Dev	t-stat	% RSD	LOD
THCCOOH	0.049	0.0057	3.75	11.9	0.022

Conclusions

The results of this study demonstrate that the TSQ 8000 Evo* GC-MS/MS system in combination with TraceFinder 3.3 software is an extremely effective tool for the routine analysis of THCCOOH in hair samples. TraceFinder software provides a workflow-oriented format that allows analysts to easily create and manage methods, acquire samples, review results and print reports.

The analytical methodology described allows the fine determination of THCCOOH at the requested cut-off of 0.05 pg/mg. The TSQ 8000 Evo* GC-MS/MS system was chosen for this assay not only because its performance exceeds that required for the analysis, but also because of its ease of use and speed of analysis relative to alternative approaches. The results show a very good linear relationship in the full calibration range of 0.05 to 1.00 pg/mg.

Moreover, thanks to the vacuum probe interlock (VPI), on the TSQ 8000 Evo* GC-MS/MS system you can change between CI and EI mode in two minutes without breaking the vacuum. The TSQ 8000 Evo* GC-MS/MS system gives unstoppable productivity to toxicology labs where some analytes require CI mode (like THCCOOH) while other analytical methods need EI (THC).

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