

Efficiently Designed Workflows Provide Accurate Results in Forensic Analysis of THC-COOH in Hair Samples

Sensitive detection of the marijuana metabolite with the Triple Quad™ 4500 LC-MS/MS System

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Marijuana is one of the most popular recreational drugs abused worldwide. Detection of its use can be done in many biological matrices, such as urine, blood, oral fluid and hair. While urine and oral fluid are very useful for determining marijuana use in short term, hair samples are extremely valuable in testing the long-term use. Additional benefits of hair testing include but are not limited to (1) sample collection being non-invasive; and (2) little risk of sample adulteration.

Presence of the main marijuana metabolite (i.e. THC-COOH) in hair indicates active drug usage. However, there are two major analytical challenges associated with detecting THC-COOH in hair samples: The concentration of THC-COOH in hair samples is very low and the high abundance of matrix interferences associated within hair samples that specifically interfere with the detection of THC-COOH.

We have previously shown the successful applicability of the QTRAP® 5500 or 6500+ systems for THC-COOH analysis in hair. Using MS/MS/MS or MRM³, very low level of THC-COOH (~ 0.1 pg/mg) in hair samples can be detected without exhaustive sample preparation.

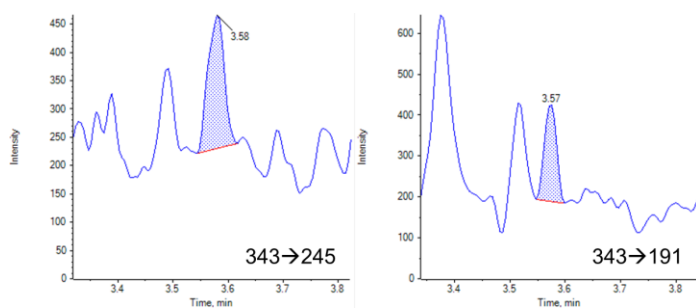
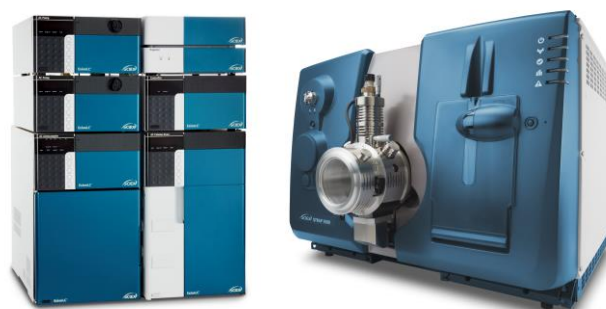


Figure 1. Detect THC-COOH in hair down to 0.2 pg/mg trace concentration levels with an efficient SPE sample preparation procedure. Using fast MRM cycle times (~100 msec) allowed the accurate quantitation (Quantifier Ion shown on left) and confirmation (Qualifier ion shown on right) of THC-COOH extracted from 25 mg of a hair sample.



In the absence of a QTRAP® System, forensic laboratories must efficiently design analysis approaches for the accurate detection of marijuana metabolite present in hair samples. In this technical note, we present a workflow that combines the Triple Quad™ 4500 LC-MS/MS System with a solid phase extraction procedure that allows the reliable and sensitive detection of trace levels of THC-COOH in hair matrix.

Features of the Triple Quad™ 4500 LC-MS/MS System for Forensic Hair Analysis

- Turbo V™ source and Curtain Gas™ interfaces delivers highly efficient desolvation for stable and sensitive performance while analyzing complex biological matrices.
- Ultra-fast MRM cycle times (With minimum dwell times of 1 msec for MRM acquisition) increasing sample throughput and data quality for trace levels of THC-COOH.
- Achieve up to 5 orders of dynamic range for high performance quantitation, reducing the needs for repeat analyses for increased productivity.
- MultiQuant™ Software allows fast data processing, with less manual intervention and quick flagging of outliers, so forensic laboratories can release results faster.

Methods

Hair Sample preparation and digestion: Hair samples were washed according to accepted laboratory procedure, dried and cut into segments of ~ 2 mm lengths. Approximately 25 mg of each hair sample was transferred into suitable and sealable container with cap. 20 µL THC-COOH-d9 internal standard solution in methanol and ~ 1.1 mL 1N potassium hydroxide solution was added, and the container was capped and gently agitated to suspend the hair segments in the solution. The containers were placed at 70°C for 1 hour with gentle agitation every 20 min (to keep hair segments fully suspended in digestion solution) for complete digestion of the hair samples. The containers were allowed to cool to room temperature. Contents of the containers were transferred to 2-mL microcentrifuge tubes for ultra-centrifugation at 15,000 rpm for 5 min.

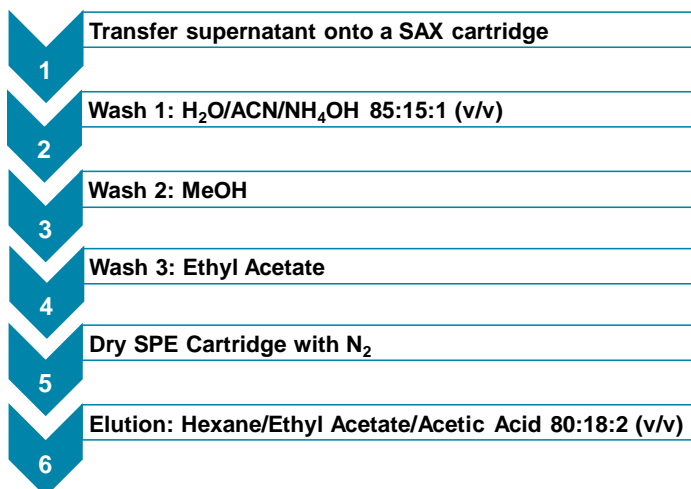


Figure 2. Strong Anion Exchange (SAX) Solid Phase Extraction workflow. A 6-step extraction protocol can be rapidly implemented and optimized for selectively extracting THC-COOH from hair samples for analysis with the Triple Quad™ 4500 LC-MS/MS System.

LC conditions: HPLC separation was performed on Phenomenex Kinetex Phenyl-hexyl column (50 × 3 mm, 2.6 µm, 00B-4495-E0) on the SCIEX ExionLC™ AC system. Mobile phase A (MPA) and mobile phase B (MPB) were 0.01% acetic acid in water and methanol, respectively. The LC flowrate was 0.75 mL/min, column temperature was held at 40°C, and the total LC runtime was 5.5 min.

MS and MS/MS conditions: Source conditions were in Table 1 and MRM conditions were listed in Table 2.

Table 1: Source Parameters

Curtain gas	25
CAD	10
Spray voltage (V)	-4500
Temperature (C)	650
GS 1	60
GS 2	60

Table 2: MRM Transitions used

Analyte	Q1	Q3	DP	CE
THC-COOH (1)	342.9	245.1	-100	-39
THC-COOH (2)	342.9	191.0	-100	-45
THC-COOH-d9 (1)	351.9	254.1	-100	-39
THC-COOH-d9 (2)	351.9	194.0	-100	-45

Data Processing: Data was acquired with Analyst 1.6.3 and processed with MultiQuant™ Software 3.0. Linear dynamic range was evaluated through calibration curves with analyte concentrations ranging from 0.2 – 2 pg/mg.

Designing an efficient sample preparation workflow to maximize THC-COOH recovery

One of the biggest challenges in getting the clean extracts of THC-COOH from hair for detection was the presence of complex matrix contents, some of which were structurally similar to THC-COOH. To remove these interferences, a SAX SPE procedure was suggested and tested.

Usually in the SAX procedure, the sample need to be basified first before being applied to the SPE cartridge, so the target analytes can bind to the oppositely charged SAX stationary phase strongly. Because hair samples usually were digested in highly alkaline solutions for more complete release of analytes from the hair samples into the extraction media, the sample solutions were already basified and can be, in theory, directly applied onto the SPE cartridge.

It was discovered that an extra ultra-centrifugation step was needed before the samples were applied to the SPE cartridge, mainly to remove the insoluble particulates in the digested hair samples. Failure to remove these particulates rendered a very long sample application step as the SPE cartridge would be clogged or partially clogged during this process.

THC-COOH: 343→245 (Top: 0.4 pg/mg; bottom: 1 pg/mg)

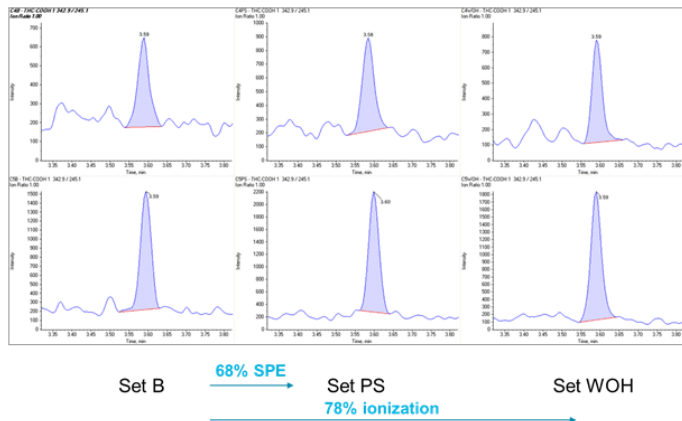


Figure 3. Obtain maximized analyte recovery performance and reduced matrix effects. The combination of SPE, LC separation and highly efficient ionization through the Turbo V™ source delivered high analyte recovery, allowing consistent quantitation of THC-COOH at low picogram levels.

To test the sample preparation recovery and matrix effects, three sets of samples were prepared:

- Set B was spiked hair samples with 0.4 and 1 pg/mg THC-COOH processed with SCX SPE.
- Set PS was similar to B but THC-COOH was not spiked before SPE step was completed, so we could measure the sample preparation recovery.
- Set WOH was also similar to B but the hair samples (in solution) were replaced with 1 N potassium hydroxide, so the signal difference between B and WOH solely indicated ion suppression or enhancement.

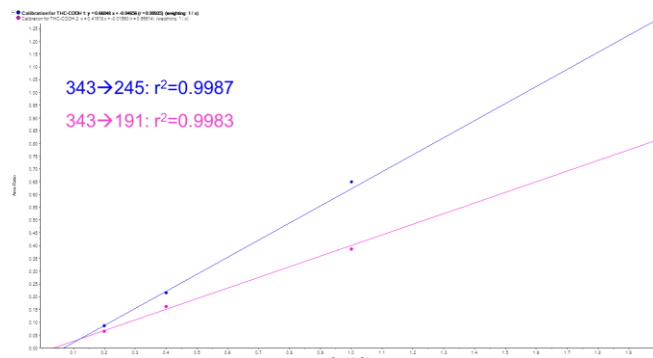
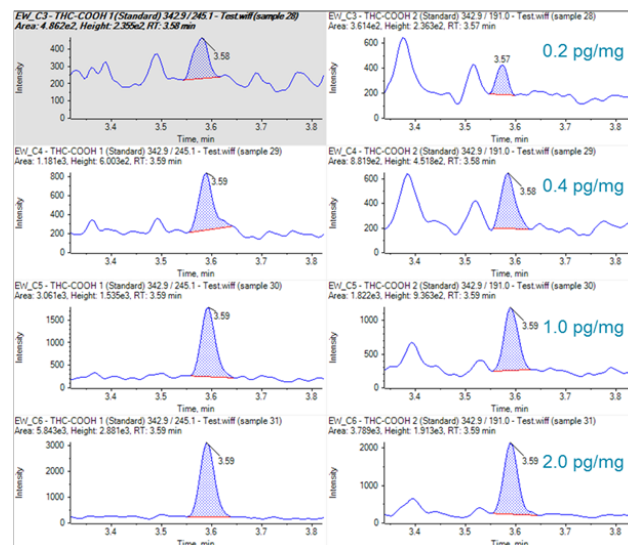
It was observed that the sample preparation recovery was at 68% and the matrix effects showed 22% loss of signal (or 78% signal recovery due to ion suppression, Figure 3). This allows the reliable quantitation of THC-COOH at low picogram concentration levels, which is only possible through the implementation of the SAX SPE procedure designed.

Evaluating the analytical sensitivity of the Triple Quad™ 4500 LC-MS/MS System

Six levels of calibrators were prepared at 0.04, 0.1, 0.2, 0.4, 1 and 2 pg/mg for THC-COOH in hair. Figure 3 showed both the quantifier (343→245) and qualifier (343→191) transitions for samples from 0.2 to 2 pg/mg samples.

343→245

343→191



Row	Component Name	Actual Concentration	Num. Values	Mean	Standard	Percent CV	Accuracy
1	THC-COOH 1	0.20	3 of 3	0.134	0.025	12.80	96.99
2	THC-COOH 1	0.40	3 of 3	0.417	0.046	10.93	104.30
3	THC-COOH 1	1.00	3 of 3	0.984	0.074	7.54	98.39
4	THC-COOH 1	2.00	3 of 3	2.004	0.136	6.78	100.19
5	THC-COOH 2	0.20	3 of 3	0.191	0.024	12.54	95.67
6	THC-COOH 2	0.40	3 of 3	0.423	0.045	10.62	105.81
7	THC-COOH 2	1.00	3 of 3	0.979	0.138	14.07	97.88
8	THC-COOH 2	2.00	3 of 3	2.004	0.087	4.35	100.21

Figure 4. Good Linear Dynamic Range, accuracy and precision was achieved for THC-COOH in hair. Calibration curves are shown as well a few representative XIC traces to demonstrate reliable quantitation from 0.2 to 2 pg/mg.

We determined 0.2 pg/mg as our LOQ for THC-COOH in hair samples. The assay showed excellent accuracy (>95%) and precision (< 15%), and the R² values for quantifier and qualifier were 0.9987 and 0.9983, respectively.

All quantitation results were processed with MultiQuant™ Software 3.0, designed for easy, quick, versatile and streamlined data processing with accurate and reliable quantitation.

Conclusions

The combination of a solid phase extraction procedure with the Triple Quad™ 4500 LC-MS/MS System allowed the efficient and sensitive detection of trace levels of THC-COOH (0.2 pg/mg) in hair samples, making the workflow to be readily adaptable into a forensic toxicology laboratory.

- A 6-step extraction protocol using SAX SPE can be rapidly implemented and optimized for selective analysis of THC-COOH.
- The design of the hair analysis workflow resulted in efficient ionization through the Turbo V™ source delivered high analyte recovery for stable and sensitive performance.
- Successful quantitation of THC-COOH was performed using MultiQuant™ Software 3.0 allowing streamlined and accurate data processing of trace level concentrations (0.2 to 2 pg/mg).

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