Application Note

Forensics

Determining Carboxy-THC in Hair Using Agilent Captiva EMR—Lipid Cleanup with LC/MS/MS

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Abstract

This application note compares two LC/MS/MS models, the Agilent 6470A triple quadrupole LC/MS and Agilent 6495C triple quadrupole LC/MS. The study summarizes sample preparation techniques available for the complex hair matrix. The study also presents the final method chosen for verification when working with Agilent Captiva EMR—Lipid in the cleanup step, to meet the cutoff limit of 0.2 ng/g of carboxy-THC in hair.

Introduction

Testing hair for drugs of abuse was introduced over 50 years ago. One advantage of hair testing is its larger window of drug detection compared to biological fluids. Many drugs are well preserved in hair. One example is that cocaine was detected in the hair of a 900-year old mummy. Applications of hair testing include criminal investigations, verifying drug use history, identifying drug-facilitated sexual assault, proving drug use in child custody cases, monitoring abstinence of parolees, drug treatment participants or employees, documenting *in utero* exposure, and determining whether a person is a regular cannabis consumer or not in the case of restitution of a driving license. Figure 1 shows the potential incorporation pathways of cannabinoids into human hair.

Workplace programs include hair testing due to its ease of collection, difficulty of adulteration, and longer detection times. Guidelines for workplace hair testing have been proposed in many countries. The guidelines establish an initial test cutoff concentration of 1 pg marijuana metabolites/mg hair. In Brazil, the limits are provided by the Society of Hair Testing (SoHT), with a confirmatory cutoff limit of 0.0002 ng for 11-nor-9-carboxy-Δ^9-tetrahydrocannbinol (THC-COOH) per mg of hair.[2] The use of sufficiently sensitive and specific analytical procedures to detect and quantify drugs in hair is important, and is the bottleneck in the workflow. This is especially relevant for cannabinoids that have low concentrations in hair. Immunoassay tests have been widely used, but sensitivity, selectivity, and cost remain challenging.

Chromatographic determinations are more specific, and are the methods of choice for confirmation of cannabinoids in hair, providing good limits of detection (LODs) and quantification. Sensitive immunoassays and gas chromatographic determinations are more specific and a good choice for confirmation of cannabinoids in hair, providing low LODs and limits of quantification (LOQs). Sensitive immunoassays, gas chromatography/mass spectrometry (GC/MS) with electron ionization (EI), GC/MS with negative chemical ionization (CI), and gas chromatography/tandem mass spectrometry (GC/MS/MS) methods for detecting cannabinoids in hair have been reported with limits 20 to 100 times lower than required by SoHT. However, a derivatization step is required.[3–10]

The most important advantage of using an LC/MS/MS method is that derivatization is not needed, plus it can be used for screening and confirmatory testing. However, the disadvantage of LC/MS/MS when compared to GC/MS/MS is that, in the detection and quantification of cannabis metabolites, GC/MS/MS presents the most abundant signals when detection limits are reached. These limitations eventually occur in the LC/MS/MS technique due to the complexity of the hair matrix, where ion suppression is observed. These effects are only noticeable for this metabolite at cutoff levels or lower. Table 1 presents the characteristics of the target analyte.

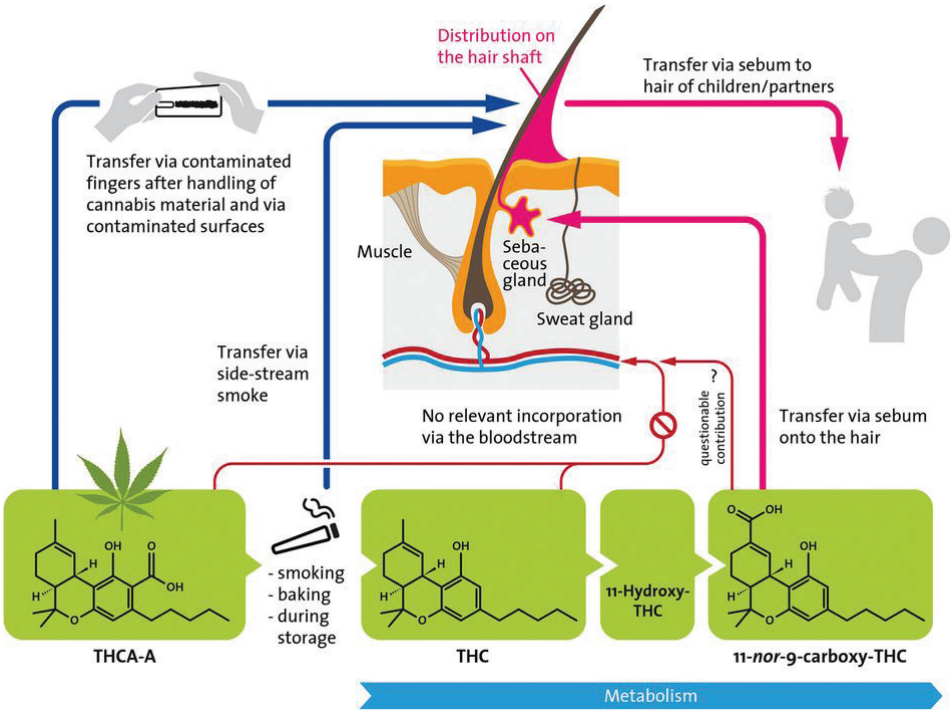


Figure 1. Potential incorporation pathways of cannabinoids into human hair.[1]

Experimental

Materials and reagents

The following materials were used:

- Agilent Captiva EMR—Lipid cartridges, 3 mL, 300 mg (p/n 5190-1003) for 40 mg hair processing, and 1 mL, 40 mg (p/n 5190-1002) or 96-well plate (p/n 5190-1000) for 25 mg hair processing
- Vac Elut 20 manifold with collection rack for 16 × 100 mm test tubes (p/n 12234103)
- Agilent InfinityLab Poroshell 120 Phenyl-Hexyl, 3.0 × 150 mm, 1.9 µm (p/n 693675-312), Poroshell 120 Phenyl-Hexyl, 3 × 5 mm, 1.9 µm, UHPLC guard (p/n 823750-943)
- Agilent 1290 Infinity II inline filter, 0.3 µm (p/n 5067-6189)

The following chemicals were used:

- Carboxy-THC and carboxy-THC-d_3 standards from Cerilliant and LGC, respectively
- GC-grade *n*-hexane and ethyl acetate (EtOAc) and LC/MS-grade methanol (MeOH) from J.T. Baker
- ACS-grade sodium hydroxide (NaOH) and acetic acid from Merck/Sigma
- Ultrapure water from Milli-Q

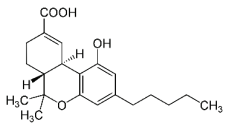
Standards and solutions

- **Sodium hydroxide 1 M (hair digestion solution):** NaOH (4 g) was weighed into a beaker. After dissolving with 50 mL of water, the solution was transferred to a 100 mL volumetric flask. The beaker was washed with a small amount of water two to three times. The solution was sonicated for one minute, and calibrated with water to the meniscus of volumetric flask. The solution was stored in a plastic-capped bottle.

Caution: heat is released when water is added to the sodium hydroxide.

- ***n*-Hexane and EtOAc (9:1 v/v) (solvent to hair extraction):** Ninety milliliters of hexane and 10 mL of ethyl acetate was combined and mixed well.
- **MeOH/water (80:20 v/v) (Captiva EMR—Lipid solvent):** Eighty milliliters of methanol and 20 mL of ultrapure water were combined and mixed well.
- **Internal standard (IS)—carboxy-THC-d_3:** The IS solution containing 8 ng/mL of carboxy-THC-d_3 was prepared in MeOH, corresponding to 8 ng/g in the matrices.
- **Calibration curve standards—carboxy-THC (CCS):** Six calibration standards were prepared in MeOH at levels of 0.15, 0.2, 0.4, 0.8, 1.6, and 3.2 ng/mL.
- **Quality control (QC) solution (carboxy-THC):** A solution at 0.2 ng/mL was used for quality control of batches. A solution at 0.04 ng/mL was injected into the LC/MS/MS to evaluate system suitability before beginning analysis.

Table 1. Target analyte, log P, molecular formula, and chemical structure.

Name	Log P	Molecular Formula	Structure
Carboxy-THC	6.21	$C_{21}H_{28}O_4$	

Sample preparation method

The hair samples were washed with water, acetone, and dichloromethane (DCM) sequentially by one minute of vortexing. The hair was dried and cut into small pieces. Afterwards, the sample preparation method included four stages: 1) hair digestion and precleanup, 2) liquid/liquid extraction (LLE), 3) Captiva EMR—Lipid cleanup, and 4) LC/MS/MS injection.

1. Hair digestion

Forty milligrams of negative hair was weighed into the headspace vial. A salinized vial is recommended to prevent hair sticking to the vial wall caused by electrostatic effects. Blank hair samples were then spiked as follows:

a) Blank – added 40 µL of IS.

b) Double blank (no IS).

c) Standards 1 to 6 were prespiked with 40 µL of calibration curve standard solutions, respectively, and 40 µL of IS solution, giving final concentrations of 0.15, 0.2, 0.4, 0.8, 1.6, and 3.2 ng/g carboxy-THC in hair.

d) QC was prespiked with 40 µL of QC solution and 40 µL of IS solution, generating a final concentration of 0.2 ng/g carboxy-THC in hair.

e) Forty microliters of IS solution was added to the rest of samples to be tested in the batch.

Spiked hair samples were equilibrated for three minutes. An aliquot of 2 mL of 1 M NaOH solution was added. Vials were capped and incubated at 100 °C for 30 minutes, then cooled to room temperature.

A 5 mL aliquot of *n*-hexane/EtOAc (9:1 v/v) was added. Samples were vortexed for one minute and centrifuged at 4,000 rpm for five minutes for clear phase separation when needed. The upper organic layer was discarded. A 2 mL aliquot of acetic acid was added, and the sample was mixed.

An alternative procedure using 25 mg of hair and a Captiva EMR—Lipid 1 mL cartridge can be used with appropriate volume adjustment. Figures 2 and 3 show the method workflows using 40 and 25 mg sample sizes.

2. Liquid-liquid extraction (LLE)

A 5 mL aliquot of *n*-hexane/EtOAc (9:1, v/v) was added. The sample was vortexed for one minute and centrifuged as needed. The upper layer was transferred to another tube. This LLE procedure was repeated two more times with the upper organic layer being transferred and combined. The combined organic extract was dried under N_2 flow at 50 °C. The dried sample was then reconstituted with 1.5 mL of MeOH/water (8:2 v/v), followed by 1.5 minutes of sonication and 30 seconds of vortexing.

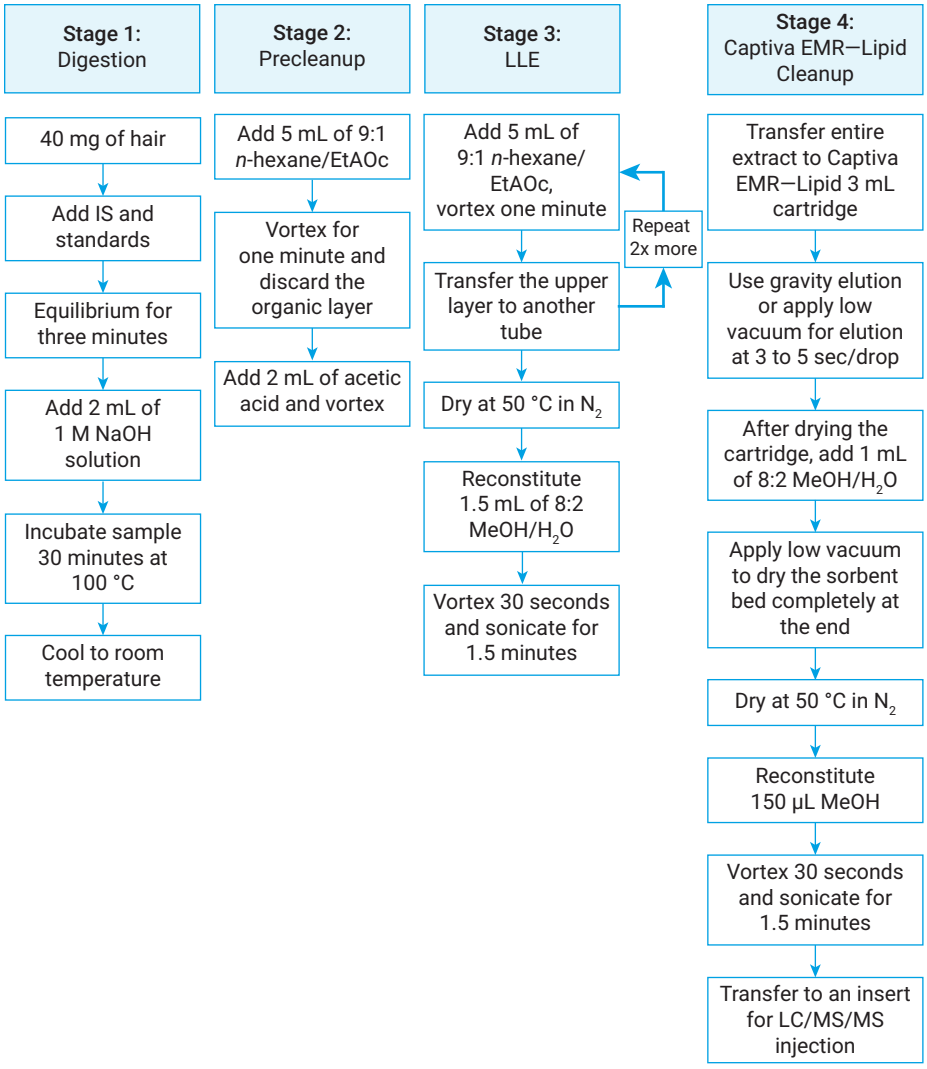


Figure 2. Sample preparation workflow for 40 mg of hair using Agilent Captiva EMR—Lipid 3 mL cartridges.

3. Captiva EMR—Lipid cleanup

The 1.5 mL of reconstituted sample was transferred to a Captiva EMR—Lipid 3 mL cartridge. Sample was eluted by gravity with a flow rate of three to five seconds per drop. Low vacuum was applied as needed. When no visible liquid was left in the cartridge, an aliquot of 1 mL MeOH/water (8:2 v/v) was added for additional elution. Vacuum was applied as needed. The eluent was dried under N_2 flow at 50 °C. The dried sample was reconstituted with 150 µL of MeOH, vortexed for 30 seconds, and sonicated for 1.5 minutes. Sample was transferred to a 2 mL vial with an insert for LC/MS/MS injection.

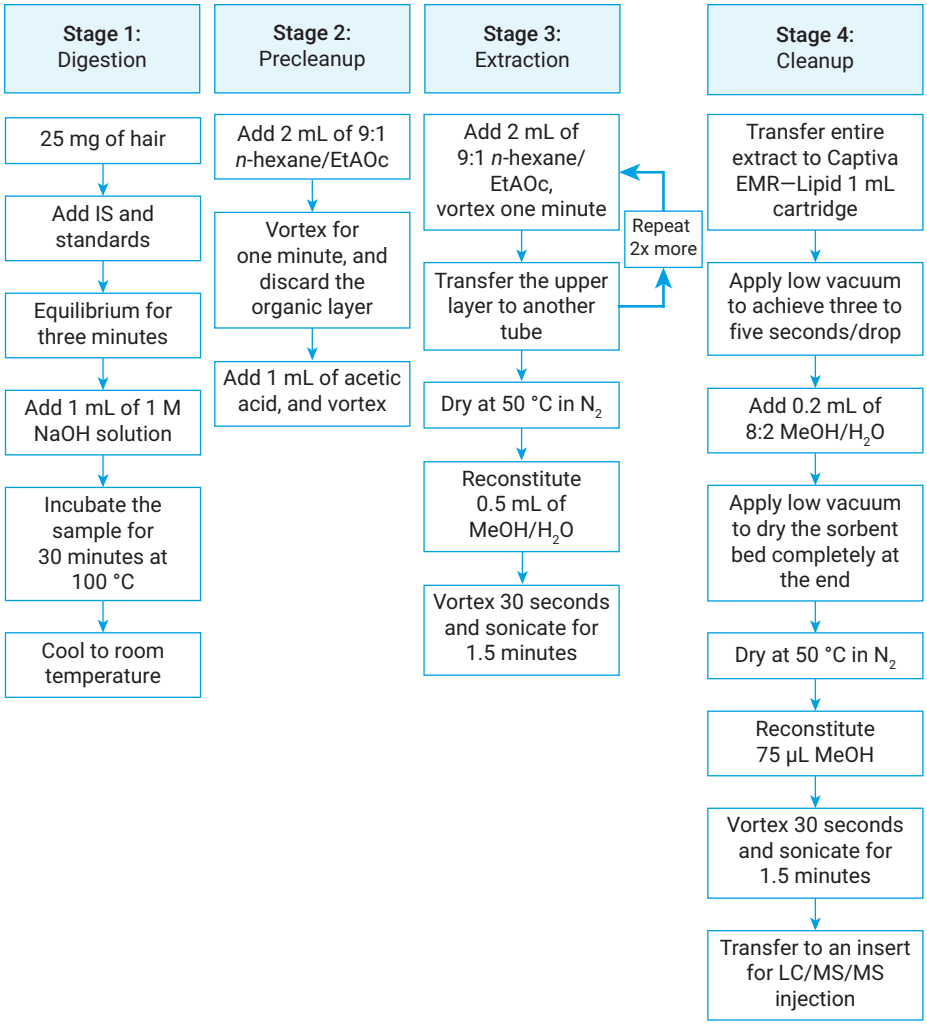


Figure 3. Sample preparation workflow for 25 mg of hair using Agilent Captiva EMR—Lipid 1 mL cartridges.

Instrument method

The samples were run on an Agilent 1290 Infinity II LC coupled to an Agilent 6495C triple quadrupole LC/MS equipped with an Agilent Jet Stream iFunnel electrospray ion source. Tables 2 and 3 show the instrument method in detail.

Prior to the daily sample analysis, a system suitability test was run by injection of a standard solution at 0.04 ng/mL in MeOH.

Table 2. LC/MS/MS conditions.

Parameter	Value
Analytical Column	Agilent InfinityLab Poroshell 120 Phenyl Hexyl (3.0 × 150 mm; 1.9 µm) and guard column. Inline filter at autosampler.
Column Temperature	50 °C
Injection Volume	5 µL
Mobile Phases	A) H_2O with 0.01% acetic acid B) MeOH with 0.01% acetic acid
Flow Rate	0.5 mL/min
Gradient	Time (min) %A %B 0.00 55 45 0.50 55 45 2.00 25 75 5.00 25 75 8.00 20 80 9.00 20 80 9.01 0 100 12.00 0 100 12.01 55 45
End Run Time	12.02
Post Time	3 minutes
Total Run Time	15 minutes

Table 3. Data acquisition parameters.

Agilent 6495C MS Conditions	
Mode	MRM
Drying Gas Temperature	300 °C
Vaporizer Temperature	450 °C
Drying Gas Flow	13 L/min
Nebulizer Pressure	50 psi
Capillary Voltage	4,500 V
Sheath Gas Flow	12 L/min
Sheath Gas Temperature	385 °C
Nozzle Voltage	2,000 V
High-Pressure RF (+)	0
High-Pressure RF (–)	130
Low-Pressure RF (+)	0
Low-Pressure RF (–)	140
Delta EMV (–)	800

Compound Name	Precursor Ion	Product Ion	CE	CAV	Polarity
Carboxy-THC	343.2	245.1	32	4	Negative
	343.2	191.1	33	3	Negative
Carboxy-THC d_3	346.2	302.2	20	3	Negative
	346.2	194.1	20	3	Negative

Results and discussion

Many types of sample preparation to extract carboxy-THC in hair have been published. These methods usually include three pretreatment steps: 1) Washing of the hair sample, 2) Cutting or pulverizing, and 3) Weighing 10 to 50 mg of hair for sample analysis. Then, hair incubation in an acidic or basic solution is applied followed with an organic solvent for extraction.

Basic digestion with NaOH was chosen in this study. The hair digestion mixture was then extracted using LLE, and followed by Captiva EMR—Lipid cartridge cleanup. The entire method provides higher recoveries and lower ion suppression, resulting from the efficient removal of salts, proteins, and lipids. Table 4 shows the comparison of typical sample preparation techniques used for hair extraction and cleanup.

The calibration curve showed linearity with R^2 >0.99 within the calibration range of 0.15 to 3.2 ng/g in hair, with quadruplicate and duplicate injections, as presented in Figure 4. Recoveries remained on average 110%, with 8.8% RSD for seven replicates at 0.2 ng/g in hair and 101% with 7.8% RSD for seven replicates at 0.8 ng/g in hair. Figure 5 shows that the 6495C triple quadrupole LC/MS provided three times higher response than the 6470A triple quadrupole LC/MS system for hair spiked with carboxy-THC at 0.2 ng/g. Figure 6 shows the hair matrix blank and five replicates at the cutoff limit on both MRMs.

Table 4. Sample preparation comparison to low quantification limits (0.0002 ng/mg) of carboxy-THC in hair matrix.

Sample Preparation Method	Advantages	Disadvantages
LLE	• Salts and protein are removed from the digestion step • Easy to apply • Wide range of compound extraction	• Co-extracted lipids with target analytes cause analytical issues on instrument • Lack of method robustness with high ion suppression
SPE-anion exchange with and without LLE	• Salts and protein are removed from the digestion step • The final extract to injection is cleaner than LLE • Higher analyte recoveries	• Although cleaner extraction than LLE, still presented high ion suppression • More steps in extraction and cleanup protocol
SPE-cation exchange with and without LLE	• Salts and protein are removed from the digestion step • The final extract to injection is cleaner than LLE • Lower ion suppression	• Cleaner extraction compared to LLE and anion exchange, but lower analyte recoveries were found • More steps in extraction and cleanup protocol
LLE with Agilent Captiva EMR—Lipid	• Salts, proteins, and lipids are removed from the digestion step • The final extract to injection is cleaner than LLE • Lower ion suppression	• More steps on extraction and cleanup protocol

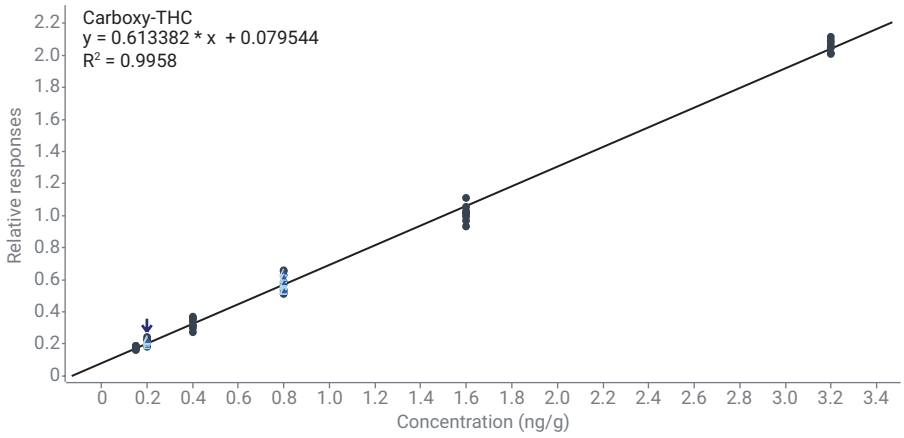


Figure 4. Calibration curve of 0.15 to 3.2 ng/g carboxy-THC in hair. Blue triangles represent seven replicate extractions at cutoff limits of 0.2 and 0.8 ng/g in hair QC samples.

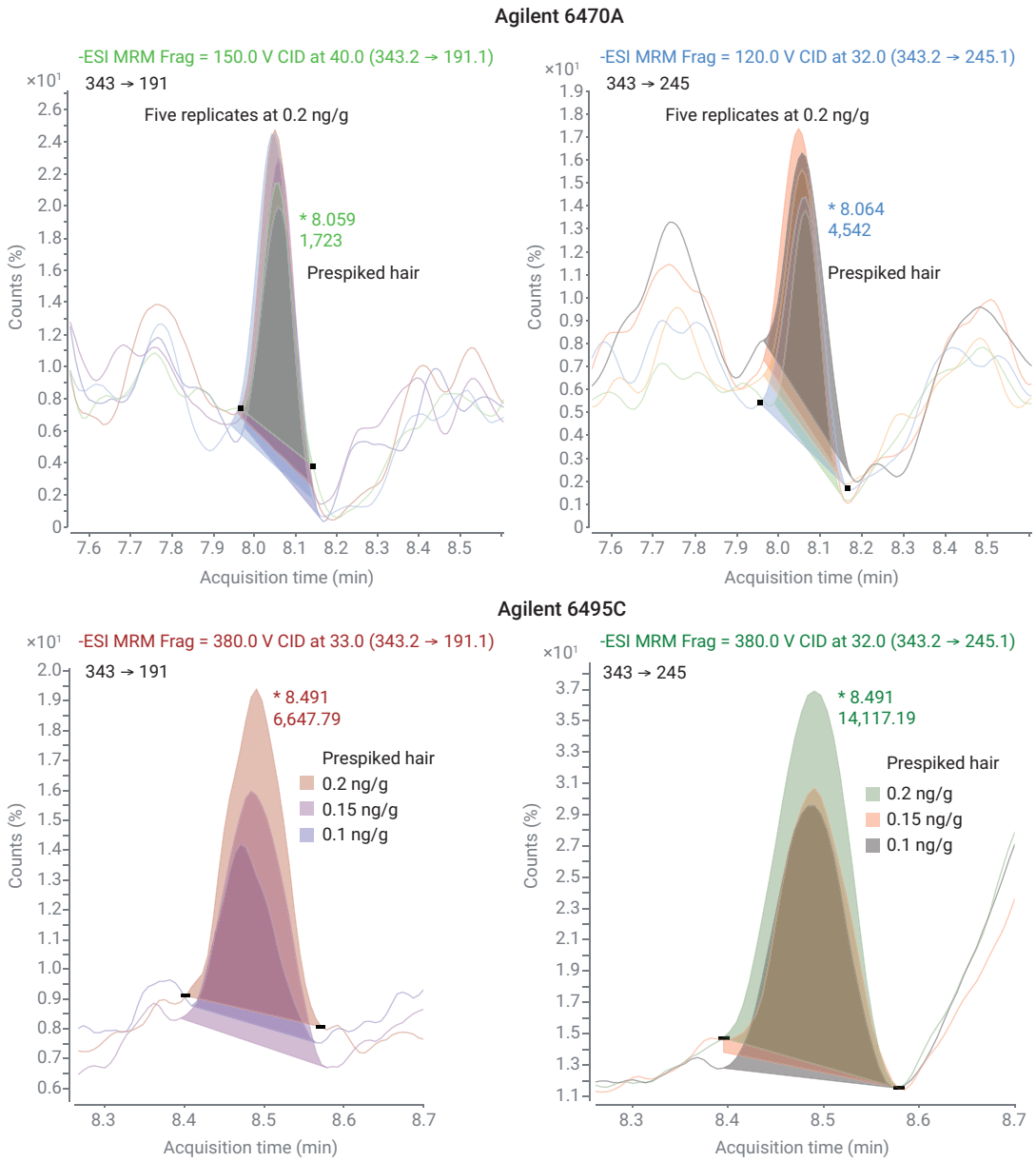


Figure 5. Results of carboxy-THC in hair at 0.2 ng/g using the Agilent 6470A and 6495C triple quadrupole LC/MS, respectively.

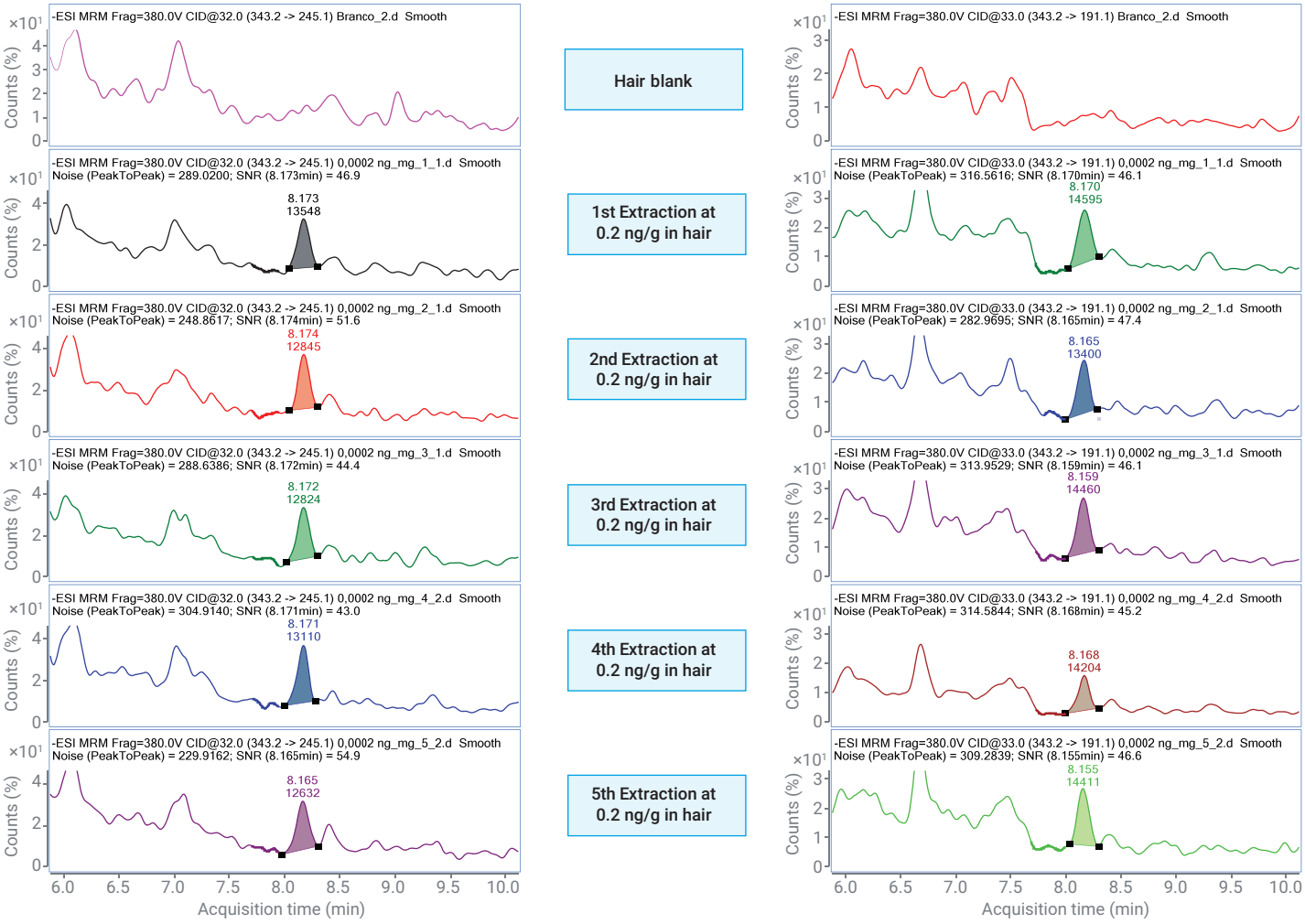


Figure 6. A hair blank and five prespiked hair samples at 0.2 ng/g, under MRM 343.2 ➔ 245.1 and 343.2 ➔ 191.1.

Conclusion

A robust sample preparation method using LLE followed by Agilent Captiva EMR—Lipid cleanup was developed and verified for carboxy-THC analysis in hair using LC/MS/MS. The hair matrix samples were also run on Agilent 6495C and 6470A triple quadrupole LC/MS systems for sensitivity comparison, with the former providing better sensitivity than the latter. The method was verified with improved method robustness and sensitivity. Hundreds of samples have already been evaluated for routine analysis using the developed method.

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