APPLICATIONS

Analyzing Drugs of Abuse from Hair

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Outside of the lab, Laura enjoys spoiling her dog Maggie and subjecting her husband to novel methods of torture, such as endless playlists of sad songs and long walks on the beach to catch Pokémon.

Overview

Hair as a sample matrix provides the unique ability to detect chronic drug use. This is in comparison with other biological matrices such as urine, blood, or oral fluid which are only suitable for monitoring relatively recent consumption. However, hair is a very complex matrix and presents significant challenges for analysis by LC-MS/MS. Typical hair extracts display a high amount of background and matrix interferences. The matrix components can also cause ion suppression and make detecting low analyte concentrations difficult. In this work, using a combination of sample preparation and chromatographic separation, we reduced the impact of matrix effects from hair samples during LC-MS/MS analysis.

Materials and Methods

Analytical reference standards were sourced from UTAK Laboratories (Santa Clarita, CA) and Cerilliant® (Round Rock, TX). Solvents were purchased from Honeywell (Muskegon, MI). All other chemicals were obtained from the Sigma-Aldrich® Company (St. Louis, MO). Water purification via Sartorius® arium® Comfort II, courtesy of Sartorius Corporation (Bohemia, NY).

Experimental Conditions

Preparing the Hair

1. Wash hair several times with methanol and water.
2. Allow hair to dry completely.
3. Clip hair into very small segments (~1 mm).

Solid-Liquid Extraction Sample Preparation (Screening & Confirmation)

Combine:	20 mg hair and 1 mL Acetone in a scintillation vial
Add:	50 µL internal standard
Shake:	Vial on a rotator overnight at room temperature (~14 hours)
Transfer:	Acetone to a tube for drying (leaving hair behind)
Add:	1-2 mL of acetone to the vial and wash the walls Transfer the solvent to same drying tube
Evaporate:	Acetone to dryness under Nitrogen at 40-50 °C
Reconstitute:	Dry residue with 100 µL of mobile phase A

LLE Sample Preparation (THC-COOH Specific Method)

Weigh:	20 mg of hair and add into a glass test tube
Add:	1N Sodium hydroxide and incubate 1 hour at 75-80 °C
Neutralize:	Add 1 mL 1N Hydrogen chloride and 6 mL Ethyl acetate extraction solvent. Vortex for approximately 2 min to allow for separation.
Centrifuge:	7 minute at 3000 rpm and flash freeze aqueous layer with dry ice and transfer supernatant
Evaporate:	Supernatant to dryness under a gentle stream of nitrogen @ 40-45 °C
Reconstitute:	Residue with 100 µL initial mobile phases

Pre-treatment

- To a glass test tube, weigh out 20 mg hair
- Add 1 mL 1N Sodium hydroxide and then add 50 µL internal standard. Incubate 1 hr @ 75-80 °C
- Dilute sample with 1 mL 50:50 Methanol/Water
- Centrifuge for 7 min @ 3000-3500 rpm, room temp.
- Collect supernatant.

SPE Sample Preparation (THC-COOH Specific Method)

96-Well Plate:	Strata®-X-A 10 mg/well
Part No.:	8E-S123-AGB
Condition:	1 mL Methanol
Equilibrate:	1 mL Methanol/Water (25:75)
Load:	Supernatant from pre-treatment
Wash 1:	1 mL Methanol/Water (25:75)
Wash 2:	1 mL Methanol
Wash 3:	1 mL Methylene Chloride
Dry:	Cartridge at max vacuum for 4-5 minutes
Elute:	2x 500 µL 5 % Formic Acid in Methanol, transfer the eluates to glass tubes for drying step
Dry Down:	Evaporate to dryness under stream of Nitrogen at 40-45 °C
Reconstitute:	Residue with 100 µL of Methanol/Water (40:60)

Revision: 0
PHEN-RUO-00025

Screening LC Method Conditions

Column: Luna® 3µm C18 (2) MercuryMS™ LC-MS Cartridge
Dimensions: 20 x 2.0 mm
Part No: 00M-4251-B0-CE
Mobile Phase: A: 10 mM Ammonium formate in Water
B: 0.1% Formic acid in Methanol

Gradient:

Time (min)	%B
0	3
0.8	97
2	97
2.01	3
2.99	3

Flow Rate: 0.75 mL/min
Col. Temp.: Ambient
Instrument: Agilent® 1260 LC + SCIEX Triple Quad™ 4500 MS

Confirmation LC Method Conditions

Column: : Kinetex® 2.6 µm Phenyl-Hexyl
Dimensions: 50 x 3.0 mm
Part No.: 00B-4495-Y0
Mobile Phase: A: 10 mM Ammonium formate in Water
B: 0.1% Formic acid in Methanol

Gradient:

Time (min)	%B
0	3
0.5	3
4.5	97
6	97
6.1	3
7.5	3

Flow Rate: 0.75 mL/min
Col. Temp.: Ambient
Instrument: Agilent 1260 LC + SCIEX Triple Quad 4500 MS

THC-COOH Specific LC Method Conditions

Column: : Kinetex 2.6 µm Phenyl-Hexyl
Dimensions: 50 x 2.1 mm
Part No.: 00B-4495-AN
Mobile Phase: A: 0.01% Acetic acid in Water
B: 0.01% Acetic acid in Methanol

Gradient:

Time (min)	%B
0	20
0.5	20
1	60
4.5	98
5.5	98
5.6	20
6.5	20

Flow Rate: 0.5 mL/min
Col. Temp.: 45°C
Instrument: Agilent 1260 LC + SCIEX API 5000™ MS

Figure 1.
Screening Method Chromatogram

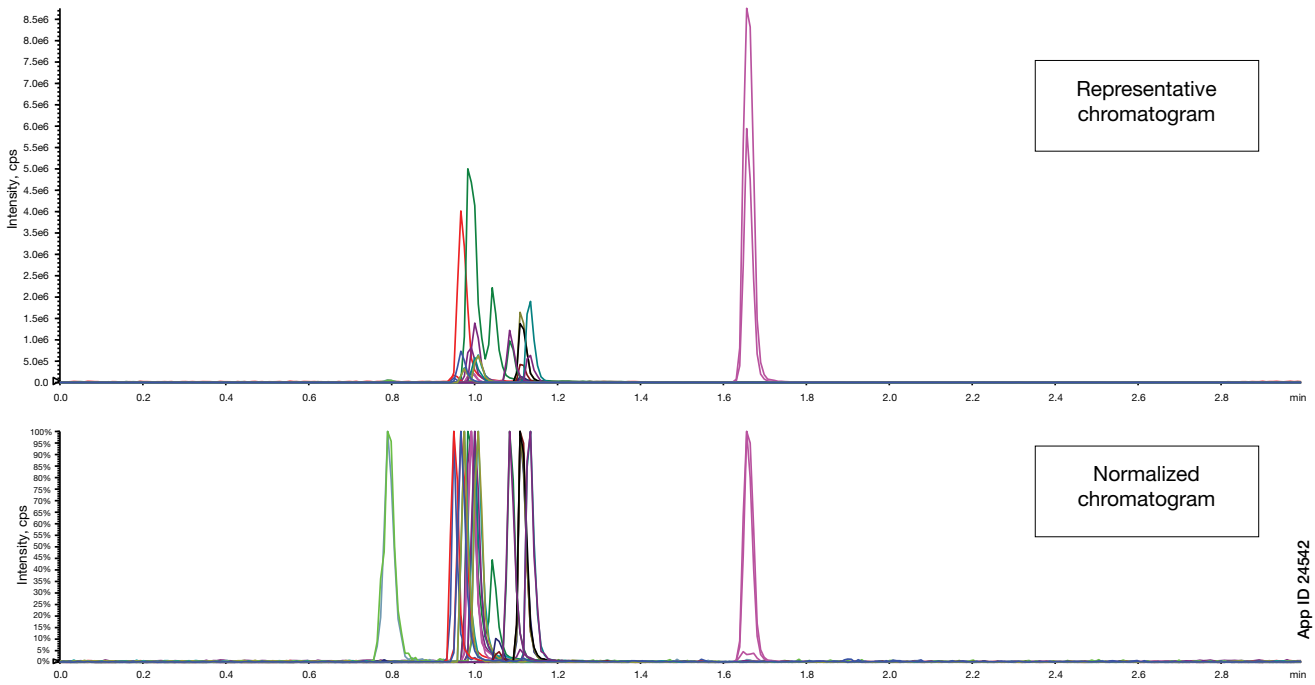


Figure 2.
Confirmation Method Chromatogram

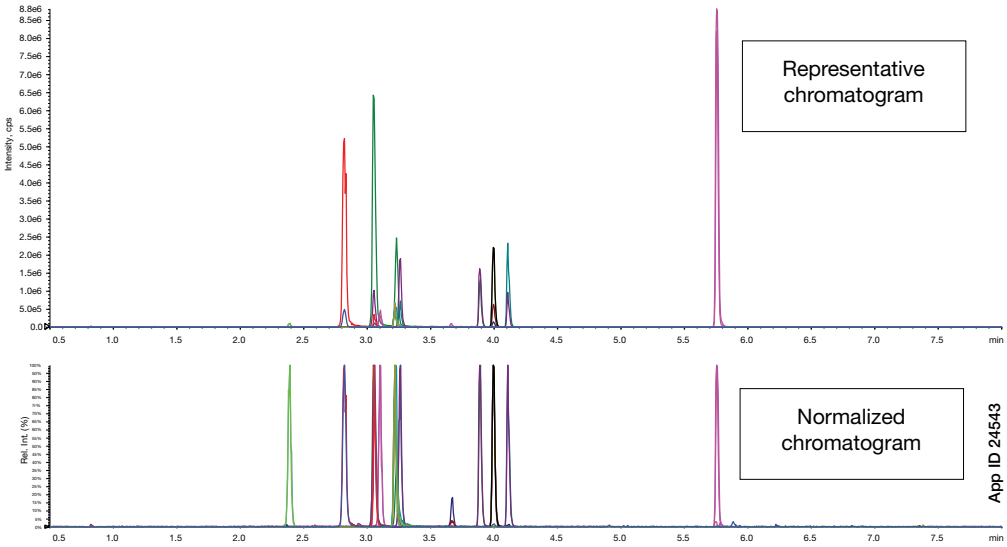


Figure 3.
THC-COOH Specific Method Chromatogram

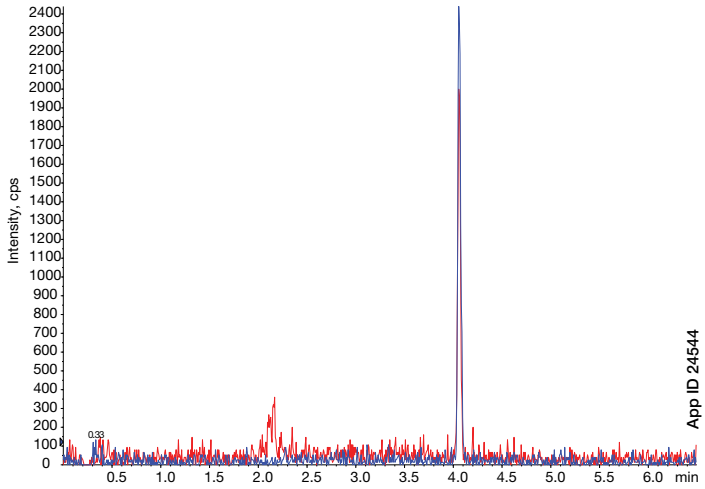


Table 1.
Analyte Retention Times

Name	Screening Method (min)	Confirmation Method (min)	THC-COOH Method (min)
Amphetamine	0.97	2.32	N/A
Methamphetamine	0.98	2.56	N/A
MDA	0.99	2.60	N/A
MDMA	1.00	2.76	N/A
Diethylpropion	1.00	2.72	N/A
Morphine	0.78	1.89	N/A
Benzoylecgonine	1.05	3.17	N/A
Norcocaine	1.11	3.49	N/A
Codeine	0.95	2.56	N/A
Cocaine	1.08	3.39	N/A
THC	1.65	5.25	N/A
Cocaethylene	1.13	3.61	N/A
6-MAM	0.97	2.72	N/A
THC-COOH	N/A	N/A	4.06

Figure 4.
Acidic & Basic Extractions for **1.6-MAM** and 2. **Cocaethylene**

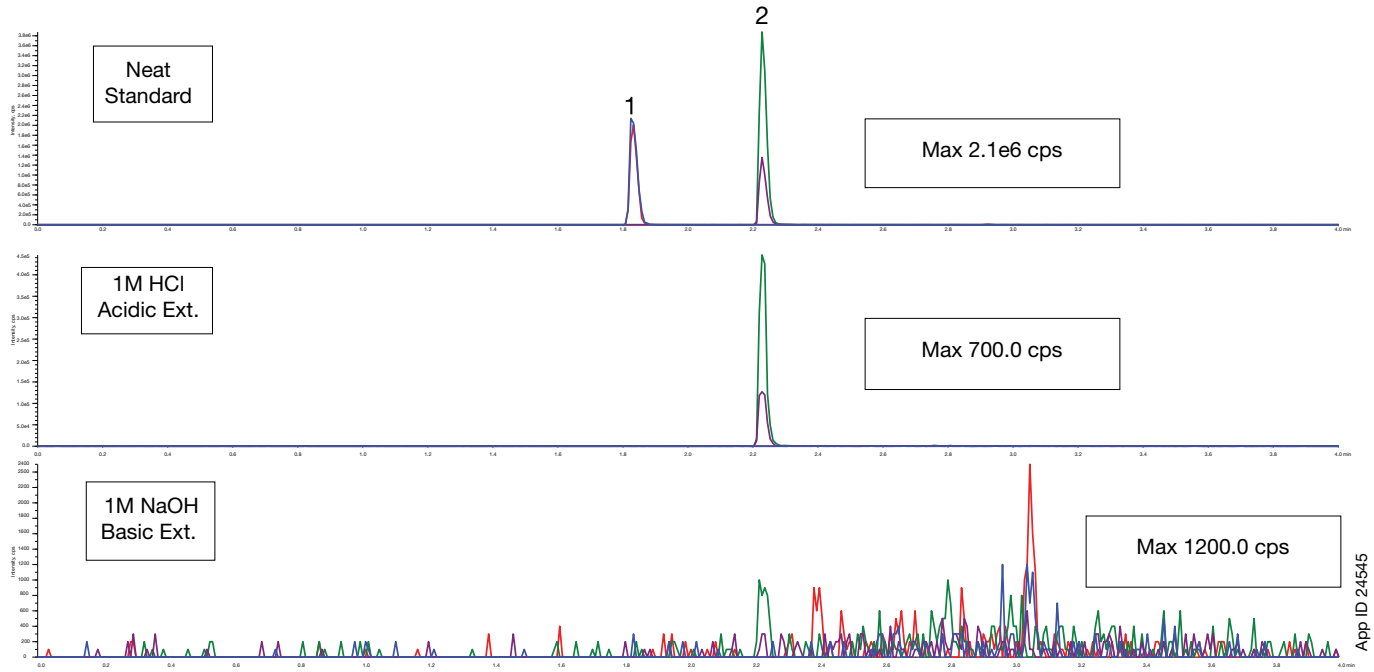


Figure 5.
Overlaid TICs of Background for Solid-Liquid Extraction Solvents

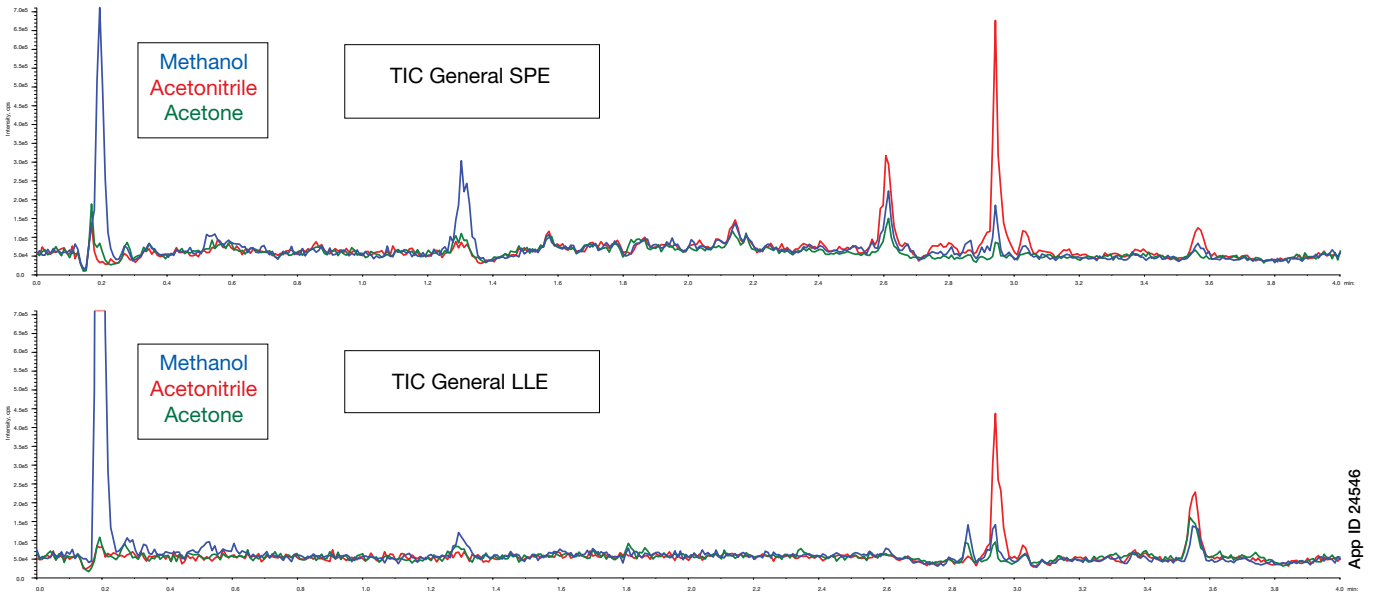


Figure 6.
Comparison of MS Transitions for SPE Extracted Solvent Spiked with Low Conc. THC-COOH

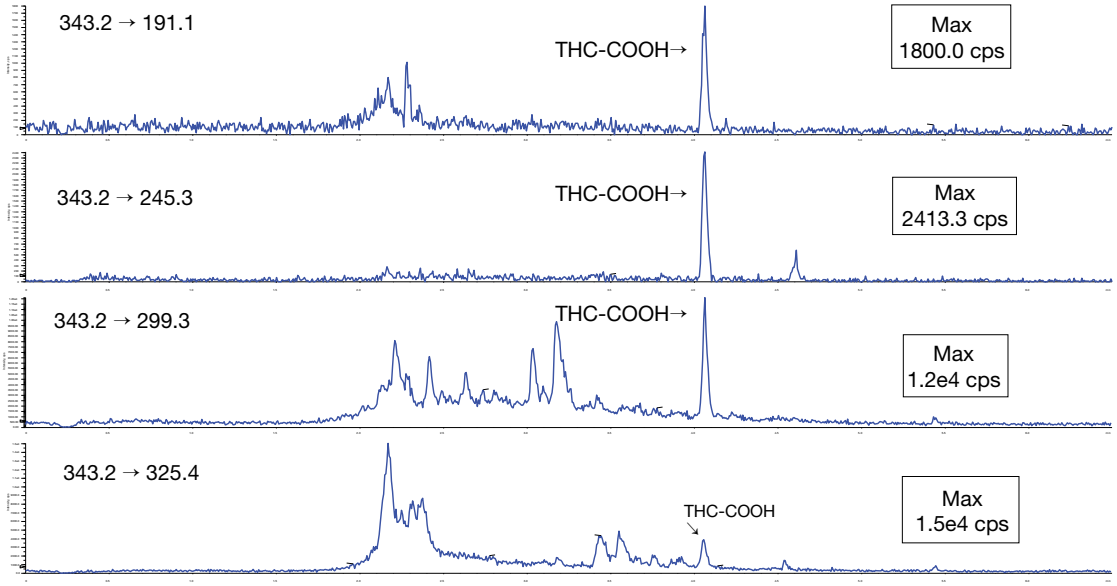


Figure 7.
LLE vs. SPE: THC-COOH Background for Extracted Black Hair

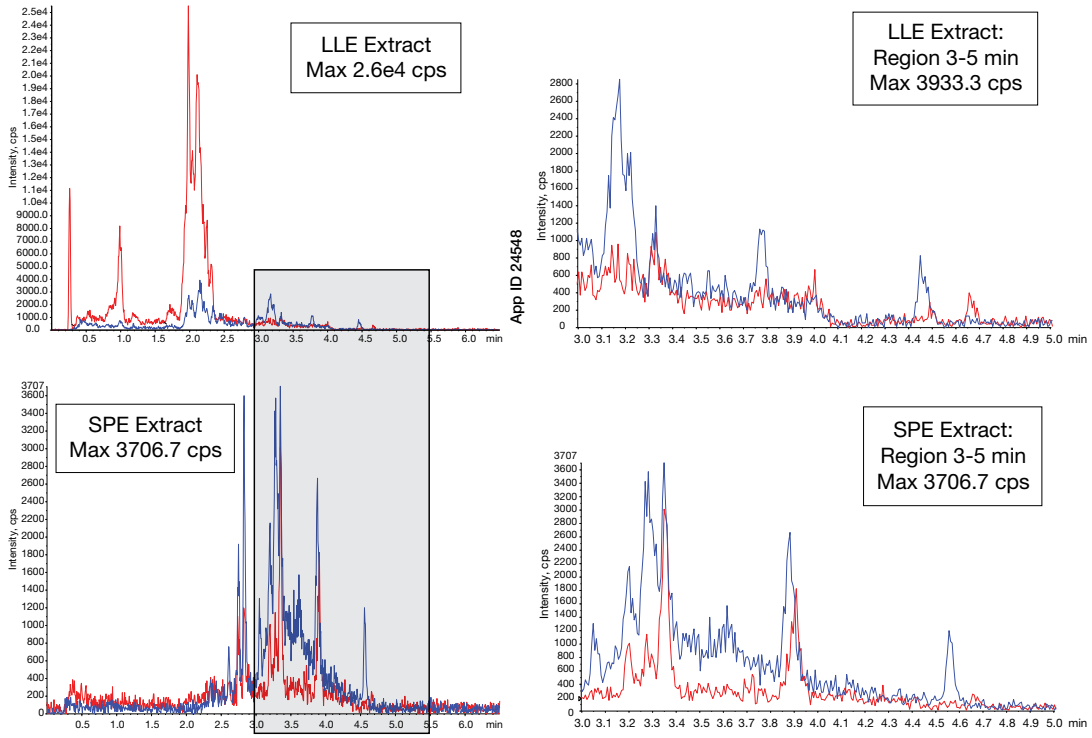


Figure 8.
THC-COOH Background for Various Hair Colors using LLE

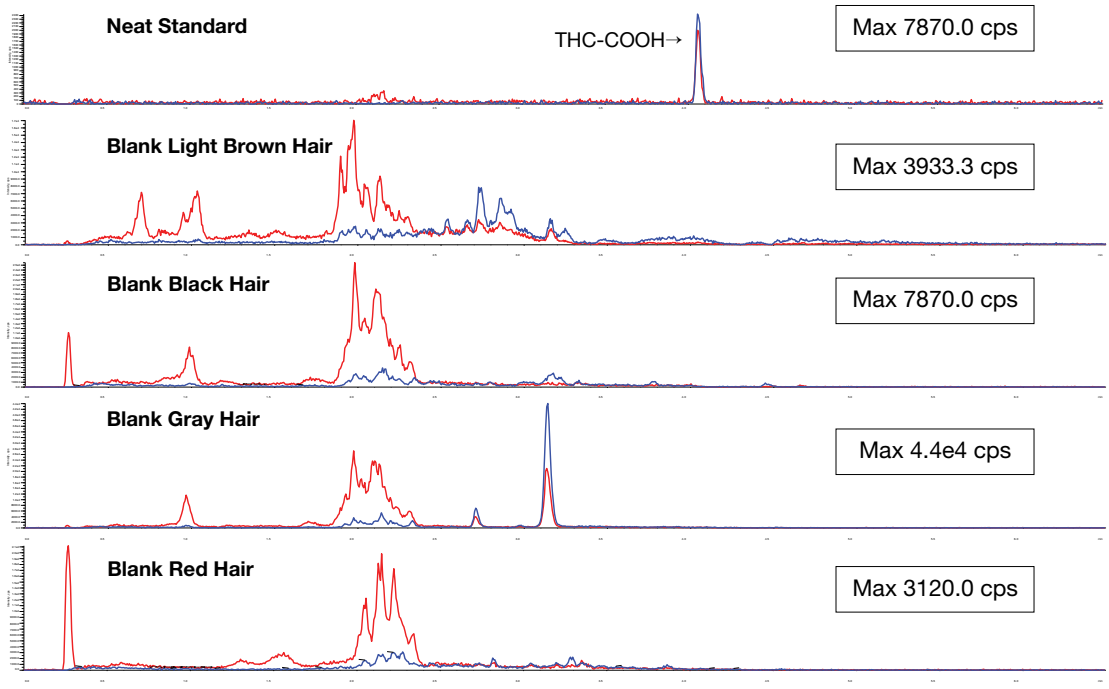
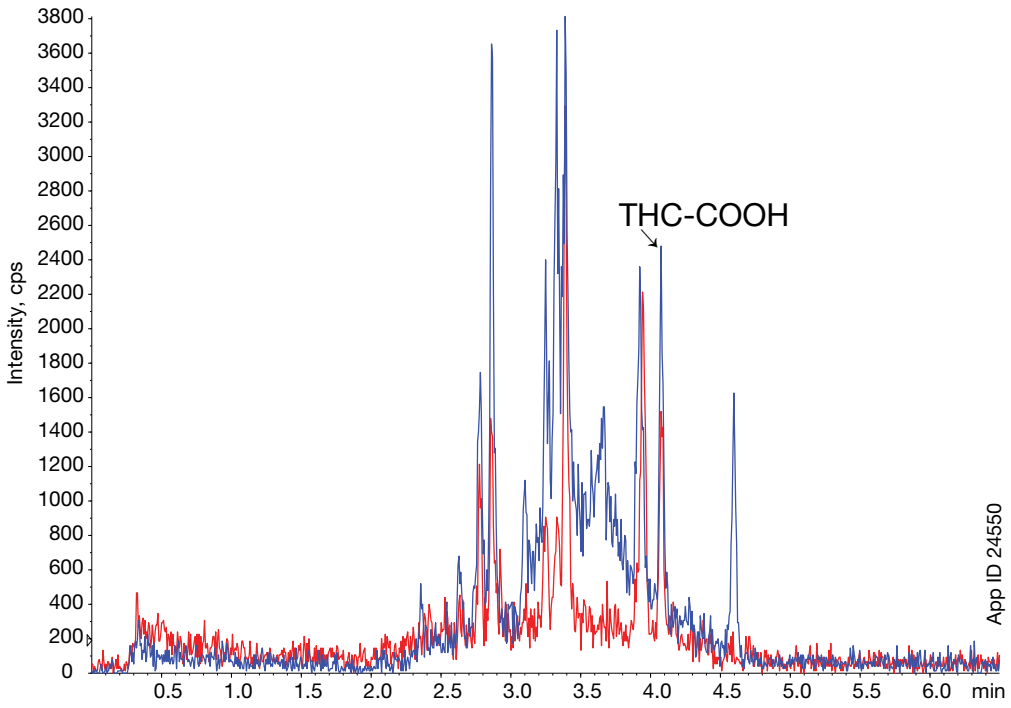


Figure 9.
SPE Extracted Black Hair, Near LOD



Results and Discussion

A 3 minute screening method was developed on the Luna® 3 µm C18(2) 20 x 2.0 mm MercuryMS™ LC-MS cartridge (**Figure 1**) and a Kinetex® 2.6 µm, 50 x 3.0 mm Phenyl-Hexyl LC column was used for a 7.5 minute confirmation method (**Figure 2**). The Kinetex 2.6 µm, 50 x 2.1 mm Phenyl-Hexyl LC column resolved THC-COOH from matrix interferences with a runtime of 6.5 minutes (**Figure 3**).

Hair samples were extracted under a variety of conditions for comparison. An acidic digestion with 1M HCl showed no recovery of 6-MAM (**Figure 4**). A basic digestion with 1M NaOH had no recovery of 6-MAM and very low recovery of cocaethylene (**Figure 4**). For a neutral solid-liquid extraction from hair, methanol, acetonitrile, and acetone were investigated as potential organic solvents. This was followed by SPE or LLE, respectively, and the TICs of the background noise were compared (**Figure 5**). Acetone extracts were found to have the lowest background and this solvent was selected for the main extraction method as it is also easily evaporated, providing both cleanliness and time advantages.

THC-COOH is present in much lower concentrations in hair than the other compounds in our panel, so although originally THC-COOH was analyzed as part of our full panel, we decided to make an optimized sample preparation and HPLC methods for this analyte alone. Four negative mode mass transitions for THC-COOH were compared for signal-to-noise (**Figure 6**). 343.2→191.1 and 343.2→245.3 were selected because of their clean background profiles. There were notable tradeoffs between selecting either LLE or SPE to follow a basic digestion with NaOH (**Figure 7**). SPE produced a cleaner sample overall, however the region near THC-COOH peak (4.06 min) is slightly less crowded for LLE. The chromatographic method effectively resolved interference peaks found in extracted light brown, black, gray, and red hair samples from THC-COOH (**Figure 8**), and interferences were resolved for SPE extracted hair samples near the limit of detection (**Figure 9**).

Conclusion

In this work we presented a simple solid-liquid extraction procedure using acetone to analyze drugs from hair, followed by screening and confirmation LC-MS/MS methods.

For THC-COOH, we were able to use a combination of MS parameter optimization, SPE or LLE, and HPLC methodologies to minimize background noise and resolve matrix interferences.

Future work should involve comparing analyte recovery of different sample preparation options and evaluating the tradeoffs between sample cleanliness and extraction efficiency.

Ordering Information

Strata®-X-A Solid Phase Extraction

Format	Sorbent Mass	Part Number	Unit
Tube			
	30 mg	8B-S123-TAK**	1 mL (100/box)
	30 mg	8B-S123-TBJ	3 mL (50/box)
	60 mg	8B-S123-UBJ	3 mL (50/box)
	100 mg	8B-S123-EBJ	3 mL (50/box)
	100 mg	8B-S123-ECH	6 mL (30/box)
	200 mg	8B-S123-FBJ	3 mL (50/box)
	200 mg	8B-S123-FCH	6 mL (30/box)
	500 mg	8B-S123-HBJ	3 mL (50/box)
	500 mg	8B-S123-HCH	6 mL (30/box)
96-Well Plate			
	10 mg	8E-S123-AGB	2 Plates/Box
	30 mg	8E-S123-TGB	2 Plates/Box
	60 mg	8E-S123-UGB	2 Plates/Box
96-Well Microelution Plate			
	2 mg	8M-S123-4GA	ea

** Tab-less tubes available. Contact Phenomenex for details.

guarantee

If Phenomenex products in this technical note do not provide at least an equivalent separation as compared to other products of the same phase and dimensions, return the product with comparative data within 45 days for a FULL REFUND.

Kinetex Core-Shell HPLC/UHPLC Columns

2.6 µm MidBore™ Columns (mm)				SecurityGuard™ ULTRA Cartridges‡
Phases	**50 x 3.0**	**100 x 3.0**	**150 x 3.0**	**3/pk**
Phenyl-Hexyl	00B-4495-Y0	00D-4495-Y0	00F-4495-Y0	AJ0-8781 for 3.0 mm ID

2.6 µm Minibore Columns (mm)			SecurityGuard ULTRA Cartridges‡
Phases	**50 x 2.1**	**100 x 2.1**	**3/pk**
Phenyl-Hexyl	00B-4495-AN	00D-4495-AN	AJ0-8788 for 2.1 mm ID

‡SecurityGuard ULTRA cartridges require holder, Part No. AJ0-9000

Luna

Luna 3 µm C18 (2) MercuryMS™ LC-MS Cartridge (mm)
20 x 2.0
00M-4251-B0-CE

MercuryMS Cartridge Holders	
Part No.	**Description**
CH0-5845	20 mm standard holder
CH0-7188	20 mm direct-connect holder

APPLICATIONS

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