APPLICATIONS

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Sample Preparation
Matt Brusius is an avid ice hockey player. He likes skating backwards and taking slapshots from the point.

Optimization of Elution Solvents for Simplified Liquid Extraction of THC and Metabolites from Urine

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Introduction

In this technical note, we develop a simplified liquid extraction (SLE) application for THC and metabolites in urine in order to identify the optimum elution solvent from 7 different elution solvent variants. Results from each extraction were then compared based on recovery as well as a general baseline cleanliness in an effort to understand which solvent(s) is most suitable for this analysis.

Materials

Δ9-Tetrahydrocannabinol (THC), 11-nor-9-Carboxy-Δ9-THC (COOH-THC), 11-Hydroxy-Δ9-THC (11-OH-THC), and 11-nor-9-Carboxy-Δ9-THC-D3 (11-OH-THC-D3) standards were purchased from Cerilliant® (Round Rock, TX). Formic acid was purchased from Sigma-Aldrich® (St. Louis, MO). HPLC-grade acetonitrile, methanol, methyl tert-butyl ether, ethyl acetate and hexane were purchased from Honeywell™ (Morris Plains, NJ). Purified water was obtained using a Sartorius® arium® comfort II filtration system (Göttingen, Germany).

Experimental Conditions

For all elution solvents, the following sample preparation, SLE, and LC conditions were followed, with the elution solvent being the only variant.

Sample Preparation

230 µL of urine was combined with 20 µL THC standards (1 µg/mL) and 200 µL of 1 % formic acid in water.

Note: When working with urine samples it is recommended to perform a hydrolysis step in place of the above sample preparation. We recommend a β-glucuronidase hydrolysis by adding 175 µL 100 mM Ammonium acetate (pH 4) and 50 µL β-glucuronidase solution (100,000 units/mL) to 200 µL of urine. Mix and vortex for 30 seconds. React at 37-40 °C for 60 minutes (with gentle shaking). Bring samples to room temperature and add 20 µL of 5M Ammonium hydroxide. Vortex/mix thoroughly.

SLE Protocol	
96-Well Plate:	Novum™ SLE MAX
Part No.:	8E-S138-5GA
Load:	Load pretreated sample and pulse vacuum (~5" Hg) for 5-10 seconds or until sample has completely entered the sorbent
Elute:	2 x 900 µL aliquots of 7 test solvents (see list below) followed by a short pulse 5" Hg to initiate flow. Allow the remaining solvent to flow via gravity. At the completion of the second aliquot, apply vacuum at 5" Hg for 15 seconds to complete the extraction
Dry down:	Evaporate eluate to dryness @ room temperature under a gentle stream of nitrogen
Reconstitute:	Reconstitute in 200 µL of Methanol/Water (50:50) containing 100 ng/mL of COOH-THC-D3

Tested Elution Solvents

1. MTBE (Methyl Tertiary Butyl Ether)
2. Ethyl Acetate
3. Hexane/Ethyl acetate (1:1)
4. Hexane/Ethyl acetate (1:3)
5. Hexane/MTBE (1:1)
6. Hexane/MTBE (1:3)
7. Hexane/Ethyl acetate (3:1)

LC Conditions

Column: Luna® Omega 1.6 µm C18
Dimensions: 50 x 2.1 mm
Part No.: 00B-4742-AN
Recommended Guard: SecurityGuard™ ULTRA Cartridges: AJ0-9502
Mobile Phase: A: 0.1 % Formic acid in Water
B: 0.1 % Formic acid in Acetonitrile

Gradient:

Time (min)	B (%)
0	65
2	100
2.10	65
4	65

Flow Rate: 0.6 mL/min
Temperature: Room temperature
Injection: 5 µL
Instrument Agilent® 1200 LC System
Detection: MS/MS API 4000™ (SCIEX), ESI+

Table 1. Average recovery and CV for seven different extraction solvents (N=3).

Elution Solvent	*THC*		*COOH-THC*		*11-OH THC*	
	Average Recovery %	*CV %*	*Average Recovery %*	*CV %*	*Average Recovery %*	*CV %*
1. MTBE	71	15	74	1	82	7
2. Ethyl acetate	85	2	86	6	79	6
3. Hexane/Ethyl acetate	62	9	81	7	90	5
4. Hexane/Ethyl acetate (1:3)	69	8	89	5	94	3
5. Hexane/MTBE (1:1)	59	10	85	6	99	6
6. Hexane/MTBE (1:3)	60	10	87	4	105	4
7. Hexane / Ethyl acetate (3:1)	58	10	85	4	101	13

Figure 1. Representative chromatogram for neat standards for THC, 11-OH-THC, COOH-THC. Peaks in order of elution: 11-OH-THC (1.01 min), COOH-THC (1.04 min), and THC (2.12 min).

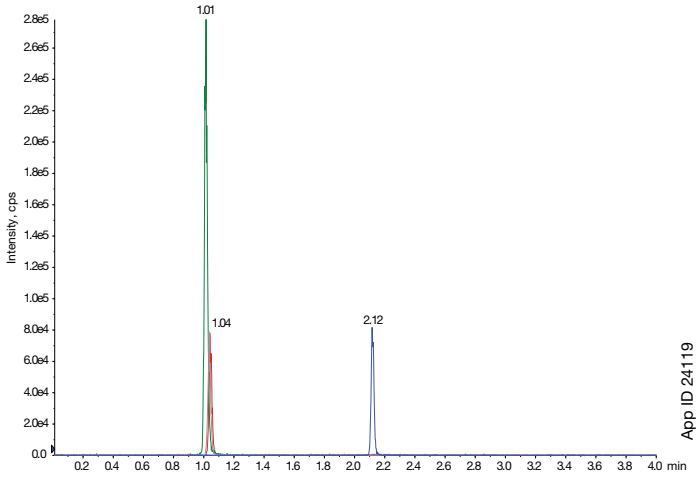


Figure 2. XIC for COOH-THC using Ethyl acetate as elution solvent. Peaks in order of elution: urine interferences (0.20 min, 0.25 min, and 0.85 min) and COOH-THC (1.05 min).

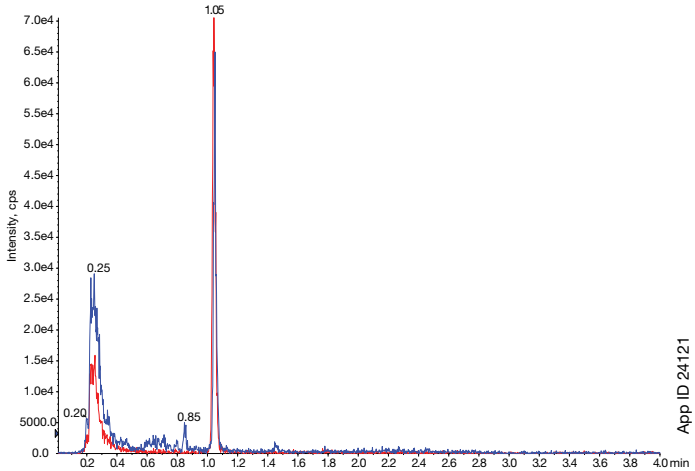
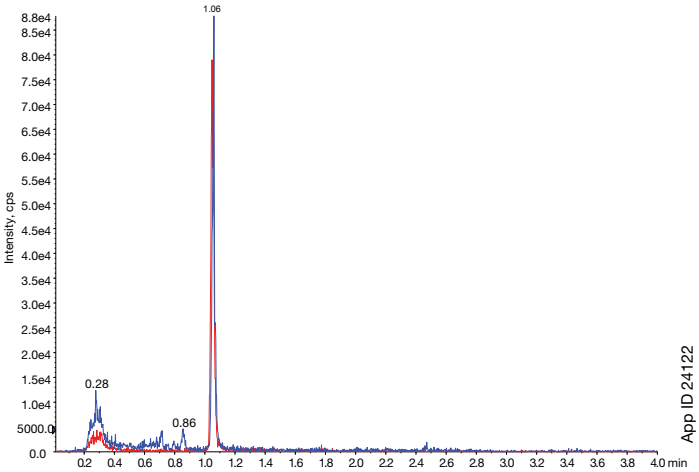


Figure 3. XIC for COOH-THC using Hexane/MTBE (1:3) as elution solvent. Peaks in order of elution: urine interferences (0.28 min and 0.86 min) and COOH-THC (1.06 min)



Results and Discussion

We first evaluated each solvent on the basis of recovery. **Table 1** shows average recovery values and coefficient of variation (CV) for three replicates. All solvents produced decent recoveries (>70 %) for COOH-THC and 11-OH-THC on the Novum™ plate. THC is generally more problematic as it is a very retentive molecule that holds to SLE sorbents stronger than other analytes. For THC, ethyl acetate appears to have the highest recovery and lowest CV. It also had above average recovery for COOH-THC and 11-OH-THC. However, a good extraction procedure should not only have high recovery, it should remove interferences. **Figure 1** is a representative chromatogram of neat standards which shows the highest possible signal-to-noise. **Figure 2** shows the extracted ion chromatogram (XIC) for COOH-THC using ethyl acetate. It shows a baseline that is a relatively dirty. By contrast, the XIC of COOH-THC extracted with Hexane/MTBE (1:3) (**Figure 3**) shows a much cleaner baseline. Additionally, its recovery for all analytes is reasonable. It also has a fast dry down time compared to ethyl acetate.

Conclusion

In this tech note we identified the best elution solvents based on analyte recovery. Next we compared top performing solvents for cleanliness. Hexane/MTBE (1:3) was determined to be the best choice based on high recoveries without sacrificing cleanliness.

Ordering Information

Luna® Omega 1.6 µm Minibore Columns (mm)					SecurityGuard™ ULTRA Cartridges‡
Phases	**30 x 2.1**	**50 x 2.1**	**100 x 2.1**	**150 x 2.1**	**3/pk**
Polar	00A-4748-AN	00B-4748-AN	00D-4748-AN	00F-4748-AN	AJ0-9505
PS C18	00A-4752-AN	00B-4752-AN	00D-4752-AN	00F-4752-AN	AJ0-9508
C18	00A-4742-AN	00B-4742-AN	00D-4742-AN	00F-4742-AN	AJ0-9502
					for 2.1 mm ID

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

Novum™ Simplified Liquid Extraction (SLE)

96-Well Plates

Part No.	Description	Unit
8E-S138-FGA	Novum SLE MINI 96-Well Plate	1/Box
8E-S138-5GA	Novum SLE MAX 96-Well Plate	1/Box

Tubes

Part No.	Description	Unit
8B-S138-FAK	Novum SLE 1 cc Tubes	100/box
8B-S138-5BJ	Novum SLE 3 cc Tubes	50/box
8B-S138-JCH	Novum SLE 6 cc Tubes	30/box
8B-S138-KDG	Novum SLE 12 cc Tubes	20/box

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.

APPLICATIONS

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