

Extraction of THC and its Metabolites from Human Nail Samples Using ISOLUTE® SLE+ Prior to UPLC-MS/MS Analysis

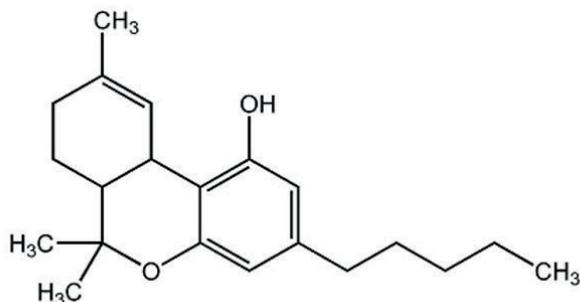


Figure 1. Structure of Δ^9 -tetrahydrocannabinol (THC).

Introduction

The testing of alternative matrices in forensic and/or clinical toxicology is gaining popularity, partly due to less invasive means of collection. Matrices such as hair or nail can provide a more rounded picture of abstinence or abuse and associated timeframes.

This application note describes a procedure for sample pre-treatment and extraction of THC and its metabolites from human nail samples, using the Biotage® Lysera for matrix pulverisation, and pre-concentration of the sample prior to clean up using ISOLUTE® SLE+ supported liquid extraction.

Manual processing protocols were developed using the Biotage® Pressure+ 96 (plate format) or 48 (column format) Positive Pressure Manifolds.

This application note contains procedures optimized for both individual column format and 96-well plate format for higher throughput applications. The methodology delivers clean extracts with analyte recoveries > 70% (plate format) or > 75% (column format) with %RSD < 10% for all analytes and LLOQ as low as 20 fg/mg of nail.

ISOLUTE SLE+ Supported Liquid Extraction columns and plates offer an efficient alternative to traditional liquid-liquid extraction (LLE) for bioanalytical sample preparation, providing high analyte recoveries, no emulsion formation and significantly reduced preparation time.

Analytes

Tetrahydrocannabinol (THC), 11-Nor-9-carboxy- Δ^9 -tetrahydrocannabinol (THC-COOH), 11-Hydroxy- Δ^9 -tetrahydrocannabinol (THC-OH), Δ^9 -tetrahydrocannabinolic acid-A (THCAA), cannabidiol (CBD), and cannabinol (CBN)

Internal Standards

Tetrahydrocannabinol -D₃ (THC- D₃), 11-Nor-9-carboxy- Δ^9 -tetrahydrocannabinol-D₃ (THC- COOH-D₃) and 11-Hydroxy- Δ^9 -tetrahydrocannabinol- D₃ (THC-OH- D₃)

These were used during the calibration process and recovery work was also done alongside the standards.

Sample Preparation Procedure

Format

ISOLUTE® SLE+ 400 μ L capacity columns (p/n 820-0055-B) or

ISOLUTE® SLE+ 400 μ L capacity 96-well plate (p/n 820-0400-P01)

Matrix Preparation

Weigh 10 mg of nail into 2 mL Biotage® Lysera tubes (p/n 19-620) containing 4 x 2.4 mm stainless steel beads (p/n 19-640).

Micropulverisation Procedure

Grind to a fine powder using Biotage® Lysera (Conditions - 8 x 6.95 m/sec for 45 seconds with a 45 second dwell.)

Add 1 mL 0.1% formic acid in methanol to each nail sample. Also add 10 μ L of a 10 ng/mL ISTD making a 10 pg/mg spike. Mix.

Centrifuge tubes for 10 minutes at 13,300 rpm (Heraeus Pico 17 Microcentrifuge (Thermo Scientific) with 24 position, 2 mL rotor).

Post Micropulverisation

Transfer a 250 μ L aliquot of supernatant into 12 x 75 mm evaporation tubes and evaporate extracts to dryness using a TurboVap® LV at 40 °C. Reconstitute in methanol:water (80:20, v/v, 250 μ L).

Supported Liquid Extraction Conditions

	ISOLUTE® SLE+ 400 µL Columns Part Number 820-0055-B	ISOLUTE® SLE+ 400 µL Plate Part number 820-0400-P01
Sample loading	Load up to 250 µL of reconstituted extract onto each ISOLUTE® SLE+ column. A pulse of pressure is not needed as the methanolic extract flows straight onto the bed. Allow the sample to absorb for 5 minutes.	Load up to 250 µL of reconstituted extract onto each ISOLUTE® SLE+ well. A pulse of pressure is not needed as the methanolic extract flows straight onto the bed. Allow the sample to absorb for 5 minutes.
Analyte Extraction	Apply MTBE (600 µL) allow to flow under gravity for 5 minutes. Apply a further aliquot of MTBE (600 µL) and allow to flow under gravity for 5 minutes. For complete removal apply a pulse of positive pressure at 10 psi (10–20 seconds).	Apply MTBE (600 µL) allow to flow under gravity for 5 minutes. Apply a further aliquot of MTBE (600 µL) and allow to flow under gravity for 5 minutes. For complete removal apply a pulse of positive pressure at 10 psi (10–20 seconds).
Collection Vessels	Collect extract in 12 x 75 mm glass tubes.	Collect extract in 96-well collection plates.
Post Elution	Evaporate extracts at 40 °C, for 30 mins at a flow rate of 1.5 L/min using a Turbovap® LV.	Evaporate extracts at 40 °C, for 30 mins at a flow rate of 20-40 L/min using the Biotage®SPE Dry-96.
Reconstitute	Reconstitute extracts in a mix of mobile phase A/mobile phase B (80:20, v/v, 200 µL). Vortex mix, transfer into a 96-well format plate and cover with a sealing mat prior to injection.	Reconstitute extracts in a mix of mobile phase A/mobile phase B (80:20, v/v, 200 µL). Vortex mix. Cover plate with a sealing mat prior to injection.

UHPLC Conditions

Instrument

Shimadzu Nexera X2 UHPLC

Column

Restek Pinnacle DB Biphenyl 1.9µm 50 x 2.1 mm combined with a Restek EXP guard holder and biphenyl guard

Mobile Phase

A: 0.01% Formic Acid (aq)

B: 0.01% Formic Acid in MeCN

Flow Rate

0.7 mL/min

Table 1. UHPLC Gradient.

Time (min)	%A	%B
0	60	40
2.5	5	95
2.6	60	40
4.1	60	40

Injection Volume

5 µL

Column Temperature

50 °C

MS/MS Conditions

Instrument

Shimadzu 8060 Triple Quadrupole MS using ES interface

Nebulizing Gas Flow

3 L/min

Drying Gas Flow

5 L/min

Heating Gas Flow

15 L/min

Interface Temperature

400 °C

DL Temperature

300 °C

Heat Block Temperature

500 °C

CID Gas Flow

270 kPa

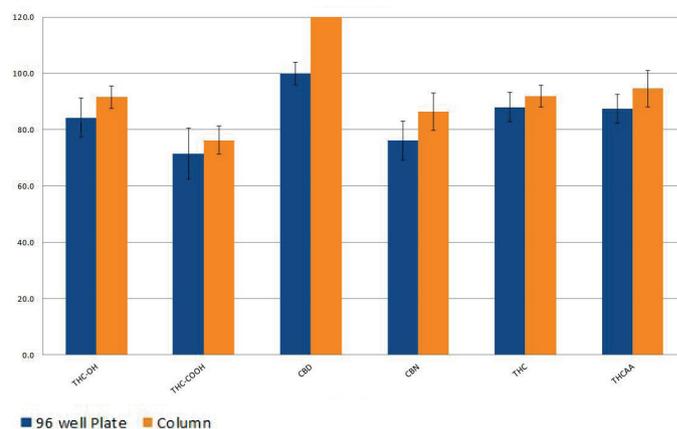
Table 2. MS conditions for target analytes in positive mode.

Analytes	MRM Transition	Collision Energy
THC-D ₃	318.0 > 196.15	-24
	318.0 > 123.2	-32
THC	315.0 > 193.10	-23
	315.0 > 123.2	-32
OH-THC-D ₃	334.0 > 316.15	-15
	334.0 > 196.25	-25
OH-THC	331.0 > 313.3	-15
	331.0 > 193.25	-26
THC-COOH-D ₃	346.3 > 302.3	22
	346.3 > 248.30	28
THC-COOH	343.3 > 299.3	22
	343.3 > 245.25	30
CBN	309.3 > 279.1	32
	309.3 > 222.05	47
CBD	315.1 > 193.1	-23
	315.1 > 123.25	-35
THCAA	357.3 > 313.3	26
	357.3 > 245.25	33

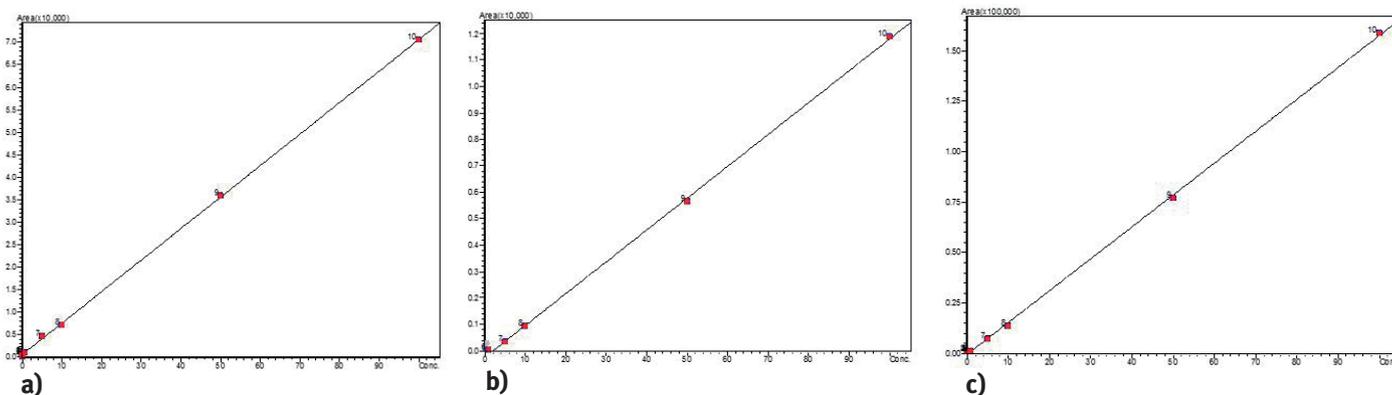
Results

This simple sample preparation method delivers clean extracts and analyte recoveries mostly greater than 75% with RSDs lower than 10% for all analytes (see fig 2). LLOQs are below 10 pg/mg and as low as 20 fg/mg for THCA-A (see table 3) for ISOLUTE® SLE+ column formats used.

Recoveries

**Figure 2.** Representative analyte recoveries.

Calibration Curves

**Figure 5.** Calibration curves for THC (a), CBN (b) and THCA-A (c) using human nail with 400 µL capacity column format (loading 250 µL of extracted sample as described).

Calibration curve performance was investigated from nails spiked between 0.01–100 pg/mg. Good linearity was observed for all analytes typically delivering r^2 values greater than 0.999. Table 3. details linearity performance and associated LOQ for each analyte using both ISOLUTE® SLE+ formats.

Table 3. Analyte calibration curve r^2 and LOQ performance.

Analyte	r^2	LLOQ (pg/mg)	r^2		LLOQ (pg/mg)	
			Column Format	Plate Format	Column Format	Plate Format
THC	0.9997	5	0.9998	10		
OH-THC	0.9997	5	0.9998	10		
THC-COOH	0.9995	1	0.9997	1		
CBN	0.9995	1	0.9992	1		
CBD	0.9992	1	0.9992	1		
THCAA	0.9995	0.02	0.9997	0.05		

Chemicals and Reagents

- » Methanol (LC-MS grade), Acetonitrile (Gradient MS) were purchased from Honeywell Research Chemicals (Bucharest, Romania).
- » All analyte standards and deuterated internal standards, and acetic acid (99.7%) (LC-MS grade) were purchased from Sigma-Aldrich Company Ltd. (Gillingham, UK).
- » Water used was 18.2 MOhm-cm, drawn daily from a Direct-Q5 water purifier.
- » Mobile phase A (0.01% Formic acid aq) was prepared by adding 50 µL acid to 500 mL of purified water.
- » Mobile phase B (0.01% Formic acid in acetonitrile) was prepared by adding 50 µL acid to 500 mL of HPLC grade acetonitrile.
- » Internal standards (10 pg/mg) were prepared from a 1 ng/µL stock solution by adding 10 µL of each of to 970 µL of MeOH. This makes a 10 ng/mL stock and then 10 µL of this solution was added to each calibration sample.

Additional Information

- » All data shown in this application note was generated using various fingernail samples, provided by healthy human volunteers.
- » THC and some of its metabolites bind to plastic, so in order to increase recoveries and reduce RSDs the pre-concentration evaporation steps should always be performed in glass tubes.
- » Biotage® Lysera hints and tips
 - » A minimum of four tubes must be loaded in the tube carriage to ensure balance during processing.
 - » Ensure vial caps are firmly tightened and Lysera locking mechanism is fully engaged.
 - » To minimize sample transfer and manipulation steps, 2 mL Lysera tubes were placed directly into the centrifuge (Heraeus Pico 17 Microcentrifuge (Thermo Scientific) with 24 position, 2 mL rotor).

Conclusion

This application note demonstrates that ISOLUTE® SLE+ columns or plates can be used in conjunction with Biotage® Lysera to provide a simple but effective sample preparation procedure for determination of THC and metabolites from human nail samples.

Ordering Information

Part Number	Description	Quantity
19-060	Biotage® Lysera	1
19-649	2 mL Reinforced Tubes with Screw Caps (Bulk pack)	1000
19-640	2.4 mm Metal Beads 500 grams	1
820-0055-B	ISOLUTE 400 µL Sample Volume Columns	50
820-0400-P01	ISOLUTE SLE+ 400 µL Capacity Plate	1
PPM-96	Biotage® PRESSURE+ 96 Positive Pressure Manifold	1
PPM-48	Biotage® PRESSURE+ 48 Positive Pressure Manifold	1
415000	TurboVap® LV	1
SD-9600-DHS-EU	Biotage® SPE Dry 96 Sample Concentrator system 220/240V	1
SD-9600-DHS-NA	Biotage® SPE Dry 96 Sample Concentrator system 100/120V	1
121-5203	Collection Plate, 2 mL Square	50
121-5204	Piercable Sealing Mat	50
C44651	Test Tubes (12 x 75 mm, Uncap)	1000

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