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

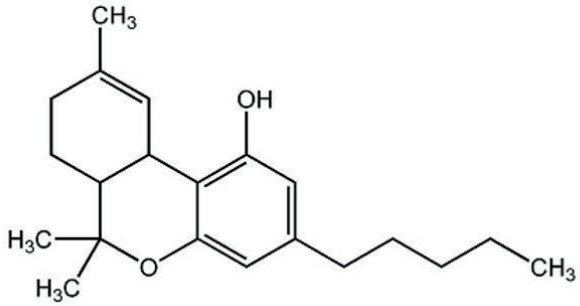


**Figure 1.** Structure of $\Delta^9$-tetrahydrocannabinol (THC).

## 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-$\Delta^9$-tetrahydrocannabinol (THC-COOH), 11-Hydroxy-$\Delta^9$-tetrahydrocannabinol (THC-OH), $\Delta$9-tetrahydrocannabinolic acid-A (THCAA), cannabidiol (CBD), and cannabinol (CBN)

## Internal Standards

Tetrahydrocannabinol –$D_3$ (THC- $D_3$), 11-Nor-9-carboxy-$\Delta^9$-tetrahydrocannabinol-$D_3$ (THC- COOH-$D_3$) and 11-Hydroxy-$\Delta^9$-tetrahydrocannabinol- $D_3$ (THC-OH- $D_3$)

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

## Sample Preparation Procedure

### Format

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

ISOLUTE® SLE+ 400 µL 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 µL 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 Micropulverisartion

Transfer a 250 µL 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 µL).



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## 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 1.9um 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<br>318.0 > 123.2 | -24<br>-32 |
| THC | 315.0 > 193.10<br>315.0 > 123.2 | -23<br>-32 |
| OH-THC-$D_3$ | 334.0 > 316.15<br>334.0 > 196.25 | -15<br>-25 |
| OH-THC | 331.0 > 313.3<br>331.0 > 193.25 | -15<br>-26 |
| THC-COOH-$D_3$ | 346.3 > 302.3<br>346.3 > 248.30 | 22<br>28 |
| THC-COOH | 343.3 > 299.3<br>343.3 > 245.25 | 22<br>30 |
| CBN | 309.3 > 279.1<br>309.3 > 222.05 | 32<br>47 |
| CBD | 315.1 > 193.1<br>315.1 > 123.25 | -23<br>-35 |
| THCAA | 357.3 > 313.3<br>357.3 > 245.25 | 26<br>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

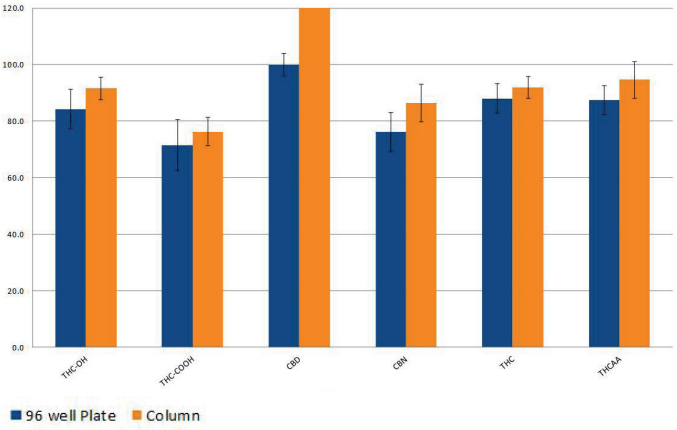


**Figure 2.** Representative analyte recoveries.

## Calibration Curves

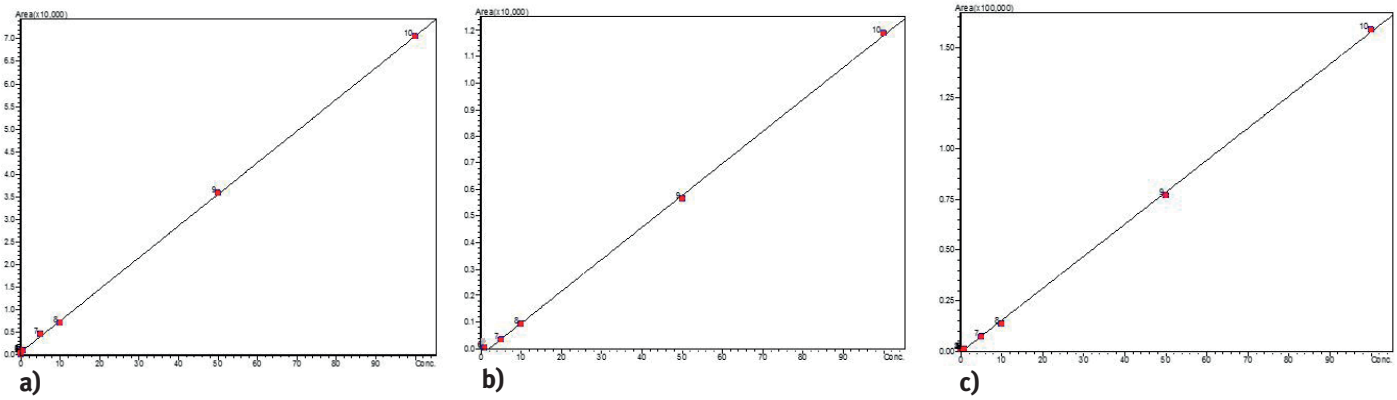


**Figure 5.** Calibration curves for THC (a), CBN (b) and THCA-A (c) using human nail with 400 µL capacity column format (loading 250 µL of extracted sample as described).

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 | |
| 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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