Simplified Sample Preparation for Low Level Determination of Cannabis Use from Hair Samples Prior to LC-MS/MS Analysis

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Introduction

Hair analysis is growing in popularity due to the non-invasive nature of the sample collection. Although not used as routinely as other matrices such as blood or urine it does have advantages in that the matrix can indicate prolonged drug exposure. This can provide valuable information with respect to therapeutic drug regimens or in abused drug abstinence cases. The low level detection required for cannabis use combined with the complexity of hair testing makes for a challenging application. This poster aims to demonstrate simplified sample preparation workflow for low level analysis of THC from hair.

Experimental

Reagents

Drug standards were purchased from LGC Standards (Teddington, UK). Acetic and formic acid were purchased from Sigma-Aldrich Company ltd. (Gillingham, UK). HPLC and LC/MS grade solvents were from Honeywell Research Chemicals (Bucharest, Romania). Water (18.2 MΩ.cm) was drawn fresh daily from a Direct-Q 5 water purifier (Merck Millipore, Watford, UK). Hair samples were kindly donated by healthy human volunteers.

Sample Preparation

ISOLUTE® SLE+ Procedure (*Figure 1.*)

ISOLUTE® SLE+ 400 µL capacity 96-well plates or columns.

Matrix Preparation:

Weigh 20 mg of hair into 2 mL Biotage® Lysera tubes containing 4 x 2.4 mm stainless steel beads. Add 1 mL of methanol to each hair sample. Internal standard added at 1 pg/mg of hair.

Micropulverisation (MPE) Procedure:

Biotage® Lysera: 3 x 1 minute cycles with 20s dwell at 5.3 m/sec.

Centrifuge extracts for 10 minutes at 13,300 rpm.

Sample Application:

200 µL of supernatant was transferred and evaporated using either: SPEdry (plate processing) or TurboVap® LV (column processing) at 40 °C. Samples were reconstituted in 200 µL 70:30 Methanol:Water and applied to the column using gravity flow.

Analyte Extraction:

MTBE (600 µL) was applied and allowed to flow under gravity for 5 minutes. A second aliquot of MTBE (600 µL) was applied and allowed to flow under gravity for 5 minutes. A pulse of positive pressure at 10 psi (10-20 seconds) allowed complete removal of the final aliquot.

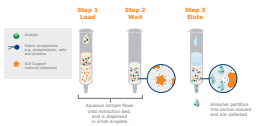


***Figure 1**. Schematic of ISOLUTE® SLE+ Supported Liquid Extraction Procedure.*

Post extraction: Extracts were evaporated at 40 °C and reconstituted with 200 µL of 70:30 Mobile phases A:B.

UHPLC Conditions

Instrument: Shimadzu Nexera x2 UHPLC (Shimadzu Europa GmbH, Duisburg, Germany)
Column: ACE EXCEL C18 50 x 2.1 mm, 1.7 µm + guard (ACT, UK)
Mobile phase: 0.01% Acetic Acid in both (aq) and MeOH
Flow rate: 0.3 mL/min **Gradient:** Shown in ***Table 1***.
Column temp: 50 °C **Injection volume:** 5 µL

***Table 1.** Gradient Parameters.*

Time (min)	% A	% B
0	50	50
0.5	20	80
2	10	90
4	10	90
4.01	50	50

Mass Spectrometry

Instrument: Shimadzu 8060 Triple Quadrupole mass spectrometer equipped with an ES interface for mass analysis (Shimadzu Europa GmbH, Duisburg, Germany). Positive or negative ions were acquired in the MRM mode (***Table 2***).
Heat Block Temp: 500° C **Interface Temp:** 400° C **DL Temp:** 300° C
Nebulizing Gas: 3 L/min **Drying Gas:** 5 L/min **Heating Gas:** 15 L/min
CID Gas: 270 kPa

***Table 2.** MRM Parameters (qual ions in parenthesis).*

Analyte	Transition (MRM)	Ionization Mode	Collision Energy (eV)
THC-D3	318.0 > 196.15 (318.0 > 123.2)	+	-24 -32
THC	315.0 > 193.10 (315.0 > 123.2)	+	-23 -32
THC-OH-D3	334.0 > 316.15 (334.0 > 196.25)	+	-15 -25
THC-OH	331.0 > 313.3 (331.0 > 193.25)	+	-15 -26
THC-COOH-D3	346.3 > 302.3 (346.3 > 248.3)	-	22 28
THC-COOH	343.3 > 299.3 (343.3 > 245.25)	-	22 30
CBN	311.0 > 223.0 (311.0 > 241.2)	+	-22 -17
CBD	313.2 > 245.15 (313.2 > 179.25)	-	24 20
THCAA	357.3 > 313.3 (357.3 > 245.25)	-	26 23

Results

Figure 2. illustrates the work-flow options investigated for hair analysis in our study. MeOH was used to swell hair samples allowing drug release when pulverization is incorporated. Aliquots were then either pre-concentrated prior to extraction or directly loaded.

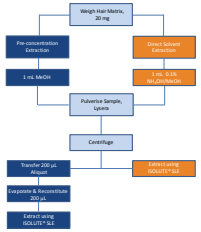


***Figure 2.** Workflow investigation for hair analysis.*

Initial investigation of the THC panel involved evaluation of non-specific binding during evaporation. As demonstrated in ***Figure 3.*** non-specific binding to collection plates was reduced by increasing methanol to above 50% during the reconstitution step.

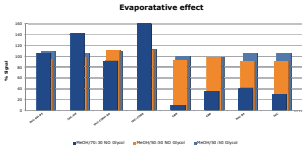


***Figure 3.** Non-specific binding profiles during evaporation from 96-well collection plates.*

Figure 4. demonstrates elution solvent performance for the analyte pre-concentration protocol. MTBE gave good reproducibility and recoveries as well as demonstrating the best matrix factors and overall signal.

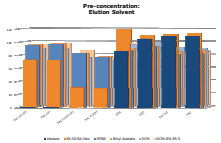


***Figure 4.** Recovery profiles from pre-concentration of pulverized hair prior to extraction.*

Both pre concentrated and direct sample protocols were considered. However, direct loading did not provide the required sensitivity to provide an LLOQ of 200 fg/mg. ***Figures 5 and 6.*** demonstrate recovery and signal profiles for pre-concentrated and direct sample loading protocols, respectively.

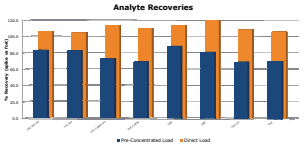
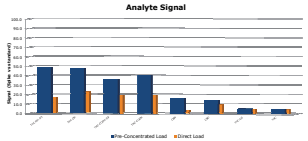


***Figure 5.** Recovery profiles comparing pre-concentrated and direct sample loads.*

***Figure 6.** Signal profiles comparing pre-concentrated and direct sample loads.*



Method performance was replicated using the ISOLUTE® SLE+ 400 µL capacity columns as shown below in ***Figure 7***.

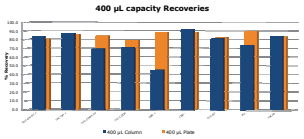


***Figure 7.** Recovery profiles comparing 400 µL capacity columns and fixed-well plates.*

0.01% Acetic acid was found to be the best LC mobile phase additive for sensitivity in both positive and negative ion. ***Figure 8***. demonstrates the chromatography achieved using 0.01% acetic acid.

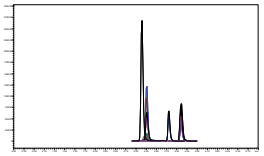


***Figure 8.** TIC using 0.01% acetic acid mobile phase composition injecting cannabis mix at a concentration of 100 pg/mg.*

Calibration curves constructed from 0.1-200 pg/mg of hair demonstrated good linearity for all analytes, returning coefficients of determination (r^2) greater than 0.99. ***Figure 9.*** demonstrates typical calibration curves for pre-concentrated hair extracts using 2 aliquots of MTBE as the elution solvent.

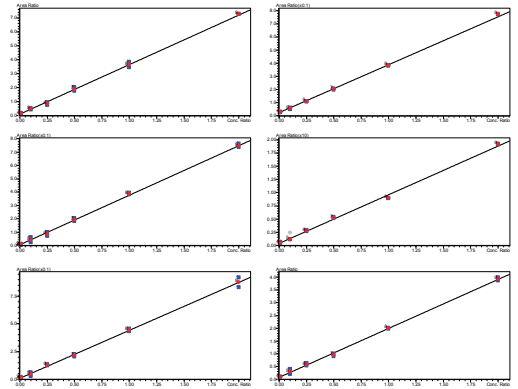


***Figure 9.** Calibration curves for THC, THC-OH, THC-COOH, CBN, CBD and THCAA respectively using pre-concentrated hair solvent extracts.*

Lower limits of quantitation (LLOQ) for the final method are summarized in ***Table 3.***

***Table 3.** Cannabis analyte LLOQ values.*

Drug Analyte	r2 (column format)	LLOQ (pg/mg) (column format)	r2 (plate format)	LLOQ (pg/mg) (plate format)
THC	0.997	10	0.998	10
OH-THC	0.997	10	0.998	10
THC-COOH	0.997	0.2	0.997	0.2
CBN	0.997	10	0.997	10
CBD	0.997	1	0.995	0.5
THCAA	0.996	1	0.995	<10

Conclusion

- This poster describes a simplified workflow for the low level analysis of cannabis from hair, utilizing bead based micro pulverization (MPE).
- Suitable cleanup and concentration of hair extracts allowed LLOQs below SoHT guidelines.

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