Comparison of Sample Preparation Approaches for the Extraction of 11-nor-9-carboxy-Δ⁹-THC from Urine Prior to GC/MS Analysis

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Introduction

GC/MS is still a mainstay in forensic analysis for drugs of abuse testing in urine. Historically silica-based solid phase extraction (SPE) columns have been used for these target analytes, the exact choice being dependent on drug functionality. The primary urinary target to prove cannabis using is the metabolite 11-nor-9-carboxy-**Δ9**-tetrahydrocannabinol. Clean up for this analyte prior to GC/MS analysis has traditionally been performed using silica-based mixed-mode anion exchange SPE columns. This poster aims to compare various sample preparation techniques for this analysis. We will aim to compare technique simplicity, streamlined workflow advantages and overall method performance using silica-based mixed-mode SPE, polymer-based SPE, both mixed-mode and reversed phase and supported liquid extraction (SLE).

Experimental

Reagents

Drug standards were purchased from LGC Standards (Teddington, UK). Sodium hydroxide, formic and acetic acids and GC derivatizing agents were purchased from Sigma-Aldrich (Dorset, UK). Negative urine was kindly donated by healthy human volunteers. All solvents were HPLC grade from Fisher Scientific (Loughborough, UK) and Milli-Q (Merck Millipore, Germany) water used throughout.

Sample Preparation

ISTD-d_9: Add 20 ng internal standard per 1 mL of urine.

Urine Hydrolysis: Add 5**0 µL of 10N** NaOH per 1 mL of urine.

Urine specimens were heated for 20 minutes at 60 °C. Samples were left to cool and pH adjusted depending on extraction mechanism.

Extractions were developed using supported liquid extraction, silica-based mixed-mode anion exchange and polymer-based SPE (reversed-phase and mixed mode) in column format.
ISOLUTE® SLE+ was used in the 1 mL capacity 6 mL column format (P/N 820-0140-CG) following a load-wait-elute procedure (***Figure 1***).

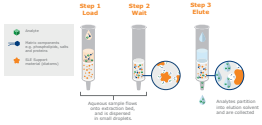

Figure 1. *Schematic of ISOLUTE® SLE+ Supported Liquid Extraction Procedure.*

EVOLUTE® EXPRESS ABN (P/N 610-0006-B) and EVOLUTE® EXPRESS AX (P/N 613-0006-B) 60mg/3mL columns.

ISOLUTE® HAX (P/N 903-0020-B) 200mg/3mL columns.

Figure 2. *Schematic of a Typical SPE Procedure.*

Full method optimization was performed for each sample preparation technique with final extraction protocols shown in ***Table 1***.

Table 1. *Optimized Extraction Protocols.*

Step	ISOLUTE® HAX	EXPRESS® ABN L-W-E	EXPRESS® AX L-W-E	ISOLUTE® SLE+
Condition	MeOH 3 mL	-	-	-
Equilibrate	Water 3 mL	-	-	-
Equilibrate	0.1 M NaOAc pH 3 (aq) 1mL	-	-	-
Urine		2 mL		1 mL
Post hydrolysis pH	1 mL acetic acid		-	60 µL acetic acid
Sample load	3.1 mL	3.1 mL	2.1 mL	1mL
Wash 1	H_2O 2 mL	H_2O 2 mL	H_2O 2 mL	-
Wash 2	95/5 0.1 M HCL/ACN 2 mL	H_2O 2 mL	H_2O 2 mL	-
Dry	***10 minutes***	-	-	-
Wash 3	Hexane 0.2 mL	-	MeOH 2 mL	-
Dry		***5 minutes***		-
Elution	50/50 Hexane/EtOAc 3 mL	50/50 Hexane/EtOAc 1 mL	1% Formic acid 50/50 Hexane/EtOAc 1 mL	50/50 Hexane/EtOAc 2 x 2.5 mL

Post extraction: Extracts were evaporated at 40 °C and reconstituted with 20 µL of ethyl acetate and 20 µL of BSTFA/1%TMCS. Derivatization was performed at 70 °C for 25 minutes prior to cooling and subsequent injection into the GC/MS.

GC/MS Conditions

GC: 7890A GC with QuickSwap (Agilent Technologies Inc.)
Column: Restek Rxi-5ms 30m x 0.25 ID x 0.25 µm

Carrier Gas: Helium 1.2 mL/min (constant flow)
Inlet: Splitless, purge flow at 50 mL/min at 1 min. Temp: 280 °C;
Injection volume: 2 **µL**
Oven conditions: Initial 125 °C, ramp 50 °C/min to 300 °C; hold 2.5 minutes, ramp 50 °C/min to 330 °C; hold for 1.4 minutes
Backflush: 2 void volumes
(Run time is 8 mins with an injection to injection time of 14 mins)
Transfer Line: 280 °C
MS: 5975C MSD (Agilent Technologies Inc.).
Source Temperature: 230 °C
Quadrupole Temperature: 150 °C
Monitored Ions: EI. signals were acquired using selected ion monitoring (SIM), as shown in ***Table 2.***

Table 2. *MS acquisition parameters.*

SIM Group	Analyte	Target (Quant) Ion	1st Qual Ion	2nd Qual Ion
1	THC-COOH-d_9	380	479	
2	THC-COOH	371	473	488

Results

Previous SLE+ work illustrated the requirement for post-hydrolysis pH control (data not shown). ***Figure 3.*** demonstrates elution solvent investigation for SLE+ extraction post acidification.

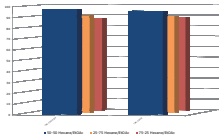

Figure 3. *THC-COOH Recovery profiles investigating elution solvent options with ISOLUTE® SLE+.*

EtOAc combinations provided optimum performance. Final selection was based on recovery, RSDs and extract cleanliness.

In order to streamline processing times, solvent use and waste SPE methods were developed using polymer based reversed phase SPE' EVOLUTE EXPRESS ABN and mixed-mode anion exchange, EVOLUTE AX. Initial SPE method development involved conversion of existing silica-based mixed-mode methods to polymer-based alternatives. ***Figure 4.*** demonstrates initial comparison data using detailed HAX method from ***Table 1.*** Direct conversion to ABN resulted in acceptable performance but less so for the AX chemistry.

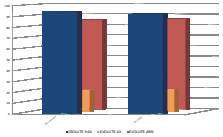

Figure 4. *Recovery profiles of THC-COOH comparing ISOLUTE HAX with EVOLUTE EXPRESS ABN and AX sorbents.*

Each chemistry was investigated and protocols optimized for recovery performance and cleanliness. Due to the water wettability of polymer-based SPE, methods were converted to the "Load-Wash-Elute" methodology. Recovery performance for final simplified methods detailed from ***Table 1.*** are demonstrated in ***Figure 5.***.

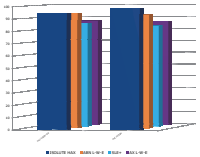

Figure 5. *Recovery profiles of THC-COOH from EVOLUTE EXPRESS ABN, EVOLUTE EXPRESS AX and ISOLUTE SLE+*

Calibration curves constructed in urine from 1-100 ng/mL demonstrated good linearity with all techniques returning coefficients of determination (r^2) greater than 0.999.

Figure 6. demonstrates calibration curve performance following extractions using ISOLUTE® SLE+, EVOLUTE® EXPRESS ABN and EVOLUTE® EXPRESS AX.

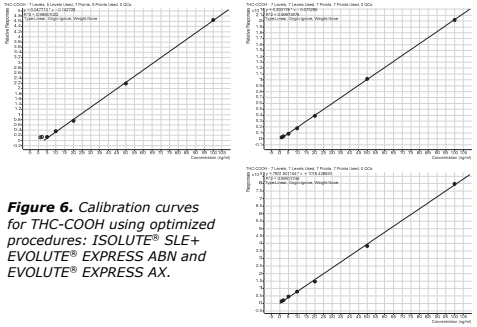

Figure 6. *Calibration curves for THC-COOH using optimized procedures: ISOLUTE® SLE+ EVOLUTE® EXPRESS ABN and EVOLUTE® EXPRESS AX.*

Figure 7. demonstrates SIM chromatograms for each technique: ISOLUTE® SLE+ at 10 ng/mL; EVOLUTE® EXPRESS ABN at 2 ng/mL and EVOLUTE® EXPRESS AX at 1 ng/mL according to LOQs, respectively.

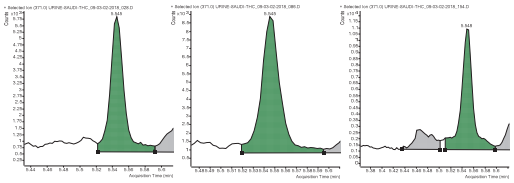

Figure 7. *SIM chromatograms for LOQ determination: ISOLUTE® SLE+ 10 ng/mL; EVOLUTE® EXPRESS ABN 2 ng/mL; and EVOLUTE® EXPRESS AX 1 ng/mL.*

Table 5. shows some efficiency gains between the various approaches. Simply moving from traditional SPE processing required for silica-based sorbents to the modified load-wash-elute protocol saves considerable time. Drying times when moving between immiscible solvents can also be a challenge with drying times being dependent on sorbent characteristics. One added benefit of polymer-based SPE is the ability to minimize bed size, resultant wash and elution volumes. The latter allows elution directly into GC vials eliminating the need to concentrate from larger tubes, transfer then concentrate prior to final derivatization.

Table 5.*Evaluation of efficiency gains with various sample preparation techniques.*

Processing Step	Traditional SPE ISOLUTE HAX	EVOLUTE® EXPRESS Load-Wash-Elute		ISOLUTE SLE+
		ABN	AX	
Bed Mass	200 mg	60 mg	60 mg	-
Processing Steps	8	4	5	3
Waste Solvent: water miscible	14.1 mL	7.1 mL	8.1 mL	-
Waste Solvent: water immiscible	0.2 mL	-	-	-
Column drying	11 minutes	5 minutes		Wait time 5 minutes
Total extraction time 48 samples	**55 minutes**	**30 minutes**	**33 minutes**	**26 minutes**
Evaporation Steps	2	1	1	2
Evaporation time	**25 minutes**	**10 minutes**		**25 minutes**

Conclusion

- This poster demonstrates a range of approaches for the extraction and cleanup of THC-COOH metabolite from hydrolyzed urine.
- Good extraction efficiency, %RSD and extract cleanliness were afforded for all sample preparation options.
- Calibration curves demonstrated excellent linearity, and r2 values > 0.99 for all three products.
- Streamlined approaches have been presented in order to save time, cost, solvent/reagent use and associated waste disposal all of which add to the overall assay cost.

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