Extraction of Δ⁹-THC, 11-hydroxy-Δ⁹-THC and 11-nor-9-carboxy-Δ⁹-THC from Whole Blood and 11-nor-9-carboxy-Δ⁹-THC from Urine using Supported Liquid Extraction (SLE) Prior to GC/MS Analysis

Katie-Jo Teehan[1], Rhys Jones[1], Lee Williams[1], Helen Lodder[1], Geoff Davies[1], Adam Senior[1], Alan Edgington[1], Claire Desbrow[1], Paul Roberts[1], Victor Vandell[2].

[1]Biotage GB Limited, Distribution Way, Dyffryn Business Park, Ystrad Mynach, Cardiff, CF82 7TS, UK.
[2]Biotage, 10430 Harris Oaks Blvd., Suite C, Charlotte North Carolina 28269, USA.

Introduction

Cannabis is one of the most widely abused substances in the world. The naturally occurring cannabinoids found in plant species bind to receptors in the brain and cause sensations of relaxation and calm. Widespread misuse has led to the necessity for rapid and reliable methods for the analysis and quantitation of cannabinoids and metabolites. Here we demonstrate a supported liquid extraction procedure and analysis of Δ⁹-THC and the metabolites; 11-hydoxy-**Δ**⁹-THC and 11-nor-9-carboxy-**Δ**⁹-THC in blood. Urine analysis only involved analysis of the 11-nor-9-carboxy-**Δ**⁹-THC metabolite.

Experimental

Reagents

Drug standards were purchased from LGC Standards (Teddington, UK). Potassium hydroxide, formic acid, beta-glucuronidase (Helix pomatia) and GC derivatizing agents were purchased from Sigma-Aldrich (Dorset, UK). Urine was kindly donated by healthy human volunteers. Blank whole blood was purchased from Sera Labs International (Sussex, UK). All solvents were HPLC grade from Fisher Scientific (Loughborough, UK) and Milli-Q (Merck Millipore, Germany) water used throughout.

Sample Preparation

ISOLUTE® SLE+ Procedure (*Figure 1.*)

Columns: ISOLUTE® SLE+ 1 mL capacity 'C' columns; 820-0140-C.

Matrix Pre-treatment:

Whole Blood: 600 µL of whole blood was pre-treated with 200 µL of 0.1% formic acid aq.

Non-hydrolyzed Urine: 500 µL of urine was pre-treated with 500 µL of H_2O.

Enzymatic Urine Hydrolysis: 500 µL of 100 mM ammonium acetate buffer at pH 5 and 50 µL of beta-glucuronidase was added per 1 mL urine.

KOH Hydrolyzed Urine: Add 100 µL of 10N KOH per 1 mL of urine.

Urine Hydrolysis:

Enzymatic: Urine specimens were heated for 2 hours at 37 °C. Samples were left to cool prior to extraction.

KOH: Urine specimens were heated for 25 minutes at 60 °C. Samples were left to cool and pH adjusted with 60 µL of glacial acetic acid (per mL urine) prior to extraction.

Sample Application:

Whole Blood 800 µL of pre-treated blood was applied to the ISOLUTE SLE+ columns.

Urine (Hydrolyzed and non-hydrolyzed): 1 mL of pre-treated urine was applied to the ISOLUTE SLE+ columns.

Analyte Extraction:

Whole Blood: 1 x 2.5 mL aliquot of MTBE followed by 1 x 2.5 mL aliquot of hexane.

Urine: 2 x 2.5 mL aliquot of MTBE.

Each aliquot was allowed to flow under gravity for 5 minutes before applying a pulse of vacuum for 10-20 seconds to completely remove the final aliquot.

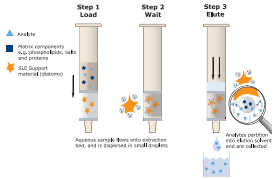

Figure 1. *Schematic of ISOLUTE® SLE+ Supported Liquid Extraction Procedure.*

Post Extraction:

The extracts were evaporated to dryness at 40 °C. Extracts were reconstituted in 40 µL EtOAc and 20 µL BSTFA:TBDMCS 99:1 and placed on a heat block for 25 minutes at 70 °C.

GC/MS Conditions

GC: 7890A GC with QuickSwap (Agilent Technologies Inc.).
Column: Phenomenex Zebron ZB-Semivolatiles, 30 m x 0.25 mm **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. Temperature: 250 °C;
Injection volume: 1 **µL**
Oven conditions: Initial 60 °C, ramp 25 °C/min to 350 °C, hold for 0.4 minutes
Backflush: 3 void volumes (2.4 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) in 3 groups, as shown in ***Table 1.***

Table 1. *MS acquisition parameters.*

SIM Group	Analyte	Target (Quant) Ion	1st Qual Ion	2nd Qual Ion
1	Δ⁹THC-D3	374	315	306
1	Δ⁹THC	371	343	386
2	THC-OH	374		
2	THC-OH-D3	371		
3	THC-COOH-D3	374	491	
3	THC-COOH	371	488	

Results

Whole Blood:

Whole blood was spiked with standards and ISTDs and allowed to bind for 1 hour before pre-treatment and subsequent processing. Early whole blood method optimization focused on pre-treatment 1:1 with buffer, loading 300 µL onto the 400 µL capacity SLE+ column. Due to the acidic moiety pH control at and below neutrality was investigated. ***Figure 2.*** demonstrates various pre-treatment options using MTBE and DCM as elution solvents.

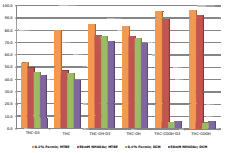

Figure 2. *Recoveries comparing pre-treatment options.*

Figure 3. demonstrates further elution solvent optimization. High reproducible recoveries, exceeding 70% were obtained for both metabolites using all solvent combinations. However, only when using an elution profile of MTBE followed by an aliquot of hexane resulted in high recoveries of parent THC.

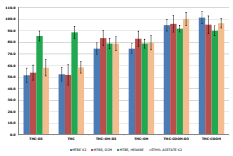

Figure 3. *Recoveries comparing elution solvent combinations.*

In order to increase sensitivity while avoiding breakthrough of blood, testing focused on pre-treatment ratios and load volumes using the 1 mL capacity SLE+ columns. We were able to modify blood pretreatment and loading volume to 800 µL with a 3:1 ratio or 1:1 ratio loading 750 µL as demonstrated in ***Figure 4.***

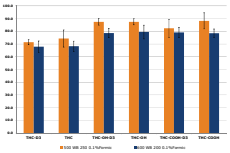

Figure 4. *Recoveries comparing 800uL 3:1 with 750uL 1:1.*

Final method optimization involved comparison of sample mixing using a vortex approach to using an OMNI BeadRuptor®24 unit. The BeadRuptor is specially designed for lysing, grinding and homogenization of various matrices before extraction. ***Figure 5.*** demonstrates the recovery comparison between the two techniques.

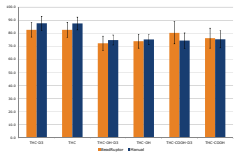

Figure 5. *Recoveries comparison of mixing technique.*

Using the above method calibration curves demonstrated increased sensitivity using the BeadRuptor. Calibration curves are demonstrated in ***Figures 6-11.*** with equivalent LLOQs detailed in ***Table 2.***

Table 2. *LLOQs comparing vortex and BeadRuptor mixing.*

Analyte	ISOLUTE® SLE+ 1mL C Column LLOQ (ng/mL)	
	Vortex	*BeadRuptor*
Δ⁹THC	1	1
THC-OH	6	1-3
THC-COOH	6	3

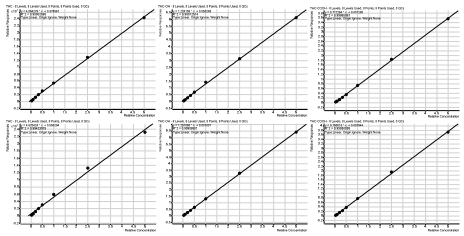

Figure 6. *Calibration curves for spiked blood from 1-150 ng/mL extracted after vortex mixing (top) and BeadRuptor (bottom) using 1 mL capacity ISOLUTE® SLE+ columns.*

Urine:

Analysis was performed investigating the primary urinary metabolite, 11-nor-9-carboxy-**Δ**⁹-THC. pH and elution solvent investigation was performed using non-hydrolyzed urine. Due to the acidic moiety on the metabolite, urine pre-treatment was investigated using H_2O or 0.1% formic acid. Elution solvent investigation focused on combinations of MTBE and DCM. ***Figure 7.*** demonstrates recovery optimization recovery results for the various combinations.

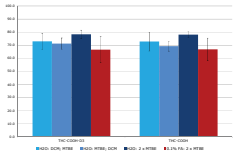

Figure 8. *Recoveries comparing KOH hydrolysis with and without post hydrolysis pH modification.*

The KOH hydrolysis procedure required pH control post hydrolysis due to the high starting pH. Initial starting pH was around 13.5 and subsequently modified to 9 and down to 5.5 with glacial acetic acid prior to loading. ***Figure 8.*** demonstrates the impact of pH on recoveries of the carboxy-THC.

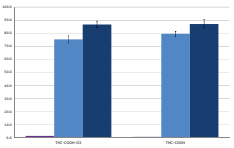

Figure 8. *Recoveries comparing KOH hydrolysis with and without post hydrolysis pH modification.*

The enzymatic hydrolysis approach using beta-glucuronidase has a working pH range of 4.5-5.5. The pH of this method did not require any post hydrolysis modification and allowed direct addition of the cooled matrix onto the SLE+ columns. Final optimized extraction recoveries for non-hydrolyzed and both hydrolyzed urine methods are demonstrated in ***Figure 9.***

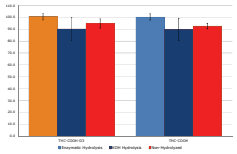

Figure 9. *Optimized urine recoveries comparing non-hydrolyzed and both hydrolysis methods.*

Calibration curves were constructed from 1-150 ng/mL and extracted using the ISOLUTE® SLE+ 1 mL C capacity columns. r^2 values > 0.997 were returned for all three extraction protocols, as demonstrated in ***Figure 10.***

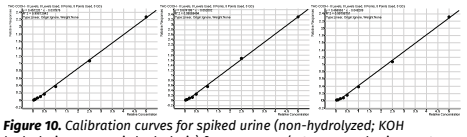

Figure 10. *Calibration curves for spiked urine (non-hydrolyzed; KOH hydrolysis; enzymatic hydrolysis) from 1-150 ng/mL extracted using 1 mL capacity ISOLUTE® SLE+ columns.*

Conclusion

- This poster demonstrates the applicability of ISOLUTE® SLE+ for the extraction of THC and relevant metabolites from whole blood and both non-hydrolyzed and hydrolyzed urine.
- Good extraction efficiency, RSDs and extract cleanliness was afforded for all matrices.
- Calibration curves demonstrated excellent linearity, and r^2 values > 0.99 for all matrices.

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