Extraction of THC and metabolites from Urine and Plasma using Supported Liquid Extraction (SLE) prior to LC-MS/MS Analysis

Rhys Jones, Lee Williams, Helen Lodder, Geoff Davies, Steve Jordan, Richard Calverley, Claire Desbrow & Gary Dowthwaite
Biotage GB Limited, Distribution Way, Dyffryn Business Park, Ystrad Mynach, Mid Glamorgan, CF82 7TS, UK.

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. The most prevalent markers in biological samples taken from cannabis abusers are Δ9-tetrahydrocannanbinol (THC), cannabidiol, cannabinol in addition to the major THC metabolites; 11-hydoxy-Δ9-THC and 11-nor-9-caroxy-Δ9-THC. Here we demonstrate a supported liquid extraction procedure for THC and its metabolites.

Experimental Procedure

Reagents

Controlled compounds Δ9-THC, cannabidiol, cannabinol, 11-hydoxy-Δ9-THC and 11-nor-9-carboxy-Δ9-THC were purchased from Lipomed (Kinesis, UK). Formic acid and ammonium hydroxide were purchased from Sigma Chemical Co. (Poole, UK). Human plasma was obtained through the Welsh Blood Service (Pontyclun, UK). Urine was obtained from a healthy human volunteer. All solvents were HPLC grade from Fisher Scientific (Loughborough, UK).

Sample Preparation

Supported Liquid Extraction Procedure

Plate: ISOLUTE SLE+ 200 Supported Liquid Extraction Plate, part number 820-0200-P01

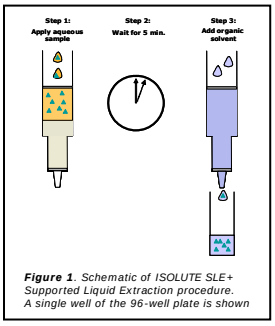

***Figure 1**. Schematic of ISOLUTE SLE+ Supported Liquid Extraction procedure. A single well of the 96-well plate is shown*

Sample pre-treatment: Plasma and urine (100 µL) were diluted 1:1 (v/v) with either 1% formic acid aq, 0.1% formic acid aq, H_2O or 0.5M NH_4OH. Spike concentrations were 400 ng/mL.

Sample Application: The pre-treated matrix (200 µL) was loaded onto the plate, a pulse of vacuum
applied to initiate flow and the samples left to absorb for 5 minutes.

Analyte Elution: Addition of 1 mL of various water immiscible extraction solvents. The extraction solvents evaluated were MTBE, Hexane, DCM and Ethyl Acetate.

Post Extraction: The eluate was evaporated to dryness and reconstituted in 500 µL of 0.1% formic acid 50:50 (v/v) H_2O/ACN.

HPLC Conditons

Instrument: Waters Acquity UPLC (Waters Assoc., Milford, MA, USA).
Column: Acquity UPLC BEH C18 column (1.7µ, 100 x 2.1 mm id) (Waters Assoc., Milford, MA, USA).
Mobile Phase: 0.1% formic acid aq and 0.1% formic acid/MeOH at a flow rate of 0.5 mL/min.
Gradient: Isocratic 10%, 0.1% (v/v) formic acid aq and 90% 0.1% formic acid/MeOH.
Injection Volume: 5 µL.
Column Temperature: 35 °C.

Mass Spectrometry

Instrument: Premier XE triple quadrupole mass spectrometer (Waters Assoc., Manchester, UK) equipped with an electrospray interface for mass analysis. ***Table 1***. shows the positive ions acquired in the multiple reaction monitoring (MRM) mode. There was no substantial signal to noise advantage acquiring the carboxylic acid metabolite in negative ion mode.
Desolvation Temperature: 450 °C
Ion Source Temperature: 150 °C
Collision Gas Pressure: 3.46 x 10^{-3} mbar
Collision Gas Pressure: 3.46 x 10^{-3} mbar

Compound	Transition	Collision Energy
Δ9-THC	315.2 -> 193.1	21
Cannabidiol	315.2 -> 135.0	20
Cannabinol	311.2 -> 223.1	20
11-OH-Δ9-THC	331.2 -> 313.3	14
11-nor-COOH-Δ9-THC	345.1 -> 327.2	16

Table 1. MRM transitions for Δ9-THC and metabolites

Results

Figures 1-4. and ***figures 5-8*** demonstrate recovery data of the cannabinoid suite from human plasma and urine, respectively. These results highlight that pH control is vital if high recoveries of all five analytes are desired. Pre-treatment of plasma with 1% formic acid yielded recoveries of >80% for all analytes when coupled with a DCM elution. Pre-treatment of urine with water (1:1) yielded recoveries for all analytes of >80% when coupled with ethyl acetate elution.

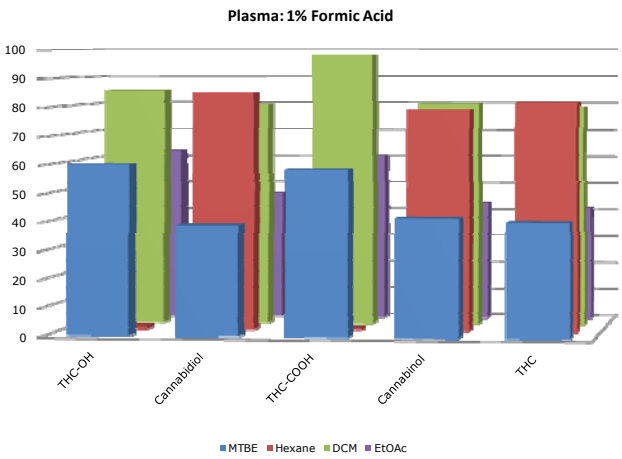

Figure 1. *THC and metabolites recovery profile from plasma using 1% formic acid pre-treatment.*

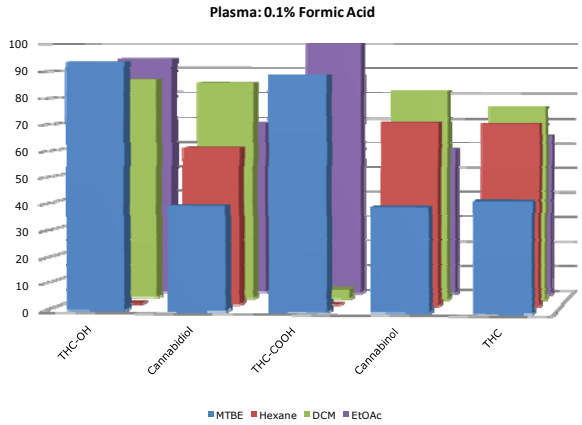

Figure 2. *THC and metabolites recovery profile from plasma using 0.1% formic acid pre treatment.*

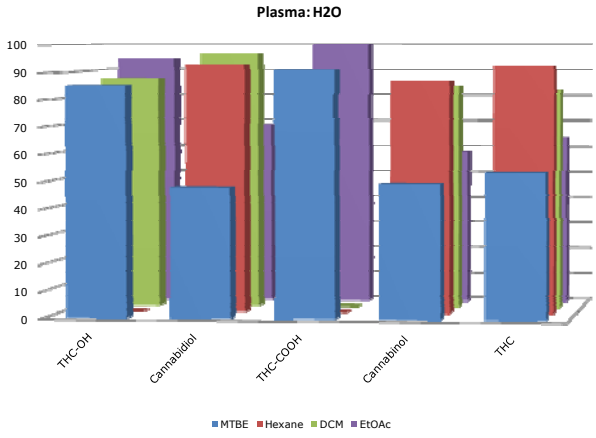

Figure 3. *THC and metabolites recovery profile from plasma using H_2O pre-treatment*

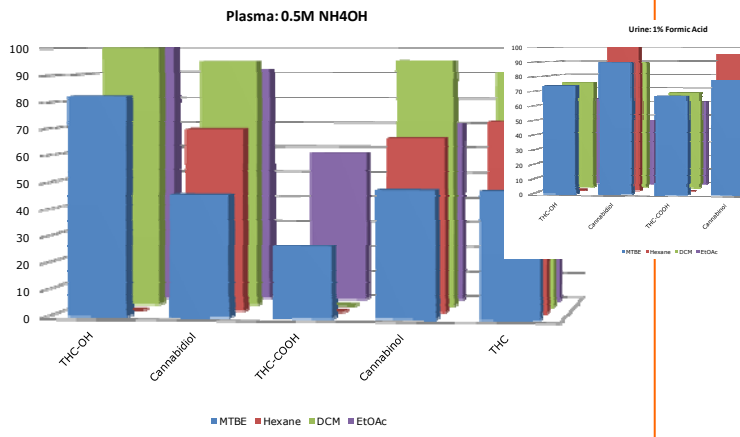

Figure 4. *THC and metabolites recovery profile from plasma using 0.5M NH_4OH pre-treatment.*

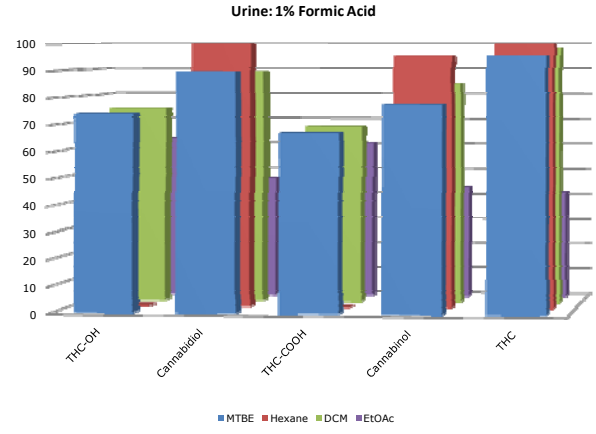

Figure 5. *THC and metabolites recovery profile from urine using 1% formic acid pre-treatment.*

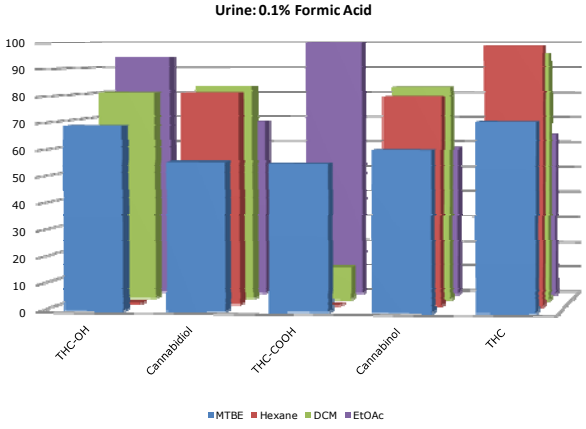

Figure 6. *THC and metabolites recovery profile from urine using 0.1% formic acid pre-treatment.*

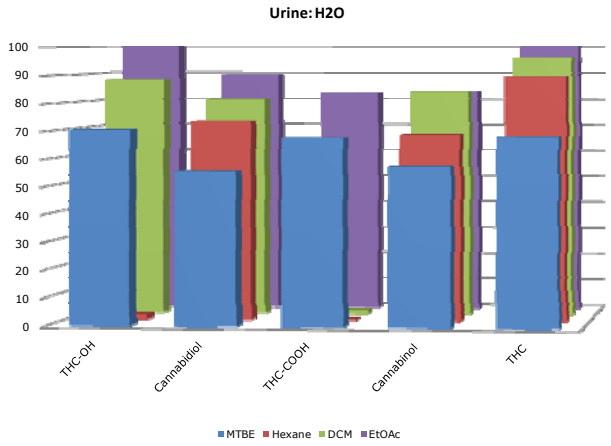

Figure 7. *THC and metabolites recovery profile from urine using H_2O pre-treatment.*

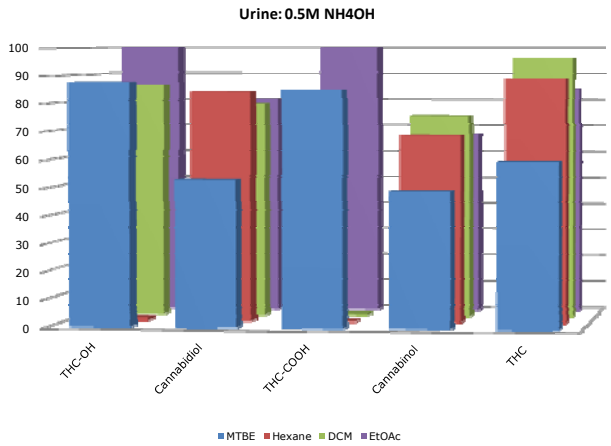

Figure 8. *THC and metabolites recovery profile from urine using 0.5M NH_4OH pre-treatment*

Conclusions

1. Recoveries of >80% were demonstrated from both plasma and urine.
2. Hexane elution yielded high recoveries of the less polar analytes whilst more polar metabolites demonstrated low extraction efficiencies.
3. EtOAc, DCM and MTBE demonstrated high extraction efficiencies for both matrices, however, matrix pH was an important factor.
4. Higher extraction efficiencies may be obtained using mixed elution solvents or subsequent elutions of hexane followed by ethyl acetate, DCM or MTBE to incorporate both the non-polar and more polar functionalities.

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United States and Canada
Tel: +1 704 654 4900
Toll-Free: +1 800 446 4752
ordermailbox@biotage.com

United Kingdom, EIRE
Biotage
Tel: +44 1992 501535
order@eu.biotage.com

Sweden
Biotage
Tel: +46 18 56 59 00
order@eu.biotage.com

Japan
Biotage
Tel: +81 422 281233
order@biotage.co.jp