Extraction of THC and Metabolites from Plasma Using ISOLUTE® SLE+

Introduction

This application note describes the extraction of THC and it's metabolites from plasma using ISOLUTE SLE+ supported liquid extraction plates followed by UPLC-MS/MS analysis.

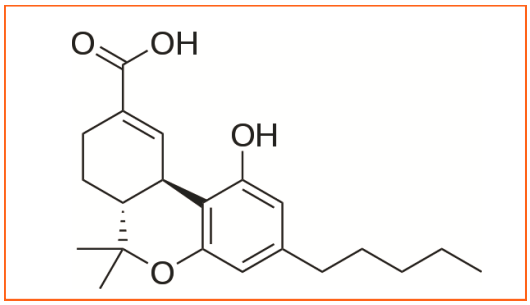

Figure 1. 11-nor-9-carboxy-Δ9-THC (THC-COOH)

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-carboxy-Δ9-THC. Here we demonstrate a supported liquid extraction procedure for THC and its metabolites.

ISOLUTE SLE+ Supported Liquid Extraction plates and columns offer an efficient alternative to traditional liquid-liquid extraction (LLE) for bioanalytical sample preparation, providing high analyte recoveries, no emulsion formation, and significantly reduced sample preparation time.

Analytes

Δ^9-tetrahydrocannanbinol (THC), cannabidiol, cannabinol, 11-hydoxy- Δ^9-THC (THC-OH) and 11-nor-9-carboxy- Δ^9-THC (THC-COOH).

SLE+ Procedure

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

Sample pre-treatment: Dilute a 100 µL plasma sample 1:1 (v/v) with 1% formic acid aq.

Sample loading: Load pre-treated plasma (200 µL) onto the plate and apply a pulse of vacuum to initiate flow. Leave the samples to absorb for 5 minutes.

Analyte Elution: Elution 1: Apply 500 µL dichloromethane, apply a short pulse of vacuum and wait 5 minutes.
Elution 2: Again apply 500 µL dichloromethane, apply a short pulse of vacuum and wait 5 minutes.

Post Extraction: Evaporate eluate to dryness and reconstitute in 500 µL of 0.1% formic acid in water / acetonitrile (v/v, 50:50).

HPLC Conditions

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: A: 0.1% formic acid aq and B: 0.1% formic acid in Methanol. Run isocratically at a flow rate of 0.5 mL/min 10%, A and 90% B.

Injection Volume: 5 µL.

Column Temperature: 35 °C.

Application Note

Mass Spectrometry Conditions

Instrument: Quattro 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.

Desolvation Temperature: 450 °C

Ion Source Temperature: 150 °C

Collision Gas Pressure: 3.46 x 10^{-3} mbar

Compound	Transition	Collision Energy (eV)	Cone Voltage (V)
Δ9-THC	315.2 –> 193.1	21	40
Cannabidiol	315.2 –> 135.0	20	30
Cannabinol	311.2 –> 223.1	20	40
11-OH-Δ9-THC	331.2 –> 313.3	14	25
11-nor-COOH-Δ9-THC	345.1 -> 327.2	16	35

Table 1. MRM transitions for Δ9-THC and metabolites

Results

All results show recoveries above 80% with %RSDs below 10%. THC-OH, Cannabidiol and THC-COOH show excellent recoveries of 95% or over. It should be noted that LOQ values can be improved by increasing the injection volume or through concentrating the sample by using a smaller reconstitution volume.

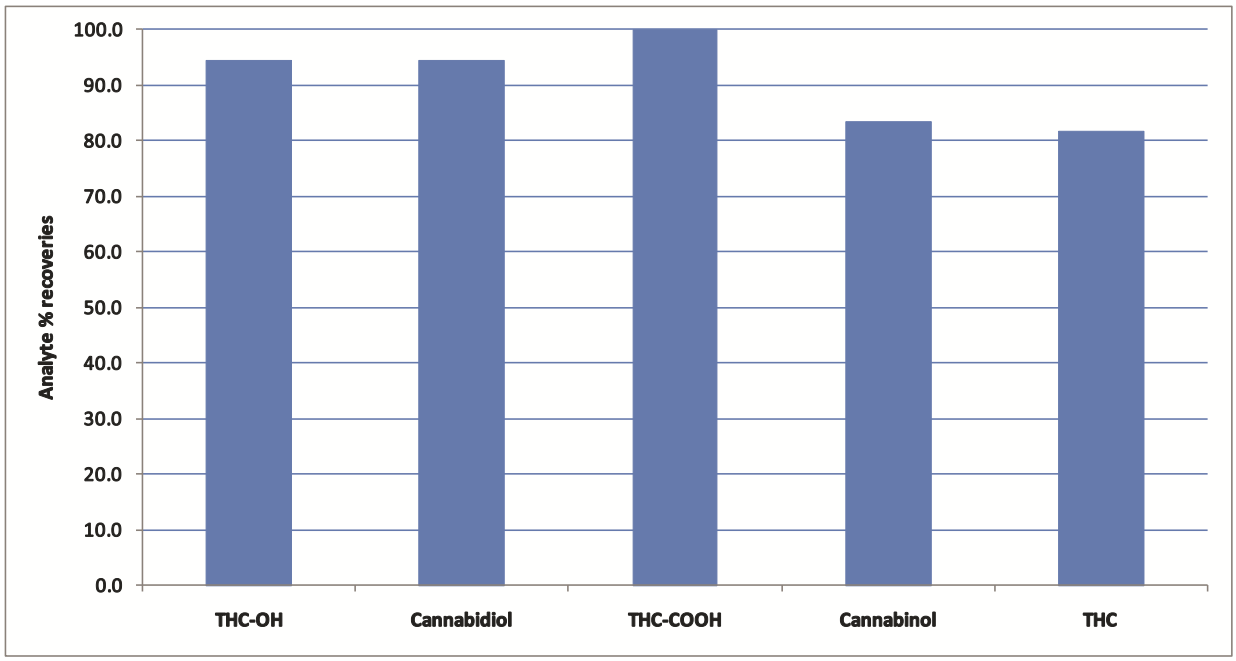

Figure 2. THC and metabolites % recoveries

References

This application note is based on the poster 'Extraction of THC and metabolites from Urine and Plasma using Supported Liquid Extraction (SLE) prior to UPLC-MS/MS Analysis', L Williams et al, presented at SOFT, Richmond, VA, October 18-22, 2010.

Additional assistance

To assist with the blowing down and subsequent concentration of samples, the TurboVap 96 Concentration Workstation is a high speed concentrator designed to work with 96-well microplates and deep-well plates. It is an efficient alternative to the constant monitoring and long evaporation times that are characteristic of conventional techniques with the added bonus of unattended operation.

Ordering information

Part number	Description	Quantity
820-0200-P01	ISOLUTE SLE+ 200 Supported Liquid Extraction Plate	1
820-0400-P01	ISOLUTE SLE+ 400 Supported Liquid Extraction Plate	1
C103263	TurboVap 96 100/120 V, 50/60 Hz	1
C103264	TurboVap 96 220/240 V, 60/60 Hz	1

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