Extraction of THC, THCA and Carboxy-THC from Oral Fluid by ISOLUTE® SLE+ after Collection with the Quantisal™ Collection Device prior to GC/MS Analysis

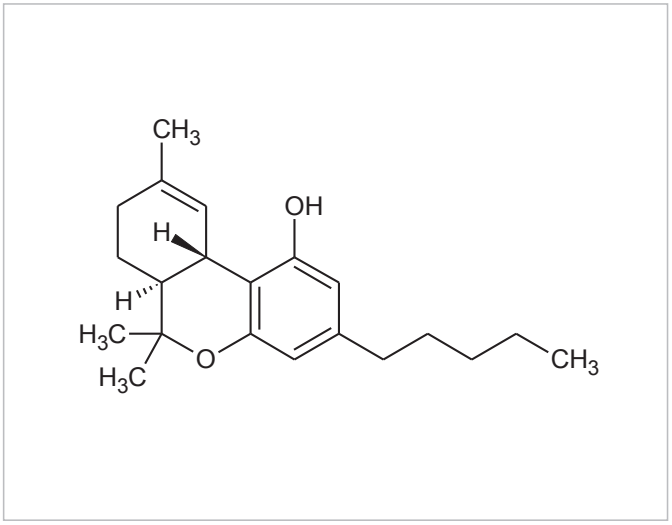

Figure 1. Structure of Δ^9 -THC (tetrahydrocannabinol)

Introduction

This application note describes the extraction of THC, THCA and Carboxy-THC from oral fluid matrix collected using the Quantisal™ (Immunalysis) device, prior to GC/MS analysis.

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.

This application note describes an effective and efficient ISOLUTE SLE+ protocol optimized for 400 µL and 1 mL sample capacity formats. The simple sample preparation procedure delivers clean extracts and analyte recoveries greater than 75% with RSDs lower than 10% for all analytes.

Analytes

THC, THCA, THC-COOH and THC-d3, THC-COOH-d3 as internal standards

Sample Preparation Procedure

Sample pre-treatment:	Following collection, add concentrated ammonium hydroxide (15 µL) to each collection device (see additional information).
Format:	**ISOLUTE® SLE+ 400 µL sample volume columns, part number 820-0055-B**
Sample loading:	Load pre-treated oral fluid (400 µL) onto the column and apply a pulse of vacuum or positive pressure (3–5 seconds) to initiate flow. Allow the sample to absorb for 5 minutes.
Analyte Extraction:	Apply dichloromethane/isopropanol, (95/5, v/v, 1 mL) and allow to flow under gravity for 5 minutes. Apply a further aliquot of DCM/IPA, (95/5, v/v, 1 mL) and allow to flow for another 5 minutes under gravity. Apply vacuum or positive pressure (5–10 seconds) to complete elution.
Format:	**ISOLUTE® SLE+ 1 mL sample volume columns, part number 820-0140-C**
Sample loading:	Load pre-treated oral fluid (1 mL) onto the column and apply a pulse of vacuum or positive pressure (3–5 seconds) to initiate flow. Allow the sample to absorb for 5 minutes.
Analyte Extraction:	Apply dichloromethane/isopropanol, (95/5, v/v, 2.5 mL) and allow to flow under gravity for 5 minutes. Apply a further aliquot of DCM/IPA, (95/5, v/v, 1 mL) and allow to flow for another 5 minutes under gravity. Apply vacuum or positive pressure (5–10 seconds) to complete elution.
Post Elution & Reconstitution:	Dry the extract in a stream of air or nitrogen using a SPE Dry (40 °C, 20 to 40 L/min) or TurboVap® (1.0 bar at 40 °C for 40 mins). Upon dryness, reconstitute with 50 µL ethyl acetate and 25 µL MTBSTFA:TBDMCS (99:1, v/v) and vortex for 20 seconds. Transfer to a high recovery glass vial. Place in a heating block set to 70 °C, for 25 minutes. Remove vial from the block and allow cooling.

Biotage®

GC Conditions

Instrument:	Agilent 7890A with QuickSwap
Column:	Phenomenex Zebron ZB-Semivolatiles, 30 m x 0.25 mm ID x 0.25 µm
Carrier	Helium 1.2 mL/min (constant flow)
Inlet:	250 °C, Splitless, purge flow: 50 mL/min at 1.0 min
Injection:	2 µL
Wash solvents:	Ethyl acetate
Oven:	Initial temperature 100 °C, hold for 1 minute
	Ramp 100 °C/min to 280 °C, hold for 10.5 minutes
	Ramp 100 °C/min to 330 °C, hold for 0.5 minutes
Post run:	Backflush for 2.4 minutes (3 void volumes)
Transfer Line:	280 °C

MS Conditions

Instrument:	Agilent 5975C
Source:	230 °C
Quadrupole:	150 °C
MSD mode:	SIM

SIM Parameters

Table 1. Ions acquired in the Selected Ion Monitoring (SIM) mode

SIM Group	Analyte	Target (Quant) Ion	1st Qual Ion	2nd Qual Ion
1	THC-d3	374	431	348
1	THC	371	428	345
2	THCA	530	631	455
3	THC-COOH-d3	416	518	575
3	THC-COOH	413	515	572

Results

The optimized ISOLUTE® SLE+ protocol demonstrated analyte recoveries ranging from 75–90% as shown in Figure 2. RSDs were below 10% for all analytes.

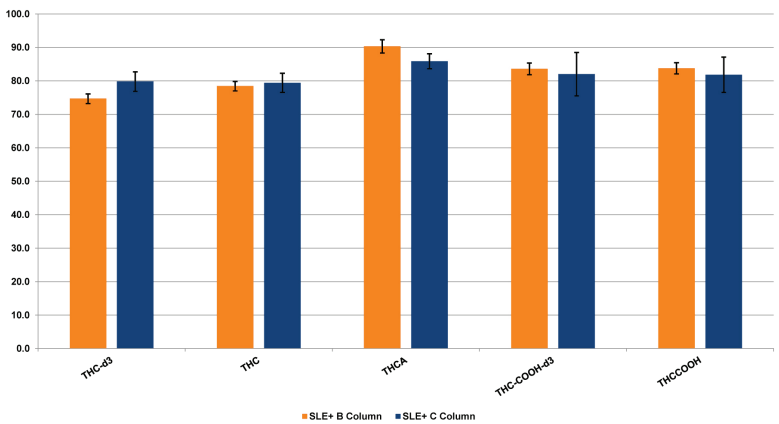

Figure 2. Typical extraction % recoveries (n=7) using the ISOLUTE® SLE+ protocol.

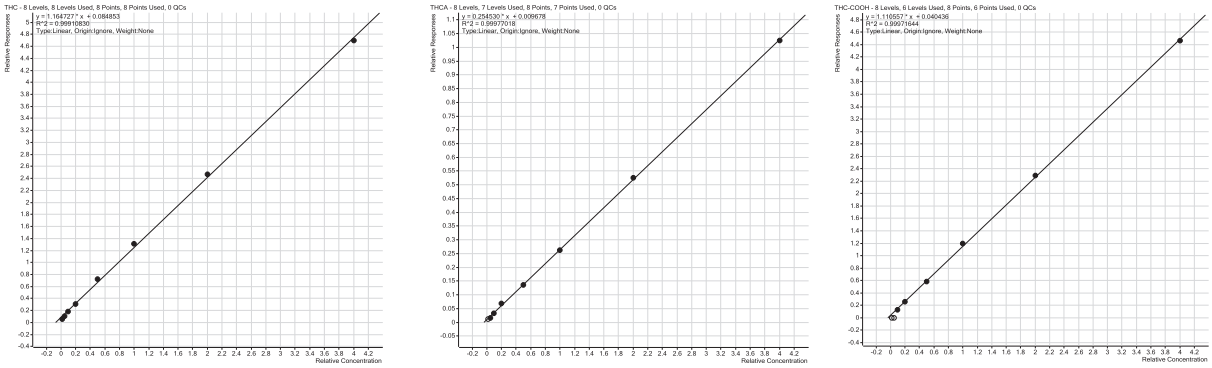

Figure 3. Calibration curves for extracted levels of spiked oral fluid after collection with Quantisal devices using 1 mL ISOLUTE® SLE+ format from 4 ng/mL to 800 ng/mL showing r^2 values of 0.9991 to 0.9997.

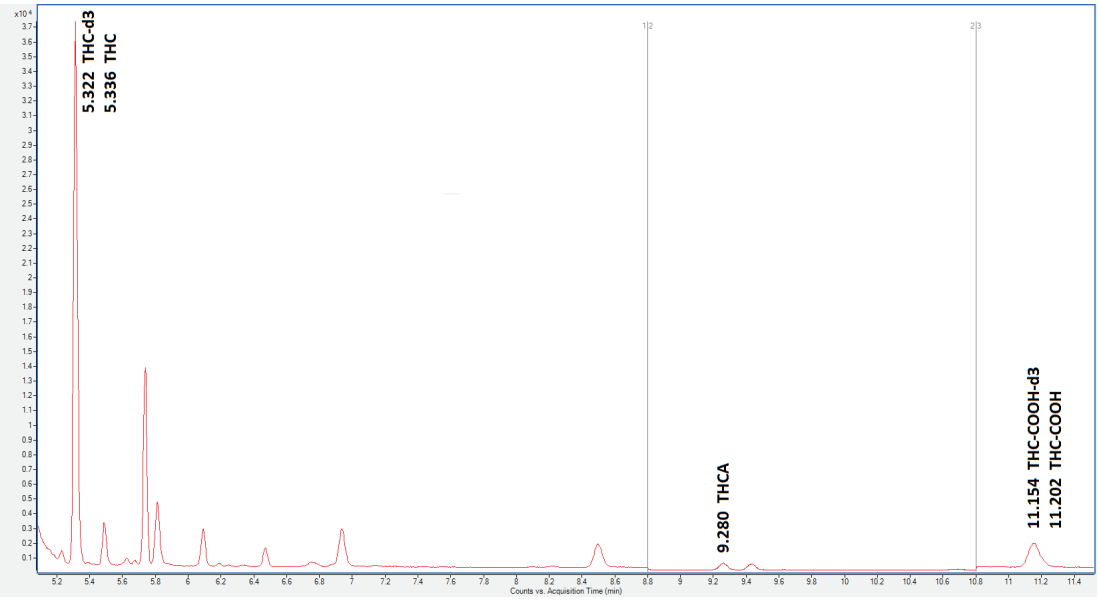

Figure 4. GC/MS chromatography for Quantisal collected oral fluid spiked at 40 ng/mL

Table 2. Lower Limits of Quantitation (LLOQ) using ISOLUTE® SLE+ procedure

Analyte	LLOQ SLE+ 1 mL column	LLOQ SLE+ 400 µL column
THC	4 ng/mL	10 ng/mL
THCA	10 ng/mL	25 ng/mL
THC-COOH	20 ng/mL	50 ng/mL

Additional Information

1. If a non-chlorinated solvent is required, MTBE (methyl-tert-butyl-ether) is a suitable substitute solvent.

2. Concentrated stock of ammonium hydroxide (28-30%) used to modify pH prior to extraction is commercially available

Ordering Information

Part Number	Description	Quantity
820-0055-B	ISOLUTE® SLE+ 400 µL Supported Liquid Extraction Columns	50
820-0140-C	ISOLUTE® SLE+ 1 mL Supported Liquid Extraction Columns	30
PPM-48	Biotage® PRESSURE+ 48 Positive Pressure Manifold 4	1
SD-9600-DHS-EU	Biotage® SPE Dry Sample Concentrator System 220/240 V	1
SD-9600-DHS-NA	Biotage® SPE Dry Sample Concentrator System 100/120 V	1
C103198	TurboVap® LV, 100/120V	1
C103199	TurboVap® LV, 220/240V	1

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