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Analysis of THC and an Extended Metabolite Suite from Oral Fluid Using ISOLUTE® SLE+ Supported Liquid Extraction Columns Prior to LC-MS/MS

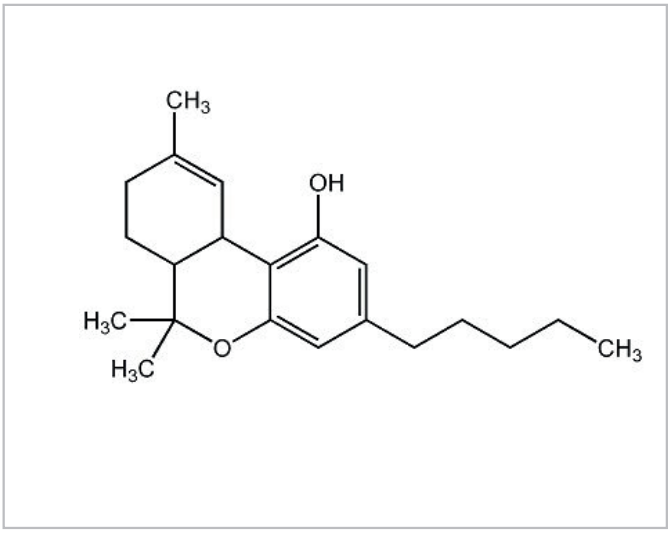

Figure 1. Structure of Δ^9-tetrahydrocannabinol (THC)

Introduction

The method described in this application note achieves high recoveries of THC and an extended suite of common metabolites in oral fluid from Quantisal (Immunalysis) oral fluid collection devices.

ISOLUTE® SLE+ products provide clean, rapid, robust, efficient , high throughput and automatable extraction solutions for a wide range of analytes.

This application note describes effective and efficient ISOLUTE SLE+ protocols optimized for sample loading volumes of either 300 µL or 800 µL. The simple sample preparation procedure delivers clean extracts and analyte recoveries greater than 64% with RSDs of ‹10% for all analytes.

Analytes

Δ^9-tetrahydrocannabinol (THC), cannabigerol, cannabidiol, Δ^9-tetrahydrocannabivarin (THCV), 11-Hydroxy- Δ^9-tetrahydrocannabinol (11-OH-THC), 11-nor-9-carboxy- Δ^9-tetrahydrocannabivarin (THC-V-COOH), 11-nor-9-carboxy- Δ^9tetrahydrocannabinol (THC-COOH), 11-nor-9-carboxy- Δ^9tetrahydrocannabinol glucuronide (THC-COOH-glucuronide), Δ^9-tetrahydrocannabinol glucuronide (THC glucuronide) and Δ^9-Tetrahydrocannabinolic Acid A (THCA-A)

Sample Preparation Procedure

Column Configuration: ISOLUTE® SLE+ 400 µL Sample Volume Columns, Part Number 820-0055-B or ISOLUTE® SLE+ 1 mL Sample Volume Columns, Part Number 820-0140-C were used.

Sample Pre-treatment: Collect saliva as per packet instructions. When required, remove the paddle from the Quantisal oral fluid collection device and add 10 µL of concentrated formic acid to adjust pH of the sample. Vortex mix thoroughly.

Format: **ISOLUTE SLE+ 400 µL Supported Liquid Extraction Columns, Part Number 820-0055-B**

Sample loading: Load pre-treated sample (300 µL) onto the ISOLUTE SLE+ bed, and apply a pulse of vacuum. Leave for 5 minutes to absorb.

Elution 1: Apply an aliquot of MTBE (750 µL), wait for 5 minutes

Elution 2: Apply a second aliquot of MTBE (750 µL), wait for 5 minutes

Elution 3: Apply a single aliquot of hexane (750 µL) and wait for 5 minutes; apply a pulse of vacuum or positive pressure to complete elution (10 seconds).

Post Elution: Dry the combined eluent in a stream of air or nitrogen using a TurboVap® LV (1.5 bar at 40 °C) for 40 mins. Reconstitute in 0.1% formic acid in H_2O/ACN ((60/40, v/v), 200 µL) and vortex mix thoroughly.

Biotage®

Format:	**ISOLUTE® SLE+ 1 mL Sample Volume Columns, Part Number 820-0140-C**
Sample loading:	Load pre-treated sample (800 µL) onto the ISOLUTE SLE+ bed, and apply a pulse of vacuum. Leave for 5 minutes to absorb.
Elution 1:	Apply an aliquot of MTBE (2 mL), wait for 5 minutes.
Elution 2:	Apply a second aliquot of MTBE (2 mL), wait for 5 minutes.
Elution 3:	Apply a single aliquot of hexane (2 mL) and wait for 5 minutes, apply a pulse of vacuum or positive pressure to complete elution (10 seconds).
Post Elution:	Dry the combined eluent in a stream of air or nitrogen using a TurboVap LV (1.5 bar at 40 °C) for 40 mins. Reconstitute in 0.1% formic acid in H_2O/ACN ((60/40, v/v), 500 µL), and vortex mix thoroughly.

HPLC Conditions

Instrument:	Waters ACQUITY UPLC with 20 µL loop
Column:	Phenomenex Kinetex XB C18 2.6 µm 100 Å 50 mm x 2.10 mm
Mobile Phase:	A: 0.1% formic acid in water B: 0.1% formic acid in ACN
Injection Volume:	15 µL (partial loop with overfill)
Flow rate:	0.35 mL/min
Injection:	5 µL, Partial Loop

Table 1. Gradient

Time	%A	%B	Flow	Curve
0.00	45	55	0.350	1
2.50	10	90	0.350	6
3.00	10	90	0.350	6
3.01	45	55	0.350	6
4.00	45	55	0.350	6

Column Temperature:	Ambient
Sample Temperaure:	20 °C

MS Conditions

Ions were selected in order to achieve maximum sensitivity using multiple reaction monitoring.

Instrument:	Waters Ultima Pt
Inonization Mode:	ESI+
Desolvation Temperature:	350 °C
Source Temperature:	100 °C

Table 2. Positive Ion Mode - MRM Parameters

Compound ID	RT	MRM Transition	Cone, (V)	CE, (V)	Collision Energy (eV)
THC-V-COOH	1.28	317.2 > 299.2	35	11	1
THC glucuronide	1.39	491.3 > 315.2	35	15	
THC-COOH glucuronide	0.88	521.2 > 345.2	35	10	
11-THC-OH	2.23	313.2 > 217.1	35	15	2
11-THC-OH d3	2.21	334.3 > 316.3	35	12	
THC-COOH	2.35	345.2 > 299.1	35	17	
THC-COOH d3	2.34	348.2 >330.2	35	12	
THCV	3.23	287.2 > 165.1	35	19	3
Cannabidiol	3.20	315.2 > 135.1	35	17	
Cannabigerol	3.17	317.2 > 193.1	35	13	
THC	4.05	315.2 > 193.1	35	19	4
THC d3	4.05	318.3 > 196.1	35	20	
THCA-A	4.50	359.2 > 219.1	35	24	
Cannabinol	3.77	311.2 > 223.1	35	17	
Dwell = 0.08 sec (all analytes), Inter channel delay 0.10 sec					

Results

The method outlined in this application note achieves high reproducible recoveries for both extraction formats, as demonstrated in tables 3 and 4.The calibration curves demonstrated excellent linearity for all analytes as shown in tables 3 and 4 and are shown for THC, 11-OH-THC, THC-COOH and THC-COOH glucuronide from ISOLUTE® SLE+ 1 mL sample volume columns in figures 4, 5, 6 and 7.

The pH of the Quantisal device buffer was measured at 6.7, and various percentages of formic acid were tested across a pH range of 3.6 to 6 to identify optimum extraction conditions. The optimum pre-treatment was 10 µL of concentrated formic acid per device resulting in a pH of 3.6. THC-COOH glucuronide was only extracted at the low pH pre-treatment conditions.

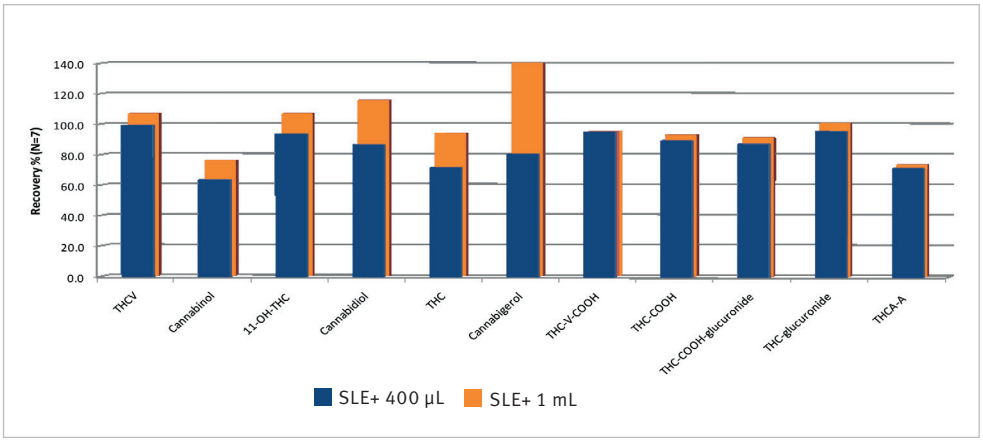

Figure 2. Typical chart of recoveries for both formats using the methods described in this application note

Table 3. Analyte performance and recovery data for THC and metabolites from Quantisal oral fluid collection device using the method described on ISOLUTE® SLE+ 400 µL sample volume columns

Analyte	r^2	% RSD	% Recovery	Estimated LOQ*
THC-V-COOH	0.994	2.8	95.3	1
THC glucuronide	0.996	4.2	95.9	1
THC-COOH glucuronide	0.994	5.3	87.6	2
11-OH-THC	0.999	5.3	93.8	2
THC-COOH	0.999	1.8	89.6	1
THCV	0.998	4.2	99.6	1
Cannabidiol	0.999	4.8	86.9	2
Cannabigerol	0.998	6.3	80.9	2
Cannabinol	0.999	2.8	63.6	1
THC	0.999	2.8	71.9	1
THCA-A	0.997	3.8	71.6	2

*based on a 5 µL injection from a reconstituted sample

Table 4. Analyte performance and recovery data for THC and metabolites from Quantisal oral fluid collection device using the method described on ISOLUTE® SLE+ 1 mL sample volume columns

Analyte	r^2	% RSD	Recovery %	Estimated LOQ*
THC-V-COOH	0.997	3.1	95.2	1
THC gluc	0.997	4.1	100.4	1
THC-COOH gluc	0.998	8.5	90.9	2
11-OH-THC	0.999	3.2	106.6	2
THC-COOH	0.999	2.9	92.8	1
THCV	0.998	4.1	106.5	1
Cannabidiol	0.999	4.9	115.4	2
Cannabigerol	0.997	5.1	147.5	2
Cannabinol	0.999	1.9	75.7	1
THC	0.999	4.2	93.8	1
THCA-A	0.999	7.5	73.2	2

*based on a 5 µL injection from a reconstituted sample

Recovery and RSD were calculated using replicate (N=7) extractions of blank Quantisal buffer pre-treated with concentrated formic acid pooled matrix spiked at 5 ng. Linearity (r^2) was calculated using a single replicate, eight point calibration from 1–200 ng/mL.

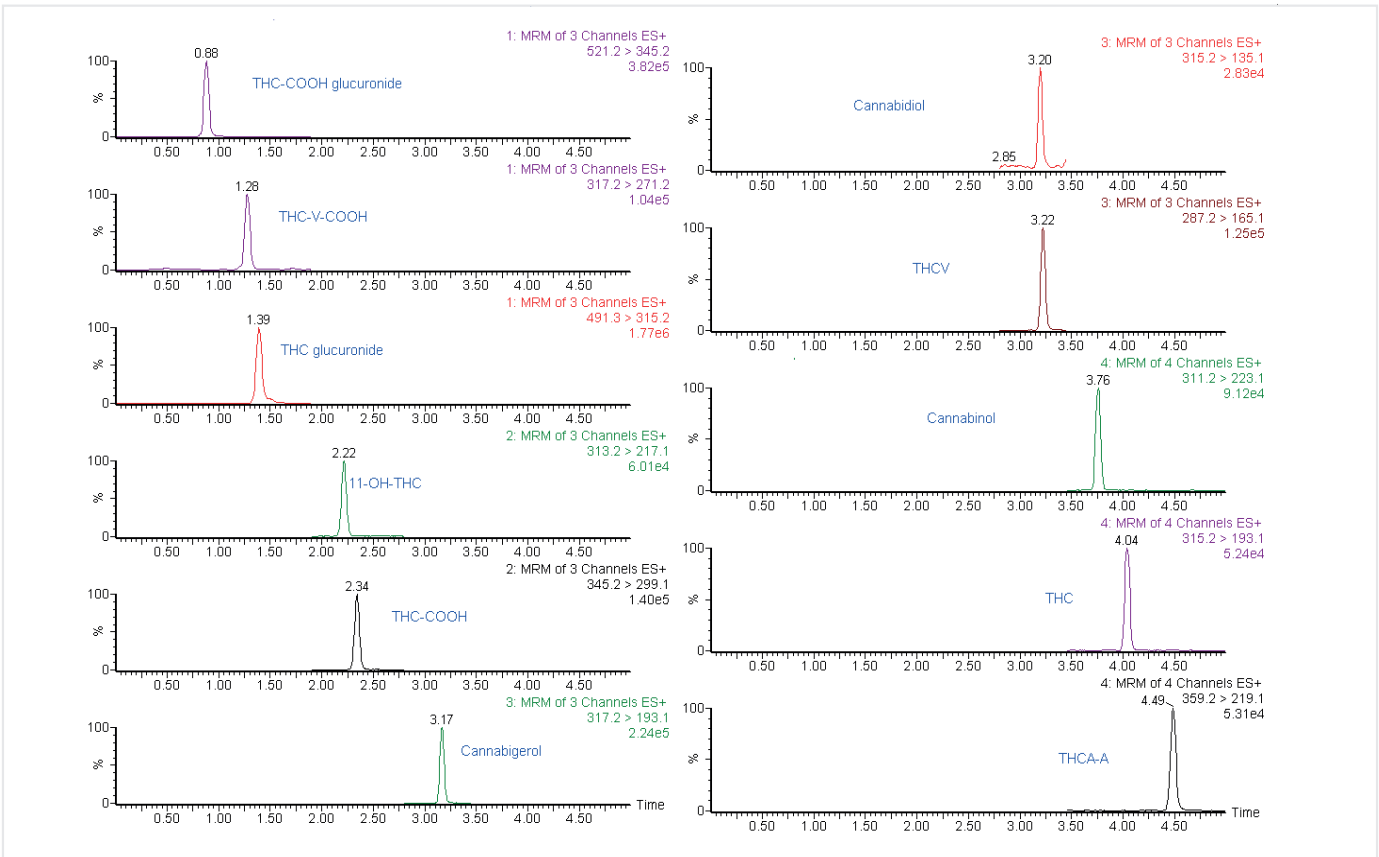

Figure 3. Typical MRM chromatogram of THC and metabolites at 5 ng extracted using the method described for ISOLUTE SLE+ 400 µL sample volume columns.

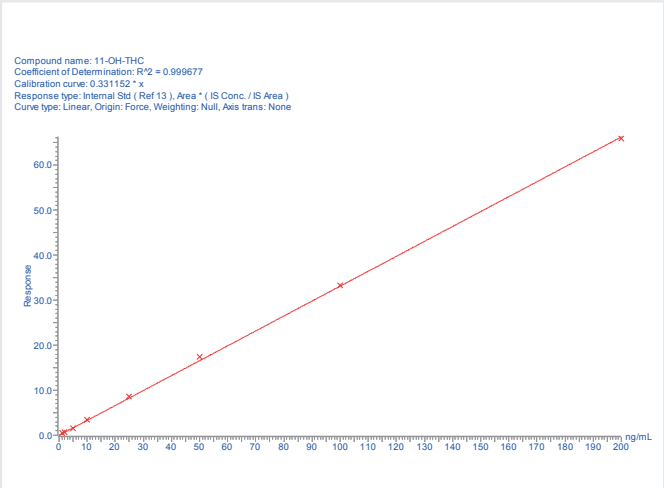

Figure 4. Typical Calibration line of 11-OH-THC at 1–200 ng/mL on ISOLUTE® SLE+ 1 mL sample volume columns

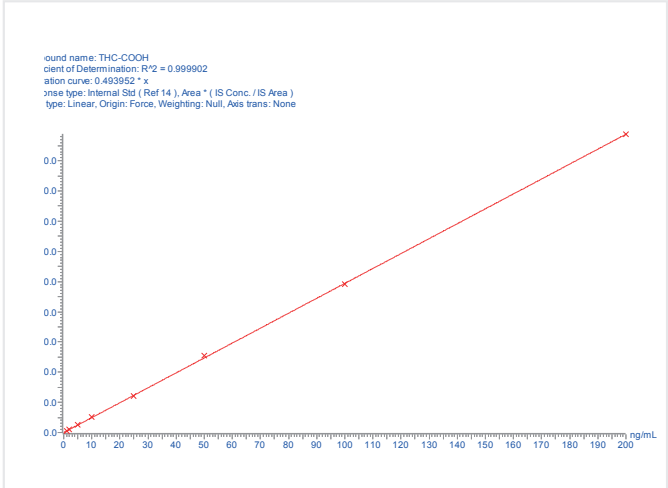

Figure 5. Typical Calibration line of THC-COOH at 1–200 ng/mL on ISOLUTE® SLE+ 1 mL sample volume columns

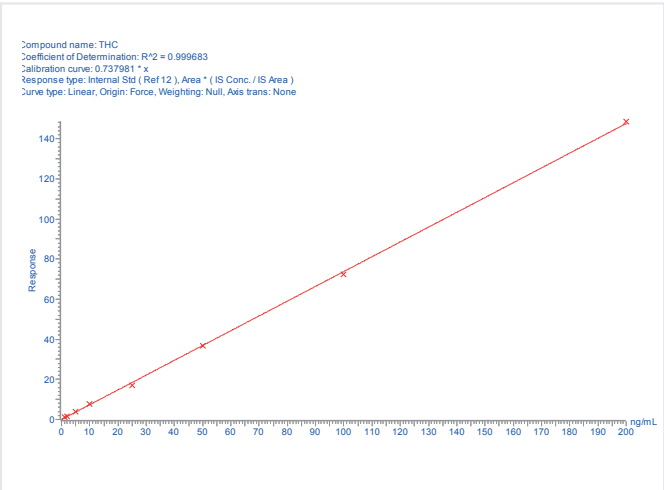

Figure 6. Typical Calibration line of THC at 1–200 ng/mL on ISOLUTE® SLE+ 1 mL sample volume columns

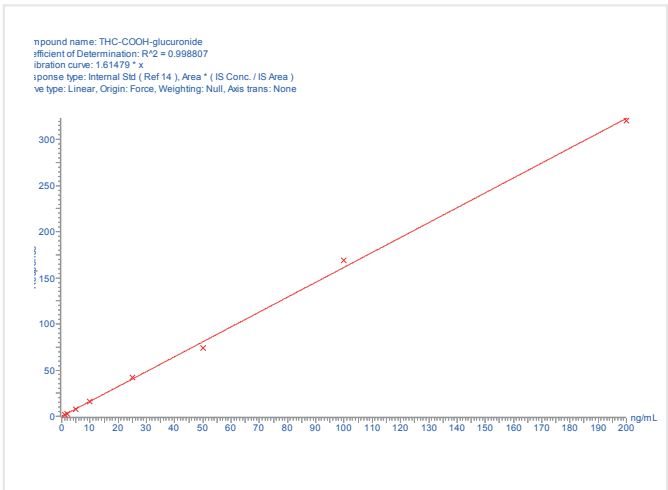

Figure 7. Typical Calibration line of THC-COOH glucuronide at 1-200 ng/mL on ISOLUTE® SLE+ 1 mL sample volume columns

Notes

This application note demonstrates LOQs down to 2 ng/mL. These LOQs were achieved using a 5 µL injection from 200 or 500 µL reconstituted sample. In order to achieve lower levels the injection volume could be increased and/or reconstitution volumes could be reduced.

Ordering Information

Part Number	Description	Quantity
820-0055-B	ISOLUTE® SLE+ 400 µL Sample Volume Columns	50
820-0140-C	ISOLUTE® SLE+ 1 mL Sample Volume Columns	30
PPM-48	Biotage® Positive Pressure Manifold 48 Position	1
C103199	TurboVap® 96 without racks 220/240V	1

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