# Extraction of THC, THCA and Carboxy-THC from Oral Fluid by ISOLUTE® SLE+ after Collection with the Intercept® Oral Fluid Drug Test Kit Prior to GC/MS Analysis

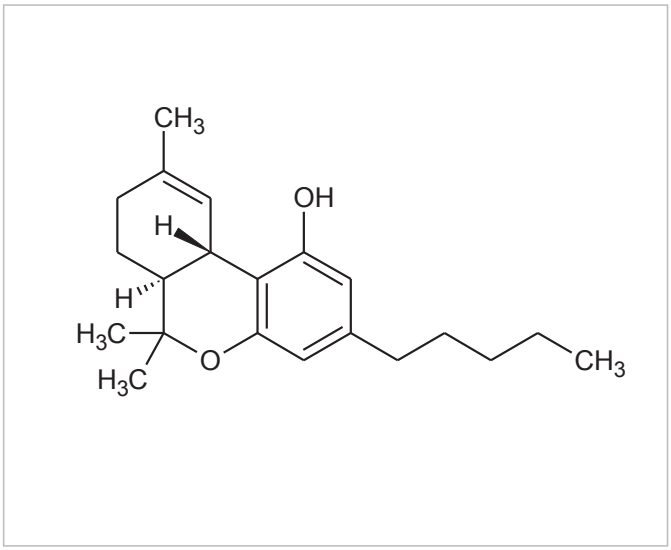


**Figure 1.** Structures of $\Delta^9$ -THC (tetrahydrocannabinol)

## Introduction

This application note describes the extraction of THC, THCA and Carboxy-THC from oral fluid matrix collected using the Intercept Oral Fluid Drug Test Kit (Orasure Technologies), prior to GC/MS analysis.

ISOLUTE® SLE+ Supported Liquid Extraction plates and columns offer an efficient alternative to traditional liquid-liquid extraction (LLE) for bio-analytical 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 80% 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 0.5% ammonium hydroxide (aq) (10 µL) to each collection device (see additional information). |
| **Format:** | **ISOLUTE® SLE+ 400 µL Supported Liquid Extraction columns, part number 820-0055-B** |
| **Sample loading:** | Load the pre-treated oral fluid (300 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 Supported Liquid Extraction columns, part number 820-0140-C** |
| **Sample loading:** | Load the complete contents of the pre-treated oral fluid device 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, 2.5 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).<br><br>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. |

## 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 |
| | 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 76-94% as shown in **Figure 2**. RSDs were below 10% for all analytes.

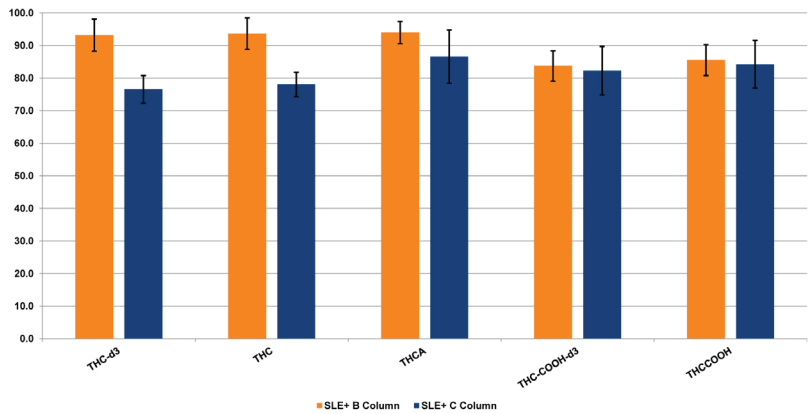


**Figure 2.** Typical extraction % recoveries (n=7) using the ISOLUTE® SLE+ protocol.

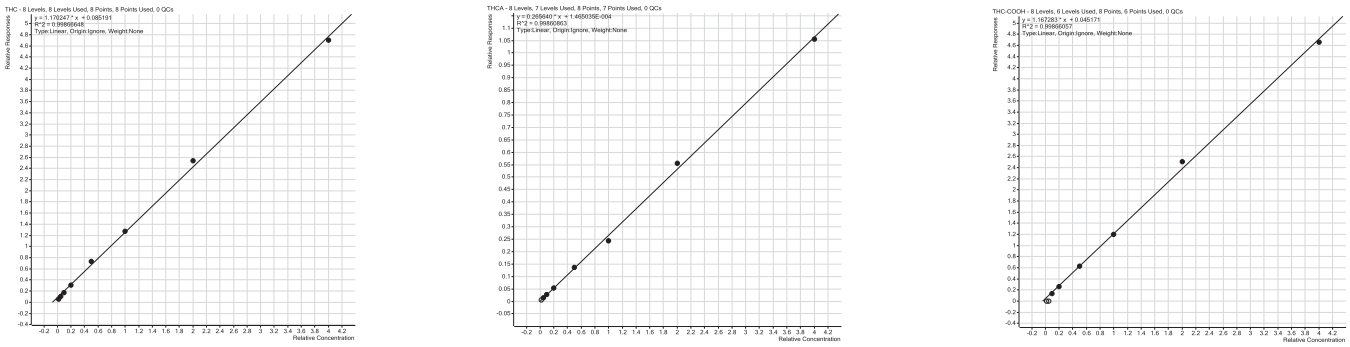

**Figure 3.** Calibration curves for extracted levels of spiked oral fluid after collection with Intercept devices, using 1 mL SLE+ format. Concentrations range from 4 ng/mL to 800 ng/mL showing $r^2$ values of approximately 0.9986 for the three analytes.

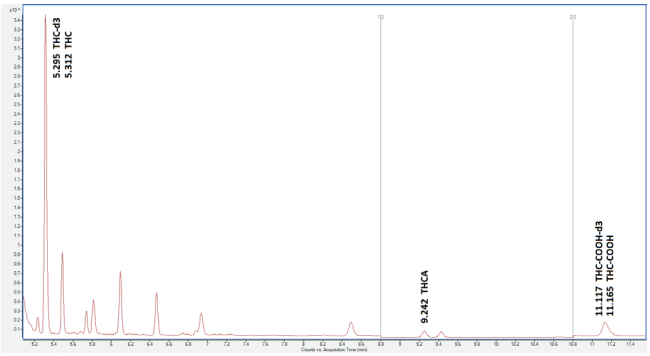


**Figure 4.** GC/MS chromatography for INTERCEPT collected oral fluid spiked at 40 ng/mL

## Additional information

1. 0.5 % ammonium hydroxide is prepared from concentrated stock (28-30%) by adding 50 µL in 10 mL HPLC grade water.
2. If a non-chlorinated solvent is required, MTBE (methyl-tert-butyl-ether) is a suitable alternative elution solvent.

**Table 3.** Lower Limits of Quantitation (LLOQ) using ISOLUTE® SLE+ procedure

| Analyte | Lower Limit Of Quantitation |
|---|---|
| THC | 4 ng/mL |
| THCA | 10 ng/mL |
| THC-COOH | 20 ng/mL |

## 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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