



## THC, THC-OH, and THC-COOH IN WHOLE BLOOD BY LC-MS/MS or GC-MS USING CLEAN SCREEN<sup>®</sup> EXTRACTION COLUMN

Part #

CSTHC206 – CLEAN SCREEN<sup>®</sup> THC 200 mg 6 mL Tube

or

CSDAU206 – CLEAN SCREEN<sup>®</sup> DAU 200 mg 6 mL Tube

SBSTFA-1-1 – SELECTRA-SIL<sup>®</sup> BSTFA w/ 1% TMCS

SMTBSTFA-1-1 - SELECTRA-SIL<sup>®</sup> MTBSTFA w/ 1% TBDMCS

SPYR-0-50 - SELECTRA-SIL<sup>®</sup> Pyridine

SLDA100ID21-5UM – Selectra<sup>®</sup> DA HPLC Column, 100 x 2.1mm, 5 µm

### 1. PREPARE SAMPLE:

To 1-2 mL of whole blood add internal standard(s)

Mix/vortex

Add dropwise while vortexing approximately 2.5 mL of **Ice Cold** acetonitrile

Centrifuge and transfer acetonitrile to a clean test tube

Adjust sample pH to 3.0± 0.5 with approx. 2 mL of 100 mM Sodium Acetate buffer

(Check pH of buffer to insure that the pH value is ~ 3.0)

Mix/Vortex

### 2. CONDITION CLEAN SCREEN<sup>®</sup> EXTRACTION COLUMN:

1 x 3 mL CH<sub>3</sub>OH.

1 x 3 mL D.I. H<sub>2</sub>O.

1 x 1 mL Acetate buffer (pH 3.0).

**NOTE:** Aspirate at full vacuum or pressure

### 3. APPLY SAMPLE:

Load at 1 to 2 mL/minute.

### 4. WASH COLUMN:

1 x 2 mL D.I. H<sub>2</sub>O

1 x 2 mL of 100 mM HCl/Acetonitrile (95:5)

**Aspirate at full vacuum or pressure for 5 minutes**

1 x 200 µL Hexane

**Aspirate at full vacuum or pressure for 5 minutes**

**Optional:** Dry column (5 minutes at greater than 10 inches HG/ Full Flow for Positive Pressure Manifold)

**Note:** The delta-9-THC (parent) will elute in hexane so special attention must be paid to not use more than 200 µL hexane in the wash/dry step. The 200 µL hexane in the wash step can be eliminated if the column is allowed to dry longer under vacuum or by positive pressure gas flow.

### 5. ELUTE THC (metabolites):

1 x 2 mL Hexane (optional, contains delta-9-THC)

1 x 3 mL Ethyl Acetate/ Hexane (50:50)

Collect eluate at 1-2 mL /minute

**Note:** Before proceeding, insure there are no water droplets at the bottom of the collection tube. This may increase drying time and decrease BSTFA derivatizing efficiency.

### 6. DRY ELUATE:

Evaporate to dryness at < 40 °C.

## 7. RECONSTITUTE / DERIVATIZE:

- **LC-MS/MS:** Reconstitute sample in 100  $\mu\text{L}$  of mobile phase  
Inject 5  $\mu\text{L}$
- **GC-MS:** Dissolve residue in 50  $\mu\text{L}$  of pyridine and 50  $\mu\text{L}$  BSTFA w/1%TMCS  
Overlay with  $\text{N}_2$  and cap. Mix/vortex  
React 30 minutes at 70°C; Cool and inject 2  $\mu\text{L}$

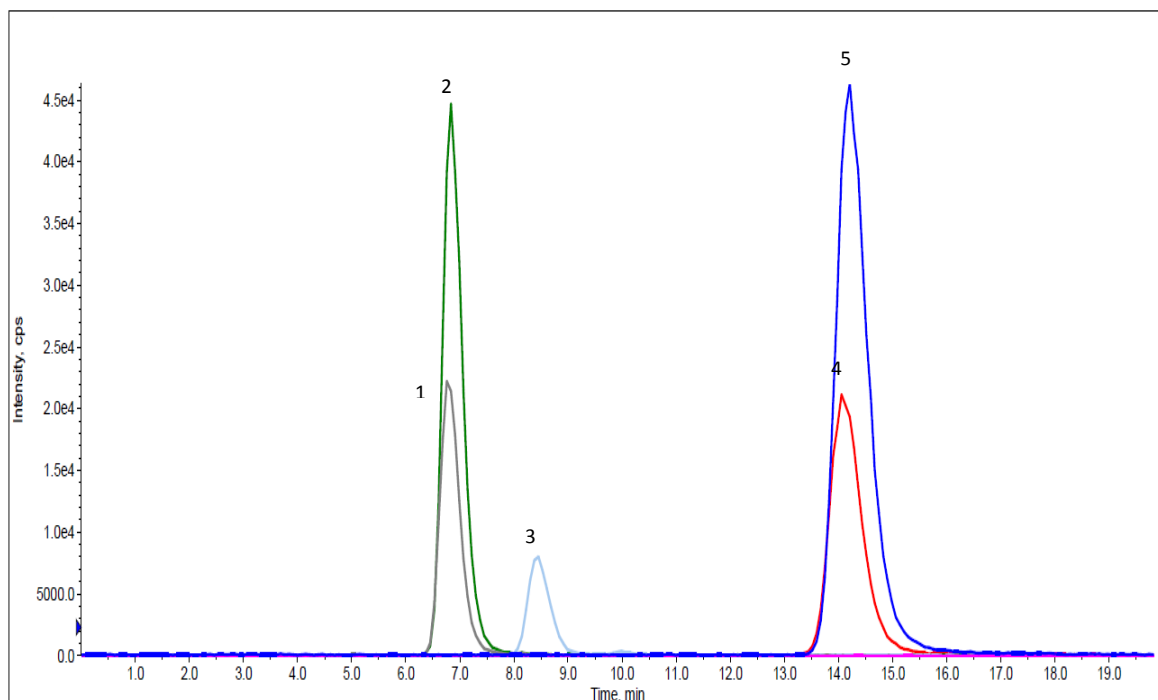
### Alternate Derivatization

#### 1. Derivatize with MTBSTFA (with 1% TBDMCS):

Dissolve residue in 50  $\mu\text{L}$  of pyridine and 50  $\mu\text{L}$  MTBSTFA w/ 1%TBDMCS  
Overlay with  $\text{N}_2$  and cap. Mix/vortex  
React 30 minutes at 70 °C; Cool and inject 2  $\mu\text{L}$

## INSTRUMENT CONDITIONS (LC-MS/MS):

### CHROMATOGRAM



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. HYDROXY DELTA 9-THC D <sub>3</sub>	334.0	316.2	6.80
2. HYDROXY DELTA 9-THC	330.9	313.2	6.88
3. CARBOXY DELTA 9-THC	343.0	299.1	8.47
CARBOXY DELTA 9-THC D <sub>3</sub> *	343.1	302.1	-
4. DELTA 9-THC D <sub>3</sub>	318.2	196.2	14.20
5. DELTA 9-THC	315.2	193.2	14.31

\*ion data provided for informational purposes only; not in run

## PARAMETERS

**Mobile Phase A:** 0.1% Formic Acid in D.I. H<sub>2</sub>O

**Flow Rate:** 0.5 mL/minute

**Reconstitute:** 100 µL

**Instrument:** API 4000 Qtrap MS/MS with Agilent 1200 Binary Pump SL

**LC Column:** Selectra<sup>®</sup> DA HPLC Column 100 x 2.1 mm 5 µm

**Mobile Phase B:** 0.1% Formic Acid in Methanol

**Polarity:** Negative/Positive

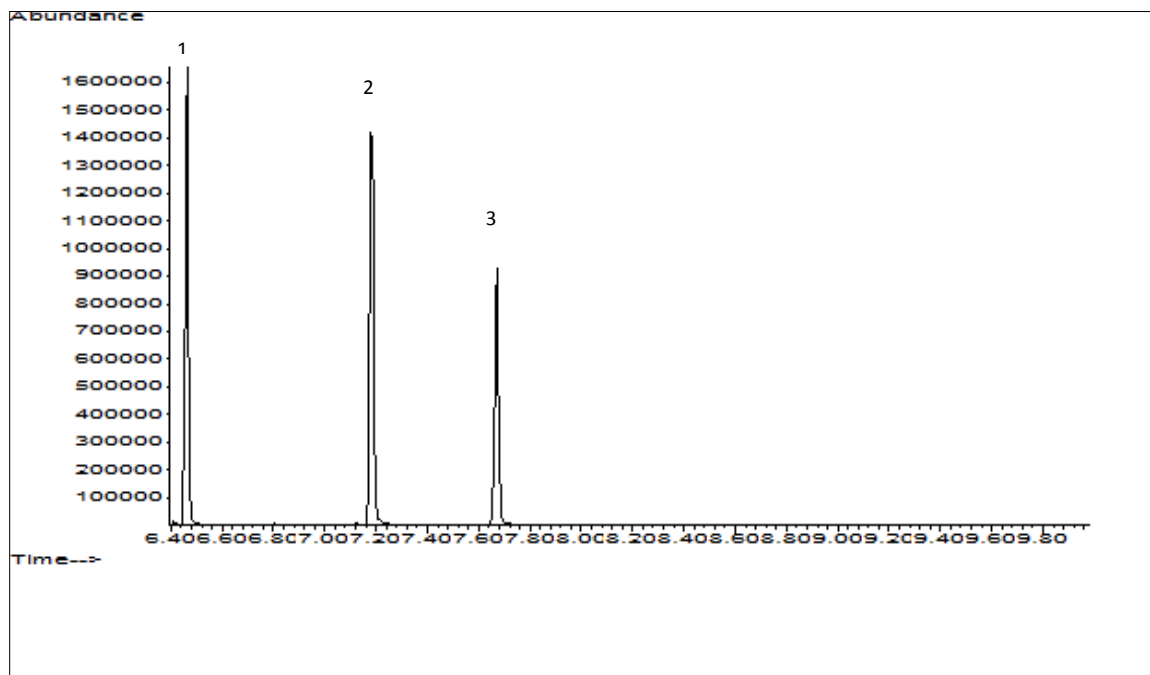
**Injection Volume:** 5 µL

**Isocratic:**

Time	%A	%B
0.00	25	75
20.00	STOP	

## **INSTRUMENT CONDITIONS (GC-MS):**

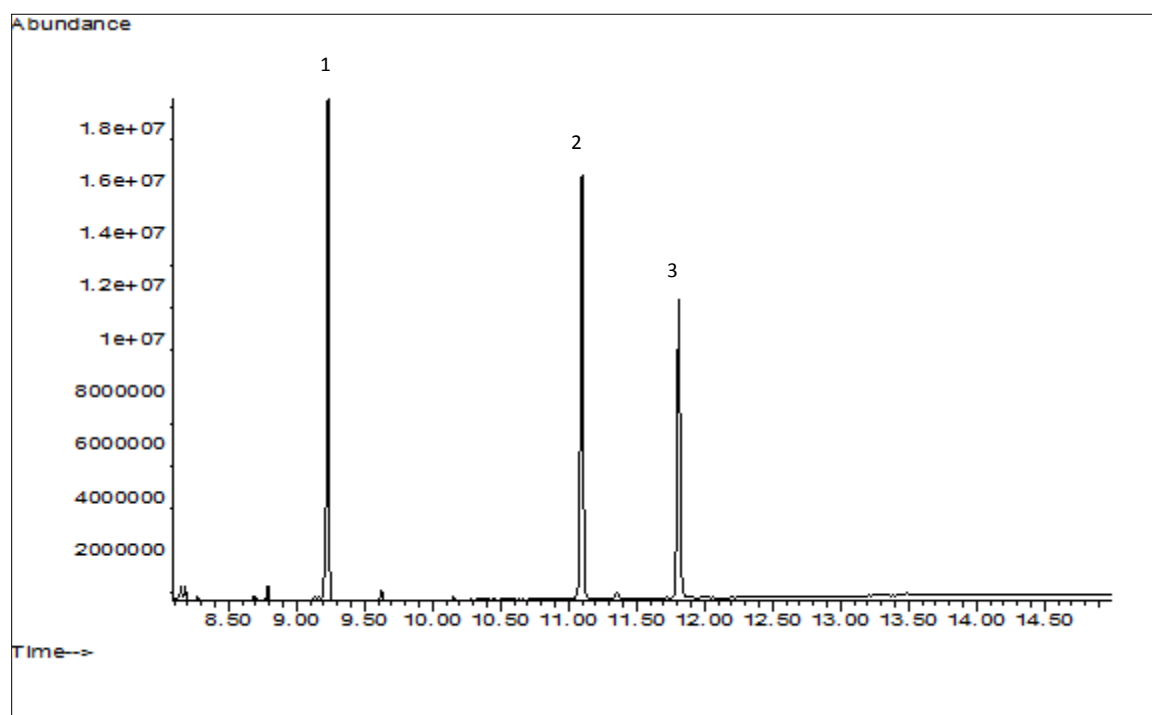
### CHROMATOGRAM



### TMS IONS

Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2	Relative Retention Time (min)
THC-D <sub>3</sub>	389.25	374.3		
1. THC	386.30	371.3	303.15	6.463
THC-OH D <sub>3</sub>	374.30	477.4		
2. THC-OH	371.3	474.4	459.3	7.178
THC-COOH D <sub>3</sub>	374.3	491.4		
3. THC-COOH	371.2	488.4	473.3	7.670

## CHROMATOGRAM



## TBDMS IONS

Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2	Relative Retention Time (min)
THC-D <sub>3</sub>	374.2	431.3		
1. THC	371.2	428.3	372.2	9.209
THC-OH D <sub>3</sub>	416.3	417.3		
2. THC-OH	413.3	369.2	414.3	11.075
THC-COOH D <sub>3</sub>	416.3	518.3		
3. THC-COOH	413.3	515.3	572.4	11.783

## PARAMETERS

**GC/MS:** Agilent - 5975C XL / 6890N GC/MS System with 7683B ALS System

**GC capillary column:** Rxi-5sil MS 30m X 0.25 mm, 0.25 µm

**Injector:** 2 µL Splitless, 250 °C

**Oven temperature program:** 100 °C for 1 min; 40 °C/min to 280 °C; 10 °C/min to 310 °C for 1.5 min

**Carrier gas:** Helium

**MSD condition:** Aux temperature: 280 °C, MS Source: 250 °C, MS Quad: 150 °C