



## THC, THC-OH, and THC-COOH CONFIRMATIONS IN WHOLE BLOOD BY LC-MS/MS or GC-MS USING 100 mg STYRESCREEN<sup>®</sup> SSTHC

Part #:

SSTHC116 – Styre Screen<sup>®</sup> THC 100 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 whole blood add appropriate internal standards prepared in alcohol  
Add drop-wise 2.5 mL **Ice Cold** acetonitrile  
Mix thoroughly and centrifuge  
Decant acetonitrile into a clean tube.  
Evaporate acetonitrile under a stream of air or nitrogen to ~ 200 µL  
Add 2 mL D.I. H<sub>2</sub>O (pH of H<sub>2</sub>O must be ~6.0-7.0)

### 2. APPLY SAMPLE:

Load at 1 to 2 mL/minute.

### 4. WASH COLUMN:

Wash with 2 mL (84: 15: 1) D.I. H<sub>2</sub>O: Acetonitrile: NH<sub>4</sub>OH (made fresh daily)  
Dry column under full vacuum or pressure for 10-15 minutes

### 5. ELUTE THC & metabolites:

1 x 3 mL Hexane/ Ethyl Acetate/ Glacial Acetic Acid (49: 49:2)  
Collect at 1-2 mL/ minute.

### 6. DRY ELUATE:

Evaporate to dryness at < 40 °C.

### 7. RECONSTITUTE / DERIVATIZE:

- **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase  
Inject 5 µL.
- **GC-MS:** Dissolve residue in 50 µL of pyridine and  
50 µL BSTFA w/ 1%TMCS  
Overlay with N<sub>2</sub> and cap. Mix/vortex  
React 30 minutes at 70 °C; Cool and inject 2 µL

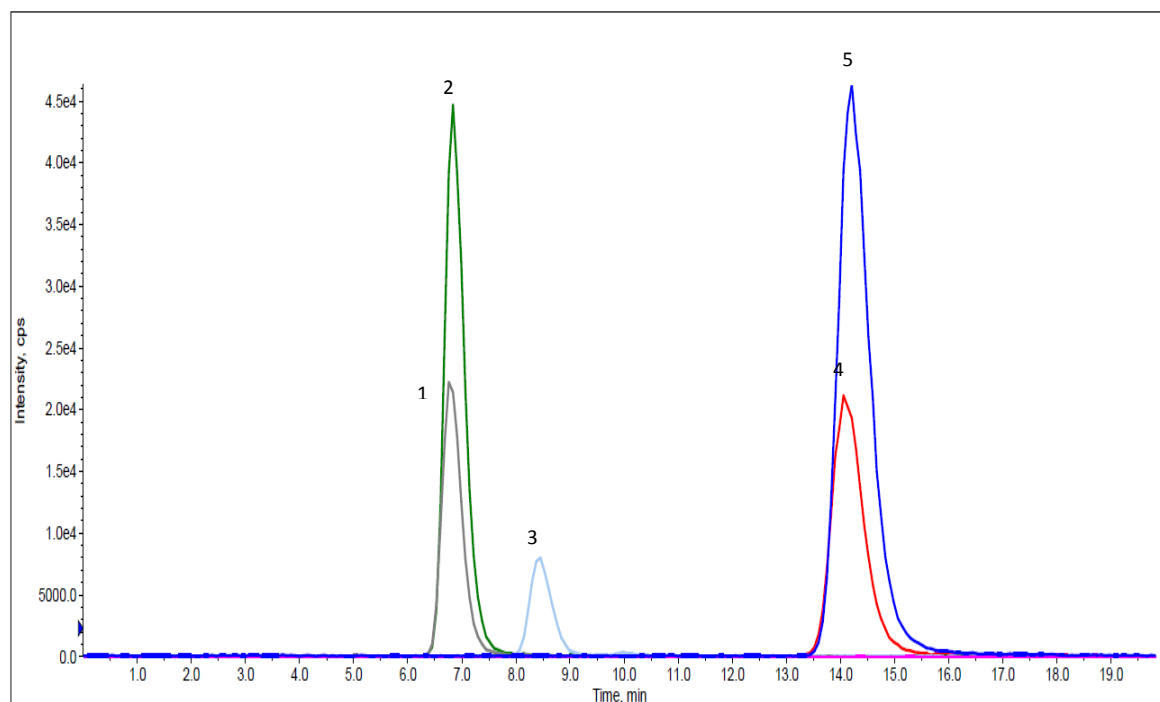
#### Alternate Derivatization

#### 1. Derivatize with MTBSTFA w/ 1% TBDMCS:

Dissolve residue in 50 µL of pyridine and 50 µL MTBSTFA w/ 1%TBDMCS  
Overlay with N<sub>2</sub> and cap. Mix/vortex  
React 30 minutes at 70°C; Cool and inject 2 µL

## 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	334.0	316.2	8.47
CARBOXY DELTA 9-THC D <sub>3</sub>	348.3	303.0	-
4. DELTA 9-THC D <sub>3</sub>	318.2	196.2	14.20
5. DELTA 9-THC	315.2	193.2	14.31

### PARAMETERS

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

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

**Flow Rate:** 0.5 mL/minute

**Polarity:** Negative/Positive

**Reconstitute:** 100 µL

**Injection Volume:** 5 µL

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

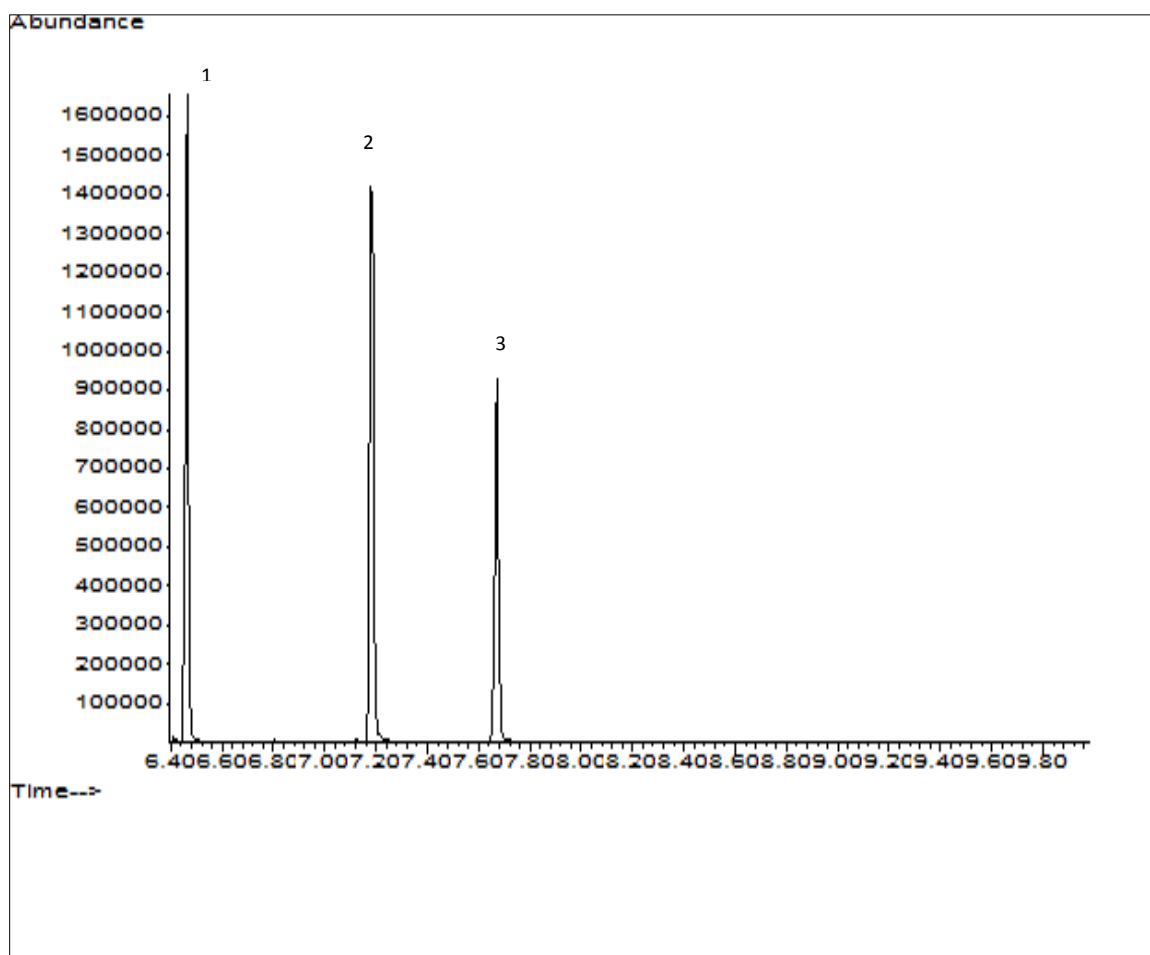
**LC Column:** Selectra<sup>®</sup> DA HPLC Column 100 x 2.1 mm 5 µm

**Isocratic:**

Time	%A	%B
0.00	25	75
20.00	STOP	

## INSTRUMENT CONDITIONS (GC-MS):

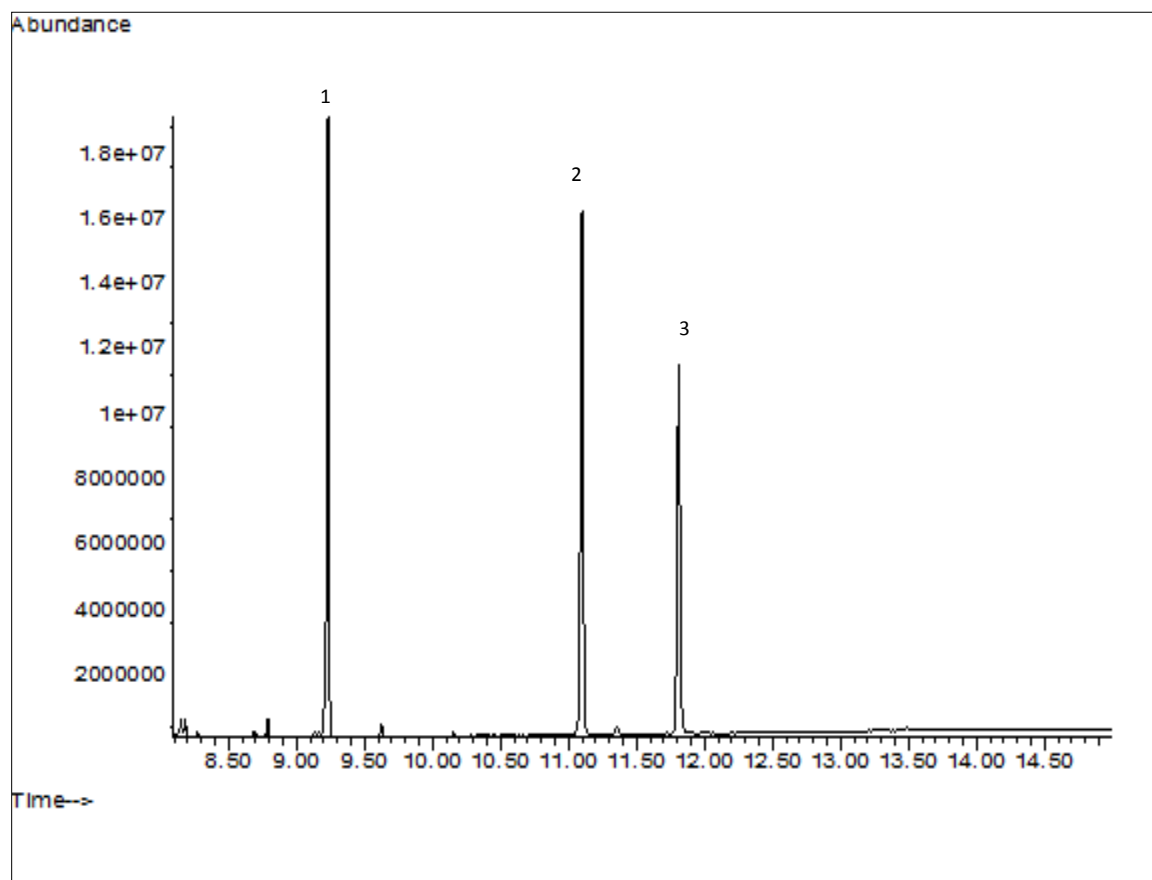
### CHROMATOGRAM



### 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