SYNTHETIC CANNABINOIDS "SPICE" DRUGS IN BLOOD, PLASMA/SERUM, URINE, TISSUE BY LC-MS/MS OR GC-MS CLEAN SCREEN® THC EXTRACTION COLUMN

Part #
CSTHC206 – CLEAN SCREEN® THC 200 mg 6 mL Tube
BETA-GLUC-10 – Selectrazyme® Beta-glucuronidase
SMSTFA-1-1 – SELECTRA-SIL® MSTFA w/ 1% TMCS
SLDA100ID21-3UM – Selectra® DA HPLC Column, 100 x 2.1 mm, 3 µm

1. **PREPARE SAMPLE:**

Blood: To 1 mL of 100 mM phosphate buffer (pH 6.0) add internal standards.
Add 1 mL of blood, plasma/ serum, or 1 g (1:4) tissue homogenate.
Mix/vortex and let stand for 5 minutes
Add 2 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex
Sample pH should be 6.0 ± 0.5.
Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate.
Centrifuge for 10 minutes at 2000 rpm and discard pellet

Urine: **PREPARE SAMPLE FOR ENZYME HYDROLYSIS OF GLUCURONIDES:**
To 1-2 mL of urine sample, add 1 mL of acetate buffer (pH 5.0) containing 5,000 units/mL of Selectrazyme® β-glucuronidase. Optionally, add 1 mL of acetate buffer and 25-50 µL of concentrated β-glucuronidase.
Vortex and heat for 1-2 hours at 65 °C.
Allow sample to cool
Do not adjust pH~ sample is ready to be added to the extraction column.

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN:**
1 x 3 mL CH_3OH.
1 x 3 mL D.I. H_2O.
1 x 3 mL 100 mM phosphate buffer (pH 6.0).

NOTE: Aspirate at full vacuum or pressure

3. **APPLY SAMPLE:**
Load at 1 to 2 mL/minute.

4. **WASH COLUMN:**
1 x 3 mL D.I. H_2O
1 x 3 mL of 100 mM phosphate buffer containing 20% Acetonitrile

Dry Column (5 minutes at >10inches Hg)

5. **ELUTE SPICE DRUGS:**
2 x 3 mL Ethyl Acetate containing 10% CH_3OH
Collect eluate at 1-2 mL /minute

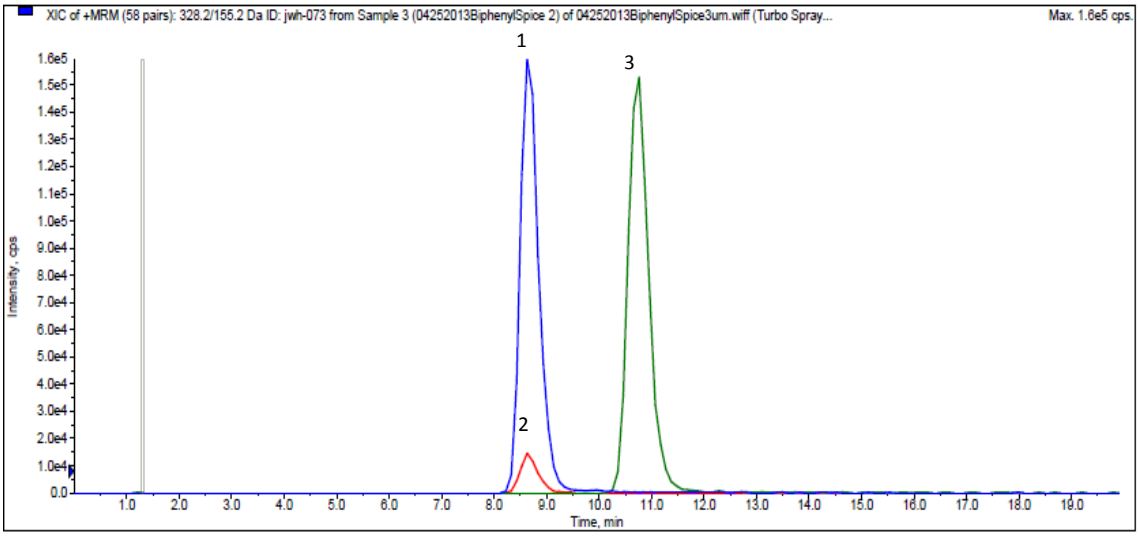
6. **DRY ELUATE:**
Evaporate to dryness at < 40 °C.

7. . RECONSTITUTE / DERIVATIZE:

- **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
  Inject 10 µL.
- **GC-MS**: Dissolve residue in 50 µL of Ethyl Acetate and
  50 µL MSTFA w/1% TMCS
  Overlay with N_2 and cap. Mix/vortex
  React 30 minutes at 70 °C; Cool and inject 1 µL

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM



PARAMETERS

Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. JWH015	**328.2**	**155.1**	**8.65**
2. JWH073	**328.2**	**155.2**	**8.66**
3. JWH018	**342.2**	**155.1**	**10.74**

Mobile Phase A: 0.1% Formic Acid in D.I. H_2O **Mobile Phase B:** 0.1% Formic Acid in Methanol

Flow Rate: 0.7 mL/minute **Polarity**: Positive

Reconstitute: 100 µL **Injection Volume**: 3 µL

LC Column: Selectra® DA HPLC Column 100 x 2.1 mm 3 µm

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

Isocratic:

Time	%A	%B
0.00	20	80
20.00	STOP	