HPLC Application

THC and Metabolites from Whole Blood on Strata-X-Drug B and Kinetex C18 by LC-MS-MS

| Column: | Kinetex® 2.6 | µm C18 100 Å, L | LC Column 50 | - x 2.1 mm, E | - Ea | | | | | | |
|---------------------------------|---|--|-----------------|------------------|--|--|--|--|--|--|--|
| Dimensions: | 50 x 2.1 mm I | | | | | | | | | | |
| Order No: | 00B-4462-AN | | | Kinetex* | | | | | | | |
| Elution Type: | Gradient | | | | Ultra-High Performance on Any LC System | | | | | | |
| Eluent A: | 1 mM ammoni | um formate with | า 0.1% FA | | | | | | | | |
| Eluent B: | 0.1% FA/meth | anol:acetonitrile | e(1:1) | | | | | | | | |
| Gradient | Step No. | Time (min) | Pct A | Pct B | | | | | | | |
| Profile: | 1 | 0 | 50 | 50 | | | | | | | |
| | 2 | 3 | 5 | 95 | Products used in this application: | | | | | | |
| | 3 | 4.5 | 5 | 95 | •• | | | | | | |
| | 4 | 4.51 | 50 | 50 | | | | | | | |
| | 5 | 6 | 50 | 50 | (KINETEX. | | | | | | |
| Flow Rate: | 0.4 mL/min | | | | <u> </u> | | | | | | |
| Col. Temp.: | ambient | | | | | | | | | | |
| Detection: | Mass Spectrometer (MS) @ amu (ambient) | | | | | | | | | | |
| Detector Info: Analyst Note: | <a <br="" target="_blank">href="https://sciex Mass spec conditior | <a <br="" target="_blank">href="https://sciex.com/products/mass-spectrometers?utm_campaign=2019%20application%20search&utm_source=phenomenex&utm_medium=referral">SCIEX Mass spec conditions: | | | | | | | | | |
| All all yes and a second | Polarity Analyte | Q1 | Q3 DP CE | | | | | | | | |
| | (+) | | 331.0 193.3 55 | | | | | | | | |
| | (+) | THC-OH | 331.0 201.2 | 55 33 | | | | | | | |
| | (+) | THC-OH | 331.0 175.3 | 55 31 | | | | | | | |
| | (+) | THC-OH-d3 | 334.0 196.3 55 | 5 31 | | | | | | | |
| | (+) | THC (*) | 315.2 193.1 60 | 50 31 | | | | | | | |
| | (+) | THC | 315.2 259.1 6 | 60 35 | | | | | | | |
| | (+) | THC-d3 | 318.2 196.1 60 | 50 25 | | | | | | | |
| | (+) | | 345.2 327.0 55 | | | | | | | | |
| | (+) | | | 49 | | | | | | | |
| | (+) | THC-COOH-d3 348 | | 29 | | | | | | | |
| | | GS2: 45 TEM: 600 CI | UR: 25 IS: 5500 | | | | | | | | |
| 19947 | * indicates quant ic | ภา | | | | | | | | | |
| 1,72,17 | | | | | | | | | | | |
| | | | | | | | | | | | |
| 5.3e5 5.0e5 | | | 6 | | | | | | | | |
| 4.5e5 | | | 5 | | | | | | | | |
| 4.0e5 | | | | | | | | | | | |
| 3.5e5 - | | | 2 | | | | | | | | |
| 3.0e5 | | | | | | | | | | | |
| 2.5e5 - | | | 4 | | | | | | | | |
| 2.0e5 | | | | | | | | | | | |
| 1.5e5 - | | | 3 | | | | | | | | |
| 1.0e5 | | | | | | | | | | | |
| 5.0e4 | | 1 | | | | | | | | | |
| 0.0 | | | All I | | | | | | | | |
| | 0.5 1.0 1 | .5 2.0 2.5 | 3.0 3.5 4.0 |) 4.5 5.0 | 5.5 min | | | | | | |

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HPLC Application ID No.: 19947



THC and Metabolites from Whole Blood on Strata-X-Drug B and Kinetex C18 by LC-MS-MS

ANALYTES:

- THC-OH 1
- 2 THC-OH-d3
- 3 THC-COOH
- THC-COOH-d3 4
- 5 THC
- THC-d3 6

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PRODUCT DESCRIPTION:

Strata[™]-X-Drug B 33 µm Polymeric Strong Cation, 60 mg / 6 mL, Tubes , 30/Pk

Order No.: 8B-S128-UCH

SOLID PHASE EXTRACTION (SPE) PRODCEDURE:

Note: The solvent volumes shown below are for a 60 mg bed mass.

The solvent volumes will need to be adjusted for a smaller or larger bed mass.

Condition:

Load:

Sample Pre-Treatment (before SPE):

Protein precipitation: To 0.5 mL whole blood (spiked with analytes), add 1.0 mL cold ACN:MeOH (85:15 v:v), vortex 1-2 mins (using a maximix II, from Barnstead) with the maximum force possible, followed by centrifugation at 10,000 rpm, for 10 mins. Discard pellet and dilute sample obtained from protein precipitation step with 3 mL of 1% Formic acid in water. Load sample directly onto cartridge - NO CONDITIONING REQUIRED

Wash:

Dry:

5 mins at 7-15 psi Nitrogen

Elute:

A

Final Prep and Analysis:

Evaporate eluant to dryness under 50 °C nitrogen. Reconstitute in 500uL of initial mobile phase. Extraction was automated and performed using a Gilson GX-274 ASPEC system

Inject: 5 µL on HPLC Mass Spectrometer (MS) @ amu (ambient)

| ANA | LYTES: | Spiked Conc. (ng/mL) | Log P | рКа | % Rec | %RSC (n=0) |
|-----|-------------|-------------------------|-------|-----|-------|---------------|
| 1 | THC-OH | 100 | | | 100.3 | |
| 2 | THC-OH-d3 | 100 | | | 100 | |
| 3 | THC-COOH | 100 | | | 103.9 | |
| 4 | THC-COOH-d3 | 100 | | | 100 | |
| 5 | THC | 100 | | | 99.3 | |
| 6 | THC-d3 | 100 | | | 100 | |
| | | | | | | |

Note: This method is designed as a convenient starting point for further investigation and can be tailored to meet your extraction goals. Call your local Phenomenex Representative for assistance in method development and optimization techniques.

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