# **HPLC Application**

ID No.: 19947



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

Kinetex® 2.6 µm C18 100 Å, LC Column 50 x 2.1 mm, Ea

50 x 2.1 mm ID **Dimensions:** Order No: 00B-4462-AN **Elution Type:** Gradient

Eluent A: 1 mM ammonium formate with 0.1% FA Eluent B: 0.1% FA/methanol:acetonitrile(1:1)

Gradient	Step No.	Time (min)	Pct A	Pct B
Profile:	1	0	50	50
	2	3	5	95
	3	4.5	5	95
	4	4.51	50	50
	5	6	50	50



Products used in this application:



0.4 mL/min Flow Rate: Col. Temp.: ambient

**Detection:** Mass Spectrometer (MS) @ amu (ambient)

**Detector Info:** <a target="\_blank"

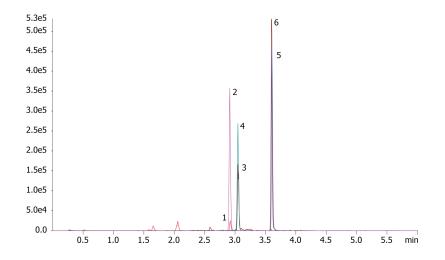
href="https://sciex.com/products/mass-spectrometers?utm\_campaign=2019%20application%20search&utm\_source=phenomenex&utm\_medium=referral">SCIEX< **Analyst Note:** 

Mass spec conditions:	•			_	 • •	_	•	_	
Polarity Analyte	01	03	DP	CE					

Polarity	Analyte		QΙ	Q3	DP	CI	=	
(+)		THC-OH (*)		331.0	193.3	55	3	31
(+)		THC-OH		33	1.0 2	01.2	55	33
(+)		THC-OH		33	1.0 1	75.3	55	31
(+)		THC-OH-d3		334.0	196.3	3 55	3	1
(+)		THC (*)		315.2	193	.1 6	0	31
(+)		THC		315.	2 259	.1 6	0	35
(+)		THC-d3		318.2	2 196	1 60	)	25
(+)		THC-COOH (	*)	345.2	327.0	55	29	)
(+)		THC-COOH	34	5.2 299	9.2 5	5 4	19	
(+)		THC-COOH-d	34	8.0 330	0.1 5	5 :	29	

CAD: 7 GS1: 60 GS2: 45 TEM: 600 CUR: 25 IS: 5500

19947



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<sup>\*</sup> indicates quant ion

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# **ANALYTES:**

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

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# **Sample Preparation Details**

for HPLC Application ID No.: 19947



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

#### **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:		
Collaition		

#### 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:		

#### **Final Prep and Analysis:**

Evaporate eluant to dryness under 50  $^{\circ}$ 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)

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