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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