

HPLC Application

ID No.: 19947

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, LC Column 50 x 2.1 mm, Ea

Dimensions: 50 x 2,1 mm ID

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 Profile:	Step No.	Time (min)	Pct A	Pct B
	1	0	50	50
	2	3	5	95
	3	4,5	5	95
	4	4,51	50	50
	5	6	50	50

Flow Rate: 0,4 mL/min

Col. Temp.: ambient

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

Detector Info: AB Sciex API 5000™

Analyst Note: Mass spec conditions:

Polarity	Analyte	Q1	Q3	DP	CE
(+)	THC-OH (*)	331.0	193.3	55	31
(+)	THC-OH	331.0	201.2	55	33
(+)	THC-OH	331.0	175.3	55	31
(+)	THC-OH-d3	334.0	196.3	55	31
(+)	THC (*)	315.2	193.1	60	31
(+)	THC	315.2	259.1	60	35
(+)	THC-d3	318.2	196.1	60	25
(+)	THC-COOH (*)	345.2	327.0	55	29
(+)	THC-COOH	345.2	299.2	55	49
(+)	THC-COOH-d3	348.0	330.1	55	29

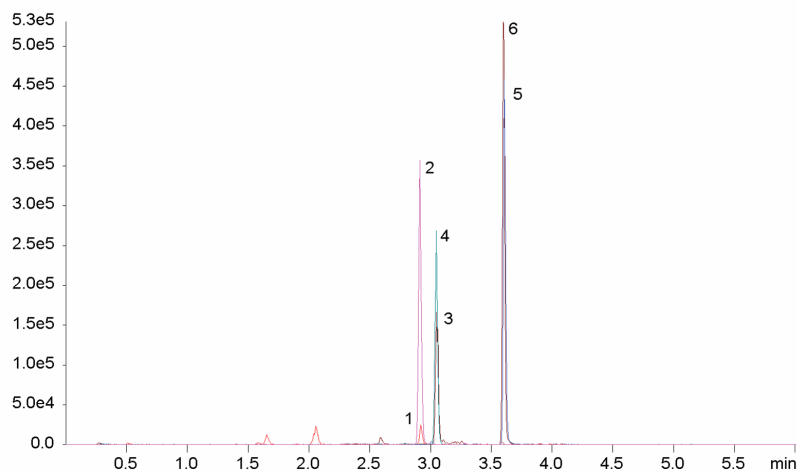
CAD: 7 GS1: 60 GS2: 45 TEM: 600 CUR: 25 IS: 5500

* indicates quant ion

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Products used in this application:



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

ANALYTES:

- 1 THC-OH (MRM: 331.0>193.3)
- 2 THC-OH-d3 (MRM: 334.0>196.3 (Internal Standard))
- 3 THC-COOH (MRM: 345.2>327.0)
- 4 THC-COOH-d3 (MRM: 348.0>330.1 (Internal Standard))
- 5 THC (MRM: 315.2>193.1)
- 6 THC-d3 (MRM: 318.2>196.1 (Internal Standard))



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:

StrataTM-X-Drug B 33 μ m Polymeric Strong Cation, 60 mg / 6 mL, Tubes , 30/Pk

Order No.: 8B-S128-UCH

SOLID PHASE EXTRACTION (SPE) PROCEDURE:

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:

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 μ L on HPLC Mass Spectrometer (MS) @ amu (ambient)

ANALYTES:	Spiked Conc. (ng / mL)	Log P	pKa	% Rec	% RSC (n = 0)
1 THC-OH	100			100,3	3,1
2 THC-OH-d3	100			100	0,1
3 THC-COOH	100			103,9	5,4
4 THC-COOH-d3	100			100	0,1
5 THC	100			99,3	3,9
6 THC-d3	100			100	0,1

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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