HPLC Application

ID No.: **24195**

Extraction of unconjugated Bile acids from Human Serum on Kinetex 2.6µm Polar C18 100x2.1 Column

Column: Kinetex® 2.6 µm Polar C18 100 Å, LC Column 100 x 2.1 mm, Ea
Dimensions: 100 x 2.1 mm ID
Order No: 00D-4759-AN
Elution Type: Gradient
Eluent A: 2mM Ammonium acetate (pH 6.9)
Eluent B: Methanol/Acetonitrile (50-50)

Gradient Profile:

Step No.	Time (min)	Pct A	Pct B
1	0	55	45
2	9	30	70
3	9.5	30	70
4	9.51	55	45
5	12	55	45

Flow Rate: 400 mL/min
Col. Temp.: 50 °C
Detection: Mass Spectrometer (MS) @ amu (50 °C)
Detector Info: <a target="_blank" href="https://sciex.com/products/mass-spectrometers?utm_campaign=2019%20application%20search&utm_source=phenomenex&utm_medium=referral">SCIEX<,

Products used in this application:

KINETEX®

Analyst Note:

Sample Prep Protocol

Dispense: 400 uL methanol into the wells of the Impact plate

Add: 100 uL of doubly stripped Serum sample (spiked with analytes at 200ng/mL) directly into the organic solvent in each well of the plate.

Vortex: 2 minutes at maximum possible speed.

Wait: Allow 5 minutes for completion of protein precipitation.

Vacuum: Place the Impact plate onto a suitable 96-well SPE manifold followed by positioning a 96-well collection plate inside, under the Impact plate. Vacuum at 5" of Hg until filtrate is collected completely.

Dilute & inject: Dispense 300 uL of mobile phase A (or water) into the collection plate, vortex for 30 secs at a high speed and inject on LC-MS-MS

Note: A doubly stripped serum sample was employed for extraction purposes to eliminate the potential bias due to presence of any endogenous bile acids, leading to erroneous analyte quantitation.

Table 1. % Absolute Recovery for Bile acids from Human Serum Extraction on an Impact Protein Precipitation Plate

Analyte	% Recovery	% CV (N=5)
UDCA	91%	1.1
GCDCA	90%	3.7
CA	88%	4.8
GDCA	90%	4.4
TDCA	94%	3.5
CDCA	90%	4.5
DCA	88	4.6
LCA	90%	6.9

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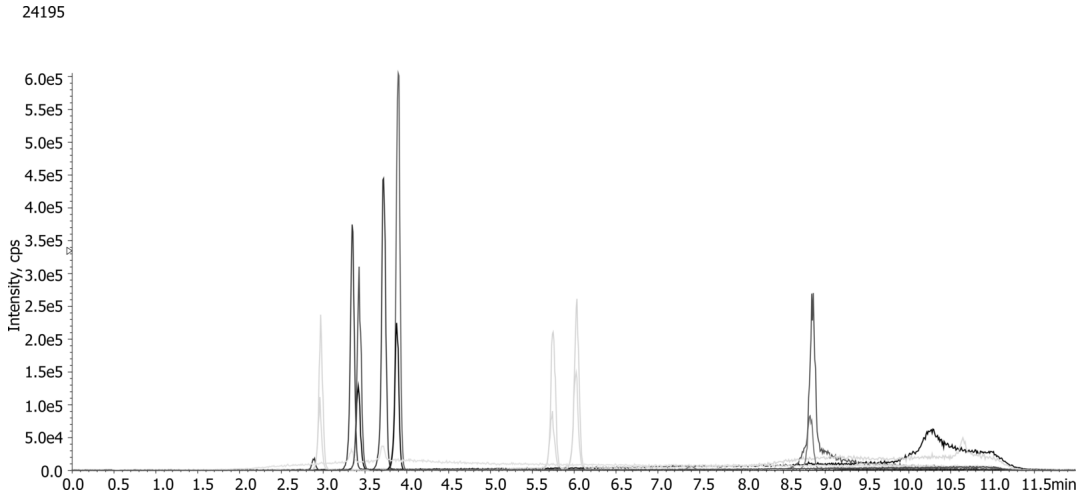

Extraction of unconjugated Bile acids from Human Serum on Kinetex 2.6µm Polar C18 100x2.1 Column

ANALYTES:

1 UDCA
2 UDCA-D4
3 GCDCA
4 GCDCA-D4
5 CA
6 CA-D4
7 GDCA
8 GDCA-D4
9 TDCA
10 TDCA-D4
11 CDCA
12 CDCA-D4
13 DCA
14 DCA-D4
15 LCA
16 LCA-D4

Sample Preparation Details

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PRODUCT DESCRIPTION:

Impact™ Protein Precipitation, 2mL Square Well Filter Plate, 2/Pk

Order No.: CE0-7565

SOLID PHASE EXTRACTION (SPE) PRODCEDURE:

Note: The solvent volumes shown below are for a Proprietary bed mass.
The solvent volumes will need to be adjusted for a smaller or larger bed mass.

Condition:

Load:

Wash:

Dry:

Elute:

Final Prep and Analysis:

Sample Prep Protocol
Dispense: 400 uL methanol into the wells of the Impact plate

Inject: 5 µL on HPLC Mass Spectrometer (MS) @ amu (50°C)

ANALYTES:	Spiked Conc. (ng/mL)	Log P	pKa	% Rec	%RSC (n=0)
1 UDCA	0			91	
2 UDCA-D4	0				
3 GCDCA	0			90	
4 GCDCA-D4	0				
5 CA	0			88	
6 CA-D4	0				
7 GDCA	0			90	
8 GDCA-D4	0				
9 TDCA	0			94	
10 TDCA-D4	0				
11 CDCA	0			90	
12 CDCA-D4	0				
13 DCA	0			88	
14 DCA-D4	0				
15 LCA	0			90	
16 LCA-D4	0				

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.*