

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"

Analyst Note: href="https://sciex.com/products/mass-spectrometers?utm_campaign=2019%20application%20search&utm_source=phenomenex&utm_medium=referral">SCIEX<

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

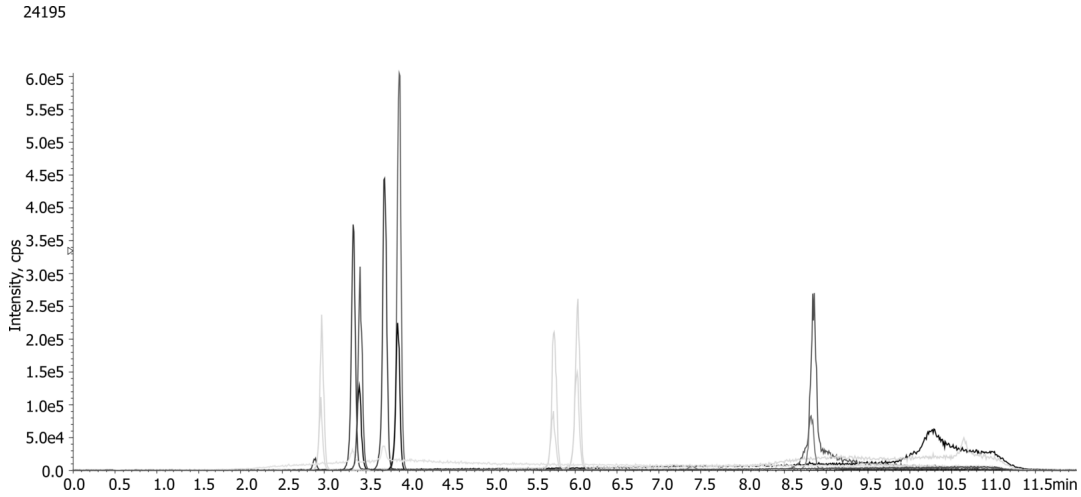
Analyte	% Recovery	% CV (N=5)
UDCA	91%	1.1
GCDCA	90%	3.7
CA	88%	4.8
GDCA	90%	4.4
TDCA	94%	3.5
CDCA	90%	4.5
DCA	88	4.6
LCA	90%	6.9



Products used in this application:



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



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

ANALYTES:

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



Sample Preparation Details

for HPLC Application ID No.: 24195

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

PRODUCT DESCRIPTION:

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

Order No.: CE0-7565

SOLID PHASE EXTRACTION (SPE) PROCEDURE:

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 µL 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	1.1
2 UDCA-D4	0				
3 GCDCA	0			90	3.7
4 GCDCA-D4	0				
5 CA	0			88	4.8
6 CA-D4	0				
7 GDCA	0			90	4.4
8 GDCA-D4	0				
9 TDCA	0			94	3.5
10 TDCA-D4	0				
11 CDCA	0			90	4.5
12 CDCA-D4	0				
13 DCA	0			88	4.6
14 DCA-D4	0				
15 LCA	0			90	6.9
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.

©2021 Phenomenex Inc. All rights reserved.

For more information contact your Phenomenex Representative at info@phenomenex.com



Phenomenex products are available worldwide.

www.phenomenex.com

info@phenomenex.com