

## 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:** Electrospray Mass Spec (ESMS) @ 0.0000000000 (50 °C)

**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

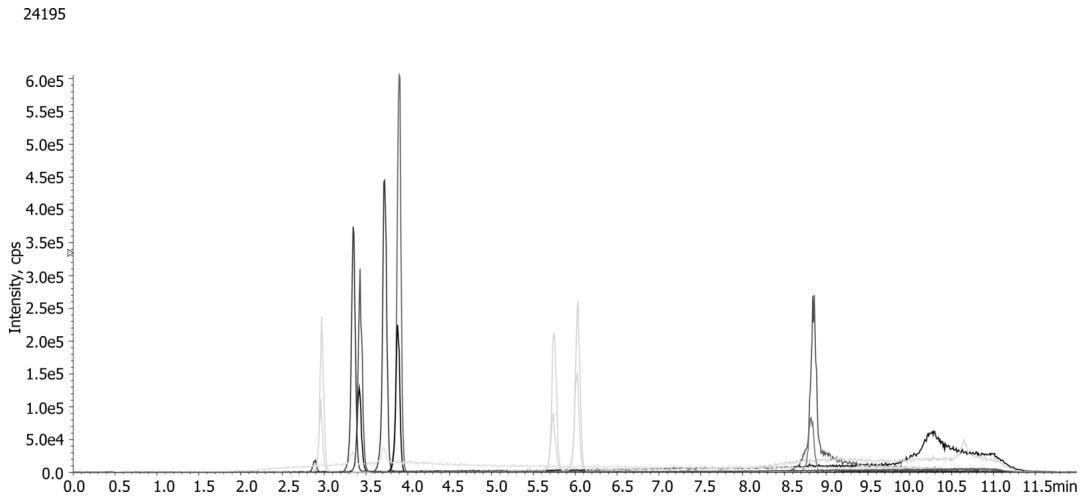
Analyte	% Recovery	% CV (N=5)
UDCA	91%	1.1
GCDCA	90%	3.7
CA	88%	4.8
GDCA	90%	4.4
TDCA	94%	3.5
CDCA	90%	4.5
DCA	88	4.6
LCA	90%	6.9



Products used in this application:



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### 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

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 Electrospray Mass Spec (ESMS) @ 0.0000000000 (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.

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For more information contact your Phenomenex Representative at [international@phenomenex.com](mailto:international@phenomenex.com).



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