RPC UPLC-QTOF Analysis of Lipids in Human Plasma and Serum - Proforma

NPC.PRO.MS003 Version 2.1

Effective Date: April 2019

1. Purpose

The purpose of this proforma is to document the Ultra Performance Liquid Chromatography (UPLC) Mass Spectrometer (MS) system reversed phase chromatographic (RPC) Lipid assay as outlined in the protocol NPC.SOP.MS003. This proforma should be used in combination with this SOP. The required sections of this proforma should be printed on the day of use, completed and then stored with all project specific documentation.

2. Proforma Approval

Prepared by Dr Verena Horneffer-van der Sluis	Date
Approved by Dr Maria Gomez-Romero	Date
Authorised by Dr Matthew Lewis	Date

Reagents

Assay specifics			
Analyst:			
Project:		**Date:**	
Chemical	**Supplier, P/N**	**Batch/lot no.**	**Opened date**
LPC(9:0)	Avanti, 855276P		
PC(11:0/11:0)	Avanti, 850330P		
FA(17:0)	Sigma, H3500		
PG(15:0/15:0)	Avanti, 840446P		
PE(15:0/15:0)	Avanti, 850704P		
PS(17:0/17:0)	Avanti, 840028P		
PA(17:0/17:0)	Avanti, 830856P		
Cer(d18:1/17:0)	Avanti, 860517P		
DG(19:0/19:0)	Sigma, 68633		
PC(23:0/23:0)	Avanti, 850372P		
TG(15:0/15:0/15:0)	Sigma, T4257		
TG(17:0/17:0/17:0)	Sigma, T2151		
LCMS grade acetic acid			
LCMS grade ammonium acetate			
LCMS grade acetonitrile			
LCMS grade isopropanol			
LCMS grade water			
Phosphoric acid (85-90%)			

PART A - Lipid Standard Mixture Preparation

Please, indicate an action has been performed by ticking the appropriate box (☐)

Assay specifics					
Analyst:					
Project:				**Date:**	
Lipid stock solutions					
Weigh each lipid standard individually into the corresponding volumetric flask outlined below and record the weight and volume (quantities can be scaled up or down depending on requirements)					☐
Make each volumetric flask up to volume with isopropanol					☐
Lipid	**Weight (mg)**	**Volume of isopropanol (mL)**	**Target stock conc. (mg/mL)**	**Weight (mg)**	**Volume (mL)**
LPC(9:0)	5	10	0.5		
PC(11:0/11:0)	5	50	0.1		
FA(17:0)	10	100	0.1		
PG(15:0/15:0)	5	500	0.01		
PE(15:0/15:0)	5	500	0.01		
PS(17:0/17:0)	20	200	0.1		
PA(17:0/17:0)	5	50	0.1		
Cer(d18:1/17:0)	5	50	0.1		
DG(19:0/19:0)	40	200	0.2		
PC(23:0/23:0)	5	50	0.1		
TG(15:0/15:0/15:0)	10	100	0.1		
TG(17:0/17:0/17:0)	10	100	0.1		
Sonicate stock solutions until sample dissolution observed					☐
Comments: n/a ☐					

Assay specifics

Analyst:	
Project:	**Date:**

Lipid standard mixture

Transfer each lipid standard stock solution prepared above to a 2 L volumetric flask according to the quantities outlined in the table below using appropriate automatic pipettes or measuring cylinders

Lipid	**Vol. of stock in mix (mL)**	**Target conc. (µg/mL) in 2 L of isopropanol**	**Final conc. (µg/mL) in 2 L**	
LPC(9:0)	1	0.25		☐
PC(11:0/11:0)	5	0.25		☐
FA(17:0)	50	2.50		☐
PG(15:0/15:0)	200	1.00		☐
PE(15:0/15:0)	50	0.25		☐
PS(17:0/17:0)	120	6.00		☐
PA(17:0/17:0)	20	1.00		☐
Cer(d18:1/17:0)	1	0.05		☐
DG(19:0/19:0)	120	12.00		☐
PC(23:0/23:0)	5	0.25		☐
TG(15:0/15:0/15:0)	50	2.50		☐
TG(17:0/17:0/17:0)	50	2.50		☐
Make the 2 L volumetric flask up to volume with isopropanol				☐
Mix the volumetric flask until the contents is visually homogenous				☐
Aliquot the Lipid standard mixture and store at -20 °C until required				☐

Comments: n/a ☐

PART B - Long Term Reference (LTR), Study Reference (SR) and SR Dilution Series Preparation - *On the Day of MS-SR preparation*

Please, indicate an action has been performed by ticking the appropriate box (☐)

Assay specifics	
Analyst:	
Project:	**Date:**
LTR	
Remove per sample batch one 11.5 mL stock plasma/serum LTR from storage at -80 °C and allow to defrost for 2 hours at 2-8 ºC (1 tube per 2 sample batches)	☐
Aliquot 450 µL of plasma/serum LTR in 25 separate Eppendorf tubes	☐
Store at -80 °C until required	☐
SR	
Aliquot 450 µL of undiluted pooled MS-SR per sample plate in _____ separate Eppendorf tubes.	☐
Aliquot 100 µL of SR into at least 7 separate Eppendorf tubes (1 set per sample batch, ≤ 1000 samples) for instrument conditioning, start/end SR, and DIDA	☐
Store at -80 °C until required	☐
Comments: n/a ☐	

Table continues

Assay specifics

Analyst:

Project:	**Date:**

No. of samples:_____ No. of batches:_____ (Sample batch is ≤1000 samples)

Total number of dilution series sets required (sample batch x (4 + 2 backup)) : _____

The table below indicates the volumes required for a single sample set (6 dilution series). This will be followed for studies consisting of 1000 samples or less. Please cross through and include an appropriate table as an attachment to this document if the number of samples in the study is more than 1000 samples

For ≤1000 samples

Dilution point	Percentage of SR (%)	Vol. of SR (µL)	Vol. of LCMS grade water (µL)	Total vol. (µL)	Vol. in aliquot (µL)	
1	100	450	0	450	70	☐
2	80	240	60	300	45	☐
3	60	150	100	250	35	☐
4	40	100	150	250	35	☐
5	20	100	400	500	45	☐
6	10	50	450	500	70	☐
7	1	10	990	1000	70	☐
Blank	0	0	500	500	70	☐

Aliquot each dilution point into 6 separate Eppendorf tubes according to the volumes detailed above (Vol. in aliquot) and store at -80 °C until required. ☐

Comments: n/a ☐

PART C - SR Dilution Series, SR and Blanks - *On the Day of Analysis*

Please, indicate an action has been performed by ticking the appropriate box (☐)

Assay specifics				
Analyst:				
Project:			**Date:**	
Remove 1 set of SR dilution series aliquots per polarity from storage at -80 °C, appropriate instrument conditioning or DIDA SR, and blanks from storage at -80°C and allow to defrost for 2 hours at 2-8 ºC				☐
Remove Lipid IPA standard mix from storage -20 °C and allow to defrost for 2 hours at 2-8 ºC.				☐
Dilution Series				
Complete all pre-project system performance checks as given NPC.SOP.MS002				☐
Add the corresponding volume of the lipid standard mixture to the SR dilution aliquot as per the table below using pipettes, then vortex mix				☐
Dilution point	**Percentage of SR (%)**	**Vol. in aliquot (µL)**	**Vol. of Lipid mix (µL)**	
1	100	70	280	☐
2	80	45	180	☐
3	60	35	140	☐
4	40	35	140	☐
5	20	45	180	☐
6	10	70	280	☐
7	1	70	280	☐
Blank	0	70	280	☐
Mix for 2 hours on a plate mixer at 1400 rpm at 4 °C				☐
Centrifuge at 3486 g for 10 minutes at 4ºC				☐
Aliquot supernatant into labelled 300 µL vials				☐
Place vials in correct positions in autosampler				☐

Table continues

Assay specifics	
Analyst:	
Project:	**Date:**
100 µL SR aliquot(s) and Lipid IPA were allowed to defrost for 2 hours at 2-8 ºC. Number of Eppendorf tube defrosted: _____	☐
Instrument Conditioning or DIDA (100% sample)	
Add 400 µL of Lipid IPA standard mixture in each tube	☐
Mix for 2 hours on a plate mixer at 1400 rpm at 2-8 °C	☐
Centrifuge at 3486 g for 10 minutes at 4 ºC	☐
Pipette supernatant into labelled 300 µL vials and seal with foil lined caps	☐
Start/End SR (50% sample)	
Add 100 µL of LCMS grade water to each tube	☐
Mix for 5 min at 1400 rpm at 2-8 °C	☐
Add 100 µL of Lipid IPA standard mixture to each tube	☐
Mix for 2 hours on a plate mixer at 1400 rpm at 2-8 °C	☐
Centrifuge at 3486 g for 10 minutes at 4 ºC	☐
Pipette supernatant into labelled 300 µL vials and seal with foil lined caps	☐
Blanks	
Pipette 100 µL of LCMS grade water in an Eppendorf	☐
Add 400 µL of Lipid IPA standard mixture	☐
Mix for 2 hours on a plate mixer at 1400 rpm at 2-8 °C	☐
Centrifuge at 3486 g for 10 minutes at 4 ºC	☐
Pipette into labelled 300 µL vials and seal with foil lined caps	☐
Comments: n/a ☐	

PART D - Sample Preparation

Please, indicate an action has been performed by ticking the appropriate box (☐)

Assay specifics		
Analyst:	**Date:**	
Project:	**Plate Identity:**	
1	Thaw sample plate, LTR, SR and Lipid IPA standard mixture at 2-8 °C for of 2 hours	☐
2	Centrifuge lipid plate at 3486 g for 1 minute at 2-8 °C	☐
3	Remove carefully seal cap mat and place side up on a clean surface	☐
4	Add 50 µL of plasma/serum LTR into column 11 of the plate	☐
5	Add 50 µL of plasma/serum SR into column 12 of the plate	☐
6	Add 50 µL of LCMS grade water into each well	☐
7	Cover the plate with the seal cap mat	☐
8	Mix for 5 min on a plate mixer at 1400 rpm at 2-8 °C	☐
9	Centrifuge lipid plate at 3486 g for 1 minute at 2-8 °C	☐
10	Remove carefully seal cap mat and discard	☐
11	Add 400 µL of the Lipid IPA standard mixture to each well	☐
12	Heat seal the sample plate with foil seal	☐
13	Mix for 2 hours on a plate mixer at 1400 rpm at 2-8 °C	☐
14	Centrifuge sample plate at 3486 g for 10 minutes at 4 °C	☐
15	Label three 96 well microplates, one for positive, one for negative and one for backup; with LPOS, LNEG, BACKUP, project name, date and barcode label	☐

Table continues

Continuation -PART D - Sample Preparation		
16	Carefully remove heat seal foil from the sample plate *(without disturbing the pelleted material)*	☐
17	Transfer 100 µL of each sample to the corresponding well in the analytical plates (LPOS, LNEG and BACKUP)	☐
18	Heat seal all analytical plates with heat seal foil	☐
19	Log the plates in LIMS	☐
20	Centrifuge LPOS and LNEG plates at 3486 g for 5 minutes at 4 ºC	☐
21	Store BACKUP plate at -80 ºC	☐
22	Place LPOS and LNEG plates in relevant autosamplers	☐
Comments: n/a ☐		

PART E – Preparation of Mobile Phases and Wash Solutions

Please, indicate an action has been performed by ticking the appropriate box (☐)

Assay specifics	
Analyst:	
Project:	**Date:**
Preparation of lipid mobile phase	
N.B. Volumes can be scaled up or down depending on requirement	
500 mM phosphoric acid solution	
Transfer 10 mL of LCMS grade water into a Duran bottle Actual Volume = _____	☐
Add 340 µL of 85 – 90% phosphoric acid using a pipette to the Duran bottle from above Actual Volume = _____	☐
Mix until the content is homogenous, seal the bottle and assign an expiry of 1 month	☐
Mobile phase A: preparation of 2 litres *(water:isopropanol:acetonitrile 2:1:1, 5 mM ammonium acetate, 0.05% acetic acid, 20 µM phosphoric acid)*	
Weigh 0.7708 g ± 0.0077 g of ammonium acetate into a weigh boat Weight = _____	☐
Transfer the ammonium acetate into a 2 L Duran bottle	☐
Using a measuring cylinder, transfer 1 L of LCMS grade water to the Duran bottle	☐
Sonicate for 5 minutes, or until the ammonium acetate has fully dissolved	☐
Add 1 mL of acetic acid to the Duran bottle using a Hamilton syringe	☐
Add 80 µL of the 500 mM phosphoric acid solution to the Duran bottle and mix until homogenous	☐
Measure the pH of the mobile phase ensuring the pH is 4.4 ± 0.1 pH = _____	☐
Using a volumetric flask, slowly transfer 500 mL of isopropanol to the Duran bottle (with mixing)	☐
Using a volumetric flask, slowly transfer 500 mL of acetonitrile to the Duran bottle (with mixing)	☐
Mix until the solution is completely homogenous	☐
Sonicate for 10 minutes	☐

Table continues

Assay specifics	
Analyst:	
Project:	**Date:**
Mobile phase B: preparation of 2 litres *(isopropanol:acetonitrile 1:1, 5 mM ammonium acetate, 0.05% acetic acid)*	
Weigh 0.7708 g ± 0.0077 g of ammonium acetate into a weigh boat Weight = _____	☐
Transfer the ammonium acetate into a 2 L Duran bottle	☐
Using a measuring cylinder, transfer 1 L of LCMS grade isopropanol to the Duran bottle	☐
Sonicate for 45 minutes, or until the ammonium acetate has fully dissolved	☐
Add 1 mL of acetic acid to the Duran bottle using a Hamilton syringe	☐
Using a volumetric flask, slowly transfer 1 L of acetonitrile to the Duran bottle (with mixing)	☐
Mix until the solution is completely homogenous	☐
Sonicate for 10 minutes	☐
Comments: n/a ☐	

Table continues

Assay specifics	
Analyst:	
Project:	**Date:**

Preparation of lipid wash solutions

N.B. Volumes can be scaled up or down depending on requirement

Seal wash *(isopropanol:water 1:9)*	
Transfer 100 mL of isopropanol into a Duran bottle	☐
Add 900 mL of LCMS grade water into the Duran bottle	☐
Mix until the content is homogenous	☐
Sonicate for 5 minutes, seal the bottle and assign an expiry of 1 month	☐
Weak needle wash *(water:isopropanol 1:4)*	
Transfer 200 mL of LCMS grade water into a Duran bottle	☐
Add 800 mL of LCMS grade isopropanol into the Duran bottle	☐
Mix until the content is homogenous	☐
Sonicate for 5 minutes, seal the bottle and assign an expiry of 1 month	☐
Strong needle wash *(isopropanol)*	
Use isopropanol as supplied and assign an expiry of 3 months	☐

Comments: n/a ☐

PART F - Acquisition

Please, indicate an action has been performed by ticking the appropriate box (☐)

Assay specifics	
Analyst:	
Project:	**Date:**
Instrument number:_____	
Column: **Acquity UPLC BEH C8 1.7µm, 2.1x100 mm; P/N:186002878** LOT: _____ Serial Number: _____	
Ionisation mode required: _____	
Instrument check performed (see separate Proforma sheet NPC.PRO.MS002)	☐
All solvent lines match the assay specific buffers and solutions	☐
Check the following against NPC.SOP.MS003:	
Tune file used: _____	
Acquisition method used: _____	
Inlet method used: _____	
Sequence loaded into Masslynx	☐
Sample plate loaded into tray position	☐
Comments: n/a ☐	