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

NPC.SOP.MS003 Version 2.1

Effective Date: April 2019

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1. Purpose

The purpose of this standard operating procedure (SOP) is to provide step-by-step instructions for the sample preparation and reversed phase chromatographic (RPC) analysis of lipids in human blood samples. This includes: details of sample preparation, mobile phase preparation and UPLC-MS analysis of the samples.

Human blood is a potentially infectious (Class 2) biofluid. The aim of this SOP is to reduce the risk from handling human blood, and to ensure accurate reproducible results through efficient and consistent sample handling. This SOP must be used alongside proforma NPC.PRO.MS003.

2. Scope

Prior to following this SOP, samples should have been sorted and formatted as detailed in NPC.SOP.CC004 and NPC.SOP.CC005.

This SOP details the methodology for the analysis of lipids in human blood (plasma and serum). After formatting, there will be a 96-well plate labelled for lipid analysis, containing 50 µL of each sample in columns 1-10, rows A-H (columns 11 and 12 are empty and are reserved for quality control (QC) samples). This SOP defines how to remove protein from the samples and include standards in each sample. This SOP details the procedure and methodology required to analyse the prepared samples by RPC UPLC-MS.

3. Analysis Notes

In addition to sample analysis, the analytical run consists of: blanks, conditioning runs, two dilution series, 5 start study reference (SR), 5 end SR, and quality control samples: SR and long term reference (LTR) samples. The SR is also used to acquire MSMS data using the data independent/dependent acquisition (DIDA) technique of choice (MS^E, DDA, SONAR or a combination of all three modes depending upon availability) for future annotation if needed.

The rationale behind the above analytical set is as follows:

The first 40 injections (10 blanks and 30 undiluted SR) are the conditioning runs to ensure that the instrument is stable and ready for sample analysis. This set of injections help to maintain consistency in the run, thus reducing the level of pre-processing sample normalisation required.

Blanks help identify any system peaks present.

The quality control samples (SR/LTR) are run throughout the analysis to monitor the analytical session and allow for normalisation of the sample set post-analysis.

The dilution series acts as a data filtering tool that gives insight into which features respond to dilution and thus which are the features that are monitored and measured post acquisition. Features that do not change are considered noise and removed. A project batch should not normally consist of more than a 1000 customer samples. A dilution series will be run at the beginning and end of each batch. The dilutions series are prepared with undiluted SR, allowing to the highly concentrated compounds in diluted samples to be encompassed.

DIDA will only be performed upon the SR and gives additional information in terms of fragmentation data/spectra, thus assisting in feature identification.

Please note that this procedure involves the handling of a Class II rated biological hazard. Please ensure you have undergone all appropriate training & read all relevant risk assessments prior to any sample handling.

4. Materials

Consumables

- Formatted blood sample plate
- Lipid study reference
- Plasma/serum long term reference
- Eppendorf 0.5 mL purple combitip
- Eppendorf 1 mL yellow combitip
- Eppendorf 2.5 mL green combitip
- Eppendorf 10 mL orange combitip
- Eppendorf tubes (1.5 or 2mL)
- Eppendorf microplate 96/U-PP
- 300 µL UPLC vials with foil lined caps
- Automatic pipette tips
- Analytical plate barcode labels
- Heat seal foil
- LCMS grade acetic acid
- LCMS grade acetonitrile
- LCMS grade ammonium acetate
- LCMS grade isopropanol
- LCMS grade leucine enkephalin
- Phosphoric acid (85-90%)
- LCMS grade water
- Lipid standards (seeTable 1)

Equipment

- Heat sealer
- Plate shaker with tube holder
- Eppendorf Multipette Xstream
- Eppendorf Xplorer 20-300 µL 12 channels pipette
- Eppendorf automatic pipettes
- Centrifuge equipped with 96-well plate rotor
- Balance (precision 0.0001 g)
- Ultrasonic bath
- Calibrated pH meter
- Hamilton syringe
- Glass weigh boat
- Glass bottles with PTFE lined caps
- -20 ºC and -80 ºC freezers
- Fridge
- Waters Xevo QTOF G2S Mass Spectrometer
- Waters Acquity binary solvent manager
- Waters Acquity column manager
- Waters 2777 sample manager
- UPLC column: Waters Acquity UPLC BEH C8, 1.7µm, 2.1 x 100 mm, P/N: 186002878

Personal Protective Equipment

- White laboratory coats
- Nitrile gloves
- Eye protection
- Class II biosafety cabinet

Before commencement of any activities described in this document, personnel must be adequately trained and must adhere to all local health and safety procedures.

5. Procedure - Samples

5.1. Lipid IPA standard mixture

PART A of Proforma NPC.PRO.MS003

5.1.1. Remove the individual lipid standards from storage at -20 °C and place in a fume cupboard.

5.1.2. Individually weigh each lipid standard into volumetric flasks using an analytical balance, and then make up to volume with isopropanol to obtain the stock concentrations specified in Table 1.

5.1.3. Sonicate each stock until the content has dissolved and is visually homogenous.

5.1.4. Store each stock solution at -20 °C until required to make the Lipid standard mixture.

5.1.5. To prepare the final Lipid standard mixture (final concentrations in Table 1), leave each stock solution at 2-8 °C till clear.

5.1.6. Transfer respective volume of each individual stock solution into a volumetric flask using automatic Eppendorf pipettes or measuring cylinders. Please see proforma NPC.PRO.MS003 for recommended volumes.

5.1.7. Make up to the chosen volume with isopropanol and mix until the solution is homogenous.

5.1.8. Although the final concentration of each Lipid standard in the mixture must be as outlined in Table 1, the weights and volumes can be scaled up or down depending on the volume of mixture required for the project (400 µL of mixture for each sample).

5.1.9. Aliquot the prepared lipid mixture into appropriately sized bottles with PTFE lined cap for storage at -20 °C until required.

Table 1. Lipid standard mixture

No.	Lipid	Supplier & P/N	Stock conc. (mg/mL)	Final conc. (µg/mL) in Lipid Mix
1	LPC(9:0)	Avanti, 855276P	0.5	0.25
2	PC(11:0/11:0)	Avanti, 850330P	0.1	0.25
3	FA(17:0)	Sigma, H3500	0.1	2.50
4	PG(15:0/15:0)	Avanti, 840446P	0.01	1.00
5	PE(15:0/15:0)	Avanti, 850704P	0.01	0.25
6	PS(17:0/17:0)	Avanti, 840028P	0.1	6.00
7	PA(17:0/17:0)	Avanti, 830856P	0.1	1.00
8	Cer(d18:1/17:0)	Avanti, 860517P	0.1	0.05
9	DG(19:0/19:0)	Sigma, 68633	0.2	12.00
10	PC(23:0/23:0)	Avanti, 850372P	0.1	0.25
11	TG(15:0/15:0/15:0)	Sigma, T4257	0.1	2.50
12	TG(17:0/17:0/17:0)	Sigma, T2151	0.1	2.50

5.2. *On the day of MS-SR preparation* – PART B of Proforma NPC.PRO.MS003

5.2.1. Long term reference (LTR) aliquotting for plate preparation

5.2.1.1. Defrost plasma/serum stock LTR at 2-8 °C (number of aliquots depends upon study size, with 1x 11.5 mL aliquot sufficient for 1000 samples).

5.2.1.2. Dispense 450 µL aliquots into 25 separate Eppendorf tubes

5.2.1.3. Store all aliquots at -80 ºC until required.

5.2.2. Study reference (SR) aliquotting for plate preparation

5.2.2.1. Per sample plate prepare, one Eppendorf tube with 450 µL aliquot of undiluted pooled MS-SR (prepared as per NPC.SOP.CC005, section 6.2.).

5.2.2.2. Store all aliquots at -80 ºC until required.

5.2.3. Instrument Conditioning, Start/End SR, and DIDA

5.2.3.1. For each batch of 1000 samples, transfer 100 µL of undiluted pooled SR into at least 7 separate Eppendorf tubes and store at -80 °C until required for conditioning, Start and End SR, and DIDA.

5.2.4. SR dilution preparation

5.2.4.1. For each polarity, prepare a master dilution series of SR for the beginning and end of every batch of 1000 samples, according to the volumes in Table 2.

5.2.4.2. Vortex mix all Eppendorf tubes containing the different SR master dilution points.

5.2.4.3. Aliquot each of the master dilution points into 6 separate sets of individual Eppendorf tubes as per the volumes in Table 2 (6 sets per<1000 sample batch: a set for start, end, and backup for each polarity).

5.2.4.4. Store each Eppendorf tube at -80 °C until required for addition of the Lipid IPA standard mixture.

Table 2. SR dilution series for one sample batch

Dilution Point	Percentage of SR (%)	Vol. of SR (µL)	Vol. of water (µL)	Total Vol. (µL)	Vol. in each aliquot (µL)	Vol. of Lipid IPA standard mixture added per aliquot (µL)
1	100	450	0	450	70	280
2	80	240	60	300	45	180
3	60	150	100	250	35	140
4	40	100	150	250	35	140
5	20	100	400	500	45	180
6	10	50	450	500	70	280
7	1	10	990	1000	70	280
Blank	0	0	500	500	70	280

N.B.: Start here with the UPLC Q-TOF system performance checks as given in 7.1.1-7.1.3 of section "7. Procedure – Acquisition" of this SOP.

5.3. *On the day of analysis* - PART C of Proforma NPC.PRO.MS003

5.3.1. Dilution series preparation

5.3.1.1. On either the first or the last day of analysis, remove 2 sets of dilution series (1 set for positive and 1 for negative) and 1 bottle of Lipid IPA standard mixture from storage and allow to defrost for 2 hours at 2 - 8 °C.

5.3.1.2. Add the corresponding volume of Lipid IPA standard mixture detailed in Table 2 to each Eppendorf tube and briefly vortex until homogenous.

5.3.2. Instrument Conditioning, DIDA and Start/End SR preparation

5.3.2.1. Remove required numbers of Eppendorf tube containing 100 µL SR for instrument conditioning (2x), Start SR (1x pos, 1x neg), End SR (1x pos, 1x neg) and DIDA (1x) from storage as needed (either the first or the last day of analysis) and allow to defrost for 2 hours at 2-8 °C.

5.3.2.2. For instrument conditioning or DIDA, add 400 µL of Lipid IPA standard mixture to each Eppendorf tube and briefly vortex until homogenous. *N.B. Use LTR for instrument conditioning, if SR volume is limited. Rescale accordingly.*

5.3.2.3. For start/end SR, add 100 µL of LCMS grade water and mix for 5 min at 1400 rpm at 2-8 °C. Then, add 800 µL of Lipid IPA standard mixture to each Eppendorf tube and briefly vortex until homogenous.

N.B.: More restart SR samples can be made in the same way when needed (i.e. when ready after instrument failure).

5.3.3. Incubation and centrifugation

5.3.3.1. Mix all Eppendorf tubes for 2 hours on a plate mixer at 1400 rpm at 2-8 ^{o}C.

5.3.3.2. Centrifuge the tubes at 3486 g for 10 minutes at 2-8 ^{o}C.

5.3.3.3. Pipette supernatant into labelled 300 µL vials and seal with foil lined caps.

5.3.4. Blanks

5.3.4.1. Pipette 100 µL of LCMS grade water in an Eppendorf tube, then add 400 µL of Lipid IPA standards mixture and vortex briefly.

5.3.4.2. Mix the Eppendorf tubes for 2 hours on a plate mixer at 1400 rpm at 2-8 ^{o}C.

5.3.4.3. Centrifuge the tubes at 3486 g for 10 minutes at 2-8 ^{o}C.

5.3.4.4. Pipette the mixture into labelled 300 µL vials and seal with foil lined caps.

5.4. Sample Preparation

PART D of Proforma NPC.PRO.MS003

N.B.: sample plates for lipid analysis have been prepared previously according to NPC.SOP.CC005

5.4.1. Remove the appropriate Lipid plate from storage, as well as one aliquot of plasma/serum LTR, one aliquot of SR and the Lipid standard mixture; thaw at 2-8 °C for 2 hours.

5.4.2. Ensure centrifuge and heat sealer are at the required temperatures of 4 °C and 160 °C, respectively.

5.4.3. Once thawed, centrifuge the sample plate, LTR and SR at 3486 g for 1 minute at 4 °C to pool sample into the bottom of the well.

5.4.4. Remove carefully the seal cap mat and place side up on a clean surface.

5.4.5. Dispense 50 µL of plasma/serum LTR into wells of column 11 and 50 µL of plasma/serum SR into wells of column 12.
(Please refer to Figure 1 for a row guide for the sample plate).

5.4.6. Add 50 µL of LCMS grade water to each well.

5.4.7. Cover the plate with the former seal cap mat and mix at 1400 rpm at 2-8 °C for 5 min.

5.4.8. Centrifuge the sample plate at 3486 g for 1 minute at 4 °C to pool sample into the bottom of the well.

5.4.9. Carefully remove seal cap mat and discard.

5.4.10. Add 400 µL of Lipid IPA standard mixture to each well.

5.4.11. Heat seal the sample plate with heat seal foil (2 seconds, followed by rotation of the plate by 180° and a further 2 seconds of sealing).

5.4.12. Mix for 2 hours on a plate mixer at 1400 rpm at 2-8 °C.

5.4.13. Centrifuge the sample plate at 3486 g for 10 minutes at 4 °C.

5.4.14. Attach unique barcode plate stickers to three 96 well microplates, one for positive (LPOS), one for negative (LNEG) and one for backup (BACKUP). In addition, label with project details, method details and date.

5.4.15. Carefully remove foil from the sample plate *(without disturbing the pelleted material)*.

5.4.16. Using a 12 channel Eppendorf Explorer pipette transfer 100 µL of each supernatant to the corresponding well in the analytical plates (LPOS, LNEG and BACKUP).

5.4.17. Heat seal the analytical plates with heat seal foil.

5.4.18. Log the plates in LIMS.

5.4.19. Centrifuge the LPOS and LNEG analytical plates at 3486 g for 5 minutes at 4 °C.

5.4.20. Transfer the LPOS and LNEG plates to the autosamplers ready for analysis. Transfer the BACKUP plate to -80 °C for storage.

	1	2	3	4	5	6	7	8	9	10	11	12
Rows A-H	S	S	S	S	S	S	S	S	S	S	LTR	SR

*S = sample, LTR = Long Term Reference, SR = Study Reference

Figure 1: Row guide for sample plate

Eppendorf Multipette Xstream pipette settings:

- LTR/SR addition: 0.5 mL CombiTip with Prog = Dis, Vol = 50 µL, No. of steps = 8, aspirate speed = max, dispense speed = max.
- Water addition: 2.5 mL CombiTip with Prog = Dis, Vol = 50 µL, No. of steps = 48, aspirate speed = max, dispense speed = max.
- Lipid IPA standard mixture addition: 10mL CombiTip with Prog = Dis, Vol = 400 µL, No. of steps = 24, aspirate speed = max, dispense speed = 4/10.
- Aliquoting: 12 channel 15-300 µL pipette with Prog = Dis, Vol = 100 µL, No. of steps = 3, aspirate speed = 2/10, dispense speed = 4/10.

6. Procedure - Mobile Phases and Wash Solutions

PART E of Proforma NPC.PRO.MS003

6.1. 500 mM phosphoric acid solution

6.1.1. Transfer 10 mL of LCMS grade water into a Duran bottle.

6.1.2. Add 340 µL of phosphoric acid (85 – 90%) to the Duran bottle using a pipette.

6.1.3. Mix until the solution is homogenous and cap the bottle.

6.1.4. Assign the solution an expiry of 1 month from the date of preparation.

***Always** add acid to water. **Never** add water to acid*

6.2. Mobile phase A *(water:isopropanol:acetonitrile 2:1:1, 5 mM ammonium acetate, 0.05% acetic acid, 20 µM phosphoric acid)*

6.2.1. To make 2 L of mobile phase A, weigh 0.7708 g ± 0.0077 g of ammonium acetate into a weigh boat.

6.2.2. Using a measuring cylinder, measure 1 L of LCMS grade water.

6.2.3. Transfer the ammonium acetate into a 2 L Duran bottle and rinse weigh boat using the measured LCMS grade water (6.2.2) into the bottle.

6.2.4. Transfer the remaining water into the 2 L Duran bottle, cap and sonicate for 5 minutes or until the ammonium acetate has fully dissolved.

6.2.5. Using a Hamilton syringe, add 1 mL of acetic acid to the Duran bottle and mix.

6.2.6. Using an appropriately sized automatic Eppendorf pipette, add 80 µL of the 500 mM phosphoric acid solution and mix until the solution is homogenous.

6.2.7. Measure the pH of the solution and record the result in the proforma NPC.PRO.MS003. The desired pH of the solution should be pH 4.4 ± 0.1.

6.2.8. Using a volumetric flask, slowly transfer 500 mL of isopropanol to the Duran bottle and 500 mL of acetonitrile while homogenising at the same time, and mix until the solution is completely homogenous.

6.2.9. Sonicate the mobile phase for 10 minutes, cap the bottle and assign an expiry date of one month from the date of preparation.

6.3. Mobile phase B *(isopropanol:acetonitrile 1:1, 5 mM ammonium acetate, 0.05% acetic acid)*

6.3.1. To make 2 L of mobile phase B, weigh 0.7708 g ± 0.0077 g of ammonium acetate into a weigh boat.

6.3.2. Using a measuring cylinder, measure 1 L of isopropanol.

6.3.3. Transfer the ammonium acetate into a 2 L Duran bottle and rinse the weigh boat using the measured isopropanol (6.3.2) into the bottle.

6.3.4. Transfer the remaining isopropanol into the 2 L Duran bottle, cap and sonicate for 45 minutes or until the ammonium acetate has fully dissolved.

6.3.5. Using a Hamilton syringe, add 1 mL of acetic acid to the Duran bottle and mix.

6.3.6. Using a volumetric flask, slowly transfer, while homogenising, 1 L of acetonitrile ensuring the ammonium acetate does not crash out, mix until the solution is completely homogenous.

6.3.7. Sonicate the mobile phase for 10 minutes, cap and assign an expiry date of one month from the date of preparation.

6.4. Seal wash *(isopropanol:water 1:9)*

6.4.1. Using a measuring cylinder, transfer 100 mL of isopropanol to a 1 L duran bottle.

6.4.2. To this, add 900 mL of LCMS grade water.

6.4.3. Cover the bottle with aluminum foil and sonicate for 5 minutes.

6.4.4. Assign an expiry date of one month from the date of preparation.

6.5. Weak needle wash *(water:isopropanol 1:4)*

6.5.1. Using a measuring cylinder, transfer 200 mL of LCMS grade water into an appropriately sized Duran bottle.

6.5.2. To this, add 800 mL of isopropanol using a measuring cylinder.

6.5.3. Mix until homogenous, then sonicate for 5 minutes and cap.

6.5.4. Assign an expiry date of one month from the date of preparation.

6.6. Strong needle wash *(100% isopropanol)*

Transfer isopropanol into the strong wash bottle and assign an expiry date of three months from the date of preparation before connecting to the autosampler strong wash line.

Please remember to purge lines when new mobile phases or solvents are added.
It is also good practice to purge all lines prior to use if the system has not been running for >1 day.

7. Procedure - Acquisition

PART F of Proforma NPC.PRO.MS003

7.1. Instrument checks and sample loading

7.1.1. Perform UPLC Q-TOF system performance check in the assay specific polarity only as per the NPC.SOP.MS002 and confirm that all checks meet acceptance criteria**.**

7.1.2. Check that all solvent lines match the assay specific buffers and solutions as outlined in Table 3.

7.1.3. Confirm that all instrument parameters and settings correspond with the values outlined in the Tables 3, 4, and 5.

7.1.4. Load the correct sequence (*SampleList.spl*) into MassLynx and confirm that sample injection order corresponds to the template in Table 6.

7.1.5. Place the samples (either vials or analytical plate) in the correct autosampler tray position.

Table 3. LC instrument parameters

Variable	Description
Mobile Phase A	Water:isopropanol:acetonitrile 2:1:1, 5 mM ammonium acetate, 0.05% acetic acid, 20 µM phosphoric acid
Mobile Phase B	Isopropanol:acetonitrile 1:1, 5 mM ammonium acetate, 0.05% acetic acid
Seal Wash	Isopropanol:water 1:9, set to run every 0.5 minutes
Weak Wash	Water:isopropanol 1:4
Strong Wash	Isopropanol
Lockspray	Leucine enkephalin 600 pg/µL (prepared in water:acetonitrile 1:1)
Column	Waters Acquity UPLC BEH C8, 1.7 µm, 2.1 x 100 mm (P/N: 186002878)
PEEK tubing	Column to sample probe: ID = 0.004 inch, length = 38 cm
Column Temp.	55 °C
Sample Loop	Positive: 1 µL, negative: 2 µL
Injection Volume	15 µL
Run Time	13.25 minutes (N.B. including auto gain each injection will be ~15 minutes)
Autosampler Settings	Syringe: 25 µL Air volume, pre-cleans, filling strokes = 0, filling & injection speed = 5 µL/sec, inject to = LC Vlv1, pre-injection delay = 500 ms, post-injection delay = 500 ms, inject 10 µL to injector, followed by 5 µL to waste Needle wash sequence = 1x weak,1x strong, 1x weak, wait time = 705 sec, Valve wash sequence = 1x weak, 2x strong, 1x weak
Sample Cooler	4 °C with low nitrogen flow to prevent condensation

LC Gradient					
#	**Time (min)**	**Flow (mL/min)**	**% A**	**% B**	**Curve**
1	Initial	0.6	99.0	1.0	Initial
2	0.10	0.6	99.0	1.0	6
3	2.00	0.6	70.0	30.0	6
4	11.50	0.6	10.0	90.0	6
5	12.00	1.0	0.1	99.9	6
6	12.50	1.0	0.1	99.9	6
7	12.55	0.9	35.0	65.0	6
8	12.65	0.8	70.0	30.0	6
9	12.75	0.7	99.0	1.0	6
10	12.95	0.6	99.0	1.0	6
11	13.25	0.6	99.0	1.0	6

Table 4. MS source parameters

Variable	Description
Sample Probe Angle	7 mm
Capillary Voltage (kV)	Positive: 2.0 / Negative: 1.5
Sampling Cone	25
Source Offset	80
Source Temperature (°C)	120
Desolvation Temperature (°C)	600
Cone Gas Flow (L/hr)	150
Desolvation Flow (L/hr)	1000
Collision Energy (V)	Off but with default set to 6
Gas	API = On, Collision = On
Detector Auto Gain, Tune Page:	Automatic Gain control ticked
Detector Auto Gain, System View:	Optimise Detector Gain Usage: Background ions = ticked, Background Ions Settings: Timeout = 70 sec, Use Max Voltage Adjust (V): ticked and set to 1.0
Stepwave 2 Offset	15
Quad Profile	Auto
Lockspray Flow (µL/min)	15
Lockspray Capillary Voltage (kV)	Positive: 3.0 / Negative:2.0

Table 5. MS instrument parameters

Variable	Description
MS Function	ToF MS
Run time	13.15 minutes
Analyser Mode	Sensitivity
Dynamic Range	Extended
Mass Range	50 – 2000 Da
Scan Time	0.1 sec
Data Format	Centroid
Lockspray function Settings	Acquire lockspray & apply correction Select appropriate Lock Spray file Scan time = 0.15 sec, interval = 60 sec, scans to average = 4, Mass window = +/- 0.5
Method Events (enabled)	At initial conditions lockspray is infused, at 12.05 minutes lockspray is refilled, at 12.45 minutes lockspray starts infusing again

7.2. Sample Acquisition

7.2.1. Export the batch sample list from LIMS. In the event of missing samples (noted during formatting), modify the sample list accordingly. The missing sample injection is replaced by the last injected SR or LTR with "_X" added at the end of the sample name.

7.2.2. The analytical batch is structured as described in Table 6.

Table 6. Acquisition order

Phase	Number of injections	Description	Acquisition Method	File Naming Convention
Start	5-10	Pre-check blanks	MS only	Project_LPOS/LNEG_TOFxx_Test01-05
	10	Blanks	MS only	Project_LPOS/LNEG_TOFxx_Blank01-10
	30	Instrument conditioning samples	MS only	Project_LPOS/LNEG_TOFxx_IC01-30
	2	Blanks (for dilution series)	MS only	Project_LPOS/LNEG_TOFxx_Blank11-12
	46	Dilution series (low to high % SR)	MS only	Project_LPOS/LNEG_TOFxx_BxSRD01-46
	3	SR conditioning samples	MS only	Project_LPOS/LNEG_TOFxx_IC31-33
	5	Batch start SR	MS only	Project_LPOS/LNEG_TOFxx_BxS01-05_SR
Sample Analysis	X	Analytical plates	MS only	Project_LPOS/LNEG_TOFxx_S*xWxx_LTR Project_LPOS/LNEG_TOFxx_S*xWxx Project_LPOS/LNEG_TOFxx_S*xWxx_SR
End	5	Batch end SR	MS only	Project_LPOS/LNEG_TOFxx_BXE01-05_SR
	46	Dilution series (high to low % SR)	MS only	Project_LPOS/LNEG_TOFxx_BXSRD92-47
	2	Blanks (for dilution series)	MS only	Project_LPOS/LNEG_TOFxx_Blank13-14
	8	DIDA conditioning samples	MS only	Project_LPOS/LNEG_TOFxx_IC34-41
	10	SR	DIDA**	Project_LPOS/LNEG_TOFxx_DIDA01-10

** S for Serum, P for Plasma, ** DIDA = data independent/dependent acquisition, for compound identification assistance. This can be DDA, MS^e, SONAR or a combination of all three modes depending upon availability.*

Start: Pre-check blanks (water:Lipid IPA standard mixture 1:4) to ensure the system is functioning as expected in terms of accuracy and sensitivity, followed by blanks (water:Lipid IPA standard mixture 1:4), conditioning SR, 2 blanks (for dilution series), dilution series run low to high % SR (see Table 7 for the number of injections of each dilution), followed by 3 conditioning SR and the 5 batch start SR.

Table 7. Number of injections for each SR dilution point

Dilution point (% SR)	Number of injections
1	10
10	10
20	5
40	3
60	3
80	5
100	10

Sample Analysis: For sample analysis each row of the 96 well plate should be analysed in the following order: one LTR, 5 samples, one SR, 5 samples. Plates are sequentially located in trays 2, 4 and 6.

End: Batch end SR, dilution series run high to low % SR (see Table 7 for the number of injections of each dilution), 2 blanks (for dilution series), 8 conditioning SR, 10 DIDA.

7.2.3. Start the acquisition of the full batch sequence (*SampleList.spl*) in MassLynx

N.B. In case of a run interruption, reset instrument, prepare fresh restart SR, check with a SR sample whether the instrument performance is still fine, and restart run with additional 8 SR samples as emergency instrument conditioning at the beginning of the remaining run.

8. Related Documents

Document Number	Title
NPC.PRO.MS003	RPC UPLC-QTOF Analysis of Lipids in Human Plasma and Serum
NPC.SOP.CC004	Sample Sorting and LIMS Logging
NPC.SOP.CC005	Formatting and Replication of Samples
NPC.SOP.MS002	UPLC and Q-ToF System Performance Check

9. Version History

Current version

Version number	Author	Changes and justification	Section(s) updated
V2.1	VHS/SC	Revision and minor changes	all

Previous versions

Version number	Author	Changes and justification	Section(s) updated
V2.0	VHS/SC	Changed for new diluted MS protocols	all
V1.1	VHS/SC/ BC	Revision and minor changes	all
V1	SC/KC	Rephrase for consolidation	Purpose & Scope

10. Responsibilities

Centre management is responsible for ensuring that laboratory technical personnel are appropriately qualified to perform the procedures outlined in this SOP. The appointed laboratory personnel are in turn responsible for conducting the procedure as outlined in accordance with health and safety standards.

Health and safety statement: before commencing any activities described in this document personnel must be adequately trained e.g. staff having completed local institution Chemical Safety Training and staff having read and understood the relevant risk assessments. Chemical, biological and general waste should be disposed of according to local policies.

11. Approval

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

End of Document