RPC UPLC-QTOF Analysis of small Molecules in Human Urine

National Phenome Centre

NPC.SOP.MS005 Version 2.1

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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 small molecules in human urine samples. This includes: details of sample preparation, mobile phase preparation, and UPLC-MS analysis of the samples.

The aim of this SOP is to reduce the risk from handling human urine, and to ensure accurate reproducible results through efficient and consistent sample handling. This SOP must be used alongside proforma NPC.PRO.MS005.

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 only details analysis of the RPC methodology for human urine samples. After formatting there will be a 96-well plate labeled for RPC analysis, containing 75 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 dilute each sample to a suitable concentration and include both internal standards to each sample and method reference standards to each QC sample. This SOP then details the procedure and methodology required to then analyse the samples by RPC UPLC-MS.

3. Analysis Notes

In addition to sample analysis the analytical run consists of: conditioning runs, blanks, quality control (QC) samples of the study reference (SR) and long term reference (LTR) samples, and two dilution series. 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 are as follows:

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

Blanks help identify any system peaks present within the sample set.

The quality control samples (SR/LTR) are run throughout the analysis and to monitor the analytical session and allow for normalisation of the sample set post-analysis should this be required due to instrumental drift for example.

The dilution series serves a different function in that it acts as a data filtering tool that gives insight into which features which respond to dilution, and thus, are the features which are monitored and measured post acquisition. Features which do not change are considered noise and removed. A project batch should not consist of more than a 1000 customer samples. 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 provide 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 and read all relevant risk assessments prior to any sample handling.

4. Materials

Consumables

- Formatted urine RPC sample plate
- RPC study reference
- Urine long term reference
- Eppendorf 2.5 mL green combitip
- Eppendorf 1 mL yellow combitip
- Eppendorf 10 mL orange combitip
- Eppendorf 300 µL orange tips
- Eppendorf 2 mL Eppendorf tubes
- Eppendorf microplate 96/U-PP
- Analytical plate barcode labels
- Heat seal foil
- LCMS grade0.1% formic acid in water
- LCMS grade 0.1% formic acid in acetonitrile
- LCMS grade isopropanol
- LCMS grade acetonitrile
- LCMS grade water
- Leucine enkephalin (prepared as per NPC.SOP.MS001)
- RPC standards (see Tables 1 and 2)
- UPLC vials

Equipment

- Heat sealer
- Plate shaker
- Eppendorf Multipette Xstream
- Eppendorf Xplorer 20-300 µL12-channel pipette
- Eppendorf automatic pipettes
- Centrifuge equipped with 96-well plate rotor
- Balance (precision 0.0001 g)
- Ultrasonic bath
- Fridge and -80ºC freezer
- Waters Xevo G2-S QTOF Mass Spectrometer
- Waters Acquity binary solvent manager
- Waters Acquity column manager
- Waters 2777 sample manager
- UPLC column: Waters Acquity UPLC HSS T3 1.8µm, 2.1 x 150 mm (P/N: 186003540)

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. RPC internal standard solution (IStd-Soln)

PART A of Proforma NPC.PRO.MS005

5.1.1. Prepare the stock solutions of the two internal standards (IStd) for RPC analysis in LCMS grade water at the concentrations outlined in Table 1.

5.1.2. Prepare the final IStd solution by mixing equal volumes of each internal standard stock solution to achieve the final concentration (as detailed in Table 1).

5.1.3. The quantity of the internal standard solution can be scaled up or down depending on the volumes required for the project (approx. 87 mL for a batch of 1000 samples).

5.1.4. Dispense the final IStd-Soln into 8.5 mL aliquots.

5.1.5. Store the prepared internal standards solution at -80 °C for a maximum of 12 months.

Table 1. IStd-Stock concentration and target concentration in final solution

Material	Supplier	Product no.	Concentration of stock		Final concentration	
			mM	mg/mL	mM	mg/mL
L-Phenylalanine-$^{13}C_9$,^{15}N	Sigma	60817	0.60	0.105	0.30	0.053
(N-Benzoyl-d_5-Glycine) Hippuric Acid-d_5	QMX	D-5588	0.50	0.093	0.25	0.047

5.2. RPC method reference solution (MR-Soln)

PART B of Proforma NPC.PRO.MS005

5.2.1. Prepare stock solutions of the method reference standards for RPC analysis in LCMS grade water at the concentrations stated in Table 2.

5.2.2. Divide stock solutions into 15 mL falcon tubes and store at -80 °C for up to 5 years after reconstitution.

5.2.3. Prepared a mixed solution containing each method reference standard to achieve the final concentration (as detailed in Table 2).

5.2.4. Cap vessel and place at -80 ^{o}C for length of study, unless being used immediately for analytical LTR and SR generation (section 5.3 and 5.4).

5.2.5. The quantity of the MR solution can be scaled up or down depending in the volumes required for the project (approx. 15 mL for a batch of 1000 samples).

Table 2. Method reference standards

Material	Supplier	Product no.	Concentration of stock		Final Concentration	
			mM	mg/mL	mM	mg/mL
L-Glutamic Acid-$^{13}C_5$	Sigma	604860	2.0	0.31	0.20	0.031
L-Isoleucine-$^{13}C_6$,^{15}N	Sigma	608092	7.5	1.04	0.15	0.021
L-Leucine-$^{13}C_6$	Sigma	605239	7.5	1.08	0.15	0.022
L-Tryptophan-$^{13}C_{11}$,$^{15}N_2$	Sigma	574597	1.5	0.33	0.15	0.033
Octanoic Acid-$^{13}C_8$	Sigma	605727	1.0	0.15	0.40	0.062
L-Glutamine-$^{13}C_5$	Sigma	605166	12.5	2.00	0.25	0.040
Creatinine-Methyl-d_3	Sigma	485446	10.0	1.00	0.20	0.020
Cytidine-5,6-d_2	QMX	D-5424	4.0	1.00	0.40	0.100
Citric Acid-$^{13}C_6$	Sigma	606081	5.0	1.00	0.10	0.020
Benzoic Acid-Ring-$^{13}C_6$	Sigma	485691	2.0	0.26	0.40	0.051

PART C of Proforma NPC.PRO.MS005 – *On the Day of MS-SR preparation*

5.3. Analytical long term reference (analytical LTR)

5.3.1. Defrost sufficient stock of urine LTR at 2-8 °C (number of aliquots depends upon study size, with 15 mL sufficient for 1000 samples).

5.3.2. Combine urine LTR with LCMS grade water and the MR-Soln together in a 1:1:1 ratio v/v *(e.g. 10 mL LTR, 10 mL LCMS grade water, and 10 mL MR-Soln*). This mixture will be called analytical LTR.

5.3.3. Vortex mix and briefly spin to collect all volume at the bottom.

5.3.4. Aliquot the analytical LTR solution into Eppendorf tubes, ensuring there is a minimum of 1 aliquot per sample plate. The volume required for each plate is 2000 µL (2 mL).

5.3.5. Store at -80 °C until required for analysis.

5.4. Analytical study reference (analytical SR)

5.4.1. Combine the RP SR stock aliquot (see NPC.SOP.CC005, section 6.3) with LCMS grade water and the MR-Soln together in a 1:1:1 ratio v/v *(e.g. 10 mL SR, 10 mL LCMS grade water, and 10 mL MR-Soln).* This mixture will be called analytical SR.

5.4.2. Vortex mix and briefly spin to collect all volume at the bottom.

5.4.1. Aliquot the analytical SR solution into Eppendorf tubes, ensuring there is a minimum of 1 aliquot per sample plate. The volume required for each plate is 2000 µL (2 mL).

5.4.2. Prepare at least two 750 µL aliquots of analytical SR solution in separate 2 mL Eppendorf tubes for Start/End SR using an automatic pipette. *N.B. It is recommended to prepare more aliquots in case of restart SR is required after instrument failure.*

5.4.3. Store at -80 °C until required for analysis.

5.5. SR containing MR (SR+MR) for instrument conditioning, dilutions series, and DIDA samples

5.5.1. To prepare SR+MR, combine RP urine stock MS- SR with MR-Soln in a ratio of 2:1 v/v. Per 1000 sample batch, recommended volumes are 3 mL SR and 1.5 mL MR-Soln.

5.5.2. Vortex mix and briefly spin to collect all volume at the bottom.

5.5.3. For instrument conditioning and DIDA, directly prepare 900 µL SR+MR + 300 µL IStd-Soln in two separate 2 mL Eppendorf tubes combine. *N.B. Use LTR for instrument conditioning, if SR volume is limited. Rescale accordingly.*

5.5.4. Vortex to mix, then briefly centrifuge.

5.5.5. Prepare 8 aliquots of 300 µL in appropriate UPLC vials (6x for instrument conditioning and 2x for DIDA).

5.5.6. Store at -80°C until required.

5.6. Blanks

5.6.1. In two separate 2 mL Eppendorf tubes combine 500 µL of LCMS grade water + 250 µL MR-Soln + 250 µL IStd-Soln

5.6.2. Vortex to mix, then briefly centrifuge.

5.6.3. Prepare 6 aliquots of 300 µL solution into appropriate UPLC vials.

5.6.4. Store aliquots at -80 °C in freezer till required.

More blanks can be made when needed. It is recommended to prepare two blanks.

5.7. Analytical SR dilution and Start/End series

5.7.1 Prior to start of analysis, preferable on the day of MS-SR preparation

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5.7.1.1. See Section 5.5 for details on how to prepare SR+MR (SR containing MR).

5.7.1.2. A dilution series of the analytical SR will be performed and run at the beginning and end of every batch of 1000 samples. Prepare the following master dilution sequence (Table 3) of the SR+MR (containing MR-Soln), IStds and water in 2 mL Eppendorf tubes using appropriately sized automatic pipettes.

5.7.1.3. For each master dilution point, the Eppendorf tubes will be vortex mixed and briefly spun down.

5.7.1.4. Once the master dilution series has been completed, each master dilution point will be divided into 6 separate sets of aliquots (two sets per polarity for the beginning and end of each batch and two sets as a spare) as per the volumes in Table 3. The dilutions will be aliquotted into appropriate UPLC vials and then be stored at -80 °C until required.

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.

5.7.2 On day of analysis

PART E of Proforma NPC.PRO.MS004

5.7.2.1 On the day of use (either first or last day of analysis), remove each dilution point from storage and allow to defrost at 2-8 °C. Vortex mix and briefly spin to pool all sample into the bottom of the UPLC vial. Each aliquot is single use only, once thawed it should be discarded not re-frozen.

5.7.2.2 On the first day of analysis remove 4x UPLC vials for instrument conditioning and 2x UPLC vials with blanks (mentioned in section 5.5 and 5.6 respectively). Allow to defrost at 2-8 °C. Vortex mix and briefly spin to pool all sample volume into the bottom of the UPLC vial. Each aliquot is single use only, once thawed it should be discarded not re-frozen.

5.7.2.3 On the last day of analysis remove, 2x UPLC vials for DIDA and 2x UPLC vials with blanks. Allow to defrost at 2-8 °C. Vortex mix and briefly spin to pool all sample volume into the bottom of the UPLC vial. Each aliquot is single use only, once thawed it should be discarded not re-frozen.

Table 3. Dilution series preparation for one sample batch (6 dilution sets)

Dilution (%)	Volume SR+MR (µL)	Volume of water (µL)	Volume of IStds (µL)	Final Volume (µL)	Aliquot volume (µL)
100	900	0	300	1200	190
80	420	105	175	700	115
60	234	156	130	520	85
40	156	234	130	520	85
20	105	420	175	700	115
10	90	810	300	1200	190
1	9	891	300	1200	190
0	0	990	330	1320	220

More SR samples can be made when needed (i.e when ready after instrument failure) as follows using a freshly thawed analytical SR prepared in 5.4:

- In an Eppendorf tube combine 750 µL analytical SR + 250 µL IStd-Soln
- Vortex to mix.
- Aliquot solution into an appropriate UPLC vial.

5.8 Sample Preparation

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

5.8.1 Remove the appropriate RPC plate, aliquot of analytical LTR and analytical SR, and RPC-IStd-Soln for the required project from storage at -80 °C and thaw at 2-8 °C for a minimum of 2 hours.

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

5.8.3 Once thawed, centrifuge the sample plate at 3486 g for 1 minute at 4ºC to pool sample into the bottom of the well.

5.8.4 Remove seal cap mat and add 150 µL of LCMS grade water to all samples (excluding the wells in column 11 and 12 designated for the analytical SR and analytical LTR) using an Eppendorf multipipette.

5.8.5 Dispense 225 µL of analytical LTR into column 11 and 225 µL of analytical SR to column 12 *(Please refer to Figure 1 for a row guide for the sample plate).*

5.8.6 Add 75 µL of RPC-IStd-Soln to each well on the plate prepared as detailed in section 4.1.

5.8.7 Seal sample plate with cap mat.

5.8.8 Mix for 2 minutes on a plate mixer at 1200 rpm at 2-8 ºC (if possible).

5.8.9 Centrifuge sample plate at 3486 g for 10 minutes at 4 ºC.

5.8.10 Label two analytical plates with a unique plate barcode label.

5.8.11 Carefully remove seal cap mat from the sample plate *(without disturbing the pelleted material)*.

5.8.12 Transfer 135 µL from each well to both analytical plates (RPOS and RNEG) using a multichannel pipette.

5.8.13 Heat seal both analytical plates with heat seal foil (2.0 seconds at 160 ºC, followed by rotation of the plate by 180° and a further 2.0 seconds of sealing) and transfer to the autosampler ready for analysis.

	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:

- Water addition: 10.0 mL CombiTip with Prog = Dis, Vol = 150 µL, No. of steps = 64, aspirate speed = max, dispense speed = max.
- Analytical SR/LTR addition: 1 mL CombiTip with Prog = Dis, Vol = 225 µL, No. of steps = 4, aspirate speed = max, dispense speed = max.
- IStd addition: 2.5 mL CombiTip with Prog = Dis, Vol = 75 µL, No. of steps = 32, aspirate speed = max, dispense speed = max.
- Aliquoting: 12 channel 15-300 µL pipette with Prog = Dis, Vol = 135 µL, No. of steps = 2, aspirate speed = 4/10, dispense speed = 4/10.

6. Procedure - Mobile Phases and Wash Solutions

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6.1. Mobile Phase A *(0.1% formic acid in water)*

0.1% formic acid in water as supplied

6.2. Mobile Phase B *(0.1% formic acid in acetonitrile)*

0.1% formic acid in acetonitrile as supplied

6.3. Preparation of seal wash and weak needle wash *(isopropanol:water 1:9)*

6.1.1.1. Using a measuring cylinder, transfer 100 mL of isopropanol to a 1 L Duran bottle.

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

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

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

6.4. Preparation of 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 H of Proforma NPC.PRO.MS005

7.1. Instrument checks and sample loading

7.1.1 Perform UPLC Q-TOF system performance check in the assay specific polarity as per 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 4.

7.1.3 Confirm that all instrument parameters and settings correspond with the values outlined in the tables on the subsequent pages.

7.1.4 Load the correct sequence (*SampleList.spl*) into MassLynx and confirm that sample injection order corresponds with the Table 7.

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

Table 4. LC instruments parameters

Variable	Description
Mobile Phase A	0.1% formic acid in water
Mobile Phase B	0.1% formic acid in acetonitrile
Seal Wash	10% isopropanol in water, set to run every 0.5 minutes
Weak Wash	10% isopropanol in water
Strong Wash	Isopropanol
Lockspray	Leucine enkephalin 200 pg/µL (prepared in 1:1 water:acetonitrile+0.05% formic ac.)
Column	Waters Acquity UPLC HSS T3 1.8µm, 2.1 x 150mm
PEEK tubing	ID = 0.004 inch, Length = 33 cm
Column Temp.	45°C
Sample Loop	2 µL
Injection Volume	15 µL
Run Time	12.65 minutes (N.B. with autogain each injection will be 15 minutes)
Autosampler cycle variables/ arguments	Injector programme: LCinj5 Pre-cleans or filling strokes = 0, Filling & injection speed = 5 µL/sec, Pre-injection delay = 500 ms, Post-injection delay = 500 ms, Inject 10 µL to injector, followed by 5 µL to waste. Needle wash sequence = Weak, strong, weak, Wait time = 720 Secs, Valve wash sequence = Weak, strong, weak.
Sample Cooler	4°C with low nitrogen flow to prevent condensation

Table continues

Table 4. LC instruments parameters - continuation

Gradient					
#	**Time (Mins)**	**Flow (ml/min)**	**% A**	**% B**	**Curve**
1	Initial	0.600	99.0	1.0	Initial
2	0.10	0.600	99.0	1.0	6
3	10.00	0.600	45.0	55.0	6
4	10.15	0.610	35.0	65.0	6
5	10.30	0.630	25.0	75.0	6
6	10.45	0.670	15.0	85.0	6
7	10.60	0.750	5.0	95.0	6
8	10.70	0.800	0.0	100.0	6
9	11.00	1.000	0.0	100.0	6
10	11.55	1.000	0.0	100.0	6
11	11.65	1.000	99.0	1.0	6
12	11.70	0.900	99.0	1.0	6
13	11.80	0.800	99.0	1.0	6
14	11.90	0.700	99.0	1.0	6
15	12.00	0.650	99.0	1.0	6
16	12.10	0.610	99.0	1.0	6
17	12.15	0.600	99.0	1.0	6
18	12.65	0.600	99.0	1.0	6

Table 5. MS instruments parameters

Variable	Description
MS Function	ToF MS
Analyser Mode	Sensitivity
Dynamic Range	Normal
Mass Range	50 – 1200 Da
Scan Time	0.1 Secs
Data Format	Centroid
Lockspray function Settings	Acquire lockspray & apply correction, Scan time = 0.1 Secs, Interval = 60 Secs, Scans to average = 4, Mass window = ±0.5.
Method Events	At initial conditions lockspray is infused, at 11.50 minutes lockspray is refilled, at 12.00 minutes lockspray starts infusing again

Table 6. MS source settings

Variable	Description **(positive/negative)*
Sample Probe Position	7
Capillary Voltage (kV)	Positive: 1.5 / Negative: 1.0
Sampling Cone	20
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 4
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 = 120 sec, Use Max Voltage Adjust (V): ticked and set to 1.0
Stepwave 2 Offset	10
Quad Profile	Auto
Lockspray Flow (µL/min)	≤15
Lockspray Capillary Voltage (kV)	Positive 3.0 / Negative: 2.0

7.2. Sample acquisition

7.2.1. Export the batch sample list from LIMS. In the event of samples missing (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 7.

Table 7. Acquisition order

Phase	Number of injections	Description	Acquisition Method	File Naming Convention
Start	5-10	Pre-check blanks	MS only	Project_RPOS/RNEG_TOFxx _test01-05
	10	Blanks	MS only	Project_RPOS/RNEG_TOFxx_Blank01-10
	30	Instrument Conditioning samples	MS only	Project_RPOS/RNEG_TOFxx_IC01-30
	2	Blanks (for dilution series)	MS only	Project_RPOS/RNEG_TOFxx_Blank11-12
	46	Dilution series (low to high % SR)	MS only	Project_RPOS/RNEG_TOFxx_BxSRD01-46
	3	SR conditioning samples	MS only	Project_RPOS/RNEG_TOFxx_IC31-33
	5	Batch Start SR	MS only	Project_RPOS/RNEG_TOFxx_BxS01-05_SR
Sample Analysis	X	Analytical plates	MS only	Project_RPOS/RNEG_ToFxx_UxWxx_LTR Project_RPOS/RNEG_ToFxx_UxWxx Project_RPOS/RNEG_ToFxx_UxWxx_SR
End	5	Batch end SR	MS only	Project_RPOS/RNEG_TOFxx_BXE01-05_SR
	46	Dilution series (high to low % SR)	MS only	Project_RPOS/RNEG_TOFxx_BXSRD92-47
	2	Blanks (for dilution series)	MS only	Project_RPOS/RNEG_TOFxx_Blank13-14
	8	DIDA conditioning samples	MS only	Project_RPOS/RNEG_TOFxx_IC34-41
	10	SR	DIDA*	Project_RPOS/RNEG_TOFxx_DIDA01-10

** 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 to ensure the system is functioning as expected in terms of accuracy and sensitivity, followed by blanks, conditioning SR, 2 blanks (for dilution series), dilution series run

low to high % SR (see Table 8 for the number of injections of each dilution), followed by 3 conditioning SR and the 5 batch start SR.

Table 8. 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 8 for the number of injections of each dilution), 2 blanks (for dilution series), 8 conditioning SR, 10 DIDA

7.2.3. Load the full batch sequence (*SampleList.spl*) into 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.MS005	RPC UPLC-MS Analysis of Human Urine - Proforma
NPC.SOP.CC005	Formatting and Replication of Samples
NPC.SOP.MS002	UPLC & Q-ToF system performance check

9. Version History

Current Version

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

Previous Version

Version number	Author	Changes and justification	Section(s) updated
V2.0	VHS/AA	Changed for new diluted MS protocols	all
V1.1	VHS/AA/ BC	Revision and minor changes	all
V1.0	AA/MD	New SOP	N/A

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