



HILIC UPLC-QTOF Analysis of Small Molecules in Human Urine - Proforma

NPC.PRO.MS006 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 hydrophilic interaction chromatographic (HILIC) assay as outlined in the protocol NPC.SOP.MS006. 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	Batch/lot no.	Opened date
L-Phenylalanine - $^{13}\text{C}_9, ^{15}\text{N}$	Sigma, 60817		
Hippuric Acid $^{13}\text{C}_6$	QMX, IS9117		
Adenosine-2-d1	CDN isotopes, D-1827		
Adenine-2-d1	CDN isotopes, D-6291		
Taurine ^{15}N	Sigma, 605956		
Creatine-(methyl-d3) monohydrate	Sigma, 616249		
L-Arginine- $^{13}\text{C}_6$ hydrochloride	Sigma, 643440		
L-Tryptophan-d5(indole-d5)	Sigma, 615862		
Uracil-2- $^{13}\text{C}, ^{15}\text{N}_2$	Sigma, 608459		
LCMS grade Ammonium formate			
LCMS grade water + 0.1% formic acid			
LCMS grade acetonitrile + 0.1% formic acid			
LCMS grade water			
LCMS grade acetonitrile			
LCMS grade isopropanol			
Comments: n/a <input type="checkbox"/>			

PART A - Internal Standard Stock (IStd-Stock) & Solution (IStd-Soln) Preparation

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

Assay specifics					
Analyst:					
Project:				Date:	
HILIC IStd-Stock					
Weigh each stock standard individually into the corresponding volumetric size outlined below and record the weight and volume (quantities can be scaled up or down depending on requirements)					<input type="checkbox"/>
Make each volumetric flask up to volume with LCMS grade water					<input type="checkbox"/>
IStd-Stock	Weight (mg)	Volume of water (mL)	Target stock conc. (mg/mL)	Weight (mg)	Volume made (mL)
L-Phenylalanine- ¹³ C ₉ , ¹⁵ N	100	5	20		
Hippuric Acid- ¹³ C ₆	37.5	25	1.5		
Sonicate stock solutions until sample dissolution observed					<input type="checkbox"/>
HILIC IStd-Soln					
Transfer each Internal Standard Stock standard prepared above into a 100mL volumetric flask according to the quantities outlined in the table below using appropriate automatic pipettes or measuring cylinders					<input type="checkbox"/>
IStd-Soln	Volume of stock in mix (mL)		Final concentration (mg/mL) in 100 mL of water		
L-Phenylalanine- ¹³ C ₉ , ¹⁵ N	1.4		0.28		<input type="checkbox"/>
Hippuric Acid- ¹³ C ₆	25		0.37		<input type="checkbox"/>
Make the 100 mL volumetric flask up to volume with LCMS grade water					<input type="checkbox"/>
Mix the volumetric flask until the content is visually homogenous					<input type="checkbox"/>
Aliquot 3 mL internal standard solution into appropriate vials and store at -80 °C until required					<input type="checkbox"/>
Comments: n/a <input type="checkbox"/>					

PART B - Method Reference Stock (MR-Stock) and Method Reference Solution (MR-Soln) Preparation

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

Assay specifics					
Analyst:					
Project:				Date:	
HILIC MR-Stock					
Weigh each HILIC standard individually into the corresponding volumetric size outlined below and record the weight and volume (quantities can be scaled up or down depending on requirements)					<input type="checkbox"/>
Make each volumetric flask up to volume with LCMS grade water					<input type="checkbox"/>
MR-Stock	Weight (mg)	Volume of water (mL)	Target stock conc. (mg/mL)	Weight (mg)	Volume made (mL)
Adenosine-2-d1	25	5	5		
Adenine-2-d1	4	5	0.8		
Taurine ¹⁵ N	120	5	24		
Creatine-(methyl-d3) monohydrate	25	5	5		
L-Arginine- ¹³ C ₆ hydrochloride	250	5	50		
L-Tryptophan-d5(indole-d5)	50	10	5		
Uracil-2- ¹³ C, ¹⁵ N ₂	50	25	2		
Sonicate stock solutions until sample dissolution observed					<input type="checkbox"/>
Comments: n/a <input type="checkbox"/>					

Table continues

Assay specifics

Analyst:

Project:

Date:

HILIC MR-Soln

Transfer each MR standard stock (MR-Stock) prepared above into a 100 mL volumetric flask according to the quantities outlined in the table below using appropriate automatic pipettes or measuring cylinders.

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MR-Soln	Volume of stock in mix (mL)	Final concentration (mg/mL) in 100 mL of water	
Adenosine-2-d1	0.86	0.043	<input type="checkbox"/>
Adenine-2-d1	1.4	0.011	<input type="checkbox"/>
Taurine ¹⁵ N	0.84	0.202	<input type="checkbox"/>
Creatine-(methyl-d3) monohydrate	0.49	0.025	<input type="checkbox"/>
L-Arginine- ¹³ C ₆ hydrochloride	0.69	0.345	<input type="checkbox"/>
L-Tryptophan-d5(indole-d5)	6.7	0.335	<input type="checkbox"/>
Uracil-2- ¹³ C, ¹⁵ N ₂	18	0.360	<input type="checkbox"/>
Make the 100 mL volumetric flask up to volume with LCMS grade water			<input type="checkbox"/>
Mix the volumetric flask until the content is visually homogenous			<input type="checkbox"/>
Aliquot into appropriate storage containers and store at -80 °C until required			<input type="checkbox"/>
Comments: n/a <input type="checkbox"/>			

PART C – Analytical Study Reference (SR), analytical Long Term Reference (LTR), SR containing MR (SR+MR) and Blanks 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:
SR and LTR	
Preparation date of MR-Soln used _____	
Remove sufficient stock urine LTR from storage at -80 °C and allow to defrost at 2-8 °C (11.5 mL sufficient for ≤ 1000 samples)	<input type="checkbox"/>
LTR (volume = _____) mixed with LCMS grade water (volume = _____) and MR-Soln (volume = _____) (ratio 1:1:1, analytical LTR)	<input type="checkbox"/>
Final volume aliquotted into _____ 700 µL aliquots.	
Store aliquots at -80 °C in freezer	<input type="checkbox"/>
SR (volume = _____) mixed with LCMS grade water (volume = _____) and MR-Soln (volume = _____) (ratio 1:1:1, analytical SR)	<input type="checkbox"/>
Final volume aliquotted into _____ 700 µL aliquots.	
Start/End SR vials: prepare 2x 150 µL aliquots of analytical SR solution	<input type="checkbox"/>
Remaining analytical SR aliquoted into _____ 150 µL aliquots.	
Store aliquots at -80 °C in freezer	<input type="checkbox"/>
SR (volume = _____) mixed with MR-Soln (volume = _____) (ratio 2:1, SR+MR)	<input type="checkbox"/>
Instrument conditioning and DIDA vials: prepare 150 µL aliquots (4 aliquots) of SR+MR (prepared above) (Sample batch is ≤1000 samples)	<input type="checkbox"/>
Store aliquots at -80 °C in freezer	<input type="checkbox"/>
Prepare 3 blanks by combining 100 µL of LCMS grade water with 50 µL of MR-Soln for each blank (Sample batch is ≤1000 samples)	<input type="checkbox"/>
Store aliquots at -80 °C in freezer	<input type="checkbox"/>
Comments: n/a <input type="checkbox"/>	

PART D - SR Dilution Series - *Prior Start of Analysis.*

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

Assay specifics							
Analyst:							
Project:					Date:		
No. of samples _____ No. of batches _____ (Sample batch is ≤1000 samples)							
Total number of dilution series sets required (sample sets x 4 + 2 backup) = _____							
The table below indicates the volumes required for a single sample batch (6 dilution series) this will be followed for studies consisting of 1000 samples or less. Please 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+MR (μL)	Vol. of LCMS grade water (μL)	Vol. of IStd-Soln	Total vol. (μL)	Vol. in aliquot (μL)	
1	100	225	0	75	300	50	<input type="checkbox"/>
2	80	105	26.3	43.8	175	29.2	<input type="checkbox"/>
3	60	57.4	38.3	31.9	127.5	21.7	<input type="checkbox"/>
4	40	38.3	57.4	31.9	127.5	21.7	<input type="checkbox"/>
5	20	26.3	105	43.8	175	29.2	<input type="checkbox"/>
6	10	22.5	202.5	75	300	50	<input type="checkbox"/>
7	1	2.3	222.8	75	300	50	<input type="checkbox"/>
Blank	0	0	247.5	82.5	300	55	<input type="checkbox"/>
Aliquot each dilution into 6 separate Eppendorf tubes containing the volumes detailed above (vol. in aliquot) and store at -80 °C until required							<input type="checkbox"/>
Comments: n/a <input type="checkbox"/>							

PART E - SR and Dilution Series - *On Day of Analysis.*

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

Assay specifics				
Analyst:				
Project:			Date:	
Complete all pre-project checks as given NPC.SOP.MS002				<input type="checkbox"/>
Remove the appropriate aliquot of SR dilution series, conditioning, blanks and DIDA SR from storage at -80°C and allow to defrost for 2 hours at 2-8 °C				<input type="checkbox"/>
Add the corresponding volume of ice cold acetonitrile to the SR dilution aliquot as per the table below using pipettes and vortex mix				<input type="checkbox"/>
Dilution point	Percentage of SR (%)	Vol. in aliquot (µL)	Vol. of Acetonitrile (µL)	
1	100	50	150	<input type="checkbox"/>
2	80	29.2	87.6	<input type="checkbox"/>
3	60	21.7	65.1	<input type="checkbox"/>
4	40	21.7	65.1	<input type="checkbox"/>
5	20	29.2	87.6	<input type="checkbox"/>
6	10	50	150	<input type="checkbox"/>
7	1	50	150	<input type="checkbox"/>
Blank	0	55	165	<input type="checkbox"/>

Table continues

Continuation - PART E - SR and Dilution Series - On Day of Analysis.

Conditioning, blanks and DIDA aliquots: thaw appropriate number of conditioning, blanks and DIDA aliquots required for study, add 50 µL of IStd-Soln and 600 µL of cold acetonitrile	<input type="checkbox"/>
Blank: Vortex mix Conditioning and DIDA: Mix for 2 minutes on a plate mixer at 1200 rpm at 4 °C	<input type="checkbox"/>
Centrifuge at 3486 g for 10 minutes at 4 °C	<input type="checkbox"/>
Aliquot supernatant into appropriate labelled UPLC vials	<input type="checkbox"/>
Place vials in correct positions in autosampler	<input type="checkbox"/>

Comments: n/a ☐

PART F- 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, analytical LTR and analytical SR at 2-8 °C for a minimum of 2 hours	<input type="checkbox"/>
2	Centrifuge sample plate at 3486 g for 1 minute at 4 °C	<input type="checkbox"/>
3	Add 50 µL of LCMS grade water to each sample well, except column 11 and 12	<input type="checkbox"/>
4	Dispense 75 µL of HILIC-LTR to column 11	<input type="checkbox"/>
5	Dispense 75 µL of project specific HILIC-SR to column 12	<input type="checkbox"/>
6	Add 25 µL of HILIC-ISTD to each well on the plate	<input type="checkbox"/>
7	Add 300 µL of ice cold acetonitrile to each well on the plate	<input type="checkbox"/>
8	Seal sample plate with heat seal foil	<input type="checkbox"/>
9	Mix for 2 minutes on a plate mixer at 1200 rpm at 4 °C	<input type="checkbox"/>
10	Centrifuge sample plate at 3486 g for 10 minutes at 4 °C	<input type="checkbox"/>
11	Label two analytical plates with a unique plate barcode label	<input type="checkbox"/>
12	Carefully remove foil from the sample plate (<i>without disturbing the pelleted material</i>)	<input type="checkbox"/>
13	Transfer 140 µL of each well to both analytical plates; HPOS and backup	<input type="checkbox"/>
14	Seal both analytical plates with heat seal foil	<input type="checkbox"/>
15	Store backup plate at -80 °C	<input type="checkbox"/>
16	Place HPOS plate in relevant autosampler position	<input type="checkbox"/>
Comments: n/a <input type="checkbox"/>		

PART G - 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 HILIC Mobile phase A and B	
N.B. Volumes can be scaled up or down depending on requirement	
Mobile phase A: preparation of 2 L (20 mM ammonium formate in 0.1% formic acid in water)	
Weigh 2.52 g of ammonium formate into a weighing boat Weight = _____	<input type="checkbox"/>
Transfer the ammonium formate into a 2 L volumetric flask	<input type="checkbox"/>
Using a measuring cylinder, transfer 1000 mL of LCMS grade water with 0.1% formic acid to the volumetric flask	<input type="checkbox"/>
Sonicate for 5 minutes or until the Ammonium formate has fully dissolved	<input type="checkbox"/>
On volumetric flask, make up remaining volume to the mark with LCMS grade water with 0.1% formic acid	<input type="checkbox"/>
Mix until the solution is completely homogenous	<input type="checkbox"/>
Measure the pH of the mobile phase ensuring the pH is 3.5 ± 0.1 pH = _____	<input type="checkbox"/>
Slowly transfer to the Duran bottle and label appropriately	<input type="checkbox"/>
Mobile phase B: preparation of 5 L (acetonitrile with 0.1% formic acid)	
Transfer LCMS grade acetonitrile in 0.1% formic acid to a 5 L Duran bottle	<input type="checkbox"/>
Label appropriately	<input type="checkbox"/>
Comments: n/a <input type="checkbox"/>	

Table continues

Assay specifics

Analyst:

Project:

Date:

Preparation of HILIC Wash Solutions

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

Seal Wash (isopropanol:water 1:9 v/v)

Transfer 100 mL of isopropanol into a Duran bottle

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Add 900 mL of LCMS grade water into the Duran bottle from above

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Mix until the content is homogenous

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Sonicate for 5 minutes, seal the bottle and assign an expiry of 1 month

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Weak needle wash (water:acetonitrile 1:3 v/v)

Transfer 125 mL of LCMS grade water into a Duran bottle

☐

Add 375 mL of LCMS grade acetonitrile into a Duran bottle from above

☐

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 H –Acquisition

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

Assay specifics	
Analyst:	
Project:	Date:
Instrument number: _____	
Column: Acquity UPLC BEH HILIC, 1.7µm, 2.1 x 150 mm, P/N: 186003462 LOT: _____ Serial Number: _____	
Ionisation mode required: _____	
Instrument check performed (Please, see separate Proforma sheet NPC.PRO.MS002)	<input type="checkbox"/>
All solvent lines match the assay specific buffers and solutions	<input type="checkbox"/>
Check the following against NPC.SOP.MS006:	
Tune file used: _____	
Acquisition method used: _____	
Inlet method used: _____	
Sequence loaded into Masslynx	<input type="checkbox"/>
Sample plate loaded into tray position	<input type="checkbox"/>
Comments: n/a <input type="checkbox"/>	