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Liquid Chromatography High-Resolution Mass Spectrometry metabolomics analysis: from preparation to injection



Forked from [Liquid-phase metabolomics analysis: from preparation to injection](#)

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External link: <https://www.laberca.org/en/analytical-platform/presentation>

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<https://dx.doi.org/10.17504/protocols.io.5jyl82odr12w/v1>



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Protocol status: In development

We are still developing and optimizing this protocol

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Protocol Integer ID: 107328

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Abstract

The purpose of this notice is to define two types of metabolomics analysis on an LC-HRMS instrument (Orbitrap or ToF) with either polar (HILIC column) or non-polar (RPLC column).

The steps cover the sample preparation, the quality control and the analytical condition.

The protocol enables samples to be obtained which can then be statistically reprocessed according to the metabolomics workflow.

Image Attribution

MELISA platform

Guidelines

Recommandations:

- Perform several blank injections at the start of analysis to check for contamination;
- Perform a system suitability test (SST) with two injections of the standard mix ("Mix metabo 2") at the beginning and end of the sequence;
- Before launching the samples injection check with "Mix Metabo 2" profiles that there has been no drift in retention times, that intensity is good and that mass accuracy is correct;
- Injection of 6 QC at start of sequence;
- Inject diluted QC (1/2, by 4 and by 8) and check that the molecules follow this decrease in concentration;
- Injection of a QC every 5 samples.

Materials

Calibrants:

- Thermo Solution: ESI+ (Reference: 88322)
- Thermo Solution: ESI- (Reference : 88324)

Pipettes and tips:

- Filter tips
- P20, P200 and P1000 pipettes

Tubing:

- Centrifuge tube
- HPLC glass vials with 200 μ L insert
- 5 mL glass tubes (except for urine preparation)
- 10 kDa filters centrifuge tubes (only for urine preparation)

Equipment lab:

- Centrifuge for tube, vials or eppendorf
- Nitrogen evaporator with racks for HPLC vials and tubes
- Refractometer (only for urine preparation)
- Universal Indicator Paper Strips for pH 1-14 (only for urine preparation)

Instruments:

- LC-HRMS : Mass spectrometer QExactive - Orbitrap - Thermo Fisher
- LC-HRMS : Mass spectrometer Exactive - Orbitrap - Thermo Fisher
- LC-QTOF : Mass spectrometer Synapt Acuity - TOF - Waters

Protocol materials

- ☒ Water LC-MS grade B&J Brand **VWR International (Avantor) Catalog #BJLC365-2.5**
- ☒ Acetonitrile **Merck MilliporeSigma (Sigma-Aldrich) Catalog #34998**
- ☒ Acetic acid glacial $\geq 99.7\%$ ACS **VWR International (Avantor) Catalog #36289.K3**
- ☒ Methanol LC-MS grade B&J Brand **VWR International (Avantor) Catalog #BJLC230-2.5**
- ☒ MTBE: Sigma Chromasolv 99.8% for HPLC 100mL (smallest available) (34875-100mL) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #34875-100ML**
- ☒ EtOH 100% at -20°C
- ☒ Ammonium Acetate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A1542-500G**
- ☒ Chloroform **Merck MilliporeSigma (Sigma-Aldrich) Catalog #366919-1L**
- ☒ Acetic acid **Fisher Scientific**

Safety warnings

- ⚠ Wear your Personal Protective Equipment (PPE) at least a lab coat, protective eyewear, gloves, long pants and closed toe shoes

Before start

- To avoid any degradation of samples, the use of plastic materials should be strictly limited. A temperature close to zero must be maintained throughout the process;
- The protocols are presented for guidance only, and it will be necessary, in the context of a new project, to carry out pre-tests in order to assess the proportions of matrix and solvents. Similarly, MS and gradient parameters can be adjusted according to matrix richness;
- Ready-to-use column: reconditioning of the column after each injection sequence.

Note

ACN and methanol solvents are **HPLC grade**, **Milli-Q** grade water and **LC-MS grade** isopropanol and chloroform.



I. Sample preparation

1 For serum, plasma or cell culture media

1.1 Triphasic preparation: Bligh and Dyer

21m 20s

Attention: The use of filter tips is essential for matrix sampling

- Thaw samples previously stored at $-20\text{ }^{\circ}\text{C}$ or $-80\text{ }^{\circ}\text{C}$ on ice
- After thawing, vortex each sample for 00:00:20
- Remove 30 μL of matrix and transfer to a 5 mL glass tube
- Add 190 μL of cold

 Methanol LC-MS grade B&J Brand **VWR International**
(Avantor) Catalog #BJLC230-2.5

and vortex for 00:00:20

- Add 390 μL of cold

 Chloroform **Merck MilliporeSigma (Sigma-Aldrich)** Catalog #366919-1L and

vortex for 00:00:20

- Add 120 μL

 Water LC-MS grade B&J Brand **VWR International**
(Avantor) Catalog #BJLC365-2.5

and vortex for 00:00:20

- Centrifuge tubes for 00:20:00 at 450 x g, 4°C
- After centrifugation, remove 190 μL of the methanolic phase and transfer to an HPLC vial (with 200 μL insert)
- Evaporate vials under nitrogen flow at $25\text{ }^{\circ}\text{C}$
- Store vials at $-20\text{ }^{\circ}\text{C}$

Equipment	
HERAEUS Multifuge x3 FR	NAME
Centrifuge	TYPE
Thermo Fisher Scientific	BRAND
HERAEUS Multifuge x3 FR	SKU
	

Equipment	
Nitrogen evaporator	NAME
Nitrogen evaporator with racks for HPLC vials and tubes	TYPE
Liebisch Labortechnik	BRAND
-	SKU

Equipment	
HPLC transparent vial with insert 0.2mL	NAME
32×11.65mm	TYPE
Thermo	BRAND
-	SKU



1.2 Triphasic preparation: MTBE

29m 20s

- Thaw samples previously stored at $-20\text{ }^{\circ}\text{C}$ or $-80\text{ }^{\circ}\text{C}$ on ice
- After thawing, vortex each sample for 00:00:20
- Place 30 μL of matrix and 220 μL of cold

Methanol LC-MS grade B&J Brand **VWR International (Avantor)** Catalog #BJLC230-2.5

in a 5 mL glass tube

- Vortex the tube for 00:00:20
- Add 750 μL of

MTBE: Sigma Chromasolv 99.8% for HPLC 100mL (smallest available) (34875-100mL) **Merck MilliporeSigma (Sigma-Aldrich)** Catalog #34875-100ML

and vortex for 00:00:20

- Place samples at $4\text{ }^{\circ}\text{C}$ and vortex every 00:02:00 for 00:06:00
- Add 188 μL of

Water LC-MS grade B&J Brand **VWR International (Avantor)** Catalog #BJLC365-2.5

, vortex for 00:00:20

- Centrifuge tubes for 00:20:00 at 450 x g
- Collect 190 μL of aqueous phase. Evaporate vials to dryness under nitrogen flow at $25\text{ }^{\circ}\text{C}$
- Store vials at $-20\text{ }^{\circ}\text{C}$

Equipment

HERAEUS Multifuge x3 FR

NAME

Centrifuge

TYPE

Thermo Fisher Scientific

BRAND

HERAEUS Multifuge x3 FR

SKU



Equipment

Nitrogen evaporator

NAME

Nitrogen evaporator with racks for HPLC vials and tubes

TYPE

Liebisch Labortechnik

BRAND

-

SKU

Equipment

HPLC vial without insert - screw vial , 1.5mL

NAME

10 mm clear WO

TYPE

Thermo Fisher Scientific

BRAND

-

SKU

1.3 Methanol extraction

20m 35s

- Thaw samples previously stored at $-20\text{ }^{\circ}\text{C}$ or $-80\text{ }^{\circ}\text{C}$ on ice
- After thawing, vortex each sample for 00:00:20
- 400 μL matrix + 1400 μL


Methanol LC-MS grade B&J Brand VWR International (Avantor) Catalog #BJLC230-2.5

in a centrifuge tube
- Vortex for 00:00:15
- Centrifuge at room temperature at 4.5 rpm for 00:20:00
- Collect 370 μL of the methanolic fraction and place in a vial without insert
- Evaporate to dryness under nitrogen at $25\text{ }^{\circ}\text{C}$

Equipment

Mini-centrifuge

NAME

Centrifuge

TYPE

Fisher

BRAND

S67601B

SKU

<https://www.fishersci.com/shop/products/fisherbrand-standard-mini-centrifuge-standard-mini-centrifuge/s67601b>

LINK

Any standard mini centrifuge with adapters for different tube sizes will suffice

SPECIFICATIONS



Equipment	
Nitrogen evaporator	NAME
Nitrogen evaporator with racks for HPLC vials and tubes	TYPE
Liebisch Labortechnik	BRAND
-	SKU

Equipment	
Centrifuge Tube	NAME
1.5mL	TYPE
Fisher Scientific	BRAND
05-408-129	SKU
https://www.fishersci.com/shop/products/fisherbrand-premium-microcentrifuge-tubes-1-5ml-natural-1-5ml-o-d-x-l-10-8-x-40-6mm/05408129	LINK
	



Equipment

HPLC vial without insert - screw vial , 1.5mL	NAME
10 mm clear WO	TYPE
Thermo Fisher Scientific	BRAND
-	SKU

2 For urine matrix

2.1 Mesuring pH and urine density

- Thaw in ice 0 °C samples previously stored at -20 °C or On ice
- Take an aliquot of 1 mL in a 5 mL glass tube
- Vortexing
- Centrifuge at 750 x g, 4°C , at least 5 min
- The pH is measured using pH paper strips. The pH value can provide information to explain why a sample may be atypical
- The urine specific gravity value is used to normalize the sample. It is measured using a refractometer. Zero measurement is carried out before each use and every 30 samples measured.



Water LC-MS grade B&J Brand **VWR International**
(Avantor) Catalog #BJLC365-2.5

is placed on the refractometer's measuring cell, the "Zero" button is pressed and the value of 1,000 is displayed. Between each measurement, rinse the cell with ,then wipe clean with paper. To obtain good measurements, make sure the cell is sufficiently covered with liquid (approximative



200 µL

liquid are required to cover the cell).

Equipment	
HERAEUS Multifuge x3 FR	NAME
Centrifuge	TYPE
Thermo Fisher Scientific	BRAND
HERAEUS Multifuge x3 FR	SKU
	

Equipment	
MQuant Supelco pH indicator strips	NAME
Universal Indicator Paper Strips for pH 1-14	TYPE
Supelco	BRAND
1.09535.0001	SKU

Equipment

AR200 Automatic Digital Refractometer	NAME
Digital Refractometer	TYPE
Reichert	BRAND
13950000	SKU
https://www.reichertai.com/products/handheld-digital/ar200-automatic-digital-refractometer/	LINK

2.2 Filtration and centrifugation

30m

- Collect  500 µL of urine and place in an centrifugation tube fitted with a 10kDa filter
- Centrifuge at  13000 x g for about  00:30:00 at about  5 °C
- Remove the filter and store the filtrate at  4 °C

2.3 Normalization

The normalization presented here is a urinary density normalization. The Excel model "normalization by urine density" can be used to perform all normalization calculations. The dilution rate depends on the type of sample analyzed (human urine, pig urine, etc.).

In the Excel "Urine Density Normalization", in order to evaluate the dilution ratio, a standard curve is created by measuring the urine density of the urine with the highest urine density at different dilution ratios. Normalization is then performed for each sample. Randomly check the new urine specific gravity value of a few samples. The excel file is available on LABERCA's Quality Management System.



Citation

Jacob CC, Dervilly-Pinel G, Deceuninck Y, Gicquiau A, Chevillon P, Bonneau M, Le Bizec B (2017)

. Urinary signature of pig carcasses with boar taint by liquid chromatography-high-resolution mass spectrometry.

<https://doi.org/10.1080/19440049.2016.1265152>

LINK

3 Preparing samples for injection

3.1 For RPLC analysis

10m 20s

- If samples have been frozen, thaw in melting ice
- Add  10 μ L of ISTD "Mix Metabo 2" mix into a clean HPLC vial with 200 μ L insert
- Evaporate to dryness under nitrogen flow at  25 °C
- Transfert  100 μ L of sample extract into the HPLC vial insert and vortex for  00:00:20 (Note: If necessary, centrifuge samples for  00:10:00 at  450 x g and  4 °C before transfer to the HPLC vial with insert)

Note

Tips: Prepare the Mix Metabo 2 SST at the same time (see 4.1).

Depending on the available sample extract really available adjust the transfered sample extract amount in order to keep a constant ISTD dilution.

If you have to dilute your sample extracts then do it with

 Water LC-MS grade B&J Brand **VWR International**
(Avantor) Catalog #BJLC365-2.5

/  Acetonitrile **Merck MilliporeSigma** (Sigma-Aldrich) Catalog #34998 **mixture**
(95:5) and vortex for  00:00:20 .

Equipment	
HERAEUS Multifuge x3 FR	NAME
Centrifuge	TYPE
Thermo Fisher Scientific	BRAND
HERAEUS Multifuge x3 FR	SKU
	

Equipment	
Nitrogen evaporator	NAME
Nitrogen evaporator with racks for HPLC vials and tubes	TYPE
Liebisch Labortechnik	BRAND
-	SKU



Equipment

HPLC transparent vial with insert 0.2mL	NAME
32×11.65mm	TYPE
Thermo	BRAND
-	SKU

3.2 For HILIC analysis

10m 20s

- If samples have been frozen, thaw in melting ice
- Add 10 μL of ISTD "Mix Metabo 2" mix into a clean HPLC vial with 200 μL insert
- Evaporate to dryness under nitrogen flow at 25 °C
- Transfert 100 μL of sample extract into the HPLC vial insert and vortex for 00:00:20 (Note: If necessary, centrifuge samples for 00:10:00 at 450 x g and 4 °C before transfer to the HPLC vial with insert)

Note

Tips: Prepare the Mix Metabo 2 SST at the same time (see 4.1).

Depending on the available sample extract really available adjust the transfered sample extract amount in order to keep a constant ISTD dilution.

If you have to dilute your sample extracts then do it with



Water LC-MS grade B&J Brand **VWR International**
(**Avantor**) Catalog #BJLC365-2.5

/



Acetonitrile **Merck MilliporeSigma (Sigma-Aldrich)** Catalog #34998

mixture

(**10:90**) and vortex for 00:00:20 .

Equipment

HERAEUS Multifuge x3 FR

NAME

Centrifuge

TYPE

Thermo Fisher Scientific

BRAND

HERAEUS Multifuge x3 FR

SKU



Equipment

HPLC transparent vial with insert 0.2mL

NAME

32×11.65mm

TYPE

Thermo

BRAND

-

SKU

II. Quality control

4 Preparation of System Suitability and Internal Standard "Mix Metabo 2"

- 4.1 A solution of standard compounds used in metabolomics, called "Mix Metabo 2" at 10 ng/μL. This solution can be used as an internal standard, but also as a standard at the start of a sequence to validate instrumental performance.
- This mix contains the following 4 standard compounds, available from Sigma-Aldrich and Cluzeau Info Labo (CIL): L-leucine-5,5,5-d3 (CAS: 87828-86-2), L-tryptophan-

2,3,3-d3 (CAS: 133519-78-5), Indole-2,4,5,6,7-d5-3-acetic acid (CAS: 76937-78-5), 1,14-tetradecanedioic-d24 acid (CAS: 130348-88-8).

In a 15mL amber vial, add the following volumes of each standard at 1mg/mL to obtain the metabo 2 mix solution at 10 ng/μL:

Name	Solvent	Volume introduce
L-tryptophan-2,3,3-d3	H2O:EtOH (25:75), 1 mg/mL	100 μL
L-leucine-5,5,5-d3	H2O:EtOH (75:25), 1 mg/mL	100 μL
Indole-2,4,5,6,7-d5-3-acetic acid	H2O:EtOH (25:75), 1 mg/mL	100 μL
1,14-tetradecanedioic-d24 acid	H2O:EtOH (25:75), 1 mg/mL	100 μL

Preparation of stock solutions

Make up to 10 mL with LC-MS

Water LC-MS grade B&J Brand VWR International
(Avantor) Catalog #BJLC365-2.5

/ EtOH 100% at -20°C (25:75, (v:v)).

Preparing Mix Metabo 2 for System Suitability Test run before starting of the sample analysis sequence:

In an HPLC vial with insert, take 100 μL of the Mix Metabo 2 mix at 10 ng/μL (or less if your instrument is sensitive enough), evaporate under nitrogen at 25 °C .

Then add into the HPLC vial 100 μL of

Acetonitrile Merck MilliporeSigma (Sigma-Aldrich) Catalog #34998 /

Water LC-MS grade B&J Brand VWR International
(Avantor) Catalog #BJLC365-2.5

(5:95, (v:v)) for RPLC analysis, or 100 μL of

Acetonitrile Merck MilliporeSigma (Sigma-Aldrich) Catalog #34998 /

Water LC-MS grade B&J Brand VWR International
(Avantor) Catalog #BJLC365-2.5

(90:10, (v:v)) for HILIC analysis.



This SST vial will be used to qualify the LC-HRMS system before any injections and after each batch analysis.

Equipment

HPLC transparent vial with insert 0.2mL	NAME
32×11.65mm	TYPE
Thermo	BRAND
-	SKU

5 Preparation of QC pool

5.1

The QC corresponds to the sample pool, and can be carried out at different stages of the manipulation. If the matrix is in liquid form, it is preferable to carry out the QC before handling, and to treat the QC pool as a sample.

In the case of a solid matrix, QC pooling can be carried out when the organic phase is sampled.

Caution: Allow for a sufficiently large volume of QC to be injected throughout the sequence, and plan for possible re-injections.

5.2 QC pool dilutions by 2, by 4 and by 8 have to be prepared.

- Transfert  100 μL of sample extract in a centrifuge tube
- Add  100 μL of the dilution solvent either



Water LC-MS grade B&J Brand **VWR International**
(Avantor) Catalog #BJLC365-2.5



Acetonitrile **Merck MilliporeSigma (Sigma-Aldrich)** Catalog #34998 mixture
(95:5) for RPLC or

20s

Water LC-MS grade B&J Brand **VWR International**
(Avantor) Catalog #BJLC365-2.5

/ Acetonitrile **Merck MilliporeSigma (Sigma-Aldrich)** Catalog #34998 **mixture**

(10:90) for HILIC then vortex for 00:00:20

- Transfert 100 µL of solution into a clean HPLC vial with insert and label it as "dil2 QC pool"
- Repeat these steps to create "dil4 QC pool" and "dil8 QC pool" vials

These vials will be used to facilitate data filtration during data analysis (e.g. search for matrix effect)

Equipment

HPLC transparent vial with insert 0.2mL	NAME
32×11.65mm	TYPE
Thermo	BRAND
-	SKU

6 Blank

- 6.1 A "handling blank" have to be carried out to assess the contamination caused by the sample handling. The handling blank follows the same protocol as that for the samples, the only difference being that the matrix is replaced by water.

7 Preparing the mobile phase (PM)

- 7.1 Preparation of mobiles phases:

For RPLC analysis: **H2O/ACN mixture (95:5)** with 0.1% Acetic Acid



- Solvent A: 1 L

Water LC-MS grade B&J Brand **VWR International (Avantor)** Catalog #BJLC365-2.5

, 1 mL Acetic acid **Fisher Scientific**

- Solvent B: 1 L

Acetonitrile **Merck MilliporeSigma (Sigma-Aldrich)** Catalog #34998 , 1 mL

Acetic acid **Fisher Scientific**

For HILIC analysis: **H₂O/ACN mixture (10:90)** with 10mM

Ammonium Acetate **Merck MilliporeSigma (Sigma-Aldrich)** Catalog #A1542-500G

- Solvent A: 990 mL

Water LC-MS grade B&J Brand **VWR International (Avantor)** Catalog #BJLC365-2.5

, 10 mL 1000 mM

Ammonium Acetate **Merck MilliporeSigma (Sigma-Aldrich)** Catalog #A1542-500G

- Solvent B: 990 mL

Acetonitrile **Merck MilliporeSigma (Sigma-Aldrich)** Catalog #34998 ,

10 mL 1000 mM

Ammonium Acetate **Merck MilliporeSigma (Sigma-Aldrich)** Catalog #A1542-500G

III. Analytical conditions

8 Instrument set-up



Create a randomized injection sequence incorporating QC injection every five samples.

Typical sequence :

- . 3*Phase Mobile (PM)
- . 2* Mix metabo 2 (SST)
- . 1* Procedural Blank
- . 6* QC



- . 3* dil8 QC pool
- . 3* dil4 QC pool
- . 3* dil2 QC pool
- . 5* samples
- . 1 QC
- . 5* samples
- ...
- . QC
- . QC with DDA or DIA MS/MS method (Positive mode)
- . QC with DDA or DIA MS/MS method (Negative mode)
- . Mix Metabo 2 (SST)
- . 3* PM

Instruments used :

- LC-HRMS : Mass spectrometer QExactive - Orbitrap - Thermo Fisher
- LC-HRMS : Mass spectrometer Exactive - Orbitrap - Thermo Fisher
- LC-QTOF : Mass spectrometer Synapt Acquity - TOF - Waters

Note

Check PM and SST injections results before going further. Look at the control chart available.

Citation

Broadhurst D, Goodacre R, Reinke SN, Kuligowski J, Wilson ID, Lewis MR, Dunn WB (2018)

. Guidelines and considerations for the use of system suitability and quality control samples in mass spectrometry assays applied in untargeted clinical metabolomic studies.

<https://doi.org/10.1007/s11306-018-1367-3>

LINK

Equipment

LC-HRMS	NAME
QExactive Mass spectrometer (MS) Orbitrap	TYPE
Thermo Fisher	BRAND
-	SKU

Equipment

LC-HRMS	NAME
Exactive Mass Spectrometer (MS) Orbitrap	TYPE
Thermo Fisher	BRAND
-	SKU

Equipment

LC-QTOF	NAME
Mass spectrometer Synapt Acquity	TYPE
Waters	BRAND
-	SKU

9 LC-HRMS instrument : LC-QExactive

9.1 *RPLC analysis*

Solvents:

- Solvent A:  1 L

 Water LC-MS grade B&J Brand **VWR International**
(Avantor) Catalog #BJLC365-2.5

,  1 mL

 Acetic acid glacial $\geq 99.7\%$ ACS **VWR International**
(Avantor) Catalog #36289.K3

- Solvent B:  1 L

 Acetonitrile **Merck MilliporeSigma (Sigma-Aldrich)** Catalog #34998 ,  1 mL

 Acetic acid glacial $\geq 99.7\%$ ACS **VWR International**
(Avantor) Catalog #36289.K3

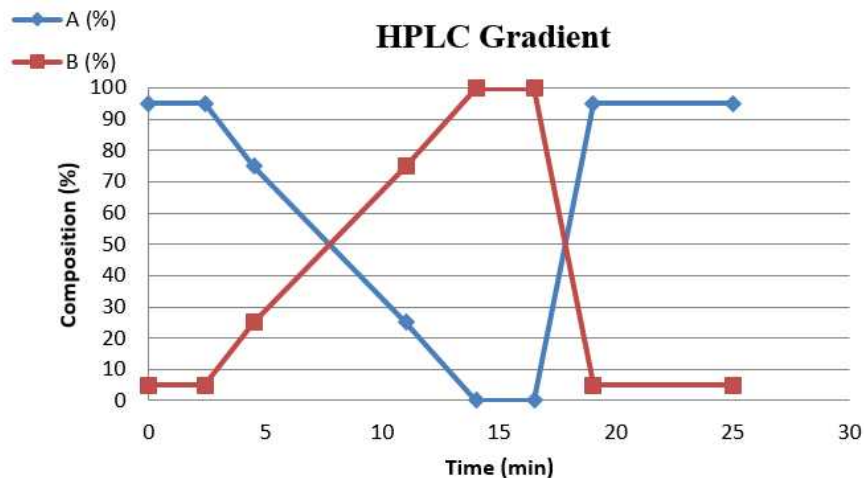
- Washing solutions depending on equipment type

Gradient:

This is a typical gradient, which can be re-evaluated depending on the type of analysis performed.

	Time (min)	A (%)	B (%)
	0	95	5
	2.4	95	5
	4.5	75	25
	11	25	75
	14	0	100
	16.5	0	100
	19	95	5
	25	95	5

Elution gradient



Wash solution:

- Rinse bottle N°1:

 Water LC-MS grade B&J Brand **VWR International**
(Avantor) Catalog #BJLC365-2.5

;

- Rinse bottle N°2:

 Water LC-MS grade B&J Brand **VWR International**
(Avantor) Catalog #BJLC365-2.5

/

 Methanol LC-MS grade B&J Brand **VWR International**
(Avantor) Catalog #BJLC230-2.5

or

 Water LC-MS grade B&J Brand **VWR International**
(Avantor) Catalog #BJLC365-2.5

/  Acetonitrile Merck MilliporeSigma (Sigma-Aldrich) Catalog #34998 **(20:80)**.

Q-Exactive:

 Water LC-MS grade B&J Brand **VWR International**
(Avantor) Catalog #BJLC365-2.5

/

 Methanol LC-MS grade B&J Brand **VWR International**
(Avantor) Catalog #BJLC230-2.5

or

 Water LC-MS grade B&J Brand **VWR International**
(Avantor) Catalog #BJLC365-2.5

/  Acetonitrile **Merck MilliporeSigma (Sigma-Aldrich)** Catalog #34998 **(95:5)**.

Column:

The column used is a **Hypersil Gold C18 (1.9µm, 100mm*2.1mm)**, Thermo Scientific. This column is used with a pre-column whose filter is changed regularly. Flow rate is 0.4 mL/min, oven temperature  35 °C .

The Hypersil Gold C18 column is reconditioned before storage in

 Acetonitrile **Merck MilliporeSigma (Sigma-Aldrich)** Catalog #34998 /

 Water LC-MS grade B&J Brand **VWR International**
(Avantor) Catalog #BJLC365-2.5

(80:20).

Tune parameters:

These are typical parameters, which can be re-evaluated according to the type of analysis performed.

	A	B
	Sheath gas (L/h)	55
	Auxiliary gas (L/h)	10
	Sweep gas (L/h)	0
	Capillary temperature (°C)	350
	Capillary voltage (V)	(+): 30 ; (-): -30
	Tube lens voltage (V)	(+): 100 ; (-): -80

Injection on Thermo brand instruments, for both ionization modes

9.2 HILIC analysis

Solvents:

- Solvent A:  990 mL

 Water LC-MS grade B&J Brand **VWR International (Avantor)** Catalog #BJLC365-2.5

,  10 mL 1000 mM

 Ammonium Acetate **Merck MilliporeSigma (Sigma-Aldrich)** Catalog #A1542-500G

- Solvent B:  990 mL

 Acetonitrile **Merck MilliporeSigma (Sigma-Aldrich)** Catalog #34998 ,

 10 mL 1000 mM

 Ammonium Acetate **Merck MilliporeSigma (Sigma-Aldrich)** Catalog #A1542-500G

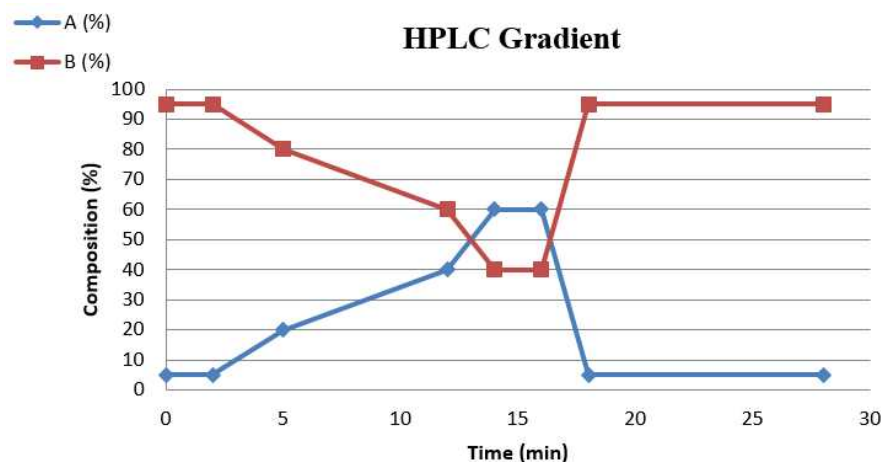
- Washing solutions depending on equipment type

Gradient:

This is an example of a gradient, which can be re-evaluated depending on the type of analysis performed.

	Time (min)	A (%)	B (%)
	0	5	95
	2	5	95
	5	20	80
	12	40	60
	14	60	40
	16	60	40
	18	5	95
	28	5	95

Elution gradient



Wash solution:

- Rinse bottle N°1:

⊗ Water LC-MS grade B&J Brand **VWR International**
(Avantor) Catalog #BJLC365-2.5

;

- Rinse bottle N°2:

⊗ Water LC-MS grade B&J Brand **VWR International**
(Avantor) Catalog #BJLC365-2.5

/

⊗ Methanol LC-MS grade B&J Brand **VWR International**
(Avantor) Catalog #BJLC230-2.5

or

⊗ Water LC-MS grade B&J Brand **VWR International**
(Avantor) Catalog #BJLC365-2.5

/ ⊗ Acetonitrile **Merck MilliporeSigma** (Sigma-Aldrich) Catalog #34998 **(20 :80)**.

Q-Exactive:

⊗ Water LC-MS grade B&J Brand **VWR International**
(Avantor) Catalog #BJLC365-2.5

/

⊗ Methanol LC-MS grade B&J Brand **VWR International**
(Avantor) Catalog #BJLC230-2.5

or

Water LC-MS grade B&J Brand **VWR International**
(Avantor) Catalog #BJLC365-2.5

/ **Acetonitrile Merck MilliporeSigma (Sigma-Aldrich) Catalog #34998 (95:5).**

Column:

The column used is a **SeQuant ZIC HILIC (5µm, 100mm*2.1mm)**, Merck KGaA. This column is used with a pre-column whose filter is changed regularly. Flow rate is 0.25 mL/min, oven temperature **25 °C**.

The ZIC HILIC column is reconditioned before storage in

Acetonitrile Merck MilliporeSigma (Sigma-Aldrich) Catalog #34998 /

Water LC-MS grade B&J Brand **VWR International**
(Avantor) Catalog #BJLC365-2.5

(80:20).

Tune parameters:

These are typical parameters, which can be re-evaluated according to the type of analysis performed.

	A	B
	Sheath gas (L/h)	55
	Auxiliary gas (L/h)	10
	Sweep gas (L/h)	0
	Capillary temperature (°C)	350
	Capillary voltage (V)	(+): 30 ; (-): -30
	Tube lens voltage (V)	(+): 100 ; (-): -80

Injection on Thermo brand instruments, for both ionization modes

	A	B
	Source temperature (°C)	120 to 150
	Sampling cone (V)	30 to 40



	A	B
	Source offset (V)	80
	Source gas flow (mL/min)	50
	Desolvatation temperature (°C)	650
	Cone gas flow (L/h)	400
	Desolvatation gas flow (L/h)	800

Injection on Synapt (Waters) type instruments, in positive ionization mode

Note

The use of solvents containing salts can lead to clogging of the tubing. To avoid this effect, perform several injections at the end of the sequence without heating the probe (HESI = 🔥 50 °C). In addition, recondition the column with salt-free solvents, taking care that the reconditioning solvents also pass into the source and not into the waste garbage can.

10 Calibration

- 10.1 Thermo Solution: ESI+ (Reference : 88322) et ESI- (Reference : 88324)
Calibration to be carried out each time you start using the project
Cone and capillary cleaning according to instrument

11 Acquisition software

- 11.1
- QExactive: Xcalibur (version 4.4) and Chromeleon for HPLC
 - Exactive: Xcalibur (version 2.1) and Aria for HPLC



Citations

Step 2.3

Jacob CC, Dervilly-Pinel G, Deceuninck Y, Gicquiau A, Chevillon P, Bonneau M, Le Bizec B. Urinary signature of pig carcasses with boar taint by liquid chromatography-high-resolution mass spectrometry.

<https://doi.org/10.1080/19440049.2016.1265152>