Pig urine for metabolomics LC-MS analysis (C18, HSS T3) (2017)

**Chemicals**

Acetonitril (ACN) (VWR)

Formic acid (FA)

Glycocholic acid (Glycine-113C)

p-chlorophenylalanine

**Solutions**

H2O/ACN/ formic acid (95/5/0.1)  
(950 ml MILLIQ-water added 50 ml acetonitril and 1 ml FA.)

ACN/IS: Internal standard solution (concentration: 0.1 mg / mL; final concentration: 0.01 mg / mL). Calculation, see bottom of document.

**Materials**

2 ml Eppendorf tubes

Pipettes and pipette tips

Centrifuge

**Intern standards (IS)**

Glycocholic acid (Glycine-113C) solution in methanol (final concentration of 1 mg/ml)

p-chlorophenylalanine solution in 2 % formic acid (final concentration 1 mg/ml)

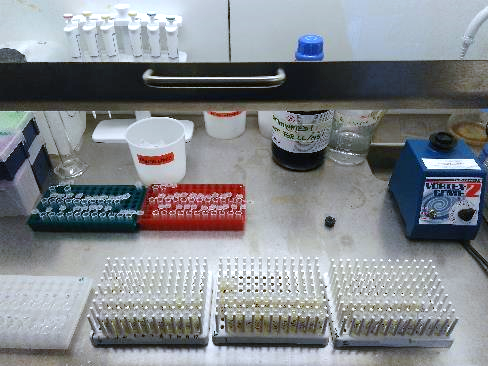
**Sample preparation**

1. 180 μl urine is mixed with 20 μl ACN/IS

Blank sample: H2O treated as a sample

QC sample: 20 μl pool of each urine sample is treated as a normal sample

Blind: 0.1% FA



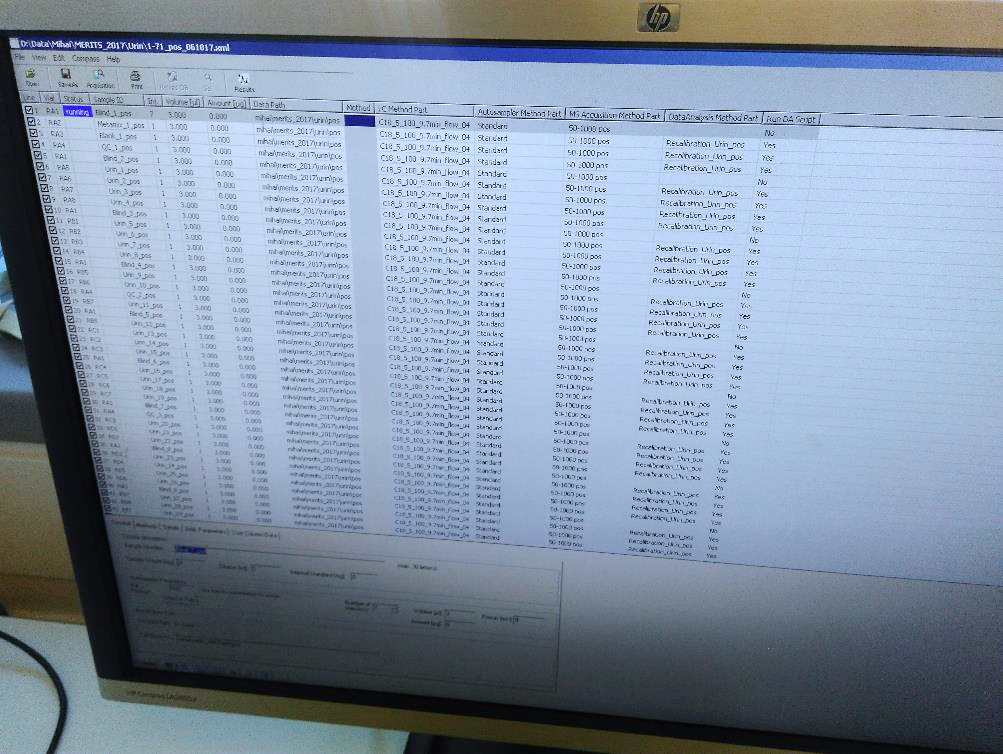
1. Protein precipitation in the fridge (20 min, 4°C).



1. Centrifugation (10 min, 13,200 rpm, 4°C).



1. The supernatant is transferred to vials with micro inserts (150 μl).
2. Analysis on UHPLC-MS with C18 column (HSS T3) according to LC-MS procedure.



Preparation of ACN and IS solution, pig urine

## Urine samples

Total samples: 20 urine samples + 2 QC + 1 blank = 23 samples

Vper sample: 25 µL

Vin total = 0,025 mL \* 23 samples = 0,575 mL -> 1 mL ACN + IS

## Internal standards in ACN

Final concentration in urine samples c2, urine + ACN = 0,01 mg/mL

V2, urine+ACN = 0,20 mL

V1, ACN = 0,02 mL

**Volume of IS in ACN**

*p-chlorophenylalanine* c1, p-chloro. = 1,0 mg/mL (evt. 2,0 mg/ml)

*Glycocholic acid* c1, glycocholic acid = 1,0 mg/mL

**Volume of ACN**

VACN = 1 mL – (0,1 mL + 0,1 mL) = **0,8 mL**

LC-MS settings

**Liquid Chromatography system**

Column: Waters HSS T3 (C18) UPLC column, 1.8 um, 100 x 2.1 mm.

Precolumn filter: Waters VanGuard Precolum, 2.1 x 5 mm.

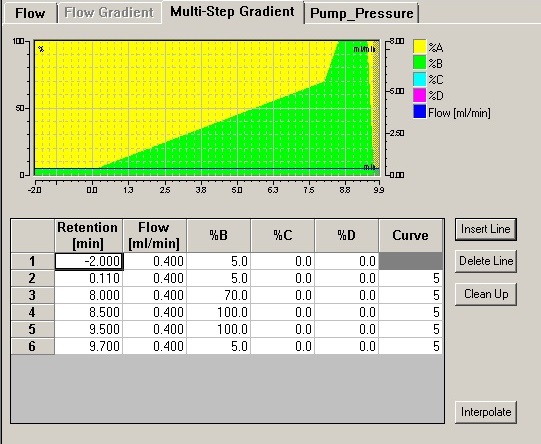
Total Run time : 9.7 min

LC flow: 0.400 µL/min

Column temperature: 30°C

Eluents: B line – ACN + 0.1% formic acid; A line – MILLIQ water + 0.1% formic acid

Gradient: Multi-step gradient: 5% B for 2 min, 5% B to 70% B for 8 minutes; 70%B to 100% B for 0.5 min; hold 100% B for 1 min; Return, 0.2 min 100% B to 5% B.



**Mass Spectrometry System; Electrospray Ionisation (ESI)**

Mode:positive and negative

Capillary voltage: POS – 4500V; NEG – 3600V

End plate offset voltage: 500V

Dry gas flow: 10 L/min

Nebulizer pressure 2.2 bar

Scan range: 50 to 1000 m/z

Sampling rate: 1 Hz

MS scan collision energy: 6 eV.

External calibrant: Li Formate clusters.