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Plasma/serum sample preparation for untargeted MS-based metabolomic

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We are still developing and optimizing this protocol

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Abstract

The following protocol describes the preparation of serum and plasma samples for non-targeted analysis by mass spectrometry coupled to liquid or gas chromatography, as proposed by the PFEM (MetaboHUB-Clermont).



Protocol materials

 ULC/MS grade methanol **Biosolve Catalog #0013684101BS** Step 5

 MilliQ water In [2 steps](#)



Sample preparation

40m

- 1 Thaw the samples according to these conditions depending on the analysis to be carried out :

LCMS	GCMS
Room temperature	4°C overnight

Samples thawing conditions

- 2 Add  100 µL of serum or plasma  sample in Eppendorf tubes.

- 3 Prepare blank sample for GCMS analysis : add  100 µL of

 MilliQ water **Contributed by users** in Eppendorf tube

- 4 Prepare  2 mL of an internal standard solution [13C1]-L-valine ( 0.2 mg/mL) in



 MilliQ water **Contributed by users** , store at  -20 °C for GCMS analysis

- 5 Extract protein by adding ice-cold methanol to each sample under the following conditions :


 ULC/MS grade methanol **Biosolve Catalog #0013684101BS**

Matrix	Vol MeOH LCMS	Vol MeOH GCMS
blank	NA	200 µL (plasma) 400 µL (serum)
plasma	200 µL	200 µL
serum	200 µL	400 µL


Methanol volume to be added to extract proteins

- 6 Mix and placed the samples at  -20 °C for  00:30:00 .

30m

- 7 Centrifuge samples  15493 x g, 4°C, 00:10:00 (Sigma 3-16PK, Fischer Bioblock Scientific).

10m

The supernatant is divided into three aliquots. A intermediate pooled QC sample is prepared by mixing  10 µL from each extracted samples (per day or per batch).

Sample Type	Vol supernatant LCMS (µL)	Vol supernatant Plasma GCMS	Vol supernatant Serum GCMS (µL)	Vol internal standard GCMS (µL)
Pool QC	45	200	NA	NA
Safety	45	200	300	10
Sample	45	200	300	10

Sample Type	Vol supernatant LCMS (μL)	Vol supernatant Plasma GCMS	Vol supernatant Serum GCMS (μL)	Vol internal standard GCMS (μL)
Blank	NA	200 (milliQ water)	300 (milliQ water)	10

Distribution of extracted samples

- 8 Dry samples completely using a EZ2.3 genevac system (Biopharma Technologies France).

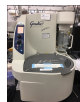
Equipment

new equipment

Genevac EZ-2

EZ-2

https://www.spscientific.com/Products/Centrifugal_Evaporators__Sample_Concentrators/G2_Series/EZ-2_Series/



The following program is used:

A	LCMS	GCMS
Program	HPLC	HPLC
Final Stage	10 min	50 min
Reduce Odour	Off	Off
Lamp	Off	On (under 30°C)

Drying program used

- 9 Store samples for LCMS analysis at -80 °C until further analysis
- 10 Derivatisation for GCMS analysis :
 - 10.1 Prepare a fresh methoxylamine solution (15 mg/mL) in pyridine. The volume of this solution depends on the number of samples to be analysed (80 μL for each samples)
 - 10.2 Dissolve the dry samples in adding 80 μL of methoxylamine solution to each vial.



10.3 Vortex vigorously for 1 min and incubate at 37°C for 24:00:00 (in order to inhibit the cyclization of reducing sugars and the decarboxylation of α -keto acids).

1d

10.4 Add 80 μL of BSTFA (1%TMCS) in the mixture and derivatize at 70°C for 01:00:00

1h

10.5 Waiting a few minutes and transfer 50 μL of derivatized mixture in a glass vial containing 100 μL of heptane prior to injection.

Store blank and biological samples at 4°C until further analysis

10.6 As well as a sample pool is formed from 10 μL of each extracted and derivatized sample to monitor the drift of the spectrometer during GCMS analysis.

Transfer 50 μL of sample pool in a glass vial containing 100 μL of heptane prior to injection, repeat this step 8 times (preprocessing analysis)

Store pool samples at 4°C until further analysis

Protocol references

CITATION

Pereira, H., Martin, JF., Joly, C. et al. (2009). Development and validation of a UPLC/MS method for a nutritional metabolomic study of human plasma. *Metabolomics* .

LINK

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Citations

Pereira, H., Martin, JF., Joly, C. et al. . Development and validation of a UPLC/MS method for a nutritional metabolomic study of human plasma
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