Sep 19, 2024

Plasma/serum sample preparation for untargeted MS-based metabolomic

This protocol is a draft, published without a DOI.

Carole MIGNÉ[1], charlotte JOLY[1], Stéphanie DURAND[1], Estelle Pujos-Guillot[2]

[1]Université Clermont Auvergne, INRAE, UNH, Plateforme d'Exploration du Métabolisme, MetaboHUB Clermont, Clermont-Ferrand, France; [2]INRAE

MetaboHUB-Clermont PF…

Estelle Pujos-Guillot
INRAE

External link: https://eng-pfem.isc.inrae.fr/

Protocol Citation: Carole MIGNÉ, charlotte JOLY, Stéphanie DURAND, Estelle Pujos-Guillot 2024. Plasma/serum sample preparation for untargeted MS-based metabolomic. **protocols.io** https://protocols.io/view/plasma-serum-sample-preparation-for-untargeted-ms-c263yhgn

Protocol status: In development
We are still developing and optimizing this protocol

Created: October 09, 2023

Last Modified: September 19, 2024

Protocol Integer ID: 89019

Keywords: serum, plasma, preparation, untargeted metabolomic analysis, mass spectrometry

Funders Acknowledgement:
Agence Nationale de la Recherche
Grant ID: ANR-11-INBS-0010
MetaboHUB
Agence Nationale de la Recherche au titre de France 2030
Grant ID: ANR- 21-ESRE-0035

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 ([M] 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 ([M] 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 incube 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
https://doi.org/10.1007/s11306-009-0188-9

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 **https://doi.org/10.1007/s11306-009-0188-9**