Sample treatment

Plasma samples were inactivated with cold (-20 °C) MeOH:EtOH (1:1, v/v). Samples were vortex-mixed for 1 min, incubated on ice for 5 min and centrifuged for 20 min at 16000 *xg* at 4 °C. The resulting supernatant was stored at -80 °C until analysis by gas chromatography-mass spectrometry (GC-MS) and capillary electrophoresis-mass spectrometry (CE-MS). Sample preparation for GC-MS and CE-MS was carried out at CEMBIO (Madrid, Spain) based on previously developed methods for plasma samples [1] [2].

For GC-MS analysis, 200 µL of frozen plasma supernatant was thawed at room temperature and 30 µL of 80 mg/L deuterated palmitic acid in MeOH was added as internal standard (IS). After samples were evaporated to dryness a two-step derivatization process was done. First, methoximation was performed by adding 20 µL of *O*-methoxyamine hydrochloride (15 mg/mL in pyridine) and vortex-mixing for 5 min. Then, 3 cycles of ultrasonication for 5 min and vortex for 5 min were done. Immediately after, vials were incubated in darkness at room temperature for 16 h. Second, silylation was carried out by adding 20 µL of BSTFA/TMCS (99:1) followed by vortex-mixing for 5 min. Then, capped vials were incubated in the oven at 70 °C for 1 h. Finally, 100 µL of heptane containing 20 mg/L of tricosane as IS was added to each vial prior to injection. For CE-MS analysis, 200 µL of frozen supernatant was thawed until room temperature and it was evaporated to dryness using a SpeedVac Concentrator System (Thermo Fisher Scientific, MA). Afterwards, 100 µL of 0.2 mM methionine sulfone (MetS) as internal standard (IS) in 0.1 M formic acid solution was added. Samples were vortex mixed for 1 min, transferred to a Millipore filter (30 kDa protein cutoff) and centrifuged for 40 min at 2000 x*g* at 4 °C. Finally, the filtrate was transferred to a CE-MS vial for analysis.

Quality control samples (QC) were prepared by adding equal volumes of plasma supernatant from each sample and were prepared as previously mentioned for GC-MS and CE-MS analysis. Blank solutions were also prepared with MeOH:EtOH (1:1, v/v). All samples were randomized during analysis.

References

1. Garcia A, Barbas C: **Gas chromatography-mass spectrometry (GC-MS)-based metabolomics**. *Methods Mol Biol* 2011, **708**:191-204.
2. Naz S, Garcia A, Rusak M, Barbas C: **Method development and validation for rat serum fingerprinting with CE-MS: application to ventilator-induced-lung-injury study**. *Anal Bioanal Chem* 2013, **405**(14):4849-4858.