Non-targeted metabolomics

Samples were analyzed by two different platforms: GC-MS, and CE-MS using previously developed methods and following the next analytical conditions [3].

An Agilent GC-MS system (8890) coupled to a single quadrupole mass spectrometer (5977B, Agilent Technologies) was used to analyze plasma samples. Two µL of derivatized samples were automatically injected in split mode (split ratio 1:10) by an Agilent autosampler (7693) into an Agilent ultra-inert deactivated glass wool split liner. Metabolite separation was carried out in a DB5-MS GC column (length, 30 m; inner diameter, 0.25 mm; and 0.25 µm film of 95% dimethyl / 5 % diphenylpolysiloxane), with a pre-column (10 m J&W integrated with Agilent 122-5532G). The flow rate of helium carrier gas was constant at 1.1359 mL/min and the injector temperature was set at 250 °C. The lock of the retention time (RTL) relative to the internal standard (methyl stearate) peak at 19.66 min was performed. The oven temperature gradient was initially set at 60 °C and was maintained for 1 min. Then it was raised by 10 °C/min until it reached 325 °C, and then was held at this temperature for 10 min before cooling down. The total analysis run time was 37.5 min. The transfer line temperature was stablished at 280 °C and the electron ionization (EI) source was operated at 70 eV and the filament source temperature was set at 200 °C. Mass spectra were collected over a mass range of 50 - 600 *m/z* at a scan rate of 2.7 scans/s. Data were acquired using the Agilent MassHunter Workstation GC/MS Data Acquisition (version 10.0).

For CE-MS analysis, prepared plasma samples were analyzed in a 7100 capillary electrophoresis (CE) system coupled to a 6230 time-of-flight mass spectrometer (TOF-MS) and equipped with an electrospray ionization (ESI) source from Agilent Technologies. Metabolite separation was performed in a fused silica capillary (100 cm; inner diameter, 50 µm, Agilent Technologies). Before each analysis, background electrolyte (BFE) (0.8 M formic acid solution in 10 % MeOH; v/v) was flushed for 5 min (950 mbar). Samples injections were performed over 50 s at 50 mbar and BGE was injected after each injection for 10 s at 100 mbar to improve reproducibility. The separation was carried out with an internal pressure of 25 mbar and 30 kV voltage with a total analytical run time of 35 min. Data were acquired in positive ionization polarity with a full scan range from 70 to 1000 *m/z* at a rate of 1.36 scan/s. The rest of MS conditions were: fragmentor set to 125 V, skimmer to 65 V, OCT RF Vpp to 750 V, drying gas temperature to 200 °C, flow rate to 10 L/min, nebulizer to 10 psig, and capillary voltage to 3500 V. The sheath liquid used for detection contained two reference masses (5 µL of purine with *m/z* 121.0509 and 5 µL of HP-0921 with *m/z* 922.0098) in MeOH/water (1/1; v/v) with 1 mM formic acid and the flow rate was set to 0.6 mL/min (split 1:100). The data acquisition was made using the Agilent MassHunter Workstation (Agilent Technologies).

QC sample was regularly analyzed through the run to assess the data quality, analytical system stability and sample treatment reproducibility. Also, a pair of blank solutions were analyzed at the beginning and at the end of each analytical sequence.