Supplemental Material

Analysis of Kynurenine/Tryptophan (KYN/TRP) Pathway Metabolites: KYN, TRP, Quinolinic Acid (QA) and Kynurenic Acid (KA)

KYN-D_4, KA-D_5, TRP-D_5, and QA-D_3 were used as internal standards (IS). Serum samples were prepared for analysis by a transfer of 10 µL sample, followed by the addition of 90 µL (QA: 40 uL) precipitation solution (KYN, KA, TRP: acetonitrile/H_2O 8:2 with 0.1% formic acid and 0.01% ascorbic acid; QA: acetonitrile/H_2O 8:2) containing IS. After vortexing, samples were centrifuged for 5 minutes at 4°C and 13,000 rpm, and 10 µL (QA: 5 µL) of supernatant was diluted (KYN, KA, TRP: 10-fold with 90 µL; QA: with 50 µL) of ultrapure water with 0.1% (v/v) formic acid and 0.01% (w/v) ascorbic acid.

Samples were injected (KYN, KA, TRP: SIL20-AD autosampler; QA: SIL 10ADvp) (Shimadzu, Japan) onto an HPLC column [KYN, TRP, KA: Atlantis T3 C18 (2.1*150 mm, 3.0 µm particle size; Waters, USA); QA: Synergi Max-RP HPLC column (3.0*100 mm, 2.5 µm particle size; Phenomenex, USA)], held at 25°C (KYN, TRP, KA) or 20°C (QA). Mobile phase A consisted of ultrapure water with 0.1% FA (KYN, KA, TRP) or 0.2% TFA (QA). Mobile phase B (KYN, KA, TRP) was acetonitrile with 0.1% FA. Elution of the compounds was performed using a linear gradient [KYN, KA, TRP: flow rate of 0.2 mL/minute using the Infinity 1290 HPLC system (Agilent, USA); QA: 0.3 mL/minute using LC-20ADxr (Shimadzu, Japan)]. The HPLC system was connected to a MS [KYN, KA, TRP: QTrap 5500®, QA: API 4000® triple quadrupole] (Sciex, USA) equipped with a Turbo Ion Spray interface.

Acquisitions on the MS were performed in positive ionization mode, with optimized settings for the analytes. The instrument was operated in multiple reaction-

monitoring mode. Data were calibrated and quantified using the Analyst™ data system (KYN, KA, TRP: Sciex, version 1.5.2; QA: 1.4.2).