Supplement 1. Detailed information on HPLC - MS/MS analysis.

Table 1. - Individually tuned MRM experiments for all analyses of interest.

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| --- | --- | --- | --- | --- | --- |
| **Substance** | **Protonated Precursor (parent) ion [M+H]+ [*m/z*]** | **Protonated Product (daughter) ion [*m/z*]** | **Collision Energy [eV] / Cone Voltage [V]** | **MRM triggered during runtime [min]** | **Function** |
| **TRP** | 205 | 146 | 18 / 30 | 1. - 1.5
 | Quantifyer |
| 188 | 10 / 30 |  | Qualifyer |
| **KYN** | 209 | 94 | 12 / 18 | 1.5 - 5.0 | Quantifyer |
| 192 | 6 / 18 |  | Qualifyer |
| **QA** | 168 | 78 | 16 / 26 | 1.0 - 3.0 | Quantifyer |
| 150 | 8 / 26 |  | Qualifyer |
| **KA** | 190 | 144 | 14 / 26 | 1.5 - 5.0 | Quantifyer |
| 172 | 10 / 26 |  | Qualifyer |
| **TRP-d5** | 210 | 150 | 18 / 30 | 1. - 1.5
 | ISTD |
| **KYN-d4** | 213 | 98 | 12 / 18 | 1.5 - 5.0 | ISTD |
| **QA-d3** | 171 | 81 | 16 / 26 | 1.0 - 3.0 | ISTD |
| **KA-d5** | 195 | 149 | 14 / 26 | 1.5 - 5.0 | ISTD |

Chemicals and reagents

For all aqueous solutions ultrapure water was obtained from a Thermo Scientific GenPure UV-TOC xCAD plus water purification system (Langenselbold, Germany). Acetonitrile and methanol of analytical grade as well as formic acid were purchased from Merck (Darmstadt, Germany). Phosphate-buffered saline (PBS, pH 7.4) buffer, which was prepared according to the manufacturer's recommendations (1 tablet dissolved in 200 mL of water), was from Sigma-Aldrich (Schnelldorf, Germany) and 5% human serum albumin (HSA) solution was from Biotest Pharma GmbH (Dreieich, Germany). Stable isotope-labeled (deuterated) D-Tryptophan-d5, Kynurenic acid-d5, L-Kynurenine-d4 and 2,3-Pyridinedicarboxylic acid-d3 which were used as internal standards (ISTD) were obtained from Toronto Research Chemicals (Toronto, CAN). Unlabeled L-Kynurenine (KYN), Kynurenic acid (KA), L-Tryptophan (TRP) and 2,3-Pyridindicarboxylic (QA) acid were further obtained from Merck.

HPLC - MS/MS

Batchwise, aliquots of 50 µL plasma (in duplicate) which were stored at -80°C until analysis, were thawed and 5 µL of ISTD mix was added. Subsequently another 200 µL of ice-cold methanol were added and samples were vigorously vortexed. Crashed proteins were removed by centrifugation (17.000 *x g*, 5 min) before 100 µL of the supernatant is transferred into HPLC glass vials to be ready for injection to the HPLC-MS system. A calibration curve was prepared including 6 concentration levels for each analyte (TRP: 0.1, 0.2, 1, 2, 10 and 20 µg/mL; QA and KYN: 0.01, 0.02, 0.1, 0.2, 1 and 2; KA: 1, 2, 10, 20, 100 and 200 ng/mL).

Analysis were performed utilizing a Waters ACQUITY UPLC® system equipped with an ACQUITY UPLC® HSS T3 analytical column (2.1 x 50 mm, 1.8 µm particles) coupled to a Xevo® TQ-XS triple quadrupole mass spectrometer (Waters, Eschborn, Germany). For chromatographic separation gradient elution was carried out using 5 mM aqueous ammonium acetate solution adjusted to pH 9 (NH4OH was added) as Eluent A and acetonitrile acidified with 2% formic acid as Eluent B. The injection volume was set to 2 µL and the overall flowrate was 300 µL/min during a total run time of 11 min. In detail, the gradient program started at 2% organic phase (B) which was increased to 20% within 6 min and further to 100% during one additional min. After two min of constant hold, the initial conditions were restored for 2 min. Positive ionization was applied by an UniSpray™ (US) ion source (US+) at an impact voltage of 3.9 kV and a desolvation temperature of 500 °C. Individually tuned multiple reaction monitoring (MRM) experiments (see Table 1) were conducted for diagnostic ion transitions (precursor ion to product ion, dwell time of 20 ms each) resulting from collision-induced dissociations (CID) triggered by the presence of Argon. For the estimation of analyte concentrations peak area ratios of the quantifying ion transition and the corresponding signal for the ISTD were used for calculation using the calibration curve equation.