Enhanced analysis of tryptophan and its metabolites in the kynurenine pathway

Tryptophan does not only function as a building block in the biosynthesis of proteins. It is also a central precursor of the kynurenine pathway, which leads to the production of nicotinamide adenine dinucleotide (NAD) due to the intermediate quinolinic acid. This is not the only reason why accurate analytical monitoring of tryptophan and its metabolites is desirable. Furthermore, a disruption of the kynurenine pathway affects the synthesis of serotonin and melatonin. In addition to a genetic cause, an inflammatory induced disruption is also associated with psychiatric disorders. Elevated kynurenine concentrations serve as peripheral markers and may reflect central nervous system inflammation.

The coordinating compounds in the pathway pose a challenge for conventional analytical setups, as non-specific absorption leads to peak tailing and reduced recovery. This Application Note outlines a robust method for the quantification of all relevant metabolites in the kynurenine pathway. The approach uses a bioinert YMC Accura Triart C18 column, designed to minimise undesired interactions and ensure reproducible performance across complex biological matrices.

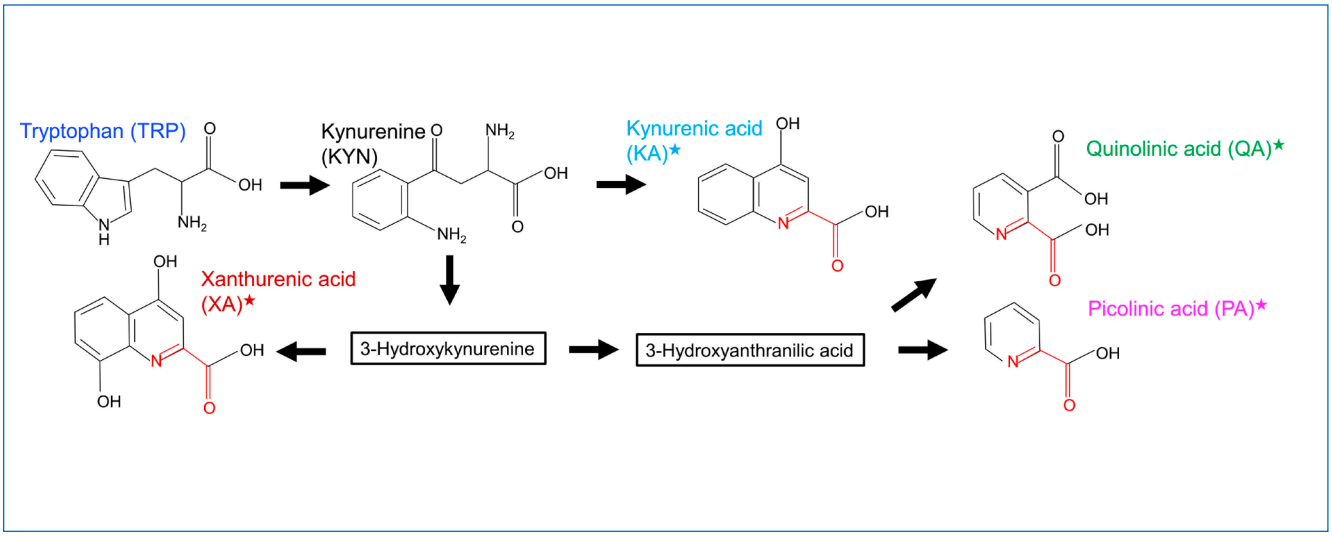

Figure 1: Tryptophan metabolites in the kynurenine pathway. Coordinating compounds are marked with stars.

Table 1: Chromatographic conditions.

Columns:	YMC Accura Triart C18 (1.9 µm, 12 nm) 50 x 2.1 mm ID (bioinert coated) YMC-Triart C18 (1.9 µm, 12 nm) 50 x 2.1 mm ID (stainless-steel)
Part Nos.:	TA12SP9-05Q1PTC TA12SP9-05Q1PT
Eluents:	A) 10 mM HCOOH-NH_4COOH (pH 3.7) B) acetonitrile
Gradient:	LC: 5–50% B (0–3 min) LC-MS/MS: 5–50% B (0–4 min)
Flow rate:	LC: 0.4 mL/min LC-MS/MS: 0.3 mL/min
Temperature:	40 °C
Detection:	UV at 254 nm ESI in positive mode
Sample:	standard samples (0.1 µg/mL) tryptophan (TRP) kynurenine (KYN) kynurenic acid (KA)* xanthurenic acid (XA)* picolinic acid (PA)* quinolinic acid (QA)* teleost brain homogenates

** coordinating compound*

Method optimisation using different column hardware types

Figure 2 illustrates the comparison between conventional stainless-steel and bioinert coated YMC Accura hardware.

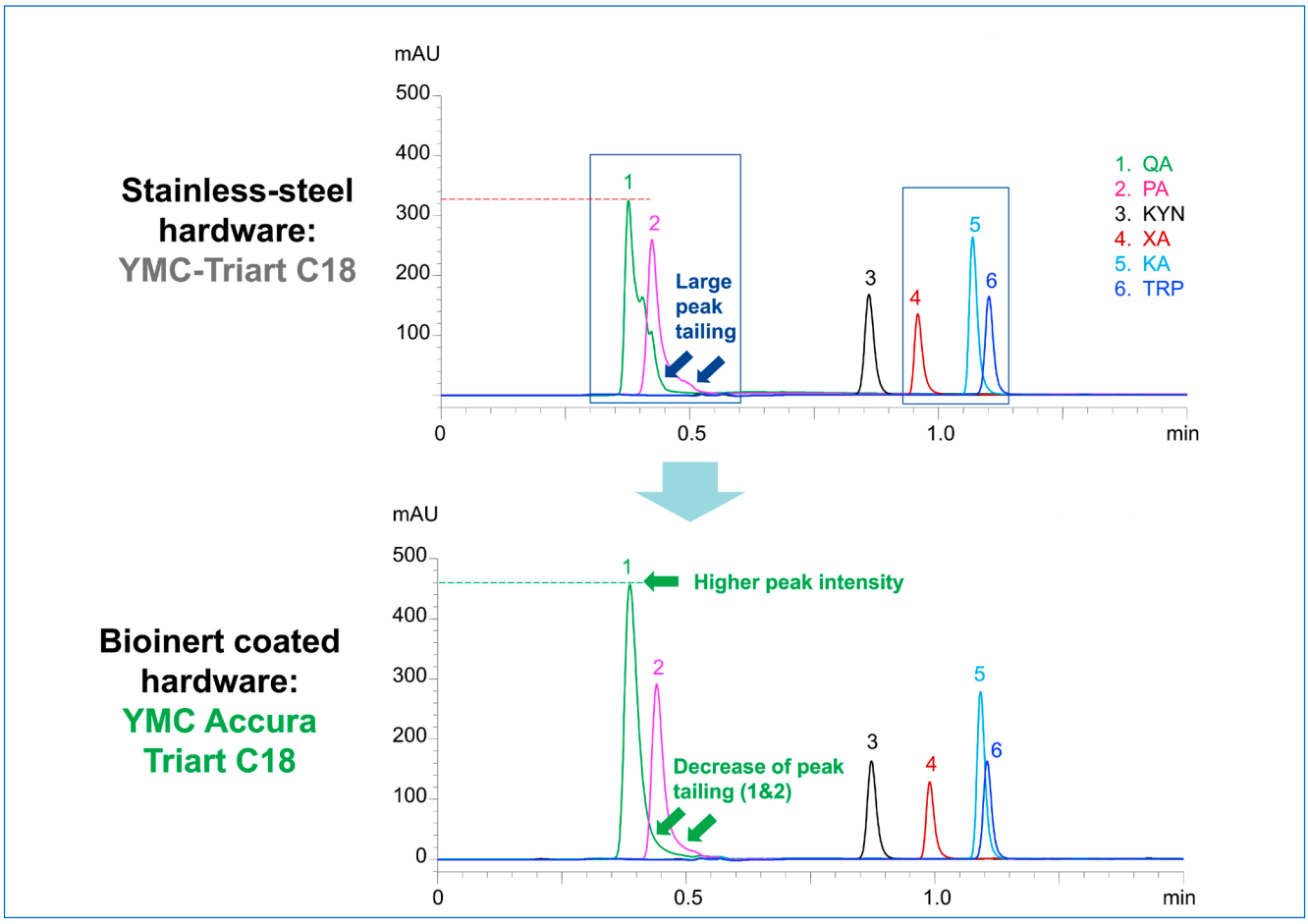

Figure 2: Analysis of tryptophan and its metabolites using stainless-steel column hardware (top) and a bioinert coated YMC Accura Triart C18 column (bottom).

While XA and KA are hardly affected by the stainless-steel hardware, QA and PA show massive peak tailing and reduced recovery. By using the bioinert coated YMC Accura Triart C18 column, peak tailing can be significantly improved and a higher peak sensitivity can be achieved. In addition, Figure 3 confirms the improved trailing factors for all coordinating compounds.

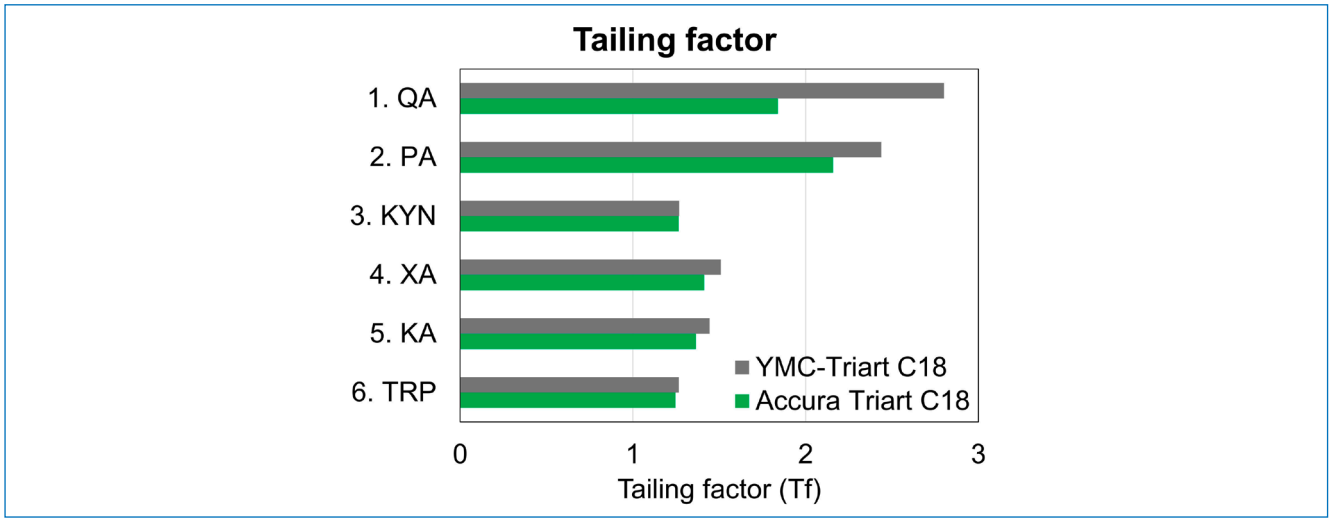

Figure 3: Tailing factors obtained with regular (grey) and bioinert coated (green) column hardware.

Quantification of tryptophan and its metabolites in teleost brain tissue using LC- MS/MS

For the application of the method to a biological matrix, teleost brain tissue was extracted with 1 mL methanol. Centrifugation removed protein components from the sample. The resulting supernatant was evaporated and reconstituted in a 50:50 mixture of eluent A and B.

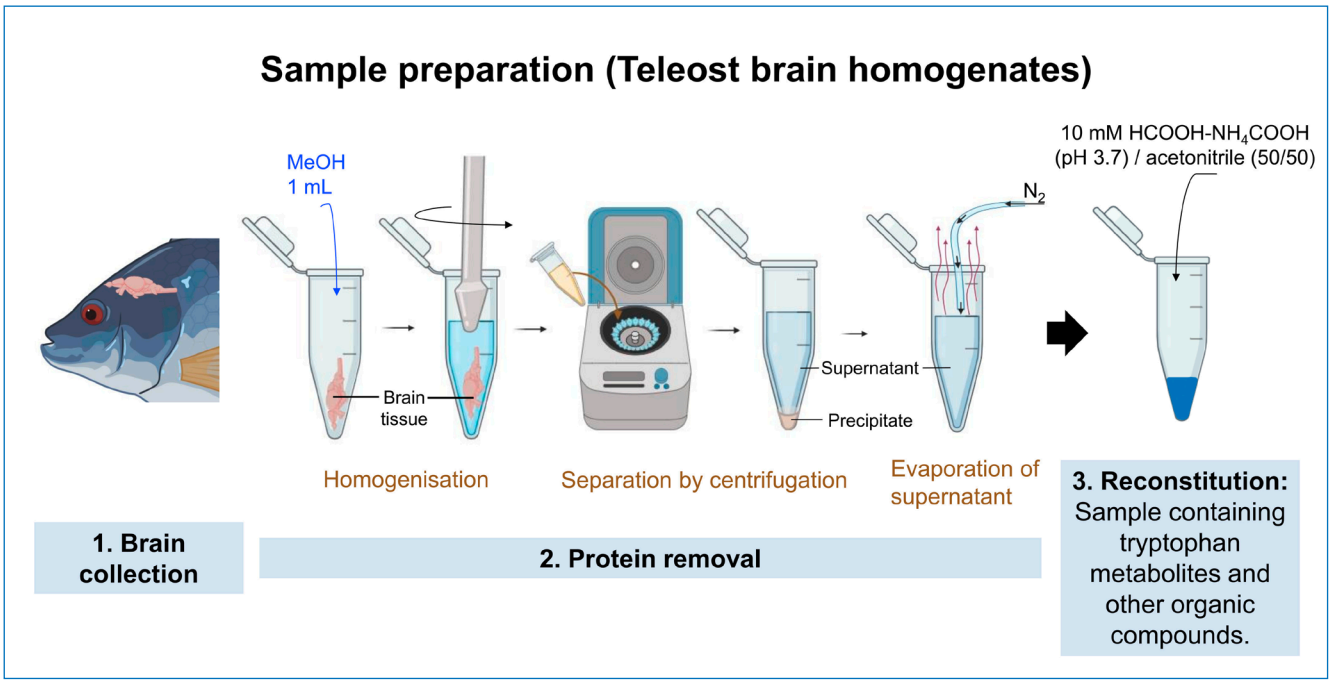

Figure 4: Sample preparation of a teleost brain tissue.

Figure 5 demonstrates that all target compounds are reliably quantified in the biological sample.

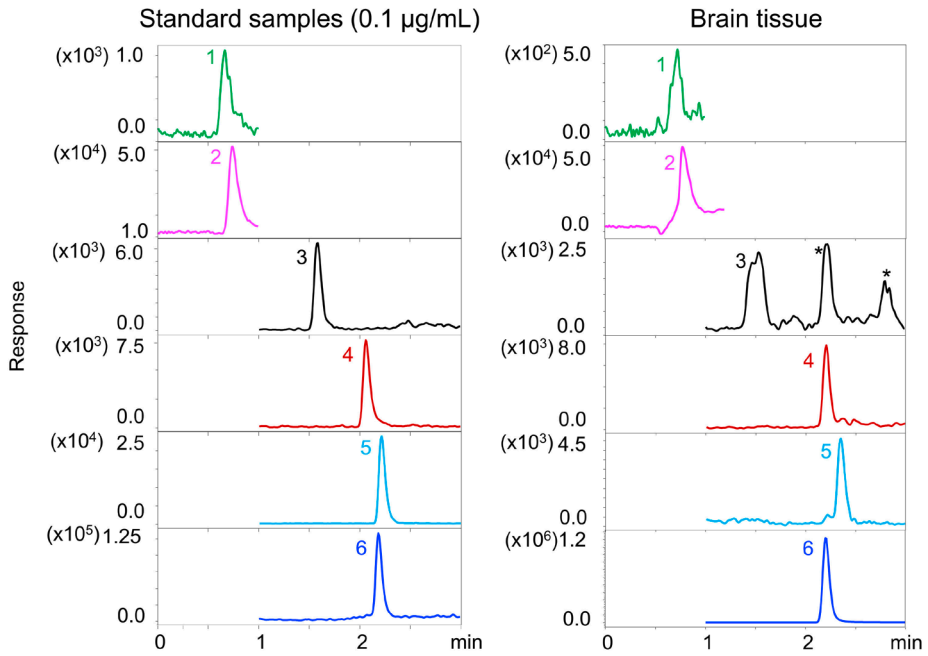

Figure 5: LC-MS/MS results of the standard samples (left) and the teleost brain homogenates (right).

Table 2 presents the quantified concentrations of all compounds in the teleost brain tissue.

Table 2: Concentrations of tryptophan and its metabolites in a teleost brain tissue.

Analytes	Concentration (pg/mg tissue)
1. QA	984.72
2. PA	1,022.59
3. KYN	751.36
4. XA	1,050.35
5. KA	259.91
6. TRP	61,848.48

Conclusion

The bioinert coated YMC Accura Triart C18 column enhances sensitivity and delivers sharper peak profiles for the analysis of tryptophan and its metabolites. This Application Note confirms the method's suitability for complex biological matrices and demonstrates its robustness under real-world conditions.