

## Introduction

### Challenges during analysis of coordinating compounds in high performance liquid chromatography

**Problems: Abnormal peak shape**

- Low peak resolution
- Difficult recovery

**Challenge when using stainless-steel HPLC columns**

Non-specific adsorption with the metal surface of the HPLC system, including HPLC column

**Our solution**

Bioinert coating

No non-specific interaction  
Good peak shape

- Increased sensitivity
- Higher resolution
- Better recovery
- Ideal for LC-MS/MS analysis

### Case study: Tryptophan metabolites in the kynurenine pathway (★ coordinating compounds)

**Objectives:**

- Elimination of peak tailing of the coordinating compounds
- Enhancement of analytical sensitivity using a bioinert coated HPLC column

## Materials and method

### Column characteristics

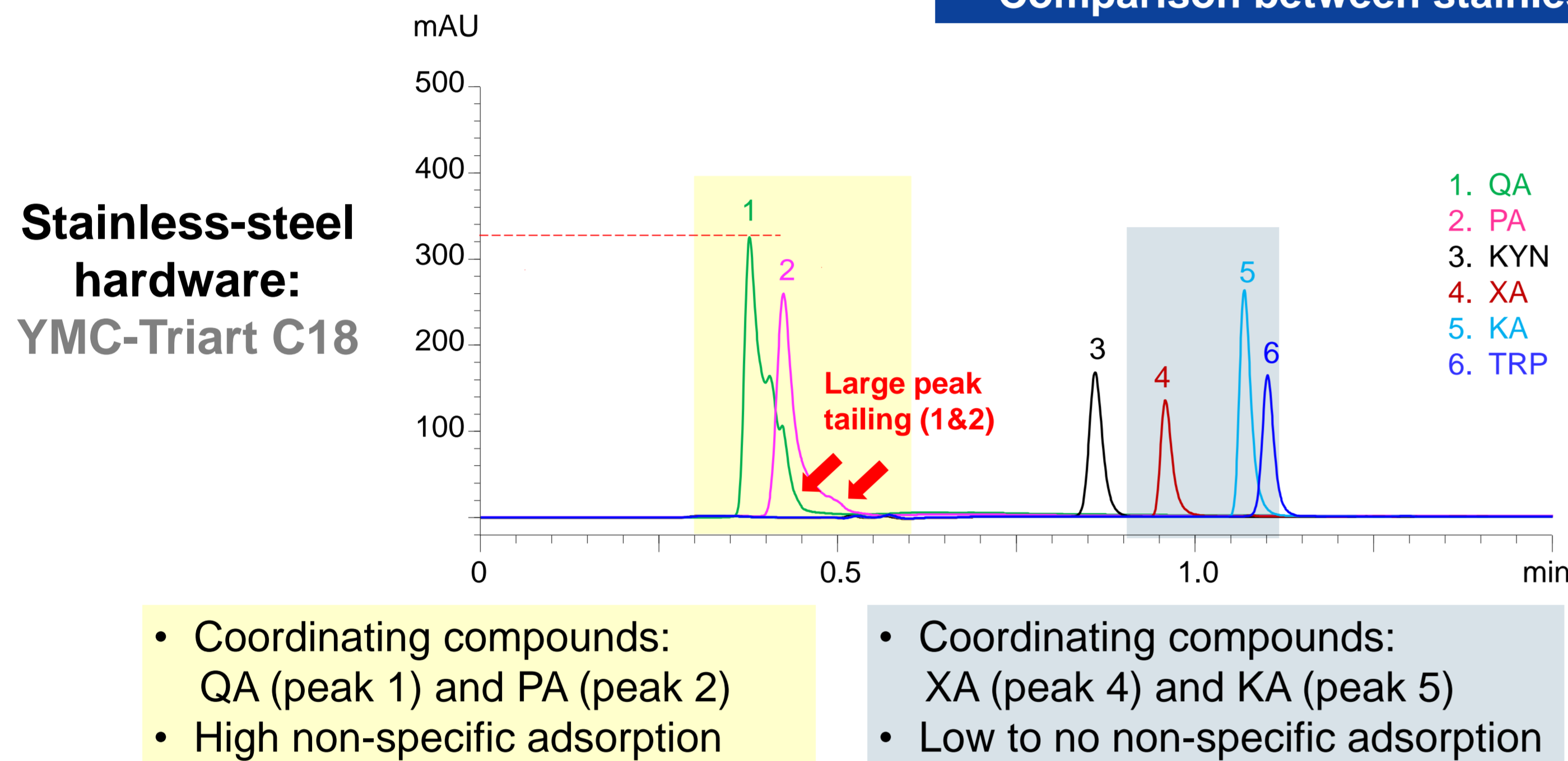
Column name	Column hardware	Packing material	Particle size (µm)	Pore size (nm)	Dimensions
YMC-Triart C18	Stainless steel	C18 bonded hybrid silica	1.9	12	50 x 2.1 mm ID
YMC Accura Triart C18	Bioinert coated	C18 bonded hybrid silica	1.9	12	50 x 2.1 mm ID

### Method conditions

	Eluents	Gradient	Gradient time	Flow rate	Column temperature	Detection
LC	A) 10 mM HCOOH-NH <sub>4</sub> COOH (pH 3.7)	5-50% B	0-3 min	0.4 mL/min	40°C	UV at 254 nm
LC-MS/MS	B) Acetonitrile	5-50% B	0-4 min	0.3 mL/min	40°C	ESI in positive mode

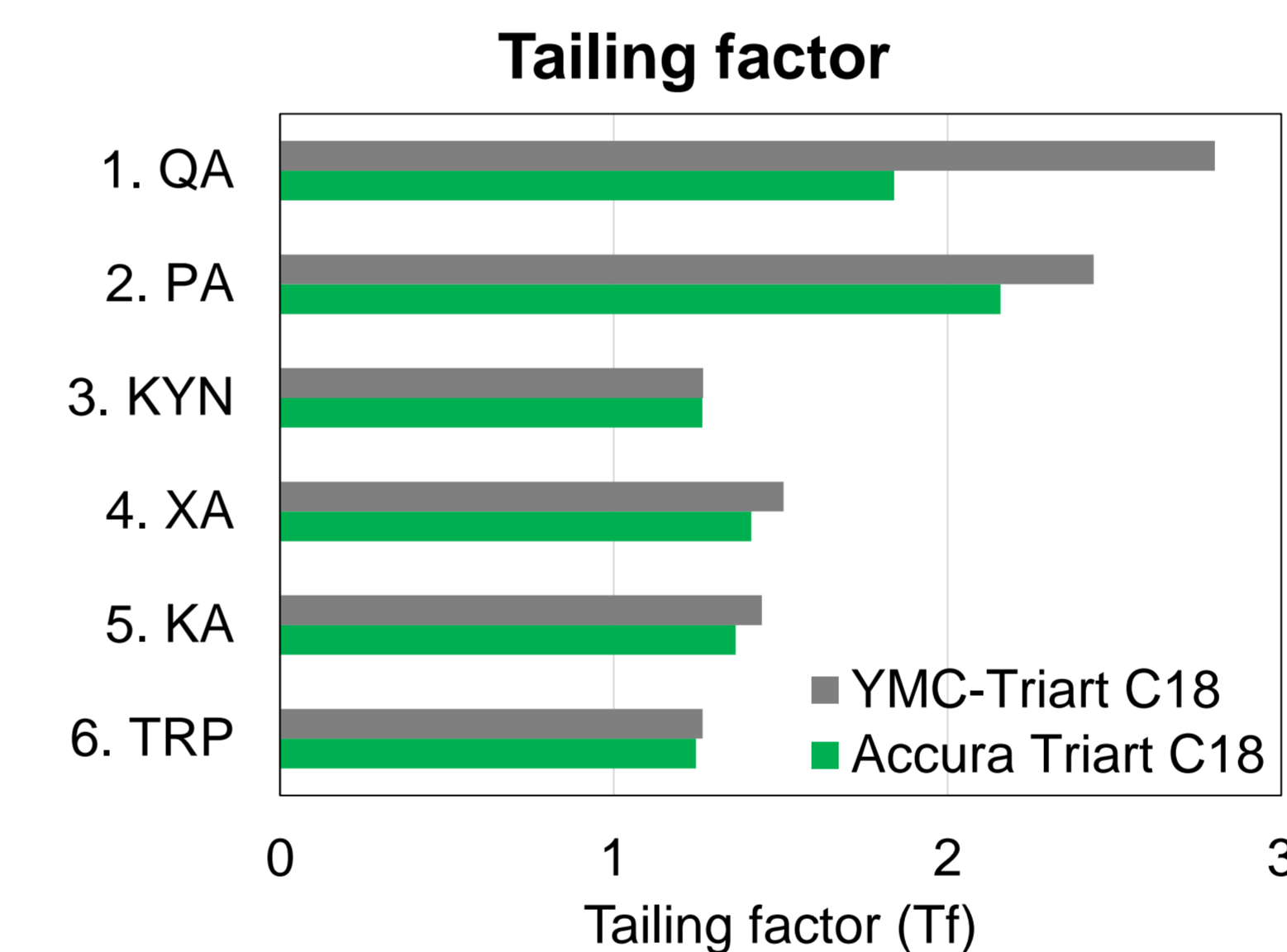
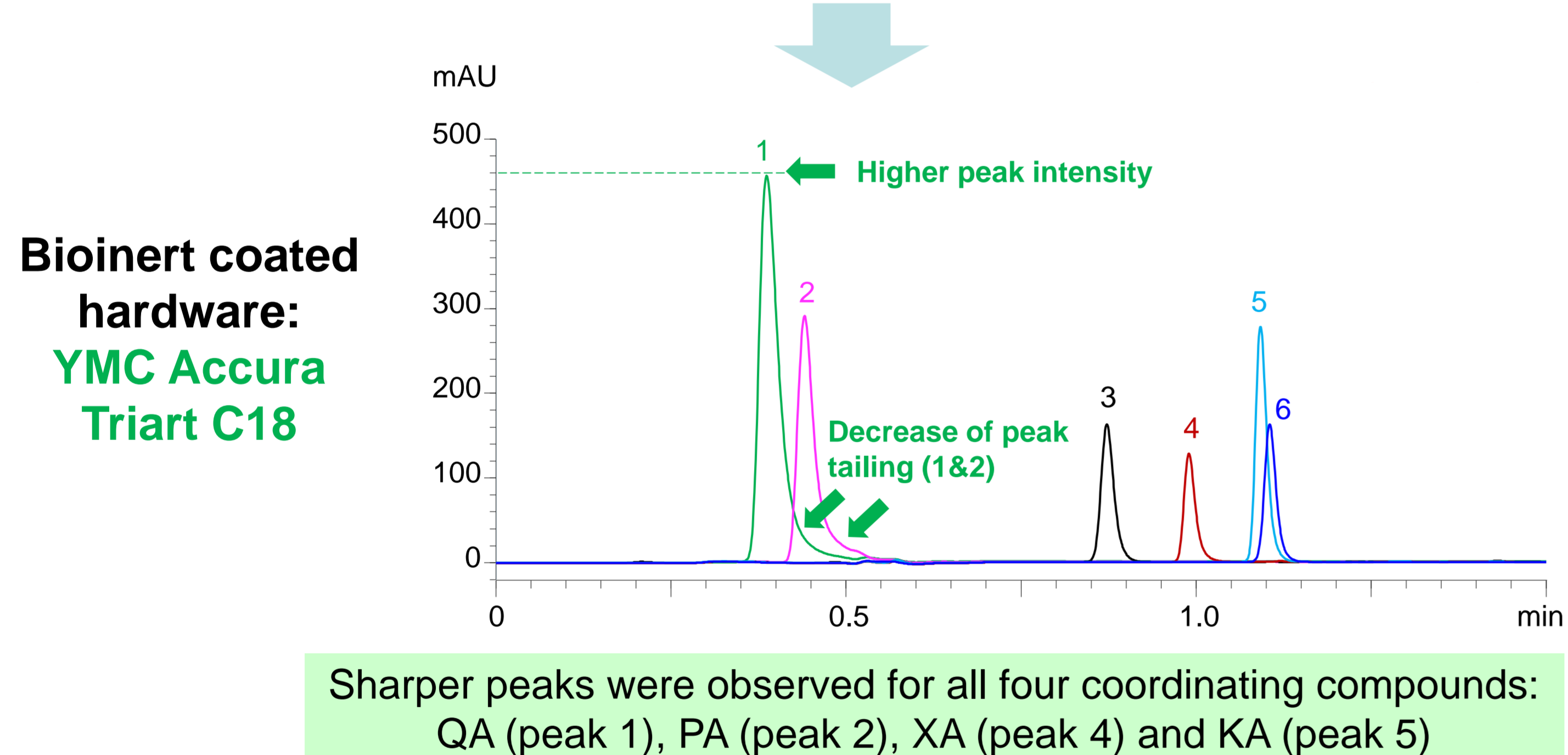
## Results and discussion

### Comparison between stainless-steel and bioinert coated hardware



Coordinating compounds	Peak area (mAU*min)		Peak height (mAU)	
	YMC-Triart C18	YMC Accura Triart C18	YMC-Triart C18	YMC Accura Triart C18
QA (peak 1)	828.43 (84%)	984.04	325.62 (71%)	456.77
PA (peak 2)	514.97 (91%)	565.70	258.50 (89%)	290.98
XA (peak 4)	162.66 (104%)	155.91	135.44 (105%)	128.92
KA (peak 5)	314.42 (96%)	326.13	262.40 (95%)	277.02
Non-coordinating compounds				
KYN (peak 3)	228.19 (91%)	251.24	167.87 (95%)	177.22
TRP (peak 6)	196.92 (99%)	197.60	163.79 (101%)	162.41

(X% : peak area ratio and peak height ratio of YMC-Triart C18 to YMC Accura Triart C18)



### Merits of YMC Accura Triart C18:

- Enhancement of the specific peak areas and peak heights of coordinating compounds, especially QA and PA.
- Elimination of non-specific adsorption of coordinating compounds resulting in significant reduction of the tailing factor of coordinating compounds, especially QA and PA.

The YMC Accura Triart C18 column effectively improved peak shapes and enhanced analytical sensitivity of coordination compounds.

### Application: Analysis of analytes in biological sample using bioinert coated column in LC-MS/MS

**Sample preparation (Teleost brain homogenates)**

- Brain collection
- Protein removal: Homogenisation, Separation by centrifugation, Evaporation of supernatant
- Reconstitution: Sample containing tryptophan metabolites and other organic compounds.

10 mM HCOOH-NH<sub>4</sub>COOH (pH 3.7) / acetonitrile (50/50)

**Standard samples (0.1 µg/mL)**

**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

← Successful quantification

Due to the merits of the YMC Accura Triart C18 column, all tryptophan and its metabolites in teleost brain homogenates were efficiently analysed.

(\*): unknown peak

## Conclusions

- Compared to a stainless-steel column, the bioinert coated YMC Accura column successfully improves the peak shapes and enhances the analytical sensitivity of coordinating compounds such as quinolinic acid, picolinic acid, xanthurenic acid, and kynurenic acid while maintaining the results for non-coordinating compounds.
- Accurate measurement of tryptophan and its metabolites in complex biological samples was achieved using a YMC Accura Triart C18 column in combination with LC-MS/MS, indicating that YMC Accura columns are ideal for the analysis of coordinating compounds when high sensitivity is required.

Due to the bioinert coating, YMC Accura Triart C18 is the ideal column for the analysis of coordinating compounds such as those in the tryptophan metabolism.