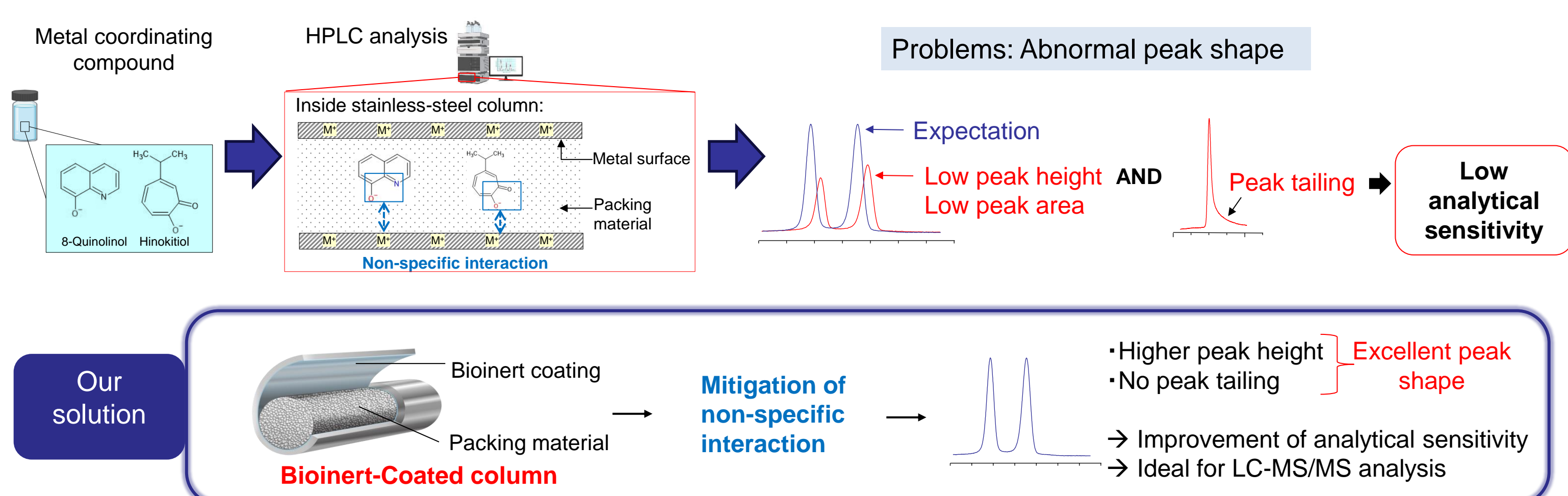
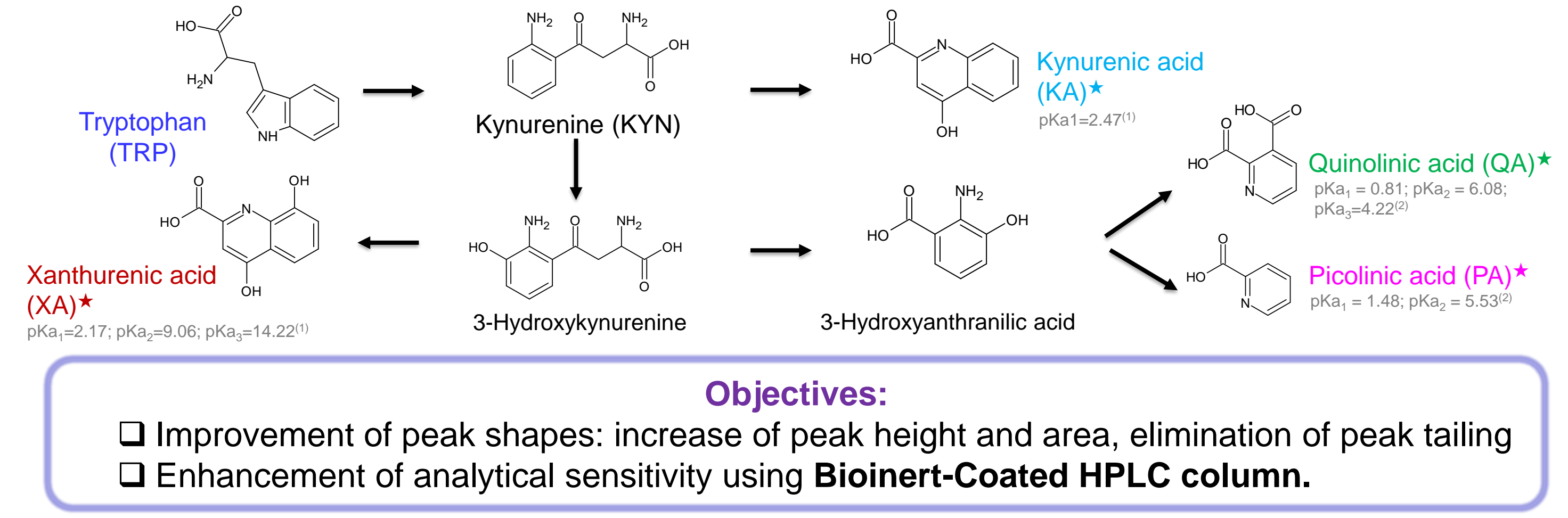


## Introduction

### Challenge during analysis of coordination compounds in High-Performance Liquid Chromatography



### Case study: Tryptophan metabolites in the kynurenine pathway (\* metal coordinating compounds)



## Materials and method

### Reversed-phase HPLC columns characteristics

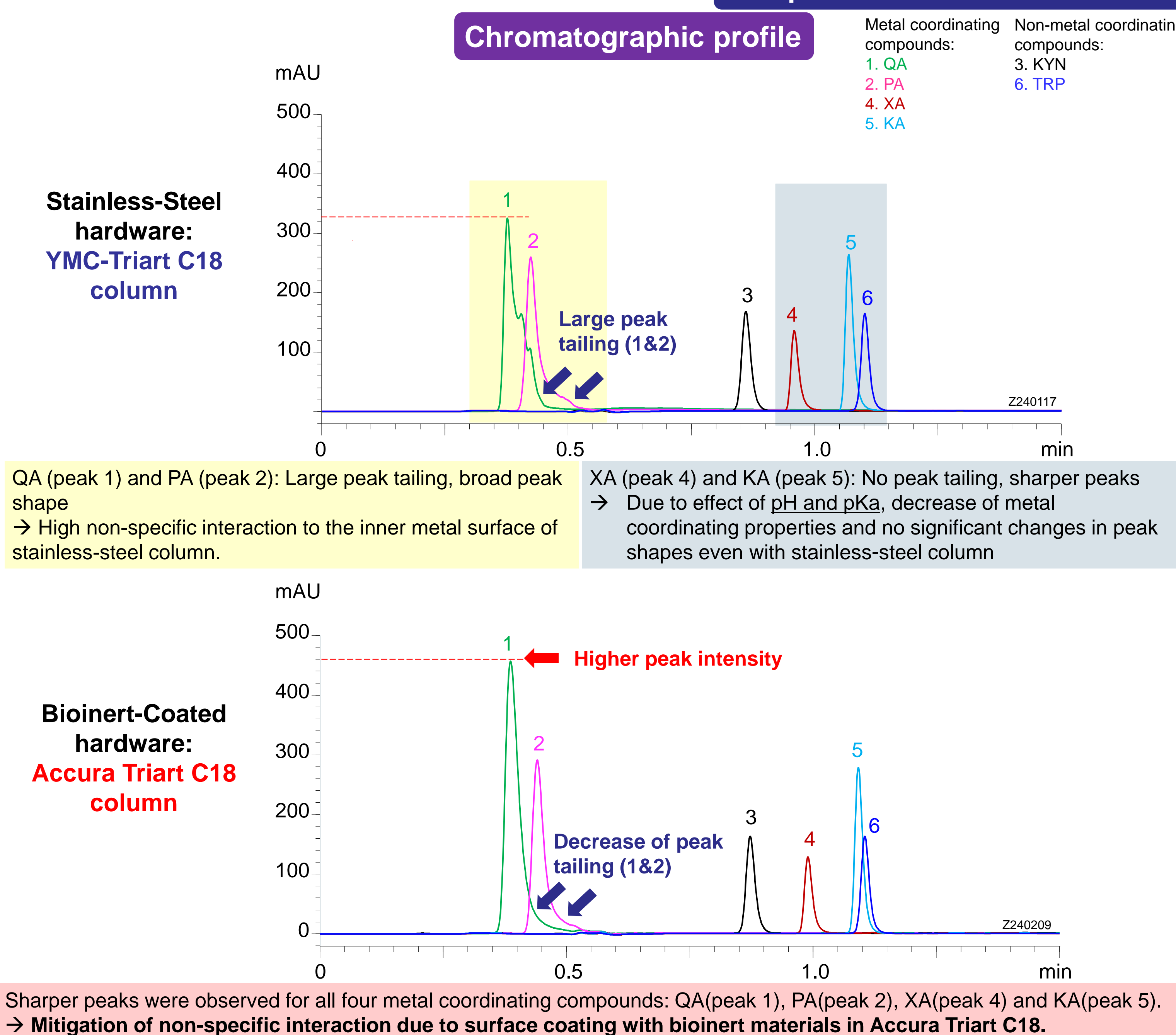
| Column name              | Hardware               | Packing material         | Particle size (μm) | Pore size (Å) | Size              |
|--------------------------|------------------------|--------------------------|--------------------|---------------|-------------------|
| YMC-Triart C18           | Stainless-Steel        | C18 bonded hybrid silica | 1.9                | 120           | 50 X 2.1 mm i. d. |
| <b>Accura Triart C18</b> | <b>Bioinert-Coated</b> | hybrid silica            |                    |               |                   |

### HPLC analysis 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                              |          | 0-4 min       | 0.3 mL/min |                    | ESI in positive mode |

## Results and discussion

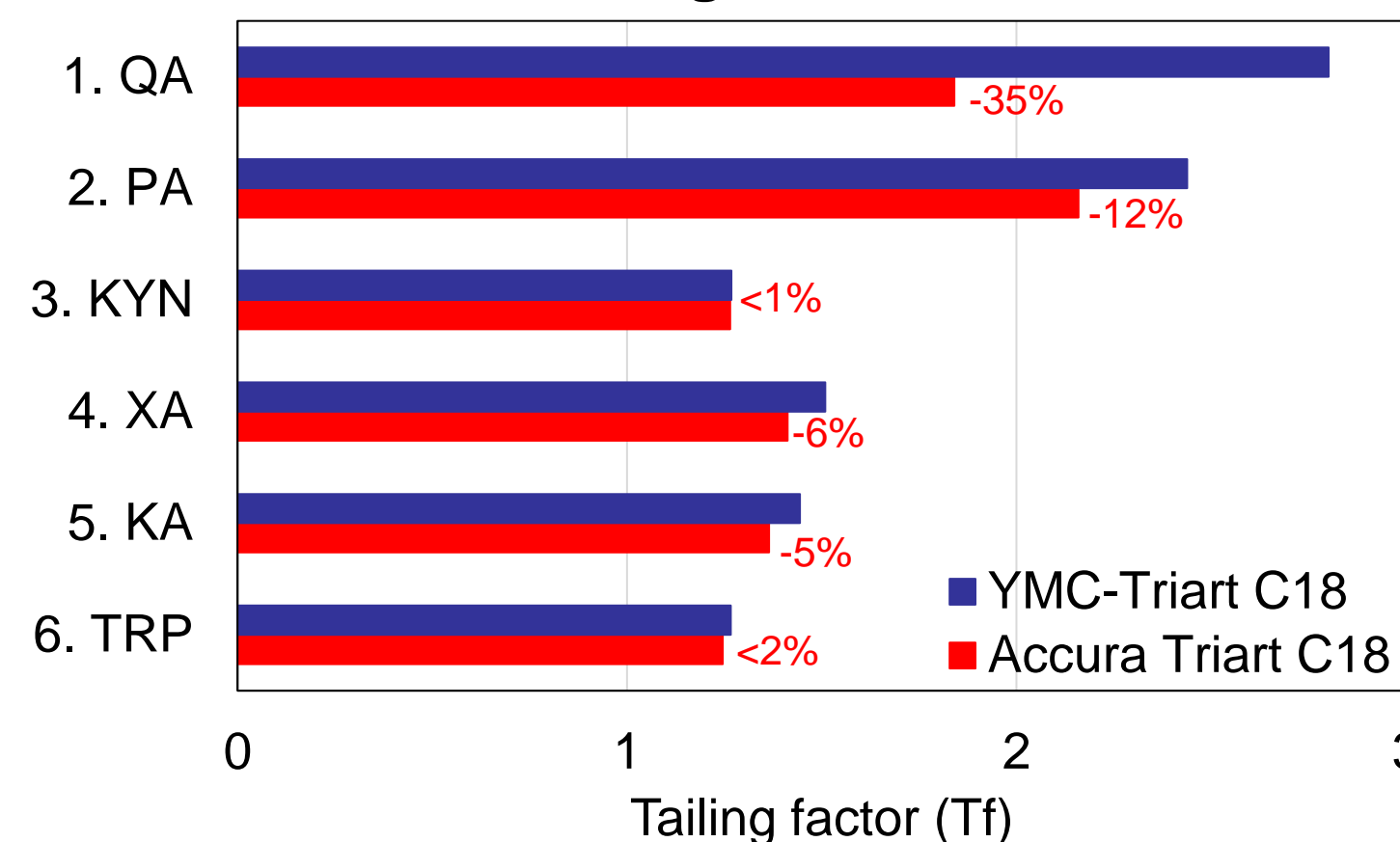
### Comparison between Stainless-Steel and Bioinert-Coated hardware



|   |             | Peak area (mAU-min) |                   | Peak height (mAU) |                   |
|---|-------------|---------------------|-------------------|-------------------|-------------------|
|   |             | YMC-Triart C18      | Accura Triart C18 | YMC-Triart C18    | Accura Triart C18 |
| <b>Metal coordinating compounds</b>     | QA(peak 1)  | 828.43              | 984.04 (+20%)     | 325.62            | 456.77 (+40%)     |
|   | PA(peak 2)  | 514.97              | 565.70 (+10%)     | 258.50            | 290.98 (+13%)     |
|   | XA(peak 4)  | 162.66              | 155.91 (-4%)      | 135.44            | 128.92 (-5%)      |
|   | KA(peak 5)  | 314.42              | 326.13 (+4%)      | 262.40            | 277.02 (+6%)      |
| <b>Non-metal coordinating compounds</b> | KYN(peak 3) | 228.19              | 251.24 (+10%)     | 167.87            | 177.22 (+6%)      |
|   | TRP(peak 6) | 196.92              | 197.60 (+0.4%)    | 163.79            | 162.41 (-0.9%)    |

(X%: increase (+) or decrease (-) of data results from Accura Triart C18 compared to those from YMC-Triart C18)

### Tailing factor

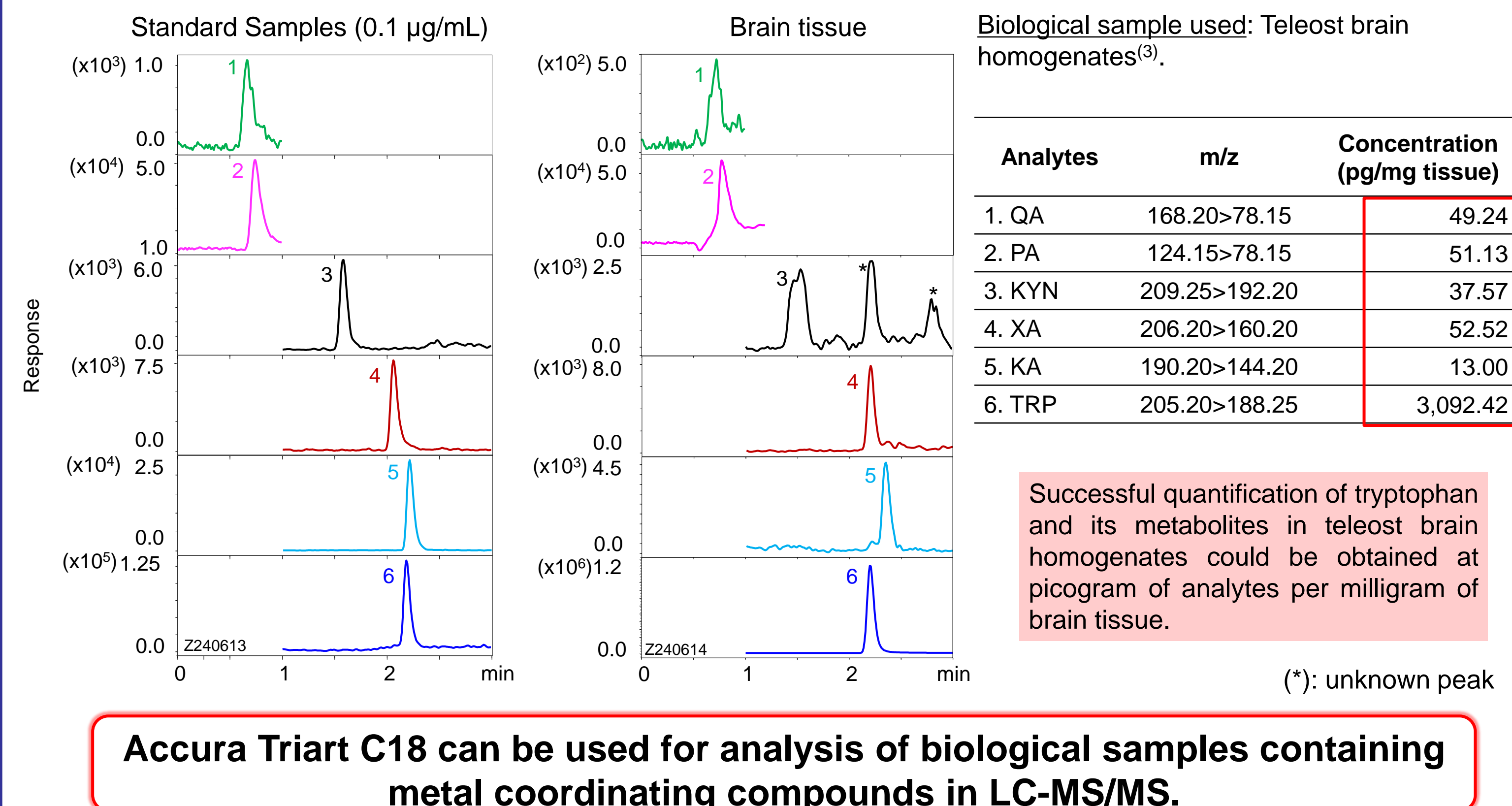
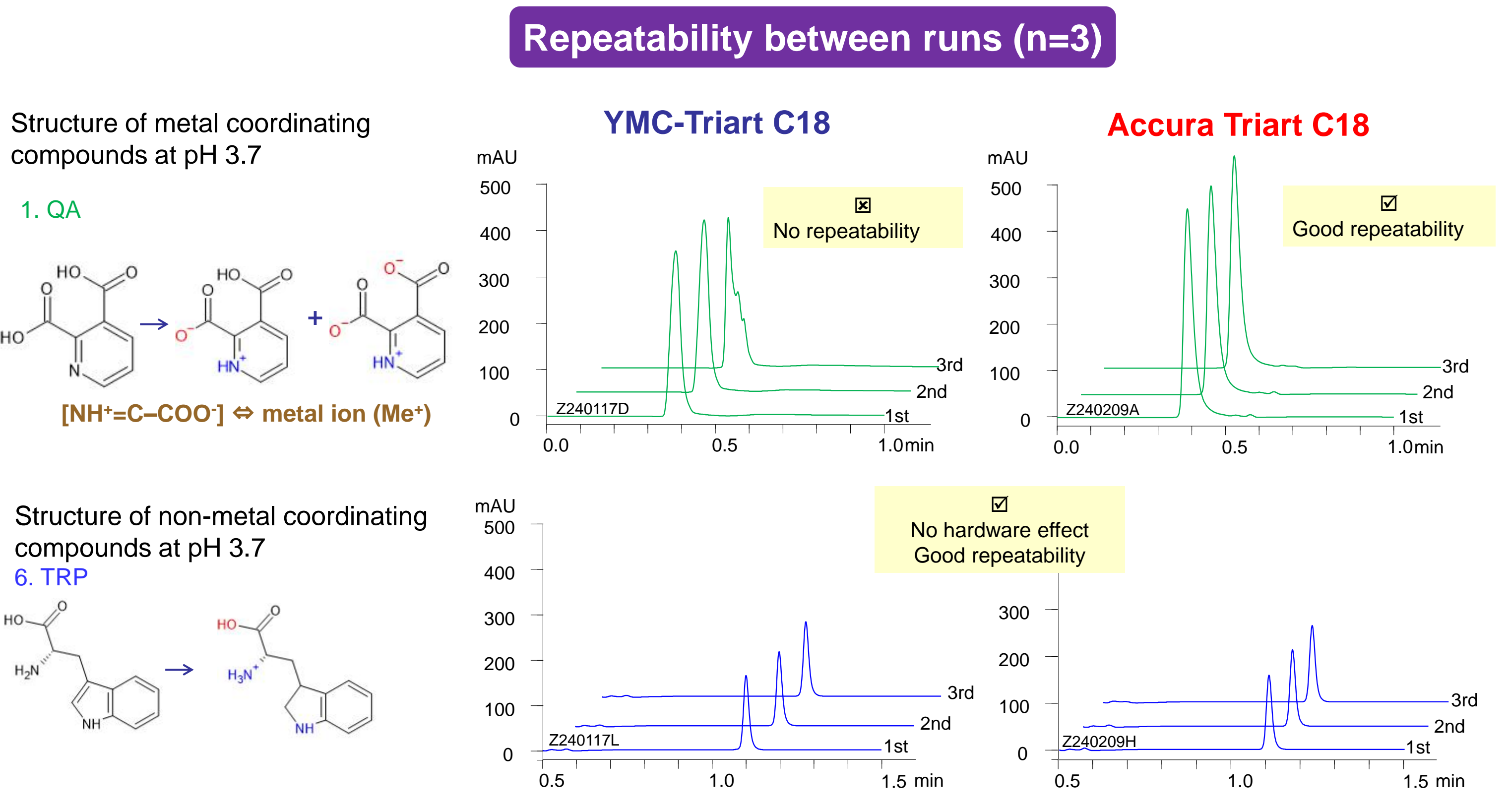


### Merits of bioinert coating in Accura Triart C18:

- Enhancement of the peak areas and peak heights of metal coordinating compounds, especially QA and PA.
- Elimination of non-specific interaction showed by significant reduction of the tailing factor of metal coordinating compounds, especially QA and PA.

**Accura Triart C18 improved peak shapes and enhanced analytical sensitivity of metal coordinating compounds.**

### Application: Analysis of biological sample using Bioinert-Coated column in LC-MS/MS



**Accura Triart C18 can be used for analysis of biological samples containing metal coordinating compounds in LC-MS/MS.**

## Conclusions

- Compared to stainless-steel column, bioinert-coated column (Accura) successfully improves the peak shapes and significantly enhances the analytical sensitivity of metal coordinating compounds such as quinolinic acid (QA) and picolinic acid (PA) while maintaining similar results for non-metal coordinating compounds.
- Analysis of biological sample containing metal coordinating compounds such as teleost brain homogenates can be achieved using Accura Triart C18 in LC-MS/MS.

**Due to the bioinert coating material on its inner surface, Accura Triart C18 is an excellent column for the analysis of metal coordinating compounds such as those in tryptophan metabolism in the kynurenine pathway.**