

A comparison of the effects of metal-free columns and bio-inert UHPLC systems on nucleotide analysis

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1. Introduction

The importance of bio-inert UHPLC systems has increased in recent years in order to improve the reliability and sensitivity of biomolecule analysis. One of the major concerns in the quantitative analysis of biomolecules is the adsorption of the molecules onto metal surfaces, which can lead to a dramatic decrease in sensitivity. [1,2] Therefore, it is essential to utilize a system with complete inertness in the entire sample flow path.

A large part of the sample flow is determined by the employed column. In fact, the column is often the part that provides the largest surface area. For this reason, it is advisable to use columns that are also inert to metal interactions. This can be achieved for example by using columns that have a protective PEEK layer between the stainless-steel body and the stationary phase particles. The advantage of combining both is that the columns show no interaction with metal-sensitive analytes but can still provide the high-pressure stability of typical metal columns used in liquid chromatography.

To investigate the effects of these inert components, we examined the applicability of inert Shimadzu Nexera XS system and metal-free Shim-pack Scepter columns for nucleotide analysis.

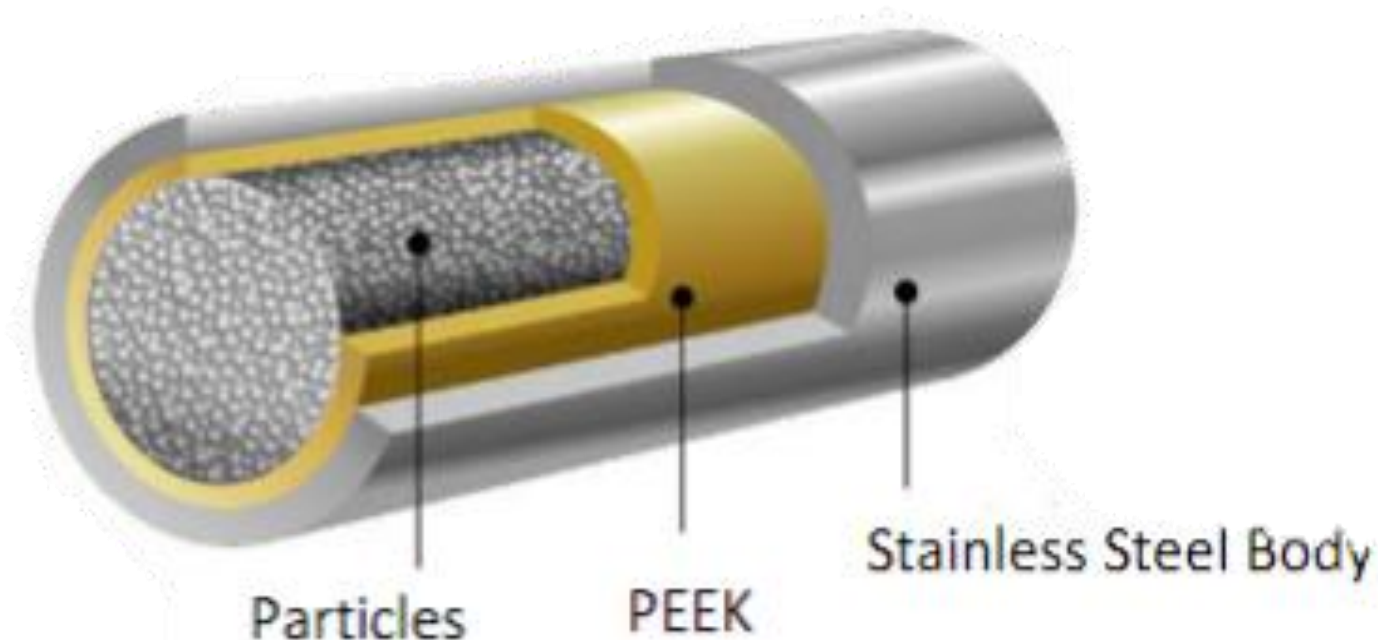


Figure 1. Schematic of a metal free (MF) column

2. Material and Method



Figure 2. Nexera XS inert system

ATP, ADP and AMP were purchased as standard substances and have been solved in water at a 50 mmol/L concentration. The standard solution has been stored in Torast™ H low adsorption vials to prevent loss of analytes to the surface of the vials.

Shimadzu UHPLC instruments Nexera XS and Nexera XS inert mounted with a photodiode array detector (SPD-M40) were used for the analysis. Each system was equipped with Shim-pack Scepter C18 and Shim-pack Scepter C18 metal free columns, yielding four distinct setups for nucleotide analysis. Detailed analytical conditions can be found in table 1.

3. Results

Metal adsorption can be determined by the fact that the components that are adsorbed display poor recovery and exhibit strong peak broadening due to the increased interaction. Indeed, the peak shape and sensitivity of ATP and ADP are poor when a conventional column and UHPLC system are combined (A) in Figure 3). All Chromatograms in figure 3 are for the third injection of the standard mixture.

Table 1. Analytical conditions

System:	Nexera XS inert or Nexera XS (Shimadzu Corporation, Japan)
Column:	Shim-pack Scepter C18 (Metal free) (3µm, 100 mm x 2.1 mm I.D.) (P/N 227-31073-02) Shim-pack Scepter C18 (3µm, 100 mm x 2.1 mm I.D.) (P/N 227-31014-05)
Mobile Phase:	Acetonitrile/Water + 50 mmol Ammonium formate = 0.5 : 99.5
Flow rate:	0.2 mL/min
Injection Volume:	2 µL
Oven Temp:	40 °C
Sample:	Equimolar (50 µg/mL) mixture of adenosine triphosphate, adenosine diphosphate and adenosine monophosphate dissolved in pure water
Vial:	Torast™-H Vial (P/N 370-04300-01)
Detector:	PDA (254 nm)
PDA Cell:	Standard (inert for Nexera XSi)

A significant improvement in performance was achieved by switching the system to an inert UHPLC (B). While the increase in sensitivity is greatest by switching from a conventional metal column to a metal-free column (C), considerable tailing can still be observed for ATP on the non-inert UHPLC system. Using an inert column and inert system gives the best results with very symmetrical peak shapes and the highest signal intensity (D).

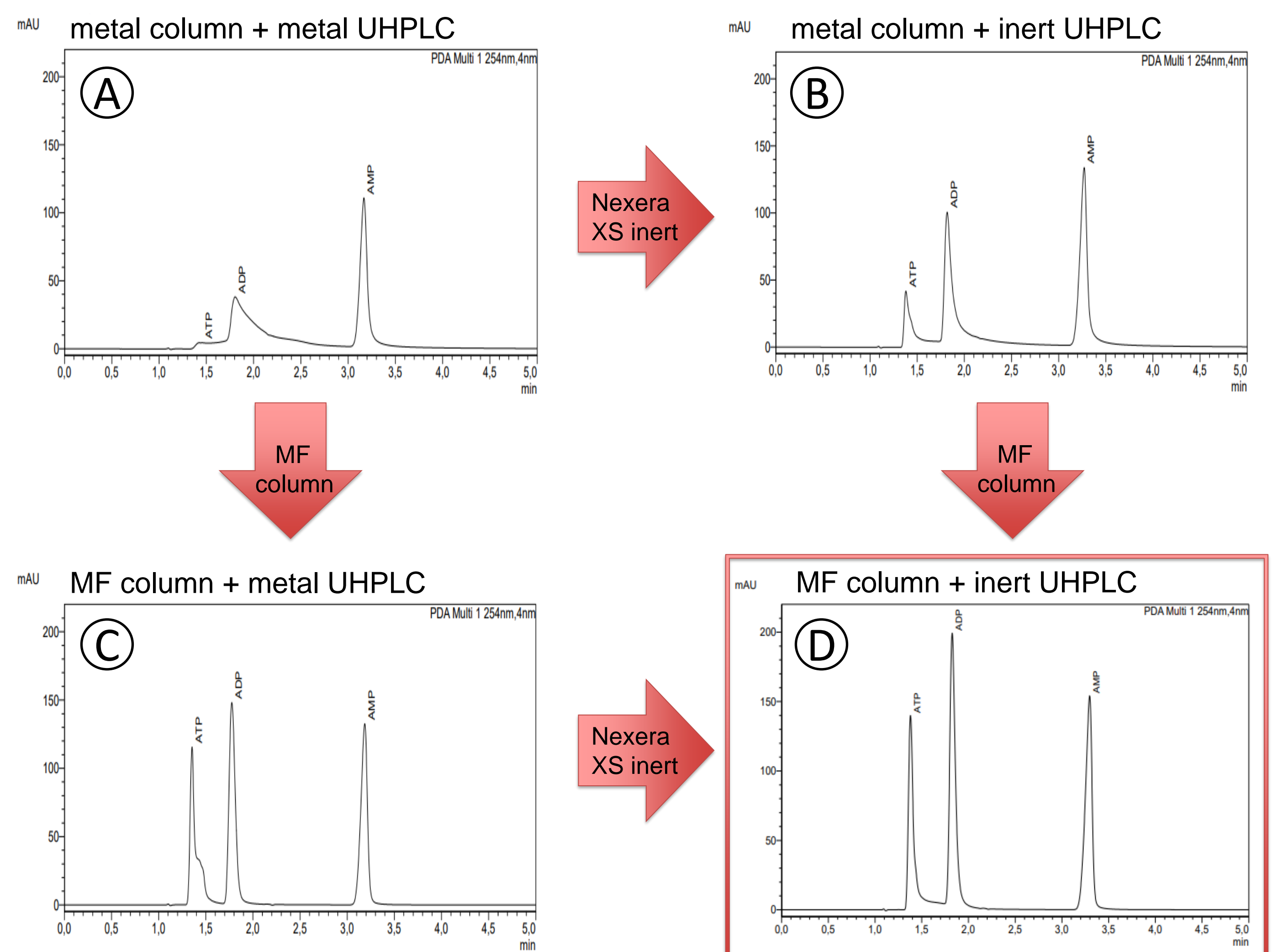


Figure 3. 3rd Injection of 50 mg/ml ATP, ADP, AMP on conventional UHPLC and column (A), Nexera XS inert and conventional column (B), MF Column and conventional UHPLC (C) and Nexera XS inert with MF column (D).

An important factor to consider, especially when working with conventional columns and systems, is that the number of injections has an impact on sensitivity and peak shape. This can be explained by the fact that with increasing number of injections the analyte forms a passivated layer with the metal surface. This is illustrated in Figure 4, which shows the tailing factor and peak height and area in dependence on the number of injections. Values that vary greatly within injection numbers indicate poor reproducibility. Therefore, the fully inert setup provides the best conditions for the analyzed nucleotides.

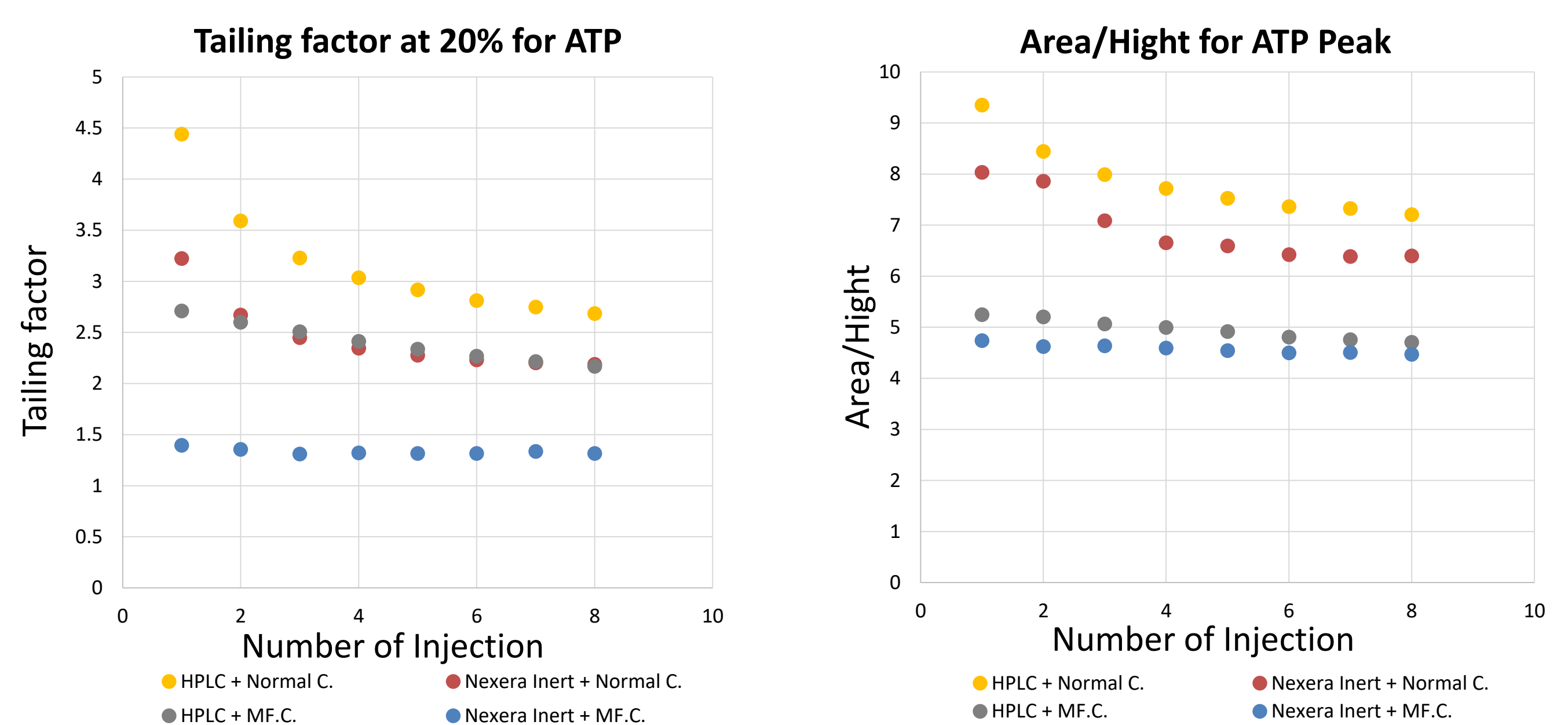


Figure 4. Tailing factor (left) and Area/Height ratio (right) for the ATP peak in dependence of the number of injections

4. Conclusions

The use of metal-free components of both, LC instrument and LC column, has significantly improved the sensitivity of nucleotide analysis. In addition, the reproducibility is superior compared to conventional systems. While the metal-free Shim-pack Scepter columns and the inert Nexera XSi system each lead to a reduction in adsorption, the best results were obtained when used in combination.

References

- [1] D'Atri, V., Murisier, A., Fekete, S., Veuthey, J.L., Guillarme, D., Current and future trends in reversed-phase liquid chromatography-mass spectrometry of therapeutic proteins, *TrAC Trends in Analytical Chemistry*, Vol. 130, 2020
- [2] Anspach, J.A., Rao, S., Rivera, B., Bioinert versus biocompatible: the benefits of different column materials in liquid chromatography separations *LCGC North Am*, Vol. 36, 2018, pp.24-29