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Application News

High Performance Liquid Chromatograph Nexera™ XS inert

Achieving Improved Sensitivity and Reliable Analytical Performances in Nucleotides Analysis

D. Fujimura, E. Ando, R. Suzuki

User Benefits

- The inert UHPLC system suppresses the interaction of compounds containing phosphate group(s) with internal surfaces.
- The combination of Nexera XS inert with a metal-free column improves peak shapes leading to better sensitivity and quantitative performance.
- The Nexera XS inert improves the overall performances by eliminating the need for conditioning procedures before the analysis.

■ Introduction

Stainless steel is commonly used in HPLC due to its pressure proof and corrosion resistant. However, it can interact with compounds containing phosphate group(s) by metallic affinity. This is a factor that negatively affecting the shape and intensity of the peak. In order to suppress metal adsorption, cleaning flow path with phosphoric acid, addition of chelating agents to mobile phase, or repeated injections of the target compounds are often performed. Nevertheless, it is not easy to obtain highly reproducible results.

This article introduces the use of the Nexera XS inert ultra-high performance liquid chromatograph, which utilizes a metal-free flow path, and a metal-free column for the accurate and reproducible analysis of nucleotides.

■ Analytical Conditions

Adenosine monophosphate (AMP), adenosine diphosphate (ADP), adenosine triphosphate (ATP) were used as compounds containing phosphate group(s). All compounds were dissolved in water, diluted and prepared. In order to evaluate the effect of metal adsorption, we compared two combinations of chromatographs and columns: one is "metal-based" and the other is "metal-free". (Table 1). The analytical conditions are same and shown in Table 2.

Table 1 Configurations

"Name"	System	Column
"metal-based"	Nexera XS	Shim-pack™ Scepter C18-120
"metal-free"	Nexera XS inert	Shim-pack Scepter C18-120 [metal-free]

Table 2 Analysis Conditions

System	: Nexera XS inert or Nexera XS
Column	: Shim-pack Scepter C18-120 [metal-free]*1 (100 mm × 2.1 mm I.D., 3 µm) Shim-pack Scepter C18-120*2 (100 mm × 2.1 mm I.D., 3 µm)
Mobile Phase	: Acetonitrile/10 mmol/L ammonium formate solution = 0.5 : 99.5
Flow Rate	: 0.2 mL/min
Column Temp.	: 40 ℃
Vial	: TORAST™ -H Bio Vial (Shimadzu GLC)*3
Injection volume	: 2 µL
Detection	: 254 nm (SPD-M40, UHPLC inert cell)

*1 P/N 227 -31073 - 02, *2 P/N 227 -31014 - 05, *3 P/N 370 - 04350 - 00

■ Comparison of Peak Shape and Reproducibility

The chromatograms of the mixed standard solutions of AMP, ADP, and ATP (50 µg/mL) are shown in Fig. 1 and Fig. 2, and the transitions in the symmetry factor and area values of the ATP peak are shown in Fig. 3 and Fig. 4 during ten analyses of the mixed standard solutions. In the "metal-based", the peak is tailing due to metal adsorption, and the symmetry factor is larger.

Moreover, it was possible to note that the peak area value of ATP increased every time the sample was injected into the "metal-based", indicating that the sample was progressively "coating" the internal surfaces due to the metal interaction. In the "metal-free", the peak shape was improved with a symmetry factor of about 1. The area value was consistently stable from the first to the tenth injection. This result highlights that the analysis using the "metal-free" suppresses metal adsorption and improves reproducibility.

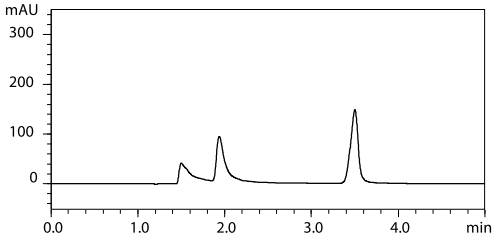

Fig. 1 Mixed standard solutions of AMP, ADP, and ATP (50 µg/mL) measured in the "metal-based"

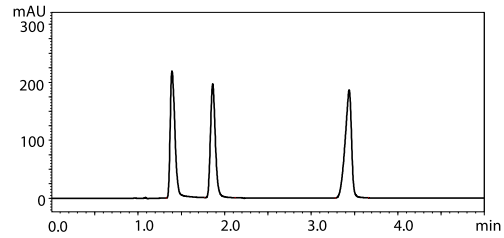

Fig. 2 Mixed standard solutions of AMP, ADP, and ATP (50 µg/mL) measured in the "metal-free"

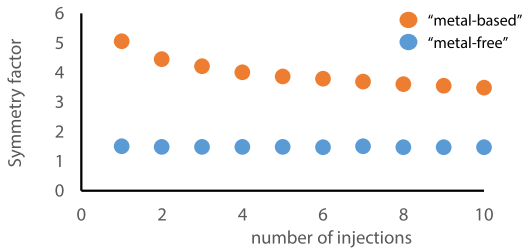

Fig. 3 Transitions in symmetry factor of ATP by the number of injections

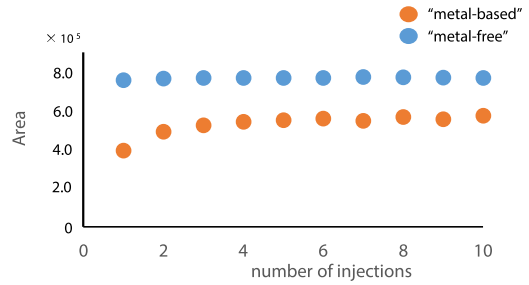

Fig. 4 Transitions in peak area of ATP by number of injections

■ Evaluation of Quantitative Accuracy in the Analysis of Nucleotide Standard Samples

The standard solutions of ATP (1, 2.5, 5, 10, 25, 50 µg/mL) were repeatedly measured 6 times under the conditions in Table 1, then a calibration curve was obtained.

In the calibration curve with the "metal-based", the linearity decreased due to metal adsorption (r^2 =0.9918, Fig. 5). Table 3 shows the accuracy and precision of each calibration point. The peak was not detected at 1 µg/mL with the "metal-based". Moreover, although it was possible to detect at 2.5 µg/mL, however, the peak intensity was not enough for quantification. Standard solutions of ATP at 2, 20, and 45 µg/mL were prepared as QC controls. These controls were quantified using the calibration curve shown in Fig. 5, and the QC results shows in Table 4. Due to metal adsorption when using the "metal-based", a large deviation in the quantitation values of QC controls was obtained, as expected by the low linearity of the calibration curve.

On the other hand, the calibration curve obtained with the "metal-free" showed excellent linearity (r^2 =0.9999, Fig. 6). Table 5 shows the accuracy and precision of each calibration point.

And Table 6 shows the quantitative results of QC controls. Excellent reproducibility was also obtained at the low-level calibration points and QC controls. These results indicate that the combination of the Nexera XS inert and the metal-free column is effective in suppressing metal adsorption and can be efficiently used to analyze compounds containing phosphate group(s) such as nucleotides.

■ Conclusion

In this article, we evaluated the effect of metal adsorption in nucleotide analysis. Compared to metal-based chromatographs and columns, metal-free flow path enabled accurate and reproducible analysis of compounds containing phosphate group(s). In the past, it was required several treatments to suppress metal adsorption. However, the combination of the Nexera XS inert and Shim-pack Scepter C18 metal-free columns provides highly sensitive, accurate, and robust analytical results.

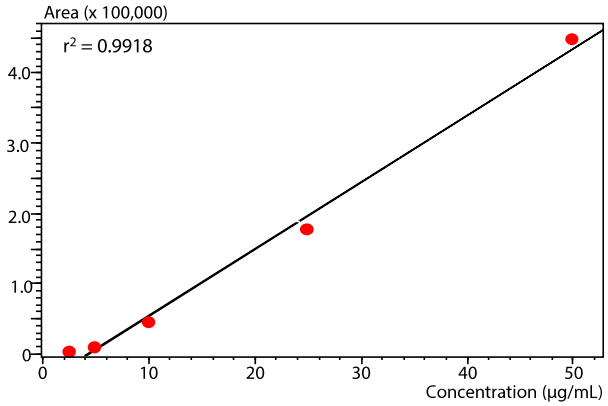

Fig. 5 Calibration Curve for ATP ("metal-based")

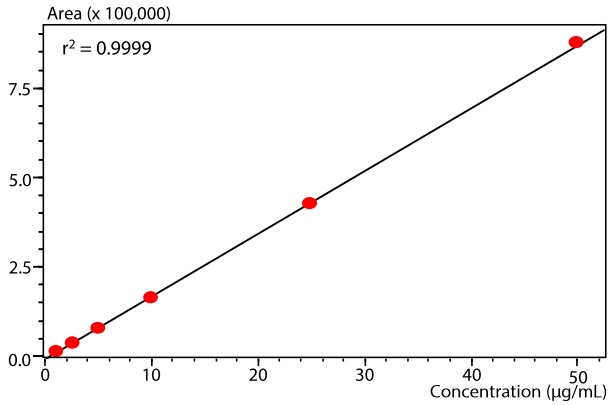

Fig.6 Calibration Curve for ATP ("metal-free")

Table 3 Quantitative values, accuracy and precision of calibration point samples ("metal-based")

Conc. (µg/mL)	Intra-Assay (n = 6)		
	Measured Conc. (µg/mL)	Accuracy (%)	Precision (%)
1	N.D. *	---	---
2.5	2.28	91.0	**110**
5	5.29	106	4.9
10	8.65	86.5	7.1
25	22.7	91.0	6.3
50	51.3	103	3.6

Table 4 Quantitative values, accuracy and precision of QC controls ("metal-based")

Conc. (µg/mL)	Intra-Assay (n = 6)		
	Measured Conc. (µg/mL)	Accuracy (%)	Precision (%)
2	**4.53**	**226**	1.6
20	18.4	91.9	5.7
45	46.4	103	3.3

Table 5 Quantitative values, accuracy and precision of QC controls ("metal-free")

Conc. (µg/mL)	Intra-Assay (n = 6)		
	Measured Conc. (µg/mL)	Accuracy (%)	Precision (%)
1	1.07	101	0.61
2.5	2.43	97.0	0.50
5	4.82	96.4	0.28
10	9.74	97.4	0.24
25	24.9	99.4	0.81
50	50.6	101	0.095

Table 6 Quantitative values, accuracy and precision of QC controls ("metal-free")

Conc. (µg/mL)	Intra-Assay (n = 6)		
	Measured Conc. (µg/mL)	Accuracy (%)	Precision (%)
2	2.00	100	0.59
20	19.6	98.2	0.081
45	44.9	99.7	0.11

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