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Ultra High-Performance Liquid Chromatograph Nexera™ XS inert

Improvement of Quantitative Performance in LC/MS Analysis of Oligonucleotides using Nexera XS inert

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■ Summary

Metal adsorption, which is caused by the interaction between the analyte and the metal surfaces in the sample flow path, is one of the major problems in the analysis of oligonucleotides. The use of a traditional LC system (stainless steel based) generally leads to poor peak shape, impaired sensitivity and quantitative performance. This article introduces an example of oligonucleotides analysis by using the Nexera XS inert, which was developed to solve the problem of metal adsorption. Sensitivity, quantitative performance, and carryover were evaluated, and the results showed an overall improvement compared to an HPLC system using stainless steel in the flow path. The Nexera XS inert exhibits optimal analytical performances for metal-coordinating compounds.

■ Introduction

Stainless steel (SUS), which is generally used in HPLC flow path, has excellent pressure resistance, but compounds with phosphate groups that coordinate with metals can adsorb by interaction with the wetted surface. Adsorption with metal negatively affects the peak shape, detection sensitivity, and reproducibility, and reduces performance in quantitative analysis. Repeated injections of highly concentrated samples can be performed to suppress adsorption, but this technique is time-consuming and expensive. Alternatively, a solution containing chelating agent can be used to suppress adsorption. However, this method is not recommended for LC/MS analysis because it may lead to contamination and decrease in sensitivity.

In this article, we present an example of a comparison between HPLC with SUS in the sample flow path and Nexera XS inert, an inert-UHPLC system that suppresses adsorption of metal-coordinating compounds, in oligonucleotide analysis.

■ Sample

Sequence : 5'-dG-dC*-dC*-dT-dC*-dA-dG-dT-dC*-dT-dG-dC*- dT-dT-dC*-dG-dC*-dA-dC*-dC*-3'

(*) Indicates 5-C or 5-U methylation
(d) 2'-Deoxy Nucleoside

Molecular weight : 6431.72

■ Analytical Conditions

In order to evaluate metal adsorption suppression, Nexera XR, which uses SUS in the sample flow path, and Nexera XS inert were used. SUS body columns and metal free columns were used. The analytical conditions are shown in Table 1. Ion-pair reagents are commonly used in the reversed-phase analysis of oligonucleotides. HFIP (1, 1, 1, 3, 3, 3-Hexafluoro -2 propanol) and DIPEA (N, N-diisopropylethylamine) were used as ion-pair reagents.

Table 1 Analytical Conditions

HPLC Conditions		
System	:	Nexera XR, Nexera XS inert
Column	:	Shim-pack™ Scepter C18-120 (100 mm x 2.1 mm I.D., 3 µm) *1 Shim-pack Scepter C18-120 [metal-free] (100 mm x 2.1 mm I.D., 3 µm) *2
Mobile phase A	:	50 mmol/L HFIP and 10 mmol/L DIPEA in water
Mobile phase B	:	Acetonitrile
Rinse solution R0	:	Water/Acetonitrile/Methanol/2-Propanol/Formic acid =30/50/10/10/0.1 (v/v/v/v/v)
Rinse solution R1	:	Water
Flow rate	:	0.3 mL/min
Gradient Program	:	B 5% (0-1 min), 5-30% (1-6 min), 95% (6.1-7 min), 5% (7.1-12 min)
MS conditions (LCMS™ -8060)		
Ionization	:	ESI (Negative mode)
Probe Voltage	:	-4 kV
Mode	:	MRM (*m/z* 803.5 > 95.0)
CID gas	:	330 kPa
Nebulizing gas flow	:	3.0 L/min
Drying gas flow	:	8.0 L/min
Heating gas flow	:	12.0 L/min
DL Temp.	:	300 ℃
Heat Block Temp.	:	450 ℃
Interface Temp	:	250 ℃

*1 P/N: 227 -31014 - 05, * 2 P/N: 227 -31073 – 02

■ Sensitivity Comparison

Fig. 1 shows chromatograms of a 10 ng/mL standard oligonucleotide solution analyzed by Nexera XR and SUS-body column, and Nexera XS inert and metal-free column. Compared to Nexera XR, Nexera XS inert showed an increase of about 1.7 times in the peak intensity.

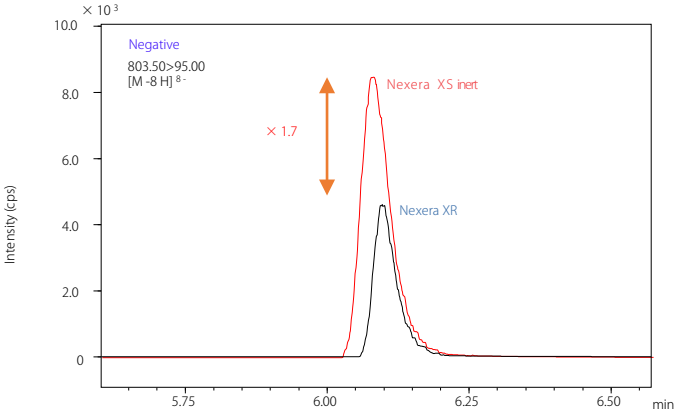

Fig. 1 MRM chromatograms of an oligonucleotide standard solution (10 ng/mL)

■ Quantitative Performance

The standard oligonucleotide solutions (0.5 -1000 ng/mL) were analyzed in each system and calibration curves were obtained. The calibration curve obtained with Nexera XR showed a decrease in linearity due to the metal adsorption, and the contribution ratio $R^2 = 0.9721$ (Fig. 2 (a)). On the other hand, the calibration curve obtained with Nexera XS inert showed excellent linearity due to the suppression of metal adsorption (Fig. 2 (b)).

Table 2 shows the accuracy at each calibration point. Using Nexera XS inert, the accuracy was clearly better than the Nexera XR. This result shows how Nexera XS inert is improving the reliability of quantitative analysis.

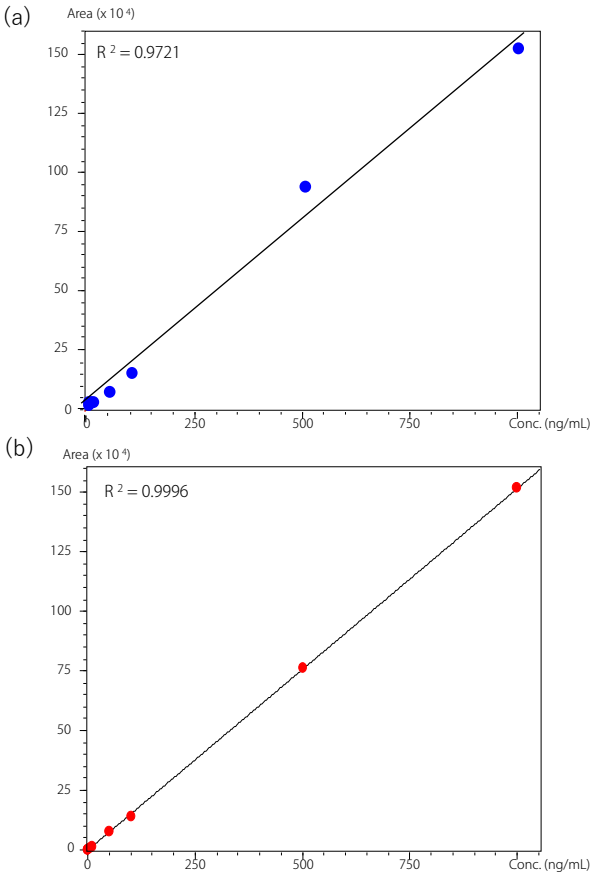

Fig. 2 Comparison of calibration curves
(a) Nexera XR, (b) Nexera XS inert

Table 2 Comparison of accuracy at each concentration

Concentration (ng/mL)	Nexera XR		Nexera XS inert	
	Calculated conc. (ng/mL)	Accuracy(%)	Calculated conc. (ng/mL)	Accuracy(%)
0.5	2.28	455.7	0.57	113.5
1	-1.04	-104.4	0.93	93.0
5	2.43	48.5	5.42	108.5
10	5.62	56.2	9.13	91.3
50	26.63	53.3	50.28	100.6
100	76.39	76.4	92.77	92.8
500	588.74	117.7	497.04	99.4
1,000	965.46	96.5	1,010.36	101.0

■ Carryover

After analyzing the oligonucleotide solution at concentration 1000 ng/mL, water, the sample solvent, was immediately injected as a blank to evaluate carryover. A chromatogram of the blank analyzed by Nexera XR is shown in Fig.3(a) and a chromatogram of the Nexera XS inert is shown in Fig. 3(b). The carryover values were 0.0790% for Nexera XR and 0.0033% for Nexera XS inert, respectively. These results indicate that Nexera XS inert suppresses metal adsorption and minimizes carryover.

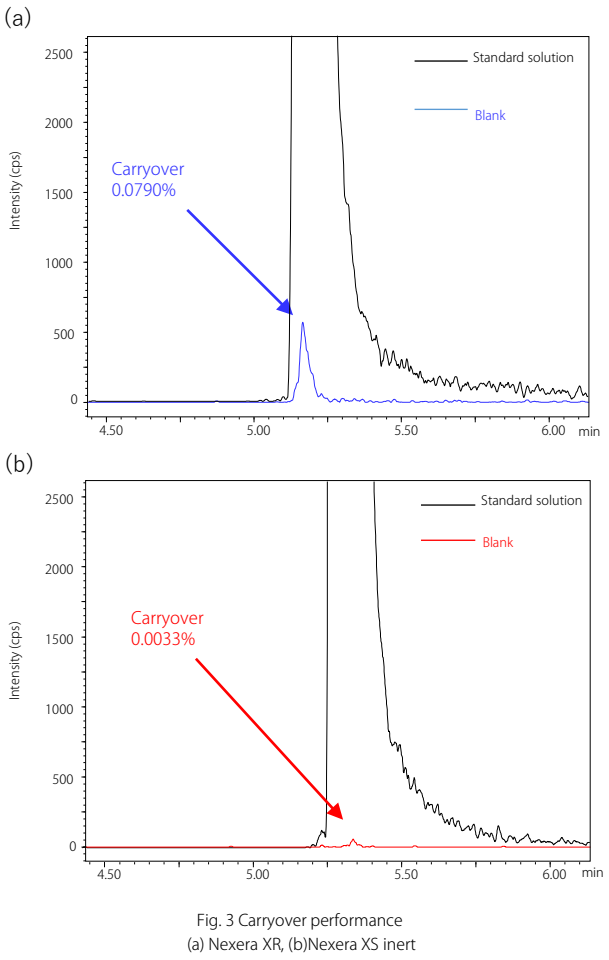

Fig. 3 Carryover performance
(a) Nexera XR, (b)Nexera XS inert

■ Summary

This paper introduced an example of performance evaluation of metal adsorption suppression using Nexera XS inert in oligonucleotide analysis. Sensitivity, quantitative performance, and carryover were evaluated, and Nexera XS inert showed a significant improvement in the analytical performances compared to a stainless-steel-based HPLC system. The Nexera XS inert is the ideal solution for the analysis of metal-sensitive compounds.

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