Excellence in Science

Application News

No. L560

High Performance Liquid Chromatography

Improvement of Peak Shape in Analysis of Basic Compounds and Reduction of Carryover by Multi-Rinse Function

Frequent hand disinfection is an effective measure against the spread of seasonal influenza. The main component of many hand disinfectants is ethanol, and some of them contain bactericidal components as well. The effective components in commercially-available disinfectants include chlorhexidine and benzethonium, both of which are strongly basic compounds.

When basic compounds are analyzed with a typical C18 column, tailing or other deterioration of the peak shape may occur due to the nonspecific interaction with remaining silanol on the surface of the packing materials. Because the Shim-pack Arata™ C18 is especially designed to suppress the interaction with silanol, a good peak shape can be expected when analyzing basic compounds, even if using a simple composition of mobile phase.

It is known that chlorhexidine is a compound that is extremely easily absorbed on the wetted parts of autosamplers, and for this reason, the results of subsequent analyses may be influenced by chlorhexidine carryover, particularly after analyzing a high concentration sample. The Nexera™ X3 system is equipped with multi-rinse function as a standard to reduce carryover by automatically rinsing the injection port, where carryover frequently occurs.

This article introduces an analysis of the two above-mentioned basic compounds using Nexera X3 and Shim-pack Arata C18, together with reduction of carryover by multi-rinse function.

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Table 1 Analytical Conditions

System	: Nexera X3
Column	: Shim-pack Arata C18 (100 mm × 3.0 mm I.D., 2.2 µm) Typical C18 column (100 mm × 3.0 mm I.D., 2.2 µm)
Mobile phase	: A) 0.1% Formic acid in water B) 0.1% Formic acid in acetonitrile
Flow rate	: 0.7 mL/min
Column temp.	: 40 °C
Injection volume	: 1 µL
Detection	: UV 258 nm

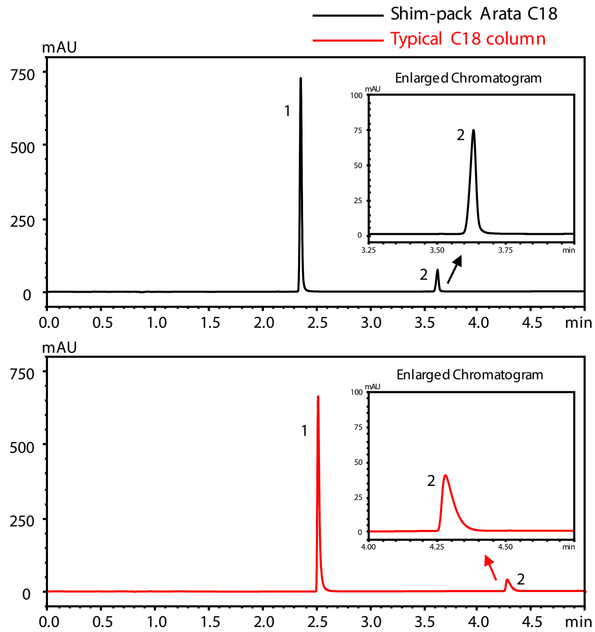
Table 2 Time Program

Time (min)	A. Conc	B. Conc
0	95	5
3	50	50
6	50	50
6.01	95	5
10	95	5

Analysis of Chlorhexidine and Benzethonium

Fig. 1 shows the chromatograms when 1 µL of mixed standard solution of chlorhexidine diacetate (50 mg/L) and benzethonium chloride (200 mg/L) was injected, and Tables 1 and 2 show the analytical conditions. Typical C18 column and Shim-pack Arata C18 were used in this experiment. Table 3 shows the symmetry factor when the mixed standard solution was analyzed using these columns.

The symmetry factors using Shim-pack Arata C18 were 1.14 for chlorohexidine and 0.98 for benzethonium, whereas those obtained with typical C18 column were 1.85 for chlorohexidine and 2.17 for benzethonium. Thus, in comparison with the typical C18 column, better peak symmetry was obtained when these basic compounds were analyzed using the Shim-pack Arata C18.



1. Chlorhexidine 2. Benzethonium

Fig. 1 Chromatograms of Mixed Standard Solution of Chlorhexidine Diacetate (50 mg/L) and Benzethonium Chloride (200 mg/L)

Table 3 Comparison of Symmetry Factors

Compound	Shim-pack Arata C18	Typical C18 column
Chlorhexidine	1.14	1.85
Benzethonium	0.98	2.17

Reduction of Carryover by Multi-Rinse Function

Multi-rinse function makes it possible to rinse the external surface of the needle with a maximum of 2 rinse solutions and the internal surface of the needle with a maximum of 3 rinse solutions, and the rinse sequence can be set as desired. Although carryover occurs easily at the injection port, automatic rinsing of this part is also possible by multi-rinse function.

Here, we compared two carryovers. One was through the conventional rinse (only external rinse of the needle), the other was through the injection port rinse contained in the multi-rinse function. Water was immediately injected as blank after injection of the standard solution, and carryover was calculated from the respective peak areas. The rinse solutions used here were a mixture of water and acetonitrile, and an acetonitrile solution containing 0.1% formic acid.

Tables 4 and 5 show the analytical conditions. Fig. 2 shows the screen capture of settings on LabSolutions™ when multi-rinse function is used. Fig. 3 shows the chromatograms when 5 µL of blank was injected following a standard solution of chlorhexidine diacetate (10,000 mg/L) for the case of only using external rinse of the needle. Fig. 4 shows the chromatograms with multi-rinse function. Table 6 shows the result of carryover for both cases. With the settings shown in Fig. 2, in addition to needle external rinse, the internal surface of the needle and the injection port are also rinsed in the sequence of rinse solution R2, R1 after the analysis is completed. Following these rinsing operations, the solvent in the sample loop is replaced with R0 and equilibration for the next analysis is conducted for a hold time of 1 min.

Table 4 Analytical Conditions

System	: Nexera X3
Column	: Shim-pack Arata C18 (100 mm × 3.0 mm I.D., 2.2 µm)
Mobile phase	: A) 0.1% Formic acid in water B) 0.1% Formic acid in acetonitrile
Flow rate	: 0.7 mL/min
Column temp.	: 40 °C
Injection volume	: 5 µL
Detection	: UV 258 nm
Rinse solution R0	: Water/acetonitrile=50/50 (v/v)
Rinse solution R1	: Water/acetonitrile=50/50 (v/v)
Rinse solution R2	: 0.1% Formic acid in acetonitrile

Table 5 Time Program

Time (min)	A. Conc	B. Conc
0	80	20
2	80	20
2.01	60	40
3	60	40
3.01	80	20
6	80	20

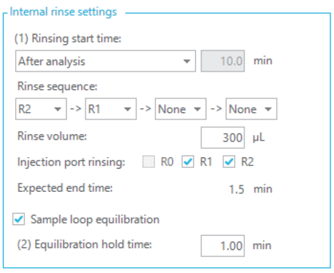


Fig. 2 Screen Capture of Needle Internal Rinse Settings (R0, R1, and R2: Types of Rinse Solutions)

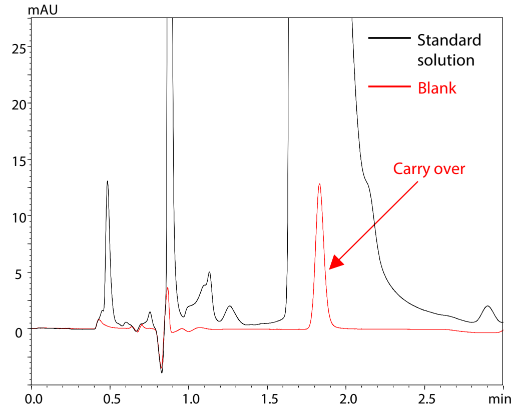


Fig. 3 Chromatograms When Only Using Needle External Rinse

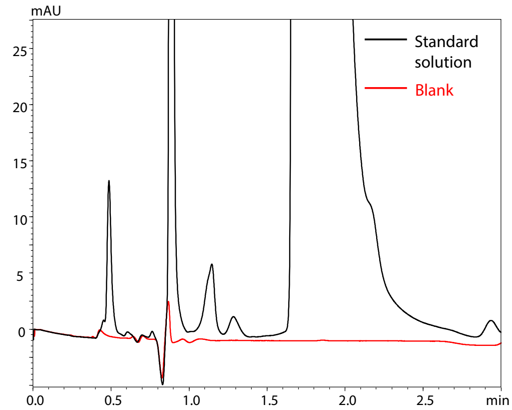


Fig. 4 Chromatograms When Using Multi-Rinse Function

The carryover when only using needle external rinse after injection of the standard solution of 10,000 mg/L of chlorhexidine diacetate was 2.31 mg/L, which is equivalent to 0.0231% of the standard solution. On the other hand, although the carryover when using needle internal/external rinse and injection port rinse was below the limit of detection, the reference concentration was 0.01 mg/L, which is equivalent to 0.0001% of the standard solution. Thus, carryover can be reduced to approximately 1/230 by multi-rinse function.

Table 6 Comparison of Carryovers

Rinse method	Concentration of carryover (mg/L)	Carryover (%)
Needle external rinse	2.31	0.0231
Needle internal/external rinse and injection port rinse	0.01	0.0001

Conclusion

When analyzing basic compounds with a simple composition of mobile phase such as 0.1% formic acid aqueous solution, an improved peak shape can be obtained by using Shim-pack Arata C18 column in comparison with that from typical C18 column. In addition, carryover can be greatly improved by multi-rinse function of Nexera. As demonstrated here, this function is a particularly useful for reducing carryover when high concentration samples are injected.

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