Minimization of carry-over for LC/MS analysis by autosampler with unique rinse function

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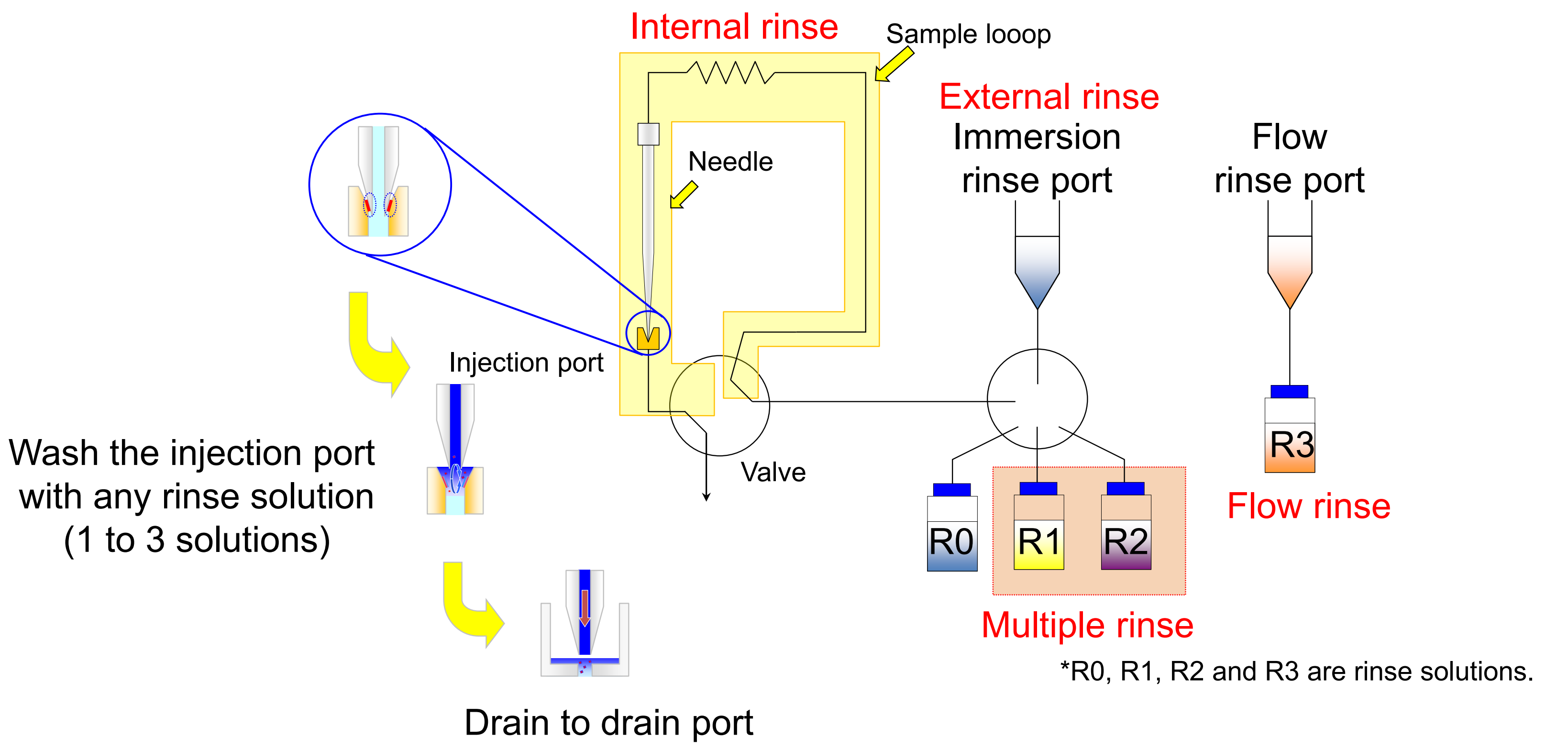
1, Introduction

With the increasing sensitivity of LC/MS in recent years, the number of cases in which extremely low concentration compounds are analyzed has increased. So-called carry-over and adsorption, in which the sample remains in the system, are more likely to become apparent in such sensitive analyses. The rinse mechanism used in many autosamplers is rinsing by immersing the sampling needle in a rinse solution, and only the surface of the sampling needle can be rinsed. However, carry-over may also occur at locations other than the surface of the sampling needle, such as sample loops and injection ports. It is difficult to take countermeasures with a normal rinse mode when it occurs in these parts. It is also important to select a rinse solution. However, due to the many limitations of the solutions that can be handled by LC/MS, it may be difficult to use rinses that match the characteristics of the compounds. Therefore, this study evaluated carry-over reduction using an autosampler with a unique rinse mechanism. The feature of this functions, called the internal rinse mode is that it is possible to rinse of the needle inner surface and injection port, etc., with multiple rinsing solutions. We also evaluated the reduction of the carry-over by using UHPLC system offering non-metal of the sample flow path.

2, Experimental

2-1, Instruments

The Nexera™ X3 (Shimadzu Corporation), a UHPLC system using stainless steel (SUS), and the Nexera XS inert (Shimadzu Corporation), a bioinert UHPLC system offering non-metal of the sample flow path, were used as HPLC systems. The autosamplers used on these systems have a unique rinse mechanism as shown in Fig. 1 in addition to the commonly adopted rinse mode. A mass spectrometer LCMS™-8060 (Shimadzu Corporation) was used as the detector. The column was used the metal-free column Mastro™ 2 C18 (Shimadzu GLC).



*R0, R1, R2 and R3 are rinse solutions.

Rinse mode	Description
External rinse	Rinse the needle outer surface (widely used in autosamplers).
Internal and external rinse	Rinse the needle outer and inner surface and rinse the injection port.
Flow rinse	Rinse the needle outer surface with the rinse pump delivering solution from the bottom to the top of the rinse port.
Multiple rinse	Perform internal rinse using multiple rinses (Up to 3 solutions R0, R1 and R2).

Fig. 1 Nexera series auto sampler rinse mechanism (schematic) and function description

2-2, Sample and analytical conditions

Butyryl coenzyme A with a phosphate group (Fig. 2) was selected as a model compound. It is well known that compounds containing phosphate groups adsorb onto metals, and the use of HPLC systems and columns using SUS can have various effects on analysis, such as deterioration of peak shape, poor sensitivity, and carry-over. The analysis conditions are shown in Tables 1 and 2.

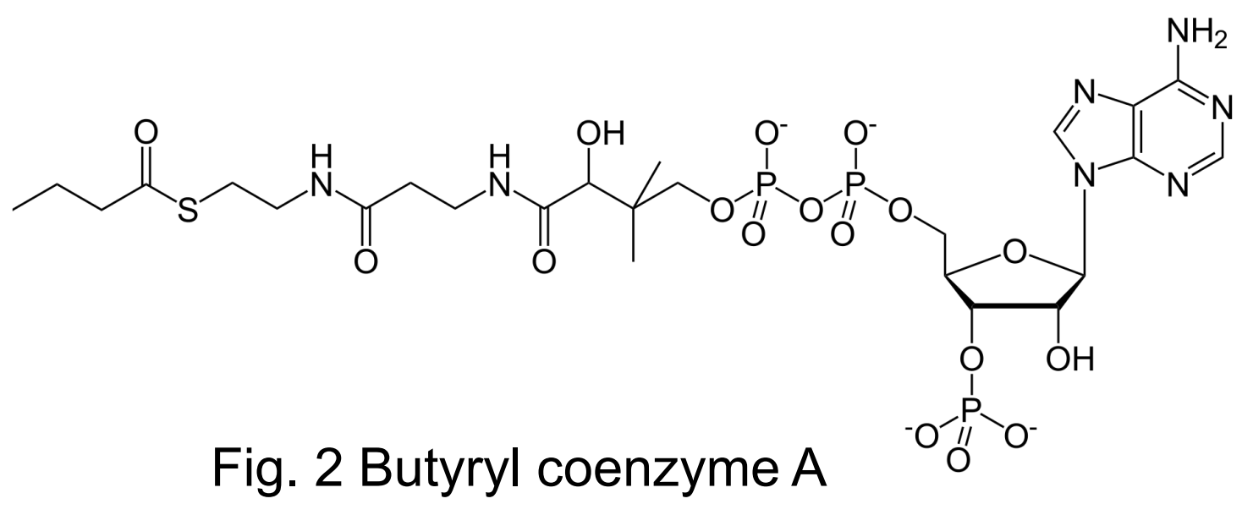


Fig. 2 Butyryl coenzyme A

Table. 1 HPLC conditions

Column : Mastro2 C18 Metal free 150 mm × 2.0 mm I.D., 3.0 μm, Shimadzu GLC
Mobile phase A : 15 mmol/L Acetic acid、10 mmol/L Dipentylamine
Mobile phase B : Methanol
Gradient : B Conc. 0% (0-0.5 min) > 15% (8 min) > 98% (12 min) > 98% (15 min) > 0% (15.10 min-20 min)
Flow rate : 0.30 mL/min
Column temp. : 40ºC
Injection vol. : 3 μL

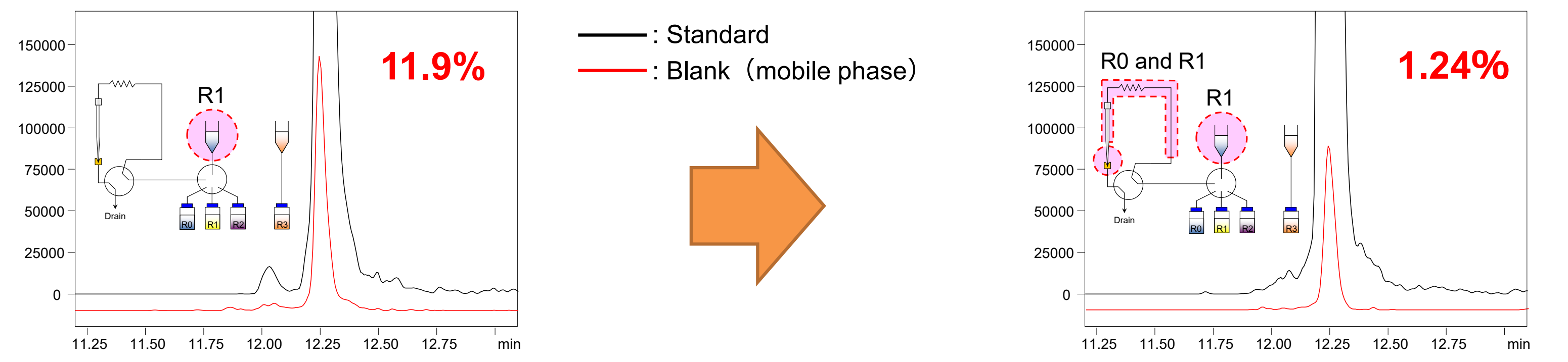
Table. 2 MS conditions

Ionization mode : IonFocus ESI nega
Mode : MRM（836.1 > 407.95, CE: 38）
Nebulizing Gas Flow : 2.0 L/min
Heating Gas Flow : 10.0 L/min
Drying Gas Flow : 10.0 L/min
Interface Temperature : 270ºC
DL Temperature : 250ºC
Heat Block Temperature : 400ºC

3, Results

3-1, Improving carry-over with internal rinse function

When the Butyryl coenzyme A was analyzed using the Nexera X3 (SUS system) with a normal rinse (external rinse mode), the results showed a very large carry-over of 11.9%. Because of the Butyryl coenzyme A showed strong retention even in reversed phase analysis, carry-over by hydrophobic adsorption seemed to occur. Therefore, in order to improve carry-over by hydrophobic adsorption first, we changed the rinse solution and used internal rinse mode. The results showed that carry-over due to hydrophobic adsorption was reduced. These results are shown in Fig. 3.



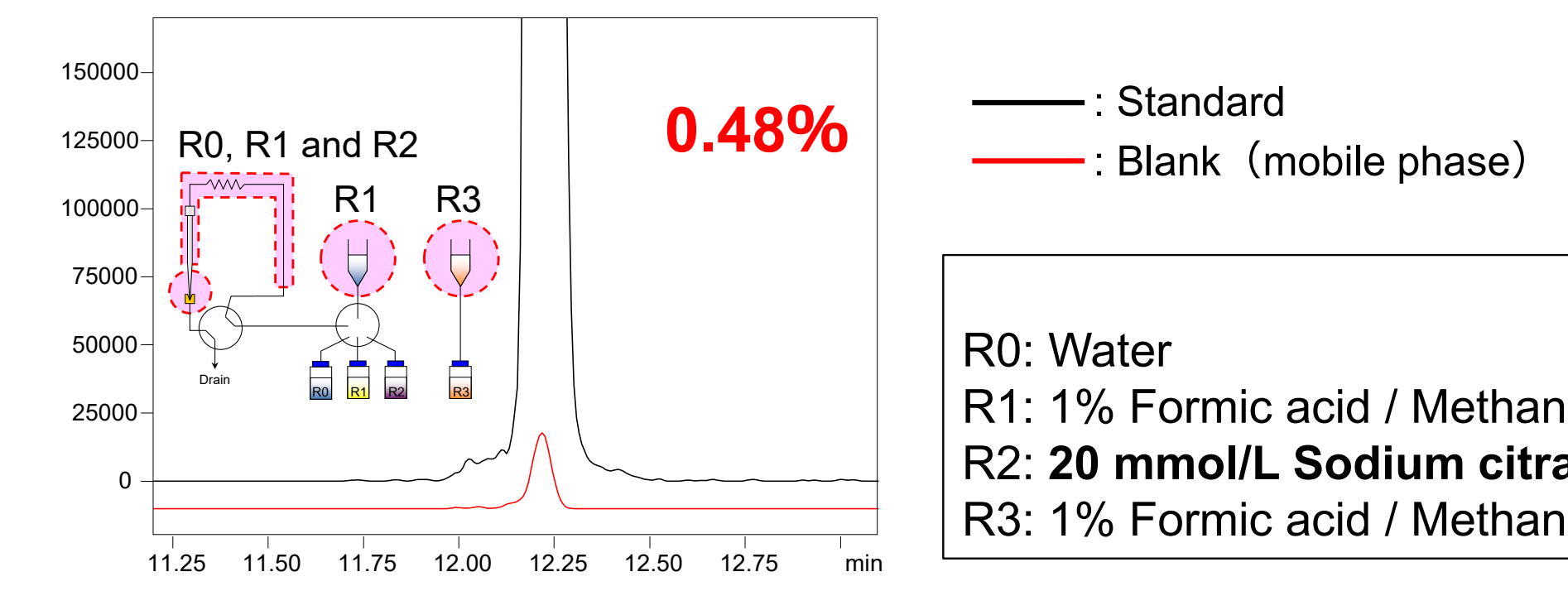
Rinse solution
R1: Water / Methanol = 1:1

Rinse solutions
R0: Water
R1: 1% Formic acid / Methanol / Acetonitrile / Isopropanol = 1:1:1:1

Fig. 3 Reduction of carry-over using internal rinse with multiple organic solutions in the Nexera X3

3-2, Carry-over reduction by using chelating agents in the rinse solution

Carry-over of Butyryl coenzyme A may also occur by adsorption to SUS in the HPLC system. Therefore, an aqueous solution of sodium citrate, a chelating agent, was used as a rinse solution. In order to prevent contamination of MS with sodium citrate as possible , a multiple rinsing function was adopted. As a result, as shown in Fig. 4, the carry-over was 0.48%, confirming a significant reduction.

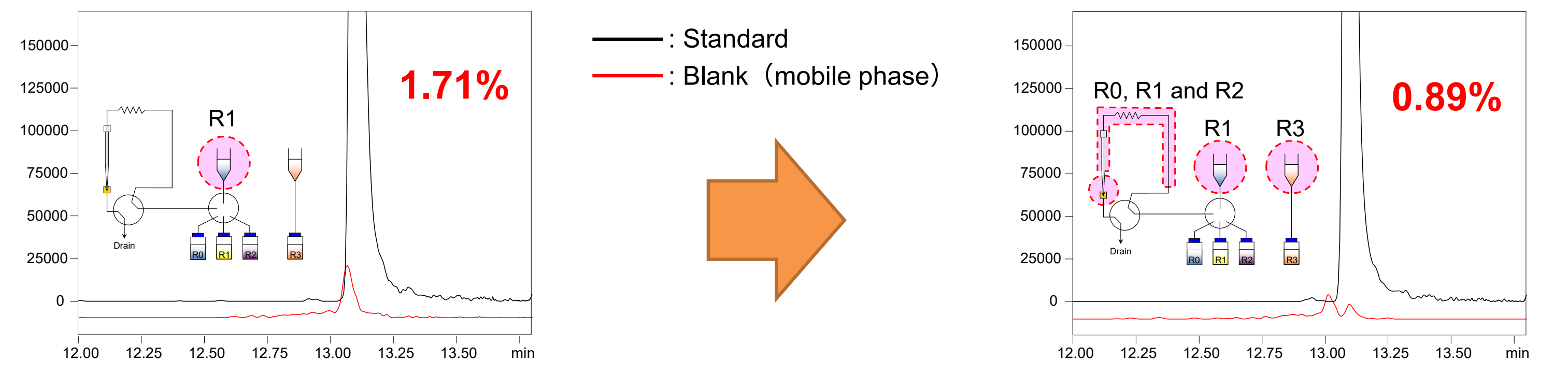


Rinse solutions
R0: Water
R1: 1% Formic acid / Methanol / Acetonitrile / Isopropanol = 1:1:1:1
R2: **20 mmol/L Sodium citrate aqueous solution**
R3: 1% Formic acid / Methanol / Acetonitrile / Isopropanol = 1:1:1:1

Fig. 4 Result of rinsing with chelating agent on the Nexera X3

3-3, Confirmation of suppression effect of metal adsorption by bioinert system

The Nexera XS inert was expected to improve carry-over caused by metal adsorption, because it consists of non-metal of the sample flow path. In order to confirm the effect of the inert system, an aqueous methanol solution was used for rinsing, and the external rinse mode was used. As a result, carry-over was 1.71%. It was a significant improvement over carry-over (11.9%) of the Nexera X3 SUS system under the same conditions, showing the benefits of using the inert system. Furthermore, by using the internal rinse mode without chelating agents, carry-over was reduced to 0.89%, and it was confirmed that carry-over due to metal adsorption can be greatly improved without using a special rinse solution by using the Nexera XS inert system. These results are shown in Fig. 5.



Rinse solution
R1: Water / Methanol = 1:1

Rinse solutions
R0, R1, R2, R3 = Water / Methanol = 1:1

Fig. 5 The Nexera XS inert results in reduced carry-over using a simple rinse solution

4, Conclusion

It was confirmed that rinsing only the outer surface of the needle is difficult to improve when the piping or injection port is the cause of the carryover. Since the injection port is also where the needle comes into contact, washing the injection port would have a significant effect on carry-over. In addition, in the SUS system, it was confirmed that the addition of a chelating agent to the rinse solution can significantly improve carry-over of metal coordination compounds. On the other hand, introducing chelating agents into the MS carries the risk of contamination. The Nexera XS inert, a bioinert UHPLC system, was found to suppress carry-over of metal coordinating compounds. Some metabolites and oligonucleotides are metal coordinating compounds, suggesting that the Nexera XS inert is highly effective in these analyses.

Carry-over occurs not only in the system but also in the column. The 0.89% (Fig. 5) may also include carry-over in the column. Therefore, when evaluating carry-over in more detail, it is necessary to correctly evaluate carry-over in the column.
