Improvement in Suppression of Carryover of Auto-sampler for High Sensitivity MS detector

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1. Introduction

As the sensitivity of the mass spectrometer has been greatly improved, the carryover of the sample remaining in the auto-sampler has become the most significant factor determining the quantitative performance of the whole LC-MSMS system. Meanwhile, as the demands arise for faster speed and higher resolution of the analysis, the higher-pressure HPLC has become available from many HPLC venders. However, users sometimes experience carryover problem in higher-pressure system that they have not experienced with conventional HPLC system pressure. We have studied how the carryover performance is affected by the system pressure, and in this paper we report how we can improve carryover in a higher-pressure application.

2. Improvement of Flow Line Structure

2.1 Improvement of Needle and Needle Seal

In the direct injection type auto-sampler, as the mobile phase flows through the interior of the sampling needle, sample sucked in the needle is sufficiently flushed by the mobile phase, but some samples may remain on the outer surface of the needle. We applied inactive metal coating and treatment to the needle surface for repellency to reduce the amount of residual compounds.

The shape of the sampling needle and needle seal have to be carefully designed so that the contact area and the dead space between the parts are minimized. This works not only for reducing the sample amount that remains in this portion, but it also increases the system pressure tolerance.

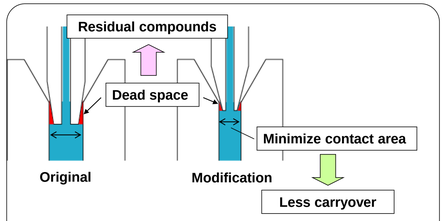


Fig. 1: Sample remaining around the needle tip

2.2 Improvement of High-Pressure Valve

The sealing surface of the high-pressure valve may also be a cause of the carryover problem. Especially when a higher pressure is applied, the samples tend to be penetrated into the dead space between the stator and rotor under higher pressure. With a conventional high-pressure valve, we observed 0.002% carryover with a caffeine sample (250 mg/L) at a backpressure of 45 MPa, while no carryover was detected at 27 MPa. We redesigned the components in the high-pressure valve's flow path so that the dead space in the sealing surface is minimized. As a result, no carryover was observed even at 74 MPa (Fig. 2, Fig. 3).

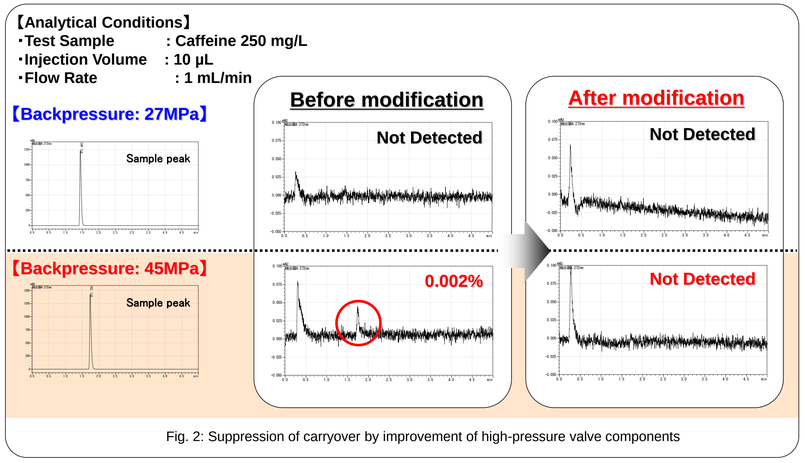


Fig. 2: Suppression of carryover by improvement of high-pressure valve components

2.3 Evaluation of Carryover at High Pressures

With the redesigned needle, needle seal and high-pressure valve described above, we performed experiments at several backpressures (46MPa, 66MPa, and 74MPa) to verify the improvements at higher pressures. Carryover from the caffeine sample (500mg/L) was not detected at either 66MPa or 74 MPa even without rinsing the outer surface of the needle. This result indicates that the redesigned flow line components are effective to suppress carryover under high-pressure conditions (Fig. 3).

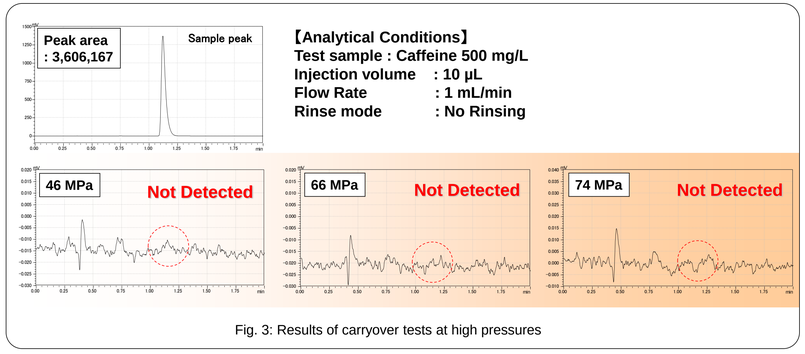
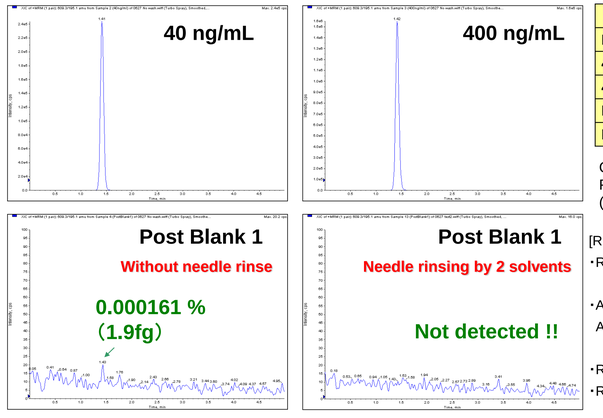


Fig. 3: Results of carryover tests at high pressures

3. Carryover Evaluation for MS Front-end HPLC System

Carryover for reserpine was tested using the modified auto-sampler with a tandem mass spectrometer. Only 0.000161% of carryover was detected, even with no rinsing mode. Moreover, no carryover was observed when the optional needle rinsing was used with an additional solvent. These results indicate that the modification applied to the auto-sampler has an excellent effect in terms of reducing carryover, and the auto-sampler equipped with this modification is well-suited for a front-end HPLC system connected to a high-sensitivity mass spectrometer.

【Analytical Conditions】
・Test Sample : Reserpine
・Mobile Phase : A：0.05% formic acid, B：Acetonitrile（A / B=6 / 4）
・Flow Rate : 0.5 mL/min
・Column : UNISON UKC18 2 mm i.d. x 50 mm L 3 µm
・Evaluation Method : 40 ng/mL reserpine injection ⇒ 400 ng/mL reserpine injection ⇒ blank injection
・Injection Volume : 3 µL
・Detection : Applied Biosystems API-5000
・Scan Type : Multiple Reaction Monitoring



	Peak area
Blank	0.0
40ng/mL	1122039.4
400ng/mL	7247763.5
PostBlank1	18.030345
PostBlank2	0.0

Carryover (%) =
Post blank peak area ÷
(peak area of 40 ng/ml x 10) x 100

[Rinse Setting]
・Rinse Solvent: Acetonitrile
(for immersion rinsing)
・Additional Rinse Solvents:
Acetonitrile / Isopropanol = 8 / 2
containing 0.5% formic acid
・Rinse Mode: Before and after aspiration
・Rinse Method:
Pump rinsing followed by immersion

Fig. 4: Carryover evaluation with a high-sensitivity tandem mass spectrometer

4. Conclusion

The direct injection type of auto-sampler delivers, by nature, lower carryover. However, when it is operated under higher pressure, some consideration should be applied to the design of fluidic components, such as the needle, needle seal and high-pressure valve, in order to keep its lower carryover nature..