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Technical Report

Improving Method Transfer by Adjustment of Gradient Delay

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Abstract:

In facilities that have number of high performance liquid chromatography (HPLC) systems, an existing method that gives proper result by one HPLC system is often applied to other HPLC systems (method transfer). However, due to the difference in system volume, pump characteristics, and liquid delivery mechanisms among systems, method transfer can yield different results even though the same method is used.

In this report, we first explain the gradient delay due to the system volume difference and the effect of the delay on separation. We then show an example of method transfer with adjustment of gradient delay using alkylphenones and UV absorbents as samples. We also describe the integrated liquid chromatograph (LC) system "i-Series" that supports to optimize method transfer and the newly designed function of "Analytical Conditions Transfer and Optimization (ACTO) " equipped in the latest version of LabSolutions software.

Keywords: HPLC, method transfer, ACTO, i-Series

1. Background

HPLC is used for the analysis of target compounds and their related impurities in a variety of applications including pharmaceutical and food products. Facilities that use HPLC systems create methods using their own original analytical conditions and/or specified testing regulations. The validated methods are then used with a number of other HPLC systems in many cases. In such situations, reproducibility (compatibility) among systems is an important factor as well as repeatability of measurements. Even when using the same method, different HPLC systems can give different chromatograms (Fig. 1). Particularly in gradient elution, retention times, resolution and other factors will be largely affected as a result of method transfer. For example, while an existing method may succeed in separating a target compound from co-existing impurities in one system, the same method may not succeed in separating these compounds in other systems. So it is often required to optimize analytical conditions for each individual system, which is an extremely time-consuming process. Such variations in retention time and separation are caused by difference in system volume and pump performance among systems (*see* section 2 for details). Especially in ultrahigh-speed analysis, even small difference in system volume can cause great difference in analysis results due to small volume of dedicated column.

Further, in pharmaceuticals, food and other fields where the test methods are specified by regulations, changes in analytical conditions are not permitted, which may be an issue.

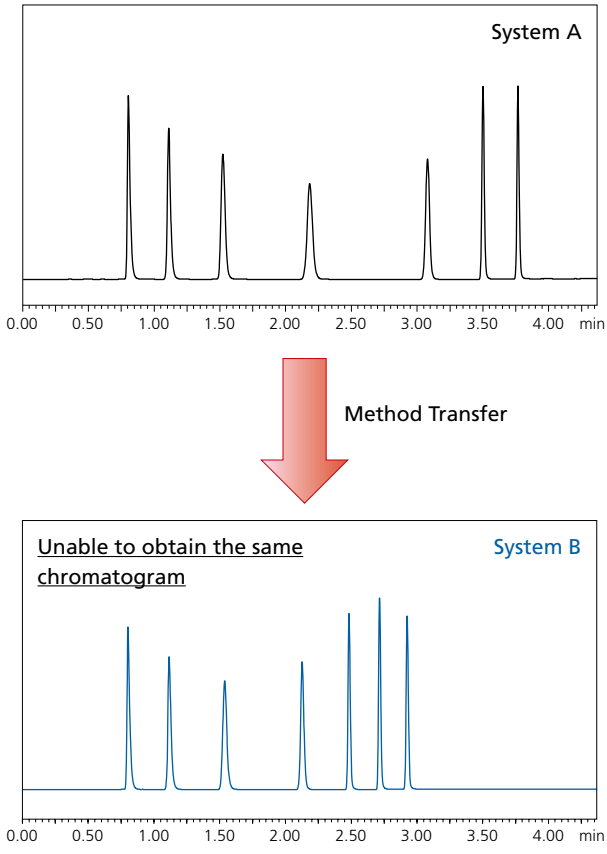


Fig. 1 Problems in Method Transfer

1 Analytical & Measuring Instruments Division

2. System Volume and Gradient Delay

System volume differences must be considered when transferring a method from one system to other systems.

Fig. 2 shows the flow line from the mobile phase reservoir to the column of LC system. Gradient delay volume means the system volume between the point where two or more eluents are mixed and the column inlet. As shown in Fig. 2, the gradient delay volumes are different for low-pressure gradient and high-pressure gradient systems. Even for the same type of gradient system, different lengths and/or internal diameters of piping can provide different gradient delay volumes.

Fig. 3 shows how gradient delay affects separation. In general, even if gradient has already started on the time program, the actual gradient start time (time to increase an organic solvent concentration) is delayed. If the result obtained from a system with a large system volume (Fig. 3, right) is compared to that from a system with a small system volume (Fig. 3, left), we can see the gradient start time is delayed more. This can cause different separation patterns on different systems.

Consequently, system volume difference must be considered when transferring a method and the gradient program must be modified by making an adjustment to the initial hold time (gradient start time). Nevertheless, gradient programs cannot be modified when the analytical conditions are strictly defined by the testing regulations.

3. Correction of Gradient Delay

We have discussed differences in chromatograms caused by method transfer and the origin of these differences. Next, we describe an example analysis and method transfer using multiple LC systems that have different system volumes.

3-1. Alkylphenone Analysis

Alkylphenones are aromatic ketones that can be analyzed by reversed phase chromatography. We analyzed alkylphenones using two systems of different system volumes and compared the chromatograms obtained from each system. We then adjusted the difference in retention time between the two systems by adding an initial hold time.

The results are shown in Fig. 4. A comparison of the two chromatograms (Fig. 4, top and middle) shows differences between the peaks in the latter half of the chromatograms. These chromatograms were obtained from analyses performed under identical conditions but different systems. The system volumes of system 1 and 2 are 1155 µL, 505 µL, respectively. Therefore, there is a difference of 650 µL between the two systems. This difference in the system volumes leads to different separation. The chromatogram at the bottom of Fig. 4 was obtained via an analysis using system 2, after adding an initial hold time equivalent to 650 µL. This chromatogram is almost identical to that obtained from system 1; this confirms that equivalent results can be obtained from different systems by correcting for system volume difference.

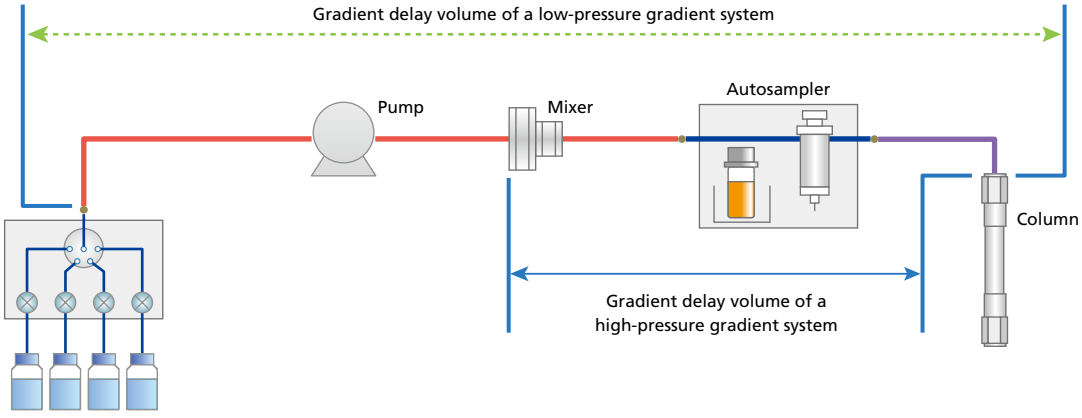


Fig. 2 Gradient Delay Volumes (System Volumes)

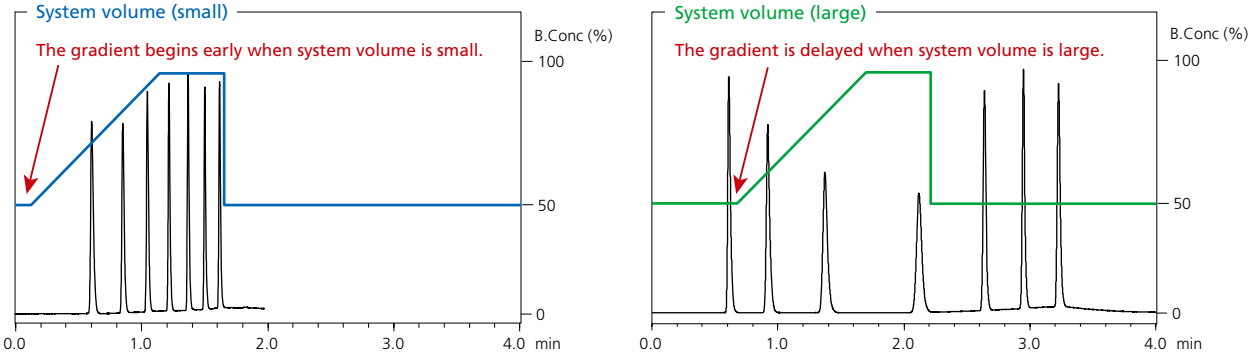
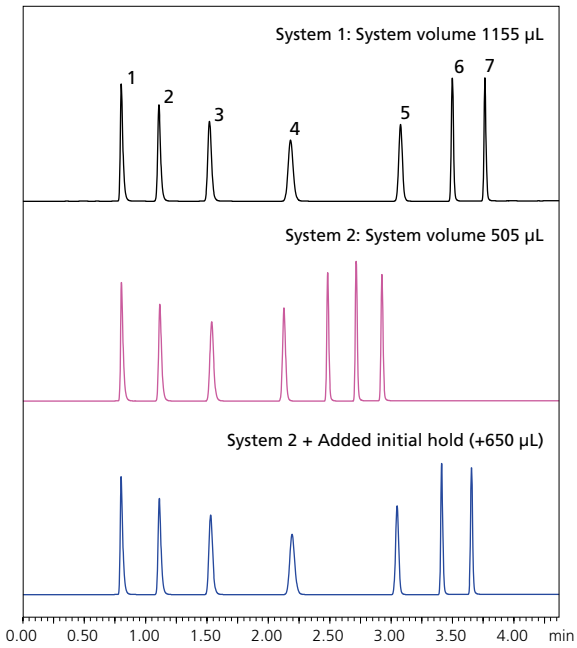


Fig. 3 System Volume and Gradient Delay



■ Peaks
1. Acetophenone, 2. Propiophenone, 3. Butyrophenone, 4. Valerophenone, 5. Hexanophenone, 6. Heptanophenone, 7. Octanophenone

■ Analytical Conditions

Column	: Shim-pack XR-ODS II (75 mm L. × 3.0 mm I.D., 2.2 µm)
Mobile phase A	: Water
Mobile phase B	: Acetonitrile
Initial concentration	: 60% B
Flow rate	: 0.8 mL/min
Temp.	: 40 °C
Detection	: Photodiode array absorbance detector
Injection volume	: 10 µL

Time program

Time	Unit	Func.	Value
0.93	Pump	B.Conc	60
1.59	Pump	B.Conc	95
3.50	Pump	B.Conc	95
3.51	Pump	B.Conc	60
6.00	Controller		Stop

Fig. 4 Different Retention Times Due to Different System Volumes and Their Correction by Addition of an Initial Hold Time (Alkylphenones)

3-2. Analysis of UV Absorbents

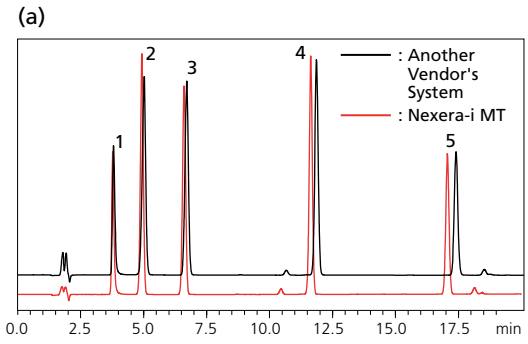
In this section, we describe an example method transfer to a Shimadzu system from another vendor's HPLC system using a sunscreen mixture.

Fig. 5a shows the chromatogram obtained from the analysis of a sunscreen mixture using Shimadzu's Nexera-i MT and another vendor's system. Although these two analyses were performed using identical methods, a huge difference in the peaks after 10 min is observed. This difference is caused by the difference in system volumes of the two systems and is similar to the analysis described earlier in this report.

Using ACTO's gradient start time adjustment function equipped in the Shimadzu LabSolutions workstation software, we adjusted the gradient start time correct the difference in system volumes and performed the analysis. As seen from Fig. 5b and the inset table in Fig. 5, the retention times were almost identical for all peaks.

Using this approach, compatibility between Shimadzu system and other vendors' system can be achieved by adjusting the gradient start time. This means that an adjustment in the gradient start time enables smoother method transfer.

The United States Pharmacopeia (USP) provides the following description to correct for errors between systems:2) "If adjustments are necessary, change in column packing (maintaining the same chemistry), the duration of an initial isocratic hold (when prescribed), and/or dwell volume adjustments are allowed." In other words, the adjustment of initial hold time (or gradient start time adjustment) does not fall under method change, and therefore, does not require revalidation.



■ Peaks
1 to 5: Sunscreen mixture

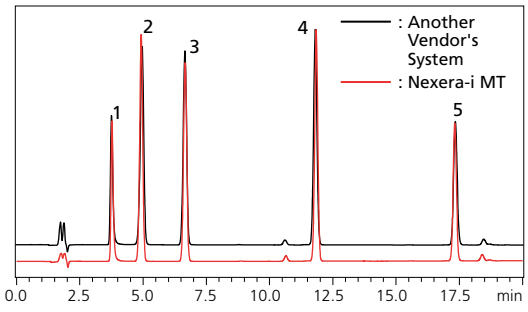
■ Analytical Conditions

Column	: Shim-pack VP-ODS (150 mm L. × 4.6 mm I.D., 5.0 µm)
Mobile phase A	: 20 mmol/L (Sodium) phosphate buffer solution (pH 2.5)
Mobile phase B	: Acetonitrile
Initial concentration	: 30% B
Flow rate	: 1.0 mL/min
Temp.	: 60 °C
Detection	: Photodiode array absorbance detector
Injection volume	: 10 µL
Flow line used	: HPLC flow line (Nexera-i MT)

Time program

Time	Unit	Func.	Value
2.00	Pump	B.Conc	30
25.00	Pump	B.Conc	70
25.10	Pump	B.Conc	30
32.00	Pump	B.Conc	30
35.00	Controller		Stop

Adjustment of gradient start time with ACTO



■ Analytical Conditions (changes only)
Gradient adjustment : +340 µL (Nexera-i MT)

Retention Time Error (%) Compared to Another Vendor's System

Component	Before gradient adjustment	After gradient adjustment
1	0.29	0.32
2	1.16	1.26
3	1.03	0.16
4	1.38	–0.38
5	1.46	–0.05

Fig. 5 Example Method Transfer with Gradient Adjustment (Sunscreen mixture)

4. i-Series Integrated Liquid Chromatograph System and the ACTO Function

This report has described examples of retention time differences caused by different system volume and adjustment of the initial hold time (adjusting the gradient start time). We now describe the Shimadzu i-Series integrated liquid chromatograph system and the ACTO function equipped in the Shimadzu LabSolutions workstation software that supports a variety of method transfers.

4-1. i-Series Integrated Liquid Chromatograph System

The i-Series is Shimadzu's product line of integrated liquid chromatographs that contain all the functions required for LC analysis in a compact unit. These functions have been optimized for ease of operation and maintenance. Using standard piping or attaching the optional compatibility kit enables the use of i-Series systems with system volumes compatible with other Shimadzu systems and other vendors' systems. This provides good reproducibility between systems when performing analyses using existing methods.

Shimadzu's workstation software also includes the ACTO function, as mentioned earlier, which is designed specifically for the i-Series and enables smooth method transfer.

Fig. 6 i-Series Integrated LC System (Nexera-i)

4-2. ACTO Function

ACTO, which is equipped in the latest version of LabSolutions, is an efficient method transfer tool provided by Shimadzu. Here we describe one of ACTO's functions called "gradient start time adjustment function."

Transferring an analytical method from an existing LC system to another system can cause differences in retention times because of the differences in system volume and specifications of solvent delivery unit. This problem can be resolved using ACTO's gradient start time adjustment function. The gradient adjustment function is configured during method creation. If a user simply enters the difference in system volume, then the corrected initial hold time is automatically added or subtracted during analysis. This enables the acquisition of identical chromatograms before and after method transfer. The function can also correct subtle errors that cannot be considered by the compatibility kit (e.g., pump characteristics and solvent delivery mechanism) and can achieve optimal compatibility. This adjustment is configured in a method separately from the time program. Thus, reconfiguration of an existing time program is unnecessary.

Consequently, using Shimadzu's i-Series instruments and the ACTO function can provide higher efficiency and reliability during method transfer in a variety of applications.

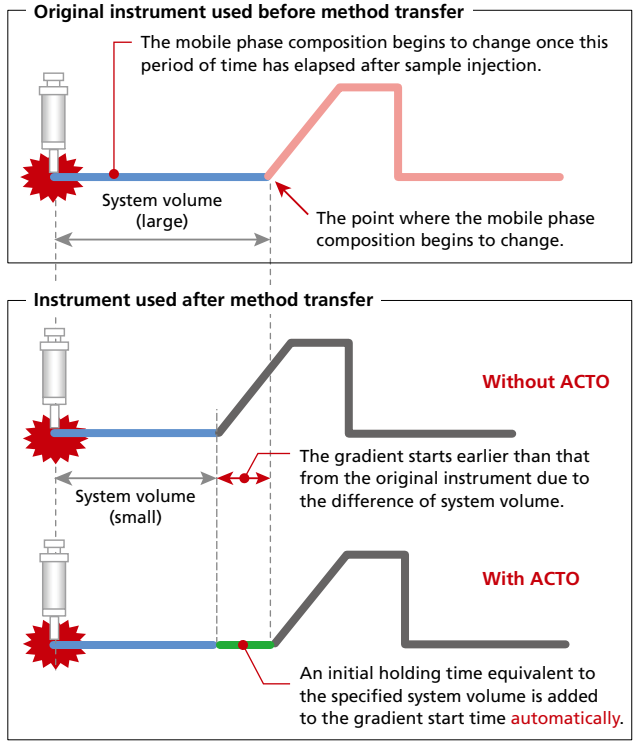


Fig. 7 Adjusting the Gradient Start Time

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