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Technical Report

Simple Method Transfer using i-Series (LC-2050/LC-2060)

Daiki Fujimura[1], Keiko Matsumoto[1]

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. Therefore, as a solution for such compatibility problems, Shimadzu offers new i-Series integrated LC systems (LC-2050/LC-2060) that are compatible with a wide variety of systems and system volumes.

This Technical Report describes an example of using an i-Series system to transfer a method by correcting for the system gradient delay volume. It also describes an actual example of using the analytical condition transfer and optimization (ACTO) function to seamlessly transfer a method.

Keywords: 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 time, 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.

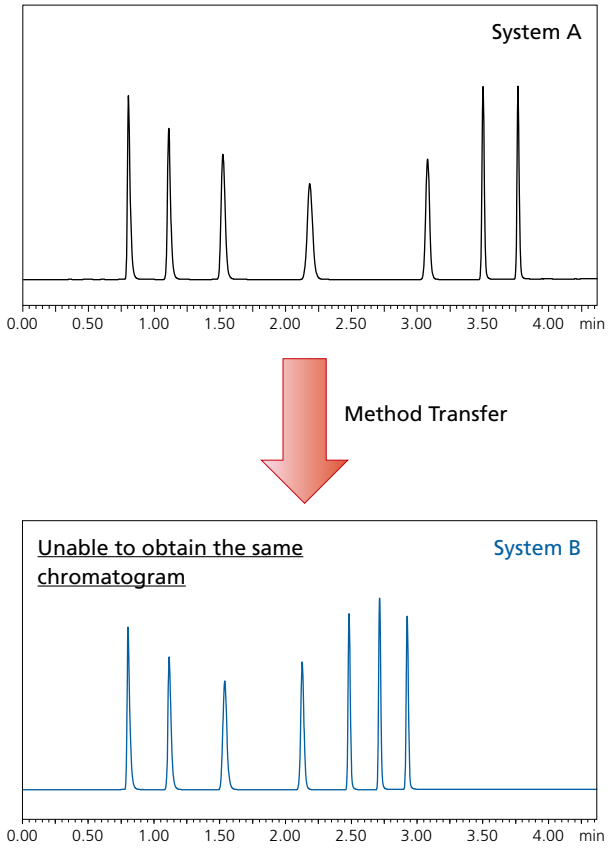


Fig. 1 Problems in Method Transfer

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. Fig. 3 shows how the gradient in a system with a larger system volume (lower chromatogram) starts later than in a system with a smaller system volume (upper chromatogram). 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.

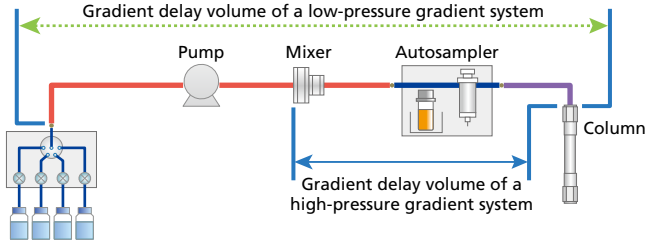


Fig. 2 Gradient Delay Volumes (System Volumes)

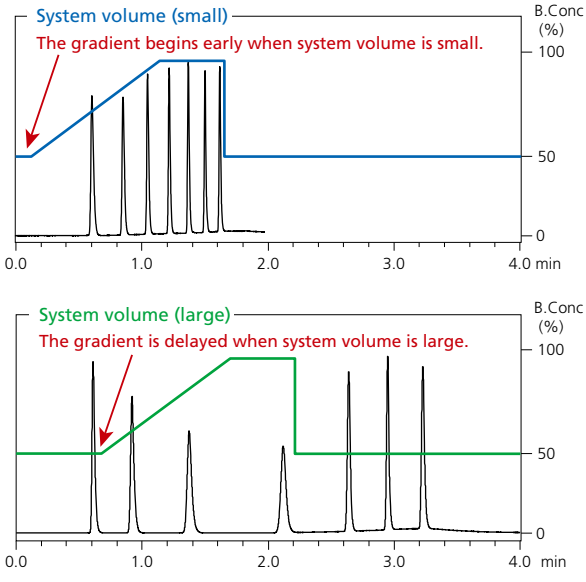


Fig. 3 Gradient Delay Volumes (System Volumes)

3. Correction of Gradient Delay

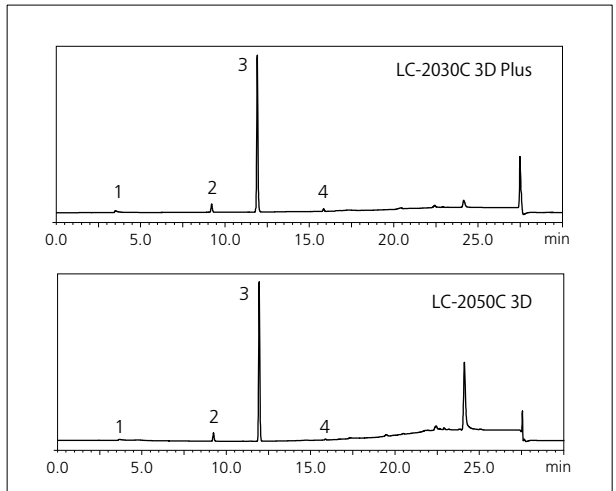
We have discussed differences in chromatograms caused by method transfer and the origin of these differences. Next, we describe an example analysis and method transfer using multiple LC systems that have different system volumes.

3-1. Example of Method Transfer with Corrected System Delay Volume

In this example, a Shimadzu LC-2050 integrated LC system was used to match the delay volume to a variety of systems and then compare chromatograms from performing the identical analysis with both systems.

First, chromatograms were compared with a previous Shimadzu LC-2030 Plus model. The results are shown in Fig. 4. That comparison confirms that given the same analytical conditions, the LC-2030 Plus, which was designed to have an equivalent system volume as the LC-2050, produces an identical chromatogram. Next, chromatograms were compared with an other vendors' LC system and a first-generation Shimadzu LC-2010C HT integrated LC system. The results are shown in Fig. 5. In this example, the system volumes of the other vendors' LC system (System A) and the LC-2050 were equivalent, so given identical analytical conditions, they produced equivalent results. However, the LC-2010C HT system has a larger system volume than the LC-2050, so a delay volume compatibility kit was added to the LC-2050 to match the system volume of both systems. That resulted in almost the same chromatogram pattern, just as in the previous case.

Thus, when transferring methods, it is important to match the system volumes so that the gradient starts at the same time.



■ Peaks
1. Salicylic acid, 2. Salicylamide, 3. Ethenzamide, 4. Methyl salicylate

■ Analytical Conditions

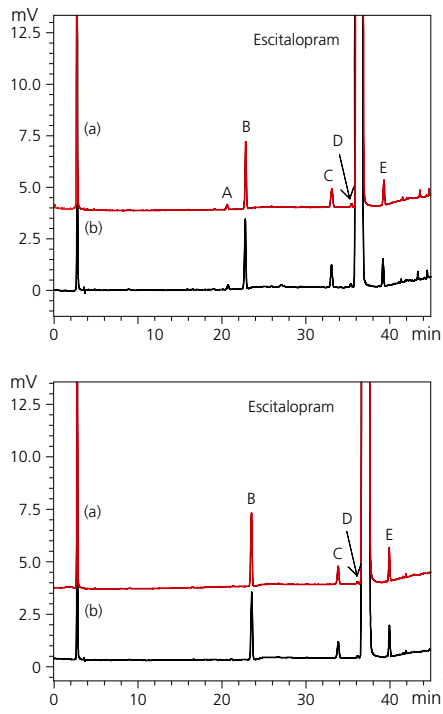
Column : Shim-pack™ GIST (150 mm L. × 4.6 mm I.D., 5 µm)

Mobile phase A	: Water
Mobile phase B	: Methanol
Initial concentration	: A/B = 90/10
Flow rate	: 1.0 mL/min
Temp.	: 40 °C
Detector	: Photodiode array absorbance detector
Injection volume	: 5 µL

Time program:

Time	Unit	Func	Value
2.00	Pump	B.Conc	10
20.0	Pump	B.Conc	95
25.0	Pump	B.Conc	95
25.1	Pump	B.Conc	10
30.0	Controller		Stop

Fig. 4 Example of Method Transfer between LC-2030 Plus and LC-2050 Systems



■ Analytical Conditions

Column	: Shim-pack VP-ODS (250 mm L. × 4.6 mm, 5 µm)
Mobile phase A	: Acetonitrile / 25 mM phosphate buffer (pH 3.0) = 1/9 (v/v)
Mobile phase B	: Acetonitrile / 25 mM phosphate buffer (pH 3.0) = 13/7 (v/v)
Initial concentration	: A/B = 95/5
Flow rate	: 1.0 mL/min (0–45 min), 2.0 mL/min (45–60 min)
Temp.	: 45 °C
Detection	: UV 237 nm
Injection volume	: 20 µL

Time program:

Time	Unit	Func	Value
0.00	Pump	B.Conc	5
35.00	Pump	B.Conc	35
45.00	Pump	B.Conc	100
60.00	Pump	B.Conc	100
60.01	Pump	B.Conc	5
68.00	Controller	Stop	

Fig. 5 Example of Method Transfer by Correction of Delay Volume of System

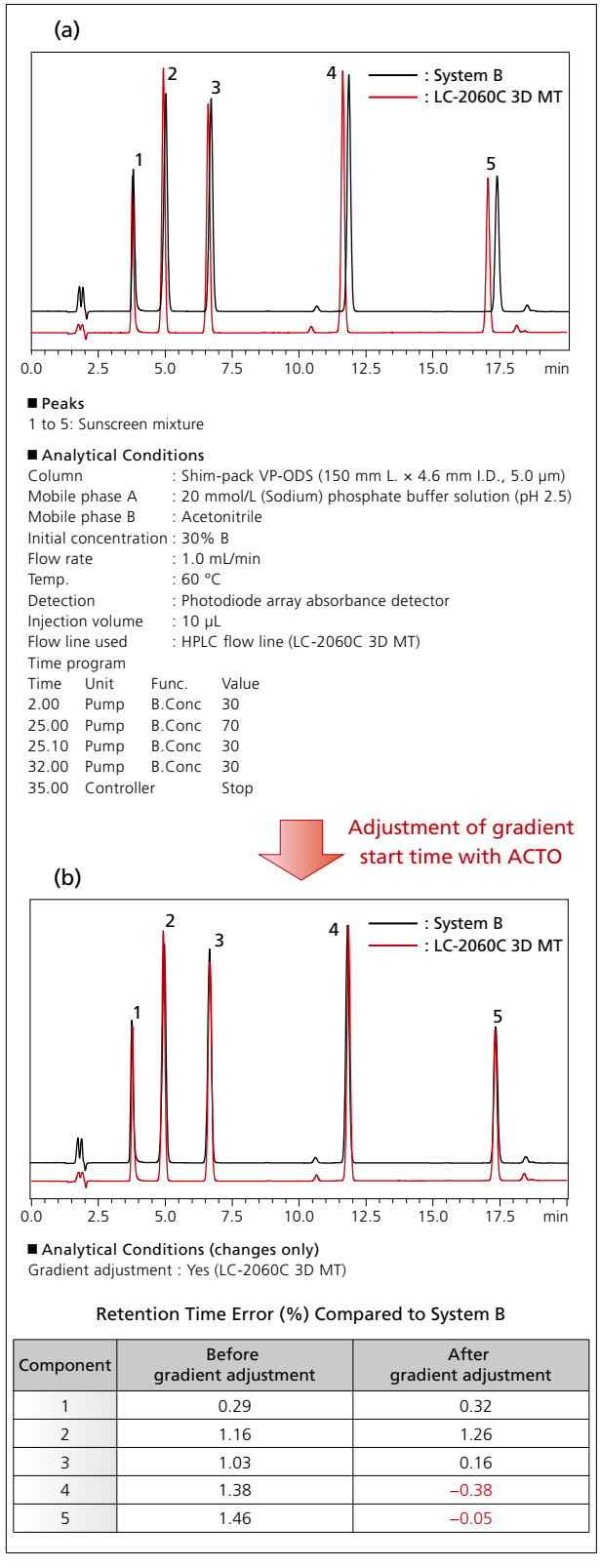
3-2. Method Transfer using ACTO Function

In this example, software functionality was used to match system delay volumes for method transfer, rather than by changing mixers or tubing. Fig. 6 (a) was obtained by analyzing a sunscreen mixture sample with a Shimadzu LC-2060C 3D MT system and a different other vendors' LC system (System B). Of the two flow lines available in the LC-2060C 3D MT system, the HPLC flow line was used.

Though the same method was used for both analyses, the peaks after 10 minutes do not match. 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. 6 (b) and the inset table in Fig. 6, 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. Note that adjusting the initial hold time is permitted by the respective pharmacopoeias, is not considered to be changing the method, and 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 (LC-2060C 3D 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

■ Analytical Conditions (changes only)
Gradient adjustment : Yes (LC-2060C 3D MT)

Retention Time Error (%) Compared to System B

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. 6 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 (LC-2050/LC-2060) 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. 7 i-Series Integrated LC System

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.

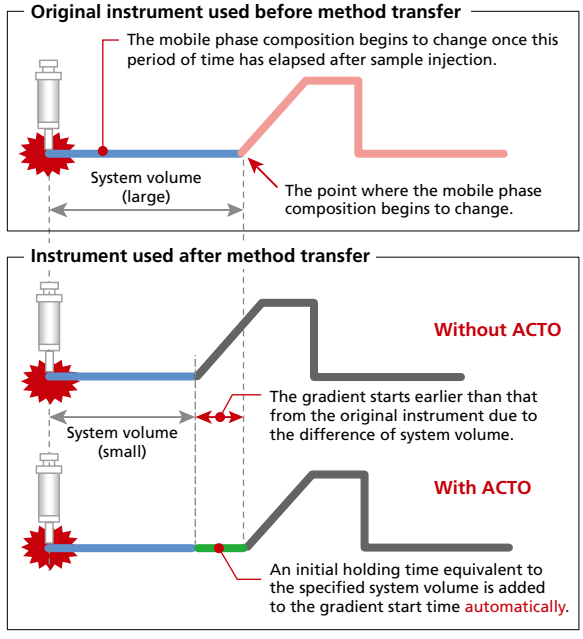


Fig. 8 Adjusting the Gradient Start Time

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