Seamless HPLC method transfer

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HPLC is used for the analysis of target compounds and their related impurities in a variety of applications, and methods are created under own original analytical conditions and/or specified testing regulations. If these validated methods are used with several different HPLC instruments, the reproducibility (comparability) among systems is an important factor as well as repeatability of measurements.

Particularly the gradient elution, retention time, resolution and other factors will be largely affected due to the 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 units.

So, it is often required to optimise analytical conditions for each instrument, which is an extremely time-consuming process. Such variations in retention time and separation are caused by diverse system volumes and pump performances among units. Especially in ultra-high speed analysis, even a small difference in system volume can cause big effects in analysis results due to a small volume of the dedicated column.

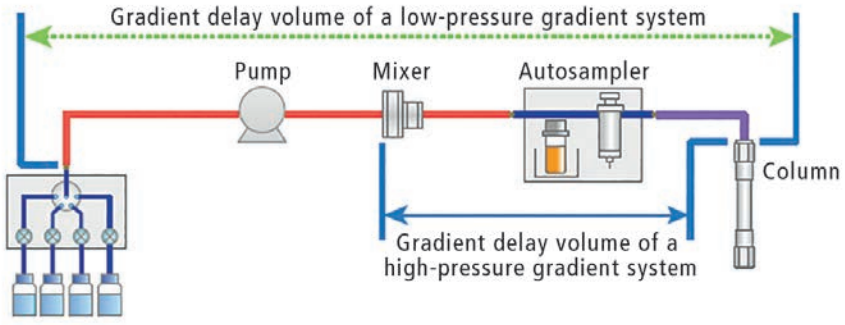


Figure 1: Gradient delay volume.

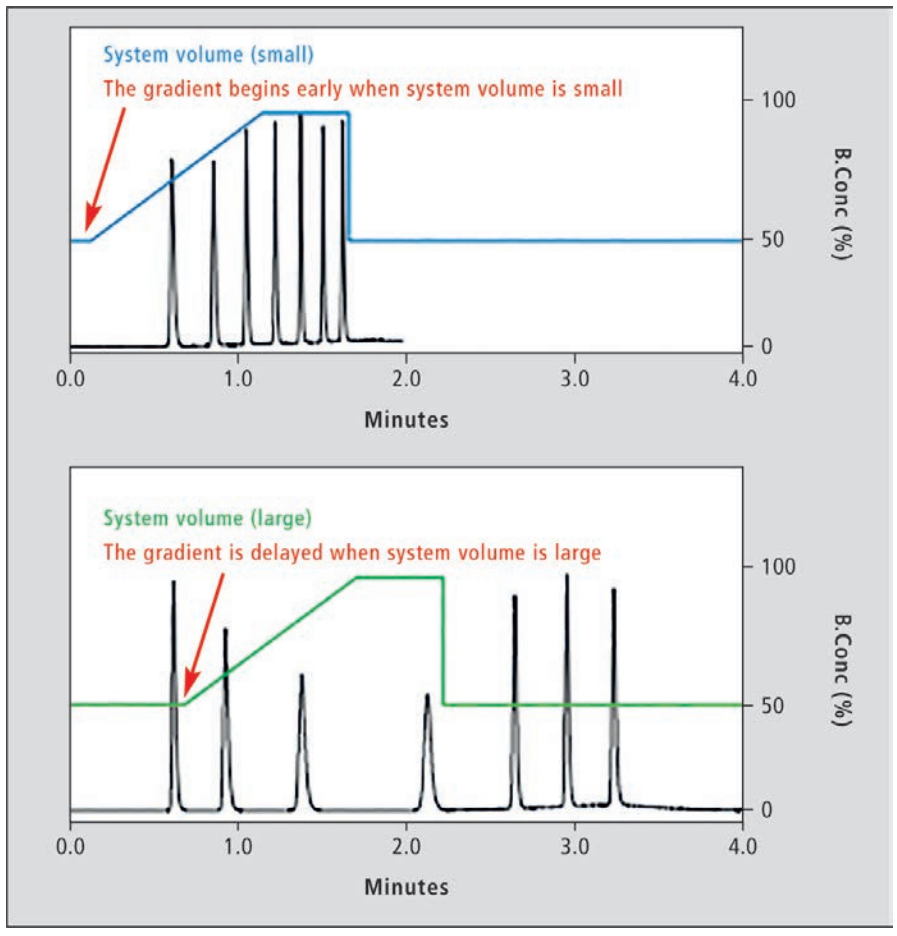
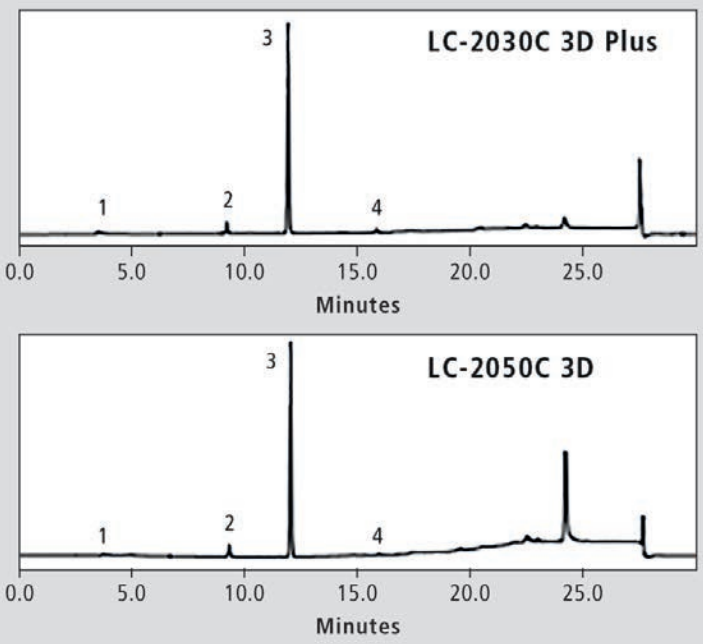


Figure 2: Gradient delay volumes (system volumes).

System volume and gradient delay

System volume differences must be considered when transferring a method from one LC unit to another. *Figure 1* shows the flow line from the mobile phase reservoir to the column of the LC instrument. Gradient delay volume refers to system volume between the point where two or more eluents are mixed, and the column inlet. As shown in *Figure 1*, gradient delay volumes are different for low-and-high-pressure gradient systems. Even for the same type, different lengths and/or internal diameters of piping can provide different gradient delay volumes.

Figure 2 shows how the gradient delay can affect separation. In general, even if the



■ **Peaks**

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

■ **Analytical Conditions**

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

Mobile phase A	: Water
Mobile phase B	: Methanol
Initial concen.	: A/B = 90/10
Flow rate	: 1.0 mL/min
Temperature	: 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	

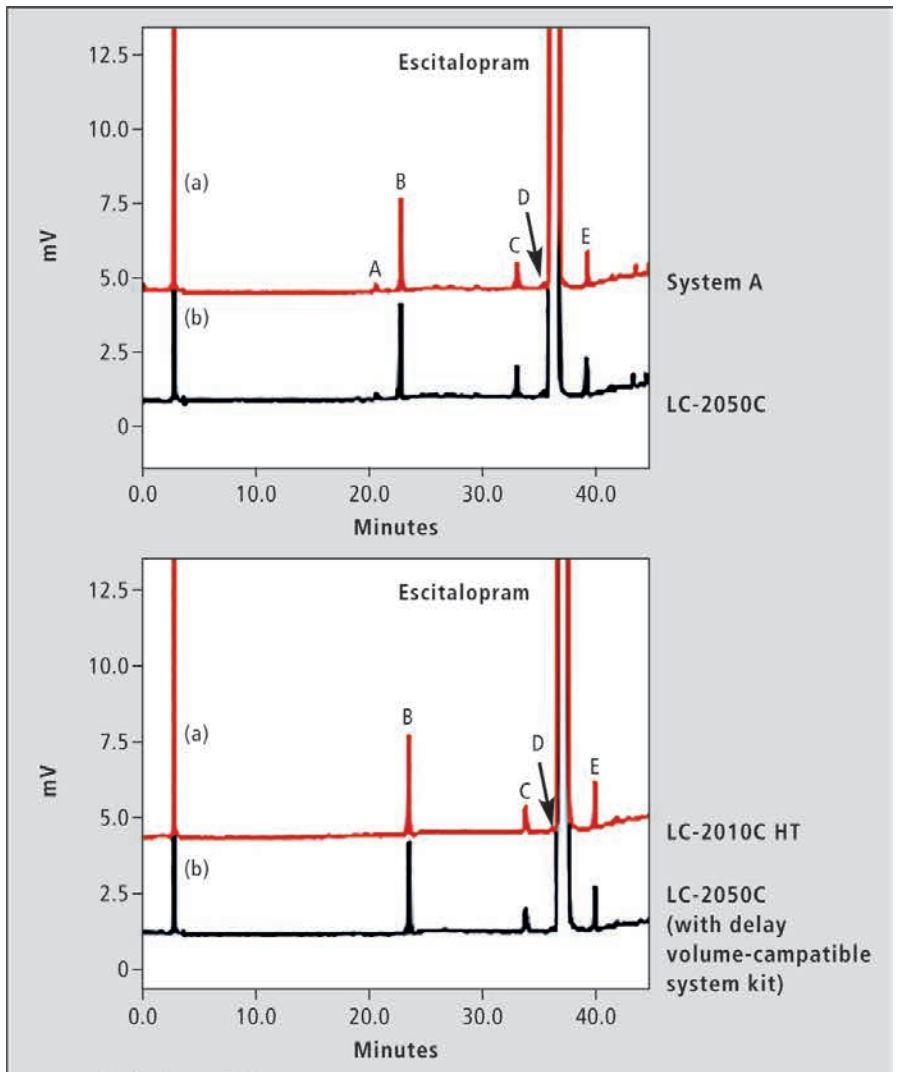
Figure 3: Example of method transfer between LC-2030 Plus and LC-2050 systems.

gradient has already started on the time program, its actual start time (time to increase an organic solvent concentration) is delayed. *Figure 3* shows how the gradient in a unit with a larger system volume (lower chromatogram) starts later than in an instrument with a smaller system volume (upper chromatogram). This can cause different separation patterns on diverse HPLCs. 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, these programs cannot just be adapted when the analytical conditions are strictly defined by the testing regulations.

Example of method transfer with corrected system delay volume

After discussing the differences in chromatograms caused by method transfer and their origin, the next part describes an example of analysis and method transfer using multiple LCs with different system volumes. A Shimadzu LC-2050 integrated LC unit was used to match the delay volume to a variety of instruments and then to compare chromatograms by performing identical analysis with different LCs.

The first part compares chromatograms with a previous Shimadzu LC-2030 Plus model. Results are shown in *Figure 3*. This comparison confirms that, given the same analytical conditions, the LC-2030 Plus, designed to have a system volume equivalent to the LC-2050, produces an identical chromatogram.



■ **Analytical Conditions**

Column : Shim-pack™ VP-ODS (250 mm L. x 4.6 mm, I.D., 5 µm)
Mobile phase A : Acetonitrile / 25 mM posphate buffer (pH 3.0) – 1/9 (v/v)
Mobile phase B : Acetonitrile / 25 mM posphate buffer (pH 3.0) – 13/7 (v/v)
Initial concen. : A/B = 95/5
Flow rate : 1.0 mL/min (0 - 45 min), 2.0 mL/min (0 - 60 min)
Temperature : 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	

Figure 4: Example of method transfer by correction of delay volume of the system.

The next chromatograms were compared with another vendor's LC system and a first-generation Shimadzu LC-2010C HT integrated LC. Results are shown in *Figure 4*. In this example, system volumes of the other vendor's LCs (System A) and the LC-2050 were equivalent, so given identical analytical conditions, they produced equivalent results.

However, compared to the LC-2050, the LC-2010C HT has a larger system volume, so a delay volume compatibility kit was added to the LC-2050 to match the system volume. This 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.

Analytical Condition Transfer and Optimisation function

Analytical Condition Transfer and Optimization (ACTO) is an efficient method transfer tool and part of the latest version of Shimadzu's LabSolutions software. One of ACTO's functions is called 'gradient start time adjustment function'.

Transferring an analytical method from an existing LC instrument to another unit 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 as shown in *Figure 5*.

The gradient adjustment function is configured during method creation. If a user simply enters the difference in system volume, the corrected initial hold time is then added or subtracted automatically during analysis. This enables the acquisition of identical chromatograms before and after the 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 separately from the time program. Reconfiguration of an existing time program is therefore unnecessary. Consequently, Shimadzu's i-Series instruments and the ACTO function can provide higher efficiency and reliability during method transfer in a variety of applications.

Method transfer using ACTO function

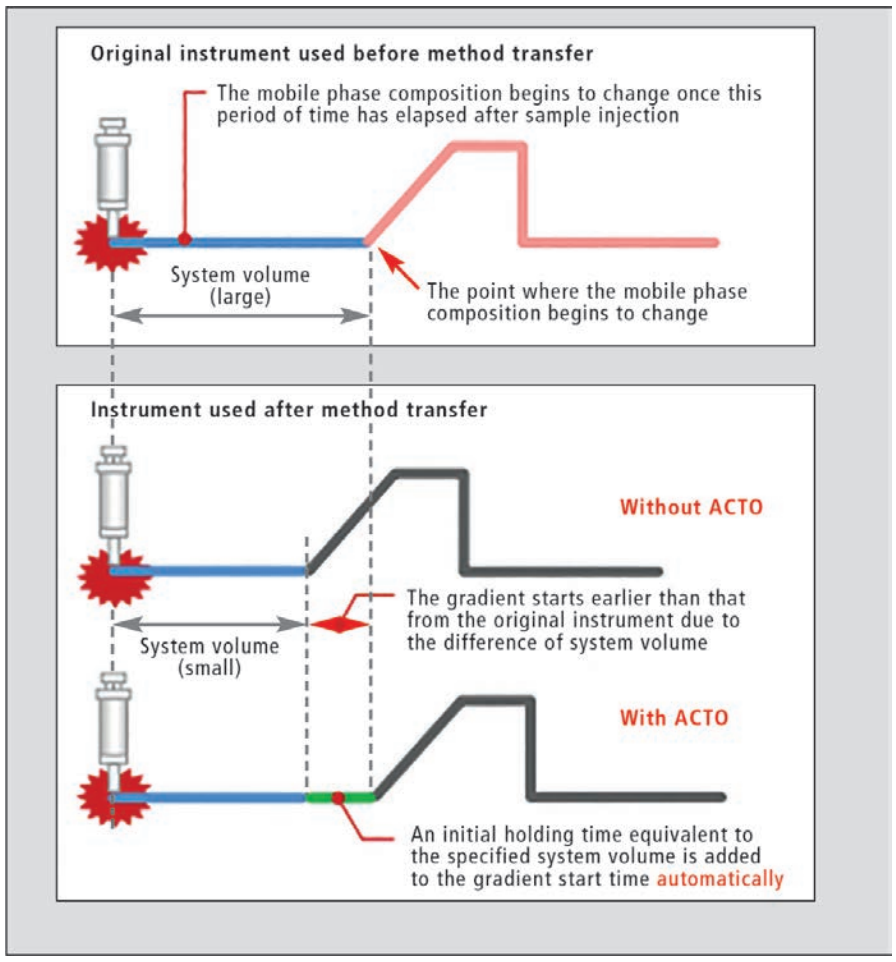
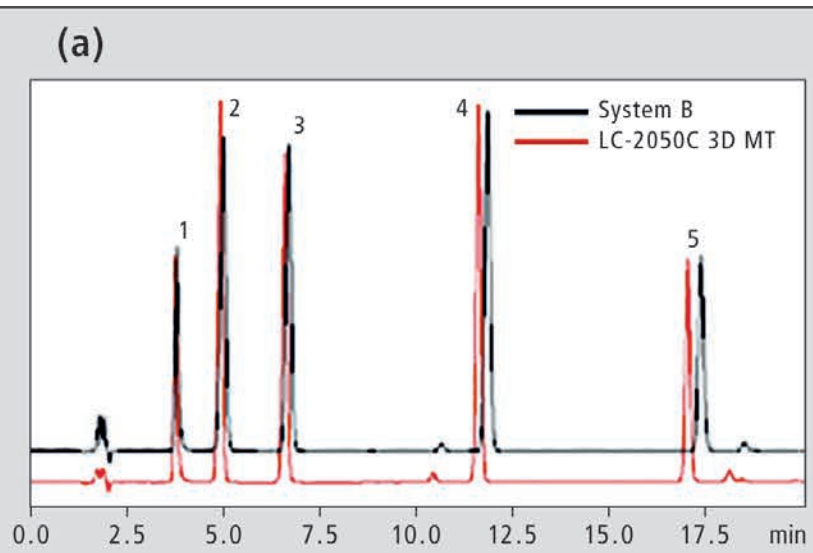


Figure 5: Adjusting the gradient start time.

In this example, the LabSolutions workstation software functionality was used to match system delay volumes for method transfer, rather than by changing mixers or tubing. In *Figure 6*, a sunscreen mixture sample was analysed with a Shimadzu LC-2060C 3D MT and a different vendor's LC instrument (System B).

Of the two flow lines available in the LC-2060C 3D MT (HPLC and UHPLC flow line), the HPLC flow line was used. Although the same method was applied on both units, the peaks after 10 minutes did not match, because of difference in system volumes of the two instruments. Using ACTO's gradient start time adjustment function from the



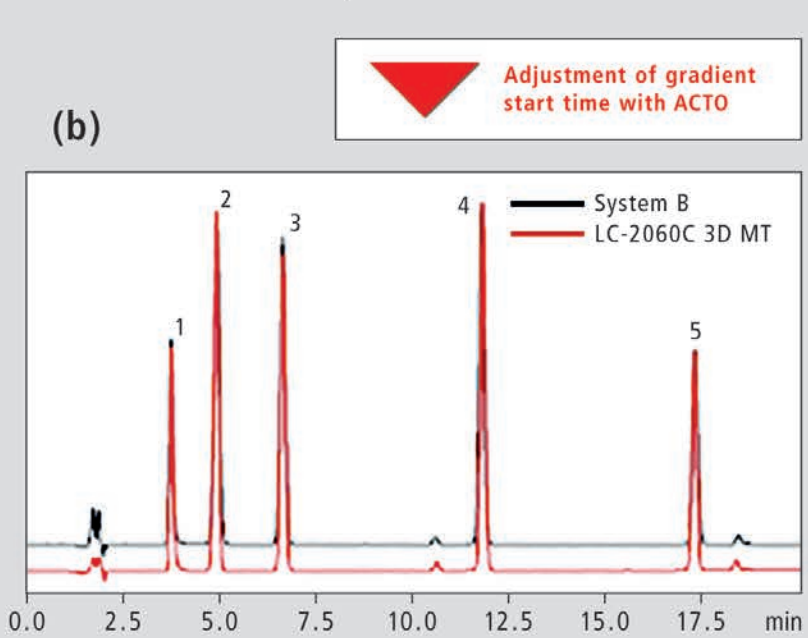
■ **Peaks**
1 to 5 Sunscreen mixture

■ **Analytical conditions**

Column	Shim-pack VP-ODS (150 mm L. x 4.6 mm I.D., 5.0 µm)
Mobile phase A	20 mmol/L (sodium) phosphate buffer solution (pH 2.5)
Mobile phase B	Actonitrile
Initial conc.	30 % B
Flow rate	1.0 mL/min
Temperature	60 °C
Detection	Photodiode array absorbence detector
Injection volume	10 µL
Flow line used	HPLC flow line (LC-2050C 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	B.Conc.	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	0.26
3	1.03	0.16
4	1.38	- 0.38
5	1.46	- 0.05

Figure 6: Example for method transfer with gradient adjustment (sunscreen mixture).

software, the gradient start time was adapted to overcome the difference in system volumes and performed the analysis.

After this and as seen in the table at the end of *Figure 6*, retention times were almost identical for all peaks. Using this approach, compatibility of two different systems could be achieved by setting the gradient start time, and this enabled smoother method transfer. Note that adjusting of initial hold time is permitted by the respective pharmacopoeias, and is not considered as changing the method, so does not normally require a revalidation.

Conclusion

The examples of retention time differences caused by different system volumes shown can be overcome easily with adjustment of initial hold time (adapting the gradient start time) or by changing the hardware to achieve the same system volume. Support of easy method transfer can be done with the ACTO function equipped in the Shimadzu LabSolutions workstation software that supports a variety of method transfer options.

References

1. C190-E263, Technical Report; January 2021, Simple Method Transfer using i-Series (LC-2050/LC-2060)

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