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Technical Report

Effective Analysis Management Achieved by Method Transfer between HPLC and UHPLC

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Abstract:

High performance liquid chromatography (HPLC) is widely used for the qualitative and quantitative analysis. In recent years, ultra-high performance liquid chromatography (UHPLC) has been developed significantly. Facilities that have both UHPLC and HPLC systems often apply newly established UHPLC method to ordinary HPLC as well as applying existing HPLC method to UHPLC. During such inter-system method application, the method must be modified properly to meet the requirements from another system. This modification is called "method transfer". Method transfer requires to modify existing parameters such as flow rate and/or time program.
Here, we describe the relationship between column dimensions including particle size of packing material and analytical conditions, then method transfer from HPLC to UHPLC for high speed analysis and that from UHPLC to HPLC for generalization of analytical conditions as well as that from Shimadzu's system to other vendor's. We also describe the Shimadzu integrated LC system "Nexera-i MT" that supports method transfer and ACTO (Analytical Conditions Transfer and Optimization) Function equipped in the latest LabSolutions LC software.

Keywords: HPLC, UHPLC, method transfer, ACTO, Nexera-i MT

1. Background

In pharmaceutical, food and various industrial fields, HPLC is widely used for the analysis of target compounds and related impurities. In recent years, UHPLC system that has more than 100 MPa of pressure tolerance affords more efficient analysis at ultra-high speed. Due to these features, UHPLC systems have been introduced in a variety of facilities where R&D departments often use UHPLC systems to develop an efficient analytical method then modify it to match HPLC analysis. The method transferred are then used by the QC department. Conversely, an existing HPLC method can be transferred into a UHPLC method to improve the speed and efficiency of analyses. In this report, we define method transfer as both directions of method modifications from HPLC to UHPLC and from UHPLC to HPLC.

We often face inadequate separation in gradient elution after a method transfer from HPLC where the separation is completed, to UHPLC (Fig.1). Furthermore, even though a method may have been created successfully using UHPLC analysis, the method transfer from UHPLC to HPLC gives different selectivity of separation, resulting in a degradation of efficiency for the entire laboratory.

Generally, a method transfer accompanied by employing different column dimensions including particle size must be required to modify analytical conditions such as flow rate and time program. However, to optimize conditions is not easy and method transfer can result in poor separation compared to those obtained prior to the method transfer. Keeping a consistent separation pattern before and after method transfer in terms of parameters, such as resolution and relative retention times, requires complex calculations based on pre- and post-transfer data, which include column dimensions (length, internal diameter, and particle size).

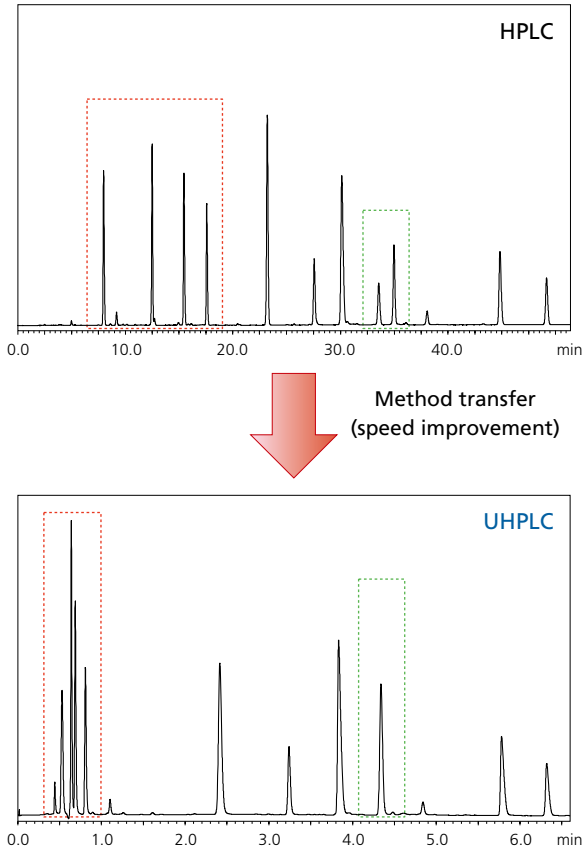


Fig. 1 Problems That Arise During Method Transfer (Analysis of cephem antibiotics)

1 Analytical & Measuring Instruments Division

2. Column Dimensions and Analytical Conditions

UHPLC analysis generally requires a column that is shorter, has smaller internal diameter, and smaller packing material size. However, once the column dimensions are modified, the existing analytical method must also be altered. This is because analytical conditions optimized for the HPLC analysis and the corresponding HPLC columns will not be optimal for the UHPLC analysis. The same is true when transferring a method from UHPLC to HPLC.

When a method is transferred, the main items that need to be changed are as follows.

- Flow rate
- **Time program**
- System delay volume
- Sampling rate
- Detector response
- Sample injection volume
- Upper pressure limit

Among these parameters, the time program is particularly important for gradient analyses. If an inappropriate gradient program is configured, it will result in an inferior separation after method transfer (*see* Fig. 1). Further, if the relative retention times and resolution are specified in testing regulations, the time program must be modified to keep the separation pattern. Due to these complications, it is much more efficient to modify a gradient program using theoretical calculations based on flow rate, column length, column internal diameter and system delay volume.

3. Example of Method Transfer

We have discussed method transfers from HPLC to UHPLC and UHPLC to HPLC. We will now describe examples of method transfer among multiple systems including Shimadzu's systems and other vendor's.

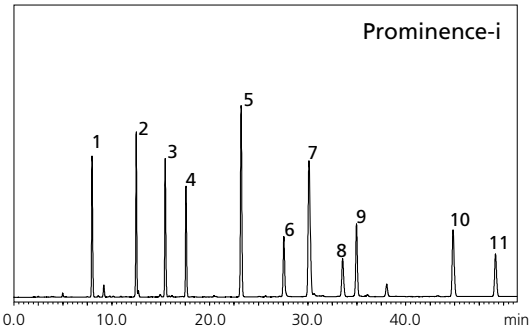
3-1. Improvement in the Speed of Analysis of Cephem Antibiotics

Cephem antibiotics are beta-lactam antibiotics that can be administered as an oral or injectable formulation. Here, we describe improving the analysis speed using a mixture of 11 cephem antibiotics as samples.

We used a Shimadzu Prominence-i system to analyze cephem antibiotics under HPLC conditions (Fig. 2a). Based on the column dimensions, we then modified the HPLC method such as the flowrate, sample injection volume, and time program for UHPLC analysis (*see* Fig. 2 for details). We used the UHPLC method to perform an analysis with a Shimadzu Nexera-i system. As seen from the results shown in Fig. 2b, we succeeded in improving the speed of the analysis of the cephem antibiotic mixture while keeping the separation pattern almost identical to that obtained from the HPLC analysis. The example shown in Fig. 1, which was a failed attempt at improving speed of analysis, also used cephem antibiotics as an analysis sample. These results show that appropriate analytical conditions must be determined during method transfer.

Improving the speed of an existing analysis leads to higher overall efficiency. For example, although quality control tests may be performed according to the test methods registered with regulatory authorities, process control tests may not be under the influence of such regulatory controls. In such cases, improving the speed of analysis during process control facilitates higher overall efficiency.

(a)



■ Peaks

1. Cefadroxil, 2. Cefapirin, 3. Cefalexin, 4. Cefradine, 5. Cefotaxime, 6. Cefazolin, 7. Cefuroxime, 8. Cefoxitin, 9. Cefoperazone, 10. Cefalothin, 11. Cefamandole.

■ Analytical Conditions

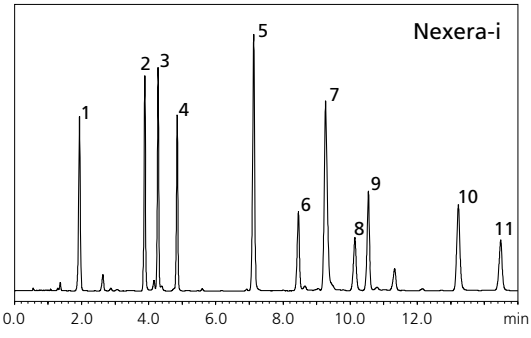
Column	: Shim-pack VP-ODS (250 mm L. × 4.6 mm I.D., 5.0 µm)
Mobile phase A	: 0.1 % formic acid
Mobile phase B	: Acetonitrile
Initial concentration	: 5% B
Flowrate	: 1.0 mL/min
Temp.	: 45 °C
Detection	: Ultraviolet-visible absorbance detector (260 nm)
Injection volume	: 10 µL

Time program

Time	Unit	Func.	Value
2.00	Pump	B.Conc	5
50.00	Pump	B.Conc	35
55.00	Pump	B.Conc	35
55.10	Pump	B.Conc	5
65.00	Controller		Stop

Method transfer
(speed improvement)

(b)



■ Analytical Conditions

Column	: Shim-pack XR-ODS II (100 mm L. × 3.0 mm I.D., 2.2 µm)
Flowrate	: 1.00 mL/min
Injection volume	: 4 µL

Time program

Time	Unit	Func.	Value
0.51	Pump	B.Conc	5
12.76	Pump	B.Conc	35
14.04	Pump	B.Conc	35
14.06	Pump	B.Conc	5
17.00	Controller		Stop

Note: Mobile phases A and B and initial concentrations are identical to those before method transfer

Fig. 2 An Example of Method Transfer (Cephem antibiotics)

3-2. Converting a Method for Sulfa Drug Analysis for General Use

Sulfa drugs (sulfonamides) is a generic term given to synthetic antibacterial drugs and chemotherapy drugs that contain a 4-aminobenzenesulfonamide moiety. These drugs are used to treat infections and for other purposes. Here, we describe an example of developing an efficient UHPLC analysis method for a sample containing nine sulfa drugs and transferring this UHPLC method to HPLC conditions. Using this HPLC method, we then performed an analysis using a Shimadzu Prominence-i system and another vendor's system and verified the compatibility of the method with these systems.

UHPLC analysis was performed using a Shimadzu Nexera-i MT system. As seen from the chromatogram in Fig. 3a, separation of the nine sulfa drugs was achieved in around 5 min.

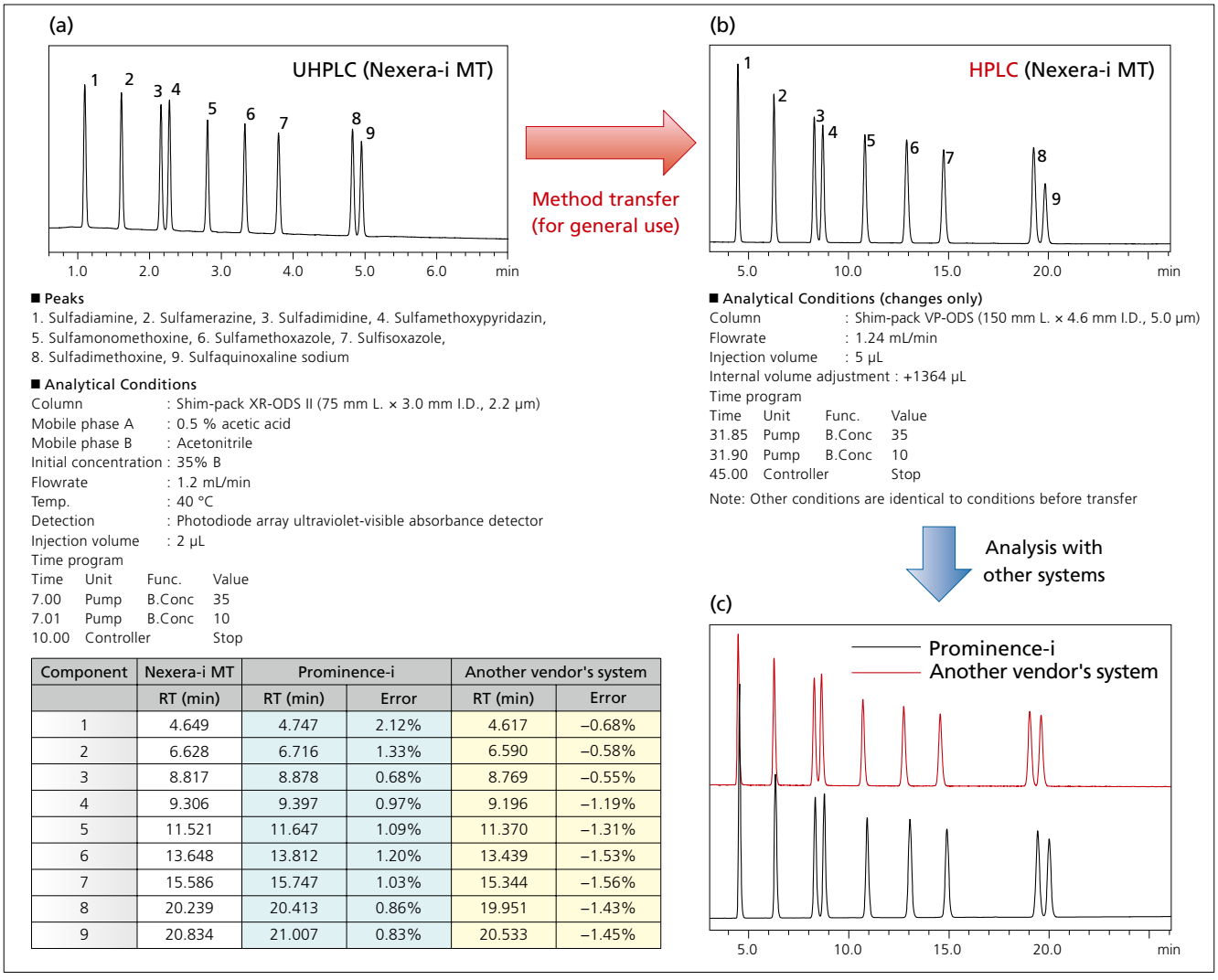
In order to transfer this method from UHPLC to HPLC, the column was changed from Shim-pack XR-ODS II (75 mm L. × 3.0 mm I.D., 2.2 µm) to Shim-pack VP-ODS (150 mm L. × 4.6 mm I.D., 5.0 µm). Based on this column information, we then modified the time program, flow rate, sampling rate, and other parameters (for method transfer to HPLC) by using the Analytical Conditions Transfer and Optimization (ACTO) function (described later).

We then performed the HPLC analysis on the sulfa drugs using the new method and using the same Nexera-i MT system. We successfully separated the sulfa drugs in around 20 min. As shown in the chromatogram in Fig. 3b, an appropriate method transfer enabled us to achieve a separation almost equivalent to that obtained with UHPLC.

Due to compatibility of Nexera-i MT system with a variety of other systems, we also performed the same analyses on other systems. Almost identical chromatograms were obtained when the analyses were performed on a Prominence-i system and another vendor's system using the same method (Fig. 3c). The table at the bottom left of Fig. 3 shows the retention times and percentage errors for each compound compared to the results obtained using the Nexera-i MT system. The retention time percentage error is within 2 % for almost all compounds.

The examples presented here suppose that methods created for UHPLC by R&D departments are transferred to methods for HPLC. In addition, they also suppose that those HPLC methods will be used in systems in other departments. As can be seen from the results, transfer can be done without any differences in the separation patterns.

Furthermore, if there is compatibility among systems, then comparable analytical results can be obtained even using other systems. For this reason, the same analysis can also be performed by departments that have different systems from R&D department.



■ Peaks
1. Sulfadiamine, 2. Sulfamerazine, 3. Sulfadimidine, 4. Sulfamethoxypyridazin,
5. Sulfamonomethoxine, 6. Sulfamethoxazole, 7. Sulfisoxazole,
8. Sulfadimethoxine, 9. Sulfaquinoxaline sodium

■ Analytical Conditions

Column	: Shim-pack XR-ODS II (75 mm L. × 3.0 mm I.D., 2.2 µm)
Mobile phase A	: 0.5 % acetic acid
Mobile phase B	: Acetonitrile
Initial concentration	: 35% B
Flowrate	: 1.2 mL/min
Temp.	: 40 °C
Detection	: Photodiode array ultraviolet-visible absorbance detector
Injection volume	: 2 µL

Time program

Time	Unit	Func.	Value
7.00	Pump	B.Conc	35
7.01	Pump	B.Conc	10
10.00	Controller		Stop

■ Analytical Conditions (changes only)

Column	: Shim-pack VP-ODS (150 mm L. × 4.6 mm I.D., 5.0 µm)
Flowrate	: 1.24 mL/min
Injection volume	: 5 µL
Internal volume adjustment	: +1364 µL

Time program

Time	Unit	Func.	Value
31.85	Pump	B.Conc	35
31.90	Pump	B.Conc	10
45.00	Controller		Stop

Note: Other conditions are identical to conditions before transfer

Component	Nexera-i MT	Prominence-i		Another vendor's system	
	RT (min)	RT (min)	Error	RT (min)	Error
1	4.649	4.747	2.12%	4.617	–0.68%
2	6.628	6.716	1.33%	6.590	–0.58%
3	8.817	8.878	0.68%	8.769	–0.55%
4	9.306	9.397	0.97%	9.196	–1.19%
5	11.521	11.647	1.09%	11.370	–1.31%
6	13.648	13.812	1.20%	13.439	–1.53%
7	15.586	15.747	1.03%	15.344	–1.56%
8	20.239	20.413	0.86%	19.951	–1.43%
9	20.834	21.007	0.83%	20.533	–1.45%

Fig. 3 Method Transfer and Compatibility with Another Vendor's System

4. Nexera-i MT and ACTO Function

We describe the Shimadzu Nexera-i MT integrated liquid chromatograph system and the ACTO function included in the Shimadzu LabSolutions workstation software, which are products that support method transfer.

4-1. Nexera-i MT Integrated Liquid Chromatography System

Nexera-i MT is an excellent method transfer system from existing LC systems. Nexera-i MT has dual flow lines, one is for HPLC and the other for UHPLC, and can switch these flow lines during a method. This system offers following two advantages:

- The ability to switch HPLC flow line and UHPLC flow line enables the transfer of UHPLC methods to general use (HPLC) methods and to transfer general use methods to UHPLC methods within a single system.
- Attaching an optional flow line to the standard HPLC flow line allows for method compatibility with liquid chromatography (LC) systems that have different system volumes.

Combining the Nexera-i system with the ACTO function described below facilitates smooth method transfer in a variety of applications.

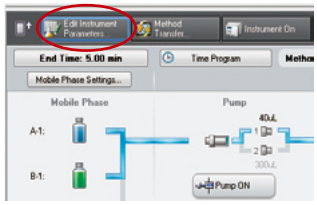
Fig. 4 Nexera-i MT

4-2. ACTO Function (Method Transfer)

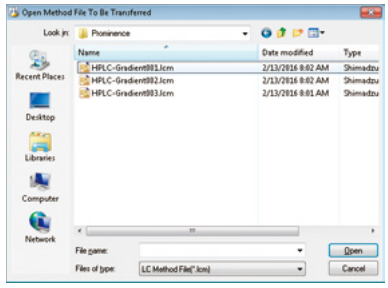
ACTO, which is included in the latest version of LabSolutions, is an efficient method transfer tool provided by Shimadzu. Here we describe one of ACTO's functions called "Method Transfer."

For improving the speed of an existing analysis performed on a HPLC system by converting it for use on a UHPLC system or for converting an analysis performed on an UHPLC system for general use with HPLC, modifications need to be made to the existing analysis methods.

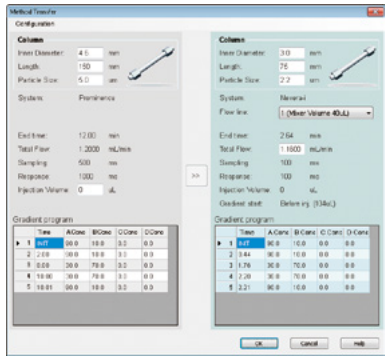
However, transferring a method requires complex calculations based on the internal diameter of the column, column length, particle diameter, and other parameters for determining an appropriate post-transfer flow rate and time program. This task can be simplified by using the "Method Transfer" tool included in LabSolutions. When this tool is used, a method is created automatically by just selecting an existing method, and entering the column information and some other parameters. Automatically created methods can be incorporated in LabSolutions software with a single click of a button; this streamlines the work flow from method conversion to analysis. Method Transfer is a powerful tool and can be utilized in a variety of situations to carry out efficient method transfers.



Step 1: Tool Startup



Step 2: Method File Selection



Step 3: Column Specification

Step 4: Save as Method File

Fig. 5 Method Transfer Work Flow

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