

# Method Transfer from HPLC to U-HPLC for Increased Speed and Resolution

L. Pereira, Thermo Fisher Scientific, Runcorn, UK

## Key Words

- Hypersil GOLD™ Column
- Accela™ High Speed LC
- European Pharmacopoeia (EP)
- Ibuprofen
- U-HPLC Method Conversion

## Overview

**Purpose:** Take the European Pharmacopoeia (EP) HPLC method for ibuprofen and related substances and transfer it to U-HPLC to reduce analysis time while maintaining chromatographic integrity.

**Results:** Analysis time is reduced by eight fold, resolution of a critical pair of impurities is maintained; and solvent consumption is reduced by fourteen fold.

## Introduction

The use of sub-2 µm particles is becoming increasingly popular for applications in either High Throughput Screening (HTS) assays or in Ultra High Pressure Liquid Chromatography (U-HPLC). Sub-2 µm particle packed columns offer advantages over the more traditional columns packed with 3 and 5 µm particles through shorter analysis times, improvements in resolving power, sensitivity and peak capacity. When transferring methods from HPLC to U-HPLC, several approaches can be taken, depending on the analytical needs.

If column dimensions are maintained and only particle size is reduced, then an improvement in efficiency, resolution, and peak capacity is obtained. A second approach is to reduce not only particle size but also column dimensions, which has the benefit of reducing analysis time. In both cases, care must be taken to ensure operating flow rate, gradient profiles, and injection volumes are scaled appropriately to obtain an equivalent or superior separation.

The work presented in this application note provides practical guidelines on scaling down separations by taking into account particle size, column ID, and column length. The sample used to demonstrate the method transfer process is ibuprofen and six impurities. The original HPLC method using a 150 × 4.6 mm 5 µm column is adapted from the EP, and simple calculation routines are used to transfer it to 100 × 2.1 mm, and 50 × 2.1 mm columns packed with 1.9 µm particles. Further method optimization to achieve the fastest analysis time is also included.

## Methods

- Columns: Column I—Hypersil GOLD 5 µm, 150 × 4.6 mm; Column II—Hypersil GOLD 1.9 µm, 100 × 2.1 mm; Column III—Hypersil GOLD 1.9 µm, 50 × 2.1 mm
- U-HPLC system: Accela High Speed LC

## Original Method

Column:	150 × 4.6 mm, 5 µm
Mobile phase:	A: 0.05% H <sub>3</sub> PO <sub>4</sub> in H <sub>2</sub> O / ACN (66:34) B: ACN
Gradient:	0 to 25 min 0% B, 55 min 85% B, 85% B for 15 min
Flow rate:	1.0 mL/min
Injection volume:	10 µL
Temperature:	30 °C
Detection:	UV at 214 nm (0.1 s rise time; 20 Hz).
Sample	(Ibuprofen and impurities): 1. Impurity K; 2. Impurity D; 3. Impurity C; 4. Ibuprofen; 5. Impurity A; 6. Impurity B; 7. Impurity E.

## Method Transfer

To transfer the method geometrically to the smaller column geometries packed with 1.9 µm particles (columns II and III) and, therefore, ensure equivalent chromatography, it is necessary to scale the flow rate, injection volume and gradient:

### Step 1. Adjust flow rate

(keep linear velocity constant between original and new method)

$$F_2 = F_1 \times (d_{c2}^2/d_{c1}^2) \times (d_{p1}/d_{p2})$$

$F_1$ —original flow rate;  
 $F_2$ —new flow rate (mL/min)  
 $d_{c1}$ —original column ID;  
 $d_{c2}$ —new column ID (mm)  
 $d_{p1}$ —original particle size;  
 $d_{p2}$ —new particle size (µm)

### Step 2. Adjust injection volume

$$V_{i2} = V_{i1} \times (d_{c2}^2 \times L_2/d_{c1}^2 \times L_1)$$

$V_{i1}$ —original injection volume;  
 $V_{i2}$ —new injection volume (mL)  
 $d_{c1}$ —original column ID;  
 $d_{c2}$ —new column ID (mm)  
 $L_1$ —original column length;  
 $L_2$ —new column length (mm)

### Step 3. Adjust gradient profile

The original method (150×4.6 mm, 5 µm column) has three segments, one isocratic segment with 100% A for 25 min, followed by a linear gradient to 85% B over 30 min and a final isocratic segment of 15 min at 85% B. To transfer these conditions geometrically to columns II and III, it is necessary to calculate the number of column volumes of mobile phase in each segment in the original method:

- Calculate volume of mobile phase in each segment, based on flow rate and segment duration in time units.

#### For segment 1:

Volume mobile phase = Flow rate × segment time =  
1 mL/min × 25 min = 25 mL

- Calculate number of column volumes in each segment, based on volume of mobile phase in segment and column volume.

$$V_c = 0.68 \times \pi \times r^2 \times L$$

$V_c$  – column volume (mL);  
 $L$  – column length (cm);  
 $r$  – column radius (cm)

$$V_{c1} = 0.68 \times 3.14 \times 0.232 \times 15 = 1.7 \text{ mL}$$

#### For segment 1:

Number of column volumes = Volume mobile phase/column volume = 25 mL / 1.7 mL = 14.7

In the U-HPLC method (100×2.1 mm, 1.9 µm column) the segments need to last for the same number of column volumes to maintain chromatographic integrity:

- Based on the number of column volumes in each segment (calculated above) and the column volume, calculate the volume of mobile phase in each segment.

#### For segment 1:

Volume mobile phase = Number of column volumes × column volume = 14.7 × 0.23 mL = 3.38 mL

- Based on the volume of mobile phase in each segment and flow rate (calculated in step 1), calculate the duration of each segment in time units.

#### For segment 1:

Segment duration = Volume mobile phase / flow rate =  
3.38 mL / 0.55 mL/min = 6.1 min

Table 1 shows the method transfer calculations from the original method on the 150×4.6 mm, 5 µm column to the U-HPLC methods on 100×2.1 mm, 1.9 µm and 50×2.1 mm, 1.9 µm columns.

## Results

The chromatographic profiles obtained for the original HPLC method and the geometrically scaled U-HPLC methods on the smaller columns packed with 1.9 µm particles are shown in Figure 1. The resolution (USP) of peaks 5 and 6 is increased from 1.6 with the HPLC method to 2.8 with the U-HPLC method on the 100×2.1 mm column. When the method is geometrically scaled to the 50×2.1 mm column the resolution of peaks 5 and 6 is maintained while analysis time is reduced by approximately seven fold. Repeatability data for this method is shown in Table 2.

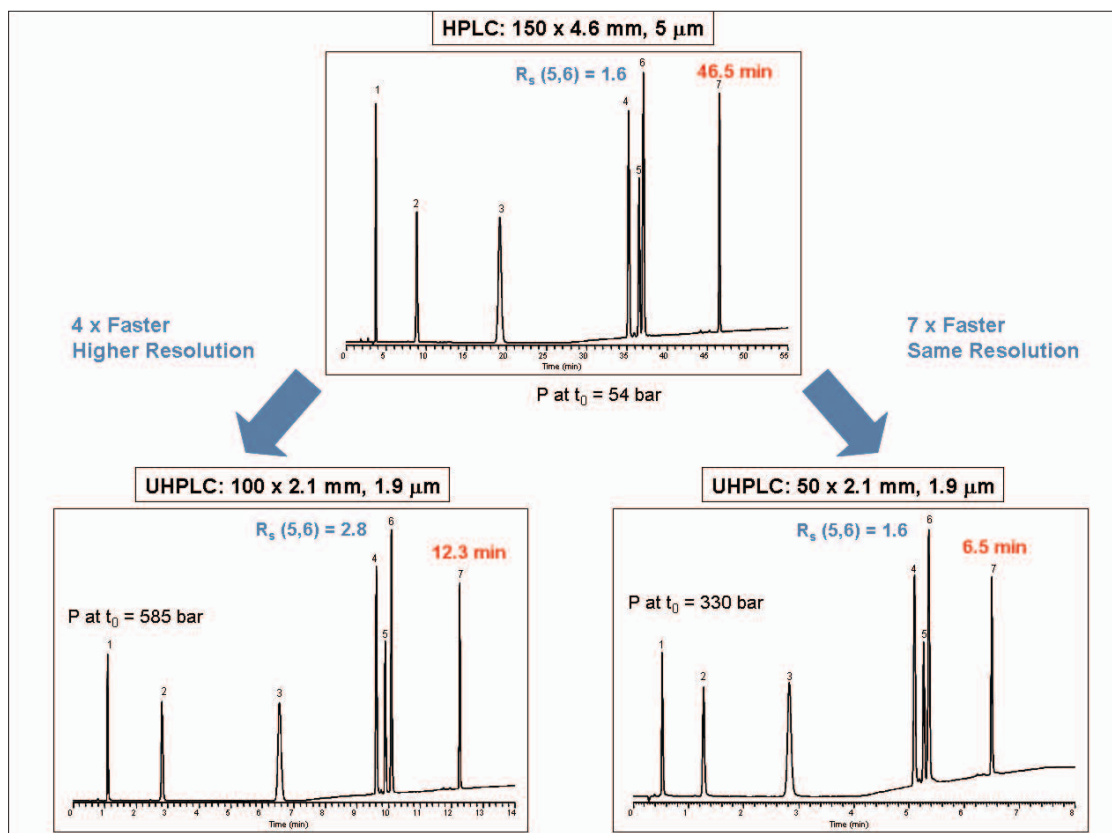


Figure 1: Chromatograms obtained with the original HPLC and geometrically scaled U-HPLC methods. Comparison of run time, USP resolution and pressure drop across the column.

<b>Original method</b> Column I : 150 x 4.6 mm, 5 µm Flow rate – 1 mL/min (Column volume = 1.7 mL ) Injection volume = 10 µL				<b>UHPLC</b> Column II : 100 x 2.1 mm, 1.9 µm Flow rate – 0.55 mL/min (Column volume = 0.24 mL ) Injection volume = 1.4 µL			<b>UHPLC</b> Column III : 50 x 2.1 mm, 1.9 µm Flow rate – 0.55 mL/min (Column volume = 0.12 mL ) Injection volume = 0.7 µL		
# of column volumes	%B	Volume of mobile phase (mL)	Gradient time (min)	Volume of mobile phase (mL)	Gradient time (min)	%B	Volume of mobile phase (mL)	Gradient time (min)	%B
0	0	0	0	0	0	0	0	0	0
14.7	0	25	25	3.5	6.4	0	1.8	3.2	0
32.4	85	55	55	7.8	14.1	85	3.9	7.1	85
41.2	85	70	70	9.9	17.9	85	4.9	8.9	85

Table 1: Method transfer conditions from HPLC (150 × 4.6 mm, 5 µm column) to U-HPLC (100 × 2.1 mm, 1.9 µm and 50 × 2.1 mm, 1.9 µm columns). Parameters in the columns in green are kept constant.

The time and solvent savings that can be gained by transferring HPLC methods to U-HPLC are illustrated in Table 3. An eight-fold reduction in analysis time and a fourteen-fold reduction in solvent consumption were observed in the analysis shown here.

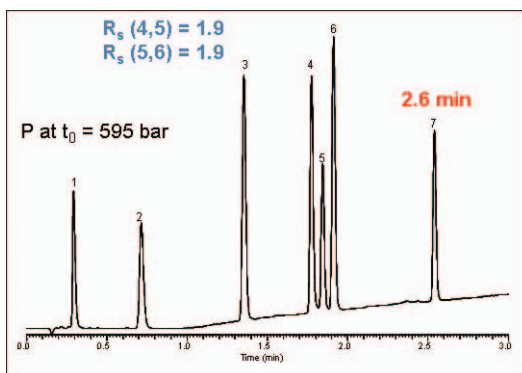
The method on the 50 × 2.1 mm, 1.9 µm column was optimized for speed by increasing the flow rate to 1 mL/min, by reducing the isocratic hold at the beginning of the run, and by making the gradient faster.

Peak number	Retention time (min)			Peak area		
	Mean	RSD	% RSD	Mean	RSD	% RSD
1	0.526	0.0006	0.116	311247	6001	1.93
2	1.275	0.0004	0.030	366600	8037	2.19
3	2.829	0.0010	0.035	763573	14055	1.84
4	5.098	0.0011	0.021	643375	10063	1.56
5	5.266	0.0011	0.020	383064	8240	2.15
6	5.358	0.0007	0.014	761428	12686	1.67
7	6.499	0.0026	0.039	457633	8570	1.87

Table 2: Repeatability data for 10 injections (no waste injection) with U-HPLC method on 50 × 2.1 mm, 1.9 µm column.

	<b>HPLC</b> 150 x 4.6 mm, 5µm	<b>UHPLC</b> 100 x 2.1 mm, 1.9µm	<b>UHPLC</b> 50 x 2.1 mm, 1.9µm
<b>Run time (min)</b>	70	18	9
<b>Run time + re-equilibration (min)</b>	87	22	11
<b>Time saving</b>	-	4 fold	8 fold
<b>Solvent used per run (mL)</b>	87	12	6
<b>Solvent saving</b>	-	7 fold	14 fold

Table 3: Time and solvent savings with U-HPLC.



Column:	Hypersil GOLD 1.9 $\mu$ m, 50 $\times$ 2.1 mm	
Mobile phase:	A: 0.05% H <sub>3</sub> PO <sub>4</sub> in H <sub>2</sub> O / ACN (66:34) B: ACN	
Gradient:	Time (min)	%B
	0	0
	0.5	0
	3	85
Flow rate:	1.0 mL/min	
Injection volume:	10 $\mu$ L	
Temperature:	30 $^{\circ}$ C	
Detection:	UV at 214 nm (0.1 s rise time; 20 Hz).	
Injection:	0.7 $\mu$ L	

Figure 2: Faster method in under three minutes. Flow rate 1 mL/min and optimized gradient.

## Conclusions

The EP HPLC method for ibuprofen and related substances was successfully transferred to U-HPLC on the Accela system running with Hypersil GOLD 1.9  $\mu$ m columns.

Transfer of the method was accomplished by geometrically scaling flow rate, injection volume, and gradient profile.

Analysis time was reduced by four fold with an improvement of 75% in the resolution of a critical pair of impurities; alternatively by using an even shorter column analysis time was reduced by eight fold, with constant resolution of the same critical pair.

The U-HPLC method offered good repeatability for retention time (% RSD <0.2) and peak area (% RSD <2.2)

Considerable solvent savings of up to fourteen fold were also measured with the U-HPLC methods.

Further optimization of the U-HPLC method allowed the separation of ibuprofen and six impurities in under three minutes.

## Additional Information

For additional information, please browse our Chromatography Resource Center which can be accessed from: [www.thermo.com/columns](http://www.thermo.com/columns)

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