Method Transfer from HPLC to UHPLC with Example of Polyphenols

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■ Introduction

We recently introduced a new series of UHPLC columns with a particle size of 1.6 µm. Here we explain how to transfer an existing HPLC method to a new UHPLC method using polyphenols as an example.

■ Steps

We previously published an HPLC method on Application Data No. 112. (https://www.develosil.net/en/wp/wp-content/uploads/2019/02/Application-Data-No.112.pdf) Entering parameters of the method and the specification of the new UHPLC column in a widely available method transfer software program generated an initial gradient table (Table 1). The software suggested a flow rate of 0.375 mL/min; this was adjusted to 0.5 mL/min, the optimum flow rate for this column.

Table 1. Starting Condition
Gradient Table

min	mL/min	%A	%B	Curve
0.00	0.5	80	20	6
1.28	0.5	45	55	6
3.20	0.5	45	55	6
3.21	0.5	80	20	6

Mobile phase:
A) Water + 0.1% formic acid
B) Acetonitrile + 0.1% formic acid

Conditions:
Column: Develosil UHPLC C18, 1.6 µm
Size: 2.0 x 50 mm
Temperature: 40°C Detection: UV at 260 nm.
System: UHPLC with a mixer of 100 µL

The first thing to decide is the type of detection. If using mass spectrometry, only volatile solvents such as formic acid can be used. In case of UV detection, acetic acid, formic acid, phosphoric acid and others can be used. We chose 0.1% formic acid, so that either detection method may be used. Since it can be prepared with a pipette alone, having advantages of time and less human error.

The tailing factor and the separation factor obtained using different acids in the mobile phase are shown in Table 2 for each analyte. Quercetin is known to have a tailing tendency with formic acid and may also show carryover due to strong ligating properties. Although 0.1% formic acid shows slight tailing with a tailing factor of 1.43, two other mobile phases showed even better results. Considering an LC/MS use, we chose 0.1% formic acid as the first candidate. For better peak shapes, a use of 0.08% formic acid + 0.02% TFA is an option.

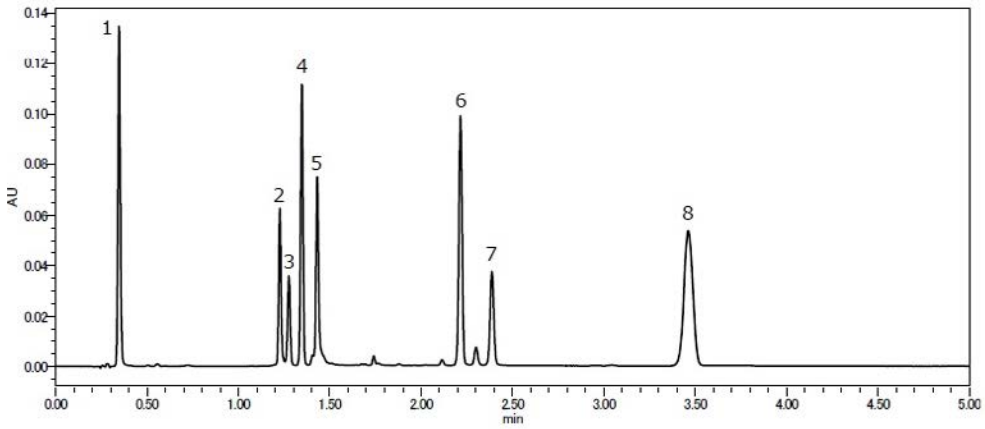

Fig 1. Chromatogram obtained as the first step.

Standards:
1. Puerarin
2. Baicalin (37.93)*
3. Resveratrol (2.00)
4. Daidzein (2.75)
5. Quercetin (3.31) “
6. Biochanin A (26.69)
7. Curcumin (4.85)
8. Ipriflavone (16.70)

*( )s indicate separation factor.

Table 2. Tailing Factors and Separation Factors in Three Types of Acids.

Compound	0.1%HCOOH		0.1%H_3PO_4		0.08%HCOOH +0.02%TFA	
	Tailing	R_S	Tailing	R_S	Tailing	R_S
Puerarin	1.11		1.22		1.11	
Baicalin	1.15	37.93	N.D.	39.98	N.D.	36.98
Resveratrol	0.95	2.00	N.D.	1.22	N.D.	1.16
Daidzein	0.99	2.75	1.02	2.68	0.96	2.53
Quercetin	1.43	3.31	1.12	3.44	1.15	3.08
Biochanin A	0.99	26.69	1.01	27.34	0.95	24.83
Curcumin	1.01	4.85	1.02	4.88	0.96	4.49
Ipriflavone	1.01	16.70	1.03	16.79	1.00	16.20

Considering separation of baicalin and resveratrol eluting very close, 0.1% formic acid showed the best separation factor. After adjustments to allow for elution of ipriflavone, we set the final gradient conditions as shown in Table 3.

Table 3. Final Gradient Condition

min	mL/min	%A	%B	Curve
0.00	0.5	80	20	6
1.28	0.5	45	55	6
3.60	0.5	45	55	6
3.61	0.5	80	20	6

Mobile phase:
A) Water + 0.1% formic acid
B) Acetonitrile + 0.1% formic acid

■ Order Information

Develosil UHPLC 1.6 μm Series

Size	C30	C18	C8	C1	HILIC
2.0 x 35 mm	201-I20035W	202-I20035W	203-I20035W	204-I20035W	205-I20035W
2.0 x 50 mm	201-I20050W	202-I20050W	203-I20050W	204-I20050W	205-I20050W
2.0 x 75 mm	201-I20075W	202-I20075W	203-I20075W	204-I20075W	205-I20075W
2.0 x 100 mm	201-I20100W	202-I20100W	203-I20100W	204-I20100W	205-I20100W
2.0 x 150 mm	201-I20150W	202-I20150W	203-I20150W	204-I20150W	205-I20150W

Develosil UHPLC 1.6 μm Metal-free Series

Size	C30	C18	C8	C1	HILIC
2.0 x 35 mm	201-I20035MFW	202-I20035MFW	203-I20035MFW	204-I20035MFW	205-I20035MFW
2.0 x 50 mm	201-I20050MFW	202-I20050MFW	203-I20050MFW	204-I20050MFW	205-I20050MFW
2.0 x 75 mm	201-I20075MFW	202-I20075MFW	203-I20075MFW	204-I20075MFW	205-I20075MFW
2.0 x 100 mm	201-I20100MFW	202-I20100MFW	203-I20100MFW	204-I20100MFW	205-I20100MFW
2.0 x 150 mm	201-I20150MFW	202-I20150MFW	203-I20150MFW	204-I20150MFW	205-I20150MFW

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