

# Welch UHPLC Column Care and Use Manual

Thank you for purchasing and using HPLC column manufactured by Welch Materials, Inc.

HPLC columns are expensive chromatographic consumables. It has strict production standards and test procedure. Please read this guide carefully before using the column, in order to maintain performance and achieve longer column life.

#### Identification of Welch Column

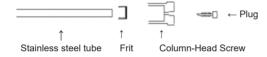
Each Welch column has a unique serial number, by which, the column can be traced back to each production procedure if any problem occurs. So when customer receives the column, please check:

- 1.If the package is intact and the label shows the same column required.
- 2.If the inside the box there is a CofA with a signature of quality inspector.
- 3.If the column has any apparent defects on our surface and two end caps are complete.
- 4.If the column has any apparent defects on surface and two end caps are complete.

### Structure and Installation

Structure:

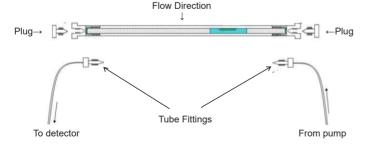
Welch HPLC column uses 316L stainless steel tube, as shown in Figure 1.



#### Installation:

To ensure highest column efficiency, use connection tubes with matched diameter. The connection of both ends must be highly fitted. The tube ends must be smooth, without any burrs or slants. Use professional tools to cut the tubes.

1. Find an arrow on the column identification label which indicates the correct direction of solvent flow. Unscrew the caps of each ends and place it in the right direction.



2.Switch the column using matched connectors (stainless steel or other materials). Make sure the column is tightly connected, without any volume or leaking when operating.

#### Glossary of Terms

**pH Range:** the pH range of mobile phase and sample solution, which is within the tolerance of the column.

Specific Surface Area: the surface area of 1g silica.

**Pore Size:** diameter of pores inside silica spheres (choose pore size according to target molecule weight) .

**Carbon load:** a parameter shows the quantity of bonded functional groups on silica sphere.

**USP Code:** general code for phases in United States Pharmacopeia Convention.

**Transition Mobile Phase:** with same or lower ratio of organic phase and water as mobile phase, but without additives like buffer salt, acid and alkali etc.

## Specification of UHPLC column

Column	USP Code	Carbon Load(%)	Pore Size(Å)	Surface Area	pH Range	Max Temp.	Max. Pressure
Xtimate UHPLC C18	L1	14%	120 Å	320 m²/g	1.0-12.5	70 C (pH < 6.5) 40 C (pH > 6.5)	15000 psi
Ultisil UHPLC XB-C18	L1	17%	120 Å	320 m²/g	1.5-10.0	60 C (pH < 6.5) 40 C (pH > 6.5)	15000 psi
Ultisil UHPLC AQ-C18	L1	12%	120 Å	320 m²/g	1.5-10.0	60 C (pH < 6.5) 40 C (pH > 6.5)	15000 psi
Ultisil UHPLC LP-C18	L1	10%	120 Å	320 m²/g	0.5-0.8	60 C (pH < 6.5) 40 C (pH > 6.5)	15000 psi
Ultisil UHPLC Polar-RP	L1	18%	120 Å	320 m <sup>2</sup> /g	1.5-10.0	60 C (pH < 6.5) 40 C (pH > 6.5)	15000 psi
Ultisil UHPLC HILIC	L3	/	120 Å	320 m²/g	2.0-8.0	60 C (pH < 6.5) 40 C (pH > 6.5)	15000 psi
Ultisil UHPLC XB-C8	L7	12%	120 Å	320 m <sup>2</sup> /g	1.5-10.0	60 C (pH < 6.5) 40 C (pH > 6.5)	15000 psi
Ultisil UHPLC XB-Phenyl	L11	13%	120 Å	320 m <sup>2</sup> /g	1.5-10.0	60 C (pH < 6.5) 40 C (pH > 6.5)	15000 psi

## Activation of New Column

Phases	Xtimate UHPLC C18 、 Ultisil UHPLC XB-C18 、 Ultisil UHPLC AQ-C18 、 Ultisil UHPLC LP-C18 、 Ultisil UHPLC Polar-RP 、 Ultisil UHPLC XB-C8 、 Ultisil UHPLC XB-Phenyl
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#### (Storage solvent: acetonitrile/water)

Activation		Transition		Note	
Column ID	≤3mm	>3mm	≤3mm	>3mm	No transition
Flow Rate	0.1ml/min	0.2ml/min	Analysis flow rate	Analysis flow rate	required for analysis mobile
Mobile Phase	80% acetonitrile		10% acetonitrile		phases without
Time	4h		1h		buffer salt
Temperature	30°C		30℃		

# Column Daily Flushing

Back flushing recommended (reverse to the normal flow direction)

Analysis	Without acid,	Containing acid,	Containing	
Mobile Phase	alkali or salts	alkali or salts	ion-pair reagents	
Flow Rate	Analysis flow rate			
Flushing Mobile Phase	80% methanol	10% methanol – 80% methanol	10% methanol - 50% methanol - 80% methanol	
Time	Column length<100mm	30min each step		
Time	Column length>100mm	40min each step		
Storage	Store in 80% methanol, kept in cool dry places			
Note	The methanol in mobile phase can be changed into acetonitrile			

## Abnormal Column Flushing

In the circumstances of high column pressure, abnormal peak shape, low column efficiency and low resolution etc, first flush off the buffer salts in column using transition mobile phase, then flush as follows; If mobile phase contains ion-pair reagents, first flush off buffer salts as above, then flush with 50% methanol, and flush as follows (back flushing recommended):

Flow Rate	1/4 analysis flow rate				
Mobile Phase	100% methanol – 100% acetonitrile – 100% isopropanol – 100% acetonitrile				
Time	Column length≤100mm 100min each step				
Time	Column length>100mm 120min each step				
Note	Isopropanol has high viscosity, causing high pressure. Please adjust the flow rate as needed.				

# Activation of New Column

	Phases	Ultisil UHPLC HILIC	
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(Storage solvent: acetonitrile)

Activation		Transition		Note	
Column ID	≤3mm	>3mm	≤3mm	>3mm	No transition
Flow Rate	0.1ml/min	0.2ml/min	Analysis flow rate	Analysis flow rate	required for analysis mobile
Mobile Phase	90% acetonitrile		Transition mobile phase		phases without
Time	4h		1h		buffer salt
Temperature	30℃		30℃		

# Column Daily Flushing

Back flushing recommended (reverse to the normal flow direction)

Analysis	Without acid	Containing acid,		
Mobile phase	alkali or salts	alkali or salts		
Flow Rate	Analysis flow rate			
Flushing	100% acetonitrile	Transition mobile phase		
Mobile Phase		-100% acetonitrile		
Time	Column length<100mm	30min each step		
Time	Column length>100mm	40min each step		
Storage	Store in 100% acetonitrile, kept in cool dry places			
Note				

# **Abnormal Column Flushing**

In the circumstances of high column pressure, abnormal peak shape, low column efficiency and low resolution etc, first flush off the buffer salts in column using transition mobile phase, then flush as follows; If mobile phase contains ion-pair reagents, first flush off buffer salts as above, then flush with 50% methanol, and flush as follows (back

Flow Rate	1/4 analysis flow rate		
Mobile Phase	100% methanol-100% acetonitrile-100% isopropanol-100% acetonitrile		
Time	Column length≤100mm	100min each step	
Time	Column length>100mm 120min each step		
Note	Isopropanol has high viscosity, causing high pressure. Please adjust the flow rate as needed.		

Welch Materials, Inc.

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