

**Title:** Dual column/polarity liquid chromatography operation for 5 minute high-resolution metabolomics, Q-Exactive HF

**SOP:** QEHF\_HRM-DC5min\_92017\_v1  
Revision: 1

**Date effective:** 27 September 2017

### **HILIC–positive chromatography method summary**

The HILIC column is operated parallel to reverse phase column for simultaneous analytical separation and column flushing through the use of a dual head HPLC pump equipped with 10-port and 6-port switching valves. During operation of HILIC separation method, the MS is operated in positive ion mode and 10  $\mu$ L of sample is injected onto the HILIC column while the reverse phase column is flushing with wash solution. Flow rate is maintained at 0.35 mL/min until 1.5 min, increased to 0.4 mL/min at 4 min and held for 1 min. Solvent A is 100% LC-MS grade water, solvent B is 100% LC-MS grade acetonitrile and solvent C is 2% formic acid (v/v) in LC-MS grade water. Initial mobile phase conditions are 22.5% A, 75% B, 2.5% C hold for 1.5 min, with linear gradient to 77.5% A, 20% B, 2.5% C at 4 min, hold for 1 min, resulting in a total analytical run time of 5 min. During the flushing phase (reverse phase analytical separation), the HILIC column is equilibrated with a wash solution of 77.5% A, 20% B, 2.5% C.

### **C<sub>18</sub>–negative chromatography method summary**

The C<sub>18</sub> column is operated parallel to the HILIC column for simultaneous analytical separation and column flushing through the use of a dual head HPLC pump equipped with 10-port and 6-port switching valves. During operation of the C<sub>18</sub> method, the MS is operated in negative ion mode and 10  $\mu$ L of sample is injected onto the C<sub>18</sub> column while the HILIC column is flushing with wash solution. Flow rate is maintained at 0.4 mL/min until 1.5 min, increased to 0.5 mL/min at 2 min and held for 3 min. Solvent A is 100% LC-MS grade water, solvent B is 100% LC-MS grade acetonitrile and solvent C is 10mM ammonium acetate in LC-MS grade water. Initial mobile phase conditions are 60% A, 35% B, 5% C hold for 0.5 min, with linear gradient to 0% A, 95% B, 5% C at 1.5 min, hold for 3.5 min, resulting in a total analytical run time of 5 min. During the flushing phase (HILIC analytical separation), the C<sub>18</sub> column is equilibrated with a wash solution of 0% A, 95% B, 5% C until 2.5 min, followed by an equilibration solution of 60% A, 35% B, 5% C for 2.5 min.

### **Chemicals Needed:**

- Positive ESI mobile phases: 1L LC-MS grade H<sub>2</sub>O (Red-A); 1L LC-MS grade acetonitrile (Red-B); 1L 5% formic acid in LC-MS grade H<sub>2</sub>O (Red-C)
- Negative ESI mobile phases: 1L LC-MS grade H<sub>2</sub>O (Green-A); 1L LC-MS grade acetonitrile (Green-B); 1L 10mM ammonium acetate in LC-MS grade H<sub>2</sub>O (Green-C)

### **Materials Needed**

- Higgins endcapped C18 stainless steel column. 2.1mm x 50mm x 3 $\mu$ m particle size, Product #TS-0521-C183

**Title:** Dual column/polarity liquid chromatography operation for 5 minute high-resolution metabolomics, Q-Exactive HF

**SOP:** QEHF\_HRM-DC5min\_92017\_v1

Revision: 1

**Date effective:** 27 September 2017

- Waters XBridge BEH Amide XP HILIC column. 2.1mm x 50mm x 2.5µm particle size. Product #186006089
- Thermo Accucore C18 guard column with holder, Product #17126-014005
- Thermo Accucore HILIC guard column with holder, Product # 17526-012105
- High-resolution Q-Exactive HF Orbitrap mass spectrometer with ESI source
- Dual LC pumps with degasser, autosampler and switching valves
- HPLC, Thermo Scientific Dionex Ultimate 3000 with refrigerated autosampler, dual channel pumps, 10-port and 6-port switching valves, with left pump set to control HILIC positive gradient and right pump set to control C18 negative gradient. Start pumps at 0.350 mL/min at initial conditions: Left pump 75% B and 2.5% C; Right pump 35% B and 5% C.

### **HILIC-positive Method**

Method filename: 20160920\_posHILIC120kres5min\_ESI\_c18negwash.meth

#### LC settings

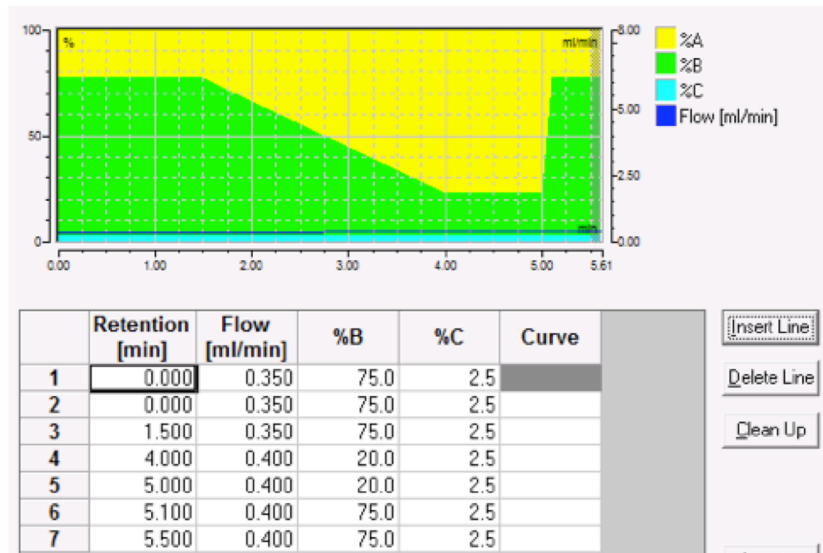
- Run length: 5.5 min
- Valve 1 position: 1\_2 at 0 min; 10\_1 at 5 min
- Valve 2 position: 1\_2
- Column oven temperature: 60°C
- Pump left: A= Water; B= Acetonitrile, C=2% formic acid in water
- Pump right: A= Water; B= Acetonitrile, C=10mM ammonium acetate in water
- Sampler: Draw speed= 2 µL/s; Draw delay= 1000 ms, Dispense speed= 25 µL/s; Dispense delay= 1000 ms; Dispense to waste= 32 µL/s; Sample height= 4mm; Inject wash= Both; Wash volume= 100 µL; Wash speed= 20 µL/s; Loop wash factor= 2; Injection mode= Normal; Drawer temperature= 8°C

**Title:** Dual column/polarity liquid chromatography operation for 5 minute high-resolution metabolomics, Q-Exactive HF

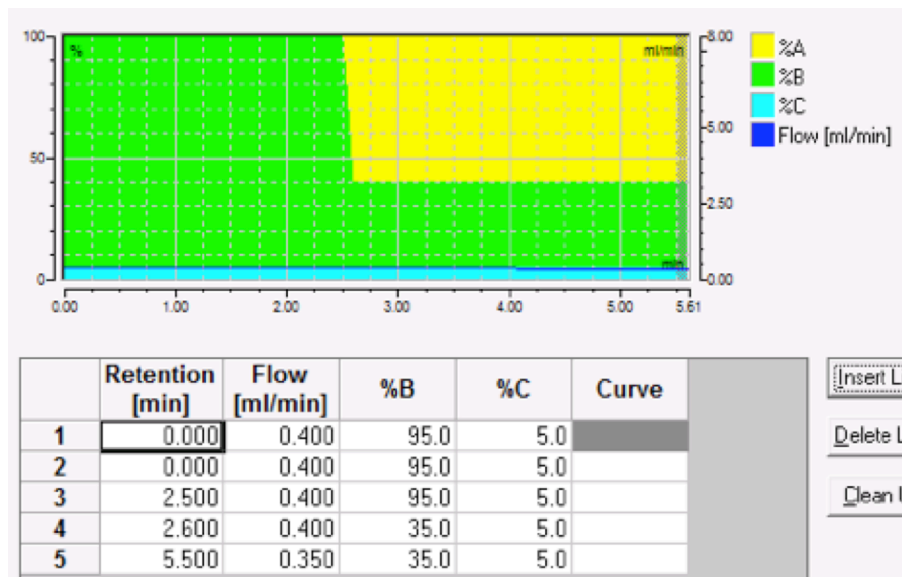
**SOP:** QEHF\_HRM-DC5min\_92017\_v1  
 Revision: 1

**Date effective:** 27 September 2017

LC gradient and flow information:



**Figure 1A:** Left pump mobile phase gradient and flow rate for HILIC-positive (HILIC analytical separation)



**Figure 1B:** Right pump mobile phase gradient and flow rate for HILIC-positive (washing C18)

**Title:** Dual column/polarity liquid chromatography operation for 5 minute high-resolution metabolomics, Q-Exactive HF

**SOP:** QEHF\_HRM-DC5min\_92017\_v1  
 Revision: 1

**Date effective:** 27 September 2017

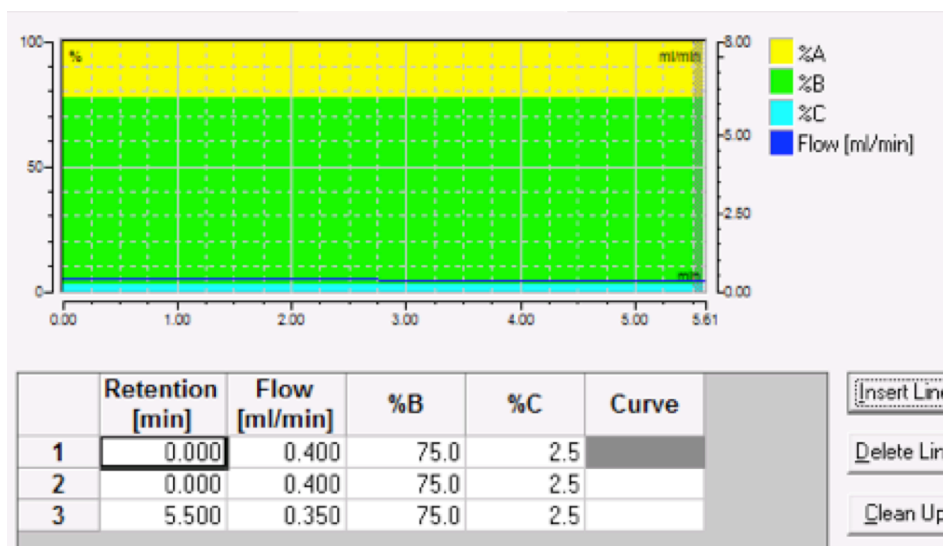
### c18-negative Method

Method filename: 20160920\_negC18120kres5min\_ESI\_HILICposwash.meth

#### LC settings

- Run length: 5.5 min
- Valve 1 position: 10\_1 at 0 min
- Valve 2 position: 6\_1
- Column oven temperature: 60°C
- Pump left: A= Water; B= Acetonitrile, C=2% formic acid
- Pump right: A= Water; B= Acetonitrile, C=10mM ammonium acetate
- Sampler: Draw speed= 2  $\mu\text{L/s}$ ; Draw delay= 1000 ms, Dispense speed= 25  $\mu\text{L/s}$ ; Dispense delay= 1000 ms; Dispense to waste= 32  $\mu\text{L/s}$ ; Sample height= 4mm; Inject wash= Both; Wash volume= 100  $\mu\text{L}$ ; Wash speed= 20  $\mu\text{L/s}$ ; Loop wash factor= 2; Injection mode= Normal; Drawer temperature= 8°C

#### LC gradient and flow information:

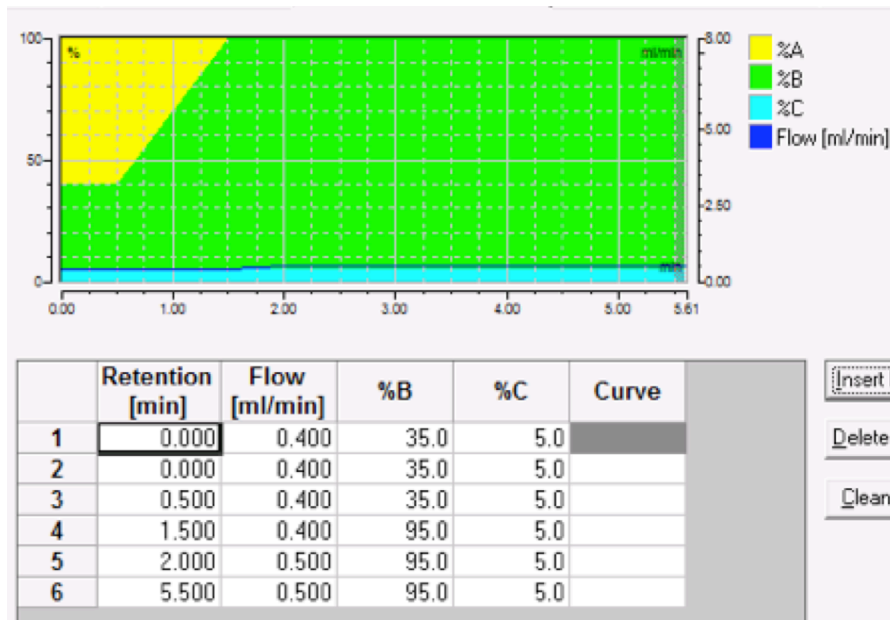


**Figure 3A:** Left pump mobile phase gradient and flow rate for C18-negative (washing HILIC)

**Title:** Dual column/polarity liquid chromatography operation for 5 minute high-resolution metabolomics, Q-Exactive HF

**SOP:** QEHF\_HRM-DC5min\_92017\_v1  
 Revision: 1

**Date effective:** 27 September 2017



**Figure 3B:** Right pump mobile phase gradient and flow rate for C18-negative (C18 analytical separation)

### Instrument maintenance schedule

The following maintenance schedule is maintained to ensure the highest data quality possible. The time limits given should never be exceeded, however, it is permissible to perform maintenance early if coincides with the start of a new study. Maintenance is to be completed by trained staff only.

- Column lifespan: 3000 injections



Department of Medicine

**Clinical Biomarkers Laboratory  
Division of Pulmonary Allergy and Critical Care Medicine  
615 Michael St. Ste. 225, Atlanta GA, 30322**

**Title:** Dual column/polarity liquid chromatography operation for 5 minute high-resolution metabolomics, Q-Exactive HF

**SOP:** QEHF\_HRM-DC5min\_92017\_v1  
Revision: 1

**Date effective:** 27 September 2017

**SOP Details and Version Information**

<b>Created by:</b> Douglas I. Walker	<b>Date:</b> 27 September 2017
<b>Reviewed by:</b> Carolyn Accardi	<b>Date:</b> 327 September 2017
<b>Approved by:</b> Dean P. Jones	<b>Date:</b> 27 September 2017

<b>Revision</b>	<b>Name</b>	<b>Reason</b>	<b>Effective date</b>
01	Douglas I. Walker	Creation of SOP	27 September 2017