EMORY UNIVERSITY SCHOOL OF MEDICINE
Department of Medicine

Clinical Biomarkers Laboratory
Division of Pulmonary Allergy and Critical Care Medicine
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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 µL 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_{18}–negative chromatography method summary
The C_{18} 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_{18} method, the MS is operated in negative ion mode and 10 µL of sample is injected onto the C_{18} 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_{18} 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_2O (Red-A); 1L LC-MS grade acetonitrile (Red-B); 1L 5% formic acid in LC-MS grade H_2O (Red-C)
- Negative ESI mobile phases: 1L LC-MS grade H_2O (Green-A); 1L LC-MS grade acetonitrile (Green-B); 1L 10mM ammonium acetate in LC-MS grade H_2O (Green-C)

Materials Needed

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

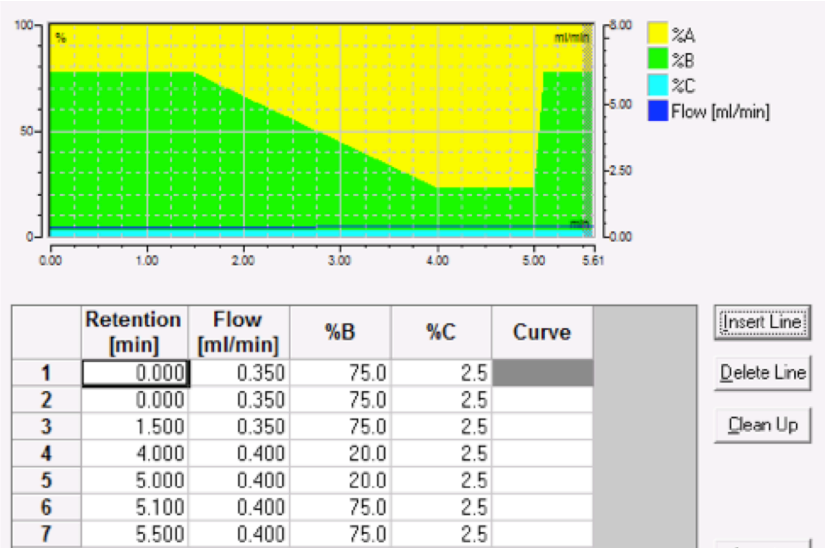
- Waters XBridge BEH Amide XP HILIC column. 2.1mm x 50mm x 2.5µm particle size. Product #186006089
- Thermo Accucore C18 guard column with holder, Product #17126-014005
- Thermo Accucore HILIC guard column with holder, Product # 17526-012105
- High-resolution Q-Exactive HF Orbitrap mass spectrometer 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

LC gradient and flow information:



	Retention [min]	Flow [ml/min]	%B	%C	Curve
1	0.000	0.350	75.0	2.5	
2	0.000	0.350	75.0	2.5	
3	1.500	0.350	75.0	2.5	
4	4.000	0.400	20.0	2.5	
5	5.000	0.400	20.0	2.5	
6	5.100	0.400	75.0	2.5	
7	5.500	0.400	75.0	2.5	

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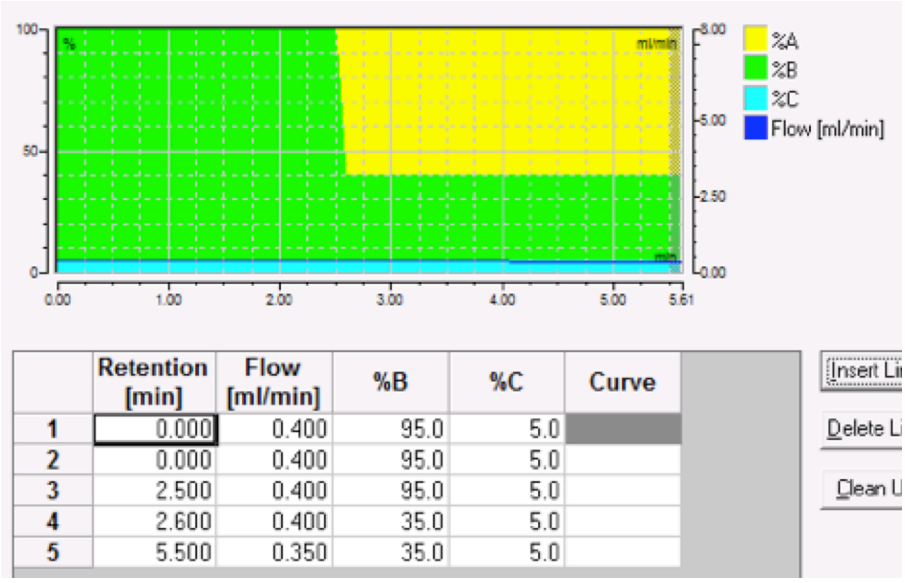
Clean Up

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



	Retention [min]	Flow [ml/min]	%B	%C	Curve
1	0.000	0.400	95.0	5.0	
2	0.000	0.400	95.0	5.0	
3	2.500	0.400	95.0	5.0	
4	2.600	0.400	35.0	5.0	
5	5.500	0.350	35.0	5.0	

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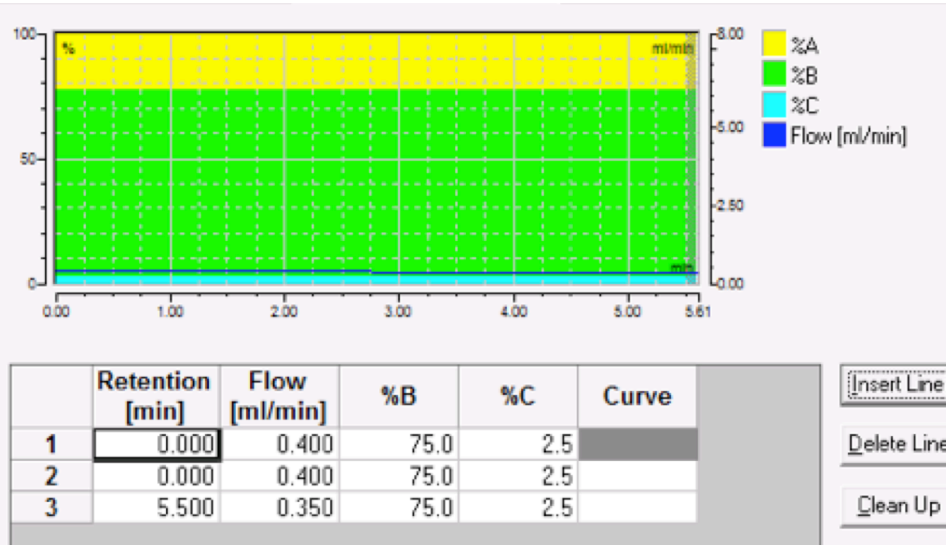
Figure 1B: Right pump mobile phase gradient and flow rate for HILIC-positive (washing C18)

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 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

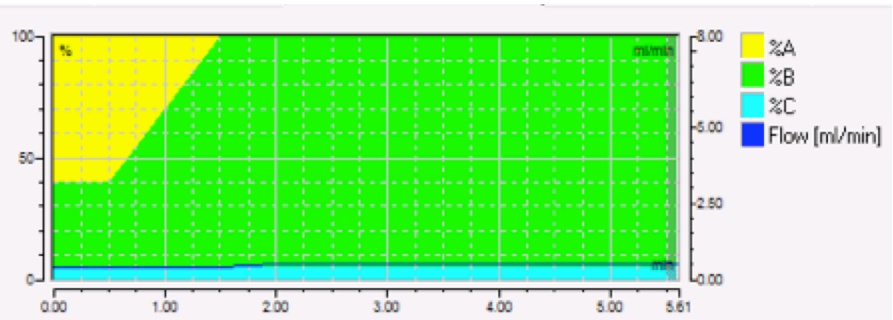
LC gradient and flow information:



	Retention [min]	Flow [ml/min]	%B	%C	Curve
1	0.000	0.400	75.0	2.5	
2	0.000	0.400	75.0	2.5	
3	5.500	0.350	75.0	2.5	

Insert Line
Delete Line
Clean Up

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



	Retention [min]	Flow [ml/min]	%B	%C	Curve
1	0.000	0.400	35.0	5.0	
2	0.000	0.400	35.0	5.0	
3	0.500	0.400	35.0	5.0	
4	1.500	0.400	95.0	5.0	
5	2.000	0.500	95.0	5.0	
6	5.500	0.500	95.0	5.0	

Insert L
Delete L
Clean

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

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SOP Details and Version Information

Created by: Douglas I. Walker	**Date:** 27 September 2017
Reviewed by: Carolyn Accardi	**Date:** 327 September 2017
Approved by: Dean P. Jones	**Date:** 27 September 2017

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