SeQuant® ZIC®-cHILIC Application Examples

Showing HPLC and LC-MS separations
of polar hydrophilic coumpounds

EMD Millipore is a division of Merck KGaA, Darmstadt, Germany

Application Index

* For more information on specifications and launch of SeQuant® ZIC®-cHILIC, please contact your EMD Millipore sales representative or visit www.emdmillipore.com/ZIC-cHILIC.

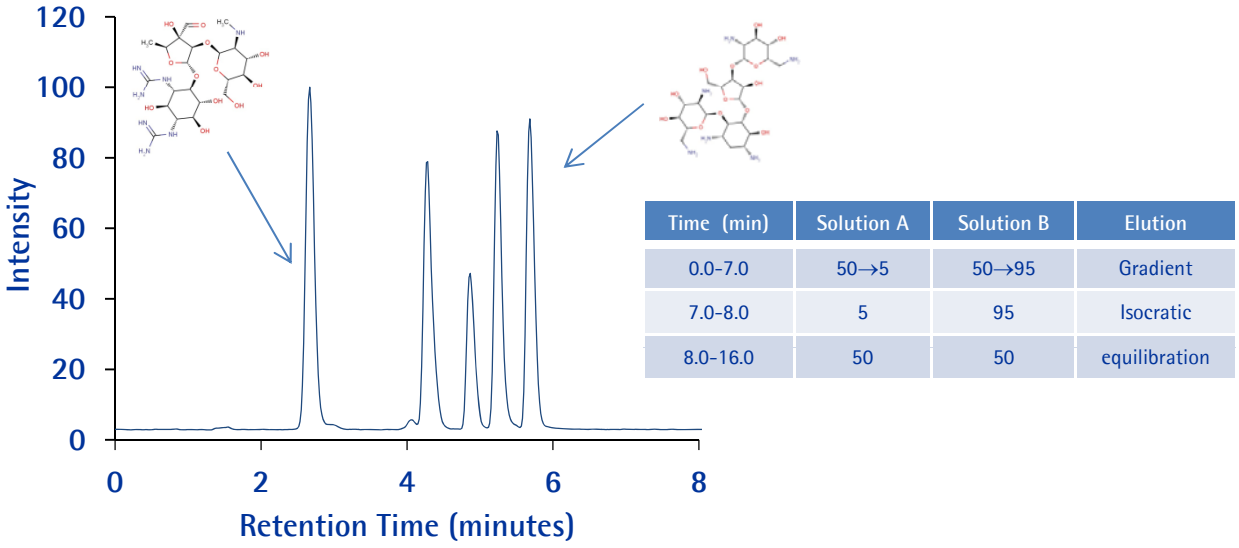
For order information, please call 1-866-645-5476 or email LEorders@emdmillipore.com.

Streptomycin, Gentamicin, Paromomycin, Tobramycin, and Neomycin

SeQuant® ZIC®-cHILIC

Chromatographic Conditions

Column:	SeQuant® ZIC®-cHILIC 100x2.1 mm, 3 µm, 100 Angstrom	1.50657.0001
Injection:	5 µl	
Detection:	Shimadzu LCMS 2012EV; Detector voltage: 2.0 kV; Heat block and CDL temp: 250 °C; SIM in positive mode: m/z 582 (STR)), 464 (GEN), 616 (PAR), 468 (TOB), and 615 (NEO)	
Flow Rate:	0.4 mL/min	
Mobile Phase (v/v):	A: Acetonitrile + 1 w% formic acid B: 100 mM ammonium acetate + 3 w% formic acid	
Gradient:	See Table	
Temperature:	50°C	
Diluent	Acetonitrile and water 30:70 (v/v)	
Sample:	Solution with 5 µg/mL of STR and 25 µg/mL of each GEN, PAR, TOB, and NEO in diluent	



Time (min)	Solution A	Solution B	Elution
0.0-7.0	50→5	50→95	Gradient
7.0-8.0	5	95	Isocratic
8.0-16.0	50	50	equilibration

Chromatographic Data

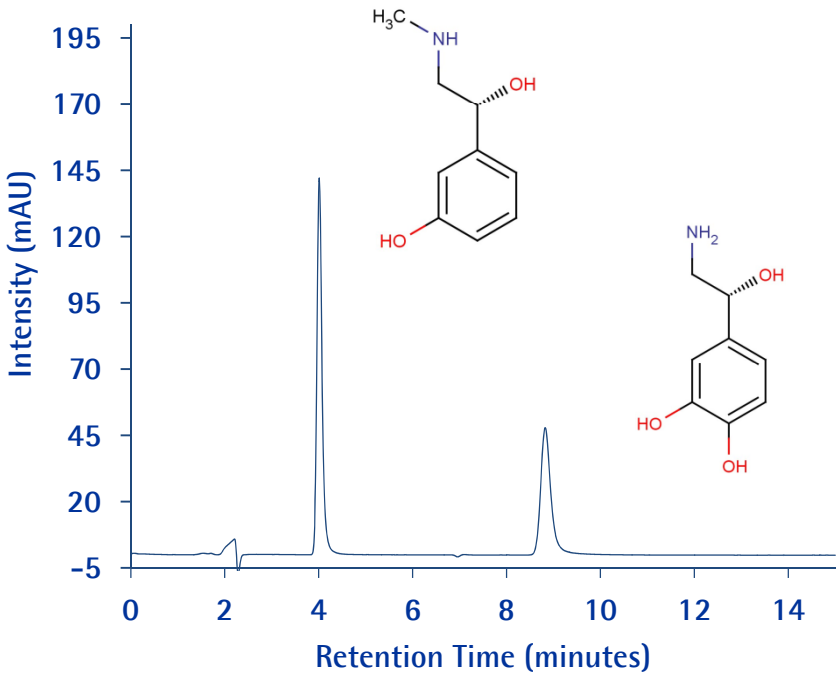
No.	Compound	Retention Time (min)	Theoretical plates	Tailing Factor
1	Streptomycin (STR)	2.7	1801	1.2
2	Gentamicin (GEN)	4.3	4667	1.4
3	Paromomycin (PAR)	4.9	6878	1.2
4	Tobramycin (TOB)	5.2	8788	1.2
5	Neomycin (NEO)	5.7	9464	1.1

Norepinephrine and Phenylephrine

SeQuant® ZIC®-cHILIC

Chromatographic Conditions

Column:	SeQuant® ZIC®-cHILIC 150x2.1 mm, 3 µm, 100 Angstrom	1.50658.0001
Injection:	10 µl	
Detection:	Agilent 1260, UV 270 nm	
Flow Rate:	0.2 mL/min	
Mobile Phase (v/v):	Acetonitrile and 100 mM ammonium acetate adjusted to pH 4.5 by glacial acetic acid (75:25). Total ionic strength in mobile phase = 25 mM.	
Gradient:	See Table	
Temperature:	30°C	
Diluent	Mobile phase	
Sample:	Solution with 15 ug/mL of norepinephrine, 20 ug/mL phenylephrine in diluent	
Pressure drop:	67 Bar (972 psi)	



Chromatographic Data

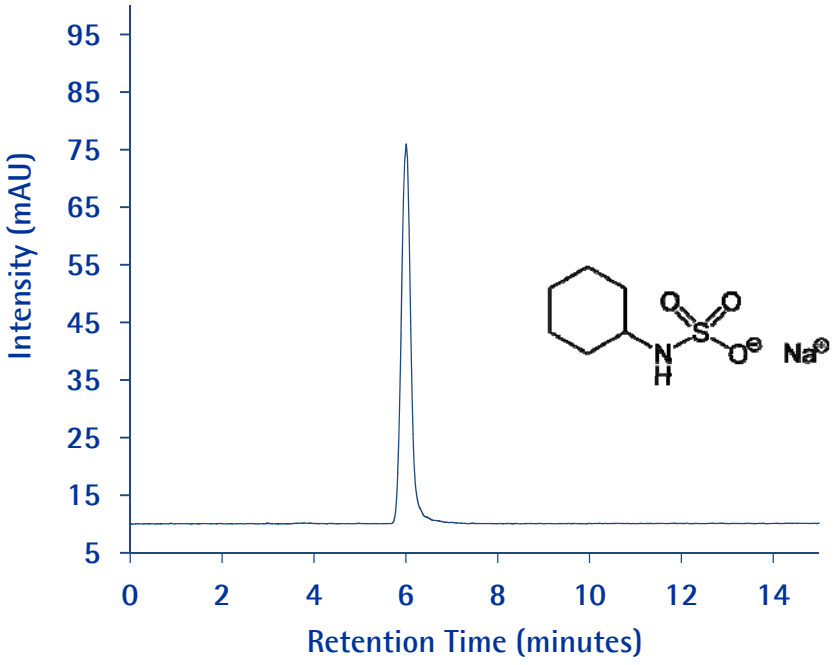
No.	Compound	Retention Time (min)	Theoretical plates	Tailing Factor
1	Phenylephrine	4.0	7120	1.3
2	Norepinephrine	8.8	8925	1.3

Sodium Cyclamate

SeQuant® ZIC®-cHILIC

Chromatographic Conditions

Column:	SeQuant® ZIC®-cHILIC 150x2.1 mm, 3 µm, 100 Angstrom	1.50658.0001
Injection:	1 µl	
Detection:	Agilent 1260, ELSD	
Flow Rate:	0.2 mL/min	
Mobile Phase (v/v):	Acetonitrile and 100 mM ammonium acetate adjusted to pH 4.5 by glacial acetic acid (75:25). Total ionic strength in mobile phase = 25 mM.	
Gradient:	See Table	
Temperature:	Ambient	
Diluent	Mobile phase	
Sample:	1mg/mL Sodium Cyclamate in diluent	
Pressure drop:	39 Bar (566 psi)	



Chromatographic Data

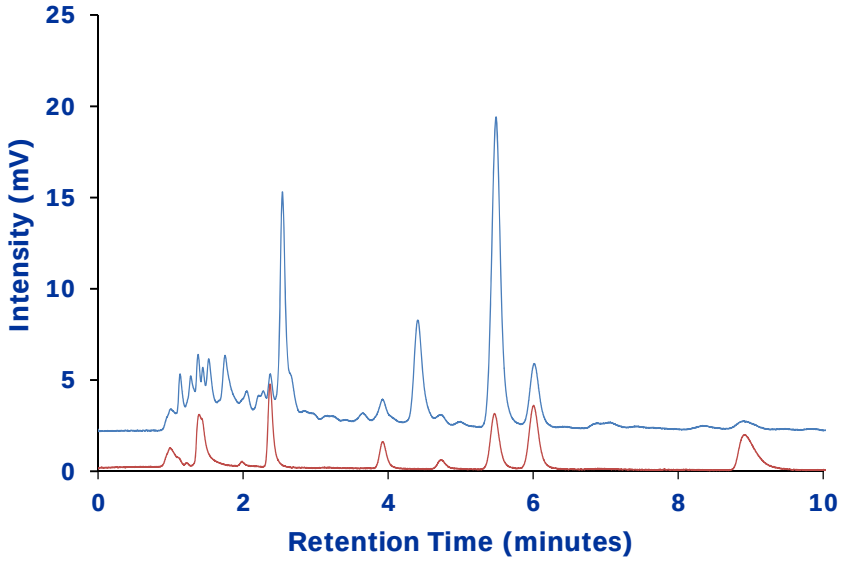
No.	Compound	Retention Time (min)	Theoretical plates	Tailing Factor
1	Cyclamate	6.0	4329	1.1

Determination of Organic Acids in wine

SeQuant® ZIC®-cHILIC

Chromatographic Conditions

Column:	SeQuant® ZIC®-cHILIC (3 µm, 100Å) PEEK 150x2.1 mm	(1.50658.0001)
Injection:	5 µL	
Detection:	UV at 200 nm. Shimadzu LC-10Vp equipped with 2.5µL semi-micro flow-cell	
Flow Rate:	0.3 mL/min.	
Mobile Phase (v/v):	Acetonitrile and 25mM Potassium Phosphate buffer pH 6.0 (75:25)	
Temperature:	30 °C	
Diluent	Mobile phase	
Sample:	Riesling wine (blue), 10ppm mix of standards (red) diluted in mobile phase	



Chromatographic Data

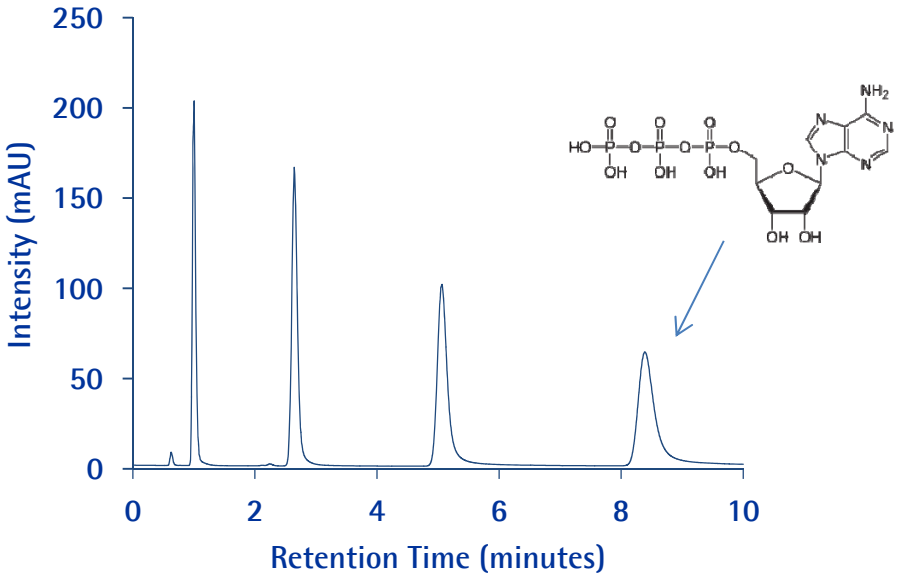
No.	Compound	Time (min)	Retention Factor
	Void volume (t0)	1	-
1	Acetic acid	2.4	1.4
2	Succinic acid	3.9	2.9
3	Malic acid	5.5	4.5
4	Tartaric acid	6.0	5.0
5	Citric acid	8.9	7.9

Adenosine and Nucleotides (AMP, ADP, ATP)

SeQuant® ZIC®-cHILIC

Chromatographic Conditions

Column:	SeQuant® ZIC®-cHILIC (3 µm, 100 Å) PEEK 150 x 2.1 mm	1.50658.0001
Injection:	2 µL	
Detection:	UV at 254 nm. Shimadzu LC-10Vp equipped with 2.5µL semi-micro flow-cell	
Flow Rate:	0.4 mL/min.	
Mobile Phase:	Acetonitrile and 100 mM ammonium acetate adjusted to pH 4.5 by glacial acetic acid (70:30). Total ionic strength in mobile phase = 30 mM.	
Temperature:	40 °C	
Diluent	Mobile phase	
Sample:	Solution with 25 µg/mL of adenosine and AMP; 50 µg/mL ADP and ATP in mobile phase	
Pressure drop:	87 bar (1253 psi)	



Chromatographic Data

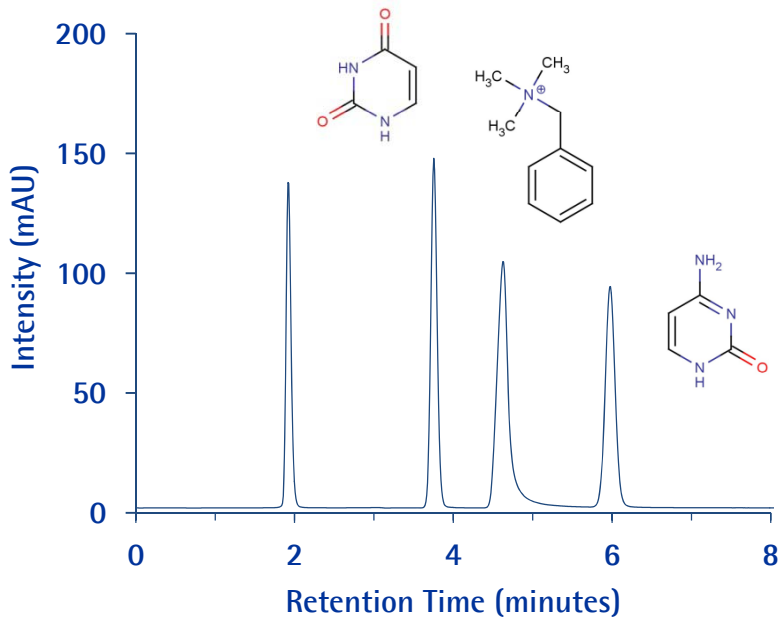
No.	Compound	Time (min)	Retention Factor
	Toluene (Void volume (t0))	0.6	-
1	Adenosine	1.0	0.6
2	AMP	2.7	3.2
3	ADP	5.1	7.1
4	ATP	8.4	12.4

Toluene, Uracil, Cytosine, and Benzyl Trimethyl Ammonium (BTMA)

SeQuant® ZIC®-cHILIC

Chromatographic Conditions

Column:	SeQuant® ZIC®-cHILIC (3 µm, 100 Å) PEEK 100 x 4.6 mm	1.50660.0001
Injection:	10 µL	
Detection:	UV at 254 nm. Shimadzu LC-10Vp equipped with 2.5µL semi-micro flow-cell	
Flow Rate:	0.5 mL/min.	
Mobile Phase (v/v):	Acetonitrile and 25 mM ammonium acetate, pH 6.8, (75/25). Total ionic strength = 6.25 mM	
Temperature:	Ambient	
Diluent	Mobile phase	
Sample:	200 µg/mL toluene, 7.5 µg/mL uracil, 15 µg/mL BTMA, and 10 µg/mL cytosine in mobile phase	



Chromatographic Data

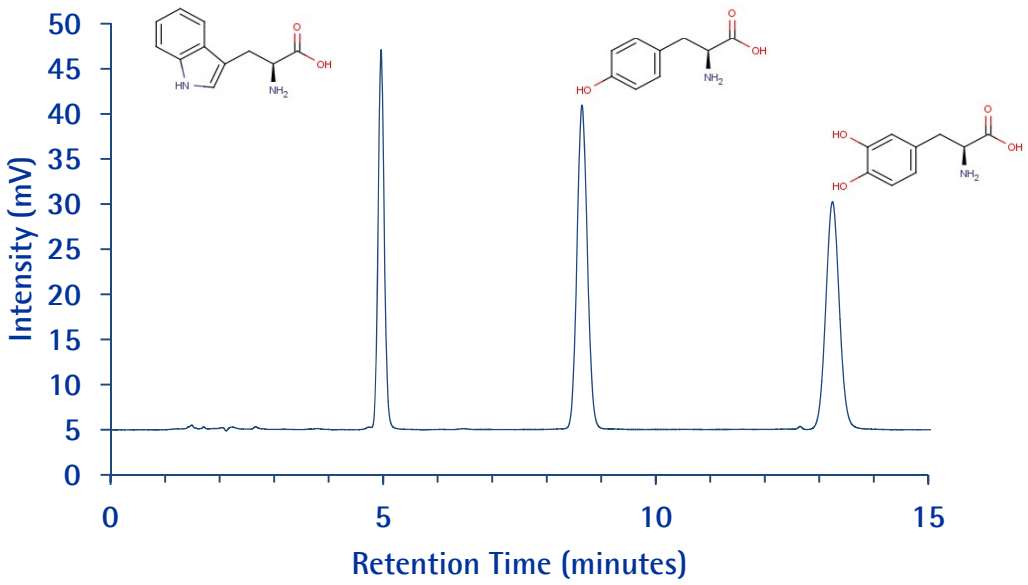
No.	Compound	Time (min)	Theoretical Plate	Tailing Factor
1	Toluene (void volume (t0))	1.9	4881	1.3
2	Uracil	3.8	10961	1.1
3	BTMA	4.6	5751	1.1
4	Cytosine	6.0	11127	1.0

Tryptophan, Tyrosine, 3,4-Dihydroxyphenylalanine

SeQuant® ZIC®-cHILIC

Chromatographic Conditions

Column:	SeQuant® ZIC®-cHILIC (3 µm, 100 Å) PEEK 100 x 4.6 mm	1.50660.0001
Injection:	1 µL	
Detection:	UV at 254 nm.	
Flow Rate:	1.0 mL/min.	
Mobile Phase (v/v):	Acetonitrile and 25 mM ammonium acetate, pH 6.8, (80/20). Total ionic strength = 5 mM	
Temperature:	23 °C	
Diluent	Mobile phase	
Sample:	Tryptophan (9 mg), Tyrosine (104.4 mg), 3,4-dihydroxyphenylalanine (97.5 mg) diluted in 100 mL diluent + 1 mL ortophosporic acid	



Chromatographic Data

No.	Compound	Time (min)	Theoretical Plate	Tailing Factor
1	Tryptophan	5.0	8060	1.2
2	Tyrosine	8.6	10220	1.1
3	3,4-dihydroxyphenylalanine	13.2	13325	1.1