Hydrophilic Interaction Chromatography (HILIC) Using Agilent Poroshell 120 HILIC

Application Note

Authors

William J. Long and Anne E. Mack

Agilent Technologies, Inc.

Introduction

Hydrophilic interaction chromatography (HILIC) is gaining popularity in liquid chromatography particularly for its ability to retain and separate small polar analytes - an area where common reversed-phase liquid chromatography (RPLC) methods often fail. This novel mode of chromatography results in unique retention mechanisms, because water is used as the strong eluting solvent and can have distinct advantages over traditional RPLC in both sample preparation and LC/MS sensitivity due to the use of highly organic mobile phases. The highly organic mobile phases do not require samples in organic solvents to be dried prior to injection, and their higher volatility than traditional RPLC mobile phases makes this technique well suited for applications with mass spectrometers [1].

HILIC retention on a silica-based column is believed to involve a combination of mechanisms. First, a water layer must be adsorbed onto the polar silica surface creating a liquid/liquid extraction system. The polar analytes can then partition into and out of this adsorbed water layer with more polar analytes having a stronger interaction with this immobilized water layer. Charged polar analytes can also undergo ion exchange with the charged silica. Elution is typically from least to most polar, the opposite of RPLC. For HILIC method development, it is important to remember that the solvent strengths are different than in RPLC. For HILIC mode, solvent strength is tetrahydrofuran < acetone < acetonitrile < isopropanol < ethanol < methanol < water, with water being the strongest solvent [2,3].

Agilent Technologies

The development of superficially porous particles has led to the possibilities of method transfer from larger 5- and 3.5-µm particles and from smaller 1.8-µm particles. HILIC mobile phases have much lower viscosities and so generate lower backpressure. The columns possess the advantage of robustness in sample preparation because they have the same 2-µm frit used with larger columns. HILIC columns deliver nearly the same efficiency as the sub-2µm columns with better robustness. In this work, several applications of HILIC are transferred from totally porous columns (Agilent ZORBAX Rx-Sil, 5 µm, or RRHD HILIC Plus, 1.8 µm) to superficially porous Agilent Poroshell 120 HILIC columns [4].

Experimental

Two systems were used in this work, an Agilent 1200 SL with a G1315C diode array detector and an Agilent 1290 Infinity LC System with an Agilent 6410A Triple Quadrupole Mass Spectrometer. The setup on the mass-spectrometer-equipped system was optimized for the lowest possible extra-column volume with short 0.075 mm id capillaries in the Agilent Ultra Low Dispersion Kit (p/n 5067-5189) and with an Agilent LC System Rack (p/n 5001-3726) [5]. The 1200 SL system was configured with the shortest possible 0.012 mm id tubing; delay volume was reduced by removing the pulse damper and mixing column and by using the automatic delay volume reduction (ADVR) for gradient analyses [5].

Columns

- Agilent ZORBAX Rx-Sil, 2.1 × 150 mm, 5 µm (p/n 883700-901)
- Agilent ZORBAX RRHD HILIC Plus, 2.1 × 100 mm, 1.8 µm (p/n 959758-901)
- Agilent Poroshell 120 HILIC, 2.1 × 100 mm, 2.7 µm (p/n 695775-901)
- Agilent Poroshell 120 HILIC, 3 × 150 mm, 2.7 µm (p/n 693975-301)
- Agilent Poroshell 120 EC-C18, 3 × 150 mm, 2.7 µm (p/n 693975-302)

Napthalene, uracil, epinephrine, dopamine, and norepinephrine were purchased as powders from Sigma Aldrich and prepared to desired concentrations in dimethyl sulfoxide (DMSO) and acetonitrile (MeCN). Morphine, normorphine, morphine-3-β-D-glucuronide (M3G), and morphine-6-β-D-glucuronide (M6G) were purchased as solutions from Cerriliant and diluted in mobile phase. Acetonitrile, methanol, and DMSO were purchased from Honeywell. Ammonium formate and formic acid were purchased from Sigma Aldrich. Water used was 18 MΩ Milli-Q water and was produced in house.

Results and Discussion

In Figure 1, a sample containing a polar analyte, uracil, and a nonpolar analyte, naphthalene, were analyzed on Poroshell 120 EC-C18 and Poroshell 120 HILIC columns. Figure 1 shows the elution order was reversed. In the case of the HILIC column, the uracil, a difficult to retain compound that is analyzed in many metabolomic studies, was strongly retained.

Conditions

Mobile phase:	90/10 MeCN/100 mM NH_4HCO_2H pH 3.2
Flow rate:	0.84 mL/min
Pressure:	220 bar Poroshell 120 HILIC; 224 bar Poroshell 120 EC-C18

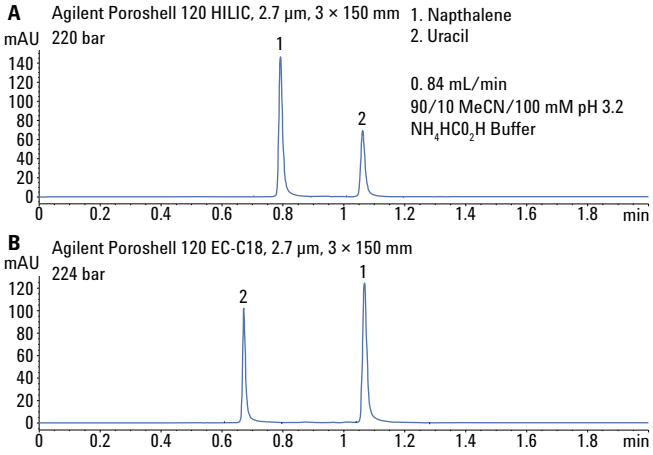

Figure 1. Comparison of the Agilent Poroshell 120 HILIC, 3 × 150 mm, 2.7 µm column (A) and the Agilent Poroshell 120 EC-C18, 3 × 150 mm, 2.7 µm reversed phase column (B).

Catecholamines are often analyzed by RPLC [6]. In Figure 2, a gradient separation of 3 catecholamines was carried out using a 5-µm ZORBAX Rx-Sil column and a shorter 2.7-µm Poroshell 120 HILIC column. Several quick observations can be made. Because the Poroshell 120 HILIC column was shorter than the original ZORBAX Rx-Sil column, 66% of the length, each segment of the gradient was reduced proportionally. Because both columns were of the same diameter, scaling was done by changing the gradient according to the length of the column. The injection volume was changed according to the volume of the column. In the case of the smaller Poroshell 120 HILIC column, the injection volume was 66% of the ZORBAX Rx-Sil column (0.5 µL versus 0.75 µL). Also evident is the higher pressure generated by the 2.7 µm Poroshell 120 HILIC particles as compared to the 5 µm ZORBAX Rx-Sil particles. This increase in pressure was due to the smaller particle size of the superficially porous particles. The shorter length of the Poroshell 120 HILIC column helped reduce the pressure. The separation achieved was similar on the 2 columns. However, the epinephrine/norepinephrine pair were slightly better separated on the Poroshell 120 HILIC column. Overall, the peaks were sharper on the Poroshell 120 HILIC column, and the last peak eluted at 3 minutes versus more than 6 minutes on the ZORBAX Rx-Sil column.

Conditions

Samples: Prepared individually in DMSO at 5 mg/mL, mixed in equal parts. ZORBAX Rx-Sil injected neat; Poroshell 120 HILIC diluted with acetonitrile to 2/3 neat injection volume.

Mobile phase:	A: 100 mM NH_4HCO_2H B: acetonitrile	
Gradient		
Agilent Poroshell		
120 HILIC:	Time	%B
	Initial	97
	4	85
	4.67	85
	5	97
	6.67	97
Gradient		
Agilent ZORBAX		
RX-Sil:	Time	%B
	Initial	97
	6	85
	7	85
	7.5	97
	10	97
Injection volume:	0.5 µL	
Pressure:	168 bar Agilent Poroshell 120 HILIC 68 bar Agilent ZORBAX Rx-Sil	
Flow rate:	0.6 mL/min	
Instrument:	Agilent 1200 SL AVR "ON" no mixer, no pulse damper	
Flow cell:	6 mm, 5 µL (p/n G1315-60025) G1315C	
Binary pump:	Agilent G1312B	
Autosampler:	Agilent G1367C Automatic Liquid Sampler	

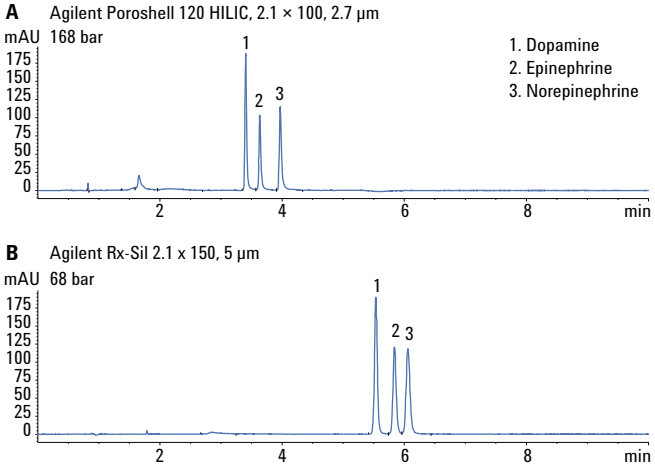

Figure 2. Overlay of HILIC separation of catecholamines on a superficially porous Agilent Poroshell 120 HILIC, 2.1 × 100, 2.7 µm column (A) and a totally porous Agilent ZORBAX Rx-Sil, 2.1 × 150, 5 µm column (B).

Figure 3 shows a gradient separation of morphine and its metabolites on a Poroshell 120 HILIC column and a ZORBAX RRHD HILIC Plus column [7]. While this separation can also be carried out using reversed phase, several advantages have been shown in using the HILIC separation with mass spectrometry detection. A fourfold increase in sensitivity with HILIC mode was produced due to more efficient spraying and desolvation in the ESI-MS source as compared to RPLC. This generated less baseline noise and a more intense MS signal. Figure 3 shows the elution order and selectivity of the compounds were the same on the superficially porous and totally porous columns. The pressure generated by the ZORBAX RRHD HILIC Plus 1.8 µm column was approximately 100 bar higher than the pressure on the Poroshell 120, 2.7 µm column. At this flow rate, the method can be run on a wide variety of instruments. The 0.075 mm id tubing from the low dispersion kit allows easy connection to the mass spectrometer with minimal peak dispersion. In reverse phase mode with its high aqueous content, this may be more of a problem due to high pressure caused by highly viscous solvents, limiting the analysis to only UHPLC systems.

Conditions

Sample:	2 µL injection of 1 µg/mL each of morphine, normorphine, morphine-3-β-D-glucuronide, and morphine-6-β-D-glucuronide. HILIC sample was prepared in CH_3CN; RPLC sample was prepared in H_2O
Mobile phase:	A: 100 mM NH_4HCO_2 pH 3.2 B: acetonitrile:100 mM NH_4HCO_2 pH 3.2 (9:1)
Gradient:	Time %B 0 100 0.88 100 3.85 55
Flow rate:	0.4 mL/min
Temp:	25 °C
MS source:	Positive ESI, capillary, 4000 V, drying gas temperature, flow rate and nebulizer pressure vary with mobile phase flow rate
MS acquisition:	Selected ion mode (SIM), delta EMV 200 V, MS dwell time varies with mobile phase flow rate
Software:	Agilent MassHunter version B.03.01 for data acquisition and qualitative analysis.

When coupled to reversed phase SPE or other sample preparation methods that would present the sample in acetonitrile, less work needs to be carried out because a HILIC method could be injected with little or no filtration.

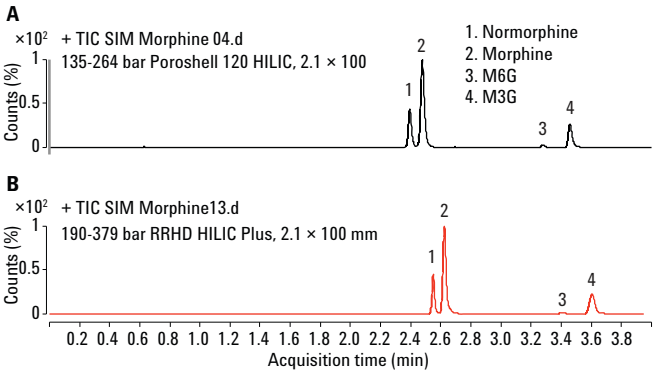

Figure 3. Morphine and metabolite separation on an Agilent Poroshell 120 HILIC 2.7 µm column (A) and and Agilent ZORBAX RRHD HILIC Plus 1.8 µm (B).

Conclusions

Elution of polar and nonpolar compounds was reversed on the Poroshell 120 HILIC column as compared to the Poroshell 120 EC-C18 column. The Poroshell 120 HILIC column was similar to the ZORBAX Rx-Sil column for many applications. Peaks were narrower and pressure was higher on the Poroshell 120 HILIC column as compared to the ZORBAX Rx-Sil column, but not sufficient to cause a problem due to the low viscosity solvents used in HILIC. The Poroshell 120 HILIC column was also similar to the ZORBAX RRHD HILIC Plus column for many applications.

The Poroshell 120 HILIC column is ideal for mass spectrometry applications especially when low dispersion tubing is used. The extra pressure generated by the tubing may be offset by the lower pressure of the Poroshell 120, 2.7 µm particles as compared to sub-2 µm particles. The Poroshell 120 HILIC column yields similar efficiencies to the sub-2 µm totally porous ZORBAX RRHD HILIC Plus column, and it may prove to be more robust in situations with moderately dirty samples due to a larger frit at the inlet of the Poroshell 120 column.

References

1. A. J. Alpert. *J. Chromatog*. **499**, 177 (1990).
2. M.R. Gama, R.G.C. Silva, C.H. Collins, C.B.G. Bottoli. *Trends in Anal. Chem*. **37**, 48 (2012).
3. D.V. McCalley, *J. Chromatog. A*. **1217**, 3408 (2010).
4. William J. Long. "Transfer of Methods between Poroshell 120 EC-C18 and ZORBAX Eclipse Plus C18". Technical Overview, Agilent Technologies, Inc., Publication Number 5990-6588EN (2011).
5. A.E. Mack. "Optimizing Performance of an Agilent ZORBAX RRHD Eclipse Plus C18 Column by Enhancing an Agilent 1290 Infinity LC System for Ultra-Low Dispersion". Application Note, Agilent Technologies, Inc., Publication Number 5990-9502EN (2011).
6. U. Huber. "Analysis of Catecholamines by HPLC". Application Note, Agilent Technologies, Inc., Publication Number 5968-2966EN (1998).
7. A.E. Mack. "Comparing HILIC and RPLC of Morphine Using Agilent ZORBAX RRHD Columns with UHPLC/MS". Application Note, Agilent Technologies, Inc., Publication Number 5990-9502EN (2011).

For More Information

These data represent typical results. For more information on our products and services, visit our Web site at www.agilent.com/chem.

www.agilent.com/chem

Printed in the USA
October 5, 2012
5991-1242EN

Agilent Technologies