



# HALO<sup>®</sup>

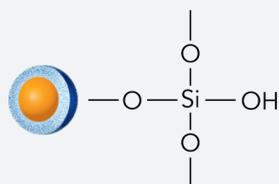
## HILIC SOLUTIONS

DESIGNED FOR RETAINING  
VERY POLAR ANALYTES

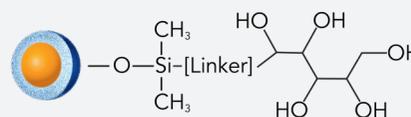


# HALO<sup>®</sup> HILIC PHASES

## HILIC Solutions: HALO<sup>®</sup> HILIC and Penta-HILIC



HALO 90 Å HILIC



HALO 90 Å Penta-HILIC

### OVERVIEW OF HILIC

- HILIC stands for Hydrophilic Interaction Liquid Chromatography, introduced in 1990
- Designed for retaining very polar analytes not well retained in reversed-phase methods
- Uses a polar stationary phase with a reversed-phase-like mobile phase
- Ideal for analytes with  $\log P \leq 0$  or  $\log D \leq 0$

### HALO<sup>®</sup> HILIC vs HALO<sup>®</sup> Penta-HILIC

- HALO HILIC is bare silica, whereas HALO Penta-HILIC is bonded silica with a proprietary ligand
- Penta-HILIC has five hydroxyl groups improving polarity and minimizing ionic interactions

### Ideal for polar compounds containing a variety of functional groups:

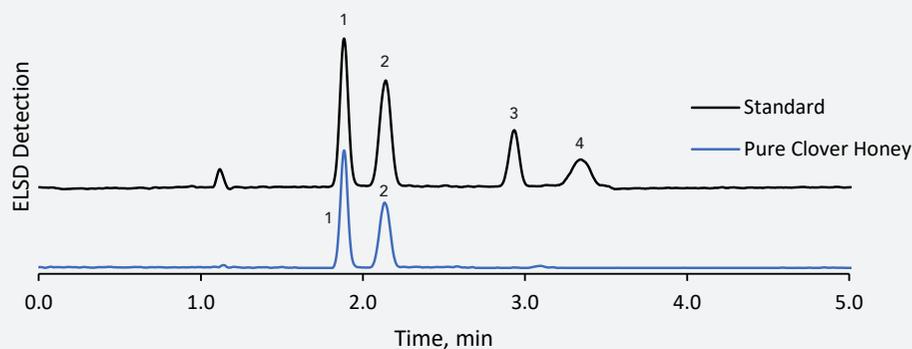
- Zwitterions
- Strong and weak acids and bases
- Amino acids, sugars, glycopeptides, nucleobases, nucleosides, nucleotides and derivatives

Characteristic	HALO <sup>®</sup> HILIC	HALO <sup>®</sup> Penta-HILIC
Ligand	N/A	proprietary penta-hydroxy ligand
Particle Size (µm)	2, 2.7, 5	2, 2.7, 5
Pore Size (Å)	90	90
USP Designation	L3	L86, L95
Carbon Load	unbonded	2.8, 3.2, 2.1
Surface Area	125, 125, 90	125, 125, 90
Endcapped	N/A	No
Low pH Limit	1	2
High pH Limit	8	9
Temp limit @ low pH	60	60
Temp limit @ high pH	40	40



## ANALYSIS OF SUGARS IN PURE HONEY USING HALO® PENTA-HILIC

Honey can significantly range in quality depending on its purity and levels of sucrose and maltose. Natural honey primarily consists of fructose and glucose, while adulterated honey can contain high levels of sucrose and maltose. A HALO® Penta-HILIC column separates the primary monosaccharides in pure honey clover showing no signs of adulteration.



### TEST CONDITIONS:

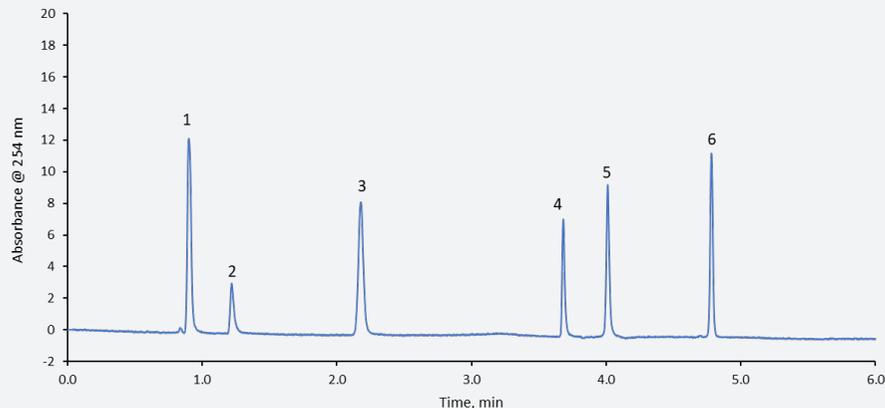
Column: HALO 90 Å Penta-HILIC, 2.7  $\mu$ m, 4.6 x 150 mm  
 Part Number: 92814-705  
 Mobile Phase A: Water  
 Mobile Phase B: Acetonitrile  
 Isocratic: 80% B  
 Flow Rate: 1.4 mL/min.  
 Initial Pressure: 213 bar  
 Temperature: 65 °C  
 Detection: ELSD, 40 °C, 3.3 bar  
 Injection Volume: 15  $\mu$ L  
 Sample Solvent: 80/20 ACN/ Water  
 Data Rate: 10 Hz  
 Response Time: 0.10 sec.  
 LC System: Shimadzu Nexera X2

**PEAK IDENTITIES:**

1. D(-) Fructose
2. D(+) Glucose
3. Sucrose
4. D(+) Maltose

## WATER-SOLUBLE VITAMINS SEPARATED ON HALO® PENTA-HILIC

An alternative method to reversed-phase for analysis of water soluble vitamins is demonstrated on a HALO® Penta-HILIC column using HILIC conditions. All of the vitamins are baseline resolved and the separation is complete in less than 5 minutes. While there can be resistance and challenges to using HILIC mode, this separation shows how useful it can be in terms of retention and resolution.



**PEAK IDENTITIES:**

1. Nicotinamide (B3)
2. Pyridoxine (B6)
3. Riboflavin (B2)
4. Thiamine (B1)
5. Ascorbic Acid (C)
6. Cyanocobalamin (B12)

### TEST CONDITIONS:

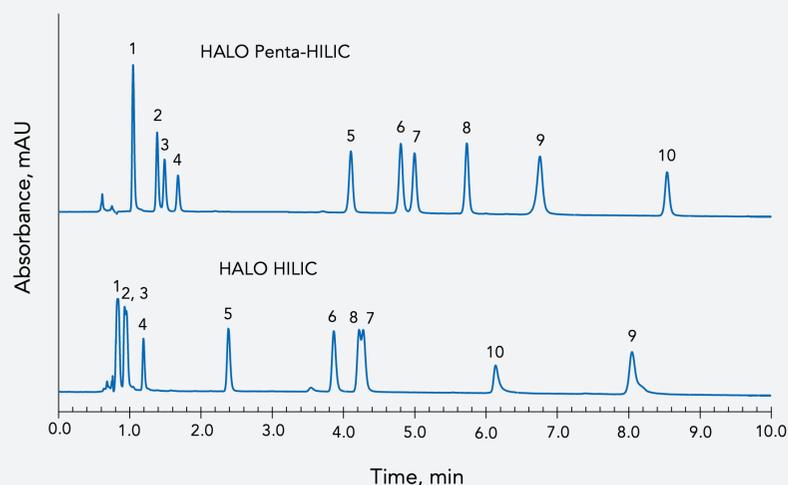
Column: HALO 90 Å Penta-HILIC, 2.7  $\mu$ m, 2.1 x 150 mm  
 Part Number: 92812-705  
 Mobile Phase A: 10 mM ammonium acetate + 0.05% acetic acid in 90/10 water/acetonitrile  
 Mobile Phase B: 10 mM ammonium acetate + 0.05% acetic acid in 10/90 water/acetonitrile  

Gradient:	Time	%B
	0.00	100
	1.00	100
	6.00	50
	7.00	100
	12.00	100

Flow Rate: 0.5 mL/min.  
 Pressure: 202 bar  
 Temperature: 40 °C  
 Detection: UV 254 nm, PDA  
 Injection Volume: .5  $\mu$ L  
 Sample Solvent: 50/50 water/acetonitrile  
 Data Rate: 40 Hz  
 Response Time: 0.05 sec.  
 Flow Cell: 1  $\mu$ L  
 LC System: Shimadzu Nexera X2

## SEPARATION OF CEPHALOSPORINS ON HALO® PENTA-HILIC AND HALO® HILIC

Analyzing these drugs using the HALO® Penta-HILIC phase offers an alternate selectivity to HALO® HILIC, highlighting improved resolution with HALO® Penta-HILIC.



### PEAK IDENTITIES:

- |                |                     |
|----------------|---------------------|
| 1. Cephalothin | 6. Cephalexin       |
| 2. Cefoxitin   | 7. Cephadrine       |
| 3. Cefotaxime  | 8. Cefadroxil       |
| 4. Cefazolin   | 9. Ceftazidime      |
| 5. Cefaclor    | 10. Cephalosporin C |

### TEST CONDITIONS:

**Columns:**  
 HALO 90 Å Penta-HILIC, 2.7 µm, 2.1 x 150 mm  
 HALO 90 Å HILIC, 2.7 µm, 2.1 x 150 mm

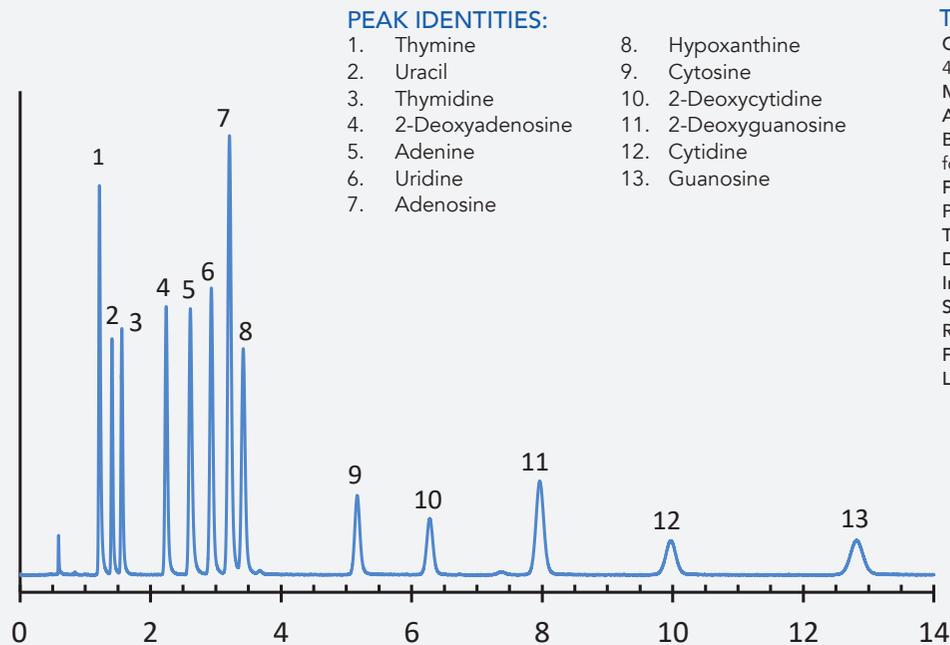
**Mobile Phase:**  
 A: 95/5 ACN/H<sub>2</sub>O with 5 mM NH<sub>4</sub>formate, pH 3.0  
 B: 50/50 ACN/H<sub>2</sub>O with 5 mM NH<sub>4</sub>formate, pH 3.0 (adj.)

**Gradient:** 85-65% B in 10 min (Penta-HILIC)  
 85-70% B in 10 min (HILIC)

**Flow Rate:** 0.5 mL/min.  
**Pressure:** 195 bar (Penta-HILIC)  
 163 bar (HILIC)  
**Temperature:** 30 °C  
**Detection:** UV 254 nm, VWD  
**Injection Volume:** 0.5 µL  
**Sample Solvent:** 50/50 ACN/water  
**Flow Cell:** 5.0 µL semi-micro  
**LC System:** Agilent 1100

## SEPARATION OF NUCLEOSIDES AND NUCLEOBASES ON HALO® PENTA-HILIC

Here, a mixture of 13 nucleosides and nucleobases are separated isocratically in a short time with excellent resolution. These bonded superficially porous 2.7 µm HALO® particles allow high resolution with modest back pressure.



### PEAK IDENTITIES:

- |                     |                      |
|---------------------|----------------------|
| 1. Thymine          | 8. Hypoxanthine      |
| 2. Uracil           | 9. Cytosine          |
| 3. Thymidine        | 10. 2-Deoxycytidine  |
| 4. 2-Deoxyadenosine | 11. 2-Deoxyguanosine |
| 5. Adenine          | 12. Cytidine         |
| 6. Uridine          | 13. Guanosine        |
| 7. Adenosine        |                      |

### TEST CONDITIONS:

**Column:** HALO 90 Å Penta-HILIC, 2.7 µm, 4.6 x 100 mm

**Mobile Phase:** 8/92 - A/B  
 A: Water  
 B: Acetonitrile with 0.01 M ammonium formate, pH 6.0 (adj.)

**Flow Rate:** 1.5 mL/min.  
**Pressure:** 99 bar  
**Temperature:** 35 °C  
**Detection:** UV 260 nm, DAD  
**Injection Volume:** 2.0 µL  
**Sample Solvent:** Mobile phase  
**Response Time:** 0.02 sec  
**Flow Cell:** 2.5 µL semi-micro  
**LC System:** Shimadzu Nexera



## PART NUMBERS

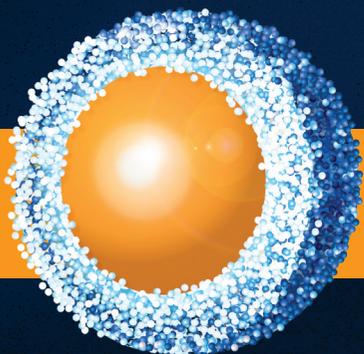
Dimensions: ID x Length (in mm)	90 Å HILIC (2 µm)	90 Å HILIC (2.7 µm)	90 Å HILIC (5 µm)	90 Å Penta-HILIC (2 µm)	90 Å Penta-HILIC (2.7 µm)	90 Å Penta-HILIC (5 µm)
0.5 x 50		98215-401			98215-405	
0.5 x 100		98215-601			98215-605	
0.5 x 150		98215-701			92815-705	
0.3 x 50		98216-401			98216-405	
0.3 x 100		98216-601			92816-605	
0.3 x 150		98216-701			92816-705	
2.1 x 20	91812-201	92812-201	95812-201	91812-205	92812-205	95812-205
2.1 x 30	91812-301	92812-301	95812-301	91812-305	92812-305	95812-305
2.1 x 50	91812-401	92812-401	95812-401	91812-405	92812-405	95812-405
2.1 x 50 INERT		P2812-401			P2812-405	
2.1 x 75	91812-501	92812-501	95812-501	91812-505	92812-505	95812-505
2.1 x 100	91812-601	92812-601	95812-601	91812-605	92812-605	95812-605
2.1 x 100 INERT		P2812-601			P2812-605	
2.1 x 150	91812-701	92812-701	95812-701	91812-705	92812-705	95812-705
2.1 x 150 INERT		P2812-701			P2812-705	
2.1 x 250	91812-901	92812-901	95812-901	91812-905	92812-905	95812-905
3.0 x 20	91813-201	92813-201	95813-201	91813-205	92813-205	95813-205
3.0 x 30	91813-301	92813-301	95813-301	91813-305	92813-305	95813-305
3.0 x 50	91813-401	92813-401	95813-401	91813-405	92813-405	95813-405
3.0 x 75	91813-501	92813-501	95813-501	91813-505	92813-505	95813-505
3.0 x 100	91813-601	92813-601	95813-601	91813-605	92813-605	95813-605
3.0 x 150	91813-701	92813-701	95813-701	91813-705	92813-705	95813-705
3.0 x 250	91813-901	92813-901	95813-901	91813-905	92813-905	95813-905
4.6 x 20		92814-201	95814-201		92814-205	95814-205
4.6 x 30		92814-301	95814-301		92814-305	95814-305
4.6 x 50		92814-401	95814-401		92814-405	95814-405
4.6 x 75		92814-501	95814-501		92814-505	95814-505
4.6 x 100		92814-601	95814-601		92814-605	95814-605
4.6 x 150		92814-701	95814-701		92814-705	95814-705
4.6 x 150 INERT		P2814-701				
4.6 x 250		92814-901	95814-901		92814-905	95814-905
10.0 x 50		92810-401	95810-401		92810-405	95810-405
10.0 x 100		92810-601	95810-601		92810-605	95810-605
10.0 x 150		92810-701	95810-701		92810-705	95810-705
10.0 x 250			95810-901			95810-905
1.0 x 50		92811-401			92811-405	
1.0 x 100		92811-601			92811-605	
1.0 x 150		92811-701			92811-705	
1.5 x 50					9281X-405	
1.5 x 100					9281X-605	
1.5 x 150					9281X-705	

### HALO® GUARD COLUMNS 3 PACK

Dimensions: ID x Length (in mm)	90 Å HILIC (2 µm)	90 Å HILIC (2.7 µm)	90 Å HILIC (5 µm)	90 Å Penta-HILIC (2 µm)	90 Å Penta-HILIC (2.7 µm)	90 Å Penta-HILIC (5 µm)
2.1 x 5	91812-101	92812-101	95812-101	91812-105	92812-105	95812-105
3.0 x 5	91813-101	92813-101	95813-101	91813-105	92813-105	95813-105
4.6 x 5		92814-101	95814-101		92814-105	95814-105
Guard Column Holder	94900-001					



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