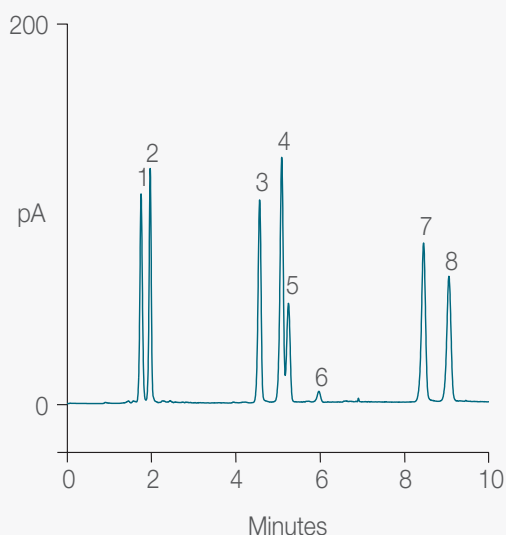


Acclaim HILIC-10 column

Separating highly hydrophilic molecules

The Thermo Scientific™ Acclaim™ HILIC-10 column is designed for separating highly hydrophilic molecules by hydrophilic interaction liquid chromatography (HILIC). This column is based on high-purity, spherical porous silica gel covalently modified with a proprietary hydrophilic layer, and is suited for use in a broad range of applications including separation of hydrophilic drugs and drug metabolites.

Separation of glycerides using the Acclaim HILIC-10 column



Column	Acclaim HILIC-10, 3 µm					
Dimensions	3.0 × 150 mm					
Mobile phase	A: Heptane B: 2-Propanol-acetic acid 99.5:0.5					
Step elution (min)	-6.0	0.0	0.5	4.0	10.0	
	%A	99	99	96	87	87
	%B	1	1	4	13	13
Flow rate	0.50 mL/min					
Temperature	25 °C					
Inj. volume	4 µL					
Detector	Corona <i>ultra</i> , nebulizer 15 °C					
Peaks	1. Tristearin					
	2. Trilaurin					
	3. Distearin isomer 1					
	4. Dilaurin isomer 1					
	5. Distearin isomer 2					
	6. Dilaurin isomer 2					
	7. Monostearin					
	8. Monolaurin					

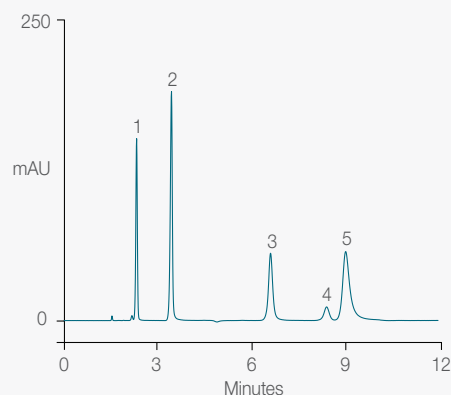
Features

- Retains highly polar molecules that are unretained by reversed-phase chromatography
- Unique selectivity, complementary to reversed-phase columns
- Hydrolytically stable
- Rugged column packing
- Broad application range

Column chemistry

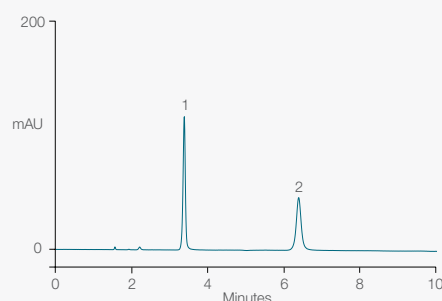
HILIC is a complementary technique to reversed-phase liquid chromatography (RPLC) with several benefits. Polar analytes that cannot be retained using RP columns can be retained and separated using the Acclaim HILIC-10 column. Traditionally, Normal-Phase Liquid Chromatography (NPLC) has been used for this type of separation, utilizing a polar stationary phase such as unbonded silica with highly organic mobile phases. The limitation of unbonded silica is that irreproducible results may be obtained due to the presence of uncontrollable significantly improved and enhanced diagnostic tools for efficient support are provided.

The advantage of the HILIC phases is that they allow the use of 5–20% aqueous mobile phase, while maintaining affinity for polar analytes. The Acclaim HILIC-10 column is rugged and reproducible with greater control of the chromatographic separation. This column is also superior for electrospray liquid chromatography-mass spectrometry (LC-MS) applications with highly polar compounds; the organic solvent-rich mobile phase used in HILIC provides a ten- to twenty-fold improvement in sensitivity. Moreover, by eliminating the need for evaporation and reconstitution of a sample dissolved in a nonaqueous solvent, sample throughput can be greatly increased.



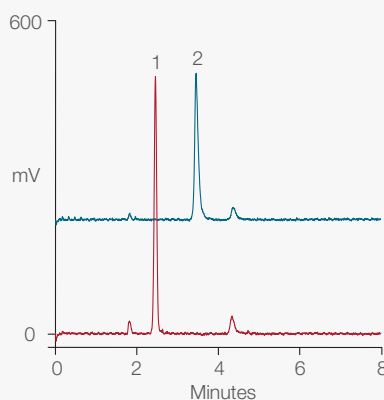
Column	Acclaim HILIC-10, 3 μ m
Dimensions	4.6 $\times$ 150 mm
Mobile phase	90/10 v/v CH ₃ CN/10 mM (total) NH ₄ OAc, pH 5
Flow rate	1 mL/min
Temperature	30 $^{\circ}$ C
Inj. volume	2 μ L
Detection	UV at 230 nm
Peaks	1. Acetaminophen 0.1 mg/mL 2. Salicylic acid 0.1 mg/mL 3. Aspirin 0.2 mg/mL 4. Penicillin G 0.1 mg/mL 5. Metformin 0.1 mg/mL

Figure 1. Separation of hydrophilic pharmaceuticals



Column	Acclaim HILIC-10, 3 μ m
Dimensions	4.6 $\times$ 150 mm
Mobile phase	90/10 v/v CH ₃ CN/10 mM (total) NH ₄ OAc, pH 5
Flow rate	1 mL/min
Temperature	30 $^{\circ}$ C
Inj. volume	1 μ L
Detection	UV at 220 nm
Peaks	1. Salicylic acid 0.1 mg/mL 2. Aspirin 0.2 mg/mL

Figure 2. Separation of aspirin and degradation product



Column	Acclaim HILIC-10, 3 μ m
Dimensions	4.6 $\times$ 150 mm
Mobile phase:	90/10 v/v CH ₃ CN/10 mM (total) NH ₄ OAc, pH 5
Flow rate	1 mL/min
Temperature	30 $^{\circ}$ C
Inj. volume	2 μ L
Detection	Corona CAD <i>ultra</i>
Peaks	1. Cyanuric acid 0.2 mg/mL 2. Melamine 0.2 mg/mL

Figure 3. Separation of melamine and cyanuric acid

Applications

The Acclaim HILIC-10 column is ideally suited for analysis of polar analytes and has demonstrated its use in a wide variety of applications. In analysis of hydrophilic pharmaceuticals and metabolites (Figures 1 and 2) good chromatographic separation is achieved in <10 min with 10% aqueous mobile phase. Separation of melamine and cyanuric acid (Figure 3) has become increasingly important in detection of food and wastewater contamination. The Acclaim HILIC-10 column can be used for the simultaneous separation of both compounds.

Monomers for industrial applications (Figures 4–6) can be eluted in the order of their hydrophilicity using the Acclaim HILIC-10 column.

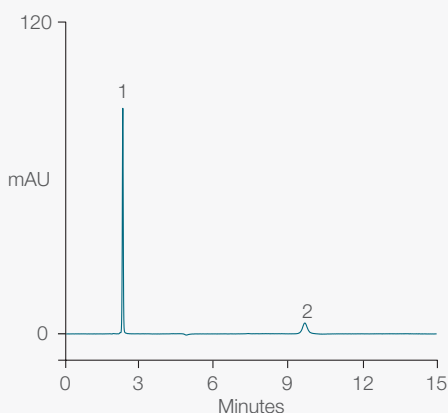


Figure 4. Separation of acrylamide and acrylic acid

Column	Acclaim HILIC-10, 3 μ m
Dimensions	4.6 $\times$ 150 mm
Mobile phase	90/10 v/v CH ₃ CN/10 mM (total) NH ₄ OAc, pH 5
Flow rate	1 mL/min
Temperature	30 $^{\circ}$ C
Inj. volume	2 μ L
Detection	UV at 230 nm
Peaks	1. Acrylamide 0.1 mg/mL 2. Acrylic acid 0.1 mg/mL

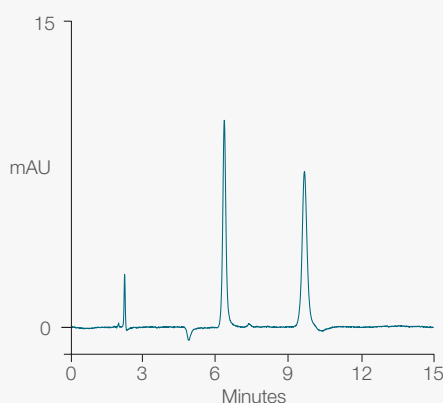


Figure 5. Separation of methacrylic acid and acrylic acid

Column	Acclaim HILIC-10, 3 μ m
Dimensions	4.6 $\times$ 150 mm
Mobile phase	90/10 v/v CH ₃ CN/10 mM (total) NH ₄ OAc, pH 5
Flow rate	1 mL/min
Temperature	30 $^{\circ}$ C
Inj. volume	2 μ L
Detection	UV at 230 nm
Peaks	1. Methacrylic acid 0.1 mg/mL 2. Acrylic acid 0.1 mg/mL

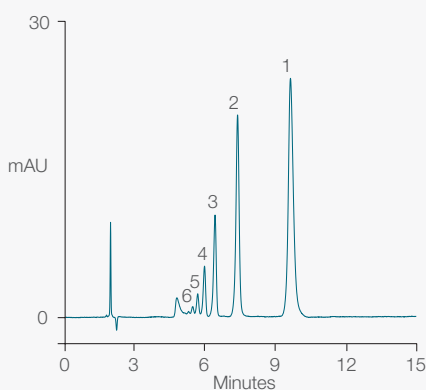


Figure 6. Separation of acrylic acid and oligomers

Column	Acclaim HILIC-10, 3 μ m
Dimensions	4.6 $\times$ 150 mm
Mobile phase	90/10 v/v CH ₃ CN/10 mM (total) NH ₄ OAc, pH 5
Flow rate	1 mL/min
Temperature	30 $^{\circ}$ C
Inj. volume	2 μ L
Detection	UV at 230 nm (5 mg/mL in mobile phase)
Peaks	1. Acrylic acid (monomer) 2. Dimer 3. Trimer 4. Tetramer 5. Pentamer 6. Hexamer

The Acclaim HILIC-10 column can be used for separation of fat-soluble vitamins. Retinyl acetate and retinyl palmitate are two forms of vitamin A that are commonly used in vitamin supplements and fortified foods. Using reversed-phase HPLC, these two compounds are widely separated and require either long run times or gradient conditions. The Acclaim HILIC-10 column provides good resolution using simple isocratic conditions as illustrated in Figure 7. Vitamin E is an antioxidant compound found in nuts, grains, and leafy green vegetables, and it protects cell membranes and other parts of the cell from damage. Deficiencies of vitamin E are believed to cause neurological problems due to poor nerve conduction. Figure 8 shows separation of vitamin E (α -tocopherol and its acetate form).

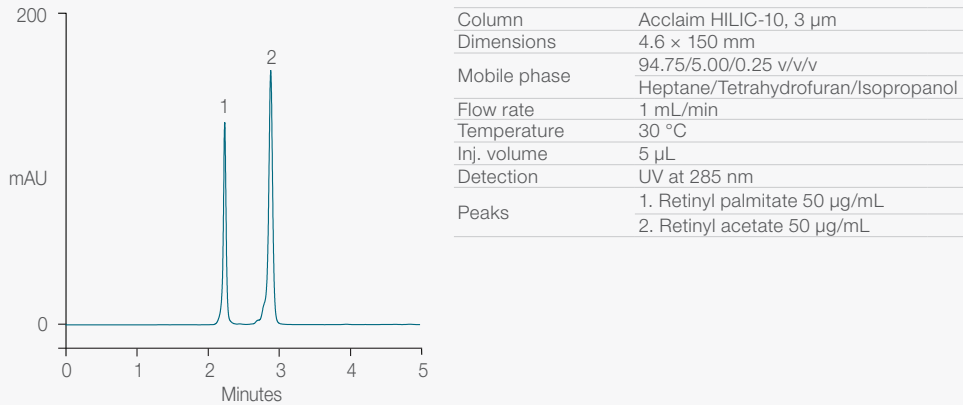


Figure 7. Separation of retinyl palmitate and retinyl acetate

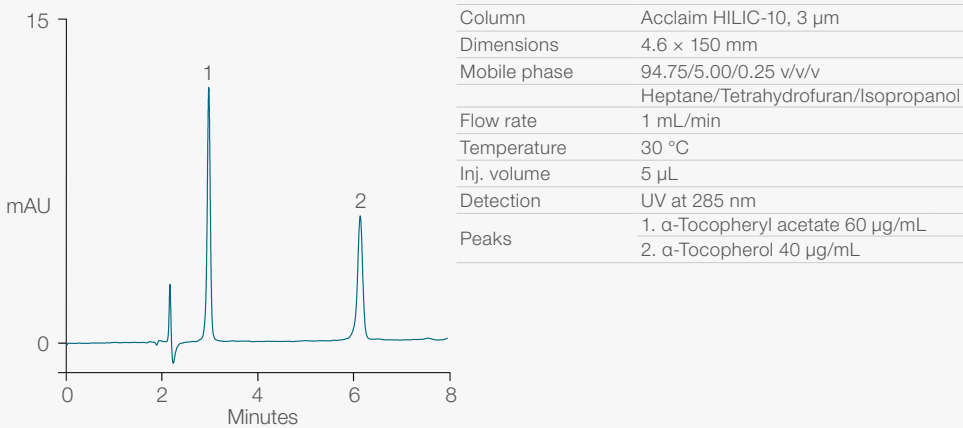


Figure 8. Separation of α -tocopherol and α -tocopheryl acetate

Excellent baseline resolution is achieved with the Acclaim HILIC-10 column for separation of Good's buffer salts (Figure 9).

The Acclaim HILIC-10 column can be operated in its primary HILIC mode or, alternately, in Normal-Phase mode. NP mode is often useful for simple class separations as shown in Figure 10 where triglycerides elute using 1% 2-propanol, diglycerides elute using 13% 2-propanol, and monoglycerides elute using 13% 2-propanol. Under these conditions, 1,2 diglycerides and 1,3 diglycerides were resolved.

Multiple column formats

The Acclaim HILIC-10 column is available in a variety of column formats: 4.6 × 150 mm, 3 µm for high-throughput routine analysis; 3.0 × 150 mm, 3 µm for high-throughput analysis with reduced solvent consumption; and 2.1 × 150 mm, 3 µm for LC-MS analysis.

Acclaim HILIC-10 columns are manufactured to stringent specifications to ensure column performance. Each column is shipped with a lot validation sheet showing the test results and specifications for the lot of bonded silica packed into the column. In addition, the columns are individually tested and shipped with a test chromatogram that validates column performance.

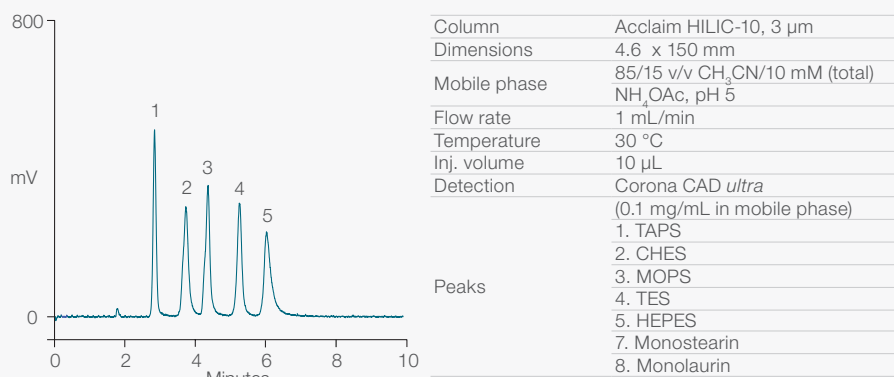


Figure 9. Separation of Good's buffer salts

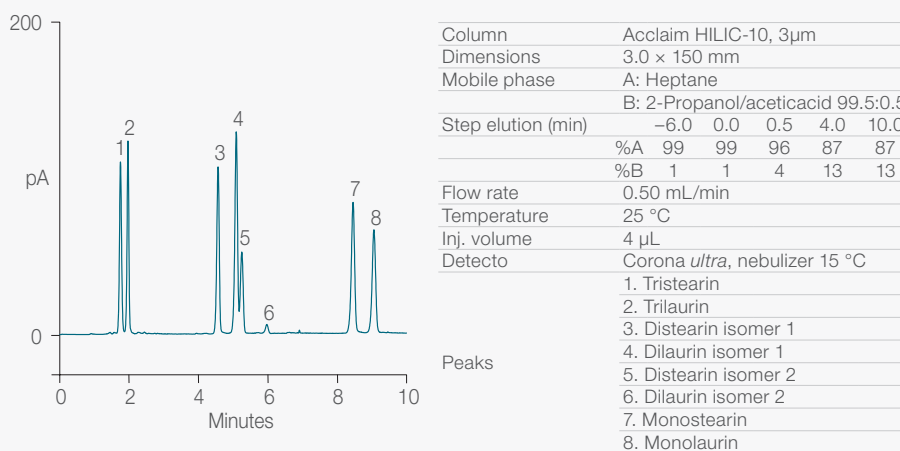


Figure 10. Separation of mono-, di-, and triglycerides

Column specifications

Specifications	
Column chemistry	Proprietary polar group
Basic silica	High purity, spherical, porous
Particle size	3 µm
Pore size	120Å
pH range	2.0 to 8.0
Temperature limit	80 °C

Ordering information

Column	Particle size (µm)	Format	Length (mm)	ID (mm)	Part number
Acclaim HILIC-10	3.0	Guard cartridge	10	2.1	074263
				3.0	074261
				4.6	074262
		HPLC column	150	2.1	074259
				3.0	074258
				4.6	074257

Acclaim Guard Holder ordering information

Guard holder	Part number
Thermo Scientific™ Acclaim™ Guard Cartridge Holder V-2	069580
Thermo Scientific™ Acclaim™ Guard Kit (Holder and coupler) V-2	069707
Guard to Analytical Column Coupler V-2	074188

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