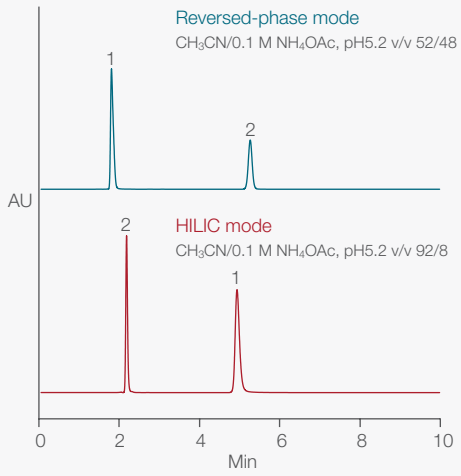
Acclaim Mixed-Mode HILIC-1 columns

A powerful tool for separating polar molecules

Thermo Scientific™ Acclaim™ Mixed-Mode HILIC-1 column utilizes a unique silica-based, mixed-mode stationary phase that combines both reversed-phase (RP) and hydrophilic interaction liquid chromatography (HILIC) properties. Unlike traditional RP or HILIC columns, this new packing features a long-chain alkyl group with a hydrophilic polar terminus, which provides great potential for separating a wide range of polar and non-polar molecules.

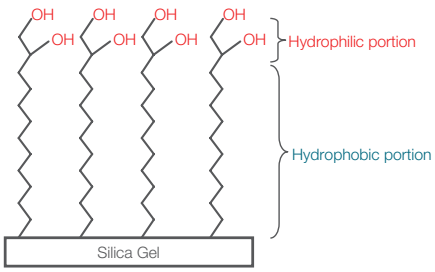
Reversed-phase and HILIC capabilities with one column



Column	Acclaim Mixed-Mode HILIC-1, 5 µm
Dimensions	4.6 x 150 mm
Mobile phase	CH_3CN/0.1 NH_4OAc, pH 5.2
Flow rate	1 mL/min
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	10 µL
Detection	UV, 254 nm
Peaks	(100 ppm each) 1. Cytosine 2. Naphthalene

Features and benefits

- Operates in both RP and HILIC modes
- Retains highly polar molecules unretained by RP columns
- Unique selectivity complementary to RP columns
- Superior performance compared to conventional diol-based columns



Surface chemistry	Alkyl Diol
Silica substrate	Spherical, high-purity
Particle size	5 µm
Surface area	300 m^2/g
Pore size	120 Å

Figure 1. Surface chemistry of the Acclaim Mixed-Mode HILIC-1 column.

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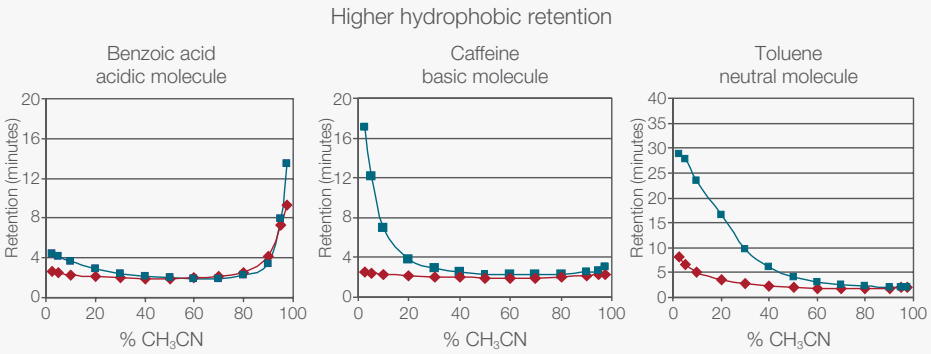
A unique column based on novel chemistry

The Acclaim Mixed-Mode HILIC-1 stationary phase combines both HILIC and RP characteristics by covalently functionalizing high-purity spherical silica with a silyl ligand consisting of both hydrophilic and hydrophobic functionalities. Although RP silica columns (e.g., C18 and C8) are widely used for small molecule separations, they are not suitable for retaining or separating highly polar compounds. HILIC columns can retain polar compounds that are unretained by RP chromatography, with additional benefits including selectivity complementary to RP columns, enhanced sensitivity for MS detection, and simplified sample preparation procedure. However, since traditional HILIC columns (e.g., unmodified silica, cyano, amino, diol phases) have hydrophilic surfaces, they are incapable of separating small molecules via hydrophobic interaction. By comparison, the Acclaim Mixed-Mode HILIC-1 column provides superior chromatographic properties and supports a broader variety of applications compared to conventional diol columns.



Columns	Acclaim Mixed-Mode HILIC-1, Conventional diol, 5 µm
Dimensions	4.6 x 150 mm
Mobile phase	CH_3CN/0.1 M NH_4OAc, pH 5.2
Flow rate	1 mL/min
Temperature	30 °C
Inj. volume	5 µL
Detection	UV, 254 nm

Figure 2. Acclaim Mixed-Mode HILIC-1 phase vs. conventional diol phase.

A mixed-mode column operating in both RP and HILIC modes

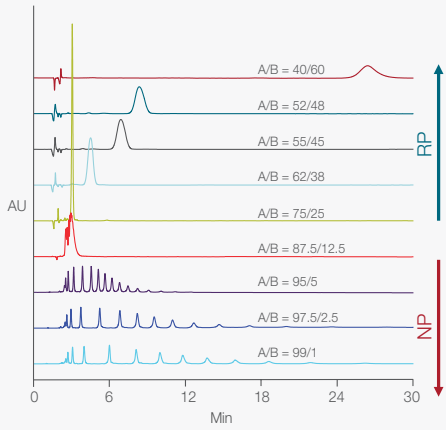
Because the Acclaim Mixed-Mode HILIC-1 column features hydrophobic alkyl chain and hydrophilic diol functionalities, it is suitable for both RP applications (in highly aqueous conditions) and HILIC applications (in highly organic conditions) as shown in Figure 1. Additionally, Figure 2 compares the retention dependency on mobile phase organic content for the Acclaim Mixed-Mode HILIC-1 column (blue squares) and a conventional diol column (red diamonds). For the acidic molecule (benzoic acid), both columns show a “U” curve—an indication of HILIC behavior. However, the Acclaim column exhibits higher retention in both RP mode and HILIC mode compared to its conventional diol counterpart.

For the basic molecule (caffeine), the conventional diol column exhibits poor retention throughout the entire range. By comparison, the Acclaim column provides substantially higher retention in highly aqueous conditions and marginally more retention in highly organic conditions. For the neutral, non-polar molecule (toluene), the Acclaim column exhibits greatly increased hydrophobic retention compared to the conventional diol column.

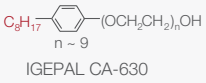
A superior HILIC column

The incorporation of hydrophobic interaction and the optimal balance between the hydrophilic and the hydrophobic moieties on the Acclaim Mixed-Mode HILIC-1 provide unique chromatographic properties compared to other commercial diol columns.

Figure 3 shows overlaid chromatograms with different mobile phase organic contents for the analysis of an alkylphenol ethoxylate (IGEPAL CA-630) using the Acclaim Mixed-Mode HILIC-1 colum. The column functions in reversed-phase mode when the mobile phase contains less than 75% acetonitrile by concentration. Retention of the hydrophobe increases as mobile phase aqueous content increases. The column functions in HILIC mode when the mobile phase concentration of acetonitrile is greater than 90%. Retention and resolution of hydrophilic oligomers increase as mobile phase organic content increases.

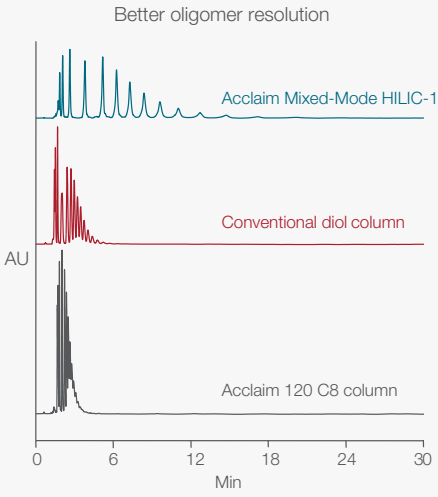


Column	Acclaim Mixed-Mode HILIC-1, 5 µm
Dimensions	4.6 x 150 mm
Mobile phase	A: CH, B: 0.1 M NH
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	10 µL
Detection	UV, 225 nm
Sample	IGEPAL CA-630 (0.1%)

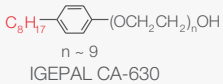


IGEPAL CA-630

Figure 3. Reversed-phase and normal phase modes on a single column.



Dimensions	4.6 x 150 mm
Mobile phase	99/1 v/v CH_3CN/0.1 M NH_4 OAc, pH 5.2
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	10 µL
Detection	UV, 225 nm
Sample	IGEPAL CA-630 (0.1%)



IGEPAL CA-630

Figure 4. Acclaim Mixed-Mode HILIC-1 phase vs. competitive diol phase.

In RP mode, selectivity of hydrophobic oligomers is suppressed, and all components with the same hydrophobe collapse into a single peak. This simplifies the chromatogram and increases the sensitivity of the analysis. The optimal balance between the hydrophilic and the hydrophobic moieties on the silica surface provides unique chromatographic properties that make the new phase useful for many applications.

Figure 4 shows a comparison of Acclaim Mixed-Mode HILIC-1, a conventional RP C8 column, and a conventional diol column for the oligomer separation (degree of ethoxylation) of IGEPAL CA-630 in the HILIC mode. The Acclaim Mixed-Mode HILIC-1 column is not only superior to the RP or diol columns; the synergy is greater than the sum of the contributions of the two phases. The result is a unique separation mode not achievable with other columns.

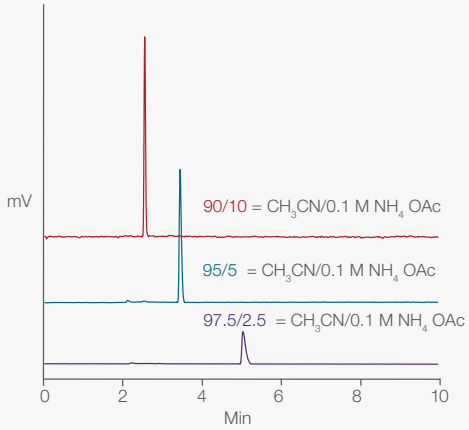
A versatile column with many applications

The Acclaim Mixed-Mode HILIC-1 column separates both polar and non-polar molecules with selectivity complementary to RP columns, and is suitable for a broad range of applications, including drug metabolites, lipids, polyethylene glycols (PEGs), ethoxylated surfactants, and more.

Like other diol columns, the Acclaim Mixed-Mode HILIC-1 retains highly polar molecules, such as urea, in the HILIC mode (Figure 5). Retention of urea increases as the mobile phase organic content increases.

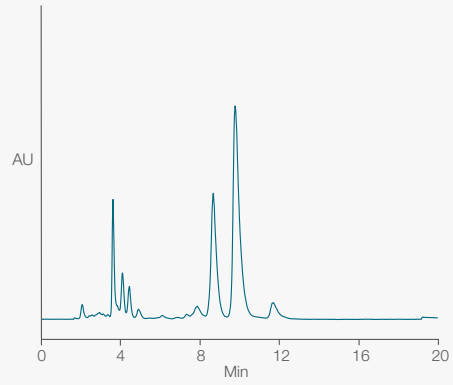
Phospholipids play an important role in life science and are present in all biological membranes. Separation of phospholipid compounds using HPLC techniques have traditionally proven difficult. The multimode characteristics of the Mixed-Mode HILIC column allow hydrophilic interaction and controlled elution by varying the organic content. Figure 6 shows conditions for determination of phospholipids.

Polyethylene glycols (PEGs) have a wide variety of uses, including medical formulations, personal care products, and industrial applications. Separation of PEGs with different molecular weights is shown in Figure 7.



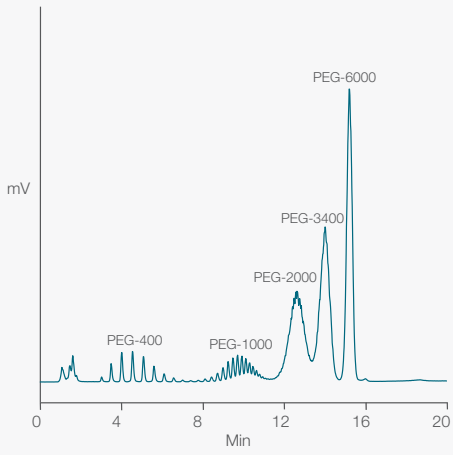
Column	Acclaim Mixed-Mode HILIC-1, 5 µm
Dimensions	4.6 x 150 mm
Mobile phase	95/5 v/v CH_3CN/0.1 M NH_4OAc, pH 4.5
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	10 µL
Detection	ELS detector
Sample	Urea (0.1% in 90% CH_3CN)

Figure 5. Mobile phase effect on urea retention.



Column	Acclaim Mixed-Mode HILIC-1, 5 µm
Dimensions	4.6 x 150 mm
Mobile phase	45/5/50 v/v/v CH_3CN/MeOH/0.1 M NH_4OAc, pH 5.2
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	20 µL
Detection	ELS detector
Sample	Lecithin (chicked egg yolk) 2 mg/mL

Figure 6. Lecithin (chicken egg yolk).



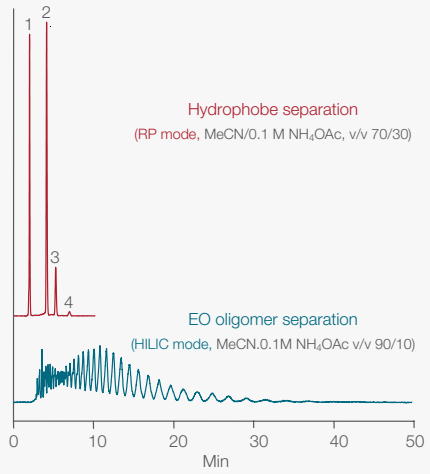
Column	Acclaim Mixed-Mode HILIC-1, 5 µm
Dimensions	4.6 x 150 mm
Mobile phase	A: MeOH, B: D.I. H_2O
Gradient	20% to 95% A in 20 min
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	25 µL
Detection	ELS detector
Sample	Various PEGs (0.04% each)

Figure 7. Analysis of polyethylene glycols (PEGs).

Nonionic ethoxylated surfactants

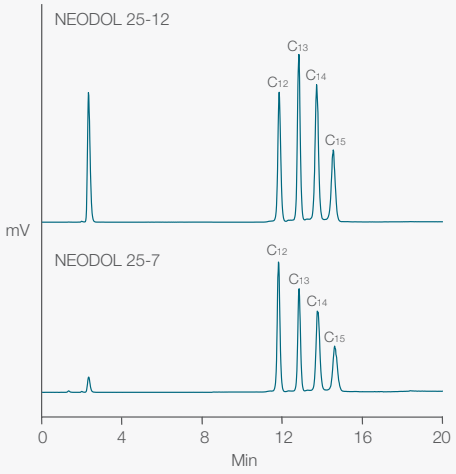
Ethoxylated surfactants (e.g. alkylphenol ethoxylates, fatty alcohol ethoxylates, etc.) are widely used in many industrial and consumer products. Determination of EO number, degree of ethoxylation, and hydrophobe distributions are important. However, despite the fact that EO number can be chromatographically measured in normal phase or HILIC conditions, determination of hydrophobe distribution is challenging. Both EO number and hydrophobe separations occur concurrently, resulting in a chromatogram that makes accurate quantification impractical.

Figure 8 illustrates chromatograms of Brij 35 (lauryl alcohol condensed with 23 moles ethylene oxide, molecular formula: $R(OCH_4CH_4)nOH$ (n~24)). In RP mode, the surfactant is separated into four single peaks, corresponding to unreacted PEGs (early eluting peak) and three ethoxylates corresponding to different alkyl chain lengths. In this mode, all EO oligomers with same hydrophobe collapse into a single peak. In HILIC mode, the separation mechanism is both hydrophobic and hydrophilic interaction, thus, degree of ethoxylation can be determined.



Column	Acclaim Mixed-Mode HILIC-1, 5 µm
Dimensions	4.6 x 150 mm
Mobile phase	See chromatogram for details
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	5 µL
Detection	ELS detector
Sample	Brij 35 (3 mg/mL)
Peaks	1. Unreacted 2. C_{12} 3. C_{14} 4. C_{16}

Figure 8. Analysis of ethoxylated fatty alcohols in both RP and HILIC modes.



Column	Acclaim Mixed-Mode HILIC-1, 5 µm
Dimensions	4.6 x 150 mm
Mobile phase	A: MeOH, B: D.I. H_2O
Gradient	60% to 90% A in 15 min, hold for 5 min
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	510 µL
Detection	ELS detector
Samples	NEODOL 25-7, NEODOL 25-12 (0.2%)

$R (OCH_2CH_2)nOH$
$R=C_{12}$ to C_{15}
NEODOL 25-12 (n~12)
NEODOL 25-7 (n~7)

Figure 9. Analysis of ethoxylated alcohols.

Figure 9 shows separations of NEODOL 25-12 and 25-7 (mixtures of C12 to C15 alcohols with average EO numbers of 7 or 12). Conventional RP columns provide both EO and hydrophobe separations, which is undesirable for accurate quantification. By comparison, the Acclaim Mixed-Mode HILIC-1 column gives four sharp, baseline resolved peaks, corresponding to four differnt alkyl chains (C12 to C15).

The Acclaim Mixed-Mode HILIC-1 is recommencded as a complement to the Thermo Scientific™ Acclaim™ Surfactant column because the balance of hydrophilicity and hydrophobicity differs.

Consistent manufacturing

Each Acclaim Mixed-Mode HILIC-1 column is manufactured according to stringent specifications to ensure column-to-column reproducibility. Each column is shipped with a test chromatogram validating column performance.

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Acclaim Mixed-Mode HILIC-1 column specifications

Specifications	
Basic silica	Spherical, high-purity
Particle sizes	3 µm and 5 µm
Pore size	120 Å
Surface area	300 m^2/g
Column chemistry	Proprietary alkyl diol

Ordering information

Column	Particle size (µm)	Format	Length (mm)	ID (mm)	Part number
Acclaim Mixed-Mode HILIC-1	3.0	HPLC column	50	3.0	071912
			150	2.1	070091
				3.0	070090
	5.0		150	2.1	066847
				4.6	066843
			250	4.6	066844
	5.0	Guard cartridge (2/pk)	10	2.1	069694
				3.0	071913
				4.6	069706

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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