

A New HILIC/RP Mixed-Mode Column and Its Applications in Surfactant Analysis

X. Liu, C. Pohl, Dionex Corporation, Sunnyvale, CA, USA

ABSTRACT

Although reversed-phase (RP) silica columns (e.g., C18 and C8) are widely used in small molecule separations, they are unsuitable for retaining or separating highly polar compounds. HILIC columns can retain highly polar compounds that are not retained by RP chromatography with additional benefits, including complementary selectivity compared to RP columns, enhanced sensitivity for MS detection, and simplified sample preparation. However, since traditional HILIC columns (i.e., silica, cyano, amino, diol phases) usually have hydrophilic surfaces with low hydrophobicity, they are incapable of separating small molecules via hydrophobic interaction.

We have developed a new mixed-mode stationary phase that combines both HILIC and RP characteristics. The new phase is based on high-purity and spherical silica functionalized with a silyl ligand consisting of both hydrophilic and hydrophobic functionalities. This new packing material can be used in either HILIC mode (in high organic conditions) or RP mode (in high aqueous conditions). The optimal balance between the hydrophilic and hydrophobic moieties on the silica surface provides unique chromatographic properties that make the new packing useful for many applications, including determination of hydrophobe distribution and degree of ethoxylation (EO number) in a broad variety of ethoxylated surfactants.

ACCLAIM MIXED-MODE HILIC-1 COLUMN

The Acclaim® Mixed-Mode HILIC-1 is a high-efficiency HILIC/RP mixed-mode silica-based stationary phase with the following benefits:

- Operates in both reversed-phase and normal phase modes
- Retains highly polar molecules that are not retained by reversed-phase chromatography
- Unique selectivity, complementary to reversed-phase columns
- Higher hydrophobic retention compared to conventional diol columns
- Broader application range than conventional diol columns

Physical Data

Bonding Chemistry:	Proprietary alkyl diol
Silica Substrate:	Spherical, high-purity
	Particle size – 5 μm
	Surface area – 300 m^2/g
	Pore size – 120 \AA

RESULTS AND DISCUSSION

Chromatographic Features

The new phase is based on high-purity and spherical silica functionalized with a silyl ligand having both hydrophilic and hydrophobic functionalities (Figure 1). The alkyl chain provides hydrophobic retention, and the dihydroxy functionality contributes to HILIC retention. Like other diol columns, the new packing material retains highly polar molecules, such as urea (Figure 2).

Figure 3 shows the dependency of retention on the mobile phase organic content for the new phase and a conventional diol packing (LiChrosorb® Diol, Merck KGaA). For an acidic molecule (benzoic acid), both columns show a “U” curve—an indication of HILIC behavior. However, the new phase exhibits higher retention in both RP (highly aqueous) and HILIC (high organic) modes. For a hydrophilic basic molecule (e.g., caffeine), the conventional diol column provides little retention. The new phase gives substantially higher retention under highly aqueous conditions, and marginally increased retention under high organic conditions. For a neutral non-polar molecule (e.g., toluene), the new phase exhibits greatly increased hydrophobic retention compared to the conventional diol column.

Due to the presence of both hydrophobic alkyl chain and hydrophilic dihydroxy functionalities, the new column can operate in either RP or HILIC mode, as shown in Figure 4. This feature facilitates a greater variety of applications compared to conventional diol columns.

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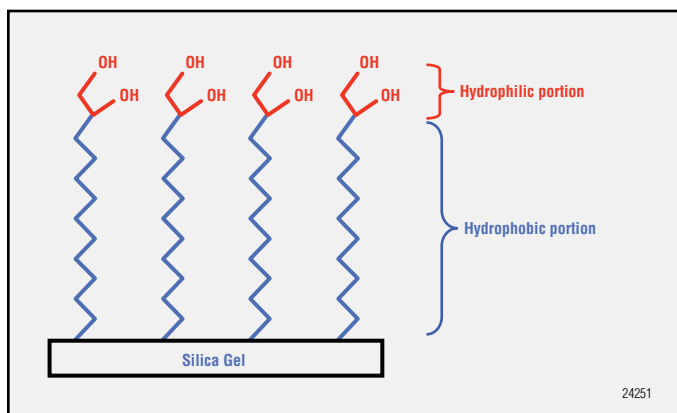


Figure 1. Surface chemistry of Acclaim Mixed-Mode HILIC-1.

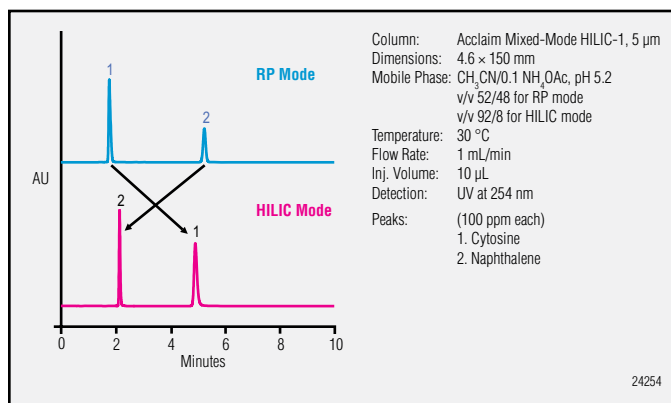


Figure 4. Dual operation modes: RP and HILIC.

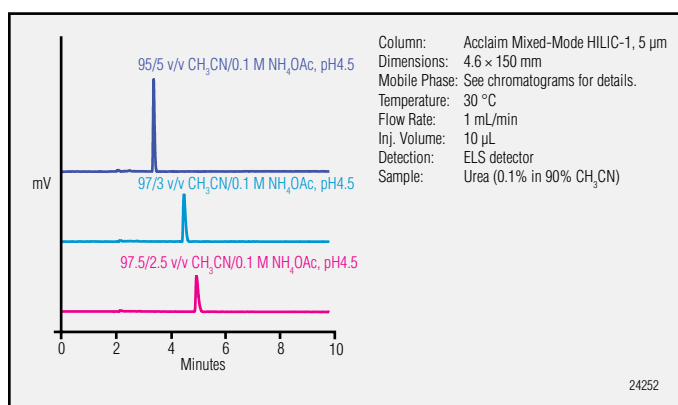


Figure 2. Analysis of urea.

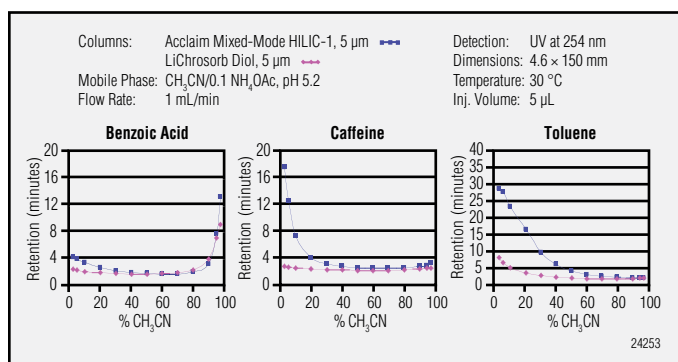


Figure 3. Dependency of retention on mobile phase organic content: comparison between the Acclaim Mixed-Mode HILIC-1 and LiChrosorb Diol.

Applications for Ethoxylated Surfactant Analyses

Ethoxylated surfactants (e.g., alkylphenol ethoxylates, fatty alcohol ethoxylates, ethoxylated alkyl sulfates, etc.) are widely used in industrial and consumer products, and account for approximately 30 percent of total surfactant consumption worldwide. Depending on circumstances, determination of both EO and hydrophobe distributions are usually desired. However, while EO distribution can be chromatographically measured in normal phase (HILIC) conditions, the determination of hydrophobe distribution, which is usually performed on a RP column, can be challenging. This is mainly because both EO number and hydrophobe separations occur concurrently on a typical RP column, leading to a complicated chromatogram, making accurate quantification impractical. The new phase creates the optimal balance between the hydrophilic and hydrophobic moieties on the silica surface, resulting in unique chromatographic properties that make it possible to achieve either an EO based separation (in HILIC mode) or a hydrophobe based separation (in RP mode), on the same column.

Alkylphenol Ethoxylates

Figure 5 illustrates a comparison of three columns: the new column, the Acclaim 120 C8, and the LiChrosorb Diol, for EO oligomer separation of an alkylphenol ethoxylate (IGEPAL CA-630) in HILIC mode. It is clear that the new column provides superior resolution as opposed to both the conventional diol and RP columns.

The chromatograms of IGEPAL CA-630 obtained on the new phase with varying mobile phase organic contents are overlaid in Figure 6. The new phase behaves as an RP column when the mobile phase contains less than 75% acetonitrile (retention increases with mobile phase aqueous content), and behaves as a HILIC column when more than 90% acetonitrile is present in the mobile phase (retention of oligomers and resolution increase with mobile phase organic content). In RP mode separations between EO oligomers are suppressed, and all components with the same hydrophobe collapse into a single peak, which simplifies the chromatogram and increases sensitivity.

Figure 7 shows an example of separating two closely-related alkylphenol ethoxylates, IGEPAL CA-630 and IGEPAL CO-630. Both surfactants have the same EO number and phenol moieties, but slightly different alkyl groups (C_8 and C_9 for CA-630 and CO-630, respectively). The new column gives two baseline-resolved single peaks corresponding to each surfactant according to their hydrophobicity differences. By comparison, on an Acclaim Surfactant column, because of the simultaneous separation of both EO oligomers and hydrophobes, determination of the individual surfactants is impossible.

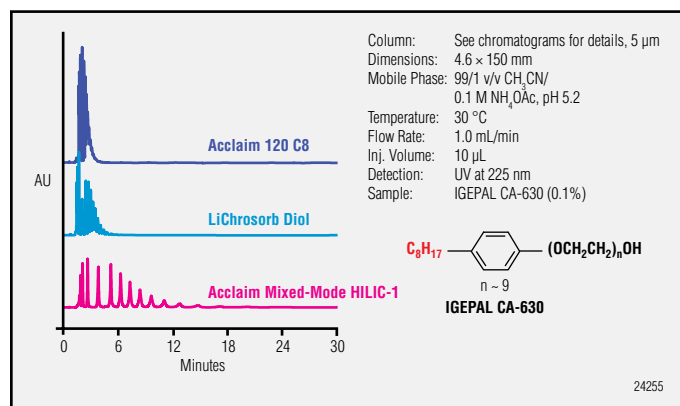


Figure 5. Analysis of an alkylphenol ethoxylate: comparison of Acclaim Mixed-Mode HILIC-1, Acclaim 120 C8, and LiChrosorb Diol.

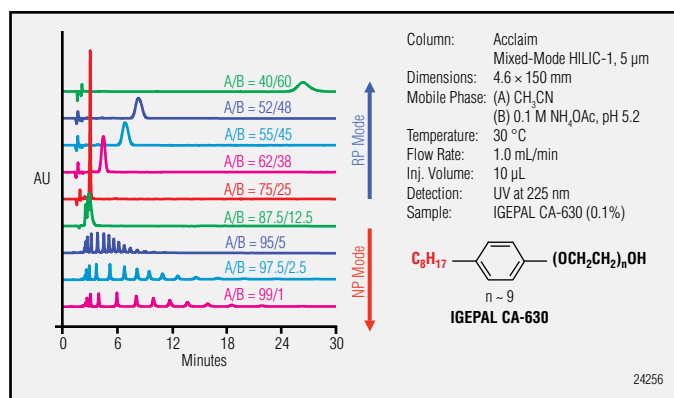


Figure 6. Dependency of separation on mobile phase organic content.

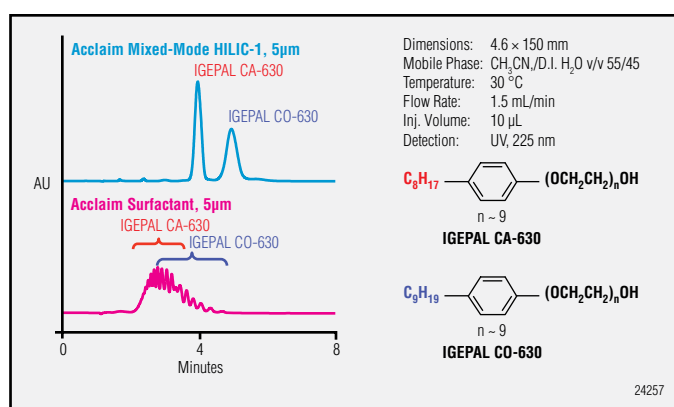


Figure 7. Separation of alkylphenol ethoxylates.

Fatty Alcohol Ethoxylates

Figure 8 illustrates chromatograms of Brij 35 [lauryl alcohol condensed with 23 moles ethylene oxide, molecular formula: $(C_2H_4O)_n C_{12}H_{26}O$]. 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. Under this condition, all EO oligomers with the same hydrophobe collapse into a single peak. In HILIC mode, on the other hand, all EO oligomers are separated in addition to the hydrophobe-based separation. Thus, the degree of ethoxylation can be determined.

Figure 9 shows the separation of NEODOL 25-12 and 25-7 (a mixture of C_{12} to C_{15} alcohols with average EO numbers of 12 and 7, respectively.) The new column gives four baseline-resolved sharp peaks according to alkyl chain lengths, regardless of EO number for each hydrophobe.

The separation of several PEGs with different molecular weights is shown in Figure 10.

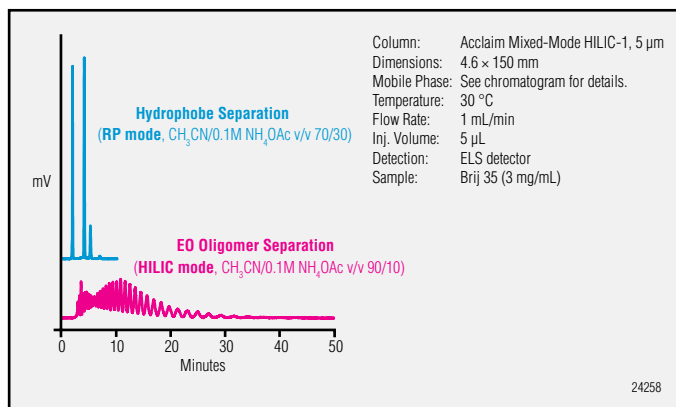


Figure 8. Analysis of ethoxylated fatty alcohols in both RP and HILIC modes.

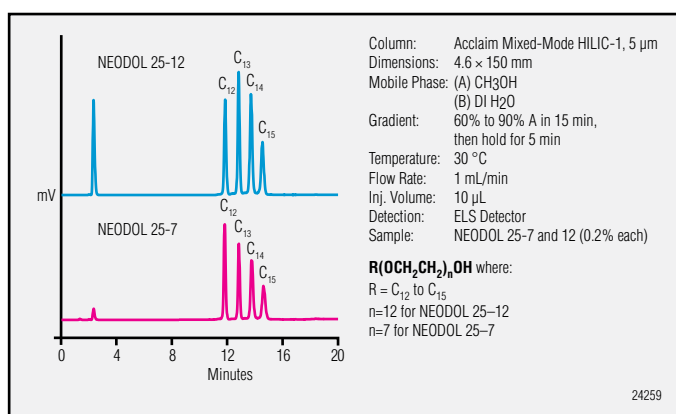


Figure 9. Analysis of NEODOL 25-12.

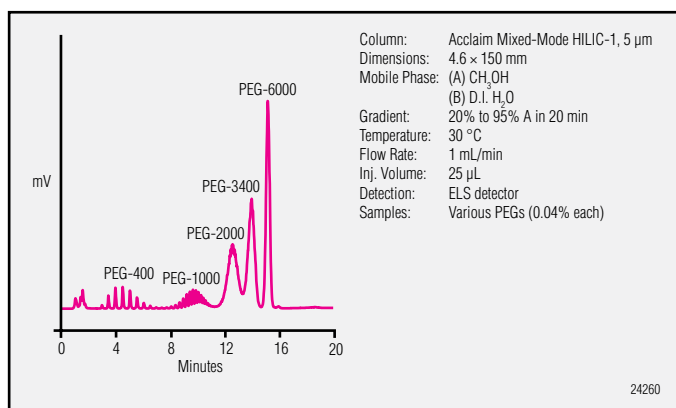


Figure 10. Separation of polyethylene glycols.

Ethoxylated Alkyl Sulfates

Ethoxylated lauryl sulfates can be separated on the new phase into two series of peaks, corresponding to two ethoxylated hydrophobes (C₁₂ and C₁₄). Within each series, the ethoxylated oligomers are also resolved. There is an overlap between two series when using acetonitrile as the organic solvent (Figure 11). If the objective is to determine the hydrophobe distribution in the surfactant, methanol should be used instead of acetonitrile in the mobile phase. This way, the surfactant is grouped into two fully separated “clusters” and the run time decreases by 50%. In comparison, the conventional C8 column only gives partially resolved clusters in its optimal condition, as shown in Figure 12.

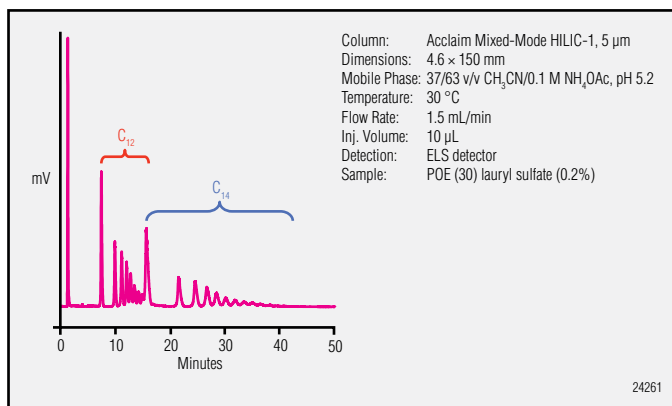


Figure 11. Analysis of ethoxylated alkyl sulfate separation based on both hydrophobe and degree of ethoxylation (EO).

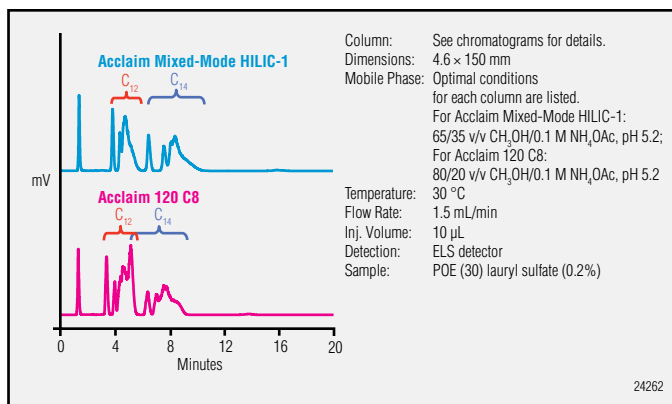


Figure 12. Hydrophobe separation of ethoxylated alkyl sulfates.

CONCLUSION

1. The Acclaim Mixed-Mode HILIC-1 column uses a new type of mixed-mode silica-based stationary phase that combines both HILIC and RP characteristics.
2. Its unique column chemistry results in several benefits, including dual operation modes (HILIC and RP), higher hydrophobic retention compared to conventional diol columns, and a wide variety of applications.
3. The new column is suitable for analyzing ethoxylated surfactants in both HILIC and RP modes. In HILIC condition, the degree of ethoxylation (EO) can be determined. In RP mode, the separation of ethoxylated oligomers is suppressed, and the hydrophobe distribution of the surfactant can be characterized.

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Dionex Corporation

1228 Titan Way
P.O. Box 3603
Sunnyvale, CA
94088-3603
(408) 737-0700

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LPN 1953-01 07/07
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