PRODUCT MANUAL

for

Acclaim® Mixed-Mode HILIC-1 Column

DIONEX

IC | HPLC | MS | EXTRACTION | PROCESS | AUTOMATION

Product Manual

for

ACCLAIM® Mixed-Mode HILIC-1 Column

5µm, 4.6 x 250mm, P/N 066844
5µm, 4.6 x 150mm, P/N 066843
5µm, 2.1 x 150mm, P/N 066847
3µm, 3.0 x 150mm, P/N 070090
3µm, 3.0 x 50mm, P/N 071912
3µm, 2.1 x 150mm, P/N 070091

ACCLAIM® Mixed-Mode HILIC-1 Guard

4.3 x 10mm (set of 2), P/N 066845
2.1 x 10mm (set of 2), P/N 066846
3.0 x 10mm (set of 2), P/N 071913
4.6 x 10mm (set of 2), P/N 069706

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TABLE OF CONTENTS

SECTION 1 – INTRODUCTION

The Acclaim Mixed-Mode HILIC-1 column is a silica-based packing material that incorporates both Reversed-Phase (RP) and Hydrophilic Interaction Liquid Chromatography (HILIC) properties. Unlike either traditional RP or HILIC stationary phases, the packing features an alkyl long chain with a hydrophilic polar terminus, and demonstrates great potentials for separating a wide range of both highly polar and non-polar molecules, in either RP mode or HILIC mode.

The Acclaim Mixed-Mode HILIC-1 columns consist of 5-µm or 3-µm high-purity, porous, spherical silica particles with 120Å diameter pores bonded with proprietary functional groups, which provide unsurpassed resolution and peak symmetry for a variety of polar and non-polar molecules.

The main features of the Acclaim HILIC-1 column include

1. Operates in both reversed-phase and normal phase modes.
2. Retains highly polar molecules that would be un-retained by reversed-phase chromatography.
3. Unique selectivity, complementary to reversed-phase columns.
4. Higher hydrophobic retention compared to the conventional Diol columns.
5. Broader application range than conventional Diol columns.

1.1. Specifications and Recommended Operating Conditions

Shipping Solution	60 / 40 Acetonitrile / 100mM Ammonium Acetate
Storage Solution:	60 / 40 Acetonitrile / 100mM Ammonium Acetate or HILIC mobile phase
Buffer pH Range:	pH 2.5 - 7.5
Temperature Range	< 50°C

Stationary Phase	Particle size	Column Dimensions	P/N	Max Recommended Pressure	Typical Flow Rate
Mixed-Mode HILIC-1	3 µm	2.1x150 mm	070091	5800 psi	0.2 - 0.5 mL/min
		3.0x50 mm	071912	4500 psi	0.4 - 1.0 mL/min
		3.0x150 mm	070090	5800 psi	0.4 - 1.0 mL/min
	5 µm	2.1x150 mm	066847	5800 psi	0.2 - 0.5 mL/min
		4.6x150 mm	066843	5800 psi	0.8- 2.0 mL/min
		4.6x250 mm	066844	5800 psi	0.8 - 2.0 mL/min

1.2. Physical

Bonding Chemistry:	Proprietary alkyl diol
Silica Substrate:	Spherical, high-purity
Particle size	5 µm and 3µm
Surface area	300 m2/g
Pore size	120 Å

1.3. Acclaim Mixed-Mode HILIC-1 Products

Acclaim Mixed-Mode HILIC-1	**Particle size**	**Column Dimensions**	**P/N**	
Analytical	3 µm	2.1x150 mm	070091	
		3.0x50 mm	071912	
		3.0x150 mm	070090	
	5 µm	2.1x150 mm	066847	
		4.6x150 mm	066843	
		4.6x250 mm	066844	
Guard	5µm	4.3 x 10mm	066845	Requires Holder p/n 059456
		2.1 x 10mm	066846	Requires Holder V-2 p/n 069580
		3.0 x 10mm	071913	Requires Holder V-2 p/n 069580
		4.6 x 10mm	069706	Requires Holder V-2 p/n 069580

SECTION 2 – INSTALLATION: STEP-BY-STEP USER GUIDE

Step 1 - Validating column performance
Dionex recommends that you perform an efficiency test on your Acclaim Mixed-Mode HILIC column before use. The purpose of column performance validation is to ensure no damage has occurred during shipping. Test the column using the conditions described on the Quality Assurance (QA) Report enclosed in the column box. Repeat the test periodically to track the column performance over time. Note that slight variations may be obtained on tow different HPLC systems due to system electronic, plumbing, operating environment, reagent quality, column conditioning, and operator technique.

Step 2 - Mobile phase preparation
Obtaining reliable, consistent and accurate results require mobile phases that are free of ionic and spectrophotometric impurities. Chemicals, solvents and de-ionized water used to prepare mobile phase should be of the highest purity available. Maintaining low trace impurities and low particle levels in mobile phases helps to protect your columns and system components. DIONEX cannot guarantee proper column performance when the quality of the chemicals, solvents and water used to prepare the mobile phase has been compromised.

De-ionized Water
The de-ionized water used to prepare the mobile phase should be Type 1 Reagent Grade Water, or HPLC Grade Water. The de-ionized water should be free of ionized impurities, organics, microorganisms and particulate matter larger than 0.2 µm. Many commercial water purifiers are designed for HPLC applications and are suitable for these applications.

NOTE ***Degas the aqueous component of the mobile phase and then add the solvent component. Avoid excessive purging or degassing of mobile phases containing solvents, if possible, since the volatile solvent can be 'boiled' off from the solution.***

Solvents
The solvents used must be free from ionic and UV-absorbing impurities. Use of ultrahigh purity solvents, HPLC grade, will usually ensure that your chromatography is not affected by impurities in the solvent.

Depending on specific application, the mobile phase system consists of an organic modifier (e.g. acetonitrile or methanol) and an aqueous portion (e.g. D.I. water, or ammonium acetate or phosphate buffer). Both pre-mixed and proportioning valve generated mobile phases give satisfactory results. The use of proportioning valve provides better flexibility in method optimization, while the pre-mixed mobile phase provides more reproducible results.

Mobile Phase for Column Performance Test:
Pre-mixed mobile phase: mix 400 g of D.I. water and 468 g of acetonitrile.
Proportioning valve generated: set up the gradient pump as 40/60 v/v D.I. water/ acetonitrile.

NOTE ***These two mobile phases could give slightly different results due to the ways they are prepared.***

Step 3 - Set up the LC system
Use a standard LC system equipped with a LC pump, a column oven, a UV detector, and an injector (or an autosampler). The system should be thoroughly primed before use.

Step 4 - Condition the column
Each new column is shipped in the mobile phase used for the column performance test. Depending on different separation modes, the column should be conditioned differently.

For HILIC mode (>70% acetonitrile in the mobile phase) applications, the column should be purged with 50% acetonitrile in D.I. water (v/v) for approximately 10 column volumes, followed by equilibration with the desirable mobile phase thoroughly (~20 to 50 column volumes depending on the aqueous content in the mobile phase) before any injection is made.

For RP mode (<70% acetonitrile in the mobile phase) applications, the column should be purged with 50% acetonitrile in D.I. water (v/v) for approximately 10 column volumes, followed by equilibration with the desirable mobile phase thoroughly (~20 column volumes) before any injection is made.

When switching to a different mobile phase, make sure that the new mobile phase is compatible with the existing mobile phase in the column to avoid column clogging due to precipitation. A good practice is to purge the column with 50% acetonitrile in D.I. water (v/v) for approximately 10 column volumes before switching to the new mobile phase.

Step 5 - Reproduce the chromatogram in the Lot Validation Report
Perform the column QA test using the conditions described in the QAR (Appendix), and compare the result with the reported values. The column should be fully equilibrated before any injection. At least three injections should be made until reproducible result is obtained.

NOTE ***Due to various reasons, such as difference of LC systems, mobile phases, oven temperature control, etc, you may observe somewhat different separation from that in the report.***

Once you are satisfied with the column performance report result, the column is ready for real applications.

SECTION 3 – CONSIDERATIONS IN METHOD DEVELOPMENT

3.1. HILIC Mode vs. Reversed-Phase Mode

The selection of separation mode depends on the type of the analyte molecules and requirement of the specific application. In general, if a highly polar molecule is the analyte of interest, HILIC mode (high organic in the mobile phase) should be considered. In addition, HILIC mode can be used to determine the degree of ethoxylation (EO) of a nonionic surfactant. For other applications, RP separation may be considered. However, it is recommended that a conventional RP column (C18, C8, or polar-embedded phase) be tried first for regular RP applications. The Acclaim HILIC column has lower hydrophobicity than C8 columns but much higher hydrophobic retention than conventional Diol columns because of its unique surface chemistry, so that selectivity different from RP columns is possible.

3.2. Selection of Organic Solvents

For HILIC applications, acetonitrile is preferred as the organic modifier.
For RP applications, both acetonitrile and methanol can be used depending on the application.
In addition, this column is compatible with traditional normal-phase solvent, such as ethanol, iso-propanol, ethyl acetate, hexane, heptane, etc.

3.3. Buffer Types

The selection of buffer depends on the detection method and pH requirement.
Ammonium acetate (or formate) buffer is the preferred buffer system because of its applicability to both RP and HILIC separation modes, compatibility with UV (> 230 nm), ELS detector and MS, high solubility in organic solvent, familiarity to most chromatographers.

Volatile organic acids, such as acetic acid, formic acid, and TFA, can also be used to control the mobile phase pH, and share the similar benefits of ammonium acetate (formate) buffer.

Phosphate buffers are ideal for applications that require low UV background, and it usually provides somewhat better peak shapes for charged molecules, compared to acetate buffer. Although there is no problem in RP application (organic content < 70%), phosphate buffers tend to precipitate in high organic condition.

3.4. Mobile Phase pH

Mobile phase pH needs to be controlled to adjust selectivity and obtain reproducible results of charged molecules.

3.5. Isocratic or Gradient Method

Isocratic methods are suitable for simple and/or well-defined application. When dealing with unknown samples, or a sample consisting of molecules with dramatically different hydrophilicity or hydrophobicity, a gradient method is often advantageous.

SECTION 4 – COLUMN CARE

4.1. Column storage

The column can be stored in the mobile phase for short period of time (e.g. overnight). If not in use for longer than one week, it is recommended to store the column in a solution with higher organic content, such as 70/30 v/v acetonitrile (or methanol)/20-100 mM ammonium acetate or D.I. water at a pH between 3.5 and 5.5.

4.2. Operating pH range: pH 2.5 to 7.5

The column lifetime depends heavily on the chromatographic condition. To obtain better column lifetime, it is highly recommended to use "silica friendlily" mobile phases, such as a buffer at a pH between pH 3 to 7. Although compatible with 100% aqueous mobile phase, it is not recommended to use this column extensively in such condition. The mobile phase pH should be controlled between 3.5 and 6 if more than 80% buffer is present in the mobile phase. If used in HILIC conditions, the column can be used in pH 2 to 8 range.

4.3. Operating temperature limit: 50 ºC

Although our experimental results indicate that the column can be used at 50 °C, a lower operating temperature (20 - 40 °C) is recommended for routine separations.

4.4. Pressure limit

It is extremely important not to impose sudden column pressure surge. The maximum recommended pressures are listed in section 1.1, the use of lower operating pressures will extend column lifetime.

4.5. Flow rate

The typical operating flow rates are listed in section 1.1, higher flow rate can be used for faster analysis as long as the pressure limit is not exceeded.

4.6. Column washing procedure

All samples should be pre-treated and filtered before being injected on the column. In the event of column washing practice is needed, the following procedure can be used as a guideline:
For a 4.6 mm i.d. column:

1. Wash the column with D.I water /acetonitrile v/v 50/50 for 10 column volumes at a flow rate between 0.5 to 1 mL/min.
2. Wash the column with 0.1 M ammonium acetate pH 4 to 5 /acetonitrile v/v 50/50 for 20 column volumes at a flow rate between 0.5 to 1 mL/min.
3. Wash the column with 0.1 M ammonium acetate pH 4 to 5 /acetonitrile v/v 10/90 for 20 column volumes at a flow rate between 0.5 to 1 mL/min.
4. Wash the column with 0.1 M ammonium acetate pH 4 to 5 /acetonitrile v/v 50/50 for 20 column volumes at a flow rate between 0.5 to 1 mL/min.
5. Before any injection is made, the column should be equilibrated with the mobile phase thoroughly.

For a 2.1 mm i.d. column, the flow rate should be reduced to 20% of that for 4.6 mm i.d column.

NOTE

If above treatment fails to revive the column, the column should be replaced.

NOTE

SECTION 5 – FREQUENT ASKED QUESTIONS

1. What is the difference between the Acclaim Mixed-Mode HILIC-1 column and conventional HILIC (Diol) column?
The Acclaim HILIC-1 column is based on a new silica-based mixed-mode stationary phase that incorporates both RP and HILIC properties. Unlike either traditional RP or HILIC stationary phases, the new packing features an alkyl long chain with a hydrophilic polar terminus, and demonstrates broader applications for separating a wide range of both highly polar and non-polar molecules, in either RP mode or HILIC mode (Figure 1). By comparison, conventional Diol columns have significantly decreased hydrophobicity (Figure 2), and have much narrower application range.

2. Why do I need an Acclaim Mixed-Mode HILIC-1 column?
The Acclaim HILIC-1 column combines both RP and HILIC characteristics. It can be used in either HILIC conditions or RP conditions the same way as conventional Diol columns or RP columns, without special requirements. More importantly, the optimal balance between the hydrophilic and hydrophobic portions on the column surface leads to unique selectivity that neither conventional Diol columns nor RP columns can provide.

3. When do I need an Acclaim Mixed-Mode HILIC-1 column?
You should consider using an Acclaim HILIC-1 column when

1) Regular RP (e.g. C18, C8, polar-embedded phase, etc) and HILIC (e.g. Diol, CN, amino, silica) columns fail to provide
   satisfactory results;
2) You are dealing with highly polar molecules, or very hydrophobic molecules;
3) You need orthogonal selectivity to complement the primary method;
4) You are analyzing ethoxylated surfactants (Figures 6 - 8).

4. What factors should I consider for method development using this column?
During method development, the following factors should be considered:

1) Separation mode
2) Type of organic modifier
3) Aqueous content in the mobile phase
4) Mobile phase pH control
5) Temperature

5. What mobile phases should I use with this column?
The new column is compatible with any mobile phases for HILIC or Normal-Phase separations provided that the pH requirement is met.
The new column is compatible with any mobile phases for Revered-Phase separations provided that the pH requirement is met. However, for typical RP applications, a RP column (C18 or C8) should be tried first.
Please refer to "Section 3 Considerations in Method Development" for more details.

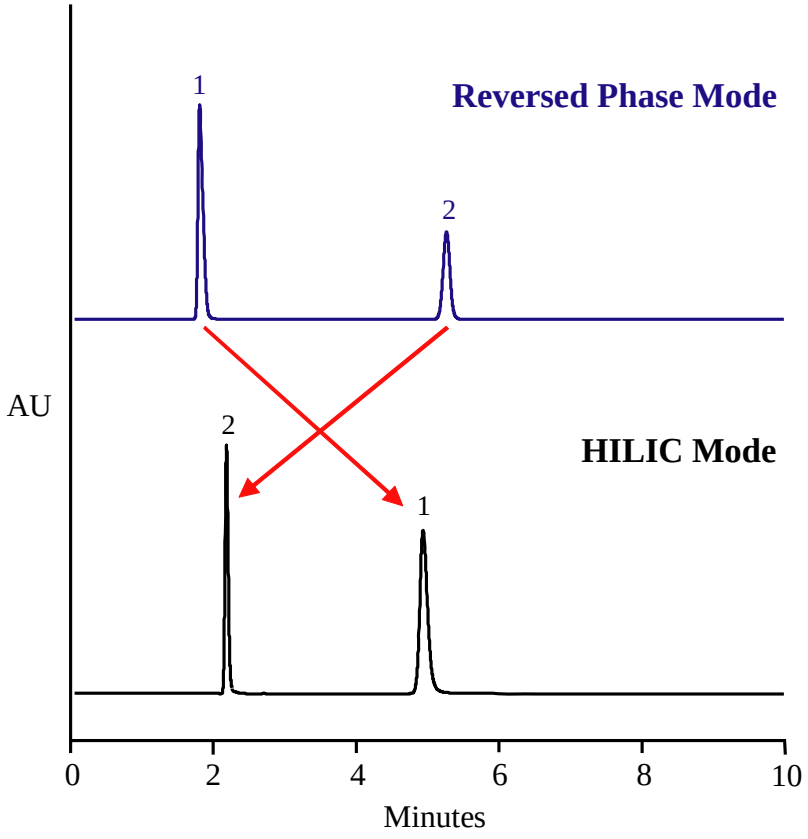
6. What should I do before starting using Acclaim Mixed-Mode HILIC-1 column?
Read this User Guide carefully, and contact Dionex Technical Support if you have any questions regarding using this column.

7. Can I use this column in normal-phase mode?
Yes. The column is compatible with traditional normal-phase solvent, such as ethanol, iso-propanol, ethyl acetate, hexane, heptane, etc.

8. How to store the column?
The column can be stored in the mobile phase for short period of time (e.g. overnight). If not in use for longer than one week, it is recommended to store the column in a solution with higher organic content, such as 70/30 v/v acetonitrile (or methanol)/20-100 mM ammonium acetate or D.I. water at a pH between 3.5 and 5.5.

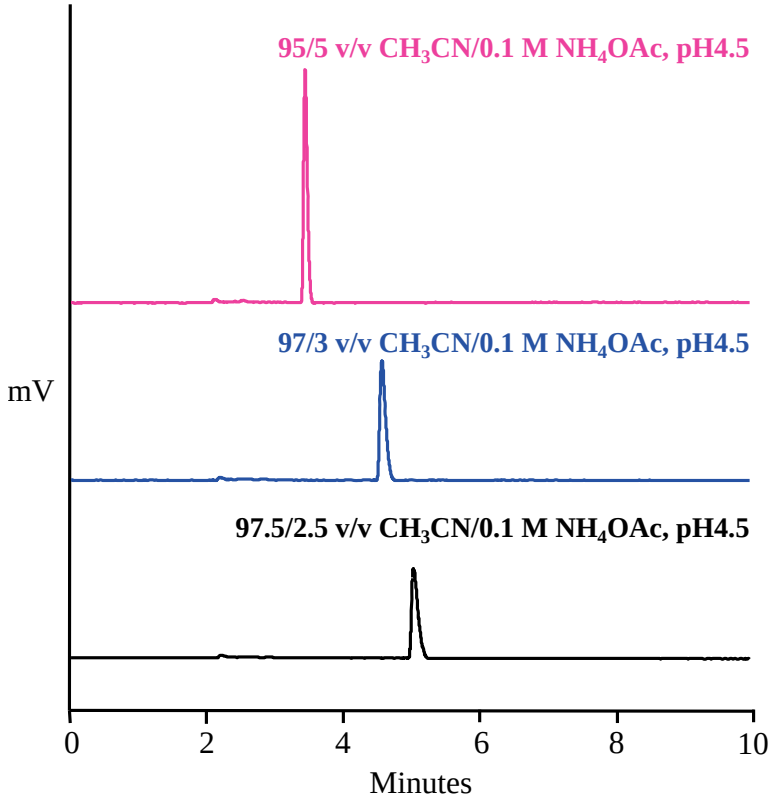
SECTION 6 – APPLICATIONS

6.1. Dual Operation Modes: Reversed-phase and HILIC



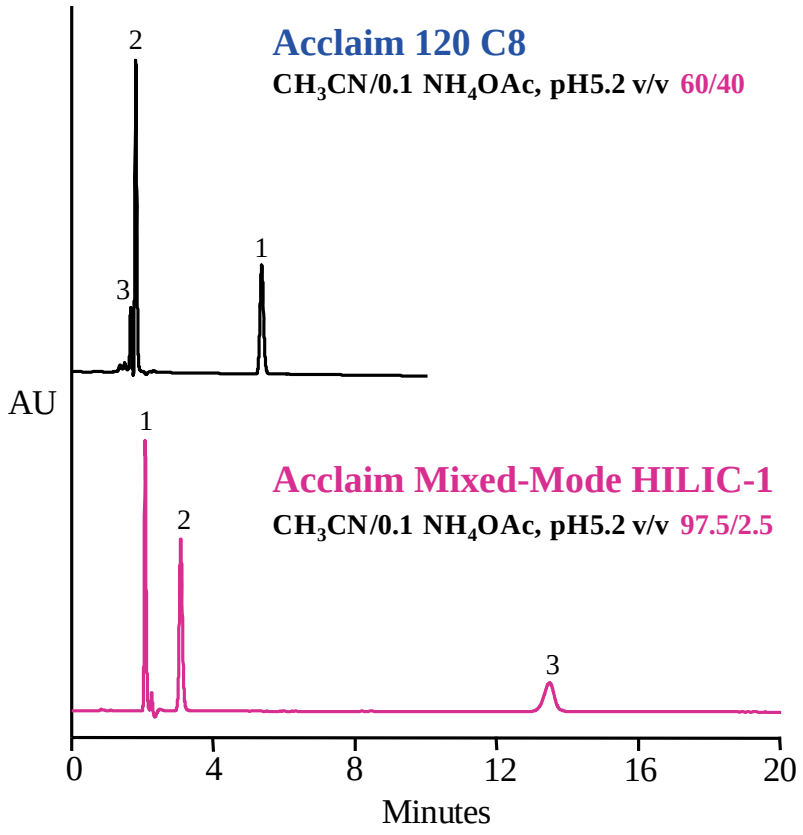
Column:	Acclaim Mixed-Mode HILIC-1, 5 µm
Dimensions:	4.6 x 150 mm
Mobile Phase:	CH_3CN/0.1 NH_4OAc, pH5.2 v/v 52/48 for RP mode; v/v 92/8 for HILIC mode
Temperature:	30 °C
Flow Rate:	1 mL/min
Inj. Volume:	10 µL
Detection:	UV @ 254 nm
Peaks:	(100 ppm each) 1. Cytosine 2. Naphthalene

6.2. Analysis of Urea



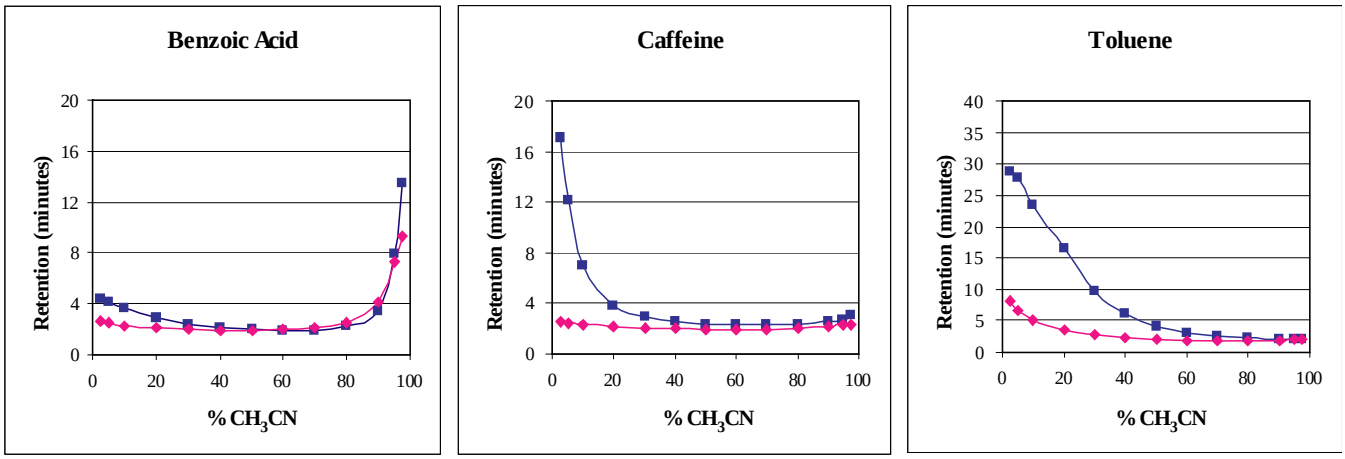
Column:	Acclaim Mixed-Mode HILIC-1, 5 µm
Dimensions:	4.6 x 150 mm
Mobile Phase:	See chromatograms for details
Temperature:	30°C
Flow Rate:	1 mL/min
Inj. Volume:	10 µL
Detection:	Sedex 85 ELS detector (gain - 9, 50ºC)
Sample:	Urea (0.1% in 90% CH_3CN)

6.3. Complementary Selectivity: Reversed-phase and HILIC



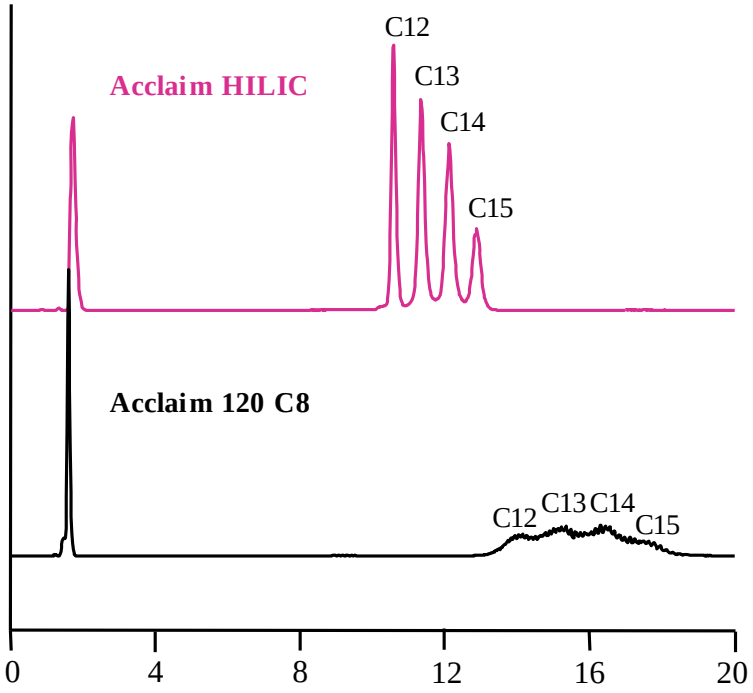
Column:	5 µm
Dimensions:	4.6 x 150 mm
Mobile Phase:	CH_3CN/0.1 NH_4OAc, pH5.2
Temperature:	30 °C
Flow Rate:	1 mL/min
Inj. Volume:	5 µL
Detection:	UV @ 254 nm
Peaks:	
	1. Toluene
	2. Caffeine
	3. Benzoic acid

6.4. Dependency of Retention on Mobile Phase Organic Content: Comparison between the Acclaim HILIC and LiChrosorb Diol



Column:	Acclaim Mixed-Mode HILIC-1, LiChrosorb Diol, 5 µm
Mobile Phase:	CH_3CN/0.1 NH_4OAc, pH5.2
Flow Rate:	1 mL/min
Detection:	UV @ 254 nm
Dimensions:	4.6 x 150 mm
Temperature:	30 °C
Inj. Volume:	5 µL

6.5. Analysis of NEODOL 25-12



$R\ (OCH_2CH_2)_nOH$

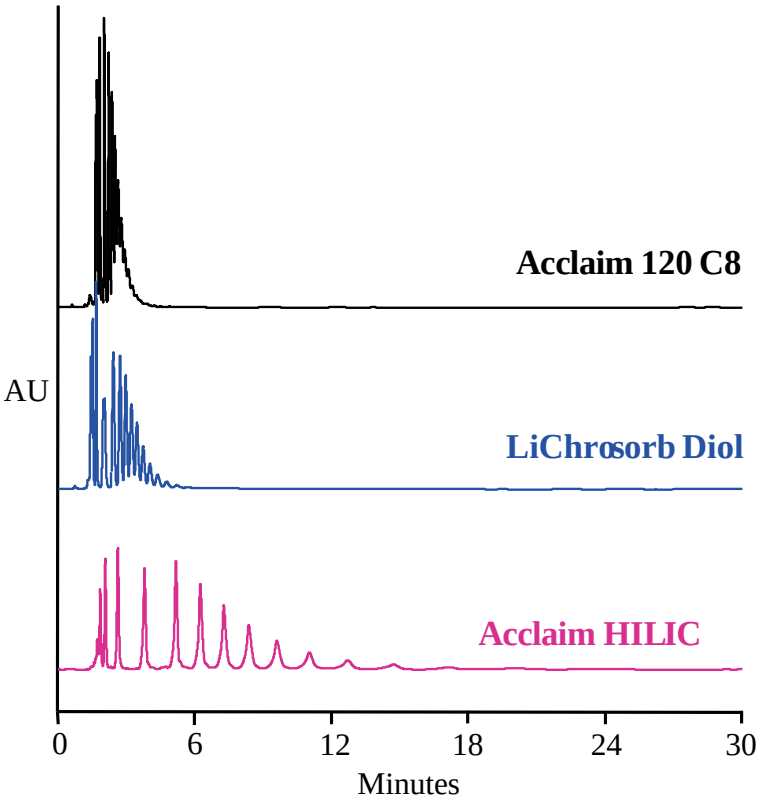
$R = C_{12}$ to C_{15}

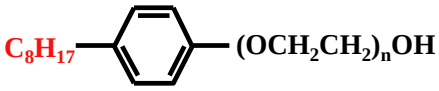
n ~

NEODOL 25–12

Column:	5 µm
Dimensions:	4.6 x 150 mm
Mobile Phase:	A - CH_3CN, B - D.I. H_2O
Gradient:	30% to 80% A in 15 min,then hold for 5 min
Temperature:	30 °C
Flow Rate:	1.5 mL/min
Inj. Volume:	10 µL
Detection:	ELS Detector
Sample:	NEODOL 25-12 (0.2%)

6.6. Analysis of Alkylphenol Ethoxylate



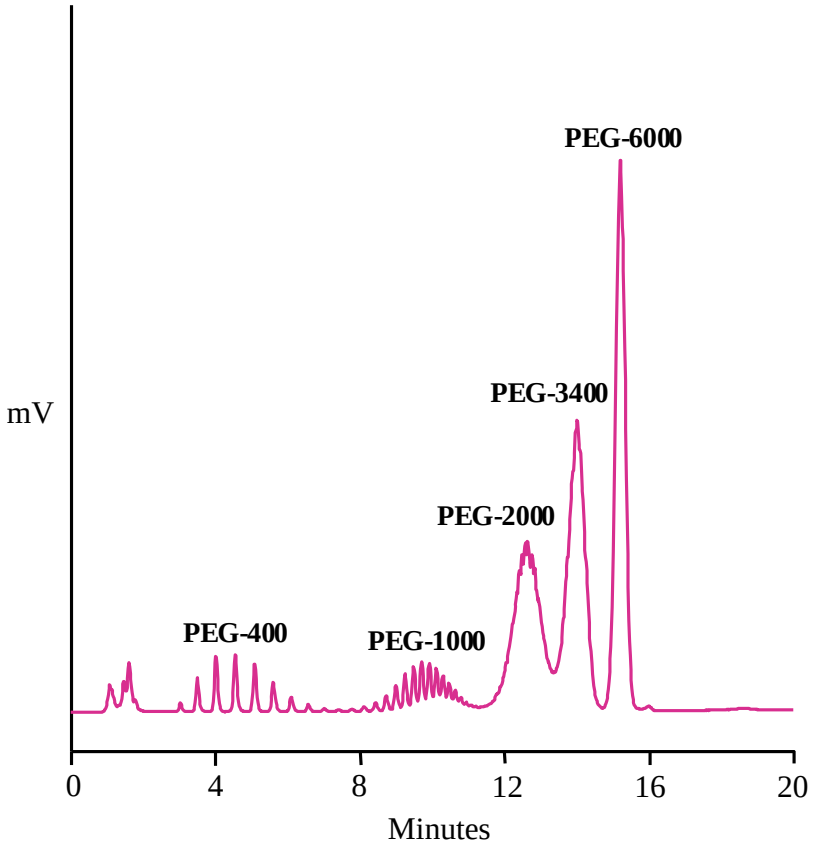


n ~ 9

IGEPAL CA-630

Column:	See chromatograms for details, 5 µm
Dimensions:	4.6 x 150 mm
Mobile Phase:	99/1 v/v CH_3CN/0.1 M NH_4OAc, pH5.2
Temperature:	30 °C
Flow Rate:	1.0 mL/min
Inj. Volume:	10 µL
Detection:	UV @ 225 nm
Sample:	IGEPAL CA-630 (0.1%)

6.7. Separation of Polyethylene Glycols



Column:	Acclaim HILIC, 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:	ELSD
Samples:	Various PEGs (0.04% each)

SECTION 7 – TROUBLESHOOTING GUIDE

The following instruction should help you to locate and eliminate problems traceable to hardware and chemistry issues. Please keep in mind that some problems may be due to other reasons, such as sample contamination, poor water quality, etc. If you cannot solve your problems with the help of this manual, please contact you local Dionex customer support specialist.

7.1. High Backpressure

7.1.1. Finding the Source of High System Pressure

If the system pressure is excessively high, determine the cause of the high pressure. The system should be used with a high-pressure in-line filter for mobile phases. The filter should be positioned between the gradient pump pressure transducer and the injection valve. Make sure you have a high-pressure in-line filter in place and that it is not contaminated.

A. Make sure that the pump is set to the correct flow rate. Higher than recommended mobile phase flow rates will cause higher pressure. Measure the pump flow rate if necessary with a stop watch and graduated cylinder.

B. Determine which part of the system is causing the high pressure. It could be a piece of tubing that has plugged, collapsed tubing walls from over tightening, an injection valve with a plugged port, a column with particulates plugging the bed support, a plugged High-pressure In-line filter, or detector cell. To identify which part of the chromatographic system is causing the problem, disconnect the pump fluid line from the injection valve and turn the pump on. Watch the pressure; it should not exceed 50psi (0.34MPa). Continue adding the system components back into the fluid path, one by one, while watching the system pressure.

7.1.2. Contaminated guard or analytical column

If the column is the cause of the back pressure, replace the guard column.

7.2. High Background or noise

7.2.1. Preparation of Mobile Phases

A. Ensure the mobile phase is made correctly.

B. Ensure the eluents are made from chemicals with the recommended purity.

7.3. Poor Peak Resolution

Poor peak resolution can be due to any or all of the following factors.

7.3.1. Loss of column Efficiency

A. Extra-column system effects can result in sample band dispersion, decreasing peak efficiencies. Make sure you are using tubing with an i.d. of no greater than 0.010" (0.007" for 2.1mm) to make all liquid line connections between the injection valve and the detector cell inlet on standard bore (4-mm) systems. Check for leaks.

B. Guard column / column needs to be replaced.

