Thermo Scientific

Acclaim HILIC-10 Columns

Product Manual

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Thermo SCIENTIFIC

Product Manual

for

Acclaim HILIC-10 Analytical Column

Acclaim HILIC-10, 3 µm, Analytical, 4.6 x 150 mm (P/N 074257)
Acclaim HILIC-10, 3 µm, Analytical, 3.0 x 150 mm (P/N 074258)
Acclaim HILIC-10, 3 µm, Analytical, 2.1 x 150 mm (P/N 074259)
Acclaim HILIC-10, 5 µm, Analytical, 4.6 x 150 mm (P/N 079693)
Acclaim HILIC-10, 5 µm, Analytical, 4.6 x 150 mm (P/N 079694)
Acclaim HILIC-10, 5 µm, Analytical, 2.1 x 150 mm (P/N 070695)
Acclaim RSLC HILIC-10, 1.9 µm, Analytical, 2.1 x 150 mm (P/N 074260)
Acclaim RSLC HILIC-10, 1.9 µm, Analytical, 2.1 x 250 mm (P/N 075709)

Acclaim HILIC-10 Guard Cartridge

Acclaim HILIC-10, 3 µm, Guard 3.0 x10 mm, 2ea (P/N 074261)
Acclaim HILIC-10, 3 µm, Guard 4.6 x10 mm, 2ea (P/N 074262)
Acclaim HILIC-10, 3 µm, Guard 2.1 x10 mm, 2ea (P/N 074263)

Revision History:

Revision 02, January, 2013, Rebranded for Thermo Scientific. Added 5 µm and 1.9 µm column formats.

For Research Use Only. Not for use in diagnostic procedures.

Safety and Special Notices

Make sure you follow the precautionary statements presented in this guide. The safety and other special notices appear in boxes.

Safety and special notices include the following:

 **SAFETY**	***Indicates a potentially hazardous situation which, if not avoided, could result in death or serious injury.***
 **WARNING**	***Indicates a potentially hazardous situation which, if not avoided, could result in damage to equipment.***
 **CAUTION**	***Indicates a potentially hazardous situation which, if not avoided, may result in minor or moderate injury. Also used to identify a situation or practice that may seriously damage the instrument, but will not cause injury.***
 **NOTE**	***Indicates information of general interest.***
IMPORTANT	***Highlights information necessary to prevent damage to software, loss of data, or invalid test results; or might contain information that is critical for optimal performance of the system.***
Tip	***Highlights helpful information that can make a task easier.***

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1. Introduction

The Thermo Scientific™ Acclaim™ HILIC-10 is a general-purpose silica based HILIC (Hydrophilic Interaction Liquid Chromatography) column for separating highly hydrophilic analytes. It is based on high-purity, spherical, porous silica gel that is covalently modified with a proprietary hydrophilic layer. HILIC is a variation of normal-phase chromatography with the advantages of being compatible with water. It is sometimes called "reverse reversed-phase" or "aqueous normal phase" chromatography. The HILIC mode of separation is used to retain very polar compounds. Stationary phase is polar and the mobile phase is highly organic (>80%) with a small amount of polar solvent (e.g, H_2O).

HILIC is a complementary technique to reversed-phase liquid chromatography (RPLC), and has several benefits over RPLC. It retains polar analytes that cannot be retained on a reversed-phase column. For electrospray LC/MS applications with very polar compounds, HILIC (using an organic-rich mobile phase) provides a ten to twenty-fold sensitivity improvement. Moreover, by eliminating the need for evaporation and reconstitution of a sample dissolved in a non-aqueous solvent, the sample analysis throughput can be greatly increased.

HILIC is useful for separating polar solutes and has been applied to a wide variety of application including carbohydrates, peptides, proteins, pharmaceuticals, as well as other highly polar molecules such as urea, biurea, choline and butyrobetaine, tromethamine, ascorbic acid and related compounds, folic acid and its metabolites, nicotine and its metabolite, saponins, aminoglycoside antibiotics, glucosinolates, ionic liquids, organophosphonate nerve agent metabolites, etc.

1.1 The main features of the Acclaim HILIC-10 columns include:

1. Ability of retaining highly polar molecules that would be un-retained by reversed-phase chromatography
2. Unique selectivity, complementary to reversed-phase columns or other HILC columns
3. Excellent chemical stability
4. Rugged column packing

1.2 Physical data

Bonding Chemistry: Proprietary covalently bonded hydrophilic layer

Silica Substrate: Spherical, high-purity

	Particle Type	Column Dimensions	P/N
Analytical	**3 µm** particle size 120 Å pore size 300 m^2/g surface area	4.6 x 150 mm	074257
		3.0 x 150 mm	074258
		2.1 x 150 mm	074259
	5 µm particle size 120 Å pore size 300 m^2/g surface area	4.6 x 250 mm	079693
		4.6 x 150 mm	079694
		2.1 x 150 mm	079695
	1.9 µm particle size 175 Å pore size 200 m^2/g surface area	2.1 x 150 mm	074260
		2.1 x 250 mm	075709
Guard (requires holder P/N 069580)	**3 µm** particle size 120 Å pore size 300 m^2/g surface area	3.0 x 10 mm	074261
		4.6 x 10 mm	074262
		2.1 x 10 mm	074263

1.3 Specifications and Recommended Operational Parameters

Shipping solution: 90/10 acetonitrile/100 mM ammonium acetate

Storage solution: 90/10 v/v acetonitrile/10-50 mM ammonium acetate or 100% acetonitrile

pH Range: pH 2 – 8

Temperature Range: < 70° C

Particle Size	Column Dimension	Maximum Recommended Pressure	Typical Flow Rate
3 µm	4.6 x 150 mm	8500 psi	0.8 – 1.5 mL/min
	3.0 x 150 mm	8500 psi	0.4 – 0.8 mL/min
	2.1 x 150 mm	8500 psi	0.2 - 0.4 mL/min
5 µm	4.6 x 250 mm	6000 psi	0.8 – 1.5 mL/min
	4.6 x 150 mm	6000 psi	0.8 – 1.5 mL/min
	2.1 x 150 mm	6000 psi	0.2 – 0.4 mL/min
1.9 µm	2.1 x 150 mm	10,000 psi	0.2 – 0.4 mL/min
	2.1 x 250 mm	15,000 psi	0.2 – 0.4 mL/min

2. Step-By-Step User Guide

Thermo Fisher Scientific recommends that you perform an efficiency test on your Acclaim HILIC-10 column before use. The purpose of column performance validation is to ensure no damage has occurred during shipping. Steps 1 – 5 below outline the necessary steps to perform this validation test. 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. Slight variations may be obtained on two different HPLC systems due to system electronic, plumbing, operating environment, reagent quality, column conditioning, and operator technique.

Step 1 – Visually inspect the column

Report any visual damage to Thermo Fisher Scientific Corporation.

Step 2 – Mobile phase preparation

Obtaining reliable, consistent and accurate results requires 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. Thermo Fisher Scientific cannot guarantee proper column performance when the quality of the chemicals, solvents and water used to prepare the mobile phase has been compromised.

Typically, the mobile phase system consists of an organic solvent (e.g. usually acetonitrile) portion and an aqueous buffer (e.g. ammonium acetate, ammonium formate, phosphate buffer, etc) portion. 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.

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.

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.

Mobile Phase for Column Performance Test (QA test):

Mobile phase can be generated either by pre-mixing or by using a proportioning valve, both give satisfactory results. The use of proportioning valve provides better flexibility in method optimization, while the pre-mixed mobile phase provides less baseline noise.

100 mM ammonium acetate, pH5.0	Weigh 7.75 ± 0.03 g ammonium acetate, 2.00 ± 0.01 g acetic acid and 998.0 ± 0.5 g of D.I. water to an eluent bottle. Mix well by sonication.
Mobile Phase A (for both Column Performance Test and Lot Qualification Test) Acetonitrile/10 mM (total) ammonium acetate, pH5, v/v 90/10	Weigh 100.0 ± 0.2 g of 100 mM ammonium acetate buffer and 704.0 ± 0.5 g acetonitrile. Mix well by sonication.

Step 3 – Set up the LC system

Use a standard LC system equipped with a LC pump, a column oven, a UV detector (or an ELS detector depending on the application), 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 column performance test (containing 90% acetonitrile). Before use, the column should be washed thoroughly with the mobile phase (~20 to 50 column volumes depending on the aqueous content in the mobile phase) 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 a new mobile phase

Step 5 – Reproduce the chromatogram in the Quality Assurance Report

Perform the column QA test using the conditions described in the Quality Assurance Report (QAR), 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 results are 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.

Step 6 – Real sample analysis

Once you are satisfied with the column performance report result, the column is ready for your application.

3. Considerations in Method Development

3.1 Selection of Organic Solvents

Acetonitrile is the preferred solvent for HILIC applications. In addition, this column is compatible with traditional normal-phase solvents, such as ethanol, iso-propanol, ethyl acetate, hexane, heptane, and dichloromethane. The column should not be exposed to ketones (e.g acetone) or aldehydes using use and storage.

3.2 Buffer Types

The selection of buffer depends on the detection method and pH requirement.

1. Ammonium acetate and ammonium formate are the preferred buffers because of their applicability in HILIC separation modes, compatibility with UV (> 230 nm), Corona CAD, ELS detector and MS, high solubility in organic solvent, and familiarity to most chromatographers.
2. 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.
3. Phosphate buffers are ideal for applications that require low UV background. However, phosphate buffers tend to precipitate in high organic condition. Thus special attention is required to prevent precipitation in the column.

3.3 Mobile Phase pH

It is required that the mobile phase be buffered and its pH be controlled for optimal and reproducible results. Depending on the nature of analytes, appropriate mobile phase should be selected for desired pH range.

3.4 Isocratic or Gradient Method

Isocratic methods are suitable for simple and/or well-defined applications. When dealing with unknown samples, or a sample consisting of molecules with dramatically different hydrophilicity or hydrophobicity, a gradient method is often advantageous. Contrary to reversed-phase application, organic solvent is the weak eluting mobile phase and aqueous buffer is strong eluting one. In a gradient method, the mobile phase organic solvent should start from the higher level and gradually decrease to a lower level. In addition, the column should be fully equilibrated before any injection is made.

3.5 Injection Volume

It is highly recommended that the injection sample be dissolved in the mobile phase (the starting condition in a gradient method) or a diluent that is weaker (containing higher organic solvent) than the mobile phase. If a stronger diluent (containing more aqueous) has to be used to dissolve the analyte, the injection volume should be minimized to a fraction of the normal injection volume. For a 4.6 x 150-mm column, the typical injection volume is 5 to 10 µL. However, an injection volume as low as 1 or 2 µL is not uncommon.

4. Column Care

4.1 Column storage

The column must be stored in 100% acetonitrile or a high acetonitrile containing buffered solution (e.g. . The column can be stored in the mobile phase for short-term storage (shorter than 24 hours). However, if an aggressive mobile phase (pH below 3 or above 7) is used, the column should be washed with acetonitrile for daily storage. For long-term storage (longer than 24 hours), it is recommended to store the column in a solution containing high organic content, such as 90/10 v/v acetonitrile/10-50 mM ammonium acetate or 100% acetonitrile.

4.2 Operating pH range: pH 2.0 to 8.0

The column lifetime depends heavily on the chromatographic condition. To obtain better column lifetime, it is recommended to use "silica friendly" mobile phases, such as using a buffer with a pH between pH 3 to 7 for the aqueous portion in the mobile phase.

4.3 Operating temperature limit: 60 ºC

Based on our experimental data, this column can be used at 60 °C. The typical operating temperatures for most applications are between 20 and 40 °C.

4.4 Pressure limit:

It is extremely important not to impose a sudden column pressure surge. The maximum pressure limit is listed in Section 1.3, and depends on the particle size and column dimensions. When used under typical conditions for HILIC applications, the operating pressure should be well below the rated limit.

4.5 Flow rate

The optimum flow rate depends on the column diameter and particle size; typical ranges are listed in Section 1.3.

4.6 Column washing procedure

All samples should be pre-treated and filtered before being injected on the column. In the event that column washing/cleaning is needed, the following procedure (for a 4.6 mm i.d. column) can be used as a guideline:

For a HILIC method that uses acetonitrile and aqueous buffer as mobile phase:

1. Wash the column with 0.1 M ammonium acetate pH 4 to 5 /acetonitrile v/v 50/50 for 20 column volumes at 50% of the typical flow rate for the column dimension.
2. Wash the column with 0.1 M ammonium acetate pH 4 to 5 /acetonitrile v/v 20/80 for 20 column volumes at 50% of the typical flow rate for the column dimension.
3. Before any injection is made, the column should be equilibrated with the mobile phase thoroughly.

For a normal phase method that uses aqueous or acetonitrile immiscible organic solvents:

1. Wash the column with iso-propanol for 10 column volumes at 20% of the typical flow rate for the column dimension Wash the column with 0.1 M ammonium acetate pH 4 to 5 /acetonitrile v/v 50/50 for 20 column volumes at 50% of the typical flow rate for the column dimension.
2. Wash the column with D.I water /acetonitrile v/v 20/80 for 20 column volumes at 50% of the typical flow rate for the column dimension.
3. Wash the column with iso-propanol for 10 column volumes at 20% of the typical flow rate for the column dimension.
4. Before any injection is made, the column should be equilibrated with the mobile phase thoroughly.

NOTE

For a 4.6 mm i.d. column, the typical flow rate for washing is 0.8 mL/min.
For a 2.1 mm i.d. column, the typical flow rate for washing is 0.15 mL/min.
For a 3.0 mm i.d. column, the typical flow rate for washing is 0.3 mL/min.

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

5. Frequently Asked Questions

5.1 What is the difference between the Acclaim HILIC-10 column and other HILIC columns?

Unlike other HILIC columns that have covalently bonded neutral hydrophilic surfaces, such as amide, diol, or cyano functionality, the Acclaim HILIC-10 column is based on a modified high-purity, spherical, porous silica with a proprietary hydrophilic ligand. As a result, the Acclaim HILIC-10 exhibits different polarity and selectivity compared to other HILIC columns.

5.2 Why do I need an Acclaim HILIC-10 column?

This column offers the following features designed for a wide range of applications:

1. Ability of retaining highly polar molecules that would be un-retained by reversed-phase chromatography
2. Unique selectivity, complementary to reversed-phase columns
3. Excellent chemical stability
4. Rugged column packing

In addition, the column chemistry is unique and different from the majority of HILIC columns in the market. Thus you will have a unique high-quality HILIC column in your method development toolbox.

5.3 When do I need an Acclaim HILIC-10 column?

When you are dealing with highly polar analytes, when the reversed-phase columns fail to provide a satisfactory solution, or your typical HILIC column fails to give you the desired results, the Acclaim HILIC-10 column might be your solution.

5.4 What factors should I consider for method development using this column?

During method development, the following factors should be considered:

1. Whether or not the application calls for a HILIC column
2. Type of organic solvent
3. Type of buffer
4. Mobile phase pH
5. Temperature

5.5 What mobile phases should I use with this column?

The Acclaim HILIC-10 column is compatible with any mobile phase commonly used for HILIC or Normal-Phase separations. For a typical HILIC application, consider 80 to 90% acetonitrile with 20 to 10% ammonium acetate buffer as the starting point.

Please refer to “Section 3 Considerations in Method Development” for more details.

5.6 What should I do before starting to use the Acclaim HILIC-10 column?

Read this Product Manual carefully, and contact Thermo Fisher Scientific Technical Support if you have any questions regarding the use of this column.

5.7 Can I use this column in normal-phase mode?

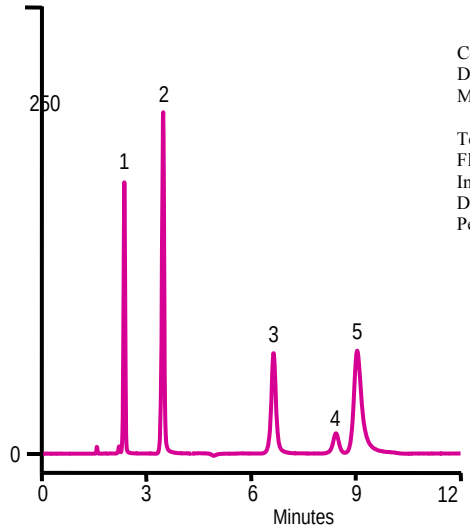
Yes. The column is compatible with traditional normal-phase solvent, such as ethanol, iso-propanol, ethyl acetate, hexane, heptane, and dichloromethane. Avoid to expose the column to ketones or aldehydes during use and storage.

5.8 How to store the column?

Refer to “Section 4.1 Column Storage” for details.

6. Applications

6.1 Hydrophilic Pharmaceuticals

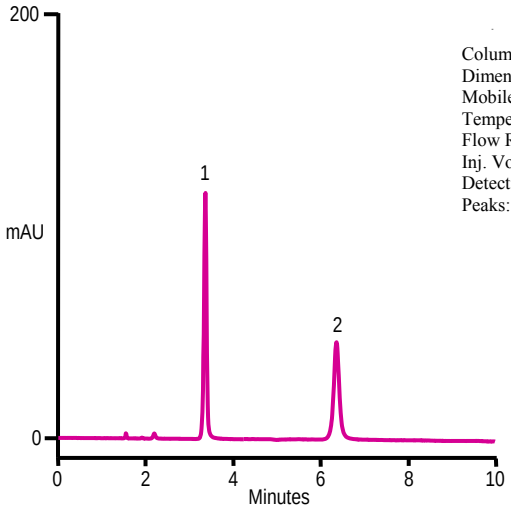


Column:	Acclaim HILIC-10, 3 µm
Dimensions:	4.6 x 150 mm
Mobile Phase:	90/10 v/v CH_3CN/10 mM (total) NH_4OAc, pH5
Temperature:	30 °C
Flow Rate:	1 mL/min
Inj. Volume:	2 µL
Detection:	UV at 230 nm

Peaks:

	mg/mL
1. Acetaminophen	0.1
2. Salicylic acid	0.1
3. Aspirin	0.2
4. Penicillin G	0.1
5. Metformin	0.1

6.2 Aspirin and Degradation Product

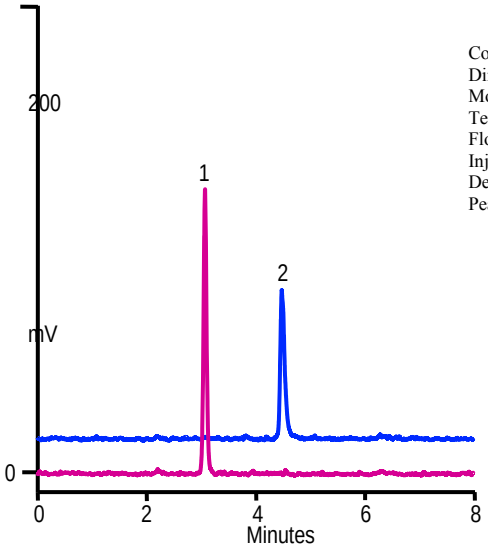


Column:	Acclaim HILIC-10, 3 µm
Dimensions:	4.6 x 150 mm
Mobile Phase:	90/10 v/v CH_3CN/10 mM (total) NH_4OAc, pH5
Temperature:	30 °C
Flow Rate:	1 mL/min
Inj. Volume:	1 µL
Detection:	UV at 220 nm

Peaks:

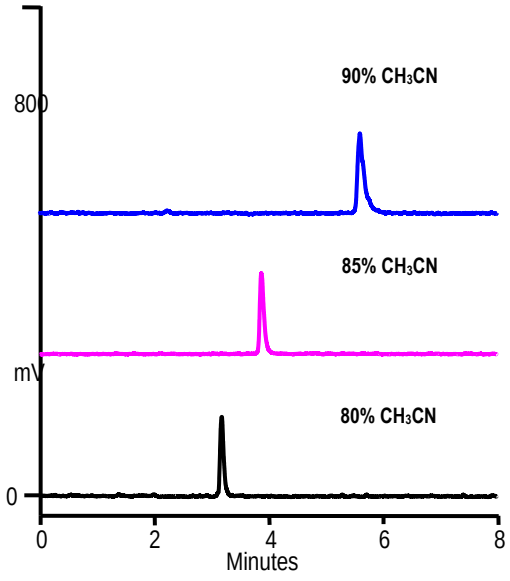
	mg/mL
1. Salicylic acid	0.1
2. Aspirin	0.2

6.3 Melamine and Cyanuric Acid



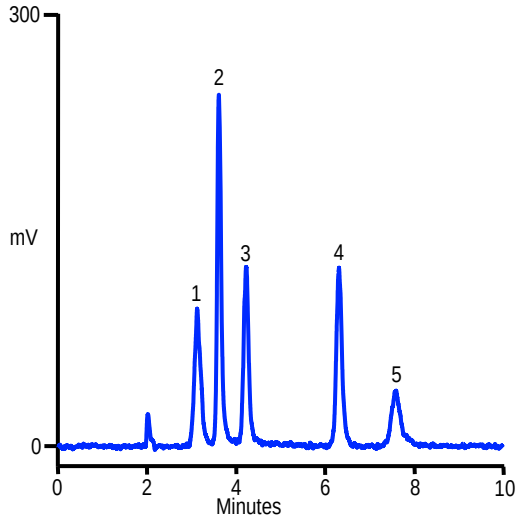
Column:	Acclaim HILIC-10, 3 µm
Dimensions:	4.6 x 150 mm
Mobile Phase:	90/10 v/v CH_3CN/10 mM (total) NH_4OAc, pH5
Temperature:	30 °C
Flow Rate:	1 mL/min
Inj. Volume:	2 µL
Detection:	ELSD
Peaks:	mg/mL
	1. Cyanuric acid 0.2
	2. Melamine 0.2

6.4 Solvent Effect of Melamine



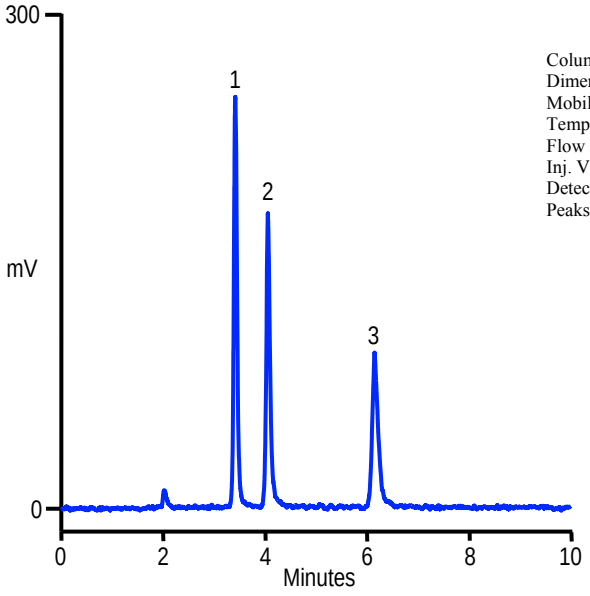
Column:	Acclaim HILIC-10, 3 µm
Dimensions:	4.6 x 150 mm
Mobile Phase:	v/v CH_3CN/100 mM NH_4OAc, pH5
Temperature:	30 °C
Flow Rate:	1 mL/min
Inj. Volume:	2 µL
Detection:	ELSD
Sample:	Melamine (0.2 mg/mL in mobile phase)

6.5 Carbohydrates



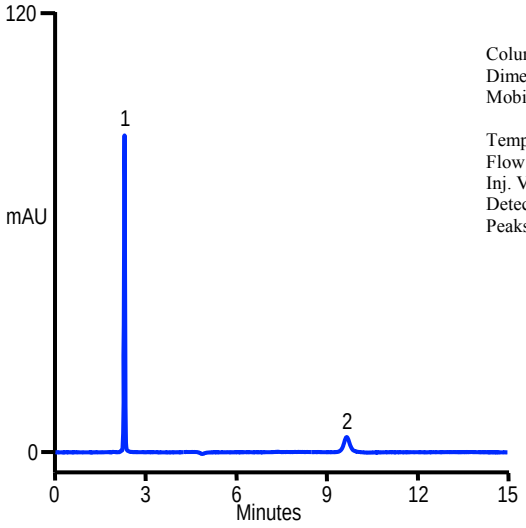
Column:	Acclaim HILIC-10, 3 µm
Dimensions:	4.6 x 150 mm
Mobile Phase:	90/10 v/v CH_3CN/10 mM (total) NH_4OAc, pH5
Temperature:	60 °C
Flow Rate:	1 mL/min
Inj. Volume:	5 µL
Detection:	ELSD
Peaks:	(0.2 mg/mL in mobile phase) 1. Fucose 2. Fructose 3. Glucose 4. Sucrose 5. Lactose

6.6 Sugar Alcohols



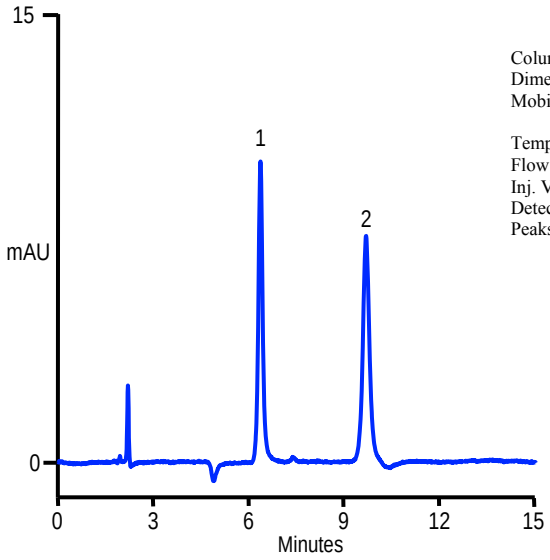
Column:	Acclaim HILIC-10, 3 µm
Dimensions:	4.6 x 150 mm
Mobile Phase:	90/10 v/v CH_3CN/10 mM (total) NH_4OAc, pH5
Temperature:	60 °C
Flow Rate:	1 mL/min
Inj. Volume:	5 µL
Detection:	ELSD
Peaks:	(0.2 mg/mL in mobile phase) 1. Xylitol 2. Sorbitol 3. Inositol

6.7 Acrylamide and Acrylic Acid



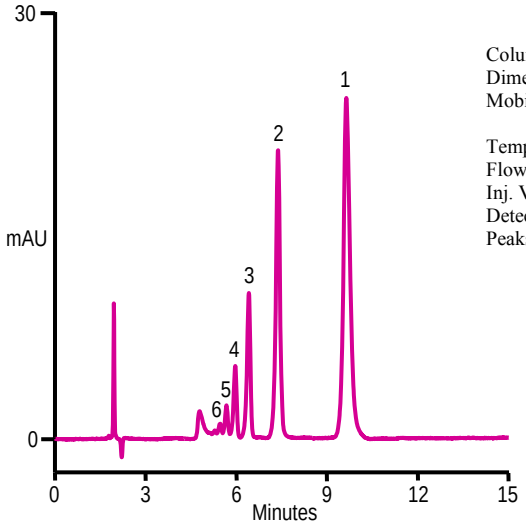
Column:	Acclaim HILIC-10, 3 µm
Dimensions:	4.6 x 150 mm
Mobile Phase:	90/10 v/v CH_3CN/10 mM (total) NH_4OAc, pH5
Temperature:	30 °C
Flow Rate:	1 mL/min
Inj. Volume:	2 µL
Detection:	UV at 230 nm
Peaks:	(0.1 mg/mL each) 1. Acrylamide 2. Acrylic acid

6.8 Methacrylic Acid and Acrylic Acid



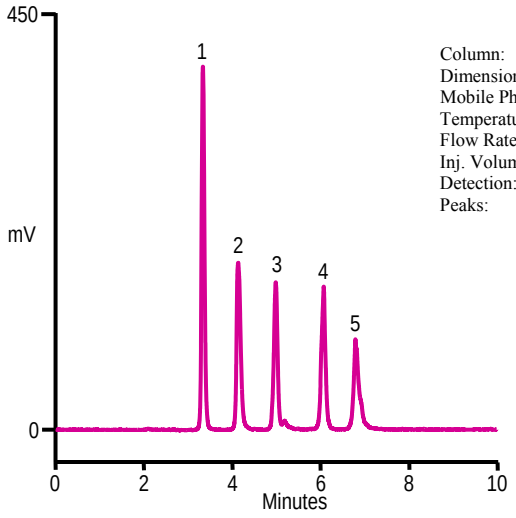
Column:	Acclaim HILIC-10, 3 µm
Dimensions:	4.6 x 150 mm
Mobile Phase:	90/10 v/v CH_3CN/10 mM (total) NH_4OAc, pH5
Temperature:	30 °C
Flow Rate:	1 mL/min
Inj. Volume:	2 µL
Detection:	UV at 230 nm
Peaks:	(0.1 mg/mL each) 1. Methacrylic acid 2. Acrylic acid

6.9 Acrylic Acid and Oligomers



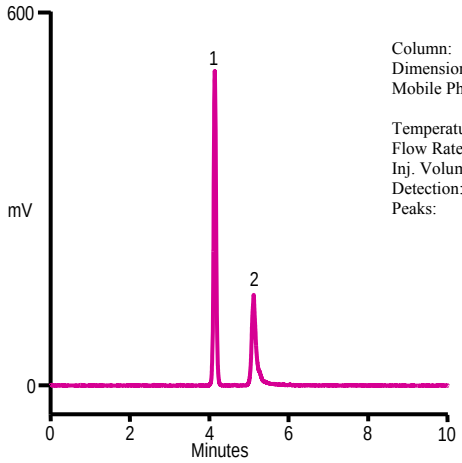
Column:	Acclaim HILIC-10, 3 µm
Dimensions:	4.6 x 150 mm
Mobile Phase:	90/10 v/v CH_3CN/10 mM (total) NH_4OAc, pH5
Temperature:	30 °C
Flow Rate:	1 mL/min
Inj. Volume:	2 µL
Detection:	UV at 230 nm
Peaks:	(5 mg/mL in mobile phase)
	1. Acrylic acid (monomer)
	2. Dimer
	3. Trimer
	4. Tetramer
	5. Pentamer
	6. Hexamer

6.10 Good's Buffer Salts



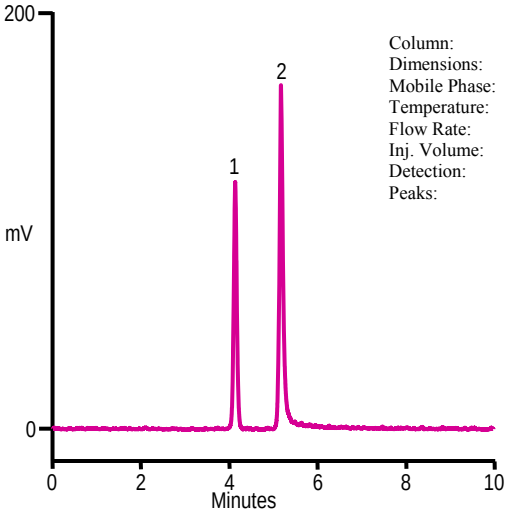
Column:	Acclaim HILIC-10, 3 µm
Dimensions:	4.6 x 150 mm
Mobile Phase:	85/15 v/v CH_3CN/10 mM (total) NH_4OAc, pH5
Temperature:	30 °C
Flow Rate:	1 mL/min
Inj. Volume:	10 µL
Detection:	ELSD
Peaks:	(0.1 mg/mL each in mobile phase)
	1. TAPS
	2. CHES
	3. MOPS
	4. TES
	5. HEPES

6.11 Sodium Chloride



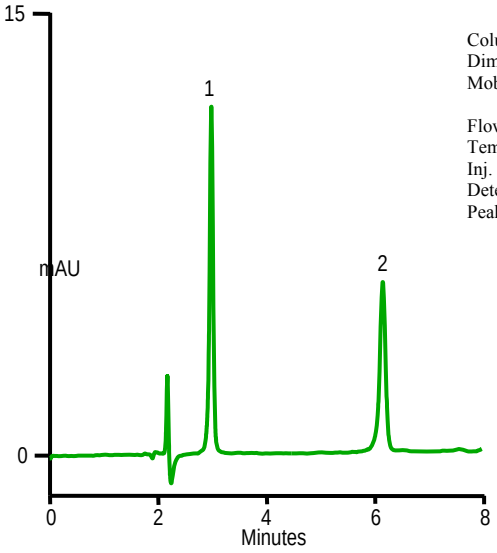
Column:	Acclaim HILIC-10, 3 µm
Dimensions:	4.6 x 150 mm
Mobile Phase:	85/15 v/v CH_3CN/10 mM (total) NH_4OAc, pH5
Temperature:	30 °C
Flow Rate:	1 mL/min
Inj. Volume:	2 µL
Detection:	ELSD
Peaks:	NaCl (0.2 mg/mL in mobile phase) 1. Cl^- 2. Na^+

6.12 Tris•HCl



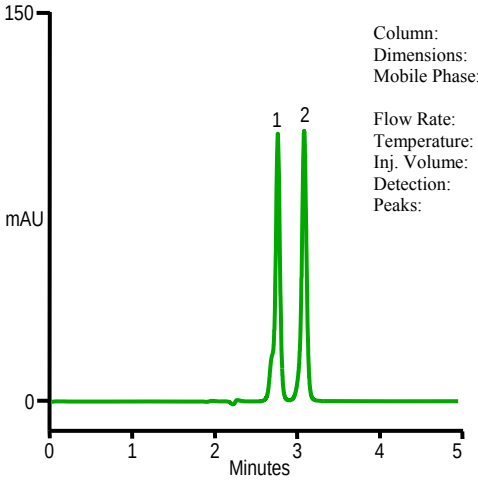
Column:	Acclaim HILIC-10, 3 µm
Dimensions:	4.6 x 150 mm
Mobile Phase:	85/15 v/v CH_3CN/10 mM (total) NH_4OAc, pH5
Temperature:	30 °C
Flow Rate:	1 mL/min
Inj. Volume:	10 µL
Detection:	ELSD
Peaks:	Tris•HCl (0.2 mg/mL in mobile phase) 1. Cl^- 2. Tris•H^+

6.13 α-Tocopherol and α-Tocopherol Acetate



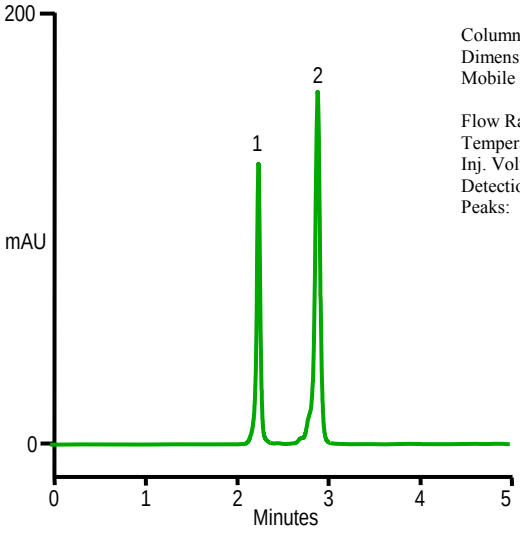
Column:	Acclaim HILIC-10, 3 µm
Dimensions:	4.6 x 150 mm
Mobile Phase:	94.75/5.00/0.25 v/v/v Heptane:Tetrahydrofuran:Isopropanol
Flow Rate:	1 mL/min
Temperature:	30 °C
Inj. Volume:	5 µL
Detection:	UVD-340U; UV 285 nm
Peaks:	µg/mL
	1. α-Tocopherol acetate 60
	2. α-Tocopherol 40

6.14 Vitamins K1 and K2



Column:	Acclaim HILIC-10, 3 µm
Dimensions:	4.6 x 150 mm
Mobile Phase:	94.75/5.00/0.25 v/v/v Heptane:Tetrahydrofuran:Isopropanol
Flow Rate:	1 mL/min
Temperature:	30 °C
Inj. Volume:	5 µL
Detection:	UVD-340U; UV 265 nm; spectra 200-400 nm
Peaks:	µg/mL
	1. Vitamin K1 50
	2. Vitamin K2 50

6.15 Retinol Palmitate and Retinol Acetate



Column:	Acclaim HILIC-10, 3 µm
Dimensions:	4.6 x 150 mm
Mobile Phase:	94.75/5.00/0.25 v/v/v Heptane:Tetrahydrofuran:Isopropanol
Flow Rate:	1 mL/min
Temperature:	30 °C
Inj. Volume:	5 µL
Detection:	UVD-340U; UV 325 nm

Peaks:

	µg/mL
1. Retinol Palmitate	50
2. Retinol Acetate	50