PRODUCT MANUAL

for

Acclaim®
Organic Acid (OA)

Now sold under the
Thermo Scientific brand

Thermo
SCIENTIFIC

DIONEX

IC | HPLC | MS | EXTRACTION | PROCESS | AUTOMATION

Product Manual

Acclaim® Organic Acid (OA) Guard Cartridge

(5 µm, 4.6 x 10mm, p/n 069700)
(5 µm, 4.3 x 10mm, p/n 062925)
(5 µm, 3.0 x 10mm, p/n 071987)

Acclaim® Organic Acid (OA) Analytical Columns

(5 µm, 4.0 x 250mm, p/n 062902)
(5 µm, 4.0 x 150mm, p/n 062903)
(3 µm, 3.0 x 150mm, p/n 070086)
(3 µm, 2.1 x 150mm, p/n 070087)

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28 July 2009

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SECTION 1 - INTRODUCTION TO ACCLAIM ORGANIC ACID COLUMN

1.1 Features of the Acclaim OA

Acclaim® OA columns were developed for the reversed-phase separation of hydrophilic aliphatic and aromatic organic acids with UV detection. The Acclaim OA columns are use-tested to ensure optimum, controlled separations. This column uses a patented polar embedded bonding chemistry that offers stable bonding which is very resistant to hydrolysis. Figure 1 shows the stability of retention times, peak efficiencies, and peak asymmetries of the Acclaim OA after long term exposure to elevated temperature and low pH. The unique bonding allows the Acclaim OA columns to be used with 100% aqueous (Figure 2) to 100% organic solvent mobile phases. This allows retention of very polar organic acids as well as the ability to elute nonpolar organic acids in a single run. Acclaim OA columns consist of 5-μm, or 3-μm high purity spherical silica particles with 120-Å diameter pores, bonded with a proprietary functional group, which provides unsurpassed resolution and efficiency of common hydrophilic organic acids, as shown in Figure 3.

Column:	Acclaim OA, 5 μm
Dimensions:	4.0 x 150 mm
Mobile Phase:	CH_3CN /1% TFA*, pH 1 v/v 50/50
Temperature:	50 °C
Flow Rate:	1.0 mL/min
Injection Volume:	5 μL
Detection:	UV, 254 nm
Analyte:	Toluene

*Trifluoroacetic acid

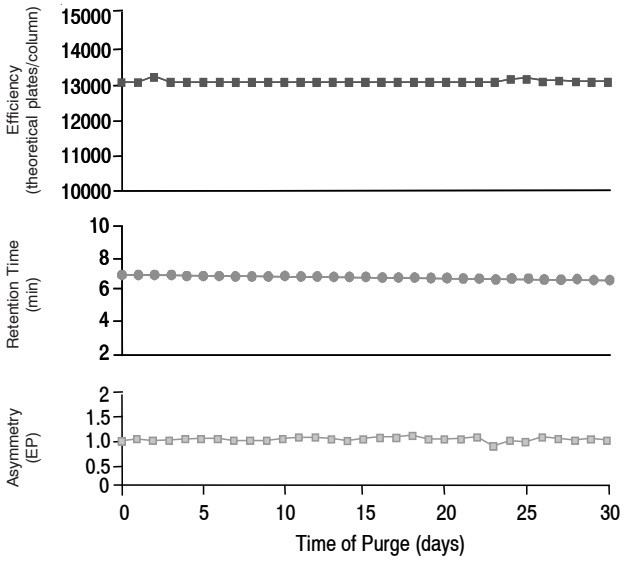
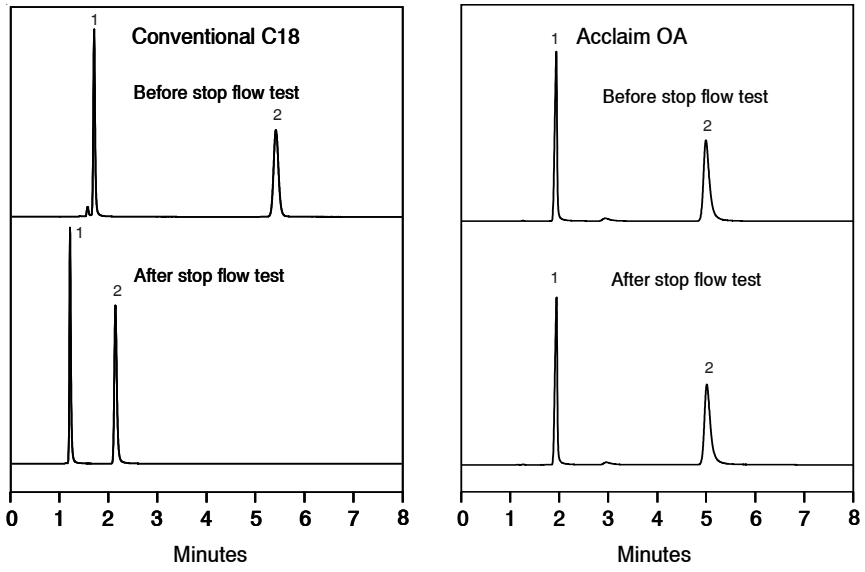


Figure 1
Hydrolytic Stability

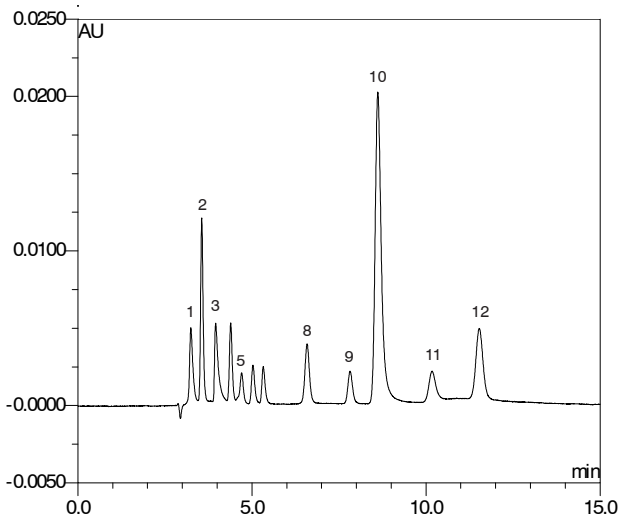
Column:	Acclaim OA, 5 μm
Dimensions:	150 x 4.0 mm
Mobile Phase:	2.5 mM methanesulfonic Acid
Flow Rate:	1 mL/min
Temperature:	30 °C
Injection Volume:	5 μL
Detection:	UV, 210 nm



Analytes
1. Tartaric acid
2. Fumaric acid

Figure 2
Resistance to Dewetting in 100% Aqueous Mobile Phase

Column:	Acclaim OA, 5 μm
Dimensions:	4 x 250 mm
Mobile Phase:	100 mM Na_2SO_4, pH 2.65 (adjusted with methanesulfonic acid)
Temperature:	30 °C
Flow Rate:	0.6 mL/min
Injection Volume:	5 μL
Detection:	UV, 210 nm



	Analytes	mg/L (ppm)
1.	Oxalic acid	15
2.	Tartaric acid	120
3.	Formic acid	180
4.	Malic acid	120
5.	*iso*-Citric acid	120
6.	Lactic acid	180
7.	Acetic acid	120
8.	Citric acid	120
9.	Succinic acid	120
10.	Fumaric acid	7
11.	*cis*-Aconitic acid	**
12.	*trans*-Aconitic acid	**

** 7 ppm total for *cis* and *trans* isomers

Figure 3
Separation of Common Hydrophilic Organic Acids

1.2 Acclaim OA Operating Limits and Specifications

Shipping Solution:	Acetonitrile, 100%
Storage Solution:	Acetonitrile, 100%
Buffers:	pH 2.0–8.0
Solvents:	Methanol, Acetonitrile 0–100%; Tetrahydrofuran 0–20%
Temperature Range:	< 60 °C

Stationary Phase	Particle size	Column dimensions	P/N	Max Recommended Pressure	Typical Back pressure (aqueous buffer)	Typical Flow Rate
Acclaim OA	3 µm	2.1x150 mm	070087	4000 psi (100% aqueous) 2000 psi (100% organic)	< 2200psi	0.2 - 0.5 mL/min
		3.0x150 mm	070086	3500 psi (100% aqueous) 2000 psi (100% organic)	< 1980 psi	0.3 - 0.8 mL/min
	5 µm	4.0x150 mm	062903	4000 psi (100% aqueous) 2000 psi (100% organic)	< 1155 psi	0.6 - 1.5 mL/min
		4.0x250 mm	062902	4000 psi (100% aqueous) 2000 psi (100% organic)	< 1650 psi	0.6 - 1.5 mL/min

Physical

Bonding:	Proprietary
Endcapping:	Yes
% C:	17
Pore size:	120 Å
Surface area:	300 m^2/g
Particle sizes:	5 µm and 3 µm

Lot Performance

Metal Activity Ratio:	0.80–1.50

1.3 Formats of the Acclaim OA

The Larger Acclaim OA columns (4.0 and 3.0 internal diameter) are packed in PEEK hardware to minimize potential metal interferences with the determinations of polyvalent carboxylic acids. The 2.1x150mm column is a stainless steel column.

1.3.1 Acclaim OA Products

Stationary Phase	Particle size	Column dimensions	P/N	
Analytical	3 µm	2.1x150 mm	070087	
		3.0x150 mm	070086	
	5 µm	4.0x150 mm	062903	
		4.0x250 mm	062902	
Guard	5µm	4.3 x 10mm	062925	Requires Holder p/n 059456
		3.0 x 10mm	071987	Requires Holder V-2 p/n 069580
		4.6 x 10mm	069700	Requires Holder V-2 p/n 069580

1.4 Acclaim OA Reversed Phase Operating Conditions

Acclaim OA columns are stable between pH 2–8 and are compatible with eluents containing 0–100% HPLC solvents such as methanol or acetonitrile. The Acclaim OA can be operated within the specification listed in section 1.2. When setting up the analytical system, check the special precautions listed in Section 3, "Installation." PEEK (polyetheretherketone) is used to make the column (4.0 and 3.0 ID) hardware. PEEK has excellent chemical resistance to most organic solvents and inorganic solutions. Tetrahydrofuran at concentrations of greater than 20% is not compatible with PEEK systems.

1.5 Key Applications of the Acclaim OA

The Acclaim OA provides excellent peak efficiencies and fast run times for determining small hydrophilic organic acids, C1 to C7 aliphatic acids, as well as hydrophilic aromatic acids. Thus it is valuable for analyzing food and beverage products, plating baths for semiconductor manufacturing and QA testing of chemicals, and chemical intermediates used in plastics and polymer manufacturing.

Applications by class of compounds:

- Small hydrophilic organic acids
- Volatile aliphatic acids
- Aromatic carboxylic acids
- Some natural amino acids and small hydrophilic peptides

Application by industries:

- Food and beverages
- Pharmaceuticals
- Chemical
- Semiconductors
- Environmental

1.6 Selecting the Best Analytical Column for Organic Acid Determinations

Dionex offers products in all five general types of columns used for organic acids analysis:

- Anion Exchange: AS11, AS11-HC, AS15, AS17, AS18
- Ion Exclusion: ICE-AS1, ICE-AS6
- Polar-embedded reversed-phase: Acclaim OA
- Ion-pairing on reversed-phase: Acclaim 120 C18, IonPac MPIC
- Mixed Mode: OmniPac PAX-500

To help you select the separation mechanism that best suits your needs consider the following questions.

- **What kind of liquid chromatography system will you use?**

A. Ion Chromatography
   1. Dionex IC systems are compatible with all five separation mechanisms. Ion exchange separations are designed for conductivity detection. Use of Acclaim OA requires a system equipped with UV or RI detection.

B. Classic HPLC
   1. Polar-embedded RP with UV or RI detection
   2. Ion-pairing on RP with UV or RI detection

- **Concentration of organic acids in your sample?**

A. 10 ppm or greater
   1. All five types can be used with appropriate dilution

B. 10 ppm or less
   1. Anion exchange or ion exclusion with suppressed conductivity detection will provide adequate sensitivity.

C. 10 ppb or less
   1. Anion exchange with mass-spectrometer detection or concentrator columns with suppressed conductivity detection are required.

- **Complexity of analysis; how many substances do you need to determine?**

A. Hydrophilic organic acids only
   1. Any of the five methods will work. Ion pair provides poorer selectivity for divalent and trivalent acids

B. Hydrophilic organic acids and neutral species or lipophilic acids
   1. Polar-embedded RP with UV
   2. Ion exclusion with UV for neutral species

C. Inorganic and organic anions
   1. Ion exchange
   2. Polar-embedded RP with UV detection for selected anions such as nitrite, nitrate, etc.

D. Organic acids and amino acids
   1. Polar-embedded RP with UV or RI for > 100 ppm
   2. Ion exchange

- **Complexity of sample; what is the matrix?**

A. Simple, well characterized matrix
   1. Any of the five methods will work

B. Moderate complexity
   1. Anion exchange; may require sample pretreatment
   2. Ion exclusion;
   3. Polar-embedded RP;

C. High MW anions (dyes, surfactants, proteins, plant pigments etc.) in sample
   1. Polar-embedded RP
   2. Ion exclusion
   3. Anion exchange after sample pretreatment. Anionic surfactants may foul the column.

D. High MW cations (dyes, surfactants) in sample
   1. Polar-embedded RP
   2. Anion exchange
   3. Ion-pairing on RP

E. Metal ions in sample
   1. Anion exchange after sample pretreatment
   2. Polar-embedded RP after sample pretreatment

F. Amino acids in sample
   1. Anion exchange
   2. Ion-pairing on RP

G. Inorganic anions at high concentration
   1. Ion exclusion
   2. Polar-embedded RP (only acceptable for UV-transparent inorganic ions)

H. Acidic sample
   1. Polar-embedded RP
   2. Ion exclusion (strong acid anions are unretained)

I. Alkaline sample
   1. Anion exchange

- **Will you need gradient elution?**

A. Isocratic elution
   1. All five methods can be used

B. Gradient elution
   1. Anion exchange (hydroxide gradient)
   2. Polar-embedded RP (organic solvent gradient)

1.7 Acclaim OA Reversed-phase Column vs. IonPac Anion Exchange or ICE Ion-exclusion Columns

If you use traditional reversed phase HPLC with UV-Vis detection for uncharged molecules plus organic acids, use the Acclaim OA columns. Reversed phase chromatography allows direct injection without sample preparation other than simple dilution of the product or reaction solution. Dyes and surfactants in the samples elute easily from the OA column. Whereas many common dyes and surfactants bind irreversibly to anion exchange or ion exclusion columns, precluding their use with such samples.

The sensitivity for the Acclaim OA method with low UV detection is in the low ppm range. If you are interested in trace organic acid determinations, you should consider anion exchange with conductivity detection or IC-MS or LC-MS.

Reversed Phase HPLC

To achieve low level sensitivity, Reversed phase HPLC with derivatization is used. Derivatizing agents such as p-bromophenacyl bromide are used to produce the strongly adsorbing phenacyl ester chromophore. Derivatization works well for monovalent organic acids; however it does not work well for polyvalent acids because multiple derivatives are formed. Detection limits are in the low ppb to high ppt range.

Ion Exclusion Chromatography

Ion exclusion or anion exchange are best suited for the separation of the more hydrophilic short-chain carboxylic acids. Ion exclusion columns are packed with highly cross-linked (approximately 30%) polystyrene divinylbenzene resins. Due to high crosslinking, these columns can be used with high levels of organic solvents. In addition to conductivity detection, UV detection at 210 nm or RI detection allows some flexibility in eluent selection. Detection limits for UV and suppressed conductivity are similar and are in the 0.5 to low ppm range. Detection limits for RI detection are in the mid-ppm range.

Ion Exclusion Columns for Organic Acids in Complex Matrices

The ICE-AS1 and ICE-AS6 columns are ion exclusion columns used for the separation of organic acids in complex matrices such as brines, wastewater, kraft liquors and soil extracts. With ion exclusion, strong acid anions such as chloride, nitrate, etc., elute in the column void volume and do not interfere in the analysis of the organic acids.

The ICE-AS1 and ICE-AS6 ion exclusion columns provide a specific solutions to the analysis of organic acids in complex matrices. Ion exchange columns such as the AS11 and AS11-HC columns do not address these high-ionic strength sample matrices without sample preparation.

Anion Exchange Chromatography

Anion Exchange columns such as the IonPac AS11 and AS11-HC columns are designed to separate monovalent carboxylic acids and to separate coeluting dicarboxylic acids including succinate/malate, malonate/tartrate, and fumarate/oxalate found in food and beverage and chemical matrices. HPLC solvents can be used for anion exchange selectivity control or to dissolve samples. These columns are designed to be used with suppressed conductivity detection, allowing gradient hydroxide elution. Method detection limits can be extended to the low ppb range.

This manual assumes that you are familiar with the installation and operation of the Dionex LC and IC systems and columns. If you do not understand the operation of the system, take the time to familiarize yourself with the various system components before beginning an analysis.

Always remember that assistance is available for any problem that may be encountered during the shipment or operation of Dionex instrumentation and columns through tnearest Dionex Office.

SECTION 2 - INSTALLATION

2.1 System Requirements

The Acclaim OA Analytical Column is designed to be run on any HPLC system with UV detection, including DIONEX IC Systems and HPLC Systems.

2.2 System Void Volume

For best performance, all of the tubing installed between the injection valve and detector should be 0.005 inch (0.12 mm) i.d. PEEK tubing (P/N 044221). Note that 0.010 inch (0.25 mm) i.d. PEEK tubing (P/N 042690) may be used but peak efficiency will be compromised which may also result in decreased peak resolution. Minimize the lengths of all connecting tubing and remove all unnecessary switching valves and couplers. If you need assistance in properly configuring your system contact the nearest Dionex Office .

2.3 The Injection Loop

DIONEX recommends a 10 μL injection loop.

2.4 Column Installation

Connect the column with the direction arrow on the label pointing toward the detector.

2.5 Column Guard

When using a guard cartridge, install the cartridge in the guard holder. Connect the guard holder to the analytical column using the column connector fitting or a 0.005 inch (0.12 mm) i.d. PEEK tubing.

NOTE

Dionex recommends the use of PEEK ferrules and bolts and PEEK tubing to install the Acclaim OA columns.

SECTION 3 - OPERATION

3.1 Standard Operating Conditions

Column:	Acclaim OA, 5 μm
Dimensions:	4 x 250 mm; 4 x 150 mm
Mobile Phase:	100 mM Na_2SO_4, pH 2.65 (adjusted with methanesulfonic acid)
Temperature:	30 °C
Flow Rate:	0.6 mL/min
Injection Volume:	10 μL
Detection:	UV, 210 nm

QuickStart for Acclaim OA

A. Prepare the mobile phase (see Section 4.8).

B. Install the mobile phase, DI water and acetonitrile on the LC pump.

C. Turn on and set the UV detector to 210 nm.

D. Prime the pump with acetonitrile.

E. Install the column.

F. Flush the column with acetonitrile (0.8 mL/min) until the UV baseline is flat (approximately 10 minutes).

G. Flush the column with DI water for approximately 7 minutes.

H. Equilibrate the column with mobile phase for at least 15 minutes.

I. Inject 5–25 μL of sample.

J. At the end of the day, flush the column with DI water for approximately 10 minutes.

K. Flush the column with acetonitrile for approximately 10 minutes.

L. Turn off the pump and detector.

M. Remove the column and plug the ends, if the system will be shut down for more than 2–3 days.

3.2 Equilibrating the Column

Equilibrate the column when it is installed for the first time. Always re-equilibrate the column prior to use following periods of storage.

Purge the column with the shipping or storage solvent until the baseline is stable. The shipping and recommended storage solvent is 100% acetonitrile. Purge the column with enough water to remove the solvent. Equilibrate the column with at least 15 column volumes of the mobile phase until a stable baseline is achieved. If the mobile phase is not fully miscible with the acetonitrile storage solvent, run a linear gradient from acetonitrile to the new solvent over approximately 10 minutes.

3.3 Validating Column Performance

Perform an efficiency test on your Acclaim OA column before you use it. Test the column using the stated conditions on the Quality Assurance 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 two different HPLC systems due to system electronic, plumbing, operating environment, reagent quality, column condition and operator technique.

Please see the example Quality Assurance Report in Appendix A.

3.4 Caring for the Column

To ensure the high performance of the Acclaim OA column, the following guidelines should be followed:

- Protect the column from contamination with an Acclaim OA guard.
- Use Dionex OnGuard® II matrix removal cartridges to clean up the sample prior to analysis.
- Filter the samples through a 0.5 μm membrane filter.
- Make sure that solvents are miscible when changing mobile phases. If the mobile phase contains a buffer, flush the column first with water and then run a solvent gradient to high organic solvent concentrations.
- Always degas and filter mobile phases through a 0.22 μm membrane filter.

3.5 Cleaning and Regenerating the Column

3.5.1 Decreased Retention or Peak Resolution

A decease in retention or resolution may indicate contamination of the column. Flushing with 20 column volumes of a neat organic solvent is usually sufficient to remove the contaminant. If the mobile phase contained a buffer, first flush the column with 10 column volumes of 18 megohm-cm reagent water before changing to the pure solvent. **For long column life and consistent performance, it is recommended that this cleaning procedure be performed on a daily basis.**

3.5.2 Peak Tailing

Severe peak tailing can be an indication of metal contamination. Treatment with a solution of a chelating agent is usually effective. EDTA at pH 4 or pentanedione in methanol are known to be effective. Recurring metal contamination may require cleaning or passivation of your LC system; refer to the operator's instructions for your LC system.

3.5.3 High Back Pressure

If system back pressure increases with usage, replace the guard cartridge.

3.6 Storing the Column

Leaving the column unused for a short period of time does not require special storage procedures. For long term storage, store the column in 100% acetonitrile and be sure that the end plugs are firmly in place. Never let the column dry out.

If the mobile phase contained a buffer, first flush the column with 10 column volumes of 18 megohm-cm reagent water before changing over to 100% acetonitrile.

3.7 Chemical Purity Requirements

Obtaining reliable, consistent and accurate results requires mobile phases that are free of ionic and spectrophotometric impurities. Chemicals, solvents and deionized water used to prepare mobile phases 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 eluents has been compromised.

3.7.1 Inorganic Chemicals

Reagent Grade inorganic chemicals should always be used to prepare ionic eluents. Whenever possible, inorganic chemicals that meet or surpass the latest American Chemical Society standard for purity should be used. These inorganic chemicals will detail the purity by having an actual lot analysis on each label.

3.7.2 Deionized Water

The deionized water used to prepare eluents should be **Type I Reagent Grade Water** with a specific resistance of 18.2 megohm-cm. The deionized 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 organic acids analysis.

3.7.3 Solvents

The solvents used must be free of ionic and UV-absorbing impurities. However, since most manufacturers of solvents do not test for ionic impurities, it is important that the highest grade of solvents available be used. Currently, several manufacturers manufacture ultrahigh purity solvents that are compatible for HPLC and spectrophotometric applications. These ultrahigh purity solvents will usually ensure that your chromatography is not affected by ionic impurities in the solvent.

When using a solvent in an ionic mobile phases, column generated back pressures will depend on the solvent used, concentration of the solvent, the ionic strength of the buffer and the flow rate used. The column back pressure will vary as the composition of water-methanol or water-acetonitrile mixture varies. The practical back pressure limit for the Acclaim OA columns is 4,000 psi (27.57 MPa).

NOTE

Since water-methanol mixtures result in much higher back-pressures, water-acetonitrile mobile phases in any ratio are preferred and recommended.

3.8 Eluent Preparation

3.8.1 100% Aqueous Mobile Phases

Standard test chromatogram mobile phase: dissolve 14.2 g Na_2SO_4 (anhydrous) in 1.0 L deionized water. Adjust pH to 2.65 ± 0.05 with methanesulfonic acid (approximately 0.55 mL/L) using a calibrated pH meter.

Alternate mobile phases: first make the aqueous solution with certain ionic strength by dissolving an inorganic salt (e.g. Na_2SO_4 or KH_2PO_4) in Type I Reagent Grade water which has been properly degassed. Then use methanesulfonic, phosphoric, or sulfuric acid to adjust to the desired pH.

3.8.2 Mobile Phases Containing Solvents

When mixing solvents with water, mix solvent with water on a volume to volume basis. For example, if a procedure requires a mobile phase of 10% acetonitrile, prepare the mobile phase by adding 100 mL of acetonitrile to a mobile phases reservoir. Then add 900 mL of the aqueous portion (D.I. water or a buffer) to the acetonitrile in the reservoir. Using this procedure to mix solvents with water will ensure that a consistent true volume/volume mobile phases is obtained. Premixing water with solvent will minimize the possibility of outgassing in the detector cell.

NOTE

Degass the aqueous component of the eluent and then add the solvent component. Avoid excessive purging or degassing of mobile phases containing solvents if possible, since a volatile solvent can be "boiled" off from the solution.

SECTION 4 – CONSIDERATIONS FOR METHOD DEVELOPMENT

4.1 pKa Values of Selected Organic Acids

The tables in Appendix B list the pKs of selected organic acids in alphabetical order and in ascending order of pK. The tables plus the example applications are designed to give the chromatographer a simple method for estimated the ability of the Acclaim OA to separate various combinations of organic acids.

4.2 Ionic Strength Effect

Mobile phase ionic strength is essential for separation: while poor resolution is observed in mobile phases without any salt additive, increasing salt concentration in the mobile phase greatly improves separation for 12 hydrophilic organic acids. In general, buffer concentration should be at or above 40 mM to achieve satisfactory separation.

Column:	Acclaim OA, 5 μm
Dimensions:	150 x 4.0 mm
Mobile Phase:	0 to 80 mM Na_2SO_4, pH 2.65 (adjusted with methanesulfonic acid)
Temperature:	30 °C
Flow Rate:	1.0 mL/min
Injection Volume:	5 μL

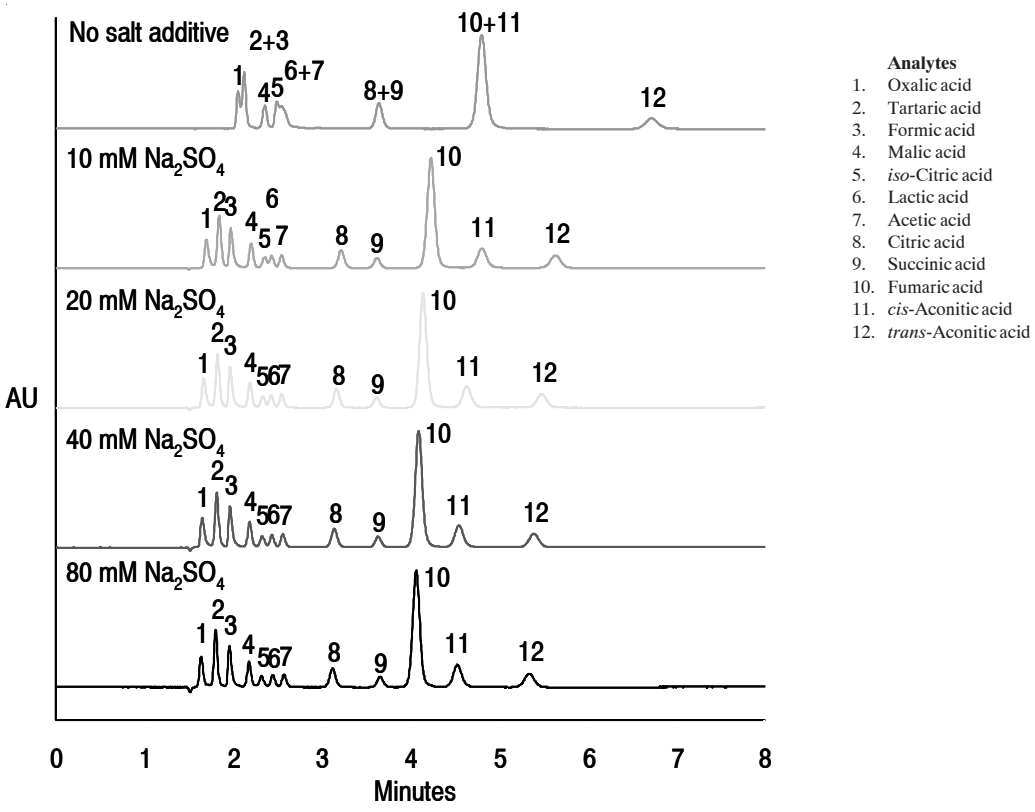


Figure 4
Ionic Strength Effect

4.3 pH Effect

Mobile phase pH determines column selectivity when analyzing organic acids. Figure 5 illustrates changes in retention times for individual acids at different pH levels. Based on different sets of organic acids of interest, the appropriate pH can be chosen to achieve the best resolution. Acclaim OA provides good separations for hydrophilic organic acids in the pH range of 2.5 to 3.2. For example, when raising the mobile phase pH to 2.8 - 3.0 range, the resolution of lactic acid and acetic acid is improved.

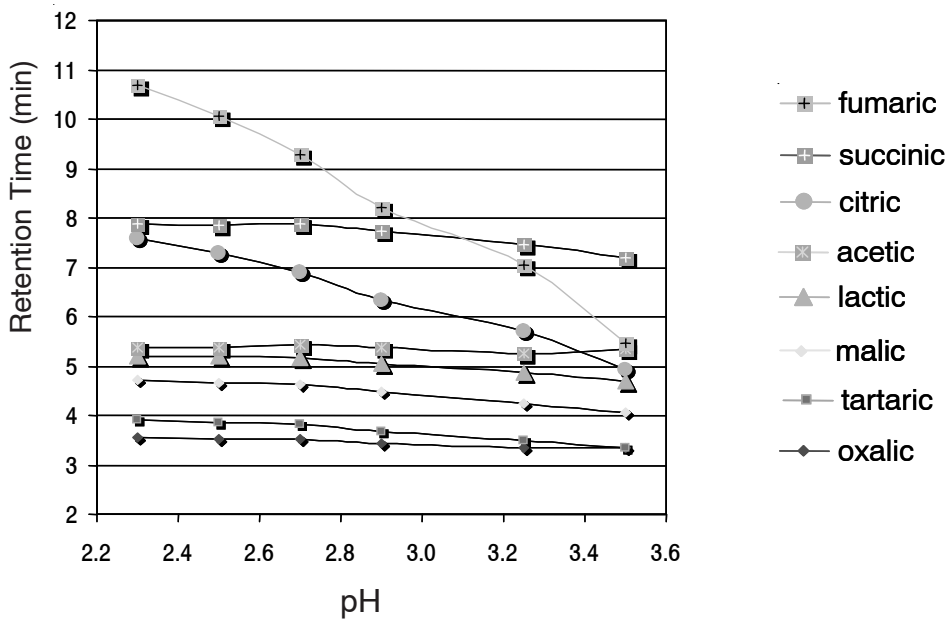


Figure 5
pH Effect

4.4 Organic Modifier Effect

As shown in Figure 6, addition of organic modifier to the mobile phase changes the retention times of organic acids, but has no effect on selectivity: the higher the organic composition, the shorter the retention time. On the other hand, the presence of organic modifier helps prolong the column life. When applicable, 1 – 2% of organic modifier such as acetonitrile can be added to the mobile phase. If your samples contain small hydrophilic aliphatic acids and aromatic acids, a buffer/organic solvent gradient method is usually required.

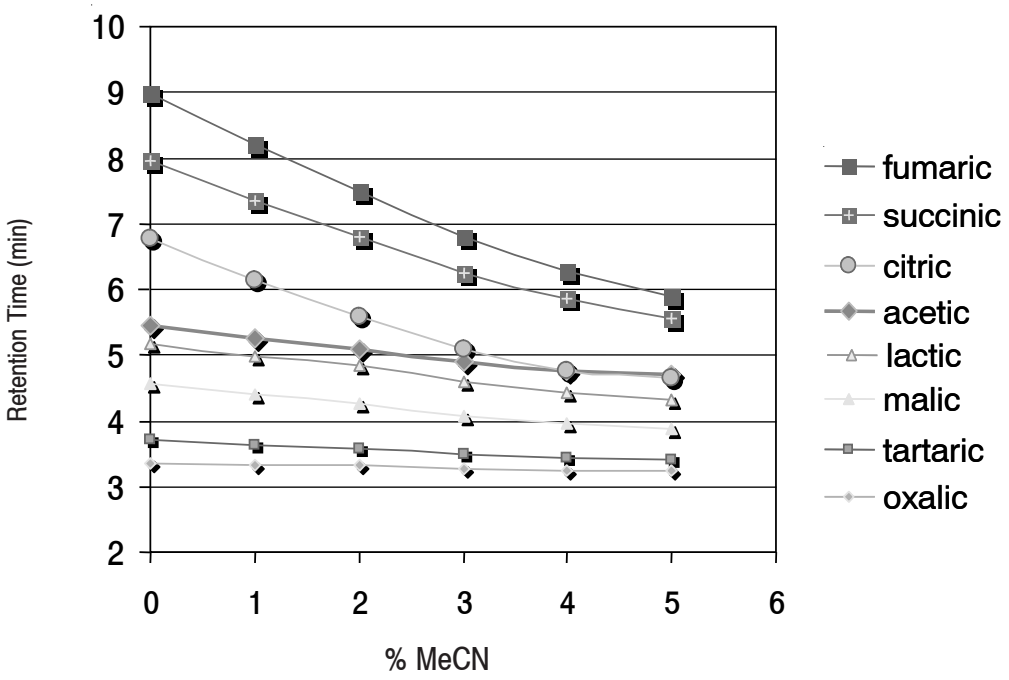


Figure 6
Organic Modifier Effect

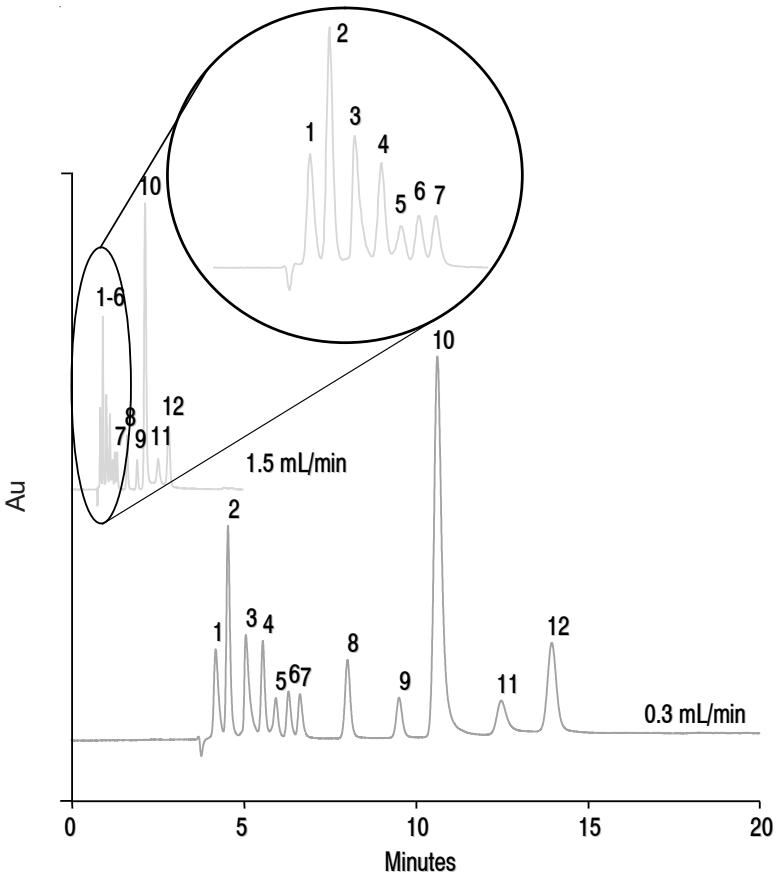
4.5 Temperature Effect

As with the reversed phase separation of other small molecules, elevating temperature shortens retention for organic acids, but in various degrees. The separation of the 12 inorganic acids in Figure 3 degrades with increasing temperature. Therefore, the recommended operating temperature is 25 °C to 35 °C.

4.6 Flow Rate Effect

When using pure buffer as the mobile phase, better column efficiencies and resolution can be obtained by lowering the flow rate. Flow rates of 0.3 to 0.5 mL/min normally result in optimum resolution in 100% aqueous mobile phases. One the other hand, fast separation of 12 hydrophilic acids can also be performed at higher flow rate (1.5 mL/min) within 3 minutes, as shown in Figure 7. The user should make the judgment to balance the resolution against sample throughput. The recommended flow rate for Acclaim OA is 0.3 to 1.5 mL/min.

Column:	Acclaim OA, 5 μm
Dimensions:	4 x 150 mm
Mobile Phase:	40 mM Na_2SO_4 pH 2.65 (adjusted with methanesulfonic acid)
Temperature:	30 °C
Flow Rate:	See chromatograms
Injection Volume:	5 μL
Detection:	UV, 210 nm



Analytes
1. Oxalic acid
2. Tartaric acid
3. Formic acid
4. Malic acid
5. *iso*-Citric acid
6. Lactic acid
7. Acetic acid
8. Citric acid
9. Succinic acid
10. Fumaric acid
11. *cis*-Aconitic acid
12. *trans*-Aconitic acid

Figure 7
Effect of Flow Rate

4.7 Mobile Phase Effect

Stable mobile phase pH and proper ionic strength are two determining factors for the separation of organic acids in terms of selectivity and resolution. Various mobile phase systems can be used to separate organic acids using the Acclaim OA column. Two proven mobile phases are Na_2SO_4 buffer, pH adjusted with methanesulfonic acid, and the phosphate buffer system. As shown in Figure 8, both buffer systems give equivalent results.

Column:	Acclaim OA, 5 μm
Dimensions:	4 x 150 mm
Mobile Phase:	see chromatograms
Temperature:	30 °C
Flow Rate:	0.5 mL/min
Injection Volume:	5 μL
Detection:	UV, 210 nm

Analytes
1. Oxalic acid
2. Tartaric acid
3. Formic acid
4. Malic acid
5. *iso*-Citric acid
6. Lactic acid
7. Acetic acid
8. Citric acid
9. Succinic acid
10. Fumaric acid
11. *cis*-Aconitic acid
12. *trans*-Aconitic acid

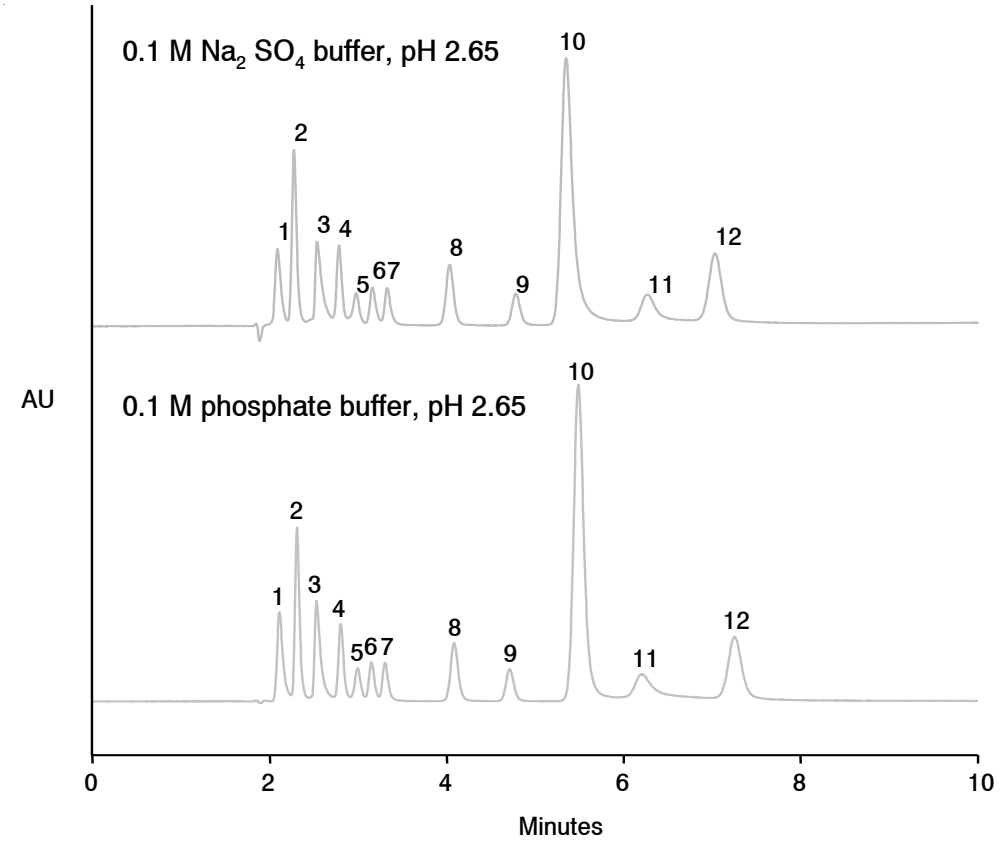
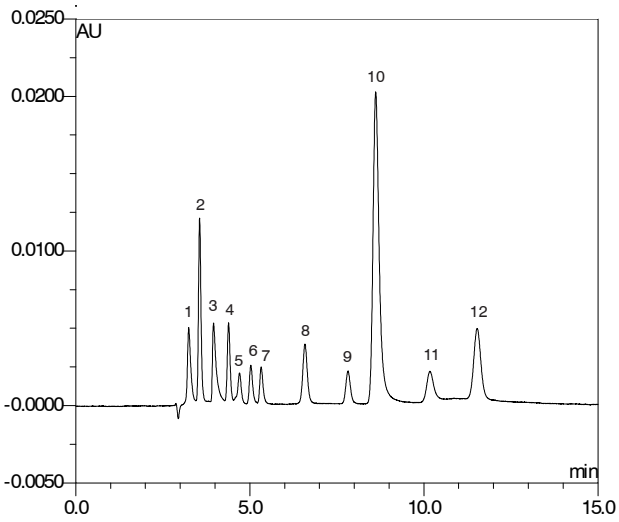


Figure 8
Mobile Phase Effect

SECTION 5 - EXAMPLE APPLICATIONS

5.1 Highly Hydrophilic Organic Acids

Column:	Acclaim OA, 5 μm
Dimensions:	4 x 250 mm
Mobile Phase:	100 mM Na_2SO_4, pH 2.65 (adjusted with methanesulfonic acid)
Temperature:	30 °C
Flow Rate:	0.6 mL/min
Injection Volume:	5 μL
Detection:	UV, 210 nm



	Analytes	mg/L (ppm)
1.	Oxalic acid	15
2.	Tartaric acid	120
3.	Formic acid	180
4.	Malic acid	120
5.	*iso*-Citric acid	120
6.	Lactic acid	180
7.	Acetic acid	120
8.	Citric acid	120
9.	Succinic acid	120
10.	Fumaric acid	7
11.	*cis*-Aconitic acid	**
12.	*trans*-Aconitic acid	**

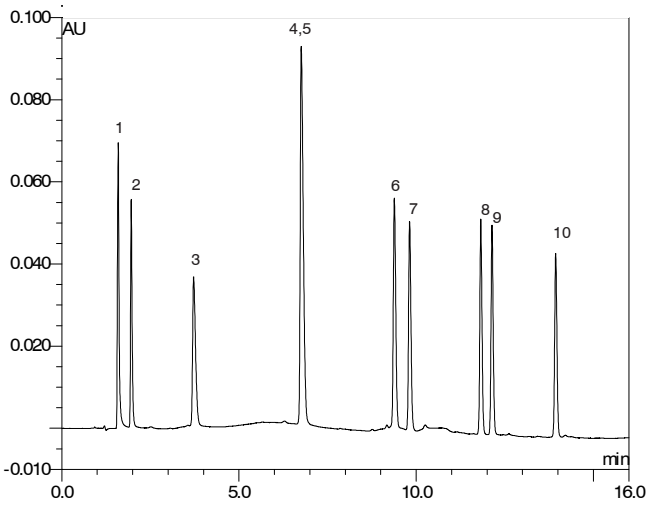
** 7 ppm total for *cis* and *trans* isomers

Figure 9
Highly Hydrophilic Organic Acids

5.2 C1 to C7 Aliphatic Acids

See Section 8.2 for plots.

Column:	Acclaim OA, 5 μm
Dimensions:	4 x 150 mm
Mobile Phase:	(A) CH_3CN
	(B) 2.5 mM methanesulfonic acid
Gradient:	Hold 100% B for 1 min
	B to A/B (45/55) in 11 min
	Hold A/B (45/55) for 4 min
Temperature:	30 °C
Flow Rate:	1.0 mL/min
Injection Volume:	15 μL
Detection:	UV, 210 nm

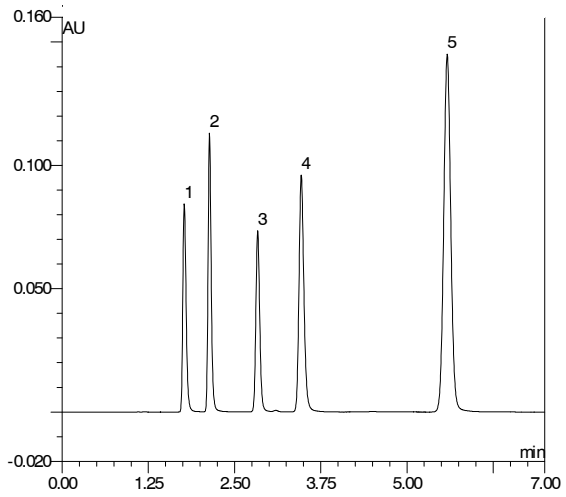


	Analytes	10 mM each
1.	Formic acid	
2.	Acetic acid	
3.	Propionic acid	
4.	Butyric acid	
5.	Isobutyric acid	
6	Isovaleric acid	
7.	n-Valeric acid	
8.	Isocaproic acid	
9.	n-Caproic acid	
10.	Heptanoic acid	

Figure 10
C1 to C7 Aliphatic Acids

5.3 Hydroxybenzoic Acids

Column:	Acclaim OA, 5 μm
Dimensions:	4 x 150 mm
Mobile Phase:	CH_3CN/5 mM methanesulfonic acid, v/v 30/70
Temperature:	30 °C
Flow Rate:	0.75 mL/min
Injection Volume:	5 μL
Detection:	UV, 230 nm

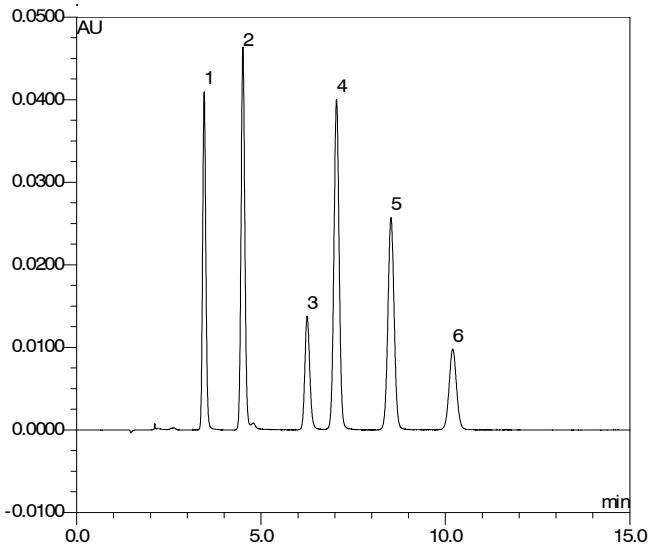


Analytes	80 mg/L (ppm) each
1. 3,4,5-Trihydroxybenzoic acid	
2. 3,5-Dihydroxybenzoic acid	
3. 4-Hydroxybenzoic acid	
4. 2,5-Dihydroxybenzoic acid	
5. Benzoic acid	

Figure 11
Hydroxybenzoic Acids

5.4 Hydroxyphenylacetic Acids

Column:	Acclaim OA, 5 μm
Dimensions:	4 x 150 mm
Mobile Phase:	CH_3CN/5 mM methanesulfonic acid, v/v 12.5/87.5
Temperature:	30 °C
Flow Rate:	0.75 mL/min
Injection Volume:	10 μL
Detection:	UV, 230 nm

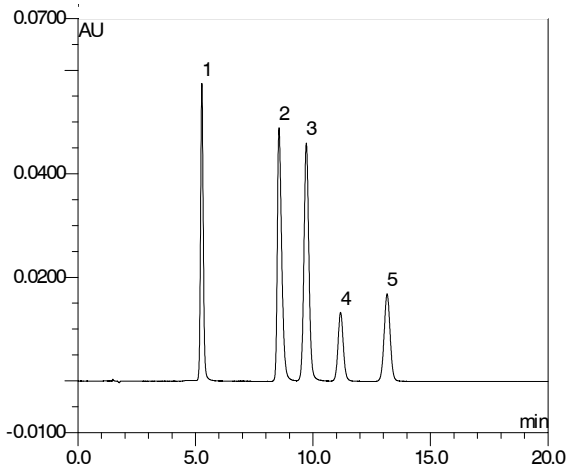


	Analytes	30 mg/L (ppm) each
1.	2,5-Dihydroxyphenylacetic acid	
2.	3,4-Dihydroxyphenylacetic acid	
3.	Mandelic acid	
4.	p-Hydroxyphenylacetic acid	
5.	m-Hydroxyphenylacetic acid	
6.	o-Hydroxyphenylacetic acid	

Figure 12
Hydroxyphenylacetic Acids

5.5 Benzenepolycarboxylic Acids

Column:	Acclaim OA, 5 μm
Dimensions:	4 x 150 mm
Mobile Phase:	CH_3CN/5 mM methanesulfonic acid, v/v 12.5/87.5
Temperature:	30 °C
Flow Rate:	0.75 mL/min
Injection Volume:	5 μL
Detection:	UV, 230 nm



	Analytes	50 mg/L (ppm) each
1.	1,2,3-Benzenetricarboxylic acid	
2.	1,2-Benzenedicarboxylic acid	
3.	1,3,5-Benzenetricarboxylic acid	
4.	1,4-Benzenedicarboxylic acid	
5.	1,3-Benzenedicarboxylic acid	

Figure 13
Benzenepolycarboxylic Acids

5.6 Natural Amino Acids

Column:	Acclaim OA, 5 μm
Dimensions:	4 x 150 mm
Mobile Phase:	40 mM Na_2SO_4, pH 2.65 (adjusted with methanesulfonic acid)
Temperature:	30 °C
Flow Rate:	0.6 mL/min
Injection Volume:	10 μL
Detection:	UV, 210 nm

Analytes
1. Lysine
2. Threonine
3. Proline
4. Valine
5. Methionine
6. Isoleucine
7. Leucine
8. Tyrosine
9. Phenylalanine

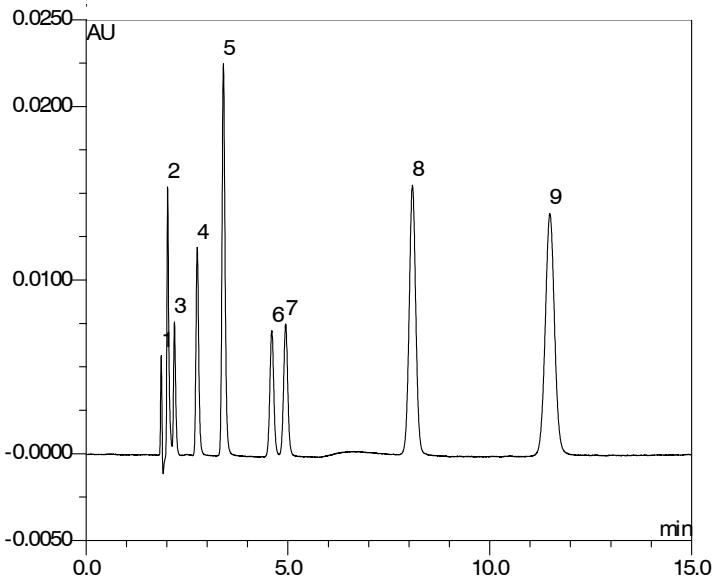
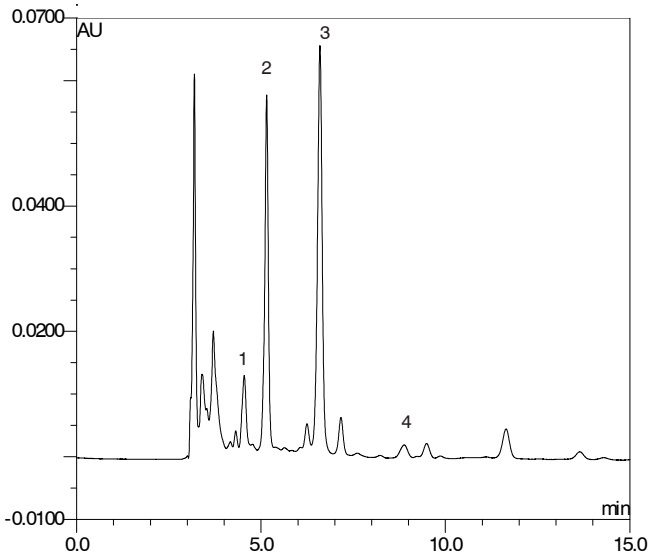


Figure 14
Natural Amino Acids

5.7 Fresh Orange Juice

Column:	Acclaim OA, 5 μm
Dimensions:	4 x 250 mm
Mobile Phase:	0.1 M Na_2SO_4, pH 2.68 (adjusted with methanesulfonic acid)
Temperature:	30 °C
Flow Rate:	0.6 mL/min
Injection Volume:	5 μL
Detection:	UV, 210 nm
Sample Preparation:	OnGuard II P, diluted 2x with D.I. water

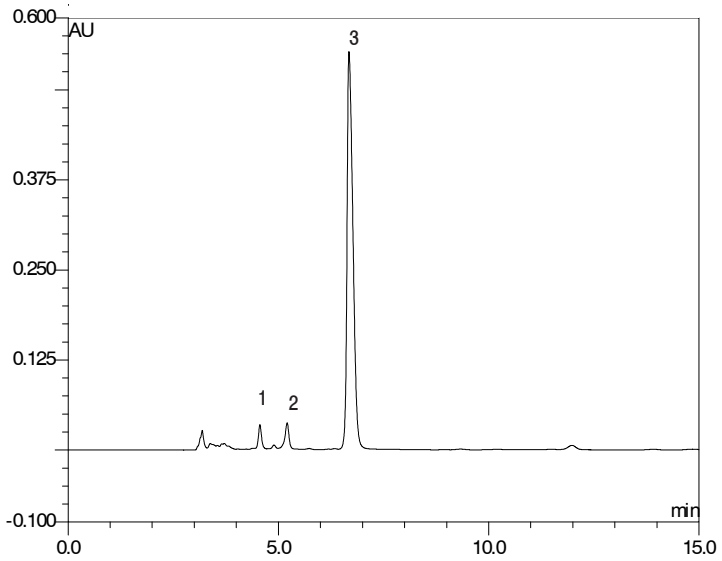


Analytes
1. Malic acid
2. Ascorbic acid (Vitamin C)
3. Citric acid
4. Fumaric acid

Figure 15
Fresh Orange Juice

5.8 Fresh Lemon Juice

Column:	Acclaim OA, 5 μm
Dimensions:	4 x 250 mm
Mobile Phase:	0.1 M Na_2SO_4, pH 2.68 (adjusted with methanesulfonic acid)
Temperature:	30 °C
Flow Rate:	0.6 mL/min
Injection Volume:	5 μL
Detection:	UV, 210 nm
Sample Preparation:	OnGuard II P, diluted 2x with D.I. water

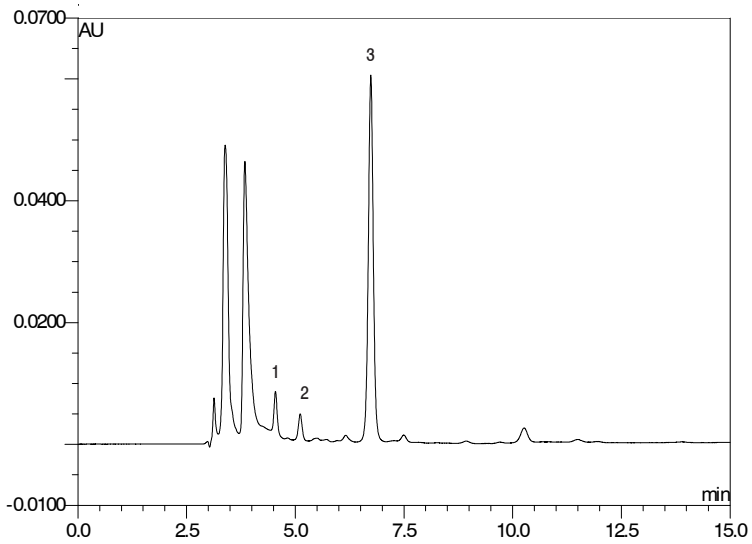


Analytes
1. Malic acid
2. Ascorbic acid (Vitamin C)
3. Citric acid

Figure 16
Fresh Lemon Juice

5.9 Juice Drink Blend

Column:	Acclaim OA, 5 μm
Dimensions:	4 x 250 mm
Mobile Phase:	0.1 M Na_2SO_4, pH 2.68 (adjusted with methanesulfonic acid)
Temperature:	30 °C
Flow Rate:	0.6 mL/min
Injection Volume:	5 μL
Detection:	UV, 210 nm
Sample Preparation:	OnGuard II P

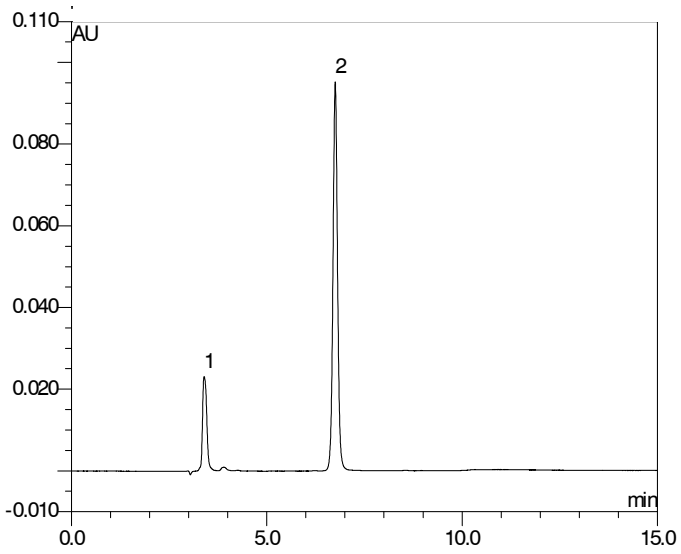


Analytes
1. Malic acid
2. Lactic acid
3. Citric acid

Figure 17
Juice Drink Blend

5.10 Sports Drink

Column:	Acclaim OA, 5 μm
Dimensions:	4 x 250 mm
Mobile Phase:	0.1 M Na_2SO_4, pH 2.68 (adjusted with methanesulfonic acid)
Temperature:	30 °C
Flow Rate:	0.6 mL/min
Injection Volume:	5 μL
Detection:	UV, 210 nm
Sample Preparation:	OnGuard II P

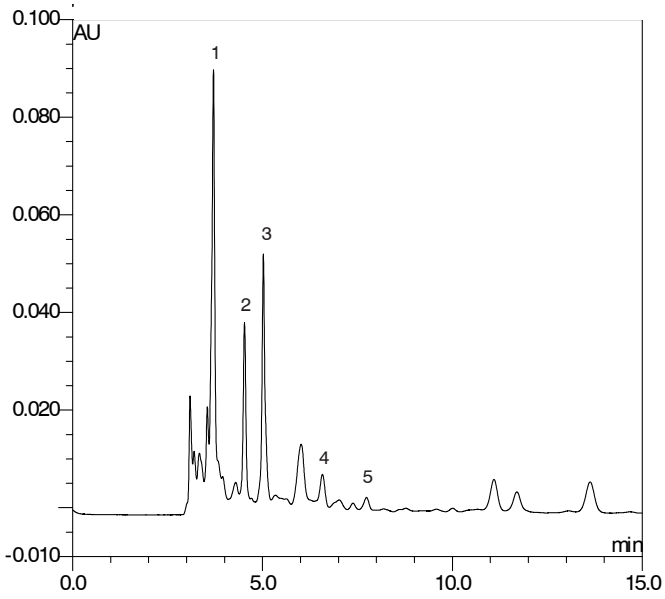


Analytes
1. Unknown
2. Citric acid

Figure 18
Sports Drink

5.11 White Wine

Column:	Acclaim OA, 5 μm
Dimensions:	4 x 250 mm
Mobile Phase:	0.1 M Na_2SO_4, pH 2.68 (adjusted with methanesulfonic acid)
Temperature:	30 °C
Flow Rate:	0.6 mL/min
Injection Volume:	5 μL
Detection:	UV, 210 nm
Sample Preparation:	OnGuard II P

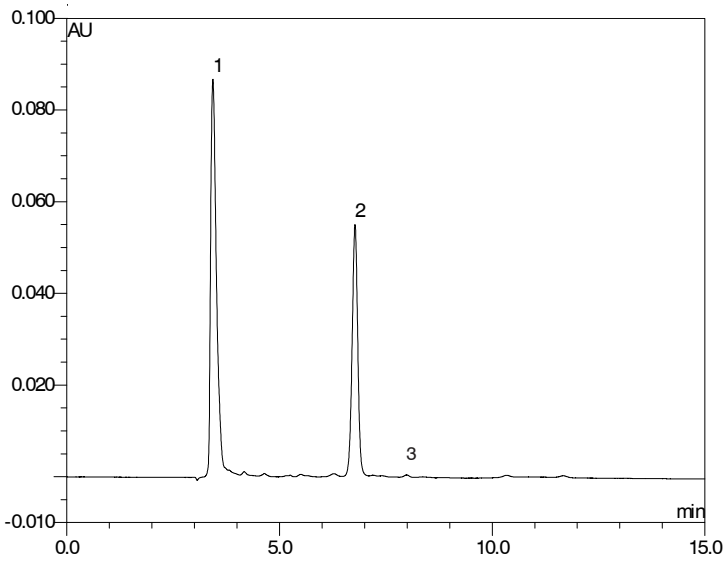


Analytes
1. Tartaric acid
2. Malic acid
3. Lactic acid
4. Citric acid
5. Succinic acid

Figure 19
White Wine

5.12 Cough Syrup (NyQuil®)

Column:	Acclaim OA, 5 μm
Dimensions:	4 x 250 mm
Mobile Phase:	0.1 M Na_2SO_4, pH 2.68 (adjusted with methanesulfonic acid)
Temperature:	30 °C
Flow Rate:	0.6 mL/min
Injection Volume:	5 μL
Detection:	UV, 210 nm
Sample Preparation:	OnGuard II P



Analytes
1. Bromide (Dextromethorphan HBr)
2. Citrate (Inactive ingredient)
3. Succinate (Doxylamine succinate)

Figure 20
Analysis of Cough Syrup (NyQuil®)

NyQuil® is a registered trademark of Procter & Gamble.

5.13 Acrylic Acid Monomer and Its Oligomers

Column:	Acclaim OA, 5 μm
Dimensions:	4 x 250 mm
Mobile Phase:	(A) CH_3CN
	(B) 2.5 mM methanesulfonic acid
Gradient:	Hold 5% A for 1 min,
	Increase to 90% A in 19 min
Temperature:	30 °C
Flow Rate:	0.75 mL/min
Injection Volume:	5 μL
Detection:	UV, 210 nm
Sample Preparation:	Diluted 1000 x with D.I. water

Analytes
1. Monomer
2. Dimer
3. Trimer
4. Tetramer
5. Pentamer
6. Hexamer
7. Heptamer

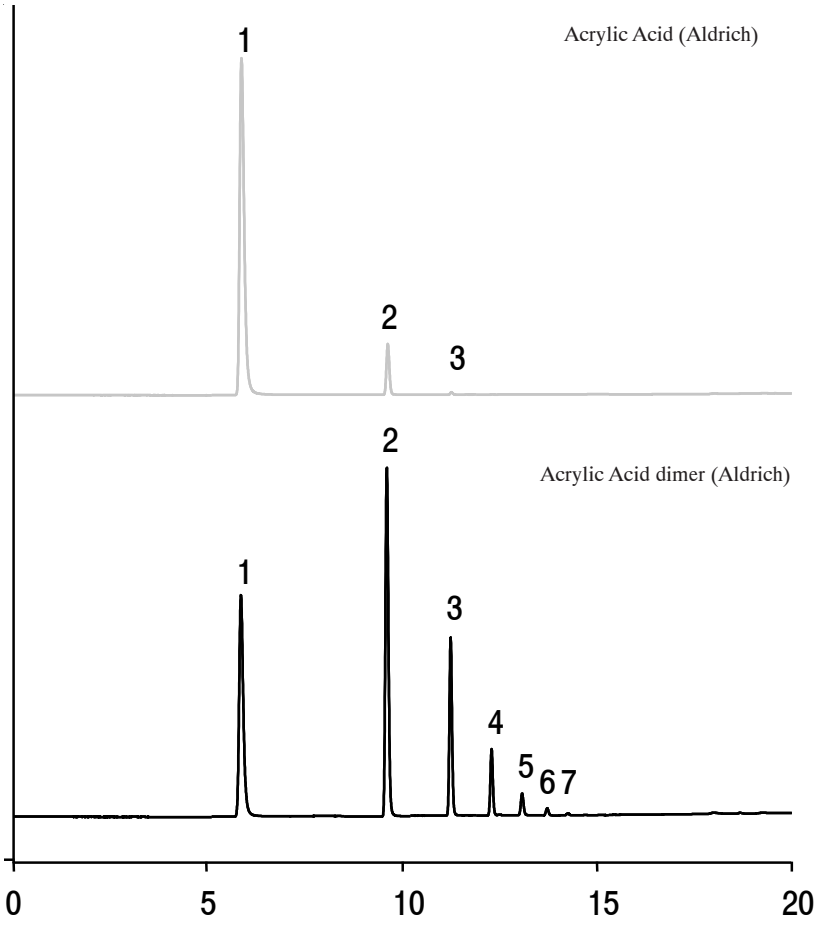


Figure 21
Analysis of Acrylic Acid Monomer and Its Oligomers

SECTION 6 - TROUBLESHOOTING GUIDE

The purpose of the Troubleshooting Guide is to help you solve operating problems that may arise while using the Acclaim OA column. If you cannot solve the problem on your own, contact the nearest DIONEX Office.

6.1 High Back Pressure

6.1.1 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 eluent 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 the detector cell. To identify which part of the chromatographic system is causing the problem, disconnect the pump eluent line from the injection valve and turn the pump on. Watch the pressure; it should not exceed 50 psi (0.34 MPa). Continue adding the system components (injection valve, column(s), the detector cell, and waste line) one by one, while watching the system pressure.

6.1.2 Replacing Column Bed Support Assemblies

If the column inlet bed support is determined to be the cause of the high back pressure, it should be replaced. To change the inlet bed support assembly, refer to the following instructions, using one of the two spare inlet bed support assemblies included in the column box.

A. Disconnect the column from the system.

B. Carefully unscrew the inlet (top) column fitting. Use two open-end wrenches. If the column packing appears soupy, carefully remove excess moisture by touching a paper towel to the edge of the packing bed. Allow water (not bonded phase) to soak into the paper towel.

C. Remove the bed support assembly. Turn the end fitting over and tap it against a bench top or other hard, flat surface to remove the bed support and seal assembly. If the bed support must be pried out of the end fitting, use a sharp pointed object such as a pair of tweezers, but be careful that you **DO NOT SCRATCH THE WALLS OF THE END-FITTING.** Discard the old bed support assembly.

D. Place a new bed support assembly into the end fitting. Make sure that the end of the column tube is clean and free of any particulate matter so that it will properly seal against the bed support assembly. Use the end of the column to carefully start the bed support assembly into the end fitting.

CAUTION

If the column tube end is not clean when inserted into the end fitting, particulate matter may obstruct a proper seal between the end of the column tube and the bed support assembly. If this is the case, additional tightening may not seal the column but instead damage the column tube or the end fitting. Carefully wipe the sealing surfaces clean before assembling.

E. Screw the end fitting back onto the column. Tighten the end fitting finger-tight, then an additional 1/4 turn (25 in x lb). Tighten further only if leaks are observed.

F. Reconnect the column to the system and resume operation.

NOTE

DO NOT attempt to remove the column outlet end fitting because the packing will extrude out of the column and ruin it.

6.2 High Background or Noise

6.2.1 Preparation of Eluents

A. Make sure that the mobile phase is made correctly.

B. Make sure that the eluents are made from chemicals with the recommended purity.

C. Make sure that the deionized water used to prepare the reagents has a specific resistance of 18.2 megohm-cm.

6.2.2 A Contaminated Guard or Analytical Column

Remove the Acclaim OA Analytical Column from the system. Connect the fluid lines to a piece of back pressure tubing. If the background decreases, then the column is the cause of the high background. Clean the column as instructed in “Cleaning and Regenerating the Column” (see Section 4.5.).

6.2.3 Contaminated Hardware

To eliminate the hardware as the source of the high background, pump deionized water with a specific resistance of 18.2 megohm-cm through the system. If it is not, check the detector cell by injecting deionized water directly into it.

6.3 Poor Peak Resolution

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

6.3.1 Loss of Column Efficiency

A. Check to see if headspace has developed in the analytical column. This may be due to improper use of the column such as submitting it to high pressures. Remove the column’s top end fitting (see Section 7.1.2, “Replacing Column Bed Support Assemblies”). If the resin does not fill the column body all the way to the top, the resin bed has collapsed, creating a headspace. Headspace of 1–2 mm is the maximum allowable before the column demonstrates significant losses of efficiency. If more than 2 mm of headspace is observed, the column must be replaced.

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

6.3.2 Poor Resolution Due to Shortened Retention Times

Even with adequate system and column efficiency, resolution of peaks will be compromised if analytes elute too fast.

A. Check the eluent flow rate. Determine if the actual flow rate is different than the flow rate specified by the analytical protocol. Measure the eluent flow rate after the column using a stopwatch and graduated cylinder. Allow the system to equilibrate for at least 5 minutes before making the measurement to allow time for the pump pressure feedback to engage.

B. Check to see if the mobile phase compositions and concentrations are correct. For isocratic analysis, a mobile phase buffer component that is too strong will cause the peaks to elute later. Prepare fresh buffer solution. If you are using a gradient pump to proportion the final mobile phase from concentrated solutions in two or three different reservoirs, the composition of the final mobile phase may not be accurate enough for the application. Use one reservoir containing the correct final mobile phases composition to see if proportioning accuracy is the problem. This may be a problem when one of the concentrated solutions is proportioned at less than 5%.

C. Column contamination can lead to a loss of column efficiency. Refer to Section 4.4, “Caring for the Column”, for recommended column cleanup procedures. Possible sources of column contamination are impurities in chemicals, in the deionized water, or from the sample matrix being used. Be especially careful to make sure that chemicals with recommended purity are used. The deionized water should have a specific resistance of at least 18.2 megohm-cm.

If you need assistance in solving resolution problems, contact the Dionex Regional Office nearest you (see, “Dionex Regional Offices”).

6.3.3 Loss of Front End Resolution

If poor resolutions and efficiencies are observed for the very early eluting peaks near the system void volume compared to the later eluting peaks, check the following:

A. Improper mobile phase concentration. Remake the mobile phase as required for your application. Ensure that the water and chemicals used are of the required purity.

B. Column overloading. Reduce the amount of sample components injected onto the analytical column by either diluting the sample or injecting a smaller sample volume onto the column. Ensure that late eluting components, present in high concentrations, are not overloading the column.

C. Sluggish operation of the injection valve. For pneumatically driven injector valves, check the air pressure and make sure there are no gas leaks. Check for partially plugged port faces. Refer to the injector valve manual for instructions.

D. Improperly swept volumes anywhere in the system prior to the guard and analytical columns. Swap components and refit tubing connections, one at a time, in the system prior to the analytical column and test for front-end resolution after every system change.

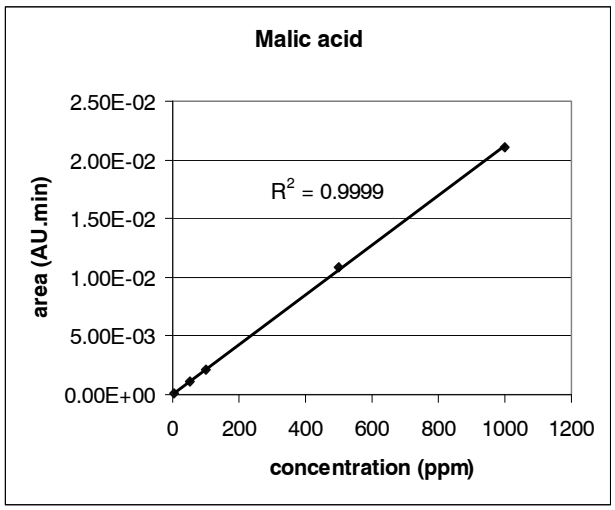
E. Sample-related problems. Too much organic solvent or extremes of pH in the sample matrix can be a problem. Dilute the sample or adjust the pH to be similar to the mobile phase.

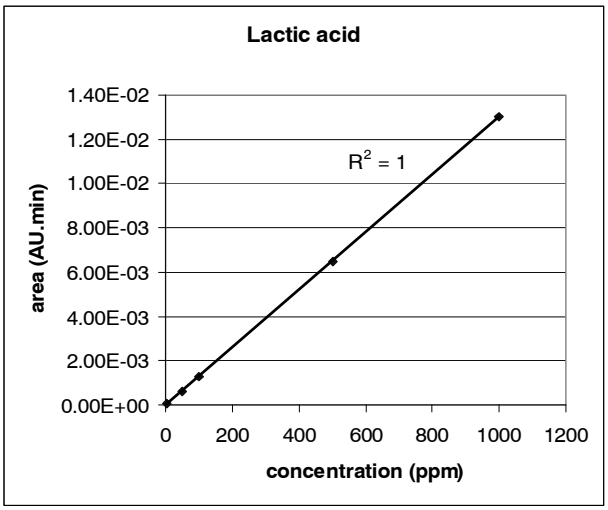
F. Guard cartridge. The guard cartridge should be replaced on at regular intervals. Keep a trending log of retention times, peak efficiencies, and resolution of key analytes. Replace the guard when these parameters indicate deterioration of the columns. A compacted or contaminated guard cartridge should be replaced.

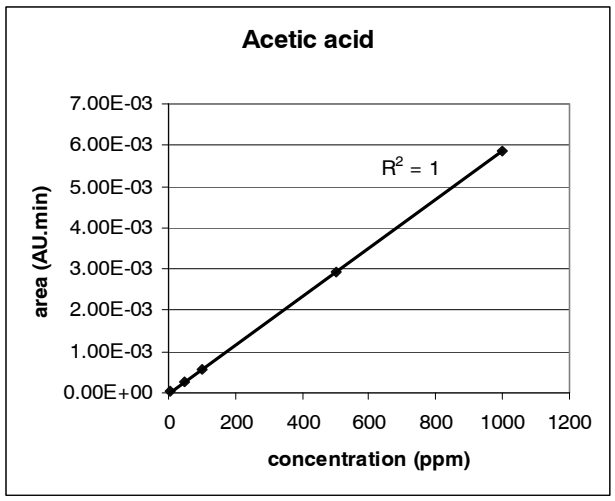
SECTION 7 – LINEARITY PLOTS

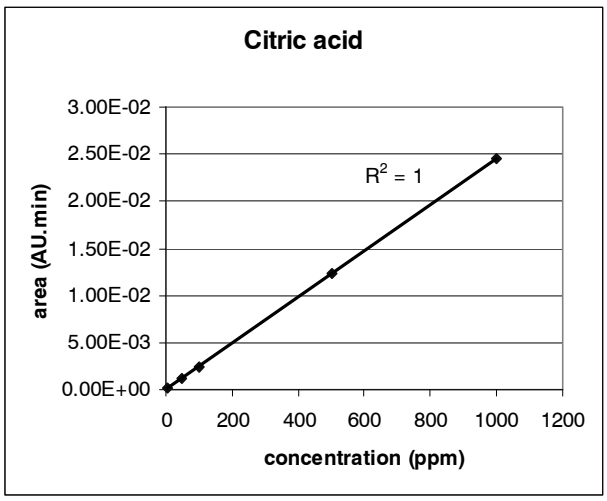
7.1 Hydroxy Carboxylic Acids

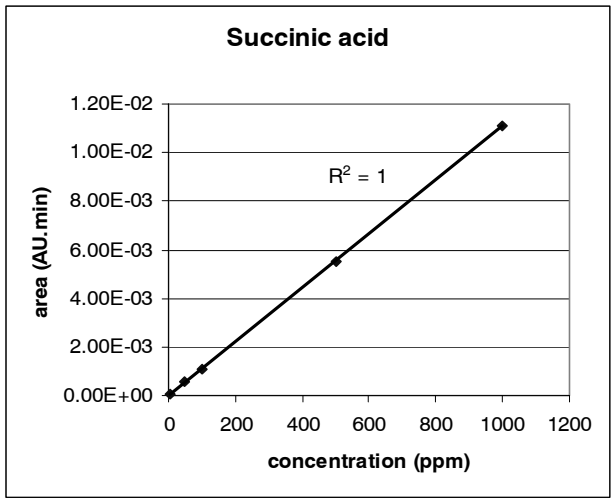
For chromatic conditions, see Section 1.1, Figure 3, Separation of Common Hydrophilic Organic Acids.





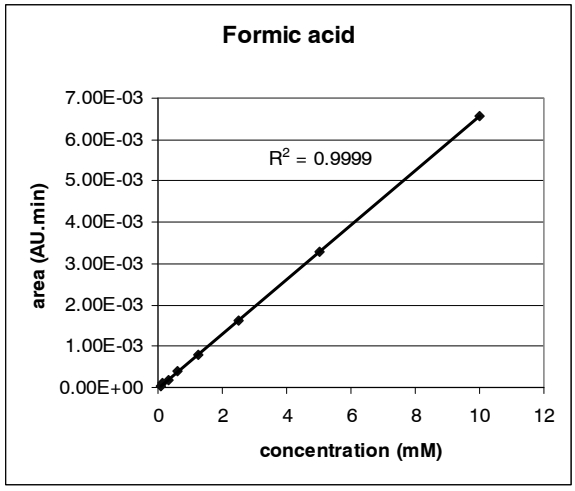


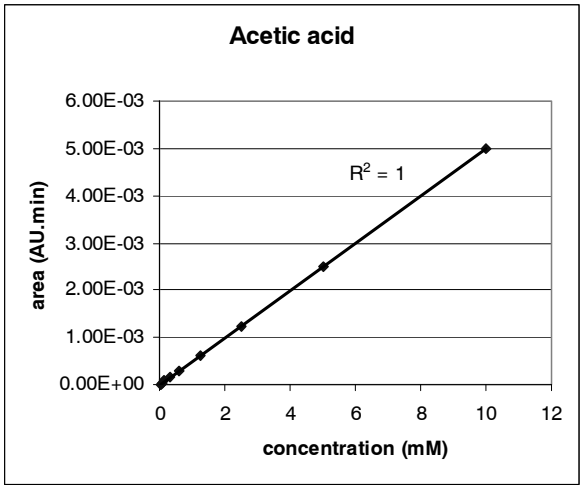


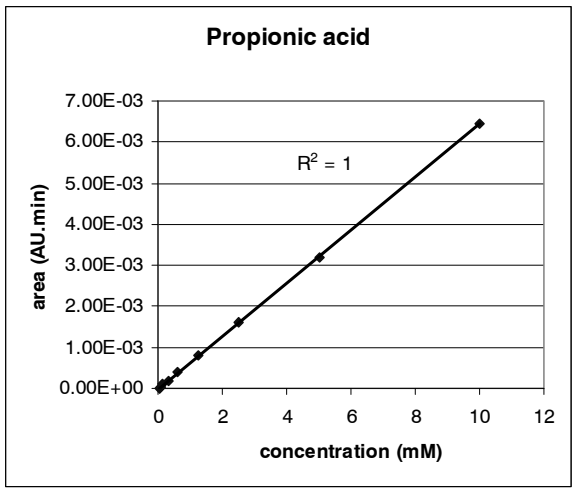


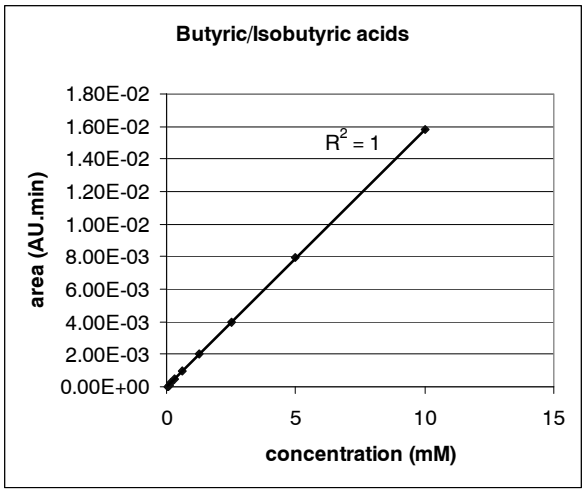
7.2 C1 to C7 Volatile Aliphatic Acids

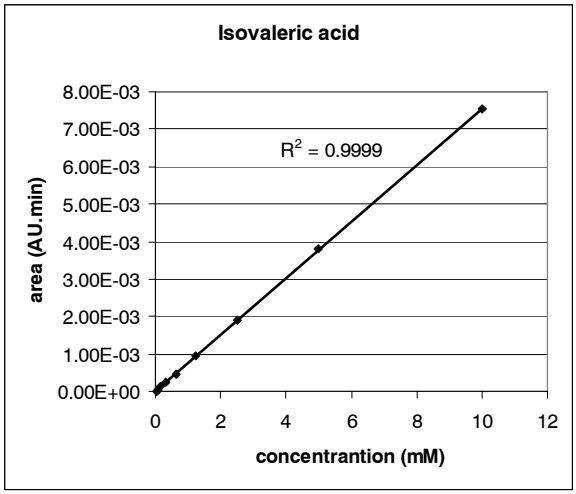
For chromatographic conditions, see Section 6.2, C1 to C7 Aliphatic Acids.

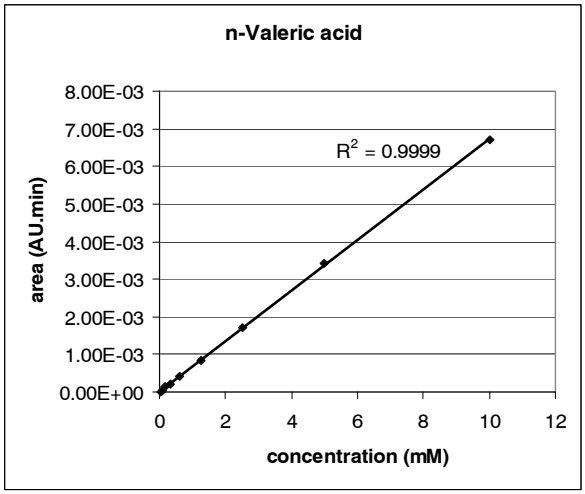


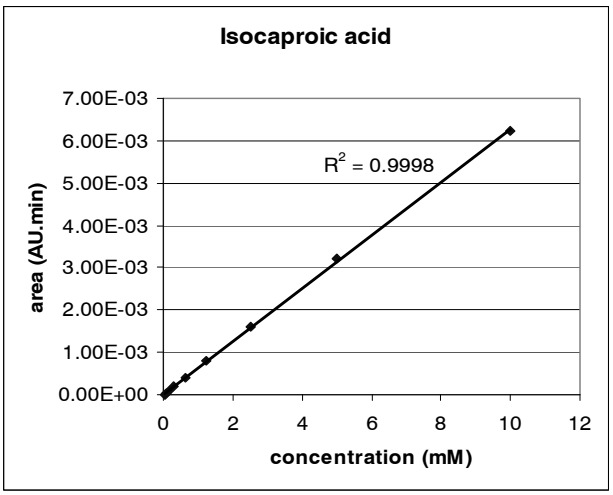











Isocaproic acid
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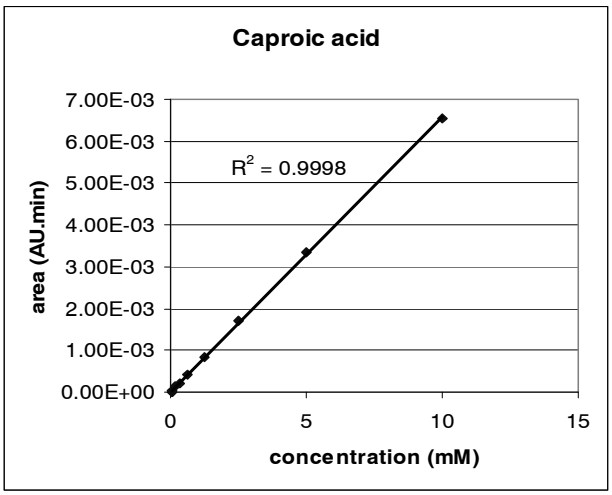
6.00E-03
5.00E-03
4.00E-03
3.00E-03
2.00E-03
1.00E-03
0.00E+00
area (AU.min)
R2 = 0.9998
0
2
4
6
8
10
12
concentration (mM)


Caproic acid
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6.00E-03
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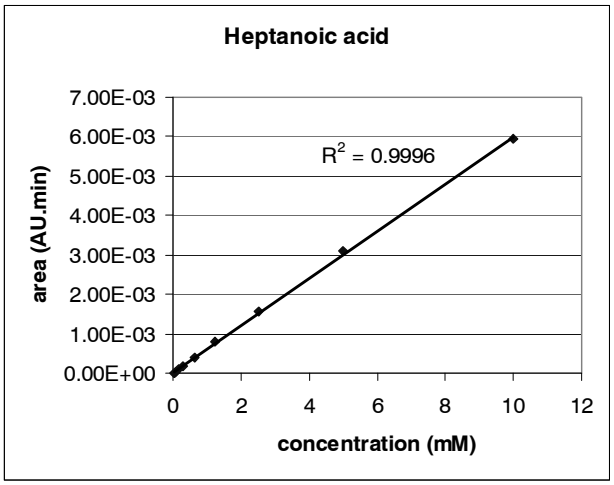
2.00E-03
1.00E-03
0.00E+00
area (AU.min)
R2 = 0.9998
0
5
10
15
concentration (mM)


Heptanoic acid
7.00E-03
6.00E-03
5.00E-03
4.00E-03
3.00E-03
2.00E-03
1.00E-03
0.00E+00
area (AU.min)
R2 = 0.9996
0
2
4
6
8
10
12
concentration (mM)

APPENDIX B - ORGANIC ACIDS

Table 3

Organic Acids with pKa Values In Order of Increasing pK_1

Compound	pK_1	pK_2	pK_3
Terephthalic			
Squaric (3,4-dihydroxy-3-cyclobutene-1,2-dione	0.40	3.10	
Trichloroacetic	0.66		
Mellitic (benzenehexacarboxylic)	0.70	2.21	3.52
Dichloroacetic	0.87		
Oxalic (ethanedioic)	1.04	3.82	
Nitroacetic	1.46		
Maleic (cis-butenedioic)	1.75	5.83	
Ketoglutaric (2-oxopentanedioic)	1.85	4.44	
Orotic (uracil-6-carboxylic)	1.96	9.34	
Citraconic (cis-methylbutenedioic)	2.20	5.60	
Pyruvic (2-oxopropanoic)	2.26		
Trimellitic (benzene-1,2,4-tricarboxylic)	2.40	3.71	5.01
Fluoroacetic	2.59		
Mesaconic (trans-methylbutene)	2.61		
Cyanoacetic	2.63		
Malonic (propanedioic)	2.65	5.28	
Chloroacetic	2.68		
Gentistic (5-hydroxysalicylic)	2.70		
Dithiotartaric (2,3-dimercaptobutanedioic)	2.71	3.48	8.89
Bromoacetic	2.72		
Phthalic (benzene-1,2-dicarboxylic)	2.75	4.93	
Diglycolic (oxydiacetic)	2.79	3.93	
Salicylic (2-hydroxybenzoic)	2.81	13.40	
Tartaric (d-2,3-dihydroxybutanedioic)	2.82	3.95	
Fumaric (trans-butenedioic)	2.85	4.10	
Citric (2-hydroxypropane-1,2,3-tricarboxylic)	2.87	4.35	5.69
Iodoacetic	2.98		
Isocitrate (dl-1-hydroxypropane-1,2,3-tricarboxylic)	3.02	4.28	5.75
Mucic	3.08	3.63	
Mandelic (l-phenylhydroxyacetic)	3.19		
Galacturonic	3.23	11.42	
Malic (l-hydroxybutanedioic)	3.24	4.71	
Thiomalic (dl-mercaptobutanedioic)	3.30	4.60	10.38
Quinic (1,3,4,5-tetrahydroxycyclohexanecarboxylic)	3.36		
Thioglycolic (mercaptoacetic)	3.42	10.11	
Thiolactic (dl-2-mercaptopropanoic)	3.48	10.08	
Hippuric (n-benzoylglycine)	3.50		
Glyceric (dl-2,3-dihydroxypropanoic)	3.52		
Formic (methanoic)	3.55		
Glycolic (hydroxyacetic)	3.63		
Lactic (d-2-hydroxypropanoic)	3.66		
Itaconic (methylenebutanedioic)	3.68	5.14	
2-Hydroxyisobutyric	3.72		
Benzoic	4.00		
Succinic (butanedioic)	4.00	5.24	
Ascorbic	4.03	11.34	
4-Hydroxybenzoic	4.10	9.96	
Vinylacetic (but-3-enoic)	4.12		
Glutaric (pentanedioic)	4.13	5.03	
Acrylic (propenoic)	4.26		
Adipic (hexanedioic)	4.26	5.03	
Pimelic (heptanedioic)	4.31	5.08	
3-Mercaptopropanoic	4.34	10.84	
Azelaic (nonanedioic)	4.39	5.12	
Anisic (4-methoxybenzoic)	4.48		
Acetic (ethanoic)	4.56		
Isovaleric (3-methylbutanoic)	4.58		
Butanoic	4.63		
Isobutyric (2-methylpropionic)	4.63		
Valeric (pentanoic)	4.64		
Propanoic	4.67		
Crotonic (trans-but-2-enoic)	4.69		
Pivalic (2,2-dimethylpropanoic)	4.83		
Caproic (hexanoic)	4.85		
Octanoic	4.89		
Uric (2,6,8-trihydroxypurine)	5.61		
Guanidine	13.54		

Table 4
Organic Acids with pKa Values in Alphabetic Order

Compound	pK_1	pK_2	pK_3
Acetic (ethanoic)	4.56		
Acrylic (propenoic)	4.26		
Adipic (hexanedioic)	4.26	5.03	
Anisic (4-methoxybenzoic)	4.48		
Ascorbic	4.03	11.34	
Azelaic (nonanedioic)	4.39	5.12	
Benzoic	4.00		
Bromoacetic	2.72		
Butanoic	4.63		
Caproic (hexanoic)	4.85		
Chloroacetic	2.68		
Citraconic (cis-methylbutenedioic)	2.20	5.60	
Citric (2-hydroxypropane-1,2,3-tricarboxylic)	2.87	4.35	5.69
Crotonic (trans-but-2-enoic)	4.69		
Cyanoacetic	2.63		
Dichloroacetic	0.87		
Diglycolic (oxydiacetic)	2.79	3.93	
Dithiotartaric (2,3-dimercaptobutanedioic)	2.71	3.48	8.89
Fluoroacetic	2.59		
Formic (methanoic)	3.55		
Fumaric (trans-butenedioic)	2.85	4.10	
Galacturonic	3.23	11.42	
Gentistic (5-hydroxysalicylic)	2.70		
Glutaric (pentanedioic)	4.13	5.03	
Glyceric (dl-2,3-dihydroxypropanoic)	3.52		
Glycolic (hydroxyacetic)	3.63		
Guanidine	13.54		
2-Hydroxyisobutyric	3.72		
4-Hydroxybenzoic	4.10	9.96	
Hippuric (n-benzoylglycine)	3.50		
Iodoacetic	2.98		
Isobutyric (2-methylpropionic)	4.63		
Isocitrate (dl-1-hydroxypropane-1,2,3-tricarboxylic)	3.02	4.28	5.75
Isovaleric (3-methylbutanoic)	4.58		
Itaconic (methylenebutanedioic)	3.68	5.14	
Ketoglutaric (2-oxopentanedioic)	1.85	4.44	
Lactic (d-2-hydroxypropanoic)	3.66		
Maleic (cis-butenedioic)	1.75	5.83	
Malic (l-hydroxybutanedioic)	3.24	4.71	
Malonic (propanedioic)	2.65	5.28	
Mandelic (l-phenylhydroxyacetic)	3.19		
Mellitic (benzenehexacarboxylic)	0.70	2.21	3.52
3-Mercaptopropanoic	4.34	10.84	
Mesaconic (trans-methylbutene)	2.61		
Mucic	3.08	3.63	
Nitroacetic	1.46		
Octanoic	4.89		
Orotic (uracil-6-carboxylic)	1.96	9.34	
Oxalic (ethanedioic)	1.04	3.82	
Phthalic (benzene-1,2-dicarboxylic)	2.75	4.93	
Pimelic (heptanedioic)	4.31	5.08	
Pivalic (2,2-dimethylpropanoic)	4.83		
Propanoic	4.67		
Pyruvic (2-oxopropanoic)	2.26		
Quinic (1,3,4,5-tetrahydroxycyclohexanecarboxylic)	3.36		
Salicylic (2-hydroxybenzoic)	2.81	13.40	
Squaric (3,4-dihydroxy-3-cyclobutene-1,2-dione	0.40	3.10	
Succinic (butanedioic)	4.00	5.24	
Tartaric (d-2,3-dihydroxybutanedioic)	2.82	3.95	
Terephthalic			
Thioglycolic (mercaptoacetic)	3.42	10.11	
Thiolactic (dl-2-mercaptopropanoic)	3.48	10.08	
Thiomalic (dl-mercaptobutanedioic)	3.30	4.60	10.38
Tricarballylic	3.48	4.50	5.82
Trichloroacetic	0.66		
Trimellitic (benzene-1,2,4-tricarboxylic)	2.40	3.71	5.01
Uric (2,6,8-trihydroxypurine)	5.61		
Valeric (pentanoic)	4.64		
Vinylacetic (but-3-enoic)	4.12		