PRODUCT MANUAL

for

Acclaim® RSLC Columns

(Rapid Separation Liquid Chromatography)

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Product Manual

for

Acclaim® RSLC Columns

(Rapid Separation Liquid Chromatography)

Acclaim RSLC 120 C18 - Analytical Column
Acclaim RSLC 120 C18, 2.2µm, 2.1 x 30 mm (P/N 071400)
Acclaim RSLC 120 C18 2.2µm, 2.1 x 50 mm (P/N 068981)
Acclaim RSLC 120 C18, 2.2µm, 2.1 x 100 mm (P/N 068982)
Acclaim RSLC 120 C18, 2.2µm, 2.1 x 150 mm (P/N 071399
Acclaim RSLC 120 C18, 2.2µm, 2.1 x 250mm (P/N 074812))
Acclaim RSLC 120 C18, 2.2µm, 3 x 30 mm (P/N 071606)
Acclaim RSLC 120 C18, 2.2µm, 3 x 50 mm (P/N 071605)
Acclaim RSLC 120 C18, 2.2µm, 3 x 100 mm (P/N 071604)
Acclaim RSLC 120 C18, 3µm, 3 x 33 mm, (P/N 066272)
Acclaim RSLC 120 C18, 3µm, 3 x 50 mm (P/N 068971)
Acclaim RSLC 120 C18, 3µm, 3 x 75 mm, (P/N 066273)

Acclaim RSLC 120, C8 - Analytical Column
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Acclaim RSLC 120, C8, 2.2µm, 2.1 x 50 mm(P/N 072615)
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Acclaim RSLC PolarAdvantage II - Analytical Column
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Acclaim RSLC PolarAdvantage II, 3µm, 3 x 50 mm (P/N 068973)
Acclaim RSLC PolarAdvantage II, 3µm, 3 x 75 mm, (P/N 066277)

Guards

Acclaim 120 C18, 5µm, 3 x 10 mm, 2ea, (P/N 071981)
Acclaim 120 C8, 5 µm, 3x10 mm, 2 ea, (P/N 071979)
Acclaim PolarAdvantage, 5µm, 3 x 10 mm, 2ea, (P/N 071983)
Acclaim PolarAdvantage II, 5µm, 3 x 10 mm, 2ea, (P/N 071988)
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SECTION 1 – INTRODUCTION

Acclaim RSLC columns are based on spherical, high-purity silica particles, and provide a simple and reliable solution for rapid separation liquid chromatography (RSLC). The columns feature a well-balanced integration of high column efficiency, excellent performance, complementary selectivity, as well as stable and rugged column packing. The RSLC columns packed with 3µm silica are designed to generate less backpressure compared to other small particle columns, and are more resistant to column fouling, making them compatible with standard HPLC instrumentation. The RSLC columns packed with 2.2µm silica are designed for very high resolution separations and are compatible with ultra-high pressure instrumentation. As the result, Acclaim RSLC columns provide rapid separation solutions in a broad range of applications, including pharmaceutical, food and beverage, environmental, chemical, consumer products, and more.

1.1. Acclaim RSLC

The Acclaim RSLC columns are silica based, 2.2µm and 3µm particle size. There are four stationary phases: Acclaim 120 C18, Acclaim 120 C8, Acclaim Polar Advantage (PA) and Polar Advantage II (PA2). The Acclaim RSLC C18 and C8, 2.2µm and 3µm, features high-density monomeric C18 and C8 chemistry (respectively) with exhaustive end-capping, and provides the same selectivity as the standard phases and extremely low silanol activity. Acclaim PA is a unique polar-embedded phase compatible with aqueous mobile phases containing no organic solvent modifiers, though maintaining selectivity similar to C18. Acclaim RSLC PA2 features amide-embedded chemistry with excellent hydrolytic stability (pH 1.5 to 10), and provides complementary selectivity.

Table 1

Acclaim RSLC Columns			
Column Chemistry	**Particle Size**	**Column Format**	**Catalog Number**
120 C18	2.2 µm	2.1 x 30 mm	071400
		2.1 x 50 mm	068981
		2.1x100 mm	068982
		2.1 x 150 mm	071399
		3 x 30 mm	071606
		3 x 50 mm	071605
		3 x 100 mm	071604
		2.1 x 250 mm	074812
	3 µm	3.0 x 33 mm	066272
		3 x 50 mm	068971
		3.0 x 75 mm	066273
120 C8	2.2 µm	2.1 x 30 mm	072614
		2.1 x 50 mm	072615
		2.1 x 100 mm	072616
		2.1 x 150 mm	072617
		3 x 30 mm	072618
		3 x 50 mm	072619
		3 x 100 mm	072620
		2.1 x 250 mm	074811
PolarAdvantage	2.2 µm	2.1 x 30 mm	072621
		2.1 x 50 mm	072622
		2.1 x 100 mm	072623
		2.1 x 150 mm	072624
		3 x 30 mm	072625
		3 x 50 mm	072626
		3 x 100 mm	072627
		2.1 x 250 mm	074813
	3 µm	3.0 x 33 mm	066274
		3 x 50 mm	068972
		3.0 x 75 mm	066275
PolarAdvantage II	2.2 µm	2.1 x 30 mm	071402
		2.1 x 50 mm	068989
		2.1 x 100 mm	068990
		2.1 x 150 mm	071401
		3 x 30 mm	071609
		3 x 50 mm	071608
		3 x 100 mm	071607
		2.1 x 250 mm	074814
	3 µm	3.0 x 33 mm	066276
		3 x 50 mm	068973
		3.0 x 75 mm	066277

The manufacture of all Acclaim columns is controlled and documented according to ISO 9001 guidelines at the Dionex Sunnyvale manufacturing facility. Certificates of Quality Assurance and Lot Validation accompany each analytical column.

1.2. Dionex RSLC Systems and Instrumentation Requirements

Acclaim RSLC columns allow accelerated separations to be run on standard LC instrumentation, provided these instruments fulfill distinct minimum requirements. In order to achieve the optimal speed of analysis and resolution, it is advantageous to use systems with lowest gradient delay volume and extra column volume. The detector must provide a minimum data rate and the flow cell needs to support these requirements for extra column volume. We suggest the use of one of Dionex's RSLC systems that meets the requirements in section 3.2.

SECTION 2 – BEFORE YOU START

2.1. The Most Important Rules

This manual describes the recommended operating conditions for the RSLC columns, and the precautions to maximize and preserve its performance.

1. Observe the recommended ranges of operating conditions
2. Keep the column clean by using HPLC-grade filtered mobile phases, by filtering the samples, by using pre-column filters.
3. Pay close attention to minimizing dead volume in the various connections, and gradient delay volume in the pump.
4. Equilibrate the column sufficiently before use, and store it properly after use.

2.2. Acclaim Column Operational Parameters

Table 2

	Recommended Ranges of Operation			
	The type 316 stainless steel column hardware is compatible with common HPLC mobile phases. To prevent corrosion, high concentrations of halide ions at pH<2.5 should be avoided.			
	Acclaim 120 C18	**Acclaim 120 C8**	**Acclaim PA**	**Acclaim PA2**
pH range	2.0—8.0	2.0—8.0	2.0—8.0	1.5—10
Temperature	60 °C	60 °C	60 °C	60 °C
Aqueous Compatibility	0—90%	0—90%	0—100%	0—100%

The pressure limits are dependent on the column geometry and particle size, and are the same for all surface chemistries.

Table 3

Particle Size	**Geometry**	**Recommended Maximum Pressure (psi)**	**Recommended Maximum Flow Rate (mL/min)**	**Typical Flow Rate (mL/min)**
3µm	3.0 x 33mm	4,500	2.5	0.4 - 1.0
	3.0 x 50mm	5,000	2.5	0.4 - 1.0
	3.0 x 75mm	6,000	2.5	0.4 - 1.0
2.2µm	3.0 x 30mm	4,500	2.5	0.6 - 1.6
	3.0 x 50mm	7,000	2.5	0.6 - 1.6
	3.0 x 100mm	10,000	2.5	0.6 - 1.6
	2.1 x 30mm	4,500	1.2	0.3 - 0.8
	2.1 x 50mm	8,500	1.2	0.3 - 0.8
	2.1 x 100mm	11,000	1.2	0.3 - 0.8
	2.1 x 150mm	11,000	1.2	0.3 - 0.8
	2.1 x 250 mm	15,000	1.2	0.3 - 0.8

SECTION 3 – OPERATION AND SYSTEM REQUIREMENTS

3.1. Chromatographic Conditions

Please refer to Dionex Technical Note 75 "Easy Method Transfer from HPLC to RSLC with the Dionex Method Speed-Up Calculator" for advice. (Search for "TN 75" at www.dionex.com)

3.2. General System Requirements

Acclaim RSLC columns can provide significant benefits to any standard HPLC system, but for the best results we recommend the UltiMate 3000 Intelligent RSLC systems. In order to use the columns in together with other vendors' LC hardware, the following minimum requirements must be fulfilled:

- System gradient delay volume not larger than 1200 µL
- Connection tubing internal diameter not wider than 250 µm (0.010 inch)
- Flow rates up to 3 mL/min for long term use
- Upper pressure limit of at least 350 bar (5000 psi)
- Column thermostatting ability with minimum extra column contribution
- Detector flow cell volume not larger than 13 µL
- Detector data collection rate of minimum 10 Hz
- Detector time constant must be settable to values of as low as 0.2 seconds

Pump: For isocratic methods, the choice of pump is not critical. For gradient elution, the gradient delay volume is a significant factor, and should be minimized. Gradient delay volume is the volume between where the solvents first mix to the head of the column. The low-pressure gradient-mixing (LPG) design is economical and flexible, but has a greater delay volume; the high-pressure gradient-mixing (HPG) design while more expensive has much lower gradient delay volume. Gradient mixing devices can make a large contribution to the delay volume, but are usually necessary for best results; therefore choose the smallest one that is consistent with good gradient mixing in the flow range of 0.4 to 1.3 mL/min.

Injector: When moving to a 3.0 mm i.d. column from 4.6 mm, you normally reduce the injection volume by a factor of 0.4. The popular split-loop autosampler design puts a relatively large sample loop in line; to reduce gradient delay volume, you may want to install a smaller loop, or program the valve to bypass the loop shortly after injection.

Detector: The flow cell makes little difference in peak shape when the peak volume is greater than 10X the flow cell volume. Since the peak volume (width at base times flow rate) is reduced to as little as 20% of the original when you accelerate a method using RSLC columns, you should consider if a smaller flow cell is appropriate. For good integration of peaks, you should have 20 - 30 data points across the narrowest peak of interest, therefore set the data collection rate and filtering constant appropriately.

Tubing connections: To minimize extra-column volume, use the recommended size or smaller capillary connections between the column and injector, and between the column and detector. Make sure that the tubing is cut squarely and cleanly, and that all fittings are correctly made. Dionex Viper™ capillary connectors are designed to make low-volume leak-proof connections for RSLC at up to 1000 bar (14,500 psi).

Recommended capillary i.d.

Column	**Capillary**
3µm, 3 mm i.d.	0.18 mm (0.007 inch)
2.2µm, 2.1 mm i.d.	0.13 mm (0.005 inch)
2.2µm, 3 mm i.d	0.18 mm (0.007 inch)

Column thermostat: This module is optional but recommended. If you are operating more than 15 °C different than ambient, you should consider a mobile phase pre-conditioner. For RSLC, a 2 µL size is appropriate.

Pre-column filter: Guard columns are not normally used for RSLC because they are relatively large compared to the analytical column. Instead, use a pre-column filter with a low-volume design. Replace the filter element regularly.

General: RSLC will demand more from your system than conventional LC. Do keep the LC system in good repair with regular maintenance. Monitor the pressure for ripple and changes in operating pressure. Fix leaks and other abnormal conditions immediately. The small cost of vigilant maintenance will be paid for many times over by the savings in time and solvents of RSLC.

SECTION 4 – INSTALLATION AND START-UP

4.1. System Configuration and Start-Up

If desired, install the optional components in the injector and detector. Install the recommended 0.18 or 0.13 mm capillaries to connect the column to the injector and detector.

4.2. System Rinse

Prepare fresh, clean mobile phases. Prime the pump with the mobile phases to make sure that all air and previous mobile phases are expelled.

4.3. Installing the Column

1. Visually inspect the column. Report any damage to your local Dionex office. Depending upon the nature of the damage, we may request that you ship the damaged column back to us for a replacement
2. The columns are shipped in an acetonitrile-water mixture, therefore be sure that the mobile phase is compatible with that. Before installing the column, purge all the pump lines with liquid to remove air and ensure the system is clean.
3. Connect the column between the injection valve and the detector, with the flow arrow on the label pointing towards the detector. The column end fittings are compatible with standard 10-32 fittings and Parker ferrule dimensions. Use the shortest lengths of tubing practical to minimize system volume. The tubing i.d. should not exceed 0.007 inches (0.18 mm) for 3.0 mm i.d. columns nor exceed 0.005 inches (0.125 mm) for 2.1mm columns.
4. Once the column is installed, pump at least 10 column volumes of mobile phase through the column, and if additives such as ion pairing reagents are present in the mobile phase, up to 200 column volumes may be needed. The column is equilibrated when the baseline is stable and several injections produce stable retention times.

4.4. Reproduce the Chromatogram in the Quality Assurance Report

Test the Acclaim column prior to its first use. Using the conditions described on the Quality Assurance Certificate, confirm that it performs as indicated. Keep a record of its performance for future reference.

SECTION 5 – PREPERATION OF ELUENTS AND STANDARDS

5.1. Mobile Phase Preparation

High purity chemicals: For best results use high-purity deionized water and HPLC-grade organic solvents; these will have the least UV background, and are pre-filtered. The Acclaim RSLC columns may be used with all the usual HPLC mobile phases.

Filtration: Buffer salts used for the preparation of mobile phases often contain insoluble particulates. If these are allowed to flow into the column, they will be trapped at the head of the column by the inlet frit, plugging the column over time. To avoid this the mobile phase should be filtered through a 0.5 µm or finer porosity filter media. In order to avoid problems associated with the loss of prime in the pump, mobile phases should be degassed prior to use.

Solvent Compatibility: The columns are compatible with all ordinary HPLC solvents, including methanol, acetonitrile, tetrahydrofuran, acetone and dichloromethane. Acclaim 120 C18 and C8 columns are compatible with highly aqueous mobile phases (up to 90% aqueous) under isocratic conditions. In gradient methods, they are compatible with up to 95% aqueous mobile phases. Dionex recommends Acclaim Polar Advantage (PA) column and Polar Advantage II (PA2) for applications that require highly aqueous mobile phases (> 90% aqueous). For control of mobile phase pH between pH 2 and pH 7, dilute acids and buffer salts such as trifluoroacetate, phosphate, formate, acetate or TRIS may be used. When making alkaline buffers for Acclaim PA2, Dionex recommends amine-type buffers such as TRIS, ammonia or diethylamine instead of phosphate buffers. When adding organic solvent to salt and buffer solutions, it is important to remember this may result in a precipitation of the salt, often in the column, leading to possible damage to the column. Always check the solubility of buffer salts in solvent-buffer mixtures before running new methods. For very fast gradient elution, greater than 40% per minute, we recommend acetonitrile as the organic solvent because of its lower viscosity.

5.2. Sample Preparation

Contamination of the column by particulate matter in the sample is a leading cause of column failure. Symptoms may include high pressure, poor symmetry, extraneous peaks, or low efficiency. Pass the sample through a 0.5 µm or smaller porosity filter before injection. Biological samples are especially troublesome due to dissolved proteins that may precipitate some time after the initial preparation.

Short columns are more sensitive to overload conditions than long columns. If the sample is dissolved in a solvent stronger than the mobile phase the chromatogram will be susceptible to "volume overload." If possible, reduce the solvent content in the prepared sample to no more than the initial composition of the mobile phase.

SECTION 6 – COLUMN CARE

6.1. Column Storage-Long Term Storage

If the column will not be used for a week or more, flush the column with 10 column volumes of unbuffered mobile phase containing at least 70% organic solvent. Do not store it with acids, salts or buffers inside. Plug the column to keep the bed moist. Do not allow the column to dry out.

6.2. Mobile Phase

Mobile phases should be freshly prepared every time. All chemicals and solvents should be at the highest available quality. All mobile phases should be filtered before use. In-line filters are recommended.

6.3. Recommended Operating pH Range

To obtain better column lifetime, it is highly recommended to use "silica friendly" mobile phases. The pH limits of the columns are listed in Table 2.

6.4. Recommended Operating Temperature Limit

The recommended temperature limits of the columns are listed in Table 2, and depend on the nature of the bonded phase. Higher temperatures accelerate the loss of bonded phase and dissolution of the silica substrate.

6.5. Flow Rate and Pressure Limit

To protect the column, observe the recommended maximum flow and pressure. Best chromatographic results are usually at more moderate flows. See Table 3 in section 2.2. It is extremely important not to impose sudden column pressure surge. Therefore increase flow rate gradually up to the desired flow rate; this feature is programmable in Dionex HPLC pumps.

6.6. Cleaning the Column

Hydrophobic compounds: If the column becomes contaminated with hydrophobic compounds, it may be safely washed with strong organic solvents such as acetone. Flush out any buffer salts with unbuffered mobile phase, then flush with methanol, then acetone, then methanol again.

Air: Very short columns can be dewetted at high linear velocity. Flushing with acetone as described above has been found to be effective at re-wetting the column.

Ion-pairing agents usually can be removed by washing with 90:10:0.1 methanol:water:formic acid. Quaternary amine agents may require extensive washing to restore the original column behavior, and it is preferable to dedicate the column to this type of mobile phase.

Particulate contaminants will cause excess backpressure and possibly loss of performance. Temporarily reverse the column, and at a reduced flow rate direct the effluent to waste until the pressure stabilizes. Prevention is the best cure: filter the samples and mobile phases, and use a pre-column filter.

Metal contamination (most commonly iron) may be removed by treatment with a chelating agent. The least damaging agent is 85:10:5 methanol:water:pentanedione. A more aggressive agent is 10 mM citrate, 10 mM EDTA at pH 4 mixed 70:30 with methanol.

Note: if above treatment fails to improve the column performance, replace it with a new one.

