

# Carbohydrate Analysis and Purification

Solutions for Challenging Applications

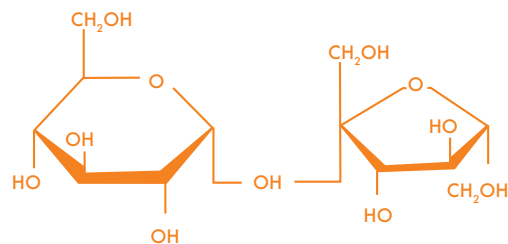


**GRACE**  
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# Challenges of Carbohydrate Analysis and Purification

## The Challenge of Separating, Detecting and Purifying Carbohydrates



Carbohydrates present a particular detection challenge because they lack chromophores. The traditional approaches to carbohydrate detection are either differential refractive index (RI) or low-wavelength UV (190 – 205nm).

The poor sensitivity and gradient incompatibility of RI detection make it less than ideal for carbohydrate analysis by HPLC.

Low-wavelength UV also lacks sensitivity and places stringent requirements on the optical properties of the mobile phase. Both techniques are subject to severe baseline shifts and long equilibration times as the detector responds to spectroscopic changes in the solvent.

Many new compounds now being synthesized in pharmaceutical and food ingredients / dietary supplement industries are being based on glycoside structures and these have the same challenge of a lack of chromophores when being analyzed or purified.

## Benefits of Evaporative Light Scattering Detection (ELSD) for Carbohydrate Analysis and Purification

- More sensitive than RI and low- $\lambda$  UV
- Gradient compatible
- Stable baselines, even with gradients
- Solvent choice not limited by UV-cutoff

The Evaporative Light Scattering Detector (ELSD) is a universal HPLC detector that detects any compound less volatile than the mobile phase to low nanogram levels. It has many advantages over RI and low-wavelength UV detection for carbohydrate analyses.

With ELSD a quiet stable baseline, and better sensitivity than with low-wavelength UV detection is obtained. Evaporation of the mobile phase before detection makes the ELSD gradient compatible for improved resolution and faster separations. In addition, ELSDs can be 10 to 1000 times more sensitive than RI detectors.

The same benefits are seen with the Reveleris® X2 flash chromatography system when purifying reaction mixtures due to its integrated, specially optimized ELS detector. The systems software switchable sample splitting, physically changes the amount of the eluent being sent to the ELSD for optimum conditions for either high or low level concentration components.

# Prevail™ Carbohydrate ES Column and Alltech® 3300 ELSD for Carbohydrate Analysis

## Benefits of Prevail™ Carbohydrate ES Column

- Longer column life-hybrid phase has ruggedness of silica with the stability of a polymer
- Quieter baselines compared to traditional amino carbohydrate columns, even with gradients
- Reduce run times with more mobile phase choices
- Compatible with gradient ELSD applications

Alltech® Prevail™ Carbohydrate ES columns are excellent for isocratic separations with common detectors, but the column's real power shows with the ELSD and a solvent gradient. Achieve powerful selectivity, reduced run times and efficient peaks from gradients while seeing the quiet, stable baselines that maximize sensitivity. Prevail™ Carbohydrate columns are versatile for mono- and oligosaccharides and sugar alcohols. They produce single peaks for reducing sugars at ambient temperatures, eliminating the need for column heating.

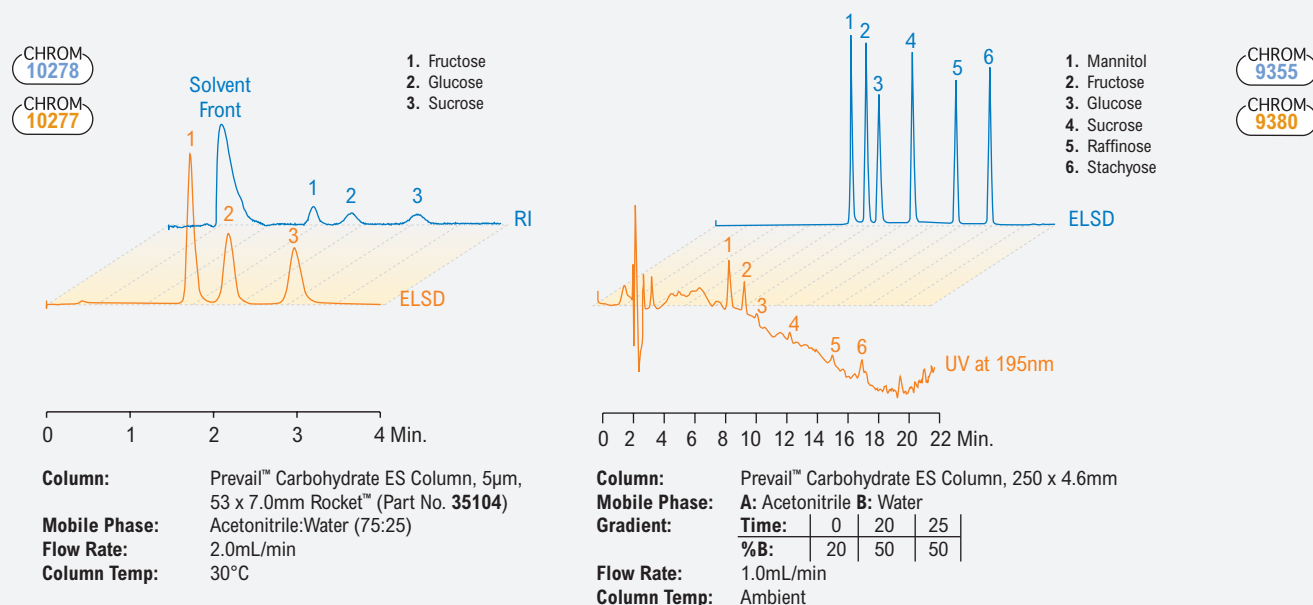
## Benefits of the Alltech® 3300 ELSD

- Low noise and excellent baseline stability
- Universal detection for a broad range of applications
- Low nanogram detection limits
- Maintain stable baselines during steep gradients

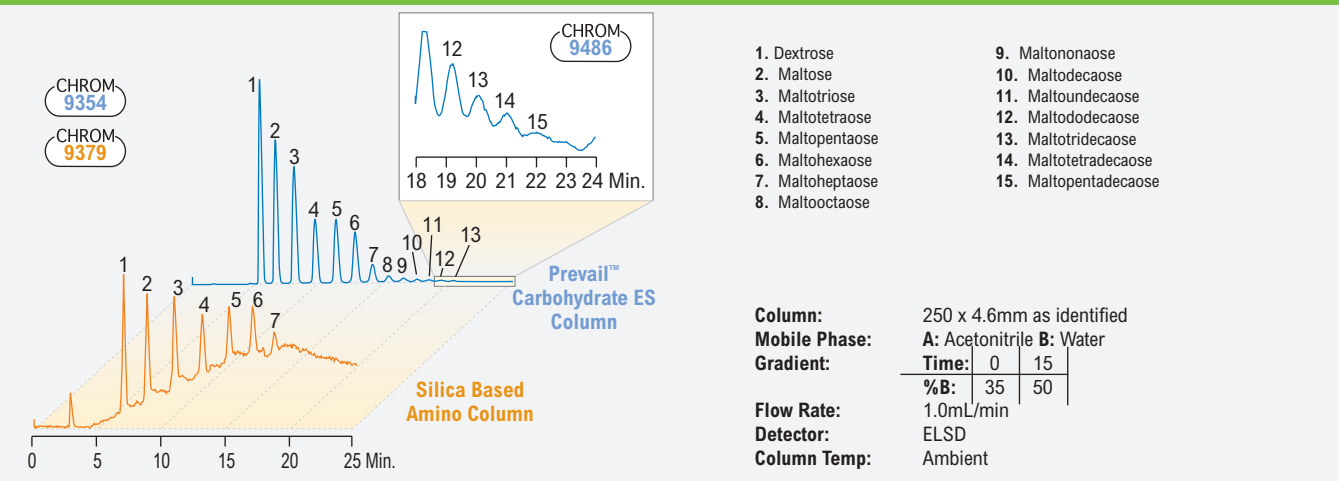


The Alltech® 3300 ELSD's high sensitivity and universal detection method are ideal for difficult compounds such as carbohydrates, lipids and nutraceuticals. It can function as a stand-alone detector or operate as a powerful complement to UV or Mass Spectrometry.

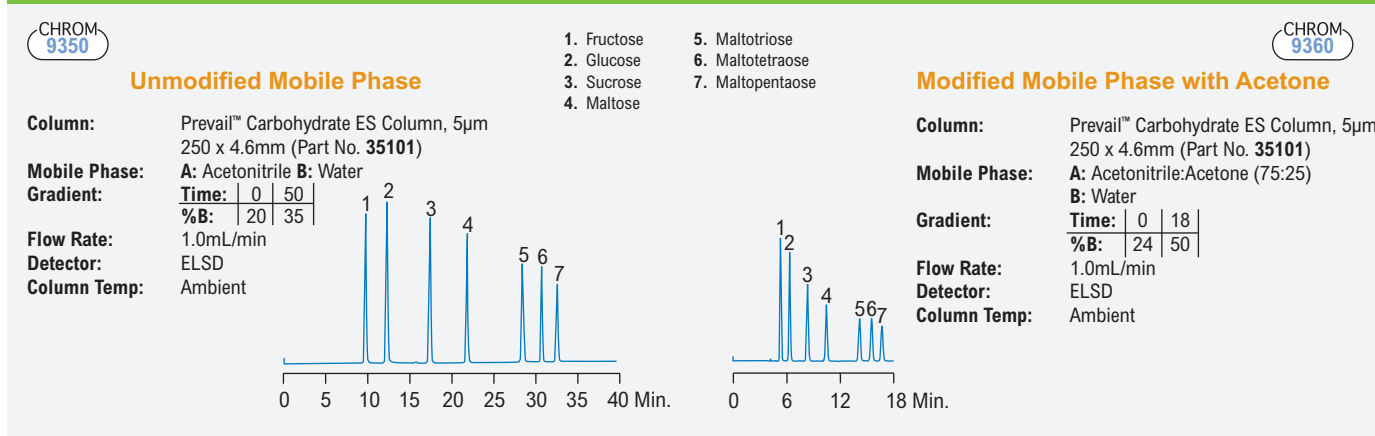
## Carbohydrate Analysis with ELSD Improves Baseline Stability and Detection Sensitivity Compared to RI and Low-Wavelength UV

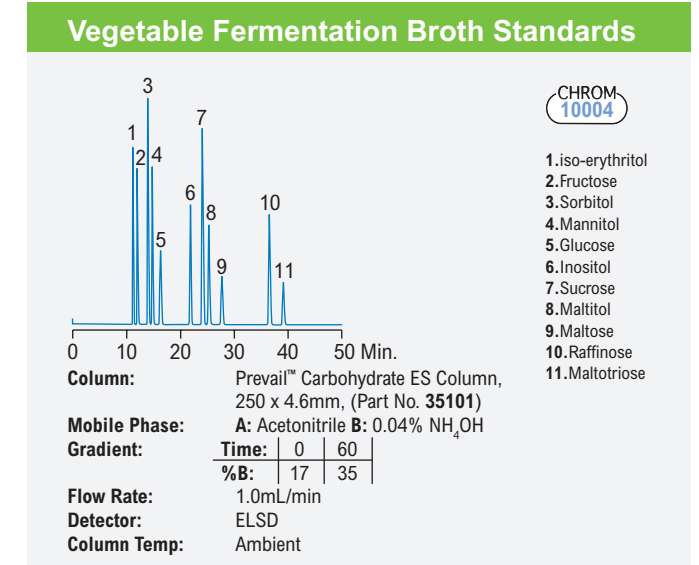
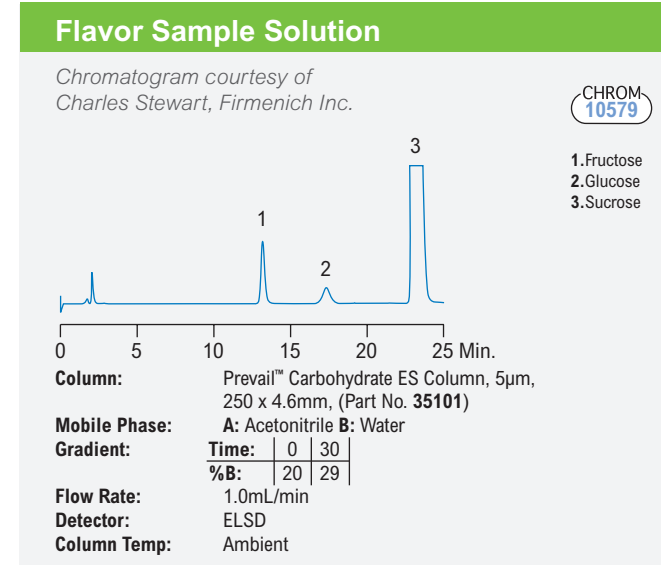
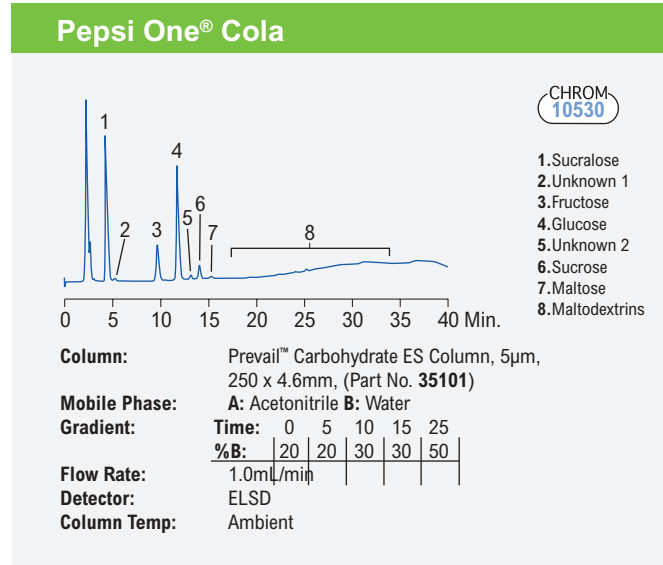
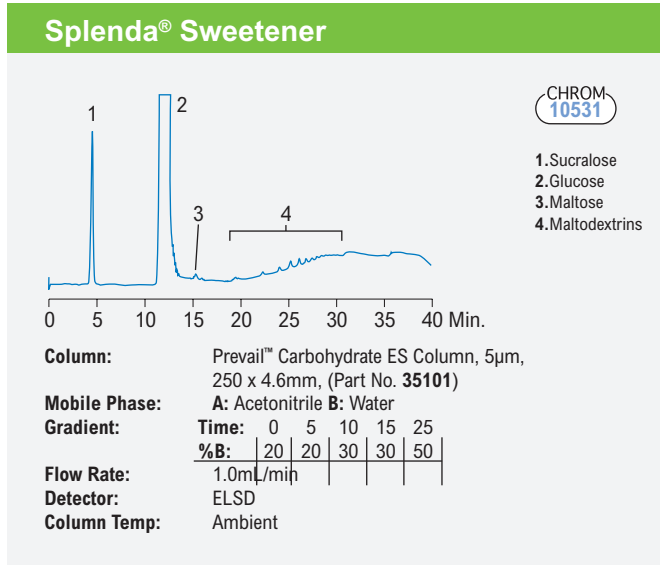
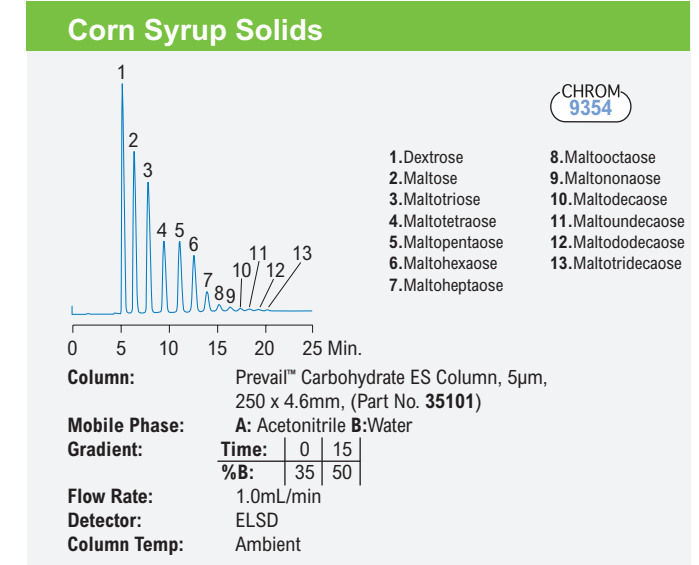
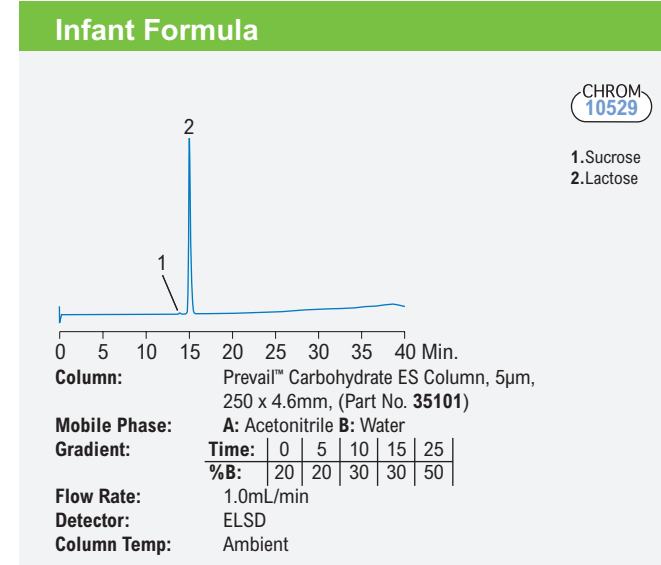
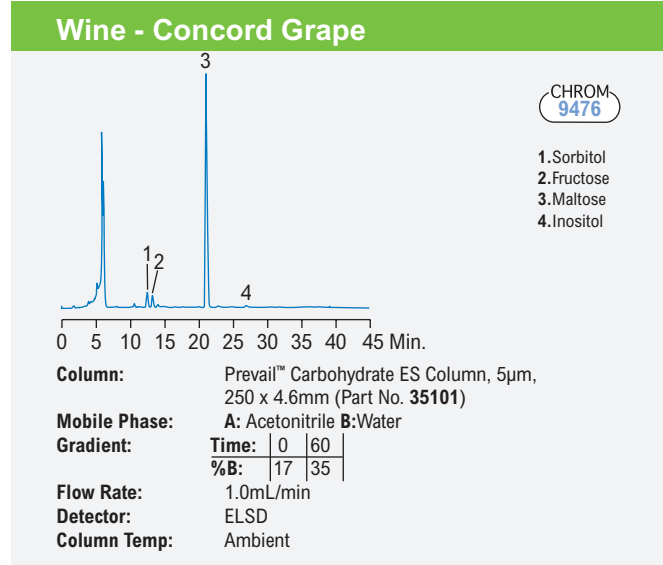
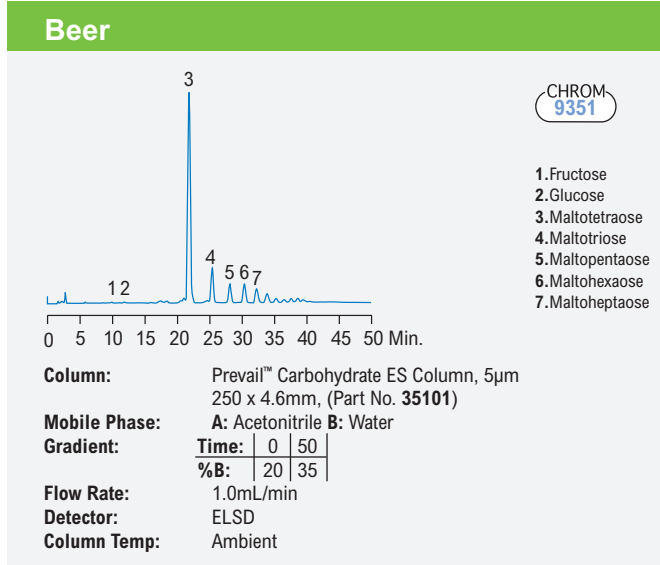
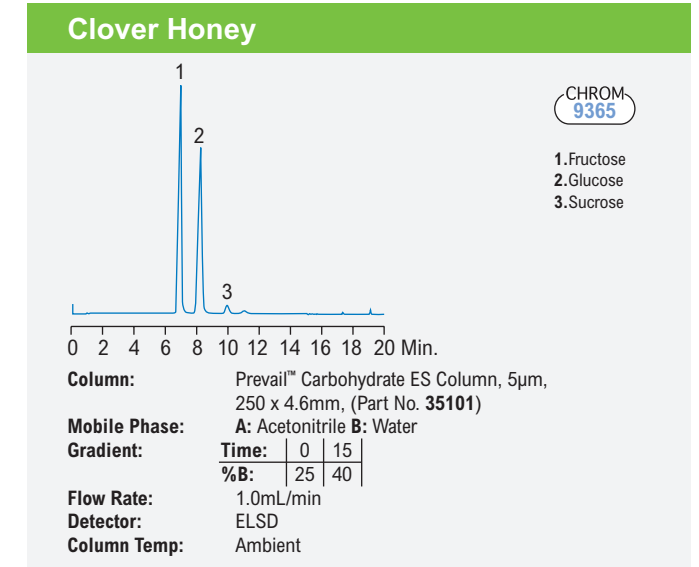
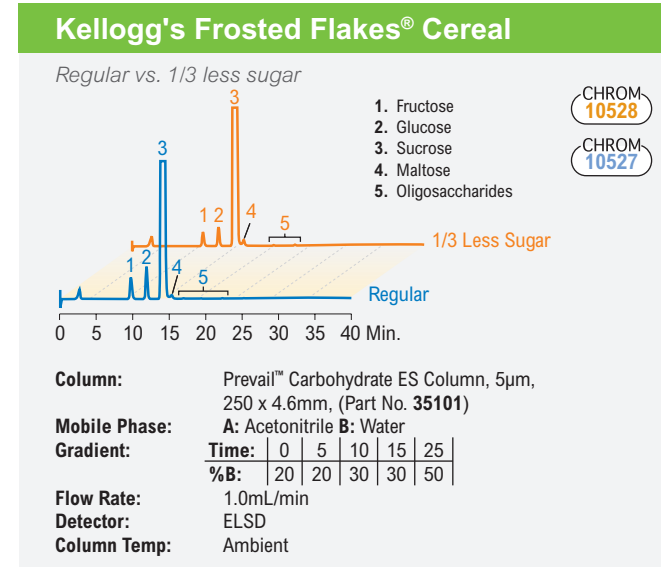
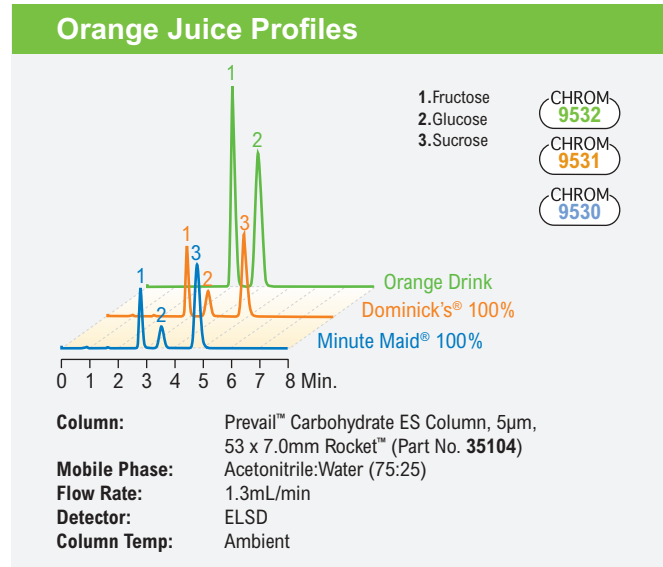
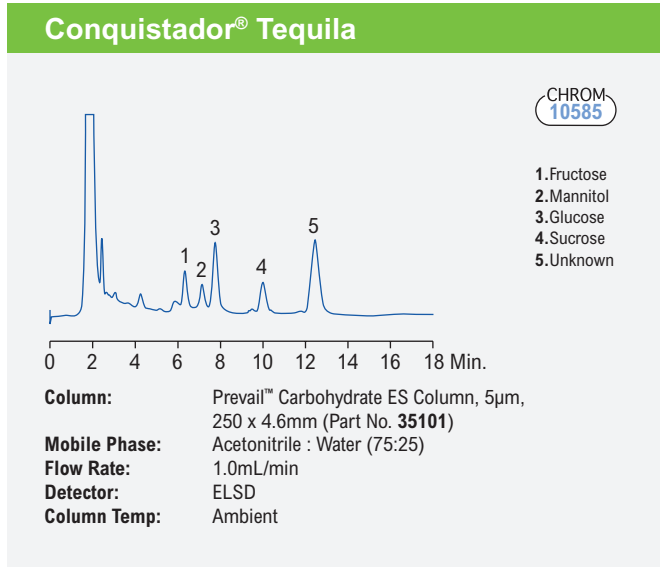


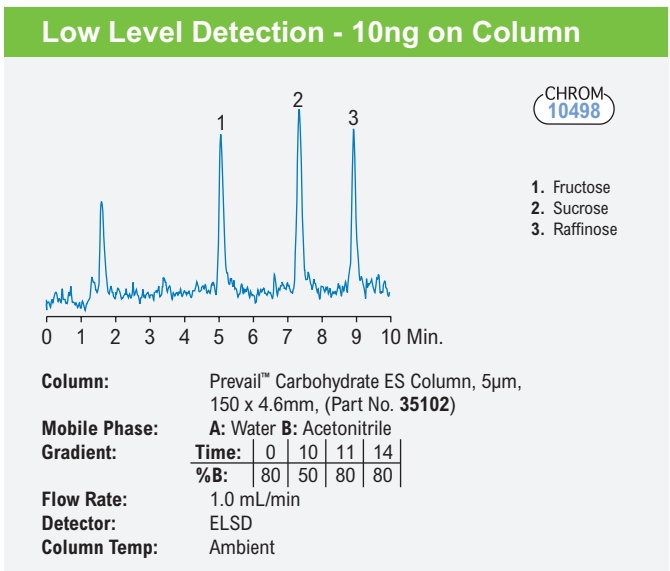
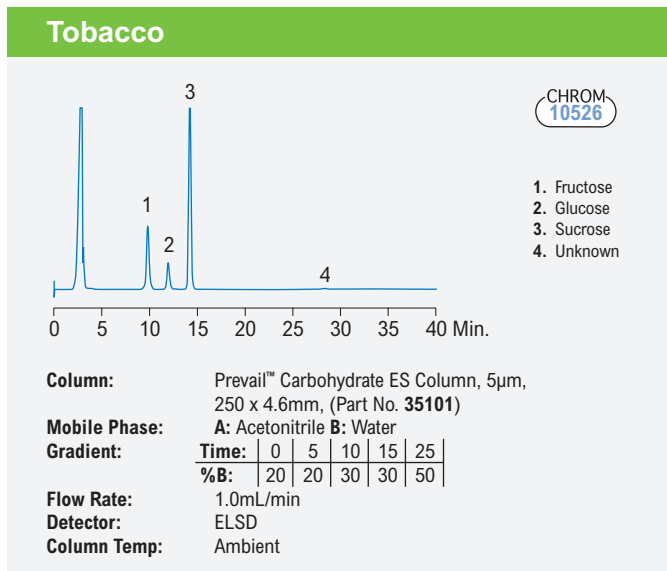
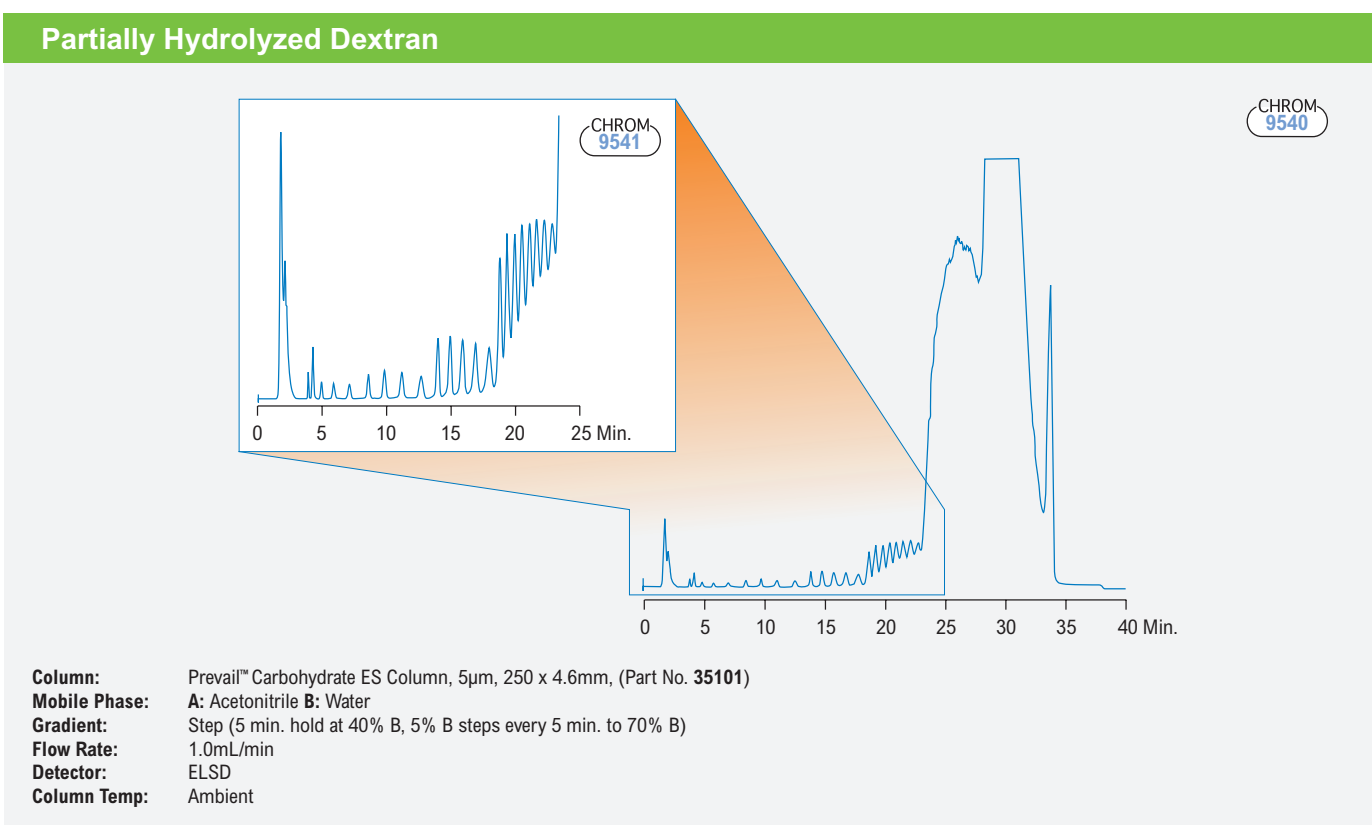
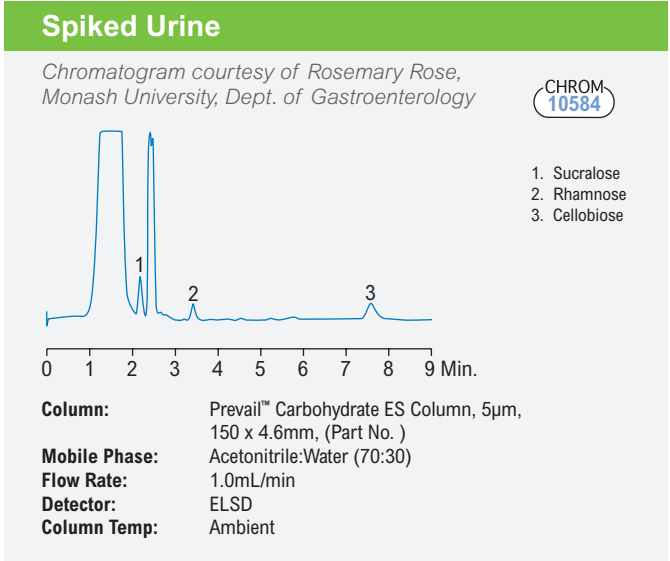
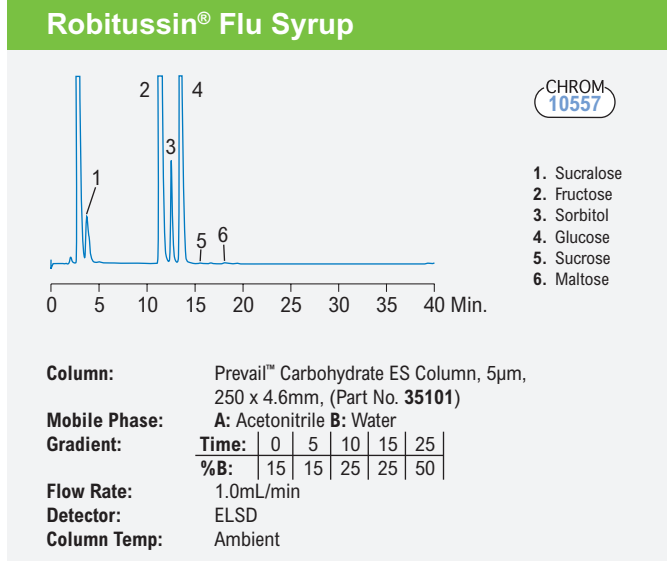
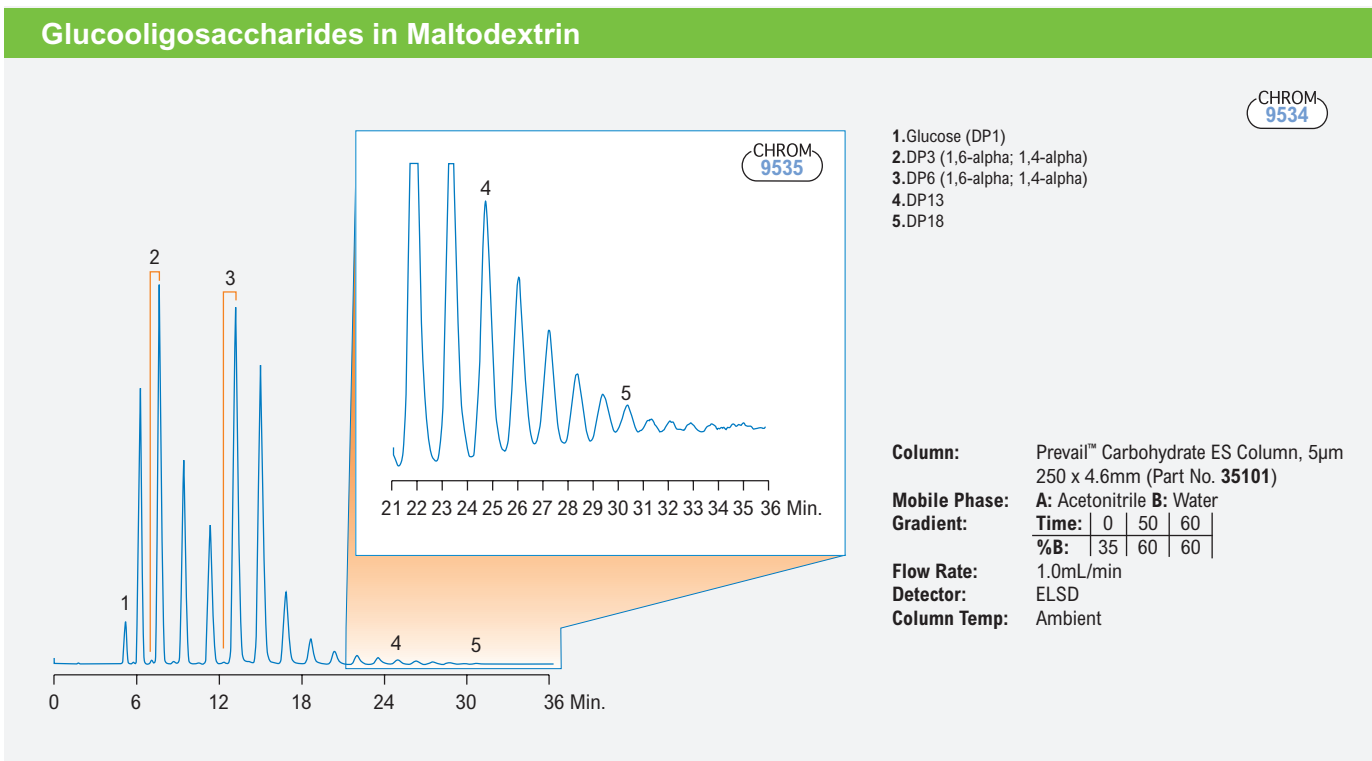
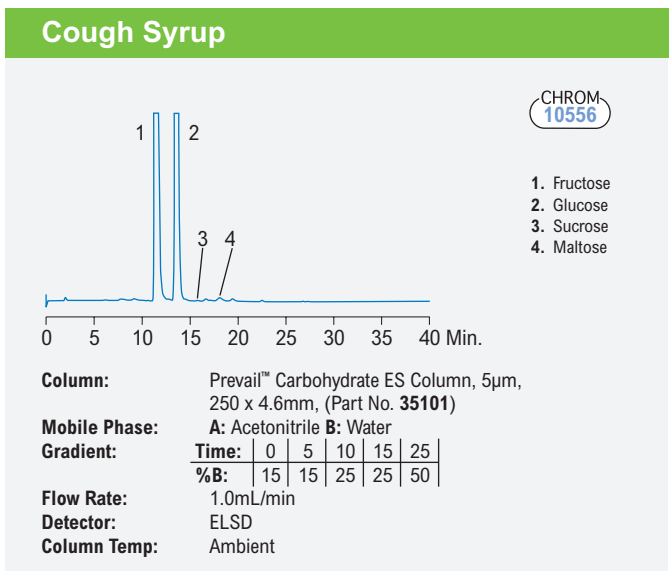
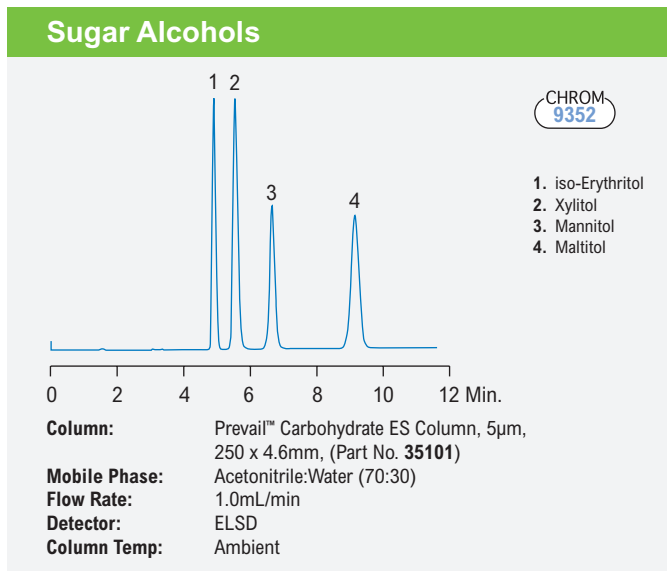
## Enhance Detector Sensitivity With Stable Gradient Baselines



## Reduce Run Times Using Alternative Solvents







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# Reveleris® X2 Flash Chromatography System for Carbohydrate Purification

## Benefits of the Reveleris® X2 Flash Chromatography System

- Detects both chromophoric and non-chromophoric compounds
- Allows use of UV interfering solvents
- No user optimization of ELSD operating conditions required
- Touch screen sensitivity switching (patent pending) changes the amount of eluent split to the ELSD for either high or low concentration components
- Minimal amount of sample sent to the ELSD reduces sample loss of less than 0.1%



## Benefits of Reveleris® Amino Flash Cartridges

- High capacity cartridges offer twice the loading capacity of other flash cartridges, helping to save time and costs
- Durable 200psi pressure limit design for greater choice of flow rate
- Auto-recognition of the Reveleris® cartridges on the Reveleris® X2 instrument, pre-populates methods with recommended operating conditions
- 40µm ultra-high purity media developed for flash applications



The Reveleris® X2 flash chromatography system is ideally suited to purification of glycoside based compounds. The Reveleris® X2 flash system incorporates UV or UV Visible detection with an ELSD detector designed for flash purifications. The patented RevealX™ detection technology synchronizes signals from the multiple detectors to determine optimum timing of fraction collection for the highest possible purity and recovery of fractions.

Reveleris® Amino flash cartridges are an ideal choice for glycoside chemistry purification for improved retention and resolution. Increased capacity for higher sample loads and durability to withstand pressures up to 200psi, allows the chemist to overcome separation challenges in purification and become more productive. When combined with the Reveleris® X2 flash chromatography system, these specialty phases allow fast, easy purification of challenging carbohydrate compounds.

## Better Together - Further Streamline Your Purifications by Combining Reveleris® Cartridges with the Most Advanced Flash System

Reveleris® Cartridge



Reveleris® X2 System



Enhanced Purification Benefits



### Plug & Play

Automatic cartridge identification populates the system for size and phase of cartridge in use

### Confidence in Results

Serial tracking of cartridges allows for tracking of cartridge use

### Simplifying Purification

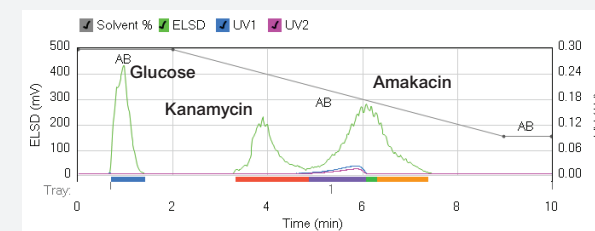
Specifically for compounds like carbohydrates and lipids, the patented RevealX™ detection technology allows maximum purity with minimal effort in a single system

### Faster Runs

Optimize purification with the Navigator software

# Carbohydrate Purification Applications

## Amakacin



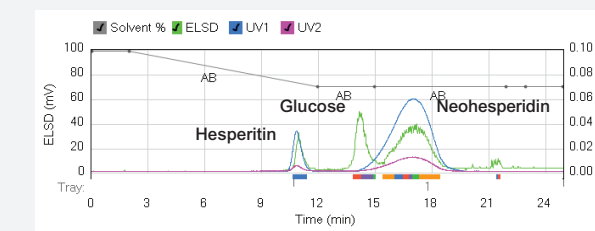
### Flash Chromatography Conditions:

Cartridge: Reveleris® C18 12g  
 Flow Rate: 36mL/min  
 Equilibration: 5.0 min  
 Run Length: 10.0 min  
 UV1 Wavelength: 220nm  
 UV2 Wavelength: 254nm  
 ELSD Carrier: Iso-propanol  
 Solvent A: Water with 0.1% TFA  
 Solvent B: Acetonitrile

### Gradient Table

Step	Time (min.)	%B
1	0.0	99
2	2.0	99
3	7.0	30
4	1.0	30

## Flavonone Glycoside



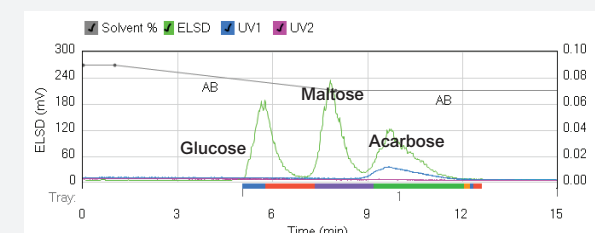
### Flash Chromatography Conditions:

Cartridge: Reveleris® amino 40g  
 Flow Rate: 40mL/min  
 Equilibration: 10.0 min  
 Run Length: 25.0 min  
 UV1 Wavelength: 220nm  
 UV2 Wavelength: 254nm  
 ELSD Carrier: Iso-propanol  
 Solvent A: Water  
 Solvent B: Acetonitrile

### Gradient Table

Step	Time (min.)	%B
1	0.0	99
2	2.0	99
3	10.0	70
4	13.0	70

## Acarbose



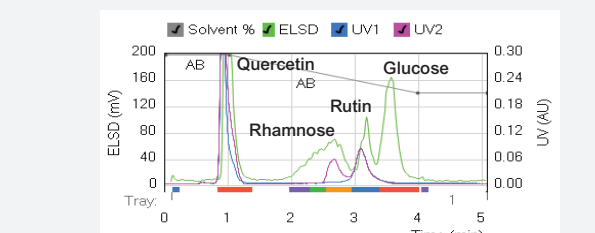
### Flash Chromatography Conditions:

Cartridge: Reveleris® amino 12g  
 Flow Rate: 36mL/min  
 Equilibration: 10.0 min  
 Run Length: 15.0 min  
 UV1 Wavelength: 220nm  
 UV2 Wavelength: 254nm  
 ELSD Carrier: Iso-propanol  
 Solvent A: Water with 0.1% TFA  
 Solvent B: Acetonitrile

### Gradient Table

Step	Time (min.)	%B
1	0.0	90
2	1.0	90
3	7.0	70
4	7.0	70

## Quercetin Rutinose



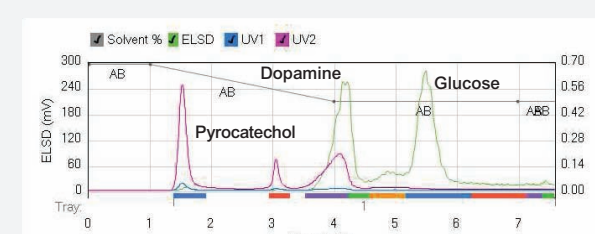
### Flash Chromatography Conditions:

Cartridge: Reveleris® amino 12g  
 Flow Rate: 36mL/min  
 Equilibration: 10.0 min  
 Run Length: 9.0 min  
 UV1 Wavelength: 265nm  
 UV2 Wavelength: 220nm  
 ELSD Carrier: Iso-propanol  
 Solvent A: Water  
 Solvent B: Acetonitrile with 0.5% TFA

### Gradient Table

Step	Time (min.)	%B
1	0.0	99
2	1.0	99
3	3.0	70
4	5.0	70

## Catechols



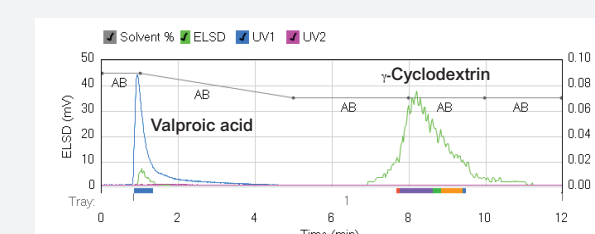
### Flash Chromatography Conditions:

Cartridge: Reveleris® amino 12g  
 Flow Rate: 36mL/min  
 Equilibration: 10.0 min  
 Run Length: 7.6 min  
 UV1 Wavelength: 254nm  
 UV2 Wavelength: 220nm  
 ELSD Carrier: Iso-propanol  
 Solvent A: Water  
 Solvent B: Acetonitrile

### Gradient Table

Step	Time (min.)	%B
1	0.0	99
2	1.0	99
3	3.0	70
4	4.0	70

## Valproic Acid



### Flash Chromatography Conditions:

Cartridge: Reveleris® amino 12g  
 Flow Rate: 36mL/min  
 Equilibration: 6.0 min  
 Run Length: 12.0 min  
 UV1 Wavelength: 212nm  
 UV2 Wavelength: 254nm  
 ELSD Carrier: Iso-propanol  
 Solvent A: Water with 0.1% TFA  
 Solvent B: Acetonitrile

### Gradient Table

Step	Time (min.)	%B
1	0.0	90
2	1.0	90
3	4.0	70
4	7.0	70

# Ordering Information

## Prevail™ Carbohydrate ES Column

PREVAIL™ HPLC COLUMNS			
Packing	Format	I.D. x Length	Part No.
Carbohydrate ES, 5µm	LC/MS	2.0 x 150mm	35116
	Analytical	4.6 x 150mm	35102
	Analytical	4.6 x 250mm	35101
	Rocket™	7.0 x 53mm	35104
	Prep	10 x 250mm	35115

PREVAIL™ ALL-GUARD™ CARTRIDGES*			
Packing	I.D. x Length	Qty	Part No.
Carbohydrate ES	4.6 x 7.5mm	3	96435
All-Guard™ Guard Cartridge Holder		1	80101

\*All-Guard™ Holder required

PREVAIL™ PREP-GUARDS*			
Packing	I.D. x Length	Qty	Part No.
Carbohydrate ES	7.6 x 50mm	ea	35105
Direct-Connect™ Column Coupler		ea	28195

\*Direct-Connect™ Column Coupler required

## Alltech® 3300 Elsd

ALLTECH® 3300 ELSD*	
Description	Part No.
3300 ELSD 110V – 240V	5135834

## Reverleris® X2 Flash Chromatography System

REVELERIS® X2 FLASH CHROMATOGRAPHY SYSTEM	
Description	Part No.
Reverleris® X2 Flash Chromatography System	5164931
Reverleris® X2 UV/Visible Wavelength, Optional Upgrade	5167301
Reverleris® X2 Navigator™ Software Optional Upgrade	5156620

REVELERIS® FLASH CARTRIDGES		
Cartridge Size	Qty.	Amino Part No.
4g	ea	5157330
12g	ea	5157331
40g	ea	5157332
80g	ea	5157333
120g	ea	5157334
330g*	ea	—



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