

Improved Purification of Carbohydrate Based Drugs in Glycochemistry Applications Using a Flash Chromatography System

**Murray Fryman, Melissa Wilcox
Jeff Horsman, Kathy Lawrence**

Grace Davison Discovery Sciences
2051 Waukegan Rd.
Deerfield IL 60015 U.S.A.
Phone: 1-800-255-8324
Website: www.discoverysciences.com

GRACE

Introduction

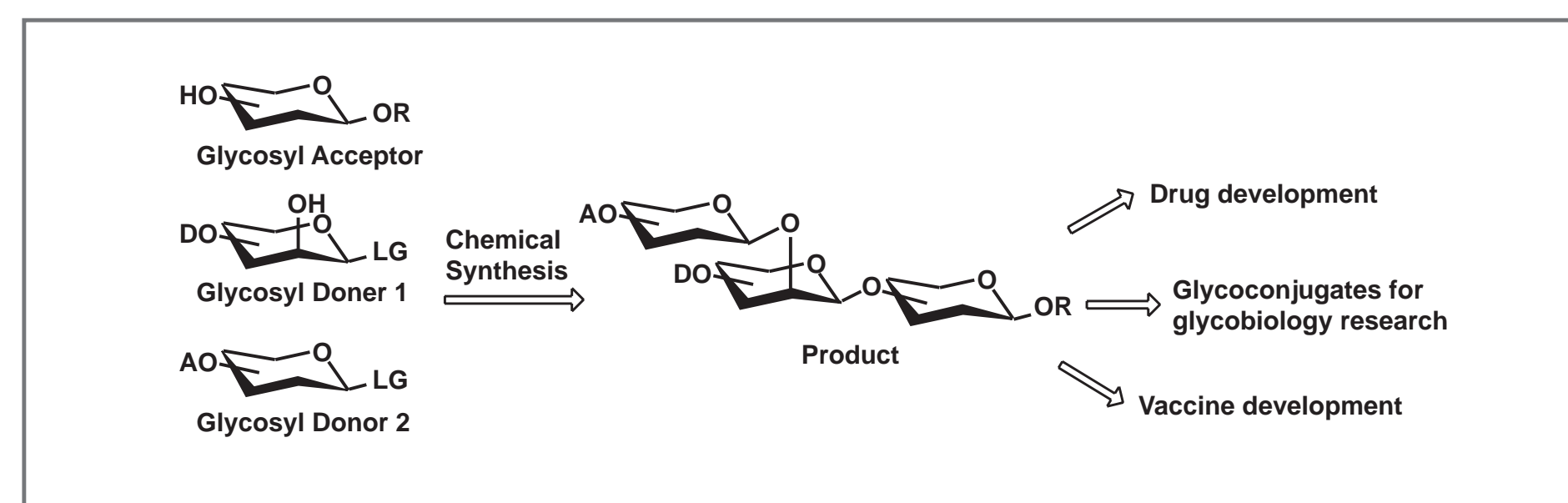
Carbohydrates are commonly found in plants, animals, and microorganisms and have considerable applications in medicine when bound to lipids, peptides, proteins, or even nucleic acids^{1,2}. Among classes of biomolecules, carbohydrates are the most structurally diverse. This heterogeneity makes isolation of pure samples in sufficient amounts, from biological sources extremely difficult. Total synthesis of bioactive natural products, aminoglycoside antibiotics, glycodrugs, and glyco-conjugate vaccines from carbohydrates have been of considerable interest in the medicinal field. Chemical synthesis offers the advantage of producing structurally targeted oligosaccharides for drug discovery. The complex nature and diverse physicochemical properties of carbohydrates, including their high polarity and lack of UV absorbance, translates to challenges in post synthesis purification. However, recent advances in flash chromatography have simplified and facilitated the separation and access to these molecules

This study identifies the specific challenges of carbohydrate isolations found during extraction from natural sources or synthetic production. We explore overcoming the drawbacks of employing traditional flash chromatography for carbohydrate purification resulting from the compound's lack of UV absorbance. Flash chromatography equipped with integrated multi-signal detection and signal processing is investigated to enable efficient separation and collection of carbohydrates and their conjugates.

Experimental and Results

Synthesis Products

Glycoconjugates, being diverse structures, require multiple step syntheses to produce. The number of monosaccharides, ring size, the different anomeric stereochemistry, and the existence of the branched-chain sugars all contribute to the complexity of the structures. Typical oligosaccharides are produced in four transformation stages: preparation of the glycosyl donors, preparation of the glycosyl acceptors with a single unprotected hydroxyl group, the coupling of them, and the deprotection process.



However, recent synthetic advances are demonstrating the ability to produce biologically interesting oligosaccharides in single-pot conversions. The regioselective protection of hydroxyl groups and the stereoselective assembly of glycosidic bonds present a number of challenges for synthetic chemists, including adding to the complexity of the crude reaction mixture. The purification challenge becomes to successfully separate the complex carbohydrates from their simple sugar building blocks, reagents, and by-products.

Flash chromatography is commonly favored by chemists since it is viewed as a very rapid and reliable technique for purification. Traditional approaches involve utilizing flash chromatographic systems equipped with ultraviolet (UV) detection, which fails to detect carbohydrates and their impurities. Consequently, chemists rely on time-based fraction collection and tedious post fraction analysis, including TLC plate staining in order to detect components in the reaction mixture, which slows isolation and subsequent downstream testing. Undoubtedly, this contradicts the perception that flash is an efficient method for purification and wastes precious research time.

The Reveleris® Flash Chromatography System

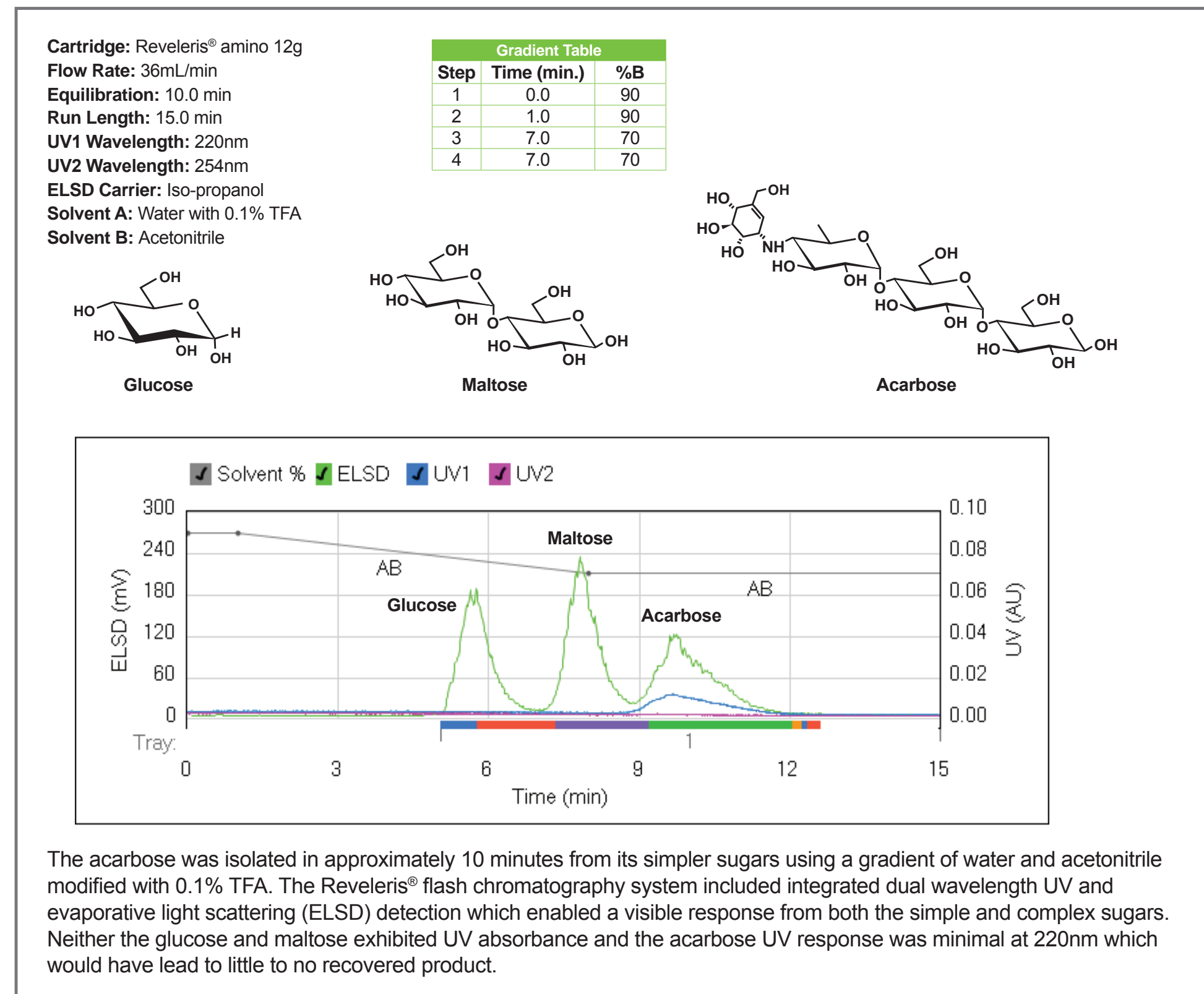


Case Studies

Acarbose

Acarbose, an inhibitor of α -glucosidase, is used in the treatment of diabetic patients to lower blood glucose. The reversed-phase separation of a pseudo-reaction mixture containing 25mg of acarbose and other sugar components was evaluated using an advanced flash chromatography system with an amino bonded silica cartridge.

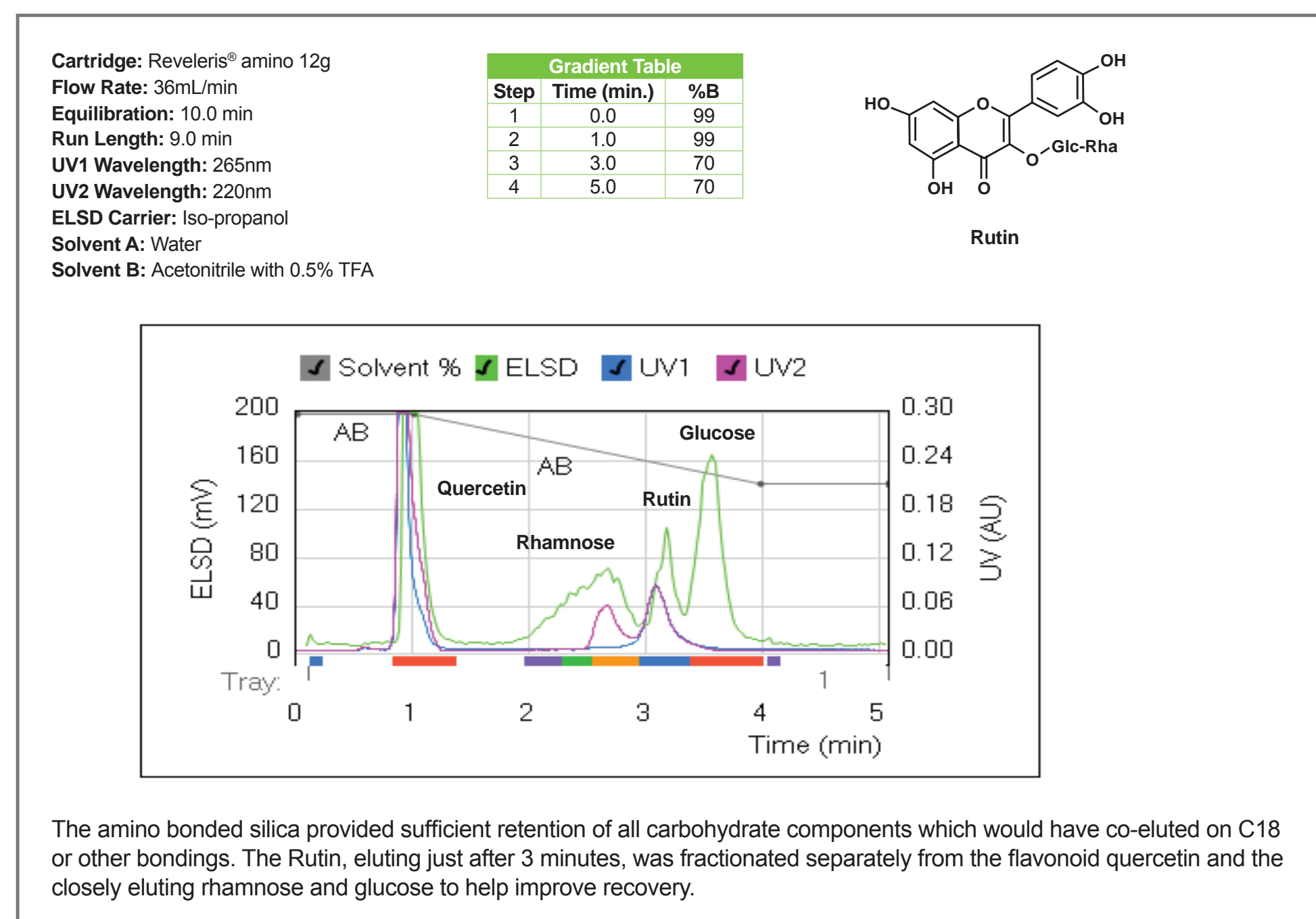
The Reveleris® iES flash chromatography system isolates the aminosugar, Acarbose, from its sugar components



Rutin

The flavanol glycoside Rutin, used in dietary supplements as Vitamin P, was examined. Besides its antioxidant property, this bioflavonoid works with vitamin C in reducing pain and intraocular pressure. It has been synthesized from the conjugation of carbohydrates such as rhamnose and glucose with the flavonoid quercetin. A post synthesis reaction mixture containing Rutin was purified using the Reveleris® flash system in less than 5 minutes.

Purification of conjugated Quercetin with Rutinose using the Reveleris® flash chromatography system is fast and efficient



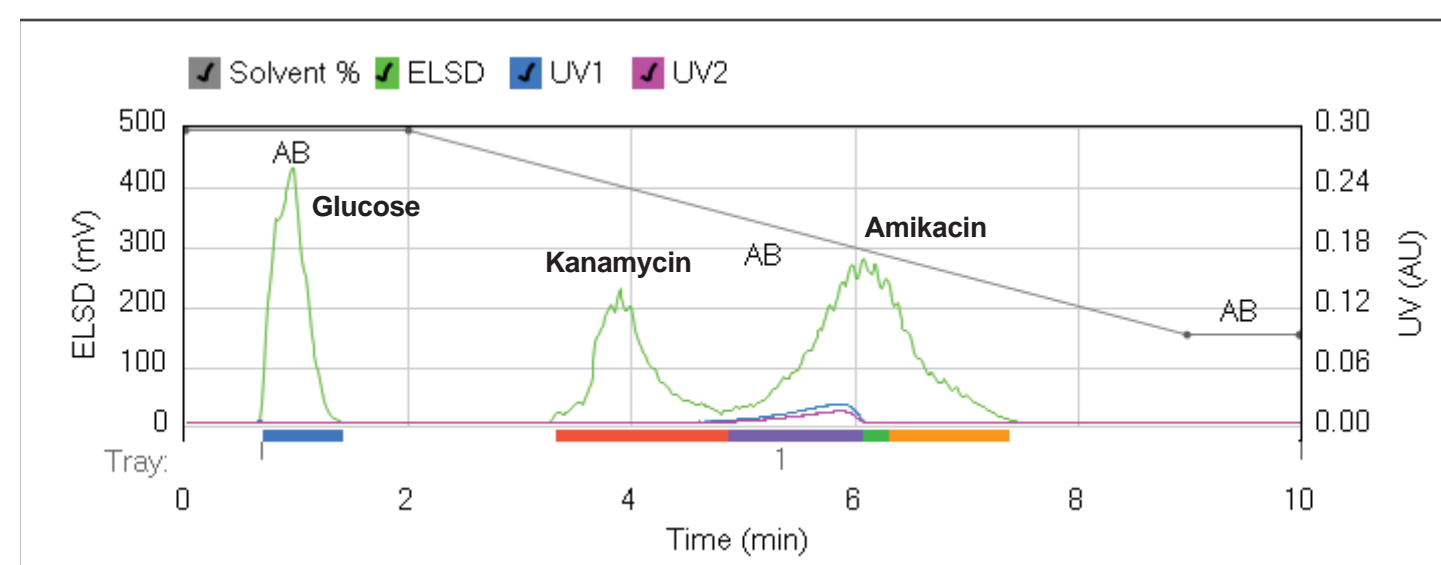
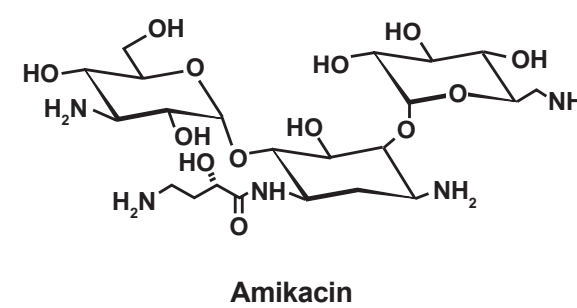
Amikacin

Bacterial resistance to aminoglycoside antibiotics has been a problem leading to clinical failures. Present as a mixture of aminoglycosides, Kanamycin A has been used as a precursor to its semi-synthetic acyl derivative, amikacin. The latter, when modified with 4-amino-2-hydroxybutyryl group, helps to protect against enzymic deactivation in the body while maintaining its activity.

Isolation of Aminoglycoside Antibiotics in the Synthesis of Amikacin is simplified with dual detection in the Reveleris® flash chromatography system

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

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



The semi-synthetic acyl derivative amikacin mixture is separated in under 8 minutes using reverse phase C18 bonded silica on the Reveleris® flash chromatography system. The amikacin is easily detected with the ELSD, well retained and separated from glucose and its precursor, Kanamycin A. A HILIC gradient method was employed to enhance the separation in which the aqueous concentration, as opposed to the organic, increases with time.

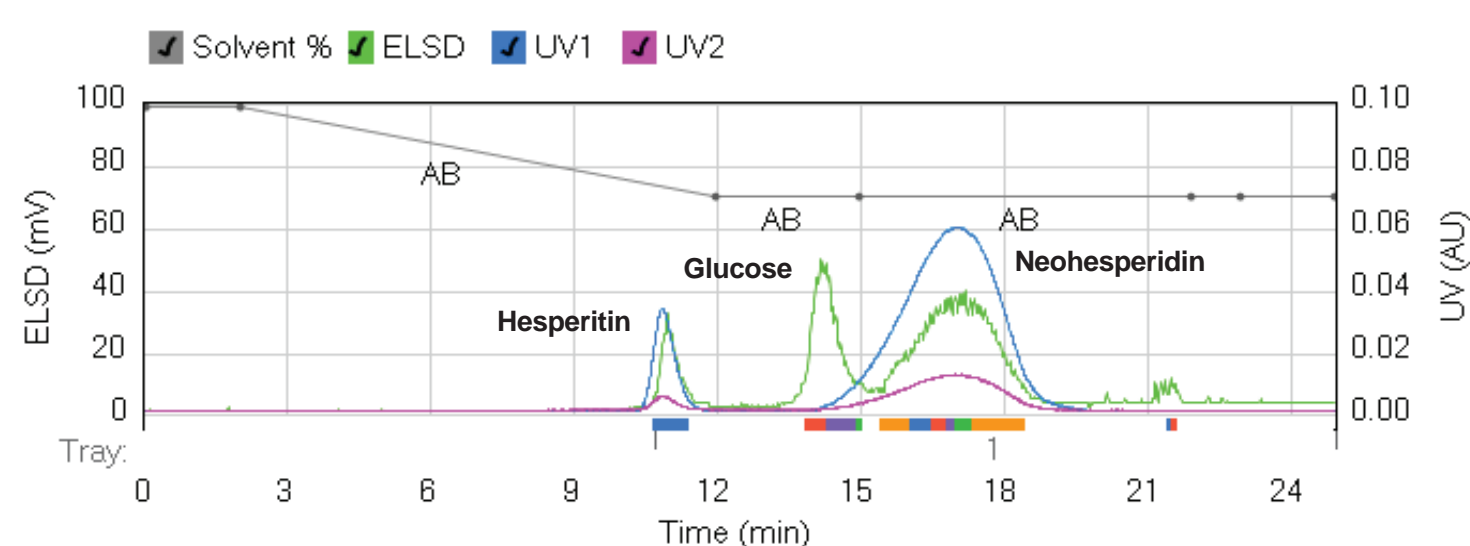
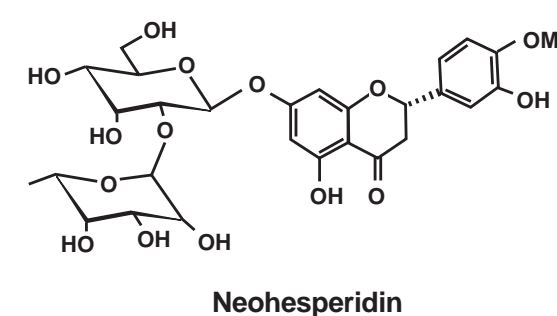
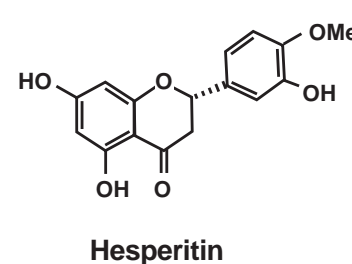
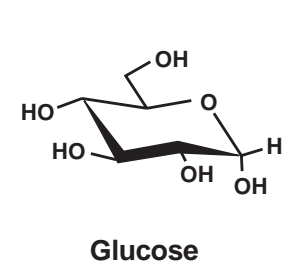
Neohesperidin

Neohesperidin, naturally derived from bitter orange, is an intensely bitter flavanone glycoside used for producing the non-sugar sweetening agent neohesperidin dihydrochalcones for diabetes related therapies. It is known to have a strong synergistic effect in combination with other sweeteners like aspartame, xylitol, and saccharin, for controlling sugar uptake in human body.

The Reveleris® flash chromatography system helps improve purification of flavanone glycosides

Cartridge: Reveleris® amino media 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

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



The Reveleris® flash chromatography system was used with an amino bonded phase and operated in a HILIC gradient mode. Unreacted hesperitin and degraded reagents yielding glucose were undetected at one or both UV wavelengths, but were isolated separately by relying on ELS detection triggering. The neohesperidin was well retained and separated from the earlier eluting hesperitin and glucose.

Conclusion

This work shows that polar sugar compounds can be rapidly separated from their reaction mixtures using a Reveleris® flash chromatography system combined with amino or C18 bonded silicas.

Purification bottlenecks encountered when using traditional flash chromatography with UV detection can be eliminated by using multi-signal detection which independently triggers fraction collection from UV and ELSD. Relying on multi-signal fraction triggering saves time and effort by foregoing the need to perform time-based collection (collect peaks only) and extensive post fractionation workup (including TLC plating/staining). The Reveleris® flash system isolates target compound fast with high purity and recovery.

References

1. Medicinal natural products; a biosynthetic approach, 3rd edition; Dewick, P.; John Wiley & Sons, Inc., Hoboken, New Jersey, 2009.
2. Glycochemistry; principles, synthesis, and applications; Wang, P. G., Bertozzi, C. R.; Marcel Dekker, Inc., New York, 2001.
3. Praveen, B., Shrivastava, P., Shrivastava, S. K.; In-Vitro release and pharmacological study of synthesized valproic acid-dextran conjugate; Acta Pharmaceutica Scientia, (2009), 51, pp. 169 – 176.
4. Vauthier, C. and Bouchemal, K.; Methods for the preparation and manufacture of polymeric nanoparticles; Pharmaceutical Research, (2008), 26, No. 5, pp. 1025 – 1058.

The information presented herein is derived from our testing and experience. It is offered for your consideration and verification. Since operating conditions vary significantly, and are not under our control, we disclaim all warranties on the results that may be obtained from the use of our products. W. R. Grace & Co.-Conn. and its subsidiaries can not be held responsible for any damage or injury occurring as a result of improper installation or use of its products. Grace reserves the right to change prices and/or specifications without prior notification.

ALLTECH® and REVELERIS® are trademarks, registered in the United States and/or other countries, of Alltech Associates, Inc. GRACE DAVISON DISCOVERY SCIENCES™ is a trademark of W. R. Grace & Co.-Conn. GRACE® and GRACE DAVISON® are trademarks, registered in the United States and/or other countries, of W. R. Grace & Co.-Conn. This trademark list has been compiled using available published information as of the publication date of this brochure and may not accurately reflect current trademark ownership or status.

Grace Davison Discovery Sciences is a product group of W. R. Grace & Co.-Conn. Alltech Associates, Inc. is a wholly owned subsidiary of W. R. Grace & Co.-Conn. © Copyright 2011 Alltech Associates, Inc. All rights reserved. Printed in the USA.