

Improved Purification of Carbohydrate Based Drugs in Glycochemistry Applications Using the Reveleris® iES Flash System

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Abstract

Carbohydrates are commonly found as constituents in plants, animals, and microorganisms. Carbohydrate units have considerable applications in medicine when bound to lipids, peptides, proteins, or even nucleic acids.^{1,2} Total synthesis of bioactive natural products, amino glycoside antibiotics, glycodrugs, and glyco-conjugate vaccines from carbohydrates have been of considerable interest in the medicinal field for a cure. Due to their therapeutic effects, conjugation of carbohydrates and their presence in antibiotics can be effectively used to find cures for the treatment of various diseases. Besides their clinical importance in glycomics, synthesizing and purifying such molecules has been a challenge to synthetic chemists.

Separation and identification of carbohydrate-based compounds is complicated due to their structure and poor detection. Such drawbacks from lack of chromophores, high sensitivity, and the compound's instability require a chromatographic system that is productive and efficient. As traditional flash chromatography is usually equipped with ultraviolet (UV) detection only, it fails to detect targets and impurities that are either present at low levels or lack chromophores. This study investigates the detection and purification of carbohydrates and glycodrug conjugates using RevealX™ detection technology, which incorporates multiple signal-processing from UV and ELSD (Evaporative Light Scattering Detection). Chemists can isolate and purify such compounds, which are non-chromophoric and used in medicinal drug discovery and development process, with speed and greater recovery.

Purpose

This study investigates the detection and purification of carbohydrates and conjugated carbohydrates using multiple signal-processing from UV and ELSD (Evaporative Light Scattering Detection). Using the RevealX™ detection technology in the Reveleris® flash chromatography system and the Reveleris® amino and reversed phase flash cartridges, chemists can isolate both chromophoric compounds and non-chromophoric sugars present in the sample matrix during a single run. Separations have been carried out to show the benefits of the amino cartridges for compounds that are polar and non-chromophoric that are not easily detectable with a traditional flash system equipped with UV detection only.

Experimental and Results

Flavanone Glycoside Purification with Reveleris® System Improves Isolation of Carbohydrates

Neohesperidin, naturally available from bitter orange, is an intensely bitter flavanone glycoside used for producing the non-sugar sweetening agent neohesperidin dihydrochalcones for diabetes related therapy.¹ It is known to have a strong synergistic effect in combination with other sweeteners, such as aspartame, xylitol, and saccharin, for controlling the sugar uptake in human body.

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

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

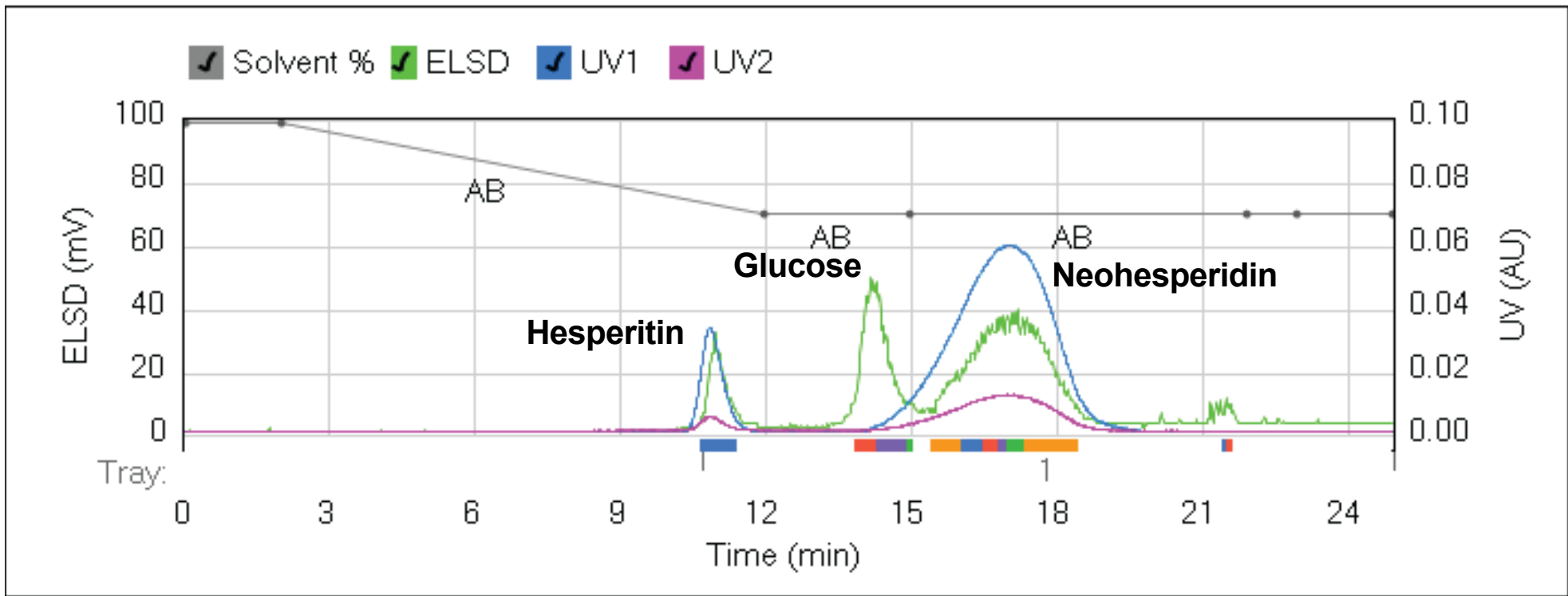
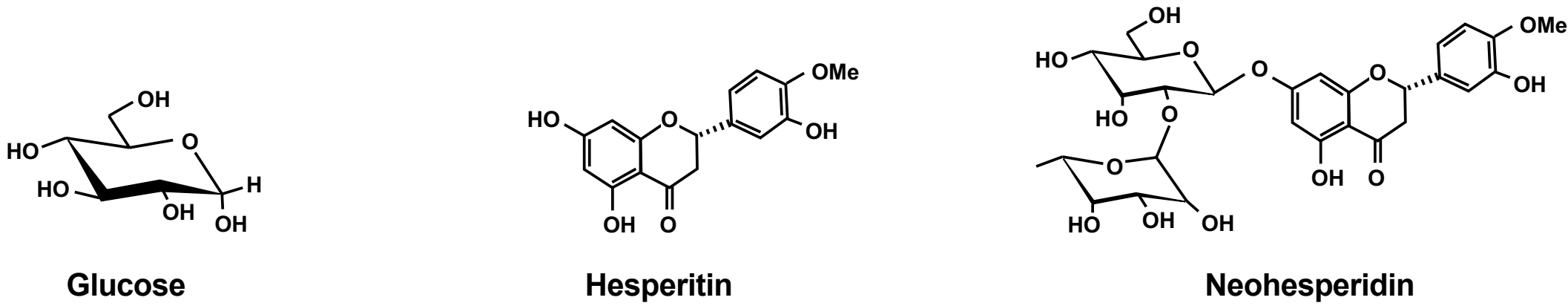
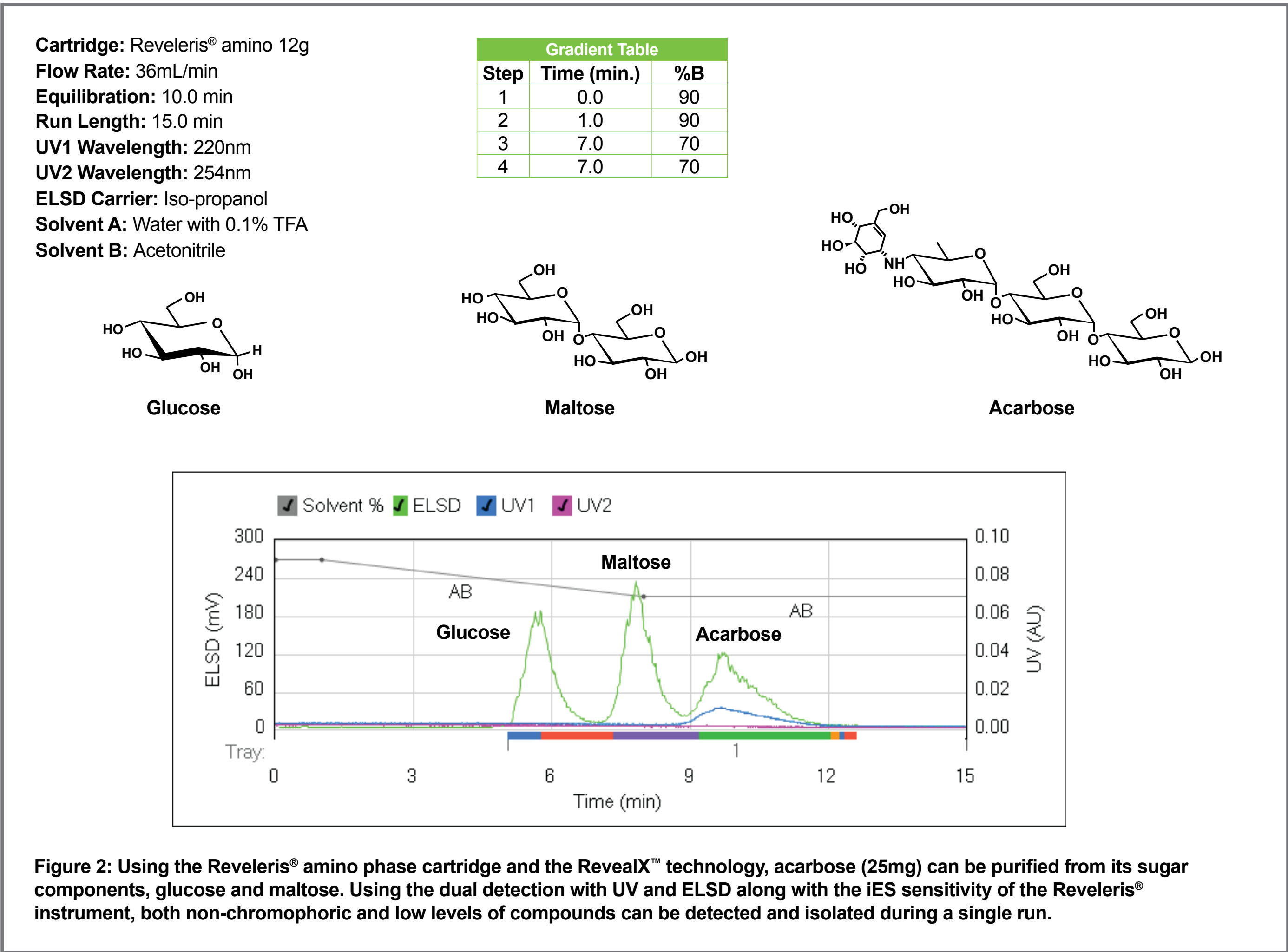


Figure 1: During the synthesis of neohesperidin, hesperitin and neo-hesperidose may be used as reagents. Using the Reveleris® system with RevealX™ technology and the high capacity Reveleris® amino cartridges, unreacted or even degraded reagents that may be undetected with UV detection can be isolated from the mixture.

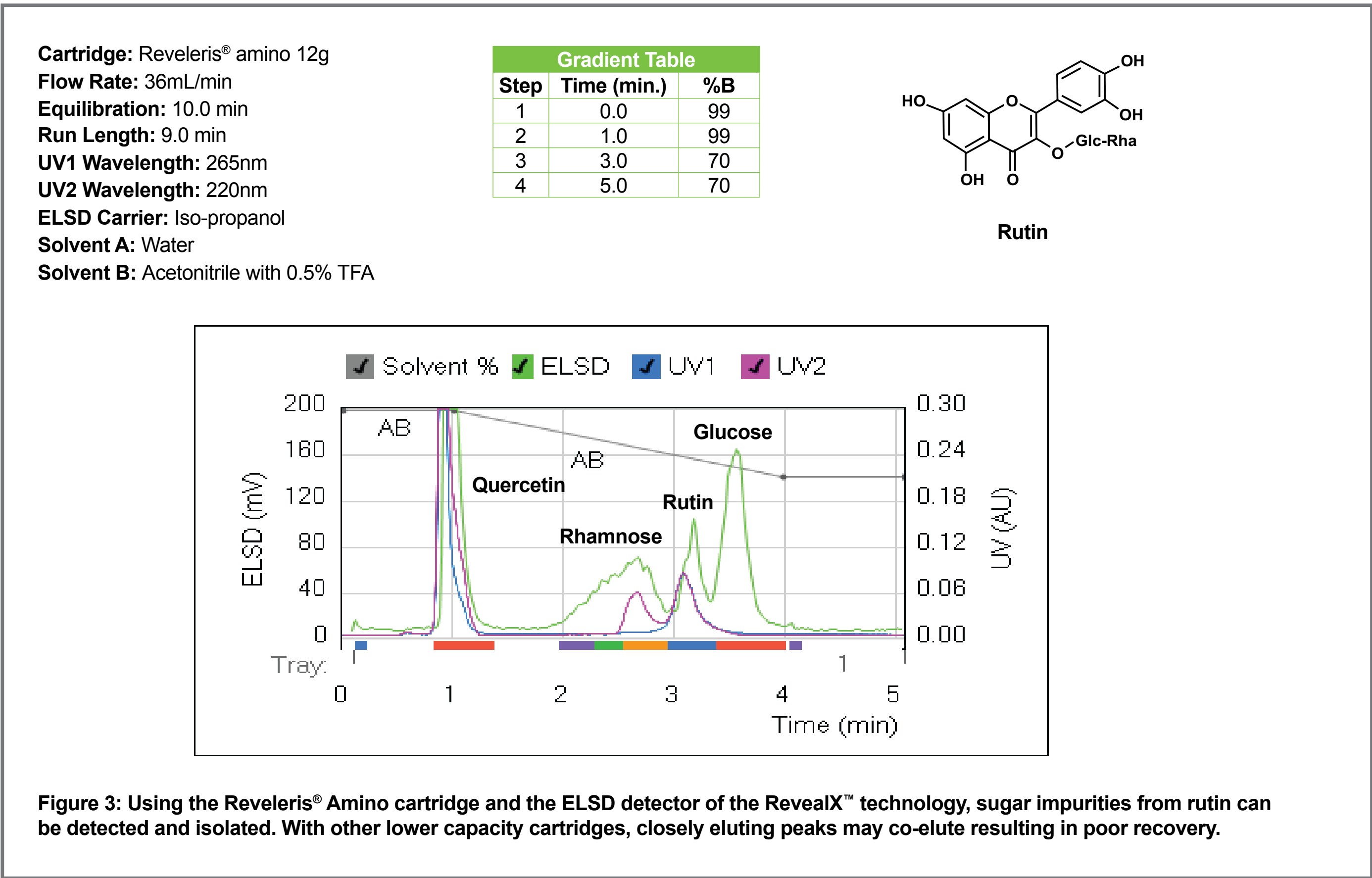
Reveleris® iES System Isolates Aminosugar, Acarbose, along with its Sugar Components

Acarbose, an inhibitor of α -glucosidase, is used in the treatment of diabetic patients due to its significant effect in lowering blood glucose.¹ Further development of this drug with voglibose than valienamine, leads to a more potent drug that has been found to be more effective as an enzyme inhibitor than acarbose.



Purification of Conjugated Quercetin with Rutinose using Reveleris® Flash System is Fast and Productive

Flavanol glycoside Rutin has been used in dietary supplements as Vitamin P for therapeutic treatments.¹ 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.

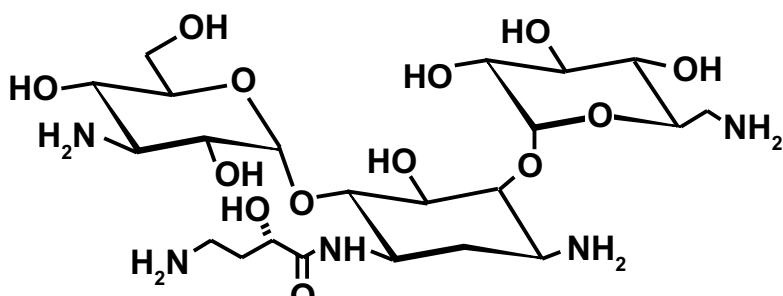


Isolation of Aminoglycoside Antibiotics in the Synthesis of Amikacin

Bacterial resistance to aminoglycoside antibiotics has been a problem leading to clinical failures.^{1,2} One such drug, kanamycin A, is a mixture of aminoglycosides and has been used as a precursor to its semi-synthetic acyl derivative, amikacin. The later when modified with 4-amino-2-hydroxybutyryl group helps to protect against the enzymic deactivation while maintaining its activity.

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



Amikacin

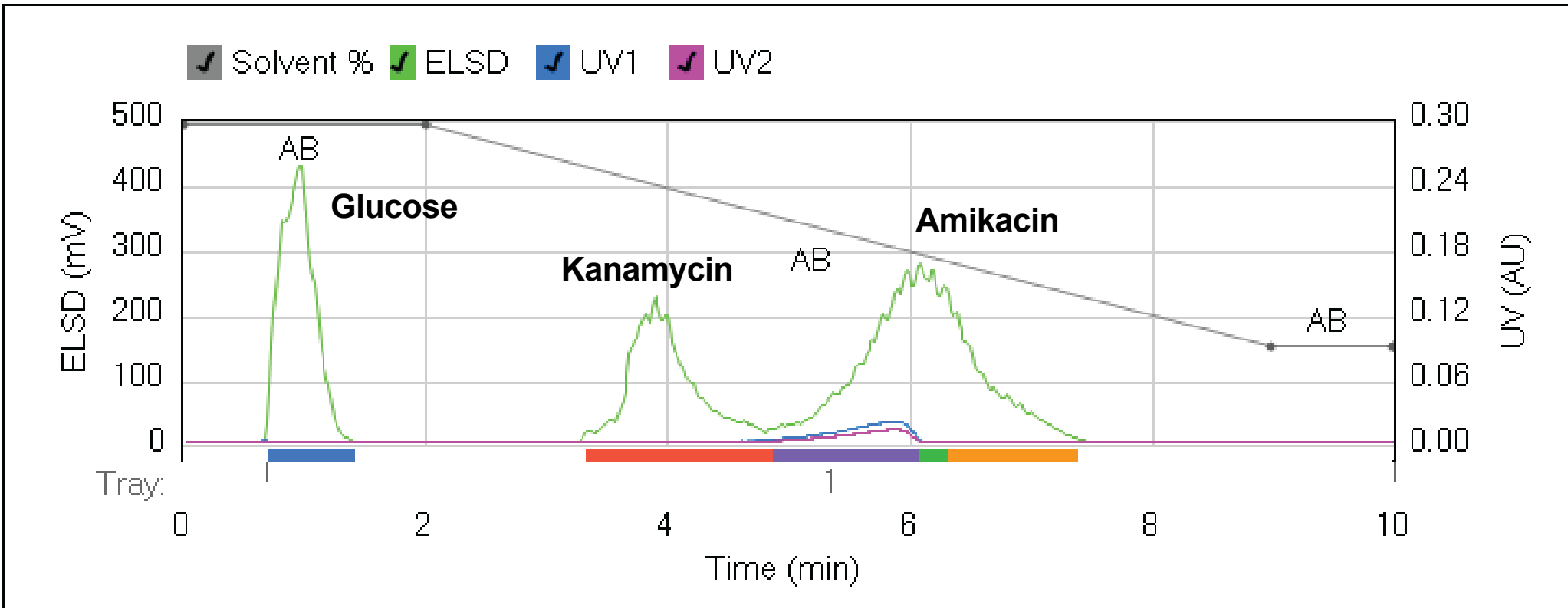


Figure 4: ELSD of RevealX™ technology detects the non-chromophoric compounds and helps to isolate kanamycin from amikacin and glucose present in the mixture. The separation was done using a C18 reversed phase Reveleris® cartridge with trifluoroacetic acid as the modifier in the mobile phase.

Purification of Catechols in the Presence of Non-chromophoric Sugars

Dopamine, a precursor to norepinephrine, is a natural catecholamine and a neurotransmitter in certain areas of the central nervous system. Its effect on the myocardium increases the heart rate and cardiac contractions. In combination with carbohydrate such as dextrose, dopamine hydrochloride may be used in treating myocardial infarction, trauma, renal failure, and even congestive heart failure.

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

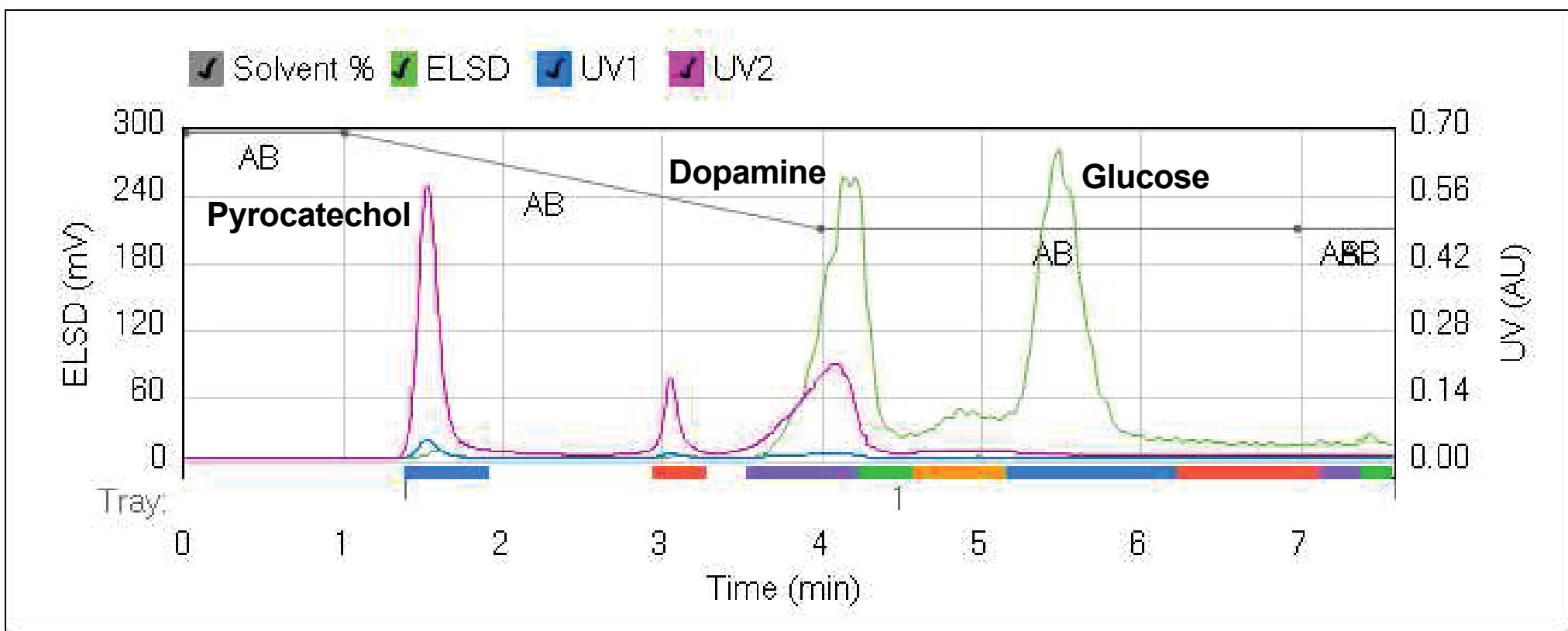


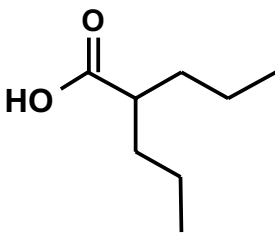
Figure 5: RevealX™ technology is able to isolate the non-chromophoric sugars such as glucose in the presence of dopamine and its precursor pyrocatechol.

RevealX™ Technology Can be Used in the Impurity Isolation of Valproic Acid from Cyclodextrin During Encapsulation and Bioavailability

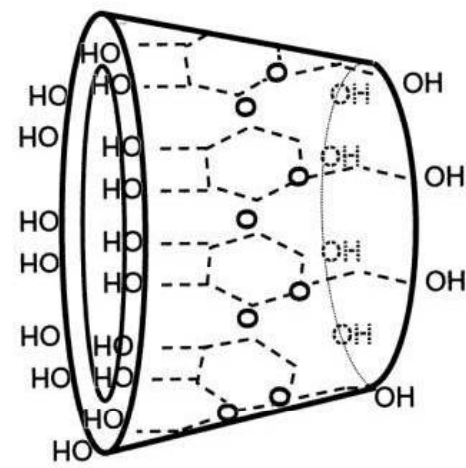
Valproic acid is one of the major antiepileptic drugs used in the treatment of different kinds of epilepsy.³ The drug has shown to be effective in treating cancer due to its antitumor properties. Although water is usually used as the reaction medium for enzymes, organic solvents or cyclodextrins have been added to improve the solubility of poorly soluble compounds. To increase its water solubility and bioavailability, the drug can be linked chemically by physical entrapment with polymers such as cyclodextrin or to polymeric carriers such as dextran.

Cartridge: Reveleris® C18 12g
Flow Rate: 36mL/min
Equilibration: 3.0 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	30
2	7.0	80



Valproic acid



γ-Cyclodextrin

Non-retention of polar compounds using standard reversed phase C18 media

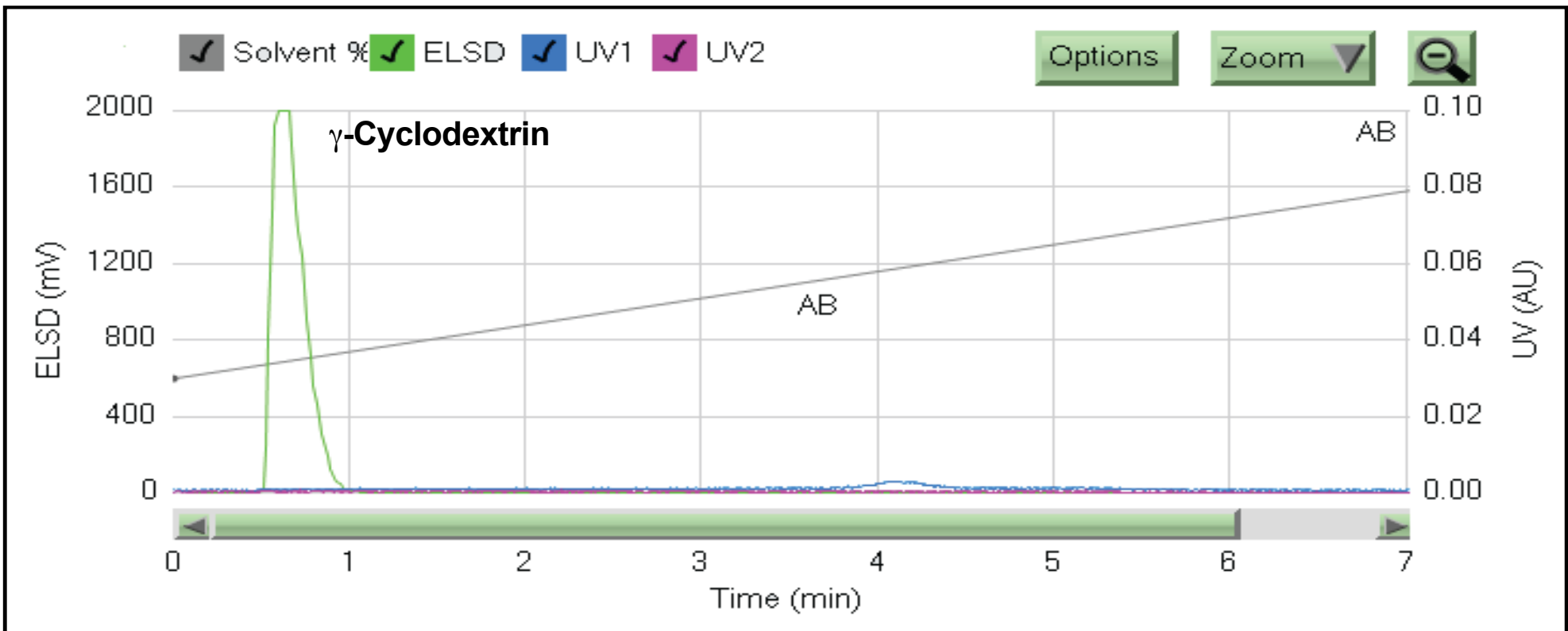


Figure 6: In this application, the separation of valproic acid from its entrapment-carrier, cyclodextrin, has been shown using the Reveleris® flash cartridge with the RevealX™ technology. Using a linear gradient with acetonitrile and water mobile phase, cyclodextrin is poorly retained on a reversed phase cartridge.

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

Improved purification of polar compounds, such as γ-Cyclodextrin, using high capacity amino phase media

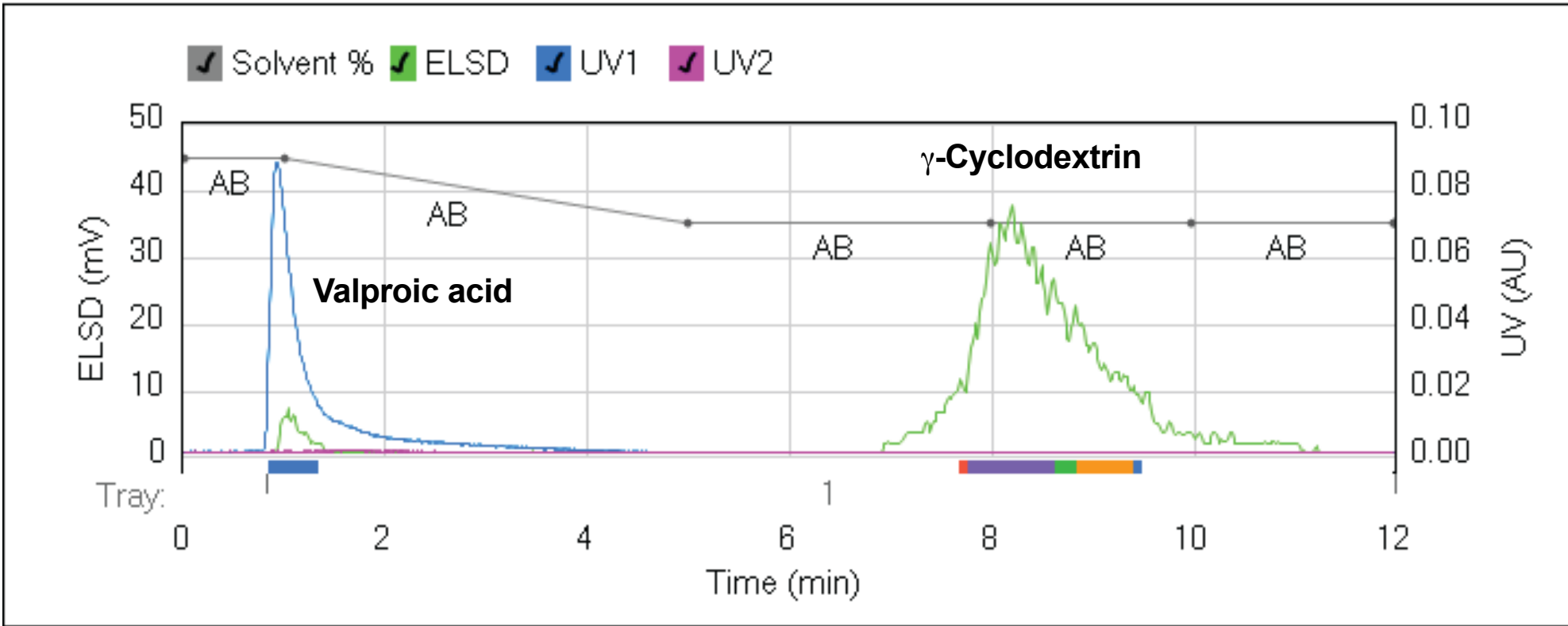


Figure 7: Compared to a reversed phase cartridge separation as shown where the retention is based on the hydrophobic interaction, the water soluble cyclodextrin has been retained and separated from the free valproic acid using an amino phase media cartridge. Amino phase cartridge retains carbohydrates that are unbound and may typically elute early on a standard reversed phase cartridge.

Polymer nanoparticles allow proper drug delivery and targeting with a wide range of properties under controlled conditions.⁴ As polymer technology plays a key role in overcoming drug delivery challenges for those undeliverable molecules, nanospheres and nanocapsules designed as drug carriers can be synthesized with drugs linked through encapsulation or conjugation for better target delivery. There needs to be a better purification technique for isolation of these compounds when the drug is poorly soluble in water while its carrier is a polar molecule. Hence such a method helps medicinal chemists isolate water based encapsulated drug from its impurity either as free or degraded and unbound compounds from the reaction mixture.

Conclusion

This work shows that by using high capacity amino and reversed phase flash cartridges along with universal RevealX™ detection, chemists can separate sugar compounds that are polar from their reaction mixture saving time and labor when doing glycochemistry.

Purification bottlenecks encountered when using flash chromatography can be eliminated by using the RevealX™ detection technology of the Reveleris® iES system, which independently triggers fraction collection from multiple detectors including UV and ELSD. Such a system is designed to detect and collect compounds for scientists whose applications demand increased lab productivity during purification of carbohydrates and glycodrugs in the drug discovery and development process.

Using a non-chromophoric detector along with UV detection can help eliminate guesswork for the user during purification of carbohydrates, saving time and sample. Isolating larger quantities of the target compound is fast with higher purity and recovery, when compared to traditional flash chromatography systems.

References

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