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Glycan Analysis using the ACQUITY UPLC H-Class Bio System

Waters Corporation

This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application brief demonstrates the successful characterization of the *N*-glycans released from fetuin using the ACQUITY UPLC H-Class Bio System in HILIC mode along with the flexibility of the novel solvent mixing technology, Auto▪Blend Plus.

Benefits

Robust analyses of the glycans of glycoproteins can be readily developed with the ACQUITY UPLC H-Class Bio System featuring Auto●Blend Plus Technology and Glycan Separation Technology Columns.

Introduction

The high complexity of glycan structure is related to its synthesis and its biological role. A complete glycosylation profile is required for various regulatory purposes. Due to the biological significance of glycoproteins, glycan analysis needs rapid, efficient, sensitive, and reproducible methods. The analysis of glycans involves efficient separations of their constituents, including *N*- and *O*-glycans.

Depending on the nature of the glycan and whether mass spectrometry detection is to follow its chromatographic separation, mobile phase formulations require adjustments. A large degree of flexibility is desired when selecting among different modifiers and ionic strengths during optimization of gradient conditions. Optimizing glycan separation conditions using a binary solvent system can be a very time-consuming process since many mobile phase compositions are used in search for the required selectivity and resolution. The parameters available for adjustment include identity and concentration of the organic component of the mobile phase as well as the pH and ionic strength.

These experiments are time-consuming and tedious, and errors can occur during preparation of the numerous mobile phases through the method development process. The quaternary-based ACQUITY UPLC H-Class Bio System, with Auto▪Blend Plus Technology, allows automatic programming of the solvent composition from up to four stock solvents, saving both preparation and analysis time while increasing reproducibility.

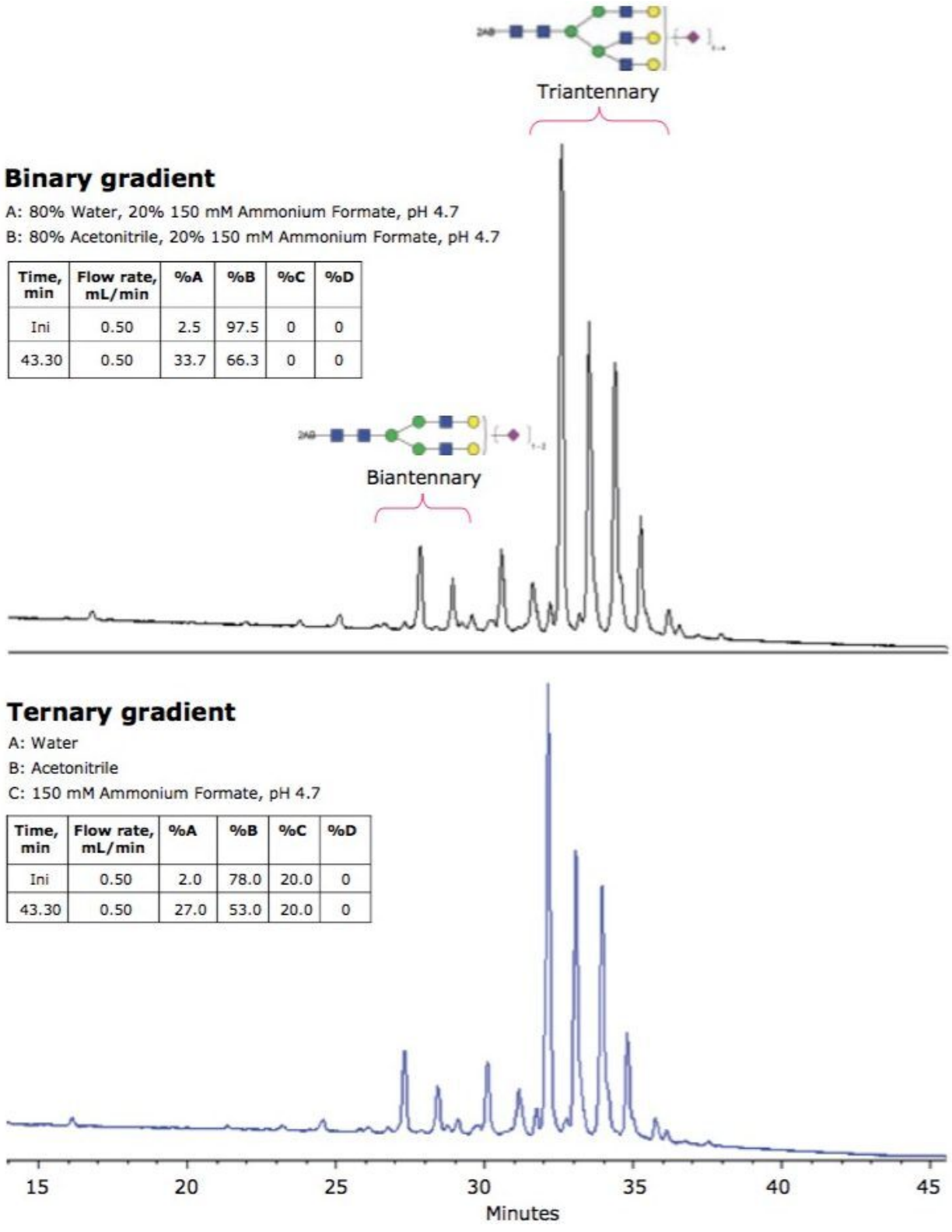
Results and Discussion

Waters Glycan Separation Technology Column chemistry, used with sub-2-µm UPLC Technology and fluorescence (FLR) detection, provides an efficient separation of protein glycans utilizing hydrophilic interaction liquid chromatography (HILIC). In HILIC mode, the oligosaccharides are retained by polar interaction and the elution is realized by the aqueous gradient.

To demonstrate the additional flexibility provided by the ACQUITY UPLC H-Class Bio System and Auto•Blend Plus Technology, this configuration was used for the characterization of the *N*-glycans isolated from fetuin and labeled with a 2-aminobenzamide (2-AB) fluorescent tag. These analytes have been separated using a high-efficiency separation with a binary gradient. This method was transferred to ternary gradient conditions. Selectivity and resolution for glycans of fetuin were identical with a ternary gradient that was generated using Auto•Blend Plus Software.

Solvent C was selected as a modifier and its concentration remained constant throughout the gradient. 150 mM ammonium formate, pH 4.7, was used as solvent C buffer, whereas pure solvents were chosen for solvent A (water) and solvent B (acetonitrile). This system configuration provides an easy approach for optimization of gradient conditions. Changing the proportion used from solvent C is all that is required for the user to introduce modifications of mobile phase composition that affect selectivity and resolution. By using Auto•Blend Plus, we can program the system to test different modifier compositions, ionic strengths, and pH to achieve the best separation conditions commonly explored during glycan analyses.

The ACQUITY UPLC H-Class Bio System provides robust, highly resolving, reproducible, and rapid separations using ternary gradient conditions as developed with Auto•Blend Plus Technology.



2-AB labeled glycans released from fetuin were separated using a standard binary HILIC method. The same separation was obtained in a ternary, Auto•Blend Plus mode where mobile phase preparation is easier and

less error-prone. The ternary method also provides an easier protocol for optimizing the method for a particular sample.

Conclusion

The ACQUITY UPLC H-Class Bio System was successfully applied to the separation of protein glycans. By allowing the biochemist to work with stock solutions to manipulate gradient compositions, Auto▪Blend Plus Technology offers a large degree of freedom to control selectivity, optimize the gradient, and manage the modifier composition independently from the rest of the solvents. This approach saves a significant amount of time for the laboratories performing glycan characterization. The high separation efficiency and reliability of ACQUITY UPLC H-Class Bio System with Auto▪Blend Plus Technology simplifies method development and provides overall laboratory productivity and efficiency.

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ACQUITY UPLC H-Class PLUS Bio System <https://www.waters.com/10166246>

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