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# Reductive Amination (2-AB and 2-AA) Dextran Calibration Ladder Standards

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## I. INTRODUCTION

Hydrophilic Interaction Liquid Chromatography (HILIC) coupled with Fluorescent Detection is widely used for fluorescent (FLR) labeled glycans. The ACQUITY UPLC® BEH Glycan Column operated in HILIC mode shows drastic improvement in separation e.g., peak resolution and the ability to separate both neutral and acidic glycans, compared to the conventional HPLC methods in HILIC mode (Ref. 1). The ACQUITY UPLC BEH Glycan Column enables scientists to get robust and reproducible glycan separation data with less time spent in method optimization.

In order to take full advantage of the BEH glycan column, Waters® created the 2-aminobenzamide (2-AB) and 2-aminobenzoic acid (2-AA) labeled Dextran Calibration Ladder Standards to assist glycan profiling: glycan profile obtained from UPLC-HILIC/FLR system can be calibrated against the 2-AB or 2-AA labeled dextran ladder and assigned with glucose unit (GU) values (Ref.2). The 2-AB and 2-AA labeled Dextran Calibration Ladder Standards are different than existing commercial offerings. The average molecular weight of the glucose homopolymer is higher (~4,500 Dalton), therefore, the "workable" GU value range is twice as much as other dextran ladder standards; the observed GU goes from 2 to 30. This feature improves the large glycan retention time assignment. The purity and structural integrity of the ladder is assessed by normal-phase HPLC and MS.

## II. STORAGE AND STABILITY

The lyophilized powder is shipped at ambient temperatures, but it is highly recommended upon receipt of the standard to store it refrigerated (4°C) until the expiration date printed on the label. Once reconstituted in should be used immediately for best results.

## III. RECONSTITUTION OF THE DEXTRAN CALIBRATION LADDER

One vial of the 2-AB and 2-AA Dextran Calibration Standards contain 200 µg of a lyophilized, solid powder. It is contained in a Waters Max Recovery Vial for supreme ease of use so that it can be directly solubilized and put on the system for analysis and injection.

For reconstitution, the sample can be diluted with 100 µL of Milli-Q water plus 100 µL of acetonitrile for a 200 µL total dilution (1µg/µL).

## IV. BENEFITS OF USING A CALIBRATION STANDARD

Each individual glycan structure has a GU value which is directly related to the number of linkage of its constituent monosaccharides. The GU value can be used to predict structures because each monosaccharide in a specific linkage adds a given amount to the GU value of a given glycan.

The elution times of glycans are expressed in glucose units (GU) by reference to a dextran ladder. This ladder is used to calibrate the LC runs against day-to-day or system-to-system changes. The GU value is calculated by fitting a fifth order polynomial distribution curve to the dextran ladder, then using this curve to allocate GU values from retention times. The GU values for neutral N-glycans are very reproducible with standard deviations of <0.3 between columns, this allows direct comparison with database values collected from a range of instruments over a period of time (Ref. 3).

## V. EXAMPLES OF USING THE DEXTRAN CALIBRATION LADDER ON THE ACQUITY UPLC

Below is a reference chromatogram using the 2-AB Dextran Calibration Ladder as an example with conditions to help provide an example of the chromatogram. If the column size is different, the gradient/ injection volume can be scaled accordingly.

LC system conditions:

| | |
|---|---|
| Injection volume: | 1.5 µL |
| Injection mode: | Partial loop needle overfill |
| Column: | ACQUITY UPLC Glycan BEH Amide Column, 1.7 µm, 2.1 x 150 mm (p/n 186004742) |
| Eluent A: | 100 mM ammonium formate buffer pH 4.5 |
| Eluent B: | Acetonitrile |
| Weak needle wash: | Acetronitrile/HPLC grade water, (90:10 v/v) |
| Strong needle wash: | Acetronitrile/HPLC grade water, (10:90 v/v) |
| Seal wash: | Acetronitrile/water (50:50 v/v) |
| Column temp.: | 60 °C |
| Detection: | Fluorescence: λex= 330 nm, λem= 420 nm |

Gradient profile:

| Time | Flow rate (µL/min) | %A | %B | Curve |
|---|---|---|---|---|
| initial | 0.50 | 25 | 75 | 6 |
| 46.5 | 0.50 | 50 | 50 | 6 |
| 48 | 0.25 | 100 | 0 | 6 |
| 49 | 0.25 | 100 | 0 | 6 |
| 50 | 0.50 | 25 | 75 | 6 |
| 63 | 0.50 | 25 | 75 | 6 |

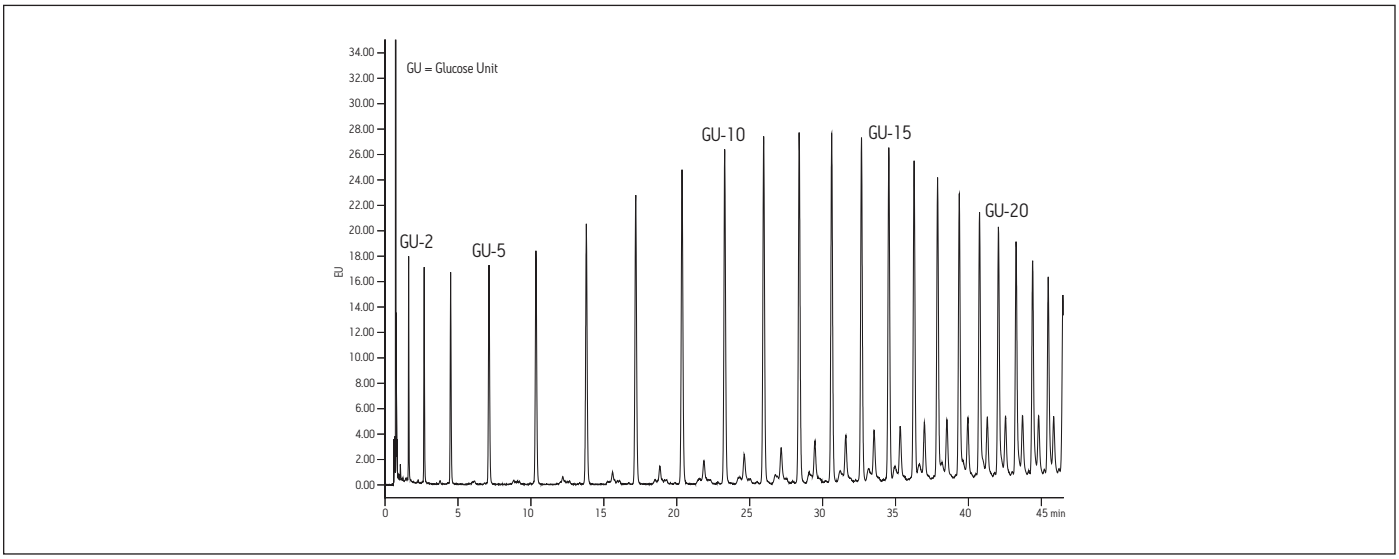


*Figure 1: Example Chromatogram of the 2-AB Dextran Calibration Ladder with glucose units (GU) labeled peaks scaled from 0.8 – 46.5 minutes.*

## IV. ORDERING INFORMATION USE HEADER 1

| Product Description | Part Number |
|---|---|
| 2-AB Dextran Calibration Ladder | 186006841 |
| 2-AA Dextran Calibration Ladder | 186007279 |
| ACQUITY UPLC Glycan BEH Amide Column, 1.7 µm, 2.1 x 50 mm | 186004740 |
| ACQUITY UPLC Glycan BEH Amide Column, 1.7 µm, 2.1 x 100 mm | 186004741 |
| ACQUITY UPLC Glycan BEH Amide Column, 1.7 µm, 2.1 x 150 mm | 186004742 |
| ACQUITY UPLC Glycan BEH Amide VanGuard Pre-column, 1.7 µm, 2.1 x 5 mm, 3/pkg | 186004739 |

### References


1. Joomi Ahn, Jonathan Bones, Ying Qing Yu, Pauline M. Rudd, Martin Gilar. "*Separation of 2-aminobenzamide labeled glycans using hydrophilic interaction chromatography columns packed with 1.7 µm sorbent*", Journal of Chromatography B, 878 (2010) 403–408.

2. Matthew P. Campbell, Louise Royle, Catherine M. Radcliffe, Raymond A. Dwek and Pauline M. Rudd. "*GlycoBase and autoGU:tools for HPLC-based glycan analysis*", BIOINFORMATICS APPLICATIONS NOTE, Vol. 24, no. 9 2008, 1214-1216.

3. Geoffrey R. Guile, Pauline M. Rudd, David R. Wing, Sally B. Prime, Raymond A. Dwek. "*A Rapid High-Resolution High-Performance Liquid Chromatographic Method for Separating Glycan Mixtures and Analyzing Oligosaccharide Profiles*" Analytical Biochemistry, 240 (1996) 210-226.


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