INVESTIGATION OF OPTIMAL HYDROPHILIC INTERACTION CHROMATOGRAPHY CONDITIONS FOR LABELED GLYCAN SEPARATION USING SUB 2 µm UPLC SORBENT

Joomi Ahn, Ying Qing Yu, Martin Gilar, and John Gebler
Waters Corporation; Milford, MA

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

OVERVIEW

- This study is focused on optimization of Hydrophilic Interaction Chromatography (HILIC) for separating labeled glycans using a UPLC-fluorescent system.
- Optimal HILIC conditions were determined using columns packed with 1.7 µm amide sorbent.
- Fast and highly resolved separations of labeled glycans released from human IgG and Ribonuclease B were achieved based on the obtained condition.
- This study provides a useful model to predict retention behavior of labeled glycans for best peak capacity and resolution.

INTRODUCTION

In this work we investigated the performance of a novel glycan column packed with 1.7 µm HILIC amide sorbent (prototype). Utilizing the peak capacity theory *(1)* and pseudo Van Deemter curves *(2)*, we measured the dependency of peak volumes and peak widths at several different flow rates and temperatures. We optimized the HILIC LC conditions to achieve the best peak capacity and resolution of labeled glycans using a 2AB-labeled glucose ladder standard. The obtained optimal conditions were utilized for fast and efficient separation of 2AB-labeled, N-linked glycans, released from human IgG and Ribonuclease B.

Better resolution and shorten analysis time were achieved with the 1.7 µm UPLC HILIC columns compared to the conventional 3.0 µm HPLC columns. The 1.7 µm sorbent is packed into 50 and 100 mm long columns with 2.1 mm internal diameter. Optimum column efficiency and peak capacity was achieved within operating pressures achievable up to 15, 000 psi with a commercially available ACQUITY® UPLC-FLR system.

(1) Peak Capacity (P) is directly calculated for measuring peak space between neighboring glucose units (GU).

$$P = 1 + \frac{(t2 - t1)}{(w2 + w1)/2}$$

t_n = retention time of peak
w = peak width at 50 % of peak height

(2) Pseudo Van Deemter model was used to predict the optimal mobile phase flow rate and the dependency of analyte sizes (MW). Because the isocratic measurement of the theoretical plate heigtht (H) for biopolymers such as glycans using Van Deemter curve is difficult, the application of peak capacity theory employing a gradient elution mode was used to plot a "Pseudo" Van Deemter curve.[1] The relation of peak volume (P_v) and H is proportional and H is inversely proportional to column efficiency (N). Therefore, the miniumum P_v^2 measured in Pseudo Van Deemter curve indicates the maxium column efficiency. P_v is measured by peak width and its retention time as described below.

$$P_v^2 = (w \times T_n)^2$$

$$H = const.(P_v)^2$$

$$N = L / H$$

L = column length

METHODS

LC condition

Waters ACQUITY UPLC™ System
Waters ACQUITY UPLC prototype glycan columns, 1.7 µm (and 3.0 µm), 2.1 x 50 and 100 mm
Mobile phase A : 100 mM ammonium formate pH 4.5
Mobile phase B : Acetonitrile
Flow : From 0.10 mL/min. to 1.0 mL/min.
Gradient : 70—40 % B for dextran ladder
75-65 % B for IgG glycans
70-60 % B for ribonuclease B glycans
Gradient time : **From 15 min. to 30 min.**
Gradient slope : From 0.8 to 8.0 % B /min.
Column Temperature : 40 and 60 °C
Weak wash : 30 % buffer A / 70 % buffer B
Strong wash : 80 % buffer A / 20 % buffer B

Waters ACQUITY UPLC Fluorescent Detector
Excitation at 360 nm, Emission at 420 nm, Scan rate at 5 Hz

Samples

2-AB labeled glucose ladder standard prepared in house
2-AB labeled glycans released from Human IgG (ProZyme)
2-AB labeled glycans released from ribonuclease B prepared in house

Waters ACQUITY UPLC system with Fluorescent detector

RESULTS

PEAK CAPACITY

Figure 1. **Flow rates varied with fixed gradient slope** (2.0%B/min.) for 2-AB labeled glucose standard separation

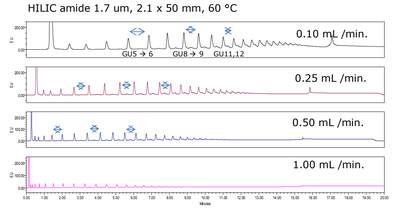


Figure 2. Peak capacity curve of glucose units at **40 and 60˚C column temperatures**

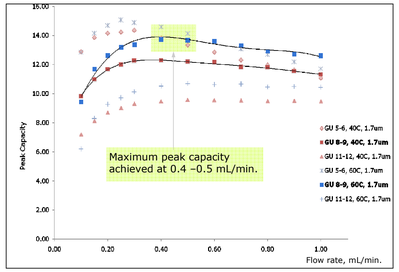
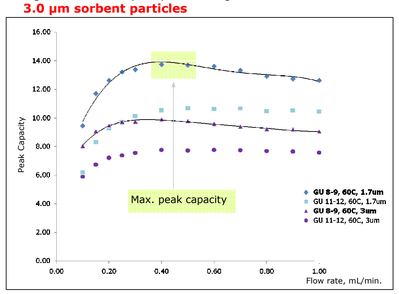


Figure 3. Peak capacity curve of glucose units for **1.7 and 3.0 µm sorbent particles**



EFFICIENCY

Figure 4. **Gradient slope varied with fixed gradient volume** (3.75 mL/min.) for 2-AB glucose standard separation

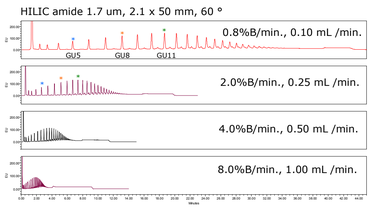


Figure 5. Column efficiency of glucose units at **40 and 60 °C column temperatures**

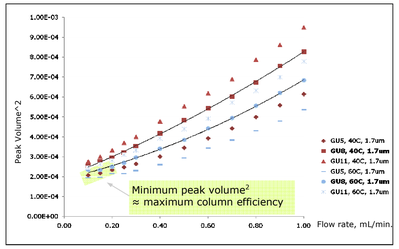


Figure 6. Column efficiency of glucose units for **1.7 and 3.0 µm sorbent particles**

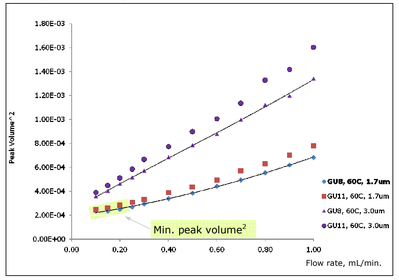


Figure 7. **2-AB labeled glycans released from human IgG** in optimized HILIC condition achieving maximum peak capacity and resolution

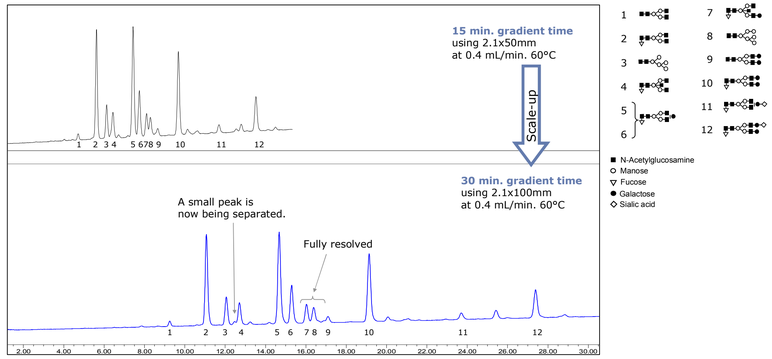
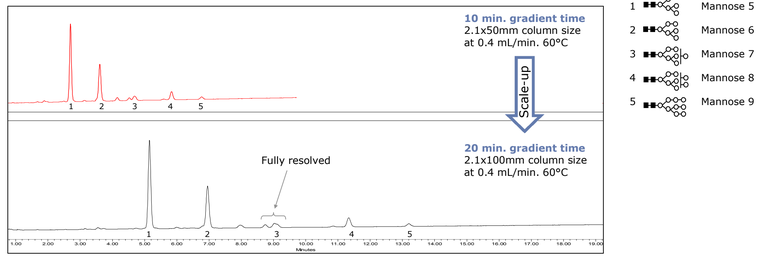


Figure 8. **2-AB labeled glycans released from ribonuclease B** in optimized HILIC condition achieving maximum peak capacity and resolution



CONCLUSION

- HILIC amide column packed with 1.7 µm sorbent achieved superior separation of labeled glycans using a UPLC-FLR system.
- Better resolution and shorten analysis time were achieved using optimal HILIC condition.
- Predicting retention time behavior of glycans in HILIC mode will lead to the improvement of peak capacity and resolution in LC-FLR method development.

References : 1. Gilar and Neue, Journal of Chromatography A, 1169(2007) 139-150

TO DOWNLOAD A COPY OF THIS POSTER, VISIT WWW.WATERS.COM/POSTERS