APPLICATION BRIEF 22014

Reverse phase analysis of free complex glycans

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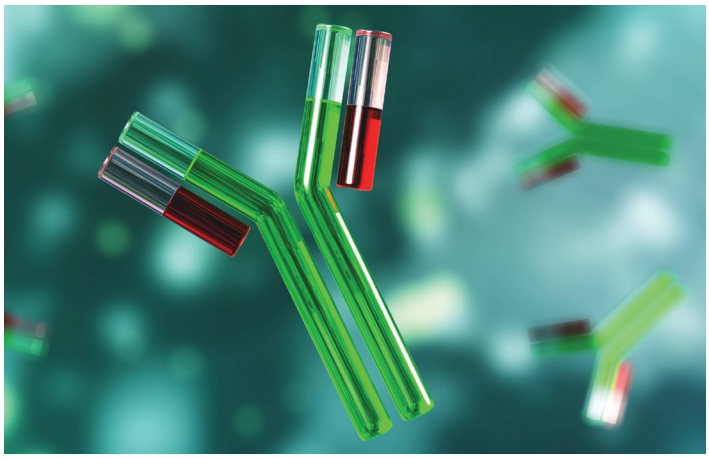
Introduction

In the last few years, biopharmaceutical analysis of complex glycans released from native proteins or protein/antibody based drugs (removal by PNGase F) has become a standard step in full protein characterization. This step involves the separation of the glycans and analysis by high resolution accurate mass analysis or matching retention times to known standards by UV/CAD/Fluorescence. Thermo Fisher Scientific has three powerful columns for glycan analysis: Thermo Scientific™ GlycanPac™ AXH-1 and Thermo Scientific™ Accucore™ Amide-HILIC for performing hydrophillic interaction chromatography and the Thermo Scientific™ GlycanPac™ AXR-1 a reverse phase column.

The GlycanPac AXR-1 discussed here is a bi-modal column with both anion-exchange and reversed phase characteristics. Alternatively, the Amide-HILIC is an excellent choice for all monoclonal antibodies and biologics with neutral glycans, which can not take advantage of the mixed mode anion exchange capabilities. This mixed mode separation initially allows for a gross separation by charge (taking advantage of sialic acids when possible) then a further fine separation of the different charged species by their differences in hydrophobicity. If mass spectrometry is used, then traditional labeling for fluorescence using 2-AB is not required.



Materials required

- Ammonium formate, 99%, ACROS Organics
- Formic Acid, 99.0+%, Optima™ LC-MS grade, Fisher Chemical™
- Water, Optima™ LC-MS grade, Fisher Chemical
- Acetonitrile, Optima™ LC-MS grade, Fisher Chemical
- High resolution mass spectrometer such as the Thermo Scientific™ Orbitrap Exploris™ 480 or Thermo Scientific™ Orbitrap Eclipse™ Tribrid™ Mass Spectrometer
- Binary or Quaternary HPLC system such as the Thermo Scientific™ Vanquish™ Horizon UHPLC System
- GlycanPac AXR-1 column, 2.1 × 150 mm, 1.9 µm (P/N 088136)
- QABio Fetuin N-Linked Glycan Library (P/N CLIBN-FETUIN-01, optional)
- Thermo Scientific™ SimGlycan™ software (P/N IQLAAEGABSFAMMMAZU, optional) and Thermo Scientific™ FreeStyle™ software

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Protocol

1. Prepare mobile phase A (7 mM Ammonium formate, pH 4.4) by adding 0.44 g of ammonium formate into 1 L of water. Stir and titrate using a pH meter to pH 4.4 using formic acid.
2. Prepare mobile phase B (75 mM Ammonium formate, pH 4.4 in 25% acetonitrile) by adding 4.73 g of ammonium formate to 750 mL of water. Add 250 mL of acetonitrile. Stir and titrate to pH 4.4 with a pH meter using formic acid.
3. Calibrate and tune the mass spectrometer in negative ion mode for a flow rate of 400 µL/min.
4. Build a liquid chromatography-mass spectrometry (LC-MS) method using a linear gradient from 0–100% B. For simple mixtures like the glycans of a single monoclonal antibody, the gradient could be as short as 10 minutes. For more complex samples like a cell lysate, the gradient can be extended to 90 minutes. The mass spectrometer should be in negative mode scanning from 300–2000 *m/z*.
5. Inject 100 pmol of glycans as a starting point. The fetuin standard is a good beginning point when first starting. Adjust parameters based on the mass spectrometer's signal response. Once the full MS scan has been observed, a background can be established, and data dependent scanning can be setup. A few injections may be needed to find optimal collision energies for identification.
6. The resulting mass spectrometer. RAW files can be processed using SimGlycan and Freestyle.

Related products

Description	Part number
Gibco™ PNGase F Glycan Cleavage Kit, for removal of the glycans	A39245
Thermo Scientific™ SOLA™ WAX 96 well SPE plate, 10 mg/2 mL; 1 pack, for cleanup of glycans	60309-005

Current versions of product instructions are available at **thermofisher.com/chromexpert**

Learn more about Bio LC columns and products at **thermofisher.com/biolc**

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