BAE HPLC Standard Operating Procedure: P Column Addendum
Last Updated: 12 December 2014

READ & UNDERSTAND THE PRIMARY HPLC SOP BEFORE BEGINNING

HPLC Instrument Information

1. Instrument: Dionex-Thermo Fisher Ultimate 3000 auto-sampler, P-680 pump, TCC-100 Column Compartment, Variable Wavelength Detector (UV); Shodex R01 Refractive Index detector
2. HPLC #1: Analytical column Bio-Rad Aminex HPX-87P (order #125-0098); Guard columns are Bio-Rad Micro-Guard De-ashing pair (cation & anion order #125-0139) & Carbo P guard column (order #125-0119). From the pump, the guard columns should be cation, anion, carbo P, and then the analytical column. The column operates at 80C
3. The 87P is a lead-form column tailored for the separation of cellulose-derived monosaccharides. It is used in analyses of pentoses and hexoses in wood products, especially cellobiose, glucose, xylose, galactose, arabinose, and mannose. It also provides excellent resolution of sucrose, lactose, and fructose in dairy products.

HPLC Operating Procedure

A. Mobile Phase & Sample Injection Volume

1. Flow rate: 0.6L/min, no gradient (isocratic), 45-70 minute run
2. Mobile phase is deionized and filtered water.
3. The standard sample injection volume is 20µL Suggest using at least 600uL in sample vial

B. Working Buffer & Flush Water Preparation for the 87P Column

1. The working buffer (mobile phase) shall be HPLC grade water. Use deionized water filtered through a 0.2 micron filter. The HPLC pump handles de-gassing.

C. ROUTINE INSTRUMENT MAINTANENCE

Note: Refer to the equipment manuals for in-depth maintenance requirements including routine and periodic maintenance for the instruments and analytical columns. See the attached Bio-Rad Use & Care guide for both analytical and guard columns (BRUC).

I. Guard & Analytical Columns

Note: Follow the primary SOP for Guard and Analytical Columns. The following bullets are specific to the 87P column:

1. Always P columns are not typically regenerated and cleaned successfully, thus it is very important to avoid contamination. See the BRUC for regeneration/cleaning solvent recommendations
2. If the resolution of standards has degraded:
    a. Run a standard over the column alone to see if one of the guards is causing the loss of resolution; if it is not a guard column causing the loss, try backwashing the column at 0.1 to 0.2 ml/min for an hour or two. This will "fluff" up the resin and fill any slight void and possibly improve resolution.
    b. If the loss is due the guard columns, reconnect one type at a time to isolate the problem source and replace the bad guard.
3. When the pressure increases and shuts down the pump, stop the analysis. Identify the source of the pressure increase by isolating the guard columns (de-ashing pair & carbo P) in succession and comparing the starting pressures recorded in the logbook; replace the failed guard column(s). If in doubt, it is preferable to replace all 3 guard columns.