

PSR SCX Fractionation Method

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Reagents

No Salt, Buffer A: 0.4% Formic Acid (FA) 25% Acetonitrile (ACN)

High Salt, Buffer B: 300mM Ammonium Formate (AF) 0.4% FA, 25% ACN

Final Elution Buffer: 500mM AF, 0.4% FA, 25% ACN

Cleaning Buffer: 0.5M KCl, 25% ACN, 0.4% FA

Storage Buffer: 25% ACN in deionized water

Salt steps

Salt steps are made by combining different amounts of the Buffer A and Buffer B to vary the concentration of AF. PSR will adjust these as necessary for a project, however the most common AF step series is: 20mM, 25mM, 30mM, 50mM, 75mM, 100mM, 200mM, 300mM (Buffer B), 500mM (Final Elution Buffer).

Sample and Column Preparation

Dry down sample and bring up in at least 100 μ L of Buffer A. Shake at 37 degrees for 10 minutes to re-suspend. It's important that the final pH be less than 3, and there are no interfering cations present in the sample.

Wash column with 0.3 mL of Cleaning Buffer

Wash column with 0.3 mL of Buffer B

(2x) Wash column with 0.3 mL of Buffer A

Sample Loading and Elution

Sample and all salt bumps should be applied slowly; the Harvard Syringe Pump is typically used at a flow rate of 100 μ L/min for this part of the procedure.

Load sample onto column while collecting the flow through.

Load the flow through onto the column, saving this second FT fraction.

Load 0.3 mL of each salt step; and collect the eluted material from each step into its own tube.

Post Elution Cleanup

Load 0.3 mL of the Cleaning Buffer, if it wasn't used as a final salt bump.

(2x) Load 0.3 mL of Storage Buffer, cap the ends of the column with parafilm and store at room temperature.

Collected fractions should be taken to dryness in the evaporator, before resuspension in 5% FA and analysis on the mass spectrometer.