

Agilent Purification System, LAB EXERCISE

LAB TITLE

Getting familiar with the creation of Load & Lock Columns.

Lab Objectives

The purpose of this LAB is to familiarize yourself with the Load & Lock Columns and systems, by packing your own high performance Preparative LC Column.

The LAB is divided into three parts:

- 1) Create the slurry
 - 2) Pack the Prep Column
 - 3) Test the Prep Column
- Appendix A: Composition of Slurry

LAB 6 – Load & Lock Columns

1) Create the Slurry

- a) Calculate the amount of media/sorbent, required for our column (2" x 25cm).

Media Density: $\rho = \frac{\text{gram}}{V_{\text{col}}}$

Column Volume: $V_{\text{col}} = \left(\frac{ID}{2}\right)^2 \times \pi \times \text{bedlength}$

Silica Mass: $G = V_{\text{col}} \cdot \rho$

- b) Weigh out the calculated Silica Mass in a beaker.
- c) Measure out the calculated volume of IPA in a cylinder
- d) Prepare the slurry by mixing the C18 media with the slurry solvents (IPA) to produce a slurry volume as described in the table in appendix A.

Note: The weight of the media indicated is usually sufficient, but it can vary with the density of the media. Packing density of most C18 bonded silica based media's is around 0.60 g / mL. (Check with below table!)

2) Pack the Prep Column

IMPORTANT: Use the Manual, slides and any other instructions in this LAB

- a) Disperse the media in the slurry solvent.
- b) Make sure that the media is free of lumps (shaken) or as recommended, ultrasonicate the slurry for approximately 5 to 10 minutes.

Note: Do not use a magnetic stirrer as this will pulverize or crush the particles.

- c) Start the assembly of the Load & Lock column according to the manual, leaving the top open.
- d) Take the homogenous slurry and pour quickly into the assembled Load & Lock column in one continuous action.
- e) Complete the assembly of the Load & Lock column.

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Note: Make sure that the outline tubing is placed into an appropriate (waste) container to catch the slurry solvent.

Note: It is important that the compression of this column is performed as rapidly as possible, to consolidate the bed and prevent polymeric media settling.

- f) Determine the compression ratio and the mechanical pressure from the table below:

Compression Ratio:
Mechanical Pressure:

Make sure you check with the trainer if the values are correct

- g) Set the compression and pressure values and operate the packing station according to the manual.

Note: Once the Load & Lock column is packed, the pump has stopped automatically. Leave the column to the packing station for 30 minutes. The column plunger should be locked in the compressed position so that the column can be operating in the Static Axial Compression (SAC) mode. The column can be “undocked” for use.

3)Test the Prep Column

- a) To run a performance efficiency test, connect the column to your solvent delivery system and flush the column with your mobile phase (3 to 4 column volumes).

Note: Make sure that the outline tubing is placed into an appropriate (waste) container to catch the solvent.

- b) Connect the column outlet (top) to the detector and continue equilibration until a stable base line has been achieved.
- c) Run the column efficiency test by using the test procedure by using 250 x 50MM (L x ID) Load & Lock Column.

Mobile: 70% ACN / 30% Water

Flow rate: 118 mL/min

Wavelength: 254nm

Test mixture 201: 1) Uracil, 2) Toluene, 3) Fluorene, 4) **Fluoranthene^(*)**

(*) Test component - you can obtain ~30,000 plates per meter by using 10-micron particle size

Note: By using 250 x 27MM the flow rate should be 34 mL/min.

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Appendix A: Composition of Slurry

	ST60 10-C18			ST150 10-C18			PLRP-S		
Packed bed density	0.59g/mL			0.49g/mL			0.33g/mL		
Slurry concentration	1g dry media plus 2mL IPA			1g dry media plus 2mL IPA			1g dry media plus 2mL ACN:water (80:20v/v)		
Load & Lock column ID	1in. (27mm)	2in. (50mm)	3in. (75mm)	1in. (27mm)	2in. (50mm)	3in. (75mm)	1in. (27mm)	2in. (50mm)	3in. (75mm)
Mechanical pressure	1000psi			1000psi			1000psi		
Ratio hydraulic to mechanical pressure	1:2.5	1.5:1	3:1	1:2.5	1.5:1	3:1	1:2.5	1.5:1	3:1
Hydraulic pressure	400psi	1500psi	3000psi	400psi	1500psi	3000psi	260psi	975psi	1950psi
Weight dry media (to pack 25cm)	84g	290g	652g	70g	241g	541g	48g	163g	365g
Volume IPA needed	170mL	580mL	1300mL	140mL	480mL	1080mL			
Volume ACN:water							210mL	750mL	1625mL