

Desalting on tC18 Sep-Pak chromatography

Date written:10/8/2018

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Reagents:

-Methanol
-HPLC grade Water
-Acetic acid

Equipment/Apparatuses:

- Sep-Pak Vac 1cc (100mg) tC18
(Waters, Cat# WAT036820)
- Glass threaded culture tube (conical bottom)
- Phenolic cap with PTFE-Faced rubber liner
- Glass pipette

Procedures:

1. Equilibrate a tC18 cartridge column (waters tC18, size 1 ml, 100 mg or comparable) using three volumes of MeOH and five volumes of 5% AcOH. Passage of the equilibrating solutions can be accelerated by application of mild positive pressure using air flow.
2. Load neutralized sample onto the equilibrated tC18 column and collect the flow through into a glass tube. Reload the flow thorough onto the tC18 column. Wash the column with 10 ml of 5% AcOH. Discard the flow thorough.
3. Elute glycolipids with 3ml of MeOH and collect the eluates in a new screw top glass tube.
4. Dry the eluate under N2 stream at 40°C.

Note:

- The dried sample can be stored at -20 °C.
- Glycolipids must be completely dried for the next step. Collecting glycolipids in a conical glass tube will maximize the recovery of glycolipids by n-hexane wash (next step), especially for small-scale sample preparation.

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