

[PROTOCOL: GCP Final Desalt (SepPak)]

Purpose

To remove salts from the samples before introduction to the mass spectrometer. Salts can dirty the instrument resulting in decreased sensitivity.

Preparation

1. Bring sample plate from Secondary Propionylation Step to room temperature
2. Prepare reagents as needed

Materials

- SepPak tC18 uElution Plate {SepPak-M01} [Waters, Cat.#186002318]
- Trifluoroacetic acid (TFA) {SepPak-M02} [Sigma-Aldrich, T6508-25ML]
- Acetonitrile (ACN) {SepPak-M03} [EMD Millipore, AX0156-1]
- HPLC-grade Water {SepPak-M04} [JT Baker, 4218-03]
- 0.75mL Micronics Vials Plate {SepPak-M05} [SOURCE NEEDED]
- Axygen Foil Seals {SepPak-M06} [Axygen, PCR-AS-200]
- Breath-EASIER seal {SepPak-M07} [BERM-2000]

Assets

- 96-well Positive Pressure Desalting Platform {SepPak-A01}
- 12-channel Multichannel pipette {SepPak-A02}
- SepPak uElution Plate Adapter {SepPak-A03}
- Desalting flow through waste plate {SepPak-A04}
- Thermo Scientific Savant SC210A Concentrator {SepPak-A05}
- Micronics Vials Adapter Rack for Positive Pressure Desalting Platform {SepPak-A07} [SOURCE NEEDED]
- SepPak uElution Elution Vials Adapter (SepPak-A08) [SOURCE NEEDED]

Reagent Mixes

ID	Name	Step	Composition	Stock Volume	Use
MIX0 1	0.1% TFA	SepPak	0.1% {SepPak-M02} in {SepPak-M04}	1 L	Resuspension of samples, desalting plate equilibration, and sample washing
MIX0 2	50% ACN/ 0.1% TFA	SepPak	50% {SepPak-M03}/0. 1% {SepPak-M02} in {SepPak-M04}	500 mL	Sample elution from the SepPak plate

Reagent Mix Preparation

MIX01- 0.1% TFA:

- Acquire 1 L glass bottle with screw top
- Add 999 mL HPLC-grade water {SepPak-M04} to bottle
- Add 1 mL TFA {SepPak-M02} to bottle
- Invert 7 times to mix

MIX02- 50% ACN/ 0.1% TFA:

- Acquire 500 mL glass bottle with screw top
- Add 249.5 mL HPLC-grade water {SepPak-M04} to bottle
- Add 250 mL ACN {SepPak-M03} to bottle
- Add 500 uL TFA {SepPak-M02} to bottle
- Invert 7 times to mix

Procedure

1. Attach the SepPak tC18 uElution Plate {SepPak-M01} to the flow-through plate {SepPak-A04}, with use of the plate adapter {SepPak-A03}. Tape the sides of assembly so no parts can separate.
2. Add 200 uL 0.1% TFA {MIX01} to each sample in the now-thawed sample plate. Recover with foil seal
3. Activate wells of SepPak plate with 200 uL 100% ACN {SepPak-M03}. Let this step flow through via gravity alone.
4. Equilibrate wells of SepPak plate with 200 uL 0.1% TFA {MIX01}.
5. Repeat step 4.

NB: At this point check volume in waste plate. Discard waste in liquids waste bucket on the bench.

6. Load sample onto the SepPak plate (200 uL/sample). With the multichannel pipette {SepPak-A02} mix samples 4 times, and on the 5th time draw up sample and load onto the SepPak plate. Draw up remaining volume and dispense into SepPak plate.
7. Wash SepPak wells with 200 uL 0.1% TFA {MIX01}
8. Repeat step 7.
9. Remove waste plate {SepPak-A04} from the SepPak desalting assembly. Transfer micronics vials that will hold eluate from their original rack to Micronics Vial Adapter {SepPak-A07}. Place adapter rack+vials on top of the waste plate (needed for height). Place the Elution Vials Adapter {SepPak-A08} on top of rack+vials. Place uElution Plate {SepPak-M01} on top of the vials adapter, make sure that cartridge ends are inside the vials. Tape assembly together so it will not separate during elution.
10. Elute samples with 100 uL 50% ACN/ 0.1% TFA. Let this step flow through via gravity, with as minimal assistance from the Positive Pressure Desalting Platform {SepPak-A01} as possible. When wells look dry, slowly dial up the pressure applied by the desalting platform {SepPak-A01}, until the rest of the volume passes into the elution vials.
11. Remove the SepPak uElution plate and vial adapter from the vials. Seal the rack with vials containing eluate with a breathable seal {SepPak-M07} followed by a foil seal {SepPak-M06}, and freeze at -80°C.
12. Create a balance plate of 50% ACN/ 0.1%TFA in the same vial+rack type as elution rack.. Seal and freeze at -80°C as well.
13. Once both sample and balance plates are frozen, remove foil seal and Speedvac {SepPak-A05} to dryness. Transfer dried vials to the original rack they came in. Label with date, initials, and study name. Store dried samples at -80°C until they are ready for resuspension and dilution, prior to mass spec analysis.

