C18 Cartridge Solid Phase Extraction (SPE) for MS Analysis

Reagents and Supplies:

**TFA (Trifluoroacetic Acid)** – Fluka Sequencing Grade or equivalent

**Water** – MilliQ or Fisher Optima LC/MS Grade

**MeOH (Methanol)** – HPLC or Fisher Optima LC/MS Grade

**AcCN (Acetonitrile)** – HPLC or Fisher Optima LC/MS Grade

**C18 SPE Cartridge** – Waters, Varian or Thermo/50 or 100mg/1ml Barrel

**Formic Acid** – Acros p.a. Grade (to adjust sample pH if necessary)

**Plasticware** – Polypropylene test tube, 4ml or larger with cap, and siliconized 1.5ml microcentrifuge tube.

Solvents:

**Conditioning Solution** – 90/10 Methanol/Water with 0.1% TFA (v/v/v)

**Equilibration /Load Solution** – 0.1% TFA in Water (v/v)

**Desalting Solution** – 5% MeOH/Water with 0.1% TFA (v/v/v)

**Elution Solution** – 50/50 AcCN/Water with 0.1% TFA (v/v/v)

Procedure:

- **Reconstitute** sample in up to 1ml 0.1-0.5% TFA. Check that pH is <3, adjust with formic acid.

- **Condition** SPE cartridge by passing 3ml Conditioning Solution through the packing bed. Do not allow air to enter the packing material.

- **Equilibrate** SPE cartridge by passing 2ml Equilibration/Load solution through the packing bed. Do not allow air to enter the cartridge packing.

- **Load** sample by passing slowly (no faster than 1 drop/second) into the cartridge packing. Do Not allow air to enter the resin bed. Complete loading by slowly passing 1ml of Equilibration/Load solution into the cartridge packing. Do not allow air to enter the packing.
Collect all cartridge effluent in a clean tube (4ml or larger).

- **Desalt** the sample by passing 1ml Desalting Solution through the packing bed. Do not allow air to enter the cartridge bed and collect the effluent in the same 4ml tube as above.

- **Elute** the sample by slowly (less than 1 drop/second) passing 1ml of Elution Solution through the cartridge bed. Collect the effluent in a clean, siliconized 1.5 ml microcentrifuge tube and carefully blow the cartridge dry.

- **Dry** the sample in Speed-Vap.

**Notes:** Procedure can be applied to SPE manifold. If very polar components (e.g. phosphopeptides) need to be retained, use 1ml of Equilibration/Load Solution for the desalting step.

Examples of SPE products:

(∗sep pak® cartridges Waters or prep sep™ cartridges Fisher scientific)∗

Sep-Pak® Cartridges (Waters)
Part.No. WAT054960
Quantity 10
Lot.No. W3324J4
Sep-Pak® Vac tC18 Icc
Capacity: 1 mg

Prep Sep™ (Fisher scientific)
Solid phase extraction products
Fisher scientific
C18 100mg/ml
Cat. # 11-131-6
Quantity: 108 Ea/Pk