Extraction of Vitamins from Serum

1. Principle

In this method fat and water-soluble vitamins are selectively extracted from serum using reverse phase SPE. An unusual two step loading procedure allows the two to be separated using one C8 SPE cartridge. In the first elution, water-soluble vitamins (niacin, Vitamin B1, B2 and B6) are collected. The fat-soluble vitamins (retinol, alpha & beta tocopherol, alpha & beta carotene and lycopene) are collected in a second elution.

This method may also be applied to any aqueous sample matrix: blood, plasma, urine, tablets, drinks, etc.

2. Materials

Strata SPE Cartridge: C8 100 mg/1mL, Part Number: 8B-S005-EAK
Strata SPE Manifold: 12 or 24 Position Vacuum Manifold, AHO- 6023 and AHO-6024, respectively.
Solvents: Methanol, Ethanol, Acetonitrile, 0.1 M NaCl, diethylamine.
Elution solvent "F": Ethanol / Acetonitrile, 1:1, containing 100 ul / liter of dietylamine.

3. Specimen Preparation

To a test tube, add:

- 200 ul serum
- 200 ul of 0.1 M NaCl
- Vortex to mix.

Note: Method is suitable for larger or smaller sample volumes. Simply choose the appropriate sorbent mass per the Phenomenex document "Choosing the Proper Sorbent Mass", then alter the processing solvent volumes per the publication "Suggested Solvent Volumes".

4. SPE Method

a) **Condition Step 1**
Sequentially pass through the cartridge to waste:

- 1 column volume of Methanol
- 1 column volume of DI Water

b) Load Step 1

Note: You will collect and save the solvent from this step as it contains the unretained fat-soluble vitamins.

- Place a fresh collection tube into the manifold.
- Slowly pass the sample through the cartridge (Approximately 30 sec.) and collect the eluent called fraction "F" (Fat Soluble). Carefully set this sample aside for later processing.

c) Elution of Water Soluble Vitamins

- Place a fresh collection tube in the manifold.
- Slowly pass 1 ml of methanol through the cartridge. This elution contains the water-soluble vitamins.
- Remove this test tube with the water-soluble fraction before proceeding

***d)* Wash Step 1**

Pass through the cartridge to waste.

- 2 column volumes of DI Water

e) Sample Application 2

Note: At this step, the Fat-soluble vitamins are retained, washed and eluted.

- To the test tube containing Fraction F, add 200 ul of acetonitrile.
- Apply diluted Fraction F back onto the Strata cartridge and pass through the cartridge. This eluent can go to waste, as the fat-soluble vitamins are retained.

f) Wash Step 2

Pass through the cartridge to waste.

- 1 column volume of DI Water

g) Dry

- Dry the cartridge under full vacuum for 30-60 seconds to insure removal of aqueous wash.

h) Elution of Fat Soluble Vitamins:

- Place a fresh test tube inside the manifold.

Pass sequentially through the cartridge:

- 400 ul of ethanol. Allow to percolate very slowly through the cartridge using little or no vacuum.
- 1.5 ml of Elution solvent F. Use vacuum to draw the remaining elution solvent through the cartridge.

5. Analysis

Analyze each fraction using the appropriate Phenomenex HPLC column. (Synergi or Luna reverse phase columns are most appropriate) Contact your local Phenomenex Technical Specialist for more specific assistance.