

## Vitamin Sample Preparation

1.) Sample prep: Mix sample 0.5g (1mL) in 10-mL of water and 10-mL of dichloromethane/hexane (2:1 v/v), vortex for 3 minutes and filter. Separate the lipid- and hydro-soluble layers in a separatory funnel. Add 50mg NaCl to the aqueous layer and mix. Dry down the lipid layer under a stream of nitrogen gas to 2-mL and reconstitute to 6-mL with hexane.

\*Vitamin stability is best with limited exposure to light, oxygen, extreme pH and heat. Best recoveries are matrix dependent, for starchy samples a hydrolysis step is commonly used and for fatty samples saponification. Hydrolysis conditions are usually low pH (OK for water solubles, but fat solubles are less stable). Saponification conditions are generally high pH (OK for fat solubles but water solubles are less stable). For the analysis of water soluble vitamins in blood plasma, the water fraction may be acidified or a polar organic solvent such as methanol or acetonitrile may be added to further precipitate proteins prior to filtration. If polar organic solvent is added it may be necessary to dry down the sample to remove the organic solvent prior to loading on the SPE cartridge.

2.) SPE Lipid Solubles: Strata Si-1(500mg/6mL): Condition: 5-mL isopropanol + 6-mL hexane. Load lipid soluble sample (3-mL/min.). Wash: 6-mL hexane and dry. Elute: 6-mL isopropanol (various ratios of isopropanol in hexane can be used for best recoveries and cleanest extract), 1mL/min. Dry under a stream of nitrogen gas and reconstitute in the mobile phase.

\*Synergi Hydro RP and Luna Si(2) columns are most commonly used for HPLC analysis, ask your Phenomenex representative for copies of these applications.

3.) SPE Hydrosolubles: Strata X(500mg/6mL): Condition: 5-mL methanol + 6-mL water. Load water soluble sample (3-mL/min.). Wash: 6-mL water and dry. Elute: 6-mL methanol, (various ratios of methanol in water can be used for best recoveries and cleanest extract), 1mL/min. Dry under a stream of nitrogen gas and reconstitute in the mobile phase.

\*For Thiamine, higher recoveries can be obtained using Strata X-C (500mg/6ml): Condition: 5-mL methanol + 6-mL 20mM sodium acetate (pH 4.6). Buffer sample with 20mM sodium acetate (pH 4.6) and load at 3-mL/min. Wash: 6-mL 20mM sodium acetate (pH 4.6) and dry. Elute with 6mL of 2M KCl adjusted to pH 1.5-2.0 with dilute HCl in water/ methanol (60/40).

\*Luna HILIC, Luna C18(2) and Synergi Hydro RP columns are most commonly used for HPLC analysis, ask your Phenomenex representative for copies of these applications.

