Extraction Procedure for Prostaglandins and Thromboxanes

Includes: Prostaglandin E_2, Prostaglandin $F_{2\alpha}$, 6-keto-Prostaglandin $F_{1\alpha}$, 11-β Prostaglandin $F_{2\alpha}$, 13,14 –dihydro-15-ket-Prostaglandin $F_{2\alpha}$, Thromboxane E_2, and 11-dehydrothromboxane B_2.

Cat #'s: EA 02, EA 03, EA 05, EA 08, EA 11, EA 20, EA 25, and EA 30

Materials Needed:

1. Methanol
2. C_{18} Sep-Pak® Columns (Waters® Corporation)
3. Deionized Water
4. Petroleum Ether
5. Methyl Formate
6. Nitrogen Gas

Reagents Needed:

1. 15% Methanol in Deionized Water

Optional Reagents:

1. 15% Methanol in 0.1 M Sodium Phosphate Buffer, pH 7.5
2. Phosphate Buffer (10 – 100 mM, pH ~7.0) for diluting

Procedure:

1. Add 0.2 mL of methanol to 1 mL of biological fluid and vortex.
2. For tissue, homogenize it in 15% methanol in 0.1 M sodium phosphate buffer, pH 7.5 (100 mg in 1 mL methanol-buffer). Centrifuge the homogenate for five (5) minutes. Collect the supernatant in a clean tube.
3. Precondition the C_{18} Sep-Pak® column (Waters® Corporation) by washing the column with 2 mL of methanol followed by 2 mL of water.
4. Apply the above sample into the column and adjust the flow rate to 1 mL per minute. Reducing the flow rate to 0.5 mL per minute may increase extraction efficiencies. Some samples may clog the column. These samples may be diluted 1:3 or 1:6 in phosphate buffer (10 to 100 mM, pH~7.0) to improve the flow rate.
5. Wash the column with 2 mL of 15% methanol in water followed by 2 mL of petroleum ether.
6. The eicosanoid is eluted by 2 mL of methyl formate.
7. Evaporate the methyl formate eluate with a stream of nitrogen gas.