Eicosanoid Mass Spectra Protocol
Updated 09/03/2004
By Ray Deems

HPLC Conditions:

1. Column Information
   Company: Vydac
   Model: 201TP52
   S/N: NE981208-3-1
   Packing: Reverse Phase C18
   Particle Size 5 um
   Diameter: 2.1 mm
   Length: 250 mm

2. Buffer A
   37% Acetonitrile
   0.02% Formic Acid
   63% Water

3. Buffer B
   50% Acetonitrile
   50% Isopropanol
   All solvents used in Buffer A and B are EMD omnisolv grade reagents (including water)

4. The column is maintained at 35 C with column heater.

Media Collection and Separation:

Cell Media Preparation Principle: The eicosanoids are separated from the other media components by purification on Strata-X columns (Phenomenex cat # 8B-S100-UBJ strata-X 33 μm Polymeric Sorbent). The 3 ml columns are used for 4 ml of media. The columns are run via a vacuum using a Supelco Visiprep 24 vacuum chamber.

Media Collection: The media is decanted off of the cells and 400 μL of 10% MeOH and 20 μL 0.5% acetic acid per 4 ml of media is added. Appropriate amounts (100 μl per sample) of internal standards are added.
The internal standard contains 0.1 ng/μl of the following deuterated eicosanoids in 50% ethanol in water:

$PGF_{2\alpha}$	D4
PGE_2	D4
PGD_2	D4
5 Hete	D8
AA	D8

Columns:
Set the manometer on the vacuum chamber to 5 mmHg.
Do not let the column run dry in steps (1) and (2).

1. Precondition columns:	Elute 2 ml MeOH, stop, and then elute 2 ml H2O.
2. Apply sample:	Load sample.
3. Wash:	Add 2 ml of 5% MeOH to the Sample vial. Vortex and apply to the column under vacuum. Allow to run dry for 30 seconds.
4. Elution:	Apply 2 ml Isopropyl alcohol to column and equilibrate for 1 min. Elute and run dry with vacuum for 30 seconds.
5. Concentration:	The solvent is removed by speed vac and the eicosanoids are redissolved in 100 μl of 50% ethanol in water.
6. Storage:	These samples can be stored at -20 C for several days at least.

All solvents are HPLC grade (e.g. EMD Omnisolv)