**Paper Reference:** Gaikwad, N. W. (2013) Ultra performance liquid chromatography-tandem mass spectrometry method for profiling of steroid metabolome in human tissue, *Anal Chem* *85*, 4951-4960.

**MS, MS/MS, and UPLC-MS/MS Analysis of Steroids:**

A Xevo-TQ triple quadruple mass spectrometer (Waters, Milford, MA, USA) recorded MS and MS/MS spectra using electro spray ionization (ESI) in positive ion (PI) and negative ion (NI) mode, capillary voltage of 3.0 kV, extractor cone voltage of 3 V, and detector voltage of 650 V. Cone gas flow was set at 50 L/h, and desolvation gas flow was maintained at 600 L/h. Source temperature and desolvation temperatures were set at 150 and 350 °C, respectively. The collision energy was varied to optimize daughter ions. The acquisition range was 20−500 Da. The test samples (compounds 1−101) at 5 μg/mL were introduced to the source at a flow rate of 5 μL/min by using acetonitrile/water (1:1) and 0.1% formic acid mixture as the carrier solution, and mass spectra were recorded. The masses of parent ion and daughter ions were obtained in the MS and MS/MS operations. MS/MS parameters were further used in the multiple reaction monitoring (MRM) method for UPLC/MS/MS operation. Analytical separations of the mixture of 101 standards were conducted on the UPLC system using an Acquity UPLC HSS T3 1.8 μm 1 × 150 mm analytical column kept at 50 °C and at a flow rate of 0.15 mL/min. The gradient started with 100% A (0.1% formic acid in H2O) and 0% B (0.1% formic acid in CH3CN), after 2 min, changed to 80% A over 2 min, and then 45% A over 5 min, followed by 20% A in 2 min. Finally, over 1 min, it was changed to the original 100% A, resulting in a total separation time of 12 min. The elutions from the UPLC column were introduced to the mass spectrometer, and resulting data were analyzed and processed using MassLynx 4.1 software.

**Sample Preparation and Analysis:**

Weighed (100 mg) breast tissue samples were ground and suspended in 4 mL of a 1:1 water/methanol mixture. The suspension was homogenized, and the resulting homogenate was cooled on ice. The precipitated material was removed by centrifuging at high speed for 5 min, and the supernatant was removed and evaporated in a SpeedVaac (Labconco Inc.) followed by lyophilizer (Labconco Inc.). The residue was suspended in 150 μL of CH3OH/H2O(1:1), filtered through a 0.2 μm ultracentrifuge filter (Millipore inc.) and subjected to UPLC/MS-MS analysis. Ten samples were run in duplicate during UPLC-MS/MS analysis. Samples were placed in an Acquity sample manager which was cooled to 5 °C to preserve the analytes. Pure standards were used to optimize the UPLC-MS/MS conditions prior to sample analysis. Also, the standard mixture was run before the first sample, after the fifth sample, and after the last (10th) sample to prevent errors due to matrix effect and day-today instrument variations. In addition, immediately after the initial standard and before the first sample, two spiked samples were run to calibrate for the drift in the retention time of all analytes due to the matrix effect. After standard and spiked sample runs, blank was injected to wash the injector and remove carry over effect.