A chromatography/tandem mass spectrometry method for the simultaneous quantitative determination of ten endogenous steroids, including adrenal precursors, progesterone, androgens and estrogens using low serum volume.

Patrick Caron, Véronique Turcotte and Chantal Guillemette[†]

Pharmacogenomics Laboratory, Centre Hospitalier Universitaire (CHU) de Québec Research Center and Faculty of Pharmacy, Laval University, Québec, Canada. [†]Canada Research Chair in Pharmacogenomics.

Measurement of a vast array of sex steroids in clinical epidemiology and laboratory research with reliable methods providing low quantification limits and using a limited volume of sample represents a significant challenge. We report a validated gas chromatography selected reaction monitoring – tandem mass spectrometry assay (GC-MS/MS) for the simultaneous quantification of ten endogenous steroids including progesterone (PROG), dehydroepiandrosterone (DHEA), androstenediol (5-diol), androstenedione (4-dione), testosterone (T), dihydrotestosterone (DHT), androsterone (ADT), 5alpha-androstan-3beta-17beta-diol (3β-diol), estrone (E_1) and estradiol (E_2). After addition of stable isotope internal standards to 250 µl of serum, the method involved the combination of liquid-liquid extraction, derivatization and solid-phase extraction for injection into the GC system and multiple reaction monitoring (MRM). The method could measure individual steroids with high sensitivity, accuracy and reproducibility. Mean recoveries in serum were 84.0% ± 5.7%. The intra-assay and inter-assay coefficients of variation were ≤ 11.5% and the bias was ≤ 10.9%. The lower limits of detection of quantification (LLOQ) were of 100 pg/ml for DHEA, 50 pg/ml for PROG, 5-diol, 4-dione and ADT, 30 pg/ml for T, 10 pg/ml for 3β-diol, 5 pg/ml for DHT and E_1, and 1 pg/ml for E_2. A cross-validation with a previous method used by our group was also performed. Finally, the applicability of the validated method to determine the concentrations of these 10 steroids was successfully tested on serum from men (n=15), premenopausal (n=10) and postmenopausal women (n=15), and is currently used for larger cancer-related epidemiology studies. One of the most considerable advantages over existing methods is the simultaneous determination of ten steroids in a limited volume of serum that will help conserve important clinical samples from existing biobanks.