Sensitive Analysis of Serum 5α-Dihydrotestosterone by 2D-LC-MS/MS

Bingfang Yue, ShuGuang Li, Daniel Anderson, WenZhe Li
NMS Labs, 3701 Welsh Rd, Willow Grove, PA 19090

Introduction: In humans, circulating androgen 5α-dihydrotestosterone (DHT) exerts major biological effects on skin and prostate. DHT is a more potent androgen than testosterone (T) and is the primary androgen in the prostate. The DHT concentration also increases with androgen replacement therapy because of T conversion to DHT. The serum DHT concentration and DHT/T ratio are clinically useful for monitoring 5α-reductase deficiency, treatment of benign prostate hyperplasia or prevention of prostate cancer by 5α-reductase inhibitors. The challenges to develop a sensitive, accurate and specific bioanalytical method for DHT include low concentration levels and endogenous T metabolites that may interfere. Radioimmunoassay for DHT measurement requires intensive sample workup and lacks specificity due to cross-reactivity. We developed a simple high-throughput assay utilizing two-dimensional liquid chromatography-tandem mass spectrometry with required performance for clinical use.

Method: Sample aliquot spiked with internal standard was extracted using a mixture of ethyl acetate and hexane. After vortex mixing, centrifugation, phase separation, complete solvent evaporation, DHT is derivatized with picolinic acid at room temperature and then injected into a 2D-LC-MS/MS system without further purification. An API-5000 triple-quadrupole mass spectrometer (AB Sciex) is coupled to a Shimadzu HPLC system of two sets of binary pumps for 2D-LC-MS/MS. The 1st D-LC uses an Agilent Zorbax 300SB-C3 guard column (12.5 x 2.1 mm) for online extraction and cleanup with 0.5% formic acid in water and methanol as mobile phase while the 2nd D-LC uses a Phenomenex Kinetex C18 (100 x 3.0 mm) for analytical separation using 0.1% formic acid in water and acetonitrile as mobile phase. A six-port switching valve is switched at 1.7 min and 2.2 min to transfer compounds of interest from 1st D to 2nd D in heart-cutting fashion without back flash. The API 5000 is operated in positive electrospray ionization and multiple reaction monitoring (MRM) mode with two MRMs monitored for each analyte or internal standard.

Results: The method was fully validated. The lower limit of quantitation (LLOQ) was validated at 5pg/mL with accuracy >93.8% and total %CV < 8.7%, while the upper limit of quantitation (ULOQ) was validated at 2500pg/mL. Within-run CVs were < 3.0% for three levels of QC samples while between-run CVs were <2.9% and a total CVs <5.6%. The extraction recovery was ~96.2% with matrix effect at ~71.3% and process efficiency of 68.6%. The correlations compared with a reference method (EP Evaluator, Deming Regression, 99% confidence interval to exclude outliers) are as follows: $Y = 1.11 * X_{\text{Reference method}} + 9.402$, r =0.9983, n=37/40, SE =14.053.

Conclusion: The 2D-LC-MS/MS setup allows extensive clean-up and transfers only a small part of elution profile of the 1st dimension containing targeted analyte to the 2nd dimension for high efficiency separation. A simple and sensitive method to accurately

quantify DHT in serum by 2D-LC-MS/MS was developed and validated, with a LLOQ of 5 pg/mL and suitable for routine clinical laboratory use.