[TECHNOLOGY BRIEF]

An LC-MS Clinical Research Method for Measuring Male Androgens in Serum

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GOAL

To demonstrate that the use of an online SPE system (ACQUITY® Online Sample Manager (OSM)) enables simultaneous and analytically sensitive measurement of a panel of androgens.

BACKGROUND

The measurement of male androgens in most clinical research laboratories is limited to only measuring testosterone. To more accurately determine androgen status in men, the measurement of other androgens such as dihydrotestosterone (DHT) and dehydroepiandrosterone (DHEA), would be useful. However, measuring other androgens can be difficult without derivatization and chromatographic separation. We report here a combined LC-MS method for the measurement of testosterone (T), androstenendione (A4), DHT, and DHEA on a small sample volume of serum.

THE SOLUTION

A clinical research method for simultaneously measuring four androgens (T, A4, DHT, and DHEA) from serum has been developed. This method takes advantage of the unique capabilities of an online SPE system (ACQUITY OSM) to enable analytically sensitive and reproducible measurements of these steroid hormones.

Online SPE has a dramatic impact on the measurement of androgens in serum

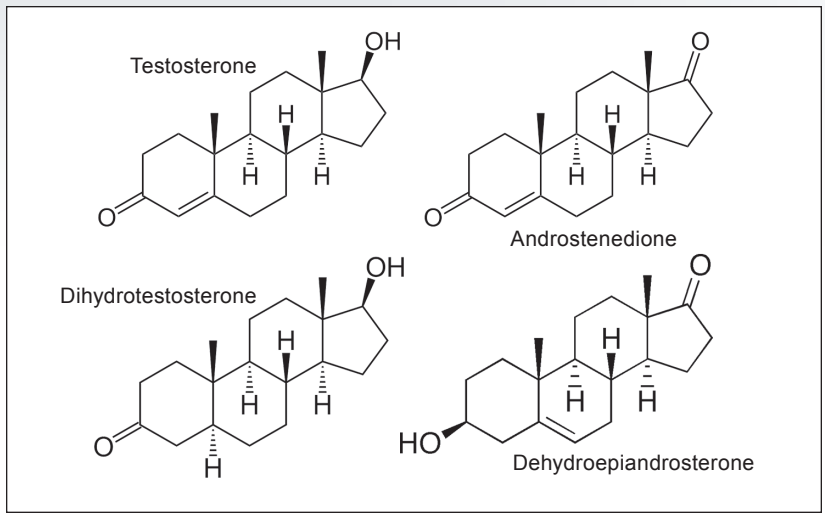

Figure 1. Structures of the steroid hormones analyzed.

The method described here enables direct measurement of these key molecules without the need for lengthy and complex derivatization and separation by complicated chromatographic methods.

ANALYTICAL METHOD DETAILS

Sample Preparation and Online SPE

Zinc sulfate (100 µL, 50 g/L) was added to 100 µL of sample. After mixing, acetonitrile (100 µL) containing internal standards (D2 T, D7 A4, D2 DHEA, and D3 DHT) was added and mixed for 1 minute. The samples were then centrifuged at 1,700 g for 10 minutes before analysis.

Sample (75 µL) was extracted using an online automated solid phase extraction using a C_{18} SPE cartridge. Cartridges were initially conditioned with methanol and equilibrated with water.

Calibrators were made in phosphate buffered saline containing 0.1% (w/v) bovine serum albumin. The measuring range was up to 50 nmol/L for T, A4, DHEA, and up to 5 nmol/L for DHT.

LC-MS/MS Analysis

- LC: ACQUITY UPLC®
- Mass Spectrometer: Xevo® TQ-S
- Column: ACQUITY UPLC HSS SB C_{18} 2.1 x 50 mm; 1.8 µm
- Gradient: 3 minute ramp of 50 – 70% methanol with 0.05% formic acid
- Flow rate: 0.45 mL/min
- Sample preparation: ACQUITY Online SPE Manager (OSM)
- SPE: MassTrak™ C_{18} OSM Cartridge
- Total analysis time: 6.5 minutes injection to injection

Eluent was directed (without stream splitting) into the ion source of a Waters Xevo TQ-S tandem quadrupole mass spectrometer operated in the positive ion mode using the following quantifier and qualifier transitions:

Compound	Quantifier m/z	Qualifier m/z
T	289.3>109.0	289.3>97.0
A4	287.3>109.0	287.3>97.0
DHT	291.3>255.1	291.3>159.0
DHEA	271.3>213.1	271.3>253.1

Table 1. Quantifier and qualifier transitions.

RESULTS

Chromatographic separation was achieved between all four androgens (see Figure 2 below). The run time was 6.5 minutes per sample. Negligible ion suppression was observed using a post-column infusion of the internal standards when extracted male and female samples (3 of each) were injected.

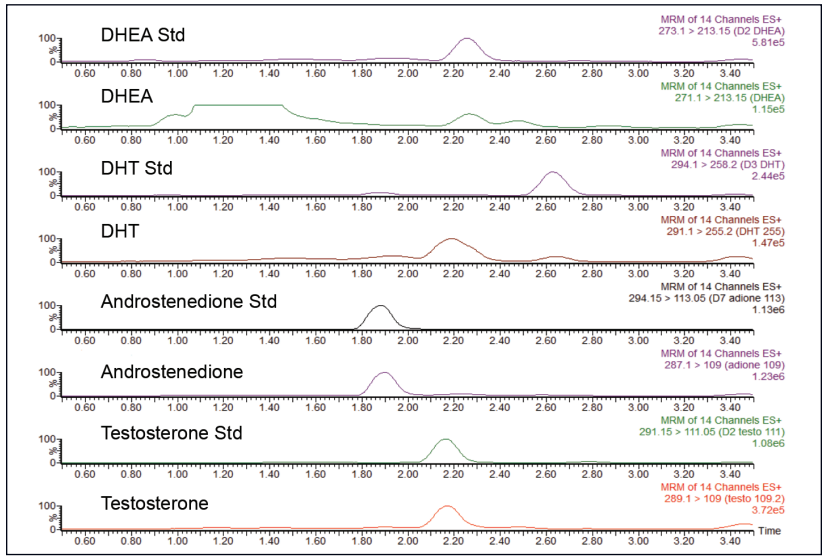

Figure 2. Example chromatogram for a serum sample containing 1.0 nmol/L testosterone, 3.9 nmol/L androstenedione, 0.13 nmol/L DHT, and 6.6 nmol/L DHEA.

Calibration lines were linear (r^2 >0.99) to at least 50 nmol/L for T, A4, DHEA, and 5 nmol/L for DHT. Table 2 shows the lower limit of quantitation for each of the androgens in both male and female samples.

Compound	LLOQ
T	0.1 nmol/L (28.8 pg/mL)
A4	0.1 nmol/L (28.7 pg/mL)
DHT	0.1 nmol/L (33 pg/mL)
DHEA	1.0 nmol/L (0.29 pg/mL)

Table 2. Androgen lower limit of of quantitation (LLOQ).

The average recovery range (n=6) for each compound was; T 98% (86 – 103%), A4 101% (83 – 107%), DHEA 94% (82 – 112%), and DHT 91% (83 – 107%).

When compared with an existing single analyte LC-MS method for measurement of these androgens, testosterone and androstenedione gave the following comparisions: Testosterone (combined) = 1.01 x existing method + 0.07 nmol/L and androstenedione (combined) = 1.09 x existing method - 0.29 nmol/L.

SUMMARY

The development of a rapid clinical research method for the LC-MS/MS measurement of testosterone, androstenedione, DHT, and DHEA in a clinical research laboratory is described here. The method requires a very small volume of sample (100 µL), and all four androgen analytes are measured simultaneously without a lengthy and complex derivatization procedure. In addition, separation of the four androgens was achieved using a relatively short (50 mm) column that was also able to separate any potential isobaric interferences (*e.g.* D2 T and DHT and DHEAS in DHEA) as shown in Figure 2.

This clinical research method is fast and relatively simple to implement, and has the potential to analyze large numbers of samples measuring multiple androgens in a single injection. These results were achieved by using a combination of efficient online SPE sample preparation in conjunction with a high analytical sensitivity mass spectrometer.

The method developed here provides:

- Simultaneous analysis of testosterone, androstenedione, dihydrotestosterone and dehydroepiandrosterone from serum
- Effective separation of all four androgens
- Efficient SPE sample preparation integrated with LC-MS in ~6.5 mins/sample
- Very good analytical sensitivity (LLOQs for all analytes in the low nanomolar ranges)
- No significant ion suppression effects with online SPE
- Excellent agreement with single analyte methods, requiring less time and resources

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