Determination of Testosterone in Human Serum using Ultivo Triple Quad LC/MS

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Introduction

In this research study, a robust, sensitive and relatively fast analytical method was developed for the quantitation of free testosterone in serum using a miniature Ultivo Triple Quadrupole LC/MS. Ultivo has been designed to address many challenges faced by routine analytical laboratories and this research study was conducted in order to assess how this novel triple quadrupole mass spectrometer (MS) could perform with a typical endogenous analyte of research interest[1]. Innovative technologies within Ultivo allow us to reduce its overall physical footprint, while generating a comparable analytical performance level to similar, but physically larger MS systems presently on the market. Instrumentation innovations, such as VacShield, Cyclone Ion Guide, Dodecapole Vortex Collision Cell and small Hyperbolic Quads were designed to maximize quantitative performance from within a miniature package and also to enhance instrument reliability and robustness.

Moreover, Ultivo reduces the need for user intervention for system maintenance, making the system operation and maintenance manageable for non-expert MS users. MassHunter Software simplifies data acquisition, method set up, data analysis and reporting, which results in the fastest possible acquisition-to-reporting time, increasing lab productivity. Herein this research study aims to outline typical confirmation performance of free testosterone in human serum using the Ultivo Tandem LC/MS. Lower limits of quantitation, chromatographic precision and calibration linearity, range and accuracy will be outlined.

Experimental

Sample Preparation:

Sample information: Testosterone was purchased from Cerilliant Corporation and stock solution was made in methanol.

Calibration curve: The 11-point calibration range of testosterone was from 0.001 to 100 ng/mL. Sufficient internal standard stock solution was added directly to each matrix standard and calibrator in order to create a consistent concentration of 25 ng/mL across all sample types injected.

Serum sample preparation: 250 µL human serum (obtained from Golden West Biologicals.) was crashed with 500 µL acetonitrile, vortexed for 1 minute and centrifuged for 4 min at 10,000 rpm. 500 µL supernatant was transferred and diluted with 500 µL of water. 20 µL is injected onto the LC-MS/MS.

LC Method:

Agilent 1290 Infinity UHPLC series binary pump, well plate sampler, thermostatted column compartment
Column: Poroshell 120 EC-C18, 2.1x50mm 2.7 µm, 600 bar
Column temperature: 55 ºC
Injection volume: 19 µL plus 1 µL of internal standard
Autosampler temp: 4 ºC
Needle wash: 100% methanol, 10 sec
Mobile phase: A = 0.1 % formic acid and 5mM ammonium acetate in water
B = methanol
Flow rate: 0.5 mL/min
Gradient: 60% B to 95% B in 4 minutes and hold at 95% B for 1 min, post run is 1 min

Experimental

MS Method:

Agilent Ultivo triple quadrupole mass spectrometer

Ion mode:	AJS positive
Gas temperature:	300 ºC
Drying gas (nitrogen):	8 L/min
Nebulizer gas (nitrogen):	50 psi
Sheath gas (nitrogen):	380 ºC
Sheath flow:	12 L/min
Capillary voltage:	3000 V
Nozzle voltage:	0 V
Cell Accelerate Voltage:	9 V
Q1/Q2 Resolution:	0.7/0.7 unit

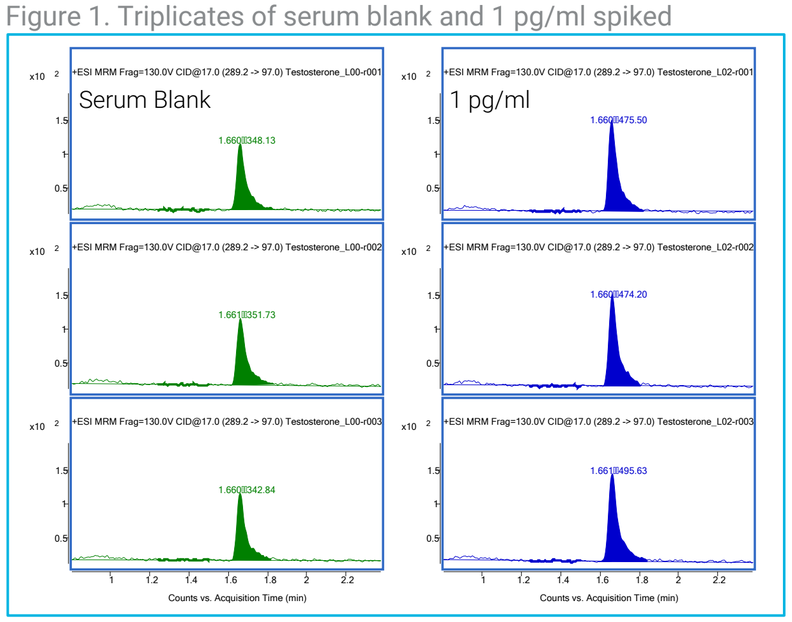
Table 1. MRM acquisition table

Compound	MRM	Dwell (msec)	Fragmentor (V)	CE (V)
Testosterone-d3	292.2>97.0	75	130	17
Testosterone	289.2>109.1	75	130	18
Testosterone	289.2>97.0	75	130	17

Results and Discussion

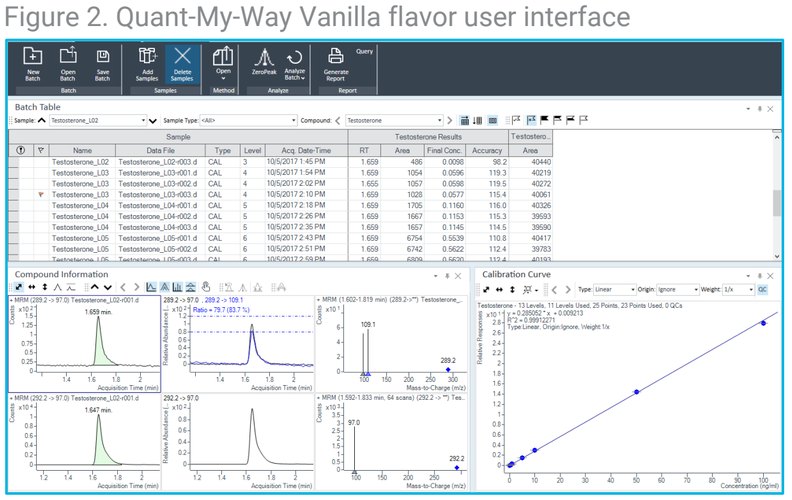
Sensitivity

Figure 1. Triplicates of serum blank and 1 pg/ml spiked



Negative serum sample blanks show measurable amounts of endogenous testosterone, the left column in Figure 1, shows a small testosterone response in blank serum. The right column, illustrates negative serum spiked with 1 pg/mL of testosterone. A significant difference in area count and signal to noise ratio can be seen and therefore the calibration curves were created and calculations undertaken by using a blank-offset feature.

Figure 2. Quant-My-Way Vanilla flavor user interface

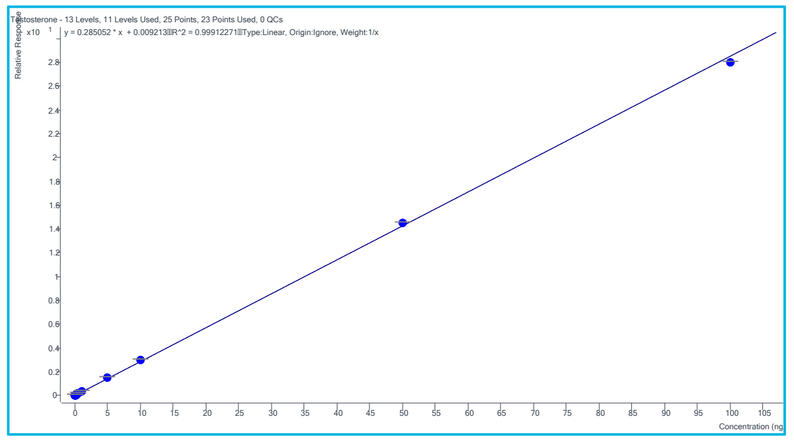


Results and Discussion

Quant-My-Way is a set of tools to designed to assist in exposing only the capabilities of interest to create a streamlined version of MassHunter Quantitative Analysis for each specific assay. The new ribbon can be customized with just the actions needed, and can hide as much or as little as the assay requires. In Figure 2. the testosterone quantitative analysis has been processed using a pre-configured Vanilla flavor user interface. The top ions are much more simplified from the original MassHunter Quantitative Analysis interface.

Calibration Curve

Figure 3. Testosterone calibration curve in serum



Excellent linearity and reproducibility were obtained with a concentration range from 1 pg/ml to 100 ng/ml (20fg on-column to 2000pg on-column) for the testosterone analyte with a linearity coefficient of >0.999; samples were prepared and analyzed in triplicate. Precision data observed over the three batches resulted with a %RSD variation of < 5% across all calibration levels in this research study.

Use Injector Program

Function	Parameter
Draw	Draw default volume from sample with default speed using default offset
Wash	Wash needle in flushport for 5 s
Draw	Draw 1 µL from location "P1-D-9" with default speed using default offset
Wash	Wash needle in flushport for 5 s
Inject	Inject

In this study, the d_3 isotopically labelled internal standard of testosterone wasn't premixed with calibrators. The above injector program was applied at the beginning of each injection. 19 µL of standard spiked calibrators were drawn, followed by needle wash. The injector then moved to the ISTD solution (pre-spiked in serum matrix) and drew 1 µL of the solution followed by needle wash again. The mixture was injected onto the column. To test the accuracy of this program, %RSD was calculated based on the response of ISTD MRM transition as 2.51.

Conclusions

This research project demonstrates that the performance of the novel Ultivo Triple Quadrupole LC/MS with the analytical methodology described herein generated excellent linearity, precision and analytical sensitivity across the range of 1 pg/ml through 100 ng/ml for free testosterone in human serum within an analysis cycle time of 6 minutes.

Future work will be required to assess potential interferences for this analytical method over a range of serum and blood matrices sourced from different suppliers and prepared for LC/MS analysis via a range of further sample preparation techniques.

References

1. Analysis of testosterone and dihydrotestosterone in mouse tissues by liquid chromatography-electro-spray tadem. *Annal Biochem.* **2010**, 15, 402(2), 121-128.

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