Evaluation of Quantitative Performance for Testosterone Analysis in Plasma on a Novel Quadrupole-Orbitrap Mass Spectrometer

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Overview

Purpose: To evaluate the quantitative performance of a novel high performance benchtop mass spectrometer powered by a quadrupole and Thermo Scientific Orbitrap technology to analyze testosterone in plasma with liquid chromatography-tandem mass spectrometry (LC-MS/MS).

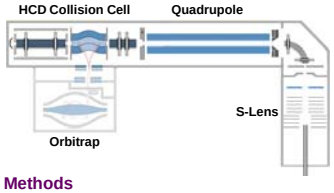
Methods: The plasma sample was processed with liquid-liquid extraction (LLE). Extracted testosterone samples were subjected to reverse phase LC gradient using a Thermo Scientific Accela UHPLC pump and a Thermo Scientific Hypersil GOLD aQ column (100 x 2.1 mm, 5 µm). The LC run time was 6 minutes. Mass spectrometry was performed on a Thermo Scientific Q Exactive quadrupole-Orbitrap™ benchtop mass spectrometer in targeted higher energy collision dissociation (HCD) mode at 70,000 resolution (FWHM) for testosterone quantitation.

Results: The novel Q Exactive™ mass spectrometer is highly sensitive and reliable.

Introduction

In recent years, high resolution accurate mass (HRAM) mass spectrometers have demonstrated significant benefits over unit resolution systems such as triple stage quadrupole and linear ion trap mass spectrometers, and are increasingly adopted for various qualitative and quantitative applications. These systems mainly include time-of-flight (TOF) and Orbitrap-based instruments. The Q Exactive mass spectrometer belongs to the latter category, which has seen great adoption for screening applications in clinical research laboratories. With the newly designed Q Exactive mass spectrometer (Figure 1), it is interesting to explore the possibility to use it as a quantitation platform for analytes with very low concentration in biological matrices. Here, testosterone in human plasma was chosen for evaluation of this novel Q Exactive mass spectrometer.

FIGURE 1. Geometry of the novel Q Exactive mass spectrometer.



Methods

Sample preparation

Samples (solvent calibrators, charcoal stripped serum calibrators, and plasma) were extracted using LLE. In brief, plasma samples were spiked with testosterone-d3 internal standard, mixed with methyl tert-butyl ether and vortexed. The mixture was centrifuged. The organic layer was dried and re-suspended in 50% methanol for the following LC-MS/MS analysis.

LC-MS Conditions

High performance liquid chromatography (HPLC) analysis was carried out on a Hypersil GOLD™ aQ column (100 x 2.1 mm, 5 µm). Mobile phase A (MPA), B (MPB) and C (MPC) are 5% methanol in water, methanol and IPA/acetonitrile/acetone, respectively.

The mass spectrometer used an atmospheric pressure chemical ionization (APCI) source and was operated in targeted MS/MS mode. The parent ions of testosterone and its internal standard were isolated through a quadrupole (mass isolation width 2 m/z), captured in the C-trap, fragmented in the HCD cell and the fragmented ions were analyzed in the Orbitrap in full scan mass range of 50 - 300 m/z. A mass extraction window of 3 ppm was applied to extract chromatograms of fragment ions.

The following are detailed LC-MS/MS settings and an exemplary acquisition method setup on the Q Exactive mass spectrometer.

Table 1. LC gradient, source parameter and acquisition settings.

Time (min)	%A	%B	%C	Flow (µL/min)
0.0	95	5	0	400
0.1	60	40	0	400
0.5	60	40	0	400
3.5	5	95	0	400
4.5	5	95	0	400
4.5	0	0	100	600
5.0	0	0	100	600
5.1	95	5	0	600
6.5	95	5	0	600

Ionization	APCI (positive)
Vaporizer temp (°C)	350
Capillary temp (°C)	320
Discharge current (µA)	5
Sheath gas (AU)	10
Auxiliary gas (AU)	20
Data acquisition mode	Targeted HCD
Q Inclusion list (m/z)	289.2, 292.2
Isolation width (in Q, m/z)	2
Collision energy (normalized)	40
AGC target	1.00E+05
Resolution	70000 at 200 m/z
Max injection time (ms)	100

Results

We observed great linearity between 10 and 500 pg/mL and very good sensitivity. Also the ion ratio between the two major fragment ions (97.0648 and 109.0648 m/z) from testosterone was consistent (97.0648 vs. 109.0648: 100%±20%) between 10 and 500 pg/mL. Figures 2 - 6 show extracted ion chromatograms of testosterone and calibration curves in difference matrices.

FIGURE 2. Extracted ion chromatograms of testosterone fragment ion (97.0648 m/z) in solvent (water, left) and charcoal stripped serum (CSS, right) calibrators.

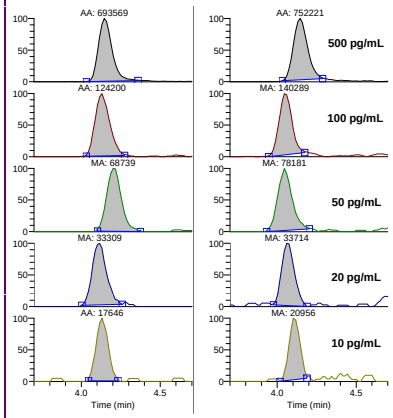


FIGURE 3 Extracted ion chromatograms of testosterone fragment ion (109.0648 m/z) in solvent (water, left) and charcoal stripped serum (CSS, right) calibrators.

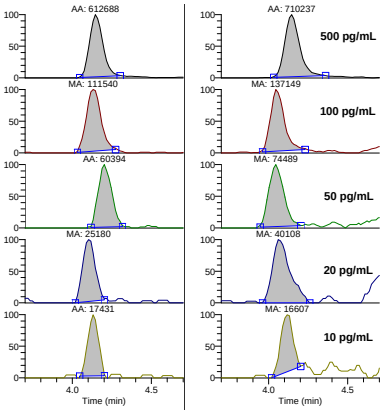


FIGURE 4. Calibration curve of testosterone (97.0648 m/z) ion for CSS calibrators.

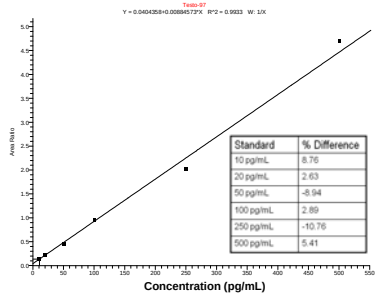


FIGURE 5. Calibration curve of testosterone (109.0648 m/z) ion for CSS calibrators.

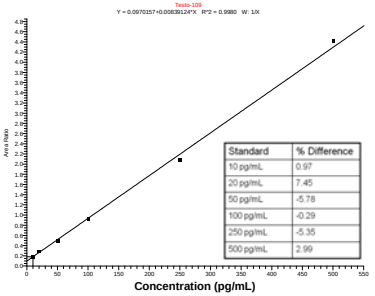
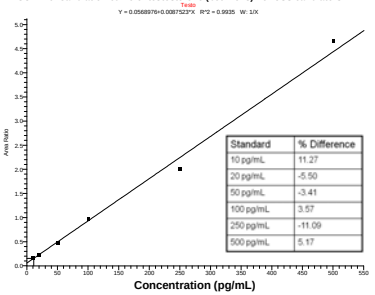


FIGURE 6. Calibration curve of testosterone (both ions) for CSS calibrators.



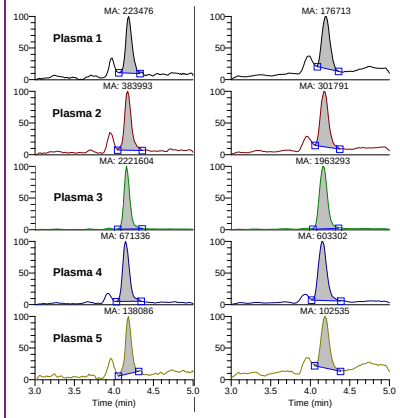
Human samples

Five human plasma samples were also tested with the Q Exactive mass spectrometer. The quantitation results and extracted ion chromatograms were summarized in Table 2 (compared with their immunoassay results) and Figure 7.

Table 2. Human sample data vs. immunoassay results.

Sample	Results by 97.0648 m/z		Results by 109.0648 m/z		Immunoassay
ng/dL	Calibrators in water	Calibrators in plasma	Calibrators in water	Calibrators in plasma	
1	15	15	13	14	21
2	17	17	19	20	38
3	156	156	156	152	178
4	34	34	37	36	62
5	8	8	8	7	< 20

FIGURE 7. Extracted ion chromatograms of testosterone fragment ions (97.0648 m/z, left, and 109.0648 m/z, right) in human plasma samples.



Conclusion

A novel Q Exactive mass spectrometer was evaluated for its quantitative performance in targeted HCD mode to analyze testosterone in plasma with LC-MS/MS.

1. Sub-3ppm mass accuracy could be easily obtained with external calibration.
2. The ion ratio for the two fragment ions used was consistently within the 20% error window in water, charcoal stripped plasma and human plasma at all levels in the calibration standards and samples.
3. Results on unknown plasma samples were identical using calibration curves created with water and charcoal stripped plasma, indicating no ion suppression.
4. Results correlated well with immunoassay.
5. The Q Exactive mass spectrometer is highly sensitive. Signal to noise (S/N) ratio of >60 was achieved with accuracy of 90 - 110% at 10 pg/mL concentration. The final LLOQ can be even lower based on the S/N we observed.