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Tomorrow's quantitation: reproducible measurement of testosterone in plasma with the TSQ Fortis MS for clinical research

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Keywords

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Goal

To develop a reliable and reproducible LC-MS/MS workflow for quantitative analysis of testosterone in plasma for clinical research using a triple quadrupole mass spectrometry coupled with ultra-high-performing liquid chromatography

Introduction

Reliable measurement of testosterone levels in plasma is critical for endocrinology clinical research. Liquid chromatography (LC) coupled to triple quadrupole mass spectrometry (QqQ) has been widely adopted as the analytical platform of choice for quantitation of testosterone in biological matrices. In this study, we report a reliable and reproducible workflow for quantitation of testosterone in plasma with LC-MS/MS using a Thermo Scientific™ Vanquish Flex™ UHPLC system, a Thermo Scientific™ TSQ Fortis™ triple quadrupole mass spectrometer, and Thermo Scientific™ TraceFinder™ 4.1 software.

Experimental

Sample preparation

To prepare the samples, a liquid-liquid extraction (LLE) process was used. First, 10 µL of spiking solution and 20 µL of internal standard (2 ng/mL testosterone-^{13}C3) were added to 600 µL of plasma. Then 3 mL of methyl tert-butyl ether (MTBE) was added, and the sample was vortexed. Post LLE, the MTBE layer was evaporated under nitrogen, and 150 µL of methanol/water (30:70) was added to reconstitute the sample. Finally, 20 µL of the sample was injected for LC-MS/MS analysis. Samples were injected in triplicate to obtain reproducibility data.

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Liquid chromatography

Chromatographic separation was performed using a Vanquish Flex Binary HPLC system equipped with a Thermo Scientific™ Accucore™ aQ C18 Polar Endcapped LC column (100 × 2.1 mm, 2.6 µm particle size, P/N 17326-102130). Mobile phases A and B were 0.5 mM ammonium fluoride in Fisher Chemical™ Optima™ grade water and pure methanol, respectively. The column temperature was 40 °C. The total run time was 9 minutes (Table 1).

Table 1. LC gradient

Number	Time	Flow (mL/min)	%B	Curve
1	0	0.25	30	5
2	1	0.25	30	5
3	1.5	0.25	55	5
4	5	0.25	85	5
5	6	0.25	100	5
6	7	0.25	100	5
7	7.01	0.25	30	5
8	9	0.25	30	5

Mass spectrometry

MS analysis was carried out on a TSQ Fortis triple quadrupole mass spectrometer equipped with a Thermo Scientific™ Ion Max NG source and heated electrospray ionization (HESI-III) probe. Table 2 shows the mass spectrometer source parameters.

Table 2. MS parameters

Ion Source	
Ion Source Type:	HESI
Spray Voltage:	Static
Positive Ion:	3500 V
Sheath Gas:	50 Arb
Aux Gas:	13 Arb
Sweep Gas:	1 Arb
Ion Tube Transfer Temperature:	275 °C
Vaporizer Temperature:	350 °C
SRM	
Use Cycle Time:	True
Cycle Time:	0.5 s
Use Calibrated Tube Lens:	False
Q1 Resolution (FWHM):	0.7
Q3 Resolution (FWHM):	1.2
CID Gas:	2 mTorr
Chromatographic Peak Width:	3 s

Two selected reaction monitoring (SRM) transitions were monitored for testosterone and its isotopically labeled internal standard to provide ion ratio confirmations (IRC). The scans were run in timed selected reaction monitoring (t-SRM) mode with a cycle time of 0.5 second. Table 3 shows the SRM properties used in this analysis.

Data analysis

Data was acquired and processed using TraceFinder 4.1 software.

Table 3. MS Method – SRM table

Compound	Polarity	Precursor (*m/z*)	Product (*m/z*)	Collision Energy (V)	Min Dwell Time (ms)	Tube Lens (V)	Source Fragmentation (V)
Testosterone	Positive	289.22	97.12	22.0	44.1	89	8.1
Testosterone	Positive	289.22	109.12	24.5	44.1	89	8.1
Testosterone-^{13}C3	Positive	292.22	100.12	22.6	44.1	89	8.1
Testosterone-^{13}C3	Positive	292.22	112.05	25.1	44.1	89	8.1

Results

The lower limit of quantitation (LLOQ) for testosterone was defined as the lowest concentration at which the back-calculated calibrator concentration on the linear calibration curve was within 20% of theoretical, the ion ratio was within 20% of target, and replicate injections had a %RSD of less than 15%. For testosterone, the LLOQ was 5 pg/mL in plasma (Figure 1). Both quantifying ion and confirming ion were clearly detected. The RSD was 2.3% on the triplicate measurements at the LLOQ level, demonstrating superior reproducibility of the LC-MS/MS workflow. The linearity range was 5–10,000 pg/mL, covering a wide range of physiological concentrations (Figure 2). The peak areas of the internal standard across 30 samples (triplicate for each calibrator) were remarkably stable with a %RSD of 6.4% (Figure 3).

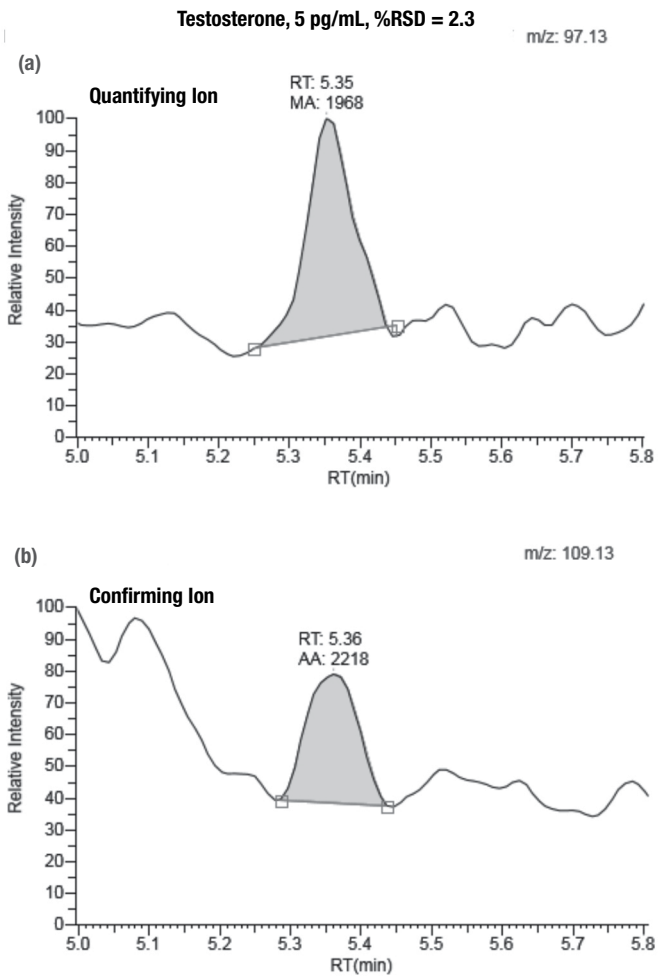

Figure 1. Chromatograms for (a) quantifying ion and (b) confirming ion at the LLOQ level, 5 pg/mL

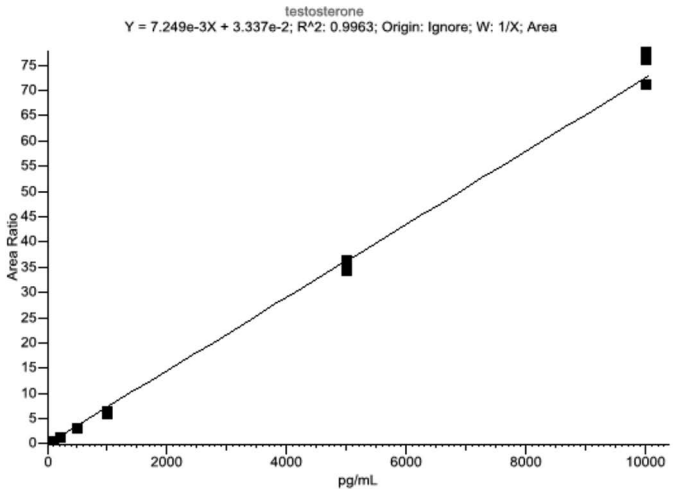

Figure 2. Calibration curve for testosterone (5–10,000 pg/mL)

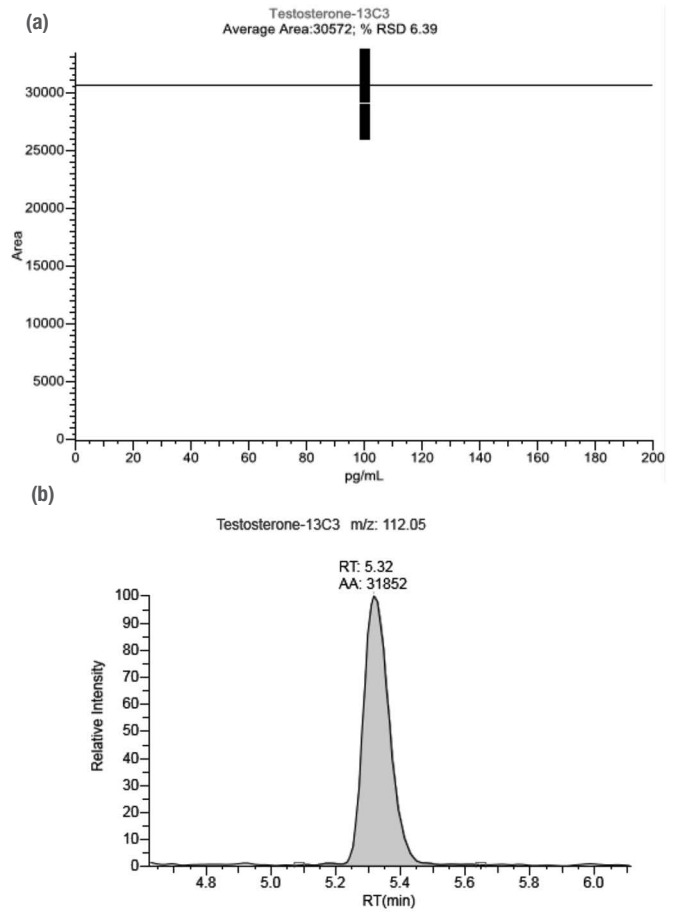

Figure 3. (a) Testosterone internal standard for reproducibility (%RSD) and (b) chromatogram for the testosterone internal standard

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Conclusion

In this study, a reliable and reproducible LC-MS/MS workflow was developed for the quantitation of testosterone in plasma. The quantitation workflow described highlights the unprecedented reliability and reproducibility that can be obtained with the Vanquish Flex Binary UHPLC and the TSQ Fortis triple quadrupole mass spectrometer.

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