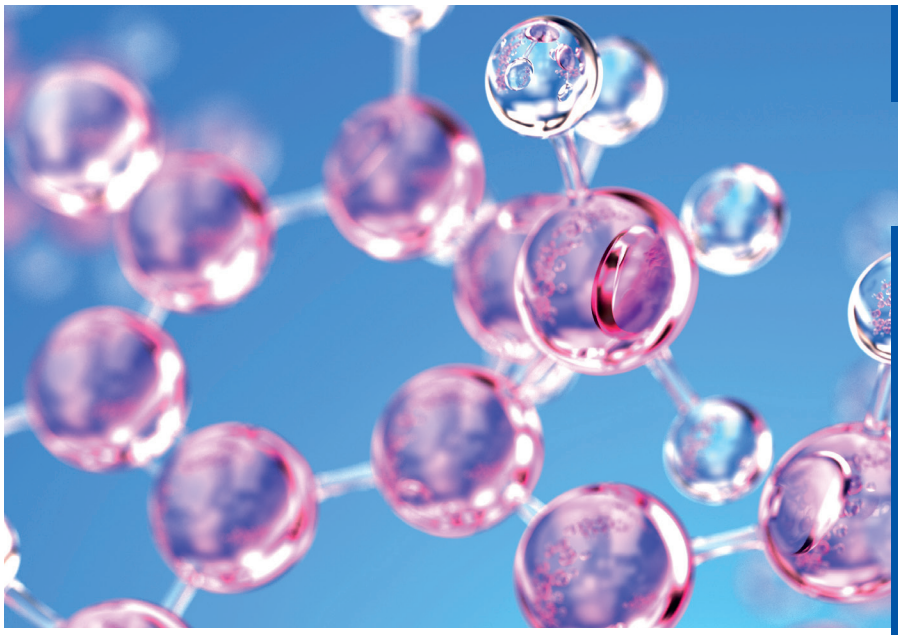

APPLICATION NOTE

1599-9831

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Testosterone Detection at Low Femtogram Levels Utilizing PerkinElmer QSight® 220 Triple Quadrupole Mass Spectrometer

Introduction

The QSight® 220 triple quadrupole mass spectrometer provides excellent sensitivity, linearity and stability and is easily able to quantify testosterone at levels necessary to perform some of the most challenging testosterone based clinical research applications.

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2. Method

Testosterone was purchased from Sigma-Aldrich (Milwaukee, WI). A stock solution (1.0 mg/mL testosterone in methanol) was prepared and stored at -15°C. Testosterone free human serum was purchased from MP Biomedical and further purified by protein precipitation with cold acetonitrile; one volume of human serum was mixed with two volumes of cold (-15°C) acetonitrile.

The mixture was vortexed for 1 min and then centrifuged for 30 min. The supernatant was transferred and used as a matrix for testosterone quantitation. The testosterone standard was spiked into clean, steroid free human serum to a concentration of 20,000 pg/mL. It was then sequentially diluted by a factor 2 to 0.61 pg/mL. There were 16 concentrations from 20,000 to 0.61 pg/mL, which were used to generate the calibration curve with three replicate injections for each concentration. All the solvents used in this application were HPLC grade.

2.1. Mass Spectrometry Conditions

Table 1: Settings used on the QSight® 220 during this method.

ESI Voltage (Volts)	5000
HSID Temp (°C)	225
Nebulizer Gas Setting	200
Drying Gas Setting	120
Heating Gas Setting	250
Source Temp. (oC)	250
Dwell Time (ms)	100
Pause Time (ms)	5

Quick Facts:

- Method for high sensitivity quantitation of testosterone in human serum using the PerkinElmer QSight® 220 triple quad mass spec.
- MRM analysis quantitates testosterone levels in the low femtogram range at 5 µL injections volumes.
- Dual Source ESI option for high throughput

The Q1 and Q2 mass filters were set to unit resolution. Table 2 provides the setting for each MRM transition of testosterone.

Table 2: Selected MRM conditions for testosterone

MRM	CCE	CCL2	CCL4	EV
289.2/ 97.1	-31	-52	-54	30
289.2 /109.1	-30	-56	-58	30

2.2. LC Conditions

Separation was done using HPLC separation and a Fortis C18 2X50 mm column (3µm) with 0.5 ml/min of 25/75 water/methanol, 0.4mM ammonium formate and 0.1% formic acid. 5µL of each sample was injected onto the column.

3. Results

3.1. Extracted Ion Chromatogram (EIC)

The EIC of LC-MS/MS for 289.2/97.1 and 289.2/109.1 in testosterone free human serum confirmed that no testosterone was detected (Figure 1A and 1C). LLOQ concentration (1.2 pg/mL in human serum), EICs for both MRM transitions are shown in Figure 1B and 1D.

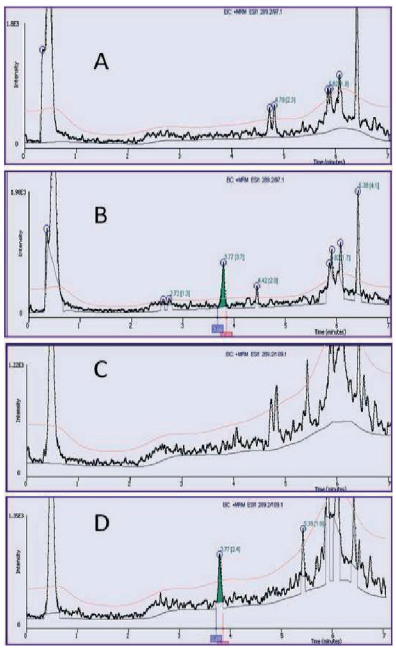


Figure 1:
(A) 289.2/97.1 EIC of human serum blank.

(B) 289.2/97.1 EIC of 1.2 pg/mL testosterone in human serum.

(C) 289.2/109.1 EIC of human serum blank.

(D) 289.2/109.1 EIC of 1.2 pg/mL testosterone in human serum.

3.2. Linearity

The calibration curve was generated with peak area based on a linear regression (a weighting factor of 1/x). The regression from 0.00061 to 1.25 ng/mL with 12 different concentrations shows good linearity with an R^2 of 0.99993 for both MRM transitions. Two calibration curves for MRM transition at 289.2 to 97.1 and 289.2 to 109.1 were shown in Figure 2.

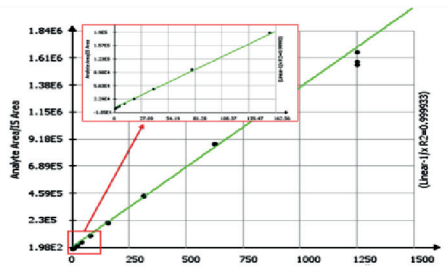
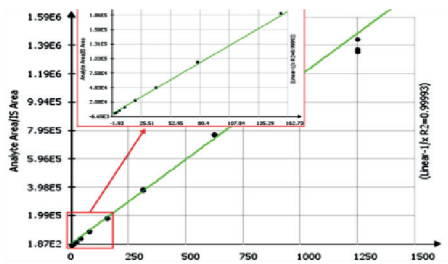


Figure 2:
(A) The calibration curve for 289.2/97.1 with a weighting factor(1/x), R^2= 0.99993



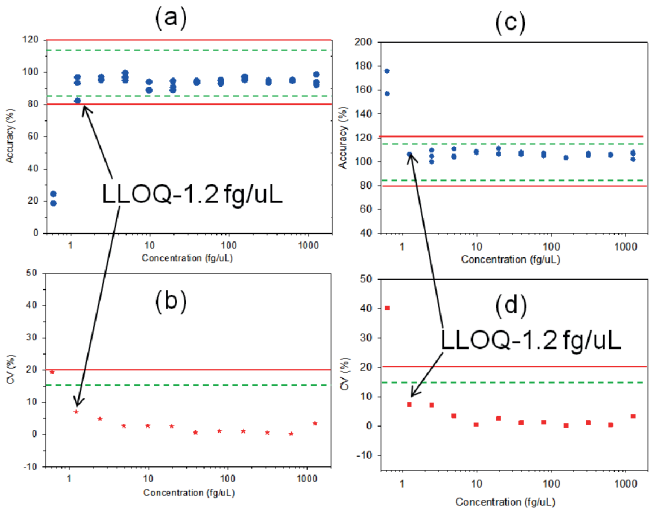
(B) The calibration curve for 289.2/109.1 with a weighting factor (1/x), R^2 = 0.99993

3.3. Quantitation Results

With the testosterone standard spiked in human serum, the LLOQ obtained from both 289.2/97.1 and 289.2/109.1 MRM transitions was 1.2 pg/mL.

Accuracy and CV for the 289.2/97.1 MRM was 90.8% and 7.1% while an accuracy of 106.2% and a CV of 7.3% were obtained for the 289.2/109.1 MRM transition.

Figure 3: (a) The accuracy changes for MRM at 289.2-97.1, (b) CV % changes for MRM at 289.2-97.1, (c) The accuracy changes for MRM at 289.2-109.1, (d) CV % changes for MRM at 289.2-109.1



The accuracy of the 289.2/97.1 MRM transition with concentrations from 2.5 pg/mL to 1250 pg/mL ranged from 88.2 to 99.0%. In the same concentration CV ranged from 0.2 to 4.8 %.

The 289.2/109.1 MRM transition had an accuracy ranging from 100 to 111% within the concentration range of 2.5 pg/mL to 1250 pg/mL. CV values for this concentration range changed from 0.2 to 3.5%, as shown in Figure 3.

4. Conclusion

A fast, sensitive LC-MS/MS method for the PerkinElmer QSight® 220 triple quadrupole mass spectrometer which achieves an LLOQ of 1.2 pg/mL for testosterone in human serum has been established. The QSight® 220 triple quadrupole mass spectrometer provides excellent sensitivity, linearity and stability and is easily able to quantify testosterone at levels necessary to perform some of the most challenging testosterone based clinical research applications.

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