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Quantification of Testosterone and Dihydrotestosterone in Human Plasma Using QTRAP 6500 Systems

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Overview

Using liquid-liquid extraction procedure, a sensitive and specific liquid chromatographic-tandem mass spectrometric (LC/MS/MS) method capable of quantifying Testosterone and Dihydrotestosterone in human plasma is described.

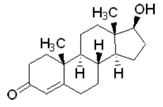
In this method, the drug was extracted from a 0.2 mL of human plasma using simple extraction method. Separation was performed on a reverse phase C8 column. Detection was achieved using a QTRAP 6500 system in the positive ion mode along with multiple reaction monitoring (MRM). The lower limit of quantitation was 0.02 ng/mL .

This method has been successfully applied to clinical pharmacokinetic studies.

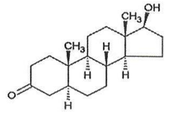
Introduction

In recent years, some analytical methods for testosterone (T) and its active metabolite dihydrotestosterone (DHT) have been developed for clinical studies, but most LC-MS/MS methods have issues due to lower specificity, higher background noise, and poor chromatograms. In this study, the positive ESI MRM mode on QTAP 6500 Systems was used to modify our previous T and DHT quantitation method in human K2 EDTA plasma with higher specificity and lower background noise.

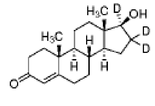
Structure



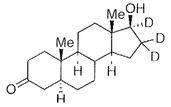
Testosterone



Dihydrotestosterone



Testosterone-d3 (IS-1)



Dihydrotestosterone-d3(IS-2)

Methods

Sample Preparation:

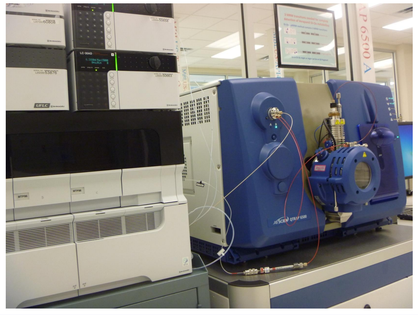
Plasma samples were extracted by using the 200-µL aliquot of plasma. After extraction, the extracts must be centrifuged at 14,000 rpm for about at least 10 minutes. The extract was then transferred to LC vials for LC-MS/MS or stay in the 96-well plate for the analysis.

Liquid Chromatography:

Pump: Shimadzu LC-30AD
Autosampler: Shimadzu SIL-30ACMP
System Controller: Shimadzu CBM-20A
Analytical Column: Kinetex, 2.6µ PFP, 100A, 100 x 2.1 mm, 5 µm
Gradient: The analytes were eluted using a gradient of mobile phase A (0.1% formic acid) and mobile phase B (Methanol:0.5mM ammonium formate in water, 90;10, v;v) from 75% to 95% mobile phase B in 3.0 min.
Injection Volume: 15 µL

Mass Spectrometry:

MS System: AB/Sciex QTRAP 6500
Condition: LC/(+)ESI-MS/MS,
MRM transition:
Testosterone: 289.3→97.0
DHT: 291.2→255.0



Results and Discussion

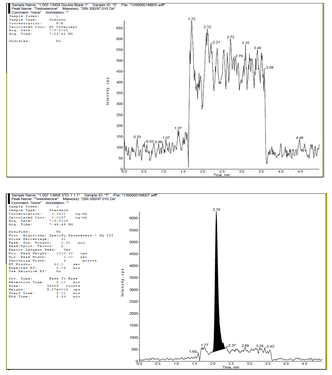


Figure 1. Ion chromatograms of blank plasma (Upper), and 0.02 ng/mL Testosterone extracted from plasma (Lower) with Unit Resolution

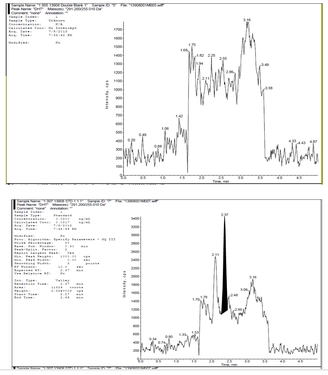


Figure 2. Ion chromatograms of blank plasma (Upper), and 0.02 ng/mL DHT extracted from plasma (Lower) with Unit Resolution

Table I. Validation Data Summary for Testosterone

Calibration Range		0.02 to 10 ng/mL		
Correlation coefficient(r^2, mean)		0.9985		
Accuracy & Precision			Accuracy	Precision
	QC	Conc. (ng/mL)	RE%	CV%
Inter-Batch (n=18)	LLOQ	0.02	3.5	5.0
	Low	0.06	-7.5	4.7
	Medium	0.4	0.3	1.2
	High	8	2.9	2.2
	Mean of internal standard normalised matrix effect			
Matrix Effect	0.98 to 0.99			
		Condition		Accuracy RE%
Freeze/Thaw		3 Cycles, <-70 ºC		<4.1
Bench-Top		4 hrs, Room Temperature		<7.2
Autosampler Extract Stability		53 hrs, Room Temperature		<8.1
Long-Term Storage Stability		31 Days, <-70 ºC		<8.9

Table II. Validation Data Summary for Dihydrotestosterone

Calibration Range		0.02 to 10 ng/mL		
Correlation coefficient(r^2, mean)		0.9966		
Accuracy & Precision			Accuracy	Precision
	QC	Conc. (ng/mL)	RE%	CV%
Inter-Batch (n=18)	LLOQ	0.5	2.0	12.2
	Low	1.5	-7.0	7.4
	Medium	40	-0.5	2.5
	High	400	2.4	2.4
	Mean of internal standard normalised matrix effect			
Matrix Effect	0.86 to 1.04			
		Condition		Accuracy RE%
Freeze/Thaw		3 Cycles, <-70 ºC		<5.5
Bench-Top		4 hrs, Room Temperature		<8.2
Autosampler Extract Stability		53 hrs, Room Temperature		<10.5
Long-Term Storage Stability		31 Days, <-70 ºC		<9.2

• Excellent linearity was obtained with a correlation coefficient ≥ 0.9957 for Testosterone and ≥ 0.9934 for Dihydrotestosterone. The high dynamic calibration range was reached due to eliminated background noise. (Table I, II Figures 1 to 3).

• For Testosterone, including LLOQ, the inter-day CV ranged from 1.2% to 5.0% and the biases of the means ranged from -7.5% to 3.5%. For Dihydrotestosterone, including LLOQ, the inter-day CV ranged from 2.4% to 12.2% and the biases of the means ranged from -7.0% to 2.4%. These results also indicate that the liquid-liquid extraction method is more suitable than protein precipitation extraction method for Testosterone and Dihydrotestosterone analysis in human K_2 EDTA plasma.

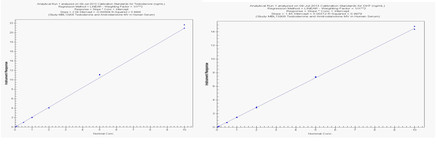


Figure 3. Typical Calibration Curve of Testosterone (Left) and Dihydrotestosterone (Right) in Human Plasma

Conclusions

A rapid, simple and specific LC-MS/MS method has been developed and validated for quantifying Testosterone and Dihydrotestosterone with a lower limit of quantitation of 0.02 ng/mL from a 0.2 mL plasma sample.