

Fast and Selective Quantification of Testosterone in Plasma using LDTD-QTrap 5500 SelexION System

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Introduction

Testosterone in plasma is a molecule that is frequently analyzed in Clinical labs. This analysis is generally performed on LC-MS/MS systems, requiring run-times as long as 15 minutes.

Laser Diode Thermal Desorption technology performs indirect sample vaporization into gas phase followed by ionization through APCI, providing fast analysis without the use of any solvent. Using such a shotgun approach for testosterone has a high potential of cross-talk interference due to similar structure and the molecular weight is a challenge. Using the SelexION™ system on AB SCIEX QTrap® 5500 we can perform “Electronic chromatography” to obtain adequate specificity for quantitation.

LDTD-MS/MS System



Figure 1 LDTD interfaced to AB SCIEX QTrap® 5500 SelexION™ System

Sample Preparation

Extraction procedure

- 100 µL plasma sample
- 20 µL IS (10 ng/mL Testosterone-d3 in MeOH)
- 300 µL NaOH (0.1N in Water)
 - Mix
- 800 µL Methyl-Tert-butyl ether (MTBE)
 - Mix and centrifuge (2 min. / 14000 rpm)
- Transfer 600 µL of upper layer
 - Evaporate to dryness
- Reconstitute with 40 µL MeOH/H₂O (75/25)
- Spot 4 µL in Lazwell plate
 - Evaporate to dryness

LDTD-MS/MS Parameters

LDTD

Gas Flow:	3 L/min	
Laser pattern:	Time (s)	Power (%)
	0	0
	2	0
	5	45
	7	45
	7.1	0
	8	0

MS/MS Method

	Transition	CE	DP
Testosterone	289->97	25	100
Testosterone-d3	292->97	25	100
Mode:	Positive		

The differential mobility separation (DMS) parameters for the source are: No modifier (MD), Separation Voltage (SV) at 4300, Compensation voltage (COV) at 14, DMS offset (DMO) at -10 and DMS temperature (DT) at high.

Results and Discussion

Linearity Results

As shown in **Figure 2**, excellent linearity ($r^2 > 0.99$) with no sign of carryover effect is achieved for Testosterone in the quantification range (0.1 to 10 ng/mL).

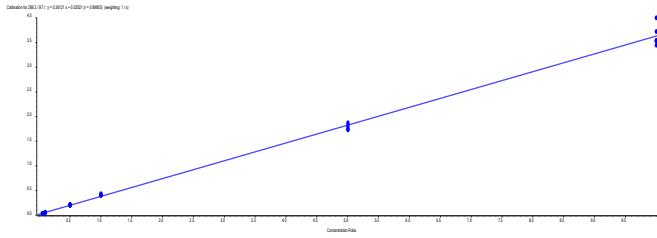


Figure 2: Testosterone standard curve

Accuracy and Precision

As shown in **Table 1**, the inter-run accuracy and precision are between 95.5 to 108.7% and 3.1 to 13.1%, respectively.

	LLOQ	QC-Low	QC-Med	QC-High	ULOQ
Conc. (ng/mL)	0.1	0.5	1	5	10
N	6	6	6	6	6
Mean (ng/mL)	0.10	0.53	1.09	4.89	10.00
%RSD	13.1	5.4	3.3	3.1	5.6
%Nom	95.5	105.3	108.7	97.9	100.0

Table 1: Inter-run precision and accuracy for Testosterone

44 real patient plasma samples have been tested with this method to correlate with LC-MS/MS results. **Figure 3** shows a correlation >95% between results using both methods.

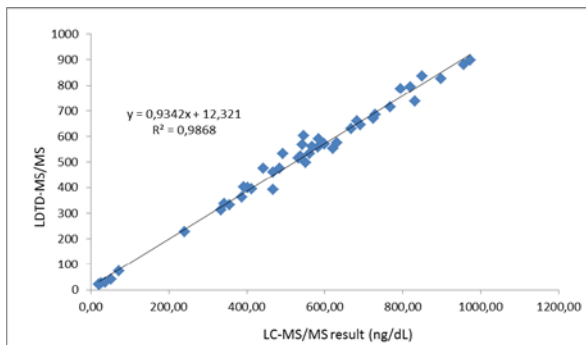


Figure 3: Correlation between Testosterone concentrations in real plasma samples obtained with LDTD-MS/MS and LC-MS/MS

Stability Verification

We analyzed a standard curve at the initial time with 5 real samples. After 6h and 24h, QC samples and real samples were re-extracted and their concentrations were determined using the initial standard curve. As shown in **Table 2**, the concentration after 6h and 24h has less than 20% difference compared to the initial value.

	Initial time	Re-extraction after 6h		Re-extraction after 24h	
	Conc (ng/mL)	Conc (ng/mL)	%Diff	Conc (ng/mL)	%Diff
QC	1	1,14	14,3	1,17	17,0
Sample 1	0,16	0,15	-4,2	0,17	6,2
Sample 2	0,16	0,15	-4,2	0,15	-9,4
Sample 3	3,75	3,93	4,7	4,03	7,3
Sample 4	0,42	0,41	-3,2	0,38	-9,5
Sample 5	3,19	3,28	2,7	3,60	12,9

Table 2: Stability Results for Testosterone

Conclusion

The High-sensitivity of the QTrap® 5500 System with the specificity offered by the SeleXION™ technology combined with the high-throughput LDTD™ Ion Source, we achieved an ultra-fast Testosterone quantification method running a sample **every 8 seconds**. Sample preparation consists of a liquid-liquid extraction. Excellent correlation of results with the LC-MS/MS method was obtained. Re-extraction and quantification using the initial curve can be done within 24h as validated.

For more information about your specific application, visit www.phytronix.com

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