A Rapid and Selective Method for the Measurement of Testosterone in Human Serum in Less than 10 Seconds using Laser Diode Thermal Desorption-Differential Ion Mobility-Tandem MS

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Michael J. Y. Jarvis[1], Evelyn L. McClure[1], Pierre Picard[2], Serge Auger[2], Gregory Blachon[2], Julianne Botelho[3] and Yuesong Wang[3]

[1]AB SCIEX, Concord, Canada; [2]Phytronix Technologies Inc., Québec City, Canada; [3]Centers for Disease Control and Prevention, Atlanta, GA, USA

INTRODUCTION

It has been well established that liquid chromatography-tandem mass spectrometry (LC-MS/MS) provides excellent accuracy, precision and sensitivity for measurements of steroids in biological matrices compared to traditional techniques such as immunoassays, which may suffer from cross-reactivity. However, a limitation of LC-MS/MS for steroid research is the comparatively low throughput of the measurements, due to the need for chromatographic separations.

An alternative sample introduction technique is the Phytronix Laser Diode Thermal Desorption (LDTD) ionization source, which combines sample introduction with APCI ionization and allows for rapid (<10 seconds sample-to-sample) analysis. Sample prepared by typical extraction procedures (e.g., SPE or LLE) is spotted in small volumes on a LazWell™ plate, allowed to dry, and then placed in the LDTD source for analysis. Using an IR laser diode, each sample is thermally desorbed from the plate well and then ionized before analysis by MS/MS. LDTD does not provide separation of sample components, and as a result the SelexION™ differential ion mobility device was used prior to the mass analyzer in order to achieve interference-free analysis of testosterone.

In this work we present a rapid method for the measurement of testosterone in human serum using a combination of Laser Diode Thermal Desorption (LDTD) ionization, differential ion mobility spectrometry, and tandem mass spectrometry. The method was successfully used to perform quantitation of testosterone in human plasma with no chromatographic separation and without interference from isobars. The data is compared directly to values obtained for the same samples analyzed using an established LC-MS/MS method.

Figure 2. The SelexION™ differential ion mobility (DMS) device provides an orthogonal means of filtering ions prior to analysis by MS/MS. DMS hardware can be installed quickly and easily and requires no tools or breaking of vacuum.

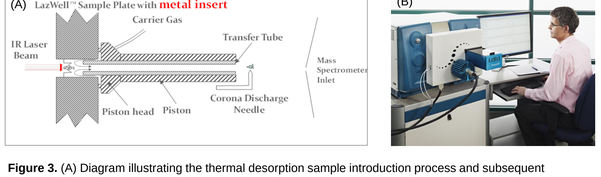


Figure 3. (A) Diagram illustrating the thermal desorption sample introduction process and subsequent ionization at the corona discharge needle. (B) The LDTD ionization source combines sample introduction and ionization to allow for rapid sample analysis.

MATERIALS AND METHODS

Sample Preparation:

- The sample preparation consisted of a simple liquid-liquid extraction, using NaOH and MtBE, followed by dry-down and reconstitution of the sample.
- 100µL of plasma was measured into a 1.5mL polypropylene microcentrifuge tube.
- 20µL of internal standard solution (Testosterone-d3) was added to each tube and the mixture was vortex mixed.
- 300µL of NaOH and 800µL of MtBE were added to each sample. After each addition, the vial was vortex mixed for 10s and 30s, respectively.
- The samples were centrifuged at 14,000 rpm for approximately 1 minute.
- 600µL of the supernatant was transferred into a clean 1.5mL microcentrifuge tube, and dried down under nitrogen gas at ambient temperature.
- The dried sample was reconstituted using 40µL of 75:25v/v methanol:water, vortexed for 10s, centrifuged for 1 min at 14, 000 rpm and then spotted on a LazWell plate (5 µL).

LDTD Conditions:

The Phytronix LDTD ionization source was operated with a transport gas flow of 3.0 L/minute and a laser pattern that consisted of a ramp from 0 to 45% laser power over 3 seconds, and a hold at 45% for 2 seconds.

SelexION™ Conditions:

The SelexION™ DMS device was operated using a Separation Voltage (SV) of 4300V and a Compensation Voltage (COV) of 14V. No solvent modifier was used for the analysis.

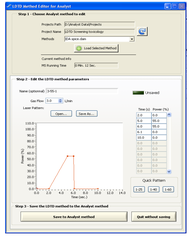
MS/MS Conditions:

MS/MS detection was accomplished using the AB SCIEX Triple Quad™ 5500 LC/MS/MS system. The Multiple Reaction Monitoring (MRM) mode of operation was used, with 2 MRM transitions monitored for testosterone. The deuterated analogue of testosterone was used as an internal standard. The MRM transitions are summarized in Table 1.

Table 1. MRM Transitions for target compounds.

Name	Q1	Q3
Testosterone 1	289.3	97.1
Testosterone 2	289.3	109.1
Testosterone -d3 1	292.2	97.1
Testosterone-d3 2	292.2	109.1

Figure 3. Example of LDTD laser pattern.



RESULTS

A rapid, 10 second LDTD-DMS-MS/MS method has been developed for the quantitation of testosterone in plasma and serum, using the AB SCIEX Triple Quad™ 5500 LC/MS/MS system , the Phytronix LDTD ionization source and the SelexION™ differential ion mobility (DMS) device.

Using an optimized ramped laser pattern to thermally desorb dried sample from a 96 well LazWell™ plate, the analyte of interest was then selectively analyzed by MS/MS following orthogonal separation in the SelexION™ differential ion mobility device. The DMS was operated at separation and compensation voltages that were optimized for testosterone. The solvent-less sample introduction and ionization process enabled the use of high separation voltages, maximizing the capability of the DMS to provide the highest possible selectivity. Analysis of a matrix blank under these operating conditions confirmed the absence of isobaric interferences (Figure 4).

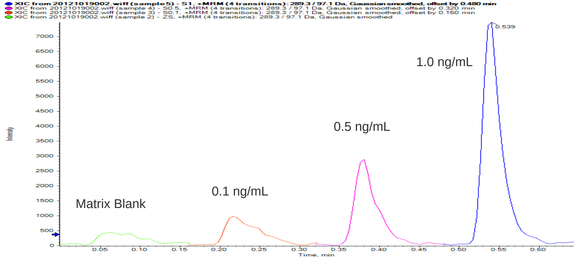


Figure 4. Representative LDTD-DMS-MS/MS data. Several calibration concentrations are shown in addition to a matrix blank , which confirms the absence of endogenous interferences.

Performance of the method was evaluated using spiked double charcoal stripped serum covering a concentration range of 0.1 – 100 ng/mL. Initial efforts yielded a LLOQ of approximated 0.5 ng/mL, which is sufficient for the analysis of typical plasma samples. Further improvements to the sample extraction procedure allowed for a decreased LLOQ of approximately 0.1 ng/mL.. A representative calibration curve is shown in Figure 5. The accuracy of the measurements for each analyte ranged from 94-104% over the entire concentration range, including at the LLOQ. The method displayed excellent linearity over the concentration range from 0.5-10 ng/mL, with r>0.999.

To demonstrate the application of this method, analysis of testosterone in 24 plasma samples was performed using the sample preparation and instrumental conditions already described. Each sample was analyzed in triplicate. Testosterone levels for these same samples had been determined previously using an established LC-MS/MS method, thus allowing for a direct comparison of the two analytical techniques.

Table 2 summarizes the concentrations (ng/mL) determined in the plasma samples by both techniques. Concentrations ranging from 0.722 – 9.73 ng/mL and 0.74 – 9.14 ng/mL were measured in the samples for the LC-MS/MS method and LDTD-DMS-MS/MS method, respectively. Agreement between the two sets of values was excellent, with accuracies between 90 – 110%. The average accuracy (n=24) was 97%, indicating a strong agreement between the two methods and demonstrating the use of the LDTD-DMS-MS/MS technology as an alternative approach for the analysis of testosterone in human plasma.

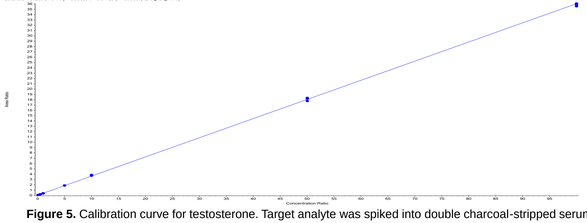


Figure 5. Calibration curve for testosterone. Target analyte was spiked into double charcoal-stripped serum samples at levels ranging from 0.5 – 100 ng/mL.

LC-MS/MS at CDC			289/97 Measured by LDTD-DMS-MS/MS (ng/mL)								
Sample ID	ng/dL	ng/mL	ID	No.Values	Mean	StdDev	%CV	Value1	Value2	Value3	Acc%
BRH532341	72.2	0.722	289.3 / 97.1	41 3 of 3	0.74	0.1	14.08	0.83	0.76	0.63	102%
BRH532342	560	5.6	289.3 / 97.1	42 3 of 3	5.29	0.32	6.02	5.45	5.5	4.93	94%
BRH532343	402	4.02	289.3 / 97.1	43 3 of 3	4.01	0.13	3.27	4.04	3.87	4.12	100%
BRH532344	582	5.82	289.3 / 97.1	44 3 of 3	5.57	0.16	2.96	5.43	5.52	5.75	96%
BRH532345	584	5.84	289.3 / 97.1	45 3 of 3	6.07	0.15	2.46	6.02	6.24	5.95	104%
BRH532346	412	4.12	289.3 / 97.1	46 3 of 3	4.01	0.26	6.5	3.96	3.78	4.29	97%
BRH532347	973	9.73	289.3 / 97.1	47 3 of 3	9.14	0.08	0.91	9.08	9.24	9.11	94%
BRH532348	546	5.46	289.3 / 97.1	48 3 of 3	5.98	0.21	3.52	6.22	5.9	5.82	110%
BRH532349	849	8.49	289.3 / 97.1	49 3 of 3	8.29	0.09	1.07	8.36	8.33	8.19	98%
BRH532350	542	5.42	289.3 / 97.1	50 3 of 3	5.6	0.23	4.13	5.86	5.49	5.44	103%
BRH532351	393	3.93	289.3 / 97.1	51 3 of 3	4.01	0.22	5.44	3.92	3.85	4.26	102%
BRH532352	794	7.94	289.3 / 97.1	52 3 of 3	7.67	0.3	3.9	8	7.41	7.6	97%
BRH532353	956	9.56	289.3 / 97.1	53 3 of 3	8.83	0.58	6.59	8.64	8.36	9.48	92%
BRH532354	334	3.34	289.3 / 97.1	54 3 of 3	3.18	0.25	7.9	2.9	3.4	3.24	95%
BRH532355	725	7.25	289.3 / 97.1	55 3 of 3	6.84	0.03	0.37	6.82	6.87	6.83	94%
BRH532356	691	6.91	289.3 / 97.1	56 3 of 3	6.48	0.05	0.8	6.47	6.54	6.43	94%
BRH532357	729	7.29	289.3 / 97.1	57 3 of 3	6.87	0.06	0.93	6.92	6.8	6.9	94%
BRH532358	551	5.51	289.3 / 97.1	58 3 of 3	4.94	0.29	5.78	5.26	4.74	4.81	90%
BRH532359	343	3.43	289.3 / 97.1	59 3 of 3	3.28	0.04	1.34	3.23	3.32	3.28	96%
BRH532360	537	5.37	289.3 / 97.1	60 3 of 3	5.33	0.12	2.23	5.35	5.44	5.2	99%
BRH532361	567	5.67	289.3 / 97.1	61 3 of 3	5.44	0.11	1.99	5.35	5.56	5.41	96%
BRH532362	600	6	289.3 / 97.1	62 3 of 3	5.64	0.44	7.74	5.27	5.52	6.12	94%
BRH532363	820	8.2	289.3 / 97.1	63 3 of 3	8.07	1.15	14.19	9.35	7.7	7.16	98%
BRH532364	321	3.21									
BRH532365	240	2.4	289.3 / 97.1	65 3 of 3	2.37	0.12	5.13	2.28	2.31	2.5	99%

Table 2. Comparison of measured testosterone levels using an established LC-MS/MS method versus a LDTD-DMS-MS/MS approach. Measured values were in close agreement between the two methods, with an average accuracy of 97% (n=24). Precision for the LDTD-DMS-MS/MS method was excellent with a %CV of less than 14% (n=3) for all measurements.

CONCLUSIONS

A rapid LDTD-DMS-MS/MS method has been developed to allow the quantitation of testosterone in plasma with a sample-to-sample analysis time of 10 seconds. Liquid-liquid extraction with NaOH/MtBE is used to clean up the plasma samples prior to analysis using the AB SCIEX Triple Quad™ 5500 LC/MS/MS system, the SelexION™ differential ion mobility device and the Phytronix Laser Diode Thermal Desorption ionization source. Comparison of measured testosterone values obtained using a conventional LC-MS/MS method and the LDTD-DMS-MS/MS method showed excellent agreement between the two. The application of the LDTD-DMS-MS/MS method to the measurement of testosterone in plasma has been demonstrated to be a selective, accurate and rapid approach for this analysis.

TRADEMARKS/LICENSING