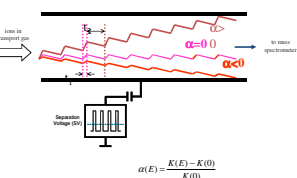


Pierre Picard¹, Jean Lacoursiere¹, Patrice Tremblay¹, Michael Jarvis², Hesham Ghobarah², Adrian Taylor²
¹Phytronix Technologies Inc., Quebec, Canada ²AB SCIEX, Ontario, Canada

OVERVIEW

In the past, the lower limit of quantification (LLOQ) of testosterone was limited by the presence of isobaric co-eluting analytes that required a significantly elongated run-time to achieve a high efficiency separation via LC-MS/MS. With the innovative combination of the Laser Diode Thermal Desorption (LDTD)TM ionization source coupled with the SelexIONTM differential ion mobility cell on a MS/MS, we have achieved a high-throughput analysis method for testosterone with a LLOQ of 0.1 ng/ml and a sample-to-sample analysis time of 7 seconds. The sample preparation for plasma consists of a liquid-liquid extraction with ethyl acetate.

- Use the dependence of ion mobility K on electric field strength E
- High-field mobility $K(E)$ and low-field mobility $K(0)$ is molecule dependent
- Molecular Trajectories are Electronically tunable
- Drift gas composition influence mobility K
- Any ion can be steered back onto the center-line, by application of a compound-specific DC Compensation Voltage (CV)
- Result: **Increased Selectivity**



Instrumentation

- LDTD model S-960, Phytronix Technologies
- QTRAP® 5500 Systems with SelexIONTM technology, AB SCIEX

LDTD Parameters

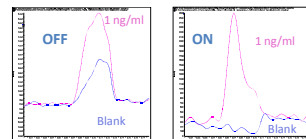
- Laser power pattern :
 - Increase laser power to 45 % in 3.0 s
 - Decrease laser power to 0 %
- Carrier gas flow : 3 L/min (Air)
- Deposited sample volume: 2 µL

MS Parameters

- APCI (+)
- Scan time : 0.025 s
- DP: 100
- CE: 30 eV
- CVM: 4 V
- SV: 3500 V
- MRM:
 - Testosterone: 289.1 → 97.1 and 109.1
 - Testosterone -d3: 292.1 → 97.1

RESULTS:

Effect of DMS on LDTD-MS/MS



- Blank interference pass from 45.6% to nearly zero
- Loss of intensity 25x but major gain in S/N

Effect of DMS: Steroid panel



INTRODUCTION

LDTDTM Ionization Source:

The LDTD uses a Laser Diode to produce and control heat on the sample support (Figure 1) which is a 96 well plate. The energy is then transferred through the sample holder to the dry sample which vaporizes prior to being carried by a gas in a corona discharge region. High efficiency protonation with strong resistance to ionic suppression characterize this type of ionization, and are the result of the absence of solvent and mobile phase. This allows for very high throughput capabilities of 7 second sample-to-sample analysis time, without carry over.

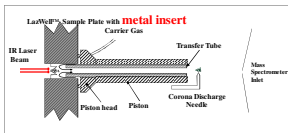


Figure 1 Schematic of the LDTD ionization source.

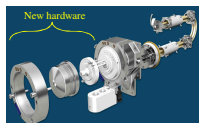


Figure 2a

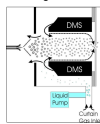


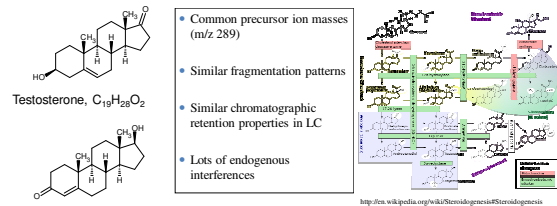
Figure 2b

SelexIONTM technology:

The SelexIONTM technology is a Differential Mobility Spectrometer (DMS) placed in front of the inlet of the mass spectrometer (Figure 2a). The ionized molecules travel into the orthogonal geometry shaped DMS (Figure 2b).

Challenges for the Analysis of Steroids

- Common precursor ion masses (m/z 289)
- Similar fragmentation patterns
- Similar chromatographic retention properties in LC
- Lots of endogenous interferences



METHOD

Solutions for shotgun approach

- Work on sample preparation:
- Similar chemical properties
 - Extensive
 - Remaining blank interference limiting LLOQ at 1 ng/ml*

OR

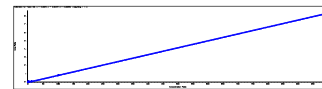
Use "Electronic chromatography" with DMS

Sample preparation

- Gain in Specificity with DMS allows a simple preparation:
- Spike standard curve in stripped plasma
 - Liquid-liquid extraction with MTBE 1:4 v/v ratio
 - Vortex 10 seconds
 - 2 µl of the upper layer directly spotted onto the Lazwell plate
 - Dry at room temperature

Plasma analysis Results

- High sensitivity with LOQ at 0.1 ng/ml (50 femtogram on plate)
- Excellent linearity, with $r^2 = 0.99972$, over 5 orders of magnitude
- Accuracy and reproducibility were within the accepted values as shown in the adjacent table.
- The sample-to-sample run time was only 7 seconds. In comparison, the equivalent analysis using conventional LC-MS/MS would typically require approximately 3-5 minutes per sample



Expected Concentration (ng/ml)	Mean Calculated Concentration	% CV	Standard Deviation	% Accuracy	Std. plant. (ng/ml)
0.1	0.0626	65.1	0.1796	62.72	0.05
1.0	1.0130	0.4	0.506	101.3	0.5
10	9.4860	2.3	0.850	94.9	5
100	113.81	1.9	7.799	113.8	50
1000	1050.3	2.2	23.46	105.0	500

CONCLUSIONS

- LDTDTM ion source coupled to SelexIONTM DMS on a MS/MS achieves a Testosterone LLOQ of 0.1 ng/ml
- SelexIONTM DMS increases selectivity and helps in removing isobaric interferences
- LDTDTM provides the High-Throughput analysis of testosterone in **7 seconds sample-to-sample**