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Improving targeted screening and quantitation performance using state-of-the art triple quadrupole mass spectrometer

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ABSTRACT

The collective goal of the project is to evaluate new ion source parameters to determine better settings to boost ionization efficiency and transmission into the Thermo Fisher Scientific™ TSQ Altis™ Plus mass spectrometer to reach the targeted limits of detection (LOD) and limits of quantitation (LOQ) for a set of steroids and anti-doping compounds in biological matrices to best simulate real world examples. The results demonstrated utilizing new parameter considerations as well as the ranges in which to test known ion source components that, when combined resulted in significant increases to the measured responses for the targeted compounds in the study. The improved transmission efficiency enabled employment of H-SRM acquisition that did show improved LODs for a portion of the compounds. The next step will be to evaluate FAIMS selectivity that could also improve signal-to-noise (S/N) despite reducing the overall transmission efficiency

INTRODUCTION

The drive to increase experimental performance for LC-MS/MS steroid screening and quantitation remains challenging due to the chemical structure and physicochemical properties. These can impact ionization efficiency or cation (e.g., Na+) clustering resulting in poorer detection and quantitation levels while still having to overcome biological matrix interference. Thus, new methods are continually being developed and evaluated to push sensitivity by employing new combination of ESI source parameters in combination with the Q1 mass analyzer with hyperbolic rods and the new Q2 collision cell with the goal to extend the detection and quantification ranges in common biological matrices.

MATERIALS AND METHODS

All experiments were performed on a Thermo Fisher Scientific™ Vanquish™ Flex Binary UHPLC system coupled to a TSQ Altis™ Plus mass spectrometer. Binary mobile phase consisted of A) 0.1% HCOOH and B) 90:10:0.1% MeOH/H2O/HCOOH and a linear gradient from 50-72.5% B in 6 minutes flowing at 0.4 mL/min. All chromatographic separations were performed using a HALO® 160 A ES-C18 column with dimensions of 2.1 x 50 mm and 2.7 µm particles.

The set of steroids included: dihydrotestosterone (DHT), testosterone, dehydroepiandrosterone, cortisol and aldosterone as well as anti-doping compounds (8 in all) spiked into neat solvents, urine and serum extracts. All of the target compounds were measured in one method. The initial analysis of the target compounds in neat solvents was performed to determine the matrix suppression per compound per matrix. All steroid standards were made into a stock solution and were diluted into the specified medium to make a standard dilution curve. Each steroid had an internal standard spiked at consistent levels throughout the entire study.

The standard DHT was used to initially evaluate the ion source conditions that were then transferred to the final method. The initial testing was conducted using the same LC injection and separation method to ensure ideal parameters for the collection of steroids. The key aspects tested for method extension (in the order applied) include: the sweep cone gas, source CID, ion transfer tube temperature, vaporizer temperature, and the auxiliary gas flow rate.

The final source settings were evaluated on comparative quantitation curves using “normal” source conditions vs. the optimized settings. Quantitative comparisons were made using unit Q1 resolution setting (0.7 Th) and H-SRM (0.2 Th) resolution to evaluate the improvement in signal-to-noise.

RESULTS

Figure 1. Components adjusted to improve ionization efficiency and overall signal

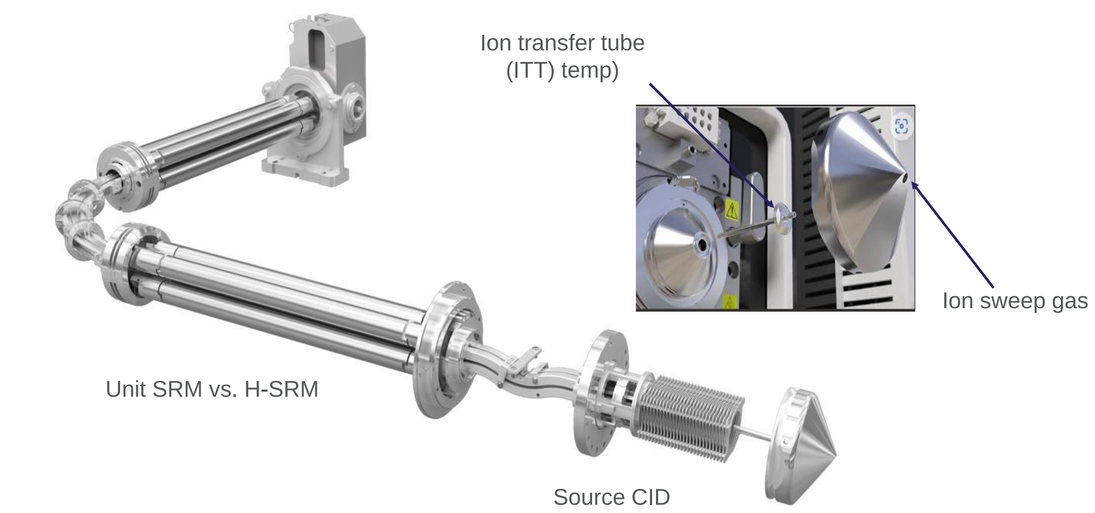


Figure 2. Evaluation of the DHT AUC response of the source CID setting. The net improvement in measured AUC values are relative to the starting parameter

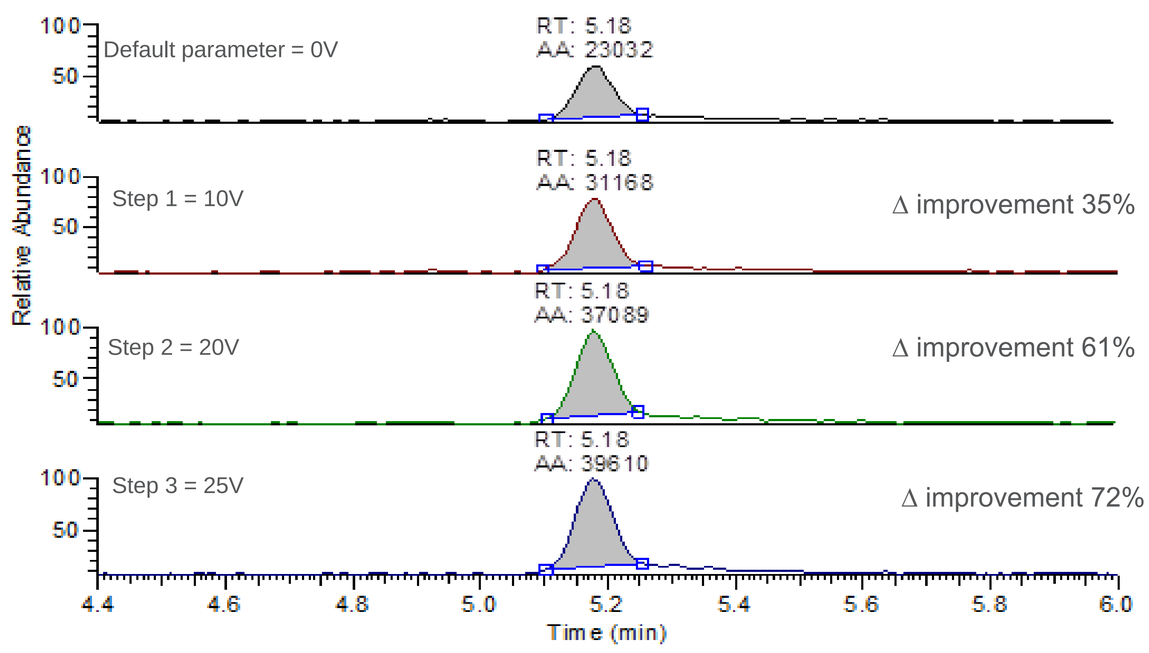
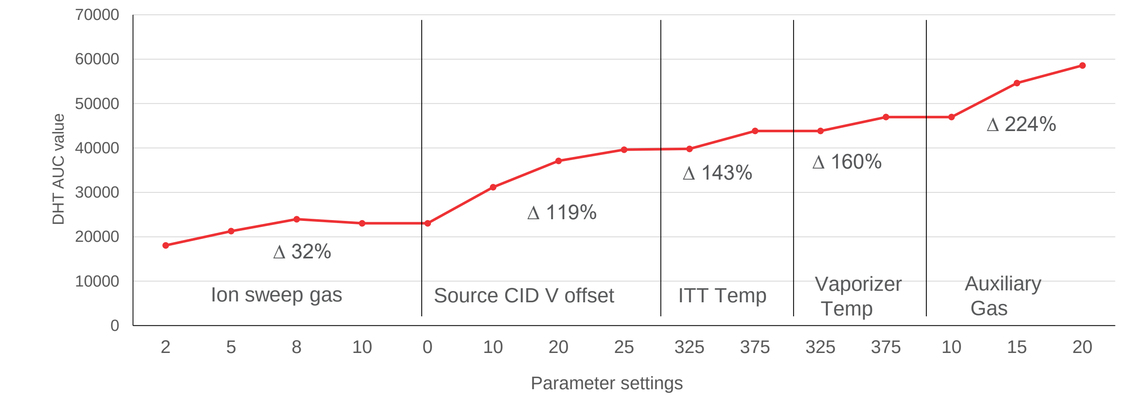


Figure 2 shows the response improvement by implementing source CID, which is generally not used to improve the signal. Steroid structures, however, are more rigid and able to make it through the source region moving from high- to low-pressure following ionization.

Figure 3. Stepwise component optimization across the five different parameters evaluated



Performing stepwise parameter optimization enables each component to boost ionization and transmission efficiency into the TSQ Altis Plus mass spectrometer. Settings used for this study go beyond the normal settings such as the ion sweep gas which is typically not set beyond 5 units. Source CID is seldom used and when it has been used is typically set no higher than 10 V, as higher settings can often result in precursor ion fragmentation. The combined parameters results in over a 3-fold increase in response.

Figure 4. Low-level DHT sample analysis in serum extract

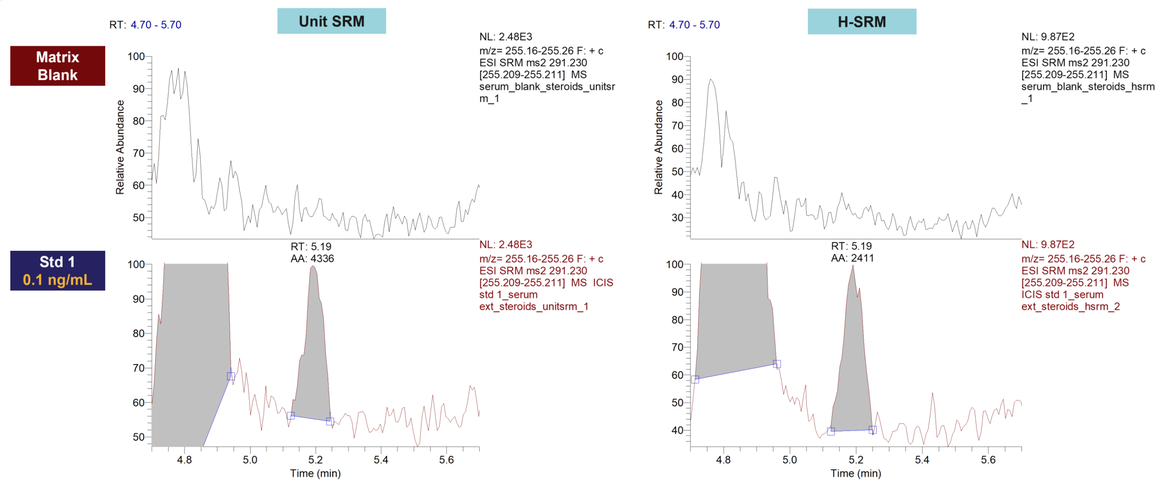
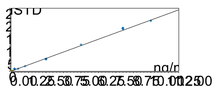


Figure 5. Quantitation curve for DHT spiked into serum extracts. The data acquisition for the samples was performed with H-SRM on Q1.



Utilizing the new source parameters boosted DHT ionization and transmission 3-fold, enabling the reliable use of H-SRM which reduces the Q1 resolution from 0.7 Th to 0.2 Th. Due to the hyperbolic surface rods used in the TSQ Altis Plus mass spectrometer, the resulting peak profile is more rectangular than gaussian thus increasing the Q1 resolution results in a 50% AUC reduction but an increase in the overall S/N despite the intense interference compound response eluting just prior to DHT.

The mass stability of the TSQ Altis Plus MS enabled the entire data set to be acquired without having the recalibrate the instrument. The set of quantitation levels were first acquired at unit resolution, followed by a series of blanks and quality control samples prior to acquiring all of the data in H-SRM for all steroids. Estimated limits of detection (LOD) for neat standards using SRM was 25 pg/mL, using H-SRM improves the estimated DHT LOD to 16.7 pg/mL.

As shown in Figures 4 and 5, H-SRM enabled linear quantitation of DHT in serum extracts down to 100 pg/mL due to matrix suppression. While SRM also demonstrated linear quantitation (data not shown), Figure 4 shows that H-SRM was able to be measured at 100 pg/mL with a S/N_{rms} of 10 as compared to a S/N_{rms} of 6 for SRM.

Figure 6. Comparative analysis for Aldosterone spiked in neat solvents (left) at the indicated concentrations and in serum extract (right) at 60.8 pM using the optimized source conditions.

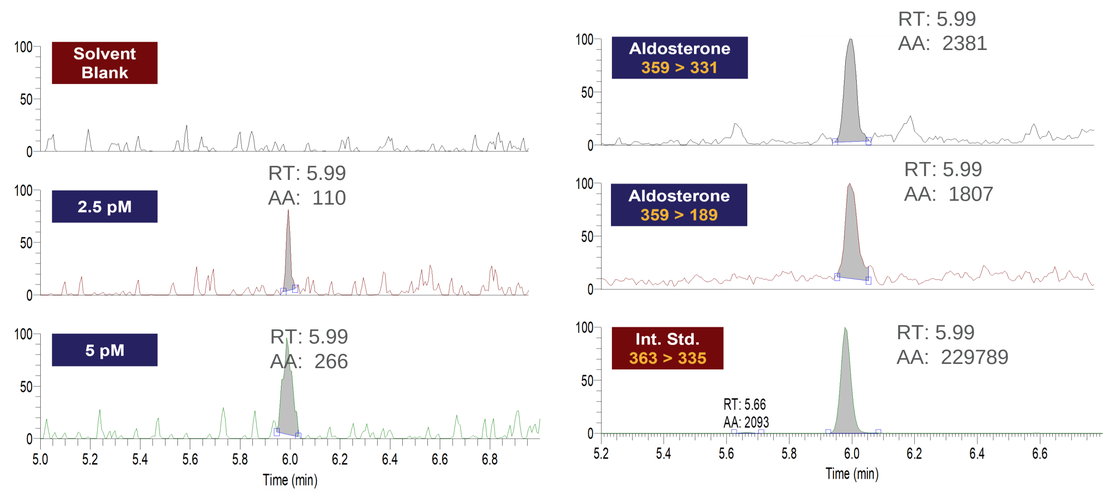
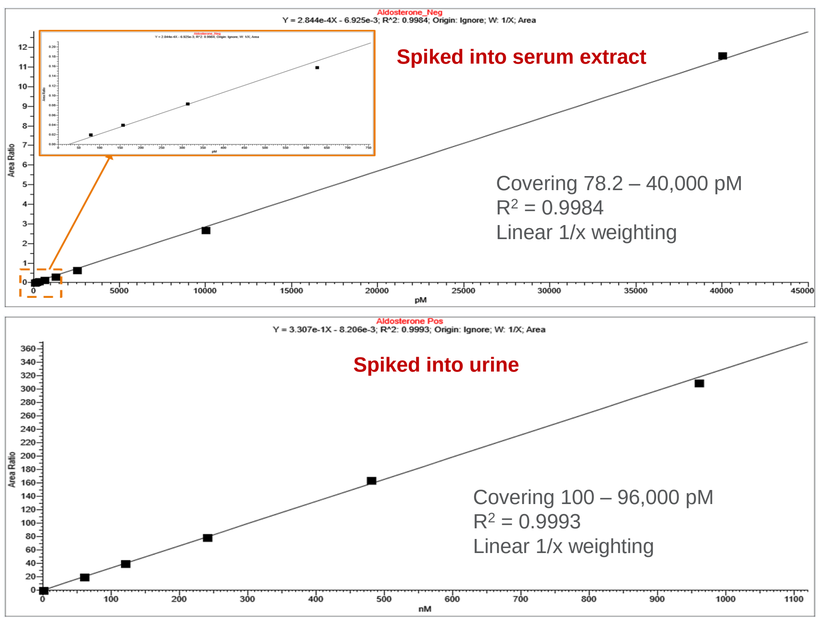


Figure 7. Aldosterone calibration curve in serum extracts using the optimized source conditions. The curve was measured over a dynamic range of ca. 3 orders of magnitude in serum extract (top) and urine (bottom).



CONCLUSIONS

- Thermo Scientifc TSQ Altis Plus mass spectrometer provides an excellent quantitative performance for measuring steroids and anti-doping compounds in complex biological matrices
- Estimated LODs for neat standards of DHT and testosterone are 25 pg/mL (0.25 pg on-column) and 1.67 pg/mL (0.17 pg), respectively, using unit resolution SRM. Employing H-SRM improves the estimated LOD for DHT to 16.7 pg/mL
- For serum extracts, all target steroids are accurately quantified at 100 pg/mL (standard 1 which is 1 pg on-column) using Unit SRM and H-SRM, including DHT which had a S/N ≥ 10
- Most anti-doping compounds analyzed from spiked urine extracts showed significant ionization suppression (>30%) relative to neat standards, raising the importance of the new source optimization parameters to help maintain, and in many instances, improve LOD/LOQ determinations

TRADEMARKS/LICENSING

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