WESTERN SLOPE LABORATORY

Anabolic Androgenic Steroids in Saliva by Turbulent Flow Orbitrap Mass Spectrometry

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Introduction

In the clinical practices of endocrinology and toxicology, anabolic androgenic steroids (AASs) are tested often. Both fields rely heavily on blood and urine levels of both exogenous and endogenous steroid and steroid hormones. However, access to blood and urine samples is not always possible. This necessitates the development of testing paradigms for alternative matrices. Oral fluid has gained in popularity as an alternative matrix due to its ease of collection, it is medically non-invasiveness, it is difficulty to adulterate, and its correlation to plasma levels.

Materials and Methods

Oral fluid samples were prepared for analysis with protein precipitation using organic solvent crash that was spiked with internal standards. The samples were centrifuged and filtered. Urine samples that were used for comparison were prepared by solid phase extraction post hydrolysis. After drying the samples are reconstituted in organic solvent. Samples were then injected onto one turbulent flow column on a Thermo Scientific TLX^{MD} system with ammonium acetate, ammonium formate, and ammonium hydroxide in water. Next, analytes were transferred to an analytical column with a guard column. Mobile phase consisted of water and methanol with ammonium formate and ammonium acetate. A flow of 350 µL/min was utilized starting with 40% organic at a gradient over two minutes to 100% organic followed by an isocratic elution for two minutes. The liquid chromatography system was coupled to an orbitrap mass spectrometer. As such, each of the nineteen compounds were analyzed using their exact mass as well as ion ratios using CID mass spectrometry, where applicable.

Compounds	
Methandrostenolone	Clenbuterol
Nandrolone	19-norandrosterone
Stanozolol	3'-hydroxystanozolol
Epitestosterone	Testosterone
Closetebol	Formestane
Tetrahydrogestrinone	DHEA
Oxymesterone	Oxandrolone
Boldenone	6-β-hydroxyboldenone
Fluoxymesterone	6-β-hydroxyfluoxymesterone

Table 1. 18 compounds tested for plus the internal standard of 3'-hydroxystanozolol-d_3

Results

Using the oral fluid turbulent flow orbitrap mass spectrometry method for AASs, we were able to improve resolution of all 19 compounds from previously published Western Slope Laboratory methods (Figure 1).

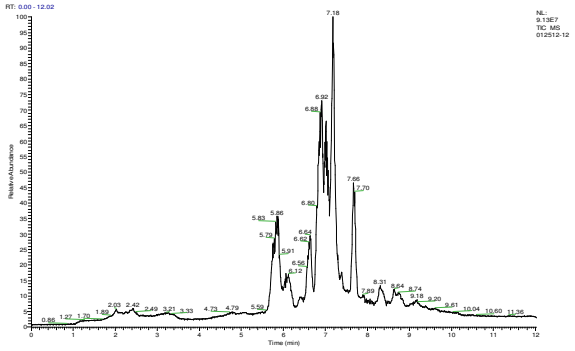

Figure 1. Total Ion Chromatogram(TIC) for 19-compound Steroid Panel.

Moreover, an increased separation of the epimers, testosterone and epitestosterone, when compared to the tandem mass spectrometry (QQQ) or time of flight mass spectrometry (TOF) methodologies previously employed, was observed. Figure 2 shows the chromatograph resolution of both compounds utilizing exact mass technology.

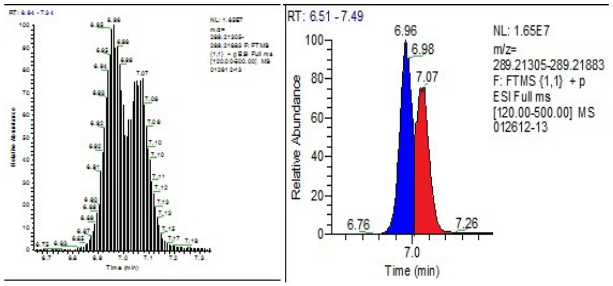

Figure 2. Separation of Testosterone from Epitestosterone.

Results Cont.

Due to the full scan analysis of the orbitrap method, compounds outside of the nineteen targeted compounds can also be qualitatively analyzed. In one unknown saliva sample, a large unknown peak was identified at approximately 7.0min (Figure 3).

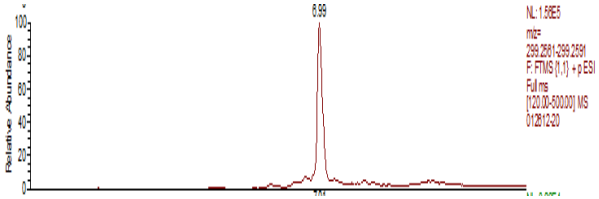

Figure 3. Unknown peak with retention time of 6.99min.

The exact mass and history of the donor determined the peak to belong to norethindrone consumption. In a targeted QQQ scan this unknown steroid would not have been detected and the accuracy of the TOF method would not have allowed for definitive qualitative determination of the compound. The orbitrap method allowed for its detection and determination. Lastly, the results from the orbitrap method were shown to correlate with the QQQ method using real urine samples run on both methods, allowing us to conclude that the turbulent flow orbitrap method for AASs can be validated for urine and saliva.

Conclusion

A oral fluid steroid method utilizing turbulent flow and exact mass technology was developed which tests for 19 compounds. The method has proven to be robust, sensitive, specific and applicable in urine and saliva. Future work will continue to improve separation, decrease method time, determine what correlations can be found between saliva and urine levels of anabolic steroids.

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American Society for Mass Spectrometry

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