Quantitative Analysis of Testosterone from Human Serum using high-flow Liquid Chromatography and FAIMS on a Triple Quadrupole Mass Spectrometer

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ABSTRACT

Purpose: Improvement of LOQ for testosterone analysis by reduction of matrix background using FAIMS technology.

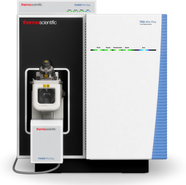
Methods: Testosterone was analyzed with and without FAIMS technology at a flow rate of 250 uL/min using identical chromatographic conditions and mass spectrometer settings. FAIMS CV was optimized on-line by injection against a matrix blank to find the optimum CV value.

Results: Matrix background was reduced significantly and LOQ of testosterone was improved 2-fold from 1 pg/mL to 0.5 pg/mL.

INTRODUCTION

Testosterone has been banned in athletic competitions because of its performance enhancing properties. Hence, quantitative determination of testosterone in humans has become important in the field of sports doping. LC/MS assays developed for the analysis of testosterone often exhibit high background signal which is difficult to eliminate by LC separation. This limits signal-to-noise ratio (S/N) and ultimately limit of quantification (LOQ) of the assay. The Thermo Scientific™ FAIMS Pro Duo interface spatially separates ions based on alternating high and low electric fields applied to a set of cylindrical electrodes, enabling attenuation of matrix signal and increasing signal-to-noise. Here we demonstrate improved LOQ for testosterone in human serum utilizing the Thermo Scientific™ FAIMS Pro Duo interface on the Thermo Scientific™ TSQ Altis™ Plus mass spectrometer (Figure 1).

Figure 1. FAIMS Pro Duo interface installed on a TSQ Altis Plus mass spectrometer.



MATERIALS AND METHODS

Sample Preparation

Testosterone was spiked into female human serum at concentrations ranging from 0.125 to 1000 pg/mL. Testosterone-d3 was added to the spiked serum at a concentration of 50 pg/mL. Liquid/liquid extraction was performed on each sample using methyl tert-butyl ether (MTBE). After evaporation, samples were reconstituted in 150 uL 70:30 water:methanol.

Test Method

A Thermo Scientific™ Vanquish™ Flex LC was used with a Thermo Scientific™ Accucore™ Vanquish™ C18+ column (P/N 27101-102130) at a flow rate of 250 uL/min. Methanol was used as mobile phase B and 0.5 mM ammonium fluoride in water was used as mobile phase A. The injection volume was 25 uL. Due to the limitation in sample volume, single injections were performed for each calibrator level. The OptaMax NG ion source HESI sprayer was positioned at L (vertical alignment) and 1 (front/back alignment) for both, runs with and without the FAIMS interface. Mass spectrometer settings were identical for runs with and without the FAIMS interface. SRM table and mass spectrometer settings are shown in Table 1 and 2, respectively.

Table 1. SRM table for the analysis of testosterone on the TSQ Altis Plus mass spectrometer.

Compound	Precursor (m/z)	Product (m/z)	Collision Energy (V)	RF Lens (V)
Testosterone	289.267	96.967	22.74	61
	289.267	108.967	24.97	61
Testosterone-d3	292.35	96.967	22.99	63

Table 2. SRM table for the analysis of testosterone on the TSQ Altis Plus mass spectrometer.

MS parameters	Value
Positive Ion	3000 V
Sheath Gas	50 Arb
Aux Gas	13 Arb
Ion Transfer Tube Temperature	340 C
Vaporizer Temperature	350 C
Q1 resolution	0.7
Q3 resolution	0.7
CID gas	2 mTorr
Source Fragmentation	0

Data Analysis

Thermo Scientific™ TraceFinder™ 5.1 was used for quantitative data analysis. For FAIMS data, a FAIMS raw file was associated with the Data Analysis Method in order to select scan filters with CV information.

FAIMS PARAMETERS

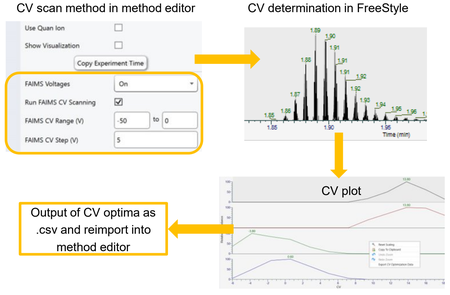
FAIMS Settings

The FAIMS Pro Duo interface was operated in high resolution mode with an inner electrode temperature of 80 °C and an outer electrode temperature of 100 °C to maximize selectivity. The carrier gas was set to 4.6 L/min which is the default value for FAIMS operation on the TSQ Altis Plus mass spectrometer.

FAIMS CV Optimization

Optimization of FAIMS CV values was performed on-line by injection using the new FAIMS CV scanning option for SRM methods in the method editor (see Figure 2).

Figure 2. On-line CV Scanning workflow for SRM methods.



A coarse CV optimization injection was performed using a CV range of -25 to +25 V with a step size of 4 V. Then, a fine optimization was performed using a CV range of +5 to +35 V with a step size of 2 V. After determining the optimum CVs for testosterone and testosterone-d3, the same fine optimization was performed on a matrix blank. In Thermo Scientific FreeStyle 1.8 SP1, CV plots were generated by using the 'CV Merge' function under 'Auto Filter' (see Figure 3) followed by the 'CV Plot' function. Sample and matrix plots were overlaid to determine the CV value which provides the best S/N (see Figure 4).

Figure 3. 'CV Merge' function under 'Auto Filter' in FreeStyle™ 1.8 SP1 which merges scan filters with different CV values into one filter.

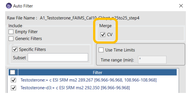
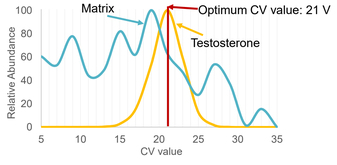


Figure 4. Overlaid CV plots of a testosterone sample and a matrix sample. The optimum CV value which maximizes testosterone signal and minimizes matrix contribution is 21 V.



RESULTS

Calibration curves for both experiments, with and without the FAIMS interface, are shown in Figure 5 and Figure 6 respectively. Using FAIMS technology, the LOQ was improved from 1 pg/mL (without FAIMS technology) to 0.5 pg/mL (with FAIMS technology). Acceptance criteria for LOQ were based on accuracy (< 20%), linearity of the calibration curve (R > 0.99), and ion ratio of the confirming ion (± 20%).

Figure 5. Calibration curve for testosterone acquired with the FAIMS Pro Duo interface: (Left) showing the full range up to 1000 pg/mL and (Right) showing the low range up to 10 pg/mL. Data points lower than the LOQ (0.5 pg/mL) were excluded because of ion ratio failure.

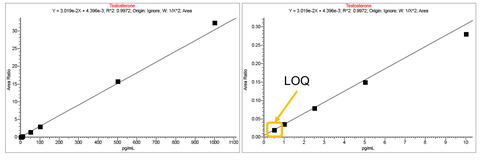
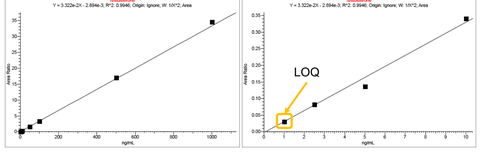


Figure 6. Calibration curve for testosterone acquired without the FAIMS Pro Duo interface: (Left) showing the full range up to 1000 pg/mL and (Right) showing the low range up to 10 pg/mL. Data points lower than the LOQ (1 pg/mL) were excluded because there was no peak distinguishable from background.



Results for back-calculated concentrations, accuracy, and ion ratios are summarized in Table 3. The RSD of the internal standard across all runs was 7.3 % with the FAIMS Pro Duo interface and 9.4 % without. The 2-fold improvement in LOQ observed with the FAIMS interface is attributed to reduction of chemical background.

Table 3. Back-calculated concentration, accuracy, and ion ratio of testosterone for each concentration both with, and without the FAIMS Pro Duo interface

Theoretical Concentration (pg/mL)	With the FAIMS Pro Duo Interface			Without the FAIMS Pro Duo Interface		
	Calculated concentration (ng/mL)	Accuracy (%)	Ion Ratio (%)	Calculated concentration (ng/mL)	Accuracy (%)	Ion Ratio (%)
0.5	0.50	-0.5	87.6	n.d.	n.d.	n.d.
1	1.03	2.9	74.8	1.01	1.7	77.6
2.5	2.48	-0.8	86.6	2.57	2.7	76.4
5	4.80	-3.9	85.4	4.21	-15.8	86.6
10	9.14	-8.6	91.0	10.3	3.4	79.6
50	48.9	-2.3	84.8	49.6	-0.8	86.8
100	101	1.3	83.5	102	2.4	87.0
500	524	4.7	84.0	511	2.2	87.0
1000	1071	7.1	84.6	1041	4.1	85.5

Figure 7 and 8 show a comparison of 0.5 and 1 pg/mL with (Figure 7) and without (Figure 8) FAIMS technology. With the FAIMS interface, background is significantly reduced, which enables the detection and reliable quantification of lower concentration levels.

Figure 7. Comparison of the quan (left) and qual (right) peaks for testosterone with the FAIMS Pro Duo interface (A) at a concentration of 0.5 pg/mL (at LOQ) and (B) 1 pg/mL.

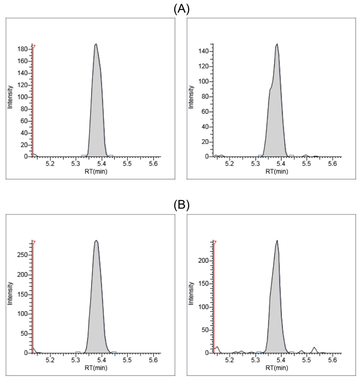
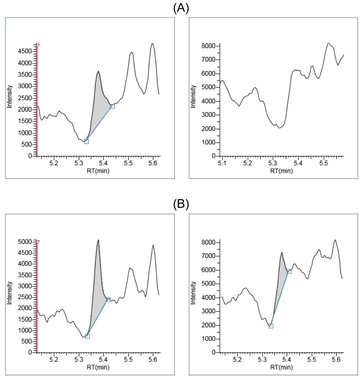


Figure 8. Comparison of the quan (left) and qual (right) peaks for testosterone without the FAIMS Pro Duo interface (A) at 0.5 pg/mL (below LOQ) and (B) 1 pg/mL (at LOQ). At a concentration of 0.5 pg/mL the confirming ion was not detected.



CONCLUSIONS

- Utilizing FAIMS technology as an additional dimension of separation can enhance LC/MS analysis by selectively transmitting analyte ions through the electrodes while attenuating signal from matrix and/or background ions.
- FAIMS technology often provides improved signal-to-noise and LOQ, particularly when dealing with complex matrices, such as human serum.
- For quantitating testosterone in human serum, use of the FAIMS Pro Duo interface improved LOQ 2-fold from 1 pg/mL to 0.5 pg/mL

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TRADEMARKS/LICENSING

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