Technical note | 000304

Mass spectrometry

Analysis of testosterone from human serum using FAIMS technology

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Keywords

TSQ Altis Plus MS, FAIMS Pro Duo interface, steroids, sports doping, on-line CV optimization

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

Goal

Improve LOQ of testosterone measured in human serum samples using the Thermo Scientific™ FAIMS Pro Duo interface on a Thermo Scientific™ TSQ Altis™ Plus mass spectrometer

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 that is difficult to eliminate by LC separation alone. This limits signal-to-noise ratio (S/N) and ultimately limit of quantification (LOQ) of the assay. The 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 FAIMS Pro Duo interface on the TSQ Altis Plus mass spectrometer (Figure 1).

Experimental

Testosterone was spiked into 2× charcoal stripped female human serum at concentrations ranging from 0.125 to 1,000 pg/mL. Testosterone-d_3 was added to the spiked serum at 50 pg/mL. Liquid/liquid extraction was performed on each sample using methyl tert-butyl ether (MTBE). After evaporation of the organic layer (supernatant), samples were reconstituted in 150 µL water:methanol (70:30 v:v). The injection volume was 25 µL.

A Thermo Scientific™ Vanquish™ Flex Binary UHPLC system was used with a Thermo Scientific™ Accucore™ Vanquish™ C18+ column 1.5 µm, 2.1 mm × 100 mm (P/N 27101-102130) installed. The column was held at 40 °C. The chromatographic conditions are shown in Table 1. Methanol was used as mobile phase B, and 0.5 mM ammonium fluoride in water was used as mobile phase A. Needle wash was performed for 3 s at a speed of 10 µL/s. The Thermo Scientific™ OptaMax NG™ ion source HESI sprayer was positioned at L (vertical alignment) and 1 (front/back alignment).

Table 1. Chromatographic conditions

Time (min)	Flow (µL/min)	%B	Curve
0.00	250	30	5
1.00	250	30	5
1.50	250	55	5
5.00	250	85	5
6.00	250	100	5
7.00	250	100	5
7.01	250	30	5
9.00	250	30	5

Mass spectrometer settings are shown in Table 2. 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. The carrier gas was set to 4.6 L/min. FAIMS CV optimization was performed on-line using the FAIMS CV scanning option for SRM methods in the method editor (Figure 2). 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-d_3, the same fine optimization was performed on a matrix blank.

Table 2. TSQ Altis Plus mass spectrometer settings for the analysis of testosterone. The same ion source and scan settings were used for runs both with and without the FAIMS interface. (A) ion source and scan settings (B) SRM table

A

MS parameters	Value
Positive ion	3,000 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

B

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-d_3	292.35	96.967	22.99	63

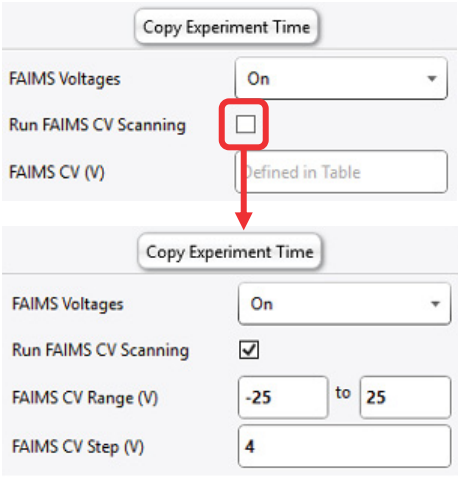

Figure 2. Settings for coarse CV optimization injection performed using the FAIMS CV Scanning option available in the method editor on compatible TSQ mass spectrometers

In Thermo Scientific™ FreeStyle™ 1.8 SP1 software, CV plots were generated by using the 'CV Merge' function under 'Auto Filter' followed by the 'CV Plot' function (Figure 3). Sample and matrix plots were overlaid to determine the CV value that provides the best S/N (Figure 4). Thermo Scientific™ TraceFinder™ 5.1 software was used for quantitative data analysis. For FAIMS data, a FAIMS raw file was associated with the Data Analysis Method to select scan filters with CV information.

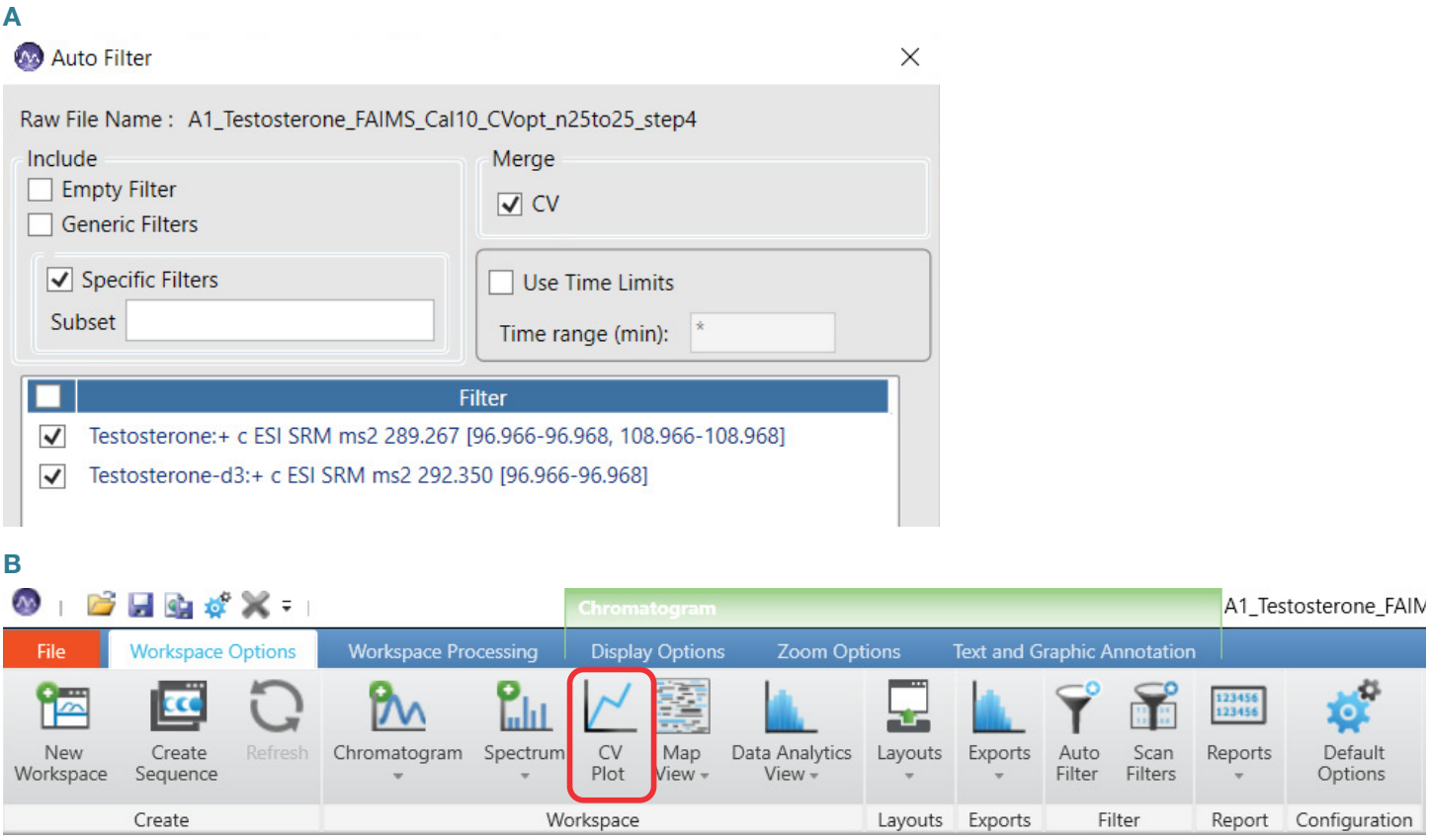

Figure 3. Generation of CV plots in FreeStyle 1.8 SP1 software: (A) CV Merge function under Auto Filter (B) CV plot function in the ribbon menu

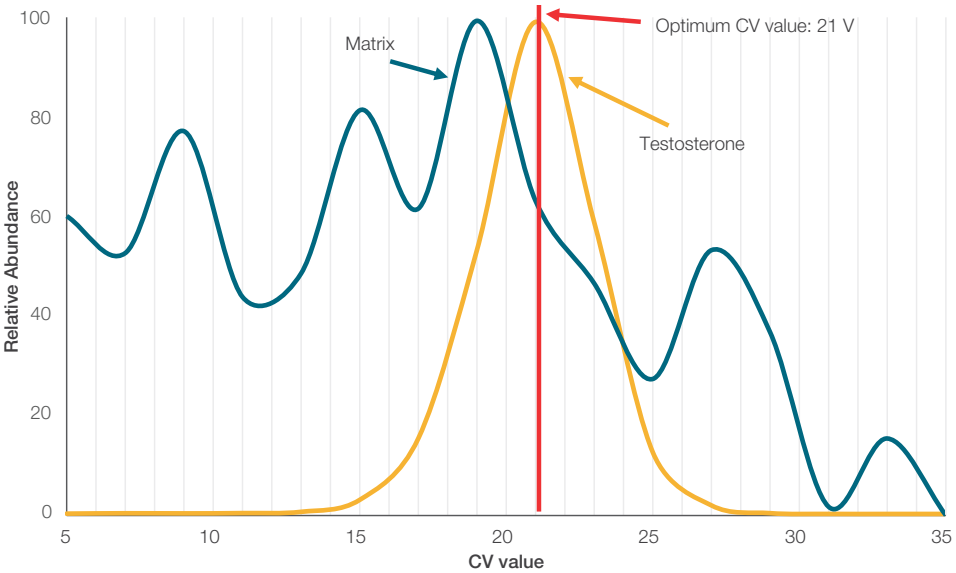

Figure 4. Overlaid CV plots of a testosterone sample and a matrix sample. The optimum CV value that maximizes testosterone signal and minimizes matrix contribution is 21 V.

Results and discussion

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^2 > 0.99), and ion ratio of the confirming ion (±20%). The linearity of the calibration curve was 0.9972 with the FAIMS Pro Duo interface and 0.9946 without the FAIMS Pro Duo interface. Results for back-calculated concentrations, accuracy, and ion ratios are summarized in Table 3. The RSD of the internal standard was 7.3% with the FAIMS Pro Duo interface and 9.4% without across all runs. The 2-fold improvement in LOQ observed with the FAIMS interface is attributed to reduction of chemical background. Figure 7 shows a comparison of 0.5 and 1 pg/mL, both, with and without FAIMS technology. With the FAIMS interface, background is significantly reduced, which enables the detection and reliable quantification of lower concentration levels.

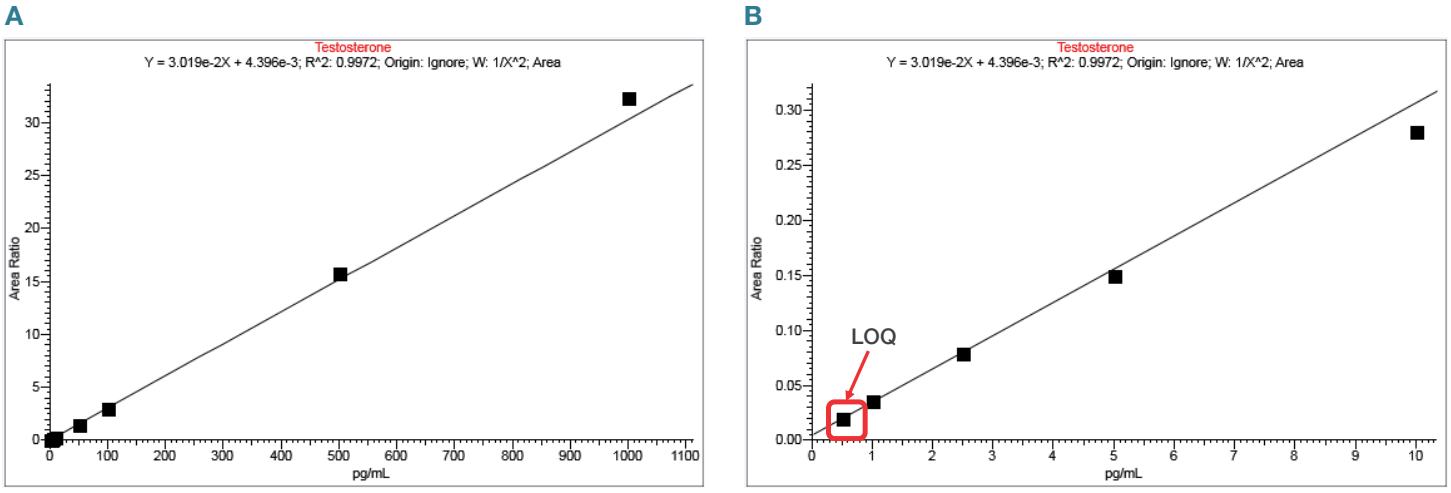

Figure 5. Calibration curve for testosterone acquired with the FAIMS Pro Duo interface: (A) showing the full range up to 1,000 pg/mL and (B) showing the low range up to 10 pg/mL

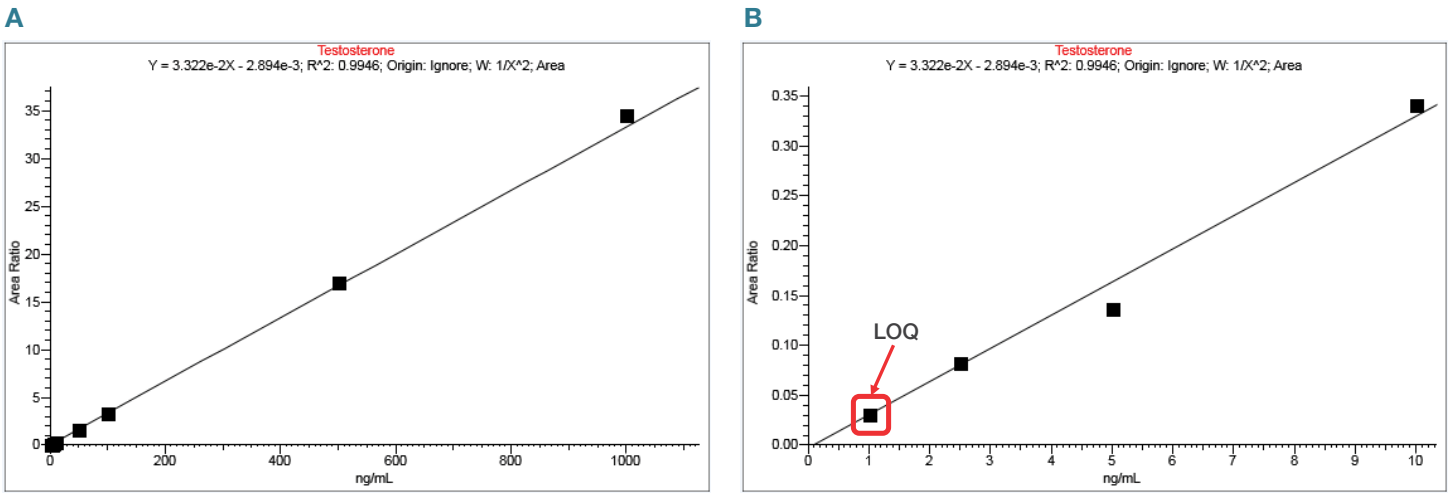

Figure 6. Calibration curve for testosterone acquired without the FAIMS Pro Duo interface: (A) showing the full range up to 1,000 pg/mL and (B) showing the low range up to 10 pg/mL

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	below LOQ	below LOQ	below LOQ
1.0	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

With the FAIMS Pro Duo interface

Without the FAIMS Pro Duo interface

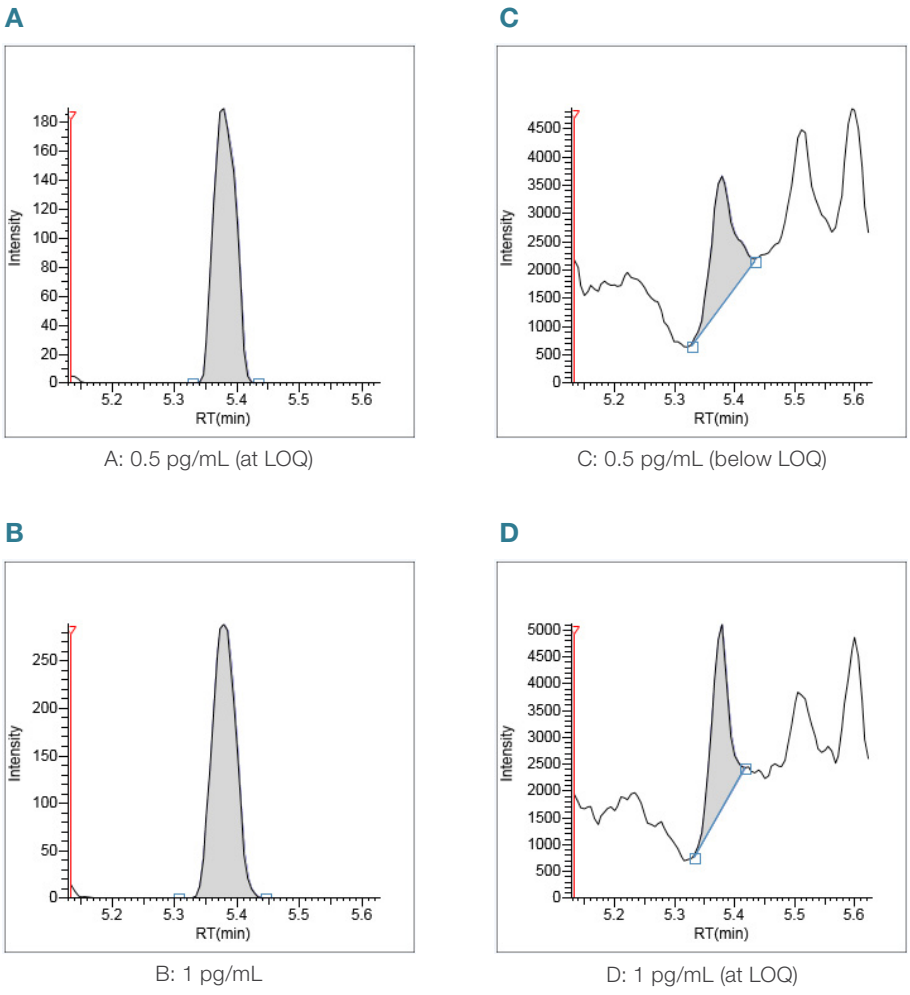

Figure 7. Comparison of the quantifying SRM transition for testosterone with the FAIMS Pro Duo interface (A) at a concentration of 0.5 pg/mL (at LOQ) and (B) 1 pg/mL, and without the FAIMS Pro Duo interface (C) at 0.5 pg/mL (below LOQ) and (D) 1 pg/mL (at LOQ)

Conclusion

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. This often results in 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 increased sensitivity 2-fold from 1 pg/mL to 0.5 pg/mL.

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