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Application of FAIMS to anabolic androgenic steroids in sport drug testing

Sven Guddat,[a]* Mario Thevis,[b] James Kapron,[c] Andreas Thomas[b] and Wilhelm Schänzer[a]

Mass spectrometric identification of anabolic androgenic steroids challenges standard doping-control methods. To reveal a doping offence the presence of prohibited anabolic androgenic steroids at trace levels in the picogram-per-millilitre range must be confirmed as reliable. Human urine samples containing epitrenbolone, metandienone metabolite (17β -hydroxymethyl-17α-methyl-18-norandrost-1,4,13-trien-3-one), stanozolol, 16β-hydroxystanozolol and 4β-hydroxystanozolol were analysed using LC-FAIMS-MS/MS. These substances are prohibited in sport according to World Anti-Doping Agency (WADA) regulations. Glucuronides were hydrolysed and prepared by liquid-liquid extraction. Excellent recovery and precision were obtained for all compounds. Linear calibration results for epitrenbolone and metandienone metabolite were obtained and concentration information could be determined in the ranges of reliable response between 750–1200 and 100–600 pg/mL, respectively. Limits of detection were estimated at 25 pg/mL (stanozolol), 50 pg/mL (metandienone metabolite, 16β-hydroxystanozolol), 100 pg/mL (4β-hydroxystanozolol) and 500 pg/mL (epitrenbolone). The assay was applied to doping-control samples. For all analytes, LC-FAIMS-MS/MS resulted in excellent interference removal, which effectively extends the post-dose detection time.

Keywords: sport drug testing; FAIMS; anabolic androgenic steroids; LC-FAIMS-MS/MS

Introduction

Anabolic androgenic steroids are the most frequently detected prohibited substances in athletes' urine.[1–4] New synthetic 'designer' steroids are constantly being developed and the anabolic agents may also be masked using innocuous substances.[5–8] The pressure to cheat is very high and so doping control scientists must ensure they are at the cutting edge of technology to keep one step ahead of the athletes.[9]

Analysis using LC-MS/MS represents a great advance over GC-MS/MS in terms of turn-around time, specificity and sensitivity for the misuse of these compounds.[1,5,10,11,12] Occasionally, samples analysed show significant interference indicating that the current state-of-the-art LC-MS/MS is not always as selective as needed. In terms of anabolic-androgenic steroids, doping control laboratories are challenged by the very low concentration levels frequently observed in athletes' urine. To meet identification criteria set by the World Anti-Doping Agency (WADA), new orthogonal separation techniques, providing increased specificity, are promising for confirmatory analysis of prohibited substances in human urine, even at the sub-pg/mL range.[4]

High-field asymmetric waveform ion mobility spectrometry (FAIMS) is a technology that separates gas-phase ions based upon their shapes at atmospheric pressure.[13] Traditional linear drift tube ion mobility spectrometry (IMS) has recently been coupled to MS for pharmaceutical characterization.[14,15] This technique is simple to comprehend in terms of ion shape but it suffers from very low ion transmission, in the order of 0.1% to 1%, despite active research.[16]

In comparison, FAIMS exploits differences in mobility as a function of very high electric fields.[17] High and low electric fields are alternately generated between concentric cylindrical electrodes. As the asymmetric waveform is applied, the ions experience a very high electric field for a short duration and are moved at high-field mobility to one of the electrodes. During the reverse polarity section of the waveform, the ions experience a longer duration of oppositely directed low-field mobility toward the other electrode. A difference in net ion movement results in a shift in trajectory to one or the other electrodes. Transmission of an ion through the device depends upon the compensation voltage (CV), which is a DC offset of the waveform. The CV adjusts the ion trajectory and keeps the ions focused in the annular space between the electrodes.[18]

Each ion type emerges from the filter at a compound-specific compensation voltage (CV). The CV actually represents a ratio of the driving forces that oscillate the ions through the mobility gas. Separation occurs on the millisecond timescale and is based on the shape and charge state of the ion. The atmospheric gas is a mixture of helium and nitrogen, which, combined with electrodes of unique cylindrical geometry, typically allows for much higher transmission efficiencies (normally 30%–50%) than traditional IMS.[19] The electrodes are temperature controlled to allow rapid thermal equilibration to operating conditions.[20]

The key benefit that this technology offers is enhancement of specificity, which assists and simplifies the detection of trace-level compounds. High-field asymmetric waveform ion mobility

* *Correspondence to: Sven Guddat, Institute of Biochemistry, German Sport University, Cologne, Germany. E-mail: S.guddat@biochem.dshs-koeln.de*

a *Institute of Biochemistry, German Sport University, Cologne, Germany*

b *Center of Preventive Doping Research, German Sport University, Cologne, Germany*

c *Thermo Fisher Scientific, 112 Colonnade Rd, Nepean, Canada, K2E 7L6*

spectrometry has demonstrated promise filtering ions post-LC and pre-MS. Examples for small molecule analysis include drug metabolism,[21] targeted metabolomics,[18,22] pharmaceutical quantitation,[23,24] and environmental studies.[25] Proteomics researchers have also found use for FAIMS both in exploratory[26,27,28] and in quantitative proteomics.[29]

High field asymmetric waveform ion mobility spectrometry has also shown utility with novel peptide sequencing technologies such as ECD and ETD.[30,31]

The specificity of the analysis is different from having only liquid (retention time) or vacuum (m/z) separation. Each compound-specific compensation voltage (CV) allows a subset of ions into the MS and filters out other ions resulting in a higher signal-to-noise ratio (S/N).[32] The higher S/N translates into greater confidence in the analysis.

This study focuses on three of the most commonly reported high potency anabolic androgenic steroids, namely stanozolol, metandienone and trenbolone. In 2007, WADA-accredited laboratories reported 281 positive findings for these compounds.[4] The assay presented converts glucuronidated metabolites to the free anabolic agents and monitors epitrenbolone (17α -hydroxy-estra-4,9,11-trien-3-one), metandienone metabolite (17β-hydroxymethyl-17α-methyl-18-norandrost-1,4,13-trien-3-one), stanozolol (17β-hydroxy-17α-methyl-5α-androst-2-eno(3,2-c)-pyrazole) and its metabolites 16β-hydroxystanozolol and 4β-hydroxystanozolol in human urine by the use of LC-FAIMS-MS/MS (Figure 1).

Experimental

Reference compounds and supplies

Reference materials for stanozolol, 16β-hydroxystanozolol, 4β-hydroxystanozolol and epitrenbolone (17α-trenbolone) were obtained from the National Measurement Institute (Pymble, Australia). 17β-Hydroxymethyl-17α-methyl-18-norandrost-1,4,13-trien-3-one (metandienone metabolite) was synthesized and characterized in-house (Sporthochschule) in cooperation with Pombiotech (Saarbrücken, Germany). Methyltestosterone was used as internal standard (IS) and was purchased from Sigma-Aldrich (Deisendorf, Germany). Structures are shown in Figure 1.

The HPLC-grade solvents were obtained from commercial sources and used without further purification. Phosphate buffer, potassium carbonate and potassium bicarbonate were obtained from Merck (Darmstadt, Germany). Helium gas was obtained from Linde (Germany). Nitrogen and compressed air were obtained via Whatman gas generators.

Beta-glucuronidase from *E. coli* (EC 3.2.1.31) was purchased from Roche Diagnostics (Mannheim, Germany).

Sample preparation

Samples were prepared using a previously published method modified for steroid analysis.[33,34]

Amounts of 5 ng IS and 1 mL of phosphate buffer (0.8M, pH 7.0) were added to aliquots of human urine (1 mL). Glucuronides were hydrolysed by the addition of beta-glucuronidase. The mixture was incubated at 50 °C for 1 hour.

Liquid-liquid extraction was performed by the addition of 750 µL of K_2CO_3/$KHCO_3$ (1 : 1) solution (20%) and 2 mL of t-butyl methyl ether at a pH of 9.6. The mixture was mechanically shaken for 5 min and then spun in a centrifuge at 1750 g for 5 min.

The organic layer (2 mL) was separated and evaporated to dryness. The residue was reconstituted in 60 µL methanol: ammonium acetate buffer (1 : 1, v : v).

Instrumental setup and sample analysis

The LC system consisted of a model 1200SL pump (Agilent, Waldbronn, Germany), and a HTS PAL autosampler (CTC Analytics, Zwingen, Switzerland). The mobile phases were composed of A: ammoniumacetate buffer (pH 3.5, 5 mM ammonium acetate, 0.1% glacial acid) and B: acetonitrile, at a total flow rate of 0.25 mL/min. The mobile phase gradient was linear beginning at 10%B until 100% over 5 minutes. Isocratic elution was carried out for 1.25 min at 100% B. The column was equilibrated for 2.5 minutes before injecting the next sample. The total run time was 8.75 min. Injection volume was 10 µL. The column was a Hypersil Gold C_{18} 50 × 2.1 mm, 1.9 µm particles from Thermo Fisher Scientific (San Jose, US). Retention times for each component are listed in Table 1.

The FAIMS system (Thermo Fisher Scientific, San Jose, US) used standard FAIMS conditions, namely, dispersion voltage −5000 V, 4 L/min equimolar nitrogen and helium, inner and outer electrode

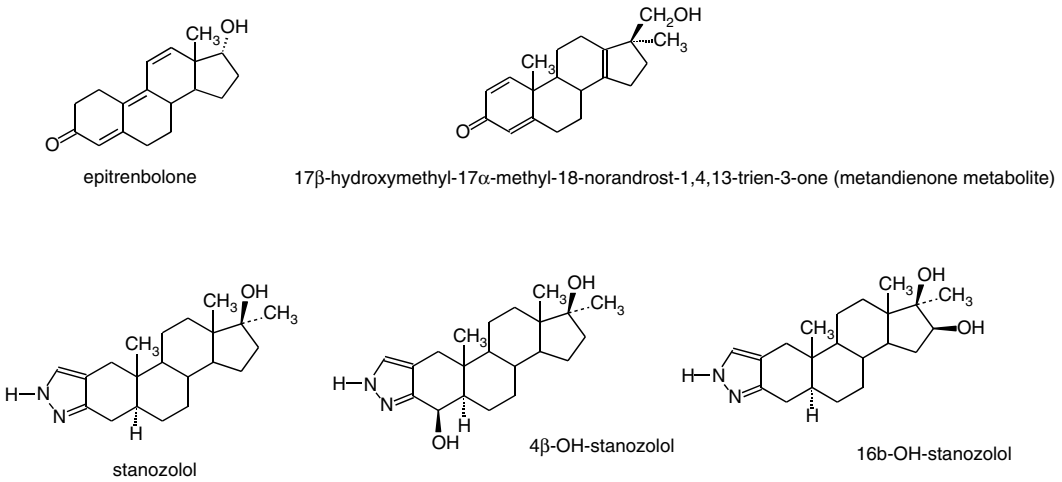

Figure 1. Chemical structures of epitrenbolone, 17β -hydroxymethyl-17α-methyl-18-norandrost-1,4,13-trien-3-one (metandienone metabolite), stanozolol, 4β-hydroxystanozolol and 16β-hydroxystanozolol.

Table 1. Compound properties. For each compound examined, the observed retention time, compensation voltage and m/z values for each precursor and product ion pairs are listed. The collision energies for each transition is included as well as the function of quantification or qualification in the assay

	Retention time (min)	CV (V)	Q1 (m/z)	Q3 (m/z)	Collision energy (eV)	Function
Epitrenbolone	3.76	−18.9	271	199	30	Quant.
			271	238	40	Qual.
			271	253	31	Qual.
Metandienone metabolite	4.15	−17.4	299	121	37	Quant.
			299	147	29	Qual.
			299	269	14	Qual.
Stanozolol	4.81	−12.3	329	81	61	Quant.
			329	95	71	Qual.
			329	109	40	Qual.
16-β-Hydroxystanozolol	4.04	−11.1	345	309	21	Quant.
			345	327	27	Qual.
			345	145	70	Qual.
4-β-Hydroxystanozolol	3.58	−13.8	345	81	66	Quant.
			345	95	61	Qual.
			345	109	61	Qual.
Methyltestosterone	4.31	−17.5	303	109	30	–

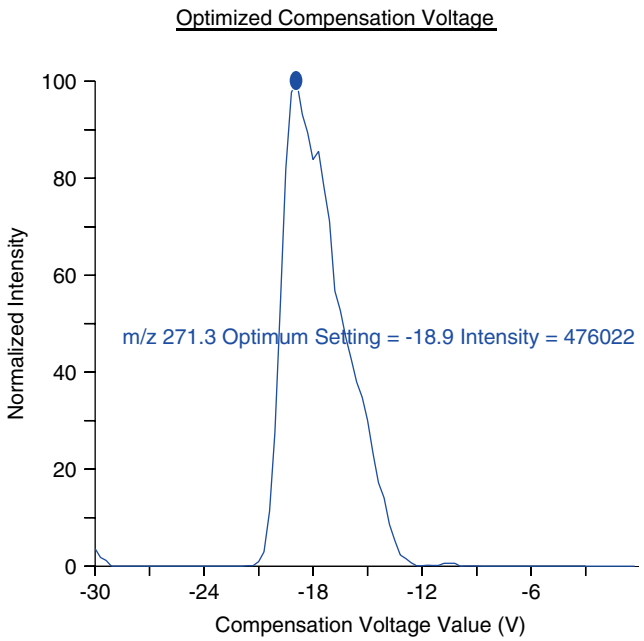

Figure 2. Representative compensation voltage (CV) scan for epitrenbolone. Infusing a reference solution of epitrenbolone and concomitant ramping of the CV resulted in optimum signal at the CV – 18.9 V. Similar results were obtained for all compounds and the CV values used are shown in Table 1.

temperatures of 70 °C and 90 °C, respectively. The resulting compensation voltages (CV) are outlined in Table 1.

Tandem mass spectrometry was performed on a TSQ Vantage (Thermo Fisher Scientific, San Jose, US) controlled by Xcalibur software (version 2.1.0, Thermo). Positive ionization mode heated electrospray (H-ESI) was used with the following conditions: ion spray voltage 4000V, vaporizer temperature 350 °C, capillary temperature 300 °C, sheath gas 276 kPa, auxiliary gas 414 kPa and collision gas (nitrogen) pressure of 0.17 Pa. Compound specific m/z values and collision energies are listed in Table 1. The MS/MS behaviour of the analytes was studied earlier and is described in detail elsewhere.[1,7,12] Resolution in Q1 was set to 0.5 FWHM (full width half maximum) and in Q3 to unit (0.7 FWHM). Analytes were monitored in two different analytical runs. In the first run epitrenbolone, metandienone metabolite and the IS were analysed and in the second stanozolol, 4β-hydroxystanozolol, 16β-hydroxystanozolol and IS were measured. In both runs three segments were used in which helium was used only during analyte elution for approximately 15 s before and after peak elution.

Data treatment

Chromatographic peak integration was performed by Xcalibur software. Peak area ratios of analyte divided by IS were plotted against nominal concentration. Concentrations were determined by back-calculating area ratios from the regression line (weighting factor $1/x^2$). All statistical calculations were performed using MicroSoft Excel Office 2003 (MicroSoft Corporation, Redmond, US). All concentrations were reported to three significant figures except for integer values and numbers greater or equal to 1000. Precision was assessed by the coefficient of variation (CV%) calculated from the measured concentrations.

Assay validation

The minimum required performance limit (MRPL) is the lowest level that a doping control laboratory must be able to reproducibly determine with acceptable accuracy and precision.[4] For the investigated anabolic agents, MRPL values are 2 ng/mL for stanozolol and metandienone and 10 ng/mL for trenbolone.

The lower limit of detection (LLOD) is the lowest concentration at which a substance may be measured with reasonable certainty at a signal-to-noise ratio greater than or equal to three. Two types of blank urine samples were prepared. One type using 10 different urine specimens was fortified only with IS (n = 10)

Table 2. Assay precision. Within and among day (intra- and inter-day, respectively) precision is presented at each of three concentrations. On each day, six replicates were run at each concentration. The inter-day precision was determined by three days' analysis

		Low	Mid	High
Epitrenbolone	Nominal concentration (pg/mL)	750	1000	1500
	Intra-day RSD (%)[a]	8.0	3.8	4.0
	Inter-day RSD (%)[b]	18.4	18.7	15.7
Metandienone metabolite	Nominal concentration (pg/mL)	100	500	1000
	Intra-day RSD (%)[a]	9.8	11.2	5.9
	Inter-day RSD (%)[b]	15.2	11.6	6.8
Stanozolol	Nominal concentration (pg/mL)	50	500	1000
	Intra-day RSD (%)[a]	8.7	7.3	6.8
	Inter-day RSD (%)[b]	19.3	16.7	14.9
16-β-hydroxystanozolol	Nominal concentration (pg/mL)	250	500	1000
	Intra-day RSD (%)[a]	8.7	10.8	10.8
	Inter-day RSD (%)[b]	16.4	19.5	17.2
4-β-hydroxystanozolol	Nominal concentration (pg/mL)	500	1000	1500
	Intra-day RSD (%)[a]	5.1	6.1	9.4
	Inter-day RSD (%)[b]	18.6	10.5	13.3

[a] n = 6 for each concentration.
[b] n = 18 for each concentration.

Table 3. Calibration statistics. For the two compounds that demonstrated linear behaviour, the slope, intercept, correlation coefficient and coefficient of determination are presented

	Slope	Intercept	Correlation coefficient (r)	Coefficient of determination (r^2)
Epitrenbolone	0.0002	0.1507	0.9944	0.9888
Metandienone metabolite	0.0001	0.012	0.9840	0.9689

and the other only with the analytes under consideration at concentrations of 50 pg/mL (stanozolol), 100 pg/mL (metandienone metabolite), 250 pg/mL (16β-hydroxystanozolol), 500 pg/mL (4β-hydroxystanozolol) and 750 pg/mL (epitrenbolone).

Intraday precision was determined by preparing and analysing six urine samples at the concentrations listed in Table 2. The intraday precision was calculated by dividing the standard deviation by the average value at each concentration level (n = 6). Interday precision was determined as for intraday precision except that samples were analyzed over three consecutive days (n = 18).

Calibration statistics from linear regression are shown in Table 3. Assay limits (Table 4) were set based upon the linearity of response from the calibration line.

Table 4. Assay limits. The limit of detection for each compound is presented. The LOD was determined using ten distinct sources of urine as a selectivity test. In the case of epitrenbolone and metandienone metabolite the lower and upper limits of quantitation are also shown

		Calibration range	
	LOD[a] *(pg/mL)*	*LLOQ*[b] *(pg/mL)*	*ULOQ*[b] *(pg/mL)*
Epitrenbolone	500	750	1200
Metandiene metabolite	50	100	600
Stanozolol	25	–	–
16-β-hydroxystanozolol	50	–	–
4-β-hydroxystanozolol	100	–	–

[a] LOD determined with n = 10.
[b] LLOQ and ULOQ determined with n = 6.

Table 5. Recovery results. Each compound was measured for recovery from urine at one concentration. In all cases pre-extraction spike and post-extraction spike samples were measured in six replicates each

	Concentration (pg/mL)	Recovery (%)[a]	Error (%)[a]
Epitrenbolone	1000	92	9.4
Metandienone metabolite	500	85	17.0
Stanozolol	500	68	16.2
16-β-hydroxystanozolol	500	68	22.2
4-β-hydroxystanozolol	1000	66	14.9

[a] n = 12.

Recovery for all analytes was determined at a mid-range concentration as listed in Table 5. Six control lots of urine were fortified with target analytes before sample preparation. In addition, six similar control lots of urine were extracted according to the sample preparation protocol, followed by addition of the analytes into the t-butyl methyl ether extract. Before evaporation, IS was added to both sets of samples. Recovery was calculated by comparison of the mean peak area ratios of analytes versus IS for samples fortified prior to and after liquid-liquid extraction.

The effect of the variation of matrix was examined by post-column continuous infusion of analyte solution and injection of six different control urine extracts.[35] In the chromatographic region where the analytes elute, negative deflection of the baseline indicates matrix suppression of ionization. Chromatographic conditions were adjusted to minimize the matrix effect during the region of elution for these analytes.

Specificity (or selectivity) was evaluated by preparing and analysing urine samples from ten distinctly different subject sources (six male/four female). Specificity was determined by comparing interference peaks at respective retention times of the analytes.

Results and Discussion

Validation

For validation the five compounds were separated into two injections on column. For the first injection, epitrenbolone and

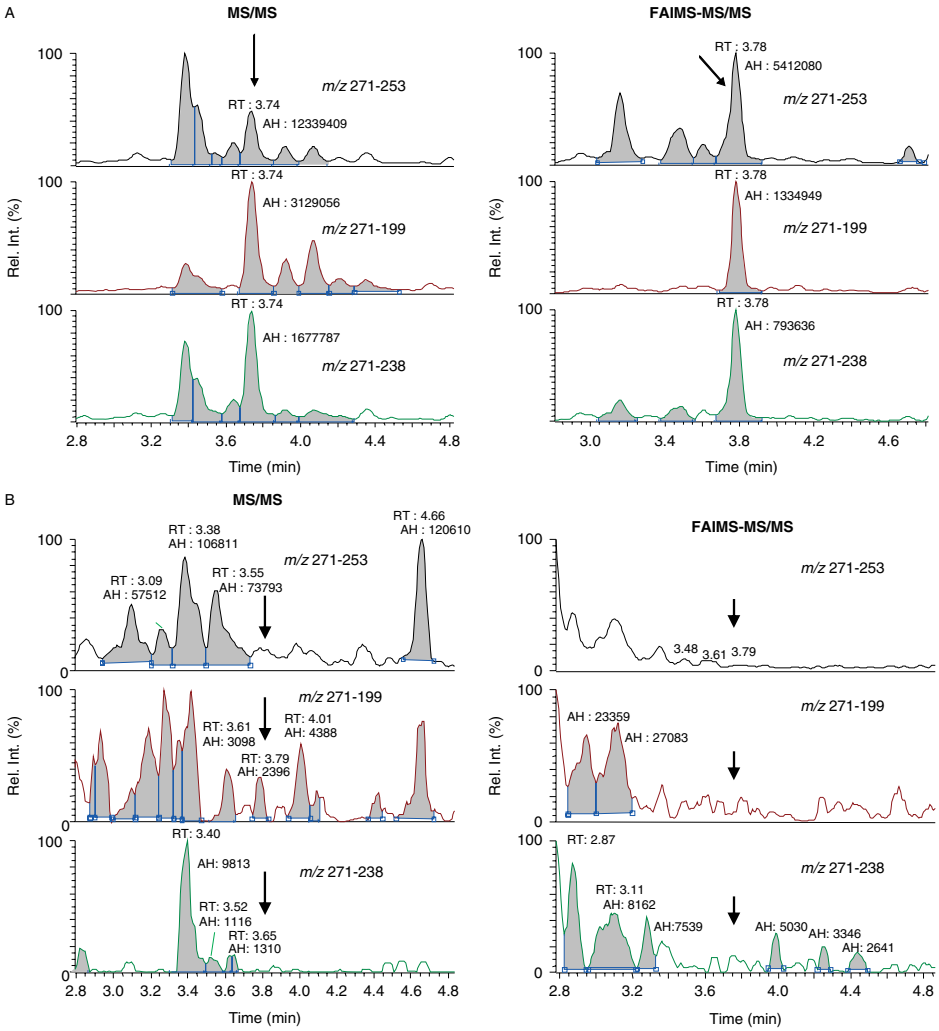

Figure 3. Representative LC-MS/MS and LC-FAIMS-MS/MS chromatograms for epitrenbolone. Panel 3a shows a positive finding at approximately 3 ng/mL. Note the improved chromatographic peak definition at retention time 3.78 min. Panel 3b shows a negative control sample. The LC-FAIMS-MS/MS trace shows fewer interferences that otherwise might be mistaken for signal.

metandienone metabolite, together with IS, were monitored at CV −17.5 V. Even though the optimum CV for epitrenbolone was determined to be −18.9 V, the validation results confirm that the system is fit for purpose under these conditions. The reason for this is due to the optimal resolution of FAIMS: resolution is high enough to exclude interference, yet low enough to allow all three compounds to emerge at this one CV. In the second injection, stanozolol, its 16β- and 4β- metabolites and IS were monitored at CV −14.0 V.

For assay validation the parameters LOD, specificity, intra- and inter-day precision, linearity and ion suppression/enhancement were investigated according to the guidelines of the International Conference on Harmonization (ICH) and WADA.[4,36] At the required signal-to-noise ratio of 3, the LODs were estimated at 25 pg/mL for stanozolol, 50 pg/mL for 16β-hydroxy stanozolol and the metandienone metabolite, 100 pg/mL for 4β-hydroxy stanozolol and at 500 pg/mL for epitrenbolone. Regarding specificity, no interfering signals were detected in the MRM chromatograms for respective analytes (Figures 3b, 4c). For all analytes, ion suppression/enhancement was not observed while different urinary matrices were injected.

Assay precision results are shown in Table 2. Intra-day precision was consistently below 12% relative standard deviation (RSD). Inter-day precision was in all cases below 20% RSD at all concentrations, which is within ICH and WADA specifications.

As presented in Table 3, concentration results are reported for epitrenbolone (750–1200 pg/mL) and for metandienone metabolite (100–600 pg/mL). In the case of stanozolol and the 16β- and 4β-hydroxymetabolites a linear correlation between analyte concentration and response was not obtained. A possible explanation for this is that methyltestosterone (the routinely used IS for anabolic steroids) was inappropriately chosen. For a qualitative identification of an exogenous doping agent linearity is not the primary validation parameter and is not obligatory according to WADA guidelines requiring no demonstration of linearity. This flexibility permits rapid method development together with a reliable to answer to the question, 'is this steroid present above the cutoff level?' Future studies on linearity will be explored with a deuterium labelled analogue of stanozolol.

Recovery results are displayed in Table 5 and show acceptable recoveries (66–92%) and errors (9–23% RSD) based on the 12 individual analyses.

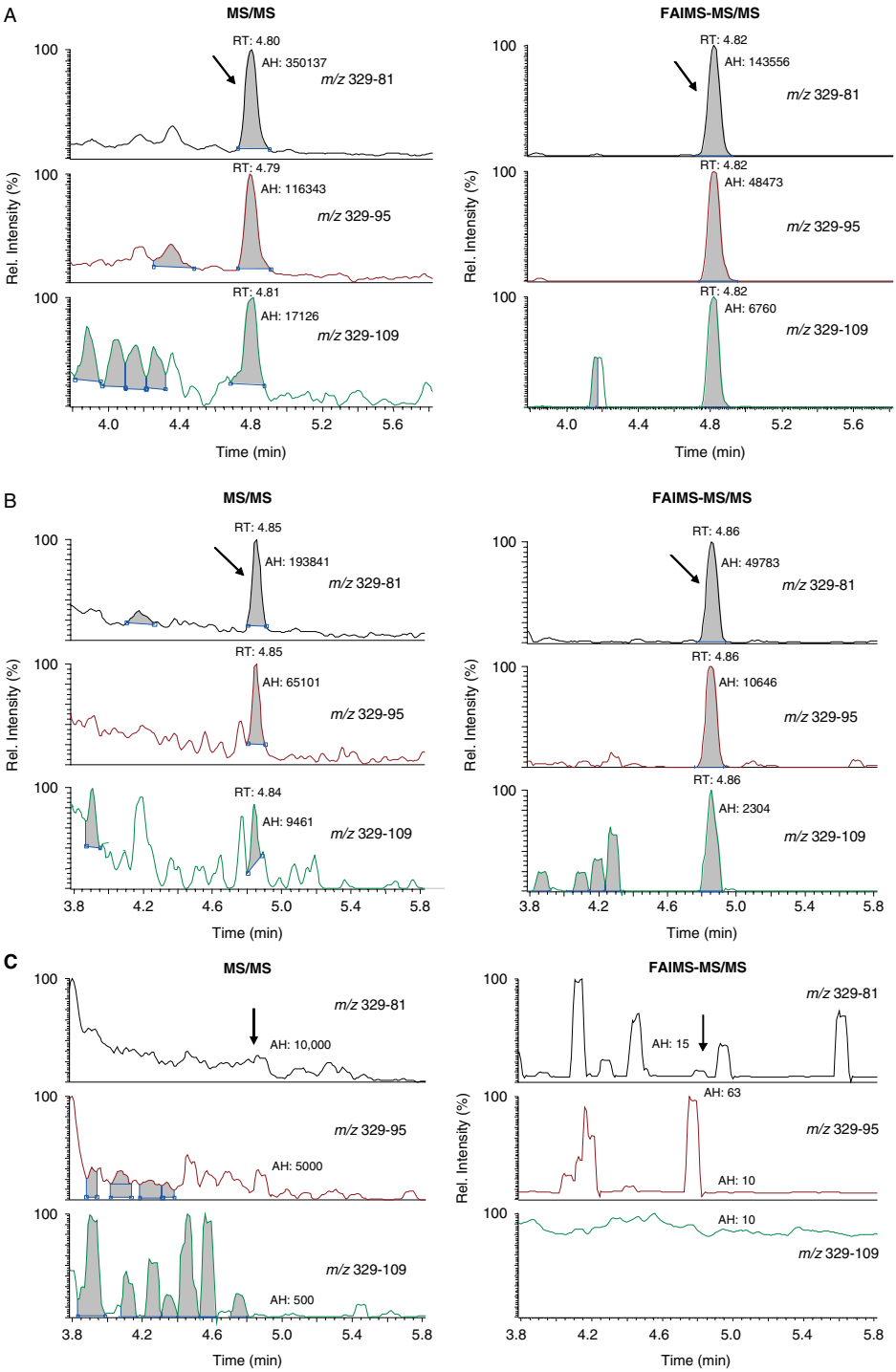

Figure 4. Representative LC-MS/MS and LC-FAIMS-MS/MS chromatograms for stanozolol. Panel 4a shows a positive finding at approximately 65 pg/mL. Signal-to-noise ratio for the LC-FAIMS-MS/MS peak at retention time 4.82 min is approximately 10-fold higher than by LC-MS/MS due to background elimination. Panel 4b is for a reference solution at LOD, 50 pg/mL. Again the signal-to-noise ratio is improved not by signal enhancement, but by reduction of the chromatographic baseline. Panel 4c demonstrates a negative control sample. The red arrows indicate the retention time at which stanozolol elutes. No signal is observed in all transitions.

LC-FAIMS-MS/MS analysis

Figure 2 shows a representative compensation voltage (CV) scan for epitrenbolone under standard FAIMS conditions. Similar results were determined for all other compounds in the study and are listed in Table 1. The maximum signal represents the optimum CV at which the compound emerges from FAIMS.

Representative chromatograms with and without FAIMS are shown in Figures 3–7. Recorded diagnostic ion-transitions of all analytes were selected based on earlier MS/MS studies and are extensively described elsewhere.[1,7,12] In each figure the left traces are LC-MS/MS chromatograms and the right are LC-FAIMS-MS/MS chromatograms. Epitrenbolone is shown in Figure 3. Figure 3a

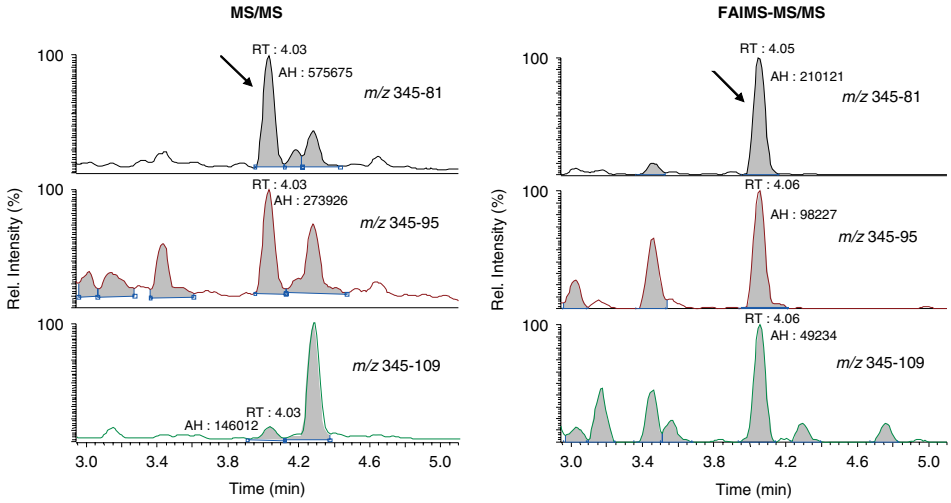

Figure 5. Representative LC-MS/MS and LC-FAIMS-MS/MS chromatograms for 16β-hydroxystanozolol showing a positive finding at approximately 500 pg/mL. Interferences near to the peak at retention time 4.03 min and a raised chromatographic baseline are removed by using LC-FAIMS-MS/MS, with corresponding improvement in signal-to-noise ratio of approximately fivefold.

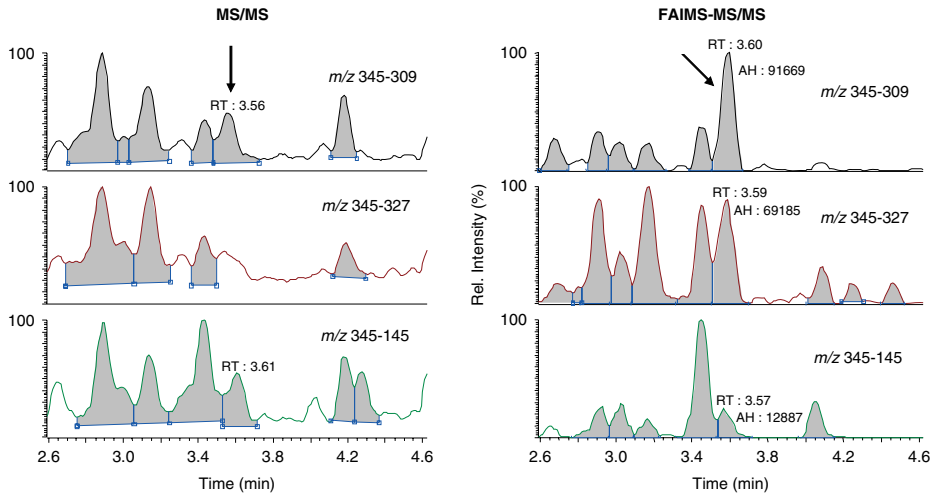

Figure 6. Representative LC-MS/MS and LC-FAIMS-MS/MS chromatograms for 4β-hydroxystanozolol. The LC-MS/MS results suggest that possibly no decision could be made because the signal-to-noise ratio is only 2. In contrast, the LC-FAIMS-MS/MS results more clearly demonstrate sharp chromatographic peaks at retention time 3.60 min and signal-to-noise ratio of approximately 20.

presents a positive finding for a sport doping control sample at a concentration of approximately 3 ng/mL. The LC-MS/MS indicated the presence of many isobaric interferences in every ion-transition and for the ion-transition *m/z* 271-253 (loss of water, −18Da) a signal-to-noise ratio (S/N) of approximately 5. The same sample analysed by LC-FAIMS-MS/MS demonstrated fewer interferences and S/N of approximately 15. This improved S/N and reduction of interference improves the confidence of the analysis and fulfils criteria for a positive finding.[29] Figure 3b is from the analysis of a control sample demonstrating the absence of epitrenbolone.

Figure 4 shows representative chromatograms for stanozolol. As presented on the right (FAIMS-MS/MS) the improvement in S/N using FAIMS is ten times for a positive doping control sample at an estimated concentration for stanozolol of 65 pg/mL (Figure 4a) and for a reference sample at the LOD (50 pg/mL, Figure 4b). In both cases the chemical background in the baseline has been removed, resulting in improved confidence regarding the finding. Utilizing FAIMS, criteria for the qualitative determination are clearly fulfilled (S/N >3) even at the sub-pg/mL level.[4]

Figure 4c demonstrates the behaviour of an undoped control sample. No interference could be observed for any ion transitions at the respective retention time for stanozolol (4.8 min).

In the case of the 16β- and 4β-hydroxystanozolol metabolites in Figures 5 and 6, respectively, there is nominally an improvement of 5× and 10× in S/N. As depicted by Figure 5, an unambiguous identification of 16β-hydroxy stanozolol (concentration approximately 500 pg/mL) is achieved using FAIMS. However, when considering confidence in determining a peak in Figure 6 for the 4β-metabolite (concentration approximately 100 pg/mL), LC-MS/MS results indicate only an indistinct peak for all ion transitions. A scientist suggesting that this athlete had this metabolite present would find it difficult to find support under scrutiny. In comparison, the LC-FAIMS-MS/MS chromatograms demonstrate an improvement in S/N for *m/z* 345–309 and 345–327;

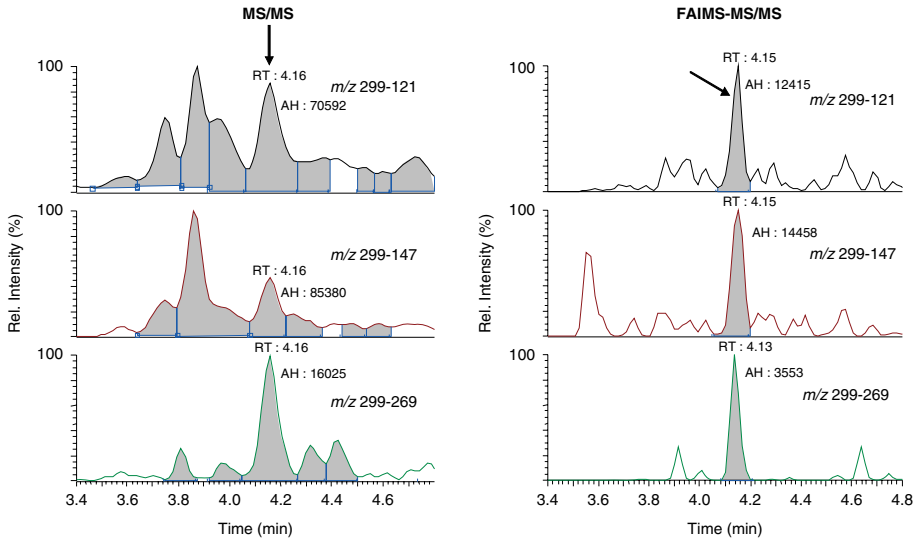

Figure 7. Representative LC-MS/MS and LC-FAIMS-MS/MS chromatograms for metandienone metabolite indicating a positive finding at approximately 200 pg/mL. The sharp chromatographic peaks of the LC-FAIMS-MS/MS analysis indicates that there are many more potential interferences in the respective LC-MS/MS chromatograms. As a result of baseline clean up the signal-to-noise ratio has improved 5-fold with a final value of 10 : 1 signal-to-noise.

nevertheless the S/N for the ion-transition *m/z* 345–145 is not sufficient.

The long-term metabolite of metandienone (17β -hydroxy-methyl-17α-methyl-18-norandrost-1,4,13-trien-3-one) shows the best reason for using this high selectivity technique to determine the presence of androgenic anabolic steroids. The analysis of this doping control sample by LC-MS/MS (Figure 7) fulfils no criteria regarding S/N (>3) to assign this sample as a positive finding. In contrast, when using LC-FAIMS-MS/MS, there is clear evidence of abuse in this sample. There is an overall improvement in S/N of 5× at the diagnostic ion-transitions *m/z* 299–121, 299-147, 299–269 and one would report a positive finding regarding the metandienone long-term metabolite 17β -hydroxymethyl-17α-methyl-18-norandrost-1,4,13-trien-3-one.

Sample preparation aspects

In this study, urine specimens were prepared according to an assay commonly used for screening analysis of anabolic steroids.[33,34] This method frequently yielded a high chemical background when used for confirmatory purposes and thus sophisticated clean-up procedures were applied for the detection of trace levels of prohibited substances in sports drug testing.[11] As this work has demonstrated, the chemical background is removed online effectively via FAIMS and special time-consuming sample preparation procedures can be avoided. Future research directions will involve exploration of ways to further simplify sample preparation.

Conclusions

There is a trend towards decreased concentration levels for banned substances in sport drug testing. This forces doping-control laboratories to improve their applied methodology continuously. Five androgenic anabolic steroid compounds were analysed by LC-FAIMS-MS/MS in human urine.

The advantage of using this technique is that a new level of selectivity is achieved. The effective removal of chemical and even isobaric interference improves detection limits and allows the unambiguous identification of all investigated prohibited substances. There are limits to this technology, including the high consumption of helium, which was mitigated by the use of method segmentation, in which this gas is used only during analyte elution.

In this work, an improvement for all investigated anabolic steroids could be achieved. Interference is removed and a positive result can be determined at progressively longer times post-dose. The implication of this finding is that athletes dosing with banned substances many weeks prior to a sporting event may be revealed and thus disqualified from competition.

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