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Applicability of routine analytical procedures to detect andarine and ostarine. A comparative study

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Abstract

The urinary excretion of selective androgen receptor modulators (SARMs) andarine and ostarine after controlled administration study was investigated. It was demonstrated that andarine is subject to extensive metabolism by desacetylation, hydroxylation and dephenylation. Importantly, most of the andarine metabolites are excreted as glucuronide and sulfate conjugates. Andarine itself is also partly conjugated with glucuronic acid. Ostarine was shown to be much more stable metabolically as its major metabolites are ostarine glucuronide and hydroxyostarine glucuronide. For the detection of andarine, ostarine and their metabolites several methods such as gas and liquid chromatography coupled to tandem mass spectrometry (GC- and LC-MS/MS) were compared. Of the analytical methods tested, LC-MS/MS of conjugated (total) fraction is preferred, though direct analysis of diluted urine is also suitable for determination of desacetylhydroxy-andarine sulfate and ostarine glucuronide. However, in the latter case the detection time window was not as long. Both SARMs were detectable in urine for about 2 weeks after a single oral dose of 60 mg of andarine and 30 mg of ostarine.

Introduction

Andarine (S-4) and ostarine (S-22) are the drug candidates belonging to the class of selective androgen receptor modulators which possess anabolic activity and promote muscle growth. Both compounds are currently advertised via the Internet [1] and could therefore be used by professional athletes. Thus, antidoping laboratories should have the analytical procedures in place to be capable of identifying these compounds. While the metabolism of andarine and ostarine has been reported earlier [2-4], the detection time window and what is the best target to detect their abuse is not yet clear enough. Therefore, the aim of present study was to investigate the urinary excretion of andarine and ostarine, compare different sample preparation protocols and select optimal detection methods.

Experimental

Three volunteers participated in this study. Single oral doses of 60 mg of andarine (male, age 53; female, age 26) and 30 mg of ostarine (female, age 33) were administered, and urine was collected up to 3 weeks. The excretion study was approved by local Ethics Committee at the Institute of Sport.
Urine samples were processed as follows: (*1*) 200 µL of urine were diluted with 800 µL of solvent mixture (0.1% formic acid and 3% methanol in water with mefruside as internal standard); (*2*) 3 mL of urine were extracted with diethyl ether in the presence of Na_2SO_4 at pH 8.5-9.5, followed by evaporation of the extract and reconstitution in 100 µL of water/methanol (60/40); (*3*) 3 mL of urine were deconjugated with a solution of β-glucuronidase from *E.coli* in phosphate buffer containing methyltestosterone as internal standard (pH 6.4, 57°C, 1h), followed by extraction and reconstitution, as in (*2*); (*4*) similar to (*3*) but after evaporation of the organic extract the residue was derivatized with 50 µL of MSTFA/NH_4I/dithiothreitol at 70°C for 30 min.
LC–MS/MS analyses were accomplished on an Acquity LC (Waters, Milford, MA, USA) coupled to a TSQ Vantage (ThermoFisher Scientific, San Jose, CA, USA). A Waters Acquity BEH C18 column (100 mm × 2.1 mm, particle size 1.7 µm) maintained at 60°C and protected by a Vanguard BEH C18 column (20 mm × 2.1 mm) was used for separation.

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GC-MS system comprised a gas chromatograph 6890N coupled to a mass spectrometer 5973inert (Agilent, Palo Alto, CA, USA). GC–MS/MS was performed using a gas chromatograph Trace GC (Thermo Scientific, Rodano, Italy) interfaced with a mass spectrometer Quantum XLS (ThermoFisher Scientific, San Jose, CA, USA). GC separation was achieved on a J&W Ultra-1 column (17 m × 0.20 mm × 0.11 µm) applying temperature programming.

Results and Discussion

Our experiments have shown that andarine is subject to extensive metabolism, which is consistent with published data [2,3]. The main pathways of its biotransformation in humans are desacetylation, hydroxylation and dephenylation. Importantly, most of the andarine metabolites are excreted as conjugates with glucuronic acid or sulfate, with parent compound being also partly conjugated with glucuronic acid.

Having compared all the methods used in our study in terms of the detection time window they provide, it became clear that:
(*1*) for reliable detection of andarine abuse the optimal target is desacetylhydroxy-andarine, which is excreted almost equally as sulfate and glucuronide. Noteworthy, the sulfate is extractable with diethyl ether and could be detected in the extraction-based procedures followed by the LC–MS/MS analysis;
(*2*) direct urine analysis, while giving a much deeper insight into the metabolism as it allows detecting intact glucuronide and sulfate conjugates (Fig. 2), is intrinsically less sensitive;
(*3*) using GC–MS or GC–MS/MS is generally unsuitable for the detection of andarine and its dephenylated metabolite due to poor chromatographic properties of these compounds and incomplete derivatization resulting in the two products upon trimethylsilylation.

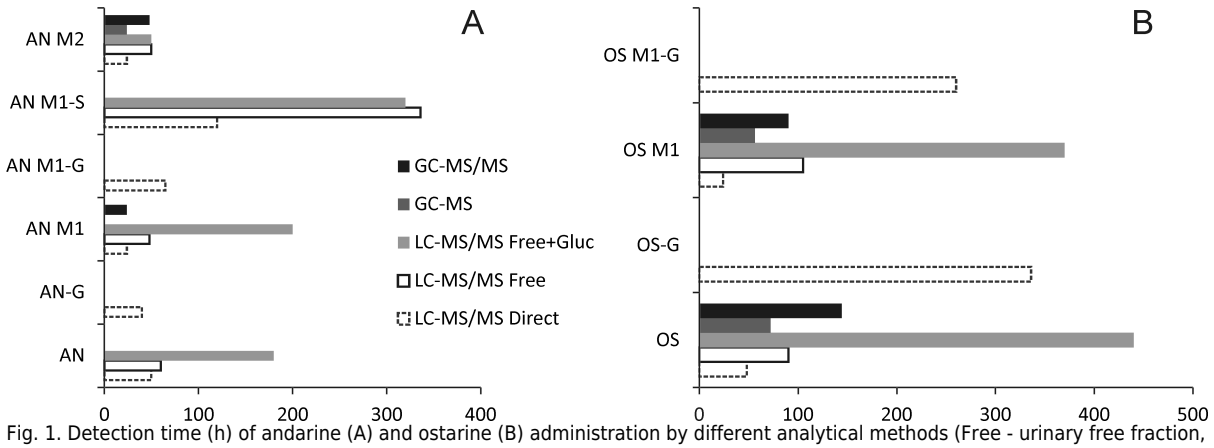

Fig. 1. Detection time (h) of andarine (A) and ostarine (B) administration by different analytical methods (Free - urinary free fraction, Free+Gluc - total fraction, Direct - analysis by "dilute and shoot").

Ostarine was found to be more stable metabolically, supporting the results reported in [4], and its major metabolites are ostarine glucuronide and hydroxyostarine glucuronide (Table 1). Therefore, the detection of deconjugated parent compound seems to be adequate for screening purposes in doping control analysis. The hydroxyostarine (also excreted as glucuronide) was generally less abundant compared to the parent. Similarly, ostarine and hydroxyostarine are more sensitively assayed by LC–MS/MS than by GC–MS or GC–MS/MS (Fig. 1B) due to the same issues as for andarine. Direct urine analysis could be used to detect intact glucuronides (Fig. 3), but the detection time window is shorter in this case.

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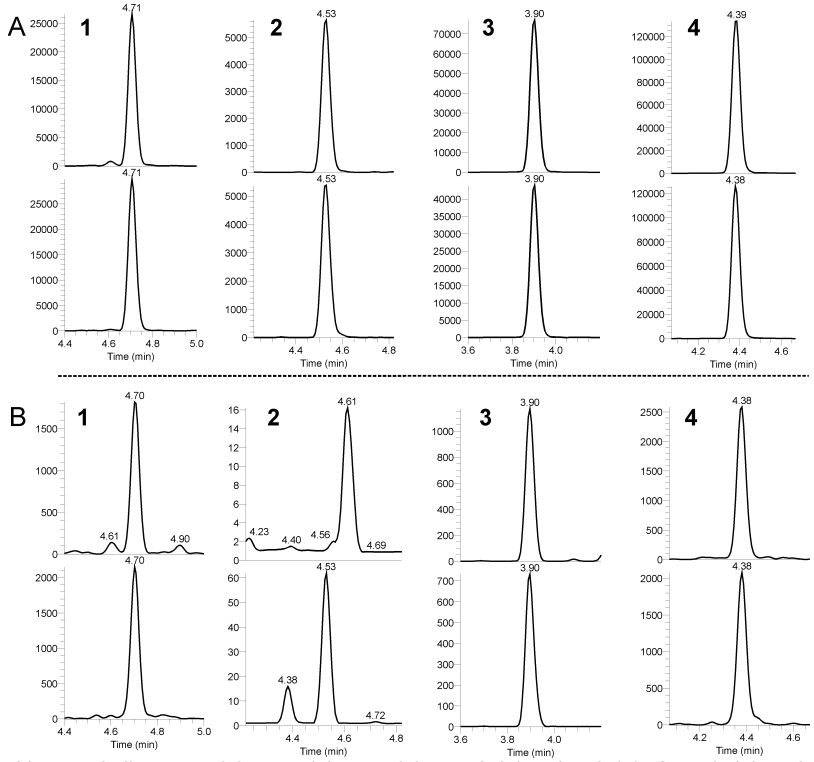

Fig. 2. Andarine (1) and its metabolites AN-G (2), M1-G (3), M1-S (4) at 24 h (A) and 58 h (B) after administration.

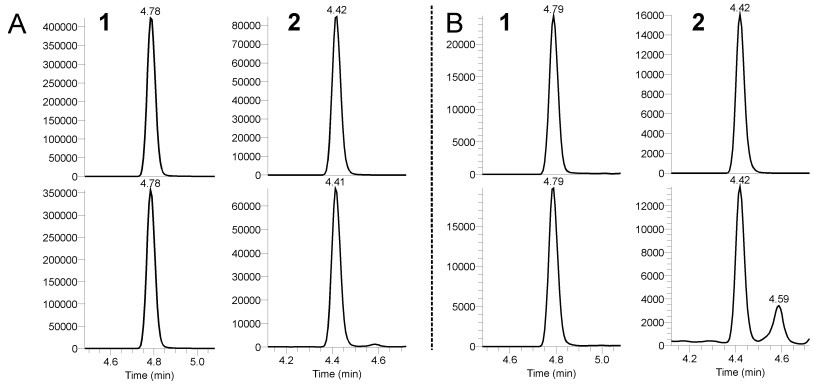

Fig. 3. Ostarine (1) and hydroxyostarine (2) glucuronides at 24 h (A) and 7 days (B) after administration.

Conclusions

LC-MS/MS with negative mode electrospray ionization is the method of choice for the detection of andarine and ostarine abuse. For andarine, the most long-term excreted metabolite seems to be desacetylhydroxy-andarine sulfate, while in case of ostarine the detection of parent compound is sufficient for screening purposes. Analysis of enzymatically deconjugated urine should be preferred, though analysis after urine dilution is also suitable for the direct assay of desacetylhydroxy-andarine sulfate and ostarine glucuronide. However, in the latter case the detection time window is not as long. Both SARMs are detectable in urine for approximately 2 weeks after a single dose of 60 mg of andarine and 30 mg of ostarine.

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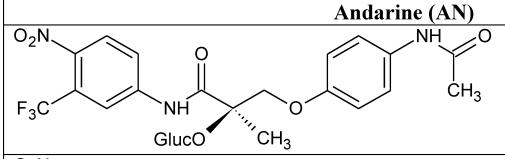
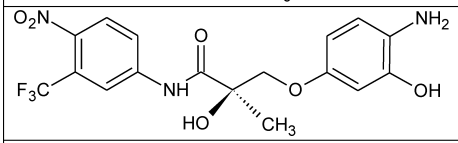
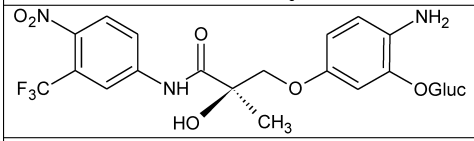
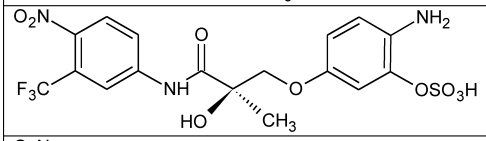
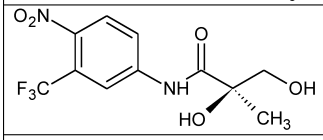
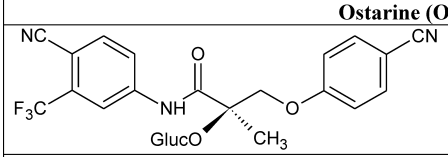
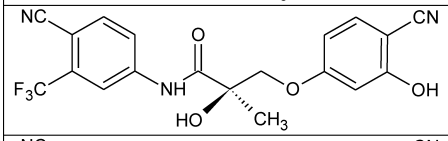
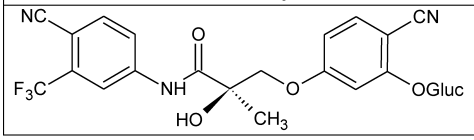
Metabolite	Name	SRM (CE)
Andarine (AN)		
	AN-G	440 > 261 (15) 440 > 150 (30)
	AN M1	414 > 261 (20) 414 > 205 (35)
	AN M1-G	590 > 261 (35) 590 > 205 (35)
	AN M1-S	494 > 261 (30) 494 > 205 (35)
	AN M2	307 > 205 (20)
Ostarine (OS)		
	OS-G	564 > 185 (40) 564 > 445 (20)
	OS M1	404 > 134 (20) 404 > 269 (20)
	OS M1-G	580 > 134 (30) 580 > 404 (20)

Table 1. Tentative structure of andarine and ostarine metabolites and SRM transitions used for their detection. Position of glucuronide and sulfate may vary.

References

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