Journal of Pharmaceutical and Biomedical Analysis 233 (2023) 115468

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.journals.elsevier.com/journal-of-pharmaceutical-and-biomedical-analysis

Equine in vivo metabolite profiling of the selective androgen receptor modulator LGD-3303 for doping control

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ARTICLE INFO

Keywords:
Mass spectrometry
Selective androgen receptor modulator
LGD-3303
Doping control
Metabolites
Equine

ABSTRACT

LGD-3303 is a Selective Androgen Receptor Modulator (SARM) that is prohibited in both equine and human sports due to its anabolic properties. The aim of this study was to investigate the equine in vivo metabolite profile of LGD-3303 and identify drug metabolites that can be suitable as new and improved analytical targets for equine doping control. This was performed by an oral administration of 0.05 mg·kg^{-1} LGD-3303 to horses, where blood and urine samples were collected up to 96 h after administration. The in vivo samples consisting of plasma, urine and hydrolyzed urine were analyzed utilizing ultra-high performance liquid chromatography hyphenated to a Q Exactive™ Orbitrap™ high resolution mass spectrometer with a heated electrospray ionization source. A total of eight metabolites of LGD-3303 were tentatively identified, including one carboxylated and several hydroxylated metabolites in combination with glucuronic acid conjugates. A monohydroxylated metabolite is suggested as an analytical target for doping control analysis of plasma and urine after hydrolysis with β-glucuronidase, due to the high intensity and prolonged detection time in comparison to parent LGD-3303.

1. Introduction

Selective Androgen Receptor Modulators (SARMs) constitute a pharmacological class of substances that have anabolic properties and are prohibited in human and equine sports, both in and out of competition, according to the International Federation of Horseracing Authorities (IFHA) and the World Anti-Doping Agency (WADA) [1–4]. Several SARMs have undergone clinical trials, but none of them have been approved and registered in a pharmaceutical product [5,6]. However, many of them are easily accessible on the Internet either advertised with the substance name or present in dietary supplements [7]. Their anabolic properties make them tempting to use illicitly for performance enhancement in sports [8–10].

When new doping agents, such as SARMs, are emerging on the black market, the drug metabolite profiles are often unknown. As demonstrated in many previously published studies, the use of a drug metabolite as analytical target often increases the detection time, since drug metabolites can be present in biological matrices such as blood and urine for an extended time [11,12]. To find and select the most suitable analytical targets for doping analysis, administration studies are preferred, but if not possible, different in vitro systems such as liver microsome incubations can also be a viable option, followed by analysis with ultra-high-performance liquid chromatography-high resolution mass spectrometry (UHPLC-HRMS) [13].

The pharmacokinetics and pharmacodynamics of the SARM LGD-3303 ([9-chloro-2-ethyl-1-methyl-3-(2,2,2-trifluoroethyl)− 3 H-pyrrolo-[3,2-f]quinolin-7(6 H)-one]) have been studied in a rat model, and it has been shown that LGD-3303 is orally bioavailable and has tissue-selective properties that are most likely due to altered molecular interactions with the androgen receptor in comparison to anabolic androgenic steroids such as testosterone [14]. Additional studies of LGD-3303 performed in rats have shown that the substance affects the sexual preference [15] and that it together with alendronate has an additive effect in treatment of osteoporosis [16]. Also, the impact of LGD-3303 on the endogenous steroid profile has been studied in vitro. There was no strong impact on the steroid profile from either LGD-3303

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https://doi.org/10.1016/j.jpba.2023.115468
Received 10 March 2023; Received in revised form 27 April 2023; Accepted 17 May 2023
Available online 18 May 2023

or the other SARMs presented in the same publication [17]. The metabolite profile of LGD-3303 has been studied in vitro by Cutler et. al in equine liver microsomes and the results showed that mainly different hydroxylation transformations had taken place [18]. There are to the best of the authors' knowledge no published in vivo metabolism studies of this compound.

The aim of this project was to investigate the equine in vivo metabolite profile of LGD-3303 and identify its metabolites by analysis of urine and plasma with UHPLC-HRMS, that can be suitable as new and improved analytical targets for equine doping control.

2. Material and methods

2.1. Chemicals

β-glucuronidase from *E. coli* K12 (80 U·mg^{-1} at 25 °C) was purchased from Roche (Basel, Switzerland). LGD-3303 (98.27%) for the administration part of the study was purchased from ChemScene (Monmouth Junction, NJ, USA). LGD-3303 (98.27%) for analysis was purchased from MedChemExpress (Monmouth Junction, NJ, USA). Formic acid (Optima LC-MS grade) and Pierce™ electrospray ionization (ESI) Positive/Negative Ion Calibration Solution were purchased from Thermo Fisher Scientific (Waltham, MA, USA). Purified water was obtained using Milli-Q® Advantage A10 with a 0.22-μm filter from Millipore (Burlington, MA, USA). All other chemicals and solvents were of analytical grade or higher.

2.2. LGD-3303 administration and sample collection

The administration study protocol has previously been described in Broberg et al. [12]. In short, two University of California-owned adult Thoroughbred, one mare and one gelding were orally administered a dose of 0.05 mg·kg^{-1} LGD-3303. Blood samples were collected in EDTA tubes immediately prior to LGD-3303 administration and 0.25, 0.50, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 8.0, 12, 24, 36, 48, 72 and 96 h post administration. The plasma was processed and transferred to separate cryovials and stored at − 20 °C until analysis. Urine samples were collected by free catch at 6, 24, 48, 72 and 96 h post LGD-3303 administration and stored at − 20 °C until analysis. One planned urine sample (6-hour sample) was not collected. The study was approved by the Institutional Animal Care and Use Committee of the University of California, Davis (Protocol #20319, date of approval January 11, 2020).

2.3. Urine sample preparation

The urine samples were aliquoted upon arrival and different sample preparation techniques were used prior to analysis. For an initial dilute and shoot analysis, the urine samples were centrifuged in Eppendorf tubes at 11,500 *g* for 10 min in a Sepatech Biofuge 15 (Heraeus, Hanau, Germany) and the supernatants were diluted 1:1 with aqueous formic acid (0.1%) and transferred to vials for analysis.

For hydrolysis with β-glucuronidase, the urine samples (2.0 mL) were added to 2.0 mL of phosphate buffer (pH 6.1; 0.1 M,) and 100 μL of β-glucuronidase was added. The samples were placed in a heating bath and incubated at 50 °C for 2 h. They were left to cool in room temperature and thereafter extracted using the solid phase extraction (SPE) method described below.

For the SPE method with the Oasis HLB 60 mg solid phase extraction cartridges from Waters (Milford, MA, USA), the unhydrolyzed urine samples were prepared with a generic method by first diluting 2.0 mL of urine with 2.0 mL of aqueous formic acid (0.1%). The hydrolyzed urine was also diluted with 2.0 mL of aqueous formic acid (0.1%). The SPE cartridges were conditioned with 6.0 mL MeOH and 2.0 mL aqueous formic acid (0.1%). The samples were loaded on to the cartridge and washed with 2.0 mL of 5% MeOH in water and thereafter eluted using 1.5 mL of MeOH. The solvent was evaporated to dryness using the vacuum centrifuge Christ RVC 2–8 (Martin Christ, Osterode am Harz, Germany). The samples were reconstituted in 600 μL of aqueous formic acid (0.1%), centrifuged at 11,500*g* for 10 min and transferred to vials for analysis.

2.4. Plasma sample preparation

Protein precipitation was performed by transferring 200 μL of each plasma sample to an Eppendorf tube. Ice cold acetonitrile (800 μL) was added and the Eppendorf tubes were mixed using a vortex mixer and stored at 5 °C for 20 min. The samples were centrifuged at 11,500*g* for 10 min and 800 μL of the supernatant was transferred to a new Eppendorf tube and evaporated to dryness using a vacuum centrifuge. The samples were thereafter reconstituted in 200 μL of aqueous formic acid (0.1%), centrifuged at 11,500*g* for 10 min and transferred to vials for analysis.

2.5. UHPLC-HRMS analysis

The chromatographic separation was performed using a Vanquish UHPLC^{+} focused binary pump F and an Vanquish Split Sampler FT autosampler from Thermo Fisher Scientific (Waltham, MA, USA) using an Acquity UPLC® HSS T3 column (2.1 × 100 mm; particle size 1.8 μm) with an HSS T3 guard column (2.1 × 5 mm; particle size 1.8 μm) from Waters (Milford, MA, USA) at 40 °C. The injection volume was 5 μL and the mobile phase consisted of 0.1% formic acid in either water (mobile phase A) or acetonitrile (mobile phase B). The flow rate was set to 0.45 mL·min^{-1} and the gradient used consisted of 3% of mobile phase B for 1 min, thereafter a linear gradient from 3% to 97% of mobile phase B over 16 min that was thereafter held at 97% for 4 min. For re-equilibration prior to the next run, the composition was held at 3% of mobile phase B for 3 min.

The UHPLC system was hyphenated to a Q Exactive™ Orbitrap™ benchtop mass spectrometer equipped with a heated electrospray ionization probe (HESI-II), all from Thermo Fisher Scientific (Waltham, MA, USA) used through the software TraceFinder™ 5.1. The spray voltage vas 3.5 kV in positive ionization, and − 3.0 kV in negative ionization. The auxiliary gas heater temperature was 400 °C and the capillary temperature was 320 °C. Nitrogen was used as collision gas, the sheath gas flow rate was 50, auxiliary gas flow rate was 10, S-lens RF level was 60 (all in arbitrary units). Data analysis was performed using the software FreeStyle™ 1.8 from Thermo Fisher Scientific. The system was operated at a resolution of 70,000 at *m/z* 200 at full width at half maximum (FWHM), in full scan mode (*m/z* 100–1000) and the data was collected in profile mode.

For analysis, several MS methods were utilized for each sample. All samples were analysed with a full scan MS. This was also used in combination with data dependent MS/MS analysis (full scan MS/ddMS^2), the data dependent selection was performed with a automated gain control (AGC) target of $5*10^4$ with a resolution of 17,500. For further analysis, data independent MS/MS analysis was performed for selected ions of interest where the inclusion window was set to ± 0.2 Da and stepped normalized collision energy (NCE) were varied between 17.5% and 90%. For metabolite investigation and data analysis, all potential metabolites were compared with results from blank urine and plasma samples from individuals not treated with LGD-3303. The metabolite search was extensive, but mainly performed by searching for theoretically possible metabolite compositions based on knowledge regarding metabolic transformations and the search for characteristic fragments from the administered substance.

3. Results and discussion

3.1. Detection of LGD-3303 and metabolites

LGD-3303 could be detected in urine and plasma as phase I and

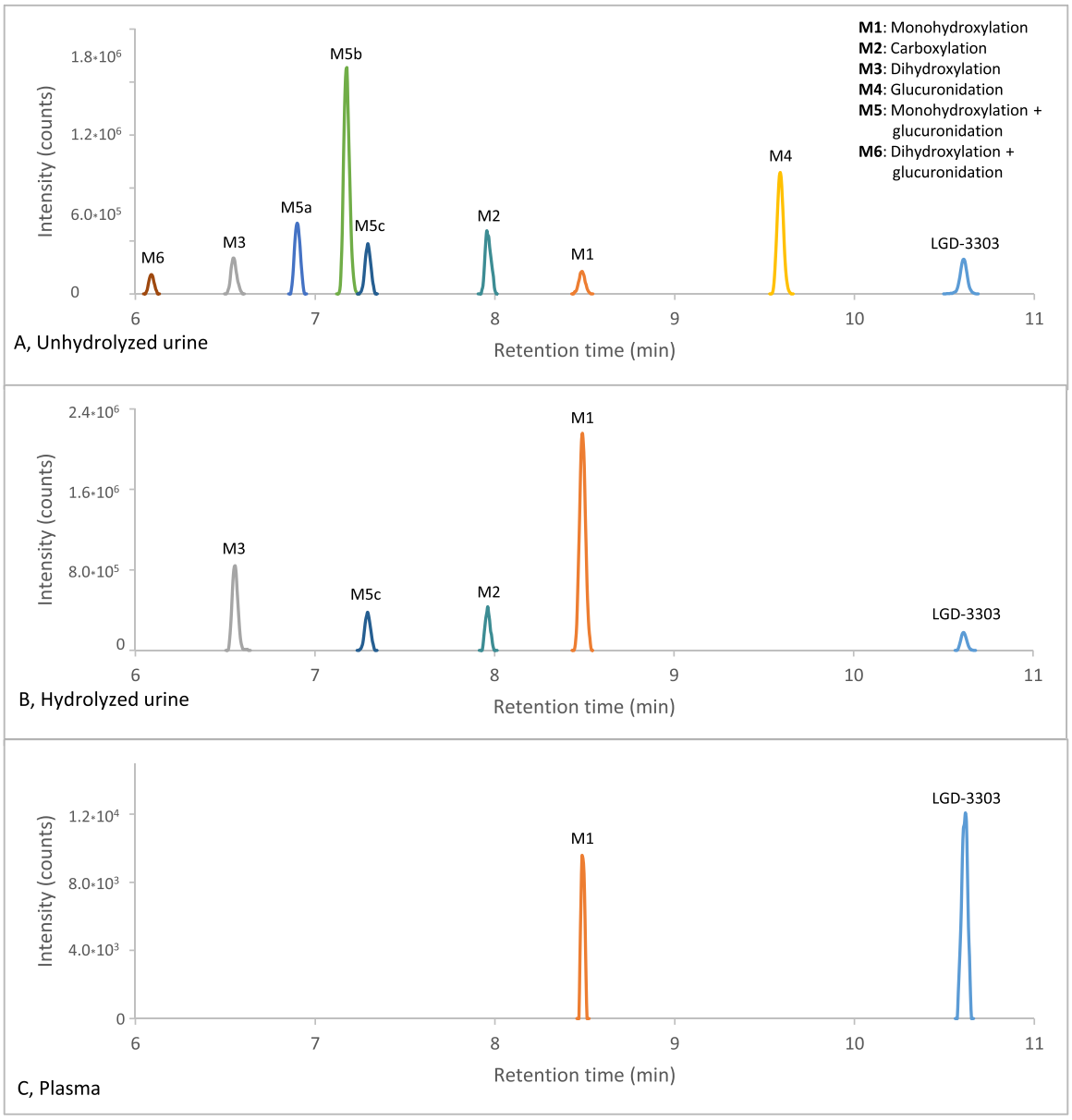

Fig. 1. Extracted ion chromatograms in positive ionization mode for metabolites from samples taken 6 h after oral administration of LGD-3303 from **A**, unhydrolyzed urine (SPE), **B**, hydrolyzed urine (SPE) and **C**, plasma (protein precipitation). Details regarding the metabolites can be found in Table 1.

phase II metabolites, but also as the parent compound, as seen in Fig. 1. The parent compound could be detected in plasma, urine and hydrolyzed urine up to 24 h after administration, in comparison to the formed metabolites that generally could be detected for a longer time after administration, some even up to 72 h. The administered substance could be identified by comparing the results with those of the reference substance regarding accurate mass, retention time and collision induced dissociation pattern.

A tentative structural identification of the metabolites was carried out using accurate masses and comparison of their dissociation patterns with that of parent LGD-3303. A total of eight metabolites of LGD-3303 were identified. In plasma, only the monohydroxylated metabolite (M1) was identified together with parent LGD-3303. The intensity of LGD-3303 was initially higher than that of the metabolite, but at 36 h after administration, only M1 was detected.

In urine, one carboxylated and several hydroxylated metabolites and glucuronides thereof were identified, which can be seen in the chromatograms in Fig. 1 and in Table 1. The highest intensity over time was from a monohydroxylated glucuronide (M5b) followed by the parent glucuronide (M4) that were both present until 48 h. After hydrolysis of urine, M1 showed the highest intensity at all time points and was detected up to 72 h. These results demonstrate the usefulness of M1 as an analytical target for doping control both for hydrolyzed urine and plasma.

In the structural elucidation based on MS/MS analysis, the comparison of product ions between LGD-3303 and M1 strongly supports the conclusion that M1 is a metabolite from LGD-3303. There are three product ions of M1 (F1, F2 and F3 in Fig. 2) that match those of LGD-3303 but with the addition of the mass of one oxygen (+16).

Structural elucidation by MS/MS of cyclic compounds such as LGD-3303 is often complicated due to the fact that the ring structures are often stable and remain intact with low collision energy or rearranges in the dissociation process at higher collision energy. The presence of a carboxylated metabolite indicated that the alkyl substituents are somewhat prone to metabolic transformations in addition to the ring structures.

Regarding glucuronide metabolites, both the glucuronidated parent compound (M4) and one of the monohydroxylated glucuronidated

Table 1
Summary of the eight tentatively identified metabolites of LGD-3303 after a single oral administration. Samples were collected from 0 to 96 h. Plasma, urine and hydrolyzed urine samples, which had been treated with β-glucuronidase, were analyzed in both positive and negative electrospray ionization mode, all identification was performed in positive ionization mode. The theoretical *m/z* (Th. *m/z*) is based on the elemental composition of the positively charged ion and the experimental *m/z* (Ex. *m/z*) is from the full scan MS analysis. The column to the right shows the detection window after administration: P, in plasma after protein precipitation, U, unhydrolyzed urine after SPE and H.U, hydrolyzed urine after SPE.

Metabolite	Ion/t_R (min)	$[M+H]^+$ Th. *m/z* Ex. *m/z* (ppm)	Product ions *m/z* (ppm)	Elemental composition	Window of detection P / U / H. U (hours)
LGD-3303 $C_{16}H_{14}ClF_3N_2O$	+H/ 10.59	343.0820 343.0820 (0.0)	328.0593 (2.4) 307.1059 (2.0) 293.0901 (1.7) 289.0951 (1.4) 275.0796 (1.8) 261.0638 (1.5) 259.0636 (1.2) 245.0481 (2.0) 227.0373 (0.9) 225.1023 (0.4) 223.0870 (1.8) 205.0764 (2.0)	$C_{15}H_{12}ClF_3N_2O^{\bullet+}$ $C_{16}H_{14}F_3N_2O^+$ $C_{15}H_{12}F_3N_2O^+$ $C_{16}H_{12}F_3N_2^+$ $C_{15}H_{10}F_3N_2^+$ $C_{14}H_8F_3N_2^+$ $C_{14}H_{12}ClN_2O^+$ $C_{13}H_{10}ClN_2O^+$ $C_{13}H_8ClN_2^+$ $C_{14}H_{13}N_2O^+$ $C_{14}H_{11}N_2O^+$ $C_{14}H_9N_2^+$	1–24 / 6–24 / 6–24
M1 Monohydroxylation $C_{16}H_{14}ClF_3N_2O_2$	+H/8.49	359.0769 359.0770 (0.3)	344.0538 (1.2) 329.0301 (0.6) 315.0508 (0.3) 305.0897 (0.3) 291.0744 (1.4) 261.0429 (1.5) 233.0478 (0.9) 211.0868 (0.9) 207.0555 (1.0)	$C_{15}H_{12}ClF_3N_2O_2^{\bullet+}$ $C_{14}H_9ClF_3N_2O_2^+$ $C_{14}H_{11}ClF_3N_2O^+$ $C_{16}H_{12}F_3N_2O^+$ $C_{15}H_{10}F_3N_2O^+$ $C_{13}H_{10}ClN_2O_2^+$ $C_{12}H_{10}ClN_2O^+$ $C_{13}H_{11}N_2O^+$ $C_{13}H_7N_2O^+$	1–36 /6–48 / 6–72
M2 Carboxylation $C_{16}H_{12}ClF_3N_2O_3$	+H/7.96	373.0561 373.0562 (0.3)	329.0662 (−0.3) 314.0431 (1.0) 293.0898 (0.7) 245.0478 (0.8)	$C_{15}H_{13}ClF_3N_2O^+$ $C_{14}H_{10}ClF_3N_2O^{\bullet+}$ $C_{15}H_{12}F_3N_2O^+$ $C_{13}H_{10}ClN_2O^+$	- / 6–24 / 6–24
M3 Dihydroxylation $C_{16}H_{14}ClF_3N_2O_3$	+H/6.55	375.0718 375.0720 (0.5)	344.0539 (1.5) 329.0304 (1.5) 261.0430 (1.9) 233.0479 (1.3) 207.0556 (1.4)	$C_{15}H_{12}ClF_3N_2O_2^{\bullet+}$ $C_{14}H_9ClF_3N_2O_2^+$ $C_{13}H_{10}ClN_2O_2^+$ $C_{12}H_{10}ClN_2O^+$ $C_{13}H_7N_2O^+$	- / 6–48 / 6–48
M4 Glucuronidation $C_{22}H_{22}ClF_3N_2O_7$	+H/9.59	519.1140 519.1140 (0.0)	343.0823 (0.9) 328.0588 (0.9) 307.1058 (1.6) 293.0901 (1.7) 289.0952 (1.7) 261.0638 (1.5) 259.0637 (1.5) 245.0480 (1.6) 225.1025 (1.3) 223.0869 (1.3) 199.0217 (2.0)	$C_{16}H_{15}ClF_3N_2O^+$ $C_{15}H_{12}ClF_3N_2O^{\bullet+}$ $C_{16}H_{14}F_3N_2O^+$ $C_{15}H_{12}F_3N_2O^+$ $C_{16}H_{12}F_3N_2^+$ $C_{14}H_8F_3N_2^+$ $C_{14}H_{12}ClN_2O^+$ $C_{13}H_{10}ClN_2O^+$ $C_{14}H_{13}N_2O^+$ $C_{14}H_{11}N_2O^+$ $C_6H_6F_3O_4^+$	- / 6–48 / -
M5a Monohydroxylation + glucuronidation $C_{22}H_{22}ClF_3N_2O_8$	+H/6.90	535.1090 535.1090 (0.0)	359.0769 (0.0) 341.0668 (1.5) 326.0433 (1.5) 305.0900 (1.3) 257.0479 (1.2) 221.0714 (2.3)	$C_{16}H_{15}ClF_3N_2O_2^+$ $C_{16}H_{13}ClF_3N_2O^+$ $C_{15}H_{10}ClF_3N_2O^{\bullet+}$ $C_{16}H_{12}F_3N_2O^+$ $C_{14}H_{10}ClN_2O^+$ $C_{14}H_9N_2O^+$	- / 6–48 / -
M5b Monohydroxylation + glucuronidation $C_{22}H_{22}ClF_3N_2O_8$	+H/7.17	535.1090 535.1090 (0.0)	359.0770 (0.3) 344.0537 (0.9) 341.0666 (0.9) 329.0302 (0.9) 315.0510 (1.0) 291.0742 (0.7) 261.0429 (1.5) 233.0479 (1.3) 211.0869 (1.4) 207.0555 (1.0)	$C_{16}H_{15}ClF_3N_2O_2^+$ $C_{15}H_{12}ClF_3N_2O_2^{\bullet+}$ $C_{16}H_{13}ClF_3N_2O^+$ $C_{14}H_9ClF_3N_2O_2^+$ $C_{14}H_{11}ClF_3N_2O^+$ $C_{15}H_{10}F_3N_2O^+$ $C_{13}H_{10}ClN_2O_2^+$ $C_{12}H_{10}ClN_2O^+$ $C_{13}H_{11}N_2O^+$ $C_{13}H_7N_2O^+$	- / 6–48 / -
M5c Monohydroxylation + glucuronidation $C_{22}H_{22}ClF_3N_2O_8$	+H/7.28	535.1090 535.1087 (−0.6)	359.0772 (0.8) 344.0538 (1.2) 329.0303 (1.2) 291.0747 (2.4) 261.0429 (1.5) 233.0480 (1.7) 211.0868 (0.9) 199.0216 (1.5)	$C_{16}H_{15}ClF_3N_2O_2^+$ $C_{15}H_{12}ClF_3N_2O_2^{\bullet+}$ $C_{14}H_9ClF_3N_2O_2^+$ $C_{15}H_{10}F_3N_2O^+$ $C_{13}H_{10}ClN_2O_2^+$ $C_{12}H_{10}ClN_2O^+$ $C_{13}H_{11}N_2O^+$ $C_6H_6F_3O_4^+$	- / 6–48 / 6–24
M6 Dihydroxylation + glucuronidation $C_{22}H_{22}ClF_3N_2O_9$	+H/6.07	551.1039 551.1036 (−0.5)	375.0723 (1.3) 344.0540 (1.7) 329.0304 (1.5) 261.0429 (1.5)	$C_{16}H_{15}ClF_3N_2O_3^+$ $C_{15}H_{12}ClF_3N_2O_2^{\bullet+}$ $C_{14}H_9ClF_3N_2O_2^+$ $C_{13}H_{10}ClN_2O_2^+$	- / 6–24 / -

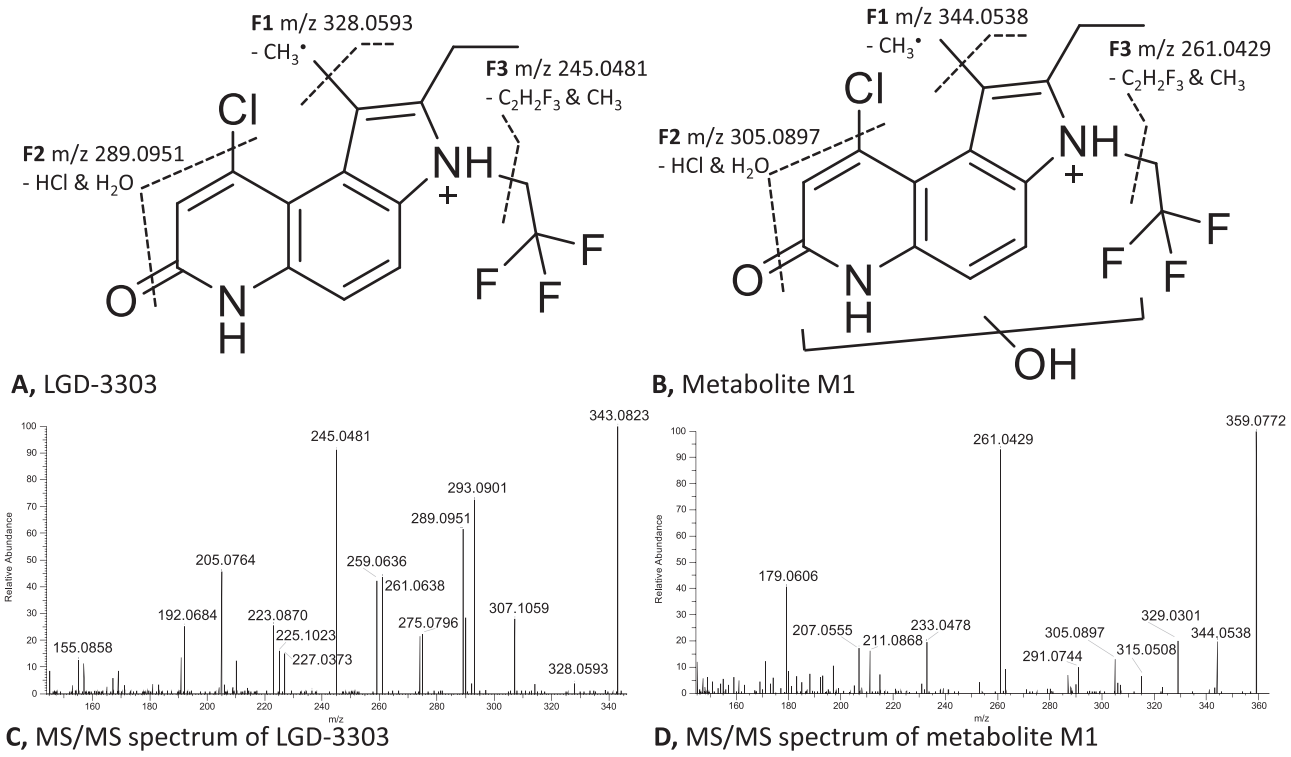

Fig. 2. Comparison of fragmentation between **A**, LGD-3303 and **B**, monohydroxylated metabolite M1. MS/MS spectrum of **C**, LGD-3303 (*m/z* 343.0820) with stepped normalized collision energy (NCE) 50, 65, 90% and **D**, monohydroxylated metabolite M1 (*m/z* 359.0769) with stepped NCE 45, 60, 85%.

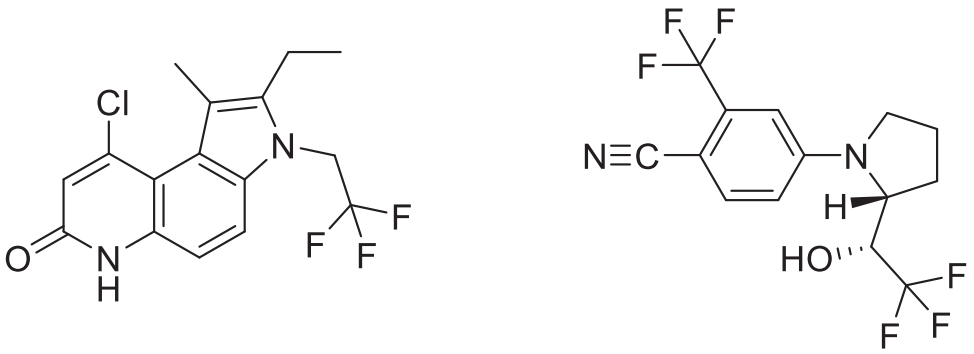

Fig. 3. The chemical structure of LGD-3303 (left) and LGD-4033 (right) without any metabolic transformations.

metabolites (M5c) created the same product ion with the elemental composition $C_6H_6F_3O_4^+$ (*m/z* 199). This elemental composition indicates that this product ion consists both of the trifluoromethyl group and parts of the glucuronic acid, likely due to the close proximity of the trifluoromethyl to the site of glucuronidation.

3.2. A glucuronide not affected by β-glucuronidase

The monohydroxylated glucuronide M5c was only affected to a very low degree by treatment with β-glucuronidase in comparison to the other phase II metabolites in this study; see Fig. 1 where M5c is detected with comparable chromatographic peak areas in both unhydrolyzed and hydrolyzed urine. This indicates that the glucuronide may not be a β-O-glucuronide, since these are expected to be sensitive to the enzyme. A possible explanation for the low effect of β-glucuronidase could be due to the electronegative properties of the trifluoromethyl group that can affect the properties of the structure. Fluorine and trifluoromethyl groups have in previous studies shown to have an inhibiting effect on β-glucuronidase [19,20]. In this study, only one isomer of hydroxy-LGD-3303 glucuronide was unaffected by the enzyme, indicating that also the position of the hydroxylation seems to affect this process. The product ion $C_6H_6F_3O_4^+$ (*m/z* 199) mentioned in Section 3.1 also supports this hypothesis, since the fragment contains the trifluoromethyl group and parts of the glucuronic acid, indicating that these structural elements are located close to each other within the structure. A similar phenomenon, where there also was a glucuronide that was only slightly affected by β-glucuronidase, has previously been observed in the equine in vivo administration study of the SARM LGD-4033 performed by Hansson et. al [21]. Both these SARMs have similar structural characteristics, such as trifluoromethyl groups seen in Fig. 3, further supporting the influence of this substituent.

4. Conclusion

A total of eight metabolites of LGD-3303 were tentatively identified. Several phase II transformations in the form of glucuronidation takes place, showing the importance of performing hydrolysis of the urine prior to analysis. The main metabolite that is suggested as an analytical

target for doping control in both plasma and hydrolyzed urine is the monohydroxylated metabolite M1, due to its overall higher intensity and the increased detection time compared to the parent compound.

Ethical declaration

This study was approved by the Institutional Animal Care and Use Committee of the University of California, Davis (Protocol #20319, date of approval 11 January 2020).

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CRediT authorship contribution statement

Malin Nilsson Broberg: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Heather Knych**: Conceptualization, Methodology, Resources, Writing – review & editing, Funding acquisition. **Ulf Bondesson**: Conceptualization, Methodology, Writing – review & editing, Supervision. **Curt Pettersson**: Conceptualization, Methodology, Writing – review & editing, Supervision. **Börje Tidstedt**: Methodology, Recourses, Writing – review & editing, Supervision. **Scott Stanley**: Conceptualization, Methodology, Writing – review & editing. **Mario Thevis**: Conceptualization, Methodology, Writing – review & editing. **Mikael Hedeland**: Conceptualization, Methodology, Recourses, Writing – review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

The data used for this manuscript, such as additional MS/MS spectra, are available via the corresponding author upon request.

References

[1] International Federation of Horseracing Authorities - The International Agreement on Breeding, Racing and Wagering, (2022). ⟨https://www.ifhaonline.org/resources/ifAgreement.pdf⟩ (Accessed 2 August 2022).
[2] World Anti-Doping Agency, The World Anti-Doping Code - The 2023 Prohibited List International Standard, (2023). ⟨https://www.wada-ama.org/en/resources/world-anti-doping-program/prohibited-list⟩ (Accessed 2 February 2023).
[3] World Anti-Doping Agency, The World Anti-Doping Code - The 2008 Prohibited List International Standard, (2008). ⟨https://www.wada-ama.org/sites/default/files/resources/files/WADA_Prohibited_List_2008_EN.pdf⟩ (Accessed 3 August 2022).
[4] M. Thevis, W. Schänzer, Detection of SARMs in doping control analysis, Mol. Cell Endocrinol. 464 (2018) 34–45, https://doi.org/10.1016/j.mce.2017.01.040.
[5] J. Kang, R. Chen, T. Tharakan, S. Minhas, Novel androgen therapies including selective androgen receptor modulators, Best. Pr. Res Clin. Endocrinol. Metab. 36 (2022), https://doi.org/10.1016/j.beem.2022.101686.
[6] Y. Xie, Y. Tian, Y. Zhang, Z. Zhang, R. Chen, M. Li, J. Tang, J. Bian, Z. Li, X. Xu, Overview of the development of selective androgen receptor modulators (SARMs) as pharmacological treatment for osteoporosis (1998–2021), Eur. J. Med Chem. 230 (2022), https://doi.org/10.1016/j.ejmech.2022.114119.
[7] N.S. Kim, H.S. Choi, N.Y. Lim, J.H. Lee, H. Kim, S.Y. Baek, Application of simultaneous analytical methods for selective androgen receptor modulator adulterated in dietary supplements advertised as muscle strengthening using UHPLC-PDA and LC–ESI–MS/MS, Chromatographia 85 (2022) 895–919, https://doi.org/10.1007/s10337-022-04170-y.
[8] E. Grata, L. Perrenoud, M. Saugy, N. Baume, SARM-S4 and metabolites detection in sports drug testing: a case report, Forensic Sci. Int 213 (2011) 104–108, https://doi.org/10.1016/j.forsciint.2011.07.014.
[9] M. Thevis, C. Görgens, S. Guddat, A. Thomas, H. Geyer, Mass spectrometry in sports drug testing—analytical approaches and the athletes' exposome, Scand. J. Med Sci. Sports (2022), https://doi.org/10.1111/sms.14228.
[10] A.T. Cawley, C. Smart, C. Greer, M. Liu Lau, J. Keledjian, Detection of the selective androgen receptor modulator andarine (S-4) in a routine equine blood doping control sample, Drug Test. Anal. 8 (2016) 257–261, https://doi.org/10.1002/dta.1867.
[11] C. Gómez, O.J. Pozo, A. Fabregat, J. Marcos, K. Deventer, P. van Eenoo, J. Segura, R. Ventura, Detection and characterization of urinary metabolites of boldione by LC-MS/MS. Part I: Phase I metabolites excreted free, as glucuronide and sulfate conjugates, and released after alkaline treatment of the urine, Drug Test. Anal. 4 (2012) 775–785, https://doi.org/10.1002/dta.1433.
[12] M.N. Broberg, H. Knych, U. Bondesson, C. Pettersson, S. Stanley, M. Thevis, M. Hedeland, Investigation of equine in vivo and in vitro derived metabolites of the selective androgen receptor modulator (SARM) ACP-105 for improved doping control, Metabolites 11 (2021) 1–17, https://doi.org/10.3390/metabo11020085.
[13] M. Thevis, T. Kuuranne, H. Geyer, Annual banned-substance review—analytical approaches in human sports drug testing 2021/2022–, Drug Test. Anal. 15 (2023) 5–26, https://doi.org/10.1002/dta.3408.
[14] E.G. Vajda, F.J. López, P. Rix, R. Hill, Y. Chen, K.J. Lee, Z. O'Brien, W.Y. Chang, M. D. Meglasson, Y.H. Lee, Pharmacokinetics and pharmacodynamics of LGD-3303 [9-chloro-2-ethyl-1- methyl-3-(2,2,2-trifluoroethyl)-3H-pyrrolo-[3,2-f]quinolin-7 (6H)-one], an orally available nonsteroidal-selective androgen receptor modulator, J. Pharmacol. Exp. Ther. 328 (2009) 663–670, https://doi.org/10.1124/jpet.108.146811.
[15] A.E. Kudwa, F.J. López, R.F. McGivern, R.J. Handa, A selective androgen receptor modulator enhances male-directed sexual preference, proceptive behavior, and lordosis behavior in sexually experienced, but not sexually naive, female rats, Endocrinology 151 (2010) 2659–2668, https://doi.org/10.1210/en.2009-1289.
[16] E.G. Vajda, A. Hogue, K.N. Griffiths, W.Y. Chang, K. Burnett, Y. Chen, K. Marschke, D.E. Mais, B. Pedram, Y. Shen, A. Van Oeveren, L. Zhi, F.J. López, M.D. Meglasson, Combination treatment with a selective androgen receptor modulator (SARM) and a bisphosphonate has additive effects in osteopenic female rats, J. Bone Miner. Res. 24 (2009) 231–240, https://doi.org/10.1359/jbmr.081007.
[17] T. Piper, S. Heimbach, M. Adamczewski, M. Thevis, An in vitro assay approach to investigate the potential impact of different doping agents on the steroid profile, Drug Test. Anal. 13 (2021) 916–928, https://doi.org/10.1002/dta.2991.
[18] C. Cutler, M. Viljanto, P. Taylor, P. Hincks, S. Biddle, P. van Eenoo, Identification of equine in vitro metabolites of seven non-steroidal selective androgen receptor modulators for doping control purposes, Drug Test. Anal. 14 (2022) 349–370, https://doi.org/10.1002/dta.3189.
[19] M. Taha, N.H. Ismail, S. Imran, F. Rahim, A. Wadood, H. Khan, H. Ullah, U. Salar, K.M. Khan, Synthesis, β-glucuronidase inhibition and molecular docking studies of hybrid bisindole-thiosemicarbazides analogs, Bioorg. Chem. 68 (2016) 56–63, https://doi.org/10.1016/j.bioorg.2016.07.008.
[20] F. Ali, K.M. Khan, U. Salar, S. Iqbal, M. Taha, N.H. Ismail, S. Perveen, A. Wadood, M. Ghufran, B. Ali, Dihydropyrimidones: as novel class of β-glucuronidase inhibitors, Bioorg. Med Chem. 24 (2016) 3624–3635, https://doi.org/10.1016/j.bmc.2016.06.002.
[21] A. Hansson, H. Knych, S. Stanley, E. Berndtson, L. Jackson, U. Bondesson, M. Thevis, M. Hedeland, Equine in vivo-derived metabolites of the SARM LGD-4033 and comparison with human and fungal metabolites, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 1074– 1075 (2018) 91–98, https://doi.org/10.1016/j.jchromb.2017.12.010.