SHORT COMMUNICATION

Detection of the selective androgen receptor modulator GSK2881078 and metabolites in urine and hair after single oral administration

Alina Rading | Patricia Anielski | Detlef Thieme | Annekathrin Martina Keiler

1Institute of Doping Analysis and Sports Biochemistry Dresden, Kreischa, Germany
2Institute of Zoology, Technical University Dresden, Dresden, Germany

Correspondence
Annekathrin Martina Keiler, Institute of Doping Analysis and Sports Biochemistry Dresden, Kreischa, Germany.
Email: a.keiler@idas-kreischa.de

Funding Information
Federal Ministry of the Interior, Building and Community of Germany

Abstract
Hair and urine concentrations of the nonsteroidal selective androgen receptor modulator GSK2881078 were examined following single oral administration to investigate its hair incorporation and estimate the general suitability of hair testing for selected androgen receptor modulators. Hair segments were collected following a single dose of 1.5 mg GSK2881078 by repeated shaving of scalp hair at Week 0 (blank), Week 1 (representing the pre-application period), Week 3 (ideally focusing the time of incorporation), and Weeks 5 and 9 (post-administration period). The intact compound and various (at least 4) hydroxy-metabolites exhibited similar elimination profiles. The peak urinary concentration (approximately 920 pg/ml) was observed after 8 h and is reduced to the detection limit (2 pg/ml) on Day 42 following administration of 760 μg GSK2881078. Correspondingly, hair concentrations of GSK2881078 (intact compound only) following a single oral dose of 1.5 mg GSK2881078 reached a peak concentration of 1.7 pg/mg in the segments collected 3 weeks post administration, representing the time of ingestion. The concentration rapidly declined to trace amounts of 0.7 (Week 5) and 0.2 pg/mg (Week 9), respectively. In conclusion, measurement of the intact compound GSK2881078 is feasible for both urine and hair analysis. However, concentrations in hair after single oral administration are in the low pg/mg range and can only be detected, if the segments cover the administration period.

KEYWORDS
doping control, GSK2881078, hair, metabolism

INTRODUCTION

Selective androgen receptor modulators (SARMs) have been developed for the prevention or alleviation of muscle wasting syndromes related to ageing or cancer cachexia. Due to their anabolic properties combined with minor androgenic effects, professional and amateur athletes might misuse SARMs for performance enhancement, muscle mass increase and muscle regeneration. Consequently, the World Anti-Doping Agency (WADA) has listed SARMs as all-time prohibited substances (other anabolic agents, class S 1.2). Mass spectrometric analyses of several SARMs in terms of doping control have been reviewed elsewhere. GSK2881078 (1-[(1R)-1-methyl-2-(methylsulfonyl)ethyl]-4-(trifluoromethyl)-1H-indole-5-carbonitrile) is a nonsteroidal SARM that so far underwent two Phase 1 and one Phase 2 clinical trial. Those trials showed safety and anabolic effects of daily doses of 0.75 to 4 mg GSK2881078 in healthy males and females as well as in cachexic...
males and females. A recent study described the mass spectrometric analyses of GSK2881078.8

Hair as matrix may provide additional information compared with urine samples in doping control. According to WADA’s International Standard for Laboratories, analysis of hair is approved but shall not be used to counter analytical findings from urine or blood.9 Due to possible long-term detection in hair, valuable methods are established to differentiate food contamination from clenbuterol abuse10 and to identify administered intact compounds, for example, testosterone esters in connection to elevated T/E ratio in urine.11 With regard to SARMs, only few studies investigated their incorporation in the hair matrix, among them LGD-4033, SARMs S4 and S22.12–14

Aim of the present study was the elucidation of the in vivo metabolism of GSK2881078 to identify metabolites as potential targets for doping analysis. After oral administration of GSK2881078 by one healthy volunteer, urine samples were collected up to 42 days post administration. Beside the excretion study, the study investigated the incorporation of GSK2881078 and its metabolites detected in urine in hair as an alternative matrix.

2 MATERIALS AND METHODS

For additional experimental details, refer to the Supporting Information. GSK2881078 with a purity of ≥98% was purchased from Cayman Chemical (Ann Arbour, Michigan, USA). LGD-4033 was received from WAADS without any certificate or purity statement. All solvents used were purchased from Chemsolute (Th Geyer, Renningen, Germany). β-Glucuronidase from Escherichia coli K12 was purchased from Roche (Mannheim, Germany).

Urine samples from a healthy male volunteer (self-administration by one of the authors) were collected over 7 days after oral ingestion of 1.5 mg GSK2881078 (resolved in 40% ethanol). Furthermore, hair samples were collected at the time point of oral self-administration as well as at Weeks 1, 3, 5, and 9 post administration by shaving newly grown hair with 4.5 mm remaining hair length.

A second self-administration with 0.76 mg GSK2881078 was performed by the same volunteer 2 months after the first oral ingestion. Urine samples were collected up to 42 days post administration of the lower dose.

2.1 Sample preparation

Urine sample extraction with and without hydrolysis was carried out as described previously.15 Hair samples were finely cut into pieces of approximately 1-mm size. For matrix calibration samples generated with blank hair from the volunteer, increasing amounts of GSK (5 to 250 pg absolute) were added to the hair samples and vortexed. Fifty milligrams of hair was mixed with 3 ml of methanol followed by an ultrasonic disintegration for 6 h at approximately 50°C (Bandelin Sonorex, RK 1028 H, Bandelin, Berlin, Germany). After ultrasonication, the samples were centrifuged for 5 min at 3100 rpm and evaporated under nitrogen. The residue was solved in 50 μl of organic solvent.

2.2 HPLC-MS measurements

High-performance liquid chromatography-mass spectrometry (HPLC-MS) measurements were performed using a HPLC (1290 Infinity II, Agilent, Waldbronn, Germany) equipped with a reversed phase column (Kinetex C18, 1.3 μm, 100 Å, 2.1 × 50 mm + KrudKatcher™ 2 μm depth filter, Phenomenex, Aschaffenburg, Germany) coupled to a quadrupole ion trap with an electrospray ionization (ESI) source (QTRAP 6500+, Sciex, Darmstadt, Germany).

Chromatographic separation was carried out using a gradient starting at 95% solvent A (solvent composition: aqueous solvent A: 5:95 acetonitrile: 0.1% acetic acid, 2 mmol/L ammonium acetate; organic solvent B: 95:5 acetonitrile: 0.1% acetic acid, 2 mmol/L ammonium acetate). The percentage of solvent B was increased to 100% over 6 min, before re-equilibration of the column to 95% solvent A. The flow rate for the chromatographic separation was set to 150 μl/min. The injection volume was 5 μl. Needle wash in between samples was performed with a mixture of 80:20 water:isopropanol.

Mass spectrometric measurements were performed in both positive and negative ESI polarity. The capillary voltage was set to +5.5 and −4.5 kV, respectively. The ESI temperature was set to 100°C in positive and to 300°C in negative ESI mode, and the source gases 1 and 2 were set to 40 and 70, respectively. The curtain gas was set to 20, and collision gas was adjusted to low.

Multiple Reaction Monitoring (MRM) screening experiments were performed in negative ESI, MS3 confirmations were established in positive ESI mode. These experiments were performed and evaluated for GSK2881078 and its metabolites. For structural interpretation, accurate fragment masses were monitored on a quadrupole time-of-flight (Q-TOF) (TripleTOF® 6600, Sciex).

3 RESULTS AND DISCUSSION

3.1 Analytical

GSK2881078 is well ionized in both polarities. Product ions observed in positive ESI are identical to those already described by Thevis and Volmer in a recent publication.8 The unusual temperature dependence in positive ESI mode—which was confirmed using different instruments and is likely to be influenced by gradient composition and ion source design—needs to be taken into consideration (Figure S1). There was a steep intensity decline of the protonated molecule observed at m/z 331 showing a minimum intensity at 500°C (i.e., typically the optimum temperature in most screening methods). This behavior is not well understood, because no formation of apparent fragments or adducts was observed at this temperature. Moreover, the conventional temperature dependence in negative ionization mode suggests a sufficient thermal stability of the molecule (Figure S1).

Collision-induced dissociation was in both polarities characterized by side-chain cleavage, losses of HF, and combinations thereof (Figure S2). Acetate adducts of GSK2881078 were formed in negative ionization at lower temperatures, but maximum intensities could be
achieved by using the in-source fragment (i.e., trifluoromethyl-indole-carbonitrile) as precursor of subsequent –HF fragmentation reactions (Figures 1B and S3). This was found to be the most sensitive approach for screening of intact GSK2881078 and its hydroxy-metabolites. To include structural information of the whole molecule and to differentiate hydroxylation position, an alternative QTrap MS3 procedure was chosen for confirmation purposes in positive ionization mode (Table 1 and Figure 2A).

Identical methods were used for urine and hair extracts, respectively. Corresponding detection limits of the negative MRM procedure (defined as signal-to-noise ratios of the qualifier ion better than three) were found to be 2 pg/ml (urine) or 0.1 pg/mg (hair). There was a good linearity of calibration over at least three decimal powers. The intraday repeatability of urinary concentrations was below 10% variation. Carry-over was not relevant in both matrices (estimated carry-over 0.03%).

### 3.2 Urine

The examination of GSK2881078 revealed immediate renal excretion of the unmodified compound. Moreover, four hydroxy-metabolites were identified based on their corresponding fragmentation pattern. Briefly, intact precursor and primary (–HF) fragments were shifted by 16 Da and secondary fragments, following side-chain cleavage, are either increased by 16 Da (in case of ring-hydroxylation) or remained identical to the corresponding GSK2881078 fragments (indicating side-chain hydroxylation, elemental composition of fragments was confirmed in high resolution) (Figure 2).

![FIGURE 1](A) Positive and (B) negative ionization and fragmentation patterns of GSK2881078, as monitored in high-resolution time-of-flight mass spectrometry (TOF-MS) mode [Colour figure can be viewed at wileyonlinelibrary.com]

There was no indication for multiple hydroxylation or Phase 2 metabolism, that is, enzymatic hydrolysis did not markedly increase urinary concentrations of GSK2881078 or its metabolites and no intact glucuronides could be detected.

The renal excretion of intact GSK2881078 reached its concentration maximum in less than 8 h, contributed to 0.02% of the total elimination within the first 18 h and remained on a similar level for several weeks. The estimated urinary half-life is 4.3 days, which is in accordance with the plasma elimination half-life described in a clinical trial. There were no signs of significant accumulation over multiple weeks. Two major hydroxy-metabolites (of total four isomers) were rapidly formed and reached peak concentrations between 3 (GSK-OH M1a) and 5 days (GSK-OH M2), respectively.

### Table 1

| Compound        | ESI polarity (method) | Transitions |
|-----------------|-----------------------|-------------|
| GSK2881078      | Positive (MS3)        | 331 → 311 → (190, 191, 204, 217, 231) |
|                 | Negative (MRM)        | 209 → 189/209 → 169 |
| GSK-OH (M1)     | Positive (MS3)        | 347 → 327 → 207 |
|                 | Negative (MRM)        | 225 → 205/225 → 185 |
| GSK-OH (M2)     | Positive (MS3)        | 347 → 327 → (191, 204, 217, 247) |
|                 | Negative (MRM)        | 209 → 189/209 → 169 |
| LGD-4033        | Positive (MRM)        | 339 → 319 |
|                 | Negative (MRM)        | 337 → 239 |

Abbreviation: ESI, electrospray ionization.

There were no signs of significant accumulation over multiple weeks. Two major hydroxy-metabolites (of total four isomers) were rapidly formed and reached peak concentrations between 3 (GSK-OH M1a) and 5 days (GSK-OH M2), respectively.
The initial administration study (oral intake of 1.5 mg GSK2881078) was repeated at reduced concentration to cover the whole elimination period within a reasonable timeframe. The total detection times after oral administration of 760 μg GSK2881078 were rather similar, that is, >42 (intact GSK2881078, M1a) and 42 days (M2) (Figure 3). There was no variation of the steroid profile observed, in particular luteinizing hormone and the ration testosterone/epi-testosterone remained constant (data not shown). This behavior is similar to pharmacokinetic observations related to the structurally and biochemically similar compound ligandrol (LGD-4033).16 Publications of administration studies and respective doping cases reported the formation of single and double-hydroxylation reactions.17,18 Detection
windows up to 21 days, following administration of 10 mg ligandrol, were observed. The dihydroxy-metabolite was assumed to be the best long-term marker for ligandrol in doping analysis.

### 3.3 Hair

Hair segments were collected following the administration of 1.5 mg GSK2881078 by repeated shaving of scalp hair at Weeks 0 (blank), 1 (representing the pre-application period), 3 (ideally focusing the time of incorporation), and 5 and 9 (post administration of 1.5 mg GSK2881078).

The assignment of hair segments to corresponding elimination times is based on the assumptions of hair growth speed as well as the temporal offset before the first collection, that is, approximately 1 week between hair formation and entering the skin surface plus approximately 4.5 mm residue length of remaining hair segments.

The individual hair growth rate was examined by comparison of segment lengths to be 1.0 cm/month (sd ~ 13%). In contrast, retrospective segmentation of long hair strands is less accurate due to misalignment of hair within the bundle and the increasing proportion of non-growing (catagen) hair. Methanol extraction was found to be the preferential sample preparation procedure, whereas sodium hydroxide disintegration of hair failed due to substance instability and acidic hydrolysis (using HCl) did not markedly improve recovery (data not shown).

Intact GSK2881078 was detected at a maximum concentration of 1.7 pg/mg in the 5 mm segments of hair collected 3 weeks post administration (i.e., perfectly covering administration period, Figure 4). None of the hydroxy-metabolites could be identified. Concentrations dropped to approximately 0.7 pg/mg in adjacent segments (collected after 1 and 5 weeks, respectively) and were above the detection limit (0.1 pg/mg) in samples collected after 9 weeks (Figure 4).

In accordance to the physicochemical similarity between neutral lipophilic steroids and GSK2881078, their incorporation rate into hair appears rather poor, as compared with basic drugs of abuse or most pharmaceuticals. A single high therapeutic oral administration of GSK2881078 was found to be detectable in hair at low pg/mg levels, if segmentation matches perfectly with administration time and superior detection limits below 1 pg/mg hair are achieved.
4 | CONCLUSION

GSK2881078 was well detectable in urine samples after oral administration of 760 μg, which was assumed to represent a single therapeutic dose. Negative MRM experiments were found to be most sensitive and suitable for screening purposes (in urine or hair). Owing to its reduced structural information (following in-source fragmentation), the confirmation should be preferentially carried out in positive ionization mode. Secondary fragmentations following the initial loss of HF (MS3 mode) was found to be sufficiently sensitive and comprehensive (e.g., to differentiate hydroxyl isomers). Temperature dependence of ionization efficiency showed unusual behavior in positive mode and needs special consideration when optimizing MS parameters. The unmodified compound could be identified in urine at concentrations larger than limit of detection (LOD) (2 pg/mg) until Day 42 post administration. Moreover, two hydroxy-metabolites were identified and found positive until Days 34 and 42, that is, exhibiting similar pharmacokinetics. An estimated urinary “half-life” of 4.3 days was observed, suggesting a long bioavailability and good pharmacologic long-term effect of the drug.

The detection in hair sample required highest sensitivity and was successful at a hair concentration 1.7 pg/mg in the segments collected 1–3 weeks post administration, that is, the 5 mm segment perfectly focusing the administration time. Adjacent segments exhibited trace amounts of 0.7 (Weeks 1 and 5) and 0.2 pg/mg (Week 9), respectively. These low hair concentrations seem to confirm the poor incorporation tendency of SARMs which is in line with its chemical structure and corresponding physicochemical properties. Inclusion of the new SARMs into a routine hair screening procedure for doping agents was accomplished and deems useful when dealing with forensic cases.

ACKNOWLEDGEMENT

This study was funded by the Federal Ministry of the Interior, Building and Community of Germany. Open access funding enabled and organized by Projekt DEAL.

ORCID

Annekathrin Martina Keller https://orcid.org/0000-0002-2157-4711

REFERENCES

1. Dalton JT, Taylor RP, Mohler ML, Steiner MS. Selective androgen receptor modulators for the prevention and treatment of muscle wasting associated with cancer. Curr Opin Support Palliat Care. 2013;7(4):345-351.
2. Morimoto M, Aikawa K, Hara T, Yamaoka M. Prevention of body weight loss and sarcopenia by a novel selective androgen receptor modulator in cancer cachexia models. Oncol Lett. 2017;14(6):8066-8071.
3. World Anti-Doping Agency (WADA). World Anti-Doping Code. Prohibited List. 2020 Jan;
4. Thevis M, Schänzer W. Detection of SARMs in doping control analysis. Mol Cell Endocrinol. 2018;15:464:34-45.
5. Clark RV, Walker AC, Andrews S, Turnbull P, Wald JA, Magee MH. Safety, pharmacokinetics and pharmacological effects of the selective androgen receptor modulator, GSK2881078, in healthy men and postmenopausal women. Br J Clin Pharmacol. 2017;83(10):2179-2194.
6. Neil D, Clark RV, Magee M, et al. GSK2881078, a SARM, produces dose-dependent increases in lean mass in healthy older men and women. J Clin Endocrinol Metab. 2018;103(9):3215-3224.
7. ClinicalTrials.gov [Internet]. Study to evaluate the safety and efficacy of 13 weeks of the selective androgen receptor modulator (SARM) GSK2881078 in chronic obstructive pulmonary disease (COPD). Identifier NCT03359473. https://clinicaltrials.gov/ct2/show/results?NCT03359473?term=gsk2881078&draw=2&rank=1. Accessed July 2020.
8. Thevis M, Volmer DA. Mass spectrometric studies on selective androgen receptor modulators (SARMs) using electron ionization and electrospray ionization/collision-induced dissociation. Eur J Mass Spectrom (Chichester, Engl). 2018;24(1):145-156.
9. World Anti-Doping Agency (WADA), World Anti-Doping Code, International Standard for Laboratories. World Anti-Doping Agency 2019 [Internet]. https://www.wada-ama.org/en/resources/laboratories/international-standard-for-laboratories-isl. Accessed September 2020
10. Krumbholz A, AIELSK P, Grérer L, et al. Statistical significance of hair analysis of clenbuterol to discriminate therapeutic use from contamination. Drug Test Anal. 2014;6(11-12):1108-1116.
11. Thieme D, AIELSK P. Chapter 10—doping, applications of hair analysis. In: Kintz P, Salomone A, Vincenti M, eds. Hair Analysis in Clinical and Forensic Toxicology. Academic Press; 2015:275-299.
12. Kintz P, Ameline A, Gheddar L, Raul J-S. LGD-4033, S-4 and MK-2866—testing for SARMs in hair: about 2 doping cases. Toxicol Anal Clin. 2019;31(1):56-63.
13. Kintz P, Gheddar L, Ameline A, Raul J-S. Identification of S22 (ostarine) in human nails and hair using LC-HRMS. Application to two authentic cases. Drug Test Anal. 2020;12(10):1508-1513. https://doi.org/10.1002/dta.2902
14. Cutler C, Viljanto M, Hincks P, Habershon-Butcher J, Muir T, Biddle S. Investigation of the metabolism of the selective androgen receptor modulator LGD-4033 in equine urine, plasma and hair following oral administration. Drug Test Anal. 2020;12(2):247-260.
15. Bräuer P, AIELSK P, Schweiger S, et al. In vitro metabolism of selected bioactive compounds of Eurycoma longifolia root extract to identify suitable markers in doping control. Drug Test Anal. 2019;11(1):86-94.
16. Hansson A, Knych H, Stanley S, et al. Equine in vivo-derived metabolites of the SARM LGD-4033 and comparison with human and fungal metabolites. J Chromatogr B. 2018;1074–1075:91-98.
17. Cox HD, Eichner D. Detection of LGD-4033 and its metabolites in athlete urine samples. Drug Test Anal. 2016;9(1):127-134.
18. Fragkaki AG, Sakellariou P, Kiousi P, et al. Human in vivo metabolism study of LGD-4033. Drug Test Anal. 2018;10(11-12):1635-1645.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Rading A, AIELSK P, Thieme D, Keller AM. Detection of the selective androgen receptor modulator GSK2881078 and metabolites in urine and hair after single oral administration. Drug Test Anal. 2021;13: 217–222. https://doi.org/10.1002/dta.2943