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Development of an analytical method for the detection of SARMs in urine and hair

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Background and objective

Selective Androgen Receptor Modulators (SARMs) are non-endogenous compounds that have similar anabolic properties to anabolic steroids. All SARMs are to this date investigational drugs, since no SARMs have passed clinical trials or are approved as human/veterinary drugs. During a police raid back in 2020, an illegal production laboratory actively producing SARMs was discovered, which led to a gained interest in these compounds regarding food safety. Due to the affordability, availability, and steroid-like effects, the use of SARMs in animal husbandry cannot be excluded. However, currently many of these SARMs are not actively monitored and are often not yet included in routine methods. Therefore, we set to develop a liquid chromatography tandem-mass spectrometry (UHPLC-MS/MS) method to detect SARMs in relevant animal matrices. As only limited information is available regarding metabolism and pharmacokinetics of these SARMs, two methods were developed for 1) bovine and porcine urine and 2) bovine hair. The urine method was successfully applied to incurred bovine urine samples.

Impounded products

Three samples were impounded during a police raid. In all three samples, ibutamoren was found.
In the capsules, the powder within contained 71% ibutamoren; about 20 times higher than the label mentioned amount.

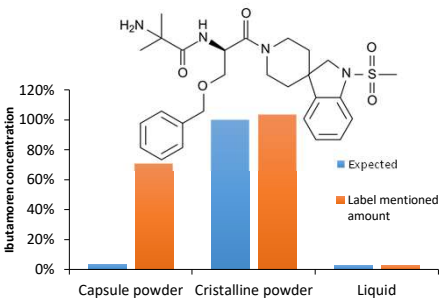
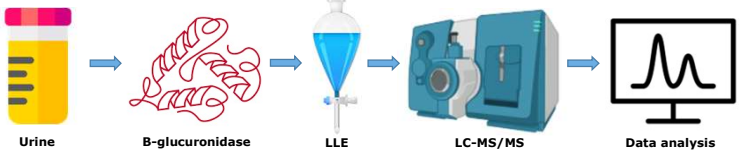


Figure 1. Ibutamoren structure together with the analysed concentrations vs the label expected amounts

Urine analysis

For urine analysis, a liquid-liquid extraction (LLE) procedure as described in literature and supported liquid extraction (SLE) were investigated with a variety of organic solvents.
Deconjugation with β–glucuronidase followed by LLE using TBME at pH 10 was deemed to be the optimal sample preparation method.
The optimized method included in total 20 SARMs, which are analysed both in positive and negative ionisation mode. With this method, SARM concentrations as low as 1 $\mu g \cdot L^{-1}$ in urine could be analysed for most of the 20 SARMs. Exceptions being BMS-564929, GLPG0492 and YK-11 which can be analysed with a concentration as low as 20 $\mu g \cdot L^{-1}$.



Application on ostarine incurred samples

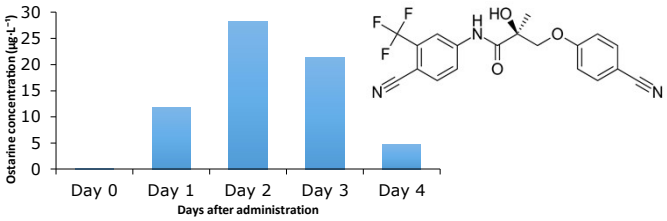
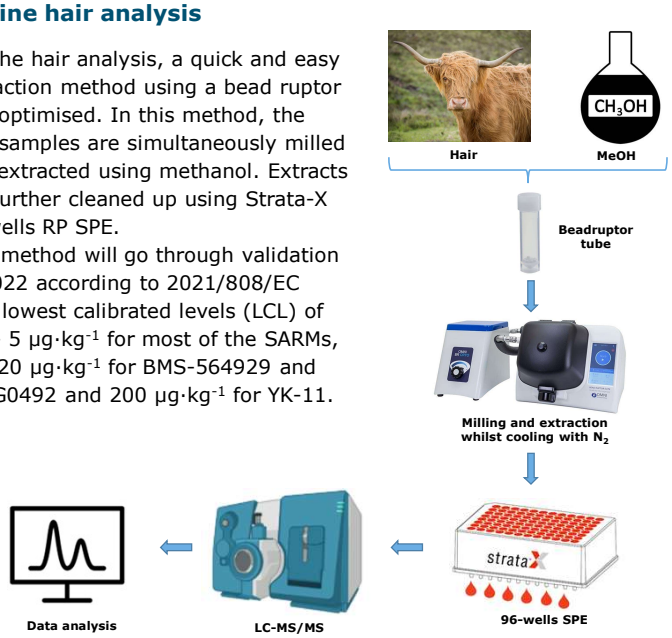


Figure 2. Ostarine structure together with the analysed concentrations in the days following administration

Incurred urine samples from an animal treated with ostarine during an earlier conducted animal study were analysed using the developed urine method. Ostarine was detected and confirmed in the days following administration, but the concentration quickly deminished over time.

Bovine hair analysis

For the hair analysis, a quick and easy extraction method using a bead ruptor was optimised. In this method, the hair samples are simultaneously milled and extracted using methanol. Extracts are further cleaned up using Strata-X 96 wells RP SPE.
This method will go through validation in 2022 according to 2021/808/EC with lowest calibrated levels (LCL) of 0.5 - 5 $\mu g \cdot kg^{-1}$ for most of the SARMs, and 20 $\mu g \cdot kg^{-1}$ for BMS-564929 and GLPG0492 and 200 $\mu g \cdot kg^{-1}$ for YK-11.



Outlook

- Urine samples will be monitored for the presence of SARMs
- The bovine hair analysis method will go through validation
  - If succesfull, bovine hair samples will also be monitored for the presence of SARMs

Acknowledgements

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