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Development and validation of an UHPLC-MS/MS analytical method for the determination and quantification of SARMs in bovine and equine urine

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Selective androgenic receptor modulators (SARMs) are considered to be an attractive alternative to anabolic-androgenic steroids due to their strong anabolic activity combined with an absence of undesirable androgenic side-effects. SARMs have not yet been approved as pharmaceutical therapeutic drugs, but many are currently undergoing evaluation in pre- and clinical trial studies. However, new drug candidates emerging from clinical development have the potential to be adopted for use in sport and food-producing animals with the aim to increase muscle growth and reduce fat mass, and for this reason SARMs have been banned by WADA and International Federation of Horseracing Authorities. Consequently, control and systematic analysis through reliable and sensitive analytical testing for prohibited substances is required both in equine sport to monitor for doping practices, and potentially in farm livestock to ensure food safety and consumer health. In this study, an UHPLC-MS/MS method was developed to detect 14 SARM compounds belonging to different families, namely: arylpropionamide (7), quinolone (1), isoquinoline (1), phenyl-pyrrolidine (1), alanine-phenyl-oxadiazole (1), alanine-tropanol (1) and hydantoin (2), in bovine and equine urine, respectively. Method development has focused on the optimization of UHPLC-MS/MS conditions as well as sample purification. Chromatographic separation was performed using a Phenomenex Luna Omega Polar C18 analytical column at 45 °C, and a binary gradient elution of 12 minutes at a flow rate of 0.4 mL/min. The mobile phase A consisted of 0.1% of acetic acid in water and mobile phase B of 0.1% of acetic acid in methanol. SARM residues, extracted with TBME without clean-up, were analysed by UHPLC-MS/MS operating in ESI+/ESI-. Validation of the confirmatory assay was carried out according to the EU Commission Decision 2002/657/EC and AORC guidelines. The following performance studies were carried out: specificity/selectivity, linearity (0.1-20 ng/mL), absolute recovery, within-laboratory repeatability/reproducibility, decision limit ($CC\alpha$) and detection capability ($CC\beta$), limit of detection (LOD) and quantification (LOQ) as well as assessment of matrix effects. Finally, the analytical method will be employed for screening and confirmatory purposes in bovine and equine urine samples to determine potential SARM abuse in stock farming and competition animals, respectively.

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