Ss. CYRIL AND METHODIUS UNIVERSITY IN SKOPJE
FACULTY OF VETERINARY MEDICINE - SKOPJE

BOOK OF ABSTRACTS

9th International Scientific Meeting Days of Veterinary Medicine 2022

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R. of North Macedonia

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P29

VALIDATION OF THE RAPID ELISA METHOD FOR DETERMINATION OF METHYLTESTOSTERONE IN URINE AND MUSCLE BASED ON THE NEW REGULATION (EU) 2021/808

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Methyltestosterone is a synthetic anabolic steroid that can be used in meat production animals to improve the weight gain and the meat/fat-ratio, but the residues of this anabolic steroid in meat present a potential risk for public health. In the European Union and most countries worldwide the use of methyltestosterone and other anabolic steroids as growth promoter in livestock production is completely banned. In this study an enzyme-linked immunosorbent assay (ELISA) screening method for detection of methyltestosterone in urine and meat has been validated according to the Regulation (EU) 2021/808. Regulation (EU) 2021/808 is a new regulation that replaces the Commission Decision 2002/657. The sample preparation procedure for urine involved hydrolysis and clean-up with solid phase extraction (SPE) using C18 cartridges, while the sample preparation for muscle included extraction with methanol, defatting with hexane and clean-up with SPE using C18 cartridges. The concentrations of the residues were measured with ELISA reader at 450 nm. In the validation procedure were included linearity, selectivity/specificity, limit of detection (LOD), decision limit (CCβ), trueness (expressed through recovery) and precision (expressed through repeatability and reproducibility). The method demonstrated excellent linearity with coefficient of variation (R^2) 0.9966 for urine and 0.9997 for muscle, in the calibration range from 0.125 to 4.5 µg/L. The results for selectivity/specificity indicated that there are not matrix interferences. LOD was 0.25 µg/l for urine and 0.25 µg/kg for muscle, while CCβ was 0.45 µg/l for urine and 0.60 µg/kg for muscle. These values for CCβ were lower than new Minimum Method Performance Requirements (MMPR) values for methyltestosterone. The new MMPR values for methyltestosterone, which were adopted in 2020, for urine is 0.5 µg/l, while for muscle is 1.0 µg/kg. The method yielded acceptable recovery rate between 70-120% in the fortified samples from urine and muscle. The coefficient of variation (CV) for repeatability was less than 15.0 %, while the CV for reproducibility was less than 20.0%. The method fulfils the criteria prescribed in Regulation (EU) 2021/808 and can be used as rapid screening method for the routine detection of methyltestosterone in urine and muscle.

Key words: methyltestosterone, urine, muscle, validation, Regulation (EU) 2021/808