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FACULTY OF VETERINARY MEDICINE - SKOPJE

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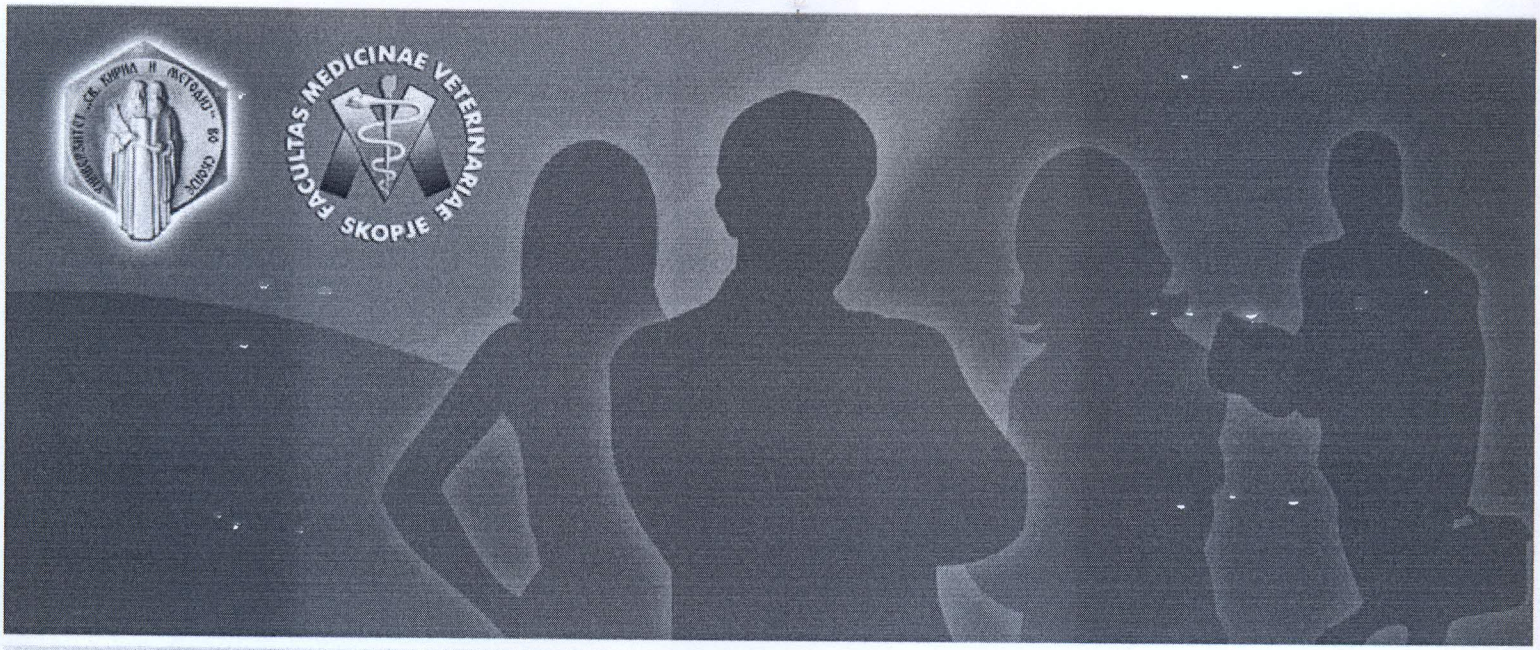

The 4th International Scientific Meeting

06-08 September 2013
Struga, Republic of Macedonia

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P22 VALIDATION OF ELISA METHOD FOR DETECTION OF TRENBOLONE IN BOVINE URINE

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ABSTRACT

Introduction

ELISA method has been developed for the determination of trenbolone residues in bovine urine. Trenbolone acetate is a synthetic anabolic hormone which is used as a feed supplement to promote the growth of bovine. Trenbolone is rapidly hydrolyzed to its metabolite 17β-trenbolone. Twenty-four hours after application, 80% of the 17β-trenbolone will be converted to 17α-trenbolone and then excreted by urine. The use of trenbolone in food producing animals is prohibited in most countries of the EU. For this reason in our study validation of ELISA method for determination of trenbolone in bovine urine is described.

Materials and Methods

The analyte was extracted with sodium acetate buffer and cleaned by C18 solid phase extraction cartridge. For determination of limit of detection we used 20 blank bovine urine samples. The method recovery was determined at three levels by spiking on blank urine (1; 1,5 and 2 ng/ml). Detection capabilities (CCβ) was evaluated by analyzing 20 spiked bovine urine on ½ of MRPL level. Precision was expressed as the Coefficient of variation (CV) of the calculated standards and sample concentrations.

Results

Detection limit for trenbolone in bovine urine was 0,20 ng/ml and CCβ was 1,08 ng/ml The overall recovery varies from 85% to 97,1% at the three target concentration. The precision (CV%) in trenbolone standards ranged from 2.0% to 8.9%. The precision in spiked cattle urine samples ranged from 2.8% to 9.2 %.

Conclusion

The method was reliable, sensitive and reproducibility, its performance can meet the requirements of the domestic and international legislation. Because of good recovery and precision, and satisfactory CCβ, it is applicable in official control laboratories as a screening method for determination of trenbolone in bovine urine. But in the case when the target analyte is clearly identified above

CCβ the sample is considered as non compliant and we must confirm the results with GC/MS, LC/MS or another confirmatory method.

Key words: trenbolone, anabolic steroid, urine, ELISA