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Detection of nortestosterone application in horses

A screening method for nortestosterone in horse serum with a possible confirmation in urine

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Introduction

Nandrolone is an anabolic steroid which is forbidden in horse as well as in human sports. The german preparation Laurabolin® is permitted for dogs leading to misuse in horses. In general a nortestosterone misuse in horses is detected by its main metabolite estrane-3 β ,17 α -diol in urine. Due to the fact that blood samples as well as urine samples have to be investigated it was necessary to develop a method for both.

As the expected amounts of nortestosterone in serum are very low the method has to be very sensitive. The proposed method for serum is a combination of immunoaffinity chromatography (IAC) and gaschromatography/high resolution mass spectrometry (GC/HRMS). After an alkaline extraction the samples are purified via IAC using an anti-nortestosterone gel. The isolated extracts are derivatized and analyzed by GC/HRMS.

Administration and sample collection

Nandrolonelaurat, 300 mg (6 ml of Laurabolin® 50, Intervet, Germany) was administered subcutaneously to a standardbred colt. Blood samples were collected 0, 0.25, 0.5, 1, 2, 3, 4, 5, 6, 7, 11, 18, 25, 31, 38, 46, 53, 67 and 82 days following administration. Urine samples were collected 0, 0.2, 1, 2, 3, 4, 5, 6, 7, 11, 12, 18, 25, 31, 38, 46, 53, 60, 67, 74, 81, 91 and 95 days following administration.

Sample preparation for the serum samples

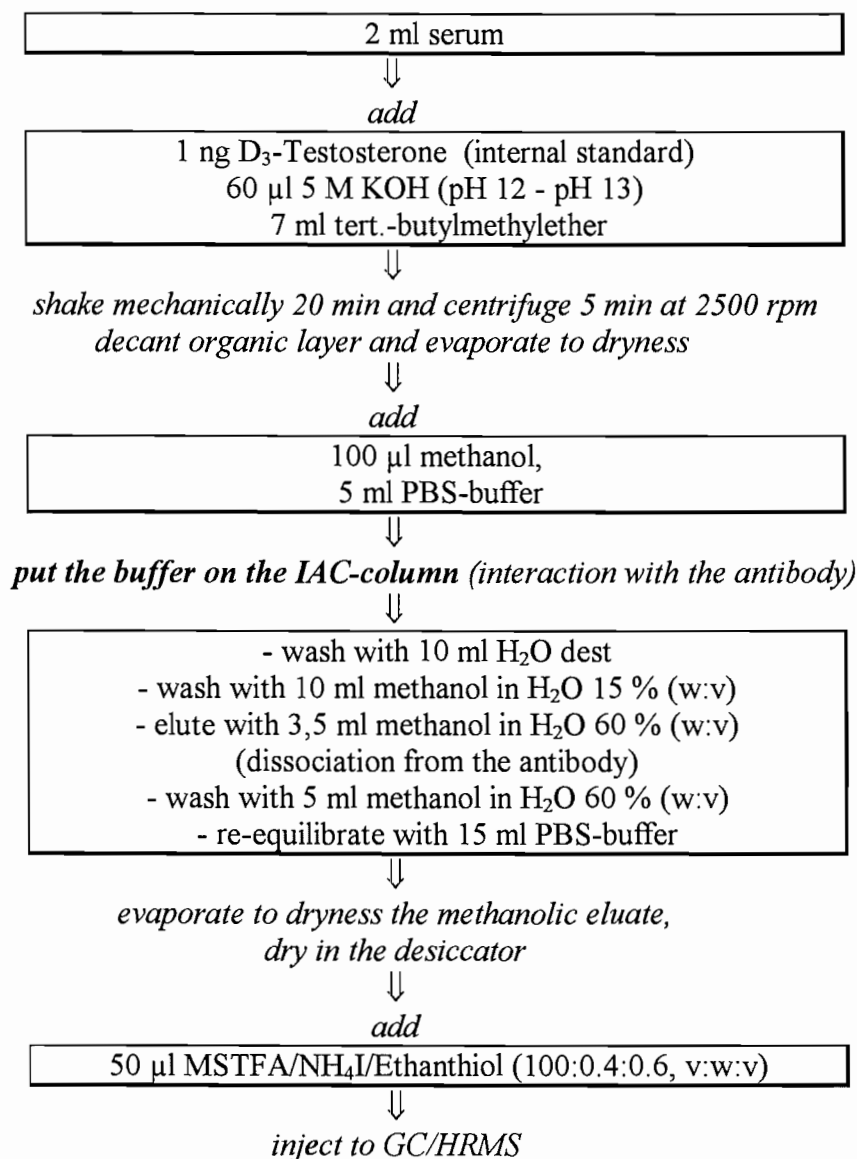


Figure 1:

GC-HRMS conditions

GC/MS-system:	Hewlett Packard 5890 / Finnigan MAT 95		
Column:	HP Ultra -1 fused silica capillary column, crosslinked methylsilicon (OV 1), 15 m, 0.2 mm i.D., 0.11 µm film thickness		
Flow parameters	carrier: He	head pressure: 12 psi	split: 10 ml/min
Injection parameters	mode: split	volume: 2 µl	temperature: 300°C
Oven temp. prog.	initial temp.: 185°C	init. time: 0	
	rate 1: 5°C/min	final temp 1: 235°C	
	rate 1: 30°C/min	final temp 1: 310°C	final time: 1.00 min
MS parameters	ionisation mode: EI	acq. mode: MID	Mass resolution: 3000
	interface temp: 300°C	source temp: 240°C	SEM: 2200 Volt

Table 1: GC-HRMS conditions

The samples are measured in HR-SIM using the masses m/z 403.2489, 404.9760 (lock mass), 418.2732, 419.2757, 423.2880, 435.3068, 454.9728 (calibr. mass).

Nortestosterone calibration curve in serum

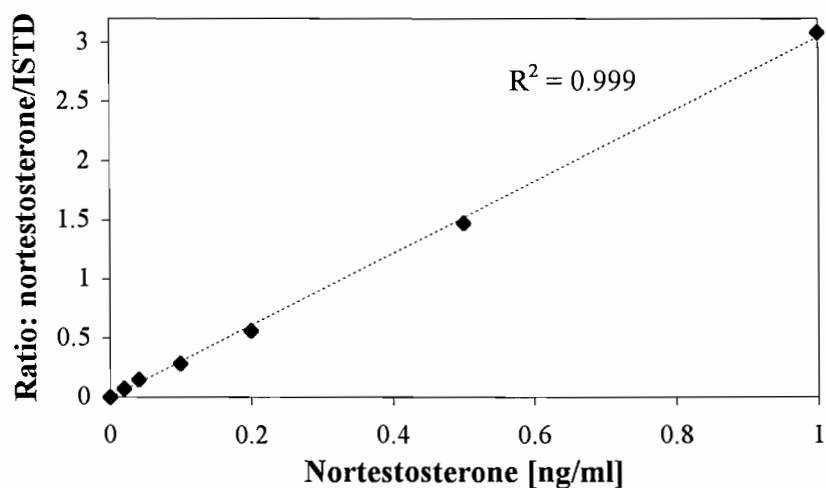


Figure 2: Nortestosterone calibration curve in serum

Results and discussion

The combination of the high purification capacity of the immunoaffinity chromatography and the high sensitivity of the high resolution mass spectrometry leads to the very low detection limit of 0.02 ng/ml.

In samples of untreated colts and mares it is normally not possible to detect nortestosterone above this detection limit. Due to the endogenous production in the testis it is possible to detect nortestosterone in the samples of most stallions.

After application of 300 mg Nandrolonelaurat to a standardbred colt, the calculated concentrations of nortestosterone in serum were very low. Nevertheless the detection for a long time period of 53 days was possible, because a depot preparation was used.

Nortestosterone and its metabolite estranediol could be detected at least 79 days after application. It is much easier to detect the nortestosterone metabolite estrane-3 β ,17 α -diol in urine than the parent compound in serum.

If nandrolone is detected in a routine screening procedure with a concentration near the detection limit, it is still enough time to collect a urine sample from the same horse to determine whether the estrane-3 β ,17 α -diol/5(10)-estren-3,17-diol ratio is higher than 1 (Fig. 5).

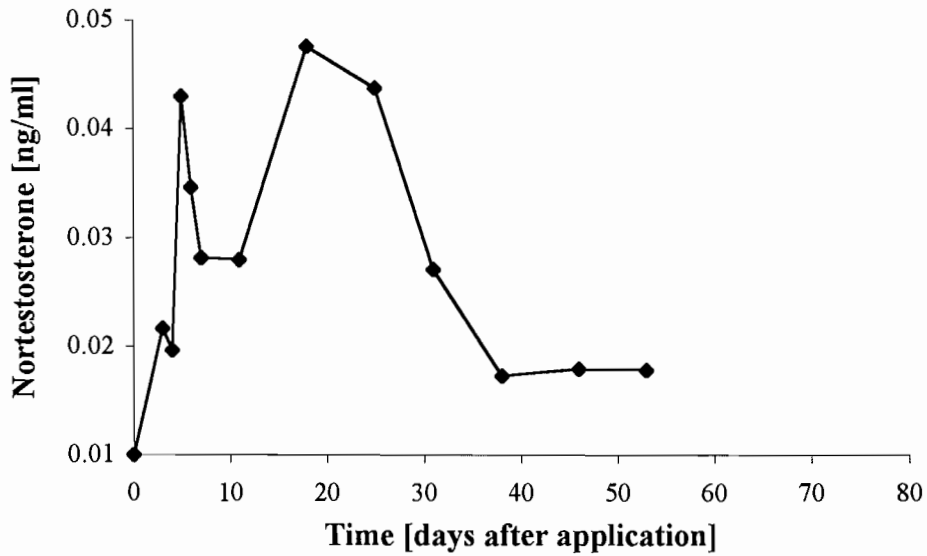


Figure 3 : Nortestosterone serum concentrations after application of 300 mg nandrolonelaurat

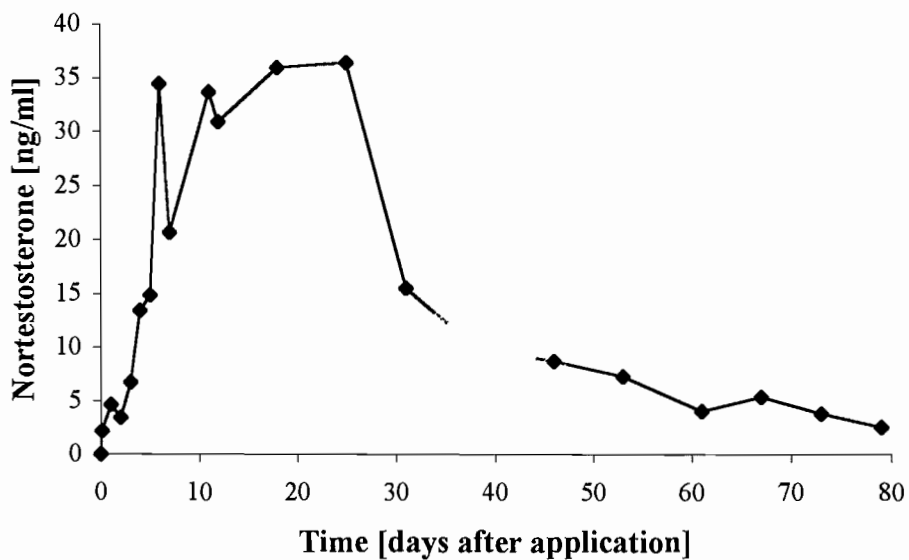


Figure 4 : Nortestosterone urine concentrations after application of 300 mg nandrolonelaurat

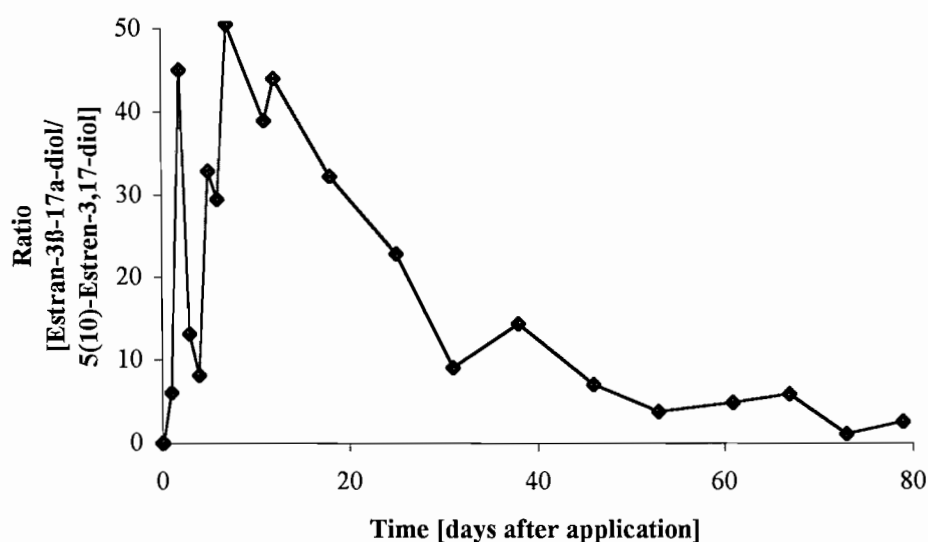


Figure 5: Estrane-3β,17α-diol and 5(10)-Estrene-3,17-diol ratio in urine after application of 300 mg nandrolonelaurat (ratio > 1 positive)

Acknowledgement

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