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# One Year Experience of Trenbolone Measurement by ELISA

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Parabolan$^{R}$ (Trenbolone-hexahydrobenzylcarbonate) is one of the steroids that exist on the black market in Sweden today. It is widely used by power lifters, bodybuilders and young boys training for fun to build up their bodies. It is also one of the steroids that doesn't fit into the general screening procedure for steroids today.
Since a year ago we have been using Ridascreen$^{R}$ enzyme linked immuno assay (ELISA) from R-Biopharm, Darmstadt, Germany. This assay is very sensitive and can be used for quantitative analysis of trenbolone and epitrenbolone in urine, bile faeces, liver and muscle. The detection limit is 100 ppt.
The bulk-ware kit contains all reagents for making 10 microtiter plates. Each plate can handle 96 samples. The coating of the plates is very easy and they can be stored in a freezer for 3 months. By using the bulk-ware kit each sample have a cost of 1.7 DM.

Since the middle of 1991 we have screened 860 samples and 658 are from weight- and power lifters, most from out-of-competition testing. During 1991 we found 10 positive samples by the ELISA method, all from power lifters.
Only one sample was confirmed by GCMS and also reported as a positive case. This is also the only positive trenbolone sample in the IOC-statistics for 1991.

At an early stage we checked the assay for crossreaction against other steroids. The assay give 100 % reaction on 17-b- and 17-a-trenbolone, trendione, methyltrenbolone and allyltrenbolone. Positive urine samples of Boldenone, Clostebol, Fluoxymesterone, Furazabol, Metandienon, Metenolone, Oxandrolone, Oxymesterone, Stanozolol, 19-nor-Androsterone and 19-nor-Etiocholanolone didn't show any crossreaction at all.

To increase the possibility to confirm more samples, we have used larger volume of urine. Using 30-40 ml of urine and clean it up by Sep-Pak$^{R}$ followed by enzymatic hydrolysis and ether extraction. The ether-phase is evaporated and the residue is dissolved in 100 $\mu$l methanol. Four times 25 $\mu$l is injected on a HPLC gradient system and the fraction containing the trenbolone and its metabolite is collected. This fraction has then been analysed by the ELISA assay again and different MS-techniques. All samples shown

positive result with the ELISA but we couldn't confirm the low concentration samples by GCMS, LCMS or Electrospray.

The ELISA method is very sensitive and need only 50 μl urine sample. A standard curve is prepared every time in a range of 0.02-5 ng/ml trenbolone.Normally we are analysing 34 samples, standard curve and a control sample.

During 1992 we have found 6 positive samples of 568. 4 samples from power lifters, 1 track and field and 1 budo. Due to the low concentration and a limit amount of urine in these samples, we have not been able to confirm the findings and report a positive case. Afterwards, we have used a larger volume by using A and B sample. All samples showed positive results after HPLC cleaning, both by ELISA and GCMS.

Today we have extended the screening to all power sports and track and field.
As a conclusion, we can say that the ELISA assay for trenbolone is very sensitive and show no crossreaction against other steroids than trenbolone analogues. One limit is that it takes about 30 hours to get the results from 34-82 samples, due to the incubation time of the microtiter-plate. Further investigations is needed to get a confirmation method and GCMS with Negative Chemical Ionisation seems to be one way.

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# Hydrolysis

50ul sample
0.5 ml blank urine
0.5 ml 50 mM acetate buffer pH 4.8
20ul ß-glucuronidase-aryl-sulfatase from
Helix Pomatia

# Extraction

0.1 ml 1 M NaOH
5 ml diethylether
Evaporate
Dissolve in 1 ml 20% methanol

# Procedure of the trenbolone kit

50 ul of conjugate solution in all the wells.
20 ul of standards and samples are pipetted
50 ul of anti-trenbolone-antibodies in all wells except in two standards (NSB).
Incubating during night at 2-6 degrees.

The next day: Pipett 50 ul substrate solution and 50 ul chromogene solution to all wells.
Incubate at room temperature for 30 min.
Add 100 ul stop solution and read the plate at 450 nm.