Waters
Lab Highlights
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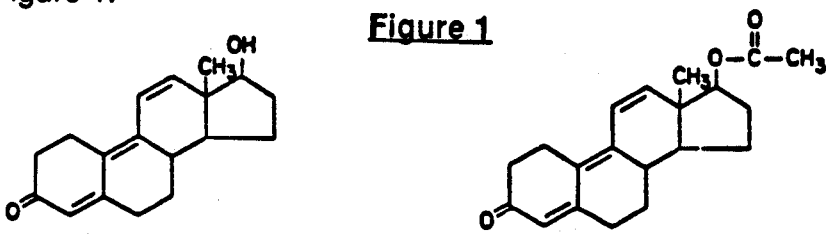
USING μBONDAPAK™ C_{18} COLUMNS FOR TRACE ANALYSIS OF TRENBOLONE ACETATE AND TRENBOLONE IN ANIMAL CHOW, WASTE WATER AND HUMAN URINE

Trenbolone acetate (TBA) is a synthetic anabolic steroid which has been used experimentally in Europe in cattle production. Before hormone toxicology studies can be initiated it was necessary to develop analytical methodology for determining residues of the synthetic steroid (TBA) and its hydrolysis product trenbolone (TBOH) in admixture in animal chow, human urine, and waste water. Formulas of TBA and TBOA are shown in Figure 1.

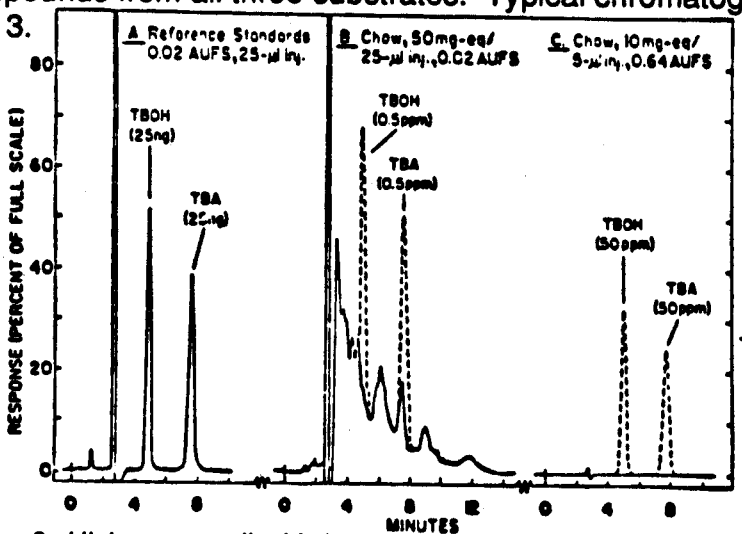
Figure 1



Trenbolone (TBOH)

Trenbolone Acetate (TBA)

The procedure which was developed (1) consisted of a benzene extract of the substrates which were subjected to liquid-liquid partitioning, further cleanup on silica gel, and direct analysis by liquid chromatography (LC) or derivatization with pentafluoropropionic (PFP) anhydride and analysis by electron capture gas chromatography (EC/GC). Satisfactory recoveries were obtained with both compounds from all three substrates. Typical chromatograms are shown in Figures 2 and 3.



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THE PERFORMANCE CHARACTERISTIC FOR THIS PROCEDURE HAS NOT BEEN ESTABLISHED.

Figure 2. High pressure liquid chromatograms. (A) is an analytical standard containing 25ng each of trenbolone and trenbolone acetate. In (B) and (C) solid lines are cleaned-up extracts of spiked chow, broken lines (superimposed) represent 0.5 and 50 ppm, respectively, of the two compounds in admixture. All injections were 25 μl in methanol.

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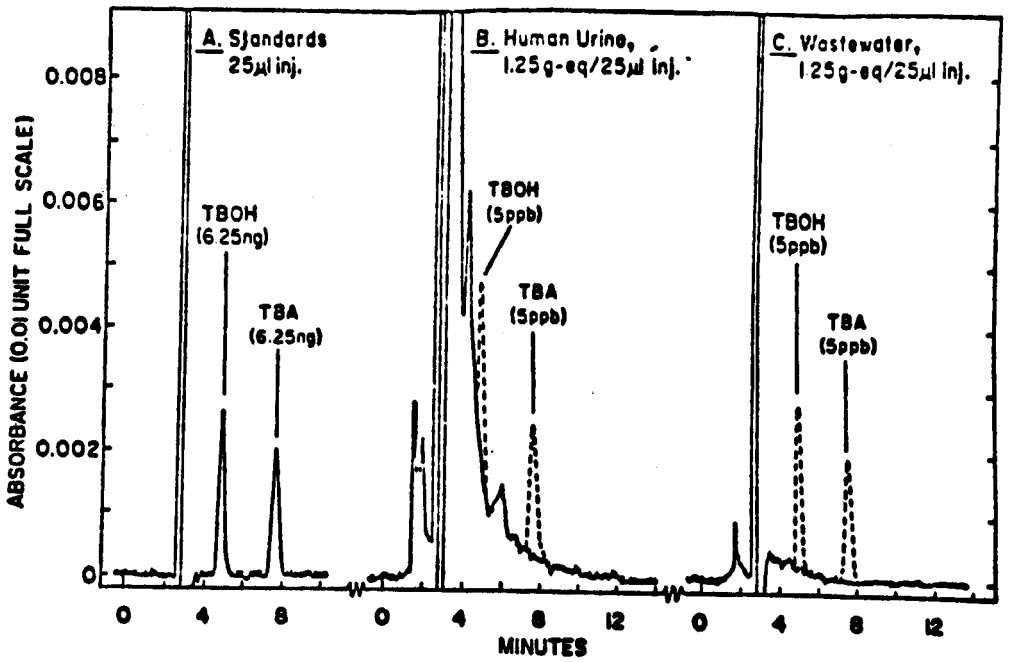

Figure 3. High pressure liquid chromatograms (A) is an analytical standard containing 6.25 ng each of trenbolone and trenbolone acetate. In B and C solid lines are cleaned-up extracts of human urine and waste water, broken lines (superimposed) represent 5 ppb amounts of trenbolone and trenbolone acetate in admixture. All injections were 25µl in methanol.

Recoveries of both compounds using LC were generally better than 80% at all levels, while those via EC/GC were consistently lower; precision of both methods was good. No attempt was made to assay the samples spiked at 0.5 ppm via EC/GC because of background interferences. The fact that no TBOH was detected in the samples spiked only with TBA is good evidence that none of the residue was hydrolyzed during the analytical process. Based on a signal which is twice that of the background (S/N=2) of unspiked chow, minimum amounts of TBOH and TBA detectable by LC were about 0.04 and 0.32 ppm respectively; by EC/GC the levels are 0.33 and 1.2 ppm. Nevertheless, quantitation of residues at the 0.5 ppm level using LC is preferred because as the report states, "Although the GC analysis of PFP derivatives of the two compounds has the potential of being more sensitive than LC, the presence of background interferences in the derivatized cleaned-up extracts precluded full use of that sensitivity; **LC is, therefore, the method of choice.** However, the GC procedures will serve well as an alternate or confirmatory procedure.

1. NCLTR Technical Report for Experiment Number 0175 (Approved for release 10/23/85) by Department of Health, Education and Welfare, Public Health Service, Food and Drug Administration, National Center for Toxicological Research, Jefferson, Arkansas 72079