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Screening Procedure for Anabolic Steroids – The Control of the Hydrolysis with Deuterated Androsterone Glucuronide and Studies with Direct Hydrolysis

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Abstract

For the deconjugation of steroid conjugates an enzymatic hydrolysis with ß-glucuronidase or ß-glucuronidase/arylsulfatase is performed. To control the hydrolysis step, we have synthesized [2,2,3,4,4-$^2H_5$]-androsterone glucuronide. This deuterated glucuronide is used as an internal standard and allows together with the unconjugated internal standard [2,2,4,4-$^2H_4$]-etiocholanolone a judgement of the completeness of the hydrolysis of steroid glucuronides. In figure 1 is shown an example for the simple visual control of the hydrolysis.
The control of the hydrolysis step makes it possible to perform a direct hydrolysis of steroid conjugates without a previous solid phase extraction (e.g. by Amberlite XAD-2 polystyrene resin). The actual procedure is presented in figure 2.
In several studies we could not find disadvantages of the direct hydrolysis regarding the detection of low amounts of anabolic steroids or quantitation of endogenous steroids.

A comprehensive publication of the presented paper is still in preparation and will be published elsewhere.

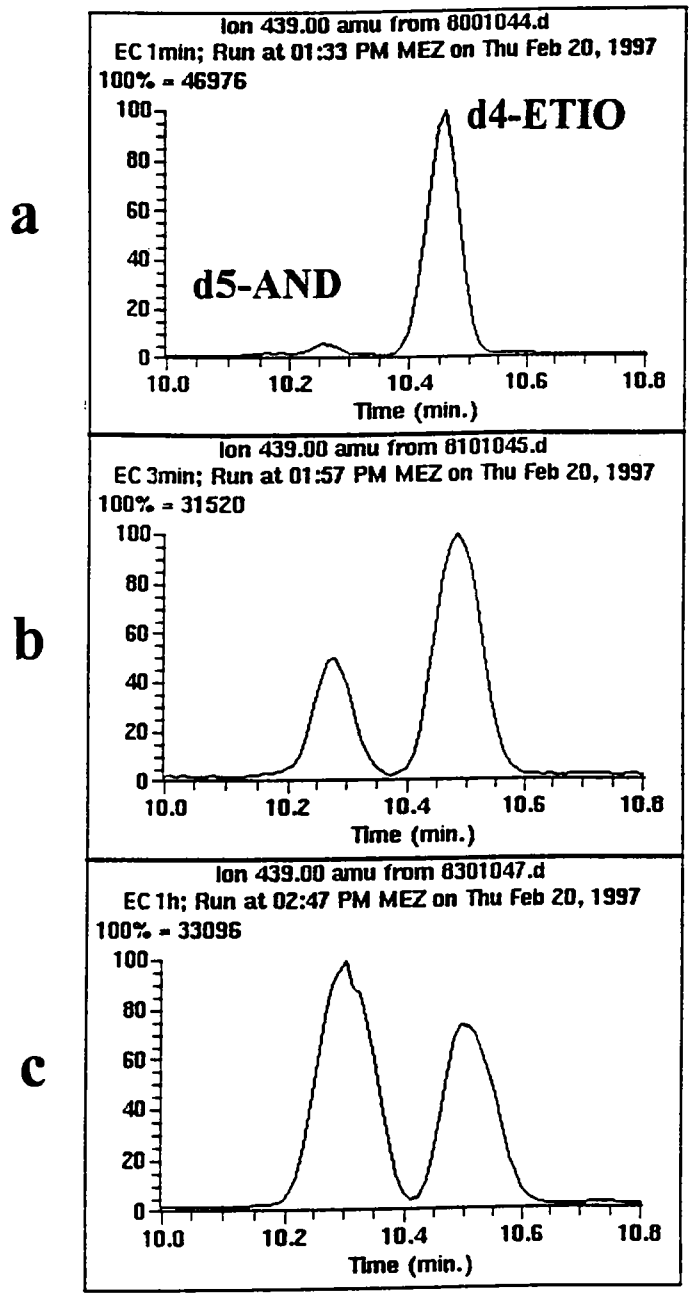

Fig. 1: Control of the hydrolysis step with [2,2,3,4,4-$^2H_5$]-androsterone glucuronide and unconjugated [2,2,4,4-$^2H_4$]-etiocholanolone 500 ng/ml urine each. Monitored is the ion m/z 439 for the molecular ion of [2,2,3,4,4-$^2H_5$]-androsterone, bis-TMS (d5-AND) and for the molecular ion plus 1 of [2,2,4,4-$^2H_4$]-etiocholanolone, bis-TMS (d4-ETIO). If the hydrolysis is complete, the signal for d5-AND is a factor 1.1-1.2 more intensive than for d4-ETIO as in example „c".

Hydrolysis of with 50 µl ß-glucuronidase from E.coli (Boehringer, Mannheim)

a) 1 min at 50°C -incomplete hydrolysis-

b) 3 min at 50°C -incomplete hydrolysis-

c) 1 h at 50°C -complete hydrolysis-

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Anabolic Steroids - Combined Fraction - Direct Hydrolysis

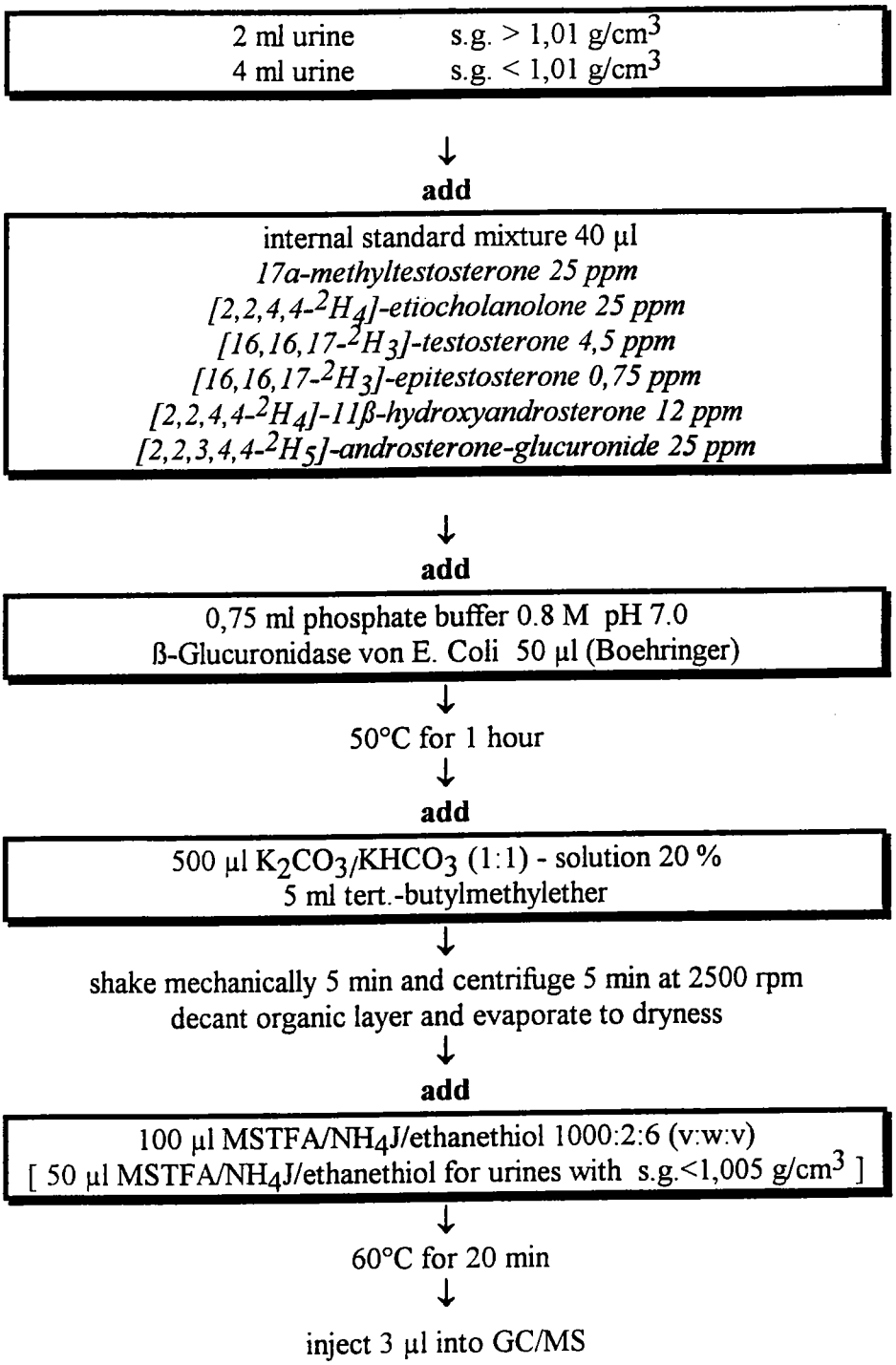

Fig. 2: Flow sheme for the sample preparation for the screening of anabolic steroids with direct hydrolysis.