In: Donike, H. Geyer, A. Gotzmann, U. Mareck-Engelke, S. Rauth (eds.)
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Improved Purification of Anabolic Steroids for GC/MS-Analysis

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Introduction

The screening procedures generally used for anabolic steroids as well as for other banned drugs are fast and have a high selectivity and sensitivity. However, sometimes normal sample pretreatment does not purify the sample enough. For these cases, more efficient purifying procedures are needed.

In our laboratory a very simple additional purification step based on solid phase extraction with amino-columns is added to the normal procedure. This extra step removes a lot of impurities which otherwise may interfere with anabolic steroids or their metabolites. The recoveries for most of the metabolites studied were satisfactory.

Experimental

Extraction - normal procedure
2.5 ml of urine was introduced into Sep-Pak C18 cartridge. Methyltestosterone was used as an internal standard. After washing with water (3 ml), the sample was eluted with 3 ml of methanol and evaporated to dryness. The residue was dissolved in 1 ml of phosphate buffer of pH 7 and hydrolysed enzymatically (ß-glucuronidase from Escherichia Coli) for 1 hour at 60 °C. After adjusting pH to 11, the sample was extracted with 5 ml of diethyl ether using salting out. The organic layer was evaporated to dryness before derivatisation.

Solid phase extraction using amino column
The residue obtained after the normal procedure (described above) was dissolved in 2 ml ethyl acetate. A 3 ml Amino (NH2) -column (Baker, the Netherlands) was conditioned by washing with 2 ml ethyl acetate.The sample dissolved in ethyl acetate was aspirated through the column and the column was washed with 2 ml ethyl acetate. Both ethyl acetate fractions were combined and then evaporated to dryness.

Derivatisation
The dry residue was derivatised with 50 µl of MSTFA/TMSI/dithioerythritol (1000:2:4) for 15 min at 60 °C.

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GC/MS parameters

Samples were analysed on HP 5995A GC/MS (Hewlett-Packard). The fused silica capillary column was Ultra-1 (Hewlett-Packard), 12 m x 0.2 mm with a 0.33 µm film thickness. Carrier gas was helium (1.1 ml/min at 100 °C). Oven was first programmed from 160 to 200 °C at 12 °C/min and then to 226 °C at 2 °C/min and finally to 300 °C at 10 °C/min. Split injection (3 µl, 1:15) was done at 280 °C. MS was operated either in SIM-mode (65 ions with dwell times of 20 msec divided into 5 different groups) or SCAN-mode (mass range 50-700 m/z).

Results

Compared with the normal extraction procedure, the additional solid phase extraction step purified the sample considerably. The effectiveness of amino column step can be seen in Figure 1 which shows total ion chromatograms of a drug-free urine extracted using both procedures.

The recoveries for the most of the main urinary metabolites of different anabolic steroids obtained in amino column solid phase extraction varied from 80 to 90 percent. The recovery was decreased when the polarity of the metabolites was increased. For example the recoveries for the metabolites of fluoxymesterone and formyldienolone were only 10 - 30 percent and the metabolites of stanozolol were totally retained. The recoveries for different anabolic steroids are listed in Table 1.

Two examples of the efficiency of the amino column solid phase extraction step are presented. Figure 2 shows how norandrosterone (the main metabolite of nandrolone) can be detected without any interfering peaks. The identification of methenolone using amino column extraction is presented in Figure 3.

Conclusions

Solid phase extraction with amino columns is very useful as an extra purification step for urinary anabolic steroids. The cleaning step is very simple and it can easily be combined with the normal extraction procedure. The recoveries for most of the main urinary metabolites of anabolic steroids are satisfactory. Many impurities which may interfere with the metabolites of anabolic steroids are removed from the sample.

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Figure 1.
Total ion chromatograms of a drug-free urine sample extracted using (A) normal procedure and (B) normal procedure combined with amino column solid phase extraction. An efficient purification of the sample is observed especially in the region where most of the metabolites of anabolic steroids are eluted.

Figure 2.
Selective ion monitoring of a nandrolone positive urine extracted using (A) normal procedure and (B) normal procedure combined with amino column solid phase extraction. A metabolite of vitamin E which normally elutes just before norandrosterone and may cause problems is totally removed using amino column extraction.

Figure 3. Identification of methenolone from a urine sample collected after administration of methenolone acetate. Total ion chromatograms with reconstructed ion profiles (m/z 195) and mass spectra from the region where bis-TMS-derivative of methenolone (molecular ion 446 and base peak 195 m/z) is eluted. (A) normal extraction procedure and (B) amino column purification. Both chromatograms and mass spectra show the efficiency of amino columns in removing impurities.

Table 1.
Recoveries for the main urinary metabolites of different anabolic steroids obtained in amino column solid phase extraction step.

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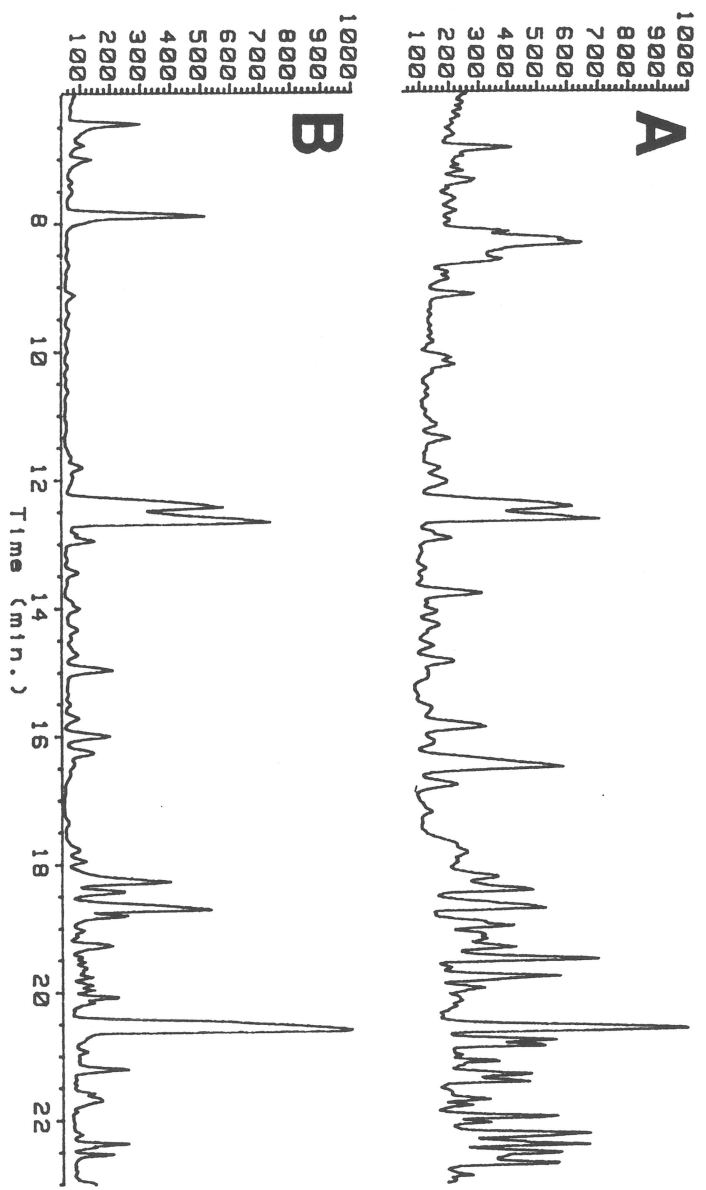

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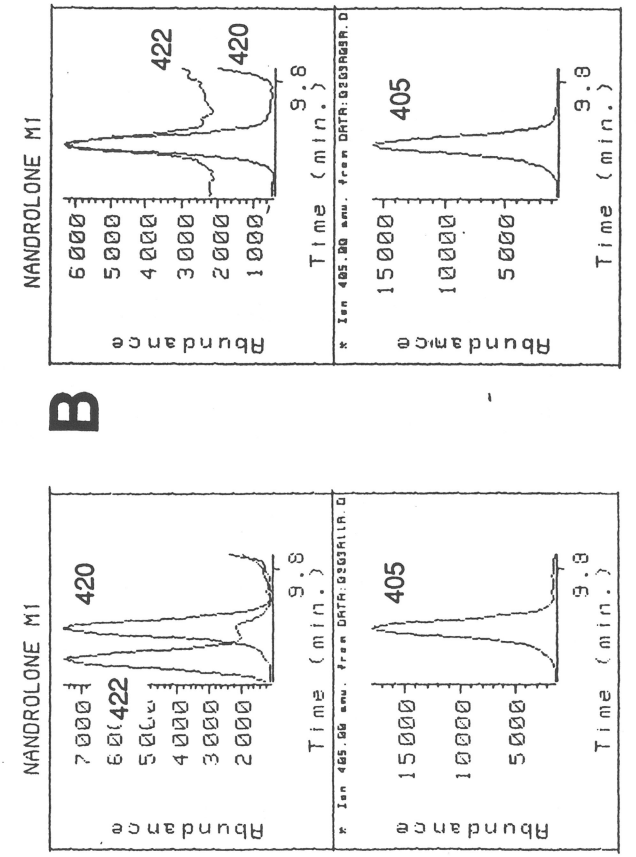

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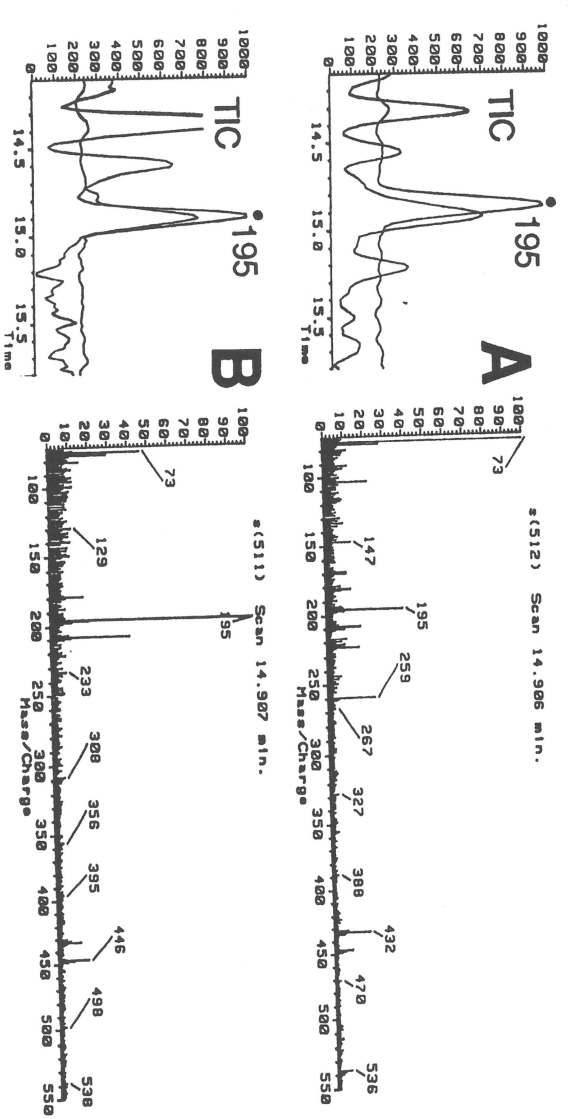

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80 - 90 %:	Bolasterone Boldenone Chlormethyltestosterone Drostanolone Methandienone Methenolone Methyltestosterone Mesterolone Nandrolone Norethandrolone Oxymesterone Testosterone and Epitestosterone
50 - 70 %:	Chlordehydromethyltestosterone Oxandrolone Oxymetholone
10 - 30 %:	Fluoxymesterone Formyldienolone
0 %:	Stanozolol

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