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RECENT ADVANCES
IN DOPING ANALYSIS
(12)

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Comprehensive Sample Preparation for Anabolic Steroids,
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Comprehensive Sample Preparation for Anabolic Steroids, Glucocorticosteroids, Beta-Receptor Blocking Agents, Selected Anabolic Androgenic Steroids and Buprenorphine in Human Urine

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Abstract

The sample preparation for anabolic steroids [1,2], glucocorticosteroids [3], beta-receptor blocking agents [1,4], selected anabolic androgenic steroids and buprenorphine in human urine was proceeded individually for each urine sample.

In order to provide a more effective doping analysis according to WADA regulations a more comprehensive sample preparation method was developed allowing an extensive screening based on conventional assays including also new sensitive techniques (LC-MS/MS).

The screening procedure for anabolic steroids was modified to include the sample preparations for glucocorticosteroids (screen 3), beta-receptor blocking agents (screen 7), selected anabolic androgenic steroids and buprenorphine (screen 9).

As shown in fig.1 the use of 3 ml of urine (instead of 2 ml of urine) and an extraction of the combined fraction after hydrolysis with 6 ml of t-butyl methyl ether (TBME) (instead of 5 ml of TBME) allows the utilisation of 2 ml of TBME (which corresponds to 1 ml of urine) for the analysis of glucocorticosteroids, beta-receptor blocking agents, selected anabolic androgenic steroids and buprenorphine.

Minimum required performance limits (MRPL) for glucocorticosteroids (30 ng/ml), beta-receptor blocking agents (0,5 µg/ml), selected anabolic androgenic steroids (10 ng/ml) and buprenorphine (10 ng/ml) according to WADA regulations are fulfilled and recoveries for beta-receptor blocking agents, selected anabolic androgenic steroids and buprenorphine are presented in tab 1 and 2. For recoveries of glucocorticosteroids please refer to [5].

The efficiency and sensitivity of the assay for anabolic steroids is not influenced in comparison to earlier validations. No increase regarding detection limits and no decrease in recoveries were observed.

This comprehensive sample preparation is a time- and urine-saving improvement including a new sensitive technique for screening purposes in doping analysis.

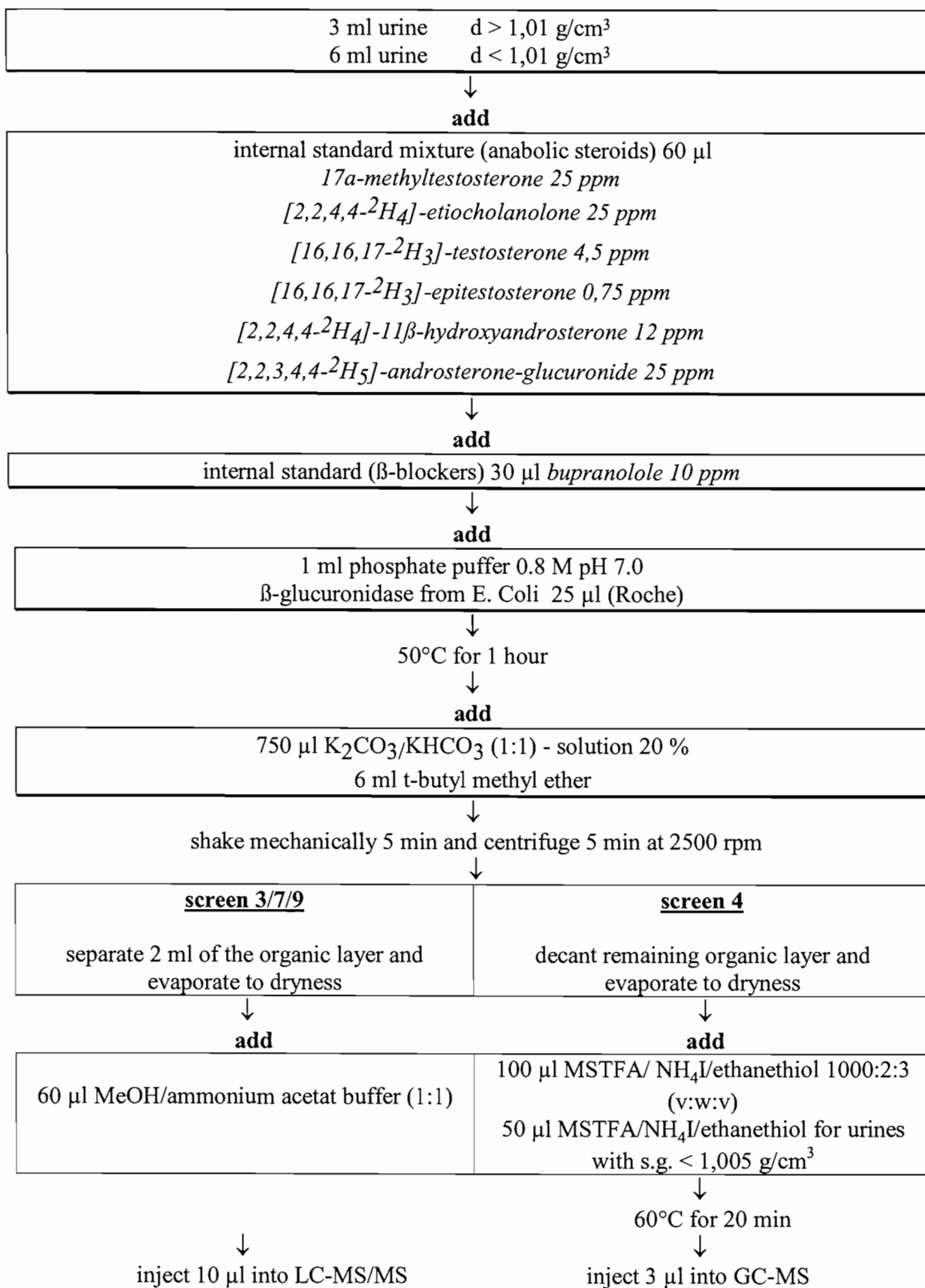


Fig.1: Flow scheme for the sample preparation for the screening of anabolic steroids / combined fraction (screen 4), glucocorticosteroids (screen 3), beta-receptor blocking agents (screen 7) and selected anabolic androgenic steroids and buprenorphine (screen 9) in human urine.

Tab 1: Recovery of beta-blockers in human urine (screen 7)

Determined at a concentration of 100 ng/ml each

Minimum required performance limit (MRPL): 0,5 µg/ml

| | recovery (%) |
|--------------|--------------|
| acebutolol | 17 |
| alprenolol | 36 |
| atenolol | 0,5 |
| betaxolol | 34 |
| bisoprolol | 32 |
| bopindolol | 26 |
| bucumolol | 27 |
| bufetolol | 29 |
| bufuralol | 33 |
| bunitrolol | 30 |
| butofildol | 32 |
| carazolol | 33 |
| carvedilol | 29 |
| celiprolol | 14 |
| esmolol | 31 |
| indenolol | 31 |
| labetalol | 28 |
| carteolol | 11 |
| metipranolol | 31 |
| metoprolol | 31 |
| levobunolol | 33 |
| cloranolol | 33 |
| penbutolol | 34 |
| mepindolol | 27 |
| morprolol | 25 |
| nadolol | 2,7 |
| nebivolol | 31 |
| nifenalol | 33 |
| oxprenolol | 30 |
| pindolol | 32 |
| propranolol | 31 |
| sotalol | 1,5 |
| talinolol | 31 |
| timolol | 29 |
| toliprolol | 32 |

Tab 2: Recovery of selected anabolic androgenic steroids and buprenorphine in human urine (screen 9)

Determined at a concentration of 20 ng/ml each

Minimum required performance limit (MRPL): 10 ng/ml

| | recovery (%) |
|----------------------|--------------|
| trenbolone | 42 |
| buprenorphine | 44 |
| tetrahydrogestrinone | 33 |
| epimethandienone | 36 |
| methandienone | 32 |
| stanozolol | 31 |
| 16-OH stanozolol | 30 |

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Nutritional Supplements – The South African Scenario

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Abstract

Introduction. Inadvertent doping through the use of nutritional supplements is a potentially important cause for the increase in positive drug tests involving high profile Olympic athletes. Results from an IOC study on nutritional supplements (13 countries excluding South Africa) showed that 94 (14.8%) samples out of 634 samples contained prohormones not listed on the label.² Nutritional supplement use is also very popular in South Africa and the turnover of the industry is approximately R1.5 billion a year (Health Products Association of South Africa Survey 1998 – 2000).

Objective. The aim of this study was to screen over-the-counter nutritional supplements for the presence of steroid or stimulant compounds banned by the International Olympic Committee (IOC) and the World Anti-Doping Agency (WADA).

Method. Thirty different nutritional supplements from 14 different manufacturers were bought at shops in Bloemfontein, South Africa and analysed for testosterone and nandrolone prohormones, various ephedrines and caffeine.

Results. Eighteen (60%) of the 30 supplements contained no prohibited substances. Of the 12 (40%) positive supplements, eight (66.7%) contained prohormones and four (33.3%) contained stimulants. Six supplements contained prohormones, which were listed on the labels, while 2 contained prohormones not listed on the labels. The stimulants were listed on the labels as Ma Huang, Guarana and Kola extracts and all contained a mixture of ephedrines and caffeine.

Conclusion. The results showed that approximately 7% of supplements tested may be mislabelled or contaminated with banned substances and that inadvertent doping through nutritional supplement use is a reality for athletes. The sporting community should therefore be aware that supplements might contain anabolic androgenic steroids and stimulants that are not declared on the labels.

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