Reprint from

RECENT ADVANCES IN DOPING ANALYSIS (11)

W. Schänzer
H. Geyer
A. Gotzmann
U. Mareck
(Editors)

Sport und Buch Strauß, Köln, 2003

A. LEINONEN, T. KUURANNE, T. KOTIAHO, R. KOSTIAINEN:
Screening of Unconjugated Anabolic Steroids in Urine by Liquid Chromatography / Mass Spectrometry
In: W. Schänzer, H. Geyer, A. Gotzmann, U. Mareck (eds.) Recent advances in doping analysis (11). Sport und Buch Strauß, Köln, (2003) 161-162

A. Leinonen[1,2], T. Kuuranne[1,2,3], T. Kotiaho[2], R. Kostiainen[2,3]

Screening of unconjugated anabolic steroids in urine by liquid chromatography/mass spectrometry

[1]Doping Control Laboratory, United Laboratories Ltd., Helsinki, Finland
[2]Viikki Drug Discovery and Technology Center, Department of Pharmacy, University of Helsinki
[3]Division of Pharmaceutical Chemistry, Department of Pharmacy, University of Helsinki

ABSTRACT

The complete article is accepted for publication in Steroids (21st October 2003):
Leinonen A., Kuuranne T., Kotiaho T., Kostiainen R. Screening of free 17-alkyl-substituted anabolic steroids in human urine by liquid chromatography / electrospray ionization – tandem mass spectrometry.

Sensitive and reliable analytical methods are needed to screen and confirm the presence anabolic androgenic steroids in urine. Due to the large number of compounds, multi-analyte methods are favored. Prevalent screening methods are mainly based on gas chromatography/electron ionization low-resolution mass spectrometry, where conjugated and unconjugated steroid-metabolites are analyzed either simultaneously or in separate procedures. To improve detectability, high-resolution mass spectrometry and tandem mass spectrometry combined with gas chromatography have also been applied.

In this study, a liquid chromatography/electrospray ionization tandem mass spectrometric method was developed for qualitative screening of several anabolic steroids or their metabolites that are excreted in urine in free form. The compounds were separated at room temperature in a reversed phase C18 column using methanol-water gradient, containing ammonium acetate and acetic acid as additives. Compounds were ionized in positive ion mode and detected using multiple reaction monitoring of two specific product ions per each analyte on a triple-stage quadrupole mass spectrometry. Either protonated molecule or ammonium adduct was selected as precursor ion.

Using the developed method, all compounds could be detected at concentration levels below 2.1 ng/ml. Chemical and biological background was repeatedly extremely low with no interfering peaks. The method showed good linearity up to 250 ng/ml with correlation coefficients higher that 0.995. Liquid chromatography/mass spectrometry has the potential to overcome some of the problems associated with the gas chromatographic methods, i.e. derivatization, adsorption of steroids and their thermal decomposing during analysis. With simple sample preparation and high degree of sensitivity and specificity, the developed method is a good alternative for gas chromatography/mass spectrometric methods in routine screening of free steroid fraction in urine.