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Liquid-phase microextraction (LPME) as a sample purification method in LC-MS/MS analysis of anabolic steroid glucuronides

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ABSTRACT

The whole article is published in:

Kuuranne, T., Kotiaho, T., Pedersen-Bjergaard, S., Rasmussen, K.E., Leinonen, A., Westwood, S., and Kostianen, R.: Feasibility of a liquid-phase microextraction sample clean-up and LC-MS/MS screening method for selected anabolic steroid glucuronides in biological samples. *J. Mass Spectrom.* 38 (2002) 16-26.

Due to extensive phase I and phase II metabolism, direct detection of glucuronide-conjugates is an attractive approach to the analysis of anabolic androgenic steroids (AAS). In the course of the liquid chromatographic-tandem mass spectrometric (LC-MS/MS) method development for AAS glucuronides in biological samples the specificity of pretreatment became critical, especially in the case with urine matrix. An alternative clean-up technique, liquid-phase microextraction (LPME) was optimized for the set of 13 steroid glucuronides, and was then compared to liquid-liquid extraction (LLE) and solid-phase extraction (SPE) procedures. In the final method ammonium acetate buffered water-acetonitrile gradient and an endcapped RP18 column were applied in LC. The AAS glucuronides were ionized in positive ion mode electrospray (ESI) and then detected by triple quadrupole MS instrument as two characteristic precursor ion - product ion pairs, which consisted mainly of the ammonium adduct of the AAS glucuronide $[M+NH_4]^+$ and dehydrated fragments of the steroid aglycone $[M+H-Glu-nH_2O]^+$, respectively. As such the method was nicely applicable for monitoring of glucuronidation of known AAS aglycones during *in vitro* metabolic studies, where the matrix is still relatively simple. Despite of enhanced performance of LPME in comparison to LLE and SPE, urine revealed the analytical problems with specificity and sensitivity. Tentative validation of the LPME-LC-MS/MS method showed

acceptable reproducibility and linearity, and for most analytes the detection limits were in the range 2-20 ng/ml, but e.g. the detection of boldenone and methyltestosterone metabolites was complicated because of co-elution of endogenous compounds and matrix interference.