

PROJECT REVIEW

“Combined screening on diuretics and heavy volatile nitrogen containing doping substances, investigations on the metabolism and synthesis of phase II-metabolites”

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In the fight against doping the laboratories are confronted with an increasing number of substances to screen on. Therefore new methods for the screening of those substances have to be implemented in the laboratories. To keep costs for doping control analysis acceptable, to ensure rapid reporting times and to lower the amount of urine needed to screen for all substances, a comprehensive screening for different classes of substances is desirable.

The project aims at combining the screening methods for diuretics, beta-2-agonists, heavy volatile stimulants and narcotics in one LC-MS/MS method. To avoid problems with the stability of diuretics during the hydrolysis, direct analysis of the excreted metabolites (where applicable as intact conjugates) is mandatory. This is also beneficial because relatively clean extracts are obtained and the time consuming hydrolysis is eliminated.

Thus the sample preparation has to be adapted, the phase II-metabolism of beta-2-agonists, narcotics and heavy volatile stimulants has to be investigated and reference substances of the metabolites have to be synthesized and characterized by nuclear magnetic resonance (NMR) and mass spectrometric (MS) techniques.

Results and Conclusions

“Combined Screening on Diuretics and Heavy Volatile Nitrogen Containing Doping Substances- investigation on the metabolism and synthesis of phase II metabolites”

In the fight against doping in sports the laboratories are confronted with screening an increasing number of substances. Therefore new methods for the screening of those substances have to be implemented. To keep costs for doping control analysis acceptable, to ensure rapid reporting times, and to lower the amount of urine needed to screen for all substances, combining ‘traditional’ screening procedures for different classes of substances is desirable.

This project aimed at combining the screening methods for diuretics, beta-2-agonists, heavy volatile stimulants and narcotics in one method. To evade the hydrolysis of the conjugates, which on the one hand is time consuming and on the other hand revealed problems with the stability of diuretics, direct analysis of the excreted metabolites (where applicable as intact conjugates) is desired.

Thus, the phase-II metabolism of beta-2 agonists, heavy volatile stimulants and narcotics was investigated and sulfoconjugates of p-Hydroxyamphetamine, p-Hydroxy-met-amphetamine, p-Hydroxyephedrine, p-Hydroxynorephedrine, Etilefrine, and Etamivan were synthesized and characterized by LC-MS/MS. The aglycons were coupled to sulfuric acid by reaction with sulfur trioxide pyridine complex. The structures of the mono-sulfates of p-Hydroxyamphetamine, p-Hydroxy-met-amphetamine, p-Hydroxyephedrine, Etilefrine, and Etamivan were confirmed by nuclear magnetic resonance.

Different materials for Solid Phase Extraction (SPE) were investigated and the best results for screening purposes were obtained with PAD I. This also allowed a simple combination with the routine procedure for diuretics used in our laboratory, which also utilizes SPE with PAD I. Validation for the sulfoconjugates and application of the whole method to real urine samples was performed. Due to the very large number of analytes with different ionization behavior, it was necessary to submit the final concentrate to two separate LC-MS/MS runs.

Presentations:

The results have been presented during the 25th Cologne Workshop in February 2007:

Parr MK, Orlovius A, Guddat S, Gütschow M, Thevis M, Schänzer W. Combined Screening on Diuretics and Heavy Volatile Nitrogen Containing Doping Substances utilizing LC-MS/MS.