PROJECT REVIEW

"Synthesis and Characterisation of Metabolites for the Integration in a Comprehensive Screening Procedure utilizing LC-MS/MS"

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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 have to be implemented by the laboratories. To keep the 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.

Following the 2005 application WADA has granted a pilot project to check for the applicability of direct LC-MS/MS measurement of sulfoconjugates of heavy volatile stimulants. As most of the beta-2 agonists and heavy volatilestimulants are conjugated to sulfuric acid in humans an extension of the method to other compounds is desirable. As reference substances of the conjugates are barely available, during the pilot project the sulfoconjugates of p-Hydroxyamphetamine, p-Hydroxymetamphetamine (Pholedrine), p-Hydroxyephedrine, p-Hydroxynorephedrine, Etilefrine and Etamivan were synthesized by coupling the aglycons to sulfuric acid by reaction with sulfur trioxide pyridine complex.

The objective of the continuation is to extend the combined screening procedure for diuretics and heavy volatile stimulants developed in the pilot project to other compounds.

Thus, studies on the metabolism have to be reviewed and relevant metabolites have to be synthesised. The structures of all relevant products will be confirmed by nuclear magnetic resonance. For the integration in a comprehensive screening procedure the analytes will be characterised by means of LC-MS/MS. To obtain acceptable selectivity and sensitivity with satisfactory run times the preliminary method has to be reviewed. The conditions for solid phase extraction will be tested and optimised and the LC-MS/MS measurement adapted for the new analytes. The method will be checked with post-administration urines and validated according to ISO 17025 regulations.

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Results and Conclusions:

The objective of the presented study was a comprehensive screening procedure for diuretics, stimulants, narcotics and β2-agonists. In order to detect these substance classes, investigations in the metabolism of the heavy volatile stimulants and β2- agonists was necessary. The investigations on the metabolism of stimulants, narcotics and beta-2 agonists were performed with special focus on the phase-II metabolism. Main fractions of these compounds are unconjugated and sulphate metabolites. Glucuronides, if any, are only excreted to a minor extend except for formoterol, morphine and sibutramine. Most of the studies reported in literature are based on separation of the metabolite fractions and separate determination of the aglycons after cleavage of the conjugates. To confirm the reported findings, direct LC-ESI-MS/MS (MRM of the anticipated metabolite transitions) analysis of intact conjugates in post administration urines was conducted.

To obtain reference material many experiences in the synthesis of the sulfoconjugates could be made and will accelerate the production of the lacking compounds. Because of failure in synthesizing the compounds selectively, unselective sulfoconjugation was performed. For characterization and the later use as reference compounds and to validate a urine-screening, elaborate clean up procedures were carried out. Column chromatography and preparative HPLC was used to separate the unconjugated compound from the desired product and side products.

Characterization (HRMS, NMR) of terbutaline sulfoconjugate and octopamine sulfoconjugate was intensively performed and for investigation of pharmacokinetics like metabolism and excretion time, detailed excretion studies with both parent compounds were carried out.

An excretion study with terbutaline was analyzed to monitor the excretion kinetics. Terbutaline sulfoconjugate could be detected for at least 4 days. Another excretion study with octopamin yielded a high amount of octopamine sulfoconjugate compared to the free compound.

Additionally, a comprehensive screening procedure was developed. Therefore, some model compounds (sulfoconjugates) from the pilot study were used to be implemented in a screening procedure with diuretics, narcotics, β2-agonists (parent compound), stimulants and several other compounds. Validation and application to post-administration urines was performed successfully.