PROJECT REVIEW

"Synthesis of Mesocarb Metabolites as Reference Compounds for Doping Analysis"

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Mesocarb (Sydnocarb®) is an amphetamine-related psychostimulant that have been used for doping purposes due to their central nervous system stimulating effects. Moreover, it is very likely that mesocarb is being used by drug addicts. Mesocarb is included in the World Anti-Doping Agency's list of substances and methods that are prohibited in sports. Mesocarb is chemically a cyclic and mesoionic N-phenylcarbamoyl-3-(beta-phenylisopropyl)sydnone imine. The dominant urinary metabolites of mesocarb are identified to be its mono-, di- and trihydroxylated isomers. The exact chemical structures of regioisomeric metabolites of mesocarb have not been solved yet. Furthermore, the metabolites are not commercially available. According to the WADA's international laboratory standard and ISO 17025 standard, well characterized pure reference materials are recommended to be used as references in the analysis.

In this project a high-yielding and selective synthesis methods for the preparation of mesocarb metabolites as reference substances will be developed. The synthesized metabolites will be purified and characterized and their chemical stability will be tested. The synthesized substances will be used to solve so far unknown regiochemical structures of the main urinary metabolites of mesocarb. Using the synthesized substances, the feasibility of various screening methods used in doping laboratories for the abuse of mesocarb will be tested, and a liquid chromatographic – tandem mass spectrometric confirmation method will be developed and validated. Finally, the synthesized mesocarb metabolites would be available free of charge as reference substances to the world's antidoping community. The pure and fully characterized reference substances would enable the reliable and legally defensible analysis of mesocarb and could also be used in quality assurance and in development of new analytical methods.

Results and Conclusions

“Synthesis of Mesocarb Metabolites as Reference Compounds for Doping Analytics”

Mesocarb (Sydnocarb®) is an N-alkylated amphetamine derivative, and has been used for doping purposes due to its central nervous system stimulating effects. It belongs to the list of prohibited substances and methods in sports of World Anti-Doping Agency (WADA). According to the WADA’s international standard for laboratories and ISO 17025 standard, well-characterized reference compounds should be used for the identification of a prohibited substance, if available. Presently, the metabolites of mesocarb are not commercially available, and additionally, the exact chemical structures of several mesocarb metabolites have not been solved yet.

In this WADA-funded research project we synthesized six potential mono-, di-, and trihydroxylated regioisomeric metabolites of mesocarb for the doping analytical purposes. The synthesized metabolites were purified, characterized and compared with the in vitro synthesized mesocarb metabolites using human liver enzymes and with the in vivo formed metabolites extracted from human urine after oral administration of mesocarb. An LC-MS/MS-method for the characterization of mesocarb and its metabolites in urine was developed.

p-Hydroxymesocarb was found to be the main (conjugated and non-conjugated) metabolite in human urine, emphasizing the importance of its synthesis and availability as a reference material with respect to doping analysis. The synthesized and fully characterized p-hydroxymesocarb enables the reliable and legally defensible confirmation analysis of mesocarb, and could also be used in quality assurance and in development of new analytical methods. The metabolite synthesized within this project will be available without charge to all WADA-accredited anti-doping laboratories.