”Development and harmonization of direct urinalysis quantitative methods for threshold substances”

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Project Overview

Recently, a few methods for urinalysis of threshold substances via LC-MS have been developed and published. These methods allow for the direct analysis of urine samples without the need for extraction. The minimal sample preparation of these methods offers several advantages besides cost effectiveness and speed. Indeed, lower sample preparation leads to a reduction in the factors contributing to the measurement uncertainty of a result. Moreover, the required volume of urine for the quantification is limited and the methodology allows for the direct quantification of Phase 2 metabolites.

Direct quantification of conjugates is preferred above hydrolysis followed by a quantification of the deconjugated substances since incomplete hydrolysis and or degradation effects (e.g. endogenous steroids) can lead to quantification errors. These errors are an important factor in the bias of current quantitative methods and of the uncertainty estimates.

The current project would develop direct LC-MS quantification methods for all WADA threshold substances with a minimal sample preparation procedure.

The developed methods would then be implemented and validated in all participating laboratories and a common measurement uncertainty estimate would be made to harmonize methodologies and decision limits (concentration above which a sample can be regarded as exceeding the threshold taking into consideration MU). As such the project would not only harmonize methodologies but also decision criteria leading to a more uniform interpretation of results. Moreover, taking into account the simplified sample preparation and the extensive harmonization of these methods among the participating laboratories it can be expected that the inter-laboratory variation will be lower than currently. Such a reduction in inter-lab variability is a primary objective for an adequate use of individual athlete passports with biometrical data.

This hypothesis will be tested in the last phase of the project during which PT-samples will be distributed for every threshold substance to all laboratories. These samples will be quantified using the procedures currently applied in these laboratories as well as with the new unified methodologies. This approach will allow for a direct comparison and evaluation of the effectiveness of the harmonization of methods.

Results and Conclusions

One of the goals of this project was to develop simple and robust quantification methods to quantify all WADA threshold substances using asimplea dilute-and-shoot approach. These methods were developed and validated and MU was estimated for each of them. All were compliant with WADA's TD's.

Every laboratory implemented the developed methods. A proficiency test was set-up and the values obtained via these methods and the laboratories own methods revealed that there were no clear differences in the quality of the data. No real benefit was obtained and no clear cut reasons could be identified when smaller differences were noticed. Perhaps, this is caused by the high quality standard WADA already demands from accredited methods/laboratories.

Publications / Presentations

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