"Rapid Multi-Residue Extraction and Analysis Method for Prohibited Substances in Urine"

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Results and Conclusions

The ultimate aim of this project was to determine whether it is possible using a single solid phase extraction (SPE) cartridge to extract the wide range of prohibited substances that are in the WADA Prohibited List, excluding the peptide hormones, in a form suitable for analysis by LC/MS/MS. The compound classes investigated include anabolic agents, beta-2 agonists, diuretics, narcotics, glucocorticosteroids, and some stimulants. Thirteen SPE protocols were evaluated and the results indicated that there were four commercially available SPE cartridges that could be suitable for this project. One cartridge was judged as the best overall in terms of both extraction efficiency and cost-effectiveness and was able to extract all the desired compounds from urine with adequate recoveries. The second part of the project was to use LC/MS/MS to analyse all the desired compounds in the SPE extract and in particular to determine if the five low level anabolic agents clenbuterol, the metabolites of nandrolone, the metabolites of stanozolol, the metabolites of methyltestosterone, and the metabolites of methandienone could be successfully detected at levels below 2 ng/mL in urine. It has been found that whilst all these compounds can be detected using the appropriate instrumentation the limits of detection for the metabolites of nandrolone and methyltestosterone are not sufficiently low to permit their reliable detection in all samples due primarily to matrix interference. The detection levels achieved for clenbuterol, and the metabolites of stanozolol and metandienone are at least equal to those achievable with GC-HRMS or GC/MS/MS. The two isotopically labelled analogues of the metabolites of methyltestosterone and methandienone which are required for their quantitative analysis by LC/MS/MS have been synthesised.

The project has shown that a wide range of 159 analytes from a single SPE extract, including steroids, beta-2 agonists, diuretics, narcotics, glucocorticosteroids, and stimulants can be detected at levels significantly below the required minimum required proficiency levels (MRPLs) using one LC/MS/MS analysis. Unfortunately there were four prohibited substances that could not be detected at the required levels using LC/MS/MS which means that analysis using GC/MS will still be required. The very low detection levels achieved for compounds such as the diuretics and glucocorticosteroids suggests that the LC/MS/MS instrumentation used in this project may be capable of detecting such compounds by direct analysis of urine.