

PROJECT REVIEW

“Derivatization of Anabolic Steroids for Low Concentration Detection by LCMS in Doping Control Analysis”

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Detection of anabolic steroids and their long term metabolites that are usually excreted in very low concentrations, remains one of the major challenges facing WADA Accredited laboratories. Though LCMS is nowadays one of the most powerful analytical techniques, its applicability on the detection of anabolic steroids is limited by the ionisation problem that anabolic steroids present with soft ionization sources like ESI or APCI.

A number of papers have been published dealing with the ionization problem of anabolic steroids. Different ionization techniques, mobile phase additives and specific transitions have been adopted by several authors in order to meet sensitivity criteria for these compounds. Despite the progress that has been done in the field, the difficulties in ionization of anabolic steroids have lead to LCMS methods that deal with limited number of analytes.

On the other hand, several authors have used derivatization, prior to LCMS analysis, in order to enhance sensitivity for difficult ionized molecules. The incorporation of an easily ionized group, like a secondary amino group, that is practically 100% ionized in acidic mobile phase, enhances by orders of magnitude the sensitivity of their detection.

This project will investigate the enhancement of sensitivity in the detection of anabolic steroids by LCMS, after derivatization, in ESI and APCI mode for doping control purposes. Several derivatization reagents and conditions will be tested in order to find the most suitable(s) for the detection of anabolic steroids by LCMS well below their MRPL. A screening method for anabolic steroids in LCMS will be developed. The sensitivity of the method will be compared with that of GCHRMS. The developed method will be validated.

The final method will be tested for its applicability in the detection of corticosteroids in order to investigate the possibility of creation of a combined LCMS screening method incorporating the whole range of both anabolic steroids and corticosteroids.

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Results and Conclusions

The current research program examined the possibility to enhance ESI LC/MS detection of anabolic steroids, a class of prohibited substances with limited soft ionisation capacity, compared to other classes of prohibited substances. The way to achieve the enhancement of sensitivity is through the enhancement of the ionisation capacity by the appropriate derivatisation. After an extensive method development stage comprising 4 phases, 3 equivalent methods of 2-steps derivatisation are proposed aiming the hydroxy and keto groups of steroidal structure. The results presented are showing significant improvement in the detection of anabolic steroids in ESI+ LC/MS. Only 3-OH stanozolol fails to comply the WADA MRPL in urine samples, but this fact should be considered with the fact that the analysis was performed on a LC ion trap MS in full scan mode without any MSMS sensitivity enhancement.