

“Quantification of synthetic glucocorticoids in dried blood spot samples for in-competition sports drug testing”

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Project Objectives:

Corticoids are prohibited in elite sport for in-competition testing and when systemically applied (oral, i.v., i.m. etc.). In contrast, local/topical administration is permitted. For urine analysis the technical document (TD2014 MRPL) recommends a MRPL of 30 ng/mL and levels below the MRPL should be reported as negative.

There are no specifications about concentrations of corticoids in blood, although blood levels directly correlate with the route of administration. Hence, quantification of synthetic corticoids in blood can provide information whether an athlete was under the influence of systemic corticosteroid influence at the time of competition or not; largely independent from the route of administration. The quantification can be performed from a drop of dried blood (dried blood spot, DBS), which can readily be taken from an athlete in addition to a urine specimen. Sampling, transport and storage of DBS is easy and the analysis is nearly completely automatable. The obtained results provide the desirable information about the amount of the active, circulating corticoid just before or shortly after the competition. This will enable a superior basis to decide whether the detected amount of the drug was of benefit to the athlete or not without causing expensive or invasive additional sampling needs.

Results and Conclusions:

Synthetic glucocorticoids belong to the classes of substances that are prohibited in-competition only and for which permissible as well as prohibited routes of administration exist. Consequently, attributing findings of corticoids in doping control samples to time-points and routes of administrations has been of particular importance but, at the same time, a considerably challenging task. In order to complement information obtained from urine analyses, the utility of dried blood spots (DBS) was assessed and a quantitative method was established allowing to determine whole blood concentrations of synthetic glucocorticoids for sports drug testing purposes. The assay was fully validated and yielded figures of merit enabling the quantification of glucocorticoids at pharmacologically relevant concentrations as demonstrated with single-dose proof-of-concept elimination studies. Dexamethasone, methylprednisolone, and prednisone (plus its metabolite prednisolone) were determined in post-administration DBS samples for 9-24 h and observed concentrations reached, depending on the administered drug, values of up to ca. 200 ng/mL. The analytical approach employed an

automated DBS extraction utilizing stable isotope-labeled dexamethasone as internal standard and subsequent liquid chromatographic-mass spectrometric detection. Additionally, stability studies were conducted over a period of 90 days in order to assess the overall suitability of DBS as alternative matrix for sports drug testing. All model compounds were found to be stable over the entire storage time independent from the parameters light, humidity, and storage atmosphere.

In consideration of the obtained results, a strategy and concept of complementary DBS sampling and testing evolved, which can significantly contribute to addressing the aforementioned challenges concerning compounds prohibited in-competition only. Given the minimal-invasive nature of DBS combined with its low-cost sampling and storage requirements, DBS can be considered as additional test matrix collected in concert with any doping control urine sample taken from athletes in-competition. In case of urinary glucocorticoid concentrations exceeding the reporting threshold of 30 ng/mL, the concurrently collected DBS sample can be analyzed to provide additional evidence concerning the presence (or absence) of pharmacologically relevant blood levels of the glucocorticoid. Depending on the data obtained from DBS analyses, i.e. if (according to blood concentrations) the athlete was under the influence of glucocorticoids at the time of competition, the decision-making process at the result management level is facilitated. The strategy can further be expanded to other banned substances such as stimulants (e.g. cocaine, amphetamine, *etc.*) as well as narcotics.