"Dried blood spots (DBS) in sports drug testing; complementary matrix providing unique features and utilities to modern anti-doping fights"

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Project overview:

The necessities generated by the high standards and quality requirements of modern sports drug testing entails numerous challenges for doping control authorities as well as accredited laboratories. Among these challenges, several prominent aspects can be approached and solved by collecting and, when indicated, testing an additional/ a complementary specimen referred to as dried blood spot (DBS).

1. An important element of efficient doping controls is the frequent and unpredictable sampling of athletes, especially out-of-competition and concerning e.g. anabolic agents, erythropoiesis-stimulating agents, etc. Here, costs associated with sample collection and analysis are particularly limiting factors.
2. With regard to in-competition controls, the differentiation whether a substance such as a stimulant or cannabinoid (i.e. not prohibited at all times) was present in the athlete's blood at pharmacologically relevant concentrations or not is required. From urine analyses, conclusions as to the blood concentration of a drug is a complex issue and has been subject of numerous studies and discussions in the past.2-5
3. The issue of instable compounds can be solved as e.g. in case of Synacthen, a substance that was confiscated in the Spanish Fuentes scandal and admittedly misused by several athletes. Due to its limited stability in blood and urine, its analysis was hampered despite the availability of analytical methods.

DBS collection and analysis is considered as a valuable means to improve currently conducted doping control strategies as it addresses all of the above mentioned aspects. DBS are substantially cheaper than conventional doping control samples and thus larger collectives can be processed; drug concentrations at the time of competition can be determined; and instable analytes can be conserved. As a result, both relevant aspects of doping controls, i.e. deterrence from doping and protecion of honest athletes will be considerably strengthened.

Result and Conclusion:

A drop of whole blood dried on filter paper (Dried Blood Spots, DBS) represents an aspiring technique for minimal-invasive sample collection in a multitude of analytical disciplines, e.g., therapeutic drug monitoring, preclinical drug development and diagnostic analysis of metabolic disorders in newborns. DBS sampling is characterized by cost-effectiveness,

straightforwardness, robustness and facilitated storage and shipment conditions.

The present investigation was conducted to highlight the opportunities arising from the implementation of DBS as complementary matrix in doping control programs. Being frequently abused, three model compounds were chosen to represent the classes of anabolic agents (stanozolol and dehydrochloromethyltestosterone) and stimulants (pseudoephedrine). A quantitative method was developed and validated for the detection of the target analytes from DBS using liquid chromatography coupled to high resolution/ high accuracy tandem mass spectrometry. The imprecision of the assay amounted to < 8% for intraday and < 18% for day-to-day measurements. Highly purified DBS sample extracts exhibited no ion suppression effects due to interfering matrix components and provided limits of detection of 20 pg/mL for stanozolol and 0.8 ng/mL for DHCMT and pseudoephedrine, respectively, notwithstanding an overall recovery of 26%. Deuterium-labeled internal standards were used to yield reliable quantitative results (accuracy 84-125%). Stability of the analytes was shown for at least 28 days at room temperature.

Proof-of-principle for the method presented was substantiated by means of the analysis of authentic specimens obtained from administration studies with stanozolol, DHCMT and pseudoephedrine. The results provided, to our best knowledge, unprecedented detection windows for the tested anabolic agents accomplished by DBS sampling to support out-of-competition control efforts for the tested anabolic agents. Furthermore, the unambiguous proof of pharmacologically relevant blood concentrations at given urinary analyte levels are noteworthy for the improvement of in-competition controls, e.g., with regard to stimulant analysis.