"Expanding the use of dried blood sports in doping controls to peptidic drugs of lower and higher molecular mass"

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PROJECT REVIEW

In sports drug testing, the use of alternative matrices such as dried blood/plasma spots (DBS/DPS), oral fluid (OF), hair, and exhaled breath (EB) can be favorable in terms of the duration, intrusiveness and invasiveness of the sampling procedure as well as analyte stability and overall costs for transportation and storage. While DBS already have become an emerging complementary matrix for a broad range of clinical and forensic applications, the utility for doping control purposes was just recently demonstrated. For a variety of low molecular weight analytes such as anabolic agents and stimulants, detection strategies were successfully developed, however, only a few protein-and peptide-based drugs (e.g. Synacthen and IGF-I) were considered so far. Therapeutic proteins have emerged to an important class of new pharmaceuticals and comprise many drug candidates with potential performance-enhancing properties. The misuse of specific protein-/peptide-based drugs in sports is prohibited and both immunological as well as mass spectrometry-based proteomic approaches are currently used for their detection from urine and/or serum samples. Within this study, analytical strategies for the analysis of peptide hormones from DBS specimens will be developed by using different proteomic approaches such as (immuno-)affinity purification, proteolytic digestion, and LC-MS/MS. As extraction, processing and analysis of DBS are automatable, a novel workstation specially designed for a standalone-preparation of DBS samples will be tested and, if successful, employed for routine doping controls. Both "classical" and novel protein and peptide therapeutics will be used as model compounds for method development and characterization: Growth hormone releasing peptides (GHRPs), a myostatin inhibiting therapeutic antibody, and erythropoiesis-stimulating Fc fusion proteins (Sotatercept, ActRIIA-Fc, ACE-011). This study will contribute to the expansion and improvement of available test methods for performance-enhancing proteins and peptides from DBS.

Results and Conclusions:

Dried blood spots (DBS) are a relatively new alternative matrix in sports drug testing, which is advantageous with regards to the invasiveness and intrusiveness of the sample collection procedure, the effort and costs for sample transportation and storage, and the analyte stability. Most of the existing doping control DBS assays include low molecular mass analytes (e.g. anabolic agents and stimulants) and only a few methods for peptidic drugs such as Synacthen and IGF-I have been developed so far.

Within this research project, a DBS detection method facilitating the analysis of insulin and its synthetic or animal analogues was established and comprehensively characterized. The successful analysis of these substances

at physiologically relevant concentrations was realized after ultrasonication-assisted extraction, immunoaffinity purification, and liquid chromatographic separation followed by high resolution mass spectrometric detection (with or without ion mobility). During method development, major challenges were an efficient purification of the target peptides from the DBS matrix in combination with the low sample volume of 20 µL. Therefore, DBS analysis of insulins at the present stage cannot reach the sensitivity and simplicity of established serum or plasma analysis. Thus, classical plasma/serum or urine analysis is still superior and recommended in case of quantitative analysis. But for the analysis of non-fasting / non-basal insulin levels, this method can provide reliable qualitative results and opens the possibility to simplify the sample collection, transfer, and storage procedures.

Additionally, two complementary LC-HRMS detection methods for the emerging erythropoiesis-stimulating agent Sotatercept (ActRIIA-Fc) from DBS were developed and validated: An initial testing and a confirmation procedure. Both methods comprise an ultrasonication-assisted extraction, affinity enrichment, proteolytic digestion, and HRMS detection by Orbitrap MS. Due to the generic extraction, the multi-analyte initial testing procedure enables the collection of retrospective data and therefore a simultaneous detection of different IgG-based drug analytes. As proof-of-concept, artificial samples fortified with the emerging protein drugs Luspatercept and Bimagrumab as well as authentic post-administration samples containing Bimagrumab were successfully analyzed.

Finally, an automated extraction method for GHRPs from DBS was set up by using the Gerstel MultiPurposeSampler (MPS). Different parameters such as the composition of the extraction buffer, the cartridge used for subsequent online solid-phase extraction (SPE), and the SPE elution buffer were optimized, enabling detection limits at ng/mL levels.

Publications:

Lange T, Walpurgis K, Thomas A, Geyer H, Thevis M. Development of two complementary LC–HRMS methods for analyzing sotatercept in dried blood spots for doping controls. *Bioanalysis* 2019;**11**:923–40