“ENHANCED URINARY STABILITY AND DETECTION WINDOW OF PEPTIDE HORMONES AND GROWTH FACTORS BY DRIED URINE MICROSAMPLING” [PROJECT 17A20LM]

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Project Overview:

Urine samples collected worldwide for anti-doping testing are not always shipped in the optimal conditions from a stability point of view, from collection sites to WADA accredited laboratories, in particular when several transport days are required. Since delays are inevitable and sample refrigeration is not always possible and effective, sound methods for preserving and stabilizing urine samples are desirable.
This Project involves the use of volumetric absorptive microsampling (VAMS) and dried urine spot (DUS) strategies for the collection of dried microsamples for high-throughput LC-MS/MS analysis of several peptide hormones and growth factors. DUS and VAMS approaches represent promising tools, in addition to the procedures currently performed by anti-doping laboratories, allowing analyte stabilization by water loss and the consequent broadening of their detection window. This innovative sampling produces logistics savings, due to the small transported volume (by air shipments and through customs) and to the possibility of storing the specimens at room temperature, with significant implications on overall analysis cost.
The ultimate goal of this project is to establish and validate feasible but reliable protocols for the collection of urine microvolumes, unlikely to be tampered, stably storable and shippable with no particular precautions.

Results and conclusions:

- Innovative microsampling and pretreatment procedures based on dried matrices (DUS and urine VAMS) were optimised for the application to urine specimens for anti-doping purposes. Important experimental parameters were studied and specifically optimised.
- Original MS, HRMS and FT-IR methods were used for peptide chemical identity confirmation; then LC-MS/MS and LC-HRMS methods were developed for the simultaneous analysis of the peptides of interest in dried urine microsamples. After suitable study of the experimental conditions, the final methods provided good, solid performance within relatively short run times.
- The LC-MS/MS and LC-HRMS methods were validated according to current guidelines, with good results for all assays and all analytes, and with similar or better performances than those of comparable published methods.
- The microsampling, pretreatment and analysis workflow was successfully applied to the study of peptide stability in dried matrices.
- Mid-term stability assays were carried out, both on dried and fluid urine-based matrices, with outstanding results. In fact, DUS and urine VAMS were remarkably stable, even though they were kept at RT, with all studied peptides recovered in the 80-95% range at the end of the study period (3 months). The stability of the chosen peptides, which are known to be prone to degradation under common storage conditions, was greatly enhanced in comparison to fluid urine stored at freezing (-20°C) and ultra-freezing (-80°C) temperatures. Analyte loss in fluid urine at -80°C was regularly 5-10% larger at study end than the loss in dried matrices, with widely worse losses (up to 35% more) for fluid urine kept at -20°C.

The use of innovative microsampling media offers significantly interesting perspectives towards the development of engineered, highly reliable devices.

Relevant symposium communications (events attended during the project duration):

M. Protti, P. Sberna, A. Sberna, R. Ferrante, R. Mandrioli, L. Mercolini
Promising alternative techniques to overcome sampling issues in anti-doping analysis
EBF Young Scientist Symposium, 4th YSS "The BioA Brain - Embracing New Ideas"
Ghent (Belgium), 15-16 March 2018

M. Protti, L. Mercolini
Enhanced stability and detection window of large and small molecules prohibited in sports by urine microsampling
(Oral Communication)
EBF Young Scientist Symposium, 4th YSS "The BioA Brain - Embracing New Ideas"
Ghent (Belgium), 15-16 March 2018

L. Mercolini, M. Protti, P. Sberna, R. Mandrioli, A. Sberna, R. Ferrante
Nuove strategie e prospettive di ricerca per l'analisi anti-doping
18° Congresso Nazionale della Società Italiana di Tossicologia - SITox 2018
Bologna (Italy), 10-13 April 2018

M. Protti, M. Larocca, L. Mercolini
Detection of peptide hormones and growth factors in dried urine microsamples: a new approach for anti-doping analysis
Chimia International Conference 2018 - New trends in applied chemistry
Constanta (Romania), 24-26 May 2018

M. Protti, M. Larocca, M.R. Battaglia, C. Marasca, L. Mercolini
Good things come in small packages: microsampling and miniaturised sample pretreatment in clinical, forensic and anti-doping settings
EBF 2018 11th Open Meeting "Raise the Anchor - Set Sail for Science"
Barcelona (Spain), 21-23 November 2018

L. Mercolini, M. Protti
Volumetric absorptive microsampling for the determination of peptide hormones: design and development of a high-throughput strategy for anti-doping analysis
3rd International Caparica Christmas Conference on Sample Treatment 2018
Caparica (Portugal), 3-6 December 2018

M. Protti, M. Larocca, R. Mandrioli, P. Sberna, L. Mercolini
Overcoming sample stability issues by means of miniaturised dried urine samples: application to human growth hormone analysis
(Oral Communication)
3rd International Caparica Christmas Conference on Sample Treatment 2018
Caparica (Portugal), 3-6 December 2018

Relevant events (organised during the project duration):

Laura Mercolini (chairperson of the national meeting)
Il doping nello sport - Problematiche e prospettive
Bologna (Italy), 17 May 2018.

Further symposium communications (events attended and to be attended after the end of the project):

L. Mercolini
Il doping nello sport: quando l'importante è vincere (ad ogni costo)
Scientific aperitif at the University of Bologna
Bologna (Italy), 10 March 2019

L. Mercolini**,** M. Protti, R. Mandrioli, P.M. Sberna, J. Rudge
Microsampling as a promising anti-doping strategy for the analysis of peptide hormones and growth factors
The 57th Annual Meeting of the International Association of Forensic Toxicologists
Birmingham (UK), 2-6 September 2019

M. Protti, P.M. Sberna, R. Mandrioli, L. Mercolini
Microsampling approaches for anti-doping analysis of hormones and growth factors
International Conference on Recent Developments in Pharmaceutical Analysis
Pescara (Italy), 8-11 September 2019

Relevant events (organised after the end of the project):

Laura Mercolini (manager of the international meeting), Michele Protti (scientific committee member of the international meeting)
European Bioanalysis Forum (EBF) - 5th Young Scientist Symposium (YSS)
in collaboration with the University of Bologna
Bologna (Italy), 21-22 March 2019

Laura Mercolini (chairperson of the national meeting)
Doping e anti-doping: azioni e reazioni in continua evoluzione
Imola (Italy), 20 June 2019