

## PROJECT REVIEW

### ***"Evaluation of Solid Phase Microextraction for Improved Multi-Residue Extraction and Analysis of Prohibited Substances by LC-MS/MS"***

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Recent advances in liquid chromatography–mass spectrometry (LC-MS) have opened the possibility of converting many of the doping control analysis methods to LC-MS platforms. This would increase flexibility and selectivity for many analyses, while obviating the need for derivatization in most cases. A limitation to this switch is the established sample preparation strategies required for the numerous doping control methods. Developed for GC-MS analysis, they are time consuming and not always appropriate for LC-MS analysis. While the LC-MS technology holds the promise of providing a universal screening tool, the varied requirements for sample preparation for the numerous tests limit this potential. Professor Pawliszyn's group has long recognized the bottle-neck that sample preparation presents for the analytical laboratory, and have made significant strides in developing solid phase microextraction (SPME) technology to simplify sample preparation for both GC-MS and LC-MS, while maintaining sensitivity and enhancing options for automation.

In this project we will evaluate automated SPME technology as a universal sample preparation strategy, coupled with LC-MS as a universal tool for identification and quantification of prohibited substances. We will develop validated methods for urine analysis for a range of prohibited substances from the S1, S3, S4, S5, S6, S7, S8, S9 and P2 categories. We will apply enzymatic deconjugation of phase II drug metabolites as required and investigate guidelines for confirmatory analysis. SPME technology is also readily applicable to plasma and whole blood analysis and has significant advantages relative to SPE. We will evaluate the performance of the system in these matrices as well. At the conclusion of the project we will have sufficient data to evaluate the suitability of automated SPME coupled with LC-MS as a universal tool in prohibited substance screening. If judged suitable, it will be immediately applicable for introduction to the screening program in WADA accredited facilities.

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### **Results and Conclusions**

- The potential of automated solid phase microextraction (SPME) in thin film geometry as simple and convenient sample preparation approach for urine, plasma and blood analysis was demonstrated.
- SPME conditions for each of the studied matrices were carefully optimized. The effect of experimental parameters such as pH, ionic strength, centrifugation and temperature was investigated.
- Protocols for the multiclass analysis of 110 and 25 prohibited substances in urine and plasma, respectively, were developed and validated.
- In the case of urine, 100 out of 110 compounds showed R<sup>2</sup> above 0.991, intra and inter day precision was below 20 % in most cases, and in terms of accuracy only 6 compounds exhibited more than 20 % deviation from their nominal concentration value. Regarding LOQ, only 15 out of 110 compounds did not meet the MRPL values stipulated by WADA; however, as presented in the final report, by using a more sensitive mass spectrometry analyzer WADA requirements can be fulfilled.
- In the method developed for plasma analysis, satisfactory results in terms of linearity (R<sup>2</sup> > 0.99), inter and intra-day accuracy (85 – 130 %), precision (<20 %) and limits of quantitation (0.25 – 10 ng/mL for most compounds) were found.
- The suitability of the automated system to incorporate a hydrolysis step before SPME extraction was also verified.
- The most important advantages of SPME in doping controls can be listed as follows:
  1. Minimum sample handling before SPME extraction. No need of centrifugation or protein precipitation (no clogging issues as in SPE).
  2. High-throughput analysis. The automated 96 Concept system allows simultaneous preparation of 96 samples.
  3. Efficient sample clean-up. Elimination or reduction of possible ionization suppression or enhancement effects.
  4. No breakthrough volume issues. The open bed geometry of SPME allows simultaneous quantitation of a wide range of substances without concerning about extraction phase saturation.

### **Publications**

- Boyaci E., Goryński K., Rodriguez-Lafuente A., Bojko B., Pawliszyn J. Introduction of solid-phase microextraction as a high-throughput sample preparation tool in laboratory analysis of prohibited substances *Anal. Chim. Acta* 809 (2014) 69.

### **Conferences**

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2. Reyes-Garcés N., Boyaci E., Goryński K., Rodríguez-Lafuente A., Bojko B., Pawliszyn J. High-Throughput SPME-UPLC-MS as a Convenient Method for the Simultaneous Determination of Various Prohibited Substances in Urine and Plasma, 62nd SMS Conference on Mass Spectrometry and Allied Topics hold on June 14-19th in Baltimore, MD, USA (poster)
3. Goryński K., Boyaci E., Rodríguez-Lafuente A., Bojko B., Pawliszyn J. Wykorzystanie zautomatyzowanej wysokowydajnej metody mikroekstrakcji do fazy stałej (SPME) przygotowania próbki do oznaczania środków dopingujących w moczu techniką chromatografii cieczowej połączonej ze spektrometrią mas (eng. Application of automated high-performance solid phase microextraction (SPME) sample preparation method for the determination of drugs in urine by LC-MS), XXII Conference of Polish Pharmaceutical Society, Białystok, Poland, 18-21.09.2013 (oral)
4. Goryński K., Bojko B., Boyaci E., Rodríguez-Lafuente A., Pawliszyn J. Solid-phase microextraction: a powerful tool for highthroughput sample preparation in laboratory analysis of prohibited substances, 24th International Symposium on Pharmaceutical & Biomedical Analysis, Bolonia, Italy, 30.06 - 03.07.2013 (oral)
5. Goryński K., Boyaci E., Rodríguez-Lafuente A., Bojko B., Pawliszyn J. Application of solid-phase microextraction for highthroughput analysis of prohibited substances in urine, Copernican's doctoral conference, Toruń, Polska, 19-21.06.2013, 1<sup>st</sup> AWARD in medical and pharmaceutical session (oral)
6. Goryński K., Boyaci E., Rodríguez-Lafuente A., Bojko B., Pawliszyn J. Development of solid phase microextraction for high-throughput sample preparation in laboratory analysis of prohibited substances, HTC-13 & HTSP-3, Bruges, Belgium, 28-31.01.2014 (poster).
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8. Boyaci E., Bessonneau V., Bojko B., Gorynski K., Jiang R., Rodríguez-Lafuente A., Pawliszyn J. Bioanalytical application of SPME and related techniques, The Chromatography Forum of Delaware Valley Annual Symposium, Ft. Washington, PA, 3 April 2014.
9. Boyaci E., Gorynski K., Rodríguez-Lafuente A., Bojko B., Pawliszyn J. Highthroughput spme in 96-blade format for analysis of complex samples and difficult analytes. 38th International Symposium on Capillary Chromatography, Riva Del Garda, Italy, 18-23 May 2014.
10. Boyaci E., Gorynski K., Rodríguez-Lafuente A., Bojko B., Pawliszyn J. Introduction of Solid-phase Microextraction as a High-throughput Sample Preparation Tool in Laboratory Analysis of Prohibited Substances. 16th International Symposium on Advances in Extraction Technologies, Chania- Crete, Greece, 25-28 May 2014.