Project Review

"Implementation of HPLC/orbitrap mass spectrometry as a general screening method for doping control"

G. Rodchenkov, E. Virus, M. Dikunets, E. Semenistay, T. Raduschina, T. Sobolevsky (Moscow Antidoping Centre, Department of Doping Control, Moscow, Russia)

Many new compounds—chemically different drugs—have recently been included in the list of prohibited substances published by the World Anti-Doping Agency. Current applications of HPLC–MS in today's anti-doping laboratories are based predominantly on the utilization of high-performance liquid chromatography/tandem mass spectrometry (HPLC-MS/MS) systems. With these systems, known drugs or metabolic products are determined by measuring precursor/product ion pairs, providing the utmost sensitivity but also restricting the technique to a limited number of compounds.

To overcome this problem, Georgakopoulos and colleagues have recently proposed the use of orthogonal-axis high-resolution time-of-flight mass spectrometry (OTOF), which allows a broader range of doping agents. Significantly improving this methodology, we have evaluated HPLC/in-source CID atmospheric pressure chemical ionization (APCI) orbitrap mass spectrometry with accurate mass measurements for its screening potential for agents with antiestrogenic activity, beta2-agonists, exogenous anabolic steroids, and other anabolic agents to take advantage of its high resolution, sensitivity, and full scan acquisition. Furthermore, we plan to refine significantly this methodology in our proposed study.

The aims of the proposed research study are the following:

Development and optimization of HPLC/ESI–, APCI–, and APPI–HRMS (MS/MS) methods with respect to eluent composition, ion source parameters, and fragmentation. The detection limits and specificity of the methods will be compared. A full description and validation as a general screening method based on SPE and HPLC/orbitrap mass spectrometry with different ionization techniques for doping agents, including anabolic steroids, beta2-adrenergic agonists, SARMs, agents with antiestrogenic activity, diuretics, stimulants, beta-blockers, and cannabinoids. Validation of the method will consist of investigation of specificity, analytical recovery, limit of detection, and repeatability.

Finally, a comparison of the HPLC/orbitrap mass spectrometry and HPLC/MS/MS methods for the qualitative screening of prohibited substances in urine will be performed.

“Implementation of HPLC/orbitrap mass spectrometry as a general screening method for doping control”

G. Rodchenkov, E. Virus, M. Dikunets, E. Semenistay, T. Raduschina, T. Sobolevsky (Moscow Antidoping Centre, Department of Doping Control, Moscow, Russia)

Results and conclusions

1) The Multipole RF Amplitude, Multipole 00, Lens 0, Multipole 1 and Front lens voltages have dramatic effect on the Orbitrap sensitivity.
2) The research results shown that at least 113 compounds(including metabolites) out of 131 can be detected in urine at concentrations corresponding the MRPL by HPLC-APCI/Orbitrap mass spectrometry.
3) It was observed that most diuretics containing double bonded sulfur atoms could not be detected by HPLC–APCI/Orbitrap mass spectrometry because they did not ionize under APCI conditions used.
4) The proposed HPLC–ESI/Orbitrap mass spectrometry with high-pH mobile phase was demonstrated to be effective for comprehensive screening of banned substances and their metabolites.
5) Ammonium hydroxide (pH=10.3) was found to be necessary for the sensitive detection of anabolic agents and other doping substances in terms of the S/N ratio.
6) Contrary to common expectations, high-pH mobile phase do not affect the responses of β-blockers and corticosteroids in positive ESI.
7) The results demonstrated that at least 121 compounds (including metabolites) out of 131 can be detected in urine at concentrations corresponding the MRPL by HPLC–ESI/Orbitrap mass spectrometry with high-pH mobile phase.
8) It was observed that steroids without any protonable function (e.g 17α-methyl-5α-androstane-3α,17β-diol) gave good photoionization yields in solvent mixtures of 2-propanol/acetonitrile/water and propanol/ethanol/water.
9) 2-propanol/acetonitrile/water (37.5:12.5:50) was found to be necessary for the sensitive detection of doping substances in terms of the S/N ratio.
10) For maximum sensitivity, the APCI probe nebulizer (shared by APPI) is titled toward the cone face as close as possible but far enough to minimize the nebulized mobile phase spraying directly on the cone face.
11) The research results shown that doping substances (including metabolites) with diverse structures and polarities can be analyzed in urine with 100% detection rate at concentrations corresponding the MRPL by HPLC-APPI/Orbitrap mass spectrometry.
12) The results demonstrated that 225 compounds (including metabolites) out of 225 can be detected in urine at concentrations corresponding the MRPL by HPLC–APPI/Orbitrap mass spectrometry with Hypercarb column.
13) Our results confirm the previous findings about the lower susceptibility to matrix effect of APPI.
14) The specificity of the method HPLC–APPI/Orbitrap mass spectrometry with hypercarb column was extremely good and did not represent a limiting factor for selectivity.

15)Our results shows that APPI is a valuable tool for day-to-day usage in doping control because it is able to successfully to ionize more compounds, with greater structural diversity, than the other two ionization techniques.

PUBLICATIONS

1. Virus E.D., Sobolevsky T.G., Rodchenkov G.M. Introduction of HPLC/Orbitrap mass spectrometry as screening method for doping control. *Journal of Mass Spectrometry* 2008; **43**: 949-957.
2. Virus E.D., Sobolevsky T.G., Rodchenkov G.M. Detection of oxandrolone and its metabolite in urine by high-performance liquid chromatography-high-resolution mass spectrometry with atmospheric pressure chemical ionization and orbitrap detection after ceasing drug administration. *Journal of Analytical Chemistry* 2009; **64**: 31-35
3. E. N. Semenistaya, M. A. Dikunets, E. D. Virus, Rodchenkov G.M. Determination of exemestane and 17-hydroxyexemestane by high-performance liquid chromatography coupled with tandem mass spectrometry and high-resolution mass spectrometry. *Journal of Analytical Chemistry* 2010; **65**: 498-506
4. Virus E.D., Sobolevsky T.G., Rodchenkov G.M. Wrong-way-round ionization and screening for doping substances in human urine by high performance liquid chromatography/orbitrap mass spectrometry. *Journal of Mass Spectrometry (manuscript accepted 20 december 2011, see the decision letter)*
5. Virus E.D., E. N. Semenistaya, Sobolevsky T.G., Rodchenkov G.M. (2009) In: Schänzer W, Geyer H., Gotzmann A., Mareck U. (eds) Recent advances in doping analysis, vol 17. Sportverlag Strauβ, Köln, Germany, pp 201.
6. Virus E.D., Sobolevsky T.G., Rodchenkov G.M. Development and Validation of a General Screening Method for Doping Substances in Human Urine by HPLC/Orbitrap Mass Spectrometry. *Proceedings of the 27th (Montreux) Symposium on Liquid Chromatography/Mass Spectrometry*, Montreux,