PROJECT **REVIEW**

"Development of a gas chromatography/mass spectrometry based method for the detection of xenon in human urine"

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The noble gas xenon has been used as a narcotic agent for more than 50 years and both effects and side-effects have been carefully studies. Besides its anesthetic properties, xenon proved to be beneficial for patients during a surgical intervention and postoperative by stimulating different relevant metabolic pathways. As the stimulations include the so called hypoxia-inducible factor 1α and as a direct consequence EPO, xenon might be of interest for high level athletes, too.

During the Winter Olympic Games first rumors spread regarding the misuse of xenon by elite athletes. As neither xenon nor any gas has ever been in the scope of doping control analysis, the challenge for WADA accredited laboratories will be to develop and validate methods suitable for the detection of xenon from different biological matrices. For blood and serum it has recently been demonstrated that xenon can be analyzed by gas chromatography/high resolution-high accuracy mass spectrometry (GC/TOF-MS) and also, much more complicated, by offline isotope ratio mass spectrometry. The sensitivity of the GC/TOF-MS approach was sufficient to detect xenon in a blood sample drawn 24 h after termination of narcosis in a patient.

Now our goals will be to investigate the suitability of urine samples to detect xenon misuse, to further increase the sensitivity by improving sample preparation and mass spectrometric conditions, to fully validate our approach to make it suitable for doping control analysis and to search for and implement a possible internal standard to enhance reproducibility of the results. This will also encompass studies on the stability of the highly volatile xenon in urine specimens in order to elucidate if and how the analysis of this special analyte can be implemented into existing routine doping control procedures.

Results and conclusions

On September 1st 2014, a modified Prohibited List as established by the World Anti-Doping Agency (WADA) has become effective featuring xenon as a banned substance categorized as hypoxia-inducible factor (HIF) activator. Consequently, the analysis of xenon from commonly provided doping control specimens such as blood and urine is desirable, and first data on the determination of xenon from urine in the context of human sports drug testing are presented.

In accordance to earlier studies utilizing plasma as doping control matrix, urine was enriched to saturation with xenon, sequentially diluted, and the target analyte was detected as supported by the internal standard d_6-cyclohexanone by means of gas chromatography/triple quadrupole mass spectrometry (GC/MS/MS) using headspace injection. Three major xenon isotopes at *m/z* 128.9, 130.9 and 131.9 were targeted in (pseudo) selected reaction monitoring mode enabling the unambiguous identification of the prohibited substance. Assay characteristics including limit of detection (LOD), intraday / interday precision, and specificity as well as analyte recovery under different storage conditions were determined. Proof-of-concept data were generated by applying the established method to urine samples collected from 5 patients before, during and after (up to 48 h) xenon-based general anesthesia.

Xenon was traceable in enriched human urine samples down to the detection limit of approximately 0.5 nmol/mL. The method's intraday and interday imprecision values were found below 25%, and specificity was demonstrated by analyzing 20 different blank urine samples that corroborated the fitness-for-purpose of the analytical approach to unequivocally detect xenon at non-physiological concentrations in human urine. The patients' urine specimens returned 'xenon-positive' test results up to 40 h post-anesthesia, indicating the limits of the expected doping control detection window. This time window was comparable to the one obtained in blood specimens.

Since xenon has been considered a prohibited substance according to WADA regulations in September 2014, its analysis from common specimens of routine sports drug testing is desirable. In previous studies, its traceability in whole blood and plasma was shown, and herein a complementary approach utilizing doping control urine samples for the GC/MS/MS analysis of xenon is reported.