New method is a thousand times more sensitive to performance-enhancing drugs

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While the world's best athletes competed during last month's winter Olympics, doctors and scientists were waging a different battle behind the scenes to make sure no one had an unfair advantage from banned performance-enhancing drugs (PEDs). Now, for the first time, researchers have unveiled a new weapon—a test for doping compounds that is a thousand times more sensitive than those used today.

The team described the approach in one of more than 10,000 presentations at the 247th National Meeting & Exposition of the American Chemical Society (ACS).

"How much of a drug someone took or how long ago they took it are beyond the analyst's control. The only thing you can control is how sensitive your method is," said Daniel Armstrong, Ph.D., who led the team. "Our goal is to develop ultra-sensitive methods that will extend the window of detection, and we have maybe the most sensitive method in the world."

Hongyue Guo, a graduate student in Armstrong's lab at the University of Texas at Arlington, explained that the new strategy is a simple variation on a common testing technique called mass spectrometry (MS). The International Olympic Committee, the U.S. Anti-Doping Agency and others routinely use MS to ensure athletes are "clean." MS separates compounds by mass, or weight, allowing scientists to determine the component parts of a mixture. In the case of PEDs, technicians use the method to find the bits left over in blood, urine or other body fluids after

the body breaks the dopants down.

Because some of the pieces, or metabolites, are small and have a negative charge, they may not produce a signal strong enough for the instrument to detect, Armstrong explained —- especially in the case of stimulants, which the body rapidly breaks down. Stimulants like amphetamine, or "speed," increase alertness and reduce an athlete's sense of fatigue.

The method Armstrong's lab has pioneered, called paired ion electrospray ionization (PIESI, pronounced "PIE-zee"), gathers several of those drug bits together, making them more obvious to the detector.

So far, Guo has used PIESI to detect different kinds of steroids and stimulants, as well as alcohol. That last one might not seem a likely choice for athletes looking to get an edge, but Armstrong explained that in shooting sports, alcohol and other depressants can help steady small muscle movements. Guo has demonstrated that the technique is often sensitive enough to detect one part per billion of the PED metabolite in urine, which he said is up to 1,000 times better than existing methods.

Testing laboratories wouldn't need to purchase new equipment to get PIESI's advantages, according to Guo. The new method only requires adding one ingredient to existing MS procedures, and Guo noted that the chemical is already commercially available and inexpensive. Today is the first time anyone has reported using PIESI on banned dopants, but Armstrong said now that the information is out there, he expects anti-doping agencies to quickly get on board.

"PIESI is going to be useful in so many different areas," Armstrong said. Already, it's being used to detect compounds crucial to human health such as phospholipids and pesticides and herbicides, as well as other environmental pollutants. In some cases, he noted, PIESI is 100,000

times more sensitive than other detection methods.

More information: Use of high performance liquid chromatography with paired ion electrospray ionization (PIESI) tandem mass spectrometry for sensitive detection of performance enhancing drugs metabolites conjugated with glucuronide and sulfate in urine:

Abstract
PEISI-MS/MS has been developed as a highly sensitive method for detecting anionic analytes. Herein, PEISI was applied to determine PEDs by detecting their glucuronide and sulfate metabolites as biomarkers. The LOD of 11 PEDs metabolites were determined with four ion pairing reagents (IPRs) both in positive SIM and SRM mode as well as negative mode. Typically, most of the analytes were detected with LODs below 40 pg level using the optimal IPR in positive mode. Chromatographic separation of these 11 compounds using HPLC-PIESI-MS/MS was developed with gradient elution in SIM mode. To further evaluate the matrix effect and lower the LOD, solid phase extraction (SPE) was used for urine spiked with 10 ng/mL, 50 ng/mL and 400ng/mL PEDs metabolite standards, which showed an average recovery ranging from 89% to 107%. The developed method is 1 to 3 orders of magnitude more than negative mode ESI-MS approaches.

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