NSF DIETARY SUPPLEMENTS

CUTTING EDGE TECHNOLOGY IS KEY TO SUPPLEMENT SCREENING

Dr. Kerri LeVanseler

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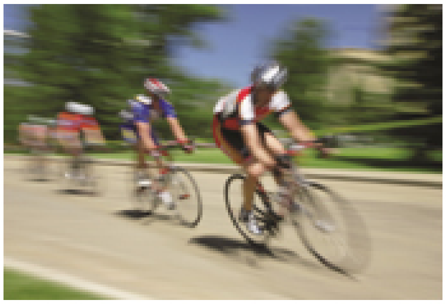

Doping in sports has secured several headlines in the last few years with major publications in the US such as *Sports Illustrated*, and *The Wall Street Journal* questioning the integrity of nutritional supplements and other sports nutrition products and their potential for causing harm to users. International athletes must be ever vigilant when consuming over-the-counter products to avoid consumption of compounds which are prohibited by professional sports organizations. More recently, there have been concerns about excessive amounts of stimulants in consumer products including both dietary supplements and food products. Warnings have spread to school age athletes. Parents and coaches are being warned about the dangers that lurk in the sports nutrition products that their young athletes consume in an attempt to improve their athletic performance.

The United States Food and Drug Administration (FDA) has issued warnings about more than 70 weight-loss supplements that include potentially dangerous ingredients. Steroids, steroid-like ingredients, stimulants and prescription drugs found in contaminated products have been reported to cause serious side effects including liver damage, stroke, and kidney failure.

With so much at stake, it is clear why the nutraceutical industry has responded with a renewed fervor for demonstrating the quality and safety of nutritional supplements. Third-party testing and certification of nutritional supplements and sports nutrition products has remained the champion for manufacturers that seek a reliable method for verifying the integrity and safety of their products. But as new designer drugs and steroid-mimicking compounds evolve, so must the technology used to test for these substances. Much of the success of screening for athletic banned substances lies on staying one step ahead of the culprits.

This means that pioneering laboratories, such as NSF International's nutritional supplement testing laboratory in Ann Arbor, Michigan, are faced with the challenge of relentlessly developing new testing methodologies and utilizing the most cutting-edge equipment around. In this article, we discuss the latest technology used to screen supplements and sports nutrition products for even the most complex of banned substances.

THE ISSUE

The World Anti Doping Agency's (WADA) list of prohibited substances is not short. It covers a veritable cornucopia of stimulants, narcotics, diuretics, beta-2 agonists, and of course, steroids. But even with so many of these performance boosting compounds identified for testing, there are more being developed. There have been several cases of dietary supplements that are laced with untested or undetectable banned substances. Clever chemistry can be deceiving by: 1) creating new compounds that act the same as banned substances, hence mimicking the same performance boosting effect, and 2) masking banned substances by making them look like the other compounds in the product or influencing the expected natural ratios between chemicals in the body.

THE METHOD

So how do testing and certification organizations like NSF International test for these banned substances? Certified nutritional supplement products are put through several complex and very rigorous testing procedures before they make it to store shelves. But the main method for identifying and measuring potential banned substances in a supplement is by using liquid chromatography and gas chromatography coupled with mass spectrometry detection (LCMS and GCMS).

THE TECHNOLOGY

Using mass spectrometry (MS) to analyze for substances in a supplement allows scientists to not only identify the compounds used, but also to verify their quantity. The chromatography system allows for the separation of the various components present in the sample. These components are passed to the mass spectrometry detector where the compounds are ionized and fragmented. Once formed, these ions are accelerated or deflected by electric and magnetic fields. Differences in the mass-to-charge ratio of the ions produced from a compound generate a total ion pattern that can be compared to reference compounds to confirm identity. In order to improve the sensitivity of the MS detector, specific masses are monitored that are the most abundant for the compounds of interest, allowing for the detection of potential contaminants amongst the multitude of intentional ingredients in the product formulations. Using triple quadrupole or tandem mass spectrometers (LCMSMS or GCMSMS systems) allow for further selectivity by monitoring specific precursor ions as they transition to product ions. A single compound may have several transitions and how unique the transitions are to that chemical will allow for the measurement of that compound in the presence of many other components. The frequency or the intensity of the signal measured for the transition is proportional to the concentration present in the sample. Therefore, a reference standard can be prepared at different concentrations to create a calibration curve and therefore the quantitative amount of a substance in a sample can be determined in comparison to the curve. But what if the analyst is looking for an unknown chemical?

Meet the LTQ Orbitrap. This type of liquid chromatography-mass spectrometry system offers an edge over the triple quadupole LCMSMS systems for the detection of unknown chemicals due to its ability to measure the exact mass for the fragment ions. Typical LCMS systems have whole unit mass resolution. Which means it can measure the mass-to-charge of the ion to within +/- 1 dalton(Da). The Orbitrap can discriminate between very small mass differences, out to 0.004 dalton(Da) with less than a two parts-per-million (ppm) mass error or ±0.0008 Da. This is crucial because the difference between the mass of an acceptable compound and a banned substance sometimes comes down to a hundredth of the molecule's mass.

The ability to identify modifications or structural changes of molecules is increasingly important as new designer drugs, steroids and steroid-like substances emerge and reference chemicals may not always be available. Knowing the exact mass of the ions is key to determining which elements may be present in that ion and in what combination. In this way, the Orbitrap data can be used to elucidate chemical structure information for unknown chemical adulterants. The Orbitrap instrument is most effective when the unknown chemicals are present in the samples at least at low part per million levels. This allows for adequate intensities of the ion peaks which results in the highest level of accuracy in the exact mass determinations and further confidence in the conclusions drawn from this data. Since banned compounds may be present in very minor concentrations, down to part per billion levels, the instrument system used needs to be well suited for the analysis goals. By combining Orbitrap technology for unknown determinations with triple-quadrupole (MSMS) systems for trace level detection of specific known banned substances, the best of both worlds in achieved in ensuring that the products evaluated are free of these unwanted contaminants.

The use of new technologies and laboratory methodologies to test supplements ushers in a new era of contamination and banned substance detection. Instruments like the LTQ Orbitrap mass spectrometer allows organizations like NSF International to not only to identify and measure compounds and banned substances in nutritional supplements; they can also use this technology to identify new banned compounds as they emerge and can help bring awareness of them to the arena of testing and enforcement.

ABOUT THE AUTHOR

Dr. Kerri LeVanseler is the Technical Manager of NSF International’s Chemistry Laboratory and has over 25 years of experience. She is an expert in a wide range of laboratory techniques and analysis of analytes (e.g. vitamins, heavy metals, pesticides, natural constituents, active ingredients and extractants) in various sample types (dietary supplement ingredients and products, food and food contact materials, sanitizers, pharmaceuticals, detergents and medical devices). She also serves on the Joint Committee for the American National Standard for Dietary Supplements (NSF/ANSI 173). Dr. LeVanseler earned a Ph.D. in Analytical Chemistry from Wayne State University, and a Bachelor of Science in Chemistry from the University of Michigan.