DOWNLOAD PDF MODERN METHODS OF STEROID ANALYSIS

Chapter 1 : Sports Drug Testing Laboratories - calendrierdelascience.com

Modern Methods of Steroid Analysis reviews modern methods of steroid analysis such as liquid column chromatography, mass spectrometry, and gas chromatography. Topics covered include qualitative and quantitative analysis of plant sterols by gas-liquid chromatography; Raman spectroscopy of steroids; nuclear magnetic resonance; and applications of.

Beta Blockers some sports, also prohibited out-of-competition in archery and shooting WADA and the Prohibited List WADA is independent of governments and sports federations, but is equally represented by all stakeholders. WADA signatories such as national anti-doping organizations and international federations are responsible for developing a sports drug testing program in accordance with the Code and must have drug testing performed by WADA-accredited laboratories. At the time of this writing there were 32 accredited laboratories worldwide, with two in the United States 4. A violation of any of the WADA anti-doping rules is considered a doping offense. These include attempting to use a prohibited substance or prohibited method, refusing to submit a sample, failing to provide whereabouts information location and availability for sample collection , failing to provide a sample, tampering or attempting to tamper with the doping control process, and possessing, trafficking, or attempting to traffic prohibited substances or prohibited methods. Although testing laboratories exist primarily to detect prohibited substances in athlete samples, they occasionally are asked to identify the contents in vials, pills, and powders confiscated from athletes suspected of possessing prohibited substances. The WADA prohibited list of substances is updated annually and new substances are typically added to the list every year 5. Prohibited substances are typically grouped based on the pharmacological effect on the body Table 1. Each class contains numerous substances; there are currently 46 exogenous steroids and 64 stimulants on the list. In the case of exogenous steroids, substances with a similar chemical structure, such as dimethazineâ€”2 molecules of methasterone linked by an azine groupâ€”are also prohibited. Some classes of compounds are prohibited at all times whereas others are prohibited only when the athlete is in competition Table 1. Alcohol and beta blockers are prohibited in certain sports during competition, but a few sports prohibit beta blocker use all the time. Prohibited methods are banned at all times and include manipulation of blood transfusions, artificially enhancing oxygen delivery and gene doping. The cap and sides of the plastic urine bottles are covered with tamper-evident tape after the lid is sealed. The red ring below the cap on the neck of the glass bottles collection kit on the right is removed and the cap is twisted onto the bottle after filling. The white teeth prevent the bottle from being opened without breaking the plastic sleeve surrounding the cap. Collection and Processing of Samples Collectors witness urine collection to help prevent samples from being adulterated or substituted with fake or drug-free urine. Urine collection kits contain either plastic or glass bottles Figure 1. Tamper-evident tape is applied to plastic bottles to spot tampering. Glass bottles, permanently sealed after filling, can only be opened by crushing the plastic sleeve surrounding the cap with a specially designed opening device. Both bottle types are placed into a box provided with the kit and are shipped to WADA-accredited laboratories, usually at ambient temperature. Chain of custody documentation begins when urine samples arrive at the laboratory. Between 5 and 7 aliquots of urine are routinely prepared for testing, with each aliquot containing from 1 to 20 mL of urine. Sample Pretreatment The amount of sample pretreatment needed varies depending on the screening method. Some urine testing methods such as hormone immunoassays for human chorionic gonadotropin and luteinizing hormone do not require pretreatment prior to testing. Sample pretreatment removes unwanted interfering substances and isolates specific compounds of interest. For instance, anabolic steroids first require an enzymatic hydrolysis step to deconjugate glucuronide moieties from the steroid molecules, followed by solid phase extraction to recover the relatively nonpolar steroids. We then add trimethylsilyl groups to steroid functional groups to improve chromatographic and mass spectral properties when these samples are analyzed by gas chromatography mass spectrometry GC-MS. The complete steroid pretreatment procedure takes approximately 6 hours. GC-MS is routinely used to detect anabolic steroids and stimulants. Exceptions include isoelectric focusing and polyacrylamide gel electrophoresis, which we use to detect recombinant

erythropoietins and analogues such as peginesatide, and isotope ratio MS, which we perform to detect exogenous testosterone and testosterone precursors. Data Analysis GC-MS detects target compounds by comparing the retention time and relative intensities of ion fragments in unknown samples to those obtained for reference compounds. Interfering peaks and background noise can complicate GC-MS data reading because screening methods are designed to detect entire classes of compounds and are not optimized for individual compounds. Furthermore, a single ion fragment may not be unique and might be shared by compounds. These issues limit the use of automated software programs for GC-MS data reading and require that experienced data readers carefully evaluate all data. The x-axis is retention time and the y-axis is ion abundance. The vertical line in each window represents the software-predicted retention time based on reference standards analyzed in the same batch of unknown urine samples. Note that the urine sample screens positive for amphetamine. GC-MS screening data for six stimulants are shown in Figure 2. The retention times of reference compounds are indicated in each window by a vertical line and the relative abundance of each ion is displayed on the y-axis. Although some of the windows have considerable background signals, the urine sample appears negative for all of the stimulants except amphetamine. Figure 3 GC-MS screening data for selected stimulants GC-MS confirmation data for the trifluoroacetyl derivative of amphetamine in a negative urine A , a positive urine control B and the urine that screened positive in figure 2 C. This data confirms the presence of amphetamine. Ion chromatograms and mass spectra of the trifluoroacetyl derivative of amphetamine are shown in Figure 3. The negative urine panel A does not contain ions at the retention time of the positive urine panel B. The confirmation data would be reviewed by two certifying scientists before being reported as an adverse analytical finding for amphetamine and must also fulfill all identification criteria in the WADA technical document 7. Other documents include a list of staff involved in the testing process, a description of the testing procedure s , negative and positive control data, instrument performance data chromatographic performance, tune reports, etc. These documents must be provided for both the initial screen and the confirmation testing. The presence or use of a prohibited substance s is considered an anti-doping rule violation. The burden of proof under the Code is to the comfortable satisfaction of the hearing panel. This is more than the mere balance of probability but less than proof beyond a reasonable doubt. The length of the sanction depends on the sporting organization; WADA signatories must follow Code guidelines which mandate a maximum of 2 years for the first violation. However, non-signatories like professional sports can enforce shorter periods of ineligibility. Athletes have the right to appeal a sanction, and as part of the appeals process accredited laboratories reporting the adverse analytical finding may be called upon to testify. Depending on the case, the laboratory may be required to provide a wide variety of documents during the discovery process. Expert testimony usually involves explaining and defending data in the documentation package, providing information as to what the drug is and why it is performance-enhancing, describing side effects, as well as when and how much the athlete took. Some of these questions can be difficult if not impossible to answer since pharmacokinetic data for many of the drugs is unknown. Another very difficult question often asked is if the drug would enhance performance or recovery given the amount detected in the urine and whether the substance was unknowingly via a contaminated supplement or intentionally ingested. External Quality Assessment Program In order to maintain WADA accreditation, each laboratory must comply with the International Standard for Laboratories document 3 and numerous technical documents that address specific issues related to the testing process. Technical documents must be integrated into the policies and procedures of each accredited laboratory and range from compound identification criteria and decision limits for threshold compounds to chain of custody documentation and preparation of documentation packages. Laboratories must also participate in the WADA external quality assessment scheme EQAS and correctly identify prohibited substances in at least 20 unknown samples received throughout the year. EQAS samples sometimes contain substances that normally are not identified in routine doping samples. In addition, EQAS samples may contain a low concentration of a prohibited substance as well as more than one prohibited substance. A typical example would be a low concentration of an anabolic steroid together with a diuretic. This quality assessment program is clearly designed to promote high quality laboratory testing procedures and raises the bar to a very high standard for WADA-accredited laboratories. The Now and Then So how big a

problem is doping in sports? Of the , samples tested in by accredited laboratories, only 1. This low percentage of positivity suggests that either doping is not a significant problem or that doping is really a problem but the cheaters are not getting caught. If one believes the media and recent doping scandals, performance-enhancing drugs are epidemic in sports like cycling and professional baseball. It is more likely that the true story lies somewhere in between: Although testing and the associated sanctions for doping violations help deter athletes from using prohibited substances, additional steps need to be taken to win the battle against doping in sports. One approach is to expand unannounced drug testing programs, develop better testing strategies including longitudinal profiling , and improve current testing methods to catch more, if not all, athletes that dope. Another approach is to provide better athlete education at an earlier age to deliver the message more effectively that doping is unethical and should not be considered or tolerated at any level of sports competition. Lastly, a change in public attitude might be needed. Perhaps a message needs to be delivered to athletes that fans will not continue supporting sports in which doping is perceived to be a major concern. Anti-doping laboratories face an enormous task in developing and validating testing methods to detect emerging compounds that become available to the sports community. The next major challenge facing laboratories will be to develop analytical methods that detect targeted gene and cell doping. Laboratorians not involved in anti-doping should at least be aware of testing methods that are currently used for detecting prohibited substances and understand new testing modalities as they become available in the fight against doping in sports.

Chapter 2 : Erich Heftmann (Author of Modern Methods of Steroid Analysis)

Modern Methods of Steroid Analysis reviews modern methods of steroid analysis such as liquid column chromatography, mass spectrometry, and gas chromatography.

Chapter 3 : Books by Erich Heftmann (Author of Modern Methods of Steroid Analysis)

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