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Mukesh Kumari
V.P.O, Birhi Kalan Bhiwani
Charkhi Dadri District
Bhiwani Haryana, India

Correspondence
Mukesh Kumari
V.P.O, Birhi Kalan Bhiwani
Charkhi Dadri District
Bhiwani Haryana, India

A study on chromatography-mass spectrometry in doping analysis

Mukesh Kumari

Abstract
Although attempts to enhance athletic performance are probably much older, the word "doping" was first mentioned in 1889 in an English dictionary. It described originally a mixed remedy containing opium, which was used to "dope" horses. In the current scenario, the misuse of drugs in human sports in an attempt to enhance performance is known as doping. Throughout history athletes have sought foods and potions to enhance physical performance. The current article discusses the chromatography-mass spectrometry in doping analysis.

Keywords: Doping, chromatography, athlete

Introduction
In the early era of modern sport, doping was mostly associated with professional cycling. Although some cyclists died from the intake of strong stimulants in the late nineteenth and early part of the twentieth century, sports authorities remained passive. It was not until a Danish cyclist died in 1960 during a road race at the Olympic Games in Rome that action was taken.
The Union Cycliste Internationale (UCI) began to develop a set of rules and in 1967, the International Olympic Committee (IOC) created a „Medical Commission" (IOC-MC) to combat the misuse of drugs in Olympic sports. Despite this long and storied history of performance enhancing drugs in sports, doping is arguably the most controversial and most talked-about issue in modern sports.
According IOC doping is the administration of or use by a competing athlete of any substance foreign to the body or any physiological substance taken in abnormal quantity or taken by an abnormal route of entry into the body with the sole purpose of increasing in an artificial and unfair manner his/her performance in competition.
The first competitive athletes charged for doping were swimmers in Amsterdam in the1860s. In the late 19th century European cyclists were using substances like caffeine and ether-coated sugar cubes to reduce pain and delay fatigue. Shortly after the Second World War, amphetamine type stimulants became very popular resulting in several lethal cases.
One of the most well-known doping victims in that period was Tom Simpson who died in 1967 on the Mont Ventoux from a combination of exhaustion, alcohol and amphetamines. The year after his death athletes were tested for the first time at the Olympic Games in Mexico City, 1968. Advances in organic chemistry in the 1950s and 1960s yielded a wide variety of pharmacologically active compounds including diuretics, beta-blockers, corticosteroids and anabolic steroids. These compounds were intensively used by athletes during the 1970s. Another way to avoid getting caught is the use of "designer" steroids.
Which new doping substances the future will bring is difficult to predict. The abuse of new doping substances or methods only reaches doping authorities by rumors or anonymous tips. Nevertheless, worldwide anti-doping efforts are being better organized, harmonized, and structured than ever. This is true not only of the rules, prohibited substances and methods, sanctions, and appeals, but also of laboratory accreditation and reporting criteria.
Testing urine is better than testing blood for most prohibited substances (small molecules, molecular weight less than 800 atomic mass units). Urine collection is noninvasive and yields a large volume of sample, with higher drug concentrations than in blood and with far fewer cells and proteins to complicate extraction. However, few of the analytes are tested in

blood viz. continuous erythropoietin receptor activator (CERA), hemoglobin based oxygen carriers (HBOCs), human growth hormone (hGH) or blood transfusions.

Chromatography-Mass Spectrometry in Doping Analysis

Since 1972 Olympics, various new mass spectrometric techniques viz. High Resolution Mass Spectrometry (Atlanta Olympics Games 1996), Isotope Ratio Mass Spectrometry (Special Olympic Winter Games, 1998) and Liquid Chromatography Mass Spectrometry (Athens Olympic Games, 2004), Time of Flight (TOF) Mass Spectrometry (2008) and Ultra Performance Liquid Chromatography coupled to High Resolution Mass Spectrometry (UPLC-HRMS) (London Olympics, 2012) have facilitated dope testing.

Each lab has their own set of testing protocols utilizing various equipment viz. GC, GC/NPD/ECD, GC/MS, HRMS, LC-MS/MS and IRMS. In combination with Gas/Liquid Chromatography and various ionization methods such as Chemical Ionization, Electro Spray Ionization, Atmospheric Chemical Ionization, Atmospheric Pressure Photo Ionization, MALDI etc numerous applications have been established enabling sensitive and selective detection of drugs in biological matrices.

GC-NPD and GC-MSD are currently standard technique for the analysis of stimulants and narcotics because of their robustness, sensitivity, selectivity for non-polar and volatile substances and high level of standardization. However, thermally labile and highly polar drugs are not suitable for GC-MS analysis due to their non-volatility and high temperature under GC. With the introduction of liquid chromatography coupled with tandem mass spectrometry (LC/MS) in doping analysis has overcome this problem. This is particularly significant for detection of metabolic products of various drugs which are excreted as polar entities in urine.

Three technologies form the core of most major drug testing laboratories are: gas chromatography (GC), liquid chromatography (LC) and mass spectrometry (MS). The purpose of present work was aimed at developing and improving analytical methods for testing of stimulants and narcotics at National Dope Testing Laboratory (NDTL), India. Different strategies were followed for developing the chemical extraction (sample pre concentration) and chromatographic-mass spectrometric methods.

The ultimate goal was to improve the existing analytical procedures and to establish high throughput, comprehensive and cost effective analytical methods for screening and confirmatory analysis. It was achieved by comparing existing extraction procedures and deconjugation process for applicability to stimulants and narcotics molecules and their metabolic products in urine; and by utilizing state of art modern dual detector capabilities of GC-NPD-MSD (instead of conventional GC-NPD and GC-MSD) and highly sensitive LC-MS/MS instruments for fast and sensitive analysis.

The outcome of the study proved useful for the dope testing of I Singapore Youth Olympic Games-2010 and XIX Commonwealth Games (CWG) held in New Delhi, India in 2010. For Singapore Youth Olympic Games, samples were received from Singapore Youth Olympic Games Organizing Committee (SYOGC) and were tested in NDTL, India. For CWG samples received from Common Wealth Games Organizing Committee (CWGOC) were tested at NDTL, India and reports were submitted in a turn-around-time (TAT) of 24 hours.

Improvement of existing methods in terms of sample preparation as well as instrumental sensitivity had been an essential requisite in view of WADA TDMRPL 2013 which requires detection of stimulants & narcotics at much lower levels. Therefore, testing protocols were further improved to make high throughput and sensitive drug testing. The decrease in total run time and limit of detection (LOD) further improved work flow in the laboratory. The excretion studies of few stimulants and narcotics were conducted to explore the metabolic profile of the drug and its metabolites for developing fool-proof confirmation methods.

Research Study

Global fight against doping in sports is supervised by World Anti-Doping Agency (WADA), which maintains the World Anti-Doping Code including the prohibited list defining the substances and methods prohibited in sports. WADA has issued stringent guidelines to implement anti-doping programs, which includes world Anti-doping code, International Standards for Laboratories (ISL) and doping education programs.

The WADA prohibited list is updated annually, imposing a considerable demand on laboratories to update their methods regularly. WADA code and ISL are revised periodically according to the requirement to maintain pace with new advancement in doping and to ensure adherence to extreme technical competence on part of doping control laboratories.

The testing of prohibited substances requires both qualitative and quantitative analysis. Though most of the banned substances require qualitative identification, for some specific compounds it is difficult to distinguish between inadvertent/therapeutic use and misuse, hence threshold concentrations have been established which require quantitative estimation of the drug.

In other cases, threshold is considered to differentiate between endogenous physiological values and exogenous administration of the drug. The threshold levels have been introduced based upon the scientific evidences of concentrations recovered in urine, pharmacokinetic profile and other pharmacological dose dependent studies.

The doping analysis is generally divided into screening and confirmatory procedures. The screening is supposed to be more comprehensive method to isolate samples suspicious for a prohibited drug. In confirmation, samples are analyzed with methods that provide unequivocal identification of the substances. The confirmation procedures for small molecules are more specific to the analytes chemistry and are based on chromatographic- mass spectrometric analysis.

The analytical procedures have to be constantly improved and updated in order to keep pace with trends in substance abuse and to fulfill technical and quality requirements. The task of anti-doping laboratories is to provide scientific evidence of the possible presence of prohibited substances, sample manipulation, or use of a prohibited method. The analytical work in doping control laboratories differs in many ways from that of other laboratories.

More than 300 drugs of abuse (parent and metabolites) are measured from complex biological matrix; concentrations of analytes vary largely sample to sample; methods and test results have to be completely valid and reliable. A false

positive result would be detrimental both for anti-doping laboratory and for the athlete's career.

The drug testing in sports was initiated in 1968 with approximately 30-40 drugs falling in IOC banned list. However, during 1972 Munich Olympic Games, for the first time chromatography-mass spectrometry was used for doping control purposes at a grand sport event. Since then, sophisticated methods based on currently prevailing techniques have been developed to cope up with the increasing number of drugs available in International markets. However, stimulants, anabolic steroids, beta blockers and narcotics were being tested on Gas Chromatography-Nitrogen Phosphorous Detector (GCNPD) and Gas Chromatography-Mass Spectrometric Detector (GCMSD).

In 2004, Olympic Summer Games in Athens, Liquid Chromatography interfaced to tandem mass spectrometry (LC-MS/MS) was utilized to identify various classes of drugs including anabolic agents, corticosteroids, narcotics -2 agonists. The technique of dope testing has improved immensely since 1972 till date by using improved extraction methods and sophisticated equipment.

Significance of the Study

Designer steroids are chemically modified anabolic steroids which were developed in the 1960s and1970s. The most public attention however was received by the substance tetra hydrogestrinone (THG), which was detected in connection with the BALCO case in 2003 and identified by the WADA-accredited laboratory in Los Angeles.

By the end of the 1970s and especially during the 1980s, biotechnology successfully advanced. Polymerase chain reactions (PCR) and genetical modification allowed for the in-vitro production of huge amounts of protein based medicines including growth hormone (GH), insulin and derivatives, adrenocorticotropin hormone (ACTH) and erythropoietin (EPO). These protein based products were helpful in the treatment of many diseases; unfortunately they found their way into doping as well.

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