Journal of Pharmaceutical and Health Sciences 2018;6(1), 39-45.

Short Review

JPHS

Sports supplements and Anabolic Androgenic Steroids

Sanaz Pashapour[1], Zahra Mousavi[1*] , Shahram Shoeibi[2]

[1]*Department of Pharmacology & Toxicology, Faculty of Pharmacy, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran - Iran (IAUPS)*

[2]*Food and Drug Reference Control Laboratories Center, Food and Drug Organization, MOH & ME, Tehran, Iran*

Abstract

Recently, the use of sport supplements has become so common among most athletes. These supplements may contain undeclared substances that are banned by such international authorities as WHO, IOC and WADA. The present study, thus, is aimed at examining the final sport supplement products in terms of banned substances and measuring the adulteration level in order to prove the safety of these products and promote public health. The Food and Drug Administration of the Ministry of Health and Medical Services of Iran confirms the safety of these products, monitors their production and import, and also marketing them at authorized places like the pharmacy.

According to the existing regulations in Iran and other countries, the safety of sport supplements and the level of their androgenic-anabolic steroids are monitored and measured by such methods as GC/MS, LC/MS/MS. Beside the well-known side effects of anabolic steroids, new problems have emerged from using sport supplements distributed in the black market which may contain banned substances not declared on the label. These effects will cause harmful and dangerous changes in levels steroids and heart's structure. Conditions pertaining to hormonal imbalances may also be caused by anabolic steroids. Due to the increasing use of sport supplements in order to achieve an effective training, the presence of adulterated, illegal and unsafe products continue to be a concern for many consumers and professional athletes alike. Since using sport supplements have become perilous, a credibility gap seems to exist between the athletes and medical communities who, it is believed can be overcome by efficient supervision and monitoring of the products.

Keywords: sport supplements, Food Safety Management System (FSMS), anabolic steroid, FDO, contaminations

***Corresponding author**: Zahra Mousavi, Department of Pharmacology & Toxicology, Faculty of Pharmacy, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran - Iran.
Tel: +982122640051-5
E-mail address: mosavi50@yahoo.com

Introduction

There are obviously a great number of sport supplements available, particularly on the internet. Most adolescents are quite unaware of the unknown health risks associated with particular supplements. (Bahrke & Yesalis, 2004; Jenkinson & Allison J, 2008) Performance-Enhancing Substances (PESs) have turned into a common and important issue in sports. Sometimes referred to as 'doping', PESS implies the consumption or manipulation of synthetic substances with the purpose of changing sports performance. (Botrè & Pavan, 2009; Eduardo, 2008) Nowadays, the use of sport supplements is much common among most sportsmen. (Dodge, 2015) Sport supplements may contain undeclared substances forbidden by such international authorities as WHO, IOC and WADA. This issue at stake certainly demands the attention of large number of interested parties, including athletes and other sport supplement consumers, national anti-doping organizations and the world anti-doping agency. The development of sport supplements industry has recently been phenomenal in the United States. Sale records indicate that a great deal of money is annually spent on sport supplements. (Baylis, Cameron-Smith & Burke, 2001; Kayser, Mauron & Miah, 2007; Lippi, Banfi, Franchini & Guidi, 2008; Mottram, 1999) Since the 1960s, the highest consumption rate of anabolic steroids has been related to sportsmen in track and field events. (Geyer, Schänzer & Thevis, 2014; Momaya, Fawal, & Estes, 2015). Nevertheless, there have recently been considerable monitoring processes on the sport supplements industry. It is acknowledged that there are a number of synthetic supplements that are deliberately combined with substances forbidden in sport (according to the Prohibited List provided by the World Anti-Doping Agency). Investigations have also shown that a high proportion of supplements may inadvertently contain forbidden substances. Recent studies have confirmed that athletes are increasingly consuming immeasurable androgenic-anabolic steroids in an attempt to gain competitive excellence. (Foster & Housner, 2004; Noppe, B., Verheyden, & De Brabander, 2008) For the same reason, many studies have been conducted on the posing health risk over sports societies. (Evans, 2004; Fuller, Junge, & Dvorak, 2012; Perret, 2017) While the sport supplements are widely consumed, their adverse effect spreads among the high-performing sportsmen population ranging from teenagers to senile people. (Knapik et al., 2016; Petroczi, Taylor, & Naughton, 2011).

Abuse of Androgenic Anabolic Steroids

The first reports of misuse of Androgenic-Anabolic Steroids (AAS) by athletes emerged in the 1950s. The abuse of AAS among athletes in athletic competitions began some years before these substances were banned in sport in 1974. In the early 1940s, scientists showed that muscle tissues grown at the presence of testosterone had a bulked for toleration act. (Eichner & Tygart, 2016; Fitch, 2008; Hambleton & Kanjee, 1995; Rashid & Ormerod, 2007) Of 4500 analytical findings presented by the World Anti-Doping Agency (WADA) via the Anti-Doping Administration and Management System (ADAMS), about 50% are anabolic agents. (Hartgens, Rietjens, Keizer, Kuipers, & Wolffenbuttel, 2004; Sagoe, Molde, Andreassen, Torsheim, & Pallesen, 2014). Concerns about the abuse of AAS and its prevention in athletes, which currently affects the public too, are compared with the efforts to prevent narcotics abuse. While the adverse effects of AAS have remained unknown, its illegal consumption still continues. (van Amsterdam, Opperhuizen, & Hartgens, 2010). Androgenic-anabolic steroids have been consumed by athletes for five decades in order to increase their activity by increasing their strength. A growing number of athletes misuse AAS to obtain a well-shaped body or increase muscular strength. Most of them take AAS for a period of 8–12 weeks several times a year. (Sjöqvist, Garle, & Rane, 2008; Urhausen, Albers, & Kindermann, 2004) In 2009, the analysis of sport supplement products demonstrated that black market products contained various peptide hormones rather than only steroid hormones. (Greydanus & Patel, 2010) The assessment of steroids originating from synthetic precursors versus their analogous natural chemicals has proved to be an important challenge for doping control laboratories validated by the WADA. Despite the laws enacted against the prescription and supply of AAS, the nonmedical consumption of its supra-physiologic doses has remained prevalent for enhancement of athletic performance. (Cawley & George, 2012; Kohler et al., 2010) Reports obtained from several

athletic communities may inform others about hormone consumption in order to help them reach an optimal and healthy anabolic hormone range. (Pieter & Cohen, 2014) The global prevalence of AAS consumption is 3.3%, with a higher rate of 6.4% in men compared to 1.6% in women. (Taylor, Padilla, & Hernández, 2017) Scientific studies play a central role in WADA's anti-doping strategies and its educational plans seem to be fostering a lasting anti-doping culture. (Christou et al., 2017) This belief has given rise to a billion-dollar industry that aggressively offers its products to sport teams as performance-enhancing agents, often without any objective or scientific document to support such pretensions. (Bishop, 2010; Morente-Sánchez & Zabala, 2013).

Toxicity of Androgenic-Anabolic Steroids

Numerous studies have been recently dedicated to a special class of AASs called Endocrine Disrupting Compounds (EDCs). (Collomp et al., 2016; Committee, 2013; Maennig, 2002) They may interfere with human hormonal systems through various mechanisms. Since 1960s, many researchers have studied the effects of these sport supplements on athletes. (Gibson & Saunders, 2014) Consumption of doping agents, particularly AASs, leads to the ignorance of their dermatological effects and thus, gonad diseases. Doping agents' consumption is no longer limited to competitive sports but, as medical studies in USA indicate, has become common in leisure sports, especially among fitness and bodybuilding sports.(Achar, Rostamian, & Narayan, 2010; Courant et al., 2008; Király, Collan, & Alén, 1987) In several studies, EDCs have been linked to the presence of behavioral disorders and infertility problems. (Attalah, Nasr, El-Gammal, & Nour El-Dien, 2016; Bahrke & Yesalis, 2004; Basaria, Wahlstrom, & Dobs, 2001; Kicman, 2008; Saseen & MacLaughlin, 1999) Abuse of AASs has also been linked to a variety of cardiovascular side effects. (Clark & Henderson, 2003; Feldman & Limbird, 2017; Johnston et al., 2016; Socas-Rodríguez, Asensio-Ramos, Hernández-Borges, Herrera-Herrera, & ÁngelRodríguez-Delgado, 2013) Inspection of fitness centers in Germany revealed that millions of hepatotoxic substances, mainly procured in the black market, have been taken by fitness workers. The occurrence of complications linked with nonmedical consumption of AAS, as performance-enhancing substances, cannot be predicted. Psychological effects of exogenous steroids' abuse including increased aggression and depression should be viewed from the safety point of view in a sociological context. (Bonetti et al., 2008; Busch et al., 2017; Hassan, Salem, & Sayed, 2009; Jakimska et al., 2013; Plotan, Elliott, Frizzell, & Connolly, 2014).

Application of Steroid Hormones Measurement Methods

Determining the concentration of exogenous steroid hormones constitutes an important aspect of clinical, epidemiological or other similar investigations.(Socas-Rodríguez et al., 2013) Anti-doping research centers apply targeted highly-sensitive chromatographically techniques next to non-targeted analyses to provide retrospective information mining. (Delanghe, Maenhout, Speeckaert, & De Buyzere, 2014) Radioimmunoassay and ELISA (Enzyme-Linked Immunosorbent Assay) have shown good sensitivity for screening AASs. However, their defect was their limited specificity due to the presence of antibody across reactivity profiles. (Abushareeda et al., 2014; Key et al., 2015). Due to their simplicity, speed and sensitivity, immunoassay tests are widely performed in laboratories. The quality of analytical results in terms of integrity and resolution must be regularly controlled through good validation methods. One of the defects of immunoassay is the need to do various tests for each steroid in a low dynamic range. (Brooks, Jeremiah, Webb, & Wheeler, 1979; Gosetti, Mazzucco, Gennaro, & Marengo, 2013) Many studies have concentrated on the measurement of Anabolic Androgenic Steroids, by means of GC–MS/MS. Overall; GC-MS is useful for unidentified molecules and non-conjugated steroids with low molecular weight (molecular weight < 500 DA). GC-MS has some limitations the most important of which are the derivatization of polar compounds and the lack of direct analysis of conjugated metabolites. Derivatization is essential for separation of hormones and significantly increases the time of analysis (it takes at least 30 min to perform the assay). (Dunn et al., 2011; Hampl & Stárka, 1979) Lately, the capability to analyze isotope distribution at natural abundance with good accuracy and high precision has increased the application of GC/MS. Accordingly, a number of sport supplements' steroid analytical protocols, based on LC-MS, have been analyzed. (Ponzetto et al., 2017) LC-MS/

MS has the capacity to analyze conjugated metabolites directly and the possibility to apply open techniques for analyzing metabolites with a common chemical structure. (Daems, Romnee, Heuskin, Froidmont, & Lognay, 2016; Gomez et al., 2014; Nielen, Hooijerink, Claassen, van Engelen, & van Beek, 2009) Extraction of steroids before using LC-MS/MS technique is mandatory in order to avoid such interference as matrix effects and the risk of signal inhibition which limit the application of this technique in a large number of samples. (Gomez et al., 2014; Nielen et al., 2009; Ponzetto et al., 2017) Therefore, due to its simplicity, no need for derivatization and also shorter application time (about 10 min), LC-MS/MS technique is simpler to use. (Adamusova, Bosakova, Coufal, & Pacakova, 2014; Daems et al., 2016; Kushnir, Rockwood, & Bergquist, 2010; Soldin & Soldin, 2009) The results of other quality controls demonstrate important differences between the results obtained by different laboratories. However, the variability of results obtained by immunoassay was less than those obtained by mass spectrometry. The majority of reference intervals have been so far established by RIA methods in the studies conducted over the last fifty years. It seems essential, thus, to determine new intervals specific to mass spectrometry. (Janssens et al., 2015; Stanczyk & Clarke, 2010; Vogeser & Parhofer, 2007; Zendjabil, Chellouai, & Abbou, 2016)

Conclusion

The consumption of sport supplements to unnaturally increase the athletic performance is called doping which is prohibited by antidoping rules and may pose a health risk to some people. Such strengthener supplements as Androgenic Anabolic Steroids (AASs) can pose significant health risks if consumed by adolescents. It is as well very clear that some of these supplements should be avoided by sportsmen at all costs. AASs are believed to have harmful effects on the health of organisms. The awareness level of people of how sport supplements become contaminated with small traces of forbidden substances has greatly increased over the last decade. Moreover, some reputable companies with appropriate quality control systems to address this problem can offer the athletes minimal-risk products. Doping detecting is more than an analytical problem. Therefore, improving pre-analytical care and a better tailoring of threshold values will increase the tests' sensitivity. In the research broadened by and oriented to UHPLC–MS/MS- GC-MS-LC-MS/MS techniques, this type of techniques are faster, more sensitive and capable of measuring steroid hormones.

References

Bahrke M, Yesalis C. Abuse of anabolic androgenic steroids and related substances in sport and exercise. Current Opinion in Pharmacology 2004; 4(6):614-620.

Jenkinson DM, Allison J H. Supplements and Sports. American family physician 2008; 78(9):1039-1046.

Botrè F, Pavan A. Enhancement drugs and the athlete. Physical Medicine and Rehabilitation Clinics of North America 2009; 20(1):133-148.

Eduardo HR. Doping in Athletes – An Update. Clinics in Sports Medicine 2008; 27(1):107-130.

Dodge T. Consumers' perceptions of the dietary supplement health and education act: implications and recommendations. Drug Testing and Analysis 2015; 8(3-4):407-409.

Lippi G, Banfi G, Franchini M, Guidi GC. New strategies for doping control. Journal of Sports Sciences 2008; 26(5):441-445.

Kayser B, Mauron A, Miah A. Current anti-doping policy: a critical appraisal. Bmc Medical Ethics 2007; 8(2).

Baylis A, Cameron-Smith D, Burke lM. Inadvertent Doping through Supplement Use by Athletes: Assessment and Management of the Risk in Australia. International journal of sport nutrition and exercise metabolism 2001; 11:365-383.

Mottram DR. Banned drugs in sport. Does the International Olympic Committee (IOC) list need updating? Sports Med 1999; 27(1):1-10.

Momaya A, Fawal M, Estes R. Performance-enhancing substances in sports: a review of the literature. Sports Med 2015; 45(4):517-531.

Geyer H, Schänzer W, Thevis M. Anabolic agents: recent strategies for their detection and protection from inadvertent doping. British journal of sports medicine 2014; 48(10):820-826.

Foster ZJ, Housner JA. Anabolic-androgenic steroids and testosterone precursors: ergogenic aids and sport. Current Sports Medicine Reports 2004; 3(4):234-241.

Noppe H, B. LB, Verheyden K, De Brabander HF. Novel analytical methods for the determination of steroid hormones in edible matrices. Analytica Chimica Acta 2008; 611(1):1-16.

Perret C. Elite-adapted wheelchair sports performance: a systematic review. Disability & Rehabilitation 2017; 39(2):164-172.

Evans NA. Current concepts in anabolic-androgenic steroids. The American Journal of Sports Medicine 2004; 32(2):534-542.

Fuller CW, Junge A, Dvorak J. Risk management: FIFA's approach for protecting the health of football players. british journal of sports medicine 2012; 46(1).

Knapik J, Steelman RA, Hoedebecke S, K.G. A, Farina EK, H.R. L. Prevalence of Dietary Supplement Use by Athletes: Systematic Review and Meta-Analysis. Sports Medicine 2016; 46(1):103-123.

Petroczi A, Taylor G, Naughton DP. Mission impossible? Regulatory and enforcement issues to ensure safety of dietary supplements. Food and Chemical Toxicology 2011; 49(2):393-402.

Eichner A, Tygart T. Adulterated dietary supplements threaten the health and sporting career of up-and-coming young athletes. Drug Testing and Analysis 2016; 8(3-4):304-306.

Fitch KD. Androgenic-anabolic steroids and the Olympic Games. Asian Journal of Andrology 2008; 10(3):384-390.

Hambleton RK, Kanjee A. European Journal of Psychological Assessment 1995; 11(3):147.
Rashid H, Ormerod SD. Anabolic androgenic steroids: what the psychiatrist needs to know. Advances in Psychiatric Treatment 2007; 13(3):203-211.

Sagoe D, Molde H, Andreassen CS, Torsheim T, Pallesen S. The global epidemiology of anabolic-androgenic steroid use: a meta-analysis and meta-regression analysis. Annals of Epidemiology 2014; 24(5):383-398.

Hartgens F, Rietjens G, Keizer HA, Kuipers H, Wolffenbuttel BH. Effects of androgenic-anabolic steroids on apolipoproteins and lipoprotein (a). british journal of sports medicine 2004; 38(3):253-259.

van Amsterdam J, Opperhuizen A, Hartgens F. Adverse health effects of anabolic-androgenic steroids. Regulatory Toxicology and Pharmacology 2010; 57(1):117-123.

Sjöqvist F, Garle M, Rane A. Use of doping agents, particularly anabolic steroids, in sports and society. Lancet 2008; 371(9627):1872-1882.

Urhausen A, Albers T, Kindermann W. Are the cardiac effects of anabolic steroid abuse in strength athletes reversible? Heart 2004; 90(5):496-501.

Greydanus DE, Patel DR. Sports doping in the adolescent: the Faustian conundrum of Hors de Combat. Pediatric Clinics of North America 2010; 57(3):729-750.

Kohler M, Thomas A, Geyer H, Petrou M, Schänzer W, Thevis M. Confiscated black market products and nutritional supplements with non-approved ingredients analyzed in the Cologne Doping Control Laboratory 2009. Drug Testing and Analysis 2010; 2(11-12):533-537.

Cawley AT, George AV. Complementary stable carbon isotope ratio and amount of substance measurements in sports anti-doping. Drug Testing and Analysis 2012; 4(12):897-911.

Pieter A, Cohen MD. Hazards of Hindsight — Monitoring the Safety of Nutritional Supplements. the New England Journal of Medicine 2014; 370:1277-1280.

Taylor MK, Padilla GA, Hernández LM. Anabolic hormone profiles in elite military men: Robust associations with age, stress, and fatigue. Steroids 2017; 124:18-22.
Christou MA, Christou PA, Markozannes G, Tsatsoulis A, Mastorakos G, Tigas S. Effects of Anabolic Androgenic Steroids on the Reproductive System of Athletes and Recreational Users: A Systematic Review and Meta-Analysis. sports Medicine 2017:1-15.

Morente-Sánchez J, Zabala M. Doping in Sport: A Review of Elite Athletes' Attitudes, Beliefs, and Knowledge. Sports Medicine 2013; 43(6):395-411.

Bishop D. Dietary supplements and team-sport performance. sports Medicine 2010; 40(12):995-1017.

Maennig W. On the Economics of Doping and Corruption in International Sports. Journal of Sports Economics 2002; 3(1):61-89.

Collomp K, Baillot A, Forget H, Coquerel A, Rieth N, Vibarel-Rebot N. Altered diurnal pattern of steroid hormones in relation to various behaviors, external

factors and pathologies: A review. Physiology & Behavior 2016; 164:68-85.

Committee ES. Scientific Opinion on the hazard assessment of endocrine disruptors: Scientific criteria for identification of endocrine disruptors and appropriateness of existing test methods for assessing effects mediated by these substances on human health and the environment. EFSA Journal 2013; 11(3):3132.

Gibson DA, Saunders PT. Endocrine disruption of oestrogen action and female reproductive tract cancers. Endocrine-Related Cancer 2014; 21(2):13-31.

Courant F, Antignac JP, Laille J, Monteau F, Andre F, Le Bizec B. Exposure assessment of prepubertal children to steroid endocrine disruptors. 2. Determination of steroid hormones in milk, egg, and meat samples. Journal of Agricultural and Food Chemistry 2008; 56(9):3176-3184.

Király CL, Collan Y, Alén M. Effect of testosterone and anabolic steroids on the size of sebaceous glands in power athletes. The American Journal of Dermatopathology 1987; 9(6):515-519.

Achar S, Rostamian A, Narayan SM. Cardiac and metabolic effects of anabolic-androgenic steroid abuse on lipids, blood pressure, left ventricular dimensions, and rhythm. The American Journal of Cardiology 2010; 106(6):893-901.

Basaria S, Wahlstrom JT, Dobs AS. Clinical review 138: Anabolic-androgenic steroid therapy in the treatment of chronic diseases. The Journal of Clinical Endocrinology and Metabolism 2001; 86(11):5108-5117.

Saseen J, MacLaughlin EJ. Appetite stimulants and anabolic steroid therapy for AIDS wasting. The AIDS Read 1999; 9(6):398,401-402,407.

Attalah E, Nasr YS, El-Gammal HA, Nour El-Dien FA. Optimisation and validation of a new analytical method for the determination of four natural and synthetic hormones using LC-ESI-MS/MS. Food Additives & Contaminants Part A, Chimistry,Analysis, Control,Exposure & Risk Assessment 2016; 33(10):1545-1556.

Kicman AT. Pharmacology of anabolic steroids. Br J Pharmacol 2008; 154(3):502-521.

Socas-Rodríguez B, Asensio-Ramos M, Hernández-Borges J, Herrera-Herrera AV, ÁngelRodríguez-Delgado M. Chromatographic analysis of natural and synthetic estrogens in milk and dairy products. TrAC Trends in Analytical Chemistry 2013; 44:58-77.

Johnston DI, Chang A, Viray M, Chatham-Stephens K, He H, Taylor E, et al. Hepatotoxicity associated with the dietary supplement OxyELITE Pro™ - Hawaii, 2013. Drug Testing and Analysis 2016; 8(3-4):319-327.

Clark AS, Henderson LP. Behavioral and physiological responses to anabolic-androgenic steroids. Neuroscience and Biobehavioral Reviews 2003; 27(5):413-436.

Feldman RD, Limbird LE. GPER (GPR30): A Nongenomic Receptor (GPCR) for Steroid Hormones with Implications for Cardiovascular Disease and Cancer. Annual Review of Pharmacology and Toxicology 2017; 57(2017):567-584.

Busch EL, Crous-Bou M, Prescott J, Chen MM, Downing MJ, Rosner BA, et al. Endometrial Cancer Risk Factors, Hormone Receptors, and Mortality Prediction. Cancer Epidemiology Biomarkers& Prevention 2017; 26(5).

Jakimska A, Huerta B, Barga ska , Kot-Wasik A, Rodríguez-Mozaz S, Barceló D. Development of a liquid chromatography-tandem mass spectrometry procedure for determination of endocrine disrupting compounds in fish from Mediterranean rivers. Journal of Chromatography A 2013; 1306:44-58.

Plotan M, Elliott CT, Frizzell C, Connolly L. Estrogenic endocrine disruptors present in sports supplements. A risk assessment for human health. Food Chemistry 2014; 159:157-165.

Hassan NA, Salem MF, Sayed MA. Doping and effects of anabolic androgenic steroids on the heart: histological, ultrastructural, and echocardiographic assessment in strength athletes. Human & Experimental Toxicology 2009; 28(5):273-283.

Bonetti A, Tirelli F, Catapano A, Dazzi D, Dei Cas A, Solito F, et al. Side Effects of Anabolic Androgenic Steroids Abuse. International Journal of Sport Medicine 2008; 29(8):679-687.

Delanghe JR, Maenhout TM, Speeckaert MM, De Buyzere ML. Detecting doping use: more than an analytical problem. Acta Clinica Belgica 2014; 69(1):25-29.

Abushareeda W, Fragkaki A, Vonaparti A, Angelis Y, Tsivou M, Saad K, et al. Advances in the detection of designer steroids in anti-doping. Bioanalysis 2014; 6(6):881-896.

Key TJ, Appleby PN, Reeves GK, Travis RC, Brinton LA, Helzlsouer KJ, et al. Steroid hormone measurements from different types of assays in relation to body mass index and breast cancer risk in postmenopausal women:

Reanalysis of eighteen prospective studies. Steroids 2015; 99(49-55).

Gosetti F, Mazzucco E, Gennaro MC, Marengo E. Ultra high performance liquid chromatography tandem mass spectrometry determination and profiling of prohibited steroids in human biological matrices. A review. Journal of chromatography B, Analytical Technologies in the Biomedical and Life Sciences 2013; 927:22-36.

Brooks RV, Jeremiah G, Webb WA, Wheeler M. Detection of anabolic steroid administration to athletes. Journal of Steroid Biochemistry 1979; 11(1C):913-917.

Dunn WB, Broadhurst D, Begley P, Zelena E, Francis-McIntyre S, Anderson N, et al. Procedures for large-scale metabolic profiling of serum and plasma using gas chromatography and liquid chromatography coupled to mass spectrometry. Nature Protocols 2011; 6(7):1060-1083.

Hampl R, Stárka L. Practical aspects of screening of anabolic steroids in doping control with particular accent to nortestosterone radioimmunoassay using mixed antisera. Journal of Steroid Biochemistry 1979; 11(1):933-936.

Ponzetto F, Boccard J, Baume N, Kuuranne T, Rudaz S, Saugy M, et al. High-resolution mass spectrometry as an alternative detection method to tandem mass spectrometry for the analysis of endogenous steroids in serum. Journal of chromatography B, Analytical Technologies in the Biomedical and Life Sciences 2017; 1052:34-42.

Gomez C, Fabregat A, J.Pozo Ó, Marcos J, Segura J, Ventura R. Analytical strategies based on mass spectrometric techniques for the study of steroid metabolism. TrAC Trends in Analytical Chemistry 2014; 53:106-116.

Nielen MW, Hooijerink H, Claassen FC, van Engelen MC, van Beek TA. Desorption electrospray ionisation mass spectrometry: A rapid screening tool for veterinary drug preparations and forensic samples from hormone crime investigations. Analytica Chimica Acta 2009; 637(1-2):92-100.

Daems F, Romnee J, Heuskin S, Froidmont É, Lognay G. Analytical methods used to quantify isoflavones in cow's milk: a review. Dairy Science & Technology 2016; 96(3):263-283.

Adamusova H, Bosakova Z, Coufal P, Pacakova V. Analysis of estrogens and estrogen mimics in edible matrices--a review. Journal of Separation Science 2014; 37(8):885-905.

Soldin SJ, Soldin OP. Steroid hormone analysis by tandem mass spectrometry. Clinical Chemistry 2009; 55(6):1061-1066.

Kushnir MM, Rockwood AL, Bergquist J. Liquid chromatography-tandem mass spectrometry applications in endocrinology. Mass Spectrometry Reviews 2010; 29(3):480-502.

Vogeser M, Parhofer KG. Liquid chromatography tandem-mass spectrometry (LC-MS/MS)--technique and applications in endocrinology. Experimental and Clinical Endocrinology & Diabetes : Official Journal, German Society of Endocrinology and German Diabetes 2007; 115(9):559-570.

Stanczyk FZ, Clarke NJ. Advantages and challenges of mass spectrometry assays for steroid hormones. The Journal of Steroid Biochemistry and Molecular biology 2010; 121(3-5):491-495.

Janssens G, Mangelinckx S, Courtheyn D, De Kimpe N, Matthijs B, Le Bizec B. Simultaneous Detection of Androgen and Estrogen Abuse in Breeding Animals by Gas Chromatography-Mass Spectrometry/Combustion/Isotope Ratio Mass Spectrometry (GC-MS/C/IRMS) Evaluated against Alternative Methods. Journal of Agricultural and Food Chemistry 2015; 63(34):7574-7581.

Zendjabil M, Chellouai Z, Abbou O. Role of mass spectrometry in steroid assays. Annales d'endocrinologie 2016; 77(1):43-48.