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Mini Review

Research progress of Electrochemical Detection of β-Agonists: a mini-review

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β-Agonists promote protein synthesis and enhance muscle content. Although these substances are banned or restricted in animal husbandry in many countries, many farmers still use them illegally to improve economic efficiency. Athletes can test positive for stimulants after ingesting food with drug residues, which can cause great losses in many aspects. In this review, we first introduce different types of stimulants. Second, detailed properties of β-agonists are highlighted. Third, several traditional analysis techniques for β-agonist determination are discussed. Finally, we summarize the recent progress of using electrochemical methods for the determination of β-agonists.

Keywords: β-agonists; Athletes; Electrochemical analysis; Sports; Food safety

1. INTRODUCTION

Most of the earliest drugs used by athletes to improve their performance are stimulants [1–7]. In the 1960s, doping tests were carried out on athletes at the 19th Olympic Games [8,9]. Competitive athletes who used any form of drug or other unusual means to improve their performance in competition were considered to be using stimulants [10–13]. There are nine types of stimulants. The first are protein assimilation agents, such as testosterone, convalescent dragon, and clenbuterol; the second are peptide hormones and related substances, such as erythropoietin, growth hormone and insulin [14–16]; the third are β-agonists, such as formotrin and albuterol; the fourth are diuretics and other masking agents, such as acetazolamide, leucuric acid, and antrexone; the fifth are psychostimulants, such as ephedrine, cocaine and amphetamine stimulants; the sixth are anaesthetic analgesics, such as codeine, demerol, and fentanyl; the seventh are anti-estrogen agents, such as linazole and anastrozole; the eighth is hemp including hashish and marijuana; and the ninth are glucocorticosteroids.

β-Agonists are a type of benzene ethanol amine synthetic drug, a category of drugs that can activate the β-adrenergic receptors [17–23]. When livestock is fed, the amount of clenbuterol, as it is commonly known in China, exceeds the treatment dose by 5-10 times, which is a significant nutritional redistribution effect. In the past decade, China's ministry of agriculture has issued several documents banning the use of β-agonists as feed additives. β-Agonists have long been used illegally and have been linked to several serious cases of food poisoning. Table 1 lists several common β-agonists.

Table 1. List of common β-agonists.

Name	Molecular weight	R_1	R_2	R_3	R_4
Clenbuterol	277	Cl	NH_2	Cl	$C(CH_3)_3$
Salbutamol	239	CH_2OH	OH	H	$C(CH_3)_3$
Cimaterol	219	CN	NH_2	H	$CH(CH_3)_2$
lsoxsuprin	301	OH	NH	OH	$CH(CH_3)(CH_2)_2C_6H_5OH$
Mabuterol	260	OH	NH_2	CF_3	$C(CH_3)_3$
Brimbuterol	366	Br	NH_2	Br	$C(CH_3)_3$
Ractopamine	301	H	OH	H	$CH(CH_3)(CH_2)_2C_6H_5OH$
Fenoterol	303	OH	H	OH	$C(CH_3)_2CH_2C_6H_5OH$

The physical and chemical properties of β-agonists are similar, and most β-agonists are white crystalline particles or powders that are odourless and bitter. Most β-agonists are chemically synthesized drugs with certain properties of chemical compounds [24–28]. In their free state, β-agonists can be dissolved in most organic polar solvents and inorganic solvents with weak acidity or alkalinity. The chlorprolenol hydrochloride, afoterol tartrate and clenbuterol hydrochloride salts are commonly used in clinical medicine. These salts are strongly polar substances, so they are easily soluble in methanol, ethanol and water, slightly soluble in acetone, but insoluble in ether. These salts do not have a nonpolar structure and are therefore insoluble in hydrophobic solutions. The insolubility property in hydrophobic solution can be used to extract pure substances. The parent molecule of a beta-stimulant is based on the benzene ring structure [29–33]. This molecule can be absorbed in the ultraviolet and visible range of wavelengths. Studies have shown that there are characteristic beta-stimulant ion peaks of ultraviolet absorption at wavelengths of 220-310 nm. For example, the ultraviolet absorption wavelength of clenbuterol hydrochloride is 243 nm and 296 nm. The phenolic hydroxyl group has fluorescence properties, and part of the beta-stimulant parent structure contains phenolic hydroxyl groups, so it also has fluorescence properties.

Excessive use of β-agonists will stimulate the activation of β-receptors, leading to abnormal changes in the human heart, resulting in heart disease caused by cardiac hypertrophy and rapid heartbeat, and even severe diseases such as ischaemia or ischaemic necrosis of local tissues in people who already have heart disease [34–39]. Animal poisoning also occurs when humans exceed the limits for beta-stimulant consumption in feedings. The symptoms of poultry poisoning are similar to those of human poisoning, including muscle tremors, difficulty exercising, muscle pain and even vomiting and dizziness. Severe poisoning with congenital heart disease may be associated with respiratory failure and shock in

severe cases. β-Agonists were first used in medicine; they bind to β-receptors, causing local muscle relaxation that helps relieve asthma and cough [40–48]. At the same time, β-agonists increase the activity of cilia in the respiratory tract, which oscillate rhythmically. In addition to their use in bronchial smooth muscle relaxation, β-agonists can also relax skeletal muscles and the uterus to ease pain.

In 2002, the department of agriculture released a list of banned drugs that included several commonly used β-agonists. In addition, the international Olympic committee medical commission banned the use of clenbuterol in daily training and sports at the 25th Barcelona Olympic Games, as well as in the treatment of diseases.

2. TRADITIONAL METHODS FOR ANALYSIS OF B-AGONISTS

At present, many analytical techniques and methods in biology, food safety, chemical technology and medical applications have difficulties meeting the needs of actual on-site detection. The detection of beta-stimulants has been continuously improved along with the severity of carnivorous animal food safety problems [49,50].

Based on the chemical structure and properties of clenbuterol, it can be extracted with water due to its solubility in water and ethanol. Clenbuterol hydrochloride contains -NH_2 functional groups in its chemical formula, so it can be identified by aromatic monoamine reactions. Since clenbuterol hydrochloride also contains -Cl ions, it can be identified by chloride reactions. At the same time, it was found by spectrophotometry that the maximum absorption peak occurred when the ultraviolet wavelength was 243 nm and 296 nm. The comprehensive analysis method is effective for the determination of primary clenbuterol residue at a basic inspection level, but its minimum detection limit is high, and false positives are likely to occur.

The separation of β-agonists by capillary electrophoresis can be carried out by capillary zone electrophoresis (CZE), micellar electrokinetic capillary electrophoresis (MECC) and capillary point chromatography (CEC). Chen et al. [51] isolated and detected clenbuterol and albuterol in feed by the CZE method with a phosphate buffer solution of 20 mM at pH 10.5. The detection limit and quantitative limit of clenbuterol were 0.95 μg/mL, 3.17 μg/mL, respectively, and those of albuterol were 1.07 μg/mL and 3.57 μg/mL, respectively; the linear range was 2.0 to 100.0 μg/mL. Shi et al. [52] used field amplified sample injection (FASI) technology and capillary zone electrophoresis to separate and detect zeitro, clenbuterol and albuterol under optimized conditions. In this method, a column of water is introduced into the capillary before sample injection to enlarge the electric field difference between the background electrolyte and the sample zone, which is conducive to sample enrichment. Compared with the traditional capillary column electrophoresis method, the sensitivity of this method is increased by more than 30 times. The separation and detection of beta doping by capillary electrophoresis integrate the functions of HPLC and CE, which not only shortens the time of sample analysis and improves the recovery rate but also achieves a better separation effect for homologues or similar structures. Fanaliet et al. [53] used vancomycin-modified silica as a capillary electrochromatographic column to separate tebutalin and other substances. Enantiomers of albuterol and clenbuterol were prepared in polar organic solvents.

Chromatography developed from the original thin layer chromatography to gas chromatography, liquid chromatography, and later high-performance liquid chromatography and capillary column gas chromatography. Due to the continuous development and improvement of chromatographic detectors, high-sensitivity detection methods such as hydrogen flame ion detection, nitrogen and phosphorus detection and mass spectrometry have appeared, greatly improving the limit of trace detection. Ji et al. [54] combined capillary electrophoresis (CE) with an immunoassay (IA) and chemiluminescence (CL) to detect clenbuterol hydrochloride in urine. According to this study, the CEIA-CL method has a high separation efficiency of CE, low sample consumption, short analysis time and ligand-specific selectivity of IA, and it has been successfully applied to the detection of clenbuterol with a detection limit of 1.2 nM. The chromatographic detection method for the detection of β-agonist residues has obvious advantages, such as high accuracy, good specificity, and high precision. However, this method requires very expensive instruments.

An enzyme immunosorbent assay for the detection of beta-stimulant residue is a method with strong specificity, high sensitivity and simple and convenient operation. Many companies have developed kits based on ELISA to detect beta-stimulant residues. Although the method is fast, it has poor reproducibility and specificity, so it can only be used for the preliminary examination of large samples. In 2007, Shen et al. [55] studied the use of a time-resolved fluorescence immunoassay to detect ractopamine residues in pig viscera tissue to further improve the sensitivity and accuracy of the immunoassay method. When the ractopamine concentration was 1 ~ 10 μg/kg, the recovery rate of ractopamine reached 90%.

Compared with the abovementioned methods, the electrochemical method has the advantages of good stability, high sensitivity, inexpensive equipment, low price, high degree of automation and easy miniaturization and is a good choice for the detection of β-agonists in field food.

3. ELECTROCHEMICAL DETERMINATION OF B-AGONISTS

Electrochemical sensors are an important type of chemical sensors and are the most widely used and developed sensors. They can provide important information for studying the chemical nature of molecules and biological phenomena. Based on the electrochemical properties of the analysed objects, the chemical information is directly or indirectly converted into electrical signals, such as current, resistance, electrical quantity and potential, to achieve the purpose of qualitative or quantitative analysis and detection.

He and co-workers demonstrated an immunosensor based on carbon nanotubes for clenbuterol determination [56]. The redox probe of $K_3Fe(CN)_6$ was used to reflect the concentration of clenbuterol. The detection limit of the clenbuterol in this study was 0.32 ng/mL. A similar study has been reported by Liu and co-workers [57]. They proposed a portable immunosensor for electrochemical determination of clenbuterol using carbon nanotubes as electrode surface modifiers. Under optimum conditions, the sensor can perform a very quick test with a detection limit of 0.1 ng/mL. In addition, Wang et al. [58] reported an electrochemical biosensor based on a silver–palladium alloy nanoparticle for the quick

determination of clenbuterol. Table 2 summarizes the recently developed electrochemical sensors for clenbuterol determination.

Table 2. Recent developed electrochemical sensors for clenbuterol determination.

Materials	Method	Reference
Graphene oxide	DPV	[59]
Nation	DPV	[60]
1,4-BDT modified gold	DPV	[61]
Melamine-Ag NPs	DPV	[62]
Magnetic nanocomposites	DPV	[63]
Aptamer-agonists	DPV	[64]
Porous carbon electrode	CV	[65]
Graphene oxide	DPV	[66]
MoS_2-Au-PEI-hemin	DPV	[67]
Nafion–Au colloids	CV	[68]
Graphene oxide–Ag	DPV	[69]
SPE	Driving potential	[70]
ZnS Qds-PANI	EIS	[71]
Multiwalled carbon nanotube-4-tert-butyl calix[6]arene	DPV	[72]
Sulfonated graphene sheets/oxygen-functionalized multi-walled carbon nanotubes	CV	[73]
Molecularly imprinted polymer	DPV	[74]
MWCNT-Nafion nanocomposite	DPV	[75]
Isopropanol–Nafion–PSS–GR	DPV	[76]

Karuwan and co-workers prepared an inkjet-printed graphene-poly(3,4-ethylenedioxythiophene)-modified SPE as an electrochemical sensor for determination of salbutamol [77]. The results demonstrated that the modified SPE showed a 150 times higher response than a bare SPE.

Table 3. Recent developed electrochemical sensors for salbutamol determination.

Materials	Method	Reference
CS-Fe_3O_4-PAMAM-GNPs	CV	[78]
Taurine/zirconia	LSV	[79]
Pd@SBA-15-ionic liquid	Amperometric responses	[80]
GCE	CV	[81]
Graphene-Au	DPV	[82]
Graphene/PEDOT	CV	[83]
Graphene-IL-AgNPs	DPV	[84]

The proposed electrochemical sensor could reach a limit of detection of 1.25 μM. Huang and co-workers prepared a PANI/PAC-Au-graphene nanocomposite for the sensitive determination of salbutamol [85]. The results indicated that the proposed electrochemical sensor can perform a linear detection between 0.08 and 1000 ng/mL with a limit of detection of 0.04 ng/mL. Similarly, Li and co-workers reported a Ag/N-doped reduced graphene oxide-based molecularly imprinted electrochemical sensor for salbutamol determination [86]. The results showed that the proposed electrochemical sensor can perform a linear detection between 0.03 and 20 μM with a limit of detection of 7 nM. Table 3 summarizes the recently developed electrochemical sensors for salbutamol determination.

Ractopamine is a synthetic β-adrenoceptor agonist that is used to treat congestive heart failure and muscular dystrophy, increasing muscle mass, reducing fat accumulation, and promoting growth in foetuses and newborns. Ractopamine is being used on some pig farms as a replacement for clenbuterol. Shen and He reported an agarose hydrogel film for fast determination of ractopamine [87]. $K_3Fe(CN)_6/K_4Fe(CN)_6$ was used as a probe. The proposed electrochemical sensor could detect and quantitatively determine trace amounts of ractopamine. Yang and co-workers used ordered mesoporous carbon-modified electrodes for the direct detection of ractopamine [88]. The results showed that the proposed electrochemical sensor can perform a linear detection between 0.085 μM and 8.0 μM with a limit of detection of 0.06 μM. They also reported a label-free electrochemical aptasensor for detection of ractopamine [89]. Table 4 summarizes the recently developed electrochemical sensors for ractopamine determination.

Table 4. Recently developed electrochemical sensors for ractopamine determination.

Materials	Method	Reference
Manganese (II) phosphate nanoflowers	EIS	[90]
Electrosynthesized o-aminothiophenol film	Amperometric responses	[91]
Ordered mesoporous carbon-AuNPs	DPV	[92]
Carbon nitride nanotubes/ionic liquid	DPV	[93]
Cu/Cu_2O-graphene oxide	EIS	[94]
Au nanoflower	CV	[95]
Poly-o-phenylenediamine/gold nanoparticle–ionic liquid–graphene	CV	[96]
MIP	CV	[97]
Mesopores cellular foam	DPV	[98]
Graphene	DPV	[99]
Acetylene black nanoparticles	DPV	[100]
Aptamer/octadecanethiol Janus particles	CV	[101]
Fe_3O_4-doped reduced graphene oxide	CV	[102]
Fe_3O_4/graphene	DPV	[103]

4. CONCLUSION

In this review, we first introduced the types of stimulants and their effects on the body. Then, we focused on the recently developed processes for detecting β-agonists. Several traditional analysis

techniques for β-agonist determination were introduced. Then, we summarized the recent progress of using electrochemical methods for the determination of β-agonists.

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