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Highly sensitive immunochromatographic assay for qualitative and quantitative control of beta-agonist salbutamol and its structural analogs in foods

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ABSTRACT

Salbutamol (SAL) is a beta-adrenoreceptor agonist (beta-agonist) and is widely used as a growth promoter in livestock and poultry. Two schemes for the immunochromatographic analysis of SAL using gold nanoparticles as labels have been developed and tested for modes of visual and instrumental detection. The direct analysis scheme was characterized by an instrumental detection limit 0.4 ng/mL, an operating range 0.6–6.8 ng/mL and a visual detection limit of 10.9 ng/mL. The analysis time was 10 min. The indirect labeling scheme (using a combination of native specific antibodies and labeled anti-species antibodies) allowed the five-fold reduction of the limit of visual detection. In the analysis of foods, the indirect scheme was characterized by a visual detection limit 4.0 ng/g for meat products and 3.0 ng/g for milk samples. The analysis time was 15 min. The developed system was characterized by a high level of cross-reactivity with terbutaline (50%) and orciprenaline (10.5%), molecules of which have similar structures to that of SAL. The assay of milk samples may be implemented without pre-treatment or dilution, proving its applicability to on-site control. Its rapidity and simplicity suggest that the proposed immunochromatographic analysis of SAL is an effective tool for the mass screening of foods.

1. Introduction

Efforts aimed at control of toxic pollutants particularly pharmaceutical residues in food stuffs are of great importance in modern society (Chiou, Leung, Lee, & Wong, 2015). One of the classes of biologically active compounds that is used to intensify animal husbandry is beta-adrenoreceptor agonists (beta-agonists) (Smith, 1998). Beta-agonists stimulate specific receptors in muscle tissue, causing an increase in protein synthesis and enhanced lipolysis, which lead to the growth of muscle fibers. This property determines the use of beta-agonists in animal husbandry to improve the efficiency of feed assimilation and biomass growth (Lu et al., 2017; Moreno & Lanusse, 2017; Smith, 1998). In addition, beta-agonists have a relaxing effect on the smooth muscles of the respiratory tract and are used to relieve bronchospasm in the treatment of bronchial asthma, chronic obstructive pulmonary disease, and respiratory diseases (Starkey, Mulla, Sammons, & Pandya, 2014).

However, the intake of this group of compounds into the human body via food (meat, meat by-products, milk, and dairy products) can adversely affect the cardiovascular and nervous systems, leading to the disruption of metabolism and the desensitization of beta-adrenoreceptors (Smith, 1998; Vidal, Wieland, Lohse, & Lorenz, 2012). In this regard, the use of beta-agonists, in particular salbutamol (SAL), in the feeding of farm animals and poultry is prohibited in a number of countries, including the Russian Federation, the EU, China, etc. (European Community, Council Directive 96/22/EC). However, cases of the illegal use of beta-agonists in livestock breeding are quite common and suggest the need for control measures.

To prevent the intake of salbutamol into the human body via food, highly sensitive, fast, and reliable analytical methods are needed. Currently, the main methods used to identify SAL are high-performance liquid chromatography (Guo et al., 2015; T.; Li, Cao, Li, Wang, & He, 2016; Shao et al., 2009; Williams, Churchwell, & Doerge, 2004), gas chromatography with mass spectrometry (Caban, Stepnowski, Kwiatkowski, Migowska, & Kumirska, 2011), and capillary electrophoresis (W. Wang, Zhang, Wang, Shi, & Ye,

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2010). However, the common drawback of these methods is the high cost of the equipment involved. The analyses should be carried out in specialized laboratories by highly qualified personnel. In addition, food products require the complex, multi-stage preparation of samples before testing (T. Li et al., 2016; Vichapong, Burakham, & Srijaranai, 2016). The introduction of solid-phase extraction (Guo et al., 2015; T.; Li et al., 2016) or ion-pair extraction (Vichapong et al., 2016) allows the purification and additional concentration of samples (the limits of detection of such integrated methods can reach 3.3 ng/kg (Guo et al., 2015)) but increases analysis time. These approaches cannot be used as out-of-laboratory methods and therefore are not suitable for mass screening of food samples.

In recent years, immunoanalytical methods due to their specificity, sensitivity, efficiency, and profitability have increasingly been used to screen foodborne toxicants (Dzantiev, Byzova, Urusov, & Zherdev, 2014; Ren, Zhang, Chen, & Yang, 2009). Among them, techniques for salbutamol detection are intensively developed. The most common form of immunoassay is ELISA, which allows the simultaneous testing of a large number of samples. Developments of salbutamol ELISA are described in a number of studies (Meng et al., 2010; Sheu, Lei, Tai, Chang, & Kuo, 2009). Commercial ELISA kits are also available: the MaxSignal® Salbutamol ELISA Kit (Bioo Scientific, Austin, USA), Salbutamol ELISA Kit (MyBiosource, San Diego, USA), Salbutamol ELISA KIT 100030 (Alpha Diagnostic, San Antonio, USA), and RIDASCREEN® β-Agonists (R-Biopharm, Darmstadt, Germany). Although, in terms of detection limit, ELISA can be compared with instrumental methods of analysis, it has a number of limitations. Specifically, it is multi-stage, time-consuming (up to 2–3 h), and cannot be used in field trials. Alternative immunoassay formats such as fluorescence polarization immunoassay (Shen et al., 2017), time-resolved fluoroimmunoassay (M. Li et al., 2017), different types of immunosensors (Dong, Tang, Zhao, Deng, & Li, 2017; Suherman, Morita & Kawaguchi, 2015; Zhang, Cai, Deng, & Li, 2014) provide high sensitivity but require complex equipment.

Immunochromatographic analysis (ICA) overcomes the shortcomings of the above techniques. It takes no more than 20 min to realize because all the reagents have already been applied to the multimembrane composite (Dzantiev et al., 2014). The first such approach to the detection of SAL was proposed by Khamta et al. (Khamta, Pattarawarapan, Nangola, & Tayapiwatana, 2009) and was based on the competitive interaction of the SAL in sample and the SAL-BSA conjugate in the test zone of the test strip with polyclonal antibodies against SAL that were conjugated to gold nanoparticles. The method was applied for detection of SAL in buffer solutions; the achieved sensitivity was 80 ng/mL. In a similar ICA system for SAL detection in swine urine, described by Xie et al., the disappearance of staining in the test zone occurred in the concentration range of 1–4 ng SAL/mL (Xie, Chen, & Yang, 2012). R. Liu et al. developed SAL ICA, which allows the detection of ten beta-agonists in the urine. Its cut-off for the detection of SAL was 5 ng/mL (R. Liu et al., 2017). B. Liu et al. (B. Liu et al., 2016) compared the performance of three kinds of labels (colloidal gold, nanogold-polyaniline-nanogold microspheres, colloidal carbon) for immunochromatographic detection of SAL. It was shown that sensitivity the colloidal gold-based strip was slightly better than the other two.

Replacing gold nanoparticles with alternative labels, such as $Ru(phen)_3^{2+}$-doped silica nanoparticles (Xu et al., 2013), fluorescent beads with various emission peaks (P. Wang, Wang, & Su, 2015), or an enzyme label with chemiluminescent detection (Gao et al., 2014), can improve ICA sensitivity. Thus, Gao and co-authors (Gao et al., 2014) developed a test system that allows the detection of SAL and ractopamine in swine urine with a sensitivity of 0.04 ng/mL. After carrying out immunochemical reactions on the test strip, the test zones were cut out and transferred to cells containing a chemiluminescent substrate, after which the signal was measured via a special detector, which significantly complicates the testing process.

The lowest sensitivity for SAL ICA, 3 pg/mL, was shown by Zhang et al. (Zhang et al., 2016), who used Au@Ag surface-enhanced Raman scattering. However, the proposed method is complicated in execution and requires a laser Raman analyzer coupled with a microscope for detecting an analytical signal. Thus, the replacement of gold nanoparticles with alternative labels is associated with the significant complication of the testing procedure and the need for special detecting devices. Consequently, these approaches are not suitable for sensitive, rapid non-laboratory analysis.

Previously, we proposed an indirect labeling technique for use in ICA that increases its sensitivity without methodological complications (Petrakova et al., 2017). The conjugate of specific antibodies and gold nanoparticles (traditional direct labeling) are replaced with a combination of native specific antibodies and labeled anti-species antibodies. This change excludes non-productive immune reactions in the system, without changing the analytical signal. On the other hand, the proposed change does not complicate the assay: the test strip contains all the necessary components for analysis (membranes, protein antigen conjugates, and colored nanoparticles), except the specific free unconjugated antibodies that are transferred to the buffer for sample dilution. Previously, indirect labeling has been demonstrated to result in a 10–20-fold increase of ICA sensitivities for various analytes, without changes in assay duration (Urusov, Zherdev, & Dzantiev, 2014; Urusov et al., 2017; Urusov, Petrakova, Zherdev, & Dzantiev, 2016). The application of this idea to the realization of highly sensitive salbutamol ICA is the goal of the presented study.

2. Materials and methods

2.1. Reagents and samples

Sodium azide, 3,3′,5,5′-tetramethylbenzidine, dimethyl sulfoxide, Triton X-100, Tween-20 and chloroauric acid were purchased from Sigma-Aldrich (St. Louis, MO, USA; sial.com). Bovine serum albumin (BSA) was purchased from MP Biomedicals (Santa Ana, CA, USA, mpbio.com). Goat anti-mouse polyclonal antibodies (GAMI) were obtained from Arista Biologicals (Allentown, PA, USA, aristabiologicals.com). Peroxidase-labeled anti-mouse immunoglobulins were obtained from the Gamaleya Institute of Microbiology and Epidemiology, Russia. The monoclonal antibodies against SAL and SAL-BSA conjugate were produced in Jiangnan University and have been previously described (Khaemba et al., 2016; Liu et al., 2017). The purity of all other reagents was of analytical grade or higher.

2.2. Microplate ELISA

A SAL-BSA conjugate (100 μL, 0.1 μg/mL) in 50 mM K-phosphate buffer, pH 7.4, containing 0.1 M NaCl (PBS) was adsorbed in the wells of a microplate at 4°C overnight. The microplate was then washed four times with PBS containing 0.05% Triton X-100 (PBST). After that, 50 μL of SAL solution in PBST were placed in the wells (concentrations ranged from 50 ng/mL to 5 pg/mL), followed by the addition of 50 μL of specific antibodies (50 ng/mL in PBST) to each well. The mixture was incubated for 1 h at 37°C; the microplate was then washed four times with PBST, and 100 μL of immunoperoxidase conjugate was added to the wells (1:6000 dilution in PBST), and the plate was incubated for 1 h 37°C. The activity of the bound peroxidase was recorded after washing (three times with PBST and once with distilled water). For this purpose, 100 μL of a 0.4 mM