Analytica Chimica Acta 917 (2016) 79–84

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

A novel immunochromatographic assay based on a time-resolved chemiluminescence strategy for the multiplexed detection of ractopamine and clenbuterol

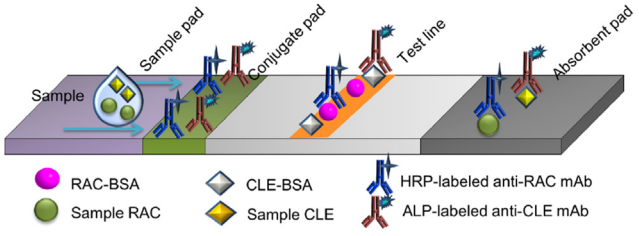
Wenwen Wang, Xiaoxiao Su, Hui Ouyang, Lin Wang, Zhifeng Fu*

Key Laboratory of Luminescence and Real-Time Analytical Chemistry, Ministry of Education, College of Pharmaceutical Sciences, Southwest University, Chongqing 400716, China

HIGHLIGHTS

- A chemiluminescent immunochromatographic assay was developed for multiplexed detection.
- This strategy could detect two β-agonists by using single test line on the nitrocellulose membrane.
- The assay can be accomplished within 20 min without complicated sample pretreatment.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 29 December 2015
Received in revised form
28 February 2016
Accepted 1 March 2016
Available online 5 March 2016

Keywords:
Ractopamine
Clenbuterol
Chemiluminescence
Multiplexed immunoassay
Immunochromatographic assay strip

ABSTRACT

A novel multiplexed immunochromatographic assay (ICA) based on a time-resolved chemiluminescence (CL) strategy was developed for quantitative detection of β-agonists, by utilizing ractopamine (RAC) and clenbuterol (CLE) as the models. Different from conventional multiplexed ICA methods which usually require two or more test lines, this strategy was developed for detection of two β-agonists by using only one test line on the nitrocellulose membrane. In this study, horseradish peroxidase and alkaline phosphatase were used as the signal probes to label RAC antibody and CLE antibody, respectively. The two CL reactions with flash type and glow type kinetics characteristics were triggered simultaneously by injecting the coreactants, then the signals for RAC and CLE detections were recorded at 3 s and 300 s after coreactants injection, respectively. Owing to the utilization of CL detection, this protocol showed ideal sensitivity for quantitation. Under the optimal conditions, the detection limits for RAC and CLE were 0.17 ng mL^{-1} and 0.067 ng mL^{-1} (S/N = 3), respectively. The whole assay process can be accomplished within 20 min without complicated sample pretreatment. The proposed method was successfully applied for the detection of RAC and CLE in spiked swine urine. It opens up a new pathway for designing a low cost, time-efficiency and multiplexed strategy for rapid screening and field assay.

1. Introduction

β-agonists are a group of phenyl ethanolamines with different substituents on the aromatic ring, and include clenbuterol (CLE), ractopamine (RAC), salbutamol, terbutaline, cimaterol, phenylethanolamine A et al. [1]. These agents are able to improve muscle mass and decrease body fat when fed to animals [2]. However, it is reported that excess uptake of these agents accumulated in animal tissues causes acute poisoning with symptoms of muscular tremor, cardiac palpitation, dizziness, nausea, vomiting, fever, chills et al. [3]. Due to its potential risk to the health of human being, β-agonists have been restricted or banned to be used in animal breeding

* Corresponding author.
E-mail address: fuzf@swu.edu.cn (Z. Fu).

http://dx.doi.org/10.1016/j.aca.2016.03.001
0003-2670/

in most countries including China, Europe Union and USA [4–6]. Accordingly, it is necessary to establish simple, rapid and sensitive approaches for detection of β-agonists at low concentrations.

To date, various assay methods such as liquid chromatography-mass spectrometry [7–9], gas chromatography-mass spectrometry [10,11], liquid chromatography [12], and capillary electrophoresis [13–15] have been well developed for detection of β-agonists. Nevertheless, in general these protocols are utilized as precise quantitative and confirmatory methods. Additionally, these methods require complicated manipulation, skilled personnel, sophisticated instrumentation and time-consuming sample pretreatment [16].

In decades, immunochromatographic assay (ICA) has attracted increasing attention owing to its low cost, minimal manipulation, short assay time, user-friendliness for rapid screening [17,18]. Conventionally, ICA protocols are applied for single analyte detection [19–23], thus multiplexed ICA strip is required when this protocol is used for multiple analytes. Recently, several ICA strips have been developed for multiplexed detection of antibiotics, proteins, and β-agonists based on fluorescent [24], electrochemical [25] and chemiluminescent (CL) [26] detection. For example, Taranova et al. [24] developed a fluorescent ICA strip with three test lines for detection of three antibiotics. Mao et al. [25] described a multiplexed electrochemical immunoassay for rabbit IgG and human IgM using ICA strip with two test lines. Similarly, Gao et al. [26] achieved simultaneous quantitation of salbutamol and RAC by using CL detection-based ICA. Since each test line can only detect single analyte for conventional ICA, these reported strip usually adopted no less than two successive test lines to achieve multiplexed detection with a spatial-resolved mode.

CL detection is usually conducted on simple and inexpensive instrumentation without external light source and optical splitting system, thus facilitates developing point-of-care test using portable detector. Nevertheless, no ICA strip based on multi-label mode and CL detection has been developed to detect multiple analytes with single test line up to now. In this study, a novel time-resolved CL multiplexed detection strategy was realized on an ICA strip with single test line co-immobilizing antigens of RAC and CLE. Due to the different kinetics characteristics [27,28], horseradish peroxidase (HRP) and alkaline phosphatase (ALP) were chosen as the CL probes to label antibodies of RAC and CLE, respectively. After the immunoreaction and the coreactants injection, CL signals from RAC and CLE were sequentially collected at different time windows. Compared with the previous time-resolved CL immunoassay on a microplate platform [29], the present method showed such merits as minimal manipulation, short assay time and user-friendliness since an ICA platform was adopted.

2. Experimental

2.1. Materials and instruments

HRP-labeled mouse monoclonal antibody for RAC (anti-RAC mAb), ALP-labeled mouse monoclonal antibody for CLE (anti-CLE mAb), RAC-bovine serum albumin (BSA) and CLE-BSA conjugates were all purchased from Guangzhou Ucando Biotechnology Co., Ltd (China). RAC and CLE standards were provided by China Institute of Veterinary Drug Control (China). Luminol and *p*-iodophenol (PIP) used as CL substrate of HRP were purchased from Sigma–Aldrich (USA). The substrate of ALP composed of disodium 3-(4-methoxyspiro{1,2-dioxetane-3,2′-(5′-chloro) tricyclo [3.3.1.1^{3,7}]decan}-4-yl) phenyl phosphate (CSPD) and Sapphire-IITM enhancer was purchased from Boson Biotech. Co., Ltd (China). SuperBlock® T20 (Thermo Fisher Scientific Inc., USA) was utilized as the blocking buffer. The dilution buffer for antibodies and antigens was 0.010 M Tris–HCl at pH 7.0. The CL substrates were prepared in 0.10 M Tris–HCl buffer at pH 8.5. All aqueous solutions were prepared using ultrapure water (18.2 MΩ) produced by an ELGA PURELAB Classic system (UK). The swine urine samples were collected from the local pig farms, and confirmed by TSQ Quantum Ultra LC-MS-MS (Thermo Finnigin Co., Ltd., USA) to be free of β-agonists. All other chemicals were of analytical reagent grade and utilized without further purification. Nitrocellulose membrane (M180), glass fiber used as sample pad and conjugate pad, and cotton pulp used as absorbent pad were purchased from Millipore Corp. (USA). The CL signals were collected by a MPI-A CL analyzer (Xi'an Remax Electronic Science & Technology Co., Ltd, China) equipped with a photomultiplier operated at −800 V.

2.2. Preparation of the ICA strip

As shown in Fig. 1, the ICA strip with a width of 4 mm consisted of an absorbent pad (17-mm length), a conjugate pad (8-mm length), a nitrocellulose membrane (25-mm length) and a sample pad (17-mm length). The sample pad and conjugate pad were pretreated with 0.010 M Tris–HCl (pH 8.0) containing 1.0% BSA and 0.10% Tween-20, then dried and stored in ambient. HRP-labeled anti-RAC mAb and ALP-labeled anti-CLE mAb were mixed at the ratio of 1:1, and used as the tracer solution. Four microliters of the above tracer solution was deposited onto the conjugate pad. Then 1.5 μL of a mixture of RAC-BSA (1.0 mg mL^{-1}) and CLE-BSA (1.0 mg mL^{-1}) was deposited onto the middle of the nitrocellulose membrane to assemble a test line. After the membrane was dried overnight at 4 °C, it was treated with 20 μL of blocking buffer for 90 min at 37 °C to minimize the non-specific adsorption. Afterward, the absorbent pad, the nitrocellulose membrane, the conjugate pad and the sample pad were assembled sequentially to fabricate the ICA strip. Each part of the ICA strip overlapped 1.5 mm with its adjacent part to enable sample solution to migrate through the whole strip.

2.3. Procedure of ICA and CL signal measurement

Eighty microliters of sample solution containing RAC and CLE was loaded onto the sample pad, and then migrated through the whole ICA strip under capillary action. The competitive immunoreactions occurred on the test line were completed within 10 min. Then the test line was cut down and placed into a reaction cell containing CL substrates consisted of PIP and luminol (90 μL for each cell). Finally, 20 μL of the freshly prepared coreactants of H_2O_2 and ALP substrate was injected. Then CL signals for RAC and CLE detections were collected at 3 s and 300 s, respectively, after the coreactants were injected.

3. Results and discussion

3.1. The principle of the multiplexed detection using ICA strip

The principle of time-resolved CL strategy for the multiplexed detection of RAC and CLE using ICA was illustrated in Fig. 1. During the assay, the sample solution containing RAC and CLE was loaded onto the sample pad and flew laterally towards the other end of the strip owing to the capillary action driven by the absorbent pad. After the sample solution reached the conjugate pad, the tracer antibodies migrated together with it through the nitrocellulose membrane. Then the competitive immunoreactions occurred between RAC and CLE in the sample solution and RAC-BSA and CLE-BSA immobilized on the test line, for binding to the tracer antibodies. In this study, HRP and ALP were chosen as the flash type and glow type CL probes to label anti-RAC mAb and anti-CLE mAb,