Talanta 129 (2014) 431–437

Contents lists available at ScienceDirect

Talanta

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

# Sulfonated polystyrene magnetic nanobeads coupled with immunochromatographic strip for clenbuterol determination in pork muscle

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ARTICLE INFO

*Article history:*
Received 2 April 2014
Received in revised form
30 May 2014
Accepted 8 June 2014
Available online 14 June 2014

*Keywords:*
Clenbuterol
Immunochromatographic strip
Magnetic solid phase extraction
Magnetic sulfonated polystyrene nanobeads

ABSTRACT

A magnetic solid-phase extraction method (MSPE) was developed to pre-concentrate and cleanup clenbuterol (CLE) from pork muscle. Novel sulfonated polystyrene magnetic nanobeads (spMNBs) were synthesized via a one-pot emulsion copolymerization method by using divinylbenzene, styrene, and sodium styrene sulfonate in the presence of oleic acid-modified and 10-undecylenic acid-modified magnetic ferrofluid. The resulting spMNBs exhibited high adsorption efficiency for CLE and for 10 other common beta-adrenergic agonists, namely, brombuterol, ractopamine, tulobuterol, bambuterol, cimbuterol, mabuterol, clorprenaline, penbutolol, salbutamol, and cimaterol. The adsorption behavior of the spMNBs for CLE was described by the Langmuir equation with a maximum adsorption capacity of 0.41 mg/g. Under the optimized parameters, the extraction of CLE from 0.5 g of pork muscle required 25 mg of the spMNBs at a shortened adsorption time (0.5 min). The proposed MSPE was coupled with colloidal gold nanoparticle-based immunochromatographic assay (MSPE–AuNPIA) for the quantitative detection of CLE residue in pork muscle. The limit of detection and limit of quantification for the pork muscle were 0.10 and 0.24 ng/g, respectively. The intra-day and inter-day assay recoveries at three CLE spiked concentrations ranged from 92.5% to 98.1%, with relative standard deviations ranging from 3.2% to 13.0%. The results of MSPE–AuNPIA were confirmed by liquid chromatography-tandem mass spectrometry (LC–MS/MS). The CLE values obtained with MSPE–AuNPIA agreed with those obtained with LC–MS/MS.



## 1. Introduction

Clenbuterol (CLE), a representative beta-adrenergic drug, has been misused as a nutrient repartitioning agent in the livestock industry in the past decades [1,2]. China, the United States, and most European countries have prohibited the use of CLE as a feed additive [3]. Various methods have been developed to detect CLE. These methods include liquid chromatography coupled with mass spectrometry (LC–MS) [4,5], gas chromatography coupled with MS [6], capillary electrophoresis with electrochemical detection [7], enzyme-linked immunosorbent assay (ELISA) [8], colloidal gold nanoparticle immunochromatographic assay (AuNPIA) [9,10], surface plasmon resonance [11], and surface-enhanced Raman scatter immunoassay [12]. Among these methods, AuNPIA is the most popular because of its simple operation, low cost, and rapidity (within 10 min); it has been successfully used to monitor CLE residue in swine urine. For the detection of tissue matrix via AuNPIA, sample pretreatments are necessary to remove matrix interference and increase CLE concentration. Classical methods for the pre-concentration and cleanup of CLE in tissue samples include extraction with perchloric acid solution, liquid–liquid extraction with isopropyl alcohol/ethyl acetate, rotary evaporator, and solid-phase extraction [4,7]. Currently, a few commercial solid-phase extraction cartridges with different surface functional groups have been widely used in CLE sample preparation. Examples of these cartridges include reversed-phase [13], strong cation-exchange [14], and mixed-mode cation-exchange [15] cartridges. Mixed-mode cartridges show higher sensitivity, better loading capacity, and better efficiencies than single-mode stationary-phase cartridges in CLE sample pretreatment because of the presence of interactions that contribute to analyte retention [16]. However, these traditional pretreatment methods cannot satisfy the demand of rapid screening analysis because of their

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http://dx.doi.org/10.1016/j.talanta.2014.06.007
0039-9140/

complicated operation, large amounts of organic pollution, and time-consuming process [17–19].

Magnetic solid-phase extraction (MSPE) based on superparamagnetic submicro-materials exhibits numerous advantages, such as fast adsorption kinetics, high adsorption/extraction efficiency, low magnetic absorbents consumption, high enrichment factor, convenient withdrawal with magnetic separator, and suitability for rapid screening operation [20–24]. Various magnetic materials that contain different surface-functionalized chemical groups have been successfully used to absorb metal ions from polluted water [25–27], phenolic compounds from environmental samples [28,29], melamine from egg samples [30], sulfonamides and tetracyclines from milk samples [31], and trace amounts of leuco-malachite green from fish tissue [32]. However, MSPE has yet to be used to extract CLE from tissue samples.

In recent years, many portable reader-based quantitative strip assays have been developed for food safety monitoring of *Brucella* [33], deoxynivalenol [34,35], fumonisins [36], enrofloxacin [37,38], etc. The ratio of the optical density (OD) of the test line ($A_T$) to that of the control line ($A_C$) is used to normalize the effects of operation temperature, immunoreaction time, matrix interference, and strip-to-strip variability [36,39]. Considering these concepts, we have previously developed a quantitative strip for the rapid detection of CLE in swine urine [40]. In the present study, novel sulfonated polystyrene magnetic nanobeads (spMNBs) that contain sulfonic acid and benzene ring groups (synthesized using a one-pot mini-emulsion copolymerization method) were used to pre-concentrate and cleanup CLE from meat samples. This spMNB-based extraction/pre-concentration method for CLE was combined with AuNPIA to develop a convenient quantitative MSPE–AuNPIA strip test for the sensitive and rapid detection of CLE residue in pork muscle. The performance of MSPE–AuNPIA for the quantitative detection of CLE was optimized in terms of sensitivity, reproducibility, accuracy, reliability, and rapidity. The results obtained with MSPE–AuNPIA for CLE detection were compared with those obtained with LC–MS/MS to confirm the accuracy of this new method.

## 2. Experimental

### 2.1. Materials and reagents

Oleic acid (OA), 10-undecylenic acid (UA), divinylbenzene (DVB), styrene, sodium p-styrenesulfonate (NaSS), potassium persulfate (KPS), CLE, ractopamine, mabuterol, salbutamol, cimbuterol, brombuterol, cimaterol, bambuterol, clorprenaline, penbutolol, and tulobuterol were purchased from Sigma-Aldrich (St. Louis, MO, USA). CLE–$D_9$ was purchased from Dr. Ehrenstorfer (Augsburg, Germany). The ELISA kit for CLE and CLE–bovine serum albumin conjugates (CLE–BSA; molar ratio of 15:1) were purchased from Wuxi Zodoboer Biotech Co., Ltd. (Wuxi, China). The BioDot XYZ platform combined with a motion controller, BioJet Quanti3000k dispenser, and AirJet Quanti3000k dispenser for solution dispensing were supplied by BioDot (Irvine, CA, USA). All other chemicals and reagents were analytical grade and purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Ultrapure water (18 MΩ/cm) produced by using a Milli-Q system (Milford, MA, USA) was used in all experiments.

A CLE stock solution (1.0 mg/mL) was prepared by dissolving CLE in methanol and stored at −20 °C until use. Working standard solutions were prepared weekly using appropriate dilutions of the stock solution. Pork muscle samples that were confirmed CLE positive or CLE free by LC–MS/MS were provided by Wuxi Zodoboer Biotech. Fortified pork muscle samples were prepared as follows for the accuracy and precision analyses. Briefly, the stock solution was diluted in methanol to obtain the intermediate CLE standard solution (200 ng/mL). CLE-free pork muscle samples (1.0 g) were fortified by adding the intermediate standard solution to produce spiked samples containing 0.25, 0.5, and 1.0 μg/kg. The spiked samples were incubated in the dark for 30 min at an

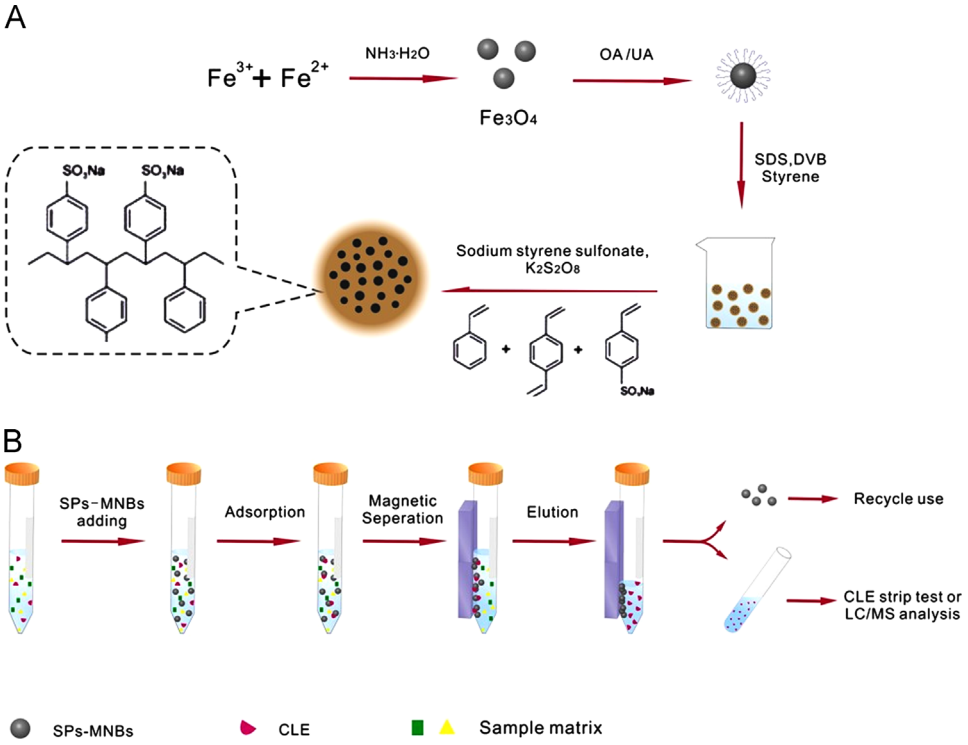


**Fig. 1.** Schematic for spMNB preparation and spMNB-based MSPE for CLE adsorption from pork muscle samples.