Meat Science 106 (2015) 55–60

Contents lists available at ScienceDirect

Meat Science

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

Development and validation of a high-performance liquid chromatography method for determination of ractopamine residue in pork samples by solid phase extraction and pre-column derivatization

Guanglong Ding [1], Deguang Li [1], Jiao Qin, Juanli Zhu, Baitao Wang, Qianqian Geng, Mingcheng Guo, Darunee Punyapitak, Yongsong Cao *

College of Agriculture and Biotechnology, China Agricultural University, Beijing, China

ARTICLE INFO

Article history:
Received 25 December 2014
Received in revised form 11 March 2015
Accepted 13 March 2015
Available online 27 March 2015

Keywords:
Ractopamine
Residue determination
Solid-phase extraction
Pre-column derivatization
High-performance liquid chromatography

ABSTRACT

Ractopamine (RAC) has been approved as a feed additive for swine, cattle or turkey, and is likely to have residue in edible animal products and may pose a potential risk for consumer health. Therefore, it is essential to establish a method to detect the residue of RAC in animal products. This work presents a rapid and sensitive HPLC method for the determination of RAC in pork samples with pre-column derivatization. The RAC derivative was separated on a kromasil C18 column and detected at 284 nm with a UV detector. The detection capability (CCβ) was 0.078 μg g^{-1} and the linearity was established over the concentration range of 0.15–100.0 μg g^{-1}. The overall mean recovery in spike range of 0.2 μg g^{-1} to 100 μg g^{-1} was 89.9% with the overall mean relative standard deviation of 4.1%. This method can be used for the quantification of RAC in pork samples and help to establish adequate monitoring of the residue of RAC.

1. Introduction

Ractopamine (RAC) is a typical β-adrenergic agonist, which has been approved as a feed additive for swine, cattle or turkey in the United States and some other countries (Shelver, Smith, & Berry, 2000; Wang, Li, Zhang, & Shen, 2006; Wang, Zhang, & Shen, 2006). The advantages of feeding animals with ractopamine include the promotion of repartitioning nutrients into muscles, increasing average daily weight gain, improving feed efficiency, increasing nitrogen retention and reducing the number of days to market (Crome et al., 1996; Engeseth et al., 1992; Gerlemann et al., 2014; Marchant-Forde, Lay, Pajor, Richert, & Schinckel, 2003; Xiong et al., 2006). However, ractopamine abuse leads to residue in edible animal products and may pose a potential risk for consumer health (Ko et al., 2012). In view of the potential adverse effects of ractopamine, several countries in Asia and Europe have banned the use of ractopamine in farm animals as a feed additive agent (Slavin & Yaeger, 2012). To ensure food safety, a rapid and effective method for the determination of ractopamine would be useful and necessary.

At present, many methods for screening and detecting ractopamine residue have been developed based on enzyme-linked immunosorbent assay (Fang et al., 2011; Pleadin, Perši, Vulić, Milić, & Vahčić, 2012; Wang, Zhang, et al., 2006). These were excellent survey tools for their high sensitivity and selectivity. However, the conventional immunoassay requires a relatively long assay time and large reagent consumption (Zhang, Zuo, & Ye, 2008). Other methods using gas chromatography–mass chromatography (Di Corcia, Morra, Pazzi, & Vincenti, 2010), high performance liquid chromatography (Burnett et al., 2012; Freire et al., 2009; Furusawa, 2013; Liu, He, Moore, Wang, & Coulter, 2009), and liquid chromatography coupled with mass spectroscopy (LC–MS, LC–MS/MS, UPLC–MS/MS) (Antignac, Marchand, Le Bizec, & Andre, 2002; Geis-Asteggiante et al., 2012; Kootstra et al., 2005; Mauro et al., 2014; Moragues & Igualada, 2009; Sakai et al., 2007; Turnipseed et al., 2014; Vulić, Pleadin, Perši, Milić, & Radeck, 2012) have also been reported. Moreover, the ultraviolet or fluorescent derivatization followed by HPLC determination of the resulting derivatives attracted more attention because it is more sensitive than HPLC determination without derivatization (Cevasco, Piątek, Scapolla, & Thea, 2010; Chen et al., 2005; Toyo'oka, 2009). HPLC with a UV detector, known for its stability, universality and low demand in terms of maintenance, is more popular than GC–MS, LC–MS, LC–MS/MS and UPLC–MS/MS in a majority of laboratories. In our previous studies, we have demonstrated the feasibility and advantages of the application of 4-chloro-3,5-dinitrobenzotrifluoride (CNBF) as the derivatization reagent for amines, which can offer adequate sensitivity using simple UV detector and the derivatization product was very stable and well-separated by the common C_{18} column in HPLC system. Meanwhile, there were no other by-products and multiple

* Corresponding author at: No. 2 Yuanmingyuan West Road, China Agricultural University, Beijing 100193, China. Tel./fax: +86 10 62734302.
E-mail address: caoysong@126.com (Y. Cao).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.meatsci.2015.03.016
0309-1740/

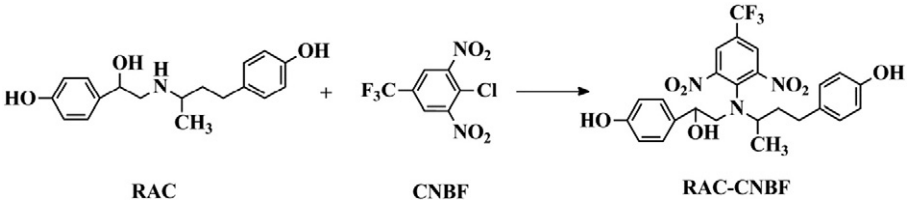

Fig. 1. Reaction scheme of CNBF with ractopamine.

derivatives apart from CNBF hydrolysis compound (CNBF-OH) in the reaction, while excess reagent and its hydrolysis product did not interfere with the separation (He et al., 2011; Qian et al., 2009; Tang et al., 2009). Additionally, CNBF is commercially available at a lower cost compared to some biochemical labeling reagents.

In this work, with the aim of monitoring the ractopamine residue, a simple, rapid, sensitive HPLC method with solid-phase extraction and pre-column derivatization for the determination of ractopamine in pork was developed. The conditions of the extraction method, solid-phase extraction clean-up, derivatization, and chromatogram were fully investigated.

2. Materials and methods

2.1. Instruments and conditions

HPLC separation was carried out using two LC-10ATvp pumps and a SPD-10Avp ultraviolet detector (Shimadzu, Japan). Chromatographic separation was performed using a reversed-phase kromasil ODS C_{18} column (AkzoNobel, Amsterdam, ZZ, Netherlands, 250 mm × 4.6 mm, 5 μm, 100 Å) linked with a guard column (kromasil ODS C_{18}, 4 mm × 3 mm, 100 Å) and Chromato Solution Light Chemstation for a LC system was applied to acquire and process chromatographic data. The Oasis HLB 500 mg cartridges, Oasis MCX 500 mg cartridges, Oasis MAX 500 mg cartridges and Widepore CBX 500 mg cartridges were purchased from J.T. Baker (Phillipsburg, NJ, USA). The values of pH were adjusted with 1 N NaOH or 1 N HCl solution and measured with a MP511 pH meter (San-Xin Instrumentation, Inc., Shanghai).

2.2. Chemicals and reagents

Ractopamine hydrochloride standard was purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). CNBF was supplied by Alfa Aesar (Ward Hill, MA, USA). Trifluoroacetic acid (TFA) and NaOH were analytical grade reagents, and purchased from Sinopharm Chemical Reagent Beijing Co., Ltd. HPLC grade methanol was supplied by J.T. Baker (Phillipsburg, NJ, USA). Ultrapure water from a Milli-Q system (Millipore, Billerica, MA, USA) was used. All other chemicals and solvents were of analytical grade and from commercial sources. Weighed and frozen portions of pork meat (castrate male pigs, Large Yorkshire, aged 120 days, body mass 115 kg) were provided by the College of Veterinary Medicine at China Agriculture University and stored at −20 °C. The pork samples (pork leg) were ground with a JJ-2 kinematica (Eltong Electric Co., Ltd, Jintan, Jiangsu). A standard solution of 3.0 mmol L^{-1} ractopamine hydrochloride was prepared in ultrapure water. The CNBF solution of

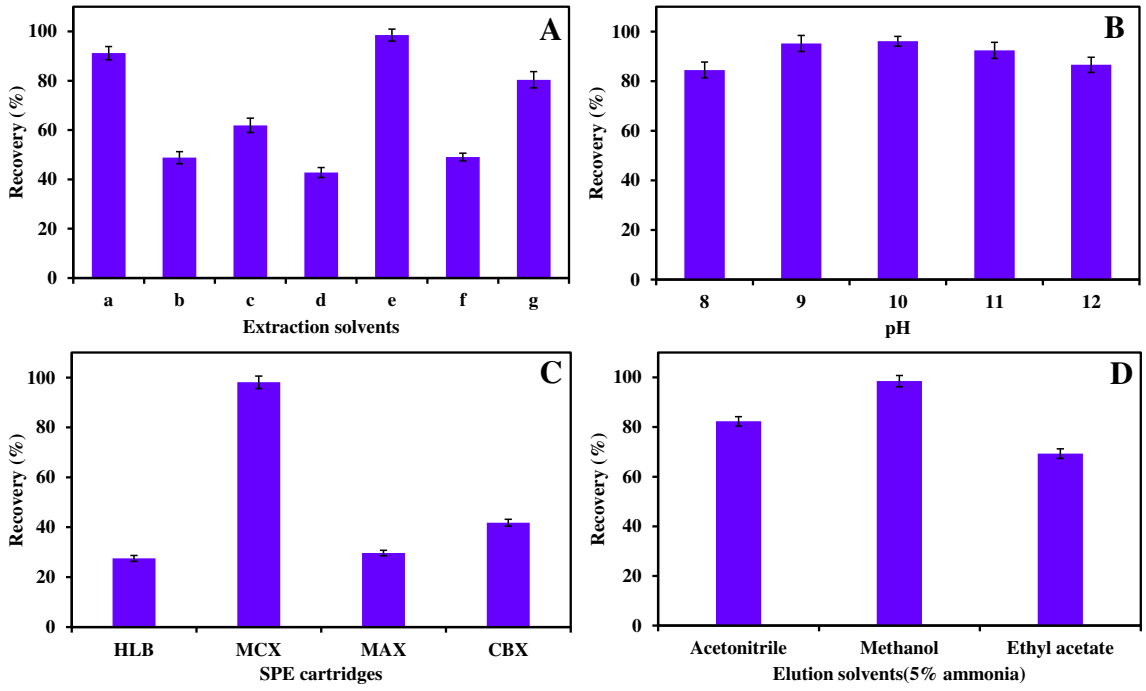

Fig. 2. Effects of different extraction solvents, pH values of the sample solutions, SPE cartridges and elution solvents on the recoveries of ractopamine. (A) Extraction solvents (a: methanol; b: acetonitrile, c: acetone, d: ethyl acetate, e: isopropanol/ethyl acetate (1/9, v/v), f: isopropanol/dichloromethane (1/9, v/v) and g: methanol/ethyl acetate (1/9, v/v); pork sample: 5 g (weighed accurately); ractopamine standard solutions: 100 μg mL^{-1}; pH values: 10; SPE cartridges: MCX; elution solvents: 5% ammoniacal methanol). (B) pH values (pork sample: 5 g (weighed accurately); ractopamine standard solutions: 100 μg mL^{-1}; extraction solvents: isopropanol/ethyl acetate (1/9, v/v); SPE cartridges: MCX; elution solvents: 5% ammoniacal methanol). (C) SPE cartridges (pork sample: 5 g (weighed accurately); ractopamine standard solutions: 100 μg mL^{-1}; extraction solvents: isopropanol/ethyl acetate (1/9, v/v); pH values: 10; elution solvents: 5% ammoniacal methanol). (D) Elution solvents (pork sample: 5 g (weighed accurately); ractopamine standard solutions: 100 μg mL^{-1}; extraction solvents: isopropanol/ethyl acetate (1/9, v/v); pH values: 10; SPE cartridges: MCX).