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Original article

Determination of ractopamine in pig hair using liquid chromatography with tandem mass spectrometric detection

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ABSTRACT

A quantitative analytical procedure for the determination of ractopamine in pig hair has been developed and validated. The hair samples were washed and incubated at 75 °C with isoxuprine and hair extraction buffer. The drug present was quantified using mixed solid-phase extraction and liquid chromatography with tandem mass spectrometric detection. The limit of quantization (LOQ) was 10 pg/mg and the intra-day precision at 25 pg/mg and 750 pg/mg was 0.49% and 2.8% respectively. Inter-day precision was 0.88% and 3.52% at the same concentrations. The hair extraction percentage recovery at 25 pg/mg and 50 ng/mL was 99.47% and 103.83% respectively. The extraction percentage recovery at 25 pg/mg and 50 ng/mg was 93.52% and 100.26% respectively. Our results showed that ractopamine residues persist in hair in 24 days of withdrawal and also showed the possibility to test ractopamine from pig hair samples.

1. Introduction

Ractopamine hydrochloride, a β-adrenoceptor agonist has been often used as growth-promoting agent to improve the feed efficiency and enhance lean meat to fat ratio in many animal species (Rickard et al., 2012). Some scientists found that reductions in carcass fat likely result in part from direct effects of ractopamine interfering with the conversion of glucose to storage triglyceride (Leick et al., 2010, Ross, Beaulieu, Merrill, Vessie, & Patience, 2011). Ractopamine mediates their cellular response through β-adrenergic receptors, activation of adenylate cyclase and protein kinase A (PKA) and affects lipogenic activity by dual mechanisms (Joost, Weber, Cushman, & Simpson, 1987, Pulce et al., 1991). Ractopamine is often misused as growth promoters to improve carcass composition by decreasing fat to the benefit of muscle mass and gain higher economic benefit (Anderson, Moody, & Hancock, 2009). Ractopamine is forbidden as a feed additive in China for its possible adverse effects on human health by carry-over from RAC-treated animals to human diet (He, Su, Zeng, Liu, & Huang, 2007, Zhang et al., 2011).

The metabolic cycle of ractopamine was very short and residual cycle was different in some animals. To avoid missing detection of ractopamine, it was necessary to establish a standard screening and confirmation method for drug residues in animals. Some analytical techniques were designed to control the use of ractopamine. Enzyme linked immunosorbent assay (ELISA), high performance liquid chromatography (HPLC) (Shelver & Smith, 2003) and/or gas chromatography mass spectrometry (GC/MS) were used for ractopamine testing (Antignac, Marchand, Le Bizec, & Andre, 2002). All these methods were designed to test ractopamine residues in urine and plasma samples taken from living animals as well as in tissue samples (muscle, liver, lung and kidney). In recent year, LC/MS/MS was also used to determine ractopamine (Li, Wu, Yang, Zhang, & Huang-Fu, 2010). Ractopamine in urine could be detected for 6 days with a 1 ng/mL cutoff (Smith, Feil, & Paulson, 2000). It was hard to test ractopamine during the withdrawal period (Liu, He, Moore, Wang, & Coulter, 2009).

Hair analysis has been used to evaluate exposure to toxic heavy metals (Castellari, Gratacós-Cubarsí, & García-Regueiro, 2009; Dunnett & Lees, 2004; Dunnett, Richardson, & Lees, 2004; Gratacós-Cubarsí, Castellari, Valero, & García-Regueiro, 2006; Kintz, 2004). It was the main method for the evaluation of individual's drug history in toxicology field (Mieczkowski, 1996). Compared to urine and blood testing, the major advantage of hair testing was its ability to have a larger surveillance window (Kintz, 2004). Literature data showed that clenbuterol could be tested in pig hair by GC/MS (Su & Shen, 2008) and ractopamine could be tested in pig retina and hair (Pleadin, Vulić, Perši, & Radeck, 2012, Vulić, Pleadin, Perši, Milić, & Radeck, 2012).

The procedure using LC/MS/MS for the analysis of ractopamine in hair was validated in our study. The procedure was developed for

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Table 1
Transitions; optimized fragment voltage; collision energy.

Drug	Precursor ion	Fragment ion	Fragment voltage (V)	Collision energy (V)	Retention time (min)
Ractopamine	302.1	164.1	120	10	4.46
Ractopamine	302.1	121.2	120	20	4.46
Isoxuprine	302.1	284.2	120	10	6.24

monitoring of ractopamine residues in pig hair and could be mandated by the regulatory agencies. Because of its high sensitivity of hair extraction and LC/MS/MS analysis, the procedure can be used to detect ractopamine residues in hair samples. The quantization limit was 10 pg/mg. The samples were prepared with hair extraction and SPE extraction, and directly analyzed by LC/MS/MS.

2. Materials and methods

2.1. Reagents and chemicals

Ractopamine hydrochloride and isoxuprine hydrochloride (ACS grade) were purchased from Sigma-Aldrich Company (Milwaukee, WI). All solvents were HPLC grade or better, and all chemicals were ACS grade. HEB (Hair Extraction Buffer, pH 4.7) was from Immunalysis Corporation (Diagnostic Apparatus, Medical in Pomona, CA).

2.2. Animals and sampling procedure

The experiment was carried out in 3 white male pigs, long and white type, aged 90 days, body mass 50 kg, farm-bred, and kept under the same zoohygienic conditions. Animals were from Golden Lake piglet breeding center in Ezhou City of Hubei Province of China. Animals were orally administered 20 mg/kg body mass ractopamine per day in the form of a pure chemical capsule admixed to feed. Hair samples were collected every other day from day 1 to day 57 during the treatment (33 days) and withdrawal period (24 days).

2.3. Equipment

Solid phase extraction columns (Clin II, 691-0353) were obtained from SPEWare (San Pedro, CA). System-48 Cerex sample concentrator and System-48 Cerex pressure processors were obtained from SPEWare (San Pedro, CA).

2.4. Calibrators

For the chromatographic calibration standards, a working solution containing internal standard was prepared in methanol at a concentration of 1000 ng/mL. Standard solutions of ractopamine were prepared in methanol at 10 ng/mL, 100 ng/mL, and 1000 ng/mL and stored at −20 °C. Ractopamine concentrations of 10, 50, 100, 1000, 7500 and 10,000 pg/mg in pig hair were prepared. Isoxuprine was used as the internal standard at a concentration of 1000 pg/mg.

2.5. Sample preparation for LC/MS/MS analysis

Hair samples were taken from pig after feeding ractopamine. Pigs were daily administered orally with 20 mg/kg ractopamine and the feeding duration was 33 days. After treatment, treated pigs were placed on control diet (without ractopamine). The white hair was collected from the back of pigs. The white hair was cut as close as possible to the scalp, fixed with string and enveloped. The samples were stored under dry conditions at room temperature until analysis. Hair samples were cut into pieces for analysis. The hair samples were washed with acetone (3 mL) and methylene chloride (3 mL) for three times to remove dirt only and dried based on the methods of Lewis, Moore, Morrissey, and Leikin (1997) with some modifications. Isoxuprine (100 μL) and 1.5 mL HEB were added to the portion of 10 mg of dry hair in a centrifuge tube. The tubes were capped and sonicated at 75 °C for 3 h. An aliquot of negative sample (10 mg) was placed in a 12 mL screw cap centrifuge tube. All samples were extracted with HEB and the extracted buffers were transferred into new tubes with addition of 1 mL sodium phosphate buffer (pH 6.0).

Solid-phase mixed mode extraction columns (Clin II, 691-0353T) were placed into a positive pressure vacuum manifold. The extracted hair buffers were then loaded to the columns conditioned with 3 mL of methanol, 3 mL of deionized water and 1 mL of phosphate buffer (pH 6.0). The columns were washed with 2 mL of deionized water, 2 mL of 1 M acetic acid and 4 mL of methanol. The columns were then allowed to dry under nitrogen pressure for about 5 min at 30 psi.

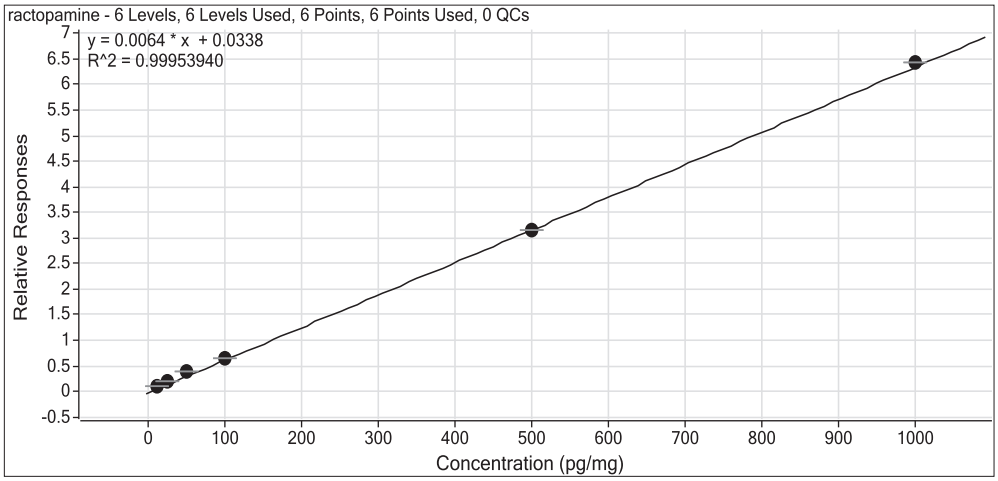

Fig. 1. Calibration for ractopamine assay in pig hair samples.