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Residue Depletion of Ractopamine and Its Metabolites in Swine Tissues, Urine, and Serum

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Ractopamine hydrochloride is a β-adrenergic leanness-enhancing agent approved for use in swine in the United States. Depletion of ractopamine and its metabolites from animal tissues, urine, and serum is of interest for the detection of illegal use. The objectives of this study were to measure the residues of ractopamine in swine incurred samples after treatment with dietary ractopamine for 28 consecutive days. An efficient and sensitive analytical method was developed for the detection of parent ractopamine and its metabolites in swine tissues, urine, and serum by HPLC-FLD. After extraction, enzymatic digestion, and solid-phase cleanup of the samples, ractopamine residues were determined by liquid chromatography (LC) with fluorescence detector. The limits of detection (LOD) for tissues, urine, and serum were 1 ng g^{-1}, 0.5 ng mL^{-1}, and 0.5 ng mL^{-1}, respectively. Recoveries ranged from 70.5 to 94.5% for samples fortified at 1−50 ng g^{-1} or ng mL^{-1}. Sixty pigs were fed twice daily for 28 consecutive days with feeds containing 18 mg kg^{-1} ractopamine HCl. The residue concentrations in urine, liver, and kidney were 650.06 ng mL^{-1}, 46.09 ng g^{-1}, and 169.27 ng g^{-1}, respectively, compared with those in muscle, fat, and serum (4.94 ng g^{-1}, 3.28 ng g^{-1}, and 7.48 ng mL^{-1}, respectively) at the feeding period of 7 days. The residue concentrations at withdrawal period of 0 days in all edible tissues were lower than tolerance values established by the FDA and MRL values listed by the JECFA. These data support the withdrawal time of 0 days established by the FDA for ractopamine used as feed additive in swine.

KEYWORDS: Ractopamine; swine; HPLC-FLD; residue depletion

INTRODUCTION

Ractopamine hydrochloride (**Figure 1**) is a kind of β-adrenergic agonist, which has a repartitioning effect (*1*). It is approved as a feed additive for swine and cattle in the United States and other countries. Some reports have shown that ractopamine reduces fat, increases average daily weight gain and lean tissue accretion, and improves feed utilization and conversion ratio (*2*, *3*). However, ractopamine has not been approved for use in other species in the United States (*4*), and use in animals other than swine and cattle would be considered off-label. Because various food poisonings have been caused by clenbuterol residues, ractopamine has not been approved for use in animal feeds by regulatory agencies in China or Europe. Therefore, control and monitoring programs mandated by various government agencies have necessitated the development of assay procedures for ractopamine and required that ractopamine depletion be conducted.

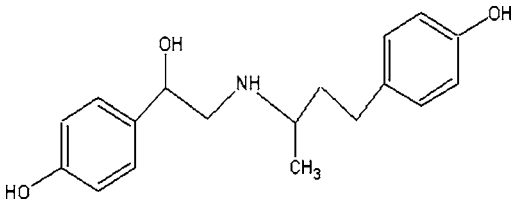

Figure 1. Structure of ractopamine.

Turberg et al. (*5*) reported the determination of ractopamine hydrochloride by LC with electrochemical detection having a limit of detection of 0.5 ng g^{-1} and recoveries of 75−100% in liver, kidney, muscle, and fat tissues. Turberg et al. (*6*) also reported a ractopamine determinative method for monkey plasma and swine serum using HPLC with electrochemical detection having a limit of detection (LOD) of 0.5 ng mL^{-1}. Confirmatory methods for β-agonists have been performed by gas chromatography−mass spectrometry (GC-MS) or tandem mass spectrometry (GC-MS/MS) (*7*, *8*) and liquid chromatography−mass spectrometry (LC-MS) or tandem mass spectrometry (LC-MS/MS) in swine tissues with a LOD of 0.5 ng g^{-1} or even lower (*2*, *9*−*11*). Depletion of ractopamine residues in

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swine tissues and urine has also been studied. In a residue depletion study (*12*), 30 pigs received a ration containing 20 mg kg^{-1} ractopamine hydrochloride for 9 days and were killed at 1, 2, 3, 4, and 5 days after the last ractopamine exposure. Livers and kidneys were collected and analyzed for residues using liquid chromatography with electrochemical detection (LOD = 5 ng g^{-1}). Smith and Shelver (*13*) have reported tissue residues and urinary excretion of ractopamine and metabolites in cattle, sheep, and ducks treated for 7 days.

The purpose of this paper was first to develop a sensitive method for the detection of parent ractopamine and its metabolites in swine tissues, urine, and serum by HPLC-FLD. The method was then applied for the quantification of ractopamine residues during its depletion from swine treated with dietary ractopamine for 28 consecutive days. Moreover, residues in swine matrices were compared to established maximum residue limits (MRLs) or tolerance values and withdrawal times established by regulatory agencies.

MATERIALS AND METHODS

Materials. Methanol and acetonitrile (chromatographic grade) were purchased from Fisher Chemicals, USA. Acetone, ethyl acetate, 1-propanol, ammonia (30%), acetic acid, sodium borate, 1-pentanesulfonic acid, and ammonium acetate (analytical grade) were obtained from Beijing Chemical Factory, Beijing, China. Ractopamine standard (99.05%) was supplied by the China Institute of Veterinary Drug Control. β-Glucuronidase/aryl sulfatase (*Helix pomatia*, 20 units mL^{-1}) was provided by Merck, Darmstadt, Germany. Demineralized water was obtained by means of a Milli-Q system (Millipore, Bedford, MA).

Instrumentation and Chromatographic Conditions. The LC system was equipped with a Waters 2475 fluorescence detector and Waters 600 pumps (Waters Co., Milford, MA). The HPLC column was a Supelcosil LC-18-DB, 250 mm × 4.6 mm, 5 μm particle size (Supelco, Bellefonte, PA). The pump was operated in an isocratic mode with a flow rate of 1 mL min^{-1}. Excitation wavelength was at 226 nm, and emission wavelength was at 305 nm. The mobile phase was composed of 80% water with 20% acetonitrile with the addition of 2 mL of glacial acetic acid and 0.7 g of 1-pentanesulfonic acid per liter. The mobile phase was filtered through a 0.2 μm membrane and sparged before use. Injection volume was 100 μL, and run time was 20 min.

Standard and Fortification Solutions. A standard stock solution was prepared by dissolving 0.01 ± 0.0005 g of ractopamine hydrochloride in 100 mL of methanol (stable for at least 3 months at 4 °C). The stock solution was diluted with appropriate volumes of 0.2% acetic acid to prepare working and fortification solutions. The standard curve for ractopamine was built in the range of 5−1000 ng mL^{-1}.

Extraction and Purification. *Tissue Samples.* Swine tissues (liver, kidney, muscle, or fat) were minced and homogenized in a homogenizer for 2 min. Five grams of homogenate was accurately weighed into a polypropylene centrifuge tube, and blank tissues were fortified with 5−250 ng of ractopamine. Then, 10 mL of acetone was added to samples, and tissues were suspended in acetone at that moment. After brief vortexing (1 min) and centrifugation (2000*g*, 10 min), the supernatant was transferred to a clean test tube and the homogenized tissue samples were further extracted twice with 10 mL of acetone. The acetone extracts were again centrifuged at 2000*g* for 10 min to further remove the precipitates. 1-Propanol (5 mL) was added to the supernatant to prevent the acetone from bumping during evaporation with a rotary evaporatory. After evaporation, the dry residue was dissolved in 1 mL of ammonium acetate buffer (25 mM, pH 5.0), and 20 μL of β-glucuronidase (0.4 unit; 20 units mL^{-1}) was added; the enzymatic hydrolysis was initialized by incubating the mixture at 65 °C for 2 h. (This step could be omitted for the purification process of control and fortified samples of β-glucuronidase untreated animals.)

After hydrolysis with β-glucuronidase, sample solutions were mixed with 2 mL of sodium borate buffer (25 mM, pH 10.3 ± 0.1) and extracted twice with 7 mL of ethyl acetate. After centrifugation (2000*g*, 5 min), the combined supernates were applied to Waters Sep-Pak Alumina A cartridges (Waters, 500 mg), previously activated with 5 mL of ethyl acetate. After the extract had passed through at a flow rate of 1−2 drops per second, the cartridges were washed with ethyl acetate and dried under vacuum for 2−3 min. The analytes were eluted with 10 mL of methanol, and the eluate was evaporated to dryness under a nitrogen stream. The solid-phase extraction method is based upon the published and validated method reported by Shishani et al. (*9*). The dry residue was reconstituted in 1 mL of 0.2% acetic acid for HPLC analysis.

Urine and Serum Samples. For urine and serum samples (5 mL), the procedures of fortification and enzyme digestion were the same as those of the tissues. Again, the hydrolysis procedure could be omitted for control and ractopamine-fortified samples. Then, 2 mL of sodium borate buffer (80 mM, pH 10.3 ± 0.1) was added to the samples, and samples were extracted twice with 20 mL of ethyl acetate. After centrifugation (2000*g* for 10 min), the collected supernatant extracts were evaporated to dryness and reconstituted in 4 mL of 1 M acetic acid. The solution was applied to an MCX cartridge (Oasis, 3 cm^3, 60 mg), previously conditioned by 3 mL of methanol and 3 mL of water. After washing with 3 mL of acetic acid (1 M) and 6 mL of acetonitrile, the analytes were eluted with 6 mL of 2% ammonia in ethyl acetate/methanol (30:70, v/v). Then, the eluate was dried under a nitrogen stream and dissolved in 1 mL of 0.2% acetic acid for analysis.

Residue Depletion. Sixty pigs (Landrace provided by Shunyi pig farm, Beijing, China; each approximately 50 kg bw) were fed twice daily (at about 10:00 a.m. and 4:00 p.m.) for 28 consecutive days with feeds (**Table 1**) containing 18 mg kg^{-1} ractopamine hydrochloride. Animals had free access to hay and water. The last feeding of ractopamine-fortified feeds was at the morning feeding period of 28 days (F28). After the ractopamine exposure period, hogs were provided with feeds containing no ractopamine during a 14 day withdrawal period. They were slaughtered during the feeding period at 7, 14, and 28 days and at 1, 2, 3, 7, 9, and 14 days of the withdrawal period. Liver, kidney, muscle, fat, urine (collected from the urinary bladder at slaughter), and serum samples were collected and stored at −20 °C until analysis. Animal corpses were buried in a pit covered with lime.

Data Analysis. Ractopamine residue concentrations in urine before and after hydrolysis with β-glucuronidase, a series of volumes of β-glucuronidase, and drug residues in matrices were expressed as mean ± SD (**Figures 3** and **4**; **Table 4**). *t* tests for main effects of treatment were calculated (**Table 5**) according to the "Committee for Veterinary Medicinal products; Approach toward harmonization of withdrawal periods" (EMEA/CVMP/036/95-FINAL). Tissue concentrations were determined at the withdrawal time of 0 days when the one-sided 95% upper tolerance limit was below the value of tolerance established by the FDA and the MRL listed by the JECFA (**Figures 5−7**).

Table 1. Composition of Swine Feed

raw material	ratio (%)
corn	54.4
smashed oats	6.4
soybean	12.0
vitamin premix	5.0
wheat bran	5.0
rice bran	4.0
silkworm chrysalis	10.0
salt	0.3
lysine	0.3
calcium carbonate	0.8
calcium phosphate dibasic	1.3
minerals	0.5
total	100.0

RESULTS

Linearity. A standard curve for ractopamine was built in the concentration range of 5−1000 ng mL^{-1}. A linear response was obtained over the entire concentration range. The standard curve equation was $Y = 1530X + 702$ with the regression coefficient of 0.9999.

Table 2. Recoveries of Ractopamine Fortified into Matrices Collected from Control Swine ($n = 6$)

matrix	concentration (ng g^{-1}/ng mL^{-1})	recovery (%)	CV[a] (%)
liver	2	81.2	3.8
	10	78.4	4.2
	20	79.7	3.3
	50	85.8	5.4
kidney	2	78.3	3.9
	10	80.6	4.8
	20	90.1	6.5
	50	92.2	8.3
muscle	2	85.4	7.8
	10	79.1	6.6
	20	80.7	7.5
	50	89.3	10.2
fat	2	70.5	2.2
	10	73.6	3.4
	20	74.7	2.5
	50	71.9	5.1
urine	1	80.0	5.0
	2	81.9	5.4
	10	76.0	6.4
	50	81.4	4.1
serum	1	88.6	5.8
	2	90.1	6.2
	10	94.5	7.9
	50	93.6	8.8

[a] Coefficient of variation.

Table 3. Between-Run Precision of the Assay for Samples Fortified with Ractopamine ($n = 3$)

matrix	fortification (ng g^{-1})	recovery (%)	CV[a] (%)
liver	2	76.3	8.9
	10	76.5	8.2
	20	77.2	9.1
	50	80.0	11.3
kidney	2	76.3	6.5
	10	79.4	8.4
	20	83.1	10.2
	50	89.7	12.1
muscle	2	80.2	10.3
	10	78.9	10.5
	20	79.5	11.1
	50	86.6	13.0
fat	2	70.0	6.5
	10	71.5	6.3
	20	72.1	7.2
	50	70.8	7.6
urine	1	78.8	5.4
	2	78.3	5.8
	10	73.9	8.3
	50	80.5	9.2
serum	1	87.5	6.7
	2	89.4	8.2
	10	92.2	9.1
	50	93.8	11.2

[a] Coefficient of variation.

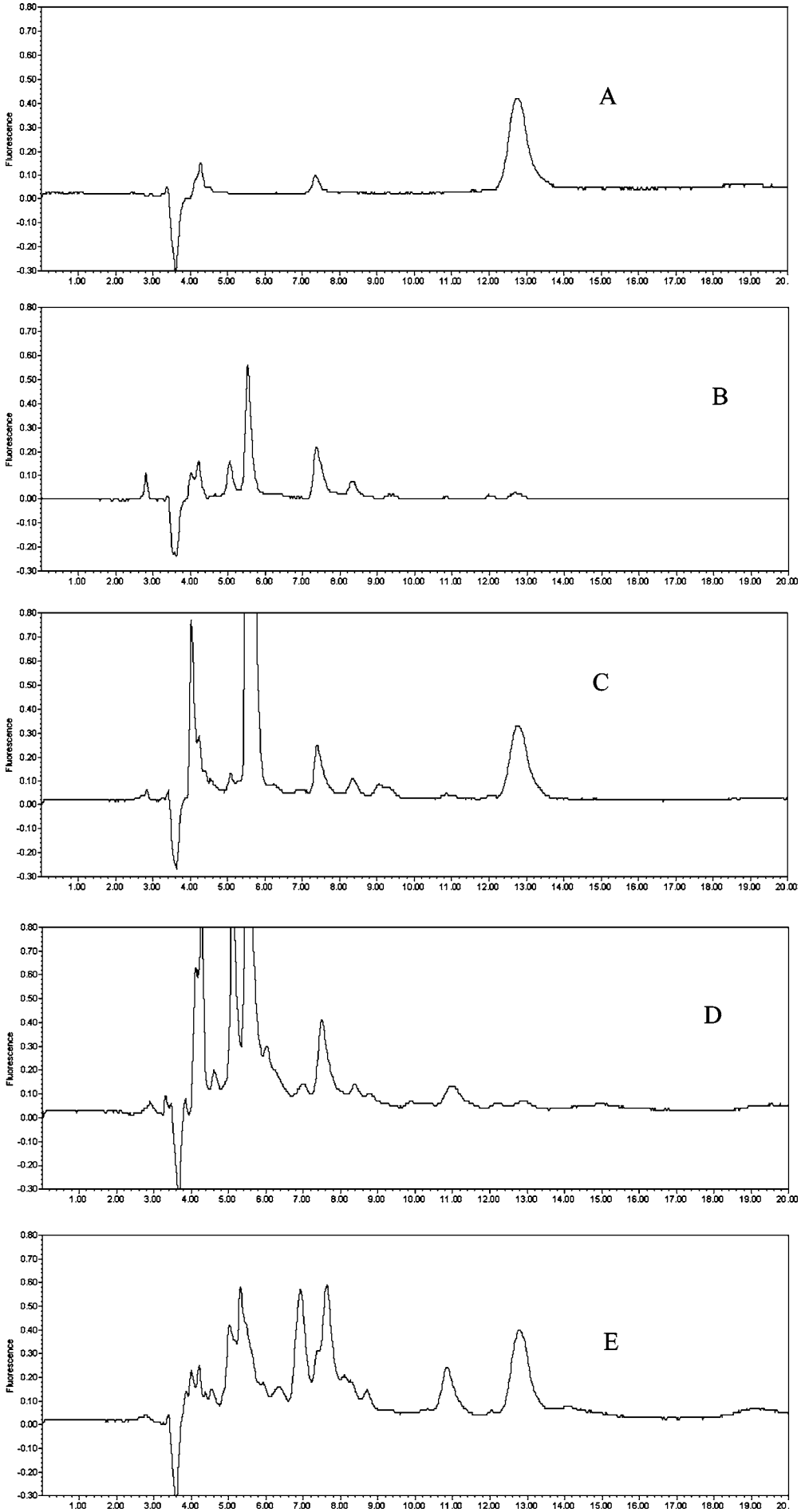
Accuracy and Precision. Method accuracy was determined by spiking control samples with an appropriate volume of a fortification solution. Tissue fortified cations, recoveries, and coefficients of variation (CV) are shown in **Table 2**. Example chromatograms of control and ractopamine-fortified samples from untreated animals for ractopamine are shown in **Figure 2**. (Representative chromatograms of ractopamine residues in muscle, fat, and serum are not shown.) The precision of the method was evaluated by calculating repeatability (between-run precision) and coefficients of variation (CV %) from fortified samples analyzed on three different days (**Table 3**). Assay of sensitivity was determined by spiking control samples with ractopamine over the range of 0.5−50 ng g^{-1} or ng mL^{-1}. The LOD, defined as a signal/noise ratio ≥ 3, was 1 ng g^{-1} for tissues and 0.5 ng mL^{-1} for urine and serum; the limit of quantification (LOQ), defined as a signal/noise ratio ≥ 6, was 2 ng g^{-1} for tissues (recovery of ractopamine in liver at 2 ng g^{-1} was 81.2% with a CV of 3.8%; in kidney, 78.3% with a CV of 3.9%; in muscle, 85.4% with a CV of 7.8%; in fat, 70.5% with a CV of 2.2%) and 1 ng mL^{-1} for urine and serum (recovery of 1 ng mL^{-1} ractopamine in urine was 80.0% with a CV of 5.0%; that in serum was 88.6% with a CV of 5.8%).

Residue Depletion of Ractopamine. The residues of ractopamine in swine tissues (liver, kidney, muscle, fat) and hydrolyzed ractopamine conjugates in urine and serum of pigs treated with β-glucuronidase during the feeding (F7, F14, F28) and withdrawal periods (W1, W2, W3, W7, W9, W14) are shown in **Table 4**. The highest level of parent ractopamine and hydrolyzed ractopamine conjugates was in urine (650.06 ng mL^{-1}) on the seventh day of the feeding period. Ractopamine residues were not detectable at the 14 day withdrawal period. Residue concentrations in kidney were relatively higher than those in liver throughout the withdrawal period, but they were less than one-third of urine at withdrawal day 1. Kidney residues were more slowly eliminated than residues in other tissues. Relative to liver and kidney, ractopamine residues were lower in muscle, fat, and serum with the highest concentrations of 4.94 ng g^{-1}, 4.71 ng g^{-1}, and 9.15 ng mL^{-1}, respectively. Residues in muscle, fat, and serum were not detectable at withdrawal day 1. Urine volumes collected from the bladder at slaughter were limited: for example, only one urine sample was determined at the 7 day collection during the feeding period, because of the lower volumes of urine in bladders.

Study on Hydrolysis by β-Glucuronidase. Although Smith and Shelver reported that about 75 and 95% of synthetic ractopamine glucuronides were hydrolyzed by β-glucuronidase after 2 and 8 h of incubation, which were run overnight at 37 °C (*15*), the enzymatic progress in this paper was accelerated by heating at 65 °C, which has commensurate effects on deconjugation progress. Meanwhile, the fortified volume of β-glucuronidase was proved by the study of a series of fortification, which is shown in **Figure 4** and **Table 5**. Ractopamine concentrations in urine at the feeding period of 28 days with 20 and 50 μL of β-glucuronidase were 125.2 ± 3.3 and 124.0 ± 3.2 ng mL^{-1} ($P > 0.05$). It can be inferred that 0.4 unit of β-glucuronidase helped ractopamine and its metabolites in swine urine to reach a steady state.

DISCUSSION

There are few published studies on the depletion of ractopamine residues from livestock species [Smith and Shelver (*13*) reported tissue residues of ractopamine and urinary excretion of ractopamine and metabolites in cattle, sheep, and ducks treated for 7 days]. We have provided additional data collected from six tissues of swine, which is a useful species for comparisons of the ractopamine residue depletion process. Because of the difficulties in preparing an electrode using an electrochemical detector and the tedious and time-consuming derivatization process for GC-MS detection, the HPLC-FLD used in this study was both practical and sensitive in a number


A
B
C
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E
Fluorescence

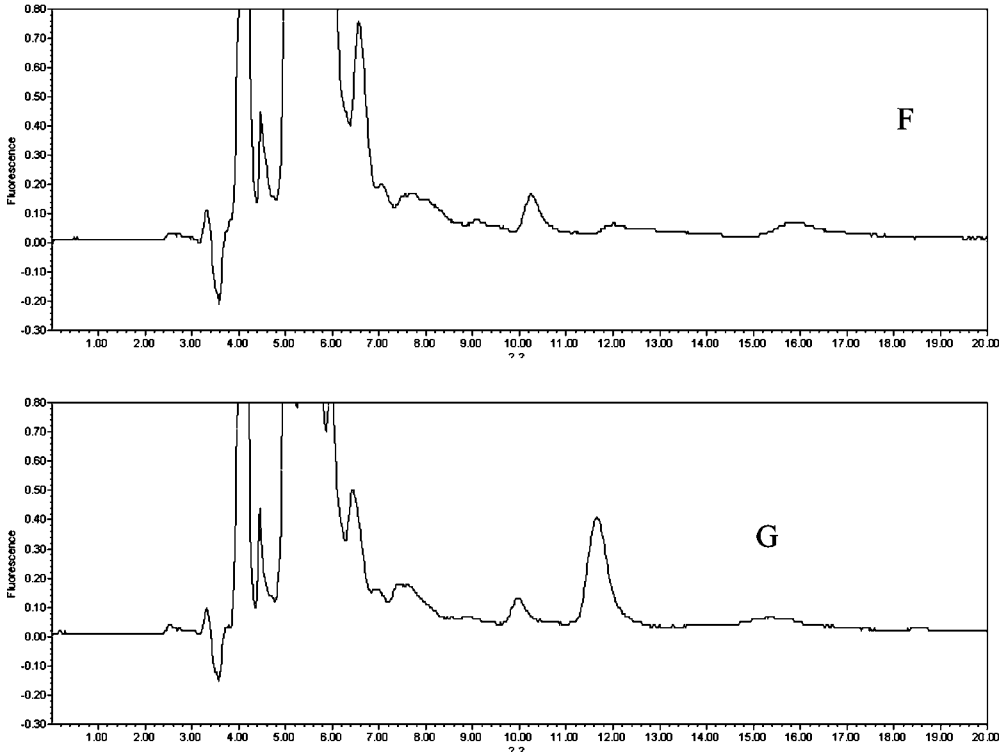

Figure 2. Representative chromatograms of swine samples fortified with ractopamine: (**A**) 10 ng mL^{-1} ractopamine standard; (**B**) control liver; (**C**) control liver fortified at 2 ng g^{-1}; (**D**) control kidney; (**E**) control kidney fortified at 2 ng g^{-1}; (**F**) control urine; (**G**) control urine fortified at 2 ng mL^{-1}.

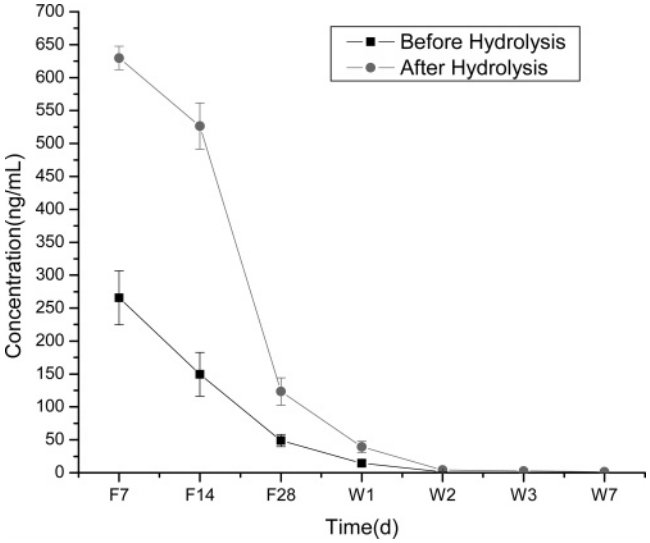

Figure 3. Ractopamine concentrations measured in urine before and after hydrolysis with glucuronidase/sulfatase.

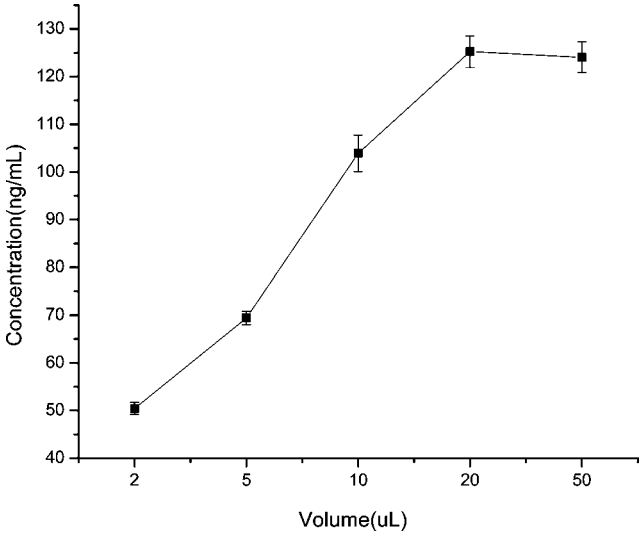

Figure 4. Series of volumes of β-glucuronidase fortified in swine urine at the feeding period of 28 days.

of practices. The LOD and recoveries in this paper were acceptable for the development and establishment for a bioanalytical method (*14*). The urinary excretion study was conducted to explore the entire progress of residue depletion in live hogs.

β-Agonist compounds are extensively metabolized to β-glucuronide and/or sulfate conjugates in humans and animals. Consequently, a deconjugation step was used during the sample preparation for the present study. The level of ractopamine and its conjugates in swine urine processed with enzyme hydrolysis was almost twice that of urine samples analyzed without enzyme hydrolysis (**Figure 3**). Attempts to liberate ractopamine conjugates in tissues after evaporation of extraction supernatants resulted in less release of interfering compounds and impurities than when conjugates were hydrolyzed prior to extraction.

The volume of fortified β-glucuronidase was optimized by a series of fortifications shown in **Figure 4**. Ractopamine concentrations in urine at the feeding period of 28 days with 20 and 50 μL of β-glucuronidase were 125.2 $\pm$ 3.3 and 124.0 $\pm$ 3.2 ng mL^{-1} ($P > 0.05$). The data indicate that 0.4 unit of β-glucuronidase helped to cause a complete release of ractopamine.

The extraction effects of acetone and other kinds of extraction liquids such as methanol and acetonitrile were also compared (**Figure 8**). Due to higher recoveries, acetone was selected as the extraction solution for swine tissues (**Figure 2**). Ractopamine is a mixture of four stereoisomers (*RS*, *SR*, *RR*, and *SS*) (*15*). In this method, these diastereoisomers were not distinguished and appeared as a single peak on the chromatograms. 1-Pentanesulfonic acid used in mobile phase helped to give the excellent

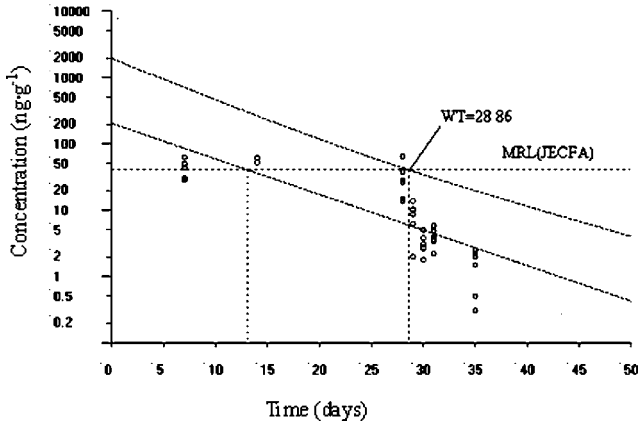

Figure 5. Plot of withdrawal time calculation for swine liver at the time when the one-sided 95% upper tolerance limit was below the JECFA MRL (40 ng g^{-1}).

Table 4. Residue Depletion of Ractopamine and Its Metabolites in Swine Tissues, Urine, and Serum Samples Treated with β-Glucuronidase ($n = 3$)[a]

matrix	no.	F7	F14	F28	W1	W2	.W3	W7	W9	W14
liver	1	43.3	62.1	14.0	13.6	2.6	4.9	ND	ND	ND
(ng g^{-1})	2	61.8	61.9	28.0	2.0	5.1	5.8	ND	ND	ND
	3	61.7	61.2	37.5	10.1	3.8	3.4	<2	ND	ND
	4	30.4	61.7	15.0	10.4	2.7	3.7	2.5	ND	ND
	5	28.6	52.4	65.7	6.3	1.8	2.2	2.2	<2	ND
	6	50.8	60.4	26.5	8.8	3.1	3.9	2.0	<2	ND
kidney	1	158.0	194.8	53.8	16.1	6.4	7.4	2.0	ND	ND
(ng g^{-1})	2	232.1	148.9	74.5	5.6	12.8	8.3	2.0	ND	<2
	3	175.9	181.0	61.0	12.2	5.7	4.9	8.7	4.36	<2
	4	172.9	177.4	53.3	37.1	9.4	6.3	4.3	<2	ND
	5	94.4	155.8	118.0	7.1	4.7	6.1	5.5	3.34	<2
	6	182.4	173.3	62.2	10.6	5.5	7.0	3.5	<2	ND
muscle	1	5.7	3.3	<2	ND	–	–	–	–	–
(ng g^{-1})	2	4.6	3.6	3.0	ND	–	–	–	–	–
	3	5.2	4.2	2.2	ND	–	–	–	–	–
	4	5.4	4.3	<2	ND	–	–	–	–	–
	5	3.4	3.3	4.2	ND	–	–	–	–	–
	6	5.3	3.6	2.1	ND	–	–	–	–	–
fat	1	1.9	5.1	ND	ND	–	–	–	–	–
(ng g^{-1})	2	2.2	5.7	<2	ND	–	–	–	–	–
	3	3.8	7.4	2.0	ND	–	–	–	–	–
	4	5.7	2.8	ND	ND	–	–	–	–	–
	5	2.3	2.5	ND	ND	–	–	–	–	–
	6	3.9	4.9	<2	ND	–	–	–	–	–
urine	1	650.1	565.3	128.6	30.4	6.1	3.7	1.6	<1	ND
(ng mL^{-1})	2	/	/	121.9	63.4	5.0	/	2.0	<1	ND
	3	/	/	/	47.1	7.3	/	2.1	<1	ND
	4	/	/	/	46.9	/	/	1.9	<1	ND
serum	1	9.6	9.3	<1	ND	ND	–	–	–	–
(ng mL^{-1})	2	9.4	8.4	<1	<1	ND	–	–	–	–
	3	5.1	9.9	1.5	ND	ND	–	–	–	–
	4	6.6	9.0	<1	<1	ND	–	–	–	–
	5	6.8	9.4	1.5	<1	ND	–	–	–	–
	6	7.5	9.0	<1	ND	ND	–	–	–	–

[a] ND, not detected; –, not measured; /, not sampled.

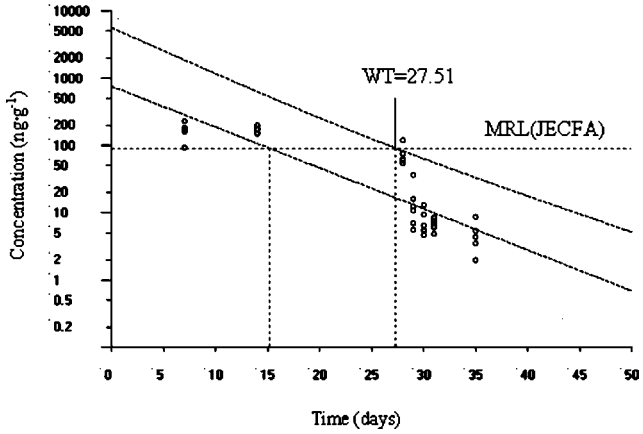

Figure 6. Plot of withdrawal time calculation for swine kidney at the time when the one-sided 95% upper tolerance limit was below the JECFA MRL (90 ng g^{-1}).

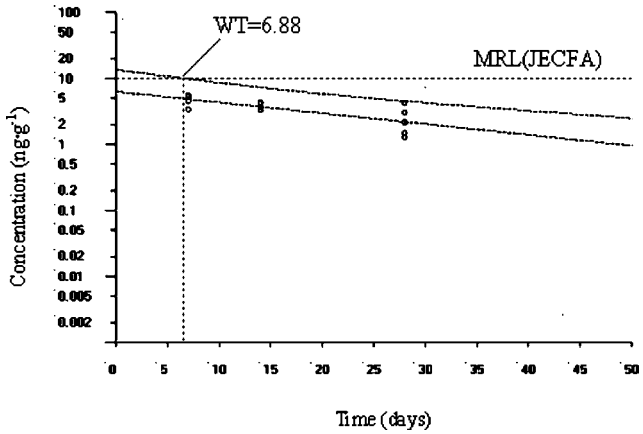

Figure 7. Plot of withdrawal time calculation for swine muscle at the time when the one-sided 95% upper tolerance limit was below the JECFA MRL (10 ng g^{-1}).

peak shape, and ractopamine was not interfered with by other impurities in the matrix (**Figure 2**). The LOD of this procedure was 1 ng g^{-1} for tissues and 0.5 ng $mL^{-1}$for urine and serum.

In Dalidowicz's depletion studies, 16 pigs received a ration containing 20 mg kg^{-1} [^{14}C]ractopamine hydrochloride for 7 days (*12*). Total radioactive residues of ractopamine (parent drug and metabolites) in kidney (116 ± 14 ng g^{-1}) were higher than those in liver (106 ± 30 ng g^{-1}) at 24 h from the last ractoamine exposure, but they were lower than those in liver after withdrawal periods of 48 and 72 h. In the present depletion study, pigs received a ration containing 18 mg kg^{-1} ractopamine for 28 days. Administration for 19−24 days is considered to be the period having maximum pharmacological effects for ractopamine, and the 28 day feeding period in this study is the longest among published studies. The residue concentrations in kidney and liver at 24 h from the last ractoamine exposure were 14.0 ± 1.0 and 8.0 ± 1.0 ng g^{-1}, both of which are lower than the results of Dalidowicz's depletion studies. Differences of the residue concentrations may be the result of two reasons.

Table 5. Comparison between Ractopamine Concentrations Measured in Urine before and after Hydrolysis with β-Glucuronidase

	ractopamine concentration in urine (ng mL^{-1}) (mean ± SD)						
	F7	F14	F28	W1	W2	W3	W7
before hydrolysis	265.7 ± 40.9	149.5 ± 33.2	48.9 ± 9.3	14.5 ± 4.0	1.3 ± 0.4		
after hydrolysis	629.8 ± 17.6[a]	526.5 ± 35.1[a]	123.4 ± 20.8[a]	39.2 ± 8.6[a]	4.3 ± 1.8[a]	2.8 ± 1.4	1.5 ± 0.4

[a] t test for treatment is significant, $P < 0.05$.

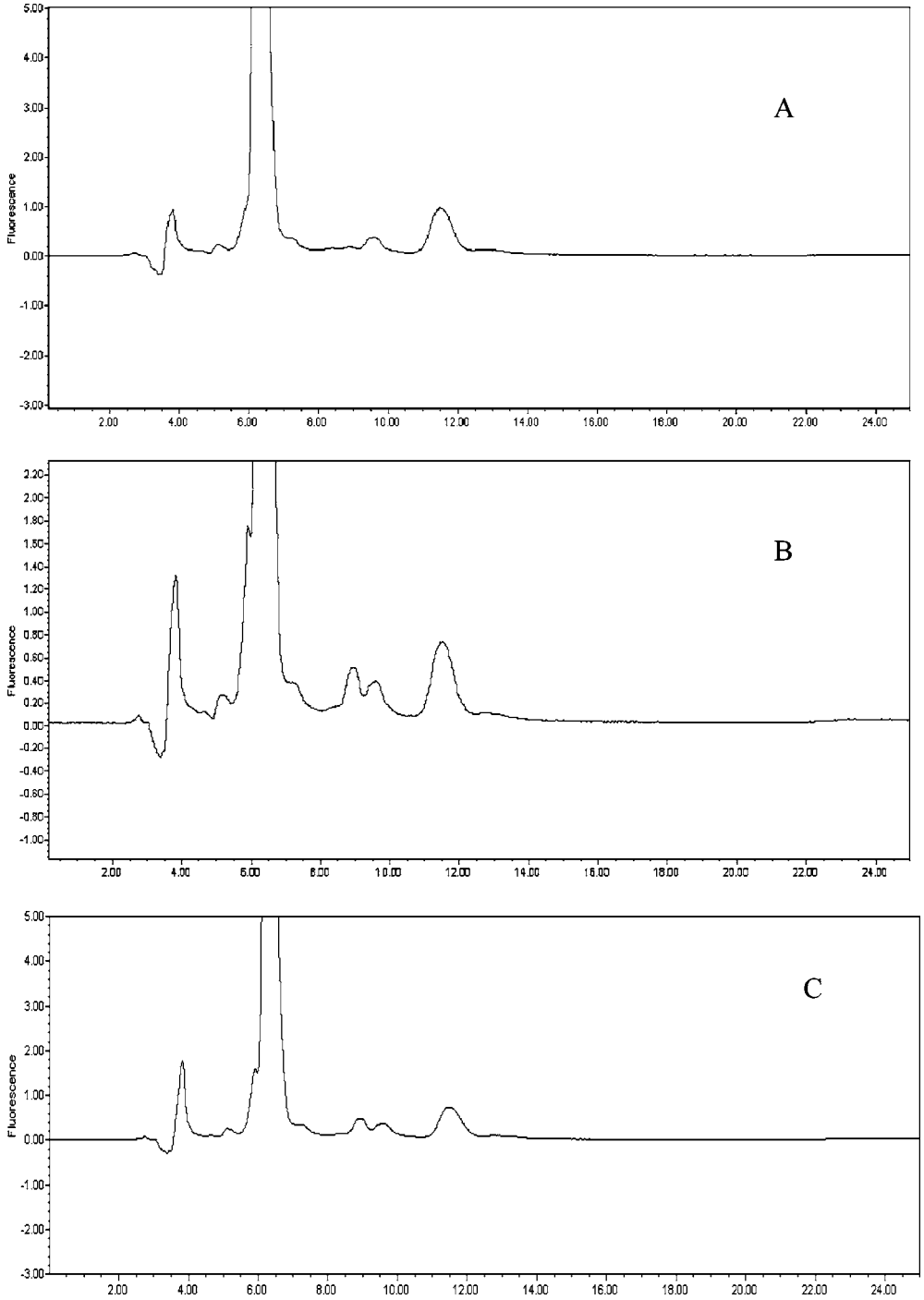

Figure 8. Representative chromatograms of swine kidney fortified with ractopamine at 10 ng g^{-1}: (**A**) kidney samples extracted by acetone; (**B**) kidney samples extracted by methanol; (**C**) kidney samples extracted by acetonitrile.

One is the physiological differences between pigs, especially under different living environments. Meanwhile, diverse compositions of feeds combined with ractopamine and amounts of feeds fed during feeding period would lead to the different results. Another is the feeding period of 28 days, which is the longest among the published literature related to the residue depletion of ractopamine in swine. Also, a few hours difference in the relative slaughter time would make a big difference in tissue residues.

Kidney residues were much greater than liver residues during the feeding period but were essentially the same as kidney during the withdrawal period, both of which showed a trend of rising at the beginning and falling afterward. The data suggest that the appropriate target tissue for a routine monitoring program is kidney (*12*). Turberg reported that for pigs fed dietary (15 mg kg^{-1}) unlabeled ractopamine for 6 days, residues in kidney, liver, muscle, and fat at 0 days following the last treatment were 45 ± 11, 26 ± 8, 5, and 1 ng g^{-1}, respectively (*12*). In this depletion study, the levels of ractopamine plus metabolites in kidney, liver, muscle, and fat at 0 days after the last treatment were 70, 31, and 3 ng g^{-1} and not detected, respectively. An additional depletion study was conducted in which 48 pigs were fed a ration containing 20 mg kg^{-1} ractopamine hydrochloride for 14 days and then slaughtered at 12 h and at 1, 2, 3, 4, and 5 days after the last ractopamine administration (*12*). Ractopamine residues in kidney and liver were not detected at the fifth day of the withdrawal period in that study, but the concentrations of ractopamine and metabolites in corresponding

Table 6. Tolerance and MRL Value Established by Regulatory Agencies

	matrix	tolerance (or MRL) (ng g^{-1})
FDA	liver	750
	kidney and fat	1500
	muscle	250
JECFA	liver	40
	kidney	90
	muscle and fat	10

tissues of this study were 4 and 2 ng g^{-1}, respectively, at the 7 day withdraw period. Ractopamine and metabolites in serum were only 1 ng mL^{-1} at the feeding period of 28 days and were not detected thereafter, which was similar to residues in fat. The concentrations of ractopamine and metabolites in swine edible tissues at the withdrawal period of 0 days were lower than the value of tolerance established by the FDA and the MRL listed by the JECFA (Joint FAO/WHO Expert Committee on Food Additives; **Table 6**; **Figures 5**−**7**). Data collected from swine edible tissues from this study support the established withdrawal time of 0 days by the FDA for ractopamine when used as a feed additive in swine.

Urine is a common matrix for the analysis of the β-agonists, although residue concentrations may be higher in liver and hair for some β-agonists (*16*). However, swine urine has not been sufficiently studied for ractopamine depletion compared to bovine urine (*2*). Dalidowicz reported that recovery of radioactivity of the entire urinary and fecal output collected from pigs over a 7-day period was 96.5% of the dosed radioactivity, of which 88.1% was via urine and 8.4% via feces (*12*). Data in **Table 4** indicate that the excretion of parent ractopamine and hydrolyzed ractopamine conjugates in swine urine is of sufficient magnitude to easily detect its use concurrently with feeding, which was highest and reached 650 ng mL^{-1} at a feeding period of 7 days. The elimination of the drug and its metabolites in urine was faster than that in kidney. If animals are provided unfortified feed, levels of the drug in urine fall rapidly, but are detectable until the ninth day of withdrawal.

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