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Simultaneous Detection of Residues of 25 β_2-Agonists and 23 β-Blockers in Animal Foods by High-Performance Liquid Chromatography Coupled with Linear Ion Trap Mass Spectrometry

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ABSTRACT: A sensitive method has been developed for the simultaneous determination of residues of 25 β_2-agonists and 23 β-blockers in animal foods by high-performance liquid chromatography coupled with linear ion trap mass spectrometry (HPLC-LIT-MS). This method is based on a new procedure of hydrolysis and extraction by 5% trichloracetic acid, and then cleaned up by mixed strong cation exchange (MCX) cartridges coupled with a novelty cleanup step by methanol. Methanol and 0.1% formic acid were used as mobile phases for gradient elution, while a Supelco Ascentis Express Rp-Amide column was used for LC separation. ESI positive ion scan mode was used with consecutive reaction monitoring (CRM, MS^3). Nine β_2-agonists labeled by the deuterium isotope were used as internal standards for quantification. The linear ranges of 48 analytes were from 5 to 200 μg/L; the coefficient of correlation was not less than 0.995. Blank pork muscle, blank liver, and blank kidney were selected as representative matrix for spiked standard recovery test. The recoveries of each compound were in the range of 46.6−118.9%, and the relative standard deviations were in the range of 1.9−28.2%. Decision limits (CCα, α = 0.01) of 48 analytes in muscles, liver, and kidney samples ranged from 0.05 to 0.49 μg/kg, and the detection capability (CCβ, β = 0.05) ranged from 0.13 to 1.64 μg/kg. This method was successfully applied to 110 real animal origin food samples including meat, liver, and kidney of pig and chicken samples.

KEYWORDS: *β_2-agonists and β-blockers, high-performance liquid chromatography, linear ion trap mass spectrometry, consecutive reaction monitoring, animal food*

INTRODUCTION

β_2-Agonists, which are well-known for their ability to improve growth rate and reduce carcass fat when fed to farm animals, were often adulterated into animal feed illegally.[1−3] Aome other similar chemicals, β-blockers, were misused during animal transportation to prevent sudden death caused by alarm reaction due to their sedation effect.[3]The residues of the misused drugs that accumulated in animal tissues can cause symptoms of acute poisoning in humans.[1−3] Therefore, the use of β_2-agonists and β-blockers in animal breeding is banned in many countries.[1−3] Furthermore, β_2-agonists and β-blockers are substances listed in the prohibited list of the World Anti-Doping Code by the World Anti-Doping Agency.[4]

In an effort to combat the illicit use of β_2-agonists and β-blockers, regulatory organizations worldwide are testing animal tissues for the presence of these drugs. During the monitoring process, various analytical methods, which were mainly focused on GC−MS [5−7] and LC−MS,[8−15] have been reported. Because of the relatively complicated operation and poor stability of derivatives, application of GC/MS for the detection of β_2-agonists and β-blockers decreased year by year.[5−7] More and more LC−MS[8−17] methods have showed up recently. For determination of β_2-agonists and β-blockers, the LC−MS method provides the advantages of time-savings, convenience, and rapidness. However, there are drawbacks when LC−MS methods were used for the determination of β_2-agonists and β-blockers. The most important is ion suppression caused by matrix effect during LC−MS detection. When using matrix-matched calibration and stable isotope dilution, multiple MS detection is very effective and feasible to overcome matrix effect. So in this study, the scan mode of Consecutive Reaction Monitoring (CRM), that is, MS^3 detection, is applied to the MS detection of β_2-agonists and β-blockers.

Mixed mode solid-phase extraction cartridges, such as C8 or C18 combined with strong cation exchange cartridge, are commonly used in the cleanup procedure of β_2-agonists or β-blockers in animal tissues.[8,10,12] To achieve the most effective purification, techniques such as sample extraction by 5% trichloracetic acid, a hexane wash step in the solid phase extraction procedure, and an additional procedure after SPE for protein and phospholipids precipitation by methanol were applied in the present method. On the basis of the MS^3 detection and newly developed purification steps, a simple, accurate, and reliable LC/MS^3 method for the determination of 25 β_2-agonists and 23 β-blockers in animal food was established.

MATERIALS AND METHODS

Reagents and Materials. Methanol and acetic acid (chromatographic pure) were purchased from Baker JT (NJ). Formic acid (99%) was purchased from Acros Organics (NJ). β-Glucuronidase/arylsulfatase (116 400 unit/mL) was purchased from Sigma-Aldrich (Saint Louis, MO). Perchloric acid, trichloracetic acid (TCA),

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ammonia, and sodium hydroxide were purchased from Beijing Chemical Reagents Co. (Beijing, China). Oasis MCX solid-phase extraction cartridges (6 mL, 150 mg) were purchased from Waters Corp. (Milford, MA). Deionized water was prepared by a Milli-Q Plus system at 18.2 M (MilliPore, Bedford, MA).

Standards. Brombuterol hydrochloride, clenisopenterol hydrochloride, clencyclohexerol, clenhexerol, cimbuterol, and mapenterol hydrochloride were purchased from WITEGA Laboratorien Berlin-Adlersh of GmbH, Germany; mabuterol and cimaterol were from Boehringer Ingekheim, Germany; salmeterol was obtained from Toronto Research Chemical Inc., U.S.; clenproperol, clenpenterol, d7-cimaterol, d6-salbutamol, d9-cimbuterol, d7-clenproperol, d5-ractopamine, d9-mabuterol, d11-mapenterol, and d6-clenbuterol were purchased from EU Reference Laboratory, Germany; metaproterenol, terbutaline, salbutamol,procaterol, fenoterol, clenbuterol, ractopamine, tulobuterol, formoterol, fumarate, bambuterol hydrochloride, ritodrine hydrochloride, metoprolol, labetalol hydrochloride, propranolol hydrochloride, betaxolol, penbutolol sulfate, sotalol hydrochloride, and esmolol hydrochloride were obtained from Sigma-Aldrich, U.S.; clorprenaline hydrochloride was purchased from the National Institute for the Control of Pharmaceutical and Biological Products, China; and NA-1141 was a gift from EU Reference Laboratory Germany. Nadolol, timolol, oxprenolol, alprenolol, bunolol, carazolol, acebutolol, celiprolol, atenolol, and carvedilol were purchased from Dr. Ehrenstorfer GmbH, Germany; metipranolol was from the British Pharmacopoeia Commission Laboratory, UK. D3-Salmeterol was purchased from Cambridge Isotope Laboratories Inc., U.S. The purity of all of these standards was not less than 96%.

Sample Extract and Cleanup. 5.0 g of homogenated animal tissue sample was weighed into a 50 mL polypropylene centrifuge tube, 50 μL of the pooled internal standard solution at the concentration of 1 mg/L was spiked into the sample, and then 10 mL of 5% trichloracetic acid solution was added. The mixture was vortexed for 30 s, and ultrasonicated for 30 min at 80 °C. After ultrasonication, centrifugation was conducted at 10 000 rpm for 10 min at 0 °C. The supernatant was transferred into a 25 mL tube. The sediment in the tube was extracted with 5 mL of 5% trichloracetic acid once again. The two extracts were combined together.

The Oasis MCX cartridge was preconditioned with 6.0 mL of methanol and 6.0 mL of water. The extract was applied to the preconditioned cartridge. The cartridge was sequentially washed with 3 mL of water, 5 mL of hexane, and 1 mL of methanol. The cartridge was dried with a vacuum for 5 min after each washing step. The analytes were eluted with 6.0 mL of methanol containing 5% ammonia into a 10 mL test tube.

The eluent was dried under a gentle nitrogen stream until nearly dry. The residue was dissolved in 1 mL of methanol, and then centrifuged at 10 000 rpm for 10 min at 4 °C. The sediment was washed with 1 mL of methanol once again. The supernatants were combined together and evaporated to dryness under a small flow of nitrogen. The residue then was dissolved in the mixed solution of 1.0 mL of methanol and 0.1% formic acid (2:8, v/v) and then filtered through a 0.22 μm nylon filter for LC−MS^3 analysis.

Sample Collection and Preparation. Three Adult ARBOR ACR ES Broilers were treated over a period of 10 days with an oral dose of terbutaline (5 mg/d) and salbutamol (0.2 mg/d). The drugs were added into the feeds, and the broilers were housed in Huadu broiler farm. All three broilers were euthanized at the end of the tenth day, and then livers, kidneys, and muscle tissues were taken as the incurred positive samples. Pork livers, pork kidneys, and chicken samples were obtained from the local markets. All of the tissue samples were homogenized and kept at −20 °C.

HPLC-LITMS Conditions. Liquid chromatographic separation was performed by an HPLC system equipped with a LC pump and an autosampler of Surveyor (Thermo-fisher, Waltham, MA) on a Supelco Ascentis Express Rp-Amide column (150 mm × 2.1 mm, 2.7 μm particle size). The column oven temperature was set at 30 °C, the flow rate of the mobile phase was 100 μL/min, and the injection volume was 10 μL. Optimal separation of the target compounds was achieved by gradient elution using methanol (A) and a 0.1% formic acid aqueous solution (B). The HPLC conditions are shown in Table 1. A 5 min equilibration was used between injections. The total chromatographic and equilibration time was 45 min for each run.

Table 1. HPLC Analysis Conditions

column	Supelco Ascentis express Rp-Amide column (150 mm × 2.1 mm, 2.7 μm particle size)		
flow rate (μL/min)	100		
needle rinse	MeOH/water (1:1, v/v)		
mobile phase A	water with 0.1% FA		
mobile phase B	MeOH with 0.1% FA		
gradient	time (min)	percentage of mobile phase A	percentage of mobile phase B
	0	80	20
	5	80	20
	15	75	25
	25	60	40
	35	40	60
	40	0	100
	41	80	20
	45	80	20
injection (μL)	10		

Mass spectrometric analysis was carried out on a linear ion trap mass spectrometer (LTQ) (Thermo-fisher, Waltham, MA) using the positive electrospray ionization mode (ESI+). The Consecutive Reaction Monitoring (CRM) scan mode, that is, MS/MS/MS (MS^3), was used. Detailed information of MS^3 is listed in Table 2. The voltage of the Ispray was set at 4.5 kV. The flow rates of the sheath gas and the auxiliary gas were 35 and 15 arb, respectively. The temperature of the capillary was 325 °C, and the capillary voltage was set at 36.7 V. High-purity helium (>99.99%) was used as collision gas. The parameters of the linear ion trap mass spectrometry were set as follows: full AGC target was 10 000.0, SIM AGC target was 5000.0, MSn AGC target was 5000.0, and Zoom AGC target was 3000.0. Figure 1 shows the total ion chromatogram and the chromatograms of the quantitative transition of MS^3 of the matrix-matched standard solution.

Calibration and Quantification. For the quantification of the 48 analytes, 9 deuterium isotope standards were used as internal standards (ISTD). The ISTD that eluted most closely was used for those compounds that were not available in the isotopically labeled form. The peak areas of the analytes (A) and the internal standards (A_i) were recorded. The calibration curve was established on the basis of the ratio of A/A_i versus the corresponding concentration of the analyte. To minimize the matrix effect, seven-point (5, 10, 20, 40, 80, 160, and 200 μg/L) matrix-matched calibration curves were constructed.

Quality Control. The quality control for the sample analysis consists of system blank and QC sample analysis. Blank animal tissue samples were selected as system blank to demonstrate low system background for each matrix to identify whether the matrix is contaminated by β_2-agonist and β-blocker drugs. QC samples were prepared at levels of low-QC (LQC, 5 μg/kg), mid-QC (MQC, 10 μg/kg), and high-QC (HQC, 20 μg/kg). A complete plate was run on each day composed of working standards, QC samples, and blank extracts.

RESULTS AND DISCUSSION

Sample Extraction. Most of the β_2-agonist and β-blocker drugs will integrate with glucuronic acid or sulfuric acid to form glcuronide or sulfuride conjugates in vivo, especially phenol-based drugs, such as salbutamol, procarterol, metaproterol, and bisoprolol. However, the conjugates cannot be directly extracted from the samples. Tissue samples should be hydrolyzed to release the analytes from conjugates. Enzymatic hydrolysis[8,11−14] and acid hydrolysis[9,15,16] are the commonly used techniques. In many articles, enzymatic hydrolysis was conducted before sample extraction.[8,11,13,14] However, enzymatic hydrolysis needs at least 2 h or even overnight. In this study, the hydrolysis

Table 2. Mass Spectrum Parameters for β_2-Agonists and β-Blockers[a]

compounds	retention time (min)	parent ion (m/z)	CE[b] (V)	daughter ion monitoring (m/z)	CE[b] (V)	granddaughter ion monitoring (m/z)
metaproterenol	3.48	212	27	194*, 152	34	152*, 177
D7-cimaterol	3.63	227	19	209*	25	161*, 146
cimaterol	3.65	220	25	202*	30	160*, 145
atenolol	3.67	267	30	225*, 190	27	208*, 190
sotalol	3.69	273	20	255*, 213	28	213*, 176
D6-salbutamol	3.70	246	20	228*, 167	25	167*, 148
salbutamol	3.72	240	21	222*, 166	26	166*, 148
terbutaline	3.94	226	30	152*, 167	35	125*, 135
D9-cimbuterol	4.30	243	20	225*, 161	20	161*
cimbuterol	4.32	234	22	216*, 160	25	160*
procaterol	5.13	291	22	273*, 232	30	231*, 214
carteolol	6.63	293	22	237*	22	202*, 164
clencyclohexerol	6.65	319	20	301*	24	203*, 188
fenoterol	6.73	304	24	286*, 135	25	135*, 107
nadolol	6.76	310	24	254*, 236	25	236*, 201
pindolol	6.92	249	29	116*, 172	40	74*, 72
NA-1141	7.39	293	18	275*	22	203*
D7-clenproperol	7.47	270	19	252*	25	204*, 189
clenproperol	7.33	263	24	245*	30	203*, 188
ritodrine	7.66	288	25	270*, 150	30	150*, 121
clorpenaline	8.59	214	23	196*	30	154*
D6-clenbuterol	10.75	283	18	265*, 204	21	204*, 203
clenbuterol	10.94	277	24	259*, 203	20	203*
metoprolol	11.65	268	34	191*, 116	30	159*, 131
timolol	11.67	317	24	261*, 244	23	244*, 188
tulobuterol	12.42	228	28	154*, 172	40	118*, 119
D5-ractopamine	12.53	307	18	289*, 167	20	167*, 121
ractopamine	12.56	302	20	284*, 164	20	164*, 121
bromchlorbuterol	13.21	323	20	305*, 249	20	249*, 207
acebutolol	14.44	337	25	319*, 260	25	244*, 260
brombuterol	15.98	367	18	349*, 293	20	293*
formoterol	16.19	345	20	327*, 149	25	149*, 121
D9-mabuterol	16.58	320	20	302*, 238	18	238*
bunolol	16.60	292	25	236*, 201	26	201*, 189
esmolol	16.64	296	26	219*, 254	28	145*, 187
levobunolol	16.67	292	22	236*	22	201*, 189
mabuterol	16.80	311	20	293*, 237	22	237*
clenpenterol	17.77	291	20	273*, 203	22	203*
oxprenolol	19.07	266	30	248*, 225	30	206*, 189
bambuterol	19.80	368	20	312*, 294	20	294*
celiprolol	20.24	380	24	307*, 306	25	251*, 233
bisoprolol	23.07	326	27	116*, 222	42	72*, 74
clenisopenterol	23.85	291	18	273*	27	217*, 188
D11-mapenterol	24.12	336	20	318*, 238	18	238*
mapenterol	24.39	325	21	307*, 237	21	237*
labetalol	26.34	329	20	311*, 207	25	207*, 294
metipranolol	27.00	310	29	233*, 191	25	191*, 233
alprenolol	27.79	250	32	116*, 173	41	74*, 56
propranolol	28.06	260	30	183*, 116	36	155*, 165
betaxolol	28.45	308	32	116*, 177	42	74*, 72
carazolol	28.47	299	29	222*, 116	36	194*, 180
clenhexerol	32.11	305	20	287*	30	188*, 217
carvedilol	36.07	407	24	283*, 224	25	210*, 212
penbutolol	37.77	292	25	236*, 201	29	201*, 168
nekivolol	38.02	406	25	388*, 151	25	208*, 224
D3-salmeterol	38.43	419	20	401*, 383	20	383*, 382
salmeterol	38.52	416	20	398*, 380	20	380*, 232

[a]"*" means the quantitative ion; collision energy (%) is the normalization energy. [b]CE means collision energy. Q value is 0.25. Isolation width (m/z) is 2.

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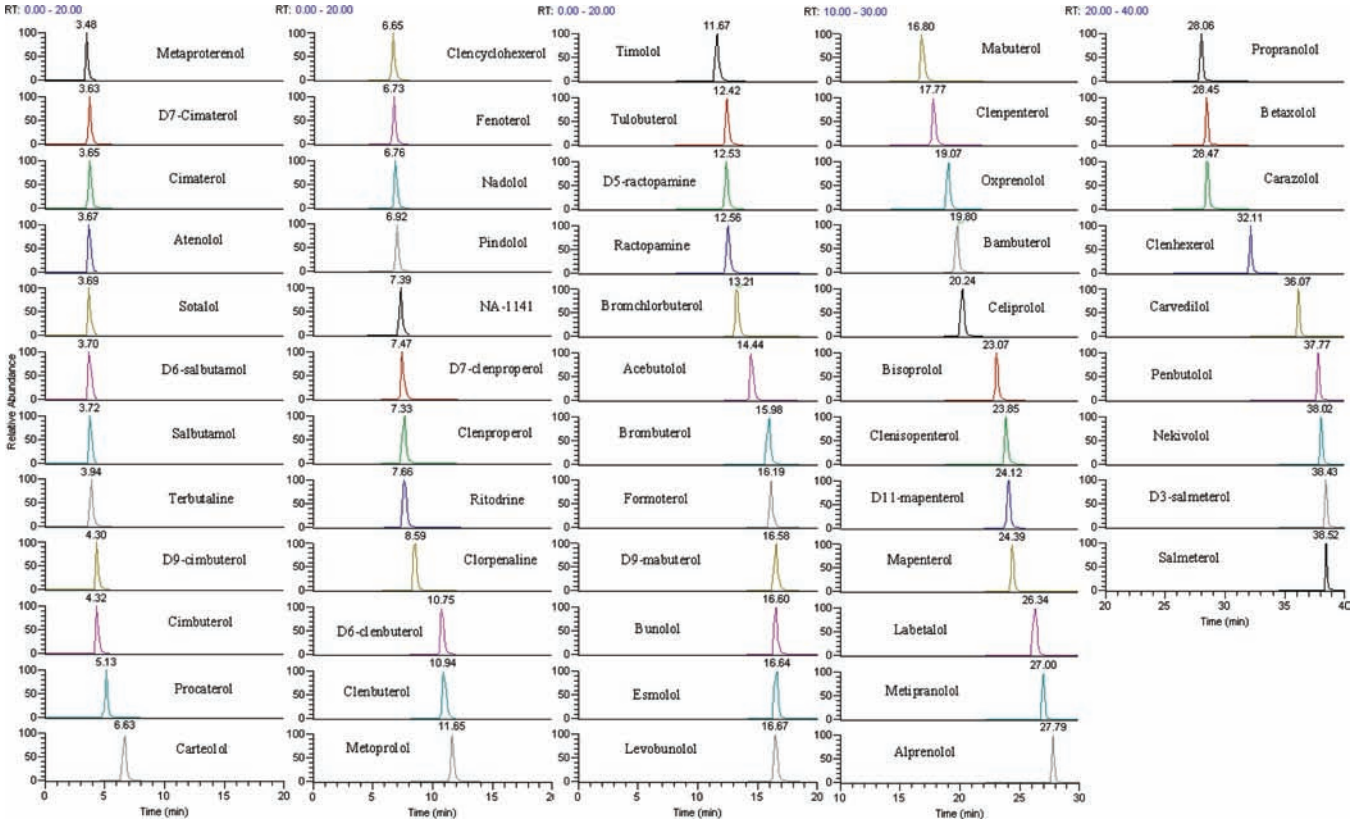

Figure 1. Chromatograms of quantification transition of MS^3 of blank porcine muscle matrix-matched standard (0.040 mg/kg).

efficiency of enzymatic hydrolysis and acid hydrolysis when coupled with MCX SPE cleanup was investigated using the incurred samples. Acid hydrolysis by 10 mL of 5% TCA (ultrsonication for 30 min) and enzymatic hydrolysis by 100 μL of β-glucuronidase/arylsulfatase (at 55 °C for 2 h) were tested, respectively. As the results show in Table 3, acid hydrolyzed

Table 3. Selection of Hydrolysis Reagent

		concentration (μg/kg)	
positive samples	hydrolysis	terbutaline	salbutamol
chicken liver	enzymolysis	16.77	8.96
	TCA acidolysis	62.44	15.41
chicken kidney	enzymolysis	161.89	15.58
	TCA acidolysis	153.63	74.41

using 5% TCA was the best choice for the present method. Furthermore, 5% TCA not only played the role of hydrolyzing the conjugates, but also served as extraction reagent for its strong acidity and denaturing agent to precipitate the protein in animal origin samples.

Various concentrations of TCA were tried, including 1%, 5%, and 10%, with 5% trichloracetic acid giving the most satisfactory results. Therefore, 10 mL of 5% trichloracetic acid solution was selected as the extraction solvent and hydrolysis reagent as well.

Sample Cleanup. The purification methods for β_2-agonists and β-blockers are mainly based on mixed phase solid-phase extraction cartridges, such as C8 or C18 combined with strong cation exchange cartridge.[8,10,12] In this study, MCX cartridges were used for sample purification. Unfortunately, there was strong matrix inhibition during the process of mass spectrometry as shown in Table 4. Moragues et al.[17] had previously applied a hexane wash step in the solid extraction procedure to remove interference in matrix. On the basis of this reference, we applied the hexane wash step in SPE procedure as well, but the phenomenon of ion suppression improved little. Also, there still was a big peak at 2.92 min in the chromatogram of the blank pork sample, which was inferred to be phospholipids from tissues. The response of metaproterenol, cimaterol, atenolol, sotalol, salbutamol, terbutaline, and cimbuterol was affected by the big peak, which were clearly showed by the ion suppression as listed in Table 4. To solve this problem, an extra cleanup procedure after MCX SPE cleanup was tried. The SPE residues were dissolved in 1 mL of methanol, and white flocculent precipitate was formed. After high-speed freezing centrifugation, the precipitate was washed with another 1 mL of methanol, the sediment after centrifugation was discarded, and the supernatants were combined and concentrated to dryness. The residue was reconstituted in 1.0 mL of methanol + 0.1% formic acid (2:8, V/V) and was injected into LC−MS. The response of the peak at 2.92 min decreased dramatically, and the background decreased as well. The effect of ion suppression improved after cleanup with methanol as shown in Table 4. So an additional methanol cleanup procedure was used after MCX cleanup.

Acid hydrolysis by 5% TCA companied by the extraction step including a novelty cleanup step by methanol was used in the present method, which significantly shortened the pretreatment progress and reduced the ion suppression of LC−MS process.

Method Validation. Validation parameters for quantification of 25 β_2-agonists and 23 β-blockers were obtained under the optimal conditions. All 48 compounds showed good linear regression in the range of 5−200 μg/L, and the correlation coefficients (r) were not less than 0.995.

Table 4. Ion Suppression (%) in Porcine Muscle Samples (n = 3)

	without methanol precipitation (μg/kg)			methanol precipitation (μg/kg)				without methanol precipitation (μg/kg)			methanol precipitation (μg/kg)		
compounds	10	40	80	10	40	80	compounds	10	40	80	10	40	80
metaproterenol	88	75	85	64	57	47	brombuterol	38	36	37	15	6	24
cimaterol	97	97	96	32	33	28	formoterol	22	16	36	11	11	7
atenolol	70	54	41	67	69	64	bunolol	15	11	18	20	5	2
sotalol	66	74	70	61	66	61	esmolol	16	4	29	11	10	18
salbutamol	89	89	87	82	74	68	levobunolol	25	17	25	41	4	6
terbutaline	98	97	95	70	47	35	mabuterol	53	17	37	20	23	20
cimbuterol	77	80	71	65	67	45	clenpenterol	29	30	34	22	19	23
procaterol	68	67	77	39	33	29	oxprenolol	14	12	19	10	21	9
carteolol	87	65	2	15	13	20	bambuterol	25	42	39	16	8	11
clencyclohexerol	53	42	53	6	15	19	celiprolol	22	22	28	3	4	13
nadolol	53	35	6	28	9	29	bisoprolol	33	11	25	18	12	15
pindolol	30	5	10	5	2	3	clenisopenterol	64	62	72	43	42	39
fenoterol	9	26	30	5	13	16	mapenterol	88	86	91	7	1	9
NA-1141	44	35	31	10	14	8	labetalol	12	9	16	3	2	17
clenproperol	44	30	24	13	7	3	metipranolol	12	3	16	9	9	19
ritodrine	32	21	21	19	29	17	alprenolol	35	23	27	18	17	21
clorpenaline	42	45	44	5	10	4	propranolol	24	22	29	25	6	20
clenbuterol	1	14	22	7	1	7	betaxolol	2	13	16	0	9	4
metoprolol	8	15	2	20	19	30	carazolol	46	72	40	23	14	19
timolol	26	22	27	5	29	30	clenhexerol	44	58	22	20	14	6
tulobuterol	24	23	23	7	12	25	carvedilol	46	46	48	5	25	24
ractopamine	24	22	28	12	21	26	penbutolol	24	10	17	13	9	10
bromchlorbuterol	27	22	29	2	21	17	nekivolol	78	45	36	41	24	30
acebutolol	17	4	14	25	3	18	salmeterol	25	22	31	23	3	8

Table 5. Recoveries of β_2-Agonists and β-Blockers in Spiked Porcine Muscle Samples

	interday (n = 6)						intraday (n = 6)	
	spike level (5 μg/kg)		spike level (10 μg/kg)		spike level (20 μg/kg)		spike level (20 μg/kg)	
compounds	average recovery (%)	RSD (%)	average recovery (%)	RSD (%)	average recovery (%)	RSD (%)	average recovery (%)	RSD (%)
metaproterenol	54.3	14.9	78.8	14	82.4	12	87	14.2
cimaterol	75.8	13.9	82.8	13.1	82.6	16.7	89	12.7
atenolol	91.1	18.7	86.3	14.6	96.1	17.4	88.4	24.2
sotalol	109.3	4.3	109.5	5.6	116.1	5.4	111.2	5.1
salbutamol	102.4	24.1	100.8	19	118.2	4.1	115.5	10.4
terbutaline	67.2	16.6	73.6	15.6	106.4	16	107.9	13.9
cimbuterol	106.7	7.1	100.7	12.4	113.5	7.6	99.5	8.6
procaterol	77.9	18.4	84.5	17.4	95.7	20.7	77.1	18.8
carteolol	81.7	12.9	71.3	19.6	104.7	13.2	106.6	12.3
nadolol	111.5	5.3	107.1	7.9	96.2	15.3	105.6	14.2
pindolol	114	23.3	96.4	19.9	56.9	28.2	101.8	25.1
fenoterol	73.6	20.3	97.2	7.1	116.9	6.8	107.8	9.2
NA-1141	114.4	3.2	92	14.9	83.5	13	83.5	24.4
clenproperol	113.3	4.4	110.1	5	118.9	1.9	111.6	5.7
ritodrine	111.5	6.2	112.3	3.2	61.2	9.9	98.9	25.7
clorpenaline	114.9	4.4	112.1	8.9	110.7	10	113.5	5.6
clenbuterol	114.9	4.4	112.1	8.9	110.7	10	113.5	5.6
metoprolol	112.7	4.2	107.2	6.5	89.1	21.6	99.7	19.4
timolol	116.6	2.8	109.7	7.2	81.3	23	106.1	27.7
tulobuterol	112	4.2	109.4	4.8	80.7	27.9	99.7	25.2
ractopamine	104.6	9.2	85.6	7.3	110.9	8.6	105.9	11.8
bromchlorbuterol	103.4	5.1	98.6	15.8	69.1	16.8	82.9	27.1
acebutolol	110.7	2.6	110.2	6.2	109.3	3.9	110.1	5.6
brombuterol	112	3.2	94.1	3.1	106.5	6.7	102.5	15.6
formoterol	102.3	3.8	107.6	3.7	113.2	6.1	117.6	6.6
bunolol	111.8	4.3	113.4	3.3	69.8	5.1	97.4	22
esmolol	112	9.6	106.6	9.3	101.5	16	111.2	13.3
levobunolol	105.1	5.7	112.4	3.2	116.4	3.2	116.5	6.3
mabuterol	117.4	4.1	114.5	6.6	116.7	6.5	114.8	7.7

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Table 5. continued

	interday (n = 6)						intraday (n = 6)	
	spike level (5 μg/kg)		spike level (10 μg/kg)		spike level (20 μg/kg)		spike level (20 μg/kg)	
compounds	average recovery (%)	RSD (%)	average recovery (%)	RSD (%)	average recovery (%)	RSD (%)	average recovery (%)	RSD (%)
clenpenterol	101.4	3.4	101.4	3.4	99.9	6.2	97.5	5.9
oxprenolol	101	11.7	96.4	8.7	101.5	11.3	99.1	7.8
bambuterol	95.4	3.8	91.8	3.8	101.3	2.5	100.8	5.1
celiprolol	112.2	4.6	104.6	8.9	113.5	9.1	115.1	6.3
bisoprolol	104.8	6.4	110.7	4.7	115.3	5.5	110.5	4.9
clenisopenterol	72.7	15.5	82.5	15.6	96.9	25.2	95.7	18.1
mapenterol	118.1	2.3	106.4	8.3	116.4	3.7	112.9	4.7
labetalol	65.4	6.5	71.7	19.7	96.9	24.7	96.2	7.2
metipranolol	113.8	7.6	112.6	2.8	117.9	5.8	115.5	7.4
alprenolol	107	15.1	110.4	10.4	112.1	5.4	113	6.1
propranolol	75.2	9.8	94.4	23.4	106.3	9.8	109.8	6.3
betaxolol	96.1	19.3	76.8	9.6	94.4	7.3	98.3	15
carazolol	113.3	6.4	112	9.6	118	4.2	109.8	7.4
clenhexerol	112.2	10.7	110.2	12.1	111.3	16.2	103.4	16.7
carvedilol	46.6	19.3	55.1	16.8	62.6	27.3	60.1	25.7
penbutolol	105.1	7	101.6	3.7	107.3	4.7	106.7	6.1
nekivolol	68.6	19.1	78.2	11.9	67.4	21.2	65.3	20.9
salmeterol	112.6	10.4	106.3	7.3	110.7	8.7	113.7	4.7

Table 6. CCα and CCβ Obtained by MS^2 and MS^3 Scan Mode for the Porcine Muscle, Kidney, and Liver Samples (μg/kg)

	MS^2						MS^3					
	muscle		liver		kidney		muscle		liver		kidney	
compounds	CCα	CCβ	CCα	CCβ	CCα	CCβ	CCα	CCβ	CCα	CCβ	CCα	CCβ
metaproterenol	0.60	1.80	0.80	2.50	0.80	2.50	0.10	0.29	0.13	0.41	0.13	0.41
cimaterol	0.60	2.00	0.90	3.00	1.50	4.00	0.10	0.32	0.15	0.49	0.24	0.66
atenolol	1.00	3.30	0.30	1.00	0.60	2.00	0.16	0.54	0.05	0.16	0.10	0.33
sotalol	0.60	2.00	2.00	5.00	2.00	5.00	0.10	0.32	0.33	0.82	0.33	0.82
salbutamol	0.30	1.00	0.50	1.50	0.90	2.50	0.05	0.16	0.08	0.24	0.15	0.41
terbutaline	0.30	1.00	0.30	1.00	0.90	2.50	0.05	0.16	0.05	0.16	0.15	0.41
cimbuterol	0.50	1.70	0.80	2.50	0.80	2.50	0.08	0.28	0.13	0.41	0.13	0.41
procaterol	1.00	3.30	2.00	5.00	3.00	10.00	0.16	0.54	0.33	0.84	0.49	1.64
carteolol	0.30	1.00	0.50	1.50	0.90	2.50	0.05	0.16	0.08	0.24	0.15	0.41
clencyclohexerol	0.50	1.70	1.50	4.00	0.80	2.50	0.08	0.28	0.25	0.66	0.13	0.41
nadolol	1.00	3.30	2.00	5.00	2.00	5.00	0.16	0.54	0.33	0.84	0.33	0.84
pindolol	1.00	3.30	1.50	5.00	0.60	2.00	0.16	0.54	0.25	0.82	0.10	0.33
fenoterol	1.00	3.30	2.00	5.00	2.00	5.00	0.16	0.54	0.33	0.84	0.33	0.84
NA-1141	0.60	2.00	2.00	5.00	0.90	2.50	0.10	0.33	0.33	0.82	0.15	0.41
clenproperol	1.00	3.30	2.00	5.00	3.00	10.00	0.16	0.54	0.33	0.84	0.49	1.64
ritodrine	0.30	1.00	1.00	3.00	1.50	4.50	0.05	0.16	0.16	0.49	0.24	0.73
clorpenaline	1.00	3.30	2.00	5.00	1.50	5.00	0.16	0.54	0.33	0.82	0.25	0.82
clenbuterol	0.30	0.80	2.00	5.00	0.80	2.50	0.05	0.13	0.33	0.82	0.13	0.41
metoprolol	0.40	1.30	0.60	2.00	0.60	2.00	0.07	0.21	0.10	0.33	0.10	0.33
timolol	0.40	1.30	0.60	2.00	0.60	2.00	0.07	0.21	0.10	0.33	0.10	0.33
tulobuterol	0.40	1.30	0.60	2.00	0.60	2.00	0.07	0.21	0.10	0.33	0.10	0.33
ractopamine	0.30	1.00	0.90	3.00	0.90	2.50	0.05	0.16	0.15	0.49	0.15	0.41
bromchlorbuterol	0.50	1.70	0.40	1.20	0.40	1.20	0.08	0.28	0.07	0.20	0.07	0.20
acebutolol	0.50	1.70	1.00	2.50	0.40	1.20	0.08	0.28	0.16	0.41	0.07	0.20
brombuterol	0.40	1.30	0.60	2.00	0.60	2.00	0.07	0.21	0.10	0.33	0.10	0.33
formoterol	0.40	1.30	0.60	2.00	0.60	2.00	0.06	0.21	0.10	0.32	0.10	0.32
bunolol	0.50	1.70	0.80	2.50	0.80	2.50	0.08	0.28	0.13	0.41	0.13	0.41
esmolol	0.30	1.00	0.50	1.50	0.30	1.00	0.05	0.16	0.08	0.24	0.05	0.16
levobunolol	0.40	1.30	0.60	2.00	0.60	2.00	0.07	0.21	0.10	0.33	0.10	0.33
mabuterol	0.50	1.70	0.80	2.50	0.80	2.50	0.08	0.28	0.13	0.41	0.13	0.41
clenpenterol	0.50	1.70	0.80	2.50	0.30	0.80	0.08	0.28	0.13	0.41	0.05	0.13
oxprenolol	0.30	1.00	2.00	5.00	0.50	1.50	0.05	0.16	0.33	0.82	0.08	0.24
bambuterol	0.50	1.70	3.00	10.00	3.00	10.00	0.08	0.28	0.49	1.64	0.49	1.64
celiprolol	0.40	1.20	0.20	0.60	0.50	1.50	0.07	0.20	0.09	0.30	0.08	0.25

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Table 6. continued

	MS^2						MS^3					
	muscle		liver		kidney		muscle		liver		kidney	
compounds	CCα	CCβ	CCα	CCβ	CCα	CCβ	CCα	CCβ	CCα	CCβ	CCα	CCβ
bisoprolol	0.40	1.20	0.50	1.50	0.50	1.50	0.07	0.20	0.08	0.25	0.08	0.25
clenisopenterol	0.40	1.20	0.50	1.50	0.50	1.50	0.07	0.20	0.08	0.25	0.08	0.25
mapenterol	0.40	1.20	0.50	1.50	0.50	1.50	0.07	0.20	0.08	0.25	0.08	0.25
labetalol	0.40	1.20	0.50	1.50	0.50	1.50	0.07	0.20	0.08	0.25	0.08	0.25
metipranolol	0.40	1.20	0.50	1.50	0.50	1.50	0.07	0.20	0.08	0.25	0.08	0.25
alprenolol	0.80	2.60	2.00	5.00	2.00	5.00	0.13	0.43	0.33	0.84	0.33	0.84
propranolol	0.80	2.60	2.00	5.00	2.00	5.00	0.13	0.43	0.33	0.84	0.33	0.84
betaxolol	0.40	1.30	0.90	3.00	0.60	2.00	0.07	0.21	0.15	0.49	0.10	0.33
carazolol	0.90	3.00	2.00	5.00	2.00	5.00	0.15	0.49	0.33	0.84	0.33	0.84
clenhexerol	0.50	1.70	2.00	5.00	1.50	5.00	0.08	0.28	0.33	0.82	0.25	0.82
carvedilol	1.00	3.30	3.00	10.00	3.00	10.00	0.16	0.54	0.49	1.64	0.49	1.64
penbutolol	0.60	2.00	0.80	2.50	0.50	1.50	0.10	0.32	0.13	0.41	0.08	0.24
nekivolol	1.00	3.30	2.00	5.00	3.00	10.00	0.16	0.54	0.33	0.84	0.49	1.64
salmeterol	1.00	3.30	1.50	4.00	2.00	5.00	0.16	0.54	0.25	0.66	0.33	0.82

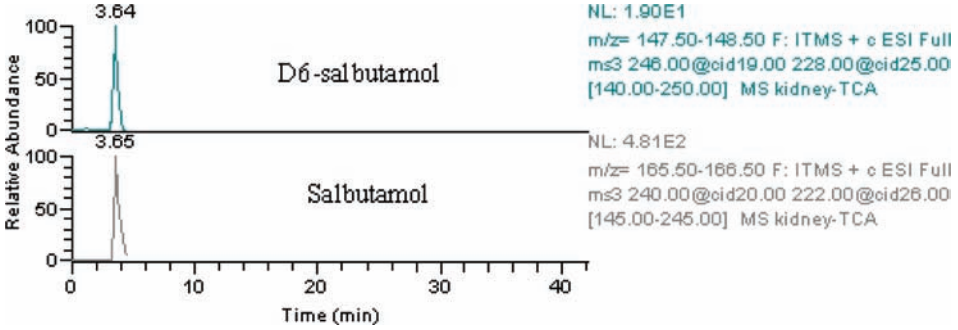

Figure 2. The chromatograms of positive porcine kidney sample.

The accuracy and precision of the method were examined by the interday and intraday reproducibilities using spiked blank porcine muscle samples. Six replicates of spiked blank samples (including LQC, MQC, and HQC) were analyzed on three separate days, respectively. The results are listed in Table 5. The interday recoveries ranged from 46.6% to 118.1%, 55.1% to 114.5%, and 56.9% to 118.9% for the three spiked levels at 5.0, 10.0, and 20.0 μg/kg with the relative standard deviations (RSD) of 2.3−24.1%, 2.8−23.4%, and 1.9−28.2%, respectively. The intraday recoveries ranged from 60.1% to 117.6%, for the spiked levels at 20.0 μg/kg, and RSD values were 4.7−27.7%. The results were satisfied except for few low recoveries and few unstable recoveries at low spiked level. The decreased recoveries on lower calibration levels may due to some adsorptive losses, for example, on the MCX cartridges. In addition, there were no suitable one-to-one deuterium isotope standards used for all of the analytes, which may be another possible reason for the unsatisfied recoveries.

The CCα and CCβ of the method were calculated (Table 6) by analyzing 20 blank samples per matrix. The 48 analytes were identified. The signal-to-noise ratio at the time window in which the analyte is expected was then calculated. The concentration at 3 times the signal-to-noise ratio was used as CCα. Twenty blank samples per matrix fortified with the analytes at the level of CCα were analyzed. The value of CCα plus 1.64 times the standard deviation (SD) of the within-laboratory reproducibility of the measured content equals the CCβ.

The CCα values of the 48 analytes in pork muscle, liver, and kidney were 0.05−0.16, 0.05−0.49, and 0.05−0.49 μg/kg, and CCβ values were 0.13−0.54, 0.16−1.64, and 0.13−1.64 μg/kg. The CCα and CCβ of this method are low enough for the supervision of 25 β_2-agonists and 23 β-blockers in animal foods. In this study, we also have compared the CCα and CCβ obtained by MS^2 and MS^3 scan mode, the compared results are also shown in Table 6, and lower CCα and CCβ were acquired when the scan mode of MS^3 was used.

Determination of β_2-Agonists and β-Blockers in Animal Foods. 110 samples including 43 porcine muscle samples, 45 porcine liver samples, and 22 porcine kidney samples were collected from local markets of eight municipal districts in Beijing. The 110 samples were analyzed using the established method along with analysis of the blank samples and QC samples. Of all of the 110 samples, only salbutamol was detected in one pork kidney sample with the concentration of 31.4 μg/kg. The chromatogram of the pork kidney sample is shown in Figure 2. Zhang et al.[18] reported the results of 174 samples including porcine muscle, porcine livers, porcine kidneys, and beef samples from three local markets in Beijing in Hebei Province. β-Blockers were rarely detected, and only metoprolol was found in one pork sample with the concentration of 3.5 μg/kg. These two results demonstrate that illegal use of β_2-agonists and β-blockers in animal breeding still happened from time to time. Therefore, it is necessary to supervise multiple residues of β_2-agonists and β-blockers in animal foods.

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