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Determination of 23 β_2-agonists and 5 β-blockers in animal muscle by high performance liquid chromatography-linear ion trap mass spectrometry

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A high performance liquid chromatography-linear ion trap mass spectrometry method using isotope dilution technique has been developed for the simultaneous determination of 23 β_2-agonists and 5 β-blockers in animal muscle tissues. Pork and chicken muscle samples were acid hydrolyzed and extracted with 5% trichloracetic acid in water, and then cleaned up using MCX solid phase extraction (SPE) cartridge. Methanol and 0.1% formic acid were used as mobile phases for gradient elution. A Waters Atlantis®T3 column was used for separation. ESI positive ion scan mode was used with selective reaction monitoring. 9 β_2-Agonists labeled by the deuterium isotope were used as internal standards for quantification. The linear ranges of 23 β_2-agonists and 5 β-blockers were 5–200 μg/L, the coefficient of correlation was not less than 0.995, and the limit of detection for each compound in the muscle tissue was below 0.2 μg/kg. The recoveries of each compound in the spiked samples at three levels 5, 10, 20 μg/kg were in the range of 47.3%–123.7%, and the relative standard deviations were in the range of 3.2%–25.7%. The developed method is sensitive and specific for the determination of β_2-agonists and β-blockers in pork and chicken muscle samples.

β_2-agonists, β-blockers, muscle, determination, HPLC-ITMS

1 Introduction

β_2-Adrenoceptor agonists (β_2-agonists) are nitrogen-containing hormones used in asthma treatment in humans, because they cause relaxation of human bronchial smooth muscle and thereby decrease airway resistance. However, in the early 1980s, a series of animal experiments showed that this type of drugs, such as clenbuterol and salbutamol, has high potencies of growth promoting and fat repartitioning in animals when the dosage is more than 5 times of the therapeutic dose for asthma [1–3]. Illegal use of β_2-agonists in animal feed with the attempt to promote growth and to increase production of muscle meat leads to the accumulation of these compounds in animal tissues, such as the liver, kidney, and muscle. Large amounts of residues of β_2-agonists in animal food may cause food poisoning in humans. Since the first case of food poisoning caused by clenbuterol from bovine livers happened in Spain in 1983, there have been more than 500 cases in Europe in the 1990s [4, 5]. After intensive control over the use of clenbuterol in animal feed, other β_2-agonists were illegally used in animal feed and drinking water [6]. Therefore, it is necessary and urgent to establish a method to determine multiple β_2-agonist residues in animal tissues. In addition, drugs of β-blockers are widely used in the prevention and treatment of sudden death of animals caused by stress response during transportation. This type of drugs is usually used a few hours before the slaughtering, so they may bring much more risks compared

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with other veterinary drugs. Long-term consumption of animal tissues containing high levels of these residues would not only cause anxiety, headaches, loss of appetite, and other psychiatric symptoms, but also cause drug-dependence in humans. Therefore, the use of β-blocker drugs in animal feed is also restricted [24]. According to the EU directive 96/23/EC, residues of β_2-agonists are prohibited in animal food. Bulletin No. 235 "The maximum residue limit in animal food" published by China's Ministry of Agriculture states that clenbuterol, salbutamol, cimaterol and their salts and esters are all prohibited as animal medicines, and all these drugs should not be detected in animal food [8].

Several analytical techniques used for β_2-agonists analysis in food materials have been published. For extraction and purification, techniques such as immunoaffinity chromatography [9, 20], solid phase extraction (SPE) [14] and matrix solid-phase extraction (MSPD) [16] are used. Gas chromatography or liquid chromatography with mass spectrometry are the most common detection method [9–18]. Some of the β_2-agonists would conjugate with glucuronate *in vivo*, so the tissue samples should be hydrolyzed by β-glycuronidase [14] or by acid [17, 18]. Immunology analyses, such as ELISA and RIA, are usually used as rapid screening method [19, 20]. Although the detection limit of ELISA could reach 0.1 μg/kg, there is few literature on multi-residue determination of β_2-agonists by ELISA. There are also few reports on the detection of β-blockers in animal foodstuffs. Dolores Hernando determined 11 β-blockers in waste water and surface water using LC-MS [21]. Lee HB determined 12 β_2-agonists and β-blockers in sewage by solid-phase extraction and liquid chromatography-tandem mass spectrometry [22].

The objective of this study is to establish a sensitive and rapid method for the simultaneous detection of multiple β_2-agonists and β-blockers in animal food to meet the requirements for the control of illegal use of β_2-agonists and β-blockers.

2 Experiments

2.1 Reagents and materials

Brombuterol-HCl (>99%), clenisopenterol-HCl (>99%), clencyclohexerol hydrochloride (>99.5%), clenhexerol (>99%), cimbuterol (>99%), and mapenterol hydrochloride (>99%) were purchased from WITEGA Laboratorien Berlin-Adlershof GmbH, Germany. Mabuterol (>99.1%) and cimaterol (>99.5%) were purchased from Boehringer Ingekheim. Salmeterol (>98%) was purchased from Toronto Research Chemical Inc., USA. Clenproperol (>99%), clenpenterol (>99%), 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 (>99.9%), terbutaline (>98%), salbutamol (>98%), procaterol (>99%), fenoterol (>98%), clenbuterol (>95%), ractopamine (>95.6%), tulobuterol (>98%), formoterol fumarate (>98%), bambuterol hydrochloride (>98%), ritodrine hydrochloride (>99%), metoprolol (>98%), labetalol hydrochloride (>98%), propranolol hydrochloride (>98%), betaxolol (>98%), and penbutolol sulfate (>98%) were purchased from Sigma-Aldrich, USA. D3-Salmeterol was purchased from Cambridge Isotope Laboratories Inc., USA.

Methanol and acetoacetate (chromatographic pure) were purchased from Baker JT (New Jersey, USA). Formic acid (99%) was purchased from Acros Organics (New Jersey, USA). Perchloric acid, trichloracetic acid, ammoniae aqua, and sodium hydroxide were purchased from Beijing Chemical Reagents Company (Beijing, China). Oasis MCX solid phase extraction cartridges (6 mL, 150 mg) were purchased from Waters Co. (Milford, MA, USA). Deionized water was prepared by a Milli-Q Plus system at 18.2 MΩ (MilliPore, Bedford, MA, USA).

2.2 Sample extraction

5.0 g of the homogenated muscle sample was weighed and put into a 50 mL polypropylene centrifuge tube and 10 mL of 5% trichloracetic acid solution was added into the tube. The mixture was vortexed for 30 s, and then ultrasonicated at 80 °C for 30 min. After ultrasonication, the centrifugation was conducted at 10000 rpm at 0 °C for 10 min. 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.

2.3 Cleanup

The Oasis MCX cartridge was preconditioned with 6.0 mL methanol and 6.0 mL water. The extract was applied to the preconditioned cartridge and followed with 2 washes of the tube with 1 mL 5% trichloracetic acid. The cartridge was washed by sequentially passing 2 mL 0.1 mol/L perchloric acid and 1 mL methanol. The analytes were eluted into a 10 mL test tube with 6.0 mL methanol containing 5% ammonia. The effluent was dried under a gentle nitrogen stream to nearly dryness. The residue was dissolved in the mixed solution of 1.0 mL methanol and 0.1% formic acid (9:1, *V*/*V*) and then filtered through a 0.22 μm nylon filter for LC-MS-MS analysis.

2.4 Liquid chromatography

Liquid chromatographic separation was performed by a HPLC system equipped with a LC pump and an autosampler of Surveyor (Thermo-fisher, Waltham MA, USA) on a Waters Atlantis®T3 column (150 mm × 2.1 mm, 3 μm particle size) coupled with a Waters Atlantis®T3 guard column

(10 mm × 2.1 mm, 3 μm particle size). The column oven temperature was set at 30 °C, the flow rate of the mobile phase was 250 μL/min, and the injection volume was 10 μL. Optimal separation of the target compounds was achieved by gradient elution using methanol (A) and the solution of 0.1% formic acid (B). The gradient elution began with 90% solution (B), then was linearly programmed to 30% in 45 min, then to 5% in 1 min to elute impurities from the column, and finally to 90% solution (B) in 1 min and kept eluting for 8 min to equilibrate the column before the next injection. The total chromatographic and equilibration time of 55 min was required for each run.

2.5 Mass spectrometry

Mass spectrometric analysis was carried out on a linear ion trap mass spectrometry (LTQ) (Thermo-fisher, Waltham MA, USA) using the positive electrospray ionisation mode (ESI+). The selective reactive monitoring (SRM) scan mode was used. The voltage of the nebulising gas was set at 4 kV. The flow rates of the sheath gas and the auxiliary gas were 35 arb and 15 arb, respectively. The temperature of the capillary was 325 °C and the capillary voltage was set at 50 V. Helium gas was used as collision gas in linear ion trap. The parameters of the linear ion trap were set as follows: full AGC target was 10000.0, SIM AGC target was 5000.0, Msn AGC target was 5000.0, and Zoom AGC target was 3000.0.

2.6 Calibration and quantification

For the quantification of the 23 β_2-agonists and 5 β-blockers, 9 deuterium isotope standards were used as internal standards. To overcome the interference of the matrix, the matrix-matched calibration curves were constructed. The concentrations of the multi-component matrix-matched standard solutions ranged from 5 to 200 μg/L, i.e., 5, 10, 20, 40, 80, 160 and 200 μg/L. The peak areas of the compounds (A) and the internal standard compounds (A_i) were recorded. A linear calibration curve was established for each compound on the basis of the ratio of A to A_i vs the corresponding concentrations of the internal standard solution.

3 Results and discussion

3.1 Optimization of the mass spectrometry conditions

The optimization of the MS parameters, such as the flow rates of the sheath gas and the auxiliary gas, the voltage of the nebulising gas and the capillary, and the temperature of the capillary and the collision energy, was achieved by injecting the standard solution (200 μg/L) with the mobile phase (methanol+0.1% formic acid (50:50, *V*/*V*)) and eluting them through the LC system into the MS system. The base peak in each compound's spectrum was the protonated molecule $[M+H]^+$ and was subsequently used as the precursor ion for the resulting SRM transitions. The optimized instrument parameters were described in Section 2.5. The retention time, collision energy, precursor ion, product ion and other information are listed in Table 1.

Analytes such as cimaterol, clencyclohexerol, clenproperol, Clenisopenterol, and Clenhexerol have only one product ion, which was 220>202, 319>301, 263>245, 291>273 and 305>287, respectively. This is different from reference [14], in which the triple-quadrupole mass spectrometry was used. The scan mode of MS/MS/MS can be applied to these compounds to acquire further information in future work.

Figures 1 and 2 show the total ion chromatogram and the chromatograms of quantitative transition of SRM of the

Table 1 Mass spectrum parameters for β_2-agonists and β-blockers

Compounds	Retention time (min)	Precursor ion (*m*/*z*)	Internal standards	Segment	Product ion 1* (*m*/*z*)	Product ion 2 (*m*/*z*)	Collision energy (%)	Scan range (*m*/*z*)
Metaproterenol	3.15	212	D7-cimaterol	1	194	152	27	149–215
D7-Cimaterol	5.03	227		2	209		19	206–230
Cimaterol	5.14	220	D7-cimaterol	2, 3	202		26	199–223
Terbutaline	6.13	226	D6-salbutamol	3	152	170, 208	26	149–230
D6-Salbutamol	6.22	246		3	228	167, 148	18	165–250
Salbutamol	6.46	240	D6-salbutamol	3	222	166	20	163–243
D9-Cimbuterol	9.71	243		4	225	161	18	155–250
Cimbuterol	9.87	234	D9-cimbuterol	4	216	160	21	155–240
Procaterol	12.14	291	D9-cimbuterol	5	273	232, 216	21	213–295
Fenoterol	12.46	304	D9-cimbuterol	5	286	135, 107	24	105–308
Ritodrine	13.09	288	D9-cimbutero	5	270	150	21	145–292
Clencyclohexerol	13.46	319	D9-cimbutero	5	301		20	298–323
D7-Clenproperol	15.28	270		6	252		17	248–273
Clenproperol	15.42	263	D7-clenproperol	6	245		22	242–266

(*To be continued on the next page*)

(*Continued*)

Compounds	Retention time (min)	Precursor ion (*m*/*z*)	Internal standards	Segment	Product ion 1* (*m*/*z*)	Product ion 2 (*m*/*z*)	Collision energy (%)	Scan range (*m*/*z*)
D6-Clenbuterol	19.43	283		7	265	204	16	201–286
Clenbuterol	19.60	277	D6-clenbuterol	7	259	203	22	75–280
D5-Ractopamine	18.99	307		7	289	167	18	165–310
Ractopamine	19.02	302	D5-ractopamine	7	284	164	21	160–305
Bromchlorbuterol	21.20	323	D5-ractopamine	8	305	249	16	246–326
Metoprolol	21.45	268	D5-ractopamine	8	191	116	34	113–271
Tulobuterol	22.15	228	D9-mabuterol	8	154	172	28	150–231
Formoterol	21.94	345	D9-mabuterol	8	327	149	22	145–350
Brombuterol	22.72	367	D9-mabuterol	8	349	293	18	290–370
D9-Mabuterol	23.13	320		8, 9	302	238	15	235–325
Clenpenterol	23.29	291	D9-mabuterol	8, 9	273	203	21	200–295
Mabuterol	24.17	311	D9-mabuterol	9	293	237	20	235–315
Bambuterol	26.61	368	D11-mapenterol	10	312	294	20	290–371
Clenisopenterol	27.07	291	D11-mapenterol	10	273		21	270–295
D11-Mapenterol	27.46	336		10	318	238	17	235–340
Mapenterol	27.73	325	D11-mapenterol	10, 11	307	237	19	235–330
Labetalol	28.00	329	D11-mapenterol	11	311	207	19	205–333
Propranolol	29.83	260	D11-mapenterol	11	183	116, 157	31	113–265
Betaxolol	31.66	308	D11-mapenterol	11, 12	116	177, 231	31	113–311
Clenhexerol	33.38	305	D11-mapenterol	12	287		17	284–310
Penbutolol	41.31	292	D3-salmeterol	13	236	201	25	197–295
D3-Salmeterol	41.71	419		13	401	383	19	380-422
Salmeterol	41.76	416	D3-salmeterol	13	398	380	18	375-420

Note: *means the quantitatve ion; collision energy (%) is the normalization energy; isolation width is 2 (*m*/*z*); *Q* value is 0.25.

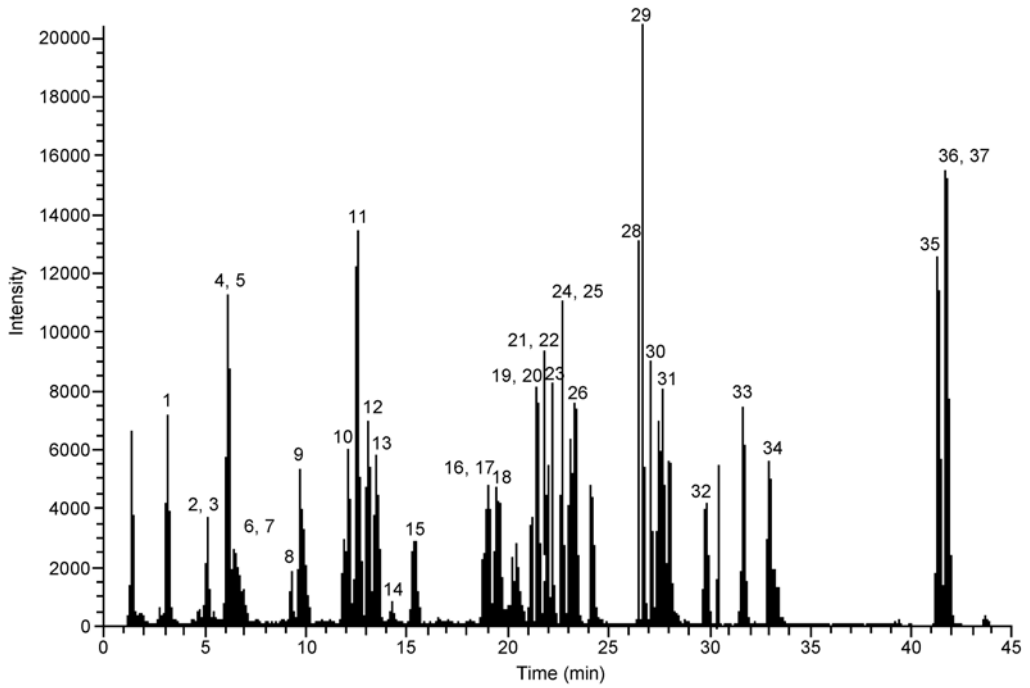

Figure 1 Total ion chromatogram of SRM of the pork matrix-matched standard solution (80 μg/kg). 1, metaproterenol; 2, cimaterol; 3, D7-cimaterol; 4, terbutaline; 5, salbutamol; 6, D6-salbutamol; 7, cimbuterol; 8, D9-cimbuterol; 9, procaterol; 10, fenoterol; 11, ritodrine; 12, clencyclohexerol; 13, clenproperol; 14, D7-clenproperol; 15, ractopamine; 16, D5-ractopamine; 17, clenbuterol; 18, D6-clenbuterol; 19, bromchlorbuterol; 20, metoprolol; 21, tulobuterol; 22, formoterol; 23, brombuterol; 24, mabuterol; 25, D9-mabuterol; 26, clenpenterol; 27, bambuterol; 28, clenisopenterol; 29, mapenterol; 30, D11-mapenterol; 31, labetalol; 32, propranolol; 33, betaxolol; 34, clenhexerol; 35, penbutolol; 36, salmeterol; 37, D3-salmeterol.

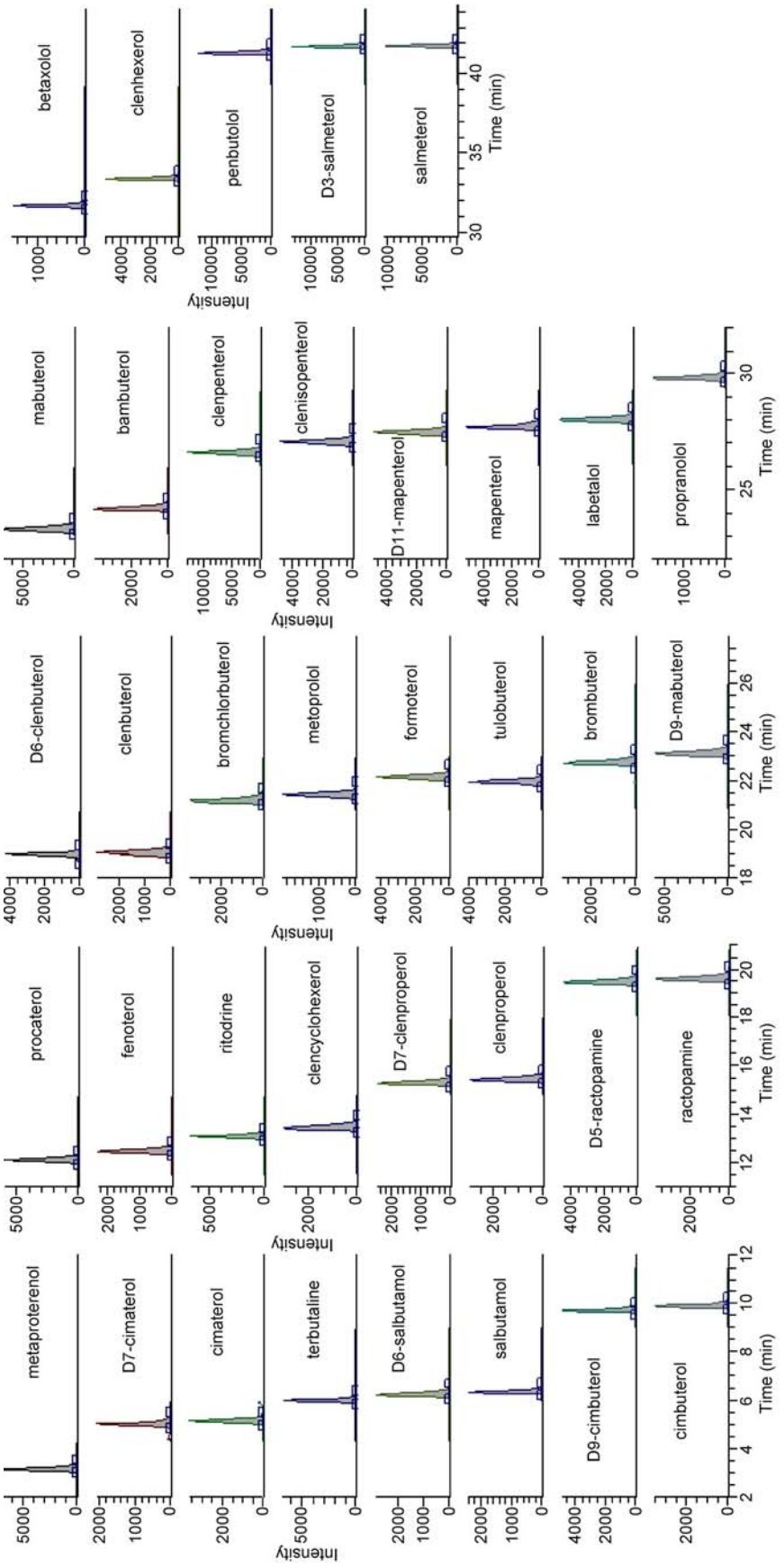

Figure 2 Chromatograms of quantification transition of SRM of the pork matrix-matched standard solution (80 μg/kg).

matrix-matched standard solution, respectively. There was no interference to any of the compounds and the well-shaped peak for each compound was obtained.

3.2 Sample extraction

It is well known that not all the β_2-agonists and β-blockers are in the form of their parent compounds in animal tissues and biological materials. Some of them, such as procarterol, metaproterol, and labetalol, are in the form of glcuronide conjugates. Therefore, the samples should be hydrolyzed first. Enzymatic hydrolysis [14], acid hydrolysis [17], and alkaline hydrolysis [18] are the commonly used techniques. In this study, trichloracetic acid was used to hydrolyze the conjugates. The trichloracetic acid not only hydrolyzed the conjugates, but also precipitated the protein in muscle tissues. The concentration of the trichloracetic acid solution was optimized. 1%, 5%, and 10% trichloracetic acids were tested as the extraction solvent, and 5% trichloracetic acid gave the most satisfactory results. Finally, 10 mL of 5% trichloracetic acid solution was selected as the extraction solvent and hydrolysis reagent as well.

3.3 Cleanup

The Oasis MCX cartridge was used for the cleanup of the extract. The pH of the extract was adjusted from 2 to 10 before the extract was applied to the cartridge. The results show that pH 4.0 gave best recoveries for almost all the analytes. The extract could be applied to the Oasis MCX cartridge directly for cleanup because the pH of the extract was just around 3.6–4.0.

3.4 Method validation

Validation parameters for quantification of 23 β_2-agonists and 5 β-blockers were obtained by using the optimal conditions for extraction, liquid chromatography and mass spectrometry. As mentioned above, the isotopic dilution technique and the matrix-matched standards were used to compensate for signal irreproducibility, matrix interference and recovery loss. Calibration curves were built from the ratio of the peak areas of the analytes vs. the peak area of the deuterium isotope internal standard. All of the 28 com pounds showed good linear regression in the range of 5–200 μg/L, and the correlation coefficients (r) were not less than 0.995. All calibration curve parameters are summarized in Table 2.

The recovery experiment of β_2-agonists and β-blockers was carried out with the spiked pork samples. The spiked levels were 5, 10 and 20 μg/kg. The results are listed in Table 3. The recoveries of the 28 compounds were 47.3%–

Table 2 Parameters of method validation

Compounds	Linear regression	Correlation coefficient (r)	Limit of detection (μg/kg)	Limit of quantification (μg/kg)
Metaproterenol	$Y = 0.0736X + 0.0736$	0.9995	0.10	0.25
Cimaterol	$Y = 0.0237X + 0.6583$	0.9958	0.20	0.50
Terbutaline	$Y = 0.0869X + 0.0854$	0.9971	0.20	0.90
Salbutamol	$Y = 0.0756X - 0.0092$	0.9972	0.05	0.20
Cimbuterol	$Y = 0.0311X + 0.0100$	0.9997	0.20	0.50
Procaterol	$Y = 0.0508X + 0.0843$	0.9957	0.20	1.00
Fenoterol	$Y = 0.0276X + 0.0048$	0.9992	0.20	1.00
Ritodrine	$Y = 0.1259X + 0.1179$	0.9980	0.15	0.50
Clencyclohexerol	$Y = 0.0364X + 1.3427$	0.9964	0.10	0.20
Clenproperol	$Y = 0.0379X + 0.0203$	0.9992	0.20	1.00
Ractopamine	$Y = 0.0606X + 0.0253$	0.9996	0.20	0.50
Clenbuterol	$Y = 0.0367X + 0.0388$	0.9970	0.20	0.50
Bromchlorbuterol	$Y = 0.0316X - 0.0057$	0.9966	0.20	1.00
Metoprolol	$Y = 0.0249X + 0.0248$	0.9991	0.10	0.25
Formoterol	$Y = 0.0695X + 0.0563$	0.9966	0.20	1.00
Tulobuterol	$Y = 0.0576X - 0.0002$	0.9993	0.10	0.20
Brombuterol	$Y = 0.0393X + 0.0412$	0.9982	0.20	1.00
Mabuterol	$Y = 0.0993X + 0.0831$	0.9994	0.20	0.50
Clenpenterol	$Y = 0.0455X + 0.0697$	0.9965	0.20	1.00
Bambuterol	$Y = 0.0826X + 0.1578$	0.9981	0.10	0.20
Clenisopenterol	$Y = 0.0457X + 0.0834$	0.9953	0.20	1.00
Mapenterol	$Y = 0.0417X + 0.0246$	0.9991	0.20	1.00
Labetalol	$Y = 0.0633X + 0.1315$	0.9961	0.20	1.00
Propranolol	$Y = 0.0307X + 0.0643$	0.9964	0.10	0.20
Betaxolol	$Y = 0.0245X + 0.0486$	0.9975	0.10	0.20
Clenhexerol	$Y = 0.0487X + 0.0912$	0.9980	0.20	1.00
Penbutolol	$Y = 0.1649X + 0.3397$	0.9969	0.20	0.50
Salmeterol	$Y = 0.1117X + 0.0671$	0.9958	0.20	1.00

Table 3 Recoveries of β_2-agonists and β-blockers in spiked samples

Compounds	Spike level (5 μg/kg)		Spike level (10 μg/kg)		Spike level (20 μg/kg)	
	recovery (%, $n=6$)	RSD (%)	recovery (%, $n=6$)	RSD (%)	recovery (%, $n=6$)	RSD (%)
Metaproterenol	101.3	9.2	70.9	5.3	109.4	7.0
Cimaterol	88.6	7.5	91.8	14.6	85.8	13.7
Terbutaline	62.3	18.1	95.8	19.1	117.7	3.2
Salbutamol	68.0	9.2	107.5	7.7	87.4	4.7
Cimbuterol	57.0	8.5	58.2	21.5	93.7	6.0
Procaterol	111.5	20.3	102.6	8.2	101.7	10.9
Fenoterol	69.8	17.3	79.3	11.3	103.5	16.0
Ritodrine	104.9	9.7	86.3	11.8	100.2	14.1
Clencyclohexerol	70.3	12.1	117.8	7.3	112.5	18.7
Clenproperol	56.2	11.8	70.9	9.9	81.2	17.2
Ractopamine	77.5	14.4	70.5	13.9	86.4	3.9
Clenbuterol	105.8	7.2	85.5	9.8	85.4	6.1
Bromchlorbuterol	98.9	17.3	77.7	5.3	88.0	13.3
Metoprolol	84.5	7.8	123.7	6.0	99.4	14.2
Tulobuterol	51.6	11.3	56.7	11.7	62.3	14.7
Formoterol	73.1	11.0	82.8	15.2	84.7	9.6
Brombuterol	47.3	8.0	67.3	10.4	79.1	5.0
Mabuterol	51.5	17.8	105.4	8.8	100.4	7.1
Clenpenterol	53.9	11.0	69.7	8.7	76.2	9.5
Bambuterol	59.6	8.8	118.6	4.8	99.2	9.9
Clenisopenterol	51.8	22.4	59.4	14.4	66.5	15.2
Mapenterol	47.9	8.5	121.6	6.0	98.4	7.7
Labetalol	54.6	9.3	83.3	10.0	76.2	7.7
Propranolol	50.5	12.0	81.0	8.6	71.5	7.7
Betaxolol	52.0	9.9	122.8	5.5	101.8	6.0
Clenhexerol	50.0	20.3	57.2	25.7	59.6	23.0
Penbutolol	50.2	14.1	69.0	11.4	66.9	6.3
Salmeterol	47.4	23.5	47.3	22.1	52.2	7.7

111.5%, 47.3%–123.7% and 52.2%–117.7% for the spiked levels of 5 μg/kg, 10 μg/kg and 20 μg/kg, respectively. The relative standard deviations (RSD) were 7.2%–23.5%, 4.8%–25.7% and 3.2%–23.0%, respectively. The results were satisfactory except for the low recoveries of salmeterol at all the three spiked levels and some analytes at the spiked level of 5 μg/kg. After the experiment was repeated for many times, it was found that the recoveries of salmeterol were much lower than those of other compounds. The recoveries of D3-salmeterol were lower than those of other internal standards, but were higher than the absolute recoveries of salmeterol. More studies need to be done to improve the low recoveries of salmeterol.

The limit of detection (LOD) and the limit of quantification (LOQ) were expressed as the concentration in the spiked samples with the ratio of signal to noise (S/N) of 3 and 10, respectively. The results are listed in Table 2. All of the LODs are in the range of 0.05–0.2 μg/kg.

3.5 Real sample analysis

This proposed method was successfully applied to the analysis of pork and chicken samples purchased from the local markets in Beijing. Among the 35 samples tested, clenbuterol and ractopamine were confirmed in one of the chicken samples at the concentration of 2.31 μg/kg and 2.17 μg/kg, respectively. The chromatograms of the quantitative transition of SRM are shown in Figure 3. No other β_2-agonists and β-blockers were detected in all of the other samples. The use of clenbuterol and ractopamine in animal breeding for human consumption is forbidden in China [8]. It could be inferred that the trend of single β_2-agonist adulteration in animal feed has gradually changed into multi-drug adulteration with lower dosage.

4 Conclusions

A sensitive high performance liquid chromatography-linear ion trap mass spectrometry (HPLC-IT/MSMS) method was developed. 23 β_2-agonists and 5 β-blockers in animal muscle samples were simultaneously identified. The method was validated and demonstrated good linearity and accuracy. The limit of detection for each compound in the muscle tissue ($\leqslant$0.2 μg/kg) was satisfying to the multi-residual analysis of β_2-agonists and β-blockers in animal muscle samples.

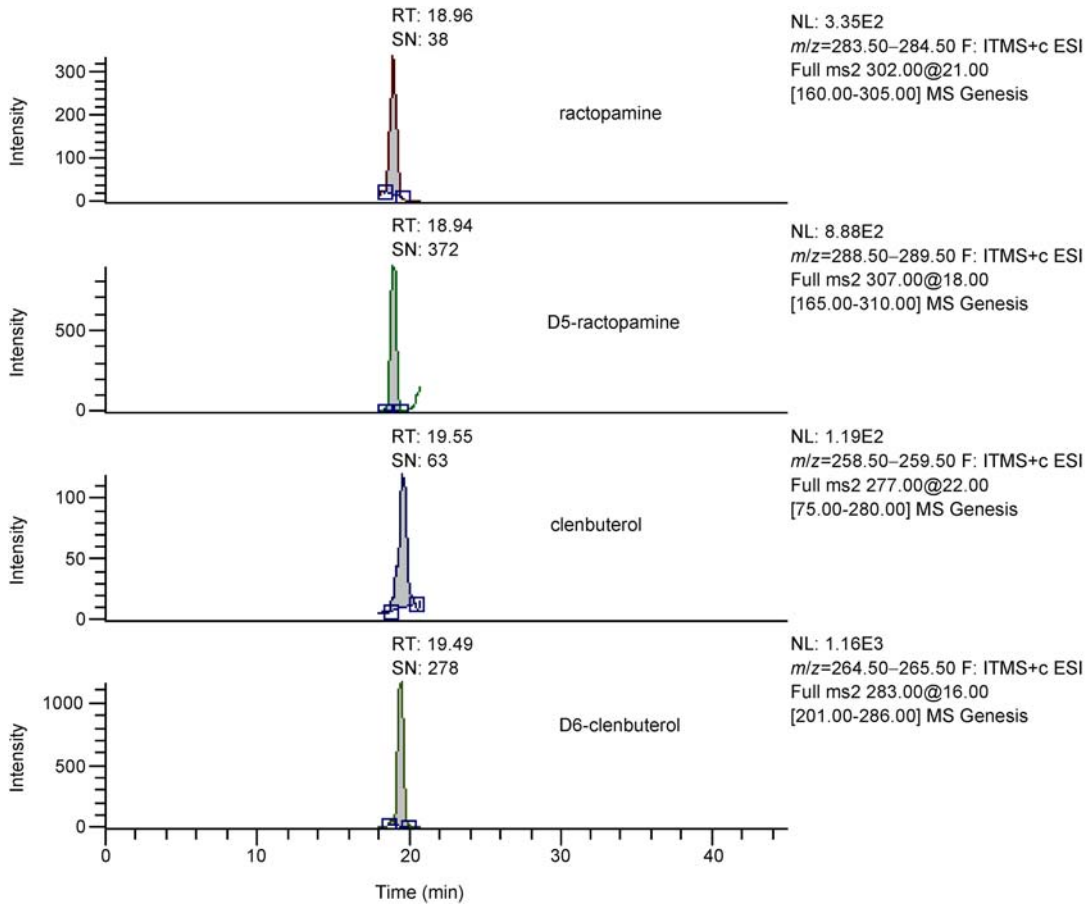

Figure 3 Chromatograms of quantification transition of SRM of the positive chicken sample.

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