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Application News

No. LCMSMS-178E

Liquid Chromatography Mass Spectrometry

Determination of Twenty-four β-Receptor Agonists in Chicken by Ultra-High-Performance Liquid Chromatography coupled with Triple Quadrupole Mass Spectrometry

Twenty-four β-receptor agonists were determined in chicken sample using Shimadzu Ultra-High-Performance Liquid Chromatograph Nexera X2 coupled with Triple Quadrupole Mass Spectrometer LCMS-8050. The calibration curves exhibited good linearity within the range of 0.1-10.0 µg/L and the RSD of retention time and peak area was 0.07-0.40% and 0.44-4.12% respectively, indicating good instrument precision. The method detection limit and limit of quantitation was determined to be 0.004-0.025 µg/kg and 0.014-0.082 µg/kg respectively.

Keywords: Ultra-high-performance liquid chromatograph; Triple quadrupole mass spectrometer; Chicken; B-receptor agonists

■ Introduction

β-receptor agonists are a group of selective β_2 adrenergic receptor agonists. The compounds are named this way due to their ability to bind to most β-receptors on histiocyte membranes in animal body. They are a class of chemically synthesized phenylethanolamines that are structurally and physiologically similar to epinephrine and norepinephrine. The regulations in the EU 96/22/EC directive have prohibited the use of these drugs. Also, the Ministry of Agriculture and the State Administration of Quality Supervision of China have prohibited the use of such drugs in animal feed or on animals in the growing phase. Therefore, it is necessary to establish a fast and highly sensitive detection technique to quantify β-receptor agonists in chicken.

With reference to the method in "GBT 22286-2008: Determination of Multiple β-receptor Agonist Residues in Animal-Derived Food – LC - tandem MS Method", a UHPLC coupled with triple quadrupole MS method was developed for the determination of 24 β-receptor agonists in chicken. The use of Shimadzu Nexera X2 UHPLC and LCMS-8050 is used in this application note and the described method serves as a reference for relevant personnel.

■ Experimental

1.1. Instruments

Shimadzu Ultra-High-Performance Liquid Chromatograph (UHPLC) Nexera X2 coupled with Triple Quadrupole Mass Spectrometer LCMS-8050 was used. The specific configuration included LC-30AD×2 infusion pumps, DGU-20A$_{5R}$ Online Degasser, SIL-30AC Autosampler, CTO-30AC Column Oven, CBM-20A System Controller, Triple Quadrupole Mass Spectrometer LCMS-8050, and LabSolutions Ver. 5.60 SP2 Chromatography Workstation.

1.2. Analytical Conditions

Liquid chromatography (LC) parameters

- Analyzer: Nexera X2
- Chromatographic column: Shimadzu Shim-pack XR-ODS III, 2.0 mm (I.D.) x 75 mm (L) x 1.6 µm
- Mobile phase: Phase A-0.1% formic acid solution; B-methanol
- Flow rate: 0.3 mL/min
- Injection volume: 5 µL
- Column temperature: 40°C
- Elution mode: gradient elution; the initial concentration of phase B was 5%, refer to Table 1 for gradient program.

Table 1: Gradient Program

Time(min)	Module	Command	Value
1.0	Pumps	Pump B Conc	5
4.0	Pumps	Pump B Conc	35
6.0	Pumps	Pump B Conc	90
8.0	Pumps	Pump B Conc	90
8.1	Pumps	Pump B Conc	5
10.0	Controller	Stop	

Mass spectrometry (MS) parameters

- Ion source: ESI, positive ion scan
- Nebulizing gas: 3 L/min
- Heating gas: 12 L/min
- Drying gas: 8 L/min
- Collision gas: Argon
- DL temperature: 250°C
- Heating module temperature: 400°C
- Scan mode: Multiple Reaction Monitoring (MRM)
- Dwell time: 15 msec
- Pause time: 3 msec
- MRM parameters: Refer to Table 2

Table 2: MRM Parameters

No.	Compound Name	CAS	Parent ion	Daughter ion	Q1 Pre Bias (V)	CE (V)	Q3 Pre Bias (V)
1	Cimaterol	54239-37-1	220.2	202.1* 143.1	-11 -26	-11 -23	-22 -27
2	Terbutaline	148-79-8	226.1	152.0* 125.1	-11 -26	-16 -23	-28 -24
3	Salbutamol	18559-94-9	240.0	222.1* 166.1	-12 -12	-11 -13	-24 -30
4	Cimbuterol	54239-39-3	234.2	143.1* 160.1	-27 -24	-25 -15	-26 -29
5	Fenoterol	13392-18-2	304.0	135.0* 286.0	-15 -15	-14 -18	-20 -25
6	Procaterol	72332-33-3	291.1	273.0* 231.2	-22 -15	-12 -21	-30 -26
7	Ritodrine	26652-09-5	288.0	270.1* 150.0	-14 -14	-13 -19	-30 -28
8	Clencyclohexerol	157877-79-7	319.1	203.0* 301.1	-15 -16	-14 -21	-21 -22
9	Hydroxymethyl-clenbuterol	38339-18-3	293.1	203.0* 275.1	-30 -15	-12 -19	-30 -22
10	Clenproperol	38339-11-6	263.1	245.0* 203.0	-13 -14	-12 -19	-27 -22
11	Clorprenaline	3811-25-4	214.1	154.1* 196.1	-21 -25	-13 -18	-21 -29
12	Clenbuterol	37148-27-9	277.1	203.0* 259.0	-28 -28	-11 -17	-28 -22
13	Formoterol	73573-87-2	345.0	149.0* 327.1	-17 -17	-14 -21	-23 -28
14	Bromochlorobuterol	78982-84-0	323.0	249.0* 305.0	-15 -15	-12 -18	-22 -27
15	Tulobuterol	41570-61-0	228.1	154.0* 118	-22 -26	-16 -28	-29 -22
16	Brombuterol	41937-02-4	367.0	349.0* 293.0	-18 -18	-13 -19	-25 -21
17	Mabuterol	56341-08-3	311.0	237.0* 293.0	-30 -15	-12 -18	-21 -26
18	Clenpenterol	37158-47-7	291.1	203.1* 273.1	-21 -22	-12 -16	-30 -22
19	Bambuterol	81732-65-2	368.0	294.1* 312.1	-18 -18	-15 -24	-22 -30
20	Clenisopenterol	157664-68-1	291.1	273.1* 188.1	-30 -15	-12 -22	-30 -21
21	Mapenterol	54238-51-6	325.1	237.0* 217.1	-16 -17	-17 -26	-26 -24
22	Phenylethanolamine A (PA)	1346746-81-3	345.1	150.1* 327.1	-18 -18	-24 -13	-28 -23
23	Clenhexerol	78982-88-4	305.1	287.1* 203.0	-30 -16	-14 -22	-20 -22
24	Salmeterol	89365-50-4	416.2	398.1* 380.1	-20 -20	-16 -20	-29 -27

Note: * represents quantifier ion

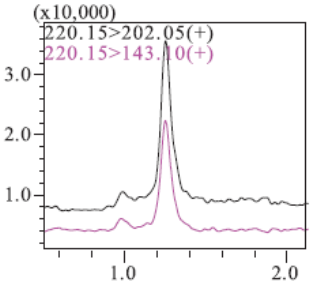
1.3. Sample Preparation

Sample was pretreated according to the sample pretreatment method stipulated in "GBT 22286-2008: Determination of Multiple β-receptor Agonist Residues in Animal-Derived Food – LC-Tandem MS Method."

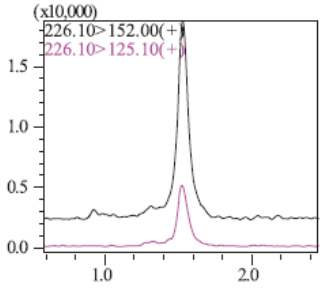
Preparation of standard solution: Mixed standard solutions containing 24 agonists at the concentration of 500 mg/L were prepared with methanol. This stock solution was sequentially diluted with methanol/water solution (5/95, V/V) to obtain a series of mixed standard working solutions at concentrations of 0.1, 0.5, 1.0, 5.0 and 10.0 µg/L.

■ Results and Discussion

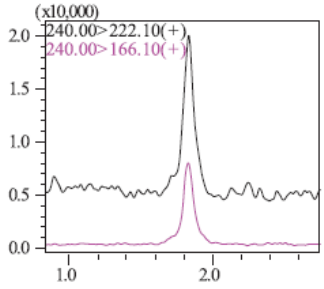
2.1. MRM Chromatograms of the Standard Samples



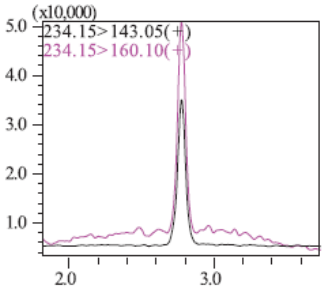
1. Cimaterol



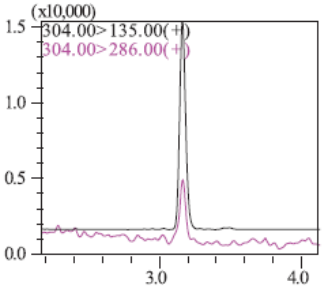
2. Terbutaline



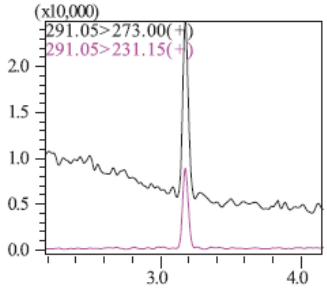
3. Salbutamol



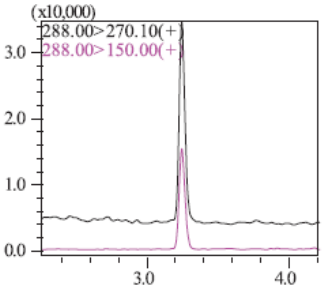
4. Cimbuterol



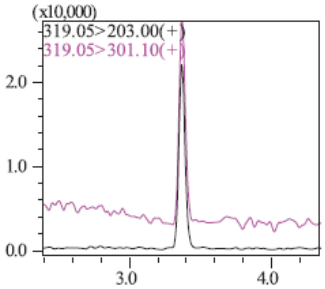
5. Fenoterol



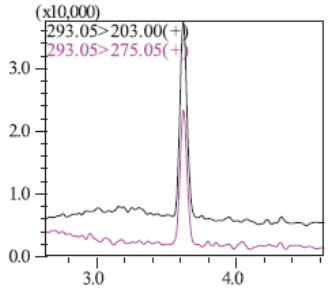
6. Procaterol



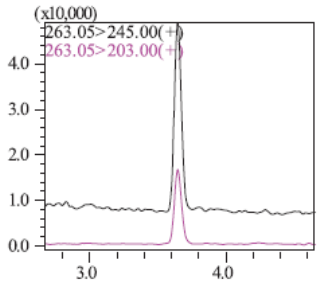
7. Ritodrine



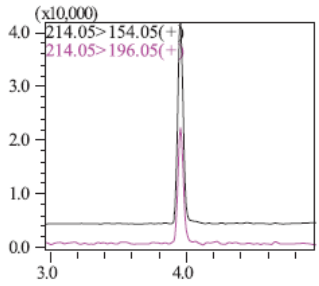
8. Clencyclohexerol



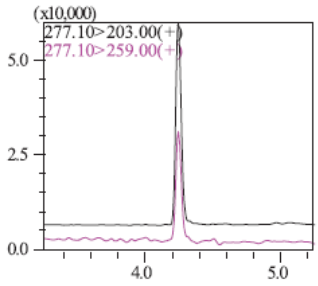
9. NA- 1411



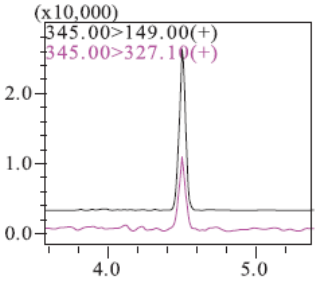
10. Clenproperol



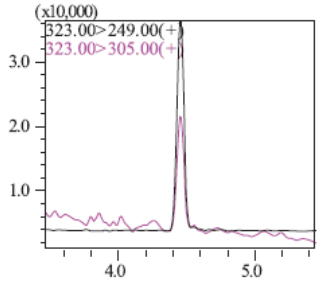
11. Clorprenaline



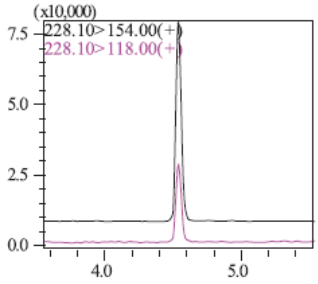
12. Clenbuterol



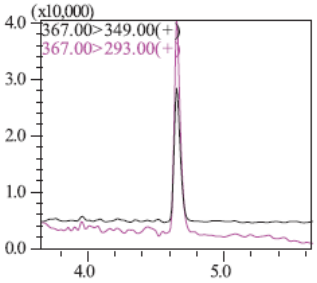
13. Formoterol



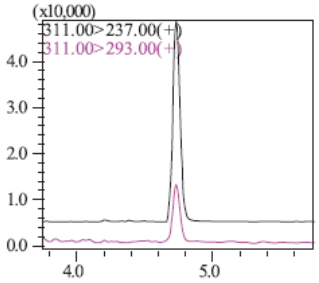
14. Bromchlorbuterol



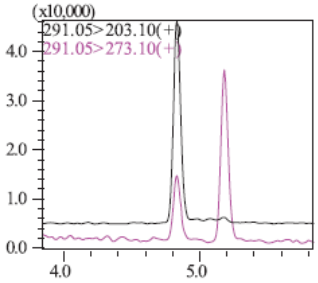
15. Tulobuterol



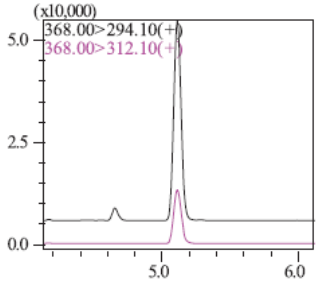
16. Brombuterol



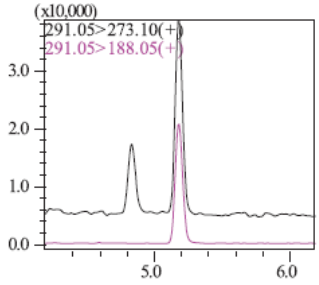
17. Mabuterol



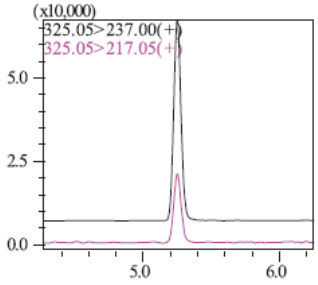
18. Clenpenterol



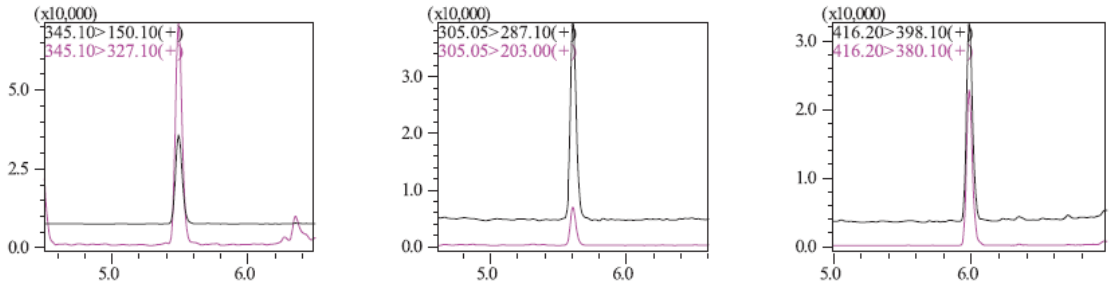
19. Bambuterol



20. Clenisopenterol



21. Mapenterol



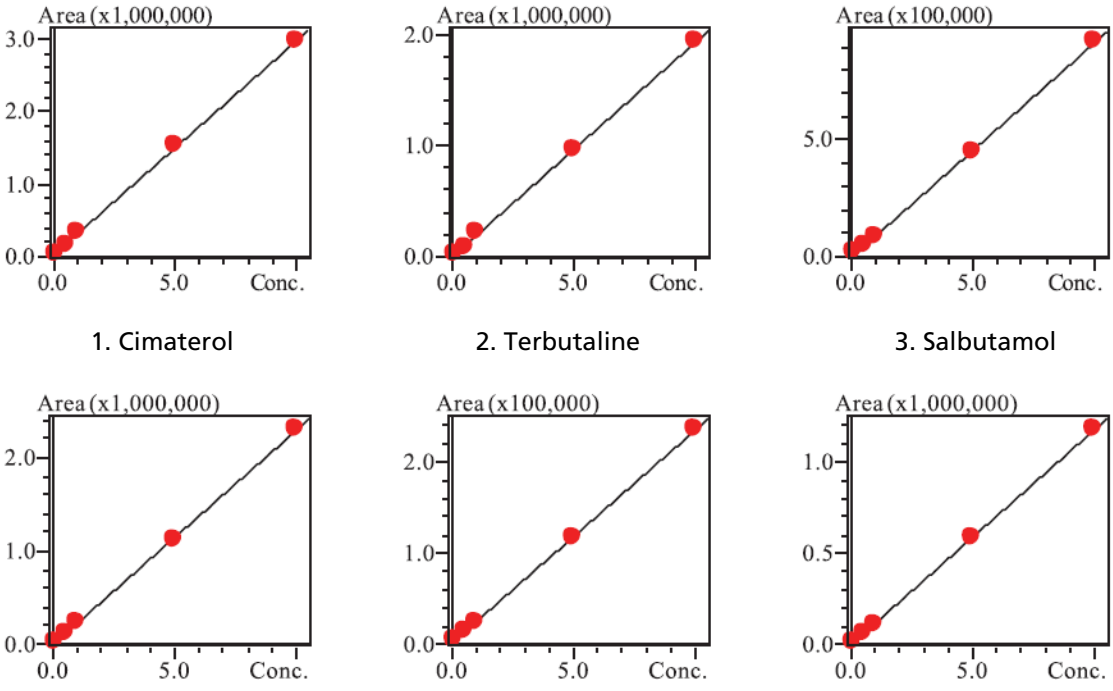
22. Phenylethanolamine A 23. Clenhexerol 24. Salmeterol

Figure 2: MRM Chromatograms of 24 Standard Samples at 0.5 µg/L

2.2. Calibration and Linearity

Calibration was performed using a 4-point curve with concentrations at 0.1, 0.5, 5 and 10 µg/L. External standard method was used to plot the calibration curves and the 24 compounds exhibited good linearity within the concentration range of 0.1-10 µg/L.

Some of the calibration curves are illustrated in Figure 3. The linear equations, correlation coefficients and linear range of all 24 compounds are shown in Table 3.



1. Cimaterol 2. Terbutaline 3. Salbutamol

4. Cimbuterol 5. Fenoterol 6. Procaterol

Figure 3: Calibration Curves of Cimaterol, Terbutaline, Salbutamol, Cimbuterol, Fenonterol and Procaterol

Table 3: Calibration Curve Parameters of 24 β-Receptor Agonists

No.	Compound Name	Calibration curve	Correlation coefficient (r)	Linear range (μg/L)
1	cimaterol	Y = (295392)X + (22965.8)	0.9996	0.1~10.0
2	terbutamol	Y = (I 93 I 58)X + (785.557)	0.9997	0.1~10.0
3	salbutamol	Y = (92096.7)X + (-4196.98)	0.9990	0.1~10.0
4	cimbuterol	Y = (228666)X + (49.6517)	0.9993	0.1~10.0
5	fenoterol	Y = (23336.1)X + (1754.60)	0.9997	0.1~10.0
6	procaterol	Y = (J I 7874)X + (-1056.10)	0.9997	0.1~10.0
7	ritodrine	Y = (168469)X + (-2714.09)	0.9998	0.1~10.0
8	clencyclohexerol	Y = (140613)X + (6007.49)	0.9981	0.1~10.0
9	NA	Y = (142095)X + (9475.16)	0.9997	0.1~10.0
10	clenproperol	Y = (276702)X + (5397.33)	0.9994	0.1~10.0
11	clorprenaline	Y = (120122)X + (2530.85)	0.9991	0.1~10.0
12	clenbuterol	Y = (165991)X + (3818.39)	0.9993	0.1~10.0
13	formoterol	Y = (63694.2)X + (967.377)	0.9991	0.1~10.0
14	bromchlorbuterol	Y = (134267)X + (5363.64)	0.9991	0.1~10.0
15	tulobuterol	Y = (395663)X + (-10450.5)	0.9997	0.1~10.0
16	brombuterol	Y = (149063)X + (613.530)	0.9995	0.1~10.0
17	mabuterol	Y = (87975 .6)X + (2167.41)	0.9994	0.1~10.0
18	clenpenterol	Y = (98120.2)X + (7456.52)	0.9998	0.1~10.0
19	bambuterol	Y = (110622)X + (-5098.32)	0.9992	0.1~10.0
20	clenisopenterol	Y = (241675)X + (3963.42)	0.9996	0.1~10.0
21	mapenterol	Y = (437827)X + (4879.97)	0.9996	0.1~10.0
22	PA	Y = (197828)X + (501.809)	0.9996	0.1~10.0
23	clenhexerol	Y = (213666)X + (5003.71)	0.9996	0.1~10.0
24	salmeterol	Y = (18969l)X + (-5054.86)	0.9999	0.1~10.0

2.3. Precision Experiment

The prepared mixed standard solutions at concentration of 0.1, 1.0 and 10.0 μg/L were determined successively six times to examine instrument precision.

The repeatability results are shown in Table 4 and the RSD of retention time of standards at low-, mid- and high- concentrations was 0.07-0.40% and 0.44-4.12% respectively, indicating good instrument precision.

Table 4: Repeatability Results of Retention Time and Peak Area (n=6)

		RSD% (0.1 μg/L)		RSD% (0.1 μg/L)		RSD% (10.0μg/L)	
No.	Sample Name	R.T	Area	R.T	Area	R.T	Area
1	cimaterol	0.40	3.90	0.24	2.18	0.29	1.08
2	terbutamol	0.35	2.93	0.13	2.44	0.23	1.17
3	salbutamol	0.11	3.85	0.19	1.87	0.18	2.49
4	cimbuterol	0.13	4.12	0.25	1.91	0.19	1.80
5	fenoterol	0.08	3.84	0.18	2.13	0.12	1.18
6	procaterol	0.07	2.74	0.19	1.95	0.08	0.44
7	ritodrine	0.13	3.80	0.12	2.88	0.09	0.87
8	clencyclohexerol	0.14	3.03	0.09	1.17	0.08	1.91
9	NA	0.11	2.10	0.12	2.09	0.13	2.13
10	clenproperol	0.16	2.87	0.10	1.80	0.14	1.85
11	clorprenaline	0.17	2.52	0.09	3.18	0.08	1.40
12	clenbuterol	0.15	3.27	0.14	1.44	0.10	1.76
13	formoterol	0.20	1.52	0.07	1.76	0.09	1.66
14	bromchlorbuterol	0.12	1.73	0.09	2.06	0.14	0.71
15	tulobuterol	0.10	2.78	0.07	1.71	0.07	1.47
16	brombuterol	0.09	2.12	0.10	1.47	0.08	1.11
17	mabuterol	0.14	2.83	0.18	1.61	0.09	2.03
18	clenpenterol	0.12	1.98	0.09	2.03	0.06	0.66
19	bambuterol	0.10	1.82	0.13	1.17	0.09	1.15
20	clenisopenterol	0.19	1.09	0.09	0.49	0.07	0.94
21	mapenterol	0.17	1.69	0.06	1.80	0.10	1.66
22	PA	0.14	0.69	0.09	1.18	0.14	1.57
23	clenhexerol	0.08	1.78	0.07	0.94	0.12	0.78
24	salmeterol	0.03	1.46	0.08	0.87	0.53	0.63

2.4. Method Performance

The matrix spike solution was prepared through dilution with chicken matrix to obtain a sample injection concentration of 0.1 µg/kg. The limit of detection (LOD, S/N=3) and limit of quantitation (LOQ, S/N=10) of these 24 β-Receptor Agonists substances are shown in Table 5.

Table 5: Limit of Detection and Limit of Quantitation of Twenty-four β-Receptor Agonists Substances

No.	Compound name	LOD (µg/kg)	LOQ (µg/kg)
1	cimaterol	0.010	0.034
2	terbutamol	0.006	0.020
3	salbutamol	0.025	0.082
4	cimbuterol	0.020	0.067
5	fenoterol	0.009	0.030
6	procaterol	0.017	0.056
7	ritodrine	0.011	0.037
8	clencyclohexerol	0.021	0.069
9	NA	0.015	0.051
10	clenproperol	0.010	0.032
11	clorprenaline	0.012	0.039
12	clenbuterol	0.009	0.031
13	formoterol	0.012	0.040
14	bromchlorbuterol	0.004	0.014
15	tulobuterol	0.006	0.020
16	brombuterol	0.011	0.036
17	mabuterol	0.008	0.027
18	clenpenterol	0.012	0.040
19	bambuterol	0.009	0.030
20	clenisopenterol	0.010	0.033
21	mapenterol	0.006	0.019
22	PA	0.004	0.DI5
23	clenhexerol	0.012	0.038
24	salmeterol	0.010	0.034

2.5. Matrix Spike and Recovery Experiment

After sample pretreatment as stated in 1.3, mixed standard stock solution was added to the blank matrix to obtain a final concentration of 1.0 µg/kg. The recovery and RSD of the 3 replicates were determined. The results are shown in Table 6, and the matrix spike recovery was in the range of 90.1-107%. The chromatogram of the blank matrix, and matrix spike sample are shown in Figures 4 and 5 respectively.

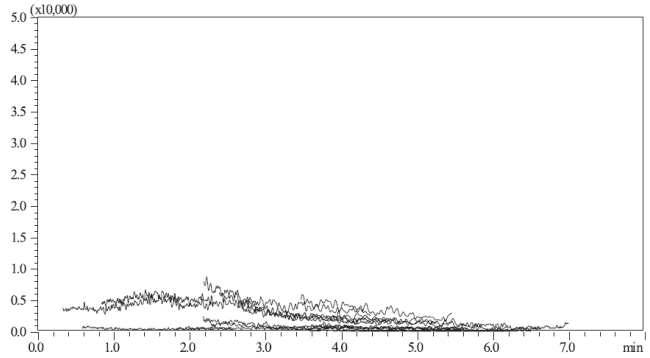


Figure 4: Chromatogram of the Blank Matrix

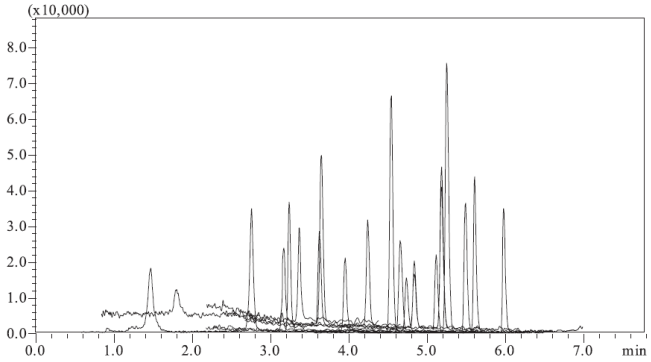


Figure 5: Chromatogram of the Matrix Spike Sample

Table 6: Recovery Results of the Matrix Spike Sample (n=3)

No.	Compound name	Recovery (%)	RSD%	No.	Compound Name	Recovery (%)	RSD%
1	Cimaterol	102.4	2.01	13	formoterol	95.3	1.93
2	terbutamol	104.7	2.09	14	bromchlorbuterol	93.1	1.71
3	salbutamol	94.8	2.02	15	tulobuterol	96.3	1.85
4	cimbuterol	91.5	1.02	16	brombuterol	94.8	0.95
5	fenoterol	99.5	2.04	17	mabuterol	102.5	1.63
6	procaterol	96.8	0.65	18	clenpenterol	99.5	1.17
7	ritodrine	98.5	1.11	19	bambuterol	96.1	1.58
8	clencyclohexerol	90.1	0.16	20	clenisopenterol	98.5	0.91
9	NA	98.6	0.74	21	mapenterol	99.1	1.53
10	clenproperol	101.9	0.92	22	PA	103.9	0.53
11	clorprenaline	97.2	0.63	23	clenhexerol	95.4	1.06
12	clenbuterol	94.1	1.14	24	salmeterol	100.3	1.79

■ Conclusion

The described method demonstrates the determination of twenty-four β-receptor agonists in chicken using Shimadzu Nexera X2 UHPLC coupled with Triple Quadrupole Mass Spectrometer LCMS-8050. This method successfully separates and quantifies 24 β-receptor agonists in 10 minutes. The RSD of retention time and peak area was 0.07-0.40% and 0.44-4.12% respectively, indicating good instrument precision. For all 24 β-receptor agonists, the calibration curves show good linearity with correlation coefficient greater than 0.9981. The LOD and LOQ was 0.004-0.025 µg/kg and 0.014-0.082 µg/kg respectively. With characteristics such as rapid, good repeatability and high sensitivity, this method demonstrates to be suitable for the detection of β-receptor agonists in chicken.

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