Application Data Set from Shimadzu

●LCMSMS Analysis LCMSMS-002

Determination of lean meat powder in pork by UFLC-triple quadrupole mass spectrometry

Abstract: A method was proposed for determination of clenbuterol, ractopamine and salbutamol in pork using Shimadzu LC-30A ultra fast liquid chromatograph and LCMS-8030 triple quadrupole mass spectrometer. Extracted samples were separated by LC-30A ultra fast liquid chromatograph, and then quantitatively assayed with LCMS-8030 triple quadrupole mass spectrometer. The calibration curves of clenbuterol, ractopamine and salbutamol were of good linearity in the concentration range of 0.05~100 μg/L with correlation coefficients higher than 0.999. Precision tests were conducted on standard solutions at concentrations of 0.1 μg/L, 0.5 μg/L, 1 μg/L, 5 μg/L and 10 μg/L. The %RSDs of retention time and peak areas in 6 successive injections were below 0.42% and 5.47%, respectively, suggesting that the system was of good precision. The method's LOQs met the requirement of 0.5 μg/kg (for clenbuterol, the LOQ requirement was 0.05 μg/kg) stipulated in SNT 1924-2007.

Key words: lean meat powder, clenbuterol, β-receptor agonist, triple quadrupole mass spectrometry, ultra fast liquid chromatography

Lean meat powder refers to a group of veterinary drugs which, when added into feedstuffs, can increase the lean meat rate of livestocks, reduce feedstuff consumption, and cut costs by marketing meat products ahead of schedule. When people refer to "lean meat powder" in China, most of the time they mean clenbuterol, a drug that was used for treatment of bronchial asthma but was later banned because of its serious side effects. At present, a category of drugs called β-receptor agonists are used for this function. Examples of these drugs include ractopamine and salbutamol. However, these drugs also pose potential safety hazard to human health because of their health-impairing effect in spite of their "lean meat rate promoting" action. For this reason, they are also banned globally. In China, β-receptor agonists were listed on the *Catalog of Drugs Prohibited*

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from Use in Feed or Drinking Water for Animals issued in 2002. It is stipulated in SNT 1924-2007, a standard issued by China Entry-Exit Inspection and Quarantine Bureau, that an LOQ requirement of 0.5 μg/kg applies to all methods for analysis of lean meat powder. The Positive List System of Japan has a stricter MRL, i.e. 0.05 μg/kg, for clenbuterol. Therefore, the LOQ requirement for product exported to Japan is even higher. In this paper, a method that meets the requirements for the assay of export food was proposed for determination of clenbuterol, ractopamine and salbutamol in pork with Shimadzu ultra-high performance liquid chromatograph-tandem mass spectrometer.

1. Experiments

1.1 Apparatus

A combined system of Shimadzu ultra fast liquid chromatograph LC-30A and triple quadrupole mass spectrometer LCMS-8030 was used in the experiment. The detailed configuration included two LC-30AD pumps, DGU-20A5 online degasser, SIL-30AC autosampler, CTO-30A column oven, CBM-20A communications bus module, LCMS-8030 triple quadrupole mass spectrometer, LabSolutions ver. 5.41 chromatography workstation.

1.2 Analytical conditions

LC conditions
Column: Shim-pack XR-ODSIII 2.0 mm I.D.×50 mm L., 1.6 μm
Mobile phase: A-0.1% aqueous solution of formic acid; B-0.1% acetonitrile solution of formic acid
Flow rate: 0.4 mL/min
Column temperature: 40 °C
Injection volume: 20 μL
Time program:

Time(min)	Module	Command	Value
0.56	Pump	B Conc.	50%
0.90	Pump	B Conc.	50%
0.95	Pump	B Conc.	10%
1.80	Controller	Stop	

MS condition
Ionization mode: ESI (+)
Ionization voltage: +4.5 kV
Nebulizing gas: Nitrogen 3.0 L/min
Drying gas: Nitrogen 15 L/min
Collision gas: Argon
DL temperature: 250 °C
Heater block temperature: 400 °C
Mode: multiple reaction monitoring (MRM)
Dwell time: 20 ms
Pause time: 3 ms
MRM parameters: see Table1

1.3 Preparation of standard solutions and pretreatment of samples

1 μg/mL multi-standard intermediate solution and 100 ng/mL multi-standard intermediate solution of isotope internal standards were prepared using methanol as solvent. The multi-standard intermediate solution was diluted with water into a series of multi-standard working solutions at concentrations of 0.05, 0.1, 0.5, 1, 5, 10, 50 and 100 ng/mL, which were then spiked with isotope internal standards at spiked level of 1.0 ng/mL.

Sample pretreatment was carried out in reference with SNT 1924-2007 *Determination of Clenbuterol, Ractopamine, Salbutamol, and Terbutalin residues in Animal Derived Food for Import and Export - HPLC-MS/MS Method,* with a modification that 5 g of sample that had been subjected to the pretreatment procedures was brought to the volume of 1 mL in the end.

Table 1 MRM parameters

Name	Precursor Ion	Product Ion	Q1 Pre Bias(V)	CE(V)	Q3 Pre Bias(V)
Salbutamol	239.95	148.10	-13.0	-20.0	-18.0
		222.10*	-13.0	-10.0	-18.0
D3-salbutamol	243.20	151.10	-13.0	-20.0	-18.0
Ractopamine	302.15	164.15	-12.0	-15.0	-13.0
		107.15*	-12.0	-30.0	-23.0
D6-ractopamine	307.90	168.15	-25.0	-15.0	-13.0
Clenbuterol	277.10	203.00	-11.0	-15.0	-16.0
		259.05*	-11.0	-10.0	-20.0
D9-clenbuterol	286.10	204.00	-15.0	-20.0	-16.0

* refers to qualitative ion.

2. Results and Discussion

2.1 Chromatograms of multi-standard working solutions

The MRM chromatograms of 1 ng/mL multi-standard working solution are shown in Figs. 1-6. The retention time data of salbutamol, D3-salbutamol, ractopamine, D6-ractopamine, clenbuterol and D9-clenbuterol were 0.476, 0.475, 0.869, 0.867, 0.911 and 0.910 minutes, respectively.

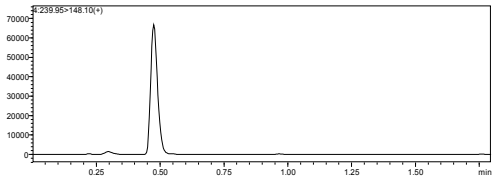

Fig.1 MRM chromatogram of 1 ng/mL salbutamol (239.95>148.10)

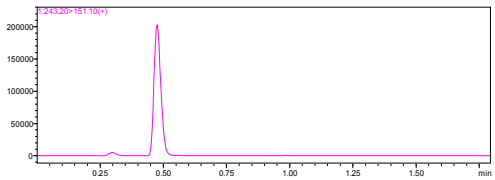

Fig.2 MRM chromatogram of 1 ng/mL D3-salbutamol (243.20>151.10)

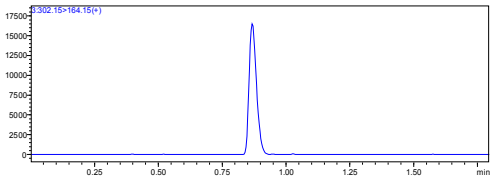

Fig.3 MRM chromatogram of 1 ng/mL ractopamine (302.15>164.15)

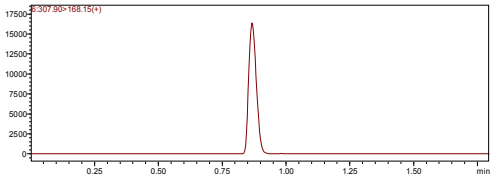

Fig.4 MRM chromatogram of 1 ng/mL D6-ractopamine (307.90>168.15)

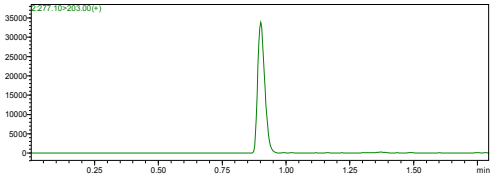

Fig.5 MRM chromatogram of 1 ng/mL clenbuterol (277.10>203.00)

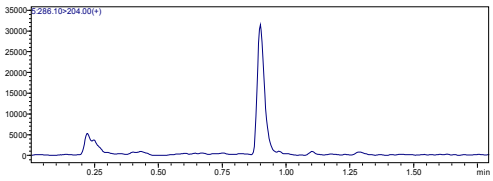

Fig.6 MRM chromatogram of 1 ng/mL D9-clenbuterol (286.10>204.00)

2.2 Linearity

Multi-standard working solutions at concentrations of 0.05, 0.1, 0.5, 1, 5, 10, 50 and 100 ng/mL were assayed using the analysis conditions under 1.2. Calibration curves were plotted using concentration ratio as abscissa and peak area ratio as ordinate. The plotted calibration curves were of satisfactory linear relation and relevant information was as shown in Table 2.

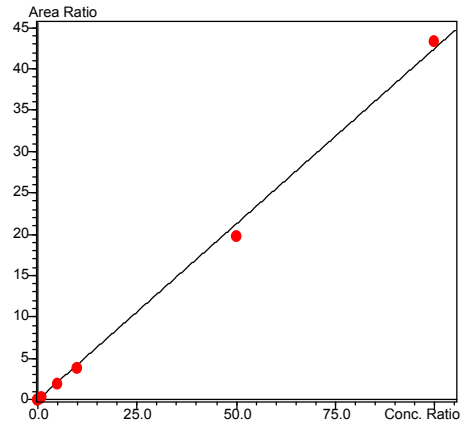

Fig.7 Calibration curve of salbutamol

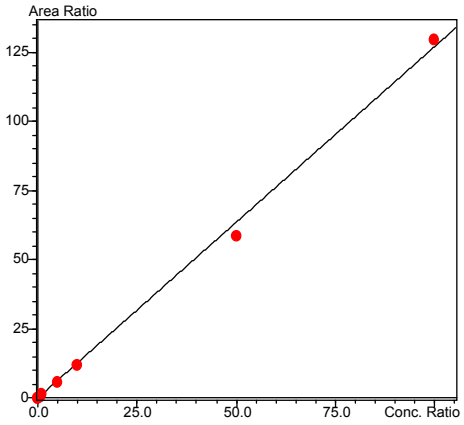

Fig.8 Calibration curve of ractopamine

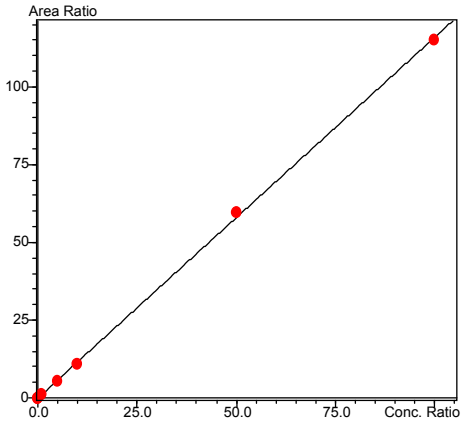

Fig.9 Calibration curve of clenbuterol

Table 2. Calibration curves of 3 β-receptor agonists

Name	Calibration Curve	Correlation Coefficient (r)
Salbutamol	Y = (0.424827)X	0.9991
Ractopamine	Y = (1.27146)X	0.9991
Clenbuterol	Y = (1.15869)X	0.9998

2.3 Precision test

Multi-standard working solutions at concentrations of 0.1, 0.5,1, 5 and 10 ng/mL were assayed with 6 consecutive injections to assess the method's precision. The repeatability of retention time and peak area is shown in Table 3.

Table 3 Repeatability of salbutamol (n=6)

Conc.(ng/mL)	%RSD (RT)	%RSD (Area)
0.1	0.12	2.43
0.5	0.18	2.98
1	0.12	0.81
5	0.07	1.26
10	0.14	1.05

Table 4 Repeatability of ractopamine (n=6)

Conc.(ng/mL)	%RSD (RT)	%RSD (Area)
0.1	0.15	5.47
0.5	0.13	2.08
1	0.10	1.89
5	0.08	1.70
10	0.06	1.91

Table 5 Repeatability of clenbuterol (n=6)

Conc.(ng/mL)	%RSD (RT)	%RSD (Area)
0.1	0.15	4.55
0.5	0.04	3.05
1	0.11	1.50
5	0.04	1.16
10	0.05	1.14

2.4 Sensitivity test

In order to assess the method's sensitivity, matrix blank samples of pork were spiked with 0.05 μg/kg clenbuterol, the resulted chromatograms are shown in Figs. 10 and 11. The method's LOQ for clenbuterol was determined to be 0.05 μg/kg.

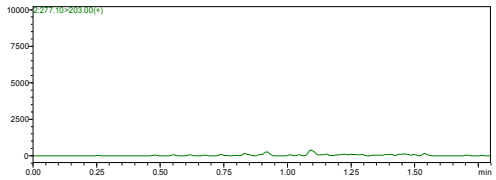

Fig.10 MRM chromatogram of pork matrix blank sample
(277.10>203.00)

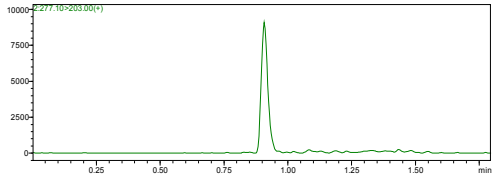

Fig.11 MRM chromatogram of pork matrix spiked with 0.05 μg/kg clenbuterol standard
(277.10>203.00)

3. Conclusion

A method was proposed for the assay of 3 β-receptor agonists in pork with Shimadzu LC-30A ultra fast liquid chromatograph and LCMS-8030 triple quadrupole mass spectrometer. The method had the merits of fast analysis speed, good precision, and wide linear range (0.05-100 ng/mL). The correlation coefficients of all calibration curves were higher than 0.999. The method's LOQ met the requirement stipulated in China's national standard (0.5 μg/kg; for clenbuterol in pork, 0.05 μg/kg). It was concluded that Shimadzu ultra fast liquid chromatograph-tandem mass spectrometer can meet the requirements for the assay of lean meat powder in food for import and export.