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LCMS-8050

Application News
SGLC-LC/MS-046EN

Determination of nine macrolide antibiotics in grass carp

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User Benefits

- Established an effective, fast and simple sample preparation method for analysis of nine macrolide antibiotics in grass carp.
- Realized simultaneous quantitative analysis of nine macrolide antibiotics.
- The method has a high recovery and good reproducibility.

■ Introduction

Macrolide antibiotics (MALs) are a class of antibiotics produced by Actinobacteria or Micromonospora. MALs has become one of the fastest growing antibiotics in terms of demand and sales worldwide. Due to its broad-spectrum antibacterial properties, MALS can resist Gram positive bacteria, Mycoplasma, and some Gram negative bacteria. Therefore, it is widely used in the treatment of respiratory and infectious diseases in pigs, cattle, sheep, shrimp, and poultry, or as a feed additive to promote animal growth and development at low doses. Residues of macrolide drugs can cause allergic reactions and lead to the spread of strains carrying resistance factors. Like other veterinary drugs, the monitoring and control of residues of macrolide drugs in animal derived foods has been highly valued by many countries. In this application, we present a complete workflow, from sample preparation using SHIMSEN Styra Al-N, to sample analysis using Shim-pack Scepter C18-120 column on Shimadzu LCMS-8050.

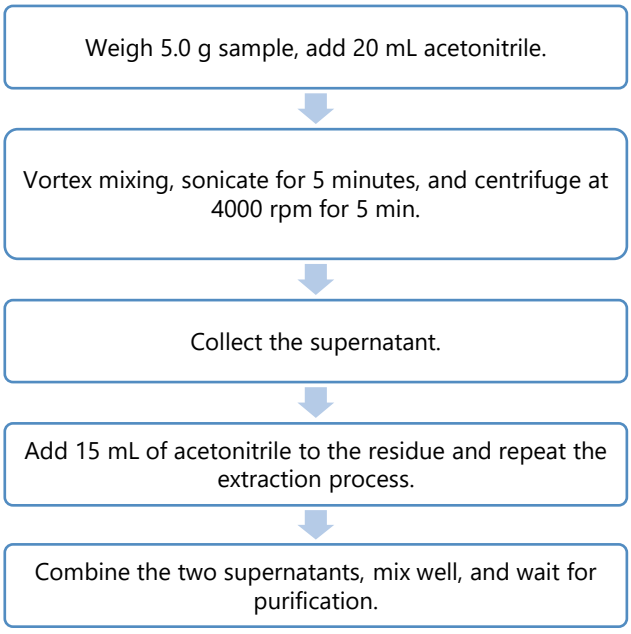

Figure 1. Sample extraction process.

■ Experimental

Materials:

SHIMSEN Styra Al-N (2 g/12 mL; P / N: 380-00865-06);
Shim-pack Scepter C18-120,1.9 µm, 100 × 2.1 mm (P/N: 227-31012-03)
Filter and vial:
SHIMSEN Disc HPTFE syringe filter (P/N: 380-00341);
LabTotal Vial (P/N: 227-34001-01)

Sample Preparation:

Sample extraction:

Weigh 5.0 g sample into a 50 mL centrifuge tube, add 20 mL acetonitrile, vortex mixing, sonicate for 5 minutes, and centrifuge at 4000 rpm for 5 min. Collect the supernatant into a 50 mL centrifuge tube. Add 15 mL of acetonitrile to the residue and repeat the extraction process. Combine the two supernatants, mix well, and wait for purification. The flowchart of sample extraction process is shown in Fig. 1.

Sample purification:

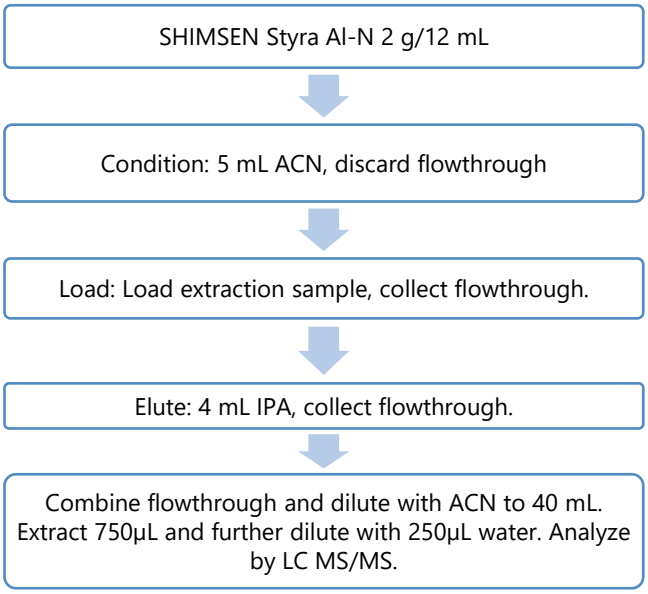

Figure 2. Purification by SPE.

Table 1. LCMS conditions

UHPLC Conditions	
LC system	Shimadzu LC-30A
Column:	Shim-pack Scepter C18-120,1.9 μm, 100 × 2.1 mm *1
Column Temp.:	40℃
Flow rate:	0.4 mL/min
Mobile phase A:	0.1% formic acid
Mobile phase B:	acetonitrile
Gradient program:	10% B (0 min) → 20%B (1.5 min) → 60% B (4 min) → 95% B (5 min) → 95% B (6 min) → 10% B (6.1 min) →10% B (8 min)
Injection volume:	10 μL
MS conditions:	
Interface:	Heated ESI (Positive)
Interface temp:	300℃
Collision gas:	Ar
Nebulizing gas:	N_2, 3 L/min
Heating gas flow:	Zero air, 10 L/min
DL temperature:	250℃
Drying gas flow:	N_2, 10 L/min
Heat block temp:	400℃
MS mode:	MRM

*1P/N:227-31012-03

Table 3. List of recovery and RSD for each macrolide antibiotic.

No.	Compounds	Spiked amount (1.0 μg / kg, n=3)	
		Avg recovery (%)	RSD(%)
1	Spiramycin	103.84	10.37
2	Tilmicosin	80.96	10.68
3	Kitasamycin	93.33	4.52
4	Erythromycin	85.43	7.13
5	Josamycine	93.85	6.98
6	Azithromycin	86.08	3.68
7	Clarithromycin	94.65	8.10
8	Desmycosin	99.06	1.26
9	Amimycin	93.71	3.14

Table 2. List of MRM used for each macrolide antibiotics.

No.	Compounds	Mode	Precursor ion	Quantifying ion	Q1 Pre Bias	CE	Q3 Pre Bias	Qualifying ion	Q1 Pre Bias	CE	Q3 Pre Bias
1	Spiramycin	+	843.20	174.15	-22.0	-35.0	-29.0	142.10	-22.0	-35.0	-12.0
2	Tilmicosin	+	869.40	174.05	-20.0	-45.0	-30.0	696.40	-20.0	-42.0	-32.0
3	Kitasamycin	+	772.20	109.00	-22.0	-42.0	-17.0	174.10	-22.0	-31.0	-15.0
4	Erythromycin	+	734.30	158.05	-20.0	-31.0	-25.0	576.30	-20.0	-21.0	-26.0
5	Josamycine	+	828.30	109.00	-20.0	-45.0	-17.0	174.20	-20.0	-33.0	-16.0
6	Azithromycin	+	749.50	591.40	-22.0	-31.0	-28.0	158.10	-22.0	-40.0	-14.0
7	Clarithromycin	+	748.50	158.20	-22.0	-29.0	-14.0	590.40	-22.0	-20.0	-26.0
8	Tylosin	+	916.60	174.20	-22.0	-39.0	-16.0	772.50	-20.0	-32.0	-36.0
9	Amimycin	+	688.40	158.20	-20.0	-27.0	-14.0	544.40	-20.0	-17.0	-24.0

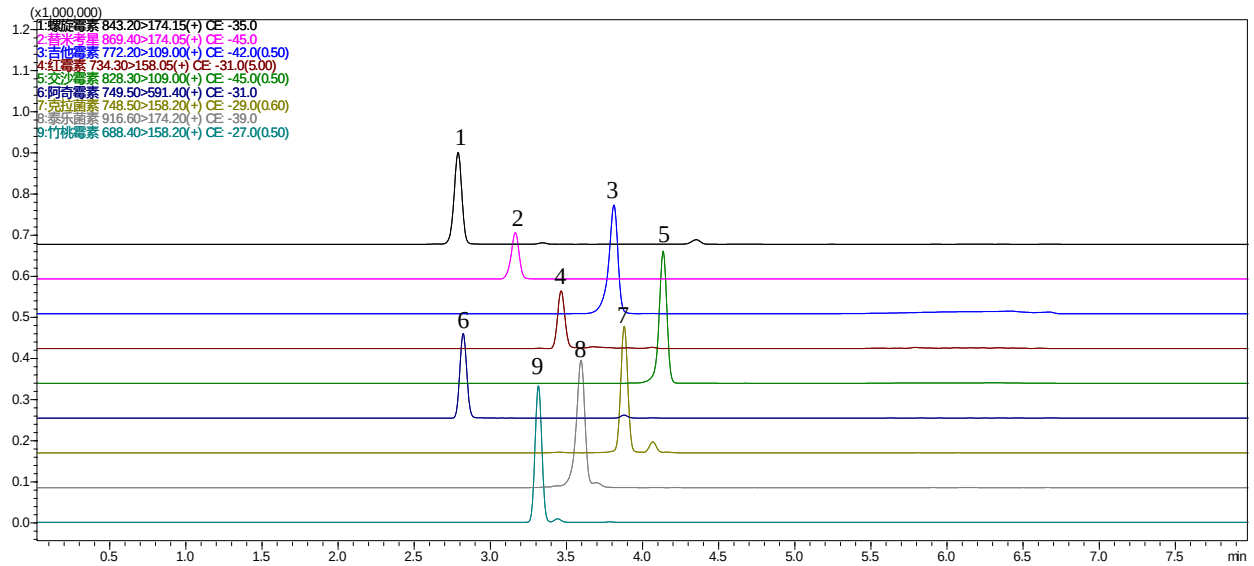

Figure 3. MRM chromatogram of nine macrolide antibiotics mixed standards solution (Concentration: 5 μg/L).
(1. Spiramycin; 2. Telmicacin; 3. Guitamycin; 4. Erythromycin; 5. Oxamycin;
6. Azithromycin; 7. Clarithromycin; 8. Thalamycin; 9. Glemonamycin)

Condition and equilibrate the SPE cartridges with 5 mL acetonitrile, discard the eluate; load the sample solution, collect the eluate; Elute with 4 mL isopropanol, collect the eluate; Combine the eluate, and add acetonitrile to 40 mL, and mix. Extract 750 uL of combined eluate, add 250 µL water, vortex to mix, filter the sample solution through a 0.22 µm micropore membrane for LC-MS/MS analysis. The flowchart of sample purification process is shown in Fig. 2.

■ Results and Discussion

Grass carp blank matrix was spiked with a standard solution of nine macrolide antibiotics to a final concentration of 1.0 µg/kg. Sample preparation by SPE purification was performed according to Figure 1 and Figure 2. Figure 3 shows the MRM chromatogram of the nine macrolide antibiotics standards. Three independent experiment was performed to determine average recovery and RSD. Results shows all compounds having a good recovery between 80%-120%. RSD of all compounds were below 20%. Recovery and RSD for all the compounds are shown in Table 3.

■ Conclusion

This study presents a method for nine macrolide antibiotics in grass carp. Shimadzu SHIMSEN Styra Al-N products were used for sample preparation, followed analysis using Shim-pack Scepter C18-120 column on Shimadzu LCMS-8050. The recovery and reproducibility was determined by 1.0 µg/kg spiked grass carp blank sample. The results shows a high recovery of 80.96%-103.84%, and a good reproducibility, with RSD of 1.26%-10.68%.

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