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Application News

No. LCMSMS-254E

Liquid Chromatography Mass Spectrometry

Determination of Sulfonamide Residues in Pork Using LCMS-8045

Abstract: This application note demonstrates a method for determination of sulfonamide residues in pork using Shimadzu Ultra-High-Performance Liquid Chromatograph (UHPLC) Nexera X2 together with the Triple Quadrupole Mass Spectrometer LCMS-8045. The linearity of the 11 sulfonamides was excellent and their correlation coefficients were all greater than 0.999. The instrument's limit of detection was 0.002 to 0.026 µg/L, and its limit of quantitation was 0.006 to 0.080 µg/L. The matrix spike recovery rate was between 86.6-119.8%. As this method meets the requirements in terms of lower limit of detection of 0.5 µg/kg as specified in the Department of Agriculture's announcement No. 1025-23-2008, it can be used to quickly and accurately determine sulfonamide residues in pork.

Key Words: Sulfonamides, Food, Ultra-High-Performance Liquid Chromatograph, Triple Quadrupole Mass Spectrometry

■ Introduction

Sulfonamides (SAs) refer to synthetic antibiotics with sulfanilamide structures and it can be used to suppress most gram-positive bacteria and some gram-negative bacteria. When used in combination with antibacterial synergists, such as trimethoprim, SAs can enhance the antibacterial effect and expand the scope of treatment. Due to their advantages of having wide antibacterial spectra, strong curative effect and low cost, SAs are widely used in the prevention and treatment of diseases. However, one of its main drawbacks is that it can easily bring about side effects such as allergies and hematopoietic disorders, thereby causing a gradual reduction in clinical applications and usage. Instead, SAs are widely used in livestock breeding and aquaculture. Most of these drugs cannot be fully metabolized in animals and these SA residues can enter human body through the food chain, thus causing harm to human health.

At present, the European Union, the United States, and Japan all list SAs as drugs with a restricted use in animal husbandry, with the maximum amount of SA residues generally limited to 50–100 µg/kg. China has also established relevant standards for detection of SA residues in animal-derived food, such as GB 29694-2013: "Determination of 13 Types of SA Residues in Animal-Derived Food Using High Performance Liquid Chromatography", GB 21316-2007: "Determination of SA Residues in Animal-Derived Food Using Liquid Chromatography-Mass Spectrometry/Mass Spectrometry", and SN/T 4057-2014: "Determination of SA Residues in Animal-Derived Food for Export Using Immunoaffinity Column Chromatography-HPLC and LC-MS/MS Method".

In reference to the sample preparation method No. 1025-23-2008 listed by the Department of Agriculture "Detection of SA Residues in Animal-Derived Food Using Liquid Chromatography-Tandem Mass Spectrometry", this application note demonstrates the use of Shimadzu UHPLC Nexera X2 together with the Triple Quadrupole Mass Spectrometer LCMS-8045 to determine SA residues in pork.

■ Experimental

1.1 Instruments

The experiment employed Shimadzu UHPLC Nexera X2 and Triple Quadrupole Mass Spectrometer LCMS-8045. The configurations are LC-30AD×2 infusion pump, DGU-20A$_{5R}$ inline degasser, SIL-30AC autosampler, CTO-30AC column oven, CBM-20A system controller, LCMS-8045 triple quadrupole mass spectrometer and LabSolutions Ver. 5.86 Chromatographic Workstation.

1.2 Analytical Conditions

Liquid Chromatography (LC) Conditions

- Column: Shim-pack XR-ODS III (2.0 mm I.D.×50 mm L., 1.6 µm)
- Mobile phase: Mobile phase A-0.1% formic acid in water, Mobile phase B-methanol
- Flow rate: 0.3 mL/min
- Column temperature: 40 °C
- Injection volume: 5 µL
- Elution method: Gradient elution with the initial concentration of Mobile Phase B at 10%. Refer to Table 1 for gradient program.

Mass Spectrometry (MS) Conditions

- Ion sources: ESI
- Nebulizing gas flow rate: 3.0 L/min
- Interface temperature: 300 °C
- Drying gas flow rate: 10.0 L/min
- DL temperature: 250 °C
- Heated gas flow rate: 10.0 L/min
- Scan mode: Multiple reaction monitoring (MRM)
- MRM parameters: Refer to Table

Table 1: Gradient program

Time (min)	Module	Command	Value (%)
0.20	Pumps	Pump B Conc.	10
1.00	Pumps	Pump B Conc.	30
2.00	Pumps	Pump B Conc.	30
4.00	Pumps	Pump B Conc.	90
5.00	Pumps	Pump B Conc.	90
5.01	Pumps	Pump B Conc.	10
8.00	Controller	Stop	

Table 2: MRM parameters

No.	Compound Name	Precursor Ion	Product Ion	Q1 Pre Bias (V)	CE (V)	Q3 Pre Bias (V)
1	Sulfathiazole	256.10	156.05*	-28.0	-16.0	-29.0
			108.10	-18.0	-25.0	-21.0
2	Sulfapyridine	250.15	156.05*	-28.0	-17.0	-29.0
			184.00	-12.0	-17.0	-19.0
3	Sulfamethiazole	271.05	156.05*	-13.0	-14.0	-29.0
			108.10	-13.0	-27.0	-21.0
4	Sulfamethazine/ sulfadimidine	279.20	186.10*	-14.0	-18.0	-19.0
			156.00	-30.0	-20.0	-28.0
5	Sulfameter/ sulfamethoxydiazine	281.15	156.05*	-30.0	-18.0	-30.0
			108.15	-20.0	-28.0	-20.0
6	Sulfamethoxypyridazine	281.15	156.10*	-30.0	-18.0	-30.0
			108.10	-20.0	-27.0	-20.0
7	Sulfachloropyridazine	285.05	156.10*	-14.0	-14.0	-29.0
			108.00	-14.0	-27.0	-21.0
8	Sulfamethoxazole	254.10	156.05*	-29.0	-16.0	-30.0
			108.10	-12.0	-24.0	-21.0
9	Sulfisoxazole	268.10	156.10*	-29.0	-14.0	-28.0
			113.15	-13.0	-15.0	-22.0
10	Sulfamethoxine	311.15	156.10*	-15.0	-23.0	-30.0
			108.05	-15.0	-30.0	-22.0
11	Sulfaquinoxaline	301.15	156.10*	-15.0	-17.0	-29.0
			108.10	-15.0	-27.0	-19.0

Note: * indicates quantifier ion

1.3 Sample Preparation

There were 11 SA substances in total, including sulfathiazole, sulfapyridine, sulfamethiazole, sulfamethazine/sulfadimidine, sulfameter/sulfamethoxydiazine, sulfamethoxypyridazine, sulfachloropyridazine, sulfamethoxazole, sulfisoxazole, sulfamethoxine, and sulfaquinoxaline.

Preparation of standard solutions:
Mixed standard stock solutions at a concentration of 10 mg/L were prepared using acetonitrile. The mixed standard stock solutions were subsequently diluted with a methanol/water solution (V/V, 10:90) to obtain mixed standard working solutions at concentrations of 0.1, 0.5, 1, 5, 10, and 50 µg/L.

Preparation of sample:
Samples were prepared and injected for analysis according to the sample extraction and clean-up method listed in the Department of Agriculture's No. 1025-23-2008 "Detection of SA Residues in Animal-Derived Food Using Liquid Chromatography-Tandem Mass Spectrometry".

■ Results and Discussion

2.1 MRM Chromatograms of Standard Samples
MRM chromatograms of mixed standard samples are shown in Figure 1.

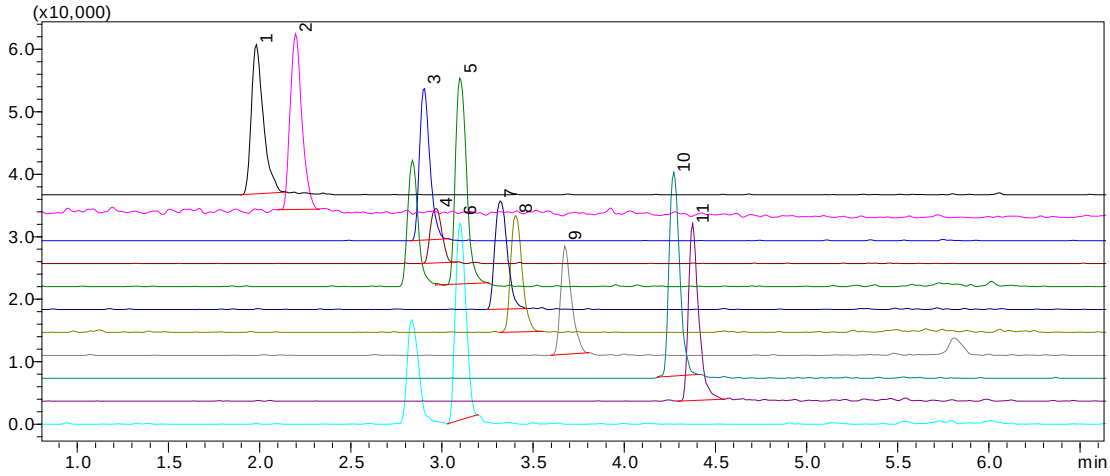

Figure 1: MRM chromatogram of mixed standard sample (0.5 µg/L)
(1. Sulfathiazole; 2. Sulfapyridine; 3. Sulfamethiazole; 4. Sulfamethazine/sulfadimidine; 5. Sulfameter/sulfamethoxydiazine; 6. Sulfamethoxypyridazine; 7. Sulfachloropyridazine; 8. Sulfamethoxazole; 9. Sulfisoxazole; 10. Sulfamethoxine; 11. Sulfaquinoxaline)

2.2 Calibration and Linearity
The mixed standard calibration solutions at concentrations of 0.1, 0.5, 1, 5, 10, and 50 µg/L were prepared and determined according to analytical conditions in Section 1.2. Linearity was good over the concentration range of 0.1-10 µg/L and 0.1-50 µg/L where the external standard method was used to generate the calibration curve. Linear equation, linear range, and coefficients of the determination are shown in Table 3.

2.3 Limit of Detection and Limit of Quantitation
Pork samples were treated according to the method specified in Section 1.3 to obtain spiked samples at a concentration of 0.5 µg/L. After injection and analysis, the lower limit of detection (LOD, S/N=3) and the lower limit of quantitation (LOQ, S/N=10) for 11 SAs were calculated using software as shown in Table 4.

2.5 Precision Test
Mixed standard solutions at various concentrations were injected consecutively 6 times to determine the instrument's precision. Repeatability results of retention time and peak area are shown in Table 5. The relative standard deviations of retention time and peak area were within ranges of 0.02 to 0.13% and 0.66 to 5.15%, respectively, indicating good instrument precision.

2.6 Matrix Spike Samples Test
Pork samples were treated according to the method specified in Section 1.3 to obtain a blank matrix, which was then used to prepare spiked samples at a concentration of 0.5 µg/L for injection and analysis. The spike recovery rate of samples ranged from 86.6 to 119.8%. The chromatogram of the blank matrix is shown in Figure 2, while the chromatogram of the spiked samples is shown in Figure 3.

Table 3: Parameters for calibration curve (linear regression, the weight coefficient was 1/C)

No.	Compound Name	Calibration Curve	Linear Range (µg/L)	Accuracy (%)	Correlation Coefficients (R)
1	Sulfathiazole	Y = (208213) X + (3090.87)	0.1~50	81.9~106.9	0.9994
2	Sulfapyridine	Y = (244596) X + (-547.080)	0.1~10	91.9~104.1	0.9998
3	Sulfamethiazole	Y = (183474) X + (-83.6554)	0.1~10	95.6~104.9	0.9999
4	Sulfamethazine/sulfadimidine	Y = (163835) X + (1324.90)	0.1~10	89.2~108.3	0.9997
5	Sulfameter/sulfamethoxydiazine	Y = (250149) X + (5313.05)	0.1~10	88.5~108.1	0.9997
6	Sulfamethoxypyridazine	Y = (242793) X + (4103.03)	0.1~10	95.2~103.3	0.9996
7	Sulfachloropyridazine	Y = (157837) X + (175.633)	0.1~10	95.0~104.5	0.9998
8	Sulfamethoxazole	Y = (154848) X + (4259.61)	0.1~50	82.0~109.0	0.9991
9	Sulfisoxazole	Y = (145057) X + (1448.37)	0.1~50	94.9~106.6	0.9999
10	Sulfamethoxine	Y = (245919) X + (11612.2)	0.1~50	97.3~103.3	0.9999
11	Sulfaquinoxaline	Y = (200233) X + (6393.09)	0.1~50	88.1~104.2	0.9997

Table 4: Limit of detection and limit of quantification for 11 SAs

No.	Compound Name	Limit of Detection (µg/L)	Limit of Quantitation (µg/L)
1	Sulfathiazole	0.002	0.006
2	Sulfapyridine	0.010	0.032
3	Sulfamethiazole	0.002	0.006
4	Sulfamethazine/sulfadimidine	0.006	0.017
5	Sulfameter/sulfamethoxydiazine	0.025	0.076
6	Sulfamethoxypyridazine	0.026	0.080
7	Sulfachloropyridazine	0.003	0.011
8	Sulfamethoxazole	0.021	0.062
9	Sulfisoxazole	0.004	0.014
10	Sulfamethoxine	0.002	0.008
11	Sulfaquinoxaline	0.009	0.028

Table 5: Repeatability results of retention time and peak area (n=6)

No.	Compound Name	RSD% (1 µg/L)		RSD% (10 µg/L)	
		R.T.	Area	R.T.	Area
1	Sulfacetamide	0.13	2.62	0.07	0.92
2	Sulfathiazole	0.08	2.47	0.05	1.28
3	Sulfapyridine	0.13	2.39	0.05	1.00
4	Sulfamethiazole	0.03	1.86	0.04	0.83
5	Sulfamethazine/sulfadimidine	0.09	4.95	0.05	0.66
6	Sulfameter/sulfamethoxydiazine	0.05	1.33	0.03	1.56
7	Sulfamethoxypyridazine	0.04	5.15	0.03	1.72
8	Sulfachloropyridazine	0.07	2.11	0.03	1.04
9	Sulfamethoxazole	0.03	3.44	0.04	0.91
10	Sulfisoxazole	0.03	2.46	0.03	1.31
11	Sulfamethoxine	0.01	1.29	0.03	1.41
12	Sulfaquinoxaline	0.06	1.91	0.02	1.26

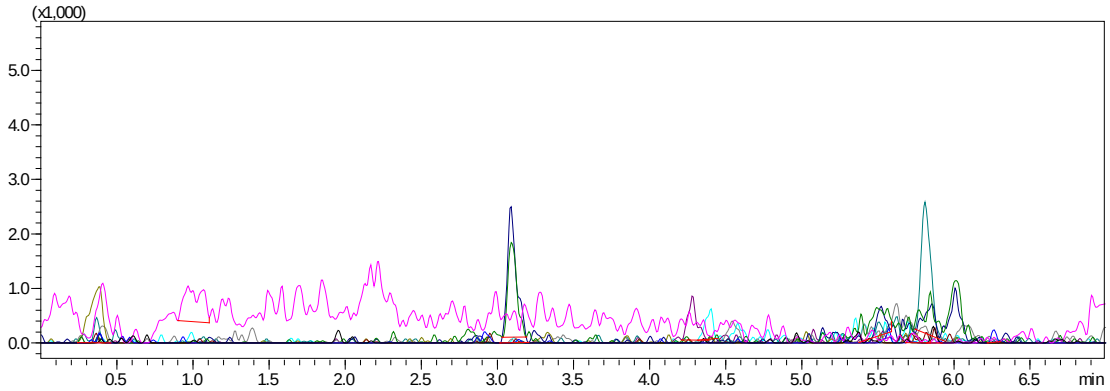

Figure 2: Chromatogram of blank matrix

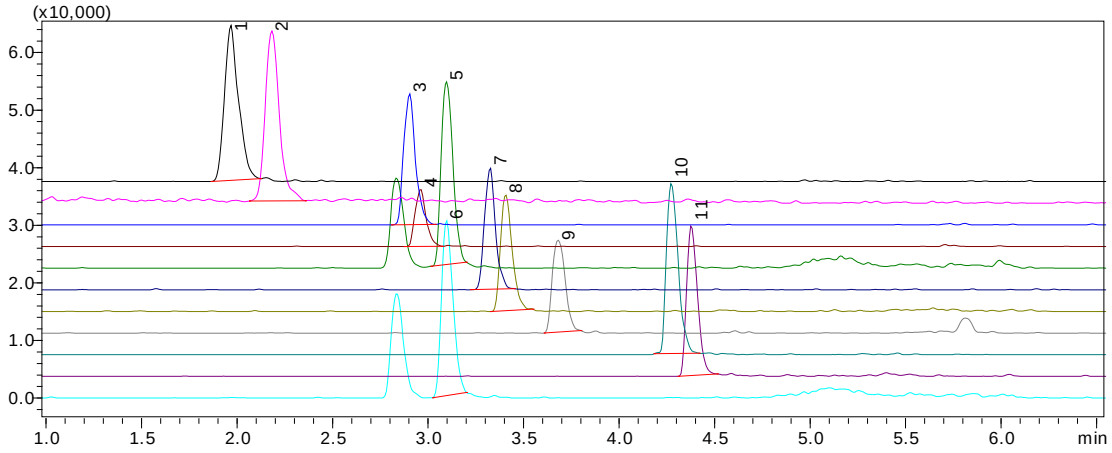

Figure 3: Chromatogram of spike recovery test
(1. Sulfathiazole; 2. Sulfapyridine; 3. Sulfamethiazole; 4. Sulfamethazine/sulfadimidine; 5. Sulfameter/sulfamethoxydiazine; 6. Sulfamethoxypyridazine; 7. Sulfachloropyridazine; 8. Sulfamethoxazole; 9. Sulfisoxazole; 10. Sulfamethoxine; 11. Sulfaquinoxaline)

Table 6: Results of spiked sample recovery

No.	Compound Name	Measured Concentration (µg/L)	Recovery (%)
1	Sulfathiazole	0.599	119.8
2	Sulfapyridine	0.553	110.6
3	Sulfamethiazole	0.509	101.8
4	Sulfamethazine/sulfadimidine	0.468	93.6
5	Sulfameter/sulfamethoxydiazine	0.494	98.8
6	Sulfamethoxypyridazine	0.508	101.6
7	Sulfachloropyridazine	0.479	95.8
8	Sulfamethoxazole	0.513	102.6
9	Sulfisoxazole	0.515	103.0
10	Sulfamethoxine	0.433	86.6
11	Sulfaquinoxaline	0.478	95.6

■ Conclusion

In the determination of SA residues in pork using Shimadzu UHPLC Nexera X2 coupled with the Triple Quadrupole Mass Spectrometer LCMS-8045, the linearity of each of these 11 SAs was good, and their correlation coefficients were all greater than 0.999. The instrument's limit of detection was 0.002 to 0.026 µg/L, and its limit of quantification was 0.006 to 0.080 µg/L. The matrix spike recovery rate was between 86.6 and 119.8%. As this method meets the requirements of lower limit of detection of 0.5 µg/kg as specified in the Department of Agriculture's No. 1025-23-2008 "Detection of SA Residues in Animal-Derived Food Using Liquid Chromatography-Tandem Mass Spectrometry", it can be used to detect SA residues in pork.

ULTRA FAST MASS SPECTROMETRY


LCMS-8040


LCMS-8045


LCMS-8050


LCMS-8060


LCMS-2020


Q-TOF LCMS-9030

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