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Application News

No.L510

High Performance Liquid Chromatography

Analysis of Residual Antimicrobials in Meat with Antimicrobial Screening System (Part 2)

Antimicrobials are a type of veterinary drug and animal feed additive, and are used for the treatment and prevention of disease in livestock and marine products. Residual antimicrobials are often found in livestock and marine products, so threshold levels for antimicrobials are set by regulation to ensure the safety of the consumer based on amounts that do not harm human health.
Due to ongoing reports of recent cases of regulatory violations in various countries and the large number of compounds targeted for testing, there is a demand for quick and simple antimicrobial screening.
While Application News No.L509 described an example of using the antimicrobial screening system for screening 12 quinolone compounds, this Application News describes an example screening analysis of 12 antimicrobial target compounds including sulfanomides.

Sample Pretreatment

Sample pretreatment for analysis of residual antimicrobials in meat usually employs liquid-liquid extraction (and sometimes solid phase extraction), but this process takes time and effort. In this article, we employed a QuEChERS method designed to be more efficient and reduce pretreatment times. The QuEChERS method is used to pretreat vegetables and fruits for residual pesticide analysis.
After using the QuEChERS method to perform extraction and fat removal, sample solutions were prepared by evaporation and redissolution steps. Table 1 shows the maximum residue limits (MRLs) of target compounds and sample solution concentrations after sample pretreatment, and Fig. 1 shows the sample pretreatment protocol. Refer to the instruction manual of the system for the details of the sample pretreatment procedure.

Table 1 Maximum Residue Limits and Sample Solution Concentration of Screening Target Compounds

	Compound	MRL (mg/kg)	Sample Solution Concentration (mg/L)
1	Sulfadiazine	0.01	0.025
2	Sulfamerazine	0.01	0.025
3	Sulfadimidine	0.01	0.025
4	Sulfamonomethoxine	0.01	0.025
5	Trimethoprim	0.01	0.025
6	Sulfamethoxazole	0.01	0.025
7	Ormetoprim	0.01	0.025
8	Sulfadimethoxine	0.01	0.025
9	Sulfaquinoxaline	0.01	0.025
10	Pyrimethamine	0.01	0.025
11	Difurazon	0.01	0.025
12	Nicarbazin*1	0.01	0.025

*1: Concentration of N, N'-Bis(4-nitrophenyl)urea, the main constituent of nicarbazin.

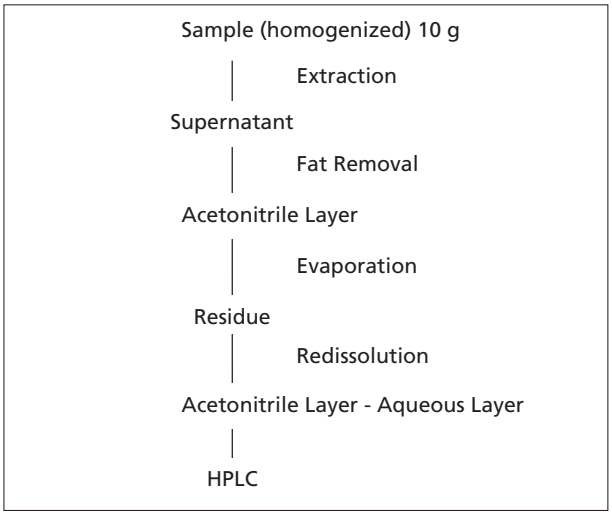

Fig. 1 Sample Pretreatment Protocol

Analysis of Antimicrobials Including Sulfonamides in Meat

Chicken and beef were used as samples. The analytical conditions are shown in Table 2. Chromatograms of the pretreated matrix solutions (blue line), matrix solutions spiked with standard solution to create matrix standard solutions (red line), and neat standard solution (black line) are shown in Fig. 2.
Standard solution was added to matrix solutions to make up antimicrobial concentrations, including sulfonamide concentrations, of 0.01 mg/kg in matrix standard solutions. Standard solutions were prepared to the sample solution concentrations listed in Table 1.
The photodiode array (PDA) detector (six-wavelength) built in the i-Series instrument was used for detecting all target compounds. Employing the analytical conditions shown, all 12 compounds were separated and eluted in approximately 25 minutes.

Table 2 Analytical Conditions

System	: LC-2040C 3D
Column	: Shim-pack FC-ODS (150 mm L. × 4.6 mm I.D., 3 µm)
Mobile Phase	: A) 20 mM (Sodium) Phosphate Buffer Containing 0.1 M Sodium Perchlorate B) Acetonitrile/Methanol=80/20
Time Program	: Gradient Elution
Flowrate	: 1.0 mL/min
Column Temp.	: 50 ˚C
Injection Volume	: 20 µL
Detection	: 240 nm 270 nm 280 nm 285 nm 350 nm 380 nm
Cell Temp.	: 40 ˚C

Similarity Calculation Using UV Spectral Library

All target compounds in this Application News can be analyzed qualitatively based on UV spectra as well as retention times. Sample spectra can be also checked for similarity against library spectra. Fig. 3 shows a UV spectrum of sulfaquinoxaline in a beef matrix spiked with a standard solution of sulfaquinoxaline at threshold concentration. Degree of similarity with the library spectrum was 0.997.

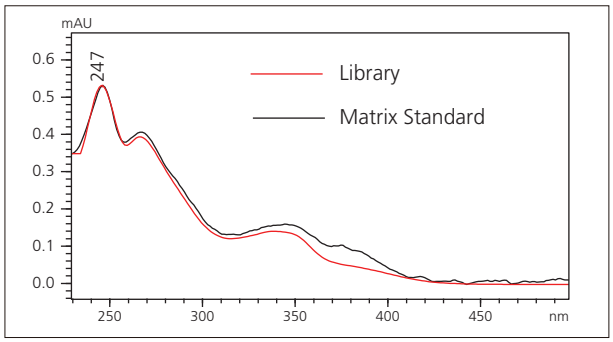

Fig. 3 Spectra of Sulfaquinoxaline

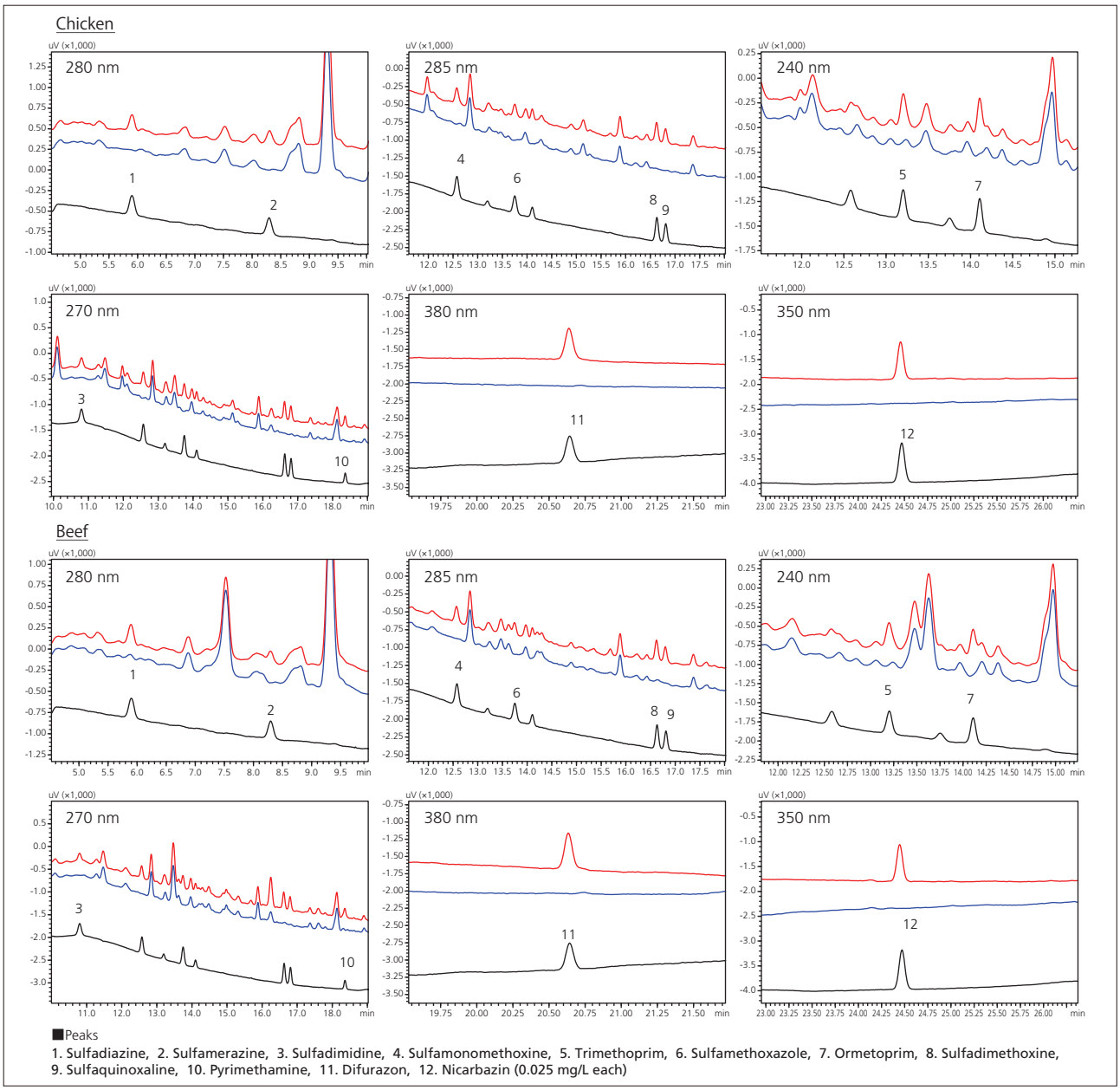

Fig. 2 Chromatograms of Chicken and Beef:
Matrix Standard Solution (Red Line), Matrix Solution (Blue Line), Neat Standard Solution (Black Line)

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