SHIMADZU
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Application News

No.L509

High Performance Liquid Chromatography

Analysis of Residual Antimicrobials in Meat with Antimicrobial Screening System (Part 1)

In May 2006, the positive list system took effect in Japan that, in principle, prohibited the sale of food products with residual levels of pesticides, animal feed additives, and veterinary drugs (collectively referred to as agricultural chemicals, etc.) above the level determined by the Minister of Health, Labour and Welfare.[1)]
Antimicrobials are a type of veterinary drug and animal feed additive, and used for the treatment and prevention of disease in livestock and marine products. Quinolones and sulfonamides are two common groups of synthetic antimicrobials.
Shimadzu's quick and simple antimicrobial screening system is capable of screening 24 antimicrobials compounds. An example screening analysis targeting 12 widely used quinolones (old quinolones, new quinolones) is described here. Application News No.L510 also describes an example screening analysis targeting 12 antimicrobials including sulfonamides (also including antifolates).

Antimicrobial Screening System

Shimadzu's antimicrobial screening system is able to determine whether levels of antimicrobials subject to regulation in various countries are above a maximum residue limit (MRL). Table 1 shows MRLs for the target quinolones.
The system uses an i-Series integrated HPLC instrument and RF-20Axs high-sensitivity fluorescence detector, and comes with a sample pretreatment method, analytical column, analytical method files, and a UV spectral library that allow for immediate operation after installation. When the analysis method capable of simultaneous component analysis is used, the system can be used for simultaneous screening of multiple components. The determination of whether MRL have been exceeded can be viewed immediately after the system completes analysis. The photodiode array (PDA) detector built into the i-Series instrument supports highly accurate screening with compound identification based on retention times as well as UV spectra.

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

	Compound	MRL (mg/kg)	Sample Solution Concentration (mg/L)
1	Marbofloxacin	0.01	0.025
2	Ofloxacin	0.01	0.025
3	Ciprofloxacin	0.01	0.025
4	Danofloxacin	0.01	0.025
5	Enrofloxacin	0.01	0.025
6	Orbifloxacin	0.01	0.025
7	Sarafloxacin	0.01	0.025
8	Difloxacin	0.01	0.025
9	Oxolinic acid	0.01	0.025
10	Nalidixic acid	0.01	0.025
11	Flumequine	0.01	0.025
12	Piromidic acid	0.01	0.025

Sample Pretreatment

Sample pretreatment was performed based on Simultaneous Analysis Method I for Veterinary Drugs by HPLC (Livestock and Marine Products).[2),3)] After acetonitrile extraction and removing fat by acetonitrile/hexane partitioning, sample solution was prepared by evaporation then redissolution. Fig. 1 shows the sample pretreatment protocol, and Table 1 shows sample solution concentrations after pretreatment. Refer to the instruction manual of the system for the details of the sample pretreatment procedure.

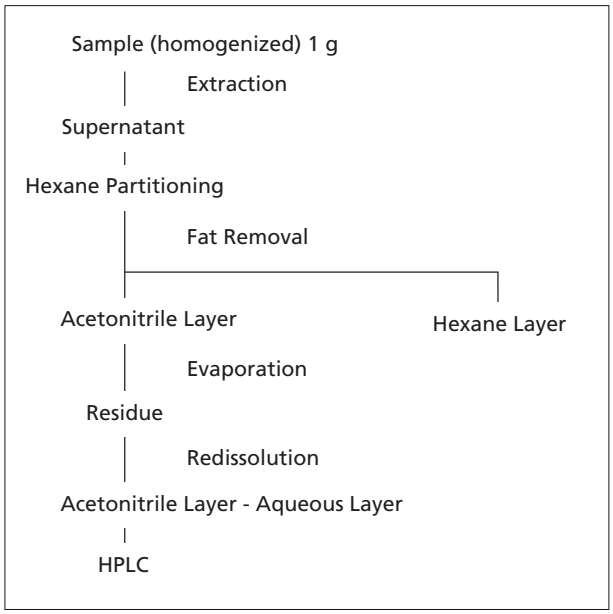


Fig. 1 Sample Pretreatment Protocol

Table 2 Analytical Conditions

System	: LC-2040C 3D, RF-20Axs
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=90/10
Time Program	: Gradient Elution
Flowrate	: 1.0 mL/min
Column Temp.	: 40 ˚C
Injection Volume	: 5 µL
Detection	: <LC-2040C 3D> 280 nm <RF-20Axs> Ex at 290 nm, Em at 495 nm Ex at 325 nm, Em at 365 nm
Cell Temp.	: 40 ˚C (PDA), 30 ˚C (RF)

Analysis of Quinolones in Meat

Chicken and pork were used as samples.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 create matrix standard solutions with quinolone concentrations of 0.01 mg/kg. The analytical conditions are shown in Table 2. Analysis was performed with the fluorescence detector in dual wavelength mode. New quinolones (compounds 1 to 8 in Table 1) were detected at an excitation wavelength of 290 nm and fluorescence wavelength of 495 nm, and old quinolones (compounds 9 to 11 in Table 1) were detected at an excitation wavelength of 325 nm and fluorescence wavelength of 365 nm. Piromidic acid (compound 12 in Table 1) differs from other quinolones in exhibiting no fluorescence characteristics, and was detected using the PDA detector. Employing the analytical conditions shown, all 12 compounds were separated and eluted in approximately 22 minutes.

Similarity Calculation Using UV Spectral Library

The PDA-detected compound (piromidic acid) can be analyzed qualitatively based on UV spectra as well as retention times. Its spectrum can be checked for similarity against the library spectra. Fig. 3 shows a UV spectrum of piromidic acid in pork matrix spiked with a standard solution of piromidic acid at threshold concentration. The degree of similarity with the library spectrum was 0.998.

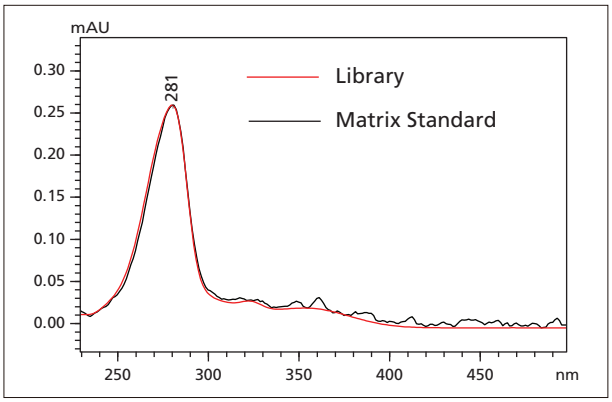
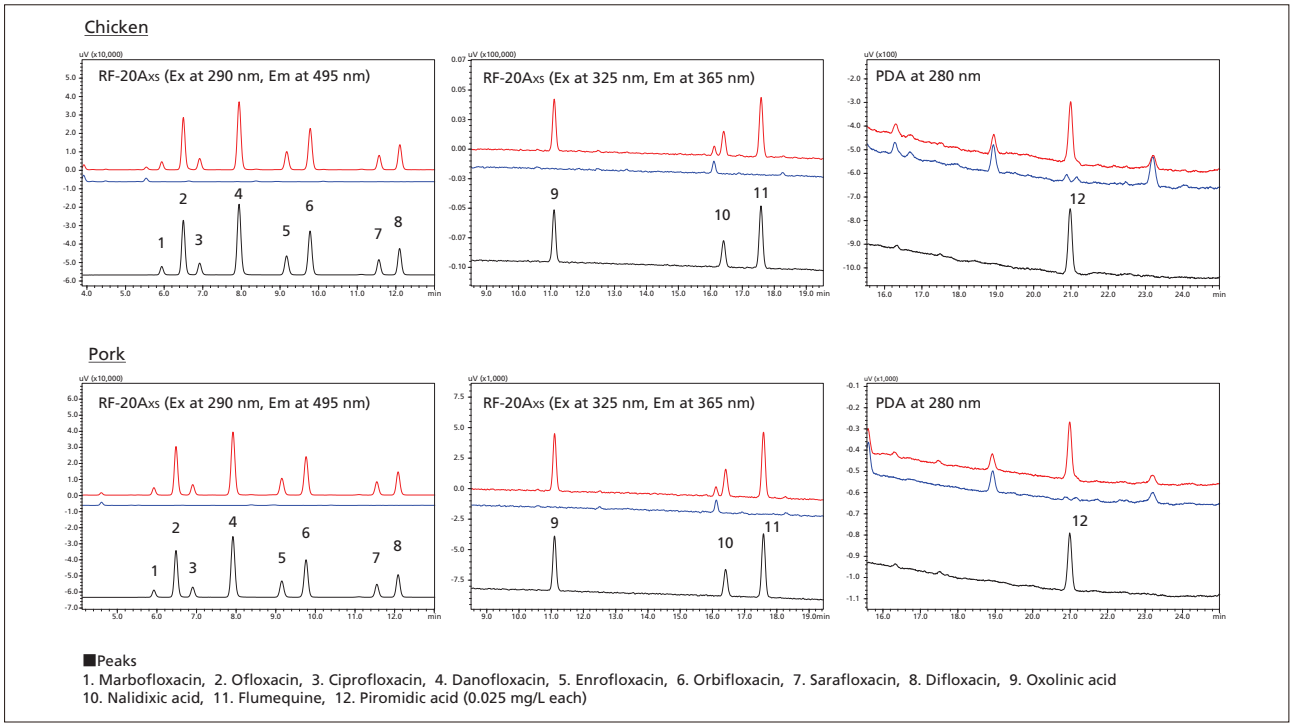


Fig. 3 Spectra of Piromidic Acid



■Peaks
1. Marbofloxacin, 2. Ofloxacin, 3. Ciprofloxacin, 4. Danofloxacin, 5. Enrofloxacin, 6. Orbifloxacin, 7. Sarafloxacin, 8. Difloxacin, 9. Oxolinic acid
10. Nalidixic acid, 11. Flumequine, 12. Piromidic acid (0.025 mg/L each)

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

<References>
1) The Japanese Positive List System for Agricultural Chemical Residues in Foods, Japan's Ministry of Health, Labour and Welfare
2) Multiresidue Method I for Veterinary Drugs, Etc. by HPLC (Animal and Fishery products)
Director Notice about Analytical Methods for Residual Compositional Substances of Agricultural Chemicals, Feed Additives, and Veterinary Drugs in Food (Syoku-An No. 0124001, January 24, 2005. Final amendments were made on May 26, 2006.), Japan's Ministry of Health, Labour and Welfare
3) "Standard methods of analysis in food safety regulation (for veterinary drugs and animal feed additives)" p.26-43, Japan Food Hygiene Association (2003), edited under the supervision of the Japan's Ministry of Health, Labour and Welfare

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