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Application News

High Performance Liquid Chromatograph Nexera™ lite

Screening Analysis for Tetracyclines Using the Autosampler's Automatic Dilution Function (2)

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User Benefits

- Troublesome pretreatments are avoided by using the autosampler's dilution function.
- The method can be applied to samples of livestock and marine products, so rapid screening analyses can be achieved.

■ Introduction

Veterinary drugs are used exclusively for animals to prevent and treat infections. Typical examples are antibacterials (antibiotics and antimicrobials). Among them, tetracyclines are a type of antibiotic that frequently appears in infringement cases in various countries.

However, if these remain unintentionally in livestock or marine products, they can lead to allergic reactions to humans or the emergence of drug-resistant strains of bacteria. To ensure that people can eat them safely, Maximum Residue Limits (MRL)[1] have been established by various countries, taking into consideration the amount of such compounds that will have no effect on human health.

Application News No. 01-00224 describes analysis of mixed tetracycline standard solution using an autosampler's automatic dilution function. This article describes screening analysis of tetracyclines applying analytical conditions previously reported to samples of livestock and marine products.

■ Sample Preparation

Samples of chicken breast, pork tenderloin, and shrimp were used. Sample preparation was done referring to "Analytical Methods for Oxytetracycline, Chlortetracycline, and Tetracycline" [2),3)] a directive from the Ministry of Health, Labour and Welfare (Japan). These analytical methods show six pretreatment processes: homogenization, extraction, fat removal, solid phase extraction, evaporation, and reconstitution. When the method in this article is used, two of these (evaporation and reconstitution) can be omitted, resulting in more efficient sample pretreatment. The pretreatment protocol is shown in Fig. 1 and the preparation method for the extracting solution is shown in Table 1. InertSep HLB FF from GL Science was used as a solid phase extraction (SPE) cartridge.

Table 1 Preparation Method for the Extracting Solution

Extracting solution	Citrate buffer containing EDTA 1st solution: Add 21.0 g of citrate acid to 1,000 mL of ultrapure water, and dissolve completely. 2nd solution: Add 71.6 g of disodium hydrogen phosphate to 1,000 mL of ultrapure water, and dissolve completely. Dissolve 1.86 g of EDTA•2Na in a mixed solution of 307 mL of the 1st solution and 193 mL of the 2nd solution.

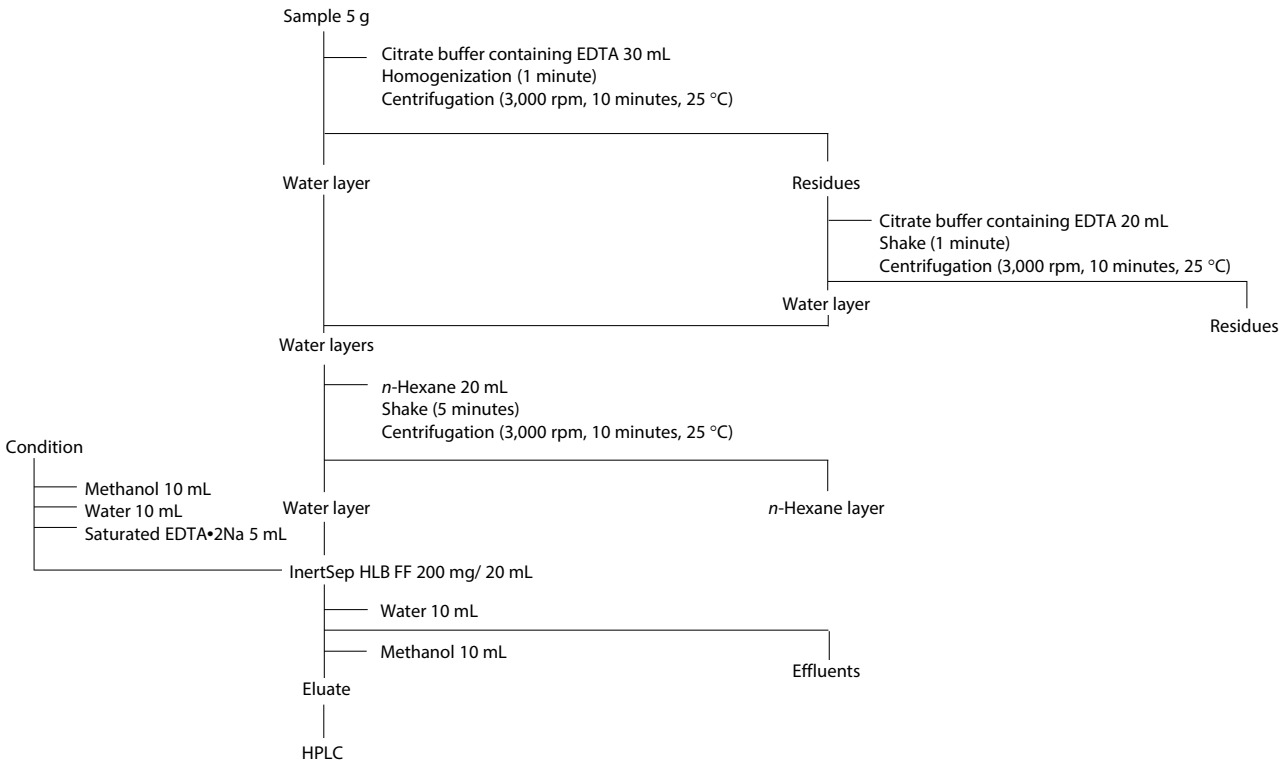

Fig. 1 Pretreatment Protocol

■ Positioning the Samples in the Autosampler

An example of the positioning within the autosampler is shown in Fig. 2. A slightly larger volume of diluent was required, so 4 mL vials were used. In addition, a sample plate for 4 mL vials was used for sample rack #3. Nexera series models are equipped with the automatic plate recognition function, so it can be used immediately by simply positioning this plate in the rack. In other words, needle position teaching is unnecessary. In this way, different capacity vials can be positioned simultaneously.

Before starting the analysis, the diluent (blue), the standard or sample solution (orange), and vials for the mixture (green) were positioned in the sample racks. 4 mL polypropylene (PP) vials were used for the diluent. 1 mL PP vials were used as the vials for the sample and mixture.

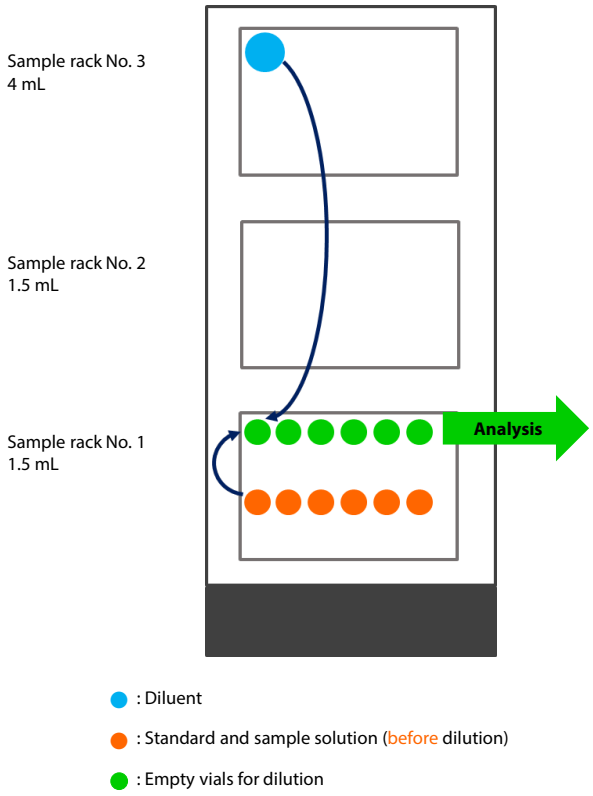

Fig. 2 Example of Positioning within the Autosampler

■ Analytical Conditions

Tetracyclines in samples were analyzed using the autosampler's automatic dilution function. The analytical conditions are shown in Table 2, and the methods of preparing the mobile phase and diluent are shown in Table 3.

The dilution factor and conditions related to the mixing process are configured using the LabSolutions™ workstation. The dilution mode settings window is shown in Fig. 3. In this article, the sample solution obtained from pretreatment shown in Fig. 1 was automatically diluted by a factor of 4 with the diluent.

Refer to Application News No. 01-00224 for the results of reproducibility from six consecutive analyses of the mixed standard solution (concentration of each compound before dilution: 100 µg/L).

Table 2 Analytical Conditions

System:	Nexera lite
Column:	Shim-pack™ FC-ODS*1 (150 mm×4.6 mm I.D., 3 µm)
Flowrate:	1.0 mL/min
Mobile Phase:	A) 1 mol/L Magnesium imidazole buffer (pH 7.2) B) Methanol A/ B = 78:22
Column Temp.:	40 °C
Injection Volume:	100 µL
Vial for Samples and Mixing:	Shimadzu Vial, LC, 1 mL, Polypropylene*2
Vial for Diluent:	Shimadzu Vial, LC, 4 mL, Polypropylene*3
Detection:	Ex: 380 nm, Em: 520 nm (RF-20AXS)

*1 P/N: 228-40511-93 *2 P/N: 228-31600-91 *3 P/N: 228-31537-91

Table 3 Preparation Method for the Mobile Phase and Diluent

Mobile Phase A	1 mol/L magnesium imidazole buffer (pH 7.2) Add 68.08 g of imidazole, 0.37 g of EDTA•2Na and 10.72 g of magnesium acetate to 800 mL of ultrapure water, and dissolve completely. Adjust the pH to 7.2 with acetic acid, and add ultrapure water to make 1000 mL using volumetric flask. Then, filter under reduced pressure with a 0.22 µm membrane filter.
Diluent	1.36 % potassium phosphate solution Add 1.36 mg of potassium dihydrogen phosphate into 100 mL of ultrapure water, and dissolve completely.

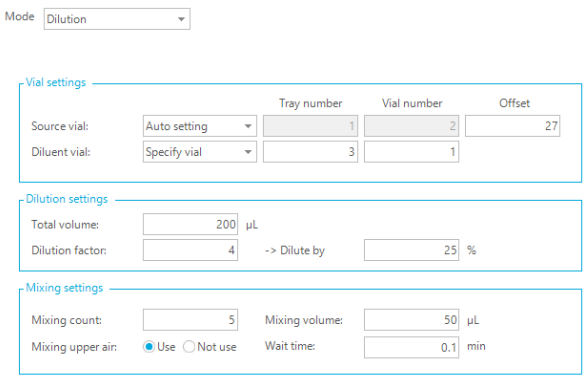

Fig. 3 Dilution Mode Settings

■ Screening Analyses of Chicken Breast, Pork Tenderloin, and Shrimp

The chromatograms for chicken breast, pork tenderloin, and shrimp are shown in Figs. 4 to 6, respectively. The chromatogram of the mixed tetracycline standard solution (prepared with methanol; concentration of each compound before dilution: 100 µg/L) was overlaid for comparison. This concentration is equivalent to the MRL (0.2 mg/kg) when the sample preparation indicated in Fig. 1 is performed. No tetracyclines were detected from any samples.

Chicken Breast

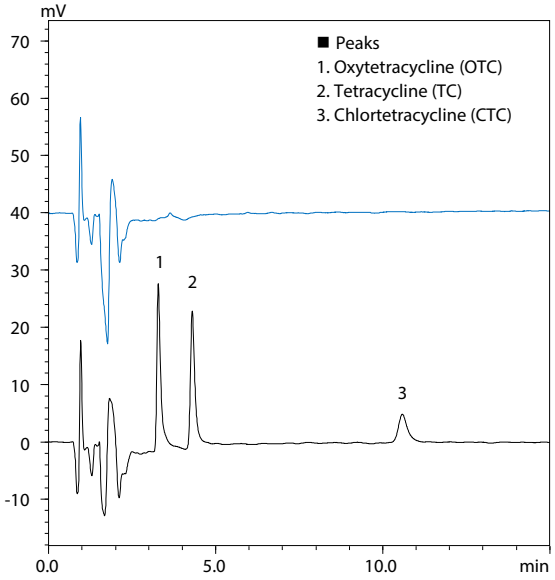

Fig. 4 Chromatogram for Chicken Breast
(Blue Line: Chicken Breast, Black Line: Standard Solution)

Pork Tenderloin

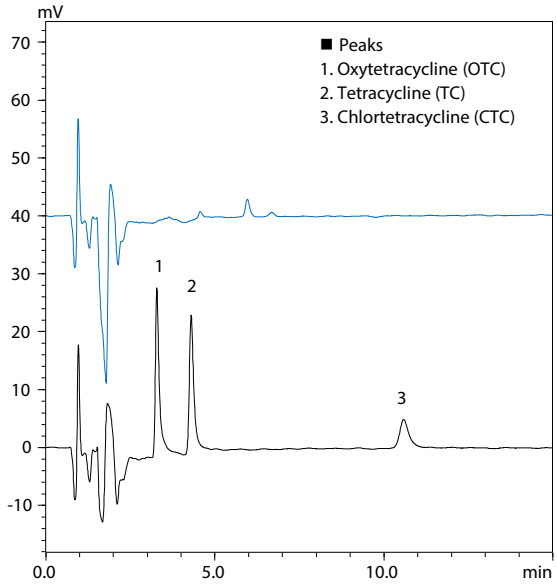

Fig. 5 Chromatogram for Pork Tenderloin
(Blue Line: Pork Tenderloin, Black Line: Standard Solution)

Shrimp

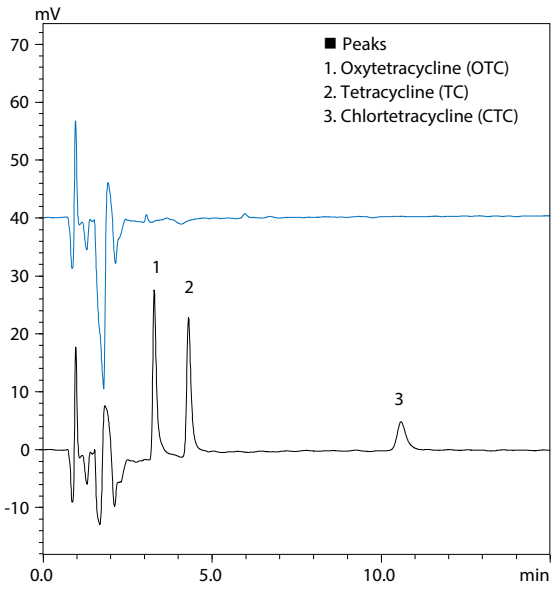

Fig. 6 Chromatogram for Shrimp
(Blue Line: Shrimp, Black Line: Standard Solution)

■ Conclusion

Performing automatic dilution using the autosampler reduced the labor for manual pretreatment. With this method, evaporation and reconstitution for actual samples (equivalent to approximately an hour) could be omitted. Accordingly, work efficiency was improved in the screening test for tetracyclines.

References

1) "New System Related to Residual Pesticides in Foods (Positive List System)" from the Ministry of Health, Labour and Welfare
2) "Analytical Methods for Oxytetracycline, Chlortetracycline, and Tetracycline" Food Safety Directive 0124001 issued by the Manager of the Food Safety Division, Pharmaceuticals and Foods Department, Ministry of Health, Labour and Welfare (January 24, 2005)
3) "Standard Methods of Analysis in Food Safety Regulation (for Veterinary Drugs and Animal Feed Additives)" Edited under the Supervision of the Japan's Ministry of Health, Labour and Welfare, pages 68 to 79, Japan Food Hygiene Association (2003)

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