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Application News

High Performance Liquid Chromatograph Nexera™ lite

# Screening Analysis for Tetracyclines Using the Autosampler's Automatic Dilution Function

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## User Benefits

- Troublesome pretreatments are avoided by using the autosampler's dilution function.
- Tailing peaks for metal complexes can be suppressed.

## ■ Introduction

Veterinary drugs are used exclusively for animals to prevent and treat infections. Typical examples are antibacterials (antibiotics and antimicrobials). 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. At the same time, infringements have been reported in various countries, and there are many compounds to test for, so LC/MS analysis is on the rise because of rapid simultaneous analysis of multiple components and high-sensitivity analysis. However, in the case of mobile phases in LC/MS analysis, tetracyclines and other metal complexes are absorbed by the metallic materials used in the LC system and columns and by residual metals on the surface of the column filler. Accordingly, even now, it is often analyzed by HPLC using a mobile phase that suppresses metal adsorption.

This article describes an example of the analysis of tetracyclines using an autosampler's automatic dilution function, with the aim of heightening the efficiency of pretreatment for food safety inspections.

## ■ Automatic Pretreatment Function

The autosampler of Nexera lite has an automatic pretreatment function. This automatic pretreatment function has three modes: dilution, reagent addition, and coinjection. The dilution mode was used in this article. The dilution mode allows automatic preparation of samples diluted by a user-defined factor, enabling them to be loaded into the analytical column. The dilution factor and conditions related to the mixing process are configured using the LabSolutions™ workstation. The window of dilution mode settings is shown in Fig. 1.

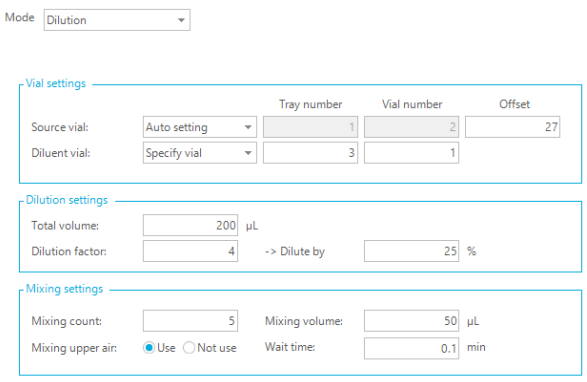


Fig. 1 Dilution Mode Settings

## ■ Analysis of a Mixed Tetracycline Standard Solution

Tetracyclines are a type of antibiotic that frequently appears in infringement cases in various countries. As indicated in "Analytical Methods for Oxytetracycline, Chlortetracycline, and Tetracycline" [2),3)] a directive from the Ministry of Health, Labour and Welfare (Japan), a mixture of an imidazole buffer solution and an organic solvent is used as the mobile phase in the HPLC analysis of tetracyclines.

In this article, tetracyclines were analyzed using the dilution mode. The analytical conditions are shown in Table 1, and the methods of preparing the mobile phase and diluent are shown in Table 2 (next page). Note that conditions of Tables 1 and 2 reference them described in the above-mentioned analytical methods. The dilution mode settings for the autosampler are shown in Fig. 1. The mixed tetracycline standard solution prepared with methanol was automatically diluted by a factor of 4 with the diluent. The chromatogram for the mixed tetracycline standard solution (prepared with methanol; concentration of each compound before dilution: 100 µg/L) is shown in Fig. 2. This concentration is equivalent to the MRL (0.2 mg/kg) when the operations up to solid phase extraction are performed within the pretreatment process indicated in the above-mentioned analytical methods. These analytical methods recommend six pretreatment processes: homogenization, extraction, fat removal, solid phase extraction, evaporation, and reconstitution. When the method in this article is used, two of them (evaporation and reconstitution) can be omitted.

On the other hand, even if an imidazole buffer solution is used as the mobile phase, adsorption to the column may not be suppressed depending on the column used. In this article, the Shim-pack™ FC-ODS column with low residual metals was used, so sharp peaks were obtained without tailing. At this time, the S/N ratio for chlortetracycline (peak 3) was 36. (Calculation method: ASTM; range: 5 to 15 minutes; interval: 0.5 minutes)

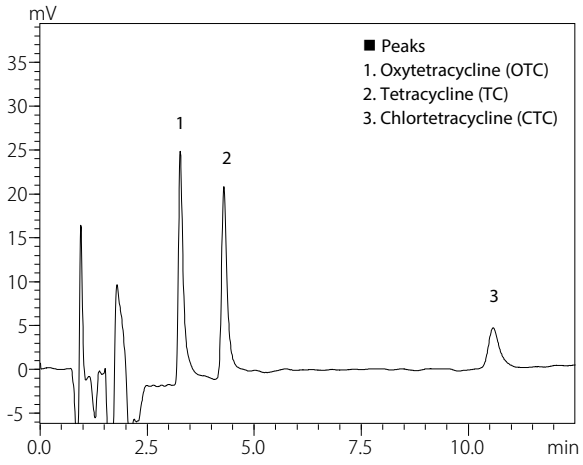


Fig. 2 Chromatogram for the Mixed Tetracycline Standard Solution (100 µg/L of each component) (Concentration of each compound after automatic dilution: 25 µg/L)

Table 1 Analytical Conditions

| | |
|---|---|
| System: | Nexera lite |
| Column: | Shim-pack FC-ODS*1<br>(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)<br>B) Methanol<br>A/ B = 78:22 |
| Column Temp.: | 40 °C |
| Injection Volume: | 100 µL |
| Vial for Samples and Mixing: | Shimadzu Vial, LC, 1mL, Polypropylene*2 |
| Vial for Diluent: | Shimadzu Vial, LC, 4mL, 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 2 Preparation Method for the Mobile Phase and Diluent

| | |
|---|---|
| Mobile Phase A | 1 mol/L magnesium imidazole buffer (pH 7.2)<br>Add 68.08 g of imidazole, 0.37 g of EDTA•2Na and 10.72 g of magnesium acetate into 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<br>Add 1.36 mg of potassium dihydrogen phosphate into 100 mL of ultrapure water, and dissolve completely. |

## ■ Reproducibility

Using the automatic dilution function, the mixed standard solution (concentration of each compound before dilution: 100 µg/L) was analyzed 6 times consecutively. The reproducibilities (%RSD) of the retention time and the peak area are shown in Table 3.

Table 3 Reproducibility (%RSD) from 6 Consecutive Analyses

| Compound | Retention time | Peak area |
|---|---|---|
| OTC | 0.06 | 5.69 |
| TC | 0.03 | 2.85 |
| CTC | 0.03 | 1.42 |

## ■ Positioning the Samples in the Autosampler

An example of the positioning within the autosampler is shown in Fig. 3. 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 lite is equipped with an 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.

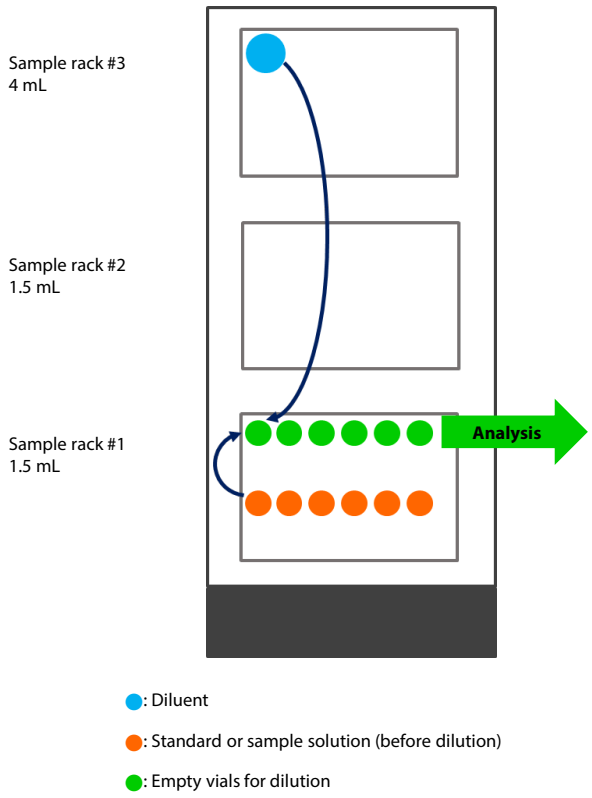


Fig. 3 Example of the Positioning within the Autosampler

## ■ Conclusion

Performing automatic dilution using the autosampler reduced the labor for manual pretreatment. With this method, evaporation and reconstitution for actual samples, could be omitted. Accordingly, work efficiency was improved when analyzing tetracyclines in screening applications.

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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