Stability studies of oxytetracycline in methanol solution

Wei WANG1, Nan WU2*, Jinghui YANG1*, Ming ZENG3, Chenshan XU2, Lun LI2, Meng ZHANG2, and Liting LI2

1College of Horticulture and Landscape, Tianjin Agricultural University, Tianjin 300384, China
2College of Engineering and Technology, Tianjin Agricultural University, Tianjin 300384, China
3College of Marine and Environmental Sciences, Tianjin University of Science and Technology, Tianjin 300457, China

Corresponding author’s e-mail address: nwu@tjau.edu.cn; jinghuiyang2@aliyun.com

Abstract. As one kind of typical tetracycline antibiotics, antibiotic residues of oxytetracycline have frequently been detected in many environmental media. In this study, the stability of oxytetracycline in methanol solution was investigated by high-performance liquid chromatography combined with UV-vis (HPLC-UV). The results show that the stability of oxytetracycline in methanol solution is highly related to its initial concentration and the preserved temperature. Under low temperature condition, the solution was more stable than under room temperature preservation. Under the same temperature preservation condition, high concentrations of stock solutions are more stable than low concentrations. The study provides a foundation for preserving the oxytetracycline-methanol solution.

1. Introduction

Antibiotics are a class of natural, semi-synthetic or synthetic compounds that are resistant to microbial activity and are widely used as antimicrobial agents in the treatment of infectious diseases in humans and animals. Besides this fundamental application, antibiotics have also been added to feed as growth promotants to promote animal growth and development [1-2]. Among veterinary antibiotics, tetracycline antibiotics such as oxytetracycline are the most commonly used types of antibiotics. However, the bioavailability of antibiotics is not high [3], most of them will be excreted in the form of the original drug with excrement and urine, and through different ways into the soil, water and other environmental media [4-7]. As one kind of typical tetracycline antibiotic, oxytetracycline is widely used in clinical treatment and animal husbandry due to its low cost and extensive activity, and antibiotic residues have been detected in environmental media such as surface water, soil and sediment [8]. The presence of antibiotics in the environment will increase the resistance of pathogens, directly threaten public health and biological safety and pollute the natural environment.

Antibiotics are a class of highly polar and nonvolatile compounds that are typically separated by liquid chromatography. Liquid chromatography combined with detectors such as UV-vis detection (UV), fluorescence detection (FLD), diode-array detection (DAD) and mass spectrometry detection (MS), are commonly used in antibiotic analysis. In recent years, high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) has gradually become the mainstream of antibiotic residue analysis. Preparation of fresh standard sample solutions on each day of analysis is...
time-consuming, so it was decided to prepare and store them before analysis. Since methanol is used in most oxytetracycline analytical work as a dissolution, extraction and elution solvent, stability studies of oxytetracycline in methanol solution are important and require investigation.

In this study, the stability of oxytetracycline in methanol solution was investigated by high-performance liquid chromatography (HPLC) combined with UV-vis detector.

2. Materials and Methods

2.1. Chemicals and Materials
Oxytetracycline powders (>99 %) were obtained from Sangon Biotech. Methanol and acetonitrile are HPLC-grade, and oxalic acid is analytical reagent grade. Distilled water was obtained from a Molgenepure water system. Two groups of stock solutions with different concentrations (10 mg/L, 50 mg/L and 100 mg/L) were prepared by dissolving oxytetracycline powders in methanol, each containing 50 ml, stored in brown glass bottles. One group of the stock solutions was stored at -20 °C, and the other group of stock solutions were stored at 25 °C. All solutions for HPLC analysis were filtered through a 0.45 μm microporous membrane (SHANGHAI XIN YA PURIFICATION EQUIPMENT CO., LTD) before use. The first analyse was performed on the day when the stock solutions were prepared, and then on the 8th, 15th, 22th, and 36th days respectively.

2.2. High performance liquid chromatography detection
Oxytetracycline in solutions was analyzed by Flexar HPLC combined with UV-vis detector (PerkinElmer, USA). The separation was performed on a Brownlee Analytical C18 column (5 μm, 4.6 mm × 250 mm, PerkinElmer, USA). Isocratic elution was carried out by using methanol-acetonitrile-0.01 M oxalic acid (8:16:76, v/v/v) as mobile phase. The flow rate was 1ml/min and detection wavelength was set as 268 nm. The injection volume was 20 µl.

2.3. Standard curve
1.00 mg of oxytetracycline powder was accurately weighed and dissolved in 10 ml of methanol, thus the concentration was 100 mg/L, then diluted with methanol to a concentration of 1 mg/L ~ 100 mg/L of the standard series, each taking 20 µl solution for injection analysis. According to the peak area and the corresponding concentrations of linear regression, the standard curve was drawed.

3. Results and Discussions

3.1. Stability of oxytetracycline-methanol solution at different concentrations

![Figure 1. Chromatogram of 10 mg/L oxytetracycline-methanol solution at the first determination](image-url)
Fig. 1 shows the chromatogram of the first determination of 10 mg/L oxytetracycline-methanol solution. Oxytetracycline retention time was about 7.950 min, and the peak shape was good. Fig. 2A shows that the concentrations of three kinds of oxytetracycline-methanol solution (10 mg/L, 50 mg/L and 100 mg/L) did not change significantly at -20 °C. Oxytetracycline concentrations at day 36 were 97% ~ 100% of the initial concentrations, which could basically be considered no degradation. On the contrary, the concentrations of three kinds of oxytetracycline-methanol solution declined significantly at 25 °C, as shown in Fig. 2B. The decreasing levels of three oxytetracycline stock solutions (10 mg/L, 50 mg/L and 100 mg/L) at day 36 were 80.2%, 35.1% and 12.6%, respectively. The higher the initial concentration, the smaller the degree of degradation. The results showed that the stability of the oxytetracycline-methanol solution was related to the initial concentrations of the stock solutions. Under the same temperature preservation condition, stock solution with higher concentration was more stable.

**Figure 2.** Changes of concentration of oxytetracycline-methanol solution at different concentrations (A. Temperature at -20 °C, B. Temperature at 25 °C)

### 3.2. Stability of oxytetracycline-methanol solution under different temperatures

Fig. 3 shows that the degradation levels of three kinds of oxytetracycline-methanol solution (10 mg/L, 50 mg/L and 100 mg/L) at -20 °C are significantly less than those at 25 °C. The concentrations at day 36 of 10 mg/L oxytetracycline-methanol solution were 3% and 80.2% lower than the initial concentrations at -20 °C and 25 °C, respectively. The concentrations at day 36 of 50 mg/L oxytetracycline-methanol solution were 2.8% and 35.1% lower than the initial concentrations at -20 °C and 25 °C, respectively. The concentration at day 36 of 100 mg/L oxytetracycline-methanol solution was the same as the initial concentration at -20 °C, and was 12.6% lower than the initial
concentration at 25 °C. The results show that the stability of oxytetracycline-methanol solution is related to the preserved temperature, and under low temperature condition the solution was more stable than under room temperature preservation.

**Figure 3.** Changes of concentration of oxytetracycline-methanol solution at different temperatures with the same initial concentration (A. Concentration of 10 mg/L, B. Concentration of 50 mg/L, C. Concentration of 100 mg/L)

4. Conclusions
According to the test results, the stability of oxytetracycline-methanol solution is highly related to its concentration and the preserved temperature. Under the same initial concentration conditions, the stock solution was more stable under low temperature preservation conditions. Under the same temperature preservation condition, high concentrations of stock solutions are more stable than low concentrations. Therefore, when the oxytetracycline-methanol solution needs to be stored, a higher concentration of the storage solution can be prepared in combination with the test conditions and stored at -20 °C for one month without degradation. However, further studies are necessary to better understand the oxytetracycline degradation process as well as the biological effects and other properties of degradation products.

Acknowledgments
This research was financially supported by National Natural Science Foundation of China (No. 21607114), National Undergraduate Training Programs for Innovation and Entrepreneurship (No. 201710061025), Natural Science Foundation of Tianjin (No. 15JCYBJC53700), and Scientific Research Foundation for the Returned Overseas Chinese Scholars.

References
[1] Halling-Sorensen B, Nors Nielsen S, Lanzky P F, et al. Occurrence, fate and effects of pharmaceutical substances in the environment-Areview [J]. Chemosphere, 1998, 36 (2) :357-393.
[2] Martinez J L. Environmental pollution by antibiotics and by antibiotic resistance determinants[J]. Environmental Pollution, 2009, 157(11) : 2893-2902.
[3] Sassman S A, Lee L S. Sorption of three tetracyclines by several soils: Assessing the role of pH and cation exchange. Environmental Science & Technology, 2005, 39(19) : 7452-7459.
[4] Sarmah A K, Meyer M T, Boxall A. A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. Chemosphere, 2006, 65(5) : 725-759.
[5] Martínez-Carballo E, González-Barreiro C, Scharf S, et al. Environmental monitoring study of selected veterinary antibiotics in animal manure and soils in Austria. Environmental Pollution, 2007, 148(2) : 570-579.
[6] Radjenovi J, Petrovi M, Barceló D. Fate and distribution of pharmaceuticals in wastewater and sewage sludge of the conventional activated sludge (CAS) and advanced membrane bioreactor (MBR) treatment. Water Research, 2009, 43(3) : 831-841.
[7] Srinivasan P, Sarmah A K, Manley-Harris M. Co-contaminants and factors affecting the sorption behaviour of two sulfonamides in pasture soils. Environmental Pollution, 2013, 180 : 165-172.
[8] Hanay O, Yildiz B, Aslan S, et al. Removal of tetracycline and oxytetracycline by microscale zerovalent iron and formation of transformation products. Environ Sci Pollut Res Int, 2014, 21(5):3774-82.