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PO-CON1415E

# Determination of Tetracyclines in Surface Water by Ultra High Performance Liquid Chromatography/ Tandem Mass Spectrometry

Pittcon 2014 1680-3P

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## Introduction

Tetracyclines (TCs) are a group of broad-spectrum antibiotics, produced by actinobacteria. Tetracyclines remain the treatment of choice for infections caused by Gram-negative bacteria, Gram-positive bacteria, chlamydia, rickettsia, brucellosis, and spirochete infections. Tetracycline antibiotics are protein synthesis inhibitors, inhibiting the binding of aminoacyl-tRNA to the mRNA-ribosome complex. They do so mainly by binding to the 30S ribosomal subunit in the mRNA translation complex. In animal husbandry, tetracycline antibiotics were widely used in veterinary medicine as additives, which pose potential threats to the environment.
In this study, a quick and sensitive analytical method was established for the determination of 7 tetracycline antibiotics minocycline, oxytetracyclin, tetracycline, demeclocycline, aureomycin, methacycline, doxycycline in surface water, using Solid Phase Extraction to enrich tetracyclines from surface water and ultra high performance liquid chromatography-electrospray tandem mass spectrometry.

## Methods and Materials

### Sample Preparation

All samples were filtered through 0.45 µm filter membrane into 1-L amber plastic bottles and stored at 4 °C until they were extracted, typically within one week. Analytes were extracted using the 200 mg/6 mL hydrophilic-lipophilic balance cartridge. The extracts were concentrated under a flow of $N_2$ to an approximate volume of 50 µL. To this, 950 µL of methanol-water (V/V, 1:9) was added. The sample solution was analyzed by LC/MS/MS.

### LC-MS/MS Analysis

#### HPLC

The analyses were performed on a Shimadzu Nexera UHPLC instrument (Kyoto, Japan) equipped with LC-30AD pump, CTO-30A column oven, DGU-30$A_5$ degasser, and SIL-30AC autosampler. The separation was carried out on a Shim-pack XR-C8 (2.0 mm I.D.× 100 mm L., 2.2 µm) with the column temperature at 35 °C. The mobile phase consisted of (A) 10 mmol/L trifluoroacetic acid-water and (B) methanol using a gradient elution of 10% B at 0-0.5 min, 10%-50% B at 0.5-1.0 min, 50%-65% B at 1.0-3.0 min, 65% B at 3.0-3.5 min, 65%-10% B at 3.5-3.6 min. The flow rate was 0.3 mL/min.

#### Mass spectrometry

A triple quadrupole mass spectrometer (Shimadzu LCMS-8040, Kyoto, Japan) was connected to the Shimadzu fast analytical UHPLC instrument via an ESI interface. The mass spectra were acquired in positive ion mode with a DL temperature at 250 °C, heat block temperature at 400 °C. The dwell time was 10 ms and pause time was 3 ms. The injection volume was 20 µL.The MRM parameters were in Table 1.

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Figure 1. Shimadzu LCMS-8040 ultra high performance liquid chromatography-electrospray tandem mass spectrometry

## Results and Discussion

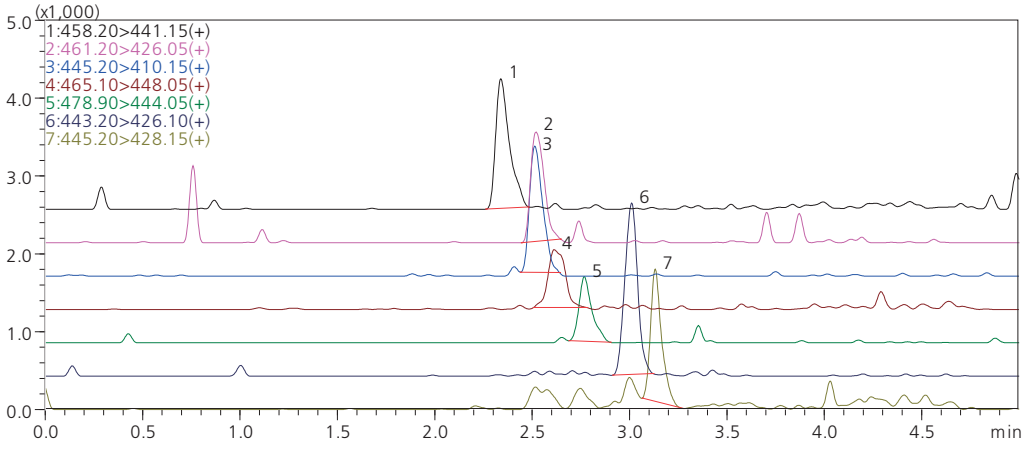
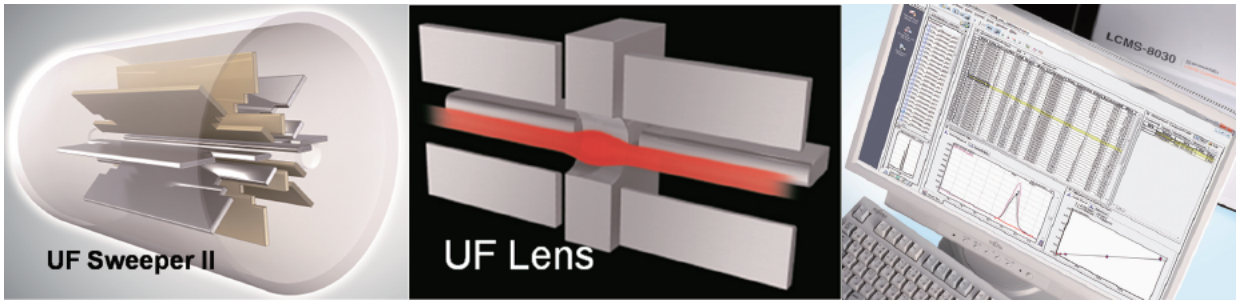



Figure 2. MRM chromatograms of 7 tetracyclines (2 µg/L)
1.minocycline; 2.oxytetracyclin; 3.tetracycline; 4.demeclocycline; 5.aureomycin; 6.methacycline; 7.doxycycline



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7 tetracyclines were separated in 5 min. The MRM chromatograms in positive ion mode of 7 tetracyclines was given in Figure 2. A linear relationship was found between peak area and different concentrations of 7 tetracyclines within 1, 3, 5, 10, 50, 100 and 500 µg/L. The calibration curves of 7 tetracyclines were constructed with correlation coefficients (r) more than 0.999, respectively, and the limits of detection (LODs) and the limits of quantitation (LOQs) for 7 tetracyclines were obtained as shown in Table 2.

Table 1. MRM parameters of 7 tetracyclines

| Compound Name | Precursor ion (*m/z*) | Product ion (*m/z*) | Q1 Pre Bais (V) | CE (V) | Q3 Pre Bais (V) |
|---|---|---|---|---|---|
| Minocycline | 458.2 | 441.2* | -22 | -21 | -30 |
| | | 283.1 | -22 | -48 | -29 |
| Oxytetracyclin | 461.2 | 426.1* | -22 | -19 | -30 |
| | | 443.1 | -22 | -12 | -22 |
| Tetracycline | 445.2 | 410.2* | -22 | -19 | -29 |
| | | 427.2 | -22 | -12 | -30 |
| Demeclocycline | 465.1 | 448.1* | -23 | -18 | -30 |
| | | 430.1 | -23 | -23 | -29 |
| Aureomycin | 478.9 | 444.1* | -24 | -21 | -30 |
| | | 462.1 | -24 | -18 | -23 |
| Methacycline | 443.2 | 426.1* | -21 | -18 | -30 |
| | | 201.1 | -21 | -36 | -21 |
| Doxycycline | 445.2 | 428.2* | -22 | -19 | -30 |
| | | 154.1 | -22 | -32 | -30 |

* for quantitation

Table 2. The calibration curve and quantitation of 7 tetracyclines

| Compound | Calibration curve | LOD (ng/L) | LOQ (ng/L) |
|---|---|---|---|
| Minocycline | Y = (11739.6)X + (-28443.4) | 5.84 | 23.37 |
| Oxytetracyclin | Y = (5044.19)X + (5932.37) | 5.76 | 23.02 |
| Tetracycline | Y = (8591.11)X + (-22142.1) | 3.77 | 15.1 |
| Demeclocycline | Y = (2883.03)X + (-20841.3) | 6.07 | 24.26 |
| Aureomycin | Y = (2549.80)X + (-8631.14) | 6.24 | 24.98 |
| Methacycline | Y = (10613.5)X + (-50409.7) | 6.29 | 25.16 |
| Doxycycline | Y = (11844.3)X + (-82438.8) | 4.46 | 17.82 |

In this study, the repeatability of 7 tetracyclines in different concentrations (20, 50 and 100 µg/L) was investigated. The %RSDs of retention time were better than 0.208 and %RSDs of peak area were less than 3.731, as show in table 3.

The mixed standard sample was spiked into the surface water at levels of 0.20 µg/L to evaluate the recovery of this method developed in this study. All the analyses were performed using above HPLC and mass spectrometry analytical conditions. A recovery rate of 61.5 % to 74.1 % was obtained for each of the compounds.

As we all known, tetracyclines are all water soluble, they have the potential to enter ground water and surface water easily. Surface water samples were collected from three public sites in Shanghai. But there were no tetracyclines detected in the surface water samples.

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Table 3. Repeatability of 7 tetracyclines in different concentrations (n=6)

| Compound | RSD% (10 µg/L) | | RSD% (50 µg/L) | | RSD% (100 µg/L) | |
|---|---|---|---|---|---|---|
| | R.T. | Area | R.T. | Area | R.T. | Area |
| Minocycline | 0.068 | 1.469 | 0.147 | 2.017 | 0.031 | 1.75 |
| Oxytetracyclin | 0.091 | 3.103 | 0.208 | 2.36 | 0.033 | 2.443 |
| Tetracycline | 0.044 | 3.731 | 0.117 | 1.165 | 0.04 | 2.037 |
| Demeclocycline | 0.117 | 3.552 | 0.161 | 1.56 | 0.021 | 2.673 |
| Aureomycin | 0.146 | 3.363 | 0.075 | 2.69 | 0.052 | 2.953 |
| Methacycline | 0.069 | 1.769 | 0.136 | 2.305 | 0.056 | 1.858 |
| Doxycycline | 0.03 | 3.361 | 0.11 | 1.319 | 0.042 | 1.682 |

# Conclusion

A LCMS/MS method has been developed for 7 tetracyclines in surface water using Shimadzu Nexera UHPLC and LCMS-8040 triple quadruple mass spectrometer. All of them were separated in 5 minutes, and the calibration curves were linear well between peak area of the selected ions and different concentrations of 7 tetracyclines with the correlation coefficient over 0.999. The limit of quantitation (LOQ) and the limit of detection (LOD) for 7 tetracyclines were based on the calibration curve of signal-to-noise ratio versus concentration. Good reproducibility on both retention time (0.208 % RSD) and peak area (3.731 % RSD) was observed. This method was established for fast and simultaneously qualitative confirmation and quantitative determination of 7 tetracyclines.

First Edition: March, 2014

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