Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 824 (2005) 57–64
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

# Determination of tetracyclines residues in honey using high-performance liquid chromatography with potassium permanganate–sodium sulfite–β-cyclodextrin chemiluminescence detection

Guo-Hui Wan [a], Hua Cui [a,*], Hai-Song Zheng [b], Jian Zhou [a], Li-Juan Liu [a], Xiao-Feng Yu [b]

[a] *Department of Chemistry, University of Science and Technology of China, Hefei, Anhui 230026, PR China*
[b] *Anhui Province Entry-Exit Inspection and Quarantine Bureau, Hefei, Anhui 230026, PR China*

Received 26 January 2005; accepted 22 June 2005
Available online 11 July 2005

**Abstract**

A novel method was developed for the simultaneous determination of tetracycline antibiotic (TCA) residues such as oxytetracycline (OTC), tetracycline (TC), and metacycline (MTC) by high-performance liquid chromatography (HPLC) coupled with chemiluminescence (CL) detection. The procedure was based on the chemiluminescent enhancement by TCAs of the potassium permanganate–sodium sulfite–β-cyclodextrin system in a phosphoric acid medium. The separation was carried out with an isocratic elution using a mixture of acetonitrile and 0.001 M phosphoric acid. For the three TCAs, the detection limits at a signal-to-noise of 3 ranged from 0.9 to 5.0 ng/ml. The relative standard deviations for the determination of TCAs ranged from 3.1 to 7.4% within a day ($n = 11$) and ranged from 2.2 to 8.6% in 3 days ($n = 9$), respectively. The method was successfully applied to the determination of TCA residues in honey samples. The possible mechanism of the CL reaction was also discussed.


*Keywords:* Tetracycline antibiotics; HPLC; Chemiluminescence; Residues; Honey

## 1. Introduction

Tetracycline antibiotics (TCAs) are commonly used both for the treatment of infectious diseases and as an additive to animal feeds for their broad-spectrum antibacterial activity and cost effectiveness. If recommendations for drug withdrawal are not respected or if veterinary drugs are used unlicensed, there is a significant risk of detecting TCA residues in honey, milk, and some edible animal tissues [1]. Moreover, TCAs can be added directly to plants in the orchard environment during blossom. The contamination of the blossom with high concentrations of antibiotic implies the risk of a carry-over of residues into honey [2]. Relatively high levels of TCA residues in food products present a potential hazard to the consumers in terms of allergic reaction and the development of bacterial resistance [3]. Therefore, regulatory authorities have established maximum residue limits (MRLs) for TCAs in food. Some countries do not have fixed MRLs for honey because TCAs are illegal for use with bees at any level, while some countries apply an action level of 50 ng/g.

Many methods have been described for the determination of TCAs such as microbiological assay [4,5], enzyme immunoassay [6], spectrophotometry [7], fluorimetry [8], electrochemical detection [9], flow-injection-chemiluminescence methods [10,11], high-performance liquid chromatography (HPLC) [12–23], and capillary electrophoresis (CE) [24]. However, few methods are applied to determine TCA residues in honey, mainly due to the lack of sufficient sensitivity for practical application and the interferences suffered from honey complex matrix. Currently, TCA residues in honey are determined mainly by microbiological assay [5], enzyme immunoassay [6], and chromatographic methods [17,21]. The official methods of assay in China are

* Corresponding author. Tel.: +86 551 3606645; fax: +86 551 3601592.
*E-mail address:* hcui@ustc.edu.cn (H. Cui).

1570-0232/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2005.06.039

the bioassay approaches. These methods are not only expensive and time consuming but they also do not distinguish among TCAs, thus their sensitivity and specificity are limited. This is why methods now developed for TCA detection in honey are focused on chromatographic methods because they offer the advantages of better sensitivity and specificity.

TCA residues in various biological matrices can be determined using reversed-phase liquid chromatography (LC) with different detection modes such as spectrophotometry [12–17], fluorescence [18,19], mass spectrometry (MS) [20–22], and electrochemical detection [23]. Mass spectrometry can detect residual TCAs with high sensitivity and selectivity, but the instrumentation is expensive. In recent years, chemiluminescence (CL) has become an attractive detection method for liquid chromatography due to its high sensitivity and wide linear working ranges, which can be obtained with relatively simple instrumentation. However, to our knowledge, there has been no published report using HPLC-CL to detect TCA residues.

It was reported that the reaction between acidic potassium permanganate and sodium sulfite could give rise to chemiluminescence from 450 to 600 nm [25]. The mechanism of the CL reaction of sodium sulfite with acidic potassium permanganate [26] was suggested to be due to electronically excited state of sulphur dioxide molecules. In the present study, we found that the reaction of potassium permanganate with sodium sulfite in the presence of β-cyclodextrin could yield intensive CL and TCAs could strongly enhance the CL of potassium permanganate–sodium sulfite–β-cyclodextrin system. On this basis, a highly sensitive method was developed for the determination of TCA residues in honey by coupling HPLC with this CL reaction.

## 2. Experimental

### 2.1. Chemicals and solutions

Acetonitrile of HPLC grade and sodium sulfite anhydrous ($Na_2SO_3$) were from Beijing Chemicals Company (Beijing, China). Potassium permanganate ($KMnO_4$), β-cyclodextrin (β-CD), and phosphoric acid ($H_3PO_4$) were obtained from Shanghai Chemicals Company (Shanghai, China). Oxytetracycline (OTC), tetracycline (TC) and metacycline (MTC) were obtained from the Institute of Pharmaceutical and Biomaterial Authentication of China (Beijing, China). All other chemicals were of analytical-reagent grade.

OTC, TC, and MTC stock standard solutions (0.1 mg/ml) were prepared weekly using redistilled water and were stored at 4 °C in a refrigerator. A stock solution of β-CD ($1 \times 10^{-3}$ M) was prepared by dissolving 0.28375 g β-CD in 250 ml redistilled water. The solution of $Na_2SO_3$ ($9.6 \times 10^{-4}$ M) was prepared by dissolving $Na_2SO_3$ in $5 \times 10^{-8}$ M β-CD. The solution of $KMnO_4$ ($1.5 \times 10^{-4}$ M) was prepared by dissolving $KMnO_4$ in 0.075 M phosphoric acid. All working solutions were freshly prepared each day with redistilled water. The HPLC mobile phases were freshly prepared each day, filtered through a 0.22-μm membrane filter (Xinya Company, Shanghai), and then degassed before use.

### 2.2. Instrumentation

The schematic diagram as described previously [27] illustrated the HPLC-CL detection system used in our experiments. The HPLC system was Agilent 1100 series (Agilent Technologies, USA), including a binary pump, a thermostat column compartment, a diode array and multiple wavelength detector (DAD), a manual sample valve injector with a 100-μl loop, and an analytical column (Zorbax Eclipse XDB-$C_{18}$, 150 mm × 2.1 mm I.D., 5 μm; Agilent Technologies, USA). CL detection was conducted on a flow-injection-chemiluminescence system (Remax, China) consisting of a model IFFM-D peristaltic pump, a mixing tee and a model IFFS-A CL detector equipped with a glass coil (used as reaction coil and detection cell), and a photomultiplier. The data from the CL detector were acquired by Agilent Interface 35900E and processed by Chemstation A.08.03 running on a DELL smartpc 100 personal computer. Fluorescence spectrum was recorded offline by LS55 fluorimeter (Hitachi, Japan) after correction. CL spectrum was obtained by inserting cut-off filters at wavelengths of 360, 380, 400, 420, 430, 470, 490, 510, 535, 550, 565, 580, 600, 630, and 650 nm (light cannot pass at wavelengths lower than these values) between the detection cell and a photomultiplier in flow injection CL system [28].

### 2.3. Procedure

TCAs were separated by XDB-$C_{18}$ column at 25 °C with an isocratic elution program at a flow rate of 0.5 ml/min. The mobile phase consisted of acetonitrile (A) and 0.001 M phosphoric acid (B). The isocratic elution program was 16% A for 11 min. The UV–vis detector was set at 274 nm for OTC and TC and 350 nm for MTC. Typical retention time of OTC, TC, and MTC was 2.6, 3.2, and 10.3 min, respectively. Solutions of $KMnO_4$ and $Na_2SO_3$ were combined with the peristaltic pump at a flow rate of 1.8 ml/min, respectively, and then mixed with the column effluent from DAD at a mixing tee via a PEEK tube (600 mm × 0.25 mm I.D., Agilent Technologies). Light emission was monitored by the photomultiplier tube. The quantitative determination was based on the relative CL intensity $\Delta I = I_S - I_0$, where $I_S$ was the CL intensity of TCA compounds and $I_0$ was the intensity of blank signal.

### 2.4. Sample preparation

For extraction of the honey sample, 50 g (accurate to 0.1 g) of the sample was weighted and dissolved in 200 ml of 2% citric acid buffer solution (pH 4.0). The pH was adjusted to 4.5 with 2% citric acid or 40% NaOH solu-