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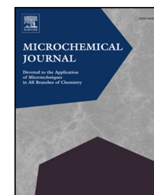

# Determination of tetracycline and cefotaxime residues in honey by micro-solid phase extraction based on electrospun nanofibers coupled with HPLC

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ABSTRACT

In the present study, electrospun polyethylene terephthalate nanofibers doped with graphene oxide nanoparticles (GO-PET) was fabricated and used as an effective adsorbent in micro-solid phase extraction (μ-SPE) of tetracycline (TC) and cefotaxime (CF) from honey samples prior to HPLC. The UV–vis spectrophotometer was utilized in the optimization step. The adsorbent was fully characterized using SEM, FT-IR, BET, and BJH methods. The specific surface area and average pore size of the adsorbent were 79.5 $m^2 g^{-1}$ and 4.83 nm, respectively. The honey samples were dissolved in water (5% *w/v*) at 50 °C for 10 min, centrifuged at 4000 rpm for 15 min and finally filtered using a filter paper before performing the extraction. The important factors of the method were studied to obtain the optimal conditions. Under the optimum conditions (sample solution, 100 mL; adsorbent, 40 mg; pH 5; extraction time, 10 min; desorption solvent; MeOH, 200 μL), the limits of detection for TC and CF were 15.3 and 3.0 μg $kg^{-1}$. The linear dynamic range (LDR) was obtained as 10–5000 μg $kg^{-1}$ with the determination coefficients ($R^2$) of 0.9939 and 0.9959, respectively. The real sample extraction recoveries were 89–94% and 95–98% for TC and CF, respectively. The intra-day and inter-day precisions were 5.6 and 4.9% (n = 3) for TC, and 3.6 and 4.5% (n = 9) for CF.

## 1. Introduction

Tetracycline (TC) is a member of tetracycline group of antibiotics (Fig. S1a). It is used in beekeeping to fight against bacterial diseases such as American foulbrood (Bacillus larvae) and European foulbrood (Streptococcus pluton). Consequently, it may remain at trace levels in honey [1]. Cefotaxime (CF) is another antibiotic from cephalosporin family that is widely used for the treatment of infections caused by gram-positive and gram-negative bacteria. CF is applied for the treatment of patients with serious infections caused by sensitive strains of the determined microorganisms. Such microorganisms involve in various diseases such as genitourinary infections, septicemia, and skin infections [2]. These antibiotics are also used as food additives to enhance the feed efficiency, and as dietary supplements for food-producing animals [3]. The residues of antibiotics in foods may lead to drug resistance and other poisonous effects in humans [4–6]. In addition, the increasing use of antibiotics results in serious risks to human and veterinary health. Moreover, when the antibiotics enter the environment, they can affect natural microbial communities [7]. Thus, determination of antibiotic residues in foodstuffs is of an importance. There are several impediments to the determination of antibiotic residuals in bee products. Honey as a known natural product has significant nutritional and medicinal properties [8]. One of the obstacles is the complex matrix of honey, which is conventionally treated by acidic hydrolysis to break the bond of sulfonamides with sugars. Nevertheless, this might decompose some of the acid sensitive antibiotics under study (in this case CF). Therefore, it requires further treatment to control the presence of antimicrobials in honey [9]. Other problems are the huge market of different varieties of honey, the availability of modern antibiotics for producers, and strict requirements for the content of antibiotics present in final products. The European Union has adopted a maximum residue limit (MRL) of 100 μg $kg^{-1}$ for tetracycline antibiotics in foodstuffs of animal origin [10]. This low MRL value demands the development of analytical methods that are sensitive enough to determine the residues in foodstuffs. Hence, it is necessary to develop a simple, rapid and efficient method for the analysis of TC and CF residues in honey samples.

Solid phase extraction (SPE), as an affordable sample preparation method, has been replaced widely to conventional liquid-liquid extraction (LLE) [11]. It has many obvious advantages, including high preconcentration factors, low consumption of organic solvents, and simple operation [12,13].

The heart of the SPE technique is the adsorbent composition

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because it affects the selectivity and sorptive capacity. The surface chemistry, porosity and specific surface area of adsorbents are the most important characteristics that are considered for extraction purposes. Electrospinning is the most promising technique to produce continuous nanofibers as adsorbent material on a large scale with tunable fiber diameter from nanometers to microns [14]. Recently, electrospun nanofibers have been successfully used as an adsorbent in solid phase extraction (SPE) [15–26]. The nanofibers can be modified by incorporating nanoparticles such as graphene oxide (GO) to produce composite nanofibers for improving their adsorption capacity. In addition, GO with oxygenated functional groups gives the hydrophobic polymeric nanofibers good dispersibility in many solvents particularly in water [27,28].

In this work, the GO-PET electrospun nanofibers were fabricated and used for extraction of TC and CF antibiotics as model compounds from honey samples. In addition, the significant parameters affecting the extraction efficiency were investigated to obtain the highest efficiency.

## 2. Materials and methods

### 2.1. Reagents and materials

Sulfuric acid (99.8%), hydrogen peroxide ($H_2O_2$, 30%), hydrochloric acid (HCl, 37%), acetone, ethanol (EtOH), sodium chloride (NaCl), sodium hydroxide, and potassium permanganate ($KMnO_4$) with the purity higher than 99.9% were purchased from Merck Chemicals (Darmstadt, Germany). Methanol (MeOH) and HPLC grade acetic acid (HAc) were obtained from Sigma Aldrich Ltd. (St Louis, USA). Graphite powder and trifluoroacetic acid (TFA, 99%) were supplied from Samchun Pure Chemical Co Ltd. (Pyeongtaek, Korea). Tetracycline and cefotaxime standards were bought from SolarBio (Beijing, China). The standard solutions of antibiotics were prepared with appropriate dissolving in water.

### 2.2. Instrumentation

A Knauer Smartline HPLC system (Berlin, Germany) with a quaternary pump, a manual injector (20 μL sample loop) and a photodiode array (PDA) detector was utilized using EZChrom Elite 3.3.2 software to control the system and for the acquisition and analysis of the data. An ultra C18 column (5 μm × 250 mm × 4.6 mm) was used for separation of the target analytes. The mobile phase composed of water, trifluoroacetic acid (5 mM, pH 3) and acetonitrile (70:30, v/v) was used in an isocratic mode (1.0 mL $min^{-1}$). The experiments were carried out at the wavelength of 260 nm for optimization step, analytical method validation, and comparison studies. A double beam Rayleigh UV-2601 (Beijing, China) UV–vis spectrophotometer using a couple of 1 cm optical path length microcuvettes (Fisher Scientific, USA) was utilized for the optimization step. Scanning electron microscopy (SEM, Zeiss DSM-960, Oberkochen, Germany) was used for surface morphology characterization of GO-PET at an accelerating voltage of 20 KV. An Equinox 55 FT-IR spectrometer (Bruker, Bremen, Germany) was used for recording infrared spectra in the 400–4000 $cm^{-1}$ region. The nanofibers were produced using an electrospinning set-up consisting of a direct current (DC) high voltage power supply. The set-up was equipped with a syringe pump (SP 1000), and a collector fabricated by Fanavaran Nano-Meghyas (FNM, Tehran, Iran). Brunauer–Emmett–Teller (BET) surface area and Barrett-Joyner-Halenda (BJH) pore size distribution analyses were determined using a Belsorb MR6 (Ozaka, Japan).

### 2.3. Fabrication of the adsorbent

Graphene oxide was synthesized by Hummers' method [29]. A portion (10 mg) of the GO was dispersed in 1 mL of TFA and subjected to ultrasound for 10 min. Then, 180 mg PET was added to the mixture and kept in the refrigerator for 24 h to obtain a homogeneous solution. Afterwards, the solution was loaded into a 2 mL plastic syringe with a needle diameter of 0.2 mm. Then, the syringe was placed on a syringe pump and the voltages +10 and −6 kV was applied to the syringe needle and an aluminum foil (as a collector), respectively. The produced electrospun fibers were collected in the aluminum foil at a distance of 15 cm from the tip of the syringe needle. The solution was electrospun for 2 h at a flow rate of 0.5 mL $h^{-1}$, and then the aluminum foil was immersed in MeOH to separate the fibrous mat from the foil before use.

### 2.4. Honey samples

The honey samples obtained from Ramsar (Mazandaran province, north of Iran), imported honey and industrial honey were purchased from a local supermarket and prepared for analysis. The honey samples were prepared as follows: A 5 g of each honey sample was dissolved in 100 mL of distilled water. Then, they were placed in a water bath at 50 °C temperature for 10 min, centrifuged at 4000 rpm for 15 min and finally filtered using a filter paper. In the reported studies on the solid phase extraction of CF and TC residues from honey [30,31], an organic wash is necessary to simplify the matrix complexity.

### 2.5. The procedure

First, 100 mL of a water-base standard solution of the selected antibiotics with its pH adjusted at 5 was placed in an Erlenmeyer flask. Then, 40 mg of the adsorbent was added to the solution and was shaken for 10 min. Afterwards, the adsorbent was removed from the solution and placed in a test tube. Next, 400 μL MeOH was added to the adsorbent, and the solution was vortexed for 1 min. Then, the extract was dried under a nitrogen gas stream and the residue was redissolved in 200 μL MeOH and was analyzed with UV–vis spectrophotometry (at 260 nm).

### 2.6. Calculation of enrichment factor and extraction recovery

The enrichment factor (EF) was calculated using the following equation:

$$EF = \frac{C_o}{C_{aq}} \tag{1}$$

Where $C_o$ and $C_{aq}$ are the final concentration of the analyte in the organic phase (desorption solvent) and the initial concentration of the analyte in the aqueous phase, respectively.

The extraction recovery (ER%) was calculated with Eq. (2):

$$ER\% = \left(\frac{C_o \times V_o}{C_{aq} \times V_{aq}}\right) \times 100 \tag{2}$$

Where $V_0$ and $V_{aq}$ are the volumes of organic solvent and an aqueous phase, respectively.

## 3. Results and discussion

### 3.1. Characterization

Fig. 1 shows FT-IR spectra of GO, electrospun PET, and GO-PET. The spectrum of GO (Fig. 1a) contains a band at 3450 $cm^{-1}$ assigned to the O—H stretching vibrations. The peaks at 1734, 1622 and 1018 $cm^{-1}$ can be ascribed to C═O and C—O functional groups, respectively. These confirm the presence of oxygen-containing functional groups on GO nanosheets. Fig. 1b (the spectrum of PET nanofibers) displays a strong band at 1735 $cm^{-1}$ related to the stretching vibrations of C═O. The bands at 730, 2965 and 3070 $cm^{-1}$ correspond to the out of plane $CH_2$ bending, aliphatic and aromatic C—H stretching vibrations,

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