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Fluorescent labelling of ciprofloxacin and norfloxacin and its application for residues analysis in surface water

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ABSTRACT

Sensitivity enhancement for residue analysis of ciprofloxacin and norfloxacin in surface water was performed by liquid chromatography with fluorescent detection (LC-FD). Labelling of both drugs were studied with fluorescent probes (e.g. Nile blue perchlorate (NBP) and 4- (N,N-Dimethylaminosulfonyl)-7-(N-chloroformylmethyl-N-methylamino)-2,1,3-benzoxadiazole (DBD-COCl). Factors affecting the derivatization (e.g. stoichiometric ratios, reaction time and base catalysts) were optimized. The derivatization was achieved in 15 min using a stoichiometric ratio between the substrate and DBD-COCl of 1:3, whereas NBP gave unsatisfactory results. Separation of the derivatives by LC was achieved (resolution (R_S) > 1.8) on a C8 column using a mobile phase consisting of 50 mM formic acid and acetonitrile (ACN) (68:32% v/v) in 20 min. The method was linear (r^2 > 0.99) in a range of 200–2,000 μg/L, precise (%RSD < 9.17) and accurate (%recovery of 102.5–122.2%) for the determination of the derivatives. The uses of fluoroquinolone molecularly imprinted polymer in conjunction with hydrophilic-lipophilic balance sorbents demonstrated an efficient procedure for sample pre-concentration and clean-up for water sample resulting in the improved percent recovery. Applications of the proposed method was shown in surface water samples in Thailand.

1. Introduction

The occurrence of active pharmaceutical ingredient (APIs) and veterinary medicine (VEMs) residues in environment is ubiquitous worldwide [1]. This results in many adverse events such as mortality of vultures in India and Pakistan [2], changing of sexual characteristics in fish due to the presence of synthetic hormones [3] an alteration of histopathological in rainbow trout fish [4] and bioaccumulation in aquatic organisms (e.g. fish, shrimp). One of the most serious issue of pharmaceutical residues is inducing antimicrobial resistance in human and animals due to the presence of antibiotics in the environment [5–7]. Several organizations such as the US Environmental Protection Agency (EPA) and European Medicines Agency (EMEA) have launched many guidelines and regulations to alleviate the drug residue problems in water [8,9]. However, the presence of the residues is still found in the environment (e.g. soil [10], sewage and sludge [11] and surface water [1,11,12] due to the increases of consumption.

Fluoroquinolones (FQs) are antibiotics, which is a fluorinated 4-quinolone ring containing carboxylic acid, fluorine and a piperazine ring substitution at position 3, 7 and 8. FQs are used for treatments of Gram-positive and Gram-negative bacterial infection. A mechanism of FQs involves the inhibition of DNA gyrase and topoisomerase IV of bacteria, which relates to bacterial cell wall synthesis [13]. Occurrences of FQ residues in water resources have been reported in publications [14–22] since FQs are heavily used in medical and veterinary practice. The presence of FQs residues is a major cause of an induction of bacterial resistance according to the World Health Organization (WHO) report in 2014 [23]. Consequently, monitoring of FQs residues in the environment is a crucial issue.

Currently, sophisticated techniques such as liquid chromatography (LC) and capillary electrophoresis (CE) coupled with the various detection techniques (e.g. ultraviolet (UV) photometric [14], fluorimetric [15–19] and mass spectrometric [18,20–22]) have been successfully used for the determination of FQ residues in surface water because of their efficiency and reliability. Limitations of these technique include cost, time and inconvenience for on-site analysis. A few publications reported uses of miniaturized systems for the analysis of residue in environment sample. Unfortunately, the uses of these devices was limited by types of detectors such as biosensors [24,25], contactless conductivity detection [26] and laser induced fluorescent (LIF) detectors [27,28].

In this work, two popular FQs, which were ciprofloxacin (CIP)

Abbreviations: CIP, ciprofloxacin; NOR, norfloxacin; DBD-COCl, 4-(N,N-Dimethylaminosulfonyl)-7-(N-chloroformylmethyl-N-methylamino)-2,1,3-benzoxadiazole; NBP, Nile Blue perchlorate; LED, light-emitting diode; LD, laser diode

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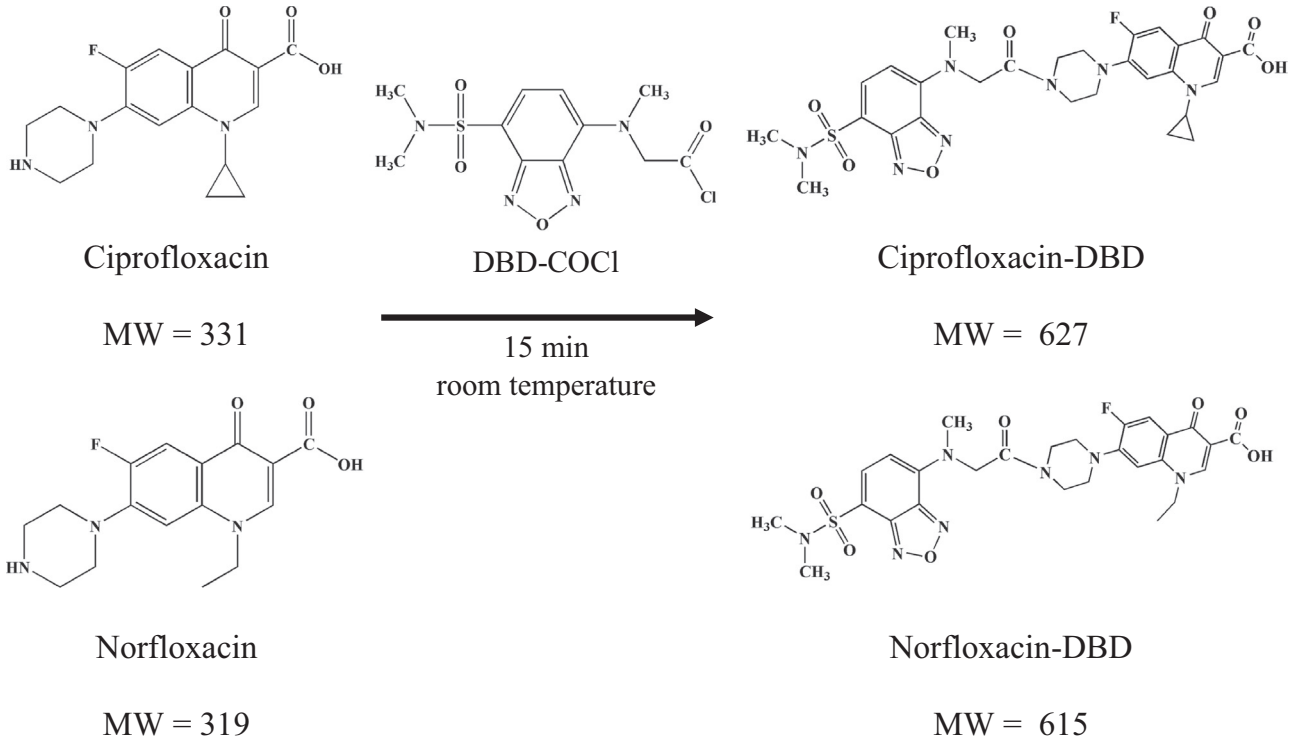

Fig. 1. Fluorescent labelling of ciprofloxacin and norfloxacin with 4-(N,N-Dimethylaminosulfonyl)-7-(N-chloroformylmethyl-N-methylamino)-2,1,3-benzoxadiazole (DBD-COCl).

and norfloxacin (NOR) (Fig. 1), were chosen for fluorescent labelling. These FQs possess chromophores and fluorophores allowing them to be determined using deep UV region (190–320 nm) detectors. To permit the detectability of FQ in a UV–visible region (320–800 nm), we investigated the fluorescent labelling of CIP and NOR. This could benefit the application of a miniaturized devices coupled with a UV–visible detector (e.g. light-emitting diode-induced fluorescence (LED-IF)) for FQ analysis [27]. Derivatization procedures were optimized in terms of stoichiometric ratios between FQ and fluorescent labelling reagents (e.g. Nile blue perchlorate (NBP) and 4-(N,N-Dimethyl aminosulfonyl)-7-(N-chloroformylmethyl-N-methylamino)-2,1,3-benzoxadiazole (DBD-COCl)) [28], base catalysts concentration and reaction time. Confirmation of the derivatives was done by mass spectrometry and analysis of the derivatives was performed by LC-FD. Sample pre-treatment was done by two clean-up steps (hydrophilic-lipophilic balance (HLB) and fluoroquinolone molecularly imprinted polymer (FQ-MIP)) to minimize interferences from sample matrices. Finally, the proposed method was validated and applied for determination of CIP and NOR residues in surface water in Thailand.

2. Material and methods

2.1. Chemicals

Ciprofloxacin (CIP) was purchased from Fluka (Buchs, Switzerland). Norfloxacin (NOR) were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). DBD-COCl was obtained from TCI (Tokyo, Japan). Acetonitrile (ACN), dichloromethane (DCM) and methanol (MeOH) (HPLC grade) and ethyl acetate (EtOAc) (AR grade) were supplied from RCI Labscan Asia (Bangkok, Thailand). Tetrabutylammonium hydroxide (TTBAOH), 1-octane sulfonate sodium (OSS), Diphenylphosphinic chloride (DPC) and Nile blue perchlorate (NBP) were from Sigma-Aldrich (St. Louis, Missouri, USA). Formic acid and ammonia (NH_4OH) were purchased from Arcos Organics (Geel, Belgium). Ethylenediaminetetraacetic acid disodium (EDTA) was obtained from Merck (Darmstadt, Germany). Triethylamine (TEA) analytical grade was obtained from Carlo Erba (Rodano, Italy). 18 MΩ water was obtained from Labconco deionized water system (Fort Scott, Kansas, USA).

Stock solutions of CIP and NOR were prepared by dissolving an accurate weight of CIP and NOR in ACN to obtain the concentration of 50 mg/L. Stock solutions were kept in refrigerator (4–8 °C) and use within one week. Ammonia (0.5% w/w) was prepared by diluting 0.2 mL of strong ammonia in 9.8 mL of methanol. EDTA (0.2% w/v, pH 4.2) was prepared by dissolving 2 g of EDTA disodium in 1 L of water and adjusted pH to 4.2 with glacial acetic acid. TEA (1% v/v) was prepared by diluting 0.1 mL of TEA in 9.9 mL of DCM.

All derivatizing reagents were freshly prepared on daily basis. DPC (20 μmol/mL) was prepared by dissolving 40 μL of DPC in 10 mL DCM. DBD-COCl (0.05% w/v) was prepared by dissolving 5 mg of DBD-COCl in 10 mL of DCM. NBP (0.5% w/v) was prepared by dissolving 25 mg of NBP in 5 mL of ACN.

2.2. Instrumentation

Thin layer chromatography (TLC) using silica gel GF254 TLC plate (Merck, Darmstadt, Germany) and mobile phase consisting of DCM and MeOH (3:1%v/v) was employed for reaction monitoring. Electrospray-ionization triple quadrupole (QqQ) mass analyzer model 6420 (Shimadzu, Tokyo, Japan) was used for product mass scanning in a positive mode. Derivatized samples were directly injected into QqQ *via* a Nexera liquid chromatographic system (Shimadzu, Tokyo, Japan) using ACN as a mobile phase at a flow rate of 0.2 mL/min.

Reaction monitoring for fluorescent labelling of CIP and NOR was performed using a liquid chromatographic system (LC10ADvp) equipped with a fluorescence detector (RF-10AXL) (Shimadzu,