Journal of Chromatography A, 1216 (2009) 5327–5332

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

# Separation of fluoroquinolones in acidic buffer by capillary electrophoresis with contactless conductivity detection

Zhongju Yang, Weidong Qin*

*College of Chemistry, Beijing Normal University, No. 19, Xinjiekou Wai Street, Beijing 100875, China*

ARTICLE INFO

*Article history:*
Received 24 January 2009
Received in revised form 6 May 2009
Accepted 8 May 2009
Available online 15 May 2009

*Keywords:*
Capillary electrophoresis
Capacitively coupled contactless conductivity detection
Fluoroquinolone
Liquid–liquid extraction
Chicken tissue

ABSTRACT

A method to determine five fluoroquinolones (FQs), namely, rufloxacin (RUF), ciprofloxacin (CIP), enrofloxacin (ENO), gatifloxacin (GAT) and moxifloxacin (MOX), in acidic buffer by capillary electrophoresis (CE)-capacitively coupled contactless conductivity detection ($C^4D$) technique is presented. Separation was carried out in a fused-silica capillary (42 cm × 50 μm) using a buffer composed of 10 mM tartaric acid, 14 mM sodium acetate and 15% (v/v) methanol at pH 3.8. The RSDs of the migration times and peak areas were 0.65% and 12.3% (intraday), 1.28% and 8.8% (interday), respectively. CE-$C^4D$ in combination with liquid–liquid extraction (LLE) as clean-up and preconcentration procedure, allows detection of the FQs in fortified chicken muscle samples with detection limits of 6.8–11.7 ng/g. This method shows potential in rapid determination of FQs in samples with complex matrix.



## 1. Introduction

Fluoroquinolones (FQs) are one of the most important classes of antibiotics with excellent activity against both Gram-negative and Gram-positive pathogenic bacteria [1]. They have been intensively used in clinical medicine and farm animals in the past decades to treat a variety of infections [2]. However, the FQ residues in animal tissue are toxic, and can cause pathogen resistance and possible allergic hypersensitivities if consumed by human being [3–5]. To ensure food safety, the European Union (EU) regulates the use of veterinary drugs through Council Regulation No. 2377/90, which was later amended by Commission Regulation 1353/2007/EC [6]. Developing and improving analytical methods for this class of antibiotics are of increasing interest because various quinolone (QN) and FQ structures have vastly different substituent groups that greatly affect their individual characteristics [7].

Several methods have been developed to detect FQs and their residues in biological samples; high performance liquid chromatography (HPLC) is the often-used technique for assaying antibiotics in real samples [8–14]. Capillary electrophoresis (CE) has been established as a powerful, multimode and effective for a variety of analytes ranging in size and character [15]. More recently, CE has been developed for the analysis of FQs, exhibiting advantages of high separation efficiency, short analysis time, low sample consumption and simplicity in operation. Different detection methods, e.g., UV [16], potential gradient [17], chemiluminescence and electrochemiluminescence [18,19], amperometry [20], laser-induced fluorescence [21], and mass spectrometry [22,23] have been hyphenated to CE to improve detection sensitivity. Capacitively coupled contactless conductivity detection ($C^4D$) is a conductometric measuring approach that is based on the conductivity differences between the sample zones and the background electrolyte (BGE) [24–29]. Sensitive detection can be achieved by using BGE with low conductivity [30]. $C^4D$ overcomes the disadvantage of short path length that is encountered by on-column optical detections. Furthermore, it avoids the direct contact of working electrodes with BGE in the conventional conductivity detection [31] and eliminates the laborious work on fabricating detection cells in potential gradient detection [17]. It prevents the potential damage of the conductometric detection cell especially in case where organic solvents are employed as additives. Additionally, the detection sensitivity of $C^4D$ could be tuned by optimizing the oscillation voltage [32]. CE-$C^4D$ has been applied in determining species including small inorganic and organic ions, peptides, proteins, oligo- and polynucleotides and nucleic acid fragments [25,26,28,29,32–40]. However, to the best of our knowledge, there is so far no report on detection of FQs by CE-$C^4D$.

Most FQs exist as zwitterions and are hydrophobic in nature; they are usually separated in alkaline BGE under counter-electroosmotic flow (EOF) mode in CE. The disadvantage of this operation mode is obvious: slight variation in EOF mobility often causes significant changes in migration times, particularly for the

* Corresponding author. Fax: +86 10 58802531.
*E-mail address:* qinwd@bnu.edu.cn (W. Qin).

0021-9673/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2009.05.014

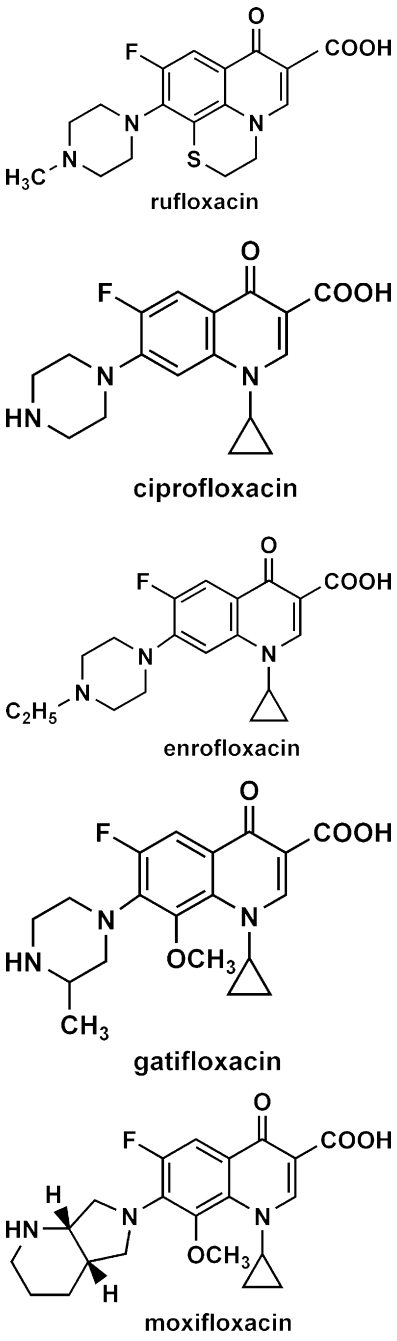


**Fig. 1.** Chemical structures of studied FQs.

long migration time species, leading to poor reproducibility. In acidic BGE, positively charged FQs move at the same direction with the strongly suppressed EOF. Variation in the weak EOF imposes less influence on the migration times than that may occur in alkaline BGE, and therefore precision of the method is improved.

The aim of this report is to develop a method to determine FQs in acidic buffer by CE-$C^4D$. Five FQs, viz., rufloxacin (RUF), ciprofloxacin (CIP), enrofloxacin (ENO), gatifloxacin (GAT) and moxifloxacin (MOX) (chemical structures shown in Fig. 1), were employed as model analytes. Parameters influencing the separation performance and detection sensitivity of the method were studied and optimized. For demonstration, a liquid–liquid extraction (LLE) method was coupled to CE-$C^4D$ in determining the FQs in fortified chicken muscle samples. The results were validated with a reported HPLC method [41].

## 2. Materials and methods

### 2.1. Reagents and solutions

RUF, CIP, ENO, GAT and MOX were purchased from the Medicinal and Biological Research Institute (Beijing, China). Sodium 1-hexadecanesulfonate (SHS) was a product of Sigma (St. Louis, MO, USA). Tartaric acid, sodium acetate, methanol, hydrochloric acid (36.0–38.0%), sulfuric acid (98%), trichloroacetic acid, citric acid, sodium hydroxide, trifluoroacetic acid (TFA), dichloromethane, *n*-hexane, and sodium dihydrogen phosphate were bought from the Beijing Chemical Plant (Beijing, China). Deionized water (Milli-Q Water System, Bedford, MA, USA) was used for preparing solutions throughout the experiments.

To prepare buffers, appropriate volumes of the stock solutions (typically of 200 mM each) and methanol were added to a 25 mL volumetric flask; the mixture was diluted with deionized water and thoroughly mixed. Individual stock solutions of the FQs were prepared in 6 mM sodium hydroxide at 10 000 μg/mL each and were kept in refrigeration. Working standards were prepared by diluting the stock solutions with deionized water to desired concentrations. All working solutions were filtered with 0.22 μm filers (Jiu Ding High Tech., Beijing, China) before use.

### 2.2. Instruments and operations

The CE-$C^4D$ system consisted of a high-voltage power supply (Sanchuan High Tech., Tianjin, China) and a laboratory-made $C^4D$ detector. The detector was designed according to the previous reports [33,42]. Briefly, two 3 mm length × 0.4 mm I.D. hypodermic needles, separated by a gap of ca. 1.5 mm, were used as excitation and pick-up electrodes, respectively. A function generator (YSD996A, Peiming, Jiangsu, China) was used to provide a sinusoidal excitation signal. The high-frequency current signal from the detection electrode was converted to voltage by an OPA627 operational amplifier (Texas Instruments, Dallas, TX, USA) via a feedback resistor of 1 MΩ. It was progressively rectified, amplified and offset against the baseline with two additional operational amplifiers (OPA627). The signal from $C^4D$ was acquired via a data acquisition unit (CT22, Qianpu, Jiangsu, China). An HW2000 chromatography station (Qianpu) was used to control the CT22 interface and for data processing.

Bare fused-silica capillary was product of Polymicro Techmologies (Phoenix, AZ, USA; 50 μm I.D. × 375 μm O.D.). The total length of the capillary was 42 cm and effective length was 32 cm. The samples were hydrodynamically introduced into the capillary by lifting the anodic end to a height of 18 cm for 10 s or 20 s as described in the text. The separation voltage was set to 13 kV. Between two consecutive runs, the capillary was flushed with the BGE for 2 min. All experiments were carried out at room temperature.

### 2.3. HPLC

HPLC analysis of FQs reported in reference [41] was modified. Experiments were performed on a Shimadzu system (Shimadzu, Kyoto, Japan) equipped with a binary pump, a gradient controller (SCL-10Avp), an on-line degasser (DGU-12A), and a Shimadzu SPD-20A UV–vis detector (set at 275 nm). Sample solutions were introduced into the system by a manual injection valve fitted with a 20 μL sample loop. The gradient elution (as described in Table 1) was carried out with a mobile phase consisting of solutions A (aqueous formic acid solution, pH 2.5) and B (acetonitrile) on a 150 mm × 4.6 mm Shimadzu $C_{18}$ column (5 μm) at a flow rate of 1.0 mL/min. The column temperature was maintained at 40 °C.