

Research Article

Determination of Norfloxacin and Ciprofloxacin in Chicken Meat Based on Matrix Solid-Phase Dispersion Extraction and Capillary Zone Electrophoresis

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This study describes an effective matrix solid-phase dispersion (MSPD) extraction method for determining norfloxacin (NOR) and ciprofloxacin (CIP) in chicken meat by capillary zone electrophoresis (CZE). The optimum conditions for separating NOR and CIP were as follows: 60 cm × 75 μm i.d. capillary, 40 mmol L⁻¹ borate buffer solution (pH 8.5), separation voltage at 16 kV, and detection wavelength at 280 nm. Before CZE determination, the chicken meat samples were purified and enriched by using an MSPD extraction step with a preconditioned C₁₈ cartridge and by eluting the compound with 3.0 mL of acetonitrile. A good linear fit curve with the concentration range of 0.10 μg g⁻¹ to 500 μg g⁻¹ for NOR and CIP was obtained, with regression coefficients of 0.9994 and 0.9986, respectively. The limits of detection of NOR and CIP were 0.04 and 0.03 μg g⁻¹, respectively. The proposed method was successfully applied to determine NOR and CIP in chicken meat.

1. Introduction

Fluoroquinolones (FQs) are a class of relatively new and entirely man-made, nonsteroidal antibiotics/antibacterials [1]. The FQ norfloxacin (NOR) is the first-choice drug for the treatment of diseases caused by *Campylobacter*, *Escherichia coli*, *Shigella*, and *Vibrio cholerae*. NOR is used to treat gonorrhoea, eye infection, and urinary tract infection [2–4]. The FQ ciprofloxacin (CIP) is used to treat systemic infections, including urinary tract, respiratory, gastrointestinal, and cutaneous infections. CIP affects the growth and reproduction of bacteria by inhibiting a subunit of bacterial DNA gyrase and an essential enzyme that maintains the super helical twists in the bacterial DNA [5]. These quinolones and antibiotics are generally used for the prophylaxis and treatment of diseases; they are also used as feed additives for mass gain promotion [6]. The widespread use of FQs presents potential hazards to human health, such as the

emergence and spread of drug-resistant bacterial strains and the possible induction of cancer [7]. Concerns about drug residues entering the food chain and contributing to bacterial resistance have prompted the European Union and China to establish maximum residue levels (MRLs) for various classes of antibiotics, including FQs, in matrices of food originating from animals of various species [8].

Several methods for the determination of NOR and CIP have been reported in recent years. These methods include high-performance liquid chromatography (HPLC) [9], flow-injection chemiluminescence (CL) [10], electrochemical method [11], and spectrophotometric method [12]. However, the application of HPLC is limited by its complicated system and maintenance, large sample requirement, and expensive reagents. CL and spectrophotometric method cannot be used to determine FQs in complex biological samples because they both lack separation capacity. Capillary electrophoresis (CE) has attracted considerable attention in

recent years for the quantitative analysis of biological samples [13, 14] because of its simplicity, high efficiency, minimum solvent and sample requirements, and relatively low cost.

A preliminary extraction step by liquid-liquid extraction (LLE) [15] or solid-phase extraction (SPE) [16] was previously employed to clean the sample in preparation for further analysis. These processes are complicated, time consuming and need large amounts of organic solvent. Matrix solid-phase dispersion (MSPD) is based on SPE in which a homogeneous dispersion of the matrix is mixed with a suitable solid adsorbent (silicagel, diatomaceous earth, alumina, C_{18} , etc.). This method has been successfully applied to extract target analytes from biological samples; compared with other processes, MSPD requires shorter analytical time, smaller sample size, and lower solvent consumption [17, 18].

This study describes a comprehensive capillary zone electrophoresis (CZE) method for the simultaneous determination of NOR and CIP residues in chicken meat. MSPD was used to extract NOR and CIP from chicken meat. Method development efforts were focused on the optimization of the MSPD, the cleanup procedure, and the avoidance of contamination. In addition, parameters affecting the separation efficiency of CZE were optimized. Finally, the developed method was validated for the analysis of NOR and CIP in real samples with better results.

2. Experimental

2.1. Chemical and Reagents. NOR and CIP were purchased from the National Institute for Food and Drug Control (Beijing, China). Analytical reagent-grade methanol, toluene, chloroform, and acetonitrile were obtained from Beijing Chemical Factory (Beijing, China). Boric acid (H_3BO_3) and sodium hydroxide (NaOH) were purchased from Tianjin General Chemical Reagent Factory (Tianjin, China). Diatomaceous earth (Florisil, 200 mesh) and silica (200 mesh) were obtained from the Chinese Medical and Biological Products Institute (Beijing, China). YWG- C_{18} - (C_{18} -) and YWG- C_8 - (C_8 -) bonded silica materials (surface area $300\text{ m}^2\text{ g}^{-1}$, average pore size: $10\ \mu\text{m}$) were obtained from Zhongkeantai Tech Co., Ltd. (Lanzhou, China). Florisil, silica, C_{18} , and C_8 were baked at 650°C for 5 h, dried overnight at 100°C for 2 h, and then stored in a desiccator for future use. Borate buffer ionic strength calculations were performed using the HP 3D CE Buffer Calculator version 1.00 (Hewlett-Packard Corp., USA). Aqueous solutions of carrier electrolytes and of standards were prepared by using $18.2\text{ M}\Omega\text{-cm}$ water from a Milli-Q water purification system (Millipore, Bedford, MA, USA) and were filtered through a $0.22\ \mu\text{m}$ membrane before use.

2.2. Procedure of MSPD. The working procedure of MSPD was the same as [19]. Approximately 0.02 g of chicken meat was homogenized in 2 mL of sodium chloride (1 mol L^{-1}), transferred into a glass mortar, and then soaked in a suitable amount of standard solution. After 10 min, 1.0 g of C_{18} (dispersion adsorbent) was mixed with the sample. The homogeneous mixture was loaded into a cartridge, which

TABLE 1: The effects of different adsorbents on recoveries of NOR and CIP ($n = 3$).

Adsorbent	NOR		CIP	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Florisil	62.4	3.9	63.6	5.6
Silica	35.4	6.8	28.4	8.4
C_8	79.4	4.5	72.6	3.6
C_{18}	85.6	4.3	89.2	3.8

was prepacked with 0.2 g of C_{18} particles. The cartridge was rinsed with 3.0 mL of methanol- H_2O (1:19, v/v) and eluted with 3.0 mL of acetonitrile. The eluates were evaporated to dryness at 50°C under a gentle stream of nitrogen gas. The dry residues were reconstituted in $200\ \mu\text{L}$ of background electrolyte (BGE) and then vortex-mixed for 20 s for CE analysis.

2.3. CE Analysis. CE analysis was performed on a Beckman Coulter Proteome Lab PA800 (Beckman Coulter, Inc., Fullerton, CA, USA) instrument equipped with an ultraviolet (UV) detector and operated in normal polarity mode. Data acquisition and instrument control were carried out using 32 Karat software 8.0 (Beckman Coulter, Inc., Fullerton, CA, USA). An untreated fused-silica capillary of $60\text{ cm} \times 75\ \mu\text{m}$ i.d., 40 cm from inlet to detector, was used for the determinations. The capillary was activated daily by rinsing it with 0.1 mol L^{-1} of NaOH for 30 min, with Milli-Q water for 30 min, and then with running buffer for 30 min. A 40 mmol L^{-1} boric acid solution adjusted with NaOH to pH 8.5 was used as the BGE. Separation was carried out by applying a voltage of 16 kV. The temperature of the capillary cartridge was maintained at 25°C by a liquid cooling system. The injection was hydrodynamic at a pressure of 1.0 psi for 2 s. Detection was performed by a UV detector at 280 nm.

3. Results and Discussion

3.1. Optimization of the MSPD Procedure. $10.00\ \mu\text{g g}^{-1}$ of NOR and CIP in drug-free chicken meat were added into a homogeneous mixture before extraction to investigate in detail the effects of the main factors in MSPD on recovery. Four MSPD adsorbents (1 g), namely, Florisil, silica, C_8 , and C_{18} , were examined. The results are given in Table 1. Comparison of the behaviour of these four adsorbents reveals that C_{18} provided an overall comparable efficiency, significantly better than that of silica, Florisil, and C_8 . C_{18} polarity was similar to the target analytes. The recoveries of two analytes (85.6% and 89.2%) were the highest when C_{18} was used as dispersion adsorbent. When the other adsorbents were used, the two analytes had lower recoveries and more peaks interfering with analyte detection. Therefore, C_{18} was chosen as the optimum dispersion adsorbent.

Since the MSPD is a complex process and according to the character of the sample (polarity, solubility, pKa, etc.), the washing and elution solvent should be carefully chosen. A

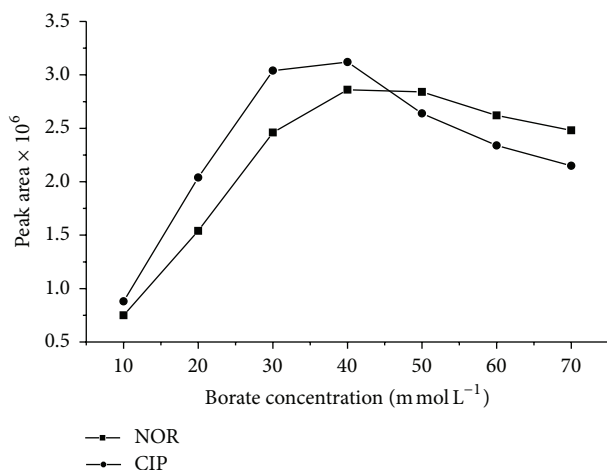


FIGURE 1: Effects of borate concentration on CZE performance.

washing step in MSPD was investigated using toluene, chloroform, methanol, and water as potential washing solvents to enhance the selectivity of MSPD and to decrease the cross-reactivity. The best results were obtained when methanol-H₂O (1:19, v/v) was used as the washing solvent. Interferences from endogenic components were observed when the volume of methanol-H₂O was lower than 3.0 mL. However, the recovery decreased when the volume of methanol-H₂O was higher than 3.0 mL. Therefore, 3.0 mL of methanol-H₂O (1:19, v/v) was used. In addition, elution solutions such as water, methanol, and acetonitrile were investigated. The best recoveries of NOR and CIP were obtained using 3.0 mL of acetonitrile as elution solution.

3.2. Optimization of CZE. Electrolyte concentration has a significant effect on the separation performance because it can influence the Joule heating, the electroosmotic flow (EOF), ionic strength, viscosity of electrolyte, and the current produced in the capillary. Therefore, the electrolyte concentration would affect the migration time and peak area. The effect of different borate concentrations (10 mmol L⁻¹ to 70 mmol L⁻¹) in BGE was studied (Figure 1). The pH was adjusted to 8.5, and the concentration of the two analytes was fixed at 10.0 μg g⁻¹. The peak area of the analytes gradually increased when the concentration of borate changed from 10 mmol L⁻¹ to 40 mmol L⁻¹. The peak area decreased and the analysis time increased significantly when the borate concentration was higher than 40 mmol L⁻¹, and migration time was prolonged. Therefore, the best borate concentration was 40 mmol L⁻¹.

Because NOR (pK_{a1} 6.1, pK_{a2} 8.5) and CIP (pK_{a1} 6.1, pK_{a2} 8.7) are zwitterionic compounds [20], buffer pH not only affects the EOF, but also affects the existence form of the two analytes. The effect of buffer pH on the separation of the two analytes was studied (Figure 2). The concentration of borate was 40 mmol L⁻¹, and the concentration of the two analytes was fixed at 10.0 μg g⁻¹. The result demonstrated that the peak area increased as the pH of BGE increased from

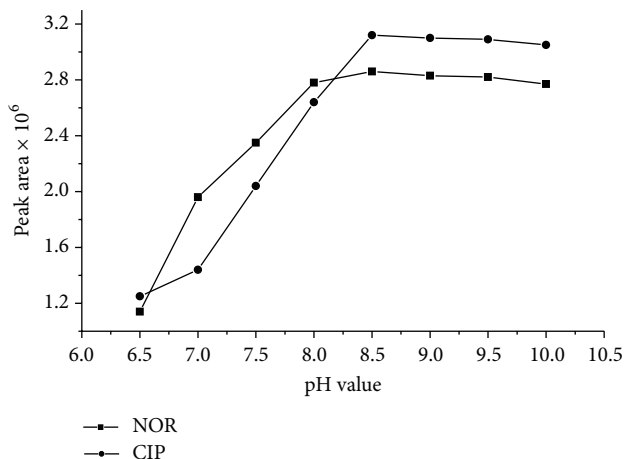


FIGURE 2: Effects of pH values on CZE performance.

6.5 to 8.5. However, the peak area was almost constant, but the migration time was prolonged when the pH was more than 8.5. This is due to the analytes existing in the form of anions: when the pH was higher than 8.5, they have the biggest absorbance at 280 nm wavelength. Thus, BGE pH was selected as 8.5 for subsequent studies.

The effect of applied voltage was examined in the range 10 kV to 20 kV using three replicate injections at each voltage. As the applied voltage was increased, the migration time was shortened and the peak area was increased, but the resolution was decreased. The applied voltage of 16 kV was selected for the experiment with satisfying sensitivity and migration time.

3.3. Linearity and LOD. The curves were prepared by adding different concentrations of NOR and CIP in drug-free samples. Linear calibration curves of NOR and CIP in chicken meat were obtained at the concentration range of 0.10 μg g⁻¹ to 500 μg g⁻¹. The correlation coefficients were 0.9994 and 0.9986, respectively. The LODs (signal-to-noise ratio of 3, S/N = 3) of NOR and CIP were 0.04 and 0.03 μg g⁻¹, and the LODs (signal-to-noise ratio of 3, S/N = 10) of NOR and CIP were 0.12 and 0.10 μg g⁻¹, respectively. The MRLs in meat established by European community for NOR and CIP are 100 μg kg⁻¹ [8]; therefore the method is sensitive enough to be applied in chicken meat.

3.4. Real Sample Analysis. Different spiked concentrations (0.50, 2.00, and 10.00 μg g⁻¹) of NOR and CIP in drug-free chicken meat were adopted to examine the recovery and precision of the method in analyzing NOR and CIP. The results of intraday and interday precision and accuracy experiments are summarized in Table 2. In our experiments, the precision of NOR was usually <5.8% at the medium, and higher concentration levels and did not exceed 6.8% at the low concentration level. Accuracy values always ranged between 86.4% and 103.2%. The precision of CIP was <4.9% at the medium and high concentration levels and did not exceed 6.2% at the low concentration level. Accuracy values always

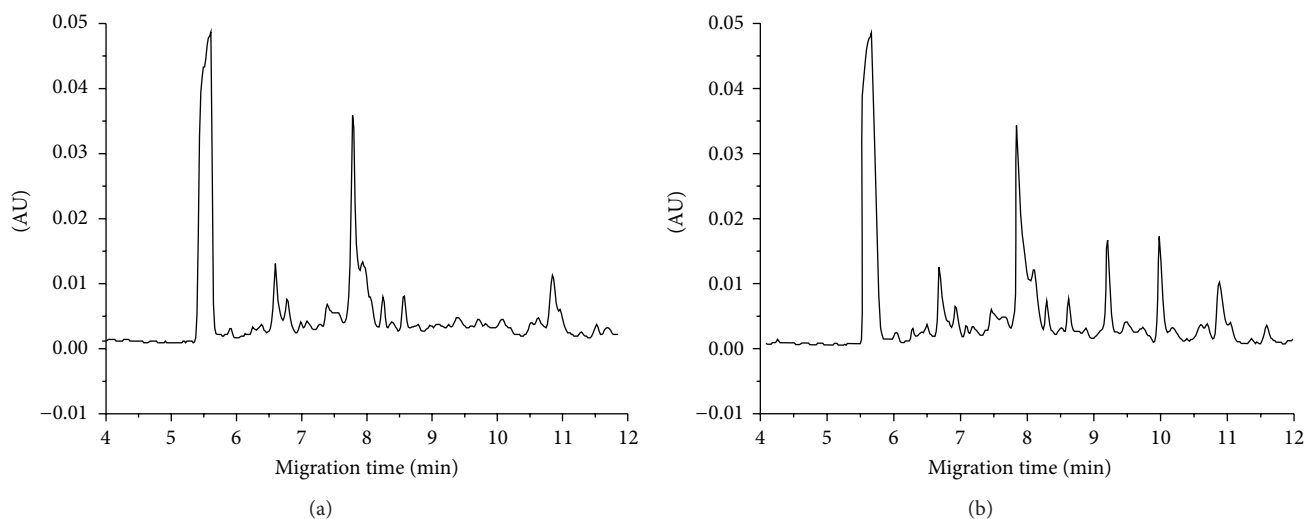


FIGURE 3: Electropherograms obtained from (a) a chicken meat sample and (b) the sample spiked with NOR and CIP at $10.00 \mu\text{g g}^{-1}$. Conditions: separation buffer, 40 mmol L^{-1} borate buffer solution (pH 8.5), separation voltage 10 kV; detection at 280 nm.

TABLE 2: Determination of NOR and CIP in chicken meat.

	Added ($\mu\text{g g}^{-1}$)	Found ($\mu\text{g g}^{-1}$)	Recovery (%)	RSD (%)
NOR				
Intraday ($n = 9$)	0.50	0.47 ± 0.025	94.0	5.4
	2.00	1.86 ± 0.085	92.8	4.6
	10.00	10.32 ± 0.506	103.2	4.9
Interday ($n = 9$)	0.50	0.43 ± 0.029	86.4	6.8
	2.00	1.95 ± 0.101	97.5	5.2
	10.00	9.58 ± 0.556	95.8	5.8
CIP				
Intraday ($n = 9$)	0.50	0.47 ± 0.023	94.2	4.8
	2.00	1.91 ± 0.067	95.4	3.5
	10.00	10.56 ± 0.401	105.6	3.8
Interday ($n = 9$)	0.50	0.45 ± 0.028	90.1	6.2
	2.00	2.09 ± 0.100	104.5	4.8
	10.00	9.84 ± 0.482	98.4	4.9

ranged between 90.1% and 105.6%. The recoveries and precision of the method were better than those from the ELISA method [21]. Figure 3 shows the typical electropherograms obtained from drug-free and spiked chicken meat samples.

4. Conclusions

This study describes a methodology for the determination and quantification of NOR and CIP in chicken meat by CZE using a preconcentration step of MSPD. This well-controlled method has generated high-quality data with acceptable levels of precision, accuracy, working range, and LOD.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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