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RESEARCH ARTICLE

# Knowledge of The HILIC Retention Behaviors of Two Quinolone Antibiotics on Sulfobetaine-Type Zwitterionic Stationary Phases

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**ABSTRACT**

Fluoroquinolones treat infections of the urinary, respiratory, gastrointestinal, skin, bone, and joint systems. This article describes the development of the hydrophilic interaction liquid chromatography (ZIC-HILIC) method to determine norfloxacin and ofloxacin retention characteristics. Separation of all analytes was accomplished using ZIC-HILIC$_1$ and ZIC-HILIC$_4$ columns. The mobile phase was 40 mM sodium acetate water solution in acetonitrile pumped at a 0.7 mL/min flow rate. The influence of acetonitrile concentration, the presence of salt in the mobile phase, and pH on the retention of norfloxacin and ofloxacin in the HILIC mode were investigated in this study. When using ZIC-HILIC columns, it was discovered that the retention factors of the analytes were inversely proportional to the amount of water present in the mobile phase, which is representative of a conventional hydrophilic partitioning mechanism. The ZIC-HILIC based strategies described in this article represent a significant step toward more sensitive drug analysis. We observed that zwitterionic materials could govern the separation of materials, as shown by the influence of the chain length between the zwitterion groups, which contributed to the explanation of the variation in the retention period of the materials to be separated.

**Keywords:** Norfloxacin, Fluoroquinolones, Ofloxacin, ZIC-HILIC, Sulfobetaine-type.

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**Conflict of interest:** None

## INTRODUCTION

Fluoroquinolones are an essential antibiotic used to treat or prevent certain bacterial infections in both humans and animals. Fluoroquinolones have been used to treat various diseases, including skin infections, gastrointestinal infections, respiratory tract infections, urinary tract infections, and bone and joint infections. Fluoroquinolone antibiotics come in four generations, with the third generation being the most often used in the clinic today. The primary three types of fluoroquinolones in the third generation commonly utilized in clinical medicine are norfloxacin, enrofloxacin, and ofloxacin.[1-5] These three fluoroquinolones continue to be widely used in various human and animal applications. The most often used fluoroquinolones are norfloxacin (NOR) and ofloxacin (OFL) (Figure 1). As a result, a worldwide effort is underway to develop quick and sensitive techniques for detecting these drugs in raw materials, pharmaceutical formulations, and biological, environmental, and dietary samples.

Reversed-phase liquid chromatography (RP-LC) is the most often used technique for NOR and OFL analysis.[6-10] Hydrophilic interaction liquid chromatography (HILIC) is an alternate chromatographic mode to RP for examining norfloxacin and ofloxacin. HILIC is an effective liquid chromatography mode for separating polar analytes that reversed-phase liquid chromatography (RPLC) insufficiently maintains. In HILIC, an organic-rich mobile phase similar to that is used in reverse-phase liquid chromatography. A few research offers insight into the retention mechanism and retention behavior in HILIC. Therefore encouraged to investigate the mechanism of NOR and OFL separation using HILIC columns. Therefore will be the study's first goal. Due to the numerous retention mechanisms that might contribute to overall solute retention, the HILIC separation mode is more complicated than RPLC. The critical interactions in HILIC include partitioning between the water-rich adsorbed layer on the stationary phase's surface and the water-deficient mobile phase and adsorption. When exposed to distinct

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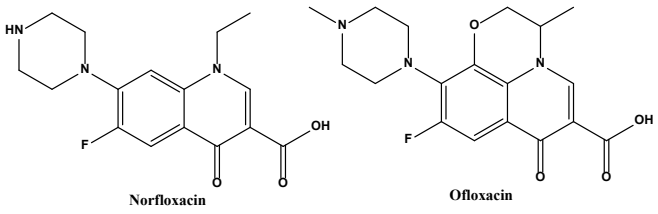


**Figure 1:** Chemical structure of norfloxacin and ofloxacin.

stationary phases and chromatographic circumstances, the mobile phase composition, the analyte characteristics, and the functional groups of the stationary phases determine the specific retention mechanism. Typically, the interactions are investigated using chromatographic procedures under various chromatographic settings (mobile phase composition, pH, and salt concentration). This work studied the separation of NOR and OFL using ZIC-HILIC$_1$ and ZIC-HILIC$_4$ columns. The length of the chain in these columns varies between inner quaternary amines and outer sulfonic acids. ZIC-HILIC$_1$ has one methylene group, and ZIC-HILIC$_4$ contains four methylene groups. As a result, the second objective of our research was to determine the influence of chain length between charges (one methylene) and the methylene group ZIC-HILIC1 and (four methylene) groups ZIC-HILIC$_4$. Rasheed and his team have presented numerous studies employing the HILIC technique in various applications, and are mentioned in the following references.[11-21]

## EXPERIMENTAL

### Chemicals

Norfloxacin and ofloxacin were purchased from Sigma-Aldrich. Acetonitrile (HPLC-grade) was obtained from Fisher Scientific. Acetic acid and sodium acetate were purchased from Merck. All studies used purified water that had been purified using a Milli-Q system (Millipore, USA).

### Chromatographic System

The analysis was conducted utilizing a Merck Hitachi HPLC system, which included an HPLC apparatus attached to a UV detector (type L-4200) and a Merck Hitachi L-6200 Pump, an in-line degasser, and a 20 µL injection loop. Chromatographic data processing was carried out using N2000 workstations. A successful analysis requires careful selection of a stationary phase. This experiment used two columns with differing chain lengths (ZIC-HILIC$_1$ and ZIC-HILIC$_4$, 100 mm 4.6 mm ID).[22] These columns have a range of functional groupings, which results in a diversity of HILIC processes. A pH 740 (WTW) was used along with an ultrasonic bath.

### Mobile Phase Preparation

Eluents with various compositions were prepared and tested in the ZIC-HILIC operating mode. Eluents were prepared by dissolving pure chemicals in Millipore water. In ZIC-HILIC mode, eluent mixtures of acetate buffer and acetonitrile were utilized. ZIC-HILC eluents were created employing the organic modifier mode with a high-pressure gradient. The mobile phase should be degassed daily to safeguard the pump and maintain a reduced detector range.

### Chromatographic Conditions

The optimum mobile phase for the analysis of NOR and OFL was a mixture of acetonitrile and an aqueous solution of acetic acid and sodium acetate. The injection volume was 20 µL. The elution was carried out at a temperature of 25°C and a flow rate of 0.7 mL/min. The study of norfloxacin and ofloxacin was carried out in the ultraviolet region at 350 nm.

### Preparation of Standard Solutions

Standard solutions for norfloxacin and ofloxacin were prepared daily. To prepare the solutions, a precisely weighed quantity of NOR and OFL (10 mg) was dissolved in 100 mL of eluent, followed by preparing a stock solution of norfloxacin and ofloxacin (100 mg/mL). The solutions were filtered using a (0.45 µm) filter. All solutions were stored at a temperature (6°C) and in opaque bottles to avoid being affected by light.

## RESULTS AND DISCUSSIONS

### Selecting Columns

The HILIC technique was created to separate highly hydrophilic chemicals that retain poorly on RP columns. Numerous HILIC columns exist, and the functional groups on their supports can alter their separation effectiveness. Two different types of stationary phase packing materials (ZIC-HILIC$_1$ and ZIC-HILIC$_4$) were evaluated during the investigation. Such an approach aimed to choose the optimum analytical conditions to make the best separation performance feasible and conduct a comparative analysis. The influence of organic modifier content, pH, and ionic strength on the retention behavior of norfloxacin and ofloxacin in the mobile phases under inquiry was assessed to investigate the separation mechanism. The chromatograms for norfloxacin and ofloxacin are shown in Figure 2. Acetate buffer (pH 4.75) with 90% ACN and a buffer concentration of 40 mM (pH 4.75).

### Study Influence of the Organic Composition

Choosing a suitable solvent is important for drug separation and estimation since acetonitrile is typically preferred over methanol due to its low viscosity, which allows for increased mobile phase flow rates. Due to the non-proteolytic nature of acetonitrile (ACN), hydrogen bonds with the stationary phase functional group or analytes are difficult to form. We studied the effect of the mobile phase (ACN) composition on

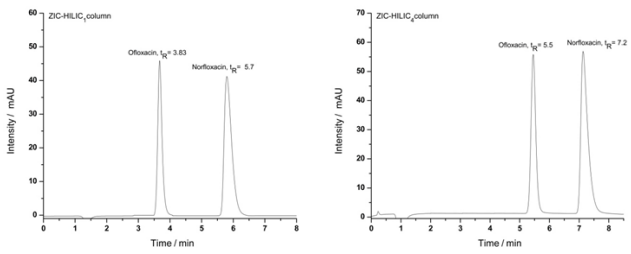


**Figure 2:** Chromatograms for norfloxacin and ofloxacin using ZIC-HILIC$_1$ and ZIC-HILIC$_4$ columns.

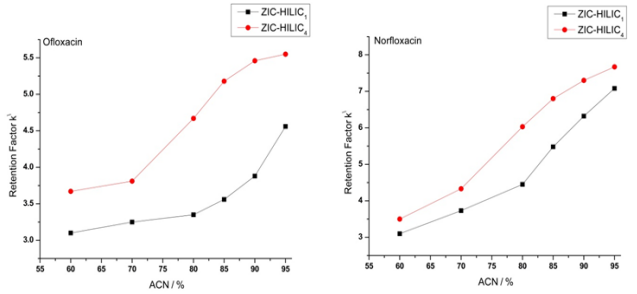


**Figure 3:** The influence of ACN % variation on NOR and OFL behavior using ZIC-HILIC$_1$ ZIC-HILIC$_4$ exchangers.

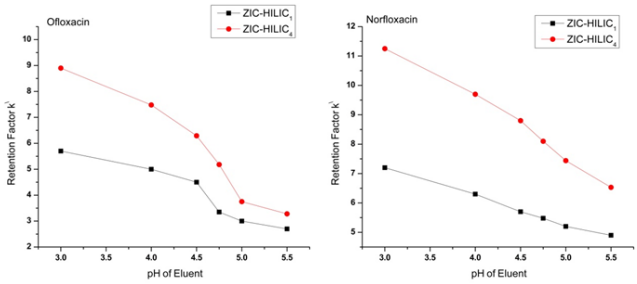


**Figure 4:** Effect of eluent pH of the buffer on NOR and OFL behavior using ZIC-HILIC$_1$ ZIC-HILIC$_4$ exchangers.

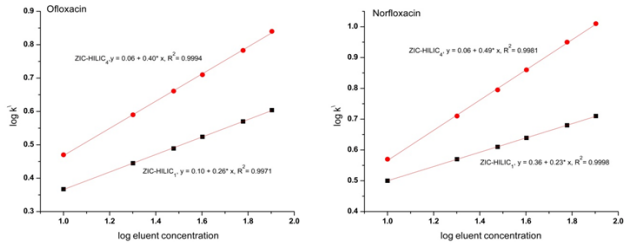


**Figure 5:** Effect of eluent concentration on NOR and OFL behavior using ZIC-HILIC$_1$ ZIC-HILIC$_4$ exchangers.

the analytical separation of norfloxacin and ofloxacin. The concentration of ACN enhanced the retention duration of norfloxacin and ofloxacin in buffer acetate (pH 4.75, 40 mM) as show in Figure 3. NOH and OFL responded similarly as a result of this effect. Another indication that norfloxacin and ofloxacin are hydrophobicity is their log $P_{OW}$ values of -1.6 and 0.09,[23] respectively. Accordingly, the prevailing mechanism of drug separation is HILIC.

### Study Influence of the pH Buffer

The pH of the mobile phase is a significant chromatographic factor because it can impact the charging state of both stationary and polar solutes. The effect of buffer pH value on the mobile phase was investigated using varied pH values of the acetate buffer (3.0–5.50) at 40 mM. As seen in Figure 4, increasing the buffer pH lowered the retention times of norfloxacin and ofloxacin. Norfloxacin and ofloxacin have isoelectric point values ranging from 7.32 to 6.61.[23] Therefore is because norfloxacin and ofloxacin are becoming more ionized. Therefore increased the electrostatic attraction between NOR and OFL and improved the stability of the zwitterionic sulfobetaine phase.

### Study the Influence of the Ionic Strength of the Buffer

Adjusting the buffer solution concentration is critical for developing a HILIC approach because, due to the HILIC mechanism's complexity, ionic strength significantly affects the chromatographic analysis. Retention results from electrostatic interactions (the functional groups of the stationary phase and the analytes have opposing charges). We investigated the influence of the eluent's NaOAc/HOAc buffer on the retention behavior of NOR and OFL at concentrations ranging from 10 to 80 mM (pH 4.75) in eluent containing 90% ACN. The results are summarized in Figure 5. Notably, increasing the concentration of NaOAc/HOAc buffer in the eluent enhances the retention factor of norfloxacin and ofloxacin for both (ZIC-HILIC$_1$ and ZIC-HILIC$_4$) columns. Therefore is due to the medicines' hydrophilicity and the ZIC-HILIC$_1$ and ZIC-HILIC$_4$ stationary phases.

## CONCLUSION

The benefits of HILIC on the separation of compounds with a wide variety of physicochemical characteristics are discussed in this paper. A novel norfloxacin and ofloxacin retention mechanism has been devised employing ZIC-HILIC columns. The basic retention mechanism in HILIC is usually based on substances trapped between the mobile phase and a water-rich stationary phase generated by multiple layers of varying compositions and mobilities between the stationary and mobile phases. Norfloxacin and ofloxacin concentrations were determined using HILIC. We explored two new zwitterionic sulfobetaine stationary phases with one and four methylene groups under HILIC conditions and discovered distinct retention characteristics for norfloxacin and ofloxacin. The geometric alignment of sulfobetaine groups and the difference in polarity account for the differential in retention behavior between these two columns.

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