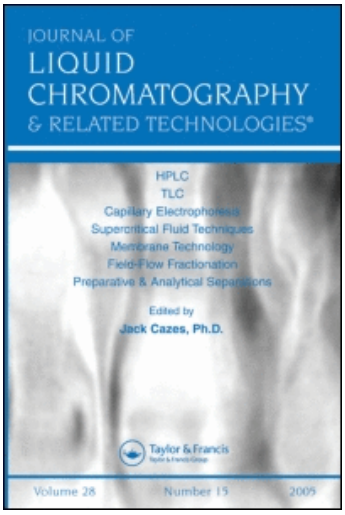


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# Influence of Perchlorate Ion on the Retention of Fluoroquinolones in RP-TLC

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# Influence of Perchlorate Ion on the Retention of Fluoroquinolones in RP-TLC

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**Abstract:** Five amphoteric piperazynyl fluoroquinolones, which are bases in acidic conditions, and flumequine, which is neutral at a low pH, were analyzed in an RP system on RP-18 W and $SiO_2$-CN plates with acidic mobile phases containing potassium perchlorate. Various retention mechanisms were taken into account, i.e., adsorption and ion-pair interaction on RP-18 W phases and chaotropic effect on cyano-silica. $R_M$ values for individual fluoroquinolones were calculated and compared for chromatographic systems with or without chaotropic ion.

**Keywords:** Chaotropic ion, Fluoroquinolones, RP-TLC

## INTRODUCTION

Ion chromatography is a form of liquid chromatography where retention is predominantly controlled by ionic interactions between solute ions and counter ions.[1,2] The chaotropic effect is mainly known in biochemistry as the process of disruption of the solvation shell of proteins and peptides in aqueous solutions.[3] Chaotropic ions are small inorganic compounds causing disruption of the water structure. They are arranged in the Hofmeiser series according to their ability to cause "chaos" of the water structure.[4] Perchlorate anion has a high position in this series due to its

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high chaotropic activity. As opposed to surfactants, it shows weak interactions with alkyl chains of bonded phase but influences interaction of basic compounds with alkyl chains, increasing their retention.[5]

The fluoroquinolones are popular chemotherapeutics used worldwide in human and veterinary treatments because of their effective pharmacokinetic properties, broad antibacterial spectrum, and good tolerability. Most of them belong to the so-called 7-piperazinylfluoroquinolones, which possess both the acidic carboxylic group, as well as the basic, amine group of piperazinyl substitution.[6] They are bases in acidic conditions and their retention in RP chromatography may be controlled by chaotropic ions, such as perchlorate. Ionic compounds are solvated in aqueous mobile phases. Chaotropic ions disrupt the solvation shell of basic analytes leading to an increase of their retention.[5,7–10]

In this paper, the influence of chaotropic perchlorate ion addition on retention of six fluoroquinolones on various sorbents with chemically bonded stationary phases were investigated.



## EXPERIMENTAL

### Equipment and Reagents

DS sandwich chambers[11] were purchased from Chromdes, Lublin, Poland. Precoated HPTLC plates RP-18 W $F_{254}$ 10 cm × 20 cm and CN $F_{254}$ 10 cm × 10 cm were purchased from E. Merck, Darmstadt, Germany. Acetonitrile was obtained from Merck, while citric acid and potassium perchlorate were from P.O.Ch. Gliwice, Poland. Fluoroquinolones were supplied by Sigma (St. Louis, MO, U.S.A).

### Mobile Phases

The following mobile phases were used:

A. acetonitrile–0.1 M citric acid–40 mM aqueous solution of $KClO_4$ (60:20:20)
B. acetonitrile–0.1 M citric acid (60:40)
C. acetonitrile–0.1 M citric acid–40 mM aqueous solution of $KClO_4$ (50:17:33)
D. acetonitrile–0.1 M citric acid–40 mM aqueous solution of $KClO_4$ (50:33:17)
E. acetonitrile–0.1 M citric acid (50:50)
F. acetonitrile–0.1 M citric acid (40:60)

G. acetonitrile–0.1 M citric acid–40 mM aqueous solution of $KClO_4$ (40:20:40)
H. acetonitrile–0.1 M citric acid–40 mM aqueous solution of $KClO_4$ (40:40:20)
I. acetonitrile–0.1 M citric acid–40 mM aqueous solution of $KClO_4$ (33:50:17)
J. acetonitrile–0.1 M citric acid (33:67)
K. acetonitrile–0.1 M citric acid (20:80)
L. acetonitrile–0.1 M citric acid–40 mM aqueous solution of $KClO_4$ (20:40:40)

## Methods

One mg portions of each of the fluoroquinolones were dissolved in 1 mL of 0.03 M NaOH, and then diluted ten times with methanol to produce 0.1 mg $mL^{-1}$ standards. The standards of fluoroquinolones were applied onto the TLC plates in 1 μL volumes using a Hamilton microsyringe (Bonaduz, Switzerland). After air-drying, fluoroquinolone spots were detected at 366 or 254 nm and flumequine only at 254 nm by UV lamp with dual-wavelength (HA-05 Haland, Warsaw, Poland).



## RESULTS AND DISCUSSION

Five 7-piperazinylflouroquinolones, i.e., sarafloxacin (1), difloxacin (2), norfloxacin (3), enrofloxacin (4), ciprofloxacin (6) and the so called acidic one–flumequine (5) were taken under consideration. The structures of the drugs are presented in Figure 1. As seen, flumequine is the only one of the chosen drugs which doesn't possess the piperazinyl group and, as a consequence, is neutral in acidic conditions ($pK_a$ is equal 6.3), in contrast to the rest of the tested compounds which are basic in low pH ($pK_{a1}$ varies from 5.5 to 6.6).[6]

Standard solutions of fluoroquinolones were applied to the TLC plates and the plates were developed with one of the phases listed in the Experimental section. All the chromatograms were obtained in triplicate. Mean $R_M$ values are presented in Table 1 for RP-18 W sorbent, and in Table 2 for Si-CN sorbent.

Figures 2–5 present dependencies between $R_M$ values for subsequent fluoroquinolones obtained for pairs of mobile phases of similar composition, differing one from another only in the presence of perchlorate ion. As mentioned earlier, all fluoroquinolones analyzed, except flumequine, are bases in acidic conditions. Hence, perchlorate chaotropic ion should

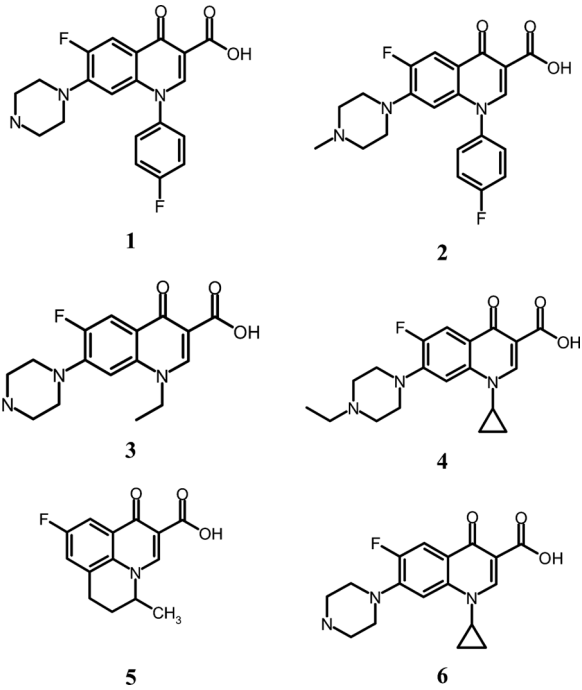


***Figure 1.*** The structures of the fluoroquinolones analyzed.



increase their retention. However, dependencies presented in the figures pointed to the opposite relationship. All phases containing perchlorate anion gave lower retentions than those without perchlorate. This phenomenon is better illustrated by $R_M$ vs. % acetonitrile relationship

***Table 1.*** Mean $R_M$ values for fluoroquinolones obtained for RP-18 W plates and phases A–J (n = 3)

| Mobile phase | Sarafloxacin 1 | Difloxacin 2 | Norfloxacin 3 | Enrofloxacin 4 | Flumequine 5 | Ciprofloxacin 6 |
|---|---|---|---|---|---|---|
| A | −0,34 | −0,18 | −0,4 | −0,17 | −0,04 | −0,4 |
| B | 0,62 | 0,8 | 0,73 | 0,75 | 0,05 | 0,46 |
| C | −0,16 | −0,04 | −0,2 | −0,04 | 0,1 | −0,21 |
| D | −0,26 | −0,14 | −0,34 | −0,13 | −0,01 | −0,33 |
| E | 0,81 | 0,85 | 0,69 | 0,85 | 0,1 | 0,73 |
| F | 0,61 | 0,65 | 0,48 | 0,73 | 0,42 | 0,54 |
| G | −0,05 | 0,02 | −0,19 | 0,01 | 0,18 | −0,16 |
| H | 0,12 | 0,23 | −0,03 | 0,12 | 0,15 | −0,01 |
| I | 0,46 | 0,54 | 0,27 | 0,49 | 0,64 | 0,29 |
| J | 0,75 | 0,83 | 0,45 | 0,70 | 0,67 | 0,52 |

***Table 2.*** Mean $R_M$ values for fluoroquinolones obtained for $SiO_2$–CN plates and phases K and L (n = 3)

| Mobile phase | Sarafloxacin 1 | Difloxacin 2 | Norfloxacin 3 | Enrofloxacin 4 | Flumequine 5 | Ciprofloxacin 6 |
|---|---|---|---|---|---|---|
| K | 0,82 | 0,92 | 0,43 | 0,67 | 1,06 | 0,46 |
| L | 1,12 | 1,19 | 0,69 | 0,87 | 0,87 | 0,73 |

obtained for separated fluoroquinolones (Figs. 6–10). As seen, for all phases without perchlorate, retention of basic fluoroquinolones does not depend on the acetonitrile content. Probably, this is caused by coexistence of two different mechanisms of sorption on C-18 wettable phases: hydrophobic, typical in RP chromatography, interactions with alkyl chains, rather weak because of the ionic character of the analytes ($R_M$ decreases with acetonitrile content) and adsorption of fluoroquinolone cations on acidic hydroxylic groups on the surface of sorbent ($R_M$ increases with acetonitrile content). It can be supposed that, in the phases containing perchlorate anion and in acidic conditions, cationic fluoroquinolones form ion pairs with perchlorate. These neutral associates can interact hydrofobically with alkyl chains what leads to a typical reaction in RP chromatography, i.e., a drop in retention with increase of acetonitrile content in the mobile phase. This behavior does not concern flumequine which, in acidic conditions, is neutral and does not associate with



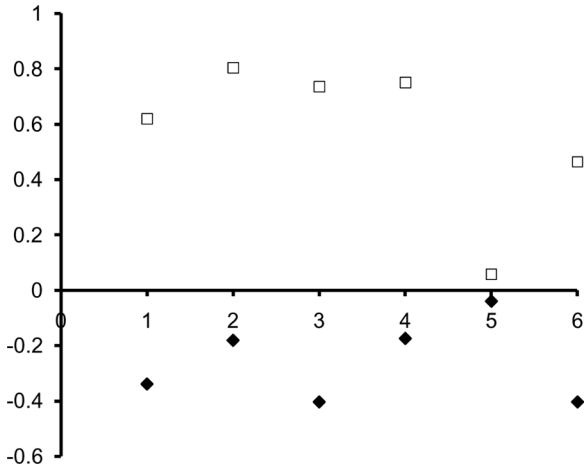


***Figure 2.*** $R_M$ values for fluoroquinolones 1–6 and the phase A (full rhombus) and B (empty square). RP-18 W plates.

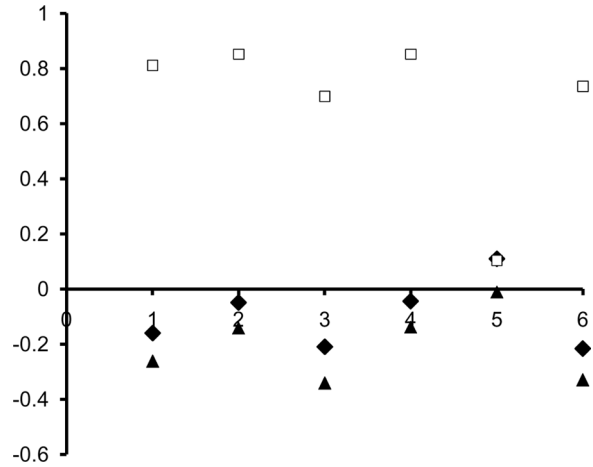


***Figure 3.*** $R_M$ values for fluoroquinolones 1–6 and the phase C (full rhombus), D (full triangle) and E (empty square). RP-18 W plates.



perchlorate ion. Then, independently of the presence of perchlorate in the mobile phase, flumequine interacts with alkyl chains in the hydrophobic mode only. Thus, for all phases used, retention of flumequine decreases with the acetonitrile content (Fig. 11).

Table 2 and Fig. 12 present $R_M$ values obtained for phases K and L on cyanopropyl silica for the analyzed fluoroquinolones. $SiO_2$-CN

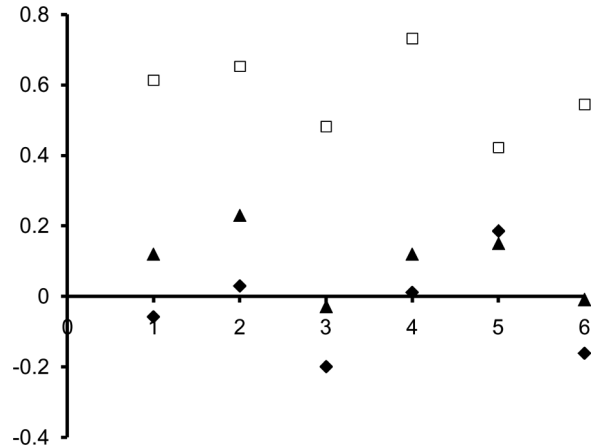


***Figure 4.*** $R_M$ values for fluoroquinolones 1–6 and the phase G (full rhombus), H (full triangle) and F (empty square). RP-18 W plates.

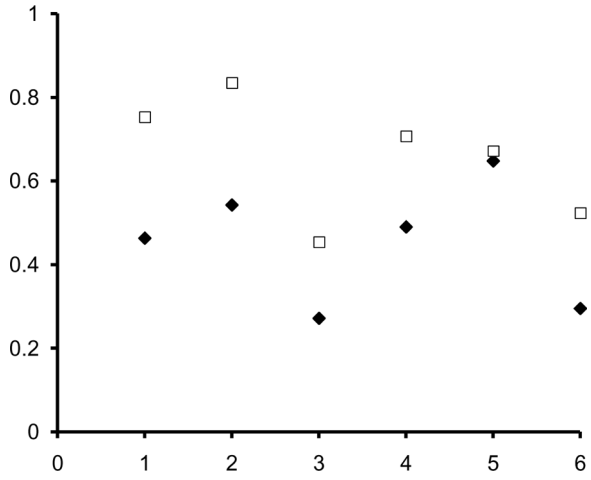


*Figure 5.* $R_M$ values for fluoroquinolones 1–6 and the phase I (full rhombus) and J (empty square). RP-18 W plates.



belongs to the so-called polar bonded phases. In spite of this, it can interact in a typically hydrophobic way when it is used with polar, aqueous mobile phases. This mechanism of retention is expected for phases K

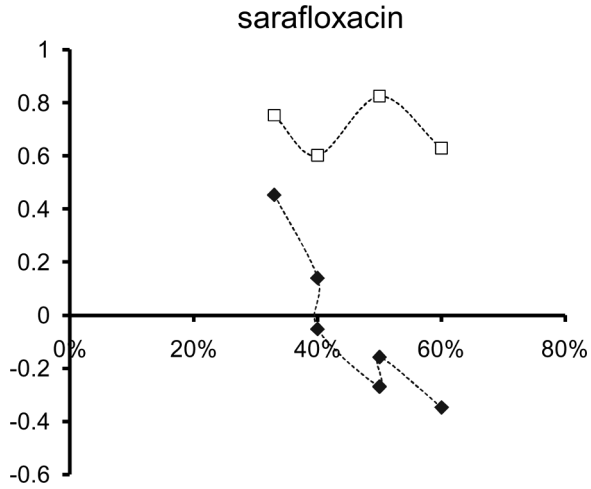


*Figure 6.* $R_M$ values of sarafloxacin for various mobile phases containing increasing concentrations of acetonitrile. The phases with an addition of $ClO_4^-$: I, H, G, D, C, A (full rhombus) and without an addition of $ClO_4^-$: J, F, E, B (empty square). RP-18 W plates.

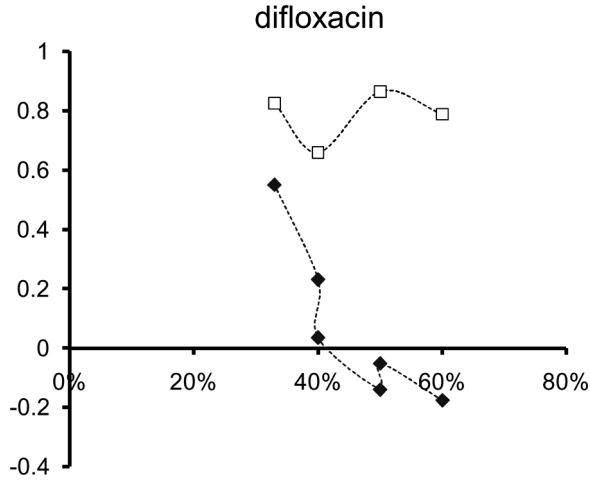


***Figure 7.*** $R_M$ values of difloxacin for various mobile phases containing increasing concentration of acetonitrile. The phases with an addition of $ClO_4^-$: I, H, G, D, C, A (full rhombus) and without an addition of $ClO_4^-$: J, F, E, B (empty square). RP-18 W plates.



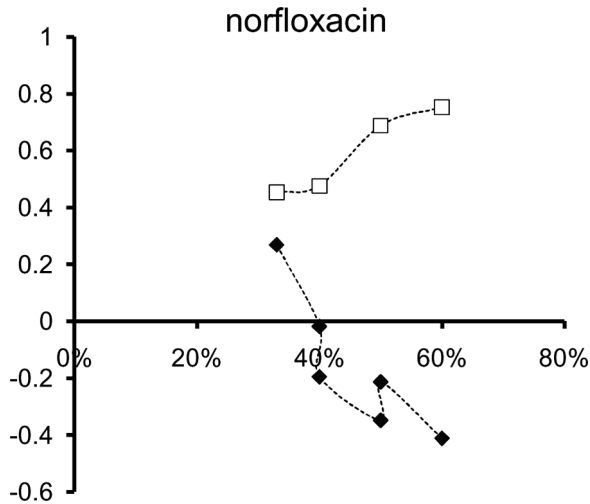


***Figure 8.*** $R_M$ values of norfloxacin for various mobile phases containing increasing concentrations of acetonitrile. The phases with an addition of $ClO_4^-$: I, H, G, D, C, A (full rhombus) and without an addition of $ClO_4^-$: J, F, E, B (empty square). RP-18 W plates.

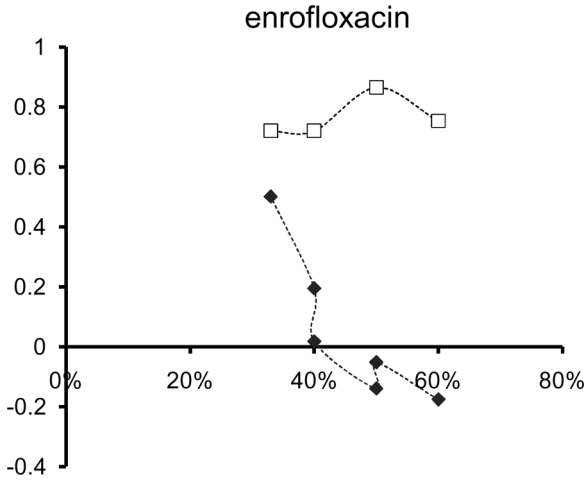


***Figure 9.*** $R_M$ values of enrofloxacin for various mobile phases containing increasing concentrations of acetonitrile. The phases with an addition of $ClO_4^-$: I, H, G, D, C, A (full rhombus) and without an addition of $ClO_4^-$: J, F, E, B (empty square). RP-18 W plates.



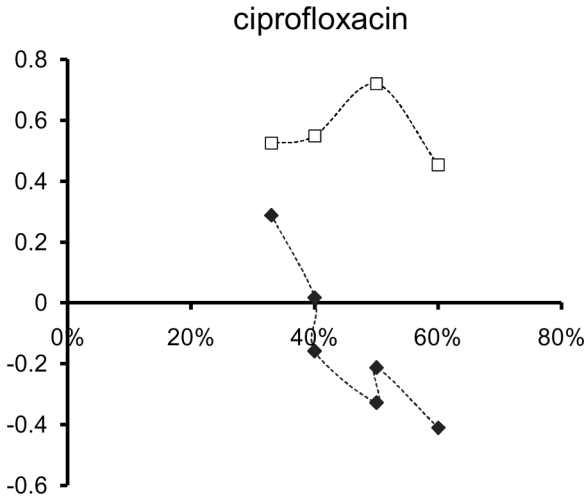


***Figure 10.*** $R_M$ values of ciprofloxacin for various mobile phases containing increasing concentrations of acetonitrile. The phases with an addition of $ClO_4^-$: I, H, G, D, C, A (full rhombus) and without an addition of $ClO_4^-$: J, F, E, B (empty square). RP-18 W plates.

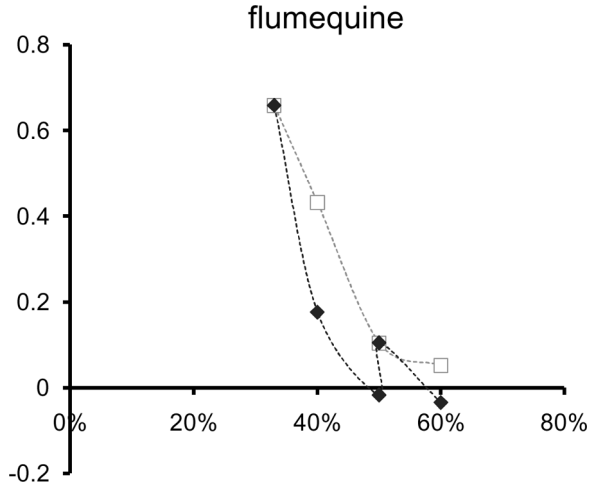


***Figure 11.*** $R_M$ values of flumequine for various mobile phases containing increasing concentrations of acetonitrile. The phases with an addition of $ClO_4^-$: I, H, G, D, C, A (full rhombus) and without an addition of $ClO_4^-$: J, F, E, B (empty square). RP-18 W plates



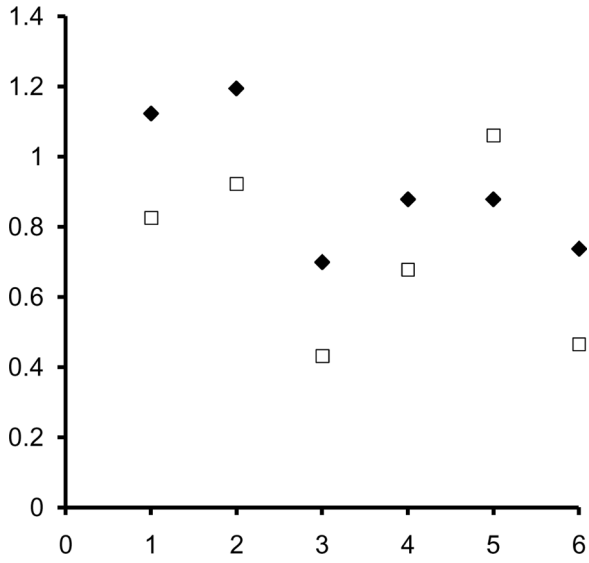


***Figure 12.*** $R_M$ values for fluoroquinolones 1–6 and the phase L (full rhombus) and K (empty square) obtained for cyanopropyl sorbent.

and L. As seen from both Table 2 and Fig. 12, the addition of perchlorate anion causes higher retention of basic fluoroquinolones, probably due to the chaotropic effect. This effect does not concern neutral at low pH flumequine.

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