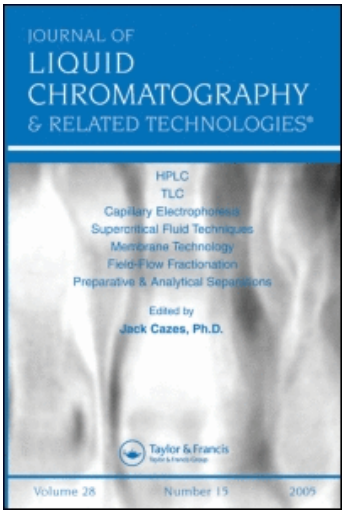

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THE INFLUENCE OF PERCHLORATE ION CONCENTRATION ON THE RETENTION OF FLUOROQUINOLONES IN RP-TLC

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□ *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 C_8 plates with acetonitrile/aqueous acidic mobile phases containing various concentrations of potassium perchlorate. Perchlorate, which is so-called chaotropic ion, i.e., making chaos in solvation shell of analytes, caused the increase in retention of basic fluoroquinolones. The retention increased with the increasing concentration of perchlorate ion in the mobile phase to achieve plateau for the mobile phases containing about 10–20 mM of chaotropic perchlorate.*

Keywords chaotropic ion, fluoroquinolones, RP-TLC

INTRODUCTION

The fluoroquinolones are popular chemotherapeutics used both in medicine and veterinary. Most of them belong to the so-called 7-piperazinylfluoroquinolones, which possess the acidic carboxylic group as well as the basic, amine group of piperazinyl substitution.[1] Ionic or ionizable compounds, like fluoroquinolones, can be separated by ion-pair chromatography. The mechanism of retention in this type of chromatography is based on formation of neutral ion-pairs between ionic solutes and lipophilic long chain counterions, which are additives to the mobile phase. Another model of ion-pair chromatography assumes that lipophilic ions first adsorb at the stationary phase forming exchange sites for an analyte. Despite the model, ion-pair chromatography leads to the growth of ions retention on nonpolar stationary phases.[2,3] Traditional long chain counterions strongly stick to the alkyl chains of stationary phase changing properties of the column. Small inorganic ions are an interesting

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alternative to long-chain ones.[4] These ions, called "chaotropic," are arranged in the Hofmeiser scale due to their ability to disrupt a water solvation shell which is connected with their polarizability, charge delocalization, and symmetry[4,5] (increasing from left to right):

$$H_2PO_4^- \langle CF_3COO^- \langle BF_4^- \langle ClO_4^- \langle PF_6^-$$

According to Kazakevich and LoBrutto theory.[4,6,7] chaotropic anions disrupt hydrogen bridges in the water shell surrounding the solute; in this way, increasing hydrophobicity of the solute. The influence of chaotropic anions on retention of cationic solutes can be also interpreted by ion-pair or dynamic ion-exchange mechanism.[8–12]

Probably the mechanism of retention is complex and related to contribution of all above mentioned models. The first attempt to explain the role of chaotropic reagents in RP-HPLC at a thermodynamic level was made by Cecchi and Passamonti.[13] Besides theoretical papers, there are a lot of examples of practical applications of the chaotropic effect in separation of various classes of basic compounds.[14–18] The majority of publications dealing with chaotropic effect concerns RP-HPLC, while there are only several examples on RP-TLC.[17,18]

Piperazinyl fluoroquinolones are bases in acidic conditions and their retention in RP chromatography may be controlled by inorganic ions, such as perchlorate, one of the strongest chaotropic ions. As can be predicted and was previously proved experimentally,[17] the chaotropic perchlorate does not change retention of flumequine, which is neutral in acidic conditions.

In this paper, the influence of chaotropic perchlorate ion concentration on retention of six fluoroquinolones on siliceous sorbents with chemically bonded C-8 phases was investigated.

EXPERIMENTAL

Equipment and Reagents

DS sandwich chambers[19] were purchased from Chromdes, Lublin, Poland. Precoated TLC plates RP-8 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 – water (40:20:40)

B. acetonitrile – 0.1 M citric acid – 0.5 mM aqueous solution of $KClO_4$ (40:20:40)
C. acetonitrile – 0.1 M citric acid – 1 mM aqueous solution of $KClO_4$ (40:20:40)
D. acetonitrile – 0.1 M citric acid – 5 mM aqueous solution of $KClO_4$ (40:20:40)
E. acetonitrile – 0.1 M citric acid – 10 mM aqueous solution of $KClO_4$ (40:20:40)
F. acetonitrile – 0.1 M citric acid – 20 mM aqueous solution of $KClO_4$ (40:20:40)
G. acetonitrile – 0.1 M citric acid – 30 mM aqueous solution of $KClO_4$ (40:20:40)
H. acetonitrile – 0.1 M citric acid – 40 mM aqueous solution of $KClO_4$ (40:20:40)

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 on 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 zwiterrionic 7-piperazinylflouroquinolones, i.e., sarafloxacin (S), difloxacin (D), norfloxacin (N), enrofloxacin (E), ciprofloxacin (C) and the so called acidic one – flumequine (F) were analyzed in RP TLC systems using ACN/water mobile phases containing constant concentration of citric acid but various concentrations of chaotropic salt, i.e., potassium perchlorate. The structures of the drugs are presented in Figure 1. As seen, flumequine is the only one of the chosen compounds which doesn't possess the piperazinyl group and, as a consequence, is neutral in acidic conditions (pK_a is equal 6.3), contrary to the rest of the tested fluoroquinolones, which are basic in low pH (pK_{a1} varies from 5.5 to 6.6).[1] In our previous paper, the influence of chaotropic perchlorate ion addition on retention of the same six fluoroquinolones on various sorbents with chemically bonded stationary phases was investigated.[17] It was proved that the influence of a chaotropic effect on retention of fluoroquinolones can be observed for cyanopropyl silica and polar mobile phases. C_{18} plates had to be excluded

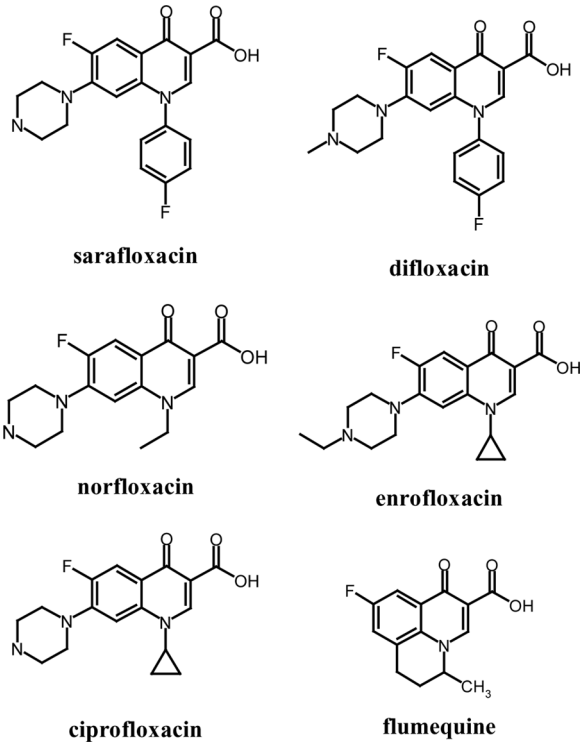

FIGURE 1 The structures of the fluoroquinolones analyzed.

from the experiments because of very poor wettability, while retention on wettable C_{18} sorbents seemed to originate from both adsorption and hydrophobic interactions, which were influenced by chaotropic effects. In the present paper, we examine retention of the fluoroquinolones on C_8 TLC plates as a function of perchlorate anion concentration.

Standard solutions of fluoroquinolones were applied onto C_8 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(log k) and k values are presented in Tables 1 and 2, respectively. Figures 2–6 present dependencies, obtained for individual fluoroquinolones, between R_m or k values and the concentration of perchlorate anion in the mobile phases of similar composition, differing one from another only in the concentration of chaotropic ion. As seen, retention is growing with the increasing concentration of perchlorate anion. The retention of flumequine, which is neutral in acidic condition, is independent from perchlorate anion concentration (Fig. 7). For comparison, Fig. 8 presents dependencies of mean R_m values vs. ClO_4^- concentration obtained for all the analyzed drugs. As was pointed earlier and shown in Fig. 7, retention

TABLE 1 Mean R_m Values for the Fluoroquinolones and Various Mobile Phases (n = 3)

	R_m					
Mobile Phase	S	D	N	E	C	F
A	−0.097	−0.048	−0.174	−0.139	−0.167	0.540
B	−0.056	−0.021	−0.128	−0.093	−0.143	0.572
C	0.046	0.049	−0.106	−0.042	−0.081	0.644
D	0.136	0.165	−0.053	0.028	−0.065	0.634
E	0.184	0.223	−0.059	0.069	−0.056	0.630
F	0.193	0.288	−0.007	0.130	−0.018	0.612
G	0.174	0.253	−0.016	0.127	−0.027	0.561
H	0.338	0.431	0.043	0.232	0.037	0.578

of flumequine does not depend on concentration of chaotropic anion. In acidic conditions, this compound is neutral, so the chaotropic effect does not influence its retention. Besides, the retention of flumequine is essentially higher than those of other drugs from the same family. All piperazynyl fluoroquinolones are protonated in acidic mobile phase and their retentions are very low in the absence of chaotropic agent. An addition of perchlorate to the mobile phase causes disruption of the solvation shell or/and formation of neutral ion-pairs which can interact much stronger with the alkyl chains of the stationary phase. The plots obtained for sarafloxacin, difloxacin, norfloxacin, enrofloxacin, and ciprofloxacin are very similar. The increase of R_m values is observed with the increase of perchlorate concentration up to a plateau at about 10–20 mM of perchlorate. Additionally, the position of plots related to the individual fluoroquinolones is connected with the hydrophobic character of the molecules. The least polar, difloxacin, interacts strongest with C_8 chains, while ciprofloxacin and norfloxacin, the most polar from the analyzed group, show the weakest interactions. Because of their similar structures, their plots are overlapped.

TABLE 2 Mean k Values for the Fluoroquinolones and Various Mobile Phases (n = 3)

	k					
Mobile Phase	S	D	N	E	C	F
A	0.811	0.900	0.677	0.733	0.686	3.492
B	0.884	0.954	0.748	0.811	0.720	3.745
C	1.114	1.120	0.784	0.908	0.836	4.521
D	1.368	1.462	0.888	1.075	0.866	4.311
E	1.558	1.679	0.878	1.180	0.889	4.289
F	1.561	1.942	0.984	1.355	0.961	4.099
G	1.493	1.792	0.964	1.339	0.940	3.637
H	2.199	2.754	1.119	1.728	1.101	3.807

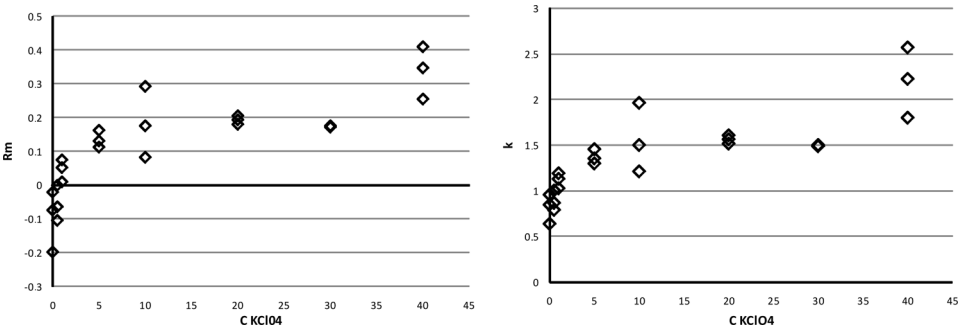

FIGURE 2 R_m and k values for sarafloxacin at various concentrations of chaotropic anion in the mobile phase.

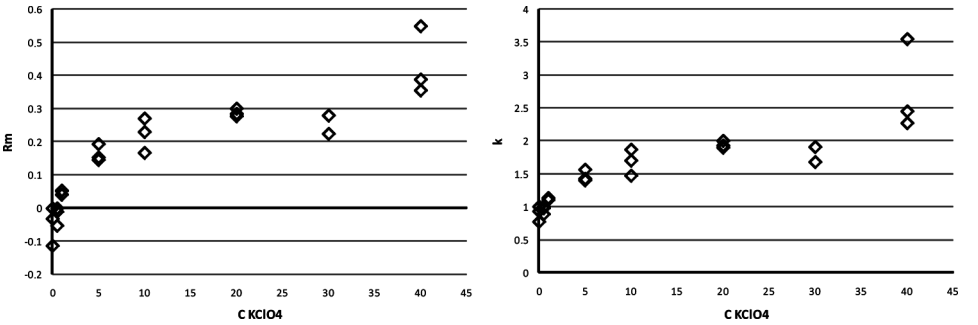

FIGURE 3 R_m and k values for difloxacin at various concentrations of chaotropic anion in the mobile phase.

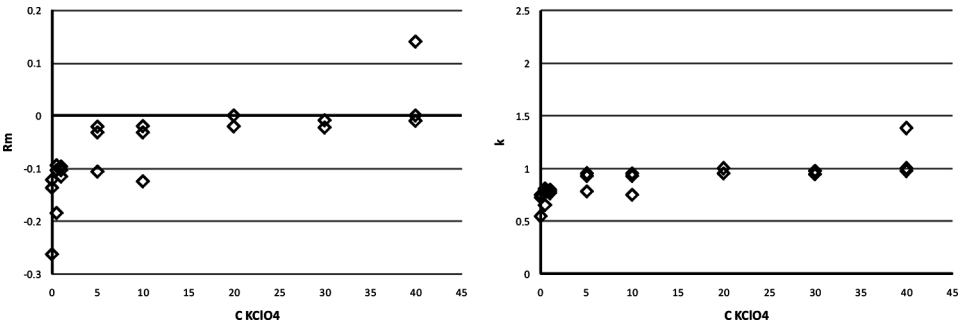

FIGURE 4 R_m and k values for norfloxacin at various concentrations of chaotropic anion in the mobile phase.

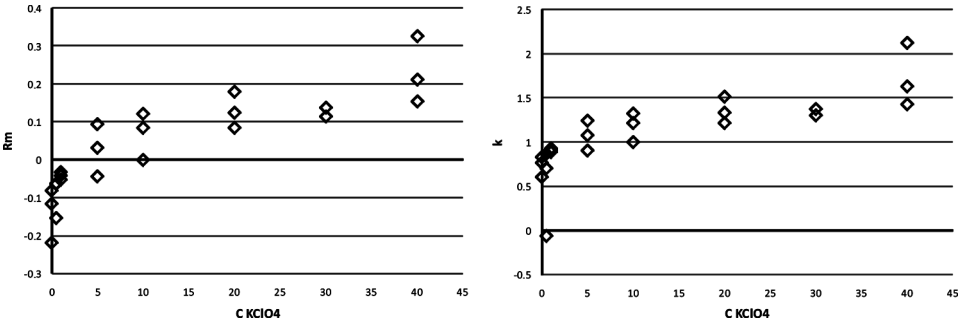

FIGURE 5 R_m and k values for enrofloxacin at various concentrations of chaotropic anion in the mobile phase.

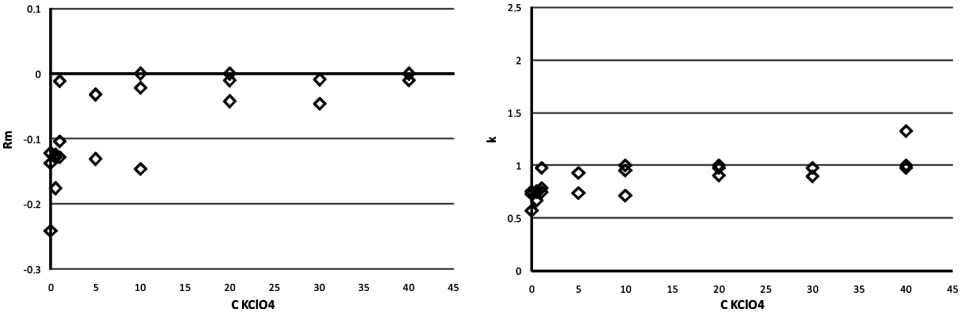

FIGURE 6 R_m and k values for ciprofloxacin at various concentrations of chaotropic anion in the mobile phase.

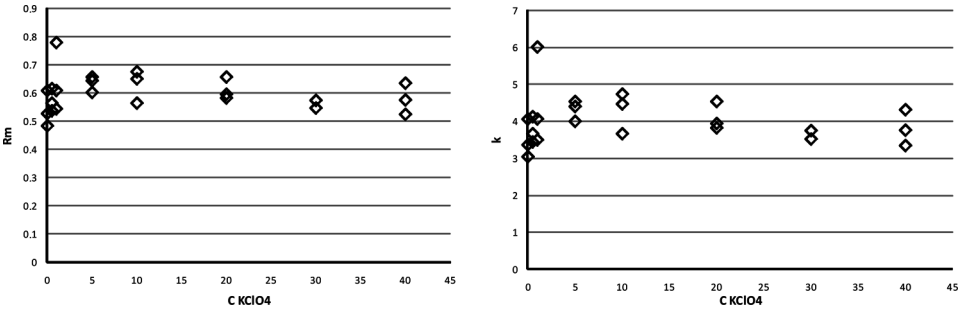

FIGURE 7 R_m and k values for flumequine at various concentrations of chaotropic anion in the mobile phase.

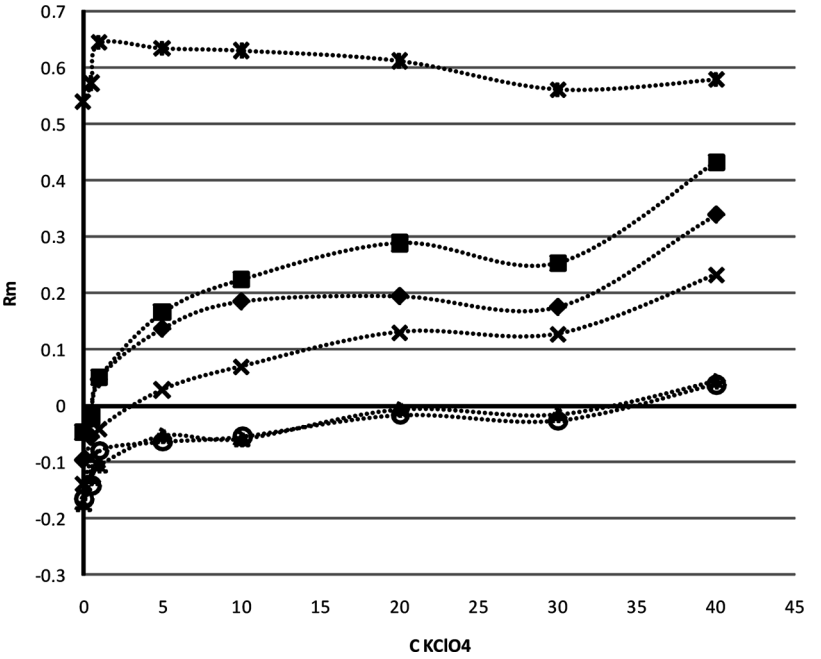

FIGURE 8 Mean R_m values for fluoroquinolones at various concentrations of chaotropic anion in the mobile phase: flumequine-asterisk, difloxacin-square, sarafloxacin-rhombus, enrofloxacin-cross, ciprofloxacin-circle, norfloxacin-triangle.

CONCLUSIONS

Perchlorate ion can be used to control retention of basic fluoroquinolones in RP chromatography, both HPLC and TLC. The higher concentration of a chaotropic ion, the stronger retention of basic compounds. The influence of chaotropic ion on retention is observed up to the concentration which allows for complete disruption of water solvation shell of the analyte.

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