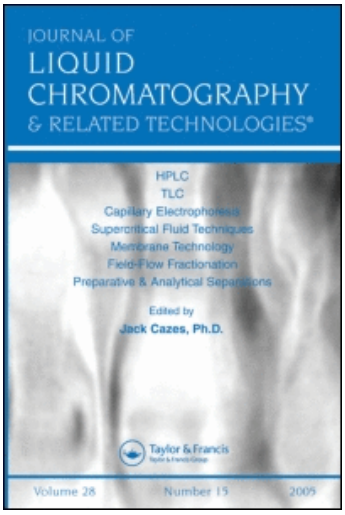

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Linear Solvation Energy Relationships in Reversed-Phase Liquid Chromatography. Prediction of Retention of Several Quinolones

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LINEAR SOLVATION ENERGY RELATIONSHIPS IN REVERSED-PHASE LIQUID CHROMATOGRAPHY. PREDICTION OF RETENTION OF SEVERAL QUINOLONES

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ABSTRACT

In this study the proportion of the organic modifier and the pH of the hydroorganic mobile phase were optimized in order to separate four important and widely used quinolones: ciprofloxacin, norfloxacin, ofloxacin and pipemidic acid. The Linear Solvation Energy Relationships (LSER) method based on the multiparameter scale developed by Kamlet and Taft can be succesfully used to select the right composition of the eluent and it has been shown that plots of log k' *versus* the Reichardt's E_T^N parameter of the mobile phase are linearly correlated for the solutes studied. Moreover, pH measurements in acetonitrile-water mixtures used as mobile phases have been made, taking into account the reference pH values previously established, in order to optimize the pH of the mobile phase for the chromatographic separation of the four quinolones studied.

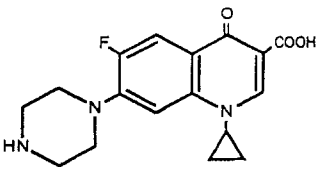
INTRODUCTION

Quinolones comprise a widely used group of antibiotics whose bacterial action is based on their anti-DNA gyrase activity [1]. Owing to their favourable

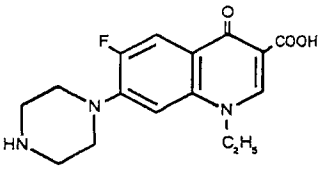
antibacterial and pharmacokinetic profile, quinolones have been used for treatment of systemic infections as well as urinary tract infections. The wide use of quinolones may lead to patients receiving multiple antimicrobials and microbiologically active metabolites of these agents may sometimes be present in biological fluids. The aim of the present study is to select the optimum eluent in order to separate four important and widely used quinolones: ciprofloxacin, norfloxacin, ofloxacin and pipemidic acid, Figure 1 [2-5]. For this purpose the proportion of the organic modifier and the pH of the hydroorganic mobile phase were optimized.

Although the structure and composition of the stationary phase plays an active role in the separation process [6] most researchers have focused attention on mobile phase optimization and with good reason since this is the easiest way to control retention and selectivity in RPLC [7]. Recently, the E_T^N scale of mobile phase polarity, proposed by Dimroth and Reichardt [8], and the multiparameter scale, developed by Kamlet and Taft [9,10], were succesfully used to study retention in RPLC. Dorsey [11-13] has shown that plots of logarithm of capacity factor, log k', *versus* the mobile phases' E_T^N solvatochromic parameter are very often more linear than plots of log k' *versus* volume fraction of organic modifier. Therefore, suitable prediction of the retention for a specific solute can be achieved from the E_T^N solvatochromic parameter values of the mobile phase and a few experimental data. However, Cheong and Carr [14] concluded that good correlations between retention and this single solvent parameter can be obtained only over a narrow range of solvent composition.

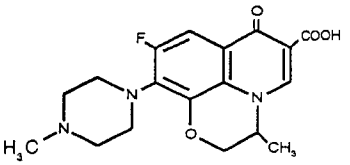
The method of Linear Solvation Energy Relationships (LSER) based on the Kamlet-Taft multiparameter scale has also been used succesfully to study retention in RPLC [15-17]. The LSER approach, when applied to chromatographic processes, correlates retention parameters of solutes to characteristic properties of mobile phases measured by the solvatochromic parameters π^*, α and β. The π^* parameter is used to evaluate solvent dipolarity/polarizability [18], and α and β scales evaluate solvent hydrogen-bond acidity [19] and solvent hydrogen-bond basicity [20] respectively.



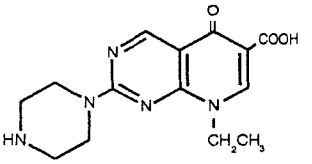
Ciprofloxacin



Norfloxacin



Ofloxacin



Pipemidic acid

FIGURE 1. Structural formula of the quinolones studied.

These approaches only allow the prediction of retention at different mobile phase compositions, but provide no information about the pH of the mobile phase, which is also critical for optimizing selectivity in RPLC. Usually, the operational pH in mixed aqueous-organic solvents is measured assuming that the mobile phase pH is the same as that of the aqueous fraction, in which case errors due to the medium effects contribute to uncertainty as to the true pH [21]. In acetonitrile-water mixtures the influence of the co-solvent on the pH is remarkable [22-24], but from the point of view of practical chromatography, it is possible to measure the activity of the hydronium ion in acetonitrile-water mixtures taking into account the reference pH values of buffer solutions in these solvents, pH_{PS}, assigned in previous works [25-29], which are used for the standardization of potentiometric sensors. Thus, pH measurements in a mixed solvent can be performed as easily as in water, using the operational definition of pH [30-32]:

$$pH_X = pH_{PS} + \frac{E_{PS} - E_X}{g} \quad (1)$$

where E_X and E_{PS} denote the electromotive force (emf) measurements in cell A on the sample solution at unknown pH_X and on the standard reference solution at known pH_{PS} respectively, and $g=(\ln 10)RT/F$.

reference electrode	salt bridge	sample solution at pH_X or standard buffer solution at pH_{PS} in solvent s	H^+ sensing electrode	(A)

In this study the proportion of the organic modifier and the pH of the hydroorganic mobile phase were optimized in order to separate four important and widely used quinolones: ciprofloxacin, norfloxacin, ofloxacin and pipemidic acid. The LSER method, based either on the multiparameter π^*, α and β scale or the single solvent parameter E_T^N and their relationships with log k', have been applied to the optimization of the mobile phase composition and to the prediction of the chromatographic behaviour of the quinolones studied. Moreover, the pH measurements in the acetonitrile-water mixtures used as mobile phases and their correlation with k' have been used in the optimization of the mobile phase pH for the separation required.

EXPERIMENTAL

Apparatus

The chromatographic equipment used consisted of an ISCO Model 2350 pump with an injection valve with a 10 μl sample loop and a variable-wavelength V^4 absorbance detector (ISCO) operating at 280 nm or at 295 nm for ofloxacin. The

chromatographic system was controlled by Chemresearch Chromatographic Data Management System Controller software (ISCO) running on a Peceman AT Supermicro personal computer. A Merck LiChrospher 100 RP-18 (5μm) column, 250 x 4 mm I.D., was used at ambient temperature. The emf values used to evaluate the pH of the mobile phase were measured with a CRISON 2002 potentiometer ($\pm$0.1 mV) using an Orion 8102 ROSS combination pH electrode. All solutions were thermostatted externally at 25$\pm$0.1° C. The electrodes were stabilized in the appropriate acetonitrile-water mixtures previous to the emf measurements, and the measurements were performed in triplicate to ensure potentiometric system stability.

Reagents

All reagents were of analytical grade. Acetonitrile (Merck) and water were of HPLC grade. The eluents were passed through a 0.22 μm nylon filter (MSI) and degassed ultrasonically before use. The quinolones (Figure 1) were obtained from various pharmaceutical firms: ciprofloxacin (Lasa), norfloxacin (Boral Química), ofloxacin (Hoescht Ibérica) and pipemidic acid (Almirall and Prodesfarma). Stock solutions of the quinolones were prepared in acetonitrile-water mixture (10:90, v/v), the concentration of these solutions was 100 mg·L^{-1}. The mixture of the four quinolones studied was prepared by diluting 5 ml of the ciprofloxacin, norfloxacin and ofloxacin solutions, and 1 ml of the pipemidic acid solution to 25 ml with acetonitrile-water mixture (10:90, v/v). The samples were passed through a 0.45 μm nylon filter (MSI).

Chromatographic Procedure

The solution used for the optimization of the mobile phase composition was 25 mM phosphoric acid adjusted to pH 3 with 0.1 M tetrabutylammonium

hydroxide [33-36] at different acetonitrile percentages, up to 30% (v/v). The flow-rate of the mobile phase was maintained at 1 ml/min. The hold-up time, t_o, was measured for every mobile phase composition by injection of 0.01% potassium bromide solution [37]. The retention times and the capacity factors for the solutes were determined from three different injections at every mobile phase composition considered.

The mobile phase used was adjusted to different pH values, between 2.5 and 6, with 0.1 M tetrabutylammonium hydroxide in order to study the influence of the eluent pH in the chromatographic separation. Quinolones have been chromatographed on reversed-phase systems by ion-pairing the acidic part of the molecule with tetrabutylammonium salts. However, k' values remain constant when the tetrabutylammonium concentration in the mobile phase is between 10 and 20 mM and , therefore, retention is not affected when tetrabutylammonium hidroxide concentration is changed to achieve higher pH values. The pH was measured in the mixed mobile phase, where the chromatographic separation takes place, taking into account the reference pH values of primary standard buffer solutions, pH_{PS}, for the standardization of potentiometric sensors in acetonitrile-water mixtures assigned in previous works [25-29] in accordance with IUPAC rules [30,38] and on the basis of the multiprimary standard scale according to the National Institute of Standard and Technology (NIST) [31,39]. In this study we have used potassium hydrogen phthalate (0.05 mol/Kg) as primary standard buffer reference solution in the acetonitrile-water mixtures studied and a combination pH electrode [32].

RESULTS AND DISCUSSION

The capacity factor values, k', for the quinolones studied were obtained at different mobile phases as shown in Table 1. Mobile phases assayed were acetonitrile-water mixtures in ratio of (5:95), (7:93), (10:90), (12:88), (15:85),

TABLE 1

Values of the Capacity Factor for the Quinolones Studied at Various Percentages of Acetonitrile in the Mobile Phase

	k'			
% Acetonitrile	Ciprofloxacin	Norfloxacin	Ofloxacin	Pipemidic acid
5	11.66	10.42	6.79	3.19
7	6.46	5.54	3.95	1.87
10	3.23	2.79	2.06	1.10
12	1.99	1.74	1.33	0.74
15	1.23	1.17	1.02	0.55
20	0.67	0.62	0.55	0.42
25	0.45	0.42	0.40	0.34
30	0.36	0.34	0.33	0.28

(20:80), (25:75) and (30:70). To optimize the composition of the mobile phase the Linear Solvation Energy Relationships (LSER) based on the Kamlet-Taft multiparameter scale were used [15-17]. The LSER approach, when applied to chromatographic processes, correlates a general solute property, such as a logarithmic capacity factor, with feature parameters of solute and both mobile and stationary phases [40]:

$$\log k' = SP_0 + M(\delta_s^2 - \delta_m^2)\overline{V}_2/100 + S(\pi_s^* - \pi_m^*)\pi_2^* + A(\beta_s - \beta_m)\alpha_2 + B(\alpha_s - \alpha_m)\beta_2 \quad (2)$$

Here, k' is the chromatographic capacity factor, SP_0 is the intercept of the regression equation, $\overline{V}_2$ is the molar volume of the solute, and δ^2 is the square of the Hildebrand solubility parameter (a measure of the work required to produce a cavity of unit volume in the solvent) and π^*, α and β are the Kamlet-Taft

solvatochromic parameters. Subscripts s and m refer to the stationary and the mobile phases respectively, and subscript 2 refers to the solute properties. The values M, S, A and B are the coefficients for this equation, they are independent of the solutes, and if the model were rigorously correct, they should be independent of the phases [14].

When a system with a fixed pair of solute and stationary phase is considered and assuming the invariance of the properties of the stationary phase with the change in the mobile phase composition [14,15] and the linear relationship between δ_m^2 and the Kamlet-Taft solvatochromic parameters of the mobile phase [14,32], equation 2 can be simplified to:

$$\log k' = (\log k')_0 + s\pi_m^* + a\alpha_m + b\beta_m \qquad (3)$$

where (log k')$_0$ depends on the parameters of the stationary and the mobile phases, s, a and b are the correlation coefficients which depend on the solute parameters, and π_m^*, α_m and β_m are the Kamlet-Taft solvatochromic parameters of the mobile phase. Values of α [41], β [42] and π^* [43] solvatochromic parameters, together with the E_T^N values [11] for all the acetonitrile-water mixtures studied were determined by interpolating literature values as shown in Table 2.

As a result of the application of the LSER method to log k' values determined in this work, equation 3, the relationships shown in Table 3 were obtained. It can be observed that log k' correlates well with the solvatochromic parameters π^*, α and β, since the average correlation coefficient value (r) was 0.999 using simple linear regression.

The relationship obtained between the chromatographic parameter log k' and the properties of the eluent mixtures π^*, α and β, allow us to predict chromatographic retention of quinolones studied for any composition of the eluent system. For this purpose the capacity factor and the separation factor values for the quinolones studied were calculated for the different compositions of the eluent system using the LSER relationships obtained. In Figure 2, plots of k' values

TABLE 2

Solvatochromic Parameters Values for the Acetonitrile-Water Mixtures Studied

% Acetonitrile	E_T^N	α	β	π^*
5	0.97	1.21	0.41	1.16
7	0.96	1.19	0.39	1.16
10	0.95	1.16	0.37	1.15
12	0.94	1.14	0.36	1.15
15	0.92	1.12	0.35	1.14
20	0.90	1.08	0.34	1.11
25	0.88	1.04	0.34	1.09
30	0.86	1.01	0.35	1.06

TABLE 3

Relationships Between log k' for the Quinolones and π^*, α and β Solvatochromic Parameters of the Eluent System in the Interval Studied Using the LSER Approach

SUBSTANCE	MULTIPARAMETER RELATION	r
Ciprofloxacin	$\log k' = -7.19 - 1.99\,\pi^* + 6.57\,\alpha + 6.32\,\beta$	0.9998
Norfloxacin	$\log k' = -7.56 - 1.03\,\pi^* + 5.63\,\alpha + 7.15\,\beta$	0.9998
Ofloxacin	$\log k' = -7.14 - 0.32\,\pi^* + 4.56\,\alpha + 6.84\,\beta$	0.9993
Pipemidic acid	$\log k' = -1.54 - 7.28\,\pi^* + 7.92\,\alpha + 2.09\,\beta$	0.9988

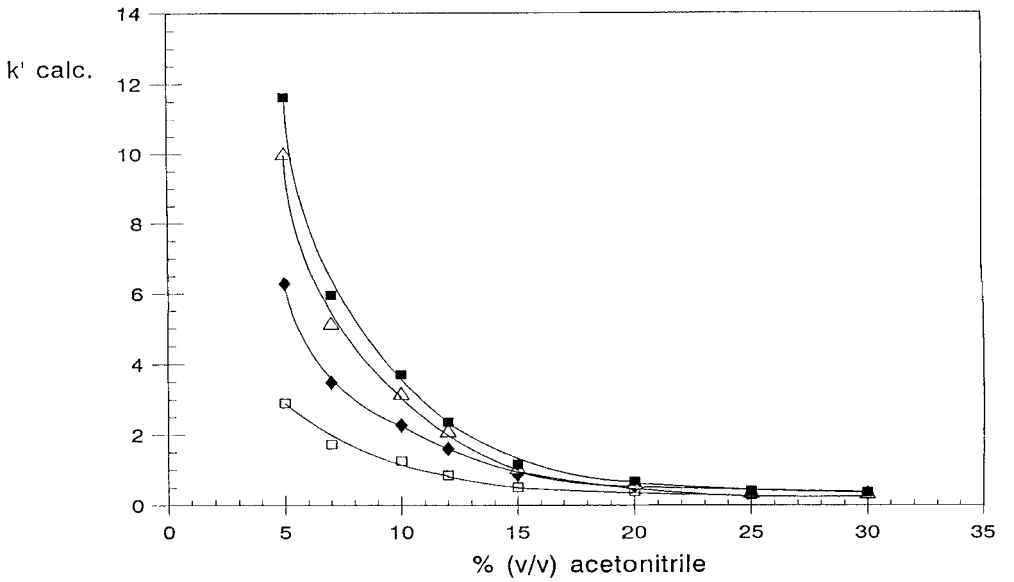


FIGURE 2. Plots of k' values calculated using LSER relationships for the quinolones studied *versus* % (v/v) of acetonitrile in the mobile phase. Ciprofloxacin (■), norfloxacin (Δ), ofloxacin (◆) and pipemidic acid (□).

calculated using LSER relationships *versus* % (v/v) acetonitrile are shown. From these values we can predict that the best chromatographic separation, in which the separation factor values for the four quinolones studied are higher, takes place when the acetonitrile contents in the mobile phase is 5 to 7 % (v/v).

Further reduction of equation 3 is not directly possible because the remaining Kamlet-Taft solvatochromic parameters measure different solvent effects, and linear correlations between them have not been demostrated. However, the structural features of acetonitrile-water mixtures shows three regions [44]. On the water-rich side there is a region in which the structure of the water remains more or less intact, the acetonitrile molecules gradually occupy the cavities between them with little disruption of the water structure. The limit of acetonitrile molar

fraction, x_{AN}, beyond which the acetonitrile molecules can no be longer accommodated within the cavities of the water structure varies with the method applied, but is ≥ 0.10. In the middle range of compositions, the acetonitrile-water mixtures show microheterogeneity; thus, there is a preference of a given water molecule for other water molecules rather than acetonitrile molecules. The same can be said to the preference of acetonitrile molecules for being in the vicinity of a given acetonitrile molecule. At $x_{AN} \geq 0.75$ the number of water clusters is low and water-acetonitrile interactions, which could be discounted in the middle range, now become important.

The difference in β values between water and acetonitrile is small [42,44]. Moreover, β values are constant over most of the composition range, which includes the microheterogeneity regions but extends beyond it on both sides [44]. Therefore, the β_m term in equation 3 can be included in the independent term. Thus, taking into acount the observed correlation: $E_T^N = 0.009 + 0.415\pi^* + 0.465\alpha$ [45], equation 3 can be reduced to:

$$\log k' = C + eE_T^N \qquad (4)$$

Log k' values of the quinolones studied *versus* the E_T^N parameter values of the acetonitrile-aqueous phase eluent system are shown in Figure 3. From Figure 3, it can be observed that the two parameters correlate linearly over the whole experimental range of acetonitrile contents studied, but there are two straight lines with different slopes, which intersect roughly at acetonitrile percentages of 15 % (v/v). All the quinolones have shown a similar elution profile. These two straight lines could be explained taking into account the two regions of acetonitrile-water mixtures studied. The slope of the plots changes in the region where acetonitrile-water mixtures show microheterogeneity. The use of equation 4 implies an important reduction of experimental work. Figure 3 indicates that good chromatographic separation can be obtained for the quinolones studied when the

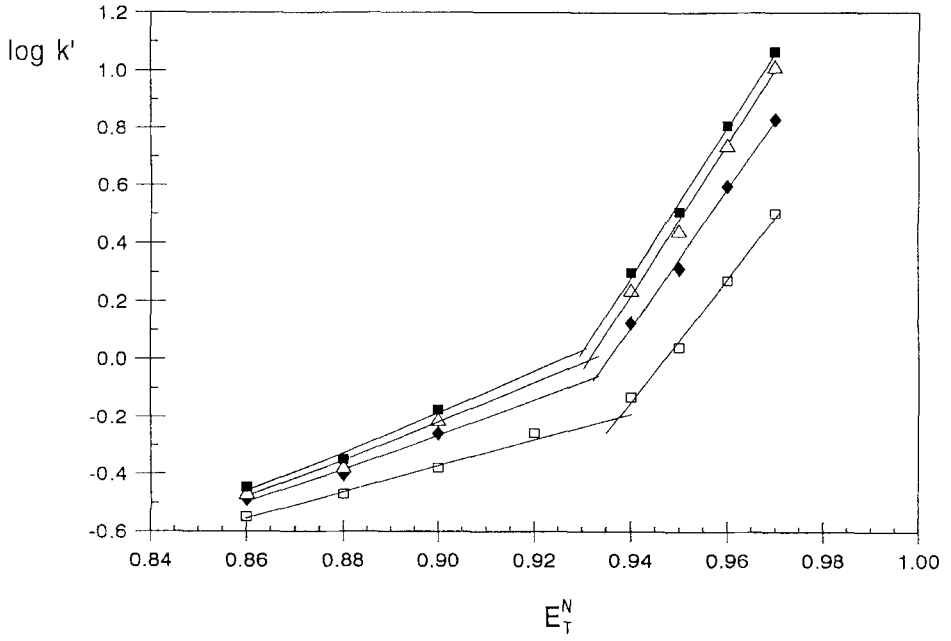


FIGURE 3. Plots of the log k' values of the quinolones *versus* the E_T^N parameters of the eluent systems. Ciprofloxacin (■), norfloxacin (Δ), ofloxacin (◆) and pipemidic acid (□).

acetonitrile contents in the mobile phase is 5 to 7 % (v/v), where the separation factor values are higher. We chose a composition of 7 % (v/v) of acetonitrile because of the shorter retention time.

The apparently contradictory results of Dorsey [12,13,46] and Cheong and Carr [14] could be explained taking into account that the E_T^N single parameter scale is an accurate descriptor of strength of mobile phase in RPLC only if all of the above conditions are true, and then equation 4 can be used. The results of Dorsey *et. al.* [12,13,46] were obtained with a large number of solutes, but practically all were in the microheterogeneity region of acetonitrile-water mixtures ($0.1 \leq x \leq 0.75$). Thus, over this range of compositions the cavity term (solvent/solvent interactions) and the solute/solvent interaction may covary and

TABLE 4

Values of the Capacity Factor for the Quinolones Studied at Various pH of the Mobile Phase

	k'			
pH	Ciprofloxacin	Norfloxacin	Ofloxacin	Pipemidic acid
2.59	5.51	4.76	3.45	1.66
3.11	6.46	5.54	3.95	1.87
4.14	6.54	5.80	4.83	1.92
5.19	10.16	8.87	15.63	2.64
6.18	22.29	18.98	-	3.81

a single parameter might be valid. The same was observed in previous papers [47,48] where the chromatographic behaviour of a series of steroids was studied in a range of acetonitrile-water mixtures from 40 to 70% (v/v). Plots of log k' values of steroids studied there *versus* the E_T^N values showed one straight line because all the data were obtained in one of the structural regions of acetonitrile-water mixtures, the microheterogeneity region.

In contrast, Cheong and Carr have studied the relations between log k' and $E_T(30)$ using acetonitrile-water systems with a wide range of acetonitrile contents, and their results were obtained in two different structural regions. Thus, correlations between measures of solvent/solute interactions and solvent/solvent interactions can change if molecular structure changes. The same can be said of our results in this work and in a recent study [49] where series of peptides have been considered and where the composition range of acetonitrile-water mixtures studied was from 5 to 40% (v/v), in both cases two different structural regions are studied. The study of higher percentages of acetonitrile in the case of quinolones and peptides is not of practical interest since the resultant k' values are subject to high errors due to the low retention, and there are difficulties in defining the column void volume.

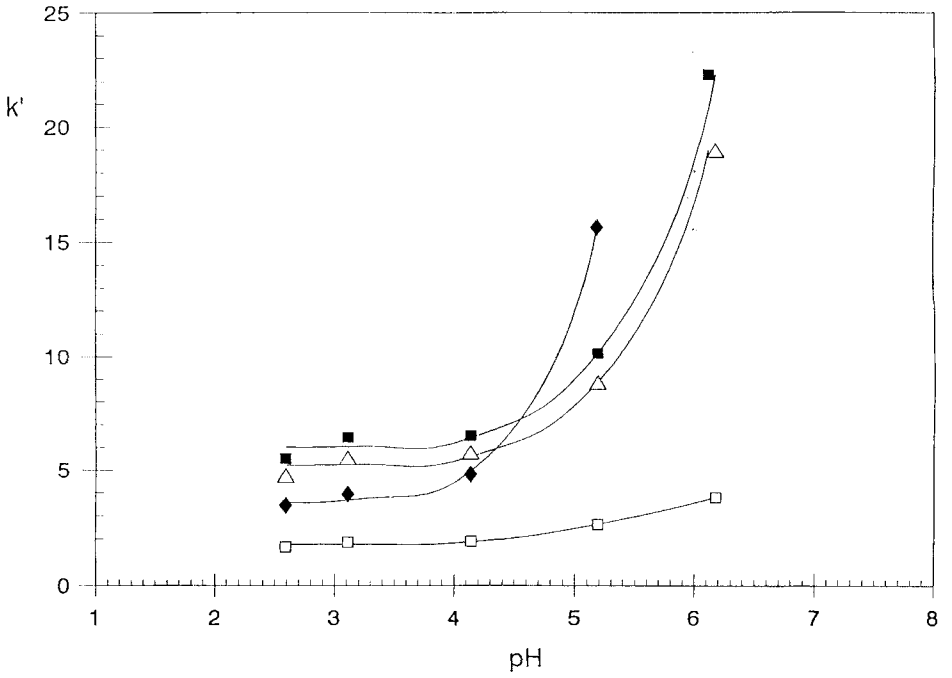


FIGURE 4. Plots of k' values of the quinolones *versus* the pH of the eluent systems. Ciprofloxacin (■), norfloxacin (△), ofloxacin (◆) and pipemidic acid (□).

It is very useful to check the linearity of the log k' values *versus* the E_T^N parameter of the mobile phase for different series of subtances in the practical range of acetonitrile-water mixtures since suitable prediction of the retention for a specific solute can be achieved from E_T^N and a few experimental data. Therefore, the optimization of the chromatographic separation for other related substances can be easily performed.

In order to study the influence of the pH of the mobile phase on the chromatographic retention, k' values for the quinolones studied at different pH of the mobile phase were determined from three different injections at every mobile phase pH considered as shown in Table 4. The National Institute of Standard and

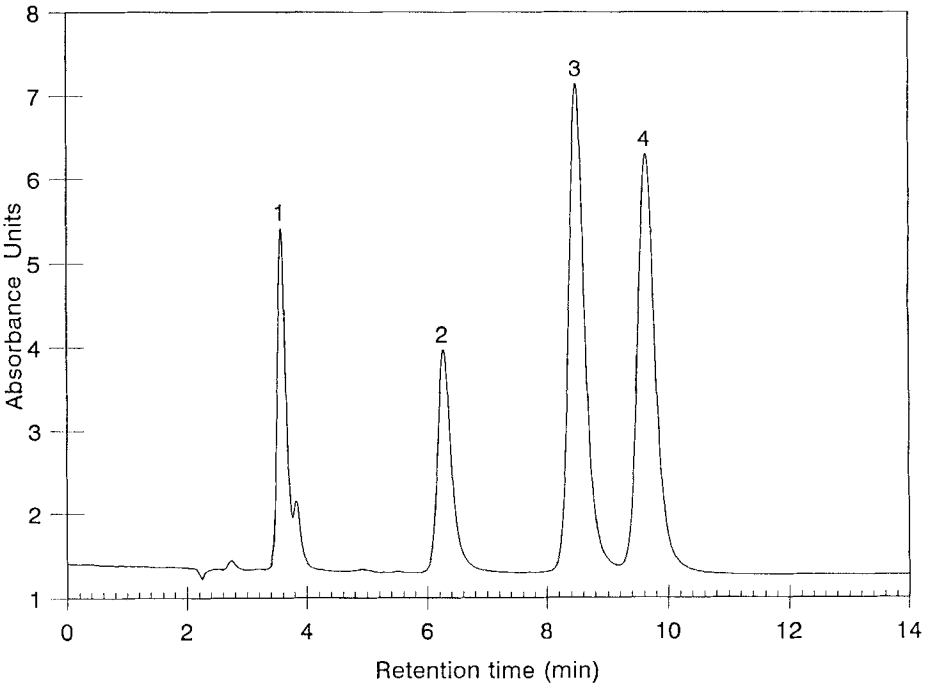


FIGURE 5. Separation of ciprofloxacin (4), norfloxacin (3), ofloxacin (2) and pipemidic acid (1) with an eluent consisting on acetonitrile-25 mM phosphoric acid adjusted to pH 3.09.

Technology (NIST) recommends choosing a standard reference solution with a pH_{PS} value as close as possible to the unknown pH_X [29,31,39]. We have used as reference solution a standard reference solution of potassium hydrogen phthalate and a commercial combination pH electrode, because, as we have shown in a previous works [32], good accuracy and precision are obtained for pH measurements in acetonitrile-water mixtures with pH values up to 7, and quick stabilization of the potentiometric system was observed. pH measurements in the hydroorganic mobile phase used permit the interpretation of chromatographic results without extrapolations of pH values from aqueous solutions. pH and pK_a values show deviations from linear dependence on the composition variations of

the mixture because of preferential solvation [24,28]. If a solute interacts with one of the solvents more strongly than with the other, then the solute is preferentially solvated by the former. On the other hand, pH measurements in the hydroorganic mixture used as mobile phase also permit the determination of pK_a values for the substances studied [49].

Plots of the k' values for the quinolones studied *versus* pH of the acetonitrile-aqueous phase eluent system are shown in Figure 4. The nonpolar stationary bonded phase used, octadecylsilica (ODS), may only be used in the pH range between 2 and 8, thus it was not possible to study the retention of quinolones as typical ampholytes because correlations between k' and the pH of the mobile phase cannot be obtained over the entire range of pH. However, k' values increase with pH, Figure 4, suggesting that the intermediate form of quinolones does not exist appreciably in zwitterion form [50]. Although it has not been shown, if zwitterion formation for the intermediate species did not occur, then a maximum in k' would be expected [50,51]. As can be shown in Figure 4, the best chromatographic separation for the quinolones studied can be performed at a pH of the mobile phase between 3 and 4. The best separation that can be obtained in these conditions was achieved at a pH of 3.09. Figure 5 shows an excellent separation of ciprofloxacin, norfloxacin, ofloxacin and pipemidic acid with an acetonitrile-aqueous phase system (7:93, v/v) adjusted to pH 3.09.

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