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Solvatochromic parameter values and pH in aqueous–organic mixtures used in liquid chromatography Prediction of retention of a series of quinolones

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Abstract

The proportion of the organic modifier and the pH of the mobile phase were optimized in order to separate six important quinolones: ciprofloxacin, norfloxacin, enoxacin, fleroxacin, ofloxacin and pipemidic acid. The mobile phase was optimized by establishing relationships between retention parameters and the Reichardt's E_T^N scale of solvent polarity, and between retention and the Kamlet–Taft multiparameter solvent scale of the eluent, using linear solvation energy relationships (LSER). In addition, the effect of liquid junction potentials was studied in cells with commercial potentiometric sensors in order to determine the highest precision and accuracy in pH measurements achievable in acetonitrile–water mixtures used as mobile phases. This pH allows the optimization of the mobile phase pH for the chromatographic separation required.

1. Introduction

Quinolones comprise a most interesting group of antibiotics whose bacterial action is based on their anti-DNA gyrase activity [1]. These drugs are suitable for the treatment of systematic infections in addition to 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. Although methods based on high-performance liquid chromatography (HPLC) are available for the detection and determination of individual quinolones, only a few methods have been described for the separation of several quinolones [2–5].

The composition of the mobile phase has a primary role in the retention of compounds in reversed-phase liquid chromatography (RPLC). Recently, the method of linear solvation energy relationships (LSER), based on the Kamlet–Taft multiparameter scale, has been used successfully to study retention in HPLC [6–8]. The solvatochromic LSER approach of Kamlet and Taft allows us to relate retention parameters of solutes to variations in characteristic properties of mobile phases such as the solvatochromic parameters α, β and π^*. The π^* parameter is used to evaluate solvent dipolarity/polarizability [9], and α and β scales evaluate solvent hydrogen-bond acidity [10] and solvent hydrogen-bond basicity [11], respectively. It is also important to examine the polarity of solvent mixtures used as mobile phases, which may serve as a measurement of their eluting strength. The polarity of

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the mobile phase is a major factor that influences solute retention in RPLC. Except in rare instances, retention in RPLC increases as the polarity of the mobile phase is increased by the addition of water. Many empirical scales of overall solvent strength have been proposed, but the most widely used is $E_T(30)$, proposed by Dimroth and Reichardt [12]. Dorsey and coworkers [13–15] have conclusively shown that plots of log k' versus the mobile phase $E_T(30)$ solvatochromic parameter are linearly correlated. This relationship can be expressed as

$$\log k' = C + e(E_T^N) \quad (1)$$

when the normalized E_T^N parameter [12] is used instead of the $E_T(30)$ values in order to use similar units to those for the other parameters. Therefore, suitable prediction of the retention for a specific solute can be achieved from the E_T^N of the mobile phase and a few experimental data. However, Cheong and Carr [16] concluded that good correlations between retention and this single solvent parameter can be obtained only over a narrow range of solvent composition.

Chromatographic retention measurements provide information about the combined nature of mobile and stationary phases. In contrast, solvatochromic measurements are performed independently of the stationary phase, thus allowing an independent examination of the effect of changing the mobile phase composition on chromatographic retention. These approaches only allow the prediction of retention at different mobile phase compositions, and provide no information about the pH of the mobile phase, which is important in understanding the retention process. The pH of the mobile phase is also critical for optimizing selectivity in RPLC, since the degree of ionization of solutes, stationary phases (e.g., ion exchangers) and mobile phase additives (e.g., ion-paring reagents) may be affected by the pH [17].

The operational pH in mixed aqueous–organic solvents is usually measured assuming that the pH of the mobile phase 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 [18]. In acetonitrile–water mixtures, the influence of the co-solvent on the pH is substantial [18–20] and therefore, for successful systematic optimization of the mobile phase pH, accurate pH measurements in the most widely used binary aqueous–organic solvent mixtures are needed.

From the point of view of practical chromatography, it is possible to measure the activity of the hydronium ion in acetonitrile–water mixtures because reference pH values of standard buffer solutions, pH_{PS}, in these solvents are known [20–22].

pH measurements in a mixed solvent can be performed as easily as in water taking into account the operational definition of pH [23,24]:

$$pH_X = pH_{PS} + \frac{E_{PS} - E_X}{g} \quad (2)$$

where E_X and E_{PS} denote the electromotive force (e.m.f.) measurements in cell A on the sample solution at unknown pH_X and on the standard reference solution at known pH_{PS}, respectively, and $g = (ln\ 10)RT/F$ (see Scheme 1).

If the liquid junction potential, E_J, changes when the reference solution, PS, is replaced by the solution at unknown pH, X, then the complete form of Eq. 2 is

$$pH_X = pH_{PS} + \frac{E_{PS} - E_X}{g} + \frac{E_{JX} - E_{JS}}{g} \quad (3)$$

where the term $E_{JX} - E_{JS}$ is the residual liquid junction potential, an important factor to be controlled in terms of uncertainty in pH measurements. Further, the pH values calculated using Eq. 3 depend on the primary standard buffer solution used as a reference owing to the different E_{JS} values achieved when different primary standard buffer solutions are used, according to the recommended approach of the

Reference electrode | Salt bridge ‖ Sample solution at pH_X or standard buffer solution at pH_{PS} in solvent S | H^+ sensing electrode

Cell A

Scheme 1.

National Institute of Standards and Technology (NIST) [25,26].

Therefore, it is necessary to study the effect of liquid junction potentials in cells with commercial potentiometric sensors in order to determine the highest precision and accuracy achievable in pH measurements in acetonitrile–water mixtures used as mobile phases. In this study we used different primary standard buffer reference solutions in acetonitrile–water mixtures containing up to 70% (w/w) of acetonitrile in order to compare the theoretical values of pH_{PS} with those obtained using different commercial electrodes, and in order to evaluate the influence of the residual liquid junction potential on the pH values measured.

The aim of this study was to select the optimum eluent in order to separate six quinolones: ciprofloxacin, norfloxacin, enoxacin, fleroxacin, ofloxacin and pipemidic acid. For this purpose, the proportion of the organic modifier and the pH of the aqueous–organic mobile phase were optimized. The LSER method based on the multiparameter π^*, α and β scale and the relationships with log k' and the single solvent parameter E_T^N were 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' were used in the optimization of the mobile phase pH for the separation required [27,28].

2. Experimental

2.1. Apparatus

The chromatographic equipment consisted of an ISCO (Lincoln, NE, USA) 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 5-μm LiChrospher 100 RP-18 (Merck, Darmstadt, Germany) column (250 × 4 mm I.D.) was used at ambient temperature. The e.m.f. values used to evaluate the pH of the mobile phase were measured with a Model 2002 potentiometer (±0.1 mV) (Crison Instruments, Barcelona, Spain) using two potentiometric systems: a Radiometer (Copenhagen, Denmark) G202B glass electrode and a Radiometer K801 reference Ag–AgCl electrode, and an 8102 ROSS combination pH electrode (Orion Research, Boston, MA, USA). All solutions were thermostated externally at 25 ± 0.1°C. The electrodes were stabilized in the appropriate acetonitrile–water mixtures prior to the e.m.f. measurements, and the measurements were performed in triplicate to ensure stability and reproducibility of the potentiometric system.

2.2. Reagents

All reagents were of analytical-reagent grade. Acetonitrile (Merck) and water were of HPLC grade. The eluents were passed through a 0.22-μm nylon filter (MSI, Westboro, MA, USA) and degassed ultrasonically. The quinolones (Fig. 1) were obtained from various pharmaceutical firms: ciprofloxacin (Lasa, Barcelona, Spain), norfloxacin (Boral Química, Barcelona, Spain), enoxacin (Almirall, Barcelona, Spain), fleroxacin (Roche, Madrid, Spain), ofloxacin (Hoechst Ibérica, Barcelona, Spain) and pipemidic acid (Almirall, and Prodesfarma, Barcelona, Spain). Stock standard solutions of the quinolones were prepared in acetonitrile–water (10:90) at concentrations of 100 mg/l. A mixture of the six quinolones studied was prepared by diluting 5 ml of the ciprofloxacin, norfloxacin, fleroxacin and ofloxacin solutions, 2 ml of the enoxacin solution and 1 ml of the pipemidic acid solution to 25 ml with acetonitrile–water (10:90). The samples were passed through a 0.45-μm nylon filter (MSI).

2.3. Chromatographic procedure

The solution used for the optimization of the mobile phase composition was 25 m*M* phos-

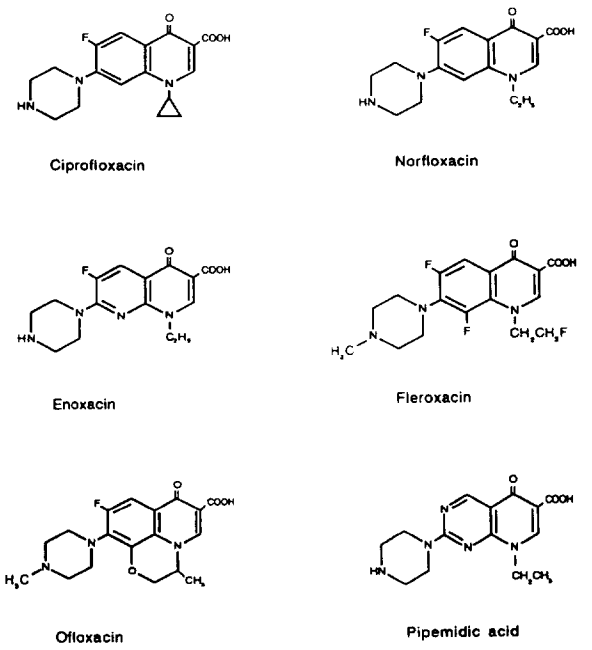

Fig. 1. Structural formulae of the quinolones studied.

phoric acid adjusted to pH 3 with 0.1 *M* tetrabutylammonium hydroxide [29–32] 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_0, was measured for every mobile phase composition by injection of 0.01% potassium bromide solution [33]. 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. 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 studies [20–22] in accordance with IUPAC rules [24,34]. For this purpose we used two primary standard buffer reference solutions in the acetonitrile–water mixtures studied: phosphate buffer (0.03043 mol/kg Na_2HPO_4 and 0.008695 mol/kg KH_2PO_4) and potassium hydrogenphthalate (0.05 mol/kg).

3. Results and Discussion

The logarithm of the capacity factor, log k', for the quinolones studied was obtained at different percentages of acetonitrile as shown in Table 1. The mobile phases studied were acetonitrile–water mixtures (5:95, 7:93, 10:90, 12:88, 15:85, 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 [6–8]. The LSER approach, when applied to phase-transfer processes, correlates a general

Table 1
Logarithm of the capacity factors for the quinolones studied at various percentages of acetonitrile in the mobile phase

Acetonitrile (%, v/v)	Log k'					
	Ciprofloxacin	Norfloxacin	Enoxacin	Fleroxacin	Ofloxacin	Pipemidic acid
5	1.067	1.018	0.854	0.847	0.832	0.504
7	0.810	0.743	0.572	0.599	0.597	0.272
10	0.509	0.445	0.290	0.339	0.313	0.041
12	0.299	0.241	0.103	0.164	0.125	−0.130
15	0.090	0.070	−0.057	0.031	0.007	−0.257
20	−0.176	−0.210	−0.287	−0.204	−0.260	−0.380
25	−0.350	−0.375	−0.420	−0.350	−0.402	−0.470
30	−0.446	−0.466	−0.499	−0.466	−0.488	−0.548

solute property (SP) such as a logarithmic capacity factor, with three types of term [35]:

$$SP = SP_0 + \text{cavity term} + \text{dipolar term} + \text{hydrogen bonding term(s)} \quad (4)$$

where the cavity term measures the free energy or enthalpy input required to separate the solvent molecules to create a suitable sized cavity for the solute, the dipolar term measures the exoergic effects of solute–solvent dipole–dipole, dipole–induced dipole and mutually induced dipole interactions and the hydrogen bonding terms measure the exoergic effects of hydrogen bonding (or Lewis acid–base) complexation between the solute and the solvent.

In the specific case of chromatographic retention, Eq. 4, with solvatochromic parameters α, β and π^* appropriately included, becomes

$$\log k' = SP_0 + M(\delta_s^2 - \delta_m^2)\bar{V}_2/100 + S(\pi_s^* - \pi_m^*)\pi_2^* + A(\beta_s - \beta_m)\alpha_2 + B(\alpha_s - \alpha_m)\beta_2 \quad (5)$$

where k' is the chromatographic capacity factor, SP_0 is the intercept of the regression equation, $\bar{V}_2$ 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). Subscripts s and m refer to the stationary and the mobile phases, respectively, and subscript 2 refers to the solute properties. 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 [16].

When a system with a fixed pair of solute and stationary phase is considered, Eq. 5 reduces to

$$\log k' = (\log k')_0 + m\delta_m^2 + s\pi_m^* + a\beta_m + b\alpha_m \quad (6)$$

where $(\log k')_0$ depends on the parameters of the stationary phase and m, s, a and b depend on the solute parameters. Invariance of the properties of the stationary phase with the change in the mobile phase composition is assumed [16,36].

Hildebrand solubility parameters are known for many pure liquids, but they have not been determined for mixtures. However, linear relationships between δ^2 and π^* have been proposed [16]. Although these correlations have been applied to pure solvents, they can also be applied to binary solvent mixtures where δ and π^* change in a limited range. Assuming that there is a linear relationship between δ_m^2 and π_m^* (and perhaps also with α_m and β_m), Eq. 6 can be simplified to

$$\log k' = (\log k')_s + s_2\pi_m^* + a_2\beta_m + b_2\alpha_m \quad (7)$$

where the independent term and the coefficients depend on the correlation of δ_m^2 with α_m, β_m and π_m^* for the mobile phase studied.

Values of α [37], β [38] and π^* [39] solvatochromic parameters, together with the E_T^N values [36] for all the acetonitrile–water mixtures studied, were obtained 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 (Eq. 7), the relationships shown in Table 3 were obtained. Log k' correlates well with the solvatochromic parameters α, β and π^*, since the average correlation coefficient (r) was 0.999 using simple linear regression for the data sets examined here.

The relationship obtained between the chromatographic parameter log k' and the properties of the eluent mixtures, α, β and π^*, allow us to predict the chromatographic retention of the quinolones studied for any composition of the

Table 2
Solvatochromic parameters values for the acetonitrile–water mixtures studied

Acetonitrile (%, v/v)	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 α, β and π^* solvatochromic parameters of the eluent system in the interval studied using the LSER approach

Substance	Multiparameter equation	r
Ciprofloxacin	$\log k' = -7.19 - 1.99\pi^* + 6.32\beta + 6.57\alpha$	0.9998
Norfloxacin	$\log k' = -7.56 - 1.03\pi^* + 7.15\beta + 5.63\alpha$	0.9998
Enoxacin	$\log k' = -5.84 - 2.75\pi^* + 6.36\beta + 5.98\alpha$	0.9997
Fleroxacin	$\log k' = -5.73 - 2.33\pi^* + 4.55\beta + 6.09\alpha$	0.9995
Ofloxacin	$\log k' = -7.14 - 0.32\pi^* + 6.84\beta + 4.56\alpha$	0.9993
Pipemidic acid	$\log k' = -1.54 - 7.28\pi^* + 2.09\beta + 7.92\alpha$	0.9988

eluent system. For this purpose, the capacity factor and the separation factor for the quinolones studied were calculated for the different compositions of the mobile phase using the LSER relationships obtained.

The separation factor of enoxacin, fleroxacin and ofloxacin was not obtained under these chromatographic conditions. Therefore, we chose ofloxacin, which is widely used, and we attempted the separation of four quinolones: ciprofloxacin, norfloxacin, ofloxacin and pipemidic acid. From the separation factors obtained using the equations given in Table 3, we can predict that the optimum chromatographic separation for these quinolones takes place when the acetonitrile content in the mobile phase is 5–7% (v/v).

Further reduction of Eq. 7 is not directly possible because the remaining Kamlet–Taft solvatochromic parameters measure different solvent effects, and linear correlations between them have not been demonstrated. However, the structural features of acetonitrile–water mixtures, explored by Marcus and Migron [40], show three regions. On the water-rich side there is a region in which the structure of the water molecules remains more or less intact and 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 it is ≥ 0.10. In the middle range of compositions, the acetonitrile–water mixtures show microheterogeneity; hence, there is a preference of a given water molecule for other water molecules rather than acetonitrile molecules. The same can be said of 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 [38,40]. Moreover, β values are constant over most of the composition range, which includes the microheterogeneity regions but extends beyond it on both sides [40]. Therefore, the β_m term in Eq. 7 can be included in the independent term. Thus, taking into account the observed correlation, $E_T^N = 0.009 + 0.415\ \pi^* + 0.465\ \alpha$ [41], Eq. 7 can be reduced to Eq. 1.

Log k' values of the quinolones studied versus the E_T^N parameter values of the acetonitrile–aqueous phase eluent system are shown in Fig. 2. Log k' and E_T^N correlate linearly over the whole experimental range of acetonitrile contents studied, but there are two straight lines with different slopes, which intersect roughly at an acetonitrile content of 15% (v/v). All the quinolones showed a similar elution profile. These two straight lines could be explained by taking into account the two region of acetonitrile–water mixtures studied. The slope of the plots changes in the region where acetonitrile–water mixtures show microheterogeneity. The use of Eq. 1 implies an important reduction of experimental work. Fig. 2 indicates that a good chromatographic separation can be obtained for the quinolones studied when the acetonitrile

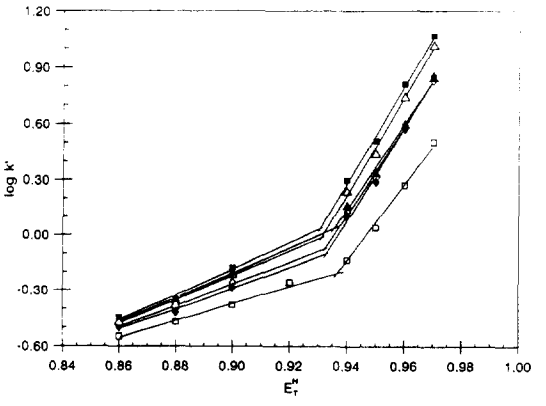

Fig. 2. Plots of log k' values of the quinolones versus the E_T^N parameters of the eluent systems. ■ = Ciprofloxacin; △ = norfloxacin; ◆ = enoxacin; ▲ = fleroxacin; ◇ = ofloxacin; □ = pipemidic acid.

content in the mobile phase is 5–7% (v/v), where the separation factor values are higher, the same result that was obtained using the LSER approach. We chose a composition of 7% (v/v) of acetonitrile because of the shorter retention time.

The apparently contradictory results of Dorsey and co-workers [13–15] and Cheong and Carr [16] 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 Eq. 1 can be used. The results of Dorsey and co-workers [13–15] were obtained with a large number of solutes, but almost all were in the microheterogeneity regions of acetonitrile–water mixtures ($0.1 \leq x \leq 0.75$). Hence, over this range of compositions the cavity term (solvent–solvent interactions) and the solute–solvent interaction may co-vary and a single parameter might be valid. The same was observed in a previous study [42] where the composition range of acetonitrile–water mixtures studied was 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.

In contrast, Cheong and Carr [16] have studied the relationships between log k' and $E_T(30)$ using acetonitrile–water systems with a high percentage of acetonitrile and their results were obtained in two different structural regions. Thus, correlations between measures of solvent–solvent interactions and solvent–solvent interactions can change if the molecular structure changes. The same can be said of the results in this work, in which two different structural regions were studied. The study of higher percentages of acetonitrile in the case of quinolones 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.

In order to study the influence of pH on chromatographic retention, accurate pH measurements in the aqueous–organic mobile phases are needed. These values permit the interpretation of chromatographic results without extrapolations of pH values from aqueous solutions. pH and pK_a values show deviations from a linear dependence on the composition variations of the mixtures because of preferential solvation [19,20]. 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 aqueous–organic mixture used as mobile phase also permit the determination of pK_a values for the substances studied.

The standardization of pH measurements in acetonitrile–water mixtures (Eq. 2) has been studied recently [20,22,43] using cells without transference and on the basis of the multiprimary standard scale according to NIST [25,26]. However, a further uncertainty arises from the standardization of pH measurements with practical cells containing glass electrodes and commercial reference electrodes, the residual liquid junction potential (Eq. 3). IUPAC recommends choosing a standard reference solution with a pH_{PS} value as close as possible to the unknown pH_X, and with a similar composition and ionic strength, so that the residual liquid junction potential term becomes small [23]. We used two different primary standard buffers as reference solutions, potassium hydrogenphthalate and phosphate buffer, and two different potentiometric systems,

a glass electrode–reference Ag–AgCl electrode and a combination pH electrode. As a result of this study we decided to use a reference solution of potassium hydrogenphthalate because good accuracy and precision were obtained for pH measurements in acetonitrile–water solutions with pH values up to 7, and a combination pH electrode because rapid stabilization of the potentiometric system was observed. In Table 4, the previously established standard values for primary reference buffer solutions, pH_{PS}, and the pH_X values obtained using a reference solution of potassium hydrogenphthalate and a combination pH electrode are compared. The difference between the theoretical values, pH_{PS}, and the experimental pH measurements, pH_X, are given in parentheses. These differences represent the errors due to the residual liquid junction potential. As can be seen in Table 4, these errors are not significant for measurements up to pH 7, a widely used pH range in RPLC with an octadecylsilica (ODS) stationary phase.

The capacity factors for the quinolones studied at different pH values of the mobile phase were determined from three different injections at every mobile phase pH considered, as shown in Table 5. Relative standard deviations lower than 2% for the k' values were obtained. The pH values were measured in the aqueous–organic mobile phase, where the chromatographic separation takes place.

The non-polar stationary bonded phase used, ODS, may only be used in the pH range 2–8, so 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 increase in pH, suggesting that the intermediate form of quinolones does not exist appreciably in zwit-

Table 4
pH_X values for primary standard reference solutions in acetonitrile–water mixtures obtained using a combination pH electrode and a 0.05 mol/kg potassium hydrogenphthalate solution as reference at 298.15 K, with differences from theoretical values, pH_{PS}, shown in parentheses

Primary standard[a]	Acetonitrile (%, w/w)									
	10		30		40		50		70	
	pH_X	pH_{PS}	pH_X	pH_{PS}	pH_X	pH_{PS}	pH_X	pH_{PS}	pH_X	pH_{PS}
KH tartrate	3.75 (−0.05)	3.802	4.26 (−0.06)	4.325	4.53 (−0.04)	4.570	4.78 (−0.07)	4.852	5.64 (−0.08)	5.723
KH_2 citrate	4.04 (+0.05)	3.994	4.50 (+0.03)	4.470	4.70 (0.00)	4.702	4.93 (−0.06)	4.995	5.59 (−0.02)	5.610
Acetate buffer	4.87 (−0.03)	4.898	5.52 (−0.01)	5.532	5.85 (−0.02)	5.875	6.17 (−0.10)	6.275		
Phosphate (buffer I)	7.11 (−0.04)	7.149	7.62 (+0.02)	7.604	7.76 (+0.09)	7.667	7.91 (−0.09)	8.002		
Phosphate (buffer II)	7.65 (−0.04)	7.697	8.16 (+0.01)	8.151	8.32 (−0.12)	8.436	8.50 (−0.15)	8.646		
Na tetraborate	9.52 (−0.08)	9.600	10.30 (−0.14)	10.437	10.63 (−0.18)	10.807	11.08 (−0.12)	11.204		
Carbonate buffer	10.78 (−0.57)	11.353	11.20 (−0.69)	11.893	12.06 (−0.06)	12.118	12.40 (−0.28)	12.676		

[a] Primary standard reference solutions: saturated (at 25°C) potassium hydrogentartrate (KH tartrate); 0.05 mol/kg potassium dihydrogencitrate (KH_2 citrate); 0.1 mol/l sodium acetate and 0.1 mol/l acetic acid (acetate buffer); 0.025 mol/kg disodium hydrogenphosphate and 0.025 mol/kg potassium dihydrogenphosphate (phosphate buffer I); 0.03043 mol/kg disodium hydrogenphosphate and 0.008695 mol/kg potassium dihydrogenphosphate (phosphate buffer II); 0.01 mol/kg sodium tetraborate (Na tetraborate); 0.025 mol/kg sodium hydrogencarbonate and 0.025 mol/kg sodium carbonate (carbonate buffer).

Table 5
Capacity factors of the quinolones studied at various pH values of the mobile phase

pH	k'					
	Ciprofloxacin	Norfloxacin	Enoxacin	Fleroxacin	Ofloxacin	Pipemidic acid
2.59	5.51	4.76	3.34	3.39	3.45	1.66
3.11	6.46	5.54	3.73	3.98	3.95	1.87
4.14	6.54	5.80	3.83	5.65	4.83	1.92
5.19	10.16	8.87	5.87	17.67	15.63	2.64
6.18	22.29	18.98	12.33	–	–	3.81

terionic form [44]. Although it has not been shown, if zwitterion formation for the intermediate species did not occur, then a maximum in k' would be expected [44,45].

From plots of k' values for the quinolones studied versus pH of the eluent system we can predict that the optimum chromatographic separation for the quinolones studied, ciprofloxacin, norfloxacin, enoxacin, fleroxacin, ofloxacin and pipemidic acid, can be achieved at a pH of the mobile phase between 3 and 4. The best separation was achieved at pH 3.89. Fig. 3 shows a chromatogram of the separation of the six substances studied with an acetonitrile–aqueous phase (7:93) system at pH 3.89; this is probably the best separation that can be obtained under these conditions.

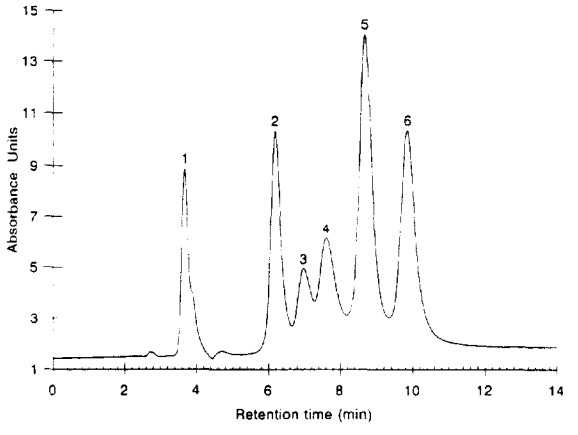

Fig. 3. Separation of (1) pipemidic acid, (2) ofloxacin, (3) fleroxacin, (4) enoxacin, (5) norfloxacin and (6) ciprofloxacin with an eluent consisting on acetonitrile–25 mM phosphoric acid adjusted to pH 3.89 with 0.1 M tetrabutylammonium hydroxide (7:93).

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