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Characterization of the acidity of residual silanol groups in microparticulate and monolithic reversed-phase columns☆

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Abstract

The residual silanol acidity and activity of several microparticulate and monolithic C18 columns has been measured from the retention of $LiNO_3$ in the columns with a methanol/buffer (1 mM in Na^+) (60:40 v/v) mobile phase buffered to different pH values. For Luna C_{18} (2) and LiChrospher RP-18 columns, at least two different types of silanols with different acidity for each packing, were observed. Purospher RP-18e and Chromolith RP-18e packings present evidence of some active silanols only at pH values close to their basic pH stability limit or higher. The results obtained have been compared with those obtained previously for Resolve C18, Resolve Silica, Symmetry C18, Symmetry Silica, XTerra MSC_{18} and Underivatized XTerra. A modification of an equation previously proposed has been applied to all columns studied and the results obtained have been used to classify the columns according to their silanol acidity and activity. The method allows the prediction of the extent of the silanol activity of the columns studied at a particular mobile phase pH.

Keywords: Monolithic columns; Stationary phases, LC; Acidity; pK_a; Silanol activity

1. Introduction

Liquid chromatography has become an indispensable tool for both routine analysis and research in the pharmaceutical, biomedical, and biotechnology industries. On an analytical level, reversed-phase liquid chromatography (RPLC) is the most widespread technique, probably due to the broad applicability of that mode of separation to a wide range of compounds and sample matrices. One distinct advantage of RPLC over other HPLC techniques, such as ion exchange or normal-phase chromatography, is the vast number of stationary phases available, which can offer a unique selectivity that can facilitate the separation and analysis of particular chemical mixtures.

The packing of stationary phases and the support employed play an important role in the separation mechanism of the analytes. In addition to partition, these packings may undergo other types of mechanisms, such as charge exclusion or ion exchange, which can significantly affect the retention and separation of analytes [1–4].

Because of its physical characteristics and versatility, silica is the most widely used support for RPLC stationary phases [5–8]. The high versatility of the silica packings is due to the easiness of bonding very different stationary phases to the particle, giving a broad range of selectivities [8].

However, silica-based materials have some disadvantages for the analysis of basic compounds because of the strong interaction between these compounds and the support [9]. The random position of the bonds generated between the phase and the support jointly with the steric hindrance, produce free residual silanols on the surface. Thus, the interaction of basic analytes with these residual silanol groups, on RP columns, are considered to be the cause of the broad and tailing peaks that are often observed [4,6–10].

Metal impurities embedded in the silica substrate can strongly enhance the acidity of the residual silanols, and thus increase the unwanted silanol interactions with the basic analytes [11–13]. Nawrocki [4] described the presence of several types of silanols (single, geminal, vicinal) in the silica surface, with different acidities, which can interact, in a different extension, with basic compounds.

☆ Dedicated to Joseph J. Kirkland for his outstanding contribution to the art and science of chromatography.

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Considerable improvements for the analysis of basic compounds, by HPLC, have been achieved by column manufacturers. In the late 1980s, one of these developments resulted in the availability of high purity silica substrates, i.e. with an absence or low content of metals [14,15], it was called by Kirkland "type B" silica [16] this term has been broadly used since then by others [17,18]. Additionally, several strategies have been developed to block, remove or decrease the number of residual silanols on the silica surface [6,8,19,20]. The most common process is column endcapping, which produces an important decrease of the number of surface silanols, although it does not completely remove them [5,6,8,20]. In order to reduce the accessibility to free silanols, new packings or stationary phases with especially designed groups, i.e. with bulky or sterically groups, bidentate, polymeric-coated, etc., have been developed [6,8,13,21–23]. Thus, the XTerra MS C_{18} of Waters, the Luna C_{18} (2) of Phenomenex and the Zorbax Extend-C_{18} of Agilent are a few examples of the commercially available new generation of columns that claim to substantially improve chemical stability.

RPLC monolithic silica phase columns have also been introduced [4,6,24–28]. These columns consist of a continuous rod-shaped porous network with a bimodal pore distribution. Its pore design avoids the high backpressure problem and allows working at high flow-rates, which lead to faster separations [8,24–28]. However, due to the relatively recent introduction of these types of phases, few studies [17,29,30] have attempted to characterize them in terms of their silanol activity (potential solute–silica interactions).

The study of the solute–silica interactions has led to the development of different characterization tests [7]. Many tests are based on the retention of amines, which usually have an aromatic part that allows UV detection [7], however, there is no commonly accepted method for carrying out such evaluations, because they are strongly dependent on the nature of the solute employed [7,31,32]. Additionally, these compounds are also bulky, which implies: (i) their interaction with the residual silanols is not only by ionic exchange, but also with the bonded phase by their hydrophobic interaction, and (ii) difficult accessibility to the smallest pores of the silica structure.

We previously proposed a method for the determination of the residual silanol acidity, based in the retention change of the lithium ions with the pH of the mobile phase, which contains a constant concentration of sodium ions [33,34]. Lithium is not retained by the organic bonded phase because of its charge and small size, which in addition allows its access to the smallest pores of the silica surface. The method was applied to the characterization of derivatized and underivatized columns: Resolve C18, Resolve Silica, Symmetry C18, Symmetry Silica, XTerra MS C_{18} and a underivatized XTerra column [34].

In this paper, we extend the application of this procedure to several other conventional microparticulate columns and to a monolithic phase. The main characteristics of the columns studied here and those reported previously [34] are given in Table 1. Lichrospher 100 RP-18 is a non-endcapped column, based on an old, less pure silica material. The other three stationary phases have also C18 packings but bonded to high purity silica materials, and they are endcapped. Purospher RP-18e and Luna C_{18} (2) are made of high purity silica particles whereas Chromolith Perfomance RP-18e is a monolithic rod column.

The results obtained in the present paper are compared with those published in a previous work for other columns [34]. We expect that this study helps to understand better the properties of these reversed-phase packing materials and to assist in the selection of suitable columns for the analysis of basic compounds.

2. Theoretical background

2.1. Cationic exchange

A silica surface, which has silanol groups partially or totally ionized, behaves as an ion exchanger [2,4]. If the mobile phase contains A^+ as the unique cation of the background electrolyte, A^+ ions are retained by the ionized silanols (R_3SiO^-) and when a cationic analyte B^+ is introduced into the column, the following ion exchange equilibrium should be observed:

$$R_3SiO^-A^+ + B^+ \Leftrightarrow R_3SiO^-B^+ + A^+$$

This equilibrium is ruled by the selectivity coefficient of B^+ in reference to A^+ ($K_{A^+}^{B^+}$), which is defined by Eq. (1):

$$K_{A^+}^{B^+} = \frac{[B^+]_S[A^+]_M}{[A^+]_S[B^+]_M} \quad (1)$$

where the subscripts S and M refer to the stationary and mobile phase, respectively.

The retention factor (k) of B^+ ion can be related with the selectivity coefficient through Eqs. (2) and (3):

$$k = \frac{[B^+]_S V_S}{[B^+]_M V_M} \quad (2)$$

where V_S and V_M are the volumes of stationary and mobile phase, respectively. By substitution of Eq. (1) into Eq. (2) gives:

$$k = K_{A^+}^{B^+} \frac{[A^+]_S V_S}{[A^+]_M V_M} = K_{A^+}^{B^+} \frac{n_{A(S)}}{n_{A(M)}} \quad (3)$$

where $n_{A(S)}$ and $n_{A(M)}$ are the number of mols of A^+ ions in the stationary and mobile phase, respectively. Since $[A^+]_M \gg [B^+]_M$, $n_{A(S)}$ is equivalent to the number of mols of ionized silanol groups, which depends on the overall number of silanol groups and on the degree of ionization of them. In previous works [33,34], we have demonstrated that at least two types of silanols with different acidities are usually present, and therefore, since there may be more than two

Table 1
Columns used in this study and in a previous work [33]; data supplied by manufacturers

	LiChrospher 100 RP-18	Purospher RP-18e	Chromolith RP-18e	Luna C_{18} (2)	Resolve C18	Resolve Silica	Symmetry C18	Symmetry Silica	XTerra MS C18	Underivatized XTerra
Particle size (μm)	5	3	–	5	5	5	5	5	5	5
Pore size, ϕ (Å)	100	120	13 nm[a]/2 mm[b]	100	90[c]	90	86[c]	90	125[c]	123
Surface area (m^2/g)	350	300	300	400	200	200	346	341	175	169
Carbon load (%)	21	16.5	18	17.5	10.2	0	19.6	0	15.5	6.8
Endcapped	No	Yes	Yes	Yes	No	No	Yes	No	Yes	No
Dimensions, length × i.d. (mm)	250 × 4.0	125 × 2.0	100 × 4.6	150 × 4.6	150 × 3.9	150 × 3.9	150 × 4.6	150 × 4.6	150 × 4.6	150 × 4.6
pH range	2–7.5	2–7.5	2–7.5	2–8	2–8	2–8	2–8	2–8	1–12	1–12
Manufacturer	Merck (Darmstadt Germany)	Merck (Darmstadt Germany)	Merck (Darmstadt Germany)	Phenomenex (Torrance, CA, USA)	Waters (Milford, MA, USA)	Waters (Milford, MA, USA)	Waters (Milford, MA, USA)	Waters (Milford, MA, USA)	Waters (Milford, MA, USA)	Waters (Milford, MA, USA)

[a] Mesopore.
[b] Macropore.
[c] Data for the packings before derivatization.

types of silanols present, we shall consider a general model for m different types of silanols. In this instance:

$$n_{A(S)} = \sum_{i=1}^{m} n_i \alpha_i \tag{4}$$

where n_i is the overall number of silanols of type i and α_i is the degree of ionization of these silanols, which is related to the acidity of the particular type of silanols (pK_{ai}) and to the pH of the mobile phase through:

$$\alpha_i = \frac{K_{ai}}{K_{ai} + [H^+]} = \frac{1}{1 + 10^{pK_{ai} - pH}} \tag{5}$$

Thus, Eq. (3) can be written as

$$k = \frac{K_{A^+}^{B^+}}{n_{A(M)}} \sum_{i=1}^{m} n_i \alpha_i \tag{6}$$

After substitution of Eq. (5) in Eq. (6) and rearrangement of terms, Eq. (7) that relates the retention of B^+ ion to the pH of the mobile phase is obtained:

$$k = \frac{K_{A^+}^{B^+} n_1}{n_{A(M)}} \sum_{i=1}^{m} \left(\frac{n_i / n_1}{1 + 10^{pK_{ai} - pH}} \right) \tag{7}$$

The retention factor of B^+ is calculated by the well-known relationship:

$$k = \frac{t_R - t_M}{t_M} \tag{8}$$

where t_R is the retention time of B^+ and t_M the elution time of an appropriate hold-up time marker.

Since $n_{A(M)}$ can be also related to t_M, through the mobile phase flow-rate in the column (F_C) and the concentration of A^+ ion in the mobile phase:

$$n_{A(M)} = [A^+]_M V_M = [A^+]_M t_M F_C \tag{9}$$

the following Eq. (10) that directly relates the retention time of the B^+ ion to the pH of the mobile phase is obtained:

$$t_R = t_M + \frac{K_{A^+}^{B^+} n_1}{[A^+]_M F_C} \sum_{i=1}^{m} \left(\frac{n_i / n_1}{1 + 10^{pK_{ai} - pH}} \right) \tag{10}$$

Eq. (10) was derived and used in the previous work [34], but in this study we shall use it in terms of the retention factor, Eq. (11), in order to get a better comparison of the retention caused by the silanols present in the different columns studied.

$$k = \sum_{i=1}^{m} \left(\frac{k_i}{1 + 10^{pK_{ai} - pH}} \right) \tag{11}$$

with

$$k_i = \frac{K_{A^+}^{B^+} n_i}{t_M [A^+]_M F_C} \tag{12}$$

where k_i is the maximum retention that can be achieved by the specific type i of silanols (with acidity pK_{ai}).

It must be noted that the degree of ionization of the silanols depends on the pK_a values of the different silanols in the particular mobile phase used and on the pH of this mobile phase. pK_a and pH values are solvent dependent parameters, and then they change with the composition of the mobile phase. We have widely discussed pH measurement in RPLC mobile phases [35–39] and recommend the use of the ${}^{s}_{w}$pH and ${}^{s}_{s}$pH scales. The ${}^{s}_{w}$pH value can be easily measured in the particular mobile phase after mixing aqueous buffer and organic modifier with an electrode system calibrated with the usual aqueous buffers. The ${}^{s}_{s}$pH value can then be obtained from the ${}^{s}_{w}$pH by means of Eq. (13), where $\delta = 0.17$ for 60% methanol [35,37,38].

$$ {}^{s}_{s}\mathrm{pH} = {}^{s}_{w}\mathrm{pH} - \delta \tag{13} $$

The acidity (pK_{ai}) values and the maximum retention factor (k_i) caused by the different types of silanols are obtained by non-linear regression from the retention factor of B^+ and the measured pH values of the mobile phase. The pK_{ai} values obtained would be ${}^{s}_{w}\mathrm{p}K_{ai}$ or ${}^{s}_{s}\mathrm{p}K_{ai}$ depending on the pH values used (${}^{s}_{w}$pH or ${}^{s}_{s}$pH, respectively).

2.2. Anionic exchange

While ionization of acidic silanols leads to ion-exchange effects with sample cations, sample anions may also undergo retention, if protonated sites exist on the column surface. These protonated sites (RH^+) may act as anionic exchangers between the anion of the background electrolite (A^-) and the anions of the sample (B^-)

$$ RH^+A^- + B^- \Leftrightarrow RH^+B^- + A^- $$

this equilibrium is governed by a selectivity coefficient $K^{B^-}_{A^-}$, equivalent to that of Eq. (1) for cationic exchange,

$$ K^{B^-}_{A^-} = \frac{[B^-]_S[A^-]_M}{[A^-]_S[B^-]_M} \tag{14} $$

As in Eq. (3), the retention factor of B^- can be given as:

$$ k = K^{B^-}_{A^-}\frac{n_{A(S)}}{n_{A(M)}} \tag{15} $$

Since there may be more than one type of protonated sites in the stationary phase, Eq. (4) can be applied, but taking into account that:

$$ \alpha_i = \frac{[H^+]}{K_{ai} + [H^+]} = \frac{1}{1 + 10^{\mathrm{pH}-\mathrm{p}K_{ai}}} \tag{16} $$

Following the same steps described for cationic exchange (Section 2.1), Eqs. (17) and (18) are obtained:

$$ t_R = t_M + \frac{K^{B^-}_{A^-}n_1}{[A^-]_M F_C}\sum_{i=1}^{m}\left(\frac{n_i/n_1}{1 + 10^{\mathrm{pH}-\mathrm{p}K_{ai}}}\right) \tag{17} $$

or

$$ k = \sum_{i=1}^{m}\left(\frac{k_i}{1 + 10^{\mathrm{pH}-\mathrm{p}K_{ai}}}\right) \tag{18} $$

It should be noticed that for anion exchange, the term pK_{ai} − pH from Eqs. (10) and (11) is replaced by pH − pK_{ai}.

3. Experimental

3.1. Apparatus

Two different equipment assemblies were used. One was an ISCO Model 2350 dual-pump system with a 10 μl loop valve connected to the following conductivity detectors: Ω Metrohm 690 or Shimadzu CDD-10Avp. The first detector was employed for the Merck LiChrospher column, like for other stationary phases analysed in a previous work [34], while the second one, was used for all the other packings in this study. In this system, data was acquired through the ISCO ChemResearch data management program. The second assembly was a Shimadzu liquid chromatograph configured with two LC-10AD pumps and a SIL-10AD auto-injector, connected to an Applied Biosystems Sciex API 150EX single quadrupole mass spectrometer (MS). For this study, we employed the electrospray in positive and negative modes. The mass spectrometer conditions were: turbo probe temperature, 80 °C; declustering potential, 30 V; nebuliser and curtain gases (nitrogen) were set at 12 and 8 arbitrary units, respectively; the ionspray voltage was maintained at −3 kV. For full-scan acquisition mode, the mass spectrometer was operated over the mass range *m/z* 30–300 in the centroid mode, at a cycle time of 1 s and with interscan time of 0.1 s. Data acquisition was conducted using PE Sciex Analyst software (version 1.1).

Our previous studies [33,34], demonstrated that mass spectrometry and conductimetric detection could be both employed to evaluate the ionic exchange between Li^+ ion and the Na^+ ions from the mobile phase retained in the column by the ionized silanols. In this study, we have employed conductimetric detection instead of MS, due to its lower cost and simplicity. The use of MS was limited to a few mobile phases to confirm peak assignments of conductimetric results.

The columns studied are detailed in Table 1, including those reported on a previous publication [34]. The C18 columns were used with their corresponding guard columns, recommended by the manufacturer. Extracolumn times were measured with the same assembly by replacing column and pre-column by a zero-volume connection.

pH measurements were performed with a Crison, MicropH 2002 potentiometer and an Orion 8102 Ross combined glass electrode.

Conventional silica supports have a stability pH range between 2 and 7.5, while some modern packings had extended this pH interval up to 10–12 [6,13]. We have studied the pH range recommended by the manufacturer for each particular column. In some cases, however, the study was extended over the specified pH range.

3.2. Chemicals

Methanol used was HPLC-grade (Merck, Darmstadt, Germany), and the deionized water from Milli-Q plus system (Millipore, Bedford, MA, USA). Buffers were prepared from hydrochloric acid (Merck, for analysis 25%), sodium acetate (Carlo Erba, Milan, Italy), trisodium phosphate (Merck), sodium tetraborate decahydrate (Aldrich, Milwaukee, WI, USA) and sodium carbonate anhydrous (Merck). Lithium nitrate was from Prolabo (Barcelona, Spain), 99% purified.

3.3. Procedure

The mobile phase used was methanol/water (60:40 v/v) buffered to different pH values. Acetate, phosphate, borate, and carbonate buffers were used. The sodium concentration was kept constant to 1 mM in the aqueous portion of the mobile phase. The mobile phase was filtered through a 0.45 μm pore-size nylon membrane (Whatman, Maidstone, Kent, UK) and degassed for 15 min by a helium stream. The mobile phase flow was 1 mL min^{-1}, except for the Purospher column, that was set at 0.3 mL min^{-1}. For MS studies, the split ratio of flow was controlled between 1/20 and 1/30.

Solutions of 0.01 mol L^{-1} $LiNO_3$ in methanol/water (60:40) were prepared and filtered through 0.45 μm nylon filter, and 10 μL of the $LiNO_3$ solution were injected into the HPLC systems. A change of sensitivity of the conductimetric detector was done just after the elution of NO_3^- because the signal of Li^+ is significantly lower than that of NO_3^-. Additionally, because of the possibility of the Shimadzu detector, the polarity was reversed for the acquisition of the Li^+ ion in positive mode. All results obtained were the mean of at least three injections.

In all experiments, the column was first equilibrated with the mobile phase for at least 30 min.

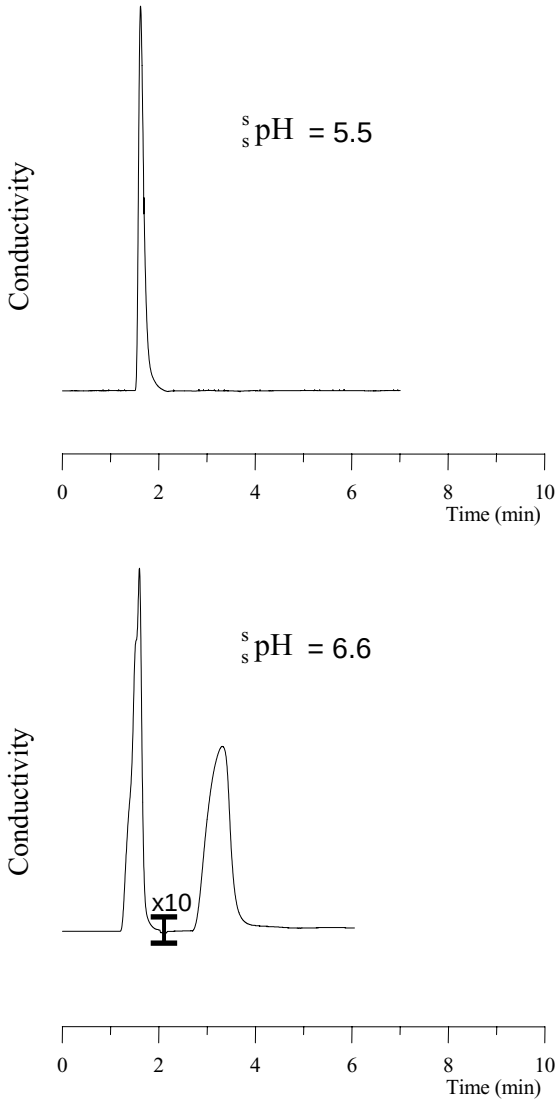

Fig. 1. Chromatograms for the elution of $LiNO_3$ from the Luna C_{18} (2) column at two mobile phase pH values obtained by conductimetric detection. Sensitivity of the detector was increased and the polarity changed just after elution of the NO_3^- peak.

4. Results and discussion

Fig. 1 presents the conductimetric chromatogram obtained for the injection of $LiNO_3$ in the Luna C_{18} (2) column with a methanol/water (60:40 v/v) mobile phase buffered at two different pH values (with a 1 mM NaAc + HCl solution). Fig. 1 shows the presence of only one peak at $^{s}_{s}$pH = 5.5, that indicates NO_3^- and Li^+ are eluted together since there is no cation exchange for Li^+ in the $^{s}_{s}$pH range between 3.0 and 5.5 in this column. However, at $^{s}_{s}$pH 6.6, Li^+ elutes after the NO_3^- peak, because of the ion exchange produced between the lithium and the sodium ions retained by the ionized silanols. Therefore, the retention time of the Li^+ ion in this column was measured and fitted to the mobile phase $^{s}_{s}$pH values through Eq. (10). The fitting parameters and statistics obtained for this and the other columns studied are presented in Table 2 and the retention plot is depicted in Fig. 2.

Table 2 contains also data for the columns studied in the previous work [34]. The results given may be slightly different than those previously published because all data have been recalculated in terms of Eq. (11) instead of Eq. (10). The new equation is more rigorous than the previous one (Eq. (10)) since the old equation computed the n_i/n_1 term, which show large standard deviations (S.D.) associated to this terms when n_1 is very small. The new equation computes directly pK_a and k associated to each type of silanols, and not related to one specific type. Thus, errors associated to these values depend on the absolute amount of silanols of the particular type. The new equation has been applied to the old data for Underivatized XTerra, which could not be fitted to Eq. (10), although the results presented for this column in Table 2 are only approximated, because of the small number of points.

Table 2
Fitting parameters and statistics for the columns studied

	Lichrospher RP-18	Lichrospher RP-18	Luna C_{18} (2)	Luna C_{18} (2)	Resolve C18	Resolve Silica	Symmetry C18	Symmetry Silica	Underivatized Xterra
t_M	1.34	1.34	1.29	1.29	0.94	0.90	1.26	1.15	1.39
pH range	2–7.5	2–7.5	2–10	2–10	2–8	2–8	2–10	2–8	1–12
m	2	3	2	3	2	2	2	2	2
$^{s}_{s}pK_1$	3.64 ± 0.08	3.09 ± 0.28			3.66 ± 0.05	3.50 ± 0.03			
k_1	4.18 ± 0.25	2.45 ± 0.56			9.86 ± 0.33	11.99 ± 0.24			
$^{s}_{s}pK_2$	5.78 ± 0.12	4.69 ± 0.26		5.65 ± 2.33				4.65 ± 0.17	
k_2	3.97 ± 0.25	3.19 ± 0.43		0.29 ± 0.67				2.41 ± 0.31	
$^{s}_{s}pK_3$		6.27 ± 0.22	7.17 ± 0.08	7.24 ± 0.17	6.45 ± 0.12	6.16 ± 0.04	7.87 ± 0.12	6.84 ± 0.07	
k_3		2.99 ± 0.38	5.23 ± 0.31	5.07 ± 0.56	10.05 ± 0.80	14.70 ± 0.31	2.19 ± 0.37	19.22 ± 1.06	
$^{s}_{s}pK_4$			9.12 ± 0.06	9.14 ± 0.07			9.39 ± 0.29		8.97 ± 0.02
k_4			11.70 ± 0.37	11.61 ± 0.42			2.65 ± 0.34		18.59 ± 0.39
r^2	0.990	0.997	0.997	0.997	0.993	0.998	0.989	0.997	0.999
S.D.	0.23	0.14	0.26	0.27	0.44	0.31	0.15	0.27	0.09
F	421	652	2374	1349	623	2583	515	2181	15035

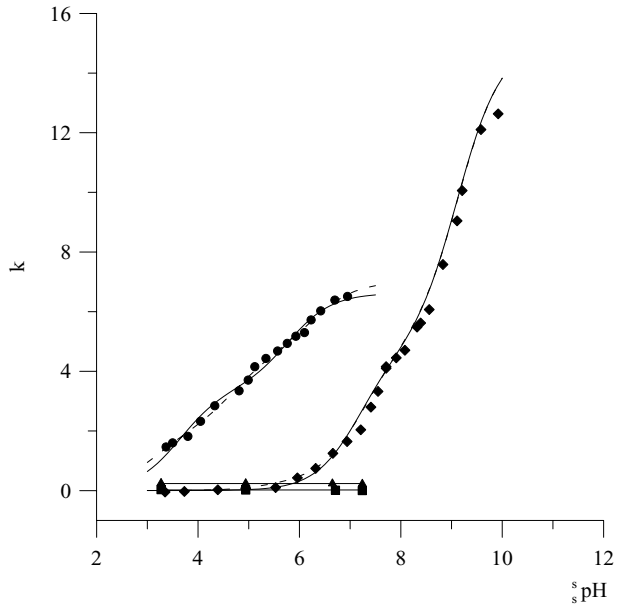

Fig. 2. Dependence of the retention factor of Li^+ in C18 columns: (◆) Luna C_{18} (2), (●) LiChrospher 100 RP-18, (■) Purospher RP-18e, and (▲) Chromolith RP-18e with the pH of the methanol—(0.001 M Na^+) (60:40 v/v) mobile phase detected by conductimetry. Fitting lines were calculated according to the model of Eq. (11) for ionic exchange assuming: (—) $m = 2$, (- - -) $m = 3$.

Since the distribution of pK_a values and types of residual silanols among columns may vary, the pK_a data and the corresponding k values have been distributed in four pH ranges. The first pH range comprises the results obtained for $^{s}_{s}pK_a$ values lower than 4 (indicated as $^{s}_{s}pK_1$ and the associated k_1). The second pH range comprises results for residual silanols with an acidity between pK_a 4 and pK_a 6 ($^{s}_{s}pK_2$ and k_2). The third pH range corresponds to the acidity between pK_a 6 and 8 ($^{s}_{s}pK_3$ and k_3). The last pH range considered is for acidity with pK_a values larger than 8 ($^{s}_{s}pK_4$ and k_4). This procedure gives a coherent distribution of silanol pK_a values that allows an easy comparison of the different columns studied.

In our previous works [33,34], we have observed that the shape of the t_R versus $^{s}_{s}$pH plot for several columns indicates that at least two different types of silanols ($m = 2$) are needed to explain the variation of the retention of Li^+ with pH. In several cases, the $m = 3$ fittings were also tested. The models with two and three different types of silanols give both good fits to the experimental data obtained here for Luna C_{18} (2) and LiChrospher 100 RP-18 columns. In fact, the two correlation coefficients and the overall standard deviations are similar in both models, while the F parameter (Fisher's test) is better for $m = 2$ than for $m = 3$, specially for the Luna column.

For the Luna column, the model fitted to two different types of silanols gives two $^{s}_{s}pK_a$ values of 7.17 and 9.12. The model $m = 3$ gives three different types of silanols, two of them with pK_a values and retention k very similar to

those obtained for $m = 2$. The presence of the third type of silanols (${}^{s}_{s}pK = 5.65$ more acidic than the other two) is uncertain since the retention associated to it is not significant ($k_1 = 0.29 \pm 0.67$). The effect of this possible type of silanol is noted as a small shoulder between pH 4 and 7 in Fig. 2.

On the other hand, the retention data of Li^+ in LiChrospher 100 RP-18 column (Fig. 2) was also fitted to pH through Eq. (11). For $m = 2$, the ${}^{s}_{s}pK_a$ values obtained were 3.64 and 5.78, which shows a fair agreement with the values found in a previous study for this column [33] with a small number of experimental data points. However the fitting of the data to the model $m = 3$, gives a first pK_a value very acidic (3.09), a second pK_a value (4.69) very close to that of Symmetry Silica (4.65) and a third pK_a value (6.27) very similar to that of Resolve Silica (6.16). For this column, all three types of silanols seem significant. In some instances it may be difficult to decide if there are two or three different types of silanols. In any case, the interpretation of the retention of Li^+ as a function of pH in terms of two or three pK_a's is only an approximation of reality. Thus, we cannot consider the residual silanols as a single and specific entity on the silica support. It probably has a continuum of pK_a values in the pH range that can be clustered to two or three types specified by the two or three pK_a values found in the fitting. The importance of the silanols clustered is measured by the associated k_i value.

The ${}^{s}_{s}pK_a$ values found for LiChrospher more acidic than those for Luna are consistent with the fact that the former corresponds to an older design, i.e. lower purity, and consequently more acidic silica; while the Luna column is a newer packing material of higher purity silica and as a result it should be a less acidic phase. It is well known that the presence of metal impurities increases the acidity of the residual silanols [6,11,12]. Thus, it is probable that the Lichrospher packing contains more metal impurities than the Luna packing.

The presence of residual silanols in the Purospher RP-18e column was also studied. Fig. 3 presents the conductivity chromatograms for this column at two different pH values. In these experiments, a single positive peak at low retention times is observed. The position of the peak practically does not change with the pH of the mobile phase for a wide interval of pH (up to pH 7, Fig. 3), which suggests that there is no ion exchange process for this packing material in this pH range. From pH 7 the presence of a small valley just after NO_3^- elution is observed (Fig. 3). However, due to the universal character of the conductimetric signal, we cannot differentiate between each ion contribution. MS detection was employed to confirm if this valley could be related to the Li^+ signal.

Mass spectrometry chromatograms (Fig. 4) show that, at ${}^{s}_{s}pH = 6.6$, Li^+ (detected as the $[LiAc_2]^-$ adduct) is eluted at the same time as NO_3^-, giving a single peak, which is in rough agreement with the single peak observed in the conductivity experiments (Fig. 3). At ${}^{s}_{s}pH = 8.7$ in Fig. 4 the retention time of Li^+ (detected as the $[LiHPO_4]^-$ adduct)

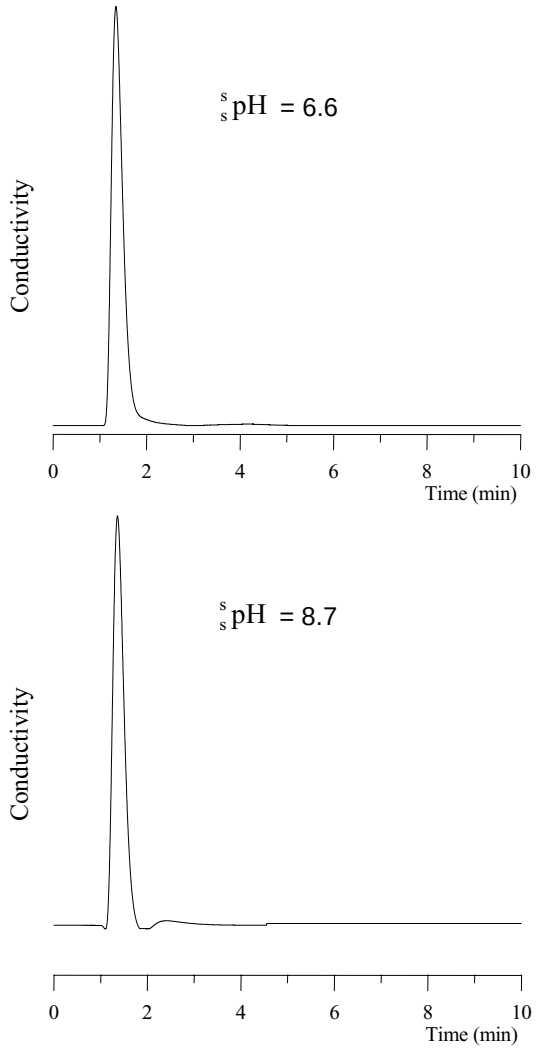

Fig. 3. Chromatograms for the elution of $LiNO_3$ from the Purospher RP-18e column at two mobile phase pH values obtained by conductimetric detection.

appears immediately after the elution of NO_3^-, which relates the valley observed in conductivity experiments, to the Li^+ signal, i.e. a small cation exchange process is demonstrated. However, the variation is so small that it is not enough to be fitted to the model. Since this pH is already above the maximum pH of use recommended by the manufacturer it may also be attributed to a small dissolution of the silica base and it has not been investigated.

A similar behaviour was observed with the Chromolith column. The conductivity and mass spectrometry chromatograms for $LiNO_3$ in this column are presented in Figs. 5 and 6, respectively. As shown in Fig. 2, the retention time of Li^+ almost does not change with the pH, which indicates no cation exchange for Li^+ in the pH range between 3 and 7, although there is a small retention of Li+ at pH >7.0. These results shed some light on the studies performed by McCalley [30] concerning to the analysis of basic compounds using this monolithic column.

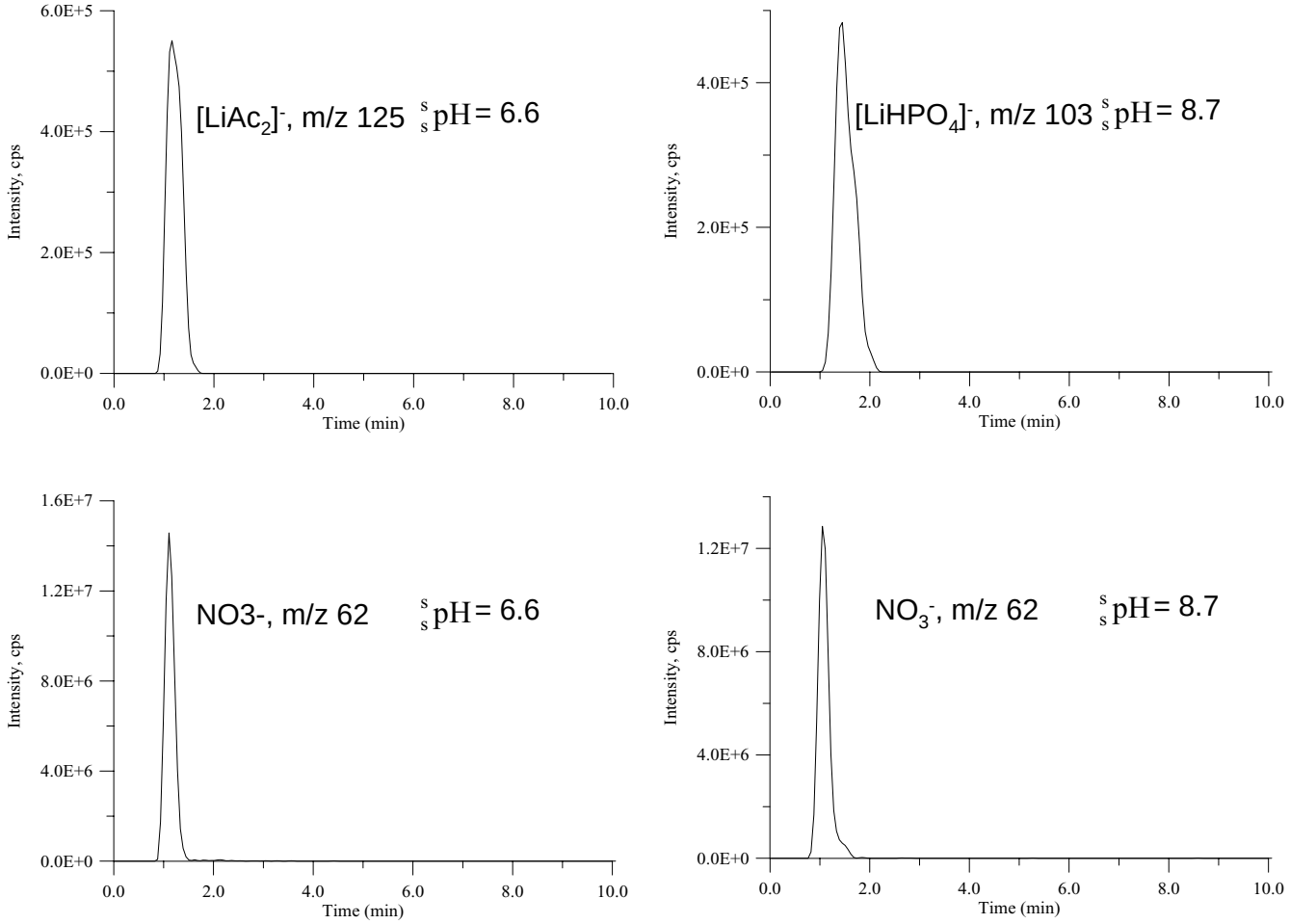

Fig. 4. Chromatograms for the elution of $LiNO_3$ from the Purospher RP-18e column at two mobile phase pH values detected by mass spectrometry. Li^+ ion has been detected as the $[LiAc_2]^-$ adduct (*m/z* 125) at $^s_spH = 6.6$, and as the $[LiHPO_4]^-$ adduct (*m/z* 112) at $^s_spH = 8.7$.

Thus, the resulting poor peak shapes obtained for strong bases in this column at pH 7 (acetonitrile/water (30:70 v/v) mobile phase buffered with phosphate) could be attributed to the different phase structure of monoliths rather than to a probable silica activity under these conditions, because the presence of residual silanols, if any, should be very low.

From the above results, we can conclude the low evidence of residual silanols for Purospher and Chromolith columns at pH values lower than 7, which confirms the higher quality

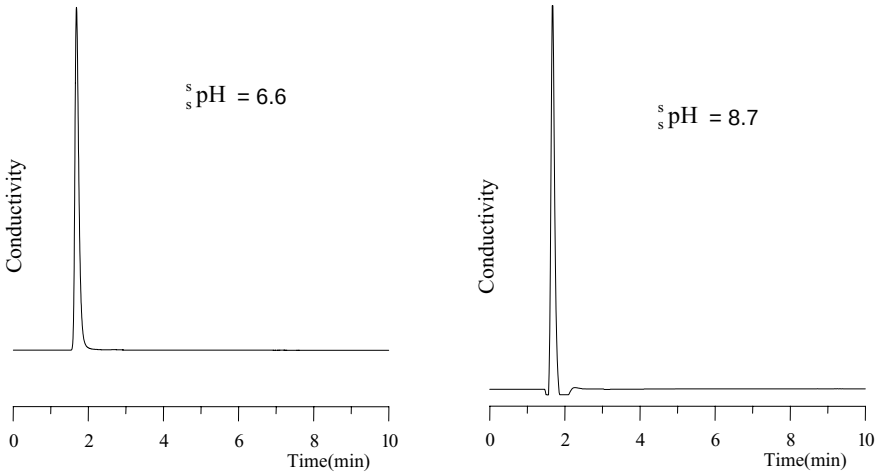

Fig. 5. Chromatograms for the elution of $LiNO_3$ from the Chromolith RP-18e column at two mobile phase pH values detected by conductimetry.

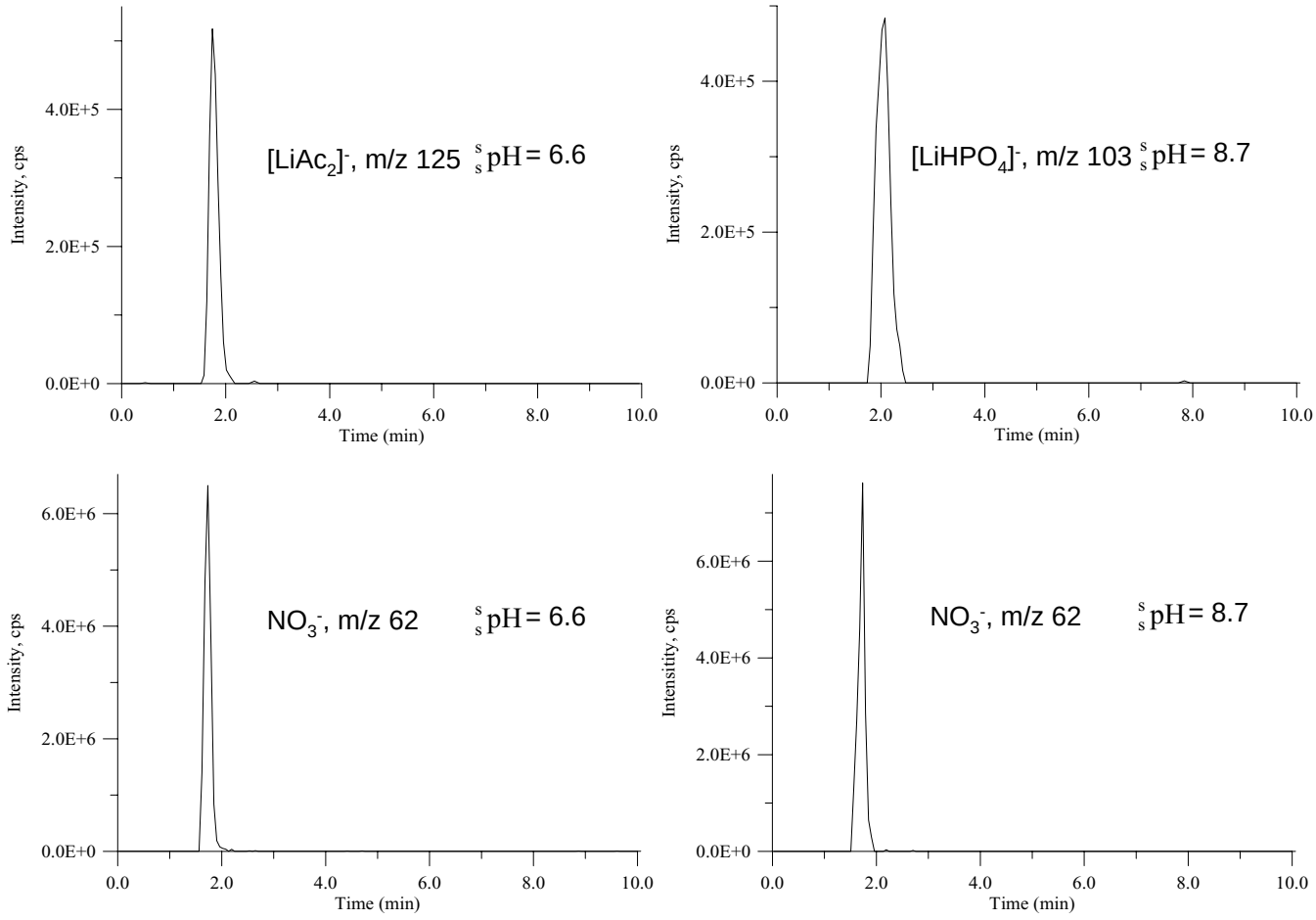

Fig. 6. Chromatograms for the elution of $LiNO_3$ from the Chromolith RP-18e column at two mobile phase pH values detected by mass spectrometry. Peak identification and other conditions as in Fig. 4.

of the silica employed in their manufacture, in conjunction with the effective end-capping of the silanol groups in these stationary phases. The presence of basic residual silanols have not been extensively investigated in both columns due to the stability pH limit of these packings, although the data obtained at ${}^{s}_{s}$pH 8.7 suggest that it may be a small number of ionized silanols at this pH value (perhaps caused by the dissolution of the silica).

The results found here for these two last columns are consistent with those previously published for Symmetry C18, Underivatized XTerra packing and XTerra MS C_{18} columns [34], where residual silanols were not detected in the range ${}^{s}_{s}$pH 3–7. Additionally, in the latter stationary phase, no evidence of residual silanols at ${}^{s}_{s}$pH values lower than 10 was observed, which confirmed the higher resistance to the hydrolysis of the hybrid support.

In the previous work [34], evidence of anion exchange was noticed in Symmetry C18 column at acidic pH values (${}^{s}_{s}pK_a = 5.4$, $k = 2.70$ according to Eq. (18)), which was attributed to residues of the base used in the bonding process. We have tested the possibility of anion exchange for Luna C_{18} (2) and LiChrospher 100 RP-18, Purospher RP-18e and Chromolith RP-18e and no evidence at all of this process was observed.

This study shows that the residual silanol activity of common C18 columns (and also silica columns) can be characterized through the model described by Eq. (11). Several types of silanols (or clusters of silanols) may contribute to the residual silanol activity. Each type of silanol is described by two parameters: its acidity (pK_a) and its effective activity (k). The acidity determines the pH value from which the interaction of cationic solutes with the silanol will be noticed. The effective activity measures the extent of these interactions, i.e. the retention of the cation that will be observed. Both parameters can be represented in an XY plot (Fig. 7) that can be used for comparison of columns. It provides information (acidity and activity) of the different types of silanols present in the different columns characterized. The plot shows that both Resolve C18 and LiChrospher 100 RP-18 have acidic silanols, below $pK_a = 7$, although the activity of Resolve C18 should be larger than the one for LiChrospher 100 RP-18. Symmetry C18 and Luna C_{18} (2) will present silanol activity only above pH 7 and the activity of Luna should be much larger than that of Symmetry. The

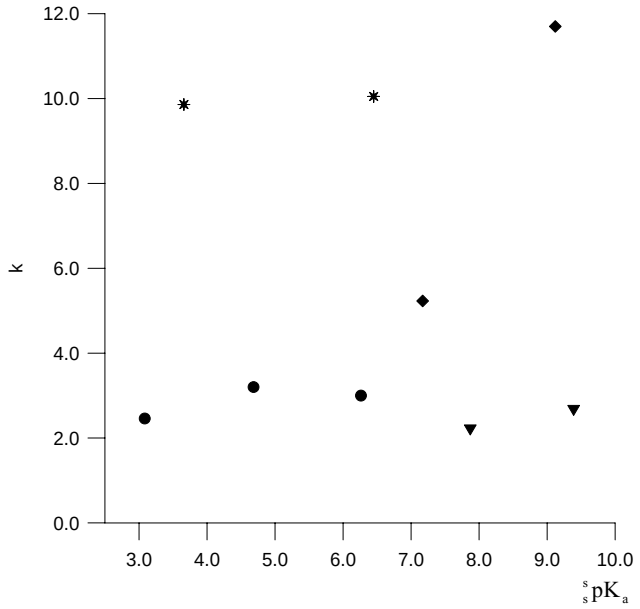

Fig. 7. Distribution of the columns in the k vs. pK_a chart. (◆) Luna C_{18} (2), (●) LiChrospher 100 RP-18, (✱) Resolve C18, (▼) Symmetry C18.

activity of the three groups of silanols in LiChrospher and the two groups of Symmetry is very similar. The two silanol groups of Resolve have also similar activities, much higher than the ones of LiChrospher and Symmetry. Luna column presents a first group of silanols with a low activity (slightly higher than the ones of LiChrospher and Symmetry) and a group of most basic silanols with a much higher activity.

An even more useful plot is presented in Fig. 8. The information given in Fig. 7 (or Table 2) has been used to calculate the k value of each studied column, including C18 and silica columns at each pH value, by using Eq. (11). This Figure allows an easy comparison of the performance, related to silanol activity, of the different columns.

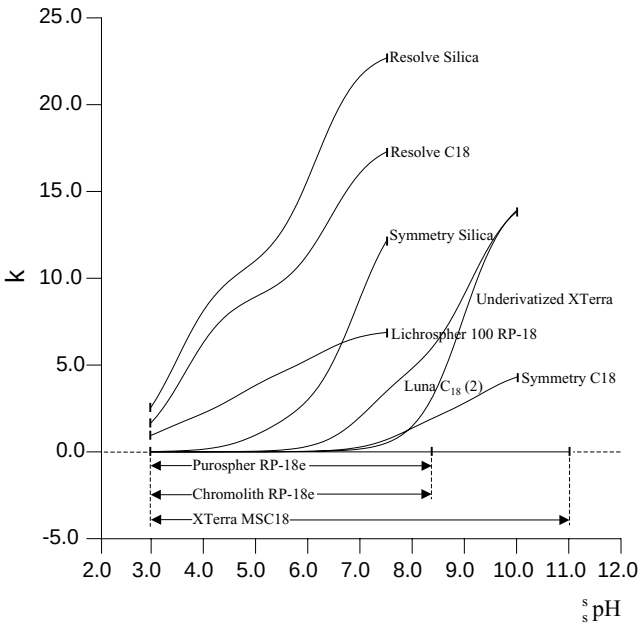

Fig. 8. Variation of silanol activity of stationary phases with the pH of the mobile phase. (|↔|) sspH range studied.

Comparison of C18 with silica columns shows that, as expected, derivatization decreases silanol activity, but in a different degree for each column. The decrease for Resolve C18 in reference to Resolve Silica is small because the column has not been end-capped and thus many silanols remain active. For end-capped and hybrid silica columns, such as Symmetry and XTerra, the reduction of silanol activity when the column is derivatized is very effective.

Among the C18 columns, Resolve C18 is the column that shows the larger silanol activity (Fig. 8), despite that Lichrospher 100 RP-18 seems to have the most acidic silanols (Fig. 7 and Table 2) although in a very low number. Lichrospher is the second most active column, followed at some distance by the more pure silica-based columns Luna C_{18} (2) and Symmetry C18. Purospher RP-18e, Chromolith RP-18e and XTerra MSC_{18} do not show any activity in the pH range of use recommended by the manufacturers.

Fig. 8 is very useful to determine what silanols activity is expected for a particular column with a mobile phase of known pH. For instance, at mobile phase pH 6, XTerra MSC_{18}, Chromolith RP-18e, Purospher RP-18e and also Symmetry C18 should not show silanol activity. Luna C_{18} (2) should show a very low silanol activity. Lichrospher 100 RP-18 will show a large silanol activity and Resolve C18 even higher silanol activity.

5. Conclusions

In this article, we have extended our method to characterize the acidity of residual silanols to several conventional microparticulate and a monolithic C18 packing. Conductivity detection has been proved to be a very useful technique to evaluate the ionic exchange between Li^+ ion and the Na^+ ions from the mobile phase retained in the column by the ionized silanols. However, mass spectrometry detection may be needed sometimes to differentiate between Li^+ and NO_3^- elution when there may be a small silanol activity.

The analysis of the retention time of Li^+ for the Luna C_{18} (2) column show that it does not present silanol interaction up to sspH = 6.0; however, at higher pH values (sspH = 9), a large proportion of silanols were evidenced. These results are in agreement with the sspK_a values found for the silanols (9 or larger) for XTerra underivatized or Symmetry C18 packing [34].

For LiChrospher 100 RP-18 column, we found that three different types of silanol groups with different acidity may be present to explain the retention behavior of Li^+, which is in good agreement with the results found for other columns studied (Resolve C18, Resolve Silica and Symmetry Silica).

Purospher RP-18e and Chromolith RP-18e packings do not present residual silanols in the pH range 3–7. These

columns may show a low level of silanols above pH 7.5, this being the stability limit of the packings. These results are similar to those previously published for Symmetry C18, Underivatized XTerra packing and XTerra MS C_{18} column [34].

Finally, we have demonstrated that this test constitutes a powerful tool not only for the chromatographic discrimination between classical silica ('type A'), high purity silica substrates ('type B') and new hybrid silica packings, but also to evaluate the different quality in these different types of materials in function of its acidity and relative population of silanols, which undoubtedly could aid in the selection of suitable stationary phases for the analysis of basic compounds.

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