separations

MDPI

Article

Hydrophilic Liquid Chromatography versus Reversed-Phase Liquid Chromatography in the Absence and the Presence of 1-Hexyl-3-methylimidazolium Chloride for the Analysis of Basic Compounds

Ester Peris-García, Raquel Burgos-Gil, María Celia García-Alvarez-Coque and María José Ruiz-Angel *

Departament de Química Analítica, Universitat de València, c/Dr. Moliner 50, 46100 Burjassot, Spain; ester.peris@uv.es (E.P.-G.); raburgil@alumni.uv.es (R.B.-G.); celia.garcia@uv.es (M.C.G.-A.-C.)

* Correspondence: Maria.J.Ruiz@uv.es

Received: 14 April 2020; Accepted: 27 May 2020; Published: 29 May 2020

Abstract: In reversed-phase liquid chromatography (RPLC), positively charged basic compounds yield broad and asymmetric peaks, as a result of ionic interactions with free silanols that remain on conventional silica-based columns. Diverse solutions have been proposed to mask the silanophilic activity, which is translated to an improved peak shape. In this work, the chromatographic performance of hydrophilic interaction liquid chromatography (HILIC) was evaluated as an alternative to the addition of an ionic liquid (IL) to the aqueous-organic mobile phase used with RPLC columns, for the analysis of eight β-adrenoceptor antagonists. ILs change the behavior of RPLC stationary phases owing to adsorption on their surface. Meanwhile, in HILIC, a layer of adsorbed water is formed on the stationary phase surface. The association of cationic basic compounds with the adsorbed additive ions, hydrophilic partitioning on the HILIC columns, and other interactions, give rise to complex retention mechanisms. The chromatographic behavior was examined in terms of retention, elution strength, selectivity, peak shape and resolution, using acetonitrile-water mobile phases buffered at pH 3. Both chromatographic modes, RPLC with added IL and HILIC, proved to be a viable solution to the problem of poor peak shape for basic compounds.

Keywords: hydrophilic interaction liquid chromatography; reversed-phase liquid chromatography; ionic liquids; basic compounds; chromatographic behavior

1. Introduction

Chromatographic analysis of basic compounds in the reversed-phase mode (RPLC) with conventional alkyl-bonded stationary phases (usually octyl or octadecylsilane) has the handicap of producing broad and asymmetric peaks, due to the direct interaction of the cationic species, formed in the acidic mobile phases, with the residual silanols in the silica supports. Residual silanols are present in a non-negligible amount on alkyl-bonded stationary phases, due to steric problems in the silica derivatization process [1,2]. These groups are weakly acidic, with an average acidity constant (pK_a) approximately in the range 4.5 to 7, which depends on the type of silica used to build the column [1]. This means that in the working pH range of typical RPLC columns ($2 < pH < 8$), silanols are negatively charged, producing ion-exchange interactions with cationic analytes, which increases the retention. As the sorption-desorption kinetics of these interactions is slow, broad and tailed peaks are obtained, with implications in peak resolution [2–4]. For this reason, the minimization and/or suppression of the "silanol effect" is a challenge in this chromatographic mode.

The reduction and even suppression of the undesirable effects of residual silanols is carried out using diverse strategies. "Ultrapure" silica has been used to synthesize a new generation of columns, where the effect of silanols on the retention of basic compounds is less significant [2,5]. However, in these columns some tailing with long analysis times is still observed. Some alternative (very extended) solutions are: (i) reducing the mobile phase pH below 3.5 in order to protonate the residual silanols, and (ii) masking the silanols with cationic, anionic, or even non-ionic reagents, added to the mobile phase, which are adsorbed on the stationary phase [6]. The use of such reagents in the mobile phase is a simple approach that does not require the use of instrumentation or special columns.

Amines, especially bulky tertiary ones, and surfactants (anionic, cationic and non-ionic) are among the most frequently assayed additives [7,8]. In the last decade, ILs have been reported as an interesting alternative [9–18]. These reagents are low melting organic salts (often melting below 100 °C). When added to the mobile phase in RPLC, cation and anion of the IL may interact with the stationary phase [17]. This creates a charged bilayer that prevents the penetration of the basic compounds through the alkyl-bonded chains to reach the silanol sites on the support. According to previous work, this effect is larger for bulky imidazolium ILs, such as those formed with the cation 1-hexyl-3-methylimidazolium [C_6C_1im], which yields highly symmetric peaks in practical analysis times [19,20]. The benefits of similar ILs cations with shorter chain are smaller. The use of ILs cations with a longer chain, such as 1-octyl-3-methylimidazolium, can be interesting, but owing to its poor solubility, they cannot be used. Different anions have been also evaluated for ILs. Chloride has the advantage of poor adsorption on RPLC columns. Anions with larger adsorption increase the retention of basic compounds [17,20]. In general, the multiple interactions with both mobile phase and stationary phase (mainly ion-pairing, electrostatic attraction and repulsion with the IL ions, together with hydrophobic partitioning), makes the interpretation of the retention mechanisms difficult. In the two last decades, hydrophilic interaction liquid chromatography (HILIC) has been demonstrated to be a useful chromatographic mode for the analysis of highly polar compounds [21–23]. HILIC is a complex chromatographic mode where partitioning of analytes with high polarity between the mobile phase and stationary phase (and consequently, the retention) is modulated by the formation of a water-rich adsorbed layer on the surface of polar stationary phases [22–25]. The hydrophilic interaction with this water layer is the main retention mechanism, which modifies the selectivity with regard to RPLC. Other secondary interactions of solutes in HILIC are hydrogen bonding, dipole–dipole and ion-exchange. In previous work [26], it was checked that the chromatographic peaks of cationic polar nucleosides with different HILIC columns are almost symmetric. This was explained by the masking effect of the activity of residual silanols by the adsorbed water layer on the stationary phase. A few reports have been published, where the possible benefits of HILIC for the chromatographic separations of basic compounds are described. McCalley et al. studied different types of HILIC columns obtaining excellent peak shape with silica phases [5,27,28]. An ACE cyano column was also used for the separation of some basic compounds, including oxprenolol and its impurities [29]. More recently, five different types of HILIC columns (two silica, two cyanopropyl and one diol) were evaluated for the analysis of six basic drugs [30]. Chromatographic analysis with HILIC columns of β-adrenoceptor antagonists, which have a basic character, has been recently suggested [31]. In this work, the behavior of eight β-adrenoceptor antagonists is explored in HILIC using a zwitterionic stationary phase, in order to study the suitability of the HILIC mode to avoid the silanol effect. The chromatographic performance with HILIC is compared with that obtained with RPLC mobile phases in the presence and absence of the IL [C_6C_1im]Cl. The behavior was examined in terms of retention, elution strength, selectivity, peak shape and resolution. β-adrenoceptor antagonists are commercialized for the treatment of various cardiac diseases [32]. The aromatic ring attached to a side alkyl chain with secondary hydroxyl and amine functional groups confer these compounds their basic character (pK_a = 9–10) [33]. Therefore, at acidic pH, they are cationic and are attracted to the free anionic residual silanols. The compounds selected for this work cover a wide range of polarities, being liable to be analyzed by both HILIC and RPLC.

2. Materials and Methods

2.1. Reagents

Eight β-adrenoceptor antagonists (acebutolol, atenolol, carteolol, esmolol, metoprolol, oxprenolol, propranolol and timolol, Sigma, St. Louis, MO, USA), were used as probe compounds. Table S1 in the Supplementary material gives information about their structures, acidity constants and octanol-water partition coefficients. All compounds were dissolved using a small amount of acetonitrile (VWR Chemicals, Radnor, PA, USA) and diluted with nanopure water (Barnstead, Sybron, Boston, MA, USA), to get a concentration of approximately 100 μg/mL. These solutions were stable during at least two months kept at 4 °C. Before injection into the chromatograph, working solutions were diluted to a final concentration of approximately 20 μg/mL, again with nanopure water for the RPLC mode and with acetonitrile for the HILIC mode.

The aqueous-organic mobile phases in RPLC contained increasing concentrations of acetonitrile: 10%, 15%, 20% and 25% *v/v*, in the absence of IL, and 10%, 12.5%, 15%, and 17.5% *v/v* acetonitrile in the presence of 10 mM $[C_6C_1im]Cl$. HILIC mobile phases contained 90%, 92.5% and 95% *v/v* acetonitrile. The pH of the mobile phases was fixed at 3.0 with a mixture of 10 mM ammonium formate (Sigma) and formic acid (Acros Organics, Geel, Belgium). The pH value was referred to the aqueous-organic mobile phase, but aqueous buffers were used to standardize the pH-meter. The above mobile phase compositions guaranteed the elution of the analytes in practical analysis times. Nylon membranes of 0.45 μm (Micron Separations, Westboro, MA, USA) were used to filter the solutions of analytes and mobile phases. All the solutions were degassed in an Elmasonic IT-H ultrasonic bath from Elma (Singen, Germany).

2.2. Instrumentation and Columns

The chromatographic system was from Agilent (Waldbronn, Germany) and was equipped with an isocratic pump (Series 1260), a solvent selector valve (Series 1290), an autosampler (Series 1260), a thermostated column compartment (Series 1260) set at 25 °C, a UV-visible wavelength detector (Series 1100), and an HPChemStation (Agilent, B.02.01) for data acquisition. The signal of the probe compounds was detected at 254 nm, except for timolol, which was measured at 300 nm due to low absorption at 254 nm. Mathematical treatment was carried out with Excel (Microsoft Office 2010, Redmond, WA, USA). Chromatographic peaks were integrated with MICHROM [34]. Triplicate injections of 20 μL were made to control the reproducibility of the measurements.

For the RPLC and HILIC modes, Zorbax Eclipse XDB-C18 (150 mm × 4.6 mm i.d., 5 μm particle size) (Agilent), and Sequant ZIC-HILIC (150 mm × 4.6 mm i.d., 5 μm particle size) (ACE, Aberdeen, Scotland, UK) columns were used, respectively. Two different columns were used for RPLC in the absence and presence of $[C_6C_1im]Cl$. A similar 30 mm pre-column was connected to the analytical column for protection. The flow-rate was 1 mL/min. Column regeneration was carried out following the manufacturer's recommendations.

3. Results and Discussion

3.1. Influence of Mobile Phase Composition on Retention

The main separation mechanisms in conventional RPLC, in the absence of additives, are the hydrophobic interaction of analytes with the alkyl-bonded layer of the stationary phase and their solubilization in the aqueous-organic mixture used as mobile phase. Additional interactions, such as ion-pair formation, salting-out effects, or ion-exchange with free silanols on the packing, also take place with cationic solutes, such as the β-adrenoceptor antagonists studied in this work, whose polarity was in the $-0.026 < \log P_{o/w} < 2.60$ range. This polarity limited the feasible acetonitrile content in the RPLC

mobile phase to 10–25% (measured as volumetric fraction), to prevent large retention times for the most hydrophobic analytes and short times for those of larger polarity.

The addition of 10 mM [C_6C_1im]Cl to the mobile phase introduces additional interactions in the chromatographic system. The adsorption of the [C_6C_1im] cation on the stationary phase is significant, whereas the chloride ion has low affinity. The strength of this interaction has been measured [35]. It has also been confirmed that there is no irreversible adsorption of the [C_6C_1im] cation, being the alkyl-bonded columns satisfactorily regenerated [35]. The adsorbed [C_6C_1im] cation gives rise to a positively charged modified layer that repels the cationic β-adrenoceptor antagonists, which decrease significantly their retention times. Therefore, the maximal content of acetonitrile in the mobile phase should be decreased (with regard to RPLC without IL) from 25% to 17.5%. The classical RPLC behavior (i.e., non-linear decrease of retention with larger amount of organic solvent) was observed with and without IL (Figure 1a,b).

In HILIC, the formation of a water-rich layer immobilized on the stationary phase surface explains solute partitioning and the separation of the basic compounds, although secondary mechanisms may take place. The zwitterionic column used in this work has a functionalized packing with a ligand exhibiting simultaneously positive charge (due to the protonated quaternary ammonium group), and negative charge (due to the sulfonate group) in a 1:1 ratio. Although, in principle, there is no net charge, the anionic sulfonate group is capable of inducing electrostatic interactions with cationic analytes, due to its position at the distal end of the ligand [36]. The selection of the zwitterionic column was based on the higher retention times, elution capability and resolution obtained in previous work for a group of polar compounds, compared to other common HILIC packages [26]. A multitude of polar stationary phases are available for HILIC applications and while there is no consensus in the literature on which is the best performing, there is growing interest in zwitterionic phases [37–39]. We found that with the zwitterionic column, the retention factors of the probe compounds gradually increased upon decreasing the concentration of water, following an almost linear trend (Figure 1c). With regard to RPLC, the elution window was narrower, with the acetonitrile content varying between 90% and 95% (water content between 5% and 10%) to avoid void volume elution. In all cases, reproducibility in retention times was measured as the RSD values, which were below 1.2% for RPLC without IL (mean value was 0.07%), 1.5% for RPLC with IL (mean value, 0.006%) and 0.4% for HILIC (mean value, 0.03%).

The elution strength (i.e., sensitivity of the retention of solutes to changes in the modifier concentration; acetonitrile in RPLC and water in HILIC) was measured based on the slope of the linear relationship of log *k* versus the concentration of each modifier (see Figure S1 in the Supplementary material). Table 1 lists the slope values for the RPLC and HILIC modes. The elution strength in RPLC varied from −0.030 to −0.097 in the absence of additive, and from –0.05 to –0.12 in the presence of [C_6C_1im]Cl. In HILIC, the elution strength of water varied from –0.009 to –0.040. The elution strength was larger (shorter retention times) in RPLC in the presence of [C_6C_1im]Cl, and smaller in the HILIC mode. On the other hand, in RPLC in the absence of additive, the elution strength increased with compound hydrophobicity. In RPLC with added [C_6C_1im]Cl, and in HILIC, there was not such clear correlation.

Table 1. Elution strength of the modifiers.

Compound	Acetonitrile Elution Strength		Water Elution Strength
	Conventional RPLC	RPLC with [C_6C_1im]Cl	HILIC
Acebutolol	−0.0948	−0.1026	−0.0405
Atenolol	−0.0295	−0.1020	−0.0342
Carteolol	−0.0789	−0.0509	−0.0392
Esmolol	−0.0929	−0.0940	−0.0253
Metoprolol	−0.0850	−0.0884	−0.0377
Oxprenolol	−0.0921	−0.0953	−0.0086
Propranolol	−0.0970	−0.1217	−0.0212
Timolol	−0.0894	−0.0986	−0.0224

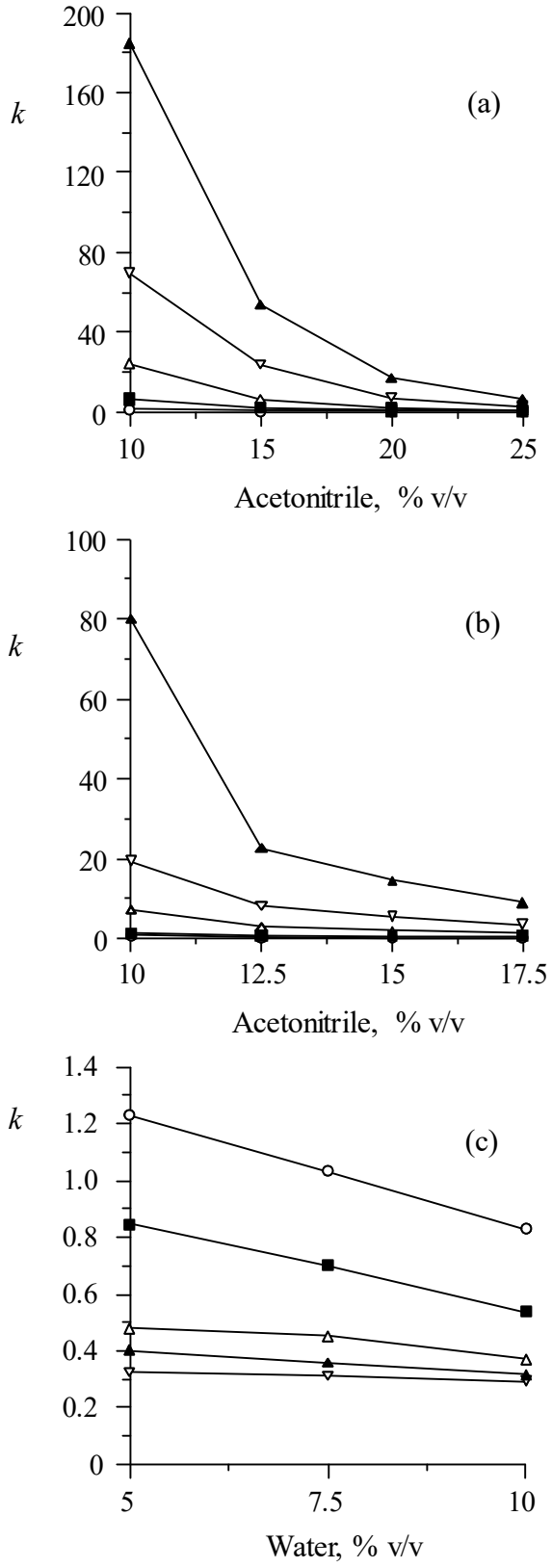

Figure 1. Changes in retention for β-adrenoceptor antagonists with increasing modifier contents in: (**a**) RPLC without IL, (**b**) RPLC with added $[C_6C_1im]Cl$, and (**c**) HILIC. Compounds: atenolol (○), carteolol (■), timolol (Δ), oxprenolol (∇), and propranolol (▲).

3.2. Selectivity

The similarities and differences between the chromatographic systems studied in this work were explored through correlations between the retention factors of the β-adrenoceptor antagonists for pairs of the studied systems, using mobile phases with the same amount of organic solvent. A high correlation between the retention behaviour of the analytes suggests similar selectivity (i.e., relative retention), although with different absolute retention. The higher scattering observed in the plots indicates, meanwhile, differences in selectivity between systems.

Figure 2 shows the correlations between different chromatographic modes for mobile phases containing all of them 10% modifier. The conclusions for other mobile phase compositions were similar. RPLC with and without [C_6C_1im]Cl showed similar selectivity (Figure 2a), whereas the selectivity differed significantly with regard to HILIC (Figure 2b,c). In fact, as expected, the correlation between RPLC (with and without [C_6C_1im]Cl) and HILIC was approximately reversed.

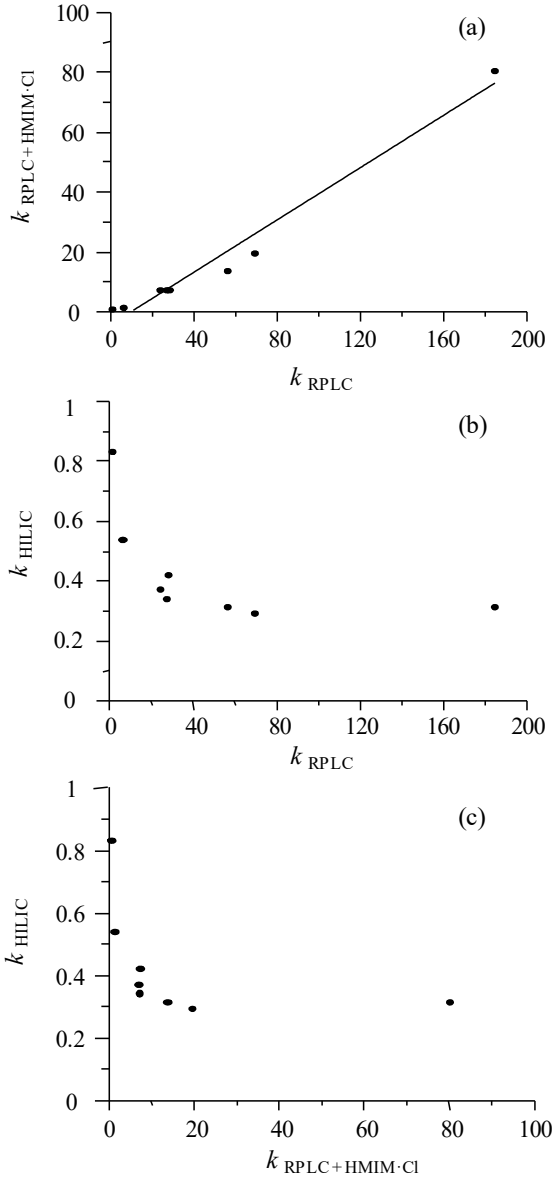

Figure 2. Comparison of selectivity for: (**a**) RPLC without additive vs. RPLC with [C_6C_1im]Cl, (**b**) RPLC without additive vs. HILIC, and (**c**) RPLC with [C_6C_1im]Cl vs. HILIC. Retention factors correspond to a mobile phase containing 10% modifier (acetonitrile in RPLC and water in HILIC).

3.3. Peak Shape

Half-width plots provide information about the peak shape for a group of compounds analyzed with a particular column [40,41]. The raw information is obtained from the chromatograms, and reveal the broadening rate and asymmetry of chromatographic peaks. These plots are built by representing the left (A) and right (B) half-widths of chromatographic peaks, or alternatively, the width ($A + B$) versus the retention times. An almost linear trend is followed, which can be modelled according to the following equations:

$$A = m_A\, t_R + A_0 \quad (1)$$

$$B = m_B\, t_R + B_0 \quad (2)$$

where m_A and m_B are the slopes of the linear correlations. The intercepts A_0 and B_0 indicate the extra-column contributions to the peak broadening. The sum of slopes ($m_A + m_B$) is directly related to the peak broadening rate inside the column, whereas their ratio (m_B/m_A) illustrates the asymmetry of peaks eluting at a time where the extra-column contribution is non-significant, provided the y-intercepts are similar. Half-width plots can be constructed using the information from the peaks of a group of compounds showing similar kinetics, obtained with a given mobile phase composition, or for only one compound eluted with several mobile phases at different compositions. Global plots can also be represented using all available data for the whole set of analyzed compounds using all mobile phase compositions.

Figure 3 depicts the global half-width plots obtained for the set of β-adrenoceptor antagonists in RPLC, in mobile phases with and without $[C_6C_1im]Cl$, and in HILIC. According to the recommendation of Foley and Dorsey [42], half-widths were measured at 10% peak height. This allows better appraising of the peak asymmetry, avoiding the higher baseline noise observed at smaller heights. In all cases, satisfactory correlations ($r^2 > 0.90$) were observed for the individual plots, especially for RPLC. Moreover, the intercepts were close to the origin (the negative intercepts should be interpreted as due to the fitting of data showing some scattering).

In RPLC without additive (Figure 3a), the slope of the right half-width (B) was significantly larger than the slope for the left half-width (A). This indicates asymmetric peaks, which are produced owing to the slow interaction of solutes with the free silanols in the stationary phase. A smaller angle between the straight-lines was observed for both half-widths, when $[C_6C_1im]Cl$ was added, informing of a significant reduction in peak asymmetry (with almost symmetric peaks, Figure 3b). The half-width plots obtained in HILIC (Figure 3c) were also almost coincident, although with some difference in the intercepts. It should be noted that the retention times for these peaks were rather short. This made the extra-column contributions to the peak retention and shape significant.

The parameters of the fitted half-width plots under all assayed chromatographic conditions are shown in Table S2 in the Supplementary material. As commented, asymmetric peaks were obtained in RPLC without additive ($m_B/m_A = 3.19$), whereas upon addition of $[C_6C_1im]Cl$, and in HILIC, the peak symmetry improved significantly ($m_B/m_A = 1.11$ and 1.04, respectively), with almost symmetric peaks. This suggests that in these conditions, the access of the cationic basic compounds to the silanols on the column is efficiently hindered, due to the adsorption of the IL cation on the stationary phase surface in RPLC and the presence of the water layer in HILIC. However, it should be noted that the sum of slopes, which indicates peak broadening as the retention increases, was smaller in RPLC without additive ($m_B + m_A = 0.054$, against 0.073 and 0.065 for RPLC with $[C_6C_1im]Cl$ and HILIC, respectively). This indicates that under the two latter conditions, the peaks are relatively wider.

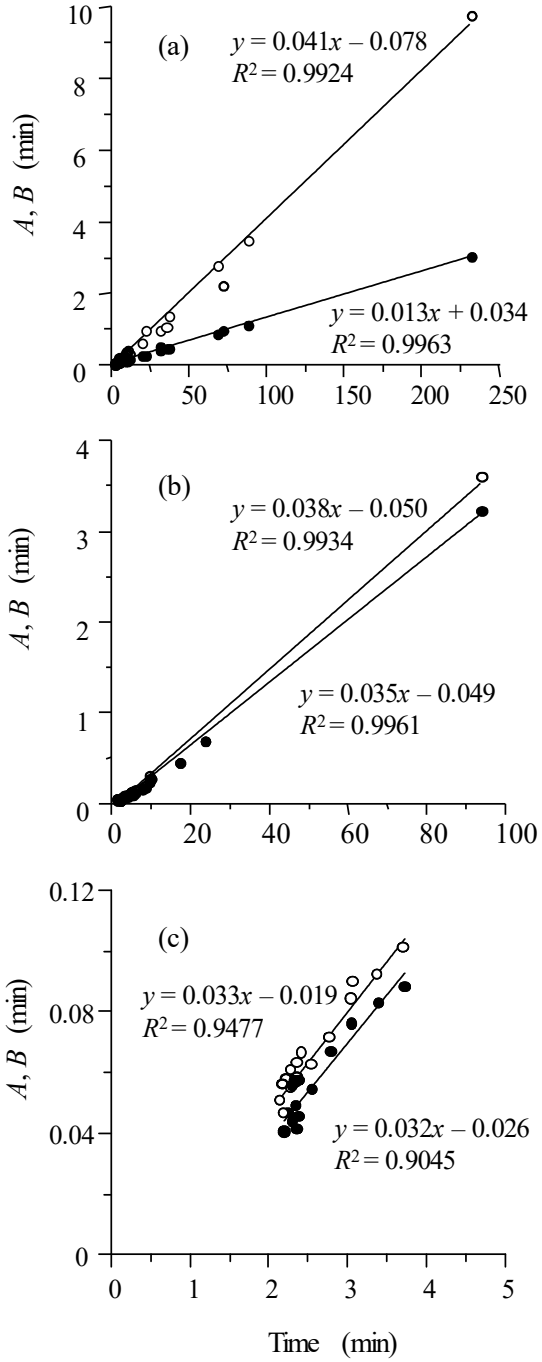

Figure 3. Half-width plots (left, *A* (•) and right, *B* (○)) built with the whole set of data for the eight basic compounds eluted with all assayed mobile phases in: (**a**) RPLC without additive, (**b**) RPLC with $[C_6C_1im]Cl$, and (**c**) HILIC.

3.4. Resolution

The possibility of resolving the mixtures of β-adrenoceptor antagonists was next examined. Figure 4 depicts the chromatograms yielding maximal resolution, as obtained with the software MICHROM for the compounds that absorb at 254 nm, where the absorption of timolol was non-significant. Timolol was detected at 300 nm, showing a peak at 12.2 and 2.6 min for RPLC using 13.8% acetonitrile and 10 mM $[C_6C_1im]Cl$)/17.5% acetonitrile, respectively, and at 2.5 min for HILIC with 6.5% water. As observed, the elution order was the same in RPLC with and without IL, except for metoprolol and acebutolol. The elution order was approximately reversed for HILIC,

except for the most hydrophobic compounds, probably due to the existence of retention mechanisms different from hydrophilic partitioning. Retention times were short in HILIC. This gave rise to poorer resolution, especially between oxprenolol, esmolol and propranolol, which eluted close to the void volume. In RPLC, with and without $[C_6C_1im]Cl$, the resolution was satisfactory, which gave rise to baseline resolved peaks. The analysis time, which was excessive using the conventional water-acetonitrile mobile phases, suffered a dramatic decrease with added IL, giving rise to symmetric peaks in practical analysis times.

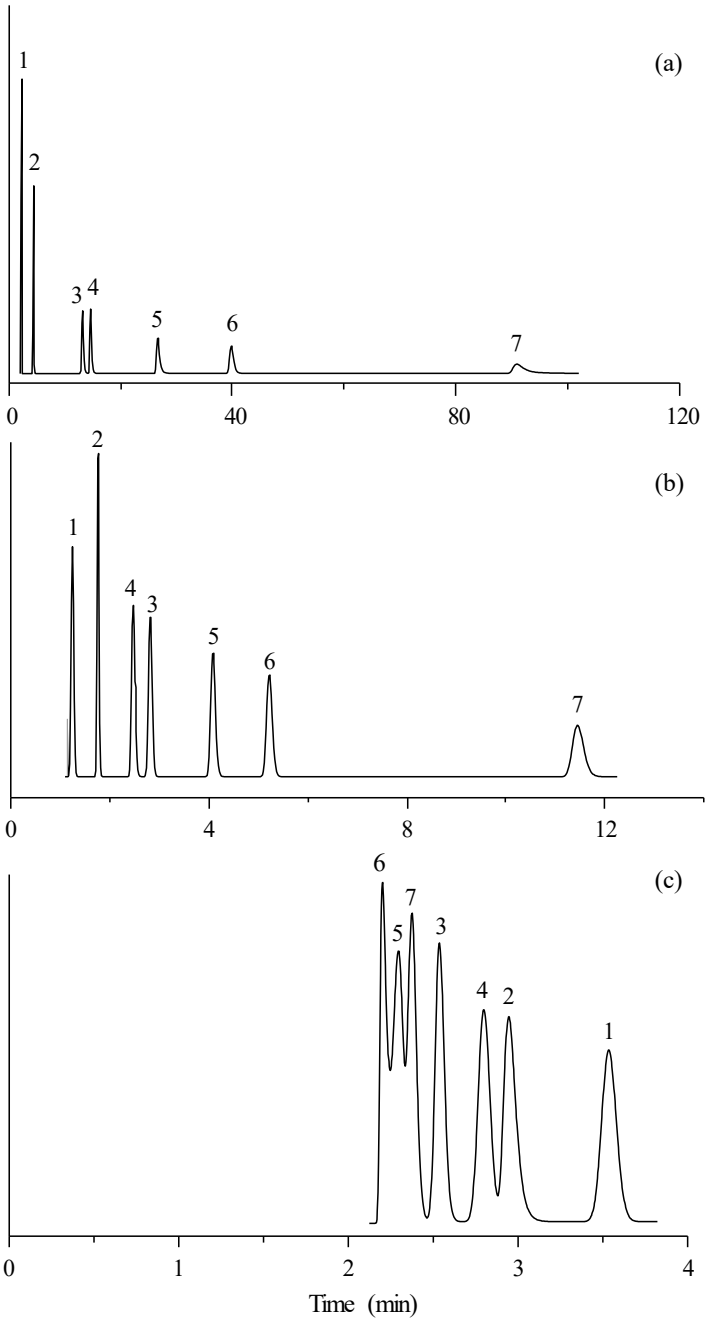

Figure 4. Chromatograms showing maximal resolution for the set of β-adrenoceptor antagonists in (mobile phase composition is given): (**a**) RPLC without additive (13.8% acetonitrile), (**b**) RPLC with IL (17.5% acetonitrile/10 mM $[C_6C_1im]Cl$, and (**c**) HILIC (6.5% water). Compounds: (1) atenolol, (2) carteolol, (3) metoprolol, (4) acebutolol, (5) esmolol, (6) oxprenolol, and (7) propranolol. Detection wavelength was 254 nm, at which timolol does not absorb.

4. Conclusions

This work compared the performance of the chromatographic behavior of β-adrenoceptor antagonists achieved in HILIC, with respect to RPLC with and without an IL. In all cases, mixtures of water-acetonitrile at different compositions were used, buffered at pH 3.0 at which the basic analytes exhibit positive charge. For this purpose, the behavior of a set of eight compounds with pharmaceutical interest was examined. It is shown that the long retention times obtained in conventional RPLC are significantly shortened, up to obtain practical analysis times, by addition of $[C_6C_1im]Cl$, or by using a polar column in HILIC conditions. The elution order, similar for the RPLC modes, was reversed in HILIC except for the most hydrophobic compounds. In general, the selectivity was significantly different in HILIC with regard to RPLC (both with and without IL), due to the particular retention mechanisms. On the other hand, peak shape was significantly enhanced in RPLC with IL and HILIC, which resulted in highly symmetric peaks. This indicates an efficient masking of the silanol effect with the adsorbed IL cation in RPLC and the water layer in HILIC.

The application of the MICHROM software (initially developed for micellar liquid chromatography) indicated the optimal separation conditions in the three chromatographic modes (RPLC without and with IL, and HILIC). It was concluded that chromatographic resolution was better for the β-adrenoceptor antagonists in RPLC, with regard to HILIC. The significant reduction of the analysis times upon addition of $[C_6C_1im]Cl$ in RPLC makes this mode preferable, with a baseline separation with an analysis time below 12 min. This can be decreased even more by applying gradient elution.

Supplementary Materials: The following are available online at http://www.mdpi.com/2297-8739/7/2/30/s1, Figure S1: Evaluation of the elution strength in RPLC without IL, RPLC with added [C6C1im]Cl, and HILIC, Table S1: Structures, acidity constants and octanol-water partition coefficients of the β-adrenoceptor antagonists, Table S2. Slopes for the left and right half-width plots, sum of slopes and slopes ratio.

Author Contributions: E.P.-G.: Investigation, methodology and writing—review; R.B.-G.: Investigation and methodology; M.J.R.-A.: Investigation, supervision, writing original draft, writing—review and editing. M.C.G.-A.-C.: Funding acquisition, project administration, resources, supervision, writing—review and editing. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by Projects CTQ2016-75644-P (Ministerio de Economía, Industria y Competitividad, Spain, and FEDER funds), PID2019-106708GB-I00 (Ministerio de Ciencia, Innovación y Universidades, Spain) and PROMETEO/2016/128 (Direcció General d'Universitat, Investigació i Ciència, Generalitat Valenciana, Spain).

Acknowledgments: Ester Peris-García thanks the University of Valencia for the pre-doctoral grant UV INV-PREDOC16F1-384313.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Nawrocki, J. The silanol group and its role in liquid chromatography. *J. Chromatogr. A* **1997**, *779*, 29–71. [CrossRef]
2. Neue, U.D.; Tran, K.; Méndez, A.; Carr, P.W. The combined effect of silanols and the reversed-phase ligand on the retention of positively charged analytes. *J. Chromatogr. A* **2005**, *1063*, 35–45. [CrossRef]
3. Reta, M.; Carr, P.W. Comparative study of divalent metals and amines as silanol-blocking agents in reversed-phase liquid chromatography. *J. Chromatogr. A* **1999**, *855*, 121–127. [CrossRef]
4. Engelhardt, H.; Blay, C.; Saar, J. Reversed phase chromatography: The mystery of surface silanols. *Chromatographia* **2005**, *62*, S19–S29.
5. McCalley, D.V. The challenges of the analysis of basic compounds by high performance liquid chromatography: Some possible approaches for improved separations. *J. Chromatogr. A* **2010**, *1217*, 858–880. [CrossRef]
6. García-Alvarez-Coque, M.C.; Navarro-Huerta, J.A.; Torres-Lapasió, J.R. Secondary chemical equilibria in reversed-phase liquid chromatography. In *Liquid Chromatography: Fundamentals and Instrumentation*, 2nd ed.; Fanali, S., Haddad, P., Poole, C.F., Riekkola, M.L., Eds.; Elsevier: Amsterdam, The Netherlands, 2017; pp. 125–146.

7. Calabuig-Hernández, S.; García-Alvarez-Coque, M.C.; Ruiz-Angel, M.J. Performance of amines as silanol suppressors in reversed-phase liquid chromatography. *J. Chromatogr. A* **2016**, *1465*, 98–106.
8. Ruiz-Angel, M.J.; Torres-Lapasió, J.R.; García-Alvarez-Coque, M.C.; Carda-Broch, S. Retention mechanisms for basic drugs in the submicellar and micellar reversed-phase liquid chromatographic modes. *Anal. Chem.* **2008**, *80*, 9705–9713. [CrossRef] [PubMed]
9. Zhang, W.; He, L.; Gu, Y.; Liu, X.; Jiang, S. Effect of ionic liquids as mobile phase additives on retention of catecholamines in reversed-phase high-performance liquid chromatography. *Anal. Lett.* **2003**, *36*, 827–838. [CrossRef]
10. Xiaohua, X.; Liang, Z.; Xia, L.; Shengxiang, J. Ionic liquids as additives in high performance liquid chromatography: Analysis of amines and the interaction mechanism of ionic liquids. *Anal. Chim. Acta* **2004**, *519*, 207–211.
11. Marszałł, M.P.; Baczek, T.; Kaliszan, R. Evaluation of the silanol-suppressing potency of ionic liquids. *J. Sep. Sci.* **2006**, *29*, 1138–1145. [PubMed]
12. Herrera-Herrera, A.V.; Hernández-Borges, J.; Rodríguez-Delgado, M.A. Ionic liquids as mobile phase additives for the high-performance liquid chromatographic analysis of fluoroquinolone antibiotics in water samples. *Anal. Bioanal. Chem.* **2008**, *392*, 1439–1446. [CrossRef] [PubMed]
13. Martín-Calero, A.; Pino, V.; Ayala, J.H.; González, V.; Afonso, A.M. Ionic liquids as mobile phase additives in high-performance liquid chromatography with electrochemical detection: Application to the determination of heterocyclic aromatic amines in meat-based infant foods. *Talanta* **2009**, *79*, 590–597. [CrossRef] [PubMed]
14. Martín-Calero, A.; Tejral, G.; Ayala, J.H.; González, V.; Afonso, A.M. Suitability of ionic liquids as mobile-phase additives in HPLC with fluorescence and UV detection for the determination of heterocyclic aromatic amines. *J. Sep. Sci.* **2010**, *33*, 182–190. [PubMed]
15. Petruczynik, A. Effect of ionic liquid additives to mobile phase on separation and system efficiency for HPLC of selected alkaloids on different stationary phases. *J. Chromatogr. Sci.* **2012**, *50*, 287–293.
16. Tang, Y.; Sun, A.; Liu, R.; Zhang, Y. Simultaneous determination of fangchinoline and tetrandrine in Stephania tetrandra S. Moore by using 1-alkyl-3-methylimidazolium-based ionic liquids as the RP-HPLC mobile phase additives. *Anal. Chim. Acta* **2013**, *767*, 148–154. [CrossRef]
17. García-Alvarez-Coque, M.C.; Ruiz-Angel, M.J.; Berthod, A.; Carda-Broch, S. On the use of ionic liquids as mobile phase additives in high-performance liquid chromatography. *Anal. Chim. Acta* **2015**, *883*, 1–21.
18. Buszewska-Forajta, M.; Markuszewski, M.J.; Kaliszan, R. Free silanols and ionic liquids as their suppressors in liquid chromatography. *J. Chromatogr A* **2018**, *1559*, 17–43. [CrossRef]
19. Fernández-Navarro, J.J.; García-Alvarez-Coque, M.C.; Ruiz-Angel, M.J. The role of the dual nature of ionic liquids in the reversed-phase liquid chromatographic separation of basic drugs. *J. Chromatogr. A* **2011**, *1218*, 398–407.
20. Ubeda-Torres, M.T.; Ortiz-Bolsico, C.; García-Alvarez-Coque, M.C.; Ruiz-Angel, M.J. Gaining insight in the behaviour of imidazolium-based ionic liquids as additives in reversed-phase liquid chromatography for the analysis of basic compounds. *J. Chromatogr. A* **2015**, *1380*, 96–103. [CrossRef]
21. Guo, Y.; Gaiki, S. Retention behavior of small polar compounds on polar stationary phases in hydrophilic interaction chromatography. *J. Chromatogr. A* **2005**, *1074*, 71–80. [CrossRef]
22. Buszewski, B.; Noga, S. Hydrophilic liquid chromatography (HILIC): A powerful separation technique. *Anal. Bioanal. Chem.* **2012**, *402*, 231–247. [CrossRef] [PubMed]
23. Jandera, P.; Janás, P. Recent advances in stationary phases and understanding of retention in hydrophilic interaction chromatography: A review. *J. Chromatogr. A* **2017**, *967*, 12–32. [CrossRef] [PubMed]
24. Cavazzini, A.; Catani, M.; Felinger, A. Hydrophilic interaction liquid chromatography. In *Liquid Chromatography: Fundamentals and Instrumentation*; Fanali, S., Haddad, P., Poole, C.F., Riekkola, M.L., Eds.; Elsevier: Amsterdam, The Netherlands, 2017; pp. 147–169.
25. McCalley, D.V. Understanding and manipulating the separation in hydrophilic interaction liquid chromatography. *J. Chromatogr. A* **2018**, *1523*, 49–71. [CrossRef] [PubMed]
26. Burgos-Gil, R.; Peris-García, E.; Ruiz-Angel, M.J.; Baeza-Baeza, J.J.; García-Alvarez-Coque, M.C. Protocol to compare column performance applied to hydrophilic interaction liquid chromatography. *Microchem. J.* **2019**, *149*, 103973.

27. McCalley, D.V. Is hydrophilic interaction chromatography with silica columns a viable alternative to reversed-phase liquid chromatography for the analysis of ionisable compounds? *J. Chromatogr. A* **1171**, *2007*, 46–55. [CrossRef]
28. McCalley, D.V. Evaluation of the properties of a superficially porous silica stationary phase in hydrophilic interaction chromatography. *J. Chromatogr A* **2008**, *1193*, 85–91. [CrossRef]
29. Al-Tannak, N.F.; Bawazeer, S.; Siddiqui, T.H.; Watson, D.G. The hydrophilic interaction like properties of some reversed phase high performance liquid chromatography columns in the analysis of basic compounds. *J. Chromatogr. A* **2011**, *1218*, 1486–1491.
30. Jovanovi, M.; Stojanovi, B.J.; Raki, T.; Malenovi, A.; Ivanovi, D.; Medenica, M. Five different columns in the analysis of basic drugs in hydrophilic interaction liquid chromatography. *Cent. Eur. J. Chem.* **2013**, *11*, 1150–1162.
31. ACE®HILIC Method Development Guide. ACE UHPLC and HPLC Columns, Advanced Chromatography Technologies, Aberdeen, Scotland. Available online: https://www.hplc.eu/Downloads/ACE_Guide_HILIC.pdf (accessed on 18 February 2020).
32. Mehvar, R.; Brocks, D.R. Stereospecific pharmacokinetics and pharmacodynamics of beta-adrenergic blockers in humans. *J. Pharm. Sci.* **2001**, *4*, 185–200.
33. Drayton, C.J. (Ed.) *Comprehensive Medicine Chemistry*; Pergamon Press: Oxford, UK, 1990; Volume 6.
34. Torres-Lapasió, J.R. *MICHROM Software*; Marcel Dekker: New York, NY, USA, 2000.
35. Berthod, A.; Ruiz-Angel, M.J.; Huguet, S. Non molecular solvents in separation methods: Dual nature of room temperature ionic liquids. *Anal. Chem.* **2005**, *77*, 4071–4080. [CrossRef]
36. Guo, Y.; Gaiki, S. Retention and selectivity of stationary phases for hydrophilic interaction chromatography. *J. Chromatogr. A* **2011**, *1218*, 5920–5938. [CrossRef] [PubMed]
37. Chirita, R.I.; West, C.; Zubrzycki, S.; Finaru, A.L.; Elfakir, C. Investigations on the chromatographic behaviour of zwitterionic stationary phases used in hydrophilic interaction chromatography. *J. Chromatogr. A* **2011**, *1218*, 5939–5963. [CrossRef] [PubMed]
38. Zhang, R.; Watson, D.G.; Wang, L.; Westrop, G.D.; Coombs, G.H.; Zhang, T. Evaluation of mobile phase characteristics on three zwitterionic columns in hydrophilic interaction liquid chromatography mode for liquid chromatography-high resolution mass spectrometry based untargeted metabolite profiling of Leishmania parasites. *J. Chromatogr. A* **2014**, *1362*, 168–179. [CrossRef] [PubMed]
39. Arase, S.; Kimura, S.; Ikegami, T. Method optimization of hydrophilic interaction chromatography separation of nucleotides using design of experiment approaches. I: Comparison of several zwitterionic columns. *J. Pharm. Biomed. Anal.* **2018**, *158*, 307–316. [CrossRef]
40. Ruiz-Angel, M.J.; Carda-Broch, S.; García-Alvarez-Coque, M.C. Peak half-width plots to study the effect of organic solvents on the peak performance of basic drugs in micellar liquid chromatography. *J. Chromatogr. A* **2010**, *1217*, 1786–1798. [CrossRef]
41. Baeza-Baeza, J.J.; Ruiz-Angel, M.J.; García-Alvarez-Coque, M.C.; Carda-Broch, S. Half width plots, a simple tool to predict peak shape, reveal column kinetics and characterise chromatographic columns in liquid chromatography: State of the art and new results. *J. Chromatogr. A* **2013**, *1314*, 142–153. [CrossRef]
42. Foley, J.P.; Dorsey, J.G. Equations for calculation of chromatographic figures of merit for ideal and skewed peaks. *Anal. Chem.* **1983**, *55*, 730–737. [CrossRef]