ELSEVIER

Journal of Chromatography A, 954 (2002) 77–87

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Change of mobile phase pH during gradient reversed-phase chromatography with 2,2,2-trifluoroethanol–water as mobile phase and its effect on the chromatographic hydrophobicity index determination

Sonia Espinosa[a], Elisabeth Bosch[a], Martí Rosés[a], Klára Valkó[b,*]

[a]*Departament de Química Analítica, Universitat de Barcelona, Diagonal 647, 08028 Barcelona, Spain*
[b]*Physicochemical Characterisation, CASS, GlaxoSmithKline, Gunnels Wood Road, Stevenage, Herts. SG1 2NY, UK*

Received 25 October 2001; received in revised form 11 February 2002; accepted 13 February 2002

Abstract

We have shown previously that using a trifluoroethanol containing mobile phase provides a unique chromatographic selectivity. This is essential to derive molecular descriptors by HPLC which requires retention data from several systems. It also requires that the ionisation is suppressed so that retention times reflect the properties of the neutral molecules. Therefore the pH change of the mobile phase during gradient elution and its effect on the solute ionisation have been studied. During gradient elution of mixtures of ammonium acetate and butylammonium formate with trifluoroethanol as an organic modifier it was found that the pH was almost constant when the gradient started with a low pH. However, when the starting mobile phase pH was above 8 the pH dropped very quickly as the trifluoroethanol concentration increased in the mobile phase. The CHI descriptor (a retention index derived directly from gradient retention times) of several basic compounds as a function of starting mobile phase pH has been measured using trifluoroethanol gradient. The effect of the trifluoroethanol on the pK_a change of the compounds has been investigated. The experimental data fit closely to a previously derived equation that describes gradient retention times as a function of mobile phase pH and analyte ionisation constant (pK_a). This equation makes it possible to predict the CHI descriptor for ionisable compounds at various pH values. We have used butylamine for high pH mobile phase preparation as is more basic than ammonia and for many basic drugs the retention of the neutral form could be obtained directly (without extrapolation).

Keywords: pH effect; Hydrophobicity index; Gradient elution; Gradient retention time; Mobile phase composition; 2,2,2-Trifluoroethanol

1. Introduction

It is very common to use generic gradient reversed-phase chromatography coupled with mass spectrometry [1] for quality control of pharmaceutical research compounds. Recently, we have proposed a method to use the gradient retention times as a measure of hydrophobicity of the compounds [2,3]. Gradient retention time and the lipophilicity of the compound depend on a great extent on the propor-

*Corresponding author. Tel.: +44-14-3876-3309; fax: +44-14-3876-3352.
E-mail address: klv39154@gsk.com (K. Valkó).

0021-9673/02/$ – see front matter
PII: S0021-9673(02)00165-6

tion of ionized forms of acidic and basic molecules. When we want to characterize the lipophilicity of the neutral form of the molecules through the gradient retention times, we have to make sure that the compound does not get ionized during the gradient chromatographic run. The HPLC retention of the neutral form of molecules can be described by the Abraham solvation equation [4] using five molecular descriptors (size, excess molar refraction, H-bond acidity–basicity and dipolarity–polarisability). Previously, we have described the standardised gradient retention times of the neutral form of the molecules by five basic molecular descriptors using various orthogonal stationary phase–mobile phase systems [5,6]. We have found that perfluorinated stationary phase with trifluoroethanol gradient represents a unique selectivity when the gradient retention is described by the Abraham solvation equation [7]. The organic modifier 2,2,2-trifluoroethanol has a strong H-bond donor property while it is weak H-bond acceptor [8]. In using this system to derive molecular descriptors for basic drug molecules it is essential to know what starting mobile phase pH is needed to keep even strong basic compounds in an unionised form (especially when they elute with a high organic phase concentration).

The variation of the isocratic retention factor of an ionisable compound with the mobile phase pH can be described with a sigmoidal function where the inflection point is at pH=pK_a [9–16]:

$$k = \frac{\left[k_{HA}10^{(pK_a - pH)} + k_A\right]}{\left[10^{(pK_a - pH)} + 1\right]} \quad (1)$$

where the observed retention factor k is an average of the retention factors of the acid (k_{HA}) and basic forms (k_A), pK_a is the acid dissociation constant of the molecule and pH is the mobile phase pH where the retention factor has been determined. Depending on the pH scale used, different values may be obtained for the pK_a parameter of Eq. (1). There are several procedures to measure the pH of the mobile phase. The most common procedure requires the calibration of the electrode system with aqueous buffers and then the measurement of the pH of the aqueous buffer before mixing it with the organic modifier. In this case we are working on the ${}^{w}_{w}$pH scale (where the subscript implies that the electrode was calibrated with aqueous buffers, and the superscript implies that we are measuring the pH of an aqueous solution.). This pH, however, changes when the organic solvent is added to the aqueous buffer. A more rigorous procedure, recommended by the IUPAC [17], is to measure the pH of the mobile phase after mixing the aqueous buffer with the organic modifier. In this case, the electrode system used to measure the pH can be calibrated either with aqueous buffer or with buffers prepared with the same composition as the mobile phase. These are the ${}^{s}_{w}$pH and the ${}^{s}_{s}$pH scales. The difference between the two scales depends on the primary medium effect and the liquid-junction potential of the electrode, and it is a constant value for each mobile phase composition (δ). These values have been published for methanol–water [18–22] and for acetonitrile–water mixtures [23]. To obtain a good fit between experimental retention factor data and mobile phase pH in order to determine pK_a values, the ${}^{s}_{w}$pH scale or the ${}^{s}_{s}$pH scale should be used. The difference between the so obtained ${}^{s}_{w}$pK and ${}^{s}_{s}$pK values should be equal to δ.

However, in gradient elution the concentration of the organic modifier is changing continuously and so is the mobile phase pH and the pK_a value of the compounds. In our previous study [24] we have measured the pH change of the mobile phase with increasing concentration of methanol and acetonitrile using ammonium acetate buffers adjusted to different pH values. The gradient retention times were measured with various starting mobile phase pH values for model compounds (acids and bases) with known pK_a values in aqueous conditions. An equation was proposed that described how the gradient retention times (t_g) depended on the starting mobile phase pH:

$$t_g = \frac{\left[t_{g_{HA}}10^{s(pK_a - pH)} + t_{g_{(A)}}\right]}{\left[10^{s(pK_a - pH)} + 1\right]} \quad (2)$$

where $t_{g(HA)}$ and $t_{g(A)}$ are the gradient retention times of the acid and basic forms, respectively; pK_a is the acid dissociation constant of the compound and s is an additional empirical parameter which improve the fit. In fact, s is directly related to the slope (first derivative) of the t_g vs. pH plot which depends on the different variation of compound ${}^{s}_{w}$pK_a and buffer ${}^{s}_{w}$pH during elution [24].

In this paper we present the results of similar studies using the unusual solvent 2,2,2-trifluoroethanol as organic modifier, and high pH stable XTerra C_{18} HPLC columns.

2. Experimental

2.1. Apparatus

Gradient retention data were measured on a Hewlett-Packard 1090 series HPLC. Data acquisition and processing was performed on a Viglen IBM-compatible PC with HP CHEMSTATION software (Hewlett-Packard, Amsterdam, The Netherlands). Gradient mixing was carried out by a low-pressure gradient mixer built into the HPLC and was controlled by the CHEMSTATION program. The reversed-phase HPLC measurements were carried out on a 5-μm XTerra™ MS C_{18} column with the dimensions of 50×4.6 mm (Waters). pH measurements were taken with a Ross semimicro Combination electrode Orion 8103 (glass electrode and a reference electrode with a 3.0 *M* KCl solution in water as a salt bridge) in a radiometer Copenhagen PHM93 reference pH meter with a precision of ±0.1 mV (±0.002 pH unit). All measurements were made in an air-conditioned room with a temperature of 26.0±0.1 °C, as measured by the HP CHEMSTATION.

2.2. Chemicals

2,2,2-Trifluoroethanol was HPLC grade from Fluka and water purified by the Milli-Q plus system from Millipore. The studied compounds were: lidocaine, nicotine, procaine, pyrilamine, diphenhydramine, 4-*tert*.-butylbenzylamine, alprenolol, propanolol, oxprenolol, metoprolol and terbutaline. The chemical structures of these compounds are shown in Fig. 1. Samples were prepared at 0.2 mg/ml in buffer–2,2,2-trifluoroethanol mixtures (1:1, v/v).

2.3. Procedure

Fast gradient retention time measurements were taken using the following gradient retention program, where the mobile phase flow-rate was 2.00 ml/min:

0.0–0.5 min, 0% organic modifier
0.5–3.0 min, 0–100% organic modifier
3.0–3.5 min, 100% organic modifier
3.5–3.7 min, 100–0% organic modifier
3.7–5.0 min, 0% organic modifier

2,2,2-Trifluoroethanol was used as organic modifier and 50 m*M* ammonium acetate or 50 m*M* butylamine were used as aqueous components of the mobile phase. The pH was adjusted by adding concentrated formic acid or ammonia solutions. Gradient retention time measurements were obtained using several different starting mobile phase pH values. All retention data were taken by triplicate and the average value was used for the calculations.

In order to reveal the pH changes during gradient, pH measurements were carried out for a set of 50 m*M* ammonium acetate buffer solutions, adjusted to different ${}^{w}_{w}$pH values (ranging from 2.68 to 9.96) by addition of concentrated formic acid or ammonia solutions and diluted with different concentrations of 2,2,2-trifluoroethanol. The same procedure was carried out for a set of 50 m*M* butylamine solutions, adjusted to different ${}^{w}_{w}$pH values (ranging from 4.11 to 11.94) by addition of concentrated formic acid. The ${}^{s}_{w}$pH values of these mixtures were also measured with the potentiometric system calibrated with aqueous buffers.

3. Results and discussions

3.1. Variation of pK_a values of compounds and pH values of buffers with the mobile phase gradient

In previous work [18,19,23–28] we have shown that the addition of organic solvent to an aqueous buffer implies a variation of the initial pH value of the solution. This fact has been studied using methanol and acetonitrile as organic solvents and similar trends have been found in both methanol–water [18,19,24,26] and acetonitrile–water mixtures [23,27,28].

In fact, when a compound with acid–base properties elutes, the variation of the mobile phase composition during gradient elution produces changes in the degree of ionization of the compound which contribute significantly to variation of retention. The change of the ionization of the compound depends on two parameters that change during

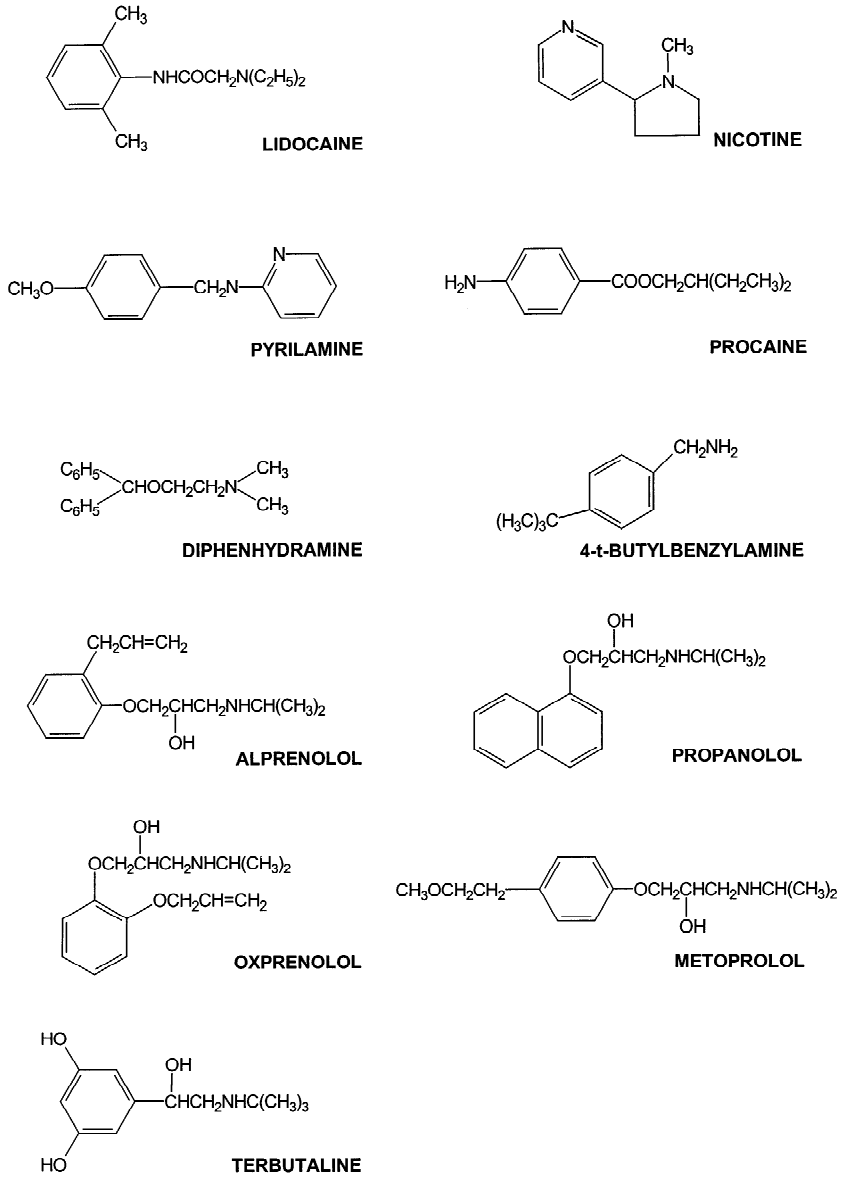

Fig. 1. Chemical structures of the basic drug compounds studied in this work.

the compound elution: the pK_a value of the compound and the pH of the mobile phase.

Unfortunately, there is no pK_a data available in the literature for 2,2,2-trifluoroethanol–water mixtures, although the pK_a values are expected to change in a similar way as they would in other alcohol–water mixtures.

Based on previous work [18,19,23–29] we expect the ${}^{s}_{w}pK_a$ value of neutral acids (e.g. acetic acid) to increase with the increase in alcohol (or acetonitrile) content, whereas the ${}^{s}_{w}pK_a$ value of cationic acids obtained by protonation of neutral bases (e.g. ammonium) decreases. However, the increase (or decrease) in the pK_a value is different for each ioniz-

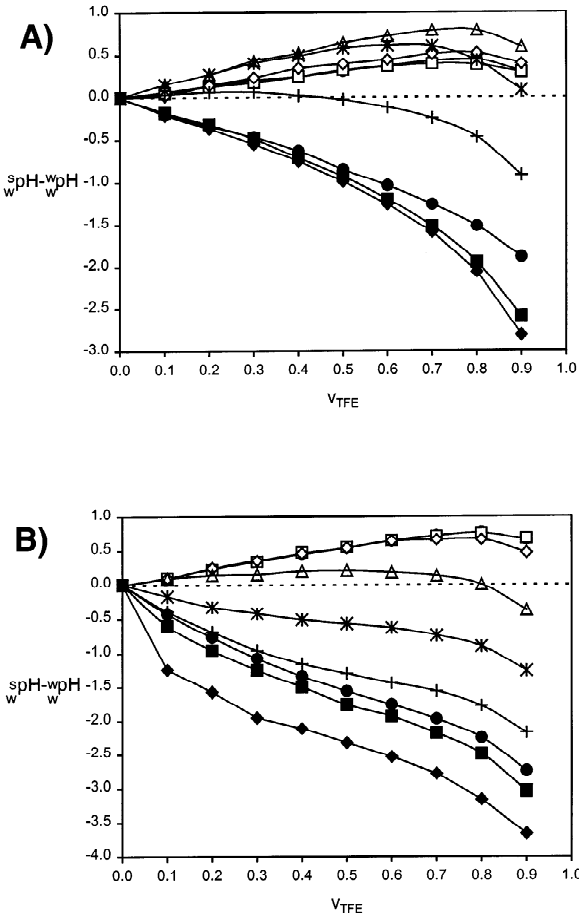

Fig. 2. Variation of the $^{s}_{w}$pH of buffers in 2,2,2-trifluoroethanol–water: (A) Ammonium acetate buffers at $^{w}_{w}$pH values: (○) 2.68, (□) 3.05, (◇) 4.04, (△) 5.05, (*) 6.01, (+) 7.09, (●) 7.98, (■) 8.97, (♦) 9.96. (B) Butylamine buffers at $^{w}_{w}$pH values: (○) 4.11, (□) 5.11, (◇) 6.10, (△) 7.07, (*) 8.02, (+) 9.02, (●) 9.99, (■) 10.98, (♦) 11.94.

able compound. The results obtained in this work confirm these variations for 2,2,2-trifluoroethanol–water mixtures.

Fig. 2 shows how the initial pH of an aqueous ammonium acetate buffer changes when 2,2,2-trifluoroethanol is added. This change has been measured for several initial aqueous buffers ($^{w}_{w}$pH values ranging from 2.68 to 9.96) to which the organic solvent was added. The pH-electrode system was calibrated with the usual aqueous buffers of pH 4.00 and 7.00, and therefore the pH readings obtained were in the absolute pH scale ($^{s}_{w}$pH). The pH values obtained are presented in Table 1.

For buffers with initial (aqueous) pH values below 7, the $^{s}_{w}$pH value of the buffer increases when the 2,2,2-trifluoroethanol content increases (at least for solutions up to 70% of 2,2,2-trifluoroethanol). As these solutions are buffered by the acetic–acetate pair, with some contribution from the formic–formate pair for the most acidic solutions, then its $^{s}_{w}pK_a$ value increases, which agrees with the behaviour of aqueous solutions made from neutral acids when increasing the methanol or acetonitrile content. However, for buffers with $^{w}_{w}$pH values above 8, the $^{s}_{w}$pH decreases because these solutions are buffered by the ammonia–ammonium pair and its $^{s}_{w}pK_a$ value decreases, which agrees with the behaviour of aqueous solutions made from cationic acids when increasing the methanol or acetonitrile content. The buffer with $^{w}_{w}pH = 7.09$ shows an intermediate behaviour. The $^{s}_{w}$pH value of this buffer shows a small variation about 50% 2,2,2-trifluoroethanol because both acid–base pairs (acetic–acetate and ammonium–ammonia) contribute to buffer the solution and the increase in

Table 1
Measured $^{w}_{s}$pH values of a 50 m*M* ammonium acetate at different 2,2,2-trifluoroethanol–water compositions

V_{TFE}	$^{s}_{w}$pH								
0.0	2.68	3.05	4.04	5.05	6.01	7.09	7.98	8.97	9.96
0.1	2.74	3.10	4.06	5.20	6.16	7.12	7.78	8.79	9.75
0.2	2.81	3.17	4.18	5.32	6.28	7.15	7.64	8.65	9.59
0.3	2.88	3.22	4.27	5.48	6.41	7.15	7.51	8.48	9.41
0.4	2.92	3.29	4.38	5.57	6.50	7.11	7.35	8.26	9.21
0.5	2.99	3.37	4.43	5.69	6.59	7.06	7.13	8.04	8.97
0.6	3.05	3.41	4.48	5.77	6.62	6.97	6.94	7.77	8.70
0.7	3.10	3.44	4.55	5.84	6.61	6.84	6.72	7.45	8.37
0.8	3.12	3.43	4.56	5.84	6.44	6.62	6.46	7.02	7.90
0.9	2.98	3.34	4.43	5.64	6.09	6.17	6.09	6.38	7.15

acetic pK_a value when 2,2,2-trifluoroethanol is added is balanced with the decrease in ammonium pK_a value, at least until 50% 2,2,2-trifluoroethanol.

Although the behaviour of the pH of the ammonium acetate buffer solution when increasing the content of 2,2,2-trifluoroethanol is as expected, the variation of pH is not so similar to the variation with methanol or acetonitrile [24]. The mobile phase pH practically does not change with the 2,2,2-trifluoroethanol gradient from low to neutral pH values (the increase is around one pH unity as a maximum) but it drops dramatically when we start from higher mobile phase pH values (almost three pH units when starting with a ${}^{w}_{w}$pH≈10 solution and adding up to 90% 2,2,2-trifluoroethanol).

In order to be able to measure accurate CHI values for the neutral form of lipophilic bases it is important to find a new buffer that can provide higher pH than the ${}^{w}_{w}$pH≈10 from the 50 m*M* ammonium acetate. A 50 m*M* butylamine solution was chosen to do the same study. We tried tetrabutylammonium hydroxide as well but we found solubility–miscibility problem with high TFE concentrations. Phosphate buffers also cause solubility problems with high concentration of organic solvents. We tried to choose buffers that are compatible with mass spectrometric detection. Fig. 2 also shows how the initial pH of an aqueous butylamine solution changes when 2,2,2-trifluoroethanol is added. This change was measured for several initial aqueous buffers (${}^{w}_{w}$pH values ranging from 4.11 to 11.94) to which the organic solvent was added. The pH values obtained are presented in Table 2. Fig. 2 shows that for initial ${}^{w}_{w}$pH values greater than about 7, the ${}^{s}_{w}$pH values decrease with increasing amounts of 2,2,2-trifluoroethanol while for initial ${}^{w}_{w}$pH lower than 7, the ${}^{s}_{w}$pH values increase with the amount of 2,2,2-trifluoroethanol in the mobile phase. These results can be explained in terms of the various buffer equilibria present in the mobile phase. Butylamine–butylammonium pair buffers solutions with ${}^{w}_{w}$pH values larger than 7 and its ${}^{s}_{w}$pK_a value decreases with increasing the amount of alcohol, which agrees again with the behaviour of aqueous solutions made from cationic acids when increasing the methanol or acetonitrile content. The solutions with ${}^{w}_{w}$pH values lower than 7 are buffered by the formic–formate pair whose ${}^{s}_{w}$pK_a value increase with the increase in the alcohol contents. The behaviour of the 50 m*M* butylamine solution is similar to the behaviour of the 50 m*M* ammonium acetate solution, but it has an advantage: a higher ${}^{w}_{w}$pH can be reached (around 12) which allows having a ${}^{s}_{w}$pH value above 9 when 70% 2,2,2-trifluoroethanol is in the mobile phase.

Fig. 2 shows that 2,2,2-trifluoroethanol decreases the ${}^{s}_{w}$pH value at all concentrations higher than 80%. This is expected for the basic pH values, but surprising for the acidic ones. We think that this pH decrease must be caused by a large negative δ value in this region. Values of δ are not known for 2,2,2-trifluoroethanol–water mixtures, but the values measured for methanol–water [18,19,22] and ethanol–water [22] show that δ values are quite low ($\pm$0.2) for alcohol contents up to 80%, but they decrease dramatically for larger alcohol concentrations (e.g. down to -2.24 for 100% methanol). Data on the Gibbs energies of transfer of H^+ from water to alcohol–water mixtures, which is directly related to δ

Table 2
Measured ${}^{w}_{s}$pH values of a 50 m*M* butylamine at different 2,2,2-trifluoroethanol–water compositions

V_{TFE}	${}^{s}_{w}$pH								
0.0	4.11	5.11	6.10	7.07	8.02	9.02	9.99	10.98	11.94
0.1	4.21	5.21	6.20	7.16	7.85	8.63	9.56	10.38	10.70
0.2	4.34	5.33	6.34	7.21	7.69	8.33	9.22	10.02	10.37
0.3	4.45	5.45	6.45	7.22	7.60	8.06	8.91	9.73	9.99
0.4	4.56	5.58	6.56	7.27	7.51	7.86	8.65	9.48	9.83
0.5	4.65	5.66	6.64	7.28	7.45	7.72	8.43	9.22	9.62
0.6	4.75	5.76	6.74	7.25	7.39	7.58	8.23	9.05	9.41
0.7	4.83	5.82	6.76	7.20	7.28	7.46	8.02	8.80	9.16
0.8	4.87	5.86	6.76	7.07	7.12	7.24	7.74	8.49	8.78
0.9	4.77	5.79	6.57	6.70	6.76	6.85	7.25	7.94	8.28

values, show that there is a strong increase to positive ΔG° values (which corresponds to large negative δ values) when neat alcohol is approached [30]. Since δ values relate ${}^{s}_{w}\mathrm{pH}$ and ${}^{s}_{s}\mathrm{pH}$ scales:

$${}^{s}_{w}\mathrm{pH} = {}^{s}_{s}\mathrm{pH} + \delta \quad (3)$$

the increase in ${}^{s}_{s}\mathrm{pH}$ for acetic–acetate and formic–formate would be overwhelmed by the large negative δ value.

3.2. Effect of the variation of the mobile phase composition in gradient elution over CHI values

In a method developed earlier [2,3] the hydrophobicity of a compound is calculated from gradient retention times measurements. Using the gradient retention program described in the experimental part, the system was standardised with a test mixture (which contains paracetamol, acetanilide, acetophenone, propiophenone, butyrophenone, valerophenone, hexanophenone, heptanophenone and octanophenone) and the chromatographic hydrophobicity indices (CHI) were derived. The CHI values approximate the percentages of organic modifier in the mobile phase at which compounds elute from the column. CHI normally ranges from 0 (hydrophilic) to 100 (lipophilic), although values outside this range are possible. It has also been pointed out that the starting mobile phase pH affects the CHI values: charged molecules have lower CHI values than their uncharged forms. Because many drug molecules have acid–base properties, the CHI lipophilicity is usually measured with three different starting mobile phase pH values (${}^{w}_{w}\mathrm{pH}=2$, ${}^{w}_{w}\mathrm{pH}=7.4$ and ${}^{w}_{w}\mathrm{pH}=10.5$). The highest CHI value obtained for the same compound at the three different pH values approximates to the hydrophobicity of the neutral molecule. However, the neutral form of the different acid–base species of these drugs cannot be achieved, for example, with very strong bases, very strong acids or amphoteric compounds. Therefore, fitting models should be used to calculate the CHI lipophilicity of the different drug species (neutral and ionic) from CHI data at different starting pH values.

Eq. (2) was successfully used in a previous work [24] to fit the gradient retention times (t_g) of a series of acids and bases to mobile phase pH (${}^{w}_{w}\mathrm{pH}$) with ammonium acetate buffers and acetonitrile and methanol as organic modifiers.

Gradient retention time is linearly related to CHI according to:

$$t_g = \mathrm{a} + \mathrm{b}\,\mathrm{CHI} \quad (4)$$

with the t_g values of the acidic and basic forms of the drug [$t_{g(HA)}$ and $t_{g(A)}$, respectively] having the same linear relation with their CHI descriptors (CHI_{HA} and CHI_{A}, respectively). Replacement of this linear relationship into Eq. (2) gives

$$\mathrm{CHI} = \frac{\left[\mathrm{CHI}_{HA}10^{s(\mathrm{p}K_a-\mathrm{pH})} + \mathrm{CHI}_{A}\right]}{\left[10^{s(\mathrm{p}K_a-\mathrm{pH})} + 1\right]} \quad (5)$$

This model has been applied to the bases studied and the results obtained are presented in Fig. 3 (for ammonia and butylamine buffers) and in Table 3.

The results presented in Table 3 show that the parameters obtained from each buffer are different. Only $\mathrm{p}K_a$, CHI_{HA} and CHI_{A} values of lidocaine show a good agreement between ammonia and butylamine buffers. Parameters of nicotine, procaine and terbutaline show a fair agreement. All other bases show a good agreement for the CHI values of the ionic form (CHI_{HA}), but CHI_{A} and $\mathrm{p}K_a$ obtained from ammonium acetate buffer are clearly overestimated.

The reason of these discrepancies is evident when one looks to Fig. 3. The plots of pyrilamine, diphenhydramine, 4-*tert.*-butylbenzylamine, alprenolol, propanolol, oxprenolol and metoprolol show an exponential trend because the protonated form of these bases predominates in the pH range covered by ammonium acetate buffers. Thus, the extrapolation leads to large CHI values for the neutral forms and to high $\mathrm{p}K_a$ values. This extrapolation produces a large uncertainty in the calculated CHI_{A} and $\mathrm{p}K_a$ values, which can be observed in the standard deviation of these parameters (values in brackets) given in Table 3. Only lidocaine, which has a low $\mathrm{p}K_a$ value, and in a minor degree nicotine and procaine, with intermediate $\mathrm{p}K_a$ values arrive at a condition where there is a predominance of the neutral form of the base.

However, butylamine buffers cover a more basic pH range and all studied bases arrive close to the plateau where there is a predominance of the neutral form in the CHI vs. pH plot (Fig. 3). Therefore, the

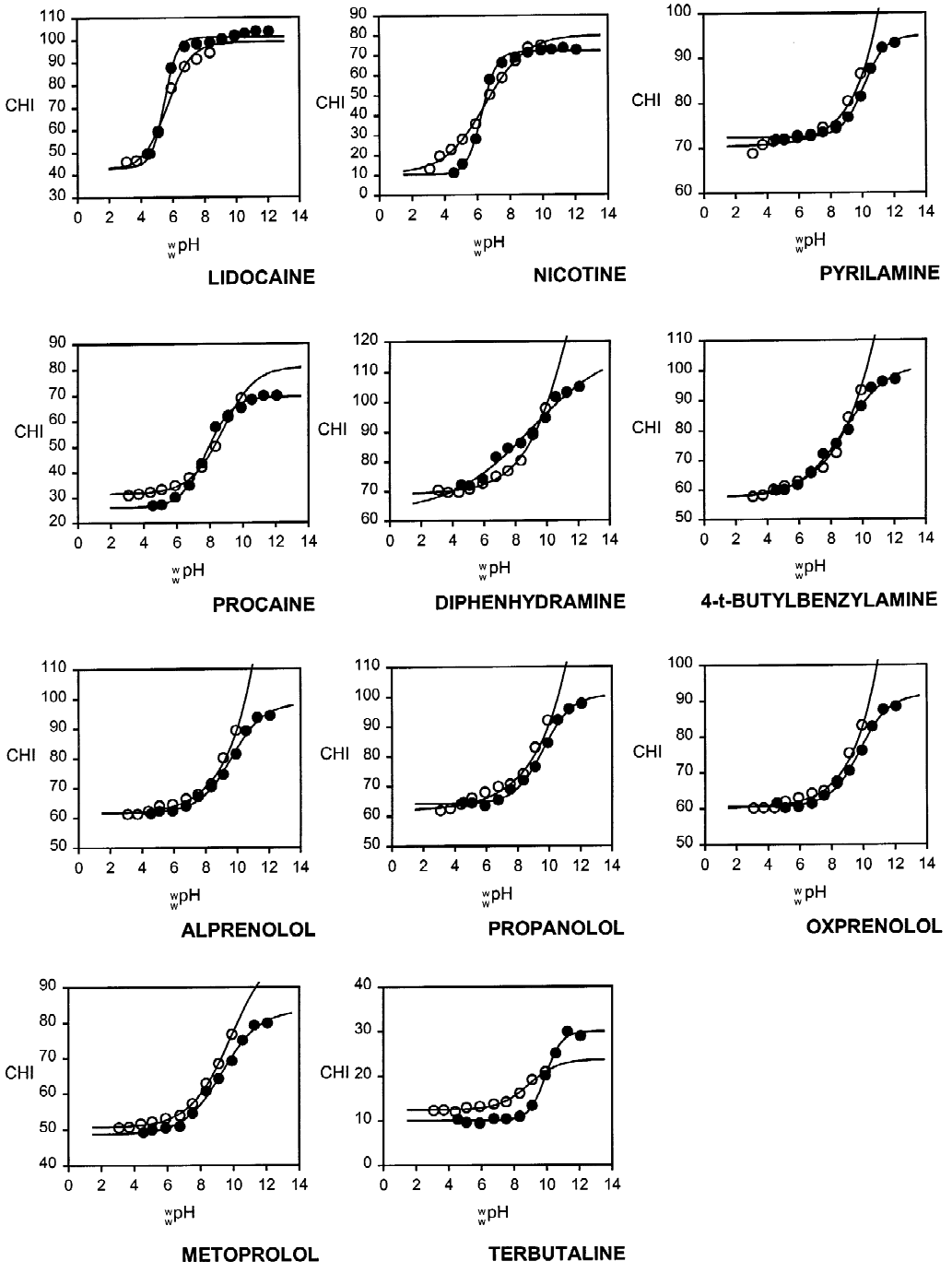

Fig. 3. Variation of CHI lipophilicity descriptor with the pH of the 2,2,2-trifluoroethanol–water mobile phase: (○) ammonium acetate buffers, (●) butylammonium formate buffers.

Table 3
Retention parameters for the studied compounds using Eq. (5)

	${}^{w}_{w}pK_a$	Ammonium acetate buffers						Butylamine buffers					
		pK_a	CHI_{HA}	CHI_A	s	F	SD	pK_a	CHI_{HA}	CHI_A	s	F	SD
Lidocaine	7.73	5.64	42.38	99.09	0.57	184	2.91	5.46	43.30	101.37	1.06	245	2.20
		(0.17)	(3.51)	(1.99)	(0.11)			(0.13)	(5.67)	(0.83)	(0.21)		
Nicotine	8.42	6.48	11.12	79.94	0.35	602	1.65	6.27	10.32	71.95	0.95	754	1.64
		(0.14)	(2.85)	(2.88)	(0.05)			(0.06)	(1.78)	(0.68)	(0.10)		
Procaine	8.90	8.75	31.52	81.27	0.44	636	0.93	7.77	26.07	69.55	0.56	630	1.31
		(0.24)	(0.60)	(5.25)	(0.06)			(0.09)	(1.15)	(0.87)	(0.06)		
Pyrilamine	8.92	17.84	70.38	2160	0.27	60	1.15	10.09	72.35	95.00	0.63	682	0.58
		(241)	(1.19)	$(3\cdot10^6)$	(0.21)			(0.08)	(0.28)	(0.89)	(0.06)		
Diphenhydramine	9.00	12.10	69.25	210.57	0.27	337	0.89	8.95	63.16	118.28	0.17	145	1.85
		(4.15)	(0.95)	(250)	(0.08)			(0.92)	(10.38)	(16.09)	(0.10)		
4-*tert*.-Butylbenzylamine	9.70	13.22	57.35	308.55	0.23	213	1.40	8.80	57.59	102.14	0.31	332	1.47
		(9.16)	(1.99)	(904)	(0.10)			(0.22)	(2.05)	(3.19)	(0.06)		
Alprenolol	10.08	14.91	61.26	639.06	0.26	258	0.99	9.62	61.85	98.46	0.43	417	1.15
		(20)	(1.10)	(6020.72)	(0.10)			(0.16)	(0.80)	(2.22)	(0.06)		
Propanolol	10.08	19.38	61.91	2805	0.21	126	1.47	9.61	64.18	100.62	0.48	555	1.03
		(172)	(2.55)	$(2\cdot10^6)$	(0.14)			(0.12)	(0.65)	(1.70)	(0.05)		
Oxprenolol	10.08	14.45	60.17	499.34	0.28	166	1.01	9.77	60.66	91.75	0.47	462	0.94
		(21)	(1.00)	(5140)	(0.12)			(0.14)	(0.58)	(1.76)	(0.06)		
Metoprolol	10.08	9.86	50.70	101.76	0.35	987	0.49	9.31	48.76	83.51	0.38	427	1.06
		(0.56)	(0.38)	(12.02)	(0.05)			(0.18)	(0.93)	(2.11)	(0.05)		
Terbutaline	12.01	8.87	12.40	23.66	0.48	189	0.39	9.92	10.02	29.98	0.87	480	0.68
		(0.40)	(0.22)	(2.15)	(0.11)			(0.07)	(0.30)	(0.67)	(0.11)		

Values in brackets are standard deviations; F, F test values; SD, overall standard deviation value.

parameters estimated for the neutral forms of strong bases with butylamine as buffer are more reliable than those estimated from the less basic ammonium acetate buffer.

Eq. (5) is a fitting equation that leads to accurate CHI_{HA} and CHI_A values provided that the experimental retention data are taken in the appropriate pH range. However, it has already been pointed out that the obtained pK_a value is not the aqueous pK_a value (${}^{w}_{w}pK_a$) of the compound [24]. The obtained pK_a value would agree with the true aqueous pK_a value of the drug only if the drug pK_a variation during gradient elution matches exactly the buffer pH variation. Table 3 shows that this is the case of diphenhydramine, which fitting pK_a value (8.95) is very close to its aqueous pK_a (9.00). Except for pyrilamine, the fitting pK_a values are lower than the ${}^{w}_{w}pK_a$ values and this shows that the pK_a of the drug decreases more than the pH of the buffer during gradient elution. A similar behaviour for basic drugs has been observed for methanol–water and acetonitrile–water mobile phases [24].

Eq. (5) can be extremely useful for estimating the CHI descriptor of the neutral forms of drugs when it is not possible to arrive at a pH basic enough to have the drug quantitatively in the neutral form. We have

Table 4
Comparison of the CHI values of the neutral forms of the drugs obtained by fitting the CHI values at several pH values to Eq. (5) and directly from the most basic butylamine buffers

	$CHI_{(A)}$ [a]	${}^{w}_{w}pH = 11.94$	
		CHI_A	Error (%)
Lidocaine	101.4	103.8	2.4
Nicotine	72.0	72.6	0.9
Procaine	69.6	69.9	0.5
Pyrilamine	95.0	93.3	1.8
Diphenhydramine	118.3	105.0	11.3
4-*tert*.-Butylbenzylamine	102.1	97.1	5.0
Alprenolol	98.5	94.3	4.2
Propanolol	100.6	97.7	2.9
Oxprenolol	91.8	88.5	3.5
Metoprolol	83.5	79.9	4.3
Terbutaline	30.0	28.9	3.5

[a] Calculated by fitting the data to Eq. (5).

tested this possibility for the studied drugs. Table 4 reports the CHI values for the neutral forms of the drugs calculated by Eq. (5) with butylamine buffers and those directly measured from the most basic starting pH which can be achieved with this buffer. The most basic butylamine buffer has a high starting pH ($^{w}_{w}$pH = 11.94) which suffices to have most of the drugs unionised during gradient elution. Therefore, for most drugs, the difference between the CHI value determined solely from this buffer and that obtained by application of Eq. (5) to the whole data at different starting pH values is small. The unique drug with a difference higher than 5% is diphenhydramine. Although this is not the most basic drug studied ($^{w}_{w}$pK_a = 9.00), it is the drug which shows the smallest s fitting parameter (0.17, see Table 3) and the largest CHI value (118.28). This means that this drug is eluted with longer retention time than the other drugs and therefore at larger trifluoroethanol concentrations. Since the average drug pK_a variation is close to the average buffer pH variation, the drug is partially ionized during the whole elution. This is evident in Fig. 3 which shows that the most basic point for diphenhydramine does not arrive at the extrapolated plateau that gives the CHI value for the unionised form. In this instance, an accurate determination of the CHI descriptor of the neutral drug requires determination of individual CHI values at different starting pH values and fitting of CHI to pH by means of Eq. (5).

4. Conclusions

The pH of ammonium acetate buffers in 2,2,2-trifluoroethanol–water mixtures ($^{s}_{w}$pH) does not change appreciably during gradient elution when the system is buffered by the acetic–acetate pair (low pH). However, at high pH value, the ammonium–ammonia pair buffers the solution and since the pK_a value of this pair strongly decreases with the increase of the trifluoroethanol concentration, the pH of the buffers drops very quickly during gradient elution.

The pH change of the buffer affects the ionization of basic drugs during gradient elution and the large pH drop in the basic pH range implies that the neutral form of many drugs cannot be achieved during the elution. The problem can be solved by replacing ammonia with a more basic compound, such as butylamine. This buffer allows determination of the lipophilicity (CHI index) of the neutral forms of basic drugs.

Eq. (5) has been shown to explain the variation of CHI retention data with the starting pH of the gradient elution. The fit provides accurate CHI values of the acid and basic forms of the drugs, and it is recommended when the buffer pH cannot be basic enough to keep the drug fully ionized during the whole gradient elution.

Acknowledgements

The work of S.E. was supported by a grant from the Catalan Government (1998FI 00639).

References

[1] I.M. Mutton, Fast generic HPLC methods, in: Handbook of Analytical Separations. Separation Methods in Drug Synthesis and Purification, Vol. 1, Elsevier, Amsterdam, 2000, p. 73.
[2] K. Valkó, C. Bevan, D. Reynolds, Anal. Chem. 69 (1997) 2022.
[3] C.M. Du, K. Valkó, C. Bevan, D. Reynolds, M.H. Abraham, Anal. Chem. 70 (1998) 4228.
[4] M.H. Abraham, Chem. Soc. Rev. 22 (1993) 73.
[5] K. Valkó, M. Plass, C. Bevan, D. Reynolds, M.H. Abraham, J. Chromatogr. A 797 (1998) 41.
[6] C.M. Du, K. Valkó, C. Bevan, D. Reynolds, M.H. Abraham, J. Chromatogr. Sci. 38 (2000) 503.
[7] K. Valkó, S. Espinosa, C.M. Du, E. Bosch, M. Rosés, C. Bevan, M.H. Abraham, J. Chromatogr. A 933 (2001) 73.
[8] R. Chitra, P.E. Smith, J. Chem. Phys. 114 (2001) 426.
[9] R.M. Lopes Marques, P.J. Shoenmakers, J. Chromatogr. 592 (1992) 157.
[10] P.J. Shoenmakers, R. Tijssen, J. Chromatogr. A 656 (1993) 577.
[11] D. Bolliet, C.F. Poole, M. Rosés, Anal. Chim. Acta 368 (1998) 129.
[12] J.P. Foley, W.E. May, Anal. Chem. 59 (1987) 102.
[13] J.P. Foley, W.E. May, Anal. Chem. 59 (1987) 110.
[14] C. Horváth, W. Melander, I. Molnár, Anal. Chem. 49 (1977) 142.
[15] P.J. Shoenmakers, S. van Molle, C.M.G. Hayes, L.G.M. Uunk, Anal. Chim. Acta 250 (1991) 1.
[16] J.A. Lewis, D.C. Lommen, W.D. Raddatz, J.W. Dolan, L.R. Snyder, I. Molnár, J. Chromatogr. 592 (1992) 183.
[17] Iupac Compendium of Analytical Nomenclature. Definitive Rules 1997, 3rd ed, Blackwell, Oxford, 1998.

[18] I. Canals, J.A. Portal, E. Bosch, M. Rosés, Anal. Chem. 72 (2000) 1802.
[19] I. Canals, F.Z. Oumada, M. Rosés, E. Bosch, J. Chromatogr. A 911 (2001) 191.
[20] C.L. De Ligny, M. Rehbach, Recl. Trav. Chim. 79 (1960) 727.
[21] R.G. Bates, M. Paabo, R.A. Robinson, J. Phys. Chem. 67 (1963) 1833.
[22] R.G. Bates, Determination of pH: Theory and Practice, 2nd ed, Wiley, New York, 1964.
[23] S. Espinosa, E. Bosch, M. Rosés, Anal. Chem. 72 (2000) 5193.
[24] I. Canals, K. Valkó, E. Bosch, A.P. Hill, M. Rosés, Anal. Chem. 73 (2001) 4937.
[25] M. Rosés, F. Rived, E. Bosch, J. Chromatogr. A 867 (2000) 45.
[26] F. Rived, I. Canals, E. Bosch, M. Rosés, Anal. Chim. Acta 439 (2001) 315.
[27] E. Bosch, S. Espinosa, M. Rosés, J. Chromatogr. A 824 (1998) 137.
[28] S. Espinosa, E. Bosch, M. Rosés, J. Chromatogr. A 947 (2002) 47.
[29] S. Espinosa, E. Bosch, M. Rosés, Anal. Chim. Acta 454 (2002) 157.
[30] C. Kalidas, G. Hefter, Y. Marcus, Chem. Rev. 100 (2000) 819.