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Prediction of peak shape as a function of retention in reversed-phase liquid chromatography

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Abstract

Optimisation of the resolution of multicomponent samples in HPLC is usually carried out by changing the elution conditions and considering the variation in retention of the analytes, to which a standard peak shape is assigned. However, the change in peak shape with the composition of the mobile phase can ruin the optimisation process, yielding unexpected overlaps in the experimental chromatograms for the predicted optimum, especially for complex mixtures. The possibility of modelling peak shape, in addition to peak position, is therefore attractive. A simple modified-Gaussian model with a parabolic variance, which is a function of conventional experimental parameters: retention time (t_R), peak height (H_0), standard deviation at the peak maximum (σ_0), and left (A) and right (B) halfwidths, is proposed. The model is a simplification of a previous equation proposed in our laboratory. Linear and parabolic relationships were found between the peak shape parameters (σ_0, A and B) and t_R, with a mean relative error of 1–5% in most cases. This error was partially due to variations in peak position and shape among injections, which in some cases were above 2%. Correlations between (σ_0, A and B) and the retention time, which is easily modelled as a function of mobile phase composition, allowed a simple and reliable prediction of chromatographic peaks. A parameter that depends on the slopes of the linear relationships for A and B versus t_R is also proposed to evaluate column efficiency. The modified-Gaussian model was used to describe the peaks of six diuretics of diverse acid–base behaviour and polarity, which were eluted with 15 mobile phases where the composition was varied between 30 and 50% (v/v) acetonitrile and the pH between 3 and 7.

Keywords: Non-Gaussian peak model; Parabolic variance; Peak shape; Efficiency; Diuretics

1. Introduction

Optimisation of the separation of multicomponent samples in HPLC is based frequently on the control of the elution of the individual compounds by modifying the composition of the mobile phase. The best conditions can be found by maximising a global resolution function built with the individual resolutions of consecutive peaks [1–4]. Quantification of the individual resolutions require the prediction of the retention behaviour of each compound by means of models that consider experimental factors, such as the percentage of organic modifier and pH. Very often, only the retention times of solutes are taken into account to evaluate the global resolution. Alternatively, peak widths and asymmetries, obtained by interpolation, are considered [3,4]. However, the inaccuracy in predicting the peak shape with changes in mobile phase composition can ruin an optimisation process, yielding unexpected overlaps, especially when complex mixtures are analysed. A useful tool is thus needed for the reliable simulation of chromatograms based on an accurate modelling of peak position and shape.

This work shows how the parameters depicting the shape (width and asymmetry) of a reversed-phase liquid chromatographic (RPLC) peak can be related with the retention time, which can be predicted with high accuracy. The relationships are demonstrated using the peaks of several diuretics, which were chromatographed isocratically in an octadecylsilane column with mobile phases of acetonitrile–water at varying pH.

There is no theoretical model for the exact description of the shape of chromatographic peaks. A number of empirical mathematical functions have been reported in the literature with different success [5–9]. The elution profiles of symmetrical chromatographic peaks are described by the

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Gaussian model. However, the assumption of this model for skewed peaks results in large errors. A useful approach is the use of a modified Gaussian equation where the standard deviation varies with time. To fit asymmetrical peaks we reported first an equation that describes peaks split in two parts of variable standard deviation [10]. This peak shape model was improved by using a standard deviation depending polynomially on the distance to the peak time (polynomially modified Gaussian model (PMG)) [11]. The PMG model can fit almost every peak, but gives a problematic baseline increase out of the peak region. Several functions have been recommended in the literature to avoid this problem [7,12]. One of them is a Gaussian-based equation whose variance is a combined parabolic-Lorentzian function (parabolic-Lorentzian modified Gaussian model, PLMG) [12]. The parabola accounts for the non-Gaussian shaped peak, whereas the Lorentzian function cancels the variance growth out of the peak region. This model makes a correct description of peaks showing a wide range of asymmetry with positive and/or negative skewness. It was applied successfully to the deconvolution of peaks in binary mixtures of highly overlapped compounds [12].

The PLMG model is, however, not adequate for prediction purposes due to its complexity. It has too many parameters (seven) which have no direct meaning in terms of peak shape characteristics. A modified Gaussian function with a parabolic variance (PVMG), which is a simplification of the PLMG model, is here proposed to describe the peak shape. This new function contains only five parameters, which can be easily related to measurable descriptors (retention time, peak height, and left and right halfwidths). The parameters in the PVMG model are also more stable against small variations in the signal (e.g. baseline, width and asymmetry) among replicate peaks than the parameters in the PLMG model. The PVMG parameters can be also related with the elution conditions as demonstrated further.

2. Theory

The PVMG model consists of a Gaussian function where the variance has a parabolic behaviour:

$$h = H_0 e^{-(1/2)t_c^2/(\sigma_0^2 + at_c + bt_c^2)} \quad (1)$$

where h is the peak height at time t, $t_0 = t - t_R$, being t_R the retention time, H_0 and σ_0 are the height and the standard deviation both at the peak maximum (i.e. for $t = t_R$), and a and b coefficients that depict the slope of the parabola at the peak maximum and its curvature, respectively. The coefficient b should be positive to make the parabola grow at times far from the minimum (i.e. avoiding negative values for the variance).

Coefficients a and b are affected by random errors and changes among replicates. It is thus convenient to relate them with more robust parameters as the left (A) and right (B) peak halfwidths, which are experimental measurable parameters giving an explicit description of peak shape. For this purpose, the following transformation of Eq. (1) was made:

$$p = -2 \ln \frac{h}{H_0} = \frac{t_c^2}{\sigma_0^2 + at_c + bt_c^2} \quad (2)$$

For $t < t_R (t_c < 0)$, a function of the left peak halfwidth at a peak height defined by p can be derived as:

$$A^2 + \frac{ap}{1 - bp} A - \frac{\sigma_0^2 p}{1 - bp} = 0 \quad (3)$$

For $t > t_R (t_c > 0)$, a similar equation is obtained for the right halfwidth:

$$B^2 - \frac{ap}{1 - bp} B - \frac{\sigma_0^2 p}{1 - bp} = 0 \quad (4)$$

The values of A and B at a given peak height are obtained by solving Eqs. (3) and (4) for the corresponding p:

$$A = -\frac{ap}{2(1 - bp)} + \sqrt{\left(\frac{ap}{2(1 - bp)}\right)^2 + \frac{\sigma_0^2 p}{(1 - bp)}} \quad (5)$$

$$B = \frac{ap}{2(1 - bp)} + \sqrt{\left(\frac{ap}{2(1 - bp)}\right)^2 + \frac{\sigma_0^2 p}{(1 - bp)}} \quad (6)$$

which results in:

$$B - A = \frac{ap}{1 - bp} \quad (7)$$

$$BA = \frac{\sigma_0^2 p}{1 - bp} \quad (8)$$

For simplicity, we took $p = 1$, that is, values of A and B measured at 60.65% of peak height. From Eqs. (7) and (8):

$$a = \frac{B - A}{BA} \sigma_0^2 \quad (9)$$

$$b = 1 - \frac{\sigma_0^2}{BA} \quad (10)$$

Therefore, peak shape can be described as a function of A and B by substituting Eqs. (9) and (10) in Eq. (1). After modelling the peak shape according to this approach, parameters A, B and σ_0 are directly obtained. Also, once these parameters are known, peak profiles can be simulated.

In practice, the proposed equation for the PVMG model can introduce distortions when the variance adopt null or negative values. We have observed this behaviour rarely and always in regions out of the peak domain. However, we suggest to avoid this problem by forcing a pseudo-exponential behaviour below a given peak height. This can be achieved by giving the variance a linear dependence with time. In this way, the Gaussian tends to an exponential as t_c^2 increases:

$$e^{-(1/2)t_c^2/(n + mt_c)} \xrightarrow{t_c \to \infty} e^{-(1/2)(t_c/m)} = e^{-kt_c} \quad (11)$$

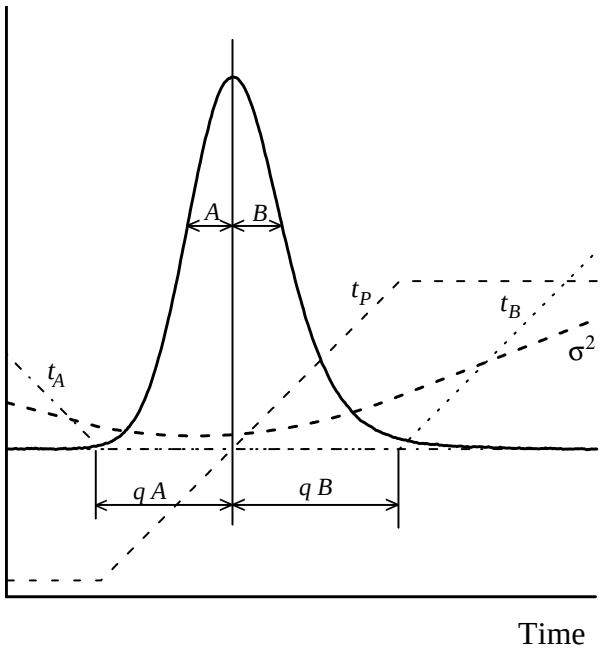

Fig. 1. Meaning of the parameters in the proposed peak shape approach. A and B are measured at 60.65% of peak height. In the figure, $q = 3$, which corresponds to 1.11% of peak height.

The transition from the parabolic to the linear behaviour can be achieved by using the following function:

$$h = H_0 e^{-(1/2)t_c^2/(\sigma_0^2 + at_P + bt_P^2 - m_A t_A + m_B t_B)} \quad (12)$$

where

$$t_P = t_c + t_A - t_B \quad (13)$$

$$t_A = \frac{\sqrt{(t_c + qA)^2} - (t_c + qA)}{2} \quad (14)$$

$$t_B = \frac{\sqrt{(t_c - qB)^2} + (t_c - qB)}{2} \quad (15)$$

Parameter q in Eqs. (14) and (15) is related to the peak height percentage at which the variance is forced to be linear. In this work, we adopted $q = 3$ ($t_c < -3A$ and $t_c > 3B$), which corresponds to 1.11% peak height. Fig. 1 and Table 1 are useful to understand the proposed approach. The figure depicts a conventional peak and how the variance (σ^2) changes with time. Note the linear behaviour of σ^2 for $t_c < -qA$ and $t_c > qB$. The linear functions representing t_P, t_A and t_B are drawn as dashed lines. As observed, t_P is constant above and below certain time values. In these regions, the variance is linear depending on t_A and t_B. On the other hand, within the peak region, $t_A = t_B = 0$ and the variance has a parabolic behaviour depending on t_P.

Table 1
Significance of the time parameters in Eqs. (13)–(15)

	t_P	t_A	t_B
$t_c < -qA$	$-qA$	$-t_c - qA$	0
$-qA < t_c < qB$	t_c	0	0
$t_c > qB$	qB	0	$t_c - qB$

The continuity of the variance function is guaranteed by giving the same slope to the parabola and linear function at the transition point (i.e. for $t_c = -qA$ and $t_c = qB$):

$$m_A = a - 2qbA \quad (16)$$

$$m_B = a + 2qbB \quad (17)$$

To assure an exponential decay for the leading and tailing edges of the peak, m_A and m_B in Eq. (12) should be positive. When these parameters are negative, they are made equal to zero ($m_A = 0$ and $m_B = 0$). According to this, Eq. (12) adopts a Gaussian behaviour for $t_c < -qA$ or $t_c > qB$, respectively.

3. Experimental

The mobile phases were prepared with acetonitrile (Scharlau, Barcelona, Spain) and the pH adjusted with 0.01 M citrate buffer, which was prepared with citric acid monohydrate and sodium hydroxide (Panreac, Barcelona). The probe compounds were: chlorthalidone (CHL) (Ciba Geigy, Barcelona), ethacrynic acid (ETH) (Merck, Sharp & Dohme, Madrid, Spain), spironolactone (SPI) (Searle, Madrid) and xipamide (XIP) (Lacer, Barcelona), which were kindly donated by the pharmaceutical laboratories, and althiazide (ALT) and benzthiazide (BEN), which were purchased from Sigma (St. Louis, MO, USA).

The HPLC system (Model HP 1050, Palo Alto, CA, USA) was equipped with an isocratic pump, an autosampler with

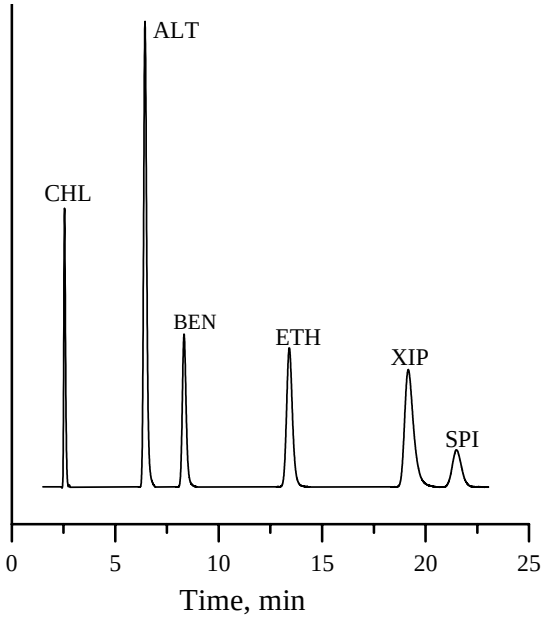

Fig. 2. Chromatogram of the six probe compounds eluted with 40% acetonitrile at pH 3.

Table 2
Retention time and asymmetry factor ranges for the probe compounds[a]

Compound	t_R (min)	B/A[a]
Chlorthalidone	1.98–4.15	1.17–1.28
Althiazide	3.47–16.0	1.08–1.19
Benzthiazide	2.80–24.84	1.06–1.21
Xipamide	1.91–41.3	1.06–1.26
Ethacrynic acid	1.96–73.5	1.17–1.44
Spironolactone	8.20–97.0	1.05–1.15

[a] Considering the 15 mobile phases.

2 ml vials (Series 1100, Model G1313A), and a UV-Vis detector. The signal was monitored at 274 nm.

All separations were carried out with a Kromasil C18 column (125 mm × 4.6 mm i.d. and 5 μm particle size) (Análisis Vínicos, Ciudad Real, Spain), which was connected to a similar 30 mm guard column (Scharlab). The probe compounds were eluted with 15 mobile phases at three acetonitrile levels (30, 40, 50% v/v) and five pH levels (3, 4, 5, 6 and 7). Chromatographic runs were carried out at room temperature. The flow-rate was 1.0 ml/min and the injection volume, 20 μl. Duplicate or triplicate injections were made.

Data acquisition was carried out with the Peak-96 software (Hewlett-Packard, Avondale, PA, USA). The data acquisition rate was 60 points per minute. All software for data treatment was implemented in our laboratory in BASIC.

4. Results and discussion

4.1. Retention behaviour of the probe compounds

Six diuretics with diverse acid–base and retention behaviour (polarity) were selected. Spironolactone is a neutral diuretic with an octanol–water partition coefficient, log $P_{o/w} = 2.71$. The other diuretics are acidic; their dissociation constants in aqueous medium (pK_a) [13,14] and log $P_{o/w}$ [15] are: chlorthalidone (9.3, 0.24), althiazide (>7,

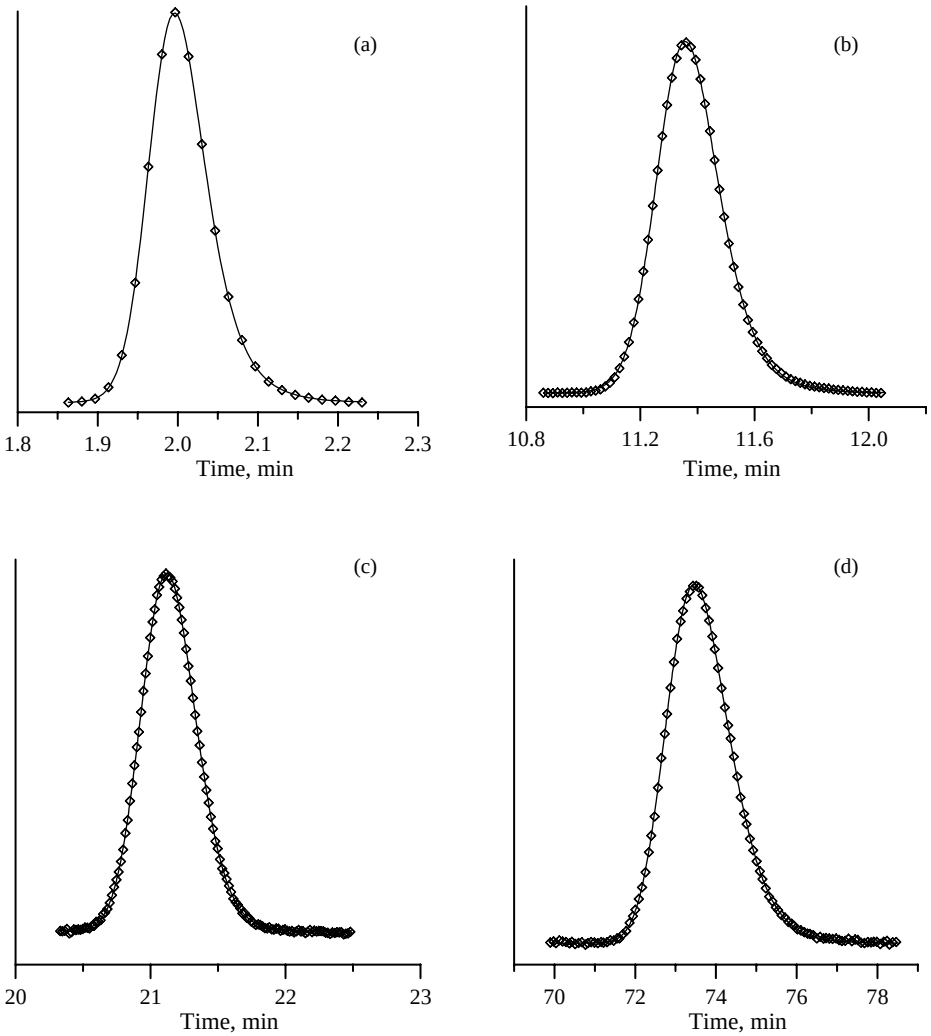

Fig. 3. Experimental (◇) and fitted (solid line) chromatographic peaks for: (a) chlorthalidone at 50% acetonitrile and pH 7; (b) xipamide at 40% acetonitrile and pH 4; (c) spironolactone at 40% acetonitrile at pH 7; and (d) ethacrynic acid at 30% acetonitrile and pH 3.

1.01), benzthiazide (6.0, 1.73), xipamide (4.8 and 10, 2.19), and ethacrynic acid (3.5, 2.20). In the aqueous-organic mixture, the dissociation constants are shifted to larger pH [16]. Table 2 gives the ranges of retention times and peak asymmetries (*B*/*A*), for the probe compounds.

Chlorthalidone elutes always at low retention times, while the retention time range for ethacrynic acid, spironolactone and xipamide is rather wide, especially for the two former. Retention at any mobile phase composition can be described according to a polynomial model:

$$\log k = c_0 + c_1\varphi + c_2\varphi^2 \quad (18)$$

where c_i are fitting coefficients.

For compounds exhibiting acid–base behaviour, the effect of pH on retention at a fixed organic modifier concentration is given as a weighted mean of the retention factors of the acidic (k_{HA}) and basic (k_A) species:

$$k = k_A\frac{1}{1+K'h} + k_{HA}\frac{K'h}{1+K'h} = \frac{k_A + k_{HA}K'h}{1+K'h} \quad (19)$$

K' being the apparent protonation constant in the aqueous–organic medium and h the hydrogen ion concentration. A sharp change in retention takes place at pH values close to the logarithm of the apparent protonation constant. Accordingly, no significant effect of pH on the retention factors was observed for the weakly acidic diuretics (althiazide and chlorthalidone) in the pH range of the chromatographic column (pH 3–7). The whole retention drop was observed for xipamide (with an aqueous log $K = 4.8$), but only a partial view of this drop appeared for the more acidic ethacrynic acid (log $K = 3.5$, retention was constant above pH 5), and the less acidic benzthiazide (log $K = 6.0$, retention was almost constant below pH 5).

Due to the diverse retention and acid–base behaviour of the probe compounds, some peak reversals took place at varying pH. Thus, at pH 3, the elution order was spironolactone > ethacrynic acid > xipamide > benzthiazide > althiazide > chlorthalidone, whereas at pH 7 the order was spironolactone > althiazide > benzthiazide > ethacrynic acid > chlorthalidone > xipamide. A chromatogram of the six compounds eluted with 40% acetonitrile at pH 3 is depicted in Fig. 2.

4.2. Accuracy of the peak model

The performance of the new modified-Gaussian model (PVMG) to describe the peak shape was first checked. For this purpose, the 90 peaks obtained for the six compounds eluted with the 15 mobile phases were fitted to Eq. (12), taking into account Eqs. (9), (10), and (13)–(17). The method of Powell was used to make the non-linear fitting of the experimental data to the proposed model [17]. Fig. 3 shows the experimental and fitted peaks for four probe compounds eluted under different elution conditions at diverse retention times, which are representative of the whole set of peaks. The accuracy of the fittings was evaluated using the regression coefficient (r) and the mean relative prediction error of the experimental points [18]:

$$\varepsilon_r(\%) = \frac{\sum |S_i^{\text{exp}} - S_i^{\text{pred}}|}{\sum |S_i^{\text{exp}}|} \times 100 \quad (20)$$

where S_i^{exp} and S_i^{pred} are the experimental and predicted signals, respectively. Mean r and ε_r for each compound (considering the peaks obtained with the 15 mobile phases) are given in Table 3. The fittings were excellent, with $r >$ 0.9999 and $\varepsilon_r <$ 1% for almost all peaks. As observed, the proposed model gives a satisfactory description of peak shape independently of the solute polarity and strength of the mobile phase. Although the relative fitting errors were always very low, it should be indicated that the best results were obtained, as expected, for the most symmetrical peaks.

Table 3
Accuracy of the proposed peak model

Compound	Mean r	Mean ε_r (%)
Chlorthalidone	0.99996 ± 0.00003	0.64 ± 0.23
Althiazide	0.99998 ± 0.00001	0.43 ± 0.16
Benzthiazide	0.99996 ± 0.00002	0.44 ± 0.17
Xipamide	0.99997 ± 0.00002	0.58 ± 0.28
Ethacrynic acid	0.99994 ± 0.00005	0.62 ± 0.31
Spironolactone	0.99974 ± 0.00023	0.31 ± 0.09

Table 4
Fitting of the peak shape parameters against retention time

Compound	Linear dependence		Parabolic dependence	
	r	ε_r (%)	r	ε_r (%)
σ_0				
Chlorthalidone	0.9840	3.2	0.9860	3.1
Althiazide	0.9994	1.4	0.9996	1.1
Benzthiazide	0.9993	2.3	0.9997	1.4
Xipamide	0.9992	3.7	0.9997	2.1
Ethacrynic acid	0.9993	4.2	0.9997	2.0
Spironolactone	0.9996	1.9	0.9998	1.2
A				
Chlorthalidone	0.9810	3.1	0.9820	3.1
Althiazide	0.9995	1.3	0.9997	0.94
Benzthiazide	0.9993	2.3	0.9998	1.2
Xipamide	0.9992	3.3	0.9997	1.9
Ethacrynic acid	0.9990	5.6	0.99991	1.2
Spironolactone	0.99990	1.2	0.99997	0.52
B				
Chlorthalidone	0.9840	3.2	0.9860	3.1
Althiazide	0.9992	1.5	0.9993	1.4
Benzthiazide	0.9993	2.0	0.9995	1.5
Xipamide	0.9992	3.7	0.9997	2.1
Ethacrynic acid	0.9993	4.2	0.9997	2.0
Spironolactone	0.9997	1.8	0.9998	1.3

4.3. Relationship between peak shape parameters and retention time

Retention times in RPLC can be predicted with high accuracy through Eqs. (18) and (19). If an adequate relationship can be established for the parameters σ_0, A and B versus t_R, peak shape will be also predicted with sufficient accuracy. Theoretically, there is a linear dependence with null intercept between the standard deviation of an ideal chromatographic peak and the retention time [19]. Therefore, we first checked the performance of linear relationships between the peak shape parameters and t_R. We further examined a parabolic dependence to include non-linear effects.

The performance of the linear and parabolic correlations is indicated in Table 4 for the five probe compounds. The linear correlation between the peak shape parameters and t_R yielded $r > 0.999$ and $\varepsilon_r = 1$–5%, except for chlorthalidone. The fittings improved when a parabolic dependence was assumed, especially for the compounds with a retention depending on pH (e.g. for ethacrynic acid ε_r decreased from 4.2 to 2.0%). For the parabolic correlation, $\varepsilon_r = 0.5$–3%. These prediction errors were partially due to variations in peak position and shape among injections, which in some cases were above 2%. Fig. 4 illustrates the dependence of σ_0, A and B with t_R for four probe compounds: two acidic (ethacrynic acid and xipamide), one weakly acidic (althiazide) and one neutral (spironolactone).

Small changes in the retention of althiazide, chlorthalidone and spironolactone were observed at varying pH. The changes were partially random (due to variations in peak position among injections as indicated above), but they may be ascribed to changes in ionic strength at varying pH (Fig. 4b and d). The linear and parabolic fittings (Table 4) were poorer for chlorthalidone. It should be noted that the retention of this compound is very low and only three retention data were in fact available (the retention of this compound was not affected by a change in pH).

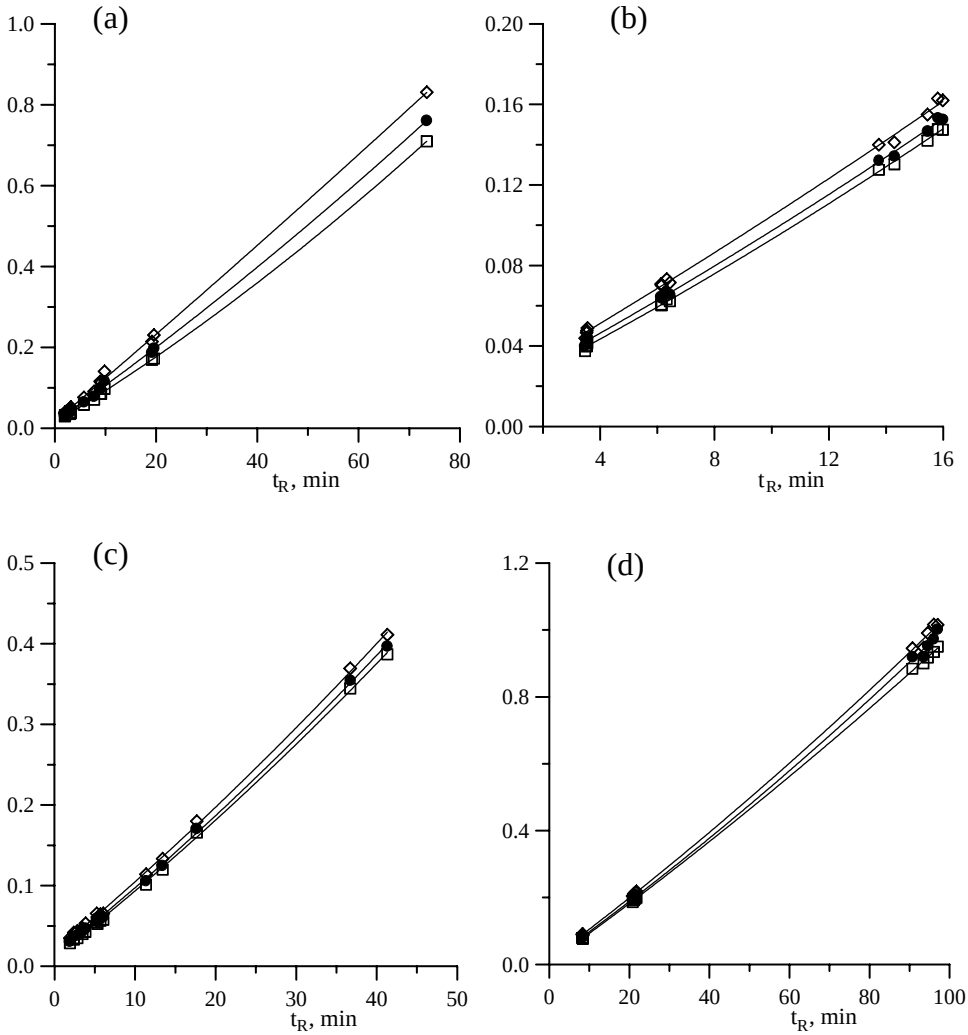

Fig. 4. Plots of the peak shape parameters σ_0 (●), A (□), and B (◇) vs. retention time for: (a) ethacrynic acid; (b) althiazide; (c) xipamide; and (d) spironolactone. The lines correspond to the parabolic fitting.

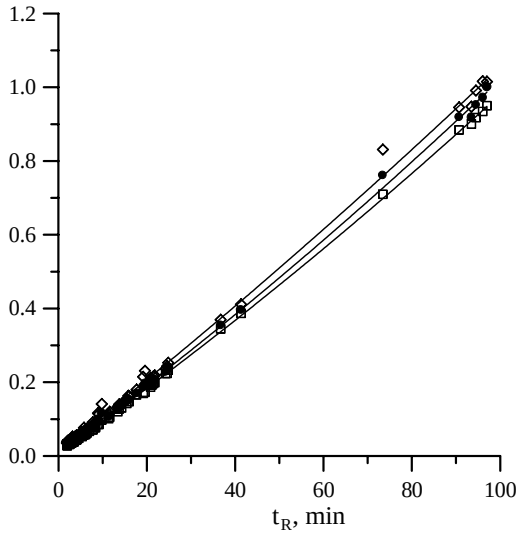

Fig. 5. Plots of the peak shape parameters σ_0 (●), A (□), and B (◇) vs. retention time considering the six probe compounds. The lines correspond to the parabolic fitting.

The results in Fig. 4 show that a parabolic dependence allows the prediction of peak shape as a function exclusively of retention time, independently of the source of change in retention time (e.g. organic modifier concentration or pH). This is confirmed in Fig. 5, where the parameters of the six probe compounds are plotted altogether. The data for all compounds follow the same parabolic trend (almost linear), within the experimental error. The regression coefficients (r) and mean prediction errors (ε_r) for the linear fittings were: 0.9991 and 5.1% for σ_0, 0.9994 and 4.4% for A, and 0.9986 and 5.5% for B, and for the parabolic fittings: 0.9996 and 2.5% for σ_0, 0.99990 and 1.8% for A, and 0.9990 and 3.8% for B. All chromatographic peaks showed a positive asymmetry factor ($B/A > 1$), corresponding to tailing peaks. This can explain the greater scattering of the right halfwidth data, B.

The proposed approach showed a very good performance in the prediction of peak shape. A chromatogram of the six probe compounds predicted for 50% acetonitrile at pH 3 is depicted in Fig. 6a together with the experimental data. The agreement is satisfactory, even for the tailing peaks of the most retained compounds. This result should be compared with that obtained considering a Gaussian peak model (Fig. 6b). Table 5 gives some more information about the accuracy of the fittings.

4.4. Measurement of column efficiency

The change in peak width with retention time, in mobile phases with diverse composition and pH, depends mainly on the characteristics of the column. As shown, the dependence of the peak shape parameters with retention time is parabolic (Fig. 5), but can be approximated to a linear trend with a positive intercept. The number of theoretical plates, N, which is a measure of the efficiency in a chromatographic system

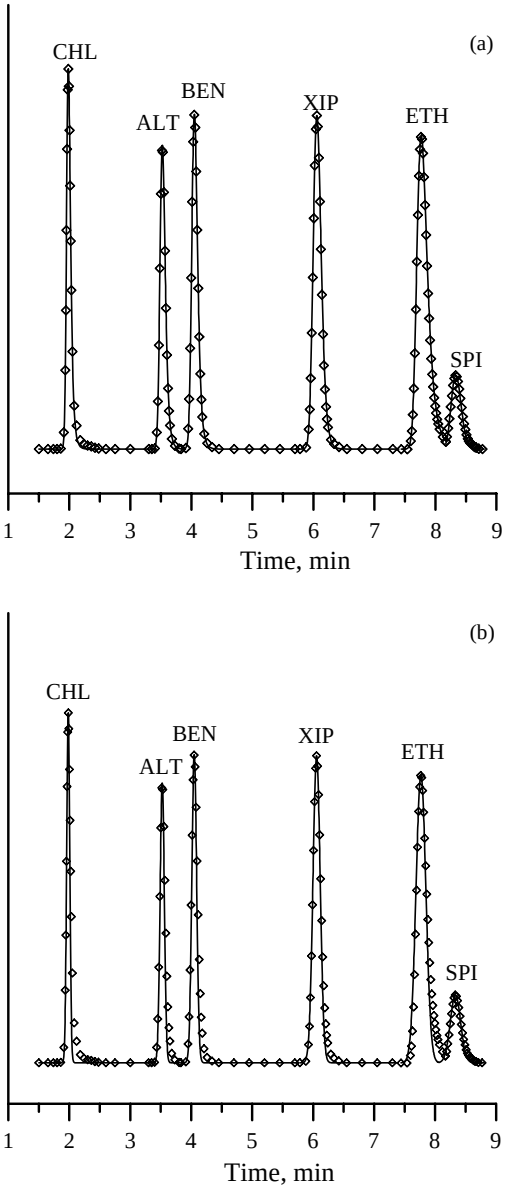

Fig. 6. Predicted chromatogram for 50% acetonitrile at pH 3. (a) proposed approach; (b) Gaussian model.

is usually defined considering a single band:

$$N = \left(\frac{t_R}{\sigma_t}\right)^2 = 16\left(\frac{t_R}{w}\right)^2 \quad (21)$$

Table 5
Prediction errors

Compound	Proposed approach		Gaussian model	
	r	ε_r (%)	r	ε_r (%)
Chlorthalidone	0.9992	2.6	0.950	19.2
Althiazide	0.9993	2.1	0.958	17.8
Benzthiazide	0.9988	3.3	0.963	18.1
Xipamide	0.9987	3.5	0.991	8.5
Ethacrynic acid	0.9985	3.9	0.968	17.5
Spironolactone	0.9973	2.9	0.991	5.1

where σ_t is the band standard deviation in time units and w the width at the peak base. Because the separation in a particular chromatographic column is linked to the time spent by the solute in the stationary phase, in practice, an effective plate number is defined [19]:

$$N_{\text{eff}} = N\left(\frac{k}{1+k}\right)^2 = \left(\frac{t_R - t_0}{\sigma_t}\right)^2 \quad (22)$$

t_0 being the dead time. The efficiency definitions expressed by Eqs. (21) and (22) give values which are independent of the retention time only when the peak width is null at $t_R = 0$. A parameter less dependent on the retention time can be established as follows:

$$N' = 16\left(\frac{dt_R}{dw}\right)^2 \quad (23)$$

We will assume (for simplicity) the following relationship:

$$w = 2(A + B) = 2(m_A + m_B)t_R + 2(n_A + n_B) \quad (24)$$

with A and B defined for $p = 1$. The non-null intercept of w versus t_R (Eq. (24)) indicates that there is a residual width that should be subtracted from the peak width to evaluate the column efficiency. From Eqs. (23) and (24):

$$N' = 16\left(\frac{\Delta t_R}{\Delta w}\right)^2 = \left(\frac{2}{m_A + m_B}\right)^2 \quad (25)$$

The efficiency parameter, N', in Eq. (25) should be constant for the chromatographic system and independent of the dead time. The calculation of N' needs at least the peak shape data of two chromatographic peaks showing different retention times to evaluate the slopes of A and B versus t_R. However, a better description is obtained using the data from a set of peaks at several retention times. Taking into account all the peaks obtained for the six probe compounds, N' ranged between 9260 and 10350 for the working column, showing a mean value and standard deviation of $N' = 9900 \pm 200$. The value of N calculated according to Eq. (21) ranged between 3100 for the peaks at the lowest retention times and 11300 for the peaks eluting above 12 min, with $N = 8000 \pm 2500$.

5. Conclusions

Knowledge of retention and peak shape is important to achieve reliable simulations of chromatograms. Both chromatographic characteristics are affected by a change in organic modifier concentration, pH, and other factors such as ionic strength. However, a correlation can be established between peak shape and retention, which can be used with prediction purposes. The simulations can be improved if peak height is also considered.

A peak shape model, which is a function of measurable experimental parameters (standard deviation and height at the peak maximum, and left and right halfwidths), is proposed. The accurate prediction of peak shape parameters is possible in RPLC by using a parabolic (almost linear) function of the retention time. The retention time, in turn, can be accurately predicted as a function of organic modifier concentration and pH.

The same experimental design used to model the retention behaviour can be applied to obtain the peak shape parameters for each compound. However, peak width depends mainly on the column performance (i.e. all compounds follow a similar trend at varying retention times). Therefore, the column can be characterised using the shape data of only a few peaks obtained for several compounds at diverse retention times.

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