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Approaches to characterise chromatographic column performance based on global parameters accounting for peak broadening and skewness

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ABSTRACT

Peak broadening and skewness are fundamental parameters in chromatography, since they affect the resolution capability of a chromatographic column. A common practice to characterise chromatographic columns is to estimate the efficiency and asymmetry factor for the peaks of one or more solutes eluted at selected experimental conditions. This has the drawback that the extra-column contributions to the peak variance and skewness make the peak shape parameters depend on the retention time. We propose and discuss here the use of several approaches that allow the estimation of global parameters (non-dependent on the retention time) to describe the column performance. The global parameters arise from different linear relationships that can be established between the peak variance, standard deviation, or half-widths with the retention time. Some of them describe exclusively the column contribution to the peak broadening, whereas others consider the extra-column effects also. The estimation of peak skewness was also possible for the approaches based on the half-widths. The proposed approaches were applied to the characterisation of different columns (Spherisorb, Zorbax SB, Zorbax Eclipse, Kromasil, Chromolith, X-Terra and Inertsil), using the chromatographic data obtained for several diuretics and basic drugs (β-blockers).

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1. Introduction

Broadening of chromatographic peaks, measured as the total peak variance in time units, σ_t^2, results from several factors of two origins: intra-columnar, σ_{col}^2, and extra-columnar, σ_{ext}^2, which are assumed to be additive [1–3]:

$$\sigma_t^2 = \sigma_{col}^2 + \sigma_{ext}^2 \quad (1)$$

Peak broadening is a fundamental factor in chromatography, since it affects the resolution capability of a chromatographic column. The interest in developing descriptors that characterise column performance related to peak broadening is, thus, not surprising, being the number of theoretical plates (plate count or efficiency, N) the most popular. This concept is based on the plate theory, described by Martin and Synge in 1941 [4]. The number of plates, obtained by considering a Poisson distribution that is approximated to a Gaussian, is given by:

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

where t_R is the retention time, and σ_t is the standard deviation of a chromatographic peak eluted in the isocratic mode. The peak standard deviation (or width) is inversely proportional to the square root of the efficiency and, thus, the narrower the peak, the higher the efficiency. According to Neue [1], the plate count can be viewed as a measure of the distribution of elution times of the analyte molecules, the relative standard deviation (in percentage) being equal to the reciprocal square root of the efficiency (×100). Thus, for example, a peak with N = 10,000 will have a standard deviation amounting 1% of the retention time.

For a pure Gaussian peak, the efficiency is often estimated in terms of total peak width (w) at a selected height, as follows:

$$N = a\left(\frac{t_R}{w}\right)^2 \quad (3)$$

where a = 4 when w is the peak width at the inflection point (60.3% peak height), a = 5.54 when measured at half-height, and a = 16 when measured at the base (4σ method, 13.4% peak height) [1]. Pure Gaussian chromatographic peaks are, however, seldom observed experimentally (i.e. the peaks are usually tailing, and in some cases, fronting), due to a number of internal and extra-column factors (mainly from the injection profile and isotherm non-linearity). Obviously, the estimation of the efficiency according to Eq. (3) will be biased for these peaks. A general solution to the calculation of efficiencies, independently of the peak skewness,

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Nomenclature

A	left half-width
A_0	left half-width for a peak eluting at the dead time
$A_{0.1}$	left half-width at 10% peak height
A_{i+1} and A_i	left half-width for two adjacent peaks
$\bar{A}_{int}$	mean left half-width according to the "integral method"
$\bar{A}_{sum}$	mean left half-width according to the "summation method"
B	right half-width
B_0	right half-width for a peak eluting at the dead time
$B_{0.1}$	right half-width at 10% peak height
B_{i+1} and B_i	right half-width for two adjacent peaks
$\bar{B}_{int}$	mean right half-width according to the "integral method"
$\bar{B}_{sum}$	mean right half-width according to the "summation method"
B/A	asymmetry factor
corrected retention time	difference between the retention time and the dead time
f_{asym}	asymmetry factor
$\bar{f}_{int}$	mean asymmetry factor according to the "integral" method
h_0	height at the peak maximum
$h(t)$	peak height at any time
integral method	method to obtain mean parameters based on a synthetic chromatogram with multiple peaks showing retention times separated in an infinitesimal distance
M_1	first moment (retention time)
M_2	second moment (peak variance)
m_σ	slope of the linear relationship between the standard deviation and the retention time
m_A	slope of the linear relationship between the left half-width and the retention time
m_B	slope of the linear relationship between the right half-width and the retention time
$m_A + m_B$	peak broadening rate
m_B/m_A	peak skewness
N	efficiency, plate count or number of plates
N_{obs}	observed efficiency
N_{col}	column efficiency calculated from the retention time (Eq. (7))
N_{eff}	effective plate number calculated from the corrected retention time (Eq. (12))
N_σ	column efficiency according to Eq. (16)
P_c	peak capacity or maximal number of resolved peaks that fit in a chromatographic window
R	regression coefficient
R^2	determination coefficient
r_{PB}	peak broadening rate inside the column
R_S	chromatographic resolution
s_0	standard deviation at the peak maximum
SDS	sodium dodecyl sulphate
"summation" method	method to obtain mean parameters based on the ideal chromatogram used to define the peak capacity concept
σ_0^2	variance of an unretained peak
σ_{col}^2	intra-column contribution to peak variance
σ_{ext}^2	extra-column contribution to peak variance
σ_t^2	total peak variance in time units
t_0	dead time
T_0	time for an ideal peak with width $A_0 + B_0$
t_1	corrected retention time for the first peak in the selected time window
t_{i+1} and t_i	retention times for two adjacent peaks
t_n	corrected retention time for the last peak in the selected time window
t_R	retention time
$\bar{t}_{R,int}$	mean retention time according to the "integral" method
$\bar{t}_{R,sum}$	mean retention time according to the "summation" method
$\bar{t}_{sum}$	mean corrected retention time according to the "summation" method
w	peak width
$w_{0.1}$	peak width at 10% peak height
z	standard score

is offered by the moment method:

$$N = \frac{M_1^2}{M_2} \tag{4}$$

where M_1 accounts for the retention time, and M_2 for the peak variance [3,5]. Compared to the moment method, the estimation of the efficiency with Eq. (3) offers overestimations, often exceeding 100% [5,6].

However, the direct numerical integration of the experimental peak profile needed to get the moments may also be affected of error arising from the limits used in the integration, the baseline drift and noise. This, together with the need of digital curve fitting, has been the reason of the proposal of other approaches based on the exponentially modified Gaussian model and measurement of the widths above the baseline [6–8], from which the most generally accepted is the Foley and Dorsey approach [6,9]. These authors developed the following expressions to estimate the second moment (the variance), and the efficiency:

$$M_2 = \frac{w_{0.1}^2}{1.764(B/A)_{0.1}^2 - 11.15(B/A)_{0.1} + 28} \tag{5}$$

$$N = \frac{41.7(t_R/w_{0.1})^2}{(B/A)_{0.1} + 1.25} \tag{6}$$

where the width ($w_{0.1}$), and the left ($A_{0.1}$) and right ($B_{0.1}$) half-widths, are measured at 10% peak height, being $w_{0.1} = A_{0.1} + B_{0.1}$, and $(B/A)_{0.1}$ the peak asymmetry (see also Fig. 1). Eqs. (5) and (6) have been reported to yield errors <1.5% for peaks showing asymmetry factors $(B/A)_{0.1}$ in the range 1.00–2.76 [5,6].

According to Eq. (1), the peak profile (i.e. the relative peak standard deviation, or observed efficiency, N_{obs}) will depend on the chromatographic instrument to which the column is connected. Also, the peak profile will depend on the retention time, since as this increases, the external contribution to the global variance becomes less significant. The efficiency for experimental peaks obtained at specific mobile phase compositions can be calculated from Eqs. (4) and (6). In some cases, however, the estimation of the efficiency at particular retention times, from which no experimental data are available, is needed. This is the case when chromatographic columns or solvents should be compared, or peak resolution optimised. For this purpose, a model that relates the peak profile (expressed as peak variance, standard deviation, or left and right half-widths), with the retention time should be useful.

A common model used to estimate the intra- and extra-column contributions to the observed peak broadening at different reten-

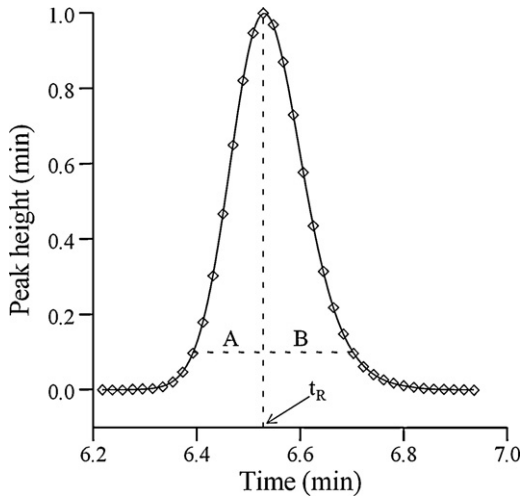

Fig. 1. Chromatographic peak fitted using the modified Gaussian model (Eqs. (8)–(10)). The retention time and peak half-widths at 10% peak height are outlined.

tion times is based on the following equation:

$$\sigma_t^2 = \frac{t_R^2}{N_{obs}} = \frac{t_R^2}{N_{col}} + \sigma_{ext}^2 \quad (7)$$

derived from Eqs. (1) and (2). It indicates that the plot of the observed variance, σ_t^2, versus t_R^2, for several peaks obtained at different retention times will yield a straight-line. Eq. (7) contains a number of assumptions: the additivity of the column and instrument variances is accepted (Eq. (1)), the instrument variance is independent of the solute retention factors, and all solutes have the same column plate height associated [10,11].

In Eq. (7), N_{obs} is the observed efficiency (which considers both intra- and extra-column contributions to the peak width, and consequently, it changes with the retention time), and N_{col} is the intrinsic column efficiency (i.e. which only takes into account the intra-column contributions, and is a fixed parameter not depending on the retention time). The observed efficiency, N_{obs}, characterises single peaks eluting at specific retention times, whereas N_{col} and σ_{ext}^2 are parameters that characterise the chromatographic system (the column behaviour and the extra-column effects, respectively). When both system (global) parameters (N_{col} and σ_{ext}^2) are known, the prediction of the peak variance (and N_{obs}) at any retention time is possible. According to Eq. (7), $N_{col} > N_{obs}$. As the retention time increases, the external contribution to the global variance is less significant, and N_{obs} becomes closer to N_{col}: the observed efficiencies of highly retained peaks will be larger than the efficiencies at shorter retention. At sufficiently high retention times: $N_{obs} = N_{col}$.

In this work, the performance of Eq. (7) and other linear equations relating the peak variance, standard deviation or half-widths, with the retention time (t_R^2 for the peak variance), are examined using information obtained in our laboratory for different compounds and columns. It will be also shown that from these equations, it is possible to derive several global parameters that describe the column performance accounting for peak broadening and skewness. The probabilities of resolution of closely eluting compounds are associated with the peak width and skewness. If the separation space is larger, reaching full resolution will be more likely. One of such global parameters characterising column performance is the column efficiency, N_{col}, described above. Another widely used global parameter is the peak capacity (i.e. maximal number of resolved peaks that fit in a chromatographic window), which considers the peak broadening produced inside the column and the extra-column effects altogether [12,13]. We give here several alternatives to these parameters, according to three approaches, to characterise the chromatographic system as a whole, or distinguish the intra- and extra-column contributions to the peak broadening. The proposed parameters should be useful for column development and selection.

2. Theory

2.1. Measurement of peak variance and half-widths

The calculation of peak moments (which are provided by several data stations) does not seem to be the best solution to obtain the peak variance (or standard deviation), since a small error in determining the baseline will influence the selected positions for the start and end of the peak, resulting in uncertain estimations. This is the reason of the wide use of the equations of Foley and Dorsey to measure the second moment and the efficiency, based on the exponentially modified Gaussian model (Eqs. (5) and (6), respectively). In these equations, the standard deviation is estimated from the half-widths at 10% peak height, where the skewness is still apparent.

In this work, the variance for each peak was obtained from Eq. (5), and from this, the standard deviation. On the other hand, the peak half-widths were estimated through fitting of the signals to a modified Gaussian model [14,15], where the variance changed with the distance to the peak maximum according to:

$$h(t) = h_0 \exp\left[-\frac{1}{2}\frac{(t-t_R)^2}{s_0^2 + a(t-t_R) + b(t-t_R)^2}\right] \quad (8)$$

where

$$a = \frac{B_{0.1} - A_{0.1}}{A_{0.1}B_{0.1}} s_0^2 \quad (9)$$

$$b = \frac{1}{4.6} - \frac{s_0^2}{A_{0.1}B_{0.1}} \quad (10)$$

$h(t)$ is the peak height at any time, and h_0 and s_0 are the height and standard deviation at the peak maximum. With this model, fitting of chromatographic peaks showing a wide range of efficiencies and asymmetries was always excellent (with $R \geq 0.999$).

2.2. First approach: estimation of column efficiency from the peak variance or standard deviation

According to Eq. (7), the intra-column peak variance is given by:

$$\sigma_{col}^2 = \frac{t_R^2}{N_{col}} \quad (11)$$

where N_{col} is the column efficiency. This concept has been also approximated from the peak broadening that occurs during the time the solute interacts with the stationary phase, which is called the effective plate number, N_{eff} [2]:

$$\sigma_{col}^2 = \frac{(t_R - t_0)^2}{N_{eff}} \quad (12)$$

t_0 being the dead time. Both definitions of column efficiency, N_{col} and N_{eff}, can be related as follows:

$$N_{eff} = N_{col}\left(\frac{t_R - t_0}{t_R}\right)^2 \quad (13)$$

The drawback of Eq. (12) is that it erroneously predicts zero peak spreading for an unretained solute. Also, note that Eq. (13) shows that N_{eff} depends on the retention time (only for sufficiently retained solutes, N_{col} and N_{eff} will be similar).

Eq. (7) allows the prediction of the observed peak broadening considering both the intra- and extra-column contributions, the former defined by Eq. (11). We propose a similar equation for the

approach based on Eq. (12), where the total variance depends on two components (σ^2_{col} and σ^2_0):

$$\sigma^2_t = \sigma^2_{col} + \sigma^2_0 = \frac{(t_R - t_0)^2}{N_{eff}} + \sigma^2_0 \quad (14)$$

The effective efficiency, N_{eff}, can be obtained from the slope of Eq. (14). The meaning of σ^2_{ext} (Eq. (7)) and σ^2_0 (Eq. (14)) is different; σ^2_{ext} refers exclusively to the extra-column contribution to the peak variance, and σ^2_0 is the variance for an unretained solute, which incorporates both intra- and extra-column components.

In spite of the widely acceptance of Eq. (7), a linear relationship has been suggested between the observed peak width (or standard deviation) and the retention time, for a variety of solutes, which also implies a constant column efficiency [16,17]:

$$w = a + bt_R \quad (15)$$

where w was measured at half peak height. We have observed that such linear relationship holds [14,18–21], and propose here the following linear equation to relate the peak standard deviation with the retention time:

$$\sigma_t = \frac{t_R - t_0}{\sqrt{N_\sigma}} + \sigma_0 = m_\sigma(t_R - t_0) + \sigma_0 \quad (16)$$

where the time the solute interacts with the stationary phase is considered again as variable, and the reverse of the square root of the efficiency, N_σ (m_σ), is the slope to make it comparable to N_{col} and N_{eff}. Eq. (16) can be expressed in terms of t_R and σ_{ext} as Eq. (7). There is no fundamental difference; only the time axis is shifted between both plots. However, we preferred using $(t_R - t_0)$ and σ_0, because they have a more straightforward meaning: σ_0 is the standard deviation associated with an unretained solute eluting at t_0. Also, quite often, owing to the experimental error, the linear equation based on t_R and σ_{ext} yields negative values for σ_{ext}.

2.3. Second approach: estimation of column peak broadening and skewness from the half-widths

Eqs. (7), (14) and (16) give an estimation of the column efficiency. These equations also allow predicting the observed peak variance or standard deviation at different retention times. However, they do not give information about the peak skewness. For this purpose, we propose the following linear relationships based on Eq. (16):

$$A = m_A(t_R - t_0) + A_0 \quad (17)$$

$$B = m_B(t_R - t_0) + B_0 \quad (18)$$

where m_A and m_B are the slopes of the equations, and A_0 and B_0 the peak half-widths at the dead time ($t_R = t_0$). These equations allow the prediction of the peak widths ($w = A + B$) and asymmetry factors (B/A). They are also useful to estimate the observed efficiency according to Eq. (6) for peaks eluting at different retention times.

It should be noted that the relationships between the peak standard deviation, or the peak half-widths, with the retention time are indeed parabolic. However, we have checked that they can be approximated to straight-lines in wide ranges of retention time [14,21]. In previous work, the usefulness of such linear simplifications was demonstrated for optimisation purposes [14], and the estimation of the peak capacity [19]. We show below that they also allow the proposal of global parameters to characterise peak broadening and skewness.

Thus, the chromatographic performance can be described by the sum of slopes ($m_A + m_B$), and their ratio (m_B/m_A). In order to understand the meaning of these parameters, consider the sum of Eqs. (17) and (18):

$$w = (m_A + m_B)(t_R - t_0) + (A_0 + B_0) = \frac{r_{PB}}{100}(t_R - t_0) + (A_0 + B_0) \quad (19)$$

where the slope $r_{PB} = (m_A + m_B) \times 100$ represents the rate of peak broadening inside the column (expressed as percentage), that is, the column peak broadening rate. For sufficiently retained peaks, the term $(A_0 + B_0)$ will be negligible, and the observed broadening should be associated only with the column.

The peak asymmetry factor at any retention time can be calculated from:

$$f_{asym} = \frac{B}{A} = \frac{m_B(t_R - t_0) + B_0}{m_A(t_R - t_0) + A_0} \quad (20)$$

which tends to be a constant value for long enough retention times, where A_0 and B_0 are negligible. Therefore, the ratio m_B/m_A represents the asymmetry factor of a highly retained compound, or the column component to peak skewness.

2.4. Third approach: estimation of mean values of the observed efficiencies and asymmetry factors to characterise the system performance

Mean values of the observed efficiencies (often estimated according to Eq. (6)) and asymmetry factors (B/A), for a set of peaks obtained with a given chromatographic system, are often reported. The peaks correspond to one or more solutes eluted at different retention times, under appropriate conditions. In principle, the larger the number of peaks and the more representative their distribution, the more significant the mean values. However, a problem arises since the width of chromatographic peaks steadily increases with the retention time. Consequently, the number of peaks eluting in a given window decreases rapidly, making the establishment of a criterion to obtain mean widths or efficiencies not straightforward.

As commented above, Eqs. (17) and (18) allow predicting the peak half-widths for peaks eluting at different retention times. This suggests new approaches to achieve mean values of the observed efficiencies and asymmetry factors, based on predictions. We suggest here two methods that consider the peaks eluting in a certain time window in a chromatogram. The simplest one, which we will call the "integral method", considers a chromatogram with multiple peaks showing retention times separated in an infinitesimal distance. In this case, the mean values are calculated by integration. For the left half-width:

$$\bar{A}_{int} = \frac{\int_{t_1}^{t_n} A(t)dt}{\int_{t_1}^{t_n} dt} = \frac{\int_{t_1}^{t_n} A(t)dt}{t_n - t_1} \quad (21)$$

where t_1 and t_n are the corrected retention times ($t_R - t_0$) for the first and last peaks in the selected window. Assuming a linear relationship between left half-width and time (Eq. (17)), the following equation results:

$$\bar{A}_{int} = \frac{m_A((t_n^2 - t_1^2)/2) + A_0(t_n - t_1)}{t_n - t_1} = m_A\frac{(t_n + t_1)}{2} + A_0 \quad (22)$$

Similarly, for the mean right half-width, from Eq. (18):

$$\bar{B}_{int} = m_B\frac{(t_n + t_1)}{2} + B_0 \quad (23)$$

The result is obvious: owing to the adopted linear relationship between the half-widths and the retention time, the mean values $\bar{A}_{int}$ and $\bar{B}_{int}$ coincide with the half-width for the peak located at the centre of the time window, whose retention time is given by:

$$\bar{t}_{R,int} = \frac{(t_n + t_1)}{2} + t_0 \quad (24)$$

From the mean half-widths (Eqs. (22) and (23)), and the mean retention time (Eq. (24)), the corresponding efficiency can be calculated according to Eq. (6). The mean asymmetry factor will be:

$$\bar{f}_{\text{int}} = \frac{\bar{B}_{\text{int}}}{\bar{A}_{\text{int}}} \tag{25}$$

The second method, which we will call the "summation method", is more complex. It is based on the ideal chromatogram used to define the peak capacity concept [19]. Accepting Eqs. (17) and (18), we can write:

$$t_{i+1} - t_i = z(A_{i+1} + B_i) = z(m_A t_{i+1} + A_0 + m_B t_i + B_0) \tag{26}$$

where t_{i+1} and t_i, and A_{i+1} and A_i (B_{i+1} and B_i) are the retention times and left (and right) half-widths for two adjacent peaks (i and $i+1$). The number z establishes the threshold defining full resolution. We have adopted the value $z = 1.4$, which corresponds to $R_S = 1.5$ or $w = 6\sigma$ (with A and B measured at 10% peak height) to guarantee sufficient resolution for asymmetrical peaks. According to Eq. (26), the time for a given peak (t_{i+1}) can be related with the time of the preceding peak (t_i) as follows:

$$t_{i+1} = \frac{1 + zm_B}{1 - zm_A} t_i + z\frac{A_0 + B_0}{1 - zm_A} = \delta t_i + \Delta_0 \tag{27}$$

From Eq. (27), the time for any peak in the chromatogram is given by (see also Ref. [19]):

$$t_i = \delta^{i-1}(t_1 + T_0) - T_0 \tag{28}$$

where

$$T_0 = \frac{A_0 + B_0}{m_A + m_B} \tag{29}$$

which is the time for an ideal peak with width $A_0 + B_0$. The mean corrected retention time in the considered time window will be:

$$\bar{t}_{\text{sum}} = \frac{\sum_{i=i}^{n} t_i}{n} = \frac{t_1 + T_0}{n} \sum_{i=i}^{n} \delta^{i-1} - T_0 = \frac{t_1 + T_0}{n} \frac{\delta^n - 1}{\delta - 1} - T_0 \tag{30}$$

where n is the peak capacity (P_c), which can be estimated with high accuracy (even for highly asymmetrical peaks with low efficiency), as follows [19]:

$$P_c = 1 + \frac{\ln((t_n + T_0)/(t_1 + T_0))}{\ln((1 + zm_B)/(1 - zm_A))} = 1 + \frac{1}{z(m_A + m_B)} \ln\frac{t_n + T_0}{t_1 + T_0} \tag{31}$$

The mean left and right half-widths can be estimated as:

$$\bar{A}_{\text{sum}} = m_A \bar{t}_{\text{sum}} + A_0 \tag{32}$$

$$\bar{B}_{\text{sum}} = m_B \bar{t}_{\text{sum}} + B_0 \tag{33}$$

where m_A, m_B, A_0 and B_0 are the parameters in Eqs. (17) and (18), and $\bar{t}_{\text{sum}}$ is calculated according to Eq. (30). The corresponding retention time is:

$$\bar{t}_{R,\text{sum}} = \bar{t}_{\text{sum}} + t_0 \tag{34}$$

Finally, the mean efficiency can be calculated with Eq. (6) from $\bar{A}_{\text{sum}}$ and $\bar{B}_{\text{sum}}$, and the mean asymmetry factor from:

$$\bar{f}_{\text{sum}} = \frac{\bar{B}_{\text{sum}}}{\bar{A}_{\text{sum}}} \tag{35}$$

3. Experimental

3.1. Reagents and columns

The discussion shown below was developed using chromatographic data (i.e. retention times, and left and right half-widths) gathered in our laboratory along several years, which constitutes a database that includes information from several thousands of peaks. We processed the data for the following sets of compounds:

(i) Alkylbenzenes: toluene (Scharlab, Barcelona, Spain), ethylbenzene and butylbenzene (Aldrich, St. Louis, MO, USA), propylbenzene (Acros Organics, Geel, Belgium), and pentylbenzene (Fluka, Buchs, Switzerland), separated with a Zorbax Eclipse XDB-C18 column (150 × 4.6 mm, 5 μm particle size, Agilent, Waldbronn, Germany). The elution was carried out with unbuffered acetonitrile–water mixtures at 25 °C.

(ii) Diuretics: benzthiazide, bumetanide, chlorothiazide, furosemide (Sigma, St. Louis, MO, USA), and xipamide (Lacer, Barcelona), separated with several microparticulate columns (5 μm particle size) and a monolithic column, all of them 4.6 mm I.D. The columns were (column length in parenthesis): unendcapped Spherisorb ODS-2 (125 mm, Scharlab), Inertsil ODS-3 (250 mm, Análisis Vínicos, Tomelloso, Spain), Kromasil C18 (150 mm, Análisis Vínicos), X-Terra MS C18 (150 mm, Waters, MA, USA), Zorbax SB C18 (150 mm, Agilent), Zorbax Eclipse XDB-C18 (150 mm, Agilent), and Chromolith Performance RP-18e (100 mm, Merck, Darmstadt, Germany). The elution was carried out with acetonitrile–water mixtures at pH 3 and 30 °C.

(iii) β-Blockers: acebutolol (Italfarmaco, Alcobendas, Madrid, Spain), atenolol, pindolol, propranolol, timolol (Sigma), carteolol (Miquel-Otsuka, Barcelona, Spain), celiprolol (Rhône-Poulenc Rorer, Alcorcón, Madrid), esmolol (Du Pont-De Nemours, Le Grand Saconnex, Switzerland), labetalol (Glaxo, Tres Cantos, Madrid), metoprolol, oxprenolol (Ciba-Geigy, Barcelona), nadolol (Squibb, Esplugues de Llobregat, Barcelona), and timolol (Merck, Sharp & Dohme, Madrid), chromatographed with the Kromasil C18, Chromolith Performance RP-18e and X-Terra MS C18 columns described above. The mobile phases contained acetonitrile, or acetonitrile and sodium dodecyl sulphate (SDS, Merck, Darmstadt, Germany) at pH 3 at 30 °C.

3.2. Apparatus

The HPLC system (Agilent, Series 1200, Waldbronn, Germany) consisted of an isocratic pump, an autosampler, a temperature controller and a variable wavelength UV–vis detector set at 254 and 225 nm, all governed by an Agilent HPChemStation B.02.01. The flow-rate and injection volume were usually 1 ml min^{-1} and 10 μl, respectively. For the diuretics chromatographed with the Zorbax SB C18 column, series run at injection volumes in the range 1–40 μl were also carried out. Triplicate injections were made in all cases.

4. Results and discussion

A usual advice to characterise chromatographic columns is to obtain the peak parameters (i.e. efficiency and asymmetry factor) for a neutral solute eluted in a selected experimental condition (i.e. mobile phase composition and temperature). In order to compare different columns, and owing to the change in efficiency with retention time (Fig. 2), it is convenient to adjust the experimental conditions to get peaks eluting at similar retention times in each column. This may involve a considerable extra work. As commented in Section 2.4, in order to characterise a column, mean values of efficiency and asymmetry factor can be also obtained by averaging the individual values for experimental peaks of several solutes eluted at different retention times.

We show below that the characterisation of columns can be made assisted by linear models that allow the prediction, ver-

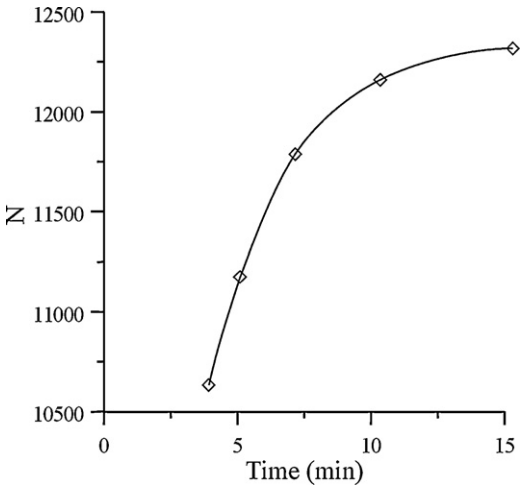

Fig. 2. Changes in efficiency with the retention time for the set of alkylbenzenes eluted with 70% acetonitrile.

sus the retention time, of parameters related to the peak width. The accuracy of the linear models is first demonstrated. Next, the global parameters according to three different approaches (outlined in Sections 2.2–2.4) are obtained for several chromatographic columns.

4.1. Peak variance and standard deviation: accuracy of the linear models versus the retention time

Sections 1 and 2.2 show three models (Eqs. (7), (14) and (16)) that can be used to predict the chromatographic peak variance (or peak standard deviation) at any retention time. These models also provide an estimation of the column efficiency, described as N_{col}, N_{eff} or N_σ respectively. The reliability of these estimations will depend on the good performance of the linear models. Therefore, we first checked the suitability of the three models to describe the peak broadening for different sets of compounds, from which the results for five alkylbenzenes (homologous compounds showing only non-polar interactions), and five diuretics (compounds of different nature and polarity) are shown. The alkylbenzenes were chromatographed in a Zorbax Eclipse XDB-C18 column with several mobile phases of acetonitrile in the range 50–100% (v/v) at 25 °C, and the diuretics in a Zorbax SB C18 column with 35% acetonitrile at pH 3 and 30 °C. For the diuretics, runs at varying injection volume were carried out.

The model parameters, determination coefficients (r^2) and relative fitting errors for Eqs. (7), (14) and (16) are given in Tables 1 and 2 for the sets of alkylbenzenes and diuretics, respectively. In all cases, the fittings were excellent. We would like to call the attention about the satisfactory fitting of the simple linear model, which relates σ_t and t_R (Eq. (16)). Note that the weight in the least-squares fitting given to the peaks that elute at higher retention time is larger for the quadratic models (Eqs. (7) and (14)) with respect to the linear model (Eq. (16)). In fact, the separation between points in the plots of σ_t^2 versus t_R^2 increases quadratically with time, which means that the data at smaller retention time are cluttered near the origin. In the linear model, the points are distributed similarly to a chromatogram, and the contribution of all peaks in the fitting is more balanced.

4.2. Half-widths: accuracy of the linear dependence with the retention time

There is a general agreement that the equation proposed by Foley and Dorsey (Eq. (6)) offers the best evaluation of chromatographic efficiency. In order to obtain this parameter, the values of peak asymmetry (B/A) and peak width ($A+B$) at 10% peak height are needed. Hence the name of "peak half-width model" is given to Eq. (6). The three models examined in Section 4.1, which describe the peak variance or standard deviation as a function of the retention time (Eqs. (7), (14) and (16)) fit satisfactorily the observed

Table 1
Accuracy of the models relating the peak variance or standard deviation with the retention time for the set of alkylbenzenes, eluted with acetonitrile–water mixtures of diverse composition from a Zorbax Eclipse XDB-C18 column.

Acetonitrile (%, v/v)	$\sigma_t^2 = \frac{1}{N_{col}} t_R^2 + \sigma_{ext}^2$			
	N_{col}	σ_{ext}^2	r^2 [a]	ε_r (%)[b]
50	10,800	−0.00310	0.9996	1.7
60	11,565	−0.00072	0.9993	2.5
70	12,560	0.000224	0.999998	0.14
80	12,170	0.000315	0.999988	0.17
90	11,595	0.000283	0.9997	0.59
Acetonitrile (%, v/v)	$\sigma_t^2 = \frac{1}{N_{eff}} (t_R - t_0)^2 + \sigma_0^2$			
	N_{eff}	σ_0^2	r^2 [a]	ε_r (%)[b]
50	10,420	−0.000973	0.9998	1.2
60	10,930	0.000529	0.9997	1.4
70	11,235	0.000875	0.9996	1.6
80	9,925	0.000730	0.9992	1.7
90	8,260	0.000572	0.9992	1.0
Acetonitrile (%, v/v)	$\sigma_t = \frac{1}{\sqrt{N_\sigma}} (t_R - t_0) + \sigma_0$			
	N_σ	σ_0	r^2 [a]	ε_r (%)[b]
50	10,445	−0.00175	0.9997	1.0
60	11,575	0.000731	0.9993	1.5
70	13,090	0.0125	0.99995	0.31
80	14,030	0.0157	0.9995	0.67
90	14,805	0.0165	0.998	0.73

[a] Determination coefficient.
[b] Relative standard deviation.

Table 2
Accuracy of the models relating the peak variance or standard deviation with the retention time for the set of diuretics, eluted with 35% acetonitrile from a Zorbax SB C18 column at diverse injection volume.

Injection volume (μl)	$\sigma_t^2 = \frac{1}{N_{col}} t_R^2 + \sigma_{ext}^2$			
	N_{col}	σ_{ext}^2	r^2 [a]	ε_r (%)[b]
1	11,865	0.00294	0.9988	2.0
5	12,000	0.00304	0.9992	1.8
10	12,095	0.00312	0.9996	1.2
20	12,210	0.00329	0.9998	0.77
40	12,335	0.00350	0.9993	1.3
Injection volume (μl)	$\sigma_t^2 = \frac{1}{N_{eff}} (t_R - t_0)^2 + \sigma_0^2$			
	N_{eff}	σ_0^2	r^2 [a]	ε_r (%)[b]
1	10,405	0.00354	0.997	3.3
5	10,570	0.00365	0.997	3.2
10	10,655	0.00372	0.998	2.6
20	10,755	0.00388	0.9991	1.9
40	10,885	0.00409	0.9996	1.2
Injection volume (μl)	$\sigma_t = \frac{1}{\sqrt{N_\sigma}} (t_R - t_0) + \sigma_0$			
	N_σ	σ_0	r^2 [a]	ε_r (%)[b]
1	18,225	0.0447	0.9992	0.98
5	18,525	0.0455	0.998	1.5
10	18,955	0.0464	0.997	1.8
20	19,775	0.0483	0.993	2.6
40	20,635	0.0504	0.986	3.8

[a] Determination coefficient.
[b] Relative standard deviation.

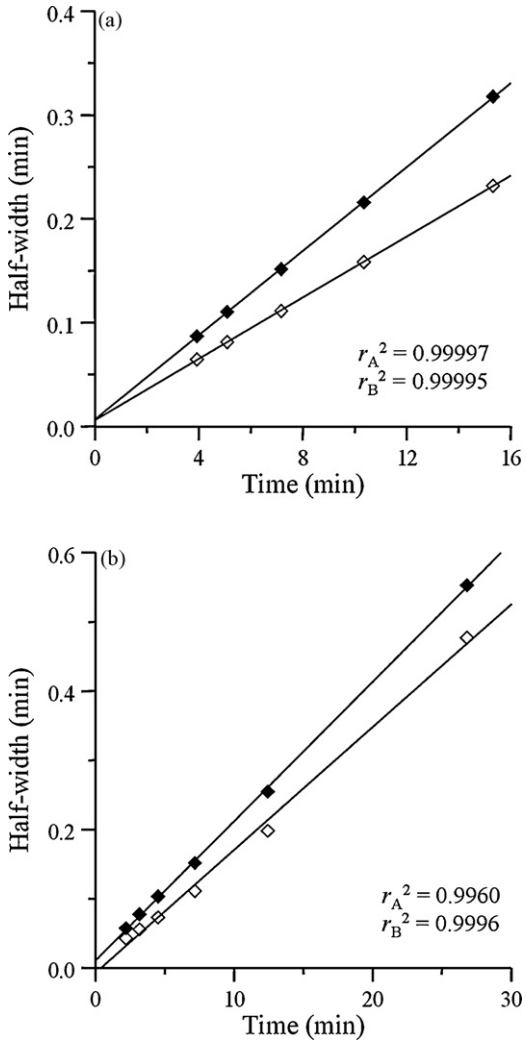

Fig. 3. Changes in the left (□) and right (■) peak half-widths with the retention time for: (a) the set of alkylbenzenes eluted with 70% acetonitrile, and (b) propylbenzene eluted with several mobile phases in the range 50–100% acetonitrile.

behaviour. However, they do not give information about the peak half-widths (*A* and *B*). In this section, we show the accuracy of Eqs. (17) and (18) to predict the peak half-widths, for three sets of compounds eluted under different experimental conditions. The half-widths were measured at 10% peak height through curve fitting, according to Eqs. (8)–(10).

Fig. 3a and b plots the half-widths versus the retention time for the set of alkylbenzenes eluted with 70% acetonitrile, and for propylbenzene eluted with several mobile phases in the range 50–100% acetonitrile, respectively. Figs. 4 and 5 depict similar plots for the set of diuretics eluted with 35% acetonitrile from different C18 columns (seven microparticulate columns and a monolithic column, the Chromolith Performance RP-18e). Fig. 6 describes the behaviour for several β-blockers eluted with mobile phases containing acetonitrile or acetonitrile and the anionic surfactant SDS [22].

As observed, the plots in Figs. 3–6 can be approximated to straight-lines. In previous work, we have observed a similar linear behaviour for sets of β-blockers [20,21] and polycyclic aromatic hydrocarbons [23], separated with microparticulate and monolithic columns. These linear plots are very practical to predict the peak half-widths and the corresponding efficiencies (through Eq. (6)) at any retention time. The increasing half-widths in the plots describe the peak broadening rate. The intercepts of the lines should be positive. However, in some cases, they are rather small and, owing to uncertainties in the measurements, may appear as negative. The intercept is usually larger for the right half-width (*B*), which makes the peaks at shorter retention time exhibiting larger asymmetry. The slope for *B* is also larger than that for the left half-width (*A*) for all columns, indicating the tailing character of the chromatographic peaks. Nevertheless, in some cases, the lines converge, suggesting the appearance of fronting peaks above a certain retention time.

The slopes for *A* and *B* are similar in most situations shown in this work, which denotes nearly symmetrical peaks. The loss in efficiency often brings collaterally a loss in peak symmetry. These effects are revealed by an increase in the slopes for the left and right half-widths, and in the angle between both lines, respectively. In Fig. 5a, we show the half-width plots for an old Spherisorb column, still kept in our laboratory, which was purchased in 1991 and used during a long time until it suffered apparent damage. The performance of this column can be compared with that for a new Spherisorb column purchased in 2008 (Fig. 5b). As observed, the half-width plots are useful to check the degree of loss of column performance due to damage or ageing.

Fig. 6 shows the behaviour of three columns used in the analysis of basic drugs (β-blockers): two new columns (Kromasil and Chromolith) bearing free silanol groups, and a deactivated column (X-Terra). The analysis of these drugs is problematic due to the severe low efficiencies and tailing peaks, produced mostly by the slow interaction of the positively charged solutes with the anionic free silanols of the packing [24]. The half-width plots for the three columns using mobile phases of acetonitrile–water are depicted in Fig. 6a, c and d. When the silanols are deactivated, the slopes and the angle between the lines for the left and right half-width plots decrease (compare Fig. 6d with Fig. 6a and c). Protection of silanol groups is also possible by the addition of the anionic surfactant SDS [22], as shown in Fig. 6b.

4.3. Characterisation of column performance based on global parameters

Half-width plots, as those depicted in Figs. 3–6, contain enough information to characterise a chromatographic system in terms of peak width and skewness. The comparison of the plots for different columns can reveal the differences or similarities in terms of peak efficiency and skewness. This information can be summarised in the four parameters m_A, m_B, A_0 and B_0 in Eqs. (17) and (18), which allow the prediction of the half-widths for chromatographic peaks eluted at any retention time, and the estimation of the corresponding efficiencies and asymmetry factors.

As commented in Section 2, the ability of a column or chromatographic system to yield narrow peaks can be also described by only one or two global parameters, which refer to the column component exclusively (Sections 2.2 and 2.3), or to both column and external components to the peak broadening and asymmetry as a whole (Section 2.4). The first approach (Section 2.2) is based on Eq. (7), which provides the intrinsic column efficiency, N_{col}. Note that σ_{ext} describes the peak width at time zero. A similar approach can be suggested for Eqs. (14) and (16) (see Tables 1 and 2), which gives rise to the parameters N_{eff} or N_σ, and σ_0. These parameters describe the column efficiency (considering only the time the solute interacts with the stationary phase), and the width at the dead time (σ_0 includes, besides the extra-column effects, intra-column effects such as dispersion, diffusion and tortuosity inside the column).

The second approach (see Section 2.3) is based on Eqs. (17) and (18). The chromatographic performance can be described by the sum of slopes ($m_A + m_B$, Eq. (19)), which indicates the peak broadening rate, and their ratio ($f_{asym} = m_A/m_B$, Eq. (20)), which describes the peak skewness, in both cases yielded inside the column.

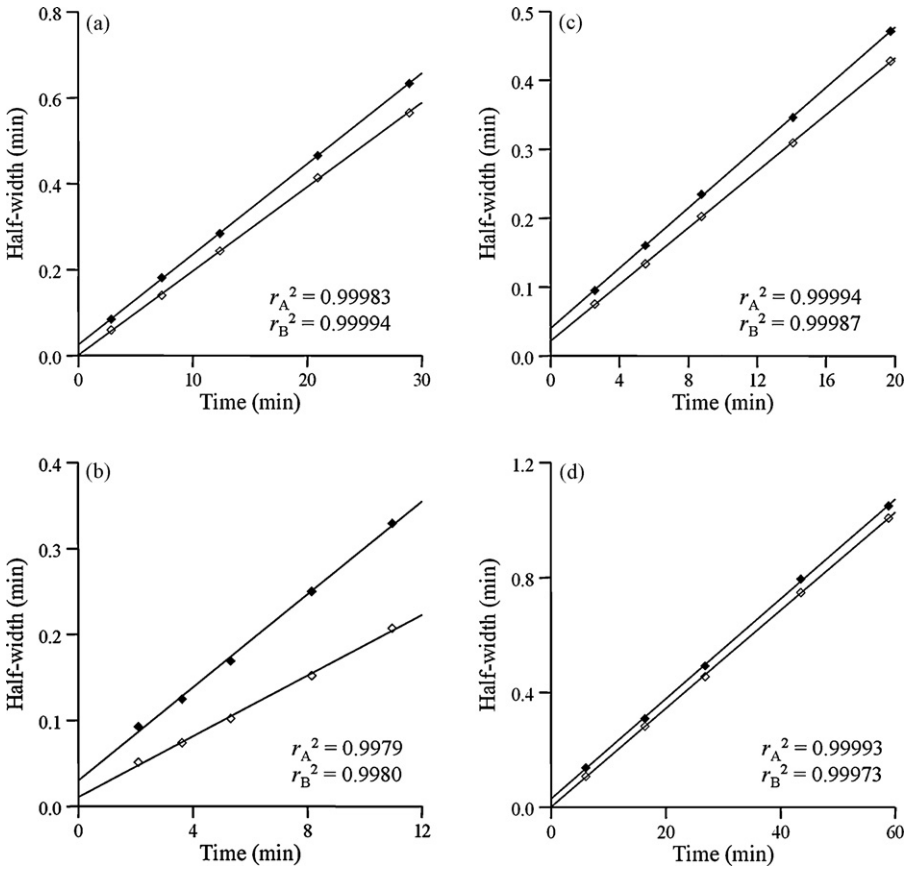

Fig. 4. Changes in the left (□) and right (■) peak half-widths with the retention time for the set of diuretics eluted with 35% acetonitrile. Columns: (a) Kromasil C18, (b) Chromolith Performance RP-18e, (c) X-Terra, and (d) Inertsil.

A different approach is the estimation of the mean values of the observed efficiencies and asymmetry factors, from the mean half-widths of peaks eluting in a selected time window (Section 2.4). These mean efficiencies and asymmetries consider both intra- and extra-column contributions to the peak shape. Two methods are suggested to calculate the mean values, which were called the "integral" and "summation" methods. The "integral" method considers a chromatogram with multiple peaks showing retention times separated in an infinitesimal distance, whereas the "summation" method is based on a chromatogram where the peaks touch each other.

The calculation of the proposed global parameters is rather simple, especially for the two first approaches. The required data are the peak half-widths and retention times for several peaks, as those for a set of compounds of increasing polarity eluted with a mobile phase at fixed composition. Note that the calculation of the efficiency for single peaks using the equation developed by Foley and Dorsey also requires the knowledge of the peak half-widths and retention times. The peak half-widths can be provided by a data station, or be obtained by other means as the fitting of the peaks to Eqs. (8)–(10) carried out for this work. The variance needed for the first approach can be calculated easily according to Eq. (5) from the width and half-widths at 10% peak height.

Table 3
Global parameters for the characterisation of several chromatographic columns or systems, obtained from the set of diuretics[a].

Column	t_0 (min)[b]	N_{col}[c]	r_{PB}[d]	m_B/m_A[e]	$\tilde{N}_{int}$[f]	$\tilde{f}_{int}$[f]	$\tilde{N}_{sum}$[f]	$\tilde{f}_{sum}$[f]	P_c[g]
Spherisorb (1991)	1.00	540	15.6	3.50	370	3.53	380	3.54	16.7
Spherisorb (2008)	0.81	10,300	4.1	0.97	9,825	1.03	9,220	1.06	49.5
Zorbax SB	1.18	12,175	3.6	0.92	9,390	1.14	8,125	1.21	39.3
Zorbax Eclipse	1.05	12,030	3.7	1.01	10,855	1.09	9,925	1.13	47.1
Kromasil	0.93	10,365	4.1	1.08	9,165	1.18	8,400	1.24	46.3
Chromolith	1.34	6,385	4.5	1.53	6,255	1.59	5,820	1.62	37.7
X-Terra	1.99	8,865	4.2	1.06	7,585	1.13	6,820	1.16	37.5
Inertsil	1.10	14,965	3.5	1.02	12,515	1.15	11,665	1.19	43.5

[a] The diuretics were eluted with 35% acetonitrile at pH 3 and 30 °C.
[b] Dead time.
[c] Column efficiency according to Eq. (7) (see Section 2.2).
[d] Peak broadening rate ($r_{PB} = (m_A + m_B) \times 100$) according to Eq. (19) (see also Eqs. (17) and (18), Section 2.3).
[e] Column component to peak asymmetry (Eq. (20), Section 2.3).
[f] Mean efficiency ($\tilde{N}$) and mean peak asymmetry ($\tilde{f}$), calculated from the mean half-widths, obtained according to the "integral" (int) and "summation" (sum) methods (see Section 2.4). The time window ranged between t_0 and $t_0 + 20$ min.
[g] Peak capacity according to Eq. (31). The time window ranged between t_0 and $t_0 + 20$ min.

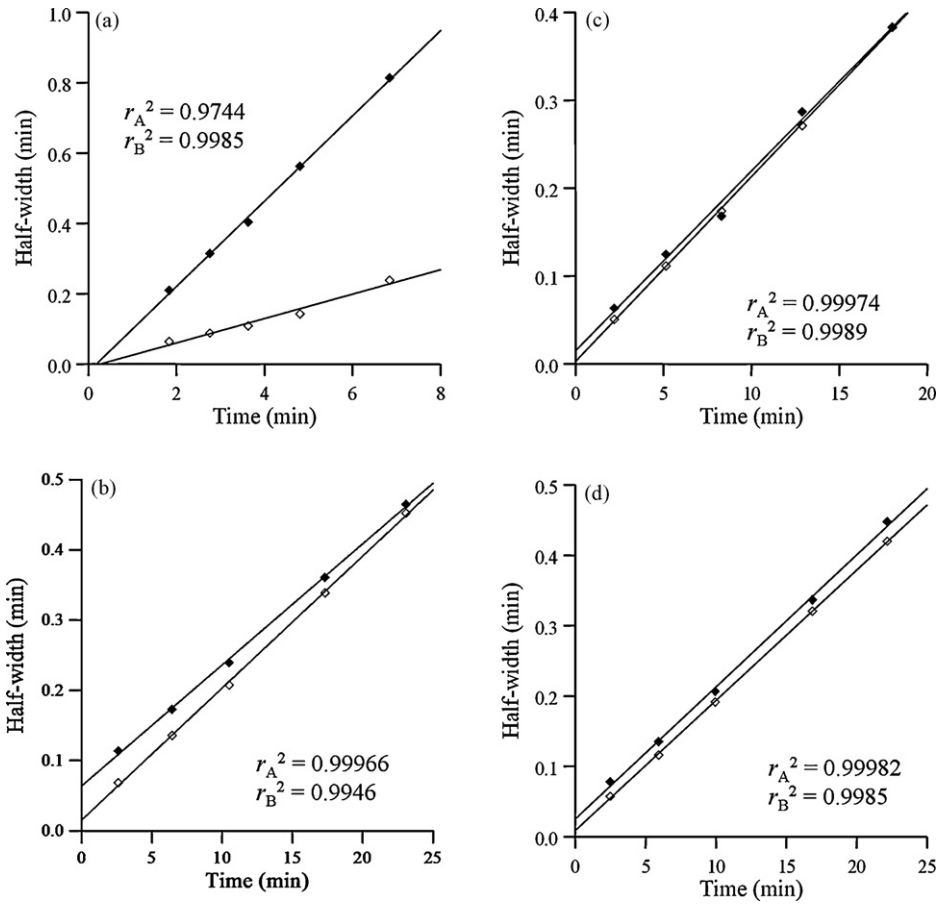

Fig. 5. Changes in the left (□) and right (■) peak half-widths with the retention time for the set of diuretics eluted with 35% acetonitrile. Columns: (a) aged Spherisorb, (b) new Spherisorb, (c) Zorbax SB C18, and (d) Zorbax Eclipse XDB-C18.

The global parameters for the first or second approaches (column efficiency, or peak broadening rate and asymmetry factor associated with the column) are derived from the slopes of the linear fittings of the peak variance or half-widths versus the retention time, according to Eq. (7) (alternatively, Eqs. (14) and (16)), or Eqs. (17) and (18), respectively. For the first approach, the column efficiency is the reverse of the slope of the fitted straight-line, and for the second approach, the peak broadening rate and the asymmetry factor are calculated as the sum and ratio of the slopes for the left and right half-widths, respectively.

The third approach consists in the estimation of the mean efficiencies and asymmetry factors, which depend on the criterion followed to select the peaks that are averaged. The simplest method is to take multiple simulated peaks within a time window, with retention times separated in an infinitesimal distance ("integral method"). In this case, the mean efficiency and asymmetry factor coincide with the values for the peak at the centre of the selected time window. The second method is based on the ideal chromatogram used in the definition of the peak capacity, where the mean efficiency (according to Eq. (6)) and asymmetry factor (Eq. (35)) are calculated from the mean half-widths obtained from Eqs. (32) and (33) (see also Eqs. (27), (29) and (30)). The "summation" method increases the weight of the narrower peaks at short retention times. As a consequence, the estimated mean efficiencies are smaller compared to the "integral" method.

The global parameters calculated according to the three approaches, using the data for the set of diuretics chromatographed in eight C18 columns (see also the plots in Figs. 4 and 5), are given in Table 3. The same instrument and tubing was used with all columns (i.e. the extra-column contributions were the same). The peak capacity estimated with Eq. (31) is also given. This parameter needs the selection of a time window, as the approaches based on the mean peak half-widths. The global parameters for the first and second approaches do not depend on the time window.

For the diuretics, the column efficiency (N_{col}) was in the range 6,000–15,000 for the set of columns, except for the aged Spherisorb column, for which it amounted 540 (Table 3). The Inertsil column showed the largest efficiencies (which is not surprising, since it was also the longest column). The observed efficiency calculated as mean values (third approach, $\bar{N}_{int}$ and $\bar{N}_{sum}$) was smaller (6,000–12,000 range), since the extra-column peak broadening is also considered. On the other hand, the asymmetry factors calculated from the mean half-widths ($\bar{f}_{int}$ and $\bar{f}_{sum}$) were larger than those obtained from the m_B/m_A ratio (which only considers the column contribution), except for the aged Spherisorb column, for which the peaks were rather broad even at low retention times (i.e. the extra-column contribution was negligible).

The global parameters for the Kromasil, Chromolith and X-Terra columns were also estimated from the chromatographic data for the set of β-blockers eluted under different conditions: with a mobile phase containing acetonitrile, and with mobile phases containing 0.15 M SDS and acetonitrile at two concentrations (Table 4, see also Fig. 6). The slow interaction of β-blockers with the free silanols in the hydro-organic mode can be evidenced by the poorer global parameters for the Kromasil and Chromolith columns, with respect to those achieved for the diuretics (Table 3). Thus, the Kromasil column yields N_{col} = 1015 versus 10365, r_{PB} = 10.8 versus 4.1, and $\bar{N}_{sum}$ = 1100 versus 8400, for the β-blockers and diuretics, respectively. In contrast, the global parameters for the deactivated X-Terra column were similar for both sets of compounds:

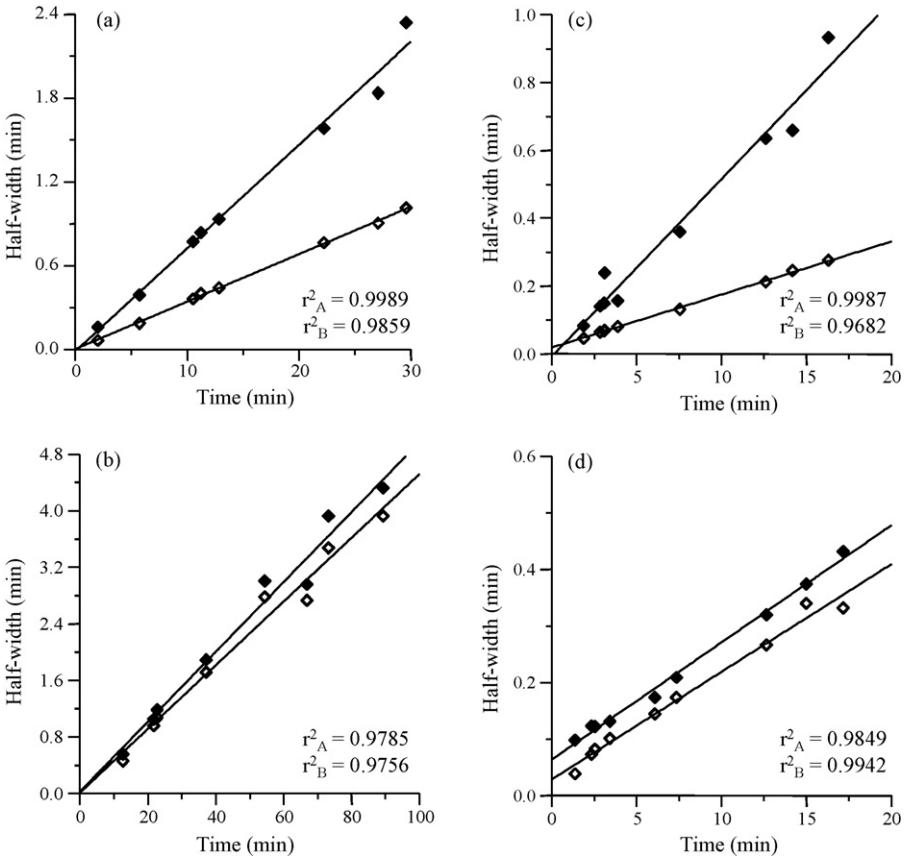

Fig. 6. Changes in the left (□) and right (■) peak half-widths with the retention time for the set of β-blockers eluted with: (a, c and d) 15% acetonitrile and (b) 0.15 M SDS/5% acetonitrile. Columns: (a and b) Kromasil C18, (c) Chromolith Performance RP-18e, and (d) X-Terra.

Table 4
Global parameters for the characterisation of several chromatographic columns or systems, obtained from the set of β-blockers[a].

Column	Mobile phase	N_{col}[b]	r_{PB}[c]	m_B/m_A[d]	$\bar{N}_{int}$[e]	$\bar{f}_{int}$[e]	$\bar{N}_{sum}$[e]	$\bar{f}_{sum}$[e]	P_c[f]
Kromasil	15% acetonitrile	1015	10.8	2.18	1080	2.12	1100	2.08	22.7
Chromolith	15% acetonitrile	2130	6.7	3.24	2040	2.99	2020	2.89	28.5
X-Terra	15% acetonitrile	8690	4.0	1.09	7280	1.22	6350	1.27	35.6
Kromasil	0.15 M SDS/5% acetonitrile	2040	9.5	1.10	1840	1.12	1770	1.14	22.3
Kromasil	0.15 M SDS/30% acetonitrile	5840	5.3	0.98	4065	1.17	3480	1.23	26.9

[a] The β-blockers were eluted with acetonitrile–water or micellar SDS–acetonitrile–water mobile phases, at pH 3 and 30 °C.
[b] Column efficiency according to Eq. (7) (see Section 2.2).
[c] Peak broadening rate ($r_{PB} = (m_A + m_B) \times 100$) according to Eq. (19) (see also Eqs. (17) and (18), Section 2.3).
[d] Column component to peak asymmetry (Eq. (20), Section 2.3).
[e] Mean efficiency ($\bar{N}$) and mean peak asymmetry ($\bar{f}$), calculated from the mean half-widths, obtained according to the "integral" (int) and summation" (sum) methods (see Section 2.4). The time window ranged between t_0 and $t_0 + 20$ min.
[f] Peak capacity according to Eq. (31). The time window ranged between t_0 and $t_0 + 20$ min.

N_{col} = 8690 versus 8865, r_{PB} = 4.0 versus 4.2, and $\bar{N}_{sum} = 6350$ versus 6820 (compare also the half-width plots for the three columns in Figs. 4 and 6 for the diuretics and β-blockers, respectively). The global parameters also revealed the protection of the silanol groups by the surfactant in the Kromasil column, but the performance of this column for β-blockers was still poorer than that shown for the diuretics. Also, the retention times were larger owing to the attraction of the cationic solutes to the stationary phase modified with the anionic surfactant (see Fig. 6b).

5. Conclusions

The resolution of chromatographic peaks depends on the selectivity (usually expressed as the ratio of the retention times of adjacent peaks), and the peak widths and skewness. Narrow symmetrical peaks do not imply necessarily the absence of overlapping, but make the separation among peaks more likely. Hence the interest is in obtaining parameters that describe the peak profile for chromatographic columns.

A common practice to characterise chromatographic columns is to estimate the efficiency and asymmetry factor for the peaks of one or more solutes arbitrarily selected and eluted with one or more mobile phases. However, owing to the extra-column contributions to the peak variance, these measurements are not robust, since they depend on the retention time. We propose several approaches that allow the estimation of global parameters to describe the column (or system) performance, which are independent of the retention time. These global parameters gather information from peaks eluted at diverse retention times, and can describe both the column and external components to the peak broadening as a whole, or the column component exclusively.

The proposed global parameters are obtained from linear relationships that can be established between the variance (or standard deviation), or the left and right half-widths, with the retention time. These equations are useful to predict the peak width for peaks eluting at any retention time, which is interesting by itself (e.g. for predicting more realistic peaks for optimisation purposes). They also allow the prediction of the peak skewness. However, it should be noted that compounds showing slow specific interactions with the stationary phase (e.g. basic compounds interacting with silanol groups) will yield different chromatographic performance and depart from the observed linear behaviour for compounds not experiencing such interactions.

Three different approaches can be outlined to describe the chromatographic performance related to the peak shape, which exhibit different characteristics. The first approach is based on the linear dependence between the variance and the squared retention time (or the standard deviation and the retention time). The second and third approaches rely on the linear relationships between the right and left half-widths and the retention time, and allow estimations of the peak skewness, as well. The first and second approaches offer global parameters related to the intrinsic column behaviour, whereas the third approach averages the observed behaviour for synthetic chromatograms containing a large number of peaks, and considers both intra- and extra-column effects, similarly to the peak capacity concept. The first and third approaches yield estimations of the efficiency (N_{col}, N_{eff}, N_{σ}, and $\bar{N}_{int}$, $\bar{N}_{sum}$, respectively), whereas the second approach gives rise to a different measurement that merits some attention, since it indicates straightforwardly the increase in peak width with the retention time inside the column (i.e. the peak broadening rate, r_{PB}). It should be noted that a better column or system performance (i.e. narrower peaks) will give rise to a larger efficiency, but a smaller peak broadening rate (see Tables 3 and 4).

All global parameters distinguish between good and poor column (or system) performance. The use of one or another approach depends on the particular interest of the chromatographer: the description of the intrinsic behaviour of a chromatographic column, or the description of the chromatographic system as a whole (i.e. the observed peak behaviour taking into account the extra- and intra-column contributions). From this point of view, N_{col}, N_{eff}, N_{σ} and r_{PB} overestimate the observed performance. Thus, for example, N_{col} = 15,000 and r_{PB} = 3.5 for the Inertsil column, but this was only able to resolve 43–44 peaks (according to the peak capacity concept), whereas N_{col} = 10,300 for the Spherisorb column, which resolved 49–50 peaks (Table 3). The reason of the disagreement between both criteria is the peak broadening at the dead time, which was larger for the Inertsil column, owing to the larger dead time value (see Eq. (19)).

Acknowledgements

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