Journal of Chromatography A, 1218 (2011) 1385–1392

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Approaches to estimate the time and height at the peak maximum in liquid chromatography based on a modified Gaussian model

J.J. Baeza-Baeza*, J.R. Torres-Lapasió, M.C. García-Álvarez-Coque

Departament de Química Analítica, Universitat de València, c/Dr. Moliner 50, 46100 Burjassot, Spain

ARTICLE INFO

Article history:
Received 26 October 2010
Received in revised form 10 January 2011
Accepted 11 January 2011
Available online 18 January 2011

Keywords:
Liquid chromatography
Peak modelling
Modified Gaussian model
Estimation of peak parameters
Time and height at the peak maximum

ABSTRACT

The time and height at the peak maximum are key parameters to describe a chromatographic peak with prediction or optimization purposes, or in the qualitative/quantitative analysis of samples. Three different approaches to estimate these parameters, using the experimental points in the peak maximum region, are here described and compared. The approaches are based on the reliable description of the peak profile using a modified Gaussian model with a parabolic variance (PVMG). In the first approach, non-linear fitting of the chromatographic data to the PVMG model is carried out to obtain the time and height at the peak maximum (Approach I). In the other two approaches, the PVMG model is linearized to carry out a linear fitting. In each case, the optimal number of processed points was assessed. The three approaches yielded highly satisfactory results, being Approach I the best in terms of accuracy and robustness. The assessment of the accuracy in the estimations was carried out using simulated peaks. These peaks were built with the parameters obtained from real peaks for several probe compounds eluted under reversed-phase liquid chromatographic (RPLC) conditions, to which noise was added.

1. Introduction

The accurate description of chromatographic peaks is an important step in the treatment of data with prediction or optimization purposes, or in the qualitative/quantitative analysis of samples, either for isolated or overlapped peaks, which are far more demanding. The most important parameters that gather all peak information (position and shape) are the time and height at the peak maximum, and the left and right half-widths. The peak half-widths (instead of the peak width) are essential to make a reliable estimation of the efficiency for asymmetrical peaks. It should be noticed that the accuracy in the measurement of the half-widths is decreased at increasing peak width and asymmetry, and depends on the accurate knowledge of the time and height at the peak maximum.

The estimation of the time and height at the peak maximum has been traditionally carried out through a polynomial fitting of the experimental points in the region close to the peak maximum [1,2]. This method is not only applied in chromatography, but also in other fields as spectroscopy, where a peak (or band) should be characterized [3]. Polynomials of different degrees have been used for the fittings. In other more complex approaches, the whole peak or the region around the peak maximum is non-linearly fitted to a function describing the peak profile [4].

A number of theoretical and empirical mathematical functions have been reported for the description of chromatographic peak profiles [5–12]. However, most functions found in the literature are not sufficiently accurate. The elution profiles of symmetrical and non-overloaded chromatographic peaks are well described by the Gaussian model. Non-ideal peaks (either tailing or fronting) are, however, quite common in practice. In this concern, polynomial modified Gaussian models (where the standard deviation or the variance changes with the distance to the peak maximum), have demonstrated a large flexibility and accuracy in the description of chromatographic peaks in a wide range of asymmetries [6,9–12].

The polynomially modified Gaussian model (PMG) (a Gaussian model with a polynomial standard deviation), which was initially proposed to describe non-ideal peaks, had associated a baseline that increased out of the peak region [6,9]. This problem was first solved by setting the height at each side of the peak region to the respective minimal value [6]. Another practical solution was a mixed exponential-PMG function, obtained by adding two exponential decays at both sides of the PMG peak at 10% height, hold to the restriction that the slopes of the Gaussian and exponential functions at the connecting points should coincide [11]. Two new modified Gaussian models including a parabolic variance (PVMG model) [12], or a combined parabolic-Lorentzian

* Corresponding author. Tel.: +34 96 354 3184; fax: +34 96 354 4436.
E-mail address: Juan.Baeza@uv.es (J.J. Baeza-Baeza).

0021-9673/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2011.01.029

function (PLMG model) [10], were proposed. In the latter model, the parabola accounted for the non-Gaussian shaped peak, whereas the Lorentzian function cancelled the variance growth out of the elution region. This model allowed the accurate description of a wide range of peaks (even with a large positive and/or negative skewness), improved with respect to other models reported in the literature.

In this work, we describe and compare three different approaches to estimate the time and height at the peak maximum from the information given by the experimental points in the region close to the peak maximum. The approaches are based on the reliable description of the peak profile using the PVMG model. The proper evaluation of the accuracy in the estimation of time and height at the peak maximum was carried out based on simulated peaks. These peaks were built with the parameters obtained from real peaks for several probe compounds, eluted under reversed-phase liquid chromatographic (RPLC) conditions, to which noise was added.

2. Experimental

2.1. Reagents

Mobile phases were prepared with acetonitrile (Scharlab, Barcelona, Spain) and water. The pH was buffered at pH 3 with 0.1 M citric acid (Panreac, Barcelona) and NaOH (Scharlab). The electrode was calibrated using aqueous buffers, and the pH of the mobile phases measured after the addition of the organic solvent.

The probe compounds were the diuretics benzthiazide, bumetanide (Sigma, St. Louis, MO, USA), bendroflumethiazide (Davur, Madrid, Spain), and xipamide (Lacer, Barcelona), and the β-blocker alprenolol (Ciba-Geigy, Barcelona). The compounds, except those from Sigma, were kindly donated by the pharmaceutical laboratories. The drugs were dissolved in a few millilitres of acetonitrile, assisted by an ultrasonic bath, and diluted with water. The concentrations of the stock and injected solutions were 100 and 10 μg/mL, respectively. The solutions were stored in the darkness at 4 °C.

Nanopure water (Barnstead, Sybron, Boston, MA, USA) was used throughout. The mobile phases and probe compound solutions were filtered through 0.45 μm Nylon membranes with a diameter of 47 mm (Magna) and 17 mm (Cameo), respectively (Osmonics, Herental, Belgium).

2.2. Apparatus, software and columns

The HPLC system (Agilent, Series 1100, Waldbronn, Germany) consisted of an isocratic pump, an autosampler, a UV–visible detector, and a temperature controller. The signals were monitored at 254 nm. Data acquisition was carried out with an HPChemStation (Agilent). The mathematical treatment was implemented in Visual Basic 6.0 and Excel XP (Microsoft, Seattle, WA, USA).

Three chromatographic columns were used: a Zorbax SB C18 (Agilent, 150 mm × 4.6 mm I.D. and 5 μm particle size), an XTerra MS C18 (Waters, MA, USA, 150 mm × 4.6 mm I.D. and 5 μm particle size), protected both with a similar C18 guard column (30 mm × 4.0 mm I.D. and 5 μm particle size), and a Chromolith Performance RP-18e (Merck, Darmstadt, Germany, 100 mm × 4.6 mm I.D.), preceded by a Chromolith guard column RP-18e (5.0 mm × 4.6 mm I.D.). The probe compounds were injected after the guard columns. The flow-rate was 1 mL/min and the injected volume 5 μL, in all cases. Triplicate injections were made.

3. Theory

3.1. Description of chromatographic peaks

The most convenient way to assess the performance of different approaches for the characterization of chromatographic peaks is to apply them to peaks with known parameters, built by simulation from mathematical models. The simulated peaks should fit as much as possible the real peaks. This is the methodology we have followed. For this purpose, we selected the experimental peaks of several probe compounds that showed diverse efficiency and asymmetry. We first fitted each elution profile to a peak model. Based on the parameters in the fitted equations, we built peaks with known profiles, to which noise was added.

We selected the PLMG model to fit and simulate the chromatographic peaks, due to its good performance. In this way, we could work with highly reliable peaks, with known parameters. The PLMG model has demonstrated to give better fittings than other models with different mathematical basis [10]. It has also the advantage that the model parameters are related to peak properties as the skewness and kurtosis.

The PLMG model is described as follows:

$$h(t) = H_0 \exp\left[-\frac{1}{2}\frac{(t - t_{max})^2}{\sigma^2}\right] \quad (1)$$

where

$$\sigma^2 = \sigma_0^2 + m\frac{(t - t_{max} + d)^2}{1 + ((t - t_{max} + r)^2/w^2)} \quad (2)$$

t being the time, $h(t)$ the peak height at different times, H_0 the height at the maximum, t_{max} the time at the peak maximum, and σ the standard deviation (which is a measurement of the peak width). The PLMG model combines a parabolic and a Lorentzian function to describe the variance profile, using the parameters σ_0, m and d for the parabolic function, and r and w for the Lorentzian function (m describes the parabola curvature and d locates its minimum; r locates the Lorentzian maximum and w accounts for its width). The parabola has a minimum at $t = t_{max} - d$, and the Lorentzian function a maximum at $t = t_{max} - r$. For tailed peaks, the minimum of the parabola is located at times $t < t_{max}$ (and therefore, $d > 0$), whereas for fronted peaks, the minimum is found at times $t > t_{max}$ ($d < 0$). The curvature of the parabola, and consequently, the variance, increases with m.

As commented, the approaches proposed in this work to obtain the time and height at the peak maximum were based on an accurate description of the peak profiles. For this purpose, we needed a reliable peak model. However, the PLMG model had the disadvantage of being too complex to be routinely applied to obtain the peak parameters. It should be noted that it makes an accurate description of the whole peak region, including the baseline at both sides of the peak. As we were interested in obtaining information associated only to the peak maximum region, we decided to eliminate the Lorentzian function in the PLMG model, and use a Gaussian model with a variance showing only a parabolic change with time (PVMG). This simplified model gives good performance in relatively narrow ranges along the peak elution [12]:

$$h(t) = H_0 \exp\left[-\frac{1}{2}\frac{(t - t_{max})^2}{\sigma_0^2 + m(t - t_{max} + d)^2}\right] \quad (3)$$

For peaks containing equally spaced points (as is the case in peak detection), the model can be re-written as follows:

$$h(t) = H_0 \exp\left[\frac{-a(\delta_i - \delta_{max})^2}{1 + b\delta_i + c\delta_i^2}\right] \quad (4)$$

where i is an index that indicates the location of the peak points with respect to the experimental point showing the highest signal (located at a time t_0, for which $i = 0$), δ_i is the distance between each point and the point at t_0, and $\delta_{max} = t_{max} - t_0$ (the distance between the point at the peak maximum and t_0). Note that since the peaks are equally spaced, the distance between a given point and the point at t_0 will be a multiple of the distance between adjacent points ($\delta_i = i\delta$).

3.2. Approaches to estimate the time and height at the peak maximum

The three approaches developed to estimate the time and height at the peak maximum are described below. All are based on Eq. (4), which gives an accurate description of the region around the peak maximum. We have assayed also a simplified model, where the $c\delta_i^2$ term in Eq. (4) was dropped:

$$h(t) = H_0 \exp\left[\frac{-a(\delta_i - \delta_{max})^2}{1 + b\delta_i}\right] \quad (5)$$

3.2.1. Approaches Ia and Ib: non-linear fitting

The non-linear fittings of the chromatographic data to Eq. (4) (which will be called Approach Ia) and Eq. (5) (without the $c\delta_i^2$ term, called Approach Ib) were first assayed. The fittings were performed using the Powell method [13], but it should be noted that the *Solver* option of the *Excel*'s spreadsheet can be used with good results.

3.2.2. Approaches IIa and IIb: polynomial fitting

We also considered the fitting of the data to a polynomial. For this purpose, Eq. (4) was rewritten in the logarithmic form:

$$y = \ln h(t) = \ln H_0 - \frac{a(\delta_i - \delta_{max})^2}{1 + b\delta_i + c\delta_i^2} \quad (6)$$

If the $b\delta_i$ and $c\delta_i^2$ terms in Eq. (6) are assumed to be sufficiently small, the expression can be approximated to a Taylor series, according to:

$$\frac{1}{1+x} \approx 1 - x \quad (7)$$

Thus:

$$y = \ln h(t) \approx \ln H_0 - a(\delta_i - \delta_{max})^2(1 - b\delta_i - c\delta_i^2)$$
$$= \alpha + \beta\delta_i + \gamma\delta_i^2 + \mu\delta_i^3 + \omega\delta_i^4 \quad (8)$$

The fourth degree polynomial in Eq. (8) constitutes the basis of Approach IIa. Approach IIb is based on the third degree polynomial obtained by dropping the $\omega\delta_i^4$ term:

$$y = \ln h(t) = \alpha + \beta\delta_i + \gamma\delta_i^2 + \mu\delta_i^3 \quad (9)$$

We will first explain Approach IIb, which is simpler. For equally spaced experimental points along the peak elution, and the same number of points (M) at both sides of the experimental point showing the maximal height in the peak (at t_0), the parameters of the third degree polynomial are given by:

$$\alpha = \frac{S_4 S_y - S_2 S_{2y}}{NS_4 - S_2^2} \quad (10)$$

$$\beta = \frac{S_6 S_{1y} - S_4 S_{3y}}{S_2 S_6 - S_4^2} \quad (11)$$

$$\gamma = \frac{NS_{2y} - S_2 S_y}{NS_4 - S_2^2} \quad (12)$$

$$\mu = \frac{S_2 S_{3y} - S_4 S_{1y}}{S_2 S_6 - S_4^2} \quad (13)$$

Table 1
Summations used in Approaches IIa and IIb to calculate the coefficients in Eqs. (8) and (9).

$S_2 = 2\delta^2 \sum_{i=1}^{M} i^2$	$S_y = y_0 + \sum_{i=1}^{M} (y_{+i} + y_{-i})$
$S_4 = 2\delta^4 \sum_{i=1}^{M} i^4$	$S_{1y} = \delta \sum_{i=1}^{M} i(y_{+i} - y_{-i})$
$S_6 = 2\delta^6 \sum_{i=1}^{M} i^6$	$S_{2y} = \delta^2 \sum_{i=1}^{M} i^2(y_{+i} + y_{-i})$
$S_8 = 2\delta^8 \sum_{i=1}^{M} i^8$	$S_{3y} = \delta^3 \sum_{i=1}^{M} i^3(y_{+i} - y_{-i})$
	$S_{4y} = \delta^4 \sum_{i=1}^{M} i^4(y_{+i} + y_{-i})$

δ is the time distance between adjacent experimental points.
i is an index that indicates the location of the peak points, with respect to the experimental point showing the highest signal, for which $i = 0$.
M is the number of points at the right or left of the point for $i = 0$.
y_{+i} and y_{-i} are the logarithm of the signals ($\log h_i$ and $\log h_{-i}$) for points at the right and left of the point for $i = 0$.

where $N = 2M + 1$.

The summation terms in Eqs. (10)–(13) are given in Table 1. The parameter δ_{max} (see Eq. (4) for meaning) can be obtained considering that, at the peak maximum, the derivative of Eq. (9) is null:

$$\beta + 2\gamma\delta_{max} + 3\mu\delta_{max}^2 = 0 \quad (14)$$

For symmetrical or nearly symmetrical peaks (for which the coefficient $\mu \approx 0$), the classical solution of the second degree equation can be problematic. Since, by definition, δ_{max} will be always small, a better solution is to calculate this parameter considering a Taylor approximation of Eq. (14):

$$\delta_{max} = \frac{-\beta}{2\gamma + 3\mu\delta_{max}} \approx \frac{-\beta}{2\gamma} + \frac{3\beta\mu}{4\gamma^2}\delta_{max} \quad (15)$$

From this equation:

$$\delta_{max} \approx \frac{-2\beta\gamma}{4\gamma^2 - 3\beta\mu} \quad (16)$$

We have applied this solution to all chromatographic peaks examined in this work, independently of their asymmetry degree. The time and height at the peak maximum were calculated as:

$$t_{max} = t_0 + \delta_{max} \quad (17)$$

$$H_0 = e^{\alpha + \beta\delta_{max} + \gamma\delta_{max}^2 + \mu\delta_{max}^3} \quad (18)$$

For Approach Ia, the parameters of the fourth degree polynomial (Eq. (8)) are given by:

$$\alpha = \frac{S_4 S_8 S_y + S_4 S_6 S_{2y} + S_2 S_6 S_{4y} - S_4^2 S_{4y} - S_6^2 S_y - S_2 S_8 S_{2y}}{NS_4 S_8 + 2S_2 S_4 S_6 - S_4^3 - NS_6^2 - S_2^2 S_8} \quad (19)$$

$$\beta = \frac{S_6 S_{1y} - S_4 S_{3y}}{S_2 S_6 - S_4^2} \quad (20)$$

$$\gamma = \frac{NS_8 S_{2y} + S_4 S_6 S_y + S_2 S_4 S_{4y} - S_4^2 S_{2y} - NS_6 S_{4y} - S_2 S_8 S_y}{NS_4 S_8 + 2S_2 S_4 S_6 - S_4^3 - NS_6^2 - S_2^2 S_8} \quad (21)$$

$$\mu = \frac{S_2 S_{3y} - S_4 S_{1y}}{S_2 S_6 - S_4^2} \quad (22)$$

$$\omega = \frac{NS_4 S_{4y} + S_2 S_6 S_y + S_2 S_4 S_{2y} - S_4^2 S_y - NS_6 S_{2y} - S_2^2 S_{4y}}{NS_4 S_8 + 2S_2 S_4 S_6 - S_4^3 - NS_6^2 - S_2^2 S_8} \quad (23)$$

Table 2
Summations used in Approaches IIIa and IIIb to calculate the coefficients in Eq. (29).

$$S_1 = 2\delta^2 \sum_{i=1}^{M} i$$

$$S_2 = \delta^2 \sum_{i=1}^{2M} i^2$$

$$S_3 = 2\delta^4 \sum_{i=1}^{M} i^3$$

$$S_{-1} = 1 + 2\sum_{i=1}^{M} (i)^{-1}$$

$$S_z = \delta \sum_{i=1}^{M} (z_i - z_{-i})$$

$$S_{1D} = \delta \sum_{i=1}^{2M} i(y_{+i} - y_{-i})$$

$$S_{2S} = \delta^2 \sum_{i=1}^{2M} i^2(y_{+i} + y_{-i})$$

$$S_{3D} = \delta^3 \sum_{i=1}^{2M} i^3(y_{+i} - y_{-i})$$

$$S_{4D2} = \delta^4 \sum_{i=1}^{2M} i^4(y_{+i} - y_{-i})^2$$

$$S_{1z} = \delta^2 \sum_{i=1}^{M} i(z_i + z_{-i})$$

$$S_{-1z} = y_0 + \sum_{i=1}^{M} \frac{z_i + z_{-i}}{i}$$

$$S_{2S2} = \delta^2 \sum_{i=1}^{2M} i^2(y_{+i} + y_{-i})^2$$

$$S_{2D2} = \delta^2 \sum_{i=1}^{2M} i^2(y_{+i} - y_{-i})^2$$

$$S_{1SD} = \delta \sum_{i=1}^{2M} i(y_{+i} + y_{-i})(y_{+i} - y_{-i})$$

$$S_{2SD} = \delta^2 \sum_{i=1}^{2M} i^2(y_{+i} + y_{-i})(y_{+i} - y_{-i})$$

$$S_{3SD} = \delta^3 \sum_{i=1}^{2M} i^3(y_{+i} + y_{-i})(y_{+i} - y_{-i})$$

$z_i = (1 + b\delta_i + c\delta_i^2)y_i$.
Other details are given in the text or Table 1.

The peak maximum will be obtained from the derivative of Eq. (8) set to zero:

$$\beta + 2\gamma\delta_{max} + 3\mu\delta_{max}^2 + 4\omega\delta_{max}^3 = 0 \tag{24}$$

After applying a treatment similar to that used for Approach IIb (Eqs. (15) and (16)), the following is obtained:

$$\delta_{max} \approx \frac{r_1}{1 + (2\omega r_1^2/\gamma)} \tag{25}$$

where

$$r_1 = \frac{r_0}{1 + (3\mu r_0/2\gamma)} \tag{26}$$

and

$$r_0 = -\frac{\beta}{2\gamma} \tag{27}$$

Finally, the retention time is calculated according to Eq. (17) and the peak height as:

$$H_0 = e^{\alpha + \beta\delta_{max} + \gamma\delta_{max}^2 + \mu\delta_{max}^3 + \omega\delta_{max}^4} \tag{28}$$

3.2.3. Approaches IIIa and IIIb: sequential fitting

We developed a third approach to obtain the time and height at the peak maximum from Eq. (4), using a linear fitting, but without making an approximation to a Taylor series (Eq. (7)). For this purpose, Eq. (6) was re-written as follows:

$$z = (1 + b\delta_i + c\delta_i^2)\ln h(t) = (1 + b\delta_i + c\delta_i^2)\ln H_0 - a(\delta_i - \delta_{max})^2 = \alpha + \beta\delta_i + \gamma\delta_i^2 \tag{29}$$

By subtracting Eq. (29) for points y_i and y_{-i}, taking into account that $\delta_{-i} = -\delta_i$:

$$y_i - y_{-i} = 2\beta\delta_i - b\delta_i(y_i + y_{-i}) - c\delta_i^2(y_i - y_{-i}) \tag{30}$$

For Approach IIIa, the $c\delta_i^2$ term in Eqs. (29) and (30) was dropped. In this case, the b coefficient can be obtained from Eq. (30) through a simple linear least-squares fitting (the summation terms are given in Table 2):

$$b = \frac{S_{2S}S_{1D} - S_2S_{1SD}}{S_2S_{2S2} - S_{2S}^2} \tag{31}$$

Once b is known, the chromatographic data can be fitted to Eq. (29) to obtain the coefficients α, β and γ as follows:

$$\alpha = \frac{S_3S_{-1z} - S_1S_{1z}}{S_3S_{-1} - S_1^2} \tag{32}$$

$$\beta = \frac{S_z}{S_1} \tag{33}$$

$$\gamma = \frac{S_{-1}S_{1z} - S_1S_{-1z}}{S_3S_{-1} - S_1^2} \tag{34}$$

The summation terms in Eqs. (32)–(34) can be also found in Table 2. For the fittings, the experimental points were weighted to increase the importance of those close to the peak maximum. The weights were $w = 1/|i|$, except for $i = 0$, for which $w = 1$.

The information that allowed obtaining the time at the peak maximum was calculated from the derivative of:

$$y = \ln h(t) = \frac{\alpha + \beta\delta_i + \gamma\delta_i^2}{1 + b\delta_i + c\delta_i^2} \tag{35}$$

obtained from Eq. (29), set to zero:

$$\delta_{max} = \frac{r}{1 + (br/2)} \tag{36}$$

where

$$r = \frac{\alpha b - \beta}{2\gamma} \tag{37}$$

The time at the peak maximum was calculated according to Eq. (17) and the height from:

$$H_0 = e^{(\alpha + \beta\delta_{max} + \gamma\delta_{max}^2)/(1 + b\delta_{max})} \tag{38}$$

For Approach IIIa, based on Eqs. (29) and (30) including the $c\delta_i^2$ term:

$$b = -\frac{S_2S_{1SD}S_{4D2} + S_{1D}S_{3SD}S_{3D} + S_{3D}S_{2S}S_{2D2} - S_{1SD}S_{3D}^2 - S_{2S}S_{1D}S_{4D2} - S_2S_{2D2}S_{3SD}}{S_2S_{2S2}S_{4D2} + S_{2S}S_{3SD}S_{3D} + S_{3D}S_{2S}S_{3SD} - S_{2S2}S_{3D}^2 - S_{2S}^2S_{4D2} - S_2S_{3SD}^2} \tag{39}$$

$$c = -\frac{S_2S_{2S2}S_{2D2} + S_{2S}S_{1SD}S_{3D} + S_{1D}S_{2S}S_{3SD} - S_{1D}S_{2S2}S_{3D} - S_{2D2}S_{2S}^2 - S_2S_{3SD}S_{1SD}}{S_2S_{2S2}S_{4D2} + S_{2S}S_{3SD}S_{3D} + S_{3D}S_{2S}S_{3SD} - S_{2S2}S_{3D}^2 - S_{2S}^2S_{4D2} - S_2S_{3SD}^2} \tag{40}$$

$$\delta_{max} = \frac{r}{1 + r((b\gamma - c\beta)/(2\gamma - 2c\alpha))} \tag{41}$$

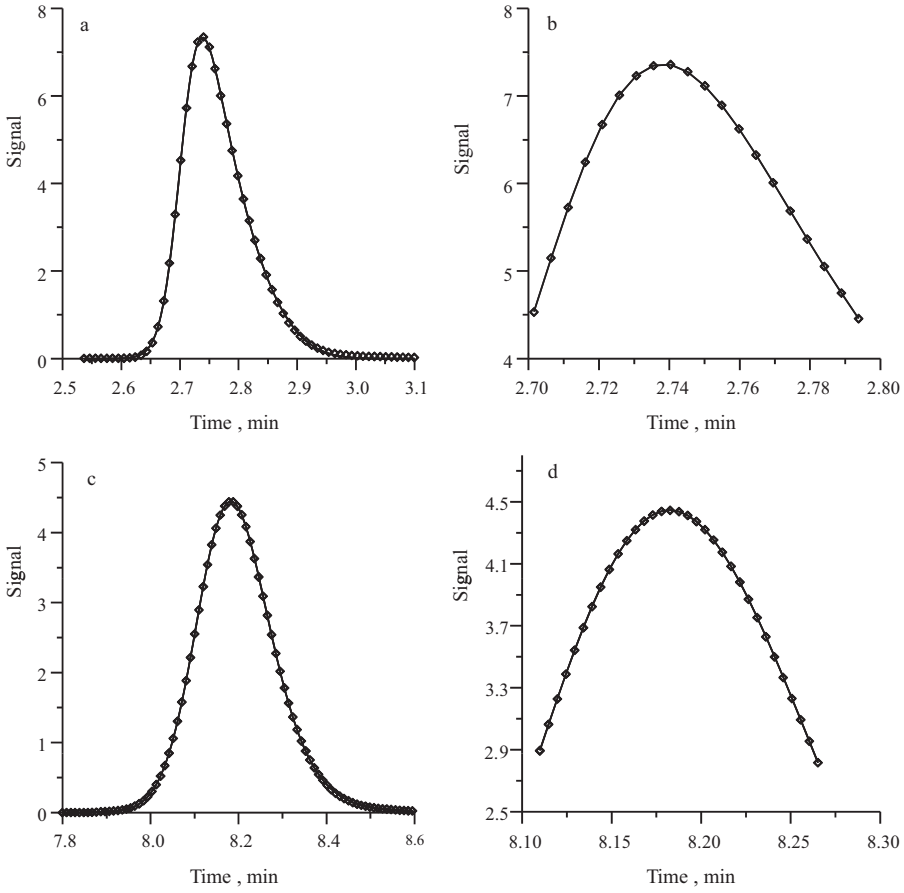

Fig. 1. Experimental (◊) and fitted (solid line) chromatographic peaks, according to the PLMG (left, Eqs. (1) and (2)) and PVMG (right, Eq. (4)) models, for: (a and b) bendroflumethiazide (peak 4 in Table 3) and (c and d) benzthiazide (peak 6), eluted at 30 and 40 °C, respectively. For the PVMG model, only the experimental points above a peak height ratio of 0.6 were fitted.

where

$$r = \frac{\alpha b - \beta}{2\gamma - 2c\alpha} \tag{42}$$

and

$$H_0 = e^{(\alpha+\beta\delta_{max}+\gamma\delta_{max}^2)/(1+b\delta_{max}+c\delta_{max}^2)} \tag{43}$$

The coefficients α, β and γ are calculated again according to Eqs. (32)–(34).

4. Results and discussion

4.1. Peak description and simulation

The experimental peaks for five probe compounds (alprenolol, bendroflumethiazide, benzthiazide, bumetanide and xipamide) were obtained for three columns and acetonitrile–water mobile phases of diverse composition at 30 or 40 °C. The peaks showed different widths and asymmetries (Table 3).

The peaks for bendroflumethiazide at 30 °C and benzthiazide at 40 °C are shown in Fig. 1. The experimental points and the fittings according to the PLMG and PVMG models are depicted. As observed, the PLMG model describes accurately the whole peak. It is thus the ideal tool to simulate peaks including the baseline. The parameters of the PLMG models and the accuracy of the fittings for eight experimental peaks are given in Table 4. The fitting quality was measured as the mean relative error calculated as:

$$\varepsilon_r\,(\%) = \frac{\sum_{i=1}^{N} \left|h_i - \hat{h}_i\right|}{\sum_{i=1}^{N} \left|h_i\right|} \times 100 \tag{44}$$

Table 3
Experimental conditions used in the elution of the probe compounds, and peak half-widths.[a]

Peak number	Compound	Column	Acetonitrile (%, v/v)	Temperature (°C)	A_{10}[b] (min)	B_{10}[b] (min)	B/A_{10}[b]
1	Alprenolol	Zorbax	45	30	0.0579	0.1461	2.52
2	Bendroflumethiazide	Zorbax	35	40	0.0602	0.1452	2.41
3	Alprenolol	Zorbax	35	40	0.0783	0.1633	2.09
4	Bendroflumethiazide	Zorbax	30	30	0.0751	0.1531	2.04
5	Bumetanide	Chromolith	35	30	0.2136	0.3442	1.61
6	Benzthiazide	Zorbax	35	40	0.1651	0.2093	1.27
7	Benzthiazide	XTerra	35	30	0.2040	0.2338	1.15
8	Xipamide	Zorbax	35	30	0.3255	0.3421	1.05

[a] The peaks are ordered according to their asymmetry degree.
[b] Right (A_{10}) and left (B_{10}) half-widths, and asymmetry degree measured at 10% peak height.

Table 4
Parameters for the peaks indicated in Table 3 obtained from the fitting to the PLMG model (Eqs. (1) and (2)) of the whole experimental peak.

Peak number[a]	t_R (min)	H_0	σ_0 (min)	m	d	r	w	RE (%)[b]
1	2.105	1.791	0.02587	0.10153	0.08962	−0.05736	0.13652	0. 80
2	2.378	10.834	0.02787	0.11261	0.07169	−0.04988	0.15189	1.5
3	3.395	1.3847	0.02525	0.04626	0.20994	−0.02802	0.27026	0.69
4	2.739	7.4012	0.035	0.10507	0.07934	−0.03946	0.16603	1.12
5	10.958	9.075	0.09953	0.05426	0.21305	−0.45298	0.4616	0.69
6	8.182	4.4297	0.07684	0.03594	0.14441	0.2835	0.99324	0.19
7	8.738	3.034	0.09303	0.01649	0.37496	0.70659	0.98955	0.55
8	16.839	6.573	0.14843	0.04014	0.1372	1.049	1.05895	0.59

[a] See Table 3 for peak identity.
[b] Relative errors were calculated according to Eq. (44).

Table 5
Parameters for the peaks indicated in Table 3 obtained from the fitting to the PVMG model (Eq. (4)) of the region around the peak maximum (above 60% of peak height).

Peak number[a]	t_R (min)	H_0	a	b	c	RE (%)[b]
1	2.1041	1.7925	389.1	13.0	118.9	0.025
2	2.3785	10.893	409.6	12.0	128.5	0.065
3	3.3953	1.3818	179.2	7.74	1.36	0.047
4	2.7384	7.3645	277.4	8.73	76.9	0.030
5	10.958	9.0723	43.9	1.28	4.20	0.036
6	8.1822	4.4459	76.4	1.30	5.56	0.016
7	8.7387	3.0365	48.2	0.58	−0.74	0.076
8	16.839	6.5724	22.1	0.16	0.51	0.033

[a] See Table 3 for peak identity.
[b] Relative errors were calculated according to Eq. (44).

where h_i and $\hat{h}_i$ are the experimental and predicted height for each point in the chromatogram and N is the number of experimental points. The mean relative fitting errors were always below 1.5%.

On the other hand, the simplified PVMG model fits accurately the upper region of the peak (Fig. 1b and d). Table 5 shows the model parameters and the high accuracy of the fittings to the PVMG model for the eight experimental peaks. The fittings correspond to the region above a peak ratio of 0.6. As observed, the mean relative errors were always below 0.1%. This demonstrates the validity of the PVMG model to gather information about the parameters related to the peak maximum.

The parameters in Table 4 were used to obtain a reliable reproduction of chromatographic peaks with known time and height at the peak maximum. The points that defined the peaks were simulated to be equally spaced, with a distance between adjacent points of $\delta = 0.004$ min. In order to consider the effect of the changes in peak location due to experimental factors, peaks with shifts in their location of +0.002, +0.001, 0, −0.001 and −0.002 min were simulated. Finally, noise (Gaussian random errors) was added to the height of each point with $\sigma = 0.005$ (a value slightly larger than the baseline noise measured in the experimental chromatograms). For each time shift, two peaks were simulated; hence, 10 peak simulations were obtained to be treated with each approach.

4.2. Performance of the approaches

As commented, the approaches developed in this work are based on the PVMG model, re-written according to Eq. (4). This model describes a modified Gaussian curve with a parabolic variance, where the coefficient a quantifies the width, whereas both b and c are related to the peak asymmetry and kurtosis.

The peaks analyzed in this work have different asymmetry degrees (Table 3). This is translated in more or less significant values for the b and c coefficients. For symmetrical peaks, $b = c = 0$. For nearly symmetrical peaks, both coefficients are rather small (e.g. see peaks 7 and 8 in Tables 3 and 5). For asymmetrical peaks, b and c are significant, but depending on the c/b ratio, two types of peaks can be recognized (see Table 5): type I (represented by peaks 1, 2 and 4), for which c is highly significant, and type II (peaks 3, 5 and 6), for which c is small. It could be thought that the $c\delta_i^2$ term can be dropped for type II peaks.

Table 6
Mean relative error (ppm) for the values of time and height at the peak maximum obtained with the three proposed approaches.[a]

Peak height ratio[b]	Ia	IIa	IIIa	Ib	IIb	IIIb
Time at the peak maximum						
0.9	34	39	24	39	36	27
0.8	25	26	16	43	27	40
0.7	20	24	17	81	22	101
0.6	18	30	22	115	33	173
0.5	17	42	40	168	44	246
0.4	19	62	80	230	65	349
Peak height						
0.9	420	423	366	428	361	336
0.8	294	324	281	378	360	313
0.7	273	299	353	712	817	467
0.6	241	285	441	1048	1547	604
0.5	237	331	584	1637	2180	890
0.4	250	437	366	2402	3253	1330

[a] Mean errors for the set of probe compounds and conditions, considering 10 simulated peaks for each compound.
[b] The chromatographic data above the indicated peak height ratio were used to fit the peak model.

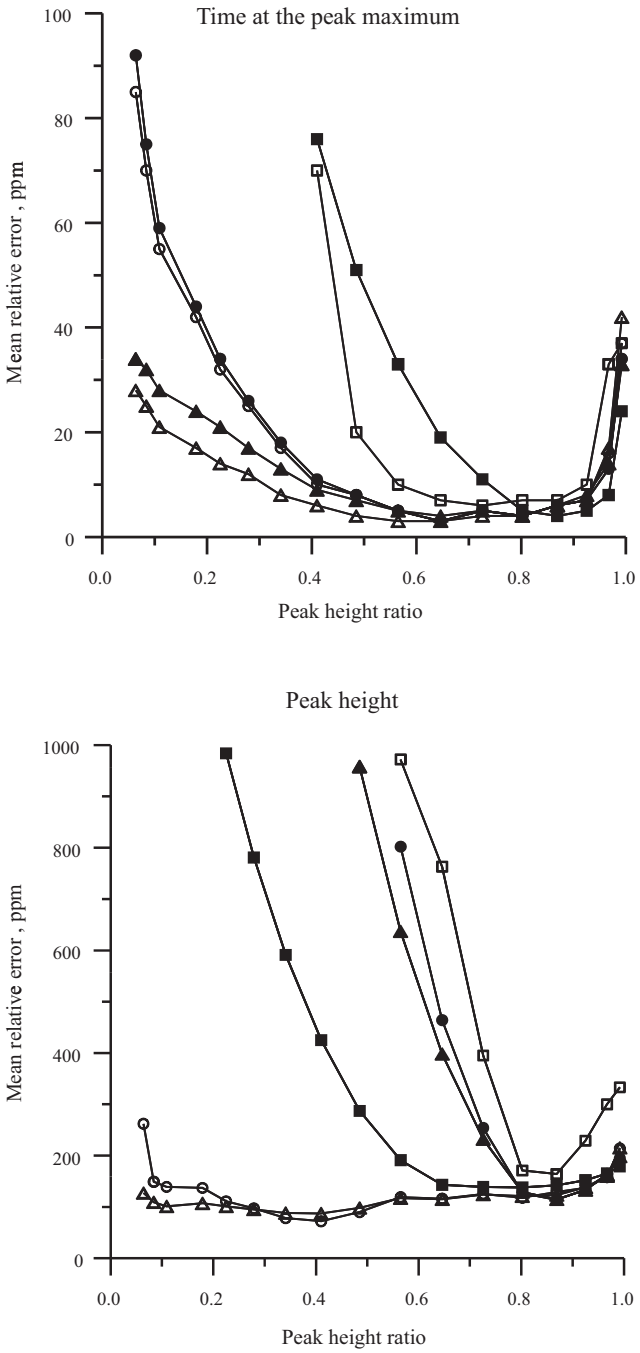

Fig. 2. Mean relative errors in the prediction of the time and height at the peak maximum for xipamide eluted at 30 °C (peak 8, B/A_{10} = 1.05), obtained by applying the approaches that consider the $c\delta_i^2$ term in Eq. (6): Ia (△), IIa (◯) and IIIa (□), and the simplified approaches: Ib (▲), IIb (●) and IIIb (■). The results correspond to 10 simulated peaks (see text).

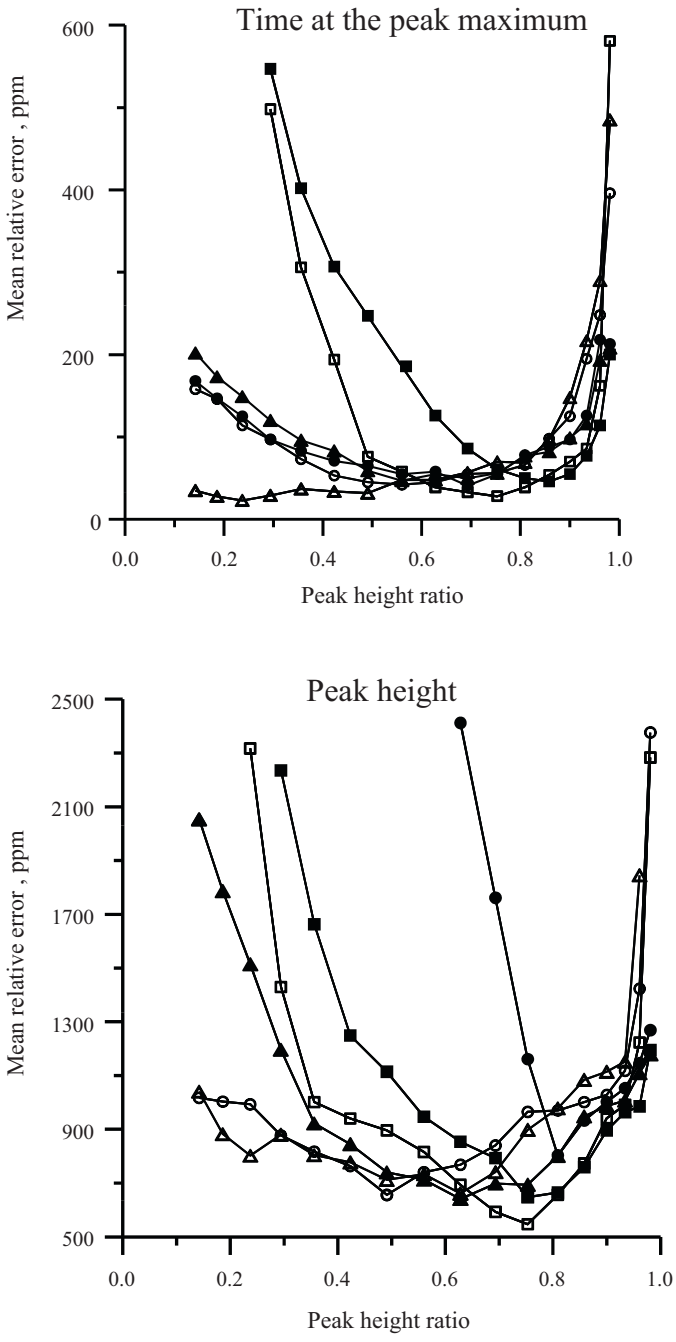

Fig. 3. Mean relative errors in the prediction of the time and height at the peak maximum for alprenolol eluted at 40 °C (peak 3, B/A_{10} = 2.09), obtained by applying Approaches Ia (△), IIa (◯), IIIa (□), Ib (▲), IIb (●) and IIIb (■). Other details are given in Fig. 2.

Figs. 2–4 depict the mean relative errors (expressed as ppm), obtained in the estimation of the time and height at the peak maximum, according to the different approaches, for the three types of peaks (see Table 5): peak 8 (Fig. 2, xipamide at 30 °C), peak 3 (Fig. 3, alprenolol at 40 °C), and peak 1 (Fig. 4, alprenolol at 30 °C). In the figures, the approaches for Eq. (4) including the $c\delta_i^2$ term (Approaches Ia, IIa and IIa, empty symbols), and the simplified approaches (Approaches Ib, IIb and IIb, full symbols) are compared. The minimal number of points needed to perform the fittings is five for the former approaches and four for the latter.

As observed, in general, the three approaches yielded rather small errors, although with differences among them. Also, the errors were significantly larger for the estimation of the peak height. For symmetrical peaks (Fig. 2), Approaches a and b gave similar values for the time at the peak maximum, but the drop of the $c\delta_i^2$ (Eq. (4)) or $\omega\delta_i^4$ (Eq. (8)) terms increased the errors in the estimation of the peak height. The results were acceptable for a wider range of peak ratios with regard to the asymmetrical peaks.

For the asymmetrical peaks (Figs. 3 and 4), Approaches IIa and IIb gave similar results for the time at the peak maximum, but it seems that the $c\delta_i^2$ term is needed for the other two types of approaches. On the other hand, the simplified approaches gave rise

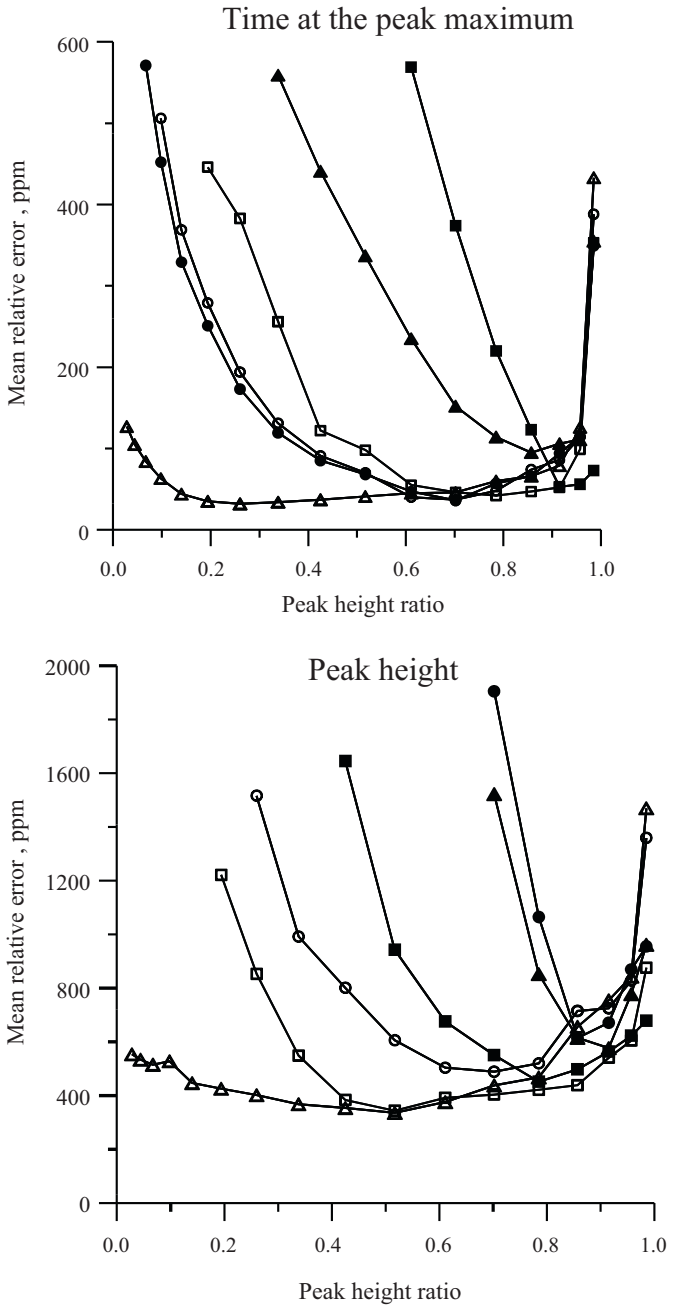

Fig. 4. Mean relative errors in the prediction of the time and height at the peak maximum for alprenolol eluted at 30 °C (peak 1, B/A_{10} = 2.52), obtained by applying Approaches Ia (△), IIa (○), IIIa (□), Ib (▲), IIb (●) and IIIb (■). Other details are given in Fig. 2.

to good results when points close to the peak maximum were processed. When more points were taken for the fittings, the errors increased since the peak was not well described. The approaches that included the $c\delta_i^2$ term (Ia, IIa and IIIa), in general, gave excellent results. They only failed when the number of points around the maximum was too small (since a minimal number of points was needed for the fittings), or when a too wide region was considered (the peak was no more well described by the PVMG model). Approach Ia was the most robust, since it offered good results in a wide time range around the peak maximum.

The mean relative errors for the estimation of the time and height at the peak maximum, considering the set of eight peaks, are given in Table 6. An increasing number of processed points were assessed (above peak height ratios of 0.9–0.4). Approach Ia (the non-linear fitting of Eq. (4)) showed clearly as the best: it offered the smallest errors and was the most robust, since the errors were maintained or even decreased when the time range width was increased. We recommend using the points above a peak height ratio of 0.6 for the fittings.

5. Conclusions

The time and height at the peak maximum can be estimated by fitting the experimental points in a chromatographic peak to an adequate model. Chromatographic peaks often do not follow the ideal Gaussian behaviour. Therefore, they should be described with models considering their skewness and kurtosis. We demonstrate here that the PVMG model (Eq. (4)) describes different types of peaks with high accuracy. This allows different approaches that offer good estimations of the time and height at the peak maximum.

Three types of approaches have been considered, based on the PVMG model: the non-linear fitting of the model (Approach I), or the linear fitting after its linearization (Approaches II and III). The non-linear fitting yields the best results in terms of accuracy and robustness of the estimations. Approaches II and III, and Eq. (4) without the $c\delta_i^2$ term or Eq. (8) without the $\omega\delta_i^4$ term, are simplifications that make the approaches less demanding in terms of computing complexity. In this work, we detail the equations to apply these approaches. It should be also noted that Approaches Ia and Ib can be tackled with the *Solver* option in the *Microsoft Excel* spreadsheet with accurate results.

Acknowledgements

This work was supported by Projects CTQ2007-61828/BQU (Ministerio de Educación y Ciencia of Spain) and CTQ2010-16010/BQU (Ministerio de Ciencia e Innovación of Spain), and FEDER funds.

References

[1] A. Felinger, Data Analysis and Signal Processing in Chromatography, Elsevier, Amsterdam, 1998.
[2] N. Dyson, Chromatographic Integration Methods, RSC, Cambridge, 1998.
[3] P.J. Cumpson, M.P. Seah, S.J. Spencer, Surf. Interface Anal. 24 (1996) 687.
[4] Z. Pápai, T.L. Pap, Analyst 127 (2002) 494.
[5] J.P. Foley, J.G. Dorsey, Anal. Chem. 55 (1983) 730.
[6] J.R. Torres-Lapasió, J.J. Baeza-Baeza, M.C. García-Álvarez-Coque, Anal. Chem. 69 (1997) 3822.
[7] V.B. Di Marco, G.G. Bombi, J. Chromatogr. A 931 (2001) 1.
[8] M.S. Jeansonne, J.P. Foley, J. Chromatogr. 594 (1992) 1.
[9] P. Nikitas, A. Pappa-Louisi, A. Papageorgiou, J. Chromatogr. A 912 (2001) 13.
[10] R.D. Caballero, M.C. García-Álvarez-Coque, J.J. Baeza-Baeza, J. Chromatogr. A 954 (2002) 59.
[11] G. Vivó-Truyols, J.R. Torres-Lapasió, A.M. van Nederkaassel, Y. Vander Heyden, D.L. Massart, J. Chromatogr. A 1096 (2005) 146.
[12] J.J. Baeza-Baeza, M.J. Ruiz-Ángel, M.C. García-Álvarez-Coque, J. Chromatogr. A 1163 (2007) 119.
[13] W.H. Press, S.A. Teukolsky, W.T. Vetterling, B.P. Flannery, Numerical Recipes in C++: The Art of Scientific Computing, 2nd ed., Cambridge University Press, New York, 1992.