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Estimation of significant solvent concentration ranges and its application to the enhancement of the accuracy of gradient predictions☆

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Abstract

The solvent concentration range actually useful for gradient predictions is significantly narrower than the total range scanned in a gradient run. This range, called "solvent informative range" (SIR), if known with the highest accuracy, allows to predict gradient retention times (t_g) with minimal error. The small size of the SIR supports the application of the linear solvent strength theory (LSST). Furthermore, LSST allows a closed-form solution to the integral required to predict gradient retention times, which eliminates numerical integration, needed with other retention models. A methodology that calculates the SIR by applying error analysis, and uses it to improve the accuracy in the prediction of t_g from isocratic experiments, is proposed. The importance of those mobile-phase compositions that do not contribute significantly to the prediction of t_g is selectively attenuated within the prediction algorithm, relying the predictions more heavily on the SIR. As a result, t_g was found to be predicted with similar accuracy using isocratic training data with regard to predictions based on gradient training data. The approach is useful for all situations where the chromatographer is able to provide predictions of retention at constant solvent concentration, and wish to predict the retention in gradient mode.

Keywords: Retention prediction; Retention models; Isocratic elution; Gradient elution; Error analysis

1. Introduction

Gradient elution is the logical choice in reversed-phase liquid chromatography (RPLC) for the separation of solute sets presenting spread polarities, which under isocratic conditions would lead to unfeasible analysis times. Practical application of gradient elution implies finding the suitable gradient program, which is usually a complex task. Straightforward trial-and-error approaches are too inefficient to be useful at routine level [1]. Fortunately, method development can be notably expedited with the application of computer-assisted optimisation strategies. The core of such techniques is always an algorithm able to predict the retention, normally based on mathematical models.

Retention models can be developed from a priori approaches, such as linear solvation energy relationships (LSER) that have been applied to the prediction of gradients from molecular descriptors [2–5]. However, their accuracy tends to be rather poor to be useful for optimisation purposes. More accurate results are obtained from experimental design approaches, allowing truly reliable predictions at low cost and effort [6–10]. In this case, a reasonably small number of experiments should be carried out to infer the retention behaviour of each compound of interest, by regressing the corresponding model parameters. The retention behaviour can be established from either isocratic or gradient experimental sets, but even in the case of using gradient experiments, there is always an underlying model relating isocratically reten-

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tion to composition (e.g. organic modifier content). Indeed, gradient predictions based on interpretive approaches always make use of models expressing these relationships.

Gradient training experiments are maximally efficient when all standards are injected at a time, within a single run, and the identity of each peak is unambiguously known. Otherwise, modelling gradient retention from isocratic experiments is often not only more reliable but also faster, since no re-equilibration time is needed. Also, isocratic data can be available from the literature or from previous data sets, and the chromatographist may wish to prospect whether a gradient separation will give satisfactory results before carrying out any gradient experiment.

This work covers two topics: (i) the estimation of the isocratic solvent concentration range actually useful for predicting the retention of a given solute under a set of gradients and (ii) the application of this concept to enhance the accuracy of predictions of gradient retention times. The potential application field of this study comprehends not only the enhancement of gradient predictions coming from isocratic experimental data, but also from molecular properties. The most frequent gradient optimisation case will be considered: the change in organic solvent content in the mobile phase. Only predictions from isocratic experimental data will be studied here.

Gradient predictions make use of experimental information from surprisingly narrow solvent concentration ranges [11,12]. Usually this range covers a small fraction of the concentrations scanned in the gradient program. The most hydrophobic solutes are scarcely affected by the lowest solvent concentrations, whereas the least hydrophobic solutes will abandon the column soon so that they will not be affected by the highest solvent concentrations of the gradient scan. On the other hand, usually the retention-to-composition relationship is rigorously linear only in narrow solvent concentration ranges. Both facts taken altogether lead to the conclusion that the linear equation is theoretically able to yield accurate predictions, provided the isocratic experiments were developed in the right solvent concentration range. However, the usual ranges scanned in isocratic mode are in practice, often too wide, since more than one solute must be eluted within adequate times under the same experimental design. The fact that the linear equation is only able to fit properly data taken in narrow concentration ranges gives rise to lack of fit when it is applied in gradient predictions. In such a case, to model the experimental behaviour, other more complex equations should be applied instead, in order to avoid biased predictions of retention.

Predictions under gradient elution imply the resolution of an integral equation, which when involves the linear retention model, presents an algebraic solution and, consequently, can be computed usually rather fast. This feature is especially interesting for optimisation purposes, where massive calculations are involved. Models lacking of algebraic solutions, which is the case of more complex equations, should be usually resolved by numerical integration. To sum up, the gradient integral including the linear model can be algebraically solved, but it lacks of accuracy, whereas gradient integrals including more complex models are more accurate, but they imply heavier calculations.

We have tried to combine both advantages: apply an equation that can be algebraically solved (i.e. the linear model), but without yielding biased predictions. In theory, this would be possible if we were able to calculate—without performing additional experiments—the narrow concentration range of organic modifier that each solute under a set of gradients requires.

2. Theory

2.1. Prediction of retention

In the isocratic mode, the RPLC retention behaviour of a given solute can be described by establishing a polynomial relationship between the logarithm of the retention factor, k, and the volume fraction of organic solvent in the aqueous–organic mobile phase, φ. This dependence has been proposed to be quadratic [13]:

$$\log k = c_0 + c_1\varphi + c_2\varphi^2 \tag{1}$$

where c_i are the regression coefficients, with characteristic values for each solute and column/solvent system. However, in narrow solvent concentration ranges, a linear dependence may also yield accurate enough results [14]:

$$\log k = c_0 + c_1\varphi = \log k_w - S\varphi \tag{2}$$

k_w being the retention factor in pure water and S is the eluent strength.

Eqs. (1) and (2) can be used to predict the retention in either isocratic or gradient modes. In the isocratic case, Eq. (1) is usually preferable, since the concentration ranges of practical interest are often wide, and in these conditions, Eq. (2) often leads to lack of fit [11]. In contrast, the solvent concentration range actually useful to predict retention in gradient elution is often narrower, which would make Eq. (2) in theory adequate.

The elution behaviour of a solute under a given gradient program is expressed by the following integral equation:

$$t_o = \int_0^{t_g - t_0} \frac{dt}{k(\varphi(t))} \tag{3}$$

where t_0 is the dead time, t_g the retention time of the solute eluted under gradient conditions, and $k(\varphi(t))$ is the equation describing the solute retention factor at the column inlet as a function of time. From this equation, the retention time can be calculated for any gradient, provided $k(\varphi(t))$ be known. This dependence is established by introducing the programmed gradient, $\varphi = f(t)$, in the retention model, $k = f(\varphi)$ (Eqs. (1) and (2)).

When the linear retention model is applied to the description of linear gradients, the integral equation has the following

solution [15–17]:

$$t_g = \frac{1}{S\varphi'} \log\left[2.3k_0 S\varphi' t_0 \left(1 - \frac{t_D}{t_0 k_0}\right) + 1\right] + t_0 + t_D \quad (4)$$

where φ' is the increment rate in organic solvent (i.e. the slope of the gradient program), t_D the time delay till the gradient formation reaches the column inlet (dwell time), and k_0 is the retention factor at the beginning of the gradient.

2.2. Use of weights in linear regression

Linear models can be expressed in matrix notation as:

$$\mathbf{y} = \mathbf{J} \cdot \boldsymbol{\beta} + \boldsymbol{\varepsilon} \quad (5)$$

where $\mathbf{y}^T = (y_1, y_2, \ldots, y_n)$ is the transposed of the column vector storing the responses of the n experiments, $\boldsymbol{\beta}^T = (\beta_1, \beta_2, \ldots, \beta_m)$ is the transposed of the column vector containing the m model coefficients, $\boldsymbol{\varepsilon}$ stores the differences between predictions and experimental results, and $\mathbf{J}$ is the $n \times m$ design matrix [18], whose value for the ith experiment and jth parameter is given by:

$$j_{i,j} = \frac{\partial y_i}{\partial \beta_j} \quad (6)$$

For non-linear models, the design matrix is usually called Jacobian matrix, and depends on the model parameters, whereas for general linear models, it does not depend on them. The regression process consists of finding the appropriate values of these parameters, in such a way that the predicted and experimental responses be maximally similar (i.e. $\boldsymbol{\varepsilon}$ should be minimal). If similarity is assessed by the least-squares criterion, the parameters can be found by:

$$\boldsymbol{\beta} = (\mathbf{J}^T \cdot \mathbf{J})^{-1} \cdot \mathbf{J}^T \cdot \mathbf{y} \quad (7)$$

In this equation, $\mathbf{J}^T$ denotes the transpose of the $\mathbf{J}$ matrix. Eq. (7) is only a valid solution of Eq. (5) if $\boldsymbol{\varepsilon}$ gathers exclusively random errors (i.e. if $\boldsymbol{\beta}$ is an unbiased solution), and there is no correlation between error and response (homoskedasticity) [19]. Once the parameters have been computed, the precision of predictions (measured as standard deviation) for a new q experiment is given by the following expression:

$$s_{y,q} = s_e \sqrt{\mathbf{j}_q (\mathbf{J}^T \cdot \mathbf{J})^{-1} \cdot \mathbf{j}_q^T} \quad (8)$$

where $\mathbf{j}_q$ is a vector containing the derivatives of the response for each of the m regressed parameters and s_e is the pure experimental error, which in the absence of bias can be approximated to:

$$s_e = \frac{\sqrt{\sum_{i=1}^{n} (y_i - \hat{y}_i)^2}}{n - m} \quad (9)$$

$\hat{y}_i$ being the predicted response for the ith experiment.

In situations where $\mathbf{J}$ does not depend on the parameters (linear regression), the solution given by Eq. (7) is unique and can be found within a single step. For this reason, any non-linear problem is faced linearly when possible. This is the case of linearisation of functions, where the original response is transformed to obtain relationships like Eq. (5) (Eqs. (1) and (2) also constitute an example of linearisation in the chromatographic field).

Predictions achieved through linearised equations are homoskedastic in the transformed response (e.g. $\log k$), but heteroskedastic in the original one (e.g. k). This is usually non-desirable, and can be compensated through weighted regression [19,20]:

$$\boldsymbol{\beta} = (\mathbf{J}^T \cdot \mathbf{W} \cdot \mathbf{J})^{-1} \cdot \mathbf{J}^T \cdot \mathbf{W} \cdot \mathbf{y} \quad (10)$$

where $\mathbf{W}$ is the weight diagonal matrix that contains the reciprocal of the $\mathbf{y}$ variance in each experiment. When weighted linear regression is applied to compensate the transformation of the responses, the diagonal terms of the $\mathbf{W}$ matrix are given by [21]:

$$w = \frac{1}{s_F^2} = \frac{1}{s_f^2 (\partial F/\partial f)^2} \propto \frac{1}{(\partial F/\partial f)^2} \quad (11)$$

where F is the transformed response, f is the original one, and s_F^2 and s_f^2 are the corresponding variances. For logarithmic transformations (e.g. $F = \log k$ and $f = k$ in Eqs. (1) and (2)), the weights are given by $w = (2.303k)^2$ [22]. Note that only the sensitivity contribution to the variance is considered in the final expression, being the constant term s_f^2 neglected. The reason is that this term affects all experimental points in a similar extent, which means that it does not have any neat influence in the regression. Provided adequate weights are applied, linear regression yields parameters identical to those found by non-linear regression.

2.3. Use of weighted linear regression when gradient retention is predicted from isocratic data

The treatment outlined in Section 2.2 solves the problem of heteroskedasticity when the function involved in the least-squares problem is different from the function that is actually predicted. In the case of concern, minimal discrepancies between predicted and actual k values (and not between $\log k$ values) are sought out. However, the approach is only adequate when the weighting of a given experimental point involves exclusively information associated to that experiment. This is not the case of the integral equation (Eq. (3)), since it implies the information of several experiments: data from two or more isocratic retention times are required to predict gradient retention times. Thus, the relationship between the original response and the predicted one goes far beyond a simple transformation. There is no straightforward equivalence linking isocratic to gradient experiments, one to one: a particular gradient scans a range of mobile-phase compositions, which means that each of the available isocratic experiments will take part—in a larger or smaller extent—in the prediction of retention for gradients.

To sum up, the problem of calculating the weights consists of measuring the importance of each isocratic composition to foresee a given set of gradients. The greater the importance of a given isocratic composition, the smaller its uncertainty, and the greater its weight. We propose obtaining the weights by outlining the problem in an inverse way: the reciprocal of the uncertainty associated to an isocratic composition predicted from gradient experiments will yield the weights of the isocratic-to-gradient prediction. Hence, the greater the uncertainty in gradient-to-isocratic prediction, the lower the weight in the isocratic-to-gradient fitting.

In previous work, a procedure to calculate these uncertainties was proposed [11]. The method was based on the calculation of two Jacobian matrices: one associated to the elution mode where the experimental data were gathered (source), and the other to the elution mode where the predictions should be performed (target). In isocratic-to-gradient predictions, the weight corresponding to the ith isocratic experiment is given by:

$$w_i = \frac{1}{s^2_{\text{grd-iso},i}} = \frac{1}{s^2_{\text{e,grd}}(\mathbf{j}_{\text{iso},i}(\mathbf{J}^{\text{T}}_{\text{grd}}\mathbf{J}_{\text{grd}})^{-1}\mathbf{j}^{\text{T}}_{\text{iso},i})} \tag{12}$$

where $s^2_{\text{grd-iso},i}$ represents the variance associated to the gradient-to-isocratic prediction for the ith experiment, $\mathbf{J}_{\text{grd}}$ the Jacobian matrix containing the derivatives of the retention times of the gradients that should be predicted and $\mathbf{J}_{\text{iso},i}$ is the row-vector containing the derivatives of the retention time for the ith isocratic composition. More details are given elsewhere [11].

The $s_{\text{e,grd}}$ term represents the pure experimental error in gradient retention time, which is unknown, since the source experimental data are isocratic. This term gathers the errors associated to the whole set of gradients. Anyway, and similarly to Eq. (11), $s_{\text{e,grd}}$ can be neglected because being constant, it modifies the significance of each experimental point in the same extent, not changing thus their relative importance.

The weights calculated with Eq. (12) do not consider the logarithmic transformation in the response. Since the fitting is not carried out by regressing the isocratic t values, but $\log k$, the weights corresponding to this additional transformation (Eq. (11)) should be included, yielding finally:

$$\begin{aligned} w_i &= \frac{1}{(\partial \log k/\partial t)^2(\mathbf{j}_{\text{iso},i}(\mathbf{J}^{\text{T}}_{\text{grd}}\mathbf{J}_{\text{grd}})^{-1}\mathbf{j}^{\text{T}}_{\text{iso},i})} \\ &= \frac{(2.303(t_i - t_0))^2}{\mathbf{j}_{\text{iso},i}(\mathbf{J}^{\text{T}}_{\text{grd}}\mathbf{J}_{\text{grd}})^{-1}\mathbf{j}^{\text{T}}_{\text{iso},i}} \end{aligned} \tag{13}$$

2.4. Enhancement of isocratic-to-gradient predictions

The weighted computation procedure outlined above can be applied to any retention model. This section presents an application of Eq. (13) to enhance gradient predictions using the linear equation (Eq. (2)). As mentioned, this model leads to an algebraic solution when used to predict gradient retention. This can save computation time, which is a very important issue for optimisation purposes.

Previous work [11] introduced the concept of "solvent informative range" (SIR). The SIR is the concentration range of organic modifier actually useful to predict a given set of target gradients, or, alternatively, the solvent concentration range for which a given gradient set extracts the maximal information about isocratic retention. The weights are directly related to this concept: the larger this information, the greater the weight. As a consequence, the SIR represents the range that isocratic experiments should sample to predict a set of gradients with maximal accuracy. Our purpose is not developing more experiments within the SIR once located, but to weight the original isocratic data to enhance the predictions using the SIR.

The size of the SIR is usually rather narrow in comparison to the increments in organic solvent between experiments in isocratic designs. In addition, the SIR depends on the considered solute. This means that each solute would require a particular experimental design to get maximal accuracy in the prediction of a given gradient. Both drawbacks altogether seem to make the direct application of weights unfeasible: if a design including a large number of experiments were available, fitting the linear equation inside the SIR without bias would be possible. Unfortunately, this is absolutely unpractical. We can, however, mimic that comprehensive hypothetical design by generating artificial experiments with an unbiased equation (the quadratic equation). The following algorithm applies this strategy (see also Fig. 1).

(i) The retention behaviour is first modelled in the whole range of the isocratic design, using an unbiased equation, such as Eq. (1). The experimental isocratic data are fitted by applying the conventional weights given by Eq. (11), which will be considered as initial weights.
(ii) Although no experimental gradient data are actually used in this computation, the set of fitted parameters in Eq. (1) will be further considered as fitted from gradient data. The weights are calculated according to Eq. (13) for a regular distribution of solvent concentrations.
(iii) The weights are made null at those solvent concentrations where the solute never elutes under any planned gradient. For determining this information, the solvent concentration at which each solute leaves the column is first computed for each target gradient using the equation fitted in (i), and the highest solute exit value found is kept. The weights corresponding to concentrations larger than this maximal value are set to zero. The weights for solvent concentrations lower than the lowest starting concentration among all planned gradients are also set to zero.
(iv) Eq. (2) is fitted with the values of retention calculated previously from Eq. (1), applying the weights computed in (ii) and (iii).

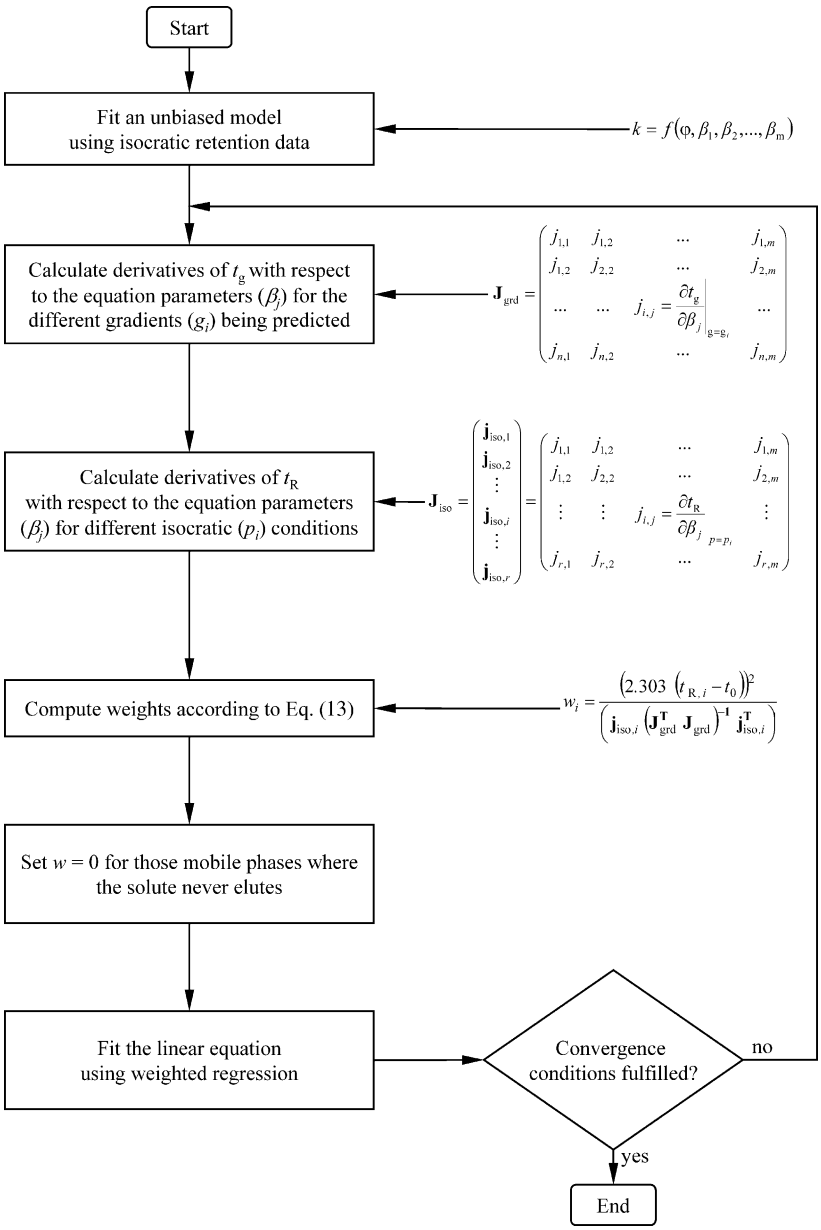

Fig. 1. Flow diagram illustrating the algorithm used for the calculation of weights (see Section 2.4).

(v) Steps (ii)–(iv) are iterated, using in (ii) the model parameters obtained in (iv) instead of Eq. (1). Convergence is usually reached within the first iteration.

3. Experimental

3.1. Reagents

Sixteen β-blockers were studied: acebutolol (Italfármaco, Alcobendas, Madrid, Spain), alprenolol, pindolol, sotalol (Sigma, St. Louis, MO), atenolol (Zeneca Farma, Madrid), bisoprolol, propranolol, practolol (ICI-Farma, Madrid), carteolol (Miquel-Otsuka, Barcelona, Spain), celiprolol (Rhône-Poulenc Rorer, Alcorcón, Madrid), esmolol (Polfa, Starogard, Poland), labetalol (Glaxo, Tres Cantos, Madrid), metoprolol, oxprenolol (Ciba-Geigy, Barcelona), nadolol (Squibb, Esplugues de Llobregat, Barcelona), and timolol (Merck, Sharp & Dohme, Madrid). Acebutolol, atenolol, carteolol, celiprolol, labetalol, metoprolol, nadolol, oxprenolol, propranolol, and timolol, were kindly donated by the cited pharmaceutical laboratories. The drugs were dissolved in a small amount of methanol and diluted with water. The concentration of the stock and injected solutions was 100 and 10 μg/ml, respectively. These solutions remained stable during at least 2 months at 4 °C.

Mobile phases were prepared with acetonitrile (Scharlab, Barcelona), buffered at pH 3 with 0.01 M di-sodium hydro-

gen phosphate and hydrochloric acid (Panreac, Barcelona). The mobile phases and drug solutions to be injected were vacuum filtered through 0.45 μm Nylon membranes (Micron Separations, Westboro, MA). Nanopure water (Barnstead, Sybron, Boston, MA) was used throughout. Acetone (Guinama, Barcelona) was used to measure the dwell time.

3.2. Apparatus

An Agilent (Model HP 1100, Waldbronn, Germany) chromatograph, equipped with a quaternary pump, a UV–vis detector, and an autosampler, was used. All components were governed by a PC. An XTerra MS C18 column (150 mm × 4.6 mm i.d., 5 μm particle size), and a guard column packed with the same material (20 mm × 3.0 mm i.d., 5 μm particle size) (Waters, MA), were used. The detection wavelength was 225 nm for all β-blockers, except for timolol, which was detected at 300 nm. The flow-rate was 1.0 ml/min, and the injection volume, 20 μl. The whole study was carried out at room temperature (25 ± 2 °C). Duplicate injections were made for each chromatogram.

The dead time (1.73 min) was measured as the first baseline deviation, and the dwell time (1.53 min) as indicated in Ref. [23], by running a blank gradient where acetone was increased from 0 to 1% in 20 min. For this determination, the times at the beginning and end of the steep increase were taken. The signal was monitored at 280 nm. Home built-in routines, written in MATLAB 6.5 (The Mathworks, Natick, MA), were developed for data treatment.

4. Results and discussion

Two experimental designs were carried out, one of them in isocratic and the other in gradient mode. The ranges of acetonitrile concentration in the mobile phases were selected to avoid retention times too close to the void volume or above 60 min. In the isocratic case, chromatographic parameters were obtained from six mobile phases containing 5, 10, 15, 20, 25, and 30% (v/v) acetonitrile at pH 3. The acidity enhanced notably peak shape, owing to protonation of column silanol groups. Due to the relatively wide range of solute polarities (octanol–water partition coefficients in the range $\log P_{o/w} = 1$–3), measurement of retention times in all mobile phases of the design was unfeasible. All available isocratic data were used to fit the retention models and predict the gradients. The repeatability of solute retention times in the isocratic mode, obtained from six replicated injections, was in the range 0.05–0.15% (relative standard deviation). A validation gradient design, including four runs where the modifier content was increased from 5 to 30% acetonitrile in 20, 30, 40, and 50 min, was carried out to test the method performance.

Table 1 shows the experimental gradient retention times for the 16 β-blockers. The errors (predicted minus experimental gradient retention times), using different strategies, are shown in Fig. 2. Boxes (A–D) and (E) correspond to isocratic-to-gradient and gradient-to-gradient predictions, respectively. (A–C) depicts the errors obtained with the linear equation and different types of weights: (A) unweighted regression, (B) weighted regression considering only the logarithmic transformation (Eq. (11)), and (C) the weighted procedure outlined in Section 2.4. For comparative purposes, two additional error boxes are shown, corresponding to: (D) the quadratic equation conventionally weighted, and (E) a straightforward prediction from gradient data (without any transference between elution modes, that is, gradient-to-gradient prediction). In the latter case, the linear equation was

Table 1
Gradient retention times (t_g, min) for different runs[a]

Compound	t_G (min)			
	20	30	40	50
Atenolol	6.22	6.88	7.06	7.22
Practolol	7.25	7.98	8.45	8.96
Sotalol	6.44	7.04	7.18	7.41
Nadolol	10.87	13.00	14.83	16.58
Carteolol	10.25	12.48	14.00	15.32
Pindolol	11.25	13.44	14.82	16.15
Timolol	14.14	17.84	20.73	23.31
Metoprolol	15.13	18.76	21.94	24.88
Acebutolol	14.39	18.14	21.22	24.21
Esmolol	17.22	22.15	26.11	29.87
Celiprolol	17.28	22.33	26.78	31.02
Labetalol	19.95	26.26	31.62	36.89
Oxprenolol	18.57	23.76	28.32	32.69
Bisoprolol	19.19	24.90	29.81	34.48
Propranolol	21.90	28.64	34.68	40.61
Alprenolol	22.16	29.19	35.33	41.21

[a] Acetonitrile concentration was increased from 5 to 30% in all cases.

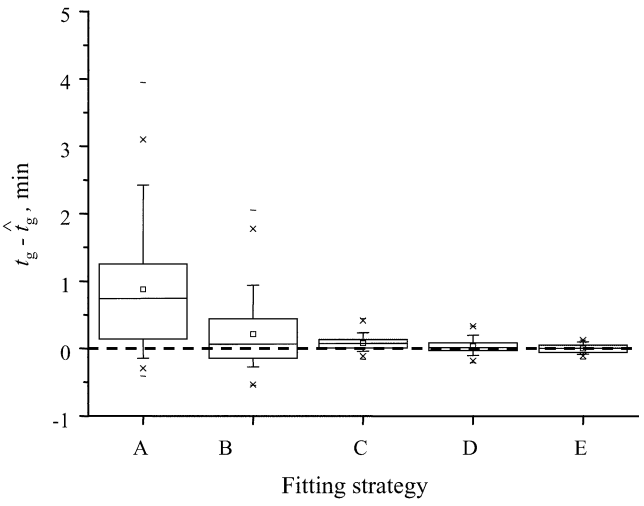

Fig. 2. Box and Whiskers plots showing the differences between experimental and predicted gradient retention times. The predicted values were obtained from isocratic experiments (A–D) as source elution mode and different fitting strategies. The fitting with the training set of gradient experiments (E) is given as reference. Eq. (2) was used in (A–C) and (E), and Eq. (1) in (D). Strategies: (A) unweighted, (B and D) conventionally weighted, and (C) weighted using the algorithm described in Section 2.4.

used. The accuracy of this equation for gradient-to-gradient predictions was checked in previous work [11].

Prediction errors obtained using the different weighting strategies confirmed that both the unweighted (A) and the traditionally weighted (B) regressions do not focus adequately the fittings to yield the minimal error in isocratic-to-gradient predictions. The more sophisticated weighted algorithm explained above (C) is needed to enhance the predictions. The residuals obtained with the parabolic equation (D) are smaller than those achieved with the linear equation (A–C). The parabolic equation was numerically integrated, although a close algebraic solution exists, but involving the error function [24]. For other retention models, which do not have any analytical solution, the proposed approach may be a real alternative. As expected, the use of gradients as source data (E) yielded the best results. Table 2 presents the relative errors, expressed as percentages, for each solute. To avoid inflated errors in the fastest gradients, a modification of the usual relative error definition was used [25]:

$$\mathrm{RE}\,(\%) = 100\frac{\sum_{i=1}^{n}|t_{g,i} - \hat{t}_{g,i}|}{\sum_{i=1}^{n} t_{g,i}} \quad (14)$$

where $t_{g,i}$ and $\hat{t}_{g,i}$ are the experimental and calculated retention times, respectively, for the ith gradient and n is the number of gradients in the validation set. As can be seen, the errors obtained with the unweighted (A) and traditionally weighted (B) regressions depend on the solute. Sotalol, nadolol, carteolol, and pindolol, which are among the fastest solutes, gave rise to the highest errors.

As commented, not all experimental conditions could be assayed for modelling the retention of all solutes, since some of them were eluted too close to the void volume or beyond 60 min. The inclusion or exclusion of a given point in the experimental design may introduce drastic changes in the fitting parameters, since a certain lack of fit remains. This does not happen when the correct equation (i.e. the quadratic one) is applied, or the linear equation is adequately corrected with weights. In the latter case, the effects of the inclusion/exclusion of a given experimental point on predictions are reduced, since the weighting function governs the importance of each experiment. Fig. 3 shows a comparison of two experimental chromatograms with the corresponding predictions performed by applying the weighted (C) and unweighted (A) approaches. As observed, the agreement is more satisfactory with the proposed approach.

Fig. 4 illustrates how the weighted fitting works. In this figure, the differences in the calculated retention factors between each studied method (A, B, C, and E) and the parabolic fitting (D), which was taken as reference, are plotted as a func-

Table 2
Relative errors in percentage according to Eq. (14), for the 16 β-blockers obtained with the strategies proposed in Section 4

Compound	Fitting strategy				
	A	B	C	D	E
Atenolol	1.1	2.9	0.98	1.05	0.93
Practolol	2.7	3.7	0.76	0.76	0.40
Sotalol	11.1	3.2	0.69	0.74	0.74
Nadolol	10.2	1.6	0.47	0.49	0.49
Carteolol	15.4	4.4	0.72	0.69	0.53
Pindolol	13.2	3.7	1.01	0.97	0.44
Timolol	3.5	0.85	0.51	0.46	0.30
Acebutolol	2.0	2.0	0.36	0.43	0.23
Esmolol	4.8	1.7	0.55	0.31	0.26
Celiprolol	3.6	0.60	0.25	0.17	0.13
Labetalol	4.5	3.3	0.91	0.67	0.25
Oxprenolol	1.7	0.30	0.33	0.45	0.27
Bisoprolol	1.7	0.40	0.66	0.34	0.15
Propranolol	1.8	1.4	0.56	0.39	0.28
Alprenolol	1.5	1.1	0.32	0.20	0.14
Mean[a]	5.3	2.1	0.61	0.54	0.37

[a] Mean error (extended to all solutes) is also given.

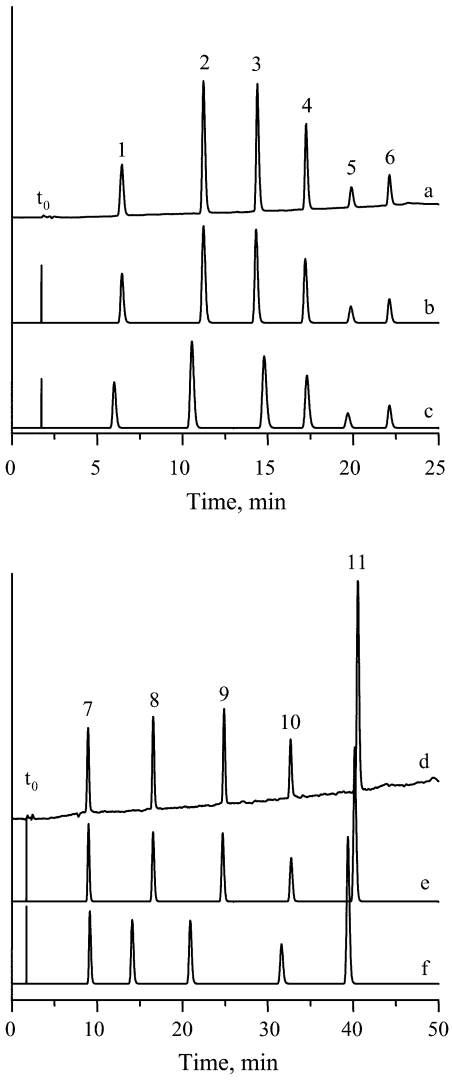

Fig. 3. Experimental (a and d) and predicted chromatograms using the proposed weighted strategy C (b and e), and the unweighted method A (c and f). Acetonitrile concentration was linearly increased from 5 to 30% in: (a–c) 20 min and (d–f) 50 min. Compounds: (1) Sotalol, (2) pindolol, (3) acebutolol, (4) celiprolol, (5) labetalol, (6) alprenolol, (7) practolol, (8) nadolol, (9) metoprolol, (10) oxprenolol, and (11) propranolol.

tion of solvent concentration, for three representative solutes: a fast (sotalol), an intermediate (acebutolol), and a slow (alprenolol) solute. The differences between the experimental and predicted values obtained with the parabolic equation are also overlaid as squares. The weighting function for method C (Section 2.4) is depicted at the top of Fig. 4a–c. The magnitude of errors with regard to the SIR location should be considered for the analysis of the results. As can be seen, linear fittings tend to produce larger errors when the total concentration range is considered. Only the parabolic fitting (which is represented as the zero line) gives low errors in the whole solvent concentration range. Both the unweighted (A) and the conventionally weighted (B) fittings produce large errors within the SIR. On the other hand, the results obtained with method C are close to those achieved using gradient source data (E), yielding smaller errors within the SIR.

Note that the weights, w, are set to zero at the largest concentration above which the solute is never eluted within the runs in the gradient experimental design (see Section 2.4, step (iii)). The plotted weights in Fig. 4 (top diagram) detect satisfactorily the SIR, which is important to describe the gradient elution behaviour. The SIR is the solvent concentration region where high values of the weighting function are found. As the polarity of solutes decreases, the SIR range is shifted to higher values of acetonitrile concentration. Thus, the SIR is located in the range 6–8% acetonitrile for sotalol, 11–17% for acebutolol, and 18–22% for alprenolol. These ranges are well located by the weighting function, which will be translated in enhanced predictions with the linear equation.

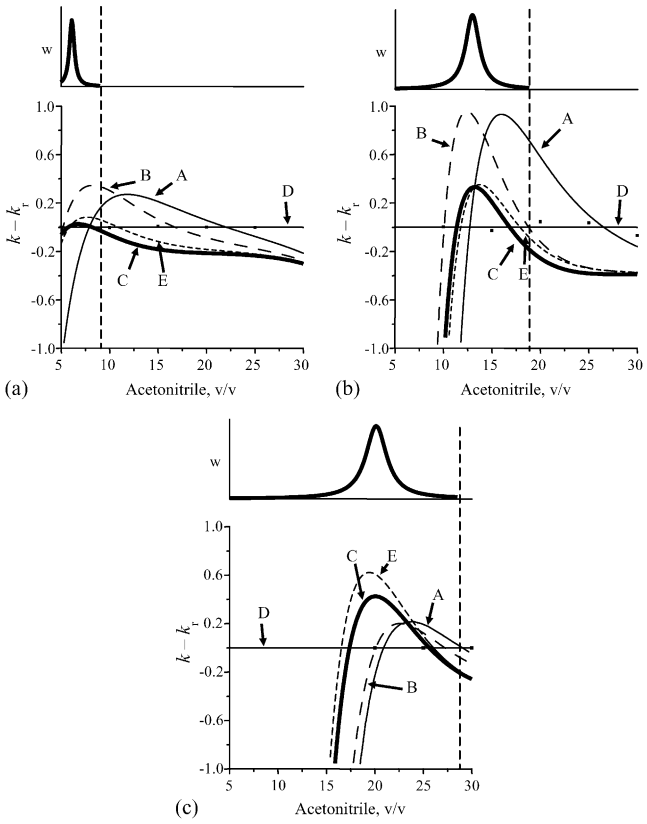

Fig. 4. Errors for the different weighted strategies as a function of solvent concentration. The lines in the bottom diagrams correspond to the differences between the retention factors (k) calculated with Eq. (2) and calculated with Eq. (1) (D), whose results were taken as reference (k_r). Five different fitting strategies (see Fig. 2 for meaning) were considered: (A) thin solid line, (B) long dashed, (C) thick solid, and (E) short dashed. The difference between the experimental points and the parabolic predictions are overlaid as squares. The corresponding weighting function is plotted at the top of the figure for method C. The vertical line indicates the maximal solvent concentration beyond which the solute never elutes in gradient mode. Compounds: (a) sotalol, (b) acebutolol, and (c) alprenolol.

5. Conclusions

The most widely applied model in gradient predictions is the linear equation (Eq. (2)), since the derived general integral can be algebraically solved under linear gradients. This may present practical advantages in situations that require massive calculations (e.g. optimisation of gradient conditions). For gradient-to-gradient predictions, where no data should be transferred between elution modes, the results are highly satisfactory. Gradients can be also predicted when an isocratic model is available. However, these predictions can be deficient, since the linear equation is often unable to fit accurately retention data from wide solvent concentration ranges. The problem is solved by weighting the regression in the isocratic domain with factors calculated from error analysis. The weights give more importance to the solvent concentrations (solvent informative range) that are significant for the desired predictions. The approach yields a quality of predictions comparable to that achieved with more complex models, but without requiring numerical integration, which represents in certain situations a considerable save in calculation time.

The concept of SIR offers a different perspective on gradient elution, providing a deep examination of the information that a given gradient extracts, which is related with isocratic information. In the considered example, where the gradients comprise an increase from 5 to 25% acetonitrile, the SIR represents ranges of acetonitrile from ca. 2% for scarcely retained solutes to 5% for retained solutes.

The aim of the proposed methodology is not to detect the SIR to include further more experiments within it, but to use the original set of experiments (that can be within or out the SIR of each solute), to fit an unbiased equation. This equation is used to calculate weights. The weights are then applied to the linear equation to obtain unbiased predictions. In the given example, typically three to five isocratic experiments by solute were developed. Problems requiring a wide scanning of modifier compositions (e.g. 0 to 90–100%) are not so frequent, except in screening studies. If the covered range is really wide, then the quadratic equation can present small bias, which can affect the accuracy of the SIR.

The bottleneck of the methodology can be the experimental work needed in isocratic mode, but previous knowledge on the components nature and a wise experimental plan can save

considerable time. Also, no experiments are required when the chromatographist is adapting isocratic data previously acquired, taken from the literature, or obtained by molecular modelling approaches. In all these cases, the outlined technique will be valid.

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