Journal of Chromatography A, 1216 (2009) 8891–8903

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Combined effect of solvent content, temperature and pH on the chromatographic behaviour of ionisable compounds. III: Considerations about robustness

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

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

ARTICLE INFO

Article history:
Received 12 June 2009
Received in revised form 7 October 2009
Accepted 13 October 2009
Available online 20 October 2009

Keywords:
Reversed-phase liquid chromatography
Ionisable compounds
Organic solvent
Temperature
pH
Optimisation
Robustness
Monte Carlo simulations

ABSTRACT

We previously reported a model able to predict the retention time of ionisable compounds as a function of the solvent content, temperature and pH [J. Chromatogr. A 1163 (2007) 49]. The model was applied further, developing an optimisation of the resolution based on the peak purity concept [J. Chromatogr. A 1193 (2008) 117]. However, we left aside an important issue: we did not consider incidental overlaps caused by shifts in the predicted peak positions, owing either to uncertainties in the source data, modelling errors, or the practical implementation in the chromatograph of the optimal mobile phase (or any other). These shifts can ruin the predicted separation, since they can easily amount several peak-width units at pH values close to the logarithm of the solutes' acid–base constants. A probabilistic optimisation is proposed here, which is able to evaluate the uncertainties associated with the model and the consequences when the optimal mobile phase is implemented in the chromatograph. This approach assumes peak fluctuations in replicated assays obtained through Monte Carlo simulations, which gives rise to a distribution of elementary peak purities. The results yielded by the conventional (i.e. non-robust), derivative-penalised, and probabilistic optimisations were compared, checking the predicted and experimental chromatograms at several critical experimental conditions. Among the three approaches, only the probabilistic one was able to appraise properly the practical difficulties of the separation problem.

1. Introduction

Part I [1] of this series was devoted to propose a general model, able to describe with accuracy the retention behaviour of ionisable compounds as a function of the organic solvent content, pH and temperature of the mobile phase. We outlined the acid–base equilibrium in the direction of protonation, being its constant (K) the reciprocal of the dissociation constant ($K = K_a^{-1}$, and therefore $\log K = \mathrm{p}K_a$). The model included eight fitting parameters and was valid for modelling the retention in wide ranges of experimental conditions:

$$\hat{t}_R = t_0(1+\hat{k}) = t_0\left[1+\exp\left(c_1+\frac{c_2}{T}+c_3P_M^N + \log\left(c_4+\frac{10^{c_5+c_6\varphi+(c_7/T)+c_8(\varphi/T)}h}{1+10^{c_5+c_6\varphi+(c_7/T)+c_8(\varphi/T)}h}(1-c_4)\right)\right)\right] \quad (1)$$

where $\hat{t}_R$ and $\hat{k}$ are the predicted retention time and retention factor, respectively, t_0 is the dead time, φ and h are the volume fraction of organic solvent and proton concentration in the hydro-organic mobile phase, T the absolute temperature, P_M^N a normalised polarity parameter that depends on the volume fraction of organic solvent [2], and c_1 to c_8, fitting parameters with specific values for each solute. The term exp refers to 10 or the number e raised to the term between parentheses (10^x or e^x). Naturally, the regression coefficients will change according to the selected base, but the predictions will be exactly the same. The coefficients c_1 to c_8 were obtained by non-linear fitting using the Powell method [3].

A linear dependence of the logarithm of the protonation constant ($\log K$) with the solvent content and the reciprocal of temperature was assumed (Eq. (1)). More complex variations in φ and T would mean a significant increase in the number of experiments (two-fold, or more). Also, the difficulties in performing the non-linear fitting and the consequences of a non-appropriate distribution of experiments within the design would limit the practical utility of such a model. It should be observed that the results presented in Part I [1], II [4] and III (this work), and in a previous work carried out at fixed temperature [5] demonstrate that the linear dependence is valid, at least for optimisation purposes.

Again for simplicity, Eq. (1) assumes a similar effect of φ and T on k_{HA} and k_A (see Part I [1]). This equation was aimed to describe weak acidic solutes, where the acidic species (HA) is the major contributor to retention (which is a weighted average of k_{HA} and k_A). Hence, the species whose retention should be described the best is

* Corresponding author at: Departament de Química Analítica, Facultad de Química, Universitat de València, c/Dr. Moliner 50, 46100 Burjassot, Spain.
Tel.: +34 963544014; fax: +34 963544436.
E-mail address: jr.torres@uv.es (J.R. Torres-Lapasió).

0021-9673/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2009.10.032

the neutral one. The retention of the anionic species is rather small and the assumption of a similar effect of φ and T on k_A has no significant consequences. Indeed, we have checked the good accuracy of the predictions. Assuming a particular dependence of k_A with φ and T would mean at least two new coefficients and colinearity problems.

Eq. (1) was valid for describing the behaviour of the basic compounds studied in this work, for which the retained species is the basic one (k_A). However, an adaptation of Eq. (1) for basic compounds can be used instead:

$$\hat{t}_R = t_0(1+\hat{k}) = t_0\left[1+\exp\left(c_1+\frac{c_2}{T}+c_3P_M^N + \log\left(1+\frac{10^{c_5+c_6\varphi+(c_7/T)+c_8(\varphi/T)}h}{1+10^{c_5+c_6\varphi+(c_7/T)+c_8(\varphi/T)}h}(c_4-1)\right)\right)\right] \quad (2)$$

Eq. (1) was applied to study the effects of the three factors (φ, pH and T), on both retention and selectivity, and prospect the magnitude of the shifts in the solute protonation constants produced by changes in solvent content and/or temperature, and the consequences of insufficiently informative experimental designs [1]. The analysis of the results suggested that the three factors exhibited strong interactions making it worthwhile to consider a combined treatment. The predictive accuracy was proved good enough to make it suitable for optimisation purposes.

Part II [4] was focused on the practical consequences of the model, examining the resolution properties of the same probe mixture considered in Part I, which included eleven ionisable compounds (nine diuretics and two β-blockers). These compounds were selected as a representative sample of the variety of protonation behaviours that one can expect to find in real chromatography. Also, the coverage of the protonation processes provided by the design was deliberately unequal: whereas the design was appropriate for some solutes, it was deficient for others, for which the acid–base transition was incomplete or even barely sampled by the design. All the same, the optimisation (based on the use of peak purities [6]) managed to succeed the full separation. Two favourable optimal regions were found, which were verified experimentally.

However, the optimisation was carried out leaving apart an important issue. It ignored the effects of incidental overlaps caused by shifts in the predicted peak positions, due either to uncertainties in the source data, modelling errors, or the practical implementation of the optimal mobile phase (or any other) in the chromatograph. These shifts, which can amount several k units at pH values close to the logarithm of the protonation constant (or pK_a) for a given solute, can ruin the predicted separation.

Robustness is an extremely important issue in analytical method validation, and concerns the capability of the method to give the same results when it is applied to a sample in conditions slightly different from the nominal ones (i.e. those specified in the protocol). Initially, it was focused on the identification of important factors that affected the stability of the results, but with the time it evolved and nowadays it is split into two concepts, one of them related to earlier stages of method validation, namely robustness as it was originally proposed, and the other more related to inter-laboratory assays, that has been called ruggedness [7].

Robustness of a method is defined as its capacity to remain unaffected by small but deliberate variations in the experimental parameters, aimed to assure the validity of the analytical procedure wherever used [8]. This implies that it should be measured by introducing changes in the working parameters, shifting them from their nominal values. There is a very efficient mathematical tool to assay systematic variations: the experimental designs. Accordingly, robustness tests are usually based (but not always) on two-level screening designs, such as fractional factorial [9–11], or Plackett-Burman designs [12,13]. Full factorial and three level designs are also applied [11,14], but they are less convenient. Robustness tests are best performed immediately after setting up the method, and are carried out in a single laboratory. Indeed, robustness insufficiencies should be detected as soon as possible. If a method does not pass an acceptable robustness threshold, it still requires basic modifications, and proceeding further is nonsense. Otherwise, time and reagents will be invested to validate a useless method that will be finally discarded in later stages.

In contrast to the pre-validating character of robustness, ruggedness is related to the mid and ending steps of validation. Assays for ruggedness explore the consequences of changes in other variables out of those strictly defining the operative conditions [15,16]. This work specifically concerns pre-validation (i.e. robustness) in chromatography. In spite of the continuous advances in instrumentation, method development in HPLC can be slow and laborious. For this reason, detecting as soon as possible that a target working condition can be risky is particularly important in this separation technique.

As mentioned, conventional robustness assays are carried out through experimental designs. This can be applied in two ways, namely, by using simulated or real experiments. In both cases, the different variables can be ranked according to their influence, but the consequences of changes in the working conditions on the implementation in the chromatograph can only be strictly evidenced from real experiments. However, simulation-based calculations are more economical, and potentially very attractive. In this case, a modified resolution is measured by evaluating the consequences of small shifts (derivatives) around the nominal values of a given candidate condition. We will call this approach "derivative-penalized" robustness. The results can be mapped as a "robust resolution surface" [17]: the highest the robust resolution value, the most suitable and reliable the separation.

This strategy ignores an essential source of error: the uncertainties associated with the modelling step. Indeed, the predicted peak positions have always associated an uncertainty band, which means that when an optimal condition is implemented in the chromatograph, the peaks will appear more or less displaced from their expected locations. The shifts can be large enough to ruin what should have been a perfect base-line separation. Eqs. (1) and (2) include a large number of parameters and three factors, one of them being as critical as pH: the consequences of shifts in these factors can be important, and should not be ignored.

We propose here the use of Monte Carlo simulations, which we have called "probabilistic approach" to estimate the uncertainties in the predicted retention for all peaks in a chromatogram, and consequently, possible overlaps not accounted using a conventional optimisation approach. The shifts in peak position arise mainly from the uncertainties in the measurement of the training data that are propagated through the model to the final predictions, and the uncertainties in the coefficients of the fitted models as a consequence of the regression process. The results obtained with the probabilistic approach are critically compared with those from a conventional optimisation and the derivative-penalised approach, by contrasting the predicted resolution with the experimental chromatograms in critical regions of the experimental domain.

2. Theory

2.1. Conventional peak purities

In Part II [4], conventional resolution was measured using the product of individual peak purities (i.e. product of non-overlapped