Correction of the deviations in the retention times with Chromolith columns associated to the flow rate: implications in the modelling of the retention behaviour

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In a previous work (*J. Sep. Sc.* 2009, *32*, 2793–2803), we reported an interpretive optimisation approach to achieve maximal resolution in minimal analysis time, based on models describing the retention and peak shape as a function of mobile phase composition and flow rate. The method was applied to the separation of a group of basic drugs in a Chromolith column. In that work, we found that the retention factors were sensitive to the flow rate. The reason of the observed deviations in retention times is the increase in the column volume at the applied pressure, which decreases the linear velocity inside the column. This behaviour forced to include a correction term in the model that described the retention. We show here how the deviations in retention times can be evaluated, allowing retention models that do not include the flow rate as variable, similarly to isocratic chromatography at fixed flow rate. The logarithm of the deviations in the retention times with flow rate are shown to correlate with the solute polarity. This correlation is compared with similar correlations for the retention factor at fixed mobile phase composition and the extrapolated retention factor in water at fixed flow rate.

Keywords: Chromoliths / Flow rate / Retention time deviations / Retention modelling / Solute polarity

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1 Introduction

Silica-based monolithic columns are becoming usual in analytical laboratories [1,2]. However, currently, only the columns developed by the group of Nakanishi [3] in cooperation with Merck KGaA (Darmstadt, Germany) are commercially available, under the trade name Chromolith [4], or Onyx (Phenomenex, Torrance, CA, USA), licensed from Merck KGaA. These columns are made of a single silica skeleton rod. Their structural characteristics and those of the conventional beds of microparticulate silica-based packing materials used in high-performance liquid chromatography (HPLC) are rather different. The most important features of Chromolith (Onyx) columns are their high external porosity, resulting from the structure of the network of macropores (with a size of around 2.2 μm), interconnected by channels through which the mobile phase flows, and the structure of the silica skeleton that consists in a network of small and thin threads of porous silica (mesopores). These structural features allow the combination of a low hydraulic resistance to the stream of mobile phase, and an enhancement in the mass transfer of sample molecules [5]. The volume of the channels (i.e. macropores) opened for percolation of the mobile phase through the bed of stationary phase in monolithic columns is larger than the volume between the particles in packed columns. Also, the structure of the monolithic channels is less tortuous than the equivalent in packed columns. This allows flow rates in the monoliths beyond those feasible for conventional microparticulate packed columns, which is of interest in the development of fast methods with common HPLC instrumentation.

Conventionally, optimisation of the resolution for a Chromolith column is made at fixed flow rate (typically 1 mL min^{-1}). In a second step, the method is speeded up at the optimal mobile phase composition by increasing the flow rate to the operational limit or to a value where enough resolution is still achieved (at increasing flow rate, peaks become relatively wider and the resolution soon deteriorates in chromatograms containing critical peak pairs).

This is usually carried out by trial-and-error. We reported an interpretive optimisation approach to achieve maximal resolution in minimal analysis time, based on models describing the retention and peak shape as a function of mobile phase composition and flow rate [6]. The method was applied to the separation of a group of basic drugs with a Chromolith Performance RP-18e column, using acetonitrile-water mixtures. The proposed approach also allowed a comprehensive exploration of the chromatographic performance upon changes in the experimental factors. In that work, we found that the retention times of the analytes were not exactly halved as the flow rate was doubled. We also observed that the longer retention times were translated into retention factors that depended on the flow rate, which forced to include a correction term in the model that described the retention.

Recently, we have discussed the possible reasons of the deviations in the retention times in Chromoliths [7]. These columns are first synthesised in a mould by polymerisation, and then encapsulated, owing to the significant shrinkage of the silica rod in the ageing and drying steps during the polymerisation process. This is why the monoliths are wrapped with a solvent- and pressure-resistant polymer (a PEEK tube), tightly melted on the outer surface of the silica rod, to which the column end fittings are attached for use in an HPLC system. An increase in flow rate is translated into an increase in the system pressure. Owing to the PEEK material of the column, maximal pressure is restricted to 200 bar (pressure that is hardly approached with these columns at the maximal operating flow rates). The Young modulus of PEEK is 3.6 GPa against 210 GPa for stainless steel, meaning that PEEK is 50 times more elastic [8]. Therefore, Chromoliths suffer larger stress with pressure than stainless steel columns that tend to inflate them and increase their volume. This decreases the linear velocity inside the column, and increases the retention at the relatively low applied pressure. In contrast, frictional heating, which is an issue for microparticulate columns and decreases the retention, seems to be small in Chromoliths, due to the moderate pressures and large permeability of these columns.

In this work, we discuss effects derived from the use of high flow rates with Chromoliths. We show the usefulness of the retention time plots versus the inverse of the delivered flow rate to correct the observed deviations in the retention times, which are more significant at higher flow rate and decreasing solute polarity. By performing this correction, the interpretive optimisation of the experimental conditions considering both mobile phase composition and flow rate can be carried out using models that do not include the flow rate as variable (only the change in retention of the marker with the flow rate is considered).

2 Experimental

2.1 Reagents and column

Eight basic drugs were used as probe compounds (logarithm of the apparent octanol-water partition coefficient at pH 3 and pK_a value is given in parenthesis) [9]: carteolol (−2.82/9.7, Miquel-Otsuka, Barcelona, Spain), nadolol (−2.53/9.4, Squibb, Esplugues de Llobregat, Barcelona), metoprolol (−2.08/9.7), and oxprenolol (−1.82/9.5, Ciba-Geigy, Barcelona), acebutolol (−2.08/9.2, Italfarmaco, Alcobendas, Madrid, Spain), alprenolol (−1.39/9.6), propranolol (−1.10/9.5, Sigma, St. Louis, MO, USA), and labetalol (−1.16/7.4, 8.7, Glaxo, Tres Cantos, Madrid). The concentration of the injected solutions was ca. 40 μg mL^{-1}.

Uracil (Acros Organics, Geel, Belgium) was chosen as dead time marker due to its small retention and negligible change in retention time with mobile phase composition [10]. Therefore, the hydrophobic interaction of this marker with a C18 stationary phase is rather small. Also, it is not affected by ionic exclusion effects. The dead time with the Chromolith column was 1.58 ± 0.02 min at 1 mL min^{-1}.

The mobile phases were prepared with acetonitrile (Scharlab, Barcelona). The pH was buffered at 3.0 (measured in the aqueous-organic mixture) with 0.01 M disodium hydrogenphosphate and addition of HCl (Panreac, Barcelona). Nanopure water (Barnstead, Sybron, Boston, MA, USA) was used throughout. Mobile phases and drug solutions were

filtered through 0.45 μm Nylon membranes (Osmonics, Herental, Belgium), with a diameter of 47 mm (Magna) and 17 mm (Cameo), respectively.

A Chromolith Performance RP-18e analytical column (100 mm × 4.6 mm I.D., Merck, Darmstadt, Germany), preceded by a Chromolith guard column RP-18e (5 mm × 4.6 mm I.D.), was used. Solute injection was carried out between the pre-column and column. Some relevant physico-chemical properties of the column are: mesopore size (130 Å), surface area (300 m^2/g), total carbon (19.5 wt %), surface coverage (3.6 $\mu mol/m^2$), and total porosity (> 0.80) [5]. According to the manufacturers, Chromoliths can be operated at flow rates up to 9–10 mL min^{-1} with acetonitrile-water mixtures. However, the pre-column or additional connections increase the operating pressure and forces to work below 6 mL min^{-1}.

2.2 Apparatus

An Agilent (Waldbronn, Germany) high pressure system was used, which contained an isocratic (Series 1200) pump. The system was equipped with an autosampler and a static air oven set at 25°C. The signal was monitored with a UV-visible detector (Series 1100) set at 225 nm, working with a sampling period of 400 ms. Triplicate injections were made with an injection volume of 20 μL. Data acquisition was carried out with an HPChemStation (Agilent), and mathematical treatment performed in MATLAB 2010a (The Mathworks, Natick, MA, USA).

The system pressure (measured with an accuracy of ±0.3 bar) ranged between 15 and 160 bar, in the working flow rate range 0.5–5 mL min^{-1} (Figure 1a). The flow rate at the column outlet was checked to be at the nominal specifications (with deviations below 1%), by measuring the volume of mobile phase with a 10-mL flask and the time to fill it to the nearest second. In order to prevent solvent evaporation in the stock mobile phases, the reservoir was carefully sealed with Parafilm (Pechiney Plastic Packaging Company, Chicago, IL, USA).

3 Results and discussion

The discussion below is based on the retention times of eight basic drugs eluted with acetonitrile-water mixtures at pH 3, using different flow rates, but is valid for other compounds retained on the Chromolith column. The elution order was usually the following: carteolol, nadolol, metoprolol, acebutolol, oxprenolol, labetalol, propranolol, and alprenolol. At each mobile phase composition, the retention times were measured at five flow rates: 1, 2, 3, 4 and 5 mL min^{-1} for 10, 15 and 20% (v/v) acetonitrile, and 0.5, 1, 1.5, 2 and 2.5 mL min^{-1} for 25% acetonitrile (due to the smaller retention times). Additional measurements were made for the least retained compounds at 0.1, 0.2, 0.3, and 0.4 mL min^{-1} for 20 and 25% acetonitrile. It should be noted that a flow-rate of 6 mL min^{-1} yielded the maximal allowed backpressure recommended by the pump manufacturer, and not always could be reached.

3.1 Deviations in retention times

Figures 2a and d depict the observed behaviour with 10 and 20% acetonitrile (similar plots were obtained with 15 and 25% acetonitrile. As reported in previous work [7], plots of retention time versus the inverse of the delivered flow rate (F_d) exhibited excellent linearity, with regression coefficients $r > 0.9999$. The linearity extended at least up to 0.1 mL min^{-1} ($F_d^{-1} = 10$), as checked with nadolol, acebutolol and oxprenolol eluted with the strongest mobile phases (20 and 25% acetonitrile). The slopes of the lines followed the ideal behaviour (i.e. in the absence of pressure effects), however, they did not cross the origin. This made the retention times not to halve as the flow rate was doubled. The relative deviations in the retention times at 10% acetonitrile are given in Table 1. As observed, these amounted up to 10%.

The straight-lines were first described by an empirical equation [7]:

$$t_R = \frac{c_1}{F_d} + c_0 \quad (1)$$

t_R being the retention time. The meaning of the parameters c_0 and c_1 in Eq. (1) can be understood by considering that the retention volume increases with the flow rate (i.e. the pressure) according to [7]:

$$V_R = V_{R0} + \frac{dV_R}{dF_d} F_d \quad (2)$$

where V_{R0} is the retention volume in the absence of pressure effects, and dV_R/dF_d is the rate of change in retention volume at increasing flow rate. Consequently:

$$t_R = \frac{V_R}{F_d} = \frac{V_{R0}}{F_d} + \frac{dV_R}{dF_d} = \frac{c_1}{F_d} + c_0 \quad (3)$$

Thus, the c_1 coefficient is the retention volume for the ideal behaviour, and c_0 the rate of change in the retention volume at increasing flow rate.

3.2 Deviations in retention factors

In HPLC, the retention is often expressed as the retention factor, which is a normalised value:

$$k = \frac{t_R - t_0}{t_0} \quad (4)$$

where t_0 is the dead time. Several reasons have been given for the extended use of the retention factors in the comparison and prediction of the retention behaviour, and in the quantification of physico-chemical phenomena in terms of linear free-energy relationships:

(i) The correlations based on the retention times are difficult to compare and interpret owing to their strong dependence on the experimental conditions and system geometry. However, 20–30 years ago, some workers chose the retention times or volumes directly as a measure of solute polarity [11–15].

(ii) The retention factor has a clear physico-chemical meaning. The dead time corresponds to a solute spending all its time in the mobile phase. Therefore, the retention factor is the ratio of the time a solute spends in the stationary phase to the time it spends in the mobile phase (provided the marker is not excluded from the pores, or at least excluded similarly to the retained solute) [16,17]. Consequently, the retention factor is directly related to the thermodynamic equilibrium constant.

(iii) It is generally accepted that the normalisation in Eq. (4) corrects the effect of flow rate.

There is a certain concern about the proper estimation of the dead time [10,18], which affects the calculation of the retention factor, but the independence from the flow rate is generally accepted. However, the deviations in the retention times with flow rate for the Chromolith column make the corresponding retention factors depend on the flow rate: the plots of k versus the flow rate are sloped, at least for the most retained compounds (Figures 2b and e). The deviations for the least retained compounds are more apparent when log k is plotted instead of k versus the flow rate (Figures 2c and f). The relative changes in the retention factors for each probe compound and flow rate with respect to the values obtained at 1 mL min^{-1} are given in Table 1.

3.3 Apparent flow rates and retention times in the ideal behaviour

The deviations in retention times for Chromoliths are mainly produced by the expansion of the column, which decreases the linear velocity of the mobile phase inside the column. The longer retention times can be explained by an apparent flow rate (F_{app}), which is smaller than the delivered flow rate. It can be estimated as follows [7]:

$$\frac{1}{F_{app}} = \frac{1}{F_d} + \frac{1}{\Delta F_c} \qquad (5)$$

where ΔF_c is the deviation in the flow rate (Figure 3). Note that the experimental errors in the delivered flow rates and retention times can yield small deviations from a perfect crossing of the lines with the F_d^{-1} axis. The apparent flow rate is obtained from:

$$F_{app} = \frac{\Delta F_c F_d}{\Delta F_c + F_d} \quad (6)$$

Figure 1b shows the apparent change in flow rate (ΔF_c) versus the delivered flow rate for 10% acetonitrile. It should be noted that for a given mobile phase composition, ΔF_c can be obtained from only a few data (e.g. the retention times of two or three non-excessively retained solutes eluted at two or three flow rates).

The c_0 coefficient in Eq. (1) can be used to infer the ideal behaviour (i.e. in the absence of pressure effects) at any flow rate. This coefficient is particular for each solute and mobile phase composition. However, it can be estimated from ΔF_c and the retention times at 1 mL min^{-1} (which matches c_1, see Eq. (1)), since $\Delta F_c = c_1/c_0$. On the other hand, the estimation of the c_0 coefficients for a group of solutes chromatographed in a range of mobile phase compositions does not require the measurement of the retention times at each particular mobile phase, since the following linear relationship holds:

$$\log c_0 = A + B\,\varphi \quad (7)$$

where φ is the volume fraction (v/v) of organic solvent in the aqueous-organic mobile phase.

Figure 4 depicts the standard deviation of the mean retention factors in the range 1–5 mL min^{-1} for the probe compounds before and after applying the correction proposed in this work. The ideal retention factor, k_i^c, was calculated as follows:

$$k_i^c = \frac{t_{R,i}^c - t_0^c}{t_0^c} = \frac{(t_{R,i} - c_{0,i}) - (t_0 - c_{0,0})}{(t_0 - c_{0,0})} \quad (8)$$

where t_0 and t_0^c are the experimental and corrected dead time at each flow rate, and $c_{0,i}$ and $c_{0,0}$ the deviations in retention time for the i^{th} retained compound and the marker, respectively.

The magnitude of the deviations can be appraised in Figure 5, which shows simulated "experimental" and ideal (corrected) chromatograms with 15% acetonitrile at 5 mL min^{-1}. For the simulations, the peak width and asymmetry for the actual peaks were used. The deviations are more important for the most retained compounds, and will be larger with weaker mobile phases and columns operating at higher backpressure.

3.4 Correlations of chromatographic retention versus octanol-water partition coefficient

We first observed that the most significant deviations in the retention times for the Chromolith column were obtained for the most retained compounds (i.e. alprenolol, labetalol and propranolol). Later, log c_0 in Eq. (1) was checked to correlate satisfactorily with the polarity of the basic drugs, expressed as the logarithm of the apparent octanol-water partition coefficient (log P_{app}), measured at pH 3 (the mobile phase pH). The correlation coefficients of the log c_0 versus log P_{app} plots for the eight basic drugs were r = 0.992 and 0.959 for 10% and 20% acetonitrile, respectively. These should be compared with the correlation coefficients for log c_1 with log P_{app}: r = 0.973 and 0.920 (remember that c_1 is the retention time at 1 mL min^{-1} for the ideal behaviour).

The performance of the above correlations can be appraised by comparison with the correlations for the logarithm of the retention factor (the corrected value, log k^c) at 10% and 20% acetonitrile and 1 mL min^{-1} (r = 0.973 and 0.942, respectively), or the logarithm of the extrapolated retention factor in water (k_0^c in Eq. (9)) at 1 and 5 mL min^{-1} (r = 0.980 and 0.976, respectively). As observed, the correlations for log c_0 at 10% acetonitrile and log k_0 at 1 mL min^{-1} were highly satisfactory. The parameters c_0 and k_0 gather information from

several conditions. Observe that these are extrapolated values with a substantial different basis. The c_0 values are obtained from retention times at a fixed mobile phase composition and varying flow rate, and would correspond to fictitious retention times at very high flow rate where the retention should be minimal (theoretically, at infinite flow rate). Meanwhile, k_0 values are obtained at a fixed flow rate and varying mobile phase composition, and correspond to the retention factors in water, where the retention times are maximal.

3.5 Modelling of the retention considering the flow rate

As indicated, our initial objective was developing an approach that allowed the prediction of the retention at any mobile phase composition and flow rate. If the retention factors are made independent of the flow rate (Eq. (8)), these should be predicted similarly to the case where the flow rate is fixed, using the following model:

$$\log k_i^{\mathrm{c}} = \log k_{0,i}^{\mathrm{c}} + a_i\,\varphi + b_i\,\varphi^2 \qquad (9)$$

where $\log k_{0,i}^{\mathrm{c}}$, a_i and b_i are regression coefficients with specific values for a given solute, i, and column/solvent system. The predicted retention times at any solvent content and flow rate, considering the effects of pressure, will be calculated as:

$$t_{\mathrm{R},i} = t_{\mathrm{R},i}^{\mathrm{c}} + c_{0,i} = t_0^{\mathrm{c}}\,(1 + k_i^{\mathrm{c}}) + c_{0,i} \qquad (10)$$

Owing to the observed dependence of the retention time with the flow rate, in previous work [6], we used the following model:

$$\log k_i = \log k_{0,i} + a_i\,\varphi + b_i\,\varphi^2 + c_i F_{\mathrm{d}} \qquad (11)$$

where k_i is the experimental (uncorrected) retention factor. This is a solution that was already proposed by other authors working at several flow rates with stainless steel columns [19–22]. In this case, the predicted retention times are obtained from:

$$t_{R,i} = t_0\,(1 + k_i) \qquad (12)$$

We have observed that the errors with Eq. (9), fitted with the retention times compensated by the flow effects ($t_R^c = t_R - c_0$) (Figure 6a), are systematically smaller with respect to Eq. (11) using the uncorrected (i.e. experimental) retention times (Figure 6b). These predictions should be also compared with those obtained with Eq. (9), using the uncorrected retention times (i.e. ignoring the flow effects) (Figure 6c).

4 Conclusions

Retention in Chromolith columns is pressure sensitive and is affected by the flow rate. The deviations in the retention time correlate with the solute polarity. Consequently, the dead time marker exhibits a deviation different from the retained solutes, making the retention factors depend on the flow rate. The deviations are larger at increasing retention and are the reason of the disagreement between predicted and experimental chromatograms when the effect of the flow rate is not considered. However, it should be noted that the resolution will be less compromised, since the deviations for solutes eluting closely will be similar.

The corrected retention times are useful when accurate retention data are needed. The algorithm proposed in this work allows the correction of the deviations, whatever their origin: produced by a change in the column volume as for Chromoliths, or by a change in the retention of solutes due to the perturbation in the retention equilibrium for solutes that vary their partial molar volume, or due to the frictional heating [7]. Obviously, its interest will be greater for large molecular weight compounds and when working with columns operating under extremely high pressures (such as sub 2-μm stainless steel columns at inlet pressures of c.a. 1000 bar), where the deviations are more significant. In all cases, the effects of pressure can be outlined using an apparent flow rate.

5 References

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FIGURE CAPTIONS

Figure 1. (a) Relationship between the delivered flow rate (F_d) and system pressure (P), and (b) apparent change in flow rate versus delivered flow rate for the Chromolith column and 10% acetonitrile (F_{app} is the apparent flow rate).

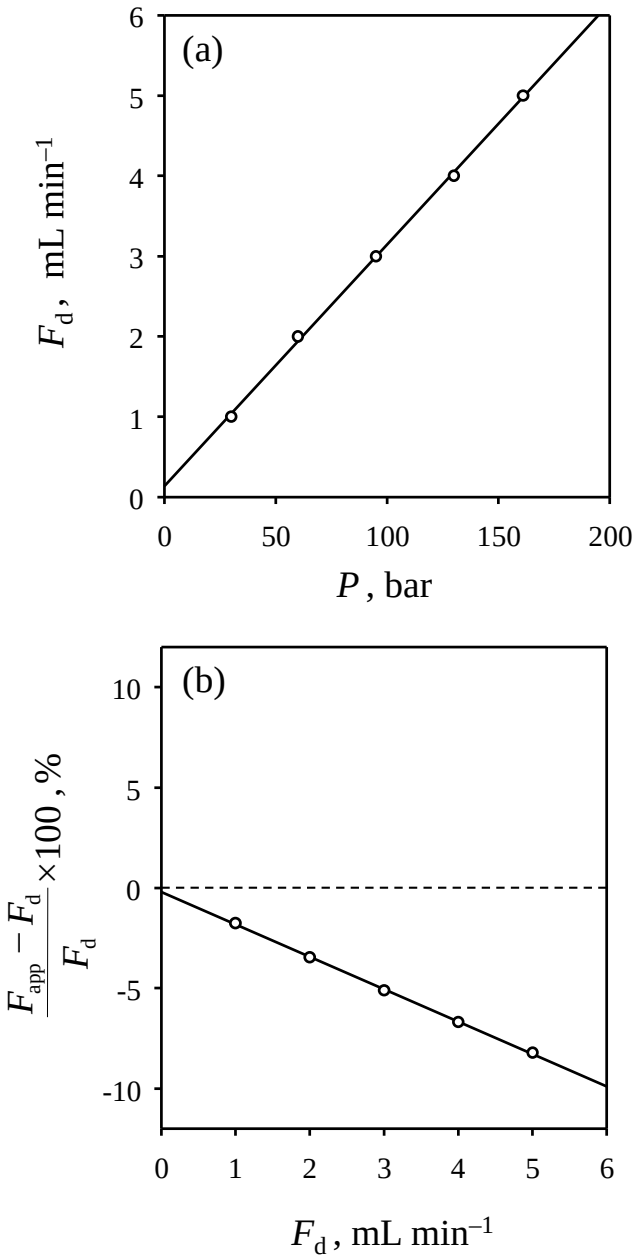
Figure 2. Dependence of the retention time (t_R) and retention factor (expressed as k or log k) on the flow rate for the set of compounds chromatographed with the Chromolith column using: (a,b,c) 10% acetonitrile, and (d,e,f) 20% acetonitrile. Compounds (from bottom to top): nadolol, metoprolol, acebutolol, oxprenolol, labetalol, and propranolol. The inner plots in (a,d) magnify the region at short retention times.

Figure 3. Estimation of the apparent flow rate (see text for symbols).

Figure 4. Variability in the retention factors in the range 1–5 mL min^{-1} (five values were averaged) for the eight probe compounds before (grey) and after (black) correcting the retention times.

Figure 5. Simulated chromatograms for a mixture eluted with 15% acetonitrile at 5 mL min^{-1}, using the Chromolith column. Compounds: (1) carteolol, (2) nadolol, (3) acebutolol, (4) metoprolol, and (5) oxprenolol. (a) Chromatogram obtained from the experimental data, and (b) chromatogram for the ideal behaviour.

Figure 6. Absolute error in the prediction of retention times (predicted minus experimental values) for the basic drugs, in the range 10–25% acetonitrile and 1–5 mL min^{-1} for: (a) Eq. (9) using the retention times corrected to the ideal behaviour, (b) Eq. (11) using the uncorrected retention times, and (c) ignoring the effects of pressure (Eq. (9) and uncorrected retention times).



Pous-Torres
Figure 1

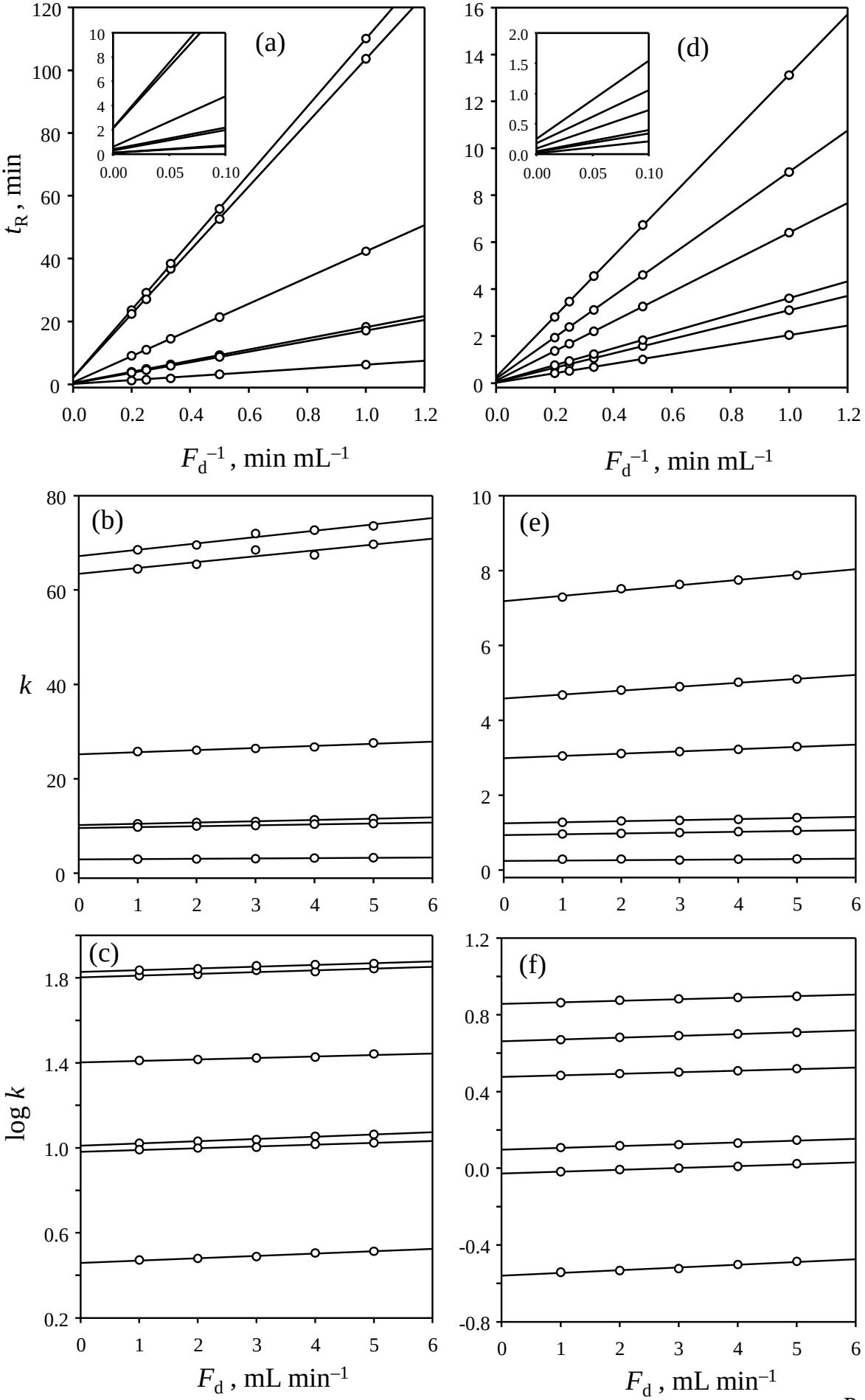


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Figure 2

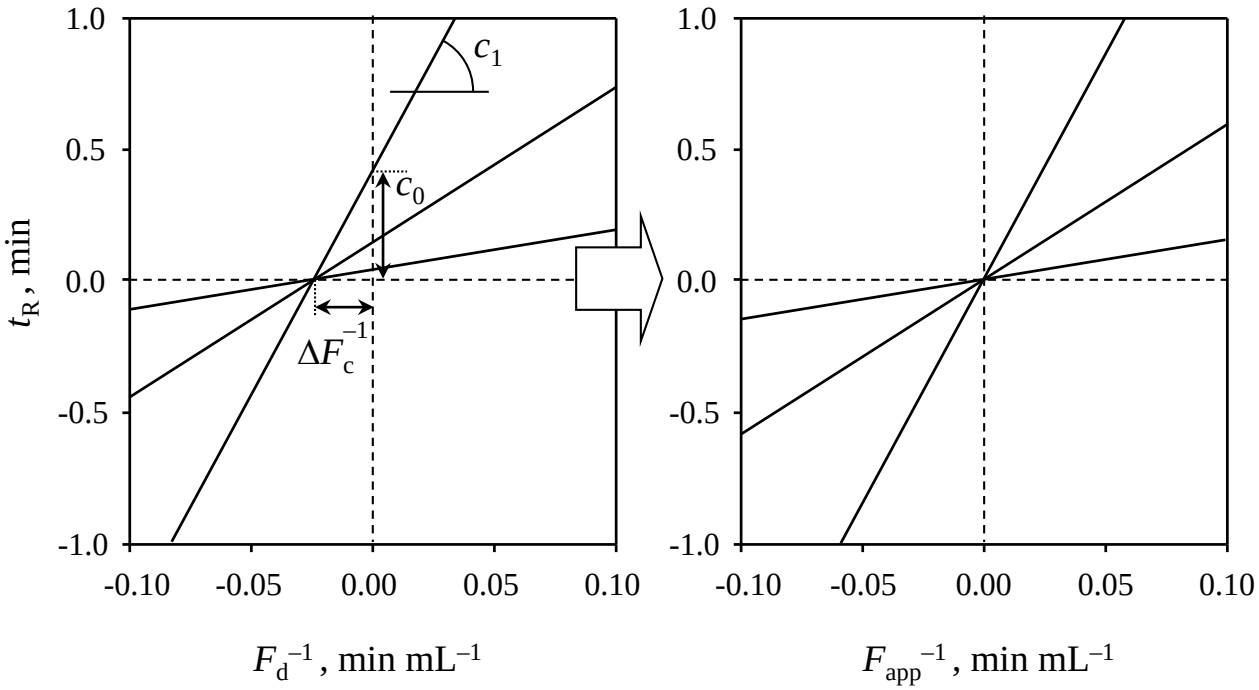

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Figure 3

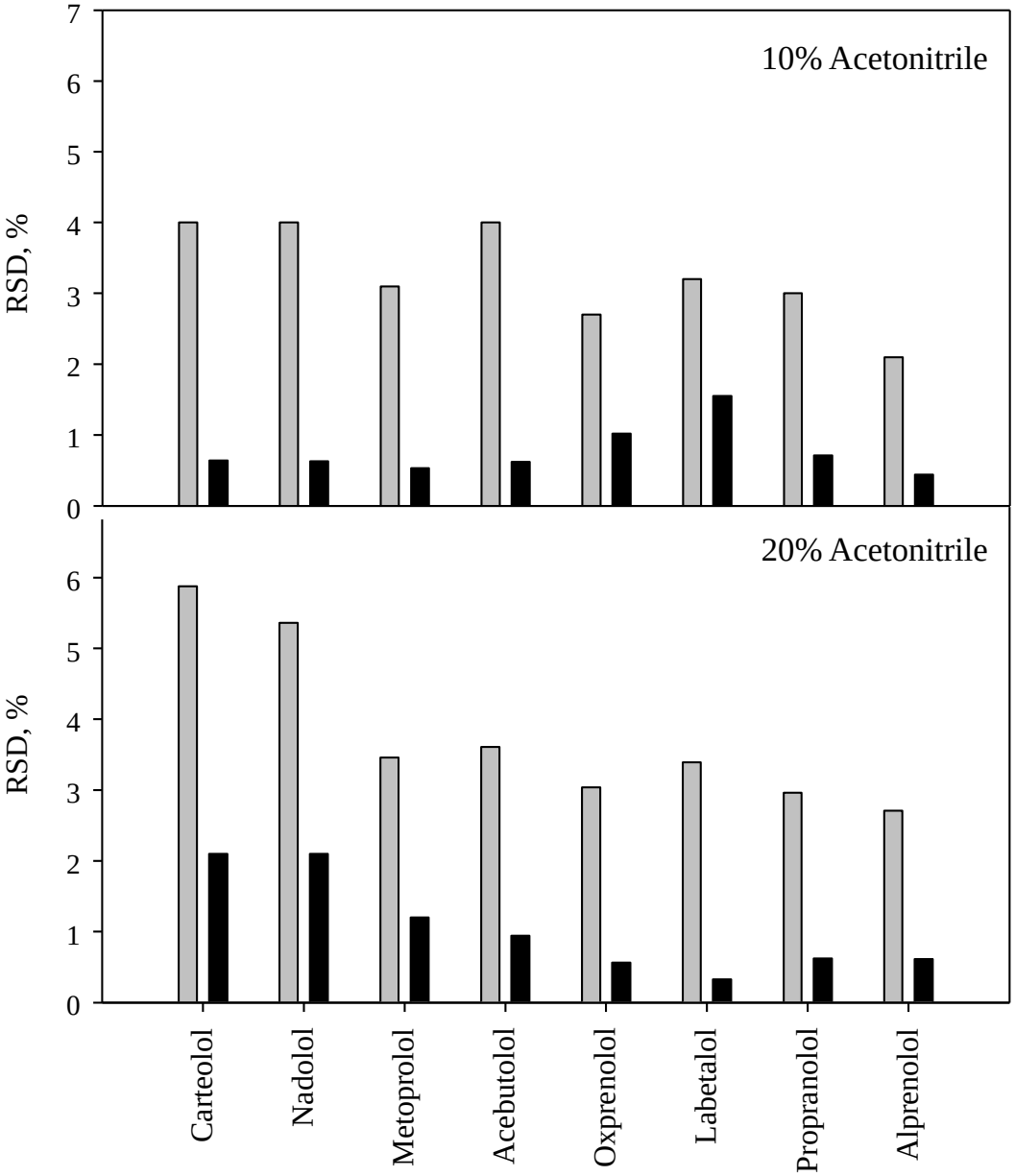

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Figure 4

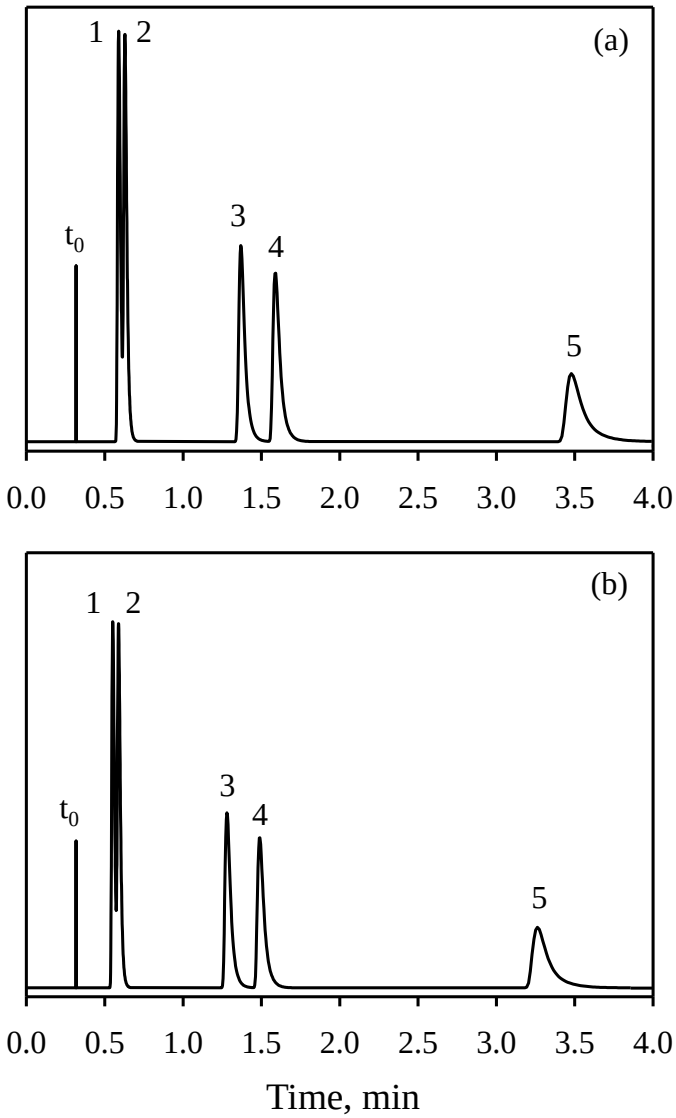

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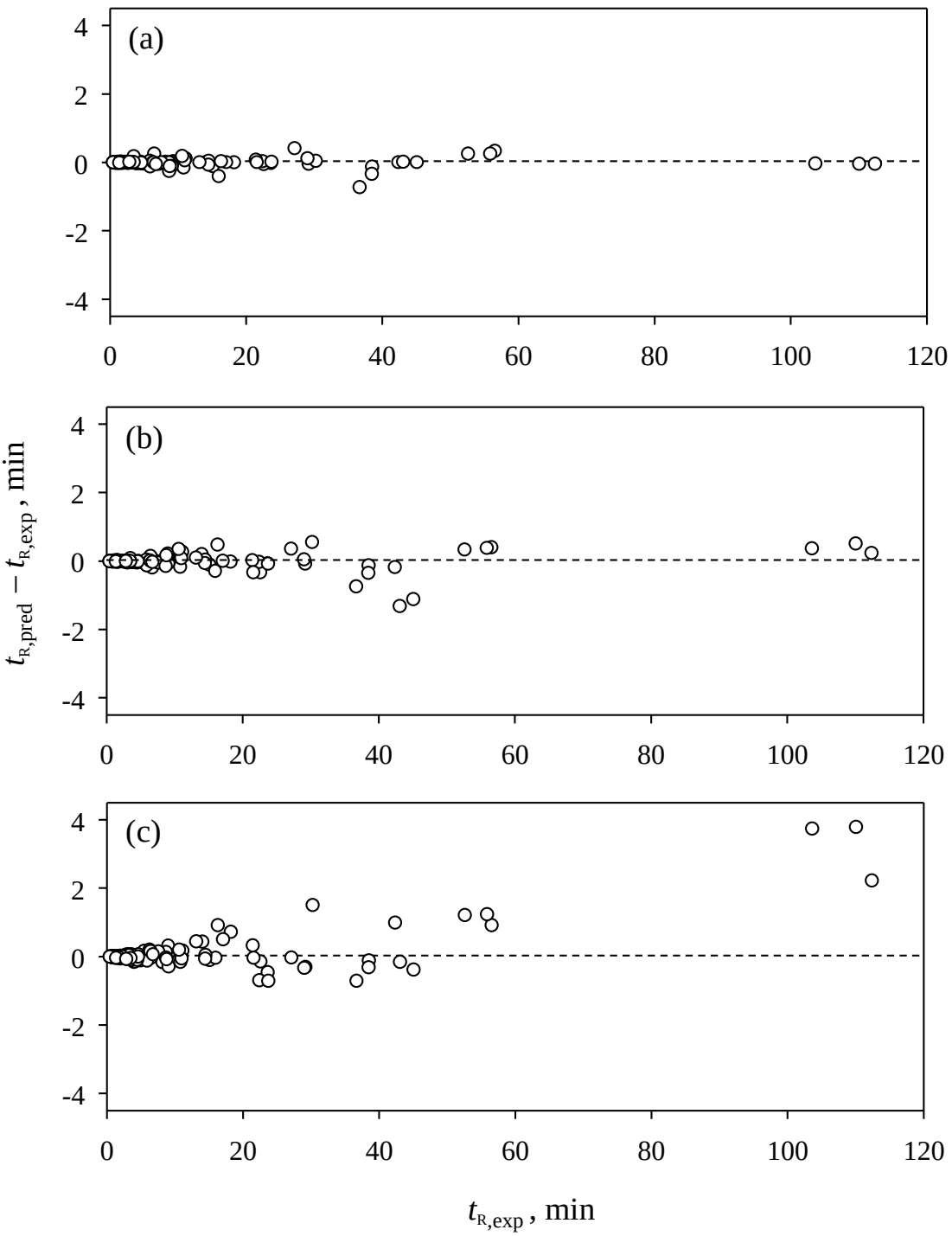
Figure 5



Pous-Torres
Figure 6

Table 1. Relative deviations (%) in the retention times and retention factors at 10% acetonitrile.

Compounds	Delivered flow rate (ml min^{-1})									
	1		2		3		4		5	
	t_R[a]	k[b]	t_R[a]	k[b]	t_R[a]	k[b]	t_R[a]	k[b]	t_R[a]	k[b]
Carteolol	1.7	–	3.5	2.0	5.3	4.1	7.0	7.9	8.7	10.1
Nadolol	1.8	–	3.7	2.0	5.5	4.0	7.3	8.1	9.2	10.0
Metoprolol	1.7	–	3.5	1.9	5.2	2.9	6.9	6.3	8.6	7.8
Acebutolol	2.2	–	4.5	2.0	6.8	4.0	8.9	7.9	11.1	10.0
Oxprenolol	1.4	–	2.8	1.1	4.3	2.6	5.7	3.7	7.0	7.1
Labetalol	2.1	–	4.2	1.6	6.1	6.3	8.5	4.6	10.5	8.1
Propranolol	2.0	–	4.0	1.5	6.0	4.9	8.0	5.5	10.0	7.8
Alprenolol	1.3	–	2.5	0.62	3.8	2.8	5.1	3.9	6.3	5.1

[a] Relative deviations were calculated as: $(c_0 / (t_R - c_0))\times100$, where t_R is the experimental retention time, and $(t_R - c_0)$ is the retention time for the “ideal” behaviour at each flow rate.

[b] Relative deviations were calculated as: $((k - k_1) / k_1)\times100$, where k is the experimental retention factor at each flow rate, and k_1 the experimental value at 1 ml min^{-1}.