

Sandra Pous-Torres
Maria-José Ruiz-Ángel
José Ramón Torres-Lapasió
Maria Celia García-Álvarez-Coque

Departament de Química
 Analítica, Universitat de
 València, Burjassot, Spain

Original Paper

Performance of a Chromolith RP-18e column for the screening of β -blockers

The chromatographic performance of a monolithic column (Chromolith RP-18e) was comprehensively examined in the isocratic separation of ten β -blockers, using ACN–water mobile phases, and compared with the performance of three microparticulate RP columns manufactured with different types of silica: Spherisorb ODS-2, Kromasil C18 and XTerra MS C18. The comparison considered the analysis time, selectivity, peak shape (column efficiency and asymmetry) and resolution, and was extended to a wide range of mobile phase compositions. The Chromolith column showed good performance for the analysis of β -blockers with regard to the packed columns. In terms of selectivity and analysis time, the greatest similarity was found between the Chromolith and XTerra columns. The addition of a silanol blocking agent (0.1% triethylamine) to both Chromolith and Spherisorb columns yielded, apparently, a similar blocking degree of the silanol groups (based on the similar peak shapes), and gave rise to similar selectivity.

Keywords: β -Blockers / Chromolith / Elution strength / Peak shape / Resolution

Received: April 27, 2009; revised: June 3, 2009; accepted: June 4, 2009

DOI 10.1002/jssc.200900291

1 Introduction

The need for obtaining high quality analysis in short times is of major importance in the pharmaceutical industry, where the number of samples to be assayed is continuously increasing [1–4]. Many pharmaceutical companies are involved with combinatorial chemistry with the purpose of searching more effective drugs. This results in thousands of new compounds, which should be analysed quickly. Also, fast separations are usually highly desirable to control a process and for quality control before releasing a batch. For this purpose, RPLC is usually employed. However, the standard separations are too time-consuming for the screening of so many samples. Thus, any development to speed up analytical methods is encouraged to drastically reduce analysis times, as this implies larger throughput. Consequently, in this decade, fast RPLC separations have constituted an area of extensive research.

The pressure limitation of commercially available chromatographic systems (usually 400 bars) clearly sets the limits for fast chromatography. But even if this is

overcome, microparticulate packed columns can be damaged by the excessive pressure associated to high flow rates [5]. Diverse approaches have been considered to face this limitation: use of microparticulate short columns at high flow rate [6–8], special packings with reduced retention [9], high temperature [10], reduced particle size at ultra-high pressure [11] and silica-based monolithic columns [12, 13]. With shorter microparticulate columns, efficiency and resolution are considerably compromised, and high temperatures or ultra-high pressures require special chromatographic equipment. In contrast, monolithic columns can be used with conventional equipment, and run at high flow rate with lower backpressure, extended column life-time and smaller loss in efficiency at increasing flow rate [14–16].

A silica-based monolithic column consists of a rod prepared inside a mould, by polymerisation of highly purified metal-free tetramethoxysilane in the presence of PEG as porogen [17]. After ageing and treatment at high temperature, the rod is removed from the mould, encased within a PEEK tube [18] and provided with the bonded chemistry for RPLC separations, followed by end-capping [19].

The highly porous monolithic rods of silica provide a unique combination of macropores ($\sim 2.2 \mu\text{m}$) and mesopores ($\sim 13 \text{ nm}$) [20]. The macropores allow rapid flow of the mobile phase at low backpressure, while the mesopores create a large uniform surface on which adsorp-

Correspondence: Professor Maria Celia García-Álvarez-Coque, Departament de Química Analítica, Universitat de València, c/ Dr. Moliner 50, 46100 Burjassot, Spain
E-mail: celia.garcia@uv.es
Fax: +34-96-3544436

Abbreviation: TEA, triethylamine

Table 1. Reports where Chromoliths are compared with microparticulate columns

Chromolith	Other columns	Compounds	Details	Reference
Prototypes of silica rod columns from Merck (100 × 4.6 mm)	Purospher RP-18e (5 µm, 125 × 4 mm, Merck)	Uracil, toluene, ethylbenzene, <i>n</i> -propylbenzene, <i>n</i> -butylbenzene and <i>n</i> -pentylbenzene	Comparison of plate height (<i>H</i>), permeability and column back pressure versus flow rate plots. Mobile phase: 73:27 ACN–water. Flow rate: 0.2–6 mL/min for the monolithic columns, 0.5–3 mL/min for Purospher	[23]
SpeedROD (50 × 4.6 mm)	Superspher 100 RP18 (125 × 3 mm, Merck)	Ochratoxin A	Comparison of elution orders and selectivities. Mobile phase: 68.5:29:2.5 methanol–water–acetic acid	[24]
Silica rod columns (100 × 4.6 and 50 × 4.6 mm)	LiChroCART Purospher RP-18e (5 µm, 125 × 4 mm, Merck), LiChroCART Purospher STAR 18e (3 µm, 55 × 4 mm, Merck)	Butylbenzene, o-terphenyl, amylbenzene and triphenylene	Comparison of elution orders and selectivities. Mobile phase: 80:20 methanol–water	[18]
Performance RP-18e (100 × 4.6 mm)	LiChrospher 100 RP-18e (5 µm, 125 × 4.6 mm, Merck), RP-Amide C16 (150 × 2.1 mm, Supelco)	Microcystins and nodularin-R	Comparison of retention times, resolutions and selectivities. Mobile phase: TFA–ACN gradients. Flow rate: 2–4 mL/min for the monolithic column, 1.0–1.5 mL/min for LiChrospher and 0.3 mL/min for RP-Amide	[25]
Performance RP-18e	Hypersyl (250 × 4.6 mm ²), Kromasil (250 × 4.6 mm), Phenomenex Luna (150 × 4.6 mm), Symmetry (150 × 3.9 mm, Waters), Vydac (250 × 4.6 mm)	Phenol and caffeine	Measurement of adsorption isotherms of solutes. Mobile phase: 30:70 methanol–water. Flow rate: 1 mL/min	[26]
Performance RP-18e, RP-8e and RP-4e	LiChrospher RP-18e (Merck), Zorbax SB300-C18 (Agilent), PurospherSTAR RP-18e (3 µm, 55 × 4.0 mm) (Merck)	Caffeine, acetone, angiotensin II, insulin and BSA	Comparison of pressure drops, efficiencies and adsorption capacities. Mobile phase: 50:50 ACN–water. Flow rate: 0.1–2.5 mL/min	[27]
SpeedROD RP-18e	Symmetry (3.5 µm, 50 × 4.6 mm, Waters), Phenomenex Luna (5 µm, 50 × 4.6 mm), XTerra (3 µm, 50 × 3.0 mm, Waters), Optimise velocity (3 µm, 50 × 4.6 mm), Varian Chrompack (3 µm, 50 × 4.6 mm)	Benzamide, N-methylbenzamide, biphenyl, acetophenone, benzyl alcohol, ethylparaben and propylparaben	Comparison of selectivities, re-equilibration times and precisions. Mobile phase: ACN–water gradients. Flow rate: 1–9 mL/min for SpeedROD	[28]
SpeedROD RP-18e and Performance RP-18e	LiChrospher 100 RP-18e (250 × 4.6 mm, Merck), Alltima C8, LiChroCART Purospher RP-18e (125 × 4 mm, Merck)	Nimesulide, tetracycline, erythromycin and phenoxymethylpenicillin	Transfer to monolithic columns of separation methods. Mobile phase: ACN–water and methanol–water mixtures. Flow rate: 1–9 mL/min for the monolithic columns	[29]
Performance RP-18e	17 conventional microparticulate columns	58 neutral compounds	Observation of similar retention properties using the solvation parameter model	[30]
Performance RP-18e and SpeedROD RP-18e	YMC C18 (5 µm, 250 × 4.6 mm and 3 µm, 150 × 4.6 mm, Waters), Zorbax C18 (3.5 µm, 250 × 4.6 mm, Agilent)	Phenol, alkylbenzenes ($C_nH_{2n} + 1C_6H_5$, $n = 0–7$) and <i>N,N</i> -dimethylaniline	Comparison of pressure drops, retentions, selectivities and asymmetries. Mobile phase: ACN–water mixtures. Flow rate: 1.5 mL/min	[2]
Performance RP-18e, SpeedROD RP-18e and Flash RP-18e (25 × 4.6 mm)	Discovery (5 µm, 250 × 3 mm, Supelco)	Methyl-, ethyl- and propylparaben, ketoprofen, estradiol, hydrocortisone and estrone	Comparison of resolutions, retention times, efficiencies and peak widths. Mobile phase: ACN–water mixtures. Flow rate: 1.0–5.0 mL/min	[31]
Performance RP-18e	YMC ODS (150 × 3 mm), LiChrospher 100 RP-18 (5 µm, 250 × 4 mm), and butylparaben Symmetry RP-18 (3.5 µm, 150 × 3 mm)	Methyl-, ethyl-, propylparaben	Comparison of resolutions, retentions, efficiencies and peak widths. Mobile phase: ACN–water gradients. Flow rate: 1–3 mL/min for the monolithic column, 1 mL/min for the microparticulate columns	[5]

Table 1. Reports where Chromoliths are compared with microparticulate columns

Chromolith	Other columns	Compounds	Details	Reference
Performance RP-18e	Luna ODS (5 µm, 250 × 4.6 mm, Phenomenex), Purospher RP-18 (5 µm, 250 × 4.6 mm, Merck), Synergi RP-MAX C12 (4 µm, 250 × 4.6 mm, Merck)	11 phenols	Comparison of van Deemter plots and asymmetries. Mobile phases: ACN–water mixtures. Flow rate: 0.5–1 mL/min for microparticulate columns and 1–4 mL/min for the monolithic column	[32]
Performance RP-18e	Pecosphere C18 (5 µm, 150 × 4.6 mm, Perkin Elmer)	Vitamins A and E	Comparison of retentions and backpressures. Mobile phase: 100% methanol. Flow rate: 2.5 mL/min for the monolithic column and 1.5 mL/min for the microparticulate column	[33]

Table 2. Physico-chemical properties of the four columns used in this work

	Chromolith RP-18e [21]	Spherisorb ODS-2 ^{a)}	Kromasil C18 [36]	XTerra MS C18 [37]
Particle size (µm)	–	5	5	5
Mesopore size (Å)	130	80	100	120
Macropore size (µm)	2	–	–	–
Surface area (m ² /g)	300	220	340	175
Total carbon (wt.%)	19.5	12	20	15
Surface coverage (µmol/m ²)	3.6	2.8	3.6	2.4
Endcapping	Yes	Yes	Yes	Yes
Dimensions (mm × mm)	100 × 4.6	125 × 4.6	150 × 4.6	150 × 4.6
Total porosity	>0.80	–	0.59	0.62
pH stability range	2.0–7.5	3.0–7.5	2.0–9.0	1.0–12.0

^{a)} www.analyticalcolumns.com.

tion takes place, thereby enabling RPLC separation. Owing to their characteristics, silica-based monolithic columns are a field of continuous research in RPLC.

However, so far, the practical use of silica-based monolithic columns in RPLC has not been as wide as expected, in spite of all their advantages. A complete understanding of their performance requires the systematic acquisition of many series of reliable experimental data in a wide range of chromatographic conditions, and a consistent analysis and interpretation of these data from different points of view [21, 22].

With the aim of characterising silica-based monolithic columns, numerous authors have compared them with microparticulate packed columns. Most authors reached the conclusion that the columns are comparable with regard to their selectivity and reproducibility [23–33], but the monolithic columns seem to be more stable than the packed ones, due to the rigid silica structure [20]. Some results from comparison studies are outlined in Table 1. In these studies, the selectivity ($\alpha = k_1/k_2$) of a few selected peak pairs is often compared. However, the comparisons are not comprehensive, as they are usually made at a unique mobile phase composition (at most, at a few mobile phase compositions), finding diverse agreement for different peak pairs. Thus, high similarity

between the Chromolith SpeedROD RP-18e and an XTerra column was observed for a test mixture of seven components showing a wide polarity range [28], and between the Chromolith Performance RP-18e and LiChrospher 100 RP-18 columns for a group of 17 aromatic compounds [30].

In this work, the Chromolith Performance RP-18e column is compared, in terms of elution strength, selectivity, peak shape and resolution capability, with three microparticulate columns (Spherisorb ODS-2, Kromasil C18 and XTerra MS C18), in the separation of ten β -blockers with ACN–water mobile phases in a wide composition range. β -Blockers are basic drugs ($pK_a \geq 8–9$) of pharmacological interest that allow probing the residual silanols on each column [34, 35].

Table 2 shows the physico-chemical properties of the four columns. Spherisorb ODS-2 is, perhaps, the most widely referenced RPLC column in the scientific literature, providing a tremendous range of validated methods and applications. Kromasil columns are a further development, very popular for both pharmaceutical and bioscience industries, and extend the packing stability from pH 7.5 to 9. XTerra belongs to more recent advances in silica-based stationary phases, and incorporates a hybrid particle that is a combination of silica and

organic (methylsilane) components that facilitate stability up to pH 12 and appears to have a low concentration of silanol groups.

Spherisorbs contain type-A silica (according to the classification proposed by Köhler and Kirkland [38]). To the best of our knowledge, Kromasils, Chromoliths and XTerra are made of highly purified type-B silica [39], which appeared over the last 20 years as the result of improved column manufacturing processes. Type-B silica allows an increase in the average surface coverage (3.2 ± 0.7 vs. $2.5 \pm 0.7 \mu\text{mol}/\text{m}^2$ for type-A C18 columns) [40]. XTerra columns are atypical type-B phases with lower bonded phase coverage ($2.4 \mu\text{mol}/\text{m}^2$). Type-A columns are more acidic than type-B, owing to the presence of isolated silanols in the silica matrix and contaminating metals (e.g. Fe and Al), which yields poorer peak shape for basic drugs.

2 Experimental

2.1 Reagents

The chromatographic behaviour of the following β -blockers was examined: acebutolol (Italfarmaco, Alcobendas, Madrid, Spain), atenolol, pindolol, propranolol, timolol (Sigma, St. Louis, MO, USA), carteolol (Miquel-Otsuka, Barcelona, Spain), esmolol (Du Pont-De Nemours, Le Grand Saconnex, Switzerland), labetalol (Glaxo, Tres Cantos, Madrid), metoprolol and oxprenolol (Ciba-Geigy, Barcelona).

The mobile phases were prepared with ACN (Scharlab, Barcelona). The pH was buffered at 3 with 0.01 M disodium hydrogenphosphate and addition of HCl (Panreac, Barcelona). Occasionally, 0.1% triethylamine (TEA, Fluka, Buchs, Switzerland) was added to the ACN–water mixtures. Nanopure water (Barnstead, Sybron, Boston, MA, USA) was used throughout. The drug solutions and mobile phases were filtered through 0.45 μm nylon membranes (Osmonics, Herental, Belgium) with a diameter of 47 mm (Magna) and 17 mm (Cameo), respectively.

2.2 Apparatus and columns

The HPLC system was equipped with an isocratic pump (Agilent 1200, Waldbronn, Germany), an autosampler and a UV-visible detector (Series 1100) set at 225 nm, except for timolol which was monitored at 300 nm. The sampling period was 400 ms. Triplicate injections were made with an injection volume of 20 μL and a flow rate of 1 mL/min. Data acquisition was carried out with an HPChemStation (Agilent), and the mathematical treatment was performed in MATLAB 6.5 (The Mathworks, Natick, MA, USA).

Four columns of different nature were used (Table 2): Chromolith Performance RP-18e (Merck), Spherisorb ODS-2 (Scharlab), Kromasil C18 (Análisis Vínicos, Ciudad

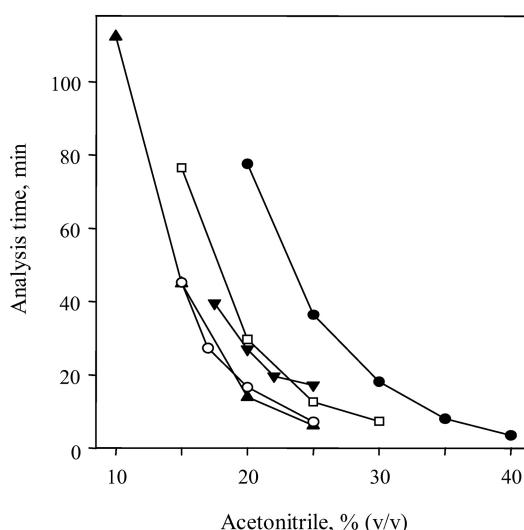


Figure 1. Analysis time *versus* mobile phase composition for the different columns: Chromolith (▲), XTerra (○), Spherisorb in the presence of TEA (▼), Kromasil (□) and Spherisorb (●).

Real, Spain) and XTerra MS C18 (Waters, MA, USA). The dead time values (t_0), measured with uracil (Acros Organics, Geel, Belgium) as marker were: Chromolith ($t_0 = 1.58$ min), Spherisorb (1.27 min), Kromasil (1.57 min) and Xterra (1.65 min).

3 Results and discussion

Monolithic columns allow the application of higher flow rates within the operational pressure limits of conventional equipment, which implies a decrease in the analysis time. The selectivity is not changed by an increase in flow rate, but the resolution of close peaks can be compromised due to the relative increase in peak width at increasing flow rate [22]. For this reason, the studies shown below were carried out at 1 mL/min.

3.1 Retention behaviour

The β -blockers were eluted with mobile phases of ACN–water at several mobile phase compositions: 10, 15, 20 and 25% ACN v/v for the Chromolith column; 20, 25, 30, 35 and 40% ACN for the Spherisorb; 15, 20, 25 and 30% ACN for the Kromasil and 15, 17, 20 and 25% ACN for the XTerra. The solvent content ranges assayed with the different columns coincided only partially, since they were selected to achieve enough retention for the β -blockers of higher polarity, and not excessively retention for the most apolar ones, in each column. Thus, the longer retention times (*i.e.* smaller elution strength) achieved for the Spherisorb column, at the same mobile phase composition, forced the use of larger ACN contents. This behaviour is illustrated in Fig. 1, where the

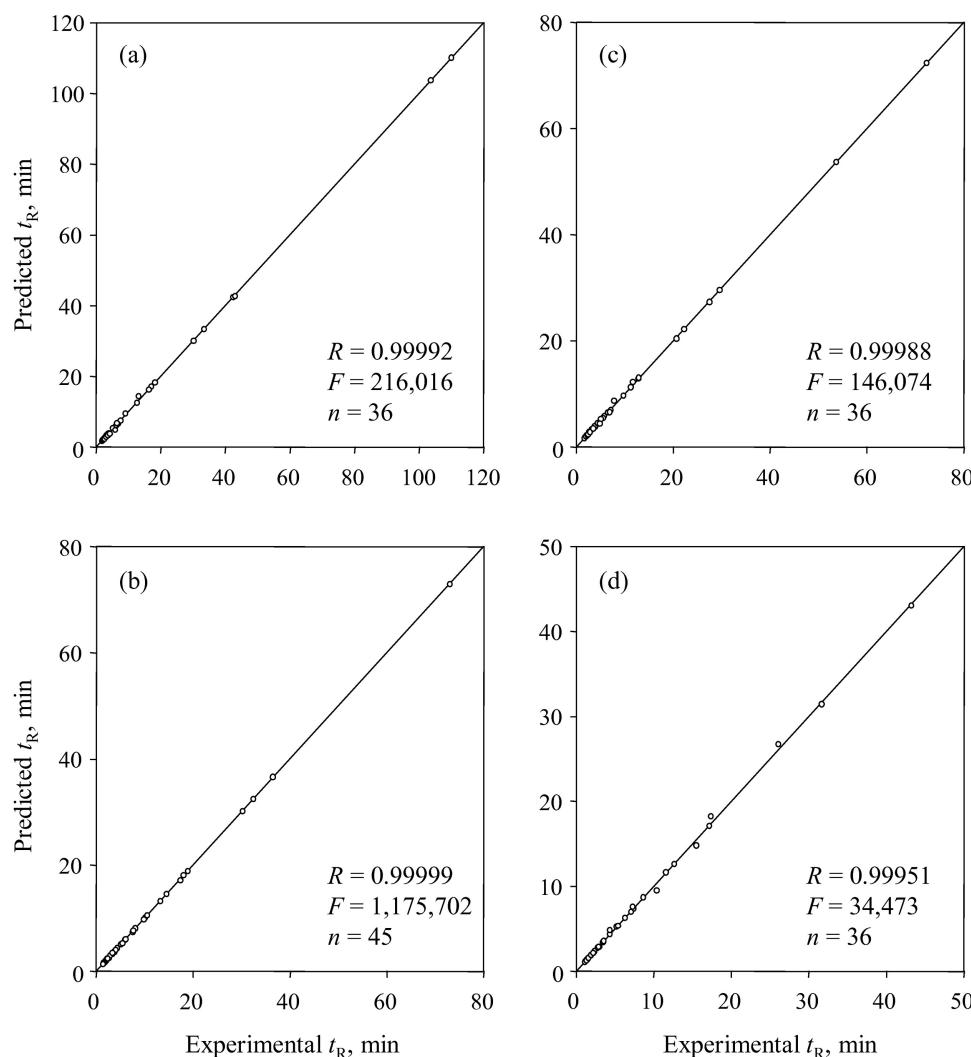


Figure 2. Accuracy in the prediction of the retention using Eq. (1) for the ten β -blockers: (a) Chromolith, (b) Spherisorb, (c) Kromasil and (d) XTerra.

analysis time (*i.e.* the retention time of the most retained compound) is correlated to the solvent content.

Several authors have reported shorter retention times with Chromoliths, compared to other columns with the same surface area and similar dimensions [2, 18], which means that less organic modifier is needed to obtain similar retention factors. This lower retention has been explained by its high porosity and low density [2]. However, Fig. 1 shows that for the β -blockers, the analysis time for the Chromolith column is similar to that of the XTerra.

In order to examine comprehensively the retention capability of the four columns in the analysis of the β -blockers, the behaviour of each compound (measured as logarithm of the retention factor, $\log k$) was modelled *versus* the volume fraction of organic solvent in the aqueous–organic mobile phase, φ , using the classical quadratic relationship:

$$\log k = c_0 + c_1\varphi + c_{11}\varphi^2 \quad (1)$$

where c_0 , c_1 and c_{11} are regression coefficients with characteristic values for a given solute and column/solvent system. Figure 2 shows the satisfactory accuracy of Eq. (1) in predicting the retention of the β -blockers for the four columns. This model was used to simulate chromatograms at different mobile phase compositions and assess the selectivity and resolution performance of each column.

3.2 Selectivity and column transfer

Figures 3–5 show the chromatograms obtained at 15, 20 and 25% ACN (only 20 and 25% for the Spherisorb column, which offered the smallest elution strength) for a mixture of nine β -blockers (timolol, which overlapped at least partially with acebutolol and metoprolol was excluded). The time scale of the chromatograms was adjusted to an equivalent size to facilitate the comparison. Note that for this purpose, the maxima of the first and last peaks were matched in all chromatograms.

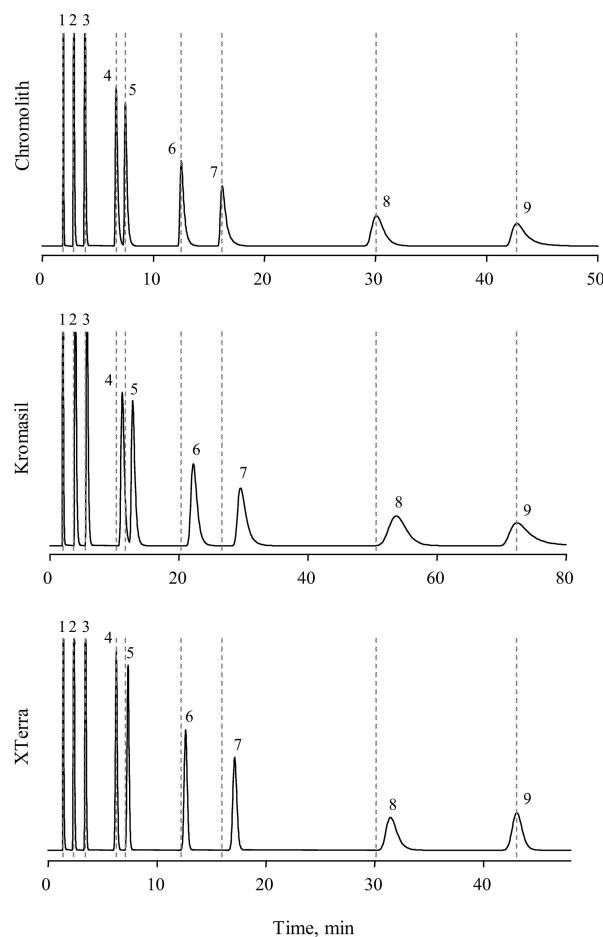


Figure 3. Chromatograms of a mixture of nine β -blockers eluted with 15% ACN using different columns. Compounds: 1, atenolol; 2, carteolol; 3, pindolol; 4, acebutolol; 5, metoprolol; 6, esmolol; 7, oxprenolol; 8, labetalol; 9, propranolol.

The elution order in the chromatograms was the same for the Chromolith, Kromasil and XTerra columns, as has been commented by other authors for different columns and compounds [5, 32]. In contrast, oxprenolol and labetalol changed their elution order with the Spherisorb column. Maximal similarity was found between the chromatograms obtained with the Chromolith and XTerra columns (Figs. 3 and 4), considering both the peak distribution (*i.e.* selectivity) and analysis time. However, at the highest solvent content (25% ACN), the peak distribution in these columns differed (Fig. 5).

The transfer from microparticulate columns to Chromoliths results in a gain in time. For some columns, this is true even at the usual flow rates (compare the behaviours of the Chromolith and Spherisorb columns in Fig. 1 at 1 mL/min), but the greater benefit with Chromoliths is achieved regarding the possibility of increasing the flow rate at high values. The interest in investigating whether separations developed on conventional C18 columns can

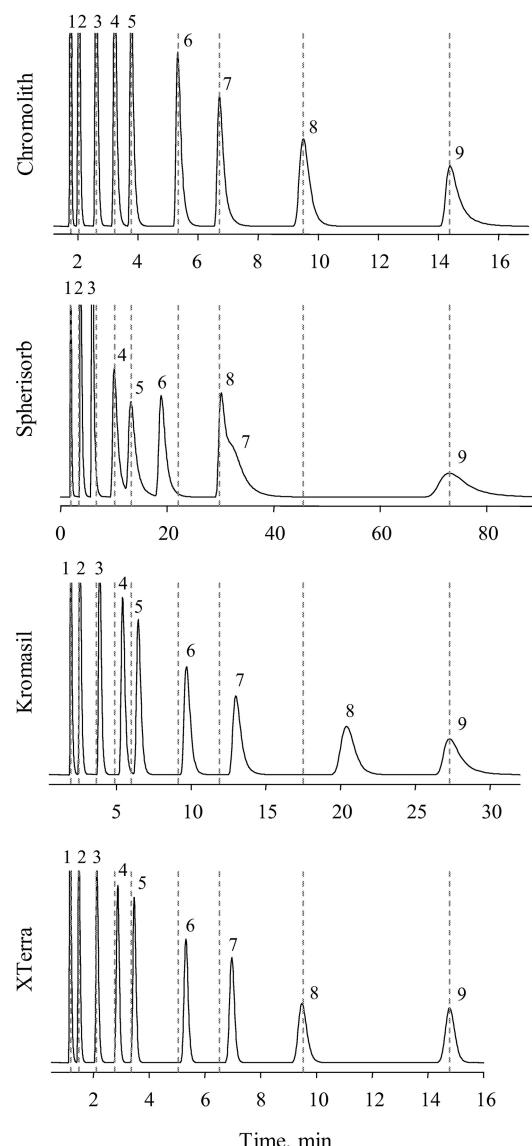


Figure 4. Chromatograms of a mixture of nine β -blockers eluted with 20% ACN using different columns. See Fig. 3 for peak identification.

be transferred to Chromoliths is, thus, not surprising [18, 20, 28–31]. However, the transfer of separations from one column to another is not always successful (even different microparticulate columns not always result in similar elution strength and selectivity). In an interesting study [29], six methods formerly developed on conventional microparticulate C8 and C18 columns for the analysis of an alkylbenzene mixture, drugs and their impurities and a green tea extract, were transferred to Chromolith Performance and Chromolith SpeedROD columns. The transferred methods succeeded only for three of the six methods.

As commented, the analysis times for the mixture of β -blockers separated with the Chromolith and XTerra col-

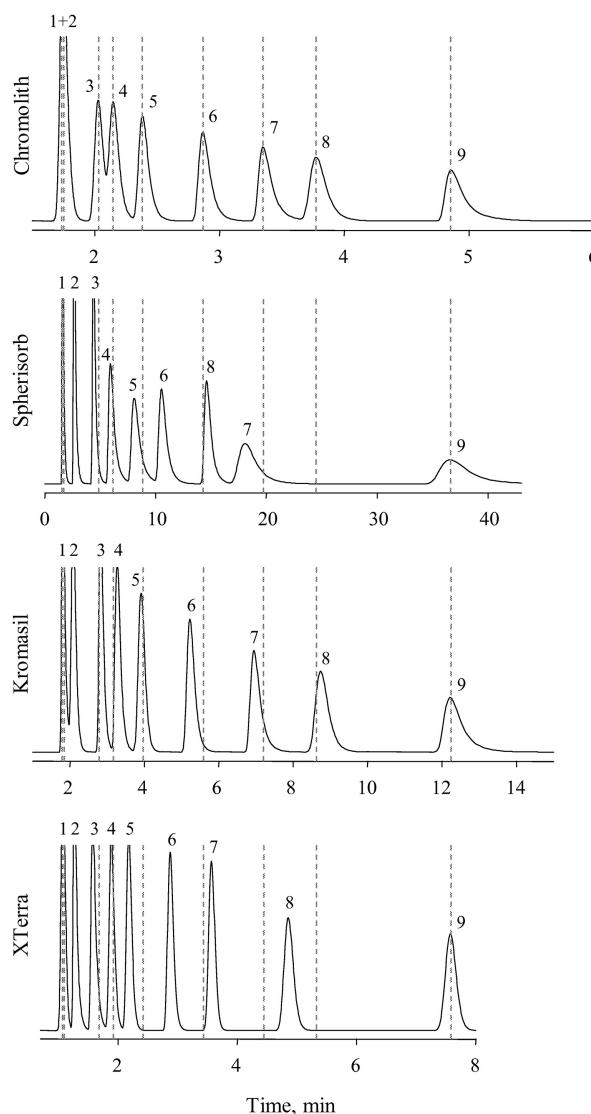


Figure 5. Chromatograms of a mixture of nine β -blockers eluted with 25% ACN using different columns. See Fig. 3 for peak identification.

umns were similar in the whole experimental domain (Fig. 1). This facilitates the direct comparison of chromatograms. In contrast, the Spherisorb column was more retentive for the assayed compounds. The analysis times for this column using 25 and 30% ACN were similar to those for the Chromolith Performance using 15 and 20% ACN (compare the corresponding chromatograms in Figs. 3 and 5). However, even considering the different elution strengths, the selectivities for the Chromolith and Spherisorb columns appear different.

In view of these results, we decided to explore the whole experimental domain in detail to assess the similarity between columns. For this purpose, the retention times of the set of β -blockers separated with the Chromo-

lith column were regressed versus the retention times for each of the three microparticulate columns, at varying mobile phase composition. The retention times for each β -blocker in each column were calculated according to Eq. (1) for 30–60 mobile phase compositions (depending on the column), evenly distributed in the solvent content range.

Figure 6 depicts contour maps showing the R^2 coefficient for the above correlations at varying mobile phase composition. This coefficient is used here as a similarity measurement of relative peak distribution. The pair Chromolith/XTerra showed the largest similarity (with $R_{\max}^2 = 0.99$), which was extended to a wide range of compositions (Fig. 6a), followed by the pair Chromolith/Kromasil (Fig. 6b) with $R_{\max}^2 = 0.98$. The similarity was appreciably smaller for Chromolith/Spherisorb (Fig. 6d) with $R_{\max}^2 = 0.95$ in a narrow region. This result can be at least partially explained by the change in elution order for oxprenolol and labetalol with the Spherisorb column. After eliminating labetalol from the mixture (Fig. 6e), the similarity increased to $R_{\max}^2 = 0.98$. On the other hand, Fig. 6c shows that the Kromasil and XTerra columns are more similar than the pair Chromolith/Kromasil ($R_{\max}^2 = 0.99$ vs. 0.98).

Therefore, an appropriate transfer of the separation of β -blockers to the Chromolith Performance RP-18e column is possible from the XTerra and Kromasil columns, especially for the former, and this is true in a wide range of mobile phase compositions. Figures 7a–c depict the correlations between the retention times of β -blockers for different pairs of columns when eluted with a mobile phase in the region of maximal similarity for each pair.

3.3 Peak shape

The efficiency and peak tailing of particular compounds are often examined to assess the performance of chromatographic columns. We measured instead the left (A) and right (B) peak half-widths at 10% height for the ten β -blockers, which were plotted versus their retention times. This correlation offered global measurements related to the peak shape capability of the columns.

Figures 8a, c, d and f depict the peak half-widths for the four columns, considering all peaks obtained for the ten β -blockers in the whole range of mobile phase compositions. As observed, there is an approximately linear relationship between the peak half-widths and the retention time:

$$A = c_{0,A} + c_{1,A} t_R \quad (2)$$

$$B = c_{0,B} + c_{1,B} t_R \quad (3)$$

The coefficients in Eqs. (2) and (3) for the four columns are shown in Table 3. The correlations between half-

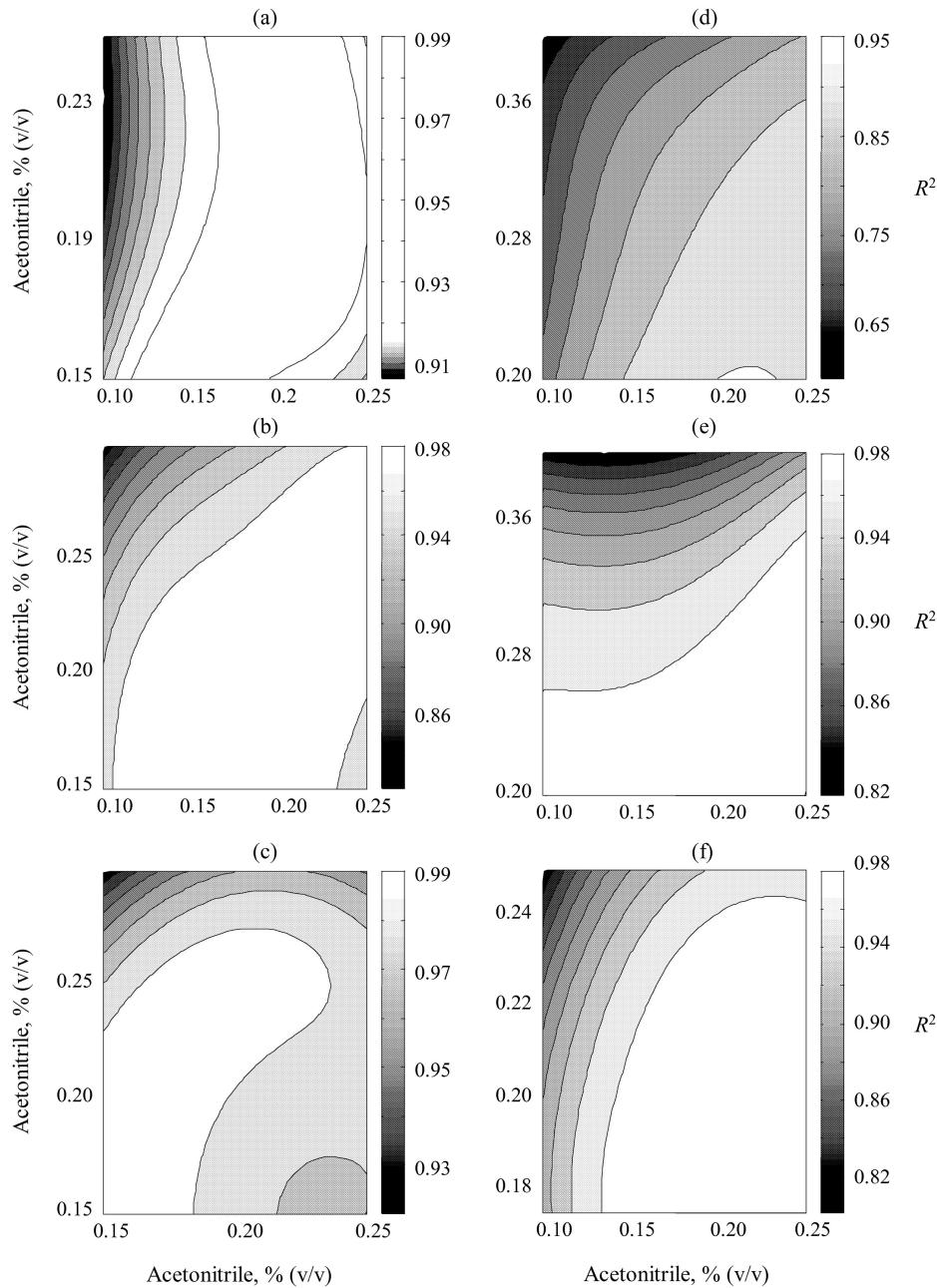


Figure 6. Similarities in selectivity between columns, expressed as the R^2 coefficients for the correlation of the retention times of nine β -blockers (those in Fig. 3) separated in a pair of columns, at varying mobile phase composition. Columns: (a) Chromolith/XTerra, (b) Chromolith/Kromasil, (c) Kromasil/XTerra, (d) Chromolith/Spherisorb, (e) Chromolith/Spherisorb (without labetalol) and (f) Chromolith/Spherisorb (both in the presence of 0.1% TEA).

widths and retention times, and between retention times and mobile phase composition through Eq. (1), are practical to predict (at least approximately) the peak shape (asymmetry, B/A , and efficiency, N [41]):

$$N = \frac{41.7 \left(\frac{t_R}{A + B} \right)}{1.25 + \frac{B}{A}} \quad (4)$$

for individual compounds eluted at a given mobile phase composition, and has been applied with success for optimisation purposes in microparticulate columns [42].

The slopes $c_{1,A}$ and $c_{1,B}$ in Eqs. (2) and (3) (Fig. 8 and Table 3) give information about the peak shape general behaviour of the columns: the sum of the slopes is related to the column efficiency, whereas their ratio ($c_{1,B}/c_{1,A}$) is a global measurement related to the peak asymmetry.

Table 3. Correlation parameters of the left (*A*) and right (*B*) half-widths with the retention time for the ten β -blockers^{a)}

Column	$c_{0,A}$	$c_{1,A}$	R_A	$c_{0,B}$	$c_{1,B}$	R_B
Chromolith RP-18e	-0.004	0.020	0.982	-0.027	0.053	0.997
Chromolith RP-18e with TEA	0.004	0.022	0.983	-0.009	0.041	0.993
Spherisorb ODS-2	-0.057	0.059	0.975	0.237	0.097	0.889
Spherisorb ODS-2 with TEA	0.023	0.023	0.983	0.056	0.042	0.944
Kromasil C18	-0.052	0.039	0.957	-0.070	0.077	0.990
XTerra MS C18	0.024	0.021	0.954	0.052	0.026	0.952

^{a)} $c_{0,A}$, $c_{1,A}$, $c_{0,B}$ and $c_{1,B}$ are the regressed coefficients in Eqs. (2) and (3), and R_A and R_B are the corresponding correlation coefficients.

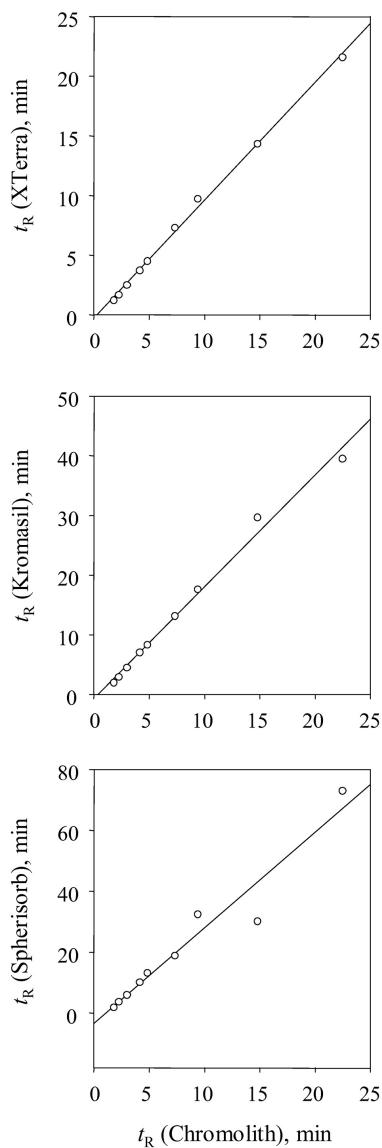


Figure 7. Correlation of the retention times for the nine β -blockers (those in Fig. 3) in a pair of columns, using a mobile phase in the region showing maximal similarity between columns (see Fig. 6). The mobile phase composition was 18% ACN for Chromolith, Kromasil and XTerra, and 20% for Spherisorb.

Leaving aside the Spherisorb column, Kromasil showed the poorest efficiencies, followed by Chromolith and XTerra. On the other hand, the larger slope for *B* indicates the tailing character of the peaks in all columns. The $c_{1,B}/c_{1,A}$ ratio was 2.65, 1.97, 1.64 and 1.24 for the Chromolith, Kromasil, Spherisorb and XTerra columns, respectively.

The reason for the tailing character of the peaks in silica-based columns is the interaction of the basic analytes with ionised silanols. In order to avoid this effect, it is common use to work at low pH (in this work, the mobile phase was buffered at pH 3.0). Since this is not sufficiently effective in many cases, another common approach is to add an amine counterion to the eluent as silanol blocker, TEA being one of the most common [43, 44]. Thus, a small improvement in the peak symmetry was reported for a basic compound, vancomycin, using a Chromolith Speed-Rod RP-18e column by addition of TEA [44].

We developed again the experimental designs for the Chromolith and Spherisorb columns, in the ranges 10–25% and 15–25% ACN, respectively, in the presence of 0.1% TEA. Figure 9 shows chromatograms obtained with a mobile phase containing 20% ACN and 0.1% TEA, for the two columns. As observed, the addition of TEA yielded an important reduction of the retention times for the Spherisorb column (see also Fig. 1), whereas the change in retention was minimal for the Chromolith column. This agrees with a previous observation that in contrast to type-A silica-based phases, the amine blocker does not have a large effect on the retention of basic compounds with the newer type-B silica-based materials [43].

In contrast, the addition of TEA produced benefits with regard to the peak shape for both columns (Chromolith and Spherisorb), but the most significant fact is that both parameters: $(c_{1,A} + c_{1,B})$ and $c_{1,B}/c_{1,A}$ became similar upon the addition of TEA ($c_{1,A} + c_{1,B} = 0.063$ and 0.065), and ($c_{1,B}/c_{1,A} = 1.86$ and 1.83), respectively (also compare Figs. 8a and d with Figs. 8b and e). There is another interesting result regarding both columns (Chromolith and Spherisorb): the similarity in the selectivities throughout the whole experimental domains was enhanced significantly upon addition of TEA (compare Figs. 6d and f).

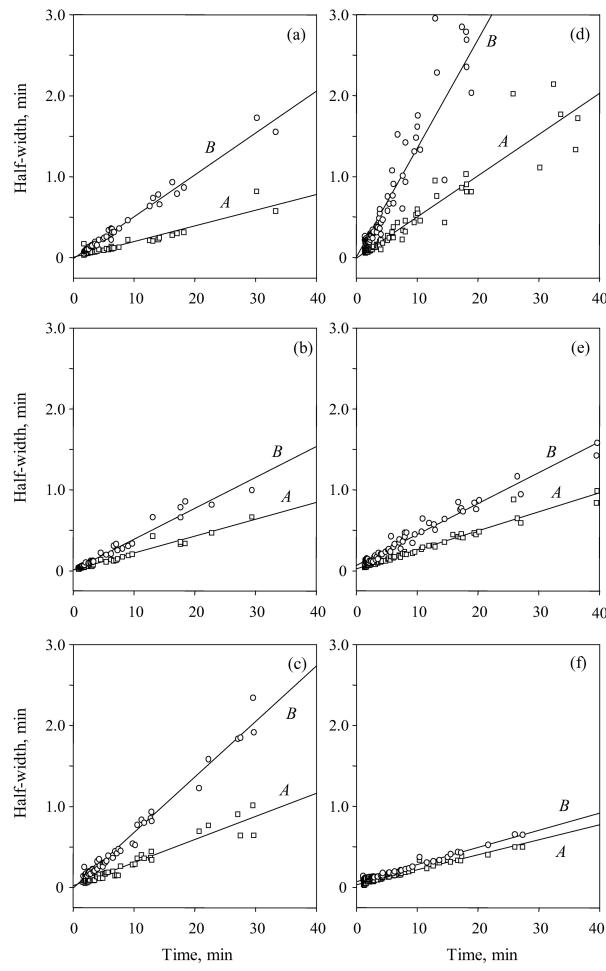


Figure 8. Correlation of the left (*A*) and right (*B*) peak half-widths with the retention time for the β -blockers, separated in: (a) Chromolith without TEA, (b) Chromolith with TEA, (c) Kromasil, (d) Spherisorb without TEA, (e) Spherisorb with TEA and (f) XTerra. Peaks obtained throughout the whole experimental design were considered for each column.

3.4 Resolution

The chromatographer is interested in achieving good resolution in sufficiently short analysis time, which depends on both the selectivity and peak shape. In order to measure (and optimise) the resolution, we used the peak purity concept, which quantifies the peak area percentage free of interference [45]. The peak purity is an objective function that takes into account the selectivity (*i.e.* peak distribution), peak shape (efficiency and asymmetry) and peak size (related to the solute concentrations and instrumental responses). The global separation in the chromatograms was evaluated using the product of elementary peak purities (*P*), which ranges from zero for full overlapping to one for full resolution [46].

Figure 10 depicts the resolution diagrams for the separation of the nine β -blockers in the whole experimental domains for the four columns (the analysis times are

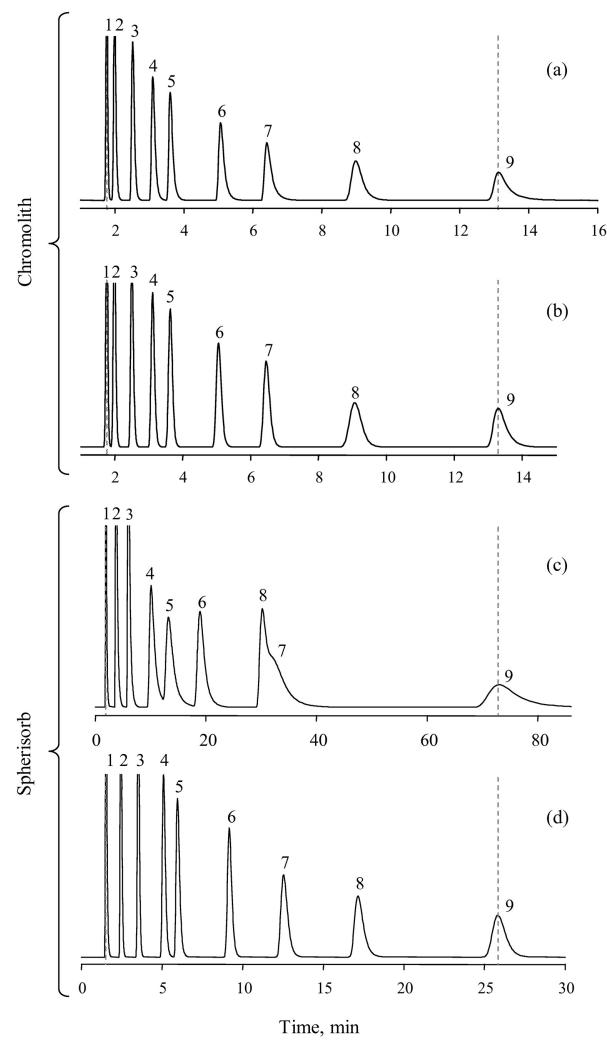


Figure 9. Chromatograms of a mixture of nine β -blockers obtained with: (a,b) Chromolith and (c,d) Spherisorb, using 20% ACN mobile phases without TEA (a,c), and in the presence of 0.1% TEA (b,d). See Fig. 3 for peak identification.

overlapped as dotted lines). As already observed in Figs. 3–5, the β -blockers exhibited good resolution in a wide range of compositions, with the Spherisorb column in the absence of TEA presenting the most critical situation followed by the Kromasil column. The resolution was complete ($P = 1$) in the whole range of working conditions for the Spherisorb column in the presence of TEA (Fig. 10e), and almost complete for the XTerra column (Fig. 10f).

Figure 11 shows the resolution diagrams for a more complex mixture that includes timolol, with a peak that, as commented, overlapped critically with those of acebutolol and metoprolol. Without TEA, only the Chromolith column was able to resolve (and only partially) the critical peaks, the maximal resolution being extremely poor for the other columns. Meanwhile, in the presence of

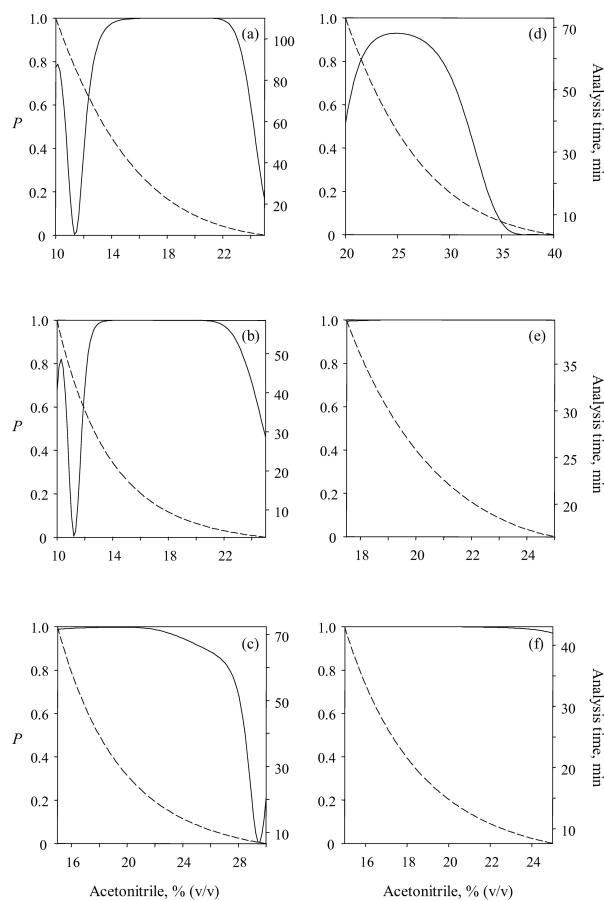


Figure 10. Resolution diagrams (full lines) and analysis times (dotted lines) for a mixture of nine β -blockers (those in Fig. 3). Columns: (a) Chromolith without TEA, (b) Chromolith with TEA, (c) Kromasil, (d) Spherisorb without TEA, (e) Spherisorb with TEA and (f) XTerra.

TEA, both Chromolith and Spherisorb succeeded in the separation of the critical pairs at low ACN content, with the analysis time of the mixture of ten β -blockers being somewhat shorter for the former column (below 30 min).

Figure 12 illustrates the situation by depicting, for the optimal mobile phase compositions, the section in the chromatograms where the three critical peaks elute. Without TEA, the columns exhibited different elution orders (it was only the same for the Kromasil and XTerra columns). However, when TEA was added, the elution order for the Chromolith and Spherisorb columns was the same, and different from that found for these columns in the absence of TEA (*i.e.* the selectivities for the critical peaks became again similar).

4 Conclusions

Numerous reports have been published, where the performance of Chromoliths are compared with one or

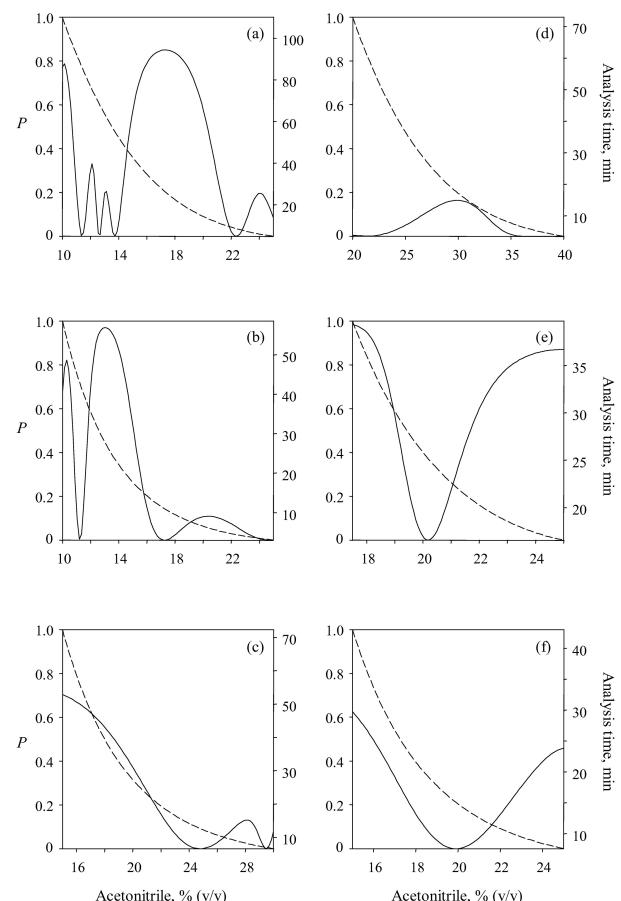


Figure 11. Resolution diagrams (full lines) and analysis times (dotted lines) for a mixture of the ten β -blockers (including timolol). Columns: (a) Chromolith without TEA, (b) Chromolith with TEA, (c) Kromasil, (d) Spherisorb without TEA, (e) Spherisorb with TEA and (f) XTerra.

more microparticulate columns of the same type, but different manufacturer. These comparisons are made usually at only one or at a few mobile phase compositions. In some cases, some similarities in selectivity have been observed among the columns. In other cases, the columns seemed to behave differently. However, the similarities may depend on the mobile phase composition. For this reason, in this work, the comparisons among columns were extended to a range of compositions. The quadratic log k versus ϕ model (Eq. 1), which yields accurate predictions in a wide range of mobile phase compositions, facilitated the study. This model can be fitted using the retention data associated to 3–4 mobile phase compositions.

There are a wide range of silica-based packed columns available in the market. Leaving aside the possibility of working at high flow rate, a Chromolith column is only one column more, with no more difference between the retention patterns with regard to a packed column, than between two packed columns of different brands. In this

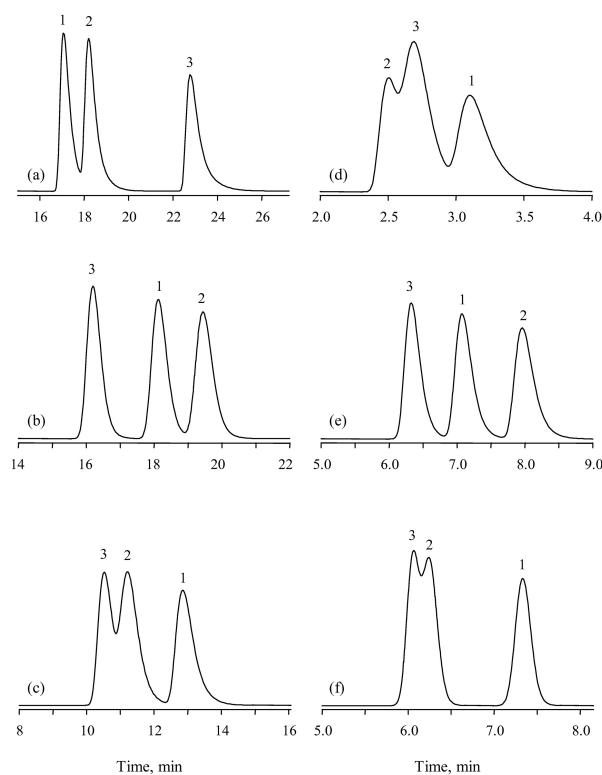


Figure 12. Optimal chromatograms for a mixture of: 1, metoprolol; 2, acebutolol; 3, timolol, obtained with: (a) Chromolith without TEA and 10% ACN ($P = 0.895$), (b) Chromolith with TEA and 10% ACN ($P = 0.981$), (c) Kromasil and 15% ACN ($P = 0.703$), (d) Spherisorb without TEA and 35.8% ACN ($P = 0.392$), (e) Spherisorb with TEA 17.5% ACN ($P = 0.983$) and (f) XTerra and 15% ACN ($P = 0.626$).

work, the Chromolith column was compared with three packed columns manufactured with different types of silica: Spherisorb (type-A silica), Kromasil (type-B) and XTerra (type-B combined with methylsilane). We were interested in examining the performance of the Chromolith column for a specific group of compounds (several β -blockers), and made a comprehensive comparison, including all the relevant chromatographic features: analysis time, selectivity, peak shape (efficiency and asymmetry) and resolution. The study showed that the Chromolith column has favourable features with regard to the other columns in the analysis of β -blockers, which are typical basic drugs. This conclusion contrasts with previous comments on the inadequacy of Chromoliths to analyse basic compounds [47]. In that report, the advantage of Chromoliths was suggested to be somewhat less for basic compounds, based on the peak shapes obtained for a few bases (pyridine, 4-ethylaniline, aniline, quinine and nortriptyline), separated in different RP-18e columns: Chromolith, Inertsil ODS-3, SymmetryShield and Purospher Star, all of them 100 \times 4.6 mm in dimension.

We have observed that the Chromolith column behaved similarly to the XTerra column in terms of anal-

ysis time and selectivity, in a wide range of mobile phase compositions, although the peak shape was improved with the XTerra column (at 1 mL/min). To a lesser extent, the selectivities for the Chromolith and Kromasil columns were also similar, but with longer analysis times for the latter. When 0.1% TEA was added to both Chromolith and Spherisorb columns, the peak shapes and selectivities, which differed significantly without TEA, became similar. This may indicate that the same blocking degree of silanol groups is achieved for both columns with TEA.

The great advantage of Chromoliths of allowing high flow rates with low backpressure should not be overlooked. The commercialisation of silica-based monolithic columns of different brands as is the case of the hundreds of packed columns in the market surely would encourage the use of these monolithic columns.

This work was supported by Project CTQ2007-61828 (Ministerio de Educación y Ciencia, MEC, of Spain) and FEDER funds. S. P. T. and M. J. R. A. thank the MEC for a FPI grant and a Ramón y Cajal contract, respectively.

The authors declared no conflict of interest.

5 References

- [1] Cabrera, K., Lubda, D., Eggenweiler, H. M., Minakuchi, H., Nakaniishi, K., *J. High Resolut. Chromatogr.* 2000, 23, 93–99.
- [2] Wu, N., Dempsey, J., Yehl, P. M., Dovletoglou, A., Ellison, D., Wyvill, J., *Anal. Chim. Acta* 2004, 523, 149–156.
- [3] Detroyer, A., Vander-Heyden, Y., Reynaert, K., Massart, D. L., *Anal. Chem.* 2004, 76, 1903–1908.
- [4] Legido-Quigley, C., Smith, N. W., *J. Chromatogr. A* 2004, 1042, 61–68.
- [5] Gerber, F., Krummen, M., Potgeter, H., Roth, A., Siffrin, C., Spoendlin, C., *J. Chromatogr. A* 2004, 1036, 127–133.
- [6] Blanco Gomis, D., Núñez, N. S., Gutiérrez-Álvarez, M. D., *J. Liq. Chromatogr. Relat. Technol.* 2006, 29, 931–948.
- [7] Yoshida, T., Majors, R. E., *J. Sep. Sci.* 2006, 29, 2421–2432.
- [8] Guillarme, D., Nguyen, D. T. T., Rudaz, S., Veuthey, J. L., *Eur. J. Pharm. Biopharm.* 2008, 68, 430–440.
- [9] Naeem, H. A., Sapirstein, H. D., *J. Cereal Sci.* 2007, 46, 157–168.
- [10] Yang, X., Ma, L., Carr, P. W., *J. Chromatogr. A* 2005, 1079, 213–220.
- [11] Wu, N., Clausen, A. M., *J. Sep. Sci.* 2007, 30, 1167–1182.
- [12] Svec, F., Huber, C. G., *Anal. Chem.* 2006, 78, 2100–2107.
- [13] Olsen, B. A., Castle, B. C., Myers, D. P., *Trends Anal. Chem.* 2006, 25, 796–805.
- [14] Tanaka, N., Kobayashi, H., Ishizuka, N., Minakuchi, H., Nakaniishi, K., Hosoya, K., Ikegami, T., *J. Chromatogr. A* 2002, 965, 35–49.
- [15] Siouffi, A. M., *J. Chromatogr. A* 2006, 1126, 86–94.
- [16] Mistry, K., Grinberg, N., *J. Liq. Chromatogr. Relat. Technol.* 2005, 28, 1055–1074.
- [17] Nakanishi, K., Minakuchi, H., Soga, N., Tanaka, N., *J. Sol-Gel Sci. Technol.* 1999, 13, 163–169.
- [18] Lubda, D., Cabrera, K., Kraas, W., Shaefer, C., Cunningham, D., *LC-GC* 2001, 19, 1186–1191.

- [19] Tanaka, H., Ishizuka, N., Hosoya, K., Kimata, K., Minakuchi, H., Nakanishi, K., Soga, N., *Kuromatogurafu* 1993, 14, 50–51.
- [20] Cabrera, K., *J. Sep. Sci.* 2004, 27, 843–852.
- [21] Miyabe, K., Guiochon, G., *J. Sep. Sci.* 2004, 27, 853–873.
- [22] Pous-Torres, S., Torres-Lapasió, J. R., Ruiz-Ángel, M. J., García-Álvarez-Coque, M. C., *J. Sep. Sci.* (in press).
- [23] Bidlingmaier, B., Unger, K. K., von Doebrin, N., *J. Chromatogr. A* 1999, 832, 11–16.
- [24] Zöllner, P., Leitner, A., Lubda, D., Cabrera, K., Lindner, W., *Chromatographia* 2000, 52, 818–820.
- [25] Spoof, L., Meriluoto, J., *J. Chromatogr. A* 2002, 947, 237–245.
- [26] Gritti, F., Guiochon, G., *Anal. Chem.* 2003, 75, 5726–5738.
- [27] Leinweber, F. C., Tallarek, U., *J. Chromatogr. A* 2003, 1006, 207–228.
- [28] Smith, J. H., McNair, H. M., *J. Chromatogr. Sci.* 2003, 41, 209–214.
- [29] van Nederkassel, A. M., Aerts, A., Dierick, A., Massart, D. L., Vander-Heyden, Y., *J. Pharm. Biomed. Anal.* 2003, 32, 233–249.
- [30] Chu, Y., Poole, C. F., *J. Chromatogr. A* 2003, 1003, 113–121.
- [31] Nováková, L., Matysová, L., Solichová, D., Koupparis, M. A., Solich, P., *J. Chromatogr. B* 2004, 813, 191–197.
- [32] Cledera-Castro, M., Santos-Montes, A., Izquierdo-Hornillos, R., *J. Chromatogr. A* 2005, 1087, 57–63.
- [33] Urbánek, L., Solichová, D., Melichar, B., Dvorák, J., Svobodová, I., Solich, P., *Anal. Chim. Acta* 2006, 573–574, 267–272.
- [34] Cruikshank, J. M., *β-Blockers in Clinical Practice*, Churchill-Livingstone, New York 1994.
- [35] Detroyer, A., Vander-Heyden, Y., Carda-Broch, S., García-Álvarez-Coque, M. C., Massart, D. L., *J. Chromatogr. A* 2001, 912, 211–221.
- [36] Gritti, F., Guiochon, G., *J. Chromatogr. A* 2004, 1038, 53–66.
- [37] Gritti, F., Guiochon, G., *J. Chromatogr. A* 2009, 1216, 1776–1788.
- [38] Köhler, J., Kirkland, J. J., *J. Chromatogr.* 1987, 385, 125–150.
- [39] Gilroy, J. J., Dolan, J. W., Snyder, L. R., *J. Chromatogr. A* 2003, 1000, 757–778.
- [40] Gilroy, J. J., Dolan, J. W., Carr, P. W., Snyder, L. R., *J. Chromatogr. A* 2004, 1026, 77–89.
- [41] Foley, J. P., Dorsey, J. G., *Anal. Chem.* 1983, 55, 730–737.
- [42] Baeza-Baeza, J. J., García-Álvarez-Coque, M. C., *J. Chromatogr. A* 2004, 1022, 17–24.
- [43] Yang, X., Dai, J., Carr, P. W., *Anal. Chem.* 2003, 75, 3153–3160.
- [44] Forlay-Frick, P., Fekete, J., *J. Liq. Chromatogr. Relat. Technol.* 2004, 27, 123–143.
- [45] López-Grío, S. J., Vivó-Truyols, G., Torres-Lapasió, J. R., García-Álvarez-Coque, M. C., *Anal. Chim. Acta* 2001, 433, 187–198.
- [46] Carda-Broch, S., Torres-Lapasió, J. R., García-Álvarez-Coque, M. C., *Anal. Chim. Acta* 1999, 396, 61–74.
- [47] McCalley, D. V., *J. Chromatogr. A* 2002, 965, 51–64.